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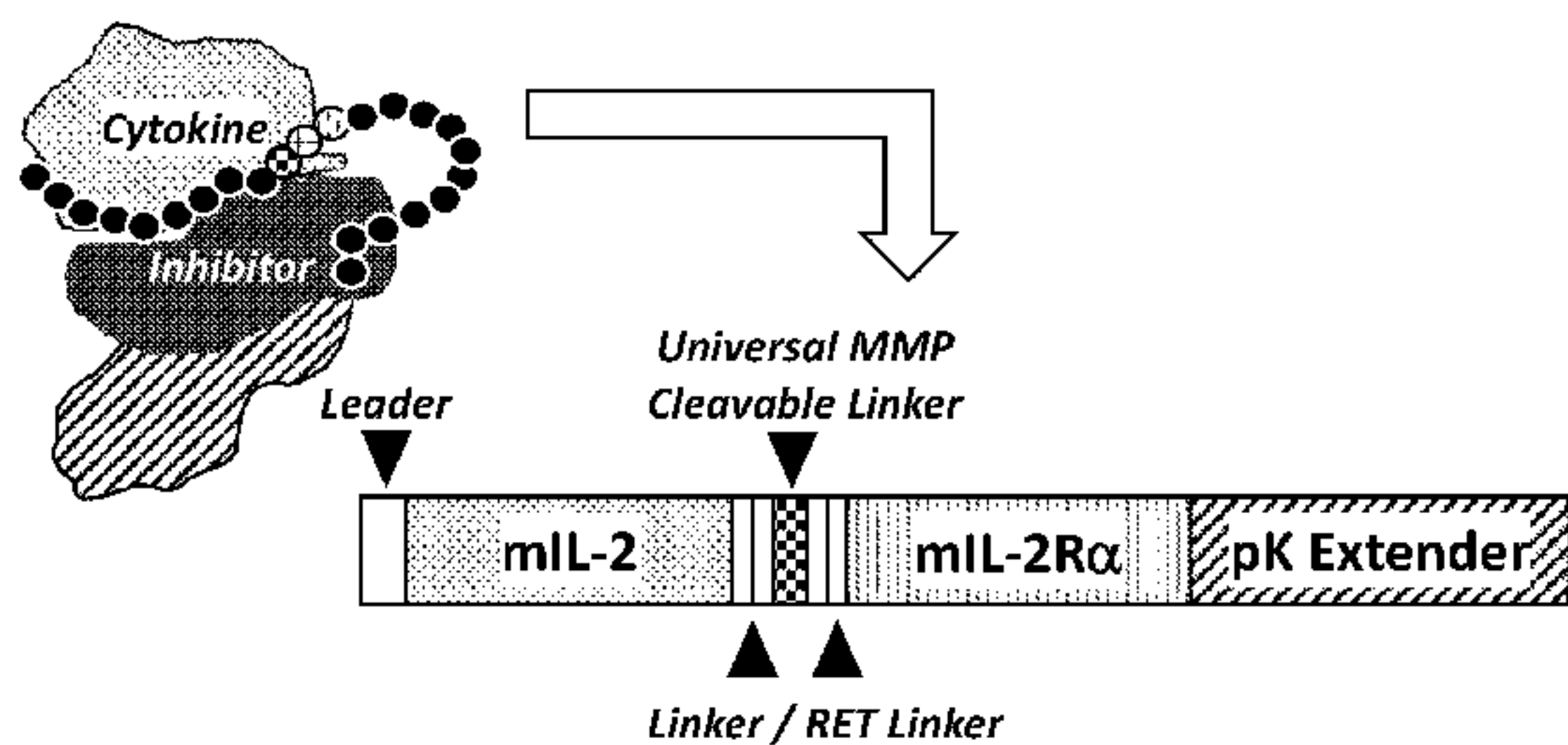
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(54) Title: CYTOKINE IL-2 PRODRUGS COMPRISING A CLEAVABLE LINKER



(57) Abstract: This disclosure relates to protease-cleavable cytokine prodrugs. In some embodiments, the prodrugs comprise a targeting sequence. In some embodiments, the prodrugs comprise a pharmacokinetic modulator.

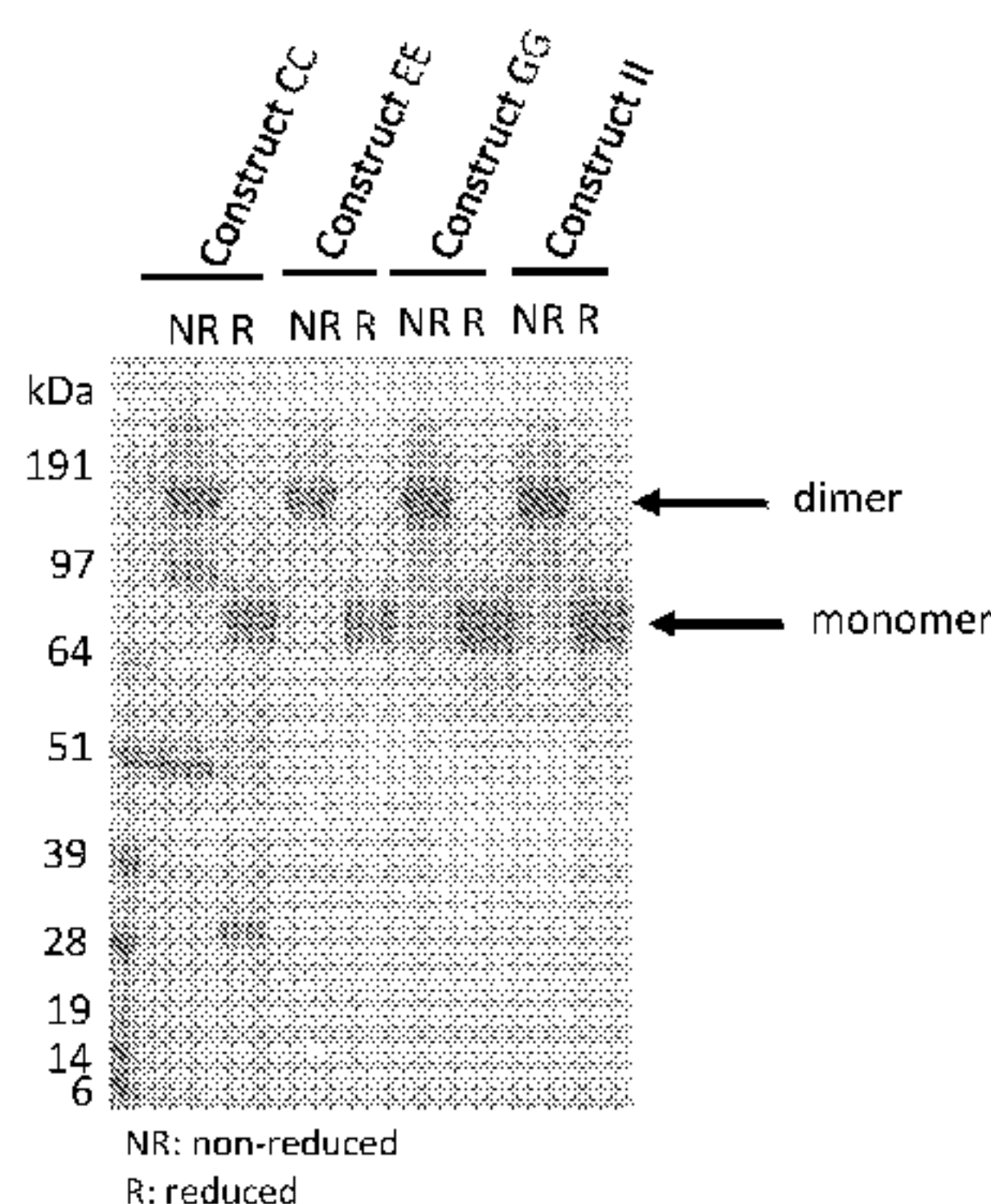


Fig. 1C

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CYTOKINE PRODRUGS COMPRISING A CLEAVABLE LINKER**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of US Provisional Patent Application No. 62/961,537, filed January 15, 2020, which is incorporated herein by reference in its entirety for all purposes.

INTRODUCTION AND SUMMARY

[0002] This disclosure relates to the field of cytokine therapeutics, particularly cytokine prodrugs comprising a cleavable linker.

[0003] Cytokines, such as IL-2, are powerful immune growth factors that play a significant role in sustaining an effective immune cell response. IL-2 has been reported to induce complete and durable regressions in cancer patients but immune related adverse effects have reduced its therapeutic potential. In some cases, however, systemic IL-2 administration can activate immune cells throughout the body. Systemic activation can lead to systemic toxicity and indiscriminate activation of immune cells, including immune cells that respond to a variety of epitopes, antigens, and stimuli. The therapeutic potential of IL-2 therapy can be impacted by these severe toxicities.

[0004] IL-2 therapies can also suffer from a short serum half-life, sometimes on the order of several minutes. Thus, the high doses of IL-2 that can be necessary to achieve an optimal immune-modulatory effect can contribute to severe toxicities.

[0005] As a result, cytokine therapeutics that overcome the hurdles of systemic or untargeted function, severe toxicity, and poor pharmacokinetics, are needed. The present disclosure aims to meet one or more of these needs, provide other benefits, or at least provide the public with a useful choice.

[0006] In some aspects, protease-activated pro-cytokines (also referred to as cytokine prodrugs) are provided, which can be administered to a subject in an inactive form. The inactive form can include a cytokine polypeptide sequence, a protease-cleavable polypeptide sequence, and an inhibitory polypeptide sequence capable of blocking an activity of the cytokine polypeptide sequence. Such prodrugs can become activated when the protease-cleavable polypeptide sequence is cleaved by a protease. Cleaving the protease-cleavable polypeptide can allow the inhibitory polypeptide sequence to dissociate from the cytokine polypeptide sequence.

[0007] Many tumors and tumor microenvironments exhibit aberrant expression of proteases. The present disclosure provides cytokine prodrugs that are activatable through proteolytic cleavage, such that they become active when they come in contact with proteases in a tumor or tumor microenvironment. In some cases, this can lead to an increase in active cytokines in and around the tumor or tumor microenvironment relative to the rest of a subject's body or healthy tissue. One exemplary advantage that can result is the formation of cytokine gradients. Such a gradient can form when a cytokine prodrug is administered and selectively or preferentially becomes activated in the tumor or tumor microenvironment and subsequently diffuses out of these areas to the rest of the body. These gradients can increase the trafficking of immune cells to the tumor and tumor microenvironment. Immune cells that traffic to the tumor can infiltrate the tumor. Infiltrating immune cells can mount an immune response against the cancer. Infiltrating immune cells can also secrete their own chemokines and cytokines. The cytokines can have either or both of autocrine and paracrine effects within the tumor and tumor microenvironment. In some cases, the immune cells include T cells, such as T effector cells or cytotoxic T cells, or NK cells.

[0008] Also described herein are methods of treatment and methods of administering the cytokine prodrugs described herein. Such administration can be systemic or local. In some embodiments, a cytokine prodrug described herein is administered systemically or locally to treat a cancer.

[0009] A further example of local administration is administration of a cytokine prodrug, such as an IL-2 cytokine prodrug, to boost T regulatory cells. In some cases, the local administration of an IL-2 cytokine prodrug is to an area of inflammation. Such a method can be used to treat chronic autoimmune and/or inflammatory diseases.

[0010] The following embodiments are encompassed.

[0011] Embodiment 1 is a protease-activated pro-cytokine comprising:

a cytokine polypeptide sequence;

a inhibitory polypeptide sequence capable of blocking an activity of the cytokine polypeptide sequence;

a linker between the cytokine polypeptide sequence and the inhibitory polypeptide sequence, the linker comprising a protease-cleavable polypeptide sequence; and

a targeting sequence, wherein the targeting sequence is configured to bind an extracellular matrix component, an integrin, or a syndecan; or is configured to bind, in a pH-sensitive manner, an extracellular matrix component, IgB (CD79b), an integrin, a cadherin, a heparan sulfate proteoglycan, a syndecan, or a fibronectin; or the targeting sequence comprises the

sequence of any one of SEQ ID NOs: 180-662 or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 180-662.

[0012] Embodiment 2 is the protease-activated pro-cytokine of the immediately preceding embodiment, further comprising a pharmacokinetic modulator.

[0013] Embodiment 3 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the pharmacokinetic modulator comprises an immunoglobulin constant domain.

[0014] Embodiment 4 is the protease-activated pro-cytokine of embodiment 2, wherein the pharmacokinetic modulator comprises an immunoglobulin Fc region.

[0015] Embodiment 5 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the immunoglobulin is a human immunoglobulin.

[0016] Embodiment 6 is the protease-activated pro-cytokine of any one of embodiments 4-5, wherein the immunoglobulin is IgG.

[0017] Embodiment 7 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IgG is IgG1, IgG2, IgG3, or IgG4.

[0018] Embodiment 8 is the protease-activated pro-cytokine of embodiment 2, wherein the pharmacokinetic modulator comprises an albumin.

[0019] Embodiment 9 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the albumin is a serum albumin.

[0020] Embodiment 10 is the protease-activated pro-cytokine of any one of embodiments 8-9, wherein the albumin is a human albumin.

[0021] Embodiment 11 is the protease-activated pro-cytokine of embodiment 2, wherein the pharmacokinetic modulator comprises PEG.

[0022] Embodiment 12 is the protease-activated pro-cytokine of embodiment 2, wherein the pharmacokinetic modulator comprises XTEN.

[0023] Embodiment 13 is the protease-activated pro-cytokine of embodiment 2, wherein the pharmacokinetic modulator comprises CTP.

[0024] Embodiment 14 is the protease-activated pro-cytokine of any one of embodiments 2-13, wherein the protease-cleavable polypeptide sequence is between the cytokine polypeptide sequence and the pharmacokinetic modulator.

[0025] Embodiment 15 is the protease-activated pro-cytokine of any one of embodiments 2-13, wherein the pharmacokinetic modulator is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence.

[0026] Embodiment 16 is the protease-activated pro-cytokine of any one of the preceding embodiments, comprising a plurality of protease-cleavable polypeptide sequences.

[0027] Embodiment 17 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the cytokine polypeptide sequence is flanked by protease cleavable polypeptide sequences.

[0028] Embodiment 18 is the protease-activated pro-cytokine of the immediately preceding embodiment, having the structure PM-CL-CY-CL-IN (from N- to C-terminus or from C- to N-terminus), where PM is the pharmacokinetic modulator, each CL independently is a protease-cleavable polypeptide sequence, CY is the cytokine polypeptide sequence, and IN is the inhibitory polypeptide sequence.

[0029] Embodiment 19 is the protease-activated pro-cytokine of any one of the preceding embodiments, comprising the targeting sequence, wherein the targeting sequence is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence or one of the protease-cleavable polypeptide sequences.

[0030] Embodiment 20 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence comprises a modification to prevent disulfide bond formation, and optionally otherwise comprises wild-type sequence.

[0031] Embodiment 21 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine polypeptide sequence or to a cytokine polypeptide sequence in Table 1.

[0032] Embodiment 22 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the cytokine polypeptide sequence is a wild-type cytokine polypeptide sequence.

[0033] Embodiment 23 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is a monomeric cytokine, or wherein the cytokine polypeptide sequence is a dimeric cytokine polypeptide sequence comprising monomers that are associated covalently (optionally via a polypeptide linker) or noncovalently.

[0034] Embodiment 24 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the inhibitory polypeptide sequence comprises a cytokine-binding domain.

[0035] Embodiment 25 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the cytokine-binding domain is a cytokine-binding domain of a cytokine receptor or a cytokine-binding domain of a fibronectin.

[0036] Embodiment 26 is the protease-activated pro-cytokine of embodiment 24, wherein the cytokine-binding domain is an immunoglobulin cytokine-binding domain.

[0037] Embodiment 27 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the immunoglobulin cytokine-binding domain comprises a light chain variable domain and a heavy chain variable domain that bind the cytokine.

[0038] Embodiment 28 is the protease-activated pro-cytokine of any one of embodiments 26-27, wherein the immunoglobulin cytokine-binding domain is an scFv, Fab, or VHH.

[0039] Embodiment 29 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by a metalloprotease, a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamate protease, a gelatinase, an asparagine peptide lyase, a cathepsin, a kallikrein, a plasmin, a collagenase, a hK1, a hK10, a hK15, a stromelysin, a Factor Xa, a chymotrypsin-like protease, a trypsin-like protease, a elastase-like protease, a subtilisin-like protease, an actinidain, a bromelain, a calpain, a caspase, a Mir 1-CP, a papain, a HIV-1 protease, a HSV protease, a CMV protease, a chymosin, a renin, a pepsin, a matriptase, a legumain, a plasmepsin, a nepenthesin, a metalloexopeptidase, a metalloendopeptidase, an ADAM 10, an ADAM17, an ADAM 12, an urokinase plasminogen activator (uPA), an enterokinase, a prostate-specific target (PSA, hK3), an interleukin-1b converting enzyme, a thrombin, a FAP (FAP-a), a dipeptidyl peptidase, or dipeptidyl peptidase IV (DPPIV/CD26), a type II transmembrane serine protease (TTSP), a neutrophil elastase, a proteinase 3, a mast cell chymase, a mast cell tryptase, or a dipeptidyl peptidase.

[0040] Embodiment 30 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 700-741, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 700-741.

[0041] Embodiment 31 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by a matrix metalloprotease.

[0042] Embodiment 32 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-1.

[0043] Embodiment 33 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-2.

[0044] Embodiment 34 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-3.

[0045] Embodiment 35 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-7.

[0046] Embodiment 36 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-8.

[0047] Embodiment 37 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-9.

[0048] Embodiment 38 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-12.

[0049] Embodiment 39 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-13.

[0050] Embodiment 40 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by MMP-14.

[0051] Embodiment 41 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by more than one MMP.

[0052] Embodiment 42 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence is recognized by two, three, four, five, six, or seven of MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, and MMP-14.

[0053] Embodiment 43 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the protease-cleavable polypeptide sequence comprises the sequence

of any one of SEQ ID NOs: 80-94 or a variant sequence having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 80-90.

[0054] Embodiment 44 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80 or a variant sequence having one or two mismatches relative thereto.

[0055] Embodiment 45 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 81 or a variant sequence having one or two mismatches relative thereto.

[0056] Embodiment 46 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 82 or a variant sequence having one or two mismatches relative thereto.

[0057] Embodiment 47 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 83 or a variant sequence having one or two mismatches relative thereto.

[0058] Embodiment 48 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 84 or a variant sequence having one or two mismatches relative thereto.

[0059] Embodiment 49 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 85 or a variant sequence having one or two mismatches relative thereto.

[0060] Embodiment 50 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 86 or a variant sequence having one or two mismatches relative thereto.

[0061] Embodiment 51 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 87 or a variant sequence having one or two mismatches relative thereto.

[0062] Embodiment 52 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 88 or a variant sequence having one or two mismatches relative thereto.

[0063] Embodiment 53 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 89 or a variant sequence having one or two mismatches relative thereto.

[0064] Embodiment 54 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 90 or a variant sequence having one or two mismatches relative thereto.

[0065] Embodiment 55 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80-89 or 90.

[0066] Embodiment 56 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 91.

[0067] Embodiment 57 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 92.

[0068] Embodiment 58 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 93.

[0069] Embodiment 59 is the protease-activated pro-cytokine of any one of embodiments 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 94.

[0070] Embodiment 60 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 180-662.

[0071] Embodiment 61 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-662.

[0072] Embodiment 62 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the targeting sequence binds to denatured collagen.

[0073] Embodiment 63 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to collagen.

[0074] Embodiment 64 is the protease-activated pro-cytokine of any one of embodiments 62-63, wherein the collagen is collagen I.

[0075] Embodiment 65 is the protease-activated pro-cytokine of any one of embodiments 62-63, wherein the collagen is collagen II.

[0076] Embodiment 66 is the protease-activated pro-cytokine of any one of embodiments 62-63, wherein the collagen is collagen III.

[0077] Embodiment 67 is the protease-activated pro-cytokine of any one of embodiments 62-63, wherein the collagen is collagen IV.

[0078] Embodiment 68 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to integrin.

[0079] Embodiment 69 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the integrin is one or more of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin, $\alpha 3\beta 1$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 5\beta 1$ integrin, $\alpha 6\beta 1$ integrin, $\alpha 7\beta 1$ integrin, $\alpha 9\beta 1$ integrin, $\alpha 4\beta 7$ integrin, $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha IIb\beta 3$ integrin, $\alpha IIIb\beta 3$ integrin, $\alpha M\beta 2$ integrin, or $\alpha IIb\beta 3$ integrin.

[0080] Embodiment 70 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to von Willebrand factor.

[0081] Embodiment 71 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to IgB.

[0082] Embodiment 72 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to heparin.

[0083] Embodiment 73 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the targeting sequence binds to heparin and a syndecan, a heparan sulfate proteoglycan, or an integrin, optionally wherein the integrin is one or more of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin, $\alpha 3\beta 1$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 5\beta 1$ integrin, $\alpha 6\beta 1$ integrin, $\alpha 7\beta 1$ integrin, $\alpha 9\beta 1$ integrin, $\alpha 4\beta 7$ integrin, $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha IIb\beta 3$ integrin, $\alpha IIIb\beta 3$ integrin, $\alpha M\beta 2$ integrin, or $\alpha IIb\beta 3$ integrin.

[0084] Embodiment 74 is the protease-activated pro-cytokine of any one of embodiments 72-73, wherein the syndecan is one of more of syndecan-1, syndecan-4, and syndecan-2(w).

[0085] Embodiment 75 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to a heparan sulfate proteoglycan.

[0086] Embodiment 76 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to a sulfated glycoprotein.

[0087] Embodiment 77 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to hyaluronic acid.

[0088] Embodiment 78 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to fibronectin.

[0089] Embodiment 79 is the protease-activated pro-cytokine of any one of embodiments 1-61, wherein the targeting sequence binds to cadherin.

[0090] Embodiment 80 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the targeting sequence is configured to bind its target in a pH-sensitive manner.

[0091] Embodiment 81 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the targeting sequence has a higher affinity for its target at a pH below normal physiological pH than at normal physiological pH, optionally wherein the pH below normal physiological pH is below 7, or below 6.

[0092] Embodiment 82 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the targeting sequence has a higher affinity for its target at a pH in the range of 5-7, e.g., 5-5.5, 5.5-6, 6-6.5, or 6.5-7, than at normal physiological pH.

[0093] Embodiment 83 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the targeting sequence comprises one or more histidines, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 histidines.

[0094] Embodiment 84 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 641-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 641-662.

[0095] Embodiment 85 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 641-662.

[0096] Embodiment 86 is the protease-activated pro-cytokine of any one of embodiments 80-86, wherein the targeting sequence is configured to bind, in a pH-sensitive manner, an extracellular matrix component, IgB (CD79b), an integrin, a cadherin, a heparan sulfate proteoglycan, a syndecan, or a fibronectin.

[0097] Embodiment 87 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the extracellular matrix component is hyaluronic acid, heparin, heparan sulfate, or a sulfated glycoprotein.

[0098] Embodiment 88 is the protease-activated pro-cytokine of embodiment 86, wherein the targeting sequence is configured to bind a fibronectin in a pH-sensitive manner.

[0099] Embodiment 89 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is an interleukin polypeptide sequence.

[00100] Embodiment 90 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD132.

[00101] Embodiment 91 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD122.

[00102] Embodiment 92 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD25.

[00103] Embodiment 93 is the protease-activated pro-cytokine of any one of the preceding embodiments, wherein the cytokine polypeptide sequence is an IL-2 polypeptide sequence.

[00104] Embodiment 94 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2 polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 1-4.

[00105] Embodiment 95 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2 polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 1-4.

[00106] Embodiment 96 is the protease-activated pro-cytokine of any one of embodiments 93-95, wherein the IL-2 polypeptide sequence is a human IL-2 polypeptide sequence.

[00107] Embodiment 97 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 1.

[00108] Embodiment 98 is the protease-activated pro-cytokine of any one of embodiments 93-95, wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 2.

[00109] Embodiment 99 is the protease-activated pro-cytokine of any one of embodiments 93-98, wherein the inhibitory polypeptide sequence comprises an IL-2 binding domain of an IL-2 receptor (IL-2R).

[00110] Embodiment 100 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the inhibitory polypeptide sequence comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 10-19.

[00111] Embodiment 101 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2R is a human IL-2R.

[00112] Embodiment 102 is the protease-activated pro-cytokine of any one of embodiments 93-98, wherein the inhibitory polypeptide sequence comprises an IL-2-binding immunoglobulin domain.

[00113] Embodiment 103 is the protease-activated pro-cytokine of any one of embodiments 93-98, wherein the IL-2-binding immunoglobulin domain is a human IL-2-binding immunoglobulin domain.

[00114] Embodiment 104 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising hypervariable regions (HVRs) HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 33, 34, and 35, respectively, and a VH region comprising HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 36, 37, and 38, respectively.

[00115] Embodiment 105 is the protease-activated pro-cytokine of any one of embodiments 102-104, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 32 and a VH region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 33.

[00116] Embodiment 106 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising the sequence of SEQ ID NO: 32 and a VH region comprising the sequence of SEQ ID NO: 33.

[00117] Embodiment 107 is the protease-activated pro-cytokine of any one of embodiments 102-104, wherein the IL-2-binding immunoglobulin domain is an scFv.

[00118] Embodiment 108 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2-binding immunoglobulin domain comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 30 or 31.

[00119] Embodiment 109 is the protease-activated pro-cytokine of the immediately preceding embodiment, wherein the IL-2-binding immunoglobulin domain comprises the sequence of SEQ ID NO: 30 or 31.

[00120] Embodiment 110 is the protease-activated pro-cytokine of embodiment 1, comprising the sequence of any one of SEQ ID NOs: 803-852.

[00121] Embodiment 111 is a pharmaceutical composition comprising the protease-activated pro-cytokine of any one of the preceding embodiments.

[00122] Embodiment 112 is the protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding embodiments, for use in therapy.

[00123] Embodiment 113 is the protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding embodiments, for use in treating a cancer.

[00124] Embodiment 114 is a method of treating a cancer, comprising administering the protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding embodiments to a subject in need thereof.

[00125] Embodiment 115 is a use of the protease-activated pro-cytokine or pharmaceutical composition of any one of embodiments 1-110 for the manufacture of a medicament for treating cancer.

[00126] Embodiment 116 is the method, use, or protease-activated pro-cytokine for use of any one of embodiments 113-115, wherein the cancer is a solid tumor.

[00127] Embodiment 117 is the method, use, or protease-activated pro-cytokine for use of the immediately preceding embodiment, wherein the solid tumor is metastatic and/or unresectable.

[00128] Embodiment 118 is the method, use, or protease-activated pro-cytokine for use of any one of embodiments 113-117, wherein the cancer is a PD-L1-expressing cancer.

[00129] Embodiment 119 is the method, use, or protease-activated pro-cytokine for use of any one of embodiments 113-118, wherein the cancer is a melanoma, a colorectal cancer, a breast cancer, a pancreatic cancer, a lung cancer, a prostate cancer, an ovarian cancer, a cervical cancer, a gastric or gastrointestinal cancer, a lymphoma, a colon or colorectal cancer, an endometrial cancer, a thyroid cancer, or a bladder cancer.

[00130] Embodiment 120 is the method, use, or protease-activated pro-cytokine for use of any one of embodiments 113-119, wherein the cancer is a microsatellite instability-high cancer.

[00131] Embodiment 121 is the method, use, or protease-activated pro-cytokine for use of any one of embodiments 113-120, wherein the cancer is mismatch repair deficient.

[00132] Embodiment 122 is a nucleic acid encoding the protease-activated pro-cytokine of any one of embodiments 1-110.

[00133] Embodiment 123 is an expression vector comprising the nucleic acid of embodiment 121.

[00134] Embodiment 124 is a host cell comprising the nucleic acid of embodiment 121 or the vector of embodiment 122.

[00135] Embodiment 125 is a method of producing a protease-activated pro-cytokine, comprising culturing the host cell of embodiment 124 under conditions wherein the protease-activated pro-cytokine is produced.

[00136] Embodiment 126 is the method of the immediately preceding embodiment, further comprising isolating the protease-activated pro-cytokine.

[00137] Embodiment 127 is a method of boosting T regulatory cells and/or reducing inflammation or autoimmune activity, comprising administering the protease-activated pro-cytokine of any one of embodiments 1-110 to an area of interest in a subject, e.g., an area of inflammation in the subject.

[00138] Embodiment 128 is a method of treating an inflammatory or autoimmune disease or disorder in a subject, comprising administering the protease-activated pro-cytokine of any one of embodiments 1-110 to an area of interest in a subject, e.g., an area of inflammation or autoimmune activity in the subject.

FIGURE LEGENDS

[00139] **FIG 1A** shows an illustration of an exemplary cytokine prodrug structure and an SDS-PAGE gel characterizing a purified cytokine prodrug (Construct B). Abbreviations: PM, pharmacokinetic modulator; HMW, high molecular weight.

[00140] **FIG 1B** shows an illustration of an exemplary cytokine prodrug structure comprising human IL-2 and IL-2R α sequences and an MMP-cleavable linker, and an SDS-PAGE gel and Western blot characterizing a purified cytokine prodrug (Construct E).

Abbreviations: Hu, human; MMP, matrix metalloprotease; other abbreviations are as above.

[00141] **FIG 1C** shows an illustration of an exemplary cytokine prodrug structure comprising murine IL-2 and IL-2R α sequences, an MMP-cleavable linker, additional linkers that include a targeting sequence ("RET Linker"), and an SDS-PAGE gel characterizing the indicated purified cytokine prodrugs.

[00142] **FIG 1D** shows an illustration of an exemplary cytokine prodrug structure comprising human IL-2 and IL-2R α sequences, an MMP-cleavable linker, additional linkers that include a targeting sequence ("RET Linker"), and an SDS-PAGE gel characterizing the indicated purified cytokine prodrugs.

[00143] **FIG 2A** illustrates a cleavage reaction of a cytokine prodrug by a protease and shows Western blot evidence of cleavage of Construct A by MMP-9 at time points of 1, 2,

and 4 hours and overnight. Each of the Western blots contains +MMP digestion lanes and -MMP mock-digestion lanes. Cleavage product was detectable at 1 hour, and the full-length cytokine prodrug was substantially undetectable at the overnight +MMP time point.

[00144] **FIG 2B** illustrates a cleavage reaction of a cytokine prodrug comprising a pharmacokinetic modulator by a protease and shows Western blot evidence of cleavage of Construct B by MMP-9 at time points of 1, 4, and 20 hours. Each of the Western blots contains +MMP digestion lanes and -MMP mock-digestion lanes. Cleavage product was detectable at 1 hour, and the full-length cytokine prodrug gave only a faint band at the 20 hour +MMP time point.

[00145] **FIGs 2C-E** illustrate cleavage reactions of a cytokine prodrug comprising a pharmacokinetic modulator by a protease and shows Western blot evidence of cleavage of Construct E by MMP-9 at time points of 1, 4, and 22 hours (2C); and cleavage of the indicated constructs at 18 hours (2D and 2E). Constructs BBB, CCC, and FFF in Fig. 2E that did not show substantial cleavage had scrambled MMP sites. Each of the Western blots contains +MMP9 digestion lanes and -MMP9 mock-digestion lanes. Cleavage product was detectable at 1 hour, and the full-length cytokine prodrug gave essentially no band at the 22 hour +MMP time point.

[00146] **FIG 3A** shows results of a CTLL-2 proliferation assay with Construct A or cleavage products thereof. Construct A was cleaved by MMP-9 and the resulting products were incubated with CTLL-2 cells. The data shows that MMP-9 treated Construct A stimulates CTLL-2 cell proliferation in a dose dependent manner and exhibits 10-fold greater activity than untreated Construct A (EC50 comparison). EC50 values are shown in nM.

[00147] **FIG 3B** shows results of a CTLL-2 proliferation assay with Construct B or cleavage products thereof. Construct B was cleaved by MMP-9 and the resulting products were incubated with CTLL-2 cells. For comparison, mIL2 was also incubated with CTLL-2 cells. The data show that MMP-9 treated Construct B stimulates CTLL-2 cell proliferation in a dose dependent manner. Uncleaved Construct B was minimally stimulatory. EC50 values are shown in nM.

[00148] **FIG 3C-FIG 3J** show HEK-Blue™ IL2 assay results. Cells were treated with various concentrations Construct E, uncleaved or cleaved with mMMP9 for 22 hours (FIG 3C); human IL2 (FIG 3D); Construct B, uncleaved or cleaved with mMMP9 for 19 hours; Construct J, Construct K, Construct F, Construct L, or Construct I, each uncleaved or cleaved with mMMP9 for 22 hours (FIGs 3E-J, respectively); and the EC50 was determined based on OD630 as a readout of IL-2 stimulation.

[00149] **FIG 3K-FIG 3L** show results of a CTLL-2 proliferation assay with Construct M, Construct N, or cleavage products thereof. Cleavage was by MMP-2 for 2 hr and the resulting products were incubated with CTLL-2 cells. The data show that MMP-2 treated Construct M and Construct N stimulate CTLL-2 cell proliferation in a dose dependent manner. EC50 values are shown in nM.

[00150] **FIG 3M** shows Coomassie-stained SDS-PAGE results comparing Construct E, Construct M, and Construct N. Construct M and Construct N showed decreased aggregation and greater stability and homogeneity.

[00151] **FIG 3N-FIG 3P** show results of a CTLL-2 proliferation assay with Construct O, Construct P, Construct Q, or cleavage products thereof. Cleavage was by MMP2 for 2 hr and the resulting products were incubated with CTLL-2 cells. The data show that MMP2 treated Construct O, Construct P, and Construct Q stimulate CTLL-2 cell proliferation in a dose dependent manner. EC50 values are shown in nM.

[00152] **FIG 3Q-FIG 3Y** show results of a HEK-Blue™ IL2 assay with the indicated construct or cleavage products thereof. Cleavage was by MMP9 for either 18 hr or 22 hr and the resulting products were incubated with HEK-Blue™ IL2 cells. EC50 was determined based on OD630 as a readout of IL-2 stimulation. The data show that MMP9 treated constructs stimulate IL-2 in a dose dependent manner. EC50 values are shown in nM.

[00153] **FIG 4** illustrates a serum stability assay using Construct B and provides results thereof indicating that Construct B was stable when incubated with serum collected from control or tumor-bearing over a time course of 72 hours. Concentrations were measured by quantitative sandwich ELISA using an mIL2 capture antibody and mIL2Rα detection antibody.

[00154] **FIG 5** shows a study design, graphical results, and pharmacokinetic (PK) parameters for Construct B in mice. PK parameters were calculated using WinNonlin 7.0 (non-compartmental model).

[00155] **FIG 6A** shows a study design and results for intratumoral dosing of Construct A in mice injected subcutaneously with MC38 cells at day -7 and then treated with Construct A, vehicle, or human IL-2 on each of days 0-4 and 7-11. Construct A substantially inhibited tumor growth. In contrast, human IL-2 adversely affected tumor control relative to vehicle. Necrosis attributable to tumor growth was observed in the control and human IL-2 groups.

[00156] **FIG 6B** shows a study design in which mice treated as in FIG 6A were re-challenged with 2×10^6 MC38 cells at day 40. Tumor growth was rejected, indicating that the treatment resulted in a durable response including anti-tumor immune memory.

[00157] **FIG 7A** shows a study design in mice injected subcutaneously with MC38 cells at day -10 where Construct B or vehicle was administered intravenously once per three days (Q3D) during a three week period (eight total administrations). Essentially no systemic toxicity was observed. Construct B-treated mice showed virtually no tumor growth after initiation of treatment, in contrast to vehicle-treated mice where tumor growth continued through day 21. Following day 21, several vehicle-treated mice were euthanized due to tumor volume exceeding 3000 mm³ and accordingly subsequent tumor volume data for vehicle-treated mice is not shown as it would be biased toward mice with smaller tumor volumes relative to the population average through day 21.

[00158] **FIG 7B** shows body weight data for the same mice as in FIG 7A. Mouse body weight was substantially constant during treatment with Construct B, consistent with lack of any apparent toxicity.

[00159] **FIG 8** shows immunohistochemistry results for tumor-infiltrating immune cells at day 21 for vehicle group tissues and at day 25 for Construct B treated tumors of the study described above for FIG 7A. Significantly more immune cells of all tested types were observed in Construct B-treated mice compared to vehicle-treated mice. Additionally, the proportion of cells with markers consistent with a effector T cell phenotype was substantially greater than the proportion of CD4+Foxp3+ (regulatory T) cells. Statistical analysis was performed using unpaired t test by Prism 5.0 software. P value between groups was calculated, and the differences with p value <0.05 were considered statistically significant. * p<0.05, ** p<0.01, *** p<0.001.

[00160] **FIG 9** shows quantification of MMP activity in the indicated tumor-bearing mouse models by fluorescence intensity over time following MMPsense 680™ injection.

[00161] **FIG 10A-FIG 10D** show tumor volume over time for mice treated with vehicle or Construct B as indicated in the indicated cancer models.

[00162] **FIG 11A-FIG 11D** show tumor volume over time (11A) and levels of the indicated enzymes (11B-D) for mice treated with vehicle or Construct B as indicated in the B16F10 melanoma model.

[00163] **FIG 12A-FIG 12D** show tumor volume over time (12A) and levels of the indicated enzymes (12B-D) for mice treated with vehicle or Construct B as indicated in the RM-1 prostate cancer model.

[00164] **FIG 13A** shows MMP activity, measured as described for FIG 9, in the indicated groups.

[00165] **FIG 13B-FIG 13C** show tumor volume over time for mice treated with vehicle or Construct B as indicated in the indicated cancer models.

[00166] **FIG 14A-B** show schematic structures of the indicated linkers and binding of MMP linker peptides containing heparin binding motifs to heparin-agarose beads.

[00167] **FIG 14C** shows cartoons of the structures of the indicated constructs and heparin binding assay results for the indicated constructs. Assays were performed at pH 7.5 unless indicated as performed at pH 6.

[00168] **FIG 14D** shows schematic structures of the indicated linkers and binding of the indicated peptides to fibronectin at the indicated pH values.

[00169] **FIG 14E** shows fibronectin binding assay results for the indicated constructs. Assays were performed at pH 7.5 unless indicated as performed at pH 6.

[00170] **FIG 14F** shows schematic structures of the indicated linkers and binding of MMP linker peptides containing collagen binding motifs to beads associated with collagen IV.

[00171] **FIG 14G** shows an anti-mIL2 Western blot of input (I), supernatant (S), collagen-bound (C) and control agarose bound (A) fractions from pulldown assays performed with the indicated constructs.

[00172] **FIG 15A** shows fluorescent images of mice treated with the indicated constructs as described in Example 15.

[00173] **FIG 15B** shows tumor-associated fluorescence measured in mice treated with the indicated constructs as described in Example 15.

[00174] **FIG 15C-H** show amounts of the indicated constructs present in tumor lysates prepared as described in Example 16. Here and throughout, mpk means mg/kg.

[00175] **FIG 15I-K** show amounts of the indicated constructs present in serum samples prepared as described in Example 16.

[00176] **FIG 16A-B** show tumor volume over time for groups treated with the indicated constructs as described in Example 17.

[00177] **FIG 17A-B** show IFN- γ levels in tumors following treatment with the indicated constructs as described in Example 18.

[00178] **FIG 18A-E** show exemplary arrangements of elements in cytokine prodrugs comprising various combinations of a cytokine polypeptide sequence (CYTOKINE), a pharmacokinetic modulator (PK EXT), a protease-cleavable polypeptide sequence in a linker (PRO-LNK), an inhibitory polypeptide sequence (INHIBITOR), and in some cases one or more targeting sequences (RET LNK) or additional linkers (LNK). The targeting sequences

are shown as white text on a dark background. In FIGs 18A and 18C, the pharmacokinetic modulator is on the same side of the protease-cleavable sequence as the inhibitory polypeptide sequence, so that it would not impact the pharmacokinetics of the cytokine polypeptide sequence. In FIGs 18B and 18D, the pharmacokinetic modulator is on the same side of the protease-cleavable sequence as the cytokine polypeptide sequence, so that it would impact the pharmacokinetics of the cytokine polypeptide sequence. In FIG 18E, a protease-cleavable sequence is present on each side of the pharmacokinetic modulator. This arrangement can produce intermediate results as the pharmacokinetic modulator would be separated from the cytokine polypeptide sequence more slowly than the inhibitory polypeptide sequence.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[00179] This specification describes exemplary embodiments and applications of the disclosure. The disclosure, however, is not limited to these exemplary embodiments and applications or to the manner in which the exemplary embodiments and applications operate or are described herein. The term “or” is used in an inclusive sense, i.e., equivalent to “and/or,” unless the context dictates otherwise. It is noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the,” and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the terms “comprise,” “include,” and grammatical variants thereof are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items. Section divisions in the specification are provided for the convenience of the reader only and do not limit any combination of elements discussed. In case of any contradiction or conflict between material incorporated by reference and the expressly described content provided herein, the expressly described content controls.

Overview

[00180] Provided herein are protease-activated pro-cytokines (also referred to herein as cytokine prodrugs) comprising a linker comprising a protease-cleavable linker and a targeting sequence described herein, e.g., a targeting sequence configured to bind an extracellular matrix component, an integrin, or a syndecan; or configured to bind an extracellular matrix component, IgB (CD79b), an integrin, a cadherin, a heparan sulfate proteoglycan, a syndecan, or a fibronectin in a pH-sensitive manner; or a targeting sequence comprising the sequence of

any one of SEQ ID NOs: 180-662. The cleavable linker can be between a cytokine polypeptide sequence and an inhibitory polypeptide sequence, such that the ability of the cytokine polypeptide sequence to activate immune cells is reduced or eliminated compared to a free cytokine polypeptide sequence. Proteolysis of the linker can liberate the cytokine so that it can activate immune cells.

[00181] In some embodiments, the protease-cleavable linker is cleavable by a protease expressed at higher levels in the tumor microenvironment (TME) than in healthy tissue of the same type. In some embodiments, the protease-cleavable linker is a matrix metalloprotease (MMP)-cleavable linker, such as any of the MMP-cleavable linkers described herein.

Without wishing to be bound by any particular theory, increased expression of proteases, including but not necessarily limited to MMPs, in the tumor microenvironment (TME) can provide a mechanism for achieving selective or preferential activation of the cytokine prodrug at or near a tumor site. Certain protease-cleavable linkers described herein are considered particularly suitable for achieving such selective or preferential activation.

[00182] In other embodiments, the cytokine prodrug comprises a targeting sequence, e.g., a targeting sequence that binds an extracellular matrix component, an integrin, or a syndecan, or is configured to bind fibronectin in a pH-sensitive manner. The targeting sequence can facilitate accumulation and/or increased residence time of the cytokine prodrug and/or the active cytokine in the ECM. In some embodiments, a targeting sequence is combined with a protease-cleavable linker cleavable by a protease expressed at higher levels in the TME and/or cleavable by an MMP.

[00183] In any of the foregoing embodiments, the cytokine prodrug may further comprise a pharmacokinetic modulator, e.g., which extends the half-life of the prodrug and which may optionally also extend the half-life of the active cytokine.

[00184] Sequences of exemplary cytokine prodrugs and components thereof are shown in Tables 1 and 2. In Table 1, “X_{Hy}” designates a hydrophobic amino acid residue. In some embodiments, the hydrophobic amino acid residue is any one of glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), and tryptophan (Trp). In some embodiments, the hydrophobic amino acid residue is any one of Ala, Leu, Val, Ile, Pro, Phe, Met, and Trp. In some embodiments, the hydrophobic amino acid residue is any one of Leu, Val, Ile, Pro, Phe, Met, and Trp. In some embodiments, the hydrophobic amino acid residue is any one of Ala, Leu, Val, Ile, Phe, Met, and Trp. In some embodiments, the hydrophobic amino acid residue is any one of Leu, Val, Ile, Phe, Met, and Trp. “(Pip)” represents piperidine. “(Hof)” represents homophenylalanine. “(Cit)”

represents citrulline. “(Et)” represents ethionine. “C(me)” represents methyleysteine. In certain sequences, underlining is used to indicate mutated positions.

[00185] This disclosure further provides uses of these cytokine prodrugs, e.g., for treating cancer. In some embodiments, the cytokine prodrug is selectively or preferentially cleaved in the tumor microenvironment, which may result in beneficial effects, e.g., improved recruitment and/or activation of immune cells in the vicinity of the tumor, and/or reduced systemic exposure to active cytokines.

Table 1. Table of Sequences of Cytokine Prodrugs and Components Thereof

SEQ ID NO	Description	Sequence	Species	Function	Notes
IL-2 sequences					
1	h IL-2	APTSSSTKKTQLQLEHLLLDLQMIILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	human	cytokine	wild-type
2	h IL-2 (C125S)	APTSSSTKKTQLQLEHLLLDLQMIILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEEELKPLEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	human	cytokine	C125 to S mutation
3	m IL-2	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLPRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ	mouse	cytokine	wild-type
4	m IL-2 (C140S)	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLPRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFSQSIISTSPQ	mouse	cytokine	C140 to S mutation
5-9	Not Used				
Blockers: IL-2R sequences					
10	h IL-2Ralpha	ELCDDDPPEIPHATFKAMAYKEGTMNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPESETSLVTTTDFIQIQTEMAATMETSIFTTEYQ	human	blocker	wild-type amino acids 1-219

11	h IL-2Ralpha (1-63)	ELCDDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRRIKSGSLYMLCTGNSSHSS WDNQCCQCTS	human	blocker	sushi domain 1 wild-type
12	h IL-2Ralpha (M25I)	ELCDDDDPPEIPHATFKAMAYKEGTILNCECKRGFRRRIKSGSLYMLCTGNSSHSS WDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPP PWENEATERIYHFVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLI CTGEMETSQFPGEEKPQASPEGRPESETSLVTTTDFQIQTEMAATMETSIFTT EYQ	human	blocker	M25 to I mutation
13	h IL-2Ralpha (L42V)	ELCDDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRRIKSGSVYMLCTGNSSHSS WDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPP PWENEATERIYHFVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLI CTGEMETSQFPGEEKPQASPEGRPESETSLVTTTDFQIQTEMAATMETSIFTT EYQ	human	blocker	L42 to V mutation
14	h IL-2Ralpha (M25I; L42V)	ELCDDDDPPEIPHATFKAMAYKEGTILNCECKRGFRRRIKSGSVYMLCTGNSSHSS WDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPP PWENEATERIYHFVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLI CTGEMETSQFPGEEKPQASPEGRPESETSLVTTTDFQIQTEMAATMETSIFTT EYQ	human	blocker	M25 to I mutation; L42 to V mutation
15	Human IL2Rgamma polypeptide sequence	LNTTILTPNGNEDTTADFFLTTMPTDSLVSSTLPLPEVQCFVFNVEYMNCTWNS SSEPQPTNLTLYWYKNSDNDKVQKCSHYLFSEETSGCQLQKKEIHL YQTFV QLQDPPRRRQATQMLKLQNLVIPWAPENLT LHKLSESQLELNWNNRFLNHCL HLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRFPNPLCGSAQHWS EWSHPIHWGSNTSKENPFLFALEA	human	blocker	

16	Human IL2Rbeta polypeptide sequence	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSQCVHAWPDRRRWNQTCCELLPVS QASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRMA PISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQ KQEWICLETLPDQTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDT	human	blocker	
17	chimeric IL- 2Ralpha	ELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKELVYMRCLGNSWSNC QCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHCREPPPWENE ATERIYHFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEM ETSQFPGEEKPQASPEGRPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQ	human/ mouse	blocker	mouse IL2Ralpha (1- 58) - hu IL2Ralpha (64-219)
18	m IL-2Ralpha	ELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKELVYMRCLGNSWSNC QCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQENLTGHCREPPPWKH EDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDE REHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETTAMTETFVLTMEYK	mouse	blocker	wild-type amino acids 1-215
19	m IL-2Ralpha (1-58)	ELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKELVYMRCLGNSWSNC QCTS	mouse	blocker	sushi domain 1 wild-type
20	h IL-2Ralpha (1-219) M25V/D4L/D5 Y	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQ IQTEMAATMETSIFTTEYQ	human	blocker	D4 to L mutation; D5 to Y mutation; M25 to I mutation
21	h IL-2Ralpha (1-219) L42V/D4L/D 5Y	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW	human	blocker	D4 to L mutation; D5 to Y mutation;

		TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQ IQTEMAATMETSIFTTEYQ			L42 to V mutation
22	h IL-2Ralpha (1-219) M25I/L42V/D 4L/D5Y	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQ IQTEMAATMETSIFTTEYQ	human	blocker	D4 to L mutation; D5 to Y mutation; M25 to I mutation; L42 to V mutation
23	h IL-2Ralpha (1- 219)D4L/D5 Y	ELCLYDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQ IQTEMAATMETSIFTTEYQ	human	blocker	D4 to L mutation; D5 to Y mutation
24	h IL-2Ralpha (1-219) SGSL ₃₉ - 42ELV	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKELVYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHCR EPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGEMETSQFPGEEKPQASPEGRPESETSCLVTTTDFQ IQTEMAATMETSIFTTEYQ	human	blocker	Wild-type residues 39- 42 replaced with ELV
25	h IL-2Ralpha (1-192)	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	Wild-type amino acids 1-192
26	h IL-2Ralpha (1-192)M25I	ELCDDDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	M25 to I mutation
27	h IL-2Ralpha (1-192)L42V	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	L42 to V mutation

28	h IL-2Ralpha (1-192)D4L/D5 Y	ELCLYDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRN ^{TT} KQVTPQPEEQKERKTT ^{EM} QSPMQPVDQASLPGHC REPPPWENEATERIYHFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	D4 to L mutation; D5 to Y mutation
29	h IL-2Ralpha (1-192) SGSL ₃₉ - 42ELV	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKELVYMLCTGNSSH SWDNQCQCTSSATRN ^{TT} KQVTPQPEEQKERKTT ^{EM} QSPMQPVDQASLPGHCR EPPPWENEATERIYHFVVGQMVYQCQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	Wild-type residues 39- 42 replaced with ELV
IL2 Blockers: anti-IL2 sequences					
30	scFv2	QSVLTQPPSVSGAPGQQRVTISCTGTSSNIGAHYDVHWYQQFPGTAPKRLIYGNN NRPSGVPARFSGSKSGTSASLAIITGLQAEDEADYYCQSYDRSLRGWVFGGGTKL TVLGEKSSGSGSEKASEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW VRQAPGKGLEWVSGISWNSGSI ^{GY} ADSVKGRFTISRDN ^{SK} N ^{TL} YLQMNSLRAED TAVYYCAKDVNWN ^Y GYFDYWGGQTLTVTSS	human	blocker	wild-type
31	scFv2 (18mer linker)	QSVLTQPPSVSGAPGQQRVTISCTGTSSNIGAHYDVHWYQQFPGTAPKRLIYGNN NRPSGVPARFSGSKSGTSASLAIITGLQAEDEADYYCQSYDRSLRGWVFGGGTKL TVLGGSTSGSGKPGSGEGSTKGEVQLVESGGGLVQPGRSLRLSCAASGFTFDDY AMHWVRQAPGKGLEWVSGISWNSGSI ^{GY} ADSVKGRFTISRDN ^{SK} N ^{TL} YLQMNSL RAEDTAVYYCAKDVNWN ^Y GYFDYWGGQTLTVTSS	human	blocker	18 mer linker between VL and VH
32	VL domain of scFv2	QSVLTQPPSVSGAPGQQRVTISCTGTSSNIGAHYDVHWYQQFPGTAPKRLIYGNN NRPSGVPARFSGSKSGTSASLAIITGLQAEDEADYYCQSYDRSLRGWVFGGGTKL TVLG	human	blocker	wild-type

33	VH domain of scFv2	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWN SGSIGYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDVNWNYYGYFF DYWGQGT LVTVSS	human	blocker	wild-type
34	scFv2 VL HVR1	TGTSSNIGAHYDVH			
35	scFv2 VL HVR2	GNNNRPS			
36	scFv2 VL HVR3	QSYDRSLRGWV			
37	scFv2 VH HVR1	DDYAMH			
38	scFv2 VH HVR2	GISWNSG SIGYADSVKG			
39	scFv2 VH HVR3	KDVNWNYYGYFDY			
747	scFv183	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSNNKNYLAWYQQKPGQPPKL LIYGASTRESWVPDRFSGSGGTDFLTISSLQAEDVAVYYCQWYYYPYTF GGGTKVEIKGGGGGGGGGGGGSEVQLLESGGGLVQPGGSLRLSCAA SGFTFSSYYMSWVRQAPGKGLEWVSDISGRGGQTNYADSVKGRFTISRDN SK NTLYLQMNSLRAEDTAVYYCARGGGSFANWGRGTL VTVSS	human	blocker	linker between VL and VH
748	VL domain of scFv183	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSNNKNYLAWYQQKPGQPPKL LIYGASTRESWVPDRFSGSGGTDFLTISSLQAEDVAVYYCQWYYYPYTF GGGTKVEIK	human	blocker	
749	VH domain of scFv183	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPGKGLEWVSDIS GRGGQTNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGGGSFA NWGRGTL VTVSS	human	blocker	
750	scFv183 VL HVR1	KSSQSVLYSNNKNYLA			

751	scFv183 VL HVR2	GASTRES				
752	scFv183 VL HVR3	QQWYYYPYT				
753	scFv183 VH HVR1	SSYYMS				
754	scFv183 VH HVR2	DISGRGGQTNYADSVKG				
755	scFv183 VH HVR3	RGGGSFAN				
Blockers: IL-2R sequences						
40	h IL-2Ralpha (1-192)M25I/D4 L/D5Y	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFBVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	D4 to L mutation; D5 to Y mutation; M25 to I mutation	
41	h IL-2Ralpha (1-192)M25I/L4 2V	ELCDDDDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFBVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	M25 to I mutation; L42 to V mutation	
42	h IL-2Ralpha (1-192) D4L/D5Y/L4 2V	ELCLYDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFBVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	D4 to L mutation; D5 to Y mutation; L42 to V mutation	
43	h IL-2Ralpha (1-192) M25I/D4L/D5 Y/L42V	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFBVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKPQASPEGRPESETSC	human	blocker	D4 to L mutation; D5 to Y mutation;	

						M25 to I mutation; L42 to V mutation
44	h IL-2Ralpha (1-178)	ELCDDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		Wild-type amino acids 1-178
45	h IL-2Ralpha (1-178) M25I	ELCDDDDPPEIPHATFKAMAYKEGT I LNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		M25 to I mutation
46	h IL-2Ralpha (1-178) L42V	ELCDDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		L42 to V mutation
47	h IL-2Ralpha (1-178) D4L/D5Y	ELCLYDPPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		D4 to L mutation; D5 to Y mutation
48	h IL-2Ralpha (1-178) SGSL ₃₉ - ₄₂ ELV	ELCDDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKELVYMLCTGNSSH SWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHCR EPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWT QPQLICTGEMETSQFPGEEKP	human	blocker		Wild-type residues 39-42 replaced with ELV
49	h IL-2Ralpha (1-178) M25I/L42V	ELCDDDDPPEIPHATFKAMAYKEGT I LNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		M25 to I mutation; L42 to V mutation
50	h IL-2Ralpha (1-178) D4L/D5Y/L42V	ELCLYDPPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCCQCTSSATRNTTKQVTPQPEEQKERKTTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGEEKP	human	blocker		D4 to L mutation; D5 to Y mutation;

						L42 to V mutation
51	h IL-2Ralpha (1-178) D4L/D5Y/M2 5I/L42V	ELCLYDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSVYMLCTGNSSH SSWDNQCQCTSSATRN TT KQVTPQP EE QKERK TT EMQSPMQPVDQASLPGHC REP PP WENEATERIYH FV VGQMVY Y QCVQGYRALHRGPAESVCKMTHGKTRW TQPQLICTGEMETSQFPGECKP	human	blocker		D4 to L mutation; D5 to Y mutation; M25 to I mutation; L42 to V mutation
52-69	Not Used					
Pharmacokinetic modulators						
70	h IgG1 Fc	DKTHTCPPCPAPELLGGPSVFLFPPKPKD TL MISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVY TL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYK TT TPPVLDSDGSFFLYSKLTVDKSRWQQGNV F SCSMHEALHNHY TQKSLSLSPG	human	half-life extension		C-terminal K residue deleted
71	Human IgG1 K392D K409D Fc domain polypeptide sequence	DKTHTCPPCPAPELLGGPSVFLFPPKPKD TL MISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVY TL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYD TT TPPVLDSDGSFFLYSDLTVDKSRWQQGNV F SCSMHEALHNHY TQKSLSLSPG	human	half-life extension		

72	Human serum albumin	RGVFRDDAHKSEVAHRFKDLGEENFKALVLI FAQYLQQCPFEDHVKL VNEVTEFAKTCVADESAENCDSKSLHTLFGDKLCT VATLRETYGEMADCCAKQEPERNECF LQHKDDNP NL PRLVRPEVDVMCTA FHDNEETFLKKYL YEIARRHPYFYAPEL LFFAKRYKAAFTTECCQAADKAACLLP KLDELREDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKA EFAEVSKLVTDLTKVHTECCHGDLLECADRADLAKYICENQDSISSKLLK ECCEKPLL EKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVF LGMFLYEYARRHPDYSVLLLR LAKTYETTLEKCCAAADPHECYAKVFDE FKPLVEEPQNL IKQNCELFEQLGEYKFQNAL LVRYTKKVPQVSTPTLVEV SRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKC CTESLVNRRPCFSALEVDETYVPKEFNAETTFHADICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMDDFAAFVEKCKKADDKETCF AE EGKKLV AASQAALGL	human	half-life extension	wild-type
73	m IgG1 Fc	GCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVT CVVVDISKDDPEVQFSWFV DDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSAAFPAPIE KTISKTKGRPKAPQVYTI PPKPEQMAKDVS L TCMITDFFPEDITV EWQWNGQP AENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLHEGLHHHTEKSL SHSPGK	mouse	half-life extension	wild-type
74	Murine IgG1 T252M Fc domain polypeptide sequence	GCKPCICTVPEVSSVFI FPPKPKDVL MITLTPKVT CVVVDISKDDPEVQFSWFV DDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSAAFPAPIE KTISKTKGRPKAPQVYTI PPKPEQMAKDVS L TCMITDFFPEDITV EWQWNGQP AENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLHEGLHHHTEKSL SHSPG	mouse	half-life extension	
75-79	Not Used				
756	IgG1 Fc (K360E/K409 W) Knob	DKTHTCPPCAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQNSTYRVSVLTVLHQDWLNGKEYCKKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TENQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNV FSCSVM HEALHNHYTQKSLSLSPG	human	half-life extension	Knob mutations

757	h IgG1 Fc (Q347R/D399 V/F405T) Hole	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SKALPAPIEKTISKAKGQPREPRVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLVSDGFTLYSKLTVDKSRWQQGNVVFSCSVM HEALHNHYTQKSLSLSPG	human	half-life extension	Hole mutations
MMP cleavable segments					
80	MMP cleavage site polypeptide sequence	GPLGVRG			
81	G112631 polypeptide sequence	GPLGVRG			
82	G112632 polypeptide sequence	GPLGLRG			
83	G112633 polypeptide sequence	GPLGLAR			
84	G112634 polypeptide sequence	GPAALVGA			
85	G112635 polypeptide sequence	GPAALIGG			

86	G112636 polypeptide sequence	GPLNLVGR				
87	G112637 polypeptide sequence	GPAGLVAD				
88	G112638 polypeptide sequence	GPANLVAP				
89	G112639 polypeptide sequence	VPLSLYSG				
90	G112640 polypeptide sequence	SGESPAYYTA				
91	MMP consensus motif	PXXX _{Hy}				
92	MMP-2 consensus motif	(L/I)XXX _{Hy}				

93	MMP-2 consensus motif	X _{Hy} SXL				
94	MMP-2 consensus motif	HXXX _{Hy}				
95-99	Not Used					
IL-2 Fusion polypeptides						
100	Construct A polypeptide sequence: mIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – 6His	APTSSSTSSSTAEAAQQQQQQQQHLEQLMDLQELLSRMENYRNKLPRL TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVT VKLKGSDNTFECQDDDESATVDFLRRIAFCQSIISTSPQSGGGSGGGGPL GVRGGGGSGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRCLGNSWSCQCTSNHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRHFLASEESQGSRNSSPESETSCPIITTTDFPQTETT AMTETFVLTMEYKHHHHH				
101	Construct B polypeptide sequence: m IL2-2x(SG4) – MMPcs1 – 2x (G4S) – IL2Ralpha – mIgG1 Fc	APTSSSTSSSTAEAAQQQQQQQQHLEQLMDLQELLSRMENYRNKLPRL TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVT VKLKGSDNTFECQDDDESATVDFLRRIAFCQSIISTSPQSGGGSGGGGPL GVRGGGGSGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRCLGNSWSCQCTSNHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRHFLASEESQGSRNSSPESETSCPIITTTDFPQTETT AMTETFVLTMEYKGCKPCICTVPEVSSVFIFPPKPKDVLITITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIIPPKQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK				

102	Construct C polypeptide sequence: mIL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – mIgG1 Fc(T252M)- 6xHIS	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLPRML TFKFYLPKQATELKDLQCLEDLGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQDDDESATVDFLRRIAFSQSIISTSPQSGGGSGGGGPL GVRGGGGSGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFI FPPKPKDVLMITLTPKVTCVWVDI SKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTI PPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGHHHHH			
103	Construct R polypeptide sequence: mIL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – hu IgG1 Fc- 6xHIS	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLPRML TFKFYLPKQATELKDLQCLEDLGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQDDDESATVDFLRRIAFSQSIISTSPQSGGGSGGGGPL GVRGGGGSGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKDKTHTCPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLN GKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGHHHHH			
104	Construct D polypeptide Sequence: mIL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – sIL2Ralpha –	APTSSSTKKTLQLEHLLLDLQMI LNINGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLTSGGGSGGGGPLGVRGGGGSGGGSEL CDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSHSSWD NQCQCTSSATRN TT KQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPW ENEATERIYHFVWQMVVYQCQGYRALHRGPAESVCKMTHGKTRWTQPQLICT GEMETSQFPGEEKPQASPEG RPESETSCLVTITDFIQIQT EMAATMETSIFTTEY QDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW			

	mIgG1 Fc(T252M)- 6xHIS	ESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNH YTQKSLSLSPGHHHHHH			
105	mIgG1 Fc - Murine IL2 - 2x(SG4) – MMPcs1 – 2x (G4S) – IL2Ralpha (long kinetic IL2 post cleavage) polypeptide Sequence	GCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVT CVVVDISKDDPEVQFSW FV DDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSAAFPAPIE KTISKTKGRPKAPQVYTI PPKQEMAKDVSLTCMITDFFPEDITVEWQWNGQP AENYKNTQPIMDTDGSYFVYSKLNQKSNWEAGNTFTCSVLHEGLHNHHTKSL SHSPGKAPTSSST`SSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRN LKLPRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFI SNIRVTVVKLKGSDNTFECQDDDESATVDFLRRWIAFCQSIISTSPQSGGGGS GGGGPLGVRGGGGGGGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRG FRRLKELVYMRCLGNWSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITTD MQKP TQSMHQENLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAIS ICKMKCGKTGWTQPQLTCVDEREHRFLASESQGSRNSSPESETSCPIITTTDF PQPTETTAMTETFVLTMEYK			
106	Construct E polypeptide sequence: HuIL2(C125S) - 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – hu IgG1 Fc – 6xHIS	APTSSSTKKTLQLEHLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEELKPLEEVNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYA DETATIVFELNRWITFSQSIISTLTSGGGSGGGGPLGVRGGGGSGGGSEL CDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSGSLYMLCTGNSSH SWD NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPPW ENEATERIYHFVVGQMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICT GEMETSQFPGEEKPQASPEGRPESETSCLVTITD FQIQTEMAATMETSIFTTEY QDKTHTCPPCPAPELLGGPSVLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNH YTQKSLSLSPGHHHHHH			

107	Construct S polypeptide sequence: hIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – hIL2Ralpha – hIgG1Fc_mut 1 (K392D; K409D)	APTSSSTKKTQLQLEHLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F C Q S I I S T L T S G G G S G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G Q M V Y Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T D F Q I Q T E M A A T M E T S I F T T E Y Q D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y D T T P P V L D S D G S F F L Y S D L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H			
108	Construct T polypeptide sequence including hIgG1 Fc_mut 2 (D356K; D399K)	D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L K S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H H			
109	hu IgG1 Fc - Hu IL2(C125S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha Polypeptide Sequence	D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G A P T S S T K T Q L Q L E H L L D L Q M I L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q I I S T L T S G G G S G G G P L G V R G G G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P W E N E A T E R I Y H F V V G Q M V Y Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T T D F Q I Q T E M A A T M E T S I F T T E Y Q			

110	hIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – hIL2Rbeta – hIgG1Fc (Construct U) polypeptide Sequence	APTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F C Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S A V N G T S Q F T C F Y N S R A N I S C V W S Q D G A L Q D T S C Q V H A W P D R R R W N Q T C E L L P V S Q A S W A C N L I L G A P D S Q K L T T V D I V T L R V L C R E G V R W R V M A I Q D F K P F E N L R L M A P I S L Q V V H V E T H R C N I S W E I S Q A S H Y F E R H L E F E A R T L S P G H T W E E A P L L T L K Q K Q E W I C L E T L T P D T Q Y E F Q V R V K P L Q G E F T T W S P W S Q P L A F R T K P A A L G K D T D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G			
111	hIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – hIL2Rgamma – hIgG1Fc polypeptide sequence	APTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F C Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S L N T T I L T P N G N E D T T A D F F L T T M P T D S L S V S T L P L P E V Q C F V F N V E Y M N C T W N S S E P Q P T N L T L H Y W Y K N S D N D K V Q K C S H Y L F S E E I T S G C Q L Q K K E I H L Y Q T F V V Q L Q D P R E P R R Q A T Q M L K L Q N L V I P W A P E N L T L H K L S E S Q L E L N W N N R F L N H C L E H L V Q Y R T D W D H S W T E Q S V D Y R H K F S L P S V D G Q K R Y T F R V R S R F N P L C G S A Q H W S E W S H P I H W G S N T S K E N P F L F A L E A D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S P G			
112- 119	Not Used				
Other					
120	Gly-Ser rich linker polypeptide sequence	SGGGGSGGGG			
121- 129	Not Used				

Fusion polypeptides (DNA coding sequences)			
130	Construct A DNA sequence (mIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – 6His)	ATGGGTTGGTCTGCATCATCCTGTTCTCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACATCATCACTCAACTTCATCTCCTCCACCGCTGAGGCTCAGCAACAA CAGCAACAACAGCAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTG CAGGAGCTGCTGTCCAGAAATGGAGAACTACCGCAAATCTGAAGTGCCAAGGATG CTGACCTTCAAGTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAG TGCCTGGAGGATGAGCTGGGCCCACTGAGGCACGTGCTGGACCTGACCCAGAGC AAGTCTTTCCAGCTGGAGGATGCTGAGAACTTTATCTCCAATATCCGGTGACC GTGGTGAAGCTGAAGGGCAGCGACAACACATTCGAGTGCCAGTTTGACGATGAG TCTGCCACCGTGGTGGAATTCCTGAGGCGGTGGATCGCTTTTGTTCAGAGCATC ATCTCCACAAGCCCTCAGTCTGGAGGAGGTGGCAGCGGAGGAGGAGGTGGCCCA CTGGCGGTGAGGGGTGGCGGCGGGCTCTGGCGGCGGGCTCCGAGCTGTGC CTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAG AACGGCACAAATCCTGAAATGCGAGTGTAAAGGGGCTTTAGACGCCCTGAAGGAG CTGGTGTACATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTGTACC TCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCAGCTGGAGCACCCAG AAGGAGCAGCAGACCACAACCGATATGCAGAAGCCACCCAGTCTATGCACCAG GAGAACTTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAGCACGAGGATAGC AAGCGCATCTATCATTTCTGTGGAGGGCCAGTCTGTGCACTACGAGTGATCCCC GGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGT GGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGGAGCAC CATCGGTTCTGGCTAGCGAGGAGTCTCAGGGCTCCCGCAACTCTTCCCCTGAG AGCGAGACATCTTGTCCAATCACAAACCACAGATTTCCACAGCCCACCCGAGACA ACCGCTATGACAGAGACCTTCGTGCTGACTATGGAAATACAAACACCCACCAC CACCACTAATGA	

131	Construct B DNA sequence: m IL2-2x(SG4) – MMPcs1 – 2x (G4S) – IL2Ralpha – mIgG1 Fc	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACATCATCAACTTCACTCTCCACCGCTGAGGCTCAGCAACAA CAGCAACAACAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTG CAGGAGCTGCTGCCAGATGGAGAACTACCGCAA TCTGAAGCTGCCAAGGATG CTGACCTTCAAGTTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAG TGCCTGGAGGATGAGCTGGGCCACTGAGGCACGTGCTGGACCTGACCCAGAGC AAGTCTTTCAGCTGGAGGATGCTGAGAACTTTATCTCCAATATCCGGTGACC GTGGTGAAGCTGAAGGCGAGCGACAACACATTCGAGTGCCAGTTTGACGATGAG TCTGCCACCGTGGTGGA TTTCTGAGGCGGTGGATCGCTTTTGT CAGAGCATC ATCTCACAAAGCCCTCAGTCTGGAGGAGGTGGCAGCGGAGGAGGTGGCCCA CTGGGCGTGAGGGGTGGCGGCGGGCTCTGGCGGCGGGCTCCGAGCTGTGC CTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAG AACGGCACAA TCTGAA TTGCGAGTGT AAGAGGGGCTTTAGACGCCCTGAAGGAG CTGGTGACATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTG TACC TCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCAGCTGGAGCACCCAG AAGGAGCAGCAGACCACAACCGATA TGCAGAAGCCACCCAGTCTATGCACCAG GAGAACTTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAGCACGAGGATAGC AAGCGCATCTATCATTTCTGTGGAGGGCCAGTCTGTGCAC TACGAGTGATCCCC GGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGT GGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGGAGCAC CATCGGTTCTGGCTAGCGAGGAGTCTCAGGGCTCCGCAACTCTTCCCCTGAG AGCGAGACATCTTGTCCAATCACAACCAACAGATTTTCCACAGCCCACCGAGACA ACCGCTATGACAGAGACCTTCGTGCTGACTATGGAA TACAAAGGATGCAAAACC TGATCTGTACCGTGCCCGAGGCTCTTCCGCTTTATTTCCCCCCCAAGCCT AAGGATGTGCTGACTATTACTCTGACCCCCCAAGGTGACATGCGTGGTGGAC ATCAGCAAGGACGATCCTGAGGTGCAGTTCTCTTGGTTTGTGGACGATGTGGAG GTGCACACCGCCAGACACAGCCAAAGGAGGAGCAGTTC AATAGCACCTTTTCGG TCCGTGAGCGAGCTGCCCATCATGCATCAGGATTGGCTGAATGGCAAGGAGTTC AAGTGCAGAGTGAACTCTGCCGCTTTTCCCGCTCTATCGAGAAGACCATCTCC AAGACAAAGGGCCGCCAAAGGCTCCACAGGTACACCATCCACCTCCAAAG GAGCAGATGGCTAAGGACAAGGTGCTCTGACCTGTATGATCACAGACTTCTTT CCTGAGGACATCACAGTGGAGTGGCAGTGGAAACGGCCAGCCTGCCGAGAACTAT AAGAA TACCCAGCCAATCATGGACACAGATGGCTCTTACTTCGTATTTCCAAG CTGAACGTGCAGAAAGTCCAA TTGGGAGGCTGGCAACACCTTTACATGTAGCGTG CTGCACGAAGGTCTGCATAACCATCATACCGAAAAATCACTGTACACTCCCCT GGAAATAATGA		
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132	Construct C DNA sequence: m IL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – mIgG1 Fc(T252M)- 6xHIS	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACATCATCAACTTCACTCTCCTCCACCGCTGAGGCTCAGCAACAA CAGCAACAACAGCAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTG CAGGAGCTGCTGTCCAGAAATGGAGAACTACCGCAA TCTGAAGCTGCCAAGGATG CTGACCTTCAAGTTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAG TGCCTGGAGGATGAGCTGGGCCACTGAGGCACGTGCTGGACCTGACCCAGAGC AAGTCTTTCAGCTGGAGGATGCTGAGAACTTTATCTCCAATATCCGGTGACC GTGGTGAAGCTGAAGGCGAGCGACAAACACATTCGAGTGCCAGTTTGACGATGAG TCTGCCACCGTGGTGGA TTTCTGAGGCGGTGGATCGCTTTTCCCAGAGCATC ATCTCACAAAGCCCTCAGTCTGGAGGAGGTGGCAGCGGAGGAGGAGGTGGCCCA CTGGGCGTGAGGGGTGGCGGCGGGCTCTGGCGGCGGGCTCCGAGCTGTGC CTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAG AACGGCACAA TCTGAA TTGCGAGTGTAAAGGGGCTTTAGACGCCCTGAAGGAG CTGGTGTA CATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTGTAAC TCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCAGCTGGAGCACCCAG AAGGAGCAGCAGACCACAACCGATA TGCAAGAGCCACCCAGTCTATGCACCAG GAGAACTTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAGCACGAGGATAGC AAGCGCATCTATCATTTCTGTGGAGGGCCAGTCTGTGCAC TACGAGTGATCCCC GGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGT GGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGGAGCAC CATCGGTTCTGGCTAGCGAGGAGTCTCAGGGCTCCCGCAACTCTTCCCCTGAG AGCGAGACATCTTGTCCAATCACAACCAACAGATTTTCCACAGCCCACCGAGACA ACCGCTATGACAGAGACCTTCGTGCTGACTATGGAA TACAAAGGATGCAAAACC TGATCTGTACCGTGCCCGAGGCTCTTCCGCTTTATTTCCCCCCCAAGCCT AAGGATGTGCTGATGATTACTCTGACCCCCCAAGGTGACATGCGTGGTGGAC ATCAGCAAGGACGATCCTGAGGTGCAGTTCTCTTGTTTGTGGACGATGTGGAG GTGCACACCGCCAGACACAGCCAAAGGAGGAGCAGTTC AATAGCACCTTTTCGG TCCGTGAGCGAGCTGCCCATCATGCATCAGGATTGGCTGAATGGCAAGGAGTTC AAGTGCAGAGTGA ACTCTGCCGCTTTTCCCGCTCTATCGAGAAGACCATCTCC AAGACAAAGGGCCGCCAAAGGCTCCACAGGTACACCATCCACCTCCAAAG GAGCAGATGGCTAAGGACAAGGTGCTCTGACCTGTATGATCACAGACTTCTTT CCTGAGGACATCACAGTGGAGTGGCAGTGGAAACGGCCAGCCTGCCGAGAACTAT AAGAA TACCCAGCCAA TCATGGACACAGATGGCTCTTACTTCGTATTTCCAAG CTGAACGTGCAGAAAGTCCAA TTGGGAGGCTGGCAACACCTTTACATGTAGCGTG CTGCACGAAGGTCTGCATAACCATCATACCGAAAAATCACTGTACACTCCCCCT GGACACCACCAACCACCACTAATGA	
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133	Construct R DNA sequence: mIL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – hu IgG1 Fc- 6xHIS	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACATCATCAACTTCACTCTCCTCCACCGCTGAGGCTCAGCAACAA CAGCAACAACAGCAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTG CAGGAGCTGCTGTCCAGAAATGGAGAACTACCGCAA TCTGAAGCTGCCAAGGATG CTGACCTTCAAGTTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAG TGCCTGGAGGATGAGCTGGGCCACTGAGGCACGTGCTGGACCTGACCCAGAGC AAGTCTTTCAGCTGGAGGATGCTGAGAACTTTATCTCCAATATCCGGTGACC GTGGTGAAGCTGAAGGCGAGCGACAAACACATTCGAGTGCCAGTTTGACGATGAG TCTGCCACCGTGGTGGA TTTCTGAGGCGGTGGATCGCTTTTCCCAGAGCATC ATCTCACAAAGCCCTCAGTCTGGAGGAGGTGGCAGCGGAGGAGGTGGCCCA CTGGGCGTGAGGGGTGGCGGCGGGCTCTGGCGGCGGGCTCCGAGCTGTGC CTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAG AACGGCACAA TCTGAA TTGCGAGTGTAAAGGGGCTTTAGACGCTTGAAGGAG CTGGTGACATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTGTAAC TCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCAGCTGGAGCACCCAG AAGGAGCAGCAGACCACAACCGATATGCAGAAGCCACCCAGTCTATGCACCAG GAGAACTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAGCACGAGGATAGC AAGCGCATCTATCATTTCTGTGGAGGGCCAGTCTGTGCAC TACGAGTGATCCCC GGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGT GGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGGAGCAC CATCGGTTCTGGCTAGCGAGGAGTCTCAGGGCTCCCGCAACTCTTCCCCTGAG AGCGAGACATCTTGTCCAATCACAACCAACAGATTTTCCACAGCCCACCGAGACA ACCGCTATGACAGAGACCTTCGTGCTGACTATGGAA TACAAAGATAAGACTCAT ACCTGTCCACCCCTGCTCCTGAACTGCTGGGGGTCTTCCGTGTTCTCTG TTCCCTCCAAACCTAAAGATACCTGATGATCTCCAGGACCCCTGAGGTGACA TGGCTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCAGGGAGGAGCAGTAC AACAGCACCTATCGGGTGGTGTCTGTGCTGACAGTGTGCAC CAGGATTGGCTG AACGGCAAGGAGTATAAGTGCAAGGTGTCTAATAAGGCCCTGCTGCTCCAATC GAGAAGACCATCTCCAAGGCCAAGGGCCAGCCAGAGAGCCTCAGGTGTACACA CTGCCCCCTAGCCGCGACGAGCTGACCAAGAACCAAGGTGCTCTGACATGCTG GTGAAGGGCTTCTATCCATCTGACATCGCTGTGGAGTGGAGTCCAATGGCCAG CCCGAGAACAA TTACAAGACCACACCCCGTGTGGACTCTGATGGCTCCTTC TTTCTGTATTCCAAGCTGACCGTGGACAAAGAGCAGATGGCAGCAGGGCAACGTG TTCTCCTGTAGCGTGATGCACGAAGCCCTGCACAACCATTACACTCAGAAAGC CTGTCCCTGTCCCTGGGCACCACCAACCACCACTAATGA		
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134	Construct D DNA Sequence: mIL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – sIL2Ralpha – mIgG1 Fc(T252M)- 6xHIS	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACATCATCAACTTCACTCTCCACCGCTGAGGCTCAGCAACAA CAGCAACAACAGCAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTG CAGGAGCTGCTGTCCAGAAATGGAGAACTACCGCAA TCTGAAGCTGCCAAGGATG CTGACCTTCAAGTTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAG TGCCTGGAGGATGAGCTGGGCCACTGAGGCACGTGCTGGACCTGACCCAGAGC AAGTCTTTCAGCTGGAGGATGCTGAGAACTTTATCTCCAATATCCGGTGACC GTGGTGAAGCTGAAGGCGAGCGACAAACACATTCGAGTGCCAGTTTGACGATGAG TCTGCCACCGTGGTGGA TTTCTGAGGCGGTGGATCGCTTTTCCCAGAGCATC ATCTCACAAAGCCCTCAGTCTGGAGGAGGTGGCAGCGGAGGAGGTGGCCCA CTGGCGGTGAGGGGTGGCGGCGGGCTCTGGCGGCGGGCTCCGAGCTGTGC CTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAG AACGGCACAAATCCTGAA TTGCGAGTGTAAAGGGGCTTTAGACGCCCTGAAGGAG CTGGTGACATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTGTAAC TCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCAGCTGGAGCACCCAG AAGGAGCAGCAGACCACAACCGATA TGCAGAAGCCACCCAGTCTATGCACCAG GAGAACTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAGCACGAGGATAGC AAGCGCATCTATCATTTCTGTGGAGGGCCAGTCTGTGCAC TACGAGTGATCCCC GGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGT GGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGGAGCAC CATCGGTTCTGGCTAGCGAGGAGTCTGGATGCAACCCTGTATCTGTACCGTG CCCAGGTCCTTCCGTC TTTATTTTCCCCCAAGCCTAAGGATGTGCTGATG ATTACTCTGACCCCCAAGGTGACATGCGTGGTGGTGGACATCAGCAAGGACGAT CCTGAGGTGCAGTTCTCTTGTTTGTGGACGATGTGGAGGTGCACACCGCCAG ACACAGCCAAGGGAGGAGCAGTTCAA TAGCACCTTTCGGTCCGTGAGCGAGCTG CCCATCATGCATCAGGATTGGCTGAATGGCAAGGAGTTCAAGTGCAGAGTGAAC TCTGCCGCTTTTCCCGCTCCTATCGAGAAGACCATCTCCAAGACAAAGGGCCGC CCAAGGCTCCACAGGTGTACACCATCCACCTCCAAGGAGCAGATGGCTAAG GACAAAGGTGCTCTGACCTGTATGATCACAGACTTCTTTCCTGAGGACATCACA GTGGAGTGGCAGTGGAAACGGCCAGCCTGCCGAGAACTATAAGAA TACCCAGCCA ATCATGGACACAGATGGCTCTTACTTCTGTTATTCCAAGCTGAACGTGCAGAAG TCCAA TTGGGAGGCTGGCAACACCTTTACATGTAGCGTGTGCACGAAGTCTG CATAACCATCATACCGAAAAATCACTGTACACTCCCTGGACACCAACCCAC CACCAC TAA TGA
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135	Construct E DNA sequence: HuIL2(C125S) - 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – hu IgG1 Fc – 6xHIS	ATGGGCTGGTCTGCATCATCTCTGTTTCTGGTGGCTACCGCCACCGGCGTGCAC TCTGCTCTACATCCTCCAGCACCAAGAAACCCAGCTGCAGTTGGAGCATCTG CTGCTGGACCTGCAGATGATCCTGAACGGCATCAACAACTACAAGAACCCCAAG CTGACCCGGATGCTGACCTTCAAGTTCTACATGCCCAAGAAGCCACCGAGCTG AAACATCTGCAGTGCCTGGAAGAGGAACTGAAGCCCTTGAAAGAAGTGCTGAAT CTGGCCCAGTCCAAGAACTTCCACCTGAGGCCTCGGGACCTGATCTCCAACATC AACGTGATCGTCTCGAGCTGAAGGGCTCCGAGACAACCTTTCATGTGCGAGTAC GCCGACGAGACAGCTACCATCGTGGAAATTCTGAACCCGGTGGATCACCTTTCAGC CAGTCCATCATCAGCACCCCTGACATCTGGCGCGGAGGATCTGGCGGAGCGGA GGACCTTTGGGAGTTCGCGGCGTGGTGGTGGCAGCGGAGTGGTGGATCTGAG CTGTGTGACGACGACCCCTCCTGAGATCCCTCACGCCACCTTTAAGGCCATGGCT TACAAAGAGGGCACCATGCTGAACTGCGAGTGCAAGAGAGGCTTCCGGCGGATC AAGTCCGGCAGCCTGTATATGCTGTGCACCGCAACTCCAGCCACTCCTCTTGG GACAAACAGTGCCAGTGCAACAGCTCTGCTACCCGGAAACACCACCAAGCAAGTG ACCCCTCAGCCTGAGGAACAGAAAGAGCGCAAGACCACCGAGATGCAGAGCCCC ATGCAGCCTGTGGATCAGGCTTCTCTGCTGGCCACTGTAGAGAGCCTCCACCT TGGAGAAATGAGGCTACCGAGAGAACTACCACTTCGTCGTGGACAGATGGTG TACTACAGTGCAGGGCTACCGGCTCTGCATAGAGGACCAGCAGAGTCC GTGTGCAAGATGACCCACGGCAAGACCAGATGGACCCAGCCTCAGCTGATCTGC ACCGGCGAGATGGAACCTCTCAGTTCCCGGCGAGGAAAGCCTCAGGCCCTCT CCTGAAGGCAGACCCGAGTCTGAGACATCCTGTCTCGTGACCACACAGACTTC CAGATCCAGACCGAGATGGCCGTACCATGGAACCCAGCATCTTCACCACCCGAG TACCAGGACAAGACCCACACCTGTCTCCATGTCTGCTCCAGAA TTGCTCGGC GGACCCCTCCGTGTTCTGTTTCCCTCAAAGCCTAAGGACACCCCTGATGATCTCT CGGACCCCTGAAGTGACCTGCGTGGTGGTCGATGTGTCACGAGGATCCCGAA GTGAAGTTCAA TTGGTACGTGGACGGCGTGGAAGTGCAACGCAAGACCAAG CCTAGAGAGGAACAGTACAACCTCCACCTACAGAGTGGTGCTCGCTGACCCGTG CTGCACCAGGATTGGCTGAATGGCAAGAGTACAAGTGCAAGGTGTCCAACAAG GCCCTGCCTGCTCCTATCGAAAGACCATCTCCAAGGCCAAGGGCCAGCCTAGG GAACCCCAAGTTTACACCTTGCCTCCA TCTCGGGACGAGCTGACCAAGAACCAG GTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAA TGGGAGTCTAATGGCCAGCCTGAAACAATTACAAGACAACCCCTCCTGTGCTG GACTCCGACGGCTCATTTCTCTGTACAGCAAGCTGACAGTGGACAAGTCCAGA TGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCA TGAGGCCCTGCACAAC CACTACACCCAGAAGTCCCTGTCTCTGTCCCCCTGGCCACCATCACCATCATCAC TGATAA	
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136	Construct S DNA sequence: hIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – hIL2Ralpha – hIgG1Fc_mut 1 (K392D; K409D)	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGCACCTACTTCAAGTTCTACAAAGAAACACACAGCTACAACTGGAGCATTTA CTTCTGGATTTACAGATGATTTTGAAATGGAAATTAATAATTACAAGAAATCCCAA CTCACAGGATGCTCACATTTAAGTTTACATGCCCAAGAGGCCACAGAACTG AAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAAT TTAGCTCAAAGCAAAACCTTTCACCTTAAGACCCAGGGACTTAATCAGCAATATC AACGTAATAGTTCTGGAACTAAAGGGATCTGAACAACATTCATGTGTGAATAT GCTGATGAGACAGCAACCATTTGTAGAAATTTCTGAACAGATGGATTACCTTTTGT CAAAGCATCATCTCAACACTGACTTCTGGTGCGGTGGCTCTGGTGCGGTGGC GGTCCCTCTGGGTGTCAGAGGTGGTGCGGTGGCTCTGGTGCGGTGGCTCTGAG CTCTGTGACGATGACCCGCCAGAGATCCACACGCCACATTCAAAGCCATGGCC TACAAGGAAGGAACCATGTTGAACTGTGAATGCAAGAGAGGTTTCCGCAGAATA AAAAGCGGTCACTCTATATGCTCTGTACAGGAAACTCTAGCCACTCGTCCTGG GACAACCAATGTCAATGCACAAGCTCTGCCACTCGGAACACACGAAACAAGTG ACACCTCAACCTGAAGAACAGAAAGAAAGAAACCCACAGAAATGCAAAGTCCA ATGCAGCCAGTGGACCAAGCGAGCCTTCCAGTCACTGCAGGGAACCTCCACCA TGGGAAATGAAGCCACAGAGAGAAATTTATCATTTCTGTTGGTGGGCAGATGGTT TATTATCAGTGCCTCCAGGGATACAGGGCTCTACACAGAGGTCTGCTGAGAGC GTCTGCAAAATGACCCACGGGAAGACAAGTGGACCCAGCCCACTCATATGC ACAGGTGAAATGGAGACCAGTCAGTTTCCAGGTGAAGAGAAGCCTCAGGCAAGC CCGAAGGCCGCTCTGAGAGTGAGACTTCTGCTCGTCTGTCAACAACAGATTTT CAAATACAGACAGAAATGGCTGCAACCATGGAGACGTCCATATTTACAACAGAG TACCAGGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACCTCCTGGGG GGACCGTCAGTCTTCTCTTCCCCCAAACCCAAAGGACACCCCTCATGATCTCC CGGACCCCTGAGGTCACATGCGTGGTGGTGACGTGAGCCACGAAGACCCCTGAG GTCAAGTTCAACTGGTACGTGGACGGGTGGAGGTGCATAATGCCAAGACAAAG CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTC CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCAACAGGTGTACACCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAG GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGCAGCCGGAGAACTACGACACACGCTCCCGTGGCTG GACTCCGACGGCTCCTTCTCTCTATAGCGACCTCACCGTGACAAAGCAGG TGGCAGCAGGGGAACGTCTTCTCATGTCTCCGTGATGCATGAGGCTCTGCACAAC CACTACACGCAGAAAGAGCCTCTCCCTGTCTCCGGTCAACCAACCAACCCAC TAATGA
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137	Construct T DNA sequence including hIgG1 Fc_mut 2 (D356K; D399K)	ATGGGTTGGTCCTGCATCATCCTGTTCTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCGACAAACTCACACATGCCCCACCGTGCCCGCAGCACCTGAACCTCTGGGGGA CCGTCAGTCTTCCTCTTCCCCCAAACCCAAAGGACACCCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCACGAAACCCCTGAGGTC AAGTTCAACTGCTACGTGGACGCGGTGGAGGTGCATAATGCCAAGACAAAGCCG CGGAGGAGCAGTACAACAGCACGTACCGTGTGTGTCAGCGTCTCACCGTCCTG CACGAGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAGCC CTCCAGCCCCCATCGAGAAACCATCTCCAAGCCAAAGGCGAGCCCCGAGAA CCACAGGTGTACACCTGCCCCCATCCCGGAAAGAGCTGACCAAGAACCCAGGTC AGCTGACCTGCCCTGGTCAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGTGAAA TCCGACGGCTCCTTCTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGG CAGCAGGGGAACGTCTTCTCATGTCTCCGTGATGCATGAGGCTCTGCACAACCAC TACACGCAGAAGAGCCTCTCCCTGTCTCCGGTCAACCACCAACCACCACTAA TGA		
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138	mIgG1 Fc - Murine IL2 - 2x(SG4) – MMPcs1 – 2x (G4S) – IL2Ralpha (long kinetic IL2 post cleavage) DNA Sequence	ATGGGTTGGTCCTGCATCATCCTGTTCTCGTGCCGACCGCCACTGGGGTCCAC TCCGGATGCAAAACCTGTATCTGTACCGTGCCCGAGGTCCTCTCCGTCCTTATT TTCCCCCAAGCCTAAGGATGTGCTGACTATTACTCTGACCCCCAAGGTGACA TGCGTGGTGGTGGACATCAGCAAGGACGATCCTGAGGTGCAGTTCTCTTGTTT GTGGACGATGTGGAGGTGCACACCGCCAGACACAGCCAAAGGAGGAGCAGTTTC AATAGCACCTTTCGGTCCGTGAGCGAGCTGCCCATCATGCATCAGGATTGGCTG AATGGCAAGGAGTTCAAGTGCAGAGTGAACTCTGCCGCTTTTCCGCTCCTATC GAGAAGACCATCTCCAAGACAAAGGGCCGCCCAAAGGCTCCACAGGTGTACACC ATCCACCTCCAAAGGAGCAGATGGCTAAGGACAAGGTGTCTCTGACCTGTATG ATCACAGACTTCTTCTCTGAGGACATCACAGTGGAGTGGCAGTGGAAACGGCCAG CCTGCCGAGAACTATAAGAAATACCCAGCCAAATCATGGACACAGATGGCTCTTAC TTCGTGTATTCCAAGCTGAACGTGAGAAGTCCAA TTGGAGGCTGGCAACACC TTTACATGTAGCGTGTGCACGAAGGTCTGCATAACCATCATACCGAAAAATCA CTGTCACACTCCCCTGGAAAGCACCTACATCATCATCAACTTCATCCTCCACC GCTGAGGCTCAGCAACAACAGCAACAACAGCAGCAGCAGCAGCATCTGGAG CAGCTGCTGATGGACCTGCAGGAGCTGCTGTCCAGAA TGAGAACTACCGCAAT CTGAAGCTGCCAAGGATGCTGACCTTCAAGTTTATCTGCCCAAGCAGGCCACA GAGCTGAAGGACCTGCAGTGCTGGAGGATGAGCTGGGCCACTGAGGCACGTG CTGGACCTGACCCAGAGCAAGTCTTTCAGCTGGAGGATGCTGAGAACTTTATC TCCAATATCCGGTGACCGTGGTGAAGCTGAAGGGCAGCGACAACACATTTCGAG TGCCAGTTTGACGATGAGTCTGCCACCGTGGTGGA TTTCTGAGCGGTGGATC GCTTTTGTGAGAGCATCATCTCCACAAGCCCTCAGTCTGAGGAGGTGCGAGC GGAGGAGGAGGTGGCCACTGGCGGTGAGGGTGGCGCGCGGCTCTGGCGGC GGCGGCTCCGAGCTGTGCTGTACGACCCCTGAGGTGCCAAATGCCACCTTC AAGGCTCTGTCTTATAAGAACGGCACAA TCCTGAA TTGCGAGTGTAAAGGGGC TTTAGACGCCCTGAAGGAGCTGGTGACATGCGGTGCTGGGCAACTCCTGGTCC AGCAATTGCCAGTGTAACCTCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACA GCCAGCTGGAGCACCAAGAGGAGCAGACACCAACCGATATGCAAGAGCCC ACCCAGTCTATGCACCAGGAGAACTGACAGGCCATTGCAGAGAGCCACCCCT TGGAAGCACGAGGATAGCAAGCGCATCTATCATTTCTGGAGGGCCAGTCTGTG CACTACGAGTGATCCCGGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCC ATCTGCAAGATGAAGTGTGGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGC GTGGACGAGAGGAGCACCATCGGTTCTCTGGCTAGCGAGGAGTCTCAGGGCTCC CGCAACTCTTCCCCTGAGAGCGAGACATCTTGTCCAATCACAACCACAGATTTT CCACAGCCCACCGAGACAACCGCTATGACAGAGACCTTCGTGCTGACTATGGAA TACAAATAATGA		
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139	hIL2- 2x(SG4) – MNPcs1 – 2x(G4S) – hIL2Rbeta – hIgG1Fc (Construct U) DNA Sequence	ATGGGTTGGTCTGCATCATCTGTTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCgcacctacttcaagttctacaaagaacacacagctacaactggagcattta cttctggatttacagatgatttgaatggaatttaataattacaagaatcccaaa ctcaccaggatgctcacatttaagttttacatgcccagaagccacagaactg aaacatcttcagtgctagaagaagaactcaaacctctggaggaagtgtaaat ttagctcaagcaaaaactttcacttaagaccagggaacttaatcagcaatatc aacgtaatagttctggaactaaagggaatctgaacaacattcatgtgaaatat gctgatgagacagcaaccattgtagaatttctgaacagatggattaccttttgt caaagcatcatctcaacactgactTCTGGTGGCGGTGGCTCTGGTGGCGTGGC GGTCCCTCTGGGTGTCAGAGGTGGTGGCGGTGGCTCTGGTGGCGGTGGCTGCG GTGAATGGCACTTCCAGTTCACATGCTTCTAACACTCGAGAGCCAACATCTCC TGTGCTGGAGCCAAGATGGGCTCTGCAGGACACTTCTGCCAAGTCCATGCC TGGCCGGACAGACGGCGGTGGAACCAACCTGTGAGCTGCTCCCCGTGAGTCAA GCATCCTGGCCTGCAACCTGATCTCGAGCCCCAGATTCTCAGAACTGACC ACAGTTGACATCGTCACCCCTGAGGGTGCTGTGCCGTGAGGGGTGCGATGGAGG GTGATGGCCATCCAGGACTTCAAGCCCTTTGAGAACCTTCGCCCTGATGGCCCCC ATCTCCCTCCAAGTTGTCCACGTGGAGACCCACAGATGCAACATAAGCTGGGAA ATCTCCCAAGCCTCCCACTACTTTGAAAGACACCTGGAGTTCGAGGCCCGGACG CTGTCCCCAGGCCACACCTGGGAGGAGGCCCTGCTGCTGACTCTCAAGCAGAAG CAGGAATGGATCTGCCCTGGAGACGCTCACCCAGACACCCAGTATGAGTTTCAG GTGCGGGTCAAGCCTCTGCAAGCGGAGTTCACGACCTGGAGCCCTGGAGCCAG CCCCTGGCCTTCAGGACAAAGCCTGCAGCCCTTGGGAAGGACACCGACAAGACC CACACCTGTCTCCATGTCTGTCTCCAGAAATGCTCGCGGACCTCCGTGTTTC CTGTTTCTCTCCAAAGCCTAAGGACACCTGATGATCTCTCGGACCCCTGAAGTG ACCTGCGTGGTGGTCGATGTGTCTACGAGGATCCCGAAGTGAAGTTCAATTGG TACGTGGACGGGTGGAAGTGCACAACGCCAAGACCAGCCTAGAGAGGAACAG TACAACTCCACCTACAGAGTGGTGCTCGTGCTGACCGTGCTGCACCAGGATTGG CTGAATGGCAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCCTGCTCCT ATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAAGTTTAC ACCTTGCCCTCCATCTCGGGACGAGCTGACCAAGAACCAGGTGTCCTGACCTGT CTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGAGTCTAATGGC CAGCCTGAAAACAATTACAAGACAACCCCTCCTGTGTGCTGGACTCCGACGGCTCA TTCTTCCTGTACAGCAAGCTGACAGTGGACAAGTCCAGATGGCAGCAGGGCAAC GTGTTCTCCTGCTCCGTGATGCATGAGGCCCTGCACAACCACCTACACCCAGAAG TCCCTGTCTCTGTCCCCCTGGCTAATGA		
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140	hIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – hIL2R _{gamma} – hIgG1Fc DNA sequence	ATGGGTTGGTCTGCATCATCTGTTCCTGGTCGCCACCGCCACTGGGGTCCAC TCCgcacctacttcaagttctacaaagaacacagctacaactggagcattta cttctggatttacagatgatttgaatggaattaataattacaagaatcccaaa ctcaccaggatgtcacatttaagttttacatgcccagaagccacagaactg aaacatcttcagtgctagaagaagaactcaaacctctggaggaagtgcataat ttagctcaagcaaaaactttcacttaagaccagggaacttaatcagcaatatc aacgtaatagtctggaactaaagggatctgaaacaacattcatgtggaatat gctgatgagacagcaaccattgtagaatttctgaacagatggattaccttttgt caaagcatcatctcaacactgactTCTGGTGGCGGTGGCTCTGGTGGCGGTGGC GGTCCCTCTGGGTGTCAGAGGTGGTGGCGGTGGCTCTGGTGGCGGTGGCTCTG AACACGACAATTCTGACGCCCAATGGGAATGAAGACACACAGCTGATTCTTC CTGACCACTATGCCCACTGACTCCCTCAGTGTTCACCTCTGCCCTCCAGAG GTTCAGTGTTTTGTGTTCAATGTCGAGTACATGAATTGCACCTTGAACAGCAGC TCTGAGCCCCAGCCTACCAACCTCACTCTGCATTATTGGTACAAGAACTCGGAT AATGATAAAGTCCAGAAGTGCAGCCACTATCTATTCTCTGAAGAAATCACTTCT GGCTGTCAAGTTGCAAAAAGGAGATCCACCTCTACCAACAATTTGTTGTTTCAG CTCCAGGACCCACGGAAACCCAGGAGACAGGCCACACAGATGCTAAAACTGCAG AATCTGGTGATCCCCCTGGGCTCCAGAGAACCTAACACTTCACAAACTGAGTGAA TCCCAGCTAGAACTGAACCTGGAACAACAGATTCTTGAACCACTGTTTGAGCAC TTGGTGCAGTACCGGACTGACTGGGACCAACAGCTGGACTGAACAATCAGTGGAT TATAGACATAAGTTCTCCTTGCCCTAGTGTGGATGGCAGAAACGCTACACGTTT CGTGTTCGGAGCCGCTTTAACCCACTCTGTGGAAGTGTCTCAGCATTTGGAGTGAA TGGAGCCACCCAAATCCACTGGGGGAGCAATACTTCAAAGAGAAATCCTTTCCCTG TTTGCAATTGGAAGCCGACAAGACCCACACCTGTCTCCATGTCTGCTCCAGAA TTGCTCGGCGGACCTCCGTGTTCTCTGTTCTCCAAAGCCTAAGGACACCCCTG ATGATCTCTCGGACCCCTGAAGTGACCTGCGTGGTGGTGCATGTGTCTCACGAG GATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAAGTGCACAACGCC AAGACCAAGCCTAGAGAGGAACAGTACAACCTCCACCTACAGAGTGGTGTCCGTG CTGACCGTGTGCAACCAGGATTGGCTGAATGGCAAAAGAGTACAAGTGCAAGGTG TCCAACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGCCAAGGGC CAGCCTAGGGAACCCAGGTTTACACCTTGCCCTCCATCTCGGACGAGCTGACC AAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGATATC GCCGTGGAATGGGAGTCTAATGGCCAGCCTGAAAACAATTACAAGACAACCCCT CCTGTGCTGGACTCCGACGGCTCATTTCTTCTGTACAGCAAGCTGACAGTGGAC AAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCATGAGGCC CTGCACAACCACTACACCCAGAAAGTCCCTGTCTGTCCCCTGGCTAATGA			
141- 149	Not Used				

Other DNA sequences				
150	Murine Ig kappa chain leader DNA sequence	ATGGGTTGGTCCTGCATCATCCTGTTCTCTGGTCGCCACCGCCACTGGGGTCCAC TCC		
151	Murine IL-2 DNA sequence	GCACCTACATCATCAACTTCATCCTCCACCCGCTGAGGCTCAGCAACAACAG CAACAACAGCAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTGCAG GAGCTGCTGCCAGAAATGGAGAACTACCGCAATCTGAAGCTGCCAAGGATGCTG ACCTTCAAGTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAGTGC CTGGAGGATGAGCTGGGCCCCACTGAGGCACGCTGGACCTGACCCAGAGCAAG TCTTTCAGCTGGAGGATGCTGAGAACTTATCTCCAATATCCGGTGACCGTG GTGAAGCTGAAGGGCAGCGACAAACACATTTCGAGTGCCAGTTTGACGATGAGTCT GCCACCGTGGTGGATTTCCTGAGGCGGTGGATCGCTTTTGTGCAGAGCATCATC TCCACAAGCCCTCAG		
152	MMP cleavage site GPLGVRG DNA sequence	GGCCCACTGGGCGTGAGGGGT		
153	Gly-Ser rich linker DNA sequence	TCTGGAGGAGGTGGCAGCGGAGGAGGAGGT		

154	Murine IL-2Ralpha DNA sequence	GAGCTGTGCCTGTACGACCCCTGAGGTGCCCAATGCCACCTTCAAGGCTCTGTCTTATAAGAACGGCACAATCCTGAATTGCGAGTGTAAAGGGGCTTTAGACGCTGAAGGAGCTGGTGTACATGCGGTGCTGGGCAACTCCTGGTCCAGCAATTGCCAGTGTACCTCTAACTCCCATGACAAGAGCAGAAAGCAGGTGACAGCCCAGCTGGAGCACGAAGGAGCAGCAGACCAACCGATATGCAGAAGCCACCCAGTCTATGCACCAGGAGAACTCTGACAGGCCATTGCAGAGAGCCACCCCTTGGAAAGCACGAGGATAGCAAGCGCATCTATCATTTCTGTGGAGGCCAGTCTGTGCACTACGAGTGTATCCCCGGCTATAAGGCCCTGCAGAGAGGCCCTGCTATCTCCATCTGCAAGATGAAGTGTGGCAAGACCGGCTGGACACAGCCTCAGCTGACCTGCGTGGACGAGAGGAGCACCATCGGTTCTGGCTAGCGAGGAGTCTCAGGGTCCCGCAACTCTTCCCCTGAGAGCGAGACATCTTGTCCAATCACAAACCACAGATTTTCCACAGCCCACCGAGACAACCGCTATGACAGAGACCTTCGTGCTGACTATGGAAATACAAA			
155	His tag DNA Sequence	CACCACCACCACCACCAC			
156	Stop codons	TAATGA			
157	Murine IL-2 C140S DNA sequence	GCACCTACATCATCAACTTCATCCTCCACCCGCTGAGGCTCAGCAACAACAGCAACAACAGCAGCAGCAGCATCTGGAGCAGCTGCTGATGGACCTGCAGGAGCTGCTGCCAGAA TGAGAACTACCGCAATCTGAAGCTGCCAAGGATGCTGACCTCAAGTTTATCTGCCCAAGCAGGCCACAGAGCTGAAGGACCTGCAGTGCCTGGAGGATGAGCTGGGCCCACTGAGGCACGTGCTGGACCTGACCCAGAGCAAGTCTTTCCAGCTGGAGGATGCTGAGAACTTTATCTCCAAATATCCGGTGACCCGTGTGAAGCTGAAGGGCAGCGACAACACATTTCGAGTGCCAGTTTGACGATGAGTCTGCCACCGTGGTGGAATTCCTGAGGCGGTGGATCGCTTTTCCAGAGCATCATCTCCACAAGCCCTCA			

158	Murine IgG1 T252M Fc domain DNA sequence	GGATGCAAAACCCTGTATCTGTACCGTGCCCGAGGTCTCTTCCGCTCTTATTTTC CCCCCAAGCCTAAGGATGTGCTGATGATTACTCTGACCCCCAAGGTGACATGC GTGGTGGTGGACATCAGCAAGGACGATCCTGAGGTGCAGTTCTCTTGGTTGTG GACGATGTGGAGTGCACACCGCCAGACACAGCCAAAGGAGGAGCAGTTCAAT AGCACCTTTCGGTCCGTGAGCGAGCTGCCCATCATGTCATCAGGATTGGCTGAAT GGCAAGGAGTTCAAGTGCAGAGTGAACCTCTGCCGCTTTTCCCGCTCCTATCGAG AAGACCATCTCCAAGACAAGGGCCGCCAAAGGCTCCACAGGTGTACACCATC CCACCTCCAAGGAGCAGATGGCTAAGGACAAGGTGTCTCTGACCTGTATGATC ACAGACTTCTTTCCTGAGGACATCACAGTGGAGTGGCAGTGGAAACGGCCAGCCT GCCGAGAACTATAAGAAATACCCAGCCAATCATGGACACAGATGGCTCTTACTTC GTGTATTCCAAGCTGAACGTGCAGAAGTCCAAATTGGGAGGCTGGCAACACCTTT ACATGTAGCGTGCTGCACGAAGGTCTGCA TAACCATCATACCGAAAAATCACTG TCACACTCCCCCTGGA		
159	Murine IgG1 Fc domain DNA sequence	GGATGCAAAACCCTGTATCTGTACCGTGCCCGAGGTCTCTTCCGCTCTTATTTTC CCCCCAAGCCTAAGGATGTGCTGACTATTACTCTGACCCCCAAGGTGACATGC GTGGTGGTGGACATCAGCAAGGACGATCCTGAGGTGCAGTTCTCTTGGTTGTG GACGATGTGGAGTGCACACCGCCAGACACAGCCAAAGGAGGAGCAGTTCAAT AGCACCTTTCGGTCCGTGAGCGAGCTGCCCATCATGTCATCAGGATTGGCTGAAT GGCAAGGAGTTCAAGTGCAGAGTGAACCTCTGCCGCTTTTCCCGCTCCTATCGAG AAGACCATCTCCAAGACAAGGGCCGCCAAAGGCTCCACAGGTGTACACCATC CCACCTCCAAGGAGCAGATGGCTAAGGACAAGGTGTCTCTGACCTGTATGATC ACAGACTTCTTTCCTGAGGACATCACAGTGGAGTGGCAGTGGAAACGGCCAGCCT GCCGAGAACTATAAGAAATACCCAGCCAATCATGGACACAGATGGCTCTTACTTC GTGTATTCCAAGCTGAACGTGCAGAAGTCCAAATTGGGAGGCTGGCAACACCTTT ACATGTAGCGTGCTGCACGAAGGTCTGCATAACCATCATACCGAAAAATCACTG TCACACTCCCCCTGGAAA		

160	Human IL-2 C125S DNA sequence	GCTCCTACATCCTCCAGCACCAAGAAACCCAGCTGCAGTTGGAGCATCTGCTG CTGGACCTGCAGATGATCCTGAACGGCATCAACAACCTACAAGAACCCCAAGCTG ACCCGGATGCTGACCTTCAAGTTCTACATGCCCAAGAAAGGCCACCGAGCTGAAA CATCTGCAGTGCCTGGAAGAGGAACTGAAGCCCTCTGGAAGAAAGTCTGAATCTG GCCAGTCCAAAGAACTTCCACCTGAGGCCCTCGGACCTGATCTCCAACATCAAC GTGATCGTGTCTGAGCTGAAGGGCTCCGAGACAACCTTCATGTGCGAGTACGCC GACGAGACAGCTACCATCGTGGAAATTTCTGAACCGGTGGATCACCTTCAGCCAG TCCATCATCAGCACCCCTGACA			
161	Human IgG1 Fc domain DNA sequence	GACAAGACCCACACCTGTCTCCATGTCTGCTCCAGAAATTGCTCGGCGGACCC TCCGTGTTCCCTGTTTCTCCAAAGCCTAAGGACACCCCTGATGATCTCTCGGACC CCTGAAGTGACCTGCGTGGTGCATGTGTCTCACGAGGATCCCGAAGTGAAG TTCAAATTGGTACGTGGACGGCGTGGAAGTGCAACAACGCCAAGACCAAGCCTAGA GAGGAACAGTACAACCTCCACCTACAGAGTGGTGTCGTGCTGACCGTGTGCAC CAGGATTGGCTGAATGGCAAGAGTACAAGTGCAAGGTGTCCAAACAAGGCCCTG CCTGCTCCTATCGAAAAGACCATCTCCAAGGCCAAGGCCAGCCTAGGGAACCC CAGGTTTACACCTTGCCCTCCATCTCGGGACGAGCTGACCAAGAACCAGGTGTCC CTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAG TCTAATGGCCAGCCTGAAAACAAATTACAAGACAACCCCTCCTGTGTGGACTCC GACGGCTCATTTCTTCTGTACAGCAAGCTGACAGTGGACAAGTCCAGATGGCAG CAGGGCAACGTGTTCTCCTGCTCCGTGATGCATGAGGCCCTGCACAACCACTAC ACCCAGAAGTCCCTGTCTGTCTCCCTGGC			
162	Human IL-2 DNA sequence	GCACCTACTTCAAGTTCTACAAAGAAACACAGCTACAACCTGGAGCATTTACTT CTGGATTTACAGATGATTTTGAATGGAAATTAATAATTACAAGAAATCCCAACTC ACCAGGATGCTCACATTTAAGTTTACATGCCCAAGAAAGGCCACAGAACTGAAA CATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTCTAAATTTA GCTCAAAGCAAAAACCTTTCACCTTAAGACCCAGGGACTTAATCAGCAATATCAAC GTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCT GATGAGACAGCAACCATTTGTAGAAATTTCTGAACAGATGGATTACCTTTTGTCAA AGCATCATCTCAACACTGACT			

163	Human IgG1 K392D K409D Fc domain DNA sequence	GACAAAACTCACACATGCCACCGTGCCAGCACCTGAACCTCCTGGGGGACCG TCAGTCTTCCTCTTCCCCCAAAACCCAAAGGACACCCCTCATGATCTCCCGGACC CCTGAGGTCACATGCGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAG TTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGG GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAAGCTCCTCACCGTCTCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGCTCTCCAACAAGCCCTC CCAGCCCCCATCGAGAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTCAGC CTGACCTGCCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGAG AGCAATGGGCAGCCGGAGAACTACGACACACGCCCTCCCGTGGACTCC GACGGCTCCTTCTCTCTATAGCGACCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGTCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAAGAGCCTCTCCCTGTCTCCGGGT			
164	Human IL- 2Raplha DNA Sequence	GAGCTCTGTGACGATGACCCGCCAGAGATCCACACGCCACATTCAAAGCCATG GCCTACAAGGAAGAACCATGTTGAACGTGAATGCAAGAGAGGTTTCCGCAGA ATAAAAAGCGGTCACCTCTATATGCTCTGTACAGGAACTCTAGCCACTCGTCC TGGGACAACCAATGTCAATGCACAAGCTCTGCCACTCGGAACACAAACGAACAA GTGACACCTCAACCTGAAGAACAGAAAGAAAGGAAACACAGAAATGCAAAGT CCAAATGCAGCCAGTGGACCAAGCGAGCCTTCCAGGTCACTGCAGGGAACCTCCA CCATGGGAAAATGAAGCCACAGAGAGAAATTTATCATTTCTGTGTGGGCAGATG GTTTATTATCAGTGCGTCCAGGGATACAGGGCTCTACACAGAGGTCTCTGTGAG AGCGTCTGCAAAATGACCCACGGGAAGACAAGGTGGACCCAGCCCCAGCTCATA TGCACAGGTGAAATGGAGACCAGTCAGTTTCCAGGTGAAGAGAAGCCTCAGGCA AGCCCCGAAGGCCGTCCTGAGAGTGAGACTTCTGCTGCTCGTCACAACAACAGAT TTTCAAAATACAGACAGAAATGGCTGCAACCAATGGAGACGTCCATATTTACAACA GAGTACCAG			
165	Gly-Ser Linker DNA sequence	TCTGGTGGCGGTGGCTCTGGTGGCGGTGGC			
166	Human MMP Cleavage Site DNA sequence	GGTCCTCTGGGTGTCAGAGGT			

167-179	Not Used								
180-700	See Table 2								
Additional Protease-cleavable sequences									
SEQ ID NO	Cleavable by	Sequence							
701	MMP7	KRALGLPG							
702	MMP7	(DE) ₈ RPLALWRS(DR) ₈							
703	MMP9	PR(S/T)(L/I)(S/T)							
704	MMP9	LEATA							
705	MMP11	GGAANLVRGG							
706	MMP14	SGRIGFLRTA							
707	MMP	PLGLAG							
708	MMP	PLGLAX							
709	MMP	PLGC(me)AG							
710	MMP	ESPAYYTA							
711	MMP	RLQLKL							
712	MMP	RLQLKAC							
713	MMP, MMP9, MMP14	EP(Cit)G(Hof)YL							
714	Urokinase plasminogen activator (uPA)	SGRSA							
715	Urokinase plasminogen activator (uPA)	DAFK							

716	Urokinase plasminogen activator (uPA)	GGRR				
717	Lysomal Enzyme	GFLG				
718	Lysomal Enzyme	ALAL				
719	Lysomal Enzyme	FK				
720	Cathepsin B	NLL				
721	Cathepsin D	PIC(Et)FF				
722	Cathepsin K	GGPRGLPG				
723	Prostate Specific Antigen	HSSKLQ				
724	Prostate Specific Antigen	HSSKLQL				
725	Prostate Specific Antigen	HSSKLQEDA				
726	Herpes Simplex Virus Protease	LVLASSSFGY				
727	HIV Protease	GVSQNYPIVG				
728	CMV Protease	GVVQASCRLA				
729	Thrombin	F(Pip)RS				
730	Thrombin	DPRSFL				
731	Thrombin	PPRSFL				
732	Caspase-3	DEVDP				
733	Caspase-3	DEVDP				

734	Caspase-3	KGSGDVEG				
735	Interleukin 1 β converting enzyme	GWEHDG				
736	Enterokinase	EDDDDKA				
737	FAP	KQEQLPGST				
738	Kallikrein 2	GKAFRR				
739	Plasmin	DAFK				
740	Plasmin	DVLK				
741	Plasmin	DAFK				
742	TOP	ALLLALL				
743-799	Not Used					
Additional fusion polypeptides						
SEQ ID NO	Description	Sequence	Species	Function	Notes	

800	TBM01	MVSKGEELFTGVVPILVELDGDVNGHKFVS GEGEGDATYGKLT LKFICT TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF FKDDGNYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNNSHN VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH YLS TQSKLSKDPNEKRDHMLLEFVTAAGITLGMDEL YKGGGSAKAQK AQAKQWKQAQKAQAKAQAKQW SGGGGGGGGLGVRGGGGGGG GGSMVSKGEELIKENMHMKLYMEGT VNNHHFKCTSEGEKPYEGTQTMRI KWVEGGPLPFAFDILATSFMYGSR TFINHTQGIPDFFKQSFPEGFTWERV TTYEDGGVL TATQDTS LQDGLIYNVKIRGVNFP SNGPVMQKKT LGWEAN TEMLYPADGGLEGRSDMAKL VGGGHLICNFKTTT YRSKPAKNLKM PGVY YVDHRLERIKEADKET YVEQHEVAVARYCDLPSKLGHK LNSGGGGGCKP CIC TVPEVSSVFI FPPKPKDVL TITLTPKVTCVVVDISKDDPEVQFSWFV DDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNSAAF APIEKTISKTKGRPKAPQVYTIPPPKEQMAKD KVS L TCMITDFFPEDITV EWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLH EGLHNHHTEKSLSHSPGK	fusion protein	tool
801	TBM02	MVSKGEELFTGVVPILVELDGDVNGHKFVS GEGEGDATYGKLT LKFICT TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF FKDDGNYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNNSHN VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH YLS TQSKLSKDPNEKRDHMLLEFVTAAGITLGMDEL YKSGGGGGGGG PLGVRGGGGGGGSMVSKGEELIKENMHMKLYMEGT VNNHHFKCTSEG EGKPYEGTQTMRIKVVEGGPLPFAFDILATSFMYGSR TFINHTQGIPDFF KQSFPEGFTWERVTTYEDGGVL TATQDTS LQDGLIYNVKIRGVNFP SNG PVMQKKT LGWEANTEMLYPADGGLEGRSDMAKL VGGGHLICNFKTT YRS KKPAKNLKM PGVYVDHRLERIKEADKET YVEQHEVAVARYCDLPSKLGH KLNSGGGGGCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNG KEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKD KVS L T CMITDFFPEDITV EWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSN WEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	fusion protein	tool

802	TBM05	MVSKGEELFTGVVPILVELDGDVNGHKFVS GEGEGDATYGKLT LKFICT TGKLPVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF FKDDGNYKTRAEVKFE GDTLVNRIELKGIDFKEDGNILGHKLEYNNSHN VYIMADKQKNGIKVNF KIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH YLS TQSKLSKDPNEKRDHMLLEFVTAAGITLGMDELYKSGGGSGGGGG PLGVRGGGGSGGGSMVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEG EGKPYEGTQIMRIKVV EGGPLPFAFDILATS FMYGSRTFINHTQGIPDFF KQSFPEGFTWERVTT YEDGGVLTATQDTS LQDGLIYNVKIRGVNFP SNG PVMQKKT LGWEANTEMLYPADGGLEGRSDMALKVGGGHLICNFKTTYRS KKPAKNLKM PGVYYVDHRLERIKEADKET YVEQHEVAVAR YCDLPSKLGH KLN GSGGGGCKPCICTVPEVSSVFI FPPKPDVLTITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNG KEFKCRVNSA AFPAPIEKTISKTKRPAQVYTI PPPKEQMAKDKVSLT CMTITDFFPEDITV EWWQNGQPAENYKNTQIMDTDGSYFVYSKLNQKSN WEAGNTFTCSVLHEGLHNHHT EKSLSHSPGKGGGSASKAQAQAKQWKQ AQAQAQAQAQAQAQAQW		fusion protein	tool
803	Construct F	APTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTFKFYMPKKATELKH LQCLEELKPLEEVLNLAQSKNFHLR PRDLISNINVI VLELKGSETTFMCEYADETAT IVEFLNRWITFSQSIIST LTSGGGGSGGGG PLGVRGGGGSGGGSELCLYDPPEVPNATFKALSYK NGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSSATRN TTQVTPQ PEEQKERKTT EMQSPMQPVDQASLP GHCREPPWENEATERIYHFVVGQM VYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEE KPQASPEG RPESETSCLVTTTDFIQIQT EMAATMETSIFTTEYQDKTHTCP PCPAPELLGGSVFLFPPKPKD TLMISRTPETCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG		fusion protein	
804	Construct G	APTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTFKFYMPKKATELKH LQCLEELKPLEEVLNLAQSKNFHLR PRDLISNINVI VLELKGSETTFMCEYADETAT IVEFLNRWITFSQSIIST LTSGGGGSGGGG PLGVRGGGGSGGGSELCDDDPPEIPHATFKAMAYK EGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSDNQCCQCTSSATRNTTK QVTPQPEEQKERKTT EMQSPMQPVDQASLP GHCREPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEGRPESETSCLVTTTDFIQIQT EMAATMETSIFTTEYQGS		fusion protein	

805	Construct H	GGGDKTHTCPPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVQLHQLDNLG KEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG			
		APTSSTKKTQLLEHL LLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVNLAAQSKNFHLRPRDLISNINVI VLELKGSE TTFMCEYADETATIVFELNRWITFSQSIISTLTSGGGSGGGGGLGVRG GGGSGGGSELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIKSG SLYMLCTGNSSSHSDNQCCCTSSATRNTTKQVTPQPEEQKERKTTEMQS PMQPVDAQSLPGHCREPPWENEATERIYHFVVGQMVYQCVQGYRALHR GPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEKPGSGGGDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVSVLTVQLHQLDNLGKEYCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPG	fusion protein		
806	Construct V	APTSSTKKTQLLEHL LLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVNLAAQSKNFHLRPRDLISNINVI VLELKGSE TTFMCEYADETATIVFELNRWITFSQSIISTLTSGGGSGGGGGLGVRG GGGSGGGSGGGGSELCDDDPPEIPHATFKAMAYKEGTM LNCEC KRGFRRIKSGSLYMLCTGNSSSHSDNQCCCTSSATRNTTKQVTPQPEEQ KERKTTEMQSPMQVDQASLPGHCREPPWENEATERIYHFVVGQMVYQ CVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEKPKQA SPEGRPESETSCLVTTTDFIQIQTEMAATMETSIFTTEYQSGGGGDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVQLHQLDNLGKEYCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG	fusion protein		
807	Construct W	APTSSTKKTQLLEHL LLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVNLAAQSKNFHLRPRDLISNINVI VLELKGSE TTFMCEYADETATIVFELNRWITFSQSIISTLTSGGGSGGGGGLGVRG GGGSGGGSGGGGSGGGGSELCDDDPPEIPHATFKAMAY KEGTM LNCECKRGFRRIKSGSLYMLCTGNSSSHSDNQCCCTSSATRNTT KQVTPQPEEQKERKTTEMQSPMQVDQASLPGHCREPPWENEATERIYH	fusion protein		

808	Construct X	FVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETS QFPGEKPKQASPEGRPESETSCLVTTTDFIQIQTEMAATMETSIFTTEYQG SGGGDKTHTCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQNSTYRVSVLTVLHQDWLN GKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG APTSSTSSSTAQAQQQQQQQQHLEQLLMDLQELLSRMENYRNLKLPRML TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFSQSIISTSPQSGGGSGGGGPA ALIGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTDFPQTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVWVDI SKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTI PPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQIMD TDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTKSLSHSPGK		fusion protein	
809	Construct Y	APTSSTSSSTAQAQQQQQQQQHLEQLLMDLQELLSRMENYRNLKL PRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENF ISNIRVTVKLKGSDNTFECQFDDDESATVDFLRRWIAFSQSIISTSPQV RIQRKKEKMKETGPLVRRGGGGGGSELCLYDPPEVPNATFKALSYK NGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQ LEHQKEQQTITDMQKPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQ SVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHRFLAS EESQGSRNSSPESETSCPIITTTDFPQTETTAMTETFVL TMEYKIEGRMD GCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVWVDISKDDPEVQF SWFVDDVEVHTAQTQPREEQFNSTFRSVSEL PIMHQDWLNGKEFKCRVNS AAFPAPIEKTISKTKGRPKAPQVYTI PPKEQMAKDVS LTCMITDFFPE DITVEWQWNGQPAENYKNTQIMD TDGSYFVYSKLVNQKSNWEAGNTFTC SVLHEGLHNHHTKSLSHSPGK		fusion protein	

810	Construct Z	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGPL GLARGGGGGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
811	Construct AA	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGFHRRIKAGPL GVRGGGGSGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
812	Construct BB	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGFHRRIKAGVR LGPGGGGSGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	

813	Construct CC	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQGGHHPHHPGPL GVRGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFFPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLNHHTEKSLSHSPGK	fusion protein	
814	Construct DD	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQGGHHPHHPGVR LGPGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFFPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLNHHTEKSLSHSPGK	fusion protein	
815	Construct EE	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGGSHWGPLG VRGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELV YMRC LGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQEN LTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGK TGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETTA MTETFVL TMEYKGCKPCICTVPEVSSVFIFFPKPKDVL TITLTPKVTCVVVDIS KDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFKC RVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFPE DITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLH EGLNHHTEKSLSHSPGK	fusion protein	

816	Construct FF	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGGSHWGVR GPGGGGGGGGSELCLYDPPPEVPNATFKALSYKNGTIINCECKRGFRRLKELV YMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQEN LTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGK TGWTQPQLTCVDEREHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETTA MTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKPKDVLITLTPKVTCVVVDIS KDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFKC RVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFPE DITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLH EGLHNHHTEKSLSHSPGK	fusion protein	
817	Construct GG	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGKLWVLPKGPL GVRGGGGGGGSELCLYDPPPEVPNATFKALSYKNGTIINCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKPKDVLITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
818	Construct HH	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGKLWVLPKGVR LGPGGGGGGGGSELCLYDPPPEVPNATFKALSYKNGTIINCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETT AMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKPKDVLITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	

819	Construct II	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLLKGSNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSLHERHLNNGPL GVRGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
820	Construct JJ	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLLKGSNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSLHERHLNNGVR LGPGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKEL VYMRLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
821	Construct KK	APTSSSTSSSTAEQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLLKGSNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQVRIQRKKEKMKET GVRLLGPGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRL KELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSM HQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKM KCGKTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPT ETTAMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVV VDISKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGK EFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITD FFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTC SVLHEGLHNHHTEKSLSHSPGK	fusion protein	

822	Construct LL	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGPL GVRGFHRRIKAGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
823	Construct MM	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGVR LGPGFHRRIKAGGSELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
824	Construct NN	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGPL GVRGGHHPHGHPHELCLYDPPEVPNATFKALSYKNGTIILNCECKRGFRRLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEEQSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFIFPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFP EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	

825	Construct OO	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGVR LPGGGHHPHGHHELCLYDPPPEVPNATFKALSYKNGTILNCECKRGFRRLLKEL VYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQE NLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCG KTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPTETT AMTETFVL TMEYKGCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVVVDI SKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFPE EDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVL HEGLHNHHTEKSLSHSPGK	fusion protein	
826	Construct PP	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGPL GVRGGWSHWGGSELCLYDPPPEVPNATFKALSYKNGTILNCECKRGFRRLLKELV YMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQEN LTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGK TGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPTETTA MTETFVL TMEYKGCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVVVDIS KDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFKC RVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFPE DITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLH EGLHNHHTEKSLSHSPGK	fusion protein	
827	Construct QQ	APTSSSTSSSTAEAAQQQQQQQQHLEQLLMDLQELLSRMENYRNKLKLPRLM TFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTV VKLKGSDNTFECQFDDDESATVDFLRRWIAFCQSIISTSPQSGGGSGGGGVR LPGGGWSHWGGSELCLYDPPPEVPNATFKALSYKNGTILNCECKRGFRRLLKELV YMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTITDMQKPTQSMHQEN LTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGK TGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTIDFPQPTETTA MTETFVL TMEYKGCKPCICTVPEVSSVFI FPPKPKDVL TITLTPKVTCVVVDIS KDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSEL PIMHQDWLNGKEFKC RVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDVS LTCMITDFFPE DITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFTCSVLH EGLHNHHTEKSLSHSPGK	fusion protein	

828	Construct RR	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGKLWVLPKGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTDFFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	fusion protein	
829	Construct SS	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGKLWVLPKGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTDFFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	fusion protein	
830	Construct TT	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGLHERHLNNGELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTDFFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	fusion protein	

831	Construct UU	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAQQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQSGGGGGGVRLLGPGHLHERHLNNGELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPKPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK	fusion protein	
832	Construct VV	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAQQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQSGGGGGHHPGLGVRGGGGGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPKPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK	fusion protein	
833	Construct WW	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAQQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVDFLRRWIAFCQSI ISTSPQGGHHPHSGGGGVLGVRGGGGGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPKPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK	fusion protein	

834	Construct XX	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGGHHPHGGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	fusion protein	
835	Construct YY	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGGGGGGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGKGHHHPH	fusion protein	
836	Construct ZZ	MGWSCIILFLVATATGVHSAPTSSSTSSSTAEAAQQQQQQQQHLEQLLMDL QELLSRMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQS KSFQLEDAENFISNIRVTVKLGSDNTFECQFDDDESATVVDFLRRWIAFCQSI ISTSPQSGGGGGGGLGVRGGGGGGGSELCLYDPPEVPNATFKALSYK NGTIILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQ KEQQTITTDMPQPTQSMHQENLTGHCREPWPWKHEDSKRIYHFVEGQSVHYECIP GYKALQRGPAISICKMKCGKTGTQPQLTCVDEREHRFLASEESQGSRNSSPE SETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKP KDVLTITLTPKVTVCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFR SVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPK EQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGKGHHHPH	fusion protein	

837	Construct I	APTSSSTKKTQLQLEHLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T I L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G Q M V Y Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T D F I Q I Q T E M A A T M E T S I F T T E Y Q D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H H		fusion protein	
838	Construct J	APTSSSTKKTQLQLEHLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S V Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G Q M V Y Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T D F I Q I Q T E M A A T M E T S I F T T E Y Q D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H H		fusion protein	
839	Construct K	APTSSSTKKTQLQLEHLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C L Y D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G Q M V Y Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T D F I Q I Q T E M A A T M E T S I F T T E Y Q D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H H		fusion protein	

840	Construct L	APTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K E L V Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q P E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G M V Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C L V T T I D F Q I Q T E M A A T M E T S I F T T E Y Q D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G H H H H H	fusion protein	
841	Construct M	APTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q P E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G M V Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G	fusion protein	
842	Construct N	APTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F H L R P R D L I S N I N V I V L E L K G S E T T F M C E Y A D E T A T I V E F L N R W I T F S Q S I I S T L T S G G G S G G G G P L G V R G G G S G G G S E L C D D D P P E I P H A T F K A M A Y K E G T M L N C E C K R G F R R I K S G S L Y M L C T G N S S H S W D N Q C Q C T S S A T R N T T K Q V T P Q P E E Q K E R K T T E M Q S P M Q P V D Q A S L P G H C R E P P P W E N E A T E R I Y H F V G M V Y Q C V Q G Y R A L H R G P A E S V C K M T H G K T R W T Q P Q L I C T G E M E T S Q F P G E E K P Q A S P E G R P E S E T S C G S G G G D K T H T C P P C A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G	fusion protein	

843	Construct O	APTSSSTKKTQLQLEHLLLDLQMIILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEEELKPLEEVNLQAQSKNFHLRPRDLISNINIVLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLTSGGGSGGGGGLGVRGGGGSGGGSEL CDDDPPEIPHATFKAMAYKEGTILNCECKRGFRRIKSGSLYMLCTGNSSHSSWD NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPPW ENEATERIYHFVGMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICT GEMETSQFPGEEKPQASPEG RPESETSCSGGGGDKTHTCPPCPAPELLGGPSV FLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG	fusion protein	
844	Construct P	APTSSSTKKTQLQLEHLLLDLQMIILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEEELKPLEEVNLQAQSKNFHLRPRDLISNINIVLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLTSGGGSGGGGGLGVRGGGGSGGGSEL CDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSVYMLCTGNSSHSSWD NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPPW ENEATERIYHFVGMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICT GEMETSQFPGEEKPQASPEG RPESETSCSGGGGDKTHTCPPCPAPELLGGPSV FLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG	fusion protein	
845	Construct Q	APTSSSTKKTQLQLEHLLLDLQMIILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEEELKPLEEVNLQAQSKNFHLRPRDLISNINIVLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLTSGGGSGGGGGLGVRGGGGSGGGSEL CLYDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWD NQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPPW ENEATERIYHFVGMVYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICT GEMETSQFPGEEKPQASPEG RPESETSCSGGGGDKTHTCPPCPAPELLGGPSV FLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG	fusion protein	

846	Construct AAA	APTSSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLLQCLEELKPLEEVLNLAQSKNFHLR PRDLISNINIVILELKGSETTFMCEYADETATVEFLNRWITFSQSIIST LTSGGSGGGGGLGVRGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTIILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFIQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPPCPAPEAAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG		fusion protein	
847	Construct BBB	APTSSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLLQCLEELKPLEEVLNLAQSKNFHLR PRDLISNINIVILELKGSETTFMCEYADETATVEFLNRWITFSQSIIST LTSGGSGGGGVRLLGPGGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTIILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFIQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPPCPAPEAAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG		fusion protein	
848	Construct CCC	APTSSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLLQCLEELKPLEEVLNLAQSKNFHLR PRDLISNINIVILELKGSETTFMCEYADETATVEFLNRWITFSQSIIST LTGHHPHGHHPHGVRLGPGGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTIILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFIQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPPCPAPEAAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR		fusion protein	

849	Construct DDD	WQQGNVFSCVMHEALHNHYTQKSLSLSPG APTSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLR PRDLISININVIVLELKGSETTFMCEYADETATIVFELNRWITFSQSIIST LTGHHPHGHHPGLGVRGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPCPAPEAAGPSVFLFPPKPKDTLMISRTPETCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGYFSPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCVMHEALHNHYTQKSLSLSPG		fusion protein	
850	Construct EEE	APTSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLR PRDLISININVIVLELKGSETTFMCEYADETATIVFELNRWITFSQSIIST LTVRIQRKKEKMKETGPLGVRGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPCPAPEAAGPSVFLFPPKPKDTLMISRTPETCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGYFSPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCVMHEALHNHYTQKSLSLSPG		fusion protein	
851	Construct FFF	APTSSTKKTQLQLEHLLLDLQMIILNGINNY KNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVLNLAQSKNFHLR PRDLISININVIVLELKGSETTFMCEYADETATIVFELNRWITFSQSIIST LTVRIQRKKEKMKETGPLGVRGGGGGGGSELCDDDPPEIPHATFKAMAYK EGTILNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCCCTSSATRNTTK QVTPQPEEQKERKTTEMQSPMPVDQASLPGHCREPPWENEATERIYHF VVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQ FPGEEKPQASPEG RPESETSCLVTTTDFQIQTEMAATMETSIFTTEYQGS GGGDKTHTCPCPAPEAAGPSVFLFPPKPKDTLMISRTPETCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG		fusion protein	

		KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG			
852	Construct GGG	APTSSSTSAEAQQQQQQQQHLEQLMDLQELLSRMENYRNCLKPRMLTFKFYLPKQATELKDLCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVKLKGSDNTFECQFDDDESATVDFLRRWIAFSQSIISTSPQSGGGSGGGVRLGPGGGGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCLGNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQTTTDMQKPTQSMHQENLTGHCREPPPWKHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHRFLASEESQGSRNSSPESETSCPIITTTDFPQPTETTAMTETFVLTMEYKGCKPCICTVPEVSSVFIFFPKPKDVLITITLTPKVTCVVVDISKDDPEVQFSWFVDDDEVHTAQTPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK		fusion protein	

Table 2. Table of Targeting Sequences

SEQ ID NO	Sequence	Binds to	Note 1	Note 2
180	(TLTYTWS)n	denatured collagen IV	binding to MMP degraded collagen	
181	(CREKA)n	denatured collagen IV	binding to MMP degraded collagen	inhibit tumor vasculature formation
182	(GXY)n	denatured Collagen	Gly = Glycine / X = Proline or modified Proline / Y = Proline or modified Proline	This peptide binds to collagen preteolytically digested by MMP
183	GHCVTDSGVVYSVGMQWLKTQGNKQMLCTCLGNGVSCQET	denatured Collagen	from Fibronectin Domain 1-6	
184	EICTTNEGVMYRIGDQWDKQHDMGHMMRCTCVGNRGWETCIAY	denatured Collagen	from Fibronectin Domain 1-7	
185	DQCIVDDITYNVNDTFHKRHEEGHMLNCTCFGQGRGRWKCDPV	denatured Collagen	from Fibronectin Domain 1-8	
186	DQCQDSETGTIFYQIGDSWEKYVHGVRYQCICYGRGIGEWHCQPL	denatured Collagen	from Fibronectin Domain 1-9	
187	SNGEPCVLPFTYNGRTFYSCCTTEGRQDGHLCWSTTSNYEQDQKYSFCTD	denatured Collagen	from Fibronectin Domain 2-1	
188	SNGALCHFPPFLYNNHNYTDCTSEGRDRDNMKWCGTTQNYDADQKFGFCPM	denatured Collagen	from Fibronectin Domain 2-2	
189	RRANAALKAGELYKSILYGC	Collagen type I	Kd 0.86uM // 860nM	Differential binding affinity to Collagen
190	RRANAALKAGELYKCILYGC	Collagen type I	Kd: 10 nM (tight binding)	Differential binding affinity to Collagen

191	MIVIELGTNPLKSSGIEN GAFQGMKK	Collagen type I	Kd 0.394 uM // 394nM	Differential binding affinity to Collagen
192	LRELHLNNN	Collagen type I	Kd 0.17uM //170nM	Differential binding affinity to Collagen
193	WREPSFCALS	Collagen type I	Kd 100 uM // 100,000nM	Differential binding affinity to Collagen
194	TKKTLRT	Collagen type I	Kd ≤100uM	Differential binding affinity to Collagen
195	CPKESCNLFVLKD	Collagen type I	Kd 0.681uM //681nM	Differential binding affinity to Collagen
196	WREPSFCALS	Collagen type I	Kd : 100uM // 100,000nM	Differential binding affinity to Collagen
197	HVWMQAPGGGK	Collagen type I	Kd 61uM // 61,000 nM	H-V-F/W-Q/ M-Q-P/A-P/K motif
198	HVWMQAPGGGC	Collagen type I		
199	WYRGRL	Collagen type II		
200	KLWVLPK	Collagen type IV		
201	RRANAALKAGELYKSIL Y	Collagen		
202	GELYKSILY	Collagen		
203	RRANAALKAGELYKCIL Y	Collagen		
204	GELYKCILY	Collagen		
205	RLDGNEIKR	Collagen		
206	AHEEISTTNEGVM	Collagen		
207	NGVFKYRPRYFLYKHAY FYPPLKRFPVQ	Collagen		
208	CQDSETRTFY	Collagen		
209	TKKTLRT	Collagen		
210	GLRSKSKKFRRPDIQYPD ATDEDITSHM	Collagen		

211	SQNPVQP	Collagen		
212	SYIRIADTNIT	Collagen		
213	KELNLVYT	Collagen		
214	GSIT	Collagen		
215	GSITTIDVPWNV	Collagen		
216	GQLYKSILY	Collagen		
217	RRANAALKAGQLYKSIL Y	Collagen		
218	WREPSFCALS	Collagen		
219	WHCTTKFPHHYCLY	Collagen		
220	AHKCPWHLYTTHYCFT	Collagen		
221	PAHKCPWHLYTHYCFT	Collagen		
222	GROGER	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
223	GMOGER	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
224	GLOGEN	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
225	GLOGER	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
226	GLKGEN	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
227	GFOGERGVGPOGPA	Collagen		O is 4-hydroxyproline (see, Raynal, N., et al., J. Biol. Chem., 2006, 281(7), 3821-3831)
228	WREPSFCALS	Collagen		Takagi, J., et al, Biochemistry, 1992, 31, 8530-8534
229	WYRGRL	Collagen		Rothenfluh D.A., et al, Nat Mater. 2008, 7(3), 748-54
230	WTCSGDEYTWHC	Collagen		
231	WTCVGDHKTWKC	Collagen		
232	QWHCTTRFPHHYCLYG	Collagen		U.S. 2007/0293656)

233	STWTWNGSAWTWNEG GK	Collagen			
234	STWTWNGTNWTRNDGG K	Collagen			WO/2014/059530
235	CVWLWEQC	Collagen			
236	CMTSPWRC	Collagen			Vanhoorelbeke, K., et al, J. Biol. Chem., 2003, 278, 37815-37821
237	CPGRVMHGLHLGDDEG PC	Collagen			Muzzard, J., et al, PLoS one. 4 (e5585) I- 10)
238	KLWLLPK	Collagen			Chan, J. M., et al, Proc Natl Acad Sci U.S.A., 2010, 107, 2213-2218)
239	CQDSETRTFY	Collagen			U.S. 2013/0243700
240	LSELRLEN	Collagen			Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10984
241	LTELHLDNN	Collagen			Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10985
242	LSELRHNN	Collagen			Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10986
243	LSELRHAN	Collagen			Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10987
244	LRELHLNHN	Collagen			Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10988
245	RVMHGLHLGDDE	Collagen			
246	RVMHGLHLGNNQ	Collagen			
747	RVMHGLHLGNNQ	Collagen			
748	GQLYKSILYGSG-4K2K	Collagen			(a 4-branch peptide) which can be conjugated to a fusion polypeptide
749	GSGQLYKSILY	Collagen			

250	GSGGQLYKSILY	Collagen		
251	KQLNLVYT	Collagen		
252	CVWLWQQC	Collagen		
253	WREPSFSALS	Collagen		
254	GHRPLDKKREEAPSLRP APPISGGGYR	Collagen		
255	GHRPLNKKRQQ APSLRPAPPISGGGYR	Collagen		
256	GELYKSILYGSG	Collagen		
257	GQLYKSILYGSG	Collagen		
258	RYPISRPRKRGSG	Collagen		
259	GELYKSILYGC	Collagen		
260	RLDGNEIKRGC	Collagen		
261	AHEEISTTNEGVMGC	Collagen		
262	GCGGELYKSILY	Collagen		
263	NGVFKYRPRYFLYKHAY FYPPLKRFPVQGC	Collagen		
264	CQDSETRTFYGC	Collagen		
265	TKKTLRTGC	Collagen		
266	GLRSKSKFRRPDIQYPD ATDEDITSHMGC	Collagen		
267	SQNPVQPGC	Collagen		
268	SYIRIADTNITGC	Collagen		
269	KELNLVYTGC	Collagen		
270	GSITTIDVPWNVGC	Collagen		
271	GCGGELYKSILYGC	Collagen		
272	RRANAALKAGELYKSIL YGSG	Collagen		
273	cyclic CVWLWENC	Collagen	cyclic peptides can be conjugated to a fusion polypeptide	

274	cyclic CVWLWEQC	Collagen	cyclic peptides can be conjugated to a fusion polypeptide	Depraetere H., et al, Blood. 1998, 92, 4207-421 1; and Duncan R., Nat Rev Drug Discov, 2003, 2(5), 347-360
275	D-amino acid EDDGLHLGHMVR	Collagen	D-amino acid-containing peptides can be conjugated to ODC	
276	D-amino acid QNNGLHLGHMVR	Collagen	D-amino acid-containing peptides can be conjugated to ODC	
277	PPTDLRFTNIGPDTMRVT WAPPPSIDLTNFLVRYSP VKNEEDVAELSISPSDNA VVLTNLLPGTEYVVSVS SVYEQHESTPLRGRQKT GLDSP	integrin	from Fibronectin Domain III-9	
278	TGIDFSDITANSFTVHWI APRATITGYRIRHHPEHF SGRPREDRVPHSRNSITL TNLTPGTEYVVSIVALN GRESPLLLIGQQSTVSD	integrin	from Fibronectin Domain III-10	
279	PGCYDNGKHYQINQQW ERTYLGNALVCTCYGGS RGNCESK	integrin	from Fibronectin Domain 1-1	
280	ETCFDKYTGNTYRVGDT YERPKDSMIWDCTCIGA GRGRISCTIA	integrin	from Fibronectin Domain 1-2	
281	NRCHEGGQSYKIGDTWR RPHETGGYMLECVCLGN GKGWETCKPI	integrin	from Fibronectin Domain 1-3	
282	EKCFDHAAGTSYVVGET WEKPYQGWMVMDCTC LGEGRITCTSR	integrin	from Fibronectin Domain 1-4	

283	NRCNDQDTRTSYRIGDT WSKKDNRGNLLQCICITG NGRGEWK CERH	integrin	from Fibronectin Domain 1-5	
284	GHCVTDSGVVYSVGMQ WLKTQGNKQMLCTCLG NGVSCQET	denatured integrin	from Fibronectin Domain 1-6	uplicated in collagen
285	EICTTNEGVMYRIGDQW DKQHDMGHMMRCTCV GNRGEWTCIAY	denatured integrin	from Fibronectin Domain 1-7	uplicated in collagen
286	DQCIVDDITYNVNDTFH KRHEEGHMLNCTCFGQ GRGRWKCDPV	denatured integrin	from Fibronectin Domain 1-8	uplicated in collagen
287	DQCQDSETGTFYQIGDS WEKYVHGVRYQCYCYG RGIGEWHCQPL	denatured integrin	from Fibronectin Domain 1-9	uplicated in collagen
288	APTDLKFTQVTP TSLSAQ WTPPNVQLTGYRVRVTP KEKTGPMKEINLAPDSSS VVVSGLMVATKYEVS YALKDTLT SRPAQGVVT TLENVSPP	integrin	from Fibronectin Domain III-14	
289	APTNLQFVNETDSTVLV RWTPPRAQITGYRLTVG LTRRGQPRQYNVGPSVS KYPLRNLQPA SEYTVSL VAIKGNQESPKATGVFT TLQPG	integrin	from Fibronectin Domain III-5	
290	KGHRGF	integrin	Derived from Collagen I	
291	GFPGER	integrin	Derived from Collagen I	
292	GTPGPQGIAGQRDVV	integrin	Derived from Collagen alpha1(I)	
293	EKGPD	integrin	Derived from Collagen II	
294	EKGPD P	integrin	Derived from Collagen II	
295	EKGPDPL	integrin	Derived from Collagen II	

296	TAGSCLRKFS TM	integrin	Derived from Collagen IV
297	TAIPSCPEGTVPLYS	integrin	Derived from Collagen alpha3(IV)-NC1
298	TDIPPCPHGWISLWK	integrin	Derived from Collagen IV
299	PHSRN	integrin	Derived from Fibronectin
300	RGD	integrin	Derived from Fibronectin
301	GRGDSP	integrin	Derived from Fibronectin
302	YRVRVTPKEKTGPMKE	integrin	Derived from Fibronectin
303	SPPRRARVT	integrin	Derived from Fibronectin
304	WQPPRARI	integrin	Derived from Fibronectin
305	KNNQKSEPLIGRKKT	integrin	Derived from Fibronectin
306	EILDVPST	integrin	Derived from Fibronectin
307	REDV	integrin	Derived from Fibronectin
308	RQVFQVAYIIKA	integrin	Derived from Laminin Alpha-1 chain
309	SINNTAVMQRLT	integrin	Derived from Laminin Alpha-1 chain
310	IKVAV	integrin	Derived from Laminin Alpha-1 chain
311	NRWHSIYTTRFG	integrin	Derived from Laminin Alpha-1 chain
312	TWYKIAFQRNRK	integrin	Derived from Laminin Alpha-1 chain
313	RKRLQVQLSIRT	integrin	Derived from Laminin Alpha-1 chain
314	KNRLTIELEVRT	integrin	Derived from Laminin Alpha-2 chain
315	SYWYRIEASRTG	integrin	Derived from Laminin Alpha-2 chain
316	DFGTVQLRNGFPFFSYD LG	integrin	Derived from Laminin Alpha-2 chain
317	GQLFHVAYILIKF	integrin	Derived from Laminin Alpha-3 chain
318	KNSFMALYLSKG	integrin	Derived from Laminin Alpha-3 chain
319	TLFLAHGRLVFM	integrin	Derived from Laminin Alpha-4 chain
320	GQVFHVAYVLIK ^F	integrin	Derived from Laminin Alpha-5 chain
321	GIIFFL	integrin	Derived from Laminin Alpha-5 chain
322	LALFLSNGHFVA	integrin	Derived from Laminin Alpha-5 chain
323	RYVVLP ^R	integrin	Derived from Laminin Beta-1 chain
324	PDSGR	integrin	Derived from Laminin Beta-1 chain

325	YIGSR		integrin	Derived from Laminin Beta-1 chain
326	KAFDITYVRLKF		integrin	Derived from Laminin Gamma-1 chain
327	RNIAEIIKDI		integrin	Derived from Laminin Gamma-1 chain
328	FRHRNRKGY		integrin	Derived from Vitronectin
329	KKQRFRRHRNRKGYRSQ		integrin	Derived from Vitronectin
330	FHRRIKA		integrin	Derived from Sialoprotein
331	KRSR		integrin	Derived from Sialoprotein
332	GLPGER		$\alpha 1\beta 1, \alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$ 7S
333	GFPGER		$\alpha 1\beta 1, \alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
334	GLSGER		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
335	DGEA		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
336	GPAGKDGEGAQG		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
337	GPKGAGGEPGKP		$\alpha 1\beta 1, \alpha 3\beta 1$	Derived from Collagen $\alpha 1(I)$
338	GAPGPKGARGSA		$\alpha 1\beta 1, \alpha 3\beta 1$	Derived from Collagen $\alpha 1(I)$
339	GPQGIAGQRGVVGLP		$\alpha 1\beta 1$	Derived from Collagen $\alpha 1(I)$
340	PKGQKGEKG		Poly(I)	Derived from Collagen $\alpha 1(I)$
341	GASGER		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
342	GQRGER		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(I)$
343	GMPGER		integrin	Derived from Collagen $\alpha 1(I)$
344	RGQPGVMGF		VWF	Derived from Collagen $\alpha 1(III)$
345	GKDGES		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(III)$
346	GLKGEN		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(III)$
347	GLPGEN		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(III)$
348	GLPGEA		$\alpha 2\beta 1$	Derived from Collagen $\alpha 1(III)$
349	GPPGDQQPPGIP		$\alpha 1\beta 1$	Derived from Collagen $\alpha 1(IV)$
350	GAKGRAGFPGLP		$\alpha 1\beta 1$	Derived from Collagen $\alpha 1(IV)$
351	MFKKPTPSTLKAGELR		integrin	Derived from Collagen $\alpha 1(IV)$
352	GFPGSRGDTGPP		integrin	Derived from Collagen $\alpha 1(IV)$
353	GVKGDKGNPGWPGAP		integrin	Derived from Collagen $\alpha 1(IV)$
354	FYFDLR		$\alpha 1\beta 1, \alpha 2\beta 1$	Derived from Collagen $\alpha 1(IV)$

355	MFKKPTPSTLKAGELR	integrin	Derived from Collagen alpha1(IV)
356	GFPGSRGDTGPP	integrin	Derived from Collagen alpha1(IV)
357	GVKGDKGNPGWPGAP	integrin	Derived from Collagen alpha1(IV)
358	FYFDLR	$\alpha 1\beta 1, \alpha 2\beta 1$	Derived from Collagen alpha1(IV)
359	RGQPGVPGVPGMKGD	integrin	Derived from Collagen alpha2(IV)
360	TDIPPCPHGWISLWK	integrin	Derived from Collagen alpha3(IV)-NC1
361	MNYYSNS	integrin	Derived from Collagen alpha3(IV)-NC1
362	CNYYSNSYSFWLASLNP ER	integrin	Derived from Collagen alpha3(IV)-NC1
363	ISRCQVCMKKRH	integrin	Derived from Collagen alpha3(IV)-NC1
364	TLGSCCLRFTM	integrin	Derived from Collagen alpha3(IV)-NC1
365	GRRGKT	integrin	Derived from Collagen alpha3(IV)-NC1
366	RGQPGRKGL	integrin	Derived from Collagen alpha3(IV)-NC1
367	MFRKPIPSTVKA	integrin	Derived from Collagen alpha3(IV)-NC1
368	IISRCQVCMKMMP	integrin	Derived from Collagen alpha3(IV)-NC1
369	LAGSCLPVFSTL	integrin	Derived from Collagen alpha4(IV)-NC1
370	TAGSCLRRFSTM	integrin	Derived from Collagen alpha5(IV)-NC1
371	NKRAHG	integrin	Derived from Collagen alpha5(IV)-NC2
372	WTPPRAQITGYRLTVGL TRR	$\alpha 5\beta 1$	Derived from Fibronectin III-5
373	KLDAPT	$\alpha 4\beta 1, \alpha 4\beta 7$	Derived from Fibronectin III-5
374	PHSRN	$\alpha 5\beta 1$	Derived from Fibronectin III-9
375	RGD	$\alpha 5\beta 1, \alpha v\beta 3$	Derived from Fibronectin III-10
376	RGDS	$\alpha II\beta\beta 3$	Derived from Fibronectin III-10
377	GRGDSP	$\alpha 5\beta 1$	Derived from Fibronectin III-10
378	EDGIHEL	$\alpha 4\beta 1, \alpha 9\beta 1$	Derived from Fibronectin EDA
379	PRARITGYIIKYEKPGSPP REVVPRPRPGV	integrin	Derived from Fibronectin III-14
380	IDAPS	$\alpha 4\beta 1$	Derived from Fibronectin IIICS-1
381	VVIDASTAIDAPSNL	$\alpha 4\beta 1$	Derived from Fibronectin IIICS-1

382	LDVPS	$\alpha 4\beta 1$	Derived from Fibronectin IIICS-1
383	REDV	$\alpha 4\beta 1$	Derived from Fibronectin IIICS-5
384	PHSRN-RGDSP	$\alpha 5\beta 1$	Derived from Fibronectin III-10
385	PLDREAI AKY	integrin	Derived from E-Cadherin EC1
386	HAVDI	integrin	Derived from E-Cadherin EC1, groove
387	LFSHAVSSNG	integrin	Derived from E-Cadherin EC1, groove
388	ADTPPV	integrin	Derived from E-Cadherin EC1, bulge
389	QGADTPPVGV	integrin	Derived from E-Cadherin EC1, bulge
390	PLDREAI AKY	integrin	Derived from E-Cadherin EC1
391	DQNDN	integrin	Derived from E-Cadherin EC1
392	HAVDI	integrin	Derived from E-Cadherin EC1
393	LRAHAVDING	integrin	Derived from E-Cadherin EC1
394	LRAHAVDVNG	integrin	Derived from E-Cadherin EC1
395	VITVKDINDN	integrin	Derived from E-Cadherin EC2
396	GLDRESYPY	integrin	Derived from E-Cadherin EC2
397	MKVSATDADD	integrin	Derived from E-Cadherin EC2
398	QDPELPDKNM	integrin	Derived from E-Cadherin EC2, bulge
399	LVVQAADLQG	integrin	Derived from E-Cadherin EC2, groove
400	NDDGGQFVVT	integrin	Derived from E-Cadherin EC3, bulge
401	LVVQAADLQG	integrin	Derived from E-Cadherin EC2, groove
402	TYRIWRDTAN	integrin	Derived from E-Cadherin EC4, bulge
403	YILHVAVTNY	integrin	Derived from E-Cadherin EC3, groove
404	YTALIIATDN	integrin	Derived from E-Cadherin EC4, groove
405	QDPELPDKNM	integrin	Derived from E-Cadherin EC2, bulge
406	RGDV	$\alpha \nu \beta 3, \alpha \nu \beta 5$	Somatomedin B
407	PQVTRGDVFTMP	$\alpha \nu \beta 3, \alpha \nu \beta 5$	Somatomedin B
408	LNRQELFPFG	integrin	Nidogen G2
409	SIGFRGDGQTC	integrin	Nidogen G2
410	TWSKVGGHLRPGIVQSG	IgB	Perlecan IV
411	VAEIDGIEL	$\alpha 9\beta 1$	Tenascin-C

412	VFDNFVLK	$\alpha 7\beta 1$	Tenascin-C	
413	VGVA PG	integrin	Elastin	
414	PGVGV	integrin	Elastin	
415	TTSWSQCSKS	$\alpha 6\beta 1$	CCN-1	
416	SVVYGLR	$\alpha 9\beta 1$	Osteopontin	
417	DGRGDSVAYG	$\alpha v\beta 3$	Osteopontin	
418	LALERKDHSG	$\alpha 6\beta 1$	Thrombospondin	
419	RGDF	$\alpha III\beta 3$	Fibrinogen	
420	KRLDGSV	$\alpha M\beta 2$	Fibrinogen	
421	HHLGGAKQAGDV	$\alpha II\beta 3$	Fibrinogen	
422	YSMKKTTMKIIPFNRLTI G	$\alpha II\beta 3$	Fibrinogen	
423	GVYYQGGTYSKAS	$\alpha M\beta 2$	Fibrinogen	
424	LWVTVRSQQRGLF	$\alpha 5\beta 1$	Laminin $\alpha 1$ LN (A3)	
425	GTNNWWQSPSIQN	$\alpha 4\beta 1, \alpha 4\beta 7$	Laminin $\alpha 1$ LN (A10)	
426	WVTVTLDLRQVFQ	$\alpha 5\beta 1$	Laminin $\alpha 1$ LN (A12)	
427	RQVFQVAYIIKA	$\alpha 1\beta 1, \alpha 2\beta 1$	Laminin $\alpha 1$ LN (A13)	
428	LTRYKITPRRGPPT	$\alpha 5\beta 1$	Laminin $\alpha 1$ LN (A18)	
429	LLEFTSARYIRL	integrin	Laminin Laminin $\alpha 1$ LN (A24)	
430	YIRLRLQIRTL	integrin	Laminin $\alpha 1$ LN (A25)	
431	RRYYYSIKDISV	integrin	Laminin $\alpha 1$ V? (A29)	
432	GGFLKYTVSYDI	integrin	Laminin $\alpha 1$ L4a (A55)	
433	RDQLMTVLANVT	integrin	Laminin $\alpha 1$ L4a (A64)	
434	VLIKGGRRARKHV	$\alpha 5\beta 1$	Laminin $\alpha 1$ L4a (A112)	
435	NLLLLLVKANLK	integrin	Laminin $\alpha 1$ L1 (A167)	
436	HRDELLWARKI	integrin	Laminin $\alpha 1$ L1 (A174)	
437	KRRARDLVHRAE	integrin	Laminin $\alpha 1$ L1 (A177)	
438	SQFQESVDNITK	integrin	Laminin $\alpha 1$ L1 (A191)	
439	PGGMREKGRKAR	integrin	Laminin $\alpha 1$ L1 (A194)	
440	MEMQANLLLDRL	integrin	Laminin $\alpha 1$ L1 (A203)	

441	LSEIKLLISRAR	integrin	Laminin α 1 L1 (A206)
442	IKVAV	α v β 3	Laminin α 1 L1 (A208)
443	AASIKVAVSADR	α v β 3	Laminin α 1 L1 (A208)
444	NRWHSIYITRFG	α 6 β 1	Laminin α 1 LG1 (AG10)
445	SSFHFDGSGYAM	integrin	Laminin α 1 LG2 (AG22)
446	IAFQRN	α 6 β 1	Laminin α 1 LG2 (AG32)
447	TWYKIAFQRNRK	α 6 β 1	Laminin α 1 LG2 (AG32)
448	SLVRNRRRVITIQ	integrin	Laminin α 1 LG2 (AG56)
449	DYATLQLQEGRLHFHMF D LG	α 2 β 1	Laminin EF-1
450	KKGSYNNIVVHV	integrin	Laminin α 2 LG (A2G2)
451	ADNLLFYLGSAK	integrin	Laminin α 2 LG (A2G4)
452	GSAKFIDFLAIE	integrin	Laminin α 2 LG (A2G5)
453	KVSFLWWVGSGV	integrin	Laminin α 2 LG (A2G7)
454	SYWYRIEASRTG	integrin	Laminin α 2 LG (A2G10)
455	ISTVMFKFRTFS	integrin	Laminin α 2 LG (A2G25)
456	KQANISIVDIDSN	integrin	Laminin α 2 LG (A2G34)
457	FSTRNESGHILL	integrin	Laminin α 2 LG (A2G48)
458	RRQTTQAYYAIF	integrin	Laminin α 2 LG (A2G51)
459	YAIFLNKGRLEV	integrin	Laminin α 2 LG (A2G52)
460	KNRLTIELEVRT	integrin	Laminin α 2 LG (A2G76)
461	GLLFYMARINHA	integrin	Laminin α 2 LG (A2G78)
462	VQLRNGFPYFSY	integrin	Laminin α 2 LG (A2G80)
463	HKIKIVRVKQEG	integrin	Laminin α 2 LG (A2G84)
464	DFGTVQLRNGFPFFSYD LG	integrin	Laminin EF-2
465	YFDGTGFAKAVG	integrin	Laminin α 2 LG (A2G94)
466	NGQWHKVTAKKI	integrin	Laminin α 2 LG (A2G103)
467	AKKIKNRLELVV	integrin	Laminin α 2 LG (A2G104)
468	GFPGGLNQFGLTTN	integrin	Laminin α 2 LG (A2G109)

469	IRSLKLTGKGK	integrin	Laminin α 2 LG (A2G111)
470	AKALELRGVQPVS	integrin	Laminin α 2 LG (A2G113)
471	GQLFHVAYILIKF	integrin	Laminin α 3 (A3-10)
472	SQRIYQFAKLNYT	integrin	Laminin α 3 LG (MA3G13)
473	NVLSLYNFKTTF	integrin	Laminin α 3 LG (MA3G22)
474	NAPFPKLSWTIQ	integrin	Laminin α 3 LG (MA3G27)
475	WTIQTTVDRGLL	integrin	Laminin α 3 LG (MA3G28)
476	DTINNGRDHMILI	integrin	Laminin α 3 LG (MA3G34)
477	MILISIGKSQKRM	integrin	Laminin α 3 LG (MA3G35)
478	PPFLMLLKWSTR	integrin	Laminin α 3 LG (A3GXX)
479	NQRLASFSAQQS	integrin	Laminin α 3 LG (MA3G57)
480	ISNVFVQRMSQSPEVLD	integrin	Laminin α 3 LG (MA3G59)
481	KARSFNVNQLLQD	integrin	Laminin α 3 LG (MA3G63)
482	KNSFMALYLSKG	integrin	Laminin α 3 LG A3G75
483	KNSFMALYLSKGRLVFA LG	integrin	Laminin α 3 LG A3G756
484	RDSFVALYLSEGHVIFAL G	integrin	Laminin EF-3
485	KPRLQFSLDIQT	integrin	Laminin α 3 LG MA3G70
486	DGQWHSVTVSIK	integrin	Laminin α 3 LG MA3G97
487	FVLYLGSKNAKK	integrin	Laminin α 4 LG (A4G4)
488	LAIKNDNLVYVY	integrin	Laminin α 4 LG (A4G6)
489	AYFSIVKIERVG	integrin	Laminin α 4 LG (A4G10)
490	DVISLYNFKHIY	integrin	Laminin α 4 LG (A4G20)
491	FFDGSSYAVVRD	integrin	Laminin α 4 LG (A4G24)
492	LHVFYDFGFSNG	integrin	Laminin α 4 LG (A4G31)
493	LKKAQINDAKYREISIIY HN	integrin	
494	RAYFNGQSFIAS	integrin	Laminin α 4 LG (A4G47)
495	SRLRGKNPTKGK	integrin	Laminin α 4 LG (A4G59)

496	LHKKGKNSSKPK	integrin	Laminin α 4 LG (A4G69)	
497	RLKTRSSHGMI	integrin		
498	GEKSQFSIRLKT	integrin	Laminin α 4 LG (A4G78)	
499	TLFLAHGRLVFM	integrin	Laminin α 4 LG (A4G82)	
500	LVFMFNVGHKKL	integrin	Laminin α 4 LG (A4G83)	
501	TLFLAHGRLVFMFNVGH KKL	integrin	Laminin α 4 LG (A4G823)	
502	DFMTLFLAHGRLVFMFN VG	integrin	Laminin EF-4	
503	HKKLKIRSQEY	integrin	Laminin α 4 LG (A4G84)	
504	GAAWKIKGPIYL	integrin	Laminin α 4 LG (A4G90)	
505	VIRDSNVVQLDV	integrin	Laminin α 4 LG (A4G107)	
506	EVNVTLDLGQVFH	α 5 β 1	Laminin Laminin α 5 LN (S1)	
507	GQVFHVAYVLIK	α 4 β 1, α 4 β 7	Laminin Laminin α 5 LN (S2)	
508	RDFTKATNIRLRFLR	α 5 β 1	Laminin Laminin α 5 LN (S6)	
509	NIRLRFLRTNTL	α 5 β 1	Laminin Laminin α 5 LN (S7)	
510	GKNTGDHFVLYM	α 5 β 1	Laminin α 5 LG1 (A5G3)	
511	VVSLYNFEQTFML	integrin	Laminin α 5 LG1 (A5G19)	
512	RFDQELRLVSYN	integrin	Laminin α 5 LG2 (A5G26)	
513	ASKAIQVFLG	integrin	Laminin α 5 LG2 (A5G33)	
514	TVFSVDQDNMLE	integrin	Laminin α 5 LG2 (A5G36)	
515	RLRGPQRVFDLH	α 5 β 1	Laminin α 5 LG3 (A5G63)	
516	SRATAQKVSRRS	integrin	Laminin α 5 LG3 (A5G66)	
517	GSLSSHLEFVGI	integrin	Laminin α 5 LG4 (A5G71)	
518	RNRLHLSMLVRP	integrin	Laminin α 5 LG4 (A5G73)	
519	APMSGRSPSLVLK	integrin	Laminin α 5 LG4 (A5G76)	
520	LALFLSNGHFVA	integrin	Laminin α 5 LG4 (A5G77)	
521	PGRWHKVSVRWE	integrin	Laminin α 5 LG4 (A5G81)	
522	VRWGMQQIQLVV	integrin	Laminin α 5 LG4 (A5G82)	
523	KMPYVSLELEMR	integrin	Laminin α 5 LG5 (A5G94)	

524	VLLQANDGAGEF	integrin	Laminin $\alpha 5$ LG5 (A5G99)
525	DGRWHRVAVIMG	integrin	Laminin $\alpha 5$ LG5 (A5G101)
526	APVNV TASVQIQ	integrin	Laminin $\alpha 5$ LG5 (A5G109)
527	KQGKALTQRHAK	integrin	Laminin $\alpha 5$ LG5 (A5G112)
528	AFGVLALWGTRV	integrin	Laminin Laminin VI (B-7)
529	IENVVTTFAPNR	integrin	Laminin Laminin VI (B-15)
530	LEAEFHFTHLIM	integrin	Laminin Laminin VI (B-19)
531	HLIMTFKTRPA	integrin	Laminin Laminin VI (B-20)
532	KTWGVYRYFAYD	integrin	Laminin Laminin VI (B-23)
533	TNLRIFVKLHT	integrin	Laminin Laminin VI (B-31)
534	REKYYAVYDMV	integrin	Laminin Laminin VI (B-34)
535	KRLVTGQR	integrin	Laminin Laminin V (B-54)
536	KDISEKVAVYST	integrin	I (B-187)
537	PDSGR	integrin	Laminin III (B-96)
538	YIGSR	$\alpha 1\beta 1, \alpha 3\beta 1$	Laminin III (B-98)
539	DPGYIGSR	$\alpha 1\beta 1, \alpha 3\beta 1$	Laminin III (B-98)
540	FALWDAIIGEL	integrin	Laminin III (B-116)
541	AAEPLKNIGILF	integrin	Laminin II (B-123)
542	DSITKYFQMSLE	integrin	Laminin II (B-133)
543	VILQQSAADIAR	integrin	Laminin I (B-160)
544	SPYTFIDSLVLMFY	integrin	Laminin Laminin IV (B-77)
545	KDISEKVAVYST	integrin	Laminin I (B-187)
546	LGTI PG	integrin	
547	LWPLLA VLA A V A	integrin	Laminin VI (C-3)
548	KAFDITYVRLKF	$\alpha v\beta 3, \alpha 5\beta 1$	Laminin VI (C-16)
549	AFSTLEGRPSAY	integrin	Laminin VI (C-25)
550	TDIRVTLNRLNTF	integrin	Laminin VI (C-28)
551	NEPKVLKSYYYAI	integrin	Laminin VI (C-30)
552	YYAISDFAVGGR	integrin	Laminin VI (C-31)
553	LPFFNDRPWRRAT	integrin	Laminin VI (C-35)

554	FDPELYRSTGHGGH	integrin	Laminin V (C-38)	
555	TNAVGYSVYDIS	integrin	Laminin V (C-50)	
556	APVKFLGNQVLSY	integrin	Laminin IV (C-57)	
557	SFSFRVDRRDTR	integrin	Laminin IV (C-59)	
558	SETTVKYIFRLHE	integrin	Laminin IV (C-64)	
559	FQKLLNNLTSIK	integrin	Laminin IV (C-67)	
560	TSIKIRGTYSER	integrin	Laminin IV (C-68)	
561	DPETGV	integrin	Laminin III (C75)	
562	TSAEAYNLLRT	integrin	Laminin II (C-118)	
563	KEAEREVTDLR	integrin	Laminin II (C102)	
564	SLLSQLNNLLDQ	integrin	Laminin II (C-155)	
565	RNIAEIIKDI	integrin	Laminin	
566	RDIAEIIKDI	integrin	Laminin	
567	GAPGER	integrin	Derived from Collagen alpha1(I)	
568	FNKHTEIIEEDTNKDKPS YQFGGHNSVDFEEDTLP KV	Fibronectin	(FAB D3: 1-37) - highest affinity	Differential binding affinity to Collagen
569	PSYQFGGHNSVDFEEDT LPK	Fibronectin	(FAB D3: 16-36) - high affinity	Differential binding affinity to Collagen
570	SYQFGGHNSVDFEEDT	Fibronectin	(FAB D3: 17-33) - medium affinity	Differential binding affinity to Collagen
571	QFGGHNSVDFEEDTLPK	Fibronectin	(FAB D3: 20-36) - medium affinity	Differential binding affinity to Collagen
572	FGGHNSVDFEEDTLPK	Fibronectin	(FAB D3: 21-36) -low affinity	Differential binding affinity to Collagen
573	NAPQPSHISKYILRWPK NSVGRWKEATIPGHLNS YTIKGLKPGVVYEGQLIS IQQYGHQEVTRFDFTTS TSTPVTSTNTVTGETTPFS	Fibronectin	Fibronectin Type III(1)	

	PLVATSESVTEITASFFV VS			
574	NAPQPSHISKYILRWPK NSVGRWKEATIPG	Fibronectin	Fibronectin Type III(1) fragment	
575	EATIPGHLNSYTIKGLKP GVVYEGQLISIQQ	Fibronectin	Fibronectin Type III(1) fragment	
576	LISIQQYGHQEVTRFDFT TTSTSTPVTSTNTV	Fibronectin	Fibronectin Type III(1) fragment	
577	VTSNTVTGETTPFSPPLVA TSESVTEITASFFVVS	Fibronectin	Fibronectin Type III(1) fragment	
578	RWSHDNGVNYKIGEKW DRQGENGQMMSSSTSLG NGKGEFKSDPHE	Fibronectin	Fibronectin Type III(1) fragment (synthetic)	
579	ATSYDDGKTYHVGQW QKEYLGAISSSTSFGGQR GWRSDNSR	Fibronectin	Fibronectin Type III(1) fragment (synthetic)	
580	DKPSYQFGGHNSVDFEE DT	Fibronectin		
581	DKPSYQFGGHNSVDFEE DTL	Fibronectin		
582	DKPSYQFGGHNSVDFEE DTLP	Fibronectin		
583	DKPSYQFGGHNSVDFEE DTLPK	Fibronectin		
584	KPSYQFGGHNSVDFEED T	Fibronectin		
585	KPSYQFGGHNSVDFEED TL	Fibronectin		
586	KPSYQFGGHNSVDFEED TLP	Fibronectin		

587	KPSYQFGGHNSVDFEED TLPK	Fibronectin		
588	PSYQFGGHNSVDFEEDT	Fibronectin		
589	PSYQFGGHNSVDFEEDT L	Fibronectin		
590	PSYQFGGHNSVDFEEDT LP	Fibronectin		
591	PSYQFGGHNSVDFEEDT LPK	Fibronectin		
592	PPFLMLLLKGSTRFNKTK TFR	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
593	RLVFALGTDGKKLRIKS KEKCNDGK	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
594	PLFLHLHKKGKNSKPKA SQNKKGKSK	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
595	TLFLAHGRLVYMFNVG HKCLKIR	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
596	TPGLGPRGLQATARKAS RRSRQPARHPACML	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
597	RQRSRPGRWHKVSVRW EKNR	Heparin / syndecans	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
598	LAGSCLARFSTM	$\alpha 2\beta 1$, Heparin	Derived from Collagen α 1(IV) HepII	
599	KGHRGF	Heparin	Derived from Collagen α 1(I)	
600	GDRGIKGHRGFSG	Heparin	Derived from Collagen α 1(I)	
601	GDLGRPGRKGRPGPP	Heparin	Derived from Collagen α 1(I)	

602	GHRGPTGRPGKRGKQG QKGDS	Heparin	Derived from Collagen alpha1(I)
603	KGIRGH	Heparin	Derived from Collagen alpha2(I)
604	GEFYFDLRLLKGDK	α 2 β 1, Heparin	Derived from Collagen alpha1(IV) HepIII
605	KYILRWRPKNS	Heparin	Derived from Fibronectin III-1
606	YRVRVTPKEKTGPMKE	Heparin	Derived from Fibronectin III-13 (FN-C/H-III)
607	SPPRRARVT	α 5 β 1, Heparin	Derived from Fibronectin III-13 (FN-C/H-IV)
608	ATETTTIS	Heparin	Derived from Fibronectin III-13
609	VSPPRRARVTDATETTIT ISWRTKTETITGFG	α 5 β 1, Heparin	Derived from Fibronectin III-13
610	KPDVRSYTTITG	α 4 β 1, Heparin	Derived from Fibronectin III-13
611	ANGQTPIQRYIK	α 4 β 1, Heparin	Derived from Fibronectin III-13
612	YEKPGSPPREVVPRPRPG V	Heparin	Derived from Fibronectin III-14 (FN-C/H-I)
613	KNNQKSEPLIGRKKT	Heparin	Derived from Fibronectin III-14 (FN-C/H-II)
614	EILDVPST	integrin	Derived from Fibronectin IIIICS-1
615	TAGSCLRKFSMT	α 2 β 1, Heparin	Derived from Collagen alpha1(IV) HepI
616	FRHRNRKGY	Heparin	HPV
617	KKQRFHRNRKGYRSQ	Heparin	HPV
618	KRSR	Heparin	Bone sialoprotein
619	FHRRIKA	Heparin, HSP	Bone sialoprotein
620	SINNTAVMQRLT	Heparin	Laminin Laminin α 1 L4a (A51)
621	ANVTHLLIRANY	Heparin	Laminin α 1 L4a (A65)
622	AGTFALRGDNPQG	integrin	Laminin α 1 L4a (A99)
623	RLVSYSGVLEFLK	Heparin	Laminin α 5 LG2 (A5G27)
624	GIIFFL	Heparin	Laminin α 5 LG2 (A5G)
625	VLVRVERATVFS	Heparin	Laminin α 5 LG2 (A5G35)
626	RIQNLLKITNLRKFVK	Heparin	Laminin Laminin VI (B-30)
627	GPGVVVVERQYI	Heparin	Laminin IV (B-62)
628	RYVVLP	Heparin	Laminin IV (B-73)

629	LSNIDYILIKAS	SDC-4	Laminin α 1 L4a (A119)	Differential binding affinity to Heparin / syndecans
630	LQQSRIANISME	SDC-4	Laminin α 1 L4a (A121)	
631	LQVQLSIR	SDC-1, -4	Laminin α 1 LG4 (AG73)	
632	RKRLQVQLSIRT	SDC-1, -4	Laminin α 1 LG4 (AG73)	
633	GLIYYVAHQNM	SDC-1, -4	Laminin α 1 LG4 (AG75)	
634	FDLHQNMGSVN	SDC-4	Laminin α 5 LG3 (A5G64)	
635	QQNLGSVNVSTG	SDC-4	Laminin α 5 LG3 (A5G65)	
636	WQPPRARI	SDC-4	Derived from Fibronectin III-14 (FN-C/H-V)	Differential binding affinity to Heparin / syndecans
637	WQPPRARITGYIIKYEKP G	SDC-4	Derived from Fibronectin III-14 (FN-C/H-V)	
638	KNSFMALYLSKGR	syndecan 2(w)	Derived from Heparin Binding Domans of Laminin	Differential binding affinity to Heparin / syndecans
639	NGRKIRMRCRAIDGD	Heparan proteoglycans	binds to HSGP with high affinity (DTx protein)	
640	DVIRDKTKTKIESLK	Heparan proteoglycans	binds to HSGP with low affinity (DTx protein)	
pH-sensitive targeting sequences				
641	GVYHREARSGKYKLTY AEAKAVCEFEFGGHLATY KGLEAARKIGFHVCAAG WMAKGRVGYPIVKPGPP NCGFGKTGHIDYGIRLNR SERWDAYCYNPHA	hyaluronic acid	pH dependent (Link_TGS6)	binds better at lower pH
642	KHAHLKKQVSDHIAVY	Heparin	binds to heparin at low pH (high affinity)	
643	TTEPSEEHNHHK	Heparin	binds to heparin at low pH (low affinity)	
644	KHAHL	Heparin	binds to heparin at low pH (lower affinity)	
645	TTEPSEEHNHHK	Heparin	binds to heparin at low pH (lower affinity)	
646	TTEPSEEHNHHKHHDK	Heparin	binds to heparin at low pH (lower affinity)	
647	HKGQHR	Heparin	binds to heparin at low pH (lower affinity)	
648	KVEHRVKKRPPTWRHN VRAKYT	Heparin	binds to heparin at low pH	

649	GGKVEHRVKKRPTWR HNVRAKYT	Heparin	binds to heparin at low pH
650	KKRPPTWRHNV	Heparin	binds to heparin at low pH
651	GTWSEW	heparin	derived from thrombospondin
652	GFWSEW	heparin	derived from thrombospondin
653	GGWSHW	Fibronectin	derived from thrombospondin (highest affinity) binds better at lower pH
654	KRFKQDGGWSHWSPWS S	Fibronectin	derived from thrombospondin (low affinity)
655	KRFKQDGGWSHWSP	Fibronectin	derived from thrombospondin (medium affinity)
656	GGWSHWSPWSS	Fibronectin	derived from thrombospondin (medium affinity)
657	WSXWS	Sulfated Glycoprotein	derived from thrombospondin (X= any amino acids)
658	WSHW	Sulfated Glycoprotein	derived from thrombospondin
659	Xaa Xaa Pro His Glu	heparin / heparan sulfate	Xaa = any amino acid
660	(H/P)(H/P)PHG	heparin / heparan sulfate	tandem repeat - pH dependent HRGP (Histidine Rich Glyco Protein)
661	HPHKHHSHEQHHPHGHP HAHHPHEHDTHRQHPH GHHPHGHHHPHGHHPHG HHPHGHHPHCHDFQDY GPCDPPPHNQGHCCCHG GPPPGHLRRRGPGKGPR PFHCRQIGSVYRLPPLRK GEVLPLPEANFPSFPLPH HKHPLKPDNQPEP	heparin / heparan sulfate	Histidine Rich Glycoprotein (Histidine Rich Domain)
662	DLHPHKHHSHEQHHPHG HPHAHHPHEHDTHRQHP H	heparin / heparan sulfate	Histidine Rich Glycoprotein (Histidine Rich Domain)
663-679	Not Used		
Control sequence			
680	QFGGHNSVDFEEDT	Fibronectin	non-binding control
681-700	Not Used		

Definitions

[00186] As used herein, a “cytokine polypeptide sequence” refers to a polypeptide sequence (which may be part of a larger sequence, e.g., a fusion polypeptide) with significant sequence identity to a wild-type cytokine and which can bind and activate a cytokine receptor when separated from an inhibitory polypeptide sequence. In some embodiments, a cytokine polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine, e.g., a wild-type human cytokine. In some embodiments, a cytokine polypeptide sequence has no more than one, two, three, four, five, six, seven, eight, nine, or ten amino acid differences from a wild-type cytokine, e.g., a wild-type human cytokine. Cytokines include but are not limited to chemokines. Exemplary cytokine polypeptide sequences are provided in Table 1. This definition applies to IL-2 polypeptide sequences with substitution of “IL-2” for “cytokine.”

[00187] As used herein, an “inhibitory polypeptide sequence” is a sequence in a cytokine prodrug that inhibits the activity of the cytokine polypeptide sequence in the prodrug. The inhibitory polypeptide sequence binds the cytokine polypeptide sequence, and such binding is reduced or eliminated by action of an appropriate protease on the protease-cleavable polypeptide sequence. Exemplary inhibitory polypeptide sequences are provided in Table 1.

[00188] As used herein, a “protease-cleavable polypeptide sequence” is a sequence that is a substrate for cleavage by a protease. The protease-cleavable polypeptide sequence is located in a cytokine prodrug such that its cleavage reduces or eliminates binding of the inhibitory polypeptide sequence to the cytokine polypeptide sequence.

[00189] As used herein, a protease-cleavable polypeptide sequence “is recognized by” a given protease or class thereof if exposing a polypeptide comprising the protease-cleavable polypeptide sequence to the protease under conditions permissive for cleavage by the protease results in a significantly greater amount of cleavage than is seen for a control polypeptide having an unrelated sequence, and/or if the protease-cleavable polypeptide sequence corresponds to a known recognition sequence for the protease (e.g., as described elsewhere herein for various exemplary proteases).

[00190] As used herein, a “pharmacokinetic modulator” is a moiety that extends the in vivo half-life of a cytokine prodrug. The pharmacokinetic modulator may be a fused domain in a cytokine prodrug or may be a chemical entity attached post-translationally. The attachment may be, but is not necessarily, covalent. Exemplary pharmacokinetic modulator

polypeptide sequences are provided in Table 1. Exemplary non-polypeptide pharmacokinetic modulators are described elsewhere herein.

[00191] As used herein, a “targeting sequence” is a sequence that results in a greater fraction of a cytokine prodrug localizing to an area of interest, e.g., a tumor microenvironment. The targeting sequence may bind an extracellular matrix component or other entity found in the area of interest, e.g., an integrin or syndecan. Exemplary targeting sequences are provided in Table 2.

[00192] As used herein, an “extracellular matrix component” refers to an extracellular protein or polysaccharide found in vivo. Integral and peripheral membrane proteins on a cell, including fibronectins, cadherins, integrins, and syndecans, are *not* considered extracellular matrix components.

[00193] As used herein, an “immunoglobulin constant domain” refers to a domain that occurs in or has significant sequence identity to a domain of a constant region of an immunoglobulin, such as an IgG. Exemplary constant domains are C_H2 and C_H3 domains. Unless indicated otherwise, a polypeptide or prodrug comprising an immunoglobulin constant domain may comprise more than one immunoglobulin constant domain. In some embodiments, an immunoglobulin constant domain has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type immunoglobulin constant domain, e.g., a wild-type human immunoglobulin constant domain. In some embodiments, an immunoglobulin constant domain has no more than one, two, three, four, five, six, seven, eight, nine, or ten amino acid differences from a wild-type immunoglobulin constant domain, e.g., a wild-type human immunoglobulin constant domain. In some embodiments, immunoglobulin constant domain has an identical sequence to a wild-type immunoglobulin constant domain, e.g., a wild-type human immunoglobulin constant domain. Exemplary immunoglobulin constant domains are contained within sequences provided in Table 1. This definition applies to C_H2 and C_H3 domains, respectively, with substitution of “C_H2” or “C_H3” for “immunoglobulin constant,” with the qualification that a C_H2 domain sequence does not have greater percent identity to a non-C_H2 immunoglobulin constant domain wild-type sequence than to a C_H2 domain wild-type sequence, and a C_H3 domain sequence does not have greater percent identity to a non-C_H3 immunoglobulin constant domain wild-type sequence than to a C_H3 domain wild-type sequence. These definitions also include domains having minor truncations relative to wild-type sequences, to the extent that the truncation does not abrogate substantially normal folding of the domain.

[00194] As used herein, a “immunoglobulin Fc region” refers to a region of an immunoglobulin heavy chain comprising a C_H2 and a C_H3 domain, as defined above. The Fc region does not include a variable domain or a C_H1 domain.

[00195] As used herein, a given component is “between” a first component and a second component if the first component is on one side of the given component and the second component is on the other component, e.g., in the primary sequence of a polypeptide. This term does not require immediate adjacency. Thus, in the structure 1-2-3-4, 2 is between 1 and 4, and is also between 1 and 3.

[00196] As used herein, a “domain” may refer, depending on the context, to a structural domain of a polypeptide or to a functional assembly of at least one domain (but possibly a plurality of structural domains). For example, a C_H2 domain refers to a part of a sequence that qualifies as such. An immunoglobulin cytokine-binding domain may comprise V_H and V_L structural domains.

[00197] As used herein, “denatured collagen” encompasses gelatin and cleavage products resulting from action of an MMP on collagen, and more generally refers to a form of collagen or fragments thereof that does not exist in the native structure of full-length collagen.

[00198] As used herein, “configured to bind ... in a pH-sensitive manner” means that a polypeptide sequence (e.g., a targeting sequence) shows differential binding affinity for its binding partner depending on pH. For example, the polypeptide sequence may have a higher affinity at a relatively acidic pH than at normal physiological pH (about 7.4). The higher affinity may occur at a pH below 7, e.g., in the range of pH 5.5-7, 6-7, or 5.5-6.5, or below pH 6.

[00199] As used herein, a “cytokine-binding domain of a cytokine receptor” refers to an extracellular portion of a cytokine receptor, or a fragment or truncation thereof that can bind a cytokine polypeptide sequence. In some embodiments, the sequence of a cytokine binding domain of a cytokine receptor has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a cytokine binding domain of wild-type cytokine receptor, e.g., a cytokine binding domain of a wild-type human cytokine receptor. Exemplary sequences of a cytokine binding domain of a cytokine receptor are provided in Table 1. This definition applies to IL-2-binding domains of an IL-2 receptor with substitution of “IL-2” for “cytokine.”

[00200] As used herein, a “cytokine-binding immunoglobulin domain” refers to one or more immunoglobulin variable domains (e.g., a V_H and a V_L domain) that can bind a

cytokine polypeptide sequence. Exemplary sequences of a cytokine-binding immunoglobulin domain are provided in Table 1. This definition applies to IL-2-binding immunoglobulin domains with substitution of “IL-2” for “cytokine.”

[00201] As used herein, “substantially” and other grammatical forms thereof mean sufficient to work for the intended purpose. The term “substantially” thus allows for minor, insignificant variations from an absolute or perfect state, dimension, measurement, result, or the like such as would be expected by a person of ordinary skill in the field but that do not appreciably affect overall performance. When used with respect to numerical values or parameters or characteristics that can be expressed as numerical values, “substantially” means within ten percent.

[00202] As used herein, the term “plurality” can be 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[00203] As used herein, a first sequence is considered to “comprise a sequence with at least X% identity to” a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence QLYV comprises a sequence with 100% identity to the sequence QLY because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[00204] As used herein, a “subject” refers to any member of the animal kingdom. In some embodiments, “subject” refers to humans. In some embodiments, “subject” refers to non-human animals. In some embodiments, “subject” refers to primates. In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In certain embodiments, the non-human subject is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, and/or a clone. In certain embodiments of the present invention the subject is an adult, an adolescent or an infant. In some embodiments, the terms “individual” or “patient” are used and are intended to be interchangeable with “subject”.

Cytokine polypeptide sequence

[00205] The cytokine polypeptide sequence may be a wild-type cytokine polypeptide sequence or a sequence with one or more differences from the wild-type cytokine polypeptide sequence. In some embodiments, the cytokine polypeptide sequence is a human cytokine polypeptide sequence (which may be wild-type or may have one or more differences). In some embodiments, the cytokine comprises a modification to prevent disulfide bond formation, and optionally otherwise comprises wild-type sequence. In some embodiments, the cytokine polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine polypeptide sequence or to a cytokine polypeptide sequence in Table 1. In some embodiments, the cytokine is a dimeric cytokine, e.g., a heterodimeric cytokine. In some embodiments, the cytokine is a homodimeric cytokine. The monomers may be linked as a fusion protein, e.g., with a linker, or by a covalent bond (e.g., disulfide bond), or by a noncovalent interaction. In some embodiments, the cytokine polypeptide sequence is an interleukin polypeptide sequence. In some embodiments, the cytokine polypeptide sequence is capable of binding a receptor comprising CD132. In some embodiments, the cytokine polypeptide sequence is capable of binding a receptor comprising CD122. In some embodiments, the cytokine polypeptide sequence is capable of binding a receptor comprising CD25.

IL-2

[00206] In some embodiments, the cytokine polypeptide sequence is an IL-2 polypeptide sequence. The IL-2 polypeptide sequence may be a wild-type IL-2 polypeptide sequence or a sequence with one or more differences from the wild-type IL-2 polypeptide sequence. In some embodiments, the IL-2 polypeptide sequence is a human IL-2 polypeptide sequence (which may be wild-type or may have one or more differences). In some embodiments, the IL-2 comprises a modification to prevent disulfide bond formation (e.g., the sequence of aldesleukin (marketed as Proleukin®), and optionally otherwise comprises wild-type sequence. In some embodiments, the IL-2 polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type IL-2 polypeptide sequence or to a IL-2 polypeptide sequence in Table 1.

Inhibitory polypeptide sequence

[00207] Various types of inhibitory polypeptide sequences may be used in a cytokine prodrug according to the disclosure. In some embodiments, the inhibitory polypeptide sequence comprises a cytokine-binding domain.

[00208] The cytokine-binding domain may be the cytokine-binding domain of a cytokine receptor. The cytokine-binding domain of a cytokine receptor may be provided as an extracellular portion of the cytokine receptor or a portion thereof sufficient to bind the cytokine polypeptide sequence of the cytokine prodrug. In some embodiments, the cytokine-binding domain of a cytokine receptor has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine-binding domain of a cytokine receptor, e.g., a wild-type cytokine-binding domain of a human cytokine receptor.

[00209] The cytokine-binding domain may be a fibronectin cytokine-binding domain. In some embodiments, the fibronectin cytokine-binding domain has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type fibronectin cytokine-binding domain of a cytokine receptor, e.g., a wild-type human fibronectin cytokine-binding domain.

[00210] The cytokine-binding domain may be an immunoglobulin cytokine-binding domain. The immunoglobulin cytokine-binding domain may be an Fv, scFv, Fab, VHH, or other immunoglobulin sequence having antigen-binding activity for the cytokine polypeptide sequence. A VHH antibody (or nanobody) is an antigen binding fragment of a heavy chain only antibody.

[00211] Additional examples of inhibitory polypeptide sequences that may be provided to inhibit the cytokine polypeptide sequence of the cytokine prodrug are anticalins, affilins, affibody molecules, affimers, affitins, alphabodies, avimers, DARPins, fynomers, kunitz domain peptides, monobodies, and binding domains based on other engineered scaffolds such as SpA, GroEL, lipocalin and CTLA4 scaffolds.

IL-2 inhibitory polypeptide sequence

[00212] In cytokine prodrugs comprising an IL-2 polypeptide sequence, the inhibitory polypeptide sequence may be an IL-2 inhibitory polypeptide sequence of any of the types described above. In some embodiments, the IL-2 inhibitory polypeptide sequence is an immunoglobulin IL-2 inhibitory polypeptide sequence. In some embodiments, the IL-2 inhibitory polypeptide sequence comprises an anti-IL-2 antibody or a functional fragment thereof. In some embodiments, the immunoglobulin IL-2 inhibitory polypeptide sequence

comprises a set of six anti-IL2 hypervariable regions (HVRs) set forth in Table 1 (e.g., SEQ ID NOs: 34-39 or 750-755). In some embodiments, the IL-2 inhibitory polypeptide sequence comprises a set of anti-IL2 VH and VL sequences having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a set of anti-IL2 VH and VL sequences set forth in Table 1, either as individual sequences or as part of an scFv. In some embodiments, the IL-2 inhibitory polypeptide sequence comprises a set of anti-IL2 VH and VL sequences having the sequence of a set of anti-IL2 VH and VL sequences set forth in Table 1, either as individual sequences or as part of an scFv. Exemplary IL-2 inhibitory polypeptide sequences include SEQ ID NOS: 10-31, 40-51, and 747, and a combination of SEQ ID NOs 32 and 33 or a combination of SEQ ID NOs 748 and 749.

Protease-cleavable sequence

[00213] The protease-cleavable sequence may be selected from sequences cleavable by various types of proteases, e.g., a metalloprotease, a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamate protease, a gelatinase, an asparagine peptide lyase, a cathepsin, a kallikrein, a plasmin, a collagenase, a hK1, a hK10, a hK15, a stromelysin, a Factor Xa, a chymotrypsin-like protease, a trypsin-like protease, a elastase-like protease, a subtilisin-like protease, an actinidain, a bromelain, a calpain, a caspase, a Mir 1-CP, a papain, a HIV-1 protease, a HSV protease, a CMV protease, a chymosin, a renin, a pepsin, a matriptase, a legumain, a plasmepsin, a nepenthesin, a metalloexopeptidase, a metalloendopeptidase, an ADAM 10, an ADAM17, an ADAM 12, an urokinase plasminogen activator (uPA), an enterokinase, a prostate-specific target (PSA, hK3), an interleukin-1b converting enzyme, a thrombin, a FAP (FAP-a), a dipeptidyl peptidase, or dipeptidyl peptidase IV (DPPIV/CD26), a type II transmembrane serine protease (TTSP), a neutrophil elastase, a proteinase 3, a mast cell chymase, a mast cell tryptase, or a dipeptidyl peptidase. In some embodiments, the protease-cleavable sequence comprises the sequence of any one of those in Table 1 (e.g., SEQ ID NOs: 80-90 or 700-741), or a variant having one or two mismatches relative to the sequence of any one of those in Table 1 (e.g., SEQ ID NOs: 80-90 or 700-741). Proteases generally do not require an exact copy of the recognition sequence, and as such, the exemplary sequences may be varied at a portion of their amino acid positions. In some embodiments, the protease-cleavable sequence comprises a sequence that matches an MMP consensus sequence, such as any one of SEQ ID NOs: 91-94. One skilled in the art will be familiar with additional sequences recognized by these types of proteases.

Matrix metalloprotease-cleavable sequence

[00214] In some embodiments, the protease-cleavable sequence is a matrix metalloprotease (MMP)-cleavable sequence. Exemplary MMP-cleavable sequences are provided in Table 1. In some embodiments, the MMP-cleavable sequence is cleavable by a plurality of MMPs and/or one or more of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, and/or MMP-14. Table 1, e.g., SEQ ID NOs: 80-90, provides exemplary MMP-cleavable sequences.

Targeting sequence

[00215] In some embodiments, the targeting sequence facilitates localization, accumulation, and/or retention of the cytokine prodrug and/or the cytokine polypeptide sequence (e.g., after proteolysis of the protease-cleavable sequence) in an area of interest, e.g., a tumor microenvironment (TME). The targeting sequence may be a sequence that binds an extracellular matrix component. Exemplary extracellular matrix components are a collagen or denatured collagen (in either case, the collagen may be collagen I, II, III, or IV), poly(I), von Willebrand factor, IgB (CD79b), heparin, a sulfated glycoprotein, or hyaluronic acid.

[00216] In other embodiments, the targeting sequence binds a target other than an extracellular matrix component. In some embodiments, the targeting sequence binds IgB (CD79b), a fibronectin, an integrin, a cadherin, a heparan sulfate proteoglycan, or a syndecan. In some embodiments, the targeting sequence binds at least one integrin, such as one or more of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin, $\alpha 3\beta 1$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 5\beta 1$ integrin, $\alpha 6\beta 1$ integrin, $\alpha 7\beta 1$ integrin, $\alpha 9\beta 1$ integrin, $\alpha 4\beta 7$ integrin, $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha I Ib\beta 3$ integrin, $\alpha III b\beta 3$ integrin, $\alpha M\beta 2$ integrin, or $\alpha I Ib\beta 3$ integrin. In some embodiments, the targeting sequence binds at least one syndecan, such as one or more of syndecan-1, syndecan-4, and syndecan-2(w). Cytokine prodrugs comprising such targeting sequences may also comprise an MMP-cleavable linker as set forth elsewhere herein, such as an MMP-cleavable linker comprising any one of SEQ ID NOs: 80-90, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 80-90.

[00217] In some embodiments, the targeting sequence comprises a sequence set forth in Table 2 (e.g., any one of SEQ ID NOs: 180-640), or a variant having one or two mismatches relative to such a sequence.

pH-sensitive targeting sequences

[00218] In some embodiments, the targeting sequence is configured to bind its target in a pH-sensitive manner. In some embodiments, the targeting sequence has a higher affinity for its target at a relatively acidic pH than at normal physiological pH (about 7.4). The higher affinity may occur at a pH below 7, e.g., in the range of pH 5.5-7, 6-7, or 5.5-6.5, or below pH 6. The presence of histidines in the targeting sequence can confer pH-sensitive binding. Without wishing to be bound by any particular theory, histidines are considered more likely to be protonated at lower pH and can render binding a negatively-charged target more energetically favorable. Accordingly, in some embodiments, a targeting sequence comprises one or more histidines, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 histidines. Including a pH-sensitive targeting sequence can enhance discrimination between tumor versus normal tissue by the cytokine prodrug, such that the cytokine prodrug is more preferentially retained in the tumor microenvironment compared to normal extracellular matrix. Thus, a pH-sensitive targeting element can further facilitate tumor specific delivery of the cytokine prodrug and thereby further reduce or eliminate toxicity that may result from cytokine activity in normal extracellular matrix.

[00219] Binding a target in a pH-sensitive manner can be useful where it is desired to localize or retain a cytokine prodrug or the cytokine polypeptide sequence thereof in an area with a pH different from normal physiological pH. For example, the tumor microenvironment may be more acidic than the blood and/or healthy tissue. As such, binding to a target in a pH-sensitive manner may improve the retention of the cytokine prodrug or the cytokine polypeptide sequence thereof in the area of interest, which can facilitate lower doses than would otherwise be needed and/or reduce systemic exposure and/or adverse effects.

[00220] In some embodiments, the targeting sequence is configured to bind any target described herein in a pH-sensitive manner. In particular embodiments, the target is an extracellular matrix component such as a hyaluronic acid, heparin, heparan sulfate, or a sulfated glycoprotein. In another particular embodiment, the target is a fibronectin.

[00221] Exemplary targeting sequences for conferring target binding in a pH-sensitive manner are provided in Table 2 (e.g., SEQ ID NOs: 641-662). In some embodiments, the targeting sequence comprises the sequence of any one of SEQ ID NOs: 641-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 641-662.

Pharmacokinetic modulators

[00222] In some embodiments, the cytokine prodrug comprises a pharmacokinetic modulator. The pharmacokinetic modulator may be covalently or noncovalently associated with the cytokine prodrug. The pharmacokinetic modulator can extend the half-life of the cytokine prodrug and optionally the cytokine polypeptide sequence, e.g., so that fewer doses are necessary and less of the prodrug needs to be administered over time to achieve a desired result. Various forms of pharmacokinetic modulator are known in the art and may be used in cytokine prodrugs of this disclosure. In some embodiments, the pharmacokinetic modulator comprises a polypeptide (see examples below). In some embodiments, the pharmacokinetic modulator comprises a non-polypeptide moiety (e.g., polyethylene glycol, a polysaccharide, or hyaluronic acid). A non-polypeptide moiety can be associated with the prodrug using known approaches, e.g., conjugation to the prodrug; for example, a reactive amino acid residue can be used or added to the prodrug to facilitate conjugation.

[00223] In some embodiments, the pharmacokinetic modulator alters the size, shape, and/or charge of the prodrug, e.g., in a manner that reduces clearance. For example, a pharmacokinetic modulator with a negative charge may inhibit renal clearance. In some embodiments, the pharmacokinetic modulator increases the hydrodynamic volume of the prodrug. In some embodiments, the pharmacokinetic modulator reduces renal clearance, e.g., by increasing the hydrodynamic volume of the prodrug.

[00224] In some embodiments, the cytokine prodrug comprising the pharmacokinetic modulator (e.g., any of the pharmacokinetic modulators described herein) has a molecular weight of at least 70 kDa, e.g., at least 75 or 80 kDa.

[00225] For further discussion of various approaches for providing a pharmacokinetic modulator, see, e.g., Strohl, *BioDrugs* 29:215-19 (2015) and Podust et al., *J. Controlled Release* 240:52-66 (2016).

Polypeptide pharmacokinetic modulators

[00226] In some embodiments, the pharmacokinetic modulator comprises a polypeptide, e.g., an immunoglobulin sequence (see exemplary embodiments below), an albumin, a CTP (a negatively-charged carboxy-terminal peptide of the chorionic gonadotropin β -chain that undergoes sialylation in vivo and in appropriate host cells), an inert polypeptide (e.g., an unstructured polypeptide such as an XTEN, a polypeptide comprising

the residues Ala, Glu, Gly, Pro, Ser, and Thr), a transferrin, a homo-amino-acid polypeptide, or an elastin-like polypeptide.

[00227] Exemplary polypeptide sequences suitable for use as a pharmacokinetic modulator are provided in Table 1 (e.g., any one of SEQ ID NOs: 70-74). In some embodiments, the pharmacokinetic modulator has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a pharmacokinetic modulator in Table 1 (e.g., any one of SEQ ID NOs: 70-74).

[00228] In any embodiment where the pharmacokinetic modulator comprises a polypeptide sequence from an organism, the polypeptide sequence may be a human polypeptide sequence.

Immunoglobulin pharmacokinetic modulators

[00229] In some embodiments, the pharmacokinetic modulator comprises an immunoglobulin sequence, e.g., one or more immunoglobulin constant domains. In some embodiments, the pharmacokinetic modulator comprises an Fc region. The immunoglobulin sequence (e.g., one or more immunoglobulin constant domains or Fc region) may be a human immunoglobulin sequence. The immunoglobulin sequence (e.g., one or more immunoglobulin constant domains or Fc region) may have at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type immunoglobulin sequence (e.g., one or more immunoglobulin constant domains or Fc region), such as a wild-type human immunoglobulin sequence. In any of such embodiments, the immunoglobulin sequence may be an IgG sequence (e.g., IgG1, IgG2, IgG3, or IgG4). Exemplary immunoglobulin pharmacokinetic modulator sequences include SEQ ID NOS: 70-74 and the combination of SEQ ID NOs 756 and 757.

Arrangement of components

[00230] The recitation of components of a cytokine prodrug herein does not imply any particular order beyond what is explicitly stated (for example, it may be explicitly stated that a protease-cleavable sequence is between the cytokine polypeptide sequence and the inhibitory polypeptide sequence). The components of the cytokine prodrug may be arranged in various ways to provide properties suitable for a particular use. The components of the cytokine prodrug may be all in one polypeptide chain or they may be in a plurality of polypeptide chains bridged by covalent bonds, such as disulfide bonds. For example, where a pharmacokinetic modulator comprises an Fc, one or more components may be bound to one

chain while one or more other components may be bound to the other chain. The Fc may be a heterodimeric Fc, such as a knob-into-hole Fc (in which one chain of the Fc comprises knob mutations and the other chain of the Fc comprises hole mutations). For an exemplary general discussion of knob and hole mutations, see, e.g., Xu et al., *mAbs* 7:1, 231-242 (2015).

Exemplary knob mutations (e.g., for a human IgG1 Fc) are K360E/K409W. Exemplary hole mutations (e.g., for a human IgG1 Fc) are Q347R/D399V/F405T. See SEQ ID NOs: 756 and 757.

[00231] For example, a pharmacokinetic modulator can be present on the same side of the protease-cleavable sequence as the cytokine polypeptide sequence, meaning that cleavage of the protease-cleavable sequence does not separate the pharmacokinetic modulator from the cytokine polypeptide sequence. Examples of such structures include CY-PM-CL-IN, IN-CL-CY-PM, and any other permutation (or variation in which additional elements are included between, before, or after the listed components) in which CL is not between CY and PM, where CY is the cytokine polypeptide sequence, PM is the pharmacokinetic modulator, CL is the protease-cleavable sequence, and IN is the inhibitory polypeptide sequence. In such embodiments, the pharmacokinetic modulator will modulate the pharmacokinetics of both the prodrug and the active cytokine polypeptide sequence. In some embodiments, the pharmacokinetic modulator is an Fc, in which case the components preceding and following PM in the exemplary structures above may be bound to the same or different chains of the Fc, as discussed above.

[00232] In some embodiments, a pharmacokinetic modulator is present on the same side of the protease-cleavable sequence as the inhibitory polypeptide sequence, meaning that cleavage of the protease-cleavable sequence does separate the pharmacokinetic modulator from the cytokine polypeptide sequence. Such embodiments can be useful to provide a longer half-life for the prodrug than for the active form.

[00233] In some embodiments, a targeting sequence can be present on the same side of the protease-cleavable sequence as the cytokine polypeptide sequence, meaning that cleavage of the protease-cleavable sequence does not separate the targeting sequence from the cytokine polypeptide sequence. Such embodiments can be useful to facilitate localizing or retaining both the prodrug and the active form in an area of interest, e.g., a tumor microenvironment. Where a pharmacokinetic modulator is used, it can be on the same side of the protease-cleavable linker as the targeting sequence (e.g., to facilitate lower and/or less frequent dosing) or on the other side (e.g., to avoid long-duration immune stimulation), depending on the desired effects.

[00234] In some embodiments, a targeting sequence is present on the same side of the protease-cleavable sequence as the inhibitory polypeptide sequence, meaning that cleavage of the protease-cleavable sequence does separate the targeting sequence from the cytokine polypeptide sequence. Such embodiments can be useful to provide a gradient of cytokine emanating from an area of interest, or to provide such a gradient more rapidly than would occur if the targeting sequence were on the same side of the protease-cleavable sequence. Where a pharmacokinetic modulator is used, it can be on the same side of the protease-cleavable linker as the targeting sequence (e.g., to minimize systemic exposure to the active form of the cytokine and/or avoid long-duration immune stimulation) or on the other side (e.g., to facilitate lower and/or less frequent dosing), depending on the desired effects.

[00235] A number of exemplary arrangements are illustrated in Figs. 9 and 10A-E. In some embodiments, the cytokine prodrug comprises components arranged according to any of the examples in Figs. 9 and 10A-E, ordered from N- to C-terminus or from C- to N-terminus, optionally with additional components inserted between any of the illustrated components.

Exemplary prodrugs

IL-2

[00236] The following table shows exemplary combinations of components according to certain embodiments of the disclosed cytokine prodrugs. The numbers indicate SEQ ID NOs for a given component. CY is the cytokine polypeptide sequence, CL is the protease-cleavable sequence, and IN is the inhibitory polypeptide sequence, and, where present, PM is the pharmacokinetic modulator. Where a range is given, any one of the listed SEQ ID NOs may be selected. Where two SEQ ID NOs are recited conjunctively (using “and”), both SEQ ID NOs are present and can function together (they may or may not be fused to each other, optionally with an intervening linker, or bridged, e.g., by a covalent bond). For example, SEQ ID NOs 32 and 33 are VL and VH domains that can function together to form a cytokine-binding immunoglobulin domain, as are SEQ ID NOs 748 and 749. SEQ ID NOs 256 and 257 are Fc polypeptide chains for forming a heterodimeric knob-into-hole Fc that can serve as a pharmacokinetic modulator. The components may be arranged in any manner consistent with the disclosure, e.g., as indicated elsewhere herein. In some embodiments, a cytokine prodrug comprises a combination of sequences as set forth in Table 3A.

Table 3A. Exemplary IL-2 prodrugs

CY	CL	IN	PM
1-2	80-90 or 201-242	10-16, 30, 31, 40-51, 747, (32 and 33), or (748 and 749)	
1-2	80-90 or 201-242	10-16, 30, 31, 40-51, 747, (32 and 33), or (748 and 749)	70-74 or (756 and 757)
1	80-90 or 201-242	10	
1	80-90 or 201-242	11	
1	80-90 or 201-242	12	
1	80-90 or 201-242	13	
1	80-90 or 201-242	14	
1	80-90 or 201-242	15	
1	80-90 or 201-242	16	
1	80-90 or 201-242	20	
1	80-90 or 201-242	21	
1	80-90 or 201-242	22	
1	80-90 or 201-242	23	
1	80-90 or 201-242	24	
1	80-90 or 201-242	25	
1	80-90 or 201-242	26	
1	80-90 or 201-242	27	
1	80-90 or 201-242	28	
1	80-90 or 201-242	29	
1	80-90 or 201-242	30	
1	80-90 or 201-242	31	
1	80-90 or 201-242	32 and 33	
1	80-90 or 201-242	40	

1	80-90 or 201-242	41	
1	80-90 or 201-242	42	
1	80-90 or 201-242	43	
1	80-90 or 201-242	44	
1	80-90 or 201-242	45	
1	80-90 or 201-242	46	
1	80-90 or 201-242	47	
1	80-90 or 201-242	48	
1	80-90 or 201-242	49	
1	80-90 or 201-242	50	
1	80-90 or 201-242	51	
1	80-90 or 201-242	747	
1	80-90 or 201-242	748 and 749	
1	80-90 or 201-242	10	70
1	80-90 or 201-242	10	71
1	80-90 or 201-242	10	72
1	80-90 or 201-242	10	73
1	80-90 or 201-242	10	74
1	80-90 or 201-242	10	756 and 757
1	80-90 or 201-242	11	70
1	80-90 or 201-242	11	71
1	80-90 or 201-242	11	72
1	80-90 or 201-242	11	73
1	80-90 or 201-242	11	74

1	80-90 or 201-242	11	756 and 757
1	80-90 or 201-242	12	70
1	80-90 or 201-242	12	71
1	80-90 or 201-242	12	72
1	80-90 or 201-242	12	73
1	80-90 or 201-242	12	74
1	80-90 or 201-242	12	756 and 757
1	80-90 or 201-242	13	70
1	80-90 or 201-242	13	71
1	80-90 or 201-242	13	72
1	80-90 or 201-242	13	73
1	80-90 or 201-242	13	74
1	80-90 or 201-242	13	756 and 757
1	80-90 or 201-242	14	70
1	80-90 or 201-242	14	71
1	80-90 or 201-242	14	72
1	80-90 or 201-242	14	73
1	80-90 or 201-242	14	74
1	80-90 or 201-242	14	756 and 757
1	80-90 or 201-242	15	70
1	80-90 or 201-242	15	71
1	80-90 or 201-242	15	72
1	80-90 or 201-242	15	73
1	80-90 or 201-242	15	74

1	80-90 or 201-242	15	756 and 757
1	80-90 or 201-242	16	70
1	80-90 or 201-242	16	71
1	80-90 or 201-242	16	72
1	80-90 or 201-242	16	73
1	80-90 or 201-242	16	74
1	80-90 or 201-242	16	756 and 757
1	80-90 or 201-242	20	70
1	80-90 or 201-242	20	71
1	80-90 or 201-242	20	72
1	80-90 or 201-242	20	73
1	80-90 or 201-242	20	74
1	80-90 or 201-242	20	756 and 757
1	80-90 or 201-242	21	70
1	80-90 or 201-242	21	71
1	80-90 or 201-242	21	72
1	80-90 or 201-242	21	73
1	80-90 or 201-242	21	74
1	80-90 or 201-242	21	756 and 757
1	80-90 or 201-242	22	70
1	80-90 or 201-242	22	71
1	80-90 or 201-242	22	72
1	80-90 or 201-242	22	73
1	80-90 or 201-242	22	74

1	80-90 or 201-242	22	756 and 757
1	80-90 or 201-242	23	70
1	80-90 or 201-242	23	71
1	80-90 or 201-242	23	72
1	80-90 or 201-242	23	73
1	80-90 or 201-242	23	74
1	80-90 or 201-242	23	756 and 757
1	80-90 or 201-242	24	70
1	80-90 or 201-242	24	71
1	80-90 or 201-242	24	72
1	80-90 or 201-242	24	73
1	80-90 or 201-242	24	74
1	80-90 or 201-242	24	756 and 757
1	80-90 or 201-242	25	70
1	80-90 or 201-242	25	71
1	80-90 or 201-242	25	72
1	80-90 or 201-242	25	73
1	80-90 or 201-242	25	74
1	80-90 or 201-242	25	756 and 757
1	80-90 or 201-242	26	70
1	80-90 or 201-242	26	71
1	80-90 or 201-242	26	72
1	80-90 or 201-242	26	73
1	80-90 or 201-242	26	74

1	80-90 or 201-242	26	756 and 757
1	80-90 or 201-242	27	70
1	80-90 or 201-242	27	71
1	80-90 or 201-242	27	72
1	80-90 or 201-242	27	73
1	80-90 or 201-242	27	74
1	80-90 or 201-242	27	756 and 757
1	80-90 or 201-242	28	70
1	80-90 or 201-242	28	71
1	80-90 or 201-242	28	72
1	80-90 or 201-242	28	73
1	80-90 or 201-242	28	74
1	80-90 or 201-242	28	756 and 757
1	80-90 or 201-242	29	70
1	80-90 or 201-242	29	71
1	80-90 or 201-242	29	72
1	80-90 or 201-242	29	73
1	80-90 or 201-242	29	74
1	80-90 or 201-242	29	756 and 757
1	80-90 or 201-242	30	70
1	80-90 or 201-242	30	71
1	80-90 or 201-242	30	72
1	80-90 or 201-242	30	73
1	80-90 or 201-242	30	74

1	80-90 or 201-242	30	756 and 757
1	80-90 or 201-242	31	70
1	80-90 or 201-242	31	71
1	80-90 or 201-242	31	72
1	80-90 or 201-242	31	73
1	80-90 or 201-242	31	74
1	80-90 or 201-242	31	756 and 757
1	80-90 or 201-242	32 and 33	70
1	80-90 or 201-242	32 and 33	71
1	80-90 or 201-242	32 and 33	72
1	80-90 or 201-242	32 and 33	73
1	80-90 or 201-242	32 and 33	74
1	80-90 or 201-242	32 and 33	756 and 757
1	80-90 or 201-242	40	70
1	80-90 or 201-242	40	71
1	80-90 or 201-242	40	72
1	80-90 or 201-242	40	73
1	80-90 or 201-242	40	74
1	80-90 or 201-242	40	756 and 757
1	80-90 or 201-242	41	70
1	80-90 or 201-242	41	71
1	80-90 or 201-242	41	72
1	80-90 or 201-242	41	73
1	80-90 or 201-242	41	74

1	80-90 or 201-242	41	756 and 757
1	80-90 or 201-242	42	70
1	80-90 or 201-242	42	71
1	80-90 or 201-242	42	72
1	80-90 or 201-242	42	73
1	80-90 or 201-242	42	74
1	80-90 or 201-242	42	756 and 757
1	80-90 or 201-242	43	70
1	80-90 or 201-242	43	71
1	80-90 or 201-242	43	72
1	80-90 or 201-242	43	73
1	80-90 or 201-242	43	74
1	80-90 or 201-242	43	756 and 757
1	80-90 or 201-242	44	70
1	80-90 or 201-242	44	71
1	80-90 or 201-242	44	72
1	80-90 or 201-242	44	73
1	80-90 or 201-242	44	74
1	80-90 or 201-242	44	756 and 757
1	80-90 or 201-242	45	70
1	80-90 or 201-242	45	71
1	80-90 or 201-242	45	72
1	80-90 or 201-242	45	73
1	80-90 or 201-242	45	74

1	80-90 or 201-242	45	756 and 757
1	80-90 or 201-242	46	70
1	80-90 or 201-242	46	71
1	80-90 or 201-242	46	72
1	80-90 or 201-242	46	73
1	80-90 or 201-242	46	74
1	80-90 or 201-242	46	756 and 757
1	80-90 or 201-242	47	70
1	80-90 or 201-242	47	71
1	80-90 or 201-242	47	72
1	80-90 or 201-242	47	73
1	80-90 or 201-242	47	74
1	80-90 or 201-242	47	756 and 757
1	80-90 or 201-242	48	70
1	80-90 or 201-242	48	71
1	80-90 or 201-242	48	72
1	80-90 or 201-242	48	73
1	80-90 or 201-242	48	74
1	80-90 or 201-242	48	756 and 757
1	80-90 or 201-242	49	70
1	80-90 or 201-242	49	71
1	80-90 or 201-242	49	72
1	80-90 or 201-242	49	73
1	80-90 or 201-242	49	74

1	80-90 or 201-242	49	756 and 757
1	80-90 or 201-242	50	70
1	80-90 or 201-242	50	71
1	80-90 or 201-242	50	72
1	80-90 or 201-242	50	73
1	80-90 or 201-242	50	74
1	80-90 or 201-242	50	756 and 757
1	80-90 or 201-242	51	70
1	80-90 or 201-242	51	71
1	80-90 or 201-242	51	72
1	80-90 or 201-242	51	73
1	80-90 or 201-242	51	74
1	80-90 or 201-242	51	756 and 757
1	80-90 or 201-242	747	70
1	80-90 or 201-242	747	71
1	80-90 or 201-242	747	72
1	80-90 or 201-242	747	73
1	80-90 or 201-242	747	74
1	80-90 or 201-242	747	756 and 757
1	80-90 or 201-242	748 and 749	70
1	80-90 or 201-242	748 and 749	71
1	80-90 or 201-242	748 and 749	72
1	80-90 or 201-242	748 and 749	73
1	80-90 or 201-242	748 and 749	74

1	80-90 or 201-242	748 and 749	756 and 757
2	80-90 or 201-242	10	
2	80-90 or 201-242	11	
2	80-90 or 201-242	12	
2	80-90 or 201-242	13	
2	80-90 or 201-242	14	
2	80-90 or 201-242	15	
2	80-90 or 201-242	16	
2	80-90 or 201-242	20	
2	80-90 or 201-242	21	
2	80-90 or 201-242	22	
2	80-90 or 201-242	23	
2	80-90 or 201-242	24	
2	80-90 or 201-242	25	
2	80-90 or 201-242	26	
2	80-90 or 201-242	27	
2	80-90 or 201-242	28	
2	80-90 or 201-242	29	
2	80-90 or 201-242	30	
2	80-90 or 201-242	31	
2	80-90 or 201-242	32 and 33	
2	80-90 or 201-242	40	
2	80-90 or 201-242	41	
2	80-90 or 201-242	42	

2	80-90 or 201-242	43	
2	80-90 or 201-242	44	
2	80-90 or 201-242	45	
2	80-90 or 201-242	46	
2	80-90 or 201-242	47	
2	80-90 or 201-242	48	
2	80-90 or 201-242	49	
2	80-90 or 201-242	50	
2	80-90 or 201-242	51	
2	80-90 or 201-242	747	
2	80-90 or 201-242	748 and 749	
2	80-90 or 201-242	10	70
2	80-90 or 201-242	10	71
2	80-90 or 201-242	10	72
2	80-90 or 201-242	10	73
2	80-90 or 201-242	10	74
2	80-90 or 201-242	10	756 and 757
2	80-90 or 201-242	11	70
2	80-90 or 201-242	11	71
2	80-90 or 201-242	11	72
2	80-90 or 201-242	11	73
2	80-90 or 201-242	11	74
2	80-90 or 201-242	11	756 and 757
2	80-90 or 201-242	12	70

2	80-90 or 201-242	12	71
2	80-90 or 201-242	12	72
2	80-90 or 201-242	12	73
2	80-90 or 201-242	12	74
2	80-90 or 201-242	12	756 and 757
2	80-90 or 201-242	13	70
2	80-90 or 201-242	13	71
2	80-90 or 201-242	13	72
2	80-90 or 201-242	13	73
2	80-90 or 201-242	13	74
2	80-90 or 201-242	13	756 and 757
2	80-90 or 201-242	14	70
2	80-90 or 201-242	14	71
2	80-90 or 201-242	14	72
2	80-90 or 201-242	14	73
2	80-90 or 201-242	14	74
2	80-90 or 201-242	14	756 and 757
2	80-90 or 201-242	15	70
2	80-90 or 201-242	15	71
2	80-90 or 201-242	15	72
2	80-90 or 201-242	15	73
2	80-90 or 201-242	15	74
2	80-90 or 201-242	15	756 and 757
2	80-90 or 201-242	16	70

2	80-90 or 201-242	16	71
2	80-90 or 201-242	16	72
2	80-90 or 201-242	16	73
2	80-90 or 201-242	16	74
2	80-90 or 201-242	16	756 and 757
2	80-90 or 201-242	20	70
2	80-90 or 201-242	20	71
2	80-90 or 201-242	20	72
2	80-90 or 201-242	20	73
2	80-90 or 201-242	20	74
2	80-90 or 201-242	20	756 and 757
2	80-90 or 201-242	21	70
2	80-90 or 201-242	21	71
2	80-90 or 201-242	21	72
2	80-90 or 201-242	21	73
2	80-90 or 201-242	21	74
2	80-90 or 201-242	21	756 and 757
2	80-90 or 201-242	22	70
2	80-90 or 201-242	22	71
2	80-90 or 201-242	22	72
2	80-90 or 201-242	22	73
2	80-90 or 201-242	22	74
2	80-90 or 201-242	22	756 and 757
2	80-90 or 201-242	23	70

2	80-90 or 201-242	23	71
2	80-90 or 201-242	23	72
2	80-90 or 201-242	23	73
2	80-90 or 201-242	23	74
2	80-90 or 201-242	23	756 and 757
2	80-90 or 201-242	24	70
2	80-90 or 201-242	24	71
2	80-90 or 201-242	24	72
2	80-90 or 201-242	24	73
2	80-90 or 201-242	24	74
2	80-90 or 201-242	24	756 and 757
2	80-90 or 201-242	25	70
2	80-90 or 201-242	25	71
2	80-90 or 201-242	25	72
2	80-90 or 201-242	25	73
2	80-90 or 201-242	25	74
2	80-90 or 201-242	25	756 and 757
2	80-90 or 201-242	26	70
2	80-90 or 201-242	26	71
2	80-90 or 201-242	26	72
2	80-90 or 201-242	26	73
2	80-90 or 201-242	26	74
2	80-90 or 201-242	26	756 and 757
2	80-90 or 201-242	27	70

2	80-90 or 201-242	27	71
2	80-90 or 201-242	27	72
2	80-90 or 201-242	27	73
2	80-90 or 201-242	27	74
2	80-90 or 201-242	27	756 and 757
2	80-90 or 201-242	28	70
2	80-90 or 201-242	28	71
2	80-90 or 201-242	28	72
2	80-90 or 201-242	28	73
2	80-90 or 201-242	28	74
2	80-90 or 201-242	28	756 and 757
2	80-90 or 201-242	29	70
2	80-90 or 201-242	29	71
2	80-90 or 201-242	29	72
2	80-90 or 201-242	29	73
2	80-90 or 201-242	29	74
2	80-90 or 201-242	29	756 and 757
2	80-90 or 201-242	30	70
2	80-90 or 201-242	30	71
2	80-90 or 201-242	30	72
2	80-90 or 201-242	30	73
2	80-90 or 201-242	30	74
2	80-90 or 201-242	30	756 and 757
2	80-90 or 201-242	31	70

2	80-90 or 201-242	31	71
2	80-90 or 201-242	31	72
2	80-90 or 201-242	31	73
2	80-90 or 201-242	31	74
2	80-90 or 201-242	31	756 and 757
2	80-90 or 201-242	32 and 33	70
2	80-90 or 201-242	32 and 33	71
2	80-90 or 201-242	32 and 33	72
2	80-90 or 201-242	32 and 33	73
2	80-90 or 201-242	32 and 33	74
2	80-90 or 201-242	32 and 33	756 and 757
2	80-90 or 201-242	40	70
2	80-90 or 201-242	40	71
2	80-90 or 201-242	40	72
2	80-90 or 201-242	40	73
2	80-90 or 201-242	40	74
2	80-90 or 201-242	40	756 and 757
2	80-90 or 201-242	41	70
2	80-90 or 201-242	41	71
2	80-90 or 201-242	41	72
2	80-90 or 201-242	41	73
2	80-90 or 201-242	41	74
2	80-90 or 201-242	41	756 and 757
2	80-90 or 201-242	42	70

2	80-90 or 201-242	42	71
2	80-90 or 201-242	42	72
2	80-90 or 201-242	42	73
2	80-90 or 201-242	42	74
2	80-90 or 201-242	42	756 and 757
2	80-90 or 201-242	43	70
2	80-90 or 201-242	43	71
2	80-90 or 201-242	43	72
2	80-90 or 201-242	43	73
2	80-90 or 201-242	43	74
2	80-90 or 201-242	43	756 and 757
2	80-90 or 201-242	44	70
2	80-90 or 201-242	44	71
2	80-90 or 201-242	44	72
2	80-90 or 201-242	44	73
2	80-90 or 201-242	44	74
2	80-90 or 201-242	44	756 and 757
2	80-90 or 201-242	45	70
2	80-90 or 201-242	45	71
2	80-90 or 201-242	45	72
2	80-90 or 201-242	45	73
2	80-90 or 201-242	45	74
2	80-90 or 201-242	45	756 and 757
2	80-90 or 201-242	46	70

2	80-90 or 201-242	46	71
2	80-90 or 201-242	46	72
2	80-90 or 201-242	46	73
2	80-90 or 201-242	46	74
2	80-90 or 201-242	46	756 and 757
2	80-90 or 201-242	47	70
2	80-90 or 201-242	47	71
2	80-90 or 201-242	47	72
2	80-90 or 201-242	47	73
2	80-90 or 201-242	47	74
2	80-90 or 201-242	47	756 and 757
2	80-90 or 201-242	48	70
2	80-90 or 201-242	48	71
2	80-90 or 201-242	48	72
2	80-90 or 201-242	48	73
2	80-90 or 201-242	48	74
2	80-90 or 201-242	48	756 and 757
2	80-90 or 201-242	49	70
2	80-90 or 201-242	49	71
2	80-90 or 201-242	49	72
2	80-90 or 201-242	49	73
2	80-90 or 201-242	49	74
2	80-90 or 201-242	49	756 and 757
2	80-90 or 201-242	50	70

2	80-90 or 201-242	50	71
2	80-90 or 201-242	50	72
2	80-90 or 201-242	50	73
2	80-90 or 201-242	50	74
2	80-90 or 201-242	50	756 and 757
2	80-90 or 201-242	51	70
2	80-90 or 201-242	51	71
2	80-90 or 201-242	51	72
2	80-90 or 201-242	51	73
2	80-90 or 201-242	51	74
2	80-90 or 201-242	51	756 and 757
2	80-90 or 201-242	747	70
2	80-90 or 201-242	747	71
2	80-90 or 201-242	747	72
2	80-90 or 201-242	747	73
2	80-90 or 201-242	747	74
2	80-90 or 201-242	747	756 and 757
2	80-90 or 201-242	748 and 749	70
2	80-90 or 201-242	748 and 749	71
2	80-90 or 201-242	748 and 749	72
2	80-90 or 201-242	748 and 749	73
2	80-90 or 201-242	748 and 749	74
2	80-90 or 201-242	748 and 749	756 and 757

[00237] Additionally, any cytokine prodrug described herein, in Table 3A or elsewhere, may further comprise a targeting sequence, such as any of the targeting sequences

described herein. In some embodiments, the targeting sequence is any one of SEQ ID NOs: 180-662.

[00238] Additionally, any one of the cytokine prodrugs described in Table 3A may comprise a consensus sequence according to any one of SEQ ID NOs: 91-94 in place of the listed protease-cleavable sequences.

[00239] Also encompassed by this disclosure are cytokine prodrugs comprising a sequence with at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of the cytokine prodrugs described above.

[00240] In some embodiments, the cytokine prodrug comprises a sequence with at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 100-111. In some embodiments, the cytokine prodrug comprises the sequence of any one of SEQ ID NOs: 100-111. In some embodiments, the cytokine prodrug comprises the sequence of any one of SEQ ID NOs: 803-852.

Combinations of a protease-cleavable sequence and a targeting sequence

[00241] Any compatible embodiment of a cytokine prodrug described herein, in Table 3A or elsewhere, may comprise a combination of a protease-cleavable sequence and a targeting sequence set forth in Table 4. Where a range is given, any one of the listed SEQ ID NOs may be selected. The components may be arranged in any manner consistent with the disclosure, e.g., as indicated elsewhere herein (e.g., Figs. 9 and 10A-E and the section regarding Arrangement of components).

Table 4. Exemplary combinations of protease-cleavable sequence and targeting sequence

Protease-cleavable sequence	Targeting sequence
80	180-662
81	180-662
82	180-662
83	180-662
84	180-662
85	180-662
86	180-662
87	180-662
88	180-662
89	180-662
90	180-662
91	180-662
92	180-662
93	180-662
94	180-662

700	180-662
701	180-662
702	180-662
703	180-662
704	180-662
705	180-662
706	180-662
707	180-662
708	180-662
709	180-662
710	180-662
711	180-662
712	180-662
713	180-662
714	180-662
715	180-662
716	180-662
717	180-662
718	180-662
719	180-662
720	180-662
721	180-662
722	180-662
723	180-662
724	180-662
725	180-662
726	180-662
727	180-662
728	180-662
729	180-662
730	180-662
731	180-662
732	180-662
733	180-662
734	180-662
735	180-662
736	180-662
737	180-662
738	180-662
739	180-662
740	180-662
741	180-662

[00242] Also encompassed by this disclosure are cytokine prodrugs comprising a sequence with at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of the cytokine prodrugs described above.

Pharmaceutical formulations

[00243] Pharmaceutical formulations of a cytokine prodrug as described herein may be prepared by mixing such cytokine prodrug having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[00244] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

Uses

[00245] In some embodiments, any one or more of the cytokine prodrugs, compositions, or pharmaceutical formulations described herein is for use in preparing a medicament for treating or preventing a disease or disorder in a subject. In some embodiments, any one or more of the cytokine prodrugs, compositions, or pharmaceutical formulations described herein is for use in a method of creating a cytokine gradient in a subject, comprising administering the protease-activated pro-cytokine or pharmaceutical composition to a subject, wherein the subject comprises a site having an abnormally high level of a protease that cleaves the protease-cleavable polypeptide sequence, optionally wherein the site comprises a cancer. In some embodiments, the abnormally high level is higher than the level of the protease in a healthy tissue of the same type as the site with the abnormally high level (*e.g.*, in the subject being treated or in a healthy subject). In some

embodiments, the abnormally high level is higher than the average level of the protease in soft tissue.

[00246] In some embodiments, a method of treating or preventing a disease or disorder in subject is provided, comprising administering to a subject any of the cytokine prodrugs or pharmaceutical compositions described herein. In some embodiments, the disease or disorder is a cancer, e.g., a solid tumor. In some embodiments, the cancer is a melanoma, a colorectal cancer, a breast cancer, a pancreatic cancer, a lung cancer, a prostate cancer, an ovarian cancer, a cervical cancer, a gastric or gastrointestinal cancer, a lymphoma, a colon or colorectal cancer, an endometrial cancer, a thyroid cancer, or a bladder cancer. The cancer (e.g., any of the foregoing cancers) may have one or more of the following features: being PD-L1-positive; being metastatic; being unresectable; being mismatch repair defective (MMRd); and/or being microsatellite-instability high (MSI-H).

[00247] In some embodiments, a method of boosting T regulatory cells and/or reducing inflammation or autoimmune activity is provided comprising administering a cytokine prodrug to an area of interest, e.g., an area of inflammation. The cytokine prodrug for use in such methods may comprise an IL-2 polypeptide sequence. In some embodiments, a method of treating an autoimmune and/or inflammatory disease is provided, comprising administering a cytokine prodrug to an area of interest, e.g., an area of inflammation or autoimmune activity. The cytokine prodrug for use in such methods may comprise an IL-2 polypeptide sequence. These methods take advantage of the ability of certain cytokines at relatively low levels to stimulate T regulatory cells, which can exert anti-inflammatory effects and reduce or suppress autoimmune activity.

[00248] The cytokine prodrugs in any of the foregoing methods and uses may be delivered to a subject using any suitable route of administration. In some embodiments, the cytokine prodrug is delivered parenterally. In some embodiments, the cytokine prodrug is delivered intravenously.

[00249] A cytokine prodrug provided herein can be used either alone or in combination with other agents in a therapy. For instance, a cytokine prodrug provided herein may be co-administered with at least one additional therapeutic agent.

[00250] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the cytokine prodrug provided herein can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

[00251] Cytokine prodrugs would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The cytokine prodrug need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of cytokine prodrug present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[00252] For the prevention or treatment of disease, the appropriate dosage of an cytokine prodrug (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of cytokine prodrug, the severity and course of the disease, whether the cytokine prodrug is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the discretion of the attending physician. The cytokine prodrug is suitably administered to the patient at one time or over a series of treatments.

Nucleic acids, host cells, and production methods

[00253] Cytokine prodrugs or precursors thereof may be produced using recombinant methods and compositions. In some embodiments, isolated nucleic acid encoding a cytokine prodrug described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the cytokine polypeptide sequence, the linker, and the inhibitory polypeptide sequence, and any other polypeptide components of the cytokine prodrug that may be present. Exemplary nucleic acid sequences are provided in Table 1. In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In some such embodiments, a host cell comprises (e.g., has been transformed with) a vector comprising a nucleic acid that encodes a cytokine prodrug according to the disclosure. In some embodiments, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In some embodiments, a method of making a

cytokine prodrug disclosed herein is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the cytokine prodrug, as provided above, under conditions suitable for expression of the cytokine prodrug, and optionally recovering the antibody from the host cell (or host cell culture medium).

[00254] For recombinant production of a cytokine prodrug, nucleic acid encoding the cytokine prodrug, e.g., as described above, is prepared and/or isolated (e.g., following construction using synthetic and/or molecular cloning techniques) and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily prepared and/or isolated using known techniques.

[00255] Suitable host cells for cloning or expression of cytokine prodrug-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, a cytokine prodrug may be produced in bacteria, in particular when glycosylation is not needed. For expression of polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. After expression, the cytokine prodrug may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[00256] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for cytokine prodrug-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of polypeptides with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[00257] Suitable host cells for the expression of cytokine prodrugs are also derived from multicellular organisms (plants, invertebrates, and vertebrates). Examples of invertebrate cells include insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[00258] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429.

[00259] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green

monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0.

[00260] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about,” to the extent they are not already so modified. “About” indicates a degree of variation that does not substantially affect the properties of the described subject matter, e.g., within 10%, 5%, 2%, or 1%. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

EXAMPLES

[00261] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1: Construction of mammalian expression vectors encoding fusion proteins.

[00262] Coding sequences for all protein domains including linker sequences were synthesized as an entire gene (Genscript, NJ). All synthetic genes were designed to contain a coding sequence for an N-terminal signal peptide (to facilitate protein secretion), a 5' Kozak sequence, and unique restriction sites at the 5' and 3' ends. These genes were then directionally cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA). Examples of fusion protein constructs are listed in table 5A. Site directed mutagenesis

was performed using standard molecular biology techniques and appropriate kit (GeneArt, Regensburg).

[00263] Table 5A. Exemplary cytokine prodrug constructs

Name	SEQ ID NO	Features
Construct A	100	mIL2- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – 6His
Construct B	101	m IL2-2x(SG4) – MMPcs1 – 2x (G4S) – IL2Ralpha – mIgG1 Fc
Construct C	102	m IL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – mIgG1 Fc(T252M)- 6xHIS
Construct D	104	m IL2(C140S)- 2x(SG4) – MMPcs1 – 2x(G4S) – soluble IL2Ralpha – mIgG1 Fc(T252M)- 6xHIS
Construct E	106	Hu IL2(C125S)- 2x(SG4) – MMPcs1 – 2x(G4S) – IL2Ralpha – hu IgG1 Fc – 6xHIS
Construct F	803	h IL2(C125S)-2x(SG4) -MMPcs1-2x(G4S)-chimeric IL2Ra(sushi mouse)-hIgG1 Fc
Construct G	804	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-219)- GSGGGG-hu IgG1 Fc
Construct H	805	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-178)- GSGGGG-hu IgG1 Fc
Construct V	806	hIL2 (C125S)-2x(SG4)-MMPcs1-4x(G4S)-hIL2Ra (1-219)- GSGGGG-hu IgG1 Fc
Construct W	807	hIL2 (C125S)-2x(SG4)-MMPcs1-6x(G4S)-hIL2Ra (1-219)- GSGGGG-hu IgG1 Fc
Construct I	837	h IL2(C125S)-2x(SG4) -MMPcs1-2x(G4S)-hIL2Ra(1-219; M25I)- hIgG1 Fc-6xHis
Construct J	838	h IL2(C125S)-2x(SG4) -MMPcs1-2x(G4S)-hIL2Ra(1-219;L42V)- hIgG1 Fc-6xHis
Construct X	808	m IL2(C140S)- 2x(SG4) – MMPik – 2x(G4S) – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct Y	809	m IL2(C140S)– VRIQRKKEKMKET- MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct Z	810	m IL2- 2x(SG4) – MMPlk – 2x(G4S) – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct AA	811	m IL2-SGG – FHRRIKA- MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct	812	m IL2-SGG – FHRRIKA- MMPscr-2x(G4S)-mIL2Ra (1-215)- mu

BB		IgG1 Fc
Construct CC	813	m IL2 – 2x(GHHPH)- MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct DD	814	m IL2 – 2x(GHHPH)- MMPscr-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct EE	815	m IL2 - SGG– GGWSHW- MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct FF	816	m IL2 -SGG– GGWSHW- MMPscr-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct GG	817	m IL2 -SGG– KLWVLPK- MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct HH	818	m IL2 -SGG– KLWVLPK- MMPscr-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct II	819	m IL2 – LHERHLNNN - MMPcs1-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct JJ	820	m IL2 – LHERHLNNN - MMPscr-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct KK	821	m IL2 – VRIQRKKEKMKET- MMPscr-2x(G4S)-mIL2Ra (1-215)- mu IgG1 Fc
Construct LL	822	m IL2- 2x(SG4) – MMPcs1 – FHRRIKAGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct MM	823	m IL2- 2x(SG4) – MMPscr – FHRRIKAGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct NN	824	m IL2 –2x(SG4)-MMPcs1-2x(GHHPH)-mIL2Ra (1-215)- mu IgG1 Fc
Construct OO	825	m IL2 –2x(SG4)-MMPscr-2x(GHHPH)-mIL2Ra (1-215)- mu IgG1 Fc
Construct PP	826	m IL2- 2x(SG4) – MMPcs1 – GGWSHWGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct QQ	827	m IL2- 2x(SG4) – MMPscr – GGWSHWGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct RR	828	m IL2- 2x(SG4) – MMPcs1 – KLWVLPKGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct SS	829	m IL2- 2x(SG4) – MMPscr– KLWVLPKGGS – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct TT	830	m IL2- 2x(SG4) – MMPcs1 – LHERHLNNNG – mIL2Ralpha (1-215)– mu IgG1 Fc
Construct	831	m IL2- 2x(SG4) – MMPscr – LHERHLNNNG – mIL2Ralpha (1-

UU		215)– mu IgG1 Fc
Construct VV	832	m IL2 – SGGGGGHHHPH- MMPcs1- 2x(G4S)-mIL2Ra- mu IgG1 Fc
Construct WW	833	m IL2 – GHHPHSGGGG- MMPcs1-2x(G4S)-mIL2Ra- mu IgG1 Fc
Construct XX	834	m IL2 –2x(SG4)-MMPcs1-GHHPHGGGGS-mIL2Ra- mu IgG1 Fc
Construct YY	835	m IL2 –2x(SG4)-MMPcs1-2x(G4S)-mIL2Ra- mu IgG1 Fc- 2x(GHHPH)
Construct ZZ	836	m IL2 –2x(SG4)-MMPcs1-2x(G4S)-mIL2Ra- mu IgG1 Fc- (GHHPH)
Construct L	840	h IL2(C125S)-2x(SG4) -MMPcs1-2x(G4S)-hIL2Ra(1-219; SGSL39-42ELV)-hIgG1 Fc-6xHis
Construct K	839	h IL2(C125S)-2x(SG4) -MMPcs1-2x(G4S)-hIL2Ra(1-219; DD4-5LY)-hIgG1 Fc- 6xHis
Construct M	841	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-192)-hu IgG1 Fc-6xHis
Construct N	842	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-192)- GSGGGG-hu IgG1 Fc -6xHis
Construct O	843	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-192/M25I)- GSGGGG-hu IgG1 Fc -6xHis
Construct P	844	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-192/L42V)- GSGGGG-hu IgG1 Fc - 6xHis
Construct Q	845	hIL2 (C125S)-2x(SG4)-MMPcs1-2x(G4S)-hIL2Ra (1-192/D4L, D5Y)-GSGGGG-hu IgG1 Fc - 6xHis
Construct AAA	846	h IL2 (C125S)– 2x(SG4)- MMPcs1-2x(G4S)-hIL2Ra(M25I)- GSGGGG- hu IgG1 Fc (LALA)
Construct BBB	847	h IL2 (C125S)– 2x(SG4)- MMPscr-2x(G4S)-hIL2Ra(M25I)- GSGGGG- hu IgG1 Fc (LALA)
Construct CCC	848	h IL2 (C125S)– 2x(GHHPH)- MMPscr-2x(G4S)-hIL2Ra(M25I)- GSGGGG- hu IgG1 Fc (LALA)
Construct DDD	849	h IL2 (C125S)– 2x(GHHPH)- MMPcs1-2x(G4S)-hIL2Ra(M25I)- GSGGGG- hu IgG1 Fc (LALA)
Construct EEE	850	h IL2 (C125S)– VRIQRKKEKMKET- MMPcs1-2x(G4S)- hIL2Ra(M25I)-GSGGGG- hu IgG1 Fc (LALA)
Construct FFF	851	h IL2 (C125S)– VRIQRKKEKMKET- MMPscr-2x(G4S)- hIL2Ra(M25I)-GSGGGG- hu IgG1 Fc (LALA)
Construct	852	m IL2(C140S)- 2x(SG4) – MMPscr– 2x(G4S) – mIL2Ralpha (1-

GGG		215)– mIgG1 Fc
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Example 2: Expression and purification of fusion proteins.

Transient expression of fusion proteins

[00264] Different mammalian cell expression systems were used to produce fusion proteins (ExpiCHO-S™, Expi293F™ and Freestyle CHO-S™, Life Technologies). Briefly, expression constructs were transiently transfected into cells following manufacturer’s protocol and using reagents provided in respective expression kits. Fusion proteins were then expressed and secreted into the cell culture supernatant. Samples were collected from the production cultures every day and cell density and viability were assessed. Protein expression titers and product integrity in cell culture supernatants were analyzed by SDS-PAGE to determine the optimal harvesting time. Cell culture supernatants were generally harvested between 4 and 12 days at culture viabilities of typically >75%. On day of harvest, cell culture supernatants were cleared by centrifugation and vacuum filtration before further use.

Purification of fusion proteins

[00265] Fusion proteins were purified from cell culture supernatants in either a one-step or two-step procedure. Briefly, Fc domain containing proteins were purified by Protein A affinity chromatography (HiTrap MabSelect SuRe, GE Healthcare). His-tagged proteins were first purified on a Nickel-agarose column (Ni-NTA Agarose, Qiagen), followed by anion ion exchange chromatography (HiTrap Capto Q ImpRes, Sigma). All purified samples were buffer-exchanged and concentrated by ultrafiltration to a typical concentration of > 1 mg/mL. Purity and homogeneity (typically >90%) of final samples were assessed by SDS PAGE under reducing and non-reducing conditions, followed by immunoblotting using an anti-His or anti-Fc antibody. Purified proteins were aliquoted and stored at -80°C until further use. Fig. 1 shows examples of successfully purified fusion proteins.

Example 3: Cleavage of fusion protein by MMP proteases

[00266] Recombinant MMP9 and/or MMP2 (R&D Systems) was first activated with p-aminophenylmercuric acetate and this activated protease or equivalent amount of activating solution without the protease was used to digest or mock digest the fusion protein for 1 hr, 2hr, 4 hr and overnight (18-22 hr) at 37 C. Cleavage assays are set up in TCNB buffer: 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% Brij-35 (w/v), pH 7.5. Digested protein was

aliquoted and stored at -80°C prior to testing. Aliquots of digests were subsequently analyzed by SDS-PAGE followed by Western blotting to evaluate the extent of cleavage. Digests were also assessed in functional assays such as CTLL-2 proliferation and HEK-Blue Interleukin reporter assays. As shown in **Figs. 2A-E**, essentially complete cleavage by MMP9 protease of the fusion proteins with functional site is seen after overnight incubation. In contrast, proteins containing a scrambled MMP cleavage site are not cut (Fig 2E).

Example 4: Detection of mouse IL-2 /IL-2Ra fusion proteins and mouse IL-2 by ELISA

[00267] We developed an ELISA assay to detect and quantify fusion proteins comprising IL-2 and IL-2Ra moieties. Wells of a 96-well plate are coated overnight with 100uL of a rat anti-mouse IL-2 monoclonal antibody (JES6-1A12; ThermoFisher) at 1 mg/ml in PBS. After washing, wells are blocked with TBS/0.05% Tween 20/ 1% BSA, then fusion proteins and/or unknown biological samples are added for 1hr at room temperature. After washing, an anti-mouse IL-2Ra biotin-labelled detection antibody (BAF2438, R&D systems) is added and binding is detected using Ultra Streptavidin HRP (ThermoFisher). The ELISA plate was developed by adding the chromogenic tetramethylbenzidine substrate (Ultra TMB, ThermoFisher). The reaction is stopped by addition of 0.5M H₂SO₄ and the absorbance is read at 450-650 nm.

[00268] We developed a second ELISA assay to detect and quantify mouse IL-2 and/or fusion proteins comprising an IL-2 moiety. Wells of a 96-well plate are coated overnight with 100uL of a rat anti-mouse IL-2 monoclonal antibody (JES6-1A12; ThermoFisher) at 1 mg/ml in PBS. After washing, wells are blocked with TBS/0.05% Tween 20/ 1% BSA, then fusion proteins and/or unknown biological samples are added for 1hr at room temperature. After washing, an anti-mouse IL-2 biotin-labelled detection antibody (JES6-5H4, ThermoFisher) is added and binding is detected using Ultra Streptavidin HRP (ThermoFisher). The ELISA plate was developed by adding the chromogenic tetramethylbenzidine substrate (Ultra TMB, ThermoFisher). The reaction is stopped by addition of 0.5M H₂SO₄ and the absorbance is read at 450-650 nm. This assay is able to simultaneously detect both free mouse IL-2 as well as mouse IL-2 in the context of pro-drug fusion proteins.

Example 5 : IL-2, IL-2Ra,6xHistidine and Fc Immunoblot analyses

[00269] Untreated and digested fusion proteins were evaluated for cleavage products by Western blot. The following monoclonal antibodies were used: rat anti-mouse IL-2 antibody (JES6-1A12; ThermoFisher), goat anti-mouse IL-2 polyclonal antibody (AF-402-NA; R&D systems), mouse anti-6xHis monoclonal antibody (MA1-21315, ThermoFisher), Anti-mIgG Fc HRP conjugated (ThermoFisher cat# A16084), and Anti-human IL2 antibody (Invitrogen, cat# MA5-17097, mouse IgG1). Detection was performed using either a goat anti-rat HRP-conjugated antibody, Donkey Anti-goat HRP-conjugated antibody or Goat Anti-mouse HRP conjugated (Jackson Immuno Research, West Grove, PA) and developed using the SuperSignal West Femto Maximum sensitivity detection reagent (ThermoFisher) following the manufacturer's recommendations.

Example 6: IL-2 functional cell-based assays

[00270] IL-2 activity was measured using either CTLL-2 cells (ATCC) or the reporter cell line HEK Blue IL2 (Invivogen, San Diego). In brief, for the CTLL-2 assay a titration of untreated and digested samples is added to 40 000 CTLL-2 cells per well in 100 ul medium in a 96-well plate and incubated at 37C in 5% CO2 for 18– 22 hr. At the end of this period, 50ug/well Thiazolyl Blue Tetrazolium Bromide (MTT) (Sigma-Aldrich) was added and the plate was incubated for 5 hr at 37C in 5% CO2. Cells were lysed with 100 ul/well 10% SDS (Sigma) acidified with HCl, incubated at 37C for 4hr, and absorbance was read at 570 nm. Recombinant human or mouse IL-2 (Peprotech and R&D systems respectively) was used as a positive control. Figs. 3A-B, 3K-L and 3N-P show examples of untreated and digested fusion proteins evaluated in CTLL-2 proliferation assay.

[00271] HEK-Blue™ IL-2 cells are specifically designed to monitor the activation of the JAK-STAT pathway induced by IL-2. Indeed, stimulation with human or murine IL-2 triggers the JAK/STAT5 pathway and induces secreted embryonic alkaline phosphatase (SEAP) production. SEAP can be readily monitored when using QUANTI-Blue™, a SEAP detection medium. These cells respond to human and murine IL-2. For the HEK Blue assay, untreated and digested samples are titrated and added to 50 000 HEK Blue cells per well in 200 ul medium in a 96-well plate and incubated at 37C in 5% CO2 for 20– 24 hr. The following day, levels of SEAP are measured by adding 20uL of cell supernatant to QuantiBlue reagent, followed by 1-3h incubation at 37C and reading absorbance at 630nm. Figures 3C-J, 3Q-Y and Table 5B-5C show results obtained from IL2 fusion proteins tested in HEK Blue IL2 assay.

[00272] Table 5B.

CANDIDATE	+ MMP EC50 (nM)	-MMP EC50 (nM)	FOLD DIFFERENCE EC50 (HEK BLUE IL2)
Construct E	0.0073	0.103	14
Construct L	0.0061	0.0453	7.3
Construct K	0.0055	0.0933	17
Construct J	0.0059	0.1264	21
Construct F	0.0078	0.1736	22
Construct I	0.0074	0.3165	43

[00273] Table 5C.

CANDIDATE	+ MMP EC50 (nM)	-MMP EC50 (nM)
Construct AA	0.01088	0.4423
Construct Y	0.0109	1.232
Construct CC	0.013	0.66
Construct EE	0.0066	1.18
Construct GG	0.0072	0.14
Construct II	0.009	0.489
Construct AAA	0.0089	0.23
Construct DDD	0.0094	0.181
Construct EEE	0.0069	0.149

[00274] Aggregation, stability, and homogeneity of Construct E, Construct M, and Construct N were compared using Coomassie-stained SDS-PAGE analysis (Fig. 3M). Construct M and Construct N showed decreased aggregation and greater stability and homogeneity, consistent with there being an improvement resulting from deletion of O-glycosylation sites.

Example 7: In vitro serum stability of fusion protein

[00275] Construct B was incubated at 37C for up to 72h with serum collected from 8 weeks old female C57BL/6 naive and MC38 tumor bearing mice respectively (n=2 per serum type, tumor volume > 3000mm³ at time of collection), in order to examine both non-specific cleavage as well as MMP-specific off-target cleavage. Samples were collected at 0h, 4h, 8h, 24h, 48h and 72h and the intact non-MMP cleaved fusion protein was quantified using an in-house developed sandwich ELISA. Results (see Fig. 4) show that the levels of fusion protein are stable in both serum types, indicating 1) a lack of off-target protein cleavage up to 72 hrs and 2) no active MMPs in circulation.

Example 8: Pharmacokinetic evaluation of fusion protein in non-tumor bearing mice

[00276] For this study, C57BL/6 8-10 weeks old female mice (Jackson Labs) were assigned to different groups (3 mice per treatment group). Mice received a single dose of fusion protein via IV injection (3,5mg/kg). 3 mice/group/time point were bled at the following time points: pre-dose (0h), 10 min, 30 min, 1h, 4h, 12h, 24h, 48h, 72h, 96h and 120h post dose. Blood samples were collected in Eppendorf tubes and processed to serum, then stored at -80C until testing. Samples were then evaluated by ELISA to quantify intact fusion protein levels. Mean serum concentrations of fusion protein were plotted over time and PK parameters were calculated using WinNonlin 7.0 (non-compartmental model) as shown in Fig 5.

Example 9: In vivo efficacy of fusion proteins in syngeneic MC38 colorectal cancer model

a. Intra-tumoral injection of Construct A

[00277] Pilot PK data indicates that Construct A is rapidly cleared from circulation (~ 30-fold drop in serum levels within 30 min of IV injection). This is common for small therapeutic proteins whose molecular weight is below the renal glomerular filtration cut-off of ~ 60-70kDa. Hence, we reasoned this fusion protein was not amenable to systemic IV dosing for our POC *in vivo* efficacy study. Instead, we chose a direct intra-tumoral delivery design with 3 arms: vehicle, recombinant human IL-2 (r hIL2) and Construct A (n=3 mice/arm). IL-2 has previously demonstrated anti-tumor activity in a variety of syngeneic models by direct tumor injection, and based on this data, we selected to dose r hIL2 at 5ug/day (equivalent to 50 000 U/day. Construct A was dosed at 70ug/day, which represents a 5 molar excess compared to recombinant IL-2 to compensate for the EC50 difference

observed in the CTLL-2 assay. All agents and vehicle were injected daily into subcutaneous MC38 tumor mass ($\sim 200\text{mm}^3$ in size upon initiation of dosing) growing on the flank of C57BL/6 mice for 12 days with 2-day holiday after first 5 injections (total of 10 injections). Tumors and body weights were measured twice a week for the duration of the study. Tumor volumes were calculated using the following equation: $(\text{longest diameter} * \text{shortest diameter}^2)/2$. As shown in Fig.6A, remarkable anti-tumor activity was observed for Construct A. Indeed, a complete elimination of tumor was observed in Construct A treatment group while no tumor regression was observed in either vehicle or r hIL2 treatment groups. When ‘cured’ Construct A-treated mice were re-inoculated with MC38 tumor cells (10^6 cells on opposite flank) on Day 40, no tumor mass was established a month after re-challenge suggesting the existence of a ‘memory’ immune response in these mice (fig. 6B).

b. Systemic IV injection of Construct B

[00278] The objective of this study is to evaluate efficacy of Construct B in the MC38-bearing female C57BL/6 mice. For this study, C57BL/6 6-8 weeks old female mice (Jackson Labs) were subcutaneously inoculated with MC38 cells (10^6 cells/animal), and when the average tumor volume reached about 80 mm^3 , animals were randomized into 2 groups based on tumor volumes (8 mice per treatment group). Animals were dosed according to the following study design:

Group	Treatment	N	Dose Route	Dosing Frequency & Duration	Dose Level (mpk)	Dose Volume (ul)
1	Vehicle Control	8	IV	Q3D for 21D	N/A	100
2	Construct B	8	IV	Q3D for 21D	10	100

[00279] Mice were dosed over a 21 day period then further observed for an additional week. Tumors and body weights were measured twice a week for the duration of the study. Tumor volumes were calculated using the following equation: $(\text{longest diameter} * \text{shortest diameter}^2)/2$. Fig.7 shows the mean tumor volume over time for both groups (Fig. 7a) and individual body weights of vehicle and treated (Fig.7B) animals.

[00280] The results showed excellent efficacy for the treatment group, with 92% inhibition of tumor growth at Day 21, while no adverse effect was observed. Remarkably, out of 8 cases, 3 complete tumor regressions (‘cures’) occurred in the colorectal cancer syngeneic setting

Example 10: Evaluation of immune cell populations by immunohistochemistry (IHC) in MC38 colorectal cancer samples

[00281] The objective of this study is to evaluate immune targets in tumor samples by IHC. See below for details:

- CD4 + Foxp3 double immunofluorescence staining
- CD8, CD25, CD3, CD4 and CD335 single IHC staining

[00282] Note that prior to performing IHC, H&E staining was ran for all control and Construct B treated tumors to check the tissue quality.

[00283] 7 tumor samples were selected from the systemic *in vivo* efficacy study and formalin-fixed paraffin embedded (FFPE) blocks were prepared following standard embedding process.

Model type: MC38

Group	Treatment	Number of FFPE blocks
1	Vehicle, IV, Q3D for 21 days	4
2	Construct B, 10 mg/kg, IV, Q3D for 21 days	3

[00284] The following antibodies were used:

Antibody	Company	Cat#	Type	Reactivity
CD4	Cell Signaling	25229	Rabbit IgG mAb	Mouse
FoxP3	Cell Signaling	12653	Rabbit IgG mAb	Mouse
CD8	Cell Signaling	98941	Rabbit IgG mAb	Mouse
CD25	abcam	ab227834	Rabbit IgG mAb	Mouse
CD3	Cell Signaling	99940	Rabbit IgG mAb	Mouse
CD335	R&D Systems	AF2225-SP	Goat IgG pAb	Mouse
Bond Polymer Refine Detection	Leica	DS9800	Anti-rabbit Poly-HRP-IgG (<25µg/mL) containing 10% (v/v) animal serum in tris-buffered saline/0.09% ProClin™ 950 (ready-to-use)	
ImmPRESS HRP Anti-Goat Ig	Vector	MP-7405	Anti-goat Poly-HRP-IgG (<25µg/mL) containing 10% (v/v) animal serum in tris-	

			buffered saline (ready-to-use) and House serum (2.5%)	
Rabbit (DA1E) mAb IgG	Cell Signaling	3900	Isotype control	
TRITC TSA(Red)	PerkinElmer		NEL742001KT	Fluorescent double staining
FITC TSA(Green)	PerkinElmer		NEL741001KT	Fluorescent double staining

[00285] FFPE blocks were sectioned with a manual rotary microtome (4 μ m thickness/section) and optimized IHC assay protocols for all the antibodies were used. All stained sections were scanned with NanoZoomer-S60 Image system with 40x magnification. High resolution picture for whole section was generated and further analyzed.

[00286] **Scoring Method:** All the images were analyzed with HALO™ Image Analysis platform. The whole slide image was analyzed and necrosis area was excluded. The total cells and IHC positive cells were counted. IHC score is presented as the ratio of the positive cell counts against the total cell numbers within whole section and shown in Fig 8. Results show that there is a significant increase in tumor infiltrating immune cells post Construct B treatment.

Example 11: *In vivo* MMP activity evaluation in diverse syngeneic tumor models.

[00287] We assessed the degree of MMP activity in the models *in vivo* utilizing an MMP-activatable fluorescent probe, MMPsense 680™. This probe is optically silent in its intact state and becomes highly fluorescent following MMP-mediated cleavage and is designed to be used as a real-time *in vivo* imaging tool (Perkin Elmer). Following a single dose IV injection of the probe to tumor-bearing mice, fluorescent images were captured over 6 days and the fluorescence intensity in tumor area, which is directly proportional to MMP activity present, was quantified (Figure 9). All models showed intrinsically different levels of MMP activity.

Example 12: *In vivo* efficacy of Construct B in diverse syngeneic tumor models.

[00288] For the efficacy studies, C57BL/6 or BALB/c mice were subcutaneously inoculated with malignant cells and when the average tumor volume reached on average 90 mm³, animals were randomized into 2 groups based on tumor volumes (n=10 mice per

treatment group). Mice were dosed intravenously every 3 days (Q3D) at 20mg/kg. Tumors, body weights and clinical observations were measured/collected twice a week for the duration of the study. Tumor volume is shown in FIGs 10A-D, 11A, 12A, and 13B-C. Robust anti-tumor activity was observed in several models, notably 49% tumor growth inhibition (TGI) was observed at D12 in the B16F10 melanoma model and 58% tumor TGI at Day 10 in the aggressive Ras/Myc transformed RM-1 prostate cancer model (Figure 10C-D and Table 6). Notably, no signs of toxicity, including body weight loss and elevated levels of liver and/or kidney enzymes, were noted and clinical observations were normal in these models. Liver and kidney enzyme results corresponding to FIGs 11A and 12A are shown in FIGs 11B-D and 12B-D, respectively.

[00289] Table 6.

Cancer type	strain	Model	Dosing Regimen	Max TGI %	T test	MMP score
Breast	BALB/c	EMT06	20mpk, IV Q3D	43 (D20)	P=0.0006	HIGH
Melanoma	C57BL/6	B16F10	20mpk, IV Q3D	49 (D12)	P=0.0004	LOW
colorectal	BALB/c	CT-26	20mpk, IV Q3D	46 (D13)	P = 0.0114	MED/HIGH
colorectal	C57BL/6	MC-38	10mpk, IV Q3D	92 (D21)	P< 0.0001	MED
prostate	C57BL/6	RM_1	20mpk, IV Q3D	58 (D10)	P< 0.0001	NOT DETERMINED

P values represent unpaired t test (graphpad prism) between vehicle and Construct B groups on Day of max TGI.

[00290] The difference in efficacy between MC38 and B16F10 models may in part be due to the lower MMP activity measured in B16F10 tumors, resulting in less functional IL-2 being released in the TME relative to the MC38 setting (Figure 13A).

Example 13: Next generation retention linker peptide binding assay

[00291] A series of peptides comprising an MMP cleavable site with or without the addition of a tumor retention sequence were synthesized and conjugated to the fluorophore

EDANS (5-((2-Aminoethyl)amino)naphthalene-1-sulfonic acid) (custom synthesis, ThermoFisher). Table 7 shows the list of peptides. These peptides were then tested for their ability to bind ECM proteins such as heparin, fibronectin and collagen which are found in abundance in the tumor stroma.

[00292] Table 7.

SEQ ID NO	Sequence	Target of retention motif
901	GGGSGGG <u>G</u> PLGVRG-*	None (1 st gen)
902	GGG <u>HHPH</u> GPLGVRG-*	pH dependent heparin
903	<i>GVRIQRKKE</i> KMKET-*	heparin
904	<i>FHRR</i> IKAGPLGVRG-*	heparin
907	GGGSGGG <u>G</u> PAALIGG-*	None (1 st gen)
913	GGG <u>WSHW</u> GPLGVRG-*	pH dependent fibronectin
914	<i>KLWVLPK</i> GPLGVRG-*	Collagen IV
915	GGGSGL <u>HERHL</u> NNN-*	Collagen I

Underlining indicates MMP cleavage site. Bold italics indicates retention motif when present. -* represents Edans fluorophore conjugated to peptide.

[00293] All binding assays were set up in 10mM TrisHCl pH 7.5 and/or 10mM TrisHCl pH 6. Peptides (20uM) were incubated on a shaker for 2hrs at room temperature with agarose cross-linked to heparin or control agarose beads (Sigma and Pierce respectively). The beads were then washed 4 times and resuspended in 100uL of binding buffer in a black 96-well plate. Peptide binding was quantified by measuring the fluorescence of samples using excitation/emission spectra of EDANS (Ex 340 / Em 490). Fig 14A shows that several next generation MMP linker peptides containing heparin binding motifs bind to the heparin-agarose beads while 1st generation MMP linkers lacking these retention sequences do not. One such peptide displays enhanced binding to heparin at pH6 (the pH of tumors) vs pH 7.5 (pH of normal tissues) (Fig 14B).

[00294] For fibronectin and collagen binding assays, streptavidin coupled magnetic beads (Mag Sepharose, Cytiva and Dynabeads, ThermoFisher, respectively) were first incubated with biotin-labelled fibronectin (Cytoskeleton) or biotin-labelled collagen IV

(Prospec) for 1 Hr with gentle shaking. Following multiple washes, the ECM-coated beads were then incubated with Edans Peptides (20uM) for 2 hours at room temperature with shaking in neutral or acidic binding buffer. Beads were then washed and resuspended in 100uL of binding buffer in a black 96-well plate. Peptide binding was quantified by measuring the fluorescence of samples using excitation/emission spectra of EDANS (Ex 340 / Em 490). Fig 14D shows that peptide 13 is able to bind fibronectin and displays enhanced binding at pH6 (the pH of tumors) vs pH 7.5 (pH of normal tissues). Fig 14F shows that peptide 14 strongly binds collagen IV while peptide 15 binds to a lesser extent.

Example 14: Next generation tumor retention IL-2 fusion protein binding assays

[00295] A series of IL-2 fusion proteins comprising tumor retention sequences in the linker regions were designed and successfully manufactured (Table 3 and Figs 1C-D). These proteins were then tested for their ability to bind ECM proteins such as heparin, fibronectin and collagen which are found in abundance in the tumor stroma.

[00296] 96-well plates were coated with 25 ug/mL of Heparin-BSA conjugate (provided by Dr. Mueller, Boehringer Ingelheim) or control BSA for 18-22h at room temperature on shaker (350rpm). After washing, wells are blocked with PBS-0.05% Tween 20/ 1% BSA for 90 min, then fusion proteins are titrated in 1% BSA/ PBS-0.05% Tween 20 pH 7.5 and/or pH 6 and added for 2hr at room temperature with shaking. After washing, an anti-mouse IL-2 biotin-labelled detection antibody (JES6-5H4, ThermoFisher) is added and binding is detected using Ultra Streptavidin HRP (ThermoFisher). The plate was developed by adding the chromogenic tetramethylbenzidine substrate (Ultra TMB, ThermoFisher). The reaction is stopped by addition of 0.5M H₂SO₄ and the absorbance is read at 450-650 nm. IL-2 fusion variants Construct Y and Construct CC at acidic pH bind heparin in dose-dependent manner and with higher affinity than Construct B (Fig 14C). Strikingly, Construct CC preferentially binds heparin at acidic pH and shows the most robust binding with EC₅₀ ~ 10nM, while Construct B's binding is much weaker with > 100-fold higher EC₅₀ value.

[00297] A similar plate-based assay was developed to interrogate binding of IL-2 fusion variants to fibronectin. 96-well plates were coated with 4 ug/mL of fibronectin (Sigma) or control BSA for 18-22h at room temperature on shaker (350rpm). After washing, wells are blocked with protein-free blocking buffer (Pierce) for 90 min, then fusion proteins are titrated in blocking buffer-0.1% Tween 20 pH 7.5 and/or pH 6 and added for 1hr at room temperature with shaking. After washing, an anti-mouse IL-2 biotin-labelled detection antibody (JES6-5H4, ThermoFisher) is added and binding is detected using Ultra Streptavidin

HRP (ThermoFisher). The plate was developed by adding the chromogenic tetramethylbenzidine substrate (Ultra TMB, ThermoFisher). The reaction is stopped by addition of 0.5M H₂SO₄ and the absorbance is read at 450-650 nm. Construct EE preferentially binds fibronectin at acidic pH and shows dose-dependent binding, while no binding is observed at pH 7.5 (Fig 14E). No significant binding of Construct B is seen in either neutral or acidic conditions.

[00298] To test binding to collagen, a pulldown assay using agarose cross-linked to collagen (Sigma) was performed. IL-2 fusion proteins were incubated with collagen-agarose or control agarose beads for 18-22h at 4C with gentle rotation in 1% BSA/ PBS-0.05% Tween 20. After washing, proteins bound to the beads were eluted by resuspending beads in SDS sample buffer (Life Technologies). Bound proteins were then separated by SDS-PAGE on 4-12% BisTris gradient gel followed by immunoblotting with goat anti-mouse IL-2 polyclonal antibody (AF-402-NA; R&D systems). Donkey Anti-goat HRP-conjugated antibody was used for detection (Jackson Immuno Research, West Grove, PA) and the blot was developed using the SuperSignal West Femto Maximum sensitivity detection reagent (ThermoFisher) following the manufacturer's recommendations. The blot image is shown in Fig 14G. Construct GG and Construct II were specifically bound by collagen-agarose beads, while no IL-2 fusion protein bound the control agarose beads. Quantitation of the blot using iBright imaging system (Invitrogen), shows that although the fraction of bound Construct GG and Construct II was low (< 1% of input), it was 2.5 and 1.4-fold higher than the fraction of bound Construct B (Table 8).

[00299] Table 8.

	Input (2%)	Bound	Bound (% input)	Normalized
Construct B	16707	2306	0.3%	1
Construct GG	15267	5191	0.7%	2.5
Construct II	12094	2277	0.4%	1.4

Example 15: Next generation retention linker IL-2 fusion proteins show greater retention in tumor in vivo.

[00300] We assessed the levels of IL-2 fusion proteins present in tumors *in vivo* by utilizing fluorescently labelled proteins and real-time whole-body imaging. Non-cleavable Construct GGG and Construct DD were conjugated to Dylight 650 probe according to the manufacturer's protocol (Dylight 650 Antibody labeling kit, ThermoFisher). We confirmed the conjugation did not significantly alter the proteins' binding to heparin. BALB/c mice were subcutaneously inoculated with EMT6 breast cancer syngeneic model and when the average tumor volume reached 240 mm³, animals were randomized into 3 groups based on tumor volumes (n=2 mice per treatment group). Table below shows study design:

Group	Treatment	N	Dose Route	Dosing Frequency & Duration	Dose Level (mg/kg)	Dose Volume (mL/kg)
1	Control -PBS	2	IV	Once	NA	4
2	Construct GGG-DY650	2	IV	Once	8	4
3	Construct DD-DY650	2	IV	Once	8	4

[00301] Following a single dose of the labeled IL-2 fusion proteins to tumor-bearing mice, fluorescent images (excitation 640 / emission 680 consistent with Dylight 650 probe ex / em spectra) were captured over 96 hrs on an IVIS system (PerkinElmer, IVIS Lumina Series III) and are shown in Figure 15A. The fluorescence intensity in tumor area was quantified across the groups, average background tumor fluorescence (group 1) was subtracted from group 2 and 3 values at each time-point, and data was normalized to the initial fluorescence intensity of same amount of each labeled protein. Figure 15B shows that the tumor-associated fluorescence with group 3 is roughly 2-fold higher than that of group 2 at each of the time-points tested. This signifies next generation retention linker Construct DD accumulates and is retained in tumors at 2-fold higher levels compared to 1st generation IL-2 fusion protein Construct GGG.

Example 16: Next generation retention MMP-Linker leads to increased levels of drug and IL2 in tumors and serum in vivo.

[00302] We quantified levels of full-length IL2-IL2Ra fusion proteins and IL-2 in tumor samples collected during pre-clinical efficacy studies comparing Construct B and retention linker IL-2 fusion drugs (see example 17).

[00303] Tumors (n=3 per group) were collected 24h after the last dose injection, flash frozen and stored at -80C until further processing. Tumor lysates were generated using tissue extraction reagent (ThermoFisher) supplemented with protease and phosphatase inhibitors and standard techniques and protein concentrations were determined using the BCA assay (Pierce).

[00304] Lysates were tested with in-house ELISAs to measure full-length IL-2 fusion proteins (IL-2 capture/ IL-2Ra detection) and IL-2 fusion proteins + free IL-2 (IL-2 capture/IL-2 detection). Free IL-2 levels in tumor were calculated by subtracting drug levels from the drug + IL-2 data set. Results were normalized to 1 mg of tumor lysate and mean values are shown in Figs 15 C-H. Levels of Construct CC (20mg/kg dose) in tumor are roughly 3-fold higher compared to Construct B levels, despite Construct B being dosed at 40mg/kg (Fig C). Drug level comparison in samples from the 10mg/kg dosing cohort shows highest levels present in collagen binding Construct GG treated tumors (Fig 15F). This indicates that retention linker technology may lead to a robust increase in drug amounts in tumor in vivo. Likewise, IL-2 levels in Construct CC, Construct GG and Construct II treated tumors are elevated compared to Construct B treated tumors (Fig 15E/H). This implies that next generation retention linker technology is able to retain in TME both full-length drug and released IL-2 post-cleavage.

[00305] The equivalent serum samples (n=3 per group) were also tested with in-house ELISAs to quantify full-length IL-2 fusion drugs and results are shown in fig 15I-K. 24 hrs after dosing, circulating drug levels of Construct B (40mg/kg) and Construct CC (20mg/kg) are roughly similar despite the dosing difference, whilst in the 10mg/kg cohort, Construct GG and Construct II drug levels in serum are roughly 5-fold and 3-fold higher than Construct B serum levels (Fig 15J). Additional serum samples collected at Day 17 (Construct B 20mg/kg) and Day 21 (Construct Y 20mg/kg), 4 days and 8 days respectively after the last IV injection, were assayed for full-length IL-2 fusion drug. Fig. 15 K shows that Construct Y drug levels in circulation are strikingly more than 10-fold higher than Construct B despite serum being collected 4 days later. Collectively, these data indicate retention linker technology leads to increased levels of drug in circulation.

Example 17: In vivo efficacy of retention linker IL-2 drugs in B16F10 syngeneic model

[00306] In a first efficacy study, C57BL/6 mice were subcutaneously inoculated with B16F10 melanoma cells and when the average tumor volume reached on average 70-90 mm³,

animals were randomized into 5 groups based on tumor volumes (n=6 mice per treatment group). Mice were dosed intravenously every 3 days (Q3D) for a total of 5 doses according to following design:

Group	Treatment	N	Dose Route	Dosing Frequency & Duration	Dose Level (mg/kg)	Dose Volume (mL/kg)
1	PBS-Vehicle	6	IV	Q3D for 14 days (5 doses)	NA	4
2	Construct B	6	IV	Q3D for 14 days (5 doses)	20	4
3	Construct B	6	IV	Q3D for 14 days (5 doses)	40	8
4	Construct Y	6	IV	Q3D for 14 days (5 doses)	20	4.45
5	Construct CC	6	IV	Q3D for 14 days (5 doses)	20	5

[00307] Tumor volumes were measured twice a week for the duration of the study. Mean tumor volume is shown in FIGs 16A. Anti-tumor activity was observed in all treatment groups, however the most robust tumor growth inhibition (TGI) was observed in the retention linker drugs Construct Y and Construct CC (77 and 78% respectively) compared to ~ 60% TGI in Construct B treated groups (regardless of dose, Table 9).

[00308] Table 9.

Group	Drug	Dose	TGI (%) D13	P value
2	Construct B	20mg/kg	61.43	0.0002
3	Construct B	40mg/kg	58.44	0.0019
4	Construct Y	20mg/kg	76.59	0.0001
5	Construct CC	20mg/kg	77.84	0.0002

P values represent unpaired t test (graphpad prism) between vehicle and Test article groups on Day 13.

[00309] In a second efficacy study in the same model, C57BL/6 mice were subcutaneously inoculated with B16F10 melanoma cells and when the average tumor volume reached on average 70-90 mm³, animals were randomized into 5 groups based on tumor volumes (n=6 mice per treatment group). Mice were dosed intravenously every 3 days (Q3D) for a total of 5 doses according to following design:

Group	Treatment	N	Dose Route	Dosing Frequency & Duration	Dose Level (mg/kg)	Dose Volume (mL/kg)
1	PBS-Vehicle	6	IV	Q3D for 14 days (5 doses)	NA	5
2	Construct B	6	IV	Q3D for 14 days (5 doses)	10	5
3	Construct EE	6	IV	Q3D for 14 days (5 doses)	10	5
4	Construct GG	6	IV	Q3D for 14 days (5 doses)	10	5
5	Construct II	6	IV	Q3D for 14 days (5 doses)	10	5

[00310] Tumor volumes were measured twice a week for the duration of the study up until Day 20, 7 days following the fifth dose. On Day 20, mice received an additional dose of drug, animals were sacrificed 24hrs later and tissues and blood (processed to serum) were collected and stored at -80C for further testing. Mean tumor volume is shown in FIGs 16B. Only modest anti-tumor activity was observed with Construct B at 10mg/kg in this aggressive model (27% TGI Day 15, Table 10). Strikingly, at equivalent dosage all retention linker IL-2 fusion drugs showed superior TGI (Table 10). In particular, collagen binding drugs Construct GG and Construct II (10mg/kg dosing) showed robust tumor control similar to what was previously observed for Construct B at twice higher dose (57% TGI Day 15 Table 10 compared to 61% TGI Day 13 Table 9 respectively). Furthermore, after a dosing holiday of 7 days Construct B showed diminished efficacy whilst all retention linker drugs maintained similar levels of tumor control at Day 20. Collectively, Figs 16A-B demonstrate that retention linker IL-2 drugs have superior anti-tumor efficacy in a pre-clinical melanoma model. This is most likely due to the higher levels of both circulating drugs in serum and resident drug in TME, which can exert prolonged anti-tumor activity even after an extended dosing holiday.

[00311] Table 10.

Group	Drug	Dose	TGI (%) D15	TGI (%) D20
2	Construct B	10mg/kg	27.43	14.02
3	Construct EE	10mg/kg	39.32	37.61
4	Construct GG	10mg/kg	57.18	64.70
5	Construct II	10mg/kg	57.68	68.52

Example 18: IFN- γ levels in tumor samples

[00312] IFN- γ cytokine levels in tumor lysates (n=3 per group) were measured using a Luminex kit according to manufacturer's protocol (Invitrogen). Results were normalized to 1 mg of lysate and mean values are shown in Fig 17A/B. Elevated levels of IFN- γ were measured in all retention linker IL-2 drug treated tumors compared to Construct B treated tumors. IFN- γ was undetectable in vehicle treated tumors.

We claim:

1. A protease-activated pro-cytokine comprising:
a cytokine polypeptide sequence;
a inhibitory polypeptide sequence capable of blocking an activity of the cytokine polypeptide sequence;
a linker between the cytokine polypeptide sequence and the inhibitory polypeptide sequence, the linker comprising a protease-cleavable polypeptide sequence; and
a targeting sequence, wherein the targeting sequence is configured to bind an extracellular matrix component, an integrin, or a syndecan; or is configured to bind, in a pH-sensitive manner, an extracellular matrix component, IgB (CD79b), an integrin, a cadherin, a heparan sulfate proteoglycan, a syndecan, or a fibronectin; or the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-662 or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 180-662.
2. The protease-activated pro-cytokine of the immediately preceding claim, further comprising a pharmacokinetic modulator.
3. The protease-activated pro-cytokine of the immediately preceding claim, wherein the pharmacokinetic modulator comprises an immunoglobulin constant domain.
4. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises an immunoglobulin Fc region.
5. The protease-activated pro-cytokine of the immediately preceding claim, wherein the immunoglobulin is a human immunoglobulin.
6. The protease-activated pro-cytokine of any one of claims 4-5, wherein the immunoglobulin is IgG.
7. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IgG is IgG1, IgG2, IgG3, or IgG4.
8. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises an albumin.
9. The protease-activated pro-cytokine of the immediately preceding claim, wherein the albumin is a serum albumin.
10. The protease-activated pro-cytokine of any one of claims 8-9, wherein the albumin is a human albumin.
11. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises PEG.

12. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises XTEN.

13. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises CTP.

14. The protease-activated pro-cytokine of any one of claims 2-13, wherein the protease-cleavable polypeptide sequence is between the cytokine polypeptide sequence and the pharmacokinetic modulator.

15. The protease-activated pro-cytokine of any one of claims 2-13, wherein the pharmacokinetic modulator is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence.

16. The protease-activated pro-cytokine of any one of the preceding claims, comprising a plurality of protease-cleavable polypeptide sequences.

17. The protease-activated pro-cytokine of the immediately preceding claim, wherein the cytokine polypeptide sequence is flanked by protease cleavable polypeptide sequences.

18. The protease-activated pro-cytokine of the immediately preceding claim, having the structure PM-CL-CY-CL-IN (from N- to C-terminus or from C- to N-terminus), where PM is the pharmacokinetic modulator, each CL independently is a protease-cleavable polypeptide sequence, CY is the cytokine polypeptide sequence, and IN is the inhibitory polypeptide sequence.

19. The protease-activated pro-cytokine of any one of the preceding claims, comprising the targeting sequence, wherein the targeting sequence is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence or one of the protease-cleavable polypeptide sequences.

20. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence comprises a modification to prevent disulfide bond formation, and optionally otherwise comprises wild-type sequence.

21. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine polypeptide sequence or to a cytokine polypeptide sequence in Table 1.

22. The protease-activated pro-cytokine of the immediately preceding claim, wherein the cytokine polypeptide sequence is a wild-type cytokine polypeptide sequence.

23. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is a monomeric cytokine, or wherein the cytokine polypeptide sequence is a dimeric cytokine polypeptide sequence comprising monomers that are associated covalently (optionally via a polypeptide linker) or noncovalently.

24. The protease-activated pro-cytokine of any one of the preceding claims, wherein the inhibitory polypeptide sequence comprises a cytokine-binding domain.

25. The protease-activated pro-cytokine of the immediately preceding claim, wherein the cytokine-binding domain is a cytokine-binding domain of a cytokine receptor or a cytokine-binding domain of a fibronectin.

26. The protease-activated pro-cytokine of claim 24, wherein the cytokine-binding domain is an immunoglobulin cytokine-binding domain.

27. The protease-activated pro-cytokine of the immediately preceding claim, wherein the immunoglobulin cytokine-binding domain comprises a light chain variable domain and a heavy chain variable domain that bind the cytokine.

28. The protease-activated pro-cytokine of any one of claims 26-27, wherein the immunoglobulin cytokine-binding domain is an scFv, Fab, or VHH.

29. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by a metalloprotease, a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamate protease, a gelatinase, an asparagine peptide lyase, a cathepsin, a kallikrein, a plasmin, a collagenase, a hK1, a hK10, a hK15, a stromelysin, a Factor Xa, a chymotrypsin-like protease, a trypsin-like protease, a elastase-like protease, a subtilisin-like protease, an actinidain, a bromelain, a calpain, a caspase, a Mir 1-CP, a papain, a HIV-1 protease, a HSV protease, a CMV protease, a chymosin, a renin, a pepsin, a matriptase, a legumain, a plasmepsin, a nepenthesin, a metalloexopeptidase, a metalloendopeptidase, an ADAM 10, an ADAM17, an ADAM 12, an urokinase plasminogen activator (uPA), an enterokinase, a prostate-specific target (PSA, hK3), an interleukin-1b converting enzyme, a thrombin, a FAP (FAP-a), a dipeptidyl peptidase, or dipeptidyl peptidase IV (DPPIV/CD26), a type II transmembrane serine protease (TTSP), a neutrophil elastase, a proteinase 3, a mast cell chymase, a mast cell tryptase, or a dipeptidyl peptidase.

30. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 700-741, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 700-741.

31. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by a matrix metalloprotease.

32. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-1.

33. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-2.

34. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-3.

35. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-7.

36. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-8.

37. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-9.

38. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-12.

39. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-13.

40. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by MMP-14.

41. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by more than one MMP.

42. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence is recognized by two, three, four, five, six, or seven of MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, and MMP-14.

43. The protease-activated pro-cytokine of any one of the preceding claims, wherein the protease-cleavable polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 80-94 or a variant sequence having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 80-90.

44. The protease-activated pro-cytokine of the immediately preceding claim, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80 or a variant sequence having one or two mismatches relative thereto.

45. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 81 or a variant sequence having one or two mismatches relative thereto.

46. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 82 or a variant sequence having one or two mismatches relative thereto.

47. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 83 or a variant sequence having one or two mismatches relative thereto.

48. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 84 or a variant sequence having one or two mismatches relative thereto.

49. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 85 or a variant sequence having one or two mismatches relative thereto.

50. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 86 or a variant sequence having one or two mismatches relative thereto.

51. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 87 or a variant sequence having one or two mismatches relative thereto.

52. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 88 or a variant sequence having one or two mismatches relative thereto.

53. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 89 or a variant sequence having one or two mismatches relative thereto.

54. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 90 or a variant sequence having one or two mismatches relative thereto.

55. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80-89 or 90.

56. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 91.

57. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 92.

58. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 93.

59. The protease-activated pro-cytokine of any one of claims 1-43, wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 94.

60. The protease-activated pro-cytokine of any one of the preceding claims, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 180-662.

61. The protease-activated pro-cytokine of the immediately preceding claim, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-662.

62. The protease-activated pro-cytokine of any one of the preceding claims, wherein the targeting sequence binds to denatured collagen.

63. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to collagen.

64. The protease-activated pro-cytokine of any one of claims 62-63, wherein the collagen is collagen I.

65. The protease-activated pro-cytokine of any one of claims 62-63, wherein the collagen is collagen II.

66. The protease-activated pro-cytokine of any one of claims 62-63, wherein the collagen is collagen III.

67. The protease-activated pro-cytokine of any one of claims 62-63, wherein the collagen is collagen IV.

68. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to integrin.

69. The protease-activated pro-cytokine of the immediately preceding claim, wherein the integrin is one or more of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin, $\alpha 3\beta 1$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 5\beta 1$ integrin, $\alpha 6\beta 1$ integrin, $\alpha 7\beta 1$ integrin, $\alpha 9\beta 1$ integrin, $\alpha 4\beta 7$ integrin, $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha IIb\beta 3$ integrin, $\alpha IIIb\beta 3$ integrin, $\alpha M\beta 2$ integrin, or $\alpha IIb\beta 3$ integrin.

70. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to von Willebrand factor.

71. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to IgB.

72. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to heparin.

73. The protease-activated pro-cytokine of the immediately preceding claim, wherein the targeting sequence binds to heparin and a syndecan, a heparan sulfate proteoglycan, or an integrin, optionally wherein the integrin is one or more of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin, $\alpha 3\beta 1$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 5\beta 1$ integrin, $\alpha 6\beta 1$ integrin, $\alpha 7\beta 1$ integrin, $\alpha 9\beta 1$ integrin, $\alpha 4\beta 7$ integrin, $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha IIb\beta 3$ integrin, $\alpha IIIb\beta 3$ integrin, $\alpha M\beta 2$ integrin, or $\alpha IIb\beta 3$ integrin.

74. The protease-activated pro-cytokine of any one of claims 72-73, wherein the syndecan is one of more of syndecan-1, syndecan-4, and syndecan-2(w).

75. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to a heparan sulfate proteoglycan.

76. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to a sulfated glycoprotein.

77. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to hyaluronic acid.

78. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to fibronectin.

79. The protease-activated pro-cytokine of any one of claims 1-61, wherein the targeting sequence binds to cadherin.

80. The protease-activated pro-cytokine of any one of the preceding claims, wherein the targeting sequence is configured to bind its target in a pH-sensitive manner.

81. The protease-activated pro-cytokine of the immediately preceding claim, wherein the targeting sequence has a higher affinity for its target at a pH below normal physiological pH than at normal physiological pH, optionally wherein the pH below normal physiological pH is below 7, or below 6.

82. The protease-activated pro-cytokine of the immediately preceding claim, wherein the targeting sequence has a higher affinity for its target at a pH in the range of 5-7, e.g., 5-5.5, 5.5-6, 6-6.5, or 6.5-7, than at normal physiological pH.

83. The protease-activated pro-cytokine of any one of the preceding claims, wherein the targeting sequence comprises one or more histidines, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 histidines.

84. The protease-activated pro-cytokine of any one of the preceding claims, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 641-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 641-662.

85. The protease-activated pro-cytokine of the immediately preceding claim, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 641-662.

86. The protease-activated pro-cytokine of any one of claims 80-86, wherein the targeting sequence is configured to bind, in a pH-sensitive manner, an extracellular matrix component, IgB (CD79b), an integrin, a cadherin, a heparan sulfate proteoglycan, a syndecan, or a fibronectin.

87. The protease-activated pro-cytokine of the immediately preceding claim, wherein the extracellular matrix component is hyaluronic acid, heparin, heparan sulfate, or a sulfated glycoprotein.

88. The protease-activated pro-cytokine of claim 86, wherein the targeting sequence is configured to bind a fibronectin in a pH-sensitive manner.

89. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is an interleukin polypeptide sequence.

90. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD132.

91. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD122.

92. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD25.

93. The protease-activated pro-cytokine of any one of the preceding claims, wherein the cytokine polypeptide sequence is an IL-2 polypeptide sequence.

94. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2 polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 1-4.

95. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2 polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 1-4.

96. The protease-activated pro-cytokine of any one of claims 93-95, wherein the IL-2 polypeptide sequence is a human IL-2 polypeptide sequence.

97. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 1.

98. The protease-activated pro-cytokine of any one of claims 93-95, wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 2.

99. The protease-activated pro-cytokine of any one of claims 93-98, wherein the inhibitory polypeptide sequence comprises an IL-2 binding domain of an IL-2 receptor (IL-2R).

100. The protease-activated pro-cytokine of the immediately preceding claim, wherein the inhibitory polypeptide sequence comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 10-19.

101. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2R is a human IL-2R.

102. The protease-activated pro-cytokine of any one of claims 93-98, wherein the inhibitory polypeptide sequence comprises an IL-2-binding immunoglobulin domain.

103. The protease-activated pro-cytokine of any one of claims 93-98, wherein the IL-2-binding immunoglobulin domain is a human IL-2-binding immunoglobulin domain.

104. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising hypervariable regions (HVRs) HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 33, 34, and 35, respectively, and a VH region comprising HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 36, 37, and 38, respectively.

105. The protease-activated pro-cytokine of any one of claims 102-104, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 32 and a VH region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 33.

106. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising the sequence of SEQ ID NO: 32 and a VH region comprising the sequence of SEQ ID NO: 33.

107. The protease-activated pro-cytokine of any one of claims 102-104, wherein the IL-2-binding immunoglobulin domain is an scFv.

108. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2-binding immunoglobulin domain comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 30 or 31.

109. The protease-activated pro-cytokine of the immediately preceding claim, wherein the IL-2-binding immunoglobulin domain comprises the sequence of SEQ ID NO: 30 or 31.

110. The protease-activated pro-cytokine of claim 1, comprising the sequence of any one of SEQ ID NOs: 803-852.

111. A pharmaceutical composition comprising the protease-activated pro-cytokine of any one of the preceding claims.

112. The protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding claims, for use in therapy.

113. The protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding claims, for use in treating a cancer.

114. A method of treating a cancer, comprising administering the protease-activated pro-cytokine or pharmaceutical composition of any one of the preceding claims to a subject in need thereof.

115. Use of the protease-activated pro-cytokine or pharmaceutical composition of any one of claims 1-110 for the manufacture of a medicament for treating cancer.

116. The method, use, or protease-activated pro-cytokine for use of any one of claims 113-115, wherein the cancer is a solid tumor.

117. The method, use, or protease-activated pro-cytokine for use of the immediately preceding claim, wherein the solid tumor is metastatic and/or unresectable.

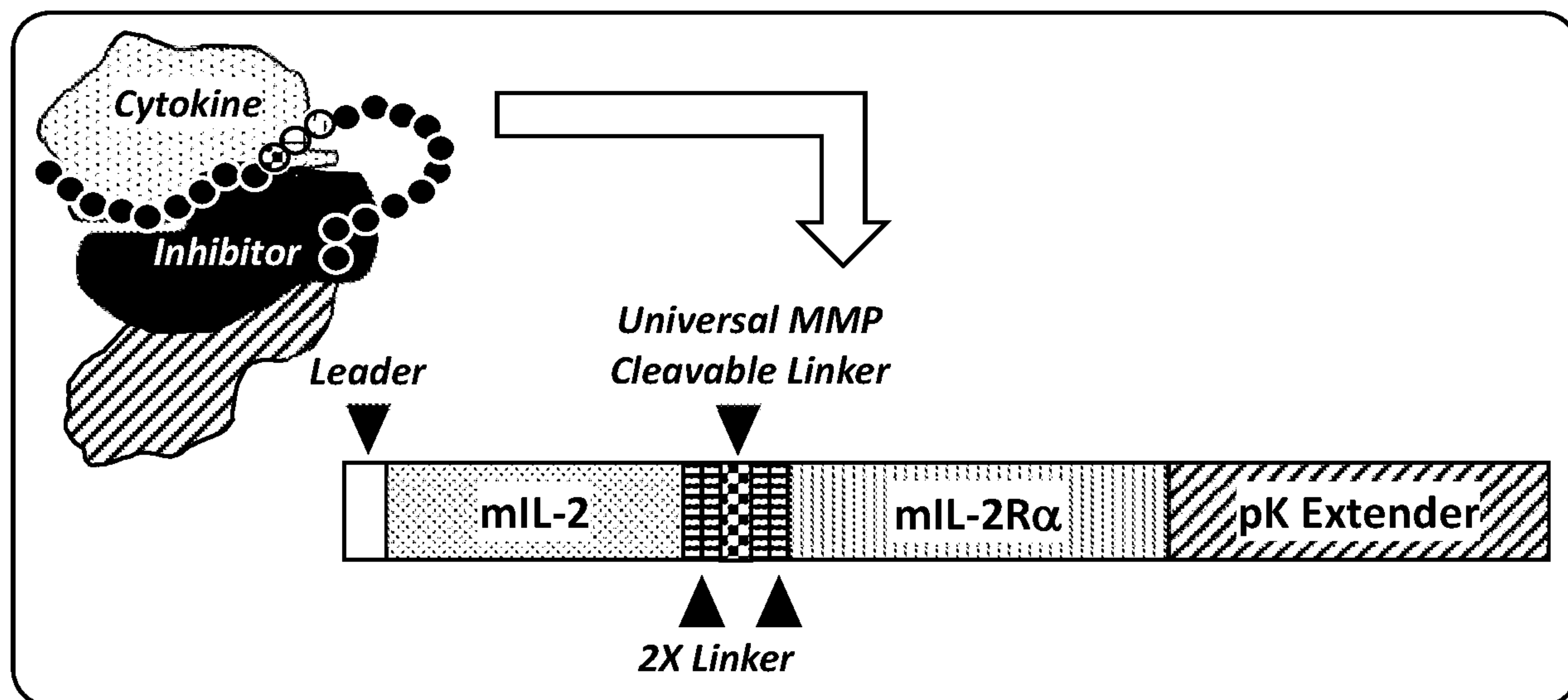
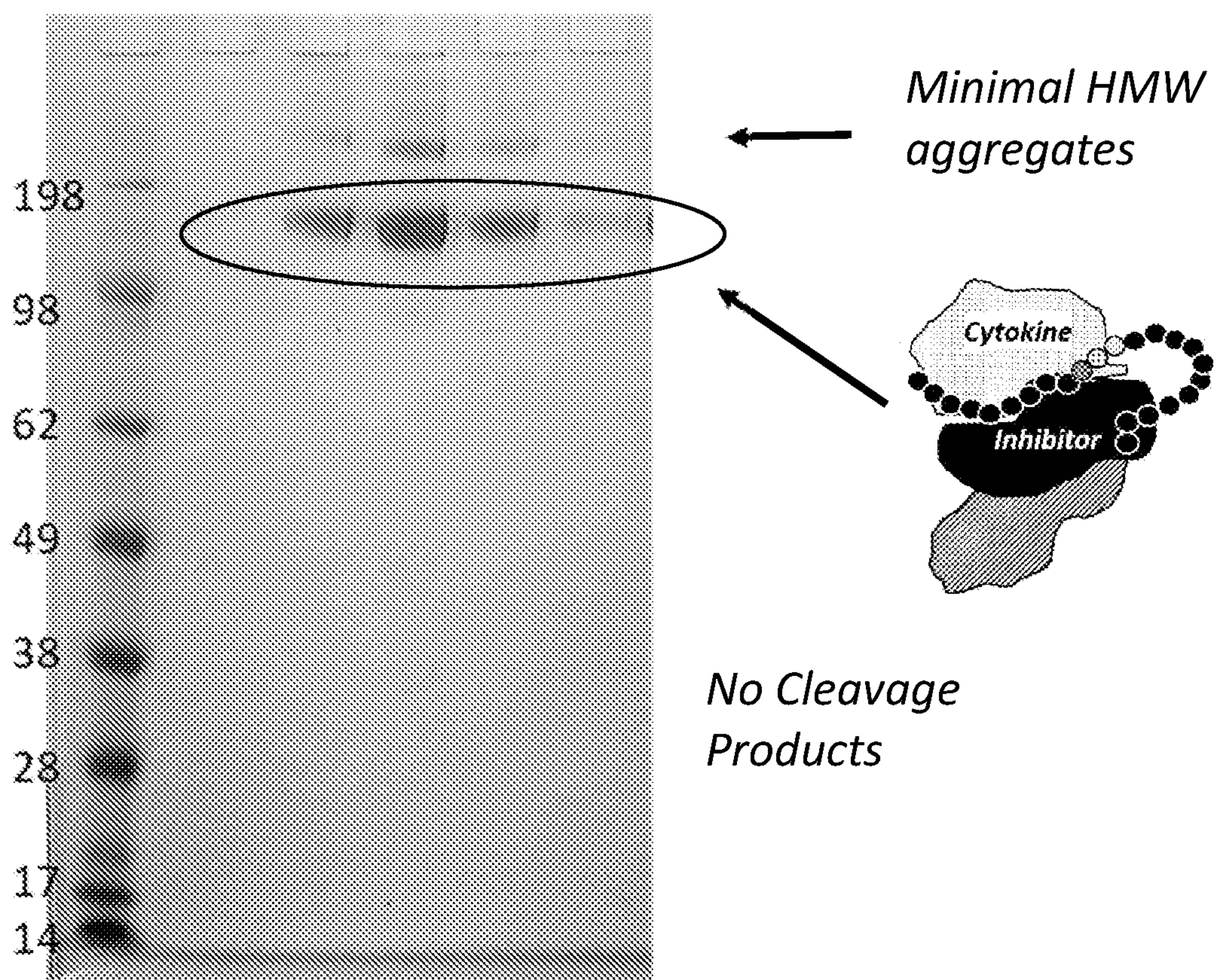
118. The method, use, or protease-activated pro-cytokine for use of any one of claims 113-117, wherein the cancer is a PD-L1-expressing cancer.

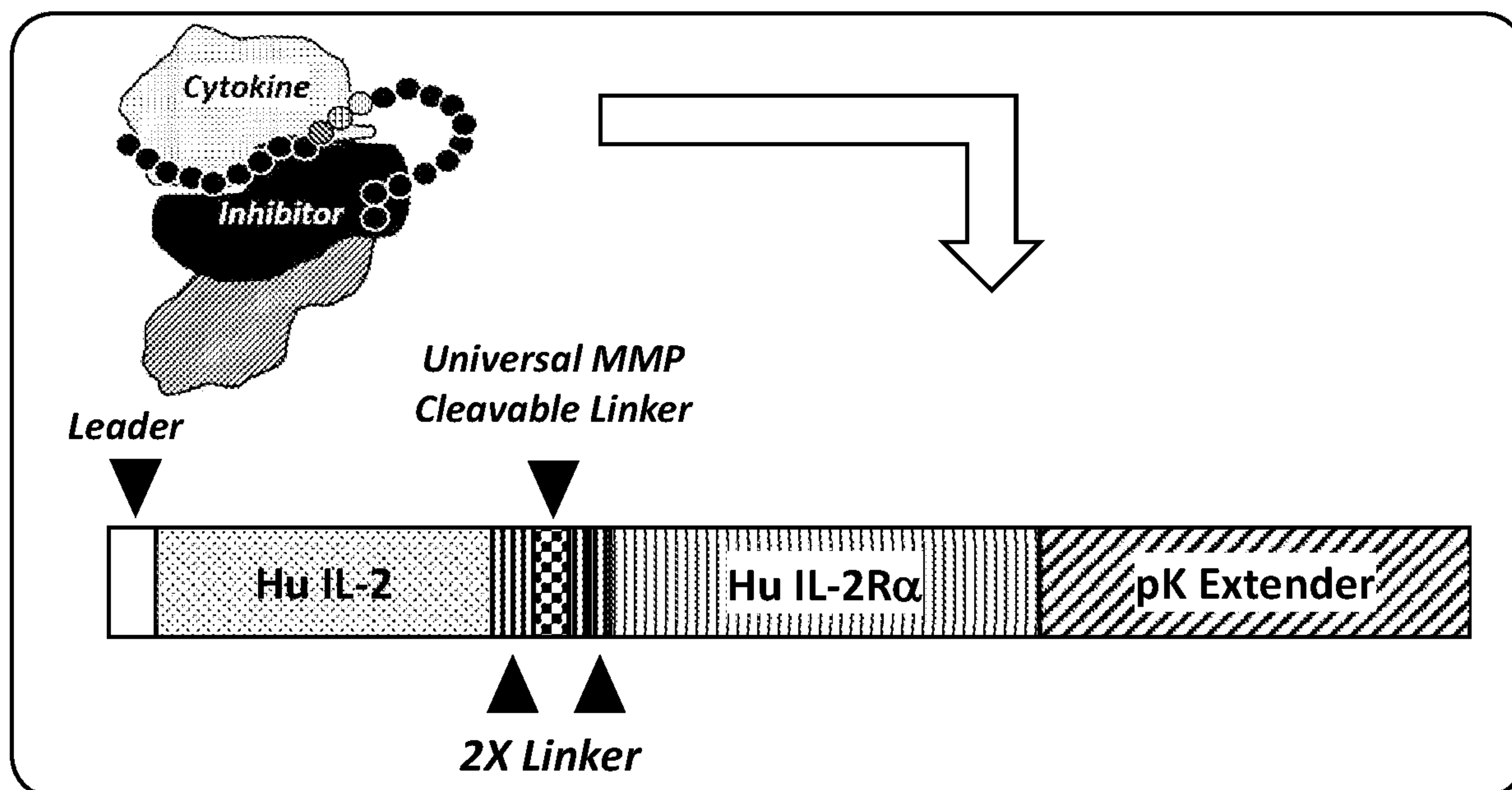
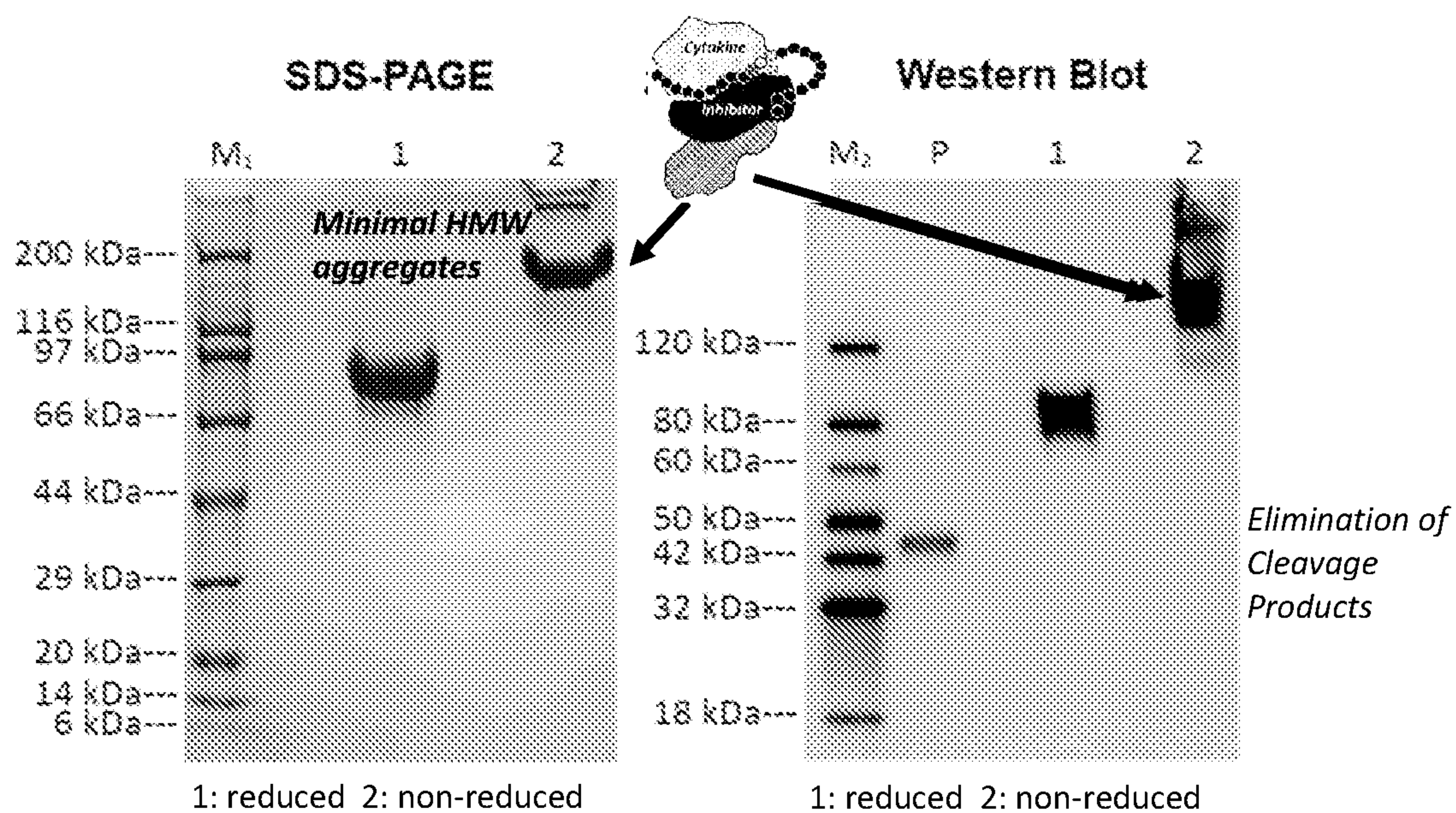
119. The method, use, or protease-activated pro-cytokine for use of any one of claims 113-118, wherein the cancer is a melanoma, a colorectal cancer, a breast cancer, a pancreatic cancer, a lung cancer, a prostate cancer, an ovarian cancer, a cervical cancer, a gastric or gastrointestinal cancer, a lymphoma, a colon or colorectal cancer, an endometrial cancer, a thyroid cancer, or a bladder cancer.

120. The method, use, or protease-activated pro-cytokine for use of any one of claims 113-119, wherein the cancer is a microsatellite instability-high cancer.

121. The method, use, or protease-activated pro-cytokine for use of any one of claims 113-120, wherein the cancer is mismatch repair deficient.

122. A nucleic acid encoding the protease-activated pro-cytokine of any one of claims 1-110.
123. An expression vector comprising the nucleic acid of claim 121.
124. A host cell comprising the nucleic acid of claim 121 or the vector of claim 122.
125. A method of producing a protease-activated pro-cytokine, comprising culturing the host cell of claim 124 under conditions wherein the protease-activated pro-cytokine is produced.
126. The method of the immediately preceding claim, further comprising isolating the protease-activated pro-cytokine.
127. A method of boosting T regulatory cells and/or reducing inflammation or autoimmune activity, comprising administering the protease-activated pro-cytokine of any one of claims 1-110 to an area of interest in a subject, e.g., an area of inflammation in the subject.
128. A method of treating an inflammatory or autoimmune disease or disorder in a subject, comprising administering the protease-activated pro-cytokine of any one of claims 1-110 to an area of interest in a subject, e.g., an area of inflammation or autoimmune activity in the subject.

**Fig. 1A**

**Fig. 1B**

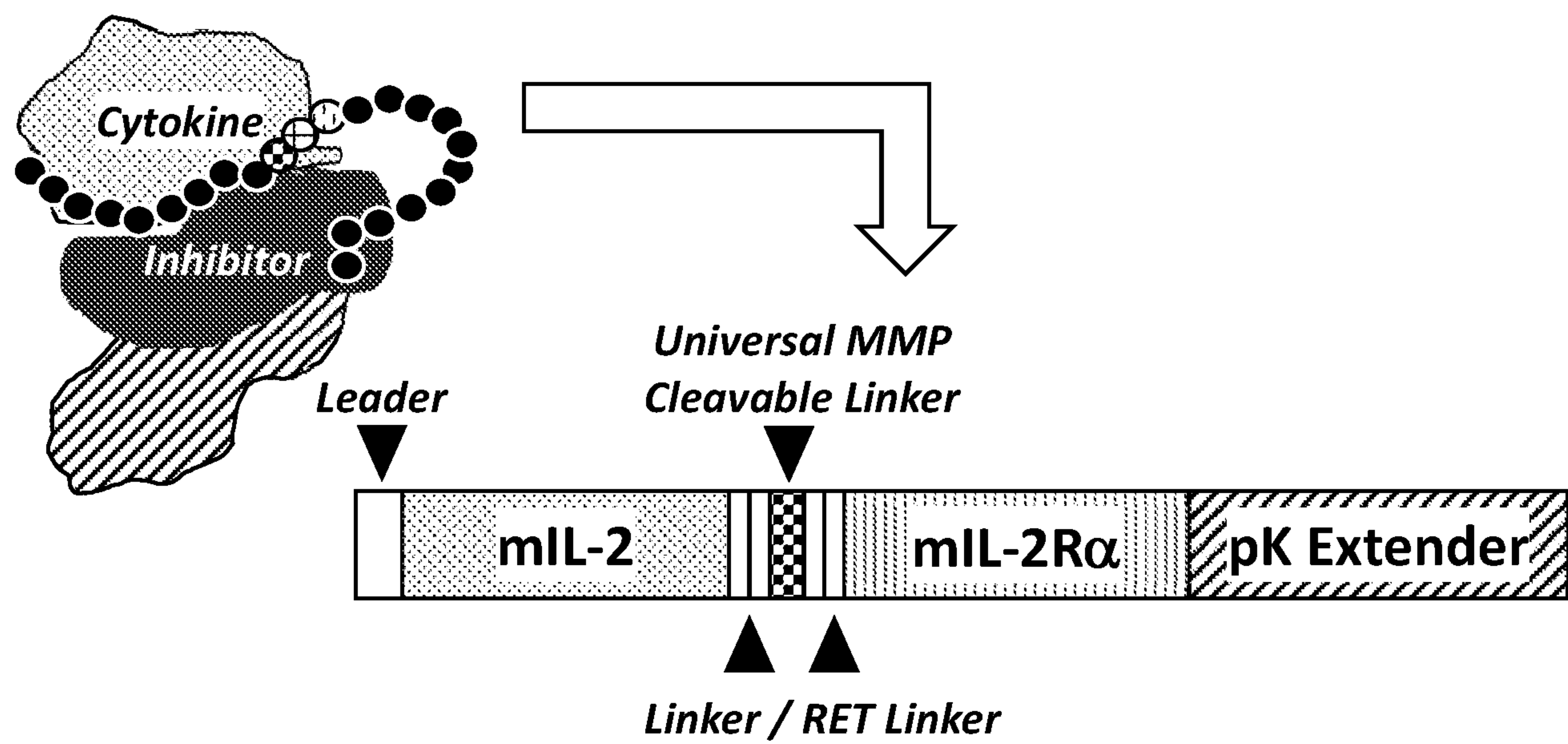
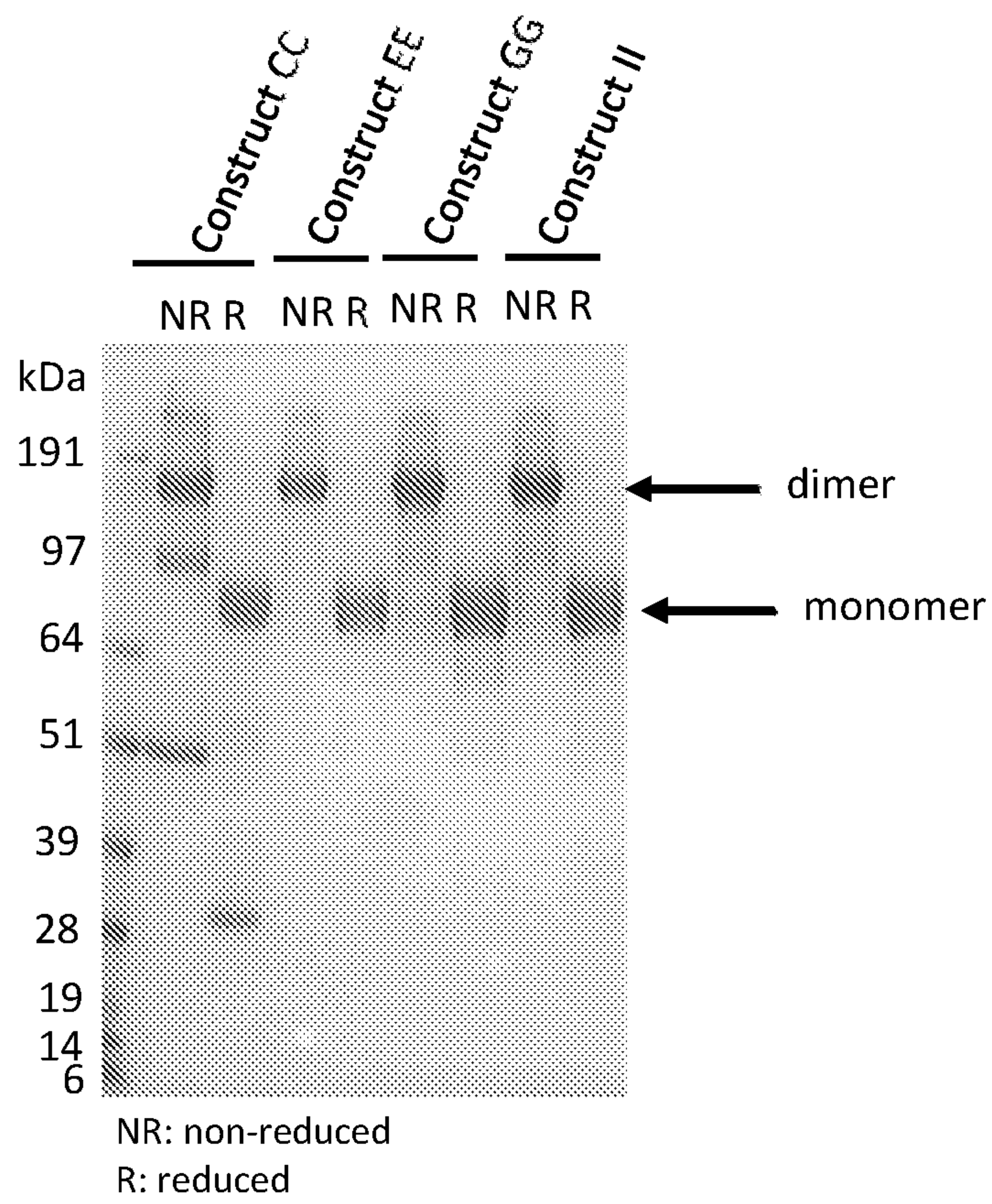


Fig. 1C

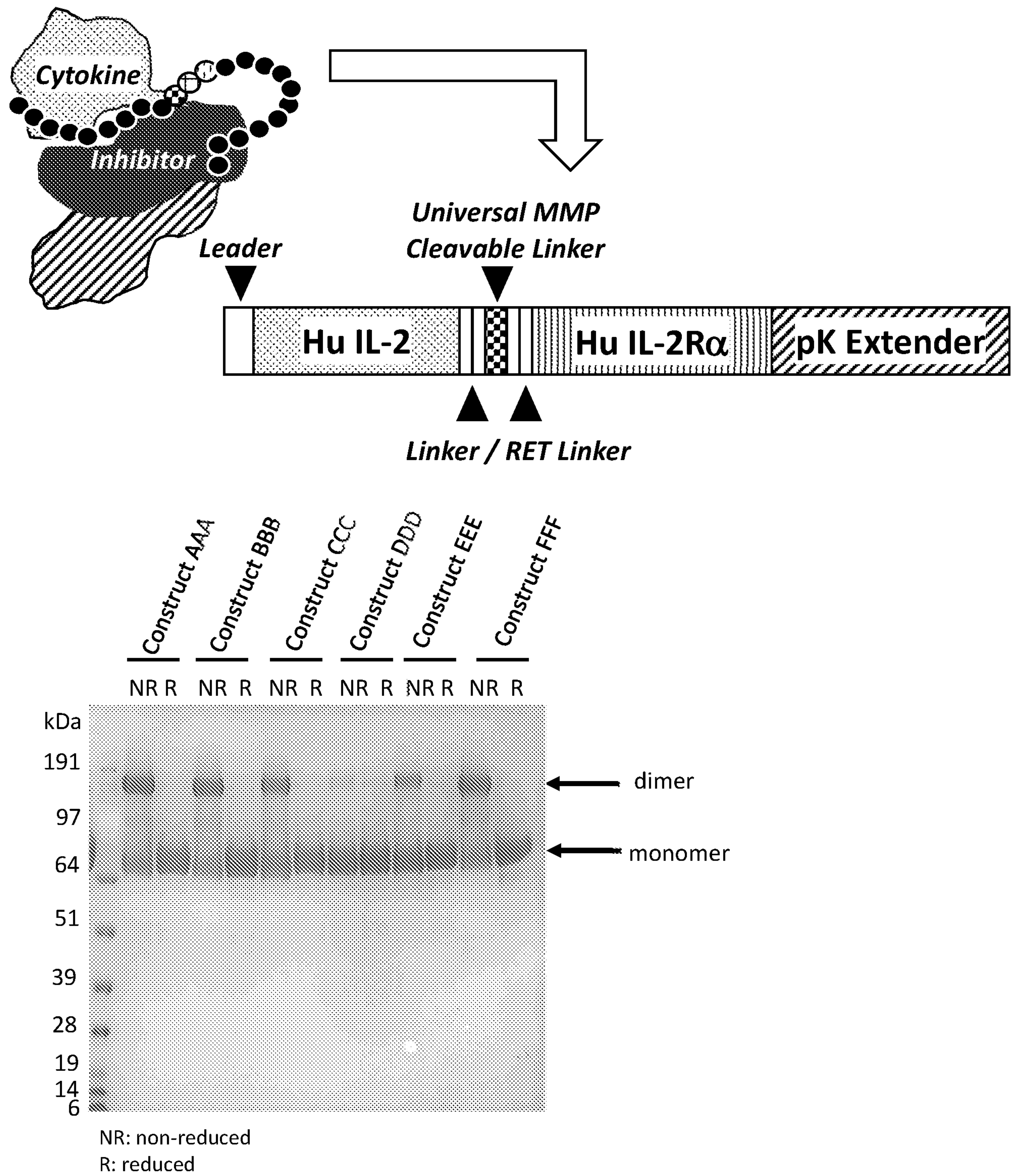


Fig. 1D

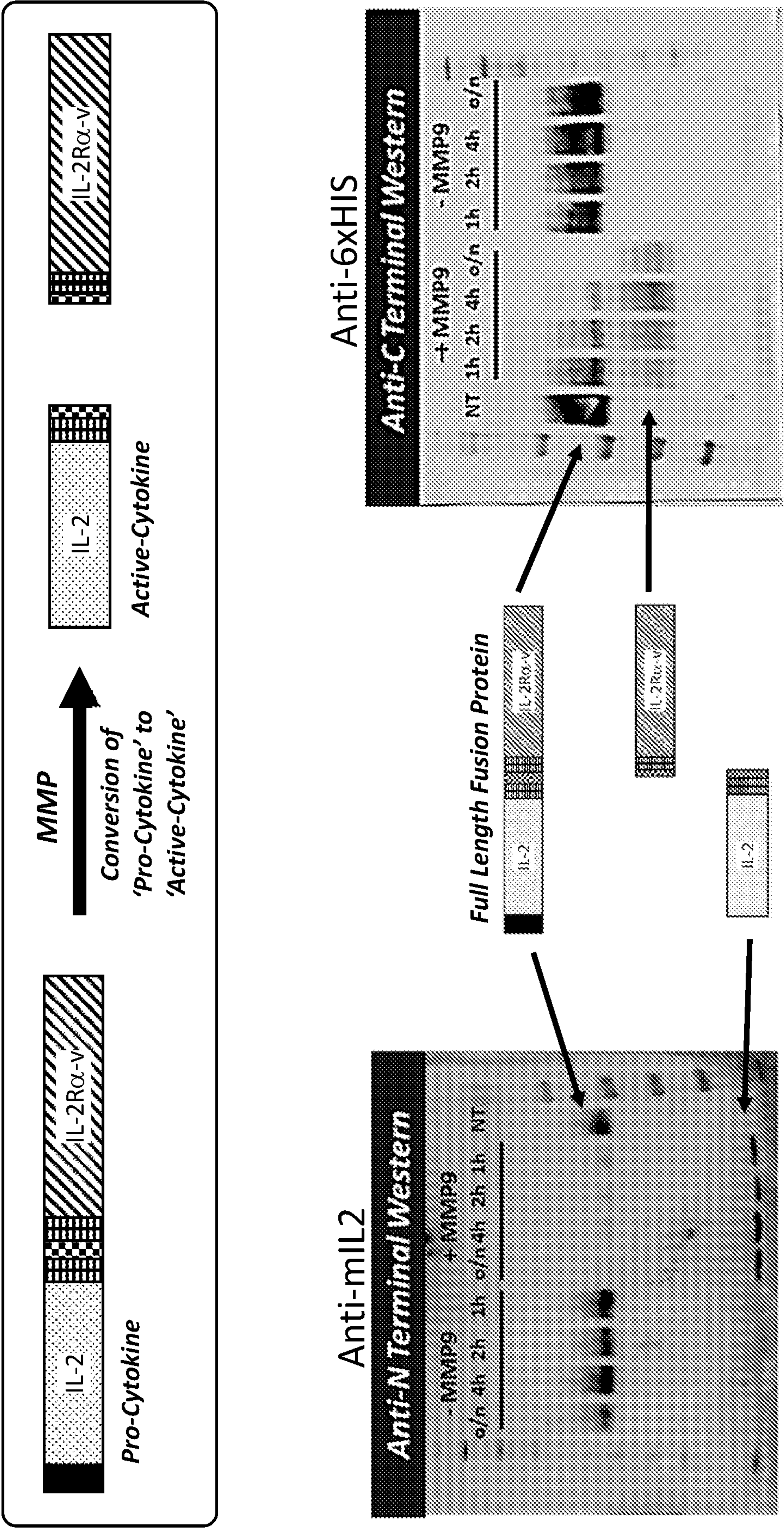
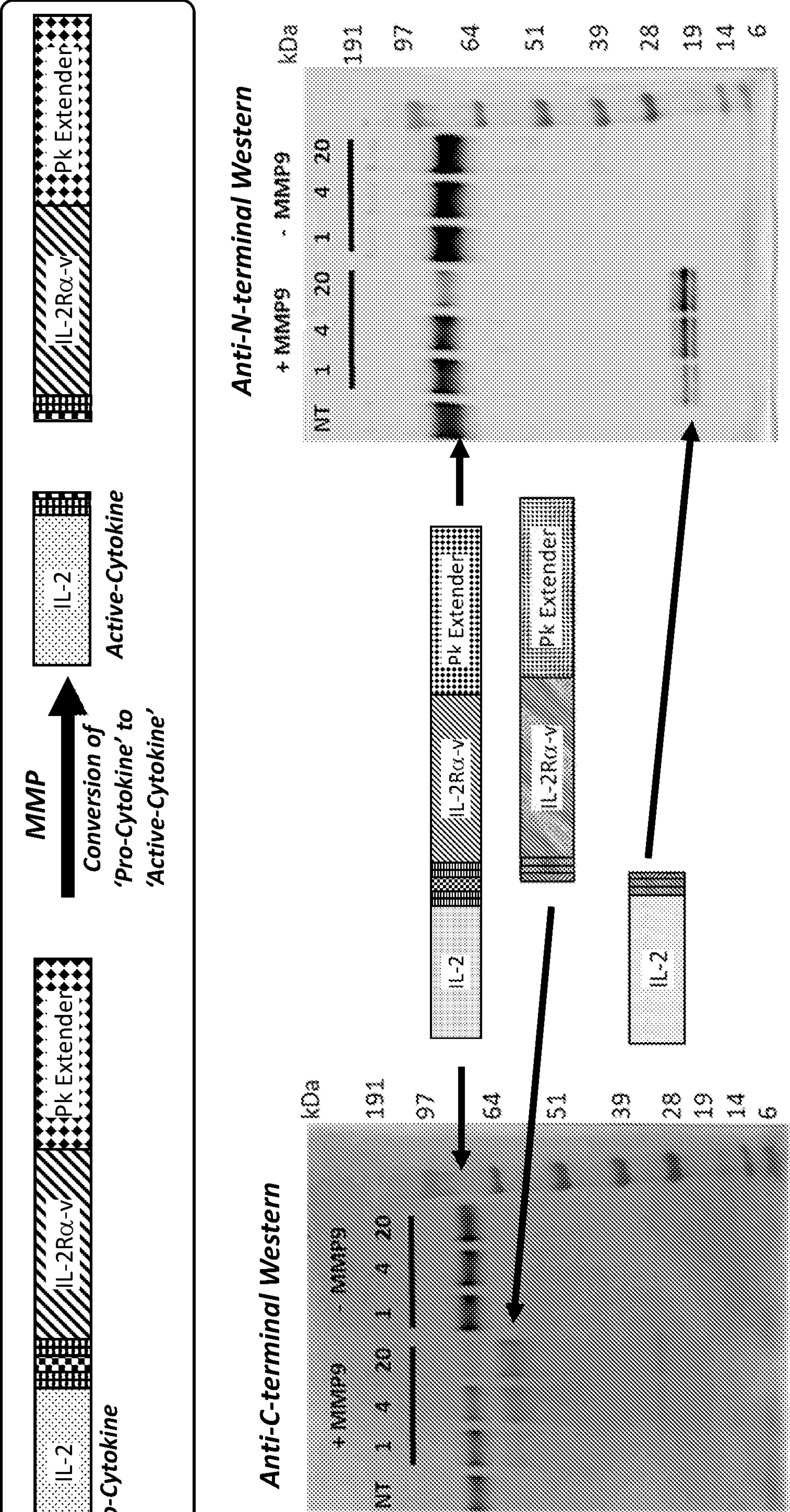


Fig. 2A



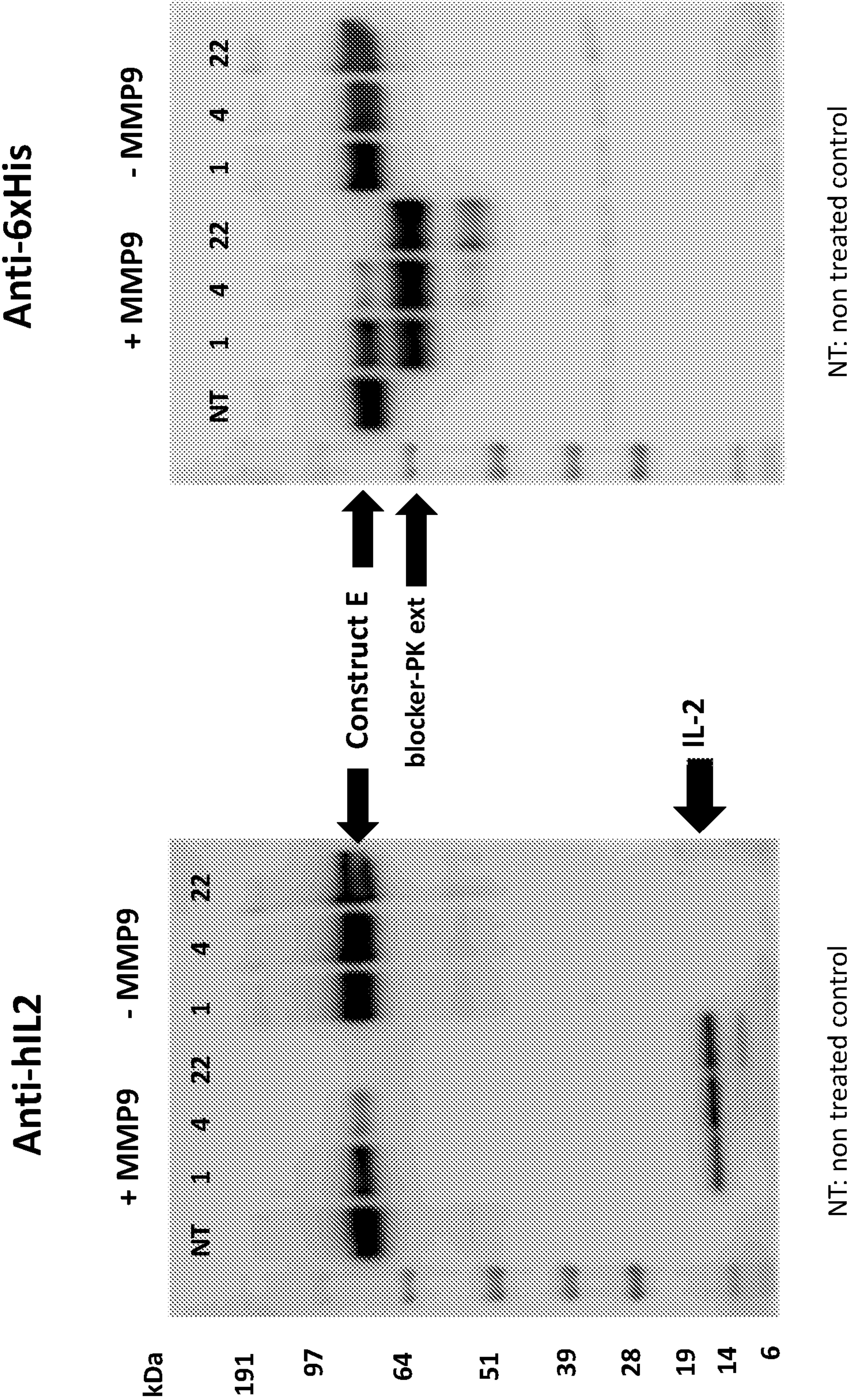


Fig. 2C

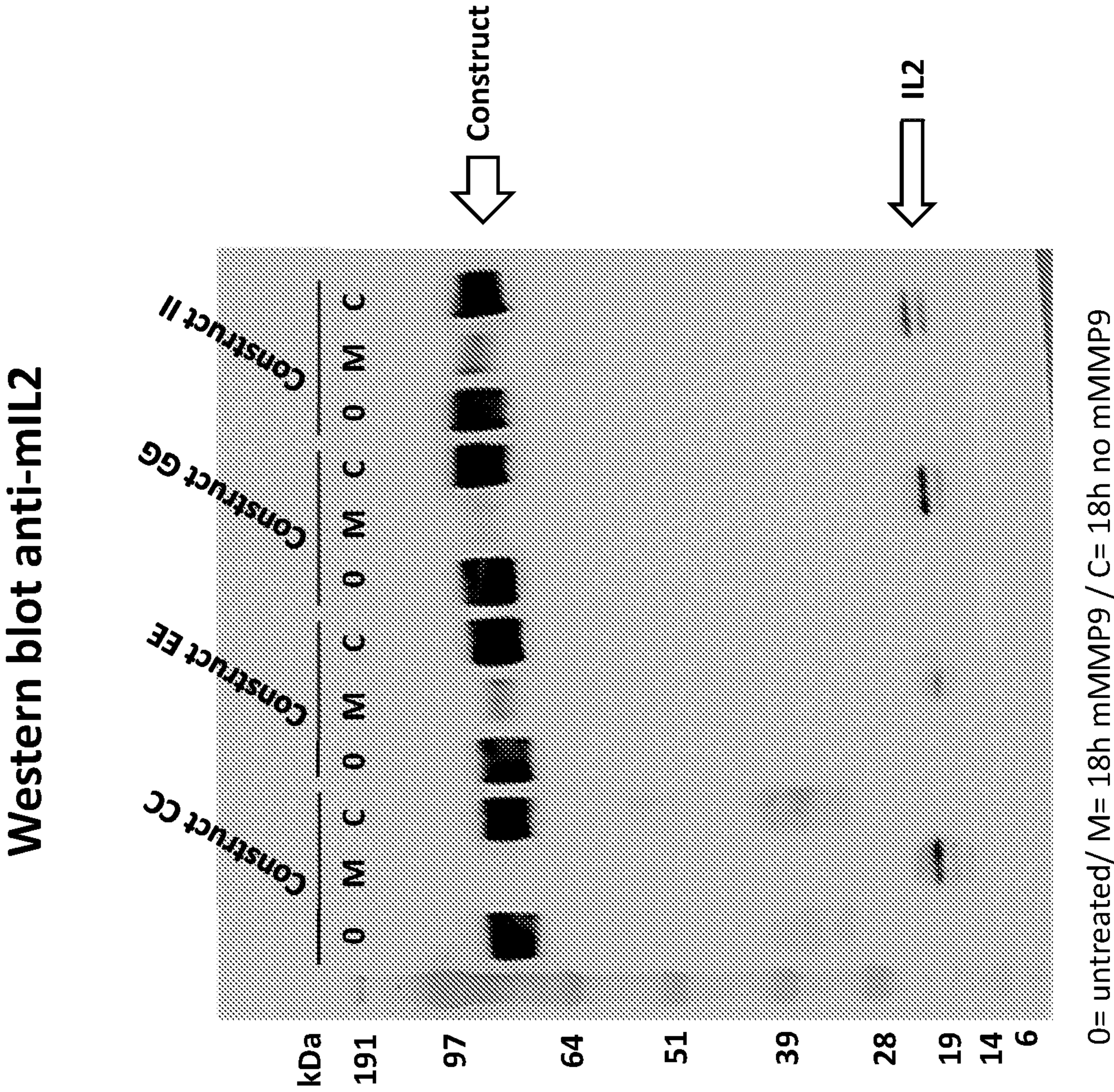


Fig. 2D

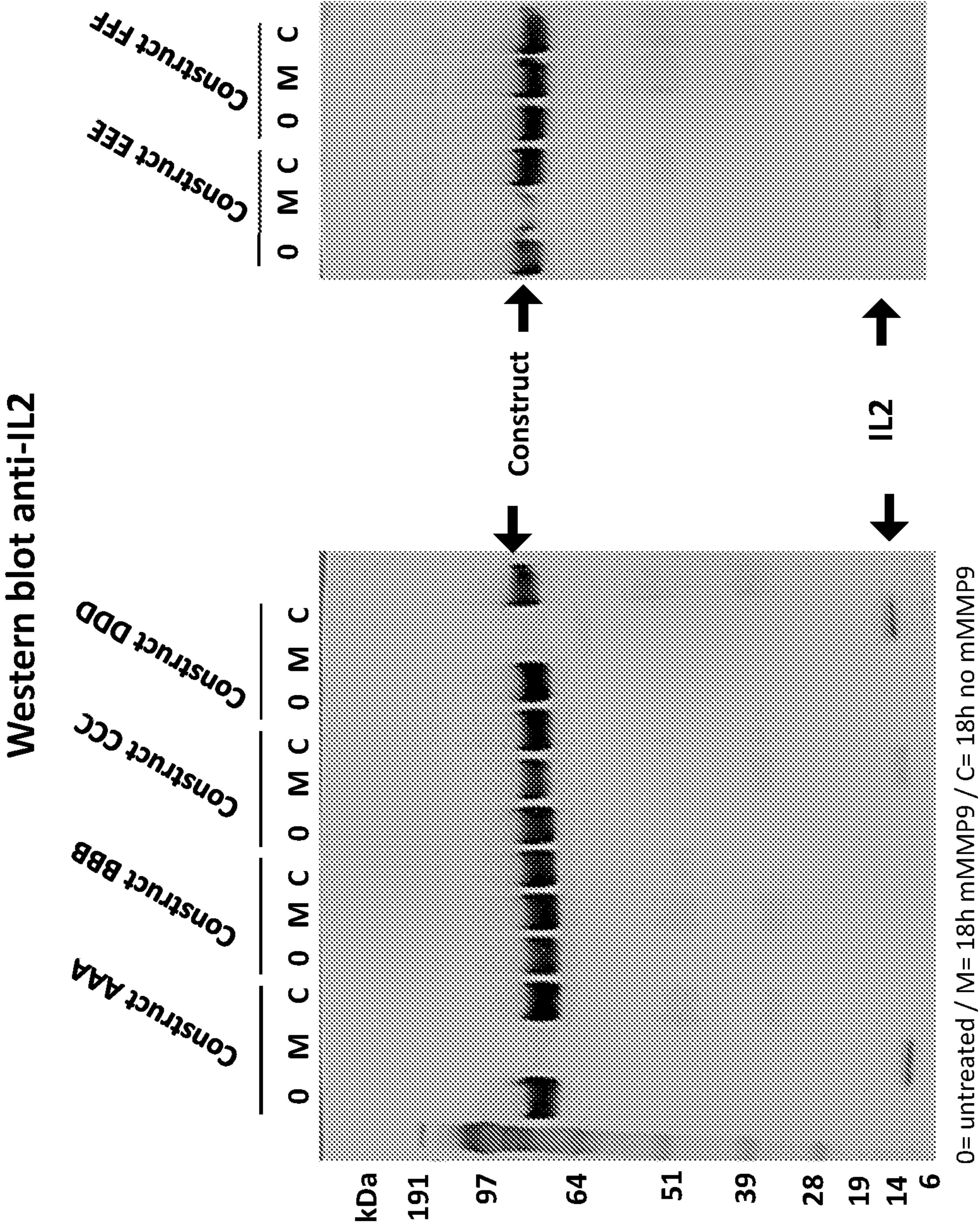


Fig. 2E

CTLL-2 proliferation Results

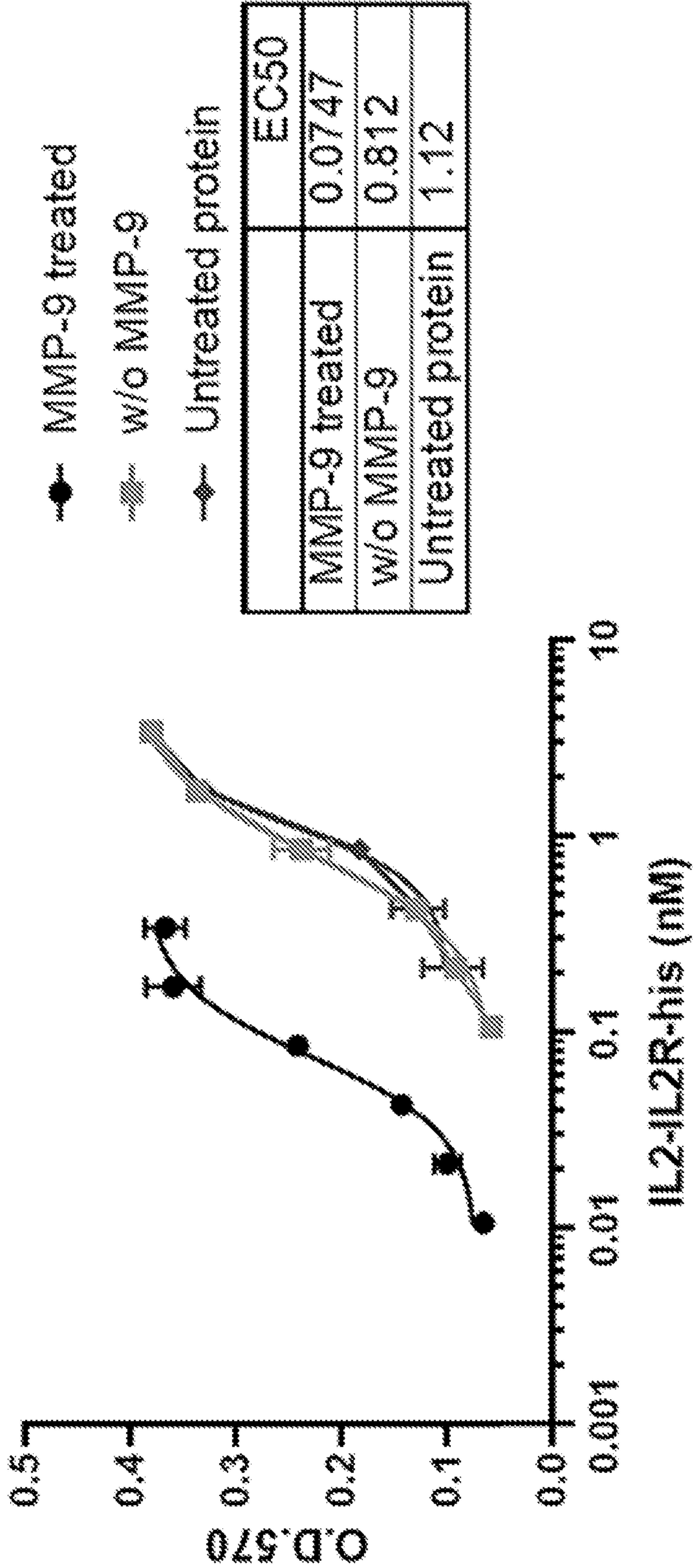
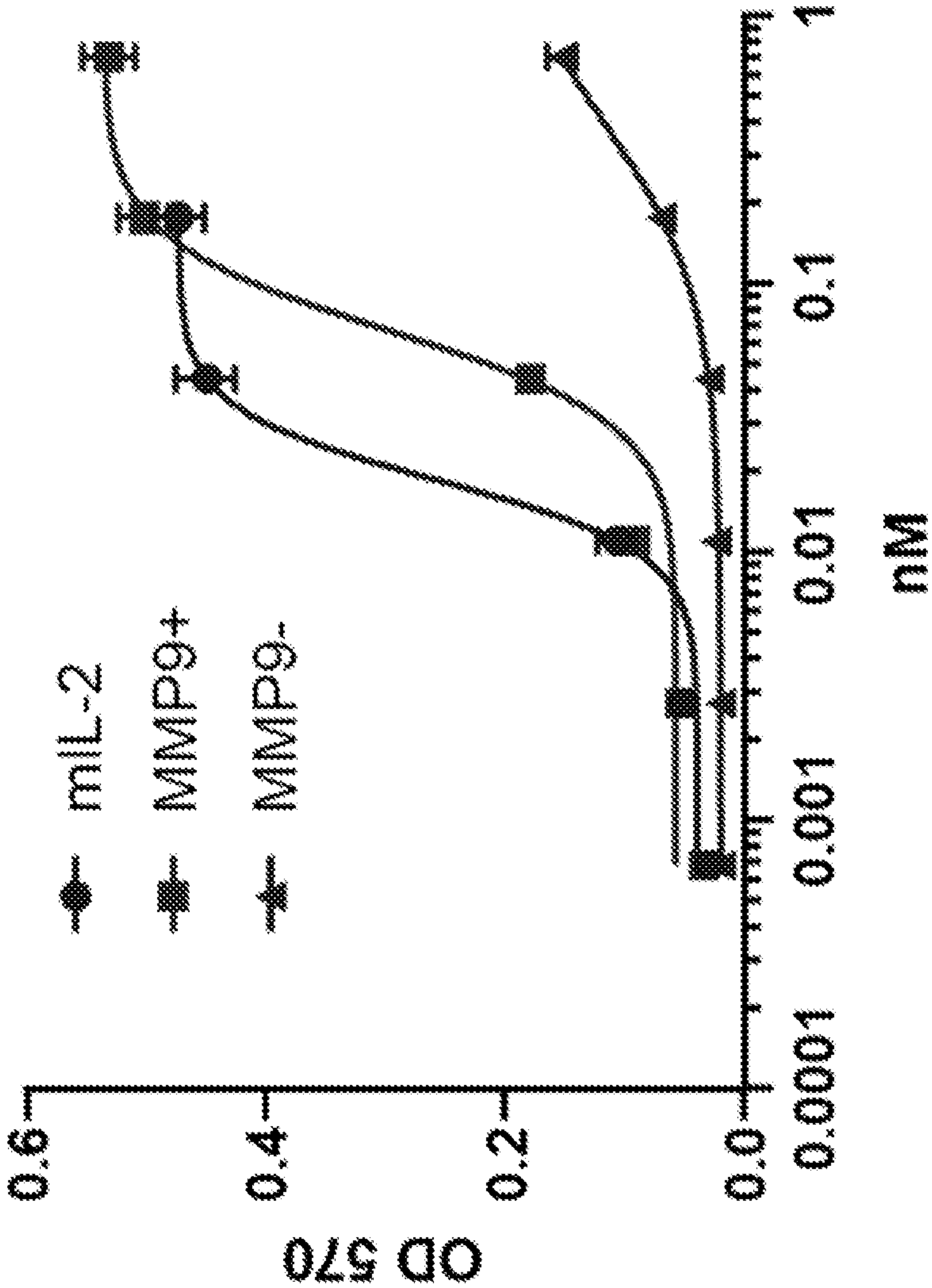


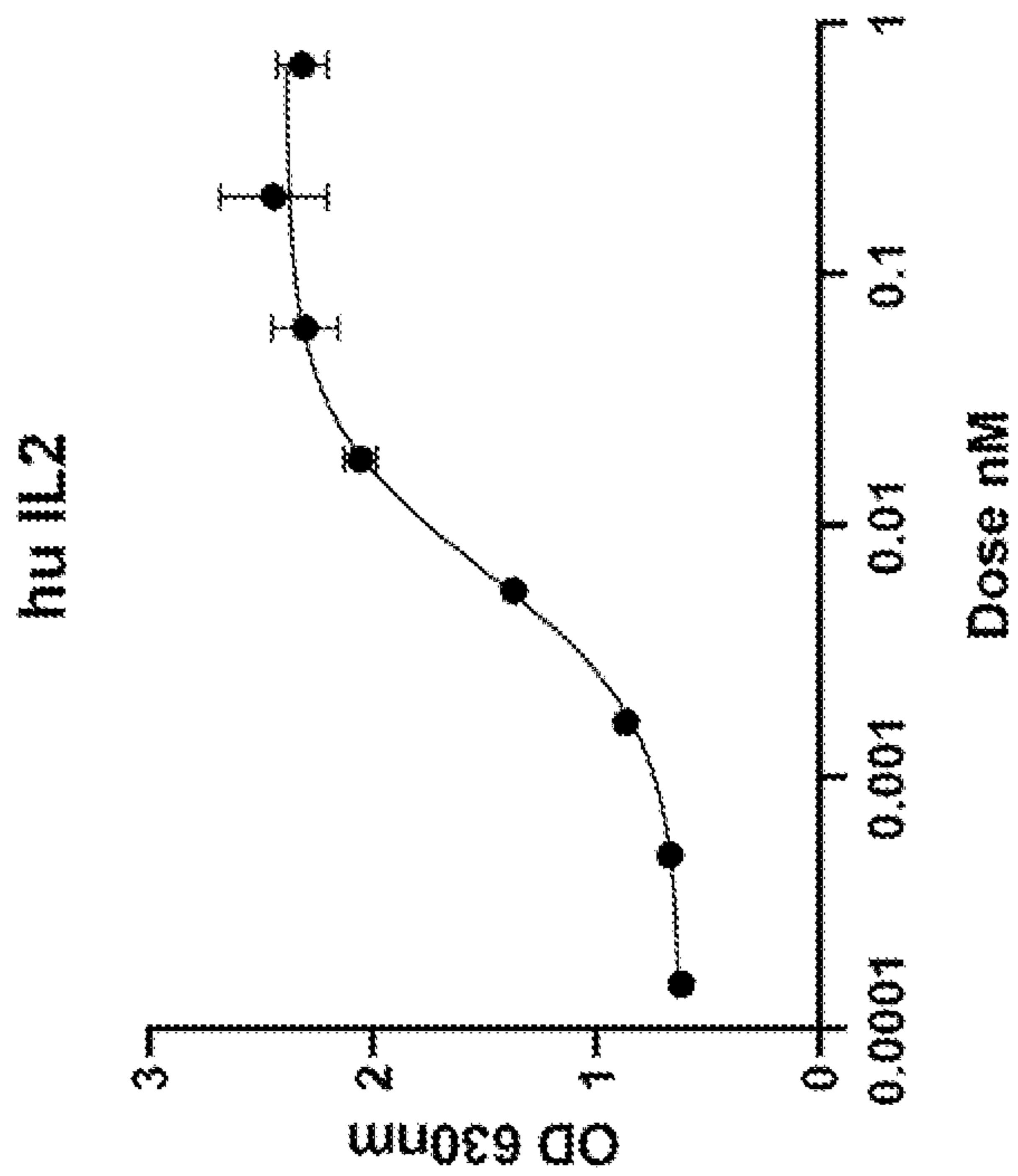
Fig. 3A

Construct B +/- MMP9 20h



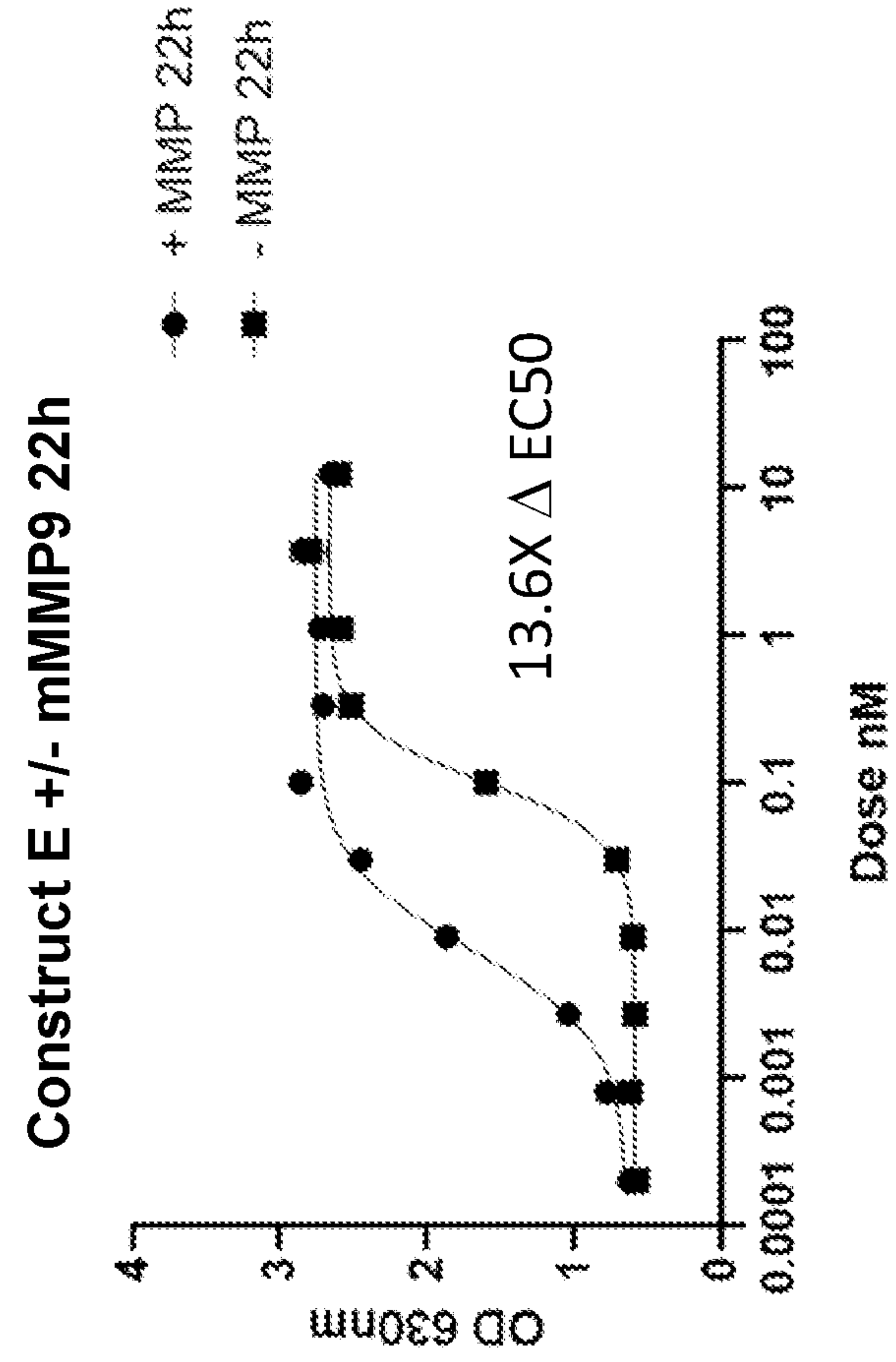
	mL-2	MMP9+
EC50	0.01836	0.06616

Fig. 3B



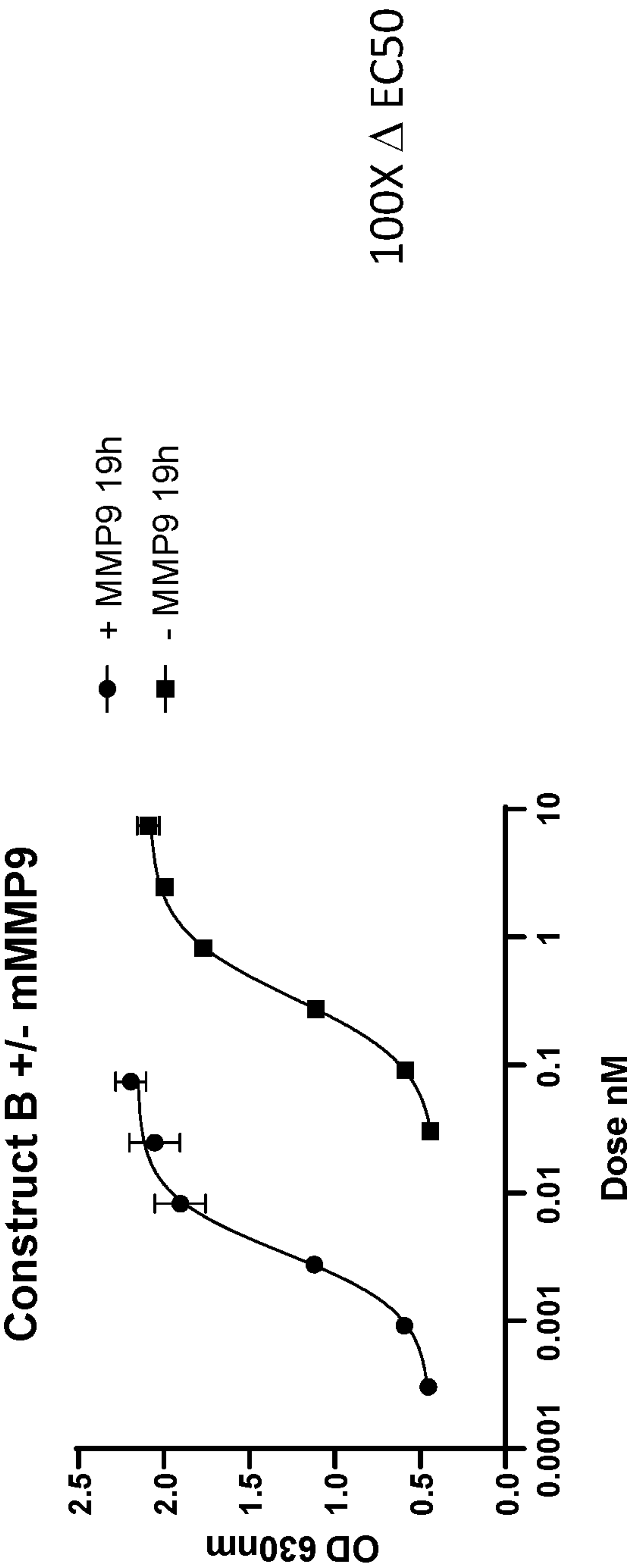
nM	hu IL2
EC50	0.0066

Fig. 3D



EC50 nM	+ MMP9	- MMP9
Construct E	0.00736	0.103

Fig. 3C



EC50 nM	+ MMP9	- MMP9
Construct B	0.0034	0.335

Fig. 3E

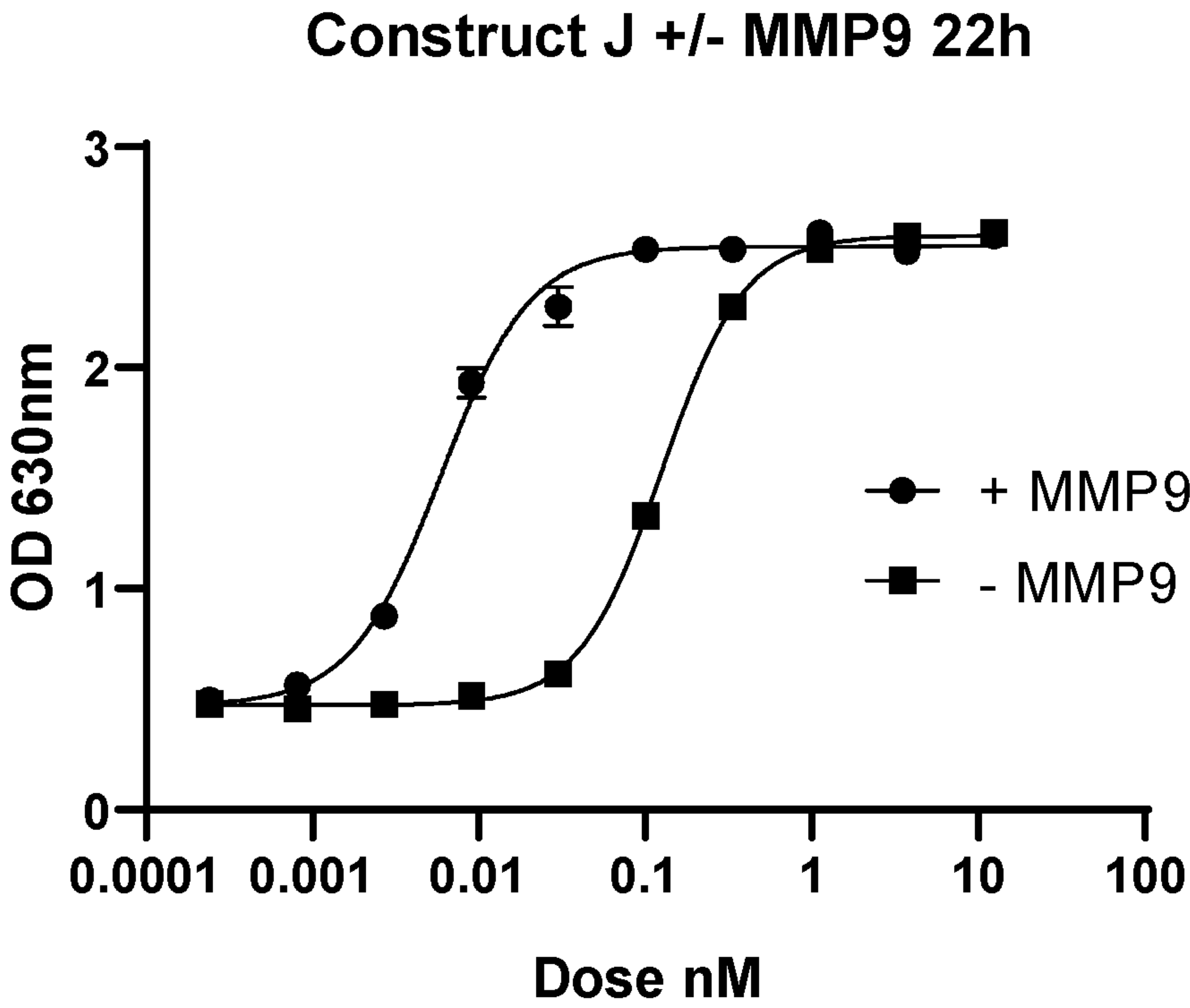


Fig. 3F

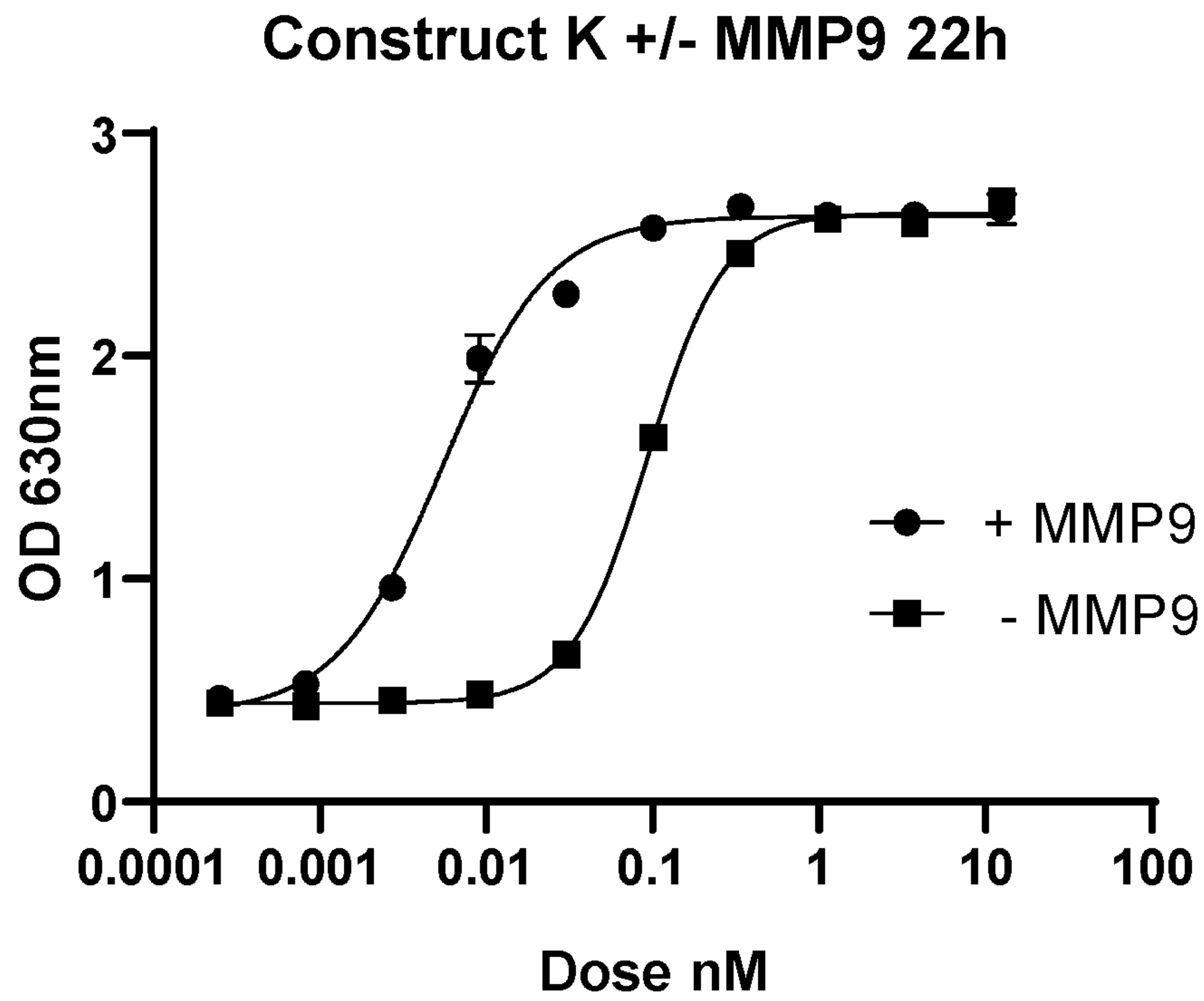


Fig. 3G

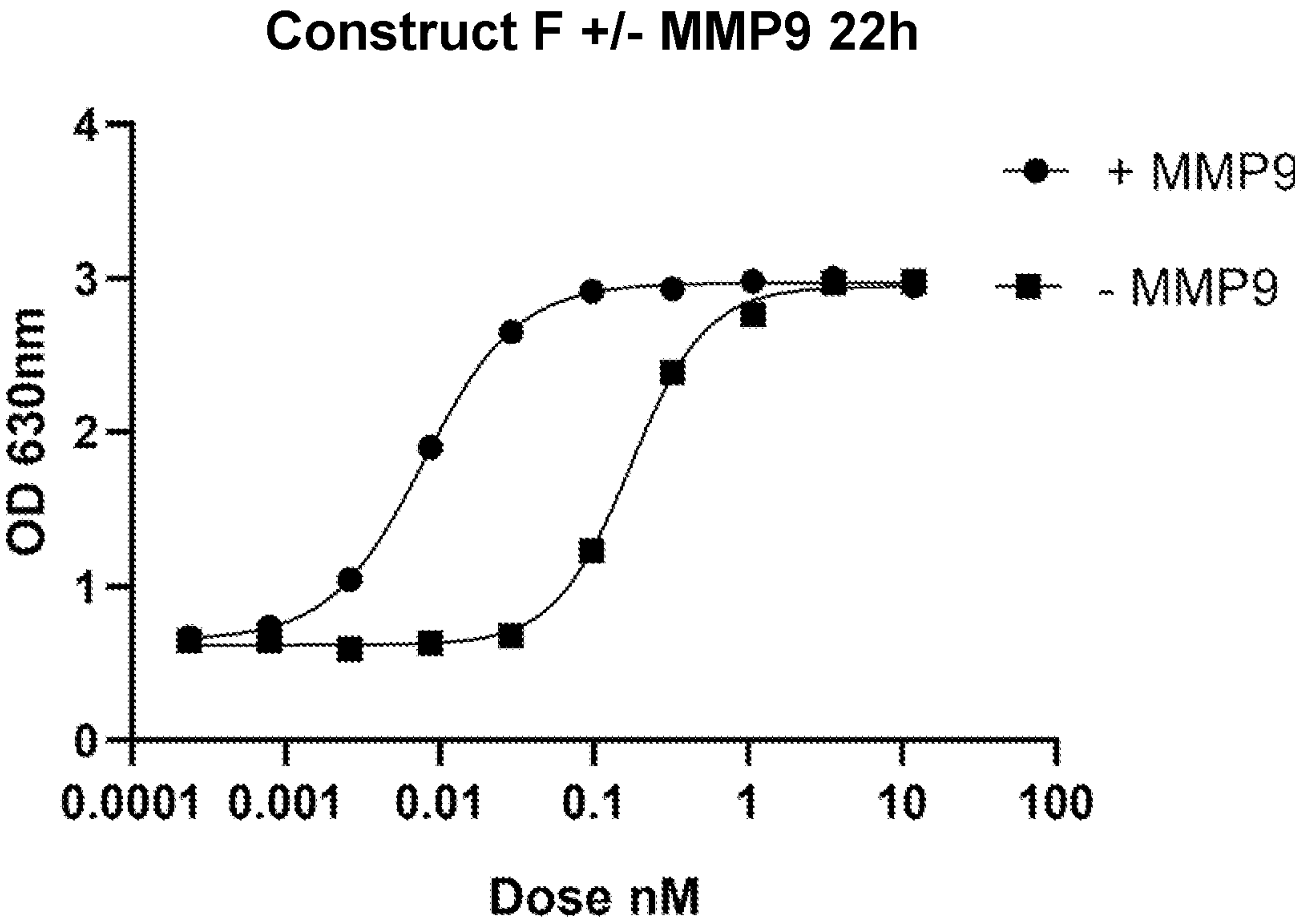


Fig. 3H

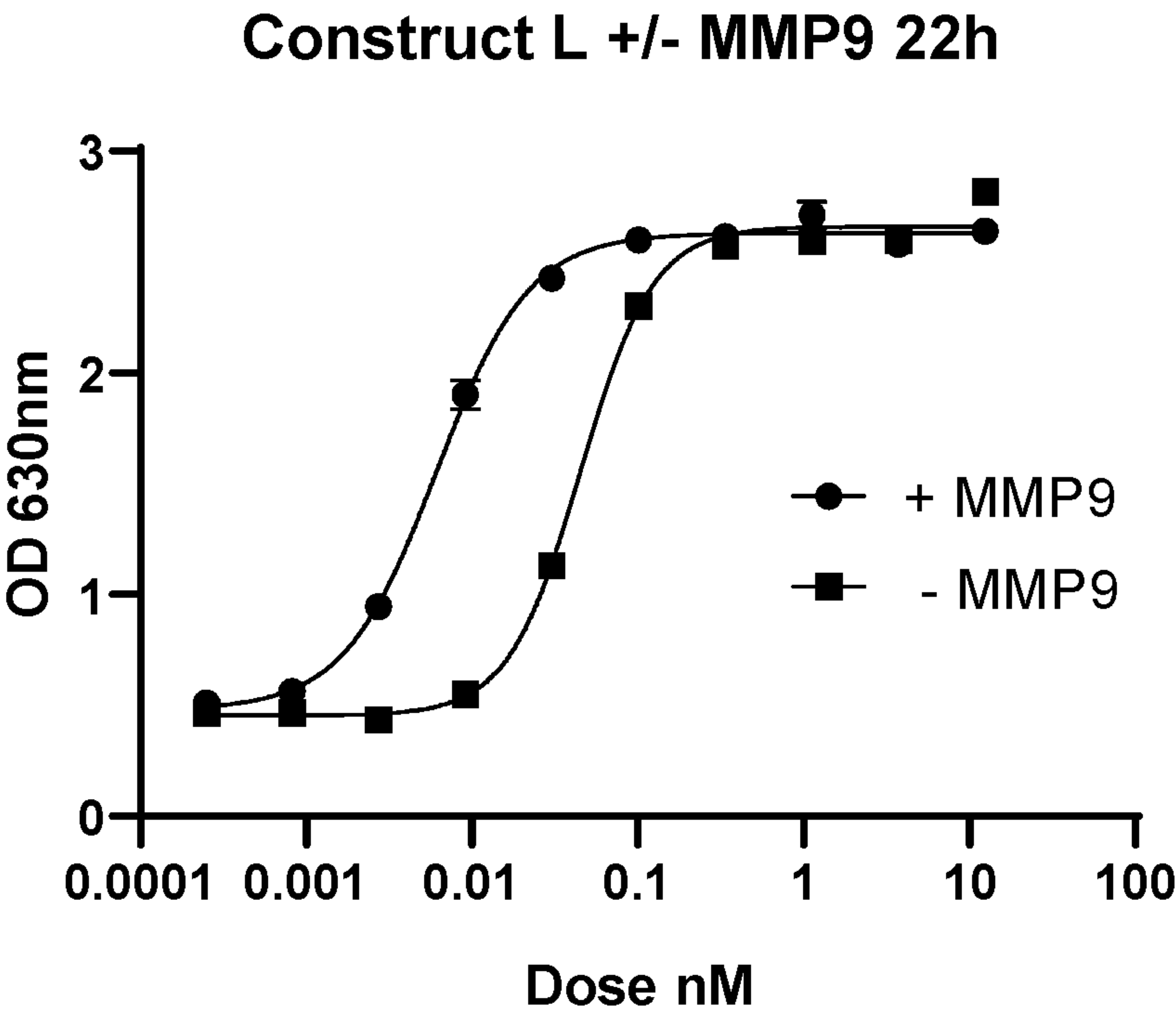


Fig. 3I

Construct I +/- MMP9 22h

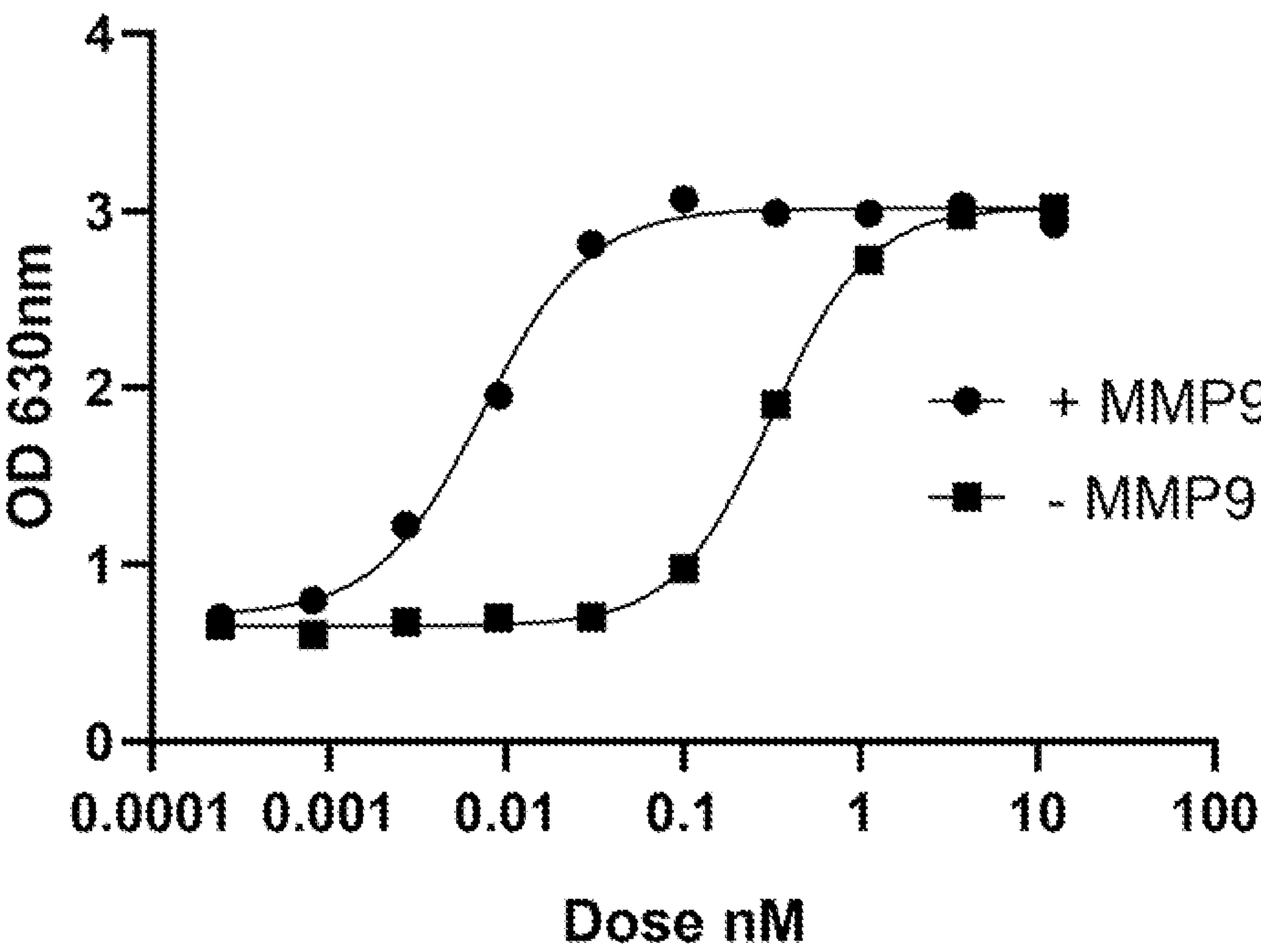
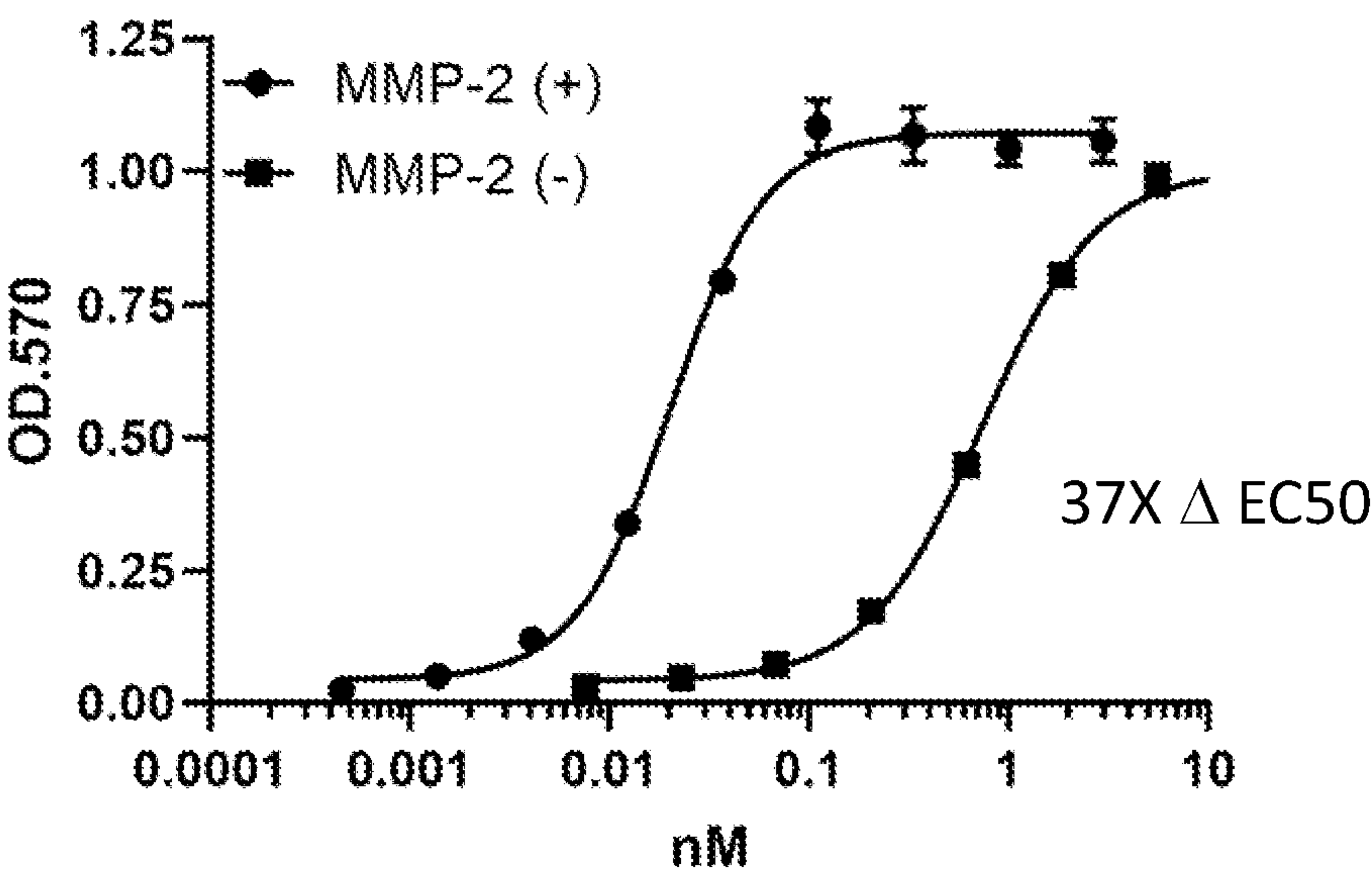


Fig. 3J

CTLL-2 assay Construct M +/- MMP2 2h



	MMP-2 (+)	MMP-2 (-)
EC50	0.02032	0.7380

Fig. 3K

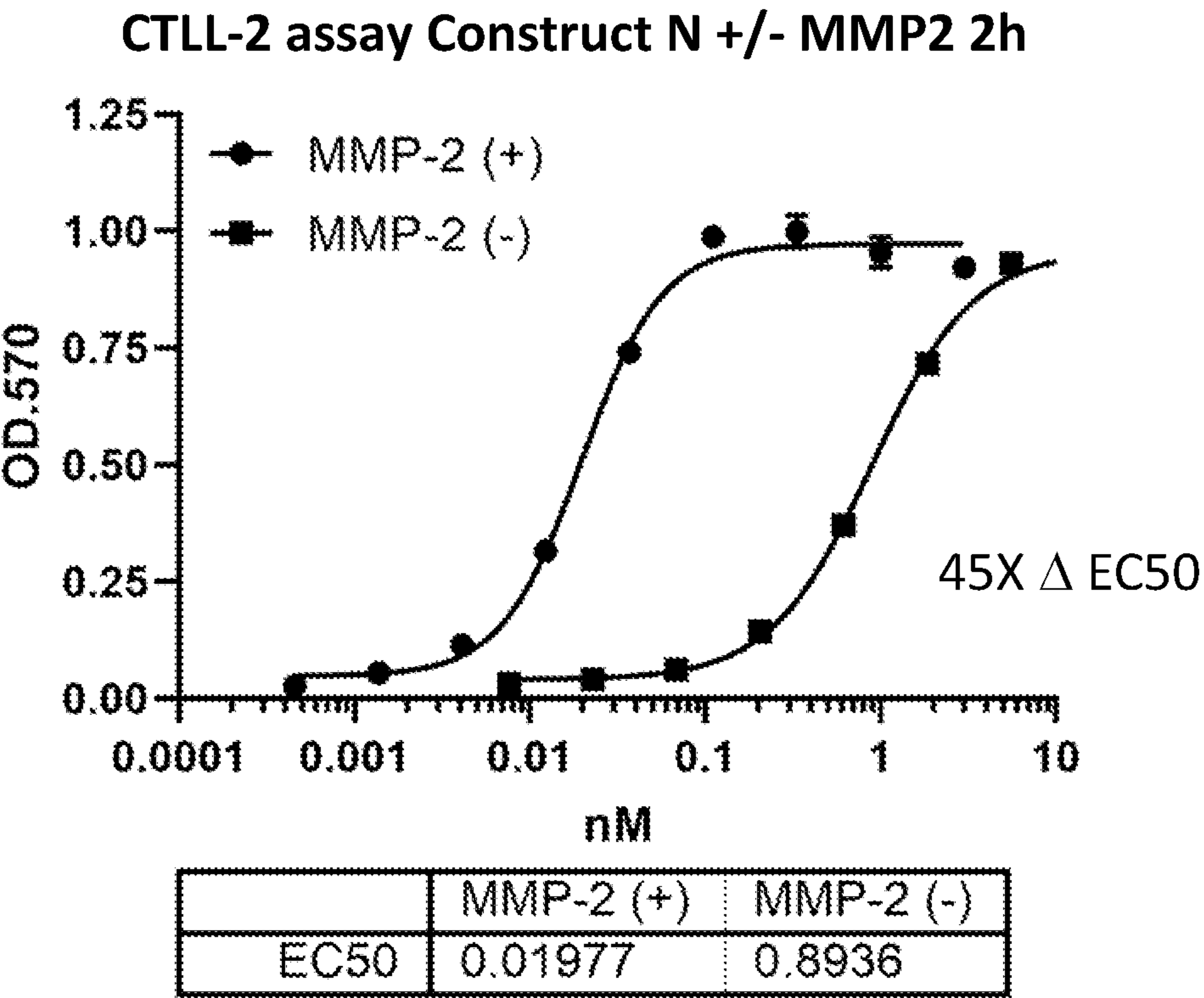


Fig. 3L

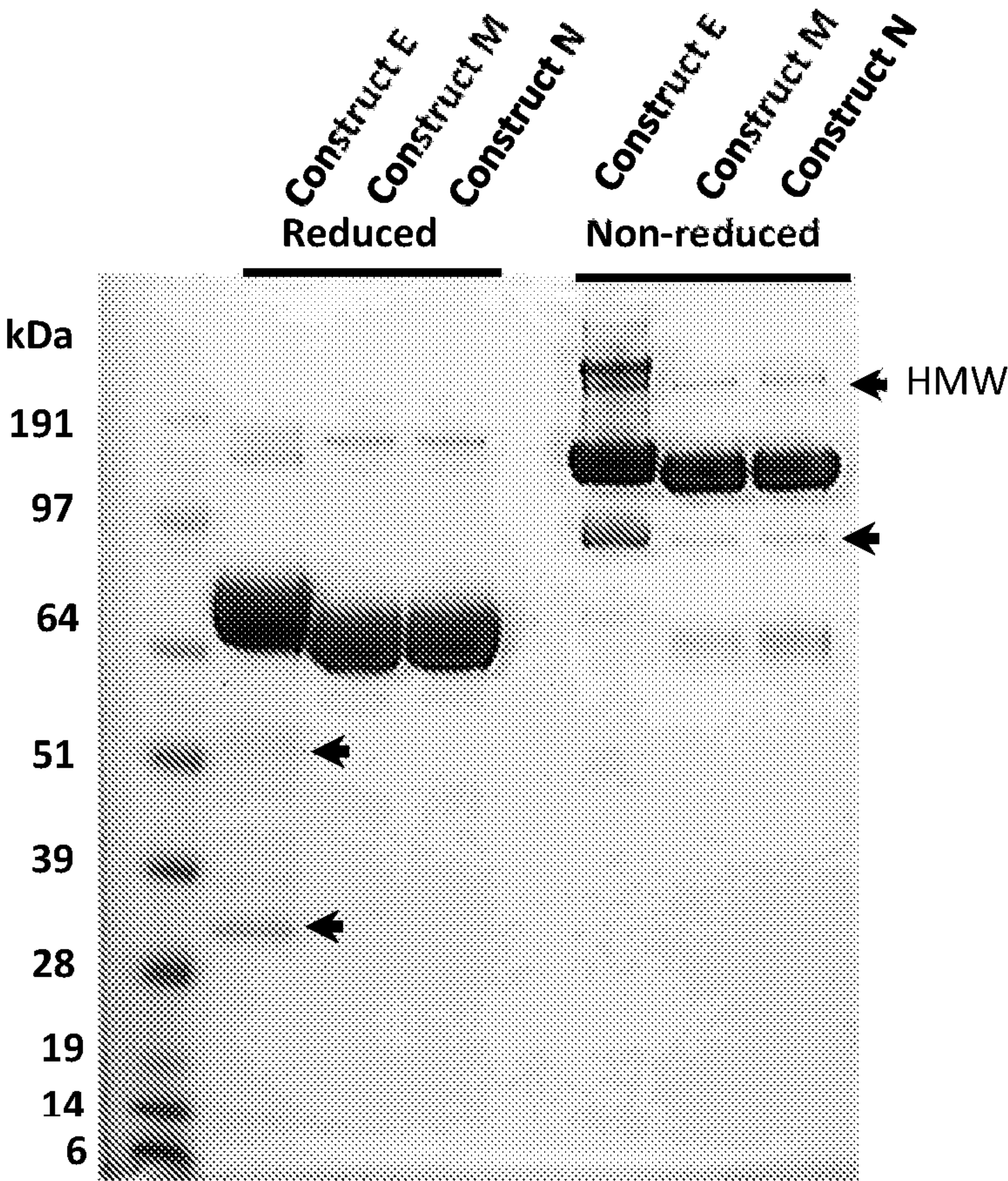


Fig. 3M

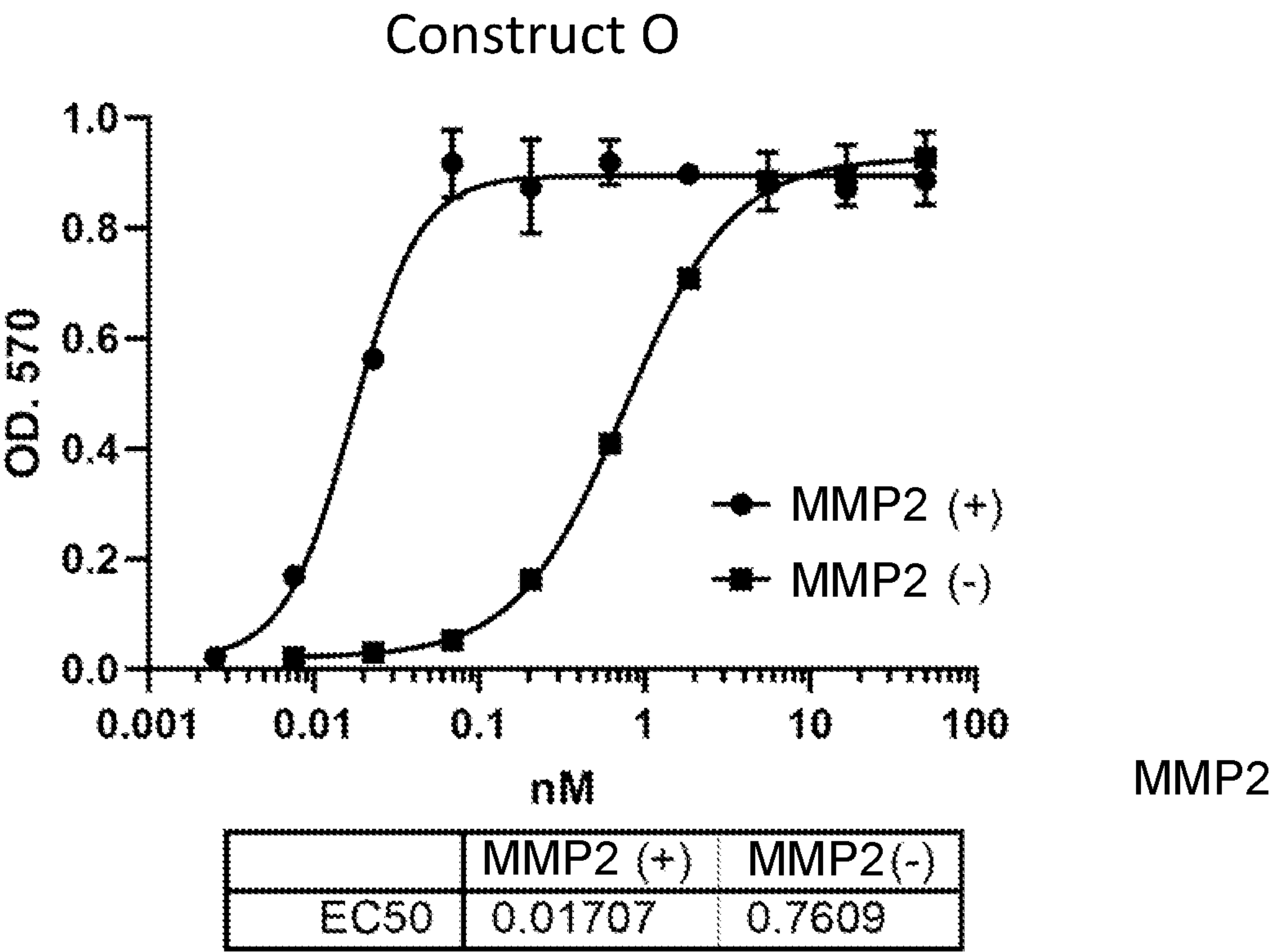


Fig. 3N

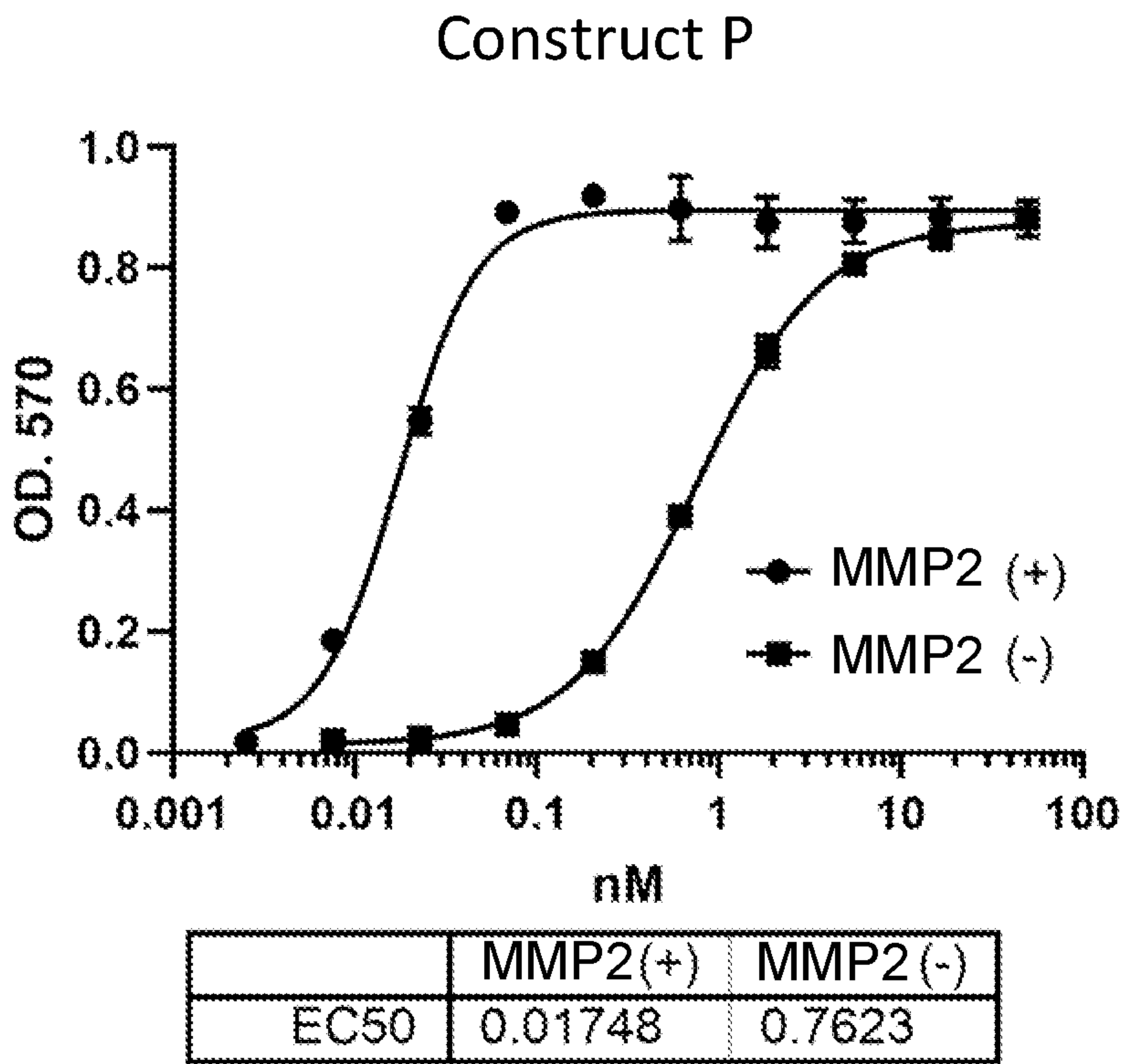


Fig. 30

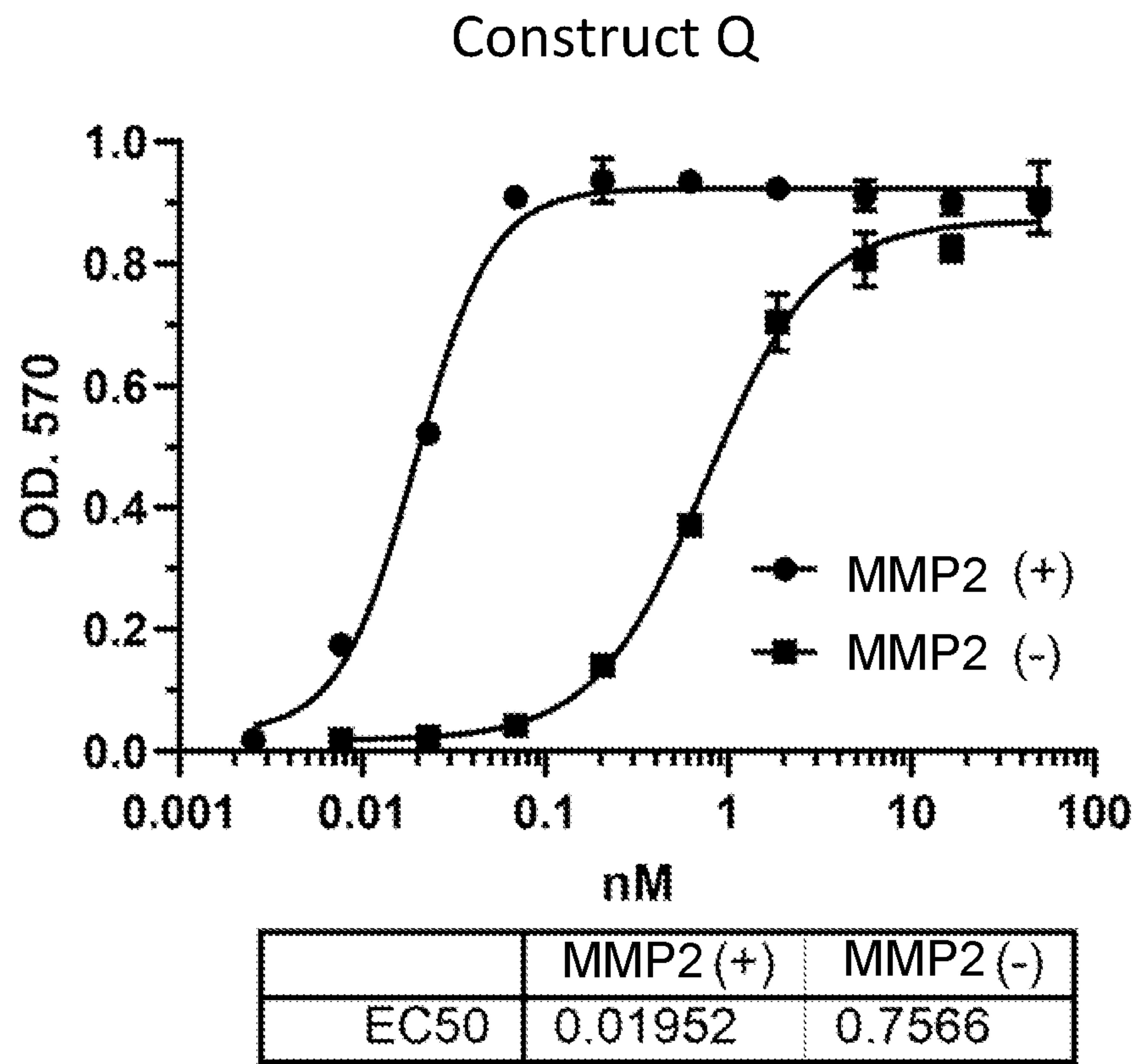


Fig. 3P

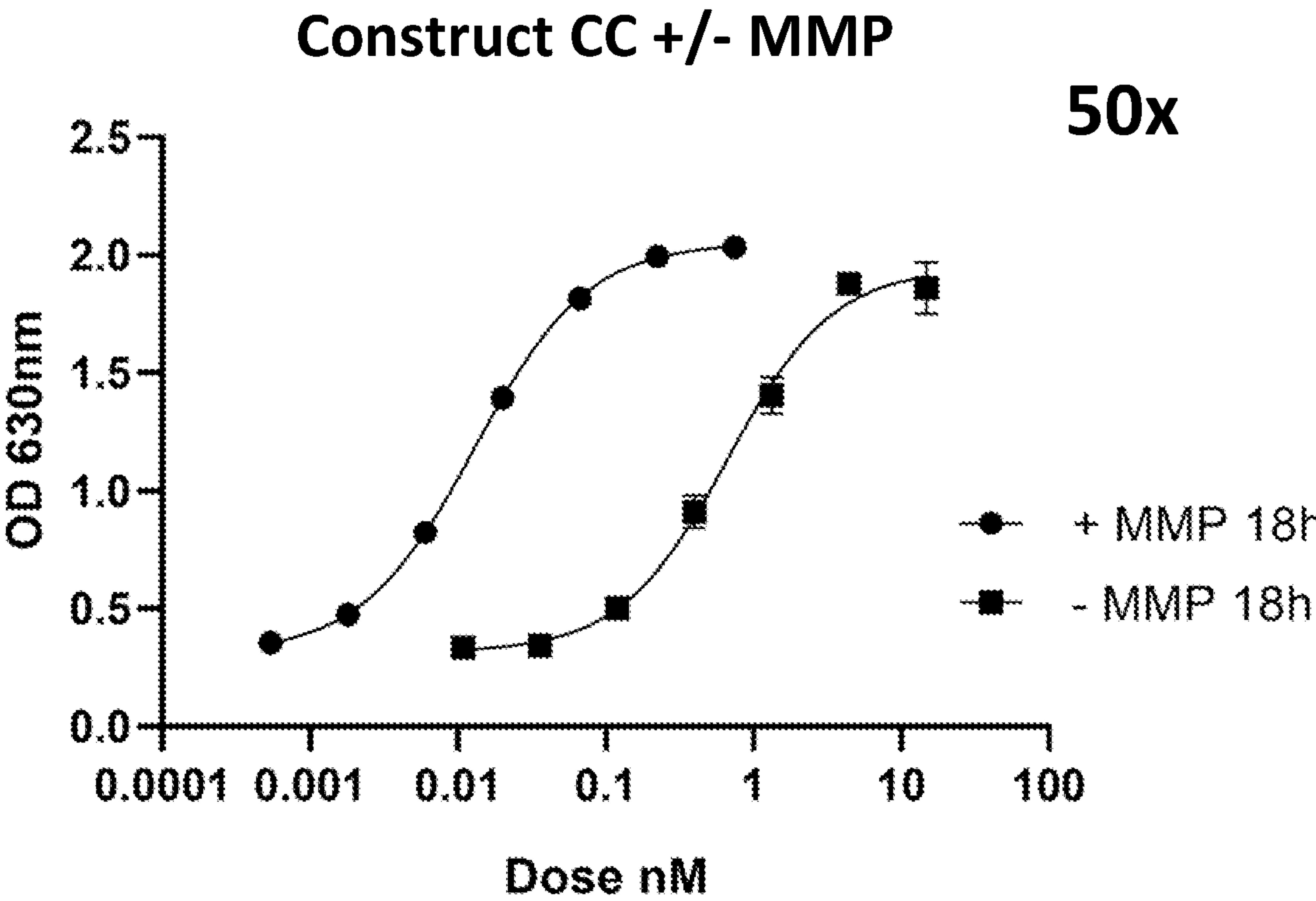


Fig. 3Q

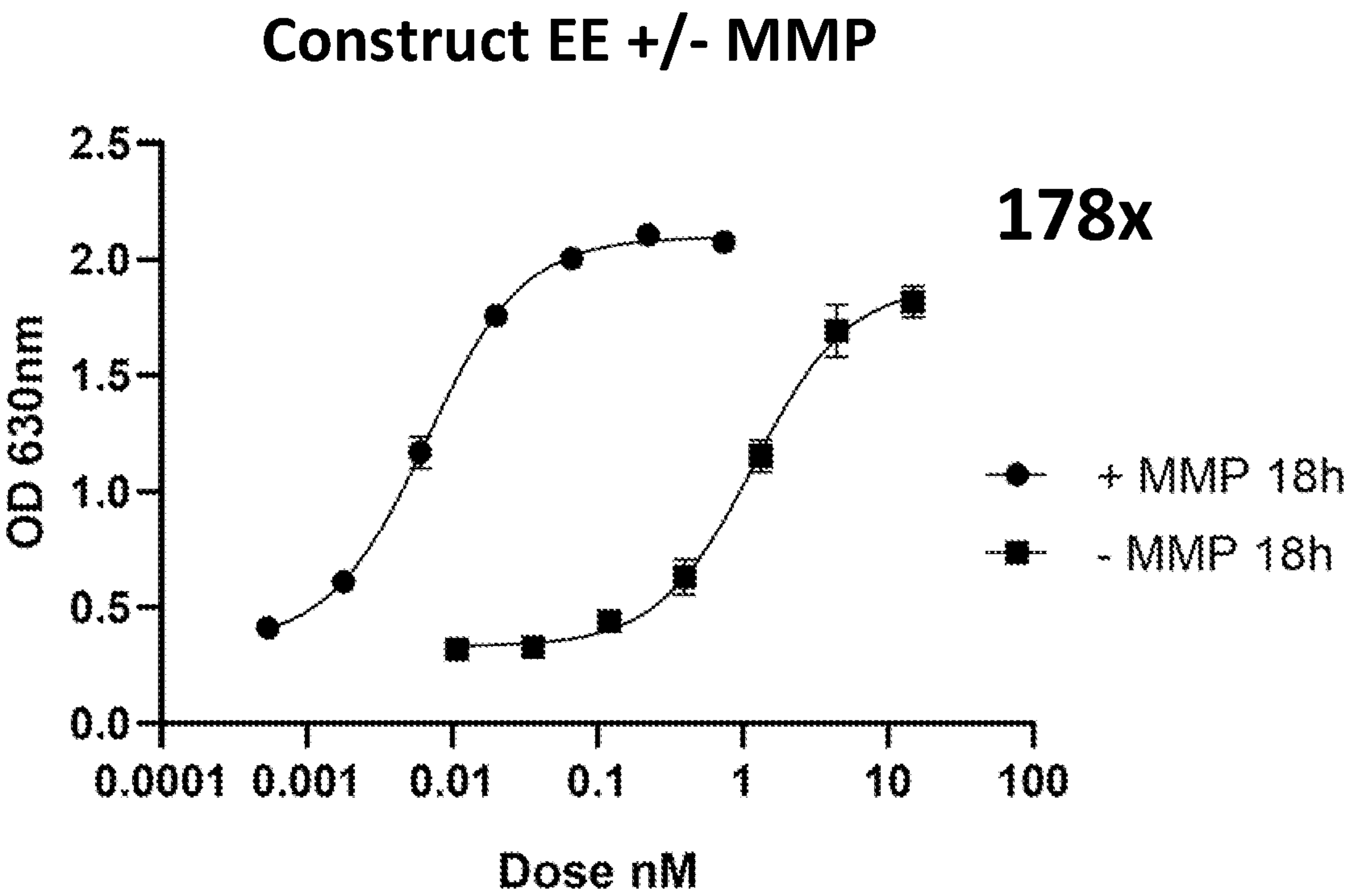


Fig. 3R

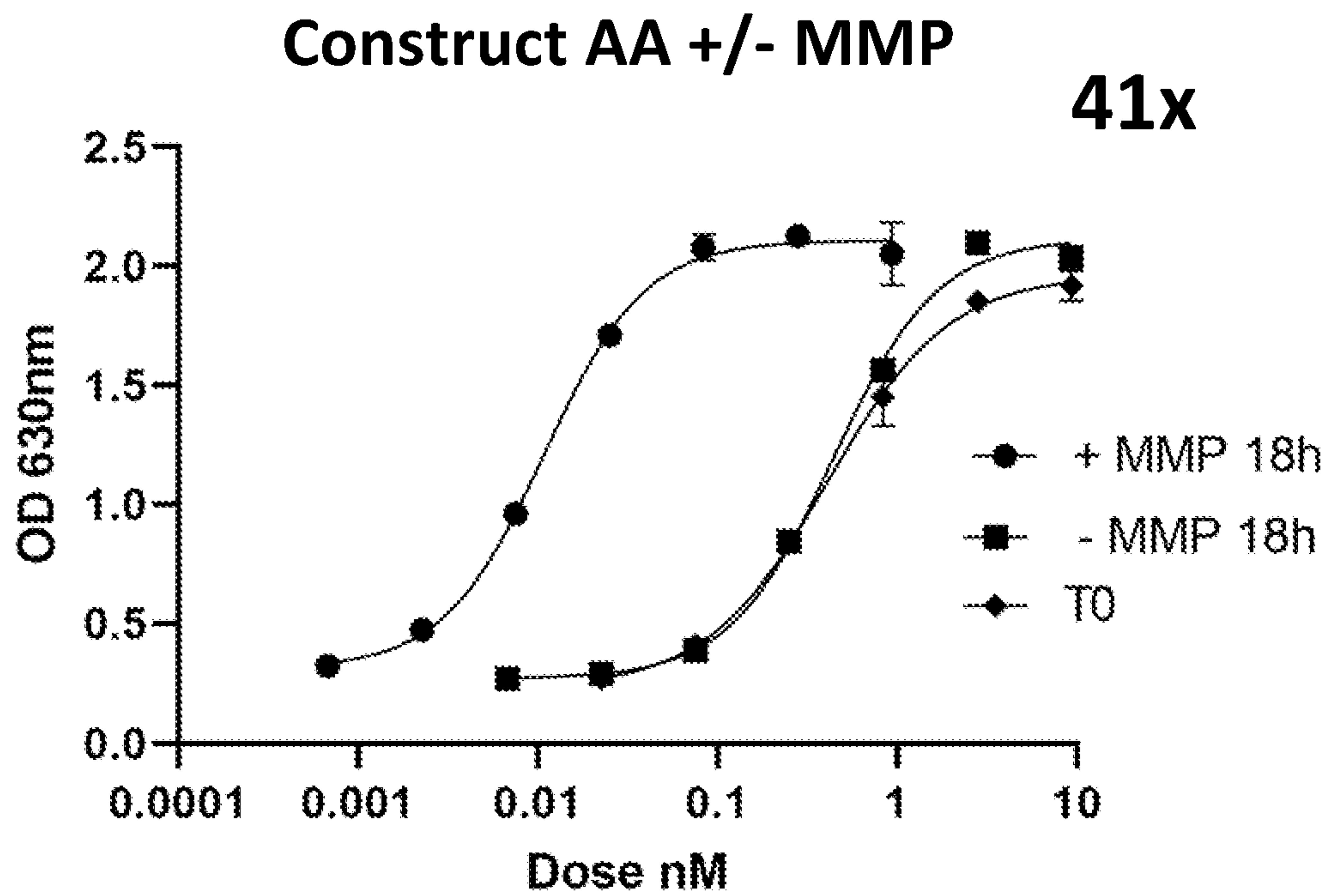


Fig. 3S

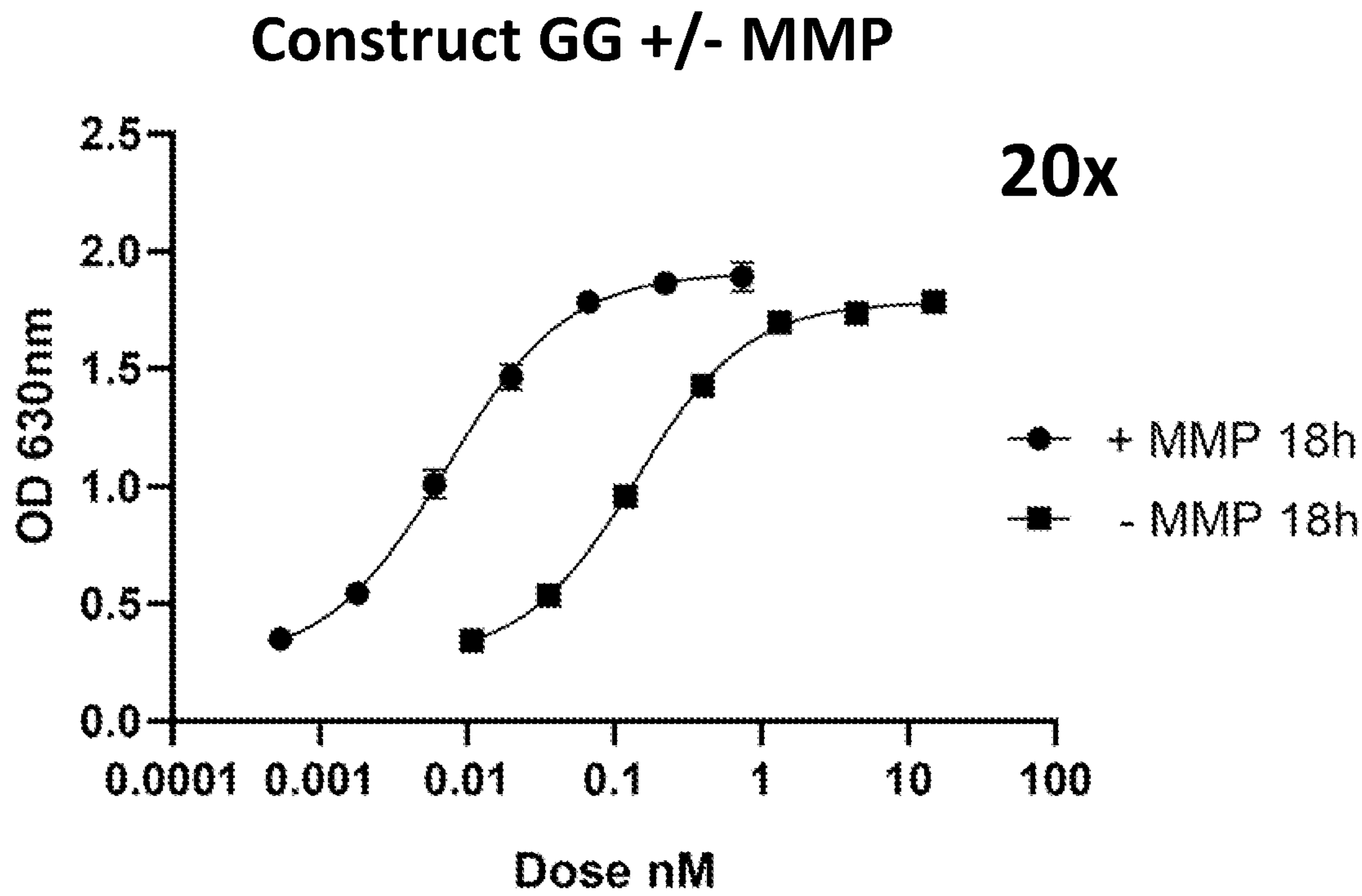


Fig. 3T

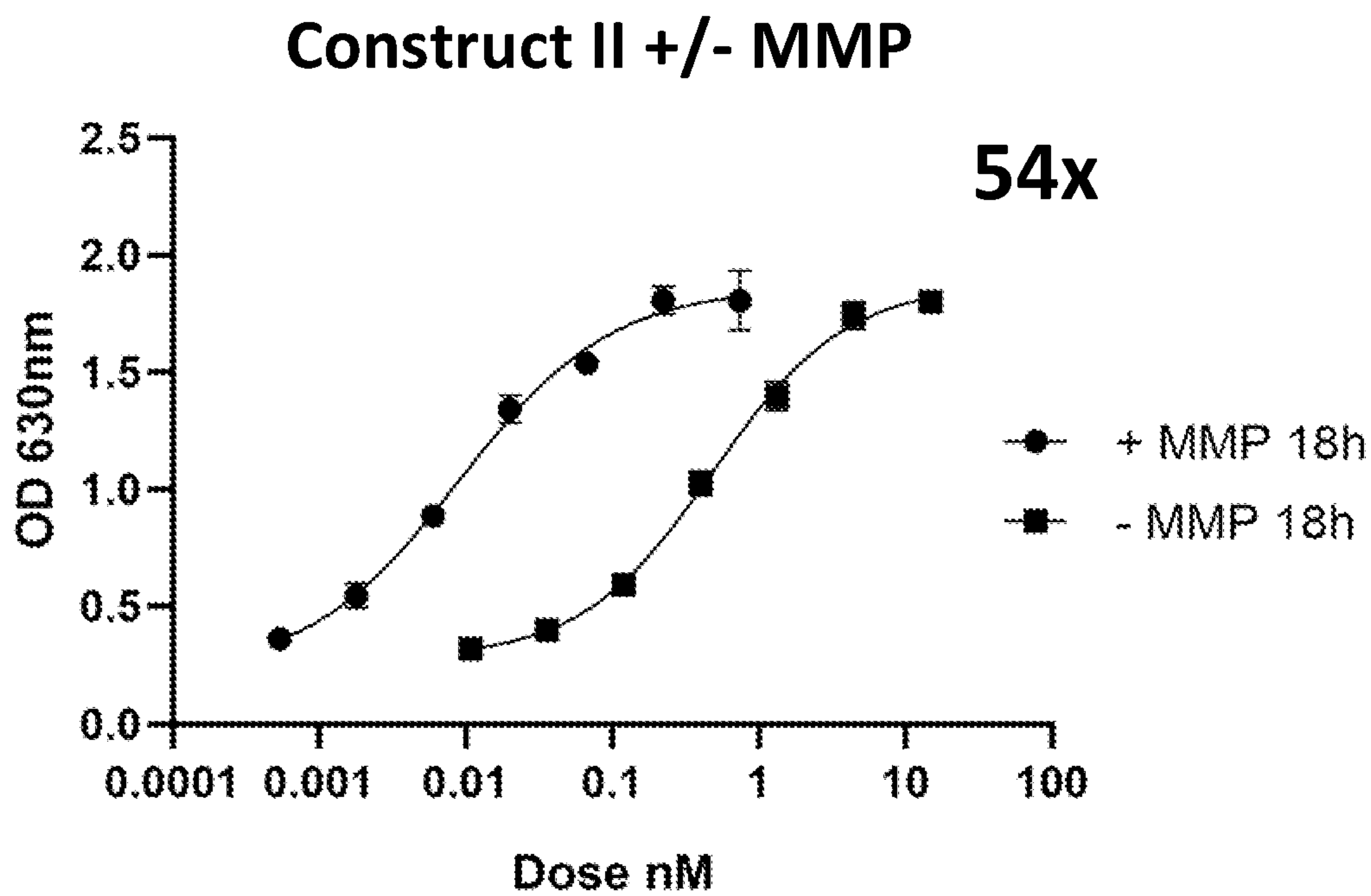


Fig. 3U

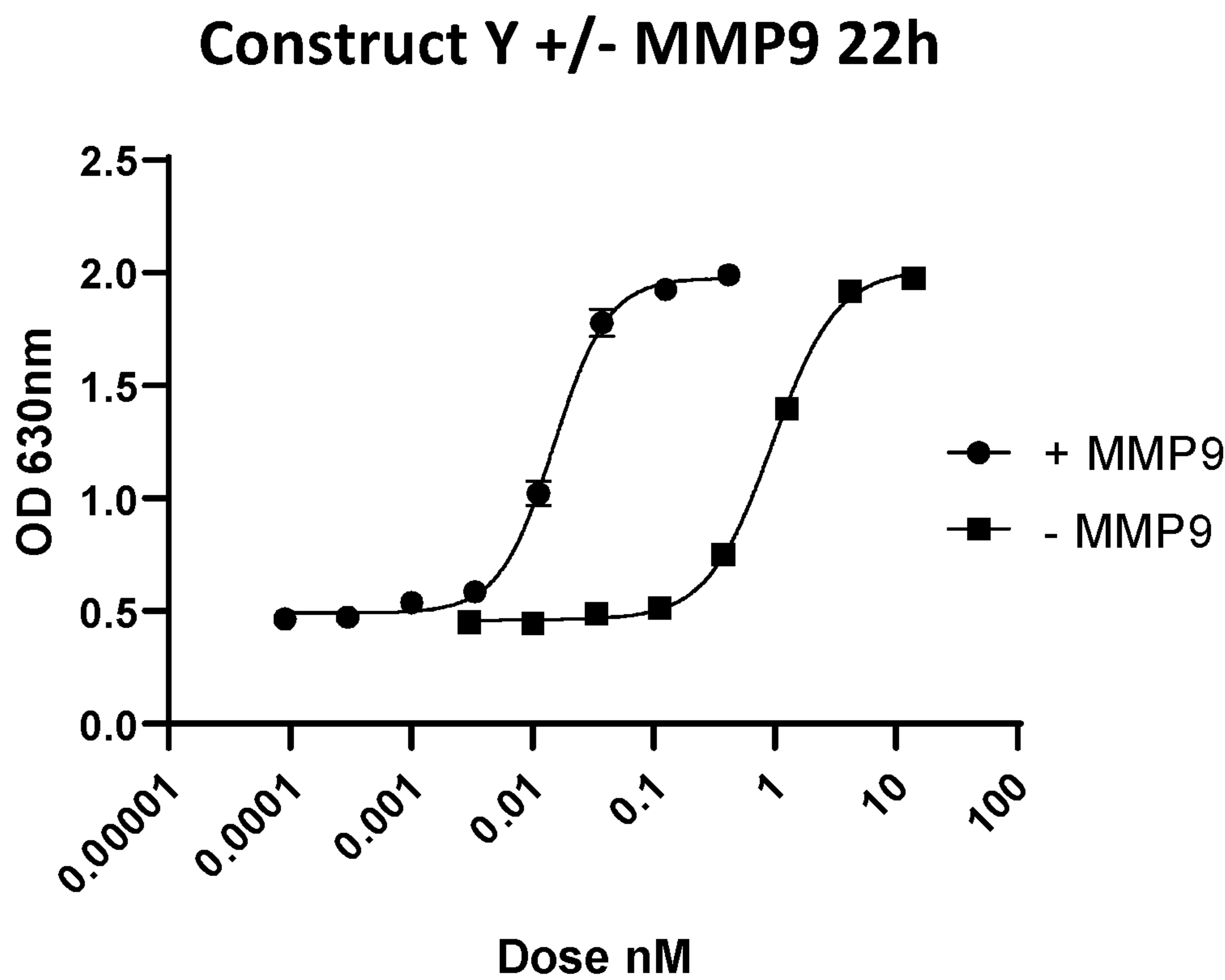


Fig. 3V

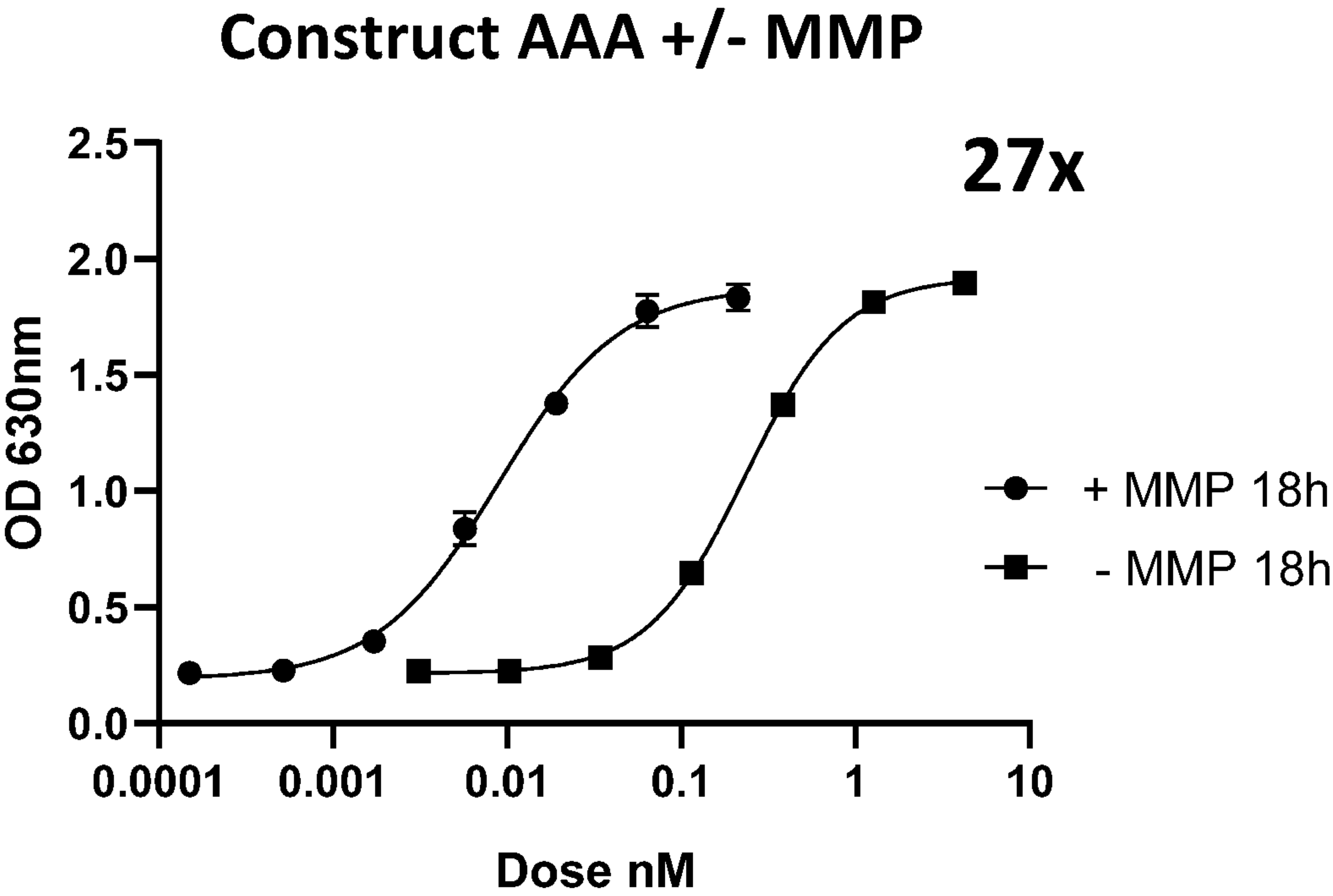


Fig. 3W

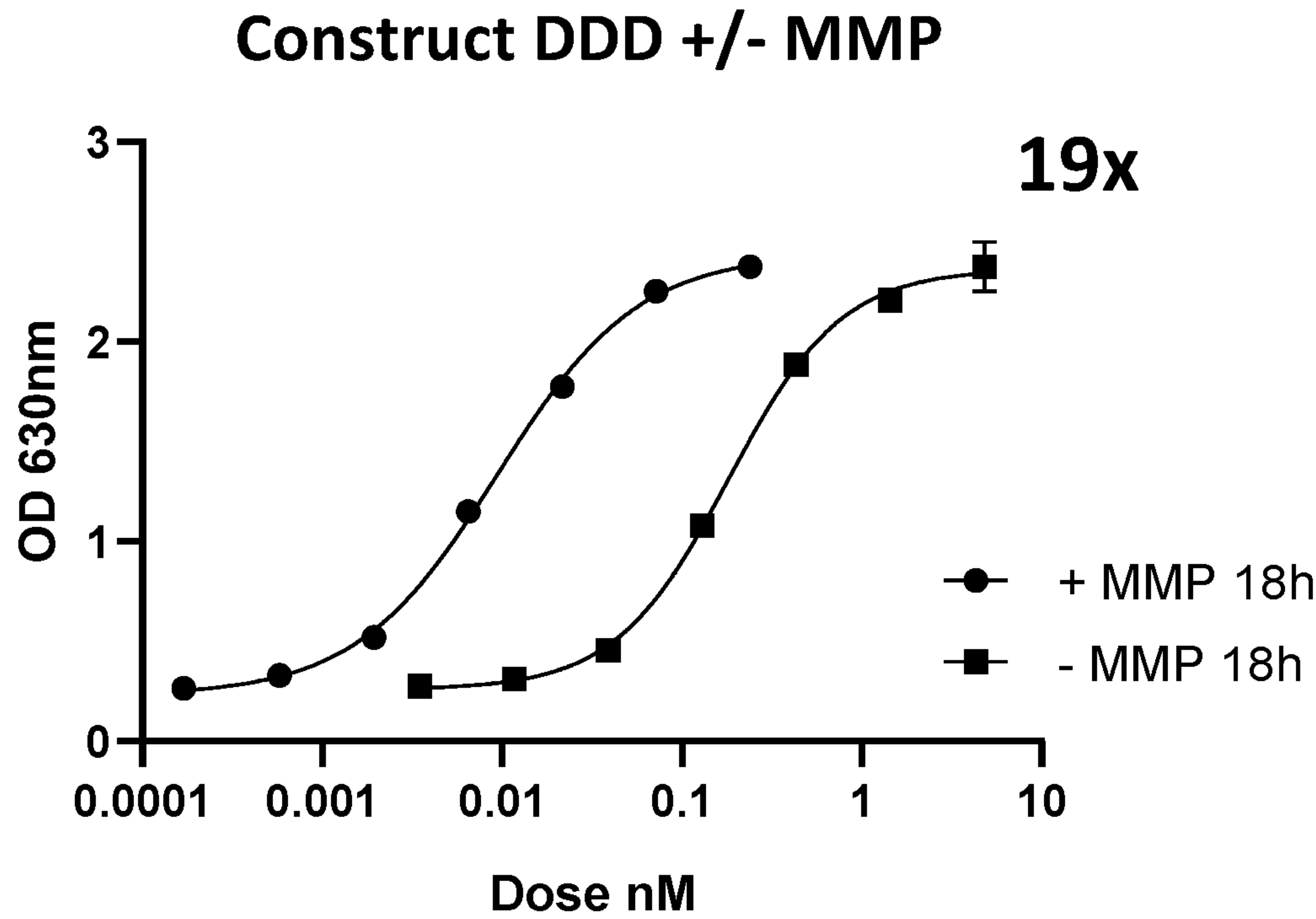


Fig. 3X

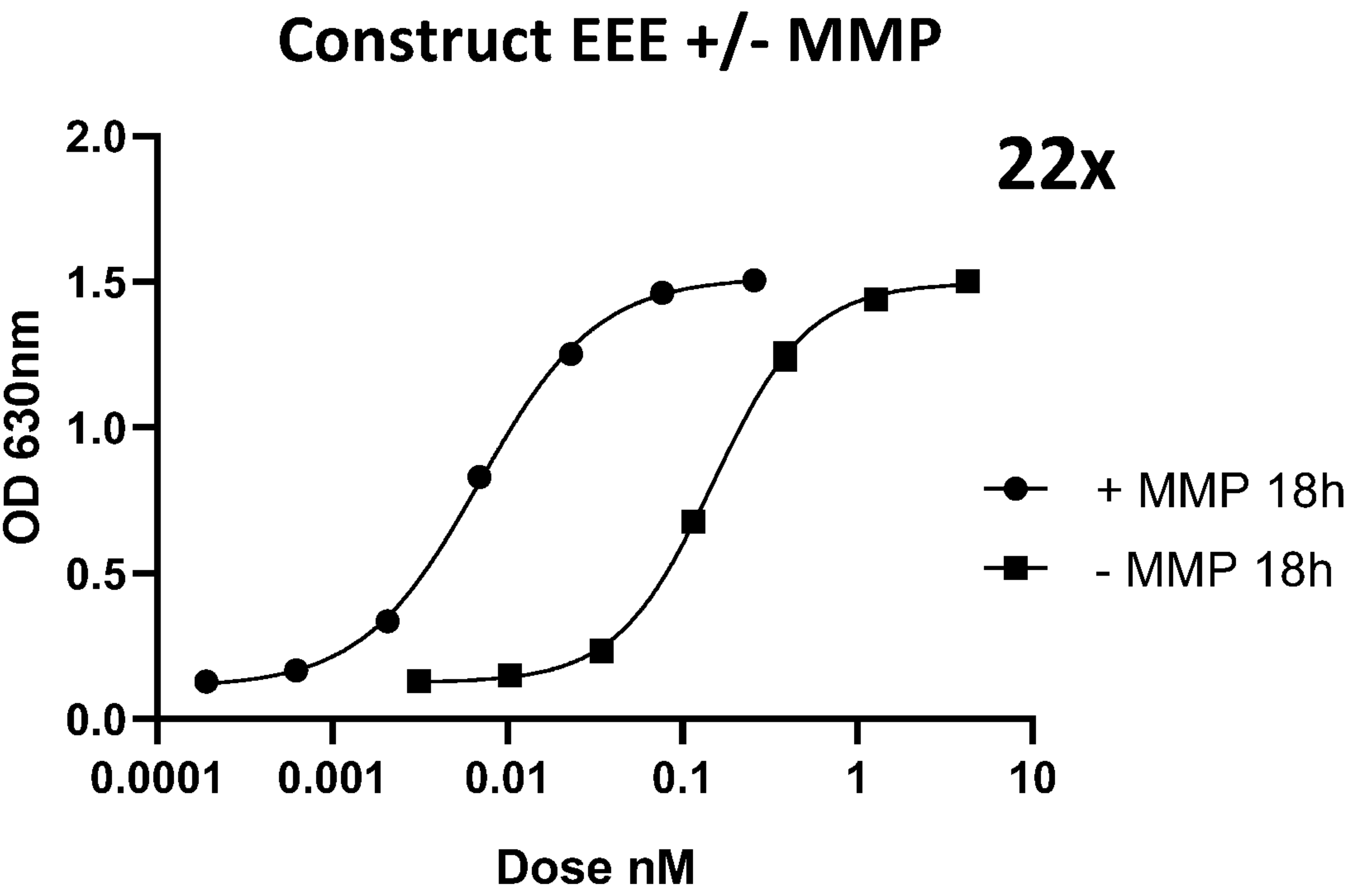


Fig. 3Y

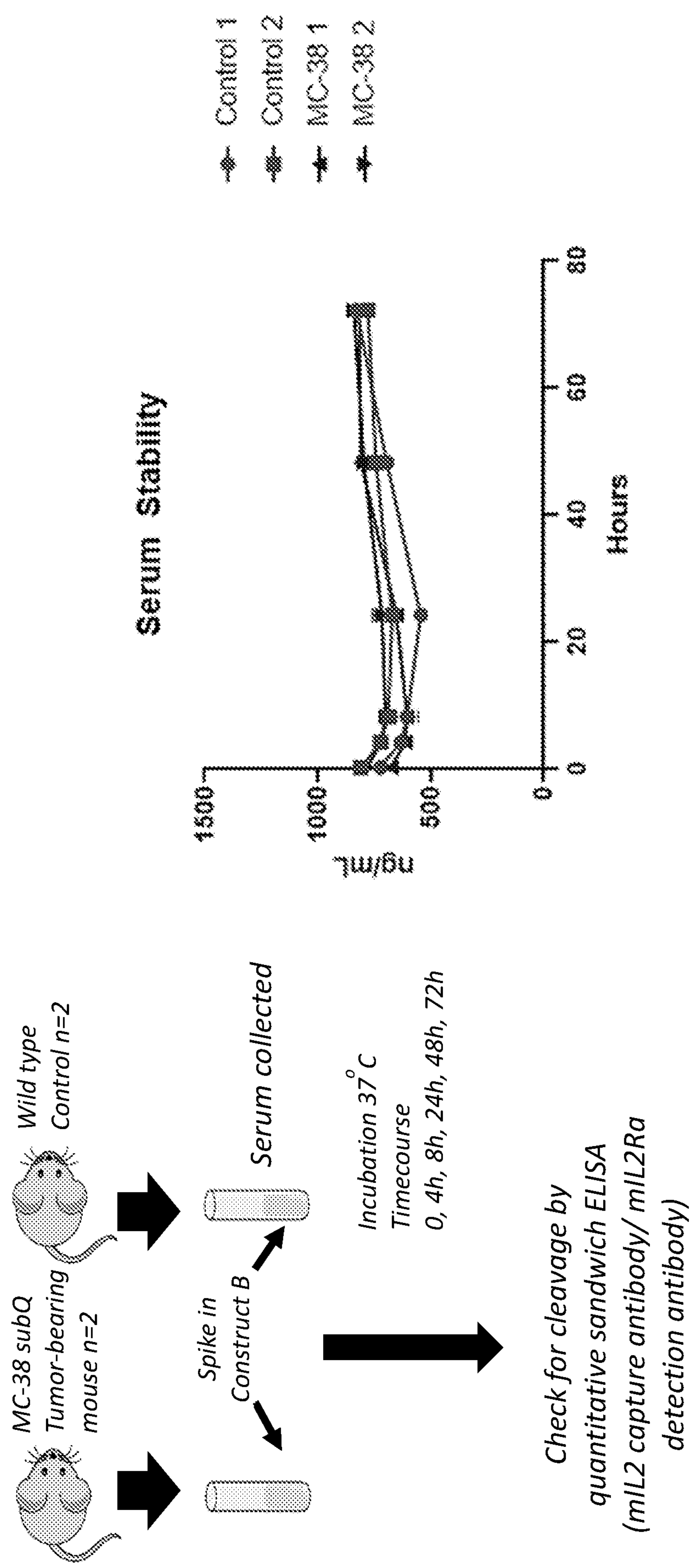
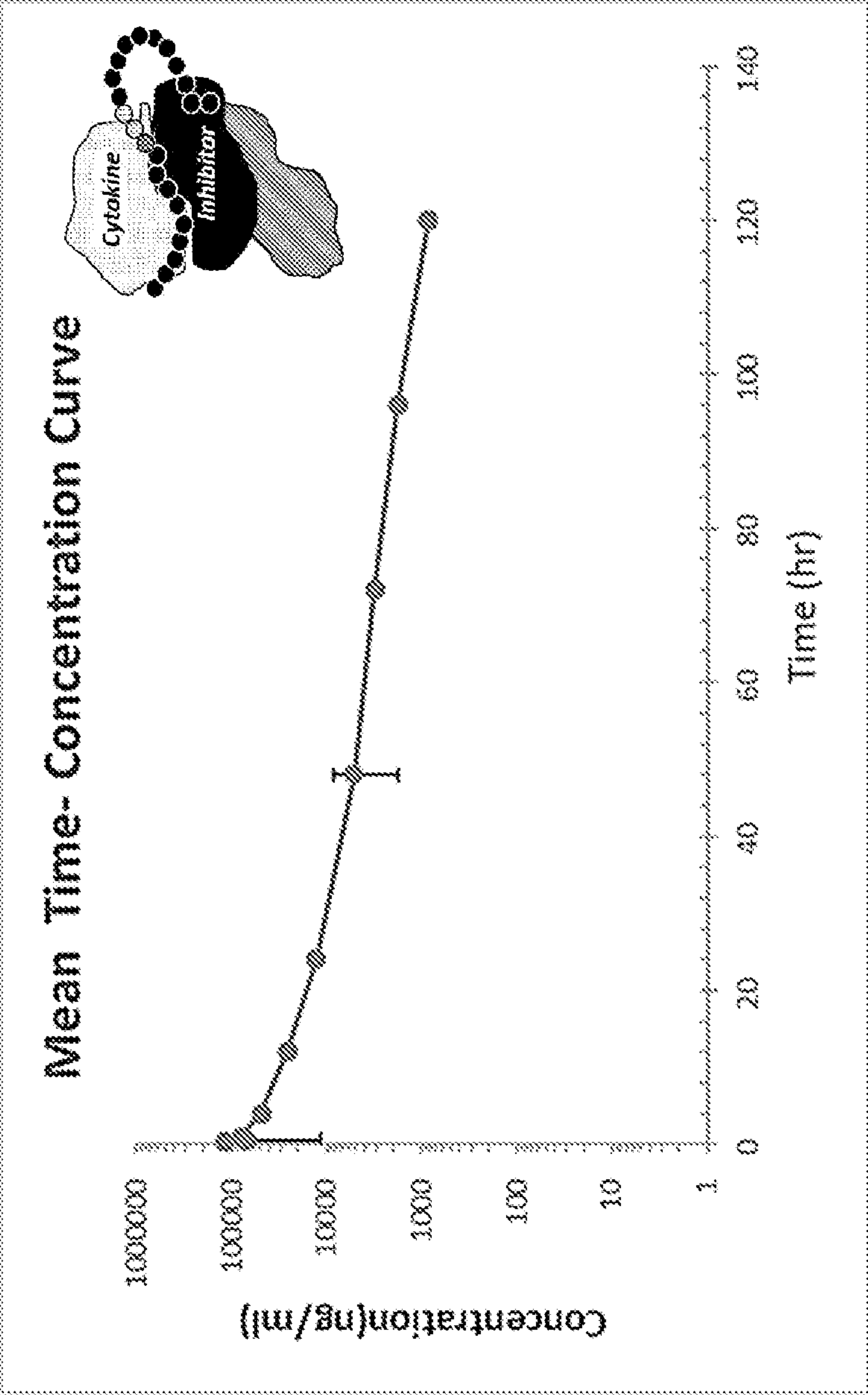


Fig. 4

Construct B serum concentrations over time (n=3 per timepoint)



	Mean serum PK parameters
t _{1/2} (hr)	27.95
T _{max} (hr)	0.2
AUC _{0-t} (ng·hr/mL)	1226407
AUC _{0-∞} (ng·hr/mL)	1260724
Cl (mL/min/kg)	0.1
Vz(L/kg)	0.21

	Study design
Strain	C57BL/6
Route /administration	IV, Single bolus Tail vein
Dose	3.5mg/kg (0.8mg/kg IL2 equivalent)
Timepoints (h)	0.2, 0.5, 1, 4, 12, 24, 48, 72, 96, 120
Sample Analysis	Quantitative ELISA (Trutino)

Fig. 5

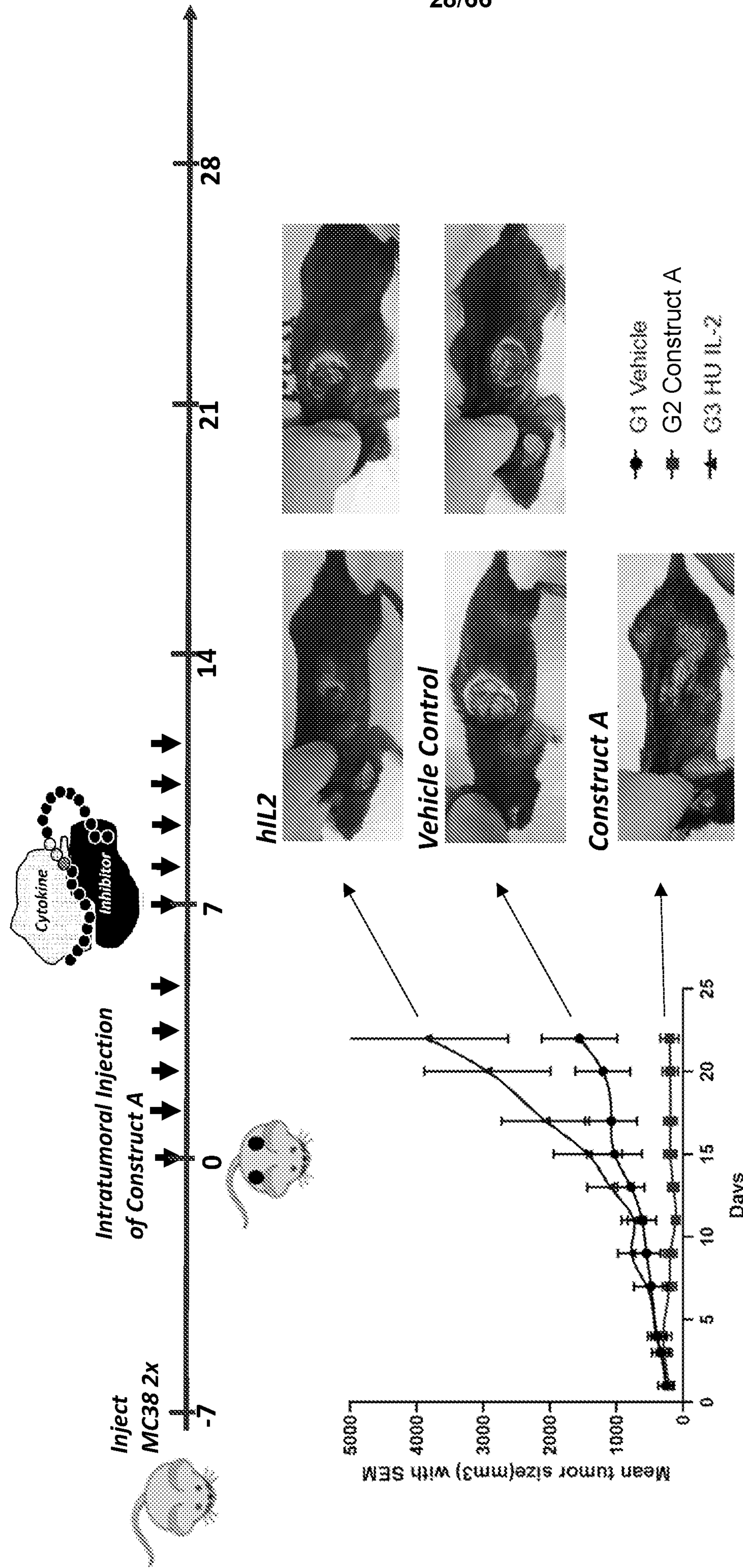


Fig. 6A

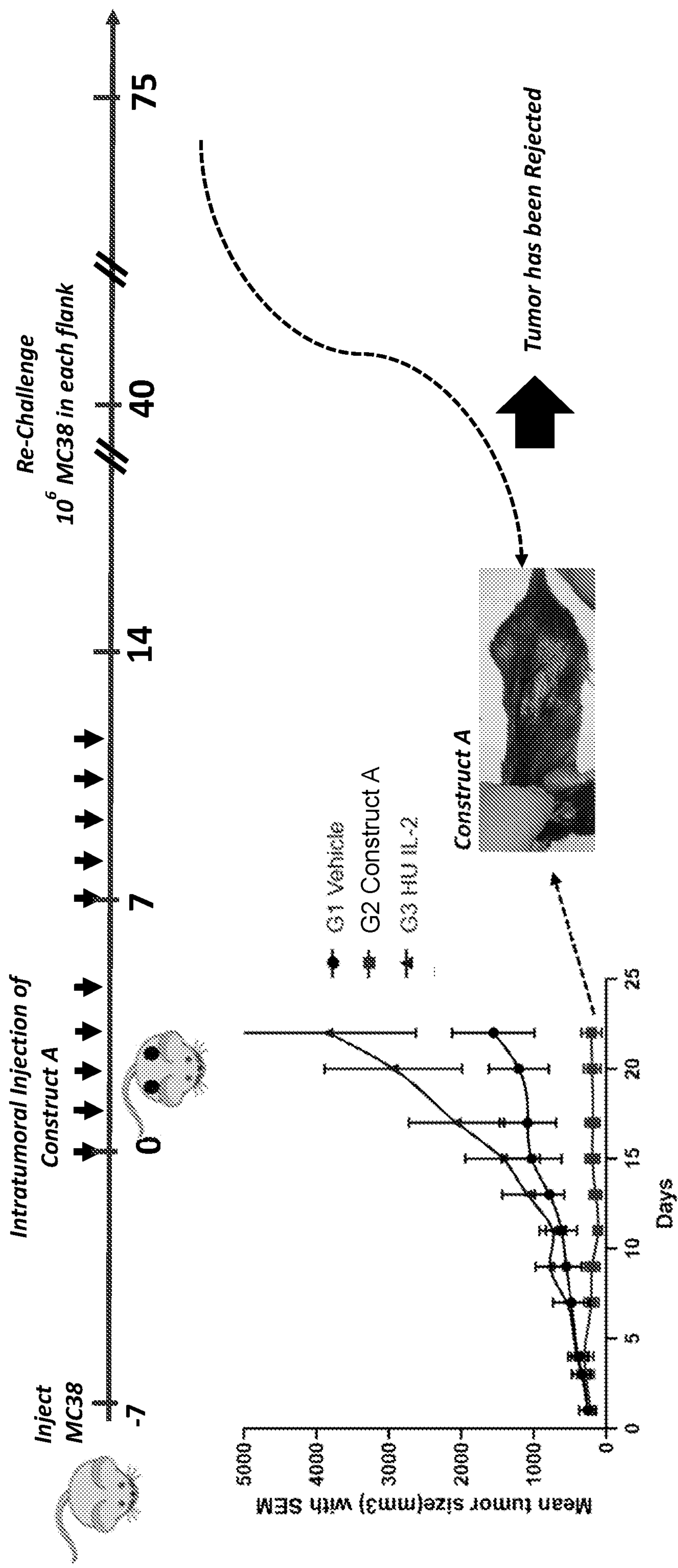


Fig. 6B

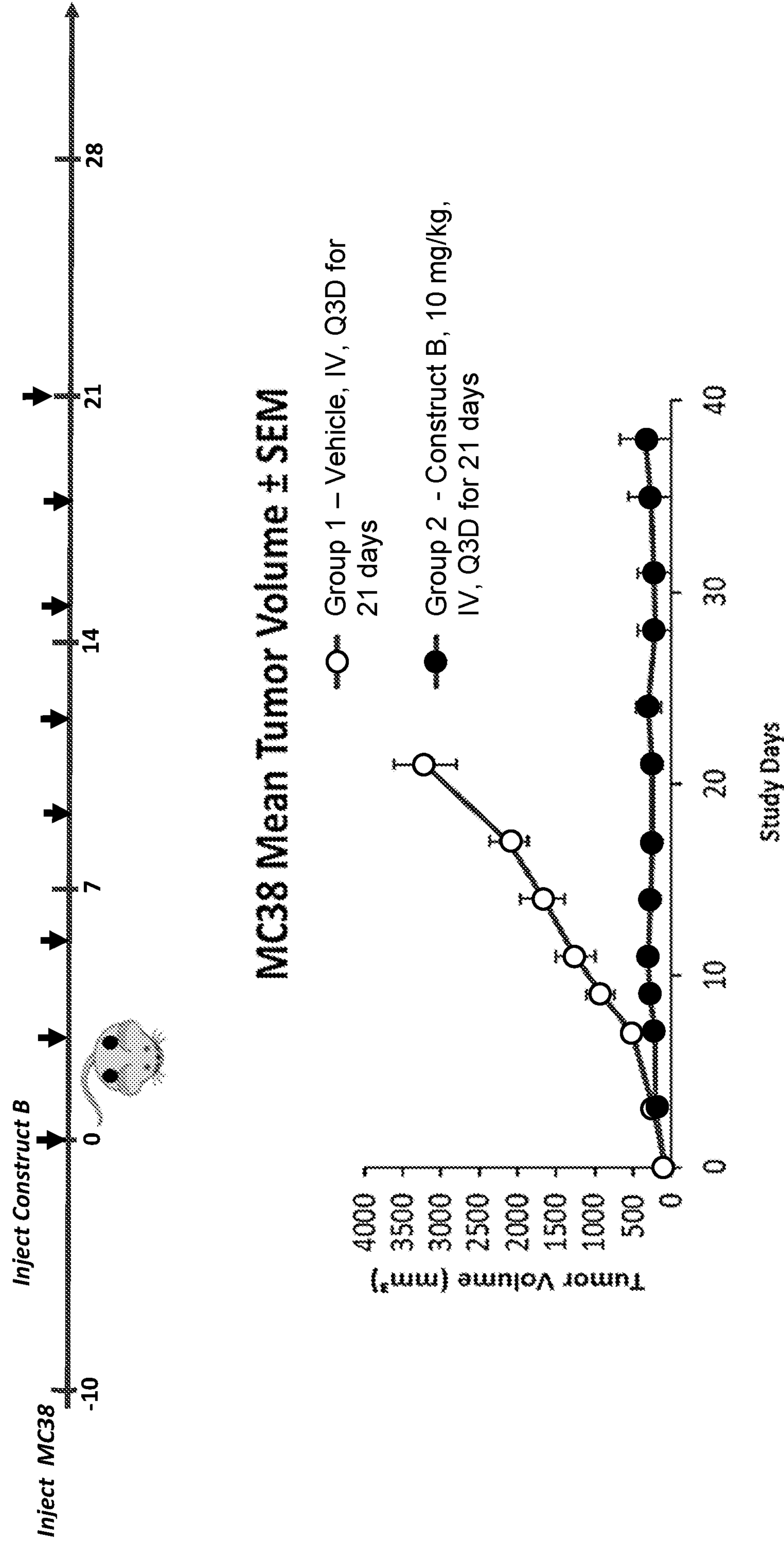


Fig. 7A

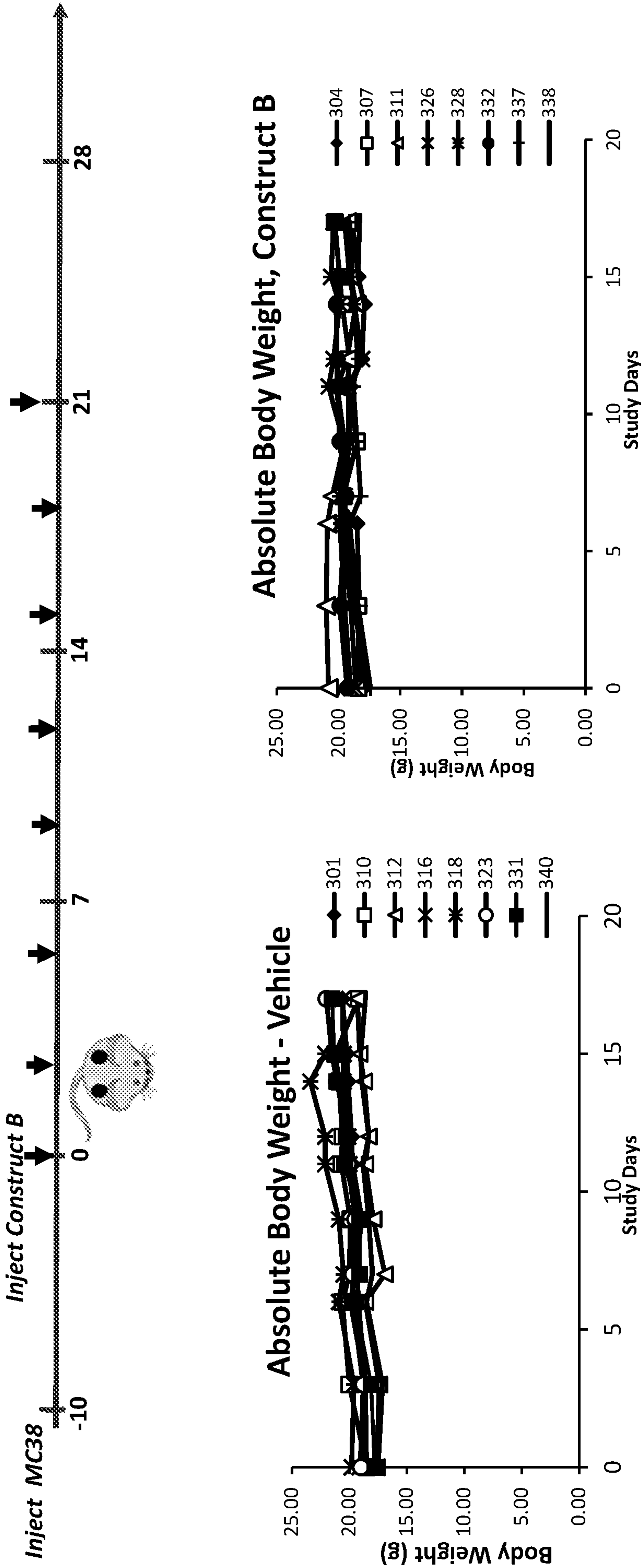


Fig. 7B

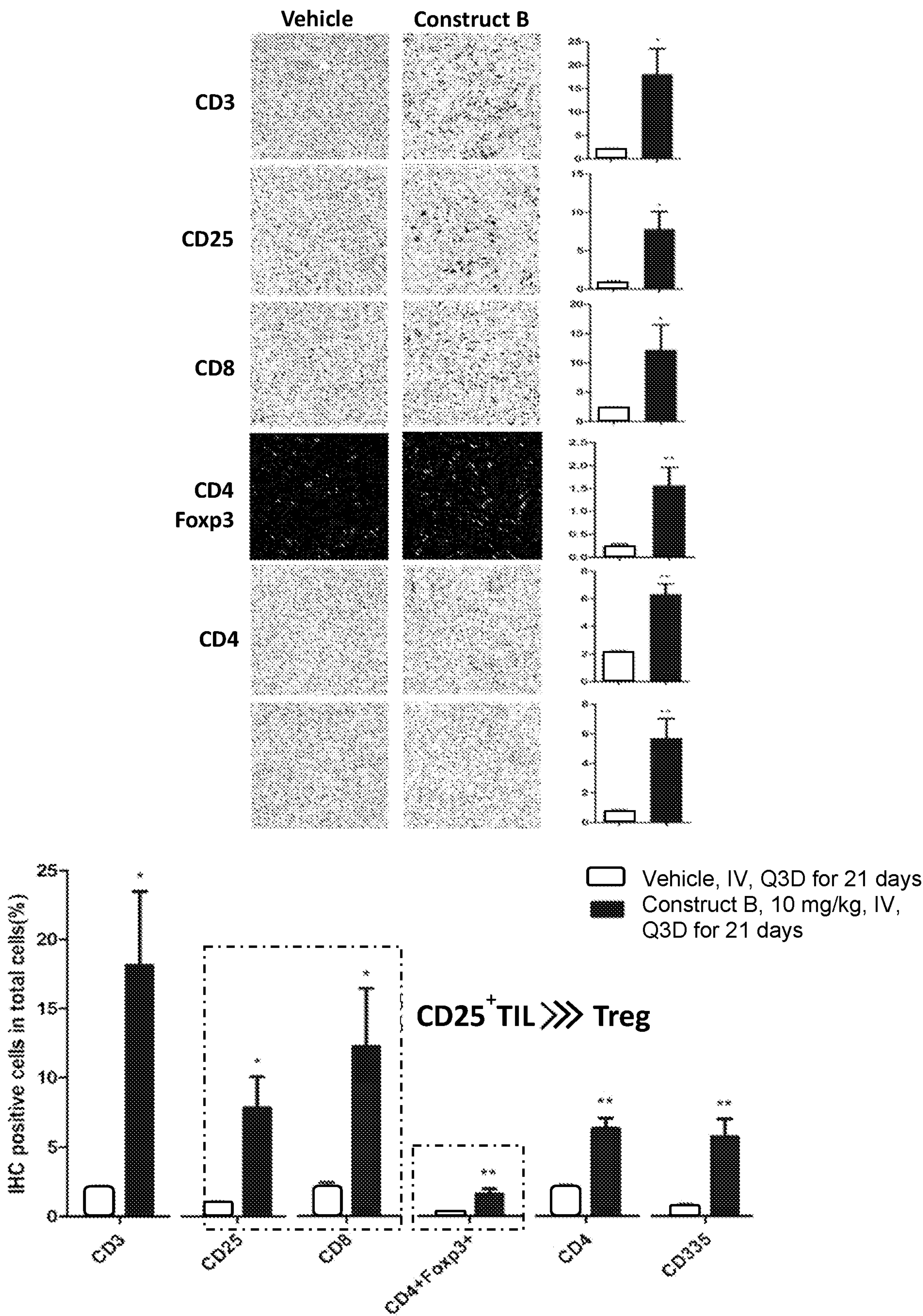


Fig. 8

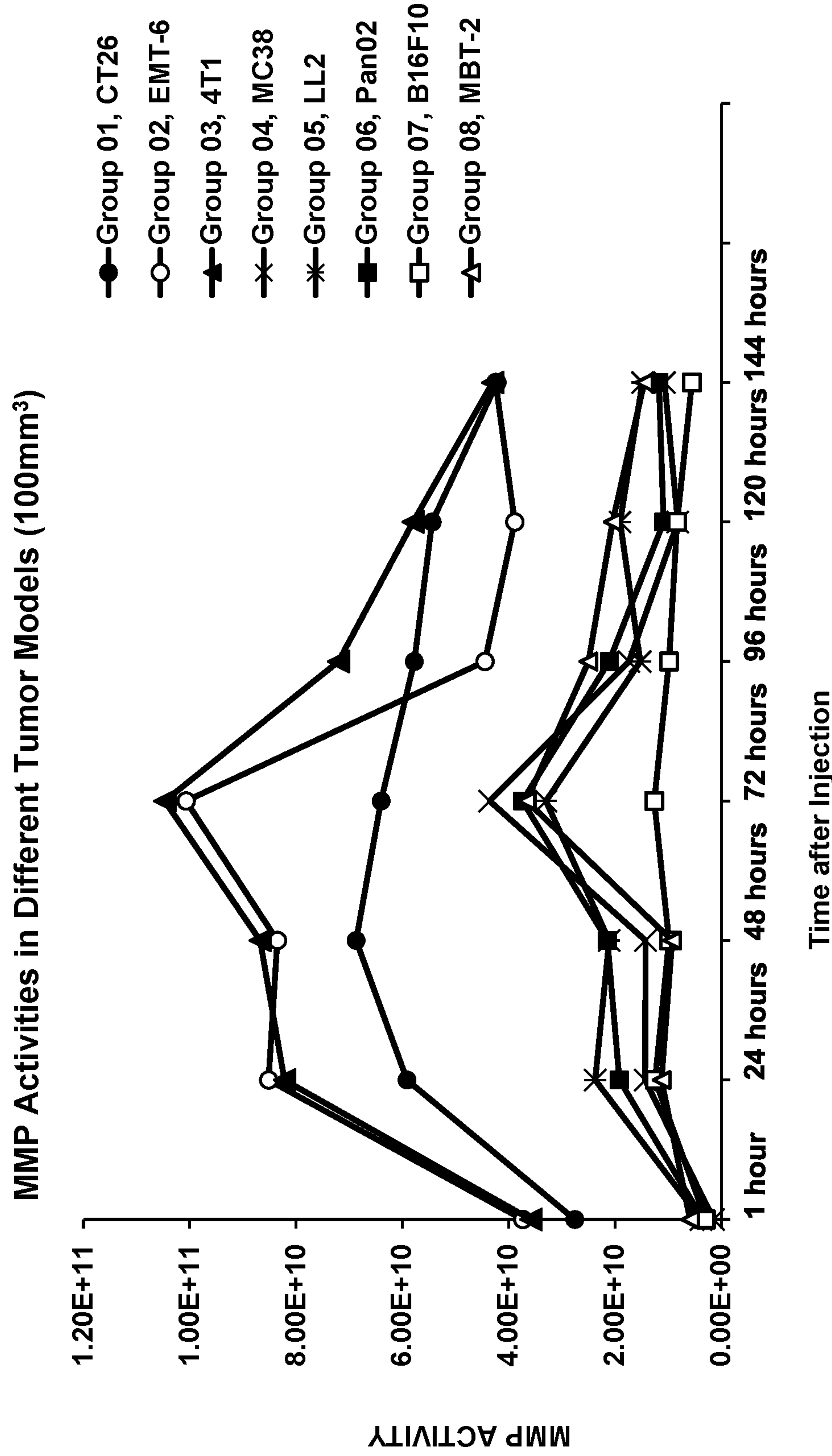


Fig. 9

- Vehicle (PBS), i.p., 10ul/g, BIW
- Construct B, 20mg/kg, i.v., 5ul/g, Q3d

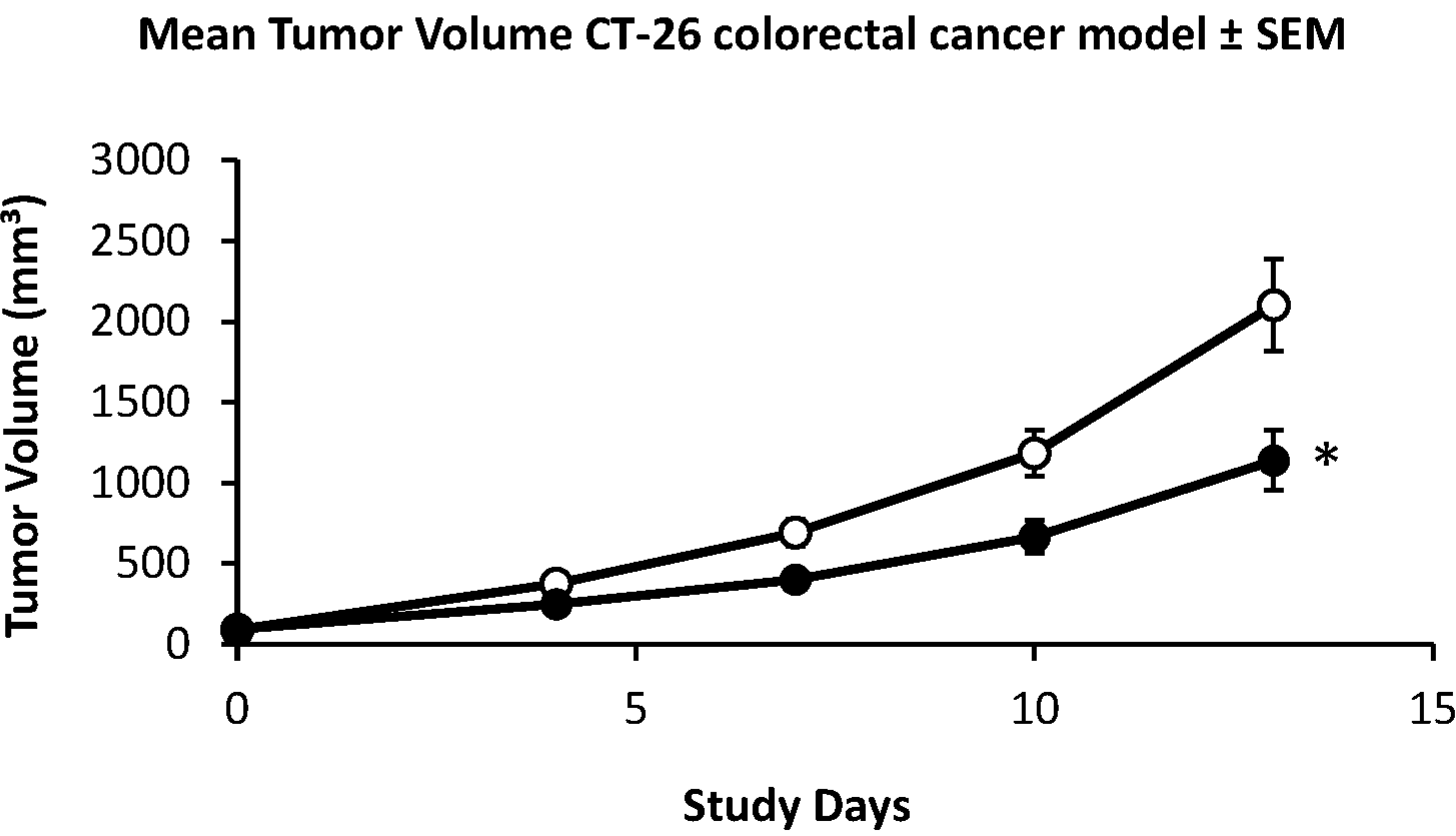


Fig. 10A

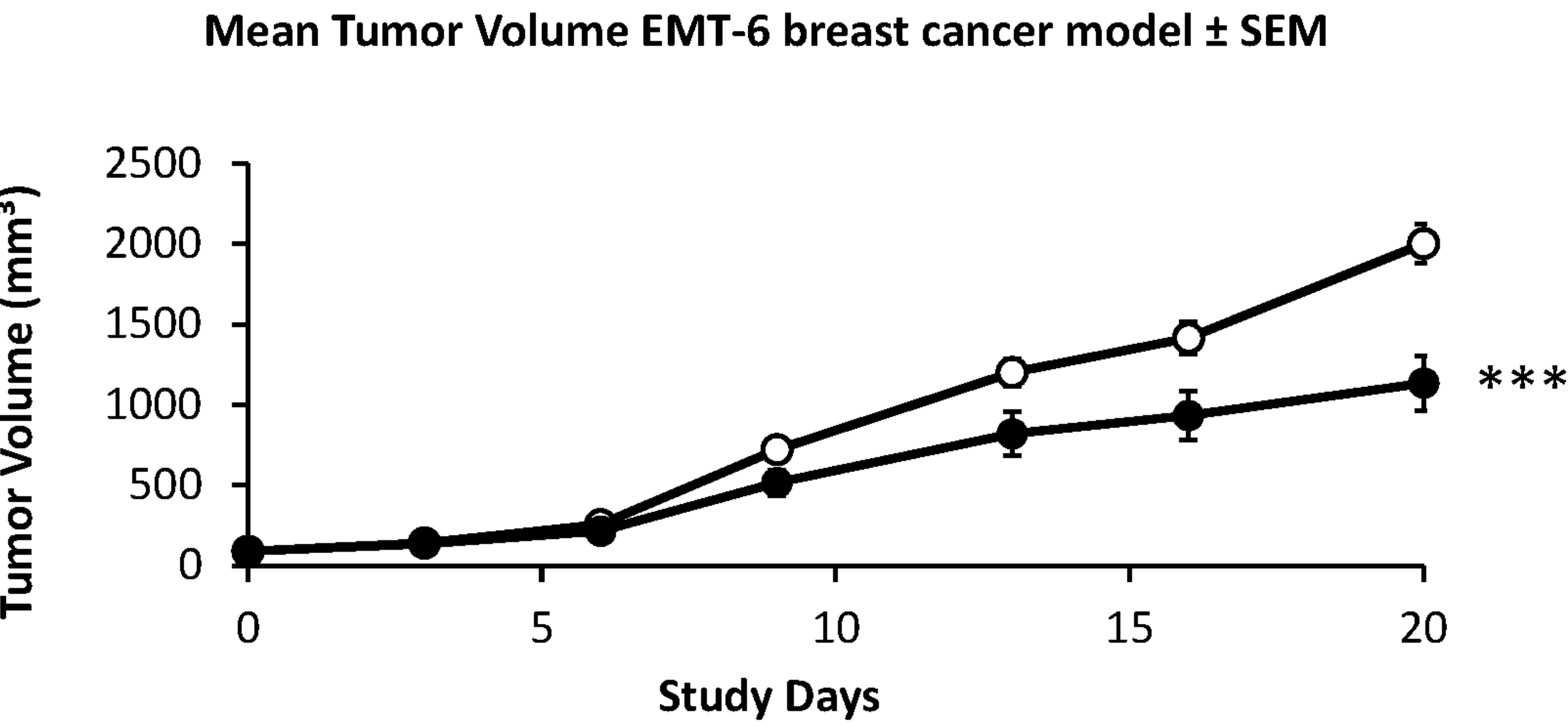


Fig. 10B

- Vehicle (PBS), i.p., 10ul/g, BIW
- Construct B, 20mg/kg, i.v., 5ul/g, Q3d

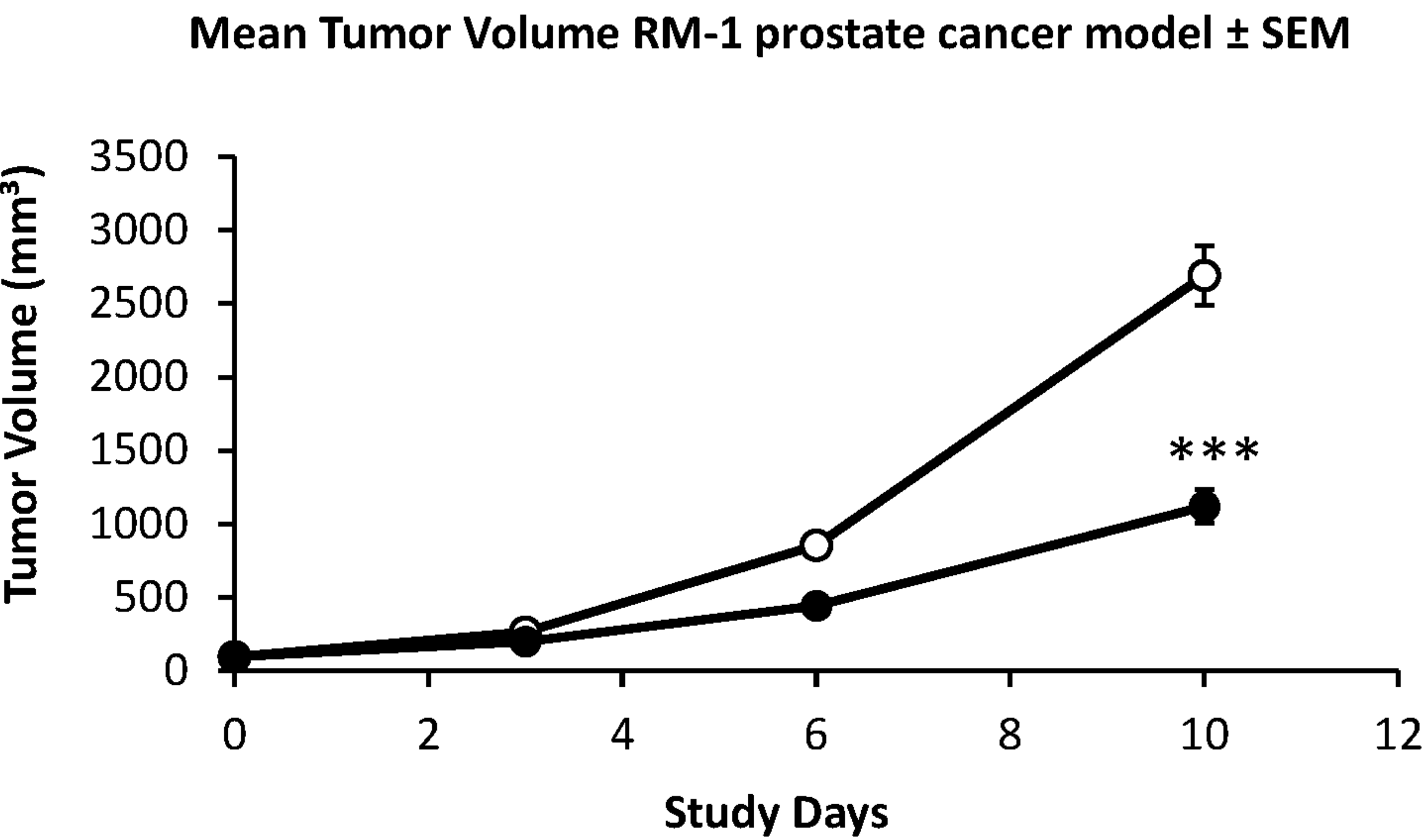


Fig. 10C

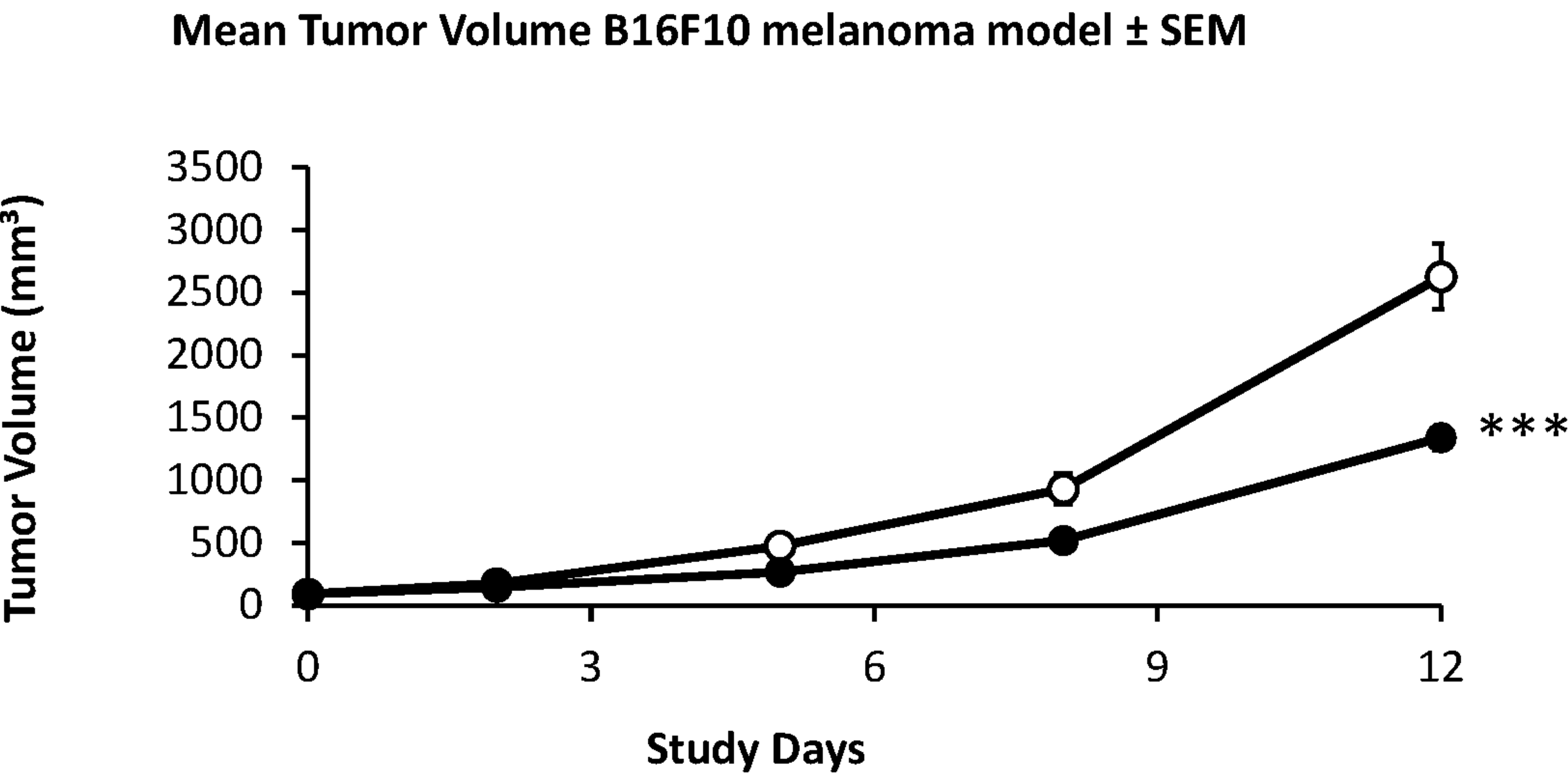


Fig. 10D

- Vehicle (PBS), i.p., 10ul/g, BIW
- Construct B, 20mg/kg, i.v., 5ul/g, Q3d

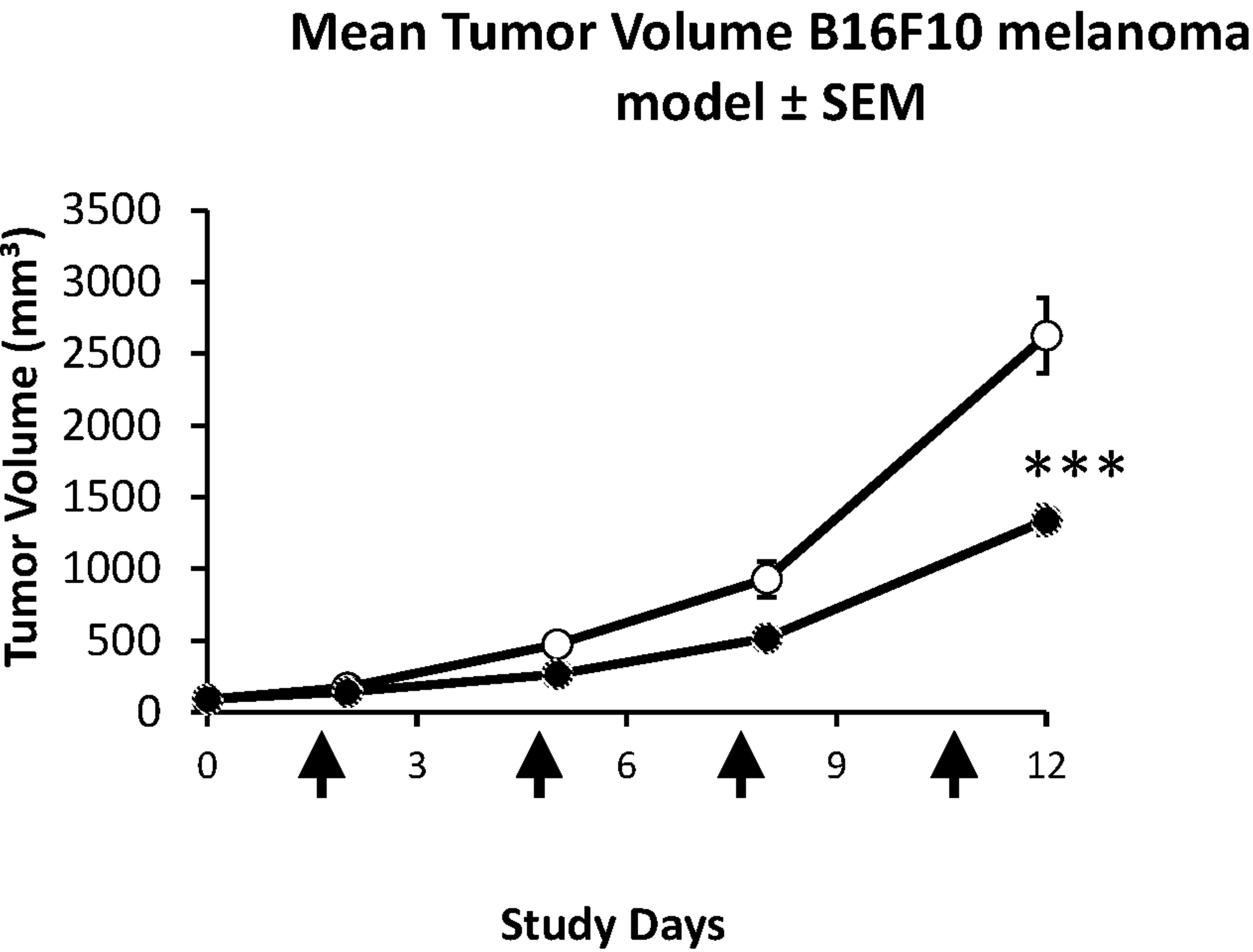


Fig. 11A

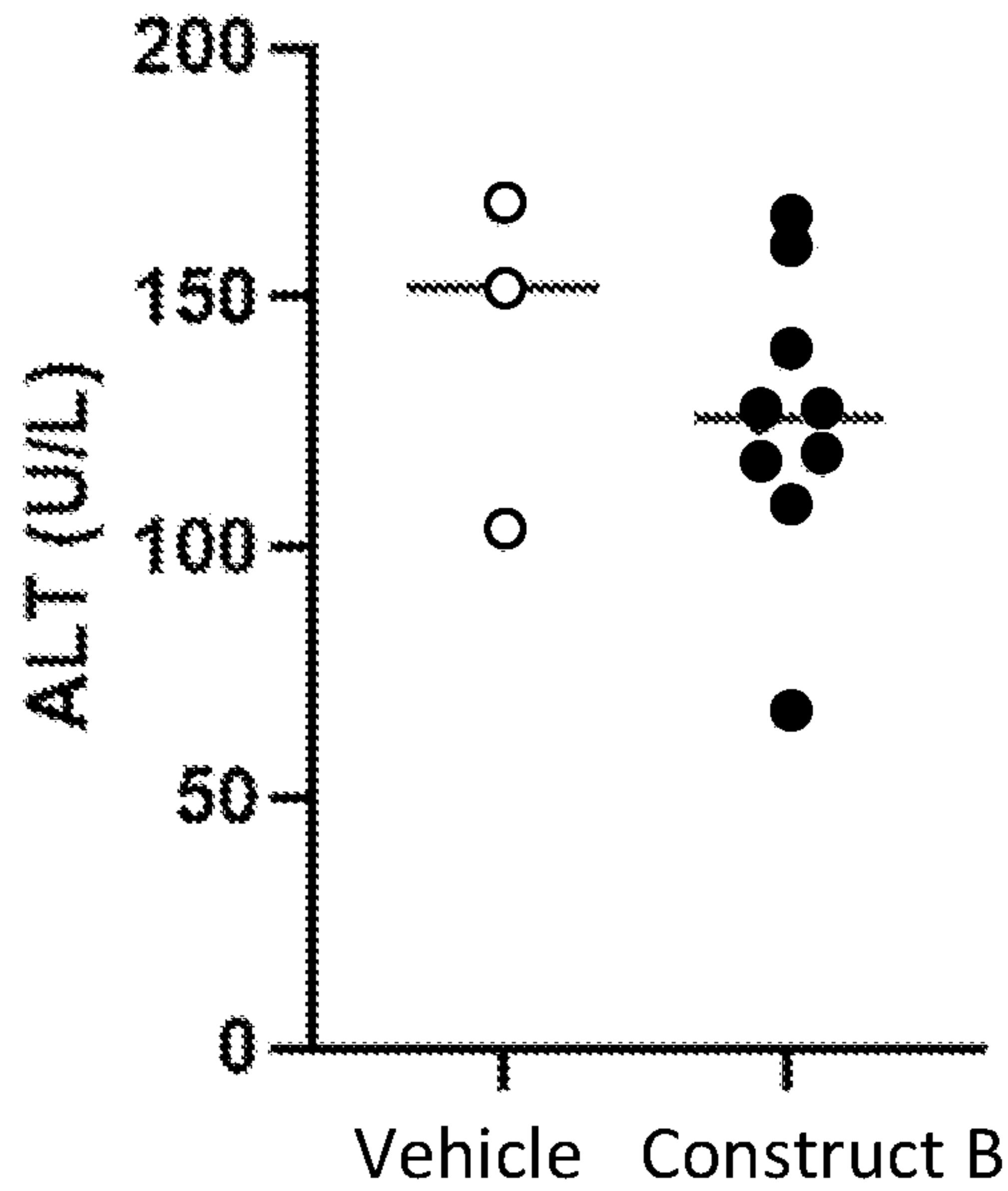


Fig. 11B

○ Vehicle (PBS), i.p., 10ul/g, BIW
● Construct B, 20mg/kg, i.v., 5ul/g, Q3d

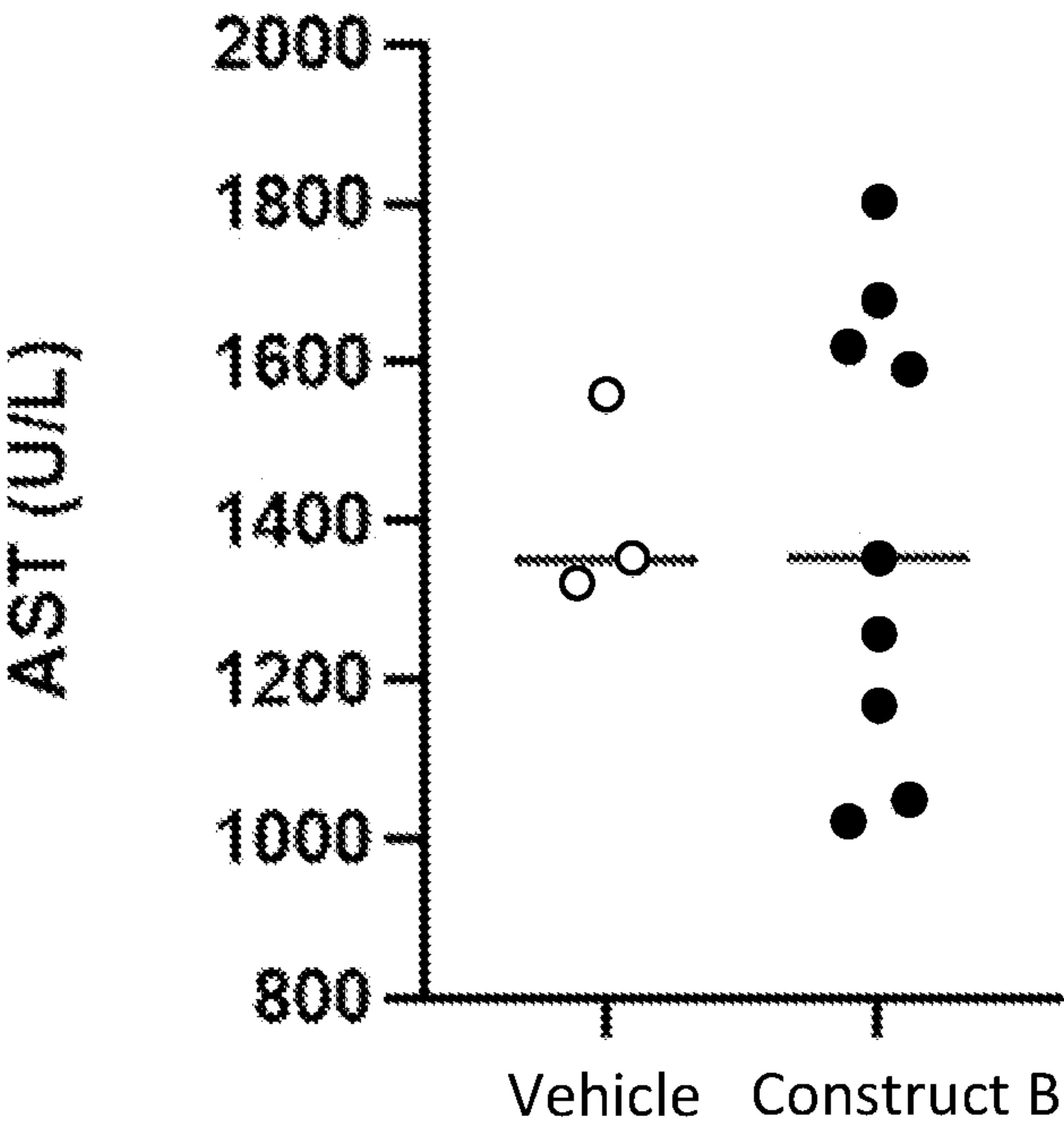


Fig. 11C

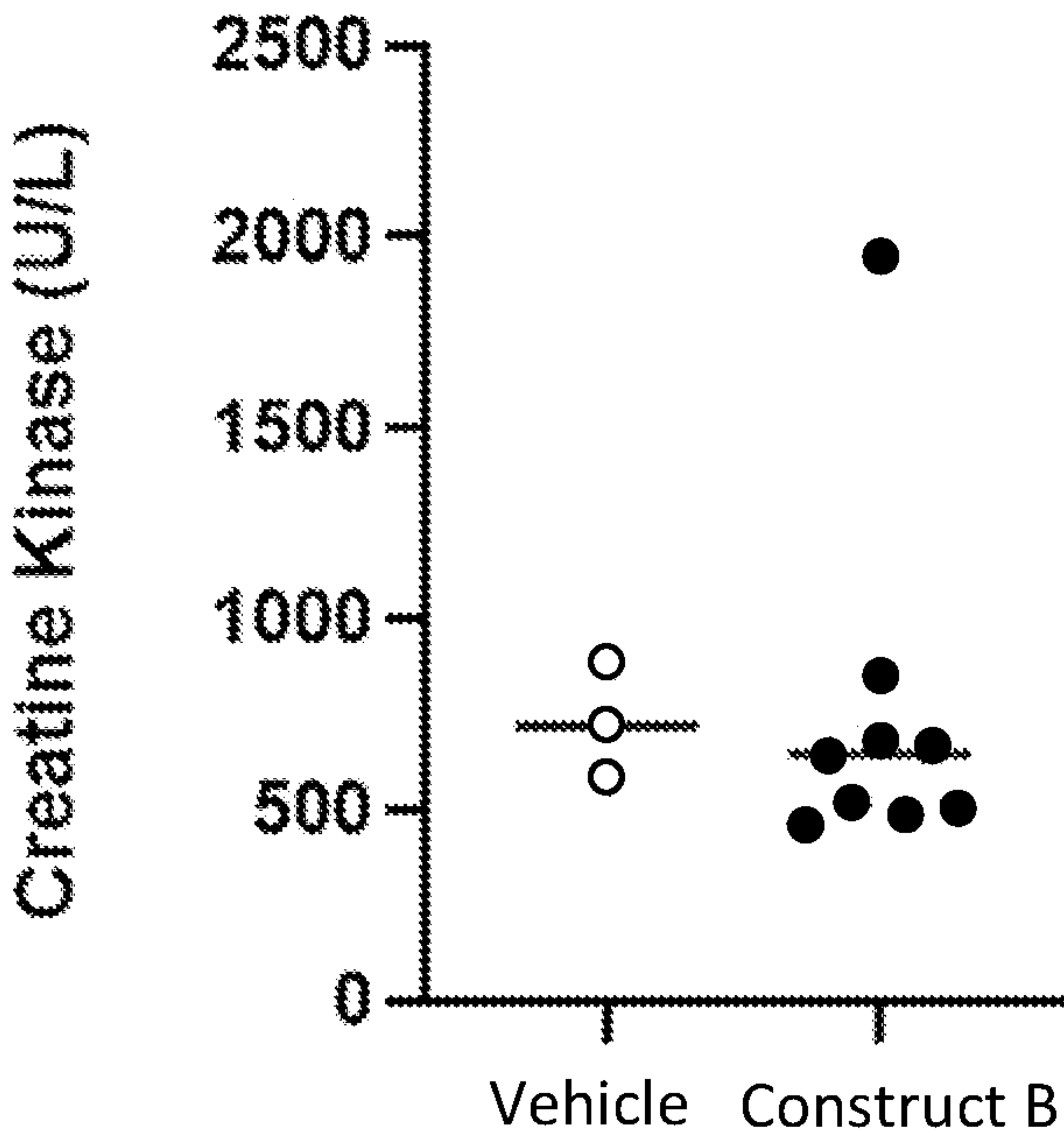


Fig. 11D

- Vehicle (PBS), i.p., 10ul/g, BIW
- Construct B, 20mg/kg, i.v., 5ul/g, Q3d

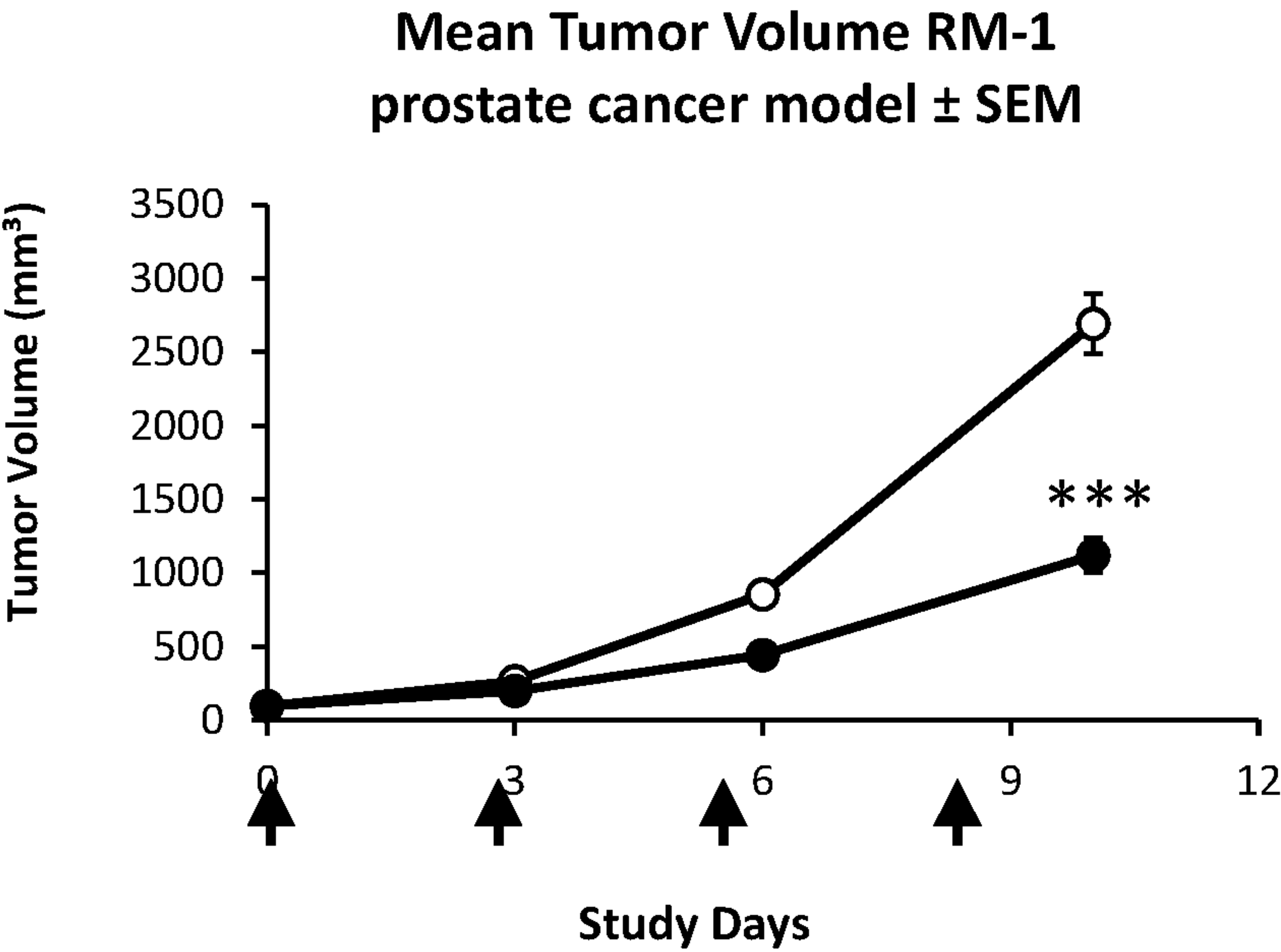


Fig. 12A

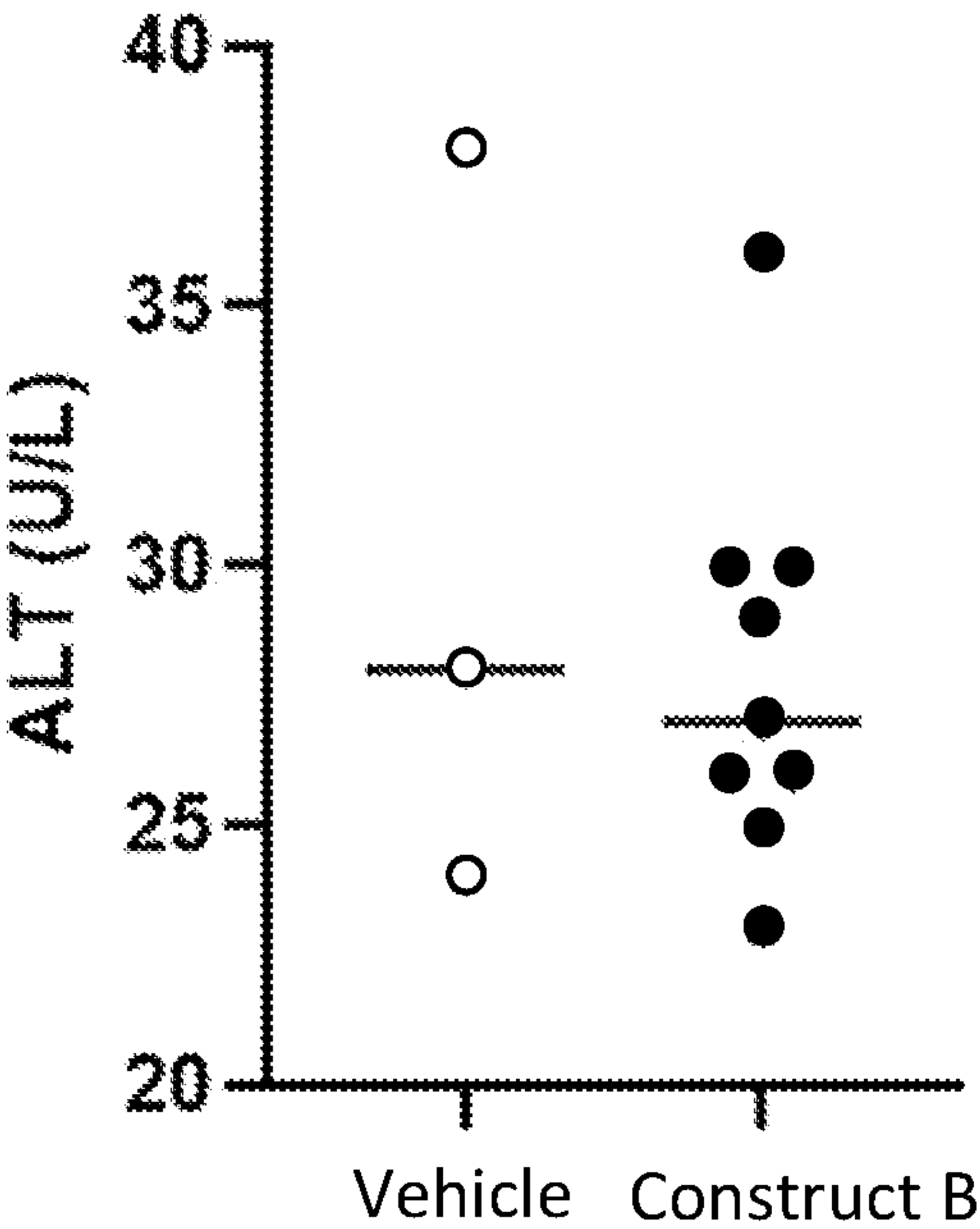


Fig. 12B

- Vehicle (PBS), i.p., 10ul/g, BIW
- Construct B, 20mg/kg, i.v., 5ul/g, Q3d

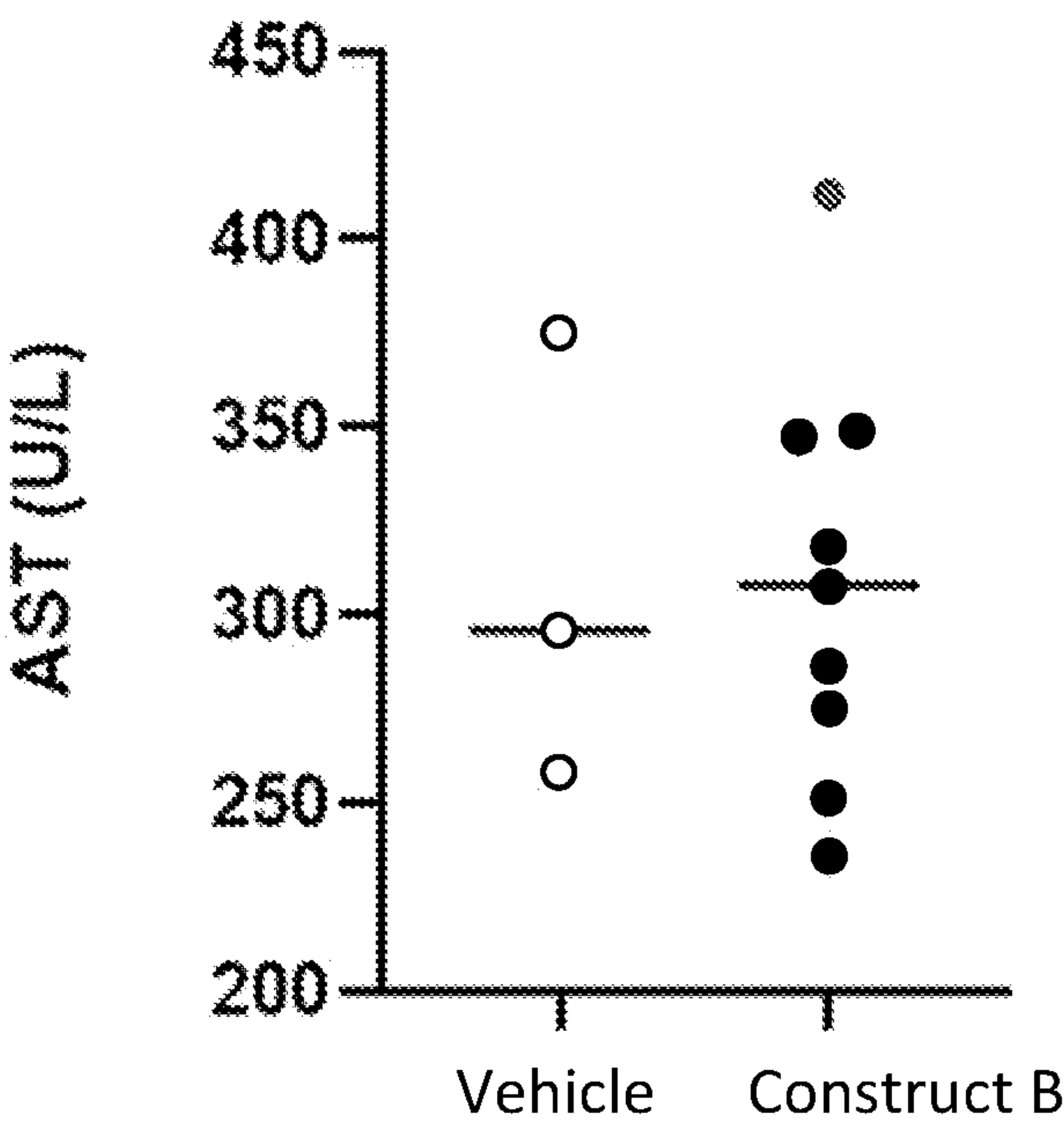


Fig. 12C

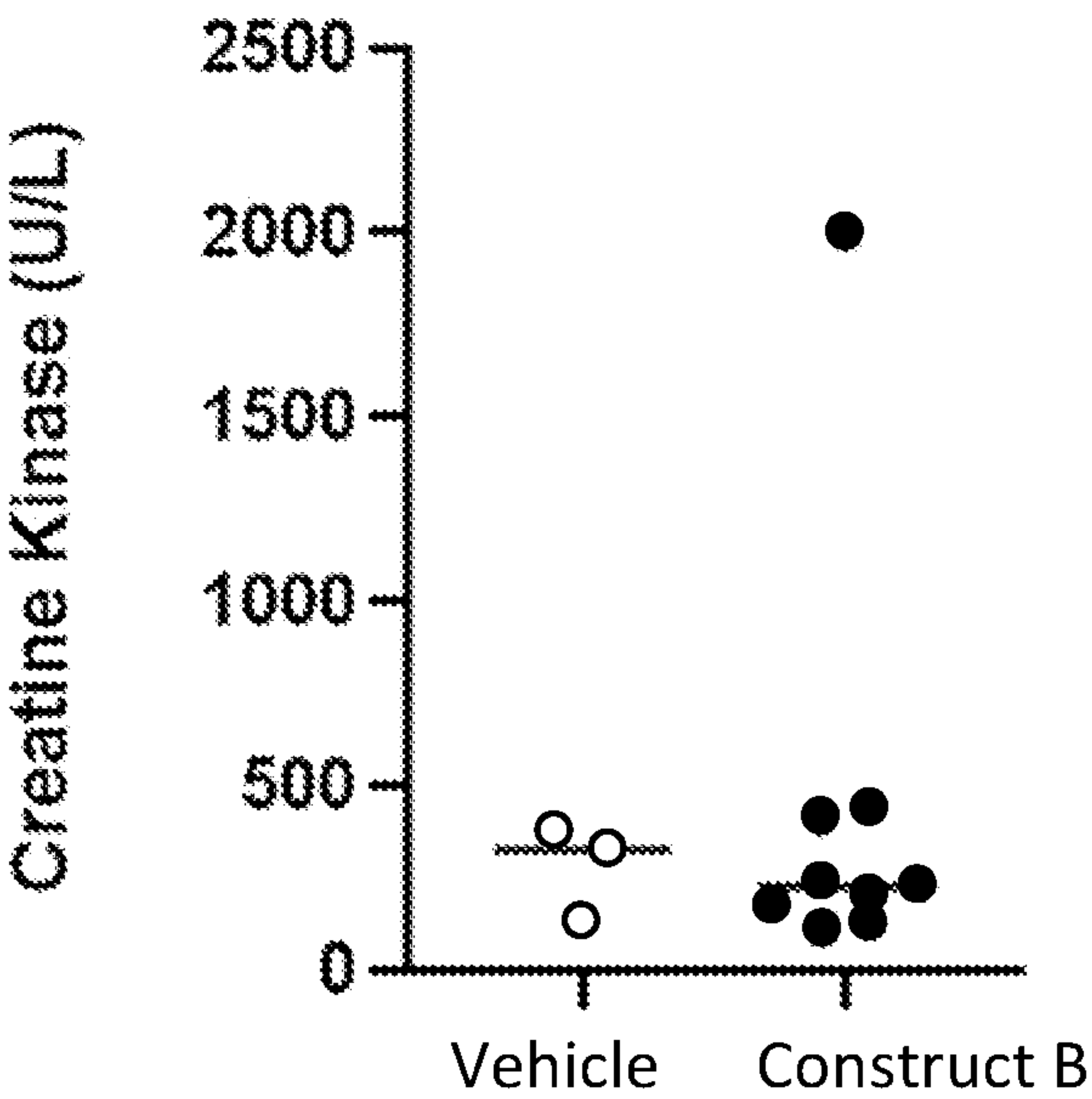


Fig. 12D

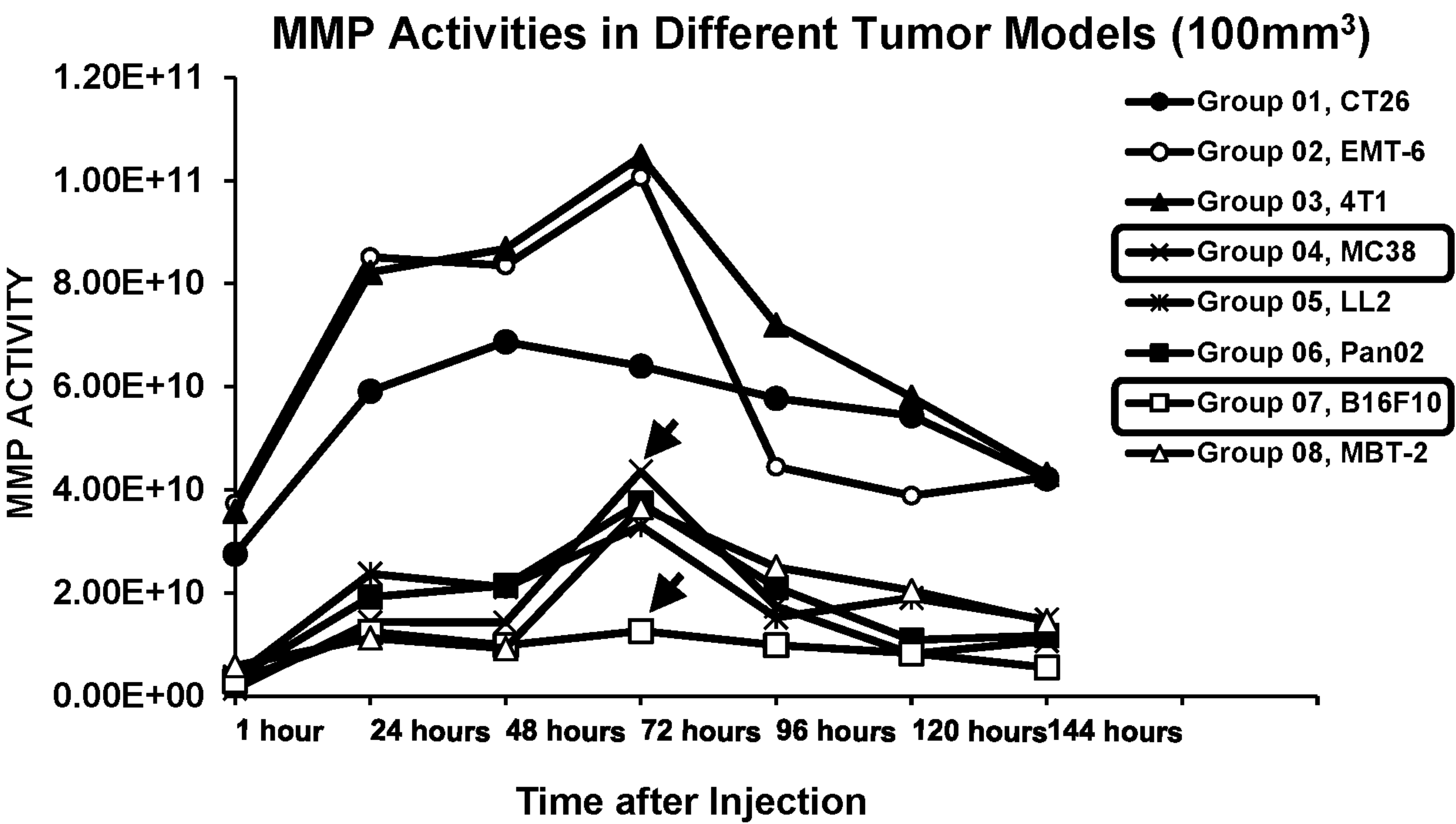


Fig. 13A

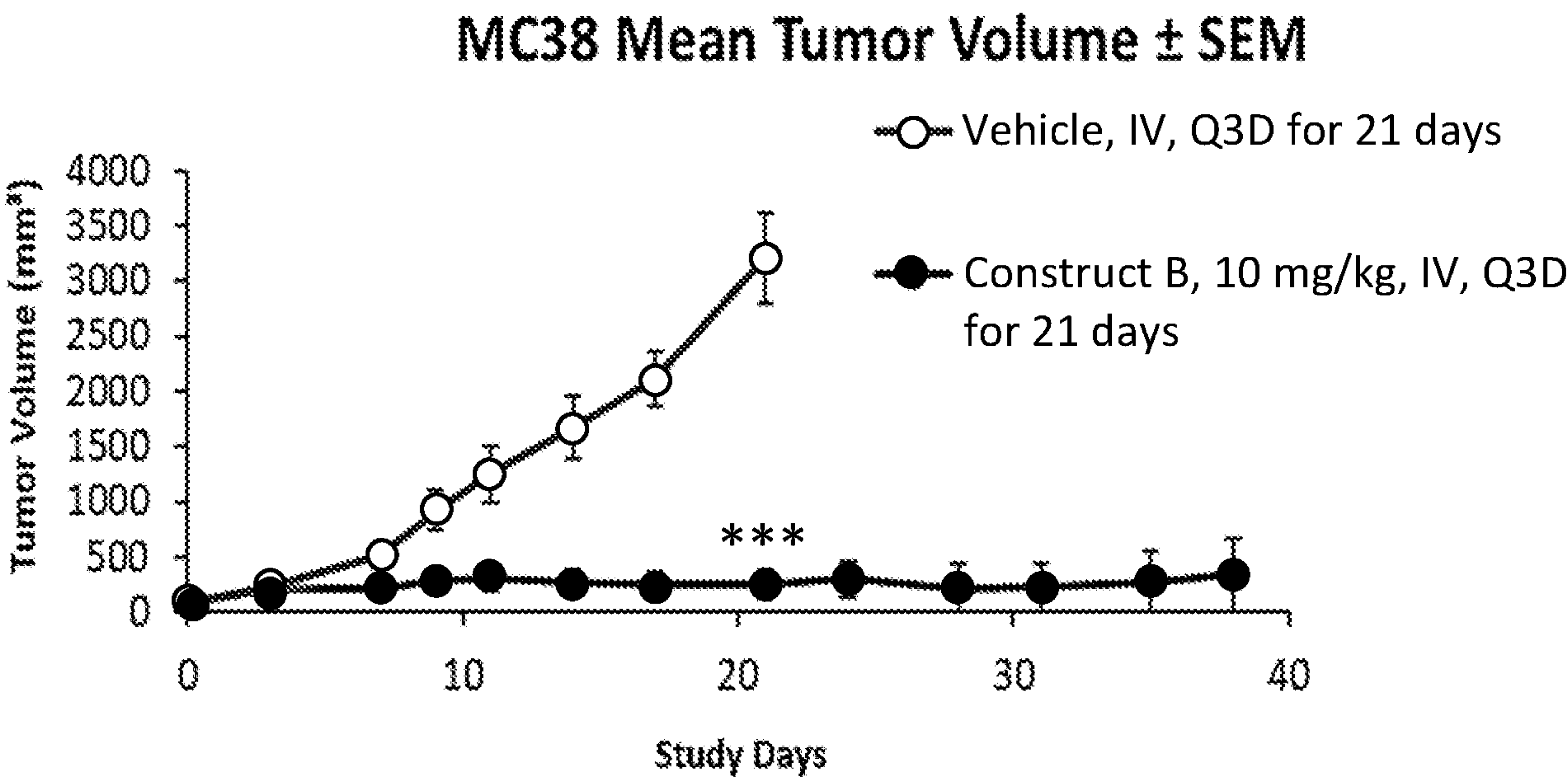


Fig. 13B

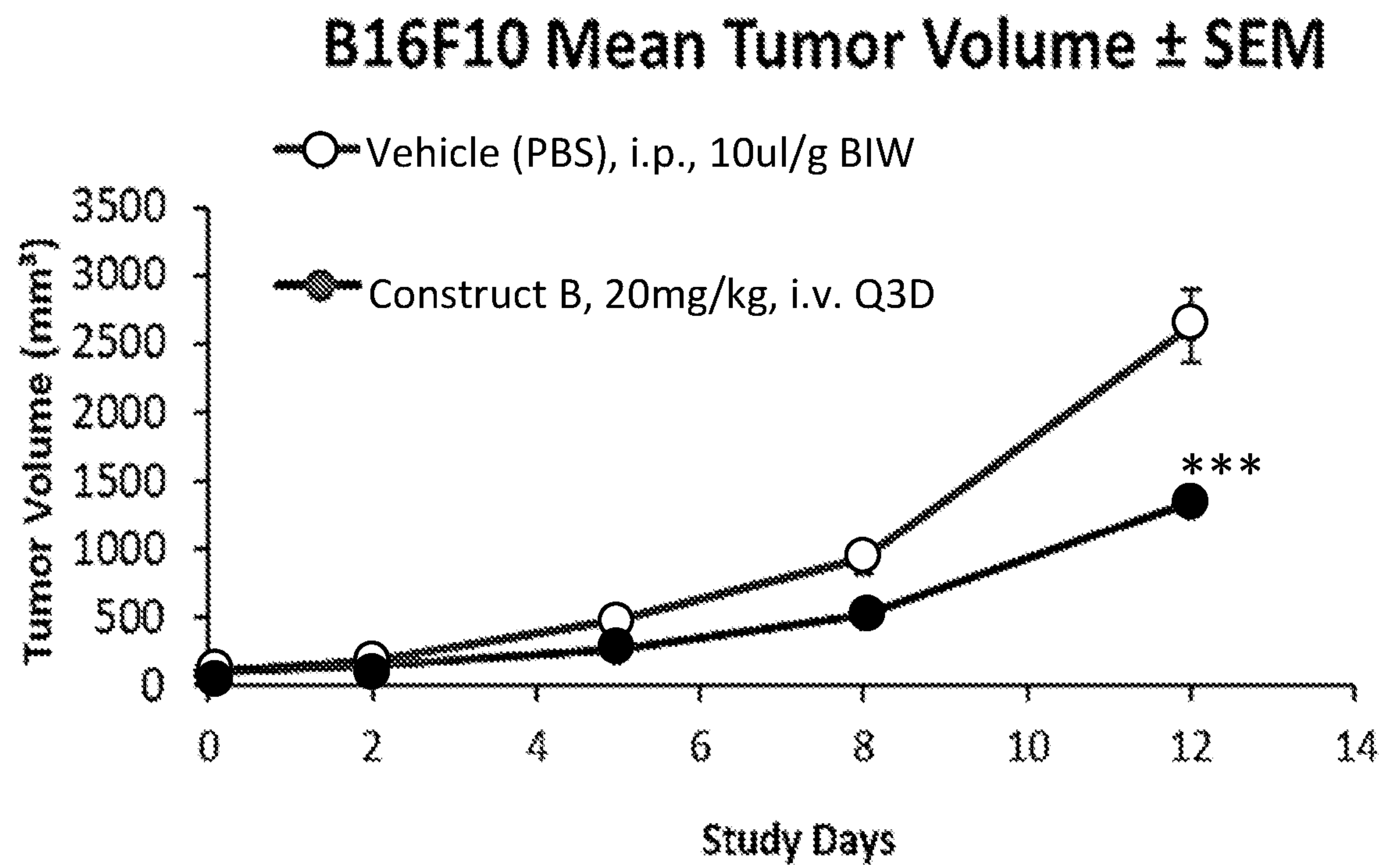


Fig. 13C

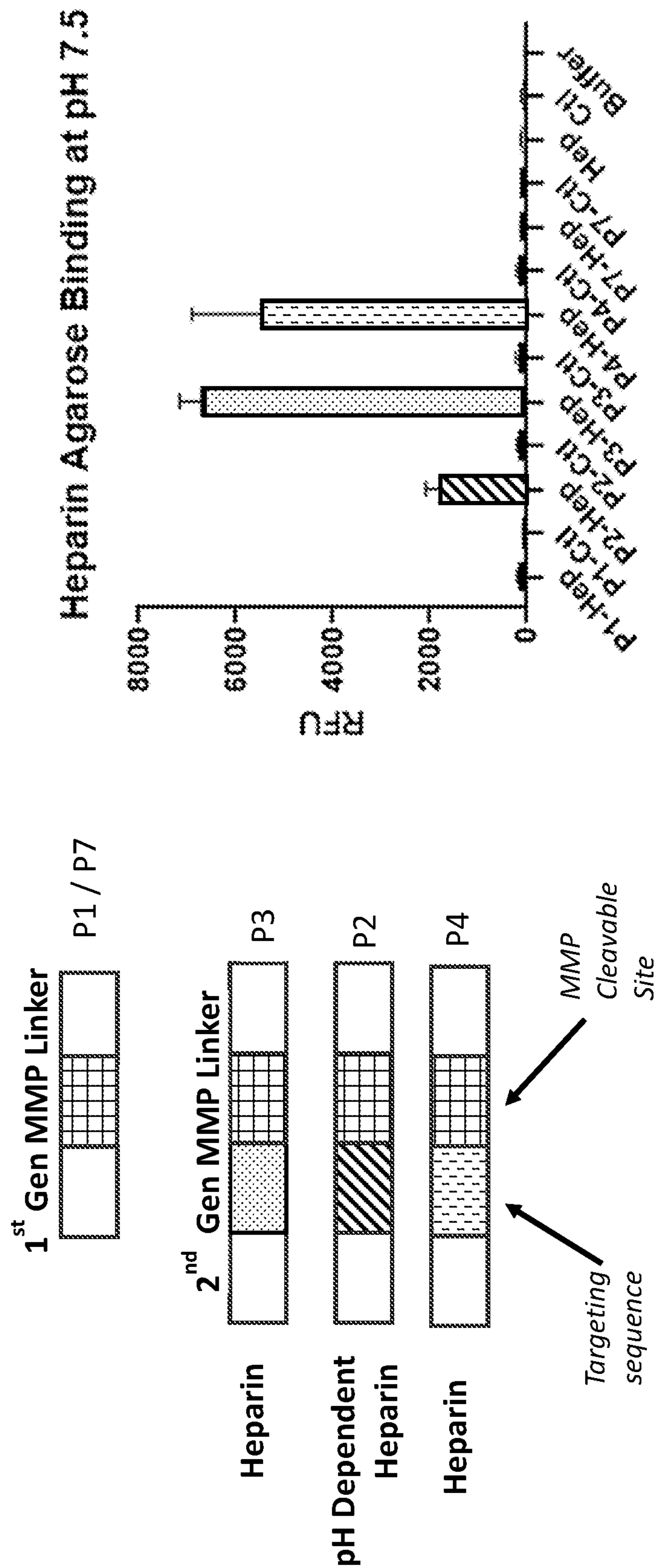


Fig. 14A

Heparin Agarose Binding pH 7.5 vs 6

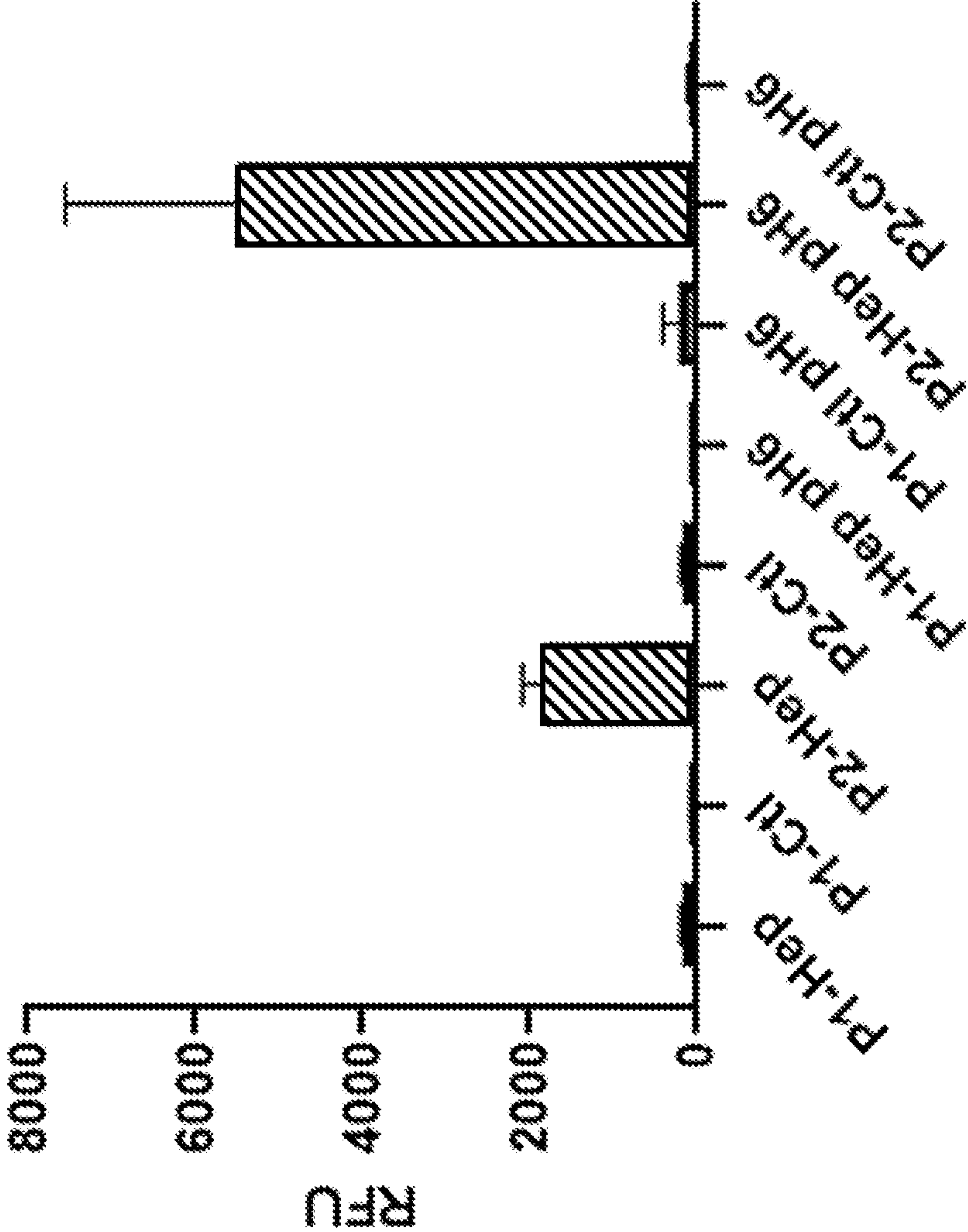


Fig. 14B

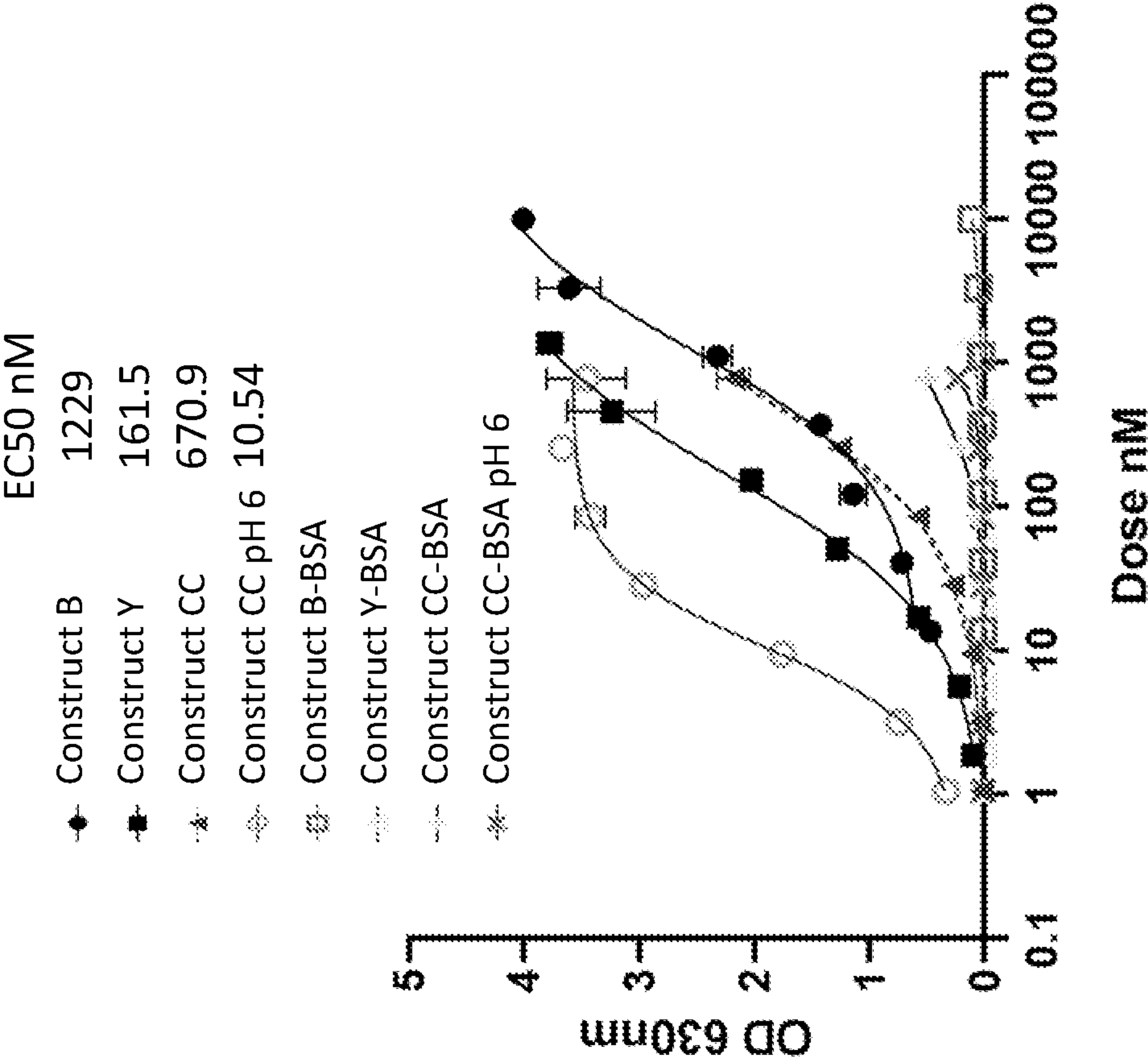
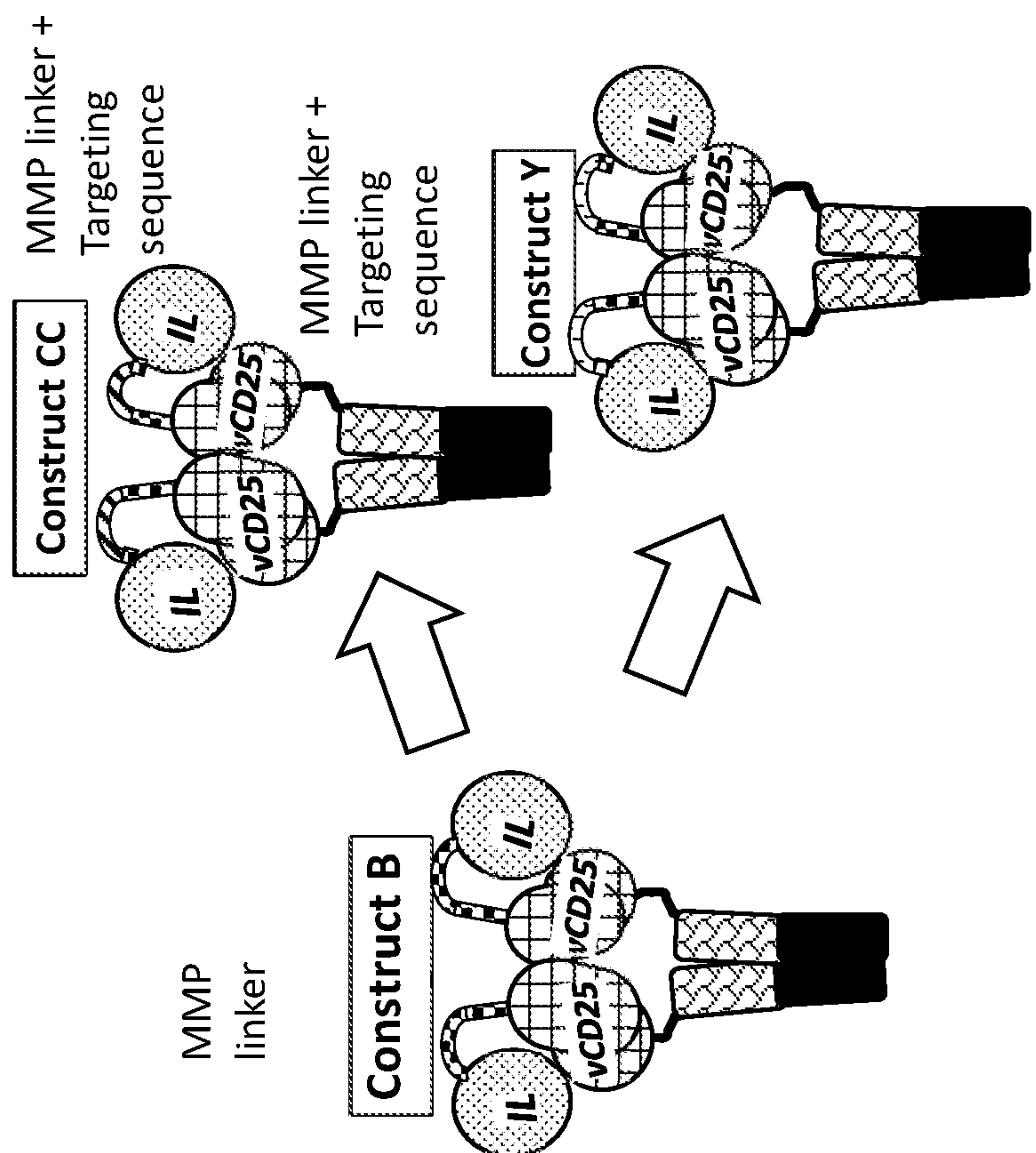


Fig. 14C



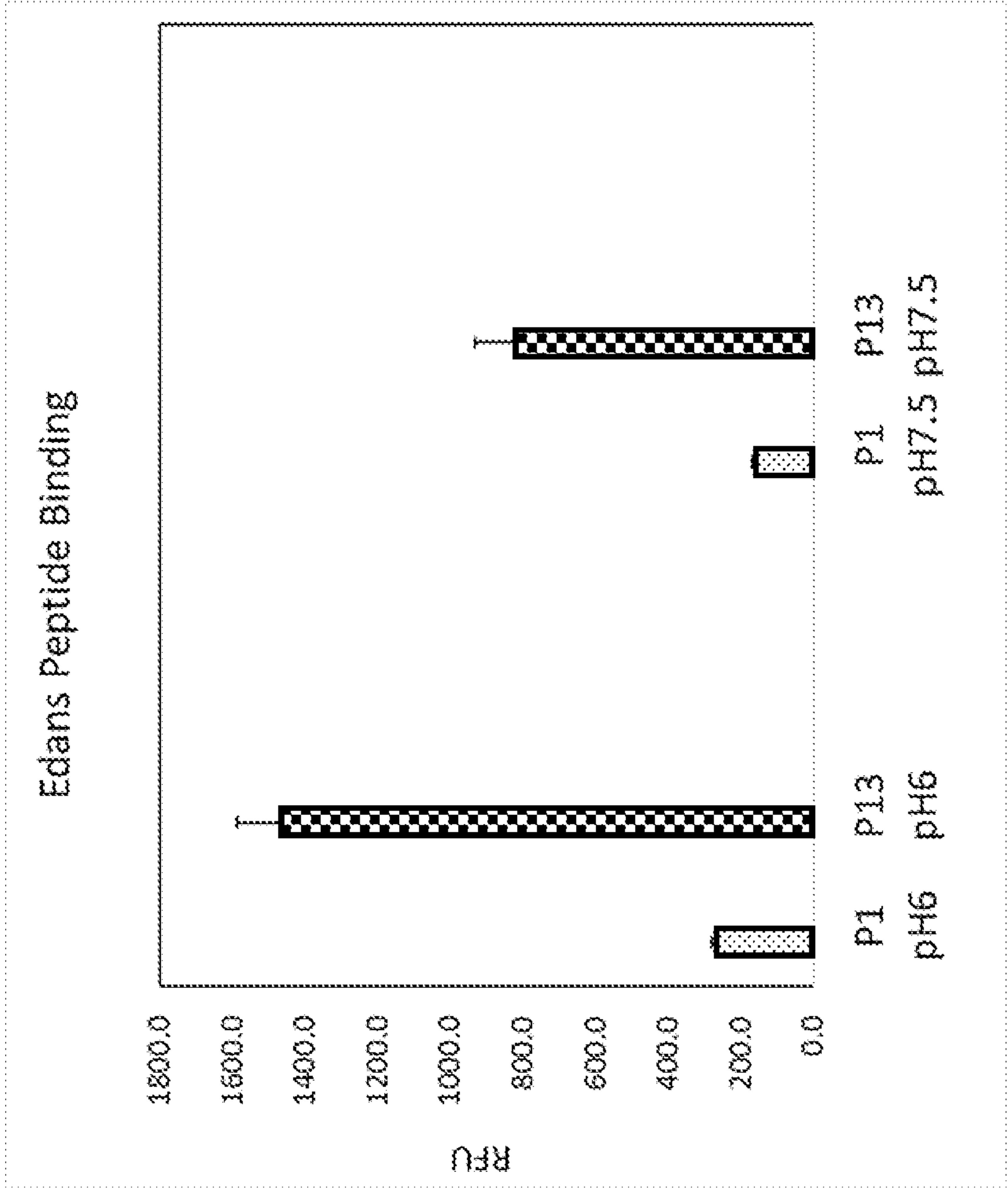
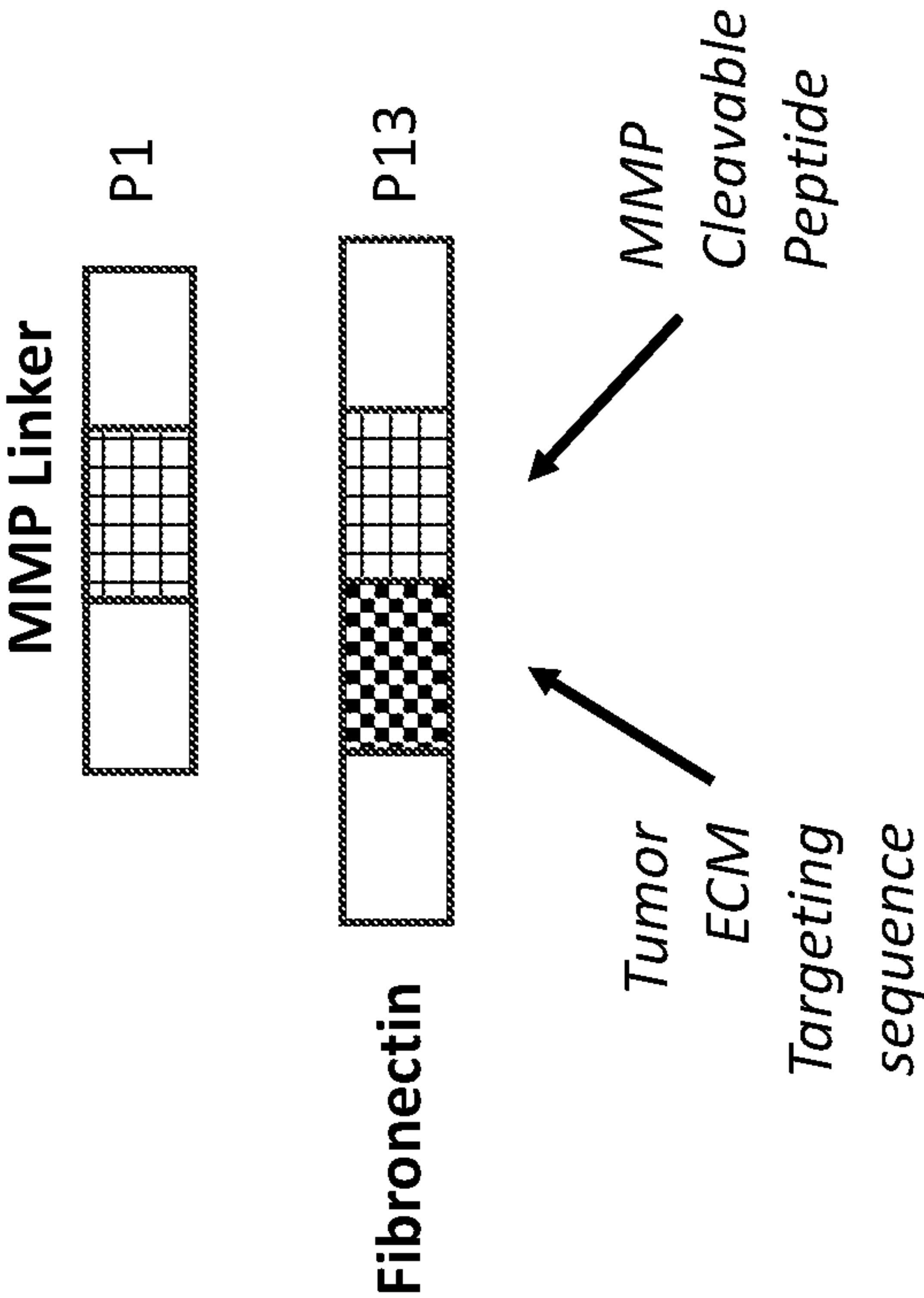


Fig. 14D



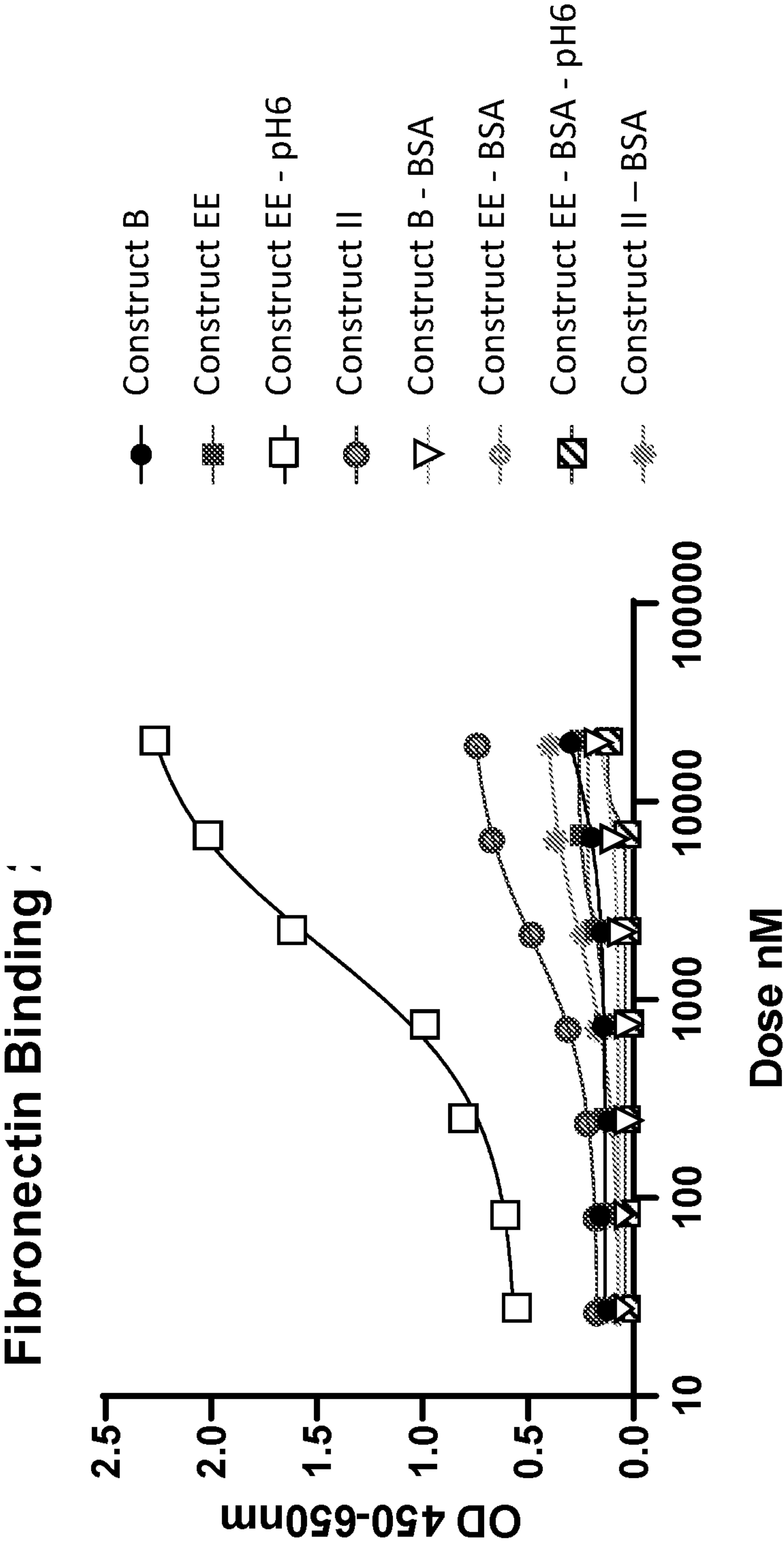


Fig. 14E

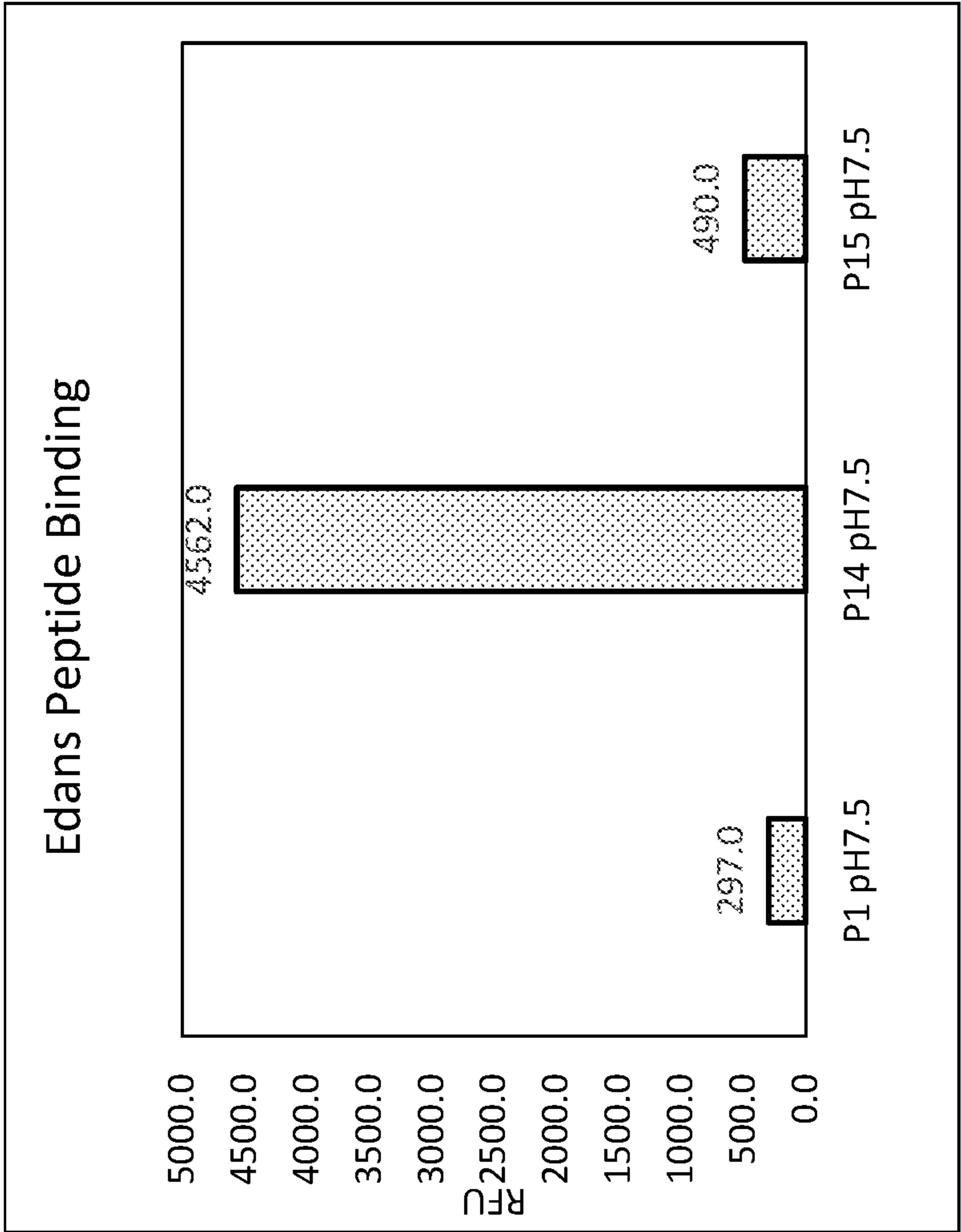
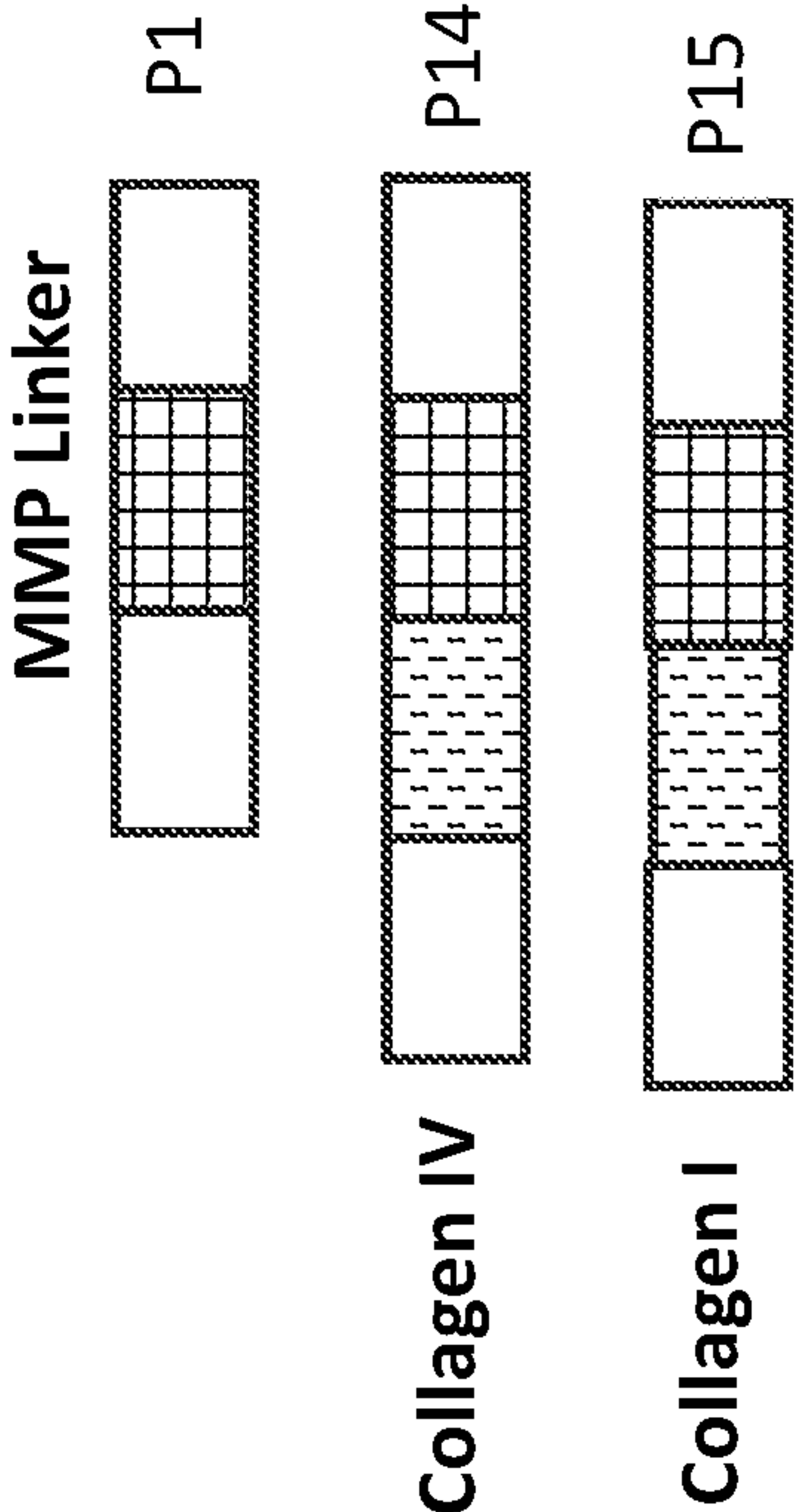


Fig. 14F



Western blot anti-mIL2

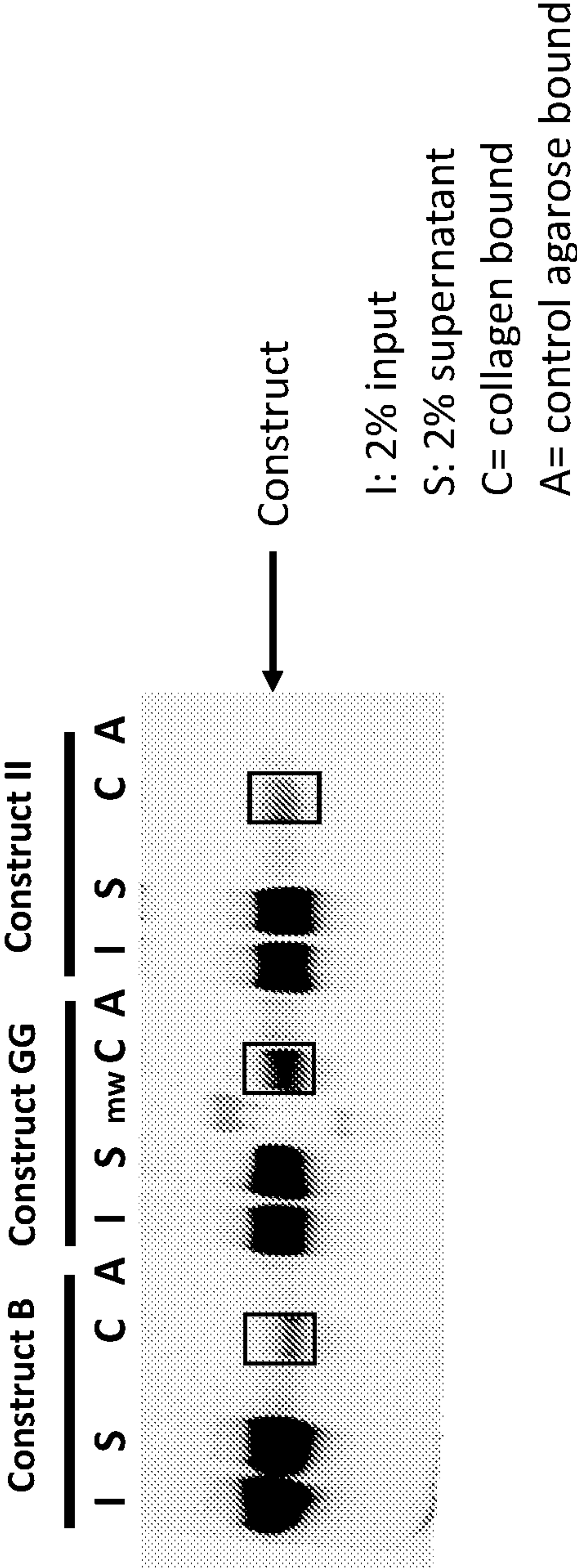


Fig. 14G

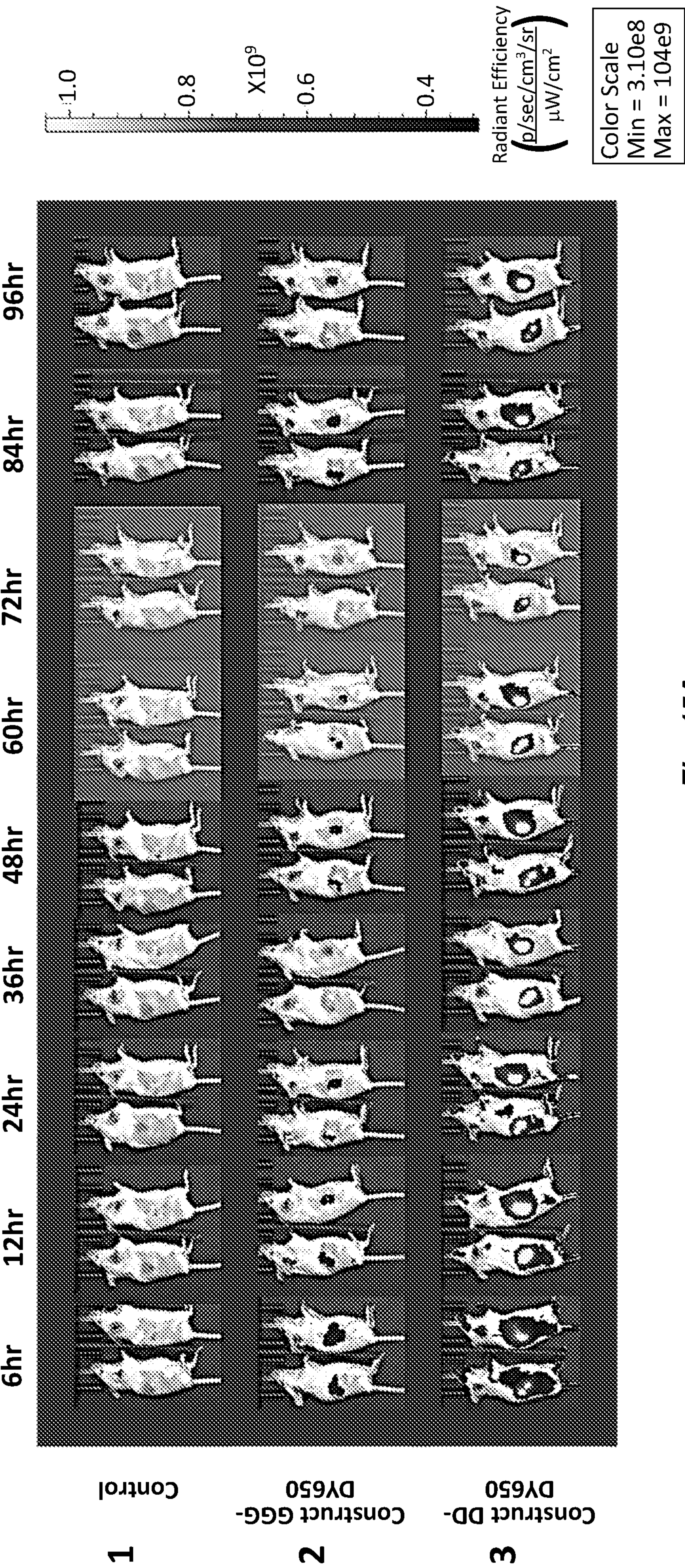


Fig. 15A

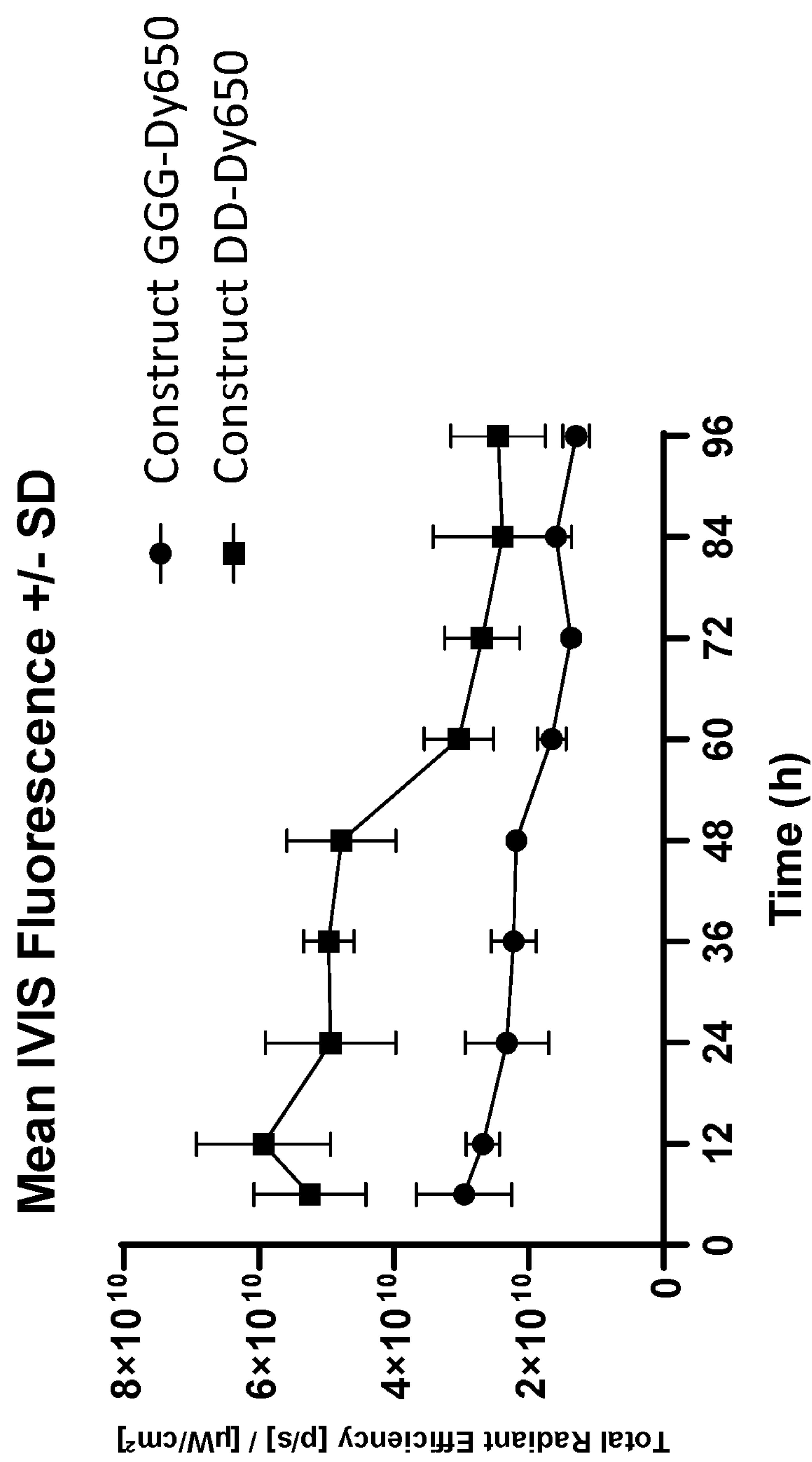


Fig. 15B

Day 14 collection
24h post final dose

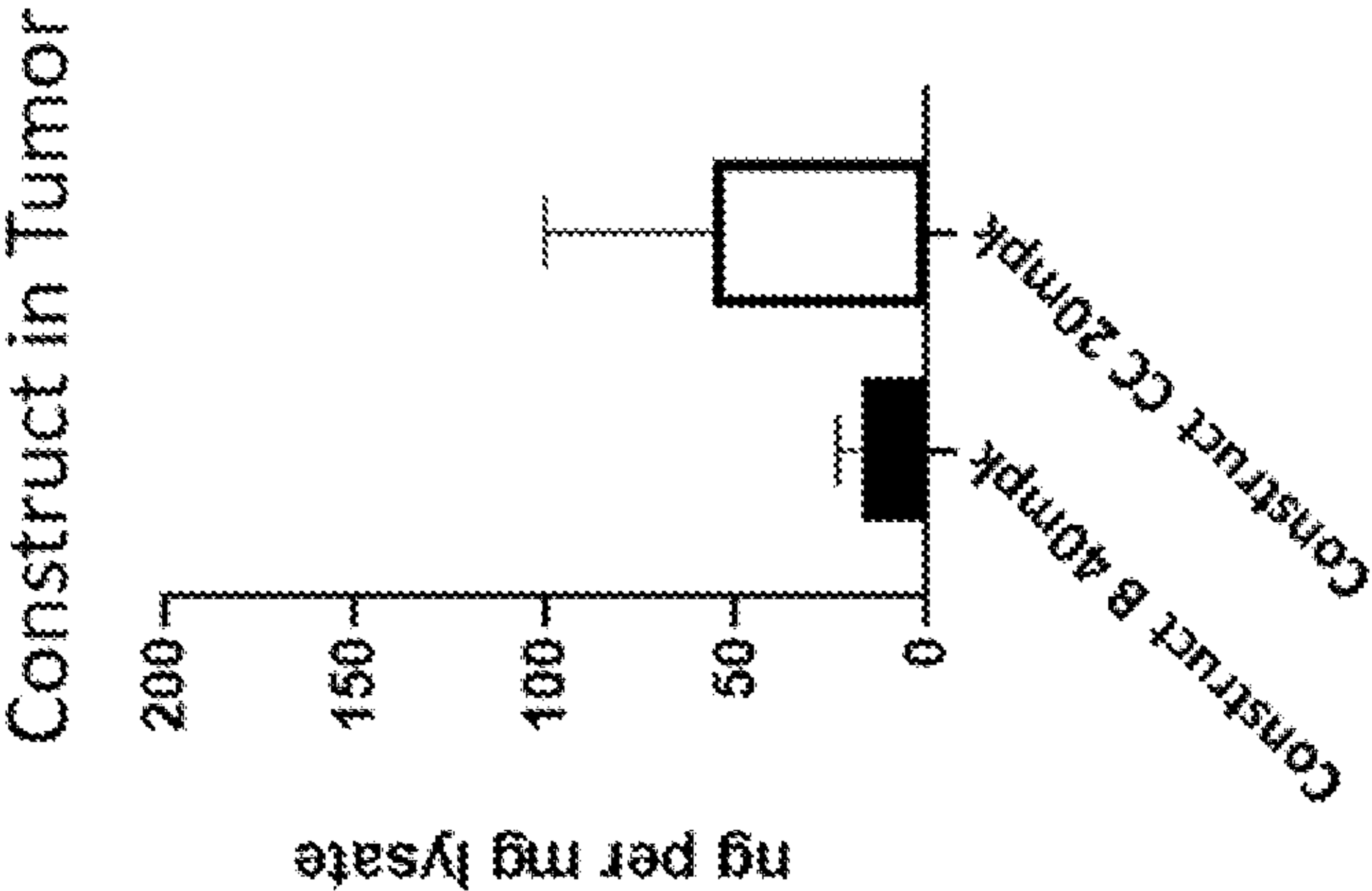


Fig. 15C

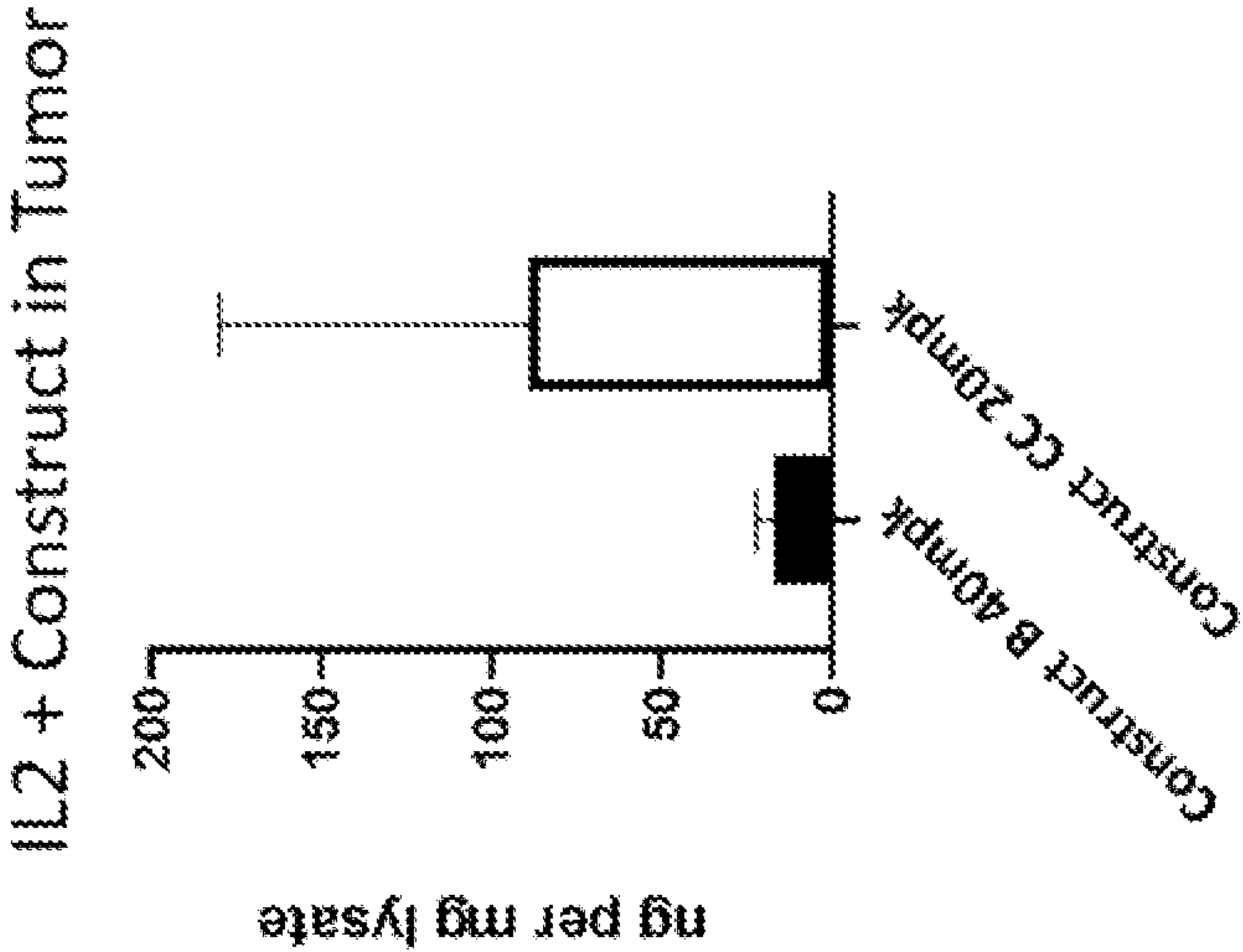


Fig. 15D

Day 21 collection
24h post final dose

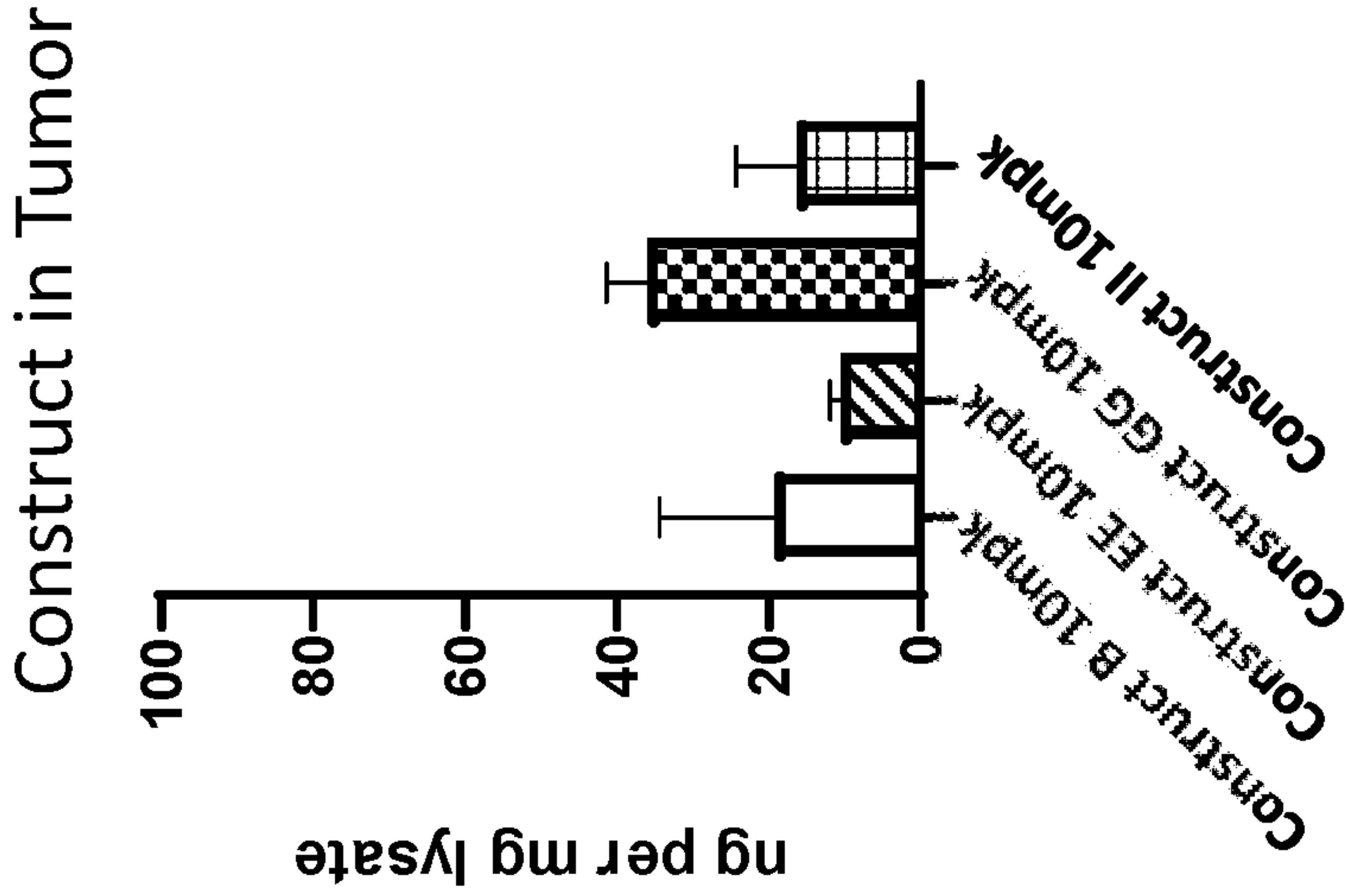


Fig. 15F

Day 14 collection
24h post final dose

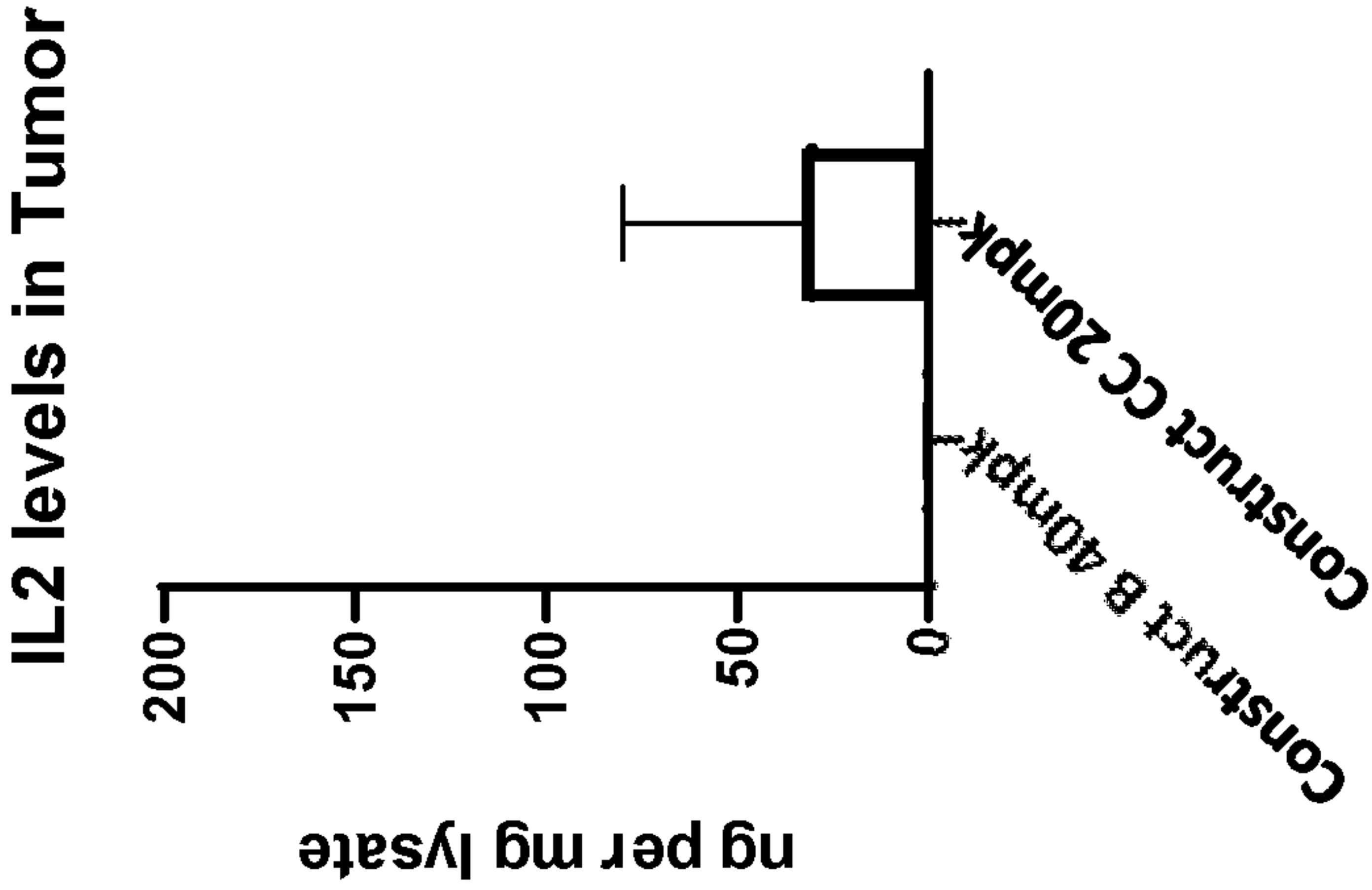


Fig. 15E

Day 21 collection
24h post final dose

IL2 + Construct in Tumor

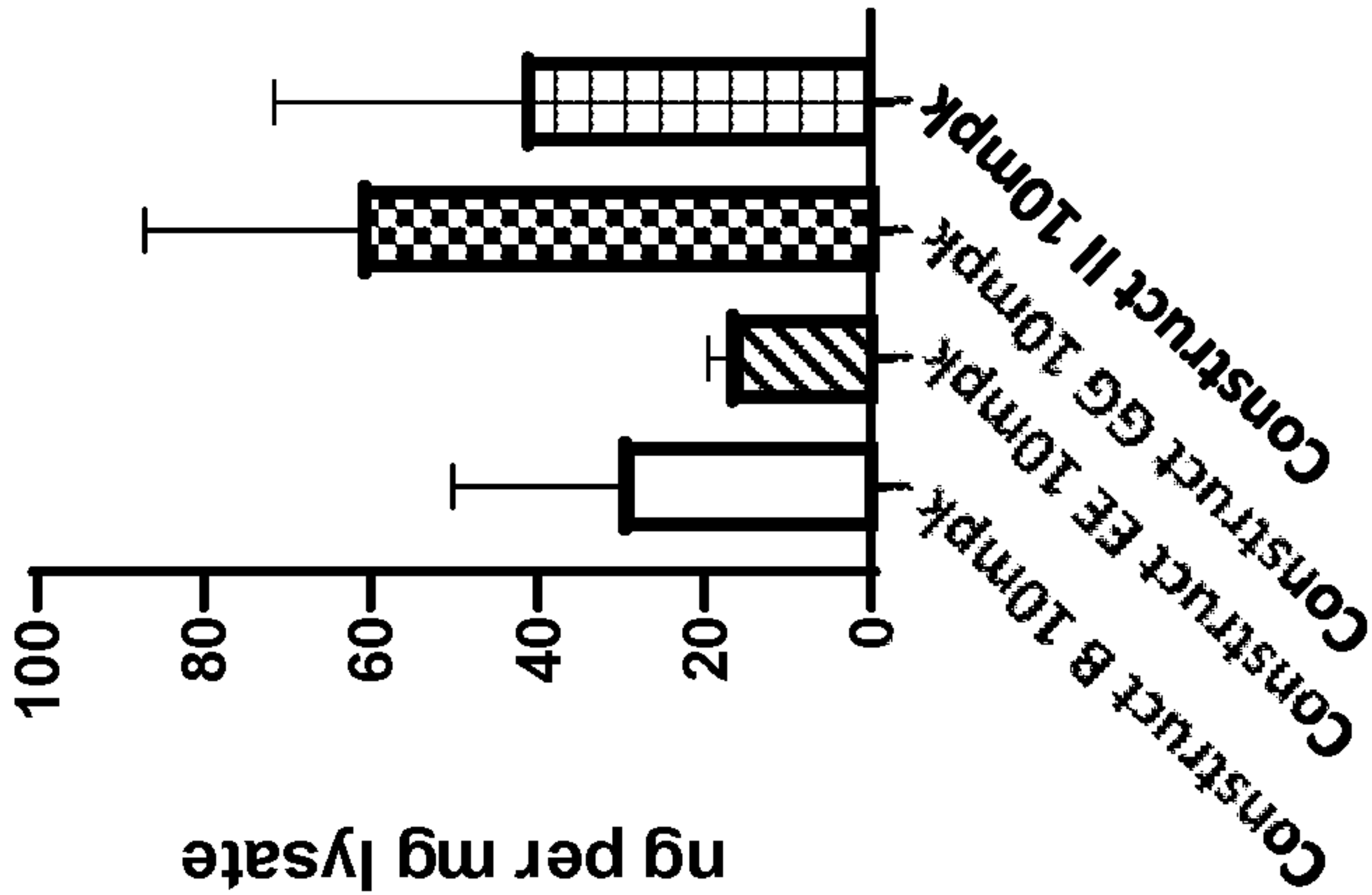


Fig. 15G

IL2 in Tumor

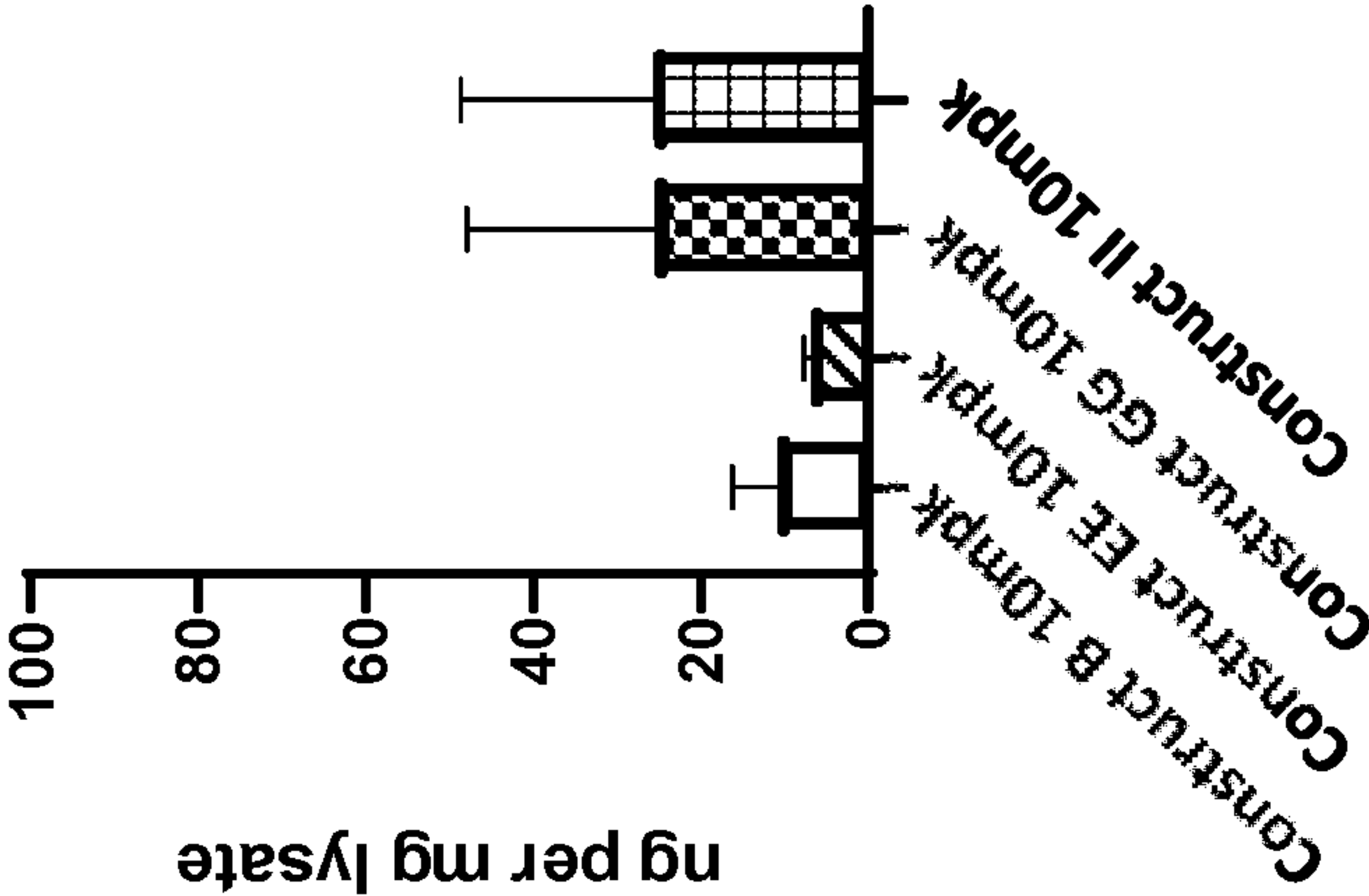


Fig. 15H

Day 14 collection
24h post final dose

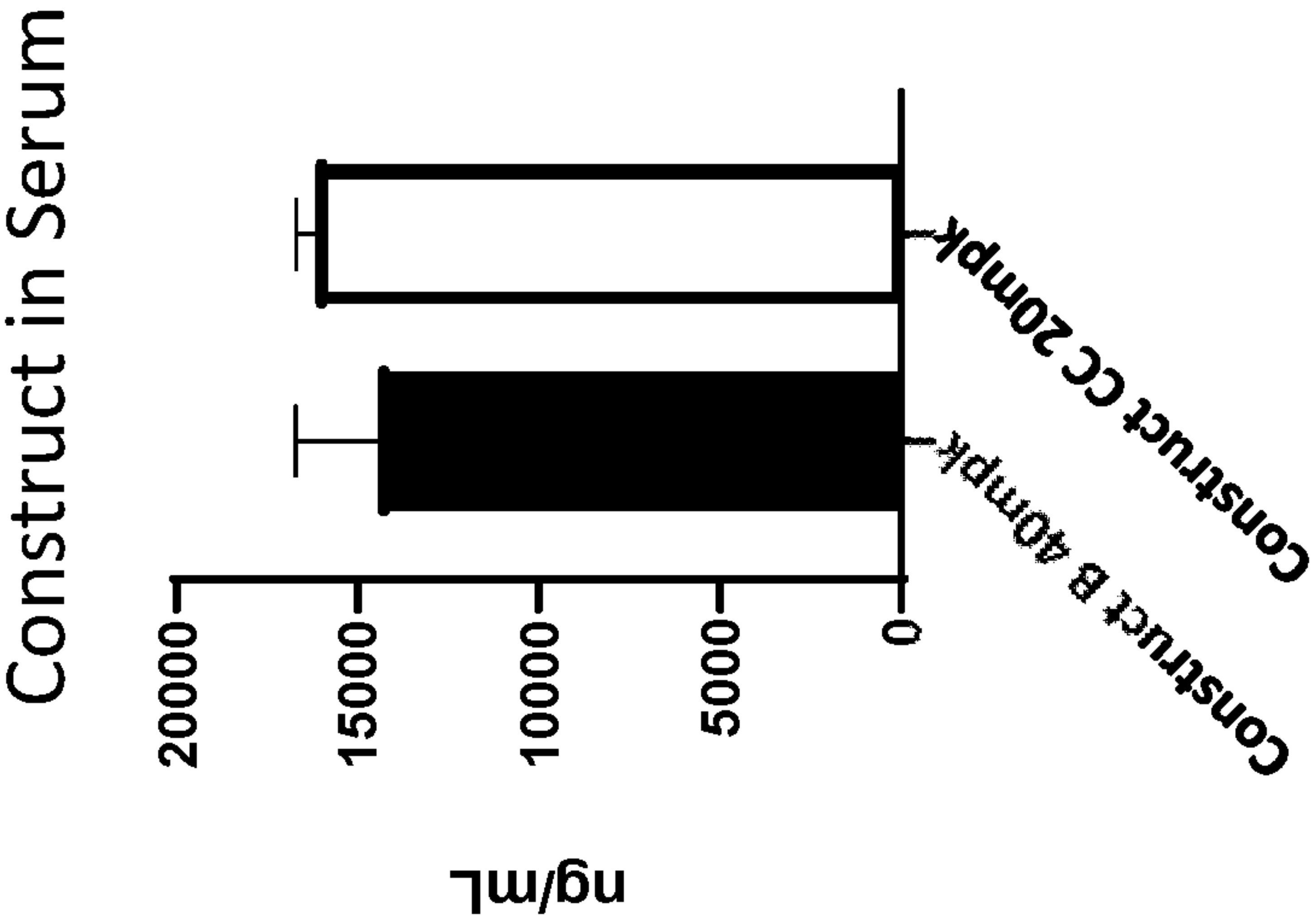


Fig. 15I

Serum collected Day 21
24h after dosing

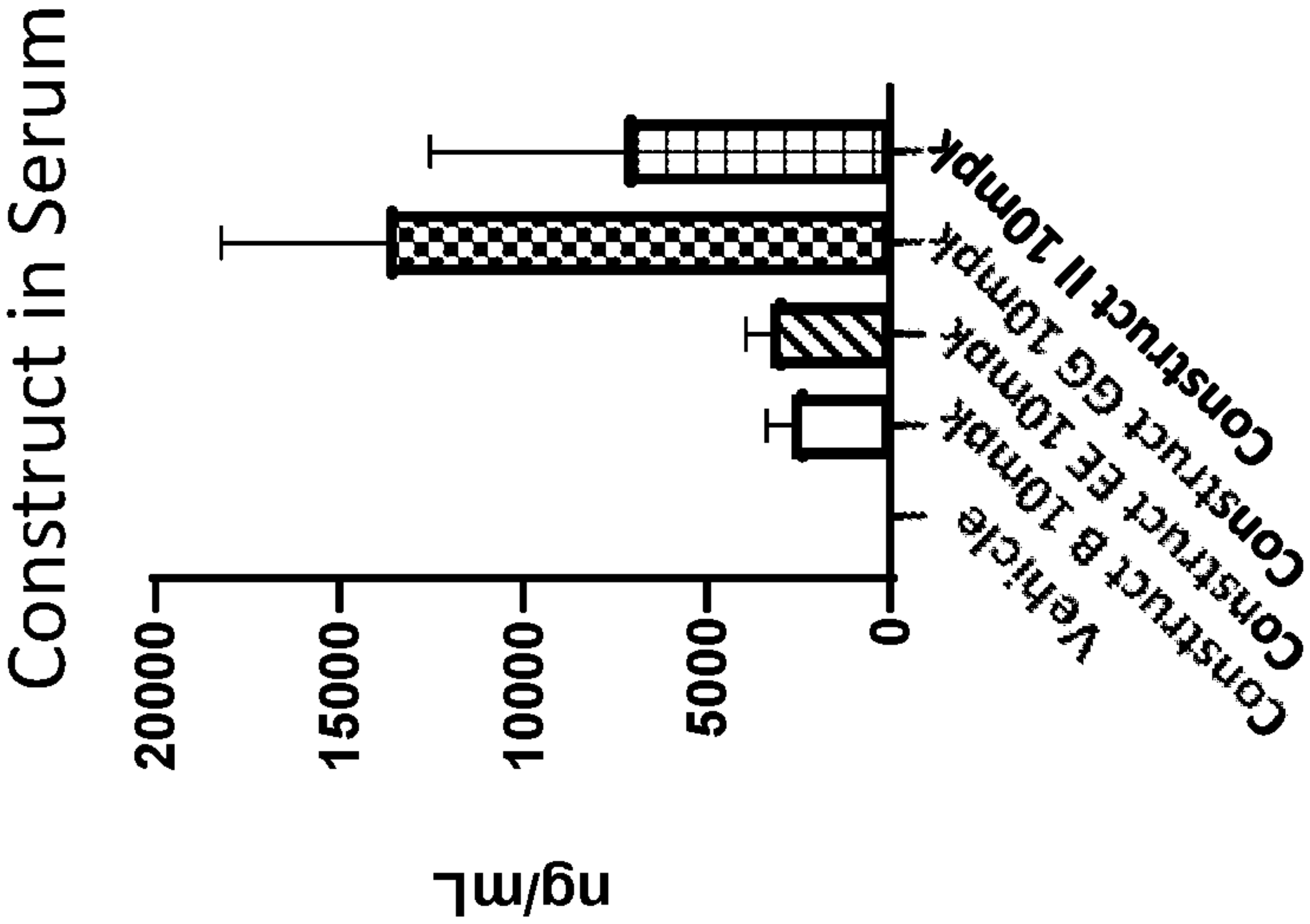


Fig. 15J

Day 17 collection TB002 - 96 h post final dose
Day 21 collection TB025 – 8 day post final dose

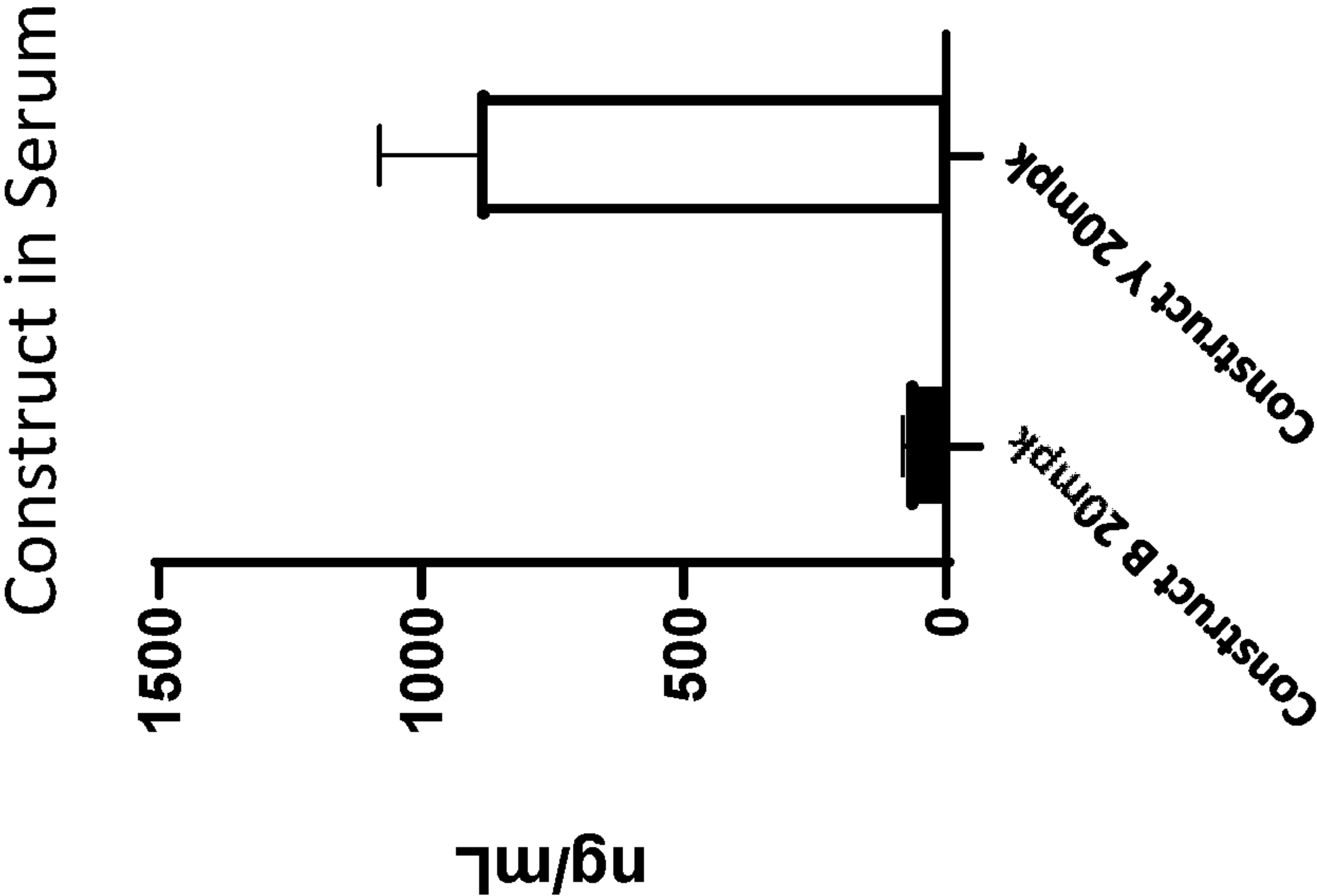


Fig. 15K

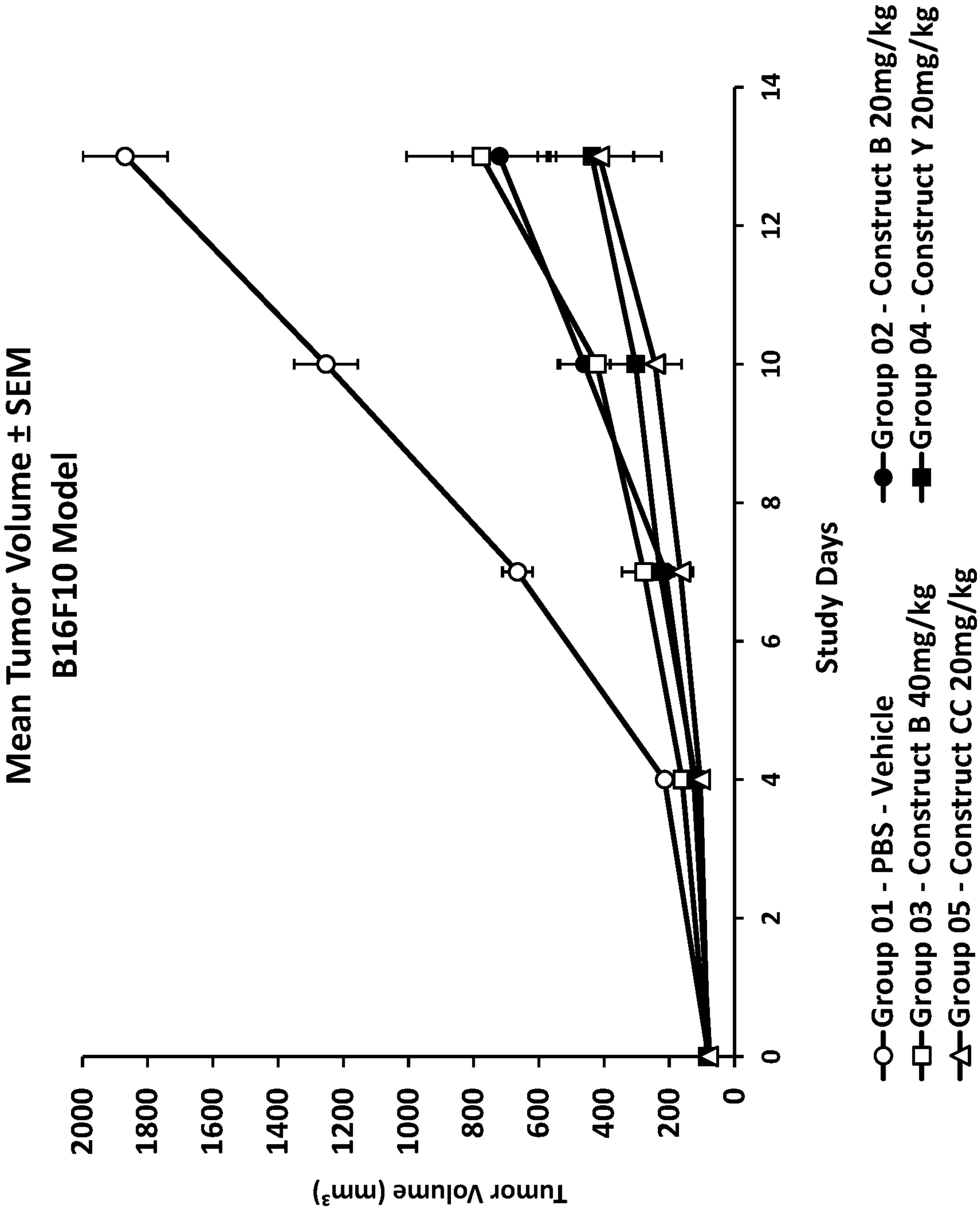


Fig. 16A

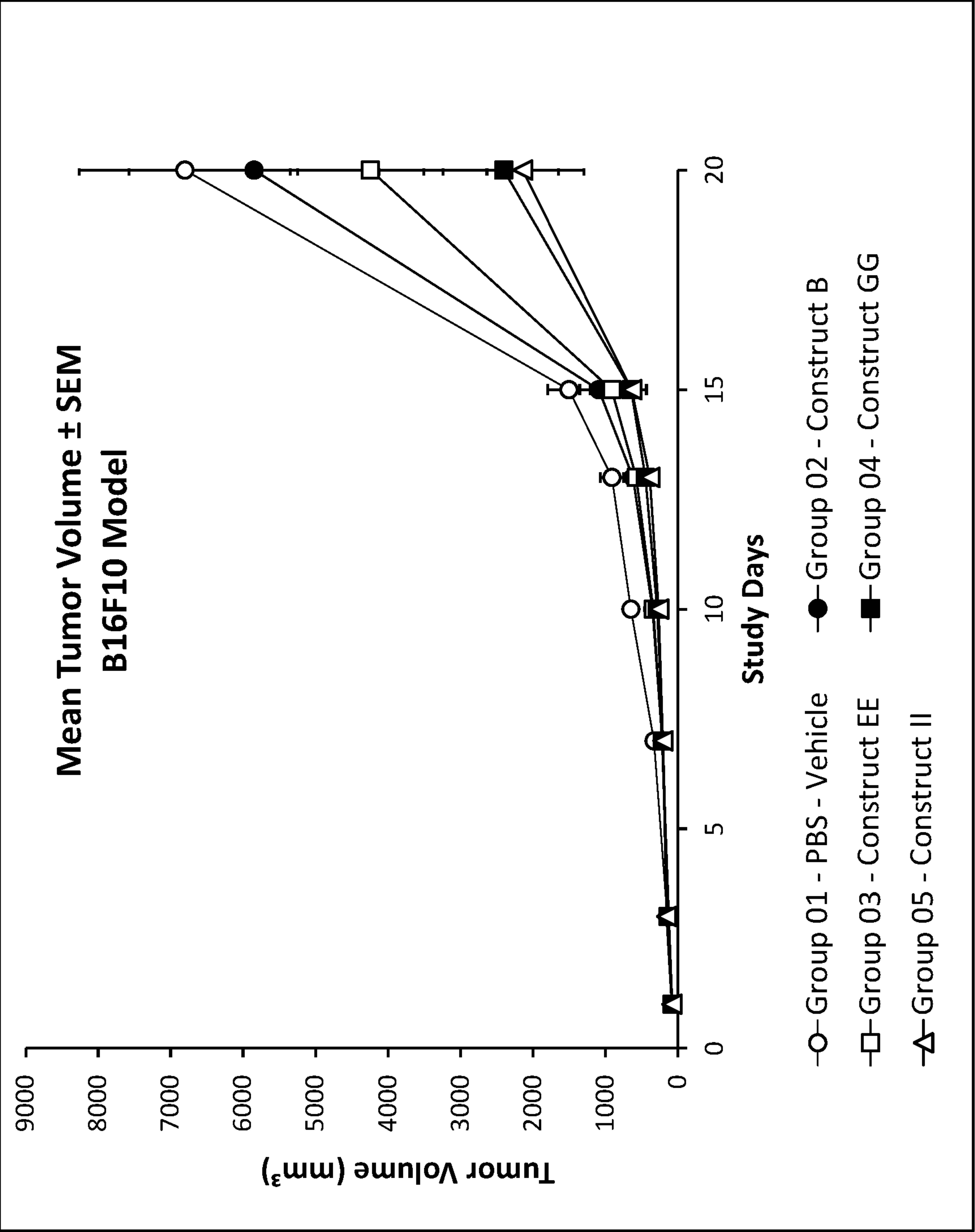


Fig. 16B

Day 17 collection Vehicle/ Construct B - 96 h post final dose
Day 21 collection Construct Y – 8 day post final dose

IFN- γ levels in Tumor

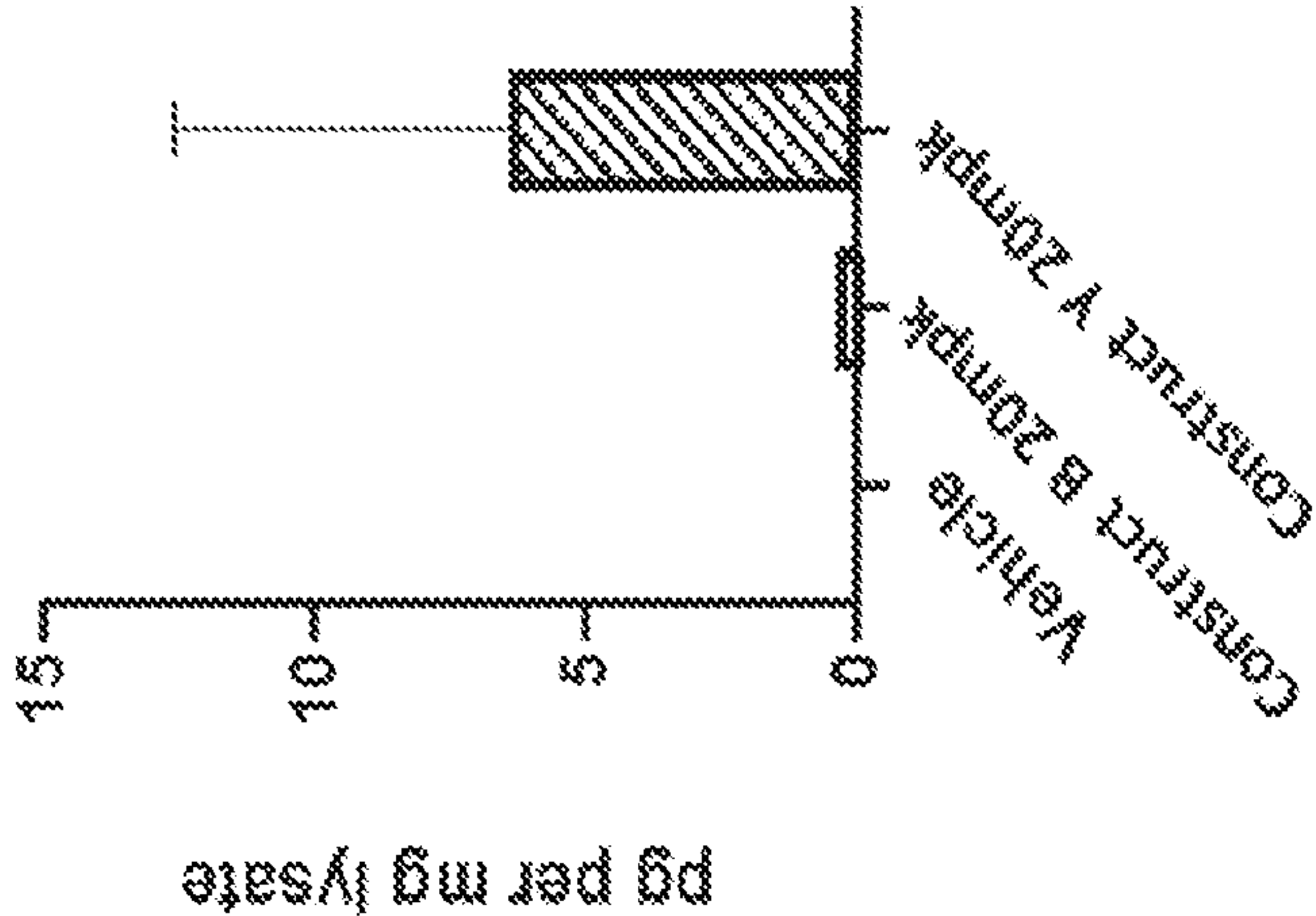


Fig. 17A

Day 21 collection
24h post final dose

IFN- γ in Tumor

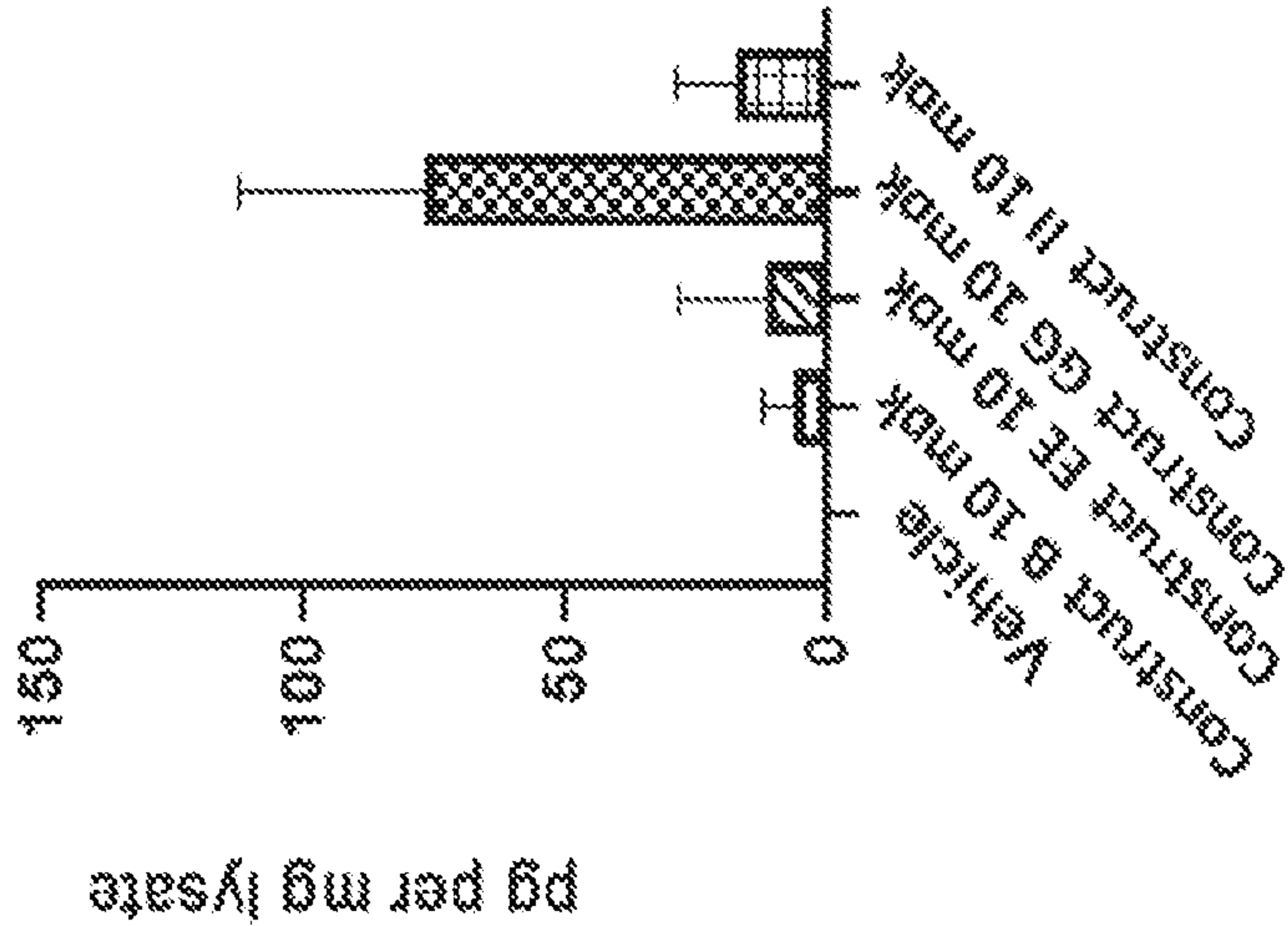


Fig. 17B

Short pK for cytokine post activation

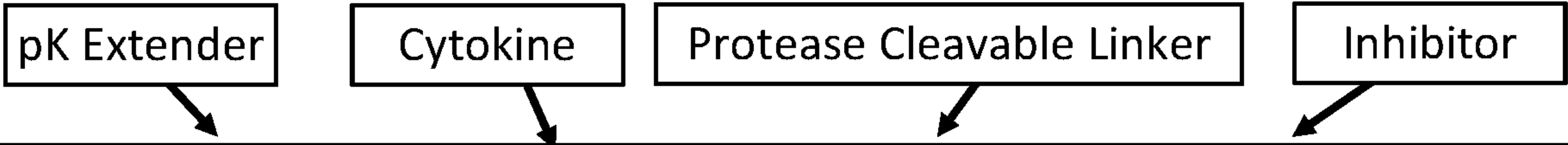
	CYTOKINE		PRO-LNK		INHIBITOR		PK EXT	
	CYTOKINE	LNK	PRO-LNK		INHIBITOR		PK EXT	
	CYTOKINE		PRO-LNK	LNK	INHIBITOR		PK EXT	
	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	
	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR		PK EXT	
	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	
	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR		PK EXT	
	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	
	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE		PRO-LNK		INHIBITOR		PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR		PK EXT	
RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR		PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	
	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	
	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
	CYTOKINE		PRO-LNK		INHIBITOR		PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK		INHIBITOR		PK EXT	RET LNK
	CYTOKINE		PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR		PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK

Fig. 18A

RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	
RET LNK	CYTOKINE		PRO-LNK		INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR		PK EXT	RET LNK
	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK	PK EXT	RET LNK

Fig. 18A (cont.)

Long pK for cytokine post activation



	PK EXT		CYTOKINE		PRO-LNK		INHIBITOR	
	PK EXT		CYTOKINE	LNK	PRO-LNK		INHIBITOR	
	PK EXT		CYTOKINE		PRO-LNK	LNK	INHIBITOR	
	PK EXT		CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	
	PK EXT		CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	
	PK EXT		CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	
	PK EXT		CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	
	PK EXT		CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	
	PK EXT		CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	
	PK EXT	RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	
	PK EXT		CYTOKINE		PRO-LNK		INHIBITOR	RET LNK
	PK EXT		CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK
	PK EXT		CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT		CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT		CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK
	PK EXT		CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT		CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK
	PK EXT		CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	PK EXT		CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE		PRO-LNK		INHIBITOR	
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK		INHIBITOR	
RET LNK	PK EXT		CYTOKINE		PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	

Fig. 18B

	PK EXT	RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	PK EXT		CYTOKINE		PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT		CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK		INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE		PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	PK EXT	RET LNK	CYTOKINE	RET LNK	PRO-LNK	RET LNK	INHIBITOR	RET LNK

Fig. 18B (cont.)

Cytokine

Protease Cleavable Linker

pK Extender

Inhibitor

	CYTOKINE		PRO-LNK	PK EXT		INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT		INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT	LNK	INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT		INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT	RET LNK	INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT		INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT		INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT	LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT		INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT		INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT		INHIBITOR	RET LNK
	CYTOKINE		PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT		INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
	CYTOKINE		PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT		INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT		INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT		INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	RET LNK	INHIBITOR	RET LNK

Fig. 18C

Cytokine

pK Extender

Protease Cleavable Linker

Inhibitor

	CYTOKINE		PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE	LNK	PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE		PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE	LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE	RET LNK	PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE	RET LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE		PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE	LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE	RET LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE		PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE		PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE		PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE		PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE	LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE		PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE		PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE		PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK

Fig. 18D

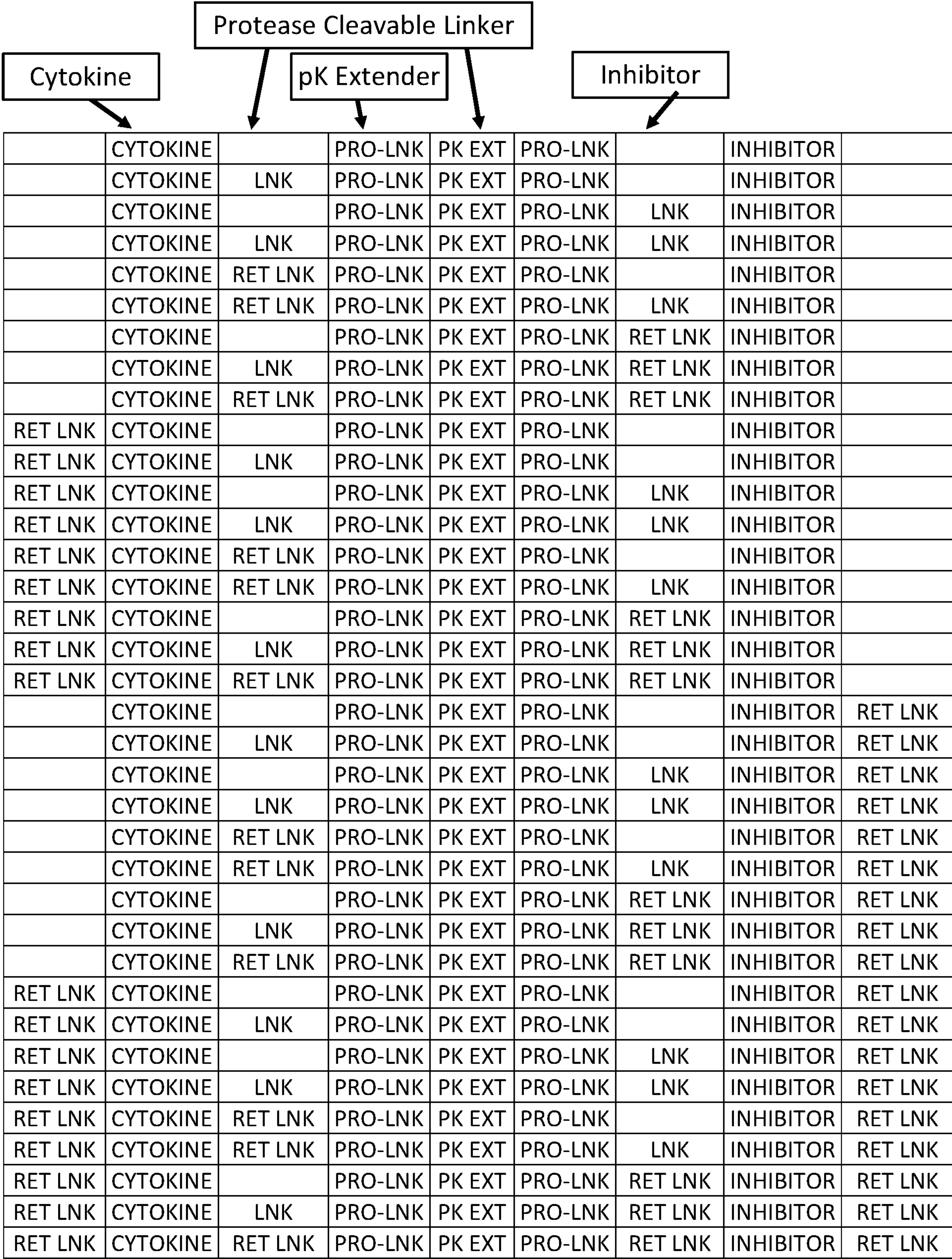


Fig. 18E

	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	
	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE		PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK		INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	LNK	INHIBITOR	RET LNK
RET LNK	CYTOKINE	RET LNK	PRO-LNK	PK EXT	PRO-LNK	RET LNK	INHIBITOR	RET LNK

Fig. 18E (cont.)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/013478

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K14/55 A61K47/64 A61K47/65 A61K47/66 A61K47/68 C07K14/715 A61P37/04 C12N15/62 ADD. According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P C07K C12N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, Sequence Search, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/246392 A1 (CUGENE INC [US]) 26 December 2019 (2019-12-26) abstract; claims 1-47; pages 1-7, 39-52, 58-72, 122-124; Example 4; Figures 1 and 2; Tables 2 and 3 -----	1-128
X	WO 2019/222295 A1 (WEREWOLF THERAPEUTICS INC [US]; WINSTON WILLIAM [US] ET AL.) 21 November 2019 (2019-11-21) abstract; pages 1-4, 16-19, 31-38; Table 3; Sequence Table; claims 1-39; Figures 1-4 -----	1-128
X	WO 2011/123683 A2 (UNIV ROCHESTER [US]; FRELINGER JOHN G [US] ET AL.) 6 October 2011 (2011-10-06) pages 8-10; Examples 1-7; figures 1A and 2A; claims 1-44 ----- -/--	1-128
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 3 May 2021		Date of mailing of the international search report 20/05/2021
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Kurz, Birgit

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/013478

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/013478

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/173832 A2 (ASKGENE PHARMA INC [US]; YU CHUNXIAO [US] ET AL.) 12 September 2019 (2019-09-12) pages 3-7, 11-23, 50-53; Tables 3 and 5; claims 1-50; Figure 3 -----	1-128
A	KI YOUNG CHOI ET AL: "Protease-Activated Drug Development", THERANOSTICS, vol. 2, no. 2, 1 January 2012 (2012-01-01) , pages 156-179, XP055263145, AU ISSN: 1838-7640, DOI: 10.7150/thno.4068 abstract; pages 158, 163-165, 168-172; Figure 1; Table 1 -----	1-128
A	RAAVÉ RENÉ ET AL: "Chemotherapeutic drug delivery by tumoral extracellular matrix targeting", JOURNAL OF CONTROLLED RELEASE, vol. 274, 1 March 2018 (2018-03-01), pages 1-8, XP055800560, AMSTERDAM, NL ISSN: 0168-3659, DOI: 10.1016/j.jconrel.2018.01.029 abstract; pages 1-6; Figure 2; Table 1 -----	1-128
A	JUN ISHIHARA ET AL: "Targeted antibody and cytokine cancer immunotherapies through collagen affinity", SCIENCE TRANSLATIONAL MEDICINE, vol. 11, no. 487, 10 April 2019 (2019-04-10), page eaau3259, XP055656045, US ISSN: 1946-6234, DOI: 10.1126/scitranslmed.aau3259 abstract; pages 1-3 and 5-7; -----	1-128
A	ADDI CYRIL ET AL: "Design and Use of Chimeric Proteins Containing a Collagen-Binding Domain for Wound Healing and Bone Regeneration", TISSUE ENGINEERING PART B, vol. 23, no. 2, 1 April 2017 (2017-04-01), pages 163-182, XP055800565, US ISSN: 1937-3368, DOI: 10.1089/ten.teb.2016.0280 abstract; pages 164, 166, 167, 169; Tables 1 and 2; Figure 1 ----- -/-	1-128

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/013478

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SIPES J M ET AL: "INHIBITION OF FIBRONECTIN BINDING AND FIBRONECTIN-MEDIATED CELL ADHESION TO COLLAGEN BY A PEPTIDE FROM THE SECOND TYPE I REPEAT OF THROMBOSPONDIN", THE JOURNAL OF CELL BIOLOGY, THE ROCKEFELLER UNIVERSITY PRESS, US, vol. 121, no. 2, 1 January 1993 (1993-01-01), pages 469-477, XP000651567, ISSN: 0021-9525, DOI: 10.1083/JCB.121.2.469 abstract; pages 468 and 470; Tables 1 to 3 -----</p>	1-128
A	<p>D.-B. BORZA ET AL: "Histidine-Proline-rich Glycoprotein as a Plasma pH Sensor: MODULATION OF ITS INTERACTION WITH GLYCOSAMINOGLYCANS BY pH AND METALS", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 10, 6 March 1998 (1998-03-06), pages 5493-5499, XP055231899, ISSN: 0021-9258, DOI: 10.1074/jbc.273.10.5493 abstract; page 5493; Figures 1 and 2 -----</p>	1-128

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2021/013478

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WO 2019246392 A1	26-12-2019	AU 2019288484 A1 CA 3102823 A1 CN 112654633 A EP 3810624 A1 KR 20210038548 A WO 2019246392 A1	21-01-2021 26-12-2019 13-04-2021 28-04-2021 07-04-2021 26-12-2019
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WO 2019173832 A2	12-09-2019	EP 3762406 A2 WO 2019173832 A2	13-01-2021 12-09-2019
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