ANTIBODIES BINDING TO VISTA AT ACIDIC pH

FIELD

The present application relates to antibodies specifically binding to the V-domain immunoglobulin-containing suppressor of T-cell activation (VISTA) at acidic pH and their use in cancer treatment.

BACKGROUND AND SUMMARY OF THE DISCLOSURE

The V-domain Ig-containing suppressor of T-cell activation, or VISTA, is a co-inhibitory member of the B7 family of immunoreceptors expressed by myelomonocytic cells and other leukocytes. However, the mechanism by which VISTA suppresses immune responses is poorly understood.

The inventors have found that unlike other known immunoreceptors, VISTA engages its counter-receptors and functions selectively at acidic pH, with little activity at physiological pH (e.g., 7.3 – 7.4). VISTA may thus suppress immune responses in acidic microenvironments, such as tumor beds or sites of inflammation, without perturbing cells circulating in blood or residing in non-inflamed, non-acidic tissues. Additionally, the inventors have found that anti-VISTA antibodies can be engineered to selectively bind to VISTA at acidic pH, with little or no binding at physiological pH, mirroring VISTA's own acidic pH selectivity. These acidic pH selective antibodies may offer desirable properties for treating diseases, such as cancer, relative to antibodies that bind VISTA at physiological pH.

The present disclosure concerns antibodies that specifically bind to the extracellular domain (ECD) of VISTA, such as human VISTA ("hVISTA" or "huVISTA") at acidic pH (e.g., in acidic conditions). The present disclosure also concerns antibodies that specifically bind to the extracellular domain (ECD) of VISTA, such as hVISTA, at acidic pH, with little or no binding at neutral or physiological pH. The inventors have noted herein that the hVISTA-ECD amino acid sequence includes a number of conserved as well as nonconserved histidine residues, and that the frequency of histidine residues in VISTA's ECD is exceptionally high relative to other B7 family members and other Immunoglobulin Superfamily members. (See Figs. 1A and 1B.) In solution, the amino acid histidine has a pK_a of about 6.5, meaning that at or below pH 6.5,

histidine residues within proteins are often protonated and thus, positively charged, while at pH higher than pH 6.5 they are increasingly unprotonated and neutral in charge. Tumor microenvironments and inflamed tissues are often acidic, and thus, VISTA proteins found in these microenvironments may be at least partially protonated at their histidine residues. The inventors, as discussed herein, have hypothesized that histidine protonation may affect the conformation, surface structure, and/or charge density of VISTA, which, in turn, may create pH-specific or pH-selective epitopes for both receptor-ligand interaction(s) and antibody binding. Targeting VISTA with antibodies that bind at acidic pH but not neutral or physiological pH may prevent target-mediated drug disposition via circulating and lymphoid organ-resident myelomonocytic cells, improving antibody PK, receptor occupancy, and activity in tumor microenvironments. Acidic pH-selective antibodies may also improve the specificity of VISTA antibodies for intratumoral, rather than circulating, target cells in the cases of therapeutic modalities such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and delivery of payloads (antibody-drug conjugates).

BRIEF DESCRIPTION OF THE FIGURES

Color versions of several of the figures herein were provided to the US Patent and Tracemark Office with the submission of the priority US provisional applications. It is presumed that, once the present application has published and the provisional applications become publicly available, the US Patent and Trademark Office will provide the color drawings upon request and payment of the necessary fee.

Figs. 1A-C show that VISTA's extracellular domain contains an exceptionally high frequency of histidine residues, that many of these histidine residues are conserved, and that at least some of these histidine residues may participate in receptor-ligand binding. **Fig. 1A** shows a graph of immunoglobulin domain-containing proteins, with the number of extracellular domain amino acid residues for each protein plotted on the x-axis, and the frequency of histidine residues within the extracellular domain for each protein plotted on the y-axis. The size of each data point corresponds to the total number of histidine residues in each protein's extracellular domain. **Fig. 1B** shows the aligned amino acid sequences of the extracellular domains of human, cynomolgus macaque, and mouse

VISTA. Signal peptide (Sig) and transmembrane domain (TMD) sequence locations are marked. Histidine residues conserved across all three species are shown in bold and underlined; histidine residues conserved across human and cynolmogus macaque are shown in bold only. **Fig. 1C** shows a model of the human VISTA immunoglobulindomain's three-dimensional structure. Histidine residues are depicted as ball and stick traces.

Figs. 2A-B show a model in which the histidine residues in VISTA's extracellular domain confer counter-receptor selectivity for acidic pH rather than physiological pH. **Fig. 2A** shows the equilibrium between the lack of, and the presence of, protonation of the pyrrole ammonium group (NH) in a histidine residue. The pKa of histidine in solution is 6.5, indicating that histidine residues are more likely to be protonated at pH 6.5 and lower, and thus, positively charged, than at higher pH. **Fig. 2B** shows shows a model in which VISTA engages P-selectin glycoprotein ligand 1 (PSGL-1) or other counter-receptors and ligands ("VISTA-R") selectively at acidic pH. Accordingly, antibody binding to VISTA's extracellular domainat at acidic pH rather at physiological pH may be critical to inhibiting or modulating VISTA activity.

Fig. 3 shows the level of VISTA surface expression (mean fluorescence intensity (MFI) of anti-VISTA antibody staining) on tumor-infiltrating macrophages, dendritic cells, neutrophils, CD4+ effector T cells, CD4+ regulatory T cells, CD8+ T cells, natural killer (NK) cells, and B cells. VISTA is expressed on many tumor-infiltrating leukocytes, particularly myeloid cells. Tumor microenvironments are often acidic, enabling VISTA to engage counter-receptors and ligands.

Figs. 4A-G show that VISTA selectively binds to leukocytes and to PSGL-1 at acidic pH, with little or no binding at neutral pH, and that this binding can be blocked by an anti-VISTA antibody. **Fig. 4A** on the left shows representative histograms of fluorescently-conjugated recombinant VISTA multimer binding to activated human CD4+ T cells. From darker gray to lighter, the filled histograms depict binding at pH 7.0, 6.5, 6.4, 6.3, 6.1, and 6.0. Some histograms are labeled with their corresponding pH. Non-VISTA control multimer binding at pH 6.0 is shown as the unfilled histogram. On the right, the mean MFI of VISTA (circles) and control (triangles) multimer binding to activated human CD4+ T cells from two donors at different pH is graphed. **Fig. 4B** shows representative histograms of recombinant VISTA multimer binding to peripheral blood

mononuclear cells (PBMC) at pH 6.0 and pH 7.4. From darker gray to lighter, the filled histograms depict binding at pH 6.0 to CD19+ B cells, CD4+ T cells, CD8+ T cells, CD56+ NK cells, and CD14+ monocytes. The unfilled, solid border and dotted border histograms depict binding at pH 7.4 to total PBMC lymphocytes and monocytes respectively. **Fig. 4C** shows representative recombinant VISTA multimer binding to activated human CD4+ T cells in the presence of an anti-VISTA blocking antibody (squares) or a non-VISTA-specific isotype-matched control antibody (circles). Antibody concentrations are plotted on log scale. Non-linear regressions are also shown. The triangle depicts the background signal from activated human CD4+ T cells that were not stained with recombinant VISTA multimers. **Fig. 4D** shows representative two-dimensional flow cytometry plots of recombinant VISTA multimer binding at pH 6.0 to heparan sulfate-deficient Chinese Hamster Ovary (CHO) cells (line pGSD-677, American Type Culture Collection) that were transfected to express human PSGL-1. Multimer binding was performed in the presence and absence of the anti-VISTA blocking antibody shown in Fig. 4C. Cells left unstained by recombinant VISTA multimers are shown as a control. PSGL-1 antibody staining is plotted on the y-axis, and VISTA multimer staining is plotted on the x-axis. **Fig. 4E** shows representative histograms of recombinant mouse VISTA-Fc fusion protein binding to mouse splenocytes at pH 6.0 and pH 7.4. From darker gray to lighter, the filled histograms depict binding at pH 6.0 to CD8+ T cells, CD11b+ myeloid cells, and CD4+ T cells. The unfilled histogram depicts binding at pH 7.4 to total splenocytes. **Figs. 4F** and **G** show representative histograms of VISTA multimer staining at pH 6.0 to human peripheral blood monocytes (Fig. 4F) and neutrophils (Fig. 4G). Cells stained at pH 7.4, with multimers not containing VISTA, or left unstained are included as controls..

Figs. 5A-D show that VISTA mediates T cell suppression and cell : cell adhesion preferentially at acidic pH, and that both effects can be reversed with an anti-VISTA blocking antibody. **Fig. 5A** shows representative cell : cell conjugate formation at pH 6.0 and 7.0 between 293T cells expressing hVISTA or vector control (plotted on the y-axes) and CHO cells endogenously expressing cell surface heparan sulfate on the x-axes. **Fig. 5B** is a graph of the frequency of cell conjugates formed at pH 6.0 between the same cells in the presence of an anti-VISTA blocking antibody, an anti-VISTA non-blocking antibody, or isotype-matched non-VISTA-specific control antibodies. **Fig. 5C** shows

representative plots of the luciferase activity generated by Jurkat (human T cell line) cells expressing an NFkB luciferase reporter after co-culture at various pH with 293T cells expressing h VISTA and a single-chain variable fragment of the anti-human T cell receptor agonist antibody OKT3 ("artificial antigen-presenting cells"). An anti-VISTA blocking antibody (squares) or an isotype-matched non-VISTA-specific control antibody (circles) were added to the co-cultured cells. In **Fig. 5D**, the data shown in Fig. 5A are plotted as fold-increase of the luciferase signal with anti-VISTA antibody treatment relative to control ("effect size").

Figs. 6A-G show that VISTA can be found in intracellular endosomes, particularly Rab11+ recycling endosomes, and can recycle to and from the cell surface via endosomal trafficking. **Fig. 6A** shows co-localization of VISTA, Rab5 (early endosome marker), Rab7 (late endosome marker), and Rab11 (recycling endosome marker) within 293T cells expressing human VISTA. **Fig. 6B** shows co-localization of VISTA and Rab11 within human monocytes. Intracellular VISTA is co-localized with Rab11+ recycling endosomes. A non-VISTA-binding control antibody of the same isotype as the VISTA antibody ("cAb") does not detectably bind the monocytes. **Fig. 6C** shows the binding of three anti-VISTA antibodies to recombinant VISTA at pH 7.4 (black), 6.7 (darker gray), and 6. (lighter gray). **Fig. 6D** shows the susceptibility of a VISTA expressing acute myeloid leukemia (AML) cell line to killing by the same anti-VISTA antibodies 1 (inverted triangles), 2 (circles), 3 (squares), or a non-VISTA-specific control antibody (triangles) bearing cathepsin B-sensitive linkers and cytotoxic payloads. Cell viability (CellTiter-Glo LU) is plotted on the y-axis and antibody concentrations are plotted on the x-axis. **Fig. 6E** compares hVISTA binding of anti-VISTA antibody 3 to that of an engineered variant ("VISTA mAb 3c") that does not exhibit impaired binding at acidic pH. **Fig. 6F** shows an antibody drug-conjugate assay comparing the potency of anti-VISTA antibody 3 (squares) and 3c (diamonds). **Fig. 6G** shows a schematic of endosome trafficking, with VISTA recycling to and from and cell surface via early endosomes and recycling endosomes.

Figs. 7A-F show how anti-VISTA antibody variant libraries were designed and screened in order to obtain acidic pH-selective antibodies. **Fig. 7A** shows amino acid substitutions that were made in VH CDR 3 of the anti-human VISTA antibody clone P1-061029 (abbreviated '029) for creating an '029 library for screening. To potentially

improve binding to VISTA's histidine-rich region at acidic pH, the libraries allowed substitutions for the negatively charged amino acids aspartate and glutamate as well as pH-responsive histidine. X=H, D or E. Bracketed sequences were removed from synthesis to avoid introducing liabilities. A total of 647 unique sequences of P1-061029 HCDR3 with 1-2 mutations were synthesized. **Fig. 7B** shows the procedure by which the '029 library is iteratively screened and selected for acidic pH-selective antibody variants. R denotes selection round. **Fig. 7C** shows representative two-dimensional flow cytometry plots data showing the variant pool after 9 rounds of selection. VISTA binding is plotted on the y-axis, and variant antibody expression is plotted on the x-axis. Binding data at various antibody concentrations and pH are shown. **Fig. 7D** shows a diagram of P1-061029 and its progeny clones binding to human VISTA at pH 6.0 and 7.4. **Fig. 7E** shows a diagram of the off-rates of P1-061029 and its progeny clones to human VISTA at pH 6.0. **Fig. 7F** shows SPR binding data of the antibodies P1-068761, P1-068767 and P1-061029 to human VISTA at pH 6.0 and pH 7.4.

Figs. 8A-F show acidic pH-selective cell binding, blocking, and effector activity of the VISTA antibodies P1-068761 and P1-068767. **Fig. 8A** and **Fig. 8B** show the mean fluorescence intensity of the acidic pH-selective antibodies P1-068761 (**Fig. 8A**) and P1-068767 (**Fig. 8B**) binding to Raji cells ectopically expressing human VISTA. The cells were stained at approximately pH 6.0 (circles; highest curve in Fig. 8A), 6.1 (squares; third highest curve), 6.2 (triangles; second highest curve), 6.4 (inverted triangles; fourth highest curve close to the pH 6.1 curve), 6.6 (diamonds; fourth curve from bottom), 7.0 (circles; third curve from bottom), 7.2 (squares; second curve from bottom), and 8.1 (unfilled triangles; bottom curve in Fig. 8A). Binding was detected with a fluorescently conjugated anti-human IgG secondary antibody. **Fig. 8C** shows P1-068767 (circles) and an isotype-matched non-specific control antibody (triangles) binding to Raji cells ectopically expressing human VISTA at 3125 ng/mL at various pH. The "pH_{50}", the pH at which 50% of P1-068767 binding is lost, is approximately 6.6. **Fig. 8D** shows the mean fluorescence intensities (MFI) of an isotype-matched non-specific control antibody (filled and unfilled circles for pH 7.0 and 6.0 respectively), anti-VISTA mAb 2 ("control", see Fig. 6C, filled and unfilled squares at pH 7.0 and 6.0 respectively), P1-068761 (filled and unfilled triangles for pH 7.0 and 6.0 respectively), and P1-068767 (filled and unfilled inverted triangles for pH 7.0 and 6.0 respectively) binding to human monocytes. Binding

was detected by a fluorescently conjugated anti-human IgG secondary antibody. **Fig. 8E** shows the comparable blocking of recombinant VISTA multimer binding to activated human CD4+ T cells at pH 6.0 by P1-061029 (squares), P1-068761 (triangles), and P1-068767 (inverted triangles), while a non-VISTA-specific control antibody (circles) did not block VISTA binding. **Fig. 8F** shows the reduced potency of P1-068761 (triangles) and P1-068767 (inverted triangles) in mediating antibody-dependent cell cytotoxicity (ADCC) at physiological pH. P1-061029 (squares), a non-VISTA-specific positive control antibody (circles), and a non-VISTA-specific negative control antibody (diamonds) are also shown. NK cell specific lysis of target cells as a percentage of total target cells is plotted on the y-axis and antibody concentrations are plotted on the x-axis. Non-linear regressions are also shown.

Fig. 9 shows enhanced pharmacokinetics (PK) of acidic pH-selective anti-VISTA antibodies in cynomolgus macaques. The figure shows serum antibody concentrations over time in cynomolgus macaques treated VISTA antibody 2 ("control", circles, see Fig. 6C), VISTA antibody 3 ("acidic pH sensitive", squares, see Fig. 6C), or P1-068767 (triangles).

Figs. 10A and 10B show the binding effects of mutations in the acidic pH-selective anti-VISTA antibodies '761 and '767. **Fig. 10A** shows kinetic binding data of P1-068761 reversion mutants at pH 7.4, pH 6.7 and pH 6.0 and the location of their reversion mutations relative to P1-068761. **Fig. 10B** shows kinetic binding data of P1-068767 reversion mutants at pH 7.4, pH 6.7 and pH 6.0 and the location of their reversion mutations relative to P1-068767.

Figs. 11A-C show epitope binning and mapping of various anti-VISTA antibodies. **Fig. 11A** shows the VISTA epitope competition for P1-068761 and P1-068767 compared to P1-061029 and VISTA antibody controls. **Fig. 11B** and **Fig. 11C** show representations of the epitopes of all the residues for blocking hVISTA antibody (Fig. 11B) as listed in Table 14 compared to a non-blocking hVISTA antibody (mAb1; Fig. 11C). Amino acid residues 66(H) and 162(A) are indicated to denote the orientation of the molecule. Histidine residues are in grey, and epitope residues are in black.

Figs. 12A-C show imaged capillary isoelectric focusing (icIEF) data for the following: **Fig. 12A**: P1-061029, **Fig. 12B**: P1-068761, and **Fig. 12C**: P1-068767. The isoelectric point of the main species (pI main) as well as pI markers are indicated.

Fig. 13A and B show alignments of variable regions for '029 and '015 progeny clones. **Fig. 13A** shows the alignment of the amino acid sequences of the variable regions of '029 and its progeny clones. **Fig. 13B** shows the alighment of the amino acid sequences of the variable regions of '015 and its progeny clones.

Fig. 14A and B show the nucleotide and amino acid sequences of 41F11 or VISTA.4 VH, showing the location of the CDRs.

Fig. 15A and B show the nucleotide and amino acid sequences of 41F11 or VISTA.4 VK1, showing the location of the CDRs.

Fig. 16A and B show the nucleotide and amino acid sequences of 41F11 or VISTA.4 VK2, showing the location of the CDRs.

Fig. 17A and B show the nucleotide and amino acid sequences of 41F11 or VISTA.4 VK3, showing the location of the CDRs.

Fig. 18A shows the cell binding of the blocking antibody VISTA.4 (pH 6.0, orange downward triangles; pH 7.0, red squares), the non-blocking antibody VISTA.5 (pH 6.0, green diamonds; pH 7.0, blue circles), and a non-VISTA-binding antibody (pH 6.0, unfilled circles; pH 7.0, unfilled upward trianges) to Raji cells ectopically expressing VISTA. Data are representative of more than four independent experiments. **Fig. 18B and 18C** show human VISTA SPR binding sensorgrams for the blocking antibody VISTA.4 (**Fig. 18B**; pH 6.0, light red; pH 6.7, red; pH 7.4, dark red) and the non-blocking antibody VISTA.5 (**Fig. 18C**; pH 6.0, light blue; pH 6.7, blue; pH 7.4, dark blue). Overlaid sensorgrams are 100 nM VISTA binding responses, normalized to the 'binding' report point. These data are representative of more than four independent experiments. **Fig. 18D** shows VISTA antibody epitope binning via VISTA.4 and VISTA.5 cross-blocking. Each row represents a unique clone, and for each clone, light grey shading indicates a lack of cross-blocking and dark grey shading indicates cross-blocking. Binding capacity at pH 6.0 relative to binding capacity at pH 7.4 is also depicted, with dark grey shading indicating a greater than 3-fold impairment in Kd at pH 6.0. These data are representative of one experiment.

Figs. 19A-C show human VISTA SPR binding sensorgrams (100 – 0.2 nM series) for VISTA.4 (pH 6.0, dotted; pH 7.4, solid) (**Figure 19A**), a pH-independent variant of VISTA.4 (pH 6.0, dotted; pH 7.4, solid) (**Figure 19B**) and an acidic pH-selective variant

of VISTA.4 (pH 6.0, dotted; pH 7.4, solid) (**Figure 19C**). These data are representative of 2 independent experiments.

Figs. 20A and 20B show the epitope of VISTA.4 as determined by MS-HDX (by MS trace Fig. 20A and sequence Fig. 20B).

Figs. 21A and 21B show VISTA multimer binding to activated human CD4+ T cells at pH 6.0 in the presence of the antibodies VISTA.4 (triangles), VISTA.5 (squares), and a non-VISTA-binding (control, circles). Figure 21B shows the blocking efficiency of each antibody relative to non-blocked T cells. One-way ANOVA with Dunnett's multiple comparisons, ***, $P < 0.001$. These data are representative of more than four independent experiments. Error bars depict the standard error of the mean.

Figs. 22A-D show that antibodies that block VISTA binding at acidic pH are functional. Effects of the blocking antibody VISTA.4 (squares), the non-blocking antibody VISTA.5 (triangles), and a non-VISTA-binding (control, circles) antibody on the proliferation (**Figure 22A**) and interferon gamma production (**Figure 22B**) of human CD4+ T cells co-cultured with 293T cells engineered to express VISTA and a TCR agonist (293T-OKT3-VISTA). Proliferation was measured as the percentage of cells exhibiting dilution of CellTrace™ Violet. One-way ANOVA with Dunnett's multiple comparisons, *, $P < 0.05$. These data are representative of three independent experiments. **Figure 22C** shows representative histograms of CellTrace™ Violet dilution on PBMC CD4+ T cells co-cultured with 293T-OKT3-VISTA cells as described in Fig. 22A. Cells were co-cultured in the presence in VISTA.4, VISTA.5, a non-VISTA-binding isotype-matched antibody, or without 293T-OKT3-VISTA cells (gray filled). As shown in the figure, the VISTA.5 and control traces closely superimpose while the VISTA.4 trace, in comparison, is shifted up and to the left. These data are representative of two independent experiments. **Figure 22D** shows results of two independent experiments in which either VISTA.4 or an isotype-matched non-VISTA-binding control antibody was added to human PBMC CD4+ T cells co-cultured with 293T-OKT3 or 293T-OKT3-VISTA cells, as described in Figure 22A. The frequency of proliferating T cells following co-culture is plotted in the figure. Paired t test, ***, $P < 0.0001$.

Fig. 23A-D show the effects of VISTA and VISTA.4 blockade on Jurkat T cell activation (by measurement of NF-kB inhibition). **Fig. 23A** shows the effects of pH on NFkB luciferase reporter Jurkat T cells co-cultured with 293T-scOKT3-VISTA cells.

Error bars depict the standard error of the mean.. These data are a composite of three independent experiments. **Fig. 23B** shows NF-kB luciferase signal in Jurkat NFkB-luciferase cells that were co-cultured with 293T-OKT3-VISTA cells as described in Figure 23 (circles). Non-co-cultured Jurkats (upward triangles) were included as controls. Data are representative of two independent experiments. Error bars depict the standard error of the mean. **Fig. 23C** shows effects of pH on VISTA suppression of human CD4+ T cells. Cells were stimulated at the indicated pH with plate coated OKT3 and VISTA-Fc in the presence of VISTA.4 (upward triangles), VISTA.5 (downward triangles), or a non-VISTA-binding antibody (antibody control, squares). Cells stimulated with plate-coated OKT3 and control IgG (VISTA control, black circles) or without OKT3 (no OKT3, grey diamond) are also shown. These data are representative of two independent experiments. **Fig. 23D** shows effects of pH on VISTA suppression of human CD8+ T cells. Cells were stimulated at the indicated pH with plate coated OKT3 and VISTA-Fc in the presence of VISTA.4 (upward triangles), VISTA.5 (downward triangles), or a non-VISTA-binding antibody (antibody control, squares). Cells stimulated with plate-coated OKT3 and control IgG (VISTA control, circles) or without OKT3 (no OKT3, gray diamond) are also shown. These data are representative of two independent experiments.

Figs. 24A-R Wildtype C57BL6 mice were implanted with MC38 tumors and treated with non-binding isotype-matched control antibodies (squares), mouse VISTA blocking antibody VISTA.10 (upward triangles), a mouse PD-1 blocking antibody (squares), or a combination of VISTA and PD-1 blocking antibodies (purple downward triangles). (See **Figs. 24A-D.**) All antibodies were mouse IgG1-D265A (Fc-inert) isotype. These data are representative of three independent experiments. **Figs. 24A-D** show the tumor volumes over time. n = 10 per group. "TF" denotes mice that rejected their tumors. **Figs. 24E-F** show the frequency of intratumoral CD8+ T cells (**Fig. 24E**) and CD4+ T cells (**Fig. 24F**) 7 days after the start of treatment. n = 5 per group. One-way ANOVA with Dunnett's multiple comparisons, P = 0.0001. **Figs. 24G and 24H** show median tumor volume over time in each group of mice (isotype control: circles; anti-PD1 D265A: squares; anti-VISTA D265A: upward pointing triangles; combination of anti-PD-1 D265A and anti-VISTA D265A: downward pointing triangles). **Fig. 24I**: VISTA knockout mice and wildtype littermates were implanted with MC38 tumors and treated with non-binding isotype-matched control antibodies (upper two curves (0/7 TF and 0/5

TF, marked with circles and downward triangles) or with a mouse PD-1 blocking antibody (lower two curves 0/5 TF and 5/8 TF, marked with squares and downward triangles). Median tumor growth and the number of mice that were tumor-free (TF) at the end of the study vs. the total number of mice are shown next to each curve (e.g, 0/7 TF). These data are representative of two independent experiments. Error bars depict the interquartile range. **Figs. 24J-M** show the tumor volumes of human VISTA knock-in (KI) mice implanted with MC38 tumors and treated with non-binding isotype-matched control antibodies (**Fig. 24J**), a mouse PD-1 blocking antibody (**Fig. 24K**), a combination of mouse PD-1 blocking antibody and the non-pH-selective human VISTA blocking antibody P1-061029 (**Fig. 24L**), or a combination of mouse PD-1 blocking antibody and the acidic pH-selective human VISTA blocking antibody P1-068767 (**Fig. 24M**). All antibodies were mouse IgG1-D265A isotype. Tumor volumes over time are shown. n = 5-8 per group. These data are representative of one independent experiment. **Fig. 24N** shows human VISTA knock-in (KI) and wildtype (WT) littermate mouse serum antibody concentrations after intravenous injection of 5 mg/kg of P1-061029 ("VISTA.16") (WT, downward triangles; KI, squares) or P1-068767 ("VISTA.18") (WT, upward triangles; KI, diamonds). The calculated serum mean residence times (MRT) for P1-061029 and P1-068767 in KI mice are estimated to be 4.1 and 71 hours respectively. n = 4 KI mice and 1-2 WT mice per antibody. These data are representative of a single experiment. **Fig. 24O** shows Cynomolgus macaque serum antibody concentrations after intravenous injection of 5 mg/kg of VISTA.4 (circles) or P1-068767 ("VISTA.18") (squares). The calculated serum mean residence times (MRT) for VISTA.4 and P1-068767 are estimated to be 7.6 hours and 717 hours respectively. n = 1 macaque per antibody. These data are representative of a single experiment. Error bars depict the standard error of the mean where not otherwise indicated. **Figs. 24P and Q** show lack of VISTA antibody single agent activity in human VISTA knock-in mice. Human VISTA knock-in mice were implanted with MC38 tumors and treated as described in Figs. 24J-M. **Fig. 24R** shows antibody blood concentration in wildtype mice were treated with a single intravenous injection of 200 µg of an anti-mouse VISTA antibody (downward triangles) or an isotype-matched control antibody (squares). n = 2 per antibody. These data are representative of two independent experiments. Error bars depict the standard error of the mean.

Figs. 25A-C show representative histograms of intratumoral CD8+ T cell expression of PD-1 (Fig. 25A), LAG-3 (Fig. 25B), and TIM-3 (Fig. 25C) 7 days after the start of treatment. Error bars depict the standard error of the mean. In each sub-part, the control, anti-PD-1, and anti-VISTA main peaks closely superimpose on the right side of each panel, while the combination main peak appears to the left.

Figs. 26A-C show that VISTA binds to PSGL-1 at acidic pH and that this interaction is blocked by VISTA antibodies P1-061029, P1-068761, P1-068767 and VISTA.4. **Fig. 26A** shows BLI binding sensorgrams for P-Selectin-Fc and VISTA-Fc binding to captured PSGL1 at pH 6.0 and pH 7.4. Figure 26B is a histogram showing that antibodies P1-061029, P1-068761, P1-068767 and VISTA.4 inhibit binding of PSGL-1 to hVISTA. **Fig. 26C** shows antibody blockade of VISTA-Fc binding to CHO-PSGL-1 cells by VISTA.4 (upward triangles) and by the anti-PSGL-1 antibody KPL-1 (circles). These data are representative of two independent experiments. Error bars depict the standard error of the mean.

Figs. 27A-E show representations of the co-crystal structure of P1-068767 Fab and hVISTA, or (in Fig. 27E) non-blocking antibody VISTA.5 and hVISTA. The VISTA IgV domain features an unusual, histidine-rich extension of its central ß-sheet. The VISTA IgV domain was co-crystallized with the P1-068767 Fragment antigen-binding (Fab). The crystal structure of the VISTA + P1-068767 complex was determined at 1.6 Å resolution. **Figure 27A** shows VISTA IgV domain : P1-068767 Fab co-crystal structure. Figure 27A shows the overall structure of the VISTA IgV domain in complex with the P1-068767 Fab (heavy chain, dark gray; light chain, light gray). **Figure 27B** shows a superimposition of the VISTA and PD-L1 IgV domains. VISTA histidine residues are depicted in stick representation. Figure 27B shows that VISTA's IgV domain possesses an unusual histidine-rich ß-sheet extension. **Figure 27C** shows the molecular surface of the P1-068767 epitope (light grey electrostatic surface) as revealed by the VISTA + P1-068767 crystal structure. Figure 27C shows that blocking antibodies bind to VISTA's histidine-rich ß-sheet extension. **Figure 27D** shows an enlarged view of the interface between VISTA (grey ribbon cartoon, with epitope residues H121, H122, and H123 depicted in stick representation) and P1-068767 (depicted as an electrostatic surface with its residues E100 and D102 in stick representation). Figure 27D shows that acidic pH-selective P1-068767 engages VISTA histidines with acidic residues. **Figure 27E** shows

that non-blocking antibody VISTA.5 binds in a different region of hVISTA from P1-068767.

FIGs. 28A-K show that VISTA : PSGL-1 binding specificity is determined by histidine and sulfotyrosine residues. As shown in **Fig 28A**, human PSGL-1 19-mer-Fc recombinant proteins were produced in cells with or without sialyl lewis X decoration (SLX+ and SLX- respectively). BLI binding magnitudes at pH 6.0 (white) and 7.4 (black) are shown for VISTA-Fc and P-selectin-Fc as indicated. Data are representative of a single independent experiment. As shown in **Fig. 28B**, human PSGL-1 19-mer-Fc glycopeptides produced with sialyl lewis X decoration were separated into fractions with greater than 90% tyrosine sulfation (sY-rich) and less than 1% tyrosine sulfation (sY-poor). BLI binding magnitudes at pH 6.0 (white) and 7.4 (black) are shown for VISTA-Fc and P-selectin-Fc as indicated. These data are representative of a single independent experiment. As provided in **Figs. 28C-28D**, human VISTA-Fc recombinant proteins were produced with the histidine residues at positions 153-155 left intact (WT VISTA) or replaced by alanine (H2A mutant), aspartic acid (H2D mutant), or arginine (H2R mutant). **Fig. 28C** shows BLI binding magnitudes for wildtype and mutant VISTA-Fc proteins binding to captured PSGL-1 at pH 6.0 and 7.4. These data are representative of a single experiment. **Fig. 28D** shows VISTA-Fc binding to CHO-PSGL-1 cells at pH 6.0 of WT VISTA (circles), H2A mutant (squares), H2D mutant (downward triangles), and H2R mutant (grey upward triangles), as well as a control (diamonds). These data are representative of two independent experiments. **Fig. 28E** shows a computational model of the PSGL-1 19-mer glycopeptide (top) in complex with VISTA's histidine-rich ligand interface (grey ribbons, bottom). VISTA residues H98, H100, H153, and H154 are marked. PSGL-1 residues Y46, Y48, E56, T57, and Y58 are also marked. **Fig. 28F** shows VISTA-Fc suppression of primary T cell activation at pH 6.8 as determined by the level of phosphor-NFkB, wherein VISTA-Fc is wildtype VISTA (second lane), VISTA H2A mutant (having His 153-155 mutated to alanine; third lane), VISTA H2D mutant (having His 153-155 mutated to aspartic acid; fourth lane) and VISTA H2R mutant (having His 153-155 mutated to arginine; fifth lane). These data are representative of two independent experiments. **Fig. 28G** and **H** show BLI binding magnitudes for captured VISTA.5 (a non-blocking antibody), **Fig. 28G**) and P1-061029 (a blocking antibody), **Fig. 28H**) binding to captured wildtype (WT, far left bars), histidine to alanine mutant

(H2A, second to left bars), histidine to aspartic acid mutant (H2D, second to right), and histidine to arginine mutant (H2R, far right) VISTA-Fc proteins at the indicated pH. These data are representative of one independent experiment. **Fig. 28I** shows BLI binding magnitudes of anti-PSGL-1 clone KPL1 to captured total, sulfotyrosine-poor, and sulfotyrosine-rich fractions of PSGL-1 19-mer-Fc at pH 6.0 (left bars) and pH 7.4 (right bars). These data are representative of one independent experiment. **Fig. 28J** shows BLI binding magnitudes of wildtype PSGL-1 19-mer-Fc (WT, left) and tyrosine to alanine mutant PSGL-1 19-mer-Fc (Y2A, right) to captured VISTA-Fc at the indicated pH. These data are representative of one independent experiment. **Fig. 28K** shows another view of the computational model of **Fig. 28E** of the PSGL-1 19-mer glycopeptide (top) in complex with VISTA's histidine-rich ligand interface (grey ribbons, bottom).

Fig. 29 is a table showing binding kinetics of mutated hVISTA protein with hPSGL-1 or anti-VISTA antibodies.

DETAILED DESCRIPTION

Definitions

In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. The terms "comprising," "including," and "having" can be used interchangeably herein. According to the present invention, an "isolated" molecule is a molecule that has been removed from its natural milieu. As such, the term "isolated" does not necessarily reflect the extent to which the molecule has been purified.

The term "polypeptide" refers to a polymer of amino acid residues, and is not limited to a minimum length. A "protein" may comprise one or more polypeptides. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" or "protein" refers to a polypeptide or protein, respectively, which includes modifications, such as

deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts that produce the proteins or errors due to PCR amplification. A protein may comprise two or more polypeptides.

"VISTA" is an abbreviation for the V-domain immunoglobulin-containing suppressor of T-cell activation protein, which is a member of the B7 family of immune checkpoint regulators. VISTA is also known as the PD-1 homolog (PD1H), B7-H5, C10orf54, differentiation of ESC-1 (Dies-1), platelet receptor Gi24 precursor, and death domain 1α (DD1α). The term "hVISTA" or "huVISTA" herein refers to the human VISTA protein. The amino acid sequence of hVISTA, including its signal peptide is provided in SEQ ID NO:1, while the sequence without the signal peptide is provided in SEQ ID NO:2. (See the Sequence Table below.) The extracellular domain or "ECD" of VISTA or the "VISTA-ECD" refers to the portion of the VISTA protein that is located in the extracellular space, which, in the case of hVISTA, comprises the amino acids 1-162 of SEQ ID NO:2. (See also Fig. 1B.) The "IgV domain" portion of hVISTA comprises residues 5-135 of SEQ ID NO:2.

The term "leader peptide" or "leader sequence" refers to a sequence of amino acid residues located at the N terminus of a polypeptide that facilitates secretion of a polypeptide from a mammalian cell. A leader sequence may be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Leader sequences may be natural or synthetic, and they may be heterologous or homologous to the protein to which they are attached.

The term "antibody" or "Ab" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. As used herein, the term refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term antibody includes, but is not limited to, fragments that are capable of binding antigen, e.g. "antigen binding fragments" or "antibody fragments," such as Fv, single-

chain Fv (scFv), Fab, Fab', and $(Fab')_2$. The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, human antibodies, and antibodies of various species such as mouse, cynomolgus monkey, *etc.*

The term "heavy chain" or "HC" refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term "full-length heavy chain" refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence, and with or without a C-terminal lysine (K).

The term "heavy chain variable region" or "VH" refers to a region comprising a heavy chain complementary determining region (CDR) 1, framework region (FR) 2, CDR2, FR3, and CDR3 of the heavy chain. In some embodiments, a heavy chain variable region also comprises at least a portion of an FR1 and/or at least a portion of an FR4. As specified below, in some embodiments, a heavy chain CDR1 comprises residues 26-35 of a VH SEQ ID NO herein; a heavy chain CDR2 comprises residues 50-66 of a VH SEQ ID NO herein, and a heavy chain CDR3 comprises residues 99-110 of a VH SEQ ID NO herein. In other embodiments, if specified, a heavy chain CDR1 corresponds to Kabat residues 31 to 35; a heavy chain CDR2 corresponds to Kabat residues 50 to 65; and a heavy chain CDR3 corresponds to Kabat residues 95 to 102. *See, e.g.*, Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, Md.). In some embodiments the heavy chain CDRs are as specified herein, such as in the sequence table below or in Table 2.

The term "light chain" or "LC" refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term "full-length light chain" refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.

The term "light chain variable region" or "VL" refers to a region comprising a light chain CDR1, FR2, HVR2, FR3, and HVR3. In some embodiments, a light chain variable region also comprises an FR1 and/or an FR4. As specified below, in some embodiments, a light chain CDR1 comprises residues 24-35 of a VL SEQ ID NO herein; a light chain CDR2 comprises residues 51-57 of a VL SEQ ID NO herein, and a light

chain CDR3 comprises residues 90-98 of a VL SEQ ID NO herein. In other embodiments, if specified, a light chain CDR1 corresponds to Kabat residues 24 to 34; a light chain CDR2 corresponds to Kabat residues 50 to 56; and a light chain CDR3 corresponds to Kabat residues 89 to 97. *See, e.g.*, Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, Md.). In some embodiments, the light chain CDRs are as specified herein such as in the sequence table.

A "chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species. In some embodiments, a chimeric antibody refers to an antibody comprising at least one variable region from a first species (such as mouse, rat, cynomolgus monkey, *etc.*) and at least one constant region from a second species (such as human, cynomolgus monkey, *etc.*). In some embodiments, a chimeric antibody comprises at least one mouse variable region and at least one human constant region. In some embodiments, a chimeric antibody comprises at least one cynomolgus variable region and at least one human constant region. In some embodiments, all of the variable regions of a chimeric antibody are from a first species and all of the constant regions of the chimeric antibody are from a second species.

A "humanized antibody" refers to an antibody in which at least one amino acid in a framework region of a non-human variable region has been replaced with the corresponding amino acid from a human variable region. In some embodiments, a humanized antibody comprises at least one human constant region or fragment thereof. In some embodiments, a humanized antibody is an Fab, an scFv, a $(Fab')_2$, *etc.*

A "human antibody" as used herein refers to antibodies produced in humans, antibodies produced in non-human animals that comprise human immunoglobulin genes, such as XenoMouse®, and antibodies selected using in vitro methods, such as phage display, wherein the antibody repertoire is based on a human immunoglobulin sequences.

A "VISTA antibody" or "anti-VISTA antibody" as used herein refers to an antibody that specifically binds to VISTA under at least some conditions such as acidic pH. In some embodiments, the antibody may be a "huVISTA antibody" or an "anti-huVISTA antibody" indicting that it specifically binds to the human VISTA protein under at least some conditions such as at acidic pH. A VISTA antibody that specifically binds

to the extracellular domain (ECD) of VISTA, for example, may be termed a "VISTA-ECD antibody."

In some embodiments, an antibody may bind with higher affinity to VISTA at acidic pH than at neutral and/or physiological pH. In some embodiments, the antibody may bind with higher affinity to VISTA at acidic pH and may only bind negligibly or nonspecifically at neutral and/or physiological pH.

A "K_D" or "dissociation constant" for binding of an antibody to a protein, e.g., a VISTA-ECD protein is a measure of the affinity or specific binding of the antibody to the protein, e.g., VISTA-ECD protein. A lower K_D indicates improved binding or affinity over a higher K_D. A K_D is composed of a ratio between an "off-rate" or k_{off} or k_d and an "on-rate" or k_{on} or k_a for the antibody and polypeptide. The off-rate and on-rate are the rates at which the two binding partners associate and dissociate in the system. Thus, a slower off-rate, where the on-rate remains roughly constant, leads to higher overall affinity and thus a lower K_D. As used herein, a k_{off} of a particular value "or less" indicates that the k_{off} or "off-rate" is as specified or is slower than the rate specified.

The terms "specific binding" or "specifically binds" or like terms signify that the K_D for the binding of two polypeptides, such as an antibody and its polypeptide target, is less than would be the case between two random polypeptides existing under the same conditions. In other words, the K_D is less than that due to nonspecific aggregation of polypeptides in the system.

In some embodiments, the antibodies specifically bind to a VISTA-ECD protein at a particular pH or pH range. An "acidic" pH herein generally refers to a pH less than 7.0, a "basic" pH generally refers to a pH higher than 7.0 and a "neutral" pH generally refers to a pH of about 7.0. A "physiological pH" herein refers to a pH in normal (i.e., non-cancerous) physiological conditions, e.g., from 7.35 to 7.45, or from 7.3 to 7.4, such as of about 7.4. Phrases such as "binding in acidic conditions" or "binding in physiological conditions" and the like herein, used in the context of binding of two molecules such as VISTA and a VISTA binding partner or VISTA and a T cell, refer to binding in acidic pH and binding in physiological pH, respectively.

When referring to an antibody that "blocks binding of" or "inhibits binding of" a ligand (or receptor) or a competing antibody to a receptor (or ligand) alone or on a cell, binding is blocked if there is an overall decrease that is statistically significant compared

to a control, e.g., an overall decrease of 50% or greater, e.g., an overall decrease of 75%, 80%, 85%, 90%, 95%, or greater. An "anti-VISTA blocking antibody," for example, is one that can block binding of VISTA to PSGL-1 or another VISTA ligand or receptor or heparan sulfate proteoglycans under at least some conditions such as at acidic pH.

A "tumor model," as used herein, refers to an *in vivo* preclinical assay, which may be used for studying the biological activity of a VISTA-ECD antibody, and includes xenograft or native mouse tumor assay systems. In some cases, a tumor model may allow for tracking of tumor size or growth upon treatment with the antibody, and/or tracking of the presence of immune cells in the tumor, such as specific types of T-cells or NK cells, in order to determine whether an antibody has triggered or enhanced an immune response.

The term "immune stimulating agent" as used herein refers to a molecule that stimulates the immune system by either acting as an agonist of an immune-stimulatory molecule, including a co-stimulatory molecule, or acting as an antagonist of an immune inhibitory molecule, including a co-inhibitory molecule. The immune-stimulatory molecule or immune inhibitory molecule may be an immune checkpoint regulator such as VISTA or another B7 family member or another molecule as described further below. An immune stimulating agent may be a biologic, such as an antibody or antibody fragment, other protein, or vaccine, or may be a small molecule drug. An "immune stimulatory molecule" includes a receptor or ligand that acts to enhance, stimulate, induce, or otherwise "turn-on" an immune response. Immune stimulatory molecules as defined herein include co-stimulatory molecules. An "immune inhibitory molecule" includes a receptor or ligand that acts to reduce, inhibit, suppress, or otherwise "turn-off" an immune response. Immune inhibitory molecules as defined herein include co-inhibitory molecules. Such immune stimulatory and immune inhibitory molecules may be, for example, receptors or ligands found on immune cells such as a T cells, or found on cells involved in innate immunity such as NK cells.

"Percent (%) amino acid sequence identity" and "homology" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for

purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

The terms "trigger" or "enhance" refer to an initiation or increase of any event (such as protein ligand binding) or to an initiation or increase of any biological activity (such as an immune response) or phenotypic characteristic or to the initiation or increase in the incidence, degree, or likelihood of that activity or characteristic. To "trigger" or "enhance" is to begin or increase an activity, function, and/or amount as compared to a reference. It is not necessary that the triggering or enhancement be complete. For example, in certain embodiments, by "enhance" is meant the ability to cause an overall increase of 20% or greater. In another embodiment, by "enhance" is meant the ability to cause an overall increase of 50% or greater. In yet another embodiment, by "enhance" is meant the ability to cause an overall increase of 75%, 85%, 90%, 95%, or greater.

The terms "inhibition" or "inhibit" more generally refer to a decrease or cessation of any event (such as protein ligand binding) or to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. To "reduce" or "inhibit" is to decrease, reduce or arrest an activity, function, and/or amount as compared to a reference. It is not necessary that the inhibition or reduction be complete. For example, in certain embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 20% or greater. In another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 50% or greater. In yet another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

"Treatment" as used herein, covers any administration or application of a therapeutic for disease in a human, and includes inhibiting the disease or progression of the disease or one or more disease symptoms, inhibiting or slowing the disease or its progression or one or more of its symptoms, arresting its development, partially or fully relieving the disease or one or more of its symptoms, or preventing a recurrence of one or more symptoms of the disease.

The terms "subject" and "patient" are used interchangeably herein to refer to a human.

The term "effective amount" or "therapeutically effective amount" refers to an amount of a drug effective for treatment of a disease or disorder in a subject, such as to partially or fully relieve one or more symptoms. In some embodiments, an effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "cancer" is used herein to refer to a group of cells that exhibit abnormally high levels of proliferation and growth. A cancer may be benign (also referred to as a benign tumor), pre-malignant, or malignant. Cancer cells may be solid cancer cells or leukemic cancer cells. The term "tumor growth" is used herein to refer to proliferation or growth by a cell or cells that comprise a cancer that leads to a corresponding increase in the size or extent of the cancer.

Examples of cancers applicable to methods of treatment herein include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive (sequential) administration in any order.

A "pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "pharmaceutical

composition" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents that can be administered in methods herein include, but are not limited to, alkylating agents such as thiotepa and Cytoxan® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (*see, e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, Adriamycin® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin,

idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, Taxol® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), Abraxane® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and Taxotere® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; Gemzar® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras, EGFR

(*e.g.*, erlotinib (Tarceva®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Further nonlimiting exemplary chemotherapeutic agents that can be administered in methods herein include anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and Fareston® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, Megase® megestrol acetate, Aromasin® exemestane, formestanie, fadrozole, Rivisor® vorozole, Femara® letrozole, and Arimidex® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (*e.g.*, Angiozyme® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, Allovectin® vaccine, Leuvectin® vaccine, and Vaxid® vaccine; Proleukin® rIL-2; Lurtotecan® topoisomerase 1 inhibitor; Abarelix® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

An "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide (including, *e.g.*, an inhibitory RNA (RNAi or siRNA)), a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent that can be administered in methods herein can include an antibody or other antagonist to an angiogenic agent, *e.g.*, antibodies to VEGF-A (*e.g.*, bevacizumab (Avastin®)) or to the VEGF-A receptor (*e.g.*, KDR receptor or Flt-1 receptor), anti-PDGFR inhibitors such as Gleevec® (Imatinib Mesylate), small molecules that block VEGF receptor signaling (*e.g.*, PTK787/ZK2284, SU6668, Sutent®/SU11248 (sunitinib malate), AMG706, or those

described in, *e.g.*, international patent application WO 2004/113304). Anti-angiogensis agents also include native angiogenesis inhibitors , *e.g.*, angiostatin, endostatin, *etc.* *See, e.g.*, Klagsbrun and D'Amore (1991) *Annu. Rev. Physiol.* 53:217-39; Streit and Detmar (2003) *Oncogene* 22:3172-3179 (*e.g.*, Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo (1999) *Nature Medicine* 5(12):1359-1364; Tonini *et al.* (2003) *Oncogene* 22:6549-6556 (*e.g.*, Table 2 listing known anti-angiogenic factors); and, Sato (2003) *Int. J. Clin. Oncol.* 8:200-206 (*e.g.*, Table 1 listing anti-angiogenic agents used in clinical trials).

A "growth inhibitory agent" as used herein refers to a compound or composition that inhibits growth of a cell (such as a cell expressing VEGF) either *in vitro* or *in vivo*. Thus, the growth inhibitory agent that can be administered in methods herein may be one that significantly reduces the percentage of cells (such as a cell expressing VEGF) in S phase. Examples of growth inhibitory agents include, but are not limited to, agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (Taxotere®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (Taxol®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "anti-neoplastic composition" refers to a composition useful in treating cancer comprising at least one active therapeutic agent. Examples of therapeutic agents include, but are not limited to, *e.g.*, chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, cancer immunotherapeutic agents, apoptotic agents, anti-tubulin agents, and other-agents to treat

cancer, such as anti-HER-2 antibodies, anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (*e.g.*, a tyrosine kinase inhibitor), HER1/EGFR inhibitor (*e.g.*, erlotinib (Tarceva®), platelet derived growth factor inhibitors (*e.g.*, Gleevec® (Imatinib Mesylate)), a COX-2 inhibitor (*e.g.*, celecoxib), interferons, CTLA4 inhibitors (*e.g.*, anti-CTLA antibody ipilimumab (YERVOY®)), PD-1 ore PD-L1 inhibitors (e.g., OPDIVO®, KEYTRUDA®, TECENTRIQ®, BAVENCIO®, IMFINZI®), TIM3 inhibitors (*e.g.*, anti-TIM3 antibodies), cytokines, antagonists (*e.g.*, neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA, CTLA4, TIM3, or VEGF receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, *etc.* Combinations thereof are also included in this disclosure.

Antibodies Specifically Binding to VISTA-ECD at Acidic pH

Because VISTA has a large number of histidine residues in its extracellular domain (ECD), its folding and overall structure, as well as the surface available for the binding of ligands such as antibodies, may differ at acidic pH compared to neutral pH, in particular, near pH 6.5, which is the pK_a for histidine. Since tumor microenvironments are generally acidic, for binding to VISTA in those microenvironments, an antibody may need to bind with specificity to VISTA at acidic pH where at least some of the surface histidine residues are more likely to be protonated.

The Sequence Table below provides the amino acid sequence of human VISTA (hVISTA) with or without signal peptide (SEQ ID NO: 1 and SEQ ID NO: 2 (mature hVISTA)), respectively. The signal peptide constitutes amino acid residues 1-32 of SEQ ID NO: 1. The extracellular domain (ECD) consists of amino acid residues 1-162 of SEQ ID NO: 2). The IgV domain constitutes amino acids residues 37-167 of SEQ ID NO: 1 and amino acid residues 5- 135 of SEQ ID NO: 2. The stalk region is at amino acid residues 172-194 of SEQ ID NO: 1 and amino acid residues 136-162 of SEQ ID NO: 2; the transmembrane domain is at amino acid residues 195-216 of SEQ ID NO: 1 and amino acid residues 163-184 of SEQ ID NO: 2. Amino acid residue 187 of SEQ ID NO: 1 and 155 of SEQ ID NO: 2 (bold and underlined) can be either D or E, which represents a polymorphism in hVISTA. That residue is shown in bold, underlining. Accordingly,

SEQ ID NO:1 and SEQ ID NO:2 encompass both of the human polymorphisms at that residue. The histidine residues in the ECD of VISTA are grey-shaded.

Anti-VISTA antibodies (Abs) may specifically bind to the VISTA-ECD or fragments thereof, e.g., comprising the IgV domain of VISTA or a region from hVISTA comprising, e.g., amino acids 20-95, 20-70 35-70, 35-95, 35-127 or 37-125 of SEQ ID NO: 2 at acidic pH. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 7.0. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 6.8. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 6.5. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 6.3. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 6.0. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 5.8. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 5.5. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 5.3. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein at a pH that is less than pH 5.0.

Certain Abs specifically bind to a VISTA-ECD protein at a pH within a range of pH 5.0-pH 7.0. Certain Abs specifically bind to the VISTA-ECD protein at a pH within a range of pH 5.0- pH 6.5. Certain Abs specifically bind to the VISTA-ECD protein at a pH within a range of pH 5.0-pH 6.0. Certain Abs specifically bind to the VISTA-ECD protein at a pH within a range of pH 5.5- pH 7.0. Certain Abs specifically bind to the VISTA-ECD protein at a pH within a range of pH 5.5-pH 6.5. Certain Abs specifically bind to the VISTA-ECD protein at a pH within a range of pH 6.0-6.5.

Provided herein are also Abs that bind to a VISTA-ECD protein, such as hVISTA-ECD or fragments thereof comprising the IgV domain of VISTA or a region from hVISTA comprising, e.g., amino acids 20-95, 20-70 35-70, 35-95, 35-127 or 37-125 of SEQ ID NO:2 at a pH of 6.5 or less, with a K_D of 10^{-6} M or less. In some embodiments, the Abs bind with a K_D of 10^{-7} M or less. In some embodiments, the Abs bind with a K_D of 10^{-8} M or less. In some embodiments, the Abs bind with a K_D of 10^{-9} M. In some embodiments, the Abs bind with a K_D of 10^{-10} M or less. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less.

Provided herein are also Abs that bind to the VISTA-ECD protein within a pH range of 6.0-6.5 with a K_D of 10^{-6} M or less. In some embodiments, the Abs bind with a K_D of 10^{-7} M or less. In some embodiments, the Abs bind with a K_D of 10^{-8} M or less. In some embodiments, the Abs bind with a K_D of 10^{-9} M. In some embodiments, the Abs bind with a K_D of 10^{-10} M or less. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less, e.g., within a pH range of 6.0-6.5, with a K_D of 10^{-7} M or less. Further, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less, e.g., within a pH range of 6.0-6.5, with a K_D of 10^{-8} M or less. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less, e.g., within a pH range of 6.0-6.5, with a K_D of 10^{-9} M or less.

Provided herein are also Abs that specifically bind to a VISTA-ECD protein, such as hVISTA-ECD or fragments thereof comprising the IgV domain of VISTA or a region from hVISTA comprising, e.g., amino acids 20-95, 20-70 35-70, 35-95, 35-127 or 37-125 of SEQ ID NO:2, e.g., at a pH of 6.5 or less, with a k_{off} of 10^{-5} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 10^{-4} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 2 10^{-4} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 5 10^{-4} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 7 10^{-4} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 10^{-3} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 2 10^{-3} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 5 10^{-3} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 7 10^{-3} s^{-1} or less at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 10^{-2} s^{-1} at either 25°C or at 37°C. In some embodiments, the Abs have a k_{off} of 10^{-1} s^{-1} or less at either 25°C or at 37°C. For example, an Ab may specifically bind to the VISTA-ECD protein at a pH of 6.5 or less with a k_{off} of 10^{-3} s^{-1} or less at either 25°C or at 37°C. An Ab may specifically bind to hVISTA-ECD at a pH of 6.5 or less with a k_{off} of 10^{-3} s^{-1} or less at either 25°C or at 37°C. Further, an Ab may specifically bind to the VISTA-ECD protein at a pH of 6.5 or less with a k_{off} of 10^{-2} s^{-1} or less at either 25°C or at 37°C.

Provided herein are Abs that bind to a VISTA-ECD protein, such as hVISTA-ECD or fragments thereof comprising the IgV domain of VISTA or a region from hVISTA comprising, e.g., amino acids 20-95, 20-70 35-70, or 35-95, 35-95, 35-127 or 37-125 of SEQ ID NO:2, e.g., at a pH of 6.5 or less, with (i) a K_D of 10^{-6} M or less, 10^{-7}

M or less, 10^{-8} M or less, 10^{-9} M or less or 10^{-10} M or less and (ii) a k_{off} rate of 10^{-5} s^{-1} or less, 10^{-4} (or 2, 5 or 7 10^{-4}) s^{-1} or less, 10^{-3} (or 2, 5 or 7 10^{-4}) s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-7} M or less and a k_{off} rate of 10^{-3} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{off} rate of 10^{-3} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{off} rate of 10^{-2} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-7} M or less and a k_{off} rate of 10^{-3} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-9} M or less and a k_{off} rate of 10^{-3} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-9} M or less and a k_{off} rate of 10^{-2} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{off} rate of 10^{-4} (or 2, 5 or 7 10^{-4}) s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{off} rate of 10^{-5} (or 2, 5 or 7 10^{-5}) s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-9} M or less and a k_{off} rate of 10^{-4} (or 2, 5 or 7 10^{-4}) s^{-1} or less, as measured, e.g., at 25°C or at 37°C. An Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-9} M or less and a k_{off} rate of 10^{-5} (or 2, 5 or 7 10^{-5}) s^{-1} or less, as measured, e.g., at 25°C or at 37°C.

Provided herein are Abs that specifically bind to the VISTA-ECD protein, e.g., at a pH of 6.5 or less, with a k_{on} of 10^4 M^{-1} s^{-1} or higher at 25°C or at 37°C. In some such embodiments, the Abs may bind with a k_{on} of 10^5 M^{-1} s^{-1} or higher. In some such embodiments, the Abs may bind with a k_{on} of 10^6 M^{-1} s^{-1} or higher. In some such embodiments, the Abs may bind with a k_{on} of 10^7 M^{-1} s^{-1} or higher. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a k_{on} of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to the ECD of hVISTA at a pH of 6.5 or less with a k_{on} of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C.

Provided herein are Abs that bind to the VISTA-ECD protein, e.g., at a pH of 6.5 or less, with (i) a K_D of 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less or 10^{-10} M or less and (ii) a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-7} M or less and a k_{on} rate of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{on} rate of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-7} M or less and a k_{on} rate of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. For example, an Ab may bind to hVISTA-ECD at a pH of 6.5 or less with a K_D of 10^{-8} M or less and a k_{on} rate of 10^6 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C.

In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-7} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, $2\ 10^{-5}$ s^{-1} or less, $5\ 10^{-5}$ s^{-1} or less, $7\ 10^{-5}$ s^{-1} or less, 10^{-4} s^{-1} or less, $2\ 10^{-4}$ s^{-1} or less, $5\ 10^{-4}$ s^{-1} or less, $7\ 10^{-4}$ s^{-1} or less, 10^{-3} s^{-1} or less, $2\ 10^{-3}$ s^{-1} or less, $5\ 10^{-3}$ s^{-1} or less, $7\ 10^{-3}$ s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-9} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C. In some such embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-10} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C.

In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-7} M or less as well as with a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less as well as with a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-9} M or less as well as with a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at

37°C. In some such embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-10} M or less as well as with a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C.

In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-7} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C, and a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-8} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C, and a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-9} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C, and a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C. In some such embodiments, an Ab may bind to the VISTA-ECD protein at a pH of 6.5 or less with a K_D of 10^{-10} M or less as well as with a k_{off} of 10^{-5} s^{-1} or less, 10^{-4} s^{-1} or less, 10^{-3} s^{-1} or less, 10^{-2} s^{-1} or less, or 10^{-1} s^{-1} or less, as measured, e.g., at 25°C or at 37°C, and a k_{on} of 10^4 M^{-1} s^{-1} or higher, 10^5 M^{-1} s^{-1} or higher, 10^6 M^{-1} s^{-1} or higher, 10^7 M^{-1} s^{-1} or higher, as measured, e.g., at 25°C or at 37°C.

As noted also above, in some of the above embodiments, the VISTA-ECD protein is hVISTA-ECD or is a portion of hVISTA-ECD such as, for example, the IgV domain. In some of the above embodiments, the Ab may bind specifically to an epitope comprising amino acids 20-95 of SEQ ID NO:2. In some of the above embodiments, the Ab may bind specifically to an epitope comprising amino acids 20-70 of SEQ ID NO:2. In some of the above embodiments, the Ab may bind specifically to an epitope comprising amino acids 35-95 of SEQ ID NO:2. In some of the above embodiments, the Ab may bind specifically to an epitope comprising amino acids 35-70 of SEQ ID NO:2. In some embodiments above, the epitope is a three-dimensional epitope that comprises not only one of the above portions of SEQ ID NO:2 from residues 20-95, 20-70, 35-95, or

35-70, but also another portion of SEQ ID NO:2, such as residues 95-105 of SEQ ID NO:2. In certain embodiments, an Ab binds to the epitope of hVISTA to which an Ab described in WO2015/097536 binds. For example, an Ab may compete or cross-compete for binding to hVISTA with an Ab disclosed in WO2015/097536. In certain embodiments, an Ab binds to a conformational epitope of human VISTA. In certain embodiments, an Ab binds to a conformational epitope that comprises, or is present within, residues 103-111 of SEQ ID NO: 2 and 136-146 of SEQ ID NO:2 for human VISTA. In certain embodiments, an Ab binds to a conformational epitope that comprises, or is present within, residues 24-36, 54-65, and 100-102 of SEQ ID NO:2 for human VISTA. In certain embodiments, an Ab binds to a conformational epitope that comprises amino acid residues in the FG loop of human VISTA. In some embodiments, an Ab binds to a polypeptide comprising amino acid residues 35 to 127 and/or 37-125 of SEQ ID NO: 2. In some embodiments, an Ab binds to a VISTA ECD polypeptide or portion thereof comprising amino acid residues 350-127 of SEQ ID NO: 2, but the antibody does not bind or binds with reduced affinity to the VISTA ECD polyptide or portion thereof comprising an amino acid substitution, wherein the substitution (1) is substitution of one of the following amino acid residues: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127 and SEQ ID NO: 2 or (2) is a substitution of one of the following amino acid residues: Y37, T39, R54, F62, Q63, H66, L115, V117, I119, S124, or E125. In some embodiments, an anti-VISTA antibody has the same binding characteristics (or significantly the same binding characteristics) as an antibody described herein, e.g., as set forth in the Examples and/or in the claims.

Some of the above antibodies may show differential binding affinity for VISTA-ECD proteins depending upon pH. Certain Abs specifically binding to a VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less, also specifically bind the VISTA-ECD protein at neutral and/or alkaline pH with similar affinity (i.e. they are "pan binders"). For example, some such Abs may bind to the VISTA-ECD protein with a K_D of 10^{-7} M or less at both pH 6.5 and at pH 7.0 (at a constant temperature, e.g., of 25°C or at 37°C) such that the K_D at pH 6.5 is within 1.5-fold of the K_D at pH 7.0. Some such Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at both pH 6.5 and at pH 7.0 (at a constant temperature, e.g., of 25°C or at 37°C) such that the K_D at pH 6.5 is within 1.5-

fold of the K_D at pH 7.0. Some such Abs may bind to hVISTA-ECD with a K_D of 10^{-8} M or less at both pH 6.5 and at pH 7.0 (at a constant temperature, e.g., of 25°C or at 37°C) such that the K_D at pH 6.5 is within 1.5-fold of the K_D at pH 7.0.

Certain Abs specifically binding to a VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less, may bind the VISTA-ECD protein at neutral, physiological, and/or alkaline conditions with lower affinity ("pH sensitive binders" or "pH sensitive Abs"). Certain Abs specifically binding to a VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less, may have non significant, e.g., nearly undetectable, binding to the VISTA-ECD protein in neutral, physiological and/or alkaline conditions. For example, in some embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D of more than 10^{-8} M at pH 7.0 and/or pH 7.4. In some such embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D at pH 7.0 and/or pH 7.4 that is more than 1.5-fold higher than that at pH 6.5. In certain embodiments, a pH sensitive Ab is provided that specifically binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold lower at pH 6.5 than at pH 7.0 (at a constant temperature, e.g., of 25°C or at 37°C). For example, in some cases an Ab binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold less at pH 6.0, relative to pH 7.0 and/or pH 7.4 or higher (at a constant temperature, e.g., of 25°C or at 37°C).

In certain embodiments, an Ab specifically binds to a VISTA-ECD protein with a k_{off} that is lower in acidic conditions relative to that in neutral, physiological, or alkaline conditions. In certain embodiments, an Ab is provided that binds to the VISTA-ECD protein in acidic conditions with a k_{off} that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, or 1000 fold lower at pH 6.5 than the k_{off} at pH 7.0 and/or pH 7.4, as measured, e.g., at 25°C or at 37°C. In other words, the off-rate is slower at acidic pH than at neutral pH. For example, in some embodiments, an Ab specifically binds to a VISTA-ECD protein with a k_{off} rate that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold or 1000 fold lower at pH 6.0, relative to pH 7.0 and/or pH 7.4, as measured, e.g., at 25°C or at 37°C. In certain embodiments, an Ab is provided that binds to the VISTA-ECD protein with a k_{off} that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20

fold, 50 fold, 100 fold or 1000 fold lower at pH 6.5 than the K_{off} at pH 7.4, as measured, e.g., at 25°C or at 37°C. In some embodiments, an Ab specifically binds to a VISTA-ECD protein with a k_{off} rate that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold or 1000 fold lower at pH 6.0, relative to pH 7.4, as measured, e.g., at 25°C or at 37°C. In certain embodiments, an Ab is provided that binds to the VISTA-ECD protein with a k_{off} that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold or 1000 fold lower at pH 6.0-6.5 than the k_{off} at pH 7.0-7.4, as measured, e.g., at 25°C or at 37°C.

In certain embodiments, an Ab that specifically binds to a VISTA-ECD protein with a k_{on} that is higher in acidic conditions relative to neutral, physiological, or alkaline conditions. In certain embodiments, an Ab is provided that binds to a VISTA-ECD protein in acidic conditions with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, or 1000 fold higher at pH 6.5 than the k_{on} at pH 7.0 and/or pH 7.4, as measured, e.g., at 25°C or at 37°C. For example, in some embodiments, an Ab specifically binds to aVISTA-ECD protein with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold or 1000 fold higher at pH 6.0 than at pH 7.0 and/or pH 7.4, as measured, e.g., at 25°C or at 37°C.

In certain embodiments, an Ab specifically binds to a VISTA-ECD protein at a pH at which at least one histidine residue, e.g., His 98 in SEQ ID NO: 1, is protonated. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein at a pH at which most histidine residues in the ECD are protonated, which is expected to be pH 6.5 or less, e.g., between pH 6.0 and pH 6.5.

Also encompassed herein are Abs that specifically bind to a VISTA-ECD protein with an affinity that is higher at neutral, physiological, or alkaline pH relative to acidic pH, provided that the affinity of binding at acidic pH remains high. For example, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at both pH 6.5 and pH 7.0 even though the Abs bind with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold lower at pH 7.0 than at pH 6.5.

Also encompassed herein are Abs that share one or more of the above properties of this section. The above properties, such as particular K_D's, k_{off}'s, k_{on}'s, specific epitopes are not to be treated in isolation. Thus, an Ab may bind to an epitope comprising one of the regions of SEQ ID NO:2 described above, and also may have pan binding or

pH sensitive or pH selective binding properties as described above, as shown by one or more of the behaviors of its K_D, k_{off}, or k_{on} at different pH's.

In any of the above embodiments, the Ab may be, for example, a full length antibody (i.e., comprising a full length heavy chain (with or without C-terminal lysine) and a full length light chain), or an antigen binding fragment such as a Fab fragment, a Fab' fragment, $(Fab')_2$ fragment, an scFv fragment, an Fv fragment, or the Ab may be a chimeric, humanized, or human antibody, or the Ab may be a bispecific or multispecific antibody.

Determining how well an Ab binds to a VISTA-ECD protein at a given pH can be conducted using several different methods. For example, by surface plasmon resonance (SPR), such as by BIACORE® assays. An exemplary SPR assay comprises capturing one or several antibodies on a CM4 sensor chip with immobilized capture reagent (e .g., using Biacore® anti-human Fc capture kit, GE Healthcare catalog # BR-1008-39, or Biacore® anti-mouse capture kit, GE Healthcare catalog #BR-1008-39), and flowing VISTA antigen as analyte in a concentration series to determine binding kinetics and affinities in a running buffer with desired pH. In one embodiment, VISTA is injected at two to five concentrations in the range of 0.1 nM to 500 nM (e.g., 0.1 nM, 1 nM, 10 nM, 100 nM, 500 nM) with a flow rate of 30 uL/min, up to four minutes association time and up to ten minutes dissociation time. Between binding cycles, the capture surface is regenerated following the manufacturer's instructions for the respective capture kit. All data is double-referenced using a reference flow cell and a blank injection. Data with simple 1:1 kinetics are fitted to a Langmuir binding model with mass transfer using the Biacore® T200 evaluation software. The SPR methods described in the Examples may also be used.

The affinity of an Ab for a VISTA ECD polypeptide may be determined using cells expressing a VISTA ECD polypeptide, PSGL-1 or heparan sulfate on their surface, which method comprises flow cytometry, and wherein binding of an Ab to cell bound VISTA-ECD is determined at a given pH, e.g., pH 6.5 or less. An exemplary flow cytometry assay comprises the following: 293T cells or other cells ectopically expressing hVISTA ECD are re-suspended in a buffer consisting of HBSS + 1% BSA adjusted to the desirable pH, e.g., pH 6.0 with MES or pH 7.4 with HEPES. Abs (e.g., human IgG) against hVISTA are serially diluted from approximately 20 μg/mLand incubated with the

re-suspended cells for 30 minutes at 4 °C. Cells are then washed twice with the same buffers, maintaining the desired pH, e.g., pH at 6.0 or 7.4, and incubated with a fluorophore-conjugated secondary antibody that recognizes the primary antibody (e.g., human IgG) and is stable at reduced pH. Cells are then washed as before and acquired immediately, without fixation, on a BD Fortessa or other flow cytometer. The affinity of an Ab for a VISTA ECD polypeptide may be determined as described in the Examples.

In certain embodiments, Abs that bind to hVISTA ECD block binding of hVISTA to its binding partner (e.g., a VISTA receptor), e.g., on cells. Inhibition or blocking may be 100% or at least 99%, 95%, 90%, 85%, 80%, 75%, or 50%. In certain embodiments, an Ab binds to a VISTA-ECD protein at acidic pH, e.g., pH 6.5 or less, and inhibits binding of VISTA to its binding partner by at least 50%, such as by at least 75%, 80%, 85%, 90%, 95%, or 100%. In certain embodiments, an Ab specifically binds to the VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 7.0. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 6.8. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 6.5. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 6.3. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 6.0. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 5.8. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 5.5. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 5.3. In certain embodiments, an Ab specifically binds to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH that is less than pH 5.0.

Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 5.0-pH 7.0.

Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 5.0- pH 6.5. Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 5.0-pH 6.0. Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 5.5- pH 7.0. Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 5.5-pH 6.5. Certain Abs specifically bind to a VISTA-ECD protein and inhibits binding of VISTA to its binding partner by at least 50% at a pH within a range of pH 6.0-6.5. Inhibition of binding can be determined as described in the Examples.

A VISTA binding partner may be PSGL-1, such as human PSGL-1. Sequences of human PSGL-1 isoforms are provided as SEQ ID NOs: 3-10 herein. VISTA binds to PSGL-1 with or without siayl lewis X. A binding partner may also be heparan sulfate proteoglycans, e.g., present on certain cells.

Inhibition of binding to a VISTA binding partner can be determined by measuring the inhibition of binding of VISTA (or VISTA ECD or VISTA IgV domain or VISTA positive cells), to cells to which VISTA binds, e.g., T cells (e.g., CD4+T cells, CD8+ T cells, either activated or not), NK cells, or other cells to which VISTA binds, in the presence and absence of the antibody. An exemplary experiment that can be used to determine if an antibody inhibits the binding of VISTA to its binding partner or T cells expressing a binding partner is a flow cytometry assay, e.g., an assay that comprises the following: human peripheral blood mononuclear cells from donor blood, buffy coat, or leukopak are re-suspended in a buffer consisting of HBSS + 1% BSA adjusted to the desirable pH, e.g., pH 6.0 with MES or pH 7.4 with HEPES. The cells are then incubated for 30 minutes at 4 °C with 20 μg/mL recombinant chimeric protein consisting of hVISTA ECD fused to human IgG1 Fc (VISTA-Fc) and with varying concentrations of candidate VISTA blocking antibodies or control antibodies. Cells are then washed twice in the same buffers, maintaining the desired pH, e.g., pH at 6.0 or 7.4, and incubated for another 30 minutes at 4 °C with a fluorophore-conjugated secondary antibody that recognizes VISTA-Fc, but not the candidate blocking antibodies or control antibodies, and is stable at reduced pH. Cells are then washed as before and acquired immediately,

without fixation, on a BD Fortessa or other flow cytometer. Inhibition of binding can be determined, e.g., as described in the Examples.

In specific embodiments, the Abs described herein may trigger or enhance an immune response, such as an antigen-specific immune response. In certain embodiments, the Abs stimulate T cell activity, particularly at acidic pH such as is found in tumor microenvironments. Stimulation of T cell activity can be measured, e.g., in a mixed lymphocyte reaction (MLR) or in an in vitro assay with an antigen presenting cell (natural or artificial) and T cells. Stimulation of T cell activity can also be measured using, e.g., the Jurkat assay described in the Examples. Stimulation of T cell activity may also be measured as described in other Examples herein, e.g., by measuring IFN-γ secretion from T cells, wherein an enhanced IFN-γ secretion indicates T cell stimulation. Secretion of other cytokines from activated T cells may also be measured. In certain embodiments, signal transduction of activated T cells is measured, such as NF-kB levels, as described, e.g., in the Examples.

In specific embodiments, the Abs described herein inhbit cell adhesion, which can be measured as described in the Examples.

Activity of anti-VISTA Abs can also be shown in monocyte assays, ADCC assays, and ADCP assays, particularly at acidic pH such as is found in tumor microenvironments.

In certain embodiments, anti-VISTA Abs inhibit tumor growth in a tumor model, e.g., a human VISTA knock-in tumor model.

As shown in the Examples herein, recycling of an anti-VISTA Ab in the endosome such as to enhance the pharmacokinetic (PK) properties, i.e., half-life, of the antibody, requires the anti-VISTA antibody to bind to VISTA in acidic conditions. Thus, anti-VISTA Abs that bind at low pH to VISTA, e.g., a pH of 6.5 or lower, as further described herein, are also expected to have a longer acceptable half-life relative to a VISTA antibody that does not bind to VISTA at acidic pH

Exemplary hVISTA-ECD binding Abs

Provided herein are Abs that bind preferentially to hVISTA (ECD) at acidic pH (e.g., in acidic conditions) relative to physiological pH or neutral pH.

In certain embodiments, an anti-hVISTA Ab comprises a heavy chain variable region ("VH") comprising VH CDR1, CDR2 and/or CDR3 of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising the VH CDR1, CDR2 and CDR3 of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising VH CDR1, CDR2 and/or CDR3 of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17. The VH CDR1, CDR2, and CDR3 of each of these species comprise amino acid positions 26-35 (VH CDR1), 50-66 (VH CDR2), and 99-110 (VH CDR3), of the VH sequences for each of the above antibody species provided in the sequence table below. The CDRs are also underlined and in bold on each of the VH sequences for the above antibody species provided in the Sequence Table below.

In certain embodiments, an anti-hVISTA Ab comprises a VL comprising VL CDR1, CDR2 and CDR3 of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VL comprising VH CDR1, CDR2 and CDR3 of one of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17. The VL CDR1, CDR2, and CDR3 of each of these species comprise amino acid positions 24-35 (VL CDR1), 51-57 (VL CDR2), and 90-98 (VL CDR3), of the VL sequences for each of the above antibody species provided in the Sequence Table below. The CDRs are also underlined and in bold on each of those sequences.

In certain embodiments, an anti-hVISTA Ab comprises a VH comprising VH CDR1, CDR2 and/or CDR3 of any of the anti-hVISTA Abs provided herein and a VL comprising CDR1, CDR2 and/or CDR3 of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising VH CDR1,

CDR2 and CDR3 of any of the anti-hVISTA Abs provided herein and a VLcomprising CDR1, CDR2 and CDR3 of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising VH CDR1, CDR2 and/or CDR3 of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17 and a VL comprising VL CDR1, CDR2 and CDR3 of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A,

P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In some embodiments, an anti-hVISTA Ab may comprise:

(a) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-061029 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-061029;

(b) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-061015 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-061015;

(c) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068757 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068757;

(d) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068759 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068759;

(e) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761;

(f) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068763 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068763;

(g) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068765 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068765;

(h) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767;

(i) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068769 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068769;

(j) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068771 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068771;

(k) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068773 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068773;

(l) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068775 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068775;

(m) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069059 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069059;

(n) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069061 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069061;

(o) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069063 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069063;

(p) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069065 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069065;

(q) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069067 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069067;

(r) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069069 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069069;

(s) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069071 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069071;

(t) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069073 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069073;

(u) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069075 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069075;

(v) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-069077 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-069077;

(w) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068736 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068736;

(x) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068738 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068738;

(y) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068740 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068740;

(z) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068742 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068742;

(aa) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068744 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068744;

(bb) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068746 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068746;

(cc) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068748 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068748;

(dd) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068750 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068750;

(ee) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068752 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068752;

(ff) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068754 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068754;

(gg) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E55A;

(hh) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_H100G and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_H100G;

(ii) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E56N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E56N;

(jj) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E55A_E56N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E55A_E56N;

(kk) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D;

(ll) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D_E55A;

(mm) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E56N_H100G and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E56N_H100G;

(nn) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D_H100G and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D_H100G;

(oo) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D_E56N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D_E56N;

(pp) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E100fF;

(qq) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E55A_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E55A_E100fF;

(rr) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_H100G_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_H100G_E100fF;

(ss) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D_E100fF;

(tt) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E56N_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E56N_E100fF;

(uu) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E32Y and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E32Y;

(vv) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E32Y_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E32Y_E55A;

(ww) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E32Y_E56N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E32Y_E56N;

(xx) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E30D_E32Y and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E30D_E32Y;

(yy) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E32Y_H100G and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E32Y_H100G;

(zz) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068761_E32Y_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068761_E32Y_E100fF;

(aaa) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_D52N_D102V and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_D52N_D102V;

(bbb) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_D52N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_D52N;

(ccc) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_D52N_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_D52N_E55A;

(ddd) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E55A_D102V and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E55A_D102V;

(eee) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_D102V and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_D102V;

(fff) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E55A;

(ggg) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E30D_D52N and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E30D_D52N;

(hhh) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E30D_D102V and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E30D_D102V;

(iii)a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E30D and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E30D;

(jjj)a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E30D_E55A and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E30D_E55A;

(kkk) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E100fF_D102V and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E100fF_D102V;

(lll) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E55A_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E55A_E100fF;

(mmm) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_D52N_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_D52N_E100fF;

(nnn) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E100fF;

(ooo) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-068767_E30D_E100fF and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-068767_E30D_E100fF;

(ppp) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of VISTA.4 and a VL comprising the VL CDR1, CDR2 and CDR3 of VISTA.4;

(qqq) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of VISTA.4_VL_A64G and a VL comprising the VL CDR1, CDR2 and CDR3 of VISTA.4_VL_A64G;

(rrr) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-070976 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-070976;

(sss) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-071799 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-071799;

(ttt) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-071801 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1- P1-071801;

(uuu) a VH comprising the amino acid sequence of the VH CDR1, CDR2 and CDR3 of P1-065333 and a VL comprising the VL CDR1, CDR2 and CDR3 of P1-065333.

Again, the Sequence Table below provides the heavy and light chain variable region sequences and full length heavy and light chain sequences of the antibodies listed above with an IgG1.3 heavy chain constant region (unless a different HC constant region is noted in the table) and notes the locations of their VH CDR1, CDR2, and CDR3 and VL CDR1, CDR2, and CDR3 by amino acid residue and with bolding and underlining of the CDRs in each VH and VL sequence. Thus, for example, VH CDR1 of P1-061029 comprises amino acids 26-35 of SEQ ID NO: 67, while VH CDR2 comprises amino acids

50-66 of SEQ ID NO: 67, and VH CDR3 comprises amino acids 99-110 of SEQ ID NO: 67, and so forth, as noted by the bolded and underlined amino acids of SEQ ID NO: 67 shown in the Sequence Table.

In certain embodiments, an anti-hVISTA Ab comprises a VH comprising the amino acid sequence of the VH of any of the anti-hVISTA Abs provided herein. The individual VH sequences for particular antibody species provided herein are listed in the Sequence Table. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising the amino acid sequence of the VH of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VH CDR1, CDR2, and CDR3 comprising the amino acid sequences of the VH CDRs of any of the anti-hVISTA Abs provided herein and comprises a VH that is at least 90%, at least 91%, at least 92%,

at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH comprising an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of the VH of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VH consisting of the amino acid sequence of the VH of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH that consists of the amino acid sequence of the VH of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065,

P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VL comprising the amino acid sequence of the VL of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VL comprising the amino acid sequence of the VL of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-

068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VL CDR1, CDR2, and CDR3 comprising the amino acid sequences of the VL CDRs of any of the anti-hVISTA Abs provided herein and comprises a VL that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VL of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VL comprising an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of the VL of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-

068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VL consisting of the amino acid sequence of the VL of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VL that consists of the amino acid sequence of the VL of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-

070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VH comprising the amino acid sequence of the VH of any of the anti-hVISTA Abs provided herein and comprises a VL comprising the amino acid sequence of the VL of any of the anti-hVISTA Abs provided herein. In certain of these embodiments, an anti-hVISTA Ab comprises a VH comprising the amino acid sequence of the VH of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF; and a VL comprising the amino acid sequence of the VL of P1-061029 or P1-061015. In certain embodiments, an anti-hVISTA Ab comprises a VH and a VL comprising the amino acid sequences of the VH and VL of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A,

P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF. In certain embodiments, the VH and VL comprise the amino acid sequences of the VH and VL of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VH CDR1, CDR2, and CDR3 comprising the amino acid sequences of the VH CDRs of any of the anti-hVISTA Abs provided herein as well as a VL CDR1, CDR2, and CDR3 comprising the amino acid sequences of the VL CDRs of any of the anti-hVISTA Abs provided herein, and also comprises a VH and a VL that are each at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the corresponding VH and VL of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises all 6 CDRs of an anti-hVISTA Ab provided herein and also comprises VH and VL amino acid sequences that are each at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the corresponding VH and VL of the anti-hVISTA Ab, such as the VH and VL of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-

068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

In certain embodiments, an anti-hVISTA Ab comprises a VH and a VL consisting of the amino acid sequence of the VH and VL of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a VH and a VL that each consist of the amino acid sequences of the VH and VL of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-

068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF. In certain embodiments, the amino acid sequences of the VH and VL each consist of the amino acid sequences of the VH and VL of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17.

An anti-hVISTA Ab may comprise:

(a) a VH comprising the amino acid sequence of the VH of P1-061029 and a VL comprising the amino acid sequence of the VL of P1-061029;

(b) a VH comprising the amino acid sequence of the VH of P1-061015 and a VL comprising the amino acid sequence of the VL of P1-061015;

(c) a VH comprising the amino acid sequence of the VH of P1-068757 and a VL comprising the amino acid sequence of the VL of P1-068757;

(d) a VH comprising the amino acid sequence of the VH of P1-068759 and a VL comprising the amino acid sequence of the VL of P1-068759;

(e) a VH comprising the amino acid sequence of the VH of P1-068761 and a VL comprising the amino acid sequence of the VL of P1-068761;

(f) a VH comprising the amino acid sequence of the VH of P1-068763 and a VL comprising the amino acid sequence of the VL of P1-068763;

(g) a VH comprising the amino acid sequence of the VH of P1-068765 and a VL comprising the amino acid sequence of the VL of P1-068765;

(h) a VH comprising the amino acid sequence of the VH of P1-068767 and a VL comprising the amino acid sequence of the VL of P1-068767;

(i) a VH comprising the amino acid sequence of the VH of P1-068769 and a VL comprising the amino acid sequence of the VL of P1-068769;

(j) a VH comprising the amino acid sequence of the VH of P1-068771 and a VL comprising the amino acid sequence of the VL of P1-068771;

(k) a VH comprising the amino acid sequence of the VH of P1-068773 and a VL comprising the amino acid sequence of the VL of -068773;

(l) a VH comprising the amino acid sequence of the VH of P1-068775 and a VL comprising the amino acid sequence of the VL of P1-068775;

(m)a VH comprising the amino acid sequence of the VH of P1-069059 and a VL comprising the amino acid sequence of the VL of P1-069059;

(n) a VH comprising the amino acid sequence of the VH of P1-069061 and a VL comprising the amino acid sequence of the VL of P1-069061;

(o) a VH comprising the amino acid sequence of the VH of P1-069063 and a VL comprising the amino acid sequence of the VL of P1-069063;

(p) a VH comprising the amino acid sequence of the VH of P1-069065 and a VL comprising the amino acid sequence of the VL of P1-069065;

(q) a VH comprising the amino acid sequence of the VH of P1-069067 and a VL comprising the amino acid sequence of the VL of P1-069067;

(r) a VH comprising the amino acid sequence of the VH of P1-069069 and a VL comprising the amino acid sequence of the VL of P1-069069;

(s) a VH comprising the amino acid sequence of the VH of P1-069071 and a VL comprising the amino acid sequence of the VL of P1-069071;

(t) a VH comprising the amino acid sequence of the VH of P1-069073 and a VL comprising the amino acid sequence of the VL of P1-069073;

(u) a VH comprising the amino acid sequence of the VH of P1-069075 and a VL comprising the amino acid sequence of the VL of P1-069075;

(v) a VH comprising the amino acid sequence of the VH of P1-069077 and a VL comprising the amino acid sequence of the VL of P1-069077;

(w) a VH comprising the amino acid sequence of the VH of P1-068736 and a VL comprising the amino acid sequence of the VL of P1-068736;

(x) a VH comprising the amino acid sequence of the VH of P1-068738 and a VL comprising the amino acid sequence of the VL of P1-068738;

(y) a VH comprising the amino acid sequence of the VH of P1-068740 and a VL comprising the amino acid sequence of the VL of P1-068740;

(z) a VH comprising the amino acid sequence of the VH of P1-068742 and a VL comprising the amino acid sequence of the VL of P1-068742;

(aa) a VH comprising the amino acid sequence of the VH of P1-068744 and a VL comprising the amino acid sequence of the VL of P1-068744;

(bb) a VH comprising the amino acid sequence of the VH f P1-068746 and a VL comprising the amino acid sequence of the VL of P1-068746;

(cc) a VH comprising the amino acid sequence of the VH of P1-068748 and a VL comprising the amino acid sequence of the VL of P1-068748;

(dd) a VH comprising the amino acid sequence of the VH of P1-068750 and a VL comprising the amino acid sequence of the VL of P1-068750;

(ee) a VH comprising the amino acid sequence of the VH of P1-068752 and a VL comprising the amino acid sequence of the VL of P1-068752;

(ff) a VH comprising the amino acid sequence of the VH of P1-068754 and a VL comprising the amino acid sequence of the VL of P1-068754;

(gg) a VH comprising the amino acid sequence of the VH of P1-068761_E55A and a VL comprising the amino acid sequence of the VL of P1-068761_E55A;

(hh) a VH comprising the amino acid sequence of the VH of P1-068761_H100G and a VL comprising the amino acid sequence of the VL of P1-068761_H100G;

(ii) a VH comprising the amino acid sequence of the VH of P1-068761_E56N and a VL comprising the amino acid sequence of the VL of P1-068761_E56N;

(jj) a VH comprising the amino acid sequence of the VH of P1-068761_E55A_E56N and a VL comprising the amino acid sequence of the VL of P1-068761_E55A_E56N;

(kk) a VH comprising the amino acid sequence of the VH of P1-068761_E30D and a VL comprising the amino acid sequence of the VL of P1-068761_E30D;

(ll) a VH comprising the amino acid sequence of the VH of P1-068761_E30D_E55A and a VL comprising the amino acid sequence of the VL of P1-068761_E30D_E55A;

(mm) a VH comprising the amino acid sequence of the VH of P1-068761_E56N_H100G and a VL comprising the amino acid sequence of the VL of P1-068761_E56N_H100G;

(nn) a VH comprising the amino acid sequence of the VH of P1-068761_E30D_H100G and a VL comprising the amino acid sequence of the VL of P1-068761_E30D_H100G;

(oo) a VH comprising the amino acid sequence of the VH of P1-068761_E30D_E56N and a VL comprising the amino acid sequence of the VL of P1-068761_E30D_E56N;

(pp) a VH comprising the amino acid sequence of the VH of P1-068761_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_E100fF;

(qq) a VH comprising the amino acid sequence of the VH of P1-068761_E55A_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_E55A_E100fF;

(rr) a VH comprising the amino acid sequence of the VH of P1-068761_H100G_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_H100G_E100fF;

(ss) a VH comprising the amino acid sequence of the VH of P1-068761_E30D_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_E30D_E100fF;

(tt) a VH comprising the amino acid sequence of the VH of P1-068761_E56N_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_E56N_E100fF;

(uu) a VH comprising the amino acid sequence of the VH of P1-068761_E32Y and a VL comprising the amino acid sequence of the VL of P1-068761_E32Y;

(vv) a VH comprising the amino acid sequence of the VH of P1-068761_E32Y_E55A and a VL comprising the amino acid sequence of the VL of P1-068761_E32Y_E55A;

(ww) a VH comprising the amino acid sequence of the VH of P1-068761_E32Y_E56N and a VL comprising the amino acid sequence of the VL of P1-068761_E32Y_E56N;

(xx) a VH comprising the amino acid sequence of the VH of P1-068761_E30D_E32Y and a VL comprising the amino acid sequence of the VL of P1-068761_E30D_E32Y;

(yy) a VH comprising the amino acid sequence of the VH of P1-068761_E32Y_H100G and a VL comprising the amino acid sequence of the VL of P1-068761_E32Y_H100G;

(zz) a VH comprising the amino acid sequence of the VH of P1-068761_E32Y_E100fF and a VL comprising the amino acid sequence of the VL of P1-068761_E32Y_E100fF;

(aaa) a VH comprising the amino acid sequence of the VH of P1-068767_D52N_D102V and a VL comprising the amino acid sequence of the VL of P1-068767_D52N_D102V;

(bbb) a VH comprising the amino acid sequence of the VH of P1-068767_D52N and a VL comprising the amino acid sequence of the VL of P1-068767_D52N;

(ccc) a VH comprising the amino acid sequence of the VH of P1-068767_D52N_E55A and a VL comprising the amino acid sequence of the VL of P1-068767_D52N_E55A;

(ddd) a VH comprising the amino acid sequence of the VH of P1-068767_E55A_D102V and a VL comprising the amino acid sequence of the VL of P1-068767_E55A_D102V;

(eee) a VH comprising the amino acid sequence of the VH of P1-068767_D102V and a VL comprising the amino acid sequence of the VL of P1-068767_D102V;

(fff) a VH comprising the amino acid sequence of the VH of P1-068767_E55A and a VL comprising the amino acid sequence of the VL of P1-068767_E55A;

(ggg) a VH comprising the amino acid sequence of the VH of P1-068767_E30D_D52N and a VL comprising the amino acid sequence of the VL of P1-068767_E30D_D52N;

(hhh) a VH comprising the amino acid sequence of the VH of P1-068767_E30D_D102V and a VL comprising the amino acid sequence of the VL of P1-068767_E30D_D102V;

(iii) a VH comprising the amino acid sequence of the VH of P1-068767_E30D and a VL comprising the amino acid sequence of the VL of P1-068767_E30D;

(jjj)a VH comprising the amino acid sequence of the VH of P1-068767_E30D_E55A and a VL comprising the amino acid sequence of the VL of P1-068767_E30D_E55A;

(kkk) a VH comprising the amino acid sequence of the VH of P1-068767_E100fF_D102V and a VL comprising the amino acid sequence of the VL of P1-068767_E100fF_D102V;

(lll) a VH comprising the amino acid sequence of the VH of P1-068767_E55A_E100fF and a VL comprising the amino acid sequence of the VL of P1-068767_E55A_E100fF;

(mmm) a VH comprising the amino acid sequence of the VH of P1-068767_D52N_E100fF and a VL comprising the amino acid sequence of the VL of P1-068767_D52N_E100fF;

(nnn) a VH comprising the amino acid sequence of the VH of P1-068767_E100fF and a VL comprising the amino acid sequence of the VL of P1-068767_E100fF; or

(ooo) a VH comprising the amino acid sequence of the VH of P1-068767_E30D_E100fF and a VL comprising the amino acid sequence of the VL of P1-068767_E30D_E100fF;

(ppp) a VH comprising the amino acid sequence of the VH of VISTA.4 and a VL comprising the VL of VISTA.4;

(qqq) a VH comprising the amino acid sequence of the VH of VISTA.4_VL_A64G and a VL comprising the VL of VISTA.4_VL_A64G;

(rrr) a VH comprising the amino acid sequence of the VH of P1-070976 and a VL comprising the VL P1-070976;

(sss) a VH comprising the amino acid sequence of the VH of P1-071799 and a VL comprising the VL of P1-071799;

(ttt) a VH comprising the amino acid sequence of the VH of P1-071801 and a VL comprising the VL of P1- P1-071801;

(uuu) a VH comprising the amino acid sequence of the VH P1-065333 and a VL comprising the VL of P1-065333.

An anti-hVISTA Ab may comprise:

(a) a VH comprising the VH CDRs of the VH of P1-061029 and a VL comprising the VL CDRs of P1-061029 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-061029;

(b) a VH comprising the VH CDRs of the VH of P1-061015 and a VL comprising the VL CDRs of P1-061015 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-061015;

(c) a VH comprising the VH CDRs of the VH of P1-068757 and a VL comprising the VL CDRs of P1-068757 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068757;

(d) a VH comprising the VH CDRs of the VH of P1-068759 and a VL comprising the VL CDRs of P1-068759 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068759;

(e) a VH comprising the VH CDRs of the VH of P1-068761 and a VL comprising the VL CDRs of P1-068761 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761;

(f) a VH comprising the VH CDRs of the VH of P1-068763 and a VL comprising the VL CDRs of P1-068763 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068763;

(g) a VH comprising the VH CDRs of the VH of P1-068765 and a VL comprising the VL CDRs of P1-068765 and VH and VL amino acid sequences that are at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068765;

(h) a VH comprising the VH CDRs of the VH of P1-068767 and a VL comprising the VL CDRs of P1-068767 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767;

(i) a VH comprising the VH CDRs of the VH of P1-068769 and a VL comprising the VL CDRs of P1-068769 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068769;

(j) a VH comprising the VH CDRs of the VH of P1-068771 and a VL comprising the VL CDRs of P1-068771 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068771;

(k) a VH comprising the VH CDRs of the VH of P1-068773 and a VL comprising the VL CDRs of P1-068773 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068773;

(l) a VH comprising the VH CDRs of the VH of P1-068775 and a VL comprising the VL CDRs of P1-068775 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068775;

(m) a VH comprising the VH CDRs of the VH of P1-069059 and a VL comprising the VL CDRs of P1-069059 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069059;

(n) a VH comprising the VH CDRs of the VH of P1-069061 and a VL comprising the VL CDRs of P1-069061 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069061;

(o) a VH comprising the VH CDRs of the VH of P1-069063 and a VL comprising the VL CDRs of P1-069063 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069063;

(p) a VH comprising the VH CDRs of the VH of P1-069065 and a VL comprising the VL CDRs of P1-069065 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069065;

(q) a VH comprising the VH CDRs of the VH of P1-069067 and a VL comprising the VL CDRs of P1-069067 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069067;

(r) a VH comprising the VH CDRs of the VH of P1-069069 and a VL comprising the VL CDRs of P1-069069 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069069;

(s) a VH comprising the VH CDRs of the VH of P1-069071 and a VL comprising the VL CDRs of P1-069071 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069071;

(t) a VH comprising the VH CDRs of the VH of P1-069073 and a VL comprising the VL CDRs of P1-069073 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069073;

(u) a VH comprising the VH CDRs of the VH of P1-069075 and a VL comprising the VL CDRs of P1-069075 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069075;

(v) a VH comprising the VH CDRs of the VH of P1-069077 and a VL comprising the VL CDRs of P1-069077 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-069077;

(w) a VH comprising the VH CDRs of the VH of P1-068736 and a VL comprising the VL CDRs of P1-068736 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068736;

(x) a VH comprising the VH CDRs of the VH of P1-068738 and a VL comprising the VL CDRs of P1-068738 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068738;

(y) a VH comprising the VH CDRs of the VH of P1-068740 and a VL comprising the VL CDRs of P1-068740 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068740;

(z) a VH comprising the VH CDRs of the VH of P1-068742 and a VL comprising the VL CDRs of P1-068742 and VH and VL amino acid sequences that are at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068742;

(aa) a VH comprising the VH CDRs of the VH of P1-068744 and a VL comprising the VL CDRs of P1-068744 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068744;

(bb) a VH comprising the VH CDRs of the VH of P1-068746 and a VL comprising the VL CDRs of P1-068746 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068746;

(cc) a VH comprising the VH CDRs of the VH of P1-068748 and a VL comprising the VL CDRs of P1-068748 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068748;

(dd) a VH comprising the VH CDRs of the VH of P1-068750 and a VL comprising the VL CDRs of P1-068750 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068750;

(ee) a VH comprising the VH CDRs of the VH of P1-068752 and a VL comprising the VL CDRs of P1-068752 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068752;

(ff) a VH comprising the VH CDRs of the VH of P1-068754 and a VL comprising the VL CDRs of P1-068754 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068754;

(gg) a VH comprising the VH CDRs of the VH of P1-068761_E55A and a VL comprising the VL CDRs of P1-068761_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E55A;

(hh) a VH comprising the VH CDRs of the VH of P1-068761_H100G and a VL comprising the VL CDRs of P1-068761_H100G and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_H100G;

(ii) a VH comprising the VH CDRs of the VH of P1-068761_E56N and a VL comprising the VL CDRs of P1-068761_E56N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E56N;

(jj) a VH comprising the VH CDRs of the VH of P1-068761_E55A_E56N and a VL comprising the VL CDRs of P1-068761_E55A_E56N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E55A_E56N;

(kk) a VH comprising the VH CDRs of the VH of P1-068761_E30D and a VL comprising the VL CDRs of P1-068761_E30D and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D;

(ll) a VH comprising the VH CDRs of the VH of P1-068761_E30D_E55A and a VL comprising the VL CDRs of P1-068761_E30D_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D_E55A;

(mm) a VH comprising the VH CDRs of the VH of P1-068761_E56N_H100G and a VL comprising the VL CDRs of P1-068761_E56N_H100G and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E56N_H100G;

(nn) a VH comprising the VH CDRs of the VH of P1-068761_E30D_H100G and a VL comprising the VL CDRs of P1-068761_E30D_H100G and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D_H100G;

(oo) a VH comprising the VH CDRs of the VH of P1-068761_E30D_E56N and a VL comprising the VL CDRs of P1-068761_E30D_E56N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D_E56N;

(pp) a VH comprising the VH CDRs of the VH of P1-068761_E100fF and a VL comprising the VL CDRs of P1-068761_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E100fF;

(qq) a VH comprising the VH CDRs of the VH of P1-068761_E55A_E100fF and a VL comprising the VL CDRs of P1-068761_E55A_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E55A_E100fF;

(rr) a VH comprising the VH CDRs of the VH of P1-068761_H100G_E100fF and a VL comprising the VL CDRs of P1-068761_H100G_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_H100G_E100fF;

(ss) a VH comprising the VH CDRs of the VH of P1-068761_E30D_E100fF and a VL comprising the VL CDRs of P1-068761_E30D_E100fF and VH and VL amino

acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D_E100fF;

(tt) a VH comprising the VH CDRs of the VH of P1-068761_E56N_E100fF and a VL comprising the VL CDRs of P1-068761_E56N_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E56N_E100fF;

(uu) a VH comprising the VH CDRs of the VH of P1-068761_E32Y and a VL comprising the VL CDRs of P1-068761_E32Y and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E32Y;

(vv) a VH comprising the VH CDRs of the VH of P1-068761_E32Y_E55A and a VL comprising the VL CDRs of P1-068761_E32Y_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E32Y_E55A;

(ww) a VH comprising the VH CDRs of the VH of P1-068761_E32Y_E56N and a VL comprising the VL CDRs of P1-068761_E32Y_E56N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E32Y_E56N;

(xx) a VH comprising the VH CDRs of the VH of P1-068761_E30D_E32Y and a VL comprising the VL CDRs of P1-068761_E30D_E32Y and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E30D_E32Y;

(yy) a VH comprising the VH CDRs of the VH of P1-068761_E32Y_H100G and a VL comprising the VL CDRs of P1-068761_E32Y_H100G and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E32Y_H100G;

(zz) a VH comprising the VH CDRs of the VH of P1-068761_E32Y_E100fF and a VL comprising the VL CDRs of P1-068761_E32Y_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068761_E32Y_E100fF;

(aaa) a VH comprising the VH CDRs of the VH of P1-068767_D52N_D102V and a VL comprising the VL CDRs of P1-068767_D52N_D102V and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_D52N_D102V;

(bbb) a VH comprising the VH CDRs of the VH of P1-068767_D52N and a VL comprising the VL CDRs of P1-068767_D52N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_D52N;

(ccc) a VH comprising the VH CDRs of the VH of P1-068767_D52N_E55A and a VL comprising the VL CDRs of P1-068767_D52N_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_D52N_E55A;

(ddd) a VH comprising the VH CDRs of the VH of P1-068767_E55A_D102V and a VL comprising the VL CDRs of P1-068767_E55A_D102V and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E55A_D102V;

(eee) a VH comprising the VH CDRs of the VH of P1-068767_D102V and a VL comprising the VL CDRs of P1-068767_D102V and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_D102V;

(fff) a VH comprising the VH CDRs of the VH of P1-068767_E55A and a VL comprising the VL CDRs of P1-068767_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E55A;

(ggg) a VH comprising the VH CDRs of the VH of P1-068767_E30D_D52N and a VL comprising the VL CDRs of P1-068767_E30D_D52N and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E30D_D52N;

(hhh) a VH comprising the VH CDRs of the VH of P1-068767_E30D_D102V and a VL comprising the VL CDRs of P1-068767_E30D_D102V and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E30D_D102V;

(iii) a VH comprising the VH CDRs of the VH of P1-068767_E30D and a VL comprising the VL CDRs of P1-068767_E30D and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E30D;

(jjj) a VH comprising the VH CDRs of the VH of P1-068767_E30D_E55A and a VL comprising the VL CDRs of P1-068767_E30D_E55A and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E30D_E55A;

(kkk) a VH comprising the VH CDRs of the VH of P1-068767_E100fF_D102V and a VL comprising the VL CDRs of P1-068767_E100fF_D102V and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E100fF_D102V;

(lll) a VH comprising the VH CDRs of the VH of P1-068767_E55A_E100fF and a VL comprising the VL CDRs of P1-068767_E55A_E100fF and VH and VL amino

acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E55A_E100fF;

(mmm) a VH comprising the VH CDRs of the VH of P1-068767_D52N_E100fF and a VL comprising the VL CDRs of P1-068767_D52N_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_D52N_E100fF;

(nnn) a VH comprising the VH CDRs of the VH of P1-068767_E100fF and a VL comprising the VL CDRs of P1-068767_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E100fF; or

(ooo) a VH comprising the VH CDRs of the VH of P1-068767_E30D_E100fF and a VL comprising the VL CDRs of P1-068767_E30D_E100fF and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-068767_E30D_E100fF;

(ppp) a VH comprising the VH CDRs of the VH of VISTA.4 and a VL comprising the VL CDRs of VISTA.4 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of VISTA.4;

(qqq) a VH comprising the VH CDRs of the VH of VISTA.4_VL_A64G and a VL comprising the VL CDRs of VISTA.4_VL_A64G and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of VISTA.4_VL_A64G;

(rrr) a VH comprising the VH CDRs of the VH of P1-070976 and a VL comprising the VL CDRs of P1-070976 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-070976;

(sss) a VH comprising the VH CDRs of the VH of P1-071799 and a VL comprising the VL CDRs of P1-071799 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-071799;

(ttt) a VH comprising the VH CDRs of the VH of P1-071801 and a VL comprising the VL CDRs of P1-071801 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-071801; or

(uuu) a VH comprising the VH CDRs of the VH of P1-065333 and a VL comprising the VL CDRs of P1-065333 and VH and VL amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the VH and VL of P1-071799.

An anti-hVISTA Ab may comprise:

(a) a VH consisting of the amino acid sequence of the VH of P1-061029 and a VL consisting of the VL of P1-061029;

(b) a VH consisting of the amino acid sequence of the VH of P1-061015 and a VL consisting of the VL of P1-061015;

(c) a VH consisting of the amino acid sequence of the VH of P1-068757 and a VL consisting of the VL of P1-068757;

(d) a VH consisting of the amino acid sequence of the VH of P1-068759 and a VL consisting of the VL of P1-068759;

(e) a VH consisting of the amino acid sequence of the VH of P1-068761 and a VL consisting of the VL of P1-068761;

(f) a VH consisting of the amino acid sequence of the VH of P1-068763 and a VL consisting of the VL of P1-068763;

(g) a VH consisting of the amino acid sequence of the VH of P1-068765 and a VL consisting of the VL of P1-068765;

(h) a VH consisting of the amino acid sequence of the VH of P1-068767 and a VL consisting of the VL of P1-068767;

(i) a VH consisting of the amino acid sequence of the VH of P1-068769 and a VL consisting of the VL of P1-068769;

(j) a VH consisting of the amino acid sequence of the VH of P1-068771 and a VL consisting of the VL of P1-068771;

(k) a VH consisting of the amino acid sequence of the VH of P1-068773 and a VL consisting of the VL of P1-068773;

(l) a VH consisting of the amino acid sequence of the VH of P1-068775 and a VL consisting of the VL of P1-068775;

(m) a VH consisting of the amino acid sequence of the VH of P1-069059 and a VL consisting of the VL of P1-069059;

(n) a VH consisting of the amino acid sequence of the VH of P1-069061 and a VL consisting of the VL of P1-069061;

(o) a VH consisting of the amino acid sequence of the VH of P1-069063 and a VL consisting of the VL of P1-069063;

(p) a VH consisting of the amino acid sequence of the VH of P1-069065 and a VL consisting of the VL of P1-069065;

(q) a VH consisting of the amino acid sequence of the VH of P1-069067 and a VL consisting of the VL of P1-069067;

(r) a VH consisting of the amino acid sequence of the VH of P1-069069 and a VL consisting of the VL of P1-069069;

(s) a VH consisting of the amino acid sequence of the VH of P1-069071 and a VL consisting of the VL of P1-069071;

(t) a VH consisting of the amino acid sequence of the VH of P1-069073 and a VL consisting of the VL of P1-069073;

(u) a VH consisting of the amino acid sequence of the VH of P1-069075 and a VL consisting of the VL of P1-069075;

(v) a VH consisting of the amino acid sequence of the VH of P1-069077 and a VL consisting of the VL of P1-069077;

(w) a VH consisting of the amino acid sequence of the VH of P1-068736 and a VL consisting of the VL of P1-068736;

(x) a VH consisting of the amino acid sequence of the VH of P1-068738 and a VL consisting of the VL of P1-068738;

(y) a VH consisting of the amino acid sequence of the VH of P1-068740 and a VL consisting of the VL of P1-068740;

(z) a VH consisting of the amino acid sequence of the VH of P1-068742 and a VL consisting of the VL of P1-068742;

(aa) a VH consisting of the amino acid sequence of the VH of P1-068744 and a VL consisting of the VL of P1-068744;

(bb) a VH consisting of the amino acid sequence of the VH f P1-068746 and a VL consisting of the VL of P1-068746;

(cc) a VH consisting of the amino acid sequence of the VH of P1-068748 and a VL consisting of the VL of P1-068748;

(dd) a VH consisting of the amino acid sequence of the VH of P1-068750 and a VL consisting of the VL of P1-068750;

(ee) a VH consisting of the amino acid sequence of the VH of P1-068752 and a VL consisting of the VL of P1-068752;

(ff) a VH consisting of the amino acid sequence of the VH of P1-068754 and a VL consisting of the VL of P1-068754;

(gg) a VH consisting of the amino acid sequence of the VH of P1-068761_E55A and a VL consisting of the amino acid sequence of the VL of P1-068761_E55A;

(hh) a VH consisting of the amino acid sequence of the VH of P1-068761_H100G and a VL consisting of the amino acid sequence of the VL of P1-068761_H100G;

(ii) a VH consisting of the amino acid sequence of the VH of P1-068761_E56N and a VL consisting of the amino acid sequence of the VL of P1-068761_E56N;

(jj) a VH consisting of the amino acid sequence of the VH of P1-068761_E55A_E56N and a VL consisting of the amino acid sequence of the VL of P1-068761_E55A_E56N;

(kk) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D;

(ll) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D_E55A and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D_E55A;

(mm) a VH consisting of the amino acid sequence of the VH of P1-068761_E56N_H100G and a VL consisting of the amino acid sequence of the VL of P1-068761_E56N_H100G;

(nn) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D_H100G and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D_H100G;

(oo) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D_E56N and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D_E56N;

(pp) a VH consisting of the amino acid sequence of the VH of P1-068761_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_E100fF;

(qq) a VH consisting of the amino acid sequence of the VH of P1-068761_E55A_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_E55A_E100fF;

(rr) a VH consisting of the amino acid sequence of the VH of P1-068761_H100G_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_H100G_E100fF;

(ss) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D_E100fF;

(tt) a VH consisting of the amino acid sequence of the VH of P1-068761_E56N_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_E56N_E100fF;

(uu) a VH consisting of the amino acid sequence of the VH of P1-068761_E32Y and a VL consisting of the amino acid sequence of the VL of P1-068761_E32Y;

(vv) a VH consisting of the amino acid sequence of the VH of P1-068761_E32Y_E55A and a VL consisting of the amino acid sequence of the VL of P1-068761_E32Y_E55A;

(ww) a VH consisting of the amino acid sequence of the VH of P1-068761_E32Y_E56N and a VL consisting of the amino acid sequence of the VL of P1-068761_E32Y_E56N;

(xx) a VH consisting of the amino acid sequence of the VH of P1-068761_E30D_E32Y and a VL consisting of the amino acid sequence of the VL of P1-068761_E30D_E32Y;

(yy) a VH consisting of the amino acid sequence of the VH of P1-068761_E32Y_H100G and a VL consisting of the amino acid sequence of the VL of P1-068761_E32Y_H100G;

(zz) a VH consisting of the amino acid sequence of the VH of P1-068761_E32Y_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068761_E32Y_E100fF;

(aaa) a VH consisting of the amino acid sequence of the VH of P1-068767_D52N_D102V and a VL consisting of the amino acid sequence of the VL of P1-068767_D52N_D102V;

(bbb) a VH consisting of the amino acid sequence of the VH of P1-068767_D52N and a VL consisting of the amino acid sequence of the VL of P1-068767_D52N;

(ccc) a VH consisting of the amino acid sequence of the VH of P1-068767_D52N_E55A and a VL consisting of the amino acid sequence of the VL of P1-068767_D52N_E55A;

(ddd) a VH consisting of the amino acid sequence of the VH of P1-068767_E55A_D102V and a VL consisting of the amino acid sequence of the VL of P1-068767_E55A_D102V;

(eee) a VH consisting of the amino acid sequence of the VH of P1-068767_D102V and a VL consisting of the amino acid sequence of the VL of P1-068767_D102V;

(fff) a VH consisting of the amino acid sequence of the VH of P1-068767_E55A and a VL consisting of the amino acid sequence of the VL of P1-068767_E55A;

(ggg) a VH consisting of the amino acid sequence of the VH of P1-068767_E30D_D52N and a VL consisting of the amino acid sequence of the VL of P1-068767_E30D_D52N;

(hhh) a VH consisting of the amino acid sequence of the VH of P1-068767_E30D_D102V and a VL consisting of the amino acid sequence of the VL of P1-068767_E30D_D102V;

(iii) a VH consisting of the amino acid sequence of the VH of P1-068767_E30D and a VL consisting of the amino acid sequence of the VL of P1-068767_E30D;

(jjj)a VH consisting of the amino acid sequence of the VH of P1-068767_E30D_E55A and a VL consisting of the amino acid sequence of the VL of P1-068767_E30D_E55A;

(kkk) a VH consisting of the amino acid sequence of the VH of P1-068767_E100fF_D102V and a VL consisting of the amino acid sequence of the VL of P1-068767_E100fF_D102V;

(lll) a VH consisting of the amino acid sequence of the VH of P1-068767_E55A_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068767_E55A_E100fF;

(mmm) a VH consisting of the amino acid sequence of the VH of P1-068767_D52N_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068767_D52N_E100fF;

(nnn) a VH consisting of the amino acid sequence of the VH of P1-068767_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068767_E100fF; or

(ooo) a VH consisting of the amino acid sequence of the VH of P1-068767_E30D_E100fF and a VL consisting of the amino acid sequence of the VL of P1-068767_E30D_E100fF;

(ppp) a VH consisting of the amino acid sequence of the VH of VISTA.4 and a VL consisting of the VL of VISTA.4;

(qqq) a VH consisting of the amino acid sequence of the VH of VISTA.4_VL_A64G and a VL consisting of the VL of VISTA.4_VL_A64G;

(rrr) a VH consisting of the amino acid sequence of the VH of P1-070976 and a VL consisting of the VL of P1-070976;

(sss) a VH consisting of the amino acid sequence of the VH of P1-071799 and a VL consisting of the VL of P1-071799;

(ttt)a VH consisting of the amino acid sequence of the VH of P1-071801 and a VL consisting of the VL of P1-071801; or

(uuu) a VH consisting of the amino acid sequence of the VH of P1-065333 and a VL consisting of the VL of P1-065333.

In certain embodiments, an anti-VISTA Ab comprises any of the variable regions and/or variable region CDRs 1-3 of the antibodies described above and elsewhere herein, such as:

(1) one or more of VH CDR1, CDR2 and CDR3 of:

(2) the VH CDR1, CDR2 and CDR3 of:

(3) the VH of:

(4) one or more of VH CDR1, CDR2 and CDR3 and one or more of VL CDR1, CDR2 and CDR3 of:

(5) the VH CDR1, CDR2 and CDR3 and VL CDR1, CDR2 and CDR3 of:

or

(6) the VH and the VLs of:

P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-

068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17; and the anti-VISTA Ab is also an IgG antibody, such as IgG1, IgG2, IgG3 or IgG4 antibody or a modified form thereof as described in the section below. In some embodiments, the constant region has effector function, and in some embodiments, the constant region is effectorless. In certain embodiments, the constant region is that of IgG1.3.

In certain embodiments, an anti-VISTA Ab comprises any of the variable regions and/or variable region CDRs 1-3 of the antibodies described above and elsewhere herein, such as:

(1) one or more of VH CDR1, CDR2 and CDR3 of:

(2) the VH CDR1, CDR2 and CDR3 of:

(3) the VH of:

(4) one or more of VH CDR1, CDR2 and CDR3 and one or more of VL CDR1, CDR2 and CDR3 of:

(5) the VH CDR1, CDR2 and CDR3 and VL CDR1, CDR2 and CDR3 of:

or

(6) the VH and the VLs of:

P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-

068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, or of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17;

and further comprises one or more of the following characteristics:

- specifically binding to hVISTA, e.g., histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at acidic pH, e.g., pH 6.0 or pH 6.5;
- lacking of significant binding to hVISTA, e.g., histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at physiological pH or neutral pH, e.g., pH 7.4 or pH 7.0;
- specifically binding to cyno VISTA, e.g., histidine rich region of the ECD, at acidic pH, e.g., pH 6.0 or pH 6.5;
- lacking of significant binding to cyno VISTA, e.g., histidine rich region of the ECD, at physiological pH or neutral pH, e.g., pH 7.4 or pH 7.0;
- having reduced binding to hVISTA-ECD having a substitution at one or more of the following amino acids: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127 relative to hVISTA ECD having SEQ ID NO: 2;

- cross-competiting for binding to hVISTA with P1-061029, P1-068761, P1-068767 and/or P1-061015;
- inhibiting binding of hVISTA to human T cells expressing VISTA (e.g., naïve or activated T cells) at acidic pH e.g., pH 6.0 or pH 6.5;
- inhibiting binding of hVISTA to PSGL-1 with or without siayl lewis X at acidic pH e.g., pH 6.0 or pH 6.5(e.g., inhibiting the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48), wherein PSGL-1 is with or without siayl lewis X, and wherein the tyrosines are preferably sulfotyrosines);
- having a mean residence time (MRT) of at least 100, 200, 300, 350, 400, 450, 500, 600, or 700 hours (e.g., at least 350 hours) in cynomolgus monkeys, measured, e.g, as described in the Examples;
- stimulating T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
- inhibiting VISTA mediated cell:cell adhesion;
- specifically binding to hVISTA in samples of human tumor cells or samples of inflamed human tissue that express VISTA;
- contacting hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;
- binding to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3: $_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;
- binding to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacting (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å) or less), such as through

hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;

- contacting hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- low or undetectable levels of TMDD;
- low or undetectable levels of neutropenia; and
- any additional characteristic set forth in the claims and/or in the Examples.

In certain embodiments, an anti-hVISTA Ab comprises a heavy chain (HC) comprising the amino acid sequence of the heavy chain of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a heavy chain comprising the amino acid sequence of the heavy chain of P1-061029 or P1-061015 or progeny thereof, as shown below in the Sequence Table, comprising an IgG1.3 heavy chain constant region, such as P1-061029.IgG1.3 (SEQ ID NO: 69), P1-068757.IgG1.3, P1-068759.IgG1.3, P1-068761.IgG1.3, P1-068763.IgG1.3, P1-068765.IgG1.3, P1-068767.IgG1.3, P1-068769.IgG1.3, P1-068771.IgG1.3, P1-068773.IgG1.3, P1-068775.IgG1.3, P1-069059.IgG1.3, P1-069061.IgG1.3, P1-069063.IgG1.3, P1-069065.IgG1.3, P1-069067.IgG1.3, P1-069069.IgG1.3, P1-069071.IgG1.3, P1-069073.IgG1.3, P1-069075.IgG1.3, P1-069077.IgG1.3, P1-061015.IgG1.3, P1-068736.IgG1.3, P1-068738.IgG1.3, P1-068740.IgG1.3, P1-068742.IgG1.3, P1-068744.IgG1.3, P1-068766.IgG1.3, P1-068748.IgG1.3, P1-068750.IgG1.3, P1-068752.IgG1.3, P1-068754.IgG1.3, P1-068761_E55A.IgG1.3, P1-068761_H100G.IgG1.3, P1-068761_E56N.IgG1.3, P1-068761_E55A_E56N.IgG1.3, P1-068761_E30D.IgG1.3, P1-068761_E30D_E55A.IgG1.3, P1-068761_E56N_H100G.IgG1.3, P1-068761_E30D_H100G.IgG1.3, P1-068761_E30D_E56N.IgG1.3, P1-068761_E100fF.IgG1.3, P1-068761_E55A_E100fF.IgG1.3, P1-068761_H100G_E100fF.IgG1.3, P1-068761_E30D_E100fF.IgG1.3, P1-068761_E56N_E100fF.IgG1.3, P1-068761_E32Y.IgG1.3, P1-068761_E32Y_E55A.IgG1.3, P1-068761_E32Y_E56N.IgG1.3, P1-068761_E30D_E32Y.IgG1.3, P1-068761_E32Y_H100G.IgG1.3, P1-068761_E32Y_E100fF.IgG1.3, P1-068767_D52N_D102V.IgG1.3, P1-068767_D52N.IgG1.3, P1-

068767_D52N_E55A.IgG1.3, P1-068767_E55A_D102V.IgG1.3, P1-068767_D102V.IgG1.3, P1-068767_E55A.IgG1.3, P1-068767_E30D_D52N.IgG1.3, P1-068767_E30D_D102V.IgG1.3, P1-068767_E30D.IgG1.3, P1-068767_E30D_E55A.IgG1.3, P1-068767_E100fF_D102V.IgG1.3, P1-068767_E55A_E100fF.IgG1.3, P1-068767_D52N_E100fF.IgG1.3, P1-068767_E100fF.IgG1.3, or P1-068767_E30D_E100fF.IgG1.3. In certain embodiments, the antibody comprises a heavy chain amino acid sequence of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17, optionally comprising an IgG1.3 heavy chain constant region.

In certain embodiments, an anti-hVISTA Ab comprises a heavy chain comprising the amino acid sequence of the heavy chain of any of the anti-hVISTA Abs provided herein, which comprise an IgG1.3 heavy chain constant region, and the amino acid sequence of the light chain of any of the anti-hVISTA Abs provided herein. In certain embodiments, an anti-hVISTA Ab comprises a heavy chain comprising the amino acid sequence of the VH of P1-061029 or P1-061015 or progeny thereof, which comprise an IgG1.3 HC constant region, such as P1-061029.IgG1.3 (SEQ ID NO: 69), P1-068757.IgG1.3, P1-068759.IgG1.3, P1-068761.IgG1.3, P1-068763.IgG1.3, P1-068765.IgG1.3, P1-068767.IgG1.3, P1-068769.IgG1.3, P1-068771.IgG1.3, P1-068773.IgG1.3, P1-068775.IgG1.3, P1-069059.IgG1.3, P1-069061.IgG1.3, P1-069063.IgG1.3, P1-069065.IgG1.3, P1-069067.IgG1.3, P1-069069.IgG1.3, P1-069071.IgG1.3, P1-069073.IgG1.3, P1-069075.IgG1.3, P1-069077.IgG1.3, P1-061015.IgG1.3, P1-068736.IgG1.3, P1-068738.IgG1.3, P1-068740.IgG1.3, P1-068742.IgG1.3, P1-068744.IgG1.3, P1-068766.IgG1.3, P1-068748.IgG1.3, P1-068750.IgG1.3, P1-068752.IgG1.3, P1-068754.IgG1.3, P1-068761_E55A.IgG1.3, P1-068761_H100G.IgG1.3, P1-068761_E56N.IgG1.3, P1-068761_E55A_E56N.IgG1.3, P1-068761_E30D.IgG1.3, P1-068761_E30D_E55A.IgG1.3, P1-068761_E56N_H100G.IgG1.3, P1-068761_E30D_H100G.IgG1.3, P1-068761_E30D_E56N.IgG1.3, P1-068761_E100fF.IgG1.3, P1-

068761_E55A_E100fF.IgG1.3, P1-068761_H100G_E100fF.IgG1.3, P1-068761_E30D_E100fF.IgG1.3, P1-068761_E56N_E100fF.IgG1.3, P1-068761_E32Y.IgG1.3, P1-068761_E32Y_E55A.IgG1.3, P1-068761_E32Y_E56N.IgG1.3, P1-068761_E30D_E32Y.IgG1.3, P1-068761_E32Y_H100G.IgG1.3, P1-068761_E32Y_E100fF.IgG1.3, P1-068767_D52N_D102V.IgG1.3, P1-068767_D52N.IgG1.3, P1-068767_D52N_E55A.IgG1.3, P1-068767_E55A_D102V.IgG1.3, P1-068767_D102V.IgG1.3, P1-068767_E55A.IgG1.3, P1-068767_E30D_D52N.IgG1.3, P1-068767_E30D_D102V.IgG1.3, P1-068767_E30D.IgG1.3, P1-068767_E30D_E55A.IgG1.3, P1-068767_E100fF_D102V.IgG1.3, P1-068767_E55A_E100fF.IgG1.3, P1-068767_D52N_E100fF.IgG1.3, P1-068767_E100fF.IgG1.3, or P1-068767_E30D_E100fF.IgG1.3; and a light chain comprising the amino acid sequence of the light chain of P1-061029 or P1-061015. In certain embodiments, the antibody comprises the heavy chain of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17 as an IgG1.3 antibody, and the light chain of the same antibody.

An anti-hVISTA Ab may comprise:

(a) a heavy chain comprising the amino acid sequence of the heavy chain of P1-061029.IgG1.3 (SEQ ID NO: 69) and a light chain comprising the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain comprising the amino acid sequence of the heavy chain of P1-061015.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-061015;

(c) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068757.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068757;

(d) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068759.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068759;

(e) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761;

(f) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068763.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068763;

(g) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068765.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068765;

(h) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767;

(i) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068769.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068769;

(j) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068771.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068771;

(k) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068773.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068773;

(l) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068775.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068775;

(m) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069059.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069059;

(n) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069061.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069061;

(o) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069063.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069063;

(p) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069065.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069065;

(q) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069067.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069067;

(r) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069069.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069069;

(s) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069071.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069071;

(t) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069073.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069073;

(u) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069075.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069075;

(v) a heavy chain comprising the amino acid sequence of the heavy chain of P1-069077.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-069077;

(w) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068736.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068736;

(x) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068738.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068738;

(y) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068740.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068740;

(z) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068742.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068742;

(aa) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068744.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068744;

(bb) a heavy chain comprising the amino acid sequence of the VH of P1-068746.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068746;

(cc) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068748.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068748;

(dd) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068750.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068750;

(ee) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068752.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068752;

(ff) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068754.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068754;

(gg) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_H100G.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E56N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E55A_E56N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E56N_H100G.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D_H100G.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D_E56N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E55A_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_H100G_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E56N_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E32Y.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E32Y_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E32Y_E56N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E30D_E32Y.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E32Y_H100G.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068761_E32Y_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_D52N_D102V.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_D52N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_D52N_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E55A_D102V.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_D102V.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E30D_D52N.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E30D_D102V.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E30D.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E30D_E55A.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E100fF_D102V.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E55A_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_D52N_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain comprising the amino acid sequence of the heavy chain of P1-068767_E30D_E100fF.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain comprising the amino acid sequence of the heavy chain of VISTA.4.IgG1.3 and a light chain comprising the light chain amino acid sequence of VISTA.4.IgG1.3;

(qqq) a heavy chain comprising the amino acid sequence of the heavy chain of VISTA.4_VL_A64G.IgG1.3 and a light chain comprising the light chain amino acid sequence of VISTA.4_VL_A64G.IgG1.3;

(rrr) a heavy chain comprising the amino acid sequence of the heavy chain of P1-070976.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-070976.IgG1.3;

(sss) a heavy chain comprising the amino acid sequence of the heavy chain of P1-071799.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-071799.IgG1.3; or

(ttt)a heavy chain comprising the amino acid sequence of the heavy chain of P1-071801.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-071801.IgG1.3;

(uuu) a heavy chain comprising the amino acid sequence of the heavy chain of P1-065333.IgG1.3 and a light chain comprising the light chain amino acid sequence of P1-065333.IgG1.3.

An anti-hVISTA Ab may comprise:

(a) a heavy chain (HC) comprising the HC CDRs of the HC of P1-061029 and a light chain (LC) comprising the LC CDRs of P1-061029 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-061029.IgG1.3, respectively;

(b) a HC comprising the HC CDRs of the HC of P1-061015 and a LC comprising the LC CDRs of P1-061015 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-061015.IgG1.3, respectively;

(c) a HC comprising the HC CDRs of the HC of P1-068757 and a LC comprising the LC CDRs of P1-068757 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068757.IgG1.3, respectively;

(d) a HC comprising the HC CDRs of the HC of P1-068759 and a LC comprising the LC CDRs of P1-068759 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068759.IgG1.3, respectively;

(e) a HC comprising the HC CDRs of the HC of P1-068761 and a LC comprising the LC CDRs of P1-068761 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761.IgG1.3, respectively;

(f) a HC comprising the HC CDRs of the HC of P1-068763 and a LC comprising the LC CDRs of P1-068763 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068763.IgG1.3, respectively;

(g) a HC comprising the HC CDRs of the HC of P1-068765 and a LC comprising the LC CDRs of P1-068765 and HC and LC amino acid sequences that are at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068765.IgG1.3, respectively;

(h) a HC comprising the HC CDRs of the HC of P1-068767 and a LC comprising the LC CDRs of P1-068767 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767.IgG1.3, respectively;

(i) a HC comprising the HC CDRs of the HC of P1-068769 and a LC comprising the LC CDRs of P1-068769 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068769.IgG1.3, respectively;

(j) a HC comprising the HC CDRs of the HC of P1-068771 and a LC comprising the LC CDRs of P1-068771 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068771.IgG1.3, respectively;

(k) a HC comprising the HC CDRs of the HC of P1-068773 and a LC comprising the LC CDRs of P1-068773 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068773.IgG1.3, respectively;

(l) a HC comprising the HC CDRs of the HC of P1-068775 and a LC comprising the LC CDRs of P1-068775 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068775.IgG1.3, respectively;

(m) a HC comprising the HC CDRs of the HC of P1-069059 and a LC comprising the LC CDRs of P1-069059 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069059.IgG1.3, respectively;

(n) a HC comprising the HC CDRs of the HC of P1-069061 and a LC comprising the LC CDRs of P1-069061 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069061.IgG1.3, respectively;

(o) a HC comprising the HC CDRs of the HC of P1-069063 and a LC comprising the LC CDRs of P1-069063 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069063.IgG1.3, respectively;

(p) a HC comprising the HC CDRs of the HC of P1-069065 and a LC comprising the LC CDRs of P1-069065 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069065.IgG1.3, respectively;

(q) a HC comprising the HC CDRs of the HC of P1-069067 and a LC comprising the LC CDRs of P1-069067 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069067.IgG1.3, respectively;

(r) a HC comprising the HC CDRs of the HC of P1-069069 and a LC comprising the LC CDRs of P1-069069 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069069.IgG1.3, respectively;

(s) a HC comprising the HC CDRs of the HC of P1-069071 and a LC comprising the LC CDRs of P1-069071 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069071.IgG1.3, respectively;

(t) a HC comprising the HC CDRs of the HC of P1-069073 and a LC comprising the LC CDRs of P1-069073 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069073.IgG1.3, respectively;

(u) a HC comprising the HC CDRs of the HC of P1-069075 and a LC comprising the LC CDRs of P1-069075 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069075.IgG1.3, respectively;

(v) a HC comprising the HC CDRs of the HC of P1-069077 and a LC comprising the LC CDRs of P1-069077 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-069077.IgG1.3, respectively;

(w) a HC comprising the HC CDRs of the HC of P1-068736 and a LC comprising the LC CDRs of P1-068736 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068736.IgG1.3, respectively;

(x) a HC comprising the HC CDRs of the HC of P1-068738 and a LC comprising the LC CDRs of P1-068738 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068738.IgG1.3, respectively;

(y) a HC comprising the HC CDRs of the HC of P1-068740 and a LC comprising the LC CDRs of P1-068740 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068740.IgG1.3, respectively;

(z) a HC comprising the HC CDRs of the HC of P1-068742 and a LC comprising the LC CDRs of P1-068742 and HC and LC amino acid sequences that are at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068742.IgG1.3, respectively;

(aa) a HC comprising the HC CDRs of the HC of P1-068744 and a LC comprising the LC CDRs of P1-068744 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068744.IgG1.3, respectively;

(bb) a HC comprising the HC CDRs of the HC of P1-068746 and a LC comprising the LC CDRs of P1-068746 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068746.IgG1.3, respectively;

(cc) a HC comprising the HC CDRs of the HC of P1-068748 and a LC comprising the LC CDRs of P1-068748 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068748.IgG1.3, respectively;

(dd) a HC comprising the HC CDRs of the HC of P1-068750 and a LC comprising the LC CDRs of P1-068750 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068750.IgG1.3, respectively;

(ee) a HC comprising the HC CDRs of the HC of P1-068752 and a LC comprising the LC CDRs of P1-068752 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068752.IgG1.3, respectively;

(ff) a HC comprising the HC CDRs of the HC of P1-068754 and a LC comprising the LC CDRs of P1-068754 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068754.IgG1.3, respectively;

(gg) a HC comprising the HC CDRs of the HC of P1-068761_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E55A.IgG1.3, respectively;

(hh) a HC comprising the HC CDRs of the HC of P1-068761_H100G.IgG1.3 and a LC comprising the LC CDRs of P1-068761_H100G and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_H100G.IgG1.3, respectively;

(ii) a HC comprising the HC CDRs of the HC of P1-068761_E56N.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E56N and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E56N.IgG1.3, respectively;

(jj) a HC comprising the HC CDRs of the HC of P1-068761_E55A_E56N.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E55A_E56N and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E55A_E56N.IgG1.3, respectively;

(kk) a HC comprising the HC CDRs of the HC of P1-068761_E30D.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E30D.IgG1.3, respectively;

(ll) a HC comprising the HC CDRs of the HC of P1-068761_E30D_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at

least 99% identical to the HC and LC of P1-068761_E30D_E55A.IgG1.3, respectively;

(mm) a HC comprising the HC CDRs of the HC of P1-068761_E56N_H100G.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E56N_H100G and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E56N_H100G.IgG1.3, respectively;

(nn) a HC comprising the HC CDRs of the HC of P1-068761_E30D_H100G.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D_H100G and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E30D_H100G.IgG1.3, respectively;

(oo) a HC comprising the HC CDRs of the HC of P1-068761_E30D_E56N.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D_E56N and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E30D_E56N.IgG1.3, respectively;

(pp) a HC comprising the HC CDRs of the HC of P1-068761_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E100fF.IgG1.3, respectively;

(qq) a HC comprising the HC CDRs of the HC of P1-068761_E55A_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E55A_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E55A_E100fF.IgG1.3, respectively;

(rr) a HC comprising the HC CDRs of the HC of P1-068761_H100G_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_H100G_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_H100G_E100fF.IgG1.3, respectively;

(ss) a HC comprising the HC CDRs of the HC of P1-068761_E30D_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E30D_E100fF.IgG1.3, respectively;

(tt) a HC comprising the HC CDRs of the HC of P1-068761_E56N_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E56N_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E56N_E100fF.IgG1.3, respectively;

(uu) a HC comprising the HC CDRs of the HC of P1-068761_E32Y.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E32Y and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E32Y.IgG1.3, respectively;

(vv) a HC comprising the HC CDRs of the HC of P1-068761_E32Y_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E32Y_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E32Y_E55A.IgG1.3, respectively;

(ww) a HC comprising the HC CDRs of the HC of P1-068761_E32Y_E56N.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E32Y_E56N and HC and LC amino acid sequences that are at least 90%,

at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E32Y_E56N.IgG1.3, respectively;

(xx) a HC comprising the HC CDRs of the HC of P1-068761_E30D_E32Y.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E30D_E32Y and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E30D_E32Y.IgG1.3, respectively;

(yy) a HC comprising the HC CDRs of the HC of P1-068761_E32Y_H100G.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E32Y_H100G and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E32Y_H100G.IgG1.3, respectively;

(zz) a HC comprising the HC CDRs of the HC of P1-068761_E32Y_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068761_E32Y_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068761_E32Y_E100fF.IgG1.3, respectively;

(aaa) a HC comprising the HC CDRs of the HC of P1-068767_D52N_D102V.IgG1.3 and a LC comprising the LC CDRs of P1-068767_D52N_D102V and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_D52N_D102V.IgG1.3, respectively;

(bbb) a HC comprising the HC CDRs of the HC of P1-068767_D52N.IgG1.3 and a LC comprising the LC CDRs of P1-068767_D52N and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_D52N.IgG1.3, respectively;

(ccc) a HC comprising the HC CDRs of the HC of P1-068767_D52N_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068767_D52N_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_D52N_E55A.IgG1.3, respectively;

(ddd) a HC comprising the HC CDRs of the HC of P1-068767_E55A_D102V.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E55A_D102V and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E55A_D102V.IgG1.3, respectively;

(eee) a HC comprising the HC CDRs of the HC of P1-068767_D102V.IgG1.3 and a LC comprising the LC CDRs of P1-068767_D102V and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_D102V.IgG1.3, respectively;

(fff) a HC comprising the HC CDRs of the HC of P1-068767_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E55A.IgG1.3, respectively;

(ggg) a HC comprising the HC CDRs of the HC of P1-068767_E30D_D52N.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E30D_D52N and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E30D_D52N.IgG1.3, respectively;

(hhh) a HC comprising the HC CDRs of the HC of P1-068767_E30D_D102V.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E30D_D102V and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E30D_D102V.IgG1.3, respectively;

(iii) a HC comprising the HC CDRs of the HC of P1-068767_E30D.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E30D and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E30D.IgG1.3, respectively;

(jjj) a HC comprising the HC CDRs of the HC of P1-068767_E30D_E55A.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E30D_E55A and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E30D_E55A.IgG1.3, respectively;

(kkk) a HC comprising the HC CDRs of the HC of P1-068767_E100fF_D102V.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E100fF_D102V and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E100fF_D102V.IgG1.3, respectively;

(lll) a HC comprising the HC CDRs of the HC of P1-068767_E55A_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E55A_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E55A_E100fF.IgG1.3, respectively;

(mmm) a HC comprising the HC CDRs of the HC of P1-068767_D52N_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068767_D52N_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_D52N_E100fF.IgG1.3, respectively;

(nnn) a HC comprising the HC CDRs of the HC of P1-068767_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E100fF.IgG1.3, respectively;

(ooo) a HC comprising the HC CDRs of the HC of P1-068767_E30D_E100fF.IgG1.3 and a LC comprising the LC CDRs of P1-068767_E30D_E100fF and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-068767_E30D_E100fF.IgG1.3, respectively

(ppp) a heavy chain (HC) comprising the HC CDRs of the HC of VISTA.4 and a light chain (LC) comprising the LC CDRs of VISTA.4 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of VISTA.4.IgG1.3, respectively;

(qqq) a heavy chain (HC) comprising the HC CDRs of the HC of VISTA.4_VL_A64G and a light chain (LC) comprising the LC CDRs of VISTA.4_VL_A64G and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of VISTA.4_VL_A64G.IgG1.3, respectively;

(rrr) a heavy chain (HC) comprising the HC CDRs of the HC of P1-070976 and a light chain (LC) comprising the LC CDRs of P1-070976 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-070976.IgG1.3, respectively;

(sss) a heavy chain (HC) comprising the HC CDRs of the HC of P1-071799 and a light chain (LC) comprising the LC CDRs of P1-071799 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-071799.IgG1.3, respectively;

(ttt)a heavy chain (HC) comprising the HC CDRs of the HC of P1-071801 and a light chain (LC) comprising the LC CDRs of P1-071801 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-071801.IgG1.3, respectively; or

(uuu) a heavy chain (HC) comprising the HC CDRs of the HC of P1-065333 and a light chain (LC) comprising the LC CDRs of P1-065333 and HC and LC amino acid sequences that are at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the HC and LC of P1-065333.IgG1.3, respectively.

In some embodiments, an anti-hVISTA Ab may comprise:

(a) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-061029.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-061029;

(b) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-061015.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-061015;

(c) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068757.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068757;

(d) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068759.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068759;

(e) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761;

(f) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068763.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068763;

(g) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068765.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068765;

(h) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767;

(i) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068769.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068769;

(j) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068771.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068771;

(k) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068773.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068773;

(l) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068775.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068775;

(m) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069059.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069059;

(n) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069061.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069061;

(o) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069063.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069063;

(p) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069065.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069065;

(q) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069067.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069067;

(r) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069069.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069069;

(s) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069071.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069071;

(t) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069073.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069073;

(u) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069075.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069075;

(v) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-069077.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-069077;

(w) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068736 and a light chain consisting of the amino acid sequence of the light chain of P1-068736;

(x) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068738.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068738;

(y) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068740.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068740;

(z) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068742.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068742;

(aa) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068744.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068744;

(bb) a heavy chain consisting of the amino acid sequence of the heavy chain f P1-068746.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068746;

(cc) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068748.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068748;

(dd) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068750.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068750;

(ee) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068752.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068752;

(ff) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068754 and a light chain consisting of the amino acid sequence of the light chain of P1-068754;

(gg) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E55A;

(hh) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_H100G.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_H100G;

(ii) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E56N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E56N;

(jj) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E55A_E56N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E55A_E56N;

(kk) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D;

(ll) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D_E55A;

(mm) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E56N_H100G.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E56N_H100G;

(nn) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D_H100G.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D_H100G;

(oo) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D_E56N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D_E56N;

(pp) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E100fF;

(qq) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E55A_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E55A_E100fF;

(rr) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_H100G_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_H100G_E100fF;

(ss) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D_E100fF;

(tt) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E56N_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E56N_E100fF;

(uu) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E32Y.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E32Y;

(vv) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E32Y_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E32Y_E55A;

(ww) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E32Y_E56N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E32Y_E56N;

(xx) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E30D_E32Y.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E30D_E32Y;

(yy) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E32Y_H100G.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E32Y_H100G;

(zz) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068761_E32Y_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068761_E32Y_E100fF;

(aaa) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_D52N_D102V.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_D52N_D102V;

(bbb) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_D52N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_D52N;

(ccc) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_D52N_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_D52N_E55A;

(ddd) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E55A_D102V.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E55A_D102V;

(eee) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_D102V.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_D102V;

(fff) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E55A;

(ggg) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E30D_D52N.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E30D_D52N;

(hhh) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E30D_D102V.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E30D_D102V;

(iii)a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E30D.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E30D;

(jjj)a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E30D_E55A.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E30D_E55A;

(kkk) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E100fF_D102V.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E100fF_D102V;

(lll)a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E55A_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E55A_E100fF;

(mmm) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_D52N_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_D52N_E100fF;

(nnn) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E100fF;

(ooo) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-068767_E30D_E100fF.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-068767_E30D_E100fF;

(ppp) a heavy chain consisting of the amino acid sequence of the heavy chain of VISTA.4.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of VISTA.4;

(qqq) a heavy chain consisting of the amino acid sequence of the heavy chain of VISTA.4_VL_A64.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of VISTA.4_VL_A64;

(rrr) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-070976.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-070976;

(sss) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-071799.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-071799;

(ttt)a heavy chain consisting of the amino acid sequence of the heavy chain of P1-071801.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-071801; or

(uuu) a heavy chain consisting of the amino acid sequence of the heavy chain of P1-065333.IgG1.3 and a light chain consisting of the amino acid sequence of the light chain of P1-065333.

In some embodiments, the disclosure contemplates anti-VISTA mAbs comprising:

a heavy chain consisting of the amino acid sequences of the heavy chain of (a) to (uuu) listed above followed by a Lys residue; and

a light chain consisting of the light chain amino acid sequence of (a) to (uuu) listed above;

wherein the heavy chain and light chain amino acid sequences are chosen from the same antibody species from among (a) to (uuu) listed above.

In some embodiments, an anti-hVISTA Ab may comprise a heavy chain amino acid sequence comprising the VH amino acid sequence of the antibody species herein, but

rather than an IgG1.3 heavy chain constant region, as provided in the HC sequences in the Sequence Table herein (and see SEQ ID NO: 163), the antibody may comprise a different heavy chain constant region sequence, such as a human wild-type IgG1 constant region such as human IgG1 allotype f (IgG1f) (SEQ ID NO: 182), or a modified human IgG1 constant region such as IgG1.1f (SEQ ID NO: 183), or a modified human IgG1 constant region such as IgG1.P238K (SEQ ID NO: 184). Accordingly, embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-061015;

(c) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068757;

(d) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068759;

(e) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761;

(f) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068763;

(g) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068765;

(h) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767;

(i) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068769;

(j) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068771;

(k) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068773;

(l) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068775;

(m) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069059;

(n) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069061;

(o) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069063;

(p) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069065;

(q) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069067;

(r) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069069;

(s) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069071;

(t) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069073;

(u) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069075;

(v) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-069077;

(w) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068736;

(x) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068738;

(y) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068740;

(z) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068742;

(aa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068744;

(bb) a heavy chain comprising the amino acid sequence of the VH of P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068746;

(cc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068748;

(dd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068750;

(ee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068752;

(ff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068754;

(gg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a

light chain comprising the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-070976;

(sss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-071799;

(ttt)a heavy chain comprising (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain comprising the light chain amino acid sequence of P1-065333.

Certain embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-061015;

(c) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068757;

(d) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068759;

(e) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761;

(f) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068763;

(g) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068765;

(h) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767;

(i) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068769;

(j) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068771;

(k) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068773;

(l) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068775;

(m) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069059;

(n) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069061;

(o) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069063;

(p) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069065;

(q) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069067;

(r) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069069;

(s) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069071;

(t) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069073;

(u) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069075;

(v) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-069077;

(w) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068736;

(x) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068738;

(y) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068740;

(z) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068742;

(aa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068744;

(bb) a heavy chain consisting of the amino acid sequence of the VH of P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068746;

(cc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068748;

(dd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068750;

(ee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068752;

(ff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068754;

(gg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a

light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-070976;

(sss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-071799;

(ttt)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 182, and a light chain consisting of the light chain amino acid sequence of P1-065333,

wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 182 form a peptidic bond.

In some embodiments, the disclosure contemplates anti-VISTA mAbs comprising:

a heavy chain consisting of the amino acid sequences of (i) a VH of (a) to (uuu) listed above, (ii) SEQ ID NO: 182, and (iii) a Lys residue, wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 182 form a peptidic bond and wherein the C-terminal amino acid of SEQ ID NO: 182 is joined to the N-terminal of the Lys; and

a light chain consisting of the light chain amino acid sequence of (a) to (uuu) listed above;

wherein the VH and light chain amino acid sequences are chosen from the same antibody species from among (a) to (uuu) listed above.

Certain embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-061015;

(c) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068757;

(d) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068759;

(e) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761;

(f) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068763;

(g) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068765;

(h) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767;

(i) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068769;

(j) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068771;

(k) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068773;

(l) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068775;

(m) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069059;

(n) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069061;

(o) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069063;

(p) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069065;

(q) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069067;

(r) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069069;

(s) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069071;

(t) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069073;

(u) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069075;

(v) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-069077;

(w) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068736;

(x) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068738;

(y) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068740;

(z) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068742;

(aa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068744;

(bb) a heavy chain comprising the amino acid sequence of the VH of P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068746;

(cc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068748;

(dd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068750;

(ee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068752;

(ff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068754;

(gg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and

a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and

a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-070976;

(sss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-071799;

(ttt)a heavy chain comprising (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain comprising the light chain amino acid sequence of P1-065333.

Certain embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-061015;

(c) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068757;

(d) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068759;

(e) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761;

(f) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068763;

(g) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068765;

(h) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767;

(i) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068769;

(j) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068771;

(k) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068773;

(l) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068775;

(m) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069059;

(n) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069061;

(o) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069063;

(p) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069065;

(q) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069067;

(r) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069069;

(s) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069071;

(t) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069073;

(u) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069075;

(v) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-069077;

(w) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068736;

(x) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068738;

(y) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068740;

(z) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068742;

(aa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068744;

(bb) a heavy chain consisting of the amino acid sequence of the VH of P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068746;

(cc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068748;

(dd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068750;

(ee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068752;

(ff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068754;

(gg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 183, and a

light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D;

(jjj) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-070976;

(sss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-071799;

(ttt)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 183, and a light chain consisting of the light chain amino acid sequence of P1-065333,

wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 183 form a peptidic bond.

In some embodiments, the disclosure contemplates anti-VISTA mAbs comprising:

a heavy chain consisting of the amino acid sequences of (i) a VH of (a) to (uuu) listed above, (ii) SEQ ID NO: 183, and (iii) a Lys residue, wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 183 form a peptidic bond and wherein the C-terminal amino acid of SEQ ID NO: 183 is joined to the N-terminal of the Lys; and

a light chain consisting of the light chain amino acid sequence of (a) to (uuu) listed above;

wherein the VH and light chain amino acid sequences are chosen from the same antibody species from among (a) to (uuu) listed above.

Further embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain comprising (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-061015;

(c) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068757;

(d) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068759;

(e) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761;

(f) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068763;

(g) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068765;

(h) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767;

(i) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068769;

(j) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068771;

(k) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068773;

(l) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068775;

(m)a heavy chain comprising (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069059;

(n) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069061;

(o) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069063;

(p) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069065;

(q) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069067;

(r) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069069;

(s) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069071;

(t) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069073;

(u) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069075;

(v) a heavy chain comprising (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-069077;

(w) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068736;

(x) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068738;

(y) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068740;

(z) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068742;

(aa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068744;

(bb) a heavy chain comprising the amino acid sequence of the VH of P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068746;

(cc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068748;

(dd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068750;

(ee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068752;

(ff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068754;

(gg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and

a light chain comprising the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and

a light chain comprising the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain comprising (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain comprising (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain comprising (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-070976;

(sss) a heavy chain comprising (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-071799;

(ttt) a heavy chain comprising (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain comprising (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain comprising the light chain amino acid sequence of P1-065333.

Yet further embodiments of this disclosure include anti-VISTA Abs comprising:

(a) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061029 (SEQ ID NO: 67) and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-061029 (SEQ ID NO: 70);

(b) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-061015 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-061015;

(c) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068757 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068757;

(d) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068759 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068759;

(e) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761;

(f) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068763 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068763;

(g) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068765 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068765;

(h) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767;

(i) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068769 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068769;

(j) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068771 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068771;

(k) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068773 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068773;

(l) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068775 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068775;

(m) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069059 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069059;

(n) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069061 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069061;

(o) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069063 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069063;

(p) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069065 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069065;

(q) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069067 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069067;

(r) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069069 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069069;

(s) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069071 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069071;

(t) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069073 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069073;

(u) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069075 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069075;

(v) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-069077 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-069077;

(w) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068736 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068736;

(x) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068738 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068738;

(y) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068740 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068740;

(z) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068742 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068742;

(aa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068744 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068744;

(bb) a heavy chain consisting of the amino acid sequence of the heavy chain f P1-068746 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068746;

(cc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068748 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068748;

(dd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068750 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068750;

(ee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068752 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068752;

(ff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068754 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068754;

(gg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A;

(hh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G;

(ii) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N;

(jj) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E56N;

(kk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D;

(ll) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E55A;

(mm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_H100G;

(nn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and

a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_H100G;

(oo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E56N;

(pp) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E100fF;

(qq) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E55A_E100fF;

(rr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_H100G_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_H100G_E100fF;

(ss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E100fF;

(tt) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E56N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E56N_E100fF;

(uu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y;

(vv) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E55A;

(ww) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E56N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E56N;

(xx) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E30D_E32Y and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E30D_E32Y;

(yy) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_H100G and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_H100G;

(zz) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068761_E32Y_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068761_E32Y_E100fF;

(aaa) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_D102V;

(bbb) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N;

(ccc) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E55A;

(ddd) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_D102V;

(eee) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_D102V;

(fff) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A;

(ggg) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D52N and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D52N;

(hhh) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_D102V;

(iii)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D;

(jjj)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E55A and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E55A;

(kkk) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF_D102V and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF_D102V;

(lll)a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E55A_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E55A_E100fF;

(mmm) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_D52N_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and

a light chain consisting of the light chain amino acid sequence of P1-068767_D52N_E100fF;

(nnn) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E100fF;

(ooo) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-068767_E30D_E100fF and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-068767_E30D_E100fF;

(ppp) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of VISTA.4;

(qqq) a heavy chain consisting of (i) the amino acid sequence of the VH of VISTA.4_VL_A64 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of VISTA.4_VL_A64;

(rrr) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-070976 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-070976;

(sss) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071799 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-071799;

(ttt) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-071801 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-071801; or

(uuu) a heavy chain consisting of (i) the amino acid sequence of the VH of P1-065333 and (ii) the amino acid sequence of SEQ ID NO: 184, and a light chain consisting of the light chain amino acid sequence of P1-065333,

wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 184 form a peptidic bond.

In certain embodiments, the amino acid sequences of the HC and LC of the antibody comprise those of a VISTA.4 (aka. 41F11)-derived antibody described in Tables 22-30 of Example 17, such as VISTA.4, VISTA.4_VL_A64G, P1-070976 (VISTA.4 VH_T28P, Y50W, S55E, D95H, L96E, P97E, Y100E; VL_A64G), P1-071799 (P1-070976_VH_H95D), P1-071801 (P1-070976_VH_E97P), or P1-065333 (VISTA.4 VH_T28P, Y50W, S55E, L96E, Y100E; VL_A64G), or another antibody disclosed in Example 17, wherein the heavy chain constant region is derived from IgG1.3, IgG1, IgG1.1f, IgG4, IgG4 S228P (EU numbering), or comprises the amino acid sequence of one of SEQ ID Nos: 182, 183, or 184. In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-070976 or comprises the amino acid sequence of the VH of P1-070976 but for 1, 2, 3, 4, or 5 amino acid substitutions, such as conservative or reversion substitutions. In some such embodiments, the antibody retains the VH CDRs and VL CDRs of antibody P1-070976, so that all of the differences in the VH sequence are restricted to the framework regions of the VH. In some embodiments, the VH is that of P1-070976, but compriseing one or both of an H95D and an E97P reversion substitution. In some embodiments, an anti-hVISTA Ab may comprise an amino acid VL sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-070976 or comprises the amino acid sequence of the VL of P1-070976, P1-071799, or P1-071801 but for 1, 2, 3, 4, or 5 amino acid substitutions, such as conservative or reversion substitutions. In some such embodiments, the antibody retains the VH CDRs and VL CDRs of antibody P1-070976, so that all of the differences in the VL sequence are restricted to the framework regions of the VL.

In some embodiments, the disclosure contemplates anti-VISTA mAbs comprising:

a heavy chain consisting of the amino acid sequences of (i) a VH of (a) to (uuu) listed above, (ii) SEQ ID NO: 184, and (iii) a Lys residue, wherein the C-terminal amino acid of VH and the N-terminal amino acid of SEQ ID NO: 184 form a peptidic bond and wherein the C-terminal amino acid of SEQ ID NO: 184 is joined to the N-terminal of the Lys; and

a light chain consisting of the light chain amino acid sequence of (a) to (uuu) listed above;

wherein the VH and light chain amino acid sequences are chosen from the same antibody species from among (a) to (uuu) listed above.

In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-061029, wherein the antibody comprises a VH CDR1, CDR2, and/or CDR3 of P1-061029 in which at least one residue has been substituted with a D, an E, or an H. In some embodiments, each of the VH CDR1, CDR2, and CDR3 of P1-061029 contains one, two, or three residues substituted with a D, E, or H. In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-061029, wherein the antibody comprises a VH CDR1 comprising one or two D or E residues at amino acid positions 4, 5, or 7 of CDR1, and/or comprises a VH CDR2 with one, two, or three D, E, or H residues at positions 3, 5, 6, or 7 of CDR2, and/or a VH CDR3 with one, two, or three D, E, or H residues at positions 6, 12, or 14 of CDR 3. (See Table 5 below for examples of antibodies falling within these embodiments.) In such cases, the light chain variable region may comprise the CDR1, CDR2, and/or CDR3 of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-

068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF, and/or the light chain variable region may be at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF.

In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-061015, wherein the antibody comprises a VH CDR1, CDR2, and/or CDR3 of P1-061015 in which at least one residue has been substituted with a D, an E, or an H. In some embodiments, each of the VH CDR1, CDR2, and CDR3 of P1-061015 contains one, two, or three residues substituted with a D, E, or H. In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or

at least 99% identical to that of P1-061015, wherein the antibody comprises a VH CDR1 comprising one or two D, E, or H residues at amino acid positions 6, 7, 8, and 9 of CDR1, and/or comprises a VH CDR2 with one, two, or three D, E, or H residues at positions 1, 2, 4, or 8-11 of CDR2, and/or a VH CDR3 with one, two, or three D, E, or H residues at positions 2, 3, 6, 7, or 12 of CDR 3. (See Table 6 below for examples os antibodies falling within these embodiments.) In such cases, the light chain variable region may comprise the CDR1, CDR2, and/or CDR3 of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF, and/or the light chain variable region may be at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of P1-061029 or P1-061015 or progeny thereof, such as P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068766, P1-068748, P1-068750, P1-068752 P1-068754, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A,

P1-068761_E56N_H100G, P1-068761_E30D_H100G, P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, or P1-068767_E30D_E100fF.

In some embodiments, an anti-hVISTA Ab may comprise an amino acid VH sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of VISTA.4, wherein the antibody comprises a VH CDR1, CDR2, and/or CDR3 of VISTA.4 in which at least one residue has been substituted with a D, an E, or an H. In some embodiments, each of the VH CDR1, CDR2, and CDR3 of VISTA.4 contains one, two, or three residues substituted with a D, E, or H. In such cases, the light chain variable region may comprise the CDR1, CDR2, and/or CDR3 of VISTA.4 or VISTA.4 A64G, and/or the light chain variable region may be at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to that of VISTA.4 or VISTA.4 A64G. Such antibodies are examples of VISTA.4 progeny.

In some embodiments, such modified anti-hVISTA P1-061029 or P1-061015 or VISTA.4 progeny possess one or more of the following characteristics:

- specifically binding to hVISTA, e.g., histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at acidic pH, e.g., pH 6.0 or pH 6.5;
- lacking of significant binding to hVISTA, e.g., histidine rich region of the ECD or a polypeptide comprising amino acid residues 35-127 of SEQ ID NO: 2, at physiological pH or neutral pH, e.g., pH 7.4 or pH 7.0;
- specifically binding to cyno VISTA, e.g., histidine rich region of the ECD, at acidic pH, e.g., pH 6.0 or pH 6.5;

- lacking of significant binding to cyno VISTA, e.g., histidine rich region of the ECD, at physiological pH or neutral pH, e.g., pH 7.4 or pH 7.0;
- having reduced binding to hVISTA-ECD having a substitution at one or more of the following amino acids: T35, Y37, K38, T39, Y41, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127 relative to hVISTA ECD having SEQ ID NO: 2;
- cross-competiting for binding to hVISTA with P1-061029, P1-068761, P1-068767, P1-061015, and/or VISTA.4;
- inhibiting binding of hVISTA to human T cells expressing VISTA (e.g., naïve or activated T cells) at acidic pH e.g., pH 6.0 or pH 6.5;
- inhibiting binding of hVISTA to PSGL-1 with or without siayl lewis X at acidic pH e.g., pH 6.0 or pH 6.5 (e.g., inhibiting the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48), wherein PSGL-1 is with or without siayl lewis X, and wherein the tyrosines are preferably sulfotyrosines;
- having a mean residence time (MRT) of at least 100, 200, 300, 350, 400, 450, 500, 600, or 700 hours in cynomolgus monkeys (e.g., at least 350 hours), measured, e.g, as described in the Examples;
- low or undetectable levels of TMDD;
- low or undetectable levels of neutropenia;
- stimulating T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
- inhibiting VISTA mediated cell:cell adhesion; and
- specifically binding to hVISTA in samples of human tumor cells or samples of inflamed human tissue that express VISTA;
- contacting hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;
- binding to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3:

$_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;

- binding to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacting (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å) or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacting hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- and any additional characteristic set forth in the claims and/or in the Examples.

Exemplary Antibody Constant Regions

In some embodiments, an antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, an antibody described herein comprises a human IgG constant region, such as an IgG1, IgG2, IgG3, or IgG4. In some embodiments, an antibody described herein comprises a human IgG4 heavy chain constant region. In some such embodiments, an antibody described herein comprises an S241P mutation in the human IgG4 constant region. In some embodiments, an antibody described herein comprises a human IgG4 constant region and a human κ light chain.

The choice of heavy chain constant region can determine whether or not an antibody will have effector function *in vivo*. Such effector function, in some embodiments, includes antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), and can result in killing of the cell to which the antibody is bound. In some methods of treatment, including methods of treating some cancers, cell killing may be desirable, for example, when the antibody binds to a cell that supports the maintenance or growth of the tumor. Exemplary cells that may support the maintenance or growth of a tumor include, but are not limited to, tumor cells themselves,

cells that aid in the recruitment of vasculature to the tumor, and cells that provide ligands, growth factors, or counter-receptors that support or promote tumor growth or tumor survival. In some embodiments, when effector function is desirable, an antibody comprising a human IgG1 heavy chain or a human IgG3 heavy chain is selected.

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.*, Wright *et al. TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibodies with certain improved properties. For example, in some embodiments an antibody may be afucosylated, for example, by mutating residues such as Asn297 that are normally glycosylated with fucose-containing glycosylations, or through other means. In some embodiments, antibodies herein may comprise an afucosylated human IgG1 constant region.

Antibodies are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibodies may have reduced fucosylation and/or improved ADCC function. Examples of such antibodies are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibodies with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibodies may have improved CDC function. Such antibodies are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

Antibodies are also provided with amino-terminal leader extensions. For example, one or more amino acid residues of the amino-terminal leader sequence are

present at the amino-terminus of any one or more heavy or light chains of an antibody. An exemplary amino-terminal leader extension comprises or consists of three amino acid residues, VHS, present on one or both light chains of an antibody.

The *in vivo* or serum half-life of human FcRn high affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice, in humans, or in non-human primates to which the polypeptides with a variant Fc region are administered. See also, *e.g.*, Petkova *et al.* *International Immunology* 18(12):1759-1769 (2006).

In some embodiments of the invention, an afucosylated antibody mediates ADCC in the presence of human effector cells more effectively than a parent antibody that comprises fucose, Generally, ADCC activity may be determined using the *in vitro* ADCC assay as herein disclosed, but other assays or methods for determining ADCC activity, *e.g.* in an animal model *etc.*, are contemplated.

In certain embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320, 322, 330, and/or 331 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

In some examples, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

In some examples, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.* In some examples, the Fc region can be modified to decrease antibody dependent cellular cytotoxicity (ADCC) and/or to decrease the affinity for an Fcγ receptor by modifying one or more amino acids at the following positions: 234, 235, 236, 238, 239, 240, 241 , 243,

244, 245, 247, 248, 249, 252, 254, 255, 256, 258, 262, 263, 264, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 299, 301, 303, 305, 307, 309, 312, 313, 315, 320, 322, 324, 325, 326, 327, 329, 330, 331, 332, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 433, 434, 435, 436, 437, 438 or 439. Exemplary substitutions include 236A, 239D, 239E, 268D, 267E, 268E, 268F, 324T, 332D, and 332E. Exemplary variants include 239D/332E, 236A/332E, 236A/239D/332E, 268F/324T, 267E/268F, 267E/324T, and 267E/268F7324T. Other Fc modifications that can be made to Fcs are those for reducing or ablating binding to FcγR and/or complement proteins, thereby reducing or ablating Fc-mediated effector functions such as ADCC, ADCP, and CDC. Exemplary modifications include but are not limited substitutions, insertions, and deletions at positions 234, 235, 236, 237, 267, 269, 325, 328, 330, and/or 331 (*e.g.*, 330 and 331), wherein numbering is according to the EU index. Exemplary substitutions include but are not limited to 234A, 235E, 236R, 237A, 267R, 269R, 325L, 328R, 330S, and 331S (*e.g.*, 330S, and 331S), wherein numbering is according to the EU index. An Fc variant can comprise 236R/328R. Other modifications for reducing FcγR and complement interactions include substitutions 297A, 234A, 235A, 237A, 318A, 228P, 236E, 268Q, 309L, 330S, 331 S, 220S, 226S, 229S, 238S, 233P, and 234V, as well as removal of the glycosylation at position 297 by mutational or enzymatic means or by production in organisms such as bacteria that do not glycosylate proteins. These and other modifications are reviewed in Strohl, 2009, *Current Opinion in Biotechnology* 20:685-691. For example, the human IgG1.3 Fc constant region contains L234A, L235E, and G237A substitutions. The IgG1fa.P238K (or IgG1.P238K) contains a P238K substitution. The IgG1.1f omprises L234A, L235E, G237A, A330S, and P331S substitutions.

Fc variants that enhance affinity for an inhibitory receptor FcγRIIb can also be used. Such variants can provide an Fc fusion protein with immunomodulatory activities related to FcγRIIb cells, including for example B cells and monocytes. In one embodiment, the Fc variants provide selectively enhanced affinity to FcγRIIb relative to one or more activating receptors. Modifications for altering binding to FcγRIIb include one or more modifications at a position selected from the group consisting of 234, 235, 236, 237, 239, 266, 267, 268, 325, 326, 327, 328, 330, 331, and 332, according to the EU index. Exemplary substitutions for enhancing FcγRllb affinity include but are not limited

to 234A, 234D, 234E, 234F, 234W, 235D, 235E, 235F, 235R, 235Y, 236D, 236N, 237A, 237D, 237N, 239D, 239E, 266M, 267D, 267E, 268D, 268E, 327D, 327E, 328F, 328W, 328Y, 330S, 331S, and 332E. Exemplary substitutions include 235Y, 236D, 239D, 266M, 267E, 268D, 268E, 328F, 328W, and 328Y. Other Fc variants for enhancing binding to FcγRIIb include 235Y/267E, 236D/267E, 239D/268D, 239D/267E, 267E/268D, 267E/268E, and 267E/328F.

Other modifications for enhancing FcγR and complement interactions include but are not limited to substitutions 298 A, 333A, 334A, 326A, 2471, 339D, 339Q, 280H, 290S, 298D, 298V, 243L, 292P, 300L, 396L, 3051, and 396L. These and other modifications are reviewed in Strohl, 2009, Current Opinion in Biotechnology 20:685-691. Fc modifications that increase binding to an Fcγ receptor include amino acid modifications at any one or more of amino acid positions 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 279, 280, 283, 285, 298, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 312, 315, 324, 327, 329, 330, 335, 337, 338, 340, 360, 373, 376, 379, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439 of the Fc region, wherein the numbering of the residues in the Fc region is that of the EU index as in Patent Publication No. WO 00/42072.

Optionally, the Fc region can comprise a non-naturally occurring amino acid residue at additional and/or alternative positions known to one skilled in the art (see, *e.g.*, U.S. Pat. Nos. 5,624,821; 6,277,375; 6,737,056; 6,194,551; 7,317,091; 8,101,720; PCX Patent Publications WO 00/42072; WO 01/58957; WO 02/06919; WO 04/016750; WO 04/029207; WO 04/035752; WO 04/074455; WO 04/099249; WO 04/063351; WO 05/070963; WO 05/040217, WO 05/092925 and WO 06/0201 14).

The affinities and binding properties of an Fc region for its ligand can be determined by a variety of *in vitro* assay methods (biochemical or immunological based assays) known in the art including but not limited to, equilibrium methods (*e.g.*, enzyme-linked immunoabsorbent assay (ELISA), or radioimmunoassay (RIA)), or kinetics (*e.g.*, BIACORE analysis), and other methods such as indirect binding assays, competitive inhibition assays, fluorescence resonance energy transfer (FRET), gel electrophoresis and chromatography (*e.g.*, gel filtration). These and other methods can utilize a label on one or more of the components being examined and/or employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels. A

detailed description of binding affinities and kinetics can be found in Paul, W. E., ed., Fundamental immunology, 4th Ed., Lippincott-Raven, Philadelphia (1999), which focuses on antibody-immunogen interactions.

In certain embodiments, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, this can be done by increasing the binding affinity of the Fc region for FcRn, For example, one or more of more of following residues can be mutated: 252, 254, 256, 433, 435, 436, as described in U.S. Pat. No. 6,277,375. Specific exemplary substitutions include one or more of the following: T252L, T254S, and/or T256F. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.* Other exemplary variants that increase binding to FcRn and/or improve pharmacokinetic properties include substitutions at positions 259, 308, 428, and 434, including for example 2591, 308F, 428L, 428M, 434S, 4341 1. 434F, 434Y, and 434X1. Other variants that increase Fc binding to FcRn include: 250E, 250Q, 428 L, 428F, 250Q/428L (Hinton *et al.* 2004, *J. Biol. Chem.* 279(8): 6213-6216, Hinton *et al.* 2006 *Journal of Immunology* 176:346-356), 256A, 272A, 286A, 305A, 307A, 307Q, 31 1A, 312A, 376A, 378Q, 380A, 382A, 434A (Shields *et al.*, *Journal of Biological Chemistry*, 2001, 276(9):6591-6604), 252F, 252T, 252Y, 252W, 254T, 256S, 256R, 256Q, 256E, 256D, 256T, 309P, 31 1 S, 433R, 433S, 4331, 433P, 433Q, 434H, 434F, 434Y, 252Y/254T/256E, 433K/434F/436H, 308T/309P/311S (Dall Acqua *et al. Journal of Immunology*, 2002, 169:5171-5180, Dall'Acqua *et al.*, 2006, *Journal of Biological Chemistry* 281:23514-23524). Other modifications for modulating FcRn binding are described in Yeung *et al.*, 2010, *J Immunol*, 182:7663-7671.

In certain embodiments, hybrid IgG isotypes with particular biological characteristics can be used. For example, an IgG1/IgG3 hybrid variant can be constructed by substituting IgG1 positions in the CH2 and/or CH3 region with the amino acids from IgG3 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody can be constructed that comprises one or more substitutions, *e.g.,* 274Q, 276K, 300F, 339T, 356E, 358M, 384S, 392N, 397M, 4221, 435R, and 436F. In some embodiments described herein, an IgG1/IgG2 hybrid variant can be constructed by substituting IgG2 positions in

the CH2 and/or CH3 region with amino acids from IgG1 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody can be constructed that comprises one or more substitutions, *e.g.,* one or more of the following amino acid substitutions: 233E, 234L, 235L, -236G (referring to an insertion of a glycine at position 236), and 327A.

Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A, which has been shown to exhibit enhanced FcγRIIIa binding and ADCC activity (Shields *et al.*, 2001). Other IgG1 variants with strongly enhanced binding to FcγRIIIa have been identified, including variants with S239D/I332E and S239D/I332E/A330L mutations which showed the greatest increase in affinity for FcγRIIIa, a decrease in FcγRIIb binding, and strong cytotoxic activity in cynomolgus monkeys (Lazar *et al.*, 2006). Introduction of the triple mutations into antibodies such as alemtuzumab (CD52-specific), trastuzumab (HER2/neu-specific), rituximab (CD20-specific), and cetuximab (EGFR- specific) translated into greatly enhanced ADCC activity *in vitro*, and the S239D/I332E variant showed an enhanced capacity to deplete B cells in monkeys (Lazar *et al.*, 2006). In addition, IgG1 mutants containing L235V, F243L, R292P, Y300L and P396L mutations which exhibited enhanced binding to FcγRIIIa and concomitantly enhanced ADCC activity in transgenic mice expressing human FcγRIIIa in models of B cell malignancies and breast cancer have been identified (Stavenhagen *et al.*, 2007; Nordstrom *et al.*, 2011). Other Fc mutants that can be used include: S298A/E333A/L334A, S239D/I332E, S239D/I332E/A330L, L235V/F243L/R292P/Y300L/ P396L, and M428L/N434S.

In certain embodiments, an Fc is chosen that has reduced binding to FcγRs. An exemplary Fc, *e.g.,* IgG1 Fc, with reduced FcγR binding comprises the following three amino acid substitutions: L234A, L235E and G237A.

In certain embodiments, an Fc is chosen that has reduced complement fixation. An exemplary Fc, *e.g.,* IgG1 Fc, with reduced complement fixation has the following two amino acid substitutions: A330S and P331S.

In certain embodiments, an Fc is chosen that has essentially no effector function, *i.e.,* it has reduced binding to FcγRs and reduced complement fixation. An exemplary Fc, *e.g.,* IgG1 Fc, that is effectorless comprises the following five mutations: L234A, L235E, G237A, A330S and P331S.

When using an IgG4 constant domain, it can include the substitution S228P, which mimics the hinge sequence in IgG1 and thereby stabilizes IgG4 molecules.

Fc modifications described in WO 2017/087678 or WO2016081746 may also be used.

One may use a constant region having effector function or depleted of effector function when the variable regions of the antibody bind to VISTA at acidic pH but not at physiological pH, as there is expected to be low TMDD and no neutropenia. However, for VISTA antibodies that are not preferably binding to hVISTA at acidic pH relative to physiological pH, it is preferable to use an effectorless contant region, e.g., IgG1.3, to avoid high TMDD and neutropenia.

In certain embodiments, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (*i.e.,* the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation can increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

Glycosylation of the constant region on N297 can be prevented by mutating the N297 residue to another residue, *e.g.,* N297A, and/or by mutating an adjacent amino acid, *e.g.,* 298 to thereby reduce glycosylation on N297.

Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example,

expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies described herein to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Led 3 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases {*e.g.*, beta(l,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17: 176-180).

Another modification of the antibodies described herein is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.*, serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. In some embodiments, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (CI-CIO) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies described herein. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

In various embodiments, an antibody binding to VISTA described herein is modified to selectively block antigen binding in tissues and environments where antigen binding would be detrimental, but allow antigen binding where it would be beneficial

("activatable antibody"). In one embodiment, a blocking peptide "mask" is generated that specifically binds to the antigen binding surface of the antibody and interferes with antigen binding, which mask is linked to each of the binding arms of the antibody by a peptidase cleavable linker. See, e.g., U.S. Pat. No. 8,518,404 to CytomX. Such constructs are useful for treatment of cancers in which protease levels are greatly increased in the tumor microenvironment compared with non-tumor tissues. Selective cleavage of the cleavable linker in the tumor microenvironment allows disassociation of the masking/blocking peptide, enabling antigen binding selectively in the tumor, rather than in peripheral tissues in which antigen binding might cause unwanted side effects. Examples of blocking peptides linked to antibodies are provided in WO 2018/08555.

Alternatively, in a related embodiment, a bivalent binding compound ("masking ligand") comprising two antigen binding domains is developed that binds to both antigen binding surfaces of the (bivalent) antibody and interfere with antigen binding, in which the two binding domains masks are linked to each other (but not the antibody) by a cleavable linker, for example cleavable by a peptidase. See, e.g., Int'l Pat. App. Pub. No. WO 2010/077643 to Tegopharm Corp. Masking ligands may comprise, or be derived from, the antigen to which the antibody is intended to bind, or may be independently generated. Such masking ligands are useful for treatment of cancers in which protease levels are greatly increased in the tumor microenvironment compared with non-tumor tissues. Selective cleavage of the cleavable linker in the tumor microenvironment allows disassociation of the two binding domains from each other, reducing the avidity for the antigen-binding surfaces of the antibody. The resulting dissociation of the masking ligand from the antibody enables antigen binding selectively in the tumor, rather than in peripheral tissues in which antigen binding might cause unwanted side effects.

Nucleic Acids and Host Cells

Also provided are nucleic acids encoding an antibody or a heavy or light chain thereof or a portion thereof. Exemplary nucleic acids are provided in the Sequence Table. Any nucleic acid that is at least 80%, 85%, 90%, 95%, 97%, 98% or 99% to a nucleic acid in the Sequence Table is encompassed herein. Compositions comprising nucleic acids encoding an antibody provided herein are also encompassed, as are cells comprising these and methods for preparing antibodies, comprising culturing a cell transformed with

a nucleic acid encoding an anti-VISTA antibody, and isolating the antibody from the medium or the cell.

Methods of Treatment using VISTA-ECD Binding Abs and Related Pharmaceutical Compositions

In certain embodiments, an anti-VISTA antibody that binds to VISTA at low pH and, e.g., lacks significant binding at neutral or physiological pH, can be a VISTA antagonist antibody, i.e., an antibody that inhibits the action of VISTA, such that an immune response is stimulated. Such antibodies may be used for treating diseases in which stimulating the immune system or an immune response is desired, such as proliferative diseases (benign or malignant), cancer, and infectious diseases (e.g., viral infections).

In certain embodiments, an anti-VISTA antibody that binds to VISTA at low pH and, e.g., lacks significant binding at neutral or physiological pH can be a VISTA agonist antibody, i.e., an antibody that increases the action of VISTA, such that an immune response is inhibited. Such antibodies may be used for treating diseases in which inhibition of the immune system or an immune response is desired, such as autoimmune diseases and inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematosus, celiac disease, Sjoigren's syndrome, Grave's disease, inflammatory bowel disease, psoriasis, ankylosing spondylitis, graft versus host disease, allergy, and asthma.

The antibodies described herein may be used, for example, for treating cancer. In some embodiments, methods for treating cancer are provided, comprising administering an effective amount of an antibody described herein to a patient. In some embodiments, the Abs may trigger or enhance an immune response in the patient, such as an antigen-specific immune response. In some embodiments, the Abs may stimulate T cell activity. In some embodiments, the Abs may inhibit the growth of at least one tumor in the patient.

Provided herein are methods for treating a subject having cancer, comprising administering to the subject a therapeutically effective amount of an anti-VISTA antibody described herein, such that the subject is treated. An anti-VISTA antibody can be used alone. Alternatively, an anti-VISTA antibody can be used in conjunction with another agent, as described further below.

Examples of cancers that may be treated with an Ab specifically binding to a VISTA-ECD protein under acidic conditions as described herein include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. Cancers that may be treated with an Ab described herein also include cancers typically responsive to immunotherapy and those that are not typically responsive to immunotherapy. Cancers that can be treated also include VISTA positive cancers, e.g., cancers having VISTA positive tumor infiltrating cells, e.g., lymphocytes, myeloid or monocytic cells. Cancers can be cancers with solid tumors or blood malignancies (liquid tumors).

Non-limiting examples of cancers for treatment include squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), nonsquamous NSCLC, glioma, gastrointestinal cancer, renal cancer (*e.g.,* clear cell carcinoma), ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer (*e.g.,* renal cell carcinoma (RCC)), prostate cancer (*e.g.,* hormone refractory prostate adenocarcinoma), thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma (glioblastoma multiforme), cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer (or carcinoma), gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma (*e.g.,* metastatic malignant melanoma, such as cutaneous or intraocular malignant melanoma), bone cancer, skin cancer, uterine cancer, cancer of the anal region, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain cancer, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally-induced cancers including those induced by asbestos, virus-related cancers or cancers of viral origin (*e.g.,* human papilloma virus (HPV-related or -originating tumors)), and hematologicd malignancies derived from either of the two major blood cell lineages, *i.e.,* the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or lymphoid cell line (which produces B, T,

NK and plasma cells), such as all types of leukemias, lymphomas, and myelomas, *e.g.*, acute, chronic, lymphocytic and/or myelogenous leukemias, such as acute leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML), undifferentiated AML (MO), myeloblastic leukemia (Ml), myeloblastic leukemia (M2; with cell maturation), promyelocytic leukemia (M3 or M3 variant [M3V]), myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]), monocytic leukemia (M5), erythroleukemia (M6), megakaryoblastic leukemia (M7), isolated granulocytic sarcoma, and chloroma; lymphomas, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), B cell hematologic malignancy, *e.g.*, B-cell lymphomas, T-cell lymphomas, lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, anaplastic (*e.g.*, Ki 1+) large-cell lymphoma, adult T-cell lymphoma/leukemia, mantle cell lymphoma, angio immunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, precursor T-lymphoblastic lymphoma, T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL), peripheral T- cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, B cell lymphoma, lymphoblastic lymphoma (LBL), hematopoietic tumors of lymphoid lineage, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, precursor B -lymphoblastic lymphoma, cutaneous T-cell lymphoma (CTLC) (also called mycosis fungoides or Sezary syndrome), and lymphoplasmacytoid lymphoma (LPL) with Waldenstrom's macroglobulinemia; myelomas, such as IgG myeloma, light chain myeloma, nonsecretory myeloma, smoldering myeloma (also called indolent myeloma), solitary plasmocytoma, and multiple myelomas, chronic lymphocytic leukemia (CLL), hairy cell lymphoma; hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; seminoma, teratocarcinoma, tumors of the central and peripheral nervous, including astrocytoma, schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for

example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) of the T-cell type; a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angiocentric (nasal) T-cell lymphoma; cancer of the head or neck, renal cancer, rectal cancer, cancer of the thyroid gland; acute myeloid lymphoma, as well as any combinations of said cancers. The methods described herein can also be used for treatment of metastatic cancers, unresectable, refractory cancers (*e.g.,* cancers refractory to previous immunotherapy, *e.g.,* with a blocking CTLA-4 or PD-1 antibody), and/or recurrent cancers.

In some embodiments, methods of treating cancer are provided, wherein the methods comprise administering an isolated antibody that binds specifically to huVISTA in acidic conditions as described herein to a subject with cancer. In some embodiments, use of an antibody described herein for treating cancer is provided.

In certain embodiments, an antibody described herein is administered to patients having a cancer that has exhibited an inadequate response to, or progressed on, a prior treatment, *e.g.,* a prior treatment with an immuno-oncology or immunotherapy drug. In some embodiments, the cancer is refractory or resistant to a prior treatment, either intrinsically refractory or resistant (*e.g.,* refractory to a PD-1 pathway antagonist), or a resistance or refractory state is acquired. For example, an antibody described herein may be administered to subjects who are not responsive or not sufficiently responsive to a first therapy or who have disease progression following treatment, *e.g.,* anti-PD-1 pathway antagonist treatment, either alone or in combination with another therapy (*e.g.,* with an anti-PD-1 pathway antagonist therapy). In other embodiments, an antibody described herein is administered to patients who have not previously received (*i.e.,* been treated with) an immuno-oncology agent, *e.g.,* a PD-1 pathway antagonist.

In certain embodiments, a method of treating cancer in a subject comprises first determining the tumor mutational burden (TMB) of a tumor in a subject, and administering an anti-VISTA antibody based on the results, e.g., to subjects found to have a high TMB.

Combinations with Immune Stimulating Agents

In some embodiments, an antibody as described herein, e.g., an antagonist VISTA antibody described herein, is administered in combination with and at least one immune stimulating agent. For example, the therapeutics may be infused together or injected at roughly the same time. In some embodiments, the antibody and the at least one immune stimulating agent are administered sequentially. For example, in some embodiments the antibody is administered sequentially before or after at least one immune stimulating agent such that the two therapeutics are administered 30 minutes, 60 minutes, 90 minutes, 120 minutes, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 3 days, 5 days, 7 days, or two weeks apart.

In some embodiments, at least one, at least two, at least three doses, at least five doses, or at least ten doses of the antibody is administered prior to administration of at least one immune stimulating agent. In some embodiments, at least one, at least two, at least three doses, at least five doses, or at least ten doses of at least one immune stimulating agent is administered prior to administration of the antibody. In some embodiments, the last dose of immune stimulating agent is administered at least one, two, three, five, days or ten, or one, two, three, five, twelve, or twenty four weeks prior to the first dose of the antibody. In some embodiments, the last dose of the antibody is administered at least one, two, three, five, days or ten, or one, two, three, five, twelve, or twenty four weeks prior to the first dose of at least one immune stimulating agent. In some embodiments, a subject has received, or is receiving, therapy with at least one immune stimulating agent and a VISTA-ECD-binding antibody is added to the therapeutic regimen.

In some embodiments, the at least one immune stimulating agent comprises an antagonist of an inhibitor of the activation of T cells, while in some embodiments, the at least one immune stimulating agent comprises an agonist of a stimulator of the activation of T cells. In some embodiments, the at least one immune stimulating agent comprises an antagonist of CTLA4, LAG-3, PD-1, PD-L1, Galectin 1, Galectin 9, CEACAM-1, BTLA, CD25, CD69, TIGIT, CD113, GPR56, VISTA, B7-H3, B7-H4, 2B4, CD48, GARP, PD1H, LAIR1, TIM1, TIM3, TIM4, ILT4, IL-6, IL-10, TGFβ, VEGF, KIR, LAG-3, adenosine A2A receptor, PI3Kdelta, or IDO. In some embodiments, the at least one immune stimulating agent comprises an agonist of B7-1, B7-2, CD28, 4-1BB (CD137), 4-

1BBL, ICOS, ICOS-L, OX40, OX40L, GITR, GITRL, CD27, CD40, CD40L, DR3, CD28H, IL-2, IL-7, IL-12, IL-15, IL-21, IFNα, STING, or a Toll-like receptor agonist such as a TLR2/4 agonist. In some embodiments, the at least one immune stimulating agent comprises an agent that binds to another member of the B7 family of membrane-bound proteins such as B7-1, B7-2, B7-H2 (ICOS-L), B7-H3, B7-H4, and B7-H6. In some embodiments, the at least one immune stimulating agent comprises an agent that binds to a member of the TNF receptor family or a co-stimulatory or co-inhibitory molecule binding to a member of the TNF receptor family such as CD40, CD40L, OX40, OX40L, GITR, GITRL, CD70, CD27L, CD30, CD30L, 4-1BBL, CD137 (4-1BB), TRAIL/Apo2-L, TRAILR1/DR4, TRAILR2/DR5, TRAILR3, TRAILR4, OPG, RANK, RANKL, TWEAKR/Fn14, TWEAK, BAFFR, EDAR, XEDAR, EDA1, EDA2, TACI, APRIL, BCMA, LTβR, LIGHT, DeR3, HVEM, VEGL/TL1A, TRAMP/DR3, TNFR1, TNFβ, TNFR2, TNFα, 1β2, FAS, FASL, RELT, DR6, TROY, or NGFβ. In some embodiments, the at least one immune stimulating agent comprises an agent that antagonizes or inhibits a cytokine that inhibits T cell activation such as IL-6, IL-10, TGFβ, VEGF. In some embodiments, the at least one immune stimulating agent comprises an agonist of a cytokine that stimulates T cell activation such as IL-2, IL-7, IL-12, IL-15, IL-21, and IFNα. In some embodiments, the at least one immune stimulating agent comprises an antagonist of a chemokine, such as CXCR2, CXCR4, CCR2, or CCR4. In some embodiments, the at least one immune stimulating agent comprises an antibody. In some embodiments, the at least one immune stimulating agent may comprise a vaccine, such as a mesothelin-targeting vaccine or attenuated listeria cancer vaccine such as CRS-207.

For example, an anti-VISTA antibody described herein could be administered with one or more of the following agents:

(1) An antagonist (inhibitor or blocking agent) of a protein that inhibits T cell activation (*e.g.*, immune checkpoint inhibitors), such as CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3, Galectin 9, CEACAM-1, BTLA, CD69, Galectin-1, TIGIT, CD113, GPR56, B7-H3, B7-H4, 2B4, CD48, GARP, PD1H, LAIR1, TIM-1,TIM-3 and TIM-4; and/or (2) An agonist of a protein that stimulates T cell activation, such as B7-1, B7-2, CD28, 4-1BB (CD137), 4-1BBL, GITR, ICOS, ICOS-L, OX40, OX40L, CD70, CD27, CD40, DR3 and CD28H.

Exemplary agents that can be combined with anti-VISTA antibodies described herein for treating cancer include: YERVOY® (ipilimumab) or Tremelimumab (to CTLA-4), galiximab (to B7.1), BMS-936558 (nivolumab; to PD-1), MK-3475 (pembrolizumab; to PD-1), atezolizumab (TECENTRIQ®), Avelumab, Durvalumab, cemiplimab, toripalimab, sintilimb, AMP224 (to B7DC), BMS-936559 (to B7-H1), MPDL3280A (to B7-H1), MEDI-570 (to ICOS), AMG557 (to B7H2), MGA271 (to B7H3), IMP321 (to LAG-3), BMS-663513 (to CD137), PF-05082566 (to CD137), CDX-1127 (to CD27), anti-OX40 (Providence Health Services), huMAbOX40L (to OX40L), Atacicept (to TACI), CP-870893 (to CD40), Lucatumumab (to CD40), Dacetuzumab (to CD40), Muromonab-CD3 (to CD3); anti-GITR antibodies MK4166, TRX518, Medi1873, INBRX-110, LK2-145, GWN-323, GITRL-Fc, or any combination thereof.

Other molecules that can be combined with anti-VISTA antibodies for the treatment of cancer include antagonists of inhibitory receptors on NK cells or agonists of activating receptors on NK cells, for example, antagonists of KIR (*e.g.*, lirilumab).

T cell activation may also be regulated by soluble cytokines. In some embodiments, anti-VISTA antibodies can be administered in combination with antagonists of cytokines that are intended to inhibit T cell activation or agonists of cytokines that stimulate T cell activation. For example, anti-VISTA antibodies can be used in combination with (i) antagonists (or inhibitors or blocking agents) of proteins of the IgSF family or B7 family or the TNF family that inhibit T cell activation or antagonists of cytokines that inhibit T cell activation (*e.g.*, IL-6, IL-10, TGF-β, VEGF; "immunosuppressive cytokines") and/or (ii) agonists of stimulatory receptors of the IgSF family, B7 family or the TNF family or of cytokines that stimulate T cell activation.

Yet other agents for combination therapies include agents that inhibit or deplete macrophages or monocytes, including but not limited to CSF-1R antagonists such as CSF-1R antagonist antibodies including RG7155 (WO11/70024, WO11/107553, WO11/131407, W013/87699, W013/119716, WO13/132044) or FPA-008 (WO11/140249; W013169264; WO14/036357).

Anti-VISTA antibodies can also be administered with agents that inhibit TGF-β signaling.

Additional agents that can be combined with an anti-VISTA antibody include agents that enhance tumor antigen presentation, *e.g.*, dendritic cell vaccines, GM-CSF

secreting cellular vaccines, CpG oligonucleotides, and imiquimod, or therapies that enhance the immunogenicity of tumor cells (*e.g.*, anthracyclines).

Yet other therapies that can be combined with an anti-VISTA antibody include therapies that deplete or block Treg cells, *e.g.*, an agent that specifically binds to CD25.

Another therapy that can be combined with an anti-VISTA antibody is a therapy that inhibits a metabolic enzyme such as indoleamine dioxigenase (IDO), dioxigenase, arginase, or nitric oxide synthetase.

Another class of agents that can be used with an anti-VISTA antibody includes agents that inhibit the formation of adenosine, *e.g.*, CD73 inhibitors, or inhibit the adenosine A2A receptor.

Other therapies that can be combined with an anti-VISTA antibody for treating cancer include therapies that reverse/prevent T cell anergy or exhaustion and therapies that trigger an innate immune activation and/or inflammation at a tumor site.

Other therapies that can be combined with an anti-VISTA antibody for treating cancer include therapies that block IL-8, *e.g.*, with HuMax®-IL8.

An anti-VISTA antibody can be combined with more than one immuno-oncology agent, and can be, *e.g.*, combined with a combinatorial approach that is intended to target multiple elements of the immune pathway, such as one or more of the following: a therapy that enhances tumor antigen presentation (*e.g.*, dendritic cell vaccine, GM-CSF secreting cellular vaccines, CpG oligonucleotides, imiquimod); a therapy that inhibits negative immune regulation *e.g.*, by inhibiting CTLA-4 and/or PD1/PD-L1/PD-L2 pathway and/or depleting or blocking Tregs or other immune suppressing cells; a therapy that stimulates positive immune regulation, *e.g.*, with agonists that stimulate the CD-137, OX-40, and/or CD40 or GITR pathway and/or stimulate T cell effector function; a therapy that increases systemically the frequency of anti-tumor T cells; a therapy that depletes or inhibits Tregs, such as Tregs in the tumor, *e.g.*, using an antagonist of CD25 (*e.g.*, daclizumab) or by *ex vivo* anti-CD25 bead depletion; a therapy that impacts the function of suppressor myeloid cells in the tumor; a therapy that enhances immunogenicity of tumor cells (*e.g.*, anthracyclines); adoptive T cell or NK cell transfer including genetically modified cells, *e.g.*, cells modified by chimeric antigen receptors (CAR-T therapy); a therapy that inhibits a metabolic enzyme such as indoleamine dioxigenase (IDO), dioxigenase, arginase, or nitric oxide synthetase; a therapy that

reverses/prevents T cell anergy or exhaustion; a therapy that triggers an innate immune activation and/or inflammation at a tumor site; administration of immune stimulatory cytokines; or blocking of immuno repressive cytokines.

Anti-VISTA antibodies described herein can be used together with one or more of agonistic agents that ligate positive costimulatory receptors, blocking agents that attenuate signaling through inhibitory receptors, antagonists, and one or more agents that increase systemically the frequency of anti-tumor T cells, agents that overcome distinct immune suppressive pathways within the tumor microenvironment (*e.g.,* block inhibitory receptor engagement (*e.g.,* PD-L1/PD-1 interactions), deplete or inhibit Tregs (*e.g.,* using an anti-CD25 monoclonal antibody (*e.g.,* daclizumab) or by *ex vivo* anti-CD25 bead depletion), inhibit metabolic enzymes such as IDO, or reverse/prevent T cell anergy or exhaustion) and agents that trigger innate immune activation and/or inflammation at tumor sites.

In certain embodiments, an anti-VISTA antibody is administered to a subject together with a BRAF inhibitor if the subject is BRAF V600 mutation positive.

Suitable PD-1 antagonists for use in the combination therapy described herein, include, without limitation, ligands, antibodies (*e.g.,* monoclonal antibodies and bispecific antibodies), and multivalent agents. In one embodiment, the PD-1 antagonist is a fusion protein, *e.g.,* an Fc fusion protein, such as AMP-244. In one embodiment, the PD-1 antagonist is an anti-PD-1 or anti-PD-L1 antibody.

An exemplary anti-PD-1 antibody is nivolumab (BMS-936558) or an antibody that comprises the CDRs or variable regions of one of antibodies 17D8, 2D3, 4H1, 5C4, 7D3, 5F4 and 4A11 described in WO 2006/121168. In certain embodiments, an anti-PD-l antibody is MK-3475 (Lambrolizumab) described in WO2012/ 145493; AMP-514 described in WO 2012/145493; or PDR001. Further known PD-1 antibodies and other PD-1 inhibitors include those described in WO 2009/014708, WO 03/099196, WO 2009/114335, WO 2011/066389, WO 2011/161699, WO 2012/145493, U.S. Patent Nos. 7,635,757 and 8,217,149, and U.S. Patent Publication No. 2009/0317368. Any of the anti-PD-1 antibodies disclosed in WO2013/173223 can also be used. An anti-PD-1 antibody that competes for binding with, and/or binds to the same epitope on PD-1 as, as one of these antibodies can also be used in combination treatments.

In some embodiments, the anti-PD-L1 antibody useful for the combination therapy is BMS-936559 (referred to as 12A4 in WO 2007/005874 and US Patent No. 7,943,743), or an antibody that comprises the CDRs or variable regions of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4, which are described in PCT Publication WO 07/005874 and US Patent No. 7,943,743. In certain embodiment an anti-PD-L1 antibody is MEDI4736 (also known as durvalumab and Anti-B7-H1), MPDL3280A (also known as atezolizumab and RG7446), MSB0010718C (also known as avelumab; WO2013/79174), or rHigM12B7. Any of the anti-PD-L1 antibodies disclosed in WO2013/173223, WO2011/066389, WO2012/ 145493, U.S. Patent Nos. 7,635,757 and 8,217,149 and U.S. Publication No. 2009/145493 can also be used. Anti-PD-L1 antibodies that compete with and/or bind to the same epitope as that of any of these antibodies can also be used in combination treatments.

In certain embodiments, the anti-VISTA antibody of the disclosure can be used with a CTLA-4 antagonist, *e.g.*, an anti-CTLA-4 antibody. In one embodiment, an anti-CTLA-4 antibody is an antibody selected from the group of: YERVOY® (ipilimumab or antibody 10D1, described in PCT Publication WO 01/14424), tremelimumab (formerly ticilimumab, CP-675,206), monoclonal or an anti-CTLA-4 antibody described in any of the following publications: WO 98/42752; WO 00/37504; U.S. Pat. No. 6,207,156; Hurwitz *et al.* (1998) *Pro. Natl. Acad. Sci. USA* 95(17): 10067-10071; Camacho *et al.* (2004) *J. Clin. Oncology* 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res.* 58:5301-5304. Any of the anti-CTLA-4 antibodies disclosed in WO2013/173223 can also be used.

In some embodiments, an anti-VISTA antibody of the disclosure is used in combination with a LAG3 antagonist. Examples of anti-LAG3 antibodies include antibodies comprising the CDRs or variable regions of antibodies 25F7, 26H10, 25E3, 8B7, 11F2 or 17E5, which are described in U.S. Patent Publication No. US2011/0150892, WO10/19570 and WO2014/008218. In one embodiment, an anti-LAG-3 antibody is BMS-986016. Other art recognized anti-LAG-3 antibodies that can be used include IMP731 and IMP-321, described in US 2011/007023, WO08/132601, and WO09/44273. Anti-LAG-3 antibodies that compete with and/or bind to the same epitope as that of any of these antibodies can also be used in combination treatments.

In some embodiments, an anti-VISTA antibody of the disclosure can be administered in combination with a CD137 (4-1BB) agonist, such as an agonistic CD137 antibody. Suitable CD137 antibodies include, for example, urelumab or PF-05082566 (W012/32433).

In some embodiments, an anti-VISTA antibody can be administered in combination with an OX40 agonist, such as an agonistic OX40 antibody. Suitable OX40 antibodies include, for example, MEDI-6383, MEDI-6469 or MOXR0916 (RG7888; WO06/029879).

In one embodiment, an anti-VISTA antibody is administered in combination with a CD40 agonist, such as an agonistic CD40 antibody. In certain embodiments, the immuno-oncology agent is a CD40 antagonist, such as an antagonistic CD40 antibody. Suitable CD40 antibodies include, for example, lucatumumab (HCD122), dacetuzumab (SGN-40), CP-870,893 or Chi Lob 7/4.

In one embodiment, an anti-VISTA antibody is administered in combination with a CD27 agonist, such as an agonistic CD27 antibody. Suitable CD27 antibodies include, for example, varlilumab (CDX-1127).

In certain embodiments, the anti-VISTA antibody is administered together with an anti-GITR antibody, *e.g.,* an antibody having the CDR sequences of 6C8, *e.g.,* a humanized antibody having the CDRs of 6C8, as described, *e.g.,* in WO2006/105021; an antibody comprising the CDRs of an anti-GITR antibody described in WO2011/028683; an antibody comprising the CDRs of an anti-GITR antibody described in JP2008278814, an antibody comprising the CDRs of an anti- GITR antibody described in WO2015/031667, WO2015/187835, WO2015/184099, WO2016/054638, WO2016/057841 or WO2016/057846 or other anti- GITR antibody described or referred to herein.

In some embodiments, an anti-VISTA antibody is administered in combination with MGA271 (to B7H3) (WO11/109400).

In some embodiments, an anti-VISTA antibody is administered in combination with a KIR antagonist, such as lirilumab.

In some embodiments, an anti-VISTA antibody is administered in combination with an IDO antagonist. Suitable IDO antagonists include, for example, INCB-024360

(WO2006/122150, WO07/75598, WO08/36653, WO08/36642), indoximod, NLG-919 (WO09/73620, WO09/1156652, WO11/56652, WO12/142237) or F001287.

In some embodiments, an anti-VISTA antibody is administered in combination with a Toll-like receptor agonist, *e.g.,* a TLR2/4 agonist (*e.g.,* Bacillus Calmette-Guerin); a TLR7 agonist (*e.g.,* Hiltonol or Imiquimod); a TLR7/8 agonist (*e.g.,* Resiquimod); or a TLR9 agonist (*e.g.,* CpG7909).

In one embodiment, an anti-VISTA is administered in combination with a TGF-β inhibitor, *e.g.,* GC1008, LY2157299, TEW7197, or IMC-TR1.

Additional Combination Therapy

The Abs herein may also be provided before, substantially contemporaneous with, or after other modes of treatment, for example, surgery, chemotherapy, radiation therapy, or the administration of a biologic, such as another therapeutic antibody. In some embodiments, the cancer has recurred or progressed following a therapy selected from surgery, chemotherapy, and radiation therapy, or a combination thereof. For example, an anti-VISTA antibody as described herein could be administered as adjunctive therapy when there is a risk that micrometastases can be present and/or in order to reduce the risk of a relapse.

For treatment of cancer, the combinations may be administered in conjunction with one or more additional anti-cancer agents, such as a chemotherapeutic agent, growth inhibitory agent, anti-cancer vaccine such as a gene therapy vaccine, anti-angiogenesis agent and/or anti-neoplastic composition. Nonlimiting examples of chemotherapeutic agent, growth inhibitory agent, anti-cancer vaccine, anti-angiogenesis agent and anti-neoplastic composition that can be used in combination with the antibodies of the present invention are provided herein under "Definitions."

In some embodiments, an anti-inflammatory drug may be administered with the combination, such as a steroid or a non-steroidal anti-inflammatory drug (NSAID). In cases where it is desirable to render aberrantly proliferative cells quiescent in conjunction with or prior to treatment with anti-VISTA antibodies described herein, hormones and steroids (including synthetic analogs), such as 17a-Ethinylestradiol, Diethylstilbestrol,Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Methylprednisolone, Methyl-testosterone,

Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, ZOLADEX®, can also be administered to the patient. When employing the methods or compositions described herein, other agents used in the modulation of tumor growth or metastasis in a clinical setting, such as antimimetics, can also be administered as desired.

Antibodies described herein can also be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al.*,(2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

In humans, some tumors have been shown to be immunogenic such as melanomas. By lowering the threshold of T cell activation via VISTA inhibition, the tumor responses in the host can be activated, allowing treatment of non-immunogenic tumors or those having limited immunogenicity.

An anti-VISTA antibody described herein, can also be combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (*see* Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C, 2000, ASCO Educational Book Spring: 300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000, ASCO Educational Book Spring: 730-738; *see also* Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology, Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, S A (1999) *Immunity* 10: 281-7). In many cases, these tumor specific antigens are

differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. VISTA inhibition can be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen can include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim *et al.* (1994) *Science* 266: 2011-2013). Tumor antigen can also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

Other tumor vaccines can include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which can be used in conjunction with VISTA inhibition is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot & Srivastava (1995) *Science* 269: 1585-1588; Tamura *et al.* (1997) *Science* 278: 117-120).

Oncolytic viruses may also be used in combination with VISTA antibodies.

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DCs can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle *et al.* (1998) *Nature Medicine* 4: 328-332). DCs can also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler *et al.* (2000) *Nature Medicine* 6:332-336). As a method of vaccination, DC immunization can be effectively combined with VISTA inhibition to activate more potent anti-tumor responses.

Infectious Disease Treatments

Methods described herein can also be used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, this disclosure also contemplates methods of treating an infectious disease in a subject comprising administering to the subject an antibody as described herein, e.g., an antagonist VISTA antibody, such that the subject is treated for the infectious disease. Similar to its application to tumors as discussed above, antibody-mediated VISTA inhibition can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach might be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. VISTA inhibition can be useful against established infections by agents such as HIV that present altered antigens over the course of the infections.

Some examples of pathogenic viruses causing infections that may be treatable by methods described herein include HIV, hepatitis (A, B, or C), herpes virus (*e.g.,* VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections that may be treatable by methods described herein include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and gonococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria.

Some examples of pathogenic fungi causing infections that may be treatable by methods described herein include Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizopus), *Sporothrix schenkii*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis* and *Histoplasma capsulatum*.

Some examples of pathogenic parasites causing infections that may be treatable by methods described herein include *Entamoeba histolytica*, *Balantidium coli*, Naegleriafowleri, *Acanthamoeba sp.*, *Giardia lambia*, *Cryptosporidium sp.*, *Pneumocystis carinii*, *Plasmodium vivax*, *Babesia microti*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii*, and *Nippostrongylus brasiliensis*.

In all of the above methods, VISTA inhibition can be combined with other forms of immunotherapy, *e.g.,* those described herein, such as cytokine treatment (*e.g.,* interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy, which may provide for enhanced presentation of tumor antigens (see, *e.g.,* Holliger (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak (1994) *Structure* 2: 1121-1123).

Routes of Administration and Carriers

In various embodiments, antibodies may be administered *in vivo* by various routes, including, but not limited to, oral, intra-arterial, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. A nucleic acid molecule encoding an antibody may be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or "gene gun," as described in the literature (see, e.g., Tang et al., *Nature* 356:152-154 (1992)). The appropriate formulation and route of administration may be selected according to the intended application.

In various embodiments, compositions comprising antibodies are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 7th ed., Lippencott Williams and Wilkins (2004); Kibbe et al., *Handbook of Pharmaceutical Excipients,* 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary

substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

In various embodiments, compositions comprising antibodies may be formulated for injection, including subcutaneous administration, by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release microcapsules, such as with biodegradable or non-biodegradable polymers. A non-limiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A non-limiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Certain methods of making such formulations are described, for example, in EP 1 125 584 A1.

Pharmaceutical packs and kits comprising one or more containers, each containing one or more doses of an antibody or combination of antibodiesare also provided. In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising an antibody or combination of antibodies, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective Ph range. Alternatively, in some embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, antibodies may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

The antibody compositions may be administered as needed to subjects. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of an antibody is administered to a subject one or more times. In various embodiments, an effective dose of an antibody is administered to the subject once a month, less than once a month, such as, for example, every two months or every three months. In other embodiments, an effective dose of an antibody is administered more than once a month, such as, for example, every three weeks, every two weeks or every week. In some embodiments, an effective dose of an antibody is administered once per 1, 2, 3, 4, or 5 weeks. In some embodiments, an effective dose of an antibody is administered twice or three times per week. An effective dose of an antibody is administered to the subject at least once. In some embodiments, the effective dose of an antibody may be administered multiple times, including for periods of at least a month, at least six months, or at least a year.

In certain embodiments, the combination of the anti-VISTA antibody and a second agent discussed herein can be administered concurrently as a single composition

in a pharmaceutically acceptable carrier, or concurrently as separate compositions with the anti-VISTA antibody and the second agent in a pharmaceutically acceptable carrier. In one embodiment, the combination of the anti-VISTA antibody and the second agent can be administered sequentially. The administration of the two agents can start at times that are, *e.g.,* 30 minutes, 60 minutes, 90 minutes, 120 minutes, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 3 days, 5 days, 7 days, or one or more weeks apart, or administration of the second agent can start, *e.g.,* 30 minutes, 60 minutes, 90 minutes, 120 minutes, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 3 days, 5 days, 7 days, or one or more weeks after the first agent has been administered.

Methods of Identifying Low pH Binding hVISTA-ECD Abs

Also provided herein are methods for identifying Abs that specifically bind to a VISTA-ECD protein in acidic (or low pH) conditions. In certain embodiments, a method for identifying an Ab that binds specifically to a VISTA-ECD protein at pH 6.5 or less comprises contacting a test Ab or plurality of test Abs with a VISTA-ECD protein at pH 6.5 or less, and selecting the test Ab if it binds to the ECD of the VISTA protein with a K_D of 10^{-7} M, 10^{-8} M, 10^{-9} M or less. In some embodiments, the method is performed at pH 6.5, while in others it is performed at pH 6.0, or at pH 5.5, or at pH 5.0. In some embodiments, the VISTA-ECD protein is a hVISTA-ECD protein, or comprises the hVISTA IgV domain, or is a polypeptide comprising amino acids 20-95 of SEQ ID NO:2, or amino acids 20-70, 35-95, or 35-70 of SEQ ID NO:2. In some embodiments, the polypeptide also comprises amino acids 95-105 of SEQ ID NO:2. In some embodiments, the polypeptide comprises amino acids 35-127 or 37-125 of SEQ ID NO: 2.

In some embodiments, the method further comprises testing binding of the test Ab or plurality of test Abs at neutral, physiological or alkaline pH, such as at pH 7.0 or pH 7.4. In some embodiments, the method further comprises selecting an antibody if it not only binds to the VISTA-ECD protein with a K_D of 10^{-7} M, 10^{-8} M, 10^{-9} M or less at pH 6.5 or lower, but also if it binds specifically to the polypeptide at pH 7.0 or pH 7.4. In some embodiments, test Abs are selected if they specifically bind to the VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less, also specifically bind the VISTA-ECD protein at neutral and/or alkaline pH with similar affinity (i.e. they are "pan binders"). For example, some such Abs may bind to the VISTA-ECD protein with a K_D of 10^{-7} M,

10^{-8} M, 10^{-9} M or less at both pH 6.5 and at pH 7.0 or pH 7.4 (at a constant temperature, e.g., of 25°C or at 37°C) such that the K_D at pH 6.5 is within 1.5-fold of the K_D at pH 7.0.

Certain Abs may be selected if they specifically bind to the VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less with higher affinity than at neutral or alkaline pH ("pH sensitive binders" or "pH sensitive Abs"). For example, in some embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D of more than 10^{-8} M at pH 7.0 or pH 7.4. In some such embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D at pH 7.0 or pH 7.4 that is more than 1.5-fold higher than that at pH 6.5 In certain embodiments, a pH sensitive Ab is selected if it specifically binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold lower at pH 6.5 than at pH 7.0 or pH 7.4 (at a constant temperature, e.g., of 25°C or at 37°C). For example, in some cases an Ab is selected if it binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold less at pH 6.0, relative to pH 7.0 or pH 7.4 or higher (at a constant temperature, e.g., of 25°C or at 37°C).

In certain embodiments, an Ab is selected if it specifically binds to the VISTA-ECD protein with a k_{off} that is lower in acidic conditions relative to that in neutral, physiological or alkaline conditions. In certain embodiments, an Ab is selected if it binds to the VISTA-ECD protein in acidic conditions with a k_{off} that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold lower at pH 6.5 than the k_{off} at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C. For example, in some embodiments, an Ab is selected if it binds to the VISTA-ECD protein with a k_{off} rate that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold lower at pH 6.0, relative to pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C.

In certain embodiments, an Ab is selected if it binds to the VISTA-ECD protein with a k_{on} that is higher in acidic conditions relative to neutral or alkaline conditions. In certain embodiments, an Ab is selected if it binds to the VISTA-ECD protein in acidic conditions with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold higher at pH 6.5 than the k_{on} at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C. For example, in some embodiments, an Ab is selected if it binds to the VISTA-ECD

protein with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold higher at pH 6.0 than at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C.

Methods of Modifying the pH Sensitivity of VISTA-ECD Binding Abs

An Ab that binds to a VISTA-ECD protein, but does not do so at pH 6.5 or less, or does not do so with a high affinity at pH 6.5 or less, can be engineered to increase its affinity of binding at pH 6.5 or lower. For example, the paratope of an Ab may be mutated, e.g., by the substitution of one or more amino acid residues. For example, in some embodiments, 1 to 8, e.g., 1 to 6, 1 to 4, 1 to 3, 1 to 2 or 1 amino acid residues in the heavy or light chain of the Ab that are contact residues with VISTA-ECD (e.g. residues in one or more of the CDRs) may be replaced with a different amino acid residue. Then, the mutated Ab may be tested for binding to the VISTA-ECD protein at pH 6.5 or less and Ab species binding with higher affinity than the parent antibody may be selected. If desired, the steps above may be repeated so that two or more rounds of mutagenesis and selection are performed on the Abs and the highest affinity binders at the acidic pH are selected. In some embodiments, such selections may improve the anti-tumor efficacy of the resulting antibody over its parent.

The above selection method may also be designed to follow the previously described general selection for VISTA-ECD protein specifically binding antibodies. Namely, in certain embodiments, the improved Ab is selected if it binds to the ECD of the VISTA protein with a K_D of 10^{-8} M or less at pH 6.5. In some embodiments, the selection is performed at pH 6.0, or at pH 5.5, or at pH 5.0 instead of at pH 6.5. In some embodiments, the VISTA-ECD protein used for the selection process is a complete hVISTA-ECD protein, or is a polypeptide that comprises the hVISTA IgV domain, or is a polypeptide comprising amino acids 20-95 of SEQ ID NO:2, or amino acids 20-70, 35-95, or 35-70 of SEQ ID NO:2. In some embodiments, the polypeptide also comprises amino acids 95-105 of SEQ ID NO:2. In some embodiment a polypeptide comprising amino acid residues 35-127of SEQ ID NO: 2 is used.

In some embodiments, a method for improving the binding of a VISTA antibody to VISTA ECD at acidic pH comprises increasing the number of glutamic acid, aspartic acid and/or histidine residues in one or more VH or VL CDRs, e.g., VH CDR1, CDR2 and CDR3 or only VH CDR1 and CDR3. In certain embodiments, a method comprises

increasing the number of glutamic acid, aspartic acid and/or histidine residues in areas of the antibody that contacts hVISTA as determined, e.g., by crystallography.

In some embodiments, the method further comprises testing binding of the selected Ab at neutral, alkaline or physiological pH, such as at pH 7.0 or 7.4. In some embodiments, the method further comprises selecting an antibody if it not only binds to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 or lower, but also if it binds specifically to the polypeptide at pH 7.0 or 7.4. In some such embodiments, Abs are selected if they specifically bind to the VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less, and also specifically bind the VISTA-ECD protein at neutral and/or alkaline or physiological pH with at similar affinity (i.e. they are "pan binders"). For example, some such Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at both pH 6.5 and at pH 7.0 (at a constant temperature, e.g., of 25°C or at 37°C) such that the K_D at pH 6.5 is within 1.5-fold of the K_D at pH 7.0 or at pH 7.4.

Certain Abs may be selected if they specifically bind to the VISTA-ECD protein in acidic conditions, e.g., at pH 6.5 or less with higher affinity than at neutral, physiological, or alkaline pH ("pH sensitive binders" or "pH sensitive Abs"). For example, in some embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D of more than 10^{-8} M at pH 7.0. In some such embodiments, Abs may bind to the VISTA-ECD protein with a K_D of 10^{-8} M or less at pH 6.5 and with a K_D at pH 7.0 that is more than 1.5-fold higher than that at pH 6.5. In certain embodiments, a pH sensitive Ab is selected if it specifically binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold lower at pH 6.5 than at pH 7.0 or pH 7.4 (at a constant temperature, e.g., of 25°C or at 37°C). For example, in some cases an Ab is selected if it binds to the VISTA-ECD protein with a K_D that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold, 100 fold, 300 fold, 500 fold, 1000 fold, or 5000 fold less at pH 6.0, relative to pH 7.0 or pH 7.4 or higher (at a constant temperature, e.g., of 25°C or at 37°C).

In certain embodiments, the method further comprises determining k_{off} at two pH values. In some such embodiments, an Ab is selected if it specifically binds to the VISTA-ECD protein with a k_{off} that is lower in acidic conditions relative to that in neutral, physiological, or alkaline conditions. In certain embodiments, an Ab is selected

if it binds to the VISTA-ECD protein in acidic conditions with a k_{off} that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold lower at pH 6.5 than the k_{off} at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C. For example, in some embodiments, an Ab is selected if it binds to the VISTA-ECD protein with a k_{off} rate that is at least 1.5 fold, 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold lower at pH 6.0, relative to pH 7.0, as measured, e.g., at 25°C or at 37°C.

In certain embodiments, the method further comprises determining k_{on} at two pH values. In some such embodiments, an Ab is selected if it binds to the VISTA-ECD protein with a k_{on} that is higher in acidic conditions relative to neutral, physiological, or alkaline conditions. In certain embodiments, an Ab is selected if it binds to the VISTA-ECD protein in acidic conditions with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold higher at pH 6.5 than the k_{on} at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C. For example, in some embodiments, an Ab is selected if it binds to the VISTA-ECD protein with a k_{on} that is at least 2 fold, 5 fold, 10 fold, 20 fold, 50 fold or 100 fold higher at pH 6.0 than at pH 7.0 or pH 7.4, as measured, e.g., at 25°C or at 37°C.

Antibodies that bind preferentially to huVISTA at acidic pH, versus neutral or physiological pH can be identified by positively screening a library of VISTA antibodies or Fabs or scFvs for binding at acidic pH, e.g., pH 6.0 or 6.5, and negatively screening the library for the lack of binding at neutral pH, e.g., pH 7.0 or physiological pH, e.g., pH 7.4. A library may be enriched in glutamic acid, aspartic acid and histidine residues, such as to select binding domains that may be charged and more likely to bind to VISTA at acidic pH. The screening may involve positive selection at acidic pH and negative selections at neutal or physiological pH. The positive and negative selections may be alternated.

Alternatively, an antibody binding to VISTA at neutral and acidic pH and can be engineered to lack binding at neutral pH and maintaining or even enhancing binding at acidic pH. For example, a library may be created by substituting VH and optionally VL amino acid residues, such as in one or more CDRs and screening the library by positive selection for antibodies that bind to hVISTA at acidic pH and negative selection for antibodies that do not bind to VISTA at neutral (or physiological) pH. A similar method may be used to engineer VISTA binding antibodies having the desired pH selective, pH dependent or pH independent VISTA binding profile.

Specific embodiments

Additional embodiments of this disclosure include the following:

1. An isolated antibody that binds specifically to human VISTA (hVISTA) in acidic conditions.
2. The isolated antibody of embodiment 1, which binds specifically to hVISTA in acidic conditions, but not significantly in neutral or physiological conditions.
3. The isolated antibody of embodiment 1 or 2, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 10 fold lower than its K_D in neutral or physiological conditions.
4. The isolated antibody of any one of embodiments 1 to 3, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 100 fold lower than its K_D in neutral or physiological conditions.
5. The isolated antibody of any one of embodiments 1-4, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 1000 fold lower than its K_D in neutral or physiological conditions.
6. The isolated antibody of any one of embodiments 1-5, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} M or more.
7. The isolated antibody of any one of embodiments 1-6, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} M or more.
8. The isolated antibody of any one of embodiments 1-7, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-3} M or more.
9. The isolated antibody of any one of embodiments 1-8, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} M or less.
10. The isolated antibody of any one of embodiments 1-9, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less.
11. The isolated antibody of any one of embodiments 1-10, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-9} M or less.
12. The isolated antibody of any one of embodiments 1-11, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} or more.

13. The isolated antibody of any one of embodiments 1-12, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} or more.
14. The isolated antibody of any one of embodiments 1-13, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 5 fold lower than its k_{off} in neutral or physiological conditions.
15. The isolated antibody of any one of embodiments 1-14, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 10 fold lower than its k_{off} in neutral or physiological conditions.
16. The isolated antibody of any one of embodiments 1-15, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 50 fold lower than its k_{off} in neutral or physiological conditions.
17. The isolated antibody of any one of embodiments 1-16, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 100 fold lower than its k_{off} in neutral or physiological conditions.
18. The isolated antibody of any one of embodiments 1-17, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7×10^{-3} s^{-1} or less.
19. The isolated antibody of any one of embodiments 1-18, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5×10^{-3} s^{-1} or less.
20. The isolated antibody of any one of embodiments 1-19 wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3×10^{-3} s^{-1} or less.
21. The isolated antibody of any one of embodiments 1-20, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-3} s^{-1} or less.
22. The isolated antibody of any one of embodiments 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7×10^{-4} s^{-1} or less.
23. The isolated antibody of any one of embodiments 1-22, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5x\ 10^{-4}$ s^{-1} or less.
24. The isolated antibody of any one of embodiments 1-23, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3×10^{-4} s^{-1} or less.
25. The isolated antibody of any one of embodiments 1-24, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less.

26. The isolated antibody of any one of embodiments 1-25, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7 x 10^{-5} s^{-1} or less.
27. The isolated antibody of any one of embodiments 1-26, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5 x 10^{-5} s^{-1} or less.
28. The isolated antibody of any one of embodiments 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3 x 10^{-5} s^{-1} or less.
29. The isolated antibody of any one of embodiments 1-28, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-5} s^{-1} or less.
30. The isolated antibody of any one of embodiments 1-29, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-3} s^{-1} or more.
31. The isolated antibody of any one of embodiments 1-30, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 3 x 10^{-3} s^{-1} or more.
32. The isolated antibody of any one of embodiments 1-31, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 5 x 10^{-3} s^{-1} or more.
33. The isolated antibody of any one of embodiments 1-32, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 7 x 10^{-3} s^{-1} or more.
34. The isolated antibody of any one of embodiments 1-33, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.
35. The isolated antibody of any one of embodiments 1-34, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 3 x 10^{-2} s^{-1} or more.
36. The isolated antibody of any one of embodiments 1-35, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 5 x 10^{-2} s^{-1} or more.
37. The isolated antibody of any one of embodiments 1-36, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 7 x 10^{-2} s^{-1} or more.

38. The isolated antibody of any one of embodiments 1-37, wherein binding of the antibody to hVISTA in neutral or physiological conditions is not detectable, e.g., via surface plasmon resonance (SPR).

39. The isolated antibody of any one of embodiments 1-38, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5×10^{-3} s^{-1} or less and in neutral or physiological conditions with a k_{off} of 7×10^{-3} s^{-1} or more.

40. The isolated antibody of any one of embodiments 1-39, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less and in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.

41. The isolated antibody of any one of embodiments 1-40, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of 5×10^{-3} s^{-1} or less and in neutral of physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of 7×10^{-3} s^{-1} or more.

42. The isolated antibody of any one of embodiments 1-41, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of 3×10^{-3} s^{-1} or less and in neutral of physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of 10^{-2} s^{-1} or more.

43. The isolated antibody of any one of embodiments 1-42, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to 5×10^{-3} s^{-1} and in neutral of physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3×10^{-3} to 10^{-2} s^{-1} or more.

44. The isolated antibody of any one of embodiments 1-43, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to 5×10^{-3} s^{-1} and in neutral of physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3×10^{-3} to 10^{-2} s^{-1} or more; and wherein the antibody binds to cyno VISTA with a K_D of 10^{-7} or less.

45. The isolated antibody of any one of embodiments 1-44, wherein the antibody binds to hVISTA with a K_D that is at least 10 fold lower at pH 6.9 than at pH 7.4 and/or a K_D that is at least 100 fold lower at pH 6.5 than at pH 7.4 and at least 1000 fold lower at pH 6.0 than at pH 7.4.

46. The isolated antibody of any one of embodiments 1-45, wherein the antibody binds specifically to cynomolgus (cyno) VISTA.

47. The isolated antibody of embodiment 46, wherein the antibody binds to cyno VISTA with higher affinity in acidic conditions relative to physiologic conditions.
48. The isolated antibody of any one of embodiments 45-47, wherein the antibody binds to cyno VISTA in acidic conditions with a K_D of 10^{-8} or less and/or a k_{off} of 10^{-2} or less and in physiologic conditions with a K_D of 10^{-6} or more and/or a k_{off} of 10^{-2} or more.
49. The isolated antibody of any one of embodiments 1-48, wherein acidic conditions are conditions having a pH of 6.5 or less.
50. The isolated antibody of any one of embodiments 1-49, wherein acidic conditions are conditions having a pH of 6.0 to 6.5.
51. The isolated antibody of any one of embodiment 1-50, wherein neutral conditions are conditions having a pH of 7.0.
52. The isolated antibody of any one of embodiments 1-51, wherein physiological conditions are conditions having a pH of 7.35 to 7.45.
53. The isolated antibody of any one of embodiments 1-52, wherein physiological conditions are conditions having a pH of 7.4.
54. The isolated antibody of any one of embodiments 1-53, wherein the antibody inhibits the binding of hVISTA to human T cells, such as human CD4+ T cells (an antagonist antibody).
55. The isolated antibody of embodiment 54, wherein the antibody inhibits the binding of hVISTA to human T cells in conditions having a pH of less than pH 7.0.
56. The isolated antibody of any one of embodiments 1-55, wherein the antibody inhibits the binding of hVISTA to human PSGL-1 (huPSGL-1) and/or the Ab competes with huPSGL1 for binding to hVISTA.
57. The isolated antibody of embodiment 56, wherein the antibody inhibits the binding of hVISTA to huPSGL-1 in conditions having a pH of less than pH 7.0.
58. The isolated antibody of any one of embodiments 1-57, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans.
59. The isolated antibody of embodiment 58, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans in conditions having a pH of less than pH 7.0.

60. The isolated antibody of any one of embodiments 55, 57 or 59, wherein a condition having a pH of less than pH 7.0 is a tumor or any diseased area having a pH of less than pH 7.0 in a subject and in which an immune stimulation is desired.
61. The isolated antibody of any one of embodiments 1-60, wherein the antibody stimulates T cell activation, as evidenced by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling; as determined, e.g., as described in the Examples.
62. The isolated antibody of embodiment 61, wherein the antibody stimulates T cell activation in conditions having a pH of less than pH 7.0.
63. The isolated antibody of any one of embodiments 1-62, wherein the antibody reduces VISTA mediated cell-cell adhesion.
64. The isolated antibody of any one of embodiments 1-63, wherein the antibody has a mean residence time (MRT) of at least 100, 200, 300, 400 or 500 days in Cynomolgus macaques.
65. The isolated antibody of any one of embodiments 1-64, wherein the antibody does not significantly bind to VISTA positive cells, e.g., neutrophils, in the peripheral blood of a subject to whom it is administered.
66. The isolated antibody of any one of embodiments 1-65, wherein the antibody does not significantly deplete VISTA positive cells, e.g., neutrophils, in peripheral blood of a subject to whom it is administered.
67. The isolated antibody of any one of embodiments 1-66, wherein the antibody has been engineered to bind to hVISTA at acidic pH, but wherein the antibody does not bind specifically to hVISTA at neutral or physiological pH.
68. The isolated antibody of any one of embodiments 1-67, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine-rich ß-sheet extension.
69. The isolated antibody of embodiment 68, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine-rich ß-sheet extension, in conditions having a pH of 6.0-6.5.
70. The isolated antibody of any one of embodiments 1-68, wherein the antibody competes or cross-competes for binding to hVISTA with one or more antibodies described herein, e.g., comprising the VH and VL of P1-061029, P1-068757, P1-

068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G,P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068748, P1-068750, P1-068752 P1-068754, VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P, (e.g., in the context of IgG1.3); as determined, e.g., by the competitive BLI epitope binning assay described in Example 15.

71. The isolated antibody of any one of embodiments 1-70, wherein the antibody bins to epitope Group A, as determined, e.g., by the competitive BLI epitope binning assay described in Example 15.

72. The isolated antibody of any one of embodiments 1-70, wherein the antibody does not bind significantly to hVISTA in which one or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

73. An isolated antibody that binds specifically to hVISTA consisting of SEQ ID NO: 1 or 2, but that does not bind significantly to hVISTA in which one or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117,

I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA; e.g., as determined by the yeast mutational analysis described in the Examples.

74. The isolated antibody of any one of embodiments 1-73, wherein the antibody does not bind significantly to hVISTA in which 2, 3, 4, 5 or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

75. The isolated antibody of any one of embodiments 72-74, wherein the antibody does not bind significantly to hVISTA in which one or more of the following residues have been mutated to one of the corresponding residues shown in Table 15: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

76. The isolated antibody of any one of embodiments 1-75, wherein the Ab binds to the amino acid region ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497), as determined by HDX-MS, as described in the Examples.

77. The isolated antibody of any one of embodiments 1-76, wherein the Ab binds to amino acid regions ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497) and also, but less strongly, to amino acids ^{57}LGPVDKGHDVTF^{68} (SEQ ID NO: 498) and ^{148}VVEIRHHHSEHRVHGAME^{165} (SEQ ID NO: 499).

78. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues of an antibody described herein, such as VISTA.4, P1-070976, P1-065333, P1-070976_H95D, P1-070976_E97P, P1-061015, P1-061029, P1-068761, or P1-068767, such as one or more amino acids selected from one of the following groups of energetically important contact residues: (i) V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127; (ii) V34, T35, Y37, T39, Y41, S52,

R54, F62, L65, H66, H68, L115, V117, I119, R120, H121, H122, S124, E125; or (iii) Y37, T39, R54, F62, H66, L115 or V117, as determined, e.g., using the yeast surface display and NGS assay described in Example 15, and wherein numbering is that of mature hVISTA.

79. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15, and wherein numbering is that of mature hVISTA.

80. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues of VISTA.4, as determined, e.g., using the yeast surface display and NGS assay described in Example 15.

81. The isolated antibody of any one of embodiments 1-80, wherein the Ab binds to the FG loop of hVISTA.

82. The isolated antibody of any one of embodiments 1-81, wherein the Ab binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples.

83. The isolated antibody of any one of embodiments 1-82, wherein the Ab contacts H121, H122 and H123 of hVISTA (distance of, e.g., 4.0 Ångströms (Å) or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples.

84. The isolated antibody of any one of embodiments 1-83, wherein the Ab contacts hVISTA through VH CDR1 and VH CDR3, and for example, not significantly through VH CDR2 and/or through a VL CDR.

85. The isolated antibody of any one of embodiments 1-84, wherein heavy chain amino acid residues 110 and 112 of the antibody form hydrogen bonds with H121 and H122 of hVISTA, respectively, and optionally, wherein an amino acid residue of the antibody forms a hydrogen bond with H123 of hVISTA, wherein amino acid numbering of the antibody is according to that used in the Examples.
86. The antibody of embodiment 85, wherein the antibody comprises one or more (e.g., 1-5, 5-10, 10-15, 10-20 or 15-20) interactions with hVISTA listed in Table 31 under VISTA.4.
87. The isolated antibody of any one of embodiments 1-86, wherein the Ab contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3.
88. The isolated antibody of any one of embodiments 1-87, wherein the Ab does not bind significantly to hVISTA at neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).
89. The isolated antibody of any one of embodiments 1-88, wherein the Ab binds to hVISTA under acidic conditions, e.g., at a pH of 6.5, with a K_D (or koff) that is at least 10 fold, 100 fold or 1000 fold lower than its K_D and/or koff of binding to hVISTA under neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).
90. The isolated antibody of any one of embodiments 1-89, wherein the Ab does not bind significantly to hVISTA at neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).
91. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab:
    - inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 (e.g., inhibits the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48);
    - enhances T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;

- contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;
- binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacts (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å)or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- binds to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3: $_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;
- competes for binding to hVISTA (two-way competition) with one or more antibodies decribed herein, e.g., P1-061015, P1-061029, P1-068761, P1-068767 and VISTA.4;
- contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- has low target mediated drug disposition, leading to mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600, or 700 hours, as measured, e.g., as described in the Examples;
- has low or undetectable levels of TMDD; and/or
- has low or undetectable levels of neutropenia.

92. An isolated antibody that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 with a K_D (and/or koff) that is at least 10 fold, 100 fold or 1000 fold lower than its K_D or koff of binding to hVISTA under neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples), wherein the Ab:

- inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 (e.g., inhibits the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48);
- enhances T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
- contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;
- binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacts (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å)or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- binds to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3: $_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;
- competes for binding to hVISTA (e.g., by two-way competition) with one or more antibodies decribed herein, e.g., P1-061015, P1-061029, P1-068761, P1-068767 and VISTA.4;
- contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- has low target mediated drug disposition, leading to mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600, or 700 hours, as measured, e.g., as described in the Examples;
- has low or undetectable levels of TMDD; and/or
- has low or undetectable levels of neutropenia.

93. The isolated antibody of any one of embodiments 1-92, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured, e.g., by icIEF.

94. The isolated antibody of any one of embodiments 1-93, wherein the antibody exhibits low aggregation, e.g., an aggregation that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples.

95. The isolated antibody of any one of embodiments 1-94, wherein the antibody exhibits a viscosity that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), as determined in the Examples.

96. The isolated antibody of any one of embodiments 1-95, wherein the antibody exhibits a hydrodynamic radius that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples.

97. The isolated antibody of any one of embodiments 1-96, wherein the antibody exhibits a melting temperature (Tm1) that is similar or higher than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples (e.g., Example 16).

98. The isolated antibody of any one of embodiments 1-97, wherein the antibody exhibits an amount of high molecular weight species that is similar to or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples (e.g., Example 16).

99. The isolated antibody of any one of embodiments 1-98, which is an IgG antibody.

100. The isolated antibody of embodiment 99, which is an IgG1, IgG2 or IgG4 antibody (IgG4 optionally with an S228P substitution (EU numbering)).

101. The isolated antibody of any one of embodiments 1-100, wherein the antibody is an effectorless antibody, e.g., lacks ADCC and/or CDC, and/or an antibody that does not significantly bind to one or more FcγRs, e.g., FcγRIII.

102. The isolated antibody of embodiment 101, wherein the constant region comprises 1-5 mutations in a wild type heavy chain constant region that reduce the effector function (e.g., ADCC and/or CDC) of the antibody and/or the ability to an bind to one or more FcγRs, e.g., FcγRIII, relative to that of the corresponding wildtype heavy chain constant region.

103. The isolated antibody of any one of embodiments 1-102, wherein the constant region of the antibody is IgG1.3, IgG1.1 or is an IgG1 with a P238K substitution (e.g., IgG1.P238K).

104. The isolated antibody of any one of embodiments 1-103, wherein the antibody has effector function and/or binds to one or more FcγRs, e.g., FcγRIII.

105. The isolated antibody of embodiment 104, wherein the antibody is afucosylated (e.g., an afucosylated IgG1 antibody).

106. The isolated antibody of embodiment 105, wherein the constant region comprises 1-5 mutations that enhance the effector function of the antibody and/or the ability to bind to one or more FcγRs, e.g., FcγRIII, relative to the corresponding wildtype constant region.

107. An isolated antibody, or an isolated antibody of any one of embodiments 1-106, wherein the antibody is 41F11 or VISTA.4 or a variant thereof.

108. The isolated antibody of any one of embodiments 1-107, wherein the variant comprises VH CDR1, CDR2, CDR3 and VL CDR1, CDR2, and CDR3 amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

109. The isolated antibody of any one of embodiments 1-108, wherein the variant comprises VH and VL amino acid sequences that are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-50, 1-40, 1-30 or 1-20, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

110. The isolated antibody of any one of embodiments 1-109, wherein the antibody comprises the VH CDR1, CDR2, and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 17 or in the Sequence Table.

111. The isolated antibody of any one of embodiments 1-110, wherein the antibody comprises the VL CDR1, CDR2, and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 1 or in the Sequence Table.

112. The isolated antibody of any one of embodiments 83-111, wherein the antibody comprises the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2 and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 17 or in the Sequence Table.

113. The isolated antibody of any one of embodiments 1-112, wherein the antibody comprises the VH amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

114. The isolated antibody of any one of embodiments 1-113, wherein the antibody comprises the VL amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

115. The isolated antibody of any one of embodiments 1-114, wherein the antibody comprises the VH and VL amino acid sequences of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

116. An isolated antibody that binds specifically to human VISTA and comprises the VH CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

117. An isolated antibody that binds specifically to human VISTA and comprises the VL CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

118. The isolated antibody of any one of embodiments 1-117, comprising the VH CDR1, CDR2 and CDR3 and the VL CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

119. The isolated antibody of any one of embodiments 1-118, wherein the antibody comprises the VH amino acid sequence of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

120. The isolated antibody of any one of embodiments 1-119, wherein the antibody comprises the VL amino acid sequence of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

121. The isolated antibody of any one of embodiments 1-120, wherein the antibody comprises the VH and VL amino acid sequences of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

122. The isolated antibody of any one of embodiments 1-121, wherein the antibody comprises the HC amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

123. The isolated antibody of any one of embodiments 1-122, wherein the antibody comprises the LC amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

124. The isolated antibody of any one of embodiments 1-123, wherein the antibody comprises the HC and LC amino acid sequences of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

125. The isolated antibody of any one of embodiments 1-124, wherein the antibody comprises the VH CDRs of VISTA.4 (SEQ ID Nos: 503-505), P1-070976 (SEQ ID Nos: 509-511), P1-065333 (SEQ ID Nos: 506-508), P1-070976_H95D (SEQ ID Nos: 512-514) or P1-070976_E97P (SEQ ID Nos: 515-517).

126. The isolated antibody of any one of embodiments 1-125, wherein the antibody comprises the VL CDRs of VISTA.4, P1-070976, P1-065333, P1-070976_H95D or P1-070976_E97P (SEQ ID Nos: 500-502).

127. The isolated antibody of any one of embodiments 1-126, wherein the antibody comprises the VH and VL CDRs of VISTA.4 (SEQ ID Nos: 503-505 and 500-502, respectively), P1-070976 (SEQ ID Nos: 509-511 and 500-502, respectively), P1-065333 (SEQ ID Nos: 506-508 and 500-502, respectively), P1-070976_H95D (SEQ ID Nos: 512-514 and 500-502, respectively), or P1-070976_E97P (SEQ ID Nos: 515-517 and 500-502, respectively).

128. The isolated antibody of any one of embodiments 1-127, wherein the antibody comprises the VH of VISTA.4 (SEQ ID NO: 492), P1-070976 (SEQ ID NO: 474), P1-065333 (SEQ ID NO: 473), P1-070976_H95D (SEQ ID NO: 477) or P1-070976_E97P (SEQ ID NO: 478).

129. The isolated antibody of any one of embodiments 1-128 , wherein the antibody comprises the VL of VISTA.4, P1-070976, P1-065333, P1-070976_H95D or P1-070976_E97P (with or without A64G) (corresponding to SEQ ID NO: 493 (without A64G) or SEQ ID NO: 479 (with A64G)).

130. The isolated antibody of any one of embodiments 1-129, wherein the antibody comprises the VH and VL (with or without A64G in VL) of VISTA.4 (SEQ ID Nos: 492 and 479 or SEQ ID Nos: 492 and 493), P1-070976 (SEQ ID Nos: 474 and 479 or SEQ ID Nos: 474 and 493), P1-065333 (SEQ ID Nos: 475

and 479 or SEQ ID Nos: 475 and 493), P1-070976_H95D (SEQ ID Nos: 477 and 479 or SEQ ID Nos: 477 and 493) or P1-070976_E97P (SEQ ID Nos: 478 and 479 or SEQ ID Nos: 478 and 493).

131. The isolated antibody of any one of embodiments 1-130, wherein the antibody comprises the HC of VISTA.4.IgG1.3 (SEQ ID NO: 491), P1-070976.IgG1.3 (SEQ ID NO: 476), P1-06533.IgG1.3 (SEQ ID NO: 475), P1-070976_H95D.IgG1.3 (SEQ ID NO: 480) or P1-070976_E97P.IgG1.3 (SEQ ID NO: 481).

132. The isolated antibody of any one of embodiments 1-131, wherein the antibody comprises the LC of VISTA.4.IgG1.3, P1-070976.IgG1.3, P1-06533.IgG1.3, P1-070976_H95D.IgG1.3 or P1-070976_E97P.IgG1.3 (with or without A64G in VL) (SEQ ID NO: 494 (without A64G) or SEQ ID NO: 482 (with A64G).

133. The isolated antibody of any one of embodiments 1-132, wherein the antibody comprises the HC and LC (with or without A64G in VL) of VISTA.4.IgG1.3 (SEQ ID NOs: 491 and 494 or SEQ ID Nos: 491 and 482), P1-070976.IgG1.3 (SEQ ID NOs: 476 and 494 or SEQ ID Nos: 476 and 482), P1-065333.IgG1.3 (SEQ ID NOs: 475 and 494 or SEQ ID Nos: 475 and 482), P1-070976_H95D.IgG1.3 (SEQ ID NOs: 480 and 494 or SEQ ID Nos: 480 and 482), or P1-070976_E97P.IgG1.3 (SEQ ID NOs: 481 and 494 or SEQ ID Nos: 481 and 482).

134. The isolated antibody of any one of embodiments 1-133, which is a full length antibody or an antibody comprising a full length heavy chain (with or without a C-terminal lysine) and a full length light chain.

135. The isolated antibody of any one of embodiments 1-130, which is an antigen binding fragment.

136. The isolated antibody of any one of embodiments 1-135, which is a multimeric (e.g., dimeric or trimeric) antibody.

137. The isolated antibody of any one of embodiments 1-136, which is linked (e.g., covalently) to another molecule.

138. The isolated antibody of embodiment 137, wherein the other molecule is a label.

139. The isolated antibody of embodiment 137 or 138, wherein the other molecule is a peptide.

140. The isolated antibody of any one of embodiments 1-139, which is an antibody drug conjugate (ADC) or an activatable antibody.

141. An isolated nucleic acid encoding an antibody of any one of embodiments 1-140.

142. An isolated nucleic acid encoding the heavy chain and/or the light chain of an antibody of any one of embodiments 1-140.

143. A composition comprising a nucleic acid encoding the heavy chain of an antibody of any one of embodiments 1-140 and a nucleic acid encoding the light chain of the antibody.

144. A cell comprising the isolated nucleic acid of any one of embodiments 141-142 or the composition of embodiment 143.

145. A method of preparing an antibody, comprising culturing the cell of embodiment 144 in conditions under which the antibody is expressed.

146. A composition comprising an isolated antibody, nucleic acid, composition or cell of any one of embodiments 1-145 and a pharmaceutically acceptable carrier.

147. The composition of embodiment 146, comprising a second therapeutic agent.

148. The composition of embodiment 147, wherein the second therapeutic agent comprises an immunostimulatory agent.

149. The composition of embodiment 148, wherein the immunostimulating agent comprises an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, a CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.

150. A method of treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of embodiments 1-149 that stimulates an immune response and/or is a VISTA antagonist antibody.

151. The method of embodiment 150, wherein the subject has VISTA positive cells, e.g., in a tumor of the cancer.

152. The method of embodiment 151, wherein the VISTA positive cells comprise VISTA positive infiltrating lymphocytic (e.g., T cells) or myelomonocytic cells.

153. The method of any one of embodiments 150-152, wherein the subject is first tested for the presence of VISTA positive cells in a tumor, and wherein the subject is treated if the subject has VISTA positive cells, e.g., in a tumor.

154. The method of any one of embodiments 150-153, wherein the method further comprises administering a second therapy.

155. The method of embodiment 154, wherein the second therapy comprises chemotherapy, radiotherapy, surgery or administration of a second agent.

156. The method of embodiment 155, wherein the second therapy comprises a second agent and the second agent is an immunostimulatory agent.

157. The method of embodiment 156, wherein the immunostimulatory agent comprises an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, an CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.

158. A method of treating an infectious disease (e.g., viral disease) in a subject, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of embodiments 1-157 that stimulates an immune response and/or is a VISTA antagonist.

159. A method of detecting VISTA in a sample, comprising contacting the sample with a VISTA antibody of any one of embodiments 1-140.

160. An isolated antibody that binds specifically to hVISTA and inhibits VISTA mediated T cell activation, wherein the antibody comprises VH CDR1, CDR2 and CDR3 sequences of a VH comprising or consisting of SEQ ID NO: 463, 473, 474, 477, 478, or , 492 (e.g. SEQ ID Nos: 503-505, 506-508, 509-511, 512-514, or 515-517).

161. The isolated antibody of embodiment 160,wherein the antibody comprises VL CDR1, CDR2 and CDR3 sequences of a VL comprising or consisting of SEQ ID NO: 464, 471, 493 or 479 (e.g., SEQ ID Nos: 500-502).

162. The isolated antibody of embodiment 160 or 161, wherein the antibody comprises a VH comprising or consisting of SEQ ID NO: 463, 473, 474, 477, 478, or 492, with or without a signal peptide.

163. The isolated antibody of any one of embodiments 160-162, wherein the antibody comprises a VL comprising or consisting of SEQ ID NO: 464, 471, 479, or 493, with or without a signal peptide.

164. The isolated antibody of any one of embodiments 160-163, comprising a heavy chain constant region comprising or consisting of a wildtype IgG1 (e.g., SEQ ID NO: 182), a wildtype IgG2, a wildtype IgG3, a wildtype IgG4, a wildtype IgG4 comprising S228P, IgG1.1 (SEQ ID NO: 183), IgG1.3 (SEQ ID NO: 163), IgG1.P238K (SEQ ID NO: 184), with or without a C-terminal lysine.

165. The isolated antibody of any one of embodiments 160-164, comprising a heavy chain comprising or consisting of SEQ ID NO: 475, 476, 491, 480, or 481, with or without signal peptide.

166. The isolated antibody of any one of embodiments 160-165, comprising a light chain comprising or consisting of SEQ ID NO: 472, 494, or 482, with or without a signal peptide.

167. The isolated antibody of embodiment 166, comprising heavy and light chains, comprising or consisting of:

(a) SEQ ID NO: 475 and SEQ ID NO: 482 or 472, respectively;

(b) SEQ ID NO: 475 and SEQ ID NO: 494, respectively;

(c) SEQ ID NO: 476 and SEQ ID NO: 482 or 472, respectively;

(d) SEQ ID NO: 476 and SEQ ID NO: 494, respectively;

(e) SEQ ID NO: 491 and SEQ ID NO: 482 or 472, respectively;

(f) SEQ ID NO: 491 and SEQ ID NO: 494, respectively;

(g) SEQ ID NO: 480) and SEQ ID NO: 482 or 472, respectively;

(h) SEQ ID NO: 480) and SEQ ID NO: 494, respectively;

(i) SEQ ID NO: 481) and SEQ ID NO: 482 or 472, respectively; or

(j) SEQ ID NO: 481) and SEQ ID NO: 494, respectively.

168. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which,

e.g., results in the increase of the number of CD4+ and CD8+ T cells, e.g., in a tumor of the subject.

169. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which, e.g., results in the reduction of the number of exhausted T cells and/or T cells expressing PD-1, LAG3 and/or TIM-3, e.g., in a tumor of the subject.

170. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which, e.g., results in the increase of the number of CD4+ and CD8+ T cells, e.g., in a tumor of the subject and a reduction of the number of exhausted T cells and/or T cells expressing PD-1, LAG3 and/or TIM-3, e.g., in a tumor of the subject and/or other features described herein.

171. The method of any one of embodiments 168-170, wherein the antibody that binds specifically to hVISTA is an antibody described herein.

Additional specific, exemplary embodiments include the following:

1. An isolated Ab that specifically binds to hVISTA, wherein the antibody comprises a heavy and a light chain, wherein (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 503, 504 and 505, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising SEQ ID NO: 463 or 492 and the light chain comprises VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 491 and the light chain comprises SEQ ID NO: 472, 482 or 494.
2. An isolated Ab that specifically binds to hVISTA, wherein the antibody comprises a heavy and a light chain, wherein (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 503, 504 and 505, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising SEQ ID NO: 463 or 492 and the light chain comprises VL comprising SEQ ID

NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 491 and the light chain comprises SEQ ID NO: 472, 482 or 494 or a variant or derivative thereof, wherein the Ab comprises one or more (or all) of the following characteristics:

a. binds to the extracellular domain of hVISTA at pH 6.0 (e.g., with a K_D of 10^{-7}M or less), and optionally does not significantly bind to the extracellular domain of hVISTA at pH 7.4;
b. inhibits binding of hVISTA to a T cell or to PSGL-1, e.g., at acidic pH;
c. binds to one or more of the following energetically important contact residues Y37, T39, R54, F62, H66, L115, V117, I119, S124 and E125, as determined by yeast surface display and NGS;
d. competes for binding to hVISTA with an antibody (i) comprising a heavy chain comprising VH comprising SEQ ID NO: 463 or 492 and a light chain comprising VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (ii) the heavy chain comprises SEQ ID NO: 491 and the light chain comprises SEQ ID NO: 472, 482 or 494;
e. binds to the amino acid regions ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497) and also, but less strongly, to amino acids ^{57}LGPVDKGHDVTF^{68} (SEQ ID NO: 498) and ^{148}VVEIRHHHSEHRVHGAME^{165} (SEQ ID NO: 499);
f. inhibits hVISTA binding to T cells, e.g., at acidic pH;
g. enhances T cell proliferation and IFN-gamma production from T cells, e.g., at acidic pH;
h. releases T cell receptor-mediated NF-kB signaling suppression by hVISTA, e.g., at acidic pH;
i. has low or undetectable levels of TMDD; and/or
j. has low or undetectable levels of neutropenia.

3. An isolated Ab that specifically binds to hVISTA, wherein the antibody comprises a heavy and a light chain, wherein (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 506, 507 and 508, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising

SEQ ID NO: 473 and the light chain comprises VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 475 and the light chain comprises SEQ ID NO: 472, 482 or 494.

4. An isolated Ab that specifically binds to hVISTA, wherein the antibody comprises a heavy and a light chain, wherein:

   a. (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 509, 510 and 511, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising SEQ ID NO: 474 and the light chain comprises VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 476 and the light chain comprises SEQ ID NO: 472, 482 or 494;

   b. (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 512, 513 and 513, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising SEQ ID NO: 477 and the light chain comprises VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 480 and the light chain comprises SEQ ID NO: 472, 482 or 494; or

   c. (i) the heavy chain comprises VH CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 515, 516 and 517, respectively, and the light chain comprises VL CDR1, CDR2 and CDR3 comprising SEQ ID Nos: 500, 501 and 502, respectively; (ii) the heavy chain comprises VH comprising SEQ ID NO: 478 and the light chain comprises VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (iii) the heavy chain comprises SEQ ID NO: 481 and the light chain comprises SEQ ID NO: 472, 482 or 494;

wherein the antibody:

(1) binds to the extracellular domain of hVISTA at pH 6.0 (e.g., with a K_D of 10^{-7}M or less), but not significantly at pH 7.4;

(2) inhibits binding of hVISTA to a T cell or to PSGL-1, e.g., at acidic pH;

(3) binds to one or more of the following energetically important contact residues Y37, T39, R54, F62, H66, L115, V117, I119, S124 and E125, as determined by yeast surface display and NGS;

(4) competes for binding to hVISTA with an antibody (i) comprising a heavy chain comprising VH comprising SEQ ID NO: 463 or 492 and a light chain comprising VL comprising SEQ ID NO: 464, 471, 479 or 482; and/or (ii) the heavy chain comprises SEQ ID NO: 491 and the light chain comprises SEQ ID NO: 472, 482 or 494;

(5) binds to the amino acid regions ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497) and also, but less strongly, to amino acids ^{57}LGPVDKGHDVTF^{68} (SEQ ID NO: 498) and ^{148}VVEIRHHHSEHRVHGAME^{165} (SEQ ID NO: 499);

(6) inhibits hVISTA binding to T cells, e.g., at acidic pH;

(7) enhances T cell proliferation and IFN-gamma production from T cells, e.g., at acidic pH;

(8) releases T cell receptor-mediated NF-kB signaling suppression by hVISTA, e.g., at acidic pH;

(9) has low or undetectable levels of TMDD; and/or

(10) has low or undetectable levels of neutropenia.

Further specific embodiments are described in the claims section of this disclosure.

EXAMPLES

The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: VISTA's extracellular domain is exceptionally rich in histidines

This example shows that VISTA's extracellular domain is exceptionally rich in histidine residues, that these histidine residues are evolutionarily conserved, and that they may contribute to receptor-ligand interactions involving VISTA.

The amino acid sequences of the extracellular domains (ECDs) of immunoglobulin domain-containing proteins were extracted from the uniprot and swiss-prot databases and analyzed for histidine content. Fig. 1A depicts the results of this analysis as a graph. For each protein, the frequency of histidine residues as a percentage of all extracellular domain amino acid residues is plotted on the y-axis, and the total number of extracellular domain amino acid residues is plotted on the x-axis. The diameter of each data point corresponds to the total number of histidine residues in the extracellular domain of each protein. VISTA (labeled) contains an exceptionally high frequency of histidine residues in its extracellular domain.

The evolutionary conservation of histidine residues in VISTA was then assessed. Fig. 1B shows the amino acid reference sequences of human, cynomolgus macaque, and mouse VISTA were aligned, excluding the signal peptides ("Sig"), transmembrane domains ("TMD") and intracellular domains. Histidine residues that are conserved across all three species are bolded and underlined. Histidine residues that are conserved across human and cyno VISTA are bolded without underlining. Many of VISTA's extracellular domain histidine residues are evolutionarily conserved, suggesting an important biological role for VISTA's high histidine content.

A three-dimensional model of the hVISTA IgV domain was created based on sequence homology analysis to available solved structures in the PDB database. The model, shown in Fig. 1C, indicates that many histidines in VISTA's ECD are exposed at the surface of the molecule, where they may play a role in ligand binding as well as in antibody recognition. Histidine residues are depicted as balls and sticks.

Example 2: Histidine protonation may regulate VISTA receptor-ligand engagement and immunosuppressive activity in tumors and other acidic microenvironments

This Example describes histidine protonation in response to physiologically relevant acidic pH, as well as a model in which VISTA extracellular domain histidines confer counter-receptor or ligand selectivity for acidic pH rather than physiological pH.

Fig. 2A shows the equilibrium between the lack of, and the presence of, protonation of the pyrrole ammonium group (NH) in a histidine residue. The pKa of histidine in solution is 6.5, indicating that histidine residues are more likely to be protonated at pH 6.5 and lower, and thus, positively charged, than at higher pH. The increase in positive charge at the surface of the VISTA ECD as a result of protonation may affect receptor or ligand binding as well as VISTA structure and/or function. Thus, changes in pH may also modify antibody binding epitopes and/or result in varied antibody affinities.

Fig. 2B shows a model in which VISTA engages PSGL-1 or other counter-receptors and ligands ("VISTA-R") selectively at acidic pH. At physiological pH, such as in the blood, histidine residues on VISTA's ECD are expected to be non-protonated. As a result, VISTA binding to PSGL-1 or other counter-receptors and ligands is neglible at physiological pH. In contrast, in locations that tend to have an acidic extracellular pH, such as tumor microenvironments or sites of inflammation, acidic pH may partially or fully drive VISTA ECD histidine protonation and thus enable VISTA engagement with PSGL-1 or other counter-receptors and ligands. Accordingly, antibodies that bind strongly to VISTA-ECD proteins at acidic pH ranges may be more effective in inhibiting VISTA activity in tumors.

Example 3: VISTA is expressed by myelomonocytic cells in tumors

This Example shows that VISTA is frequently expressed by myelomonocytic cells in tumors, including macrophages, dendritic cells, and granulocytes.

Surgically resected non-small cell lung carcincoma, renal clear cell carcinoma, melanoma, colorectal carcinoma, and other tumor samples were washed in ice-cold PBS, cut into approximately 15 mm^3-sized pieces, and suspended in ice-cold RPMI-1640 media (Fisher Scientific catalog number 11875093) supplemented with 2% heat-inactivated FBS and 2 mM EDTA (Fisher Scientific 15575020). Each sample was transferred to a large clearance glass dounce (Tenbroeck Tissue Grinders) and ground until the tissue pieces were visually disassociated. The suspensions were filtered through 70 μM nylon mesh and centrifuged. The supernatants were discarded and the cell pellets were re-suspended in room-temperature PBS supplemented with 0.1% bovine serum albumin and 250 mg/mL sterile-filtered DNase 1 (grade II, from bovine pancreas, Roche

catalog number 10104159001) for 3 minutes at room temperature. The cells were then washed in ice-cold supplemented RPMI and re-suspended in ice-cold PBS. A cell viability dye was added and the cells were incubated on ice in the dark. After 20 minutes, non-specific antibody staining was blocked by adding 4% normal rat serum, 4% normal mouse serum, 20% human serum from AB plasma, and 1:125 diluted Human TruStain FcX™ (Biolegend catalog number 422302). The cells were stained with fluorophore-conjugated antibodies against HLA-DR (BD Biosciences catalog number 564040), CD8 (Fisher Scientific catalog number 46-0087-42), CD14 (Biolegend catalog number 325620), CD45 (Biolegend catalog number 304017), CD4 (BD Biosciences catalog number 563875), CD11c (BD Biosciences catalog number 744439), CD15 (BD Biosciences catalog number 563142), PD-1 (BD Biosciences catalog number 565299), CD3 (BD Biosciences catalog number 565515), CD56 (Fisher Scientific catalog number 61-0567-42), CD19 (BD Biosciences catalog number 564977), and VISTA (VISTA antibody 3 conjugated to AlexaFluor™ 647, Fisher Scientific catalog number A20186) suspended in Brilliant Stain Buffer (BD Biosciences catalog number 562794) for 30 minutes on ice in the dark. The stained cells were washed in ice-cold PBS, fixed (Fisher Scientific catalog number 00-5523-00), and acquired on a flow cytometer. Data were analyzed using FlowJo™ software (BD Biosciences). As shown in Fig. 3, VISTA cell surface expression was highest on macrophages and granulocytes, moderate on dendritic cells, and low on T cells, natural killer cells, and B cells.

Example 4: VISTA cell binding exhibits acidic pH selectivity

This Example shows that multimerized human VISTA ECD binds more efficiently to stimulated human CD4+ T cells and human peripheral blood mononuclear cells at acidic pH than at neutral or physiological pH, and that this binding can be blocked by an anti-human VISTA locking antibody. Acidic pH-selective-dimerized mouse VISTA ECD binding to mouse splenocytes is also shown.

Human CD4+ T cells were enriched from healthy donor blood by RosetteSep™ (Stemcell catalog number 15062) and stimulated *in vitro* for approximately four days with Human T-Activator CD3/CD28 Dynabeads™ (Fisher Scientific catalog number 111.32D) and recombinant human IL-2 (Peprotech catalog number 200-02) in RPMI-1640 supplemented with 10% heat-inactivated FBS, Glutamax™ (Fisher Scientific catalog

number 35050061), non-essential amino acids (Fisher Scientific 11140050), sodium pyruvate (Fisher Scientific catalog number 11360070), and 2-mercaptoethanol (Fisher Scientific 21985023). The activated CD4+ T cells were stained with monobiotinylated hVISTA ECD molecules (Phe 33 - Ala 194 (Accession # AAH20568)-polyhistidine; AcroBiosystems, Inc. B75- H82F3) loaded at a 28:1 molar ratio onto Phycoerythrin (PE)-conjugated streptavidin dextramers (catalog number DX01-PE) diluted into Hank's Buffered Salt Solution (HBSS, Fisher Scientific catalog number 14025134) acidified to various pH with mM MES (Sigma, 1317-100ML) for 30 minutes at room temperature. As a control, activated CD4+ T cells were stained with PE-conjugated streptavidin dextramers that were not loaded with hVISTA. The stained cells were washed with HBSS + MES and acquired on a flow cytometer. Data were analyzed using FlowJo™ software (BD Biosciences). The results, depicted in Fig. 4A, show that hVISTA did not bind CD4+ T cells better than the control at pH > 6.5. In contrast, hVISTA exhibited progressively stronger binding to CD4+ T cells at pH < 6.5. Left, from darker gray to lighter, the filled histograms depict binding at pH 7.0, 6.5, 6.4, 6.3, 6.1, and 6.0. Some histograms are labeled with their corresponding pH. Non-VISTA control multimer binding at pH 6.0 is shown as the unfilled histogram. Right, graphed PE mean fluorescence intensities (MFI) of CD4+ T cells stained with hVISTA-loaded dextramers (blue circles) or with non-loaded dextramers (triangles) at various pH.

Peripheral blood mononuclear cells (PBMC) were enriched from healthy donor blood by ficoll gradient centrifugation (Ficoll-Paque Plus, GE Life Sciences catalog number 17144003) and stained with hVISTA-loaded dextramers (also referred to as multimers) and fluorophore-conjugated diluted in HBSS + MES buffers as described above. Fig. 4B shows filled histograms that depict, from darker gray to lighter, binding at pH 6.0 to CD19+ B cells, CD4+ T cells, CD8+ T cells, CD56+ NK cells, and CD14+ monocytes. The unfilled, solid border and dotted border histograms depict binding at pH 7.4 to total PBMC lymphocytes and monocytes respectively. The results show that hVISTA can bind many leukocytes at acidic pH but not significantly at physiological pH.

Activated human CD4+ T cells were stained with hVISTA multimers at pH 6 in the presence of titrated anti-human VISTA antibody or an isotype-matched non-VISTA-specific antibody. The results, graphed in Fig. 4C, show VISTA multimer MFI relative to antibody concentration. Anti-hVISTA antibody (VISTA antibody 3; squares), but not the

non-VISTA-specific control antibody (circles), blocked hVISTA binding to activated CD4+ T cells in a concentration-dependent manner. The PE MFI of CD4+ T cells that were not stained with hVISTA-loaded multimers is included as a control (single triangle).

Fig. 4D shows representative two-dimensional flow cytometry plots of VISTA multimer staining at pH 6.0 to heparan sulfate-mutant Chinese Hamster Ovary (CHO) cells (line pGSD-677, American Type Culture Collection) that were transfected to express full length human PSGL-1 (SEQ ID NO: 3; nucleic acid NM_003006.4). Staining was performed in the presence or absence of a titrated anti-VISTA blocking antibody (mAb 3). Cells left unstained by VISTA multimers are shown as a control. PSGL-1 antibody (BD Biosciences catalog number 562758) staining is plotted on the y-axis, and VISTA multimer staining is plotted on the x-axis.

Splenocytes were collected from C57BL6/J mice (Jackson Laboratory catalog number 000664) and stained with mVISTA ECD / human IgG Fc (Fragment, crystallizable) chimeric fusion proteins followed by fluorophore-conjugated anti-human IgG Fc secondary antibodies (Jackson Immunoresearch catalog number 109-065-098) at pH 6.0 or 7.4. The results, depicted by histogram in Fig. 4E, show that mVISTA binds murine splenocytes more efficiently at pH 6.0 than at physiological pH (approximately pH 7.4). From darker gray to lighter, the filled histograms depict binding at pH 6.0 to CD8+ T cells, CD11b+ myeloid cells, and CD4+ T cells. The unfilled histogram depicts binding at pH 7.4 to total splenocytes. Figs. 4F and G show that VISTA multimer binds to monocytes and neutrophils, respectively, and does so more strongly at pH 6.0 than at pH 7.4.

Example 5: VISTA mediates cell : cell adhesion and immune suppression selectively at acidic pH

This Example shows that VISTA mediates cell : cell adhesion and suppresses T cell activation more potently at acidic pH than at neutral or physiological pH.

An acidic pH-compatible flow cytometry-based cell/cell conjugate assay was established. 293T cells (an immortalized human embryonic kidney cell line, ATCC catalog number CRL-3216) ectopically expressing full-length human VISTA or vector were labeled with CFSE (Carboxyfluorescein succinimidyl ester; Fisher Scientific catalog number C34554). CHO cells were labeled with CellTrace™ Far Red (Fisher Scientific

catalog number C34564). The Vector or VISTA 293T cells were then mixed at a 1:1 ratio with CHO cells in pH7.0 or pH6.0 buffers and incubated for 1 hour at room temperature. The formation of CHO and 293T cell/cell conjugates was assessed by flow cytometry. The results shown in Figs. 5A-B demonstrate that VISTA-expressing 293T cells preferentially adhere to CHO cells at acidic pH and that inclusion of an anti-VISTA blocking antibody (VISTA mAb 3; red bars) inhibits VISTA mediated cell/cell adhesion.

An acidic pH-compatible T cell suppression assay was established. Jurkat cells (an immortalized human T cell line, ATCC catalog number TIB-152) expressing an NFkB promoter driven luciferase reporter were co-cultured in HBSS + MES buffers of various pH with 293T cells (an immortalized human embryonic kidney cell line, ATCC catalog number CRL-3216) ectopically expressing full-length human VISTA and a single-chain variable fragment of the anti-human T cell receptor agonist antibody clone OKT3 at a 10 : 1 Jurkat : 293T cell ratio. An anti-VISTA blocking antibody (VISTA mAb 3) or an isotype-matched non-VISTA-specific control antibody were added at 10 μg/mL to the co-cultures. After incubation, Jurkat T cell activation was quantified by measuring luciferase activity (1 second interval Promega catalog number G7940). The results are shown in Fig. 5C-D. Fig. 5C shows a plot of luciferase units in Jurkats treated with anti-VISTA (red squares) or control antibody (blue circles) at different pH. Fig. 5D shows a plot of the luciferase signal in anti-VISTA antibody-treated co-cultures divided by the luciferase signal in control antibody-treated co-cultures at each pH tested. The results show that VISTA-mediated T cell suppression is most potent at acidic pH.

Example 6: VISTA trafficks through intracellular recycling endosomes

This Example shows that VISTA can be found in intracellular endosomes, particularly Rab11+ recycling endosomes, and can recycle to and from the cell surface via endosomal trafficking. The strength with which an anti-VISTA antibody binds VISTA at acidic pH influences its capacity to remain bound to VISTA during endosomal trafficking.

Monocytes were isolated from PBMCs by magnetic activated cell sorting. Both monocytes and 293T cells were then fixed in 4% paraformaldehyde and stained intracellularly for Rab5, Rab7 or Rab11, and with an anti-VISTA or control antibody. The control antibody ("cAb"), which is a non-VISTA-binding antibody of the same isotype as

the anti-VISTA antibody, does not detectably bind monocytes or 293T cells expressing human VISTA. Anti-VISTA and control antibodies were directly labeled with Alexa488. Rab antibodies were detected using an Alexa594 anti-rabbit Ig secondary antibody. Hoescht 33342 staining was performed to identify cell nuclei. Images were captured using a spinning disk confocal microscope. **Fig. 6A** shows co-localization of VISTA, Rab5 (early endosome marker), Rab7 (late endosome marker), and Rab11 (recycling endosome marker) within 293T cells expressing human VISTA. **Fig. 6B** shows co-localization of VISTA and Rab11 within human monocytes. Intracellular VISTA is co-localized with Rab11+ recycling endosomes.

To assess VISTA's capacity to recycle through endosomes, an endolysosome-dependent antibody drug conjugate killing assay was performed with three anti-hVISTA antibodies (VISTA mAb 1, 2 and 3) with varying VISTA binding properties at physiological and acidic pH. An SPR assay was performed first to compare hVISTA binding profiles for all three VISTA antibodies at pH 7.4, 6.7 and 6.0. VISTA antibodies were captured on a Biacore® T100 (GE Healthcare) CM5 biosensor containing immobilized Protein A, then 100nM hVISTA-ECD (amino acids 32-193 of SEQ ID NO: 1 with a 7xHis tail, i.e., AFKVATPYSL YVCPEGQNVT LTCRLLGPVD KGHDVTFYKT WYRSSRGEVQ TCSERRPIRN LTFQDLHLHH GGHQAANTSH DLAQRHGLES ASDHHGNFSI TMRNLTLLDS GLYCCLVVEI RHHHSEHRVH GAMELQVQTG KDAPSNCVVY PSSSQESENI TAHHHHHHH; SEQ ID NO: 325) was flowed in PBST running buffer at the indicated pH at 37°C. Reference-subtracted sensorgrams were normalized to the 'binding' report point and plotted. VISTA antibody 3, "mAb 3", (Fig. 6C, top) exhibited the greatest degree of VISTA binding impairment at acidic pH, followed by VISTA antibody 2 , "mAb 2," (Fig. 6C, middle), which was only moderately impaired. VISTA antibody 1, "mAb 1," maintained strong VISTA binding at acidic and physiological pH conditions (Fig. 6C, bottom).

The endolysosome-dependent antibody drug conjugate killing assay was performed as follows. AML3 cells (an immortalized human monocyte cell line, ATCC CRL-9589), which endogenously express human VISTA, were cultured with titrated anti-VISTA antibodies or a non-VISTA-specific control antibody and an anti-human IgG secondary antibody that was conjugated to a cathepsin B-sensitive linker and a cytotoxic tubulysin payload. Because Cathepsin B is predominantly active in late endosomes and

lysosomes, anti-VISTA antibodies that recycle with VISTA through early endosomes and recycling endosomes will experience low levels of linker cleavage and as a result low levels of the cytotoxic payload release and cell death. Anti-VISTA antibodies which become disassociated from VISTA in acidic endosomes and sorted into late endosomes and lysosomes will experience higher levels of linker cleavage. Cell viability was measured by Cell Titer Glo® (Promega catalog number G7573) after five days in culture. Fig. 6D shows the results of this assay, with AML3 viability (Cell Titer Glo) plotted on the y-axis and primary antibody concentrations plotted on the x-axis. Calculated EC50s for primary antibodies: VISTA antibody 1, inverted triangles, 0.485 μg/mL; VISTA antibody 2, circles, 0.092 μg/mL; VISTA antibody 3, squares, 0.006 μg/mL; Control, triangles, 1.085 μg/mL. Antibody potency was inversely correlated with anti-VISTA antibody binding at acidic pH.

To confirm that binding at acidic pH was responsible for the differences in potency, VISTA antibody 3 was affinity optimized such that its ability to bind VISTA at acidic pH was improved. Fig. 6E shows an SPR assay comparing the hVISTA antibody binding profiles of VISTA antibody 3 with this variant, VISTA antibody 3c, using the assay conditions described for Fig. 6C. VISTA antibody 3 again exhibited VISTA binding impairment at acidic pH, whereas the variant VISTA antibody 3c exhibited comparable VISTA binding at acidic and physiological pH. Fig. 6F shows the activity of VISTA antibody 3c (diamonds) in the killing assay described for Fig. 6D. The pH independent variant of VISTA antibody 3 exhibited a 31-fold lower potency than that of the original antibody, indicating that impaired anti-VISTA antibody binding at acidic pH results in a loss of antibody binding during VISTA recycling.

Based on these findings, a recycling model is proposed in which VISTA gets recycled to and from the cell surface via early endosomes and recycling endosomes. This model is depicted in Fig. 6G. Anti-VISTA antibodies can recycle with VISTA through these endosomes, maintaining target engagement. However, VISTA antibodies with impaired VISTA binding at acidic pH, particularly those with a fast off-rate at acidic pH, may disassociate from VISTA during recycling and become trapped or degraded inside cells, resulting in poor target engagement and continual consumption of circulating antibodies. In contrast, antibodies which bind and remain bound to VISTA at acidic pH

may maintain higher levels of target engagement, particularly in acidic microenvironments such as tumors, and exhibit longer mean residence times *in vivo*.

Example 7: Superiority of VISTA antibodies lacking binding at physiological pH

The inventors have shown that VISTA is an acidic pH-selective immunoreceptor, demonstrating the importance and utility of targeting VISTA with antibodies that bind well at acidic pH. Additionally, antibodies that do not bind or negligibly bind to VISTA at physiological pH are advantageous for several reasons. First, due to the relatively abundant expression of VISTA on circulating myelomonocytic cells, particularly monocytes and neutrophils, antibodies that bind VISTA at physiological pH are subject to high levels of target-mediated drug disposition (TMDD) in blood. This effect is exacerbated by the propensity of VISTA to recycle through intracellular endosomes, leading to anti-VISTA antibody internalization and degradation. This secondary effect is particularly problematic for antibodies which have impaired binding at acidic pH, as can be observed for antibodies that bind VISTA's histidine-rich ligand interface. Both effects will reduce the amount of anti-VISTA antibody in circulation, reducing the amount of antibody that will reach the tumor and thus the intended biology activity of the antibody. Second, antibodies that bind VISTA at physiological pH and which possess effector functions such as induction of antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP), or delivery of an immunomodulatory payload will subject circulating myelomonocytic cells to those effector functions, potentially resulting in undesirable effects such as circulating neutrophil depletion or activation. Thus, the inventors discovered that antibodies binding to huVISTA at acidic pH, but negligibly at physiological pH, have the double advantage of (1) better exposure in relevant sites such as tumors and (2) reduced toxicities in the case of antibodies with effector functions such as ADCC, ADCP, or delivery of an immunomodulatory payload. Additionally, because VISTA itself is an acidic pH-selective immunoreceptor, blockade of VISTA's ligand interface at physiological pH is likely unnecessary to modulate VISTA receptor-ligand activity. Therefore, antibodies that bind to huVISTA at acidic pH, but not significantly at physiological pH were generated as described below.

Example 8: Isolation of anti-VISTA antibodies binding preferentially to human VISTA at acidic pH over physiological pH

This Example describes the generation of antibodies that bind preferentially to human VISTA at low (acidic) pH relative to neutral or physiological pH.

A library of anti-VISTA antigen binding fragments of antibodies was constructed and screened as follows. Antibody libraries were created using genetic material isolated from HuMab mice immunized with full length human VISTA (hVISTA). These antibodies were formatted as scFv and were selected against full length hVISTA binding at low pH (pH 6.0) via mRNA display (Xu L et al. (2002) *Chemistry & Biology* 9: 933; Roberts RW and JW Szostak (1997) *Proc. Natl. Acad. Sci. USA* 94:12297; Kurz et al. (2000) *Nucleic Acids Res.* 28(18):E83). Selection output was analyzed via next generation sequencing (NGS), and library members that demonstrated an enrichment to VISTA binding at low pH were identified, reformatted as IgG 1.3 (an effectorless IgG1 constant region consisting of an IgG1 Fc having amino acid mutations L234A, L235E, and G237A), and screened for binding to VISTA by SPR.

Surface plasmon resonance (SPR) analysis was performed to measure the association rates (defined as ka or k_{on}, 1/Ms units), dissociation rates (defined as kd or k_{off}, s^{-1} units) and affinity constants (defined as K_D, M units) for VISTA Abs at acidic and physiological pHs using a Biacore® T200 instrument (GE Healthcare). Protein A (Fisher Scientific catalog #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 6,000 RU immobilization density of Protein A per flow cell. SPR experiments were conducted at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4 and 6.0. Antibodies were diluted to 20 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 50 seconds. A concentration series of 50 – 0.2 nM monovalent hVISTA-ECD (SEQ ID NO: 325) was prepared in pH 7.4 and 6.0 running buffers, and was injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants k_a (k_{on}) and k_d (k_{off}) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200

Evaluation Software v.2.0. For each VISTA antibody, the ratio of k_{off} at pH 6 / k_{off} at pH 7.4 was calculated to identify antibodies exhibiting slow off-rates at acidic pH and fast off-rates at physiological pH.

Six antibodies, reformatted as IgG1.3 antibodies, demonstrated near equivalent affinity at both pH 6 and pH 7.4. In particular, two antibodies had a slower off rate at pH 6.0 than at pH 7.4 (i.e., faster k_{off} at pH 7.4 than pH 6.0). The variable regions of these two huVISTA antibodies are referred to as P1-061015 and P1-061029 and the antibodies comprising these variable regions and formatted as IgG1.3 antibodies are referred to as P1-061015.IgG1.3 and P1-061029.IgG1.3, respectively. The k_{off} rates of P1-061015.IgG1.3 and P1-061029.IgG1.3 are provided in Table 1.

Table 1: k_{off} of selected antibodies at pH 6.0 and pH 7.0

Antibody name	pH 6 k_{off} (s^{-1})	pH 7 k_{off} (s^{-1})	pH 6/pH 7 k_{off}
P1-061015.lgG1.3	1.4×10^{-3}	2.3×10^{-3}	0.6
P1-061029.lgG1.3	4.8×10^{-3}	9.1×10^{-3}	0.5

The heavy and light chain CDR1, CDR2 and CDR3 sequences of P1-061015 and P1-061029 are provided in Table 2 below and are also shown in the Sequence Table following the Examples section of the disclosure.

Table 2: Amino acid sequences of huVISTA antibodies binding to huVISTA preferentially at pH 6.0 than pH 7.4

P1 ID	VH-gene	VH CDR1	VH CDR2	VH CDR3
P1-061015.lgG1.3	3-33	GFTFSSYAMH (SEQ ID NO: 95 Residues 26-35)	IIWYDGSNKYYADSVKG (SEQ ID NO: 95 Residues 50-66)	DSGFYSSYYFDY (SEQ ID NO: 95 Residues 99-110)
P1-061029.lgG1.3	3-09	GFTLDDYAMH (SEQ ID NO: 67 Residues 26-35)	GINWNSANIGYADSVKG (SEQ ID NO: 67 Residues 50-66)	VPGYSGGWIDAFDV (SEQ ID NO: 67 Residues 99-112)
	VL-gene	VL CDR1	VL CDR2	VL CDR3
P1-061015.lgG1.3	L6	RASQSVSSSYLA (SEQ ID NO: 96 Residues 24-35)	DASNRAT (SEQ ID NO: 96 Residues 51-57)	QQYNSYPYT (SEQ ID NO: 96 Residues 90-98)
P1-061029.lgG1.3	A27	RASQSVSSSYLA (SEQ ID NO: 68 Residues 24-35)	GASSRAT (SEQ ID NO: 68 Residues 51-57)	QQYGSSPFT (SEQ ID NO: 68 Residues 90-98)

Example 9: Further engineering of the P1-061015 and P1-061029 anti-VISTA Abs to develop acidic pH-selective antibodies

This Example describes the further engineering of variable regions P1-061015 and P1-061029 identified in Example 2 to obtain anti-huVISTA variable regions that have a higher k_{off} ratio between binding at pH 6.0 relative to pH 7.4.

Two libraries were built by introducing specific mutations in the VH CDRs of P1-061015 and P1-061029, respectively. The libraries allowed only for amino acid substitutions that were the most likely to improve binding at low pH, i.e., aspartate, glutamate and histidine. The library also allowed for single and double amino acid substitutions in each CDR and for recombinations across CDRs (maximum of 6 amino acid substitutions per chain). Fig. 7A shows the mutations that were introduced into the heavy chain CDR3 amino acid sequences of P1-061029 to form the P1-061029 library. The figure indicates that specific sequences were excluded to avoid introducing liabilities (e.g., DG).

The '029 and '015 libraries were screened by several rounds of binding to full length hVISTA at pH 6.0 via yeast surface display. Further rounds of selection were conducted by toggling between positive (pH 6.0 binding to huVISTA) and negative (pH 7.4 binding to huVISTA) (shown in Fig. 7B) selections, where library members that did not bind to VISTA at pH 7.4 were collected in the negative selection rounds. The selection output was analyzed by NGS. The '029 library members that bound to huVISTA at pH 6.0 after round 9 of selection were analyzed for binding to human VISTA at pH 6.0 and pH 7.4 via flow cytometry. Fig. 7C shows representative two-dimensional flow cytometry plots showing the variant pool after 9 rounds of selection. VISTA binding is plotted on the y-axis, and variant antibody expression is plotted on the x-axis. Binding data at various antibody concentrations and pH are shown. The results demonstrated very strong pH 6-selective binding to human VISTA, particularly at 20 nM.

Additional progeny clones of the '029 were isolated from the '029 library using a different method. Some clones were the same as those identified by the first method, and nine additional clones were isolated.

The 19 clones isolated from the '029 library selected for further analysis were reformatted as IgG1.3 antibodies. The amino acid differences in the heavy chain CDRs of these clones relative to those of the '029 VH CDRs are shown in Table 5.

Table 5: VH CDR1, CDR2 and CDR3 amino acid sequences (separated by an underscore) of antibodies derived from the '029 parent antibody

NAME	CDR1 (pos 26-35)	CDR2 (pos 50-66)	CDR3 (pos 99-110)	SEQ ID NO
P1-061029	GFT**LD**D**Y**AMH	GI**N**WNS**AN**IGYADSVKG	VPGYS**G**GWIDA**F**D**V**	67
P1-068757	----E-E---	------EE---------	----------E-D	71
P1-068759	----E-E---	--D---E---------	----------E-D	87
P1-068761	----E-E---	------EE---------	-----H-----E--	51
P1-068763	----E-----	--D---E---------	-----H-----E--	91
P1-068765	---DE-----	------EE---------	----------E-D	63
P1-068767	----E-----	--D---E---------	----------E-D	55
P1-068769	----E-E---	------DH---------	----------E-D	83
P1-068771	----E-E---	------HE---------	----------E-D	75
P1-068773	----E-----	--D---D---------	----------E-D	59
P1-068775	----E-E---	--D---EE---------	-----H-----E-D	79
P1-069059	----E-----	------DH---------	----------E-D	11
P1-069061	----E-----	-------E---------	----------E-D	15
P1-069063	----E-----	-------E---------	----------D-E	19
P1-069065	----E-E---	------DD---------	----------	23
P1-069067	----------	------EE---------	----------D-E	27
P1-069069	----------	------EE---------	----------D--	31
P1-069071	----E-E---	-------D---------	-----E--------	35
P1-069073	----E-----	--D---D---------	----------E-D	39
P1-069075	----E-----	----D--E---------	-----H-----E--	43
P1-069077	----E-E---	------DE---------	----------	47

Binding of several preparations of each of the '029 progeny clones and of the parent '029 antibodies, formatted as IgG1.3 antibodies, to human VISTA at pH 6.0 and 7.4 was measured by Surface plasmon resonance (SPR). SPR analysis was performed to measure koff and K_D binding affinity measurements for VISTA Abs at acidic and neutral pHs using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. SPR experiments were conducted at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween® 20)

running buffer at pH 7.4 and 6.0. Antibodies were diluted to 25 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 60 seconds. A concentration series of 50 – 5 nM monovalent hVISTA-ECD (SEQ ID NO: 325) was prepared in pH 7.4 and 6.0 running buffers, and was injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants ka (k_{on}) and kd (k_{off}) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0. The affinity constant, K_D was calculated as the ratio of rate constants k_{off}/k_{on} for each VISTA antibody.

The maximal (or magnitude) human VISTA binding response is defined as the reference-subtracted 'binding' report point response at the end of the 50nM VISTA injection for each antibody, and is reported in response units (RUs). The maximal human VISTA binding response (RUs) to each antibody is plotted in Fig. 7D. The mean average binding response (between two to four replicate antibodies) is plotted, and error bars represent the standard deviation. The results indicate that the selected progeny clones of '029 bind to hVISTA at pH 6.0, but not at pH 7.4 (empty circles representing binding at pH 7.4 are all located at the bottom of the graph except for the parent '029 clone).

The k_{off} rates at pH 6.0 of the '029 and its progeny was determined by SPR using the method described above, and are represented in Fig. 7E. The dashed line in the figure represent the k_{off} rate of '029, and clones to the left of the dashed line have a slower k_{off} rate at pH 6.0 relative to that of the parental '029 antibody, whereas those on the right side have a faster k_{off} rate at pH 6.0 relative to that of the parental '029 antibody.

Representative hVISTA SPR binding sensorgrams to the '029, '761 and '767 antibodies at neutral and acidic pH are shown in Fig. 7F. Reference-subtracted 50 nM and 5 nM huVISTA sensorgrams are plotted. At neutral pH, <10 RU VISTA binding signal was observed for '761 and '767, thus in order to adequately measure and compare the k_{off} and K_D for '761 and '767 to '029, a SPR kinetics assay utilizing μM VISTA concentrations at physiological pH was required.

For this assay, '029, '761 and '767 were reformatted as hIgG1f isotype and were expressed as both standard hIgG1f and in hIgG1f afucosylated formats to compare against the hIgG1.3f Fc. An SPR kinetics assay was conducted to measure koff and K_D binding

affinity measurements for VISTA Abs at acidic and physiological pH using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog #21181) was diluted to 20 μg/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. SPR experiments were conducted at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4 and 6.0. Antibodies were diluted to 25 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 45 seconds. A concentration series of 1600 – 0.78 nM (pH 7.4) and 100 – 0.78 nM (pH 6.0) monovalent hVISTA-ECD (SEQ ID NO: 325) was prepared running buffer, and was injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants ka (k_{on}) and kd (k_{off}) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0. The affinity constant, K_D was calculated as the ratio of rate constants k_{off}/k_{on} for each VISTA antibody. Ratios of k_{off} and K_D at pH 7.4 / pH 6.0 were calculated to compare off-rate and affinity improvement at acidic pH relative to physiological pH. While the neutral pH binding rate constants were not previously able to be determined for '761 and '767 using 50 nM hVISTA (Figs. 7D and 7F), inceasing the neutral pH VISTA concentration range to 1.6 μM resulted in binding responses (> 10 RU) for these clones that fit to a 1:1 binding model. Kinetic data for these acidic-selective VISTA antibodies is shown in Table 6. The '029 parent exhibits equivalent k_{off} at both pHs, while '761 and '767 exhibit over 10-fold selectivity for pH 6 over pH 7.4 in k_{off} and over 2000-fold selectivity for pH 6 over pH 7.4 in K_D. Human VISTA binding rate constants are conserved across hIgG1.3f, hIgG1f and afucosylated hIgG1f isotype variants.

Table 6: Binding characteristics of VISTA antibodies as determined by SPR

Antibody	Isotype	pH 7.4			pH 6.0			kd ratio (7.4/6)	KD ratio (7.4/6)
		ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)		
P1-061029	hIgG1.3f	1.6E+05	6.8E-03	4.2E-08	1.1E+06	7.9E-03	7.2E-09	0.9	5.8
	hIgG1f	1.7E+05	7.4E-03	4.2E-08	1.1E+06	8.0E-03	7.1E-09	0.9	5.9
	hIgG1f afucosylated	1.7E+05	7.2E-03	4.1E-08	1.1E+06	7.8E-03	6.9E-09	0.9	5.9
P1-068761	hIgG1.3f	3.8E+03	4.2E-02	1.1E-05	3.7E+05	1.6E-03	4.3E-09	26.3	2558.1
	hIgG1f	1.2E+03	4.2E-02	3.5E-05	3.6E+05	1.5E-03	4.2E-09	28.0	8333.3
	hIgG1f afucosylated	5.1E+03	4.2E-02	8.2E-06	3.7E+05	1.5E-03	4.1E-09	28.0	2000.0
P1-068767	hIgG1.3f	1.9E+03	3.6E-02	1.9E-05	3.3E+05	2.6E-03	7.8E-09	13.8	2435.9
	hIgG1f	1.5E+03	3.2E-02	2.2E-05	3.2E+05	2.6E-03	8.0E-09	12.3	2750.0
	hIgG1f afucosylated	1.3E+03	3.3E-02	2.4E-05	3.3E+05	2.6E-03	7.9E-09	12.7	3038.0
α-VISTA acidic pH sensitive	hIgG1.3f	2.2E+05	7.8E-04	3.6E-09	2.8E+06	9.0E-02	3.2E-08	0.01	0.1

Human VISTA binding kinetics of P1-061029 (" '029"), P1-068761 (" '761") and P1-068767 (" '767") (as IgG1.3 antibodies) were measured at pH values between pH 7.4 and pH 6.0, i.e., at pH 6.9 and pH 6.45 using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. The assay was conducted at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4, 6.9, 6.45 and 6.0. Antibodies were

diluted to 25 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 45 seconds. A concentration series of 100 – 0.78 nM monovalent hVISTA-ECD (SEQ ID NO: 325) was prepared pH 7.4, 6.9, 6.45 and 6.0 running buffers, and was injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants ka (k_{on}) and kd (k_{off}) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0. The affinity constant, K_D was calculated as the ratio of rate constants k_{off}/k_{on} for each VISTA antibody. Ratios of k_{off} and K_D at each pH relative to pH 6.0 were calculated to evaluate how the VISTA k_{off} and K_D change as the buffer pH shifts to physiological, and are shown in Table 7. The '029 parent exhibited consistent k_{off} at each pH tested, while the '761 and '767 progeny exhibited at least 10-fold faster VISTA k_{off} and 100-fold weaker VISTA K_D at pH 6.9 compared to pH 6.0. As the buffer pH shifts from acidic to physiological, VISTA k_{off} and K_D both weaken for '761 and '767. Physiological pH data for '761 and '767 for comparison is referenced from Table 7 and noted with an asterisk (*).

Table 7: Kinetic binding characteristics of '029, '761 and '767 antibodies at different pH values

Antibody	pH	ka (1/Ms)	kd (1/s)	KD (M)	koff ratio to pH 6.0	KD ratio to pH 6.0
P1-061029 (parent)	6.0	2.9E+06	5.7E-03	2.0E-09	1.0	1.0
	6.45	7.4E+05	4.0E-03	5.3E-09	0.7	2.7
	6.9	4.1E+05	5.7E-03	1.4E-08	1.0	7.1
	7.4	2.5E+05	6.4E-03	2.6E-08	1.1	13.2
P1-068761	6.0	6.0E+05	6.6E-04	1.1E-09	1.0	1.0
	6.45	1.1E+05	2.1E-03	2.0E-08	3.2	18.4
	6.9	4.8E+04	8.9E-03	1.9E-07	13.4	170
	7.4 *	3.8E+03	4.2E-02	1.1E-05	~ 63.6	~10000
P1-068767	6.0	5.6E+05	1.9E-03	3.4E-09	1.0	1.0
	6.45	1.3E+05	4.8E-03	3.8E-08	2.5	11.0
	6.9	7.4E+04	2.9E-02	4.0E-07	15.3	115.1
	7.4 *	1.9E+03	3.6E-02	1.9E-05	~ 19.0	~5000

The data in Table 7 indicates that an at least 10 fold lower affinity of binding of '761 and '767 to hVISTA at pH 6.45 compared to pH 6.0; an at least 100 fold lower affinity of binding of '761 and '767 to hVISTA at pH 6.9 compared to pH 6.0; and an at least 1000 fold lower affinity of binding of '761 and '767 to hVISTA at pH 7.4 compared to pH 6.0.

The '015 library also demonstrated a slight preference for pH 6-selective binding to VISTA. The amino acid differences of the progeny clones relative to the '015 VH CDRs is shown in Table 8. Binding of several preparations of each of the '015 progeny clones and of the parent '015 (all as IgG1.3 antibodies) to human VISTA at pH 6.0 and 7.4 was measured via SPR using the identical method described for the '029 analysis above, and is shown in Table 8.

Table 8: VH CDR1, CDR2 and CDR3 amino acid sequences of antibodies (separated by underscore) derived from the '015 parent antibody

NAME	CDR1 (pos 26-35)	CDR2 (pos 50-66)	CDR3 (pos 99-110)	SEQ ID NO
P1-061015	GFTFS**S**YA**M**H	IIW**Y**DGS**NKYY**ADSVKG	DSGFY**SS**YYFDY	95
P1-068736	-----E-----	-D-------D-------	-----D-----D	107
P1-068738	-----E--H-	---D----H--------	-----ED-----	131
P1-068740	-----D----	-------D-D-------	-----D-----D	115
P1-068742	-----D----	-------D-D-------	-----ED-----	119
P1-068744	-----E-----	H---------E------	-----E-----E	103
P1-068746	----------	--------HH-------	-----D------	123
P1-068748	-----HH---	--------DD-------	-----D------	99
P1-068750	-----D-D--	E--D-----------	-EE---------	127
P1-068752	----------	E--------D-------	-----D-----E	111
P1-068754	-----D-D--	E--D----------	----H-D-----	135

A summary of the kinetics of binding of '029 and '015 and their progeny clones to huVISTA at pH 6.0 and 7.4, as determined by SPR, is shown in Tables 9 and 10.

Table 9: huVISTA kinetics summary and VH CDR sequences of the ‘029 clone and their progeny

ID	Avg 7.4 ka (1/Ms)	Avg 7.4 kd (1/s)	Avg 7.4 KD (M)	Avg 6.0 ka (1/Ms)	Avg 6.0 kd (1/s)	Avg 6.0 KD (M)	VH CDR 1 (pos 26-35)	VH CDR 2 (pos 50-66)	VH CDR 3 (pos 99-110)	SEQ ID NO
P1-069077	6.0E+04	1.9E-03	3.1E-08	6.3E+05	1.2E-04	1.9E-10	....E.E...	......DE.....	.....	47
P1-069065	5.9E+04	2.3E-03	3.9E-08	5.7E+05	2.2E-04	3.8E-10	....E.E...	......DD.....	.....	23
P1-069075	1.3E+05	2.3E-03	1.8E-08	1.3E+06	2.9E-04	2.2E-10	....E.....	....D..E.....	.....H.....E..	43
P1-069071	4.3E+04	4.0E-03	9.3E-08	7.0E+05	5.1E-04	7.3E-10	....E.E...	.......D.....	.....E.....	35
P1-069061	Weak, fast kd			4.3E+05	1.1E-03	2.5E-09	....E.....	.......E.....	.....E.D	15
P1-069069	9.0E+04	7.5E-03	8.4E-08	1.4E+06	1.2E-03	8.6E-10	.....	......EE.....	.....D..	31
P1-068761	Weak, fast kd			3.8E+05	1.4E-03	3.8E-09	....E.E...	......EE.....	.....H.....E..	51
P1-069059	Weak, fast kd			3.4E+05	1.6E-03	4.8E-09	....E.....	......DH.....	.....E.D	11
P1-068767	Weak, fast kd			3.4E+05	2.6E-03	7.6E-09	....E.....	..D...E.....	.....E.D	55
P1-068773	Weak, fast kd			3.0E+05	2.9E-03	9.4E-09	....E.....	..D...D.....	.....E.D	59
P1-069063	1.2E+05	2.7E-02	2.3E-07	1.9E+06	4.4E-03	2.4E-09	....E.....	.......E.....	.....D.E	19
P1-069067	1.0E+05	2.7E-02	2.9E-07	1.7E+06	4.5E-03	2.7E-09	.....	......EE.....	.....D.E	27
P1-069073	Weak, fast kd			6.1E+05	5.8E-03	9.4E-09	....E.E...	.......E.....	.....E..	39
P1-061029	2.9E+05	5.6E-03	1.9E-08	1.6E+06	5.8E-03	3.6E-09	GFTLDDYAMH	GINWNSANIGYADSVKG	VPGYSGGWIDAFDV	67
P1-068765	No binding			3.7E+05	7.0E-03	1.9E-08	...DE.....	......EE.....	.....E.D	63
P1-068757	No binding			8.9E+05	1.7E-02	1.9E-08	....E.E...	......EE.....	.....E.D	71

ID	Avg 7.4 ka (1/Ms)	Avg 7.4 kd (1/s)	Avg 7.4 KD (M)	Avg 6.0 ka (1/Ms)	Avg 6.0 kd (1/s)	Avg 6.0 KD (M)	VH CDR 1 (pos 26-35)	VH CDR 2 (pos 50-66)	VH CDR 3 (pos 99-110)	SEQ ID NO
P1-068771	No binding			7.6E+05	1.8E-02	2.5E-08	....E.E...	......HE.....	..........E.D	75
P1-068769	No binding			8.1E+05	4.0E-02	5.5E-08	....E.E...	......DH.....	..........E.D	83
P1-068775	No binding			1.8E+06	4.7E-02	2.3E-08	....E.E...	..D...EE.....	.....H.....E.D	79
P1-068759	No binding			1.3E+06	8.0E-02	6.0E-08	....E.E...	..D...E.....	..........E.D	87

Table 10: huVISTA kinetics summary and VH CDR sequences of the '015 clone and their progeny

Clone	Avg 7.4 ka (1/Ms)	Avg 7.4 kd (1/s)	Avg 7.4 KD (M)	Avg 6.0 ka (1/Ms)	Avg 6.0 kd (1/s)	Avg 6.0 KD (M)	HCDR Sequence (pos 26-35) (pos 50-66) (pos 99-110)	SEQ ID NO
P1-061015	2.3E+05	2.0E-03	8.8E-09	1.8E+06	9.5E-04	5.4E-10	GFTFSSYAMH_IIWYDGSNKYYADSVKG_DSGFYSSYYFDY	95
P1-068748	No binding			1.4E+06	1.5E-03	1.0E-09	.....HH..._........DD....._.....D.....	99
P1-068744				1.3E+06	1.8E-03	1.3E-09	.....E...._H.........E....._.....E.....E	103
P1-068736				8.4E+06	9.5E-03	1.1E-09	.....E...._.D.......D....._.....D.....D	107
P1-068752				6.1E+06	3.4E-02	5.6E-09	..........E........D....._.....D.....E	111
P1-068740				Too fast	4.7E-02	ND	.....D...._.......D.D....._.....D.....D	115
P1-068742				Too fast	>1E-02	ND	.....D...._.......D.D....._.....ED.....	119
P1-068746				Too fast	>1E-02	ND	..........._........HH....._.....D.....	123
P1-068750				Weak			.....D.D.._E..D............._.EE.........	127

Thus, several '029 progeny clones were identified which either maintained or improved k_{off} to VISTA-ECD at pH 6.0 compared to the '029 parent, and also demonstrated weaker k_{off} or a loss of binding to VISTA at physiologic pH. The '015 progeny exhibited acidic pH selective binding to VISTA-ECD, with no binding detected to VISTA at neutral pH, but all '015 progeny assayed yielded a faster k_{off} at pH 6.0 compared to the '015 parent.

Example 10: Acidic pH-selective '029 progeny demonstrate acidic pH-dependent cell binding and effector function while maintaining VISTA blocking activity

pH-dependent binding of clones '761 and '767 to Raji cells engineered to ectopically express full length human VISTA (SEQ ID NO: 1 with D187E substitution) was measured. For this experiment, '761 and '767 were formatted as IgG1.3 antibodies, and binding was measured by an anti-human IgG secondary antibody (Jackson Immunoresearch catalog number 109-065-098). The results, which are shown in Figs. 8A-8B, indicate that the '761 (Fig. 8A) and '767 clones (Fig. 8B) bind poorly at pH 7.2 and 8.1, but better at acidic pH, particularly at pH 6.0, 6.1, 6.2, and 6.4. Binding MFIs are plotted on the y-axis and primary antibody concentrations are plotted on the x-axis in log scale. Non-linear regressions are also shown.

Fig. 8C shows data from the experiment described in Figs. 8A-B measuring P1-068767 (circles) and an isotype-matched non-specific control antibody (triangles) binding to Raji cells expressing human VISTA at 3125 ng/mL at different pH. The "pH50", the pH at which 50% of P1-068767 binding is lost, is approximately 6.6. Binding MFIs are plotted on the y-axis, and buffer pH is plotted on the x-axis. Non-linear regressions are also shown.

Fig. 8D shows the MFI of an isotype-matched non-specific control antibody (filled and unfilled circles for pH 7.0 and 6.0 respectively), anti-VISTA mAb 2 ("control", see Fig. 6C, filled and unfilled squares at pH 7.0 and 6.0 respectively), P1-068761 (filled and unfilled triangles for pH 7.0 and 6.0 respectively), and P1-068767 (filled and unfilled inverted triangles for pH 7.0 and 6.0 respectively) binding to human monocytes. Binding was detected as described in Figs. 8A-B. The non-pH-selective VISTA control antibody (mAb 2) bound monocytes at both pH. Both engineered acidic pH-selective antibodies bound monocytes well at pH 6.0, but did not bind better than the non-specific isotype-matched control at pH 7.0.

Thus, clones '761 and '767 have weak binding or no binding to VISTA at neutral pH, and instead bind VISTA on cells selectively at acidic pH.

Fig. 8E shows the comparable blocking of recombinant VISTA multimer binding to activated human CD4+ T cells at pH 6.0 by '029 (squares), '761 (triangles), and '767 (inverted triangles), while a non-VISTA-specific control antibody (circles) did not block VISTA binding. This blocking assay was performed as described in Example 4. These data show that engineered acidic pH-selective VISTA antibodies are still capable of blocking VISTA receptor-ligand binding at acidic pH.

NK cell specific lysis of target cells (the same Raji cells expressing human VISTA described in Figs. 8A-B) via antibody-dependent cell cytotoxicity (ADCC) at physiological pH was measured for the P1-061029.IgG1f, P1-068761.IgG1f, P1-068767.IgG1f antibodies, a non-VISTA-specific antibody and a non-VISTA-specific negative control antibody, all expressed as afucosylated IgG1 antibodies. NK cells were enriched from PBMC via negative bead selection (StemCell Technologies catalog number 19055) and cultured overnight in Myelocult™ media (StemCell Technologies catalog number 05150) supplemented with 1 μM hydrocortisone (StemCell Technologies catalog number 07904) and 500 U/mL recombinant human IL-2 (Peprotech catalog number 200-02). On the day of the assay, Raji cells ectopically expressing human VISTA (described in Fig. 8A-B) were labeled with Calcein AM (Life Technologies catalog number C3100MP) and co-cultured with the cultured NK cells at a 10:1 NK : target cell ratio and with P1-061029.IgG1f, P1-068761.IgG1f, P1-068767.IgG1f antibodies, a non-VISTA-specific antibody and a non-VISTA-specific negative control antibody for 2 hours at physiological pH. Specific lysis was interpolated from the supernatant fluorescence signal (EnVision™ plate reader). The spontaneous lysis signal obtained from co-culture without antibodies, and the maximal lysis signal was determined by lysis of target cells with Delfia® lysis buffer (PerkinElmer catalog number 4005-0010). Antibody-specific lysis was calculated to be the percentage of lysis observed divided by (the maximal lysis signal minus the spontaneous lysis signal).

The results, which are provided in Fig. 8F, show the reduced potency of P1-068761.IgG1f and P1-068767.IgG1f, relative to P1-061029 and the positive control in mediating antibody-dependent cell cytotoxicity (ADCC) at physiological pH.

Example 11: Cyno PK of VISTA antibodies

Human antibody-naïve cynomolgus macaques were injected intravenously with a single 5 mpk dose of either a VISTA antibody which binds comparably at acidic and neutral pH ("control"; mAb2), a VISTA antibody with impaired binding at acidic pH ("acidic pH sensitive", mAb3), or acidic pH-selective antibody '767, to determine the cyno PK of these antibodies.

The SPR binding kinetics of the antibodies used in this Example are provided in Table 11, and were determined as follows. Cyno VISTA cross-reactivity for acidic pH-selective and control anti-VISTA antibodies was evaluated at acidic and neutral pH. Binding affinity measurements for VISTA Abs were conducted using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog number #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. The assay was run at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4 and 6.0. Antibodies (formatted as IgG1.3 antibodies) were diluted to 25 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 45 seconds. Concentration series of 1600 – 0.78 nM (pH 7.4) and 100 – 0.78 nM (pH 6.0) monovalent hVISTA-ECD (SEQ ID NO: 325) and cyno VISTA-ECD (AFKVATLYSL YVCPEGQNVT LTCRVFGPVD KGHDVTFYKT WYRSSRGEVQ TCSERRPIRN LTFQDLHLHH GGHQAANTSH DLAQRHGLES ASDHHGNFSI TMRNLTLLDS GLYCCLVVEIRHHHSEHRVH GAMELQVQTG KDAPSSCVAY PSSSQESENI TAHHHHHHH; (SEQ ID NO: 326) were prepared running buffer, and were injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants ka (k_{on}) and kd (k_{off}) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0. The affinity constant, K_D was calculated as the ratio of rate constants koff/kon for each VISTA antibody. Ratios of k_{off}

and K_D at pH 7.4 / pH 6.0 were calculated to compare off-rate and affinity differences at acidic pH relative to neutral pH, and are shown in Table 11. All anti-VISTA antibodies tested exhibited comparable (within 2-fold) human and cyno VISTA kinetic binding parameters at both pHs tested, confirming cross-reactivity with cyno VISTA. Both anti-VISTA control antibodies exhibited improved kd and stronger K_Ds at physiological pH compared to acidic pH, and the acidic pH-sensitive control exhibited a faster VISTA kd at acidic pH compared to the control antibody.

Table 11: SPR binding kinetics of VISTA antibodies to cyno VISTA

Antibody	VISTA	pH 7.4			pH 6.0			kd ratio (7.4/6)	KD ratio (7.4/6)
		ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)		
P1-061029	human	1.2E+05	7.5E-03	6.2E-08	9.8E+05	6.6E-03	6.8E-09	1.1	9.1
	cyno	1.4E+05	6.7E-03	4.7E-08	6.2E+05	6.2E-03	1.0E-08	1.1	4.7
P1-068761	human	4.3E+03	3.7E-02	8.7E-06	3.5E+05	1.4E-03	4.1E-09	26.4	2122.0
	cyno	6.5E+03	3.6E-02	5.5E-06	2.1E+05	1.7E-03	7.9E-09	21.2	696.2
P1-068767	human	1.6E+03	3.5E-02	2.3E-05	3.2E+05	2.4E-03	7.5E-09	14.6	3066.7
	cyno	1.3E+03	3.4E-02	2.6E-05	1.9E+05	2.5E-03	1.3E-08	13.6	2000.0
α-VISTA control (mAb 3)	human	4.4E+05	1.3E-03	3.0E-09	9.6E+05	6.0E-03	6.2E-09	0.2	0.5
	cyno	4.9E+05	1.7E-03	3.4E-09	5.5E+05	7.1E-03	1.3E-08	0.2	0.3
α-VISTA acidic pH sensitive (mAb 2)	human	1.8E+05	7.8E-04	4.3E-09	1.8E+06	5.0E-02	2.8E-08	0.02	0.2
	cyno	1.9E+05	6.8E-04	3.5E-09	1.2E+06	5.2E-02	4.4E-08	0.01	0.08

Human antibody-naïve cynomolgus macaques were injected intravenously with a single 5 mpk dose of either VISTA mAb2 ("control"), VISTA mAb3 ("acidic pH sensitive"), or P1-068767.IgG1.3. The serum concentration of each antibody following injection is shown in Fig. 9. Mean residence times for P1-068767.IgG1.3 and the control anti-VISTA antibody were 717 and 22 hours respectively, indicating that acidic pH selectivity greatly reduced VISTA antibody Target Mediated Drug Disposition (TMDD). Although the control antibody (mAb 2) and the acidic pH sensitive antibody (mAb 3) bind VISTA comparably at physiological pH, the acidic pH sensitive antibody had a lower mean residence time of 7.6 hours, demonstrating the importance of acidic pH binding to VISTA antibody recycling as described in Examples 6 and 7. The results show that acidic pH selective antibodies have

superior PK and will thus more easily achieve target engagement in tumors or other microenvironments.

Example 12: pH selective '029 progeny do not bind non-specifically to high pI proteins

The binding specificity of the '029, '761 and '767 clones to VISTA and to other high pI proteins was evaluated by SPR at neutral and acidic pH using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM3 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 800 RU immobilization density of Protein A per flow cell. SPR experiments were conducted at 25°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4 and 6.0. Antibodies (formatted as IgG1.3 antibodies) were diluted to 50 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 60 seconds. A concentration series of 100 – 10 nM monovalent hVISTA-ECD (SEQ ID NO: 325), avidin (ThermoFisher Scientific catalog #21128), cytochrome C (Sigma catalog #C2867), BSA (Calbiochem catalog #126593), and monovalent control antigen ("Ag") were prepared in pH 7.4 and 6.0 running buffers, and were injected over the captured antibodies at 50 ul/min to evaluate binding specificity. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Reference flow cell and 0 nM blank-subtracted sensorgrams were inspected using Biacore® T200 Evaluation Software v.2.0. The results are shown in Table 12.

Table 12: Binding of VISTA clones to proteins having a high pI

Sample	Isoel. pt (pI)	'029		'761		'767		Anti-Ag		PBS (no Ab)	
		pH 6	pH 7.4	pH 6	pH 7.4	pH 6	pH 7.4	pH 6	pH 7.4	pH 6	pH 7.4
huVISTA -His	6.9										
Avidin	10										
Cyto-Chrome C	10.7										
BSA	4.7										
Ag-His	6.5										

In Table 12, specific binding, defined as >10 RU SPR binding responses at the end of the sample injection is indicated by filled gray boxes. "Anti-Ag" is an VISTA binding control Ab. The '029 clone was specific for VISTA at acidic and neutral pH. The '029 progeny clones '761 and '767 were specific for VISTA at acidic pH, while the control antibody also maintained antigen specificity. Non-specific binding ("NSB") of the pI control proteins to the Protein A reference surface was not observed in this assay.

Thus, the charged amino acids introduced to the VH CDRs of '761 and '767 do not cause these antibodies to bind electrostatically to other high pI proteins, such as avidin and cytochrome C, or low pI proteins, such as BSA.

Example 13: Release of VISTA mediated inhibition of T cell activation by antibodies '761 and '767

This Example describes an assay that can be conducted to determine the ability of antibodies '761 and '767 to block hVISTA inhibition of Jurkat T cell activation.

The same assay as described in Example 5 is used. Briefly, Jurkat (human T cell line) cells expressing an NFkB luciferase reporter are co-cultured at various pH with 293T cells expressing human VISTA and a single-chain variable fragment of the anti-human T cell receptor agonist antibody OKT3. Anti-VISTA antibodies '761 and '767 or an isotype-matched non-VISTA-specific control antibody are added to the co-cultured cells. Jurkat activation is shown as luciferase units and as the fold-increase of the luciferase signal with anti-VISTA treatment relative to control.

Example 14: Mutational analysis identified key residues conferring pH dependent binding properties to VISTA antibodies

Antibodies P1-068761.IgG1.3 and P1-068767.IgG1.3 contain 5-6 mutations from P1-061029 (Table 7). A mutational analysis was conducted in order to identify key residues important for conferring the pH dependent properties of the VISTA antibodies. As such, a panel of N-1 (1 amino acid reversion to P1-061029) and N-2 (2 amino acid reversion to P1-

061029) variants of P1-068761 and P1-068767 were synthesized, expressed as IgG1.3, and analyzed for their binding to huVISTA at pH 6, pH 6.7, and pH 7.4.

Binding kinetics were measured using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. The assay was conducted at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4, 6.7 and 6.0. Antibodies were diluted to 25 nM in PBST pH 7.4, and were captured across active biosensor flow cells at 5 ul/min for 40 seconds. A concentration series of 100 – 10 nM monovalent hVISTA-ECD (SEQ ID NO: 325) was prepared in pH 7.4, 6.7 and 6.0 running buffers, and was injected over the captured antibodies at 40 ul/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants ka (kon) and kd (koff) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0.

The affinity constant, K_D was calculated as the ratio of rate constants k_{off}/k_{on} for each VISTA antibody. The %Rmax was calculated to compare how pH affects an antibody's binding capacity for VISTA, and represents the measured maximal VISTA binding response relative to the expected maximal VISTA binding response. The %Rmax is defined as the ratio of the reference-subtracted 'binding' report point response at the end of the 100 nM VISTA injection for each antibody (Rmax) relative to the expected VISTA binding response (Rexp). Rexp is calculated as Rexp = [(VISTA-ECD molecular weight / mAb molecular weight) x (mAb 'capture' report point response (RUs)] x 2 binding sites per mAb.

The SPR results obtained for P1-068761 reversion variants are shown in Fig. 10A, which is ranked by the pH 6.0 k_{off}, slowest to fastest. In the table, antibodies that exhibited weak or no binding response (< 10 RU) to 100 nM hVISTA were categorized as non-binding (NB). The results indicate that E at positions 32 (i.e., amino acid residue 7 in VH CDR1 of '761) and 100f (amino acid residue 12 in VH CDR3 of '761) are both required for

maintaining acidic pH selectivity, since these reversions to P1-061029 parent sequence allowed for significant hVISTA binding at physiological pH (%Rmax > 10). Further analysis revealed that variants with E55A (amino acid residue 6 in VH CDR2 of '761) reversion maintained acidic pH-selectivity and exhibited comparable binding kinetics within 2-fold of P1-068761. In contrast, while variants with H100G, E56N and E30D (amino acid residues 12 of VH CDR3, 7 of VH CDR2 and 4 of VH CDR1 of '761, respectively) reversions maintained acidic pH selectivity, these mAbs also exhibited ~3-fold faster k_{off} at acidic pHs compared to P1-068761, bringing the off-rates of these mutants at acidic pH closer to the P1-061029 parent. Thus, adding G100H, N56E and/or D30E mutations to the original P1-061029 clone contributed to acidic pH VISTA affinity improvements observed in the acidic selective P1-068761 clone.

The SPR results obtained for P1-068767 reversion variants are shown in Fig. 10B, which is ranked by the pH 6.0 k_{off}, slowest to fastest. The results indicate that D at position 102 (amino acid residue 14 in VH CDR3 of '767) was required for maintaining acidic pH selectivity, and D102V reversion back to P1-061029 parent sequence allowed for significant hVISTA binding at neutral pH (%Rmax > 10). Further analysis revealed that variants with E30D, D52N and E55A (amino acid residues 4 of VH CDR1, 3 of VH CDR2 and 6 of VH CDR3 of '767, respectively) reversions maintained acidic pH-selectivity and exhibited comparable pH 6.0 binding kinetics within 2-fold of P1-068767. In contrast, variants with E100fF (amino acid residue 12 of VH CDR3 of '767) reversion maintained acidic pH selectivity, albeit with > 3-fold faster k_{off} at acidic pH compared to P1-068767. Notably, variants with E100fF reversion exhibited even faster k_{off} at acidic pH compared to the parent mAb P1-061029.

Thus, a summary of the reversion mutants of P1-068761 and P1-068767 (acidic pH-selective) relative to P1-061029 (pH-tolerant) is as follows in Table 13 (HCDR1, HCDR2, and HCDR3 are separated by an underscore).

Table 13: Seq Id.

P1-061029	GFTLDDYAMH_GINWNSANIGYADSVKG_VPGYSGGWIDAFDV	67
P1-068761	....E.**E**..._......EE........._.....H.....**E**..	51
P1-068767	....E....._..D...E........._..........E.**D**	55
	aa pos. 26-35 50-66 99-110	

The above VH CDR sequences for P1-061029, P1-068761, and P1-068767 in Table 13 are at amino acids ("aa pos.") 26-35, 50-66, and 99-110 of SEQ ID NOs: 67, 51, 55, respectively. Key mutations required for acid pH-selectivity are indicated in bold, and mutations with over 3 fold impact on pH 6.0 k_d compared to P1-068761 and P1-068767 are underlined.

Example 15: Mapping of VISTA antibody epitopes

The hVISTA epitopes of '015, '029, '761 and '767, formatted as IgG1.3 antibodies, were determined by 2 different methods: BLI (bio-layer interferometry) competition and yeast surface display.

Competitive BLI epitope binning assays were conducted to evaluate whether acidic pH-selective VISTA antibodies P1-068761 and P1-068767 retained similar or overlapping epitopes on VISTA compared to the P1-061029 parent, P1-061015 and relevant VISTA control antibodies 1, 2 and 3. Sandwich and tandem format binning assays were performed on an OctetRed384 BLI instrument (PALL/ForteBio). All assay steps were performed at 30°C at 1000 rpm shake speed, and the buffer used was acidic (pH 6.0) or neutral (pH 7.4) PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20). For the sandwich format, anti-human IgG-Fc sensors (AHC, PALL/ForteBio) first captured the VISTA antibody panel at pH 7.4, then the anti-human capture sensors were blocked with total human IgG (Jackson #009-000-002). Human VISTA-ECD was captured next at pH 6.0, and finally competition for all possible antibody combinations was assessed at pH 6.0. In the tandem format assay, streptavidin-coated biosensors (SAX, PALL/ForteBio) first captured biotinylated hVISTA-ECD at pH 7.4, then the sensors captured the full VISTA antibody panel at pH 6.0, ensuring complete binding saturation of each antibody on VISTA before evaluating competition with all possible antibody combinations at pH 6.0.

The results of the Competitive BLI epitope binning assays are summarized by a competition matrix, Figure 11A. In this figure, the first antibody captured is listed by row, and its binding or blocking activity to the (second) competitor antibodies are shown in each column. The competition matrix was identical for both assay formats. For the sandwich assay, binding (light gray) of the competitor antibody was defined by a signal ranging between 0.4 – 1.2 nm, and blocked antibodies (black) exhibited non-binding signal < 0.1 nm. For the tandem assay, binding of the competitor antibody was defined by a signal ranging between 0.3 – 0.8 nm, and blocked antibodies exhibited non-binding signal < 0.2 nm. While 'VISTA mAb 3' exhibited a fast acidic pH dissociation from hVISTA-ECD by SPR at 37°C (Figure 6C), it did not rapidly dissociate in either BLI assay format (conducted at 30°C). These competitive assays indicated that P1-061015, P1-061029, acidic-pH selective antibodies P1-068761 and P1-068767 and VISTA antibodies 2 and 3 all compete with one another for similar or overlapping epitopes on VISTA. However, VISTA antibody 1 binds to a separate and distinct epitope. Thus, the charged amino acid mutations introduced to the VH CDRs of P1-061029 to generate the acidic-selective clones P1-068761 and P1-068767 have not signigicantly altered the VISTA binding epitope.

The epitopes of antibodies '029, '015, '761, and '767 were also mapped using yeast surface display and NGS according to the method of Chao *et al.* (2004) *J. Mol. Biol.* 342:539-550, Oliphant *et al.* (2006) *J. Virol.* 80:12149–12159, and Kowalsky *et al.* (2015) *J. Biol. Chem.* 290:26457–26470. Briefly, a saturation mutagenesis library of single point mutants of the VISTA ECD was generated and displayed on the surface of yeast. VISTA mutants that lost binding to the antibody being mapped but retained binding to a non-blocking antibody (mAb1) were sorted and sequenced. Since they retained binding to mAb1, these mutants were likely correctly folded, and the loss of binding seen to the antibody being mapped was likely due to the loss of an energetically important contact residue. The positions of these mutations were designated as energetically important residues in the antibody's epitope and are shown in Table 14.

Table 14: Residues of huVISTA that are identified as epitope residues of anti-VISTA mAbs

mAb	T 36	Y 37	K 38	T 39	Y 41	R 54	T 61	F 62	Q 63	L 65	H 66	L 67	H 68	R 69	F 97	L 112	V 117	I 119	H 121	H 122	S 124	E 126	R 127
Pt. 061015		x	x	x		x		x	x	x	x				x	x	x	x			x	x	
Pt. 061029		x		x	x	x	x	x	x	x	x		x	x		x	x	x			x	x	x
Pt. 068761	x	x		x		x	x	x	x		x	x	x	x		x	x	x			x	x	x
Pt. 068767		x		x	x	x	x	x	x	x	x					x	x	x	x	x	x	x	x

Table 15 includes detailed data from Table 14, and lists the amino acid residues of hVISTA that are likely to reduce binding of each antibody listed, based on residue frequency observed in the yeast surface display/NGS method

Table 15: VISTA amino acid substitutions likely to reduce binding of the listed antiobodies

	P1-061015 pH 6	P1-061015 pH 7	P1-061029 pH 6	P1-061029 pH 7	P1-068761 pH 6	P1-068767 pH 6
T35					P, Y, W	
Y37	P, G, A, S, T, K, R, H, N, D, E, Q	P, G, S, N, D, E Q	Y, S, T, V, L, I, M, K, R, N, D, Q	P, G, S, T, V, L, I, M, K, R, N, D, E, Q	P, G, A, S, T, V, L, I, M, K, R, N, D, E, Q	G, T, V, L, I, M, K, R, N, Q
K38	P, G, A, S, V					
T39	G, M, R, H, F, Y, W, N, D, E, Q	M, K, R, H, F, Y, W, D, E, Q	G, A, S, M, Y, W, N, D, E, Q	G, A, S, V, L, M, R, H, F, Y, W, N, D, E, Q	G, Y, D, E	G, A, S, H, Y, W, N, D, E, Q
Y41				A, S, T, I, M		P, I, M, H
R54	L, M, F, Y, E	M, E	P, A, T, V, I, M, F, Y, N, D, E, Q	P, G, A, S, T, V, L, I, M, H, F, Y, W, N, D, E, Q	A, T, V, L, I, M, K, F, Y, E, Q	P, A, S, T, V, L, I, M, F, Y, W, D, E, Q
T61			G, L, R, H, F, Y, D, E, Q	V, L, K, R, H, F, Y	G, V, H, Y, D	L, R, H, F, Y, D, E
F62	G, A, S, M, K, R, N, D, E, Q	G, K, R, D, E, Q	P, G, A, S, T, V, I, M, H, Y, W, D, E, Q	P, G, A, S, T, V, L, I, M, K, R, H, Y, W, N, D, E, Q	P, G, A, S, T, V, M, H, Y, W, D, E, Q	P, G, A, S, T, V, L, M, H, Y, W, N, D, E, Q
Q63	G, R, W, D, E	W, D, E	G, A, S, T, V, K, R, H, Y, W, N, D, E	P, G, S, T, L, M, K, R, H, F, Y, W, N, D, E	G, S, T, K, H, Y, N, D, E	P, G, A, S, T, V, L, I, M, K, H, F, Y, W, N, D, E
L65	P, G, A, S, T, K, R, H, W, N, D, E, Q	P, G, S, K, W, D, E, Q	G, T, Y, D, E, Q	P, G, A, S, T, H, Y, W, N, D, E, Q,		P, G, S, H, D, E, Q
H66	P, T, V, L, I, M, K, R, F, Y, W	P, T, V, L, I, M, K, R, F, Y, W	T, V, L, I, Y, D, E, Q	G, S, T, V, L, I, M, K, R, W, N, D, E, Q	T, I, K, W, D	T, V, I, K, W, D, E
L67					G, A	
H68			L, I, M, F, E	L, I, E	G, T, V, L, I, Y, W, D, E, Q	
F97	G, D, E					

	P1-061015 pH 6	P1-061015 pH 7	P1-061029 pH 6	P1-061029 pH 7	P1-068761 pH 6	P1-068767 pH 6
L115	R, W		A, T, K, N, Q	A, T, K, F, N, Q	A, T, M, K, F, N	A, T, K, F, N, Q
V117	M, K, N, D	M, K, R, W, E	T, M, K, R, W, E	T, L, I, M, K, R, W, E	T, I, M, K, W	T, L, I, M, K, R, W, E
I119	F, P	P, N	P, M, E	P, M, E	M, H	P, M, H, F, N, E
H121						V, E, Q
H122						P, Y, N, D
S124	P, V, L, I, K, F, D, E	L, I, M, H, W, Q	L, I, M, H, W, Q		L, I, M, Q	L, I, M
E125	A, S, T, L, M, K, H, Y, D	A, T, V, I, M, K, H, F, Y, W, N, D	T, V, I, M, H, F, Y, W	G, T, K, H, Y, W, N, D	V, I, H, N	T, V, I, F, Y, W, N
R127			S, V, M, H	P, S, V, M, K, H, N	P, V, M, N	P, S, V, M, H, N

Fig. 11B and Fig. 11C show a representation of an epitope encompassing all the residues for blocking hVISTA antibody as listed in Table 14 (Fig. 11B) compared to the epitope of a non-blocking hVISTA antibody (mAb1; Fig. 11C). Amino acid residues 66(H) and 162(A) are indicated to denote the orientation of the molecule. H66 and A162 residues are in grey, and epitope residues are in black. Notably, all blocking anti-VISTA mAbs occupy the same epitope region, in agreement with the octet binning data (showing that they competed with each other), with subtle residue differences among the queried antibodies. In contrast, the non-blocking hVISTA antibody (mAb1) occupies a distinct epitope region on the hVISTA molecule, and this is further supported by the octet binning data shows that none of the blocking mAbs competed with mAb1.

Example 16: Biophysical properties of '761 and '767

The physical and chemical properties of the P1-068761 and P1-068767 were compared to that of the parent P1-061029 (all with an IgG1.3 constant region) by the following analytical and biophysical techniques.

Analytical SEC data were acquired using an Agilent 1260 HPLC instrument using a Shodex™ KW403-4F column (4.6mmID X 300mmL), in buffer containing 100mM Sodium Phosphate, 150mM Sodium Chloride, pH7.3(0.2 um filtered) running at a flow rate of 0.30 mL/min. Data were collected by an Agilent 1260 Infinity Diode Array Detector set to collect at 280nm and analyzed by Agilent Chemstation software (Agilent, Santa Clara, CA).

Imaged capillary isoelectric focusing (icIEF) data were acquired on a ProteinSimple iCE3 Instrument with Alcott 720NV Autosampler. Antibody samples were mixed with a separation mixture to yield final concentrations of 0.2 mg/ml antibody, 0.35% methyl cellulose, 2.0 M Urea, 1% v/v pharmalyte pI 5-8, and 3% v/v pharmalyte pI 8-10.5. These samples were analyzed using a pre-focus time of 1min at 1500V, and focusing time of 10min at 3000V, in a ProteinSimple cIEF cartridge FC-coated (product # 101701). Data were analyzed using iCE CFR Software V4.3.1.5352.

Antibody hydrodynamic size was determined by dynamic light scattering (DLS) and thermal stability was characterized by fluorescence spectroscopy and static light scattering (SLS) using an UNcle molecular characterization instrument (Unchained Labs).

Antibodies P1-061029, P1-068761, and P1-068767 were prepared at a concentration of 2mg/ml in 1X PBS buffer, and then diluted 1:1 with either 40 mM Tris in 1X PBS, or 40 mM citrate in 1X PBS, at different pH to yield final samples of 1mg/ml antibody in either 20mM Tris / 1X PBS, or 20mM citrate / 1X PBS at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 or 9.0. These samples were loaded into a UNi cuvette cartridge and analyzed within 1 hour of dilution into the different pH formulations. DLS data were collected at 25oC, using 4 acquisitions of 5 s each. Intensity autocorrelation functions were fitted using UNcle analysis software version V2.0. Thermal denaturation data was obtained by scanning samples from 25oC to 90oC at a scan rate of 0.5o/min, with excitation at 266 nm and 473 nm. Fluorescence data was acquired over a range of 250 nm – 720 nm. Fluorescence and SLS data were analyzed using UNcle analysis software version V2.0.

The apparent viscosity of antibodies P1-061029, P1-068761, and P1-068767 was measured on an UNcle molecular characterization instrument (Unchained Labs), using a bead-based DLS method which measures the diffusion rate of polystyrene beads in the presence of formulated antibody solutions, as per Unchained Labs recommended protocol. Briefly, a 10% solution of 100 nm polystyrene beads (Thermo Scientific cat#3100A) was prepared in formulation buffer containing 0.5% tween 80. 3ul of this polystyrene bead mixture was added to 30ul of formulated antibody (various Ab concentrations in 20 mM histidine, 260 mM sucrose pH 6.0,) and the resulting protein/bead mixture was loaded into 3 separate lanes of a UNi cuvette cartridge (9 ul each lane) for triplicate analysis. Data were analyzed using UNcle analysis software version V2.0, using a reference viscosity of 1.3 cP.

The physical stability of antibodies P1-061029, P1-068761, and P1-068767 was studied under accelerated stress conditions by preparing 50 mg/ml antibody samples in 20 mM histidine, 260 mM sucrose pH 6.0 and exposing to 40oC thermal stress for 4 weeks. Aliquots were removed immediately prior to 40oC incubation (time zero = t0), as well as after 1 week (1 w) and 4 weeks (4 w) of thermal stress, and samples were diluted to 2 mg/ml using formulation buffer and analyzed by aSEC. The aSEC data was acquired using an Agilent 1260 HPLC instrument using a Shodex KW403-4F column (4.6mmID X 300mmL), in buffer containing 100mM Sodium Phosphate, 150mM Sodium Chloride, pH7.3(0.2 um filtered) running at a flow rate of 0.30 mL/min. Data were collected by an

Agilent 1260 Infinity Diode Array Detector set to collect at 280nm and analyzed by Agilent Chemstation software (Agilent, Santa Clara, CA).

The resuts are as follows. Analytical size exclusion chromatography (aSEC) data showed that all three antibodies could be purified to high purity, with each antibody sample consisting of more than 99.3% monomer (main peak), less than 0.7% high molecular weight (HMW) species, and undetectable levels of low molecular weight (LMW) species, Table 16.

Table 16: Analytical SEC data for anti-VISTA antibodies, showing the percentage of high molecular weight species (%HMW), percentage of monomeric/main species (%Main) and percentage of low molecular weight species (%LMW).

Sample Name	%HMW	%Main	%LMW
P1-061029	0.4	99.6	0.0
P1-068761	0.6	99.4	0.0
P1-068767	0.5	99.5	0.0

The charge variant profile as determined by imaged capillary isoelectric focusing (icIEF) for antibody P1-061029 showed the presence of a main species (69.4%) with isoelectric point (pI) of 8.56, and 30.6% acidic species. (Fig. 12A-C.) P1-068761 demonstrated a main species (66.4%) with pI of 6.69 and 33.6% acidic species. P1-068767 demonstrated a main species (61.4%) with pI of 6.63 and 38.6% acidic species. Therefore, the distribution of acidic, basic and main species is similar for the three antibodies but the engineered antibodies P1-068761 and P1-068767 have significantly lower isoelectric point than the parental P1-061029 antibody.

The oligomeric state of P1-061029, P1-068761, and P1-068767 wasere determined over the pH range of 3-9 using dynamic light scattering (DLS) in buffers of different pH. All hydrodynamic radius (Rh) values for each antibody were in the range of 4.8 – 5.7 nM, which is typical for monomeric antibody samples, Table 17. This suggests that these antibodies do not form detectable levels of high molecular weight aggregated species at 1 mg/ml within the first hour after dilution into formulations having pH between 3 – 9.

Table 17: The hydrodynamic radius as determined by DLS for 1 mg/ml samples of anti-VISTA antibodies across the pH range of pH3 – pH9.

pH	Buffer	Rh (nm) P1-061029	Rh (nm) P1-068761	Rh (nm) P1-068767
9	20mM Tris / 1 X PBS	5.2	4.8	5.2
8	20mM Tris / 1 X PBS	5.2	5.2	5.2
7	20mM Tris / 1 X PBS	4.8	5.2	5.2
7	20mM citrate / 1 X PBS	4.8	5.2	5.7
6	20mM citrate / 1 X PBS	5.2	5.2	4.8
5	20mM citrate / 1 X PBS	5.2	4.8	5.2
4	20mM citrate / 1 X PBS	4.8	4.8	5.2
3	20mM citrate / 1 X PBS	5.2	5.2	5.2

The thermal stability of P1-061029, P1-068761, and P1-068767 was measured over the pH range of 3-9 by monitoring fluorescence and static light scattering as a function of temperature in buffers of different pH. The first thermal denaturation transition (Tm1) which typically represents denaturation of the CH2 domain of IgG1 antibodies was determined by fluorescence and is shown in Table 18, and the onset of aggregation (Tagg) which typically represents denaturation of the FAB domain of IgG1 antibodies was measured by static light scattering and is shown in Table 19. At neutral pH (pH 7.0) in Tris/PBS formulation the Tm1 values for the three antibodies were P1-061029 (67.4 °C), P1-068761 (67.0 °C), and P1-068767 (65.3 °C), with Tagg values of P1-061029 (67.8 °C), P1-068761 (67.5 °C), and P1-068767 (65.8 °C). The Tm1 for each antibody in Citrate/PBS formulation at the same neutral pH 7.0 or slightly more acidic pH of 6.0 were all within 0.7° of the Tris/PBS pH 7.0 values. However, Tm1 values were slightly lower (between 0.3° – 1.1° lower) at more basic pH of 8-9, and significantly lower at more acidic pH of 3-5, for each antibody. Compared to neutral pH, the Tagg for P1-061029 was within 0.1° of pH 7.0 value at more basic pH 8.0 – 9.0, was 1.0° lower at pH 5.0, and much lower (6.1° – 19.6° lower) at the most acidic pH conditions pH 3.0 – 4.0. The Tagg for P1-068761 and P1-068767 were also significantly lower at pH 3.0 – 4.0. However at pH 5.0 the Tagg for P1-068761 was only 0.2° lower than Tagg at pH 6.0, whereas Tagg for P1-068767 was 2.2° lower at pH 5.0 than at pH 6.0, demonstrating some differences in Tagg for each antibody, Table 19.

Table 18: Thermal stability (Tm1 values) for P1-061029, P1-068761, P1-068767 across the pH range of pH3 – pH 9 as determined by fluorescence spectroscopy

pH	Buffer	Tm1 (°C) P1-061029	Tm1 (°C) P1-068761	Tm1 (°C) P1-068767
9	20mM Tris / 1 X PBS	66.6	65.9	65.0
8	20mM Tris / 1 X PBS	67.0	66.5	64.8
7	20mM Tris / 1 X PBS	67.4	67.0	65.3
7	20mM citrate / 1 X PBS	67.2	66.9	64.8
6	20mM citrate / 1 X PBS	67.6	67.5	65.0
5	20mM citrate / 1 X PBS	64.4	64.7	62.1
4	20mM citrate / 1 X PBS	51.8	52.0	50.8
3	20mM citrate / 1 X PBS	30.7	28.1	28.7

Table 19: Thermal stability (Tagg values) for P1-061029, P1-068761, P1-068767 across the pH range of pH3 – pH 9 as determined by static light scattering

pH	Buffer	Tagg (°C) P1-061029	Tagg (°C) P1-068761	Tagg (°C) P1-068767
9	20mM Tris / 1 X PBS	67.7	67.1	66.0
8	20mM Tris / 1 X PBS	67.8	67.5	65.8
7	20mM Tris / 1 X PBS	67.8	68.2	65.9
7	20mM citrate / 1 X PBS	67.8	68.1	65.7
6	20mM citrate / 1 X PBS	68.1	68.9	65.6
5	20mM citrate / 1 X PBS	66.8	68.7	63.7
4	20mM citrate / 1 X PBS	61.7	63.6	56.9
3	20mM citrate / 1 X PBS	48.2	48.8	41.0

The apparent viscosity of P1-061029, P1-068761, and P1-068767 was measured using a bead-based DLS method which measures the diffusion rate of polystyrene beads in the presence of formulated antibody solutions. Comparison of all three antibodies at 44 mg/ml shows similar viscosity for both engineered antibodies as the parent antibody under these conditions, Table 20. In a second study additional protein material for P1-068761 and P1-068767 was concentrated to higher concentrations to analyze viscosity at 136 mg/ml, 100 mg/ml, and 50 mg/ml. These data show increased apparent viscosity at higher antibody concentrations, with maximum apparent viscosity of 5.7 ± 0.7 for P1-068761 and 5.3 ± 0.6 for P1-068767 at 136 mg/ml.

Table 20: Apparent viscosity (in cP) for antibodies in 20mM histidine, 260mM sucrose pH 6.0 at 25°C as determined by bead based DLS method. Values represent the average and standard deviation of data from three UNi lanes

Antibody	Apparent Viscosity (cP) @ 136 mg/ml	Apparent Viscosity (cP) @ 100 mg/ml	Apparent Viscosity (cP) @ 50 mg/ml	Apparent Viscosity (cP) @ 44 mg/ml
P1-061029				1.6 ± 0.1
P1-068761	5.7 ± 0.7	3.1 ± 0.0	1.4 ± 0.2	1.5 ± 0.4
P1-068767	5.3 ± 0.6	3.0 ± 0.3	1.7 ± 0.2	1.6 ± 0.2

The physical stability of 50 mg/ml samples of P1-061029, P1-068761, and P1-068767 in 20 mM histidine, 260 mM sucrose pH 6.0 was studied under accelerated stress conditions of 40°C for 4 weeks. The oligomeric state of the antibodies was monitored by aSEC for samples immediately prior to 40°C incubation (time zero = t0), as well as after 1 week (1w) and 4 weeks (4w) of 40°C stress. These data show that all three antibodies remain more than 96% monomeric after 4 weeks at 40°C, with low levels of HMW species (< 1.6 % HMW) and low levels of LMW species (< 2.0 % LMW), Table 21.

Table 21: aSEC data for anti-VISTA antibody accelerated stability samples, showing the percentage of high molecular weight species (%HMW), percentage of monomeric/main species (%Main) and percentage of low molecular weight species (%LMW), for t0, 1w, and 4w samples

Antibody	Sample	% HMW	% Main	% LMW
P1-061029	t0	0.4	99.7	0.0
	1w	0.5	99.4	0.2
	4w	0.8	97.2	2.0
P1-068761	t0	0.6	99.4	0.0
	1w	0.9	98.8	0.3
	4w	1.6	96.4	2.0
P1-068767	t0	0.5	99.5	0.0
	1w	0.8	98.9	0.3
	4w	1.6	96.4	2.0

Example 17: Engineering of anti-VISTA antibodies to reduce binding at neutral pH

This Example describes the selection of an anti-human VISTA antibody that binds to human VISTA at pH 7.0, and its engineering to obtain antibodies that bind to human VISTA at acidic pH.

An anti-human VISTA antibody was isolated from hybridomas obtained from huMab mice immunized with human VISTA. This Ab, which is referred to 41F11, is of the IgG1 isotype, and its heavy and light chain variable region amino acid and nucleotide sequences are provided in Figs 14A, 14B, 15A,15B, 16A, 16B, 17A and 17B and In the Sequence Table below. Although three potential light chains (VK1, VK2 and VK3) were identified, VK1 (Figs 15A and B) appears to be the functional light chain, and was used in recombinant expression of this antibody, unless specified otherwise. For this particular antibody, and any variants thereof, HCDR1 is defined according to the AbM nomenclature, and all other five heavy and light chain CDRs are defined according to Kabat nomenclature.

A recombinant antibody comprising VH and VL (VK1) of 41F11 and the heavy chain constant region IgG1.3 (and light chain of 41F11) was generated, and is referred to as VISTA.4.IgG1.3. It was observed that amino acid 64 of the VK1 light chain is a germ line mutation (germline amino acid residue at that position is a G), and therefore, a VK

light chain was generated in which this amino acid was changed to a G to mirror the germline. This antibody is referred to as VISTA.4.A64G.IgG1.3.

The binding kinetics of VISTA.4 to human and cyno VISTA were determined using a Biacore® T100 instrument (GE Healthcare). Protein A (ThermoFisher Scientific catalog number #21181) was diluted to 20 ug/ml in 10mM sodium acetate pH 4.5 and immobilized onto flow cells of a CM5 biosensor following the manufacturer's amine coupling protocol (GE Healthcare), targeting 2,000 RU immobilization density of Protein A per flow cell. The assay was run at 37°C using PBST (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20) running buffer at pH 7.4 and 6.0. VISTA.4 IgG1.3 was diluted to 25 nM in PBST pH 7.4, and was captured across active biosensor flow cells at 10 ul/min for 30 seconds. Concentration series of 1600 – 0.78 nM (pH 7.4) and 100 – 0.78 nM (pH 6.0) monovalent hVISTA-ECD (SEQ ID NO: 325) and cyno VISTA-ECD (SEQ ID NO: 326) were prepared in running buffer, and were injected over the captured antibodies at 40 µl/min to measure association and dissociation. Two 15 second injections of 10 mM glycine pH 1.5 were used to regenerate the Protein A capture surface between assay cycles. Rate constants k_a (kon) and k_d (koff) were derived from reference flow cell and 0 nM blank-subtracted sensorgrams, and were fit to a 1:1 binding model in Biacore® T200 Evaluation Software v.2.0. The affinity constant, K_D was calculated as the ratio of rate constants koff/kon, and the results are provided in Table 22.The VISTA.4 antibody exhibited comparable (within 2-fold) human and cyno VISTA kinetic binding parameters at both pHs tested, confirming cross-reactivity with cyno VISTA. For VISTA.4, the measured k_d is ~100-fold weaker at acidic pH than neutral pH, while the K_D is also ~10-fold weaker.

Table 22: Binding kinetics of VISTA.4 to human and cyno VISTA

Sample	pH 7.4			pH 6.0		
	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
huVISTA-His	3.1E+05	8.9E-04	2.9E-09	3.4E+06	7.8E-02	2.3E-08
cyVISTA-His	3.1E+05	7.7E-04	2.5E-09	1.9E+06	6.3E-02	3.4E-08

VISTA.4 binds with higher affinity to human VISTA at pH 7.4 than at pH 6.0. More specifically, VISTA.4 had far faster off-rates at acidic pH than at pH 7.4, while the

off-rate of the non-blocking antibody VISTA.5 (mAb 1) was unaffected by pH (Figure 18B). VISTA.4 also exhibited a lower Y_{max} value at acidic pH than at pH 7.4 in cell-binding assays (Figure 18A). We hypothesized that antibodies against VISTA's receptor-ligand interface might be capable of distinguishing between inactive (less or no histidine protonation) and active (more histidine protonation) states. Thus, variants of VISTA.4 were generated to identify those with improved affinity at pH 6, while maintaining the binding at pH 7. This was done by screening via yeast surface display a mutational scan library of VISTA.4, in which each variant in the library has a single mutation in the loops, and each position in the loops is substituted with all possible amino acid residues. The library was screened with hVISTA-His. Analysis of the mutational scan data led to identification of mutations with improved binding at pH 6.0. These led to designed VISTA.4 variants in which each variant contains a combination of a few improved mutations within and across CDRs. Variants selected are set forth in Table 23, and the amino acid variations in these variants are set forth in Table 24. The binding kinetics to human VISTA protein of the variants at neutral and acidic pHs were analyzed using the SPR method described in Example 9 above, and results are reported below in Table 23.

Table 23: Kinetics of VISTA.4 variants with enhanced binding at pH 6.0

P1-ID	pH 6.0				pH 7.4				KD ratio (pH 7.4/ pH 6.0)	Mutations compared to VISTA.4 Ab (41F11)
	ka (1/Ms)	kd (1/s)	KD (M)	Comment	ka (1/Ms)	kd (1/s)	KD (M)	Comment		
P1-064532-1				Weak RU response				Weak RU response		41F11_VH_F27E/D31E/Y50W/S55E/L96E/Y100E
P1-065320-1	6.94E+05	3.87E-02	5.58E-08		1.38E+04	3.52E-02	2.54E-06	Weak RU response	45.52	41F11_VH_L96EF100aG_VK_A64G
P1-065329-1	6.63E+05	7.91E-03	1.19E-08		1.80E+03	8.58E-04	4.78E-07	Weak RU response	40.17	41F11_VH_D31E/S35Y/Y50W/S55E/A60H/Y100E/F100aG VK_A64G
P1-064508-1				Weak RU response				Weak RU response		41F11_Vk_A25EQ90DR91AR96YA64G
P1-064510-1	1.24E+06	3.12E-03	2.51E-09		6.22E+03	5.27E-04	8.48E-08		33.78	41F11_Vk_A25E/V29D/A64G/R91T
P1-064548-1				Weak RU response				Weak RU response		41F11_VH_T28P/S35Y/Y50W/S55E/A60H/Y100E/F100aG
P1-064490-1	2.09E+05	7.26E-03	3.48E-08		7.18E+03	2.66E-03	3.70E-07	Weak RU response	10.63	41F11_VH_F27EY50EL96E
P1-061528-2	6.16E+05	2.37E-03	3.86E-09		6.26E+03	1.90E-04	3.04E-08		7.88	41F11 NGS mut VK_A64G/R91A
P1-065333-1	3.28E+06	1.42E-03	4.34E-10		2.78E+05	8.83E-04	3.17E-09		7.30	41F11_VH_T28P/Y50W/S55E/L96E/Y100E VK_A64G
P1-065313-1				Weak RU response				Weak RU response		41F11_VH_S35Y Vk_A64G
P1-064542-1	3.27E+06	1.15E-03	3.51E-10		2.71E+05	6.17E-04	2.28E-09		6.50	41F11_VH_T28P/Y50W/S55E/L96E/Y100E
P1-064488-1	9.32E+05	3.27E-02	3.50E-08		1.35E+05	2.71E-02	2.02E-07	Weak RU response	5.77	41F11_VH_L96EF100aG
P1-065326-1	3.52E+06	6.03E-04	1.72E-10		2.89E+05	2.85E-04	9.86E-10		5.73	41F11_VH_D31E/Y50W/S55E/L96E/Y100E VK_A64G

P1-ID	pH 6.0				pH 7.4				KD ratio (pH 7.4/ pH 6.0)	Mutations compared to VISTA.4 Ab (41F11)
	ka (1/Ms)	kd (1/s)	KD (M)	Comment	ka (1/Ms)	kd (1/s)	KD (M)	Comment		
P1-065318-1	1.96E+06	1.54E-02	7.84E-09		1.09E+05	4.20E-03	3.85E-08		4.91	41F11_VH_L96E VK A64G
P1-064528-1	3.49E+06	5.84E-04	1.67E-10		2.96E+05	2.27E-04	7.68E-10		4.60	41F11_VH_D31E/Y50 W/S55E/L96E/Y100E
P1-064514-1				Weak RU response				Weak RU response		41F11_Vk_V29D/A64 G/Q90D/R91T
P1-065323-1				Weak RU response				Weak RU response		41F11_VH_F27E/D31 E/Y50W/S55E/Y100E VK_A64G
P1-064484-1	2.13E+06	1.53E-02	7.18E-09		1.35E+05	3.53E-03	2.61E-08		3.64	41F11_VH_L96E
P1-065327-1				Weak RU response				Weak RU response		41F11_VH_D31E/Y50 W/S55E/Y100E/F100 aG VK_A64G
P1-064490-2	2.60E+05	8.18E-03	3.14E-08		2.46E+04	2.30E-03	9.32E-08	Weak RU response	2.97	41F11_VH_F27EY50E L96E
P1-064516-1	2.28E+06	1.78E-02	7.81E-09		1.17E+05	2.23E-03	1.91E-08		2.45	41F11_Vk_V29D/A64 G/R91T/R96Y
P1-065335-1				Weak RU response				Weak RU response		41F11_VH_F27E/T28 P/Y50W/S55E/L96E/ Y100E VK_A64G
P1-065317-1				Weak RU response				Weak RU response		41F11_VH_Y50EA60H VK A64G
P1-065328-1				Weak RU response				Weak RU response		41F11_VH_F27E/D31 E/Y50W/S55E/L96E/ Y100E VK_A64G
P1-064534-1	7.17E+05	6.49E-03	9.05E-09		4.17E+04	6.75E-04	1.62E-08	Weak RU response	1.79	41F11_VH_D31E/S35 Y/Y50W/S55E/A60H/ Y100E/F100aG
P1-065325-1	7.99E+06	1.43E-03	1.79E-10		6.18E+05	1.58E-04	2.55E-10		1.42	41F11_VH_D31E/Y50 W/S55E/A60H/Y100E VK A64G
P1-064474-1				Weak RU response				Weak RU response		41F11 VH S35Y
P1-065336-1				Weak RU response				Weak RU response		41F11_VH_T28P/S35 Y/Y50W/S55E/A60H/ Y100E/F100aG VK_A64G

P1-ID	pH 6.0				pH 7.4				KD ratio (pH 7.4/ pH 6.0)	Mutations compared to VISTA.4 Ab (41F11)
	ka (1/Ms)	kd (1/s)	KD (M)	Comment	ka (1/Ms)	kd (1/s)	KD (M)	Comment		
P1-065334-1				Weak RU response				Weak RU response		41F11_VH_T28P/Y50W/S55E/Y100E/F100aG_VK_A64G
P1-065330-1				Weak RU response				Weak RU response		41F11_VH_F27E/T28P/Y50W/S55E/Y100E_VK_A64G
P1-065332-1	5.41E+06	2.21E-03	4.09E-10		5.17E+05	2.36E-04	4.56E-10		1.11	41F11_VH_T28P/Y50W/S55E/A60H/Y100E_VK_A64G
P1-064540-1	7.16E+06	1.92E-03	2.68E-10		5.53E+05	1.58E-04	2.85E-10		1.06	41F11_VH_T28P/Y50W/S55E/A60H/Y100E
P1-063001-5	2.61E+06	1.68E-03	6.44E-10		1.95E+05	1.30E-04	6.65E-10		1.03	41F11_LC_Mutant: V29D/A64G/R91T
P1-063125-5	3.47E+06	1.24E-03	3.56E-10		4.88E+05	1.78E-04	3.65E-10		1.03	41F11_HC_Mutant: T28P/Y50W/S55E/Y100E
P1-063001-6	2.28E+06	1.71E-03	7.51E-10		1.63E+05	1.20E-04	7.37E-10		0.98	41F11_LC_Mutant: V29D/A64G/R91T
P1-064536-1				Weak RU response				Weak RU response		41F11_VH_F27E/T28P/Y50W/S55E/Y100E
P1-064522-1				Weak RU response				Weak RU response		41F11_VH_F27E/D31E/Y50W/S55E/Y100E
P1-060879-18	2.40E+06	3.42E-02	1.43E-08		7.05E+04	8.73E-04	1.24E-08		0.87	41F11
P1-064544-1				Weak RU response				Weak RU response		41F11_VH_T28P/Y50W/S55E/Y100E/F100aG
P1-065319-1	1.32E+06	3.85E-02	2.91E-08		2.09E+05	5.14E-03	2.46E-08		0.85	41F11_VH_F100aG_VK_A64G
P1-064526-1	3.84E+06	9.40E-04	2.45E-10		5.55E+05	1.09E-04	1.96E-10		0.80	41F11_VH_D31E/Y50W/S55E/A60H/Y100E
P1-063125-4	2.77E+06	1.20E-03	4.35E-10		4.41E+05	1.41E-04	3.20E-10		0.74	41F11_HC_Mutant: T28P/Y50W/S55E/Y100E
P1-063153-5	8.19E+06	1.14E-03	1.39E-10		6.85E+05	6.90E-05	1.01E-10		0.73	41F11 HC mutant: D31E/Y50W/S55E/Y100E

P1-ID	pH 6.0				pH 7.4				KD ratio (pH 7.4/ pH 6.0)	Mutations compared to VISTA.4 Ab (41F11)
	ka (1/Ms)	kd (1/s)	KD (M)	Comment	ka (1/Ms)	kd (1/s)	KD (M)	Comment		
P1-060879-19	2.18E+06	2.85E-02	1.30E-08		1.02E+05	7.84E-04	7.69E-09		0.59	41F11
P1-064502-1	4.06E+06	3.32E-01	8.17E-08	Weak RU response	3.01E+05	1.23E-02	4.08E-08		0.50	41F11_Vk_R96YA64G
P1-064492-1	1.00E+06	1.40E-01	1.40E-07	Weak RU response	1.97E+05	1.36E-02	6.89E-08		0.49	41F11_VH_S35YA60H F100aG
P1-064500-1	1.67E+06	1.57E-01	9.41E-08	Weak RU response	3.25E+05	1.45E-02	4.47E-08		0.48	41F11_Vk_Q90DA64G
P1-064512-1				Weak RU response				Weak RU response		41F11_Vk_V29D/A34K/A64G/R91T
P1-064486-1	3.07E+06	1.05E-01	3.43E-08		2.23E+05	3.41E-03	1.53E-08		0.45	41F11_VH_F100aG
P1-063153-4	4.07E+06	9.09E-04	2.23E-10		6.00E+05	4.05E-05	6.75E-11		0.30	41F11 HC mutant: D31E/Y50W/S55E/Y100E
P1-065312-1	2.01E+06	4.49E-02	2.23E-08		1.98E+05	1.31E-03	6.61E-09		0.30	41F11_VH_F27E VK_A64G
P1-064530-1				Weak RU response				Weak RU response		41F11_VH_D31E/Y50W/S55E/Y100E/F100aG
P1-064494-1	1.36E+06	4.85E-02	3.56E-08		2.00E+05	2.07E-03	1.03E-08		0.29	41F11_Vk_A25EA64G
P1-065316-1	3.86E+06	5.61E-02	1.45E-08		2.42E+05	9.95E-04	4.12E-09		0.28	41F11_VH_A60H VK A64G
P1-061520-10	2.31E+06	4.03E-02	1.74E-08		2.31E+05	1.03E-03	4.48E-09		0.26	41F11 Vk:A64G
P1-061520-11	2.37E+06	3.95E-02	1.66E-08		2.50E+05	1.05E-03	4.19E-09		0.25	41F11 Vk:A64G
P1-060879-17	3.03E+06	4.08E-02	1.35E-08		3.17E+05	9.14E-04	2.88E-09		0.21	41F11
P1-064472-1	2.29E+06	4.11E-02	1.80E-08		2.74E+05	9.87E-04	3.60E-09		0.20	41F11_VH_F27E
P1-064480-1	2.32E+06	3.29E-02	1.42E-08		3.20E+05	7.55E-04	2.36E-09		0.17	41F11_VH_A60H
P1-064546-1				Weak RU response				Weak RU response		41F11_VH_F27E/T28P/Y50W/S55E/L96E/Y100E
P1-064478-1	1.26E+05	1.21E-02	9.65E-08		5.54E+04	3.81E-04	6.89E-09		0.07	41F11_VH_Y50E

P1-ID	pH 6.0				pH 7.4				KD ratio (pH 7.4/ pH 6.0)	Mutations compared to VISTA.4 Ab (41F11)
	ka (1/Ms)	kd (1/s)	KD (M)	Comment	ka (1/Ms)	kd (1/s)	KD (M)	Comment		
P1-064482-1	6.39E+04	1.36E-02	2.13E-07		7.16E+04	3.40E-04	4.75E-09	Weak RU response	0.02	41F11 VH Y50EA60H
P1-064476-1				No binding				No binding	ND	41F11_VH_F27ES35Y
P1-064496-1				No binding				Weak RU response	ND	41F11 Vk A34KA64G
P1-064498-1				No binding				No binding	ND	41F11_Vk_A25EA34K A64G
P1-064504-1				Weak RU response				Weak RU response	ND	41F11_Vk_Q90DR91A R96YA64G
P1-064506-1				No binding				No binding	ND	41F11_Vk_A25EA34K R96YA64G
P1-064518-1				No binding				No binding	ND	41F11_Vk_A25E/V29 D/A34K/A64G/R91T/ R96Y
P1-064520-1				No binding				No binding	ND	41F11_Vk_A25E/V29 D/A64G/Q90D/R91T/ R96Y
P1-064524-1				No binding				No binding	ND	41F11_VH_D31E/S35 Y/Y50W/S55E/Y100E
P1-064538-1				No binding				No binding	ND	41F11_VH_T28P/S35 E/Y50W/S55E/Y100E
P1-065314-1				No binding				No binding	ND	41F11_VH_F27ES35Y VK A64G
P1-065315-1				No binding				No binding	ND	41F11_VH_Y50E VK A64G
P1-065321-1				No binding				No binding	ND	41F11_VH_F27EY50E L96E VK A64G
P1-065322-1				No binding				Weak RU response	ND	41F11_VH_S35YA60H F100aG VK A64G
P1-065324-1				No binding				No binding	ND	41F11_VH_D31E/S35 Y/Y50W/S55E/Y100E VK A64G
P1-065331-1				No binding				No binding	ND	41F11_VH_T28P/S35 E/Y50W/S55E/Y100E VK A64G
P1-058268-102				No binding				No binding	ND	IgG1.3 isotype

Table 24: Amino acid substitutions in VISTA.4 variants of Table 23 compared to VISTA.4 Ab

Name	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	64	LCDR3
P1-0645 32-1	.E...E....	W.....E.....	.E...E...	.....	.....	A	.....
P1-0653 20-1	.....	.....	.E....G..	.....	.....	G	.....
P1-0653 29-1	.....E...Y	W.....E....H.....	.....EG..	.....	.....	G	.....
P1-0645 08-1	.....	.....	.....	.E.....	.....	G	.DA....Y.
P1-0645 10-1	.....	.....	.....	.E...D.....	.....	G	..T.....
P1-0645 48-1	..P.....Y	W.....E....H.....	.....EG..	.....	.....	A	.....
P1-0644 90-1	.E.....	E.....	.E.....	.....	.....	A	.....
P1-0615 28-2	.....	.....	.....	.....	.....	G	..A.....
P1-0653 33-1	..P.....	W.....E.....	.E...E...	.....	.....	G	.....
P1-0653 13-1	.....Y	.....	.....	.....	.....	G	.....
P1-0645 42-1	..P.....	W.....E.....	.E...E...	.....	.....	A	.....
P1-0644 88-1	.....	.....	.E....G..	.....	.....	A	.....
P1-0653 26-1	.....E....	W.....E.....	.E...E...	.....	.....	G	.....
P1-0653 18-1	.....	.....	.E.....	.....	.....	G	.....
P1-0645 28-1	.....E....	W.....E.....	.E...E...	.....	.....	A	.....
P1-0645 14-1	.....	.....	.....	.....D.....	.....	G	.DT.....
P1-0653 23-1	.E...E....	W.....E.....	.....E...	.....	.....	G	.....
P1-0644 84-1	.....	.....	.E.....	.....	.....	A	.....
P1-0653 27-1	.....E....	W.....E.....	.....EG..	.....	.....	G	.....
P1-0644 90-2	.E.....	E.....	.E.....	.....	.....	A	.....

Name	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	64	LCDR3
P1-0645 16-1	....	....	....	.....D.....	....	G	..T....Y.
P1-0653 35-1	.EP.......	W.....E..........	.E...E...	....	....	G	....
P1-0653 17-1	....	E..........H.....	....	....	....	G	....
P1-0653 28-1	.E...E....	W.....E..........	.E...E...	....	....	G	....
P1-0645 34-1	.....E...Y	W.....E....H.....	.....EG..	....	....	A	....
P1-0653 25-1	.....E....	W.....E....H.....	.....E...	....	....	G	....
P1-0644 74-1	.........Y	....	....	....	....	A	....
P1-0653 36-1	..P......Y	W.....E....H.....	.....EG..	....	....	G	....
P1-0653 34-1	..P.......	W.....E..........	.....EG..	....	....	G	....
P1-0653 30-1	.EP.......	W.....E..........	.....E...	....	....	G	....
P1-0653 32-1	..P.......	W.....E....H.....	.....E...	....	....	G	....
P1-0645 40-1	..P.......	W.....E....H.....	.....E...	....	....	A	....
P1-0630 01-5	....	....	....	.....D.....	....	G	..T......
P1-0631 25-5	..P.......	W.....E..........	.....E...	....	....	A	....
P1-0630 01-6	....	....	....	.....D.....	....	G	..T......
P1-0645 36-1	.EP.......	W.....E..........	.....E...	....	....	A	....
P1-0645 22-1	.E...E....	W.....E..........	.....E...	....	....	A	....
P1-0608 79-18	GFTFSDYYMS	YISNSGSPIYYADSVKG	DLPGWYFDL	RASQSVSSYLA	DASNRAT	A	QQRNNWPRT
P1-0645 44-1	..P.......	W.....E..........	.....EG..	....	....	A	....
P1-0653 19-1	....	....	......G..	....	....	G	....
P1-0645 26-1	.....E....	W.....E....H.....	.....E...	....	....	A	....

Name	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	64	LCDR3
P1-06312 5-4	..P.....	W.....E.....	.....E...	.....	.....	A	.....
P1-06315 3-5	.....E....	W.....E.....	.....E...	.....	.....	A	.....
P1-06087 9-19	GFTFSDYYMS	YISNSGSPIYYADSVKG	DLPGWYFDL	RASQSVSSYLA	DASNRAT	A	QQRNNWPRT
P1-06450 2-1	.....	.....	.....	.....	.....	G	.....Y.
P1-06449 2-1	.....Y	.....H.....	.....G..	.....	.....	A	.....
P1-06450 0-1	.....	.....	.....	.....	.....	G	.D.....
P1-06451 2-1	.....	.....	.....	.....D....K	.....	G	..T.....
P1-06448 6-1	.....	.....	.....G..	.....	.....	A	.....
P1-06315 3-4	.....E....	W.....E.....	.....E...	.....	.....	A	.....
P1-06531 2-1	.E.....	.....	.....	.....	.....	G	.....
P1-06453 0-1	.....E....	W.....E.....	.....EG..	.....	.....	A	.....
P1-06449 4-1	.....	.....	.....	.E.....	.....	G	.....
P1-06531 6-1	.....	.....H.....	.....	.....	.....	G	.....
P1-06152 0-10	.....	.....	.....	.....	.....	G	.....
P1-06152 0-11	.....	.....	.....	.....	.....	G	.....
P1-06087 9-17	GFTFSDYYMS	YISNSGSPIYYADSVKG	DLPGWYFDL	RASQSVSSYLA	DASNRAT	A	QQRNNWPRT
P1-06447 2-1	.E.....	.....	.....	.....	.....	A	.....
P1-06448 0-1	.....	.....H.....	.....	.....	.....	A	.....
P1-06454 6-1	.EP.....	W.....E.....	.E...E...	.....	.....	A	.....
P1-06447 8-1	.....	E.....	.....	.....	.....	A	.....

Name	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	64	LCDR3
P1-0644 82-1	....	E.........H.....	....	....	....	A	....
P1-0644 76-1	.E.......Y	.....	....	....	....	A	....
P1-0644 96-1	....	.....	....	.........K	....	G	....
P1-0644 98-1	....	.....	....	.E........K	....	G	....
P1-0645 04-1	....	.....	....	....	....	G	.DA....Y.
P1-0645 06-1	....	.....	....	.E........K	....	G	.......Y.
P1-0645 18-1	....	.....	....	.E...D....K	....	G	..T....Y.
P1-0645 20-1	....	.....	....	.E...D.....	....	G	.DT....Y.
P1-0645 24-1	.....E...Y	W.....E.....	.....E...	....	....	A	....
P1-0645 38-1	..P......Y	W.....E.....	.....E...	....	....	A	....
P1-0653 14-1	.E.......Y	.....	....	....	....	G	....
P1-0653 15-1	....	E.....	....	....	....	G	....
P1-0653 21-1	.E........	E.....	.E.......	....	....	G	....
P1-0653 22-1	.........Y	..........H.....	......G..	....	....	G	....
P1-0653 24-1	.....E...Y	W.....E.....	.....E...	....	....	G	....
P1-0653 31-1	..P..E....	W.....E.....	.....E...	....	....	G	....

To confirm measured kinetic parameters obtained for progeny exhibiting pH-agnostic binding to VISTA at acidic and neutral pH, an SPR kinetics assay was repeated for selected antibodies of Tables 23 and 24 against a human VISTA concentration series spanning 50-0.39 nM at pH 7.4 and 6.0. The k_d and K_D ratios were calculated as described in prior examples, and the results are shown in Table 25.

Table 25: Kinetic analysis of VISTA.4 variants

P1 ID	pH 6.0				pH 7.4				pH 6.0/7.4 kd	pH 6.0/7.4 KD	Details
	ka (1/Ms)	kd (1/s)	kd improvement vs parent	KD (M)	ka (1/Ms)	kd (1/s)	kd improvement vs parent	KD (M)			
P1-060879-17	5.7E+06	1.1E-01	1.0	1.9E-08	5.2E+05	8.8E-04	1.0	1.7E-09	121.5	11.3	41F11 parent
P1-063153-5	3.6E+06	4.6E-04	233.1	1.3E-10	7.8E+05	1.2E-04	7.4	1.5E-10	3.9	0.8	41F11 HC mutant: D31E/Y50W/S55E/Y100E
P1-065059-1	3.4E+06	4.8E-04	221.5	1.4E-10	7.2E+05	1.0E-04	8.5	1.4E-10	4.6	1.0	41F11 HC mutant: D31E/Y50W/S55E/Y100E
P1-065326-1	2.9E+06	3.6E-04	294.0	1.2E-10	4.4E+05	3.3E-04	2.7	7.6E-10	1.1	0.2	41F11_VH_D31E/Y50W/S55E/L96E/Y100E VK_A64G
P1-064391-1	5.1E+06	3.3E-02	3.3	6.4E-09	4.3E+05	1.6E-02	0.1	3.7E-08	2.1	0.2	41F11_HC_Mutant: D31E/Y50W/S55E/Y100E 41F11_LC_Mutant: V29D/A64G/R91T
P1-064392-1	4.8E+06	8.6E-03	12.4	1.8E-09	4.7E+05	7.0E-03	0.1	1.5E-08	1.2	0.1	41F11_HC_Mutant: D31E/Y50W/S55E/Y100E 41F11_LC_Mutant: V29D/A64G/Q89E/R91T
P1-063125-4	3.1E+06	7.9E-04	136.0	2.5E-10	7.7E+05	2.0E-04	4.3	2.7E-10	3.9	0.9	41F11_HC_Mutant: T28P/Y50W/S55E/Y100E
P1-065061-1	1.5E+06	2.2E-04	495.4	1.5E-10	3.7E+05	9.7E-05	9.1	2.6E-10	2.2	0.6	41F11_HC_Mutant: T28P/Y50W/S55E/Y100E
P1-065333-1	3.4E+06	1.3E-03	81.7	3.8E-10	4.8E+05	9.3E-04	1.0	1.9E-09	1.4	0.2	41F11_VH_T28P/Y50W/S55E/L96E/Y100E VK_A64G
P1-063001-5	1.3E+06	1.3E-03	81.7	1.0E-09	3.4E+05	1.3E-04	6.8	3.8E-10	10.2	2.7	41F11_LC_Mutant: V29D/A64G/R91T
P1-065064-1	1.3E+06	1.3E-03	80.5	1.1E-09	3.3E+05	1.2E-04	7.4	3.6E-10	11.2	3.0	41F11_LC_Mutant: V29D/A64G/R91T
P1-064510-1	1.2E+06	3.1E-03	35.0	2.6E-09	1.5E+05	6.4E-04	1.4	4.1E-09	4.8	0.6	41F11_Vk_A25E/V29D/A64G/R91T

P1 ID	pH 6.0				pH 7.4				pH 6.0/7.4 kd	pH 6.0/7.4 KD	Details
	ka (1/Ms)	kd (1/s)	kd improvement vs parent	KD (M)	ka (1/Ms)	kd (1/s)	kd improvement vs parent	KD (M)			
P1-061528-2	4.5E+05	2.1E-03	51.4	4.7E-09	1.2E+05	1.7E-04	5.1	1.4E-09	12.0	3.3	41F11 NGS mut VK A64G/R91A
P1-064490-1	6.0E+05	1.3E-02	8.2	2.2E-08	4.3E+04	3.2E-03	0.3	7.5E-08	4.0	0.3	41F11_VH_F27E/Y50E/L96E

This initial campaign led to pH-independent VISTA.4 variants with near equivalent k_{off} at pH 6 and pH 7. In order to obtain an antibody that binds to hVISTA with high affinity at pH 6.0 and low affinity at pH 7.0, a new library based on consensus sequence of pH-independent VISTA.4 variants was designed and constructed, with amino acid residues in the loops substituted for charge amino acid residues (see Example 9), and screened via yeast surface display (see Example 9). The library was analyzed as previously described (see Example 9) and selected variants were reformatted as IgG1.3 for further analysis (Tables 26 and 27). SPR analysis was conducted on these variants using the conditions previously described in Example 9.

Table 26: Kinetics of 41F11_VH_T28P/Y50W/S55E/L96E/Y100E; VK_A64G and variants with enhanced binding at pH 6.0

ID	pH 7.4			pH 6.0			
	Avg ka (1/Ms)	Avg kd (1/s)	Avg KD (M)	Avg ka (1/Ms)	Avg kd (1/s)	Avg KD (M)	Name
P1-070976	No binding at 100nM			1.9E+05	2.8E-03	1.5E-08	41F11_VH_T28P/Y50W /S55E/D95H/L96E/P97E /Y100E; Vk_A64G
P1-065333	4.3E+05	9.4E-04	2.2E-09	3.8E+06	1.8E-03	4.7E-10	41F11_VH_T28P/Y50W /S55E/L96E/Y100E; VK_A64G
P1-061520	5.5E+05	9.3E-04	1.7E-09	2.3E+06	8.6E-02	3.8E-08	41F11 VK_A64G FW reversion
VISTA.4*	6.0E+05	1.0E-03	1.8E-09	7.3E+05	3.5E-02	4.8E-08	
P1-064510*	1.1E+05	3.9E-04	3.4E-09	7.5E+05	3.6E-03	4.8E-09	41F11_Vk_A25E/V29D; VK_A64G/R91T
P1-065326	4.5E+05	3.2E-04	7.2E-10	2.4E+06	5.1E-04	2.1E-10	41F11_VH_D31E/Y50W /S55E/L96E/Y100E; VK_A64G
P1-064391	6.3E+05	1.9E-02	3.1E-08	2.7E+06	2.7E-02	1.0E-08	41F11_VH_D31E/Y50W /S55E/Y100E; Vk_V29D_A64G/R91T
P1-064392	7.7E+05	7.8E-03	1.1E-08	7.7E+06	1.2E-02	1.6E-09	

Table 27: Amino acid substitutions in VISTA.4 variants of Table 26

ID	LCDR1	LCDR3	HCDR1	HCDR2	HCDR3
P1-070976	.....	.....	..P.....	W.....E.....	HEE..E...
P1-065333	.....	.....	..P.....	W.....E.....	.E...E...
P1-061520	RASQSVSSYLA	QQRNNWPRT	GFTFSDYYMS	YISNSGSPIYYADSVKG	DLPGWYFDL
VISTA.4*	RASQSVSSYLA	QQRNNWPRT	GFTFSDYYMS	YISNSGSPIYYADSVKG	DLPGWYFDL
P1-064510*	.E...D.....	..T.....	.....	.....	.....
P1-065326	.....	.....	.....E....	W.....E.....	.E...E...
P1-064391	.....D.....	..T.....	.....E....	W.....E.....	.....E...
P1-064392	.....D.....	E.T.....	.....E....	W.....E.....	.....E...

From the above screenings, a single VISTA.4 variant (41F11_VH_T28P/Y50W/S55E/D95H/L96E/P97E/Y100E; VK_A64G (P1-070976)) was identified as binding with high affinity to hVISTA at pH 6.0 and not binding significantly to it at pH 7.4. Further binding kinetic analyses to hVISTA and cynoVISTA were conducted on this variant, and compared to several anti-hVISTA antibodies described in previous Examples. Binding of each antibody to avidin (high pI specificity control protein) at pH 6.0 and 7.4 was analysed and found to be undetectable.

Table 28:

Antibody	Details	Sample	pH 7.4			pH 6.0		
			ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
VISTA.4 hIgG1.3f	VISTA.4	huVISTA-His	3.1E+05	8.9E-04	2.9E-09	3.4E+06	7.8E-02	2.3E-08
		cyVISTA-His	3.1E+05	7.7E-04	2.5E-09	1.9E+06	6.3E-02	3.4E-08
P1-070976-7	41F11_VH_T28P/Y50W/S55E/D95H/L96E/P97E/Y100E; Vk_A64G	huVISTA-His	6.1E+04	1.4E-01	2.3E-06	2.3E+05	2.8E-03	1.3E-08
		cyVISTA-His	4.4E+03	2.6E-02	5.9E-06	1.6E+05	3.8E-03	2.4E-08
P1-072315-1	41F11_VH_T28P/Y50W/S55E/L96E/Y100E VK_A64G	huVISTA-His	3.2E+05	7.9E-04	2.5E-09	4.2E+06	1.5E-03	3.5E-10
		cyVISTA-His	3.5E+05	6.8E-04	1.9E-09	2.7E+06	1.6E-03	6.0E-10

As shown in Table 28, antibody 41F11_VH_T28P/Y50W/S55E/D95H/ L96E/P97E/Y100E; VK_A64G (also referred to as P1-070976) binds with high affinity to both human and cyno VISTA at pH 6.0 and with low affinity to both human and cyno VISTA at pH 7.4.

Some of the VISTA.4 mutants described above and new mutants were tested for binding to hVISTA, according to the methods described in Example 9. The results, which are provided in Table 29 (amino acid sequences of the CDRs are provided in Table 30), indicate that most mutants did not exhibit binding at either pH 6.0 or 7.4. Antibody 41F11_VH_T28P/Y50W/S55E/D95H/ L96E/P97E/Y100E;VK_A64G (P1-070976) is the only VISTA.4 derivative that is pH selective.

Two mutants of this pH selective VISTA.4 derivative were created to determine whether all substitutions that were made to VISTA.4 are necessary for pH selectiveness: P1-070976_H95D and P1-070976_E97P (both with a VK_A64G light chain), in which either H95 or E97 were reverted back to the residue, D and P, respectively, in VISTA.4. As shown in Table 29 (which shows the average rates of two experiments), H95 is not necessary for the pH selectivity as its reversion to D did not significantly affect the binding kinetics. However, E97 is important for pH selectivity, as its reversion to P resulted in the loss of the pH selectivity.

Table 29:

ID	Description	Avg pH 7.4 ka (1/Ms)	Avg pH 7.4 kd (1/s)	Avg pH 7.4 KD (M)	Avg pH 6.0 ka (1/Ms)	Avg pH 6.0 kd (1/s)	Avg pH 6.0 KD (M)
P1-071799	P1-070976_H95D	No binding at 100nM			1.7E+06	2.7E-02	1.5E-08
P1-071801	P1-070976_E97P	2.9E+04	6.1E-04	2.1E-08	4.6E+05	1.6E-03	3.5E-09
P1-072996	41F11_VH_D31E_Y32D_S35E_Y50W_S53E_G54D_S55E_L96H_W99E_Y100E; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-072997	41F11_VH_F27H_S35E_Y50E_G54D_Y58E_D95H_L96E_Y100E; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-072998	41F11_VH_D31H_Y33E_Y50E_Y58D_Y59H_L96H_W99H_Y100E; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-072999	41F11_VH_D31E_Y32D_S35E_Y50E_S53H_Y59E_L96E_W99H_Y100D; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073001	41F11_VH_D31E_Y33D_S35E_Y50W_S52E_S55E_Y59H_L96H_Y100E_L102H; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073002	41F11_VH_F29E_D31E_S35E_Y50E_S52E_Y59D_D95H_L96E_P97E_Y100E; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073003	41F11_VH_Y33E_Y50E_S52E_Y58E_L96H_Y100E_F100aD; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073004	41F11_VH_S30H_D31E_M34H_Y50W_I51D_S52D_S55E_L96E_Y100E_D101E; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073005	41F11_VH_F29D_D31E_S35D_Y50W_S55H_Y59H_L96E_W99D_Y100E_D101H; Vk A64G	No binding at 100nM			No binding at 100nM		
P1-073006	41F11_VH_T28E_S30E_D31E_Y50W_S55D_P56E_L96E_P97E_Y100E_F100aE; Vk A64G	No binding at 100nM			No binding at 100nM		

ID	Description	Avg pH 7.4 ka (1/Ms)	Avg pH 7.4 kd (1/s)	Avg pH 7.4 KD (M)	Avg pH 6.0 ka (1/Ms)	Avg pH 6.0 kd (1/s)	Avg pH 6.0 KD (M)
P1-065333	VISTA.4 pH-independent progeny	2.4E+05	7.8E-04	3.2E-09	2.7E+06	1.5E-03	5.6E-10
P1-070976	acidic pH selective VISTA.4 progeny	No binding at 100nM			1.1E+05	2.6E-03	2.3E-08
P1-061520	VISTA.4 A64G	2.3E+05	9.1E-04	4.0E-09	8.3E+05	3.3E-02	4.0E-08

Table 30:

ID	Description	HCDR1	HCDR2	HCDR3
P1-071799	P1-070976_H95D	..P.....	W.....E.....	.EE..E...
P1-071801	P1-070976_E97P	..P.....	W.....E.....	HE...E...
P1-072996	41F11_VH_D31E_Y32D_S35E_Y50W_S53E_G54D_S55E_L96H_W99E_Y100E; Vk A64G	.....ED..E	W...EDE.....	.H..EE...
P1-072997	41F11_VH_F27H_S35E_Y50E_G54D_Y58E_D95H_L96E_Y100E; Vk A64G	.H.......E	E....D...E.....	HE...E...
P1-072998	41F11_VH_D31H_Y33E_Y50E_Y58D_Y59H_L96H_W99H_Y100E; Vk A64G	.....H.E..	E........DH.....	.H..HE...
P1-072999	41F11_VH_D31E_Y32D_S35E_Y50E_S53H_Y59E_L96E_W99H_Y100D; Vk A64G	.....ED..E	E...H.....E.....	.E..HD...
P1-073001	41F11_VH_D31E_Y33D_S35E_Y50W_S52E_S55E_Y59H_L96H_Y100E_L102H; Vk A64G	.....E.D.E	W.E...E...H.....	.H...E..H
P1-073002	41F11_VH_F29E_D31E_S35E_Y50E_S52E_Y59D_D95H_L96E_P97E_Y100E; Vk A64G	...E.E...E	E.E.......D.....	HEE..E...
P1-073003	41F11_VH_Y33E_Y50E_S52E_Y58E_L96H_Y100E_F100aD; Vk A64G	.......E..	E.E......E.....	.H...ED..
P1-073004	41F11_VH_S30H_D31E_M34H_Y50W_I51D_S52D_S55E_L96E_Y100E_D101E; Vk A64G	....HE..H.	WDD...E.....	.E...E.E.
P1-073005	41F11_VH_F29D_D31E_S35D_Y50W_S55H_Y59H_L96E_W99D_Y100E_D101H; Vk A64G	...D.E...D	W.....H...H.....	.E..DE.H.
P1-073006	41F11_VH_T28E_S30E_D31E_Y50W_S55D_P56E_L96E_P97E_Y100E_F100aE; Vk A64G	..E.EE....	W.....DE.....	.EE..EE..
P1-065333	VISTA.4 pH-independent progeny	..P.....	W.....E.....	.E...E...
P1-070976	acidic pH selective VISTA.4 progeny	..P.....	W.....E.....	HEE..E...

ID	Description	HCDR1	HCDR2	HCDR3
P1-061520	VISTA.4 A64G	**GFTFSDYYMS**	**YISNSGSPIYYADSVKG**	**DLPGWYFDL**
ID	**Description**	**LCDR1**	**LCDR2**	**LCDR3**
P1-071799	P1-070976_H95D	.....	.....	.....
P1-071801	P1-070976_E97P	.....	.....	.....
P1-072996	41F11_VH_D31E_Y32D_S35E_Y50W_S53E_G54D_S55E_L96H_W99E_Y100E; Vk A64G	.....	.....	.....
P1-072997	41F11_VH_F27H_S35E_Y50E_G54D_Y58E_D95H_L96E_Y100E; Vk A64G	.....	.....	.....
P1-072998	41F11_VH_D31H_Y33E_Y50E_Y58D_Y59H_L96H_W99H_Y100E; Vk A64G	.....	.....	.....
P1-072999	41F11_VH_D31E_Y32D_S35E_Y50E_S53H_Y59E_L96E_W99H_Y100D; Vk A64G	.....	.....	.....
P1-073001	41F11_VH_D31E_Y33D_S35E_Y50W_S52E_S55E_Y59H_L96H_Y100E_L102H; Vk A64G	.....	.....	.....
P1-073002	41F11_VH_F29E_D31E_S35E_Y50E_S52E_Y59D_D95H_L96E_P97E_Y100E; Vk A64G	.....	.....	.....
P1-073003	41F11_VH_Y33E_Y50E_S52E_Y58E_L96H_Y100E_F100aD; Vk A64G	.....	.....	.....
P1-073004	41F11_VH_S30H_D31E_M34H_Y50W_I51D_S52D_S55E_L96E_Y100E_D101E; Vk A64G	.....	.....	.....
P1-073005	41F11_VH_F29D_D31E_S35D_Y50W_S55H_Y59H_L96E_W99D_Y100E_D101H; Vk A64G	.....	.....	.....
P1-073006	41F11_VH_T28E_S30E_D31E_Y50W_S55D_P56E_L96E_P97E_Y100E_F100aE; Vk A64G	.....	.....	.....
P1-065333	VISTA.4 pH-independent progeny	.....	.....	.....
P1-070976	acidic pH selective VISTA.4 progeny	.....	.....	.....
P1-061520	VISTA.4 A64G	**RASQSVSSYLA**	**DASNRAT**	**QQRNNWPRT**

Thus, this Example identified anti-human VISTA antibodies that bind to human VISTA with 200-10,000 fold greater affinity at acidic pH than at physiological pH (Figure 19A). In cell binding assays, these acidic pH-selective VISTA antibodies exhibited an inflection point in binding intensity at approximately pH 6.5, similar to what was observed for the binding of VISTA to T cells.

Example 18: Epitope mapping of VISTA.4

VISTA.4 was used in the competitive BLI epitope binning assay described in Example 15. The results indicate that VISTA.4 competes for binding to human VISTA with the antibodies described above P1-061015, P1-061029, P1-068761, and P1-068767, and thus belongs to the same epitope group as these antibodies (Group A). VISTA.4 does not compete for binding to human VISTA with VISTA mAb 1 (VISTA.5; see Fig. 11A).

The epitope of VISTA.4 was also mapped using yeast surface display and NGS, as described in Example 15 for antibodies P1-061015, P1-061029, P1-068761, and P1-068767. VISTA mutants that lost binding to the antibody being mapped but retained binding to a non-blocking antibody (mAb1) were sorted and sequenced. Since they retained binding to mAb1, these mutants were likely correctly folded, and the loss of binding seen to the antibody being mapped was likely due to the loss of an energetically important contact residue. The positions of the mutations that resulted in loss of binding, and which were designated as energetically important residues in the antibody's epitope, and are shown in Table 31, along with the energetically important contact residues of antibodies P1-061015, P1-061029, P1-068761, and P1-068767 (also shown in Table 14 above).

Table 31: Energetically important contact residues of antibodies VISTA.4, ‘029, ‘015, ‘761, and ‘767

mAb	V 34	T 35	Y 37	K 38	T 39	Y 41	S 52	R 54	T 61	F 62	Q 63	L 65	H 66	L 67	H 68	H 69	F 97	L 115	V 117	E 118	I 119	R 120	H 121	H 122	S 124	E 125	R 127
VISTA.4	x	x	x		x	x	x	x		x		x	x		x			x	x		x	x	x	x	x	x	
‘015			x	x	x			x		x	x	x	x				x	x	x		x				x	x	
‘029			x		x	x		x	x	x	x	x	x		x	x		x	x		x				x	x	x
‘761		x	x		x			x	x	x	x		x	x	x	x		x	x		x				x	x	x
‘767			x		x	x		x	x	x	x	x	x					x	x		x		x	x	x	x	x

Hydrogen/deuterium exchange mass spectrometry (HDX-MS) was utilized to probe binding epitopes of human VISTA with mAb VISTA.4. HDX-MS probes protein conformation and conformational dynamics in solution by monitoring the rate and extent of deuterium exchange of backbone amide hydrogen atoms (Huang and Chen (2014) Analytical and Bioanalytical Chemistry 406, 6541-6558; Wei, et al. Drug Discovery Today (2014) 19, 95-102). The level of HDX depends on the solvent accessibility of backbone amide hydrogen atoms and the protein hydrogen bonds. The mass increase of the protein upon HDX can be precisely measured by MS. When this technique is paired with enzymatic digestion, structure features at the peptide level can be resolved, enabling differentiation of surface exposed peptides from those folded inside, or from those sequestered at the interface of a protein-protein complex. Typically, the deuterium labeling and subsequent quenching experiments are performed, followed by enzymatic digestion, peptide separation, and MS analysis.

Prior to epitope mapping experiments, non-deuteriated experiments were carried out to generate a list of common peptides for recombinant human VISTA (15 μM) and protein complexes of VISTA with mAb VISTA.4 (1:1 molar ratio). In the HDX-MS experiment, 5 μL of each sample (VISTA or VISTA with mAb VISTA.4) was diluted into 55 μL of D_2O buffer (10 mM phosphate buffer, D_2O, pH7.0) to start the labeling reactions. The reactions were carried out for different periods of time: 1 min, 10 min and 240 min. By the end of each labeling reaction period, the reaction was quenched by adding quenching buffer (100 mM phosphate buffer with 4M GdnCl and 0.4M TCEP, pH 2.5, 1:1, v/v) and 50 μL of quenched sample was injected into Waters HDX-MS system for analysis. The deuterium uptake levels of common peptic peptides were monitored in the absence/presence of VISTA.4. The obtained sequence coverage was 82%.

HDX-MS experiments provided 85% sequence coverage for human VISTA. As shown in Figure 20A, the HDX-MS data analysis on VISTA.4 in human VISTA indicates that VISTA.4's epitope is comprised of three regions of human VISTA, with region 2 being the primary epitope (residue numbers correspond to native human VISTA sequence, Figure 20B):

Region 1: ^{57}LGPVDKGHDVTF^{68} (SEQ ID NO: 498)

Region 2: ^{86}RRPIRNLTFQDL^{97} (SEQ ID NO: 497)

Region 3: ^{148}VVEIRHHHSEHRVHGAME^{165} (SEQ ID NO: 499)

As described in the above Examples, antibody VISTA.4 bound equally well at acidic and neutral pH (Figure 19B). Further rounds of selection yielded a variant that bound VISTA with 200-fold higher affinity at pH 6.0 than at pH 7.4 (Figure 19C). Similar efforts with VISTA blocking antibodies produced variants with up to 10,000-fold selectivity for pH 6.0 relative to pH 7.4 (see Examples before Example 17). VISTA.5 and other non-blocking antibodies were largely insensitive to acidic pH in cell-based and biophysical assays (Figs. 18 and 19). Fig. 18D shows that VISTA blocking antibodies are frequently sensitive to acidic pH.

We used these antibodies to map VISTA's receptor-ligand binding interface at acidic and neutral pH. pH-independent, neutral pH-selective, and acidic pH-selective VISTA blocking antibodies bound nearly identical epitopes, suggesting that histidine protonation alone, without marked conformational changes, controls VISTA's ability to engage its counter-receptor at acidic pH. This epitope-specific pH sensitivity suggested that antibodies can distinguish the active (acidic pH) and inactive (neutral pH) states of VISTA's ligand interface.

Example 19: VISTA.4 inhibits VISTA binding to T cells at acidic pH

This Example shows that VISTA.4 and other antibodies in epitope group A blocked VISTA binding to T cells at acidic pH, whereas VISTA.5 (mAb1) and other antibodies in epitope group B did not (Figure 21A and B).

This example was conducted essentially as described in Example 4.

Example 20: VISTA.4 enhances T cell proliferation and IFN-γ production

This Example describes the effects of VISTA blocking and non-blocking antibodies (VISTA.4 and VISTA.5, respectively) on CD4+ cells co-cultured at physiological pH with 293T cells engineered to express VISTA and a single chain variable fragment of the CD3 agonist antibody OKT3 (293T-scOKT3-VISTA). This experiment was conducted by adding VISTA antibodies to CD4+ T cells co-cultured with 293T cells engineered to express human VISTA and a single chain variable fragment of the T cell receptor agonist antibody OKT3 (293T-OKT3-VISTA). CD4+ T cells were enriched from healthy donor blood by negative selection (StemCell RosetteSep) and labeled with the proliferation dye CellTrace Violet (ThermoFisher). 293T cells were

engineered to ectopically express a single chain variable fragment of the agonistic CD3 monoclonal antibody OKT3 and human VISTA ("293T-OKT3-VISTA"). CD4+ T cells and irradiated 293T-OKT3-VISTA cells were co-cultured at a ratio of 4:1 in RPMI-1640 supplemented with 10% V/V heat-inactivated fetal calf serum, 2 mM L-glutamine (Gibco), 2 mM non-essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), 55 uM B-mercaptoethanol, and titrated human anti-human VISTA or isotype-matched non-VISTA-binding control antibodies for 5 days. Proliferation was calculated as the percentage of CD4+ T cells undergoing CellTrace Violet dye dilution, as determined by flow cytometry.

The results show that VISTA blocking Abs (epitope Group A) moderately enhanced T cell proliferation and IFN-γ production (Fig. 22). VISTA.5 (mAb1), which is not a blocking antibody, did not enhance T cell proliferation or IFN-γ production. VISTA.4 had no effect when T cells were co-cultured with 293T-OKT3 cells that did not express VISTA.

Example 21: VISTA suppressed T cell receptor-mediated NF-kB signaling

This Example was conducted to further assess the effects of pH on VISTA function, and shows that VISTA suppressed T cell receptor-mediated NF-kB signaling more potently at acidic pH than at neutral pH, though a modest level of activity was maintained above pH 7.0 (Figure 23). Maximal suppression was reached below pH 6.5, similar to VISTA : T cell binding (Figure 23 and Figure 4A).

NF-kB signaling was measured using NFkB-reporting Jurkat T cells, essentially as described in Examples 5 and 13. Jurkat cells were engineered to express luciferase under the control of an NF-kB-inducible promoter. These Jurkat NFkB-luciferase reporter cells were co-cultured with non-irradiated 293T-OKT3-VISTA cells at a ratio of 4:1 in HBSS (ThermoFisher) acidified to various pH with MES and human anti-human VISTA antibodies for 4 hours. Jurkat cell activation was measured by luciferase substrate assay (Promega).

Similarly, plate-coated recombinant VISTA suppressed CD4+ and CD8+ T cell NFkB phosphorylation most effectively below pH 7.0 (Fig 23C and D). For these experiments, tissue culture-treated 96-well flat bottom plates were coated with OKT3 (0.5 µg/mL) and either human VISTA-Fc (comprising a hVISTA extracellular domain

comprising amino acids 33-194 of SEQ ID NO: 1 linked to an Fc domain) or isotype-matched control antibody (5.0 μg/mL) in PBS at 37 C for approximately 2 hours. The wells were then washed with PBS and pre-incubated with human anti-human VISTA or non-VISTA-binding control antibodies diluted to 5.0 μg/mL in HBSS acidified to various pH with MES for 30 minutes. T cells were suspended in the same HBSS + MES buffers, added to the wells, centrifuged, and cultured at 37° C for 15 minutes. The cells were then fixed (Cytofix buffer, BD Biosciences), permeabilized (Phosflow Permeabilization Buffer III, BD Biosciences), and stained with anti-pNFkB S529 (BD Biosciences) and anti-mIgG secondary antibodies (Jackson Immunoresearch) before being acquired on a flow cytometer. NFkB phosphorylation was calculated as a percentage of the pNFkB MFI for cells stimulated at pH 7.4 in wells that had been coated with OKT3 and isotype-matched control and pre-incubated with soluble non-binding isotype-matched control antibodies. These results indicate that VISTA is more potent at acidic pH than at neutral pH, and that its function is reversed by the same antibodies that block VISTA binding to T cells at acidic pH. Thus, these data and those of Example 20 suggest that blockade of VISTA's acidic pH-selective receptor-ligand interface could reverse immunosuppression.

Example 22: Anti-VISTA antibody and anti-PD-1 antibody act synergistically to elicit tumor rejection

To characterize the effects of blocking VISTA's acidic pH-selective ligand interface in a tumor, a mouse surrogate antibody, VISTA.10, was produced, which blocks mouse VISTA binding to mouse T cells at acidic pH. (VISTA.10 also binds mVISTA at physiological pH.) Anti-mouse VISTA antibodies were produced in VISTA knockout mice immunized with recombinant mouse VISTA. Splenocytes from immunized mice were fused with the Sp2/0 myeloma cell line. Hybridoma supernatants were screened for reactivity to recombinant mouse VISTA by ELISA and to cell surface mouse VISTA by flow cytometry. VISTA.10 was selected, and, to avoid Fc receptor engagement and any subsequent effector functions, VISTA.10 was converted to an IgG1 isotype with a point mutation, D265A, to avoid Fc receptor engagement and effector functions (Clynes et al. (2000) Nat Med 6, 443-446). MC38 tumors were implanted subcutaneously in mice, and when the tumors reached about 70mm^2, the following treatments were administered every three days to the mice: Group 1: 4 doses of anti-KLH mIgG1-D265A at 30 mpk; Group 2:

two doses of anti-PD-1 mIgG1-D265A at 5 mg/kg; Group 3: 4 doses of anti-VISTAmIgG1-D265A at 30 mg/kg; and Group4: nti-PD-1 + anti-VISTA combination. Combination treatment of VISTA.10 and a PD-1 blocking antibody elicited tumor rejection in approximately70% of mice implanted with MC38 colorectal adenocarcinoma tumors (Figure 24A-D and G-H). Treatment with anti-PD-1 or VISTA.10 as single agents slightly delayed, but did not prevent, tumor progression (Figure 24HB, C, G and H).

Consistent with these results, e*x vivo* analysis of tumors from mice treated for 7 days with VISTA.10 and anti-PD-1 therapy had 5- 10-fold increases in tumor-infiltrating CD8+ T cells and CD4+ T cells relative to control mice (Figure 24E-F). Combination therapy also resulted in significantly lower expression of PD-1, LAG-3 and TIM-3, all markers of T cell exhaustion and dysfunction (inhibitory receptors associated with T cell dysfunction), on tumor-infiltrating CD8+ T cells (Figure 25A-C). Consistent with the efficacy data, treatment with PD-1 or VISTA antibodies alone had smaller effects on T cell frequencies and phenotypes (Figures 24A-F). Intratumoral myeloid cell subset frequencies, including those of macrophages, monocytes (e.g., monocytic myeloid-derived suppressor cells (MDSC)), and granulocytes (e.g., granulocytic MDSC), were largely unaffected by VISTA antibody treatment.

To contextualize VISTA antibody activity, VISTA knockout mice were implanted with MC38 tumors and treated with PD-1 blocking or control antibodies. As shown in Fig. 24I, in the control treatment groups, MC38 tumors grew comparably in VISTA knockout mice and their wildtype littermates.VISTA knockout mice exhibited increased responsiveness to anti-PD-1, reminiscent of VISTA and PD-1 combination efficacy. This responsiveness was again correlated with increased intratumoral CD4+ and CD8+ T cells. These data indicate that antibodies that block VISTA binding at acidic pH can reverse VISTA-mediated immune suppression and phenocopy VISTA knockout mice.

Because VISTA preferentially binds and suppresses T cells at acidic pH in vitro, we hypothesized that VISTA-mediated suppression of anti-tumor responses occurs predominantly within the tumor microenvironment. We tested this hypothesis using the acidic pH-selective human VISTA blocking antibody P1-068767 (’767) and its non-pH-selective parent antibody P1-061029 (’029) in transgenic mice expressing the human VISTA extracellular domain in place of the endogenous VISTA extracellular domain

(human VISTA knock-in mice, genOway). In human VISTA knock-in mice implanted with MC38 tumors, treatment with anti-PD-1 alone elicited tumor rejection in 13% of mice and delayed tumor progression in approximately 20% of mice (**Fig 24 J-M**). Treatment with '029 or '767 alone had little therapeutic benefit (**Fig. 24 N and O**). In combination with anti-PD-1, '029 treatment increased the rate of tumor rejection to 50% (**Fig 24 J-M**). Strikingly, '767 combination treatment efficacy was nearly identical to that of '029 (56% rejection rate, **Fig 24 J-M**). These results suggest that VISTA's immunosuppressive activity occurs primarily in acidic tumor microenvironments, rather than in the blood or other non-acidic tissues.

The half-life of P1-068767 (acidic pH selective) and P1-061029 (non pH-selective) in the human VISTA knock-in mice was measured. In wildtype mice, high VISTA expression on myeloid cells in blood and lymphoid organs subjects VISTA antibodies to target-mediated drug disposition (TMDD) and shortened serum mean residence times (MRT Fig 24R). Similarly, '029 has a short MRT in human VISTA knock-in mice (4.1 hours, Fig. 24N). As shown in Fig. 24N, P1-068767 exhibited a nearly 20-fold longer mean residence time (MRT) than did P1-061029, indicative of weak binding to VISTA at pH 7.4 (e.g., in circulating blood) and consequently reduced TMDD (71 hours and 4.1 hours respectively).

To assess antibody engagement of peripheral VISTA in a non-transgenic model, we treated cynomolgus macaques with P1-068767 and the neutral pH-preferring antibody VISTA.4 as follows. VISTA.4 and P1-068767 were evaluated following 10 minute intravenous infusions into protein-naive cynomolgus monkeys at a dose of 5 mg/kg (n = 1 per antibody). Serial blood samples were collected at 0.17, 0.5, 2, 4, 6, 24, 48, 72, 168, 216, 240, 336 hours post-infusion. Subsequently, serum samples were obtained for antibody concentration analysis using a ligand-binding assay that employed the recombinant VISTA as a capturing agent and an anti-human IgG Fc mAb as a detecting agent. The lower limit of quantification for the assay was 1 ng/mL. Mean residence times were estimated by non-compartment analysis of the serum mAb concentration-time data using Kinetica software (Version 5.0, Thermo Fisher Scientific). The results show that P1-068767 again exhibited a much longer MRT (717 hours and 7.6 hours respectively, Fig. 24O). These data show that '767 retains its acidic pH selectivity

in vivo. These results suggest that VISTA blockade in the tumor microenvironment, rather than in the blood and non-acidic tissues, drives anti-tumor efficacy.

Example 23: VISTA.4 inhibits VISTA binding to PSGL-1

Imunoreceptor P-selectin glycoprotein ligand-1 (PSGL-1) was identified previously as a VISTA ligand (see, WO2018132476). PSGL-1 is a receptor for selectins, particularly P-selectin, and binding to its primary ligand, P-selectin, is a well characterized facilitator of adhesion interactions between leukocytes, platelets, and endothelial cells (Carlow, D.A., et al., PSGL-1 function in immunity and steady state homeostasis. Immunol Rev, 2009. 230(1): p. 75-96, and Abadier, M. and K. Ley, P-selectin glycoprotein ligand-1 in T cells. Curr Opin Hematol, 2017. 24(3): p. 265-273. 18). PSGL-1 has also been identified as a negative regulator of T cell responses in contexts of chronic viral infection, cancer tumor immunity, and some autoimmune diseases (Angiari, S., et al., Regulatory T cells suppress the late phase of the immune response in lymph nodes through P-selectin glycoprotein ligand-1. J Immunol, 2013. 191(11): p. 5489-500; Matsumoto, M., M. Miyasaka, and T. Hirata, P-selectin glycoprotein ligand-1 negatively regulates T-cell immune responses. J Immunol, 2009. 183(11): p. 7204-11; Nunez-Andrade, N., et al., P-selectin glycoprotein ligand-1 modulates immune inflammatory responses in the enteric lamina propria. J Pathol, 2011. 224(2): p. 212-21; Perez-Frias, A., et al., Development of an autoimmune syndrome affecting the skin and internal organs in P-selectin glycoprotein ligand 1 leukocyte receptor-deficient mice. Arthritis Rheumatol, 2014. 66(11): p. 3178-89; Tinoco, R., et al., PSGL-1 Is an Immune Checkpoint Regulator that Promotes T Cell Exhaustion. Immunity, 2016. 44(5): p. 1190-203). This immunosuppressive function appears to be independent of known PSGL-1 ligands (Tinoco, R., et al., PSGL-1: A New Player in the Immune Checkpoint Landscape. Trends Immunol, 2017. 38(5): p. 323-335).

In cell-based assays, recombinant PSGL-1 and recombinant P-selectin were shown to both be capable of blocking of VISTA multimer binding to activated human CD4+ T cells. Deletion of PSGL-1 from activated CD4+ T cells by CRISPR also ablated VISTA multimer binding. In addition, ectopic expression of PSGL-1 was shown to be sufficient to enable VISTA binding to CHO cells at acidic pH, and VISTA expression was sufficient to enable PSGL-1 binding to 293T cells at acidic pH. In addition,

isothermal titration calorimetry (ITC) confirmed hPSGL1 and hVISTA interaction and confirmed that the interaction occurs at pH 6.0, but not significantly at pH 7.4 ITC also indicated that the stoichiometry is close to 1:1 for PSGL1-Fc with VISTA-Fc and that the KD at pH 6.0 is in the high nM range (2 measurements at 0.65 μM and 0.85 μM). An approximately 4 times weaker binding was observed at 37°C compared to 25°C. When using VISTA-His instead of VISTA-Fc, a KD of 2.73μM was observed, which is about four fold weaker than with VISTA-Fc, presumably due to lack of avidity for His-tagged VISTA.

This Example also shows that PSGL-1 bound P-selectin comparably at acidic and physiological pH, but bound VISTA only at acidic pH (Figure 26A). The experiment was conducted using Octet biosensor assays with VISTA, P-selectin, and the minimal PSGL-1 glycopeptide (amino acids 1-19, with both sulfotyrosine and siayl lewis X carbohydrate post-translational modifications) previously shown to support high affinity P-selectin binding (Sako, D., et al., A sulfated peptide segment at the amino terminus of PSGL-1 is critical for P-selectin binding. Cell, 1995. 83(2): p. 323-319).

PSGL-1's ligand interface relies on negatively charged sulfotyrosine and siayl Lewis-X post-translational modifications to bind P-selectin with high affinity (Sako et al. 1995 Cell 83(2): p. 323-319), and naïve T cells, which express non-siayl-lewis-X decorated PSGL-1, are consequently unable to engage P-selectin efficiently. Siayl lewis X-decorated PSGL-1 is constitutively expressed on circulating monocytes and neutrophils, and inducibly expressed on activated T cells, consistent with strong VISTA binding to these cell types at acidic pH. However, VISTA was found to bind to both naïve and activated T cells, suggesting that unlike P-selectin, VISTA binds PSGL-1 independently of siayl lewis X. In additional Octet biosensor assays, it was found that, while VISTA and P-selectin both bound preferentially to PSGL-1 glycopeptides with siayl lewis X decoration, only VISTA bound PSGL-1 glycopeptides without siayl lewis X. In addition, PSGL-1 produced in cells not expressing the enzymes glucosaminyl (N-acetyl) transferase (GCNT1) and alpha (1,3)-fucosyltransferase-7 enzymes (FUT7) lacked sialyl lewis X decoration and bound poorly to P-selectin (Fig 29A). In contrast, VISTA bound PSGL-1 independently of sialyl lewis X (Fig 29A). This result is consistent with VISTA, but not P-selectin, binding to naïve T cells which lack sialyl lewis X.

Also similar to P-selectin, VISTA bound modestly to heparan sulfate at acidic pH.

Additionally, PSGL-1 antibodies that block P-selectin binding did not block VISTA binding. These data indicate that VISTA binds a PSGL-1 interface that is similar but distinct from that bound by P-selectin.

This Example further shows that antibodies P1-061029, P1-068761, P1-068767 and VISTA.4, which block VISTA binding to T cells, also blocked VISTA binding to the PSGL-1 glycopeptide. Competitive Octet assays were conducted to evaluate whether acidic pH-selective α-VISTA antibodies P1-068761 and P1-068767, the P1-061029 pH-independent parent, and acidic pH-sensitive VISTA.4 blocked VISTA binding to PSGL1. Binding assays were performed on an OctetRed384 bio-layer interferometry (BLI) instrument (PALL/ForteBio). All assay steps were performed at 30°C at 1000 rpm shake speed, and the buffer used was PBST, pH 6.0 (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM phosphate buffer, 0.05% Tween 20). Human VISTA-Fc (R&D Systems # 7126-B7) was diluted to 400 nM in PBST pH 6.0 and premixed for 30 minutes with a 0 nM, 40 nM, and 400 nM titration series of P1-068761, P1-068767, P1-061029 and VISTA.4. The human PSGL1 19-mer-huFc protein, consisting of the amino-terminal 19 amino acids of mature PSGL1 fused to human Fc, was captured onto anti-human IgG-Fc sensors (AHC, PALL/ForteBio). The anti-human capture sensors were blocked next with total human IgG (Jackson #009-000-002). Binding of the captured PSGL1 to VISTA-Fc/α-VISTA antibody mixture was measured next to assess whether the α-VISTA antibodies prevented VISTA from binding to PSGL1. For each antibody titration series, the magnitude of VISTA binding to PSGL1 (nm shift) was normalized to the 0nM unblocked VISTA:PSGL1 response, set at 100%. The results from this assay are summarized in Figure 26B. In this assay, the 400 nM concentrations of P1-061029, P1-068761, P1-068767 and VISTA.4 all demonstrated blocking activity. The VISTA-Fc protein was prevented from binding to the captured human PSGL1-19-mer-huFc protein, as indicated by the reduced VISTA binding observed.

Additionally, it was demonstrated that VISTA binding to CHO-PSGL-1 cells was blocked by both VISTA.4 and the P-selectin-blocking PSGL-1 antibody KPL-1 (Fig. 26C). In PSGL-1 antibody blocking assays, the cells were pre-incubated with KPL-1 (BD Biosciences or Biolegend) or PL2 (MBL) prior to labeling with 32 nM-loaded VISTA multimers or VISTA-Fc chimeric proteins. VISTA-Fc binding was detected by anti-IgG

(Jackson ImmunoResearch) or anti-6xhis (Columbia Biosciences) antibodies. Cells were acquired by flow cytometry or homogenous time resolved fluorescence (HTRF).

Example 24: Crystal structure of '767 bound to hVISTA

To characterize VISTA's structure and the molecular determinants of VISTA antibody binding, a co-crystal of the hVISTA IgV domain with the P1-068767 Fragment antigen-binding (Fab) was made. The structure of the resulting complex was determined at 1.6 Å resolution (Figure 27A-D). The VISTA IgV domain is generally characteristic of its family, with some resemblance to PD-L1 (Figure 24B). However, unlike PD-L1 and most other B7 family or immunoglobulin superfamily members, the VISTA IgV domain's two C-terminal β-strands contain multiple additional residues, resulting in an unusually elongated and histidine-rich central β-sheet (Figure 27B). P1-068767, a blocking antibody, binds VISTA at this β-sheet extension (Figure 27C), while the non-blocking antibody VISTA.5 binds a different region (Figure 27E). VISTA's β-sheet extension is capped by a loop connecting the β-strands, which loop includes the three histidine residues: H121, H122, and H123. The blocking antibody '767 binds this region, while the non-blocking antibody VISTA.5 binds a different region of the IgV domain (Fig 27C and E, respectively). P1-068767 heavy chain residues E110 and D112 (shown as E100 and D102 in Fig. 27D) form hydrogen bonds with VISTA residues H121 and H122 respectively (Figure 27D). These interactions fit well with the findings described in previous Examples that P1-068767 residues E110 and D112 are necessary and sufficient for conferring acidic pH-selectivity to the antibody. In the cocrystal, VISTA residue H123 interacts with a sulfate molecule from the precipitant and forms a salt bridge with P1-068767 residue E1; though it is possible that VISTA H123 can form a bona fide hydrogen bond with P1-068767 in the absence of sulfate (Figure 27D). Additional interactions are provided in Table 32. These data suggest that the VISTA IgV domain's unusual, histidine-rich β-sheet extension is a key component of VISTA's acidic pH-selective receptor-ligand interface. Histidine protonation, particularly at residues H153, H154, and H155 (H121, H122, and H123, respectively, in mature hVISTA), may drive pH-selective VISTA binding to its counter-receptor.

Table 32 details the distances in Ångströms (Å), between '767 Fab HC atoms within 4 Å of VISTA atoms.

Table 32:

##	'767 Fab HC	Dist.	VISTA
1	H:GLU 1 [OE1]	3.1	V:HIS 123 [NE2]
2	H:VAL 2 [N]	3.2	V:HIS 123 [O]
3	H:GLY 26 [O]	3.1	V:GLU 125 [N]
4	H:GLU 30 [O]	3.3	V:ARG 54 [NH2]
5	H:GLU 30 [OE1]	3.8	V:ARG 127 [NE]
6	H:GLU 30 [OE1]	3.2	V:ARG 127 [NH2]
7	H:GLU 30 [OE2]	3.4	V:ARG 127 [NH1]
8	H:GLU 30 [OE2]	3.5	V:ARG 127 [NH2]
9	H:ASP 31 [OD1]	2.8	V:ARG 54 [NH1]
10	H:ASP 31 [OD1]	2.7	V:ARG 54 [NH2]
11	H:ASP 31 [OD1]	2.8	V:ARG 127 [NH1]
12	H:ASP 31 [OD2]	3.8	V:ARG 127 [NE]
13	H:ASP 31 [OD2]	3.1	V:ARG 127 [NH1]
14	H:TYR 32 [OH]	2.6	V:GLU 125 [OE1]
15	H:GLU 110 [OE2]	2.8	V:HIS 122 [N]
16	H:GLU 110 [OE1]	2.7	V:HIS 121 [ND1]
17	H:GLU 110 [OE2]	3.8	V:HIS 121 [ND1]
18	H:GLU 110 [OE2]	3.5	V:HIS 122 [ND1]
19	H:ASP 111 [OD1]	3.6	V:HIS 122 [NE2]
20	H:ASP 112 [OD1]	3.5	V:HIS 122 [ND1]

Additional characteristics regarding the crystallography are provided in Table 33 below:

Table 33: data collection and refinement statistics

	VISTA + VISTA 18
Data collection	
Space group	
Cell dimensions	
a, *b*, *c* (Å)	66.17, 125.86, 192.04
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å)	1.61–96.02 (1.61–1.81)*
R_{sym} or R_{merge}	0.049 (0.724)
$I / \sigma I$	18.75 (2.17)
Completeness (%)	88.6 (73.9)
Redundancy	6.46 (5.57)
Refinement	
Resolution (Å)	1.61–96.02 (1.61–1.73)
No. reflections	55883 (1063)
R_{work} / R_{free}	18.2 / 22.4 (24.3 / 25.8)
No. atoms	
Protein	4281
Ligand/ion	84
Water	758
B-factors	
Protein	33.97
Ligand/ion	82.40
Water	42.13
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.07

*Values in parentheses are for highest-resolution shell.

Example 25: VISTA : PSGL-1 binding specificity is determined by histidine and sulfotyrosine residues

To characterize PSGL-1 binding specificity, in addition to that provided by sialyl lewis X (described in Example 23), we examined tyrosine sulfation post-translational modifications that contribute to P-selectin binding. To test the role of tyrosine sulfation, PSGL-1 glycopeptides were fractioned into sulfotyrosine-rich (> 90%) and sulfotyrosine-

poor (< 1%) peaks by anion exchange liquid chromatography. Neither VISTA nor P-selectin bound detectably to sulfotyrosine-poor PSGL-1 (Fig. 28B and I). VISTA was also unable to bind PSGL-1 glycopeptides in which tyrosines were substituted with alanines (Fig. 28J). These results indicate that sulfotyrosine residues are key mediators of PSGL-1 binding to VISTA.

We hypothesized that VISTA binding specificity is mediated by the same histidine residues found within the VISTA blocking antibody epitope: H153, H154, and H155 (numbered in hVISTA with signal sequence, and corresponding to H121, H122 and H123, respectively, in mature human VITA; see Example 24). Replacement of these histidine residues with negatively charged aspartic acid (H2D) significantly reduced VISTA binding to recombinant PSGL-1 and CHO-PSGL-1 cells (Figs. 29C-D). In contrast, replacement with positively charged arginine (H2R) residues (positively charged side chain mimics that of protonated histidine) left VISTA binding and function at acidic pH intact, though did not confer binding at pH 7.4 (Fig. 28C-D and F). Replacement with noncharged alanine (H2A) had intermediate effects (Fig 8-D). Functional testing recapitulated these binding data; H2R mutant VISTA suppressed T cell activation at acidic pH as potently as did wildtype VISTA, while H2D mutant VISTA had little effect (Fig 28F). VISTA blocking and non-blocking antibodies bound comparably to wildtype and all mutant VISTA proteins (Figs. 28G and H). These results suggest that protonation of VISTA residues H153, H154, H155, and potentially other histidines, is required for PSGL-1 binding.

VISTA-Fc proteins with H153, H154, and H155 residues mutated to alanine, aspartic acid, or arginine were produced by transient transfection of Expi293 cells.

We then used the solved structures of PSGL-1 bound to P-selectin (Somers, W. S., Tang, J., Shaw, G. D. & Camphausen, R. T. Cell 103, 467-479 (2000) and VISTA bound to P1-068767 Fab (Fig. 27) to develop a computational model of the PSGL-1 19-mer glycopeptide docked to VISTA (Fig. 28E and K). In this model, sulfated PSGL-1 tyrosine residues Y46 and Y48 make ionic interactions with VISTA histidine residues H153 and H154 (2.5 – 3.0 Å distances). PSGL-1 E56 and sulfated Y51 residues are more distant from VISTA, but may interact with a flexible, histidine-rich loop spanning VISTA residues 99-110. PSGL-1 Y51 is more distant from VISTA (~ 4.5 Å), but may interact meaningfully with VISTA H100. PSGL-1 E56 also forms ionic interactions with VISTA

H98 and H100. The hydroxyl group of PSGL-1 T57, which can be decorated with sialyl lewis X, points away from VISTA, consistent with the negligible influence of sialyl lewis X on VISTA : PSGL-1 binding. Taken together, these data and modeling suggest that VISTA binding to PSGL-1 at acidic pH is driven primarily by the VISTA histidine residues H153, H154, and H155, and by the PSGL-1 sulfated tyrosine (sulfotyrosine) residues Y46 and Y48. These results suggest that protonated VISTA histidine residues and sulfated PSGL-1 tyrosine residues drive binding.

VISTA was recently reported to bind V-set Immunoglobulin domain containing 3 (VSIG-3), a surface receptor expressed in brain, testis, and some cancer tissues (Wang, J. et al. VSIG-3 as a ligand of VISTA inhibits human T-cell function. Immunology 156, 74-85 (2019)). Consistent with this report, we observed recombinant VISTA binding to recombinant VSIG-3. VISTA : VSIG-3 binding was moderately enhanced at acidic pH. However, recombinant VSIG-3 did not bind specifically to VISTA-expressing cells, and recombinant VISTA did not bind specifically to VSIG-3-expressing cells. VSIG3 was also unable to compete with PSGL-1 for binding to VISTA. These data suggest that VSIG-3 is not likely to impair VISTA : PSGL-1-mediated T cell binding.

In VSIG-3 binding assays, CHO and HEK293 cells were engineered to ectopically express human VSIG-3 and VISTA respectively. VSIG-3 expression was confirmed by flow cytometry using anti-VSIG-3 (pAb AF4915, R&D Systems). VISTA expression was confirmed by flow cytometry using anti-VISTA (clone 740804, R&D Systems). Cell binding assays were performed in PBS buffers containing 0.9 mM CaCl2, 0.05 mM MgCl2, and 0.5% BSA that were adjusted to the indicated pH by varying the ratios of Na2HPO4 and KH2PO4. VISTA-Fc and VSIG-3-Fc were used at 10 μg/mL. Binding was detected with anti-human IgG Fab'2 PE (Invitrogen).

VISTA has also been reported to engage in homotypic binding (Yoon, K. W. et al. Control of signaling-mediated clearance of apoptotic cells by the tumor suppressor p53. Science 349, 1261669 (2015)), though we are unable detect this interaction at acidic or neutral pH.

To further look into the potential role of H98 and H100 of VISTA in binding to PSGL1 and VISTA antibodies, the impact of histidine to alanine, arginine and aspartic acid mutations at H98 and H100 were determined individually and in combination with H153, H154 and H155 mutations in hVISTAFc (human Fc). These Fc-fusion samples

were produced in transiently transfected Expi293 cells, normalized to about 250nM in pH 6 or 7.4 buffer. Binding responses, which are shown in Figure 29, are reference (blocked sensor)-subtracted, and are the responses to 500nM huPSGL1, or 250nM each indicated antibody.

The data, which is shown in Figure 29, implicates H98/H100 of hVISTA in binding to PSGL1, and (as expected based on the yeast surface display assay described above) is also important in binding of the acidic-selective antibodies P1-068761 ('761) and P1-068767 ('767). Consistent with prior data, VISTA.5 and P1-061029 bound to all mutants. As with the H153R/H154/H155R mutant, H98R/H100R mutant retained PSGL1 binding, as did the quintuple mutant combination. Thus, this data further confirms the role of histidine residues 98 and 100 in hVISTA in mediating binding to PSGL1 and to the pH selective antibodies '761 and '767.

Sequence Table

The following is a table of certain sequences referred to in this application. In SEQ ID NO: 2, amino acid position 187 may be either a D or an E.

In the antibody sequences below, the VH CDR1, CDR2, and CDR3 sequences are located at amino acid positions comprising amino acids 26-35, 50-66, and 99-110, respectively, and the VL CDR1, CDR2, and CDR3 sequences are located at amino acid positions comprising amino acids 24-35, 51-57, and 90-98, respectively. The VH CDR1 is numbered according to AbM (AA 26-35; Abhinandan and Martin (2008) Mol. Immunol. 45:3832-3839; Swindells et al. (2017) J. Mol. Biol. 429:356-364) and all other CDRs (VH CDR2, VH CDR3, VL CDR1-3) are numbered according to Kabat. The CDR sequences of particular antibody species are bold and underlined below on their VH and VL sequences.

SEQ ID NO	Name	Sequence
1	hVISTA (with leader sequence)	MGVPTALEAG SWRWGSLLFA LFLAASLGPV AAFKVATPYS LYVCPEGQNV TLTCRLLGPV DKGHDVTFYK TWYRSSRGEV QTCSERRPIR NLTFQDLHLH HGGHQAANTS HDLAQRHGLE SASDHHGNFS ITMRNLTLLD SGLYCCLVVE IRHHHSEHRV HGAMELQVQT GKDAPSNCVV YPSSSQ**D**SEN ITAAALATGA CIVGILCLPL ILLLVYKQRQ AASNRRAQEL VRMDSNIQGI ENPGFEASPP AQGIPEAKVR HPLSYVAQRQ PSESGRHLLS EPSTPLSPPG PGDVFFPSLD PVPDSPNFEV I
2	hVISTA (no leader sequence)	FKVATPYSLY VCPEGQNVTL TCRLLGPVDK GHDVTFYKTW YRSSRGEVQT CSERRPIRNL TFQDLHLHHG GHQAANTSHD LAQRHGLESA SDHHGNFSIT MRNLTLLDSG LYCCLVVEIR HHHSEHRVHG AMELQVQTGK DAPSNCVVYP SSSQ**[D/E]**SENIT AAALATGACI VGILCLPLIL LLVYKQRQAA SNRRAQELVR MDSNIQGIEN PGFEASPPAQ GIPEAKVRHP LSYVAQRQPS ESGRHLLSEP STPLSPPGPG DVFFPSLDPV PDSPNFEVI
3	Human PSGL-1 isoform 2 precursor, with signal peptide	MPLQLLLLLI LLGPGNSLQL WDTWADEAEK ALGPLLARDR RQATEYEYLD YDFLPETEPP EMLRNSTDTT PLTGPGTPES TTVEPAARRS TGLDAGGAVT ELTTELANMG NLSTDSAAME IQTTQPAATE AQTTQPVPTE AQTTPLAATE AQTTRLTATE AQTTPLAATE AQTTPPAATE AQTTQPTGLE AQTTAPAAME AQTTAPAAME AQTTPPAAME AQTTQTTAME AQTTAPEATE AQTTQPTATE AQTTPLAAME ALSTEPSATE ALSMEPTTKR GLFIPFSVSS VTHKGIPMAA SNLSVNYPVG APDHISVKQC LLAILILALV ATIFFVCTVV LAVRLSRKGH MYPVRNYSPT EMVCISSLLP DGGEGPSATA NGGLSKAKSP GLTPEPREDR EGDDLTLHSF LP
4	Human PSGL-1 isoform 2, without signal peptide	LQL WDTWADEAEK ALGPLLARDR RQATEYEYLD YDFLPETEPP EMLRNSTDTT PLTGPGTPES TTVEPAARRS TGLDAGGAVT ELTTELANMG NLSTDSAAME

SEQ ID NO	Name	Sequence
		IQTTQPAATE AQTTQPVPTE AQTTPLAATE AQTTRLTATE AQTTPLAATE AQTTPPAATE AQTTQPTGLE AQTTAPAAME AQTTAPAAME AQTTPPAAME AQTTQTTAME AQTTAPEATE AQTTQPTATE AQTTPLAAME ALSTEPSATE ALSMEPTTKR GLFIPFSVSS VTHKGIPMAA SNLSVNYPVG APDHISVKQC LLAILILALV ATIFFVCTVV LAVRLSRKGH MYPVRNYSPT EMVCISSLLP DGGEGPSATA NGGLSKAKSP GLTPEPREDR EGDDLTLHSF LP
5	Human PSGL-1 isoform 2 ECD, with signal peptide	MPLQLLLLLI LLGPGNSLQL WDTWADEAEK ALGPLLARDR RQATEYEYLD YDFLPETEPP EMLRNSTDTT PLTGPGTPES TTVEPAARRS TGLDAGGAVT ELTTELANMG NLSTDSAAME IQTTQPAATE AQTTQPVPTE AQTTPLAATE AQTTRLTATE AQTTPLAATE AQTTPPAATE AQTTQPTGLE AQTTAPAAME AQTTAPAAME AQTTPPAAME AQTTQT
6	Human PSGL-1 isoform 2 ECD, without signal peptide	LQL WDTWADEAEK ALGPLLARDR RQATEYEYLD YDFLPETEPP EMLRNSTDTT PLTGPGTPES TTVEPAARRS TGLDAGGAVT ELTTELANMG NLSTDSAAME IQTTQPAATE AQTTQPVPTE AQTTPLAATE AQTTRLTATE AQTTPLAATE AQTTPPAATE AQTTQPTGLE AQTTAPAAME AQTTAPAAME AQTTPPAAME AQTTQT
7	Human PSGL-1 ECD (N-terminal positions 42 to 295 of a full length Human PSGL-1 Accession No. AAC50061)	QATEYEYLD YDFLPETEPP EMLRNSTDTT PLTGPGTPES TTVEPAARRS TGLDAGGAVT ELTTELANMG NLSTDSAAME IQTTQPAATE AQTTPLAATE AQTTRLTATE AQTTPLAATE AQTTPPAATE AQTTQPTGLE AQTTAPAAME AQTTAPAAME AQTTPPAAME AQTTQTTAME AQTTAPEATE AQTTQPTATE AQTTPLAAME ALSTEPSATE ALSMEPTTKR GLFIPFSVSS VTHKGIPMAA SNLSV
8	HumPSGL-1 isoform 1 precursor, with signal peptide NP_001193538	MAVGASGLEG DKMAGAMPLQ LLLLLILLGP GNSLQLWDTW ADEAEKALGP LLARDRRQAT EYEYLDYDFL PETEPPEMLR NSTDTTPLTG PGTPESTTVE PAARRSTGLD AGGAVTELTT ELANMGNLST DSAAMEIQTT QPAATEAQTT QPVPTEAQTT PLAATEAQTT RLTATEAQTT PLAATEAQTT PPAATEAQTT QPTGLEAQTT APAAMEAQTT APAAMEAQTT PPAAMEAQTT QTTAMEAQTT APEATEAQTT QPTATEAQTT PLAAMEALST EPSATEALSM EPTTKRGLFI PFSVSSVTHK GIPMAASNLS VNYPVGAPDH ISVKQCLLAI LILALVATIF FVCTVVLAVR LSRKGHMYPV RNYSPTEMVC ISSLLPDGGE GPSATANGGL SKAKSPGLTP EPREDREGDD LTLHSFLP
9	Human PSGL-1, without signal peptide	LQLWDTW ADEAEKALGP LLARDRRQAT EYEYLDYDFL PETEPPEMLR NSTDTTPLTG PGTPESTTVE PAARRSTGLD AGGAVTELTT ELANMGNLST DSAAMEIQTT QPAATEAQTT QPVPTEAQTT PLAATEAQTT RLTATEAQTT PLAATEAQTT PPAATEAQTT QPTGLEAQTT APAAMEAQTT APAAMEAQTT PPAAMEAQTT QTTAMEAQTT APEATEAQTT QPTATEAQTT PLAAMEALST EPSATEALSM EPTTKRGLFI PFSVSSVTHK GIPMAASNLS VNYPVGAPDH ISVKQCLLAI LILALVATIF FVCTVVLAVR LSRKGHMYPV RNYSPTEMVC ISSLLPDGGE GPSATANGGL SKAKSPGLTP EPREDREGDD LTLHSFLP
10	Human PSGL-1 ECD, with signal peptide	MAVGASGLEG DKMAGAMPLQ LLLLLILLGP GNSLQLWDTW ADEAEKALGP LLARDRRQAT EYEYLDYDFL PETEPPEMLR NSTDTTPLTG PGTPESTTVE PAARRSTGLD AGGAVTELTT ELANMGNLST DSAAMEIQTT QPAATEAQTT QPVPTEAQTT PLAATEAQTT RLTATEAQTT PLAATEAQTT PPAATEAQTT QPTGLEAQTT APAAMEAQTT APAAMEAQTT PPAAMEAQTT QT

SEQ ID NO	Name	Sequence
11	P1-069059 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSDHIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
12	P1-069059 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
13	P1-069059 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSDHIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
14	P1-069059 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
15	P1-069061 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
16	P1-069061 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
17	P1-069061 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
18	P1-069061 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
19	P1-069063 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDADDE**WGQGTMVTVSS
20	P1-069063 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
21	P1-069063 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDADDEWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED

SEQ ID NO	Name	Sequence
		PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
22	P1-069063 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
23	P1-069065 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSDDIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
24	P1-069065 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
25	P1-069065 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSDDIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
26	P1-069065 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
27	P1-069067 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDADDE**WGQGTMVTVSS
28	P1-069067 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
29	P1-069067 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDADDEWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
30	P1-069067 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
31	P1-069069 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDADDV**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
32	P1-069069 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
33	P1-069069 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDADDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
34	P1-069069 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
35	P1-069071 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSADIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSEGWIDAFDV**WGQGTMVTVSS
36	P1-069071 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
37	P1-069071 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSADIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSEGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
38	P1-069071 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
39	P1-069073 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
40	P1-069073 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
41	P1-069073 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

SEQ ID NO	Name	Sequence
		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
42	P1-069073 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
43	P1-069075 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWDSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
44	P1-069075 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
45	P1-069075 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWDSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
46	P1-069075 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
47	P1-069077 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSDEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
48	P1-069077 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
49	P1-069077 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSDEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
50	P1-069077 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
51	P1-068761 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
52	P1-068761 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
53	P1-068761 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
54	P1-068761 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
55	P1-068767 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
56	P1-068767 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
57	P1-068767 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
58	P1-068767 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
59	P1-068773 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSDNIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
60	P1-068773 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
61	P1-068773 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSDNIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

SEQ ID NO	Name	Sequence
		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
62	P1-068773 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
63	P1-068765 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTDEDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
64	P1-068765 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
65	P1-068765 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTDEDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
66	P1-068765 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
67	P1-061029 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
68	P1-061029 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
69	P1-061029 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
70	P1-061029 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
71	P1-068757 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
72	P1-068757 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
73	P1-068757 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
74	P1-068757 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
75	P1-068771 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSHEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
76	P1-068771 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
77	P1-068771 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSHEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
78	P1-068771 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
79	P1-068775 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GIDWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDD**WGQGTMVTVSS
80	P1-068775 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
81	P1-068775 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGIDWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

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		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
82	P1-068775 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
83	P1-068769 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSDHIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
84	P1-068769 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
85	P1-068769 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSDHIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
86	P1-068769 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
87	P1-068759 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
88	P1-068759 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
89	P1-068759 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
90	P1-068759 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
91	P1-068763 VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
92	P1-068763 VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
93	P1-068763 IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
94	P1-068763 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
95	P1-061015 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYAMH**WVRQAPGKGLE WVA**IIWYDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYSSYYFDY**WGQGTLVTVSS
96	P1-061015 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
97	P1-061015 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAIIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYSSYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
98	P1-061015 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
99	P1-068748 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSHHAMH**WVRQAPGKGLE WVA**IIWYDGSNDDYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYDSYYFDY**WGQGTLVTVSS
100	P1-068748 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
101	P1-068748 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSHHAMHWVRQAPGKGLE WVAIIWYDGSNDDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYDSYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS

SEQ ID NO	Name	Sequence
		LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
102	P1-068748 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
103	P1-068744 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSEYAMH**WVRQAPGKGLE WVA**HIWYDGSNKYEADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYESYYFDE**WGQGTLVTVSS
104	P1-068744 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
105	P1-068744 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSEYAMHWVRQAPGKGLE WVAHIWYDGSNKYEADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYESYYFDEWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
106	P1-068744 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
107	P1-068736 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSEYAMH**WVRQAPGKGLE WVA**IDWYDGSNKDYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYDSYYFDD**WGQGTLVTVSS
108	P1-068736 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
109	P1-068736 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSEYAMHWVRQAPGKGLE WVAIDWYDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYDSYYFDDWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
110	P1-068736 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
111	P1-068752 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYAMH**WVRQAPGKGLE WVA**EIWYDGSNKDYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYDSYYFDE**WGQGTLVTVSS

SEQ ID NO	Name	Sequence
112	P1-068752 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
113	P1-068752 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAEIWYDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYDSYYFDEWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
114	P1-068752 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
115	P1-068740 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSDYAMH**WVRQAPGKGLE WVA**IIWYDGSDKDYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYDSYYFDD**WGQGTLVTVSS
116	P1-068740 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
117	P1-068740 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYAMHWVRQAPGKGLE WVAIIWYDGSDKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYDSYYFDDWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
118	P1-068740 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
119	P1-068742 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSDYAMH**WVRQAPGKGLE WVA**IIWYDGSDKDYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYEDYYFDY**WGQGTLVTVSS
120	P1-068742 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
121	P1-068742 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYAMHWVRQAPGKGLE WVAIIWYDGSDKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYEDYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS

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		LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
122	P1-068742 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
123	P1-068746 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSSYAMH**WVRQAPGKGLE WVA**IIWYDGSNHHYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYDSYYFDY**WGQGTLVTVSS
124	P1-068746 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
125	P1-068746 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAIIWYDGSNHHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYDSYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
126	P1-068746 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
127	P1-068750 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSDYDMH**WVRQAPGKGLE WVA**EIWDDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DEEFYSSYYFDY**WGQGTLVTVSS
128	P1-068750 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
129	P1-068750 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYDMHWVRQAPGKGLE WVAEIWDDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDEEFYSSYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
130	P1-068750 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
131	P1-068738 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSEYAHH**WVRQAPGKGLE WVA**IIWDDGSNHYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFYEDYYFDY**WGQGTLVTVSS

SEQ ID NO	Name	Sequence
132	P1-068738 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
133	P1-068738 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSEYAHHWVRQAPGKGLE WVAIIWDDGSNHYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFYEDYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
134	P1-068738 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
135	P1-068754 VH	QVQLVESGGGVVQPGRSLRLSCAAS**GFTFSDYDMH**WVRQAPGKGLE WVA**EIWDDGSNKYYADSVKG**RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR**DSGFHSDYYFDY**WGQGTLVTVSS
136	P1-068754 VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFATYYC**QQY NSYPYT**FGQGTKLEIK
137	P1-068754 IgG1.3 HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYDMHWVRQAPGKGLE WVAEIWDDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDSGFHSDYYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
138	P1-068754 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFATYYCQQY NSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
139	P1-069293 VH (P1-068761.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
140	P1-069293 VL (P1-068761.IgG1f)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
141	P1-069293 HC (P1-068761.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

SEQ ID NO	Name	Sequence
		KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
142	P1-069293 LC (P1-068761.IgG1f)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
143	P1-069298 VH (P1-068767.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
144	P1-069298 VL (P1-068767.IgG1f)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
145	P1-069298 HC (P1-068767.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
146	P1-069298 LC (P1-068767.IgG1f)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
147	P1-069302 VH (P1-061029.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
148	P1-069302 VL (P1-061029.IgG1f)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
149	P1-069302 HC (P1-061029.IgG1f)	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
150	P1-069302 LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN

SEQ ID NO	Name	Sequence
	(P1-061029.IgG1f)	NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
151	P1-069312 VH (P1-068761.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
152	P1-069312 VL (P1-068761.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
153	P1-069312 HC (P1-068761.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
154	P1-069312 LC (P1-068761.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
155	P1-069309 VH (P1-068767.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
156	P1-069309 VL (P1-068767.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
157	P1-069309 HC (P1-068767.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO	Name	Sequence
158	P1-069309 LC (P1-068767.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
159	P1-069307 VH (P1-061029.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
160	P1-069307 VL (P1-061029.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
161	P1-069307 HC (P1-061029.IgG1f afucosylated)	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
162	P1-069307 LC (P1-061029.IgG1f afucosylated)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
163	IgG1.3 (or IgG1.3f) heavy chain constant region (L234A, L235E, G237A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPG
164	Exemplary light chain constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
165	Human VISTA NP_071436.1 (Fig. 1B)	See Fig. 1B
166	Cyno VISTA XP_005565644.1 (Fig. 1B)	See Fig. 1B
167	Mouse VISTA NP_083008.1 (Fig. 1B)	See Fig. 1B
168	P1-061029 HDR3 chip oligo (Fig. 7A)	**X**PGYSGGWIDAFDV

SEQ ID NO	Name	Sequence
169	P1-061029 HDR3 chip oligo (Fig. 7A)	XXGYSGGWIDAFDV
170	P1-061029 HDR3 chip oligo (Fig. 7A)	XPXYSGGWIDAFDV
171	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGXSGGWIDAFDV
172	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYXGGWIDAFDV
173	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSXGWIDAFDV
174	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGXWIDAFDV
175	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGXIDAFDV
176	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWXDAFDV
177	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWIXAFDV
178	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWIDXFDV
179	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWIDAXDV
180	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWIDAFXV
181	P1-061029 HDR3 chip oligo (Fig. 7A)	XPGYSGGWIDAFDX
182	IgG1f (human wild-type allotype f) heavy chain constant region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPG
183	IgG1.1f Heavy chain constant region (L234A, L235E, G237A, A330S, P331S)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

SEQ ID NO	Name	Sequence
		TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPG
184	IgG1fa.P238K (or IgG1.P238K) heavy chain constant region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGKSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPG
185	P1-070864 P1-068761_E30D VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
186	P1-070864 P1-068761_E30D VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
187	P1-070864 P1-068761_E30D IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
188	P1-070864 P1-068761_E30D LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
189	P1-070866 P1-068761_E32Y VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
190	P1-070866 P1-068761_E32Y VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
191	P1-070866 P1-068761_E32Y IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
192	P1-070866 P1-068761_E32Y LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO	Name	Sequence
193	P1-070868 P1-068761_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
194	P1-070868 P1-068761_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
195	P1-070868 P1-068761_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
196	P1-070868 P1-068761_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
197	P1-070870 P1-068761_E56N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
198	P1-070870 P1-068761_E56N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
199	P1-070870 P1-068761_E56N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
200	P1-070870 P1-068761_E56N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
201	P1-070872 P1-068761_H100G VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
202	P1-070872 P1-068761_H100G VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
203	P1-070872 P1-068761_H100G IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED

SEQ ID NO	Name	Sequence
		PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
204	P1-070872 P1-068761_H100G LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
205	P1-070874 P1-068761_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAFDV**WGQGTMVTVSS
206	P1-070874 P1-068761_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
207	P1-070874 P1-068761_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
208	P1-070874 P1-068761_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
209	P1-070876 P1-068761_E30D_E32Y VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
210	P1-070876 P1-068761_E30D_E32Y VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
211	P1-070876 P1-068761_E30D_E32Y IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
212	P1-070876 P1-068761_E30D_E32Y LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO	Name	Sequence
213	P1-070878 P1-068761_E55A_E56N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
214	P1-070878 P1-068761_E55A_E56N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
215	P1-070878 P1-068761_E55A_E56N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
216	P1-070878 P1-068761_E55A_E56N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
217	P1-070880 P1-068761_H100G_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
218	P1-070880 P1-068761_H100G_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
219	P1-070880 P1-068761_H100G_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
220	P1-070880 P1-068761_H100G_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
221	P1-070882 P1-068761_E32Y_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
222	P1-070882 P1-068761_E32Y_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
223	P1-070882 P1-068761_E32Y_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
224	P1-070882 P1-068761_E32Y_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
225	P1-070884 P1-068761_E32Y_E56N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
226	P1-070884 P1-068761_E32Y_E56N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
227	P1-070884 P1-068761_E32Y_E56N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
228	P1-070884 P1-068761_E32Y_E56N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
229	P1-070886 P1-068761_E32Y_H100G VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
230	P1-070886 P1-068761_E32Y_H100G VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
231	P1-070886	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA

SEQ ID NO	Name	Sequence
	P1-068761_E32Y_H100G IgG1.3 HC	LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
232	P1-070886 P1-068761_E32Y_H100G LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
233	P1-070888 P1-068761_E32Y_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAFDV**WGQGTMVTVSS
234	P1-070888 P1-068761_E32Y_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
235	P1-070888 P1-068761_E32Y_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
236	P1-070888 P1-068761_E32Y_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
237	P1-070890 P1-068761_E30D_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDEAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
238	P1-070890 P1-068761_E30D_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
239	P1-070890 P1-068761_E30D_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDEAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

SEQ ID NO	Name	Sequence
		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
240	P1-070890 P1- 068761_E30D_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
241	P1-070892 P1- 068761_E30D_E56N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDEAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAEDV**WGQGTMVTVSS
242	P1-070892 P1- 068761_E30D_E56N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
243	P1-070892 P1- 068761_E30D_E56N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDEAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
244	P1-070892 P1- 068761_E30D_E56N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
245	P1-070894 P1- 068761_E30D_H100 G VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
246	P1-070894 P1- 068761_E30D_H100 G VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
247	P1-070894 P1- 068761_E30D_H100 G IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
248	P1-070894 P1- 068761_E30D_H100 G	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN

SEQ ID NO	Name	Sequence
	LC	NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
249	P1-070896 P1-068761_E30D_E100 fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDEAMH**WVRQAPGKGLE WVS**GINWNSEEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAFDV**WGQGTMVTVSS
250	P1-070896 P1-068761_E30D_E100 fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
251	P1-070896 P1-068761_E30D_E100 fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDEAMHWVRQAPGKGLE WVSGINWNSEEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
252	P1-070896 P1-068761_E30D_E100 fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
253	P1-070898 P1-068761_E55A_E100 fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSAEIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAFDV**WGQGTMVTVSS
254	P1-070898 P1-068761_E55A_E100 fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
255	P1-070898 P1-068761_E55A_E100 fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSAEIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
256	P1-070898 P1-068761_E55A_E100 fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO	Name	Sequence
257	P1-070900 P1-068761_E56N_H100G VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
258	P1-070900 P1-068761_E56N_H100G VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
259	P1-070900 P1-068761_E56N_H100G IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
260	P1-070900 P1-068761_E56N_H100G LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
261	P1-070902 P1-068761_E56N_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDEAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSHGWIDAFDV**WGQGTMVTVSS
262	P1-070902 P1-068761_E56N_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
263	P1-070902 P1-068761_E56N_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDEAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSHGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
264	P1-070902 P1-068761_E56N_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
265	P1-070904 P1-068767_E30D VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
266	P1-070904 P1-068767_E30D VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
267	P1-070904 P1-068767_E30D IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
268	P1-070904 P1-068767_E30D LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
269	P1-070906 P1-068767_D52N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
270	P1-070906 P1-068767_D52N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
271	P1-070906 P1-068767_D52N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
272	P1-070906 P1-068767_D52N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
273	P1-070908 P1-068767_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
274	P1-070908 P1-068767_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
275	P1-070908 P1-068767_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

SEQ ID NO	Name	Sequence
		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
276	P1-070908 P1-068767_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
277	P1-070910 P1-068767_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDD**WGQGTMVTVSS
278	P1-070910 P1-068767_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
279	P1-070910 P1-068767_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
280	P1-070910 P1-068767_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
281	P1-070912 P1-068767_D102V VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
282	P1-070912 P1-068767_D102V VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
283	P1-070912 P1-068767_D102V IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
284	P1-070912 P1-068767_D102V LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
285	P1-070914 P1-068767_E30D_D52N VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS

SEQ ID NO	Name	Sequence
286	P1-070914 P1-068767_E30D_D52N VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
287	P1-070914 P1-068767_E30D_D52N IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
288	P1-070914 P1-068767_E30D_D52N LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
289	P1-070916 P1-068767_D52N_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
290	P1-070916 P1-068767_D52N_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
291	P1-070916 P1-068767_D52N_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
292	P1-070916 P1-068767_D52N_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
293	P1-070918 P1-068767_E55A_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDD**WGQGTMVTVSS
294	P1-070918 P1-068767_E55A_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
295	P1-070918	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA

SEQ ID NO	Name	Sequence
	P1-068767_E55A_E100fF IgG1.3 HC	LYYCAKVPGYSGGWIDAFDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
296	P1-070918 P1-068767_E55A_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
297	P1-070920 P1-068767_E100fF_D102V VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMHW**VRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDV**WGQGTMVTVSS
298	P1-070920 P1-068767_E100fF_D102V VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
299	P1-070920 P1-068767_E100fF_D102V IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
300	P1-070920 P1-068767_E100fF_D102V LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
301	P1-070922 P1-068767_E30D_E55A VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GIDWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDD**WGQGTMVTVSS
302	P1-070922 P1-068767_E30D_E55A VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
303	P1-070922 P1-068767_E30D_E55A IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGIDWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

SEQ ID NO	Name	Sequence
		VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
304	P1-070922 P1-068767_E30D_E55A LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
305	P1-070924 P1-068767_E30D_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDD**WGQGTMVTVSS
306	P1-070924 P1-068767_E30D_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYL**AWYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
307	P1-070924 P1-068767_E30D_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
308	P1-070924 P1-068767_E30D_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
309	P1-070926 P1-068767_E30D_D102V VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLDDYAMH**WVRQAPGKGLE WVS**GIDWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAEDV**WGQGTMVTVSS
310	P1-070926 P1-068767_E30D_D102V VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
311	P1-070926 P1-068767_E30D_D102V IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLDDYAMHWVRQAPGKGLE WVSGIDWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
312	P1-070926	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY

SEQ ID NO	Name	Sequence
	P1-068767_E30D_D102V LC	GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
313	P1-070928 P1-068767_D52N_E100fF VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAFDD**WGQGTMVTVSS
314	P1-070928 P1-068767_D52N_E100fF VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
315	P1-070928 P1-068767_D52N_E100fF IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAFDDWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
316	P1-070928 P1-068767_D52N_E100fF LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
317	P1-070930 P1-068767_D52N_D102V VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GINWNSENIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAED**VWGQGTMVTVSS
318	P1-070930 P1-068767_D52N_D102V VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
319	P1-070930 P1-068767_D52N_D102V IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGINWNSENIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
320	P1-070930 P1-068767_D52N_D102V LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO	Name	Sequence
321	P1-070932 P1-068767_E55A_D102 V VH	EVQLVESGGGLVQPGKSLRLSCAAS**GFTLEDYAMH**WVRQAPGKGLE WVS**GIDWNSANIGYADSVKG**RFTISRDNAKNSLYLQMNSLRTEDTA LYYCAK**VPGYSGGWIDAED**VWGQGTMVTVSS
322	P1-070932 P1-068767_E55A_D102 V VL	EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQY GSSPFT**FGPGTKVDIK
323	P1-070932 P1-068767_E55A_D102 V IgG1.3 HC	EVQLVESGGGLVQPGKSLRLSCAASGFTLEDYAMHWVRQAPGKGLE WVSGIDWNSANIGYADSVKGRFTISRDNAKNSLYLQMNSLRTEDTA LYYCAKVPGYSGGWIDAEDVWGQGTMVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
324	P1-070932 P1-068767_E55A_D102 V LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
325	hVISTA-ECD 6-His tag	AFKVATPYSL YVCPEGQNVT LTCRLLGPVD KGHDVTFYKT WYRSSRGEVQ TCSERRPIRN LTFQDLHLHH GGHQAANTSH DLAQRHGLES ASDHHGNFSI TMRNLTLLDS GLYCCLVVEI RHHHSEHRVH GAMELQVQTG KDAPSNCVVY PSSSQESENI TAHHHHHHH
326	Cyno VISTA-ECD 6-His tag	AFKVATLYSL YVCPEGQNVT LTCRVFGPVD KGHDVTFYKT WYRSSRGEVQ TCSERRPIRN LTFQDLHLHH GGHQAANTSH DLAQRHGLES ASDHHGNFSI TMRNLTLLDS GLYCCLVVEI RHHHSEHRVH GAMELQVQTG KDAPSSCVAY PSSSQESENI TAHHHHHHH
327	P1-069059 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGACCATATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
328	P1-069059 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
329	P1-069061 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA

SEQ ID NO	Name	Sequence
		TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
330	P1-069061 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
331	P1-069063 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGACGATGAATGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
332	P1-069063 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
333	P1-069065 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TGAAGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGACGACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
334	P1-069065 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
335	P1-069067 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA

SEQ ID NO	Name	Sequence
		CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGACGATGAATGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
336	P1-069067 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
337	P1-069069 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGACGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
338	P1-069069 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
339	P1-069071 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TGAAGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGAAGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
340	P1-069071 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
341	P1-069073 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TGAAGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG

SEQ ID NO	Name	Sequence
		ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
342	P1-069073 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
343	P1-069075 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGGACAGTGCTGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
344	P1-069075 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
345	P1-069077 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAAGA TGAAGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGACGAAATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
346	P1-069077 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
347	P1-068761 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A

SEQ ID NO	Name	Sequence
348	P1-068761 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
349	P1-068767 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
350	P1-068767 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
351	P1-068773 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGATAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
352	P1-068773 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
353	P1-068765 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGATGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
354	P1-068765 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG

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		CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
355	P1-061029 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
356	P1-061029 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
357	P1-068757 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
358	P1-068757 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
359	P1-068771 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTCATGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
360	P1-068771 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT

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		GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
361	P1-068775 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
362	P1-068775 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
363	P1-068769 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGATCACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
364	P1-068769 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
365	P1-068759 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
366	P1-068759 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA

SEQ ID NO	Name	Sequence
367	P1-068763 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
368	P1-068763 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
369	P1-061015 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAG CTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGTATGATGGAAGTAATAAATACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACTCCTCGTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
370	P1-061015 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
371	P1-068748 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTCA CCATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGTATGATGGAAGTAATGACGACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGACTCGTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
372	P1-068748 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
373	P1-068744 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA GTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCACATATATGGTATGATGGAAGTAATAAATACGAGGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA

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		CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGAATCGTACTACT TTGACGAGTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
374	P1-068744 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
375	P1-068736 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA GTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTGATTGGTATGATGGAAGTAATAAAGACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGACTCGTACTACT TTGACGACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
376	P1-068736 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
377	P1-068752 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAG CTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAGAGATATGGTATGATGGAAGTAATAAAGACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGACTCGTACTACT TTGACGAGTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
378	P1-068752 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
379	P1-068740 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA CTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGTATGATGGAAGTGATAAAGACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGACTCGTACTACT TTGACGACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
380	P1-068740 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG

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		CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
381	P1-068742 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA CTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGTATGATGGAAGTGATAAAGACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGAAGATTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
382	P1-068742 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
383	P1-068746 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAG CTATGCCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGTATGATGGAAGTAATCACCACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGACTCGTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
384	P1-068746 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
385	P1-068750 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA CTATGACATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAGAGATATGGGATGATGGAAGTAATAAATACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATGAGGAATTTTACTCCTCGTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
386	P1-068750 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA

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387	P1-068738 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA GTATGCCCATCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATGGGATGATGGAAGTAATCACTACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTTACGAAGATTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
388	P1-068738 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
389	P1-068754 VH DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGA GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTGA CTATGACATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAGAGATATGGGATGATGGAAGTAATAAATACTATGCAG ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCT GTGTATTACTGTGCGAGAGATAGTGGTTTTCACTCCGATTACTACT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
390	P1-068754 VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCA GGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGCCTGGAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTAT AATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCA AA
391	P1-070864 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
392	P1-070864 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
393	P1-070866 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA

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		CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
394	P1-070866 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
395	P1-070868 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
396	P1-070868 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
397	P1-070870 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
398	P1-070870 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
399	P1-070872 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG

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		ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
400	P1-070872 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
401	P1-070874 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
402	P1-070874 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
403	P1-070876 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
404	P1-070876 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
405	P1-070878 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A

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406	P1-070878 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
407	P1-070880 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
408	P1-070880 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
409	P1-070882 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
410	P1-070882 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
411	P1-070884 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
412	P1-070884 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG

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		CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
413	P1-070886 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
414	P1-070886 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
415	P1-070888 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
416	P1-070888 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
417	P1-070890 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
418	P1-070890 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT

SEQ ID NO	Name	Sequence
		GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
419	P1-070892 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
420	P1-070892 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
421	P1-070894 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
422	P1-070894 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
423	P1-070896 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
424	P1-070896 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA

SEQ ID NO	Name	Sequence
425	P1-070898 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTGAGATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
426	P1-070898 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
427	P1-070900 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
428	P1-070900 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
429	P1-070902 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TGAGGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCCATGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
430	P1-070902 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
431	P1-070904 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG

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		TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
432	P1-070904 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
433	P1-070906 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
434	P1-070906 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
435	P1-070908 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
436	P1-070908 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
437	P1-070910 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC

SEQ ID NO	Name	Sequence
		TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
438	P1-070910 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
439	P1-070912 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
440	P1-070912 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
441	P1-070914 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
442	P1-070914 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
443	P1-070916 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A

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444	P1-070916 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
445	P1-070918 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
446	P1-070918 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
447	P1-070920 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
448	P1-070920 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
449	P1-070922 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
450	P1-070922 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG

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		CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
451	P1-070924 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
452	P1-070924 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
453	P1-070926 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGATGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
454	P1-070926 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
455	P1-070928 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTTTTGATGACTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
456	P1-070928 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT

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		GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
457	P1-070930 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAATTGGAACAGTGAAAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
458	P1-070930 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
459	P1-070932 VH DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCA AGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTTGAGGA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTGATTGGAACAGTGCTAACATAGGCTATGCGG ACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAA CTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACGGCC TTGTATTACTGTGCAAAAGTTCCTGGGTATAGCGGTGGCTGGATTG ACGCTGAAGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTC A
460	P1-070932 VL DNA	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGG CTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACA GGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAG CAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTAT GGTAGCTCACCATTCACTTTCGGCCCTGGGACCAAAGTGGATATCA AA
461	IgG1.3 heavy chain constant region DNA	GCGTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCA AGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGA CTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG CACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACA CATGCCCACCGTGCCCAGCACCTGAAGCCGAAGGGGCCCCGTCAGT CTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACC CTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAT CGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGC CGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC

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		ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCA AGCTCACCGTGGCGTCGACCAAGGGCCCATCGGTCTTCCCCCTGGC ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACT CAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGC CCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGA CAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCGAAGGG GCCCCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAG CCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCC GAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGAC CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT CCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCCCCGGGTTGA
462	Exemplary light chain constant region DNA	CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATG AGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAA CTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGC AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTT AG
463	VISTA.4 VH 41F11 Signal sequence is underlined	MEFGLSWVFLVAIIKGVQCQVQLVESGGGLVKPGGSLRLSCAASGF TFSDYYMSWIRQAPGKGLEWVSYISNSGSPIYYADSVKGRFTISRD NAKNSLYLQMNSLRAEDTAVYYCARDLPGWYFDLWGRGTLVTVSS
464	VISTA.4 VK1 VL 41F11 Signal sequence is underlined	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCRAS QSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSASGSGTDFT LTISSLEPEDFAVYYCQQRNNWPRTFGQGTKVEIK
465	VISTA.4 VK2 VL 41F11 Signal sequence is underlined	MDMRVLAQLLGLLLLCFPGARCDIQMTQSPSSLSASVGDRVTITCR ASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK
466	VISTA.4 VK3 VL 41F11 Signal sequence is underlined	METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSLSPGERATLSCRAS QSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK
467	VISTA.4 VH DNA 41F11 Signal sequence is underlined	ATGGAGTTTGGGCTGAGCTGGGTTTTCCTTGTTGCTATTATAAAAG GTGTCCAGTGTCAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGT CAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTC ACCTTCAGTGACTATTACATGAGCTGGATCCGCCAGGCTCCAGGGA AGGGGCTGGAGTGGGTTTCATACATTAGTAATAGTGGTAGTCCCAT ATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGAC AACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCG

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		AGGACACGGCCGTGTATTACTGTGCGAGAGATCTCCCGGGCTGGTA CTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCCTCA
468	VISTA.4 VK1 VL DNA Signal sequence is underlined	ATGGAAGCCCCAGCTCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC CAGATACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCT GTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGT CAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAACCTGGCC AGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGG CATCCCAGCCAGGTTCAGTGCCAGTGGGTCTGGGACAGACTTCACT CTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAGTTTATTACT GTCAGCAGCGTAACAACTGGCCTCGGACGTTCGGCCAAGGGACCAA GGTGGAAATCAAA
469	VISTA.4 VK2 VL DNA Signal sequence is underlined	ATGGACATGAGGGTCCTCGCTCAGCTCCTGGGGCTCCTGCTGCTCT GTTTCCCAGGTGCCAGATGTGACATCCAGATGACCCAGTCTCCATC CTCACTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGTCGG GCGAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTATCAGCAGAAAC CAGAGAAAGCCCCTAAGTCCCTGATCTATGCTGCATCCAGTTTGCA AAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAT TTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTT ATTACTGCCAACAGTATAATAGTTACCCTCGGACGTTCGGCCAAGG GACCAAGGTGGAAATCAAA
470	VISTA.4 VK3 VL DNA Signal sequence is underlined	ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC CAGATACCACCGGAGAAATTGTGTTGACGCAGTCTCCAGGCACCCT GTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGT CAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTG GCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCAC TGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTC ACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATT ACTGTCAGCAGTATGGTAGCTCACCGTGGACGTTCGGCCAAGGGAC CAAGGTGGAAATCAAA
471	VISTA.4.A64G VK1 VL Signal sequence is underlined	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCRAS QSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSLEPEDFAVYYCQQRNNWPRTFGQGTKVEIK
472	VISTA.4.A64G LC Signal sequence is underlined	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCRAS QSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSLEPEDFAVYYCQQRNNWPRTFGQGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
473	P1-065333 VH 41F11_VH_T28P/Y50W/S55E/L96E/Y100E VH	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDEPGWEFDLWGRGTLVTVSS
474	P1-070976 VH 41F11_VH_T28P/Y50W/S55E/D95H/L96E/P97E/Y100E VH	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARHEEGWEFDLWGRGTLVTVSS
475	P1-065333 HC 41F11_VH_T28P/Y50W/S55E/L96E/Y100E HC	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDEPGWEFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC

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		KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
476	P1-070976 HC 41F11_VH_T28P/Y50W/S55E/D95H/L96E/P97E/Y100E HC	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARHEEGWEFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
477	P1-071799 VH P1-070976_H95D VH	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDEEGWEFDLWGRGTLVTVSS
478	P1-071801 VH P1-070976_E97P VH	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARHEPGWEFDLWGRGTLVTVSS
479	VISTA.4.A64G VK1 VL P1-071799 VL P1-070976_H95D VL P1-070976_E97P VL P1-070976_A64G VL	EIVLTQSPATLSLSPGERATLSC**RASQSVSSYLA**WYQQKPGQAPRL LIY**DASNRAT**GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC**QQRN NWPRT**FGQGTKVEIK
480	P1-071799 HC P1-070976_H95D HC (IgG1.3)	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDEEGWEFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
481	P1-071801 HC P1-070976_ E97P HC (IgG1.3)	QVQLVESGGGLVKPGGSLRLSCAASGFPFSDYYMSWIRQAPGKGLE WVSWISNSGEPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARHEPGWEFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
482	VISTA.4.A64G VK1 LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRN

SEQ ID NO	Name	Sequence
	P1-071799 LC P1-070976_H95D LC P1-070976_E97P LC P1-070976_A64G LC	NWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
483	P1-071799 VH P1-070976_H95D VH DNA	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAG GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCCCTTCAGTGA CTATTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTTTCATGGATTAGTAATAGTGGTGAGCCCATATACTACGCAG ACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAA CTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTGTATTACTGTGCGAGAGATGAAGAGGGCTGGGAGTTCGATCTCT GGGGCCGTGGCACCCTGGTCACTGTCTCCTCA
484	P1-071801 VH P1-070976_E97P VH DNA	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAG GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCCCTTCAGTGA CTATTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTTTCATGGATTAGTAATAGTGGTGAGCCCATATACTACGCAG ACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAA CTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTGTATTACTGTGCGAGACATGAACCGGGCTGGGAGTTCGATCTCT GGGGCCGTGGCACCCTGGTCACTGTCTCCTCA
485	P1-071799 VL P1-070976_H95D VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTC CTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGT TCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAG CCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAAC AACTGGCCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
486	P1-071801 VL P1-070976_E97P VL DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTC CTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGT TCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAG CCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAAC AACTGGCCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
487	P1-071799 HC P1-070976_H95D HC DNA	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAG GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCCCTTCAGTGA CTATTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTTTCATGGATTAGTAATAGTGGTGAGCCCATATACTACGCAG ACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAA CTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTGTATTACTGTGCGAGAGATGAAGAGGGCTGGGAGTTCGATCTCT GGGGCCGTGGCACCCTGGTCACTGTCTCCTCAGCGTCGACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC CGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAG AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAAGCCGAAGGGGCCCCGTCAGTCTTCCTCTTCCCCC CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC

SEQ ID NO	Name	Sequence
		ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCT CACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC CCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC CCCGGGTTGA
488	P1-071799 HC P1-070976_ E97P HC DNA	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAG GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCCCTTCAGTGA CTATTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTTTCATGGATTAGTAATAGTGGTGAGCCCATATACTACGCAG ACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAA CTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTGTATTACTGTGCGAGACATGAACCGGGCTGGGAGTTCGATCTCT GGGGCCGTGGCACCCTGGTCACTGTCTCCTCAGCGTCGACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC CGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAG AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAAGCCGAAGGGGCCCCGTCAGTCTTCCTCTTCCCCC CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCT CACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC CCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC CCCGGGTTGA
489	P1-071799 LC P1-070976_H95D LC DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTC CTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGT TCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAG CCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAAC AACTGGCCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC GTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAA

SEQ ID NO	Name	Sequence
		GGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGG GCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTA G
490	P1-071799 LC P1-070976_ E97P LC DNA	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAG GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG CTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTC CTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGT TCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAG CCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAAC AACTGGCCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC GTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAA GGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGG GCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTA G
491	VISTA.4 HC (IgG1.3)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLE WVSYISNSGSPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDLPGWYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
492	VISTA.4 VH 41F11 without Signal sequence	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLE WVSYISNSGSPIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDLPGWYFDLWGRGTLVTVSS
493	VISTA.4 VK1 VL without Signal sequence	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSASGSGTDFTLTISSLEPEDFAVYYCQQRN NWPRTFGQGTKVEIK
494	VISTA.4 LC without Signal sequence	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSASGSGTDFTLTISSLEPEDFAVYYCQQRN NWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
495	VISTA.5 VH	AVQLQESGPGLVRPSQSLSLTCTVTDYSITSDYAWNWIRQFPGSKL EWLGFIGYSGNTNYNPSLESRISITRHTSKNQFFLHLNSMTTEDTA TYYCARSLYGGSHWYFDVWGAGTTVTVSS
496	VISTA.5 VK1 VL	DIVLTQSPASLAVSLGQRATISCRGSESVEYYGTILMQWYQQKPGQ PPKLLIYGASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC QQSRKVPWTFGGGTKLEIK
497	hVISTA residues 86 to 97	RRPIRNLTFQDL
498	hVISTA residues 57 to 68	LGPVDKGHDVTF
499	hVISTA residues 148 to 165	VVEIRHHHSEHRVHGAME
500	VL CDR1 VISTA.4	RASQSVSSYLA
501	VL CDR2 VISTA.4	DASNRAT

SEQ ID NO	Name	Sequence
502	VL CDR3 VISTA.4	QQRNNWPRT
503	VH CDR1 VISTA.4	GFTFSDYYMS
504	VH CDR2 VISTA.4	YISNSGSPIYYADSVKG
505	VH CDR3 VISTA.4	DLPGWYFDL
506	VH CDR1 P1-065333	GFPFSDYYMS
507	VH CDR2 P1-065333	WISNSGEPIYYADSVKG
508	VH CDR3 P1-065333	DEPGWEFDL
509	VH CDR1 P1-070976	GFPFSDYYMS
510	VH CDR2 P1-070976	WISNSGEPIYYADSVKG
511	VH CDR3 P1-070976	HEEGWEFDL
512	VH CDR1 P1-070976 H95D (P1-071799)	GFPFSDYYMS
513	VH CDR2 P1-070976 H95D (P1-071799)	WISNSGEPIYYADSVKG
514	VH CDR3 P1-070976 H95D (P1-071799)	DEEGWEFDL
515	VH CDR1 P1-070976 E97P (P1-071801)	GFPFSDYYMS
516	VH CDR2 P1-070976 E97P (P1-071801)	WISNSGEPIYYADSVKG
517	VH CDR3 P1-070976 E97P (P1-071801)	HEPGWEFDL

CLAIMS

1. An isolated antibody that binds specifically to human VISTA (hVISTA) in acidic conditions.
2. The isolated antibody of claim 1, which binds specifically to hVISTA in acidic conditions, but not significantly in neutral or physiological conditions.
3. The isolated antibody of claim 1 or 2, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 10 fold lower than its K_D in neutral or physiological conditions.
4. The isolated antibody of any one of claims 1 to 3, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 100 fold lower than its K_D in neutral or physiological conditions.
5. The isolated antibody of any one of claims 1-4, wherein the antibody binds to hVISTA in acidic conditions with a K_D that is at least 1000 fold lower than its K_D in neutral or physiological conditions.
6. The isolated antibody of any one of claims 1-5, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} M or more.
7. The isolated antibody of any one of claims 1-6, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} M or more.
8. The isolated antibody of any one of claims 1-7, wherein the antibody binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-3} M or more.
9. The isolated antibody of any one of claims 1-8, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} M or less.
10. The isolated antibody of any one of claims 1-9, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less.
11. The isolated antibody of any one of claims 1-10, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-9} M or less.
12. The isolated antibody of any one of claims 1-11, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-4} or more.
13. The isolated antibody of any one of claims 1-12, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-7} or less and binds to hVISTA in neutral or physiological conditions with a K_D of 10^{-5} or more.

14. The isolated antibody of any one of claims 1-13, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 5 fold lower than its k_{off} in neutral or physiological conditions.

15. The isolated antibody of any one of claims 1-14, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 10 fold lower than its k_{off} in neutral or physiological conditions.

16. The isolated antibody of any one of claims 1-15, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 50 fold lower than its k_{off} in neutral or physiological conditions.

17. The isolated antibody of any one of claims 1-16, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} that is at least 100 fold lower than its k_{off} in neutral or physiological conditions.

18. The isolated antibody of any one of claims 1-17, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7×10^{-3} s^{-1} or less.

19. The isolated antibody of any one of claims 1-18, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5×10^{-3} s^{-1} or less.

20. The isolated antibody of any one of claims 1-19 wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3×10^{-3} s^{-1} or less.

21. The isolated antibody of any one of claims 1-20, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-3} s^{-1} or less.

22. The isolated antibody of any one of claims 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7×10^{-4} s^{-1} or less.

23. The isolated antibody of any one of claims 1-22, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of $5x\ 10^{-4}$ s^{-1} or less.

24. The isolated antibody of any one of claims 1-23, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3×10^{-4} s^{-1} or less.

25. The isolated antibody of any one of claims 1-24, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less.

26. The isolated antibody of any one of claims 1-25, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 7×10^{-5} s^{-1} or less.

27. The isolated antibody of any one of claims 1-26, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5×10^{-5} s^{-1} or less.

28. The isolated antibody of any one of claims 1-21, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 3 x 10^{-5} s^{-1} or less.
29. The isolated antibody of any one of claims 1-28, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-5} s^{-1} or less.
30. The isolated antibody of any one of claims 1-29, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-3} s^{-1} or more.
31. The isolated antibody of any one of claims 1-30, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 3 x 10^{-3} s^{-1} or more.
32. The isolated antibody of any one of claims 1-31, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 5 x 10^{-3} s^{-1} or more.
33. The isolated antibody of any one of claims 1-32, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 7 x 10^{-3} s^{-1} or more.
34. The isolated antibody of any one of claims 1-33, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.
35. The isolated antibody of any one of claims 1-34, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 3 x 10^{-2} s^{-1} or more.
36. The isolated antibody of any one of claims 1-35, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 5 x 10^{-2} s^{-1} or more.
37. The isolated antibody of any one of claims 1-36, wherein the antibody binds to hVISTA in neutral or physiological conditions with a k_{off} of 7 x 10^{-2} s^{-1} or more.
38. The isolated antibody of any one of claims 1-37, wherein binding of the antibody to hVISTA in neutral or physiological conditions is not detectable, e.g., via surface plasmon resonance (SPR).
39. The isolated antibody of any one of claims 1-38, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 5 x 10^{-3} s^{-1} or less and in neutral or physiological conditions with a k_{off} of 7 x 10^{-3} s^{-1} or more.
40. The isolated antibody of any one of claims 1-39, wherein the antibody binds to hVISTA in acidic conditions with a k_{off} of 10^{-4} s^{-1} or less and in neutral or physiological conditions with a k_{off} of 10^{-2} s^{-1} or more.
41. The isolated antibody of any one of claims 1-40, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of 5 x 10^{-3} s^{-1} or

less and in neutral of physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of 7 x 10^{-3} s^{-1} or more.

42. The isolated antibody of any one of claims 1-41, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-8} M or less and a k_{off} of 3 x 10^{-3} s^{-1} or less and in neutral of physiological conditions with a K_D of 10^{-6} M or more and a k_{off} of 10^{-2} s^{-1} or more.

43. The isolated antibody of any one of claims 1-42, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to 5 x 10^{-3} s^{-1} and in neutral of physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3 x 10^{-3} to 10^{-2} s^{-1} or more.

44. The isolated antibody of any one of claims 1-43, wherein the antibody binds to hVISTA in acidic conditions with a K_D of 10^{-12} to 10^{-8} M and a k_{off} of 10^{-4} to 5 x 10^{-3} s^{-1} and in neutral of physiological conditions with a K_D of 10^{-7} to 10^{-4} M and a k_{off} of 3 x 10^{-3} to 10^{-2} s^{-1} or more; and wherein the antibody binds to cyno VISTA with a K_D of 10^{-7} or less.

45. The isolated antibody of any one of claims 1-44, wherein the antibody binds to hVISTA with a K_D that is at least 10 fold lower at pH 6.9 than at pH 7.4 and/or a K_D that is at least 100 fold lower at pH 6.5 than at pH 7.4 and at least 1000 fold lower at pH 6.0 than at pH 7.4.

46. The isolated antibody of any one of claims 1-45, wherein the antibody binds specifically to cynomolgus (cyno) VISTA.

47. The isolated antibody of claim 46, wherein the antibody binds to cyno VISTA with higher affinity in acidic conditions relative to physiologic conditions.

48. The isolated antibody of any one of claims 45-47, wherein the antibody binds to cyno VISTA in acidic conditions with a K_D of 10^{-8} or less and/or a k_{off} of 10^{-2} or less and in physiologic conditions with a K_D of 10^{-6} or more and/or a k_{off} of 10^{-2} or more.

49. The isolated antibody of any one of claims 1-48, wherein acidic conditions are conditions having a pH of 6.5 or less.

50. The isolated antibody of any one of claims 1-49, wherein acidic conditions are conditions having a pH of 6.0 to 6.5.

51. The isolated antibody of any one of claim 1-50, wherein neutral conditions are conditions having a pH of 7.0.
52. The isolated antibody of any one of claims 1-51, wherein physiological conditions are conditions having a pH of 7.35 to 7.45.
53. The isolated antibody of any one of claims 1-52, wherein physiological conditions are conditions having a pH of 7.4.
54. The isolated antibody of any one of claims 1-53, wherein the antibody inhibits the binding of hVISTA to human T cells, such as human CD4+ T cells (an antagonist antibody).
55. The isolated antibody of claim 54, wherein the antibody inhibits the binding of hVISTA to human T cells in conditions having a pH of less than pH 7.0.
56. The isolated antibody of any one of claims 1-55, wherein the antibody inhibits the binding of hVISTA to human PSGL-1 (huPSGL-1) and/or the Ab competes with huPSGL1 for binding to hVISTA.
57. The isolated antibody of claim 56, wherein the antibody inhibits the binding of hVISTA to huPSGL-1 in conditions having a pH of less than pH 7.0.
58. The isolated antibody of any one of claims 1-57, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans.
59. The isolated antibody of claim 58, wherein the antibody inhibits the binding of hVISTA to heparan sulfate proteoglycans in conditions having a pH of less than pH 7.0.
60. The isolated antibody of any one of claims 55, 57 or 59, wherein a condition having a pH of less than pH 7.0 is a tumor or any diseased area having a pH of less than pH 7.0 in a subject and in which an immune stimulation is desired.
61. The isolated antibody of any one of claims 1-60, wherein the antibody stimulates T cell activation, as evidenced by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling; as determined, e.g., as described in the Examples.
62. The isolated antibody of claim 61, wherein the antibody stimulates T cell activation in conditions having a pH of less than pH 7.0.
63. The isolated antibody of any one of claims 1-62, wherein the antibody reduces VISTA mediated cell-cell adhesion.

64. The isolated antibody of any one of claims 1-63, wherein the antibody has a mean residence time (MRT) of at least 100, 200, 300, 400 or 500 days in Cynomolgus macaques.

65. The isolated antibody of any one of claims 1-64, wherein the antibody does not significantly bind to VISTA positive cells, e.g., neutrophils, in the peripheral blood of a subject to whom it is administered.

66. The isolated antibody of any one of claims 1-65, wherein the antibody does not significantly deplete VISTA positive cells, e.g., neutrophils, in peripheral blood of a subject to whom it is administered.

67. The isolated antibody of any one of claims 1-66, wherein the antibody has been engineered to bind to hVISTA at acidic pH, but wherein the antibody does not bind specifically to hVISTA at neutral or physiological pH.

68. The isolated antibody of any one of claims 1-67, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine-rich ß-sheet extension.

69. The isolated antibody of claim 68, wherein the antibody binds at or near the histidine rich region of hVISTA, such as the histidine-rich ß-sheet extension, in conditions having a pH of 6.0-6.5.

70. The isolated antibody of any one of claims 1-68, wherein the antibody competes or cross-competes for binding to hVISTA with one or more antibodies described herein, e.g., comprising the VH and VL of P1-061029, P1-068757, P1-068759, P1-068761, P1-068763, P1-068765, P1-068767, P1-068769, P1-068771, P1-068773, P1-068775, P1-069059, P1-069061, P1-069063, P1-069065, P1-069067, P1-069069, P1-069071, P1-069073, P1-069075, P1-069077, P1-069077, P1-068761_E55A, P1-068761_H100G, P1-068761_E56N, P1-068761_E55A_E56N, P1-068761_E30D, P1-068761_E30D_E55A, P1-068761_E56N_H100G,P1-068761_E30D_H100G, or P1-068761_E30D_E56N, P1-068761_E100fF, P1-068761_E55A_E100fF, P1-068761_H100G_E100fF, P1-068761_E30D_E100fF, P1-068761_E56N_E100fF, P1-068761_E32Y, P1-068761_E32Y_E55A, P1-068761_E32Y_E56N, P1-068761_E30D_E32Y, P1-068761_E32Y_H100G, P1-068761_E32Y_E100fF, P1-068767_D52N_D102V, P1-068767_D52N, P1-068767_D52N_E55A, P1-068767_E55A_D102V, P1-

068767_D102V, P1-068767_E55A, P1-068767_E30D_D52N, P1-068767_E30D_D102V, P1-068767_E30D, P1-068767_E30D_E55A, P1-068767_E100fF_D102V, P1-068767_E55A_E100fF, P1-068767_D52N_E100fF, P1-068767_E100fF, P1-068767_E30D_E100fF, P1-061015, P1-068736, P1-068738, P1-068740, P1-068742, P1-068744, P1-068748, P1-068750, P1-068752 P1-068754, VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P, (e.g., in the context of IgG1.3); as determined, e.g., by the competitive BLI epitope binning assay described in Example 15.

71. The isolated antibody of any one of claims 1-70, wherein the antibody bins to epitope Group A, as determined, e.g., by the competitive BLI epitope binning assay described in Example 15.

72. The isolated antibody of any one of claims 1-70, wherein the antibody does not bind significantly to hVISTA in which one or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

73. An isolated antibody that binds specifically to hVISTA consisting of SEQ ID NO: 1 or 2, but that does not bind significantly to hVISTA in which one or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA; e.g., as determined by the yeast mutational analysis described in the Examples.

74. The isolated antibody of any one of claims 1-73, wherein the antibody does not bind significantly to hVISTA in which 2, 3, 4, 5 or more of the following amino acid residues have been mutated: V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

75. The isolated antibody of any one of claims 72-74, wherein the antibody does not bind significantly to hVISTA in which one or more of the following residues have been mutated to one of the corresponding residues shown in Table 15: V34, T35,

Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127, wherein numbering is that of mature hVISTA.

76. The isolated antibody of any one of claims 1-75, wherein the Ab binds to the amino acid region ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497), as determined by HDX-MS, as described in the Examples.

77. The isolated antibody of any one of claims 1-76, wherein the Ab binds to amino acid regions ^{86}RRPIRNLTFQDL^{97} of hVISTA (SEQ ID NO: 497) and also, but less strongly, to amino acids ^{57}LGPVDKGHDVTF^{68} (SEQ ID NO: 498) and ^{148}VVEIRHHHSEHRVHGAME^{165} (SEQ ID NO: 499).

78. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues of an antibody described herein, such as VISTA.4, P1-070976, P1-065333, P1-070976_H95D, P1-070976_E97P, P1-061015, P1-061029, P1-068761, or P1-068767, such as one or more amino acids selected from one of the following groups of energetically important contact residues: (i) V34, T35, Y37, K38, T39, Y41, S52, R54, T61, F62, Q63, L65, H66, L67, H68, H69, F97, L115, V117, I119, H121, H122, S124, E125, R127; (ii) V34, T35, Y37, T39, Y41, S52, R54, F62, L65, H66, H68, L115, V117, I119, R120, H121, H122, S124, E125; or (iii) Y37, T39, R54, F62, H66, L115 or V117, as determined, e.g., using the yeast surface display and NGS assay described in Example 15, and wherein numbering is that of mature hVISTA.

79. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using

the yeast surface display and NGS assay described in Example 15, and wherein numbering is that of mature hVISTA.

80. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab inhibits the interaction between VISTA and (a) T cells or (b) PSGL-1, and wherein the Ab contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues of VISTA.4, as determined, e.g., using the yeast surface display and NGS assay described in Example 15.

81. The isolated antibody of any one of claims 1-80, wherein the Ab binds to the FG loop of hVISTA.

82. The isolated antibody of any one of claims 1-81, wherein the Ab binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples.

83. The isolated antibody of any one of claims 1-82, wherein the Ab contacts H121, H122 and H123 of hVISTA (distance of, e.g., 4.0 Ångströms (Å) or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples.

84. The isolated antibody of any one of claims 1-83, wherein the Ab contacts hVISTA through VH CDR1 and VH CDR3, and for example, not significantly through VH CDR2 and/or through a VL CDR.

85. The isolated antibody of any one of claims 1-84, wherein heavy chain amino acid residues 110 and 112 of the antibody form hydrogen bonds with H121 and H122 of hVISTA, respectively, and optionally, wherein an amino acid residue of the antibody forms a hydrogen bond with H123 of hVISTA, wherein amino acid numbering of the antibody is according to that used in the Examples.

86. The antibody of claim 85, wherein the antibody comprises one or more (e.g., 1-5, 5-10, 10-15, 10-20 or 15-20) interactions with hVISTA listed in Table 31 under VISTA.4.

87. The isolated antibody of any one of claims 1-86, wherein the Ab contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3.

88. The isolated antibody of any one of claims 1-87, wherein the Ab does not bind significantly to hVISTA at neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).

89. The isolated antibody of any one of claims 1-88, wherein the Ab binds to hVISTA under acidic conditions, e.g., at a pH of 6.5, with a K_D (or koff) that is at least 10 fold, 100 fold or 1000 fold lower than its K_D and/or koff of binding to hVISTA under neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).

90. The isolated antibody of any one of claims 1-89, wherein the Ab does not bind significantly to hVISTA at neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples).

91. An isolated antibody (Ab) that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 (as measured, e.g., by one of the assays described in the Examples), wherein the Ab:

- inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 (e.g., inhibits the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48);
- enhances T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
- contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;
- binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacts (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å)or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;

- binds to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3: $_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;
- competes for binding to hVISTA (two-way competition) with one or more antibodies decribed herein, e.g., P1-061015, P1-061029, P1-068761, P1-068767 and VISTA.4;
- contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- has low target mediated drug disposition, leading to mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600, or 700 hours, as measured, e.g., as described in the Examples;
- has low or undetectable levels of TMDD; and/or
- has low or undetectable levels of neutropenia.

92. An isolated antibody that binds specifically to hVISTA under acidic conditions, e.g., at a pH of 6.5 with a K_D (and/or koff) that is at least 10 fold, 100 fold or 1000 fold lower than its K_D or koff of binding to hVISTA under neutral or physiological pH (as measured, e.g., by one of the assays described in the Examples), wherein the Ab:
    - inhibits the interaction between hVISTA and (a) T cells and/or (b) PSGL-1 (e.g., inhibits the interaction between H153 and H154 of hVISTA having SEQ ID NO: 1 and PSGL-1 tyrosines Y46 and Y48);
    - enhances T cell activation by, e.g., enhancing T cell proliferation; enhancing IFN-γ production from T cells; and/or stimulating T cell receptor mediated NF-kB signaling;
    - contacts hVISTA through one or more (e.g., at least 1-3, 1-5, 1-10, 5-10, 5-15 or all) energetically important contact residues Y37, T39, R54, F62, H66, V117, I119 or S124, as determined, e.g., using the yeast surface display and NGS assay described in Example 15; and wherein numbering is that of mature hVISTA;

- binds to the histidine-rich β-sheet extension of hVISTA, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- contacts (i) H121, H122 and/or H123 or (ii) H66, H68, H121, H122 and/or H123 of mature hVISTA (distance of 4.0 Ångströms (Å)or less), such as through hydrogen bonds, as determined, e.g., by crystallography, as described, e.g., in the Examples;
- binds to Region 1: $_{57}$LGPVDKGHDVTF$_{68}$ (SEQ ID NO: 498); Region 2: $_{86}$RRPIRNLTFQDL$_{97}$ (SEQ ID NO: 497); and Region 3: $_{148}$VVEIRHHHSEHRVHGAME$_{165}$ (SEQ ID NO: 499) of hVISTA having SEQ ID NO: 1, and optionally wherein the binding is strongest to Region 2, as determined by MS-HDX as described in Example 18;
- competes for binding to hVISTA (e.g., by two-way competition) with one or more antibodies decribed herein, e.g., P1-061015, P1-061029, P1-068761, P1-068767 and VISTA.4;
- contacts hVISTA through at least one or more glutamic acid, aspartic acid or histidine residue that is located in VH CDR1, CDR2 or CDR3;
- has low target mediated drug disposition, leading to mean residence time (MRT) of at least 100, 200, 300, 400, 500, 600, or 700 hours, as measured, e.g., as described in the Examples;
- has low or undetectable levels of TMDD; and/or
- has low or undetectable levels of neutropenia.

93. The isolated antibody of any one of claims 1-92, wherein the antibody has an isoelectric point (pI) between 6.5 and 6.8, as measured, e.g., by icIEF.

94. The isolated antibody of any one of claims 1-93, wherein the antibody exhibits low aggregation, e.g., an aggregation that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples.

95. The isolated antibody of any one of claims 1-94, wherein the antibody exhibits a viscosity that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), as determined in the Examples.

96. The isolated antibody of any one of claims 1-95, wherein the antibody exhibits a hydrodynamic radius that is similar or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples.

97. The isolated antibody of any one of claims 1-96, wherein the antibody exhibits a melting temperature (Tm1) that is similar or higher than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples (e.g., Example 16).

98. The isolated antibody of any one of claims 1-97, wherein the antibody exhibits an amount of high molecular weight species that is similar to or lower than that of VISTA.4 or a derivative thereof, such as P1-070976, P1-065333, P1-070976_H95D, or P1-070976_E97P (e.g., in the context of IgG1.3 and with VK1.A64G), e.g., as determined in the Examples (e.g., Example 16).

99. The isolated antibody of any one of claims 1-98, which is an IgG antibody.

100. The isolated antibody of claim 99, which is an IgG1, IgG2 or IgG4 antibody (IgG4 optionally with an S228P substitution (EU numbering)).

101. The isolated antibody of any one of claims 1-100, wherein the antibody is an effectorless antibody, e.g., lacks ADCC and/or CDC, and/or an antibody that does not significantly bind to one or more FcγRs, e.g., FcγRIII.

102. The isolated antibody of claim 101, wherein the constant region comprises 1-5 mutations in a wild type heavy chain constant region that reduce the effector function (e.g., ADCC and/or CDC) of the antibody and/or the ability to an bind to one or more FcγRs, e.g., FcγRIII, relative to that of the corresponding wildtype heavy chain constant region.

103. The isolated antibody of any one of claims 1-102, wherein the constant region of the antibody is IgG1.3, IgG1.1 or is an IgG1 with a P238K substitution (e.g., IgG1.P238K).

104. The isolated antibody of any one of claims 1-103, wherein the antibody has effector function and/or binds to one or more FcγRs, e.g., FcγRIII.

105. The isolated antibody of claim 104, wherein the antibody is afucosylated (e.g., an afucosylated IgG1 antibody).

106. The isolated antibody of claim 105, wherein the constant region comprises 1-5 mutations that enhance the effector function of the antibody and/or the ability to bind to one or more FcγRs, e.g., FcγRIII, relative to the corresponding wildtype constant region.

107. An isolated antibody, or an isolated antibody of any one of claims 1-106, wherein the antibody is 41F11 or VISTA.4 or a variant thereof.

108. The isolated antibody of any one of claims 1-107, wherein the variant comprises VH CDR1, CDR2, CDR3 and VL CDR1, CDR2, and CDR3 amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

109. The isolated antibody of any one of claims 1-108, wherein the variant comprises VH and VL amino acid sequences that are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-50, 1-40, 1-30 or 1-20, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

110. The isolated antibody of any one of claims 1-109, wherein the antibody comprises the VH CDR1, CDR2, and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 17 or in the Sequence Table.

111. The isolated antibody of any one of claims 1-110, wherein the antibody comprises the VL CDR1, CDR2, and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 1 or in the Sequence Table.

112. The isolated antibody of any one of claims 83-111, wherein the antibody comprises the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2 and CDR3 amino acid sequences of an anti-VISTA antibody disclosed in Eample 17 or in the Sequence Table.

113. The isolated antibody of any one of claims 1-112, wherein the antibody comprises the VH amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

114. The isolated antibody of any one of claims 1-113, wherein the antibody comprises the VL amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

115. The isolated antibody of any one of claims 1-114, wherein the antibody comprises the VH and VL amino acid sequences of an anti-VISTA antibody disclosed in Example 17 or in the Sequence Table.

116. An isolated antibody that binds specifically to human VISTA and comprises the VH CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

117. An isolated antibody that binds specifically to human VISTA and comprises the VL CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

118. The isolated antibody of any one of claims 1-117, comprising the VH CDR1, CDR2 and CDR3 and the VL CDR1, CDR2 and CDR3 amino acid sequences of 41F11 or VISTA.4 or amino acid sequences that, combined, are at least 75%, 80%, 85%, 90%, 95%, or 98% identical to those of 41F11 or VISTA.4, or differ therefrom in 1-20, 1-15, 1-10 or 1-5 amino acid residues, such as amino acid substitutions (e.g., substitutions to D, E or H).

119. The isolated antibody of any one of claims 1-118, wherein the antibody comprises the VH amino acid sequence of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

120. The isolated antibody of any one of claims 1-119, wherein the antibody comprises the VL amino acid sequence of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or

comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

121. The isolated antibody of any one of claims 1-120, wherein the antibody comprises the VH and VL amino acid sequences of 41F11 or VISTA.4 or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

122. The isolated antibody of any one of claims 1-121, wherein the antibody comprises the HC amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

123. The isolated antibody of any one of claims 1-122, wherein the antibody comprises the LC amino acid sequence of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

124. The isolated antibody of any one of claims 1-123, wherein the antibody comprises the HC and LC amino acid sequences of an anti-VISTA antibody disclosed in Example 17 or the Sequence Table or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, 99% or 100% identical thereto or comprises 1-50, 1-40, 1-30, 1-20, 1-10 or 1-5 amino acid changes, e.g., substitutions thereto.

125. The isolated antibody of any one of claims 1-124, wherein the antibody comprises the VH CDRs of VISTA.4 (SEQ ID Nos: 503-505), P1-070976 (SEQ ID Nos: 509-511), P1-065333 (SEQ ID Nos: 506-508), P1-070976_H95D (SEQ ID Nos: 512-514) or P1-070976_E97P (SEQ ID Nos: 515-517).

126. The isolated antibody of any one of claims 1-125, wherein the antibody comprises the VL CDRs of VISTA.4, P1-070976, P1-065333, P1-070976_H95D or P1-070976_E97P (SEQ ID Nos: 500-502).

127. The isolated antibody of any one of claims 1-126, wherein the antibody comprises the VH and VL CDRs of VISTA.4 (SEQ ID Nos: 503-505 and 500-502, respectively), P1-070976 (SEQ ID Nos: 509-511 and 500-502, respectively),

P1-065333 (SEQ ID Nos: 506-508 and 500-502, respectively), P1-070976_H95D (SEQ ID Nos: 512-514 and 500-502, respectively), or P1-070976_E97P (SEQ ID Nos: 515-517 and 500-502, respectively).

128. The isolated antibody of any one of claims 1-127, wherein the antibody comprises the VH of VISTA.4 (SEQ ID NO: 492), P1-070976 (SEQ ID NO: 474), P1-065333 (SEQ ID NO: 473), P1-070976_H95D (SEQ ID NO: 477) or P1-070976_E97P (SEQ ID NO: 478).

129. The isolated antibody of any one of claims 1-128 , wherein the antibody comprises the VL of VISTA.4, P1-070976, P1-065333, P1-070976_H95D or P1-070976_E97P (with or without A64G) (corresponding to SEQ ID NO: 493 (without A64G) or SEQ ID NO: 479 (with A64G)).

130. The isolated antibody of any one of claims 1-129, wherein the antibody comprises the VH and VL (with or without A64G in VL) of VISTA.4 (SEQ ID Nos: 492 and 479 or SEQ ID Nos: 492 and 493), P1-070976 (SEQ ID Nos: 474 and 479 or SEQ ID Nos: 474 and 493), P1-065333 (SEQ ID Nos: 475 and 479 or SEQ ID Nos: 475 and 493), P1-070976_H95D (SEQ ID Nos: 477 and 479 or SEQ ID Nos: 477 and 493) or P1-070976_E97P (SEQ ID Nos: 478 and 479 or SEQ ID Nos: 478 and 493).

131. The isolated antibody of any one of claims 1-130, wherein the antibody comprises the HC of VISTA.4.IgG1.3 (SEQ ID NO: 491), P1-070976.IgG1.3 (SEQ ID NO: 476), P1-06533.IgG1.3 (SEQ ID NO: 475), P1-070976_H95D.IgG1.3 (SEQ ID NO: 480) or P1-070976_E97P.IgG1.3 (SEQ ID NO: 481).

132. The isolated antibody of any one of claims 1-131, wherein the antibody comprises the LC of VISTA.4.IgG1.3, P1-070976.IgG1.3, P1-06533.IgG1.3, P1-070976_H95D.IgG1.3 or P1-070976_E97P.IgG1.3 (with or without A64G in VL) (SEQ ID NO: 494 (without A64G) or SEQ ID NO: 482 (with A64G).

133. The isolated antibody of any one of claims 1-132, wherein the antibody comprises the HC and LC (with or without A64G in VL) of VISTA.4.IgG1.3 (SEQ ID NOs: 491 and 494 or SEQ ID Nos: 491 and 482), P1-070976.IgG1.3 (SEQ ID NOs: 476 and 494 or SEQ ID Nos: 476 and 482), P1-065333.IgG1.3 (SEQ ID NOs: 475 and 494 or SEQ ID Nos: 475 and 482), P1-

070976_H95D.IgG1.3 (SEQ ID NOs: 480 and 494 or SEQ ID Nos: 480 and 482), or P1-070976_E97P.IgG1.3 (SEQ ID NOs: 481 and 494 or SEQ ID Nos: 481 and 482).

134. The isolated antibody of any one of claims 1-133, which is a full length antibody or an antibody comprising a full length heavy chain (with or without a C-terminal lysine) and a full length light chain.

135. The isolated antibody of any one of claims 1-130, which is an antigen binding fragment.

136. The isolated antibody of any one of claims 1-135, which is a multimeric (e.g., dimeric or trimeric) antibody.

137. The isolated antibody of any one of claims 1-136, which is linked (e.g., covalently) to another molecule.

138. The isolated antibody of claim 137, wherein the other molecule is a label.

139. The isolated antibody of claim 137 or 138, wherein the other molecule is a peptide.

140. The isolated antibody of any one of claims 1-139, which is an antibody drug conjugate (ADC) or an activatable antibody.

141. An isolated nucleic acid encoding an antibody of any one of claims 1-140.

142. An isolated nucleic acid encoding the heavy chain and/or the light chain of an antibody of any one of claims 1-140.

143. A composition comprising a nucleic acid encoding the heavy chain of an antibody of any one of claims 1-140 and a nucleic acid encoding the light chain of the antibody.

144. A cell comprising the isolated nucleic acid of any one of claims 141-142 or the composition of claim 143.

145. A method of preparing an antibody, comprising culturing the cell of claim 144 in conditions under which the antibody is expressed.

146. A composition comprising an isolated antibody, nucleic acid, composition or cell of any one of claims 1-145 and a pharmaceutically acceptable carrier.

147. The composition of claim 146, comprising a second therapeutic agent.

148. The composition of claim 147, wherein the second therapeutic agent comprises an immunostimulatory agent.

149. The composition of claim 148, wherein the immunostimulating agent comprises an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, a CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.

150. A method of treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of claims 1-149 that stimulates an immune response and/or is a VISTA antagonist antibody.

151. The method of claim 150, wherein the subject has VISTA positive cells, e.g., in a tumor of the cancer.

152. The method of claim 151, wherein the VISTA positive cells comprise VISTA positive infiltrating lymphocytic (e.g., T cells) or myelomonocytic cells.

153. The method of any one of claims 150-152, wherein the subject is first tested for the presence of VISTA positive cells in a tumor, and wherein the subject is treated if the subject has VISTA positive cells, e.g., in a tumor.

154. The method of any one of claims 150-153, wherein the method further comprises administering a second therapy.

155. The method of claim 154, wherein the second therapy comprises chemotherapy, radiotherapy, surgery or administration of a second agent.

156. The method of claim 155, wherein the second therapy comprises a second agent and the second agent is an immunostimulatory agent.

157. The method of claim 156, wherein the immunostimulatory agent comprises an antagonist of an immunosuppressive molecule, e.g., the PD-1/PD-L1, an CTLA-4 and LAG-3, or an agonist of an immunostimulating molecule, e.g., GITR and OX40.

158. A method of treating an infectious disease (e.g., viral disease) in a subject, comprising administering to the subject a therapeutically effective amount of a composition or isolated antibody of any one of claims 1-157 that stimulates an immune response and/or is a VISTA antagonist.

159. A method of detecting VISTA in a sample, comprising contacting the sample with a VISTA antibody of any one of claims 1-140.

160. An isolated antibody that binds specifically to hVISTA and inhibits VISTA mediated T cell activation, wherein the antibody comprises VH CDR1, CDR2 and CDR3 sequences of a VH comprising or consisting of SEQ ID NO: 463, 473, 474, 477, 478, or , 492 (e.g. SEQ ID Nos: 503-505, 506-508, 509-511, 512-514, or 515-517).

161. The isolated antibody of claim 160,wherein the antibody comprises VL CDR1, CDR2 and CDR3 sequences of a VL comprising or consisting of SEQ ID NO: 464, 471, 493 or 479 (e.g., SEQ ID Nos: 500-502).

162. The isolated antibody of claim 160 or 161, wherein the antibody comprises a VH comprising or consisting of SEQ ID NO: 463, 473, 474, 477, 478, or 492, with or without a signal peptide.

163. The isolated antibody of any one of claims 160-162, wherein the antibody comprises a VL comprising or consisting of SEQ ID NO: 464, 471, 479, or 493, with or without a signal peptide.

164. The isolated antibody of any one of claims 160-163, comprising a heavy chain constant region comprising or consisting of a wildtype IgG1 (e.g., SEQ ID NO: 182), a wildtype IgG2, a wildtype IgG3, a wildtype IgG4, a wildtype IgG4 comprising S228P, IgG1.1 (SEQ ID NO: 183), IgG1.3 (SEQ ID NO: 163), IgG1.P238K (SEQ ID NO: 184), with or without a C-terminal lysine.

165. The isolated antibody of any one of claims 160-164, comprising a heavy chain comprising or consisting of SEQ ID NO: 475, 476, 491, 480, or 481, with or without signal peptide.

166. The isolated antibody of any one of claims 160-165, comprising a light chain comprising or consisting of SEQ ID NO: 472, 494, or 482, with or without a signal peptide.

167. The isolated antibody of claim 166, comprising heavy and light chains, comprising or consisting of:

(a) SEQ ID NO: 475 and SEQ ID NO: 482 or 472, respectively;

(b) SEQ ID NO: 475 and SEQ ID NO: 494, respectively;

(c) SEQ ID NO: 476 and SEQ ID NO: 482 or 472, respectively;

(d) SEQ ID NO: 476 and SEQ ID NO: 494, respectively;

(e) SEQ ID NO: 491 and SEQ ID NO: 482 or 472, respectively;

(f) SEQ ID NO: 491 and SEQ ID NO: 494, respectively;

(g) SEQ ID NO: 480) and SEQ ID NO: 482 or 472, respectively;

(h) SEQ ID NO: 480) and SEQ ID NO: 494, respectively;

(i) SEQ ID NO: 481) and SEQ ID NO: 482 or 472, respectively; or

(j) SEQ ID NO: 481) and SEQ ID NO: 494, respectively.

168. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which, e.g., results in the increase of the number of CD4+ and CD8+ T cells, e.g., in a tumor of the subject.

169. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which, e.g., results in the reduction of the number of exhausted T cells and/or T cells expressing PD-1, LAG3 and/or TIM-3, e.g., in a tumor of the subject.

170. A method of treating cancer in a subject, comprising administering to the subject an antibody that binds specifically to hVISTA and inhibits the activity of hVISTA (e.g., T cell activation) and a PD1/PD-L1 pathway antagonist, which, e.g., results in the increase of the number of CD4+ and CD8+ T cells, e.g., in a tumor of the subject and a reduction of the number of exhausted T cells and/or T cells expressing PD-1, LAG3 and/or TIM-3, e.g., in a tumor of the subject and/or other features described herein.

171. The method of any one of claims 168-170, wherein the antibody that binds specifically to hVISTA is an antibody described herein.