

## Antibodies binding to GPRC5D

### Field of the Invention

The present invention generally relates to antibodies that bind to GPRC5D, including bispecific antigen binding molecules e.g. for activating T cells. In addition, the present invention relates to polynucleotides encoding such antibodies, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the antibodies, and to methods of using them in the treatment of disease.

### Background

Affecting ~75,000 new patients every year in the EU and US, multiple myeloma (MM) is one of the most common hematological malignancies, which remains a high unmet medical need. Multiple myeloma is characterized by terminally differentiated plasma cells that secrete non-functional monoclonal immunoglobulins. In the short-term, the immunomodulatory drugs such as lenalidomide and pomalidomide, and proteasome inhibitors such as carfilzomib or bortezomib may remain the backbone of 1<sup>st</sup> line therapy for multiple myeloma (Moreau, P. and S.V. Rajkumar, multiple myeloma-translation of trial results into reality. Lancet, 2016. 388(10040): p. 111-3). However, these drugs do not target specifically the diseased tumor cells e.g. diseased plasma cells (PC). Efforts have been made towards selectively depleting the plasma cells in multiple myeloma. The lack of surface proteins that specifically mark plasma cells has hampered the development of antibodies or cellular therapies for multiple myeloma. So far, there are few cases of successful biologics, including daratumumab (anti-CD38) and elotuzumab (anti-CD319), with the caveat that these two molecules are not uniquely expressed by plasma cells. Therefore, novel targets from plasma cells in multiple myeloma were identified using RNA-sequencing, such as the G protein-coupled receptor class C group 5 member D (GPRC5D). that is differentially expressed by plasma cells in multiple myeloma versus plasma cells from healthy donors. It has been reported that GPRC5D is associated with prognosis and tumour load in multiple myeloma patients (Atamaniuk, J., et al., Overexpression of G protein-coupled receptor 5D in the bone marrow is associated with poor prognosis in patients with multiple myeloma. Eur J Clin Invest, 2012. 42(9): p. 953-60; and

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Cohen, Y., et al., GPRC5D is a promising marker for monitoring the tumour load and to target multiple myeloma cells. *Hematology*, 2013. 18(6): p. 348-51).

GPRC5D is an orphan receptor with no known ligand and largely unknown biology in men in general and in cancer specifically. The GPRC5D encoding gene, which is mapped on chromosome 12p13.3, contains three exons and spans about 9.6 kb (Brauner-Osborne, H., et al., Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D. *Biochim Biophys Acta*, 2001. 1518(3): p. 237-48). The large first exon encodes the seven-transmembrane domain. It has been shown that GPRC5D is involved in keratin formation in hair follicles in animals (Gao, Y., et al., Comparative Transcriptome Analysis of Fetal Skin Reveals Key Genes Related to Hair Follicle Morphogenesis in Cashmere Goats. *PLoS One*, 2016. 11(3): p. e0151118; and Inoue, S., T. Nambu, and T. Shimomura, The RAIG family member, GPRC5D, is associated with hard-keratinized structures. *J Invest Dermatol*, 2004. 122(3): p. 565-73).

WO 2018/017786 A2 discloses GPRC5D-specific antibodies or antigen-binding fragments.

Given that all standard-of-care treatments are not able to cure multiple myeloma patients, there is a clear need to develop potent and specific new therapies. One of this approaches includes antibodies that bind GPRC5D, in particular bispecific antibodies that bind GPRC5D on target cells and an activating T-cell antigen such as CD3 on T-cells. Upon simultaneous binding of such an antibody to both of its targets, a T-cell synapse will be formed, leading to activation of the (cytotoxic) T cell and subsequent lysis of the target cell.

The present invention provides novel antibodies, including bispecific antibodies that specifically bind human GPRC5D. Particularly, the T-cell bispecific antibodies according to the invention targeting GPRC5D have the potency to treat multiple myeloma.

### Summary of the Invention

The present inventors have developed bispecific antigen binding molecules that bind to GPRC5D and an activating T cell antigen, incorporating the novel GPRC5D antibody.

In a first aspect the present invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a (i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; (ii) a heavy chain variable region

(VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; (iii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; (iv) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; or (v) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; and (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety comprises (i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 29, a HCDR 2 of SEQ ID NO: 30, and a HCDR 3 of SEQ ID NO: 31, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 32, a LCDR 2 of SEQ ID NO: 33 and a LCDR 3 of SEQ ID NO: 34; (i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; or (ii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 106, a HCDR 2 of SEQ ID NO: 107, and a HCDR 3 of SEQ ID NO: 108, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 109, a LCDR 2 of SEQ ID NO: 110 and a LCDR 3 of SEQ ID NO: 111.

In another embodiment, (i) the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 13, and the VL of the first antigen binding moiety comprises an amino acid sequence

that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 14; or (ii) the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 15, and the VL of the first antigen binding moiety comprises an amino acid sequence

5 that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 16; or (iii) the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 48, and the VL of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of

10 SEQ ID NO: 53; or (iv) wherein the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 49, and wherein the VL of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52; or (v) the VH of the first antigen binding moiety comprises an

15 amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 57, and the VL of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 64; or (vi) the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the

20 sequence of SEQ ID NO: 58, and the VL of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63. In another embodiment, the VH of the second antigen binding moiety comprises an amino acid sequence (i) that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35, and the VL of the second antigen

25 binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36; (ii) that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 104, and the VL of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:

30 105; or (iii) that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 112, and the VL of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 113.

In an embodiment, the first and/or the second antigen binding moiety is a Fab molecule. This means, either the first antigen binding moiety may be a Fab molecule, or the second antigen binding moiety may be a Fab molecule, or the first antigen binding moiety and the second antigen binding moiety may be Fab molecules. In another embodiment, the second antigen binding moiety

5 is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other. In another embodiment, the first antigen binding moiety is a Fab molecule wherein in the constant domain the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at

10 position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index). In another embodiment, the first and the

15 second antigen binding moiety are fused to each other, optionally via a peptide linker. In another embodiment, the first and the second antigen binding moiety are each a Fab molecule and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab

20 heavy chain of the second antigen binding moiety. In another embodiment, the bispecific antigen binding molecule comprises a third antigen binding moiety. In another embodiment, the third antigen moiety is identical to the first antigen binding moiety. In another embodiment, the bispecific antigen binding molecule comprises an Fc domain composed of a first and a second subunit. In another embodiment, the first, the second and, where present, the third antigen binding

25 moiety are each a Fab molecule; and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain

30 of the second antigen binding moiety and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and wherein the third antigen binding moiety, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. In another embodiment, the

Fc domain is an IgG Fc domain. In another embodiment, the Fc domain is an IgG1 Fc domain. In yet another embodiment, the Fc domain is a human Fc domain. In another embodiment, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. In another embodiment, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In another aspect, the invention provides one or more isolated polynucleotide encoding the bispecific antigen binding molecule as described herein. In a further aspect, the invention provides one or more vector, particularly expression vector, comprising the polynucleotide(s) as described herein. In another aspect, the invention provides a host cell comprising the polynucleotide(s) or the vector(s) as described herein.

In another aspect of the invention a method of producing a bispecific antigen binding molecule that binds to GPRC5D, comprising the steps of a) culturing the host cell as described herein under conditions suitable for the expression of the bispecific antigen binding molecule and b) optionally recovering the bispecific antigen binding molecule.

In another aspect, the invention provides a bispecific antigen binding molecule that binds to GPRC5D, produced by the method of claim 21.

In another aspect, the invention provides a pharmaceutical composition comprising the bispecific antigen binding molecule as described herein and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a bispecific antigen binding molecule as disclosed herein or a pharmaceutical composition as disclosed herein for use as a medicament.

In another aspect, the invention provides a bispecific antigen binding molecule as disclosed herein or a pharmaceutical composition as disclosed herein for use in the treatment of a disease.

In another aspect, the invention provides a bispecific antigen binding molecule or pharmaceutical composition as disclosed herein, wherein the disease is cancer or an autoimmune disease.

In another aspect, the invention provides a bispecific antigen binding molecule or pharmaceutical composition as disclosed herein, wherein the disease is multiple myeloma.

In a further aspect, the invention provides use of a bispecific antigen binding molecule as disclosed herein in the manufacture of a medicament for the treatment of a disease.

In another aspect the invention relates to a method of treating a disease, particularly cancer, more particularly multiple myeloma, in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising a bispecific antigen binding molecule as described herein in a pharmaceutically acceptable form. Alternatively, the disease is an autoimmune disease, such as systemic lupus erythematosus and/or rheumatoid arthritis. In any of the above embodiments the individual preferably is a mammal, particularly a human.

### Brief Description of the Drawings

**Figures 1A-Z.** Exemplary configurations of the bispecific antigen binding molecules of the invention. (Fig. 1A, Fig. 2D) Illustration of the “1+1 CrossMab” molecule. (Fig. 1B, Fig. 1E) Illustration of the “2+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (Fig. 1C, Fig. 1F) Illustration of the “2+1 IgG Crossfab” molecule. (Fig. 1G, Fig. 1K) Illustration of the “1+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (Fig. 1H, Fig. 1L) Illustration of the “1+1 IgG Crossfab” molecule. (Fig. 1I, Fig. 1M) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs. (Fig. 1J, Fig. 1N) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs and alternative order of Crossfab and Fab components (“inverted”). (Fig. 1O, Fig. 1S) Illustration of the “Fab-Crossfab” molecule. (Fig. 1P, Fig. 1T) Illustration of the “Crossfab-Fab” molecule. (Fig. 1Q, Fig. 1U) Illustration of the “(Fab)<sub>2</sub>-Crossfab” molecule. (Fig. 1R, Fig. 1V) Illustration of the “Crossfab-(Fab)<sub>2</sub>” molecule. (Fig. 1W, Fig. 1Y) Illustration of the “Fab-(Crossfab)<sub>2</sub>” molecule. (Fig. 1X, Fig. 1Z) Illustration of the “(Crossfab)<sub>2</sub>-Fab” molecule. Black dot: optional modification in the Fc domain promoting heterodimerization. ++, --: amino acids of opposite charges optionally introduced in the CH1 and CL domains. Crossfab molecules are depicted as comprising an exchange of VH and VL regions, but may – in embodiments wherein no charge modifications are introduced in CH1 and CL domains – alternatively comprise an exchange of the CH1 and CL domains.

**Figure 2.** Analysis of gene expression of tumor targets on plasma cells and B-cells by RNAseq.

**Figure 3.** Exemplary configurations of the 5E11-bispecific antigen binding molecules of the invention. Black dot: optional modification in the Fc domain promoting heterodimerization. ++, --: amino acids of opposite charges optionally introduced in the CH1 and CL domains.

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**Figures 4A-C.** Binding analysis of bispecific antigen binding molecules 5F11-TCB (Fig. 4A) and 5E11-TCB (Fig. 4B) and control antibody ET150-5-TCB (Fig. 4C) to GPRC5D-expressing multiple myeloma cell lines AMO-1, L636, NCI-H929, RPMI-8226, OPM-2 and control cells WSU-DLCL2.

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**Figures 5A-E.** Analysis of GPRC5D-TCB mediated T cell cytotoxicity on multiple myeloma cell lines AMO-1 (Fig. 5A), NCI-H929 (Fig. 5B), RPMI-8226 (Fig. 5C) and L363 (Fig. 5D). Control cell line is WSU-DL CL2 (Fig. 5E). Tested molecules: 5E11-TCB, 5F11-TCB. Control molecules: DP47-TCB (untargeted) and ET150-5-TCB.

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**Figure 6.** Analysis of GPRC5D-TCB activated T cell engagement with multiple myeloma cell lines NCI-H929 and negative control cell line WSU-DLCL2 upregulating CD25 and CD69.

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**Figures 7A-J.** T-cell activation, as determined by up-regulation of CD25 on CD8+ T-cells, upon incubation of T-cells with increasing concentrations of GPRC5D-TCBs or negative control DP47-TCB in presence of AMO-1 (Fig. 7A), NCI-H929 (Fig. 7B), RPMI-8226 (Fig. 7C), L363 (Fig. 7D) and WSU-DLCL2 (Fig. 7E); and as determined by up-regulation of CD69 on CD8+ T-cells upon incubation of T-cells with increasing concentrations of GPRC5D-TCBs or negative control DP47-TCB in presence of either AMO-1 (Fig. 7F), NCI-H929 (Fig. 7G), RPMI-8226 (Fig. 7H), L363 (Fig. 7I) and WSU-DLCL2 (Fig. 7J).

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**Figures 8A-B.** Visualization of antibody localization and internalization by Fluorescence Confocal Microscopy (Fig. 8A) and analysis of signal intensities of membrane vs cytoplasm (Fig. 8B).

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**Figure 9.** Binding of different anti-GPRC5D antibodies to human, cynomolgus and murine GPRC5D was assessed by ELISA, using stably transfected CHO clones expressing

either human GPRC5D (clone 12) or cynomolgus GPRC5D (clone 13), murine GPRC5D (clone 4) or human GPRC5A (clone 30).

**Figure 10A-G.** T-cell mediated lysis of various Multiple Myeloma (MM) cell lines induced by different GPRC5D- or BCMA-targeting T-cell bispecific molecules during 20 hours of co-incubation (E:T = 10:1, human pan T cells). Depicted are duplicates with SD.

**Figure 11A-F.** T-cell activation induced by different GPRC5D- or BCMA-targeting T-cell bispecific molecules (5E11-TCB in Fig. 11A; 5F11-TCB in Fig. 11B; 10B10-TCB in Fig. 11C; BCMA-TCB in Fig. 11D; B72-TCB in Fig. 11E; DP47-TCB in Fig. 11F) during ~20 hours of co-incubation of allogenic pan human T cells and unprocessed Bone Marrow cells from healthy donors (E:T = 10:1, human pan T cells). Depicted are FACS dot plots from one representative donor, showing up-regulation of the activation marker CD69 on CD4 (upper row) or CD8 T-cells (lower row) as percent positive cells among all CD4 respective CD8 T-cells.

**Figures 12A-B.** T-cell activation induced by different GPRC5D- or BCMA-targeting T-cell bispecific molecules during ~20 hours of co-incubation of allogenic pan human T cells and unprocessed Bone Marrow cells from healthy donors (E:T = 10:1, human pan T cells). Depicted is the summary of all four assessed donors, showing up-regulation of the activation marker CD69 on CD8 T-cells at the selected fixed dose of either 50 nM of the TCB (Fig. 12A) or 5 nM (Fig. 12B).

**Figure 13A-D.** In vivo efficacy induced by different GPRC5D-targeting T-cell bispecific molecules (5F11-TCB in Fig. 13A; BCMA-TCB in Fig. 13B; B72-TCB in Fig. 13C; Vehicle in Fig. 13D), as depicted by tumor growth kinetics over time in a model of humanized NSG mice, engrafted with NCI-H929 tumor cells. Plotted are spider graphs with each line referring to a single mouse.

**Figures 14A-D.** In vivo efficacy induced by different GPRC5D-targeting T-cell bispecific molecules (5F11-TCB in Fig. 14A; 5E11-TCB in Fig 14B; B72-TCB in Fig. 14C; vehicle in Fig. 14D), as depicted by tumor growth kinetics over time in a model of humanized NSG mice, engrafted with OPM-2 tumor cells. Plotted are spider graphs with each line referring to a single mouse.

**Figures 15A-B.** PGLALA-CAR-J activation after roughly 16 hours of incubation, as determined by luminescence. The latter is induced upon simultaneous binding of the GPRC5D IgGs (5F11-IgG in Fig. 15A; 5E11-IgG in Fig. 15B) to the GPRC5D-expressing multiple myeloma cell line L-363 and of the PGLALA-modified Fc domain to Jurkat-NFAT reporter cells, which were genetically engineered to express a TCR-directed against the PGLALA mutation in the Fc part of these IgG molecules. Depicted are duplicates with SD.

**Figures 16A-D.** Binding of humanized TCB molecules vs. parental TCBs to human GPRC5D on NCI-H929 cells (Figures 16A and 16B) and human CD3 on Jurkat cells (Figure 16C and 16D) expressed on cells.

**Figure 17A-G.** Jurkat-NFAT activation assay in presence of different GPRC5D $\times$ CD3 bispecific TCB molecules (Fig. 17A – G) versus untargeted control TCBs, as indicated.

**Figure 18A-D.** Tumor Cell Lysis assay comparing GPRC5D-TCB molecules as presented herein and molecules known in the art targeting GPRC5D or BCMA versus an untargeted reference TCB molecule.

**Figure 19.** Activation of autologous T-cells upon incubation of a primary MM sample with different CD3-engaging bispecific molecules. GPRC5D-TCBs as presented herein were compared to molecules known in the art targeting GPRC5D or BCMA versus an untargeted reference TCB molecule.

**Figure 20A-D.** Depletion of B cells upon incubation of PBMCs from healthy donors with different CD3-engaging bispecific molecules. GPRC5D-TCBs as presented herein were compared to molecules known in the art targeting either GPRC5D or BCMA versus an untargeted reference TCB molecule. Antibodies were used at concentrations of 50 nM (Fig. 20A), 5 nM (Fig. 20B), 0.5 nM (Fig. 20C) and 0.05 nM (Fig. 20D).

**Figure 21A-B.** Activation of T-cells upon incubation of bone marrow samples from healthy donors with different CD3-engaging bispecific molecules. GPRC5D-TCBs as presented herein were compared to molecules known in the art. Activation was determined by detection of the percent of either CD69<sup>+</sup>CD8<sup>+</sup> T cells (Fig. 21A) and CD69<sup>+</sup>CD4<sup>+</sup>T cells (Fig. 21B) were used among all CD8<sup>+</sup> respective CD4<sup>+</sup> T-cells.

**Figure 22A-B.** Cytokine release in human whole blood of healthy donors (TNF $\alpha$  readouts in Fig.22A; IL6 readouts in Fig.22B). GPRC5D-TCBs as described herein and

positive (Gazyva, Lemtrada) and negative (Erbix) reference molecules were compared.

**Figure 23A-G.** In vivo efficacy of different GPRC5D $\times$ CD3 bispecific TCB molecules in NCI-H929 (hNSG mice), including average tumor volume per treatment group over the course of therapy (Fig.23A), tumor volume at day 37 (Fig.23B), and tumor growth for the molecules with each line representing a single mouse (vehicle: Fig.23C; 6623: Fig.23D; 6624: Fig.23E, 6625: Fig.23F, 6626: Fig.23G;).

**Figure 24.** *in vivo* SDPK in hFcRn Tg and KO mice and clearance data of the indicated TCB molecules.

**Figure 25A-I.** Representative Examples of the binding analysis of bispecific antigen binding molecule 5E11(6625)-TCB to human GPRC5D-expressing multiple myeloma cell lines OPM-2 (Fig.25A, Fig.25D, Fig.25G), NCI-H929 (Fig.25B, Fig.25E, Fig.25H) and RPMI-8226 (Fig.25C, Fig.25F, Fig.25I). The number of GPRC5D antibody binding sites (ABS) per cell line are given in brackets and were determined by QSC previously (Quantum Simply Cellular, BangsLabs). Depicted are relative median fluorescence valus (MFI) from triplicates with SD. EC50 values of binding were calculated by GraphPadPrism and are summarized in Table 14.2.

## Detailed Description of the Invention

### Definitions

Terms are used herein as generally used in the art, unless otherwise defined in the following.

As used herein, the term "antigen binding molecule" refers in its broadest sense to a molecule that specifically binds an antigenic determinant. Examples of antigen binding molecules are immunoglobulins and derivatives, e.g. fragments, thereof.

The term "bispecific" means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. Typically, a bispecific antigen binding molecule comprises two antigen binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

The term "valent" as used herein denotes the presence of a specified number of antigen binding sites in an antigen binding molecule. As such, the term "monovalent binding to an antigen" denotes

the presence of one (and not more than one) antigen binding site specific for the antigen in the antigen binding molecule.

An "antigen binding site" refers to the site, i.e. one or more amino acid residues, of an antigen binding molecule which provides interaction with the antigen. For example, the antigen binding site of an antibody comprises amino acid residues from the complementarity determining regions (CDRs). A native immunoglobulin molecule typically has two antigen binding sites; a Fab molecule typically has a single antigen binding site.

As used herein, the term "antigen binding moiety" refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one embodiment, an antigen binding moiety is able to direct the entity to which it is attached (e.g. a second antigen binding moiety) to a target site, for example to a specific type of tumor cell bearing the antigenic determinant. In another embodiment an antigen binding moiety is able to activate signaling through its target antigen, for example a T cell receptor complex antigen. Antigen binding moieties include antibodies and fragments thereof as further defined herein. Particular antigen binding moieties include an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In certain embodiments, the antigen binding moieties may comprise antibody constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes:  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , or  $\mu$ . Useful light chain constant regions include any of the two isotypes:  $\kappa$  and  $\lambda$ .

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope", and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM). The proteins referred to as antigens herein (e.g. GPRC5D, CD3) can be any native form of the proteins from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. In a particular embodiment the antigen is a human protein. Where reference is made to a specific protein herein, the term encompasses the "full-length", unprocessed protein as well as any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g. splice variants or allelic variants. An exemplary human protein useful as antigen is CD3,

particularly the epsilon subunit of CD3 (see UniProt no. P07766 (version 185), NCBI RefSeq no. NP\_000724.1, SEQ ID NO: 40 for the human sequence; or UniProt no. Q95LI5 (version 69), NCBI GenBank no. BAB71849.1, SEQ ID NO: 41 for the cynomolgus [*Macaca fascicularis*] sequence), or GPRC5D (see UniProt no. Q9NZD1 (version 115); NCBI RefSeq no. NP\_061124.1, SEQ ID NO: 45 for the human sequence). In certain embodiments the antibody or bispecific antigen binding molecule of the invention binds to an epitope of CD3 or GPRC5D that is conserved among the CD3 or GPRC5D antigens from different species. In particular embodiments, the antibody or bispecific antigen binding molecule of the invention binds to human GPRC5D.

By "specific binding" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed e.g. on a BIAcore instrument) (Liljeblad et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain embodiments, an antigen binding moiety that binds to the antigen, or an antigen binding molecule comprising that antigen binding moiety, has a dissociation constant ( $K_D$ ) of  $\leq 1 \mu\text{M}$ ,  $\leq 100 \text{ nM}$ ,  $\leq 10 \text{ nM}$ ,  $\leq 1 \text{ nM}$ ,  $\leq 0.1 \text{ nM}$ ,  $\leq 0.01 \text{ nM}$ , or  $\leq 0.001 \text{ nM}$  (e.g.  $10^{-8} \text{ M}$  or less, e.g. from  $10^{-8} \text{ M}$  to  $10^{-13} \text{ M}$ , e.g., from  $10^{-9} \text{ M}$  to  $10^{-13} \text{ M}$ ).

"Affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., an antigen binding moiety and an antigen, or a receptor and its ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ( $K_D$ ), which is the ratio of dissociation and association rate constants ( $k_{\text{off}}$  and  $k_{\text{on}}$ , respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well-established methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

"Reduced binding", for example reduced binding to an Fc receptor, refers to a decrease in affinity for the respective interaction, as measured for example by SPR. For clarity, the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete

abolishment of the interaction. Conversely, “increased binding” refers to an increase in binding affinity for the respective interaction.

An “activating T cell antigen” as used herein refers to an antigenic determinant expressed on the surface of a T lymphocyte, particularly a cytotoxic T lymphocyte, which is capable of inducing T cell activation upon interaction with an antigen binding molecule. Specifically, interaction of an antigen binding molecule with an activating T cell antigen may induce T cell activation by triggering the signaling cascade of the T cell receptor complex. In a particular embodiment the activating T cell antigen is CD3, particularly the epsilon subunit of CD3 (see UniProt no. P07766 (version 144), NCBI RefSeq no. NP\_000724.1, SEQ ID NO: 40 for the human sequence; or UniProt no. Q95LI5 (version 49), NCBI GenBank no. BAB71849.1, SEQ ID NO: 41 for the cynomolgus [*Macaca fascicularis*] sequence).

“T cell activation” as used herein refers to one or more cellular response of a T lymphocyte, particularly a cytotoxic T lymphocyte, selected from: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers. Suitable assays to measure T cell activation are known in the art and described herein.

A “target cell antigen” as used herein refers to an antigenic determinant presented on the surface of a target cell, for example a cell in a tumor such as a cancer cell or a cell of the tumor stroma. In a particular embodiment, the target cell antigen is GPRC5D, particularly human GPRC5D according to SEQ ID NO: 45.

As used herein, the terms “first”, “second” or “third” with respect to Fab molecules etc., are used for convenience of distinguishing when there is more than one of each type of moiety. Use of these terms is not intended to confer a specific order or orientation of the bispecific antigen binding molecule unless explicitly so stated.

By “fused” is meant that the components (e.g. a Fab molecule and an Fc domain subunit) are linked by peptide bonds, either directly or via one or more peptide linkers.

A “Fab molecule” refers to a protein consisting of the VH and CH1 domain of the heavy chain (the “Fab heavy chain”) and the VL and CL domain of the light chain (the “Fab light chain”) of an immunoglobulin.

By a “crossover” Fab molecule (also termed “Crossfab”) is meant a Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other), i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable domain VL and the heavy chain constant domain 1 CH1 (VL-CH1, in N- to C-terminal direction), and a peptide chain composed of the heavy chain variable domain VH

and the light chain constant domain CL (VH-CL, in N- to C-terminal direction). For clarity, in a crossover Fab molecule wherein the variable domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain constant domain 1 CH1 is referred to herein as the "heavy chain" of the (crossover) Fab molecule. Conversely, in a crossover Fab molecule wherein the constant domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain variable domain VH is referred to herein as the "heavy chain" of the (crossover) Fab molecule.

In contrast thereto, by a "conventional" Fab molecule is meant a Fab molecule in its natural format, i.e. comprising a heavy chain composed of the heavy chain variable and constant domains (VH-CH1, in N- to C-terminal direction), and a light chain composed of the light chain variable and constant domains (VL-CL, in N- to C-terminal direction).

The term "immunoglobulin molecule" refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 Daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a light chain variable region, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called  $\alpha$  (IgA),  $\delta$  (IgD),  $\epsilon$  (IgE),  $\gamma$  (IgG), or  $\mu$  (IgM), some of which may be further divided into subtypes, e.g.  $\gamma_1$  (IgG1),  $\gamma_2$  (IgG2),  $\gamma_3$  (IgG3),  $\gamma_4$  (IgG4),  $\alpha_1$  (IgA1) and  $\alpha_2$  (IgA2). The light chain of an immunoglobulin may be assigned to one of two types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

The term "antibody" 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.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprised in the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation,

such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

An “isolated” antibody is one which has been separated from a component of its natural environment, i.e. that is not in its natural milieu. No particular level of purification is required. For example, an isolated antibody can be removed from its native or natural environment. Recombinantly produced antibodies expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant antibodies which have been separated, fractionated, or partially or substantially purified by any suitable technique. As such, the antibodies and bispecific antigen binding molecules of the present invention are isolated. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies. For a review of certain antibody fragments, see Hudson et al., *Nat Med* 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')<sub>2</sub>

fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

The term "antigen binding domain" refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain comprises an antibody light chain variable domain (VL) and an antibody heavy chain variable domain (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6<sup>th</sup> ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity. As used herein in connection with variable region sequences, "Kabat numbering" refers to the numbering system set forth by Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., *Sequences of Proteins of Immunological Interest*, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), referred to as "numbering according to Kabat" or "Kabat numbering" herein. Specifically the Kabat numbering system (see pages 647-660 of Kabat, et al., *Sequences of Proteins of Immunological Interest*, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) is used for the light chain constant domain CL of kappa and lambda isotype and the Kabat EU index numbering system (see pages 661-723) is used for the heavy chain

constant domains (CH1, Hinge, CH2 and CH3), which is herein further clarified by referring to “numbering according to Kabat EU index” in this case.

The term “hypervariable region” or “HVR”, as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence (“complementarity determining regions” or “CDRs”; CDRs of the heavy chain variable region/domain are abbreviated e.g. as HCDR1, HCDR2 and HCDR3; CDRs of the light chain variable region/domain are abbreviated e.g. as LCDR1, LCDR2 and LCDR3 ) and/or form structurally defined loops (“hypervariable loops”) and/or contain the antigen-contacting residues (“antigen contacts”). Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
- (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
- (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996));
- and
- (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following order in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. Such variable domains are referred to herein as “humanized variable region”. A humanized antibody

optionally may comprise at least a portion of an antibody constant region derived from a human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity. A “humanized form” of an antibody, e.g. of a non-human antibody, refers to an antibody that has undergone humanization. Other forms of “humanized antibodies” encompassed by the present invention are those in which the constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

10 A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. In certain embodiments, a human antibody is derived from a non-human transgenic mammal, for example a mouse, a rat, or a rabbit. In certain embodiments, a human antibody is derived from a hybridoma cell line. Antibodies or antibody fragments isolated from human antibody libraries are also considered human antibodies or human antibody fragments herein.

The “class” of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

The term “Fc domain” or “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore, an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain (also referred to herein as a “cleaved variant heavy chain”). This may

be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to Kabat EU index). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (K447), of the Fc region may or may not be present. Amino acid sequences of heavy chains including Fc domains (or a subunit of an Fc domain as defined herein) are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise. In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, comprised in an antibody or bispecific antigen binding molecule according to the invention, comprises an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, comprised in an antibody or bispecific antigen binding molecule according to the invention, comprises an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat). Compositions of the invention, such as the pharmaceutical compositions described herein, comprise a population of antibodies or bispecific antigen binding molecules of the invention. The population of antibodies or bispecific antigen binding molecules may comprise molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain. The population of antibodies or bispecific antigen binding molecules may consist of a mixture of molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain, wherein at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the antibodies or bispecific antigen binding molecules have a cleaved variant heavy chain. In one embodiment of the invention a composition comprising a population of antibodies or bispecific antigen binding molecules of the invention comprises an antibody or bispecific antigen binding molecule comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment of the invention a composition comprising a population of antibodies or bispecific antigen binding molecules of the invention comprises an antibody or bispecific antigen binding molecule comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat). In one embodiment of the invention such a composition comprises a population of antibodies or bispecific antigen binding molecules comprised of molecules comprising a heavy chain including a subunit of an Fc domain as specified herein; molecules comprising a heavy chain including a subunit of a Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat); and molecules comprising a heavy chain including a subunit of an Fc domain as specified herein

- with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (see also above). A “subunit” of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.
- 10 A “modification promoting the association of the first and the second subunit of the Fc domain” is a manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. A modification promoting association as used herein particularly includes separate modifications made to each of the two Fc domain subunits
- 15 desired to associate (i.e. the first and the second subunit of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable, respectively. Thus, (hetero)dimerization occurs between a polypeptide comprising the
- 20 first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical in the sense that further components fused to each of the subunits (e.g. antigen binding moieties) are not the same. In some embodiments the modification promoting association comprises an amino acid mutation in the Fc domain, specifically an amino acid substitution. In a particular embodiment, the modification promoting association comprises a separate amino acid
- 25 mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.
- The term “effector functions” refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular
- 30 phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

As used herein, the terms “engineer, engineered, engineering”, are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches.

The term “amino acid mutation” as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., reduced binding to an Fc receptor, or increased association with another peptide. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. Particular amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an Fc region, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are particularly preferred. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution from proline at position 329 of the Fc domain to glycine can be indicated as 329G, G329, G<sub>329</sub>, P329G, or Pro329Gly.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference 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, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being

compared. For purposes herein, however, % amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448; W. R. Pearson (1996) "Effective protein sequence comparison" Meth. Enzymol. 266:227- 258; and Pearson et. al. (1997) Genomics 46:24-36, and is publicly available from [http://fasta.bioch.virginia.edu/fasta\\_www2/fasta\\_down.shtml](http://fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml). Alternatively, a public server accessible at [http://fasta.bioch.virginia.edu/fasta\\_www2/index.cgi](http://fasta.bioch.virginia.edu/fasta_www2/index.cgi) can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

The term "polynucleotide" refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA). The term "nucleic acid molecule" refers to any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By "isolated" nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

"Isolated polynucleotide (or nucleic acid) encoding [e.g. an antibody or bispecific antigen binding molecule of the invention]" refers to one or more polynucleotide molecules encoding antibody heavy and light chains (or fragments thereof), including such polynucleotide molecule(s) in a

single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette comprises polynucleotide sequences that encode antibodies or bispecific antigen binding molecules of the invention or fragments thereof.

The term "vector" or "expression vector" refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated in a cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression vector is inside the cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode antibodies or bispecific antigen binding molecules of the invention or fragments thereof.

The terms "host cell", "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the antibodies or bispecific antigen binding molecules of the present invention. Host cells include cultured cells, *e.g.* mammalian cultured cells, such as HEK cells, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

An “activating Fc receptor” is an Fc receptor that following engagement by an Fc domain of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Human activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and FcαRI (CD89).

- 5 Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or derivatives thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. As used herein, the term “reduced ADCC” is defined as either a reduction in the number of target cells that are lysed in a given time, at a given  
10 concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or an increase in the concentration of antibody in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The reduction in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard production, purification,  
15 formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example, the reduction in ADCC mediated by an antibody comprising in its Fc domain an amino acid substitution that reduces ADCC, is relative to the ADCC mediated by the same antibody without this amino acid substitution in the Fc domain. Suitable assays to measure ADCC are well known in the art (see e.g. PCT publication no. WO 2006/082515 or PCT  
20 publication no. WO 2012/130831).

An “effective amount” of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

- A “therapeutically effective amount” of an agent, e.g. a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic  
25 or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

- An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). Particularly, the individual or subject  
30 is a human.

The term “pharmaceutical composition” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains

no additional components which are unacceptably toxic to a subject to which the composition would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies or bispecific antigen binding molecules of the invention are used to delay development of a disease or to slow the progression of a disease.

The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

### **Detailed Description of the Embodiments**

The invention provides antibodies and bispecific antigen binding molecules that bind GPRC5D, particularly human GPRC5D. In addition, the molecules have other favorable properties for therapeutic application, e.g. with respect to efficacy and/or safety as well as produceability.

### **GPRC5D antibody**

In a first aspect the present invention provides an antibody that binds to GPRC5D, wherein the antibody comprises (i) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; (ii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ

ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; (iii) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a  
5 LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; (iv) a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; or (v) a heavy chain variable  
10 region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97.

In some embodiments, the antibody is a humanized antibody. In one embodiment, the VH is a  
15 humanized VH and/or the VL is a humanized VL. In one embodiment, the antibody comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

In a particular embodiment, (i) the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 13, and the VL  
20 comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 14; or (ii) the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 15, and the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 16; or (iii) the VH  
25 comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 48, and the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 53; or (iv) the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 49, and the VL comprises an amino  
30 acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52; or (v) the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 57, and the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100%

identical to the amino acid sequence of SEQ ID NO: 64; or (vi) the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 58, and the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63.

- 5 In a particular embodiment, the antibody comprises (i) a VH that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence of SEQ ID NO: 13, and a VL that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 14; or (ii) a VH that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 15, and a VL that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 16; or (iii) a VH that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 48, and the VL is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 53; or (iv) the VH is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 49, and the VL is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 52; or (v) the VH is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 57, and the VL is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 64; or (vi) the VH is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 58, and the VL is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 63.

In another embodiment, the antibody is an IgG, particularly an IgG1, antibody. In one embodiment, the antibody is a full-length antibody. In another embodiment, the antibody is an antibody fragment selected from the group of an Fv molecule, a scFv molecule, a Fab molecule, and a F(ab')<sub>2</sub> molecule. In one embodiment, the antibody is a multispecific antibody.

- 25 In certain embodiments, a VH or VL sequence having at least 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an antibody comprising that sequence retains the ability to bind to GPRC5D. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 13 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 14 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 15 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 16 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 48 and/or a total of 1 to 10 amino

acids have been substituted, inserted and/or deleted in SEQ ID NO: 53 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 49 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 52 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 57 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 64 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 58 and/or a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 63.

In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the antibody comprises the VH sequence in SEQ ID NO: 13 and/or the VL sequence in SEQ ID NO: 14, including post-translational modifications of that sequence. Optionally, the antibody comprises the VH sequence in SEQ ID NO: 15 and/or the VL sequence in SEQ ID NO: 16, including post-translational modifications of that sequence. Optionally, the antibody comprises the VH sequence in SEQ ID NO: 448 and/or the VL sequence in SEQ ID NO: 53, including post-translational modifications of that sequence. Optionally, the antibody comprises the VH sequence in SEQ ID NO: 49 and/or the VL sequence in SEQ ID NO: 52, including post-translational modifications of that sequence. Optionally, the antibody comprises the VH sequence in SEQ ID NO: 57 and/or the VL sequence in SEQ ID NO: 64, including post-translational modifications of that sequence. Optionally, the antibody comprises the VH sequence in SEQ ID NO: 58 and/or the VL sequence in SEQ ID NO: 63, including post-translational modifications of that sequence.

In one embodiment, the antibody comprises a VH comprising an amino acid sequence selected from the group of SEQ ID NO: 13 and SEQ ID NO: 15, and a VL comprising the amino acid sequence of SEQ ID NO: 14.

In one embodiment, the antibody comprises a VH sequence selected from the group of SEQ ID NO: 13 and SEQ ID NO: 12, and the VL sequence of SEQ ID NO: 16.

In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 13 and a VL comprising the amino acid sequence of SEQ ID NO: 14. In a particular embodiment, the antibody comprises the VH sequence of SEQ ID NO: 13 and the VL sequence of SEQ ID NO: 14.

In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 15 and a VL comprising the amino acid sequence of SEQ ID NO: 16. In a particular

embodiment, the antibody comprises the VH sequence of SEQ ID NO: 15 and the VL sequence of SEQ ID NO: 16.

In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 48 and a VL comprising the amino acid sequence of SEQ ID NO: 53. In a particular  
5 embodiment, the antibody comprises the VH sequence of SEQ ID NO: 48 and the VL sequence of SEQ ID NO: 53.

In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 49 and a VL comprising the amino acid sequence of SEQ ID NO: 52. In a particular  
10 embodiment, the antibody comprises the VH sequence of SEQ ID NO: 49 and the VL sequence of SEQ ID NO: 52.

In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 57 and a VL comprising the amino acid sequence of SEQ ID NO: 64. In a particular  
embodiment, the antibody comprises the VH sequence of SEQ ID NO: 57 and the VL sequence of SEQ ID NO: 64.

15 In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 58 and a VL comprising the amino acid sequence of SEQ ID NO: 63. In a particular  
embodiment, the antibody comprises the VH sequence of SEQ ID NO: 58 and the VL sequence of SEQ ID NO: 63.

In one embodiment, the antibody comprises a human constant region. In one embodiment, the  
20 antibody is an immunoglobulin molecule comprising a human constant region, particularly an IgG class immunoglobulin molecule comprising a human CH1, CH2, CH3 and/or CL domain.  
Exemplary sequences of human constant domains are given in SEQ ID NOs 37 and 38 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 39 (human IgG1 heavy chain  
constant domains CH1-CH2-CH3). In some embodiments, the antibody comprises a light chain  
25 constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%,  
99% or 100% identical to the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 39,  
particularly the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibody  
comprises a heavy chain constant region comprising an amino acid sequence that is at least about  
95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 39.  
30 Particularly, the heavy chain constant region may comprise amino acid mutations in the Fc domain  
as described herein.

In one embodiment, the antibody is a monoclonal antibody.

In one embodiment, the antibody is an IgG, particularly an IgG<sub>1</sub>, antibody. In one embodiment, the antibody is a full-length antibody.

In one embodiment, the antibody comprises an Fc domain, particularly an IgG Fc domain, more particularly an IgG1 Fc domain. In one embodiment the Fc domain is a human Fc domain. The Fc domain of the antibody may incorporate any of the features, singly or in combination, described herein in relation to the Fc domain of the bispecific antigen binding molecule of the invention.

In another embodiment, the antibody is an antibody fragment selected from the group of an Fv molecule, a scFv molecule, a Fab molecule, and a F(ab')<sub>2</sub> molecule; particularly a Fab molecule.

In another embodiment, the antibody fragment is a diabody, a triabody or a tetrabody.

- 10 In a further aspect, the antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in the sections below.

#### Glycosylation variants

- 15 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 oligosaccharide 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 antibody variants with certain improved properties.

- 25 In one embodiment, antibody variants are provided having a non-fucosylated oligosaccharide, i.e. an oligosaccharide structure that lacks fucose attached (directly or indirectly) to an Fc region. Such non-fucosylated oligosaccharide (also referred to as "afucosylated" oligosaccharide) particularly is an N-linked oligosaccharide which lacks a fucose residue attached to the first GlcNAc in the stem of the biantennary oligosaccharide structure. In one embodiment, antibody variants are provided having an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a native or parent antibody. For example, the proportion of non-fucosylated

oligosaccharides may be at least about 20%, at least about 40%, at least about 60%, at least about 80%, or even about 100% (i.e. no fucosylated oligosaccharides are present). The percentage of non-fucosylated oligosaccharides is the (average) amount of oligosaccharides lacking fucose residues, relative to the sum of all oligosaccharides attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2006/082515, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about  $\pm 3$  amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such antibodies having an increased proportion of non-fucosylated oligosaccharides in the Fc region may have improved Fc $\gamma$ RIIIa receptor binding and/or improved effector function, in particular improved ADCC function. See, e.g., US 2003/0157108; US 2004/0093621.

Examples of cell lines capable of producing antibodies with reduced fucosylation include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US 2003/0157108; and WO 2004/056312, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87:614-622 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107), or cells with reduced or abolished activity of a GDP-fucose synthesis or transporter protein (see, e.g., US2004259150, US2005031613, US2004132140, US2004110282).

In a further embodiment, antibody variants are provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function as described above. Examples of such antibody variants are described, e.g., in Umana et al., *Nat Biotechnol* 17, 176-180 (1999); Ferrara et al., *Biotechnol Bioeng* 93, 851-861 (2006); WO 99/54342; WO 2004/065540, WO 2003/011878.

Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

### 30 Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues.

In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein.

- 5 Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541, 8,30,930, 7,855,275, 9,000,130, or WO2016040856.

### Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable  
10 for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random  
15 copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the  
20 antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

- 25 In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a  
30 temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

### Immunoconjugates

The invention also provides immunoconjugates comprising an anti-GPRC5D antibody as described herein conjugated (chemically bonded) to one or more therapeutic agents such as cytotoxic agents, chemotherapeutic agents, drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more of the therapeutic agents mentioned above. The antibody is typically connected to one or more of the therapeutic agents using linkers. An overview of ADC technology including examples of therapeutic agents and drugs and linkers is set forth in *Pharmacol Review* 68:3-19 (2016).

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include  $\text{At}^{211}$ ,  $\text{I}^{131}$ ,  $\text{I}^{125}$ ,  $\text{Y}^{90}$ ,  $\text{Re}^{186}$ ,  $\text{Re}^{188}$ ,  $\text{Sm}^{153}$ ,  $\text{Bi}^{212}$ ,  $\text{P}^{32}$ ,  $\text{Pb}^{212}$  and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example  $\text{tc99m}$  or  $\text{I}^{123}$ , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis-(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be

prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

#### Multispecific antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites, i.e., different epitopes on different antigens or different epitopes on the same antigen. In certain embodiments, the multispecific antibody has three or more binding specificities. In certain embodiments, one of the binding specificities is for GPRC5D and the other (two or more) specificity is for any other antigen. In certain embodiments, bispecific antibodies may bind to two (or more) different epitopes of GPRC5D. Multispecific (e.g., bispecific) antibodies may also be used to localize cytotoxic agents or cells to cells which express GPRC5D. Multispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)) and “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168, and Atwell et al., *J. Mol. Biol.* 270:26 (1997)). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (see, e.g., WO 2009/089004); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992) and WO 2011/034605); using the common light chain technology for

circumventing the light chain miss-pairing problem (see, e.g., WO 98/50431); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. 5 *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more antigen binding sites, including for example, "Octopus antibodies," or DVD-Ig are also included herein (see, e.g. WO 2001/77342 and WO 2008/024715). Other examples of multispecific antibodies with three or more antigen binding sites can be found in WO 2010/115589, WO 2010/112193, WO 2010/136172, WO2010/145792, and WO 10 2013/026831. The bispecific antibody or antigen binding fragment thereof also includes a "Dual Acting FAB" or "DAF" comprising an antigen binding site that binds to GPRC5D as well as another different antigen, or two different epitopes of GPRC5D (see, e.g., US 2008/0069820 and WO 2015/095539).

Multi-specific antibodies may also be provided in an asymmetric form with a domain crossover in one or more binding arms of the same antigen specificity, i.e. by exchanging the VH/VL domains 15 (see e.g., WO 2009/080252 and WO 2015/150447), the CH1/CL domains (see e.g., WO 2009/080253) or the complete Fab arms (see e.g., WO 2009/080251, WO 2016/016299, also see Schaefer et al, PNAS, 108 (2011) 1187-1191, and Klein et al., MAbs 8 (2016) 1010-20). Asymmetrical Fab arms can also be engineered by introducing charged or non-charged amino acid 20 mutations into domain interfaces to direct correct Fab pairing. See e.g., WO 2016/172485.

Various further molecular formats for multispecific antibodies are known in the art and are included herein (see e.g., Spiess et al., *Mol Immunol* 67 (2015) 95-106).

A particular type of multispecific antibodies, also included herein, are bispecific antibodies designed to simultaneously bind to a surface antigen on a target cell, e.g., a tumor cell, and to an 25 activating, invariant component of the T cell receptor (TCR) complex, such as CD3, for retargeting of T cells to kill target cells. Hence, in certain embodiments, an antibody provided herein is a multispecific antibody, particularly a bispecific antibody, wherein one of the binding specificities is for GPRC5D and the other is for CD3.

Examples of bispecific antibody formats that may be useful for this purpose include, but are not 30 limited to, the so-called "BiTE" (bispecific T cell engager) molecules wherein two scFv molecules are fused by a flexible linker (see, e.g., WO2004/106381, WO2005/061547, WO2007/042261, and WO2008/119567, Nagorsen and Bäuerle, *Exp Cell Res* 317, 1255-1260 (2011)); diabodies (Holliger et al., *Prot Eng* 9, 299-305 (1996)) and derivatives thereof, such as tandem diabodies

(“TandAb”; Kipriyanov et al., J Mol Biol 293, 41-56 (1999)); “DART” (dual affinity retargeting) molecules which are based on the diabody format but feature a C-terminal disulfide bridge for additional stabilization (Johnson et al., J Mol Biol 399, 436-449 (2010)), and so-called triomabs, which are whole hybrid mouse/rat IgG molecules (reviewed in Seimetz et al., Cancer Treat Rev 5 36, 458-467 (2010)). Particular T cell bispecific antibody formats included herein are described in WO 2013/026833, WO2013/026839, WO 2016/020309; Bacac et al., Oncoimmunology 5(8) (2016) e1203498.

### **Bispecific antigen binding molecules that bind to GPRC5D and a second antigen**

10 The invention also provides a bispecific antigen binding molecule, i.e. an antigen binding molecule that comprises at least two antigen binding moieties capable of specific binding to two distinct antigenic determinants (a first and a second antigen).

According to particular embodiments of the invention, the antigen binding moieties comprised in the bispecific antigen binding molecule are Fab molecules (i.e. antigen binding domains composed 15 of a heavy and a light chain, each comprising a variable and a constant domain). In one embodiment, the first and/or the second antigen binding moiety is a Fab molecule. In one embodiment, said Fab molecule is human. In a particular embodiment, said Fab molecule is humanized. In yet another embodiment, said Fab molecule comprises human heavy and light chain constant domains.

20 Preferably, at least one of the antigen binding moieties is a crossover Fab molecule. Such modification reduces mispairing of heavy and light chains from different Fab molecules, thereby improving the yield and purity of the bispecific antigen binding molecule of the invention in recombinant production. In a particular crossover Fab molecule useful for the bispecific antigen binding molecule of the invention, the variable domains of the Fab light chain and the Fab heavy 25 chain (VL and VH, respectively) are exchanged. Even with this domain exchange, however, the preparation of the bispecific antigen binding molecule may comprise certain side products due to a so-called Bence Jones-type interaction between misspaired heavy and light chains (see Schaefer et al, PNAS, 108 (2011) 11187-11191). To further reduce mispairing of heavy and light chains from different Fab molecules and thus increase the purity and yield of the desired bispecific antigen 30 binding molecule, charged amino acids with opposite charges may be introduced at specific amino acid positions in the CH1 and CL domains of either the Fab molecule(s) binding to the first antigen (GPRC5D), or the Fab molecule binding to the second antigen (e.g. an activating T cell antigen such as CD3), as further described herein. Charge modifications are made either in the

conventional Fab molecule(s) comprised in the bispecific antigen binding molecule (such as shown e.g. in Figures 1 A-C, G-J), or in the VH/VL crossover Fab molecule(s) comprised in the bispecific antigen binding molecule (such as shown e.g. in Figure 1 D-F, K-N) (but not in both). In particular embodiments, the charge modifications are made in the conventional Fab molecule(s) comprised in the bispecific antigen binding molecule (which in particular embodiments bind(s) to the first antigen, i.e. GPRC5D).

In a particular embodiment according to the invention, the bispecific antigen binding molecule is capable of simultaneous binding to the first antigen (i.e. GPRC5D), and the second antigen (e.g. an activating T cell antigen, particularly CD3). In one embodiment, the bispecific antigen binding molecule is capable of crosslinking a T cell and a target cell by simultaneous binding GPRC5D and an activating T cell antigen. In an even more particular embodiment, such simultaneous binding results in lysis of the target cell, particularly a GPRC5D expressing tumor cell. In one embodiment, such simultaneous binding results in activation of the T cell. In other embodiments, such simultaneous binding results in a cellular response of a T lymphocyte, particularly a cytotoxic T lymphocyte, selected from the group of: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers. In one embodiment, binding of the bispecific antigen binding molecule to the activating T cell antigen, particularly CD3, without simultaneous binding to GPRC5D does not result in T cell activation. In one embodiment, the bispecific antigen binding molecule is capable of re-directing cytotoxic activity of a T cell to a target cell. In a particular embodiment, said re-direction is independent of MHC-mediated peptide antigen presentation by the target cell and and/or specificity of the T cell. Particularly, a T cell according to any of the embodiments of the invention is a cytotoxic T cell. In some embodiments the T cell is a CD4<sup>+</sup> or a CD8<sup>+</sup> T cell, particularly a CD8<sup>+</sup> T cell.

#### First antigen binding moiety

The bispecific antigen binding molecule of the invention comprises at least one antigen binding moiety, particularly a Fab molecule, that binds to GPRC5D (first antigen). In certain embodiments, the bispecific antigen binding molecule comprises two antigen binding moieties, particularly Fab molecules, which bind to GPRC5D. In a particular such embodiment, each of these antigen binding moieties binds to the same antigenic determinant. In an even more particular embodiment, all of these antigen binding moieties are identical, i.e. they comprise the same amino acid sequences including the same amino acid substitutions in the CH1 and CL domain as described herein (if

any). In one embodiment, the bispecific antigen binding molecule comprises not more than two antigen binding moieties, particularly Fab molecules, which bind to GPRC5D.

In particular embodiments, the antigen binding moiety(ies) which bind to GPRC5D is/are a conventional Fab molecule. In such embodiments, the antigen binding moiety(ies) that binds to a second antigen is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other.

In alternative embodiments, the antigen binding moiety(ies) which bind to GPRC5D is/are a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other. In such embodiments, the antigen binding moiety(ies) that binds a second antigen is a conventional Fab molecule.

The GPRC5D binding moiety is able to direct the bispecific antigen binding molecule to a target site, for example to a specific type of tumor cell that expresses GPRC5D.

The first antigen binding moiety of the bispecific antigen binding molecule may incorporate any of the features, singly or in combination, described herein in relation to the antibody that binds GPRC5D, unless scientifically clearly unreasonable or impossible.

Thus, in one aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a

first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6, and (b) a second antigen binding moiety that binds to a second antigen. In another aspect, the invention provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region

(LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12, and (b) a second antigen binding moiety that binds to a second antigen.

In some embodiments, the first antigen binding moiety is (derived from) a humanized antibody.

In one embodiment, the VH is a humanized VH and/or the VL is a humanized VL. In one

5 embodiment, the first antigen binding moiety comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

In one embodiment, the VH of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence

10 selected from the group of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and the VL of the first antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

15 In one embodiment, the first antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

In one embodiment, the first antigen binding moiety comprises a VH comprising an amino acid sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and a VL comprising the amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53,

25 SEQ ID NO: 63 and SEQ ID NO: 64.

In one embodiment, the first antigen binding moiety comprises a VH sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and the VL sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

30 In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 13 and a VL comprising the amino acid sequence of SEQ ID NO: 14. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 13 and the VL sequence of SEQ ID NO: 14.

- In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 15 and a VL comprising the amino acid sequence of SEQ ID NO: 16. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 15 and the VL sequence of SEQ ID NO: 16.
- 5 In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 48 and a VL comprising the amino acid sequence of SEQ ID NO: 53. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 48 and the VL sequence of SEQ ID NO: 53.
- In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 49 and a VL comprising the amino acid sequence of SEQ ID NO: 52. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 49 and the VL sequence of SEQ ID NO: 52.
- 10 In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 57 and a VL comprising the amino acid sequence of SEQ ID NO: 64. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 57 and the VL sequence of SEQ ID NO: 64.
- 15 In a particular embodiment, the first antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 58 and a VL comprising the amino acid sequence of SEQ ID NO: 63. In a particular embodiment, the first antigen binding moiety comprises the VH sequence of SEQ ID NO: 58 and the VL sequence of SEQ ID NO: 63. In one embodiment, the first antigen binding moiety comprises a human constant region. In one embodiment, the first antigen binding moiety is a Fab molecule comprising a human constant region, particularly a human CH1 and/or CL domain. Exemplary sequences of human constant domains are given in SEQ ID NOs 37 and 38 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 39 (human IgG<sub>1</sub> heavy chain constant domains CH1-CH2-CH3). In some embodiments, the first antigen binding moiety comprises a light chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38, particularly the amino acid sequence of SEQ ID NO: 37. Particularly, the light chain constant region may comprise amino acid mutations as described herein under “charge modifications” and/or may comprise deletion or substitutions of one or more (particularly two) N-terminal amino acids if in a crossover Fab molecule. In some embodiments, the first antigen binding moiety comprises a heavy chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the CH1 domain sequence
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- 25
- 30

comprised in the amino acid sequence of SEQ ID NO: 39. Particularly, the heavy chain constant region (specifically CH1 domain) may comprise amino acid mutations as described herein under “charge modifications”.

#### Second antigen binding moiety

- 5 The bispecific antigen binding molecule of the invention comprises at least one antigen binding moiety, particularly a Fab molecule that binds to a second antigen (different from GPRC5D). In particular embodiments, the antigen binding moiety that binds the second antigen is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced
- 10 by each other. In such embodiments, the antigen binding moiety(ies) that binds to the first antigen (i.e. GPRC5D) is preferably a conventional Fab molecule. In embodiments where there is more than one antigen binding moiety, particularly Fab molecule, that binds to GPRC5D comprised in the bispecific antigen binding molecule, the antigen binding moiety that binds to the second antigen preferably is a crossover Fab molecule and the antigen binding moieties that bind to
- 15 GPRC5D are conventional Fab molecules.
- In alternative embodiments, the antigen binding moiety that binds to the second antigen is a conventional Fab molecule. In such embodiments, the antigen binding moiety(ies) that binds to the first antigen (i.e. GPRC5D) is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy
- 20 and light chains are exchanged / replaced by each other. In embodiments where there is more than one antigen binding moiety, particularly Fab molecule, that binds to a second antigen comprised in the bispecific antigen binding molecule, the antigen binding moiety that binds to GPRC5D preferably is a crossover Fab molecule and the antigen binding moieties that bind to the second antigen are conventional Fab molecules.
- 25 In some embodiments, the second antigen is an activating T cell antigen (also referred to herein as an “activating T cell antigen binding moiety, or activating T cell antigen binding Fab molecule”). In a particular embodiment, the bispecific antigen binding molecule comprises not more than one antigen binding moiety capable of specific binding to an activating T cell antigen. In one embodiment the bispecific antigen binding molecule provides monovalent binding to the
- 30 activating T cell antigen.
- In particular embodiments, the second antigen is CD3, particularly human CD3 (SEQ ID NO: 40) or cynomolgus CD3 (SEQ ID NO: 41), most particularly human CD3. In one embodiment the

second antigen binding moiety is cross-reactive for (i.e. specifically binds to) human and cynomolgus CD3. In some embodiments, the second antigen is the epsilon subunit of CD3 (CD3 epsilon).

In one embodiment, the second antigen binding moiety comprises a HCDR 1 of SEQ ID NO: 29, a HCDR 2 of SEQ ID NO: 30, a HCDR 3 of SEQ ID NO: 31, a LCDR 1 of SEQ ID NO: 32, a LCDR 2 of SEQ ID NO: 33 and a LCDR 3 of SEQ ID NO: 34. In one embodiment, the second antigen binding moiety comprises a VH comprising a HCDR 1 of SEQ ID NO: 29, a HCDR 2 of SEQ ID NO: 30, and a HCDR 3 of SEQ ID NO: 31, and a VL comprising a LCDR 1 of SEQ ID NO: 32, a LCDR 2 of SEQ ID NO: 33 and a LCDR 3 of SEQ ID NO: 34. In some embodiments, the second antigen binding moiety is (derived from) a humanized antibody. In one embodiment, the VH is a humanized VH and/or the VL is a humanized VL. In one embodiment, the second antigen binding moiety comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In one embodiment, the second antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35. In one embodiment, the second antigen binding moiety comprises a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36. In one embodiment, the second antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35, and a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36. In one embodiment, the VH of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35, and the VL of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36. In one embodiment, the second antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 35, and a VL comprising the amino acid sequence of SEQ ID NO: 36. In one embodiment, the second antigen binding moiety comprises the VH sequence of SEQ ID NO: 35, and the VL sequence of SEQ ID NO: 36.

In one embodiment, the second antigen binding moiety comprises a HCDR 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, a HCDR 3 of SEQ ID NO: 100, a LCDR 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103. In one embodiment, the second antigen binding moiety comprises a VH comprising a HCDR 1 of SEQ ID NO: 98, a HCDR 2 of

SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a VL comprising a LCDR 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103. In some embodiments, the second antigen binding moiety is (derived from) a humanized antibody. In one embodiment, the VH is a humanized VH and/or the VL is a humanized VL. In one embodiment, the second antigen binding moiety comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In one embodiment, the second antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 104. In one embodiment, the second antigen binding moiety comprises a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 105. In one embodiment, the second antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 104, and a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 105. In one embodiment, the VH of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 104, and the VL of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 105. In one embodiment, the second antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 104, and a VL comprising the amino acid sequence of SEQ ID NO: 105. In one embodiment, the second antigen binding moiety comprises the VH sequence of SEQ ID NO: 104, and the VL sequence of SEQ ID NO: 105.

In one embodiment, the second antigen binding moiety comprises a HCDR 1 of SEQ ID NO: 106, a HCDR 2 of SEQ ID NO: 107, a HCDR 3 of SEQ ID NO: 108, a LCDR 1 of SEQ ID NO: 109, a LCDR 2 of SEQ ID NO: 110 and a LCDR 3 of SEQ ID NO: 111. In one embodiment, the second antigen binding moiety comprises a VH comprising a HCDR 1 of SEQ ID NO: 106, a HCDR 2 of SEQ ID NO: 107, and a HCDR 3 of SEQ ID NO: 108, and a VL comprising a LCDR 1 of SEQ ID NO: 109, a LCDR 2 of SEQ ID NO: 110 and a LCDR 3 of SEQ ID NO: 111.

In some embodiments, the second antigen binding moiety is (derived from) a humanized antibody. In one embodiment, the VH is a humanized VH and/or the VL is a humanized VL. In one embodiment, the second antigen binding moiety comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In one embodiment, the second antigen binding

moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 112. In one embodiment, the second antigen binding moiety comprises a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 113. In one embodiment, the second antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 112, and a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 113. In one embodiment, the VH of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 112, and the VL of the second antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 113. In one embodiment, the second antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 112, and a VL comprising the amino acid sequence of SEQ ID NO: 113. In one embodiment, the second antigen binding moiety comprises the VH sequence of SEQ ID NO: 112, and the VL sequence of SEQ ID NO: 113.

In one embodiment, the second antigen binding moiety comprises a human constant region. In one embodiment, the second antigen binding moiety is a Fab molecule comprising a human constant region, particularly a human CH1 and/or CL domain. Exemplary sequences of human constant domains are given in SEQ ID NOs 37 and 38 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 39 (human IgG<sub>1</sub> heavy chain constant domains CH1-CH2-CH3). In some embodiments, the second antigen binding moiety comprises a light chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38, particularly the amino acid sequence of SEQ ID NO: 37. Particularly, the light chain constant region may comprise amino acid mutations as described herein under “charge modifications” and/or may comprise deletion or substitutions of one or more (particularly two) N-terminal amino acids if in a crossover Fab molecule.. In some embodiments, the second antigen binding moiety comprises a heavy chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the CH1 domain sequence comprised in the amino acid sequence of SEQ ID NO: 39. Particularly, the heavy chain constant region (specifically CH1 domain) may comprise amino acid mutations as described herein under “charge modifications”.

In some embodiments, the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other (i.e. according to such embodiment, the second antigen binding moiety is a crossover Fab molecule wherein the variable or constant domains of the Fab light chain and the Fab heavy chain are exchanged). In one such embodiment, the first (and the third, if any) antigen binding moiety is a conventional Fab molecule.

In one embodiment, not more than one antigen binding moiety that binds to the second antigen (e.g. an activating T cell antigen such as CD3) is present in the bispecific antigen binding molecule (i.e. the bispecific antigen binding molecule provides monovalent binding to the second antigen).

#### Charge modifications

The bispecific antigen binding molecules of the invention may comprise amino acid substitutions in Fab molecules comprised therein which are particularly efficient in reducing mispairing of light chains with non-matching heavy chains (Bence-Jones-type side products), which can occur in the production of Fab-based bi-/multispecific antigen binding molecules with a VH/VL exchange in one (or more, in case of molecules comprising more than two antigen-binding Fab molecules) of their binding arms (see also PCT publication no. WO 2015/150447, particularly the examples therein, incorporated herein by reference in its entirety). The ratio of a desired bispecific antigen binding molecule compared to undesired side products, in particular Bence Jones-type side products occurring in bispecific antigen binding molecules with a VH/VL domain exchange in one of their binding arms, can be improved by the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH1 and CL domains (sometimes referred to herein as “charge modifications”).

Accordingly, in some embodiments wherein the first and the second antigen binding moiety of the bispecific antigen binding molecule are both Fab molecules, and in one of the antigen binding moieties (particularly the second antigen binding moiety) the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other,

i) in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index); or

- ii) in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index).

The bispecific antigen binding molecule does not comprise both modifications mentioned under i) and ii). The constant domains CL and CH1 of the antigen binding moiety having the VH/VL exchange are not replaced by each other (i.e. remain unexchanged).

In a more specific embodiment,

- i) in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- In one such embodiment, in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- In a further embodiment, in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- In a particular embodiment, in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H)

(numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a more particular embodiment, in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In an even more particular embodiment, in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In particular embodiments, if amino acid substitutions according to the above embodiments are made in the constant domain CL and the constant domain CH1 of the first antigen binding moiety, the constant domain CL of the first antigen binding moiety is of kappa isotype.

Alternatively, the amino acid substitutions according to the above embodiments may be made in the constant domain CL and the constant domain CH1 of the second antigen binding moiety instead of in the constant domain CL and the constant domain CH1 of the first antigen binding moiety. In particular such embodiments, the constant domain CL of the second antigen binding moiety is of kappa isotype.

Accordingly, in one embodiment, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen

binding moiety the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further embodiment, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In still another embodiment, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one embodiment, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In another embodiment, in the constant domain CL of the second antigen binding moiety the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second antigen binding moiety the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In a particular embodiment, the bispecific antigen binding molecule of the invention comprises (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region

- (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89, and
- (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- 15 In a particular embodiment, the bispecific antigen binding molecule of the invention comprises
- (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region
- 20 (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89, and
- (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- 25 wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- 30

- In a particular embodiment, the bispecific antigen binding molecule of the invention comprises
- (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97, and
- (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).
- In a particular embodiment, the bispecific antigen binding molecule of the invention comprises
- (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97, and
- (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H)

(numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, the bispecific antigen binding molecule of the invention comprises (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97, and (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;

wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, the bispecific antigen binding molecule of the invention comprises (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6, and

(b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;  
wherein in the constant domain CL of the first antigen binding moiety the amino acid at position  
5 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid  
10 at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, the bispecific antigen binding molecule of the invention comprises  
(a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D  
15 and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12, and  
20 (b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;  
wherein in the constant domain CL of the first antigen binding moiety the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according  
25 to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in a particular embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first antigen binding moiety the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering  
30 according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

#### Bispecific antigen binding molecule formats

The components of the bispecific antigen binding molecule according to the present invention can be fused to each other in a variety of configurations. Exemplary configurations are depicted in **Figures 1A-Z**.

In particular embodiments, the antigen binding moieties comprised in the bispecific antigen binding molecule are Fab molecules. In such embodiments, the first, second, third etc. antigen binding moiety may be referred to herein as first, second, third etc. Fab molecule, respectively.

In one embodiment, the first and the second antigen binding moiety of the bispecific antigen binding molecule are fused to each other, optionally via a peptide linker. In particular embodiments, the first and the second antigen binding moiety are each a Fab molecule. In one such embodiment, the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety. In another such embodiment, the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety. In embodiments wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety, additionally the Fab light chain of the first antigen binding moiety and the Fab light chain of the second antigen binding moiety may be fused to each other, optionally via a peptide linker.

A bispecific antigen binding molecule with a single antigen binding moiety (such as a Fab molecule) capable of specific binding to a target cell antigen such as GPRC5D (for example as shown in **Figures 1A, 1D, 1G, 1H, 1K, 1L**) is useful, particularly in cases where internalization of the target cell antigen is to be expected following binding of a high affinity antigen binding moiety. In such cases, the presence of more than one antigen binding moiety specific for the target cell antigen may enhance internalization of the target cell antigen, thereby reducing its availability. In other cases, however, it will be advantageous to have a bispecific antigen binding molecule comprising two or more antigen binding moieties (such as Fab molecules) specific for a target cell antigen (see examples shown in **Figures 1B, 1C, 1E, 1F, 1I, 1J, 1M or 1N**), for example to optimize targeting to the target site or to allow crosslinking of target cell antigens.

Accordingly, in particular embodiments, the bispecific antigen binding molecule according to the present invention comprises a third antigen binding moiety.

In one embodiment, the third antigen binding moiety binds to the first antigen, i.e. GPRC5D. In one embodiment, the third antigen binding moiety is a Fab molecule.

In one embodiment, the third antigen moiety is identical to the first antigen binding moiety.

The third antigen binding moiety of the bispecific antigen binding molecule may incorporate any of the features, singly or in combination, described herein in relation to the first antigen binding moiety and/or the antibody that binds GPRC5D, unless scientifically clearly unreasonable or impossible.

5 In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87,  
10 a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87,  
15 a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94,  
20 a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94,  
25 a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94,  
30 a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 4, and a light chain variable region

(VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 5, a LCDR 2 of SEQ ID NO: 6 and a LCDR 3 of SEQ ID NO: 7.

In one embodiment, the third antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a  
5 HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12.

In some embodiments, the third antigen binding moiety is (derived from) a humanized antibody. In one embodiment, the VH is a humanized VH and/or the VL is a humanized VL. In one  
10 embodiment, the third antigen binding moiety comprises CDRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

In one embodiment, the VH of the third antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence  
15 selected from the group of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and the VL of the third antigen binding moiety comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

20 In one embodiment, the third antigen binding moiety comprises a VH sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15 SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and a VL sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16,  
25 SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

In one embodiment, the third antigen binding moiety comprises a VH comprising an amino acid sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15 SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and SEQ ID NO: 58, and a VL comprising the amino acid sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53,  
30 SEQ ID NO: 63 and SEQ ID NO: 64.

In one embodiment, the third antigen binding moiety comprises a VH sequence selected from the group of SEQ ID NO: 13, SEQ ID NO: 15 SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 57 and

SEQ ID NO: 58, and the VL sequence selected from the group of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 63 and SEQ ID NO: 64.

In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 13 and a VL comprising the amino acid sequence of SEQ ID

5 NO: 14. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 13 and the VL sequence of SEQ ID NO: 14.

In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 15 and a VL comprising the amino acid sequence of SEQ ID

10 NO: 16. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 15 and the VL sequence of SEQ ID NO: 16.

In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 48 and a VL comprising the amino acid sequence of SEQ ID

NO: 53. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 48 and the VL sequence of SEQ ID NO: 53.

15 In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 49 and a VL comprising the amino acid sequence of SEQ ID

NO: 52. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 49 and the VL sequence of SEQ ID NO: 52.

In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 57 and a VL comprising the amino acid sequence of SEQ ID

20 NO: 64. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 57 and the VL sequence of SEQ ID NO: 64.

In a particular embodiment, the third antigen binding moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 58 and a VL comprising the amino acid sequence of SEQ ID

25 NO: 63. In a particular embodiment, the third antigen binding moiety comprises the VH sequence of SEQ ID NO: 58 and the VL sequence of SEQ ID NO: 63.

In one embodiment, the third antigen binding moiety comprises a human constant region. In one embodiment, the third antigen binding moiety is a Fab molecule comprising a human constant region, particularly a human CH1 and/or CL domain. Exemplary sequences of human constant

30 domains are given in SEQ ID NOs 37 and 38 (human kappa and lambda CL domains, respectively) and SEQ ID NO: 39 (human IgG<sub>1</sub> heavy chain constant domains CH1-CH2-CH3). In some embodiments, the third antigen binding moiety comprises a light chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the

- amino acid sequence of SEQ ID NO: 37 or SEQ ID NO: 38, particularly the amino acid sequence of SEQ ID NO: 37. Particularly, the light chain constant region may comprise amino acid mutations as described herein under “charge modifications” and/or may comprise deletion or substitutions of one or more (particularly two) N-terminal amino acids if in a crossover Fab molecule. In some embodiments, the third antigen binding moiety comprises a heavy chain constant region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the CH1 domain sequence comprised in the amino acid sequence of SEQ ID NO: 39. Particularly, the heavy chain constant region (specifically CH1 domain) may comprise amino acid mutations as described herein under “charge modifications”.
- 10 In particular embodiments, the third and the first antigen binding moiety are each a Fab molecule and the third antigen binding moiety is identical to the first antigen binding moiety. Thus, in these embodiments the first and the third antigen binding moiety comprise the same heavy and light chain amino acid sequences and have the same arrangement of domains (i.e. conventional or crossover)). Furthermore, in these embodiments, the third antigen binding moiety comprises the
- 15 same amino acid substitutions, if any, as the first antigen binding moiety. For example, the amino acid substitutions described herein as “charge modifications” will be made in the constant domain CL and the constant domain CH1 of each of the first antigen binding moiety and the third antigen binding moiety. Alternatively, said amino acid substitutions may be made in the constant domain CL and the constant domain CH1 of the second antigen binding moiety (which in particular
- 20 embodiments is also a Fab molecule), but not in the constant domain CL and the constant domain CH1 of the first antigen binding moiety and the third antigen binding moiety.

- Like the first antigen binding moiety, the third antigen binding moiety particularly is a conventional Fab molecule. Embodiments wherein the first and the third antigen binding moieties are crossover Fab molecules (and the second antigen binding moiety is a conventional Fab molecule) are, however, also contemplated. Thus, in particular embodiments, the first and the third
- 25 antigen binding moieties are each a conventional Fab molecule, and the second antigen binding moiety is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other. In other embodiments, the first and the third antigen binding
- 30 moieties are each a crossover Fab molecule and the second antigen binding moiety is a conventional Fab molecule.

If a third antigen binding moiety is present, in a particular embodiment the first and the third antigen moiety bind to GPRC5D, and the second antigen binding moiety binds to a second antigen, particularly an activating T cell antigen, more particularly CD3, most particularly CD3 epsilon.

In particular embodiments, the bispecific antigen binding molecule comprises an Fc domain  
5 composed of a first and a second subunit. The first and the second subunit of the Fc domain are capable of stable association.

The bispecific antigen binding molecule according to the invention can have different configurations, i.e. the first, second (and optionally third) antigen binding moiety may be fused to each other and to the Fc domain in different ways. The components may be fused to each other  
10 directly or, preferably, via one or more suitable peptide linkers. Where fusion of a Fab molecule is to the N-terminus of a subunit of the Fc domain, it is typically via an immunoglobulin hinge region.

In some embodiments, the first and the second antigen binding moiety are each a Fab molecule and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-  
15 terminus of the first or the second subunit of the Fc domain. In such embodiments, the first antigen binding moiety may be fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety or to the N-terminus of the other one of the subunits of the Fc domain. In particular such embodiments, said first antigen binding moiety is a conventional Fab molecule, and the second antigen binding moiety is a crossover Fab molecule as  
20 described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other. In other such embodiments, said first Fab molecule is a crossover Fab molecule and the second Fab molecule is a conventional Fab molecule.

In one embodiment, the first and the second antigen binding moiety are each a Fab molecule, the  
25 second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety. In a specific embodiment, the bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first  
30 and a second subunit, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain. Such a configuration

is schematically depicted in Figures 1G and 1K (with the second antigen binding domain in these examples being a VH/VL crossover Fab molecule). Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

- 5 In another embodiment, the first and the second antigen binding moiety are each a Fab molecule and the first and the second antigen binding moiety are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain. In a specific embodiment, the bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more
- 10 peptide linkers, wherein the first and the second Fab molecule are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain. Such a configuration is schematically depicted in Figures 1A and 1D (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first antigen binding moiety being a conventional Fab molecule). The first and the second Fab molecule may be fused
- 15 to the Fc domain directly or through a peptide linker. In a particular embodiment the first and the second Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region, particularly where the Fc domain is an IgG<sub>1</sub> Fc domain.

- In some embodiments, the first and the second antigen binding moiety are each a Fab molecule
- 20 and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain. In such embodiments, the second antigen binding moiety may be fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety or (as described above) to the N-terminus of the other one of the subunits of the Fc domain. In particular such embodiments, said
- 25 first antigen binding moiety is a conventional Fab molecule, and the second antigen binding moiety is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other. In other such embodiments, said first Fab molecule is a crossover Fab molecule and the second Fab molecule is a conventional Fab molecule.

- 30 In one embodiment, the first and the second antigen binding moiety are each a Fab molecule, the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain, and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first

antigen binding moiety. In a specific embodiment, the bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain. Such a configuration is schematically depicted in Figures 1H and 1L (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first antigen binding moiety being a conventional Fab molecule). Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In some embodiments, a third antigen binding moiety, particularly a third Fab molecule, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In particular such embodiments, said first and third Fab molecules are each a conventional Fab molecule, and the second Fab molecule is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other. In other such embodiments, said first and third Fab molecules are each a crossover Fab molecule and the second Fab molecule is a conventional Fab molecule.

In a particular such embodiment, the second and the third antigen binding moiety are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In a specific embodiment, the bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. Such a configuration is schematically depicted in Figure 1B and 1E (in these examples with the second antigen binding moiety being a VH/VL crossover Fab molecule, and the first and the third antigen binding moiety being a conventional Fab molecule), and Figure 1J and 1N (in these examples with the second antigen binding moiety being a conventional Fab molecule, and the first and the third antigen binding

moiety being a VH/VL crossover Fab molecule). The second and the third Fab molecule may be fused to the Fc domain directly or through a peptide linker. In a particular embodiment the second and the third Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region, particularly where the Fc domain is an IgG<sub>1</sub> Fc domain. Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In another such embodiment, the first and the third antigen binding moiety are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety. In a specific embodiment, the bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. Such a configuration is schematically depicted in Figure 1C and 1F (in these examples with the second antigen binding moiety being a VH/VL crossover Fab molecule, and the first and the third antigen binding moiety being a conventional Fab molecule) and in Figure 1I and 1M (in these examples with the second antigen binding moiety being a conventional Fab molecule, and the first and the third antigen binding moiety being a VH/VL crossover Fab molecule). The first and the third Fab molecule may be fused to the Fc domain directly or through a peptide linker. In a particular embodiment the first and the third Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG<sub>1</sub> hinge region, particularly where the Fc domain is an IgG<sub>1</sub> Fc domain. Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In configurations of the bispecific antigen binding molecule wherein a Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of each of the subunits of the Fc domain through an immunoglobulin hinge regions, the two Fab molecules, the hinge regions and the Fc domain essentially form an immunoglobulin molecule. In a particular embodiment the

immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG<sub>1</sub> subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG<sub>4</sub> subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a  
5 chimeric immunoglobulin or a humanized immunoglobulin. In one embodiment, the immunoglobulin comprises a human constant region, particularly a human Fc region.

In some of the bispecific antigen binding molecule of the invention, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule are fused to each other, optionally via a peptide linker. Depending on the configuration of the first and the second Fab molecule, the  
10 Fab light chain of the first Fab molecule may be fused at its C-terminus to the N-terminus of the Fab light chain of the second Fab molecule, or the Fab light chain of the second Fab molecule may be fused at its C-terminus to the N-terminus of the Fab light chain of the first Fab molecule. Fusion of the Fab light chains of the first and the second Fab molecule further reduces mispairing of unmatched Fab heavy and light chains, and also reduces the number of plasmids needed for  
15 expression of some of the bispecific antigen binding molecules of the invention.

The antigen binding moieties may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example, (G<sub>4</sub>S)<sub>n</sub>, (SG<sub>4</sub>)<sub>n</sub>, (G<sub>4</sub>S)<sub>n</sub> or G<sub>4</sub>(SG<sub>4</sub>)<sub>n</sub> peptide linkers. "n" is generally an integer  
20 from 1 to 10, typically from 2 to 4. In one embodiment said peptide linker has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is (G<sub>x</sub>S)<sub>n</sub> or (G<sub>x</sub>S)<sub>n</sub>G<sub>m</sub> with G=glycine, S=serine, and (x=3, n= 3, 4, 5 or 6, and m=0, 1, 2 or 3) or (x=4, n=2, 3, 4 or 5 and m= 0, 1, 2 or 3), in one embodiment x=4 and n=2 or 3, in a further embodiment x=4 and n=2. In one embodiment said  
25 peptide linker is (G<sub>4</sub>S)<sub>2</sub>. A particularly suitable peptide linker for fusing the Fab light chains of the first and the second Fab molecule to each other is (G<sub>4</sub>S)<sub>2</sub>. An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second Fab fragments comprises the sequence (D)-(G<sub>4</sub>S)<sub>2</sub> (SEQ ID NOs 43 and 44). Another suitable such linker comprises the sequence (G<sub>4</sub>S)<sub>4</sub>. Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly  
30 where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule

shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-CH2-CH3(-CH4)), and  
5 a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(1)</sub>-CH1<sub>(1)</sub>-CH2-CH3(-CH4)). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain  
10 polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab  
15 molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(2)</sub>-CL<sub>(2)</sub>-CH2-CH3(-CH4)), and a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(1)</sub>-CH1<sub>(1)</sub>-CH2-CH3(-CH4)). In some embodiments  
20 the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

25 In some embodiments, the bispecific antigen binding molecule comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond  
30 with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>-CH2-CH3(-CH4)). In other embodiments, the bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light

chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond  
5 with an Fc domain subunit (VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VL<sub>(2)</sub>-CH1<sub>(2)</sub>-CH2-CH3(-CH4)).

In some of these embodiments the bispecific antigen binding molecule further comprises a crossover Fab light chain polypeptide of the second Fab molecule, wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>), and the Fab light chain  
10 polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In others of these embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain polypeptide of the first Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>-VL<sub>(1)</sub>-CL<sub>(1)</sub>), or a  
15 polypeptide wherein the Fab light chain polypeptide of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>-VH<sub>(2)</sub>-CL<sub>(2)</sub>), as appropriate.

The bispecific antigen binding molecule according to these embodiments may further comprise (i)  
20 an Fc domain subunit polypeptide (CH2-CH3(-CH4)), or (ii) a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(3)</sub>-CH1<sub>(3)</sub>-CH2-CH3(-CH4)) and the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

25 In some embodiments, the bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond  
30 with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(2)</sub>-CL<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>-CH2-CH3(-CH4)). In other embodiments, the bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy

chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond  
5 with an Fc domain subunit (VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VH<sub>(2)</sub>-CL<sub>(2)</sub>-CH2-CH3(-CH4)).

In some of these embodiments the bispecific antigen binding molecule further comprises a crossover Fab light chain polypeptide of the second Fab molecule, wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>), and the Fab light chain  
10 polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In others of these embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain polypeptide of the first Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-VL<sub>(1)</sub>-CL<sub>(1)</sub>), or  
15 a polypeptide wherein the Fab light chain polypeptide of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>-VL<sub>(2)</sub>-CH1<sub>(2)</sub>), as appropriate.

The bispecific antigen binding molecule according to these embodiments may further comprise (i)  
20 an Fc domain subunit polypeptide (CH2-CH3(-CH4)), or (ii) a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH<sub>(3)</sub>-CH1<sub>(3)</sub>-CH2-CH3(-CH4)) and the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

25 In certain embodiments, the bispecific antigen binding molecule does not comprise an Fc domain. In particular such embodiments, said first and, if present third Fab molecules are each a conventional Fab molecule, and the second Fab molecule is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other. In other such  
30 embodiments, said first and, if present third Fab molecules are each a crossover Fab molecule and the second Fab molecule is a conventional Fab molecule.

In one such embodiment, the bispecific antigen binding molecule essentially consists of the first and the second antigen binding moiety, and optionally one or more peptide linkers, wherein the

first and the second antigen binding moiety are both Fab molecules and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety. Such a configuration is schematically depicted in Figures 1O and 1S (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first antigen binding moiety being a conventional Fab molecule).

In another such embodiment, the bispecific antigen binding molecule essentially consists of the first and the second antigen binding moiety, and optionally one or more peptide linkers, wherein the first and the second antigen binding moiety are both Fab molecules and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety. Such a configuration is schematically depicted in Figures 1P and 1T (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first antigen binding moiety being a conventional Fab molecule). In some embodiments, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the bispecific antigen binding molecule further comprises a third antigen binding moiety, particularly a third Fab molecule, wherein said third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In certain such embodiments, the bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. Such a configuration is schematically depicted in Figures 1Q and 1U (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first and the antigen binding moiety each being a conventional Fab molecule), or Figures 1X and 1Z (in these examples with the second antigen binding domain being a conventional Fab molecule and the first and the third antigen binding moiety each being a VH/VL crossover Fab molecule).

In some embodiments, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the bispecific antigen binding molecule further comprises a third antigen binding moiety, particularly a third Fab molecule, wherein said third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the first Fab molecule. In certain such embodiments, the bispecific antigen binding molecule essentially consists of the first, the second and the third Fab

- molecule, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the first Fab molecule. Such a configuration is schematically depicted in Figures 1R and 1V (in these examples with the second antigen binding domain being a VH/VL crossover Fab molecule and the first and the antigen binding moiety each being a conventional Fab molecule), or Figures 1W and 1Y (in these examples with the second antigen binding domain being a conventional Fab molecule and the first and the third antigen binding moiety each being a VH/VL crossover Fab molecule).
- 10 In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VL<sub>(2)</sub>-CH1<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>).
- 15 20 In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>).
- 25 30 In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy

chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH<sub>(3)</sub>-CH1<sub>(3)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VL<sub>(2)</sub>-CH1<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>). In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab

molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region) (VH<sub>(3)</sub>-CH1<sub>(3)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VH<sub>(2)</sub>-CL<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule

5 further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>).

10 In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a

15 carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of a third Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>-VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VH<sub>(3)</sub>-CH1<sub>(3)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the

20 second Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-CL<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>).

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule

25 shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of a third Fab molecule (VH<sub>(2)</sub>-CL<sub>(2)</sub>-

30 VH<sub>(1)</sub>-CH1<sub>(1)</sub>-VH<sub>(3)</sub>-CH1<sub>(3)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL<sub>(2)</sub>-CH1<sub>(2)</sub>) and the Fab light chain polypeptide of the first Fab molecule (VL<sub>(1)</sub>-

CL<sub>(1)</sub>). In some embodiments the bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL<sub>(3)</sub>-CL<sub>(3)</sub>).

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the second Fab molecule shares a  
5 carboxy-terminal peptide bond with the Fab light chain variable region of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the first Fab molecule (i.e. the first Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of a third Fab molecule,  
10 which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH<sub>(2)</sub>-CH1<sub>(2)</sub>-VL<sub>(1)</sub>-CH1<sub>(1)</sub>-VL<sub>(3)</sub>-CH1<sub>(3)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the first Fab molecule  
15 shares a carboxy-terminal peptide bond with the Fab light chain constant region of the first Fab molecule (VH<sub>(1)</sub>-CL<sub>(1)</sub>) and the Fab light chain polypeptide of the second Fab molecule (VL<sub>(2)</sub>-CL<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (VH<sub>(3)</sub>-  
20 CL<sub>(3)</sub>).

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of  
25 the first Fab molecule (i.e. the first Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of a third Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein  
30 the heavy chain constant region is replaced by a light chain constant region) (VH<sub>(2)</sub>-CH1<sub>(2)</sub>-VH<sub>(1)</sub>-CL<sub>(1)</sub>-VH<sub>(3)</sub>-CL<sub>(3)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the first Fab

molecule (VL<sub>(1)</sub>-CH1<sub>(1)</sub>) and the Fab light chain polypeptide of the second Fab molecule (VL<sub>(2)</sub>-CL<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (VL<sub>(3)</sub>-CH1<sub>(3)</sub>).

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the first Fab molecule (i.e. the first Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the second Fab molecule (VL<sub>(3)</sub>-CH1<sub>(3)</sub>-VL<sub>(1)</sub>-CH1<sub>(1)</sub>-VH<sub>(2)</sub>-CH1<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the first Fab molecule (VH<sub>(1)</sub>-CL<sub>(1)</sub>) and the Fab light chain polypeptide of the second Fab molecule (VL<sub>(2)</sub>-CL<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (VH<sub>(3)</sub>-CL<sub>(3)</sub>).

In certain embodiments the bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the first Fab molecule (i.e. the first Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the second Fab molecule (VH<sub>(3)</sub>-CL<sub>(3)</sub>-

VH<sub>(1)</sub>-CL<sub>(1)</sub>-VH<sub>(2)</sub>-CH1<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the first Fab molecule (VL<sub>(1)</sub>-CH1<sub>(1)</sub>) and the Fab light chain polypeptide of the second Fab molecule (VL<sub>(2)</sub>-CL<sub>(2)</sub>). In some embodiments the bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (VL<sub>(3)</sub>-CH1<sub>(3)</sub>).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the

second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain

- composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under

a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen  
5 is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6; b) a second antigen  
10 binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID  
15 NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-  
20 terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of  
25 the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen  
30 is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of

SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ

ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

- 5 In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region  
10 (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other,  
15 and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second  
20 subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

- In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D  
25 and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding  
30 moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy

chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen

binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein

(i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7,

- a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein (i) the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).
- 15 In all of the different configurations of the bispecific antigen binding molecule according to the invention, the amino acid substitutions described herein, if present, may either be in the CH1 and CL domains of the first and (if present) the third antigen binding moiety/Fab molecule, or in the CH1 and CL domains of the second antigen binding moiety/Fab molecule. Preferably, they are in the CH1 and CL domains of the first and (if present) the third antigen binding moiety/Fab molecule.
- 20 In accordance with the concept of the invention, if amino acid substitutions as described herein are made in the first (and, if present, the third) antigen binding moiety/Fab molecule, no such amino acid substitutions are made in the second antigen binding moiety/Fab molecule. Conversely, if amino acid substitutions as described herein are made in the second antigen binding moiety/Fab molecule, no such amino acid substitutions are made in the first (and, if present, the third) antigen binding moiety/Fab molecule. Amino acid substitutions are particularly made in bispecific antigen binding molecules comprising a Fab molecule wherein the variable domains VL and VH1 of the Fab light chain and the Fab heavy chain are replaced by each other.
- In particular embodiments of the bispecific antigen binding molecule according to the invention, particularly wherein amino acid substitutions as described herein are made in the first (and, if present, the third) antigen binding moiety/Fab molecule, the constant domain CL of the first (and, if present, the third) Fab molecule is of kappa isotype. In other embodiments of the bispecific antigen binding molecule according to the invention, particularly wherein amino acid substitutions as described herein are made in the second antigen binding moiety/Fab molecule, the constant
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domain CL of the second antigen binding moiety/Fab molecule is of kappa isotype. In some embodiments, the constant domain CL of the first (and, if present, the third) antigen binding moiety/Fab molecule and the constant domain CL of the second antigen binding moiety/Fab molecule are of kappa isotype.

- 5 In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86; and a light chain variable region
- 10 (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a
- 15 heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain
- 20 CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the
- 25 amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second
- 30 antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a

HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3 and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen

binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100,

and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by

lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In one embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the

- amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or (ii) the second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light
- 15 chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a
- 25 first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light
- 15 chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first
- 25 antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light
- 15 chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first
- 25 antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light
- 15 chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first
- 25 antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain
- 15 variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a
- 25 first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In a particular embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain
- 15 variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule
- 20 comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) a third antigen binding moiety that binds to the first antigen and is identical to the first antigen binding moiety; and d) an Fc domain composed of a
- 25 first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and
- 30 wherein in the constant domain CH1 of the first antigen binding moiety under a) and the third antigen binding moiety under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein (i) the first antigen

- binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or (ii) the
- 5 second antigen binding moiety under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety under a), and the first antigen binding moiety under a) and the third antigen binding moiety under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).
- 10 In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 84, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region
- 15 (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a
- 20 heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain
- 25 CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the
- 30 amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

- In another embodiment, the invention provides a bispecific antigen binding molecule comprising
- a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 83, a HCDR 2 of SEQ ID NO: 85, and a HCDR 3 of SEQ ID NO: 86, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 87, a LCDR 2 of SEQ ID NO: 88 and a LCDR 3 of SEQ ID NO: 89; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).
- In another embodiment, the invention provides a bispecific antigen binding molecule comprising
- a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain

and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 91, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 96 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and

wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 90, a HCDR 2 of SEQ ID NO: 92, and a HCDR 3 of SEQ ID NO: 93, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 94, a LCDR 2 of SEQ ID NO: 95 and a LCDR 3 of SEQ ID NO: 97; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region

(VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 of SEQ ID NO: 3, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 4, a LCDR 2 of SEQ ID NO: 5 and a LCDR 3 of SEQ ID NO: 6; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In another embodiment, the invention provides a bispecific antigen binding molecule comprising a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is GPRC5D and the first antigen binding moiety is a Fab molecule comprising a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 7, a HCDR 2 of SEQ ID NO: 8, and a HCDR 3 of SEQ ID NO: 9, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 10, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 12; b) a second antigen binding moiety that binds to a second antigen, wherein the second antigen is CD3, and the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, and wherein the Fab molecule comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 98, a HCDR 2 of SEQ ID NO: 99, and a HCDR 3 of SEQ ID NO: 100,

and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 101, a LCDR 2 of SEQ ID NO: 102 and a LCDR 3 of SEQ ID NO: 103; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein the first antigen binding moiety under a) and the second antigen binding moiety under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

According to any of the above embodiments, components of the bispecific antigen binding molecule (e.g. Fab molecules, Fc domain) may be fused directly or through various linkers, particularly peptide linkers comprising one or more amino acids, typically about 2-20 amino acids, that are described herein or are known in the art. Suitable, non-immunogenic peptide linkers include, for example,  $(G_4S)_n$ ,  $(SG_4)_n$ ,  $(G_4S)_n$  or  $G_4(SG_4)_n$  peptide linkers, wherein n is generally an integer from 1 to 10, typically from 2 to 4.

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the

amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional)

- Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 64; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).
- 20 In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by

arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 48 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is

GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

In a particular aspect, the invention provides a bispecific antigen binding molecule comprising a) a first and a third antigen binding moiety that binds to a first antigen; wherein the first antigen is GPRC5D and wherein the first and the second antigen binding moiety are each a (conventional) Fab molecule comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63; b) a second antigen binding moiety that binds to a second antigen; wherein the second antigen is CD3 and wherein the second antigen binding moiety is Fab molecule wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105; c) an Fc domain composed of a first and a second subunit; wherein in the constant domain CL of the first and the third antigen binding moiety under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is

substituted by lysine (K) or arginine (R) (numbering according to Kabat) (most particularly by arginine (R)), and wherein in the constant domain CH1 of the first and the third antigen binding moiety under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index); and wherein further the first antigen binding moiety under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety under b), and the second antigen binding moiety under b) and the third antigen binding moiety under a) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c).

- 10 In one embodiment according to this aspect of the invention, in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A)  
15 (numberings according to Kabat EU index).

In a further embodiment according to this aspect of the invention, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the  
20 second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index).

In still a further embodiment according to this aspect of the invention, in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A)  
25 and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index).

In still a further embodiment according to this aspect of the invention, the Fc domain is a human IgG1 Fc domain.

In particular specific embodiment, the bispecific antigen binding molecule comprises a  
30 polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99%

identical to the sequence of SEQ ID NO: 17, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 18, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 19, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 20. In a further particular specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 17, a polypeptide comprising the amino acid sequence of SEQ ID NO: 18, a polypeptide comprising the amino acid sequence of SEQ ID NO: 19 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 20.

10 In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 21, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 24. In a further specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 21, a polypeptide comprising the amino acid sequence of SEQ ID NO: 22, a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 24.

In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 114, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 115, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 116, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 117. In a further specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 114, a polypeptide comprising the amino acid sequence of SEQ ID NO: 115, a polypeptide comprising the amino acid sequence of SEQ ID NO: 116 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 117.

In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 118, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 119, a polypeptide  
5 comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 120, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 121. In a further specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 118, a polypeptide comprising the amino acid sequence  
10 of SEQ ID NO: 119, a polypeptide comprising the amino acid sequence of SEQ ID NO: 120 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 121.

In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 122, a polypeptide comprising an amino acid sequence that is at least  
15 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 123, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 124, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 125. In a further specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising  
20 the amino acid sequence of SEQ ID NO: 122, a polypeptide comprising the amino acid sequence of SEQ ID NO: 123, a polypeptide comprising the amino acid sequence of SEQ ID NO: 124 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 125.

In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the  
25 sequence of SEQ ID NO: 126, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 127, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 128, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 129. In a further  
30 specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 126, a polypeptide comprising the amino acid sequence of SEQ ID NO: 127, a polypeptide comprising the amino acid sequence of SEQ ID NO: 128 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 129.

In another specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 130, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 131, a polypeptide  
5 comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 132, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 133. In a further specific embodiment, the bispecific antigen binding molecule comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 130, a polypeptide comprising the amino acid sequence  
10 of SEQ ID NO: 131, a polypeptide comprising the amino acid sequence of SEQ ID NO: 132 and a polypeptide comprising the amino acid sequence of SEQ ID NO: 133.

### **Fc domain**

In particular embodiments, the bispecific antigen binding molecule of the invention comprises an  
15 Fc domain composed of a first and a second subunit. It is understood, that the features of the Fc domain described herein in relation to the bispecific antigen binding molecule can equally apply to an Fc domain comprised in an antibody of the invention.

The Fc domain of the bispecific antigen binding molecule consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of  
20 an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other. In one embodiment, the bispecific antigen binding molecule of the invention comprises not more than one Fc domain.

In one embodiment, the Fc domain of the bispecific antigen binding molecule is an IgG Fc domain.  
25 In a particular embodiment, the Fc domain is an IgG<sub>1</sub> Fc domain. In another embodiment the Fc domain is an IgG<sub>4</sub> Fc domain. In a more specific embodiment, the Fc domain is an IgG<sub>4</sub> Fc domain comprising an amino acid substitution at position S228 (Kabat EU index numbering), particularly the amino acid substitution S228P. This amino acid substitution reduces *in vivo* Fab arm exchange of IgG<sub>4</sub> antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)).  
30 In a further particular embodiment, the Fc domain is a human Fc domain. In an even more particular embodiment, the Fc domain is a human IgG<sub>1</sub> Fc domain. An exemplary sequence of a human IgG<sub>1</sub> Fc region is given in SEQ ID NO: 42.

### Fc domain modifications promoting heterodimerization

Bispecific antigen binding molecules according to the invention comprise different antigen binding moieties, which may be fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain are typically comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to  
5 several possible combinations of the two polypeptides. To improve the yield and purity of bispecific antigen binding molecules in recombinant production, it will thus be advantageous to introduce in the Fc domain of the bispecific antigen binding molecule a modification promoting the association of the desired polypeptides.

Accordingly, in particular embodiments, the Fc domain of the bispecific antigen binding molecule  
10 according to the invention comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

There exist several approaches for modifications in the CH3 domain of the Fc domain in order to  
15 enforce heterodimerization, which are well described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012058768, WO 2013157954, WO 2013096291. Typically, in all such approaches the CH3 domain of the first subunit of the Fc domain and the CH3 domain of the second subunit of the Fc domain are both engineered in a complementary  
20 manner so that each CH3 domain (or the heavy chain comprising it) can no longer homodimerize with itself but is forced to heterodimerize with the complementarily engineered other CH3 domain (so that the first and second CH3 domain heterodimerize and no homodimers between the two first or the two second CH3 domains are formed). These different approaches for improved heavy chain heterodimerization are contemplated as different alternatives in combination with the heavy-light  
25 chain modifications (e.g. VH and VL exchange/replacement in one binding arm and the introduction of substitutions of charged amino acids with opposite charges in the CH1/CL interface) in the bispecific antigen binding molecule which reduce heavy/light chain mispairing and Bence Jones-type side products.

In a specific embodiment said modification promoting the association of the first and the second  
30 subunit of the Fc domain is a so-called “knob-into-hole” modification, comprising a “knob” modification in one of the two subunits of the Fc domain and a “hole” modification in the other one of the two subunits of the Fc domain.

The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance  
5 can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine  
10 or threonine).

Accordingly, in a particular embodiment, in the CH3 domain of the first subunit of the Fc domain of the bispecific antigen binding molecule an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second  
15 subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

Preferably said amino acid residue having a larger side chain volume is selected from the group  
20 consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W).

Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V).

The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis.

25 In a specific embodiment, in (the CH3 domain of) the first subunit of the Fc domain (the “knobs” subunit) the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in (the CH3 domain of) the second subunit of the Fc domain (the “hole” subunit) the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In one embodiment, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with  
30 a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index).

In yet a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position

356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index). Introduction of these two cysteine residues results in formation of a disulfide  
5 bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

In a particular embodiment, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).  
10 In a particular embodiment the antigen binding moiety that binds to the second antigen (e.g. an activating T cell antigen) is fused (optionally via the first antigen binding moiety, which binds to GPRC5D, and/or a peptide linker) to the first subunit of the Fc domain (comprising the “knob” modification). Without wishing to be bound by theory, fusion of the antigen binding moiety that binds a second antigen, such as an activating T cell antigen, to the knob-containing subunit of the  
15 Fc domain will (further) minimize the generation of antigen binding molecules comprising two antigen binding moieties that bind to an activating T cell antigen (steric clash of two knob-containing polypeptides).

Other techniques of CH3-modification for enforcing the heterodimerization are contemplated as alternatives according to the invention and are described e.g. in WO 96/27011, WO 98/050431,  
20 EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954, WO 2013/096291.

In one embodiment, the heterodimerization approach described in EP 1870459, is used alternatively. This approach is based on the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3 domain interface between the two  
25 subunits of the Fc domain. One preferred embodiment for the bispecific antigen binding molecule of the invention are amino acid mutations R409D; K370E in one of the two CH3 domains (of the Fc domain) and amino acid mutations D399K; E357K in the other one of the CH3 domains of the Fc domain (numbering according to Kabat EU index).

In another embodiment, the bispecific antigen binding molecule of the invention comprises amino  
30 acid mutation T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the

Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (numberings according to Kabat EU index).

In another embodiment, the bispecific antigen binding molecule of the invention comprises amino acid mutations S354C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations Y349C, T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, or said bispecific antigen binding molecule comprises amino acid mutations Y349C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations S354C, T366S, L368A, Y407V in the CH3 domains of the second subunit of the Fc domain and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (all numberings according to Kabat EU index).

In one embodiment, the heterodimerization approach described in WO 2013/157953 is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutation T366K and a second CH3 domain comprises amino acid mutation L351D (numberings according to Kabat EU index). In a further embodiment, the first CH3 domain comprises further amino acid mutation L351K. In a further embodiment, the second CH3 domain comprises further an amino acid mutation selected from Y349E, Y349D and L368E (preferably L368E) (numberings according to Kabat EU index).

In one embodiment, the heterodimerization approach described in WO 2012/058768 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment the second CH3 domain comprises a further amino acid mutation at position T411, D399, S400, F405, N390, or K392, e.g. selected from a) T411N, T411R, T411Q, T411K, T411D, T411E or T411W, b) D399R, D399W, D399Y or D399K, c) S400E, S400D, S400R, or S400K, d) F405I, F405M, F405T, F405S, F405V or F405W, e) N390R, N390K or N390D, f) K392V, K392M, K392R, K392L, K392F or K392E (numberings according to Kabat EU index). In a further embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366V, K409F. In a further embodiment, a first CH3 domain comprises amino acid mutation Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment, the second CH3 domain further comprises amino acid mutations K392E, T411E, D399R and S400R (numberings according to Kabat EU index).

In one embodiment, the heterodimerization approach described in WO 2011/143545 is used alternatively, e.g. with the amino acid modification at a position selected from the group consisting of 368 and 409 (numbering according to Kabat EU index).

In one embodiment, the heterodimerization approach described in WO 2011/090762, which also  
5 uses the knobs-into-holes technology described above, is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutation T366W and a second CH3 domain comprises amino acid mutation Y407A. In one embodiment, a first CH3 domain comprises amino acid mutation T366Y and a second CH3 domain comprises amino acid mutation Y407T (numberings according to Kabat EU index).

10 In one embodiment, the bispecific antigen binding molecule or its Fc domain is of IgG<sub>2</sub> subclass and the heterodimerization approach described in WO 2010/129304 is used alternatively.

In an alternative embodiment, a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of  
15 one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable. In one such embodiment, a first CH3 domain comprises amino acid substitution of K392 or N392 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K392D or N392D) and a second CH3 domain  
20 comprises amino acid substitution of D399, E356, D356, or E357 with a positively charged amino acid (e.g. lysine (K) or arginine (R), preferably D399K, E356K, D356K, or E357K, and more preferably D399K and E356K). In a further embodiment, the first CH3 domain further comprises amino acid substitution of K409 or R409 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K409D or R409D). In a further embodiment the first CH3  
25 domain further or alternatively comprises amino acid substitution of K439 and/or K370 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D)) (all numberings according to Kabat EU index).

In yet a further embodiment, the heterodimerization approach described in WO 2007/147901 is used alternatively. In one embodiment, a first CH3 domain comprises amino acid mutations  
30 K253E, D282K, and K322D and a second CH3 domain comprises amino acid mutations D239K, E240K, and K292D (numberings according to Kabat EU index).

In still another embodiment, the heterodimerization approach described in WO 2007/110205 can be used alternatively.

In one embodiment, the first subunit of the Fc domain comprises amino acid substitutions K392D and K409D, and the second subunit of the Fc domain comprises amino acid substitutions D356K and D399K (numbering according to Kabat EU index).

Fc domain modifications reducing Fc receptor binding and/or effector function

- 5 The Fc domain confers to the bispecific antigen binding molecule (or the antibody) favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time, it may, however, lead to undesirable targeting of the bispecific antigen binding molecule (or the antibody) to cells expressing Fc receptors rather than to the preferred antigen-bearing cells.
- 10 Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with the T cell activating properties (e.g. in embodiments of the bispecific antigen binding molecule wherein the second antigen binding moiety binds to an activating T cell antigen) and the long half-life of the bispecific antigen binding molecule, results in excessive activation of cytokine receptors and severe side effects upon systemic administration. Activation of (Fc
- 15 receptor-bearing) immune cells other than T cells may even reduce efficacy of the bispecific antigen binding molecule (particularly a bispecific antigen binding molecule wherein the second antigen binding moiety binds to an activating T cell antigen) due to the potential destruction of T cells e.g. by NK cells.

- Accordingly, in particular embodiments, the Fc domain of the bispecific antigen binding molecule
- 20 according to the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG<sub>1</sub> Fc domain. In one such embodiment the Fc domain (or the bispecific antigen binding molecule comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG<sub>1</sub> Fc domain (or a bispecific antigen
- 25 binding molecule comprising a native IgG<sub>1</sub> Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG<sub>1</sub> Fc domain (or a bispecific antigen binding molecule comprising a native IgG<sub>1</sub> Fc domain). In one embodiment, the Fc domain (or the bispecific antigen binding molecule comprising said Fc domain) does not substantially bind to an Fc receptor and/or induce effector
- 30 function. In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically

human Fc $\gamma$ RIIIa, Fc $\gamma$ RI or Fc $\gamma$ RIIa, most specifically human Fc $\gamma$ RIIIa. In one embodiment the effector function is one or more selected from the group of CDC, ADCC, ADCP, and cytokine secretion. In a particular embodiment, the effector function is ADCC. In one embodiment, the Fc domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG<sub>1</sub> Fc domain. Substantially similar binding to FcRn is achieved when the Fc domain (or the bispecific antigen binding molecule comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG<sub>1</sub> Fc domain (or the bispecific antigen binding molecule comprising a native IgG<sub>1</sub> Fc domain) to FcRn.

10 In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In particular embodiments, the Fc domain of the bispecific antigen binding molecule comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each

15 of the two subunits of the Fc domain. In one embodiment, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid

20 mutations may reduce the binding affinity of the Fc domain to an Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the bispecific antigen binding molecule comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to a bispecific antigen binding molecule comprising a non-engineered Fc domain. In a particular embodiment,

25 the Fc receptor is an Fc $\gamma$  receptor. In some embodiments, the Fc receptor is a human Fc receptor. In some embodiments, the Fc receptor is an activating Fc receptor. In a specific embodiment, the Fc receptor is an activating human Fc $\gamma$  receptor, more specifically human Fc $\gamma$ RIIIa, Fc $\gamma$ RI or Fc $\gamma$ RIIa, most specifically human Fc $\gamma$ RIIIa. Preferably, binding to each of these receptors is reduced. In some embodiments, binding affinity to a complement component, specifically binding

30 affinity to C1q, is also reduced. In one embodiment, binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the bispecific antigen binding molecule comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a

non-engineered form of the Fc domain (or the bispecific antigen binding molecule comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or bispecific antigen binding molecules of the invention comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments, the Fc domain of the bispecific antigen binding molecule is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced crosslinking of target-bound antibodies, reduced dendritic cell maturation, or reduced T cell priming. In one embodiment, the reduced effector function is one or more selected from the group of reduced CDC, reduced ADCC, reduced ADCP, and reduced cytokine secretion. In a particular embodiment, the reduced effector function is reduced ADCC. In one embodiment the reduced ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or a bispecific antigen binding molecule comprising a non-engineered Fc domain).

In one embodiment, the amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function is an amino acid substitution. In one embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some embodiments, the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain is an IgG<sub>1</sub> Fc domain, particularly a human IgG<sub>1</sub> Fc domain. In one embodiment, the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment, the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment, the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In a more specific embodiment, the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments, the Fc domain comprises

- amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments, the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA", "PGLALA" or "LALAPG"). Specifically, in particular embodiments, each subunit of the Fc domain comprises the amino acid substitutions
- 5 L234A, L235A and P329G (Kabat EU index numbering), i.e. in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index).
- 10 In one such embodiment, the Fc domain is an IgG<sub>1</sub> Fc domain, particularly a human IgG<sub>1</sub> Fc domain. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor (as well as complement) binding of a human IgG<sub>1</sub> Fc domain, as described in PCT publication no. WO 2012/130831, which is incorporated herein by reference in its entirety. WO 2012/130831 also describes methods of preparing such mutant Fc domains and methods for
- 15 determining its properties such as Fc receptor binding or effector functions.
- IgG<sub>4</sub> antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG<sub>1</sub> antibodies. Hence, in some embodiments, the Fc domain of the bispecific antigen binding molecules of the invention is an IgG<sub>4</sub> Fc domain, particularly a human IgG<sub>4</sub> Fc domain. In one embodiment, the IgG<sub>4</sub> Fc domain comprises amino acid substitutions at position
- 20 S228, specifically the amino acid substitution S228P (numberings according to Kabat EU index). To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment, the IgG<sub>4</sub> Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E (numberings according to Kabat EU index). In another embodiment, the IgG<sub>4</sub> Fc domain comprises an amino acid substitution at position P329,
- 25 specifically the amino acid substitution P329G (numberings according to Kabat EU index). In a particular embodiment, the IgG<sub>4</sub> Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G (numberings according to Kabat EU index). Such IgG<sub>4</sub> Fc domain mutants and their Fcγ receptor binding properties are described in PCT publication no. WO 2012/130831, incorporated herein by
- 30 reference in its entirety.
- In a particular embodiment, the Fc domain exhibiting reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG<sub>1</sub> Fc domain, is a human IgG<sub>1</sub> Fc domain comprising the amino acid substitutions L234A, L235A and optionally P329G, or a human

IgG<sub>4</sub> Fc domain comprising the amino acid substitutions S228P, L235E and optionally P329G (numberings according to Kabat EU index).

In certain embodiments, N-glycosylation of the Fc domain has been eliminated. In one such embodiment, the Fc domain comprises an amino acid mutation at position N297, particularly an amino acid substitution replacing asparagine by alanine (N297A) or aspartic acid (N297D) (numberings according to Kabat EU index).

In addition to the Fc domains described hereinabove and in PCT publication no. WO 2012/130831, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056) (numberings according to Kabat EU index). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. Alternatively, binding affinity of Fc domains or bispecific antigen binding molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor.

Effector function of an Fc domain, or a bispecific antigen binding molecule comprising an Fc domain, can be measured by methods known in the art. Examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be

assessed *in vivo*, e.g. in an animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

In some embodiments, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have  
5 reduced effector function, said reduced effector function includes reduced CDC. C1q binding assays may be carried out to determine whether the Fc domain, or the bispecific antigen binding molecule comprising the Fc domain, is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol  
10 Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006); WO  
15 2013/120929).

### **Polynucleotides**

The invention further provides isolated polynucleotides encoding an antibody or bispecific antigen binding molecule as described herein or a fragment thereof. In some embodiments, said fragment is an antigen binding fragment.

20 The polynucleotides encoding antibodies or bispecific antigen binding molecules of the invention may be expressed as a single polynucleotide that encodes the entire antibody or bispecific antigen binding molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional antibody or bispecific antigen binding  
25 molecule. For example, the light chain portion of an antibody or bispecific antigen binding molecule may be encoded by a separate polynucleotide from the portion of the antibody or bispecific antigen binding molecule comprising the heavy chain of the antibody or bispecific antigen binding molecule. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antibody or bispecific antigen binding molecule. In another  
30 example, the portion of the antibody or bispecific antigen binding molecule comprising one of the two Fc domain subunits and optionally (part of) one or more Fab molecules could be encoded by a separate polynucleotide from the portion of the antibody or bispecific antigen binding molecule

comprising the other of the two Fc domain subunits and optionally (part of) a Fab molecule. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In some embodiments, the isolated polynucleotide encodes the entire antibody or bispecific antigen binding molecule according to the invention as described herein. In other embodiments,

5 the isolated polynucleotide encodes a polypeptide comprised in the antibody or bispecific antigen binding molecule according to the invention as described herein.

In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

10

### **Recombinant Methods**

Antibodies or bispecific antigen binding molecules of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production.

For recombinant production one or more polynucleotide encoding the antibody or bispecific

15 antigen binding molecule (fragment), e.g., as described above, is isolated and inserted into one or

more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct

20 expression vectors containing the coding sequence of an antibody or bispecific antigen binding

molecule (fragment) along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y.

25 (1989); and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid,

virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the antibody or bispecific antigen binding molecule (fragment) (i.e. the coding region) is cloned in operable association with a promoter and/or other

30 transcription or translation control elements. As used herein, a "coding region" is a portion of

nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites,

transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the antibody or bispecific antigen binding molecule (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit  $\beta$ -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters

inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the antibody or bispecific antigen binding molecule is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding an antibody or bispecific antigen binding molecule of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.* an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse  $\beta$ -glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (*e.g.* a histidine tag) or assist in labeling the antibody or bispecific antigen binding molecule may be included within or at the ends of the antibody or bispecific antigen binding molecule (fragment) encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (*e.g.* has been transformed or transfected with) one or more

vector comprising one or more polynucleotide that encodes (part of) an antibody or bispecific antigen binding molecule of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the antibody or bispecific antigen binding molecule of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of antibodies or bispecific antigen binding molecules are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the antibody or bispecific antigen binding molecule for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, *Nat Biotech* 22, 1409-1414 (2004), and Li et al., *Nat Biotech* 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES<sup>TM</sup> technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J Gen Virol* 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol Reprod* 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., *Annals N.Y. Acad Sci* 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary

(CHO) cells, including dhfr<sup>-</sup> CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells  
5 include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., YO, NS0, Sp20 cell).

10 Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain.

In one embodiment, a method of producing an antibody or bispecific antigen binding molecule  
15 according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the antibody or bispecific antigen binding molecule, as provided herein, under conditions suitable for expression of the antibody or bispecific antigen binding molecule, and optionally recovering the antibody or bispecific antigen binding molecule from the host cell (or host cell culture medium).

20 The components of the bispecific antigen binding molecule (or the antibody) of the invention may be genetically fused to each other. The bispecific antigen binding molecule can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different  
25 components of bispecific antigen binding molecules are provided herein. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

The antibody or bispecific antigen binding molecule of the invention generally comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can  
30 form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-

peptide synthesis, can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

Any animal species of antibody, antibody fragment, antigen binding domain or variable region  
5 may be used in the antibody or bispecific antigen binding molecule of the invention. Non-limiting antibodies, antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the antibody or bispecific antigen binding molecule is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can  
10 also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b)  
15 grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further  
20 described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68  
25 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling). Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993));  
30 human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions

derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolation from human antibody libraries, as described herein.

Antibodies useful in the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. Methods for screening combinatorial libraries are reviewed, e.g., in Lerner et al. in *Nature Reviews* 16:498-508 (2016). For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Frenzel et al. in *mAbs* 8:1177-1194 (2016); Bazan et al. in *Human Vaccines and Immunotherapeutics* 8:1817-1828 (2012) and Zhao et al. in *Critical Reviews in Biotechnology* 36:276-289 (2016) as well as in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and in Marks and Bradbury in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al. in *Annual Review of Immunology* 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al. in *EMBO Journal* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter in *Journal of Molecular Biology* 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent Nos. 5,750,373; 7,985,840; 7,785,903 and 8,679,490 as well as US Patent Publication Nos. 2005/0079574, 2007/0117126, 2007/0237764 and 2007/0292936. Further examples of methods known in the art for screening combinatorial libraries for antibodies with a desired activity or activities include ribosome and mRNA display, as well as methods for antibody display and selection on bacteria, mammalian cells, insect cells or yeast cells. Methods for yeast surface display are reviewed, e.g., in Scholler et al. in *Methods in Molecular Biology* 503:135-56 (2012) and in Cherf et al. in *Methods in Molecular biology* 1319:155-175 (2015) as well as in the Zhao et al. in *Methods in Molecular Biology* 889:73-84 (2012). Methods for ribosome display are described, e.g., in He et al. in *Nucleic Acids Research* 25:5132-5134 (1997) and in Hanes et al. in *PNAS* 94:4937-4942 (1997).

Antibodies or bispecific antigen binding molecules prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification, an antibody, ligand, receptor or antigen can be used to which the antibody or bispecific antigen binding molecule binds. For example, for affinity chromatography purification of antibodies or bispecific antigen binding molecules of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an antibody or bispecific antigen binding molecule essentially as described in the Examples. The purity of the antibody or bispecific antigen binding molecule can be determined by any of a variety of well-known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like.

15

### Assays

Antibodies or bispecific antigen binding molecules provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

20

### Affinity assays

The affinity of the antibody or bispecific antigen binding molecule for an Fc receptor or a target antigen can be determined for example by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of antibodies or bispecific antigen binding molecules for different receptors or target antigens may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following.

30 According to one embodiment,  $K_D$  is measured by surface plasmon resonance using a BIAcore® T100 machine (GE Healthcare) at 25 °C.

To analyze the interaction between the Fc-portion and Fc receptors, His-tagged recombinant Fc-receptor is captured by an anti-Penta His antibody (Qiagen) immobilized on CM5 chips and the

bispecific constructs are used as analytes. Briefly, carboxymethylated dextran biosensor chips (CM5, GE Healthcare) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Anti Penta-His antibody is diluted with 10 mM sodium acetate, pH 5.0, to 40 µg/ml before  
5 injection at a flow rate of 5 µl/min to achieve approximately 6500 response units (RU) of coupled protein. Following the injection of the ligand, 1 M ethanolamine is injected to block unreacted groups. Subsequently the Fc-receptor is captured for 60 s at 4 or 10 nM. For kinetic measurements, four-fold serial dilutions of the antibody or bispecific antigen binding molecule (range between 500 nM and 4000 nM) are injected in HBS-EP (GE Healthcare, 10 mM HEPES, 150 mM NaCl,  
10 3 mM EDTA, 0.05 % Surfactant P20, pH 7.4) at 25 °C at a flow rate of 30 µl/min for 120 s.

To determine the affinity to the target antigen, antibodies or bispecific antigen binding molecules are captured by an anti-human Fab specific antibody (GE Healthcare) that is immobilized on an activated CM5-sensor chip surface as described for the anti Penta-His antibody. The final amount of coupled protein is approximately 12000 RU. The antibodies or bispecific antigen binding  
15 molecules are captured for 90 s at 300 nM. The target antigens are passed through the flow cells for 180 s at a concentration range from 250 to 1000 nM with a flowrate of 30 µl/min. The dissociation is monitored for 180 s.

Bulk refractive index differences are corrected for by subtracting the response obtained on reference flow cell. The steady state response was used to derive the dissociation constant  $K_D$  by  
20 non-linear curve fitting of the Langmuir binding isotherm. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) are calculated using a simple one-to-one Langmuir binding model (BIAcore® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_D$ ) is calculated as the ratio  $k_{off}/k_{on}$ . See, e.g., Chen et al., J Mol Biol 293, 865-881 (1999).

25

#### Activity assays

Biological activity of the bispecific antigen binding molecules (or antibodies) of the invention can be measured by various assays as described in the Examples. Biological activities may for example include the induction of proliferation of T cells, the induction of signaling in T cells, the induction  
30 of expression of activation markers in T cells, the induction of cytokine secretion by T cells, the induction of lysis of target cells such as tumor cells, and the induction of tumor regression and/or the improvement of survival.

**Compositions, Formulations, and Routes of Administration**

In a further aspect, the invention provides pharmaceutical compositions comprising any of the antibodies or bispecific antigen binding molecules provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises any of the

5 antibodies or bispecific antigen binding molecules provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises any of the antibodies or bispecific antigen binding molecules provided herein and at least one additional therapeutic agent, e.g., as described below.

Further provided is a method of producing an antibody or bispecific antigen binding molecule of

10 the invention in a form suitable for administration in vivo, the method comprising (a) obtaining an antibody or bispecific antigen binding molecule according to the invention, and (b) formulating the antibody or bispecific antigen binding molecule with at least one pharmaceutically acceptable carrier, whereby a preparation of antibody or bispecific antigen binding molecule is formulated for administration in vivo.

15 Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of antibody or bispecific antigen binding molecule dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when

20 administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains an antibody or bispecific antigen binding molecule and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human)

25 administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g. antibacterial

30 agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's

Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

- An immunoconjugate of the invention (and any additional therapeutic agent) can be administered  
5 by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.
- 10 Parenteral compositions include those designed for administration by injection, e.g. subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the antibodies or bispecific antigen binding molecules of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain  
15 formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the antibodies or bispecific antigen binding molecules may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the antibodies or bispecific antigen binding molecules of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated  
20 below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying  
25 techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It  
30 will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl

ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic  
5 polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection  
10 suspensions may contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents  
15 or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleates or triglycerides, or liposomes.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug  
20 delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which  
25 matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

In addition to the compositions described previously, the antibodies or bispecific antigen binding  
30 molecules may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the antibodies or bispecific antigen binding molecules may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an

acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the antibodies or bispecific antigen binding molecules of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. The antibodies or bispecific antigen binding molecules may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

## 20 **Therapeutic Methods and Compositions**

Any of the antibodies or bispecific antigen binding molecules provided herein may be used in therapeutic methods. Antibodies or bispecific antigen binding molecules of the invention may be used as immunotherapeutic agents, for example in the treatment of cancers.

For use in therapeutic methods, antibodies or bispecific antigen binding molecules of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

30 In one aspect, antibodies or bispecific antigen binding molecules of the invention for use as a medicament are provided. In further aspects, antibodies or bispecific antigen binding molecules of the invention for use in treating a disease are provided. In certain embodiments, antibodies or bispecific antigen binding molecules of the invention for use in a method of treatment are provided.

In one embodiment, the invention provides an antibody or bispecific antigen binding molecule as described herein for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides an antibody or bispecific antigen binding molecule for use in a method of treating an individual having a disease comprising administering to the individual

5 a therapeutically effective amount of the antibody or bispecific antigen binding molecule. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. In further embodiments, the invention

10 provides an antibody or bispecific antigen binding molecule as described herein for use in inducing lysis of a target cell, particularly a tumor cell. In certain embodiments, the invention provides an antibody or bispecific antigen binding molecule for use in a method of inducing lysis of a target cell, particularly a tumor cell, in an individual comprising administering to the individual an effective amount of the antibody or bispecific antigen binding molecule to induce lysis of a target

15 cell. An "individual" according to any of the above embodiments is a mammal, preferably a human. In certain embodiments the disease to be treated is an autoimmune disease particularly systemic lupus erythematosus and/or rheumatoid arthritis. Production of pathogenic autoantibodies by self-reactive plasma cells is a hallmark of autoimmune diseases. Therefore, GPRC5D can be used to target self-reactive plasma cells in autoimmune diseases.

20 In a further aspect, the invention provides for the use of an antibody or bispecific antigen binding molecule of the invention in the manufacture or preparation of a medicament. In one embodiment the medicament is for the treatment of a disease in an individual in need thereof. In a further embodiment, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective amount of the medicament. In certain

25 embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In one embodiment, the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. In a further embodiment, the medicament is for inducing lysis of a target cell, particularly a tumor cell. In still a further embodiment, the medicament is for

30 use in a method of inducing lysis of a target cell, particularly a tumor cell, in an individual comprising administering to the individual an effective amount of the medicament to induce lysis of a target cell. An "individual" according to any of the above embodiments may be a mammal, preferably a human.

In a further aspect, the invention provides a method for treating a disease. In one embodiment, the method comprises administering to an individual having such disease a therapeutically effective amount of an antibody or bispecific antigen binding molecule of the invention. In one embodiment a composition is administered to said individual, comprising the antibody or bispecific antigen binding molecule of the invention in a pharmaceutically acceptable form. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above  
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embodiments may be a mammal, preferably a human.

In a further aspect, the invention provides a method for inducing lysis of a target cell, particularly a tumor cell. In one embodiment the method comprises contacting a target cell with an antibody or bispecific antigen binding molecule of the invention in the presence of a T cell, particularly a cytotoxic T cell. In a further aspect, a method for inducing lysis of a target cell, particularly a  
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tumor cell, in an individual is provided. In one such embodiment, the method comprises administering to the individual an effective amount of an antibody or bispecific antigen binding molecule to induce lysis of a target cell. In one embodiment, an "individual" is a human.

In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer. Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that may be treated using an antibody or bispecific antigen binding molecule of the present invention include, but are not limited to neoplasms located  
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in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of kidney  
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cancer, bladder cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer and prostate cancer. In one embodiment, the cancer is prostate cancer. A skilled artisan readily recognizes that in many cases the antibody or bispecific antigen binding molecule may not provide a cure but may only provide partial benefit. In some embodiments, a

physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of antibody or bispecific antigen binding molecule that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

In some embodiments, an effective amount of an antibody or bispecific antigen binding molecule of the invention is administered to a cell. In other embodiments, a therapeutically effective amount of an antibody or bispecific antigen binding molecule of the invention is administered to an individual for the treatment of disease.

For the prevention or treatment of disease, the appropriate dosage of an antibody or bispecific antigen binding molecule of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of antibody or bispecific antigen binding molecule, the severity and course of the disease, whether the antibody or bispecific antigen binding molecule is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the antibody or bispecific antigen binding molecule, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The antibody or bispecific antigen binding molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (e.g. 0.1  $\text{mg/kg}$  – 10  $\text{mg/kg}$ ) of antibody or bispecific antigen binding molecule can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or bispecific antigen binding molecule would be in the range from about 0.005  $\text{mg/kg}$  to about 10  $\text{mg/kg}$ . In other non-limiting examples, a dose may also comprise from about 1 microgram/kg body weight, about 5 microgram/kg body weight, about 10 microgram/kg body weight, about 50 microgram/kg body weight, about 100 microgram/kg body weight, about

200 microgram/kg body weight, about 350 microgram/kg body weight, about 500 microgram/kg body weight, about 1 milligram/kg body weight, about 5 milligram/kg body weight, about 10 milligram/kg body weight, about 50 milligram/kg body weight, about 100 milligram/kg body weight, about 200 milligram/kg body weight, about 350 milligram/kg body weight, about 500 milligram/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 microgram/kg body weight to about 500 milligram/kg body weight, etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or bispecific antigen binding molecule). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The antibodies or bispecific antigen binding molecules of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the antibodies or bispecific antigen binding molecules of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC<sub>50</sub> as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the antibodies or bispecific antigen binding molecules which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved

by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

In cases of local administration or selective uptake, the effective local concentration of the antibodies or bispecific antigen binding molecules may not be related to plasma concentration.

- 5 One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the antibodies or bispecific antigen binding molecules described herein will generally provide therapeutic benefit without causing substantial toxicity.

- 10 Toxicity and therapeutic efficacy of an antibody or bispecific antigen binding molecule can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD<sub>50</sub> (the dose lethal to 50% of a population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Antibodies or bispecific antigen binding molecules that exhibit large therapeutic
- 15 indices are preferred. In one embodiment, the antibody or bispecific antigen binding molecule according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon a variety of
- 20 factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

- 25 The attending physician for patients treated with antibodies or bispecific antigen binding molecules of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder
- 30 of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

### Other Agents and Treatments

The antibodies and bispecific antigen binding molecules of the invention may be administered in combination with one or more other agents in therapy. For instance, an antibody or bispecific antigen binding molecule of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers. In a particular embodiment, the additional therapeutic agent is an anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent. Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of antibody or bispecific antigen binding molecule used, the type of disorder or treatment, and other factors discussed above. The antibodies or bispecific antigen binding molecules are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the antibody or bispecific antigen binding molecule of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or bispecific antigen binding molecules of the invention may also be used in combination with radiation therapy.

### Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the

container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or bispecific antigen binding molecule of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or bispecific antigen binding molecule of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

## 20 **Methods and Compositions for Diagnostics and Detection**

In certain embodiments, any of the anti-GPRC5D antibodies provided herein is useful for detecting the presence of GPRC5D in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as prostate tissue.

25 In one embodiment, an anti-GPRC5D antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of GPRC5D in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-GPRC5D antibody as described herein under conditions permissive for binding of the anti-GPRC5D antibody to GPRC5D, and detecting whether a complex is formed between the anti-GPRC5D antibody and GPRC5D. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-GPRC5D antibody is used to select subjects eligible for therapy with an anti-GPRC5D antibody, e.g. where GPRC5D is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include cancer, particularly multiple myeloma.

In certain embodiments, labeled anti-GPRC5D antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes <sup>32</sup>P, <sup>14</sup>C, <sup>125</sup>I, <sup>3</sup>H, and <sup>131</sup>I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

A further aspect of the invention relates to an antibody (10B10) that binds GPRC5D comprising a variable heavy chain region (VL), wherein the VL may comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 81. The antibody may comprises a light chain variable region (VL), wherein the VL comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 82. The antibody may comprises a VH and a VL, wherein the VL may comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 81 and wherein the VL comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 82. Preferably, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 81 and a VL comprising the amino acid sequence of SEQ ID NO: 82.

A further aspect of the invention relates to an antibody (10B10-TCB). The antibody may comprise a first light chain, wherein the first light chain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 67. The antibody may comprise a second light chain, wherein the second light chain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID

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NO: 68. The antibody may comprise a first heavy chain, wherein the first heavy chain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 69. The antibody may comprise a second heavy chain, wherein the second heavy chain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 70. In a preferred embodiment, the antibody comprises a first light chain comprising the amino acid sequence of SEQ ID NO: 67, a second light chain comprising the amino acid sequence of SEQ ID NO: 68, a first heavy chain comprising the amino acid sequence of SEQ ID NO: 69 and a second heavy chain comprising the amino acid sequence of SEQ ID NO: 70.

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### Amino Acid Sequences

|               | Amino acid sequence                                                                                                         | SEQ ID NO |
|---------------|-----------------------------------------------------------------------------------------------------------------------------|-----------|
| 5E11-VH-HCDR1 | GFTFSKYAMA                                                                                                                  | 1         |
| 5E11-VH-HCDR2 | STGGVNTYYRDSVKA                                                                                                             | 2         |
| 5E11-VH-HCDR3 | HTGDYFDY                                                                                                                    | 3         |
| 5E11-VL-LCDR1 | ASQSVSISGINLMN                                                                                                              | 4         |
| 5E11-VL-LCDR2 | HASILAS                                                                                                                     | 5         |
| 5E11-VL-LCDR3 | QQTRESPLT                                                                                                                   | 6         |
| 5F11-VH-HCDR1 | GFSFSNYGMA                                                                                                                  | 7         |
| 5F11-VH-HCDR2 | STGGGNTYYRDSVKG                                                                                                             | 8         |
| 5F11-VH-HCDR3 | HDRGGLY                                                                                                                     | 9         |
| 5F11-VL-LCDR1 | RSSKSLHSHNGITYVY                                                                                                            | 10        |
| 5F11-VL-LCDR2 | RMSNLAS                                                                                                                     | 11        |
| 5F11-VL-LCDR3 | GQLLENPYT                                                                                                                   | 12        |
| 5E11-VH       | ELQLEQSGGGLVQPGGSLTSCAASGFTFSKYAMAWVRQAPTKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDNANTQYLQMDSLRS EDTATYYCA<br>THTGDYFDYWGQGVMTVSS | 13        |
| 5E11-VL       | DIVLTQSPALAVSPGQRATISCRASQSVSISGINLMNWWYQQKPGQPKLLI<br>YHASILASGIPTFRFSGSGGTDFLTIDPVQADDIATYYCQQTRESPLTFGS<br>GTNLEIK       | 14        |
| 5F11-VH       | EVQLVESGGGLVQGRSLKLSCAASGFSFSNYGMAWVRQAATKGLEWV<br>ASISTGGGNTYYRDSVKGRFIVSRDNANTQYLQMDSLRS EDTATYYCT<br>RHDRGGLYWGQGVMTVSS  | 15        |

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |    |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 5F11-VL                  | DIVMTQAPLSVSVTPGESASISCRSSKSLLSNGITYVYWFQKPGKSPQV<br>LIYRMSNLASGVDPDRFSGSGSETDFTLKISRVEAEDVGIYHCGQLLENPYT<br>FGAGTELELK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 16 |
| 5E11-TCB-<br>LC1(GPRC5D) | DIVLTQSPALAVSPGQRATISCRASQSVSISGINLMNWYQQKPGQQPKLLI<br>YHASILASGIPTRFSGSGSGTDFTLTIDPVQADDIATYYCQQTRESPLTFGS<br>GTNLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKV<br>DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQ<br>GLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 17 |
| 5E11-TCB-<br>LC2(CD3)    | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 18 |
| 5E11-TCB-HC<br>hole      | ELQLEQSGGGLVQPGGSLTLSCAASGFTFSKYAMAWVRQAPTKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDNANKNTQYLQMDSLRSEDATATYYCA<br>THTGDYFDYWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ<br>YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKISKAKGQPR<br>EPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT<br>PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SPGK                                                                                                                                                                                                                                            | 19 |
| 5E11-TCB-HC<br>knob      | ELQLEQSGGGLVQPGGSLTLSCAASGFTFSKYAMAWVRQAPTKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDNANKNTQYLQMDSLRSEDATATYYCA<br>THTGDYFDYWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQAVVTQEPSLTVSPG<br>GTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTARF<br>SGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSAS<br>TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDDKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 20 |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |    |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 5F11-TCB-LC1(GPRC5D)    | DIVMTQAPLSVSVTPGESASISCRSSKSLLSHNGITYVYWFQKPGKSPQV<br>LIYRMSNLASGVDPDRFSGSGSETDFTLKISRVEAEDVGIYHCGQLLENPYT<br>FGAGTELELKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEV<br>THQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 21 |
| 5F11-TCB-LC2(CD3)       | EVQLLESGGGLVQPGGSLRLSCAASGFTFTSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 22 |
| 5F11-TCB-HC hole        | EVQLVESGGGLVQGRSLKLSCAASGFSFSNYGMAWVRQAATKGLEWV<br>ASISTGGGNTYYRDSVKGRFIVSRDNAKNTQYLQMDSLRSEDATYYCT<br>RHDRGGGLYWGGQVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIKAKGQPRE<br>PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTP<br>PVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK                                                                                                                                                                                                                                                 | 23 |
| 5F11-TCB-HC knob        | EVQLVESGGGLVQGRSLKLSCAASGFSFSNYGMAWVRQAATKGLEWV<br>ASISTGGGNTYYRDSVKGRFIVSRDNAKNTQYLQMDSLRSEDATYYCT<br>RHDRGGGLYWGGQVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQA VVTQEPSLTVSPGG<br>TVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFS<br>GSLGGAALTLGAQPEDEAEYYCALWYSLWVFGGGTKLTVLSSAST<br>KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF<br>PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKTIKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTPPVLDSGDSFFLYSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 24 |
| ET150-5-TCB-LC1(GPRC5D) | QSVLTQPPSASGTPGQRVTISCSGSRSNVGGNYVFWYQQVPGATPKLLIY<br>RSNQRPSGVPDRFAGSKSGSSASLAISGLRSEDEADYYCATWDDSLSGFV<br>FGTGKVTVLGQPKAAPSVTLFPPSSKKLQANKATLVCLISDFYPGAVTV<br>AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRYSYSCQV<br>THEGSTVEKTVAPTECS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 25 |

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| ET150-5-TCB-LC2(CD3) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 26 |
| ET150-5-TCB-HC hole  | EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVS<br>YISSSGSTIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARG<br>YGKAYDQWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED<br>YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI<br>CNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS<br>TYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQ<br>VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPV<br>LDSGGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG<br>K                                                                                                                                                                                                                                              | 27 |
| ET150-5-TCB-HC knob  | EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVS<br>YISSSGSTIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARG<br>YGKAYDQWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED<br>YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI<br>CNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQAVVTQEPSLTVSPGGT<br>VTLCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSG<br>SLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTK<br>GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP<br>AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCD<br>KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE<br>VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY<br>KCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV<br>KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLVSKLTVDKSRWQ<br>QGNVFSCSVMHEALHNHYTQKSLSLSPGK | 28 |
| CD3-VH-HCDR1         | TYAMN                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 29 |
| CD3-VH-HCDR2         | RIRSKYNNYATYYADSVKG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 30 |
| CD3-VH-HCDR3         | HGNFGNSYVSWFAY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 31 |
| CD3-LH-LCDR1         | GSSTGAVTTSNYAN                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 32 |
| CD3-LH-LCDR2         | GTNKRAP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 33 |
| CD3-LH-LCDR3         | ALWYSNLWV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 34 |
| CD3-VH               | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 35 |

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| CD3-VL                                               | QAVVTQEPSLTVSPGGTVLTCGSSTGAVTTSNYANWVQEKPGQAFRGLI<br>GGTNKRAPGTPARFSGSLLGGKAALTLGAQPEDEAEYYCALWYSNLW<br>VFGGGTKLTVL                                                                                                                                                                                                                                           | 36 |
| Human kappa CL domain                                | RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG<br>NSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK<br>SFNRGEC                                                                                                                                                                                                                                            | 37 |
| Human lambda CL domain                               | QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKA<br>GVETTTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTV<br>APTECS                                                                                                                                                                                                                                              | 38 |
| Human IgG1 heavy chain constant region (CH1-CH2-CH3) | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV<br>HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP<br>KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH<br>EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN<br>GKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT<br>CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQGNVFSCSVMHEALHNHYTQKSLSLSP | 39 |
| hCD3                                                 | MQSGTHWRVLGLCLLSVGWVGQDGNEMGGITQTPYKVSISGTTVILTC<br>PQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCY<br>PRGSKPEDANFYLYLRARVCENCMEMDVMSVATIVIVDICITGLLLL VY<br>YWSKNRKAKAKPVTRGAGAGGRQGRQNKERPPVPNPDIPIRKGGQRD<br>LYSGLNQRRI                                                                                                                                 | 40 |
| cynoCD3                                              | MQSGTRWRVLGLCLLSIGWVGQDGNEMGSITQTPYQVSISGTTVILTCS<br>QHLGSEAQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSPED<br>ASHHLYLKARVCENCMEMDVMAVATIVIVDICITLGLLLL VYYWSKNRK<br>AKAKPVTRGAGAGGRQGRQNKERPPVPNPDIPIRKGGQDLYSGLNQR<br>RI                                                                                                                                           | 41 |
| hIgG1 Fc region                                      | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV<br>KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ<br>QGNVFSCSVMHEALHNHYTQKSLSLSP                                                                                                              | 42 |
| linker                                               | GGGGSGGGGS                                                                                                                                                                                                                                                                                                                                                    | 43 |
| linker                                               | DGGGGSGGGGS                                                                                                                                                                                                                                                                                                                                                   | 44 |

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| Human GPRC5D | MYKDCIESTGDYFLLCDAEGPWGIILESLAILGIVVTILLLLAFLFLMRKIQ<br>DCSQWNVLPTQLLFLLSVLGLFGLAFAFIIELNQQTAPVRYFLFGVLFALC<br>FSCLLAHASNLVKLVRGCVSFSWTTILCIAIGCSLLQIIIATEYVTLIMTRG<br>MMFVNMTPCQLNVDFVLLVYVLFLMALTFFVSKATFCGPCENWKQHG<br>RLIFITVLFSSIIWVWVISMILLRGNPQFQRQPQWDDPVVCIALVTNAWVFL<br>LLYIVPELCILYRSCRQECPLQGNACPVTAYQHSFQVENQELSRARDSDG<br>AEEDVALTSYGTPIQPQTVDPTQECFIPQAKLSPQQDAGGV | 45 |
| 5E11_VH1a    | EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDN SKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGMVTVSS                                                                                                                                                                                                                                                     | 46 |
| 5E11_VH1b    | ELQLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDN AKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGMVTVSS                                                                                                                                                                                                                                                      | 47 |
| 5E11_VH1c    | EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGMVTVSS                                                                                                                                                                                                                                                     | 48 |
| 5E11_VH1d    | ELQLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGMVTVSS                                                                                                                                                                                                                                                      | 49 |
| 5E11_VL1a    | DIVMTQSPDSLAVSLGERATINCRASQSVSISGINLMNWHYQQKPGQPKL<br>LIYHASILASGVPRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRESPLTF<br>GQGTRLEIK                                                                                                                                                                                                                                                            | 50 |
| 5E11_VL1c    | DIVMTQSPDSLAVSLGERATINCKSSQSVSISGINLMNWHYQQKPGQPKL<br>LIYHASILASGVPRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRESPLTF<br>GQGTRLEIK                                                                                                                                                                                                                                                            | 51 |
| 5E11_VL2a    | EIVLTQSPGTLSPGERATLSCRASQSVSISGINLMNWHYQQKPGQPKLLI<br>YHASILASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIK                                                                                                                                                                                                                                                             | 52 |
| 5E11_VL2b    | EIVLTQSPGTLSPGERATLSCRASQSVSISGINLMNWHYQQKPGQPKLLI<br>YHASILASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIK                                                                                                                                                                                                                                                             | 53 |
| 5E11_VL3a    | DIQMTQSPSSLSASVGDRVTITCRASQSVSISGINLMNWHYQQKPGKQPKLL<br>IYHASILASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTRESPLTFG<br>QGTRLEIK                                                                                                                                                                                                                                                           | 54 |
| 5E11_VL3b    | DIQMTQSPSSLSASVGDRVTITCRASQSVSISGINLMNWHYQQKPGQPKLL<br>IYHASILASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTRESPLTFG<br>QGTRLEIK                                                                                                                                                                                                                                                            | 55 |

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| 5F11_VH1a     | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTR<br>HDRGGLYWGGQTMVTVSS                                                                                                          | 56 |
| 5F11_VH1b     | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTVSS                                                                                                          | 57 |
| 5F11_VH1c     | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTVSS                                                                                                          | 58 |
| 5F11_VH1d     | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTVSS                                                                                                          | 59 |
| 5F11_VH2b     | EVQLVESGGGLVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTVSS                                                                                                          | 60 |
| 5F11_VH2d     | EVQLVESGGGLVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTVSS                                                                                                          | 61 |
| 5F11_VL1a     | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGQSPQV<br>LIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK                                                                                                                | 62 |
| 5F11_VL1b     | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGKSPQV<br>LIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK                                                                                                                | 63 |
| 5F11_VL2a     | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGQSPQL<br>LIYRMSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK                                                                                                                | 64 |
| 5F11_VL2b     | DIVMTQSPDSLAVSLGERATINCKSSKSLLSNGITYVYWYQKPGQPPK<br>LLIYRMSNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYHCGQLLENPY<br>TFGQGTKLEIK                                                                                                                 | 65 |
| 5F11_VL2c     | EIVLTQSPGTLSPGERATLSCRASKSLLSNGITYVYWYQKPGQAPRL<br>LIYRMSNLASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYHCGQLLENPYTF<br>GQGTKLEIK                                                                                                                   | 66 |
| 10B10_TCB_LC1 | DIQLTQSPHLSASLGETVSI ECLASEGISNYLAWFHQKPGKSPQLLIYYAS<br>SLQDGVPSRFSGSGSGTQYSLKISNMQPEDEGVYYCQQGYKYPLTFGSGT<br>KLEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN<br>ALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLS<br>SPVTKSFNRGEC | 67 |

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| 10B10 TCB_LC2            | EVQLLESGGGLVQPGGSLRLSCAASGFTFTSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 68 |
| 10B10<br>TCB_HC(Fc hole) | EVQLVESGGGLVQPGRSMKLSAASGFTFTNFYMAWVRQAPTKALEWV<br>ASINTGGGYTYRDSVKGRFTVSRDNRSTLYLQMDSLRSEETATYYCA<br>RHLTYYGRIYYFDYWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGTAA<br>LGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSST<br>LGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFL<br>FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP<br>REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK<br>GQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN<br>YKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK<br>SLSLSPGK                                                                                                                                                                                                                                               | 69 |
| 10B10<br>TCB_HC(Fc knob) | EVQLVESGGGLVQPGRSMKLSAASGFTFTNFYMAWVRQAPTKALEWV<br>ASINTGGGYTYRDSVKGRFTVSRDNRSTLYLQMDSLRSEETATYYCA<br>RHLTYYGRIYYFDYWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGTAA<br>LGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSST<br>LGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSQAVVTQEPSL<br>TVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPG<br>TPARFSGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTV<br>LSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALT<br>GVHTFPAVLQSSGLYSLSSVTVPSSTLGTQTYICNVNHKPSNTKVDEK<br>EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDV<br>SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL<br>NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVS<br>LWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDK<br>SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 70 |
| 07A04 IgG_LC             | DVQMTQSPYNLAASPGESVSINCKASKSISKYLAWYQQKPGKANKLLIY<br>DGSTLQSGIPSRFSGSGGTDFTLTIRSLPEDFGLYYCQQHNEYPLTFGSG<br>TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD<br>NALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQG<br>LSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 71 |

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| 07A04 IgG_HC | QVTLKESGPGILQPSHTLSLTCFSGFSLSSTYGMGVNWIRQPSGKGLEWL<br>ASIWWNGNTYNNPSLKSRLTVSKDTSNNQAFKVTSDTADTATYYCVH<br>TRGIIRGRGLFFDYWGQGVMTVSSASTKGPSVFPLAPSSKSTSGGTAAL<br>GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS<br>LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFL<br>FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP<br>REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK<br>GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN<br>YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK<br>SLSLSPGK | 72 |
| B72-TCB_HC1  | QTVVTQEPSTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLI<br>GGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLW<br>VFGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT<br>VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK<br>PSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT<br>PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS<br>VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPP<br>CRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG<br>SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK                | 73 |
| B72-TCB_LC1  | EVQLVESGGGLVQPGGSLRLSCAASGFTFTNYAMNWVRQAPGKGLEWV<br>ARIRSKYNNYATYYAASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYY<br>CARHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSST<br>LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                 | 74 |
| B72-TCB_HC2  | QVQLVQSGAEVKKPGASVKVSCKASGYSTGYTMNWVRQAPGQGLEW<br>MGLINPYNSDTNYAQLKQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYC<br>ARVALRVALDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGC<br>LVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT<br>QTYICNVNHKPSNTKVDKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP<br>KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE<br>QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP<br>REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT<br>TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS<br>LSPGK      | 75 |
| B72-TCB_LC2  | DIQMTQSPSSLSASVGDRVTITCKASQNVATHVGWYQQKPGKAPKRLIYS<br>ASYRYSRVPSRFSGSGSGTEFTLTISNLPEDFATYYCQQYNRYPYTFGGQ<br>TKLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD<br>NALQSGNSQESVTEQDSKDSYSTLSSTLTLKADYEKHKVYACEVTHQG<br>LSSPVTKSFNRGEC                                                                                                                                                                                                                                                                    | 76 |

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| BCMA-TCB-HC1<br>(hole) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVS<br>AITASGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR<br>YWPMSLWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDY<br>FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC<br>NVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT<br>LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST<br>YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV<br>CTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVL<br>DSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK                                                                                                                                                                                                                                                     | 77 |
| BCMA-TCB-LC1           | EIVLTQSPGTLSPGERATLSCRASQSVSAYYLAWYQQKPGQAPRLMY<br>DASIRATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYCQQYERWPLTFGQ<br>GTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKV<br>DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQ<br>GLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 78 |
| BCMA-TCB-HC2<br>(knob) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVS<br>AITASGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR<br>YWPMSLWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDY<br>FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC<br>NVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQA VVTQEPSLTVSPGGTV<br>TLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSL<br>LGGAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKGP<br>SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV<br>LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT<br>HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK<br>FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC<br>KVSNAKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG<br>FYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLVSKLTVDKSRWQQG<br>NVFSCFSVMHEALHNHYTQKSLSLSPGK | 79 |
| BCMA-TCB-LC2           | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHGNFGNSYVSWFAYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 80 |
| 10B10_VH               | DIQLTQSPHLSASLGETVSI ECLASEGISNYLAWFHQKPGKSPQLLIYYAS<br>SLQDGVPSRFSGSGGTQYSLKISNMQPEDEGVYYCQQGYKYP LTFGSGT<br>KLEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 81 |
| 10B10_VL               | EVQLVESGGGLVQPGSRMKLSCAASGFTFTNFYMAWVRQAPTKALEWV<br>ASINTGGGYTYRDSVKGRFTVSRDNTRSTLYLQMDSLRSEETATYYCA<br>RHLTYYGRIYYFDYWGGQVMVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 82 |

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| 5E11_P1AE5706_<br>PARENT-VH-<br>HCDR1   | GFTFSKYAMA        | 83 |
| 5E11_P1AE5706_<br>PARENT-VH-<br>HCDR2   | SISTGGVNTYYRDSVKA | 84 |
| 5E11_P1AE5723_<br>P1AE5728_VH-<br>HCDR2 | SISTGGVNTYYADSVKG | 85 |
| 5E11_P1AE5706_<br>PARENT-VH-<br>HCDR3   | HTGDYFDY          | 86 |
| 5E11_P1AE5706_<br>PARENT-VL-<br>LCDR1   | RASQSVSISGINLMN   | 87 |
| 5E11_P1AE5706_<br>PARENT-VL-<br>LCDR2   | HASILAS           | 88 |
| 5E11_P1AE5706_<br>PARENT-VL-<br>LCDR3   | QQTRESPLT         | 89 |
| 5F11_P1AE5733_<br>PARENT-VH-<br>HCDR1   | GFSFSNYGMA        | 90 |
| 5F11_P1AE5733_<br>PARENT-VH-<br>HCDR2   | SISTGGGNTYYRDSVKG | 91 |
| PF11_P1AE5745_<br>VL-HCDR2              | SISTGGGNTYYADSVKG | 92 |
| 5F11_P1AE5733_<br>PARENT-VH-<br>HCDR3   | HDRGGLY           | 93 |
| 5F11_P1AE5733_<br>PARENT-VL-<br>LCDR1   | RSSKSLHLSNGITYVY  | 94 |
| 5F11_P1AE5733_<br>PARENT-VL-<br>LCDR2   | RMSNLAS           | 95 |

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| 5F11_P1AE5741_<br>VL_LCDR2            | RMSNRAS                                                                                                                                  | 96  |
| 5F11_P1AE5733_<br>PARENT-VL-<br>LCDR3 | GQLLENPYT                                                                                                                                | 97  |
| CD3-CI22-VH-<br>HCDR1                 | SYAMN                                                                                                                                    | 98  |
| CD3-CI22-VH-<br>HCDR2                 | RIRSKYNNYATYYADSVKG                                                                                                                      | 99  |
| CD3-CI22-VH-<br>HCDR3                 | HTTFPSSYVSYYGY                                                                                                                           | 100 |
| CD3-CI22-VH-<br>LCDR1                 | GSSTGAVTTSNYAN                                                                                                                           | 101 |
| CD3-CI22-VH-<br>LCDR2                 | GTNKRAP                                                                                                                                  | 102 |
| CD3-CI22-VH-<br>LCDR3                 | ALWYSNLWV                                                                                                                                | 103 |
| CD3-CI22-VH                           | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQA<br>PGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNT<br>LYLQMNSLRAEDTAVYYCVRHTTFPSSYVSYYGYWGQGT<br>LTVSS | 104 |
| CD3-CI22-VL                           | QAVVTQEPLTVSPGGTVLTCGSSTGAVTTSNYANWVQE<br>KPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLGAQ<br>PEDEAEYYCALWYSNLWVFGGGTKLTVL                       | 105 |
| CD3-V9-VH-<br>HCDR1                   | GYSFTGYTMN                                                                                                                               | 106 |
| CD3-V9-VH-<br>HCDR2                   | LINPYKGVSTYNQKFKD                                                                                                                        | 107 |
| CD3-V9-VH-<br>HCDR3                   | SGYYGSDWYFDV                                                                                                                             | 108 |
| CD3-V9-VH-<br>LCDR1                   | RASQDIRNYLN                                                                                                                              | 109 |
| CD3-V9-VH-<br>LCDR2                   | YTSRLES                                                                                                                                  | 110 |
| CD3-V9-VH-<br>LCDR3                   | QQGNTLPWT                                                                                                                                | 111 |
| CD3-V9-VH                             | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWV<br>ALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCA<br>RSGYYGSDWYFDVWGQGT LTVSS        | 112 |

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| CD3-V9-VL                                                       | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYY<br>TSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGGG<br>TKVEI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 113 |
| 5F11(VH1b +<br>VL2a)-CI22-TCB-<br>LC1(antiGPRC5D)<br>(P1AE6623) | DIVMTQSPLSLPVTPGEPASISCRSSKSLLSNGITYVYWYLQKPGQSPQL<br>LIYRMSNRASGVPRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEV<br>THQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 114 |
| 5F11(VH1b +<br>VL2a)-CI22-TCB-<br>LC2(antiCD3)<br>(P1AE6623)    | EVQLLESGGGLVQPGGSLRLSCAASGFSYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHTTFPSSYVSYYGYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLT<br>LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 115 |
| 5F11(VH1b +<br>VL2a)-CI22-TCB-<br>HC1(Fc hole)<br>(P1AE6623)    | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGGLYWGGQTMVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKISKAKGQPRE<br>PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPP<br>VLDSGDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS<br>PGK                                                                                                                                                                                                                                           | 116 |
| 5F11(VH1b +<br>VL2a)-CI22-TCB-<br>HC2(Fc knob)<br>(P1AE6623)    | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGGLYWGGQTMVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQAVVTQEPSTLTPSPGG<br>TVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFS<br>GSLGGAALTLGAQPEDEAEYCALWYSLWVFGGGTKLTVLSSAST<br>KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF<br>PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 117 |

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| 5F11(VH1c+VL1b)<br>)-CI22-TCB-<br>LC1(antiGPRC5D)<br>(P1AE6624) | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGKSPQV<br>LIYRMSNLASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEV<br>THQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 118 |
| 5F11(VH1c+VL1b)<br>)-CI22-TCB-<br>LC2(antiCD3)<br>(P1AE6624)    | EVQLLESGLLVQPGGSLRLSCAASGFSYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHTTFPSSYVSYYGYWGQGTLTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 119 |
| 5F11(VH1c+VL1b)<br>)-CI22-TCB-<br>HC1(Fc hole)<br>(P1AE6624)    | QVQLVESGGGVVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>NSTYRVVSVLTVLHQDWLNGKEYCKVSNKALGAPIEKTIKAKGQPRE<br>PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTP<br>PVLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>PGK                                                                                                                                                                                                                                              | 120 |
| 5F11(VH1c+VL1b)<br>)-CI22-TCB-<br>HC2(Fc knob)<br>(P1AE6624)    | QVQLVESGGGVVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQAVVTQEPSLTVSPGG<br>TVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFS<br>GSLGGAALTLGAQPEDEAEYYCALWYSLWVFGGGTKLTVLSSAST<br>KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF<br>PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKTIKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTPPVLDSGDSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 121 |
| 5E11(VH1c+VL2b)<br>)-CI22-TCB-<br>LC1(antiGPRC5D)<br>(P1AE6625) | EIVLTQSPGTLSLSPGERATLSCRASQSVSISGINLMNWYQQKPGQPKLLI<br>YHASILASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKV<br>DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQ<br>GLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 122 |

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| 5E11(VH1c+VL2b)<br>)-CI22-TCB-<br>LC2(antiCD3)<br>(P1AE6625)    | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHTTFPSSYVSYYGYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 123 |
| 5E11(VH1c+VL2b)<br>)-CI22-TCB-<br>HC1(Fc hole)<br>(P1AE6625)    | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGLMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ<br>YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPR<br>EPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT<br>PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SPGK                                                                                                                                                                                                                                               | 124 |
| 5E11(VH1c+VL2b)<br>)-CI22-TCB-<br>HC2(Fc knob)<br>(P1AE6625)    | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGLMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQA VVTQEPSLTVSPG<br>GTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARF<br>SGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSAS<br>TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 125 |
| 5E11(VH1d+VL2a)<br>)-CI22-TCB-<br>LC1(antiGPRC5D)<br>(P1AE6626) | EIVLTQSPGTLSPGERATLSCRASQSVSISGINLMNWYQQKPGQQRLLI<br>YHASILASGIPDRFSGSGGTDFTLISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKV<br>DNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQ<br>GLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 126 |
| 5E11(VH1d+VL2a)<br>)-CI22-TCB-<br>LC2(antiCD3)<br>(P1AE6626)    | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQAPGKGLEWV<br>SRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY<br>CVRHTTFPSSYVSYYGYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGT<br>ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST<br>LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 127 |

|                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |     |
|---------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 5E11(VH1d+VL2a)<br>)-CI22-TCB-<br>HC1(Fc hole)<br>(P1AE6626)  | ELQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP<br>KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ<br>YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKISKAKGQPR<br>EPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT<br>PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SPGK                                                                                                                                                                                                                                              | 128 |
| 5E11(VH1d+VL2a)<br>)-CI22-TCB-<br>HC2(Fc knob)<br>(P1AE6626)  | ELQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV<br>EDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT<br>YICNVNHKPSNTKVDEKVEPKSCDGGGSGGGGSQAVVTQEPSLTVSPG<br>GTVTLTCGSSTGAVTTSNYANWVQEPGQAFRGLIGGTNKRAPGTPARF<br>SGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSAS<br>TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP<br>EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK | 129 |
| 5F11(VH1c+VL1b)<br>)-V9-TCB-<br>LC1(antiGPRC5D)<br>(P1AF1336) | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGKSPQV<br>LIYRMSNLASGVDRFSGSGGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEV<br>THQGLSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 130 |
| 5F11(VH1c+VL1b)<br>)-V9-TCB-<br>LC2(antiCD3)<br>(P1AF1336)    | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWV<br>ALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCA<br>RSGYYGDSWDYFDVWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGTAS<br>VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLT<br>LSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 131 |

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|                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |     |
|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 5F11(VH1c+VL1b)-V9-TCB-HC1(Fc hole) (P1AF1336) | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGGLYWGGQTMVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK<br>DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE<br>PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP<br>PVLDSGGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK                                                                                                                                                                                                                                                   | 132 |
| 5F11(VH1c+VL1b)-V9-TCB-HC2(Fc knob) (P1AF1336) | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGGLYWGGQTMVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVE<br>DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY<br>ICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSDIQMTQSPSSLSASVGD<br>RVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSG<br>TDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTKGPSVFP<br>LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS<br>GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP<br>PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN<br>KALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP<br>SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF<br>SCSVMHEALHNHYTQKSLSLSPGK | 133 |

### Examples

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

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#### Example 1

##### Expression of tumor targets

To identify the differential genes expressed by multiple myeloma over the normal plasma cells,  
 10 RNAseq was performed for 10 samples derived from patients with multiple myeloma (MM) and  
 10 plasma cells (PCs) derived from bone marrow of healthy donors. The RNA was extracted using  
 the RNeasy Micro kit (Qiagen) according to manufacturer's instructions. The genomic DNA was

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removed using the RNase free DNase set (Qiagen) during the RNA extraction. The quality of the extracted RNA was controlled on Agilent Eukaryote Total RNA pico chips (Agilent Technologies). SMARTer ultra-low RNA kit for Illumina sequencing (Clontech) was used to prepare and amplify cDNA from 1.6 ng of total RNA according to the manufacturer's instructions.

5 Then, 1 ng of amplified cDNA was subjected to Nextera XT library preparation (Illumina) according to the manufacturer's instructions. Sequencing libraries were quantified using the Kapa Library Quantification kit (Kapa Biosystems) and quality controlled by capillary electrophoresis on a Bioanalyzer using High Sensitivity chips (Agilent Technologies). The libraries were sequenced on a HiSeq2500 sequencer (Illumina) for 2 x 50 cycles using version 4 cluster

10 generation kits and version 4 sequencing reagents (Illumina).

B-cell maturation antigen (BCMA) is a cell surface protein, which is expressed on malignant plasma cells and thus recognized as multiple myeloma target (Tai YT & Anderson KC, Targeting B-cell maturation antigen in multiple myeloma, Immunotherapy. 2015 Nov; 7(11): 1187–1199). Using the RNAseq technology, in-depth analysis indicated that GPRC5D is expressed as highly

15 as BCMA in plasma cells from multiple myeloma patients (Figure 2). More importantly, the differential expression of GPRC5D between plasma cells from multiple myeloma patients and healthy plasma cells is approximately 20 fold. In contrast, differential expression of BCMA between plasma cells from multiple myeloma patients and healthy plasma cells is only 2-fold. The overall expression of GPRC5D is much high than the expression of other known multiple myeloma

20 target molecules such as SLAMF7, CD138 and CD38. In addition, GPRC5D is hardly expressed by healthy naive or memory B cells.

## Example 2

### Generation of GPRC5D binders and preparation of T cell bispecific (TCB) antibodies

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GPRC5D binders were generated by DNA immunization of rats, followed by hybridoma generation, screening and sequencing of hybridoma. Screening for specific binding was measured by ELISA by its binding to GPRC5D-expressing transfectant. Two GPRC5D binders were identified referred to as 5E11 (SEQ ID Nos 13 and 14) and 5F11 (SEQ ID NOs 15 and 16) in the

30 following. Once the specific binders were identified, the IgGs were converted into T cell bispecific antibodies. The principles of converting binders into T cell bispecific antibodies are exemplified and described in the art, e.g. in PCT publication no. WO 2014/131712 A1, which is incorporated herein by reference in its entirety. The T cell bispecific antibodies comprise two GPRC5D-binding

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moieties and one CD3-binding moiety (anti-GPRC5D/anti-CD3 T cell bispecific antibodies) as illustrated in Figure 3. The following anti-GPRC5D/anti-CD3 T cell bispecific antibodies were prepared: i) 5E11-TCB (SEQ ID NOs 17, 18, 19 and 20); ii) 5F11-TCB (SEQ ID NOs 21, 22, 23 and 24); iii) ET150-5-TCB (SEQ ID NOs 25, 26, 27 and 28); iv) B72-TCB (SEQ ID NOs: 73, 74, 5 75 and 76); and v) BCMA-TCB (SEQ ID NOs: 77, 78, 79 and 80). The ET150-5 GPRC5D binding moiety is described in PCT publication no. WO 2016/090329A2. The term “ET-150-5” is synonymically used for the term “ET150-5” herein, and vice versa. As negative control the untargeted DP47-TCB was prepared. DP47-TCB is an untargeted T cell bispecific antibody, which only binds to CD3 but not to GPRC5D. DP47-TCB is described in PCT publication no. WO 10 2014/131712 A1, which is incorporated herein by reference in its entirety. The B72-TCB derives from the GCDB72 antibody disclosed in Table 23 of WO 2018/0117786 A2 and comprises the GPRC5D binding moiety of GCDB72. B72-TCB was generated in the crossmab 1+1 Format (SEQ ID NOs: 73, 74, 75 and 76). The BCMA-TCB derives from WO 2016/166629 A1 and comprises the GPRC5D binding moiety of A02\_Rd4\_6nM\_C01 as disclosed therein. BCMA-TCB was 15 generated in the crossmab 2+1 Format (SEQ ID NOs: 77, 78, 79 and 80).

### Example 3

#### Binding of T cell bispecific antibodies to multiple myeloma cell lines

To measure the binding to GPRC5D, we performed FACS based binding assay on reported 20 multiple myeloma cell lines (Lombardi et al., Molecular characterization of human multiple myeloma cell lines by integrative genomics: insights into the biology of the disease; Genes Chromosomes Cancer. 2007 Mar;46(3):226-38.). The cell lines AMO-1, L363 and OPM-2 were cultured in RPMI 1640 + Glutamax medium (Gibco) supplemented with 20% Heat-Inactivated Fetal Bovine Serum (FBS, Gibco) and 1% Penicillin – Streptomycin 100X (Gibco). The cell line 25 WSU-DLCL2 (negative control) was cultured with the same medium supplemented with only 10% FBS. The cell lines NCI-H929 and RPMI-8226 were also cultured with the same medium supplemented with 50 µM Mercaptoethanol (Gibco) and 1 mM Sodium Pyruvate (Gibco). The cell lines were cultured in 75 cm<sup>2</sup> flasks (TPP) with two passages per week.

The binding of different anti-human GPRC5D-TCBs antibodies (5E11-TCB, 5F11-TCB and 30 ET150-5 TCB) was evaluated using an indirect staining. The cells were incubated with the anti-human GPRC5D-TCB constructs 5E11-TCB, 5F11-TCB or ET150-5 TCB in the range from 10 µg/ml to 0.00064 µg/ml using serial dilution with a factor of 0.2, or no construct in 100 µL of Phosphate Buffer Saline (PBS; Gibco) for 1 hour at 4°C. The cells were stained with Live blue

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dye (Life Technologies) diluted 1:800 in PBS for 20 min at 4°C before staining with PE conjugated Goat anti-human IgG, Fc $\gamma$  fragment specific (Jackson Laboratories) diluted 1/300 in Flow cytometry staining buffer (eBioscience) incubated for 30 min at 4°C. Flow cytometry acquisition was performed on a custom-designed BD Biosciences Fortessa and analyzed using FlowJo software (Tree Star, Ashland, OR) and GraphPad Prism software.

Figures 4A-C show that both 5E11-TCB and 5F11-TCB bind all of the tested multiple myeloma cell lines in a dose-dependent manner. In contrast, ET150-5-TCB binds much weaker to the tested cell lines. There was no binding to WSU-DLCL2 cells (GPRC5D<sup>+</sup> cell lines of non-Hodgkin lymphoma) observed by the anti-GPRC5D-TCBs.

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#### Example 4

##### anti-GPRC5D-TCB mediated T cell cytotoxicity

To measure the functionality of the anti-GPRC5D-TCB antibodies, an *in-vitro* T cell cytotoxicity assay was performed. Briefly, AMO-1, L363 and OPM-2 cell lines were cultured in RPMI 1640 + Glutamax medium (Gibco) supplemented with 20% Heat-Inactivated Fetal Bovine Serum (FBS; Gibco) and 1% Penicillin – Streptomycin 100X (PS; Gibco). The cell line WSU-DLCL2 was cultured with the same medium supplemented with only 10% FBS. The cell lines NCI-H929 and RPMI-8226 were cultured the same medium supplemented with 50  $\mu$ M Mercaptoethanol (Gibco) and 1 mM Sodium Pyruvate (Gibco). The cell lines were cultured in 75 cm<sup>2</sup> Flask (TPP) with two passages per week.

The cell lines were co-cultured at a ratio Target:Effector of 1:10 with 3.105 allogeneic T cells isolated from peripheral blood mononuclear cells (PBMCs) (Buffy coat from Blutspende Schlieren) using a human Pan T cell Isolation kit (Miltenyi Biotec) in IMDM Medium (Gibco) supplemented with 10% FBS (Gibco) + 1% PS (Gibco). Anti-human GPRC5D-TCB antibodies (5E11-TCB, 5F11-TCB, ET150-5 TCB or DP47-TCB) were added to the co-culture at different concentration, in the range from 1  $\mu$ g/ml to 0.000001 $\mu$ g/ml with serial dilution of factor 0.1 or 0  $\mu$ g/ml. After 20 hours of incubation at 37°C with 5% CO<sub>2</sub>, 75  $\mu$ l of supernatant per well were transferred into a 96-well white plate (Greiner bio-one) with 25  $\mu$ l per well of CytoTox-Glo Cytotoxicity Assay (Promega). Luminescence acquisition was performed on the PerkinElmer EnVision after 15 min incubation at room temperature and analyzed using GraphPad Prism and XL fit software. Data are plotted as the Luminescence signal for LDH release.

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Figures 5A-E show that both 5E11-TCB and 5F11-TCB mediated strong T cell cytotoxicity on the multiple myeloma cell lines, particularly NCI-H929 (Fig. 5B), RPMI-8226 (Fig. 5C), L363 and (Fig. 5D) AMO-1 (Fig. 5A), whereas no killing was observed on the negative control line WSU-DLCL2 (Fig. 5E). In contrast, ET150-5-TCB mediated little or significantly lower killing on the tested multiple myeloma cell lines. Table 1 summarizes the EC<sub>50</sub> values derived from the data shown in Figure 5A-E. EC<sub>50</sub> value was calculated using XLfit add-on feature in Excel by plotting the raw data of the signals against the titrated TCBs.

**Table 1.** EC<sub>50</sub> of anti-GPRC5D-TCB mediated killing

|             | NCI-H929 | RPMI-8226 | L363     | AMO-1     | WSI-DLCL2 |
|-------------|----------|-----------|----------|-----------|-----------|
| 5E11-TCB    | 0.007 nM | 0.024 nM  | 0.012 nM | 0.014 nM  | /         |
| 5F11-TCB    | 0.001 nM | 0.002 nM  | 0.001 nM | 0.003 nM  | /         |
| ET150-5-TCB | 0.833 nM | 0.797 nM  | 0.768 nM | 0.0835 nM | /         |

10

### Example 5

#### anti-GPRC5D-TCB mediated T cell activation

To mechanistically address the modes of action of the anti-GPRC5D-TCBs, the activation of T cells after co-culturing with target multiple myeloma cell lines in the presence of anti-GPRC5D-TCBs was measured. Similar to the experiment described in Example 4 and Figures 5A-E, the cell lines were co-cultured at ratio Target:Effector of 1:10 with 3.105 allogeneic T cells isolated from PBMCs (Buffy coat from Blutspende Schlieren) using a human Pan T cell Isolation kit (Miltenyi Biotec) in IMDM Medium (Gibco) supplemented with 10% FBS (Gibco) + 1% PS (Gibco). Anti-human GPRC5D-TCB antibodies (5E11-TCB, 5F11-TCB, ET150-5-TCB or DP47-TCB) were added to the co-culture at different concentration, in the range from 1 µg/ml to 0.000001 µg/ml with serial dilution of factor 0.1 or 0 µg/ml. After 20 hours of incubation at 37°C with 5% CO<sub>2</sub>, the cells were stained to evaluate T cell activation. The cells were first stained with Live blue dye (Life Technologies) diluted 1:800 in PBS (Gibco) for 20 min at 4°C. Afterwards, the cells were stained with AF700 anti-human CD4 (clone OKT4), BV711 anti-human CD8 (clone SK1), BV605 anti-human CD25 (clone BC96), APC-Cy7 anti-human CD69 (clone FN50) all from BioLegend and PE-Cy5.5 anti-human CD3 (clone SK7; eBioscience) in Flow cytometry staining buffer (eBioscience) for 30 min at 4°C. Flow cytometry acquisition was performed on a custom-designed BD Biosciences Fortessa and analyzed using FlowJo software (Tree Star, Ashland, OR) and GraphPad Prism software.

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Figure 6 shows that 5F11-TCB induces T cell activation in co-cultures with NCI-H929 cells by upregulating the activation marker CD25 and CD69, whereas the controls, e.g. untargeted DP47-TCB and without any TCB, did not induce T cell activation. As another negative control, 5F11-TCB treated T cells were co-cultured with WSU-DLCL2 cells, wherein T cells were also not activated. These activation profiles were consistent across multiple cell lines we studied, e.g. AMO-1, NCI-H929, RPMI-8226, L363 (Figures 7A-J). In line with the poor killing potency, ET150-5-TCB did not induce T cell activation except at the highest tested concentration of 1 mg/kg.

### Example 6

#### 10                    **Localization and internalization of anti-GPRC5D-TCB**

NCI-H929 cells were stained with CMFDA (Invitrogen) and seeded on Poly-L-Lysine (Sigma) coated round coverslips in 24 well plates. Antibodies (5E11-IgG, 5E11-TCB, 5F11-IgG, 5F11-TCB) were labeled with an Alexa Fluor 647 Succinimidyl Ester (Invitrogen, cat#A201106) at a molar ratio of 2.5. Cells were allowed to adhere overnight at 37 °C before fluorescently-tagged antibodies (Alexa Fluor 647 labeled-5E11-IgG, -5E11-TCB, -5F11-IgG, -5F11-TCB) were added directly into growth media for different durations and temperatures (30 min on ice, 1 hour at 37 °C and 3 hours at 37 °C). Cold PBS (Lonza) was used to quench the reaction and to wash off unbound antibodies after each time point. Cells were then fixed with Cytofix (BD) for 20 minutes at 4 °C and washed twice with PBS. Coverslips were then transferred and mounted on glass slides with Fluoromount G (eBioscience) and kept in the dark at 4 °C overnight before imaging. Fluorescence confocal microscopy was performed with an inverted LSM 700 from Zeiss with a 60x oil objective. Images were collected using Zen software (Zeiss) coupled to the microscope and visualized on the IMARIS software (Bitplane). Figure 8A shows that all antibodies stained the surface (plasma membrane) of the multiple myeloma cell line at 4 °C or 37 °C. If antibodies are internalized by the cells, then the fluorescent staining will appear in the cytoplasm when cultured at 37 °C. No internalization of the GPRC5D-binding-IgGs or GPRC5D-binding-TCBs by the GPRC5D<sup>+</sup> cell lines was observed. It was further confirmed by applying the intensity sum from membrane and cytoplasm defined regions of interest of cells (at three hours). The IMARIS software was used for analysis and quantification of the signal ratio of membrane to cytoplasm. Figure 8B indicates that 3 hours after incubation with the different antibodies, the ratio of membrane to cytoplasmic intensity was unchanged at ~4, meaning the fluorescent signals concentrate at the surface, not in the cytoplasm.

**Example 7****Characterizing GPRC5D binders: recombinant cell binding by ELISA**

Stable transfected CHO clones expressing either human GPRC5D or cynomolgus GPRC5D or murine GPRC5D or human GPRC5A were used to analyze the binding of potential lead candidate antibodies as IgGs. In detail,  $10^4$  cells (viability  $\geq 98\%$ ) were seeded into 384 well-microtiter plates (BD Poly D-Lysin, #356662, volume: 25  $\mu$ l/well) using fresh culture medium. After overnight incubation at 37 °C, 25  $\mu$ l/well dilutions of antibodies were added (15 x 1:3 dilutions in 1xPBS, assay conc. starts at 30 $\mu$ g/ml) to the cells for 2 hours at 4°C. After one washing step using 90  $\mu$ l/well PBST (10x PBS, Roche, #11666789001 + 0,1% Tween 20), cells were subsequently fixed by the addition of 50  $\mu$ l/well 0.05 % glutaraldehyde (Sigma Cat.No: G5882 in 1xPBS) for 10 min at room temperature (RT). After three additional washing steps using 90  $\mu$ l/well PBST, secondary antibodies were added for detection: for human antibodies goat anti-human Ig  $\kappa$  chain antibody HRP conjugate (Millipore #AP502P) diluted 1:2000 in blocking buffer (1x PBS (Roche # 11666789001) + 2% BSA (Bovine Serum Albumin Fraction V, fatty acid free, Roche, # 10735086001) + 0,05% Tween 20) was used (25  $\mu$ l/well). For rat antibodies a mixture of Goat anti-Rat IgG1 Antibody HRP Conjugated (Bethyl #A110-106P), Goat anti-Rat IgG2a Antibody HRP Conjugated (Bethyl #A110-109P) and Goat anti-Rat IgG2b Antibody HRP Conjugated (Bethyl #A110-111P) was used in a 1:10000 dilution of each antibody in blocking buffer (25  $\mu$ l/well). After incubation for 1 h at RT and three additional washing steps using 90  $\mu$ l/well PBST, 25  $\mu$ l/well TMB substrate was added (Roche order no. 11835033001) for 10 min and color development to final ODs was determined by measurement at 370 nm/492 nm.

All tested antibodies showed positive binding to human GPRC5D with  $EC_{50}$  values (reflecting avidity) in the pM range. Only the rat IgGs 10B10 and 07A04 showed cross-reactivity on CHO cells expressing the cynomolgus GPRC5D with  $EC_{50}$  values comparable to the human version of the receptor (Figure 9). Cynomolgus crossreactivity was also detected for all other antibodies but at lower levels compared to 10B10 and 07A04 (Figure 9). No significant binding to CHO cells expressing murine GPRC5D and no binding to CHO cells expressing the human version of GPRC5A was detected (Figure 9). The  $EC_{50}$  values of binding are summarized in Table 2.

**Table 2.** ELISA based binding properties to GPRC5D across species

|              | human GPRC5D+ CHO |                | cyno GPRC5D+ CHO  |                |
|--------------|-------------------|----------------|-------------------|----------------|
| IgG-Antibody | $EC_{50}$ (ng/ml) | $EC_{50}$ (nM) | $EC_{50}$ (ng/ml) | $EC_{50}$ (nM) |

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|       |       |       |        |       |
|-------|-------|-------|--------|-------|
| 5E11  | 29.57 | 0.198 | -      | -     |
| 5F11  | 21.67 | 0.144 | -      | -     |
| 10B10 | 16.34 | 0.109 | 12     | 0,080 |
| 07A04 | 24.26 | 0.162 | 114.54 | 0,764 |

### Example 8

#### GPRC5D binders: recombinant GPRC5D-TCB mediates T cell cytotoxicity on MM cell lines

5 To compare the functionality of the GPRC5D-TCB or other targeted TCBs, we performed an in vitro T cell cytotoxicity assay on multiple MM cell lines: MOLP-2 (Fig. 10B), AMO-1 (Fig. 10C), EJM (Fig. 10D) and NCI-H929 (Fig. 10G). Briefly, cell lines were cultured in RPMI 1640 + Glutamax medium (Gibco) supplemented with 20% Heat-Inactivated Fetal Bovine Serum (FBS; Gibco) and 1% Penicillin – Streptomycin 100X (PS; Gibco). MOLP-2 was cultured with this  
10 medium supplemented with GlutaMax 1X (Gibco). OPM-2 (Fig. 10A), RPMI-8226 (Fig. 10E) and L-363 (Fig. 10F) cell line was cultured with this medium supplemented with only 10% FBS. NCI-H929 was cultured with this medium supplemented with 50  $\mu$ M Mercaptoethanol (Gibco), 1mM Sodium Pyruvate (Gibco) and GlutaMax 1X (Gibco). EJM was cultured in IMDM (Gibco) + 10% FBS (Gibco) and 1% PS (Gibco). All the cell lines were cultured in 75 cm<sup>2</sup> Flask (TPP) with two  
15 passages per week.

Cell lines were co-cultured at Effector to Target ratio of 10 to 1, using 0.3 million allogeneic T cells isolated from PBMCs (Buffy coat from Blutspende Schlieren) using a human Pan T cell Isolation kit (Miltenyi Biotec) in RPMI Medium (Gibco) supplemented with 10% FBS (Gibco) + 1% PS (Gibco). Anti-human GPRC5D TCB construct (5E11-TCB, 5F11-TCB, 10B10-TCB, B72-  
20 TCB, BCMA-TCB and DP47-TCB) were added to the co-culture at different concentration, from 12.5 nM to 0.0000125 nM with serial dilution 1/10 and compared to untreated samples. After 20 hours of incubation at 37 °C with 5% CO<sub>2</sub>, 75  $\mu$ l of supernatant per well were transferred into a 96-well white plate (Greiner bio-one) with 25  $\mu$ l per well of CytoTox-Glo Cytotoxicity Assay (Promega). Luminescence acquisition was performed on the PerkinElmer EnVision after 15 min  
25 incubation at room temperature and analyzed using GraphPad Prism and XL fit software. Data were plotted as the Luminescence signal for LDH release (Figure 10). Figures 10A-G summarizes the data showing that both 5E11-TCB and 5F11-TCB mediated stronger T cell cytotoxicity on the MM cell lines than BCMA-TCB, 10B10-TCB and B72-TCB. The EC<sub>50</sub> of TCB mediated killing

is shown in table 3, and is calculated as average from different experiments with different donor T cells (n=2 or n=3).

**Table 3.** EC<sub>50</sub> values on in vitro killing assay

| EC <sub>50</sub> (pM) | n=3                 |                  |                   |                 | n=2            |                  |                 |
|-----------------------|---------------------|------------------|-------------------|-----------------|----------------|------------------|-----------------|
| Cell lines            | NCI-H929 (Fig. 10G) | AMO-1 (Fig. 10C) | MOLP-2 (Fig. 10B) | L363 (Fig. 10F) | EJM (Fig. 10D) | OPM-2 (Fig. 10A) | RPMI (Fig. 10E) |
| 5F11-TCB              | 4                   | 6                | 1                 | 3               | 3              | 1                | 6               |
| 5E11-TCB              | 7                   | 8                | 18                | 17              | 11             | 2                | 64              |
| 10B10-TCB             | 56                  | 84               | 160               | 34              | 79             | 28               | 965             |
| B72-TCB               | 58                  | 109              | 124               | 58              | 60             | 171              | 193             |
| BCMA-TCB              | 311                 | 518              | 32                | 127             | 132            | 33               | 11              |

5

### Example 9

#### In vitro T cell activation in healthy human bone marrow cells

Fresh unprocessed Bone Marrow of four different healthy donors (Lonza #1M-105, lot 0000739254; 0000739255; 0000739256 and 0000734008) were processed 1 or 2 days after sampling. After a quick red blood cell lysis using BD Pharm Lysis buffer (BD #555899; 1X in sterile water) for 5 minutes at room temperature; cells were washed 2 times by centrifugation and buffer exchange at 126g and 443g respectively. Cells were counted and resuspended at 300 000 cells/mL in RPMI 1640 Glutamax + 20% HI Fetal Bovine Serum + 2% human serum + 1% Penicillin /Streptomycin (all from Gibco) and 100 µL of cell suspension were seeded per well in a 96-well plate round bottom (TPP). 50 µL of medium or medium supplemented with B72-TCB, 5F11-TCB, 5E11-TCB, BCMA-TCB, 10B10-TCB or DP47-TCB from 200 nM (4X) to 20 pM with serial dilution 1/10 were added per well. Finally, 50 µL of allogeneic T cell isolated using Pan T cell (Miltenyi Biotec, # 130-096-535) from healthy donor PBMCs were added at 6 Mio/mL (effector T to healthy bone marrow target cell ratio of 10:1). After overnight incubation at 37 °C in a humidified incubator, cells were washed once with PBS and stained for 20 minutes at 4°C with 50 µL of Live blue (Invitrogen, # L23105) diluted 1/800 in PBS. After a wash, cells were incubated for 30 minutes at 4 °C with the following mix of antibodies diluted in FACs buffer (PBS 1X, 2% Fetal Bovine Serum; 1% 0.5m EDTA PH 8; 0.25% NaN<sub>3</sub> Sodium azide (20%)): CD25 BV605, CD69 APC-Cy7, BCMA BV421, CD38 BV510, CD138 FITC, FcRH5 PE diluted 1/100 and CD8 BV711, CD3 PE-Cy5 and CD4 AlexaFluor 700 diluted 1/300 (all from BioLegend) and GPRC5D

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AlexaFluor 647 (in house, clone 5E11 IgG). After a wash, cells were resuspended in 100  $\mu$ L of FACs buffer and acquired with Fortessa (BD Biosciences).

Data presented in Figures 11A-F illustrate that the B72-TCB induced unspecific activation of T cells (as measured by upregulation of CD69) in the healthy bone marrow, but not by any of the other tested TCBs. As indicated, the unspecific activation induced by the B72-TCB was a concentration dependent effect and more pronounced at 50 nm than at 5 nm (Figures 12A and 12B).

### Example 10

#### In vivo efficacy of TCBs

10 In the efficacy study different TCB constructs (GPRC5D 5F110-TCB, 5E11-TCB, BCMA-TCB and B72-TCB) were compared in terms of tumor regression in multiple myeloma bearing fully humanized NSG mice. NCI-H929 cells were originally obtained from ATCC and OPM-2 cells from DSMZ. Both cell lines were expanded. Cells were cultured in RPMI containing 10% FCS and 2 mM L-Glutamine, 10 mM HEPES, 1 mM Sodiumpyruvate. The cells were cultured at 37 °C  
15 in a water-saturated atmosphere at 5 % CO<sub>2</sub>. 2.5 x10<sup>6</sup> NCI-H929 and 5 x10<sup>6</sup> OPM-2 cells per animal were injected subcutaneously into the right flank of the animals in RPMI cell culture medium (Gibco) and GFR matrigel (1:1, total volume of 100ul) at a viability of > 95.0%.

Female NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice, age 4-5 weeks at start of the experiment (bred at Charles River, Lyon, France) were maintained under specific-pathogen-free condition  
20 with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (ROB-55.2-2532.Vet\_03-16-10). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

25 According to the protocol, female NSG mice were injected i.p. (intraperitoneal) with 15 mg/kg of Busulfan followed one day later by an i.v. injection of 1x10<sup>5</sup> human hematopoietic stem cells isolated from cord blood. At week 16-20 after stem cell injection mice were bled and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups  
30 (n=10/group). At that time, mice were injected with tumor cells subcutaneously. as described above and treated once weekly with the compounds or PBS (Vehicle) when tumor size reached

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approximately 200 mm<sup>3</sup>. All mice were injected intravenously with different doses of TCB molecules (see Figures 13A-D and 14A-D).

To obtain the appropriate amount of compounds stock solutions were diluted with Histidine buffer (20 mM histidine, 140 mM NaCl, pH 6.0). Tumor growth was measured twice weekly using a caliper and tumor volume was calculated as followed:

$$T_v: (W^2/2) \times L \quad (W: \text{Width}, L: \text{Length})$$

The study was terminated and all mice were sacrificed after four injections of the compounds and tumors were explanted and weighted.

Figures 13A-D show the tumor growth kinetics in all animals, which had received NCI-H929 injections, after the treatment. 5F11-TCB induced complete tumor remission in all animals at either 1 mg/kg or 0.1 mg/kg (Fig. 13A), whereas B72-TCB only induced partial tumor remission when used at 1 mg/kg, with no effect at 0.1 mg/kg (Fig. 13C). BCMA-TCB also induced partial tumor remission at 1 mg/kg (Fig. 13B).

Figures 14A-D show the tumor growth kinetics in all animals, which had received OPM-2 injections, after the treatment. 5F11-TCB (Fig. 14A, top panel) and 5E11-TCB (Fig. 14B, top panel) induced complete tumor remission in the majority of animals at 0.1 mg/kg whereas B72-TCB (Fig. 14C, top panel) at 0.1 mg/kg was less potent in controlling tumor growth. At 0.01 mg/kg 5F11-TCB (Fig. 14A, bottom panel) and 5E11-TCB (Fig. 14B, bottom panel) were more potent in inhibiting tumor growth as compared to B72-TCB (Fig. 14C, bottom panel).

## Example 11

### Humanization of anti-GPRC5D antibodies

Suitable human acceptor frameworks were identified by querying a BLASTp database of human V- and J-region sequences for the murine input sequences (cropped to the variable part). Selective criteria for the choice of human acceptor framework were sequence homology, same or similar CDR lengths, and the estimated frequency of the human germline, but also the conservation of certain amino acids at the VH-VL domain interface. Following the germline identification step, the CDRs of the murine input sequences were grafted onto the human acceptor framework regions. Each amino acid difference between these initial CDR grafts and the parental antibodies was rated for possible impact on the structural integrity of the respective variable region, and “back mutations” towards the parental sequence were introduced whenever deemed appropriate. The

structural assessment was based on Fv region homology models of both the parental antibody and the humanization variants, created with an in-house antibody structure homology modeling protocol implemented using the BIOVIA Discovery Studio Environment, version 17R2. In some humanization variants, “forward mutations” were included, i.e., amino acid exchanges that change the original amino acid occurring at a given CDR position of the parental binder to the amino acid found at the equivalent position of the human acceptor germline. The aim is to increase the overall human character of the humanization variants (beyond the framework regions) to further reduce the immunogenicity risk.

An *in silico* tool developed in-house was used to predict the VH-VL domain orientation of the paired VH and VL humanization variants (as WO 2016/062734A1, which is incorporated by reference in its entirety). The results were compared to the predicted VH-VL domain orientation of the parental binders to select for framework combinations which are close in geometry to the original antibodies. The rationale is to detect possible amino acid exchanges in the VH-VL interface region that might lead to disruptive changes in the pairing of the two domains that in turn might have detrimental effects on the binding properties.

#### Choice of acceptor framework and adaptations thereof for the GPRC5D binder 5E11

The acceptor frameworks were chosen according to the following table 4.

**Table 4.** Acceptor frameworks for the GPRC5D binder 5E11

|                | <b>Murine (<i>Rattus norvegicus</i>)<br/>V-region germline</b> | <b>Choice of human acceptor<br/>V-region germline</b> |
|----------------|----------------------------------------------------------------|-------------------------------------------------------|
| <b>VH1abcd</b> | IGHV5S13*01                                                    | IGHV3-23*03                                           |
| <b>VL1ac</b>   | IGKV3S18*01                                                    | IGKV4-1*01_human                                      |
| <b>VL2ab</b>   |                                                                | IGKV3-20*01_human                                     |
| <b>VL3ab</b>   |                                                                | IGKV1-39*01_human                                     |

Post-CDR3 framework regions were adapted from human IGHJ germline IGHJ3\*02 (DAFDIWGQGTMVTVSS) and human IGKJ germline IGKJ5\*01 (ITFGQGTRLEIK). The part relevant for the acceptor framework is indicated in bold script.

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Based on structural considerations, back mutations from the human acceptor framework to the amino acid in the parental binder were introduced at certain positions of the 5E11 humanization variants (Table 5 and 6). Furthermore, some positions were identified as promising candidates for forward mutations, where the amino acid in a CDR of the parental binder is substituted by the amino acid found in the human acceptor germline. The changes are detailed in the table below.

Note: Back mutations are prefixed with **b**, forward mutations with **f**, e.g., bS49A refers to a back mutation (human germline amino acid to parental antibody amino acid) from serine to alanine at position 49. All residue indices given in Kabat numbering.

**Table 5.** List of VH/VL 5E11 humanization variants

| Variant Name                                   | Identity to human V-region germline (BLASTp) |
|------------------------------------------------|----------------------------------------------|
| 5E11_VH1a (bS49A_bK94T)                        | 89.7                                         |
| 5E11_VH1b (bV2L_bS49A_bS74A_bK94T)             | 87.6                                         |
| 5E11_VH1c (bS49A_fR60A_fA65G_bK94T)            | 91.8                                         |
| 5E11_VH1d (bV2L_bS49A_fR60A_fA65G_bS74A_bK94T) | 89.7                                         |
| 5E11_VL1a (bP43Q)                              | 80.2                                         |
| 5E11_VL1c (fR24K_fA25S_bP43Q)                  | 82.2                                         |
| 5E11_VL2a (bA43Q)                              | 86.2                                         |
| 5E11_VL2b (bA43Q_bR45K)                        | 85.1                                         |
| 5E11_VL3a (bA43Q)                              | 83.8                                         |
| 5E11_VL3b (bK42Q_bA43Q)                        | 82.8                                         |

10

**Table 6.** Sequences of VH/VL 5E11 humanization variants

| name      | aa sequence                                                                                                                     | SEQ ID NO. |
|-----------|---------------------------------------------------------------------------------------------------------------------------------|------------|
| 5E11_VH1a | EVQLLES GGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDN SKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWQGQTMVTVSS | 46         |

|           |                                                                                                                             |    |
|-----------|-----------------------------------------------------------------------------------------------------------------------------|----|
| 5E11_VH1b | ELQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYRDSVKARFTISRDNKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGTMTVSS | 47 |
| 5E11_VH1c | EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGTMTVSS | 48 |
| 5E11_VH1d | ELQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWV<br>ASISTGGVNTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCA<br>THTGDYFDYWGQGTMTVSS | 49 |
| 5E11_VL1a | DIVMTQSPDSLAVSLGERATINCRASQSVSISGINLMNWHYQQKPGQPKL<br>LIYHASILASGVPRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRESPLTF<br>GQGTREIK       | 50 |
| 5E11_VL1c | DIVMTQSPDSLAVSLGERATINCKSSQSVSISGINLMNWHYQQKPGQPKL<br>LIYHASILASGVPRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRESPLTF<br>GQGTREIK       | 51 |
| 5E11_VL2a | EIVLTQSPGTLSPGERATLSCRASQSVSISGINLMNWHYQQKPGQPKLLI<br>YHASILASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIK       | 52 |
| 5E11_VL2b | EIVLTQSPGTLSPGERATLSCRASQSVSISGINLMNWHYQQKPGQPKLLI<br>YHASILASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQ<br>GTRLEIK       | 53 |
| 5E11_VL3a | DIQMTQSPSSLSASVGRVTITCRASQSVSISGINLMNWHYQQKPGKPKLL<br>IYHASILASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTRESPLTFG<br>QGTREIK        | 54 |
| 5E11_VL3b | DIQMTQSPSSLSASVGRVTITCRASQSVSISGINLMNWHYQQKPGQPKLL<br>IYHASILASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTRESPLTFG<br>QGTREIK        | 55 |

### Choice of acceptor framework and adaptations thereof for the GPRC5D binder 5F11

The acceptor frameworks were chosen according to the following table 7.

**Table 7.** Acceptor frameworks for the GPRC5D binder 5F11

|                | Murine ( <i>Rattus norvegicus</i> ) V-region<br>germline | Choice of human acceptor V-region<br>germline |
|----------------|----------------------------------------------------------|-----------------------------------------------|
| <b>VH1abcd</b> | IGHV5S13*01                                              | IGHV3-30*03                                   |
| <b>VH2bd</b>   |                                                          | IGHV3-23*04                                   |

|                    |             |             |
|--------------------|-------------|-------------|
| <b>VL1ab, VL2a</b> | IGKV2S17*01 | IGKV2-28*01 |
| <b>VL2b</b>        |             | IGKV4-1*01  |
| <b>VL2c</b>        |             | IGKV3-20*01 |

Post-CDR3 framework regions were adapted from human IGHJ germline IGHJ3\*02 (DAFDIWGQGTMVTVSS) and human IGKJ germline IGKJ2\*01 (YTFGQGTKLEIK). The part relevant for the acceptor framework is indicated in bold script.

Based on structural considerations, back mutations from the human acceptor framework to the amino acid in the parental binder were introduced at certain positions of the 5F11 humanization variants (Table 8 and 9). Furthermore, some positions were identified as promising candidates for forward mutations, where the amino acid in a CDR of parental binder is substituted by the amino acid found in the human acceptor germline. The changes are detailed in the table below.

Note: Back mutations are prefixed with **b**, forward mutations with **f**, e.g., bA93T refers to a back mutation (human germline amino acid to parental antibody amino acid) from alanine to threonine at position 93. All residue indices given in Kabat numbering.

**Table 8.** List of VH/VL 5F11 humanization variants

| Variant Name                              | Identity to human V-region germline (BLASTp) |
|-------------------------------------------|----------------------------------------------|
| 5F11_VH1a (bA93T)                         | 89.8                                         |
| 5F11_VH1b (bQ1E_bS74A_bA93T)              | 87.8                                         |
| 5F11_VH1c (fR60A_bA93T)                   | 90.8                                         |
| 5F11_VH1d (bQ1E_fR60A_bS74A_bA93T)        | 88.8                                         |
| 5F11_VH2b (bS49A_bS74A_bA93T_bK94R)       | 86.7                                         |
| 5F11_VH2d (bS49A_fR60A_bS74A_bA93T_bK94R) | 87.8                                         |
| 5F11_VL1a (bL46V_bY87H)                   | 86.0                                         |
| 5F11_VL1b (bQ42K_bL46V_bY87H)             | 85.0                                         |
| 5F11_VL2a (bY87H)                         | 88.0                                         |
| 5F11_VL2b (bY87H)                         | 80.2                                         |

|                         |      |
|-------------------------|------|
| 5F11_VL2c (fS25A_bY87H) | 80.0 |
|-------------------------|------|

**Table 9.** Sequences of VH/VL 5F11 humanization variants

| variant   | aa sequence                                                                                                                  | SEQ ID NO. |
|-----------|------------------------------------------------------------------------------------------------------------------------------|------------|
| 5F11_VH1a | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTR<br>HDRGGLYWGGQTMVTSS | 56         |
| 5F11_VH1b | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTSS | 57         |
| 5F11_VH1c | QVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTSS | 58         |
| 5F11_VH1d | EVQLVESGGGVVQPGRSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTSS | 59         |
| 5F11_VH2b | EVQLVESGGGLVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYRDSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTSS | 60         |
| 5F11_VH2d | EVQLVESGGGLVQPGGSLRLSCAASGFSFSNYGMAWVRQAPGKGLEWV<br>ASISTGGGNTYYADSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVYYCT<br>RHDRGGLYWGGQTMVTSS | 61         |
| 5F11_VL1a | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGQSPQV<br>LIYRMSNLA SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK     | 62         |
| 5F11_VL1b | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGKSPQV<br>LIYRMSNLA SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK     | 63         |
| 5F11_VL2a | DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSNGITYVYWYLQKPGQSPQL<br>LIYRMSNRA SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYHCGQLLENPY<br>TFGQGTKLEIK     | 64         |
| 5F11_VL2b | DIVMTQSPDSLAVSLGERATINCKSSKSLLSNGITYVYWYQKPGQPPK<br>LLIYRMSNLA SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYHCGQLLENPY<br>TFGQGTKLEIK      | 65         |

|           |                                                                                                                        |    |
|-----------|------------------------------------------------------------------------------------------------------------------------|----|
| 5F11_VL2c | EIVLTQSPGTLSPGERATLSCRASKSLLSHNGITYVYVYQQKPGQAPRL<br>LIYRMSNLAAGIPDRFSGSGSGTDFTLTISRLEPEDFAVYHCGQLLENPYTF<br>GQGTELEIK | 66 |
|-----------|------------------------------------------------------------------------------------------------------------------------|----|

### Characterization of humanization variants by ELISA

For the characterization of the humanization variants of the VH and VL domains of the GPCR5D binders the ELISA protocol as described above was used (see Example 7). The data are

- 5 summarized in the table 10 for the humanization variants of 5E11 and in table 11 for the humanization variants of 5F11. Table 12 shows CDR sequences of the parental 5E11 and parental 5E11 and of selected humanization variants.

**Table 10.** Characterization of humanization variants of 5E11

| Tapir           | Sort     | Humanness       |                 |         |         |         | CHO hu GPCR5D       |                  | CHO cy GPCR5D       |
|-----------------|----------|-----------------|-----------------|---------|---------|---------|---------------------|------------------|---------------------|
|                 |          | VH              | VL              | VH huID | VL huID | Fv huID | EC/IC50 rel (ng/ml) | EC/IC50 rel (nM) | EC/IC50 rel (ng/ml) |
| <b>P1AE5706</b> | <b>1</b> | <b>parental</b> | <b>parental</b> |         |         |         | <b>10,4</b>         | <b>0,07</b>      | <b>na</b>           |
| P1AE5707        | 2        | VH1a            | VL1a            | 89,7    | 80,2    | 84,95   | 14,2                | 0,09             | na                  |
| P1AE5708        | 3        | VH1a            | VL1c            | 89,7    | 82,2    | 85,95   | 12,0                | 0,08             | na                  |
| P1AE5709        | 4        | VH1a            | VL2a            | 89,7    | 86,2    | 87,95   | 19,1                | 0,13             | na                  |
| P1AE5710        | 5        | VH1a            | VL2b            | 89,7    | 85,1    | 87,4    | 10,1                | 0,07             | na                  |
| P1AE5712        | 6        | VH1a            | VL3a            | 89,7    | 83,8    | 86,75   | 13,1                | 0,09             | na                  |
| P1AE5713        | 7        | VH1a            | VL3b            | 89,7    | 82,8    | 86,25   | 16,5                | 0,11             | na                  |
| P1AE5714        | 8        | VH1b            | VL1a            | 87,6    | 80,2    | 83,9    | 12,9                | 0,09             | na                  |
| P1AE5715        | 9        | VH1b            | VL1c            | 87,6    | 82,2    | 84,9    | 21,1                | 0,14             | na                  |
| P1AE5716        | 10       | VH1b            | VL2a            | 87,6    | 86,2    | 86,9    | 15,1                | 0,10             | na                  |
| P1AE5717        | 11       | VH1b            | VL2b            | 87,6    | 85,1    | 86,35   | 13,9                | 0,09             | na                  |
| P1AE5718        | 12       | VH1b            | VL3a            | 87,6    | 83,8    | 85,7    | 12,1                | 0,08             | na                  |
| P1AE5719        | 13       | VH1b            | VL3b            | 87,6    | 82,8    | 85,2    | 16,6                | 0,11             | na                  |
| P1AE5720        | 14       | VH1c            | VL1a            | 91,8    | 80,2    | 86      | 21,7                | 0,14             | na                  |
| P1AE5721        | 15       | VH1c            | VL1c            | 91,8    | 82,2    | 87      | 18,3                | 0,12             | na                  |
| P1AE5722        | 16       | VH1c            | VL2a            | 91,8    | 86,2    | 89      | 19,7                | 0,13             | na                  |
| P1AE5723        | 17       | VH1c            | VL2b            | 91,8    | 85,1    | 88,45   | 6,0                 | 0,04             | na                  |
| P1AE5724        | 18       | VH1c            | VL3a            | 91,8    | 83,8    | 87,8    | 5,3                 | 0,04             | na                  |
| P1AE5725        | 19       | VH1c            | VL3b            | 91,8    | 82,8    | 87,3    | 5,1                 | 0,03             | na                  |
| P1AE5726        | 20       | VH1d            | VL1a            | 89,7    | 80,2    | 84,95   | 7,6                 | 0,05             | na                  |
| P1AE5727        | 21       | VH1d            | VL1c            | 89,7    | 82,2    | 85,95   | 8,7                 | 0,06             | na                  |
| P1AE5728        | 22       | VH1d            | VL2a            | 89,7    | 86,2    | 87,95   | 7,9                 | 0,05             | na                  |
| P1AE5729        | 23       | VH1d            | VL2b            | 89,7    | 85,1    | 87,4    | 10,4                | 0,07             | na                  |
| P1AE5730        | 24       | VH1d            | VL3a            | 89,7    | 83,8    | 86,75   | 8,0                 | 0,05             | na                  |
| P1AE5731        | 25       | VH1d            | VL3b            | 89,7    | 82,8    | 86,25   | 5,2                 | 0,03             | na                  |

**Table 10.** Continued

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| Tapir           | Sort     | Humanness       |                 |            |            |            | CHO hu<br>GPCR5A       | CAR-J2 EC50 |
|-----------------|----------|-----------------|-----------------|------------|------------|------------|------------------------|-------------|
|                 |          | VH              | VL              | VH<br>huID | VL<br>huID | Fv<br>huID | EC/IC50 rel<br>(ng/ml) | [ng/mL]     |
| <b>PIAE5706</b> | <b>1</b> | <b>parental</b> | <b>parental</b> |            |            |            | <b>na</b>              | <b>3,9</b>  |
| PIAE5707        | 2        | VH1a            | VL1a            | 89,7       | 80,2       | 84,95      | na                     | 42,4        |
| PIAE5708        | 3        | VH1a            | VL1c            | 89,7       | 82,2       | 85,95      | na                     | 455,8       |
| PIAE5709        | 4        | VH1a            | VL2a            | 89,7       | 86,2       | 87,95      | na                     | 59,4        |
| PIAE5710        | 5        | VH1a            | VL2b            | 89,7       | 85,1       | 87,4       | na                     | 3,5         |
| PIAE5712        | 6        | VH1a            | VL3a            | 89,7       | 83,8       | 86,75      | na                     | 16,1        |
| PIAE5713        | 7        | VH1a            | VL3b            | 89,7       | 82,8       | 86,25      | na                     | 76,9        |
| PIAE5714        | 8        | VH1b            | VL1a            | 87,6       | 80,2       | 83,9       | na                     | 5,5         |
| PIAE5715        | 9        | VH1b            | VL1c            | 87,6       | 82,2       | 84,9       | na                     | 3,6         |
| PIAE5716        | 10       | VH1b            | VL2a            | 87,6       | 86,2       | 86,9       | na                     | 3,3         |
| PIAE5717        | 11       | VH1b            | VL2b            | 87,6       | 85,1       | 86,35      | na                     | 79,9        |
| PIAE5718        | 12       | VH1b            | VL3a            | 87,6       | 83,8       | 85,7       | na                     | 105,4       |
| PIAE5719        | 13       | VH1b            | VL3b            | 87,6       | 82,8       | 85,2       | na                     | 2,8         |
| PIAE5720        | 14       | VH1c            | VL1a            | 91,8       | 80,2       | 86         | na                     | 6,3         |
| PIAE5721        | 15       | VH1c            | VL1c            | 91,8       | 82,2       | 87         | na                     | 25          |
| PIAE5722        | 16       | VH1c            | VL2a            | 91,8       | 86,2       | 89         | na                     | 4,6         |
| PIAE5723        | 17       | VH1c            | VL2b            | 91,8       | 85,1       | 88,45      | na                     | 3,7         |
| PIAE5724        | 18       | VH1c            | VL3a            | 91,8       | 83,8       | 87,8       | na                     | 3,6         |
| PIAE5725        | 19       | VH1c            | VL3b            | 91,8       | 82,8       | 87,3       | na                     | 10,9        |
| PIAE5726        | 20       | VH1d            | VL1a            | 89,7       | 80,2       | 84,95      | na                     | 37,8        |
| PIAE5727        | 21       | VH1d            | VL1c            | 89,7       | 82,2       | 85,95      | na                     | 6,3         |
| PIAE5728        | 22       | VH1d            | VL2a            | 89,7       | 86,2       | 87,95      | na                     | 5,6         |
| PIAE5729        | 23       | VH1d            | VL2b            | 89,7       | 85,1       | 87,4       | na                     | 61,3        |
| PIAE5730        | 24       | VH1d            | VL3a            | 89,7       | 83,8       | 86,75      | na                     | 3,5         |
| PIAE5731        | 25       | VH1d            | VL3b            | 89,7       | 82,8       | 86,25      | na                     | 2,3         |

**Table 11.** Characterization of humanization variants of 5F11

|                 | Sort     | Humanness       |                 |         |         |         | CHO hu GPCR5D          |                  |
|-----------------|----------|-----------------|-----------------|---------|---------|---------|------------------------|------------------|
|                 |          | VH              | VL              | VH huID | VL huID | Fv huID | EC/IC50 rel<br>(ng/ml) | EC/IC50 rel (nM) |
| <b>PIAE5733</b> | <b>1</b> | <b>parental</b> | <b>parental</b> |         |         |         | <b>5,8</b>             | <b>0,04</b>      |
| PIAE5734        | 2        | VH1a            | VL1a            | 89,8    | 86      | 87,9    | 5,5                    | 0,04             |
| PIAE5735        | 3        | VH1a            | VL1b            | 89,8    | 85      | 87,4    | 6,6                    | 0,04             |
| PIAE5736        | 4        | VH1a            | VL2a            | 89,8    | 88      | 88,9    | 3,7                    | 0,02             |
| PIAE5737        | 5        | VH1a            | VL2b            | 89,8    | 80,2    | 85      | 5,9                    | 0,04             |
| PIAE5738        | 6        | VH1a            | VL2c            | 89,8    | 80      | 84,9    | 4,1                    | 0,03             |
| PIAE5739        | 7        | VH1b            | VL1a            | 90,8    | 86      | 88,4    | 4,0                    | 0,03             |
| PIAE5740        | 8        | VH1b            | VL1b            | 90,8    | 85      | 87,9    | 6,3                    | 0,04             |
| PIAE5741        | 9        | VH1b            | VL2a            | 90,8    | 88      | 89,4    | 6,7                    | 0,04             |
| PIAE5742        | 10       | VH1b            | VL2b            | 90,8    | 80,2    | 85,5    | 6,1                    | 0,04             |
| PIAE5743        | 11       | VH1b            | VL2c            | 90,8    | 80      | 85,4    | 7,6                    | 0,05             |
| PIAE5744        | 12       | VH1c            | VL1a            | 90,8    | 86      | 88,4    | 8,6                    | 0,06             |
| PIAE5745        | 13       | VH1c            | VL1b            | 90,8    | 85      | 87,9    | 9,7                    | 0,06             |
| PIAE5746        | 14       | VH1c            | VL2a            | 90,8    | 88      | 89,4    | 10,7                   | 0,07             |
| PIAE5747        | 15       | VH1c            | VL2b            | 90,8    | 80,2    | 85,5    | 9,0                    | 0,06             |

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|          |    |      |      |      |      |      |      |      |
|----------|----|------|------|------|------|------|------|------|
| P1AE5749 | 16 | VH1c | VL2c | 90,8 | 80   | 85,4 | 7,4  | 0,05 |
| P1AE5750 | 17 | VH1d | VL1a | 89,8 | 86   | 87,9 | 9,4  | 0,06 |
| P1AE5751 | 18 | VH1d | VL1b | 89,8 | 85   | 87,4 | 12,4 | 0,08 |
| P1AE5752 | 19 | VH1d | VL2a | 89,8 | 88   | 88,9 | 6,2  | 0,04 |
| P1AE5753 | 20 | VH1d | VL2b | 89,8 | 80,2 | 85   | 10,4 | 0,07 |
| P1AE5754 | 21 | VH1d | VL2c | 89,8 | 80   | 84,9 | 9,0  | 0,06 |
| P1AE5755 | 22 | VH2b | VL1a | 90,8 | 86   | 88,4 | 7,8  | 0,05 |
| P1AE5756 | 23 | VH2b | VL1b | 90,8 | 85   | 87,9 | 7,8  | 0,05 |
| P1AE5757 | 24 | VH2b | VL2a | 90,8 | 88   | 89,4 | 2,9  | 0,02 |
| P1AE5758 | 25 | VH2b | VL2b | 90,8 | 80,2 | 85,5 | 2,6  | 0,02 |
| P1AE5759 | 26 | VH2b | VL2c | 90,8 | 80   | 85,4 | 3,1  | 0,02 |
| P1AE5760 | 27 | VH2d | VL1a | 89,8 | 86   | 87,9 | 4,0  | 0,03 |
| P1AE5761 | 28 | VH2d | VL1b | 89,8 | 85   | 87,4 | 3,7  | 0,02 |
| P1AE5762 | 29 | VH2d | VL2a | 89,8 | 88   | 88,9 | 4,6  | 0,03 |
| P1AE5763 | 30 | VH2d | VL2b | 89,8 | 80,2 | 85   | 6,0  | 0,04 |
| P1AE5764 | 31 | VH2d | VL2c | 89,8 | 80   | 84,9 | 4,5  | 0,03 |

Table 11. Continued.

|                 |          | Humanness       |                 |         |            |         | CHO cy<br>GPCR5D       | CHO hu<br>GPCR5A       | CAR-J2<br>EC50 |
|-----------------|----------|-----------------|-----------------|---------|------------|---------|------------------------|------------------------|----------------|
|                 | Sort     | VH              | VL              | VH huID | VL<br>huID | Fv huID | EC/IC50<br>rel (ng/ml) | EC/IC50 rel<br>(ng/ml) | [ng/mL]        |
| <b>P1AE5733</b> | <b>1</b> | <b>parental</b> | <b>parental</b> |         |            |         | <b>na</b>              | <b>na</b>              | <b>2,65</b>    |
| P1AE5734        | 2        | VH1a            | VL1a            | 89,8    | 86         | 87,9    | na                     | na                     | 41,53          |
| P1AE5735        | 3        | VH1a            | VL1b            | 89,8    | 85         | 87,4    | na                     | na                     | 82,08          |
| P1AE5736        | 4        | VH1a            | VL2a            | 89,8    | 88         | 88,9    | na                     | na                     | 1,38           |
| P1AE5737        | 5        | VH1a            | VL2b            | 89,8    | 80,2       | 85      | na                     | na                     | 2,95           |
| P1AE5738        | 6        | VH1a            | VL2c            | 89,8    | 80         | 84,9    | na                     | na                     | 397,43         |
| P1AE5739        | 7        | VH1b            | VL1a            | 90,8    | 86         | 88,4    | na                     | na                     | 23,54          |
| P1AE5740        | 8        | VH1b            | VL1b            | 90,8    | 85         | 87,9    | na                     | na                     | 8,96           |
| P1AE5741        | 9        | VH1b            | VL2a            | 90,8    | 88         | 89,4    | na                     | na                     | 1,4            |
| P1AE5742        | 10       | VH1b            | VL2b            | 90,8    | 80,2       | 85,5    | na                     | na                     | 61,93          |
| P1AE5743        | 11       | VH1b            | VL2c            | 90,8    | 80         | 85,4    | na                     | na                     | 583,32         |
| P1AE5744        | 12       | VH1c            | VL1a            | 90,8    | 86         | 88,4    | na                     | na                     | 3,63           |
| P1AE5745        | 13       | VH1c            | VL1b            | 90,8    | 85         | 87,9    | na                     | na                     | 1,62           |
| P1AE5746        | 14       | VH1c            | VL2a            | 90,8    | 88         | 89,4    | na                     | na                     | 514,19         |
| P1AE5747        | 15       | VH1c            | VL2b            | 90,8    | 80,2       | 85,5    | na                     | na                     | 182,79         |
| P1AE5749        | 16       | VH1c            | VL2c            | 90,8    | 80         | 85,4    | na                     | na                     | 82,59          |
| P1AE5750        | 17       | VH1d            | VL1a            | 89,8    | 86         | 87,9    | na                     | na                     | 20,58          |
| P1AE5751        | 18       | VH1d            | VL1b            | 89,8    | 85         | 87,4    | na                     | na                     | 6,44           |
| P1AE5752        | 19       | VH1d            | VL2a            | 89,8    | 88         | 88,9    | na                     | na                     | 508,96         |
| P1AE5753        | 20       | VH1d            | VL2b            | 89,8    | 80,2       | 85      | na                     | na                     | 30,03          |
| P1AE5754        | 21       | VH1d            | VL2c            | 89,8    | 80         | 84,9    | na                     | na                     | 8,89           |
| P1AE5755        | 22       | VH2b            | VL1a            | 90,8    | 86         | 88,4    | na                     | na                     | 151,74         |
| P1AE5756        | 23       | VH2b            | VL1b            | 90,8    | 85         | 87,9    | na                     | na                     | 170,2          |
| P1AE5757        | 24       | VH2b            | VL2a            | 90,8    | 88         | 89,4    | na                     | na                     | 144,74         |
| P1AE5758        | 25       | VH2b            | VL2b            | 90,8    | 80,2       | 85,5    | na                     | na                     | 189,51         |
| P1AE5759        | 26       | VH2b            | VL2c            | 90,8    | 80         | 85,4    | na                     | na                     | 15,7           |
| P1AE5760        | 27       | VH2d            | VL1a            | 89,8    | 86         | 87,9    | na                     | na                     | 189,94         |
| P1AE5761        | 28       | VH2d            | VL1b            | 89,8    | 85         | 87,4    | na                     | na                     | 74,56          |

|          |    |      |      |      |      |      |    |    |       |
|----------|----|------|------|------|------|------|----|----|-------|
| P1AE5762 | 29 | VH2d | VL2a | 89,8 | 88   | 88,9 | na | na | 84,16 |
| P1AE5763 | 30 | VH2d | VL2b | 89,8 | 80,2 | 85   | na | na | 5,47  |
| P1AE5764 | 31 | VH2d | VL2c | 89,8 | 80   | 84,9 | na | na | 78,22 |

**Table 12.** CDR sequences of a selection of humanization variants

|               | HCDR1           | HCDR2           | HCDR3           | LCDR1           | LCDR1           | LCDR3           |
|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 5E11 parental | SEQ ID<br>NO:83 | SEQ ID<br>NO:84 | SEQ ID<br>NO:86 | SEQ ID<br>NO:87 | SEQ ID<br>NO:88 | SEQ ID<br>NO:89 |
| 5E11_P1AE5723 | SEQ ID<br>NO:83 | SEQ ID<br>NO:85 | SEQ ID<br>NO:86 | SEQ ID<br>NO:87 | SEQ ID<br>NO:88 | SEQ ID<br>NO:89 |
| 5E11_P1AE5728 | SEQ ID<br>NO:83 | SEQ ID<br>NO:85 | SEQ ID<br>NO:86 | SEQ ID<br>NO:87 | SEQ ID<br>NO:88 | SEQ ID<br>NO:89 |
| 5F11 parental | SEQ ID<br>NO:90 | SEQ ID<br>NO:91 | SEQ ID<br>NO:93 | SEQ ID<br>NO:94 | SEQ ID<br>NO:95 | SEQ ID<br>NO:97 |
| 5F11_P1AE5741 | SEQ ID<br>NO:90 | SEQ ID<br>NO:91 | SEQ ID<br>NO:93 | SEQ ID<br>NO:94 | SEQ ID<br>NO:96 | SEQ ID<br>NO:97 |
| 5F11_P1AE5745 | SEQ ID<br>NO:90 | SEQ ID<br>NO:92 | SEQ ID<br>NO:93 | SEQ ID<br>NO:94 | SEQ ID<br>NO:95 | SEQ ID<br>NO:97 |

**Example 12****5 In vitro activation of CAR-J cells in presence of different humanization variants of selected anti-GPRC5D IgGs**

- The capacity of the different humanized anti-GPRC5D IgGs to activate PGLALA-CAR-J effector cells was assessed as described in the following. GPRC5D-expressing multiple
- 10 Myeloma target cells L363 (Diehl et al., Blut 36: 331-338 (1978)) were co-cultured with anti-PGLALA-CAR-J effector cells (Jurkat-NFAT human acute lymphatic leukemia reporter cell line expressing a TCR directed against the PGLALA (P329G L234A L235A) mutation in the Fc part of IgG molecules and containing a NFAT promoter, as disclosed in PCT application no PCT/EP2018/086038 and PCT application No. PCT/EP2018/086067. Upon simultaneous
- 15 binding of the IgG molecule to the GPRC5D on L363 cells and PGLALA-CAR-J cells, the NFAT promoter is activated and leads to expression of active firefly luciferase.
- For the assay, the humanized IgG variants were diluted in RPMI 1640 medium (containing Glutamax, 15% HI Fetal Bovine Serum, 1% Penicillin-Streptomycin; all from GIBCO) and transferred into round-bottom-96 well plates (final concentration range of 0.2 pg/ml till 10 µg/ml).
- 20 20 000 L363 cells per well and anti-PGLALA-CAR-J effector cells were added to obtain a final effector (anti-PGLALA-CAR-J) to target (L363) cell ratio of 5:1 and a final volume of 200 µl per well. Cells were incubated for roughly 16 h at 37°C in a humidified incubator. At the end of the incubation time, 100 µl/well of the supernatant were transferred to a white flat bottom 96-well plate (Costar) and incubated with another 100 µl/well of ONE-Glo luciferase substrate (Promega)

for 5 min before luminescence was read using PerkinElmer Envision. The row data was plotted as relative luminescence signals (RLUs) against the IgG concentration using GraphPad Prism and the EC<sub>50</sub> were calculated using XL-fit software.

As shown in Figures 15A-B and Table 13, all evaluated GPRC5D IgGs induce CAR-J activation upon simultaneous binding to GPRC5D-expressing target cells and anti-PGLALA-CAR-J cells. For both anti-GPRC5D binder 5F11 and 5E11, humanization variants could be identified with similar or even improved EC<sub>50</sub> values as compared to parental antibodies pre-humanization. For binder 5F11, the strongest activation could be induced by molecule P1AE5741 (Fig. 15A). For binder 5E11, the strongest activation could be induced by molecule P1AE5730 and P1AE5723 (Fig. 15B).

**Table 13.** EC<sub>50</sub> values of CAR-J activation

|                             | Binder 5F11                |              |              |              |              | Binder 5E11                |              |              |              |              |
|-----------------------------|----------------------------|--------------|--------------|--------------|--------------|----------------------------|--------------|--------------|--------------|--------------|
|                             | P1AE573<br>3<br>(parental) | P1AE574<br>1 | P1AE574<br>5 | P1AE574<br>4 | P1AE576<br>3 | P1AE570<br>6<br>(parental) | P1AE572<br>3 | P1AE572<br>8 | P1AE573<br>0 | P1AE571<br>8 |
| EC <sub>50</sub><br>(ng/ml) | 2.65                       | 1.4          | 1.62         | 3.63         | 5.47         | 3.9                        | 3.7          | 5.6          | 3.5          | 105.4        |

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### Example 13

#### Preparation of further T cell bispecific antibodies

The principles of converting binders into T cell bispecific antibodies are exemplified and described in the art, e.g. in PCT publication no. WO 2014/131712 A1, which is incorporated herein by reference in its entirety. The T cell bispecific antibodies comprise two GPRC5D-binding moieties and one CD3-binding moiety (anti-GPRC5D/anti-CD3 T cell bispecific antibodies) as illustrated in Figure 3. The following anti-GPRC5D/anti-CD3 T cell bispecific antibodies were prepared: i) 6623 (SEQ ID NOs 114, 115, 116 and 117); ii) 6624 (SEQ ID NOs 118, 119, 120 and 121); iii) 6625 (SEQ ID NOs 122, 123, 124 and 125); iv) 6626 (SEQ ID NOs: 126, 127, 128 and 129). DP47-TCB (the “untargeted TCB”) is described in PCT publication no. WO 2014/131712 A1, which is incorporated herein by reference in its entirety. The B72 TCB derives from the GCDB72 antibody disclosed in Table 23 of WO 2018/0117786 A2 and comprises the GPRC5D binding

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moiety of GCDB72 (Example 7). The term “B72 TCB” also refers to the term “B72” herein. The BCMA-TCB derives from WO 2016/166629 A1 and comprises the GPRC5D binding moiety of A02\_Rd4\_6nM\_C01 as disclosed therein. BCMA-TCB was generated in the crossmab 2+1 Format (SEQ ID Nos 77, 78, 79, 80) as described for Example 2. The terms “5F11-TCB” and “5F11p-CH2527” are used interchangeable herein. The terms “5E11-TCB” and “5E11p-CH2527” are used interchangeable herein.

### Example 14.1

#### Binding of T cell bispecific antibodies to multiple myeloma cell lines and Jurkat-NFAT cells

To measure the binding to GPRC5D, we performed a flow cytometry-based binding assay on reported multiple myeloma cell lines (Lombardi et al., Molecular characterization of human multiple myeloma cell lines by integrative genomics: insights into the biology of the disease; Genes Chromosomes Cancer. 2007 Mar;46(3):226-38.). The cell line NCI-H929 (ATCC® CRL-9068) was cultured in RPMI 1640 with Glutamax medium (Gibco) supplemented with 10% FBS, 1x Penicillin/Streptomycin (Gibco), 1x Sodium Pyruvate (Gibco) and 50  $\mu$ M beta-Mercaptoethanol (Gibco). Jurkat-NFAT reporter cells (a CD3-expressing human acute lymphatic leukemia reporter cell line with a NFAT promoter, GloResponse Jurkat NFAT-RE-luc2P, Promega #CS176501) were cultured in RPMI 1640, containing 2g/l Glucose, 2 g/l NaHCO<sub>3</sub>, 10 % FCS, 25 mM HEPES, 2 mM L-Glutamine, 1 x NEAA, 1 x Sodium-pyruvate and 200  $\mu$ g/ml Hygromycin B.

0.1 Mio cells per well of a 96-round-bottom-well plate were incubated with 100 nM to 1.3 pM (serial dilutions of 1:5) of the indicated GPRC5D-TCB constructs 5E11p-CH2527, 6625, 6626, 5F11p-CH2527, 6623 or 6624 or no construct for 30 min at 4 °C. The cells were washed with FACS buffer (PBS, 2% Fetal Bovine Serum; 1% 0.5m EDTA pH 8; 0.25% NaN<sub>3</sub> Sodium azide (20%)) twice and stained with PE-conjugated Goat anti-human IgG, Fc $\gamma$  fragment specific (Jackson Laboratories, 109-606-008) diluted 1/100 in FACS buffer, for another 30 min at 4 °C.

Flow cytometry acquisition was performed on a custom-designed BD Biosciences Fortessa and analyzed using BD Diva. EC50 values were calculated, using GraphPad Prism software.

Figure 16 shows that all TCB molecules are able to bind both, human GPRC5D, as well as human CD3 in a concentration-dependent manner. Briefly, both humanized versions of the 5E11p-CH2527, namely 6625 and 6626, show enhanced binding to human GPRC5D compared to their

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parental TCB, which results in lower EC50 values of binding as well (Figure 16A and Table 14.1). In addition, 6624 shows slightly enhanced binding to human GPRC5D compared to 5F11p-CH2527 and 6623 (Figure 16B). In general, all 5F11-based molecules show better binding to human GPRC5D than the 5E11-based molecules. All 5E11-based TCB molecules show comparable concentration-dependent binding to human CD3 (Figure 16C), whereas both humanization variants of the 5F11p-CH2527, namely the 6623 and 6624 show stronger overall binding signals at the highest antibody concentrations compared to the parental one, when incubated with human CD3-expressing Jurkat-NFAT cells (Figure 16D).

- 10 **Table 14.1:** EC50 values (nM) for binding of the indicated GPRC5D-TCB molecules to either human GPRC5D expressed on NCI-H929 or to human CD3 expressed on Jurkat cells.

| EC50 (nM) | 5E11p-CH2527 | 6625  | 6626 | 5F11p-CH2527 | 6623 | 6624 |
|-----------|--------------|-------|------|--------------|------|------|
| NCI-H929  | 3.36         | 1.15  | 0.85 | 0.18         | 0.36 | 0.1  |
| Jurkat    | 3.67         | 11.47 | 7.8  | 2.31         | n.c. | n.c. |

### Example 14.2

#### 15 Binding of T cell bispecific antibodies to multiple myeloma cell lines

As the data presented in Example 14.1 were falsely calculated by a factor of 10, the EC50 values are too low. Therefore and to reassess the binding to GPRC5D a, we performed a series of FACS based binding assay on reported multiple myeloma cell lines (Lombardi et al., Molecular characterization of human multiple myeloma cell lines by integrative genomics: insights into the biology of the disease; Genes Chromosomes Cancer. 2007 Mar;46(3):226-38.). The cell line OPM-2 was cultured in RPMI 1640 + 1 % Glutamax medium (Gibco) supplemented with 20 % Heat-Inactivated Fetal Bovine Serum (FBS, Gibco). The cell line NCI-H929 was cultured in RPMI 1640 + 1 % Glutamax medium (Gibco) supplemented with 10% Heat-Inactivated Fetal Bovine Serum (FBS, Gibco), 50 µM Mercaptoethanol (Gibco) and 1 mM Sodium Pyruvate (Gibco), and RPMI-8226 were cultured in RPMI 1640 + 1 % Glutamax medium (Gibco) supplemented with 10 % Heat-Inactivated Fetal Bovine Serum (FBS, Gibco). The cell lines were cultured in 75 cm<sup>2</sup> flasks (TPP) with two passages per week.

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Briefly, suspension cells were harvested, counted and assessed for viability. All subsequent steps were performed at 4 °C.

Cells were re-suspended in PBS at 0.5 Mio cells per ml. Next, 0.05 Mio cells were plated per well of a round-bottom 96-well-plate, centrifuged and supernatants were discarded. Cells were stained with zombie aqua viability stain (BioLegend #423102, pre-diluted 1:400), containing Fc blocking (BioLegend #422302, prediluted 1:400) for 20 minutes in a total volume of 50 ul per well. Cells were washed with FACS buffer and incubated with increasing concentrations of the 5E11(6625)-TCB, also referred to as 6625 herein, (0.7 nM - 500 nM, total volume of 25 ul per well) for 30 minutes at 4°C. Assay plates were centrifuged and supernatants were discarded.

Thereafter, cells were resuspended by smooth vortexing and incubated for another 30 min at 4 °C in a total of 25 µl per well, containing 500 nM of the secondary antibody (aPGLALA mulG2b Alexa 647, produced inhouse) in FACS buffer. Cells were washed once and analyzed on a BD flow cytometer, equipped with FACS Diva. Binding curves and EC50 values were obtained using GraphPadPrism6. The EC50 values of assay replicate 1 correspond to the graphs shown in Fig.25A, Fig.25B and Fig.25C. The EC50 values of assay replicate 2 correspond to the graphs shown in Fig.25D, Fig.25E and Fig.25F. The EC50 values of assay replicate 3 correspond to the graphs shown in Fig.25G, Fig.25H and Fig.25I.

Figure 25 shows concentration-dependent binding of the 5E11(6625)-TCB to MM cell lines expressing various levels of human GPRC5D. The EC50 of binding ranges from 20 nM to 158 nM and shows some assay variation due to variation in target expression levels on cells.

**Table 14.2.** EC50 values Binding of 5E11(6625)-TCB to human GPRC5D expressed on different established MM cell lines

| EC50 (nM)         | OPM-2 | NCI-H929 | RPMI-8226 |
|-------------------|-------|----------|-----------|
| Assay replicate 1 | 34.5  | 75.8     | 26.8      |
| Assay replicate 2 | 75.5  | ~ 123.2  | 20.4      |
| Assay replicate 3 | 54.2  | ~ 158.2  | 55        |

### Example 15

The capacity of the GPRC5D-TCBs to induce CD3-mediated activation of Jurkat-NFAT effector cells upon simultaneous binding to human CD3 and human GPRC5D, was assessed using co-cultures of RPMI-8226 (ATCC® CCL-155) cells and Jurkat-NFAT reporter cells (Promega #CS176501). Upon simultaneous binding of the TCB molecule to the human GPRC5D on RPMI-8226 cells and the human CD3 antigen on Jurkat-NFAT reporter cells, the NFAT promoter is activated and leads to expression of active firefly luciferase. The intensity of luminescence signal (obtained upon addition of luciferase substrate) is proportional to the intensity of CD3 activation and signaling.

- 10 For the assay, 20 000 RPMI8226 cells were plated per well of a 96-well-plate and the indicated TCB molecules were added to obtain a final concentration range of 50 nM to 5 fM, using serial dilution steps of 1:10 in in RPMI, containing 20% FBS and 1% Pen/Strep. 50 000 Jurkat-NFAT cells were added per well to obtain a final E:T ratio of 2.5:1. After an overnight incubation at 37 °C 5% CO<sub>2</sub>, 100 µl of ONE-Glo reagent (Promega) were added to equivalent volumes of the assay
- 15 supernatant and incubated for 5 minutes at room temperature, protected from light. Luminescence was analyzed using a Perkin Elmer plate reader.

As depicted in Figure 17A, all evaluated GPRC5D TCB molecules induce Jurkat-NFAT activation in a dose-dependent manner, whereas no significant signal was obtained in the presence of any of the two untargeted DP47 TCB control molecules. The untargeted DP47 TCB 1 comprises a CD3 binder comprising the VH of SEQ ID NO: 104 and the VL of SEQ ID NO: 105. The untargeted DP47 TCB 2 comprises a CD3 binder comprising the VH of SEQ ID NO: 35 and the VL of SEQ ID NO: 36. The corresponding EC<sub>50</sub> values for Jurkat activation were calculated using GraphPadPrism6, and are given in Table 15. Taking into account both EC<sub>50</sub>, as well as AUC (see Table 15) the ranking of the molecules is as follows: 6624 > 6623 > 5F11p-CH2527 > 6626 ~6625

25 > 5E11p-CH2527.

Similar assays were conducted in the presence of additional (Multiple Myeloma) cell lines, expressing various levels of human GPRC5D, as determined by flow (Quantum Simply Cellular, Bangslabs) and indicated as GPRC5D binding site numbers in brackets next to the cell line name. EC<sub>50</sub> and AUC were calculated, using GraphPadPrism and are plotted on x- versus y-axis (Figure

30 17B-G).

As depicted in Figure 17B-G), all molecules show concentration-dependent Jurkat-activation in presence of cell lines with a broad range of relative GPRC5D expression; the ranking of the molecules is similar, independent of the target cell line present.

**Table 15:** EC50 values (pM) or area under the curve (AUC), calculated from GPRC5D-TCB-mediated activation of Jurkat-NFAT reporter cells in presence of RPMI-8226 cells, as measured by luminescence after overnight incubation (~20h).

|                  | <b>5E11p-<br/>CH2527</b> | <b>6625</b> | <b>6626</b> | <b>5F11p-<br/>CH2527</b> | <b>6623</b> | <b>6624</b> |
|------------------|--------------------------|-------------|-------------|--------------------------|-------------|-------------|
| <b>EC50 (pM)</b> | 157                      | 40          | 49.7        | 14.0                     | 8.9         | 4.5         |
| <b>AUC</b>       | 265 250                  | 571 600     | 582 400     | 393 460                  | 778 140     | 880 720     |

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### Example 16

#### GPRC5D-TCB mediated T cell cytotoxicity

10 To further measure the functionality of the anti-GPRC5D-TCB antibodies, an *in-vitro* tumor cell lysis assay was performed. Briefly, AMO-1 (DSMZ ACC 538), NCI-H929 ATCC® CRL-9068, LP-1 (DSMZ ACC 41) and IM-9 (ATCC® CCL-159) cell lines were co-cultured with human pan T as effector cells at a final effector to target ratio of 10:1. Human pan T cells were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors, using a human Pan T cell

15 Isolation kit (Miltenyi Biotec). The indicated GPRC5D- (6625 and B72) or BCMA-targeting T-cell engaging bispecific molecules were added at decreasing concentrations (range from 50 nM to 5 pM, with dilution steps of 1:10). As negative control, the untargeted TCB was included.

After 20 hours of incubation at 37 °C with 5% CO<sub>2</sub>, cell death was determined by quantification of the luminescence signal (CytoTox-Glo Cytotoxicity Assay, Promega), following the

20 manufacturers' manual. Depicted are relative luminescence signals (RLUs) as direct measurement of determined cell death. EC50 and AUC were calculated, using GraphPadPrism and are summarized in Table 16.

Figure 18A-D shows that all TCB molecules are able to induce concentration-dependent lysis of a wide range of tumor cell lines with varying relative expression level of human GPRC5D and

25 BCMA, respectively. The direct comparison of the 6625 and the B72 suggests an increased efficacy and potency of the 6625 molecule. The comparison of the 6625 and the BCMA-TCB reveals better in vitro efficacy and potency of the 6625 in presence of AMO-1 (Figure 18A), NCI-

H929 (Figure 18B) and LP-1 (Figure 18C), whereas the BCMA-TCB is inducing stronger tumor cell lysis of IM-9 (Figure 18D), which express rather low levels of GPRC5D. The different ranking of the 6625 and the BCMA-TCB on the tested cell lines can likely be explained by different relative expression levels of the GPRC5D versus the BCMA on these cell lines.

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**Table 16:** EC50 values (pM), calculated from GPRC5D- or BCMA-TCB-mediated tumor cell lysis in presence of the indicated cell lines and as determined by luminescence after overnight incubation (~20h).

| EC50 (pM) | Tumor Indication                   | 6625  | B72   | BCMA-TCB |
|-----------|------------------------------------|-------|-------|----------|
| AMO-1     | plasmacytoma                       | 8.3   | 79.9  | 137.1    |
| NCI-H929  | MM                                 | 0.597 | 26.95 | 171      |
| LP-1      | MM                                 | 7.99  | 48.95 | 76.36    |
| IM-9      | B lymphoblastoid from a MM patient | 266.9 | 197.5 | 55.69    |

10

**Table 17:** Area under the curve values, calculated from GPRC5D- or BCMA-TCB-mediated tumor cell lysis in presence of the indicated cell lines and as determined by luminescence after overnight incubation (~20h).

| AUC      | Tumor Indication                   | 6625     | B72      | BCMA-TCB |
|----------|------------------------------------|----------|----------|----------|
| AMO-1    | plasmacytoma                       | 80117761 | 54494020 | 47326610 |
| NCI-H929 | MM                                 | 17872547 | 10854134 | 10684467 |
| LP-1     | MM                                 | 15438497 | 11504124 | 11100680 |
| IM-9     | B lymphoblastoid from a MM patient | 9268093  | 8600921  | 10994563 |

15

### Example 17

#### **anti-GPRC5D-TCB mediated T cell activation in presence of a primary MM sample**

To evaluate the activity of GPRC5D TCB molecules on primary Multiple Myeloma samples, a frozen unprocessed Bone Marrow sample (Proteogenex) was thawed and a quick red blood cell lysis was performed, using BD Pharm Lysis buffer (#555899). Thereafter, cells were washed, resuspended in RPMI 1640 Glutamax, including 20% heat-inactivated Fetal Bovine Serum, 2% human serum and 1% Penicillin /Streptomycin (all from Gibco) and 100  $\mu$ L of cell suspension (30 000 cells) were seeded per well in a 96-well plate round bottom (TPP). Autologous T-cells were added to obtain a final ratio of 10 T-cells per cells of the mixed BM sample. The indicated molecules were added to obtain a final concentration range of 50 nM to 0.05nM (1:10 dilution steps) in a total volume of 200  $\mu$ l per well of a 96-well-plate.

After overnight incubation at 37 °C in a humidified incubator, cells were washed with PBS once and stained for 20 minutes at 4 °C with 50  $\mu$ L of Live blue (Invitrogen, # L23105) to discriminate between live and dead cells. Surface staining was performed using a mixture of the following antibodies based on the manufacturers' suggestions: CD25 BV605, CD69 APC-Cy7, CD38 BV510, CD138 FITC, CD8 BV711, CD3 PE-Cy5 and CD4 AlexaFluor700 (all from BioLegend). For the final analysis, cells were resuspended in 100  $\mu$ L of FACs buffer and acquired using a Fortessa (BD Biosciences). Figure 19 shows percent of T-cell activation, as determined by the percent of living CD8 T cells, being positive for the early activation marker CD69. EC50 of T-cell activation was calculated by Graph Pad Prism and is summarized in Table 18. Both representative GPRC5D-targeting bispecific molecules, namely 6624 and 6625, are able to induce concentration-dependent T-cell activation with an EC50 of 1.06 pM and 14.8 pM, respectively, whereas no T-cell activation was induced in presence of an untargeted TCB control. In the depicted case, the BCMA-TCB activated T-cells to a lesser extent than both of the evaluated GPRC5D TCB molecules. A potential reason might be differences in relative expression levels of GPRC5D and BCMA (not assessed). Significant T-cell activation was induced by the B72 molecule only at much higher concentrations than it was observed with the 6624 and 6625, however the B72 led to a higher overall activation at the two highest concentrations measured. As we have observed T-cell activation at similar concentrations of the B72 in bone marrow samples of healthy donors, it is not clear, if the observed effect of B72 in presence of the primary MM samples is purely target-dependent (see Figure 21).

**Table 18:** EC<sub>50</sub> values (pM), calculated from GPRC5D- or BCMA-TCB-mediated activation of autologous T-cells, incubated with a primary MM sample and quantified by flow cytometric analysis of CD69 on CD8 T cells after roughly 24h.

|                             | <b>6624</b> | <b>6625</b> | <b>B72</b> | <b>BCMA-TCB</b> |
|-----------------------------|-------------|-------------|------------|-----------------|
| <b>EC<sub>50</sub> (pM)</b> | 1.06        | 14.8        | ~ 612.5    | 52.54           |

5

### Example 18

#### Depletion of B cells upon incubation of PBMCs from healthy donors

Human PBMCs were isolated from the blood of healthy donors by classical density gradient centrifugation. 200 000 PBMCs were plated per well of a 96-well-plate in RPMI 1640 medium, containing 10% FBS and 1% Pen/Strep. The indicated bispecific molecules were added to have a final concentration of 50 nM, 5 nM, 0.5 nM or 0.05 nM in a total volume of 200 µl per well. After incubation for 48h at 37 °C in a humidified incubator, cells were washed with FACS buffer and Fc receptors were blocked by incubation of cells with the Human TruStain FcX™ (Fc block, BioLegend), according to the manufacturers' protocols. Live blue (Invitrogen, # L23105) was used to discriminate between live and dead cells (see Example 17). Surface expression of the following markers was performed for 30 min at 4 °C: CD19, CD45, CD4, CD38, CD8, CD138 (all from BioLegend). For the absolute quantification of B cells per well, 10 µl per well of CountBright absolute counting beads (Invitrogen #C36950) were added prior the flow cytometric analysis with BD FACS Fortessa. Figure 20A-D shows the summary of 5 different healthy donors, that have been assessed with the indicated bispecific molecules at different antibody concentrations, namely 50 nM (Figure 20A), 5 nM (Figure 20B), 0.05 nM (Figure 20C) and 0.05 nM (Figure 20D). Depicted is the B cell count, normalized to untreated controls, based on duplicates with SD (per donor). Significant depletion of healthy B cells was observed for both, the BCMA-TCB, as well as the GPRC5D-TCB 6626, whereas none of the other GPRC5D-targeting TCBs, including the B72, significantly depleted B cells in the majority of donors. The B-cell depleting effect observed with 6626 was limited to concentrations of above ~5 nM, whereas the BCMA-TCB depleted healthy B-cells already at concentrations of 0.05 nM. In summary, this suggests, that GPRC5D-targeting molecules seem to have a much lower risk to deplete healthy B cells, which might be a safety advantage.

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### Example 19

#### Impact on Activation of T-cells upon incubation of bone marrow samples from healthy donors

- 5 Unprocessed Bone Marrow of healthy donors (Lonza) was evaluated 1 day after sampling. After a quick red blood cell lysis using (BD Pharm Lysis buffer #555899 cells were washed, resuspended in RPMI 1640 Glutamax, including 20% heat-inactivated Fetal Bovine Serum, 2% human serum and 1% Penicillin /Streptomycin (all from Gibco) and 100  $\mu$ L of cell suspension (30 000 cells) were seeded per well in a 96-well plate round bottom (TPP). The indicated molecules were added
- 10 to obtain a final concentration range of 5 nM to 0.05nM (1:10 dilution steps) in a total volume of 200  $\mu$ l per well of a 96-well-plate.

- After overnight incubation at 37 °C in a humidified incubator, cells were washed with PBS once and stained for 20 minutes at 4°C with 50  $\mu$ L of Live blue (Invitrogen, # L23105) to discriminate between live and dead cells. Surface staining was performed using a mixture of the following
- 15 antibodies based on the manufacturers' suggestions: CD25 BV605, CD69 APC-Cy7, CD38 BV510, CD138 FITC, CD8 BV711, CD3 PE-Cy5 and CD4 AlexaFluor700 (all from BioLegend). For the final analysis, cells were resuspended in 100  $\mu$ L of FACs buffer and acquired using a Fortessa (BD Biosciences). Figure 21 shows T-cell activation, as determined as percent of either CD69-positive CD8<sup>+</sup> (A) or CD4<sup>+</sup> T cells (B), upon the indicated treatments.
- 20 A clear concentration-dependent T-cell activation in the bone marrow sample was detected with BCMA-TCB or B72, but not with 6624 or 6625. This illustrates a potential safety advantage of molecules such as the 6624 and 6625 over BCMA-TCB or B72, when used at higher doses.

### Example 20

#### 25 Cytokine Release in human whole blood from healthy donors

- Whole blood from 6 healthy donors was collected in BD Vacutainer Lithium-Heparin tubes and assayed within 3 hours. GPRC5D-TCBs 6624 and 6625, as well as the untargeted TCB control molecule were diluted in PBS (Gibco #14190) and 5  $\mu$ L were added to 195  $\mu$ L of whole blood in a round-bottomed 96-well plate (Corning # Costar 3799) to reach final concentrations of 50, 0.5
- 30 and 0.005 nM. The monoclonal antibodies Gazyva (obinutuzumab) and Lemtrada (alemtuzumab) were similarly assayed at 50, 0.5 and 0.005 nM, while Erbitux (cetuximab) was tested at 50 nM. PBS only served as vehicle control. After 24 h incubation at 37°C, the plate was centrifuged at 1800 g (3000 rpm) for 5 min. The plasma supernatants (~70  $\mu$ l) were collected and

stored at -80°C before multiplex cytokine detection was performed using a Millipore kit (HCYTOMAG-60K) and a Luminex reader LX 200, according to the manufacturers' suggestions. As summarized in Figure 22A (human TNFa) and Figure 22B (human IL-6), 6624 induced secretion of low levels of TNFa and IL-6 in a similar range to Gazyva, whereas 6625 induced even lower levels of the evaluated cytokines, suggesting that 6624 may display a favourable safety profile in terms of cytokine release.

### Example 21

#### In vivo efficacy of different GPRC5DxCD3 bispecific TCB molecules

##### 10 in NCI-H929 (hNSG mice)

To further evaluate the efficacy of the GPRC5D TCB molecules 6623, 6624, 6625 and 6626, their potential to induce tumor regression in multiple myeloma bearing fully humanized NSG mice was assessed.  $2.5 \times 10^6$  NCI-H929 cells with a viability of > 95.0 % were re-suspended in RPMI cell culture medium (Gibco) and GFR matrigel (1:1, total volume of 100  $\mu$ l) and injected subcutaneously into the right flank of humanized female NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice.

Humanization of the mice was conducted as follows: aged 4-5 weeks at start of the experiment (bred at Charles River, Lyon, France) the mice were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (ROB-55.2-2532.Vet\_03-16-10). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis. According to the protocol, female NSG mice were injected i.p. (intraperitoneal) with 15 mg/kg of Busulfan followed one day later by an i.v. injection of  $1 \times 10^5$  human hematopoietic stem cells isolated from cord blood. At week 16-20 after stem cell injection mice were bled and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups (n=10/group).

At that time, mice were injected with tumor cells subcutaneously. as described above and treated once weekly with the compounds or PBS (Vehicle) when tumor size reached a median size of 308 mm<sup>3</sup> (range 92 - 841 mm<sup>3</sup>). All mice were injected intravenously with 0.05 mg/kg and 0.005 mg/kg of the indicated TCB molecules (see Fig. 23A and 23B).

To obtain the appropriate amount of compounds, stock solutions were diluted with Histidine buffer (20 mM histidine, 140 mM NaCl, pH 6.0). Tumor growth was measured twice weekly using a caliper and tumor volume was calculated as followed:

$$T_v: (W^2/2) \times L \quad (W: \text{Width}, L: \text{Length})$$

- 5 The study was terminated at day 41 after tumor cell inoculation and all mice were sacrificed after three injections of the compounds and tumors were explanted and weighted. Statistics were performed according to Two way anova, Tukey test.

As illustrated in Figure 23A and 23B, at the low dose of 0.005 mg/kg none of the evaluated GPRC5D-TCB molecule showed efficient tumor growth inhibition. In contrast, at 0.05 mg/kg, we  
10 saw significant anti-tumor growth responses by all four GPRC5D TCB molecules assessed compared to the vehicle group. Figure 23C-G further demonstrates the tumor growth inhibition of the single mice per treatment group. There were no significant differences among the four molecules assessed though, validating the high preclinical efficacy of all four GPRC5D TCB molecules.

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### Example 22

#### **in vivo SDPK in hFcRn Tg and KO mice**

To evaluate PK properties of the GPRC5D TCB molecules 6623, 6624, 6625 and 6626, the respective molecules were administered intravenously (bolus) via tail vein at a dose of 1mg/kg  
20 into either -/- huFcRn Tg line 32 (B6.Cg-Fcgrt<tm1Dcr> Tg(FCGRT)32Dcr) mice or -/- muFcRn (B6.129X1-Fcgrttm1Dcr/DcrJ) (JAX laboratories, Bar harbor, USA). All studies were conducted with the approval of the local veterinary authority in strict adherence to the Swiss federal regulations on animal protection and to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Blood was collected by  
25 venous puncture (tail vein) at different time points to obtain serum for analysis at the indicated time points after dosing: 0.083, 7, 24, 48, 72, 168, 336, 504 and 672 post dose for the -/- huFcRn Tg line and 32, 0.083, 2, 7, 24, 31, 48, 72 and 96 hours post dose for the -/- muFcRn line. Blood was stored for 20 minutes at room temperature for clotting and serum was obtained by centrifugation at 15 000 rpm for 5 min at 4 °C and frozen immediately. All serum samples were stored at -20 °C  
30 until they were analyzed by electro-chemiluminescence immunoassay (ECLIA), a method specific for the human Fab moiety of the administered antibody and its variants.

Briefly, samples, prediluted with assay buffer, were incubated with capture and detection molecules for 9 min at 37 °C. Biotinylated mAb<H-Fab(kappa)>M-IgG-Bi was used as capture molecule and a ruthenium(II)tris(bipyridyl)<sub>3</sub>2+ labeled mAb<H-Fab(CH1)>M-1.19.31-IgG-S-Ru mouse monoclonal antibody was used for detection. Streptavidin-coated magnetic microparticles were added and incubated for additional 9 min at 37 °C to allow complex formation due to biotin–streptavidin interactions. Complexes were magnetically captured on an electrode and a chemiluminescent signal generated using the co-reactant tripropylamine (TPA) was measured by a photomultiplier detector. All serum samples and positive or negative control samples were analyzed in quadruplicates and calibrated against the corresponding antibody that was administered.

As illustrated in Figure 24 and Table 19, all four GPRC5D TCB molecules exhibit an acceptable PK profile in hFcRn tg32 mice in the range of the one from a classical IgG. The data generated in FcRn ko mice are considered to be relevant for evaluating unspecific cellular uptake, which might be linked to immunogenicity. As summarized in Figure 10B, 6625 and 6626 show comparable clearance rates, whereas the one for 6623 and 6624 are elevated, suggesting a slightly higher potential for unspecific cellular uptake.

**Table 19:** Clearance, respective half-life calculated from SDPK studies run in hFcRn Tg32 or FcRn KO mice

|          | hFcRn Tg32 mice     |               | FcRn KO mice        |               |
|----------|---------------------|---------------|---------------------|---------------|
| Molecule | Clearance (mL/d/kg) | Half-life (d) | Clearance (mL/d/kg) | Half-life (d) |
| 6623     | 13.6                | 5.6           | 97.3                | 0.3           |
| 6624     | 22.5                | 6.2           | 143                 | 0.6           |
| 6625     | 13.0                | 7.2           | 61.3                | 1.2           |
| 6626     | 9.6                 | 7.3           | 57.8                | 1.1           |

\* \* \*

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.