CD47 pathway in regulating myeloid cells with potential applications in cancer immunotherapy.

SUMMARY

[0007] In certain examples, the present disclosure provides agents that down-regulate SIRPA, *e.g.,* anti-SIRPA antibodies. Such agents can be used for treating, preventing, or reducing risk of a disease or pathology associated with SIRPA expression, activity, or signaling. In some examples, the disclosure relates to the identification of anti-SIRPA antibodies that are capable of downregulating, *i.e.*, decreasing levels of, SIRPA on human macrophages and dendrocytes, as well as cell lines that express SIRPA. In some examples, the disclosure relates to anti-SIRPA antibodies that antagonize the immune suppressive SIRPA-CD47 interaction and facilitate phagocytosis of CD47-expressing tumor cells. In a further example, the present disclosure provides unique SIRPA-specific antibodies that disrupt CD47 binding through non-competitive inhibition.

[0008] Thus, in one example, the disclosure relates to a SIRPA antibody that selectively binds SIRPA and down-regulates SIRPA expressed on the cell surface. In some examples, the anti-SIRPA antibody decreases cell surface levels of SIRPA, decreases intracellular levels of SIRPA, decreases total levels of SIRPA , or any combination thereof. In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody induces SIRPA degradation, SIRPA cleavage, SIRPA internalization, SIRPA shedding, downregulation of SIRPA expression, or any combination thereof. In some examples, which may be combined with any of the preceding examples, the antibody decreases cellular levels of SIRPA *in vivo*. In some embdoiments that may be combined with any of the preceding examples, the anti-SIRPA antibody inhibits cell surface clustering of SIRPA. In further embdodiments that may be combined with any of the preceding embdoiments, the anti-SIRPA antibody inhibits one or more SIRPA activities; or counteracts, one or more SIRPA activities, which may be selected from the group sonsiting of: (a) SIRPA binding to one or more SIRPA ligands, optionally wherein the one or more SIRPA ligands are selected from the group consisting of CD47, surfactant protein A and D and any combination thereof; (b) decreasing proliferation of one or more cells selected from the group consisting of dendritic cells, bone marrow-derived dendritic cells, macrophages, neutrophils, NK cells, M1 macrophages, M1 neutrophils, M1 NK cells, activated M1 macrophages, activated M1 neutrophils, activated M1 NK cells, M2 macrophages, M2 neutrophils, M2 NK cells, monocytes, osteoclasts, T cells, T helper cells, cytotoxic T cells, granulocytes,

tumor cell proliferation activity of one or more of microglia, macrophages, neutrophils, NK cells, dendritic cells, bone marrow-derived dendritic cells, neutrophils, T cells, T helper cells, or cytotoxic T cells; (h) modulated expression of one or more inflammatory receptors, optionally wherein the one or more inflammatory receptors comprise CD86 and the one or more inflammatory receptors are expressed on one or more of microglia, macrophages, neutrophils, NK cells, dendritic cells, bone marrow-derived dendritic cells, neutrophils, T cells, T helper cells, or cytotoxic T cells; (i) promoting or rescuing functionality of one or more of immunosuppressor dendritic cells, immunosuppressor macrophages, immunosuppressor neutrophils, immunosuppressor NK cells, myeloid-derived suppressor cells, tumor-associated macrophages, tumor-associated neutrophils, tumor-associated NK cells, and regulatory T cells; (j) increasing infiltration of one or more of immunosuppressor dendritic cells, immunosuppressor macrophages, immunosuppressor neutrophils, immunosuppressor NK cells, myeloid-derived suppressor cells, tumor-associated macrophages, tumor-associated neutrophils, tumor-associated NK cells, non-tumorigenic CD45+CD14+ myeloid cells, and regulatory T cells into tumors; (k) increasing the number of tumor-promoting myeloid/granulocytic immune-suppressive cells and/or non-tumorigenic CD45+CD14+ myeloid cells in a tumor, in peripheral blood, or other lymphoid organ; (l) enhancing tumor-promoting activity of myeloid-derived suppressor cells and/or non-tumorigenic CD45+CD14+ myeloid cells; (m) enhancing survival of non-tumorigenic myeloid-derived suppressor cells and/or non-tumorigenic CD45+CD14+ myeloid cells; (n) decreasing activation of tumor-specific T lymphocytes with tumor killing potential; (o) decreasing infiltration of tumor-specific NK cells with tumor killing potential; (p) increasing tumor volume; (q) increasing tumor growth rate; and (r) decreasing efficacy of one or more immune-therapies that modulate anti-tumor T cell responses, optionally wherein the one or more immune-therapies are immune-therapies that target one or more target proteins selected from the group consisting of PD1/PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, TREM1, TREM2, CD39, CD73, CSF-1 receptor, and any combination thereof, or of one or more cancer vaccines. In some examples that may be combined with any of the preceding examples, the anti-SIRPA antibody induces one or more of the activities that are selected from the group consisting of: (a) increasing the number of tumor infiltrating CD3+ T cells; (b) decreasing cellular levels of SIRPA in non-tumorigenic CD14+myeloid cells, optionally wherein the non-tumorigenic CD14+ myeloid cells are tumor infiltrating cells or optionally

wherein the non-tumorigenic CD14+ myeloid cells are present in blood; (c) reducing the number of non-tumorigenic CD14+ myeloid cells, optionally wherein the non-tumorigenic CD14+ myeloid cells are tumor infiltrating cells or optionally wherein the non-tumorigenic CD14+ myeloid cells are present in blood; (d) reducing PD-L1 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (e) reducing PD-L2 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (f) reducing B7-H2 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (g) reducing B7-H3 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (h) reducing CD200R levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (i) reducing CD163 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (j) reducing CD206 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (k) decreasing tumor growth rate of solid tumors; (l) reducing tumor volume; (m) increasing efficacy of one or more PD-1 inhibitors; (n) increasing efficacy of one or more checkpoint inhibitor therapies and/or immune-modulating therapies, optionally wherein the one or more checkpoint inhibitor therapies and/or immune-modulating therapies target one or more of CTLA4, the adenosine pathway, PD-L1, PD-L2, OX40, TIM3, LAG3, or any combination thereof; (o) increasing efficacy of one or more chemotherapy agents, optionally wherein the one or more of the chemotherapy agents are gemcitabine, capecitabine, anthracyclines, doxorubicin (Adriamycin®), epirubicin (Ellence®), taxanes, paclitaxel (Taxol®), docetaxel (Taxotere®), 5-fluorouracil (5-FU), cyclophosphamide (Cytoxan®), carboplatin (Paraplatin®), and any combination thereof; (p) increasing proliferation of T cells in the presence of non-tumorigenic myeloid-derived suppressor cells (MDSC); (1) inhibiting differentiation, survival, and/or one or more functions of non-tumorigenic myeloid-derived suppressor cells (MDSC); and (r) killing CD33-expressing immunosuppressor non-tumorigenic myeloid cells and/or non-tumorigenic CD14-expressing cells in solid tumors and associated blood vessels when conjugated to a chemical or radioactive toxin.

[0009] In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody inhibits interaction between SIRPA and one or more

SIRPA ligands. In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody decreases cellular levels of SIRPA and inhibits interaction between SIRPA and one or more SIRPA ligands. In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody blocks binding of CD47 to human SIRPA.

[009a] In one aspect, there is provided an isolated anti-signal regulatory protein α (SIRPA) antibody, wherein the anti-SIRPA antibody comprises a heavy chain variable region (VH region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:9, a CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a CDR3 comprising the amino acid sequence of SEQ ID NO:11; and a light chain variable region (VL region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8.

[009b] In one aspect, there is provided an isolated anti-signal regulatory protein α (SIRPA) antibody, wherein the anti-SIRPA antibody comprises: a heavy chain variable region (VH region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:15, a CDR2 comprising the amino acid sequence of SEQ ID NO:16, and a CDR3 comprising the amino acid sequence of SEQ ID NO:17; and a light chain variable region (VL region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:12, a CDR2 comprising the amino acid sequence of SEQ ID NO:13, and a CDR3 comprising the amino acid sequence of SEQ ID NO:14.

[0010] In some examples, which may be combined with any of the preceding examples, the antibody selectively binds human SIRPA and does not substantially block binding of CD47 binding to human SIRPA expressed on cells and further, wherein binding to human SIRPA decreases the level of SIRPA on the cell surface. In some examples, the antibody binds to the D1 domain of SIRPA, *e.g.*, human SIRPA. In some examples, the antibody binds to the D2 domain of SIRPA *e.g.*, human SIRPA. In some examples, the antibody binds to the D3 domain of SIRPA, *e.g.,* human SIRPA. In some examples, such an anti-SIRPA antibody competes with an antibody comprising a V_H sequence comprising the amino acid sequence of SEQ ID NO:2 and a V_L sequence comprising the amino acid sequence of SEQ ID NO:3. In some examples, such an anti-SIRPA antibody comprises a V_H region comprising: a CDR3 comprising the amino acid sequence of SEQ ID NO:11, a CDR1 comprising the amino acid sequence of SEQ ID NO:9, or a CDR2 comprising the amino acid sequence of SEQ ID NO:10. In some examples, the anti-SIRPA antibody comprises a V_H region comprising: a) a CDR1 that comprises the amino acid sequence of SEQ ID NO:9, a CDR1 that comprises the amino acid sequence of SEQ ID NO:9 with no more than two amino acid substitutions, or a CDR1 having at least about 90% identity to the amino acid sequence of SEQ ID NO:9; (b) a CDR2 that comprises the amino acid sequence of SEQ ID NO:10 or a CDR2 that comprises the amino acid sequence of SEQ ID NO:10 with no more than two amino acid substitutions; or a CDR2 having at least about 90% identity to the amino acid sequence of SEQ ID NO:10; and (c) a CDR3 that comprises the amino acid sequence of SEQ ID NO:11, a CDR3 that comprises the amino acid sequence of SEQ ID NO:11 with no more than two amino acid substitutions; or a CDR3 having at least about 90% identity to the amino acid sequence of SEQ ID NO:11. In some examples, the anti-SIRPA comprises a V_H region comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:9 or a CDR1 comprising the amino acid sequence of SEQ ID NO:9 with no more than one amino acid substitution; a CDR2 comprising the amino acid sequence of SEQ ID NO:10 or a CDR2 comprising the amino acid sequence of SEQ ID NO:10 with no more than one amino acid substitution; and a CDR3 comprising the amino acid sequence of SEQ

ID NO:11 or a CDR3 comprising the amino acid sequence of SEQ ID NO:11 with no more than one amino acid substitution. In some examples, the anti-SIRPA antibody comprises a VH region that comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:9, a CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a CDR3 comprising the amino acid sequence of SEQ ID NO:11. In some examples that may be combined with any of the preceding examples, the antibody comprises a V_H region comprising the amino acid sequence of a V_H region shown in Figure 14Aor comprises a V_H region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at leaset 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of a V_H region of Figure 14A. In some embodimments, which may be combined with any of the preceding examples, the anti-SIRPA antibody comprises a V_L region that comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:8, a CDR1 comprising the amino acid sequence of SEQ ID NO:6, or a CDR2 comprising the amino acid sequence of SEQ ID NO:7. In some embodimemnts, the V_L region comprises: (a) a CDR1 compirsing the amino acid sequence of SEQ ID NO:6, a CDR1 comprising the amino acid sequence of SEQ ID NO:6 with no more than two amino acid substitutions, or a CDR1 having at least about 90% identity to the amino acid sequence of SEQ ID NO:6; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO:7, a CDR2 comprising the amino acid sequence of SEQ ID NO:7 with no more than two amino acid substitutions, or a CDR2 having at least about 90% identity to the amino acid sequence of SEQ ID NO:7; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO:8, a CDR3 comprising the amino acid sequence of SEQ ID NO:8 with no more than two amino acid substitutions, or a CDR3 having at least about 90% identity to the amino acid sequence of SEQ ID NO:8. In some examples, the V_L region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:6 or a CDR1 comprising the amino acid sequence of SEQ ID NO:6 with no more than one amino acid substitution; a CDR2 comprising the amino acid sequence of SEQ ID NO:7 or a CDR2 comprising the amino acid sequence of SEQ ID NO:7 with no more than one amino acid substitution; and a CDR3 comprising the amino acid sequence of SEQ ID NO:8 or a CDR3 comprising the amino acid sequence of SEQ ID NO:8 with no more than one amino acid substitution. In some examples, the V_L region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. In some examples, which may be combined with any of the preceding examples, the V_L region comprises the amino acid sequence of a V_L region shown in Figure 14B; or comprises

a V_L region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at leaset 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of a V_L region of Figure 14B. In some examples, the antibody comprises an Fc region that decreases the levels of FcγR expressed on the surface of cells In some examples, the antibody comprises an Fc region that decreases the levels of FcγRBII on the surface of cells.

[0011] In some examples, which may be combined with any of the preceding examples, the antibody selectively binds human SIRPA, but not murine SIRPA, and does not substantially block binding of CD47 binding to human SIRPA expressed on cells and further, wherein binding to human SIRPA decreases the level of SIRPA on the cell surface. In some examples, such an anti-SIRPA antibody competes with an antibody comprising a V_H sequence comprising the amino acid sequence of SEQ ID NO:2 and a V_L sequence comprising the amino acid sequence of SEQ ID NO:3. In some examples, the antibody binds to the D1 domain of SIRPA, *e.g.*, human SIRPA. In some examples, the antibody binds to the D2 domain of SIRPA *e.g.*, human SIRPA. In some examples, the antibody binds to the D3 domain of SIRPA, *e.g.,* human SIRPA. In some examples, the anti-SIRPA antibody comprises a V_H region that comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:17, a CDR1 comprising the amino acid sequence of SEQ ID NO:15, or a CDR2 comprising the amino acid sequence of SEQ ID NO:16. In some examples, the V_H region comprises: a) a CDR1 comprising the amino acid sequence of SEQ ID NO:15, a CDR1 comprising the amino acid sequence of SEQ ID NO:15 with no more than two amino acid substitutions, or a CDR1 having at least about 90% identity to the amino acid sequence of SEQ ID NO:15; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO:16, a CDR2 comprising the amino acid sequence of SEQ ID NO:16 with no more than two amino acid substitutions, or a CDR2 having at least about 90% identity to the amino acid sequence of SEQ ID NO:16; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO:17, a CDR3 comprising the amino acid sequence of SEQ ID NO:17 with no more than two amino acid substitutions, or a CDR3 having at least about 90% identity to the amino acid sequence of SEQ ID NO:17. In some examples, the V_H region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:15 or a CDR1 comprising the amino acid sequence of SEQ ID NO:15 with no more than one amino acid substitution; a CDR2 comprising the amino acid sequence of SEQ ID NO:16 or a CDR2 comprising the amino acid sequence of SEQ ID NO:16 with no more than one amino acid substitution; and a CDR3 comprising the

amino acid sequence of SEQ ID NO:16 or a CDR3 comprising the amino acid sequence of SEQ ID NO:16 with no more than one amino acid substitution. In some examples, the V_H region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:15, a CDR2 comprising the amino acid sequence of SEQ ID NO:16, and a CDR3 comprising the amino acid sequence of SEQ ID NO:17. In some examples, which may be combined with any of the preceding examples, the antibody comprises a V_H region that comprises the amino acid sequence of a V_H region of Figure 14C; or comprises a V_H region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at leaset 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of a V_H region of Figure 14C. In some examples, which may be combined with any of the preceding examples, the V_L region comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:14, a CDR1 comprising the amino acid sequence of SEQ ID NO:12, or a CDR2 comprising the amino acid sequence of SEQ ID NO:13. In some examples, the V_L region comprises: a) a CDR1 compirsing the amino acid sequence of SEQ ID NO:12, a CDR1 comprising the amino acid sequence of SEQ ID NO:12 with no more than two amino acid substitutions, or a CDR1 having at least about 90% identity to the amino acid sequence of SEQ ID NO:12; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO:13, a CDR2 comprising the amino acid sequence of SEQ ID NO:13 with no more than two amino acid substitutions, or a CDR2 having at least about 90% identity to the amino acid sequence of SEQ ID NO:13; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO:14, a CDR3 comprising the amino acid sequence of SEQ ID NO:14 with no more than two amino acid substitutions, or a CDR3 having at least about 90% identity to the amino acid sequence of SEQ ID NO:14. In some examples, the V_L region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:12 or a CDR1 comprising the amino acid sequence of SEQ ID NO:12 with no more than one amino acid substitution; a CDR2 comprising the amino acid sequence of SEQ ID NO:13 or a CDR2 comprising the amino acid sequence of SEQ ID NO:13 with no more than one amino acid substitution; and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 or a CDR3 comprising the amino acid sequence of SEQ ID NO:14 with no more than one amino acid substitution. In some examples, the V_L region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8. In some examples, which may be combined with any of the preceding examples, the V_L region comprises the amino acid sequence of a VL region of Figure 14D; or comprises a V_L region having at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at leaset 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of a V_L region of Figure 14D. In some examples, the antibody comprises an Fc region that decreases levels of FcγR on the surface of cells. In some examples, the antibody comprises an Fc region that decreases levels of FcγRBII on the surface of cells.

[0012] In a further example, which may be combined with any of the preceding examples, an isolated anti-SIRPA of the present disclosure competes with one or more antibodies selected from the group consisting of 3F9, 9C2, 8A9, 8F4, 1E2, 7H9, and 4D8 for binding to SIRPA. In some examples, the antibody binds to the D1 domain of SIRPA, *e.g.*, human SIRPA. In some examples, the antibody binds to the D2 domain of SIRPA *e.g.*, human SIRPA. In some examples, the antibody binds to the D3 domain of SIRPA, *e.g.,* human SIRPA. In some examples, the isolated anti-SIRPA antibody binds to essentially the same epitope as one or more antibodies selected from the group consisting of 3F9, 9C2, 8A9, 8F4, 1E2, 7H9, and 4D8. In some examples, the isolated anti-SIRPA antibody comprises a V_H region and a V_L region, wherein the V_H region, the V_L region, or both, comprise at least one, two, three, four, five, or six CDRs of a monoclonal antibody selected from the group consisting of 3F9, 9C2, 8A9, 8F4, 1E2, 7H9, and 4D8.

[0013] In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is a monoclonal antibody. In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is a humanized antibody. In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is an Fab, Fab', Fab'-SH, $F(ab')_2$, Fv or scFv fragment; or a multivalent antibody, an antibody is of the IgG class, the IgM class, or the IgA class.

[0014] In some examples that may be combined with any of the preceding examples, the anti-SIRPA antibody is of the IgG class the IgM class, or the IgA class. In some examples that may be combined with any of the preceding examples, the anti-SIRPA antibody has an IgG1, IgG2, IgG3, or IgG4 isotype. In some examples that may be combined with any of the preceding examples, the antibody binds an inhibitory Fc receptor. In some examples that may be combined with any of the preceding examples, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγRIIB). In some examples, the antibody decreases the level of FcγRIIB on the cell surface. In some

examples that may be combined with any of the preceding examples: (a) the anti-SIRPA antibody has a human or mouse IgG1 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: N297A, D265A, D270A, L234A, L235A, G237A, P238D, L328E, E233D, G237D, H268D, P271G, A330R, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering, or comprises an amino acid deletion in the Fc region at a position corresponding to glycine 236; (b) the anti-SIRPA antibody has an IgG1 isotype and comprises an IgG2 isotype heavy chain constant domain 1(CH1) and hinge region, optionally wherein the IgG2 isotype CH1 and hinge region comprises the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGVHTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVERKCCVECPPCP (SEQ ID NO:34), and optionally wherein the antibody Fc region comprises a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, wherein the numbering of the residues is according to EU numbering; (c) the anti-SIRPA antibody has an IgG2 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: P238S, V234A, G237A, H268A, H268Q, V309L, A330S, P331S, C214S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, H268E, N297A, N297Q, A330L, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; (d) the anti-SIRPA antibody has a human or mouse IgG4 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, E233P, F234V, L234A/F234A, S228P, S241P, L248E, T394D, N297A, N297Q, L235E, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; or (e) the anti-SIRPA antibody has a hybrid IgG2/4 isotype, and optionally wherein the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU or, Kabat numbering. In some examples that may be combined with any of the preceding examples: (a) the anti-SIRPA antibody has a human or mouse IgG1 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: N297A, N297Q, D270A, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V,

P238A, A327Q, A327G, P329A, K322A, L234F, L235E, P331S, T394D, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; (b) the anti-SIRPA antibody has an IgG2 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: P238S , V234A, G237A, H268A, H268Q, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; or (c) the anti-SIRPA antibody has an IgG4 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: E233P, F234V, L234A/F234A, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering. In some examples that may be combined with any of the preceding examples: (a) the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of A330L, L234F; L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; (b) the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of M252Y, S254T,T256E, and any combination thereof, wherein the numbering of the residues is according to EU or Kabat numbering; or (c) the Fc region further comprises a S228P amino acid substitution according to EU or Kabat numbering. In some examples that may be combined with any of the preceding examples, the antibody has an IgG4 isotype. In some examples that may be combined with any of the preceding examples, the anti-SIRPA antibody comprises an S228P amino acid substitution at residue position 228, an F234A amino acid substitution at residue position 234, and an L235A amino acid substitution at residue position 235, wherein the numbering of the residue position is according to EU or Kabat numbering.

[0015] In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is a bispecific antibody. In some examples, the anti-SIRPA antibody recognizes a first and a second antigen, wherein the first antigen is SIRPA and the second antigen is: (a) an antigen facilitating transport across the blood-brain-barrier; (b) an antigen facilitating transport across the blood-brain-barrier selected from the group consisting of transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and

2), diphtheria toxin receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an angiopep peptide, and ANG1005; (c) a disease-causing agent selected from the group consisting of disease-causing peptides or proteins or, disease-causing nucleic acids, wherein the disease-causing nucleic acids are antisense GGCCCC (G2C4) repeat-expansion RNA, the disease-causing proteins are selected from the group consisting of amyloid beta, oligomeric amyloid beta, amyloid beta plaques, amyloid precursor protein or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, C9orf72 (chromosome 9 open reading frame 72), c9RAN protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, ataxin 1, ataxin 2, ataxin 3, ataxin 7, ataxin 8, ataxin 10, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, ubiquitin, and proline-arginine (PR) repeat peptides; and (d) ligands and/or proteins expressed on immune cells, wherein the ligands and/or proteins selected from the group consisting of PD1/PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, CD73, and phosphatidylserine; and a protein, lipid, polysaccharide, or glycolipid expressed on one or more tumor cells.

[0016] In some examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is a conjugated antibody. For example, the anti-SIRPA antibody may be conjugated to a detectable marker, a toxin, or a therapeutic agent. In some examples, the anti-SIRPA antibody is conjugated to a toxin selected from the group consisting of ricin, ricin A chain, doxorubicin, daunorubicin, a maytansinoid, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, alpha sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, Saponaria officinalis inhibitor, glucocorticoid, auristatin, auromycin, yttrium, bismuth, combrestatin, duocarmycins, dolastatin, cc1065, and a cisplatin.

[0017] In further examples, which may be combined with any of the preceding examples, the anti-SIRPA antibody is used in combination with one or more antibodies that specifically bind a disease-causing protein selected from the group consisting of amyloid beta, oligomeric amyloid beta, amyloid beta plaques, amyloid precursor protein or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, C9orf72 (chromosome 9 open reading frame 72), prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, ataxin 1, ataxin 2, ataxin 3, ataxin 7, ataxin 8, ataxin 10, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, ubiquitin, and proline-arginine (PR) repeat peptides, and any combination thereof; or with one or more antibodies that bind an immunomodulatory protein selected from the group consisting of: PD1/PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, CD73, TREM1, TREM2, CD33, Siglec-5, Siglec-7, Siglec-9, Siglec-11, phosphatidylserine, disease-causing nucleic acids, antisense GGCCCC (G2C4) repeat-expansion RNA, and any combination thereof.

[0018] In a further example, the disclosure provides a method of decreasing the activity, functionality, or survival of regulatory T cells, tumor-imbedded immunosuppressor dendritic cells, tumor-imbedded immunosuppressor macrophages, myeloid-derived suppressor cells, tumor-associated macrophages, acute myeloid leukemia (AML) cells, chronic lymphocytic leukemia (CLL) cell, or chronic myeloid leukemia (CML) cells in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an agent that binds or interacts with SIRPA, *e.g.,* an antibody of any of the examples described above.

[0019] In an additional example, the disclosure provides a method of inducing or promoting the survival, maturation, functionality, migration, or proliferation of one or more immune cells in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an agent, *e.g.,* an antibody of any of the examples described above, that decreases cellular levels of SIRPA, inhibits interaction between SIRPA

and one or more SIRPA ligands, or both. In some embodimetns, the one or more immune cells are selected from the group consisting of dendritic cells, macrophages, neutrophils, NK cells, microglia, T cells, T helper cells, cytotoxic T cells, and any combination thereof.

[0020] In another example, the disclosure provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an anti-SIRPA antibody of any of the examples described above to a patient that has a tumor the expresses CD47.

[0021] Disclosed is a method of treating cancer, the method comprising administering a therapeutically effective amount of an agent, *e.g.,* an anti-SIRPA antibody of any of the examples described above, that decreases the cellular levels of SIRPA. In some examples, the method further comprises administering a therapeutic agent that inhibitis PD1, PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, CTLA4, PD-L2, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, or CD73. In some examples, the therapeutic agent is an antibody that inhibits PD1, PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, CTLA4, PD-L2, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, or CD73.

[0022] Disclosed is a method of treating cancer, the method comprising administering a therapeutically effective amount of an agent, *e.g.,* an anti-SIRPA antibody of any of the examples described above, that decreases the cellular levels of SIRPA. In some examples, the method further comprises administering to the individual at least one antibody that specifically binds to an inhibitory checkpoint molecule, and/or one or more standard or investigational anti-cancer therapies. Insa eom examples, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is administered in combination with the anti-SIRPA antibody. In some examples, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is selected from the group consisting of an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-PD-L2 antibody, an anti-PD-1 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, and anti-HVEM antibody, an anti- B- and T-lymphocyte attenuator (BTLA) antibody, an anti-Killer inhibitory receptor (KIR) antibody, an anti-GAL9 antibody, an anti-TIM-1 antibody, an anti-TIM3 antibody, an anti-TIM-4 antibody, an anti-A2AR antibody, an anti-CD39 antibody, an anti-CD73 antibody, an anti-LAG-3 antibody, an anti-phosphatidylserine

antibody, an anti-CD27 antibody, an anti-CD30 antibody, an anti-TNFa antibody, an anti-CD33 antibody, an anti-Siglec-5 antibody, an anti-Siglec-7 antibody, an anti-Siglec-9 antibody, an anti-Siglec-11 antibody, an antagonistic anti-TREM1 antibody, an antagonistic anti-TREM2 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-CD2 antibody, an anti-CD5 antibody, and any combination thereof. In some examples, which may be combined with any of the preceding examples, the one or more standard or investigational anti-cancer therapies are selected from the group consisting of radiotherapy, cytotoxic chemotherapy, targeted therapy, imatinib therapy, trastuzumab therapy, etanercept therapy, adoptive cell transfer (ACT) therapy, chimeric antigen receptor T cell transfer (CAR-T) therapy, vaccine therapy, and cytokine therapy.

[0023] In some examples, which may be combined with any of the preceding method examples, the method further comprises administering to the individual at least one antibody that specifically binds to an inhibitory cytokine. In some emodiments, the at least one antibody that specifically binds to an inhibitory cytokine is administered in combination with an anti-SIRPA antibody of any one of the preciding examples. In some examples, the at least one antibody that specifically binds to an inhibitory cytokine is selected from the group consisting of an anti-CCL2 antibody, an anti-CSF-1 antibody, an anti-IL-2 antibody, and any combination thereof. In some examples that may be combined with any of the preceding examples, the method further comprises administering to the individual at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein. In some examples, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is administered in combination with an anti-SIRPA antibody of any of the preceding embdoiments. In some examples, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is selected from the group consisting of an agonist anti-CD40 antibody, an agonist anti-OX40 antibody, an agonist anti-ICOS antibody, an agonist anti-CD28 antibody, an agonistic anti-TREM1 antibody, an agonistic anti-TREM2 antibody, an agonist anti-CD137/4-1BB antibody, an agonist anti-CD27 antibody, an agonist anti-glucocorticoid-induced TNFR-related protein GITR antibody, an agonist anti-CD30 antibody, an agonist anti-BTLA antibody, an agonist anti-HVEM antibody, an agonist anti-CD2 antibody, an agonist anti-CD5 antibody, and any combination thereof. In some examples, which may be combined with any of the preceding embodimetns, the method further comprises administering to the individual at least one stimulatory cytokine. In some embdoiments, the stimulatory cytokine

is selected from the group consisting of IFN-α4, IFN-β, IL-1β, TNF-α, IL-6, IL-8, CRP, IL-20 family members, LIF, IFN-γ, OSM, CNTF, GM-CSF, IL-11, IL-12, IL-15, IL-17, IL-18, IL-23, CXCL10, IL-33, MCP-1, MIP-1-beta, and any combination thereof.

[0024] In a further example, the disclosure provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an anti-SIRPA antibody of any one of the preceding examples to a subject that has cancer cells of a myeloid lineage that expresses SIRPA.

[0025] In another example, the disclosure provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an anti-SIRPA antibody of any one of the preceding examples to a subject that has a cancer, wherein the cancer is selected from the group consisting of sarcoma, bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, kidney cancer, renal pelvis cancer, leukemia, lung cancer, melanoma, lymphoma, pancreatic cancer, prostate cancer, ovarian cancer, and fibrosarcoma; or wherein the cancer is selected from the group consisting of glioblastoma multiforme; renal clear cell carcinoma; adrenocortical carcinoma; bladder urothelial carcinoma; diffuse large B-cell lymphoma; lung adenocarcinoma; pancreatic adenocarcinoma, renal cell cancer, non-Hodgkin's lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, breast invasive carcinoma, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, head and neck squamous cell carcinoma, kidney chromophobe, renal papillary cell carcinoma, lower grade glioma, hepatocellular carcinoma, lung squamous cell carcinoa, mesothelioma, ovarian serous cystadenomcarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarconimo, rectal adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thyumoma, uterine corpus endometrial carcinoma, uternine carcinosarcoma, and uveal melanoma. In some examples, the anti-SIRPa antibody is conjugated to a cytotoxic agent and/or induces ADCC.

[0026] In another aspect, there is provided a pharmaceutical composition comprising an anti-SIRPA antibody described herein and a physiologically acceptable carrier. In some examples, the disclosure provides an anti-

SIRPA antibody of any one of the preceding examples for use in the treatment of cancer; and/or for use in a method of preparing a medicament for the treatment of cancer.

[0027] In a further example, the disclosure provides a method of preventing, reducing risk, or treating a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer's disease, vascular dementia, mixed dementia, taupathy disease, Parkinon's disease, mutliple sclerosis, amyotrophic lateral sclerosis, traumatic brain injury, stroke, frontotemporal dementia, spinal cord injury, Huntington's disease, infections, and cancer comprising administering to an individual in need thereof a therapeutically effective amount of an agent that decreases cellular levels of SIRPA, inhibits interaction between SIRPA and one or more SIRPA ligands, or both. In some examples, the disease, disorder, or injury is cancer and wherein the agents inhibits one or more SIRPA activities selected from the group consisting of: (a) promoting proliferation, maturation, migration, differentiation, and/or functionality of one or more of immunosuppressor dendritic cells, immunosuppressor macrophages, immunosuppressor neutrophils, immunosuppressor NK cells, myeloid derived suppressor cells, tumor-associated macrophages, tumor-associated suppressor neutrophils, tumor-associated suppressor NK cells, non-tumorigenic CD14+ myeloid cells, and regulatory T cells; (b) enhancing infiltration of one or more of immunosuppressor dendritic cells, immunosuppressor macrophages, immunosuppressor neutrophils, immunosuppressor NK cells, myeloid derived suppressor cells, tumor-associated macrophages, tumor-associated suppressor neutrophils, tumor-associated suppressor NK cells, and regulatory T cells into tumors; (c) increasing number of tumor-promoting myeloid/granulocytic immune-suppressive cells and/or non-tumorigenic CD14+ myeloid cells in a tumor, in peripheral blood, or other lymphoid organ; (d) enhancing tumor-promoting activity of myeloid-derived suppressor cells (MDSC) and/or non-tumorigenic CD14+ myeloid cells; (e) increasing expression of tumor-promoting cytokines in a tumor or in peripheral blood, optionally wherein the tumor-promoting cytokines are TGF-beta or IL-10; (f) increasing tumor infiltration of tumor-promoting FoxP3+ regulatory T lymphocytes; (g) decreasing activation of tumor-specific T lymphocytes with tumor killing potential; (h) decreasing infiltration of tumor-specific T lymphocytes with tumor killing potential; (i) decreasing infiltration of tumor-specific NK cells with tumor killing potential; (j) decreasing the tumor killing potential of NK cells; (k) decreasing infiltration of tumor-specific B lymphocytes with potential to enhance immune response; (l) increasing tumor volume; (m) increasing tumor growth rate; (n) increasing metastasis; (o) increasing rate of tumor

recurrence; (p) decreasing efficacy of one or more immune-therapies that modulate anti-tumor T cell responses, optionally wherein the one or more immune-therapies are immune-therapies that target one or more target proteins selected from the group consisting of PD1/PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, CD73, and any combination thereof, or one or more cancer vaccines; (q) inhibition of PLCγ/PKC/calcium mobilization; and (r) inhibition of PI3K/Akt, Ras/MAPK signaling. In some examples, which may be combined with any of the preceding examples, the disease, disorder, or injury is cancer, and wherein the agent exhibits one or more SIRPA activities selected from the group consisting of: (a) increasing the number of tumor infiltrating CD3+ T cells; (b) decreasing cellular levels of CD33 in non-tumorigenic CD14+myeloid cells, optionally wherein the non-tumorigenic CD14+ myeloid cells are tumor infiltrating cells or optionally wherein the non-tumorigenic CD14+ myeloid cells are present in blood; (c) reducing the number of non-tumorigenic CD14+ myeloid cells, optionally wherein the non-tumorigenic CD14+ myeloid cells are tumor infiltrating cells or optionally wherein the non-tumorigenic CD14+ myeloid cells are present in blood; (d) reducing PD-L1 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (e) reducing PD-L2 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (f) reducing B7-H2 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (g) reducing B7-H3 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (h) reducing CD200R levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (i) reducing CD163 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (j) reducing CD206 levels in one or more cells, optionally wherein the one or more cells are non-tumorigenic myeloid-derived suppressor cells (MDSC); (k) decreasing tumor growth rate of solid tumors; (l) reducing tumor volume; (m) increasing efficacy of one or more PD-1 inhibitors; (n) increasing efficacy of one or more checkpoint inhibitor therapies and/or immune-modulating therapies, optionally wherein the one or more checkpoint inhibitor therapies and/or immune-modulating therapies target one or more of CTLA4, the adenosine pathway, PD-L1, PD-L2, OX40, TIM3, LAG3, or any combination thereof; (o) increasing efficacy of one or more chemotherapy agents, optionally wherein the

one or more of the chemotherapy agents are gemcitabine, capecitabine, anthracyclines, doxorubicin (Adriamycin®), epirubicin (Ellence®), taxanes, paclitaxel (Taxol®), docetaxel (Taxotere®), 5-fluorouracil (5-FU), cyclophosphamide (Cytoxan®), carboplatin (Paraplatin®), and any combination thereof; (p) increasing proliferation of T cells in the presence of non-tumorigenic myeloid-derived suppressor cells (MDSC); (q) inhibiting differentiation, survival, and/or one or more functions of non-tumorigenic myeloid-derived suppressor cells (MDSC); and (r) killing CD33-expressing immunosuppressor myeloid cells and/or CD14-expressing cells in solid tumors and associated blood vessels when conjugated to a chemical or radioactive toxin. In some examples, the cancer expresses SIRPA or one or more SIRPA ligands.

[0028] In a further example, the disclosure provides a method of treating, preventing or decreasing risk of a disease, disorder, or injury, comprising an agent that down-regulates SIRPA, wherein the disease, disorder, or injury is selected from the group consisting of dementia, frontotemporal dementia, Alzheimer's disease, vascular dementia, mixed dementia, taupathy disease, Parkinon's disease, mutliple sclerosis, amyotrophic lateral sclerosis, traumatic brain injury, stroke, frontotemporal dementia, spinal cord injury, and Huntington's disease. In some examples, the agent is an anti-SIRPA antibody, *e.g.,* of any one of the preceding examples, that downregulates SIRPA.

[0029] In another aspect, there is provided a polynucleotide comprising a nucleic acid sequence encoding a V_H region and a V_L region of an anti-SIRPA antibody described herein. In a further example, the disclosure provides: a polynucleotide comprising a nucleic acid sequence encoding a V_H region of an anti-SIRPA antibody of any one of the examples described herein; and/or a polynucleotide comprising a nucleic acid sequence encoding a V_L region of an anti-SIRPA antibody of any one of the examples described herein. In another aspect, there is provided an expression vector comprising the polynucleotide described herein. In a further example, the disclosure provides an expression vector comprising the polynucleotide comprising the nucleic acid sequence encoding the V_H region or an expression vector comprising the polynucleotide comprising the nucleic acid sequence encoding the V_L region. In some examples, the disclosure provides an expression vector that comprises the polynucleotide encoding the V_H region and the polynucleotide encoding the V_L region. In another aspect, there is provided a host cell comprising the polynucleotide described herein. In an additional example, the disclosure provides a host cell comprising the polynucleotide comprising the nucleic acid sequence encoding the V_H region or host cell comprising the polynucleotide comprising the nucleic acid sequence encoding the V_L region. In some examples, the disclosure provides a host cell that comprises the polynucleotide encoding the V_H region and the polynucleotide encoding the V_L region. In another aspect, there is provided a host cell comprising an expression vector described herein.

In another aspect, there is provided a host cell that expresses the anti-SIRPA antibody described herein. In another aspect, there is provided a method of producing an anti-SIRPA antibody, the method comprising culturing a host cell described herein under conditions in which the antibody is expressed. In some examples, the host cell is a mammalian host cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1A shows an amino acid sequence alignment between the two most common alleles of human SIRPA protein (v1 (SEQ ID NO:1 and v2 (SEQ ID NO:45)) depicting the divergent residues within the ligand-binding domain. Accession numbers are NP542970 and CAA71403, respectively.

[0031] FIG. 1B shows an amino acid sequence alignment between the human SIRPA v1 protein (SEQ ID NO:1) and the human SIRPB1 protein (SEQ ID NO:46), depicting the homology between the two proteins. Accession numbers are NP542970 and O00241, respectively.

[0032] FIG. 2 shows an amino acid sequence alignment between the human SIRPA protein (SEQ ID NO:1) and the mouse SIRPA protein (SEQ ID NO:47), depicting the homology between the two proteins. Accession numbers are NP542970 and Q6P6I8, respectively.

[0033] FIG. 3A shows FACS histograms on the left panel of selected SIRPA antibodies binding to the rodent Chinese hamster ovary cell line (CHO) expressing either human SIRPA (HuSIRPA) or mouse SIRPA (MuSIRPA). Shaded histograms represent the CHO-MuSIRPA cells. Black outlined histograms represent the CHO-HuSIRPA cells. The right panel presents the relative MFI values of SIRPA antibodies binding HuSIRPA compared to MuSIRPA. Results are expressed as fold over background. The background level is set to 1 on y-axis. Antibody mIgG is the isotype negative control. FIG. 3B shows FACS histograms of selected SIRPA antibodies binding to primary human macrophages. Antibody mIgG represents a negative isotype control. Shaded histograms represent the cells stained with anti-mouse IgG secondary antibody only. Black outlined histograms represent the SIRPA positive cell population. FIG. 3C shows surface plasmon resonance sensorgrams of indicated anti-SIRPA antibodies binding to recombinant soluble HuSIRPA protein. An anti-mouse IgG antibody immobilized on a CM5 chip captured anti-SIRPA antibodies and serial dilutions of His-tagged soluble HuSIRPA protein flowed over the antibody. Kd values were determined by curve fitting analysis. FIG. 3D shows binding of increasing concentrations of anti-SIRPA

WHAT IS CLAIMED IS:

1. An isolated anti-signal regulatory protein α (SIRPA) antibody, wherein the anti-SIRPA antibody comprises a heavy chain variable region (VH region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:9, a CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a CDR3 comprising the amino acid sequence of SEQ ID NO:11; and a light chain variable region (VL region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8.

2. The anti-SIRPA antibody of claim 1, wherein the VH region comprises the amino acid sequence of hSB-3F9-H1 or hSB-3F9-H2 in Figure 14A; or comprises a VH region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of hSB-3F9-H1 or hSB-3F9-H2 in Figure 14A.

3. The anti-SIRPA antibody of claim 1 or claim 2, wherein the VL region comprises the amino acid sequence of hSB-3F9-L1, hSB-3F9-L2, or hsB-3F9-L3 in Figure 14B; or comprises a VL region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of hSB-3F9-L1, hSB-3F9-L2, or hsB-3F9-L3 in Figure 14B.

4. An isolated anti-signal regulatory protein α (SIRPA) antibody, wherein the anti-SIRPA antibody comprises: a heavy chain variable region (VH region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:15, a CDR2 comprising the amino acid sequence of SEQ ID NO:16, and a CDR3 comprising the amino acid sequence of SEQ ID NO:17; and a light chain variable region (VL region) comprising: a CDR1 comprising the amino acid sequence of SEQ ID NO:12, a CDR2 comprising the amino acid sequence of SEQ ID NO:13, and a CDR3 comprising the amino acid sequence of SEQ ID NO:14.

5. The anti-SIRPA antibody of claim 4, wherein the VH region comprises the amino acid sequence of hSB-9C2-H1, hSB-9C2-H2, hSB-9C2-H3, or hSB-9C2-H4 in Figure 14C; or comprises a VH region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to

the amino acid sequence of hSB-9C2-H1, hSB-9C2-H2, hSB-9C2-H3, or hSB-9C2-H4 in Figure 14C.

6. The anti-SIRPA antibody of claim 4 or claim 5, wherein the VL region comprises the amino acid sequence of hSB-9C2-L1, hSB-9C2-L2, hSB-9C2-L3, or hSB-9C2-L4 in Figure 14D; or comprises a VL region having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of hSB-9C2-L1, hSB-9C2-L2, hSB-9C2-L3, or hSB-9C2-L4 in Figure 14D.

7. The isolated anti-SIRPA antibody of any one of claims 1 to 6, wherein the antibody does not substantially block binding of CD47 to SIRPA expressed on cells, wherein binding of the antibody to SIRPA decreases the level of SIRPA expressed on the cell surface, and/or wherein the antibody binds to the D2 or D3 domain of SIRPA.

8. The anti-SIRPA antibody of any one of claims 1 to 7, wherein the antibody is a monoclonal antibody.

9. The anti-SIRPA antibody of any one of claims 1 to 8, wherein the antibody is a humanized antibody.

10. The anti-SIRPA antibody of any one of claims 1 to 9, wherein the antibody is an Fab, Fab', Fab'-SH, F(ab')2, Fv or scFv fragment.

11. The anti-SIRPA antibody of any one of claims 1 to 9, wherein the anti-SIRPA antibody has an IgG1, IgG2, IgG3, or IgG4 isotype.

12. The anti-SIRPA antibody of claim 11, wherein the antibody binds to an inhibitory Fc receptor.

13. The anti-SIRPa antibody of claim 12, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγRIIB).

14. The anti-SIRPA antibody of claim 13, wherein:

(a) the anti-SIRPA antibody has a human IgG1 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: N297A, D265A, D270A, L234A, L235A, G237A, P238D, L328E, E233D, G237D, H268D, P271G, A330R, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, and any combination

thereof, wherein the numbering of the residues is according to EU numbering, or comprises an amino acid deletion in the Fc region at a position corresponding to glycine 236;

(b) the anti-SIRPA antibody has an IgG1 isotype and comprises an IgG2 isotype heavy chain constant domain 1(CH1) and hinge region, optionally wherein the IgG2 isotype CH1 and hinge region comprises the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGVHTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVERKCCVECPPCP (SEQ ID NO:34), and optionally wherein the antibody Fc region comprises a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, wherein the numbering of the residues is according to EU numbering;

(c) the anti-SIRPA antibody has an IgG2 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: P238S, V234A, G237A, H268A, H268Q, V309L, A330S, P331S, C214S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, H268E, N297A, N297Q, A330L, and any combination thereof, wherein the numbering of the residues is according to EU numbering;

(d) the anti-SIRPA antibody has a human IgG4 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, E233P, F234V, L234A/F234A, S228P, S241P, L248E, T394D, N297A, N297Q, L235E, and any combination thereof, wherein the numbering of the residues is according to EU numbering; or

(e) the anti-SIRPA antibody has a hybrid IgG2/4 isotype, and optionally wherein the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU numbering.

15. The anti-SIRPA antibody of claim 13, wherein:

(a) the anti-SIRPA antibody has a human IgG1 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: N297A, N297Q, D270A, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V, P238A, A327Q, A327G, P329A, K322A, L234F, L235E, P331S, T394D, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering;

(b) the anti-SIRPA antibody has an IgG2 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: P238S, V234A, G237A, H268A, H268Q, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering; or

(c) the anti-SIRPA antibody has an IgG4 isotype and comprises one or more amino acid substitutions in the Fc region at a residue position selected from the group consisting of: E233P, F234V, L234A/F234A, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

16. The anti-SIRPA antibody of claim 15, wherein:

(a) the Fc region comprises one or more amino acid substitutions at a position selected from the group consisting of A330L, L234F; L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU numbering;

(b) the Fc region comprises one or more amino acid substitutions at a position selected from the group consisting of M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering; or

(c) the Fc region comprises a S228P amino acid substitution according to EU numbering.

17. The anti-SIRPA antibody of claim 11, wherein the antibody has an IgG4 isotype, and wherein the anti-SIRPA antibody comprises an S228P amino acid substitution at residue

position 228, an F234A amino acid substitution at residue position 234, and an L235A amino acid substitution at residue position 235, wherein the numbering of the residue position is according to EU numbering.

18. The anti-SIRPA antibody of any one of claims 1 to 9, wherein the anti-SIRPA antibody has a human IgG1 isotype and comprises substitution P331S; or substitutions L234A, L235A, and P331S; or substitutions S267E and L328F, wherein the numbering of the residues is according to EU numbering.

19. The anti-SIRPA antibody of any one of claims 1 to 18, wherein the antibody is a bispecific antibody.

20. The anti-SIRPA antibody of claim 19, wherein the bispecific antibody recognizes a first and a second antigen, wherein the first antigen is SIRPA and the second antigen is:

(a) an antigen facilitating transport across the blood-brain-barrier;

(b) an antigen facilitating transport across the blood-brain-barrier selected from the group consisting of transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an angiopep peptide, and ANG1005;

(c) a disease-causing agent selected from the group consisting of disease-causing peptides or proteins or, disease-causing nucleic acids, wherein the disease-causing nucleic acids are antisense GGCCCC (G2C4) repeat-expansion RNA, the disease-causing proteins are selected from the group consisting of amyloid beta, oligomeric amyloid beta, amyloid beta plaques, amyloid precursor protein or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, C9orf72 (chromosome 9 open reading frame 72), c9RAN protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, ataxin 1, ataxin 2, ataxin 3, ataxin 7, ataxin 8, ataxin 10, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine

(GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, ubiquitin, and proline-arginine (PR) repeat peptides; and

(d) ligands and/or proteins expressed on immune cells, wherein the ligands and/or proteins selected from the group consisting of PD1/PDL1, CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, LIGHT, BTLA, CD30, TIGIT, VISTA, KIR, GAL9, TIM1, TIM3, TIM4, A2AR, LAG3, DR-5, CD2, CD5, CD39, CD73, and phosphatidylserine; and a protein, lipid, polysaccharide, or glycolipid expressed on one or more tumor cells.

21. The anti-SIRPA antibody of any one of claims 1 to 20, wherein the anti-SIRPA antibody is conjugated to a detectable marker, a toxin, or a therapeutic agent.

22. The anti-SIRPA antibody of claim 21, wherein the anti-SIRPA antibody is conjugated to a toxin selected from the group consisting of ricin, ricin A chain, doxorubicin, daunorubicin, a maytansinoid, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, alpha sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, Saponaria officinalis inhibitor, glucocorticoid, auristatin, auromycin, yttrium, bismuth, combrestatin, duocarmycins, dolastatin, cc1065, and a cisplatin.

23. A pharmaceutical composition comprising an anti-SIRPA antibody of any one of claims 1 to 22 and a physiologically acceptable carrier.

24. A polynucleotide comprising a nucleic acid sequence encoding a VH region and a VL region of an anti-SIRPA antibody of any one of claims 1 to 22.

25. An expression vector comprising the polynucleotide of claim 24.

26. A host cell comprising the polynucleotide of claim 24.

27. A host cell that expresses the anti-SIRPA antibody of any one of claims 1 to 22.

28. A host cell comprising the expression vector of claim 25.

29. A method of producing an anti-SIRPA antibody, the method comprising culturing a host cell of any one of claims 26 to 28 under conditions in which the antibody is expressed.

30. The method of claim 29, wherein the host cell is a mammalian host cell.