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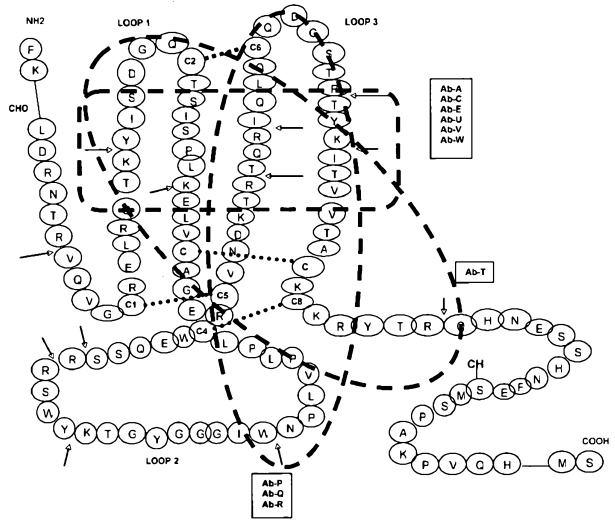
[Continued on next page]

(54) Title: WISE BINDING ANTIBODIES AND EPITOPES

(57) Abstract: The present invention relates to binding agents for WISE such as antibodies, and includes for their manufacture and use.

Figure 27



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WISE BINDING AGENTS AND EPITOPES

[0001] This application claims the benefit of U.S. Provisional Application No. 61/004,037, filed November 21, 2007, which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0001a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[0002] Fibrosis is generally defined as the development of extra connective tissue as part of the healing process and includes a diverse set of symptoms. Excessive fibrosis is a grievous problem that has few therapeutic options.

[0003] Cystine knot-containing proteins are typically important regulators of key functions and affect diverse cell types. WISE (USAG-1, SOSTDC1) is a secreted, cystine knot-containing protein and is expressed primarily in the kidney, lungs and epithelial cells. WISE KO mice are fertile and their kidneys have normal function. However when challenged to develop kidney injury either by unilateral ureteral obstruction (UUO) or injection of chemotoxic agent Cisplatin, the WISE KO mice are protected (Yanagita *et al.*, J. Clin Invest. 2006 January 4; 116(1): 70–79). In the UUO model, there is much less fibrosis in the affected kidney in WISE KO mice and expressed much less aSMA, a marker of myofibroblast activation, and preserved the expression of epithelial cell marker E-cadherin. In a Cisplatin model for kidney injury, WISE deletion protected the animal from tubular injury and reduced mortality (Tanaka *et al.*, Kidney International advance online publication 17 October 2007). In addition, when WISE KO mice (aka USAG-1 KO mice) were bred with Col4a3 KO mice, the double knockout mice had significantly less proteinuria and developed less end stage renal disease relative to the Col4a3 KO mice with WT WISE gene. At 4 weeks of age, USAG-1+/+, 3(IV)-/- mice already showed severe proteinuria with extensive splitting of glomerular basement membrane (GBM), while double KO mice showed normal

structure of GBM. At 10 weeks of age, USAG-1+/+, 3(IV)-/- mice developed end-stage renal disease, while double KO mice showed significantly preserved renal function with less renal histological changes. (Abstract TH-FC059 2008 ASN meeting).

[0004] These data suggest that WISE could be a regulator of adult kidney function. However these studies were limited to knock out mice lacking WISE for their entire development cycle, accordingly it was unpredictable whether acute inhibition of WISE activity using an inhibitor such as an antibody could provide therapeutic benefit to preserve kidney function under pathological conditions associated with various fibrotic diseases.

[0005] The present inventors demonstrate it is possible to treat lung and kidney disorders associated with damage and repair including fibrosis and organ dysfunction using binding agents that target WISE.

BRIEF SUMMARY OF THE INVENTION

[0005a] According to a first aspect, the present invention provides an isolated antibody or fragment thereof that binds to a T49 epitope, or a T56.1 epitope of WISE.

[0005b] According to a second aspect, the present invention provides a method of treating fibrosis comprising administering an antibody according to any one of the preceding claims that has an affinity of at less than $1x10^{-7}M$ and inhibits WISE activity.

[0005c] According to a third aspect, the present invention provides a method of treating proteinuria comprising administering an antibody according to the first aspect that has an affinity of at less than $1x10^{-7}M$ and inhibits WISE activity.

[0005d] According to a fourth aspect, the present invention provides a pharmaceutical composition comprising the antibody or fragment according to the first aspect.

[0005e] According to a fifth aspect, the present invention provides an immunogenic polypeptide of WISE suitable for use in producing inhibitory antibodies, the peptide corresponding to a T49 epitope or a T56.1 epitope of WISE.

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[0005f] According to a sixth aspect, the present invention provides use of an antibody according to the first aspect in the manufacture of a medicament for the treatment of fibrosis; or for the treatment of proteinuria.

[0005g] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

[0006] Disclosed herein are compositions and methods that can be used to prevent or treat kidney and lung fibrosis as well as prevent or treat lung and kidney damage, disease and/or injury, and may be used to treat a wide variety of diseases and disorders described herein.

[0007] The invention further relates to regions of human WISE recognized by the binding agents disclosed herein, methods of using these regions, and methods of making such regions.

[0008] The invention also relates to epitopes specific to the region of WISE identified as the cystine knot domain, and binding agents which specifically bind to that region.

[0009] The invention relates to binding agents, such as antibodies, that specifically bind to WISE. The binding agents can be characterized by their ability to cross-block the binding of at least one antibody disclosed herein to WISE and/or to be cross-blocked from binding WISE by at least one of said

antibodies. The antibodies and other binding agents of the invention can also be characterized by their binding pattern to human WISE peptides in a human WISE peptide epitope competition binding assay as disclosed herein.

[0010] In certain embodiments, the invention relates to binding agents such as antibodies that inhibit WISE activity and that can decrease tissue injury and associated fibrosis in tissues such as the kidneys, lungs, skin, eye, liver and heart. In addition, the invention relates to binding agents that inhibit proteinuria which is associated with various immunological and non-immune mediated renal diseases such as in patients with diabetic nephropathy, glomerolonephritis, membrane nephropathy, lupus, transplantation and other renal diseases involving manifestation of increased proteinuria. Furthermore the invention relates to binding agents that improve the function of organs or delay the loss of function in organs mentioned above that are impacted due to either fibrosis and/or proteinuria including but not limited to diseases such as chronic kidney diseases, chronic allograft nephropathy, idiopathic pulmonary fibrosis, cardiomyopathy, glaucoma (lens cell fibrosis) and scleroderma (skin fibrosis). In addition, as tumor metastasis also using similar mechanisms to those used in tissue fibrosis, WISE binding agent may also have utility in delaying tumor metastasis and /or cancer progression.

[0011] In other embodiments, the invention relates to binding agents, such as antibodies, that can block the inhibitory effect of WISE in a cell based assay.

[0012] The invention further relates in part to polypeptide constructs comprising two, three, or four polypeptide fragments linked by at least one disulfide bond, representing a core region of the cystine-knot of WISE, and antibodies capable of specifically binding thereto.

[0013] In one embodiment, the invention relates to methods of obtaining epitopes suitable for use as immunogens for generating, in mammals, binding agents, such as antibodies capable of binding specifically to WISE; in certain embodiments the binding agents generated are capable of neutralizing WISE activity in vitro and/or in vivo.

[0014] In another embodiment, the invention relates to a composition for eliciting an antibody specific for WISE when the composition is administered to an animal, the composition comprising a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, and 8.

[0015] In other embodiments, the invention also relates to a composition for eliciting an antibody specific for WISE when the composition is administered to an animal, the composition comprising at least one polypeptide consisting essentially of the amino acid sequence of human, mouse, rat or cynomolgus WISE (SEQ ID NOs: 2, 4, 6, or 8).

[0016] In a specific embodiment, the invention also relates to polypeptide consisting essentially of a multiply truncated human WISE protein of SEQ ID NO: 2, wherein amino acids 1 to 70, 113 to 126, and 171 to 206 of SEQ ID NO: 2 are absent from the polypeptide; this polypeptide may be obtained by recombinant expression of fragments of the protein, tryptic digestion of human WISE, and the protein may be isolated by HPLC fractionation among other methods.

[0017] In another specific embodiment, the invention further relates to an immunogenic portion of the cystine knot of human WISE comprising amino acids 71 to 112 and 127 to 170 of SEQ ID NO:2, wherein the immunogenic portion comprises at least one of:

[0018] (a) a disulfide bond between amino acids C1 and C5;

[0019] (b) a disulfide bond between amino acids C2 and C6; and

[0020] (c) a disulfide bond between amino acids C3 and C7;

[0021] the immunogenic portion may have at least two of these disulfide bonds; and the immunogenic portion may have all three disulfide bonds.

[0022] In one embodiment, the invention relates to a method of generating an antibody capable of specifically binding to WISE, comprising: (a) immunizing an animal with a composition comprising a polypeptide having amino acids 24-206 of SEQ ID NO: 2, a polypeptide having amino acids 24-206 of SEQ ID NO: 4, a polypeptide having amino acids 24-206 of SEQ ID NO: 6, and a polypeptide

having amino acids 24-206 of SEQ ID NO: 8; (b) collecting sera from the animal; and (c) isolating from the sera an antibody capable of specifically binding to and inhibiting the biological activity of WISE.

[0023] In additional embodiments, the invention also relates to a method of generating an antibody capable of specifically binding to WISE, the method comprising: (a) immunizing an animal with a composition comprising a cystine knot-containing fragment of WISE or a derivative thereof; (b) collecting sera from the animal; and (c) isolating from the sera an antibody capable of specifically binding to and inhibiting the biological activity of WISE.

[0024] In further embodiments, the invention further relates to a method of detecting an anti-WISE antibody in a biological sample, comprising the steps of (a) contacting the biological sample with a polypeptide consisting essentially of a polypeptide having amino acids 24 to 206 of SEQ ID NO: 2, a polypeptide having amino acids 24 to 206 of SEQ ID NO: 4, a polypeptide having amino acids 24 to 206 of SEQ ID NO: 6, and a polypeptide having amino acids 24 to 206 of SEQ ID NO: 8 under conditions allowing a complex to form between the antibody and the polypeptide; and (b) detecting the presence or absence of the complex, wherein the presence of the complex indicates that the biological sample contains an anti-WISE antibody.

[0025] In other embodiments, the invention comprises a method of detecting an anti-WISE antibody in a biological sample, comprising the steps of (a) contacting the biological sample with a composition comprising a cystine knot-containing fragment of WISE under conditions allowing a complex to form between the antibody and the polypeptide; and (b) detecting the presence or absence of the complex, wherein the presence of the complex indicates that the biological sample contains an anti-WISE antibody.

[0026] In certain embodiments, the invention relates to a WISE binding agent, such as an antibody that cross-blocks the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, and Ab-X

to a WISE protein. In other embodiments, the invention relates to a WISE binding agent, such as an antibody that cross-blocks the binding of at least one of antibodies Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 to a WISE protein.

[0027] The WISE binding agent may also be cross-blocked from binding to WISE by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, and Ab-X. The WISE binding agent may also be cross-blocked from binding to WISE by at least one of antibodies Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9.

[0028] In these embodiments, the invention further relates to a WISE binding agent, such as an antibody, that is cross-blocked from binding to WISE by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, and Ab-X. In these embodiments, the invention further relates to a WISE binding agent, such as an antibody, that is cross-blocked from binding to WISE by at least one of antibodies Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9.

[0029] In yet other embodiments, the invention relates to a binding agent, such as an isolated antibody that exhibits a similar binding pattern to human WISE peptides in a "human WISE peptide epitope competition binding assay" as that exhibited by at least one of the antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9; the isolated antibody, or an antigen-

binding fragment thereof, may be a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, or a chimeric antibody.

[0030] The invention still further relates to a method for treating a renal and/or lung fibrotic disease or disorder in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-WISE binding agent sufficient to decrease symptoms associated with the disorder, wherein the anti-WISE binding agent comprises an antibody, or WISE-binding fragment thereof.

[0031] Provided herein are antibodies that specifically bind to human WISE. The antibodies of the invention are characterized by their ability to cross-block the binding of at least one antibody disclosed herein to human WISE and/or to be cross-blocked from binding human WISE by at least one antibody disclosed herein. The invention also provides is an isolated antibody, or an antigen-binding fragment thereof, that can block the effect of WISE in a cell based assay.

[0032] Also provided is a binding agent, such as an antibody, that specifically binds to WISE and comprises at least one CDR sequence selected from SEQ ID NOs: 123, 124, 125, 127, 128, 129, 131, 132, 133, 135, 136, 137, 139, 140, 141, 143, 144, 145, 147, 148, 149, 151, 152, 153, 155, 156, 157, 158, 159, 160, 164, 167, 168, 170, 171, 173, 174, 175, 177, 178, 179, 181, 182, 183, 185, 186, 187, 189, 190, 191, 193, 194, 195, 197, 198, 199, 201, 202, 203, 205, 206, 207, 209, 210, 211, 213, 214, 215, 217, 218, 219, 221, 222, 223, 225, 226, 227, 229, 230, 231, 233, 234, 235, 237, 238, 239, 241, 242, 243, 245, 246, 247, 249, 250, 251, 253, 254, 255, 257, 258, 259, 261, 262, 263, 273, 274, 275, 277, 278 and 279, and variants thereof.

[0010] In another embodiment the invention contemplates a binding agent comprising three CDR sequences selected from the groups consisting of: CDR sequences of SEQ ID NOs: 123, 124, and 125; CDR sequences of SEQ ID NOs: 127, 128, and 129; CDR sequences of SEQ ID NOs: 131, 132, and 133; CDR sequences of SEQ ID NOs: 135, 136, and 137; CDR sequences of SEQ ID NOs: 139, 140, and 141; CDR sequences of SEQ ID NOs: 143, 144, and 145; CDR

sequences of SEQ ID NOs: 147, 148, and 149; CDR sequences of SEQ ID NOs: 151, 152, and 153; CDR sequences of SEQ ID NOs: 155, 156, and 157; CDR sequences of SEQ ID NOs: 158, 159, and 160; CDR sequences of SEQ ID NOs: 161, 162, and 163; CDR sequences of SEQ ID NOs: 164, 165, and 166; CDR sequences of SEQ ID NOs: 167, 168, and 169; CDR sequences of SEQ ID NOs: 170, 171, and 172; CDR sequences of SEQ ID NOs: 173, 174, and 175; CDR sequences of SEQ ID NOs: 177, 178, and 179; CDR sequences of SEQ ID NOs: 181, 182, and 183; CDR sequences of SEQ ID NOs: 185, 186 and 187; CDR sequences of SEQ ID NOs: 189, 190, and 191; CDR sequences of SEQ ID NOs: 193, 194, and 195; CDR sequences of SEQ ID NOs: 197, 198, and 199; CDR sequences of SEQ ID NOs: 201, 202, and 203; CDR sequences of SEQ ID NOs: 205, 206 and 207; CDR sequences of SEQ ID NOs: 209, 210, and 211; CDR sequences of SEQ ID NOs: 213, 214, and 215; CDR sequences of SEQ ID NOs: 217, 218, and 219; CDR sequences of SEQ ID NOs: 221, 222, and 223; CDR sequences of SEQ ID NOs: 225, 226, and 227; CDR sequences of SEQ ID NOs: 229, 230, and 231; CDR sequences of SEQ ID NOs: 233, 234, and 235; CDR sequences of SEQ ID NOs: 237, 238, and 239; CDR sequences of SEQ ID NOs: 241, 242, and 243; CDR sequences of SEQ ID NOs: 245, 246, and 247; CDR sequences of SEQ ID NOs: 249, 250, and 251; CDR sequences of SEQ ID NOs: 253, 254, and 255; CDR sequences of SEQ ID NOs: 257, 258, and 259; CDR sequences of SEQ ID NOs: 261, 262 and 263; CDR sequences of SEQ ID NOs: 273, 274 and 275; or CDR sequences of SEQ ID NOs: 277, 278 and 279.

[0034] In one embodiment the invention contemplates a binding agent comprising six CDR sequences selected from the groups consisting of: SEQ ID NOs: 123, 124, and 125 and CDR sequences of SEQ ID NOs: 127, 128, and 129; CDR sequences of SEQ ID NOs: 131, 132, and 133 and CDR sequences of SEQ ID NOs: 135, 136, and 137; CDR sequences of SEQ ID NOs: 139, 140, and 141 and CDR sequences of SEQ ID NOs: 143, 144, and 145; CDR sequences of SEQ ID NOs: 147, 148, and 149 and CDR sequences of SEQ ID NOs: 151, 152, and 153; CDR sequences of SEQ ID NOs: 155, 156, and 157 and CDR sequences of SEQ ID NOs: 158, 159, and 160; CDR sequences of SEQ ID NOs: 161, 162, and

163 and CDR sequences of SEQ ID NOs: 164, 165, and 166; CDR sequences of SEQ ID NOs: 167, 168, and 169 and CDR sequences of SEQ ID NOs: 170, 171, and 172; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 173, 174, and 175; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 177, 178, and 179; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 181, 182, and 183; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 185, 186 and 187; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 189, 190, and 191; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 193, 194, and 195; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 197, 198, and 199; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 201, 202, and 203; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 205, 206 and 207; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 209, 210, and 211; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 213, 214, and 215; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 217, 218, and 219; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 221, 222, and 223; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 225, 226, and 227; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 229, 230, and 231; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 233, 234, and 235; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 237, 238, and 239; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 241, 242, and 243; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 245, 246, and 247; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 249, 250, and 251; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 253, 254, and 255; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences

of SEQ ID NOs: 257, 258, and 259; CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 261, 262 and 263; or CDR sequences of SEQ ID NOs: 273, 274 and 275 and CDR sequences of SEQ ID NOs: 277, 278 and 279.

[0035] Also provided is a binding agent, such as an antibody, that specifically binds to WISE and has at least one CDR sequence derived from SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 60, 62, 64, 66, 68, 70, 72, 74, 266 and 268 and variants thereof, wherein the antibody or antigen-binding fragment thereof neutralizes WISE in vitro and/or in vivo.

[0036] Also provided is an antibody that specifically binds to WISE where the heavy chain is selected from Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, and Ab-X and the light chain has been identified via screening, such as use of phage display. Examples of such light chain sequences are depicted in SEQ ID NOs: 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118 and 120.

[0037] These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entireties as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] FIG. 1: Bioactivity of antibodies in neutralizing WISE activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is human WISE with no antibody, and for each of the four antibodies 0.3 ug/ml of human WISE is mixed with the antibody before being added to the testing well.

[0039] FIG. 2: WISE dose-dependently inhibited Wnt-induced luciferase expression in MC3T3-E1 STF cells.

[0040] FIG. 3: Direct binding of anti-WISE Abs (Ab-C, Ab-E, Ab-A) to human WISE.

[0041] FIG. 4: Direct binding of anti-WISE Abs (Ab-A, Ab-B, Ab-G, Ab-I) to human WISE.

[0042] FIG. 5: Direct binding of anti-WISE Abs (Ab-C, Ab-D, Ab-J) to human WISE.

[0043] FIG. 6: Direct binding of anti-WISE Abs (Ab-C, Ab-J, Ab-N, Ab-O) to human WISE.

[0044] FIG. 7: Competition assay showing binding of Ab-C can be dose dependently cross-blocked by Ab-C, Ab-J, Ab-N and Ab-O.

[0045] FIG. 8: Bioactivity of various antibodies (Ab-C, Ab-J, Ab-N, Ab-D, Ab-P, Ab-R) in neutralizing WISE activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is human WISE with no antibody, and for each of the six antibodies 0.5 ug/ml of human WISE is mixed with the antibody before being added to the testing well.

[0046] FIG. 9A: Competition assay showing binding of digested human WISE peptides to mature antibodies Ab-A, Ab-C, and Ab-E.

[0047] FIG. 9B: Competition assay showing binding of digested human WISE peptides to mature antibody Ab-P.

[0048] FIG. 10: Binding assay shows that the impact of specific mutation in the human WISE protein on the binding of four anti-WISE Abs (Ab-A, Ab-C, Ab-E and Ab-P) to wild type human WISE protein.

[0049] FIG. 11: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-R to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5

ug/ml of human WISE mixed with the antibody R (Ab-R) before being added to the testing well.

[0050] FIG. 12: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-C to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody C (Ab-C) before being added to the testing well.

[0051] FIG. 13: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-A to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody A (Ab-A) before being added to the testing well.

[0052] FIG. 14: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-E to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody E (Ab-E) before being added to the testing well.

[0053] FIG. 15: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-U to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody U (Ab-U) before being added to the testing well.

[0054] FIG. 16: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-V to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody V (Ab-V) before being added to the testing well.

[0055] FIG. 17: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-W to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody W (Ab-W) before being added to the testing well.

[0056] FIG. 18: Cell-based assay shows that the impact of change of individual amino acid to Ala at each of the designated position on the ability of Ab-T to neutralize mutant WISE protein activity in MC3T3-E1 SuperTopFlash (STF) cells. For each set of columns, the first column shows no treatment, the second is 0.5 ug/ml human WISE or WISE mutant with no antibody, and the third is 0.5 ug/ml of human WISE mixed with the antibody T (Ab-T) before being added to the testing well.

[0057] FIG. 19: Diagram showing that the position of each mutation in the loop1 or loop3 of human WISE protein.

[0058] FIG. 20: Competition ELISA binding assay shows that the binding of Antibody S to human WISE can be cross-blocked by itself and Ab-P.

[0059] FIG. 21: Competition ELISA binding assay shows that the binding of Antibody C to human WISE can be cross-blocked completely by itself, Ab-A, Ab-E and partially by Ab-P.

[0060] FIG. 22: Competition ELISA binding assay shows that the binding of Antibody E to human WISE can be cross-blocked completely by itself, Ab-A, Ab-C and partially by Ab-P.

[0061] FIG. 23: Competition ELISA binding assay shows that the binding of Antibody A to human WISE can be cross-blocked completely by itself, Ab-C, Ab-E and partially by Ab-P.

[0062] FIG. 24: Competition ELISA binding assay shows that the binding of Antibody P to human WISE can be cross-blocked completely only by itself and partially by Ab-S.

[0063] FIG. 25: Competition ELISA binding assay shows that the binding of Antibody T to human WISE can be cross-blocked completely by itself, Ab-A, Ab-C, Ab-P and Ab-S.

[0064] FIG. 26: Competition ELISA binding assay shows that the binding of Antibody P and C to human WISE can be cross-blocked by Ab-T completely or partially respectively.

[0065] FIG. 27: The diagram shows the potential epitope areas covered by different antibodies based on competition binding assay and cell-based neutralizing assay. Arrows indicate the cleavage sites upon trypsin digestion.

[0066] FIG. 28: Prophylactic anti-WISE Mab Treatment reduced lung injury in Bleomycin induced lung injury model.

[0067] FIG. 29: Prophylactic anti-WISE Mab Treatment reduced collagen deposition in Bleomycin induced lung injury model.

[0068] FIG. 30: Prophylactic anti-WISE Mab Treatment reduced aSMA expression in Bleomycin induced lung injury model.

[0069] FIG. 31: Prophylactic anti-WISE Mab Treatment reduced serum OPN level in Bleomycin induced lung injury model.

[0070] FIG. 32: Prophylactic Treatment with Anti-WISE Mab Reduced Collagen Production in a Mouse Model of Bleomycin-induced lung fibrosis.

[0071] FIG. 33: Prophylactic Treatment with Anti-WISE Mab Reduced BAL OPN expression in a Mouse Model of Bleomycin-induced lung fibrosis.

[0072] FIG. 34: Histological analysis trends towards a moderate reduction in lung injury with WISE Ab.

[0073] FIG. 35: Histological analysis trends towards a moderate reduction in Sirius Red score with WISE Ab.

[0074] FIG. 36: Histological analysis trends towards a moderate reduction in fibrotic marker FSP1 expression with WISE Ab.

[0075] FIG. 37: Histological analysis trends towards a moderate reduction in fibrotic markeraSMA expression with WISE Ab.

[0076] FIG. 38: WISE Ab treatment moderately decreased serum OPN levels.

[0077] FIG. 39: WISE Antibody treatment reduced 24 hr Urinary Total Protein (UTP) in Col4a3 KO mice.

DETAILED DESCRIPTION

[0078] The present invention relates in part to regions of the WISE protein that contain epitopes recognized by antibodies that also bind to full-length WISE, and methods of making and using these epitopes. The invention also provides binding agents (such as antibodies) that specifically bind to WISE or portions of WISE, and methods for using such binding agents. The binding agents are useful to block or impair the binding of human WISE to one or more ligand(s) and its biological activity.

[0079] As used herein, the term human WISE is intended to include the protein of SEQ ID NO: 2 and allelic variants thereof. Orthologs of WISE are also described and include mouse, rat and cynomolgus (SEQ ID NOs: 4, 6, and 8, respectively). WISE can be purified from host cells that have been transfected by a gene encoding WISE by elution of filtered supernatant of host cell culture fluid.

The preparation and further purification are described in the Examples. Human WISE is described in U.S. Patent No. 5,780,263.

[0080] It will be understood by one of skill in the art that there is a high degree of sequence identity between the orthologs of WISE. Accordingly, binding agents to human WISE will be expected to bind to the mouse, rat or cynomolgus WISE in cases where the recognition site of the binding agent, e.g., an antibody binding site such as an epitope, is highly conserved and in particular nearly or completely identical to the human sequence . Thus, when the term "specific binding to WISE" is used, it is understood to include binding to multiple species of WISE where the sequences between species are conserved.

[0081] Examples of binding agents according to the invention include the following antibodies: Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9.

[0082] As used herein, Ab-A is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 9 and 11. Ab-B is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 13 and 15. Ab-C is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 17 and 19. Ab-D is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 21 and 23. Ab-E is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 25 and 27. Ab-F is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 29 and 31. Ab-G is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 13 and 33. Ab-H is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 21 and 35. Ab-I is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 37 and 39. Ab-J is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 41 and 43. Ab-K is comprised of the

polypeptides expressed by the nucleotides shown in SEQ ID NOs: 45 and 23. Ab-L is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 45 and 35. Ab-M is comprised of the mature polypeptides of SEQ ID NOs: 271 and 272. Ab-N is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 121 and 23. Ab-O is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 41 and 23. Ab-P is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 49 and 47. Ab-Q is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 55 and 57. Ab-R is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 53 and 51. Ab-S is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 61 and 59. Ab-T is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 265 and 267. Ab-U is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 65 and 63. Ab-V is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 69 and 67. Ab-W is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 73 and 71. Ab-X is comprised of the mature polypeptides of SEQ ID NOs: 269 and 270.

[0083] Ab-1 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 75 and 23. Ab-13 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 77 and 23. Ab-16 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 79 and 23. Ab-18 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 81 and 23. Ab-23 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 83 and 23. Ab-24 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 85 and 23. Ab-28 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 87 and 23. Ab-29 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 89 and 23. Ab-48 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 91 and 23. Ab-60 is comprised of the polypeptides expressed by the

nucleotides shown in SEQ ID NOs: 93 and 23. Ab-62 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 95 and 23. Ab-63 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 97 and 23. Ab-65 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 99 and 23. Ab-66 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 101 and 23. Ab-67 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 103 and 23. Ab-69 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 105 and 23. Ab-7 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 107 and 23. Ab-70 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 109 and 23. Ab-72 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 111 and 23. Ab-74 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 113 and 23. Ab-75 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 115 and 23. Ab-76 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 117 and 23. Ab-9 is comprised of the polypeptides expressed by the nucleotides shown in SEQ ID NOs: 119 and 23.

[0084] Binding agents of the invention are typically antibodies or fragments thereof, as defined herein. The term "antibody" refers to an intact antibody, or a binding fragment thereof. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include F(ab') 2, Fab, Fab', Fv, Fc, and Fd fragments, and can be incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (See e.g., Hollinger and Hudson, 2005, Nature Biotechnology, 23, 9, 1126-1136). Antibody polypeptides are also disclosed in U.S. Pat. No. 6,703,199, including fibronectin polypeptide monobodies. Other antibody polypeptides are disclosed in U.S. patent Publication 2005/0238646, which are single-chain polypeptides. As used herein, the isolated antibody or an

antigen-binding fragment thereof may be a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody or the like.

[0085] Antigen binding fragments derived from an antibody can be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of whole antibodies according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment termed F(ab')2. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. No. 4,331,647, Nisonoff et al., Arch. Biochem. Biophys. 89:230, 1960; Porter, Biochem. J. 73:119, 1959; Edelman et al., in Methods in Enzymology 1:422 (Academic Press 1967); and by Andrews, S. M. and Titus, J. A. in Current Protocols in Immunology (Coligan J. E., et al., eds), John Wiley & Sons, New York (2003). pages 2.8.1-2.8.10 and 2.10A.1-2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light-heavy chain fragments (Fd), further cleaving of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[0086] An antibody fragment may also be any synthetic or genetically engineered protein. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (scFv proteins).

[0087] Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units", or "hypervariable region") can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., Methods: A Companion to Methods in Enzymology 2:106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies. Production, Engineering and Clinical Application, Ritter et al. (eds.), page 166 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch et al., (eds.), page 137 (Wiley-Liss, Inc. 1995)).

[0088] Thus, in one embodiment, the binding agent comprises at least one CDR as described herein. The binding agent may comprise at least two, three, four, five or six CDR's as described herein. The binding agent further may comprise at least one variable region domain of an antibody described herein. The variable region domain may be of any size or amino acid composition and will generally comprise at least one CDR sequence responsible for binding to human WISE, for example CDR-H1, CDR-H2, CDR-H3 and/or the light chain CDRs specifically described herein and which is adjacent to or in frame with one or more framework sequences. In general terms, the variable (V) region domain may be any suitable arrangement of immunoglobulin heavy (VH) and/or light (VL) chain variable domains. Thus, for example, the V region domain may be monomeric and be a VH or VL domain, which is capable of independently binding human WISE with an affinity at least equal to $1x10^{-7}$M or less as described below. Alternatively, the V region domain may be dimeric and contain VH-VH, VH-VL, or VL-VL, dimers. The V region dimer comprises at least one VH and at least one VL chain that may be non-covalently associated (hereinafter referred to as FV). If desired, the chains may be covalently coupled either directly, for example via a disulfide

bond between the two variable domains, or through a linker, for example a peptide linker, to form a single chain Fv (scFV).

[0089] The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

[0090] The variable region domain may be covalently attached at a C-terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a VH domain that is present in the variable region domain may be linked to an immunoglobulin CH1 domain, or a fragment thereof. Similarly a VL domain may be linked to a CK domain or a fragment thereof. In this way, for example, the antibody may be a Fab fragment wherein the antigen binding domain contains associated VH and VL domains covalently linked at their C-termini to a CHI and CK domain, respectively. The CH1domain may be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody CH2 and CH3 domains.

[0091] As described herein, binding agents comprise at least one of these CDRs. For example, one or more CDR may be incorporated into known antibody framework regions (IgG1, IgG2, etc.), or conjugated to a suitable vehicle to enhance the half-life thereof. Suitable vehicles include, but are not limited to Fc, polyethylene glycol (PEG), albumin, transferrin, and the like. These and other suitable vehicles are known in the art. Such conjugated CDR peptides may be in monomeric, dimeric, tetrameric, or other form. In one embodiment, one or more

water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a binding agent.

[0092] In certain embodiments, a binding agent comprises one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. See, e.g., U.S. Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. In certain embodiments, a derivative binding agent comprises one or more of monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of such polymers. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains. In certain embodiments, PEG can act to improve the therapeutic capacity for a binding agent, such as an antibody. Certain such methods are discussed, for example, in U.S. Pat. No. 6,133,426, which is hereby incorporated by reference for any purpose.

[0093] It will be appreciated by one of skill in the art that a binding agent of the present invention may have at least one amino acid substitution, providing that the binding agent retains binding specificity. Therefore, modifications to the binding agent structures are encompassed within the scope of the invention. These may include amino acid substitutions, which may be conservative or non-conservative and that do not destroy the WISE binding capability of a binding agent. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. A conservative amino acid substitution may also involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position.

[0094] Non-conservative substitutions may involve the exchange of a member of one class of amino acids or amino acid mimetics for a member from another class with different physical properties (e.g. size, polarity, hydrophobicity, charge). Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

[0095] Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. Such testing can be done on the target of the binding agent as described below in the examples or on the therapeutic binding agent of the invention. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[0096] A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[0097] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or

structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0098] A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou et al., Biochemistry, 13(2):222-245 (1974); Chou et al., Biochemistry, 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47: 45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

[0099] Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-19 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, supra (1999), and Brenner, supra (1997)).

[00100] In certain embodiments, variants of binding agents include glycosylation variants wherein the number and/or type of glycosylation site has

been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the parent amino acid sequence. Cysteine variants may be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

[00101] Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to WISE, or to increase or decrease the affinity of the antibodies to WISE described herein.

[00102] According to certain embodiments, preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physiochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino

acid substitutions) may be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W.H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. Nature 354:105 (1991), which are each incorporated herein by reference.

[00103] In certain embodiments, binding agents of the invention may be chemically bonded with polymers, lipids, or other moieties.

[00104] The binding agents may comprise at least one of the CDRs described herein incorporated into a biocompatible framework structure. In one example, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a conformationally stable structural support, or framework, or scaffold, which is able to display one or more sequences of amino acids that bind to an antigen (e.g., CDRs, a variable region, etc.) in a localized surface region. Such structures can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

[00105] Typically the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostain, cytochrome b, CP1 zinc finger, PST1, coiled coil, LAC1-D1, Z domain and tendramisat domains may be

used (See e.g., Nygren and Uhlen, 1997, Current Opinion in Structural Biology, 7, 463-469).

[00106] In preferred embodiments, it will be appreciated that the binding agents of the invention include the humanized antibodies described herein. Humanized antibodies such as those described herein can be produced using techniques known to those skilled in the art (Zhang, W., et al., Molecular Immunology. 42(12): 1445-1451, 2005; Hwang W. et al., Methods. 36(1):35-42, 2005; Dall'Acqua W F, et al., Methods 36(1):43-60, 2005; and Clark, M., Immunology Today. 21(8):397-402, 2000).

[00107] Additionally, one skilled in the art will recognize that suitable binding agents include portions of these antibodies, such as one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as specifically disclosed herein. At least one of the regions of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 may have at least one amino acid substitution, provided that the binding agent retains the binding specificity of the non-substituted CDR. The non-CDR portion of the binding agent may be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to WISE and/or neutralizes WISE. The non-CDR portion of the binding agent may be a non-protein molecule in which the binding agent exhibits a similar binding pattern to human WISE peptides in a "human WISE peptide epitope competition binding assay" as that exhibited by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 and/or neutralizes WISE. The non-CDR portion of the binding agent may be composed of amino acids, wherein the binding agent is a recombinant binding protein or a synthetic peptide, and the recombinant binding protein cross-blocks the binding of an antibody disclosed herein to WISE and/or neutralizes WISE. The non-CDR portion of the binding agent may be composed of amino acids,

wherein the binding agent is a recombinant binding protein, and the recombinant binding protein exhibits a similar binding pattern to human WISE peptides in the human WISE peptide epitope competition binding assay (described hereinbelow) as that exhibited by at least one of the antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9, and/or neutralizes WISE.

[00108] In one embodiment, it is contemplated that one can use the antibody heavy chain as 'bait' in a library screen where the library is composed of human antibody light chains, to identify complementing human light chains where the reconstituted antibody binds to WISE. In this embodiment, the heavy chain is from an antibody specific to WISE and is mouse, chimeric or humanized. Ab-K heavy chain was used for this type of screen and several human light chain partners were identified that restored affinity for WISE, and importantly also inhibitory activity that was found in the parent mouse antibody. Such light chains are found in the examples below where the heavy chain shown in SEQ ID NO: 24 was used as bait, and these light chains are shown in SEQ ID NOs: 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, and 122.

[00109] Where an antibody comprises one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as described above, it may be obtained by expression from a host cell containing DNA coding for these sequences. A DNA coding for each CDR sequence may be determined on the basis of the amino acid sequence of the CDR and synthesized together with any desired antibody variable region framework and constant region DNA sequences using oligonucleotide synthesis techniques, site-directed mutagenesis and polymerase chain reaction (PCR) techniques as appropriate. DNA coding for variable region frameworks and constant regions is widely available to those skilled in the art

from genetic sequences databases such as GenBank.RTM. Each of the above-mentioned CDRs will be typically located in a variable region framework at positions 31-35 (CDR-H1), 50-65 (CDR-H2) and 95-102 (CDR-H3) of the heavy chain and positions 24-34 (CDR-L1), 50-56 (CDR-L2) and 89-97 (CDR-L3) of the light chain according to the Kabat numbering system (Kabat et al., 1987 in Sequences of proteins of Immunological Interest, U.S. Department of Health and Human Services, NIH, USA).

[00110] Once synthesized, the DNA encoding an antibody of the invention or fragment thereof may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision, ligation, transformation, and transfection using any number of known expression vectors. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as Escherichia coli (see, e.g., Pluckthun et al., 1989 Methods Enzymol. 178:497-515). In certain other embodiments, expression of the antibody or a fragment thereof may be preferred in a eukaryotic host cell, including yeast (e.g., Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Pichia pastoris), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma (such as a mouse NSO line), COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells.

[00111] One or more replicable expression vectors containing DNA encoding an antibody variable and/or constant region may be prepared and used to transform an appropriate cell line, for example, a non-producing myeloma cell line, such as a mouse NSO line or a bacteria, such as E. coli, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operatively linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well-known and routinely used. For example, basic molecular biology procedures are described by Maniatis et al. (Molecular

Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, New York, 1989; see also Maniatis et al, 3rd ed., Cold Spring Harbor Laboratory, New York, (2001)). DNA sequencing can be performed as described in Sanger et al. (PNAS 74:5463, (1977)) and the Amersham International plc sequencing handbook, and site directed mutagenesis can be carried out according to methods known in the art (Kramer et al., Nucleic Acids Res. 12:9441, (1984); Kunkel Proc. Natl. Acad. Sci. USA 82:488-92 (1985); Kunkel et al., Methods in Enzymol. 154:367-82 (1987); the Anglian Biotechnology Ltd handbook). Additionally, numerous publications describe techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors, and transformation and culture of appropriate cells (Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews (ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK); "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed.), Wiley Interscience, New York).

[00112] Where it is desired to improve the affinity of antibodies according to the invention containing one or more of the above-mentioned CDRs can be obtained by a number of affinity maturation protocols including maintaining the CDRs (Yang et al., J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutation strains of E. coli. (Low et al., J. Mol. Biol., 250, 350-368, 1996), DNA shuffling (Patten et al., Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al., J. Mol. Biol., 256, 7-88, 1996) and sexual PCR (Crameri, et al., Nature, 391, 288-291, 1998). All of these methods of affinity maturation are discussed by Vaughan et al. (Nature Biotechnology, 16, 535-539, 1998).

[00113] Other antibodies according to the invention may be obtained by conventional immunization and cell fusion procedures as described herein and known in the art. Monoclonal antibodies of the invention may be generated using a variety of known techniques. In general, monoclonal antibodies that bind to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler et al., Nature 256:495, 1975; Coligan et al. (eds.),

Current Protocols in Immunology, 1:2.5.12.6.7 (John Wiley & Sons 1991); U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.) (1980); and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); Picksley et al., "Production of monoclonal antibodies against proteins expressed in E. coli," in DNA Cloning 2: Expression Systems, 2nd Edition, Glover et al. (eds.), page 93 (Oxford University Press 1995)). Antibody fragments may be derived therefrom using any suitable standard technique such as proteolytic digestion, or optionally, by proteolytic digestion (for example, using papain or pepsin) followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques as described herein.

[00114] Monoclonal antibodies can be obtained by injecting an animal, for example, a rat, hamster, a rabbit, or preferably a mouse, including for example a transgenic or a knock-out, as known in the art, with an immunogen comprising human WISE of SEQ ID NO: 2, or a fragment thereof, according to methods known in the art and described herein. The presence of specific antibody production may be monitored after the initial injection and/or after a booster injection by obtaining a serum sample and detecting the presence of an antibody that binds to human WISE or peptide using any one of several immunodetection methods known in the art and described herein. From animals producing the desired antibodies, lymphoid cells, most commonly cells from the spleen or lymph node, are removed to obtain B-lymphocytes. The B lymphocytes are then fused with a drug-sensitized myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (e.g., inability to express endogenous Ig gene products, e.g., P3X63-Ag 8.653 (ATCC No. CRL 1580); NSO, SP20) to produce hybridomas, which are immortal eukaryotic cell lines. The lymphoid (e.g., spleen) cells and the myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low

density on a selective medium that supports the growth of hybridoma cells but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks, colonies of cells are observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to human WISE, using any one of a variety of immunoassays known in the art and described herein. The hybridomas are cloned (e.g., by limited dilution cloning or by soft agar plaque isolation) and positive clones that produce an antibody specific to WISE are selected and cultured. The monoclonal antibodies from the hybridoma cultures may be isolated from the supernatants of hybridoma cultures. An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (e.g., pristane-primed) to promote formation of ascites fluid containing the monoclonal antibody. Monoclonal antibodies can be isolated and purified by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)," in Methods in Molecular Biology, Vol. 10, pages 79-104 (The Humana Press, Inc. 1992)). Monoclonal antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the antibody (e.g., heavy or light chain isotype, binding specificity, etc.). Examples of a suitable ligand, immobilized on a solid support, include Protein A, Protein G, an anticonstant region (light chain or heavy chain) antibody, an anti-idiotype antibody, and a TGF-beta binding protein, or fragment or variant thereof.

[00115] An antibody of the present invention may also be a human monoclonal antibody. Human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include, but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (e.g., containing B lymphocytes), in vitro

immunization of human B cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein. For example, human monoclonal antibodies may be obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green et al., Nature Genet. 7:13, 1994; Lonberg et al., Nature 368:856, 1994; Taylor et al., Int. Immun. 6:579, 1994; U.S. Pat. No. 5,877,397; Bruggemann et al., 1997 Curr. Opin. Biotechnol. 8:455-58; Jakobovits et al., 1995 Ann. N. Y Acad. Sci. 764:525-35. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci (see also Bruggemann et al., Curr. Opin. Biotechnol. 8:455-58 (1997)). For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo B cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. Human monoclonal antibodies may be obtained by immunizing the transgenic mice, which may then produce human antibodies specific for WISE. Lymphoid cells of the immunized transgenic mice can be used to produce human antibody-secreting hybridomas according to the methods described herein. Polyclonal sera containing human antibodies may also be obtained from the blood of the immunized animals.

[00116] Another method for generating human antibodies of the invention includes immortalizing human peripheral blood cells by EBV transformation. See, e.g., U.S. Pat. No. 4,464,456. Such an immortalized B cell line (or lymphoblastoid cell line) producing a monoclonal antibody that specifically binds to WISE can be identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning-techniques. The stability of the lymphoblastoid cell line producing an anti-WISE antibody may be improved by fusing the transformed cell line with a murine myeloma to produce a

mouse-human hybrid cell line according to methods known in the art (see, e.g., Glasky et al., Hybridoma 8:377-89 (1989)). Still another method to generate human monoclonal antibodies is in vitro immunization, which includes priming human splenic B cells with human WISE, followed by fusion of primed B cells with a heterohybrid fusion partner. See, e.g., Boerner et al., 1991 J. Immunol. 147:86-95.

[00117] In certain embodiments, a B cell that is producing an anti-human WISE antibody is selected and the light chain and heavy chain variable regions are cloned from the B cell according to molecular biology techniques known in the art (WO 92/02551; U.S. Pat. No. 5,627,052; Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-48 (1996)) and described herein. B cells from an immunized animal may be isolated from the spleen, lymph node, or peripheral blood sample by selecting a cell that is producing an antibody that specifically binds to WISE. B cells may also be isolated from humans, for example, from a peripheral blood sample. Methods for detecting single B cells that are producing an antibody with the desired specificity are well known in the art, for example, by plaque formation, fluorescence-activated cell sorting, in vitro stimulation followed by detection of specific antibody, and the like. Methods for selection of specific antibody-producing B cells include, for example, preparing a single cell suspension of B cells in soft agar that contains human WISE. Binding of the specific antibody produced by the B cell to the antigen results in the formation of a complex, which may be visible as an immunoprecipitate. After the B cells producing the desired antibody are selected, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA according to methods known in the art and described herein.

[00118] An additional method for obtaining antibodies of the invention is by phage display. See, e.g., Winter et al., 1994 Annu. Rev. Immunol. 12:433-55; Burton et al., 1994 Adv. Immunol. 57:191-280. Human or murine immunoglobulin variable region gene combinatorial libraries may be created in phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or

multimers thereof) that bind specifically to TGF-beta binding protein or variant or fragment thereof. See, e.g., U.S. Pat. No. 5,223,409; Huse et al., 1989 Science 246:1275-81; Sastry et al., Proc. Natl. Acad. Sci. USA 86:5728-32 (1989); Alting-Mees et al., Strategies in Molecular Biology 3:1-9 (1990); Kang et al., 1991 Proc. Natl. Acad. Sci. USA 88:4363-66; Hoogenboom et al., 1992 J. Molec. Biol. 227:381-388; Schlebusch et al., 1997 Hybridoma 16:47-52 and references cited therein. For example, a library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a variant thereof, in frame with the sequence encoding a phage coat protein. A fusion protein may be a fusion of the coat protein with the light chain variable region domain and/or with the heavy chain variable region domain. According to certain embodiments, immunoglobulin Fab fragments may also be displayed on a phage particle (see, e.g., U.S. Pat. No. 5,698,426).

[00119] Heavy and light chain immunoglobulin cDNA expression libraries may also be prepared in lambda phage, for example, using lambda ImmunoZap TM (H) and lambda ImmunoZap TM (L) vectors (Stratagene, La Jolla, Calif.). Briefly, mRNA is isolated from a B cell population, and used to create heavy and light chain immunoglobulin cDNA expression libraries in the lambda ImmunoZap(H) and lambda ImmunoZap(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse et al., supra; see also Sastry et al., supra). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from E. coli.

[00120] In one embodiment, in a hybridoma the variable regions of a gene expressing a monoclonal antibody of interest are amplified using nucleotide primers. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. (See, e.g., Stratagene (La Jolla, Calif.), which sells primers for mouse and human variable regions including, among others, primers for VHa, VHb, VHc, VHd, CHI, VL and CL

regions.) These primers may be used to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP TM H or ImmunoZAP TM (Stratagene), respectively. These vectors may then be introduced into E. coli, yeast, or mammalian-based systems for expression. Large amounts of a single-chain protein containing a fusion of the VH and VL domains may be produced using these methods (see Bird et al., Science 242:423-426, 1988).

[00121] Once cells producing antibodies according to the invention have been obtained using any of the above-described immunization and other techniques, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA therefrom according to standard procedures as described herein. The antibodies produced therefrom may be sequenced and the CDRs identified and the DNA coding for the CDRs may be manipulated as described previously to generate other antibodies according to the invention.

[00122] Preferably the binding agents specifically bind to WISE. As with all binding agents and binding assays, one of skill in this art recognizes that the various moieties to which a binding agent should not detectably bind in order to be therapeutically effective and suitable would be exhaustive and impractical to list. Therefore, for a binding agent disclosed herein, the term "specifically binds" refers to the ability of a binding agent to bind to WISE, preferably human WISE, with greater affinity than it binds to an unrelated control protein. Preferably the control protein is hen egg white lysozyme. Preferably the binding agents bind to WISE with an affinity that is at least, 50, 100, 250, 500, 1000, or 10,000 times greater than the affinity for a control protein. A binding agent may have a binding affinity for human WISE of less than or equal to $1x10^{-7}$M, less than or equal to $1x10^{-8}$M, less than or equal to $1x10^{-9}$M, less than or equal to $1x10^{-10}$ M, less than or equal to $1x10^{-11}$ M, or less than or equal to $1x10^{-12}$ M.

[00123] Affinity may be determined by an affinity ELISA assay. In certain embodiments, affinity may be determined by a BIAcore assay. In certain embodiments, affinity may be determined by a kinetic method. In certain

embodiments, affinity may be determined by an equilibrium/solution method. Such methods are described in further detail herein or known in the art.

[00124] WISE binding agents of the present invention preferably modulate WISE function in the cell-based assay described herein and/or the in vivo assay described herein and/or bind to one or more of the epitopes described herein and/or cross-block the binding of one of the antibodies described in this application and/or are cross-blocked from binding WISE by one of the antibodies described in this application. Accordingly such binding agents can be identified using the assays described herein.

[00125] In certain embodiments, binding agents are generated by first identifying antibodies that bind to one more of the epitopes provided herein and/or neutralize in the cell-based and/or in vivo assays described herein and/or cross-block the antibodies described in this application and/or are cross-blocked from binding WISE by one of the antibodies described in this application. The CDR regions from these antibodies are then used to insert into appropriate biocompatible frameworks to generate WISE binding agents. The non-CDR portion of the binding agent may be composed of amino acids, or may be a non-protein molecule. The assays described herein allow the characterization of binding agents. Preferably the binding agents of the present invention are antibodies as defined herein.

[00126] It will be understood by one skilled in the art that some proteins, such as antibodies, may undergo a variety of posttranslational modifications during expression and secretion from host cells. The type and extent of these modifications often depends on the host cell line used to express the protein as well as the culture conditions. Such modifications may include variations in glycosylation, methionine or tryptophan oxidation, diketopiperizine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of Chromatography 705:129-134, 1995). Once the proteins have been expressed and

processed they are in a 'mature' form. Thus it is understood that the invention includes mature antibodies that result from expression of the DNAs of the invention.

[00127] Antibodies disclosed herein bind to regions of human WISE which are important for the in vivo activity of the protein thereby inhibiting the activity of WISE. Binding of an antibody to WISE can be correlated with changes in biomarkers associated with kidney function, for example urinary levels of albumin or 24 hours total urinary protein excretion. Methods of constructing and expressing antibodies and fragments thereof comprising CDR's of the present invention are known to those of skill in the art.

[00128] An oligopeptide or polypeptide is within the scope of the invention if it has an amino acid sequence that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to at least one of the CDR's depicted in Table 1; and/or to a CDR of a WISE binding agent that cross-blocks the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9to WISE, and/or is cross-blocked from binding to WISE by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9; and/or to a CDR of a WISE binding agent wherein the binding agent can block the inhibitory effect of WISE in a cell based assay (i.e. a WISE neutralizing binding agent); and/or to a CDR of a WISE binding agent that binds to a cystine knot domain epitope.

[00129] WISE binding agent polypeptides and antibodies are within the scope of the invention if they have amino acid sequences that are at least 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a variable region of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9, and cross-block the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 to WISE, and/or are cross-blocked from binding to WISE by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9; and/or can block the inhibitory effect of WISE in a cell based assay (i.e. a WISE neutralizing binding agent); and/or bind to a cystine knot domain epitope.

[00130] Polynucleotides encoding WISE binding agents are within the scope of the invention if they have polynucleotide sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polynucleotide encoding a variable region of at least one of antibodies Ab-A, Ab-B, and Ab-C, and wherein the encoded WISE binding agents cross-block the binding of at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 to WISE, and/or are cross-blocked from binding to WISE by at least one of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13,

Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9; and/or can block the inhibitory effect of WISE in a cell based assay (i.e. a WISE neutralizing binding agent); and/or bind to a cystine knot domain epitope.

[00131] The affinity of a binding agent such as an antibody or binding partner, as well as the extent to which a binding agent (such as an antibody) inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard et al. (Ann. N.Y. Acad. Sci. 51:660-672 (1949)) or by surface plasmon resonance (SPR; BIAcore, Biosensor, Piscataway, N.J.). For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al., Cancer Res. 53:2560-65 (1993)).

[00132] An antibody according to the present invention may belong to any immunoglobin class, for example IgG, IgE, IgM, IgD, or IgA. It may be obtained from or derived from an animal, for example, fowl (e.g., chicken) and mammals, which includes but is not limited to a mouse, rat, hamster, rabbit, or other rodent, cow, horse, sheep, goat, camel, human, or other primate. The antibody may be an internalizing antibody. Production of antibodies is disclosed generally in U.S. patent Publication No. 2004/0146888 A1.

Characterization Assays

[00133] In the methods described herein to generate antibodies according to the invention, including the manipulation of the specific Ab-A, Ab-B, Ab-C, Ab-D,

Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 CDRs into new frameworks and/or constant regions, appropriate assays are available to select the desired antibodies or binding agents (i.e. assays for determining binding affinity to WISE; cross-blocking assays; Biacore-based "human WISE peptide epitope competition binding assay;" MC3T3-E1 cell based assay; in vivo assays).

Epitope Binding Assays

[00134] The unprocessed human WISE is 206 amino acids with the signal peptide and the mature form of human WISE is a 183 amino acid glycoprotein containing a cystine-knot motif. Due to conservation of key amino acid residues, particularly the cysteines, it is believed that WISE has a structure similar to previously described cysteine knot proteins. This structure includes, in addition to the cystine-knot motif, three loops designated as Loop 1, Loop 2 and Loop 3. As used herein, the positions of the loops are defined as approximately at amino acids 75 to 104 of SEQ ID NO: 2 for Loop 1; Loop 2 is approximately at amino acids 105 to 132; and Loop 3 is approximately at amino acids 134 to 170 of SEQ ID NO:2. It is understood that approximate positions mean that the relative positions could be plus or minus 2 amino acids carboxy terminal or amino terminal of the stated positions.

[00135] Human WISE was subjected to proteolytic digestion to produce fragments. Briefly, using different proteases, including trypsin, aspN, and lysC, fragments with various cleavage sites and sizes were generated. The sequences and mass for various human WISE peptides were determined. Antibody protection was evaluated to determine the effect on accessibility for proteolysis, including clipped site masking and peptide shifting. Finally, a BIAcore-based "human WISE peptide epitope competition assay" was performed.

[00136] One such fragment called T49 consists essentially of a multiply truncated human WISE protein of SEQ ID NO:2, wherein amino acids 1 to 70, 113 to 126, and 171 to 206 of SEQ ID NO:2 are absent from the polypeptide; this polypeptide may be obtained by tryptic digestion of human WISE, and the protein may be isolated by HPLC fractionation. This fragment is an immunogenic portion of the cystine knot of human WISE comprising amino acids 71 to 112 and 127 to 170 of SEQ ID NO:2, wherein the immunogenic portion comprises at least one of: (a) a disulfide bond between amino acids C1 and C5; (b) a disulfide bond between amino acids C2 and C6; and (c) a disulfide bond between amino acids C3 and C7; the immunogenic portion may have at least two of these disulfide bonds; and the immunogenic portion may have all three disulfide bonds.

[00137] Another fragment called T56.1 consists essentially of a multiply truncated human WISE protein of SEQ ID NO: 2, wherein amino acids 1 to 70, 122 to 126, and 171 to 206 of SEQ ID NO: 2 are absent from the polypeptide; this polypeptide may be obtained by tryptic digestion of human WISE, and the protein may be isolated by HPLC fractionation. This fragment is an immunogenic portion of the cystine knot of human WISE comprising amino acids 71 to 121 and 127 to 170 of SEQ ID NO: 2, wherein the immunogenic portion comprises at least one of: (a) a disulfide bond between amino acids C1 and C5; (b) a disulfide bond between amino acids C2 and C6; and (c) a disulfide bond between amino acids C3 and C7; the immunogenic portion may have at least two of these disulfide bonds; and the immunogenic portion may have all three disulfide bonds.

[00138] One group of antibodies exhibits a specific pattern of binding to certain epitopes as evidenced by a Biacore-based "human WISE peptide epitope competition binding assay." Briefly, the antibody is preincubated with the epitope to be tested, at concentrations that will saturate the epitope-binding sites on the antibody. The antibody is then exposed to WISE bound to a chip surface. After the appropriate incubation and washing procedures, a pattern of competitive binding is established.

[00139] Cross-Blocking Assays

[00140] The terms "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to WISE.

[00141] The extent to which an antibody or other binding agent is able to interfere with the binding of another to WISE, and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One particularly suitable quantitative assay uses a Biacore machine which can measure the extent of interactions using surface plasmon resonance technology. Another suitable quantitative cross-blocking assay uses an ELISA-based approach to measure competition between antibodies or other binding agents in terms of their binding to WISE.

Biacore Cross-Blocking Assay

[00142] The following generally describes a suitable Biacore assay for determining whether an antibody or other binding agent cross-blocks or is capable of cross-blocking according to the invention. For convenience reference is made to two antibodies, but it will be appreciated that the assay can be used with any of the WISE binding agents described herein. The Biacore machine (for example the Biacore 3000) is operated in line with the manufacturer's recommendations.

[00143] Thus in one cross-blocking assay, WISE is coupled to a CM5 Biacore chip using standard amine coupling chemistry to generate a WISE-coated surface. Typically 200-800 resonance units of WISE would be coupled to the chip (an amount that gives easily measurable levels of binding but that is readily saturable by the concentrations of test reagent being used).

[00144] The two antibodies (termed A* and B*) to be assessed for their ability to cross-block each other are mixed at a one to one molar ratio of binding sites in a suitable buffer to create the test mixture. When calculating the concentrations on a binding site basis the molecular weight of an antibody is assumed to be the

total molecular weight of the antibody divided by the number of WISE binding sites on that antibody.

[00145] The concentration of each antibody in the test mix should be high enough to readily saturate the binding sites for that antibody on the WISE molecules captured on the Biacore chip. The antibodies in the mixture are at the same molar concentration (on a binding basis) and that concentration would typically be between 1.00 and 1.5 micromolar (on a binding site basis).

[00146] Separate solutions containing antibody A* alone and antibody B* alone are also prepared. Antibody A* and antibody B* in these solutions should be in the same buffer and at the same concentration as in the test mix.

[00147] The test mixture is passed over the WISE-coated Biacore chip and the total amount of binding recorded. The chip is then treated in such a way as to remove the bound antibodies without damaging the chip-bound WISE. Typically this is done by treating the chip with 30 mM HCl for 60 seconds.

[00148] The solution of antibody A* alone is then passed over the WISE-coated surface and the amount of binding recorded. The chip is again treated to remove all of the bound antibody without damaging the chip-bound WISE.

[00149] The solution of antibody B* alone is then passed over the WISE-coated surface and the amount of binding recorded.

[00150] The maximum theoretical binding of the mixture of antibody A* and antibody B* is next calculated, and is the sum of the binding of each antibody when passed over the WISE surface alone. If the actual recorded binding of the mixture is less than this theoretical maximum then the two antibodies are cross-blocking each other.

[00151] Thus, in general, a cross-blocking antibody or other binding agent according to the invention is one which will bind to WISE in the above Biacore cross-blocking assay such that during the assay and in the presence of a second antibody or other binding agent of the invention the recorded binding is between 80% and 0.1% (e.g. 80% to 4%) of the maximum theoretical binding, specifically

between 75% and 0.1% (e.g. 75% to 4%) of the maximum theoretical binding, and more specifically between 70% and 0.1% (e.g. 70% to 4%) of maximum theoretical binding (as just defined above) of the two antibodies or binding agents in combination.

[00152] The Biacore assay described above is an assay used to determine if antibodies or other binding agents cross-block each other according to the invention. On rare occasions particular antibodies or other binding agents may not bind to WISE coupled via amine chemistry to a CM5 Biacore chip (this usually occurs when the relevant binding site on WISE is masked or destroyed by the coupling to the chip). In such cases cross-blocking can be determined using a tagged version of WISE, for example N-terminal His-tagged WISE. In this particular format, an anti-His antibody would be coupled to the Biacore chip and then the His-tagged WISE would be passed over the surface of the chip and captured by the anti-His antibody. The cross blocking analysis would be carried out essentially as described above, except that after each chip regeneration cycle, new His-tagged WISE would be loaded back onto the anti-His antibody coated surface. In addition to the example given using N-terminal His-tagged WISE, C-terminal His-tagged WISE could alternatively be used. Furthermore, various other tags and tag binding protein combinations that are known in the art could be used for such a cross-blocking analysis (e.g. HA tag with anti-HA antibodies; FLAG tag with anti-FLAG antibodies; biotin tag with streptavidin).

Elisa-Based Cross-Blocking Assay

[00153] The following generally describes an ELISA assay for determining whether an anti-WISE antibody or other WISE binding agent cross-blocks or is capable of cross-blocking according to the invention. For convenience, reference is made to two antibodies, but it will be appreciated that the assay can be used with any of the WISE binding agents described herein.

[00154] The general principal of the assay is to have an anti-WISE antibody coated onto the wells of an ELISA plate. An excess amount of a second, potentially cross-blocking, anti-WISE antibody is added in solution (i.e. not bound to the ELISA plate). A limited amount of WISE is then added to the wells. The coated antibody and the antibody in solution compete for binding of the limited number of WISE molecules. The plate is washed to remove WISE that has not been bound by the coated antibody and to also remove the second, solution phase antibody as well as any complexes formed between the second, solution phase antibody and WISE. The amount of bound WISE is then measured using an appropriate WISE detection reagent. An antibody in solution that is able to cross-block the coated antibody will be able to cause a decrease in the number of WISE molecules that the coated antibody can bind relative to the number of WISE molecules that the coated antibody can bind in the absence of the second, solution phase, antibody.

[00155] This assay is described in more detail further below for Ab-A, Ab-C and Ab-E. In the instance where Ab-A is chosen to be the immobilized antibody, it is coated onto the wells of the ELISA plate, after which the plates are blocked with a suitable blocking solution to minimize non-specific binding of reagents that are subsequently added. An excess amount of Ab-C is then added to the ELISA plate such that the moles of Ab-C WISE binding sites per well are at least 10 fold higher than the moles of Ab-A WISE binding sites that were used, per well, during the coating of the ELISA plate.

[00156] WISE is then added such that the moles of WISE added per well are at least 25-fold lower than the moles of Ab-A WISE binding sites that were used for coating each well. Following a suitable incubation period the ELISA plate is washed and a WISE detection reagent is added to measure the amount of WISE specifically bound by the coated anti-WISE antibody (in this case Ab-A). The background signal for the assay is defined as the signal obtained in wells with the coated antibody (in this case Ab-A), second solution phase antibody (in this case Ab-B), WISE buffer only (i.e. no WISE) and WISE detection reagents. The

positive control signal for the assay is defined as the signal obtained in wells with the coated antibody (in this case Ab-A), second solution phase antibody buffer only (i.e. no second solution phase antibody), WISE and WISE detection reagents. The ELISA assay needs to be run in such a manner so as to have the positive control signal at least 3 times the background signal.

[00157] To avoid any artifacts (e.g. significantly different affinities between Ab-A and Ab-B for WISE) resulting from the choice of which antibody to use as the coating antibody and which to use as the second (competitor) antibody, the cross-blocking assay needs to be run in two formats: 1) format 1 is where the first antibody is the antibody that is coated onto the ELISA plate and second antibody is the competitor antibody that is in solution and 2) format 2 is where the first and second antibody are reversed in coating and solution.

Cell Based Neutralization Assay

[00158] MC3T3-E1 SuperTopFlash (STF) reporter cells are used to determine whether WISE protein can modulate Wnt signaling. The activation of TCF-dependent signaling in MC3T3-E1 STF cells can be triggered using either endogenous Wnt signaling induced by switching culturing medium to differentiation medium or by adding exogenous Wnt such as Wnt3a. Recombinant WISE protein derived from either E coli or mammalian cell can dose-dependently inhibit Wnt signaling in MC3T3-E1 STF cells.

[00159] Luciferase assay: a vial of MC3T3-E1/STF cells is plated into a culture flask in expansion medium. When the cells are confluent they are trypsinized and cells in expansion medium plated into each well in 96 well plate. Next day all expansion medium is removed and replaced with 100 ul of freshly prepared differentiation medium.

[00160] Half of the differentiation medium (50 ul) was replaced with freshly prepared differentiation medium every day for the next four days. After five days of differentiation, all medium is replaced with testing samples in the fresh

differentiation medium in 100 ul volume. The plates are then allowed to incubate for 24 hours before luciferase signal are measured. Luciferase signal is measured upon removal of medium from testing plates and addition of 20 ul of 1x lysis buffer that has been equilibrated to room temperature. The plate is sealed and rocked for 30 minutes at room temperature and 100 ul of luciferase assay reagent was added to each well and the signal was captured using Luminometer (LMAX, Molecular Device) according to manufacturer's instruction.

In Vivo Neutralization Assay

[00161] Increases in various parameters associated with, or that result from, renal protection or pulmonary protection can be measured as an output from in vivo testing of WISE binding agents in order to identify those binding agents that are able to neutralize WISE and provide a therapeutic benefit. Such parameters include various renal / pulmonary markers and histomorphometric markers of renal / pulmonary health. A WISE neutralizing binding agent is defined as one capable of causing a statistically significant increase, as compared to vehicle treated animals, in any parameter associated with, or that results from, the stimulation of renal / pulmonary protection. Such in vivo testing can be performed in any suitable mammal (e.g. mouse, rat, monkey).

Formulation and Delivery of Therapeutics

[00162] Pharmaceutical compositions are provided, comprising one of the above-described binding agents such as at least one of antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-E, Ab-F, Ab-G, Ab-H, Ab-I, Ab-J, Ab-K, Ab-L, Ab-M, Ab-N, Ab-O, Ab-P, Ab-Q, Ab-R, Ab-S, Ab-T, Ab-U, Ab-V Ab-W, Ab-X, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9 to human WISE, along with a pharmaceutically or physiologically acceptable carrier, excipient, or diluent.

[00163] The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., subcutaneous, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

[00164] In certain applications, the pharmaceutical compositions disclosed herein may be delivered via oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

[00165] In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein subcutaneously, parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U.S. Pat. No. 5,543,158; U.S. Pat. No. 5,641,515 and U.S. Pat. No. 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

[00166] Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U.S. Pat. No. 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol,

polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00167] In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, Remington's Pharmaceutical Sciences, 15th ed., pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

[00168] In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric,

mandelic, and the like. 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, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[00169] The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

[00170] In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

[00171] The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, Trends Biotechnol. 16(7):307-21, 1998; Takakura, Nippon Rinsho 56(3):691-95, 1998; Chandran et al., Indian J. Exp. Biol. 35(8):801-09, 1997; Margalit, Crit. Rev. Ther. Drug Carrier Syst. 12(2-3):233-61, 1995; U.S. Pat. No. 5,567,434; U.S. Pat. No. 5,552,157; U.S. Pat. No. 5,565,213; U.S. Pat.

No. 5,738,868 and U.S. Pat. No. 5,795,587, each specifically incorporated herein by reference in its entirety). The use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery. In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)).

[00172] Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero et al., Drug Dev. Ind. Pharm. 24(12):1113-28, 1998). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 um) may be designed using polymers able to be degraded in vivo. Such particles can be made as described, for example, by Couvreur et al., Crit. Rev. Ther. Drug Carrier Syst. 5(1):1-20, 1988; zur Muhlen et al., Eur. J. Pharm. Biopharm. 45(2):149-55, 1998; Zambaux et al., J Controlled Release 50(1-3):31-40, 1998; and U.S. Pat. No. 5,145,684.

[00173] In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

[00174] The dose administered may range from 0.01 mg/kg to 200 mg/kg of body weight. Typical dosages are between 30 mg/kg and 75 mg/kg. However, as will be evident to one of skill in the art, the amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and

so forth. Typically, the compositions may be administered by a variety of techniques, as noted above.

Method of Treatment Using WISE Binding Agents

[00175] "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

[00176] "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[00177] As used in the context of treating renal disorders or diseases, the phrase "therapeutically effective amount" is meant to refer to an amount of therapeutic or prophylactic WISE antibody that provides a reduction in renal damage or deterioration, or that provides a reduction in the severity or progression of symptoms associated with renal disease, such as fibrosis and proteinuria (i.e. that provides "therapeutic efficacy"). As used in the context of treating fibrosis the phrase "therapeutically effective amount" is meant to refer to an amount of therapeutic or prophylactic WISE antibody that provides a reduction in fibroid elements or their precursors, and/or that provides a reduction in the severity or progression of symptoms associated with fibrotic disease (i.e. that provides "therapeutic efficacy"), e.g., proteinuric glomerula disease.

[00178] In one embodiment, the compositions of the invention are contemplated to be useful for treating, reducing and/or preventing renal dysfunction including those selected from the group consisting of proteinuric glomerula disease, end stage renal disease, chronic renal disease, IgA nephropathy, Bartter's syndrome, Gitelman syndrome, nephrolithiasis, renal amyloidosis, hypertension, primary aldosteronism, Addison's disease; renal

failure; glomerulonephritis and chronic glomerulonephritis: tubulointerstitial nephritis; cystic disorders of the kidney and dysplastic malformations such as polycystic disease, renal dysplasias, and cortical or medullary cysts; inherited polycystic renal diseases (PRD), such as recessive and autosomal dominant PRD; medullary cystic disease; medullary sponge kidney and tubular dysplasia; Alport's syndrome; non-renal cancers which affect renal physiology, such as bronchogenic tumors of the lungs or tumors of the basal region of the brain; multiple myeloma; adenocarcinomas of the kidney; metastatic renal carcinoma; in addition, nephrotoxic disorders include any functional or morphologic change in the kidney produced by any pharmaceutical, chemical, or biological agent that is ingested, injected, inhaled, or absorbed. Some broad categories of common nephrotoxic agents include but are not limited to immune suppressants, such as calcineurin inhibitors, heavy metals, all classes of antibiotics, analgesics, solvents, oxalosis-inducing agents, anticancer drugs, herbicides and pesticides, botanicals and biologicals, and antiepileptics.

[00179] The phrase "fibrotic-reducing activity" is meant to refer to the ability to inhibit, fully or partially, fibroid formation or to remove or reduce existing fibrosis. Thus, in one embodiment the compositions of the present invention are contemplated to be useful for treat fibrotic diseases, including pathological fibrosis or scarring (including endocardial sclerosis), idiopathic interstitial fibrosis, interstitial pulmonary fibrosis, perimuscular fibrosis, Symmers' fibrosis, pericentral fibrosis, hepatitis, dermatofibroma, billary cirrhosis, alcoholic cirrhosis, acute pulmonary fibrosis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, kidney fibrosis/glomerulonephritis, kidney fibrosis/diabetic nephropathy, scleroderma/systemic, scleroderma/local, keloids, hypertrophic scars, severe joint adhesions/arthritis, myelofibrosis, corneal scarring, cystic fibrosis, muscular dystrophy (duchenne's), cardiac fibrosis, muscular fibrosis/retinal separation, esophageal stricture and payronles disease. Further fibrotic disorders may be induced or initiated by surgery, including scar revision/plastic surgeries, glaucoma, cataract fibrosis, corneal scarring, joint adhesions, graft vs. host disease (e.g., in transplant patients), tendon surgery,

nerve entrapment, dupuytren's contracture, OB/GYN adhesions/fibrosis, pelvic adhesions, peridural fibrosis, restenosis. It is also contemplated that fibrotic conditions where deposition of fibronectin is a causative factor can be treated according to the invention. Idiopathic pulmonary fibrosis, bleomycin lung, cystic fibrosis, and glomerular nephropathy, including disease characterized by fibronectin deposits in the kidneys ultimately leading to renal failure are examples of conditions which can also be treated in accordance with the present invention.

[00180] The invention also contemplates an antibody that has an affinity of at less than $1x10^{-7}M$ to WISE and inhibits WISE activity for use in a method for treating a medical condition associated with fibrosis, wherein the fibrosis can be associated with a disease discussed above including lung disease or kidney disease. Furthermore, also contemplated is an antibody that has an affinity of at less than $1x10^{-7}M$ to WISE and inhibits WISE activity suitable for use in a method for treating a medical condition associated with proteinuria.

[00181] The invention also provides for combination therapies where the compositions of the invention are administered to a patient in need thereof with additional therapeutic agents that either treat the underlying disease or reduce symptoms associated with the disease being treated. These additional therapies can be administered simultaneously, before or after the administration of the composition of the present invention. Additional therapies for use in combination with the compositions of the present invention include ACE inhibitors, angiotensin receptor blockade (ARB), erythropoietin (e.g., Aranesp® (darbepoetin), Epogen® (erythropoietin alfa), calcineurin inhibitors, steroids, beta blockers and the like.

[00182] The invention also provides a diagnostic kit comprising at least one anti-WISE binding agent according to the present invention. The binding agent may be an antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the one or more binding agent(s) for screening, diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labeled binding partner to the anti-WISE binding

agent(s); (3) a solid phase (such as a reagent strip) upon which the anti-WISE binding agent(s) is immobilized; and (4) a label or insert indicating regulatory approval for screening, diagnostic, prognostic or therapeutic use or any combination thereof. If no labeled binding partner to the binding agent(s) is provided, the binding agent(s) itself can be labeled with one or more of a detectable marker(s), e.g. a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

[00183] The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

[00184] A human WISE clone was used as template for PCR to generate huWise-MYC expression cassette by PCR. The resulting product was assembled into a vector after confirming the sequence, the fragment from clone was then cloned into a pCDNA3.1 vector. huWise-MYC/pCDNA3.1 was used as template to generate a huWise expression cassette using PCR. The PCR product sequence was confirmed, and subsequently cut and then sub-cloned into an expression vector. The DNA sequence of huWise is depicted in SEQ ID NO:1. The polypeptide sequence of human WISE is depicted in SEQ ID NO:2

Cloning of mouse, rat and cynomolgus Wise

[00185] Mouse WISE was used as a template for PCR by using primers to generate a NSP-mWise expression construct (NSP - native signal peptide). The PCR product was cloning into vector for sequence confirming, the DNA fragment with right sequence was then cloned into a gene expression vector. The DNA sequence of mWISE is depicted in SEQ ID NO:3. The polypeptide sequence of mouse WISE/pTT5 is depicted in SEQ ID NO:4. Rat Wise was cloned using PCR, and cloned directly into a vector. DNA sequence of ratWise is depicted in SEQ ID NO:5. The polypeptide sequence of rat WISE is depicted in SEQ ID

NO:6. Cynomolgous Wise was also cloned and inserted into an expression vector. The DNA sequence of cynoWise is depicted in SEQ ID NO:7. The polypeptide sequence of Cyno WISE is depicted in SEQ ID NO:8.

Expression and Purification of Mouse and Human WISE Protein in *E. coli.*

[00186] Cells that have been transformed with a WISE expression vector were grown to an optical density of 8 to 11 at 600 nm and then induced and harvested by centrifugation six hours later. Frozen cell paste was thawed and re-suspended into buffer with a homogenizer until the slurry was homogeneous. The cell slurry was then homogenized to break open the cells and release inclusion bodies. The resulting homogenate is then centrifuged at 5,000 x g for an hour at 5C to harvest the inclusion bodies as a pellet, leaving the cytoplasmic contaminants in the discarded supernatant. The residual cytoplasm is washed from the inclusion bodies by homogeneously re-suspending the pellet to the original homogenate volume using chilled water and a homogenizer at high speed followed by centrifugation as before. The resulting pellet, washed inclusion bodies (WIBS), is then frozen at -80C.

[00187] A sufficient amount of WIBS and guanidine hydrochloride (GnHCl) are used in a reducing-solubilization to result in approximately 1 mg/ml reduced product and 0.18 M final concentrations. The solubilization was then added with stirring to refold solution. The refold was allowed to gently stir and air-oxidize for 72 hours at 6 C. Precipitation was removed by 0.45 um filtration leaving the filtrate (AP). The AP was then adjusted to 20 mM Tris, pH 8.5 using 1 M Tris HCl to generate a second slight precipitate that is removed by centrifugation at 5 K x g for 1 hour at 5C.

[00188] For the purification of mouse WISE, a reverse phase HPLC column was equilibrated and loaded with the supernatant from the acid-base precipitation step followed by a wash with equilibration buffer until a baseline ultraviolet (UV) trace was achieved. Product was eluted and fractions were collected and

subsequent pooling was determined by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide electrophoresis) of the fractions.

[00189] Following protein folding human WISE was purified using column chromatography. Purification processes were carried out at room temperature. The purification scheme used cation exchange chromatography followed by reversed phase chromatography. The fractions were then assayed by Coomassie-stained SDS-PAGE to identify fractions containing a polypeptide that migrates at the predicted size of WISE. The appropriate fractions from the column were combined to make the SPHP pool. Following purification, the WISE was formulated in PBS by dialysis. Following formulation, the preparation was filtered through a sterile 0.2 μm filter and stored at 4°C or frozen.

Expression And Purification Of Mouse And Human WISE In Mammalian Cells

[00190] One vial of stock culture was inoculated into 10 ml culture medium in Shake Flask (125 ml, Plastic), the culture was continued for 2-3 days; then the culture was expanded from 10 mL into 100 mL shake flask and again from 100 ml into 500 ml volume culture. For transfection, were seeded into 1 liter culture medium and grown until appropriate cell density.

[00191] Transfection mix was prepared, the cells were transfected using standard techniques and 24 hours post-transfection a feed was added to the cells. The culture was then continued for another 48 hours and conditioned medium was harvested by spinning at 4000 rpm for 30 minutes and then filter through a 0.2 uM filter. A small sample (1 ml) was then taken for western and the rest was frozen down for purification. The host cell culture fluid (CCF) was centrifuged to remove cell debris. The CCF supernatant was then filtered.

[00192] A Heparin column was loaded with protein then washed with PBS until the absorbance at 280 nm of the flow-through returned to baseline. WISE protein was then eluted from the column using a linear gradient from 150 mM to 2 M

sodium chloride in PBS and fractions collected. The fractions were then assayed by Coomassie-stained SDS-PAGE to identify fractions containing a polypeptide that migrates at the predicted size of WISE. The appropriate fractions from the column were combined to make the Heparin pool.

[00193] The WISE protein eluted from the Heparin column was further purified by reversed phase chromatography. The Heparin pool was made 22% ethanol and adjusted to pH 5.0 with acetic acid. The pool was filtered. The filtered Heparin pool was then loaded onto a equilibrated column. After loading, the column was washed until the absorbance at 280 nm of the flow-through returned to baseline. The WISE protein was then eluted from the column.

[00194] Following purification, the WISE was formulated in PBS by dialysis. Following formulation the WISE was filtered through a sterile 0.2 μm filter and stored at 4°C or frozen.

In Vitro Bioactivity of recombinant WISE protein

[00195] MC3T3-E1 STF reporter cells were used to determine whether WISE protein can modulate Wnt signaling. The activation of TCF-dependent signaling in MC3T3-E1 STF cells can be triggered using either endogenous Wnt signaling induced by switching culturing medium to differentiation medium or by adding exogenous Wnt such as Wnt3a. Recombinant WISE protein derived from either E coli or mammalian cell can dose-dependently inhibit Wnt signaling in MC3T3-E1 STF cells (Figure 2).

[00196] Luciferase assay: a vial of MC3T3-E1/STF cells was plated into a culture flask in expansion medium. When the cells get confluent they were trypsinized and cells in 100 ul expansion medium were plated into each well in 96 well plate. Next day all expansion medium was removed and replaced with 100 ul of freshly prepared differentiation medium.

[00197] Half of the differentiation medium (50 ul) was replaced with freshly prepared differentiation medium every day for the next four days. After five days

of differentiation, all medium was replaced with testing samples in the fresh differentiation medium in 100 ul total volume. The plates were then allowed to incubate for 24 hours before luciferase signal are measured. Luciferase signal was measured upon removal of medium from testing plates and addition of 20 ul of 1x lysis buffer that has been equilibrated to room temperature. The plate was sealed and rocked for 30 minutes at room temperature and 100 ul of luciferase assay reagent was added to each well and the signal was captured using Luminometer (LMAX, Molecular Device) according to manufacturer's instruction.

Generation and Characterization of Monoclonal Antibodies

[00198] Murine and human WISE protein from bacterial as well as mammalian sources was conjugated to a PADRE peptide. Unreacted cross-linker was removed via dialysis followed by addition of the PADRE peptide in molar amounts equal to the amount of cross-linker added. This WISE-PADRE derivatized antigen was emulsified using various combinations of adjuvants then immunized sub-cutaneously and intraperitoneally into normal C57BL/6, BDF1, and 129x BL/6 F1 mice (Jackson Labs). In addition, Brown Norway rat was also used for immunization either with PADRE-WISE conjugates or native murine WISE protein. Immunization occurred at least every 2 weeks.

[00199] 4 days prior to fusion, each mouse was boosted intraperitoneally with WISE-PADRE antigen in PBS. On the fusion day, the spleen was removed aseptically and the organ was processed into a single cell suspension. After washing, the cells were suspended in fusion buffer and this mixture was loaded into fusion chambers then subjected to electrofusion conditions.

[00200] The cell suspensions were removed from the chambers and suspended in cell growth media. 20µl per well of this cell suspension was plated into 384 well cell culture plates and incubated overnight in an incubator. The following day, 20µl of the above mentioned growth media containing 2X HAT (Sigma) was added to each well of the plates. The cultures were incubated for 7 days then the

growth media was aspirated out of the wells and exchanged for fresh growth media. Screening of hybridoma supernatants commenced 2-3 days after the media change.

[00201] High-binding clear polystyrene 384 well plates were coated with 25 µl/well of a 1ug/ml solution consisting of goat anti-mouse IgG, Fc specific pAb (Pierce) in PBS. The plates were incubated with coating solution overnight 4°c then washed once on an automatic plate washer using PBS +.05% Tween 20 (Sigma). Block solution was added to each well and incubated overnight at 4°c. 5 µl of hybridoma supernatant was transferred to each well of the ELISA plate and allowed to incubate for 60 min at room temp. The plates were then washed 2 times using the method described above. 20 µl/well of a 20 ng/ml solution of biotinylated WISE protein diluted in blocking solution that had been premixed with a 1:10,000 dilution of Nuetravidin-HRP (Pierce) was then added to each well of the plate.

[00202] After the addition of the WISE antigen, the ELISA plates were allowed to incubate for 60 min at RT. They were then washed. Finally, 20 µl/well of TMB (Pierce) was added to each well and the plates were read on an absorbance plate reader. Cells from the ELISA positive hybridoma wells were subsequently expanded in cell culture for further characterization studies.

[00203] Single cells from the ELISA positive hybridoma wells were isolated using FACS sorting and placed into 384-well plates (1 cell per well). These cells were allowed to grow for 3 days. Once the adequate cell mass was reached, supernatant from each well was collected and re-screened for antigen binding ability (see screening).

[00204] From each 384-well plate, two clones with highest antigen binding activity were identified and expanded further into 96-well plates (Falcon) with 150ul of hybridoma growth medium per well. After 3 days, cells from 96-well plates were transferred to 24-well plates with medium and allowed to grow for 3 additional days. Once 24 well plates were confluent, cells were transferred to 6-well plates. After 5 days of incubation, a portion of the cells were frozen down.

The remainder of the cells were transferred into a flask and allowed to expand. Once the flasks were confluent, half of cells were frozen down (3 vials per clone) in for additional backup. The other half was allowed to expand further in flasks with medium for antibody production.

[00205] Isotypes were determined using standard methodologies. WISE monoclonal antibodies (mAbs) were purified from hybridoma cell culture as follows. All purification processes were carried out at 4°C. One purification scheme was used to purify the various mAbs and used affinity chromatography.

[00206] The host cell culture fluid (CCF) was centrifuged to remove cell debris. The CCF supernatant was then filtered, diluted and then loaded onto Protein G chromatography media in the form of a column, Protein G High Perfomance (GE Healthcare) and equilibrated.

[00207] After loading, the Protein G column was washed until the absorbance at 280 nm of the flow-through returned to baseline. The WISE mAb was then eluted from the column using glycine, pH 2.5 and immediately neutralized by adding 50 μL of a stock solution of 1 M Tris Base per mL of elution volume. The absorbance at 280 nm of the eluate was monitored and fractions containing protein were collected to make the Protein G pool.

[00208] Following purification, the WISE mAbs were formulated in PBS by dialysis using 10,000 MWCO membranes (Pierce Slide-A-Lyzer or dialysis tubing). Following formulation the WISE mAbs were filtered.

[00209] Among the top 140 hybridoma CM tested, 40 showed good binding to recombinant muWISE protein. These hybridoma clones were selected for expansion and purification. To screen hybridoma clones that produce neutralizing antibody against mouse WISE, the ability of hybridoma CM to reverse the inhibition of TCF-luciferase signaling by 300 ng/ml recombinant WISE protein was tested.

[00210] Both mouse, rat, cyno and human WISE proteins inhibited Wnt-induced TCF-luciferase signal with similar potency (IC50~200 ng/ml). At 300

ng/ml muWISE consistently inhibits approximately 60% of the signal induced by differentiation. WISE protein alone or mWISE protein and hybridoma CM (1:1 dilution) pre-incubated mixture was added to the culture that have been differentiated for 5 days and then luciferase signal was measured at 24 hour post-treatment. Among the 40 top binding antibodies tested in cell-based assay, mature antibodies Ab-A, Ab-C and Ab-E showed potent neutralizing activities against mouse WISE.

[00211] The result was also confirmed using purified antibodies from CMs of the top hybridoma clones. In this case, Wise protein was either mixed with different amounts of purified antibodies or PBS for one hour at 37°C before adding to the culture that have been differentiated for 5 days and luciferase signal was measured 24 hours later. These antibodies also neutralized the activity of human, rat and cynomolgus WISE in MC3T3-E1 STF-Luciferase assay and results for the human activity are shown in Figures 1. Comparable results were seen in rat and cynomolgus assays.

Cross-Competition ELISA

[00212] Clear polystyrene plates (Corning #3708) were coated with 25 µl/well of a 2ug/ml Mab solution in PBS consisting of one of the 40 mouse anti-WISE antibodies. The plates were incubated with coating solution overnight 4°c then washed once on an automatic plate washer using PBS + 0.05% Tween 20 (Sigma). 50µl of block solution consisting of PBS + 1% BSA + 1% normal goat serum +0.5% Tween 20 (Sigma) was added to each well and incubated overnight at 4°c. 25 ul of competitive antibodies in blocking solution starting from 30 ug/ml and then 3 fold serial dilution were then added to each well of the plate followed by adding 25 µl/well of a 1 ng/ml solution of biotinylated WISE protein premixed with Nuetravidin-HRP (Pierce) in blocking solution.

[00213] After the addition of the antigen-antibody mix, the ELISA plates were allowed to incubate for reaching equilibrium for overnight at 4°C. They were

then washed 4X with PBS +.05% Tween20. Finally, 25 µl/well of luminescent substrate (Pierce) was added to each well and the plates were read on a luminescence plate reader. Based on complete set of data, there are multiple antibody bins. Examples of the data are shown in Figures 20-26.

Epitope mapping

[00214] To determine whether WISE antibodies bind to linear or conformational epitopes, the ability of select antibodies to bind to reduced or non-reduced antigen using western blot was tested. If the antibody binds to a linear epitope, it will bind to WISE protein whether or not it has been reduced or not. Otherwise, it is binding to a conformational epitope. Briefly, both human (1ug/ul) and mouse (0.25ug/ul) WISE proteins made in mammalian cells are denatured under either reduced or non-reduced conditions (65°C for 10 minutes with or without b-ME respectively).

[00215] For each lane 100 ng of the denatured proteins in Laemmli sample buffer were loaded onto NuPAGE Bis-Tris 4-12% gel and subjected to SDS-polyacrylamide gel electrophoresis and western blotting using nitrocellulose membrane. Upon blocking, each testing antibody at 120 ng/ml was incubated in PBS and 0.05%Tween with the membrane for 1 hr at RT with gentle agitation.

[00216] The bound antibody was detected using HRP labeled secondary antibody against mouse IgG-Fc (Cat # 31439, PIERCE) used at 1:10000 dilution for 1 hr at room temperature. The signal was detected using ECL substrates and exposed onto film. Among the antibodies tested, all antibodies are binding to conformational epitope.

[00217] To determine the epitope of the different antibodies, the tryptic peptide HPLC profile derived from trypsin digestion of WISE protein either in the presence or absence of individual antibodies was compared. Mammalian cell derived human recombinant Wise protein (10 ug) was mixed with individual antibody (32 ug) with a molar ratio of appr. 1:1 in 200ul of 0.1M Tris-HCl buffer

(pH 7.5). The mixture was incubated for 30 minutes at room temperature. Trypsin (Roche) (2ug) was added and the digestion was allowed to proceed for 24 hours at 37 C. Under these conditions, the antibody against human Wise is comparatively stable against the proteolytic digestion, particularly the tryptic digestion. The trypsin-treated samples were directly subjected to reversed phase HPLC using the TFA-acetonitrile system. A reverse phase HPLC column was used for separation of the peptides. The peptides were eluted by a linear gradient from 2% solvent B to 35 % solvent B for 30 min with a flow rate of 0.25ml/min, monitoring at 215 nm absorbance. Using this approach it was found that several groups of antibodies that generated similar tryptic peptide profile including the mature forms of Ab-A, Ab-C and Ab-E.

[00218] To obtain sequence identity of individual peptides derived from tryptic digests, WISE protein (100ug) was digested with trypsin (Roche) (2ug) for 24 hours at 37 C and the digestion was allowed to proceed to further 24 hours after addition of 2ug of trypsin in order to complete the proteolysis. The peptides were purified by reversed phase HPLC. Linear gradient conditions were used as follows: a column was equilibrated with 2% solvent B. After sample injection, a linear gradient from 2% solvent B to 35% solvent B for 60 min was performed with a flow rate of 0.25ml/min. The HPLC peptide peaks were manually collected and dried. After reconstitution as described above, the samples (0.7-1ul) were subjected to MALDI mass spectrometry (Micromass, Waters) and the remaining sample was kept for sequence analysis. One aliquot of the sample was loaded on stainless steel MALDI plate with a matrix, alpha-cyano 4-hydroxy cinnamic acid (4-HCCA). Peptide sequence was also determined.

[00219] To identify which peptides compete with WISE for binding to the antibodies of the invention, rhuWise was immobilized on a CM5 surface with high density. 3nM of mature antibodies Ab-A, Ab-C and Ab-E were pre-incubated with and without 30nM peptides and huWise (as control), then injected over the huWise surfaces. As shown in Figure 9A and B both T49 and T56.1 peptides blocked the binding of the antibodies to huWISE. As these antibodies

bind to a conformational epitope as demonstrated by western blot, it is postulated that these antibodies' binding sites involve domains formed by loop 1 and /or loop 3 as well as residues from the cytine knot.

Affinity Measurement of Selected Antibodies

[00220] Binding of muAbs with rhuWise and rmuWise were tested on KinExA. Azlactone beads (Pierce) was pre-coated with rhuWise/rmuWise at pH9.0 and blocked with ethanolamine, then further washed with sample buffer (0.1mg/mL Heparin in BSA/P20/PBS solution). 10 pM and 100pM of Ab-A, Ab-C and Ab-E were incubated with various concentrations (0.1 pM – 3 nM) of rhuWise/rmuWise at room temperature for at least 10 hours before run through the rhuWise/rmuWise coated beads.

[00221] The amount of the bead-bound muAbs were quantified by fluorescent (Cy5) labeled goat anti-mouse IgG (H+L) antibody (Jackson Immuno Research). The binding signal is proportional to the concentration of free muAbs at binding equilibrium. Equilibrium dissociation constant (Kd) was obtained from nonlinear regression of the competition curves using a two-curve one-site homogeneous binding model (KinExA^{TM} Pro software). Mature antibodies Ab-A, Ab-C and Ab-E have similar affinity for mouse and human WISE, and the Kd measure for human WISE is approximately 2 pM for Ab-A, approximately 4 pM for Ab-C, and approximately 18 pM for Ab-E.

[00222] From the foregoing, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims. All publications, published patent applications, and patent documents disclosed herein are hereby incorporated by reference.

[00223] Humanization of Ab-A, Ab-C, Ab-E, Ab-P and Ab-T

[00224] Each of the variable domains of Ab-A and Ab-C were cloned onto a human IgG2 constant domain to make chimeric antibodies (Ab-I and Ab-J) and the appropriate CDR regions of SEQ ID NOs: 10, 12, 18 and 20 were grafted into either human kappa light chains (CDRs of SEQ ID NOs :10 and 18) or a human IgG2 framework (CDRs of SEQ ID NOs: 12 and 20) to give antibodies Ab-B, Ab-D, and Ab-G, Ab-H, Ab-K, Ab-L, Ab-N and Ab-O..

[00225] Each of the variable domains of Ab-P and Ab-T were cloned onto a human IgG2 constant domain to make chimeric antibodies (Ab-Q and Ab-X) and the appropriate CDR regions of SEQ ID NOs: 48, 50, 266, 268 were grafted into either human kappa light chain (CDRs of SEQ ID NO: 50) or lambda chain (CDRs of SEQ ID NO: 266) respectively; or a human IgG2 framework (CDRs of SEQ ID NOs: 48, 268) to give rise to humanized antibodies Ab-R and Ab-M.

[00226] The ability of the antibodies to bind human WISE was determined using the following binding assay. 96 well plates were coated with 50 µl/well of a 2 µg/ml solution of each testing antibody in coating buffer for two hours at room temperature. The plates were then washed once using PBS +.05% Tween 20 (Sigma). Block solution was added to each well and incubated overnight at 4°c. A serial dilution of biotinylated human WISE protein starting at 30 ng/ml or 100 ng/ml in blocking solution that had been premixed with a 1:10,000 dilution of Nuetravidin-HRP (Pierce) was then added to each well of the plate and incubated overnight at 4°c. After incubation plates were washed. Finally, 50 µl/well of TMB (Pierce) was added to each well and the plates were read on an absorbance plate reader. Data were plotted PRISM software. Data is shown in Figures 3, 4 and 5, 6.

[00227] The ability of the humanized antibodies to croos-block the binding of Ab-C to human WISE was determined using competition binding assay. 96 well plates were coated with 50 µl/well of a 1 µg/ml solution of antibody C (Ab-C) in coating buffer for two hours at room temperature. The plates were then washed once using PBS +.05% Tween 20 (Sigma). Block solution was added to each well and incubated overnight at 4°c. A two fold serial dilution of testing antibodies

Ab-C, Ab-J, Ab-N and Ab-O, were added to each well along with biotinylated human WISE protein at 1 ng/ml in blocking solution that had been premixed with a 1:10,000 dilution of Nuetravidin-HRP (Pierce) was then added to each well of the plate and incubated overnight at 4°c. After incubation plates were washed. Finally, 50 μl/well of TMB (Pierce) was added to each well and the plates were read on an absorbance plate reader. Data were plotted PRISM software. Data is shown in Figure 7.

[00228] The ability of the chimeric or humanized antibodies to neutralize WISE activity relative to that of the original rodent antibodies were determined using a cell-based assay with MC3T3-E1 SuperTopFlash (STF) cells. The cells were differentiated first for five days according to the detailed procedures described in this invention and then they were either treated with PBS only or human WISE protein only at 0.5 ug/ml or 0.5 ug/ml human WISE proteins that has been premixed with a two fold serial dilution of the testing antibodies starting at 5 ug/ml, Twenty four hours later the luciferase signal was determined using Luminometer (LMAX, Molecular Device) according to manufacturer's instruction. Data were plotted PRISM software. Data is shown in Figure 8.

[00229] **Cloning of anti-WISE humanized antibodies**:

[00230] These anti-WISE antibodies are humanized antibodies that were generated by complementarity determining region (CDR) grafting of mouse or rat CDRs into human germline acceptor framework sequences.

[00231] Full-length rodent cDNAs encoding both antibody light and heavy chains were isolated from single cell cloned hybridoma cells using RACE followed by PCR. The cDNAs for the variable regions of the chosen lead candidate murine antibodies Ab-C, Ab-A, and rat antibody Ab-P and Ab-T, were used as design templates for CDR grafting.

[00232] Antibody C was humanized by CDR grafting and mutagenesis to generate several light chain and heavy chain variants. The light chain depicted in

SEQ ID NO: 22, variant 1, was generated by grafting the Ab-C light chain CDRs into human germline acceptor frameworks VK1 O2 and JK2. Residue 22 (Kabat numbering) was maintained as the murine serine residue.

[00233] The light chain depicted in SEQ ID NO: 46, light chain variant 2, was generated by grafting the Ab-C light chain CDRs into human germline acceptor frameworks VK4 B3 and JK2. Residue 22 (Kabat numbering) was again maintained as in the murine serine residue.

[00234] The light chain depicted in SEQ ID NO: 122, light chain variant 3, was generated by making Y36F, Y87F mutations in SEQ ID NO: 22.

[00235] The heavy chain depicted in SEQ ID NO: 24, heavy chain variant 1, was generated by grafting the murine heavy chain CDRs into human germline acceptor frameworks VH1 1-69 and JH4. The heavy chain depicted in SEQ ID NO: 36, heavy chain variant 2, was generated by grafting the murine heavy chain CDRs into human germline acceptor frameworks VH1 1-69 and JH4 and back mutate the following select CDR2 residues into their original murine residues: M48I, G49A, R66K, V67A, .T68Q and I69L.

[00236] Antibody A (SEQ ID NO: 10 and 12) was humanized by CDR grafting and mutagenesis to generate a humanized light chain and several humanized heavy chain variants.

[00237] The humanized light chain variant 1 depicted in SEQ ID NO: 14 was generated by CDR grafting the light chain murine CDRs into human germline acceptor framework VK2 A17 for framework 1 and human germline acceptor frameworks VK2 A19 and JK4 for frameworks 2, 3 and 4 to maintain as much homology as possible between the murine and humanized versions. The amino acid at Kabat position 87 was maintained as the murine phenylalanine due to it's proximity to CDR3 and potential involvement in loop structure and antigen binding (SEQ ID NO:14).

[00238] Humanized heavy chain variant 1 depicted in SEQ ID NO: 16 was generated by grafting the murine heavy chain CDRs into human germline acceptor

framework VH1 1-02 for frameworks 1 and 2 and human germline acceptor sequence VH7 7-4.1 and JH4 for frameworks 3 and 4. The naturally occurring cysteine residue at position 82a was replaced with a serine to avoid heterogeneous disulfide bond formation (SEQ ID NO: 16).

[00239] Humanized heavy chain variant 2 depicted in SEQ ID NO: 34 was generated by reversion of CDR2 and CDR3 proximal residues: R38K, Y91F and A93V (SEQ ID NO: 34).

[00240] Antibody P was humanized by CDR grafting. The light chain depicted inSEQ ID NO: 54 was generated by grafting the rat light chain CDRs into human germline acceptor framework VK3 L2 and JK4 (SEQ ID NO: 54).

[00241] The heavy chain depicted in SEQ ID NO: 52 was generated by grafting the rat heavy chain CDRs into human germline acceptor framework VH3 3-33 and JH4. Murine residues that were kept in place due to proximity to CDRs 1 and 3 included proline 28 (P28), threonine 93 (T93) and serine 94 (S94) (SEQ ID NO: 52).

[00242] Antibody T was humanized by CDR grafting. The light chain depicted in SEQ ID NO: 271 was designed by grafting the rat light chain CDRs into human germline acceptor framework VL6 6a and JL2. Rat residues that were kept in place due to their importance as canonical or interface residues included glutamine 1 (Q1), valine 2 (V2), glutamic acid 40 (E40), arginine 42 (R42) and phenylalanine 87 (F87).

[00243] The heavy chain depicted in SEQ ID NO: 272 was designed by grafting the rat heavy chain CDRs into human germline acceptor framework VH4 4-59 and JH3. Rat residues that were kept in place due to proximity to CDRs 1 and 3 or their importance as interface and canonical residues included phenylalanine 27 (F27), leucine 29 (L29), threonine 30 (T30), valine 37 (V37), arginine 71 (R71) and valine 93 (V93).

[00244] Phage Derived Anti-Wise Ab-C Light Chain

[00245] Part 1. Construction of LC-Shuffling Library

[00246] 3.5 x10^{11} pfu (ten times of library size) of 310Fab library (Dyax) and 4x10^{11} pfu of TQ library (Target Quest) were used to infect 2L of TG1 culture in log phase (OD600 at 0.6) at 37 °C for 30 minutes. Infected cells were spun down, re-suspended and plated on ten 2XYT-CG 245 mm square plates. After overnight incubation at 37 °C, cells were scraped off and spun down. The cell pellets were used for megaprep of phagemid plasmids of libraries.

[00247] Phagemid plasmids of 310Fab library and TQ library were digested with SfiI and NotI. Digested samples were run on preparative 0.5% agarose gel to separate the VH-CH1 fragment pool and the pCES1-LC fragment pool. The large vector-LC fragment bands (5242 bp for TQ library and 4517 bp for 310Fab library) were excised and DNA eluted using QIAquick Gel Extraction kit (Qiagen).

[00248] Humanized anti-WISE Ab-C VH-CH1 fragment was amplified with primers 5104-91 (CCG TTC GTG GCC CAG CCG GCC TCT GCT CAG GTT CAG CTG GTG CAG TCT G; SEQ ID NO: 281) and 5104-93 (GTG ATG GTG ATG ATG ATG TGC GGC CGC ACA TTT GCG CTC AAC TGT CTT GTC; SEQ ID NO: 282) using humanized anti-WISE Ab-C IgG2 gamma chain (SEQ ID NO: 24) as the template. Amplified humanized Ab-C heavy chain VH-CH1 fragment (676 bp) was digested with SfiI and NotI and purified using PCR purification kit from Qiagen.

[00249] Humanized Ab-C VH-CH1 SfiI/NotI fragment was ligated to pCES1-LC SfiI/NotI fragment pool at 4:1 and 3:1 ratio, respectively. Ligated DNA was cleaned up by phenol chloroform extraction and ethanol precipitation. 20 ug of ligated DNA from TQ library and 93 ug of ligated DNA from 310Fab library were used to transform electro-competent TG1 cells (Stratagene, #200123) at 500 ng DNA per 100 ul cells and 300 ul/cuvette by electroporation in BioRad Gene Pulser using 0.22CM cuvettes (BioRad, Catalog #1652086) at 25uF, 200ohm, 2500V. Electroporated cells were diluted 3X with SOC and incubated at 37 °C for 1 hour. Transformed cells were titered and plated on 2XY-CG 245 mm square

plates and incubated overnight at 37 °C. Cells of transformant colonies were scraped off and used for phage rescue. The library size of the constructed TQ-LC shuffling library was 6.6x106 and the library size of the constructed 310 Fab-LC shuffling library was 2.1x109.

[00250] Phagemid phages were rescued from LC-shuffling libraries separately. 2XYT-CG media were inoculated with 10X library size inoculums from LC-shuffling libraries, respectively, to OD600 0.1. The cultures were grown to OD600 0.5, then infected with KO7 helper phage (Invitrogen) at MOI of 20. After 30 minutes of incubation at 37 °C, cells were spun down, re-suspended into 2XY-CK, and incubated at 30 °C overnight. The cells were spun down by centrifugation. The phage supernatants were transferred into fresh tubes. 1/5th volume of 20%PEG/1.5M NaCl was added to the phage supernatant to precipitated the phage. The mixture was incubated on ice for 1-3 hour. Precipitated phage was spun down by centrifugation at 14K for 30 minutes. Phage pellet was re-suspended into 1 ml of PBS and centrifuged at 14 K for 10 minutes to remove cell debris. Phage precipitation was repeated as described above. Final phage pellet was re-suspended into 1 ml of PBS/1% BSA.

[00251] Part 2. LC-shuffling library panning

[00252] 1x1011 pfu of rescued phage from TQ-LC shuffling library and 1x1012 pfu of rescued phage from 310Fab-LC shuffling library were used for panning against bionitylated hu-WISE coated on Streptavidin M-280 Dynabeads (Dynal Biotech, # 112.06). Panning procedures are: three 30-minutes Negative selections with biotinylated FGF23 coated streptavidin Dynabeads, followed by 60-minute Positive selection, then washing the beads 6x with 3%BSA / 3%MPBT (0.1% Tween-20), 6X with PBST, and 2x with PBS, and finally elution of bound phages with 1ml of 0.1M TEA followed by neutralization with 0.5 ml of 1M Tris HCl. For each round, panning was done at two antigen coating concentrations and a 10- or 20-fold reduction of coating concentrations in the subsequent rounds except for RD4 as shown below: RD1A (3.3 ug/ml);RD2B (0.33 ug/ml);RD3D (0.015 ug/ml);RD4D (0.015 ug/ml) and RD1B (0.33 ug/ml);RD2C (0.033

ug/ml);RD3E (0.0033 ug/ml);RD4E (0.0033 ug/ml). For RD3 panning, two washing protocols were implemented. Besides the regular washing, additional overnight washing steps were carried out for a separate set of RD3 panning.

[00253] Part 3: Phagemid clonal analysis and clone selection

[00254] Eluted RD2, RD3 and RD4 phagemid clones were screened in phage ELISA on Neutravidin plates coated with biotinylated human WISE at 3.3 ug/ml and 0.33 ug/ml. One 96-well plate of individual clones from each RD2, RD3 and RD4 eluted pool were screened. Clones that showed similar binding signals at both antigen concentrations were cherry-picked and sequenced. A total of 77 unique phagemid clones were identified. Two of them were dropped due to the presence of an amber codon in the framework. All of the remainders were converted to IgG2 into corresponding pTT5 vectors with Vκ1|O12-O2 signal peptide sequence (MDMRVPAQLLGLLLLWLRGARC; amino acids 1-22 of SEQ ID NO: 58) by inserting the variable region of HC and lambda LC as BssHII/BsMBI fragments and the variable region of kappa LC as BssHII/BwiI fragments.

[00255] Part 4: Transient transfection of LC mutant IgG2

[00256] Equal amount of humanized anti-WISE Ab-C IgG2 gamma chain vector and the light chain vector at final concentration of 0.5 ug/ml were used to transfect 50 ml of 293 6E cells at $1x10^6$ cell/ml using PEI as the transfectant. Trypton was added on Day 2 of the transfection. Conditioned medium was collected on Day 7. The polypeptides of the light chains identified in this example are: L1 (SEQ ID NO: 76), L13 (SEQ ID NO: 78), L16 (SEQ ID NO: 80), L18 (SEQ ID NO: 82), L23 (SEQ ID NO: 84), L24 (SEQ ID NO: 86), L28 (SEQ ID NO: 88), L29, (SEQ ID NO: 90), L48 (SEQ ID NO: 92), L60 (SEQ ID NO: 94), L62 (SEQ ID NO: 96), L63 (SEQ ID NO: 98), L65 (SEQ ID NO: 100), L66 (SEQ ID NO: 102), L66 (SEQ ID NO: 104), L67 (SEQ ID NO: 106), L69 (SEQ ID NO: 108), L7 (SEQ ID NO: 110), L70 (SEQ ID NO: 112), L72 (SEQ ID NO: 114), L74 (SEQ ID NO: 116), L75 (SEQ ID NO: 118), L76 (SEQ ID NO: 120), and L9 (SEQ ID NO: 122) and when paired with the heavy chain depicted in SEQ ID NO:

24 are active in binding WISE protein and inhibiting WISE activity in various assays including MC3T3-E1 SuperTopFlash (STF) assay.

[00257] Fine Epitope Mapping by site-directed mutagenesis

[00258] Alanine scanning was utilized to determine contact points of the antibodies with the WISE protein. Site directed single amino acid mutations in the first and third loops of the WISE protein were introduced using PCR with Stratagene's QuikChange site-directed mutagenesis kit. The DNA constructs were sequence confirmed and transfected into 293 cells for transient production of mutated proteins. The effect of single amino acid mutation was found to have no effect for protein expression. The supernatants and purified proteins were tested and all mutants retained their ability to inhibit Wnt signaling as the wild type protein in MC3T3-E1 STF reporter cell line.

[00259] Relative capture of individual WISE mutant proteins or wild type proteins by either neutralizing antibodies such as Ab-C, Ab-E, Ab-A or Ab-P or non-neutralizing antibody Ab-S were compared to assess whether any of these mutations affects binding of antibodies to WISE proteins. The bound WISE proteins were then detected using HRP-conjugated affinity-purified polyclonal antibodies against WISE.

[00260] Individual mutant proteins were tested for functional activity to assess whether any of the residues required for binding is also critical for WISE protein activity. The single mutants retained the activity of inhibiting TCF-Luc expression in the above described cell-based assay.

[00261] The relative neutralizing activity of individual antibodies was obtained by comparing the ability of the test antibody to reverse the inhibition of TCF-Luc activity either by wild type human WISE or mutant WISE proteins. The data are shown in Figures 11-18 for Ab-R, Ab-C, Ab-A, Ab-E, Ab-U, Ab-V, Ab-W, and Ab-T.

[00262] In vivo activities of anti-WISE Mab in mouse model of bleomycin induced lung fibrosis

[00263] The effect of WISE inhibition on disease progression and fibrosis was evaluated using a mouse model of bleomycin induced lung fibrosis. Female C57Bl/6 mice of 8-10 weeks old (Jackson Lab) were pre-treated twice (Days -5 and -3) with injections of WISE Ab, sTGF-beta Receptor-muFc, and mIgG2b control isotype by intraperitoneal route. On Day 0, a single dose of intratracheal bleomycin (3.75 u/kg or saline control) was given to each mice followed by a M,W,F, dosing regimen of each treatment for 2 weeks. Two weeks (day 14) post-bleomycin, mice was anesthetized using Avertin and blood/tissues will be collected as follows: Right lungs were snap frozen for Sircol collagen assay, the left lung from each mouse was inflated with and then placed in 10% neutral-buffered formalin in preparation for light microscopy. Sections of lungs were stained with hematoxylin and eosin (H&E) and Sirius red (demonstration of collagen) stains and by immunohistochemical methods to demonstrate alpha smooth muscle actin (αSMA) and fibroblast specific protein 1 (FSP-1 or A100S4), and examined by routine light microscopy. In addition serum or bronchoalveolar lavage fluid from each mouse was collected for the measurement of osteopontin level using ELISA (R&D system).

[00264] H&E-stained tissue sections were scored for the amount of the lung tissue that was characterized as alveolar collapse/consolidation on a semi-quantitative scale where 0 = not present, 1 = ≤ 10% of lung affected, 2 = 11%-33% of lung affected, 3 = 34%-67% of lung affected, and 4 = >67% of lung affected. Additionally, the severity of the alveolar and interstitial changes in areas of lung not affected by alveolar collapse/consolidation was scored for severity according to a semi-quantitative scale where 0 = not present, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. The total H&E score represented the sum of these 2 scores. All slides were examined without knowledge of treatment group on 2 separate occasions separated by at least 2 days. The final H&E score was taken as the average of the scores determined on these 2 days.

[00265] Lung sections stained by Sirius red and by immunohistochemical methods to demonstrate αSMA or FSP-1 were evaluated for the extent of specific staining and assigned scores according to a semi-quantitative scale where 0 = not present, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. All slides were examined without knowledge of treatment group on 2 separate occasions separated by at least 2 days. The final score for each parameter was taken as the average of the scores determined on these 2 days.

[00266] In the first study, two anti-WISE antibodies Ab-C (20 mg/kg n=10) and Ab-E (20 mg/kg, n=10) were used and both mIgG1 (n=10) and IgG2b (n=10) control IgG were used. The results for Ab-C and Ab-E are similar and thus data were pooled as anti-WISE Mab; and the results for two IgG controls are similar and thus pooled as IgG control. The results are shown in Figure 28, 29, 30 31. Prophylactic treatment of mice with anti-WISE antibody significantly reduced lung injury based on H&E score (Figure 28), collagen deposition measured by Sircol collagen assay (Figure 29), the expression of myofibroblast marker alpha smooth muscle actin (aSMA by immunohistochemistry, Figure 30), and finally the level of osteopontin in serum measured by ELISA (Figure 31). Similarly, Sirius red staining and the expression of FSP1 were also significantly reduced by WISE antibody treatment. These parameters were also reduced by sTGFbR_mFc at 5 mg/kg but the effect was less than what was observed with anti-WISE antibodies.

[00267] In the 2nd study, anti-WISE Ab-C (20 mg/kg every two days, n=20) and mIgG2b (20 mg/kg every two days, n=20) control IgG were used. The soluble TGFbeta-Receptor mouse Fc protein (3 mg/kg every two days) was used as positive control. The results are shown in Figure 32-33. The goal is to assess the impact on collagen deposition using a large number animals in light of the variation in collagen deposition observed during the first study. Prophylactic treatment of mice with anti-WISE antibody C significantly reduced collagen deposition (about 50% relative to IgG2b control) measured by Sircol collagen assay (Figure 32). Brochoalveolar lavarge fluid from a subset of mice was also collected and as shown in Figure 33, anti-WISE Ab-C significantly reduced the

BAL OPN level. In addition, several souble markers of tissue injury or fibrosis were found elevated upon belomycin injection but were significantly reduced in BAL fluid from Ab-C treated mice versus those treated with IgG2b control, those include MMP9, VEGF, IP10, MIP-2, MIP-1gamma and IgA. Because mice with BAL fluid harvest don't have good morphological preservation, not all lung samples were collected for histological study and thus a third study was conducted to evaluate that.

[00268] In the 3rd study, anti-WISE antibody Ab-C (20 mg/kg, n=20) was used and mIgG2b (n=20) control IgG was used. Histological assessment using H&E, Sirius red staining and IHC of aSMA, FSP1 were performed. Prophylactic treatment of mice with anti-WISE antibody C resulted in lower lung inury based on H&E score (Figure 34), collagen deposition measured by Sirius Red (Figure 35), the expression of myofibroblast marker FSP1 (Figure 36) and alpha smooth muscle actin (aSMA by immunohistochemistry, Figure 37), and lower serum osteopontin level measured by ELISA(Figure 38). It is noted that the collagen deposited in the IgG control group measured by sircol collagen assay was twice as much as what was reported in the 1st and 2nd study and thus these mice have more server disease than those in the previous two studies (a new batch bleomycin was used).

[00269] These data showed that anti-WISE antibody treatment prophylactically can reduce lung injury, collagen deposition and myofibroblast markers, and osteopontin level in both BAL fluid and serum in the bleomycin induced lung fibrosis model. It is interesting to note that OPN is highly upregulated in human IPF samples (Pardo et al PLOS Medicine 2005 Volume 2, Page 0891-0903; Kadota et al. Respiratory Medicine Volume 99, Issue 1, January 2005, Pages 111-117) and knockout of OPN itself resulted in reduced fibrosis in several fibrosis models including bleomycin model and unilateral ureteral obstruction (UUO) induced renal fibrosis model, cyclosporine induced renal toxicity model. In addition, Opn knockout mice did not develop albuminuria in response to LPS injection, and Opn knockout mice were protected from diabetes-induced

albuminuria and mesangial expansion. (Takahashi et al. Am. J. Respir. Cell Mol. Biol., Volume 24, Number 3, March, 2001 264-271; Berman et al *Am J Physiol Lung Cell Mol Physiol* 286: L1311-L1318, 2004; Mazzali et al *Kidney International* (2002) 62, 78–85; Yoo et al. *Kidney International* (2006) 70, 1735–1741; Lorenzen *JASN*, Vol. 19, No. 5. (May 2008), pp. 884-890) Thus WISE Ab treatment may have utility in treating a variety of disorders involving kidney and lung injury and related fibrosis.

[00270] In addition, elevated osteopontin (OPN) plasma levels are highly prognostic in advanced non-small cell lung cancer (NSCLC) (2006 ASCO Annual Meeting, Abstract 7198) and it has been shown that OPN can promote integrin activation and cancer cell survival (Lee et al Cancer Research 2007 Mar 1;67(5):2089-97). OPN can also promote tumor metastasis and elevated osteopontin levels in various cancer types are associated with poor prognosis (reviewed by El-Tanani MK Front Biosci. 2008 May 1;13:4276-84; Johnston et al Front Bioscience. 2008 May 1: 13: 4361-4372). Thus, WISE binding agents may also reduce cancer cell survival and metastasis through its ability to decrease osteopontin expression in tumor.

[00271] The Effect of Anti-WISE Antibody Treatment On Proteinuria In Col4a3 KO Mice

[00272] Col4a3 KO mice (129-Col4a3<tm1Dec>/J) were obtained from the Jackson Laboratory. Col4a3 mice develop server proteinuria starting age of week 5 and gradually develop end stage renal disease at week 10 or later. To evaluate the impact of anti-WISE treatment after proteinuria has been developed, treatments were started at age of day 43 (week 6) with 20 mg/kg of anti-WISE antibody Ab-C or Ab-E or vehicle every other day (IP). Treatments were continued for 14 days. Each group (n=12) received six injections during the study period. Urinary samples were collected in metabolic cages on day 42 (1 day before treatment), day 48, day 52 and day 57. Twenty four hour total urinary protein (UTP) excretion were measured using Albuwell M (Exocell Inc.)

according to manufacturer's instruction and adjusted for urine volume. Figure 39 shows that 24 hr total urinary protein (UTP) was statistically significantly lower in anti-WISE treated groups compared to vehicle treated groups. Along with the observation reported for WISE KO mice, this data suggests potential utility of anti-WISE agents in reducing proteinuria which is a common manifestation in various kidney diseases such as glomerulonephritis, membranous nephropathy, diabetic nephropathy and transplantation related nephropathy.

CLAIMS

1. An isolated antibody or fragment thereof that binds to a T49 epitope, or a T56.1 epitope of WISE.

2. The antibody or fragment thereof of claim 1 that comprises at least one CDR sequence having at least 90% identity to a CDR selected from SEQ ID NOs: 123, 124, 125, 127, 128, 129, 131, 132, 133, 135, 136, 137, 139, 140, 141, 143, 144, 145, 147, 148, 149, 151, 152, 153, 155, 156, 157, 158, 159, 160, 164, 167, 168, 170, 171, 173, 174, 175, 177, 178, 179, 181, 182, 183, 185, 186, 187, 189, 190, 191, 193, 194, 195, 197, 198, 199, 201, 202, 203, 205, 206, 207, 209, 210, 211, 213, 214, 215, 217, 218, 219, 221, 222, 223, 225, 226, 227, 229, 230, 231, 233, 234, 235, 237, 238, 239, 241, 242, 243, 245, 246, 247, 249, 250, 251, 253, 254, 255, 257, 258, 259, 261, 262, 263, 273, 274, 275, 277, 278 and 279 and binds to human WISE.

3. The antibody or fragment thereof of claim 2 comprising six of said CDR's.

4. The antibody or fragment thereof according to any one of claims 1 to 3 wherein said percent identity is 95%.

5. The antibody or fragment thereof according to claim 2 comprising: a. CDR sequences of SEQ ID NOs: 123, 124, and 125; b. CDR sequences of SEQ ID NOs: 127, 128, and 129; c. CDR sequences of SEQ ID NOs: 131, 132, and 133; d. CDR sequences of SEQ ID NOs: 135, 136, and 137; e. CDR sequences of SEQ ID NOs: 139, 140, and 141; f. CDR sequences of SEQ ID NOs: 143, 144, and 145; g. CDR sequences of SEQ ID NOs: 147, 148, and 149; h. CDR sequences of SEQ ID NOs: 151, 152, and 153; i. CDR sequences of SEQ ID NOs: 155, 156, and 157; j. CDR sequences of SEQ ID NOs: 158, 159, and 160; k. CDR sequences of SEQ ID NOs: 161, 162, and 163; l. CDR sequences of SEQ ID NOs: 164, 165, and 166; m. CDR sequences of SEQ ID NOs: 167, 168, and 169; n. CDR sequences of SEQ ID NOs: 170, 171, and 172; o. CDR sequences of SEQ ID NOs: 173, 174, and 175; p. CDR sequences of SEQ ID NOs: 177, 178, and 179; q. CDR sequences of SEQ ID NOs: 181, 182, and 183; r. CDR sequences of SEQ ID NOs: 185, 186 and 187; s. CDR sequences of SEQ ID NOs: 189, 190, and 191; t.

CDR sequences of SEQ ID NOs: 193, 194, and 195; u. CDR sequences of SEQ ID NOs: 197, 198, and 199; v. CDR sequences of SEQ ID NOs: 201, 202, and 203; w. CDR sequences of SEQ ID NOs: 205, 206 and 207; x. CDR sequences of SEQ ID NOs: 209, 210, and 211; y. CDR sequences of SEQ ID NOs: 213, 214, and 215; z. CDR sequences of SEQ ID NOs: 217, 218, and 219; aa. CDR sequences of SEQ ID NOs: 221, 222, and 223; bb. CDR sequences of SEQ ID NOs: 225, 226, and 227; cc. CDR sequences of SEQ ID NOs: 229, 230, and 231; dd. CDR sequences of SEQ ID NOs: 233, 234, and 235; ee. CDR sequences of SEQ ID NOs: 237, 238, and 239; ff. CDR sequences of SEQ ID NOs: 241, 242, and 243; gg. CDR sequences of SEQ ID NOs: 245, 246, and 247; hh. CDR sequences of SEQ ID NOs: 249, 250, and 251; ii. CDR sequences of SEQ ID NOs: 253, 254, and 255; jj. CDR sequences of SEQ ID NOs: 257, 258, and 259; kk. CDR sequences of SEQ ID NOs: 261, 262 and 263; ll. CDR sequences of SEQ ID NOs: 273, 274 and 275; or mm. CDR sequences of SEQ ID NOs: 277, 278 and 279.

6. The antibody or fragment thereof according to claim 2 comprising: a. CDR sequences of SEQ ID NOs: 123, 124, and 125 and CDR sequences of SEQ ID NOs: 127, 128, and 129; b. CDR sequences of SEQ ID NOs: 131, 132, and 133 and CDR sequences of SEQ ID NOs: 135, 136, and 137; c. CDR sequences of SEQ ID NOs: 139, 140, and 141 and CDR sequences of SEQ ID NOs: 143, 144, and 145; d. CDR sequences of SEQ ID NOs: 147, 148, and 149 and CDR sequences of SEQ ID NOs: 151, 152, and 153; e. CDR sequences of SEQ ID NOs: 155, 156, and 157 and CDR sequences of SEQ ID NOs: 158, 159, and 160; f. CDR sequences of SEQ ID NOs: 161, 162, and 163 and CDR sequences of SEQ ID NOs: 164, 165, and 166; g. CDR sequences of SEQ ID NOs: 167, 168, and 169 and CDR sequences of SEQ ID NOs: 170, 171, and 172; h. CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 173, 174, and 175; i. CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 177, 178, and 179; j. CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 181, 182, and 183; k. CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 185, 186 and 187; l. CDR sequences of SEQ ID NOs: 135, 136 and 137 and CDR sequences of SEQ ID NOs: 189, 190, and 191; m. CDR sequences of SEQ ID NOs: 135, 136 and 137 and

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7. The antibody or fragment thereof according to claim 1 comprising a heavy chain wherein said heavy chain comprises a polypeptide having at least 85% identity to the sequence given in SEQ ID NO: 24; SEQ ID NO: 16; SEQ ID NO: 52 or SEQ ID NO: 272.

8. The antibody or fragment thereof according to claim 1 comprising both a heavy chain and a light chain wherein the heavy chain comprises a polypeptide having at least 85% identity to the sequence given in SEQ ID NO: 24 and the light chain comprises a polypeptide having at least 85% identity to the sequence selected from the sequence in SEQ ID NOs: 22, 46, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, and 122.

9. A method of treating fibrosis comprising administering an antibody according to any one of the preceding claims that has an affinity of at less than $1x10^{-7}M$ and inhibits WISE activity.

10. The method according to claim 9 wherein the fibrosis is associated with lung disease.

11. The method according to claim 9 wherein the fibrosis is associated with kidney disease.

12. A method of treating proteinuria comprising administering an antibody according to any one of claims 1 to 8 that has an affinity of at less than $1x10^{-7}M$ and inhibits WISE activity.

13. The method of claim 12 wherein the antibody is selected from group consisting of Ab-M, Ab-N, Ab-O, Ab-R, Ab-1, Ab-13, Ab-16, Ab-18, Ab-23, Ab-24, Ab-28, Ab-29, Ab-48, Ab-60, Ab-63, Ab-65, Ab-66, Ab-67, Ab-69, Ab-7, Ab-70, Ab-72, Ab-74, Ab-75, Ab-76, and Ab-9.

14. A pharmaceutical composition comprising the antibody or fragment according to any one of claims 1 to 8.

15. The antibody or fragment thereof according to any one of claims 1 to 8 in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

16. The antibody or fragment thereof according to any one of claims 1 to 8 conjugated to at least one of Fc, PEG, albumin, and transferrin.

17. An immunogenic polypeptide of WISE suitable for use in producing inhibitory antibodies, the peptide corresponding to a T49 epitope or a T56.1 epitope of WISE.

18. Use of an antibody according to any one of claims 1 to 8 in the manufacture of a medicament for the treatment of fibrosis; or for the treatment of proteinuria.

19. An isolated antibody or fragment thereof according to claim 1; a method of treating fibrosis according to claim 9; a method of treating proteinuria according to claim 12; a pharmaceutical composition according to claim 14; an immunogenic polypeptide of WISE according to claim 17; or use according to claim 18, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

Figure 1

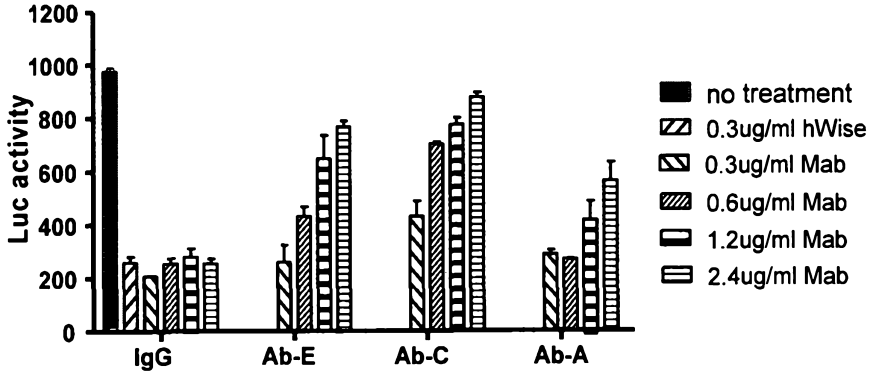
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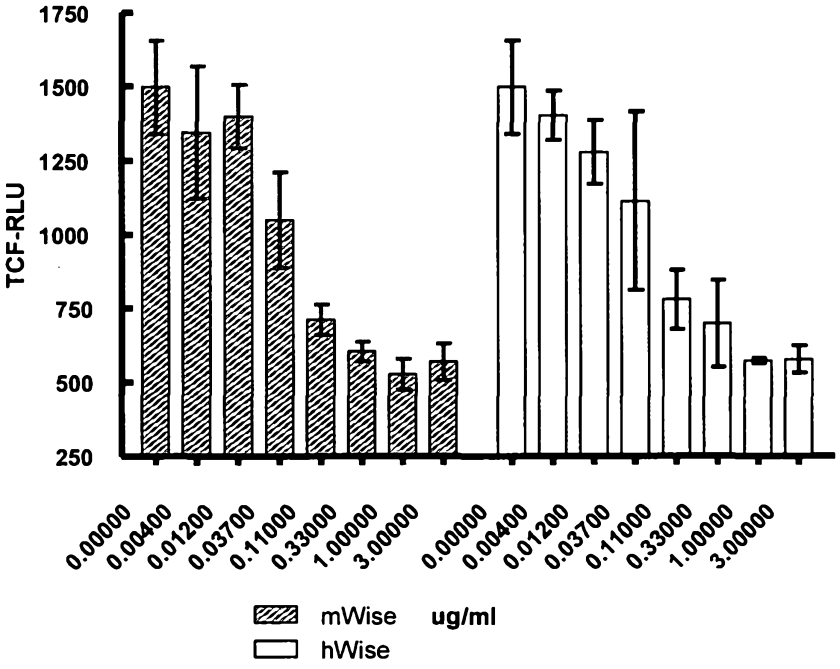
Figure 2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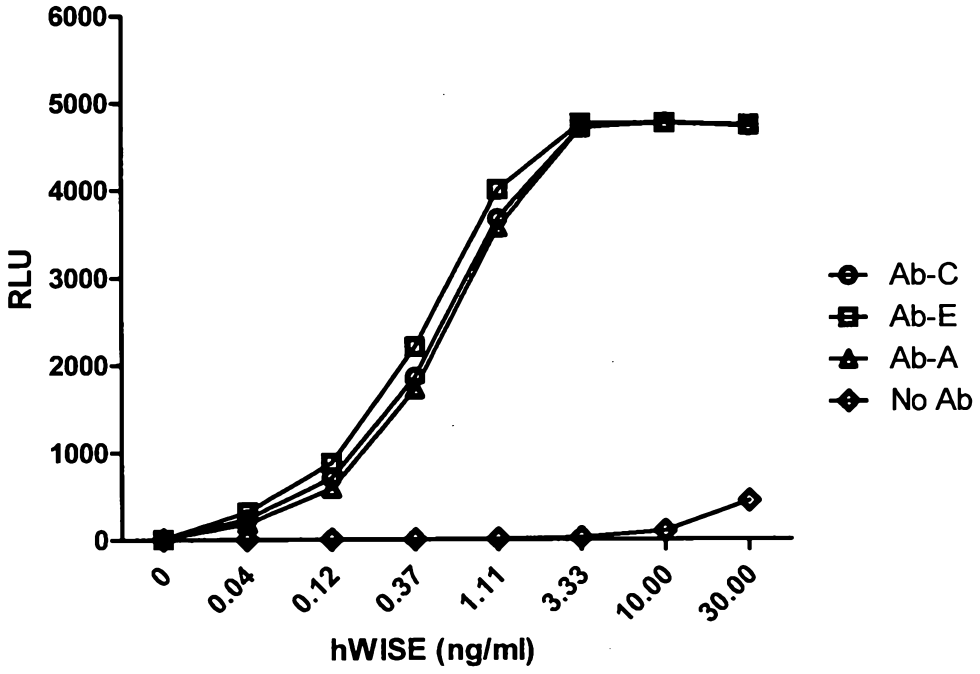
mWise
hWise
ug/ml

Figure 3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Ab-C
Ab-E
Ab-A
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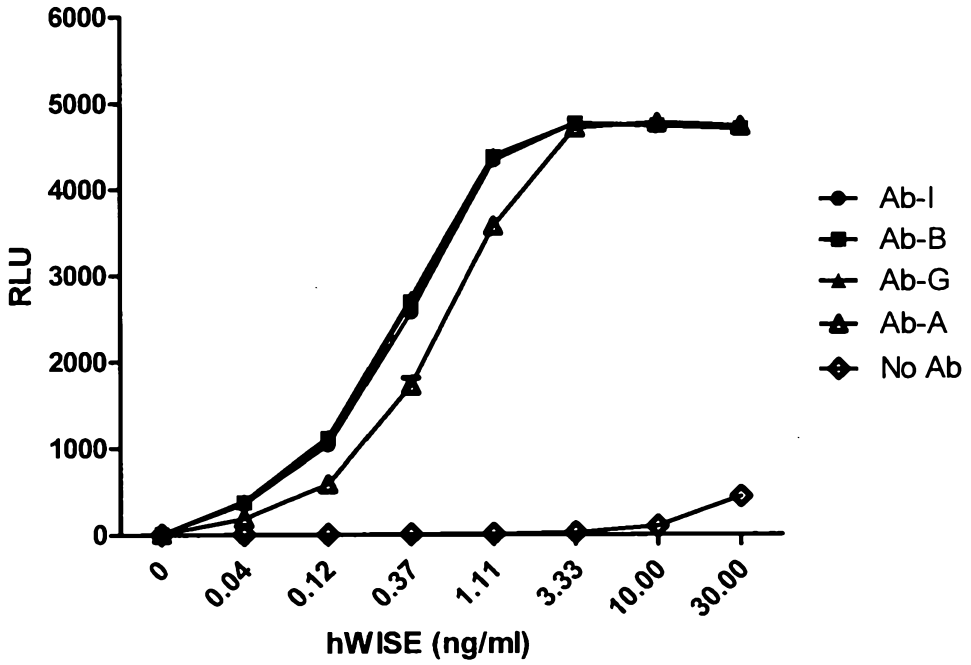
Figure 4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Ab-A
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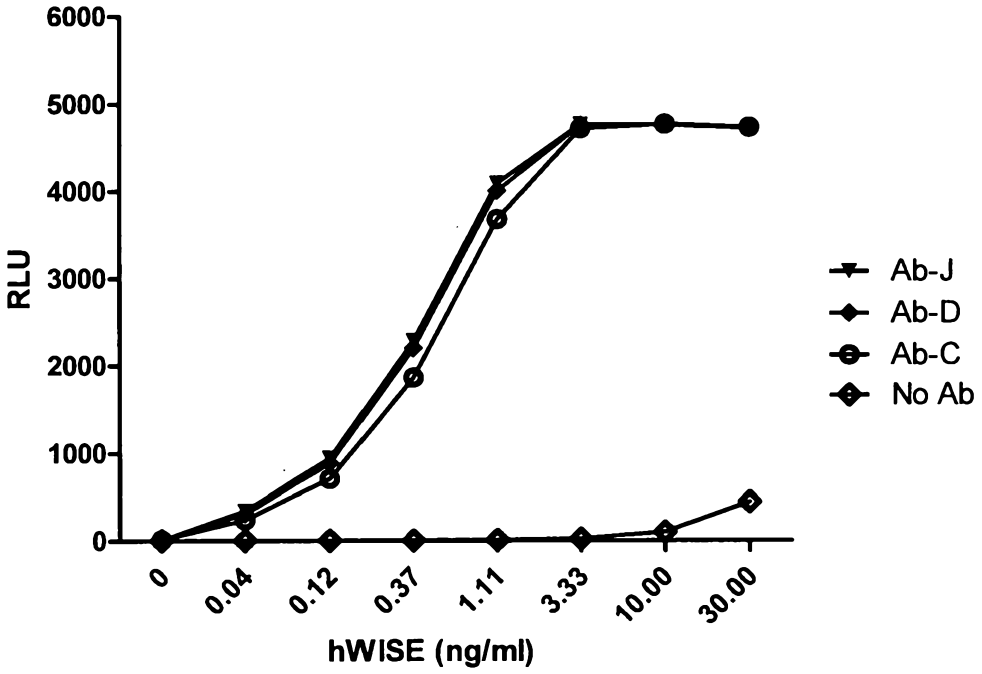
Figure 5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hWISE (ng/ml)
Ab-J
Ab-D
Ab-C
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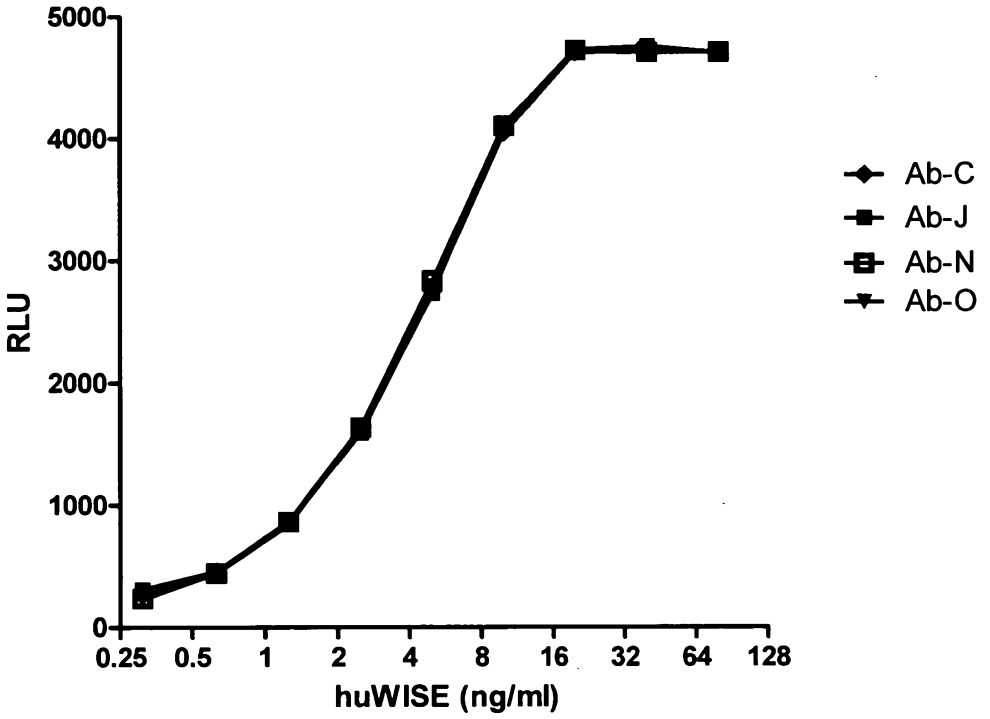
Figure 6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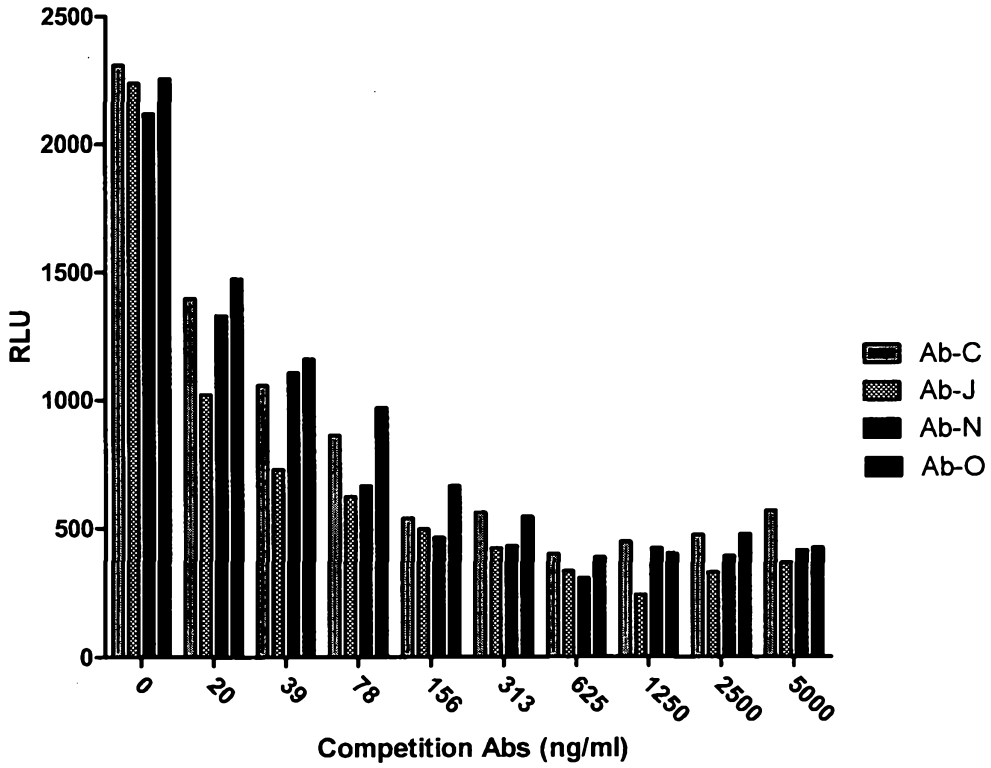
Ab-C
Ab-J
Ab-N
Ab-O

Figure 7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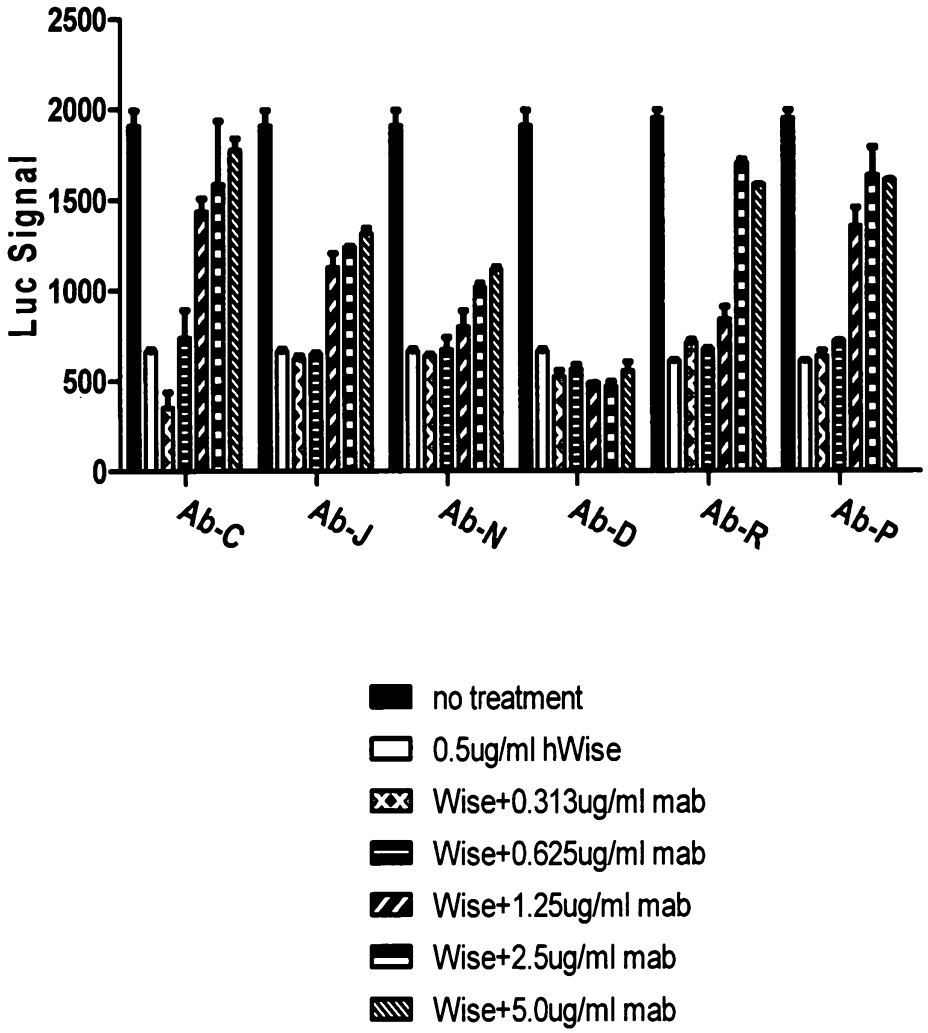
Ab-J
Ab-N
Ab-O

Figure 8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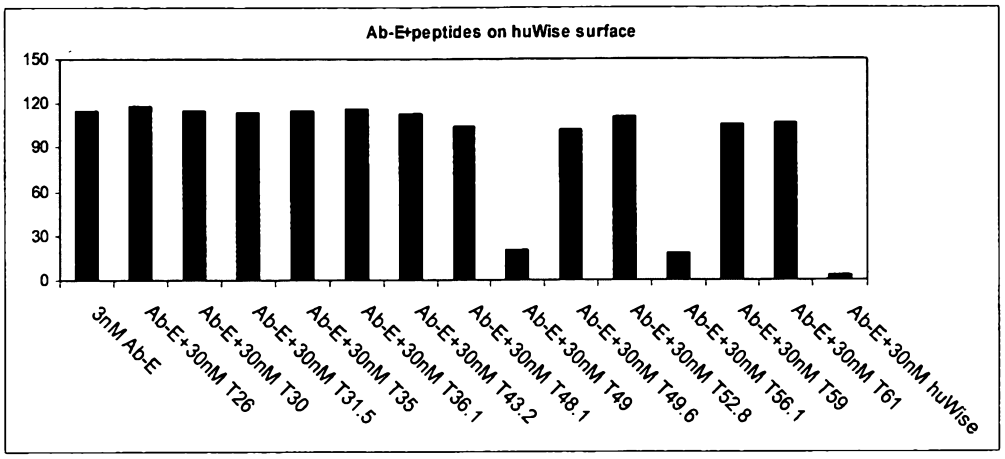
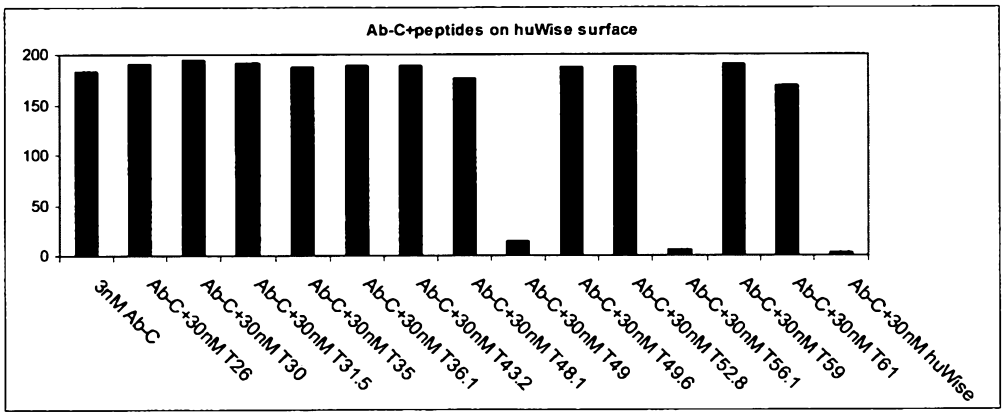
Wise+0.313ug/ml mab
Wise+0.625ug/ml mab
Wise+1.25ug/ml mab
Wise+2.5ug/ml mab
Wise+5.0ug/ml mab

Figure 9A


Ab-E+peptides on huWise surface
150
120
90
60
30
0
3nM Ab-E
Ab-E+30nM T26
Ab-E+30nM T30
Ab-E+30nM T31.5
Ab-E+30nM T35
Ab-E+30nM T36.1
Ab-E+30nM T43.2
Ab-E+30nM T48.1
Ab-E+30nM T49
Ab-E+30nM T49.6
Ab-E+30nM T52.8
Ab-E+30nM T56.1
Ab-E+30nM T59
Ab-E+30nM T61
Ab-E+30nM huWise


Ab-C+peptides on huWise surface
200
150
100
50
0
3nM Ab-C
Ab-C+30nM T26
Ab-C+30nM T30
Ab-C+30nM T31.5
Ab-C+30nM T35
Ab-C+30nM T36.1
Ab-C+30nM T43.2
Ab-C+30nM T48.1
Ab-C+30nM T49
Ab-C+30nM T49.6
Ab-C+30nM T52.8
Ab-C+30nM T56.1
Ab-C+30nM T59
Ab-C+30nM T61
Ab-C+30nM huWise

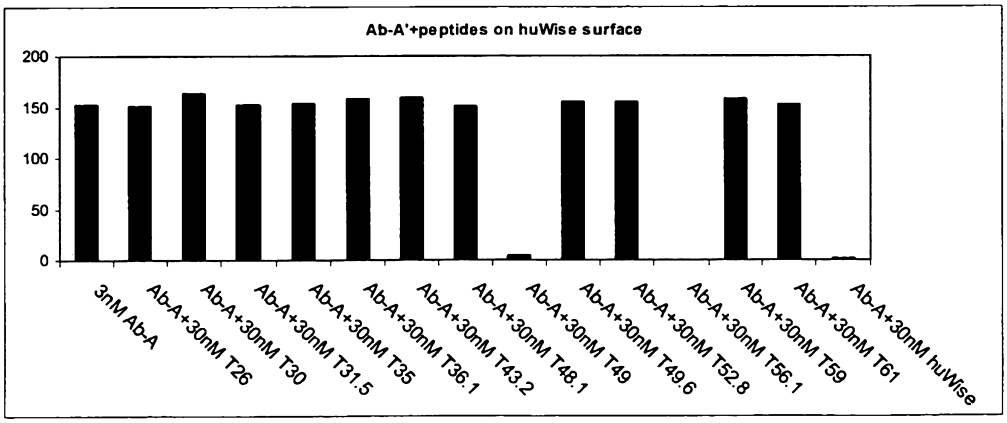

Ab-A'+peptides on huWise surface
200
150
100
50
0
3nM Ab-A
Ab-A+30nM T26
Ab-A+30nM T30
Ab-A+30nM T31.5
Ab-A+30nM T35
Ab-A+30nM T36.1
Ab-A+30nM T43.2
Ab-A+30nM T48.1
Ab-A+30nM T49
Ab-A+30nM T49.6
Ab-A+30nM T52.8
Ab-A+30nM T56.1
Ab-A+30nM T59
Ab-A+30nM T61
Ab-A+30nM huWise

Figure 9B

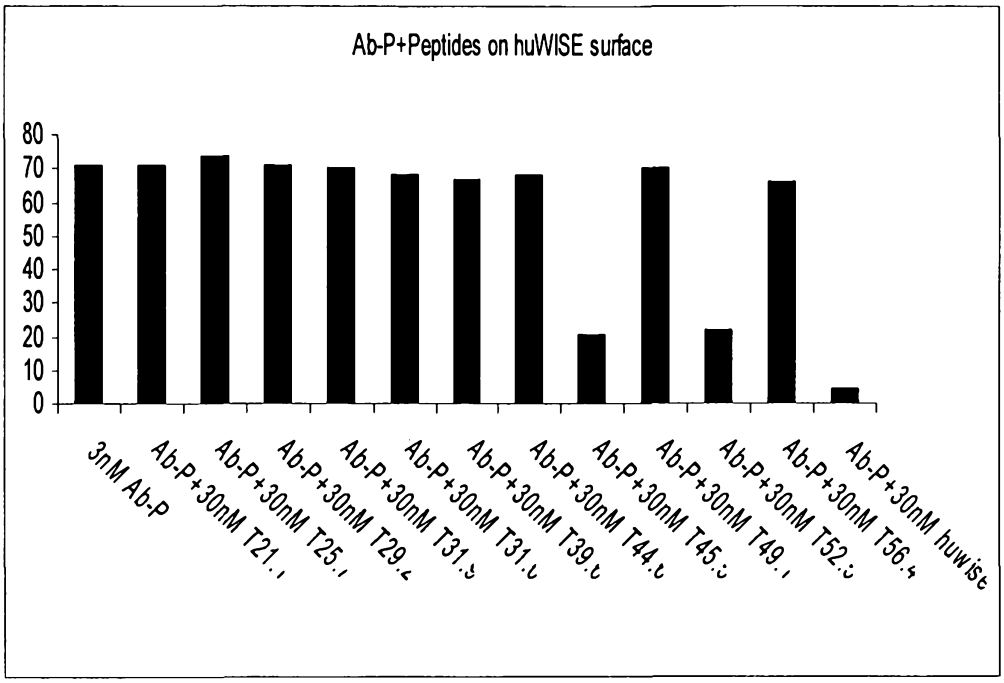

Ab-P+Peptides on huWISE surface
80
70
60
50
40
30
20
10
0
3nM Ab-P
Ab-P+30nM T21.1
Ab-P+30nM T25.1
Ab-P+30nM T29.2
Ab-P+30nM T31.3
Ab-P+30nM T31.4
Ab-P+30nM T39.4
Ab-P+30nM T44.4
Ab-P+30nM T45.3
Ab-P+30nM T49.1
Ab-P+30nM T52.3
Ab-P+30nM T56.4
Ab-P+30nM huWISE

Figure 10

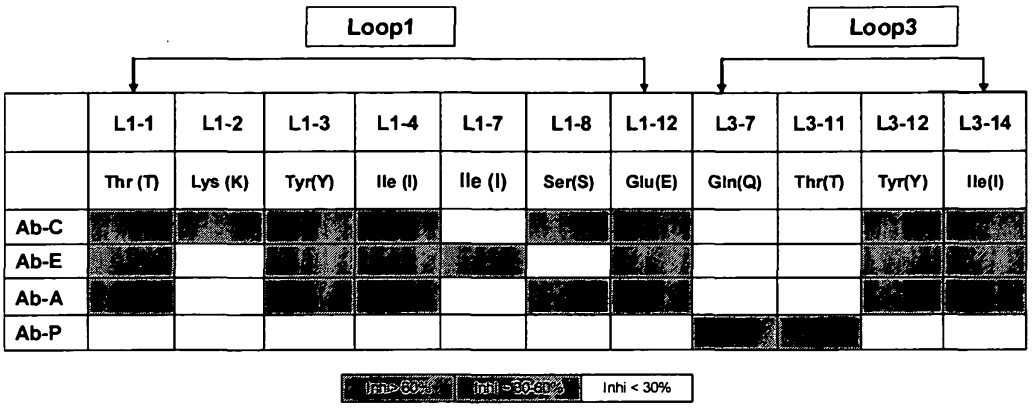

Loop1
Loop3
L1-1
L1-2
L1-3
L1-4
L1-7
L1-8
L1-12
L3-7
L3-11
L3-12
L3-14
Thr (T)
Lys (K)
Tyr(Y)
Ile (I)
Ile (I)
Ser(S)
Glu(E)
Gln(Q)
Thr(T)
Tyr(Y)
Ile(I)
Ab-C
Ab-E
Ab-A
Ab-P
Inhi < 30%

Figure 11

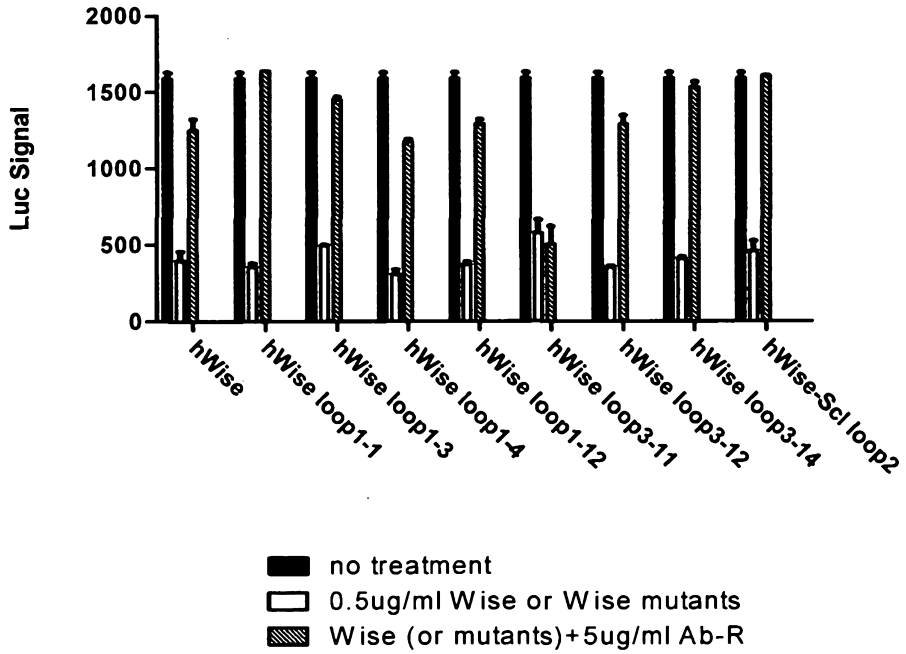

Luc Signal
2000
1500
1000
500
0
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5ug/ml Ab-R

Figure 12

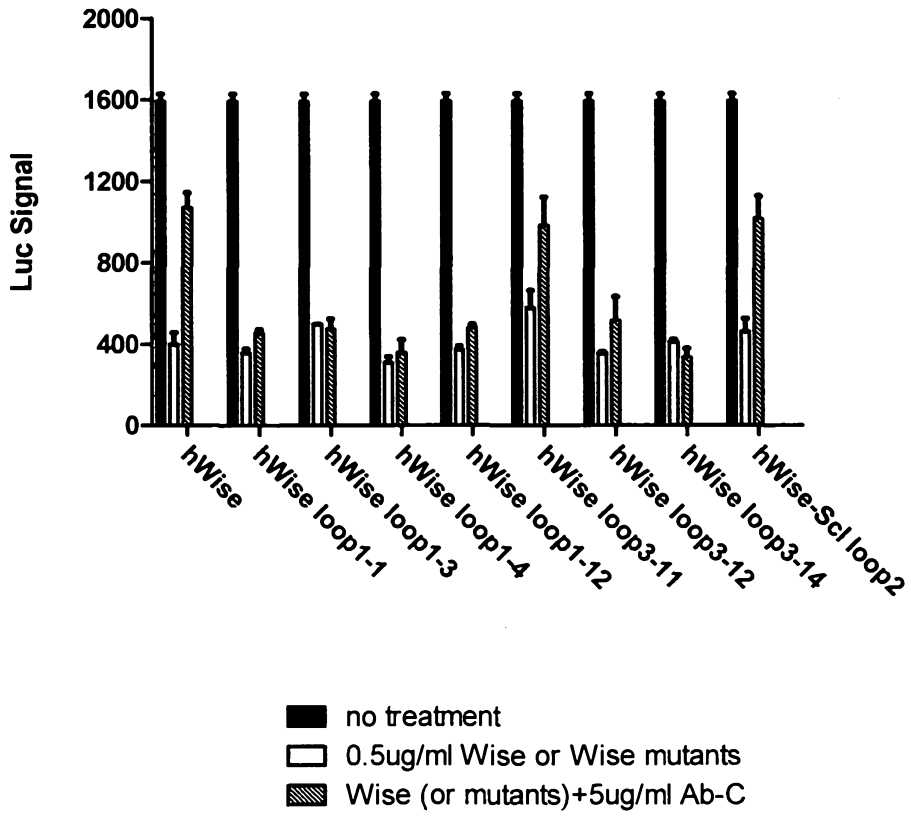

Luc Signal
2000
1600
1200
800
400
0
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5ug/ml Ab-C

Figure 13

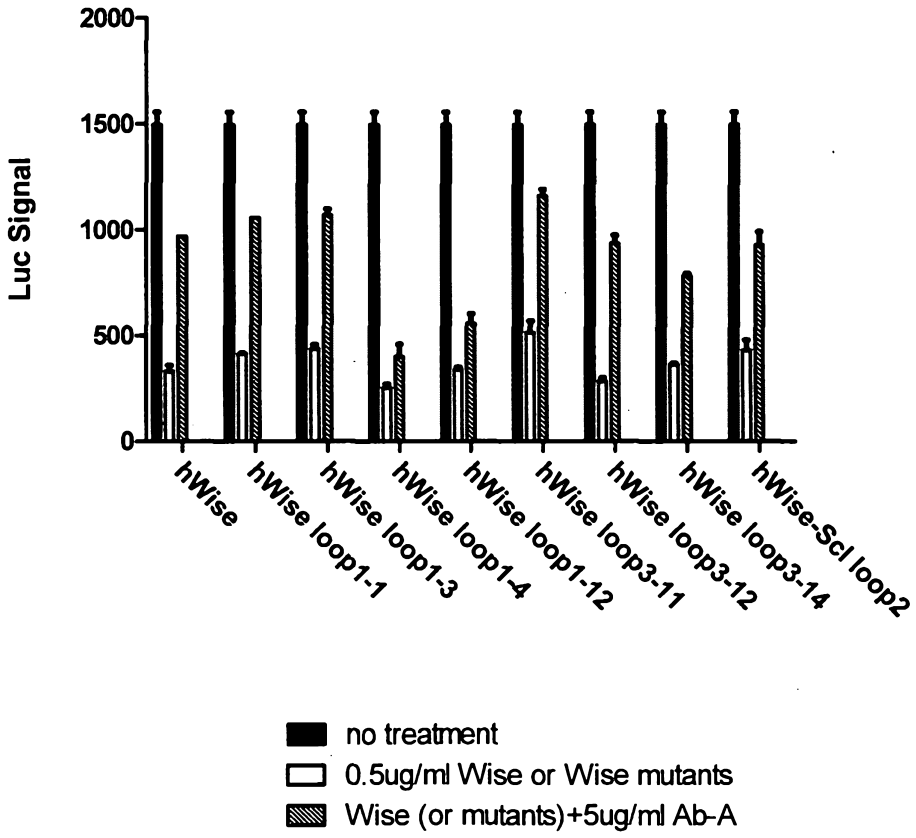

2000
1500
1000
500
0
Luc Signal
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5ug/ml Ab-A

Figure 14

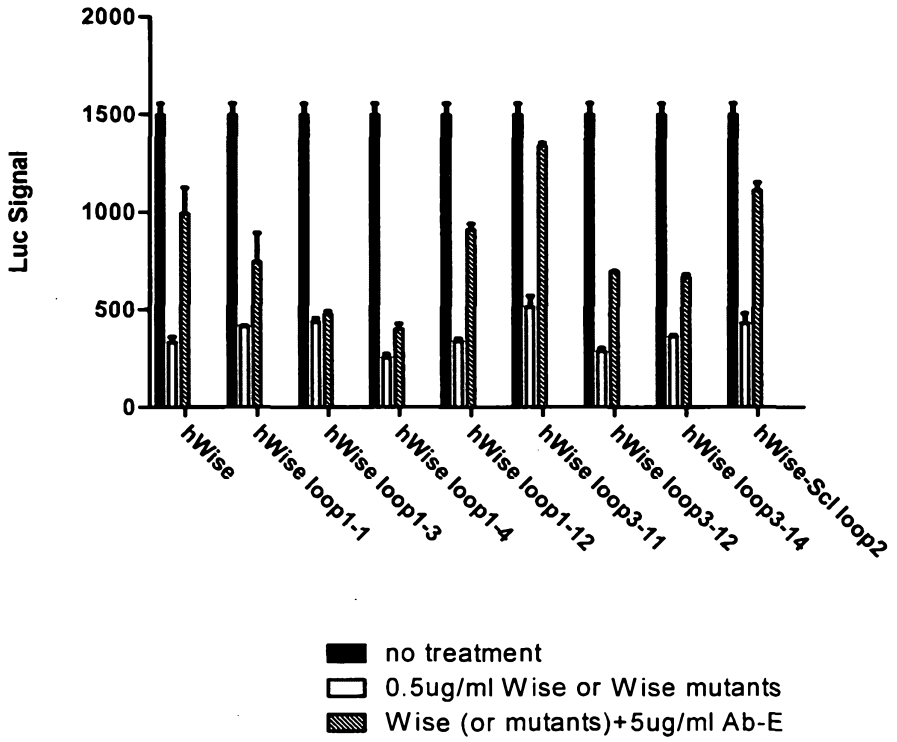

2000
1500
1000
500
0
Luc Signal
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5ug/ml Ab-E

Figure 15

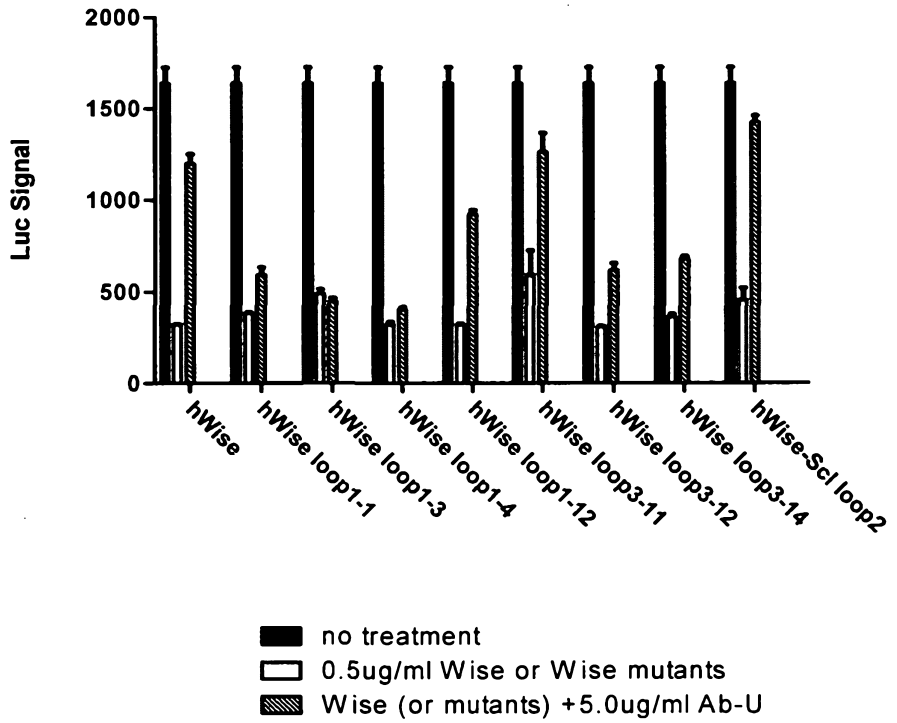

2000
1500
1000
500
0
Luc Signal
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants) +5.0ug/ml Ab-U

Figure 16

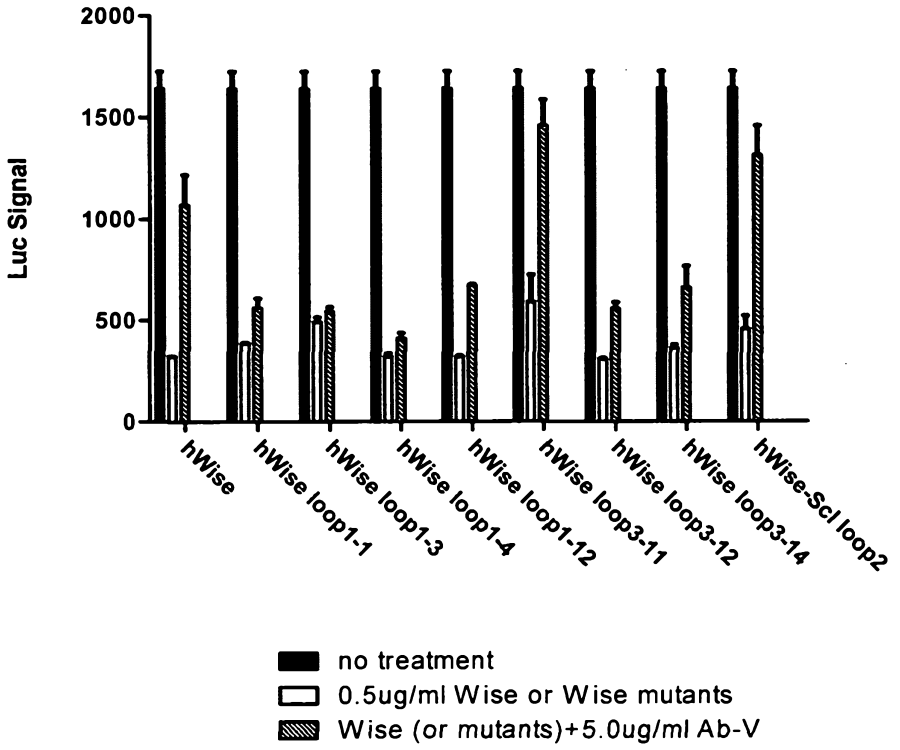

2000
1500
1000
500
0
Luc Signal
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5.0ug/ml Ab-V

Figure 17

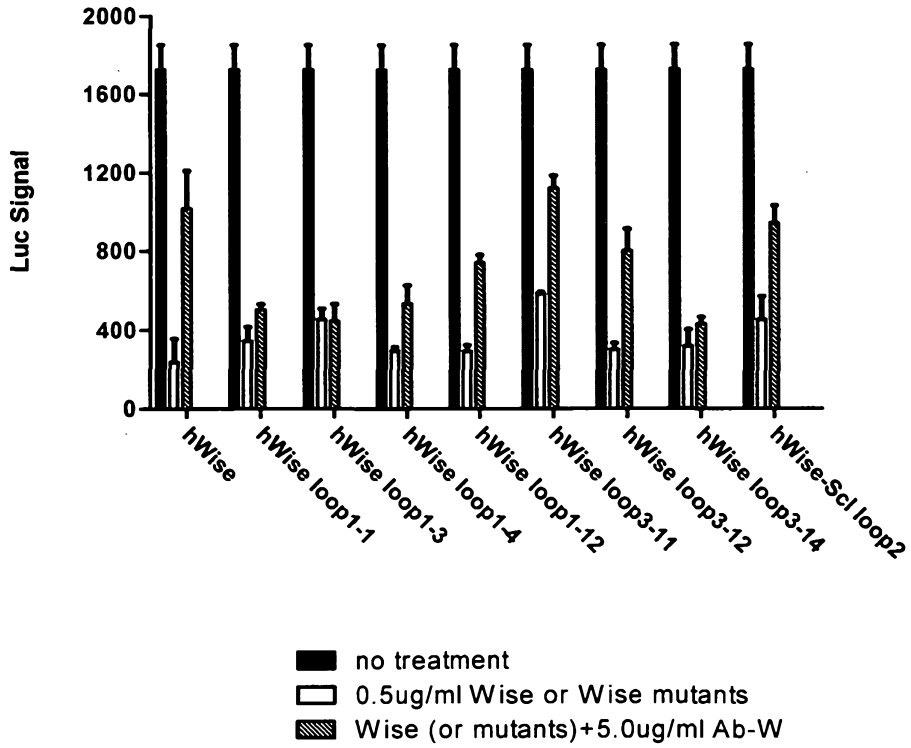

Luc Signal
2000
1600
1200
800
400
0
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5.0ug/ml Ab-W

Figure 18

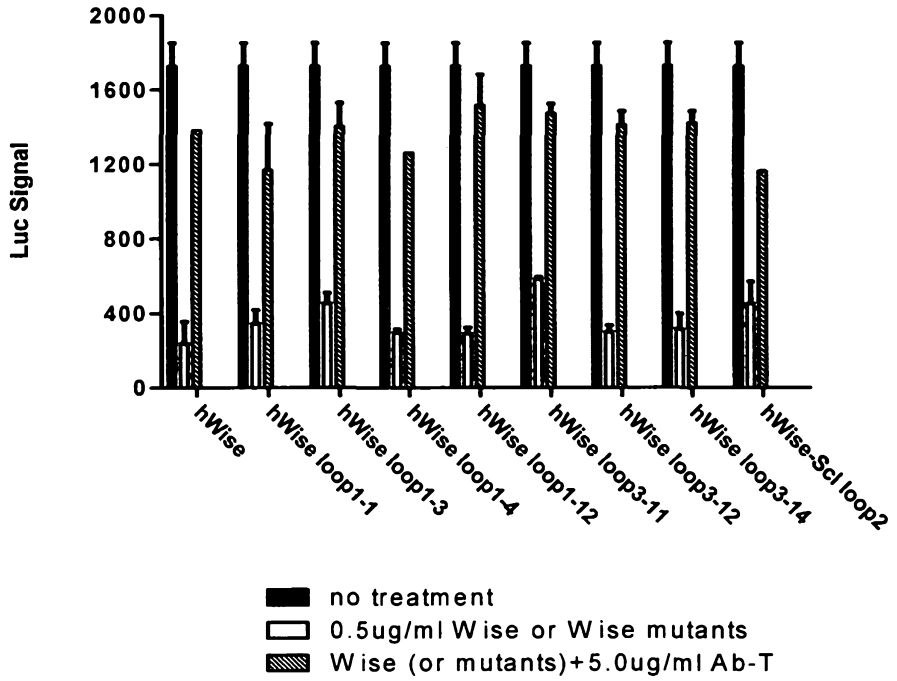

Luc Signal
2000
1600
1200
800
400
0
hWise
hWise loop1-1
hWise loop1-3
hWise loop1-4
hWise loop1-12
hWise loop3-11
hWise loop3-12
hWise loop3-14
hWise-Scl loop2
no treatment
0.5ug/ml Wise or Wise mutants
Wise (or mutants)+5.0ug/ml Ab-T

Figure 19

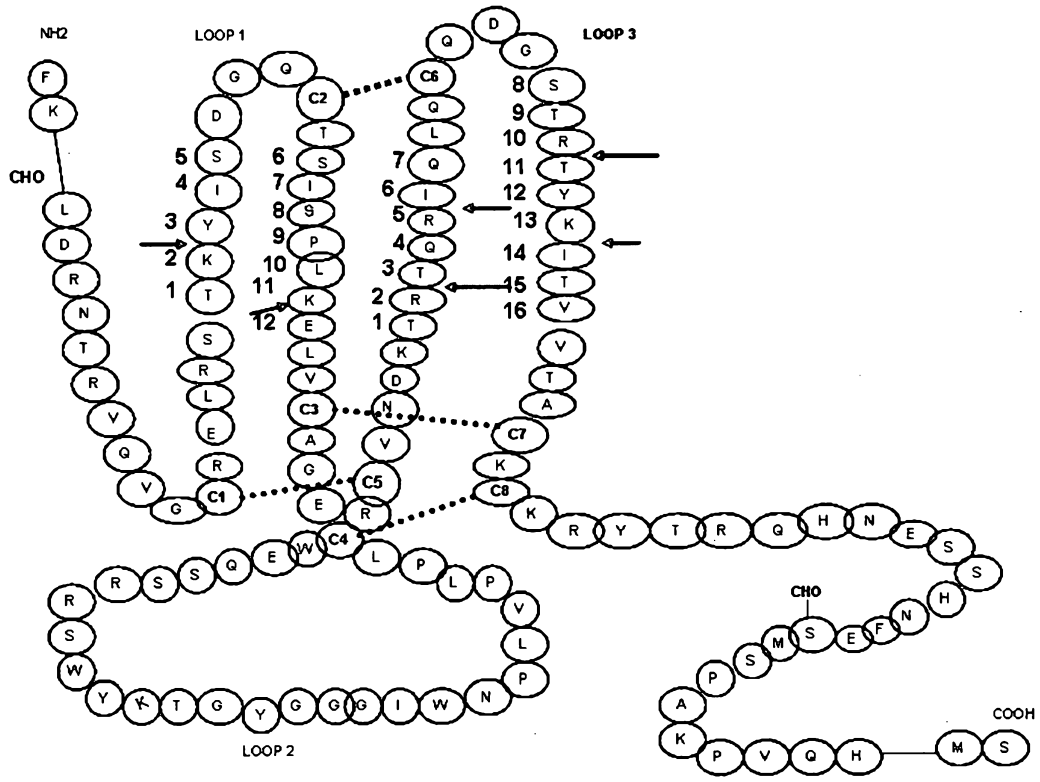


Figure 20

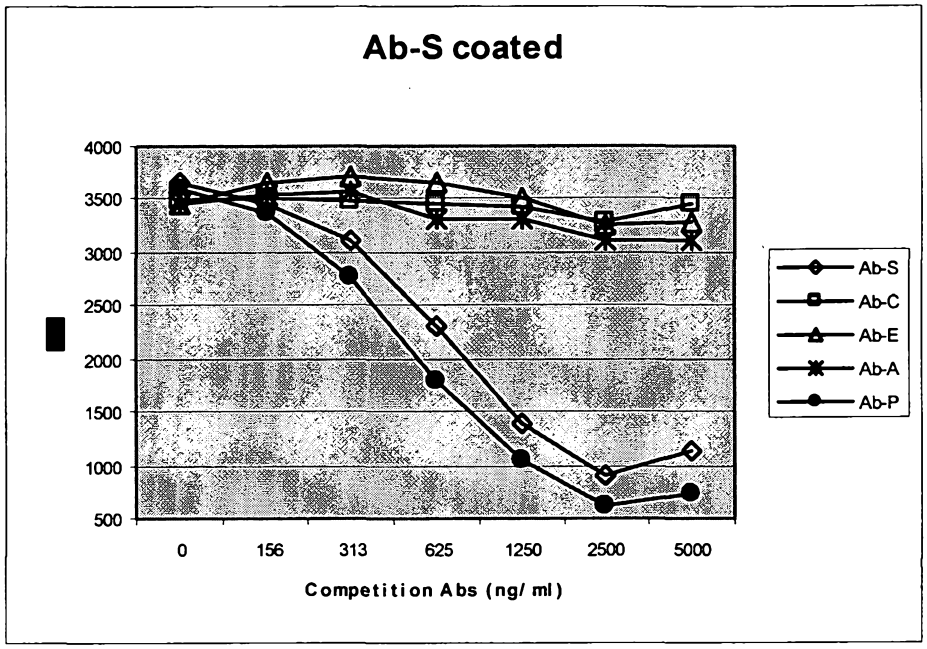

Ab-S coated
4000
3500
3000
2500
2000
1500
1000
500
0
156
313
625
1250
2500
5000
Competition Abs (ng/ ml)
Ab-S
Ab-C
Ab-E
Ab-A
Ab-P

Figure 21

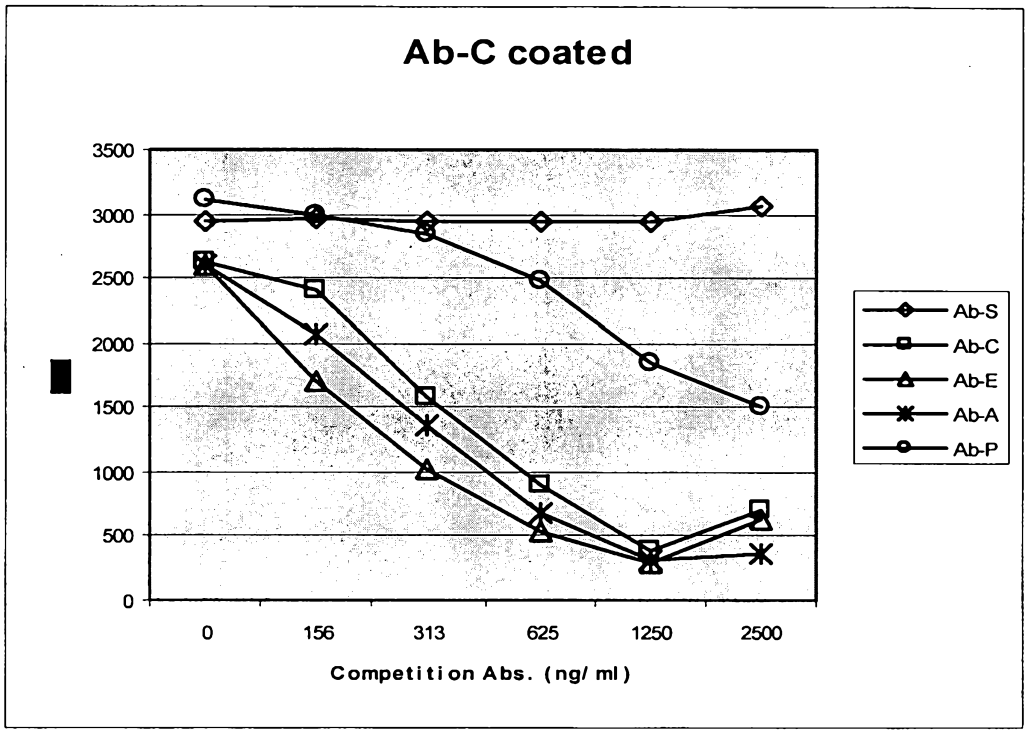

Ab-C coated
3500
3000
2500
2000
1500
1000
500
0
0
156
313
625
1250
2500
Competition Abs. (ng/ml)
Ab-S
Ab-C
Ab-E
Ab-A
Ab-P

Figure 22

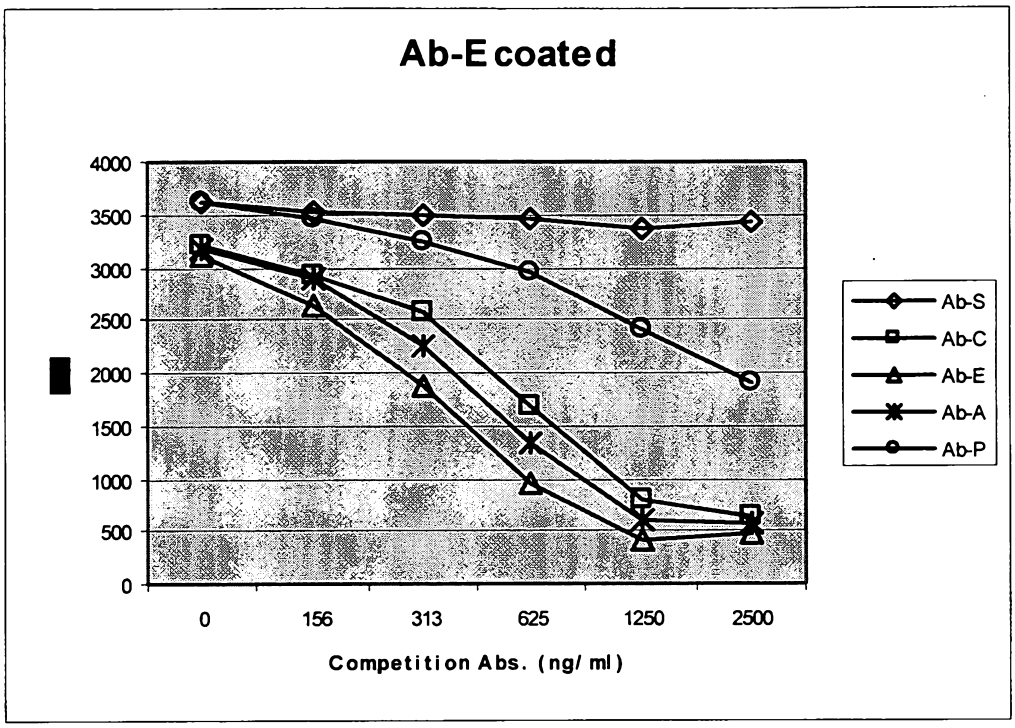

Ab-E coated
4000
3500
3000
2500
2000
1500
1000
500
0
0
156
313
625
1250
2500
Competition Abs. (ng/ml)
Ab-S
Ab-C
Ab-E
Ab-A
Ab-P

Figure 23

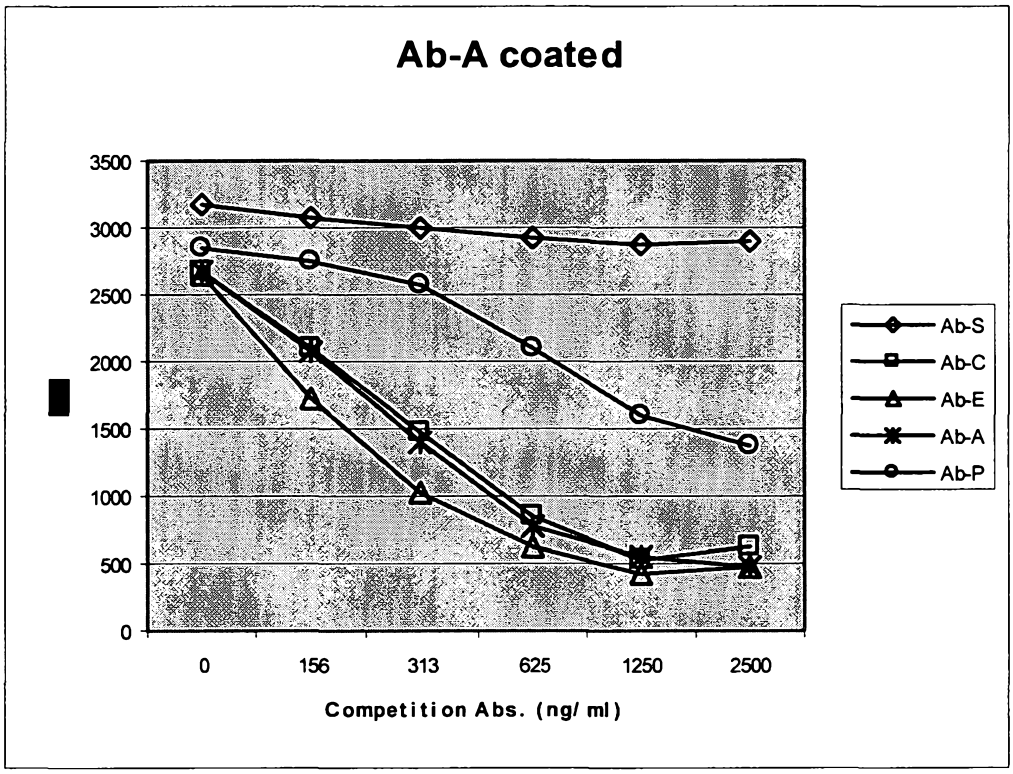

Ab-A coated
3500
3000
2500
2000
1500
1000
500
0
0
156
313
625
1250
2500
Competition Abs. (ng/ml)
Ab-S
Ab-C
Ab-E
Ab-A
Ab-P

Figure 24

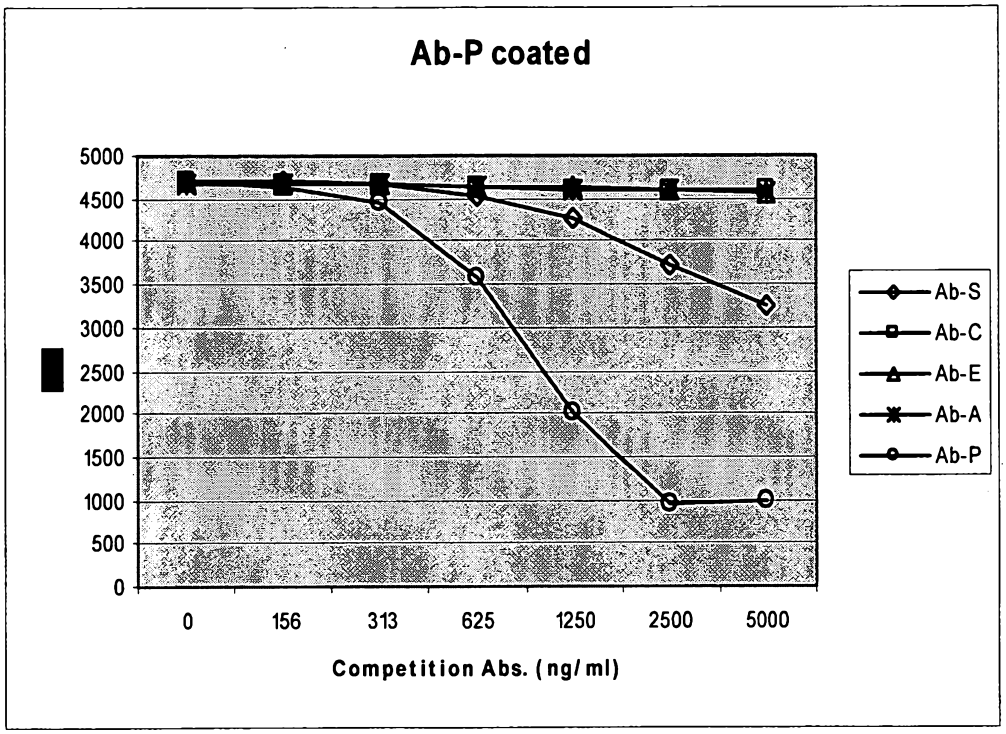

Ab-P coated
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
156
313
625
1250
2500
5000
Competition Abs. (ng/ml)
Ab-S
Ab-C
Ab-E
Ab-A
Ab-P

Figure 25

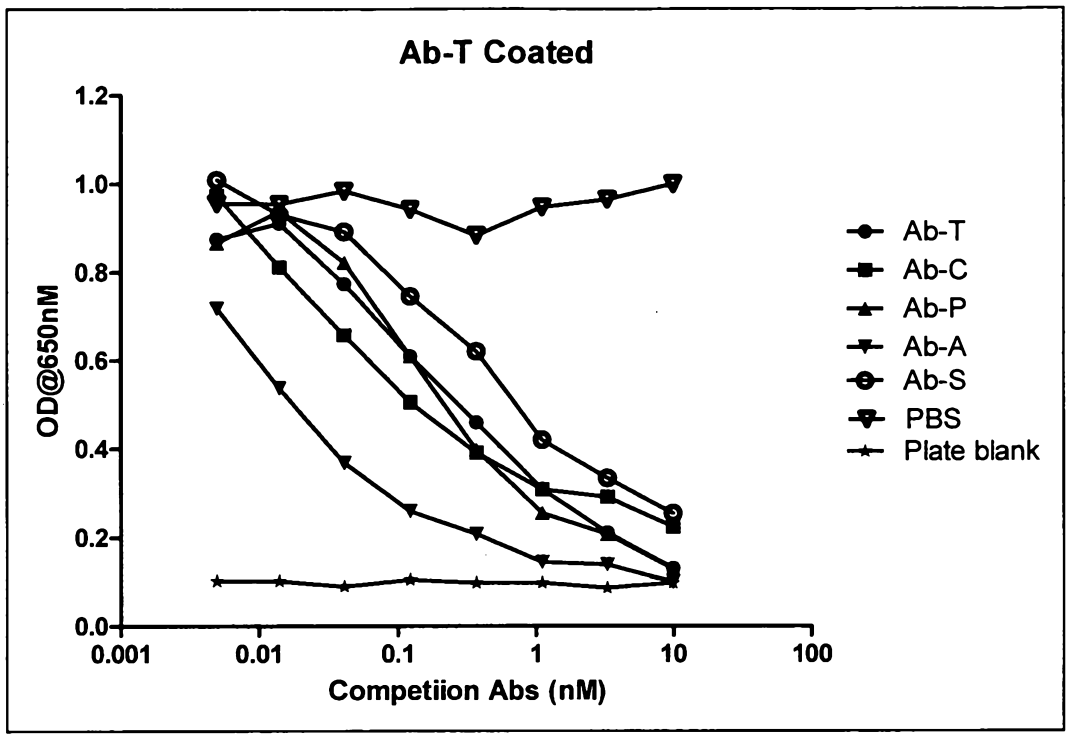

Ab-T Coated
OD@650nM
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
100
Competiion Abs (nM)
Ab-T
Ab-C
Ab-P
Ab-A
Ab-S
PBS
Plate blank

Figure 26

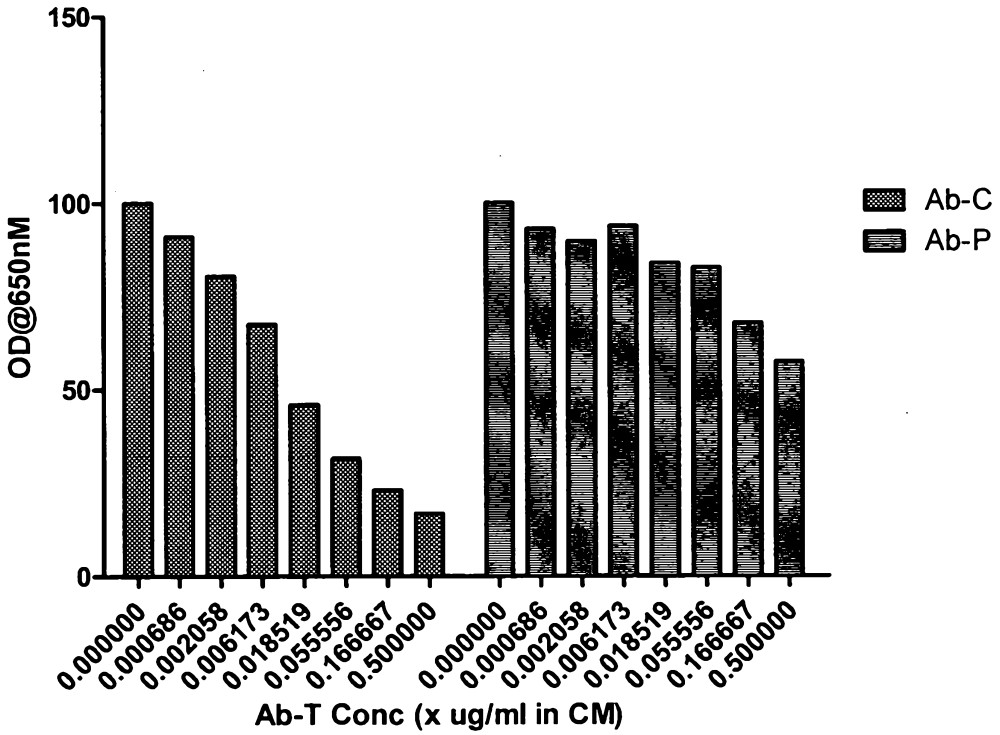

150
100
50
0
OD@650nM
Ab-C
Ab-P
0.000000
0.000686
0.002058
0.006173
0.018519
0.055556
0.166667
0.500000
0.000000
0.000686
0.002058
0.006173
0.018519
0.055556
0.166667
0.500000
Ab-T Conc (x ug/ml in CM)

Figure 27

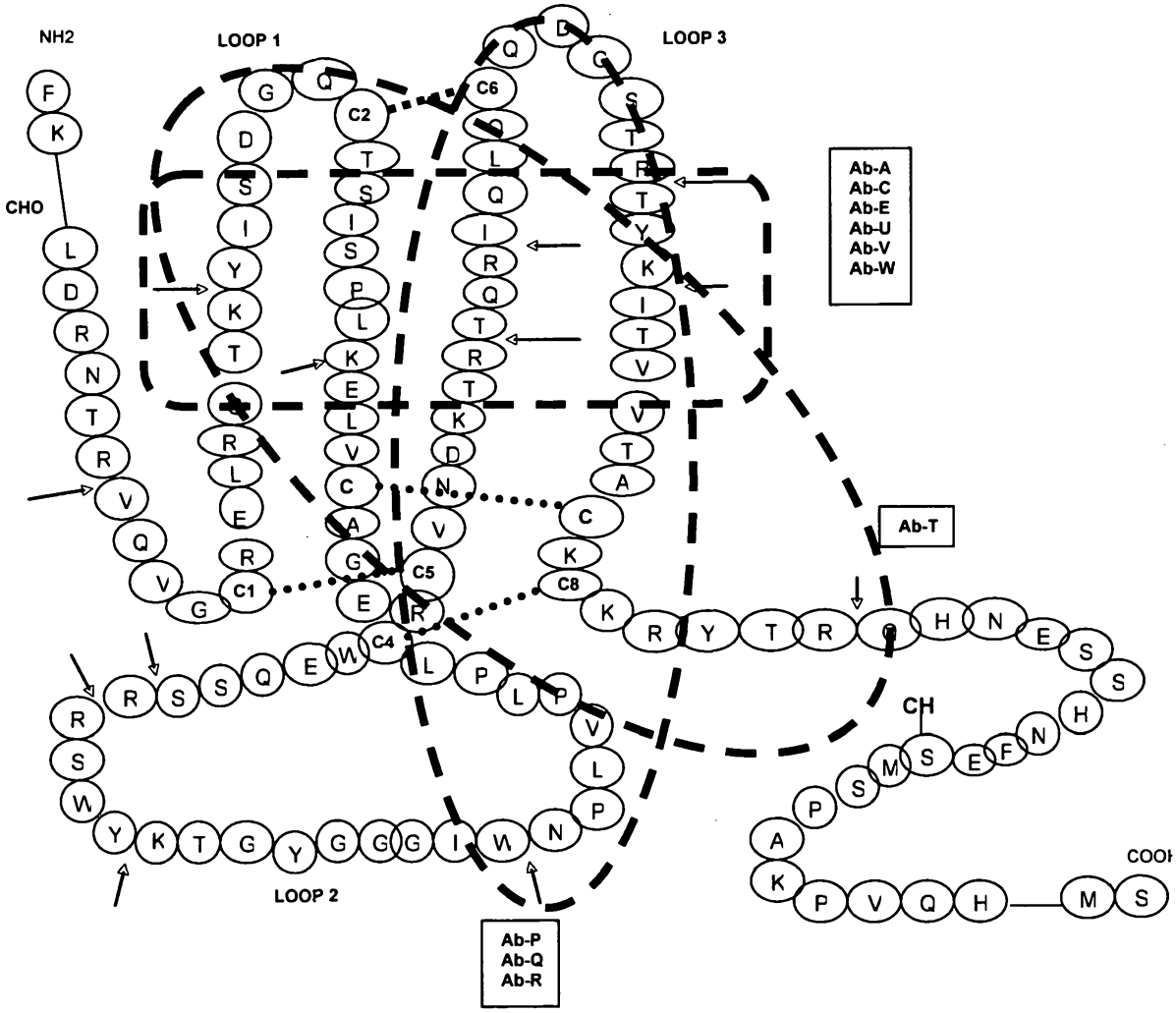

NH2
LOOP 1
LOOP 3
CHO
Ab-A
Ab-C
Ab-E
Ab-U
Ab-V
Ab-W
Ab-T
Ab-P
Ab-Q
Ab-R
LOOP 2
C1
C2
C4
C5
C6
C8
CH
COOH

Figure 28

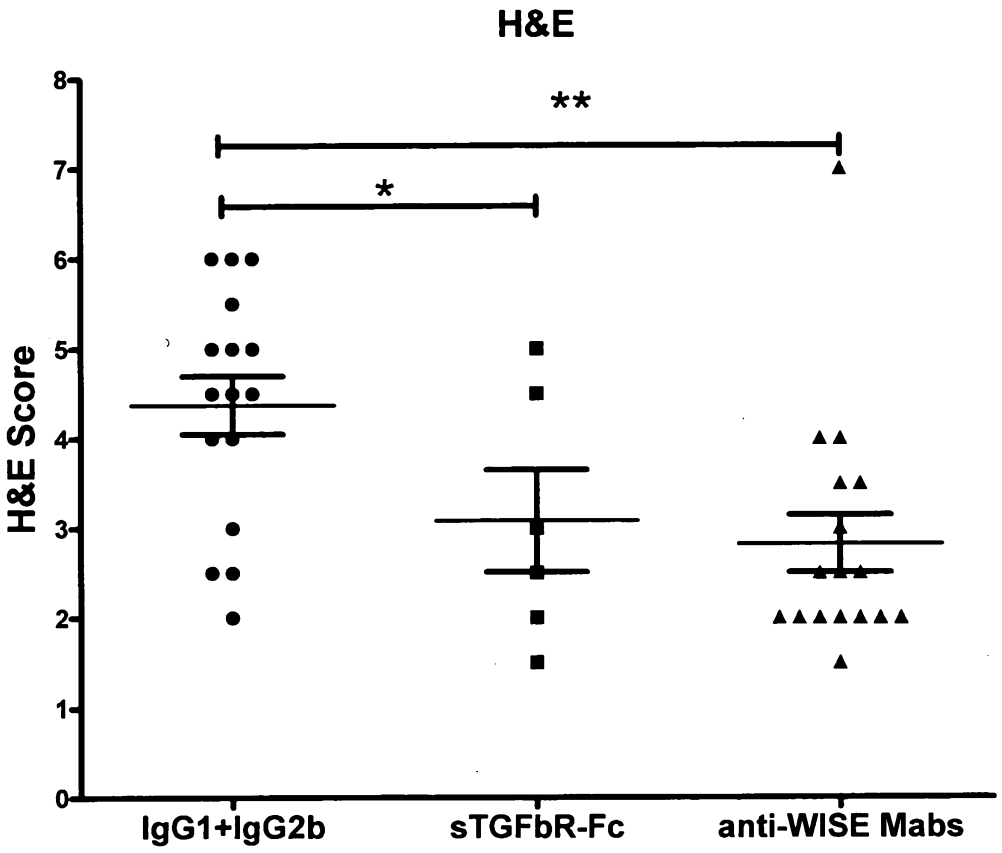


Figure 29

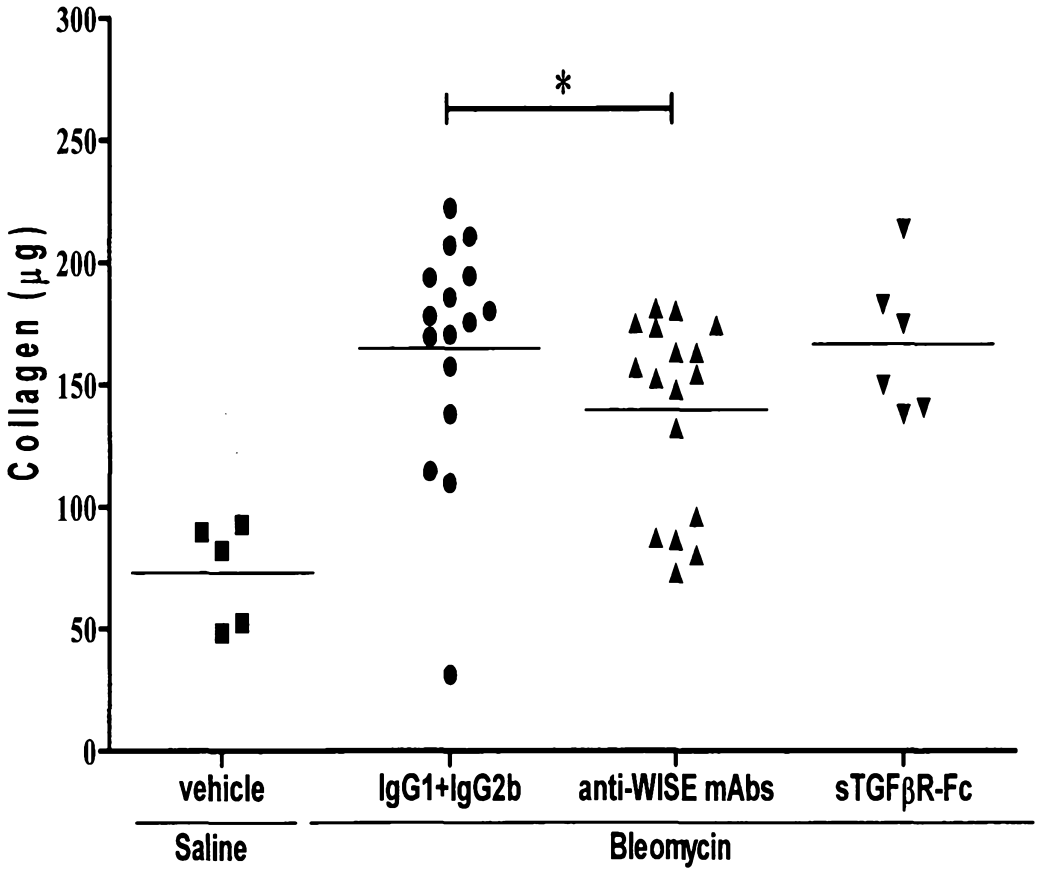

300
250
200
150
100
50
0
Collagen (μg)
*
vehicle
IgG1+IgG2b
anti-WISE mAbs
sTGFβR-Fc
Saline
Bleomycin

Figure 30

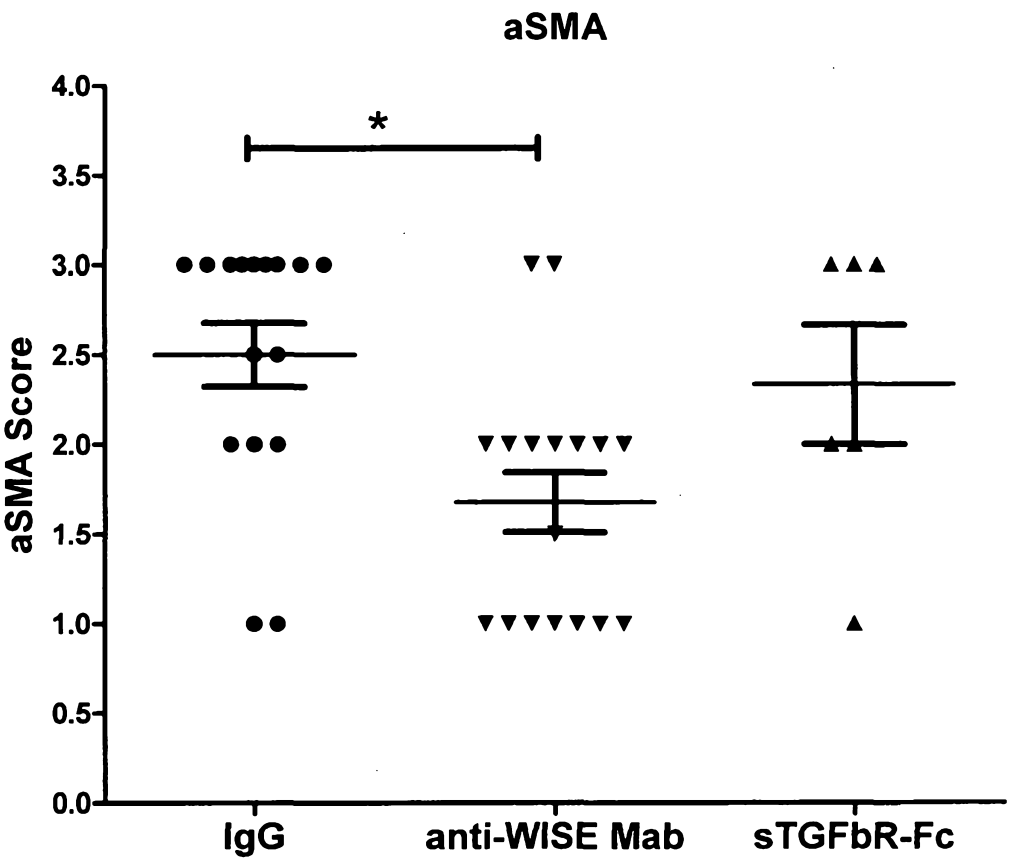

aSMA
aSMA Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
IgG
anti-WISE Mab
sTGFbR-Fc

Figure 31

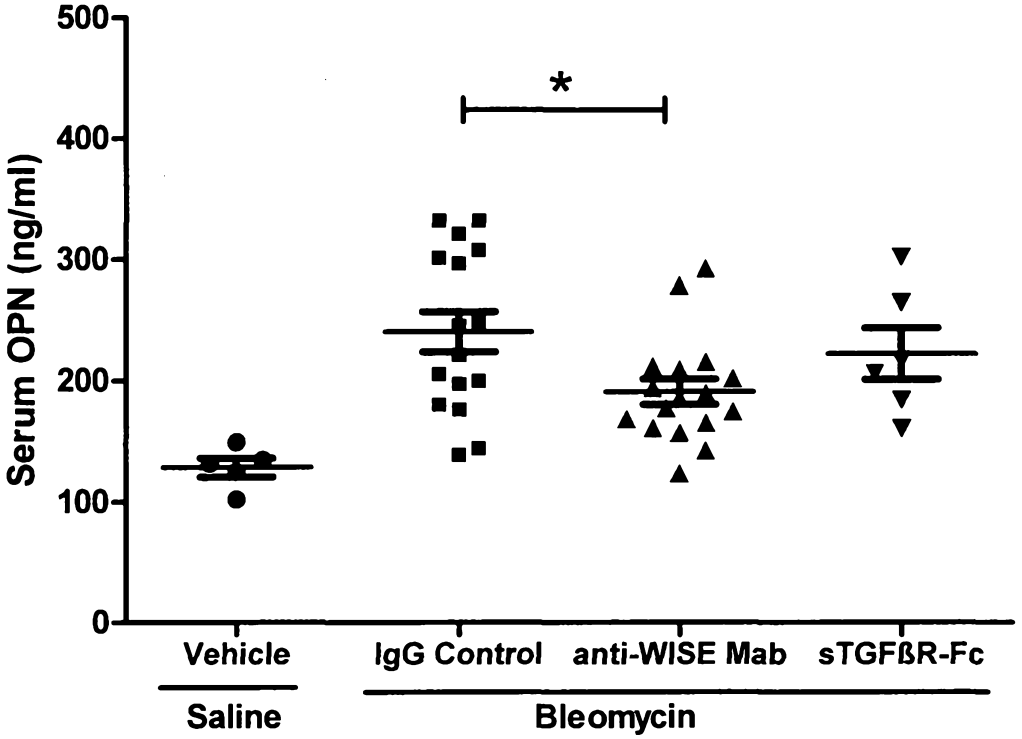

500
400
300
200
100
0
Serum OPN (ng/ml)
*
Vehicle
IgG Control
anti-WISE Mab
sTGFßR-Fc
Saline
Bleomycin

Figure 32

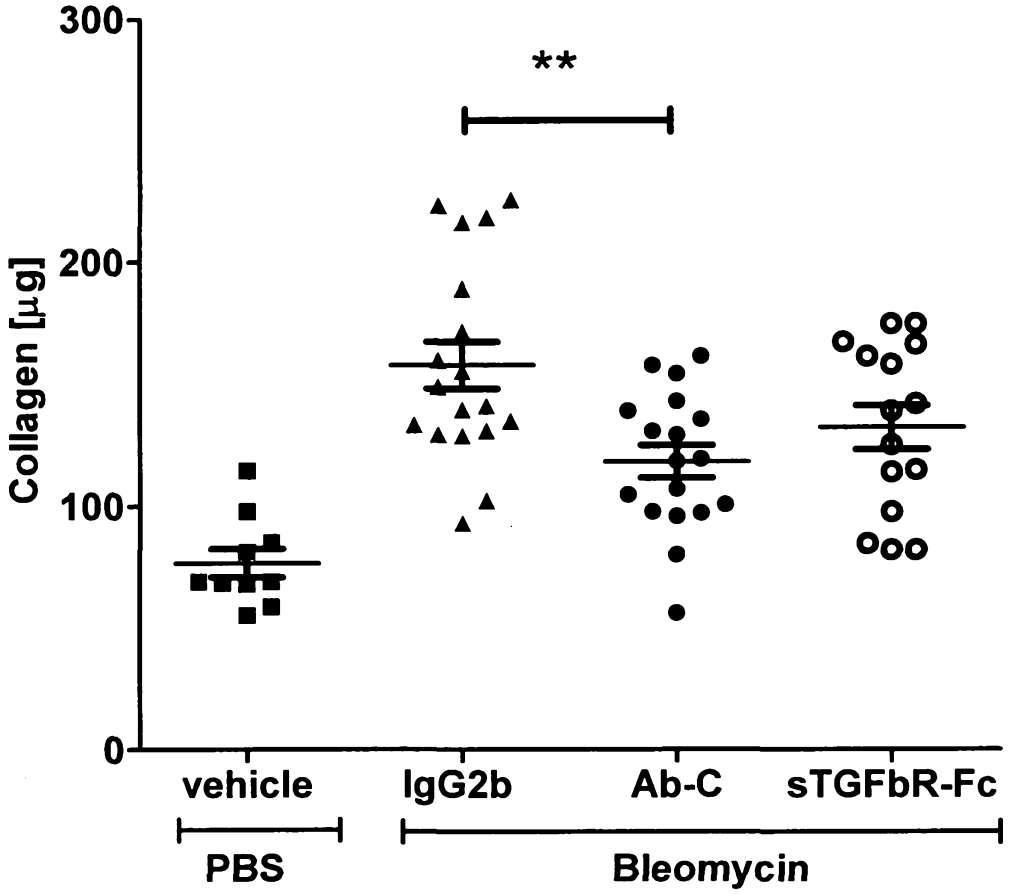

300
200
100
0
Collagen [µg]
**
vehicle
IgG2b
Ab-C
sTGFbR-Fc
PBS
Bleomycin

Figure 33

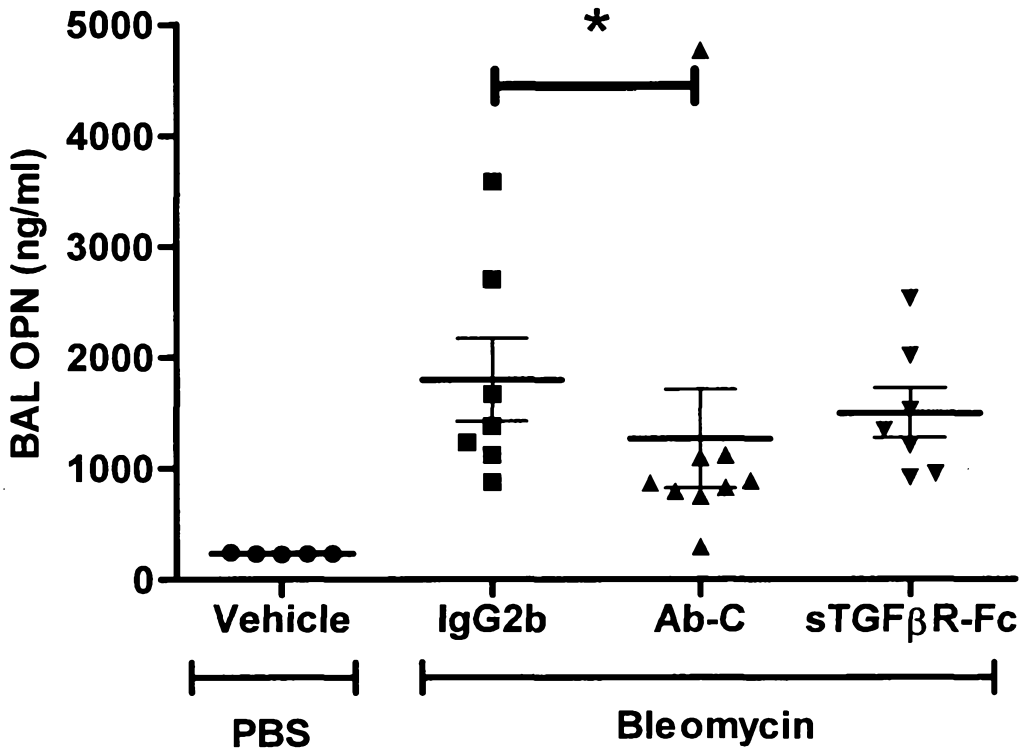

5000
4000
3000
2000
1000
0
BAL OPN (ng/ml)
*
Vehicle
IgG2b
Ab-C
sTGFβR-Fc
PBS
Bleomycin

Figure 34

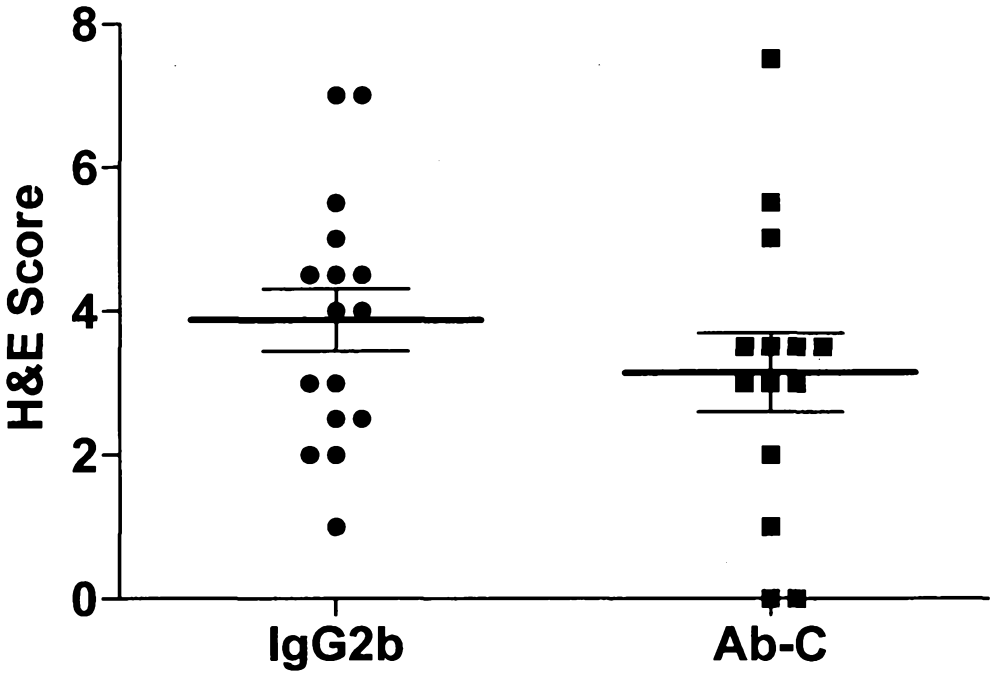


Figure 35

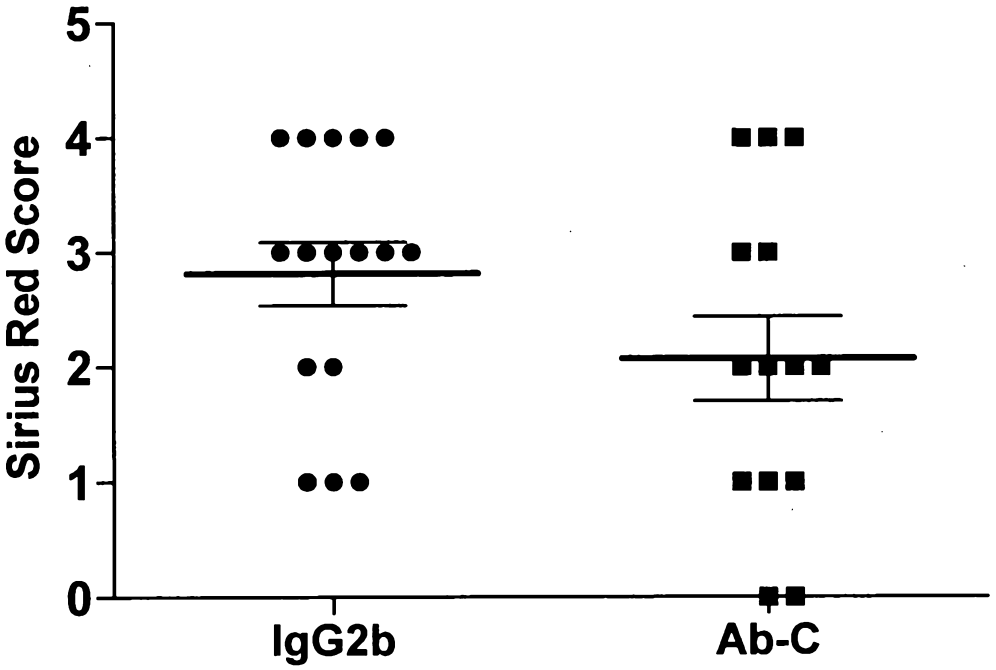


Figure 36

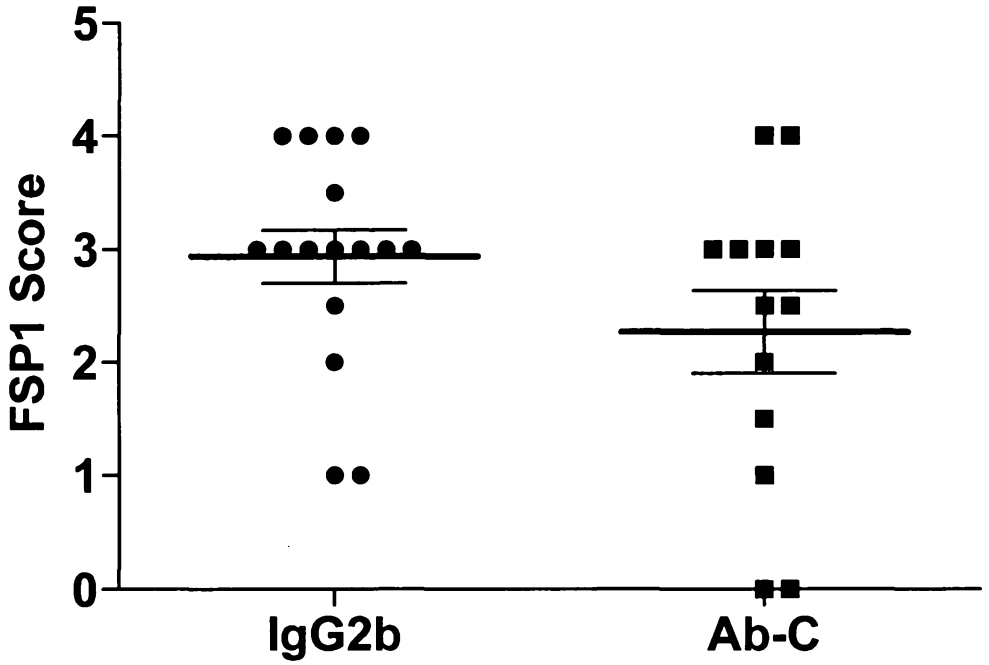


Figure 37

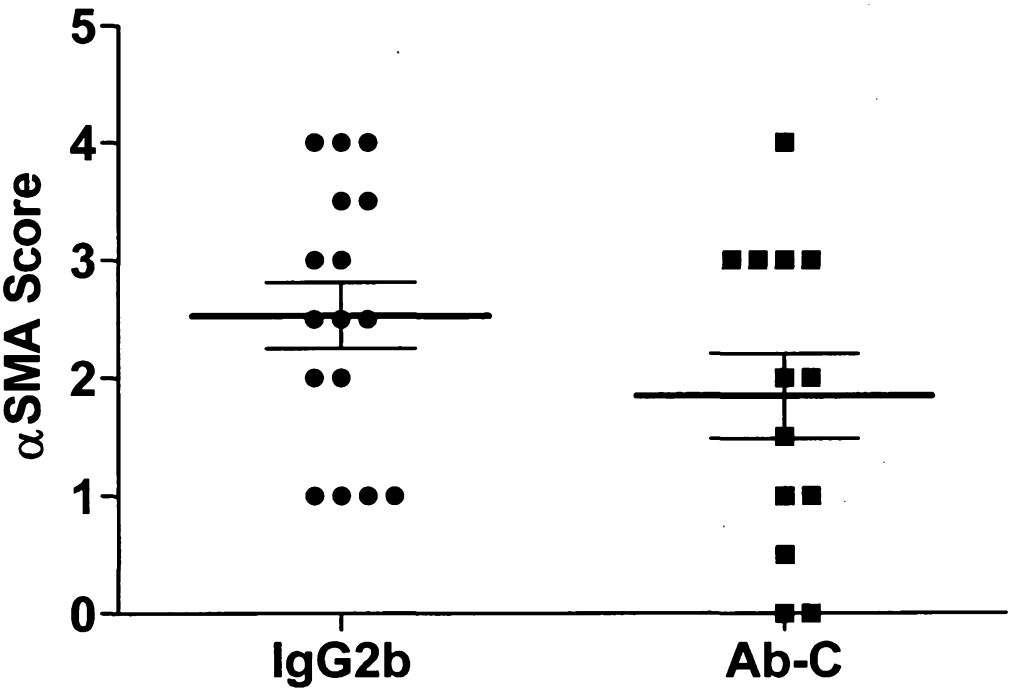


Figure 38

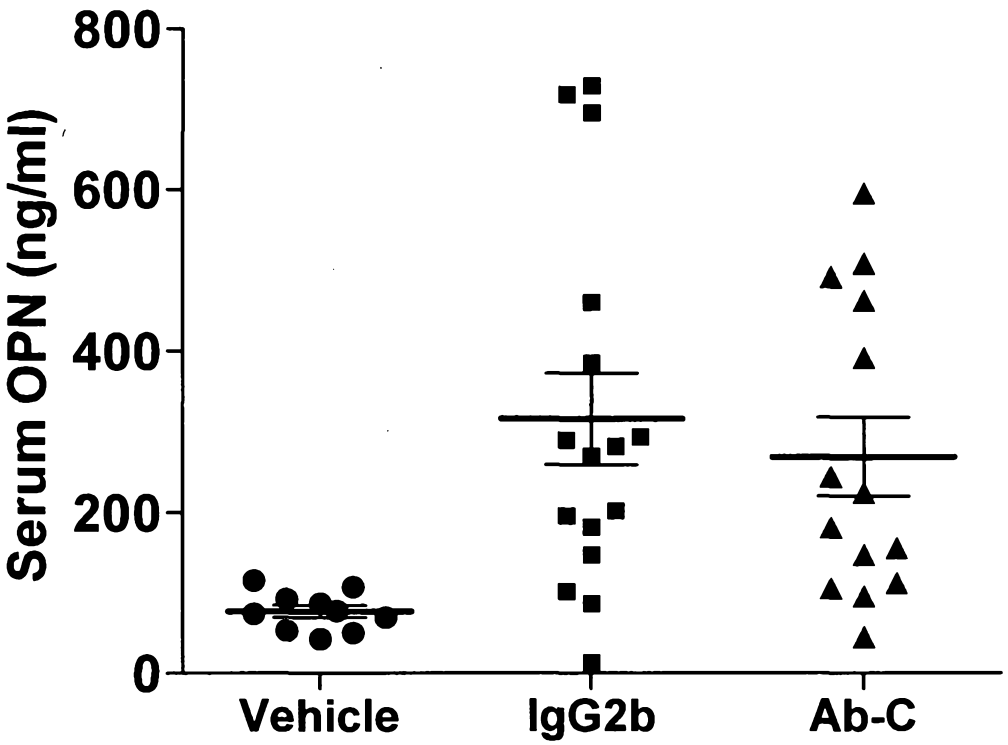
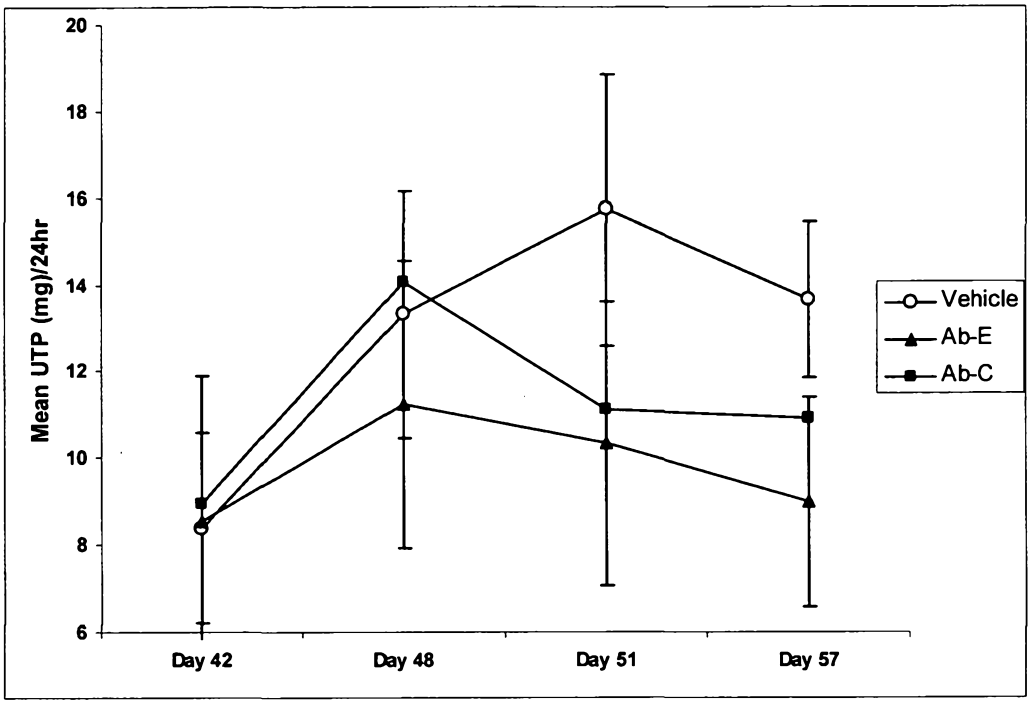


Figure 39


Mean UTP (mg)/24hr
20
18
16
14
12
10
8
6
Day 42
Day 48
Day 51
Day 57
Vehicle
Ab-E
Ab-C