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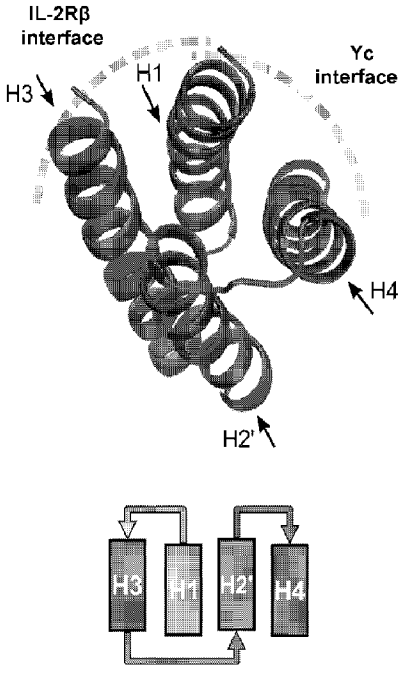
(54) Title: DE NOVO DESIGN OF POTENT AND SELECTIVE INTERLEUKIN MIMETICS

FIG. 3E
Neo-2/15



(57) Abrégé/Abstract:
De novo designed polypeptides that bind to IL-2 receptor ß? c heterodimer (IL- 2Rß? c), IL-4 receptor a? cheterodimer (IL-4Ra? c), or IL-13 receptor a subunit (IL-13Ra) are disclosed, as are methods for using and designing the polypeptides.

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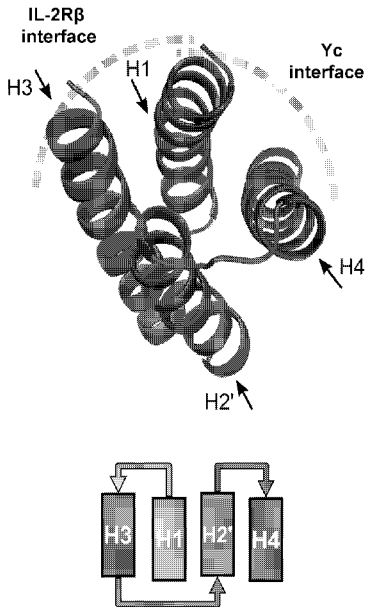
(54) Title: DE NOVO DESIGN OF POTENT AND SELECTIVE INTERLEUKIN MIMETICS

(57) Abstract: De novo designed polypeptides that bind to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$), IL-4 receptor $\alpha\gamma_c$heterodimer (IL-4R$\alpha\gamma_c$), or IL-13 receptor α subunit (IL-13Rα) are disclosed, as are methods for using and designing the polypeptides.

FIG. 3E

Neo-2/15



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DE NOVO DESIGN OF POTENT AND SELECTIVE INTERLEUKIN MIMETICS

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This application claims priority to U.S. Provisional Patent Application Serial Nos. 62/689,769 filed June 25, 2018 and 62/768,733 filed November 16, 2018, each incorporated by reference herein in its entirety.

Background

The considerable potential of central immune cytokine interleukins such as IL-2 and IL-4 for cancer treatment has sparked numerous efforts to improve their therapeutic properties by mutation and/or chemical modification. However, because these approaches are closely tied to native IL-2 or IL-4, they cannot eliminate undesirable properties such as low stability and binding to the IL-2 receptor α subunit (IL-2Rα), to IL-4 receptor $\alpha\gamma_c$heterodimer (IL-4R$\alpha\gamma_c$), or to IL-13 receptor α subunit (IL-13Rα).

Summary

In one aspect, a method is provided. A computing device determines a structure for a plurality of residues of a protein where the structure of the plurality of residues provides a particular receptor binding interface. The computing device determines a plurality of designed residues using a mimetic design protocol provided by the computing device, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues.

The computing device determines one or more connecting helix structures that connect the plurality of designed residues. The computing device determines a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations. The computing device designs a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone. The computing device generates an output related to at least the second protein backbone.

Also included are non-naturally occurring proteins prepared by the methods described herein. The non-naturally occurring proteins can be cytokines, for example, non-naturally occurring IL-2 or IL-4 (also referred to herein as IL-2, IL-2/15 mimetics or IL-4 mimetics).

In another aspect, a computing device is provided. The computing device includes one or more processors; and data storage that is configured to store at least computer-readable instructions that, when executed by the one or more processors, cause the computing device to perform functions. The functions include: determining a structure for a plurality of residues of a protein that provides a particular receptor binding interface; determining a plurality of designed residues using a mimetic design protocol, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues; determining one or more connecting helix structures that connect the plurality of designed residues; determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations; designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone; and generating an output related to at least the second protein backbone for the protein.

In another aspect, a non-transitory computer-readable medium is provided. The non-transitory computer-readable medium is configured to store at least computer-readable instructions that, when executed by one or more processors of a computing device, cause the computing device to perform functions. The functions include: determining a structure for a plurality of residues of a protein that provides a particular receptor binding interface; determining a plurality of designed residues using a mimetic design protocol, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues; determining one or more connecting helix structures that connect the plurality of designed residues; determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations; designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone; and generating an output related to at least the second protein backbone for the protein.

In another aspect, a device is provided. The device includes: means for determining a structure for a plurality of residues of a protein that provides a particular receptor binding interface; means for determining a plurality of designed residues using a mimetic design protocol, wherein the plurality of designed residues provide the particular receptor binding

interface, and wherein the plurality of designed residues differ from the plurality of residues; means for determining one or more connecting helix structures that connect the plurality of designed residues; means for determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations; means for designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone; and means for generating an output related to at least the second protein backbone for the protein.

In another aspect, non-naturally occurring polypeptides are provided comprising domains X1, X2, X3, and X4, wherein:

(a) X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to EHALYDAL (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence at least 25%%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to YAFNFELI (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to ITILQSWIF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$), IL-4 receptor $\alpha\gamma_c$ heterodimer (IL-4R$\alpha\gamma_c$), or IL-13 receptor α subunit (IL-13Rα).

In other aspects are provided pharmaceutical compositions comprising one or more polypeptide disclosed herein and a pharmaceutically acceptable carrier, recombinant nucleic acids encoding a polypeptide disclosed herein, expression vectors comprising the recombinant nucleic acids disclosed herein, and recombinant host cells comprising one or more expression vector disclosed herein. In a further aspect, methods for treating cancer are provided, comprising administering to a subject having cancer one or more polypeptide, recombinant nucleic acid, expression vector comprising the recombinant nucleic acid, and/or recombinant host cells disclosed herein or a pharmaceutical composition thereof in an amount effective to treat the tumor.

Description of the Drawings

The following figures are in accordance with example embodiments:

Figure 1A-1D. Computational design of *de novo* cytokine mimetics. FIG. 1A) The designed mimetics have four helices; three mimetic IL-2 interactions with hIL-2Rβγ_c, while the fourth holds the first three in place. *Top*: in the first generation of designs, each of the core elements of IL-2 (helices H1-H4) were independently idealized using fragment-assembly from a clustered ideal fragment database (size: 4 a.a.); *bottom*: in the second generation of designs the core elements were instead built using parametric equations that recapitulate the shape of each disembodied helix, allowing changes in the length of each helix by +/- 8 a.a. ; **FIG. 1B)** Pairs of helices were reconnected using ideal loop fragments (size: 4 a.a. or 7 a.a., for gen-1 and gen-2 respectively, see Methods), representative examples are shown with newly built elements connecting each pair of helices; **FIG. 1C)** The helix hairpins generated in FIG. 1B were assembled in all possible combinations to generate fully connected protein backbones; **FIG. 1D)** The designs and experimentally matured versions were tested for binding by yeast display, and those exhibiting high affinity binding were recombinantly expressed (*E. coli*) and tested for binding using surface plasmon resonance and IL-2 like phospho-STAT5 (pSTAT5) signaling. The results for 3 designs of the first generation and 10 designs from the second generation are shown in the 2D-plot in solid symbols. The open star is Neoleukin-2/15, the arrow originates in its parent (unoptimized) design.

Figure 2A-2C. Characterization of neoleukin-2/15. FIG. 2A) From *top* to *bottom*: In surface plasmon resonance experiments, neoleukin-2/15 does not bind human or murine IL-2Rα, but binds both human and murine IL-2Rβ with similar affinity (K_d ~ 11.2 nM and 16.1 nM, for human and mice receptor, respectively). Like natural IL-2, neoleukin-2/15 binds poorly to the γ_c receptor, and exhibits cooperative binding for both human and murine IL-2Rβγ_c (K_d ~ 18.8 nM and 38.4 nM, for the human and mice heterodimeric receptor, while the Kd of native hIL-2 and Super-2 are ~ 193.6 nM and 300.9 nM, see Table E1). **FIG. 2B)** *top*: *In-vitro* pSTAT5 signaling studies demonstrate that neoleukin-2/15 elicits IL-2-like signaling in human cells (EC_{50}), and activates with ~identical potency (EC_{50} ~ 73.0 pM and 49.2 pM on CD25+ and CD25- cells, respectively) human YT-1 NK cells with or without IL-2Rα expression (CD25); *bottom*: similarly *ex vivo* experiments in murine CD4+ primary cells demonstrate that neoleukin-2/15 can also elicit potent IL-2 like signaling in murine cells, and is independent of IL-2Rα expression (EC_{50} ~ 24 pM and 129 pM on CD25+ and CD25- cells, respectively); **FIG. 2C)** *top*: binding experiments (OCTET) show that neoleukin-2/15 can be

incubated for 2 hours at 80°C without any noticeable loss of binding, whereas human and murine IL-2 quickly lose activity; *bottom*: an *ex vivo* experiment on cultured murine splenocytes that require IL-2 for survival, demonstrates that neoleukin-2/15 incubated at 95°C for 1 hour still drives cell survival effectively (~70% relative luminescence, at 10 ng/ml), while mIL2 and Super-2 are virtually inactive (~10% and 0.1%, respectively at 10 ng/ml).

Figure 3A-3E. Structure of neoleukin-2/15 (Neo-2/15) and its ternary complex with mIL-2Rβγc. FIG. 3A) *Top*: structural alignment of neoleukin-2/15 (Neo-2/15) chain A with the design model (r.m.s.d. 1.11 Å for 100 Cα atoms); *bottom*: detail of interface helices H1, H3 and H4 (numbered according to hIL-2, see Fig 1). The interface side chains are shown in sticks; **FIG. 3B)** crystallographic structure of the ternary complex of Neo-2/15 with mIL-2Rβ and γ_c (r.m.s.d 1.27 Å for the 93 modeled Cα atoms of Neo-2/15 in the ternary complex); **FIG. 3C)** structural alignment of monomeric Neo-2/15 (chain A) with Neo-2/15 in the ternary complex (r.m.s.d 1.71 Å for the 93 modeled Cα atoms in the ternary complex). Helix H4 shows an approximately 4.0 + shift of helix H4 in the ternary-complex structure compared to the monomeric crystal structure; **FIG. 3D)** crystallographic structure of: hIL-2 (cartoon representation). The regions that interact with the IL-2Rβ and γ_c are denoted. The loop-rich region from hIL-2 that interacts with IL-2Rα does not exist in the *de novo* mimetic Neo-2/15. **FIG. 3E)**: crystallographic structure of neoleukin-2/15 from the ternary complex in "b)" (cartoon representation). The regions that interact with the IL-2Rβ and γ_c are denoted. The loop-rich region from hIL-2 that interacts with IL-2Rα does not exist in the *de novo* mimetic Neo-2/15.

Figure 4A-4G. Immunogenicity, immunostimulatory and therapeutic activity of neoleukin-2/15. FIG. 4A) Dose escalation effect of neoleukin-2/15 (Neo-2/15) in naive mice T cells. Naive C57BL/6 mice were treated daily with neoleukin-2/15 or mIL-2 at the indicated concentrations (n=2-3 per group). After 14 days, spleens were harvested and analyzed by flow cytometry using the indicated markers. The bar plot shows that mIL-2 enhanced CD4+ Treg expansion in a dose dependent fashion, while Neo-2/15 had little or no effect in expansion of Treg cells. Neoleukin-2/15 drove a higher CD8+:Treg ratio compared to mIL-2; **FIG. 4B)** Effect of Neo-2/15 in mice in an airway inflammation model (20 µg/day/mouse, 7 days). Similar to naive mice, Neo-2/15 does not increase the frequency of antigen-specific CD4+ Foxp3+ T_{regs} in the lymphoid organs, and is comparably effective to mIL-2 in increasing the frequency of lung resident (Thy1.2- by intravascular labeling) CD8+ T cells; **FIG.4C)** Neoleukin-2/15 does not have detectable immunogenicity. C57BL/6 mice

were inoculated with $5x10^5$ B16F10 cells by subcutaneous injection. Starting on day 1, mice were treated daily with neoleukin-2/15 (10 μg) or equimolar mIL-2 by intraperitoneal (i.p.) injection (n=10 for each group). After 14 days, serum (antiserum) was collected and IgG was detected by ELISA in plates coated with fetal bovine serum (FBS 10%, negative control), neoleukin-2/15, mIL-2, hIL-2, or Ovalbumin (OVA) as negative control (the dotted line shows the average of the negative control). Anti-Neo-2/15 polyclonal antibody was used as positive control (black, n=2) and did not cross react with mIL-2 or h-IL2; **FIG. 4D)** C57BL/6 mice were immunized with 500 μg KO Neo-2/15 in complete Freund's adjuvant and boosted on days 7 and 15 with 500 μg KO Neo-2/15 in incomplete Freund's adjuvant. Reactivity against KO Neo-2/15 and native Neo-2/15, as well as cross-reactivity with mouse IL-2 were determined by incubation of serum (diluted 1:1,000 in PBS) with plate-bound KO Neo-2/15, Neo-2/15 or mouse IL-2 as indicated. Serum binding was detected using an anti-mouse secondary antibody conjugated to HRP followed by incubation with TMB. Data are reported as optical density at 450 nm. *Top*, naive mouse serum; *bottom*, immunized mouse serum. **FIG. 4E-4G)** Therapeutic efficacy of Neoleukin-2/15: **FIG. 4E)** BALB/C mice were inoculated with CT26 tumors. Starting on day 6, mice were treated daily with i.p. injection of mIL-2 or neoleukin-2/15 (10 μg), or were left untreated (n = 5 per group). Tumor growth curves (*top*, show only data for surviving mice). Survival curves (*bottom*). Mice were euthanized when weight loss exceeded 10% of initial weight or when tumor size reached 1,300 mm^3. **FIG. 4F)** C57BL/6 mice were inoculated with B16 tumors as in "a)". Starting on day 1, mice were treated daily with i.p. injection of neoleukin-2/15 (10 μg) or equimolar mIL-2 (n = 10 per group). Twice-weekly treatment with TA99 was added on day 3. Mice were euthanized when weight loss exceeded 10% of initial weight or when tumor size reached 2,000 mm^3. Tumor growth curves (*top* and *bottom left*). Survival curve, inset shows average weight change (*top right*). Quantification of cause of death (*bottom right*). **FIG. 4G)** Neo-2/15 elicits a higher CD8+:Treg ratio than mouse IL-2. C57BL/6 mice were inoculated with B16 tumors and treated by daily i.p. injection as indicated. Treatment with TA99 (*bottom plot*) was started on day 5 and continued twice-weekly. Tumors were harvested from mice when they reached 2,000 mm^3 and analyzed by flow cytometry. The CD8:Treg cell ratio was calculated by dividing the percentage $CD45^+$ $CD3^+$ cells that were $CD8^+$ by the percentage that were $CD4^+$ $CD25^+$ $FoxP3^+$.

Figure 5A-5D. Therapeutic effect of neoleukin-2/15 on colon cancer. FIG. 5A) BALB/C mice were inoculated with CT26 tumors. Starting on day-9 and ending on day-14, mice were treated daily with i.p. injection of mIL-2 or neoleukin-2/15 at the specified

concentrations, or were left untreated (n = 5 per group). Tumor growth curves (*top*, show only data for surviving mice). Survival curves (*bottom*). Mice were euthanized when weight loss exceeded 10% of initial weight or when tumor size reached 1,300 mm^3. **FIG. 5B-5D)** The bar-plots compare the T cell populations for BALB/C mice (n=3 per group) that were inoculated with CT26 tumors and treated starting from day-6 with by daily i.p. injection of 10μg of Neolukin-2/15 or 10μg mIL-2 or no-treatment (No Tx). On day-14 the percentage of Treg cells ($CD4^+$ $CD45^+$ $FoxP3^+$, *top* graph) and CD8:Treg cell ratio (($CD45^+$ $CD3^+$ $CD8^+$)/ Treg, *bottom* graph) was assessed in: **FIG. 5B)** tumors, **FIG. 5C)** neighboring inguinal lymph node (LN), and **FIG. 5D)** spleen.

Figure 6A-6D. Therapeutic effect of neoleukin-2/15 on melanoma. FIG. 6A-6E) Tumor growth curves (*bottom*) and survival curves (*top)* for C57BL/6 mice that were inoculated with B16 tumors and treated with low (1 μg/mice/day, a-b) or high doses of neoleukin-2/15 (10 μg/mice/day, c-d). Starting on day 1, mice (n = 5 per group) were treated daily with i.p. injection of **FIG. 6A):** single agent neoleukin-2/15 at 1 μg/mice or equimolar mIL-2 (n = 5 per group), or **FIG. 6B):** the same treatments in combination with a twice-weekly treatment with TA99 (started on day 5). Mice were euthanized when tumor size reached 2,000 mm^3. C57BL/6 mice were inoculated with B16 tumors and treated by daily i.p. injection as indicated. **FIG. 6C-6D)** Similar to "a-b)", but starting on day 4, mice were treated daily with i.p. injection of 10 μg/mouse of neoleukin-2/15, or equimolar mIL-2, either alone **FIG. 6C)** or in combination with twice-weekly TA99 started on day 4 **FIG. 6D)**. Mice were euthanized when tumor size reached 2,000 mm^3. The therapeutic effect of Neoleukin-2/15 is dose dependent (higher doses are better) and is potentiated in the presence of the antibody TA99. The experiments were performed once. In all the growth curves, data are mean ± s.e.m. Results were analysed by one-way ANOVA (95% confidence interval), except for survival curves that were assessed using the Mantel–Cox test (95% confidence interval).

Figure 7A-7C. Reengineering of neoleukin-2/15 into a human interleukin-4 (hIL-4) mimetic (neoleukin-4). FIG. 7A) Neo-2/15 structurally aligned into the structure of IL-4 in complex with IL-4Rα and γ_c (from PDB code 3BPL). Fourteen IL-4 residues that contact IL-4Rα and that were grafted into Neo-2/15 are labeled. **FIG. 7B)** Neoleukin-4 (Neo-4), a new protein with sixteen amino acid mutations compared to Neo-2/15. These mutations are labeled; thirteen of these were derived from the IL-4 residues depicted in panel "a)" that mediate contact with IL-4Rα, and three of them (H8M, K68I and I98F, underlined in the figure) were introduced by directed evolution using random mutagenesis and screening for high binding affinity variants. **FIG. 7C)** Biolayer interferometry data showing that Neo-4,

like IL-4, binds to IL-4Rα alone, has no affinity for γ_c alone, but binds to γ_c when IL-4Rα is present in solution.

Figure 8A-8B. Stimulatory effect of Neoleukin-2/15 on human CAR-T cells. FIG. 8A) Anti-CD3/CD28 stimulated or **FIG. 8B)** unstimulated human primary CD4 (*top*) or CD8 (*bottom*) T cells were cultured in indicated concentrations of human IL2 or neoleukin-2/15. T cell proliferation is measured as fold change over T cells cultured without IL2 supplement. Neo-2/15 is as effective as native IL-2 at inducing proliferation of stimulated CAR-T cells, and more effective than native IL-2 at inducing proliferation of unstimulated CAR-T cells, particularly of unstimulated CD8 CAR-T cells.

Figure 9A-9D. Overall sequence conservation in binding residues for each of the four common helices, combining information from three different de novo-designed IL-2 mimics. Sequence logos were generated using combined data from binding experiments (using the heterodimeric mouse IL-2Rβγc) from three independent SSM mutagenesis libraries for G2_neo2_40_1F_seq27, G2_neo2_40_1F_seq29 and G2_neo2_40_1F_seq36 (Figs. 11-13). All of these proteins are functional high-affinity *de novo* mimetics of mouse and human IL-2, some having topologies that differ from that of Neo-2/15, but all containing the four Helices H1 (**FIG. 9A**; Neo-2/15 1-22 is SEQ ID NO:248, IL-2 6-27 is SEQ ID NO:249, IL-15 1-15 is SEQ ID NO:250), H3 (**FIG. 9B**; Neo-2/15 34-55 is SEQ ID NO:251, IL-2 82-103 is SEQ ID NO:252, IL-15 59-80 is SEQ ID NO:253), H2′ (**FIG. 9C**; Neo-2/15 58-76 is SEQ ID NO:254, IL-2 50-68 is SEQ ID NO:255, IL-15 34-52 is SEQ ID NO:256) and H4 (**FIG. 9D**; Neo-2/15 80-100 is SEQ ID NO:257, IL-2 111-131 is SEQ ID NO:258, IL-15 93-113 is SEQ ID NO:259). The logos show the combined information for each helix independently. Below each logo, a line graph shows the probability score (higher means more conserved) for each amino acid in the Neo-2/15 sequence. The solid horizontal line highlights positions where the Neo-2/15 amino acid has a probability score ≥30% (that is, these amino acids contribute more generally to receptor binding as they are globally enriched in the binding populations across all of the de novo IL-2 mimics tested). The topology of each helix in Neo-2/15 is shown left of each logo. The sequences of the Neo-2/15 helices and those of the corresponding helices (structurally aligned) in human IL-2 and IL-15 are shown below the graphs, highlighting the distinctiveness of the Neo-2/15 helices and binding interfaces.

Figure 10A-10D. Experimental optimization of G1_neo2_40. FIG. 10A-10C) Heatmaps for G1_neo2_40 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of increasing selection with **FIG. 10A)** 50 nM, **FIG. 10B)** 2 nM, and **FIG. 10C)** 0.1 nM IL-2Rβγ_c heterodimer. Based on these enrichment data, a

combinatorial library was designed with nucleotide diversity 1.5×10^6. **FIG. 10D)** Amino acid residues available in the initial combinatorial library are depicted indicating residues predicted to be advantageous (shown above the original sequence) and deleterious (shown below the original sequence; in the depiction of the original sequence, black indicates residues that are represented in the combinatorial library and gray, residues not represented in the combinatorial library.

Figure 11A-11E. Experimental optimization of G2_neo2_40_1F_seq27. Heatmaps for G2_neo2_40_1F_seq27 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of increasing selection with **FIG. 11A)** 10 nM, **FIG. 11B)** 1 nM, **FIG. 11C)** 0.1 nM, and **FIG. 11D)** 0.1 nM IL-2Rβγ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 5.3×10^6. **FIG. 11E)** Amino acid residues available in the initial combinatorial library are depicted indicating residues predicted to be advantageous; black indicates residues in the starting sequence represented in the combinatorial library.

Figure 12A-12E. Experimental optimization of G2_neo2_40_1F_seq29. Heatmaps for G2_neo2_40_1F_seq29 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of increasing selection with **FIG. 12A)** 10 nM, **FIG. 12B)** 1 nM, **FIG. 12C)** 0.1 nM, and **FIG. 12D)** 0.1 nM IL-2Rβγ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 2.9×10^6. **FIG. 12E)** Amino acid residues available in the initial combinatorial library are depicted indicating residues predicted to be advantageous; black indicates residues in the starting sequence represented in the combinatorial library.

Figure 13A-13E. Experimental optimization of G2_neo2_40_1F_seq36. Heatmaps for G2_neo2_40_1F_seq36 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of increasing selection with **FIG. 13A)** 10 nM, **FIG. 13B)** 1 nM, **FIG. 13C)** 0.1 nM, and **FIG. 13D)** 0.1 nM IL-2Rβγ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 2.7×10^6. **FIG. 13E)** Amino acid residues available in the initial combinatorial library are depicted indicating residues predicted to be advantageous; black indicates residues in the starting sequence represented in the combinatorial library.

Figure 14A-14B. Circular Dichroism (CD) thermal denaturation experiments for multiple IL-2/IL-15 *de novo* designed mimetics, generation-1. **FIG. 14A)** Thermal denaturation curves and **FIG. 14B)** wavelength scans.

Figure 15A-15B. Circular Dichroism (CD) thermal denaturation experiments for multiple IL-2/IL-15 de novo designed mimetics, generation-1 experimentally optimized. **FIG. 15A)** Thermal denaturation curves and **FIG. 15B)** wavelength scans.

Figure 16A-16D. Circular dichroism thermal melts for IL-2/IL-15 mimetic designs generation-2. **FIG. 16A** and **FIG. 16C)** Thermal denaturation curves and **FIG. 16B** and **FIG. 16D)** wavelength scans.

Figure 17A-17C. Expression, purification, and thermal denaturation characterization of neoleukin-2/15. FIG. 17A) SDS Tris-Tricine gel electrophoresis showing expression and purification over affinity column. **FIG. 17B)** Circular dichroism at 222 nm during thermal melting from 25°C to 95°C, showing robust temperature stability. **FIG. 17C)** Circular dichroism wavelength scans at 25°C, 95°C and then again 25°C, showing that neoleukin-2/15 does not fully melt at 95°C and refolds fully after cooling back to 25°C.

Figure 18A-18D. Single disulfide stapled variants of neoleukin-2/15 with higher thermal stability. Structural model of disulfide stabilized variants of Neoleukin-2/15 are shown with positions of the mutated residues labeled and the disulfide bond shown. Two strategies were used to generated the disulfide variants: **FIG. 18A)** internal placement at residues 38 and 75 and terminal linkage; **FIG. 18B)** for the terminal variant, three residues were added to each terminus in order to limit any distortions to the starting structure that would otherwise be required to form the disulfide bond. CD spectra at 25°C, 95°C and 25°C after cooling for the internal and terminal disulfide variants are shown below their structural models. Both variants show very little signal loss at 95°C and complete refolding upon cooling; **FIG. 18C)** thermal melts of each variant were performed by monitoring CD signal at 222.0 nm over a range of temperatures. Each of the disulfide variants shows improved stability relative the native; **FIG. 18D)** binding strength of each variant to IL-2Rβγc was measured by biolayer interferometry. Contrary to disrupting the binding interaction, these data show the introduction of the disulfide bond improves the binding of the mimetics to IL-2Rβγc. Both disulfide-bonded variants exhibit an improvement in binding IL-2Rβγc (Kd ~ 1.3 ± 0.49 and 1.8 ± 0.26 nM, for the internal and external disulfide-staples, respectively, compared to 6.9 ± 0.61 nM for Neo-2/15 under the same experimental conditions), which is consistent with the expected effect of disulfide-induced stabilization of the protein's binding site.

Figure 19A-19B. Robustness of neoleukin-2/15 to single-point cysteine mutants on non-binding interface positions. FIG. 19A) Schematic showing point mutant positions in neolukin-2/15 that can individually be mutated to cysteine without interfering with

expression of the protein or binding to IL-2Rβγc. Positions were chosen to avoid interference with receptor binding. **FIG. 19B)** Association kinetics of Neolukin-2/15 cysteine mutants with IL-2Rβγc measured using biolayer interferometry. All of the variants associate with receptor approximately similarly to Neo-2/15.

Figure 20A-20C. Expression, purification, and thermal denaturation characterization of neoleukin-4. FIG. 20A) SDS Tris-Tricine gel electrophoresis showing expression and purification over affinity column. **FIG. 20B)** Circular dichroism at 222 nm during thermal melting from 25°C to 95°C, showing robust temperature stability. **FIG. 20C)** Circular dichroism wavelength scans at 25°C, 95°C and then again 25°C, showing that neoleukin-4 does not fully melt at 95°C and refolds fully after cooling back to 25°C.

Figure 21A-21D. Cytokine levels in non-human primates response to Neo-2/15 or Neo-2/15-PEG. Two non-human primates (NHP) per group, one male and one female per group, were assigned to treatment with either vehicle, Neo-2/15 or Neo-2/15-PEG (comprising Neo-2/15 with a single cysteine mutation of E62C conjugated to PEG40K). Animals were administered either 0 (vehicle), 0.1, 0.2 or 0.3 mg/kg of Neo-2/15, or 0.05, 0.10 or 0.15 mg/kg of Neo-2/15-PEG, by intravenous bolus. Animals treated with Neo-2/15 PEG were administered by intravenous bolus. Cytokine samples were taken 0, 4, 8 and 24 hours post dose. Cytokine serum samples were prepared and frozen at <-70°C and shipped for analysis where samples were analyzed through a Luminex multiplex immunoassays system. Several cytokines, including IL-10 **(FIG. 21A-21B)** and IL-15 **(FIG.21C-21D)** demonstrated marked differences in the time course of cytokine production, consistent with a more sustained pharmacodynamic effect for the PEGylated molecule.

Figure 22: A block diagram of an example computing network.

Figure 23A: A block diagram of an example computing device.

Figure 23B: A block diagram of an example network of computing devices arranged as a cloud-based server system.

Figure 24: A flowchart of a method.

Detailed Description

As used herein and unless otherwise indicated, the terms "a" and "an" are taken to mean "one", "at least one" or "one or more". Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to". Words using the singular or plural number also include the plural or singular number, respectively. Additionally, the words "herein," "above" and "below" and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of this application.

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

In one aspect, the invention provides non-naturally occurring polypeptides comprising domains X1, X2, X3, and X4, wherein:

(a) X1 is a peptide comprising the amino acid sequence at least 25% identical to EHALYDAL (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence at least 25% identical to YAFNFELI (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence at least 25% identical to ITILQSWIF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$) IL-4 receptor $\alpha\gamma_c$ heterodimer (IL-4R$\alpha\gamma_c$), or IL-13 receptor α subunit (IL-13Rα). In various embodiments, the polypeptides bind IL-2R$\beta\gamma_c$ or IL-4R$\alpha\gamma_c$ with a binding affinity of 200 nM or less, 100 nM or less, 50 nM or less or 25 nM or less.

In one aspect, the invention provides non-naturally occurring polypeptides comprising domains X1, X2, X3, and X4, wherein:

(a) X1 is a peptide comprising the amino acid sequence at least 85% identical to EHALYDAL (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence at least 85% identical to YAFNFELI (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence at least 85% identical to ITILQSWIF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$). In various embodiments, the polypeptides bind IL-2R$\beta\gamma_c$ with a binding affinity of 200 nM or less, 100 nM or less, 50 nM or less or 25 nM or less.

In one aspect, the invention provides non-naturally occurring polypeptides comprising domains X1, X2, X3, and X4, wherein:

(a) X1 is a peptide comprising the amino acid sequence IHALYDAL (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence YAFNFELI (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence ITILQSWIF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$). In various embodiments, the polypeptides bind IL-2R$\beta\gamma_c$ with a binding affinity of 200 nM or less, 100 nM or less, 50 nM or less or 25 nM or less.

As shown in the examples that follow, the polypeptides of the disclosure are (a) mimetics of IL-2 and interleukin-15 (IL-15) that bind to the IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$), but have no binding site for IL-2Rα or IL-15Rα, or (b) mimetics of IL-4 that bind to the IL-4 receptor $\alpha\gamma_c$ heterodimer (IL-4R$\alpha\gamma_c$) or IL-13 receptor α subunit (IL-13Rα) (natural IL-4 and the IL-4 mimetics described herein cross-react with IL-13 receptor, forming an IL-4Rα/IL13Rα heterodimer). The designs are hyper-stable, bind to human and mouse IL-2R$\beta\gamma_c$ or IL-4R$\alpha\gamma_c$ with higher affinity than the natural cytokines, and elicit downstream cell signaling independent of IL-2Rα and IL-15Rα, or independent of IL-13Rα. The polypeptides can be used, for example, to treat cancer.

The term protein mimetic as used herein refers to a protein that imitates certain aspects of the function of another protein. The two proteins typically have different amino acid sequence and/or different structures. Provided herein, among other things, are de novo mimetics of IL-2 and IL-15. The aspects of the function of IL-2 and IL-15 that these mimetics imitate is the induction of heterodimerization of IL-2RβγC, leading to phosphorylation of STAT5. Because IL-2 and IL-15 both signal through heterodimerization of IL-2RβγC, these mimetics imitate this biological function of both IL-2 and IL-15. These mimetics may be referred to herein as mimetics of IL-2, of IL-15, or of both IL-2 and IL-15.

Also provided are de novo mimetics of IL-4. These mimetics are capable of imitating certain functions of IL-4. The function of IL-4 that these mimetics imitate is the induction of heterodimerization of IL-4Rαγ_c (and/or heterodimerization of IL-4Rα/IL-13Rα).

Native hIL-2 comprises four helices connected by long irregular loops. The N-terminal helix (H1) interacts with both the beta and gamma subunits, the third helix (H3) interacts with the beta subunit, and the C-terminal helix (H4) with the gamma subunit; the alpha subunit interacting surface is formed by the irregular second helix (H2) and two long loops, one connecting H1 to H2 and the other connecting H3 and H4. Idealized proteins were designed and produced in which H1, H3 and H4 are replaced by idealized structural domains, including but not limited to helices and beta strands (referred to as domains X1, X3 and X4, respectively) displaying an IL-2Rβγ_c or IL-4Rαγ_c interface inspired by H1, H3 and H4, and in which H2 is replaced with an idealized helix (referred to as domain X2) that offers better packing. As shown in the examples, extensive mutational studies have been carried out, demonstrating that the amino acid sequence of each peptide domain each can be extensively modified without loss of binding to the IL-2 or IL-4 receptor, and that the domains can be placed in any order while retaining binding to the IL-2 or IL-4 receptor. The polypeptides may comprise L amino acids and glycine, D- amino acids and glycine, or combinations thereof.

Thus, X1, X2, X3, and X4 may be in any order in the polypeptide; in non-limiting embodiments, the ordering may be X1-X2-X3-X4; X1-X3-X2-X4; X1-X4-X2-X3; X3-X2-X1-X4; X4-X3-X2-X1; X2-X3-X4-X1; X2-X1-X4-X3; etc.

The domains may be separated by amino acid linkers of any length of amino acid composition. There is no requirement for linkers; in one embodiment there are no linkers present between any of the domains. In other embodiments, an amino acid linker may be present between 1, 2, or all 3 junctions between domains X1, X2, X3, and X4. The linker may be of any length as deemed appropriate for an intended use.

In various embodiments, X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:1. In other embodiments, X3 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%m 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:2. In further embodiments, X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:3.

In one embodiment, the polypeptides are IL-2/15 mimetics and (i) X1 includes one or both of the following: H at residue 2 and Y at residue 5; and/or (ii) X3 includes 1, 2, 3, 4, or all 5 of the following: Y at residue 1, F at residue 3, N at residue 4, L at residue 7, and I at residue 8. In a further embodiment, (iii) X4 includes I at residue 8.

In another embodiment, the polypeptides are IL-4 mimetics, and (i) X1 includes E at residue 2 and K at residue 5; and (ii) X3 includes F at residue 1, K at residue 3, R at residue 4, R at residue 7, and N at residue 8. In a further embodiment, (iii) X4 includes F at residue 8.

In all of these embodiments, X1, X3, and X4 may be any suitable length, meaning each domain may contain any suitable number of additional amino acids other than the peptides of SEQ ID NOS:1, 2, and 3, respectively. In one embodiment, X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide PKKKIQLHA[H]A[L,Y][I]A[L]MILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length the peptide [L]ED[Y]AF[N]FE[L][L]EE[A]RLFESG (SEQ ID NO:5); and X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide EDEQEEMANAI[T]IL[Q]S[W]IFS(SEQ ID NO:6).

In one embodiment, X1 is a peptide comprising the amino acid sequence at least 80% identical along its length to the peptide PKKKIQLHA[H]A[L,Y][I]A[L]MILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence at least 80% identical along its length the peptide [L]ED[Y]AF[N]FE[L][L]EE[A]RLFESG (SEQ ID NO:5); and X4 is a peptide comprising

the amino acid sequence at least 80% identical along its length to the peptide EDEQEEMANAIITILQSWIFS(SEQ ID NO:6).

In one embodiment, X1 is a peptide comprising the amino acid sequence at least 85% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence at least 85% identical along its length the peptide LEDYAFNFELILEEIARLFESG (SEQ ID NO:5); and X4 is a peptide comprising the amino acid sequence at least 85% identical along its length to the peptide EDEQEEMANAIITILQSWIFS(SEQ ID NO:6).

In one embodiment, X1 is a peptide comprising the amino acid sequence at least 90% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence at least 90% identical along its length the peptide LEDYAFNFELILEEIARLFESG (SEQ ID NO:5); and X4 is a peptide comprising the amino acid sequence at least 90% identical along its length to the peptide EDEQEEMANAIITILQSWIFS(SEQ ID NO:6).

In one embodiment, X1 is a peptide comprising the amino acid sequence at least 95% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence at least 95% identical along its length the peptide LEDYAFNFELILEEIARLFESG (SEQ ID NO:5); and X4 is a peptide comprising the amino acid sequence at least 95% identical along its length to the peptide EDEQEEMANAIITILQSWIFS(SEQ ID NO:6).

In one embodiment, X1 is a peptide comprising the amino acid sequence 100% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4); X3 is a peptide comprising the amino acid sequence 100% identical along its length to the peptide LEDYAFNFELILEEIARLFESG (SEQ ID NO:5); and X4 is a peptide comprising the amino acid sequence 100% identical along its length to the peptide EDEQEEMANAIITILQSWIFS(SEQ ID NO:6).

In one embodiment, the polypeptides are IL-2/15 mimetics and (i) X1 includes 1, 2, 3, 4, or all 5 of the following: L at residue 7, H at residue 8, H at residue 11, Y at residue 14; M at residue 18; and/or (ii) X3 includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following: D at residue 3, Y at residue 4, F at residue 6, N at residue 7, L at residue 10, I at residue 11, E at residue 13, or E at residue 14. In a further embodiment, (iii) X4 includes I at residue 19.

In one embodiment of IL-2 mimetics, amino acid substitutions relative to the reference peptide domains (i.e.: SEQ ID NOS: 1, 2, 3, 4, 5, or 6) do not occur at AA residues marked in bold font.

In another embodiment, the polypeptides are IL-4/IL-13 mimetics, and

X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide PKKKIQIMA**EEALKDAL**SILNI (SEQ ID NO: 8);

X3 is a peptide comprising the amino acid sequence at least 37% 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length the peptide LER**FAKRFERN**LWGIARLFESG (SEQ ID NO: 9); and

X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide EDEQEEMANA**IITILQSWFFS** (SEQ ID NO: 10).

wherein

(i) X1 includes I at residue 7, T or M at residue 8, E at residue 11, K at residue 14 and S at residue 18; and

(ii) X3 includes R at residue 3, F at residue 4, K at residue 6, R at residue 7, R at residue 10, N at residue 11, W at residue 13, and G at residue 14.

In a further embodiment, (iii) X4 includes F at residue 19.

In one embodiment, amino acid substitutions relative to the reference peptide domains are conservative amino acid substitutions. As used herein, "conservative amino acid substitution" means a given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g. antigen-binding activity and specificity of a native or reference polypeptide is retained. Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic:

Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into H is; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

In one embodiment, amino acid residues in X1 relative to SEQ ID NO:4 are selected from the group consisting of:

Position 01:	A	F	I	L	M	P	Q	R	S	W
Position 02:	A	D	E	G	V	K				
Position 03:	D	E	F	W	K					
Position 04:	D	E	K	N	P	R	W			
Position 05:	D	E	H	I	K	L	M	S		
Position 06:	A	D	E	G	L	P	S	W	Q	
Position 07:	D	E	L	Q	Y	I				
Position 08:	A	F	H	W	Y	M	T			
Position 09:	C	F	P	A						
Position 10:	C	D	E	F	K	P				
Position 11:	D	F	H	E						
Position 12:	A	D	E	P	S	T	V			
Position 13:	H	I	L	M	P	R	V	W		
Position 14:	F	R	W	Y	K					
Position 15:	D	E	N	Y						
Position 16:	A	C	L	M	S					
Position 17:	F	I	L	M	P	R				
Position 18:	G	M	Q	Y	S					
Position 19:	I	L	M	P	Q	V				
Position 20:	A	K	L	M	Q	R	S			
Position 21:	G	K	N	P	R	S	W			
Position 22:	D	E	I	K	M	N	W	Y		

In one embodiment the polypeptides are IL-4 mimetics, and position 7 is I, position 8 is M or T, position 11 is E, position 14 is K, and position 18 is S.

In another embodiment the polypeptides are IL-2 mimetics, and 1, 2, 3, 4, or 5 of the following are not true: position 7 is I, position 8 is M or T, position 11 is E, position 14 is K, and position 18 is S.

In another embodiment, amino acid residues in X3 relative to SEQ ID NO:5 are selected from the group consisting of:

Position 01:	A	L								
Position 02:	D	E	G	K	M	T				
Position 03:	D	E	N	Y	R					
Position 04:	C	D	G	T	Y	F				
Position 05:	A	F	H	S	V	W	Y			
Position 06:	A	F	I	M	T	V	Y	K		
Position 07:	D	K	N	S	T	R				
Position 08:	A	C	G	L	M	S	V	F		
Position 09:	C	H	K	L	R	S	T	V	E	
Position 10:	F	I	L	M	Y	R				
Position 11:	I	L	N	T	Y					
Position 12:	F	K	L	M	S	V				
Position 13:	A	D	F	G	I	N	P	Q	S	T	E	W
Position 14:	A	E	F	G	H	S	V			
Position 15:	C	I	L	M	V	W				
Position 16:	A	D	G	S	T	V				
Position 17:	H	K	L	N	R					
Position 18:	C	D	G	I	L	Q	R	T	W	
Position 19:	D	F	M	N	W					
Position 20:	A	C	E	F	G	M	S	Y		
Position 21:	D	E	G	H	L	M	R	S	T	V	W
Position 22:	A	D	G	K	N	S	Y			

In another embodiment, the polypeptides are IL-4/IL-13 mimetics and position 3 is R, position 4 is F, position 6 is K, position 7 is R, position 10 is R, position 11 is N, position 13 is W, and position 14 is G.

In another embodiment, the polypeptides are IL-2 mimetics and 1, 2, 3, 4, 5, 6, 7, or all 8 of the following are not true: position 3 is R, position 4 is F, position 6 is K, position 7 is R, position 10 is R, position 11 is N, position 13 is W, and position 14 is G.

In any of such embodiments, the polypeptide further allows for a cysteine at position 17 relative to SEQ ID NO:5 in addition to the amino acid residues of H, K, L, N and R. Accordingly, amino acid residues in X3 relative to SEQ ID NO:5 can be selected from the group consisting of:

Position 01:	A	L									
Position 02:	D	E	G	K	M	T					
Position 03:	D	E	N	Y	R						
Position 04:	C	D	G	T	Y	F					
Position 05:	A	F	H	S	V	W	Y				
Position 06:	A	F	I	M	T	V	Y	K			
Position 07:	D	K	N	S	T	R					
Position 08:	A	C	G	L	M	S	V	F			
Position 09:	C	H	K	L	R	S	T	V	E		
Position 10:	F	I	L	M	Y	R					
Position 11:	I	L	N	T	Y						
Position 12:	F	K	L	M	S	V					
Position 13:	A	D	F	G	I	N	P	Q	S	T	E W
Position 14:	A	E	F	G	H	S	V				
Position 15:	C	I	L	M	V	W					
Position 16:	A	D	G	S	T	V					
Position 17:	H	K	L	N	R	C					
Position 18:	C	D	G	I	L	Q	R	T	W		
Position 19:	D	F	M	N	W						
Position 20:	A	C	E	F	G	M	S	Y			
Position 21:	D	E	G	H	L	M	R	S	T	V	W
Position 22:	A	D	G	K	N	S	Y				

In another embodiment, amino acid residues in X4 relative to SEQ ID NO:6 are selected from the group consisting of:

Position 01:	D	E	G	K	V				
Position 02:	D	I	M	S					
Position 03:	E	G	H	K					
Position 04:	E	G	I	K	Q	R	S		
Position 05:	A	D	E	G	H	S	V		
Position 06:	C	D	E	G	I	M	Q	R	T V

Position 07:	C	E	L	M	P	R	T		
Position 08:	A	F	L	M	W				
Position 09:	A	G	L	N	Q	R	T		
Position 10:	A	C	D	E	F	H	I	W	
Position 11:	I	M	N	S	V	W			
Position 12:	I	K	L	S	V				
Position 13:	C	L	M	R	S	T			
Position 14:	I	L	P	T	Y				
Position 15:	F	G	I	L	M	N	V		
Position 16:	H	K	Q	R					
Position 17:	C	F	K	S	W	Y			
Position 18:	K	Q	T	W					
Position 19:	C	G	N	I					
Position 20:	C	F	G	L	Y				
Position 21:	A	F	G	H	S	Y			

In another embodiment, the polypeptides are IL-4/IL-13 mimetics and position 19 is I. In another embodiment, the polypeptides are IL-2 mimetics and position 19 is not I.

In any of such embodiments, the polypeptide further allows for a cysteine at position 3 relative to SEQ ID NO:6 in addition to the amino acid residues of E, G, H and K.

Accordingly, amino acid residues in X4 relative to SEQ ID NO:6 can be selected from the group consisting of:

Position 01:	D	E	G	K	V				
Position 02:	D	I	M	S					
Position 03:	E	G	H	K	C				
Position 04:	E	G	I	K	Q	R	S		
Position 05:	A	D	E	G	H	S	V		
Position 06:	C	D	E	G	I	M	Q	R	T	V
Position 07:	C	E	L	M	P	R	T		
Position 08:	A	F	L	M	W				
Position 09:	A	G	L	N	Q	R	T		
Position 10:	A	C	D	E	F	H	I	W	
Position 11:	I	M	N	S	V	W			
Position 12:	I	K	L	S	V				
Position 13:	C	L	M	R	S	T			

Position 14:	I	L	P	T	Y		
Position 15:	F	G	I	L	M	N	V
Position 16:	H	K	Q	R			
Position 17:	C	F	K	S	W	Y	
Position 18:	K	Q	T	W			
Position 19:	C	G	N	I			
Position 20:	C	F	G	L	Y		
Position 21:	A	F	G	H	S	Y	

As noted herein, domain X2 is a structural domain, and thus any amino acid sequence that connects the relevant other domains (depending on domain order) and allows them to fold can be used. The length required will depend on the structure of the protein being made and can be 8 amino acids or longer. In one exemplary and non-limiting embodiment, X2 is a peptide comprising the amino acid sequence at least 20%, 27%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to KDEAEKAKRMKE\\MKRIKT (SEQ ID NO:7). In a further embodiment, amino acid residues in X2 relative to SEQ ID NO:7 are selected from the group consisting of:

Position 01:	A	H	L	M	R	S	V	K		
Position 02:	A	D	E	Q	R	S	T	V	W	Y
Position 03:	C	E	G	K	L	N	Q	R	W	
Position 04:	A	F	G	N	S	T	V	Y		
Position 05:	A	E	G	I	M	R	V			
Position 06:	C	E	K	L	N	R	V			
Position 07:	A	C	E	I	L	S	T	V	W	
Position 08:	H	K	L	M	S	T	W	Y		
Position 09:	A	I	L	M	Q	S	R			
Position 10:	A	I	M	S	W	Y				
Position 11:	C	I	K	L	S	V				
Position 12:	C	E	K	L	P	Q	R	T		
Position 13:	A	D	H	N	W					
Position 14:	A	C	G	I	L	S	T	V	M	
Position 15:	A	E	G	I	K	L	M	R	V	
Position 16:	G	H	L	R	S	T	V			

Position 17:	A	I	L	V						
Position 18:	A	C	D	E	G	H	I	K	M	S
Position 19:	D	E	G	L	N	V	T			

In another embodiment, the polypeptides are IL-4/IL-13 mimetics and position 11 is I. In another embodiment, the polypeptides are IL-2 mimetics and position 11 is not I.

In any of such embodiments, the polypeptide further allows for a cysteine at positions 5 or 16 relative to SEQ ID NO:7.

Alternatively, in any of such embodiments, the polypeptide further allows for a cysteine at positions 1, 2, 5, 9 or 16 relative to SEQ ID NO:7

Accordingly, amino acid residues in X2 relative to SEQ ID NO:7 can be selected from the group consisting of:

Position 01:	A	H	L	M	R	S	V	K	C	
Position 02:	A	D	E	Q	R	S	T	V	W	Y	C
Position 03:	C	E	G	K	L	N	Q	R	W	
Position 04:	A	F	G	N	S	T	V	Y		
Position 05:	A	E	G	I	M	R	V	C		
Position 06:	C	E	K	L	N	R	V			
Position 07:	A	C	E	I	L	S	T	V	W	
Position 08:	H	K	L	M	S	T	W	Y		
Position 09:	A	I	L	M	Q	S	R	C		
Position 10:	A	I	M	S	W	Y				
Position 11:	C	I	K	L	S	V				
Position 12:	C	E	K	L	P	Q	R	T		
Position 13:	A	D	H	N	W					
Position 14:	A	C	G	I	L	S	T	V	M	
Position 15:	A	E	G	I	K	L	M	R	V	
Position 16:	G	H	L	R	S	T	V	C		
Position 17:	A	I	L	V						
Position 18:	A	C	D	E	G	H	I	K	M	S
Position 19:	D	E	G	L	N	V	T			

In another embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid

sequence selected from the group consisting of the following polypeptides (i.e.: SEQ ID NOS:11-94, 103-184, 190-243, and 245-247). Underlined residues are linkers and are optional and each residue of the linker, when present, may comprise any amino acid. For each variant below, two SEQ ID NOS are provided: a first SEQ ID NO: that includes the linker positions as optional and variable, and a second SEQ ID NO: that lists the sequence as shown below.

G1_neo2_33	H1->H4->H2'->H3	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDLDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 11) STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDLDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 103)
G1_neo2_34	H1->H4->H2'->H3	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSCISTGKCDLDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 12) STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSCISTGKCDLDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 104)
G1_neo2_35	H1->H4->H2'->H3	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDCDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAC (SEQ ID NO: 13) STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDCDKAEDIRRNSDQARR EAEKRGIDVRDLISNAQVILLEAC (SEQ ID NO: 105)
G1_neo2_36	H1->H4->H2'->H3	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 14) STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 106)
G1_neo2_37	H1->H4->H2'->H3	STKKLQLQAEHFLLDVQMILNESPEPNEELNRCITDAQSWISTGKIDLDRAEECARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 15) STKKLQLQAEHFLLDVQMILNESPEPNEELNRCITDAQSWISTGKIDLDRAEECARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 107)
G1_neo2_38	H1->H4->H2'->H3	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSCISTGKCDLDRAEELARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 16) STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSCISTGKCDLDRAEELARNLEKVRD EALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 108)
G1_neo2_39	H1->H4->H2'->H3	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELCRNLEKVRD EALKRGIDVRDLVSNACVIALELK (SEQ ID NO: 17) STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELCRNLEKVRD EALKRGIDVRDLVSNACVIALELK (SEQ ID NO: 109)
G1_neo2_40	H1->H4->H2'->H3	STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSWISTGKIDLDGAKELAKEVEELRQ EAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 18) STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSWISTGKIDLDGAKELAKEVEELRQ EAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 110)
G1_neo2_41	H1->H4->H2'->H3	STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSCISTGKCDLDGAKELAKEVEELRQ EAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 19) STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSCISTGKCDLDGAKELAKEVEELRQ EAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 111)
G1_neo2_42	H1->H4->H2'->H3	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMAKEAEKIRK EMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 20) STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMAKEAEKIRK EMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 112)

G1_neo2_43	H1->H4->H2'->H3	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSCISTGKCDLDNAQEMAKEAEKIRK EMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 21) STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSCISTGKCDLDNAQEMAKEAEKIRK EMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 113)
G1_neo2_44	H1->H4->H2'->H3	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMCKEAEKIRK EMEKRGIDVRDLISNICVILLELS (SEQ ID NO: 22) STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMCKEAEKIRK EMEKRGIDVRDLISNICVILLELS (SEQ ID NO: 114)

G1_neo2_40_1A	H1->H4->H2'->H3	STKKTQLLAEHALLDAFMMLNVVPEPNEKLNRIITTMQSWIYTGKIDADGAKELAKEVEELEQE YEKRGIDVEDDASNLKVILLELA (SEQ ID NO: 23) STKKTQLLAEHALLDAFMMLNVVPEPNEKLNRIITTMQSWIYTGKIDADGAKELAKEVEELEQE YEKRGIDVEDDASNLKVILLELA (SEQ ID NO: 115)
G1_neo2_40_1B	H1->H4->H2'->H3	STKKTQLLAEHALLDAHMMLNMLPEPNEKLNRIITTMQSWIHTGKIDGDGAQELAKEVEELEQE YEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 24) STKKTQLLAEHALLDAHMMLNMLPEPNEKLNRIITTMQSWIHTGKIDGDGAQELAKEVEELEQE YEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 116)
G1_neo2_40_1C	H1->H4->H2'->H3	STKKTQLLAEHALLDAFMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELEQE FEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 25) STKKTQLLAEHALLDAFMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELEQE FEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 117)
G1_neo2_40_1D	H1->H4->H2'->H3	STKKTQLLAEHALLDALMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE LEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 26) STKKTQLLAEHALLDALMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE LEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 118)
G1_neo2_40_1E	H1->H4->H2'->H3	STKKTQLLAEHALLDAHMMLNVVPEPNEKLNRIITTMQSWIYTGKIDRDGAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 27) STKKTQLLAEHALLDAHMMLNVVPEPNEKLNRIITTMQSWIYTGKIDRDGAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 119)
G1_neo2_40_1F	H1->H4->H2'->H3	STKKTQLLAEHALLDALMMLNLLPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE HEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 28) STKKTQLLAEHALLDALMMLNLLPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE HEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 120)
G1_neo2_40_1G	H1->H4->H2'->H3	STKKTQLLAEHALLDAYMMLNMVPEPNEKLNRIITTMQSWILTGKIDSDGAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 29) STKKTQLLAEHALLDAYMMLNMVPEPNEKLNRIITTMQSWILTGKIDSDGAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 121)
G1_neo2_40_1H	H1->H4->H2'->H3	STKKTHLLAEHALLDAYMMLNVMPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELEQE FEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 30) STKKTHLLAEHALLDAYMMLNVMPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELEQE FEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 122)
G1_neo2_40_1I	H1->H4->H2'->H3	STKKTQLLAEHALLDAYMMLNLVPEPNEKLNRIITTMQSWIFTGKIDADGAQELAIEVEELEQE YEKRGIDVDDYASNLKVILLELA (SEQ ID NO: 31) STKKTQLLAEHALLDAYMMLNLVPEPNEKLNRIITTMQSWIFTGKIDADGAQELAIEVEELEQE YEKRGIDVDDYASNLKVILLELA (SEQ ID NO: 123)
G1_neo2_40_1J	H1->H4->H2'->H3	STKKTQLMAEHALLDAFMMLNVLPEPNEKLNRIITTMQSWIFTGKIDGDDAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 32) STKKTQLMAEHALLDAFMMLNVLPEPNEKLNRIITTMQSWIFTGKIDGDDAQELAKEVEELEQE LEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 124)
G1_neo2_40_1F_H1	H1->H4->H2'-	STKKTQLLIEHALLDALDMSRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQQLAKEVEELEQE HEKRGEDVEDEASNLKVILLELA (SEQ ID NO: 33)

	>H3	STKKTQLLIEHALLDALDMSRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQQLAKEVEELEQE HEKRGEDVEDEASNLKVILLELA (SEQ ID NO: 125)
G1_neo2_40_1F_H2	H1->H4->H2'->H3	STKKTQLLLEHALLDALHMRRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE HEKRGRDVEDDASNLKVILLELA (SEQ ID NO: 34) STKKTQLLLEHALLDALHMRRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQELAKEVEELEQE HEKRGRDVEDDASNLKVILLELA (SEQ ID NO: 126)
G1_neo2_40_1F_H3	H1->H4->H2'->H3	STKKTQLLIEHALLDALNMRKKLPEPNEKLSRIITDMQSWIFTGKIDGDGAQQLAKEVEELEQE HEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 35) STKKTQLLIEHALLDALNMRKKLPEPNEKLSRIITDMQSWIFTGKIDGDGAQQLAKEVEELEQE HEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 127)
G1_neo2_40_1F_H4	H1->H4->H2'->H3	STKKTQLLLEHALLDALHMSRELPEPNEKLNRIITDMQSWIFTGKIDGDGAQDLAKEVEELEQE HEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 36) STKKTQLLLEHALLDALHMSRELPEPNEKLNRIITDMQSWIFTGKIDGDGAQDLAKEVEELEQE HEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 128)
G1_neo2_40_1F_H5	H1->H4->H2'->H3	STKKTQLLIEHALLDALHMSRKLPEPNEKLSRIITTMQSWIFTGKIDGDGAQHLAKEVEELEQE HEKRGGEVEDEASNLKVILLELA (SEQ ID NO: 37) STKKTQLLIEHALLDALHMSRKLPEPNEKLSRIITTMQSWIFTGKIDGDGAQHLAKEVEELEQE HEKRGGEVEDEASNLKVILLELA (SEQ ID NO: 129)
G1_neo2_40_1F_H6	H1->H4->H2'->H3	STKKTQLLIEHALLDALHMKRKLPEPNEKLNRIITNMQSWIFTEKIDGDGAQDLAKEVEELEQE HEKRGQDVEDYASNLKVILLELA (SEQ ID NO: 38) STKKTQLLIEHALLDALHMKRKLPEPNEKLNRIITNMQSWIFTEKIDGDGAQDLAKEVEELEQE HEKRGQDVEDYASNLKVILLELA (SEQ ID NO: 130)
G1_neo2_40_1F_M1	H1->H4->H2'->H3	STEKTQLAAEHALRDALMLKHLLNEPNEKLARIITTMQSWQFTGKIDGDGAQELAKEVEELQQE HEVRGIDVEDYASNLKVILLHLA (SEQ ID NO: 39) STEKTQLAAEHALRDALMLKHLLNEPNEKLARIITTMQSWQFTGKIDGDGAQELAKEVEELQQE HEVRGIDVEDYASNLKVILLHLA (SEQ ID NO: 131)
G1_neo2_40_1F_M2	H1->H4->H2'->H3	STKNTQLAAEDALLDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQQE HEERGIDVEDYASNLKVILLQLA (SEQ ID NO: 40) STKNTQLAAEDALLDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQQE HEERGIDVEDYASNLKVILLQLA (SEQ ID NO: 132)
G1_neo2_40_1F_M3	H1->H4->H2'->H3	STEKTQHAAEDALRDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQQE HEVRGIDVEDYASNLKVILLQLA (SEQ ID NO: 41) STEKTQHAAEDALRDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQQE HEVRGIDVEDYASNLKVILLQLA (SEQ ID NO: 133)

G2_neo2_40_1F_seq02	H1->H4->H2'->H3	TQKKQQLLAEHALLDALMILNMLKTSSEAVNPMITIAQSWIFTGTSNPEEAKEMIKMAEQAEEE ARREGVDTEDYVSNLKVILKEIA (SEQ ID NO: 42) TQKKQQLLAEHALLDALMILNMLKTSSEAVNRMITIAQSWIFTGTSNPEEAKEMIKMAEQAEEE ARREGVDTEDYVSNLKVILKEIA (SEQ ID NO: 134)
G2_neo2_40_1F_seq03	H1->H4->H2'->H3	TTKKYQLLVEHALLDALMMLNLSSESNEKMNRIITTMQSWIFTGTFDPDQAEELAKLVEELREE FRKRGIDTEDYASNLKVILKELS (SEQ ID NO: 43) TTKKYQLLVEHALLDALMMLNLSSESNEKMNRIITTMQSWIFTGTFDPDQAEELAKLVEELREE FPKRGIDTEDYASNLKVILKELS (SEQ ID NO: 135)
G2_neo2_40_1F_seq04	H1->H4->H2'->H3	TTKKIQLLVEHALLDALMILNLSSESNEKLNRIITTLQSWIFPGEIDPDPARRELAKLLEEIREE MRKRGIDTEDYVSNMIVIIRELA (SEQ ID NO: 44) TTKKIQLLVEHALLDALMILNLSSESNEKLNRIITTLQSWIFRGEIDPDRARRELAKLLEEIREE MRKRGIDTEDYVSNMIVIIRELA (SEQ ID NO: 136)
G2_neo2_40_1F_seq05	H1->H4->H2'->H3	TKKKIQLLAEHVLLDLLMMLNLSSESNEKMNRLITIVQSWIFTGTIDPDQAEEMAKWVEELREE FPKRGIDTEDYASNVKVILKELS (SEQ ID NO: 45) TKKKIQLLAEHVLLDLLMMLNLSSESNEKMNRLITIVQSWIFTGTIDPDQAEEMAKWVEELREE FRKRGIDTEDYASNVKVILKELS (SEQ ID NO: 137)

G2_neo2_40_1F_seq06	H1->H4->H2'->H3	TKKKYQLLIEHLLLDALMVLNMSSESNEKLNRIITILQSWIFTGTWDPDLAEEMEKLMQEIEEE LRRRGIDTEDYMSNMRVIIKELS (SEQ ID NO: 46) TKKKYQLLIEHLLLDALMVLNMSSESNEKLNRIITILQSWIFTGTWDPDLAEEMEKLMQEIEEE LRRPGIDTEDYMSNMRVIIKELS (SEQ ID NO: 138)
G2_neo2_40_1F_seq07	H1->H4->H2'->H3	TKKKLQLLVEHLLLDMLMILNMSSESNEKLNRLITELQSWIFRGEIDPDKAEEMWKIMEEIEKE LPERGIDTEDYMSNAKVIIKELS (SEQ ID NO: 47) TKKKLQLLVEHLLLDMLMILNMSSESNEKLNRLITELQSWIFPGEIDPDKAEEMWKIMEEIEKE LRERGIDTEDYMSNAKVIIKELS (SEQ ID NO: 139)
G2_neo2_40_1F_seq08	H1->H4->H2'->H3	TSKKQQLLAEHALLDALMILNISSESSEAVNPAITWLQSWIFKGTVNPDQAEEMRKLAEQIREE MRKPGIDTEDYVSNLEVIAKELS (SEQ ID NO: 48) TSKKQQLLAEHALLDAIMILNISSESSEAVNRAITWLQSWIFKGTVNPDQAEEMRKLAEQIREE MRKRGIDTEDYVSNLEVIAKELS (SEQ ID NO: 140)
G2_neo2_40_1F_seq09	H1->H4->H2'->H3	TKKKYQLLIEHLLLDLLMVLNMSSESNEKINRLITWLQSWIFTGTYDPDLAEEMYKILEELREE MRERGIDTEDYMSNMRVIVKELS (SEQ ID NO: 49) TKKKYQLLIEHLLLDLLMVLNMSSESNEKINPLITWLQSWIFTGTYDPDLAEEMYKILEELREE MPERGIDTEDYMSNMPVIVKELS (SEQ ID NO: 141)
G2_neo2_40_1F_seq10	H1->H4->H2'->H3	TKKKWQLLIEHLLLDLLMILNLSSESNEKLNRLITWLQSWIFTGTYDPDLAEEMKKMMDEIEDE LRERGIDTEDYMSNAKVIIKELS (SEQ ID NO: 50) TKKKWQLLIEHLLLDLLMILNLSSESNEKLNRLITWLQSWIFTGTYDPDLAEEMKKMMDEIEDE LREPGIDTEDYMSNAKVIIKELS (SEQ ID NO: 142)
G2_neo2_40_1F_seq11	H1->H4->H2'->H3	TKKKIQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELSKLVEEIREE MPKRGIDTEDYVSNLKVILDELS (SEQ ID NO: 51) TKKKIQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELSKLVEEIREE MRKRGIDTEDYVSNLKVILDELS (SEQ ID NO: 143)
G2_neo2_40_1F_seq12	H1->H4->H2'->H3	TEKKLQLLVEHALLDAIMILNLWSESNEKLNRIITTMQSWIFTGRIDPDKAEELAKLVEELREE ARERGIDTEDYVSNLKVILKELS (SEQ ID NO: 52) TEKKLQLLVEHALLDAIMILNLWSESNEKLNRIITTMQSWIFTGRIDPDKAEELAKLVEELREE ARERGIDTEDYVSNLKVILKELS (SEQ ID NO: 144)
G2_neo2_40_1F_seq13	H1->H4->H2'->H3	TKKKYQLLMEHLLLDLLMVLNMSSESNEKLNRLITIIQSWIFTGTWDPDKAEEMAKMLKEIEDE LRERGIDTEDYMSNMIVIMKELS (SEQ ID NO: 53) TKKKYQLLMEHLLLDLLMVLNMSSESNEKLNPLITIIQSWIFTGTWDPDKAEEMAKMLKEIEDE LRERGIDTEDYMSNMIVIMKELS (SEQ ID NO: 145)
G2_neo2_40_1F_seq14	H1->H4->H2'->H3	TTKKIQLLVEHALLDAIMLLNLSSESNEKMNRIITTMQSWIFEGRIDPDQAQELAKLVEELREE FRKRGIDTEDYVSNLKVILEELS (SEQ ID NO: 54) TTKKIQLLVEHALLDAIMLLNLSSESNEKMNRIITTMQSWIFEGRIDPDQAQELAKLVEELPEE FRKPGIDTEDYVSNLKVILEELS (SEQ ID NO: 146)
G2_neo2_40_1F_seq15	H1->H4->H2'->H3	TKKKIQLLVEHALLDAIMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELAKLVRELREE FRKRGIDTEDYASNLEVILRELS (SEQ ID NO: 55) TKKKIQLLVEHALLDAIMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELAKLVRELREE FRKRGIDTEDYASNLEVILPELS (SEQ ID NO: 147)
G2_neo2_40_1F_seq16	H1->H4->H2'->H3	TKKKIQLLVEHALLDALMILNLSSKSNEKLNRIITTMQSWIFNGTIDPDRARELAKLVEEIRDE MEKNGIDTEDYVSNLKVILEELA (SEQ ID NO: 56) TKKKIQLLVEHALLDAIMILNLSSKSNEKLNRIITTMQSWIFNGTIDPDRAPELAKLVEEIRDE MEKNGIDTEDYVSNLKVILEELA (SEQ ID NO: 148)
G2_neo2_40_1F_seq17	H1->H4->H2'->H3	TKKKYQLLIEHVLLDLLMLLNLSSESNEKMNRLITILQSWIFTGTYDPDKAEEMAKLLKELREE FRERGIDTEDYISNAIVILKELS (SEQ ID NO: 57) TKKKYQLLIEHVLLDLLMLLNLSSESNEKMNPLITILQSWIFTGTYDPDKAEEMAKLLKELREE FRERGIDTEDYISNAIVILKELS (SEQ ID NO: 149)
G2_neo2_40_1F_seq18	H1->H4->H2'->H3	TKKKIQLLVEHALLDAIMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLVEELREE FRKRGIDTEDYASNLKVILKELS (SEQ ID NO: 58) TKKKIQLLVEHALLDAIMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLVEELREE

		FPKRGIDTEDYASNLKVILKELS (SEQ ID NO: 150)
G2_neo2_40_1F_seq19	H1->H4->H2'->H3	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITTMQSWIFNGTIDPDQARELAKLVEELREE FRKRGIDTEDYASNLKVILEELA (SEQ ID NO: 59) TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITTMQSWIFNGTIDPDQARELAKLVEELREE FRKRGIDTEDYASNLKVILEELA (SEQ ID NO: 151)
G2_neo2_40_1F_seq20	H1->H4->H2'->H3	TKKKLQLLVEHALLDAIMLLNLSSESNEKLNRIITTMQSWIFTGTVDPDQAEELAKLVEEIREE LRKRGIDTEDYVSNLKVILKELS (SEQ ID NO: 60) TKKKLQLLVEHALLDAIMLLNLSSESNEKLNRIITTMQSWIFTGTVDPDQAEELAKLVEEIREE LRKPGIDTEDYVSNLKVILKELS (SEQ ID NO: 152)
G2_neo2_40_1F_seq21	H1->H4->H2'->H3	TTKKYQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTFDPDQAEELAKLVREIREE MRKRGIDTEDYVSNLEVILRELS (SEQ ID NO: 61) TTKKYQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTFDPDQAEELAKLVREIREE MRKRGIDTEDYVSNLEVILRELS (SEQ ID NO: 153)
G2_neo2_40_1F_seq22	H1->H4->H2'->H3	TKKKIQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLVREIREE MRKPGIDTEDYVSNLEVILRELS (SEQ ID NO: 62) TKKKIQLLVEHALLDAIMILNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLVREIREE MPKRGIDTEDYVSNLEVILRELS (SEQ ID NO: 154)
G2_neo2_40_1F_seq23	H1->H4->H2'->H3	TKKKYQLLIEHLLLDLIMILNLSSESNEKLNRLITWLQSWIFPGEWDPDKAEEWAKILKEIREE LRERGIDTEDYMSNAIVIMKELS (SEQ ID NO: 63) TKKKYQLLIEHLLLDLLMILNLSSESNEKLNPLITWLQSWIFRGEWDPDKAEEWAKILKEIREE LREPGIDTEDYMSNAIVIMKELS (SEQ ID NO: 155)
G2_neo2_40_1F_seq24	H1->H4->H2'->H3	TDKKLQLLVEHLLLDLLMMLNLSSKSNEKMNRLITIAQSWIFTGKVDPDLAREMIKLLEETEDE NRKNGIDTEDYVSNARVIAKELE (SEQ ID NO: 64) TDKKLQLLVEHLLLDLLMMLNLSSKSNEKMNRLITIAQSWIFTGKVDPDLAREMIKLLEETEDE NRKNGIDTEDYVSNARVIAKELE (SEQ ID NO: 156)
G2_neo2_40_1F_seq25	H1->H4->H2'->H3	TKKKIQLLVEHALLDALMLLNLSSESNEKMNPIITTMQSWIFTGTIDPDQAEELAKLVEELKEE FKKRGIDTEDYVSNLKVILKELS (SEQ ID NO: 65) TKKKIQLLVEHALLDAIMLLNLSSESNEKMNRIITTMQSWIFTGTIDPDQAEELAKLVEELKEE FKKRGIDTEDYVSNLKVILKELS (SEQ ID NO: 157)
G2_neo2_40_1F_seq26	H1->H4->H2'->H3	TKKKYQLLIEHALLDALMILNLWSESNEKLNRIITTMQSWIFTGTYDPDKAEELEKLAKEIEDE AREPGIDTEDYMSNLRVILKELS (SEQ ID NO: 66) TKKKYQLLIEHALLDALMILNLWSESNEKLNPIITTMQSWIFTGTYDPDKAEELEKLAKEIEDE APERGIDTEDYMSNLRVILKELS (SEQ ID NO: 158)
G2_neo2_40_1F_seq27	H1->H4->H2'->H3	TKKKAQLLAEHALLDALMLLNLSSESNEPLNRIITWLQSIIFTGTYDPDMVKEAVKLADEIEDE MRKRGIDTEDYVSNLRVILQELA (SEQ ID NO: 67) TKKKAQLLAEHALLDALMLLNLSSESNERLNRIITWLQSIIFTGTYDPDMVKEAVKLADEIEDE MRKPGIDTEDYVSNLRVILQELA (SEQ ID NO: 159)
G2_neo2_40_1F_seq28	H1->H4->H2'->H3	TQKKNQLLAEHLLLDALMVLNQSSESSEVANRIITWAQSWIFEGRVDPNKAEEAKKLAKKLEEE MPKRGIDMEDYISNMKVIAEEMS (SEQ ID NO: 68) TQKKNQLLAEHLLLDALMVLNQSSESSEVANRIITWAQSWIFEGRVDPNKAEEAKKLAKKLEEE MRKRGIDMEDYISNMKVIAEEMS (SEQ ID NO: 160)
G2_neo2_40_1F_seq29	H3->H2'->H4->H1	EDYYSNLKVILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYI QSQIFEISEPIRETDQEKKEESWKKWQLLLEHALLDVLMLLND (SEQ ID NO: 69) EDYYSNLKVILEELAPEMERNGLSDKAEEWRQWKKIVERIRQIPSNNSDLNEAKELLNRLITYI QSQIFEISERIRETDQEKKEESWKKWQLLLEHALLDVLMLLND (SEQ ID NO: 161)
G2_neo2_40_1F_seq30	H1->H3->H2'->H4	PEKKRQLLLEHILLDAIMLLNLXXXXXXNTESKFEDYISNAEVIAEELAKLMESXXLSDEAEKF KKIKQWLREVWRIWXXXZWSTLEDKARELLNRIITTIQSQIFY (SEQ ID NO: 70) PEKKRQLLLEHILLDAIMLLNLLETNPQNTESKFEDYISNAEVIAEELAKLMESLGLSDEAEKF KKIKQWLPEVWRIWSSTNWSTLEDKARELLNRIITTIQSQIFY (SEQ ID NO: 162)
G2_neo2_40_	H1->H3-	PEKKRQLLLEHILLDLLMILNMXXXXXXNTESEMEDYWSNVPVILRELAPLMEEXXKELSELM

1F_seq31	>H2'- >H4	EPMRKIVEKIRQIVTXXXXLDTAREWLNPLITWIQSLIFR (SEQ ID NO: 71) PEKKPQLLLEHILLDLLMILNMIETNRENTESEMEDYWSNVRVILRELARLMEELNYKELSELM ERMPKIVEKIPQIVTNNSSLDTAREWLNRLITWIQSLIFR (SEQ ID NO: 163)
G2_neo2_40_ 1F_seq32	H1->H3- >H2'- >H4	PEKKRQLLAEHALLDAIMLLNIIETNSKNTESKMEDYVSNLEVILTEFKKLAEKLNFSEEAERA EPMKRWARKAYQMMTLDLSLDKAKEMLNRIITILQSIIFN (SEQ ID NO: 72) PEKKRQLLAEHALLDAIMLLNIIETNSKNTESKMEDYVSNLEVILTEFKKLAEKLNFSEEAEPA ERMKPWARKAYQMMTLDLSLDKAKEMLNRIITILQSIIFN (SEQ ID NO: 164)
G2_neo2_40_ 1F_seq33	H1->H3- >H2'- >H4	PEKKRQLLAEHLLLDVLMMLNGNASLKDYASNAQVIADEFRELARELGLTDEAKKAEKIIEALE RAREWLLNNKDKEKAKEALNRAITIAQSWIFN (SEQ ID NO: 73) PEKKRQLLAEHLLLDVLMMLNGNASLKDYASNAQVIADEFRELARELGLTDEAKKAEKIIEALE PAREWLLNNKDNEKAKEALNPAITIAQSWIFN (SEQ ID NO: 165)
G2_neo2_40_ 1F_seq34	H1->H3- >H2'- >H4	PEKKRQLLLEHLLLDLLMILNMLRTNPKNIESDWEDYMSNIEVIIEELPKIMESLGRSEKAKEW KRMKQWVRRILEIVKNNSDLEEAKEWLNRLITIVQSEIFE (SEQ ID NO: 74) PEKKRQLLLEHLLLDLLMILNMLRTNPKNIESDWEDYMSNIEVIIEELRKIMESLGRSEKAKEW KPMKQWVRRILEIVKNNSDLEEAKEWLNRLITIVQSEIFE (SEQ ID NO: 166)
G2_neo2_40_ 1F_seq35	H1->H3- >H2'- >H4	WEKKRQLLLEHLLLDLLMILNMWRTNPQNTESLMEDYMSNAKVIVEELARMMRSQGLEDKAREW EEMKKRIEEIRQIIQNNSSKEPAKEELNPLITYVQSEIFR (SEQ ID NO: 75) WEKKPQLLLEHLLLDLLMILNMWRTNPQNTESLMEDYMSNAKVIVEELARMMRSQGLEDKAREW EEMKKRIEEIPQIIQNNSSKERAKEELNRLITYVQSEIFR (SEQ ID NO: 167)
G2_neo2_40_ 1F_seq36	H1->H3- >H2'- >H4	PKKKIQLLAEHALLDAIMILNIVKTNSQNAEEKLEDYASNVEVILEEIARLMESGDQKDEAEKA KPMKEWMKRIKTTASEDEQEEMANRIITLLQSWIFS (SEQ ID NO: 76) PKKKIQLLAEHALLDAIMILNIVKTNSQNAEEKLEDYASNVEVILEEIAPLMESGDQKDEAEKA KRMKEWMKRIKTTASEDEQEEMANRIITLLQSWIFS (SEQ ID NO: 168)
G2_neo2_40_ 1F_seq37	H1->H3- >H2'- >H4	PEKKPQLLAEHALLDAIMILNXXXXXXQNAEEKLEDYMSNVEVIMEEFARMMRXXXXSEEAENA ERIKKWVRKASSXXXSEEQREMMNPAITLMQSWIFE (SEQ ID NO: 77) PEKKRQLLAEHALLDAIMILNILQTNPQNAEEKLEDYMSNVEVIMEEFARMMRNGDRSEEAENA ERIKKWVRKASSTASSEEQREMMNRAITLMQSWIFE (SEQ ID NO: 169)
G2_neo2_40_ 1F_seq38	H1->H3- >H2'- >H4	PEKKPQLLAEHLLLDALMVLNMXXXXXXNTEEKLEDYISNMKVIIKEMIELMRSLXXXEEAEKW KEALKAVEKIXXXXDSETARELANPIITLAQSAIFY (SEQ ID NO: 78) PEKKRQLLAEHLLLDALMVLNMLTTNSKNTEEKLEDYISNMKVIIKEMIELMRSLGPLEEAEKW KEALKAVEKIGSRMDSETARELANRIITLAQSAIFY (SEQ ID NO: 170)
G2_neo2_40_ 1F_seq39	H1->H3- >H2'- >H4	PEKKRQLLAEHALLDALMFLNLXXXXXXQAEEKIEDYASNLPVIAEELARLFENLXXXDEAQKA KDIKELAERAPSXXSSEKRKEAMNPAITILQSMIFR (SEQ ID NO: 79) PEKKRQLLAEHALLDALMFLNLVETNPDQAEEKIEDYASNLRVIAEELARLFENLGRLDEAQKA KDIKELAERARSRVSSEKRKEAMNRAITILQSMIFR (SEQ ID NO: 171)
G2_neo2_40_ 1F_seq40	H1->H3- >H2'- >H4	PEKKRQLLAEHALLDALMILNIIRTNSDNTESKLEDYISNLKVILEEIARLMESLGLSDEAEKA KEAMPLADKAGSTASEEEKKEAMNRVITWAQSWIFN (SEQ ID NO: 80) PEKKRQLLAEHALLDALMILNIIRTNSDNTESKLEDYISNLKVILEEIARLMESLGLSDEAEKA KEAMRLADKAGSTASEEEKKEAMNRVITWAQSWIFN (SEQ ID NO: 172)
G2_neo2_40_ 1F_seq41	H1->H3- >H2'- >H4	PEKKRQLLAEHALLDALMMLNILRTNPDNAEEKLEDYWSNLIVILREIAKLMESLGLTDEAEKA KEAARWAEEARTTASKDQRRELANRIITLLQSWIFS (SEQ ID NO: 81) PEKKPQLLAEHALLDALMMLNILRTNPDNAEEKLEDYWSNLIVILREIAKLMESLGLTDEAEKA KEAARWAEEAPTTASKDQRRELANPIITLLQSWIFS (SEQ ID NO: 173)
G2_neo2_40_ 1F_seq42	H1->H3- >H2'- >H4	PEKKRQLLAEHLLLDALMILNIIETNEQNAESKLEDYISNAKVILDEFREMAPDLGLLDEAKKA EKMKRWLEKMRSNASSDERREWANRMITTAQSWIFN (SEQ ID NO: 82) PEKKRQLLAEHLLLDALMILNIIETNEQNAESKLEDYISNAKVILDEFREMARDLGLLDEAKKA EKMKRWLEKMRSNASSDERREWANRMITTAQSWIFN (SEQ ID NO: 174)

G2_neo2_40_1F_seq27_S3	H1->H4->H2'->H3	TNKKAQLHAEFALHDALMLLNLSSESNERLNRIITWLQSIIFYGTYDPDMVKEAVKDADEIEDE MRKRGIDTEDYVSNLRLILQELA (SEQ ID NO: 83) TNKKAQLHAEFALHDALMLLNLSSESNERLNRIITWLQSIIFYGTYDPDMVKEAVKDADEIEDE MRKRGIDTEDYVSNLRLILQELA (SEQ ID NO: 245)
G2_neo2_40_1F_seq27_S18	H1->H4->H2'->H3	TNKEAQLHAEFALYDALMLLNLSSESNERLNRIITWLQSIIFYETYDPDMVKEAVKLADEIEDE MRKRKIDTEDYVVNLRLILQELA (SEQ ID NO: 84) TNKEAQLHAEFALYDALMLLNLSSESNERLNRIITWLQSIIFYETYDPDMVKEAVKLADEIEDE MRKRKIDTEDYVVNLRLILQELA (SEQ ID NO: 175)
G2_neo2_40_1F_seq27_S22	H1->H4->H2'->H3	TKKDAELLAEFALYDALMLLNLSSESNERLNEIITWLQSIIFYGTYDPDMVKEAVKLADEIEDE MRKRGIDTEDYVSNLRLILQELA (SEQ ID NO: 85) TKKDAELLAEFALYDALMLLNLSSESNERLNEIITWLQSIIFYGTYDPDMVKEAVKLADEIEDE MRKRGIDTEDYVSNLRLILQELA (SEQ ID NO: 176)
G2_neo2_40_1F_seq27_S24	H1->H4->H2'->H3	TNKKAQLHAEFALYDALMLLNLSSESNERLNDIITWLQSIIFTGTYDPDMVKEAVKLADEIEDE MRKRKIDTEDYVVNLRYILQELA (SEQ ID NO: 86) TNKKAQLHAEFALYDALMLLNLSSESNERLNDIITWLQSIIFTGTYDPDMVKEAVKLADEIEDE MRKRKIDTEDYVVNLRYILQELA (SEQ ID NO: 177)
G2_neo2_40_1F_seq29_S6	H3->H2'->H4->H1	EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYI QSQIFEVLHGVGETDQEKKEESWKKWDLLLEHALLDVLMLLND (SEQ ID NO: 87) EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYI QSQIFEVLHGVGETDQEKKEESWKKWDLLLEHALLDVLMLLND (SEQ ID NO: 178)
G2_neo2_40_1F_seq29_S7	H3->H2'->H4->H1	EDYYSNLKVILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNELITYI QSQIFEVIEREGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 88) EDYYSNLKVILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNELITYI QSQIFEVIEREGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 179)
G2_neo2_40_1F_seq29_S8	H3->H2'->H4->H1	EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYI QSQIFEVLEGVGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 89) EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYI QSQIFEVLEGVGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 180)
Neoleukin-2/15 (i.e. G2_neo2_40_1F_seq36_S11)	H1->H3->H2'->H4	PKKKIQLHAEHALYDALMILNIVKTNSPPAEEKLEDYAFNFELILEEIARLFESGDQKDEAEKA KRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 90) PKKKIQLHAEHALYDALMILNIVKTNSPPAEEKLEDYAFNFELILEEIARLFESGDQKDEAEKA KRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 181)
G2_neo2_40_1F_seq36_S12	H1->H3->H2'->H4	PKKKIQLLAEHALFDLLMILNIVKTNSQNAEEKLEDYAYNAGVILEEIARLFESGDQKDEAEKA KRMKEWMKRIKDTASEDEQEEMANEIITILQSWNFS (SEQ ID NO: 91) PKKKIQLLAEHALFDLLMILNIVKTNSQNAEEKLEDYAYNAGVILEEIARLFESGDQKDEAEKA KRMKEWMKRIKDTASEDEQEEMANEIITILQSWNFS (SEQ ID NO: 182)
Neoleukin-2/15-H8Y-K33E	H1->H3->H2'->H4	PKKKIQLYAEHALYDALMILNIVKTNSPPAEEELEDYAFNFELILEEIARLFESGDQKDEAEKA KRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 94) PKKKIQLYAEHALYDALMILNIVKTNSPPAEEELEDYAFNFELILEEIARLFESGDQKDEAEKA KRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 246)
Neoleukin-2/15 (K32 is considered to be a residue of the optional	H1->H3->H2'->H4	PKKKIQLHAEHALYDALMILNIVKTNSPPAEE**K**LEDYAFNFELILEEIARLFESGDQKDEAEK AKRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 247)

linker in this depicted sequence)		

IL4_G2_neo2_40_1F_seq36_S11	PKKKIQITAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLFESGDQKDEAEKAKRMKE WMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 92) PKKKIQITAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLFESGDQKDEAEKAKRMKE WMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 183)
Neoleukin-4 (i.e. IL4_G2_neo2_40_1F_seq36_S11_MIF)	PKKKIQIMAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLFESGDQKDEAEKAKRMIE WMKRIKTTASEDEQEEMANAIITILQSWFFS (SEQ ID NO: 93) PKKKIQIMAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLFESGDQKDEAEKAKRMIE WMKRIKTTASEDEQEEMANAIITILQSWFFS (SEQ ID NO: 184)

For each variant below, two SEQ ID NOs are provided: a first SEQ ID NO: that lists the sequence as shown below, and a second SEQ ID NO: that includes the linker positions as optional and variable.

>Neoleukin-2/15_R50C (SEQ ID NO: 190)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIACLFESG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R50C (SEQ ID NO: 217)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIACLFESG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C(SEQ ID NO: 191)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFCSG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C(SEQ ID NO: 218)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFCSG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C(SEQ ID NO: 192)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C(SEQ ID NO: 219)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C(SEQ ID NO: 193)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**CDEAEKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C(SEQ ID NO: 220)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**CDEAEKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C(SEQ ID NO: 194)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KCEAEKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C(SEQ ID NO: 221)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KCEAEKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C(SEQ ID NO: 195)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEACKAKRMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C(SEQ ID NO: 222)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEACKAKRMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C(SEQ ID NO: 196)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKCMK
EWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C(SEQ ID NO: 223)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKCMK
EWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E69C(SEQ ID NO: 197)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
CWMKRIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E69C(SEQ ID NO: 224)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
CWMKRIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R73C(SEQ ID NO: 198)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R73C(SEQ ID NO: 225)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_T77C(SEQ ID NO: 199)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
EWMKRIKTCASEDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_T77C(SEQ ID NO: 226)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
EWMKRIKTCASEDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E82C(SEQ ID NO: 200)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E82C(SEQ ID NO: 227)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E85C(SEQ ID NO: 201)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDEQECMANAIITILQSWIFS*

>Neoleukin-2/15_E85C(SEQ ID NO: 228)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDEQECMANAIITILQSWIFS*

>Neoleukin-2/15_R50C_R73C(SEQ ID NO: 202)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIACLFESG**DQ**KDEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R50C_R73C(SEQ ID NO: 229)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIA**C**LFESG**XX**KDEAEKAKRMK
EWMK**C**IKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C_R73C(SEQ ID NO: 203)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFCSG**DQ**KDEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C_R73C(SEQ ID NO: 230)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFCSG**XX**KDEAEKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C_R73C(SEQ ID NO: 204)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C_R73C(SEQ ID NO: 231)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C_R73C (SEQ ID NO:205)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**CDEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C_R73C (SEQ ID NO:232)
PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**CDEAEKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C_R73C(SEQ ID NO: 206)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KCEAEKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C_R73C(SEQ ID NO: 233)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KCEAEKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C_R73C(SEQ ID NO: 207)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEACKAKRMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C_R73C(SEQ ID NO: 234)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEACKAKRMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C_R73C(SEQ ID NO: 208)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKCMK
EWMKCIKT**TAS**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C_R73C(SEQ ID NO: 235)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKCMK
EWMKCIKT**XXX**EDEQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R50C_E82C(SEQ ID NO: 209)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIACLFESG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R50C_E82C(SEQ ID NO: 236)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIACLFESG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C_E82C(SEQ ID NO: 210)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFCSG**DQ**KDEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E53C_E82C(SEQ ID NO: 237)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFCSG**XX**KDEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C_E82C(SEQ ID NO: 211)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D56C_E82C(SEQ ID NO: 238)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESGCQKDEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C_E82C(SEQ ID NO: 212)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**CDEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_K58C_E82C(SEQ ID NO: 239)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**CDEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C_E82C(SEQ ID NO: 213)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KCEAEKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_D59C_E82C(SEQ ID NO: 240)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KCEAEKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C_E82C(SEQ ID NO: 214)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEACKAKRMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E62C_E82C(SEQ ID NO: 241)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEACKAKRMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C_E82C(SEQ ID NO: 215)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKCMK
EWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_R66C_E82C(SEQ ID NO: 242)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKCMK
EWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E69C_E82C(SEQ ID NO: 216)

PKKKIQLHAEHALYDALMILNI**VKTNSPPAEEK**LEDYAFNFELILEEIARLFESG**DQ**KDEAEKAKRMK
CWMKRIKT**TAS**EDCQEEMANAIITILQSWIFS*

>Neoleukin-2/15_E69C_E82C(SEQ ID NO: 243)

PKKKIQLHAEHALYDALMILNI**XXXXXXXXXXX**LEDYAFNFELILEEIARLFESG**XX**KDEAEKAKRMK
CWMKRIKT**XXX**EDCQEEMANAIITILQSWIFS*

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:90, 181, and 247.

In another embodiment, the polypeptide comprises a polypeptide identical to the amino acid sequence of SEQ ID NO:90, 181, or 247, wherein the polypeptide (i) does not bind to human or murine IL-2Ralpha, (ii) binds to human IL2RB with an affinity of about

11.2 nM (iii) binds to murine IL2RB with an affinity of about 16.1 nm (iv) binds to human IL-2R$\beta\gamma_c$ with an affinity of about 18.8 nM and (v) binds to murine IL-2R$\beta\gamma_c$ with an affinity of about 3.4 nM.

In any of these embodiments of the full length polypeptides, the polypeptide may be an IL-4/IL-13 mimetic, wherein position 7 is I, position 8 is T or M, position 11 is E, position 14 is K, position 18 is S, position 33 is Q, position 36 is R, position 37 is F, position 39 is K, position 40 is R, position 43 is R, position 44 is N, position 46 is W, and position 47 is G. In a further embodiment, position 68 is I and position 98 is F.

In any of these embodiments of the full length polypeptides, the polypeptide may be an IL-2 mimetic, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following are not true: position 7 is I, position 8 is T or M, position 11 is E, position 14 is K, position 18 is S, position 33 is Q, position 36 is R, position 37 is F, position 39 is K, position 40 is R, position 43 is R, position 44 is N, position 46 is W, and position 47 is G. In a further embodiment, one or both of the following are not true: position 68 is I and position 98 is F.

In one embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein have a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms, less than 1.5 Angstroms, or less than 1 Angstrom when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-2.

In another embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein have a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms, less than 1.5 Angstroms, or less than 1 Angstrom when superimposed on backbone atoms or alpha carbon atoms of a three dimensional structure having the structural coordinates of Table E2.

In a further embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, when in ternary complex with the mouse IL-2 receptor $\beta\gamma_c$, have a three dimensional structure wherein the structural coordinates of common residue backbone atoms or alpha carbon atoms have a root mean square deviation of less than 2.5 Angstroms, less than 1.5 Angstroms, or less than 1 Angstrom when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-2 when in ternary complex with the mouse IL-2 receptor $\beta\gamma_c$.

In another embodiment, the IL-4 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein have a three dimensional structure with structural coordinates comprising a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms less than 1.5 Angstroms, or less than 1 Angstrom when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-4.

In each of these embodiments, the three dimensional structure of the polypeptide may be determined using computational modeling or alternatively, the three dimensional structure of the polypeptide is determined using crystallographically-determined structural data.

In one embodiment of any embodiment or combination of embodiments disclosed herein, X1, X2, X3, and X4 are alpha-helical domains. In another embodiment, the amino acid length of each of X1, X2, X3 and X4 is independently at least about 8, 10, 12, 14, 16, 19, or more amino acids in length. In other embodiments, the amino acid length of each of X1, X2, X3 and X4 is independently no more than 1000, 500, 400, 300, 200, 100, or 50 amino acids in length. In various further embodiments, the amino acid length of each of X1, X2, X3 and X4 is independently between about 8-1000, 8-500, 8-400, 8-300, 8-200, 8-100, 8-50, 10-1000, 10-500, 10-400, 10-300, 10-200, 10-100, 10-50, 12-1000, 12-500, 12-400, 12-300, 12-200, 12-100, 12-50, 14-1000, 14-500, 14-400, 14-300, 14-200, 14-100, 14-50, 16-1000, 16-500, 16-400, 16-300, 16-200, 16-100, 16-50, 19-1000, 19-500, 19-400, 19-300, 19-200, 19-100, or about 19-50 amino acids in length.

In another embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, X1 binds to the beta and the gamma subunit of the human IL-2 receptor. In another embodiment of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, X2 does not bind to the human IL-2 receptor. In another embodiment, of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, X3 binds to the beta subunit of the human IL-2 receptor. In a further embodiment of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, X4 binds to the gamma subunit of the human IL-2 receptor. In another embodiment or the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptide does not bind to the alpha subunit of the human or murine IL-2 receptor. In one embodiment, binding to the receptors is specific binding as determined by surface plasmon resonance at biologically relevant concentrations. In another embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein that bind to the IL-2 receptor

$\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$) do so with a binding affinity of 200 nm or less, 100 nm or less, 50 nM or less, or 25 nM or less. In a further embodiment of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptide's affinity for the human and mouse IL-2 receptors is about equal to or greater than that of native IL-2.

In one embodiment of the IL-4 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein that bind to the IL-4 receptor $\alpha\gamma_c$heterodimer (IL-4R$\alpha\gamma_c$) do so with a binding affinity of 200 nm or less, 100 nm or less, 50 nM or less, or 25 nM or less. In another embodiment of the IL-4 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptide's affinity for the human and mouse IL-4 receptors is about equal to or greater than that of native IL-4.

In one embodiment of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptide stimulates STAT5 phosphorylation in cells expressing the IL-2 receptor with potency about equal to or greater than native IL-2. In another embodiment of the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptide stimulates STAT5 phosphorylation in cells expressing the IL-2 receptor with potency about equal to or greater than native IL-2 in cells expressing IL-2 receptor $\beta\gamma_c$ heterodimer but lacking the IL-2 receptor α.

In another embodiment, the IL-2 mimetic polypeptides of any embodiment or combination of embodiments disclosed herein demonstrate thermal stability about equal to or greater than the thermal stability of native IL-2.

In a further embodiment, the polypeptides of any embodiment or combination of embodiments disclosed herein, the polypeptides maintain or recover at least 70%, 80%, or 90% of their folded structure after thermal stability testing, and/or maintain or recover at least 80% of their ellipticity spectrum after thermal stability testing, and/or maintain or recover at least 70% or 80% of their activity after thermal stability testing. In one embodiment, such activity is determined by a STAT5 phosphorylation assay. In another embodiment, thermal stability is measured by circular dichroism (CD) spectroscopy at 222 nM. In a further embodiment, the thermal stability test comprises heating the polypeptide from 25 degrees Celsius to 95 degrees Celsius in a one hour time frame, cooling the polypeptide to 25 degrees Celsius in a 5 minute time frame and monitoring ellipticity at 222 nm.

The polypeptides described herein may be chemically synthesized or recombinantly expressed (when the polypeptide is genetically encodable). The polypeptides may be linked to other compounds, such as stabilization compounds to promote an increased half-life in

vivo, including but not limited to albumin, PEGylation (attachment of one or more polyethylene glycol chains), HESylation, PASylation, glycosylation, or may be produced as an Fc-fusion or in deimmunized variants. Such linkage can be covalent or non-covalent. For example, addition of polyethylene glycol ("PEG") containing moieties may comprise attachment of a PEG group linked to maleimide group ("*PEG*-MAL") to a cysteine residue of the polypeptide. Suitable examples of *PEG*-MAL are methoxy *PEG*-MAL 5 kD; methoxy *PEG*-MAL 20 kD; methoxy (*PEG*)2-MAL 40 kD; methoxy *PEG*(MAL)2 5 kD; methoxy *PEG*(MAL)2 20 kD; methoxy *PEG*(MAL)2 40 kD; or any combination thereof. See also US Patent No. 8,148,109. In other embodiments, the PEG may comprise branched chain PEGs and/or multiple PEG chains.

In one embodiment, the stabilization compound, including but not limited to a PEG-containing moiety, is linked at a cysteine residue in the polypeptide. In another embodiment, the cysteine residue is present in the X2 domain. In some embodiments, the cysteine residue is present, for example, in any one of a number of positions in the X2 domain. In some such embodiments, the X2 domain is at least 19 amino acids in length and the cysteine residue is at positions 1, 2, 5, 9 or 16 relative to those 19 amino acids. In a further embodiment, the stabilization compound, including but not limited to a PEG-containing moiety, is linked to the cysteine residue via a maleimide group, including but not limited to linked to a cysteine residue present at amino acid residue 62 relative to SEQ ID NO:90.

In some aspects, the polypeptide is a Neo-2/15 polypeptide and an amino acid of Neo-2/15 is mutated to a cysteine residue for attachment of a stabilization moiety (e.g., PEG-containing moiety) thereto. In some aspects, the polypeptide is a Neo-2/15 polypeptide and the amino acid at positions 50, 53, 62, 69, 73, 82, 56, 58, 59, 66, 77, or 85 or a combination thereof relative to SEQ ID NO:90, 181, or 247 is mutated to a cysteine residue for attachment of a stabilization moiety (e.g., PEG-containing moiety) thereto. Accordingly, in a further embodiment, the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence of SEQ ID NO:90, 181, or 247 [Neo-2/15], and wherein one, two, three, four, five, or all six of the following mutations are present:

R50C;

E53C;

E62C;

E69C;

R73C; and/or

E82C.

In a further embodiment, the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence of SEQ ID NO:90, 181, or 247, and wherein one, two, three, four, five, six, seven, eight, nine, ten, eleven, or all twelve of the following mutations are present

D56C;

K58C;

D59C;

R66C;

T77C;

E85C;

R50C;

E53C;

E62C;

E69C;

R73C; and/or

E82C.

In a further embodiment, the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 190-243.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:190 and 217. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:190.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:191 and 218. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%,

55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:191.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:192 and 219. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:192 .

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:193 and 220. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:193.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:194 and 221. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:194.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:195 and 222. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:195.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:196 and 223. In one aspect, the

polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:196.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:197 and 224. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:197.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:198 and 225. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:198.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:199 and 226. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:199.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence selected from the group consisting of SEQ ID NO:200 and 227. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:200.

In one embodiment, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid

sequence selected from the group consisting of SEQ ID NO:201 and 228. In one aspect, the polypeptide comprises a polypeptide at least at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical along its length to the amino acid sequence of SEQ ID NO:201.

In another embodiment, the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence selected from the group consisting of SEQ ID NO:195, 207, 214, 222, 234, and 241; or wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence selected from the group consisting of SEQ ID NO:195, 207, and 214.

In a further embodiment, the polypeptide further comprises a targeting domain. In this embodiment, the polypeptide can be directed to a target of interest. The targeting domain may be covalently or non-covalently bound to the polypeptide. In embodiments where the targeting domain is non-covalently bound to the polypeptide, any suitable means for such non-covalent binding may be used, including but not limited to streptavidin-biotin linkers.

In another embodiment, the targeting domain, when present, is a translational fusion with the polypeptide. In this embodiment, the polypeptide and the targeting domain may directly abut each other in the translational fusion or may be linked by a polypeptide linker suitable for an intended purpose. Exemplary such linkers include, but are not limited, to those disclosed in WO2016178905, WO2018153865 (in particular, at page 13), and WO 2018170179 (in particular, at paragraphs [0316]-[0317]). In other embodiments, suitable linkers include, but are not limited to peptide linkers, such as GGGGG (SEQ ID NO: 95), GSGGG (SEQ ID NO: 96), GGGGGG (SEQ ID NO: 97), GGSGGG (SEQ ID NO: 98), GGSGGSGGGSGGSGSG (SEQ ID NO: 99), GSGGSGGGSGGSGSG (SEQ ID NO: 100), GGSGGSGGGSGGSGGGGSGGSGGGSGGGGS (SEQ ID NO: 101), and $[GGGGX]_n$ (SEQ ID NO: 102), where X is Q, E or S and n is 2-5.

The targeting domains are polypeptide domains or small molecules that bind to a target of interest. In one non-limiting embodiment, the targeting domain binds to a cell surface protein; in this embodiment, the cell may be any cell type of interest that includes a surface protein that can be bound by a suitable targeting domain. In one embodiment, the cell surface proteins are present on the surface of cells selected from the group consisting of tumor cells, tumor vascular component cells, tumor microenvironment cells (e.g. fibroblasts, infiltrating immune cells, or stromal elements), other cancer cells and immune cells

(including but not limited to CD8+ T cells, T-regulatory cells, dendritic cells, NK cells, or macrophages). When the cell surface protein is on the surface of a tumor cell, vascular component cell, or tumor microenvironment cell (e.g. fibroblasts, infiltrating immune cells, or stromal elements), any suitable tumor cell, vascular component cell, or tumor microenvironment cell surface marker may be targeted, including but not limited to EGFR, EGFRvIII, Her2, HER3, EpCAM, MSLN, MUC16, PSMA, TROP2, ROR1, RON, PD-L1, CD47, CTLA-4, CD5, CD19, CD20, CD25, CD37, CD30, CD33, CD40, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, B7-H3, B7-DC, HLD-DR, carcinoembryonic antigen (CEA), TAG-72, MUC1, folate-binding protein, A33, G250, prostate-specific membrane antigen (PSMA), ferritin, GD2, GD3, GM2, Le^y, CA-125, CA19-9, epidermal growth factor, p185HER2, IL-2 receptor, EGFRvIII (de2-7 EGFR), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, avB3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, MAGE A3, p53 nonmutant, NY-ESO-1, MelanA/MART1, Ras mutant, gp100, p53 mutant, PR1, bcr-abl, tyronsinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B 1, polysialic acid, MYCN, RhoC, TRP-2, fucosyl GM1, mesothelin (MSLN), PSCA, MAGE Al, sLe(animal), CYP1B1, PLAV1, GM3, BORIS, Tn, GloboH, ETV6-AML, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TESL Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, Legumain, Tie 3, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, ROR2, TRAIL1, MUC16, MAGE A4, MAGE C2, GAGE, EGFR, CMET, HER3, MUC15, CA6, NAPI2B, TROP2, CLDN6, CLDN16, CLDN18.2, CLorf186, RON, LY6E, FRA, DLL3, PTK7, STRA6, TMPRSS3, TMPRSS4, TMEM238, UPK1B, VTCN1, LIV1, ROR1, and Fos-related antigen 1.

In other embodiments, when the cell surface protein is on the surface of a tumor cell, vascular component cell, or tumor microenvironment cell (e.g. fibroblasts, infiltrating immune cells, or stromal elements), any suitable tumor cell, vascular component cell, or tumor microenvironment cell surface marker may be targeted, including but not limited to targets in the following list:

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM.sub.--001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM.sub.--003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM.sub.--012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM.sub.--005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM.sub.--006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm. 42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM.sub.--017763);

(11) STEAP2 (HGNC.sub.--8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM.sub.--017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP.sub.--003203 or NM.sub.--003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR(C3d/Epstein Barr virus receptor) or Hs. 73792, Genbank accession no. M26004);

(15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM.sub.--000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_--030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20R.alpha. (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) Ephb2R (Genbank accession no. NM_--004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763);

(26) BAFF-R (Genbank accession no. NP_--443177.1);

(27) CD22 (Genbank accession no. NP-001762.1);

(28) CD79a (CD79A, CD79.alpha., immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_--001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_--001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_--002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_--002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_--001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_--005573.1);

(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_--443170.1); or

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_--112571.1).

In another embodiment, the targeting domain binds to immune cell surface markers. In this embodiment, the target may be cell surface proteins on any suitable immune cell, including but not limited to CD8+ T cells, T-regulatory cells, dendritic cells, NK cells or macrophages. The targeting domain may target any suitable immune cell surface marker

(whether an endogenous or an engineered immune cell, including but not limited to engineered CAR-T cells), including but not limited to CD3, CD4, CD8, CD19, CD20, CD21, CD25, CD37, CD30, CD33, CD40, CD68, CD123, CD254, PD-1, B7-H3, and CTLA-4. In another embodiment, the targeting domain binds to PD-1, PDL-1, CTLA-4, TROP2, B7-H3, CD33, CD22, carbonic anhydrase IX, CD123, Nectin-4, tissue factor antigen, CD154, B7-H3, B7-H4, FAP (fibroblast activation protein) or MUC16, and/or wherein the targeting domain binds to PD-1, PDL-1, CTLA-4, TROP2, B7-H3, CD33, CD22, carbonic anhydrase IX, CD123, Nectin-4, tissue factor antigen, CD154, B7-H3, B7-H4, FAP (fibroblast activation protein) or MUC16.

In all these embodiments, the targeting domains can be any suitable polypeptides that bind to targets of interest and can be incorporated into the polypeptide of the disclosure. In non-limiting embodiments, the targeting domain may include but is not limited to an scFv, a F(ab), a F(ab')$_2$, a B cell receptor (BCR), a DARPin, an affibody, a monobody, a nanobody, diabody, an antibody (including a monospecific or bispecific antibody); a cell-targeting oligopeptide including but not limited to RGD integrin-binding peptides, de novo designed binders, aptamers, a bicycle peptide, conotoxins, small molecules such as folic acid, and a virus that binds to the cell surface.

In another embodiment, the polypeptides include at least one disulfide bond (i.e.: 1, 2, 3, 4, or more disulfide bonds). Any suitable disulfide bonds may be used, such as disulfide bonds linking two different helices. In one embodiment, the disulfide bonds include a disulfide bond linking helix 1 (X1) and helix 4 (X4). The disulfide bond may, for example, improve the thermal stability of the polypeptide as compared to a substantially similar polypeptide with no disulfide bond linking two domains together.

The polypeptides and peptide domains of the invention may include additional residues at the N-terminus, C-terminus, or both that are not present in the polypeptides or peptide domains of the disclosure; these additional residues are not included in determining the percent identity of the polypeptides or peptide domains of the disclosure relative to the reference polypeptide. Such residues may be any residues suitable for an intended use, including but not limited to detection tags (i.e.: fluorescent proteins, antibody epitope tags, etc.), adaptors, ligands suitable for purposes of purification (His tags, etc.), other peptide domains that add functionality to the polypeptides, etc. Residues suitable for attachment of such groups may include cysteine, lysine or p-acetylphenylalanine residues or can be tags, such as amino acid tags suitable for reaction with transglutaminases as disclosed in U.S. Patent Nos. 9,676,871 and 9,777,070.

In a further aspect, the present invention provides nucleic acids, including isolated nucleic acids, encoding a polypeptide of the present invention that can be genetically encoded. The isolated nucleic acid sequence may comprise RNA or DNA. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the polypeptides of the invention.

In another aspect, the present invention provides recombinant expression vectors comprising the isolated nucleic acid of any aspect of the invention operatively linked to a suitable control sequence. "Recombinant expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the invention are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors include but are not limited to, plasmid and viral-based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In various embodiments, the expression vector may comprise a plasmid, viral-based vector (including but not limited to a retroviral vector or oncolytic virus), or any other suitable expression vector. In some embodiments, the expression vector can be administered in the methods of the disclosure to express the polypeptides *in vivo* for therapeutic benefit. In non-limiting embodiments, the expression vectors can be used to transfect or transduce cell therapeutic targets (including but not limited to CAR-T cells or tumor cells) to effect the therapeutic methods disclosed herein.

In a further aspect, the present disclosure provides host cells that comprise the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably engineered to incorporate the expression vector of the invention, using techniques including but not limited to bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press); *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY)). A method of producing a polypeptide according to the invention is an additional part of the invention. The method comprises the steps of (a) culturing a host according to this aspect of the invention under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide. The expressed polypeptide can be recovered from the cell free extract, but preferably they are recovered from the culture medium.

In a further aspect, the present disclosure provides antibodies that selectively bind to the polypeptides of the disclosure. The antibodies can be polyclonal, monoclonal antibodies, humanized antibodies, and fragments thereof, and can be made using techniques known to those of skill in the art. As used herein, "selectively bind" means preferential binding of the antibody to the polypeptide of the disclosure, as opposed to one or more other biological molecules, structures, cells, tissues, etc., as is well understood by those of skill in the art.

In another aspect, the present disclosure provides pharmaceutical compositions, comprising one or more polypeptides, nucleic acids, expression vectors, and/or host cells of the disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the disclosure can be used, for example, in the methods of the disclosure described below. The pharmaceutical composition may comprise in addition to the polypeptide of the disclosure (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer.

In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the

pharmaceutical composition includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleaste, or a combination thereof. The pharmaceutical composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical composition additionally includes a stabilizer, e.g., a molecule which, when combined with a protein of interest substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The polypeptides, nucleic acids, expression vectors, and/or host cells may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable for an intended use.

In a further aspect, the present disclosure provides methods for treating and/or limiting cancer, comprising administering to a subject in need thereof a therapeutically effective amount of one or more polypeptides, nucleic acids, expression vectors, and/or host cells of the disclosure, salts thereof, conjugates thereof, or pharmaceutical compositions thereof, to treat and/or limit the cancer. When the method comprises treating cancer, the one or more polypeptides, nucleic acids, expression vectors, and/or host cells are administered to a subject that has already been diagnosed as having cancer. As used herein, "treat" or "treating" means accomplishing one or more of the following: (a) reducing the size or volume of tumors and/or metastases in the subject; (b) limiting any increase in the size or volume of tumors and/or metastases in the subject; (c) increasing survival; (d) reducing the severity of symptoms associated with cancer; (e) limiting or preventing development of symptoms associated with cancer; and (f) inhibiting worsening of symptoms associated with cancer.

When the method comprises limiting development of cancer, the one or more polypeptides, nucleic acids, expression vectors, and/or host cells are administered prophylactically to a subject that is not known to have cancer, but may be at risk of cancer. As used herein, “limiting” means to limit development of cancer in subjects at risk of cancer,

including but not limited to subjects with a family history of cancer, subjects genetically predisposed to cancer, subjects that are symptomatic for cancer, etc.

The methods can be used to treat or limit development of any suitable cancer, including but not limited to colon cancer, melanoma, renal cell cancer, head and neck squamous cell cancer, gastric cancer, urothelial carcinoma, Hodgkin lymphoma, non-small cell lung cancer, small cell lung cancer, hepatocellular carcinoma, pancreatic cancer, Merkel cell carcinoma colorectal cancer, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, ovarian cancer, cervical cancer, and any tumor types selected by a diagnostic test, such as microsatellite instability, tumor mutational burden, PD-L1 expression level, or the immunoscore assay (as developed by the Society for Immunotherapy of Cancer).

The subject may be any subject that has or is at risk of developing cancer. In one embodiment, the subject is a mammal, including but not limited to humans, dogs, cats, horses, cattle, etc.

In a further aspect, the present disclosure provides methods for modulating an immune response in a subject by administering to a subject a polypeptide, recombinant nucleic acid, expression vector, recombinant host cell, or the pharmaceutical composition of the present disclosure.

As used herein, an "immune response" being modulated refers to a response by a cell of the immune system, such as a B cell, T cell (CD4 or CD8), regulatory T cell, antigen-presenting cell, dendritic cell, monocyte, macrophage, NKT cell, NK cell, basophil, eosinophil, or neutrophil, to a stimulus. In some embodiments, the response is specific for a particular antigen (an "antigen-specific response"), and refers to a response by a CD4 T cell, CD8 T cell, or B cell via their antigen-specific receptor. In some embodiments, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. Such responses by these cells can include, for example, cytotoxicity, proliferation, cytokine or chemokine production, trafficking, or phagocytosis, and can be dependent on the nature of the immune cell undergoing the response. In some embodiments of the compositions and methods described herein, an immune response being modulated is T-cell mediated.

In some aspects, the immune response is an anti-cancer immune response. In some such aspects, an IL-2 mimetic described herein is administered to a subject having cancer to modulate an anti-cancer immune response in the subject.

In some aspects, the immune response is a tissue reparative immune response. In some such aspects, an IL-4 mimetic described here is administered to a subject in need thereof to modulate a tissue reparative immune response in the subject.

In some aspects, the immune response is a wound healing immune response. In some such aspects, an IL-4 mimetic described here is administered to a subject in need thereof to modulate a wound healing immune response in the subject.

In some aspects, methods are provided for modulating an immune response to a second therapeutic agent in a subject. In some such aspects, the method comprises administering a polypeptide of the present disclosure in combination with an effective amount of the second therapeutic agent to the subject. The second therapeutic agent can be, for example, a chemotherapeutic agent or an antigen-specific immunotherapeutic agent. In some aspects, the antigen-specific immunotherapeutic agent comprises chimeric antigen receptor T cells (CAR-T cells). In some aspects, the polypeptide of the present disclosure enhances the immune response of the subject to the therapeutic agent. The immune response can be enhanced, for example, by improving the T cell response (including CAR-T cell response), augmenting the innate T cell immune response, decreasing inflammation, inhibiting T regulatory cell activity, or combinations thereof.

In some aspects, a cytokine mimetic of the present invention, e.g., an IL-4 mimetic as described herein, will be impregnated to or otherwise associated with a biomaterial and the biomaterial will be introduced to a subject. In some aspects, the biomaterial will be a component of an implantable medical device and the device will be, for example, coated with the biomaterial. Such medical devices include, for example, vascular and arterial grafts. IL-4 and/or IL-4 associated biomaterials can be used, for example, to promote wound healing and/or tissue repair and regeneration.

As used herein, a "therapeutically effective amount" refers to an amount of the polypeptide, nucleic acids, expression vectors, and/or host cells that is effective for treating and/or limiting cancer. The polypeptides, nucleic acids, expression vectors, and/or host cells are typically formulated as a pharmaceutical composition, such as those disclosed above, and can be administered via any suitable route, including but not limited to orally, by inhalation spray, ocularly, intravenously, subcutaneously, intraperitoneally, and intravesicularly in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. In one particular embodiment, the polypeptides, nucleic acids, expression vectors, and/or host cells are administered mucosally, including but not limited to intraocular, inhaled, or intranasal administration. In another particular embodiment, the

polypeptides, nucleic acids, expression vectors, and/or host cells are administered orally. Such particular embodiments can be administered via droplets, nebulizers, sprays, or other suitable formulations.

Any suitable dosage range may be used as determined by attending medical personnel. Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). A suitable dosage range for the polypeptides may, for instance, be 0.1 ug/kg-100 mg/kg body weight; alternatively, it may be 0.5 ug/kg to 50 mg/kg; 1 ug/kg to 25 mg/kg, or 5 ug/kg to 10 mg/kg body weight. In some embodiments, the recommended dose could be lower than 0.1 mcg/kg, especially if administered locally. In other embodiments, the recommended dose could be based on weight/m^2 (i.e. body surface area), and/or it could be administered at a fixed dose (e.g., .05-100 mg). The polypeptides, nucleic acids, expression vectors, and/or host cells can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by an attending physician.

The polypeptides, nucleic acids, expression vectors, and/or host cells made be administered as the sole prophylactic or therapeutic agent, or may be administered together with (i.e.: combined or separately) one or more other prophylactic or therapeutic agents, including but not limited to tumor resection, chemotherapy, radiation therapy, immunotherapy, etc.

Example Computing Environment

Figure 22 is a block diagram of an example computing network. Some or all of the above-mentioned techniques disclosed herein, such as but not limited to techniques disclosed as part of and/or being performed by software, the Rosetta software suite, RosettaScripts, PyRosetta, Rosetta applications, and/or other herein-described computer software and computer hardware, can be part of and/or performed by a computing device. For example, FIG X1 shows protein design system 102 configured to communicate, via network 106, with client devices 104a, 104b, and 104c and protein database 108. In some embodiments, protein design system 102 and/or protein database 108 can be a computing device configured to perform some or all of the herein described methods and techniques, such as but not limited to, method 300 and functionality described as being part of or related to Rosetta. Protein database 108 can, in some embodiments, store information related to and/or used by Rosetta.

Network 106 may correspond to a LAN, a wide area network (WAN), a corporate intranet, the public Internet, or any other type of network configured to provide a

communications path between networked computing devices. Network 106 may also correspond to a combination of one or more LANs, WANs, corporate intranets, and/or the public Internet.

Although Figure 22 only shows three client devices 104a, 104b, 104c, distributed application architectures may serve tens, hundreds, or thousands of client devices. Moreover, client devices 104a, 104b, 104c (or any additional client devices) may be any sort of computing device, such as an ordinary laptop computer, desktop computer, network terminal, wireless communication device (e.g., a cell phone or smart phone), and so on. In some embodiments, client devices 104a, 104b, 104c can be dedicated to problem solving / using the Rosetta software suite. In other embodiments, client devices 104a, 104b, 104c can be used as general purpose computers that are configured to perform a number of tasks and need not be dedicated to problem solving / using the Rosetta software suite. In still other embodiments, part or all of the functionality of protein design system 102 and/or protein database 108 can be incorporated in a client device, such as client device 104a, 104b, and/or 104c.

Computing Environment Architecture

Figure 23A is a block diagram of an example computing device (e.g., system) In particular, computing device 200 shown in Figure 23A can be configured to: include components of and/or perform one or more functions of some or all of the herein described methods and techniques, such as but not limited to, method 300 and functionality described as being part of or related to Rosetta. Computing device 200 may include a user interface module 201, a network-communication interface module 202, one or more processors 203, data storage 204, and protein synthesis device 220, all of which may be linked together via a system bus, network, or other connection mechanism 205.

User interface module 201 can be operable to send data to and/or receive data from external user input/output devices. For example, user interface module 201 can be configured to send and/or receive data to and/or from user input devices such as a keyboard, a keypad, a touch screen, a computer mouse, a track ball, a joystick, a camera, a voice recognition module, and/or other similar devices. User interface module 201 can also be configured to provide output to user display devices, such as one or more cathode ray tubes (CRT), liquid crystal displays (LCD), light emitting diodes (LEDs), displays using digital light processing (DLP) technology, printers, light bulbs, and/or other similar devices, either now known or later developed. User interface module 201 can also be configured to generate audible output(s), such as a speaker, speaker jack, audio output port, audio output device, earphones, and/or other similar devices.

Network-communications interface module 202 can include one or more wireless interfaces 207 and/or one or more wireline interfaces 208 that are configurable to communicate via a network, such as network 106 shown in Figure 22. Wireless interfaces 207 can include one or more wireless transmitters, receivers, and/or transceivers, such as a Bluetooth transceiver, a Zigbee transceiver, a Wi-Fi transceiver, a WiMAX transceiver, and/or other similar type of wireless transceiver configurable to communicate via a wireless network. Wireline interfaces 208 can include one or more wireline transmitters, receivers, and/or transceivers, such as an Ethernet transceiver, a Universal Serial Bus (USB) transceiver, or similar transceiver configurable to communicate via a twisted pair, one or more wires, a coaxial cable, a fiber-optic link, or a similar physical connection to a wireline network.

In some embodiments, network communications interface module 202 can be configured to provide reliable, secured, and/or authenticated communications. For each communication described herein, information for ensuring reliable communications (*i.e.*, guaranteed message delivery) can be provided, perhaps as part of a message header and/or footer (*e.g.*, packet/message sequencing information, encapsulation header(s) and/or footer(s), size/time information, and transmission verification information such as CRC and/or parity check values). Communications can be made secure (*e.g.*, be encoded or encrypted) and/or decrypted/decoded using one or more cryptographic protocols and/or algorithms, such as, but not limited to, DES, AES, RSA, Diffie-Hellman, and/or DSA. Other cryptographic protocols and/or algorithms can be used as well or in addition to those listed herein to secure (and then decrypt/decode) communications.

Processors 203 can include one or more general purpose processors and/or one or more special purpose processors (*e.g.*, digital signal processors, application specific integrated circuits, etc.). Processors 203 can be configured to execute computer-readable program instructions 206 contained in data storage 204 and/or other instructions as described herein. Data storage 204 can include one or more computer-readable storage media that can be read and/or accessed by at least one of processors 203. The one or more computer-readable storage media can include volatile and/or non-volatile storage components, such as optical, magnetic, organic or other memory or disc storage, which can be integrated in whole or in part with at least one of processors 203. In some embodiments, data storage 204 can be implemented using a single physical device (*e.g.*, one optical, magnetic, organic or other memory or disc storage unit), while in other embodiments, data storage 204 can be implemented using two or more physical devices.

Data storage 204 can include computer-readable program instructions 206 and perhaps additional data. For example, in some embodiments, data storage 204 can store part or all of data utilized by a protein design system and/or a protein database; *e.g.*, protein designs system 102, protein database 108. In some embodiments, data storage 204 can additionally include storage required to perform at least part of the herein-described methods and techniques and/or at least part of the functionality of the herein-described devices and networks.

In some examples, computing device 200 includes protein synthesis device 220. Protein synthesis device can synthesize (or generate polypeptides based on input data provided to protein synthesis device 220 using commands and/or data provided by processors 203 and/or data storage 204. For example, part or all of the functionality of protein synthesis device 220 can be performed by a semi-automated or an automated peptide synthesizer.

Figure 23B depicts a network 106 of computing clusters 209a, 209b, 209c arranged as a cloud-based server system in accordance with an example embodiment. Data and/or software for protein design system 102 can be stored on one or more cloud-based devices that store program logic and/or data of cloud-based applications and/or services. In some examples, protein design system 102 can be a single computing device residing in a single computing center. In other examples, protein design system 102 can include multiple computing devices in a single computing center, or even multiple computing devices located in multiple computing centers located in diverse geographic locations.

In some examples, data and/or software for protein design system 102 can be encoded as computer readable information stored in tangible computer readable media (or computer readable storage media) and accessible by client devices 104a, 104b, and 104c, and/or other computing devices. In some examples, data and/or software for protein design system 102 can be stored on a single disk drive or other tangible storage media, or can be implemented on multiple disk drives or other tangible storage media located at one or more diverse geographic locations.

Figure 23B depicts a cloud-based server system in accordance with an example embodiment. In Figure 23B, the functions of protein design system 102 can be distributed among three computing clusters 209a, 209b, and 209c. Computing cluster 209a can include one or more computing devices 200a, cluster storage arrays 210a, and cluster routers 211a connected by a local cluster network 212a. Similarly, computing cluster 209b can include one or more computing devices 200b, cluster storage arrays 210b, and cluster routers 211b connected by a local cluster network 212b. Likewise, computing cluster 209c can include one

or more computing devices 200c, cluster storage arrays 210c, and cluster routers 211c connected by a local cluster network 212c.

In some examples, each of the computing clusters 209a, 209b, and 209c can have an equal number of computing devices, an equal number of cluster storage arrays, and an equal number of cluster routers. In other examples, however, each computing cluster can have different numbers of computing devices, different numbers of cluster storage arrays, and different numbers of cluster routers. The number of computing devices, cluster storage arrays, and cluster routers in each computing cluster can depend on the computing task or tasks assigned to each computing cluster.

In computing cluster 209a, for example, computing devices 200a can be configured to perform various computing tasks of protein design system 102. In one example, the various functionalities of protein design system 102 can be distributed among one or more of computing devices 200a, 200b, and 200c. Computing devices 200b and 200c in computing clusters 209b and 209c can be configured similarly to computing devices 200a in computing cluster 209a. On the other hand, in some examples, computing devices 200a, 200b, and 200c can be configured to perform different functions.

In some examples, computing tasks and stored data associated with protein design system 102 can be distributed across computing devices 200a, 200b, and 200c based at least in part on the processing requirements of protein design system 102, the processing capabilities of computing devices 200a, 200b, and 200c, the latency of the network links between the computing devices in each computing cluster and between the computing clusters themselves, and/or other factors that can contribute to the cost, speed, fault-tolerance, resiliency, efficiency, and/or other design goals of the overall system architecture.

The cluster storage arrays 210a, 210b, and 210c of the computing clusters 209a, 209b, and 209c can be data storage arrays that include disk array controllers configured to manage read and write access to groups of hard disk drives. The disk array controllers, alone or in conjunction with their respective computing devices, can also be configured to manage backup or redundant copies of the data stored in the cluster storage arrays to protect against disk drive or other cluster storage array failures and/or network failures that prevent one or more computing devices from accessing one or more cluster storage arrays.

Similar to the manner in which the functions of protein design system 102 can be distributed across computing devices 200a, 200b, and 200c of computing clusters 209a, 209b, and 209c, various active portions and/or backup portions of these components can be distributed across cluster storage arrays 210a, 210b, and 210c. For example, some cluster

storage arrays can be configured to store one portion of the data and/or software of protein design system 102, while other cluster storage arrays can store a separate portion of the data and/or software of protein design system 102. Additionally, some cluster storage arrays can be configured to store backup versions of data stored in other cluster storage arrays.

The cluster routers 211a, 211b, and 211c in computing clusters 209a, 209b, and 209c can include networking equipment configured to provide internal and external communications for the computing clusters. For example, the cluster routers 211a in computing cluster 209a can include one or more internet switching and routing devices configured to provide (i) local area network communications between the computing devices 200a and the cluster storage arrays 201a via the local cluster network 212a, and (ii) wide area network communications between the computing cluster 209a and the computing clusters 209b and 209c via the wide area network connection 213a to network 106. Cluster routers 211b and 211c can include network equipment similar to the cluster routers 211a, and cluster routers 211b and 211c can perform similar networking functions for computing clusters 209b and 209b that cluster routers 211a perform for computing cluster 209a.

In some examples, the configuration of the cluster routers 211a, 211b, and 211c can be based at least in part on the data communication requirements of the computing devices and cluster storage arrays, the data communications capabilities of the network equipment in the cluster routers 211a, 211b, and 211c, the latency and throughput of local networks 212a, 212b, 212c, the latency, throughput, and cost of wide area network links 213a, 213b, and 213c, and/or other factors that can contribute to the cost, speed, fault-tolerance, resiliency, efficiency and/or other design goals of the moderation system architecture.

Example Methods of Operation

Figure 24 is a flow chart of an example method 300. Method 300 can be carried out by a computing device, such as computing device 200 described in the context of at least FIG 2A. At least the examples of method 300 mentioned below are discussed above.

Method 300 can begin at block 310, where the computing device can determine a structure for a plurality of residues of a protein using a computing device, where the structure of the plurality of residues provides a particular receptor binding interface. As will be understood by the skilled practitioner, the determining of a structure for a plurality of residues of a protein where the structure of the plurality of residues provides a particular receptor binding interface is typically the identification of the original residues of a native protein that bind to a particular receptor binding interface whereas the plurality of designed residues are identified residues that can bind to the same receptor binding interface.

At block 320, the computing device can determine a plurality of designed residues using a mimetic design protocol, where the plurality of designed residues provide the particular receptor binding interface, and where the plurality of designed residues differ from the plurality of residues.

In some examples, determining the plurality of designed residues using the mimetic design protocol can include determining an idealized residue using a database of idealized residues, where the idealized residue is related to a designed residue of the plurality of designed residues. In some of these examples, determining the idealized residue using the database of idealized residues can include: retrieving one or more idealized fragments related to the idealized residue from the database of idealized residues; and determining the idealized residue by reconstructing the related designed residue using the one or more idealized fragments. In some of these examples, reconstructing the related designed residue using the one or more idealized fragments can include: reconnecting pairs of the one or more idealized fragments by: use of combinatorial fragment assembly of the pairs of the one or more idealized fragments; and using Cartesian-constrained backbone minimization to determine whether the pairs of the one or more idealized fragments link two or more of the plurality of designed residues. In some of these examples, reconstructing the related designed residue using the one or more idealized fragments can include: verifying that overlapping fragments of the idealized residue are idealized fragments using the database of idealized residues; verifying whether the idealized residue does not clash with a target receptor associated with the particular receptor binding interface; and after verifying that the idealized residue does not clash with a target receptor associated with the particular receptor binding interface, determining a most probable amino acid at each position of the idealized residue using the database of idealized residues. In some of these examples, determining the first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over the plurality of combinations can include: recombining the pairs of the one or more idealized fragments by combinatorially recombining the pairs of the one or more idealized fragments; and determining the first protein backbone for the protein using the recombined pairs of the one or more idealized fragments. In some of these examples, combinatorially recombining the pairs of the one or more idealized fragments can include ranking the pairs of the one or more idealized fragments based on an interconnection length between idealized fragments of the pairs of the one or more idealized fragments.

In other examples, determining the plurality of designed residues using the mimetic design protocol can include: determining an idealized residue using one or more parametric

equations that represent a shape of a designed residue of the plurality of designed residues; and determining a single fragment that closes the idealized residue with at least one designed residue of the plurality of designed residues. In some of these examples, the designed residue can include a helical structure, and the one or more parametric equations can include an equation related to phi and psi angles of the helical structure. In some of these examples, the equation related to phi and psi angles of the helical structure can include one or more terms related to an angular pitch of the phi and psi angles of the helical structure.

At block 330, the computing device can determine one or more connecting helix structures that connect the plurality of designed residues.

At block 340, the computing device can determine a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations.

At block 350, the computing device can design a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone.

At block 360, the computing device can generate an output related to at least the second protein backbone. In some examples, generating the output related to the second protein backbone for the protein can include designing one or more molecules based on the second protein backbone for the protein.

In other examples, generating the output related to the second protein backbone for the protein can include: generating a synthetic gene for the protein that is based the second protein backbone for the protein; expressing a particular protein *in vivo* using the synthetic gene; and purifying the particular protein. In some of these examples, expressing the particular protein sequence *in vivo* using the synthetic gene can include expressing the particular protein sequence in one or more *Escherichia coli* that include the synthetic gene.

In other examples, generating the output related to the second protein backbone for the protein can include generating one or more images that include at least part of the second protein backbone for the protein.

In other examples, the computing device can include a protein synthesis device; then, generating the output related to at least the second protein backbone for the protein can include synthesizing at least the second protein backbone for the protein using the protein synthesis device.

In one embodiment, the methods are for designing a protein mimetic, as exemplified herein.

Also included are non-naturally occurring proteins prepared by the computational methods described herein. The non-naturally occurring proteins can be cytokines, for example, non-naturally occurring IL-2 or IL-4 mimetics.

The particulars shown herein are by way of example and for purposes of illustrative discussion of embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

The above definitions and explanations are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

The above description provides specific details for a thorough understanding of, and enabling description for, embodiments of the disclosure. However, one skilled in the art will understand that the disclosure may be practiced without these details. In other instances, well-known structures and functions have not been shown or described in detail to avoid unnecessarily obscuring the description of the embodiments of the disclosure. The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

All of the references cited herein are incorporated by reference. Aspects of the disclosure can be modified, if necessary, to employ the systems, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated

with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

The above detailed description describes various features and functions of the disclosed systems, devices, and methods with reference to the accompanying figures. In the figures, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, figures, and claims are not meant to be limiting. Other embodiments can be utilized, and other changes can be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein.

With respect to any or all of the ladder diagrams, scenarios, and flow charts in the figures and as discussed herein, each block and/or communication may represent a processing of information and/or a transmission of information in accordance with example embodiments. Alternative embodiments are included within the scope of these example embodiments. In these alternative embodiments, for example, functions described as blocks, transmissions, communications, requests, responses, and/or messages may be executed out of order from that shown or discussed, including substantially concurrent or in reverse order, depending on the functionality involved. Further, more or fewer blocks and/or functions may be used with any of the ladder diagrams, scenarios, and flow charts discussed herein, and these ladder diagrams, scenarios, and flow charts may be combined with one another, in part or in whole.

A block that represents a processing of information may correspond to circuitry that can be configured to perform the specific logical functions of a herein-described method or technique. Alternatively or additionally, a block that represents a processing of information may correspond to a module, a segment, or a portion of program code (including related data). The program code may include one or more instructions executable by a processor for implementing specific logical functions or actions in the method or technique. The program code and/or related data may be stored on any type of computer readable medium such as a storage device including a disk or hard drive or other storage medium.

The computer readable medium may also include non-transitory computer readable media such as computer-readable media that stores data for short periods of time like register

memory, processor cache, and random access memory (RAM). The computer readable media may also include non-transitory computer readable media that stores program code and/or data for longer periods of time, such as secondary or persistent long term storage, like read only memory (ROM), optical or magnetic disks, compact-disc read only memory (CD-ROM), for example. The computer readable media may also be any other volatile or non-volatile storage systems. A computer readable medium may be considered a computer readable storage medium, for example, or a tangible storage device. Moreover, a block that represents one or more information transmissions may correspond to information transmissions between software and/or hardware modules in the same physical device. However, other information transmissions may be between software modules and/or hardware modules in different physical devices.

Numerous modifications and variations of the present disclosure are possible in light of the above teachings.

EXAMPLES

A computational approach for designing *de novo* cytokine mimetics is described that recapitulate the functional sites of the natural cytokines, but otherwise are unrelated in topology or amino acid sequence. This strategy was used to design *de novo* mimetics of IL-2 and interleukin-15 (IL-15) [15] that bind to the IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$) [16,17], but have no binding site for IL-2Rα or IL-15Rα. The designs are hyper-stable, bind to human and mouse IL-2R$\beta\gamma_c$ with higher affinity than the natural cytokines, and elicit downstream cell signaling independent of IL-2Rα and IL-15Rα. Crystal structures of an experimentally optimized mimetic, neoleukin-2/15, are very close to the design model and provide the first structural information on the murine IL-2R$\beta\gamma_c$ complex. Neoleukin-2/15 has highly efficacious therapeutic activity compared to IL-2 in murine models of melanoma and colon cancer, with reduced toxicity and no signs of immunogenicity. This strategy for building hyper-stable *de novo* mimetics can be readily applied to a multitude of natural cytokines and other signaling proteins, enabling the creation of superior therapeutic candidates with enhanced clinical profiles.

Because of the potent biological activity of natural protein hormones and cytokines, there have been extensive efforts to improve their potential therapeutic efficacy through protein engineering. Such efforts have sought to simplify manufacturing, extend half-life, and modulate receptor interactions [18–20]. However, there are inherent challenges to the

development of a new therapeutic when starting with a naturally occurring bioactive protein. First, most natural proteins are only marginally stable [21–25], hence amino acid substitutions aimed at increasing efficacy can decrease expression or cause aggregation, making manufacturing and storage difficult. More substantial changes, such as the deletion or fusion of functional or targeting domains, are often unworkable and can dramatically alter pharmacokinetic properties and tissue penetration [19]. Second, any immune response against the engineered variant may cross-react with the endogenous molecule [26–35] with potentially catastrophic consequences. A computational design approach was developed to generate analogues of natural proteins with improved therapeutic properties that circumvent these challenges, focusing effort on engineering *de novo* cytokine mimetics displaying specific subsets of the receptor binding interfaces optimal for treating disease.

Many cytokines interact with multiple different receptor subunits [15,16,36–39], and like most naturally occurring proteins, contain non-ideal structural features that compromise stability but are important for function. A computational protocol was developed in which the structural elements interacting with the desired receptor subunit(s) are fixed in space, and an idealized globular protein structure is built to support these elements. Previous efforts were extended using combinatorial fragment assembly to support short linear epitopes with parametric construction of disembodied helices coupled with knowledge-based loop closure (Figure 1a-b). The approach was tested by attempting to *de novo* design stable idealized proteins with interaction surfaces mimicking those of human IL-2 (hIL-2) and human IL-15 (hIL-15) for the human IL-2Rβγ$_c$ (hIL-2Rβγ$_c$), but entirely lacking the IL-2 receptor alpha (IL-2Rα) interaction surface. Previous efforts at removing the alpha interaction region in hIL-2, by either mutation [9,44,45] (e.g. F42A mutation of Super-2, also known as H9 [9]) or PEGylation (e.g. NKTR-214 [9,13]), have resulted in markedly reduced stability, binding and/or potency of the cytokine on the hIL-2Rβγ$_c$ receptor while failing to completely eliminate the alpha interaction.

Computational design of IL-2/IL-15 mimetics that bind and activate IL-2Rβγ$_c$: Native hIL-2 comprises four helices connected by long irregular loops. The N-terminal helix (H1) interacts with both the beta and gamma subunits of the IL-2 receptor, the third helix (H3) interacts with the beta subunit, and the C-terminal helix (H4) with the gamma subunit; the alpha subunit interacting surface is formed by the irregular second helix (H2) and two long loops, one connecting H1 to H2 and the other connecting H3 and H4. An idealized protein was designed that recapitulates the interface formed by H1, H3 and H4 with beta and gamma and to replace H2 with a regular helix that offers better packing. The helices H1, H3

and H4 (see Figure 1a) were used as a template for the binding site, while helix H2 was reconstructed (H2') using a database off highly-represented clustered-fragments (see Methods). Pairs of helices were connected with loops extracted from the same database (see Figure 1b), the resulting helical hairpins combined into fully connected backbones (see Figure 1c), and Rosetta [46–48] combinatorial flexible backbone sequence design calculations were carried out in the presence of hIL-2R$\beta\gamma_c$ (see Methods). The top four computational designs and eight single-disulfide stapled variations (see Table S1) were selected for experimental characterization by yeast display (see Methods). Eight designs were found to bind fluorescently-tagged beta-gamma chimeric IL-2 receptor at low-nanomolar concentrations. The best non-disulfide design (G1_neo2_40) was subjected to site saturation mutagenesis followed by selection and combination of affinity-increasing substitutions for the murine IL-2R$\beta\gamma_c$ (mIL-2R$\beta\gamma_c$, see Figure 10). Optimized designs (were expressed recombinantly in *E. coli* and found to elicit pSTAT5 signaling *in vitro* on IL-2-responsive murine cells at low-nanomolar or even picomolar concentrations (see Table E1), but had relatively low thermal stability (Tm ~<45°C, see Figures 14 and 15). To improve stability, the computational design protocol was repeated starting from the backbone of the highest affinity first round design (G1_neo2_40_1F, topology: H1->H4->H2'->H3), coupling the loop building process with parametric variation in helix length (+/- 8 amino acids, see Figure 1a bottom panel). This second approach improved the quality of the models by enabling the exploration of substantially more combinations of loops connecting each pair of helices. The fourteen best designs of the second generation, along with twenty-seven Rosetta sequence redesigns of G1_neo2_40_1F (see Table S3), were experimentally characterized and all but one were found to bind IL-2 receptor at low-nanomolar concentrations (Figure 1d, Table E1, and Figure 16). The three highest affinity and stability designs (one sequence redesign and two new mimetics) were subjected to site saturation mutagenesis for mIL-2R$\beta\gamma_c$ binding (Figures 11-13), followed by selection and combination of affinity-increasing substitutions for both human and mouse IL-2R$\beta\gamma_c$. The matured designs (see Table S4) showed enhanced binding while retaining hyper-stability (see Table E1). The top design, neoleukin-2/15 (also referred to herein as Neo-2/15), is a 100 residue protein with a new topology and sequence quite different from human or murine IL-2 (29% sequence identity to hIL-2 over 89 residues, and 16% sequence identity to mIL-2 over 76 aligned residues, in structural topology-agnostic based alignment, see Table E1).

Functional characterization of neoleukin-2/15: Neoleukin-2/15 binds with high affinity to human and mouse IL-2R$\beta\gamma_c$ (Kd ~38 nM and ~19 nM, respectively), but does not

interact with IL-2Rα (Figure 2a). The affinities of Neoleukin-2/15 for the human and mouse IL-2 receptors (IL-2Rβ and IL-2Rβγc) are significantly higher than those of the corresponding native IL-2 cytokines. In contrast with native IL-2, Neoleukin-2/15 elicits IL-2Rα-independent signaling in both human and murine IL-2-responsive cells (Figure 2b, *top*), and in murine primary T cells (Figure 2b, *bottom*). Neoleukin-2/15 activates IL-2Rα- cells more potently than native human or murine IL-2 in accordance with its higher binding affinity. In primary cells, neoleukin-2/15 is more active on IL-2Rα- cells and less active on IL-2Rα+ compared to Super-2, presumably due to its complete lack of IL-2Rα binding. Neoleukin-2/15 is hyper-stable (see Figure 17) and does not lose binding affinity for hIL-2Rβγc following incubation at 80°C for 2 hours, while hIL-2 and Super-2 are completely inactivated after 10 minutes (half-inactivation time = ~4.2 min and ~2.6 min, respectively, Figure 2c). Similarly, in *ex vivo* primary cell cultures, neoleukin-2/15 drove T cell survival effectively after being boiled for 60 minutes at 95°C, while these conditions inactivated both IL-2 and Super-2 (Figure 2c, bottom). Thermal denaturation studies were carried out on many other of the designed mimetics, demonstrating their thermal stability as well (see Figure 14-16). This unprecedented stability for a cytokine-like molecule, beyond eliminating the requirement for cold chain storage, suggests a robustness to mutations (see Figure 13 and 18-19), genetic fusions and chemical modification greatly exceeding that of native IL-2, which could contribute to the development of improved or new therapeutic properties (see Figure 7).

Structure of monomeric neoleukin-2/15 and ternary complex with mIL-2Rβγc: The X-ray crystal structure of neoleukin-2/15 was determined and found it to be very close to the computational design model ($r.m.s.d._{C\alpha}$ = 1.1-1.3 Å for the 6 copies in the asymmetric unit, Figure 3a). The crystal structure of neoleukin-2/15 in a ternary complex with murine IL-2Rβγc (Figure 3b, Table E2) was solved; this may be the first example in which a *de novo* designed protein enabled the structural determination of a previously unsolved natural receptor complex. The neoleukin-2/15 design model and crystal structure align with the mouse ternary complex structure with $r.m.s.d._{C\alpha}$ of 1.27 and 1.29 Å, respectively (Figure 3c). The order of helices in Neoleukin-2/15 (in IL-2 numbering) is H1->H3->H2'->H4 (see Figures 1a and 3a,d). The H1-H3 loop is disordered in the ternary complex, but helix H3 is in close agreement with the predicted structure; there is also an outward movement of helix H4 and the H2'-H4 loop compared to the monomeric structure (Figure 3c). Neoleukin-2/15 interacts with mIL-2Rβ via helices H1 and H3, and with γc via the H1 and H4 helices (Figure 3c), and these regions align closely with both the computational design model (Figure 3a) and

the monomeric crystal structure (Figure 3c). Structural alignment to the previously reported crystal structure of the hIL-2 receptor complex [49] reveals a close agreement between the helical backbones of Neoleukin-2/15 and hIL-2 in the binding site, despite the different topology of the two proteins (Figure 3d-e). Some side chain interactions between neoleukin-2/15 and mIL-2Rβγc are present in the hIL-2 - hIL-2Rβγ complex, while others such as L19Y, arose during the computational design process.

Therapeutic applications of neoleukin-2/15: The clinical use of IL-2 has been mainly limited by toxicity [50–52]. Although the interactions responsible for IL-2 toxicity in humans are incompletely understood, in murine models toxicity is T cell independent and ameliorated in animals deficient in the IL-2Rα chain (CD25+). Thus, many efforts have been directed to reengineer IL-2 to weaken interactions with IL-2Rα, but mutations in the CD25 binding site can be highly destabilizing [6]. The inherent low stability of IL-2 and its tightly evolved dependence on CD25 have been barriers to the translation of reengineered IL-2 compounds. Other efforts have focused on IL-15 [53,54], since it elicits similar signaling to IL-2 by dimerizing the IL-2Rβγc but has no affinity for CD25. However, IL-15 is dependent on trans presentation by the IL-15α (CD215) receptor that is displayed primarily on antigen-presenting cells and natural killer cells. The low stability of native IL-15 and its dependence on trans presentation have also been substantial barriers to reengineering efforts [53–55].

Dose escalation studies on naive mice show that mIL-2 preferentially expands regulatory T cells, consistent with preferential binding to CD25+ cells [41,56,57], while neoleukin-2/15 primarily drives expansion of $CD8^+$ T cells (Figure 4a) and does not *induce or minimally induces* expansion of regulatory T cells only at the highest dose tested. Similarly, in a murine model of airway inflammation, which normally induces a small percentage of tissue resident CD8+ T cells, neoleukin-2/15 produces an increase in $Thy1.2^+$ $CD44^+$ $CD8^+$ T cells without increasing $CD4^+$ $Foxp3^+$ antigen-specific Tregs in the lymphoid organs (Figure 4b).

De novo protein design allows the circumvention of the structural limitations of native cytokines, but there is a possibility of eliciting anti-drug antibodies. To test whether neoleukin-2/15 elicits an anti-drug response, tumor-bearing mice were treated daily with neoleukin-2/15 over a period of 2 weeks, and no evidence of anti-drug antibodies was observed in any of the treated animals (Figure 4c, left panel; a similar lack of immune response was observed for other de novo design therapeutic candidates [41]). Polyclonal antibodies against neoleukin-2/15 were produced by vaccinating mice with an inactive neoleukin-2/15 mutant (K.O. neoleukin) in complete Freund's adjuvant. These polyclonal

anti-neoleukin-2/15 antibodies did not cross react with human or mouse IL-2 (Figure 4c). The absence of binding to native IL-2 suggests that even if there is an immune response to neoleukin-2/15, this response is unlikely to cross-react with endogenous IL-2. Furthermore, since the sequence identity between neoleukin-2/15 and hIL-2 is low (<30%, see Table E1), an autoimmune response against host IL-2 is much more likely with previous engineered hIL-2 variants (e.g. Super-2, see Table E1) which differ from endogenous IL-2 by only a few mutations.

The therapeutic efficacy of neoleukin-2/15 was tested in the poorly immunogenic B16F10 melanoma and the more immunogenic CT26 colon cancer mouse models. Single agent treatment with neoleukin-2/15 led to dose-dependent delays in tumour growth in both cancer models. In CT26 colon cancer, single agent treatment showed improved efficacy to that observed for recombinant mIL-2 (Figure 4d and Figure 5). In B16F10 melanoma, co-treatment with the anti-melanoma antibody TA99 (anti-TRP1) led to significant tumour growth delays, while TA99 treatment alone had little effect (Figure 4e and Figure 6). In long term survival experiments (8 weeks), neoleukin-2/15 in combination with TA99 showed substantially reduced toxicity and an overall superior therapeutic effect compared to mIL-2 (Figure 4e). Mice treated with the combination mIL-2 and TA99 steadily lost weight and their overall health declined to the point of requiring euthanasia, whereas little decline was observed with the combination of neoleukin-2/15 and TA99 (Figure 4e). Consistent with a therapeutic benefit, neoleukin-2/15 treatment led to a significant increase in intratumoral CD8:T_{reg} ratios (see Figure 4f and Figure 5), which has been previously correlated with effective antitumor immune responses [58]. The increases of CD8:T_{reg} ratios by neoleukin-2/15 are dose and antigen dependent (Figure 4f); optimum therapeutic effects were obtained at higher doses and in combination with other immunotherapies (see Figure 6). Altogether, these data show that neoleukin-2/15 exhibits the predicted homeostatic benefit derived from its IL-2 like immunopotentiator activity, but without the adverse effects associated with $CD25^+$ preferential binding. These enhanced properties and low-toxicity may allow the routine use of neoleukin-2/15 for other immunotherapies where recombinant IL-2 is not broadly used. As an example of such a use, the potential application of neoleukin-2/15 to enhance CAR-T cell therapy (see Figure 8) was investigated. NSG mice inoculated with 0.5×10^6 RAJI tumor cells were left untreated, were treated with 0.8×10^6 anti-CD19 CAR-T cells (infused 7 days after inoculation of tumor cells), or were similarly treated with anti-CD19 CAR-T cells plus 20 μg/day of either human IL-2 or neoleukin-2/15 on days 8-14 after tumor inoculation. As expected, Neoleukin-2/15 significantly enhanced the anti-tumor effect of

CAR-T cell therapy in this model, slowing growth of the tumor and extending the survival of the mouse (data not shown).

De novo design of protein mimetics has the potential to transform the field of protein-based therapeutics, enabling the development of biosuperior molecules with enhanced therapeutic properties and reduced side-effects, not only for cytokines, but for virtually any biologically active molecule with known or accurately predictable structure. Because of the incremental nature of current traditional engineering approaches (e.g. 1-3 amino acid substitutions, chemical modification at a single site), most of the shortcomings of the parent molecule are inevitably passed on to the resulting engineered variants, often in an exacerbated form. By building mimetics *de novo*, these shortcomings can be completely avoided: unlike recombinant IL-2 and engineered variants of hIL-2, neoleukin-2/15 can be solubly expressed in *E. coli* (see Fig 17), retains activity at high temperature, does not interact with IL-2Rα and is robust to substantial sequence changes that allow the engineering of new functions (Fig. 7). Likely because of the small size and high stability of *de novo* designed proteins, immunogenicity appears to be low, and in contrast to incremental variants of hIL-2, any antibody response to the mimetic is unlikely to cross react with the natural parent cytokine. Because of their high stability and robustness, and their tailored interaction surfaces, designed mimetics are likely to be particularly powerful in next generation therapeutics which combine different protein functionalities, for example targeted versions of neoleukin-2/15.

Robust modularity of neoleukin-2/15. Disulfide-stapling and reengineering into an IL-4 mimetic: Neoleukin-2/15 is highly modular, allowing to easily tune its properties, such as increasing its stability or modify its binding preference. This modularity and robustness was taken advantage of by introducing, by computational design, stability enhancing single-disulfide staples that preserve the function of neoleukin-2/15 [59]. For this, two orthogonal strategies were used. First, a disulfide bridge was introduced by searching pairs of positions with favorable geometrical arrangements followed by flexible backbone minimization. The final design introduced a single disulfide between residues 38 and 75, which stabilizes helices H3 and H2. In the second approach, the N- and C-terminus of neoleukin-2/15 was remodeled to allow the introduction of a single-disulfide staple that encompasses the entire protein (added sequences CNSN (SEQ ID NO:260) and NFQC (SEQ ID NO:261), for N- and C-termini, respectively after removing terminal P and S residues, see Figure 18). Both disulfide stapling strategies increased the stability of neoleukin-2/15 (Tm > 95°C), while retaining its sequence and function mostly unaffected (see Figure 18). The

modularity properties of neoleukin-2/15 were used to modify its binding preference. All cytokines in the interleukin-2 family interact with the γ_c and share a common architecture. Therefore, it was hypothesized that neoleukin-2/15 could be transformed into another cytokine mimetic of the IL-2 family by changing only amino acids in the half of the binding-site that interacts with IL-2Rβ (helices H1 and H3). As proof of a concept, human interleukin-4 (hIL-4) was chosen as target, since it shares extensive structural homology with IL-2 and has potential applications in regenerative medicine [60,61]. Neo-2/15 was modified to bind to the human IL-4 receptor (comprising IL-4Rα and γ_c) and not to the human IL-2 receptor (comprising IL-2Rβ and γ_c) by aligning the Neo-2/15 model into the structure of human IL-4 bound to its IL-4 receptor, and mutating 14 residues in Neo-2/15 to match the amino-acids of IL-4 at those structural positions that mediate interactions between IL-4 and IL4r (Figure 7). Binding was further optimized by directed evolution using random mutagenesis and screening for high binding affinity variants, which introduced two additional amino acid substitutions and modified one of the fourteen original residues grafted from the IL-4 protein, thereby creating a new protein Neoleukin-4 with a total of sixteen mutations from Neoleukin-2/15. The resulting optimized design, neoleukin-4 (see Table S5), was recombinantly expressed and purified from *E. coli* and tested for binding. Neoleukin-4 binds with high affinity to IL-4Rα receptor, binds cooperatively to IL-4Rαγ_c (see Figure 7), and does not bind with any affinity to the IL-2 receptor (data not shown) Neoleukin-4 retains the superior thermostable properties of neoleukin-2/15 (see Figure 20b,c), and binds to the IL-13 receptor as expected given the natural cross-reactivity of IL-4 to IL-13 receptor (data not shown). Altogether, this shows that neoleukin-2/15 is robust enough to act as a modular scaffold where significant rational sequence changes can be introduced to modify its function or physical properties in a highly predictable way

Methods

Computational design of de novo cytokine mimetics: The design of *de novo* cytokine mimetics began by defining a the structure of hIL-2 in the quaternary complex with the IL-2Rβγ_c receptor as template for the design. After inspection, the residues composing the binding-site were defined as hotspots using Rosetta's metadata (PDBInfoLabels). The structure was feed into the new mimetic design protocol that is programmed in PyRosetta, and which can automatically detect the core-secondary structure elements that compose the target-template and produce the resulting de novo mimetic backbones with full RosettaScripts

compatible information for design. Briefly, the mimetic building algorithm works as follows. For the first generation of designs, each of the core-elements was idealized by reconstruction using loops from a clustered database of highly-ideal fragments (fragment-size 4 amino acids). After idealization, the mimetic building protocol aims to reconnect the idealized elements by pairs in all possible combinations. To do this it uses combinatorial fragment assembly of sequence-agnostic fragments from the database, followed by cartesian-constrained backbone minimization for potential solutions (i.e. where the N- and C- ends of the built fragment are close enough to link the two secondary structures). After minimization, the solutions are verified to contain highly ideal fragments (i.e. that every overlapping fragment that composes the two connected elements is also contained within the database) and no backbone clashes with the target (context) receptor. Passing backbone solutions were then profiled using the same database of fragments in order to determine the most probable amino acids at each position (this information was encoded in metadata on the design). Next, solutions for pairs of connected secondary structures were combinatorially recombined to produce fully connected backbones by using graph theory connected components. Since the number of solutions grows exponentially with each pair of elements, at each fragment combination step we ranked the designs to favor those with shorter interconnections between pairs of core elements, and kept only the top solutions to proceed to the next step. Fully connected solutions were then profiled by layer (interface, core, non-core-surface, surface), in order to restrict the identities of the possible amino acids to be layer-compatible. Finally, all the information on hotspots, compatible built-fragment amino acids and layers were combined (hotspot has precedence to amino acid probability, and amino acid probability took precedence to layer). These fully profiled backbones were then passed to RosettaScripts for flexible backbone design and filtering (see rosetta-script in Appendix A). For the second generation of designs, two approaches were followed. In the first approach, sequence redesigns of the best first generation optimized design were executed (G1_neo2_40_1F, see Appendix B). In the second approach new mimetics were engineered using G1_neo2_40_1F as the target template. The mimetic design protocol in this second generation was similar to the one described for the first generation, but with two key differences. Firstly, the core-fragments were no longer built from fragments, but instead by discovering parametric equations of repetitive phi and psi angles (omega fixed to 180°) that result in repetitive secondary structures that recapitulated each of the target helices as close as possible, a "pitch" on the phi and psi angles was allowed every X-amino acids in order to allow the helices the possibility to have curvature (final parameters: H1:, H2:, H3, H4), the sue of these

parametric equations allowed to change the size of each of the core-elements in the target structure at will (either increase or decrease the size), which was coupled (max/min 8.a.a.) with the loop building process, and reductions in the size of the core elements were not allowed to remove hotspots from the binding site. The second difference in the second generation designs, is that instead of reconnecting the secondary structure core-elements we used a fragment-size of 7 amino acids, and no combinatorial assembly of more than one fragment was allowed (i.e. a single fragment has to be able to close a pair of secondary structures). The rest of the design algorithm was in essence similar to the one followed in the generation one (see Appendix C). The Rosetta energy functions used were "talaris2013" and "talaris2014", for the first and second generation of designs, respectively.

The databases of highly ideal fragments used for the design of the backbones for the *de novo* mimetics were constructed with the new Rosetta application "kcenters_clustering_of_fragments" using an extensive database of non-redundant publicly available protein structures from the RCSB protein data bank, which was comprised of 16767 PDBs for the 4-mer database used for the first generation designs, and 7062 PDBs for the 7-mer database used for the second generation designs.

Yeast display: Yeast were transformed with genes encoding the proteins to be displayed together with linearized pETcon3 vector. The vector was linearized by 100 fold overdigestion by NdeI and XhoI (New England Biolabs) and then purified by gel extraction (Qiagen). The genes included 50 bases of overlap with the vector on both the 5' and 3' ends such that homologous recombination would place the genes in frame between the AGA2 gene and the myc tag on the vector. Yeast were grown in C-Trp-Ura media prior to induction in SGCAA media as previously described. 12-18 hours after induction, cells were washed in chilled display buffer (50mM $NaPO_4$ pH 8, 20mM NaCl, 0.5% BSA) and incubated with varying concentrations of biotinylated receptor (either human or murine IL-2Rα, IL-2Rβ, IL-2Rγ, or human IL-4Rα) while being agitated at 4°C. After approximately 30 minutes, cells were washed again in chilled buffer, and then incubated on ice for 5 minutes with FITC-conjugated anti-c-Myc antibody (1 uL per $3x10^6$ cells) and streptavidin-phycoerythrin (1 uL per 100 uL volume of yeast). Yeast were then washed and counted by flow cytometry (Accuri C6) or sorted by FACS (Sony SH800). For experiments in which the initial receptor incubation was conducted with a combination of biotinylated IL-2Rγ and non-biotinylated IL-4Rα, the non-biotinylated receptor was provided in molar excess.

Mutagenesis and affinity maturation: For error-prone PCR based mutagenesis, the design to be mutated was cloned into pETcon3 vector and amplified using the MutaGene II

mutagenesis kit (Invitrogen) per manufacturer's instructions to yield a mutation frequency of approximately 1% per nucleotide. 1 μg of this mutated gene was electroporated into EBY100 yeast together with 1 μg of linearized pETcon3 vector, with a transformation efficiency on the order of 10^8. The yeast were induced and sorted multiple times in succession with progressively decreasing concentrations of receptor until convergence of the population. The yeast were regrown in C-Trp-Ura media between each sort.

Site-saturation mutagenesis (SSM) libraries were constructed from synthetic DNA from Genscript. For each amino acid on each design template, forward primers and reverse primers were designed such that PCR amplification would result in a 5' PCR product with a degenerate NNK codon and a 3' PCR product, respectively. Amplification of "left" and "right" products by COF and COR primers yielded a series of template products each consisting of a degenerate NNK codon at a different residue position. For each design, these products were pooled to yield the SSM library. SSM libraries were transformed by electroporation into conditioned Saccharomyces cerevisiae strain EBY100 cells, along with linearized pETCON3 vector, using the protocol previously described by Benatuil et al.

Combinatorial libraries were constructed from synthetic DNA from Genscript containing ambiguous nucleotides and similarly transformed into linearized pETCON3 vector.

Protein expression: Genes encoding the designed protein sequences were synthesized and cloned into pET-28b(+) *E. coli* plasmid expression vectors (GenScript, N-terminal 6xHis tag and thrombin cleavage site). Plasmids were then transformed into chemically competent *E. coli* Lemo21 cells (NEB). Protein expression was performed using Terrific Broth and M salts, cultures were grown at 37°C until OD^{600} reached approximately 0.8, then expression was induced with 1 mM of isopropyl β-D-thiogalactopyranoside (IPTG), and temperature was lowered to 18 °C. After expression for approximately 18 hours,, cells were harvested and lysed with a Microfluidics M110P microfluidizer at 18,000 psi, then the soluble fraction was clarified by centrifugation at 24,000 g for 20 minutes. The soluble fraction was purified by Immobilized Metal Affinity Chromatograpy (Qiagen) followed by FPLC size-exclusion chromatography (Superdex 75 10/300 GL, GE Healthcare). The purified neoleukin-2/15 was characterized by Mass Spectrum (MS) verification of the molecular weight of the species in solution (Thermo Scientific), Size Exclusion - MultiAngle Laser Light Scattering (SEC-MALLS) in order to verify monomeric state and molecular weight (Agilent, Wyatt), SDS-PAGE, and endotoxin levels (Charles River).

Human and mouse IL-2 complex components including hIL-2 (a.a. 1-133), hIL-2Rα (a.a. 1-217), hIL-2Rβ (a.a. 1-214) hIL-2Rγ (a.a. 1-232), mIL-2 (a.a. 1-149), mIL-2Rα ectodomain (a.a. 1-213), mIL-2Rβ ectodomain (a.a. 1-215), and $m\gamma_c$ ectodomain (a.a. 1-233) were secreted and purified using a baculovirus expression system, as previously described [17,49]. All proteins were purified to >98% homogeneity with a Superdex 200 sizing column (GE Healthcare) equilibrated in HBS. Purity was verified by SDS-PAGE analysis. For expression of biotinylated human IL-2 and mouse IL-2 receptor subunits, proteins containing a C-terminal biotin acceptor peptide (BAP)-LNDIFEAQKIEWHE (SEQ ID NO:262) were expressed and purified as described via Ni-NTA affinity chromatography and then biotinylated with the soluble BirA ligase enzyme in 0.5 mM Bicine pH 8.3, 100 mM ATP, 100 mM magnesium acetate, and 500 mM biotin (Sigma). Excess biotin was removed by size exclusion chromatography on a Superdex 200 column equilibrated in HBS.

Neoleukin-2 crystal and co-crystal structures: C-terminally 6xHis-tagged endoglycosidase H (endoH) and murine IL-2Rβ and IL-2Rγ were expressed separately in Hi-five cells using a baculovirus system as previously described. IL-2Rγ was grown in the presence of 5 μM kifunensin. After approximately 72 hours, the secreted proteins were purified from the media by passing over a Ni-NTA agarose column and eluted with 200 mM imidazole in HBS buffer (150 mM NaCl, 10 mM HEPES pH 7.3). EndoH was exchanged into HBS buffer by diafiltration. mIL-2Rγ was deglycosylated by overnight incubation with 1:75 (w/w) endoH. mIL-2Rβ and mIL-2Rγ were further purified and buffer exchanged by FPLC using an S200 column (GE Life Sciences).

Monomeric neoleukin-2/15 was concentrated to 12 mg/ml and crystallized by vapor diffusion from 2.4 M sodium malonate pH 7.0, and crystals were harvested and flash frozen without further cryoprotection. Crystals diffracted to 2.0 Å resolution at Stanford Synchrotron Radiation Laboratory beamline 12-2 and were indexed and integrated using XDS (Kabsch, 2010). The space group was assigned with Pointless (Evans, 2006), and scaling was performed with Aimless (Evans and Murshudov, 2013) from the CCP4 suite (Winn et al., 2013). Our predicted model was used as a search ensemble to solve the structure by molecular replacement in Phaser (McCoy et al., 2007), with six protomers located in the asymmetric unit. After initial rebuilding with Autobuild (Terwilliger et al., 2008), iterative cycles of manual rebuilding and refinement were performed using Coot (Emsley et al., 2010) and Phenix (Adams et al., 2010).

To crystallize the ternary neoleukin:mIL-2Rβ:mIL-2Rγ complex, the three proteins were combined in equimolar ratios, digested overnight with 1:100 (w/w) carboxypeptidases A and B to remove purification tags, and purified by FPLC using an S200 column; fractions containing all three proteins were pooled and concentrated to 20 mg/ml. Initial needlelike microcrystals were formed by vapor diffusion from 0.1 M imidazole pH 8.0, 1 M sodium citrate and used to prepare a microseed stock for subsequent use in microseed matrix screening (MMS, (D'Arcy et al., 2014)). After a single iteration of MMS, crystals grown in the same precipitant were cryoprotected with 30% ethylene glycol, harvested and diffracted anisotropically to 3.4 Å x 3.8 Å x 4.1 Å resolution at Advanced Photon Source beamline 23ID-B. The structure was solved by molecular replacement in Phaser using the human IL-2Rβ and IL-2Rγ structures (pdb ID 2B5I) as search ensembles. This produced an electron density map into which two poly-alanine alpha helices could be manually built. Following rigid body refinement in Phenix, electron density for the two unmodeled alpha helices, along with the BC loop and some aromatic side chains, became visible, allowing docking of the monomeric neoleukin. Two further iterations of MMS and use of an additive screen (Hampton Research) produced crystals grown by vapor diffusion using 150 nl of protein, 125 nl of well solution containing 0.1 M Tris pH 7.5, 5% dextran sulfate, 2.1 M ammonium sulfate and 25 nl of microseed stock containing 1.3 M ammonium sulfate, 50 mM Tris pH 7.5, 50 mM imidazole pH 8.0, 300 mM sodium citrate. Crystals cryoprotected with 3 M sodium malonate were flash frozen and diffracted anisotropically to 2.5 Å x 3.7 Å x 3.8 Å at Advanced Light Source beamline 5.0.1. After processing the data with XDS, an elliptical resolution limit was applied using the STARANISO server (Bruhn et al., 2017). Rapid convergence of the model was obtained by refinement against these reflections using TLS and target restraints to the higher resolution human receptor (PDB id 2B5I) and neoleukin-2/15 structures in Buster (Smart et al., 2012; Bricogne et al., 2016), with manual rebuilding in Coot, followed by a final round of refinement in Phenix with no target restraints. Structure figures were prepared with PyMol (Schrodinger, LLC. 2010. The PyMOL Molecular Graphics System, Version 2.1.0). Software used in this project was installed and configured by SBGrid (Morin et al., 2013).

Cell Lines: Unmodified YT-1[64] and IL-2Rα$^+$ YT-1 human natural killer cells [65] were cultured in RPMI complete medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, minimum non-essential amino acids, sodium pyruvate, 25 mM HEPES, and penicillin-streptomycin [Gibco]). CTLL-2 cells purchased from ATCC

were cultured in RPMI complete with 10% T-STIM culture supplement with ConA (Corning). All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. The subpopulation of YT-1 cells expressing IL-2Rα was purified via magnetic selection as described previously [17]. Enrichment and persistence of IL-2Rα expression was monitored by analysis of PE-conjugated anti-human IL-2Rα (Biolegend) antibody binding on an Accuri C6 flow cytometer (BD Biosciences). .

Circular dichroism (CD): Far-ultraviolet CD measurements were carried out with an AVIV spectrometer model 420 in PBS buffer (pH 7.4) in a 1 mm path-length cuvette with protein concentration of ~0.20 mg/ml (unless otherwise mentioned in the text). Temperature melts where from 25 to 95 °C and monitored absorption signal at 222 nm (steps of 2 °C/min, 30 s of equilibration by step). Wavelength scans (195-260 nm) were collected at 25°C and 95°C, and again at 25°C after fast refolding (~5 min).

Binding studies: Surface plasmon resonance (SPR): For IL-2 receptor affinity titration studies, biotinylated human or mouse IL-2Rα, IL-2Rβ, and IL-2Rγ receptors were immobilized to streptavidin-coated chips for analysis on a Biacore T100 instrument (GE Healthcare). An irrelevant biotinylated protein was immobilized in the reference channel to subtract non-specific binding. Less than 100 response units (RU) of each ligand was immobilized to minimize mass transfer effects. Three-fold serial dilutions of hIL-2, mIL-2, Super-2, or engineered IL-2 mimetics were flowed over the immobilized ligands for 60 s and dissociation was measured for 240 s. For IL-2Rβγ_c binding studies, saturating concentrations of hIL-2Rβ (3 uM) or mIL-2Rβ (5 uM) were added to the indicated concentrations of hIL-2 or mIL-2, respectively. Surface regeneration for all interactions was conducted using 15 s exposure to 1 M MgCl2 in 10 mM sodium acetate pH 5.5. SPR experiments were carried out in HBS-P+ buffer (GE Healthcare) supplemented with 0.2% bovine serum albumin (BSA) at 25°C and all binding studies were performed at a flow rate of 50 L/min to prevent analyte rebinding. Data was visualized and processed using the Biacore T100 evaluation software version 2.0 (GE Healthcare). Equilibrium titration curve fitting and equilibrium binding dissociation (KD) value determination was implemented using GraphPad Prism assuming all binding interactions to be first order. Biolayer interferometry: binding data were collected in a Octet RED96 (ForteBio, Menlo Park, CA) and processed using the instrument's integrated software using a 1:1 binding model. Biotinylated target receptors, either human or murine IL-2Rα, IL-2Rβ, IL-2Rγ, or human IL-4Rα, were functionalized to streptavidin coated biosensors (SA ForteBio) at 1μg/ml in binding buffer (10 mM HEPES [pH 7.4], 150 mM

NaCl, 3 mM EDTA, 0.05% surfactant P20, 0.5% non-fat dry milk) for 300 seconds. Analyte proteins were diluted from concentrated stocks into binding buffer. After baseline measurement in binding buffer alone, the binding kinetics were monitored by dipping the biosensors in wells containing 100 nM of the designed protein (association) and then dipping the sensors back into baseline wells (dissociation). For binding experiments in which either IL-2Rβ or IL-4Rα were supplemented in solution while IL-2Rγ was bound to the sensor, the supplemental proteins were provided in 2.5 fold molar excess

STAT5 phosphorylation studies: *In vitro* studies: Approximately $2x10^5$ YT-1, IL-$2R\alpha^+$ YT-1, or CTLL-2 cells were plated in each well of a 96-well plate and re-suspended in RPMI complete medium containing serial dilutions of hIL-2, mIL-2, Super-2, or engineered IL-2 mimetics. Cells were stimulated for 15 min at 37°C and immediately fixed by addition of formaldehyde to 1.5% and 10 min incubation at room temperature. Permeabilization of cells was achieved by resuspension in ice-cold 100% methanol for 30 min at 4°C. Fixed and permeabilized cells were washed twice with FACS buffer (phosphate-buffered saline [PBS] pH 7.2 containing 0.1% bovine serum albumin) and incubated with Alexa Fluor® 647-conjugated anti-STAT5 pY694 (BD Biosciences) diluted in FACS buffer for 2 hours at room temperature. Cells were then washed twice in FACS buffer and MFI was determined on a CytoFLEX flow cytometer (Beckman-Coulter). Dose-response curves were fitted to a logistic model and half-maximal effective concentration (EC_{50} values) were calculated using GraphPad Prism data analysis software after subtraction of the mean fluorescence intensity (MFI) of unstimulated cells and normalization to the maximum signal intensity. Experiments were conducted in triplicate and performed three times with similar results. Ex vivo studies: Spleens and lymph nodes were harvested from wild-type C57BL/6J or B6;129S4-Il2ra^{tm1Dw} (CD25KO) mice purchased from The Jackson Laboratory and made into a single cell suspension in sort buffer (2% Fetal Calf Serum in pH 7.2 phosphate-buffered saline). CD4+ T cells were enriched through negative selection by staining the cell suspension with biotin-conjugated anti-B220, CD8, NK1.1, CD11b, CD11c, Ter119, and CD19 antibodies at 1:100 for 30 min on ice. Following a wash with sort buffer, anti-biotin MicroBeads (Miltenyi Biotec) were added to the cell suspension at 20 µL per 10^7 total cells and incubated on ice for 20 minutes. Cells were washed, resuspended and negative selection was then performed using EasySep Magnets (STEMCELL Technologies). Approximately 1 $x10^5$ enriched cells were added to each well of a 96-well plate in RPMI complete medium with 5% FCS with 10-fold serial dilutions of mIL-2, Super-2, or Neoleukin-2/15. Cells were stimulated for 20 minutes at

37°C in 5% CO_2, fixed with 4% PFA and incubated for 30 minutes at 4°C. Following fixation, cells were harvested and washed twice with sort buffer and again fixed in 500 µL 90% ice-cold methanol in dH_2O for 30 minutes on ice for permeabilization. Cells were washed twice with Perm/Wash Buffer (BD Biosciences) and stained with anti-CD4-PerCP in Perm/Wash buffer (1:300), anti-CD44-Alexa Fluor 700 (1:200), anti-CD25-PE-Cy7 (1:200), and 5 µL per sample of anti-pSTAT5-PE pY694 for 45 min at room temperature in the dark. Cells were washed with Perm/Wash and re-suspended in sort buffer for analysis on a BD LSR II flow cytometer (BD Biosciences).

***In vivo* murine airway inflammation experiments:** C57BL/6J were purchased from The Jackson Laboratory. Mice were inoculated intranasally with 20µL of whole house dust mite antigen (Greer) resuspended in PBS to a total of 23µg Derp1 per mouse. From Days 1-7, mice were given a daily intraperitoneal injection of 20µg mIL-2 in sterile PBS (pH 7.2), a molar equivalent of Neoleukin-2/15 in sterile PBS, or no injection. On Day 8, circulating T cells were intravascularly labeled and tetramer positive cells were enriched from lymph nodes and spleen or lung as previously described (Hondowicz, Immunity, 2016). Both the column flow-through and bound fractions were saved for flow cytometry analysis. Cells were surface stained with antibodies and analyzed on a BD LSR II flow cytometer (BD Biosciences). Animal models: C57BL/6 mice were purchased from The Jackson Laboratory or bred in house and. BALB/c mice were purchased from Charles River. Animals were maintained according to protocols approved by Dana–Farber Cancer Institute (DFCI) Institutional Animal Care and Use Committee, Direção Geral de Veterinária and iMM Lisboa ethical committee.

Colorectal carcinoma *in vivo* mice experiments: CT26 cells were sourced from Jocelyne Demengeot's research group at IGC (Instituto Gulbenkian de Ciência), Portugal. On day 0, 5 x 10^5 cells were injected subcutaneously (s.c.) into the flanks of BALB/c mice with 50 µL of a 1:1 mixture of Dulbecco's modified Eagle medium (Gibco) with Matrigel (Corning). Starting on day 6, when tumour volume reached around 100mm3, neoleukin-2/15 and mIL-2 (Peprotech) were administered daily by intraperitoneal (i.p.) injection in 50 µL of PBS (Gibco). Treatment with anti-PD-1 antibody (Bio X Cell) was performed twice a week by i.p. injection of 200 µg per mouse in PBS. Mice were sacrificed when tumour volume reached 1,300 mm3.

Melanoma in vivo experiments: B16F10 cells were purchased from ATCC. On day 0, 5×10^5 cells were inoculated by s.c. injection in 500 µL of Hank's Balanced Salt Solution (Gibco). Starting on day 1, neoleukin-2/15 and mIL-2 (Peprotech) were administered daily by

intraperitoneal (i.p.) injection in 200 µL of LPS-free PBS (Teknova). Treatment with TA99 (a gift from Noor Momin and Dane Wittrup, Massachusetts Institute of Technology) at 150 µg/mouse was added several days later as indicated. Mice were sacrificed when tumor volume reached 2,000 mm3.

Flow cytometry: Excised tumors were minced, enzymatically digested (Miltenyi Biotec), and passed through a 40-µm filter. Cells from spleens and tumor-draining lymph nodes were dispersed into PBS through a 40-µm cell strainer using the back of a 1-mL syringe plunger. All cell suspensions were washed once with PBS, and the cell pellet was resuspended in 2% inactivated fetal calf serum containing fluorophore-conjugated antibodies. Cells were incubated for 15 minutes at 4°C then fixed, permeabilized, and stained using a BioLegend FoxP3 staining kit. Samples were analyzed on a BD Fortessa flow cytometer. Antibodies (BioLegend) used in melanoma experiments were: CD45-BV711 (clone 30-F11), CD8-BV650 (53-6.7), CD4-BV421 (GK1.5), TCRβ-BV510 (H57-597), CD25-AF488 (PC61), FoxP3-PE (MF-14). Antibodies (eBioscience) used in colon carcinoma experiments were: CD45-BV510 (30-F11), CD3-BV711 (17A2), CD49b-FITC (DX5), CD4-BV605 (GK1.5), CD8-PECy7 (53-6.7), Foxp3-APC (FJK-16s). Fixable Viability Dye eFluor 780 (eBioscience) was used to exclude dead cells.

Generation of anti-neoleukin-2/15 polyclonal antibody: Mice were injected i.p . with 500 µg of K.O. neoleukin in 200 µL of a 1:1 emulsion of PBS and Complete Freund's Adjuvant. Mice were boosted on days 7 and 15 with 500 µg of K.O. neoleukin in 200 µL of a 1:1 emulsion of PBS and Incomplete Freund's Adjuvant. On day 20, serum was collected and recognition of neoleukin-2/15 was confirmed by ELISA.

Enzyme-linked immunosorbent assay (ELISA): High-binding 96-well plates (Corning) were coated overnight at 4°C with 100 ng/mL of neoleukin-2/15, mIL-2 (Peprotech), hIL-2 (Peprotech), or ovalbumin (Sigma-Aldrich) in carbonate buffer. Antibody binding to target proteins was detected using HRP-conjugated sheep anti-mouse IgG (GE Healthcare) at 75 ng/mL. Plates were developed with tetramethylbenzidine and HCl. Absorbance was measured at 450 nm with an EnVision Multimode Plate Reader (PerkinElmer).

T cell proliferation assay: Cells were isolated from a mouse spleen using an EasySep T Cell Isolation Kit (Stemcell Technologies). They were plated in RPMI in 96-well culture plates at a density of 10,000 cells/well. Media were supplemented with regular or heat-treated neoleukin-2/15, rmIL-2, or Super-2. After 5 days of incubation at 37°C cell

survival and proliferation were measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega).

Statistical and power analyses: In vivo murine airway inflammation experiments: MIKEL. *In vivo* murine Colon cancer experiments: CARLOS. *In vivo* murine Melanoma experiments: Comparisons of the survival of tumor-bearing mice were performed using the log-rank (Mantel-Cox) test. Comparisons of weight loss in tumor-bearing mice were performed using a two-tailed t test. A P value less than 0.05 was considered to be significant. The minimum group size was determined using G*Power for an expected large effect size (Cohen's d = 1.75).

Biolayer Interferometry analysis of a Mouse Serum Albumin (MSA) fusion to Neoleukin-2/15. Genetic fusion of Neoleukin-2/15 to MSA for extended half-life and preserves intact binding affinity of the cytokine mimetic to murine IL-2RBeta and IL-2RGamma (33.5 ± 0.2 nM) (data not shown). The construct utilized in this study was as follows:

Optional: (HisTag TEV cleavage site in parentheses)
Mouse serum albumin (italicized)
Linker
Neo2/15 (bold font)

(GSDGGSHHHHHHGSGSENLYFQGSG)*EAHKSEIAHRYNDLGEQHFKGLVLIAFSQYLQKCS YDEHAKLVQEVTDFAKTCVADESAANCDKSLHTLFGDKLCAIPNLRENYGELADCCTKQEPE RNECFLQHKDDNPSLPPFERPEAEAMCTSFKENPTTFMGHYLHEVARRHPYFYAPELLYYAE QYNEILTQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKFGERAFKAWAVARLSQ TFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAELAKYMCENQATISSKLQTCCDKPL LKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSL LLRLAKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAI LVRYTQKAPQVSTPTLVEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRVCLLHEKTPV SEHVTKCCSGSLVERRPCFSALTVDETYVPKEFKAETFTFHSDICTLPEKEKQIKKQTALAE LVKHKPKATAEQLKTVMDDFAQFLDTCCKAADKDTCFSTEGPNLVTRCKDALA*GGGSGGSGG GSGGSGSG**PKKKIQLHAEHALYDALMILNIVKTNSPPAEEKLEDYAFNFELILEEIARLFES GDQKDEAEKAKRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO:244)**

Biotin-mIL2Gamma was immobilized on a Streptavidin biosensor, MSA-Neo2 concentration was titrated from 729 to 1nM in presence of saturating concentrations of mIL2Beta. Biolayer interferometry was carried out as above: binding data were collected in a Octet RED96 (ForteBio, Menlo Park, CA) and processed using the instrument's integrated software using a 1:1 binding model. Biotinylated target receptors, either human or murine IL-2Rα, IL-2Rβ, IL-2Rɣ, or human IL-4Rα, were functionalized to streptavidin coated

biosensors (SA ForteBio) at 1μg/ml in binding buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, 0.05% surfactant P20, 0.5% non-fat dry milk) for 300 seconds. Analyte proteins were diluted from concentrated stocks into binding buffer. After baseline measurement in binding buffer alone, the binding kinetics were monitored by dipping the biosensors in wells containing 100 nM of the designed protein (association) and then dipping the sensors back into baseline wells (dissociation).

CAR-T cell in vivo experiments: In vitro T cell proliferation assay. Primary human T cells were obtained from healthy donors. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll-Hypaque (Sigma). T cells were isolated using EasySep™ CD8 or CD4 negative isolation kits (STEMCELL Technologies). To stimulate T cells, T cells were thawed and incubated with anti-CD3/CD28 Dynabeads (Gibco) at 1:1 ratio in media supplemented with 50 IU/ml (3.1ng/ml) of IL2. Beads were removed after four days of incubation. Stimulated or freshly thawed unstimulated T cells were plated at 30000 or 50000 cells/well, respectively, in 96 well format and cultured in indicated concentrations of IL2 or neoleukin-2/15 in triplicate. Three days later, proliferation was measured using CellTiter-Glo 2.0. (Promega).

In vivo RAJI experiment: Six- to eight-week old NSG mice were obtained from the Jackson Laboratory. 0.5*10^6 RAJI tumor cells transduced with ffluc/eGFP were tail vein injected into the NSG mice. Seven days post tumor inject, lentiviral transduced anti-CD19 CAR T cells (0.4*10^6 CD4, 0.4*10^6 CD8) prepared as described in (Liu et al, 2016) were infused i.v. into mice. hIL2 or neoleukin-2/15 at 20μg/mouse were given i.p. from day 8 to 16 post tumor injection.

Preparation of PEGylated polypeptides: Neo-2/15 stocks with either single or dual cysteine mutations were dialyzed into phosphate buffer, pH7.0 and adjusted to 1.0-2.0mg/ml. TCEP was added at a molar ratio of 10:1 to protein and incubated for 10 minutes at RT to reduce disulfides. Maleimide-modified PEG40k (PEG40k-MA) or PEG30k (PEG30k-MA) powder was added directly to the reduced protein solution at a molar ratio of 10:1 PEG:cysteine and incubated for 2 hours with stirring. Aliquots for SDS-PAGE were taken directly from the reaction mixture. These data demonstrate the rapid, spontaneous, and near-quantitative formation of covalent linkages between PEG40k-MA or PEG30k-MA and Neo-2/15 cysteine mutants in the expected stoichiometry.

Treatment with Neo-2/15 and PEGylated Neo-2/15-E62C (Neo-2/15-PEG) demonstrated changes in the levels of multiple inflammatory markers: Two non-human primates (NHP), one male and one female per group, were assigned to treatment with either

vehicle (group 1), Neo-2/15 (w/o PEG) (groups 2-4) or Neo-2/15 PEG (groups 5-7; single cysteine mutation of E62C and PEG40K). Animals treated with vehicle or Neo-2/15 (w/o PEG) were dosed by intravenous (IV) bolus on study days 1, 2, 3, 4, 5, 6 and 7 (once daily for one week) at dose levels of either 0 (vehicle) or dose adjusted values of 0.07, 0.21 or 0.14 mg/kg/day Neo 2/15 (w/o PEG) (groups 2, 3 and 4, respectively). Animals treated with Neo-2/15 PEG were dosed by IV bolus on study days 1 and 7 at dose levels of 0.05, 0.15 or 0.10 mg/kg/day Neo-2/15PEG (groups 5, 6 and 7, respectively). Cytokine samples were taken on day 1 and 7 at timepoints of 0, 4, 8 and 24 hours post dose. Cytokine serum samples were prepared and frozen at <-70°C and shipped for analysis where samples were analyzed through a Luminex multiplex immunoassays system. Several cytokines, including IL-15 and IL-10 demonstrated marked differences in the time-course of cytokine production, consistent with a more sustained pharmacodynamic effect for the PEGylated molecule.

Targeted Neo-2/15 fusions retained their IL-2R binding affinity and demonstrated anti-tumor effects. Select targeting domains were fused to the N- or C-termini of Neo-2/15 via peptide linkers and were tested *in vitro* to characterize their binding affinity to human and mouse IL-2R by Biolayer Interferometry. The results confirmed that fusions to Neo-2/15 at either the N or C termini did not hinder its ability to bind IL-2R. Subsequent in vitro Flow Cytometry studies confirmed that the fusion proteins were capable of binding a target receptor on the surface of a cell. The efficacy of the targeted constructs was evaluated in *in vivo* mouse experiments, in which it was demonstrated that a targeted Neo-2/15 moiety to tumor cells or immune cells has a beneficial anti-tumor effect over a non-targeted control (data not shown).

Fusions that were tested include but are not limited to: (i) a fusion of an anti-CD47 nanobody to the C terminus of Neo 2/15 via the linker of SEQ ID NO:100; (b) a fusion of an anti-CD47 nanobody to the N terminus of Neo 2/15 via the linker of SEQ ID NO:100;(c) a fusion of an anti-CTLA4 nanobody to the C terminus of Neo 2/15 via the linker of SEQ ID NO:100; (d) a fusion of anti-CTLA4 nanobody to the N terminus of Neo 2/15 via the linker of SEQ ID NO:100; (e) a fusion of an anti-PDL-1 nanobody to the C terminus of Neo 2/15 via the linker of SEQ ID NO:100; and (f) a fusion of an anti-PDL-1 nanobody to the N terminus of Neo 2/15 via the linker of SEQ ID NO:100.

Fusions of albumin to Neo -2 /15 maintained IL-2R binding affinity. Mouse serum albumin (MSA) was fused to the N-terminus of Neo 2/15 via a peptide linker and was tested *in vitro* to characterize its binding affinity to mouse IL-2R by Biolayer Interferometry. Biotin-mIL2Gamma was immobilized on a Streptavidin biosensor, MSA-Neo2 concentration

was titrated from 729 to 1nM in presence of saturating concentrations of mIL2Beta. The fusions maintained IL-2R binding capacity (data not shown).

PEGylated and non-PEGylated Neo-2/15 does not elicit a meaningful anti-drug antibody (ADA) response in non-human primates (NHPs). The potential of PEGylated and non-PEGylated Neo-2/15 (for PEGylated Neo-2/15: single cysteine mutation of E62C and PEG40K) to elicit ADAs was tested in non-human primates. Animals were administered intravenously with either compound for 1 week: PEGylated Neo-2/15on days 1 and 7; wild-type Neo-2/15 on days 1-7. Blood was drawn at various times thereafter and analyzed for the presence of antibodies specific for the administered compound. Each dose group consisted of 1 male and 1 female macaque. Non-PEGylated Neo-2/15 was administered via daily iv bolus injection for 7 consecutive days at 0.1m/kg, 0.2mg/kg, or 0.3mg/kg. PEGylated Neo-2/15 was administered via iv bolus injection at 0.015mg/kg, 0.050mg/kg, and 0.10mg/kg on days 1 and 7. An equivalent volume of saline was administered daily to a vehicle control group for 7 consecutive days. Approximately 750ul of blood was collected from each animal for ADA analysis on study Days 1 (pre-dose), 22, 29, and 43 via the cephalic or saphenous vein. Serum was extracted from blood using a serum separator tube on wet ice and subsequently stored at -80C until analysis. All cynomolgus macaques receiving either vehicle or PEGylated Neo-2/15 tested negative for ADAs on days 22, 29, and 43 demonstrating that PEGylated Neo-2/15 did not elicit a detectable immune response, even after repeat dosing, despite being a computationally-designed protein that is entirely foreign to the macaque immune system. Both (1 male; 1 female) macaques receiving vehicle control tested negative for ADAs against wild-type Neo-2/15 on days 1, 15, 22, and 28. All animals (3 males; 2 females) in the groups receiving non-PEGylated Neo-2/15 tested negative for ADAs on day 1 (pre-dose). Of these, 3 out of 5 (60%) remained negative for ADAs on days 22, 29, and 43. The remaining two animals subsequently tested positive for ADAs on days 22, 29, or 43. One subject tested positive on days 22 and 29, but returned negative by day 43. For that subject, the ADA response was low and transient, suggesting minimal clinical significance. Another subject tested positive on days 22, 29, and 43. For that subject, the measured ADA concentrations were well below 100ng/ml and thus of unclear clinical relevance.

Data Tables

Table E1. Characterization of several de novo designed mimetics of IL-2/IL-15. The table shows the Kd of *de novo* IL-2/IL-15 mimetics and reference cytokines for: mIL-2Rβ, mIL-2RβɣC, EC_{50}, the sequence similarity by structural alignment (MICAN [63]) against hIL-2 (PDB: 2B5I) and mIL-2 (PDB:), the parent of each molecule, its amino acid length, and the sequences for the *de novo* IL-2 mimetics. "N/S" stands for non-significant and "N/A" for non-available.

Table E1

Binding affinity (Kd) to *Hs*IL-2RβXc, and cell signaling in human NK (YT, CD25-) cells								
Name	Kd *Hs*IL-2RβXc (nM)	Kd *Hs*IL-2Rβ (nM)	EC50 (CD25-) pSTAT5p (nM) / (exp i.d.)	Seq identity to *Hs*IL-2 (% / (num a.a. algn))	Seq identity to *Mm*IL-2 (% / (num a.a. algn))	Exp. optimized	Parent molecule	a.a. length
*Hs*IL-2	*193.6*	*326.9*	*0.41 / (a)*	*100.0 / (120)*	*54.5 / (112)*	-	-	133
*Mm*IL-2	*8034.0*	*4950.0*	*39.05 / (a)*	*54.5 / (112)*	*100 / (122)*	-	-	130
Super-2 / Superkine (PDB: 3QAZ)	*300.9*	*2.0*	*0.07 / (a)*	*94.9 / (117)*	*50.9 / (114)*	*Y*	*Hs/L-2*	133
G1_neo2_40	260.0	1457.0	0.14 / (b)	47.7 / (86)	30.4 / (79)	N	-	87
G1_neo2_41	187.0	720.6	0.07 / (b)	47.7 / (86)	30.4 / (79)	N	-	87
G1_neo2_43	533.4	2861.0	0.21 / (b)	50.0 / (86)	32.9 / (79)	N	-	87
G1_neo2_40_1F	2.3	2.6	0.09 / (c)	44.2 / (86)	26.6 / (79)	Y	G1_neo2_40	87
G2_neo2_40_1F_dsn36	113.9	27.6	0.12 / (a)	33.7 / (89)	17.6 / (85)	N	*De novo* mimetic design inspired on template:	100

							G1_neo2_40_1F	
Neoleukin-2/15 (G2_neo2_40_1F_dsn36_opt)	**18.8**	**11.2**	**0.05 / (a)**	29.2 / (89)	15.7 / (83)	Y	**G2_neo2_40_1F_dsn36**	100

Binding affinity (Kd) to *Mm*IL-2RβXc, and cell signaling (EC50) in murine T (CTLL-2, CD25+) cells

Name	Kd *Mm*IL-2RβXc (nM)	Kd *Mm*IL-2Rβ (nM)	EC50 (CD25+) pSTAT5 (nM) / (exp i.d.)	Seq identity to *Hs*IL-2 (% / (num a.a. algn))	Seq identity to *Mm*IL-2 (% / (num a.a. algn))	Exp. optimized	Parent molecule	a.a. length
*Hs*IL-2	*492.2*	*8106.0*	*0.002 / (d)*	*see top table				
*Mm*IL-2	*126.2*	*1496.0*	*0.003 / (e)*	*see top table				
Super-2 / Superkine (PDB: 3QAZ)	*312.2*	*214.0*	*N/A*	*see top table				
G1_neo2_40_1F	7.9	485.5	0.2 / (e)	*see top table				
G1_neo2_40_1F_H1	2654.0	6799.0	37.38 / (d)	39.5 / (86)	25.0 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_H2	963.7	68300.0	9.38 / (d)	40.7 / (86)	26.2 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_H3	3828.0	N/S	35.2 / (d)	39.5 / (86)	25.0 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_H4	391.8	10070.0	0.93 / (d)	41.9 / (86)	26.2 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_H5	5123.0	45300.0	84.69 / (d)	39.5 / (86)	23.8 / (80)	Y	G1_neo2_40_1F	87

G1_neo2_40_1F_M1	4.3	213.9	0.007 / (d)	36.0 / (86)	25.0 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_M2	886.3	2599.0	3.11 / (d)	37.2 / (86)	25.0 / (80)	Y	G1_neo2_40_1F	87
G1_neo2_40_1F_M3	64.8	402.3	0.08 / (d)	34.9 / (86)	25.3 / (79)	Y	G1_neo2_40_1F	87
G2_neo2_40_1F_seq04	80.0	N/A	1.95 / (f)	38.4 / (86)	23.8 / (80)	N	Sequence redesign of G1_neo2_40_1F	87
G2_neo2_40_1F_seq12	39.1	N/A	1.74 / (f)	38.4 / (86)	25.3 / (79)	N	Sequence redesign of G1_neo2_40_1F	87
G2_neo2_40_1F_seq16	71.5	N/A	2.20 / (f)	34.9 / (86)	22.5 / (80)	N	Sequence redesign of G1_neo2_40_1F	87
G2_neo2_40_1F_seq26	27.8	N/A	1.06 / (f)	39.5 / (86)	25.3 / (79)	N	Sequence redesign of G1_neo2_40_1F	87
G2_neo2_40_1F_seq27	13.6	N/A	0.24 / (f)	36.0 / (86)	25.0 / (80)	N	Sequence redesign of G1_neo2_40_1F	87
G2_neo2_40_1F_dsn29	38.2	N/A	0.48 / (f)	36.6 / (82)	8.9 / (90)	N	*De novo* mimetic design using template: G1_neo2_40_1F	107
G2_neo2_40_1F_dsn30	925.0	N/A	7.61 / (f)	33.0 / (97)	23.4 / (94)	N	*De novo* mimetic design using template: G1_neo2_40_1F	107
G2_neo2_40_1F_dsn36	568.5	2432.0	1.36 / (e)	*see top table				
G2_neo2_40_1F_dsn40	69.2	N/A	0.50 / (f)	33.7 / (89)	17.9 / (84)	N	*De novo* mimetic design inspired on template: G1_neo2_40_1F	100
Neoleukin-2/15 (G2_neo2_40_1F_dsn36_opt)	**38.4**	**16.1**	**0.07 / (e)**	***see top table**				

Table E2. Crystallographic data table for neoleukin-2/15 and neoleukin-2/15 quaternary complex with mIL-2Rβγc.

	Neoleukin-2/15 (6DG6)	Neoleukin-2/15 ternary complex with IL-2R (6DG5)
Wavelength		
Resolution range	39.28 - 1.999 (2.07 - 1.999)	47.005 - 2.516 (2.826 – 2.516)
	-	3.687 (0.065 a* + 0.998 c*)
Ellipsoidal resolution limit (Å) (direction)	-	3.756 (0.884 a* + 0.468 c*)
	-	2.516 (0.132 a* + 0.859 b* + 0.495 c*)
Space group	P 21 21 21	P 21 2 21
Unit cell (Å, °)	73.73, 86.8, 92.31, 90, 90, 90	65.125, 67.914, 172.084, 90, 90, 90
Total reflections	351741 (32344)	132356 (7834)
Unique reflections	40650 (3977)	13961 (698)
Multiplicity	8.7 (8.1)	9.5 (11.2)
Completeness (spherical) (%)	92.58 (77.83)	52.3 (9.0)
Completeness (ellipsoidal) (%)		93.2 (77.2)
Mean I/sigma(I)	12.19 (1.25)	6.8 (1.3)
Wilson B-factor	34.54	39.86
R-merge	0.1027 (1.709)	0.359(2.516)
R-meas	0.1094 (1.824)	0.380 (2.636)
R-pim	0.0369 (0.6252)	0.122 (0.780)
CC1/2	0.999 (0.557)	0.987 (0.445)
CC*	1 (0.846)	0.993 (0.328)
Resolution range used in refinement	39.28 - 1.999 (2.07 - 1.999)	43.82 - 2.516 (2.606 - 2.516)
Reflections used in refinement	37747 (3125)	13923 (138)
Reflections used for R-free	1840 (143)	1366 (14)
R-work	0.2037 (0.3137)	0.2211 (0.3271)
R-free	0.2260 (0.3377)	0.2658 (0.4429)
Number of non-hydrogen atoms	4791	4100
macromolecules	4735	3949
ligands	-	138
solvent	56	13
Protein residues	597	492
RMS(bonds)	0.005	0.004
RMS(angles)	0.88	0.94
Ramachandran favored (%)	97.41	97.1
Ramachandran allowed (%)	2.59	2.9
Ramachandran outliers (%)	0	0
Rotamer outliers (%)	1.26	4.5
Clashscore	2.14	4.55
Average B-factor	52.56	47.05
macromolecules	52.54	46.39
ligands	-	67.79
solvent	54.21	27.31
Number of TLS groups	20	3

*Statistics for the highest-resolution shell are shown in parentheses.

Table S1: Amino acid sequences for the best twelve first-round designs. Ten of the designs were (G1_neo2_35-44) were experimentally characterized by yeast display and all but two (G1_neo2_35 and G1_neo2_44) were found to bind fluorescently labeled chimeric ILRβγc at low nanomolar concentrations via flow cytometry screening of designed first-round protein binders. Designs indicated were expressed on yeast and incubated with 2 nM hIL-2Rβγc or 0 nM IL-2Rβγc (data not shown).

Design	Sequence
G1_neo2_33	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDLDKAEDIRRNSDQARREAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 103)

G1_neo2_34	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSCISTGKCDLDKAEDIRRNSDQ ARREAEKRGIDVRDLISNAQVILLEAR (SEQ ID NO: 104)
G1_neo2_35	STKKWQLQAEHALLDWQMALNKSPEPNENLNRAITAAQSWISTGKIDCDKAEDIRRNSDQ ARREAEKRGIDVRDLISNAQVILLEAC (SEQ ID NO: 105)
G1_neo2_36	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELARNLEK VRDEALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 106)
G1_neo2_37	STKKLQLQAEHFLLDVQMILNESPEPNEELNRCITDAQSWISTGKIDLDRAEECARNLEK VRDEALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 107)
G1_neo2_38	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSCISTGKCDLDRAEELARNLEK VRDEALKRGIDVRDLVSNAKVIALELK (SEQ ID NO: 108)
G1_neo2_39	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELCRNLEK VRDEALKRGIDVRDLVSNACVIALELK (SEQ ID NO: 109)
G1_neo2_40	STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSWISTGKIDLDGAKELAKEVEE LRQEAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 110)
G1_neo2_41	STKKLQLQAEHALLDAQMMLNRSPEPNEKLNRIITTMQSCISTGKCDLDGAKELAKEVEE LRQEAEKRGIDVRDLASNLKVILLELA (SEQ ID NO: 111)
G1_neo2_42	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMAKEAEK IRKEMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 112)
G1_neo2_43	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSCISTGKCDLDNAQEMAKEAEK IRKEMEKRGIDVRDLISNIIVILLELS (SEQ ID NO: 113)
G1_neo2_44	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMCKEAEK IRKEMEKRGIDVRDLISNICVILLELS (SEQ ID NO: 114)

Table S2. Amino acid sequences for the experimentally optimized first-round designs.

Design	Sequence
G1_neo2_40_1A	STKKTQLLAEHALLDAFMMLNVVPEPNEKLNRIITTMQSWIYTGKIDADGAKELAKEVEELE QEYEKRGIDVEDDASNLKVILLELA (SEQ ID NO: 115)
G1_neo2_40_1B	STKKTQLLAEHALLDAHMMLNMLPEPNEKLNRIITTMQSWIHTGKIDGDGAQELAKEVEELE QEYEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 116)
G1_neo2_40_1C	STKKTQLLAEHALLDAFMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELE QEFEKRGIDVEDEASNLKVILLELA (SEQ ID NO: 117)
G1_neo2_40_1D	STKKTQLLAEHALLDALMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELE QELEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 118)
G1_neo2_	STKKTQLLAEHALLDAHMMLNVVPEPNEKLNRIITTMQSWIYTGKIDRDGAQELAKEVEELE

40_1E	QELEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 119)
G1_neo2_40_1F	STKKTQLLAEHALLDALMMLNLLPEPNEKLNRIITTMQSWIFTGKIDGDGAQELAKEVEELE QEHEKRGIDVEDYASNLKVILLELA (SEQ ID NO: 120)
G1_neo2_40_1G	STKKTQLLAEHALLDAYMMLNMVPEPNEKLNRIITTMQSWILTGKIDSDGAQELAKEVEELE QELEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 121)
G1_neo2_40_1H	STKKTHLLAEHALLDAYMMLNVMPEPNEKLNRIITTMQSWIFTGKIDGDGAKELAKEVEELE QEFEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 122)
G1_neo2_40_1I	STKKTQLLAEHALLDAYMMLNLVPEPNEKLNRIITTMQSWIFTGKIDADGAQELAIEVEELE QEYEKRGIDVDDYASNLKVILLELA (SEQ ID NO: 123)
G1_neo2_40_1J	STKKTQLMAEHALLDAFMMLNVLPEPNEKLNRIITTMQSWIFTGKIDGDDAQELAKEVEELE QELEKRGIDVDDDASNLKVILLELA (SEQ ID NO: 124)
G1_neo2_40_1F_H1	STKKTQLLIEHALLDALDMSRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQQLAKEVEELE QEHEKRGEDVEDEASNLKVILLELA (SEQ ID NO: 125)
G1_neo2_40_1F_H2	STKKTQLLLEHALLDALHMRRNLPEPNEKLSRIITTMQSWIFTGKIDGDGAQELAKEVEELE QEHEKRGRDVEDDASNLKVILLELA (SEQ ID NO: 126)
G1_neo2_40_1F_H3	STKKTQLLIEHALLDALNMRKKLPEPNEKLSRIITDMQSWIFTGKIDGDGAQQLAKEVEELE QEHEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 127)
G1_neo2_40_1F_H4	STKKTQLLLEHALLDALHMSRELPEPNEKLNRIITDMQSWIFTGKIDGDGAQDLAKEVEELE QEHEKRGGDVEDYASNLKVILLELA (SEQ ID NO: 128)
G1_neo2_40_1F_H5	STKKTQLLIEHALLDALHMSRKLPEPNEKLSRIITTMQSWIFTGKIDGDGAQHLAKEVEELE QEHEKRGGEVEDEASNLKVILLELA (SEQ ID NO: 129)
G1_neo2_40_1F_H6	STKKTQLLIEHALLDALHMKRKLPEPNEKLNRIITNMQSWIFTEKIDGDGAQDLAKEVEELE QEHEKRGQDVEDYASNLKVILLELA (SEQ ID NO: 130)
G1_neo2_40_1F_M1	STEKTQLAAEHALRDALMLKHLLNEPNEKLARIITTMQSWQFTGKIDGDGAQELAKEVEELQ QEHEVRGIDVEDYASNLKVILLHLA (SEQ ID NO: 131)
G1_neo2_40_1F_M2	STKNTQLAAEDALLDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQ QEHEERGIDVEDYASNLKVILLQLA (SEQ ID NO: 132)
G1_neo2_40_1F_M3	STEKTQHAAEDALRDALMLRNLLNEPNEKLARIITTMQSWQFTEKIDGDGAQELAKEVEELQ QEHEVRGIDVEDYASNLKVILLQLA (SEQ ID NO: 133)

Table S3: Amino acid sequences for second-round designs. G2_neo2_40_1F_seq02 to G2_neo2_40_1F_seq28 correspond to the 27 Rosetta sequence redesigns of G1_neo2_40_1F; G2_neo2_40_1F_seq29 to G2_neo2_40_1F_seq42 represent the 14 new *de novo* mimetic designs.

Design	Sequence
G2_neo2_40_1F_seq02	TQKKQQLLAEHALLDALMILNMLKTSSEAVNRMITIAQSWIFTGTSNPEEAKEMIKMA EQAEEEARREGVDTEDYVSNLKVILKEIA (SEQ ID NO: 134)
G2_neo2_40_1F_seq03	TTKKYQLLVEHALLDALMMLNLSSESNEKMNRIITTMQSWIFTGTFDPDQAEELAKLV EELREEFRKRGIDTEDYASNLKVILKELS (SEQ ID NO: 135)
G2_neo2_40_1F_seq04	TTKKIQLLVEHALLDALMILNLSSESNEKLNRIITTLQSWIFRGEIDPDRARELAKLL EEIREEMRKRGIDTEDYVSNMIVIIRELA (SEQ ID NO: 136)
G2_neo2_40_1F_seq05	TKKKIQLLAEHVLLDLLMMLNLSSESNEKMNRLITIVQSWIFTGTIDPDQAEEMAKWV EELREEFRKRGIDTEDYASNVKVILKELS (SEQ ID NO: 137)
G2_neo2_40_1F_seq06	TKKKYQLLIEHLLLDALMVLNMSSESNEKLNRIITILQSWIFTGTWDPDLAEEMEKLM QEIEEELRRRGIDTEDYMSNMRVIIKELS (SEQ ID NO: 138)
G2_neo2_40_1F_seq07	TKKKLQLLVEHLLLDMLMILNMSSESNEKLNRLITELQSWIFRGEIDPDKAEEMWKIM EEIEKELRERGIDTEDYMSNAKVIIKELS (SEQ ID NO: 139)
G2_neo2_40_1F_seq08	TSKKQQLLAEHALLDALMILNISSESSEAVNRAITWLQSWIFKGTVNPDQAEEMRKLA EQIREEMRKRGIDTEDYVSNLEVIAKELS (SEQ ID NO: 140)
G2_neo2_40_1F_seq09	TKKKYQLLIEHLLLDLLMVLNMSSESNEKINRLITWLQSWIFTGTYDPDLAEEMYKIL EELREEMRERGIDTEDYMSNMRVIVKELS (SEQ ID NO: 141)
G2_neo2_40_1F_seq10	TKKKWQLLIEHLLLDLLMILNLSSESNEKLNRLITWLQSWIFTGTYDPDLAEEMKKMM DEIEDELRERGIDTEDYMSNAKVIIKELS (SEQ ID NO: 142)
G2_neo2_40_1F_seq11	TKKKIQLLVEHALLDALMILNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELSKLV EEIREEMRKRGIDTEDYVSNLKVILDELS (SEQ ID NO: 143)
G2_neo2_40_1F_seq12	TEKKLQLLVEHALLDALMILNLWSESNEKLNRIITTMQSWIFTGRIDPDKAEELAKLV EELREEARERGIDTEDYVSNLKVILKELS (SEQ ID NO: 144)
G2_neo2_40_1F_seq13	TKKKYQLLMEHLLLDLLMVLNMSSESNEKLNRLITIIQSWIFTGTWDPDKAEEMAKML KEIEDELRERGIDTEDYMSNMIVIMKELS (SEQ ID NO: 145)
G2_neo2_40_1F_seq14	TTKKIQLLVEHALLDALMLLNLSSESNEKMNRIITTMQSWIFEGRIDPDQAQELAKLV EELREEFRKRGIDTEDYVSNLKVILEELS (SEQ ID NO: 146)
G2_neo2_40_1F_seq15	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDQAEELAKLV RELREEFRKRGIDTEDYASNLEVILRELS (SEQ ID NO: 147)
G2_neo2_40_1F_seq16	TKKKIQLLVEHALLDALMILNLSSKSNEKLNRIITTMQSWIFNGTIDPDRARELAKLV EEIRDEMEKNGIDTEDYVSNLKVILEELA (SEQ ID NO: 148)
G2_neo2_40_1F_seq17	TKKKYQLLIEHVLLDLLMLLNLSSESNEKMNRLITILQSWIFTGTYDPDKAEEMAKLL KELREEFRERGIDTEDYISNAIVILKELS (SEQ ID NO: 149)
G2_neo2_40_1F_seq18	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLV EELREEFRKRGIDTEDYASNLKVILKELS (SEQ ID NO: 150)

G2_neo2_40_1F_seq19	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITTMQSWIFNGTIDPDQARELAKLVEELREEFRKRGIDTEDYASNLKVILEELA (SEQ ID NO: 151)
G2_neo2_40_1F_seq20	TKKKLQLLVEHALLDALMLLNLSSESNEKLNRIITTMQSWIFTGTVDPDQAEELAKLVEEIREELRKRGIDTEDYVSNLKVILKELS (SEQ ID NO: 152)
G2_neo2_40_1F_seq21	TTKKYQLLVEHALLDALMILNLSSESNEKLNRIITTMQSWIFTGTFDPDQAEELAKLVREIREEMRKRGIDTEDYVSNLEVILRELS (SEQ ID NO: 153)
G2_neo2_40_1F_seq22	TKKKIQLLVEHALLDALMILNLSSESNEKLNRIITTMQSWIFTGTIDPDRAEELAKLVREIREEMRKRGIDTEDYVSNLEVILRELS (SEQ ID NO: 154)
G2_neo2_40_1F_seq23	TKKKYQLLIEHLLLDLLMILNLSSESNEKLNRLITWLQSWIFRGEWDPDKAEEWAKILKEIREELRERGIDTEDYMSNAIVIMKELS (SEQ ID NO: 155)
G2_neo2_40_1F_seq24	TDKKLQLLVEHLLLDLLMMLNLSSKSNEKMNRLITIAQSWIFTGKVDPDLAREMIKLLEETEDENRKNGIDTEDYVSNARVIAKELE (SEQ ID NO: 156)
G2_neo2_40_1F_seq25	TKKKIQLLVEHALLDALMLLNLSSESNEKMNRIITTMQSWIFTGTIDPDQAEELAKLVEELKEEFKKRGIDTEDYVSNLKVILKELS (SEQ ID NO: 157)
G2_neo2_40_1F_seq26	TKKKYQLLIEHALLDALMILNLWSESNEKLNRIITTMQSWIFTGTYDPDKAEELEKLAKEIEDEARERGIDTEDYMSNLRVILKELS (SEQ ID NO: 158)
G2_neo2_40_1F_seq27	TKKKAQLLAEHALLDALMLLNLSSESNERLNRIITWLQSIIFTGTYDPDMVKEAVKLADEIEDEMRKRGIDTEDYVSNLRVILQELA (SEQ ID NO: 159)
G2_neo2_40_1F_seq28	TQKKNQLLAEHLLLDALMVLNQSSESSEVANRIITWAQSWIFEGRVDPNKAEEAKKLAKKLEEEMRKRGIDMEDYISNMKVIAEEMS (SEQ ID NO: 160)
G2_neo2_40_1F_seq29	EDYYSNLKVILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYIQSQIFEISERIRETDQEKKEESWKKWQLLLEHALLDVLMLLND (SEQ ID NO: 161)
G2_neo2_40_1F_seq30	PEKKRQLLLEHILLDALMLLNLLETNPQNTESKFEDYISNAEVIAEELAKLMESLGLSDEAEKFKKIKQWLREVWRIWSSTNWSTLEDKARELLNRIITTIQSQIFY (SEQ ID NO: 162)
G2_neo2_40_1F_seq31	PEKKRQLLLEHILLDLLMILNMIETNRENTESEMEDYWSNVRVILRELARLMEELNYKELSELMERMRKIVEKIRQIVTNNSSLDTAREWLNRLITWIQSLIFR (SEQ ID NO: 163)
G2_neo2_40_1F_seq32	PEKKRQLLAEHALLDALMLLNIIETNSKNTESKMEDYVSNLEVILTEFKKLAEKLNFSEEAERAERMKRWARKAYQMMTLDLSLDKAKEMLNRIITILQSIIFN (SEQ ID NO: 164)
G2_neo2_40_1F_seq33	PEKKRQLLAEHLLLDVLMMLNGNASLKDYASNAQVIADEFRELARELGLTDEAKKAEKIIEALERAREWLLNNKDKEKAKEALNRAITIAQSWIFN (SEQ ID NO: 165)
G2_neo2_40_1F_seq34	PEKKRQLLLEHLLLDLLMILNMLRTNPKNIESDWEDYMSNIEVIIEELRKIMESLGRSEKAKEWKRMKQWVRRILEIVKNNSDLEEAKEWLNRLITTIVQSEIFE (SEQ ID NO: 166)

G2_neo2_40_1F_seq35	WEKKRQLLLEHLLLDLLMILNMWRTNPQNTESLMEDYMSNAKVIVEELARMMRSQGLE DKAREWEEMKKRIEEIRQIIQNNSSKERAKEELNRLITYVQSEIFR (SEQ ID NO: 167)
G2_neo2_40_1F_seq36	PKKKIQLLAEHALLDALMILNIVKTNSQNAEEKLEDYASNVEVILEEIARLMESGDQK DEAEKAKRMKEWMKRIKTTASEDEQEEMANRIITLLQSWIFS (SEQ ID NO: 168)
G2_neo2_40_1F_seq37	PEKKRQLLAEHALLDALMILNILQTNPQNAEEKLEDYMSNVEVIMEEFARMMRNGDRS EEAENAERIKKWVRKASSTASSEEQREMMNRAITLMQSWIFE (SEQ ID NO: 169)
G2_neo2_40_1F_seq38	PEKKRQLLAEHLLLDALMVLNMLTTNSKNTEEKLEDYISNMKVIIKEMIELMRSLGRL EEAEKWKEALKAVEKIGSRMDSETARELANRIITLAQSAIFY (SEQ ID NO: 170)
G2_neo2_40_1F_seq39	PEKKRQLLAEHALLDALMFLNLVETNPDQAEEKIEDYASNLRVIAEELARLFENLGRL DEAQKAKDIKELAERARSRVSSEKRKEAMNRAITILQSMIFR (SEQ ID NO: 171)
G2_neo2_40_1F_seq40	PEKKRQLLAEHALLDALMILNIIRTNSDNTESKLEDYISNLKVILEEIARLMESLGLS DEAEKAKEAMRLADKAGSTASEEEKKEAMNRVITWAQSWIFN (SEQ ID NO: 172)
G2_neo2_40_1F_seq41	PEKKRQLLAEHALLDALMMLNILRTNPDNAEEKLEDYWSNLIVILREIAKLMESLGLT DEAEKAKEAARWAEEARTTASKDQRRELANRIITLLQSWIFS (SEQ ID NO: 173)
G2_neo2_40_1F_seq42	PEKKRQLLAEHLLLDALMILNIIETNEQNAESKLEDYISNAKVILDEFREMARDLGLL DEAKKAEKMKRWLEKMRSNASSDERREWANRMITTAQSWIFN (SEQ ID NO: 174)

Table S4. Amino acid sequences for the experimentally optimized second-round designs.

Design	Sequence
G2_neo2_40_1F_seq27_S18	TNKEAQLHAEFALYDALMLLNLSSESNERLNRIITWLQSIIFYETYDPDMVKEAV KLADEIEDEMRKRKIDTEDYVVNLRLILQELA (SEQ ID NO: 175)
G2_neo2_40_1F_seq27_S22	TKKDAELLAEFALYDALMLLNLSSESNERLNEIITWLQSIIFYGTYDPDMVKEAV KLADEIEDEMRKRGIDTEDYVSNLRLILQELA (SEQ ID NO: 176)
G2_neo2_40_1F_seq27_S24	TNKKAQLHAEFALYDALMLLNLSSESNERLNDIITWLQSIIFTGTYDPDMVKEAV KLADEIEDEMRKRKIDTEDYVVNLRYILQELA (SEQ ID NO: 177)
G2_neo2_40_1F_seq29_S6	EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKE LLNRLITYIQSQIFEVLHGVGETDQEKKEESWKKWDLLLEHALLDVLMLLND (SEQ ID NO: 178)
G2_neo2_40_1F_seq29_S7	EDYYSNLKVILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKE LLNELITYIQSQIFEVIEREGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 179)
G2_neo2_40_1F_seq29_S8	EDYYSNLKLILEELAREMERNGLSDKAEEWRQWKKIVERIRQIRSNNSDLNEAKE LLNRLITYIQSQIFEVLEGVGETDQEKKEESWKKWELHLEHALLDVLMLLND (SEQ ID NO: 180)

Neolukin-2/15 (i.e. G2_neo2_40_1F_seq36_S11)	PKKKIQLHAEHALYDALMILNIVKTNSPPAEEKLEDYAFNFELILEEIARLFESG DQKDEAEKAKRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 181)
G2_neo2_40_1F_seq36_S12	PKKKIQLLAEHALFDLLMILNIVKTNSQNAEEKLEDYAYNAGVILEEIARLFESG DQKDEAEKAKRMKEWMKRIKDTASEDEQEEMANEIITILQSWNFS (SEQ ID NO: 182)

Neoleukin-2/15-H8Y-K33E: H1->H3->H2'->H4
PKKKIQLYAEHALYDALMILNIVKTNSPPAEEELEDYAFNFELILEEIARLFESGDQKDEAEKAKRMKEWMKRIKTTAS
EDEQEEMANAIITILQSWIFS (SEQ ID NO: 94)

Binding of Neoleukin-2/15-H8Y-K33E to the IL2 receptor was measured by biolayer interferometry, and it was found to have higher binding affinity than Neoleukin-2 for IL2-Rbeta, both when tested against IL2Rbeta alone and when tested against the IL2Rbeta-gamma complex. This increased affinity was attributable mostly to an improved off-rate from IL2-Rbeta.

Table S5. Amino acid sequences for the interleukin-4 mimetic designs based on reengineering of neolukin-2/15.

Design	**Sequence**
IL4_G2_neo2_40_1F_seq36_S11	PKKKIQITAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLF ESGDQKDEAEKAKRMKEWMKRIKTTASEDEQEEMANAIITILQSWIFS (SEQ ID NO: 183)
Neoleukin-4 (i.e. IL4_G2_neo2_40_1F_seq36_S11_MIF)	PKKKIQIMAEEALKDALSILNIVKTNSPPAEEQLERFAKRFERNLWGIARLF ESGDQKDEAEKAKRMIEWMKRIKTTASEDEQEEMANAIITILQSWFFS (SEQ ID NO: 184)

Appendix A. RosettaScripts XML protocol for sequence design of mimetics generation-1.

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        <IncludeCurrent name="inclcur"/>
        <LimitAromaChi2 name=limitchi2 />
        DisallowIfNonnative name="not_aa_H" disallow_aas="H"/>
        <ReadResfile name="resfile" filename="./input.resfile"
/>
    </TASKOPERATIONS>

    <MOVERS>
        <Dssp name=dssp/>
        <FastDesign name="fdesign"
task_operations="init,resfile,limitchi2" scorefxn="SFXN6dA"
allow_design="1" only_design_worst_region="0"
design_by_psipred="0" design_by_frag_qual="0" repeats="2"
clear_designable_residues="0"  max_redesigns="2000" />
        <FastRelax name=relax />
        <ParsedProtocol name="complexDesign" >
```

```
            <Add mover_name="fdesign" />
            <Add mover_name="relax" />
            <Add mover_name="dssp" />
        </ParsedProtocol>
        <LoopOver name="fastDesignProtein"
mover_name="complexDesign" filter_name=filt drift=0
iterations="10" ms_whenfail=FAIL_DO_NOT_RETRY/>
    </MOVERS>

    <APPLY_TO_POSE>
    </APPLY_TO_POSE>

    <PROTOCOLS>
        <Add mover_name=fastDesignProtein />
        <Add filter_name=sspred />
        <Add filter_name=pack />
        <Add filter_name=score />
        <Add filter_name=score_res />
        <Add filter_name=holes />
        <Add filter_name=rama />
    </PROTOCOLS>
</ROSETTASCRIPTS>
```

Appendix B. RosettaScripts XML protocol for sequence redesigns of G1_Neo2_40_1F (i.e. generation-2).

```
<ROSETTASCRIPTS>
    <SCOREFXNS>
        <SFXN6_vanilla weights="./talaris2014_cart.wts"
symmetric=0 />
        <SFXN6dA_vanilla
weights="./talaris2014_cart_downAla.wts" symmetric=0 />
        <SFXN6dA_norep_elect
weights="./talaris2014_cart_downAla.wts" symmetric=0 >
                <Reweight scoretype=fa_rep  weight=0.05 />
                <Reweight scoretype=fa_elec weight=1.0 />
        </SFXN6dA_norep_elect >
        <SFXN6dA_elect
weights="./talaris2014_cart_downAla.wts" symmetric=0 >
                <Reweight scoretype=fa_elec weight=2.0 />
        </SFXN6dA_elect >
    </SCOREFXNS>

    <MOVERS>
        <SwitchChainOrder name="keep_only_chain_A"
chain_order="1"/>
    </MOVERS>

    <FILTERS>
    <!--Not Enabled-->
        <Holes name="holes_disabled" threshold="1.2"
confidence="0"/>
        <ScoreType name="score_disabled"
scorefxn="SFXN6_vanilla" score_type="total_score"
threshold=0.0 confidence="0" />
        <ResidueCount name="nres_disabled" confidence="0" />
        <PackStat name=packstat_disabled threshold="0.65"
repeats="3" confidence="0" />
        <SSPrediction name="sspred_disabled"
cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.85
confidence="0"/>
        <BuriedUnsatHbonds name="unsat_core_disabled" cutoff=0
task_operations="only_core_residues" jump_number=0
confidence="0"/>
        <RmsdSimple name="rmsd1_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
        <RmsdSimple name="rmsd2_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
```

```
        <RmsdSimple name="rmsd3_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
        <CalculatorFilter name="score_res_disabled"
equation="SCORE/NRES" threshold="-1.8" confidence="0">
            <SCORE name="SCORE" filter_name="score_disabled"
/>
            <NRES name="NRES" filter_name="nres_disabled" />
        </CalculatorFilter>

        <!--Enabled-->
        <PackStat name="packstat_enabled" threshold="0.65"
repeats="3" confidence="1" />
        <SSPrediction name="sspred_enabled"
cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.85
confidence="1"/>
        <CalculatorFilter name="score_res_enabled"
equation="SCORE/NRES" threshold="-1.8" confidence="1">
            <SCORE name="SCORE" filter_name="score_disabled"
/>
            <NRES name="NRES" filter_name="nres_disabled" />
        </CalculatorFilter>
        <CavityVolume name="cav_vol_disabled" />
        <BuriedUnsatHbonds name="unsat_core_enabled" cutoff=0
task_operations="only_core_residues" jump_number=0
confidence="1"/>
        <RmsdSimple name="rmsd1_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <RmsdSimple name="rmsd2_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <RmsdSimple name="rmsd3_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <CompoundStatement name=all_enabled_filters >
            <AND filter_name=sspred_enabled />
            <AND filter_name=score_res_enabled />
            <AND filter_name=packstat_enabled />
            <AND filter_name=unsat_core_enabled />
        </CompoundStatement>
    </FILTERS>

    <FILTERS>
        <!--Chain A Filters-->
        <!--Not Enabled-->
        <MoveBeforeFilter name="packstat_chainA_disabled"
mover="keep_only_chain_A" filter=packstat_disabled
confidence="0"/>
```

```
        <MoveBeforeFilter name="sspred_chainA_disabled"
mover="keep_only_chain_A" filter=sspred_disabled
confidence="0"/>
        <MoveBeforeFilter name="score_res_chainA_disabled"
mover="keep_only_chain_A" filter=score_res_disabled
confidence="0"/>
        <MoveBeforeFilter name="cav_vol_chainA_disabled"
mover="keep_only_chain_A" filter=cav_vol_disabled
confidence="0"/>
        <MoveBeforeFilter name="unsat_core_chainA_disabled"
mover="keep_only_chain_A" filter=unsat_core_disabled
confidence="0"/>
        <!--Enabled-->
        <MoveBeforeFilter name="packstat_chainA_enabled"
mover="keep_only_chain_A" filter=packstat_enabled
confidence="1"/>
        <MoveBeforeFilter name="sspred_chainA_enabled"
mover="keep_only_chain_A" filter=sspred_enabled
confidence="1"/>
        <MoveBeforeFilter name="score_res_chainA_enabled"
mover="keep_only_chain_A" filter=score_res_enabled
confidence="1"/>
        <MoveBeforeFilter name="all_enabled_filters_chainA"
mover="keep_only_chain_A" filter=all_enabled_filters
confidence="1"/>
        <MoveBeforeFilter name="unsat_core_chainA_enabled"
mover="keep_only_chain_A" filter=unsat_core_enabled
confidence="1"/>
    </FILTERS>

    <RESIDUE_SELECTORS>
        <ResiduePDBInfoHasLabel name="hotspots"
property="HOTSPOTB" />
    </RESIDUE_SELECTORS>

    <TASKOPERATIONS>
        <InitializeFromCommandline name="init"/>
        <IncludeCurrent name="inclcur"/>
        <LimitAromaChi2 name=limitchi2 />
        <DisallowIfNonnative name="only_native_H"
disallow_aas="H"/>
        <ReadResfile name="resfile" filename="./input.resfile"
/>
        <PreventChainFromRepacking name="not_chain_B"
chain="2" />
        <PreventChainFromRepacking name="not_chain_C"
chain="3" />
        <PreventChainFromRepacking name="not_chain_D"
chain="4" />
        <!--Select designable residues by sasa and packable by
flag-->
```

```
        <SelectBySASA name="only_core_residues" mode="mc"
probe_radius=2.0 core_asa=20.0 surface_asa=30.0 core=1
boundary=0 surface=0 verbose=1 />
        <!--Restrict Hotspots to Repacking-->
        <OperateOnResidueSubset name="hotspot_onlyrepack"
selector="hotspots" >
            <RestrictToRepackingRLT/>
        </OperateOnResidueSubset>
        <!--Layer Design as Tom Helped to set omit operations.
Thanks Tom L. :)-->
        <LayerDesign name="layer_all" layer="all"
use_sidechain_neighbors="True" pore_radius="2.0"
verbose="true" >
            <NoRepackDisulfides name="disulf" >
                    <all aa="c" specification="fixed"
operation="omit" />
            </NoRepackDisulfides>
            <OperateOnResidueSubset
name="hotspot_onlyrepack_layerdesignOmit" selector="hotspots"
>
                <PreventRepackingRLT/>
                <all specification="fixed" operation="omit" />
            </OperateOnResidueSubset>
            <ReadResfile name="resfile_layerdesignOmit"
filename="./input_fix.resfile" >
                <all specification="fixed" operation="omit" />
            </ReadResfile>
            <core>
                    <all append="M" />
            </core>
            <boundary>
                    <all append="M" />
            </boundary>
        </LayerDesign>
        <LayerDesign name="layer_boundary_surface"
layer="boundary_surface" use_sidechain_neighbors="True"
pore_radius="2.0" verbose="true" >
            <NoRepackDisulfides name="disulf" >
                    <all aa="c" specification="fixed"
operation="omit" />
            </NoRepackDisulfides>
            <OperateOnResidueSubset
name="hotspot_onlyrepack_layerdesignOmit" selector="hotspots"
>
                <PreventRepackingRLT/>
                <all specification="fixed" operation="omit" />
            </OperateOnResidueSubset>
            <ReadResfile name="resfile_layerdesignOmit"
filename="./input_fix.resfile" >
                <all specification="fixed" operation="omit" />
            </ReadResfile>
```

```
            <core>
                    <all append="M" />
            </core>
            <boundary>
                    <all append="M" />
            </boundary>
        </LayerDesign>
    </TASKOPERATIONS>

    <MOVERS>
        <SavePoseMover name="save_RMSDreference_conformation"
reference_name="reference_conformation"/>
        <AddConstraintsToCurrentConformationMover
name=constrainCA
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" CA_only=1 />
        <ClearConstraintsMover name=clearAllConstraints />
        <PackRotamersMover name="design_all_norep"
scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" />
        <PackRotamersMover name="design_onlyCore_norep"
scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,only_core_residues,not_chain_B,no
t_chain_C,not_chain_D" />
        <TaskAwareMinMover name="min_vanilla_SC"
scorefxn="SFXN6_vanilla"  bb="0" chi="1" jump="1"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" />
        <TaskAwareMinMover name="min_vanilla_BBSC"
scorefxn="SFXN6_vanilla"  bb="1" chi="1" jump="1"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" />
        <FastDesign name="fdesign_all_elec"
scorefxn="SFXN6dA_vanilla"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D"  only_design_worst_region="0" design_by_psipred="0"
design_by_frag_qual="0" repeats="3"
clear_designable_residues="0"  max_redesigns="2000" >
        <FastRelax name=fast_relax_vanilla
scorefxn="SFXN6dA_vanilla">
            <MoveMap name="mappyfr">
                <Chain number=1 chi=1 bb=1/>
                <Chain number=2 chi=0 bb=0/>
                <Chain number=3 chi=0 bb=0/>
```

```
                <Chain number=4 chi=0 bb=0/>
                <Jump number=1 setting=0/>
            </MoveMap>
        </FastRelax>
        <ParsedProtocol name="design_all_w_minimize_vanilla" >
            <Add mover_name="constrainCA" /> <!-- START CA-
contraints -->
            <Add mover_name="design_all_norep" />
            <Add mover_name="min_vanilla_SC" />
            <Add mover_name="min_vanilla_BBSC" />
            Add filter_name="rmsd_chainA_enabled" /> <!--
Check RMSD -->
            <Add mover_name="clearAllConstraints" /> <!-- END
CA-contraints -->
        </ParsedProtocol>
        <GenericSimulatedAnnealer name="SA_DesignProtein"
            mover_name="design_onlyCore_norep" trials="100"
            periodic_mover="design_all_w_minimize_vanilla"
eval_period="20" history="10"
            bolz_rank="1" recover_low="1" preapply="0"
drift="1"
            checkpoint_file="mc" keep_checkpoint_file="0"
            filter_name="cav_vol_chainA_disabled"
temperature="1.5" sample_type="low"
            stopping_condition="all_enabled_filters_chainA" >
            <Filters>
                <AND filter_name=unsat_core_chainA_disabled
sample_type="low" temperature=0.05 />
                <AND filter_name="score_res_chainA_disabled"
sample_type="low" temperature=0.05 />
            </Filters>
        </GenericSimulatedAnnealer>
        <GenericMonteCarlo name="MC_FastDesignProtein"
            mover_name="fdesign_all_elec"
            filter_name="cav_vol_chainA_disabled"
            sample_type="low"
            trials="3" preapply="0"
            stopping_condition="all_enabled_filters_chainA" >
            <Filters>
                <AND filter_name="unsat_core_chainA_disabled"
sample_type="low"  />
                <AND filter_name="score_res_chainA_disabled"
sample_type="low" />
            </Filters>
        </GenericMonteCarlo>
    </MOVERS>

    <APPLY_TO_POSE>
    </APPLY_TO_POSE>

    <PROTOCOLS>
```

```
            <Add mover_name=save_RMSDreference_conformation />
            <Add mover_name=SA_DesignProtein />
            <Add  filter_name=rmsd1_chainA_enabled />
            <Add mover_name=MC_FastDesignProtein />
            <Add  filter_name=rmsd2_chainA_enabled />
            <Add mover_name=fast_relax_vanilla />
            <Add  filter_name=rmsd3_chainA_enabled />
            <Add  filter_name=unsat_core_chainA_enabled />
            <Add  filter_name=score_res_chainA_enabled />
            <Add  filter_name=sspred_chainA_enabled />
            <Add  filter_name=packstat_chainA_disabled />
            <Add  filter_name=cav_vol_chainA_disabled />
        </PROTOCOLS>
    </ROSETTASCRIPTS>
```

Appendix C. RosettaScripts XML protocol for sequence design of new mimetics generation-2 (i.e. mimetics based on G1_Neo2_40_1F as template).

```
<ROSETTASCRIPTS>
    <SCOREFXNS>
        <SFXN6_vanilla weights="./talaris2014_cart.wts"
symmetric=0 />
        <SFXN6dA_vanilla
weights="./talaris2014_cart_downAla.wts" symmetric=0 />
        <SFXN6dA_norep_elect
weights="./talaris2014_cart_downAla.wts" symmetric=0 >
                <Reweight scoretype=fa_rep  weight=0.05 />
                <Reweight scoretype=fa_elec weight=1.0 />
        </SFXN6dA_norep_elect >
        <SFXN6dA_elect
weights="./talaris2014_cart_downAla.wts" symmetric=0 >
                <Reweight scoretype=fa_elec weight=2.0 />
        </SFXN6dA_elect >
    </SCOREFXNS>

    <MOVERS>
        <SwitchChainOrder name="keep_only_chain_A"
chain_order="1"/>
    </MOVERS>

    <FILTERS>
    <!--Not Enabled-->
        <Holes name="holes_disabled" threshold="1.2"
confidence="0"/>
        <ScoreType name="score_disabled"
scorefxn="SFXN6_vanilla" score_type="total_score"
threshold=0.0 confidence="0" />
        <ResidueCount name="nres_disabled" confidence="0" />
        <PackStat name=packstat_disabled threshold="0.65"
repeats="3" confidence="0" />
        <SSPrediction name="sspred_disabled"
cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.85
confidence="0"/>
        <BuriedUnsatHbonds name="unsat_core_disabled" cutoff=0
task_operations="only_core_residues" jump_number=0
confidence="0"/>
        <RmsdSimple name="rmsd1_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
        <RmsdSimple name="rmsd2_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
        <RmsdSimple name="rmsd3_chainA_disabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="0"/>
```

```
        <CalculatorFilter name="score_res_disabled"
equation="SCORE/NRES" threshold="-1.8" confidence="0">
            <SCORE name="SCORE" filter_name="score_disabled"
/>
            <NRES name="NRES" filter_name="nres_disabled" />
        </CalculatorFilter>

        <!--Enabled-->
        <PackStat name="packstat_enabled" threshold="0.65"
repeats="3" confidence="1" />
        <SSPrediction name="sspred_enabled"
cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.85
confidence="1"/>
        <CalculatorFilter name="score_res_enabled"
equation="SCORE/NRES" threshold="-1.8" confidence="1">
            <SCORE name="SCORE" filter_name="score_disabled"
/>
            <NRES name="NRES" filter_name="nres_disabled" />
        </CalculatorFilter>
        <CavityVolume name="cav_vol_disabled" />
        <BuriedUnsatHbonds name="unsat_core_enabled" cutoff=0
task_operations="only_core_residues" jump_number=0
confidence="1"/>
        <RmsdSimple name="rmsd1_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <RmsdSimple name="rmsd2_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <RmsdSimple name="rmsd3_chainA_enabled"
reference_name="reference_conformation" chain="1" align="1"
threshold="1.0" confidence="1"/>
        <CompoundStatement name=all_enabled_filters >
            <AND filter_name=sspred_enabled />
            <AND filter_name=score_res_enabled />
            <AND filter_name=packstat_enabled />
            <AND filter_name=unsat_core_enabled />
        </CompoundStatement>
    </FILTERS>

    <FILTERS>
        <!--Chain A Filters-->
        <!--Not Enabled-->
        <MoveBeforeFilter name="packstat_chainA_disabled"
mover="keep_only_chain_A" filter=packstat_disabled
confidence="0"/>
        <MoveBeforeFilter name="sspred_chainA_disabled"
mover="keep_only_chain_A" filter=sspred_disabled
confidence="0"/>
```

```
        <MoveBeforeFilter name="score_res_chainA_disabled"
mover="keep_only_chain_A" filter=score_res_disabled
confidence="0"/>
        <MoveBeforeFilter name="cav_vol_chainA_disabled"
mover="keep_only_chain_A" filter=cav_vol_disabled
confidence="0"/>
        <MoveBeforeFilter name="unsat_core_chainA_disabled"
mover="keep_only_chain_A" filter=unsat_core_disabled
confidence="0"/>
        <!--Enabled-->
        <MoveBeforeFilter name="packstat_chainA_enabled"
mover="keep_only_chain_A" filter=packstat_enabled
confidence="1"/>
        <MoveBeforeFilter name="sspred_chainA_enabled"
mover="keep_only_chain_A" filter=sspred_enabled
confidence="1"/>
        <MoveBeforeFilter name="score_res_chainA_enabled"
mover="keep_only_chain_A" filter=score_res_enabled
confidence="1"/>
        <MoveBeforeFilter name="all_enabled_filters_chainA"
mover="keep_only_chain_A" filter=all_enabled_filters
confidence="1"/>
        <MoveBeforeFilter name="unsat_core_chainA_enabled"
mover="keep_only_chain_A" filter=unsat_core_enabled
confidence="1"/>
    </FILTERS>

    <RESIDUE_SELECTORS>
        <ResiduePDBInfoHasLabel name="hotspots"
property="HOTSPOTB" />
    </RESIDUE_SELECTORS>

    <TASKOPERATIONS>
        <InitializeFromCommandline name="init"/>
        <IncludeCurrent name="inclcur"/>
        <LimitAromaChi2 name=limitchi2 />
        <DisallowIfNonnative name="only_native_H"
disallow_aas="H"/>
        <ReadResfile name="resfile" filename="./input.resfile"
/>
        <PreventChainFromRepacking name="not_chain_B"
chain="2" />
        <PreventChainFromRepacking name="not_chain_C"
chain="3" />
        <PreventChainFromRepacking name="not_chain_D"
chain="4" />
        <!--Select designable residues by sasa and packable by
flag-->
        <SelectBySASA name="only_core_residues" mode="mc"
probe_radius=2.0 core_asa=20.0 surface_asa=30.0 core=1
boundary=0 surface=0 verbose=1 />
```

```
        <!--Restrict Hotspots to Repacking-->
        <OperateOnResidueSubset name="hotspot_onlyrepack"
selector="hotspots" >
            <RestrictToRepackingRLT/>
        </OperateOnResidueSubset>
        <!--Layer Design as Tom Helped to set omit operations.
Thanks Tom L. :)-->
        <LayerDesign name="layer_all" layer="all"
use_sidechain_neighbors="True" pore_radius="2.0"
verbose="true" >
            <NoRepackDisulfides name="disulf" >
                    <all aa="c" specification="fixed"
operation="omit" />
            </NoRepackDisulfides>
            <OperateOnResidueSubset
name="hotspot_onlyrepack_layerdesignOmit" selector="hotspots"
>
                <PreventRepackingRLT/>
                <all specification="fixed" operation="omit" />
            </OperateOnResidueSubset>
            <ReadResfile name="resfile_layerdesignOmit"
filename="./input_fix.resfile" >
                <all specification="fixed" operation="omit" />
            </ReadResfile>
            <core>
                    <all append="M" />
            </core>
            <boundary>
                    <all append="M" />
            </boundary>
        </LayerDesign>
        <LayerDesign name="layer_boundary_surface"
layer="boundary_surface" use_sidechain_neighbors="True"
pore_radius="2.0" verbose="true" >
            <NoRepackDisulfides name="disulf" >
                    <all aa="c" specification="fixed"
operation="omit" />
            </NoRepackDisulfides>
            <OperateOnResidueSubset
name="hotspot_onlyrepack_layerdesignOmit" selector="hotspots"
>
                <PreventRepackingRLT/>
                <all specification="fixed" operation="omit" />
            </OperateOnResidueSubset>
            <ReadResfile name="resfile_layerdesignOmit"
filename="./input_fix.resfile" >
                <all specification="fixed" operation="omit" />
            </ReadResfile>
            <core>
                    <all append="M" />
            </core>
```

```
            <boundary>
                    <all append="M" />
            </boundary>
        </LayerDesign>
    </TASKOPERATIONS>

    <MOVERS>
        <SavePoseMover name="save_RMSDreference_conformation"
reference_name="reference_conformation"/>
        <AddConstraintsToCurrentConformationMover
name=constrainCA
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" CA_only=1 />
        <ClearConstraintsMover name=clearAllConstraints />
        <PackRotamersMover name="design_all_norep"
scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" />
        <PackRotamersMover name="design_onlyCore_norep"
scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,only_core_residues,not_chain_B,no
t_chain_C,not_chain_D" />
        <TaskAwareMinMover name="min_vanilla_SC"
scorefxn="SFXN6_vanilla"  bb="0" chi="1" jump="1"
task_operations="init,resfile,inclcur,limitchi2,only_native_H,
layer_all,hotspot_onlyrepack,not_chain_B,not_chain_C,not_chain
_D" />
        <TaskAwareMinMover name="min_vanilla_BBSC"
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```
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```

We claim

1. A non-naturally occurring polypeptide comprising domains X1, X2, X3, and X4, wherein:

(a) X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to §HA§_YD§A§_ (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to §AF§FE§_§ (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to §TIL§S§§IF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$), IL-4 receptor $\alpha\gamma_c$heterodimer (IL-4R$\alpha\gamma_c$), or IL-13 receptor α subunit (IL-13Rα).

2. The polypeptide of claim 1, wherein:

(i) X1 includes one or both of the following: H at residue 2 and Y at residue 5; and/or

(ii) X3 includes 1, 2, 3, 4, or all 5 of the following: Y at residue 1, F at residue 3, N at residue 4, L at residue 7, and I at residue 8.

3. The polypeptide of claim 1 or 2, wherein:

(iii) X4 includes I at residue 8.

4. The polypeptide of claim 1, wherein:

(i) X1 includes E at residue 2 and K at residue 5; and

(ii) X3 includes F at residue 1, K at residue 3, R at residue 4, R at residue 7, and N at residue 8.

5. The polypeptide of claim 1 or 4, wherein

(iii) X4 includes F at residue 8.

6. The polypeptide of any one of claims 1-5, wherein:

X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4) ;

X3 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length the peptide LEDYAFNFELILEELARLFESG (SEQ ID NO:5) ; and

X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide EDEQEEMANAIITILQSWIFS (SEQ ID NO:6) .

7. The polypeptide of claim 6, wherein:

(i) X1 includes 1, 2, 3, 4, or all 5 of the following: L at residue 7, H at residue 8, H at residue 11, Y at residue 14; M at residue 18; and/or

(ii) X3 includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following: D at residue 3, Y at residue 4, F at residue 6, N at residue 7, L at residue 10, I at residue 11, E at residue 13, or E at residue 14.

8. The polypeptide of claim 6 or 7, wherein:

(iii) X4 includes I at residue 19.

9. The polypeptide of claim 6, wherein:

X1 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide PKKKIQIMAEEALKDALSILNI (SEQ ID NO: 8);

X3 is a peptide comprising the amino acid sequence at least 37% 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length the peptide LERFAKRFERNLWGIARLFESG (SEQ ID NO: 9); and

X4 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to the peptide EDEQEEMANAIITILQSWFFS (SEQ ID NO: 10).

wherein

(i) X1 includes I at residue 7, T or M at residue 8, E at residue 11, K at residue 14 and S at residue 18; and

(ii) X3 includes R at residue 3, F at residue 4, K at residue 6, R at residue 7, R at residue 10, N at residue 11, W at residue 13, and G at residue 14.

10. The polypeptide of claim 9, wherein

(iii) X4 includes F at residue 19.

11. The polypeptide of any one of claims 1-7, wherein amino acid substitutions relative to the reference peptide domains do not occur at AA residues marked in bold font.

12. The polypeptide of any one of claims 1-11, wherein amino acid substitutions relative to the reference peptide domains are conservative amino acid substitutions.

13. The polypeptide of any one of claims 1-12, wherein the domains are arranged N-terminal to C-terminal in an arrangement selected from the group consisting of X1-X2-X3-X4; X1-X3-X2-X4; X1-X4-X2-X3; X3-X2-X1-X4; X4-X3-X2-X1; X2-X3-X4-X1; and X2-X1-X4-X3.

14. The polypeptide of any one of claims 6-13, wherein amino acid residues relative to SEQ ID NO:4 are selected from the group consisting of:

Position 01:	A	F	I	L	M	P	Q	R	S	W
Position 02:	A	D	E	G	V	K				
Position 03:	D	E	F	W	K					
Position 04:	D	E	K	N	P	R	W			
Position 05:	D	E	H	I	K	L	M	S		
Position 06:	A	D	E	G	L	P	S	W	Q	
Position 07:	D	E	L	Q	Y	I				
Position 08:	A	F	H	W	Y	M	T			
Position 09:	C	F	P	A						
Position 10:	C	D	E	F	K	P				
Position 11:	D	F	H	E						
Position 12:	A	D	E	P	S	T	V			
Position 13:	H	I	L	M	P	R	V	W		

Position 14:	F	R	W	Y	K					
Position 15:	D	E	N	Y						
Position 16:	A	C	L	M	S					
Position 17:	F	I	L	M	P	R				
Position 18:	G	M	Q	Y	S					
Position 19:	I	L	M	P	Q	V				
Position 20:	A	K	L	M	Q	R	S			
Position 21:	G	K	N	P	R	S	W			
Position 22:	D	E	I	K	M	N	W	Y		

15. The polypeptide of claim 14, wherein position 7 is I, position 8 is M or T, position 11 is E, position 14 is K, and position 18 is S.

16. The polypeptide of claim 14, wherein 1, 2, 3, 4, or 5 of the following are not true: position 7 is I, position 8 is M or T, position 11 is E, position 14 is K, and position 18 is S.

17. The polypeptide of any one of claims 6-16, wherein amino acid residues relative to SEQ ID NO:5 are selected from the group consisting of:

Position 01:	A	L								
Position 02:	D	E	G	K	M	T				
Position 03:	D	E	N	Y	R					
Position 04:	C	D	G	T	Y	F				
Position 05:	A	F	H	S	V	W	Y			
Position 06:	A	F	I	M	T	V	Y	K		
Position 07:	D	K	N	S	T	R				
Position 08:	A	C	G	L	M	S	V	F		
Position 09:	C	H	K	L	R	S	T	V	E	
Position 10:	F	I	L	M	Y	R				
Position 11:	I	L	N	T	Y					
Position 12:	F	K	L	M	S	V				
Position 13:	A	D	F	G	I	N	P	Q	S	T	E	W
Position 14:	A	E	F	G	H	S	V			
Position 15:	C	I	L	M	V	W				

Position 16:	A	D	G	S	T	V					
Position 17:	H	K	L	N	R						
Position 18:	C	D	G	I	L	Q	R	T	W		
Position 19:	D	F	M	N	W						
Position 20:	A	C	E	F	G	M	S	Y			
Position 21:	D	E	G	H	L	M	R	S	T	V	W
Position 22:	A	D	G	K	N	S	Y				

18. The polypeptide of any one of claims 6 to 17 comprising a cysteine substitution at position 17 or 20 relative to SEQ ID NO:5.

19. The polypeptide of claim 17 or 18, wherein position 3 is R, position 4 is F, position 6 is K, position 7 is R, position 10 is R, position 11 is N, position 13 is W, and position 14 is G.

20. The polypeptide of claim 17 or 18, wherein 1, 2, 3, 4, 5, 6, 7, or all 8 of the following are not true: position 3 is R, position 4 is F, position 6 is K, position 7 is R, position 10 is R, position 11 is N, position 13 is W, and position 14 is G.

21. The polypeptide of any one of claims 6-20, wherein amino acid residues relative to SEQ ID NO:6 are selected from the group consisting of:

Position 01:	D	E	G	K	V				
Position 02:	D	I	M	S					
Position 03:	E	G	H	K					
Position 04:	E	G	I	K	Q	R	S		
Position 05:	A	D	E	G	H	S	V		
Position 06:	C	D	E	G	I	M	Q	R	T	V
Position 07:	C	E	L	M	P	R	T		
Position 08:	A	F	L	M	W				
Position 09:	A	G	L	N	Q	R	T		
Position 10:	A	C	D	E	F	H	I	W	
Position 11:	I	M	N	S	V	W			
Position 12:	I	K	L	S	V				
Position 13:	C	L	M	R	S	T			
Position 14:	I	L	P	T	Y				

Position 15:	F	G	I	L	M	N	V
Position 16:	H	K	Q	R			
Position 17:	C	F	K	S	W	Y	
Position 18:	K	Q	T	W			
Position 19:	C	G	N	I			
Position 20:	C	F	G	L	Y		
Position 21:	A	F	G	H	S	Y	

22. The polypeptide of any one of claims 6 to 21 comprising a cysteine substitution at position 3 relative to SEQ NO:6.

23. The polypeptide of claim 21 or 22 where position 19 is I.

24. The polypeptide of claim 21 or 22 where position 19 is not I.

25. The polypeptide of any one of claims 1-24, wherein X2 is a peptide comprising the amino acid sequence at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical along its length to KDEAEKAKRMKEWMKRIKT (SEQ ID NO:7) .

26. The polypeptide of claim 25, wherein amino acid residues relative to SEQ ID NO:7 are selected from the group consisting of:

Position 01:	A	H	L	M	R	S	V	K		
Position 02:	A	D	E	Q	R	S	T	V	W	Y
Position 03:	C	E	G	K	L	N	Q	R	W	
Position 04:	A	F	G	N	S	T	V	Y		
Position 05:	A	E	G	I	M	R	V	C		
Position 06:	C	E	K	L	N	R	V			
Position 07:	A	C	E	I	L	S	T	V	W	
Position 08:	H	K	L	M	S	T	W	Y		
Position 09:	A	I	L	M	Q	S	R			
Position 10:	A	I	M	S	W	Y				
Position 11:	C	I	K	L	S	V				

Position 12:	C	E	K	L	P	Q	R	T		
Position 13:	A	D	H	N	W					
Position 14:	A	C	G	I	L	S	T	V	M	
Position 15:	A	E	G	I	K	L	M	R	V	
Position 16:	G	H	L	R	S	T	V	C		
Position 17:	A	I	L	V						
Position 18:	A	C	D	E	G	H	I	K	M	S
Position 19:	D	E	G	L	N	V	T			

27. The polypeptide of claim 25 or 26 comprising a cysteine substitution at position 1, 2, 5, 9, 12, or 16 relative to SEQ NO:7.

28. The polypeptide of claim 26 or 27, wherein position 11 is I.

29. The polypeptide of claim 26 or 27, wherein position 11 is not I.

30. The polypeptide of any one of claims 1-29, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:11-94, 103-184, 190-243, and 245-247.

31. The polypeptide of any one of claims 1-29, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:90 and 181.

32. The polypeptide of any one of claims 1-31, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence of SEQ ID NO:90, and wherein one, two, or more of the following mutations are present:

R50C;

E53C;

E62C;

E69C;

R73C; and/or
E82C.

33. The polypeptide of any one of claims 1-31, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence of SEQ ID NO:181, and wherein one, two, or more of the following mutations are present
D56C;
K58C;
D59C;
R66C;
T77C;
E85C;
R50C;
E53C;
E62C;
E69C;
R73C; and/or
E82C.

34. The polypeptide of claim 32 or 33, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 190-243; or wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence selected from the group consisting of SEQ ID NO:195 and 222; or wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the full length of the amino acid sequence of SEQ ID NO:195.

35. The polypeptide of any one of claims 1-34, wherein the polypeptide is linked to a stabilization compound, including but not limited to a polyethylene glycol ("PEG") containing moiety.

36. The polypeptide of claim 35, wherein the stabilization compound, including but not limited to a PEG-containing moiety, is linked at a cysteine residue in the polypeptide.

37. The polypeptide of claim 36, wherein the cysteine residue is present in X2.

38. The polypeptide of any one of claims 35-37, wherein the stabilization compound, including but not limited to a PEG-containing moiety, is linked to the cysteine reside via a maleimide group, including but not limited to linked to a cysteine residue present at amino acid residue 62 relative to SEQ ID NO:90.

39. The polypeptide of any one of claims 1-38, wherein the polypeptide comprises a polypeptide at least 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:92, 93, 183, and 184.

40. The polypeptide of any one of claims 30-39, wherein position 7 is I, position 8 is T or M, position 11 is E, position 14 is K, position 18 is S, position 33 is Q, position 36 is R, position 37 is F, position 39 is K, position 40 is R, position 43 is R, position 44 is N, position 46 is W, and position 47 is G.

41. The polypeptide of claim 40, wherein position 68 is I and position 98 is F.

42. The polypeptide of any one of claims 30-39, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all1 4 of the following are not true: position 7 is I, position 8 is T or M, position 11 is E, position 14 is K, position 18 is S, position 33 is Q, position 36 is R, position 37 is F, position 39 is K, position 40 is R, position 43 is R, position 44 is N, position 46 is W, and position 47 is G.

43. The polypeptide of claim 42, wherein one or both of the following are not true: position 68 is I and position 98 is F.

44. The polypeptide of claim 1, wherein the polypeptide comprises a polypeptide at least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:11-94, 103-184, 190-243, and 245-247.

45. The polypeptide of claim 44, wherein the polypeptide comprises a polypeptide at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:11-94, 103-184, 190-243, and 245-247.

46. The polypeptide of claim 44 or 45 wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$).

47. The polypeptide of claim 1, wherein:

(a) X1 is a peptide comprising the amino acid sequence at least 85% identical to EHALYDAL (SEQ ID NO:1);

(b) X2 is a helical-peptide of at least 8 amino acids in length;

(c) X3 is a peptide comprising the amino acid sequence at least 85% identical to YAFNFELI (SEQ ID NO:2);

(d) X4 is a peptide comprising the amino acid sequence at least 85% identical to ITILQSWIF (SEQ ID NO:3);

wherein X1, X2, X3, and X4 may be in any order in the polypeptide;

wherein amino acid linkers may be present between any of the domains; and

wherein the polypeptide binds to IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$).

48. The polypeptide of claim 1, wherein:

X1 is a peptide comprising the amino acid sequence at least 80% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4) ;

X3 is a peptide comprising the amino acid sequence at least 80% identical along its length to the peptide LEDYAFNFELILEEIARLFESG (SEQ ID NO:5) ; and

X4 is a peptide comprising the amino acid at least 80% identical along its length to the peptide EDEQEEMANAITILQSWIFS (SEQ ID NO:6) .

49. The polypeptide of claim 48, wherein:

X1 is a peptide comprising the amino acid sequence at least 90% identical along its length to the peptide PKKKIQLHAEHALYDALMILNI (SEQ ID NO: 4) ;

X3 is a peptide comprising the amino acid sequence at least 90% identical along its length to the peptide LEDVAFNFELLEELARLFESG (SEQ ID NO:5) ; and

X4 is a peptide comprising the amino acid at least 90% identical along its length to the peptide EDEQEEMANAITILQSWIFS (SEQ ID NO:6) .

50. The polypeptide of any one of claims 47 to 49, wherein X2 is a peptide comprising the amino acid sequence at least 90% identical along its length to KDEAEKAKRMKEWMKRIKT (SEQ ID NO:7) .

51. The polypeptide of any one of claims 44-50, wherein the polypeptide is linked to a polyethylene glycol ("PEG") containing moiety at a cysteine residue in X2 of the polypeptide.

52. The polypeptide of claim 51 wherein the polypeptide comprises a polypeptide at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:195 and 222 and wherein the PEG-containing moiety, is linked to the cysteine reside at amino acid residue 62 relative to SEQ ID NO:195.

53. The polypeptide of any one of claims 44 to 52, wherein the domains are arranged N-terminal to C-terminal in an arrangement selected from the group consisting of X1-X3-X2-X4.

54. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-17, 20-21, 24-26, 29-38, 42-43 and 47 -53 having a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-2.

55. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-17, 20-21, 24-26, 29-38, 42-44 and 47-53 having a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than

2.5 Angstroms when superimposed on backbone atoms or alpha carbon atoms of a three dimensional structure having the structural coordinates of Table E2.

56. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-17, 20-21, 24-26, 29-38, 42-45, and 47 -53, wherein when in ternary complex with the mouse IL-2 receptor $\beta\gamma_c$, the polypeptide has a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-2 when in ternary complex with the mouse IL-2 receptor $\beta\gamma_c$.

57. The polypeptide of any one of claims 54-56 wherein the root mean square deviation is less than 1.5 Angstroms.

58. The polypeptide of claim 57 wherein the root mean square deviation is less than 1 Angstrom.

59. The polypeptide of any one of claims 4-6, 9-15, 17-19, 21-23, 25-28, 30, and 39-41 having a three dimensional structure with structural coordinates having a root mean square deviation of common residue backbone atoms or alpha carbon atoms of less than 2.5 Angstroms when superimposed on backbone atoms or alpha carbon atoms of the three dimensional structure of native IL-4.

60. The polypeptide of claim 59 wherein the root mean square deviation is less than 1.5 Angstroms.

61. The polypeptide of claim 59 or 60 wherein the root mean square deviation is less than 1 Angstrom.

62. The polypeptide of any one of claims 54-61 wherein the three dimensional structure of the polypeptide is determined using computational modeling.

63. The polypeptide of any one of claims 1-62, wherein X1, X2, X3, and X4 are alpha-helical domains.

64. The polypeptide of any one of claims 1-63 wherein the amino acid length of each of X1, X2, X3 and X4 is at least about 8 amino acids in length.

65. The polypeptide of any one of claims 1-64 wherein the amino acid length of each of X1, X2, X3 and X4 is at least about 19 amino acids in length.

66 The polypeptide of any one of claims 1-65 wherein the amino acid length of each of X1, X2, X3 and X4 is no more than 1000 amino acids in length, no more than 500 amino acids in length, no more than 400 amino acids in length, no more than 300 amino acids in length, no more than 200 amino acids in length, no more than 100 amino acids in length, or amino acids in length 50 amino acids in length.

67. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-66, wherein X1 binds to the beta and the gamma subunit of the human IL-2 receptor.

68. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-555, and 62-67 wherein X2 does not bind to the human IL-2 receptor.

69. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-68, wherein X3 binds to the beta subunit of the human IL-2 receptor.

70. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-69, wherein X4 binds to the gamma subunit of the human IL-2 receptor.

71. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-70 that does not bind to the alpha subunit of the human or murine IL-2 receptor.

72 The polypeptide of any one of claims 67-71 wherein the binding is specific binding as determined by surface plasmon resonance at biologically relevant concentrations.

73. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-72 that binds to that binds to the IL-2 receptor $\beta\gamma_c$ heterodimer (IL-2R$\beta\gamma_c$) with a binding affinity of 200 nm or less, 100 nm or less, 50 nM or less, or 25 nM or less.

74. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-73 wherein the polypeptide's affinity for the human and mouse IL-2 receptors is about equal to or greater than that of native IL-2.

75. The polypeptide of any one of claims 4-6, 9-15, 17-19, 21-23, 25-28, 30, 35-37, 39-41, and 59-66 that binds to that binds to the IL-4 receptor $\alpha\gamma_c$heterodimer (IL-4R$\alpha\gamma_c$) with a binding affinity of 200 nm or less, 100 nm or less, 50 nM or less, or 25 nM or less.

76. The polypeptide of any one of claims 4-6, 9-15, 17-19, 21-23, 25-28, 30, 35-37, 39-41, 59-66, and 75, wherein the polypeptide's affinity for the human and mouse IL-4 receptors is about equal to or greater than that of native IL-4.

77. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, and 62-74, wherein the polypeptide stimulates STAT5 phosphorylation in cells expressing the IL-2 receptor with potency about equal to or greater than native IL-2.

78. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, 62-74, and 77 wherein the polypeptide stimulates STAT5 phosphorylation in cells expressing the IL-2 receptor with potency about equal to or greater than native IL-2 in cells expressing IL-2 receptor $\beta\gamma_c$ heterodimer but lacking the IL-2 receptor α.

79. The polypeptide of any one of claims 2-3, 6-8, 11-14, 16-18, 20-22, 24-27, 29-38, 42-55, 62-74, and 77-78, wherein the polypeptide demonstrates thermal stability about equal to or greater than the thermal stability of native IL-2.

80. The polypeptide of any one of claims 1-79, that maintains or recovers at least 70% of its folded structure after thermal stability testing.

81. The polypeptide of any one of claims 1-80 that maintains or recovers at least 80% of its folded structure after thermal stability testing.

82. The polypeptide of any one of claims 1-81 that maintains or recovers at least 90% of its folded structure after thermal stability testing.

83. The polypeptide of any one of claims 1-82 that maintains or recovers at least 80% of its ellipticity spectrum after thermal stability testing.

84. The polypeptide of any one of claims 1-83 that maintains or recovers at least 70% activity after thermal stability testing.

85. The polypeptide of any one of claims 1-84 that maintains or recovers at least 80% activity after thermal stability testing.

86 The polypeptide of any one of claims 84 or 85 wherein activity is determined by a STAT5 phosphorylation assay.

87. The polypeptide of any one of claims 79-85 wherein thermal stability is measured by circular dichroism (CD) spectroscopy at 222 nM

88. The polypeptide of claim 87 wherein the thermal stability test comprises heating the polypeptide from 25 degrees Celsius to 95 degrees Celsius in one hour time frame , cooling the polypeptide to 25 degrees Celsius in a 5 minute time frame and monitoring ellipticity at 222 nm.

89. The polypeptide of claim 1, wherein the polypeptide comprises a polypeptide at least 90% identical, at least 95% identical or 100% identical to the amino acid sequence of SEQ ID NO:90, 181, or 247 wherein the polypeptide (i) does not bind to human or murine IL-2Ralpha, (ii) binds to human IL2RB with an affinity of about 11.2 nM (iii) binds to murine IL2RB with an affinity of about 16.1 nm (IV) binds to human IL-2R$\beta\gamma_c$ with an affinity of about 18.8 nM and (V) binds to murine IL-2R$\beta\gamma_c$ with an affinity of about 3.4 nM.

90. The polypeptide of any one of claims 1-89, wherein the polypeptide includes at least one disulfide bond.

91. The polypeptide of claim 90 wherein the disulfide bond links two of the X1, X2, X3, and X4 domains together.

92. The polypeptide of claim 90 or 91 wherein the disulfide bond links the X1 domain to the X4 domain.

93. The polypeptide of claim 90-92 wherein the disulfide linkage improves the thermal stability of the polypeptide as compared to a substantially similar polypeptide with no disulfide bond linking two domains together.

94. The polypeptide of any one of claims 1-93, wherein the polypeptide further comprises a targeting domain.

95. The polypeptide of claim 94, wherein the targeting domain is a translational fusion with the polypeptide.

96. The polypeptide of claim 94 or 95 wherein the targeting domain binds to a cell surface protein.

97. The polypeptide of claim 96, wherein the cell surface protein is present on the surface of cells selected from the group consisting of tumor cells, vascular components, tumor microenvironment (e.g. fibroblasts, infiltrating immune cells, or stromal elements), other cancer cells and immune cells (including but not limited to CD8+ T cells, T-regulatory cells, dendritic cells, NK cells, or macrophages), such immune cell surface markers including but not limited to CD3, CD4, CD8, CD19, CD20, CD21, CD25, CD37, CD30, CD33, CD40, CD68, CD123, CD254, PD-1, B7-H3, and CTLA-4.

98. The polypeptide of claim 96, wherein the targeting domain binds to a tumor cell, tumor vascular component cell, or tumor microenvironment cell surface marker.

99. The polypeptide of claim 98, wherein the cell surface marker is selected from the group including but not limited to EGFR, EGFRvIII, Her2, HER3, EpCAM, MSLN, MUC16, PSMA, TROP2, ROR1, RON, PD-L1, CD47, CTLA-4,CD5, CD19, CD20, CD25, CD37, CD30, CD33, CD40, CD45, CAMPATH-1, BCMA, CS-1, PD-L1, B7-H3, B7-DC, HLD-

DR, carcinoembryonic antigen (CEA), TAG-72, EpCAM, MUC1, folate-binding protein, A33, G250, prostate-specific membrane antigen (PSMA), ferritin, GD2, GD3, GM2, Le^y, CA-125, CA19-9, epidermal growth factor, p185HER2, IL-2 receptor, EGFRvIII (de2-7 EGFR), fibroblast activation protein, tenascin, a metalloproteinase, endosialin, vascular endothelial growth factor, avB3, WT1, LMP2, HPV E6, HPV E7, Her-2/neu, MAGE A3, p53 nonmutant, NY-ESO-1, MelanA/MART1, Ras mutant, gp100, p53 mutant, PR1, bcr-abl, tyronsinase, survivin, PSA, hTERT, a Sarcoma translocation breakpoint protein, EphA2, PAP, ML-IAP, AFP, ERG, NA17, PAX3, ALK, androgen receptor, cyclin B 1, polysialic acid, MYCN, RhoC, TRP-2, fucosyl GM1, mesothelin (MSLN), PSCA, MAGE Al, sLe(animal), CYP1B1, PLAV1, GM3, BORIS, Tn, GloboH, ETV6-AML, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TESL Sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, Legumain, Tie 3, VEGFR2, MAD-CT-1, PDGFR-B, MAD-CT-2, ROR2, TRAIL1, MUC16, MAGE A4, MAGE C2, GAGE, EGFR, CMET, HER3, MUC15, CA6, NAPI2B, TROP2, CLDN6, CLDN16, CLDN18.2, CLorf186, RON, LY6E, FRA, DLL3, PTK7, STRA6, TMPRSS3, TMPRSS4, TMEM238, UPK1B, VTCN1, LIV1, ROR1, Fos-related antigen 1, and the markers listed below:

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM.sub.--001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM.sub.--003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM.sub.--012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM.sub.--005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM.sub.--006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm. 42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM.sub.--

017763);

(11) STEAP2 (HGNC.sub.--8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM.sub.--017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP.sub.--003203 or NM.sub.--003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR(C3d/Epstein Barr virus receptor) or Hs. 73792, Genbank accession no. M26004);

(15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM.sub.--000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM.sub.--030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20R.alpha. (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) Ephb2R (Genbank accession no. NM.sub.--004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763);

(26) BAFF-R (Genbank accession no. NP.sub.--443177.1);

(27) CD22 (Genbank accession no. NP-001762.1);

(28) CD79a (CD79A, CD79.alpha., immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP.sub.--001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP.sub.--001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and

presents them to CD4+ T lymphocytes, Genbank accession No. NP.sub.--002111.1);
(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP.sub.--002552.2);
(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP.sub.--001773.1);
(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP.sub.--005573.1);
(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP.sub.--443170.1); and
(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP.sub.--112571.1).

100. The polypeptide of claim 96, wherein the targeting domain binds to an immune cell surface marker (including but not limited to CD8+ T cells, T-regulatory cells, dendritic cells, NK cells, or macrophages), wherein the immune cell surface marker may include but is not limited to CD3, CD4, CD8, CD19, CD20, CD21, CD25, CD37, CD30, CD33, CD40, CD68, CD123, CD254, PD-1, B7-H3, and CTLA-4, and/or wherein the targeting domain binds to PD-1, PDL-1, CTLA-4, TROP2, B7-H3, CD33, CD22, carbonic anhydrase IX, CD123, Nectin-4, tissue factor antigen, CD154, B7-H3, B7-H4, FAP (fibroblast activation protein) or MUC16.

101. The polypeptide of any one of claims 94-100, wherein the targeting domain may include but is not limited to an scFv, a F(ab), a $F(ab')_2$, a B cell receptor (BCR), a DARPin, an affibody, a monobody, a nanobody, diabody, an antibody (including a monospecific or bispecific antibody); a cell-targeting oligopeptide including but not limited to RGD integrin-binding peptides, de novo designed binders, aptamers, a bicycle peptide, conotoxins, small molecules such as folic acid, and a virus that binds to the cell surface.

102. A recombinant nucleic acid encoding the polypeptide of any one of claims 1-101.

103. An expression vector comprising the recombinant nucleic acid of claim 102 operatively linked to a promoter.

104. A recombinant host cell comprising the nucleic acid of claim 102 and/or the expression vector of claim 103.

105. A pharmaceutical composition, comprising the polypeptide of any one of claims 1-101, the recombinant nucleic acid of claim 102, the expression vector of claim 103, or the recombinant host cell of claim 104, and a pharmaceutically acceptable carrier.

106. A method for treating cancer, comprising administering to a subject having cancer the polypeptide of any one of claims 1-101, the recombinant nucleic acid of claim 102, the expression vector of claim 103, the recombinant host cell of claim 104, or the pharmaceutical composition of claim 105 in an amount effective to treat the tumor.

107. The method of claim 106, where the cancer is selected from the group consisting of colon cancer, melanoma, renal cell cancer, head and neck squamous cell cancer, gastric cancer, urothelial carcinoma, Hodgkin lymphoma, non-small cell lung cancer, small cell lung cancer, hepatocellular carcinoma, pancreatic cancer, Merkel cell carcinoma, colorectal cancer, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, ovarian cancer, cervical cancer, and any tumor types selected by a diagnostic test, such as microsatellite instability, tumor mutational burden, PD-L1 expression level, or the immunoscore assay (as developed by the Society for Immunotherapy of Cancer).

108. The polypeptide of any one of claims 1-101, the recombinant nucleic acid of claim 102, the expression vector of claim 103, the recombinant host cell of claim 104, or the pharmaceutical composition of claim 105 for use as a medicament for treating cancer and/or for modulating an immune response in a subject.

109. A method for modulating an immune response in a subject comprising administering to the subject the polypeptide of any one of claims 1-101, the recombinant nucleic acid of claim 102, the expression vector of claim 103, the recombinant host cell of claim 014, or the pharmaceutical composition of claim 105.

110. The method of claim 109 wherein the immune response is an anti-cancer immune response, a tissue reparative immune response or a wound healing immune response.

111. The method of claim 109 or 110, wherein the polypeptide, recombinant nucleic acid, expression vector, recombinant host cell, or pharmaceutical composition is administered as a component of a biomaterial.

112. The method of any one of claims 109-111 further comprising administering a second therapeutic agent to the subject.

113. The method of claim 112 wherein the polypeptide enhances the subject's immune response to the second therapeutic agent.

114. The method of any one of claims 112-113 wherein the second therapeutic agent comprises a chemotherapeutic agent or an antigen-specific immunotherapeutic agent including but not limited to a chimeric antigen receptor T cell.

115. A method, comprising:

determining a structure for a plurality of residues of a protein using a computing device, wherein the structure of the plurality of residues provides a particular receptor binding interface;

determining a plurality of designed residues using a mimetic design protocol provided by the computing device, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues;

determining one or more connecting helix structures that connect the plurality of designed residues using the computing device;

determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations using the computing device;

designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone using the computing device; and

generating an output related to at least the second protein backbone.

116. The method of claim 115, wherein determining the plurality of designed residues using the mimetic design protocol comprises determining an idealized residue using a database of idealized residues, wherein the idealized residue is related to a designed residue of the plurality of designed residues.

117. The method of claim 116, wherein determining the idealized residue using the database of idealized residues comprises:

retrieving one or more idealized fragments related to the idealized residue from the database of idealized residues; and

determining the idealized residue by reconstructing the related designed residue using the one or more idealized fragments.

118. The method of claim 117, wherein reconstructing the related designed residue using the one or more idealized fragments comprises:

reconnecting pairs of the one or more idealized fragments by: use of combinatorial fragment assembly of the pairs of the one or more idealized fragments; and

using Cartesian-constrained backbone minimization to determine whether the pairs of the one or more idealized fragments link two or more of the plurality of designed residues.

119. The method of claim 118, wherein reconstructing the related designed residue using the one or more idealized fragments comprises:

verifying that overlapping fragments of the idealized residue are idealized fragments using the database of idealized residues;

verifying whether the idealized residue does not clash with a target receptor associated with the particular receptor binding interface; and

after verifying that the idealized residue does not clash with a target receptor associated with the particular receptor binding interface, determining a most probable amino acid at each position of the idealized residue using the database of idealized residues.

120. The method of claim 118, wherein determining the first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over the plurality of combinations comprises:

recombining the pairs of the one or more idealized fragments by combinatorially recombining the pairs of the one or more idealized fragments; and

determining the first protein backbone for the protein using the recombined pairs of the one or more idealized fragments.

121. The method of claim 120, wherein combinatorially recombining the pairs of the one or more idealized fragments comprises ranking the pairs of the one or more idealized fragments based on an interconnection length between idealized fragments of the pairs of the one or more idealized fragments.

122. The method of claim 115, wherein determining the plurality of designed residues using the mimetic design protocol comprises:

determining an idealized residue using one or more parametric equations that represent a shape of a designed residue of the plurality of designed residues; and

determining a single fragment that closes the idealized residue with at least one designed residue of the plurality of designed residues.

123. The method of claim 122, wherein the designed residue comprises a helical structure, and wherein the one or more parametric equations comprise an equation related to phi and psi angles of the helical structure.

124. The method of claim 123, wherein the equation related to phi and psi angles of the helical structure includes one or more terms related to an angular pitch of the phi and psi angles of the helical structure

125. The method of claim 115, wherein generating the output related to the second protein backbone for the protein comprises designing one or more molecules based on the second protein backbone for the protein.

126. The method of claim 115, wherein generating the output related to the second protein backbone for the protein comprises:

generating a synthetic gene for the protein that is based the second protein backbone for the protein;

expressing a particular protein *in vivo* using the synthetic gene; and

purifying the particular protein.

127. The method of claim 126, wherein expressing the particular protein sequence *in vivo* using the synthetic gene comprises expressing the particular protein sequence in one or more *Escherichia coli* that include the synthetic gene.

128. The method of claim 115, wherein generating the output related to the second protein backbone for the protein comprises generating one or more images that include at least part of the second protein backbone for the protein.

129. The method of claim 115, wherein the computing device comprises a protein synthesis device, and wherein generating the output related to at least the second protein backbone for the protein comprises synthesizing at least the second protein backbone for the protein using the protein synthesis device.

130. The method of any one of claims 115 to 129 wherein the method is for designing a protein mimetic.

131. A non-naturally occurring protein mimetic identified by the method of claim 130.

132. The non-naturally protein of claim 131 that is a cytokine mimetic.

133. The non-naturally protein of claim 122 that is an IL-2 mimetic or an IL-4 mimetic.

134. A computing device, comprising:

one or more processors; and

data storage, configured to store at least computer-readable instructions that, when executed, cause the computing device to perform functions comprising:

determining a structure for a plurality of residues of a protein that provides a particular receptor binding interface;

determining a plurality of designed residues using a mimetic design protocol, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues;

determining one or more connecting helix structures that connect the plurality of designed residues;

determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations;

designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone; and

generating an output related to at least the second protein backbone for the protein.

135. The computing device of claim 134, wherein determining the plurality of designed residues using the mimetic design protocol comprises determining an idealized residue using a database of idealized residues, wherein the idealized residue is related to a designed residue of the plurality of designed residues.

136. The computing device of claim 134, wherein determining the plurality of designed residues using the mimetic design protocol comprises:

determining an idealized residue using one or more parametric equations that represent a shape of a designed residue of the plurality of designed residues; and

determining a single fragment that closes the idealized residue with at least one designed residue of the plurality of designed residues.

137. The computing device of claim 135, wherein the computing device further comprises a protein synthesis device, and wherein generating the output related to at least the second

protein backbone for the protein comprises synthesizing at least the second protein backbone for the protein using the protein synthesis device.

138. A non-transitory computer-readable medium, configured to store at least computer-readable instructions that, when executed by one or more processors of a computing device, cause the computing device to perform functions comprising:

determining a structure for a plurality of residues of a protein that provides a particular receptor binding interface;

determining a plurality of designed residues using a mimetic design protocol, wherein the plurality of designed residues provide the particular receptor binding interface, and wherein the plurality of designed residues differ from the plurality of residues;

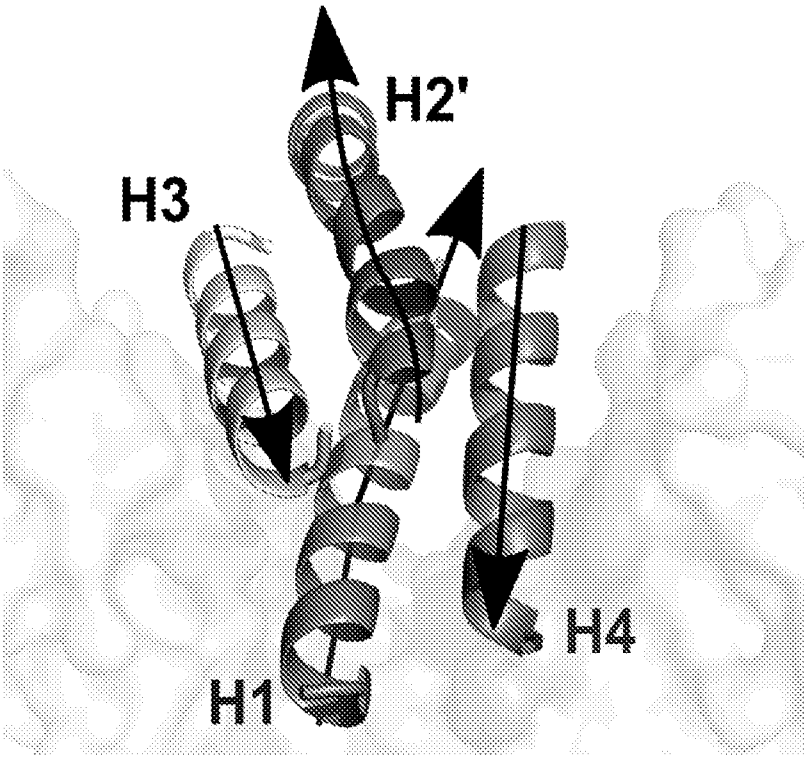
determining one or more connecting helix structures that connect the plurality of designed residues;

determining a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations;

designing a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone; and

generating an output related to at least the second protein backbone for the protein.

FIG. 1A



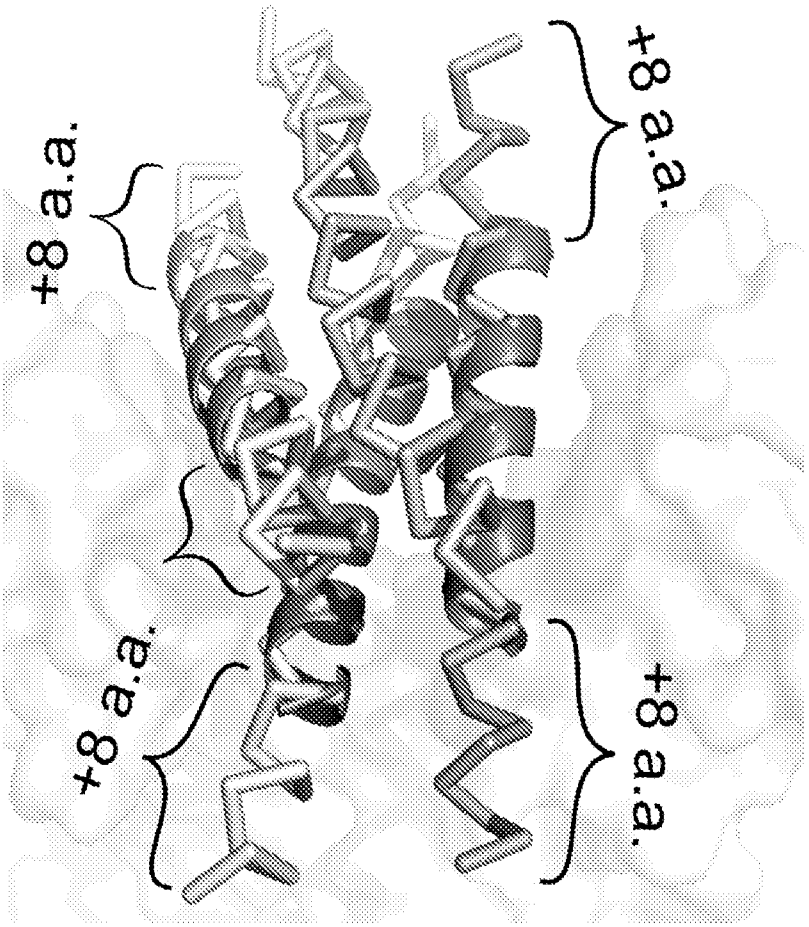
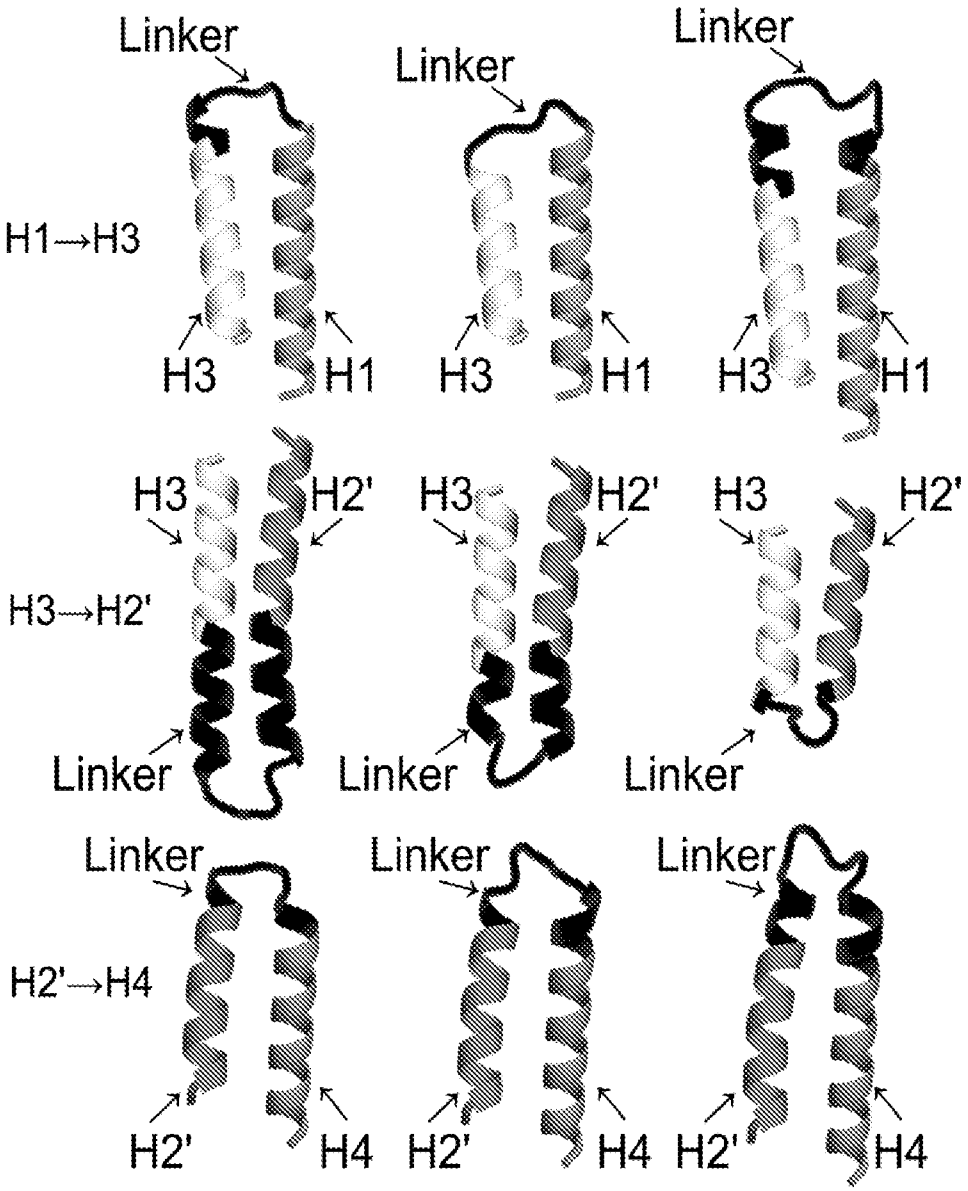


FIG. 1B


Linker
Linker
Linker
H1→H3
H3
H1
H3
H1
H3
H1
H3
H2'
H3
H2'
H3
H2'
H3→H2'
Linker
Linker
Linker
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Linker
Linker
H2'→H4
H2'
H4
H2'
H4
H2'
H4

FIG. 1C

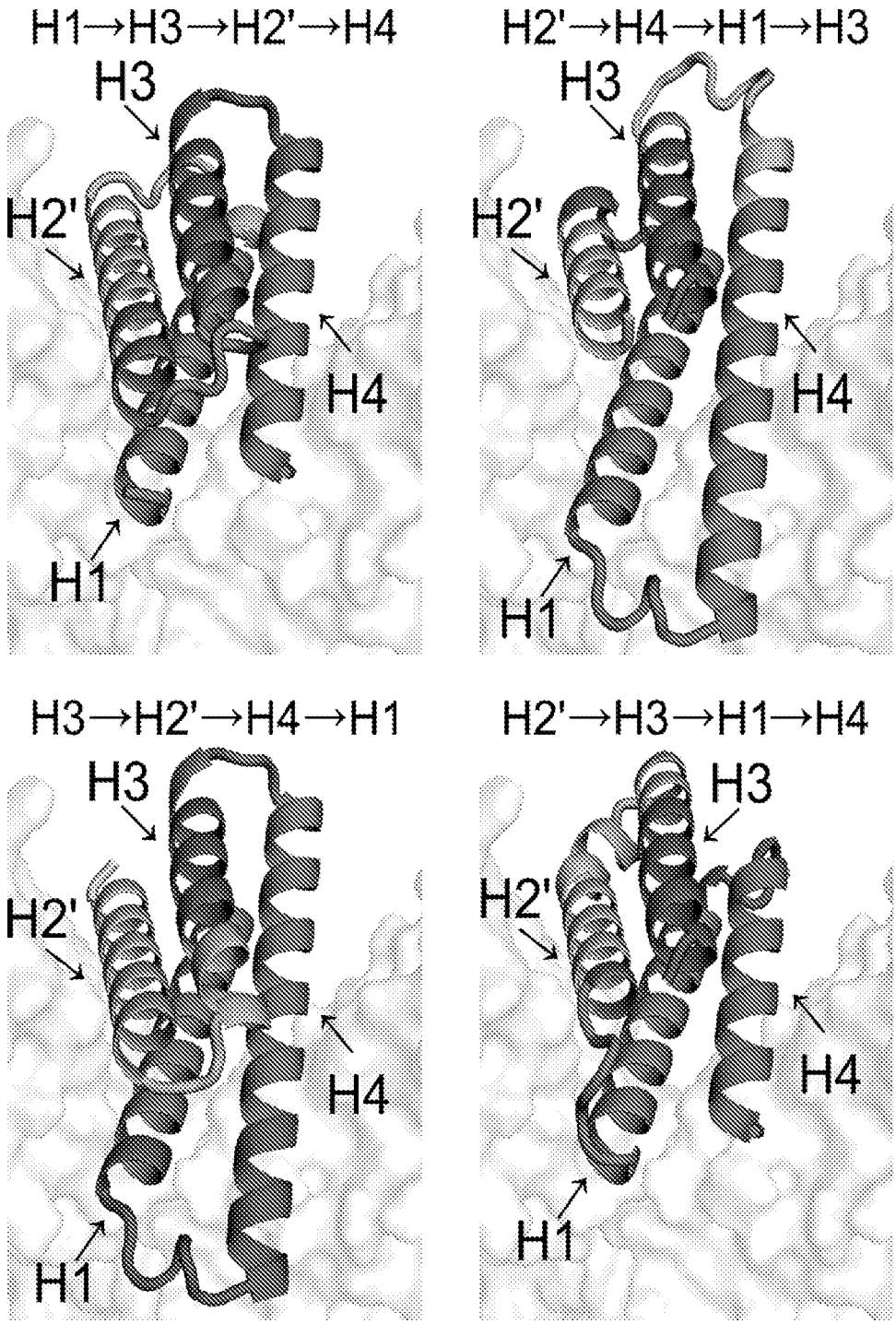

H1→H3→H2'→H4
H3
H2'
H4
H1
H2'→H4→H1→H3
H3
H2'
H4
H1
H3→H2'→H4→H1
H3
H2'
H4
H1
H2'→H3→H1→H4
H3
H2'
H4
H1

FIG. 1D

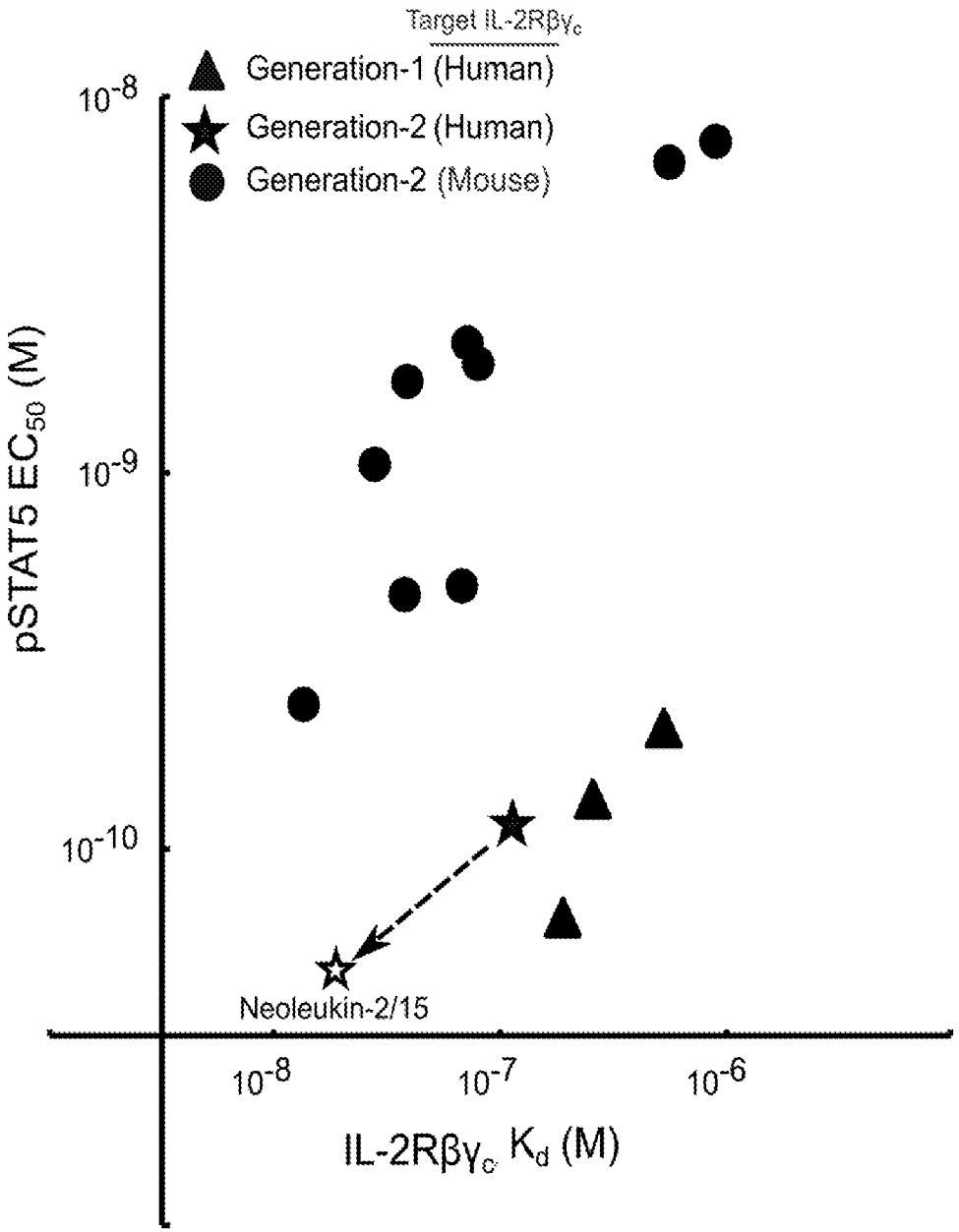

Target IL-2Rβγc
Generation-1 (Human)
Generation-2 (Human)
Generation-2 (Mouse)
pSTAT5 EC50 (M)
10-8
10-9
10-10
Neoleukin-2/15
10-8
10-7
10-6
IL-2Rβγc Kd (M)

FIG. 2A

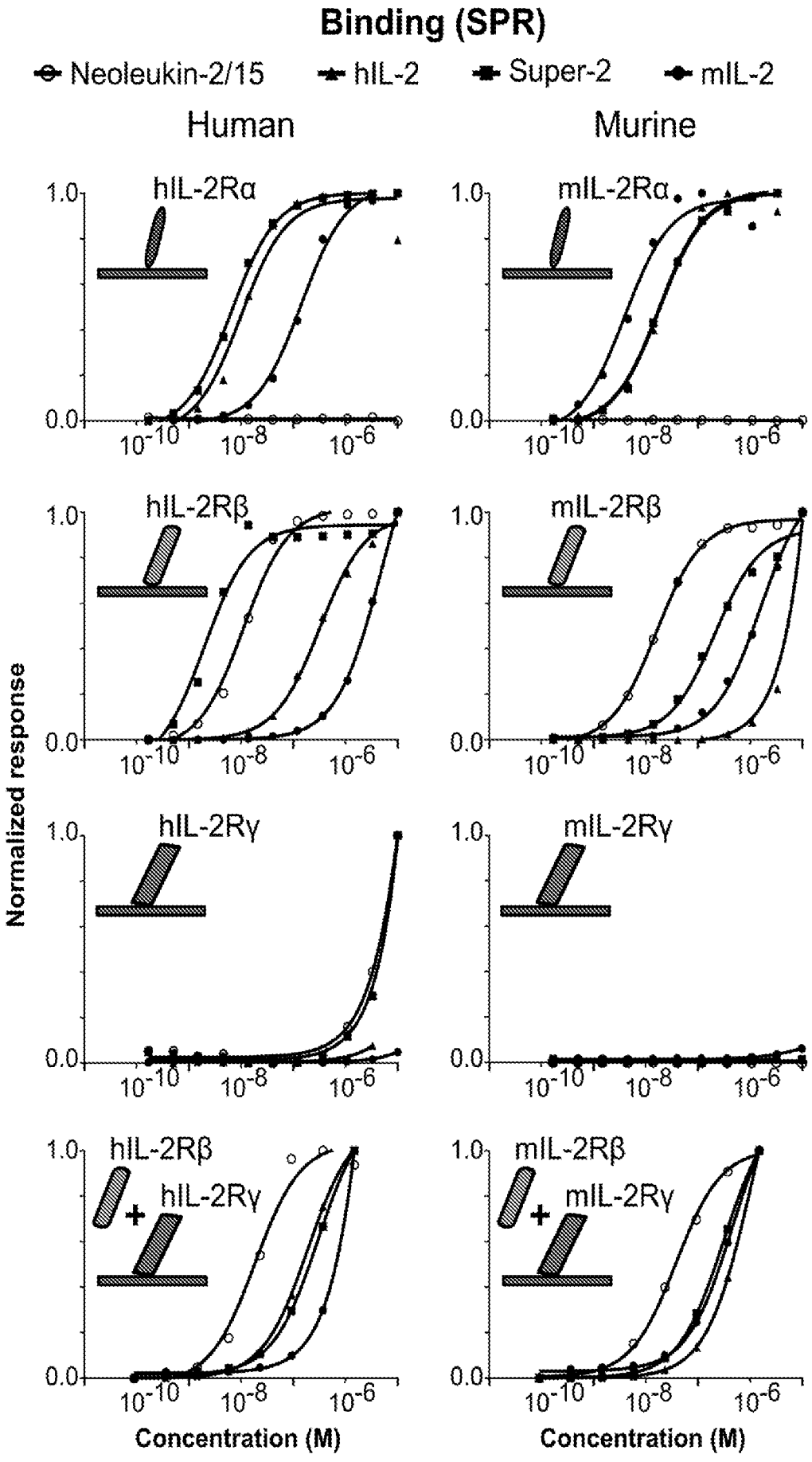

Binding (SPR)
Neoleukin-2/15
hIL-2
Super-2
mIL-2
Human
Murine
hIL-2Rα
mIL-2Rα
hIL-2Rβ
mIL-2Rβ
hIL-2Rγ
mIL-2Rγ
hIL-2Rβ
hIL-2Rγ
mIL-2Rβ
mIL-2Rγ
Normalized response
1.0
0.0
10-10
10-8
10-6
Concentration (M)

FIG. 2B

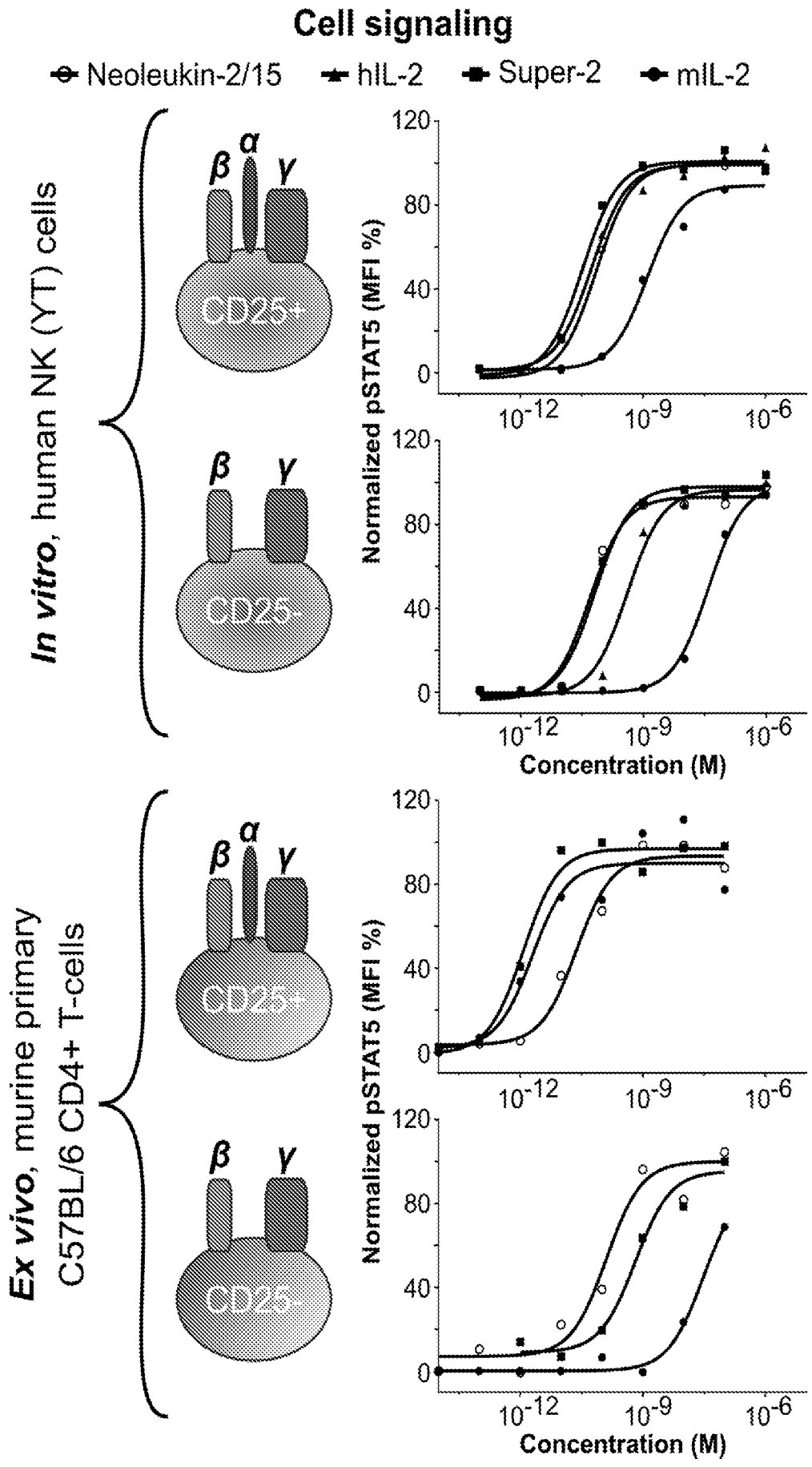

Cell signaling
Neoleukin-2/15
hIL-2
Super-2
mIL-2
In vitro, human NK (YT) cells
Ex vivo, murine primary C57BL/6 CD4+ T-cells
α
β
γ
CD25+
CD25-
Normalized pSTAT5 (MFI %)
120
80
40
0
10-12
10-9
10-6
Concentration (M)

FIG. 2C

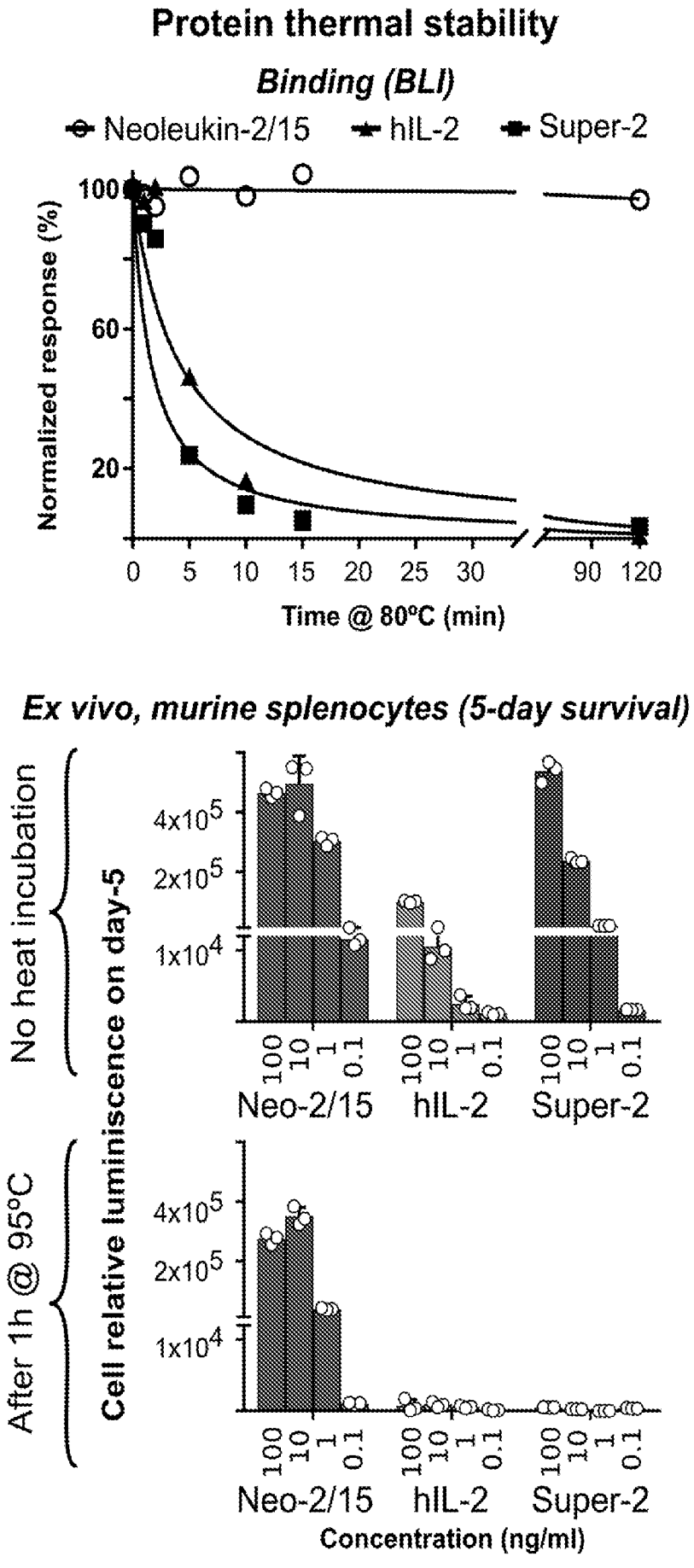

Protein thermal stability
Binding (BLI)
Neoleukin-2/15
hIL-2
Super-2
Normalized response (%)
100
60
20
0
5
10
15
20
25
30
90
120
Time @ 80°C (min)
Ex vivo, murine splenocytes (5-day survival)
No heat incubation
After 1h @ 95°C
Cell relative luminiscence on day-5
4x10^5
2x10^5
1x10^4
100
10
1
0.1
Neo-2/15
hIL-2
Super-2
Concentration (ng/ml)

FIG. 3A

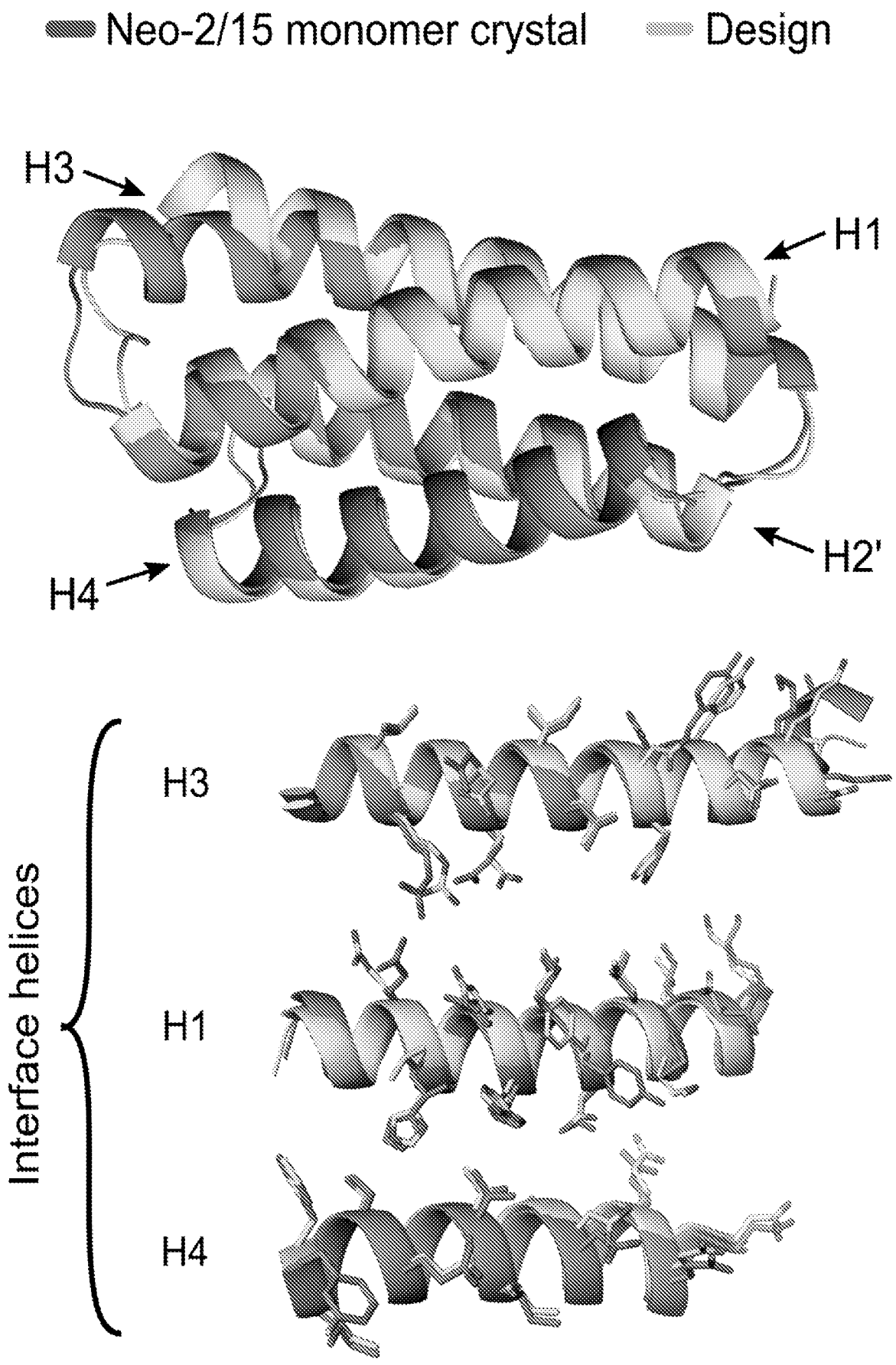

Neo-2/15 monomer crystal
Design
H3
H1
H4
H2'
Interface helices
H3
H1
H4

FIG. 3B

Neo-2/15 ternary crystal (with mIL-2βγc)

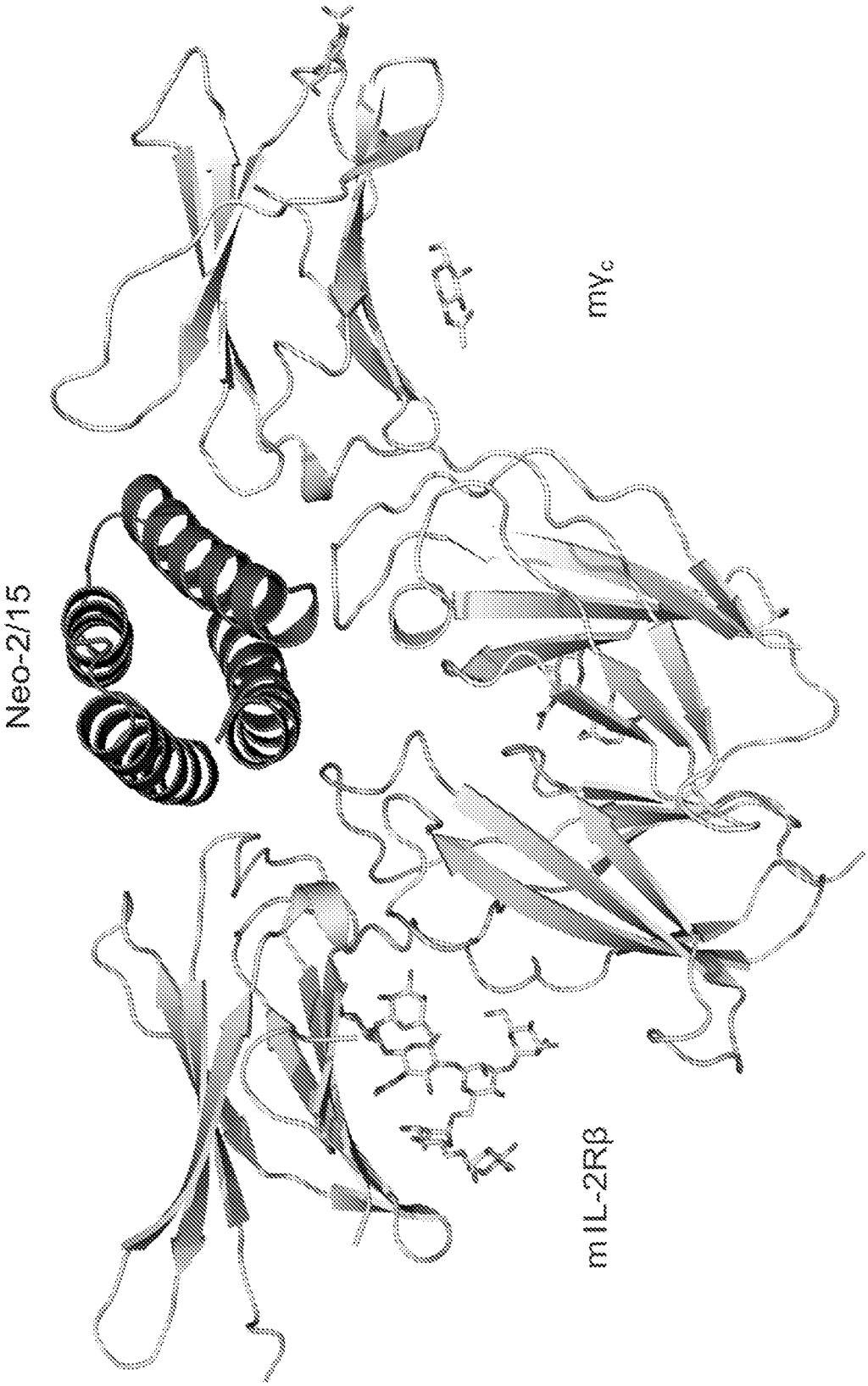


FIG. 3C

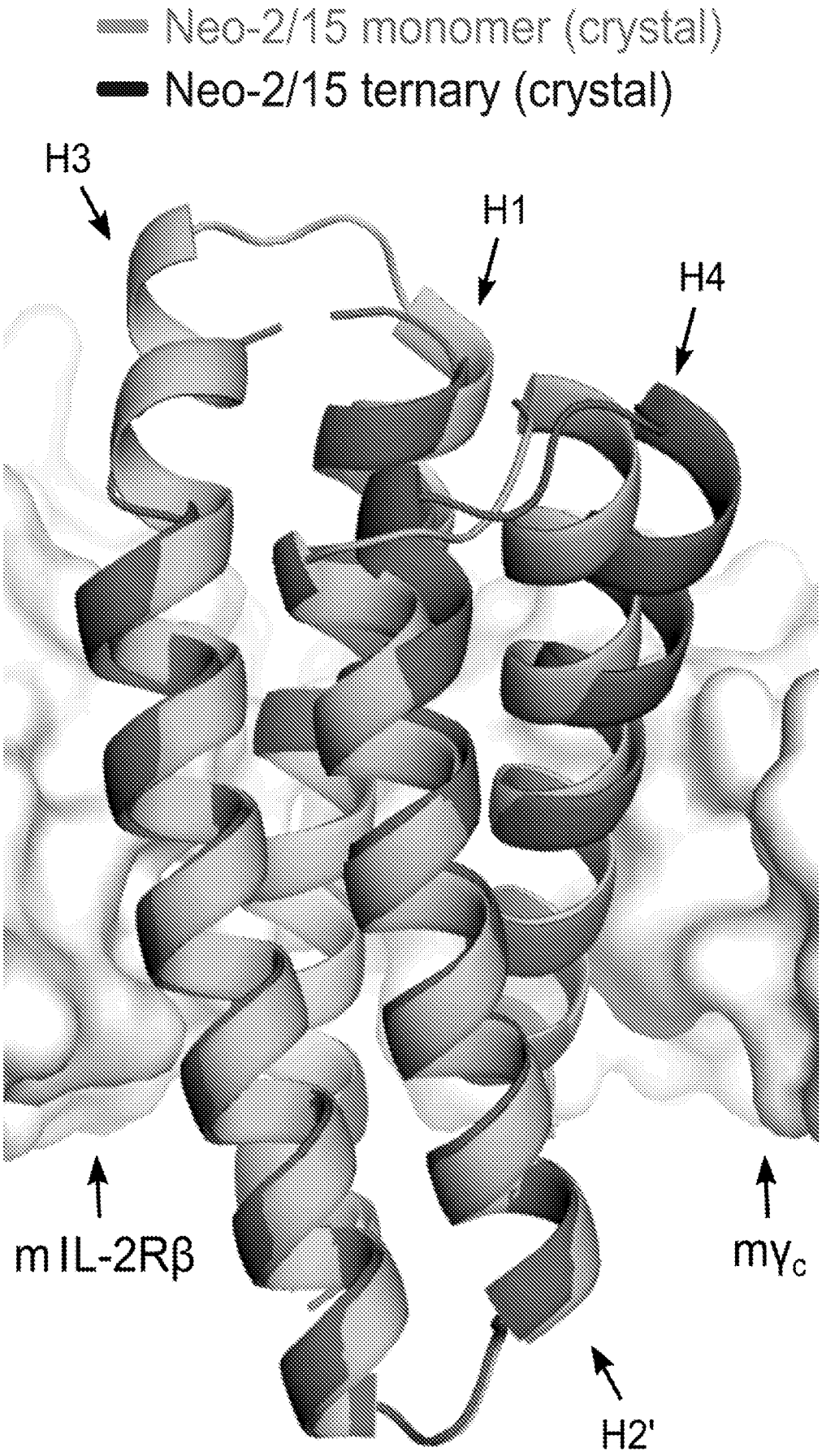

Neo-2/15 monomer (crystal)
Neo-2/15 ternary (crystal)
H3
H1
H4
mIL-2Rβ
mγc
H2'

FIG. 3D

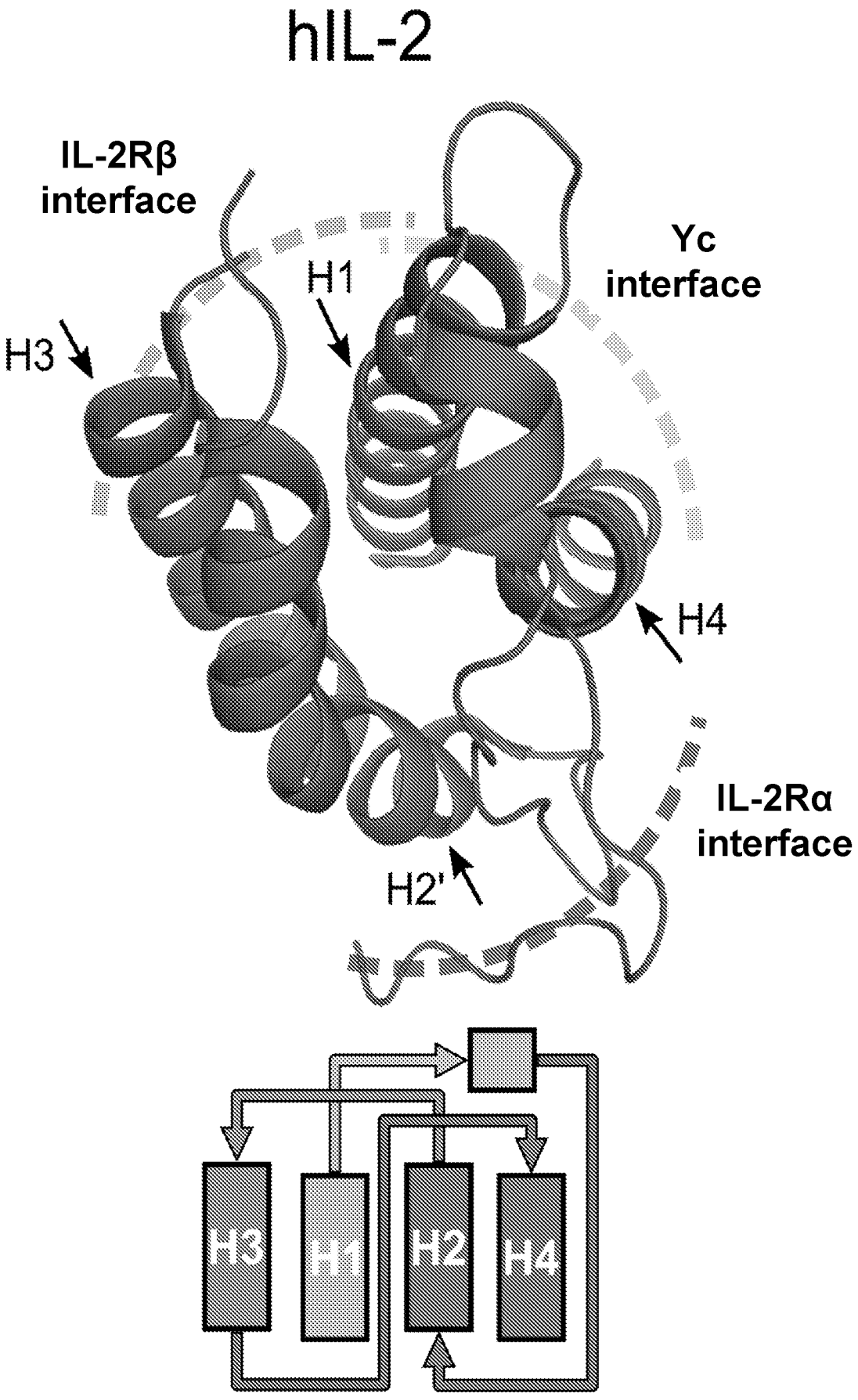
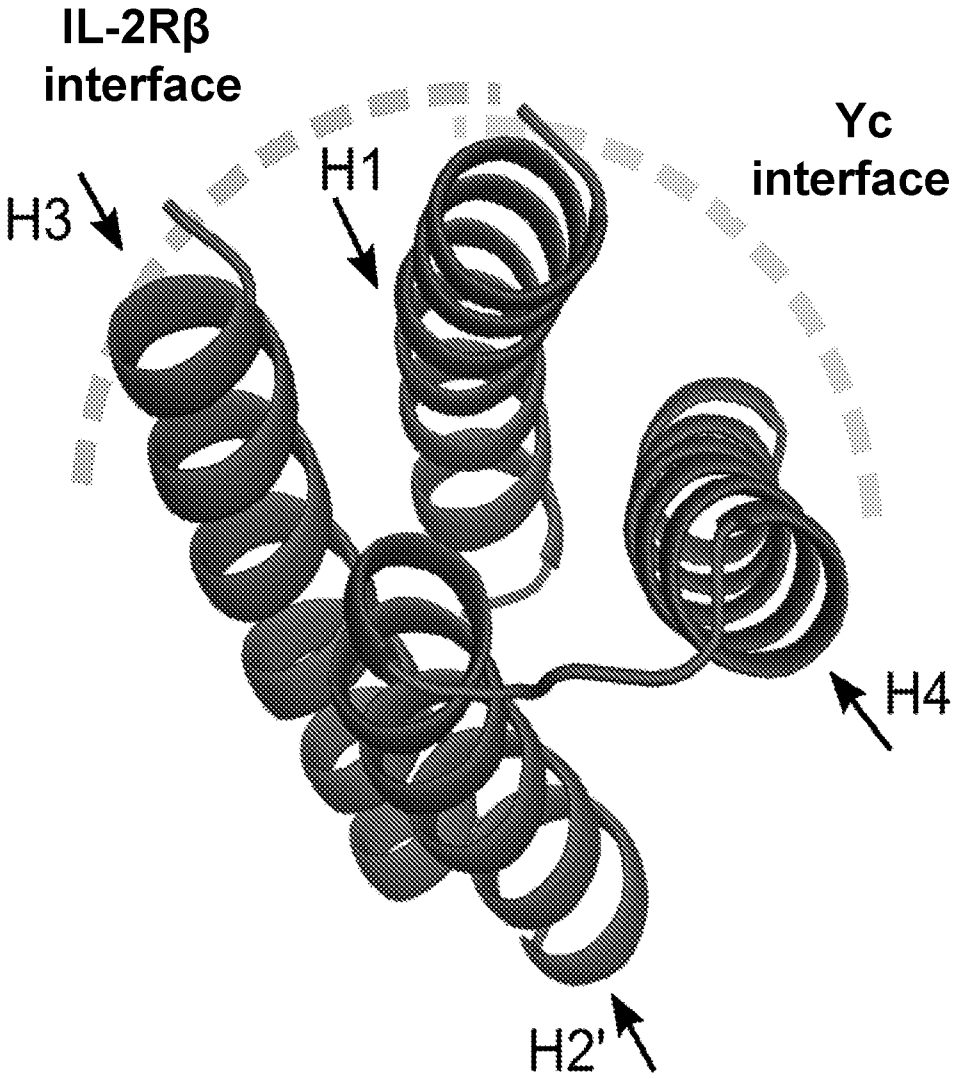

hIL-2
IL-2Rβ interface
Yc interface
H1
H3
H4
IL-2Rα interface
H2'
H3
H1
H2
H4

FIG. 3E

Neo-2/15



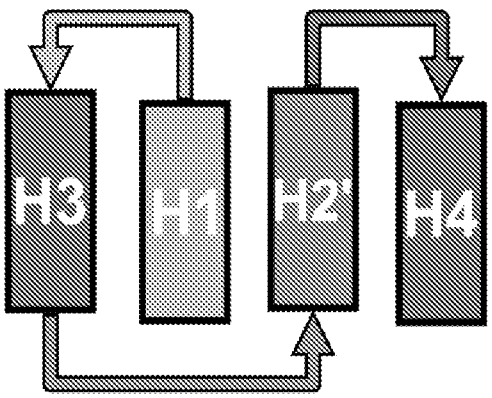


FIG. 4A

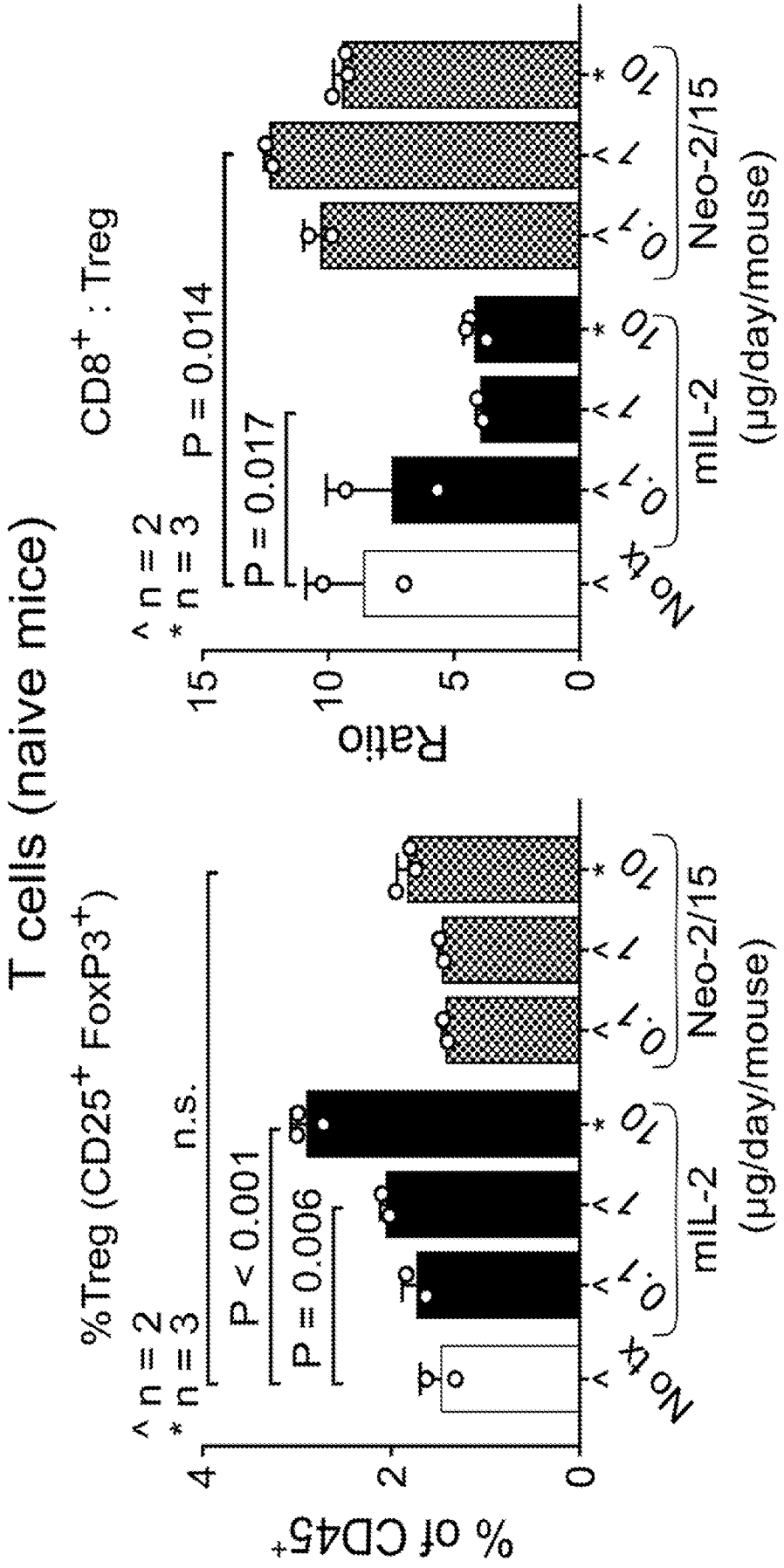

T cells (naive mice)
% of CD45+
%Treg (CD25+ FoxP3+)
^ n = 2
* n = 3
n.s.
P < 0.001
P = 0.006
CD8+ : Treg
P = 0.017
P = 0.014
Ratio
No tx
mIL-2
Neo-2/15
0.1
1
10
(µg/day/mouse)

FIG. 4B

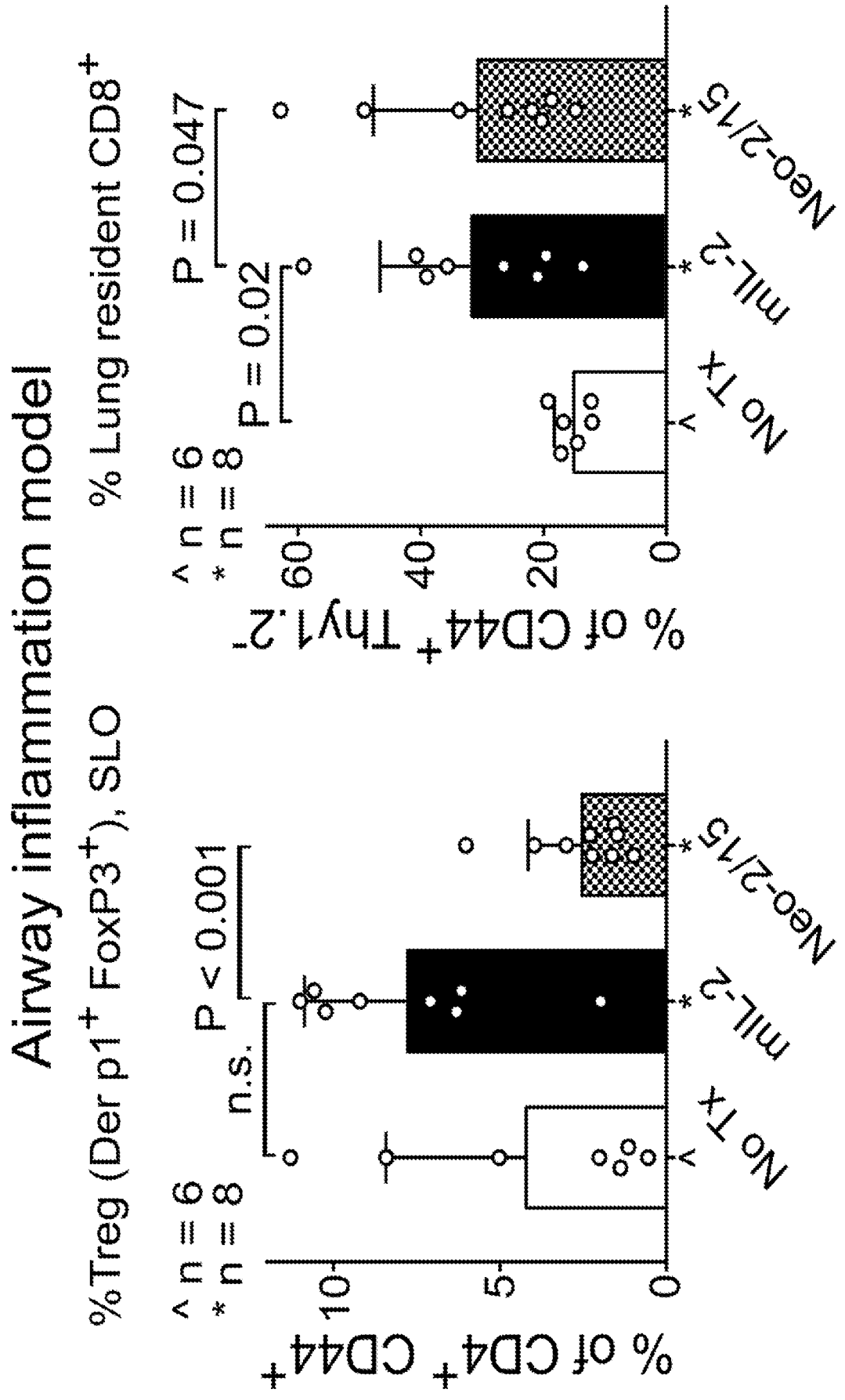

Airway inflammation model
%Treg (Der p1+ FoxP3+), SLO
% Lung resident CD8+
% of CD44+
% of CD44+ Thy1.2-
n.s.
P < 0.001
P = 0.02
P = 0.047
^ n = 6
* n = 8
No Tx
mIL-2
Neo-2/15

FIG. 4C

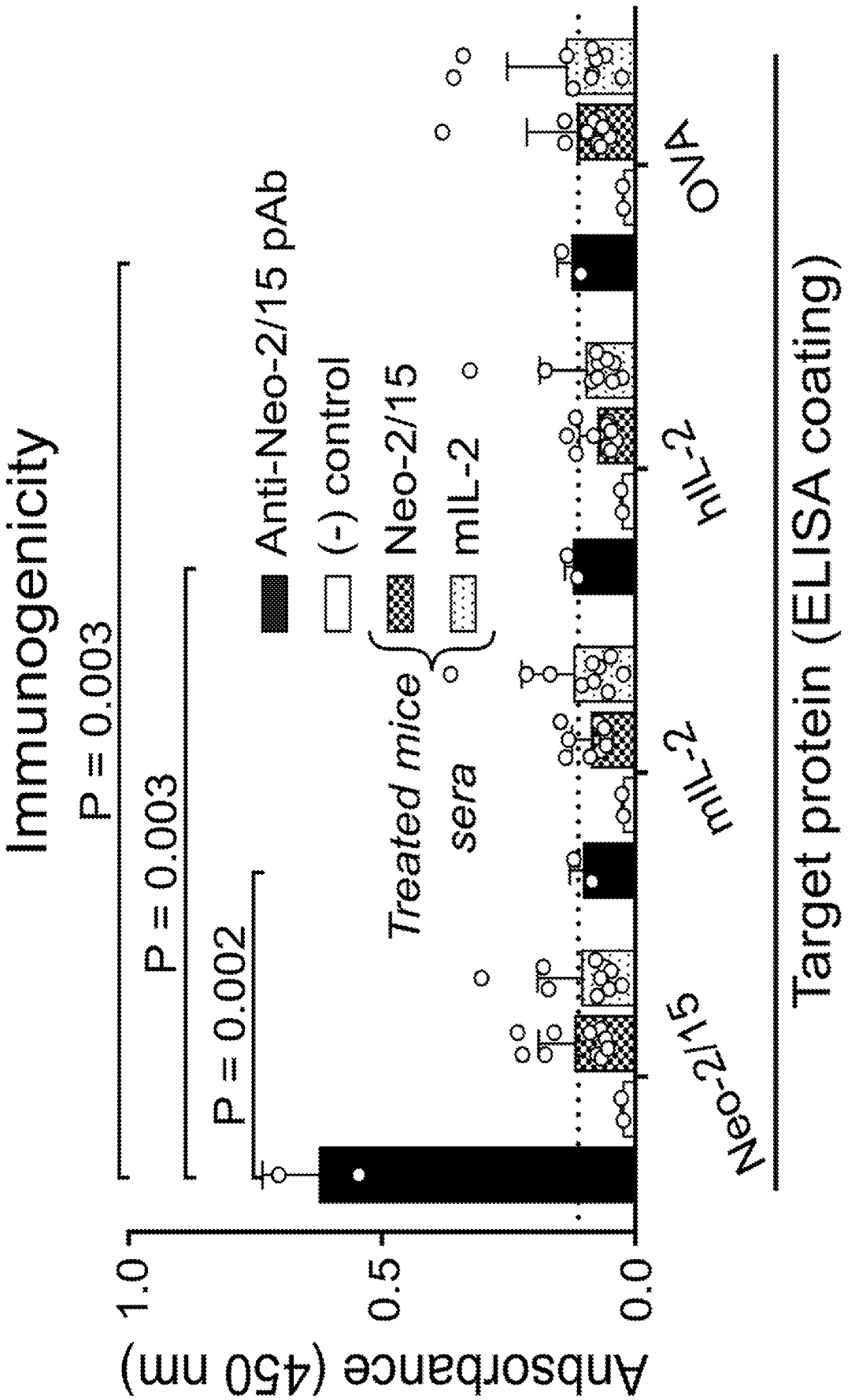

Immunogenicity
P = 0.003
P = 0.003
P = 0.002
Anti-Neo-2/15 pAb
(-) control
Neo-2/15
mIL-2
Treated mice sera
Absorbance (450 nm)
1.0
0.5
0.0
Neo-2/15
mIL-2
hIL-2
OVA
Target protein (ELISA coating)

FIG. 4D

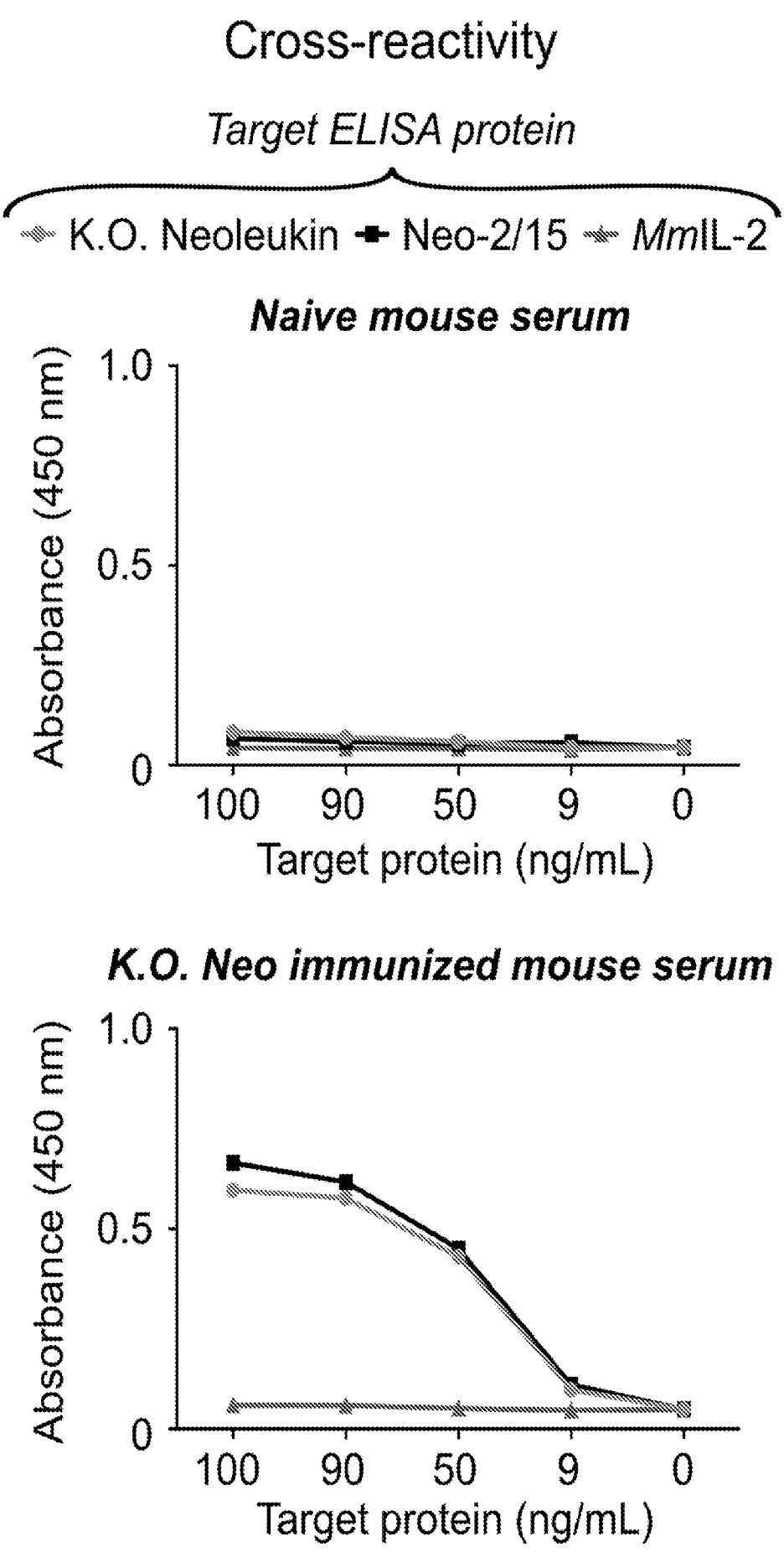

Cross-reactivity
Target ELISA protein
K.O. Neoleukin
Neo-2/15
MmIL-2
Naive mouse serum
Absorbance (450 nm)
1.0
0.5
0
100
90
50
9
0
Target protein (ng/mL)
K.O. Neo immunized mouse serum
Absorbance (450 nm)
1.0
0.5
0
100
90
50
9
0
Target protein (ng/mL)

FIG. 4E

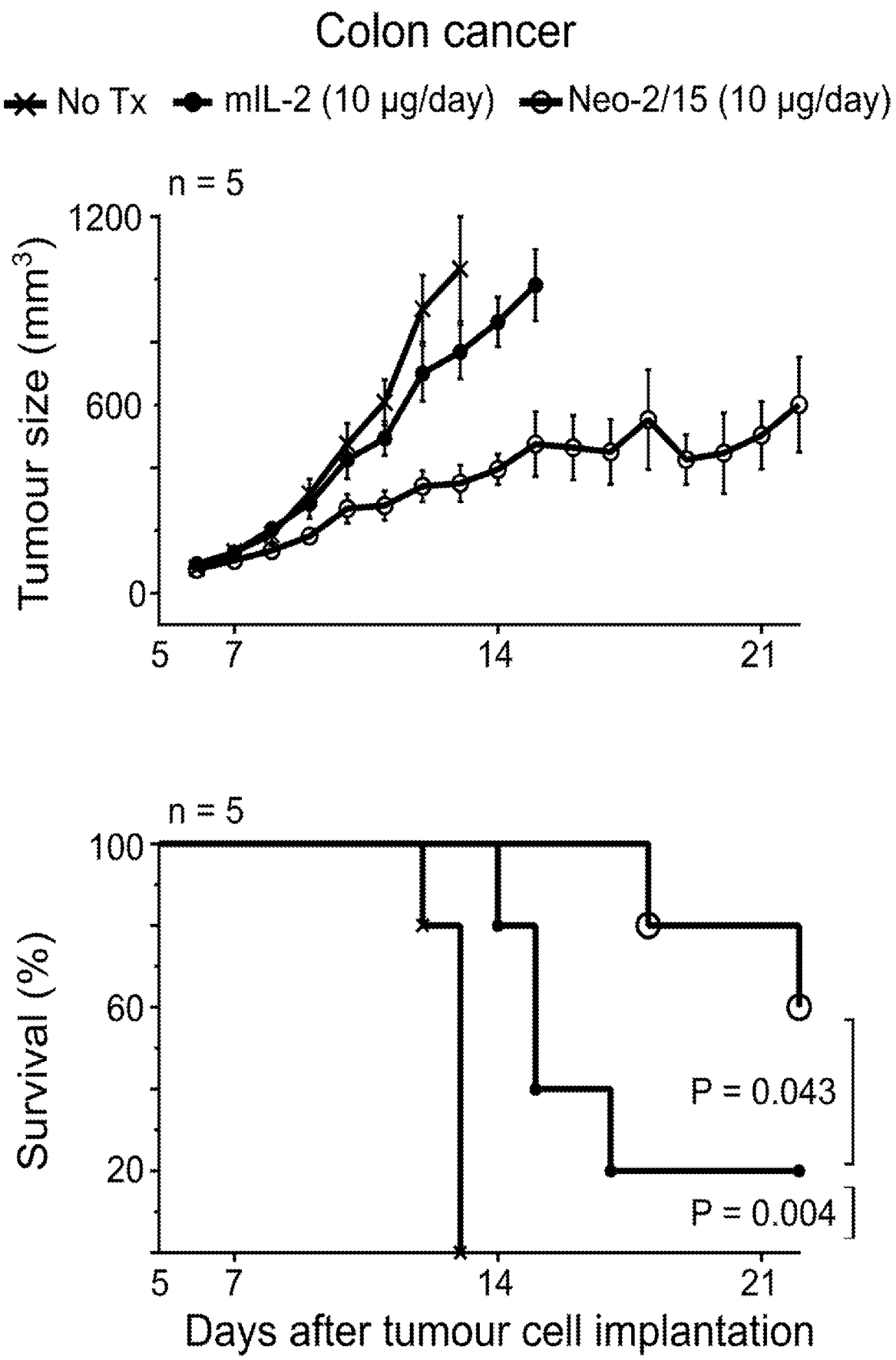

Colon cancer
No Tx
mIL-2 (10 µg/day)
Neo-2/15 (10 µg/day)
n = 5
Tumour size (mm3)
1200
600
0
5
7
14
21
n = 5
Survival (%)
100
60
20
P = 0.043
P = 0.004
5
7
14
21
Days after tumour cell implantation

FIG. 4F
Melanoma

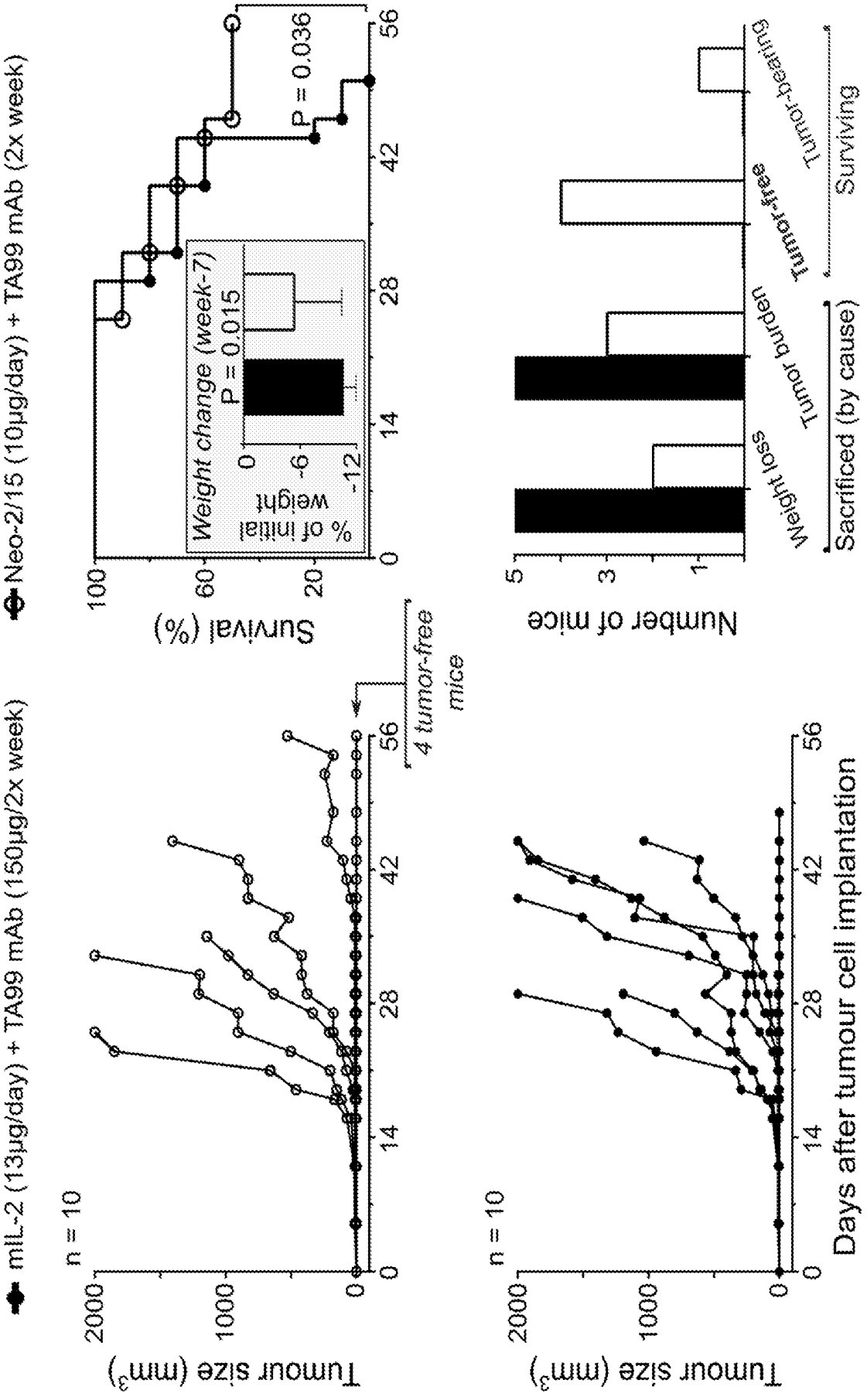


FIG. 4G

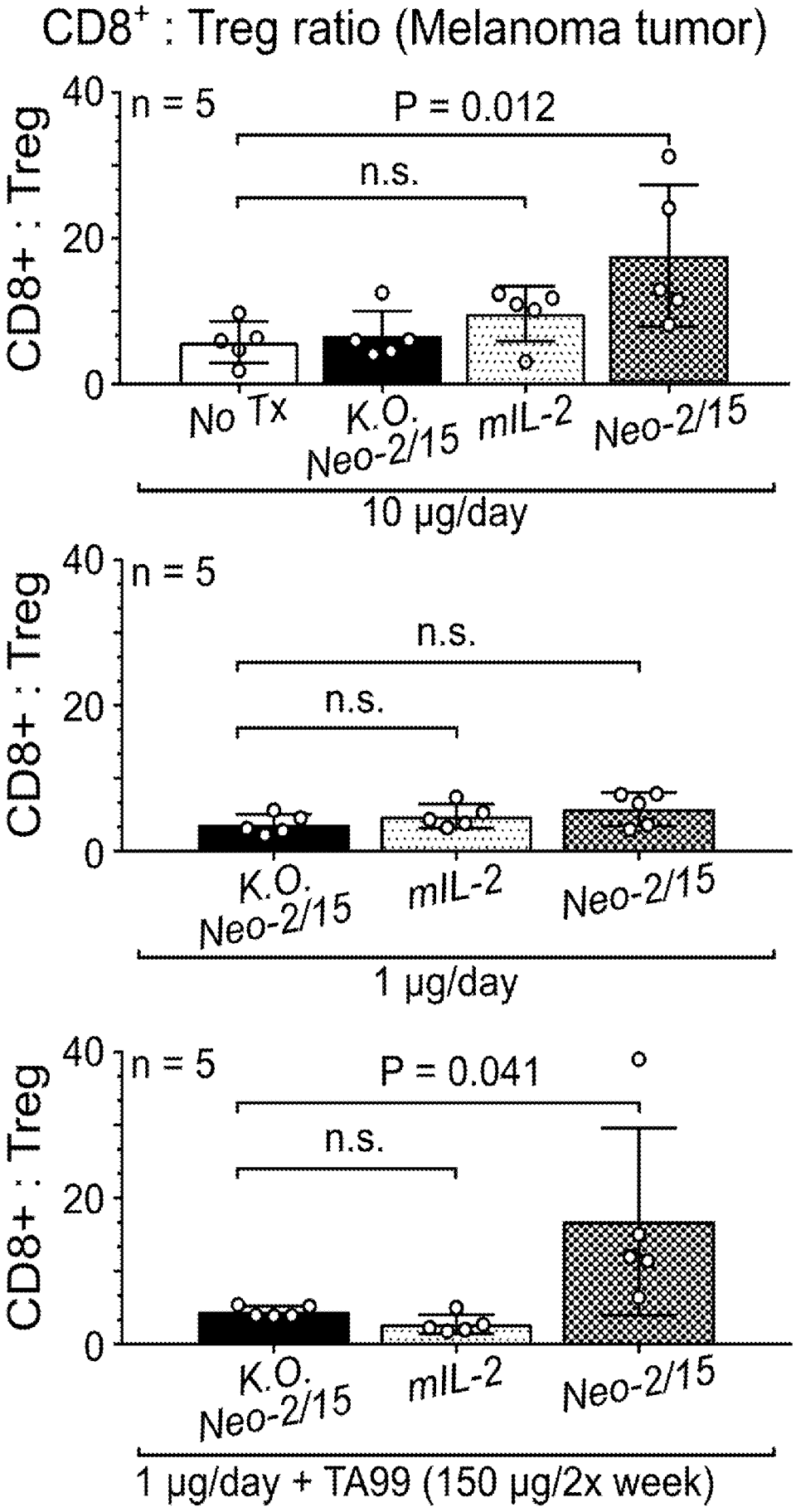

CD8+ : Treg ratio (Melanoma tumor)
CD8+ : Treg
40
20
0
n = 5
P = 0.012
n.s.
No Tx
K.O. Neo-2/15
mIL-2
Neo-2/15
10 µg/day
n = 5
n.s.
n.s.
K.O. Neo-2/15
mIL-2
Neo-2/15
1 µg/day
n = 5
P = 0.041
n.s.
K.O. Neo-2/15
mIL-2
Neo-2/15
1 µg/day + TA99 (150 µg/2x week)

FIG. 5A

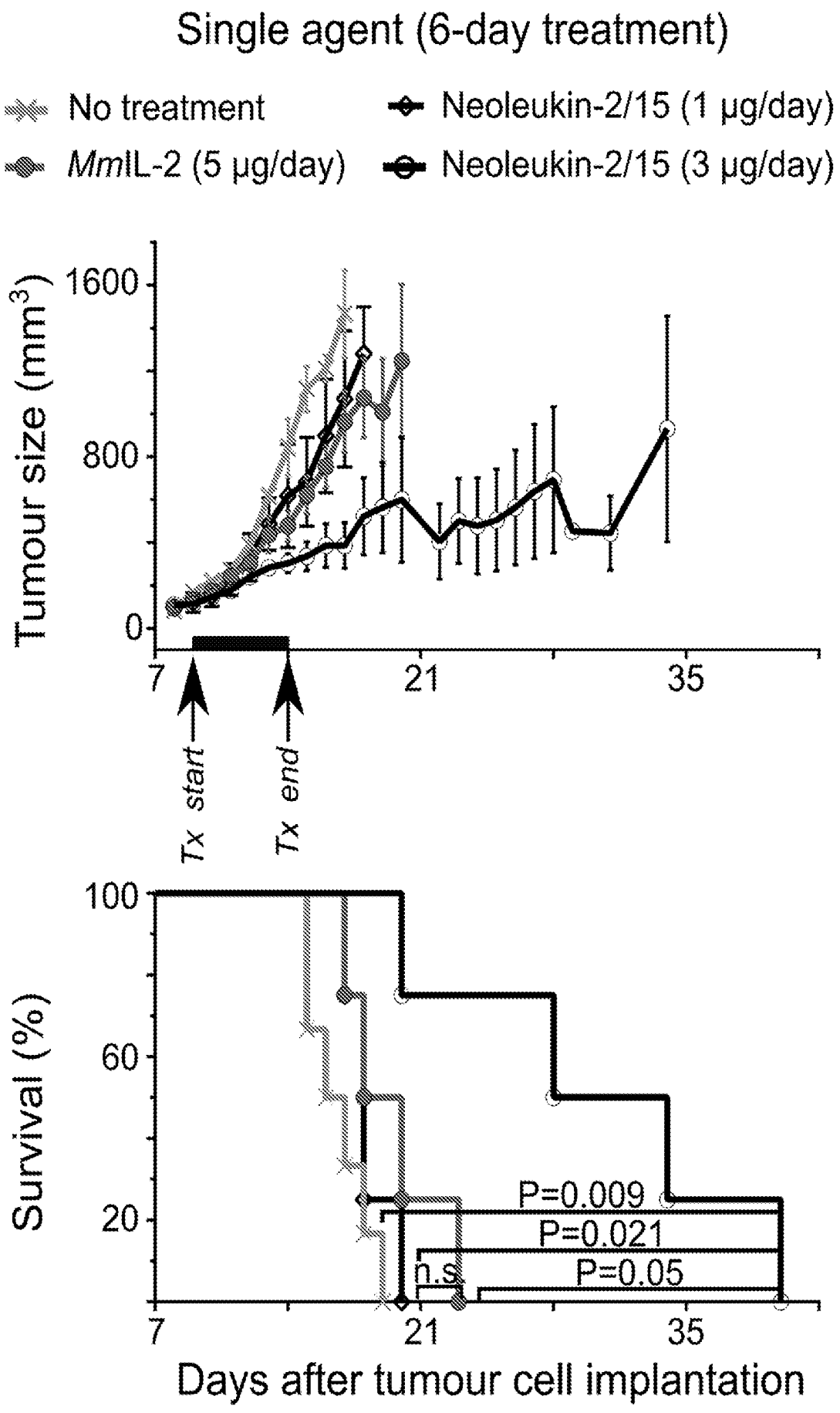

Single agent (6-day treatment)
No treatment
Neoleukin-2/15 (1 µg/day)
MmIL-2 (5 µg/day)
Neoleukin-2/15 (3 µg/day)
Tumour size (mm3)
1600
800
0
7
21
35
Tx start
Tx end
Survival (%)
100
60
20
P=0.009
P=0.021
n.s.
P=0.05
7
21
35
Days after tumour cell implantation

FIG. 5B

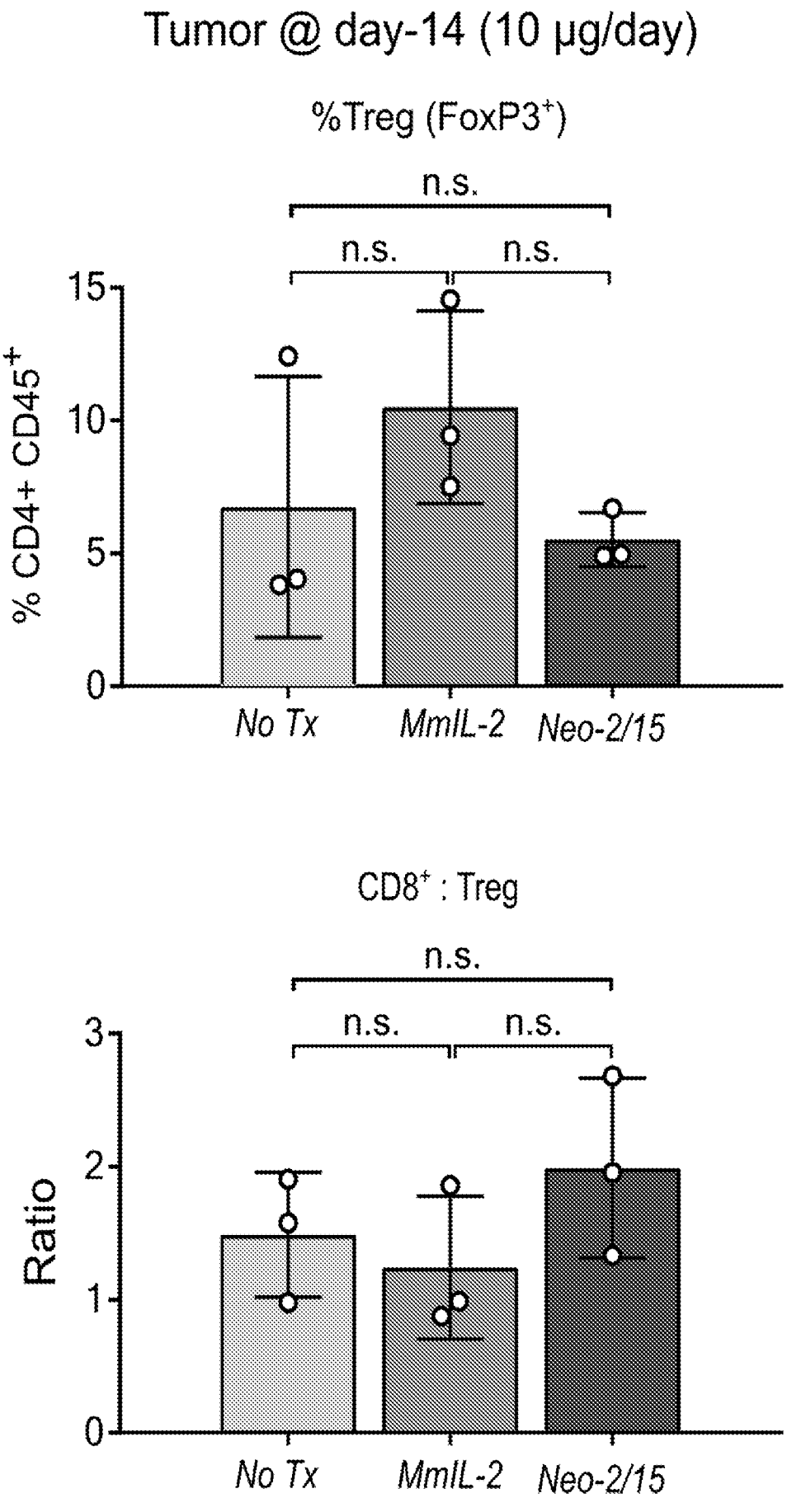

Tumor @ day-14 (10 µg/day)
%Treg (FoxP3+)
n.s.
n.s.
n.s.
% CD4+ CD45+
15
10
5
0
No Tx
MmIL-2
Neo-2/15
CD8+ : Treg
n.s.
n.s.
n.s.
Ratio
3
2
1
0
No Tx
MmIL-2
Neo-2/15

FIG. 5C

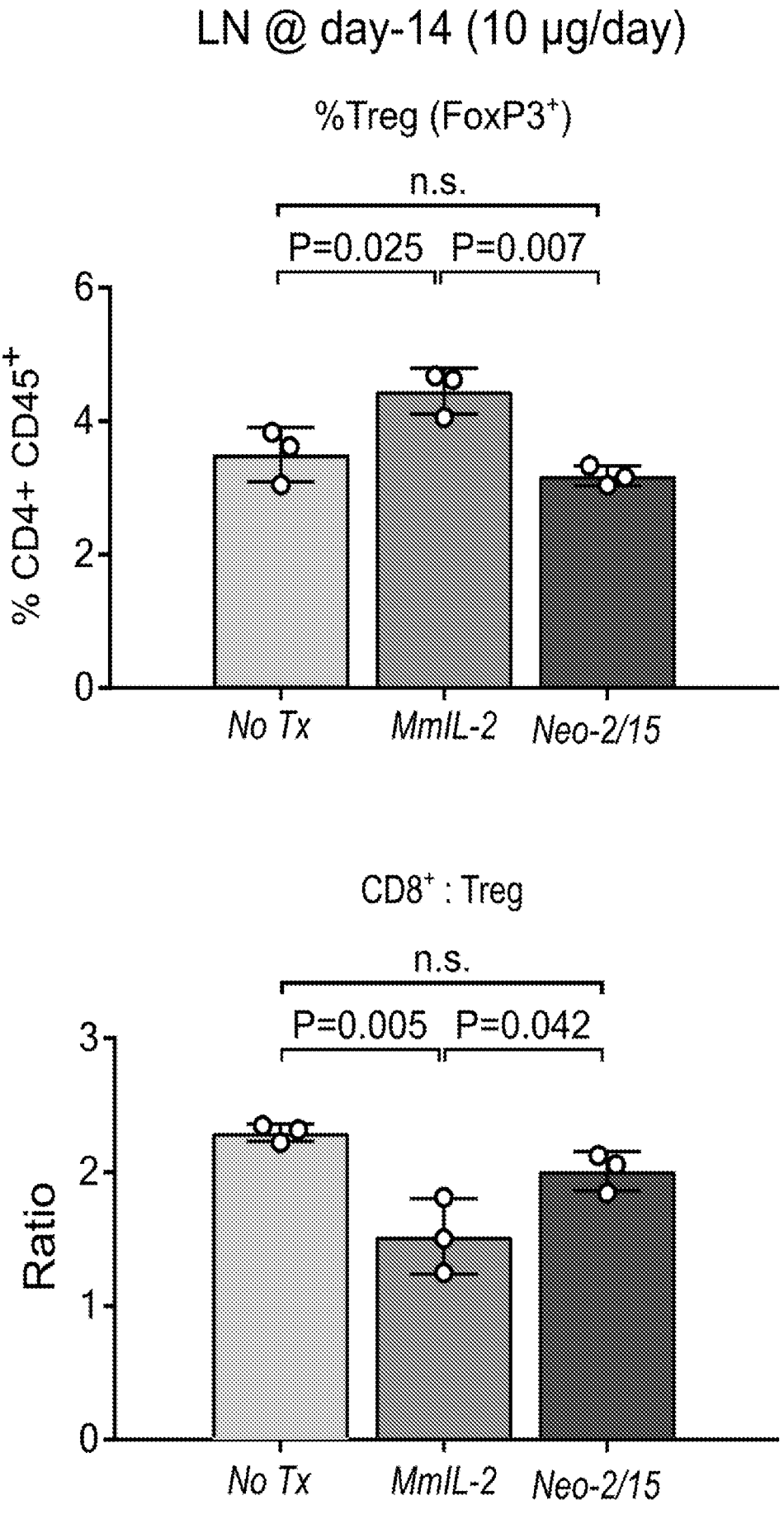

LN @ day-14 (10 µg/day)
%Treg (FoxP3+)
n.s.
P=0.025 P=0.007
% CD4+ CD45+
6
4
2
0
No Tx
MmIL-2
Neo-2/15
CD8+ : Treg
n.s.
P=0.005 P=0.042
Ratio
3
2
1
0
No Tx
MmIL-2
Neo-2/15

FIG. 5D

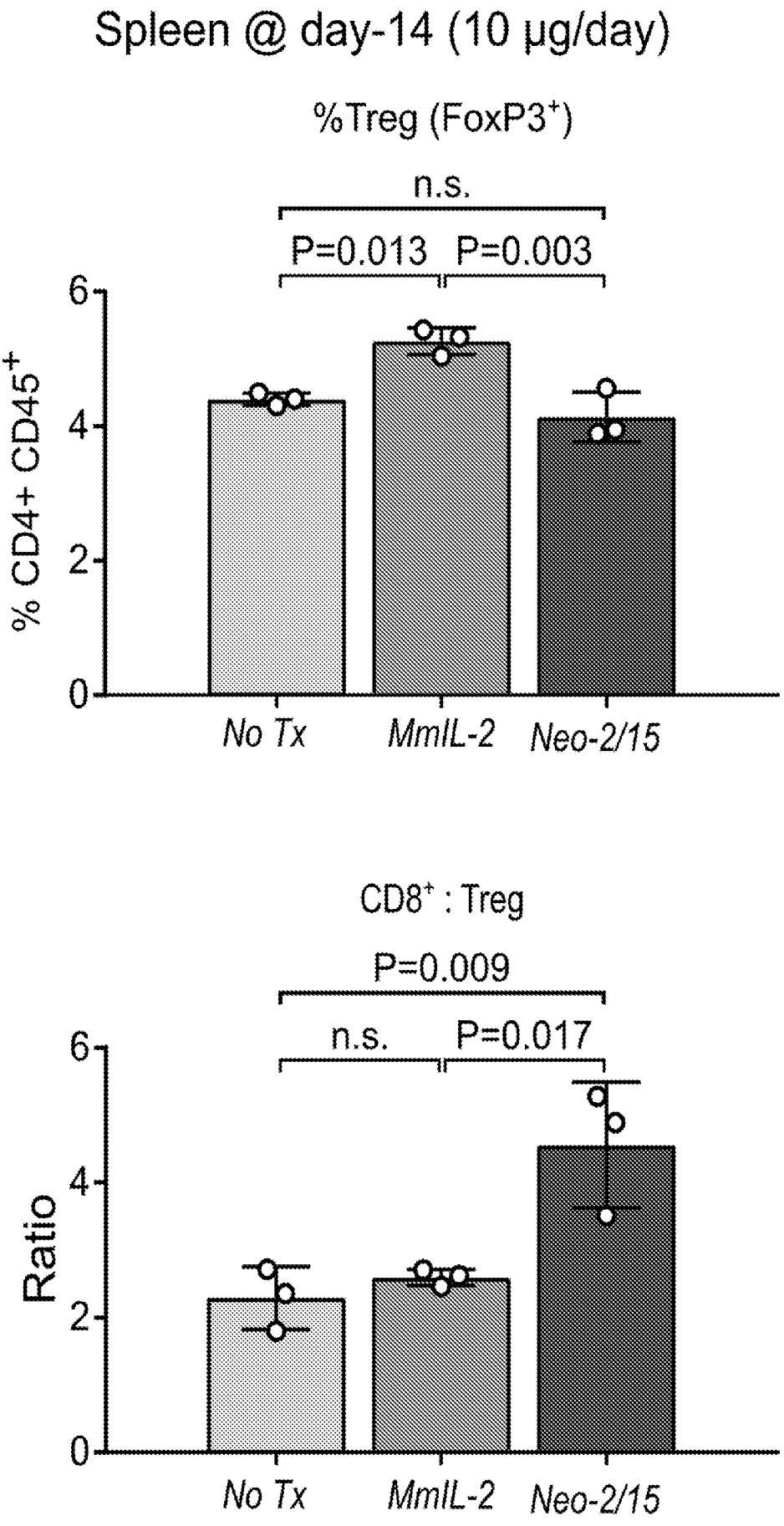

Spleen @ day-14 (10 µg/day)
%Treg (FoxP3+)
n.s.
P=0.013
P=0.003
% CD4+ CD45+
6
4
2
0
No Tx
MmIL-2
Neo-2/15
CD8+ : Treg
P=0.009
n.s.
P=0.017
Ratio
6
4
2
0
No Tx
MmIL-2
Neo-2/15

FIG. 6A

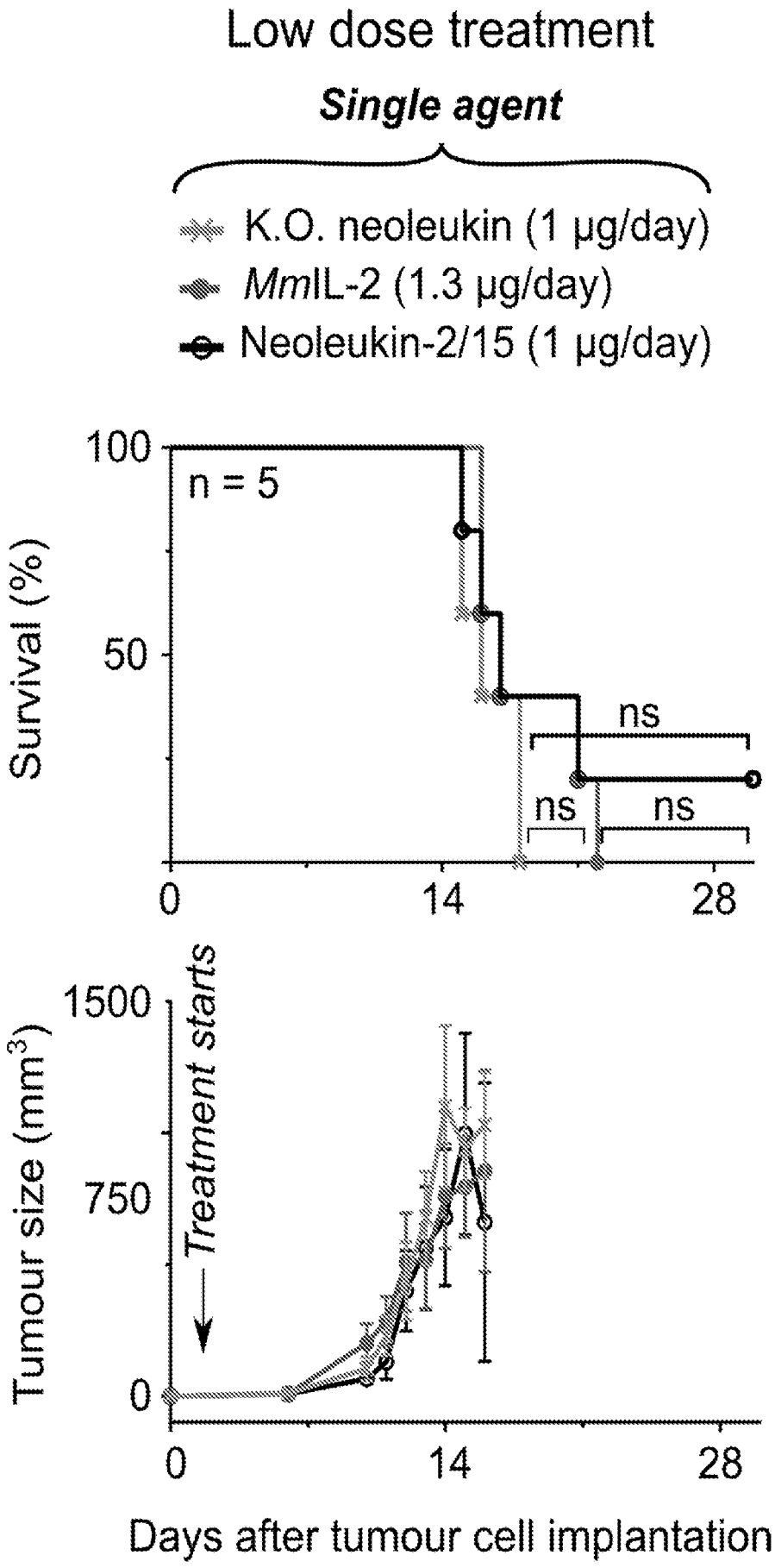

Low dose treatment
Single agent
K.O. neoleukin (1 µg/day)
MmIL-2 (1.3 µg/day)
Neoleukin-2/15 (1 µg/day)
n = 5
Survival (%)
100
50
0
14
28
ns
ns
ns
Tumour size (mm3)
1500
750
0
Treatment starts
0
14
28
Days after tumour cell implantation

FIG. 6B

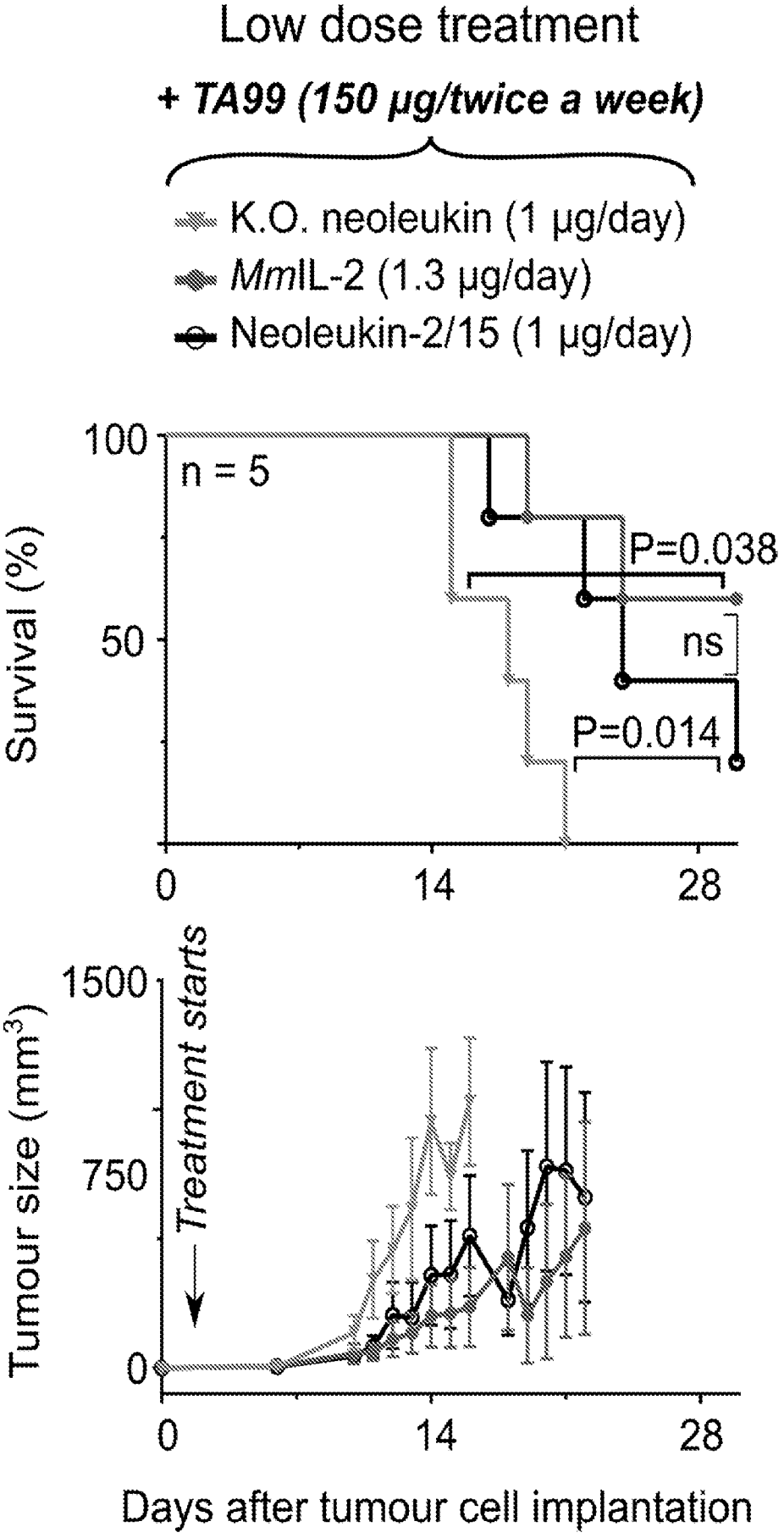

Low dose treatment
+ TA99 (150 µg/twice a week)
K.O. neoleukin (1 µg/day)
MmIL-2 (1.3 µg/day)
Neoleukin-2/15 (1 µg/day)
Survival (%)
100
50
n = 5
P=0.038
ns
P=0.014
0
14
28
Tumour size (mm3)
1500
750
0
Treatment starts
0
14
28
Days after tumour cell implantation

FIG. 6C

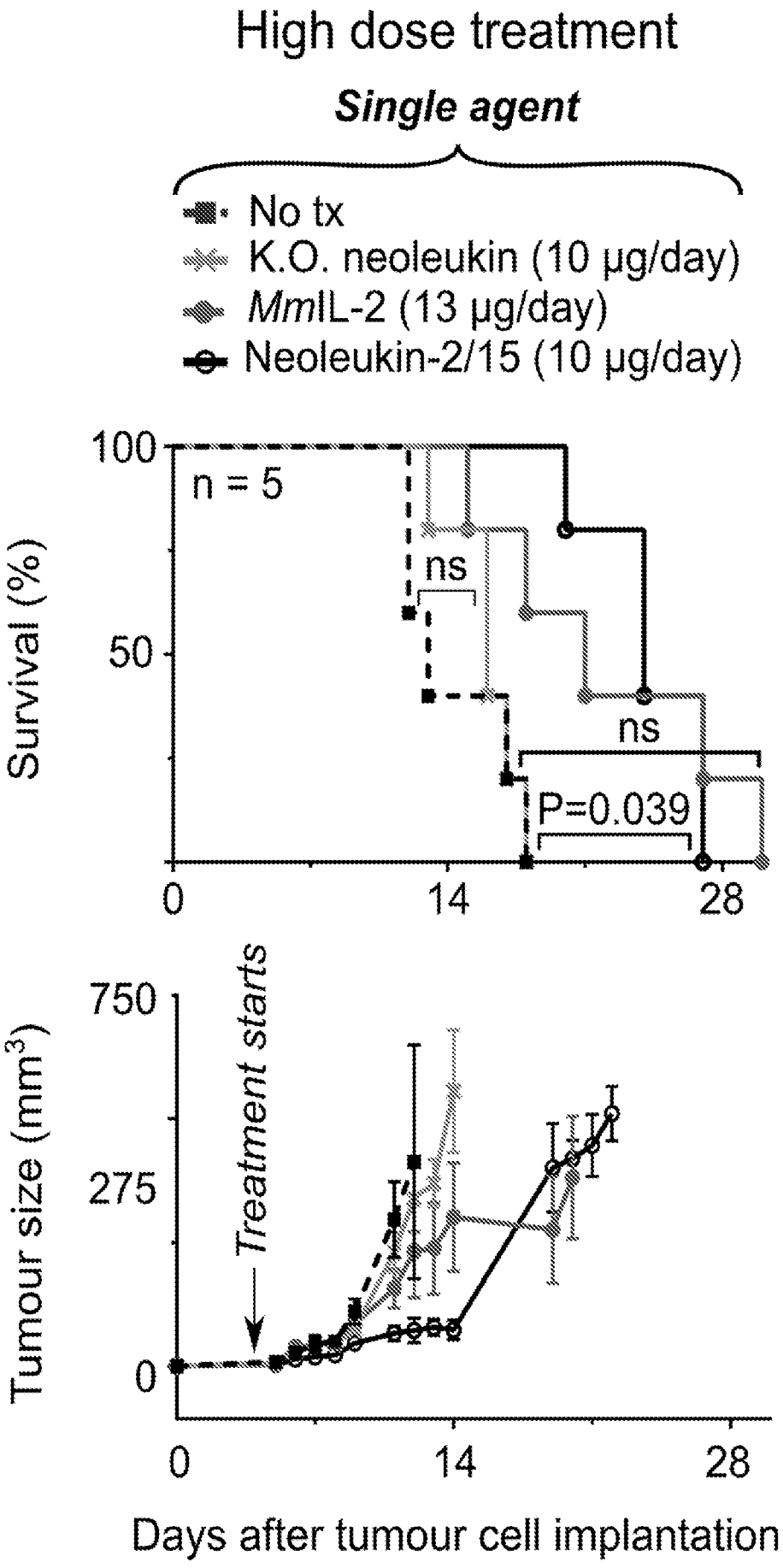

High dose treatment
Single agent
No tx
K.O. neoleukin (10 µg/day)
MmIL-2 (13 µg/day)
Neoleukin-2/15 (10 µg/day)
Survival (%)
100
50
0
n = 5
ns
ns
P=0.039
14
28
Tumour size (mm3)
750
275
0
Treatment starts
0
14
28
Days after tumour cell implantation

FIG. 6D

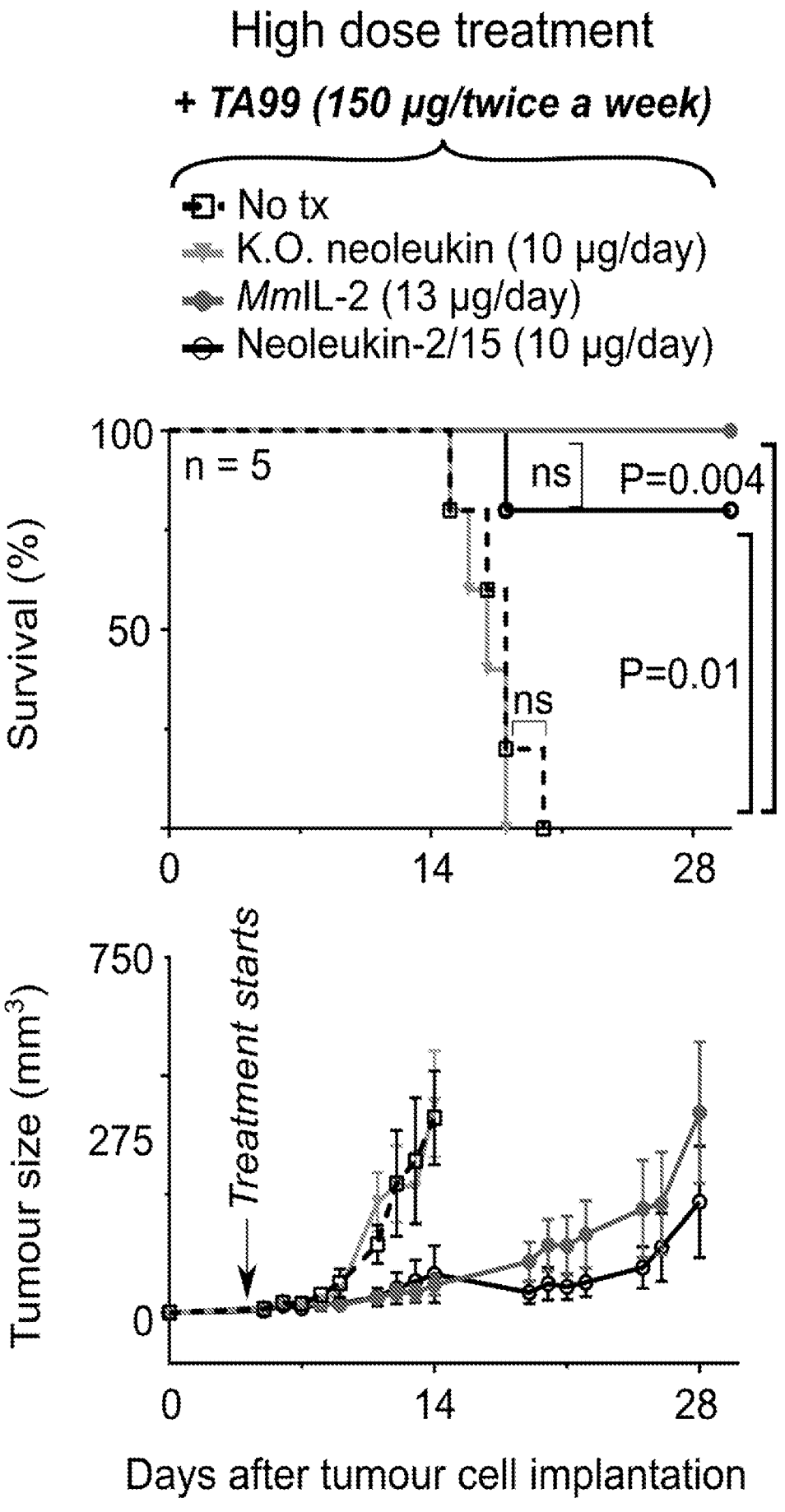

High dose treatment
+ TA99 (150 µg/twice a week)
No tx
K.O. neoleukin (10 µg/day)
MmIL-2 (13 µg/day)
Neoleukin-2/15 (10 µg/day)
Survival (%)
100
50
n = 5
ns
P=0.004
P=0.01
ns
0
14
28
Tumour size (mm3)
750
275
0
Treatment starts
0
14
28
Days after tumour cell implantation

FIG. 7A

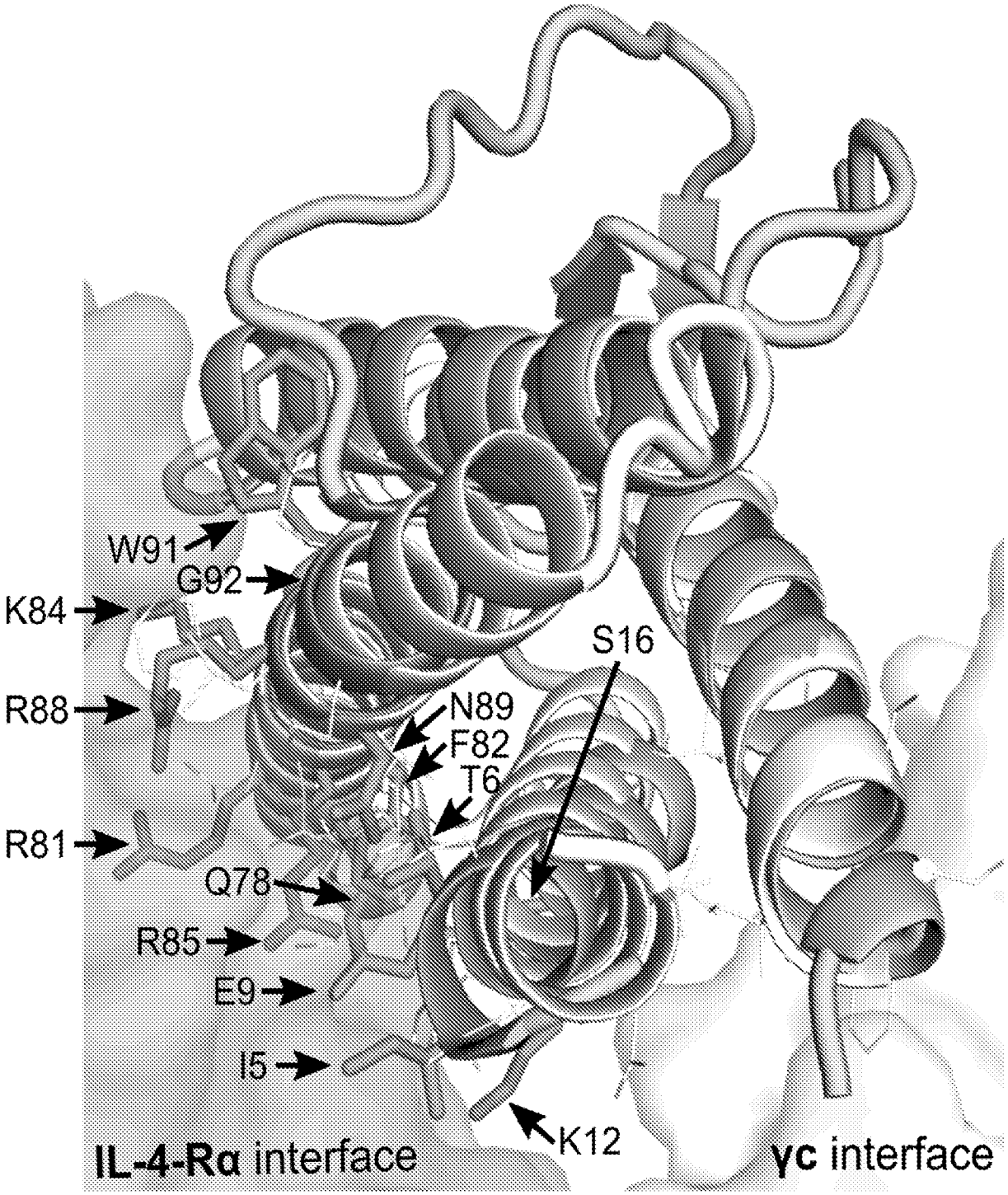

W91
G92
K84
S16
R88
N89
F82
T6
R81
Q78
R85
E9
I5
K12
IL-4-Rα interface
γc interface

FIG. 7B

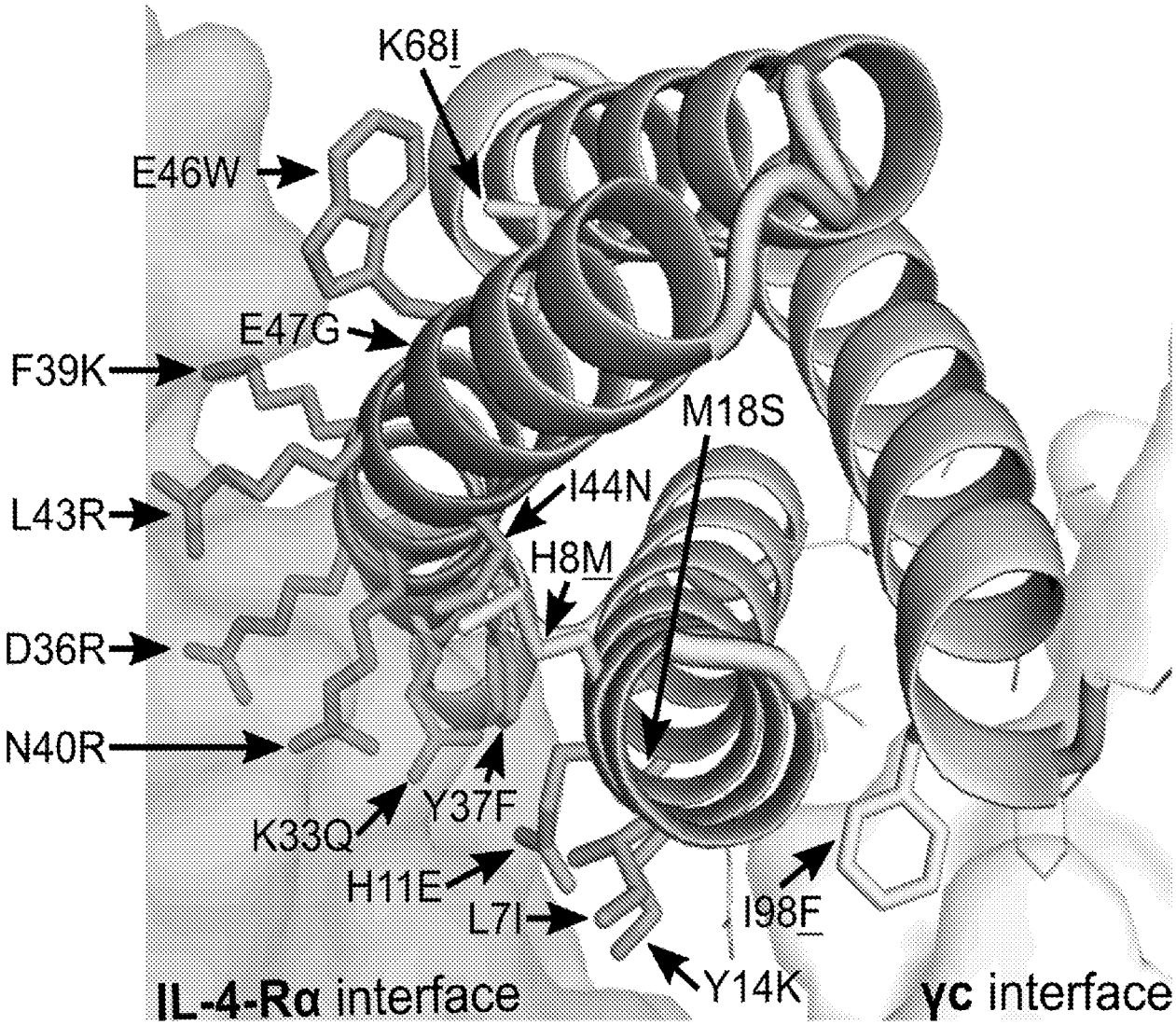

K68I
E46W
E47G
F39K
M18S
I44N
L43R
H8M
D36R
N40R
Y37F
K33Q
H11E
L7I
I98F
Y14K
IL-4-Rα interface
γc interface

FIG. 7C

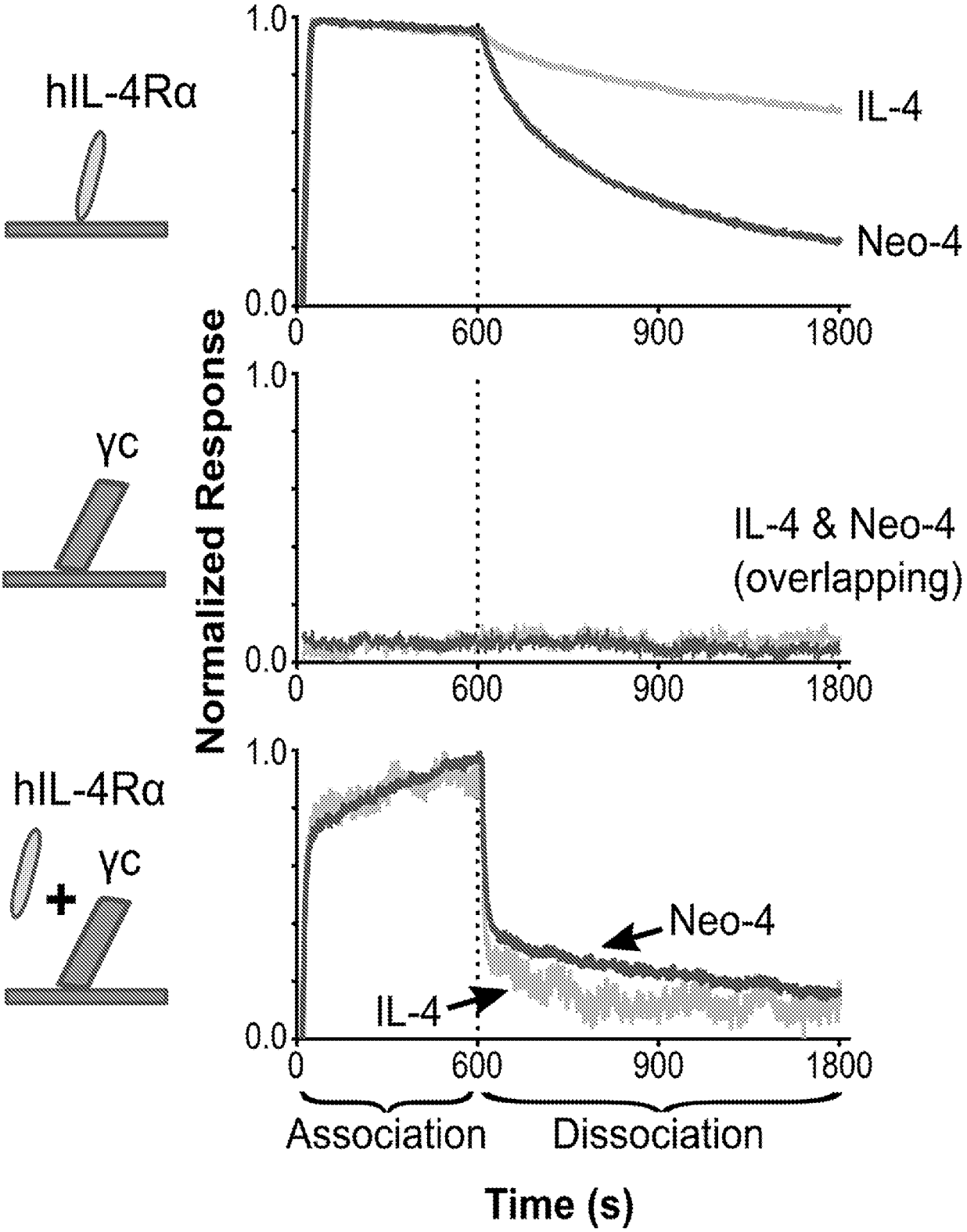

hIL-4Rα
γc
hIL-4Rα
+
γc
1.0
0.0
0
600
900
1800
IL-4
Neo-4
IL-4 & Neo-4
(overlapping)
Normalized Response
Neo-4
IL-4
Association
Dissociation
Time (s)

FIG. 8A

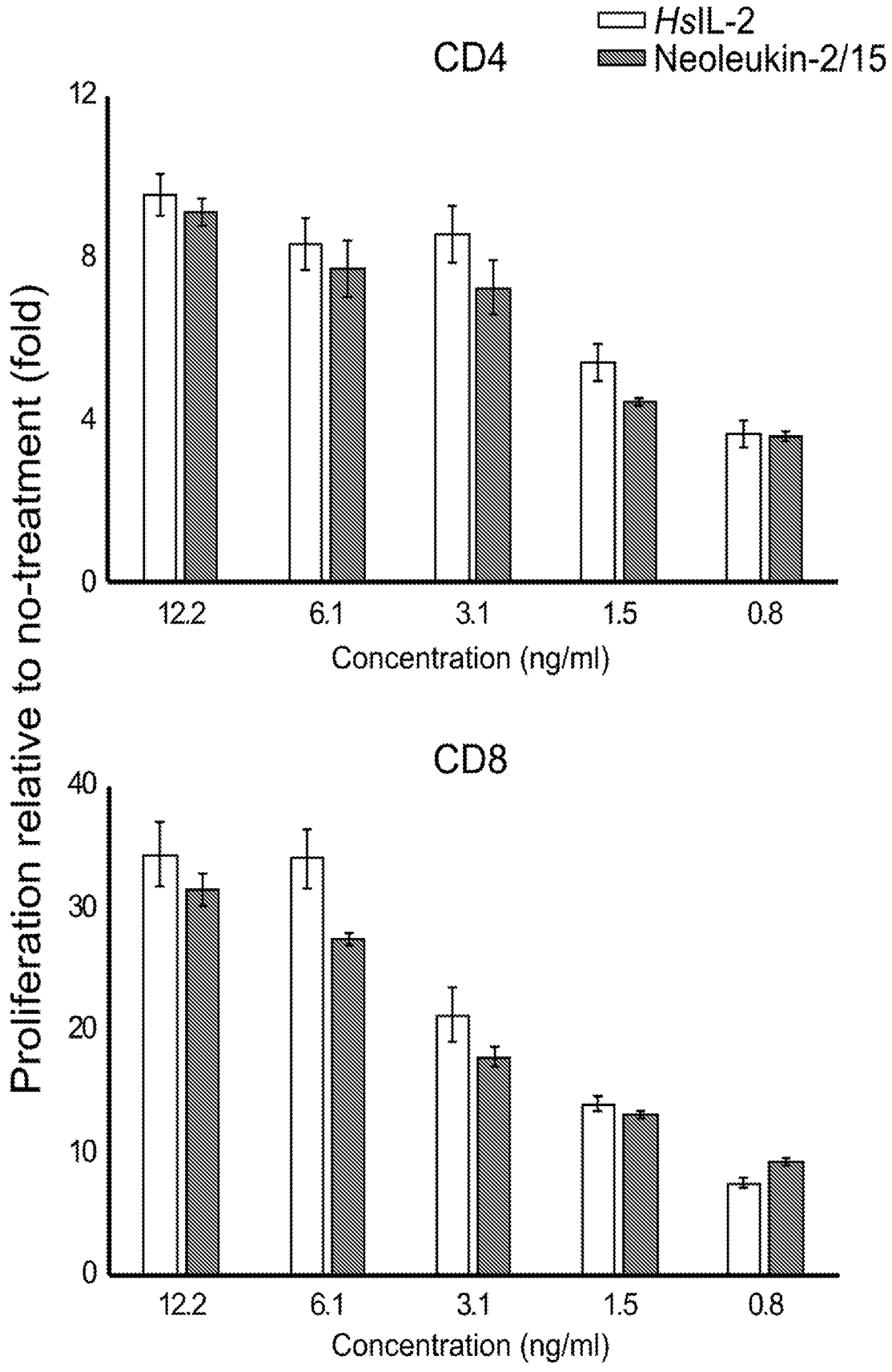

(in vitro) proliferation of **stimulated** T-cells
HsIL-2
Neoleukin-2/15
CD4
CD8
Proliferation relative to no-treatment (fold)
0
4
8
12
0
10
20
30
40
12.2
6.1
3.1
1.5
0.8
Concentration (ng/ml)

FIG. 8B

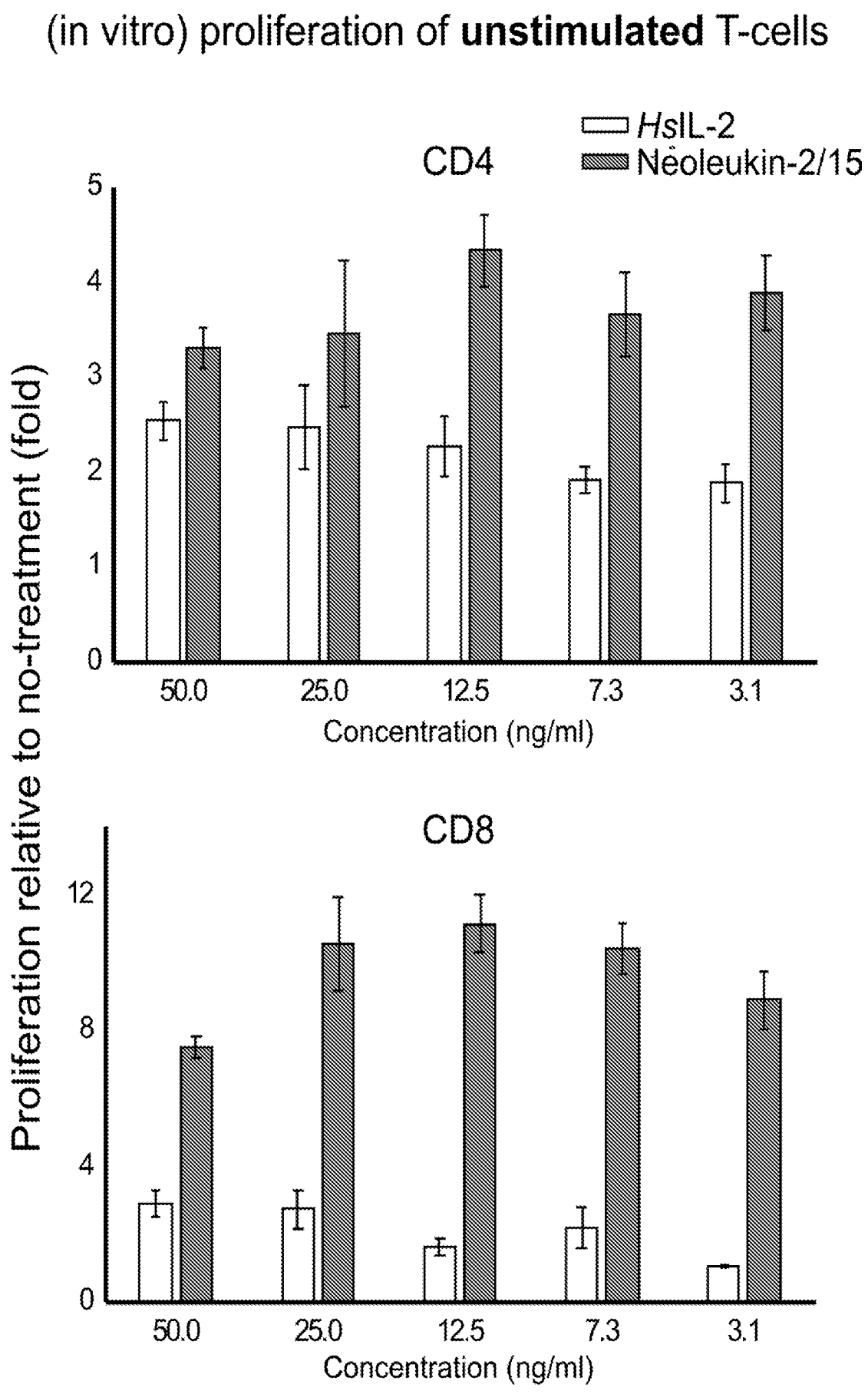

(in vitro) proliferation of **unstimulated** T-cells
HsIL-2
Neoleukin-2/15
CD4
CD8
Proliferation relative to no-treatment (fold)
0
1
2
3
4
5
8
12
50.0
25.0
12.5
7.3
3.1
Concentration (ng/ml)

FIG. 9A

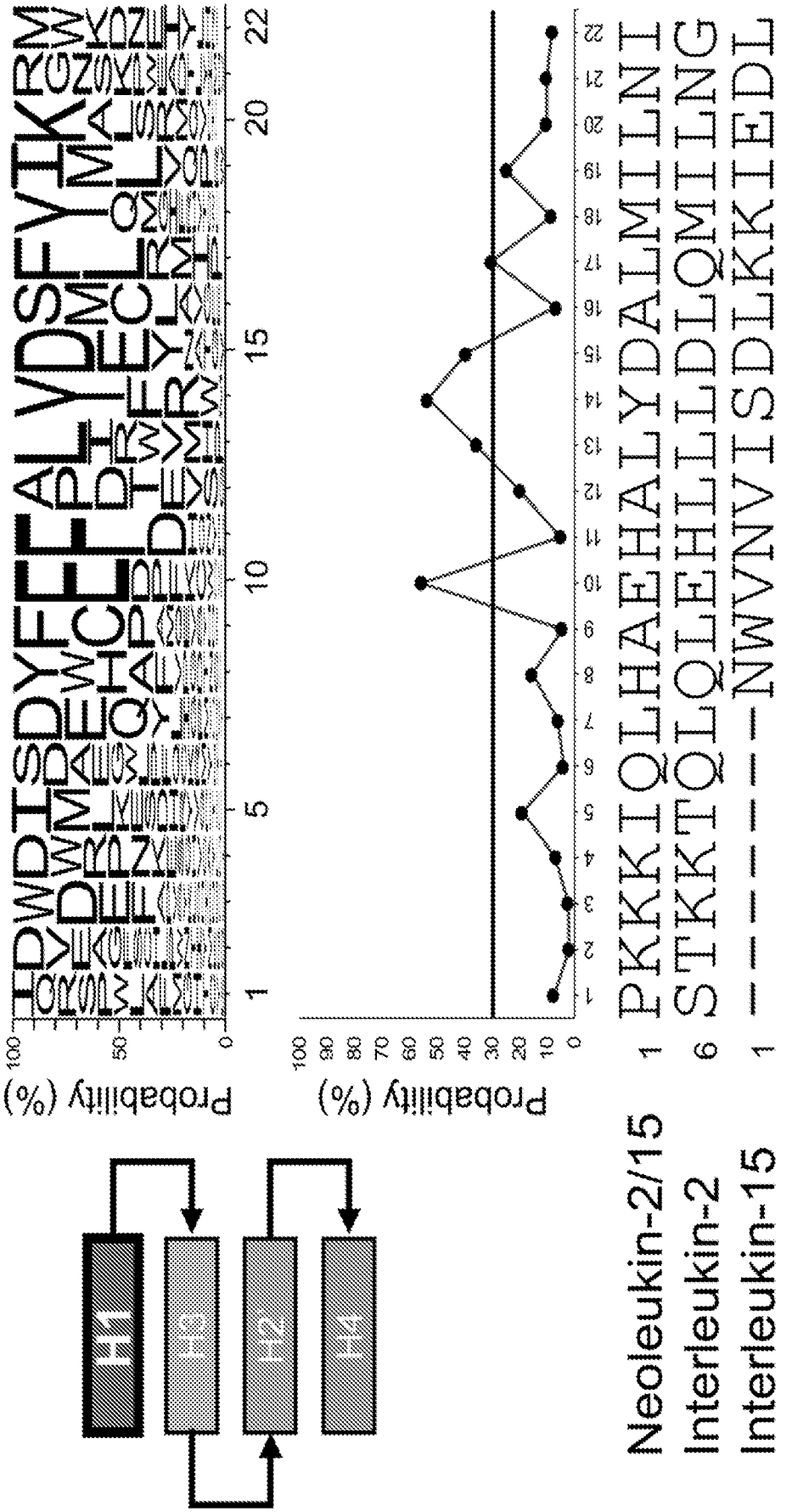

Helix 1 consensus
Probability (%)
100
50
0
1
5
10
15
20
22
Probability (%)
100
90
80
70
60
50
40
30
20
10
0
H1
H3
H2
H4
Neoleukin-2/15 1 PKKIQLHAEHALYDALMILNI 22
Interleukin-2 6 STKKTQLQLEHLLLDLQMILNG 27
Interleukin-15 1 ---------NWVNVISDLKKIEDL 15

FIG. 9B

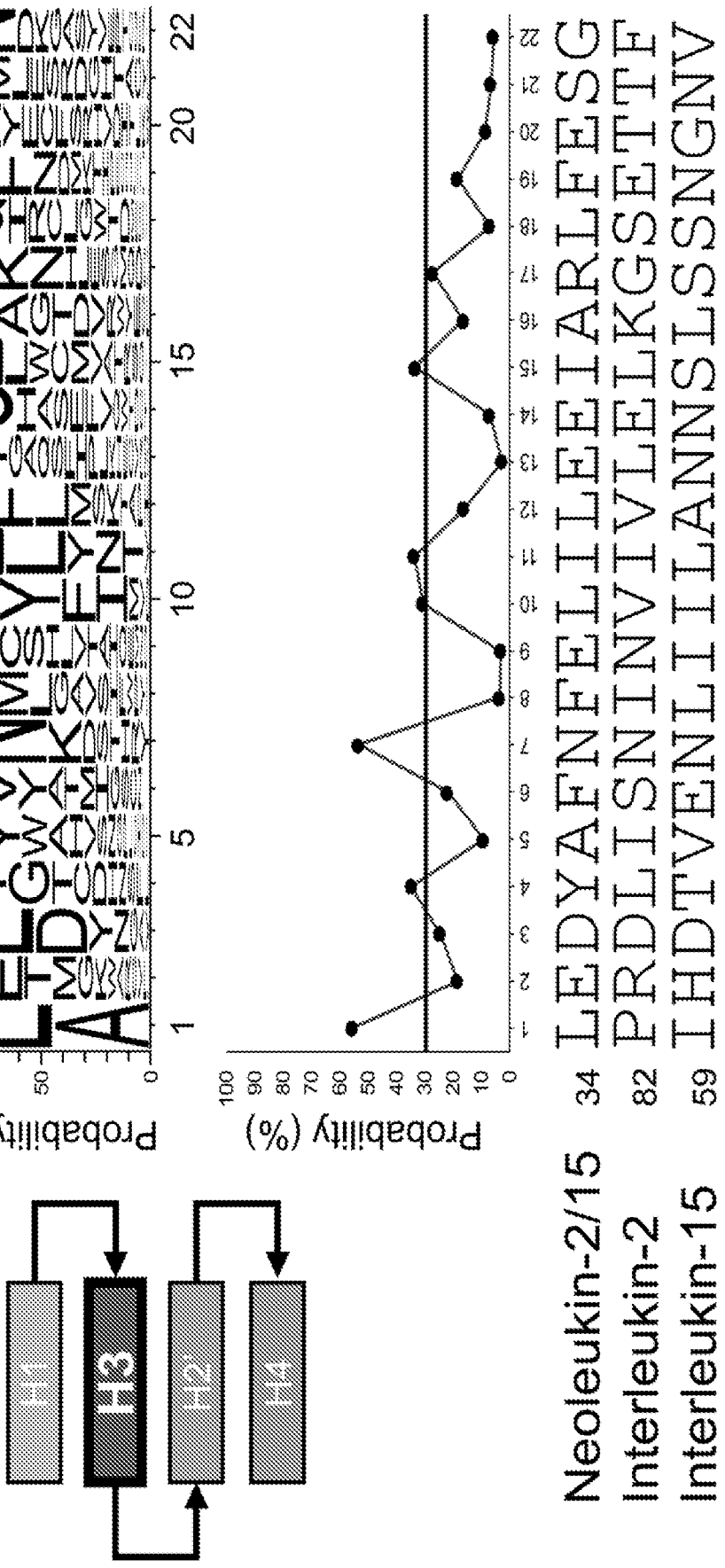

Helix 3 consensus
Probability (%)
100
50
0
1
5
10
15
20
22
H1
H3
H2
H4
Probability (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Neoleukin-2/15 34 LEDYAFNEELILEEIARLFESG 55
Interleukin-2 82 PRDLISNINVIVLELKGSETTF 103
Interleukin-15 59 THDTVENLILLANNSLSSNGNV 80

FIG. 9C

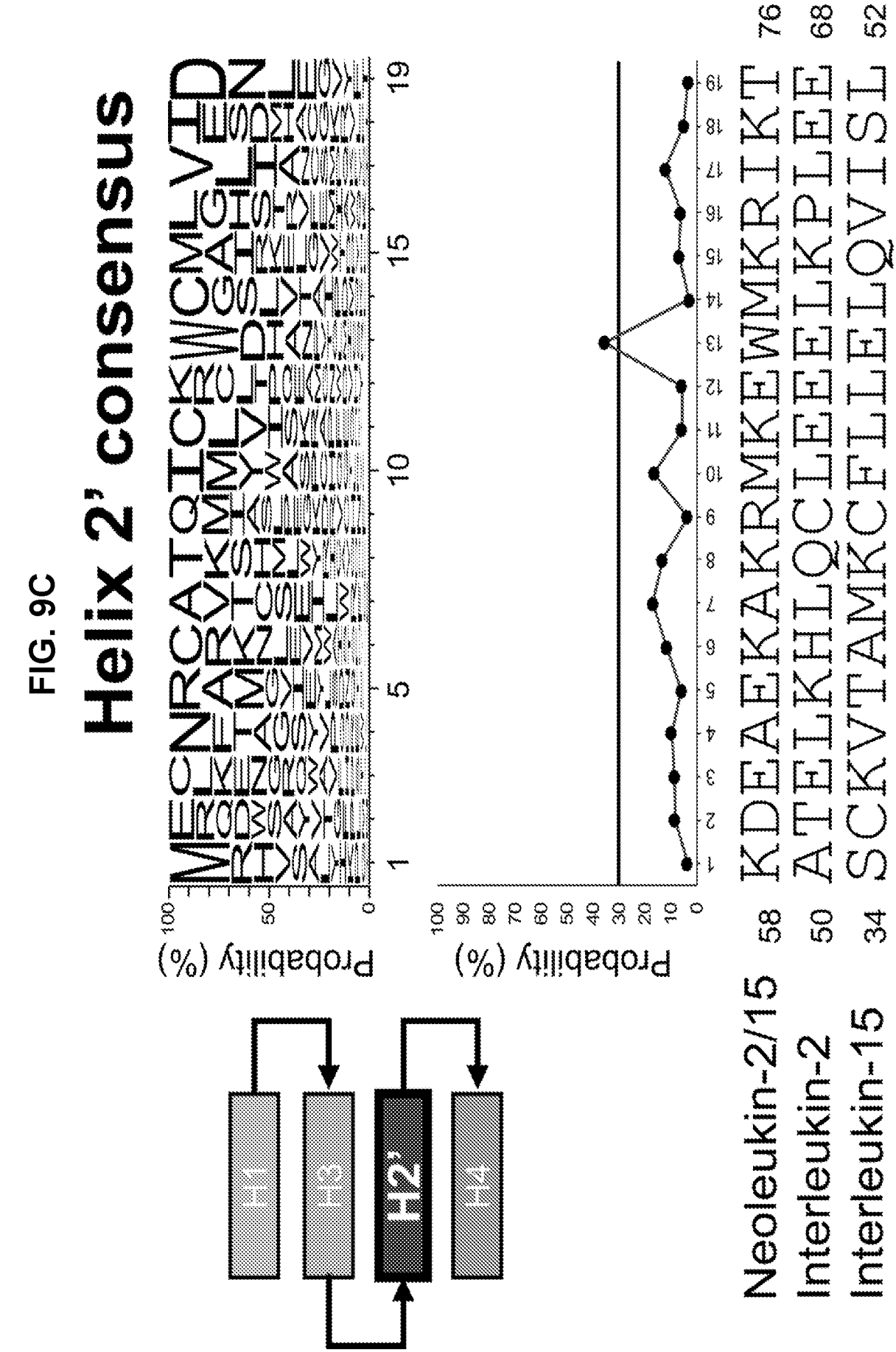

Helix 2' consensus
Probability (%)
100
50
0
1
5
10
15
19
Probability (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
H1
H2
H2'
H4
Neoleukin-2/15 58 KDEAEKAKRMKEWMKRIKT 76
Interleukin-2 50 ATELKHLQCLEEELKPLEE 68
Interleukin-15 34 SCKVTAMKCFLLELQVISL 52

FIG. 9D

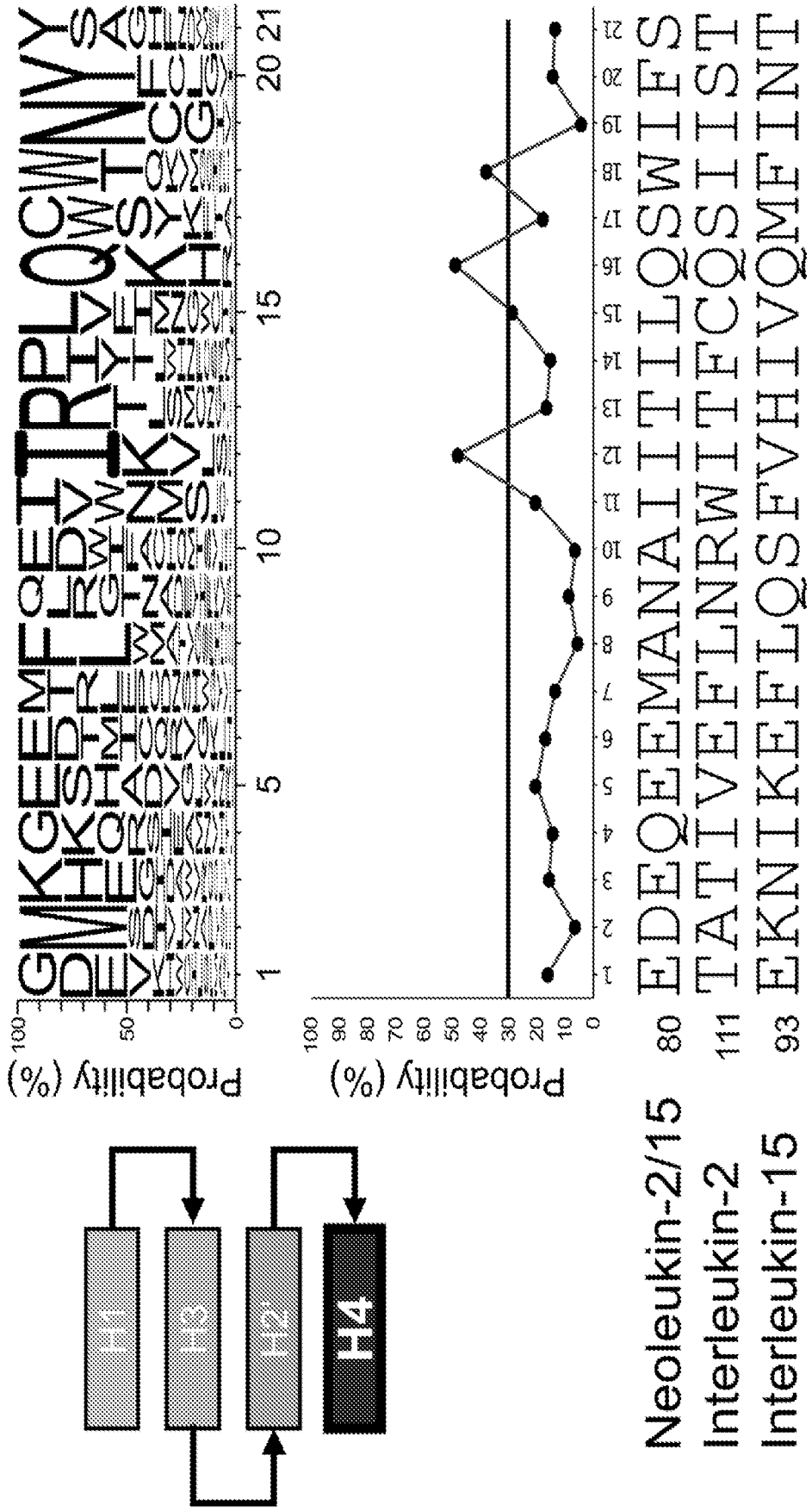

Helix 4 consensus
Probability (%)
1
5
10
15
20 21
H1
H2
H3
H4
Probability (%)
Neoleukin-2/15 80 EDEQEEMANAIITILQSWIFS 100
Interleukin-2 111 TATIVEFLNRWITFCQSIIST 131
Interleukin-15 93 EKNIKEFLQSFVHIVQMFINT 113

FIG.10A

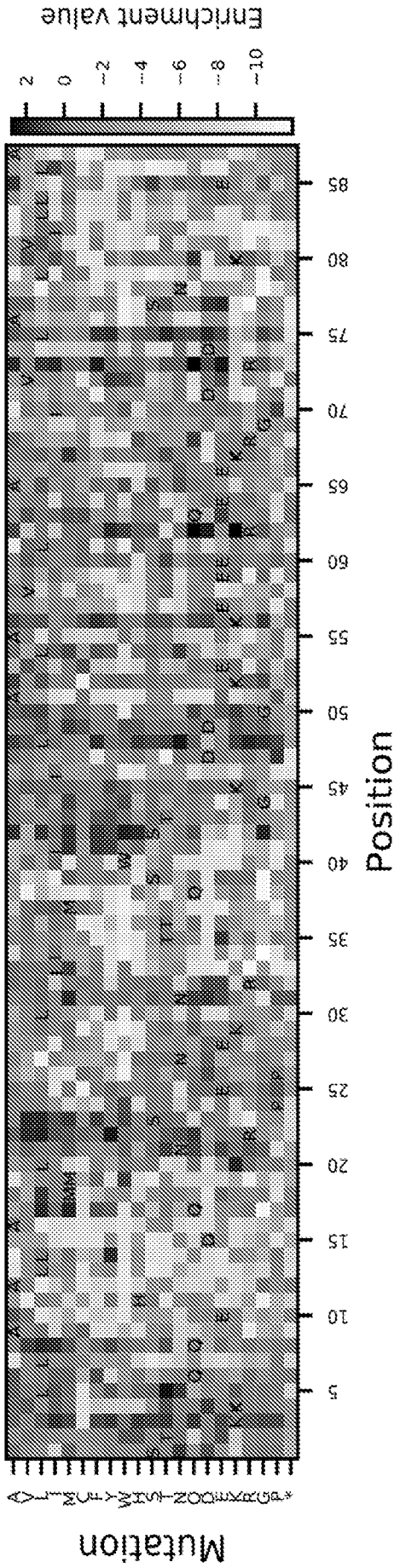

Enrichment value
2
0
−2
−4
−6
−8
−10
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG.10B

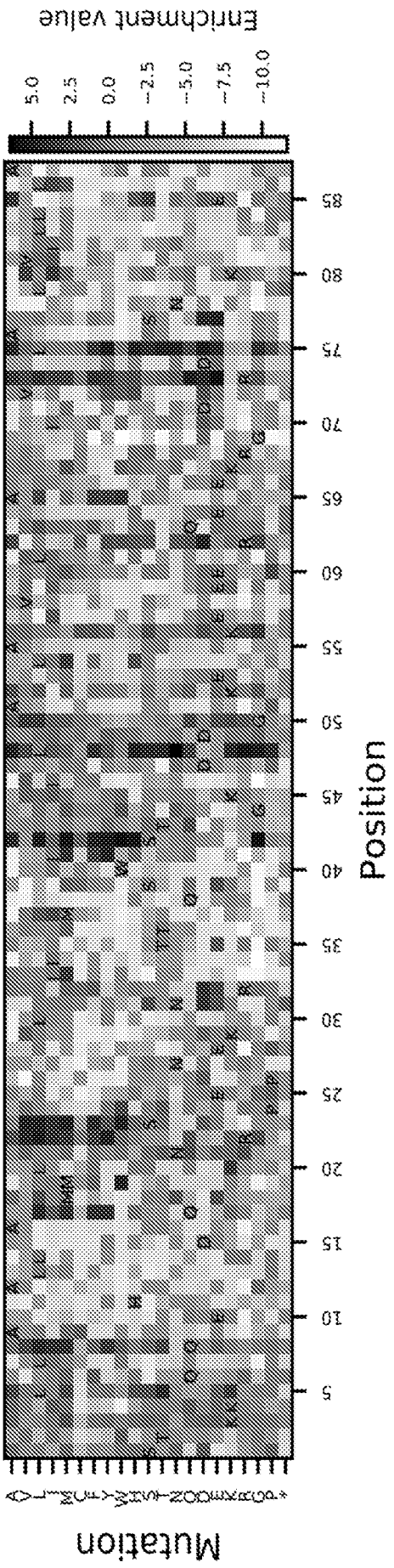

Enrichment value
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG.10C

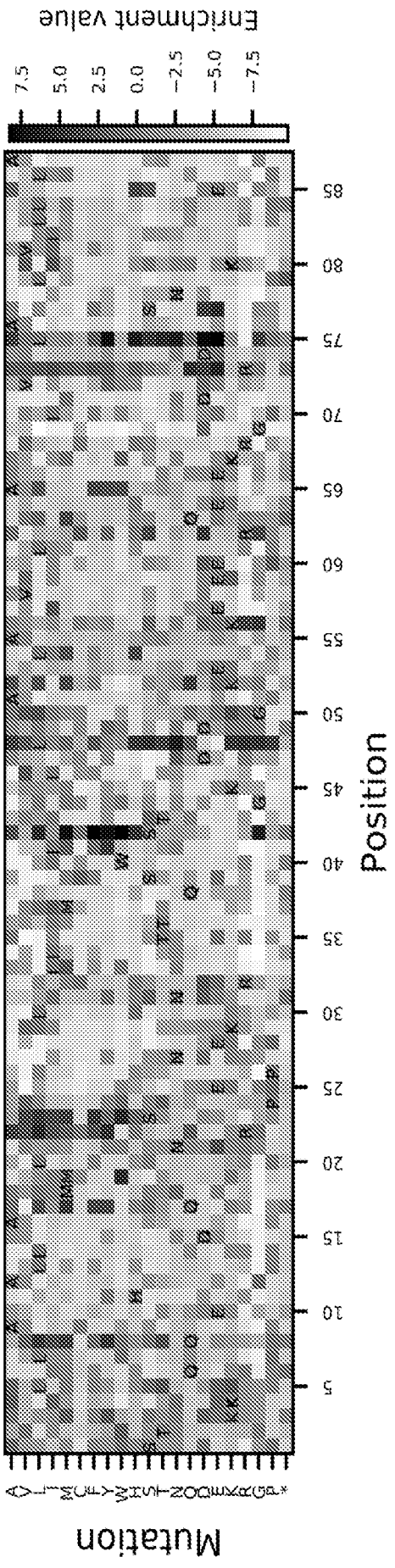

Enrichment value
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG.10D

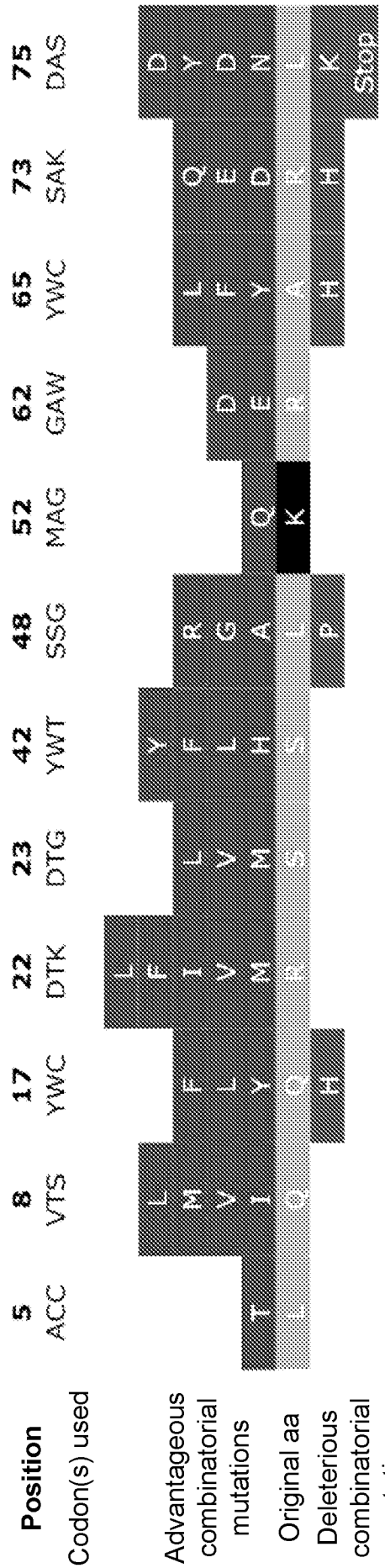

Position
Codon(s) used
Advantageous combinatorial mutations
Original aa
Deleterious combinatorial mutations
5 ACC
8 VTS
17 YWC
22 DTK
23 DTG
42 YWT
48 SSG
52 MAG
62 GAW
65 YWC
73 SAK
75 DAS
Stop

FIG. 11A

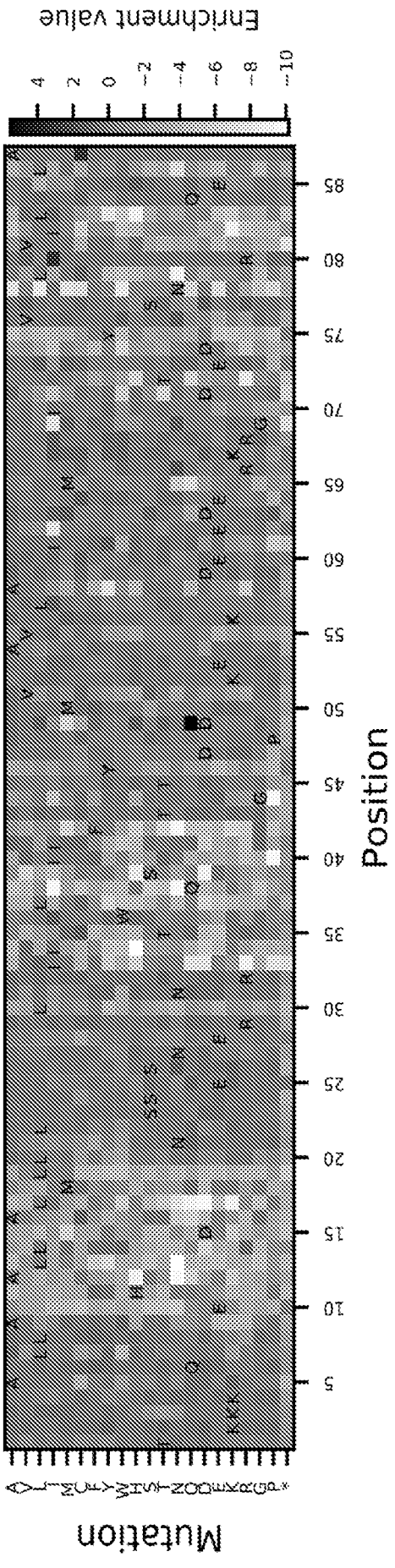

Enrichment value
4
2
0
-2
-4
-6
-8
-10
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG. 11B

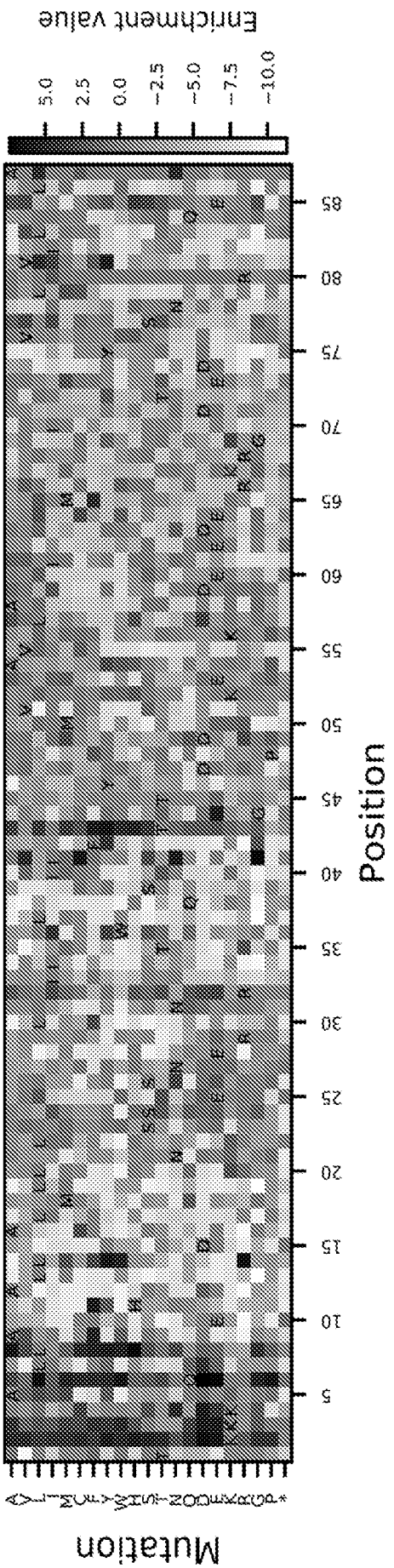

Enrichment value
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG. 11C

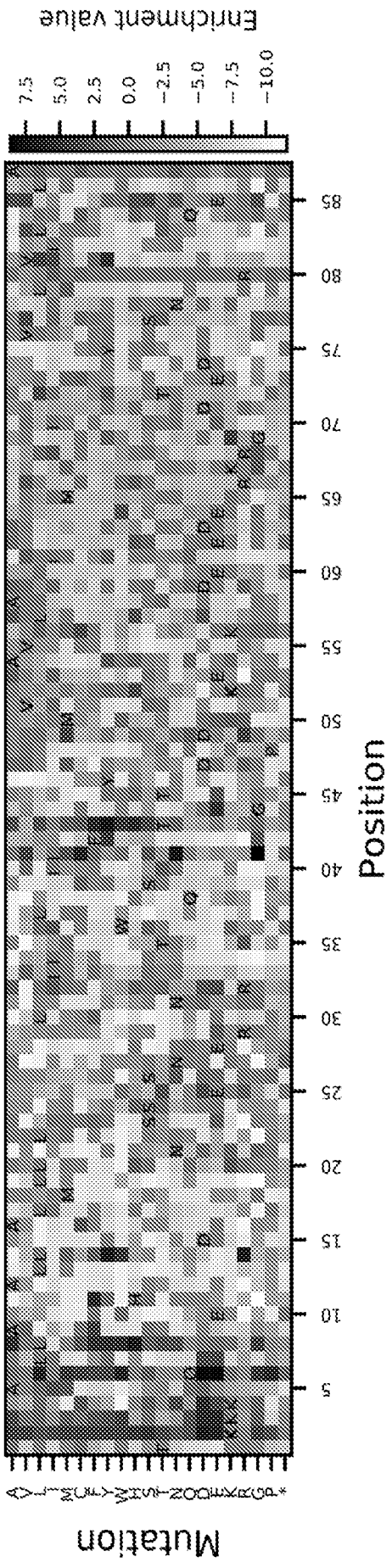

Enrichment value
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Mutation
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85

FIG. 11D

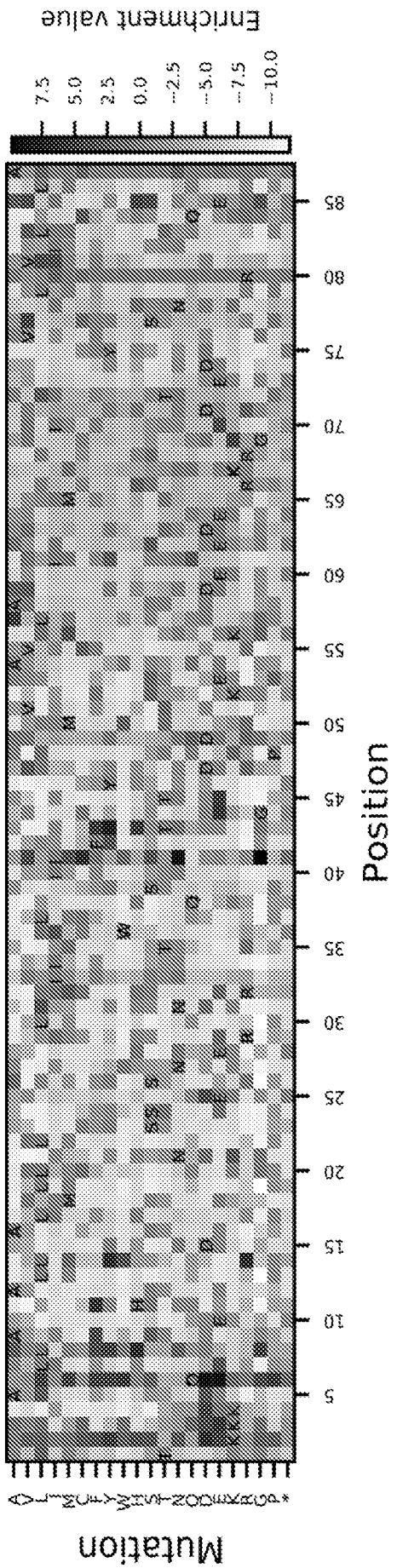

Enrichment value
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Mutation

FIG. 11E

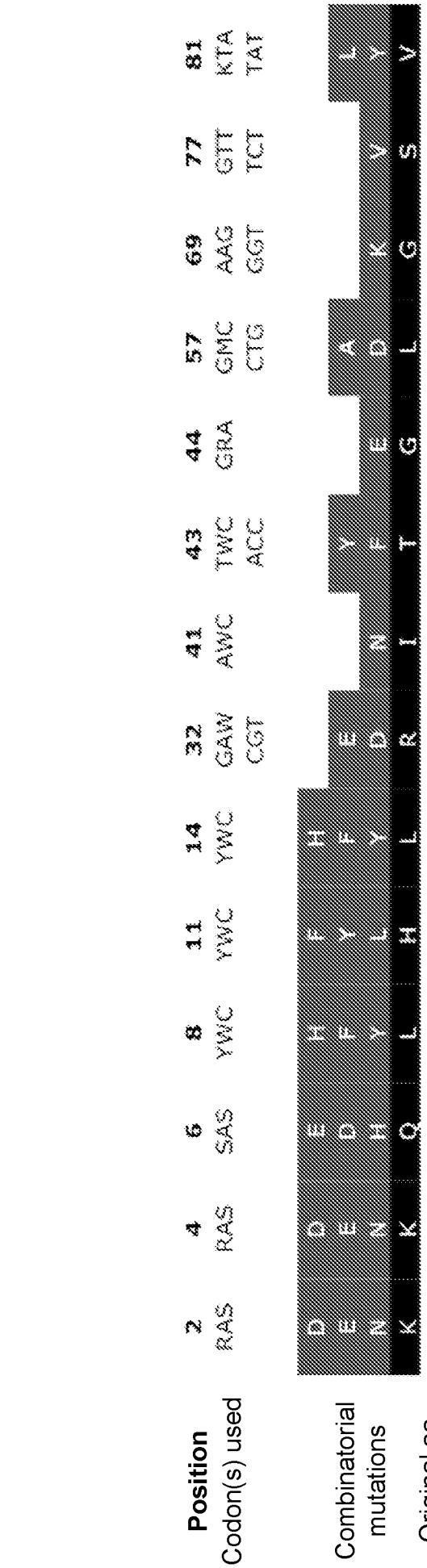

Position
Codon(s) used
Combinatorial mutations
Original aa
2 RAS
4 RAS
6 SAS
8 YWC
11 YWC
14 YWC
32 GAW CGT
41 AWC
43 TWC ACC
44 GRA
57 GMC CTG
69 AAG GGT
77 GTT TCT
81 KTA TAT
D E N K
D E N K
E D H Q
H F Y L
F Y L H
H F Y L
E D N T
Y F T
E G
A D L
K G
V S
L Y V

FIG. 12A

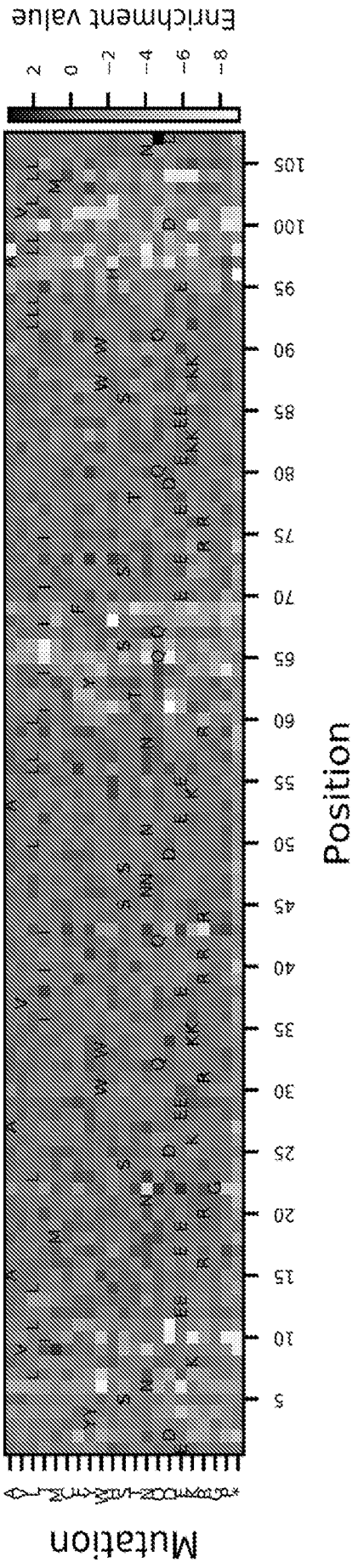

Enrichment value
2
0
−2
−4
−6
−8
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
Mutation

FIG. 12B

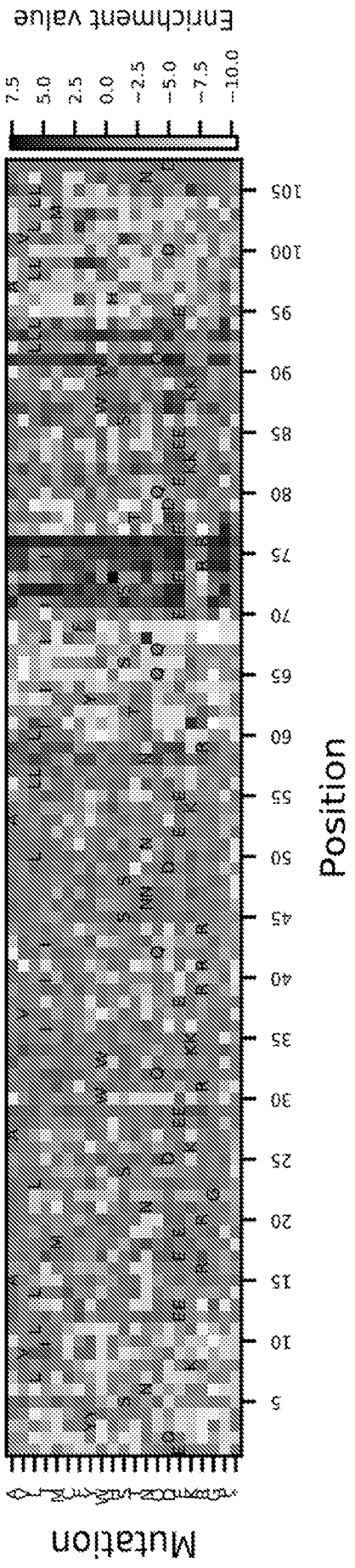

Enrichment value
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
Mutation

FIG. 12C

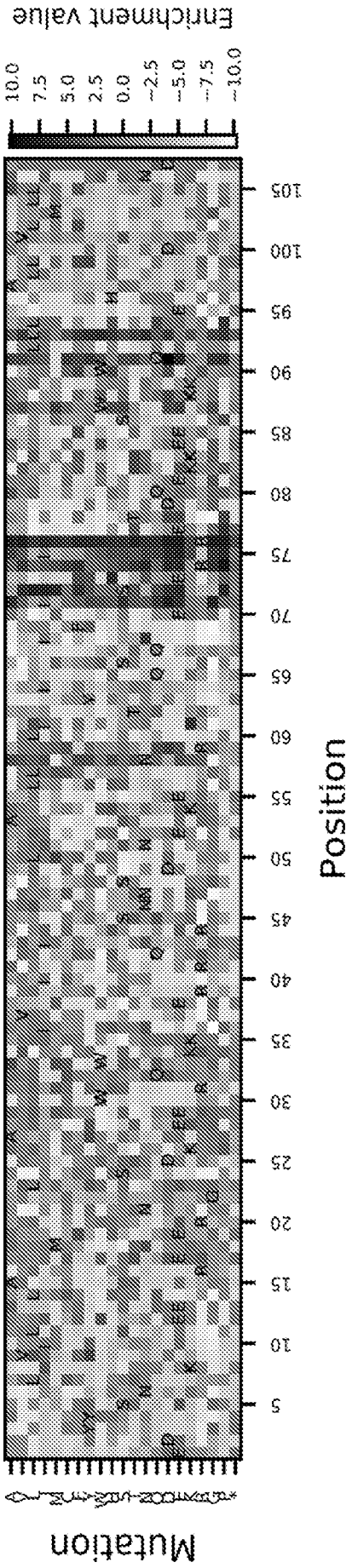

Enrichment value
10.0
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
Mutation

FIG. 12D

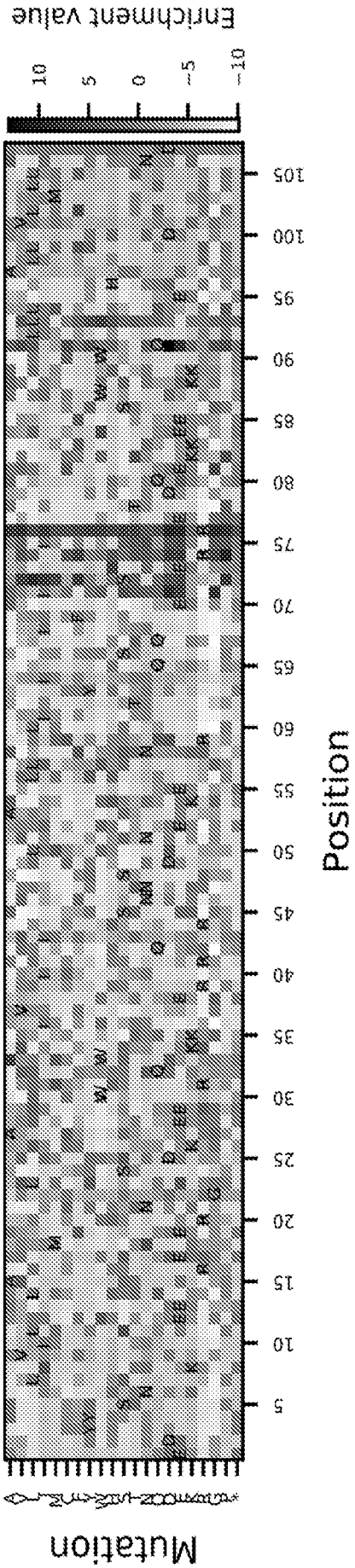

Enrichment value
10
5
0
−5
−10
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
Mutation

FIG. 12E

Position	9	59	68	71	72	73	74	75	76	91	93	99
Codon(s) used	KTA TAT	GAW CGT	AWC	RWC	HYA	GAA CAT	SST	RWA	SSA	SAW	YWC	YWC
Combinatorial mutations	Y L	E D	N	D N V	I P L T	H	P G A	E K V	G P A	D E H	Y F H	Y F H
Original aa	V	R	I	I	S	E	R	I	R	Q	L	L

FIG. 13A

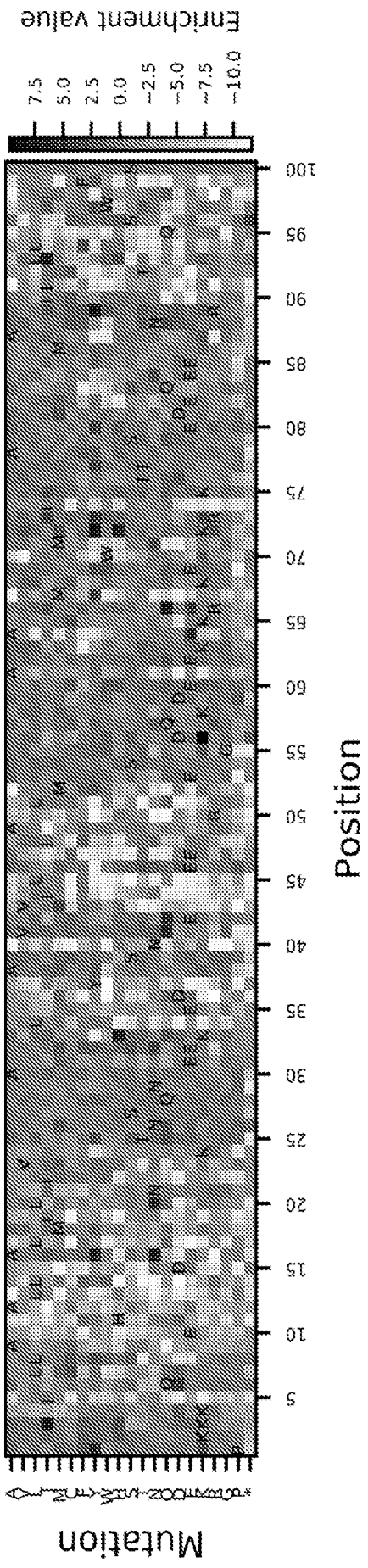

Enrichment value
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
−10.0
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Mutation

FIG. 13B

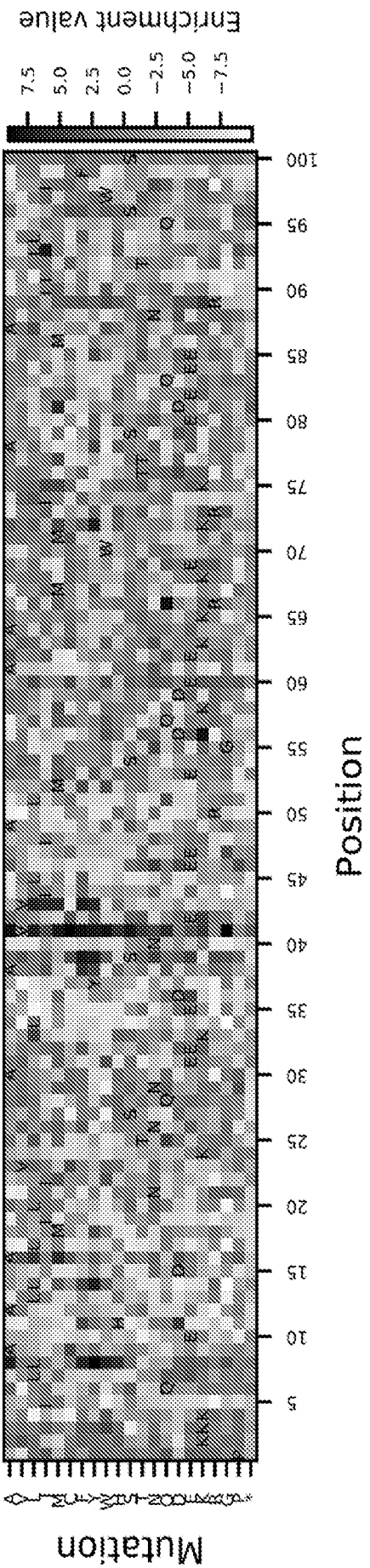


FIG. 13C

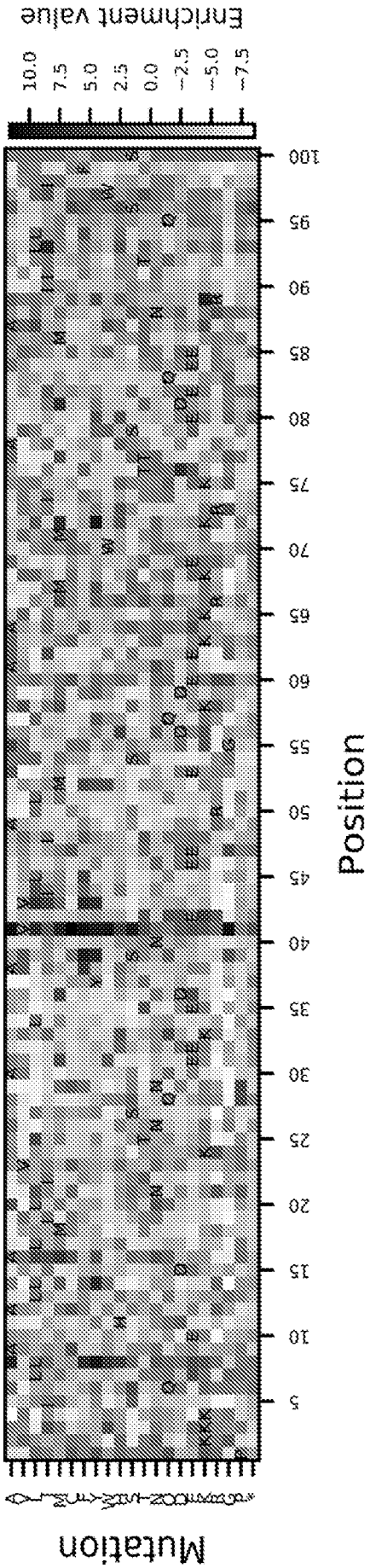

Enrichment value
10.0
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Mutation

FIG. 13D

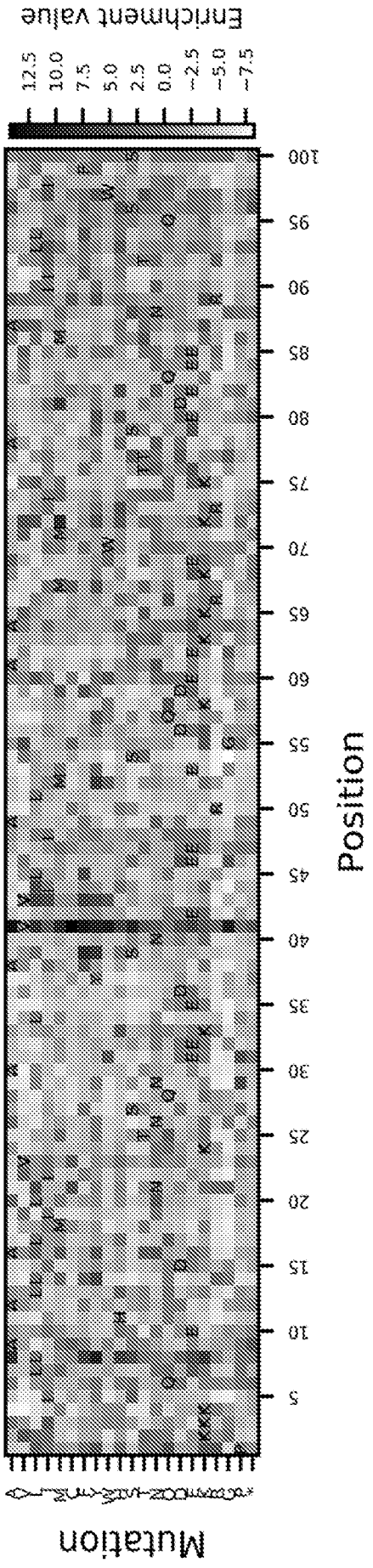

Enrichment value
12.5
10.0
7.5
5.0
2.5
0.0
−2.5
−5.0
−7.5
Position
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Mutation

FIG. 13E

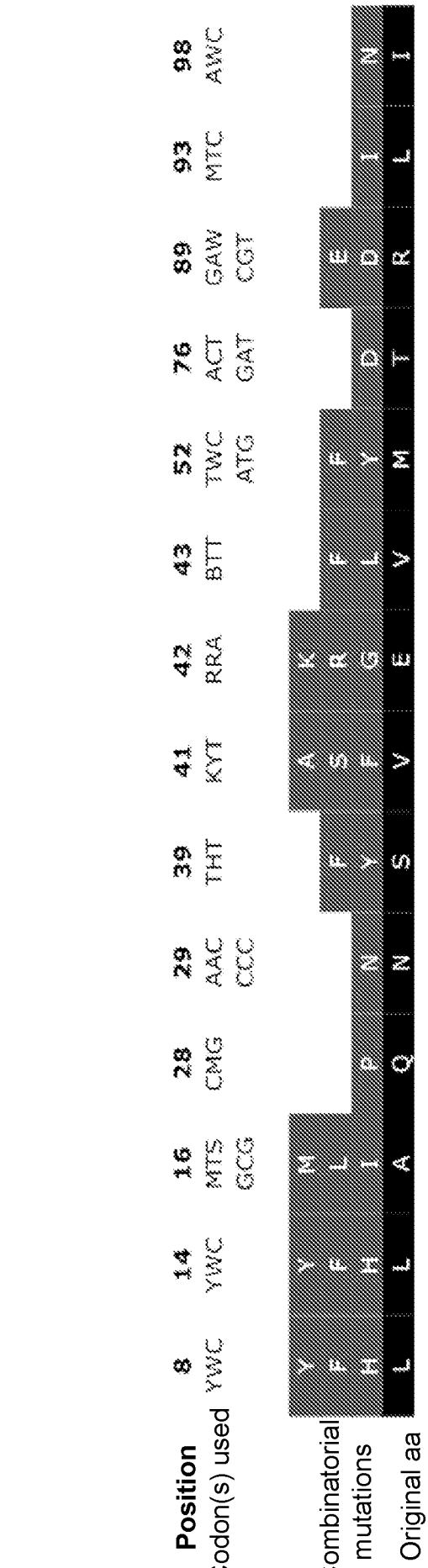

Position
Codon(s) used
Combinatorial mutations
Original aa
8 14 16 28 29 39 41 42 43 52 76 89 93 98
YWC YWC MTS CMG AAC THT KYT RRA BTT TWC ACT GAW MTC AWC
GCG CCC ATG GAT CGT

FIG. 14A

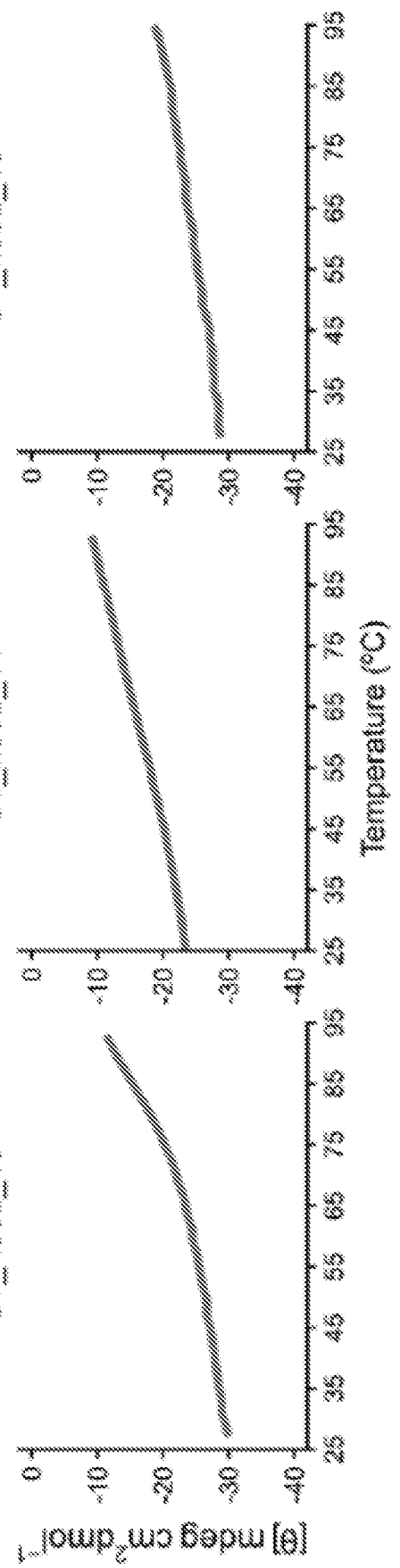


FIG. 14B

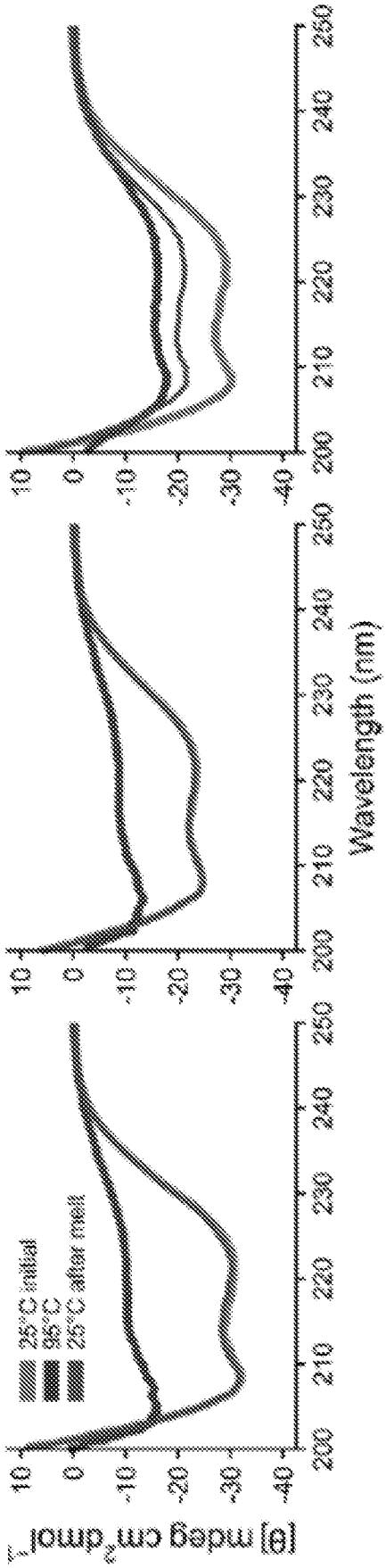


FIG. 15A

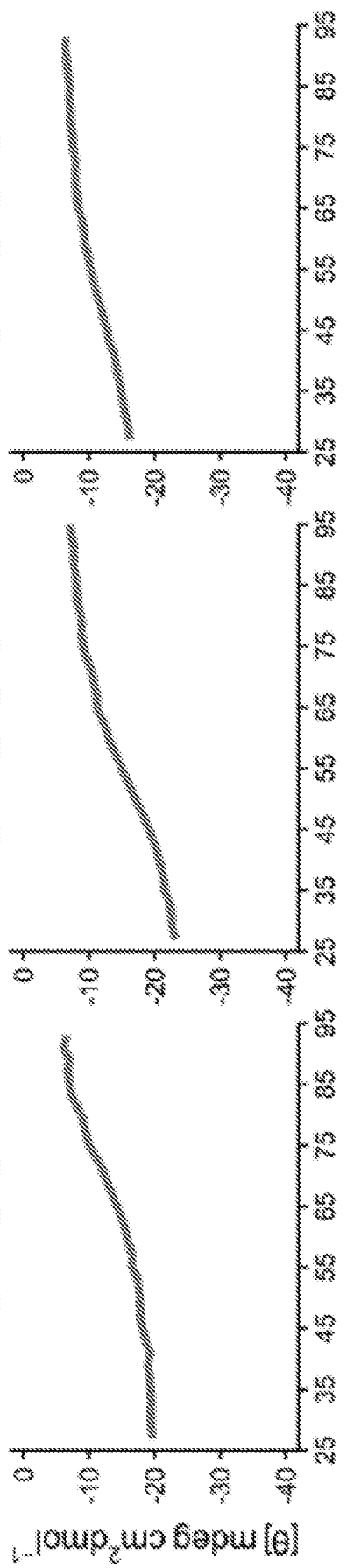


FIG. 15B

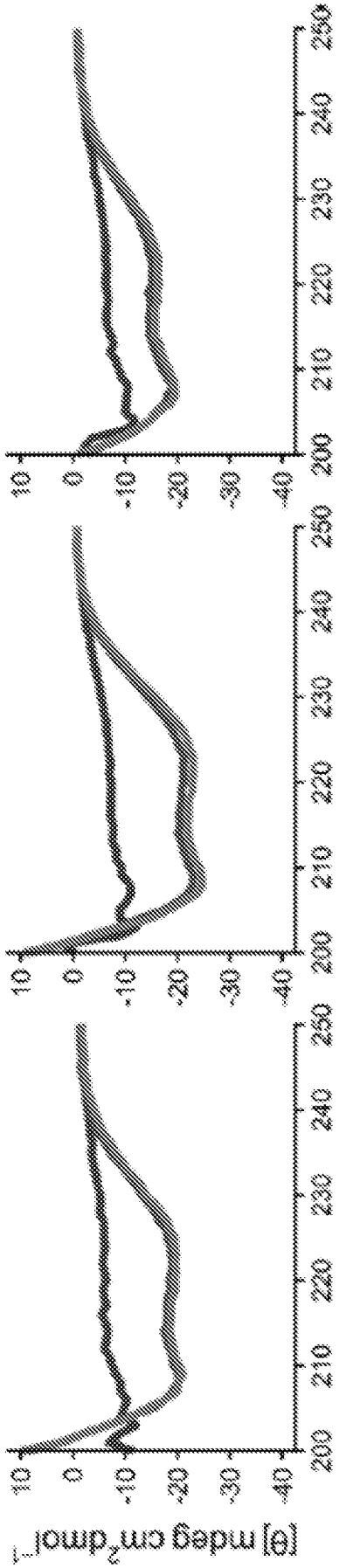


FIG. 16A

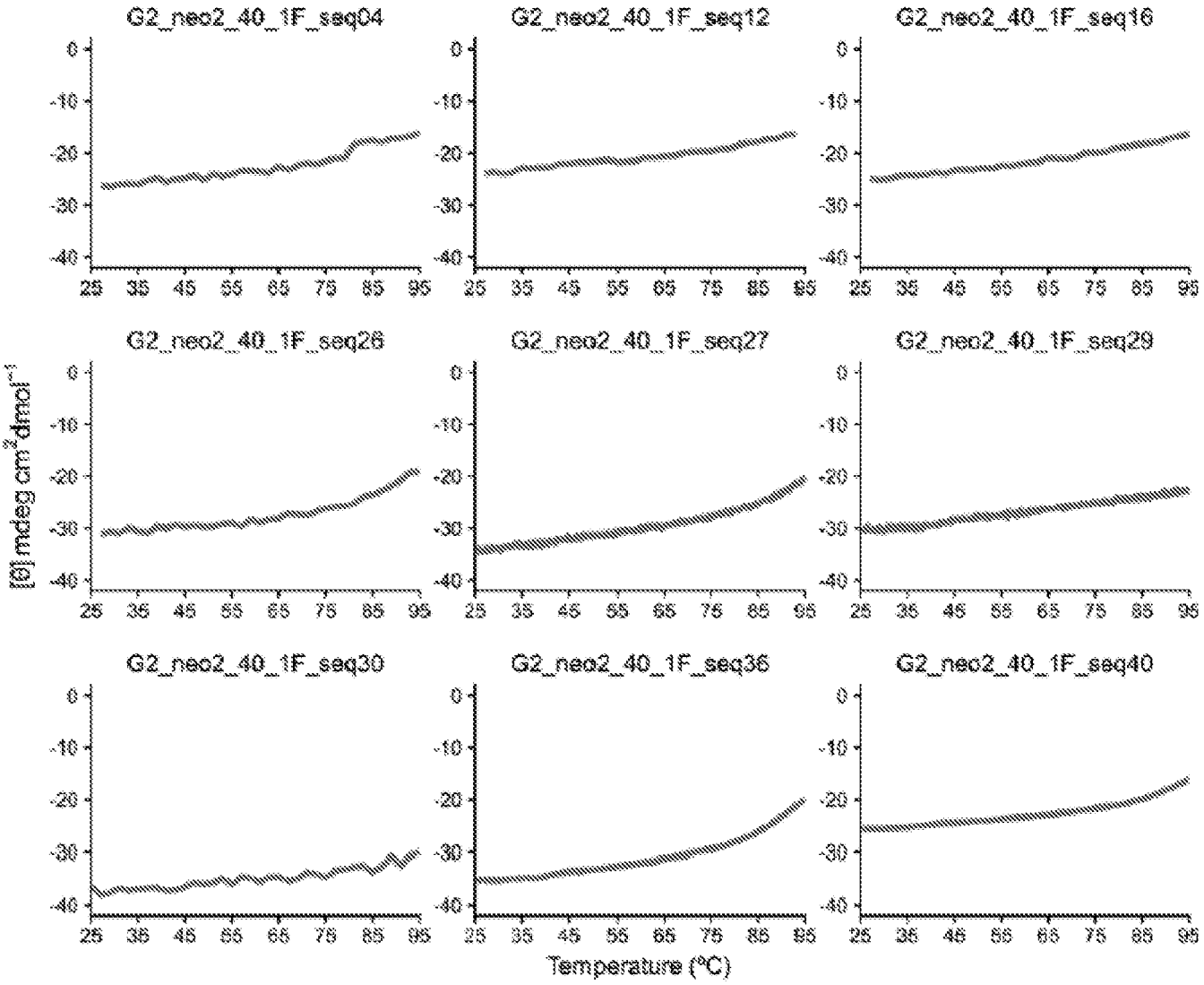

G2_neo2_40_1F_seq04
G2_neo2_40_1F_seq12
G2_neo2_40_1F_seq16
G2_neo2_40_1F_seq26
G2_neo2_40_1F_seq27
G2_neo2_40_1F_seq29
G2_neo2_40_1F_seq30
G2_neo2_40_1F_seq36
G2_neo2_40_1F_seq40
[θ] mdeg cm²dmol⁻¹
Temperature (°C)

FIG. 16B

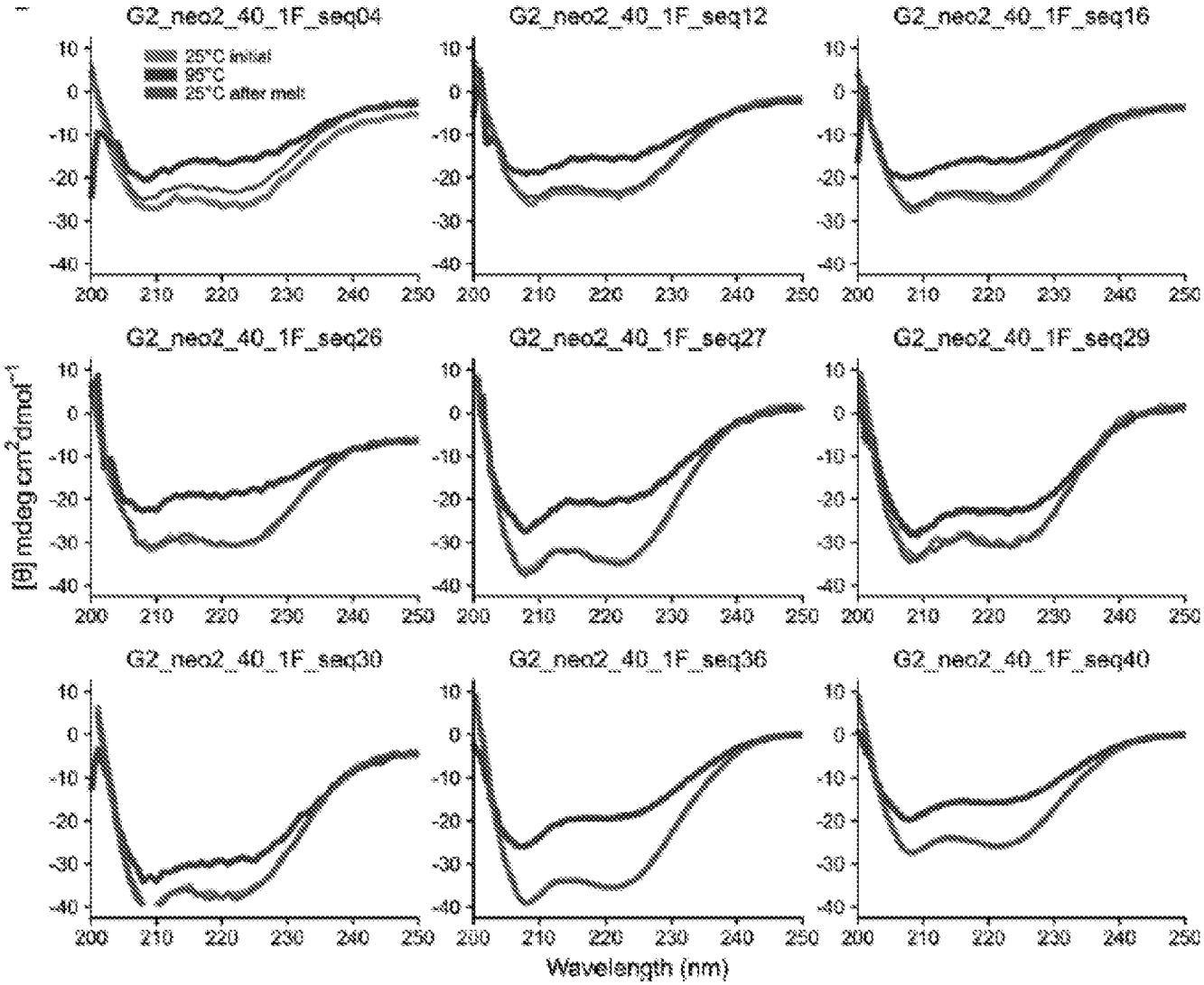

G2_neo2_40_1F_seq04
G2_neo2_40_1F_seq12
G2_neo2_40_1F_seq16
G2_neo2_40_1F_seq26
G2_neo2_40_1F_seq27
G2_neo2_40_1F_seq29
G2_neo2_40_1F_seq30
G2_neo2_40_1F_seq36
G2_neo2_40_1F_seq40
25°C initial
95°C
25°C after melt
[θ] mdeg cm2dmol-1
Wavelength (nm)

FIG. 16C

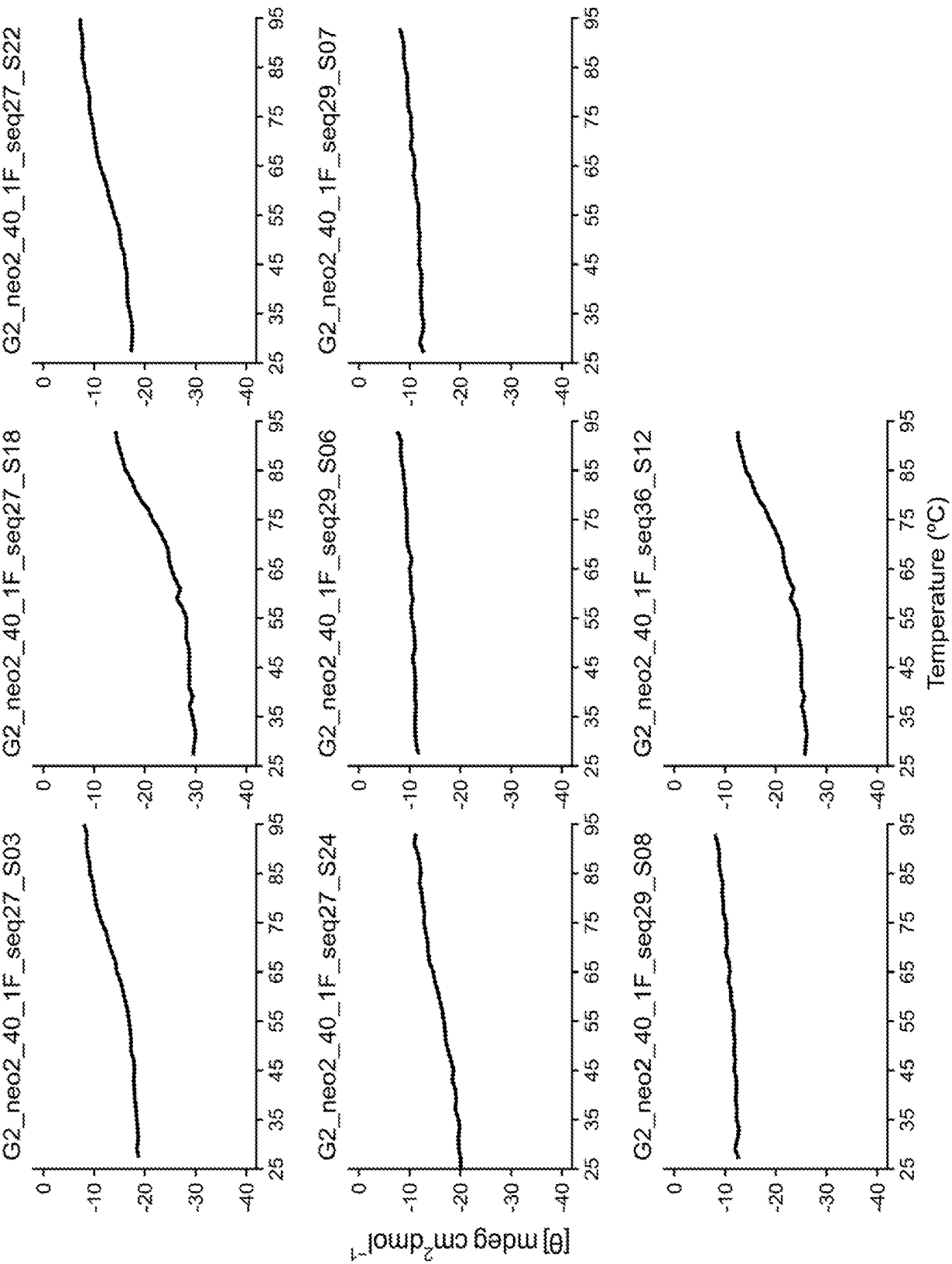

G2_neo2_40_1F_seq27_S03
G2_neo2_40_1F_seq27_S18
G2_neo2_40_1F_seq27_S22
G2_neo2_40_1F_seq27_S24
G2_neo2_40_1F_seq29_S06
G2_neo2_40_1F_seq29_S07
G2_neo2_40_1F_seq29_S08
G2_neo2_40_1F_seq36_S12
[θ] mdeg cm²dmol⁻¹
Temperature (°C)

FIG. 16D

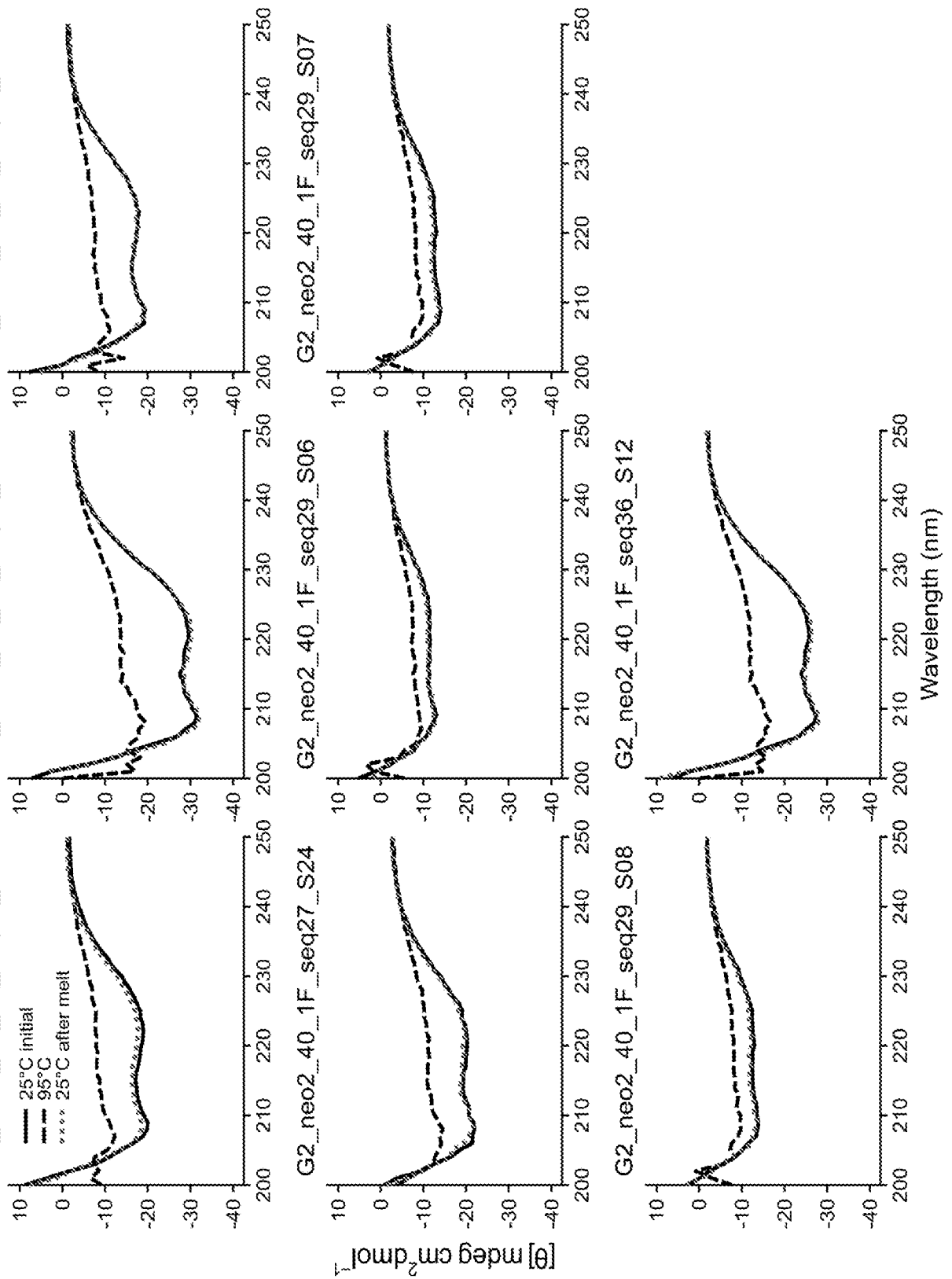

G2_neo2_40_1F_seq27_S03
G2_neo2_40_1F_seq27_S18
G2_neo2_40_1F_seq27_S22
G2_neo2_40_1F_seq27_S24
G2_neo2_40_1F_seq29_S06
G2_neo2_40_1F_seq29_S07
G2_neo2_40_1F_seq29_S08
G2_neo2_40_1F_seq36_S12
25°C initial
95°C
25°C after melt
[θ] mdeg cm²dmol⁻¹
Wavelength (nm)

FIG. 17A

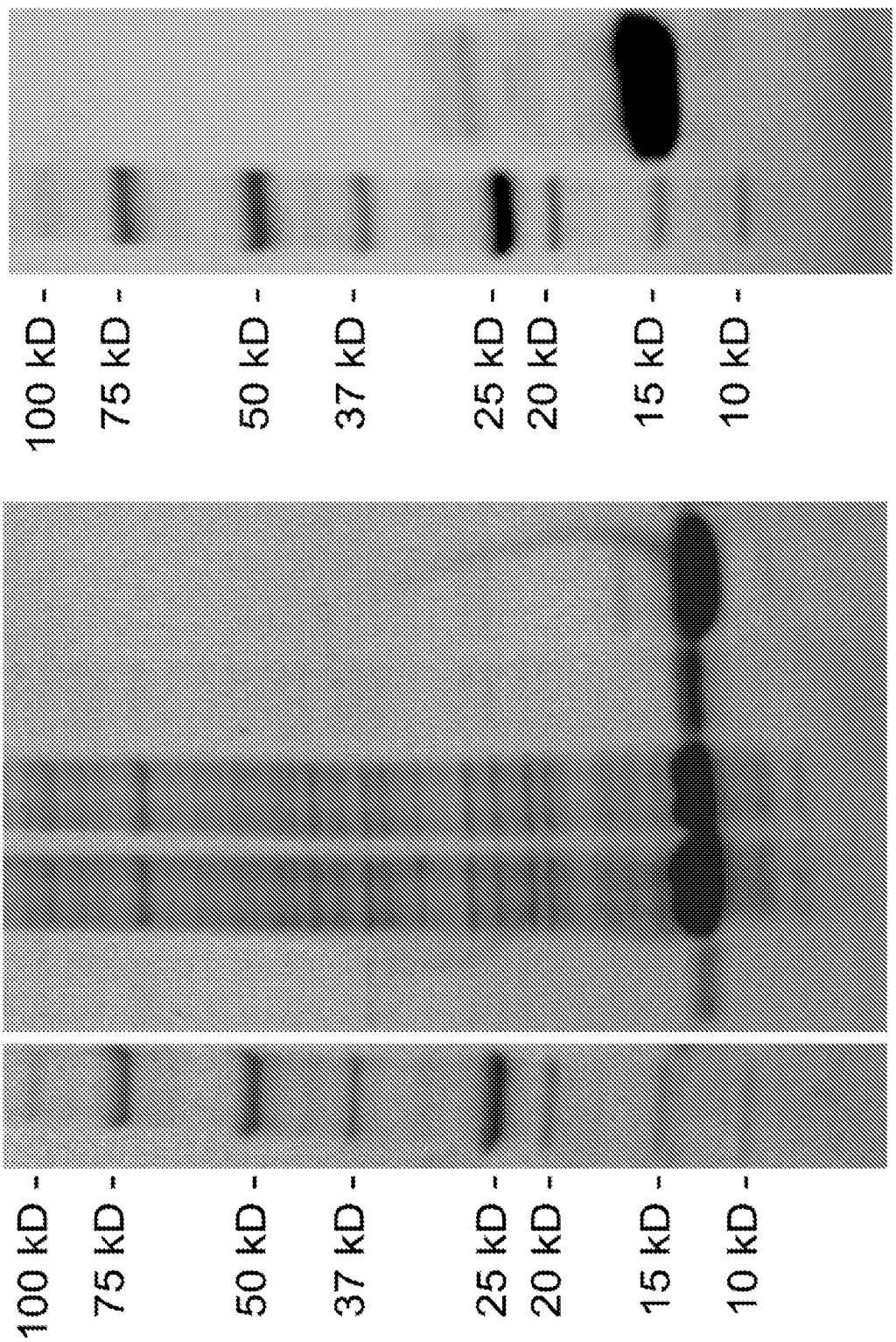

Ladder
Insoluble
Soluble
Flowthrough
Wash
Elution
Ladder
Neo-2/15
100 kD -
75 kD -
50 kD -
37 kD -
25 kD -
20 kD -
15 kD -
10 kD -

FIG. 17B

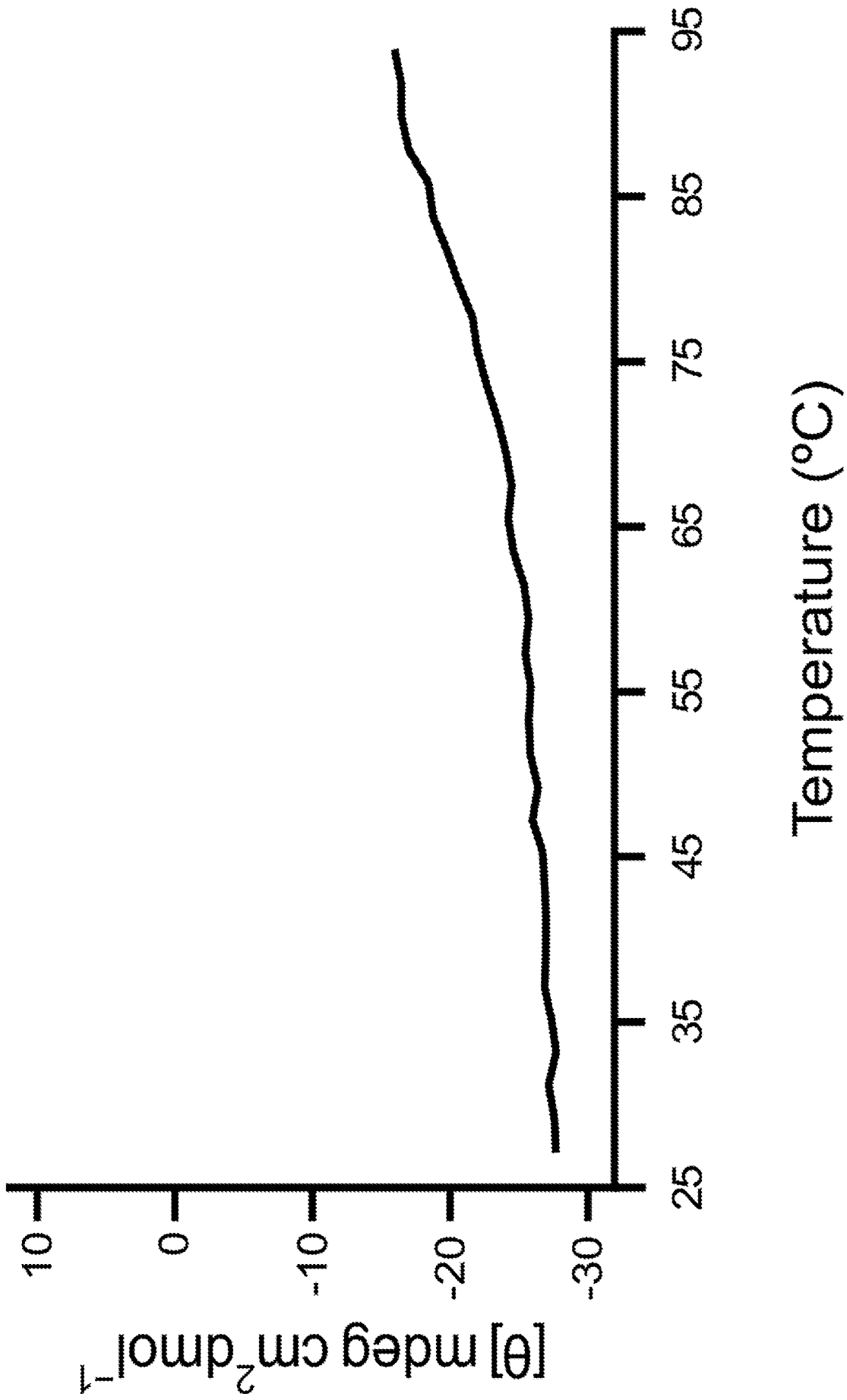

[θ] mdeg cm²dmol⁻¹
10
0
-10
-20
-30
25
35
45
55
65
75
85
95
Temperature (°C)

FIG. 17C

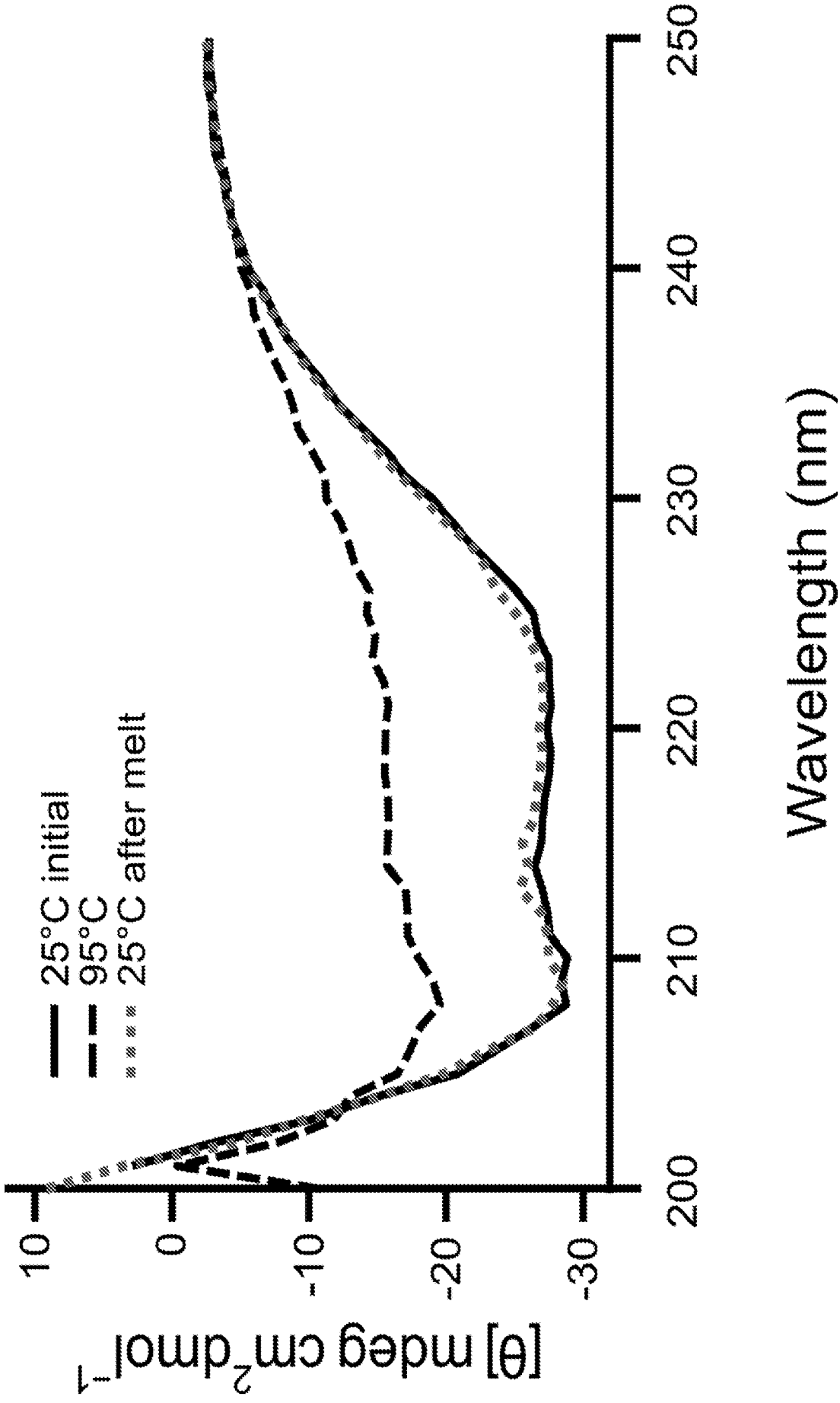

25°C initial
95°C
25°C after melt
[θ] mdeg cm²dmol⁻¹
10
0
-10
-20
-30
200
210
220
230
240
250
Wavelength (nm)

FIG. 18A

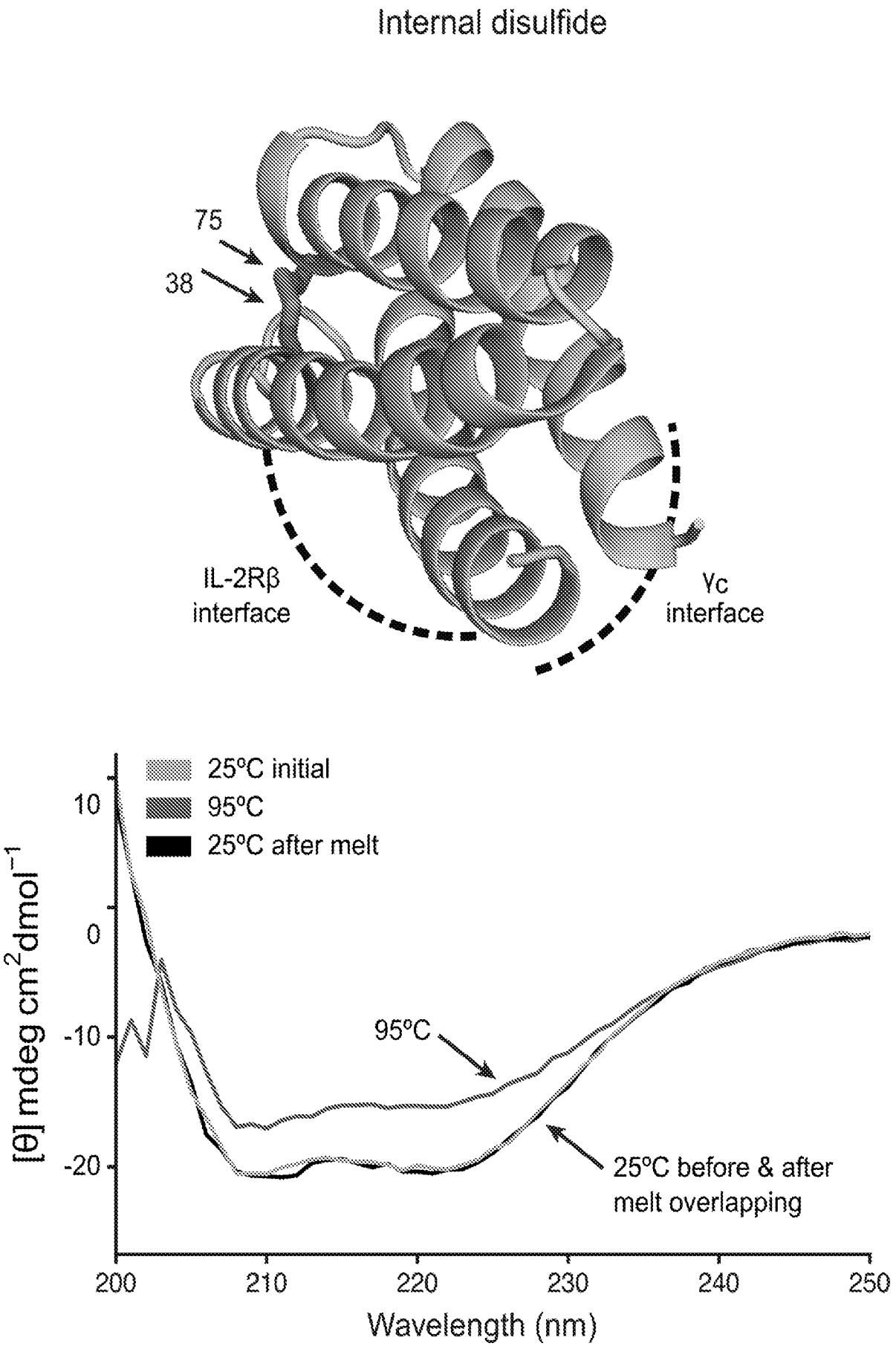

Internal disulfide
75
38
IL-2Rβ interface
γc interface
25°C initial
95°C
25°C after melt
10
0
-10
-20
[θ] mdeg cm^{2}dmol^{-1}
95°C
25°C before & after melt overlapping
200
210
220
230
240
250
Wavelength (nm)

FIG. 18B

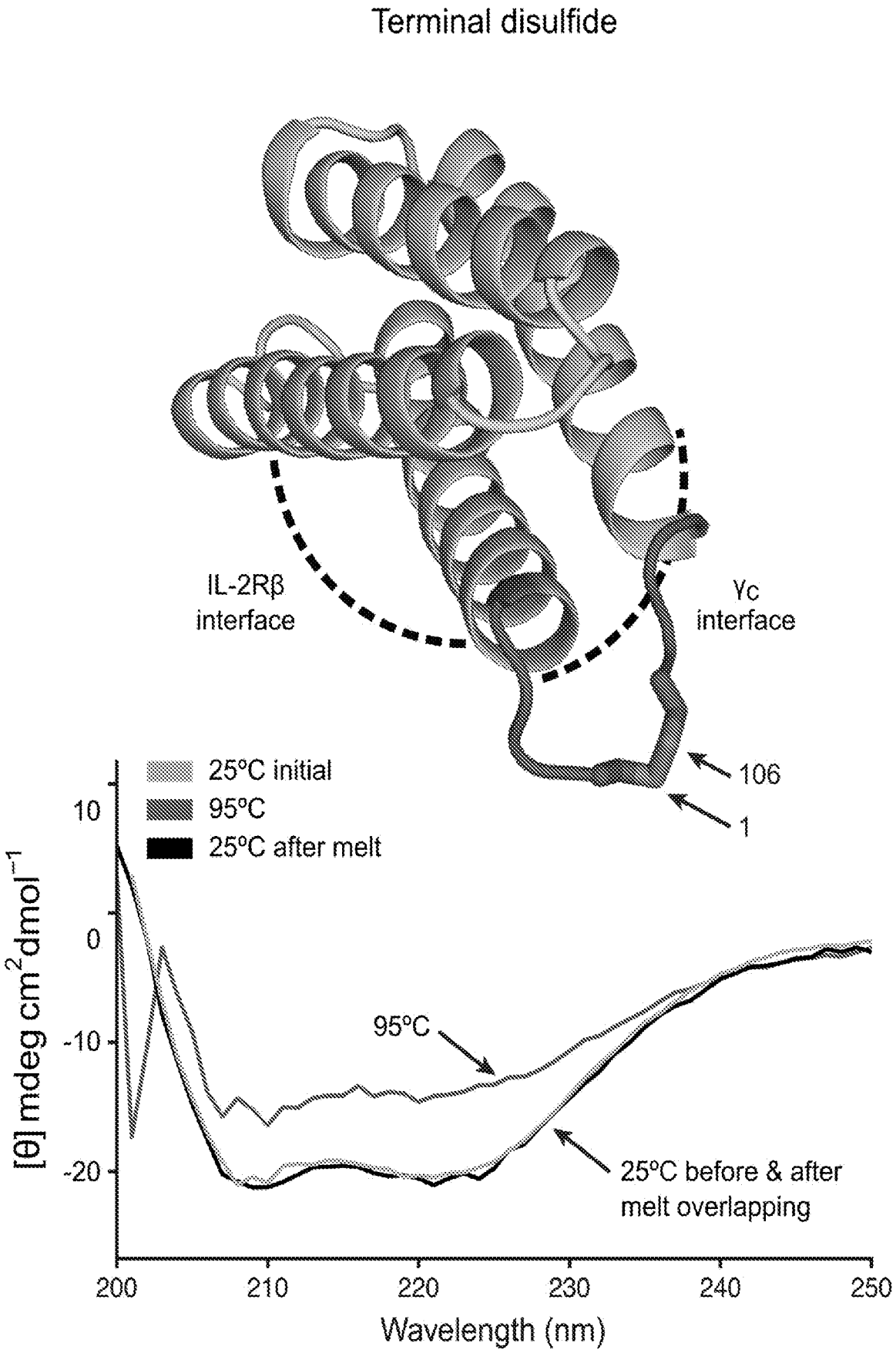

Terminal disulfide
IL-2Rβ interface
γc interface
106
1
25°C initial
95°C
25°C after melt
10
0
-10
-20
[θ] mdeg cm^{2}dmol^{-1}
95°C
25°C before & after melt overlapping
200
210
220
230
240
250
Wavelength (nm)

FIG. 18C

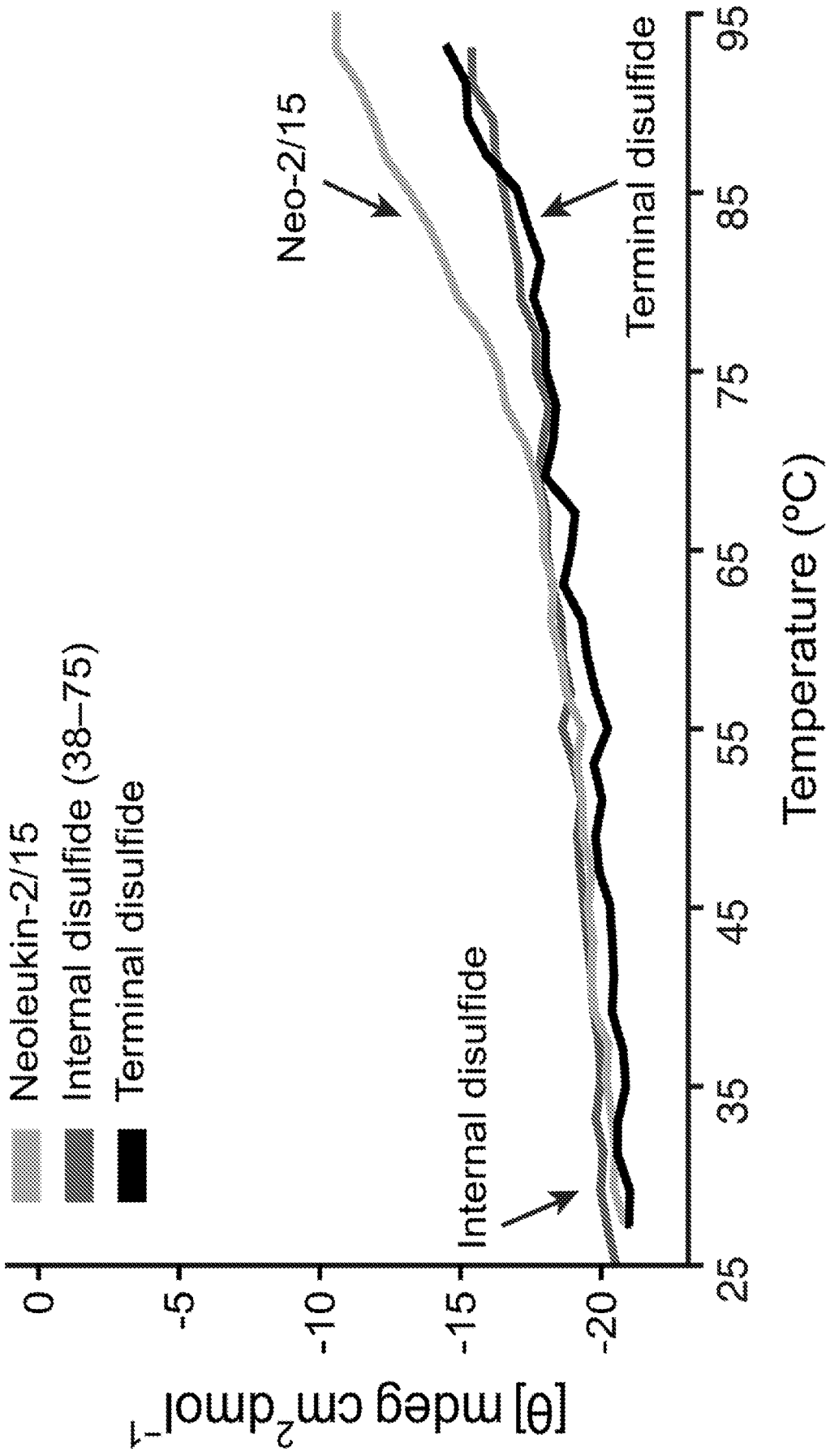

Neoleukin-2/15
Internal disulfide (38–75)
Terminal disulfide
Neo-2/15
Terminal disulfide
Internal disulfide
[θ] mdeg cm²dmol⁻¹
0
-5
-10
-15
-20
25
35
45
55
65
75
85
95
Temperature (°C)

FIG. 18D

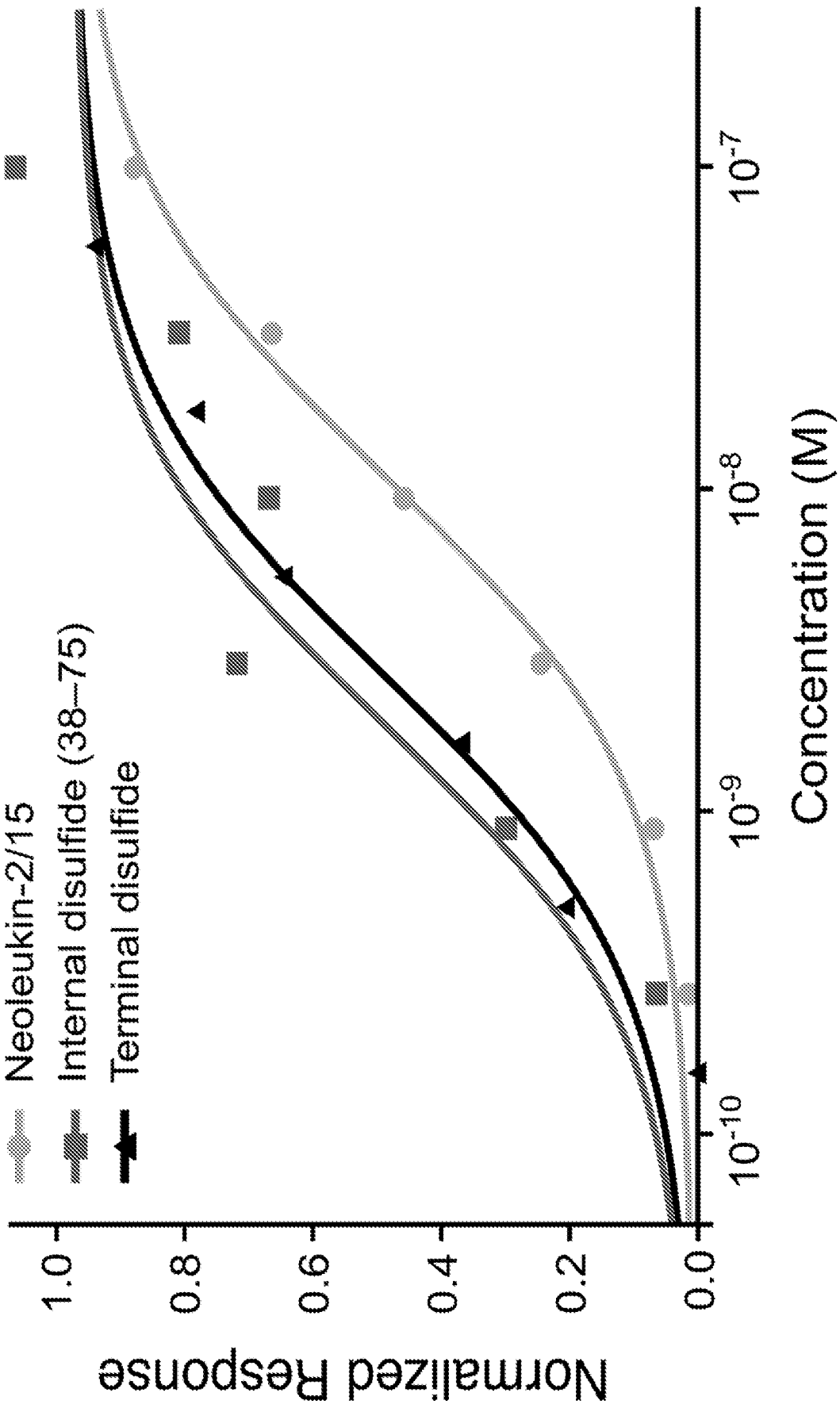

Neoleukin-2/15
Internal disulfide (38–75)
Terminal disulfide
Normalized Response
1.0
0.8
0.6
0.4
0.2
0.0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Concentration (M)

FIG. 19A

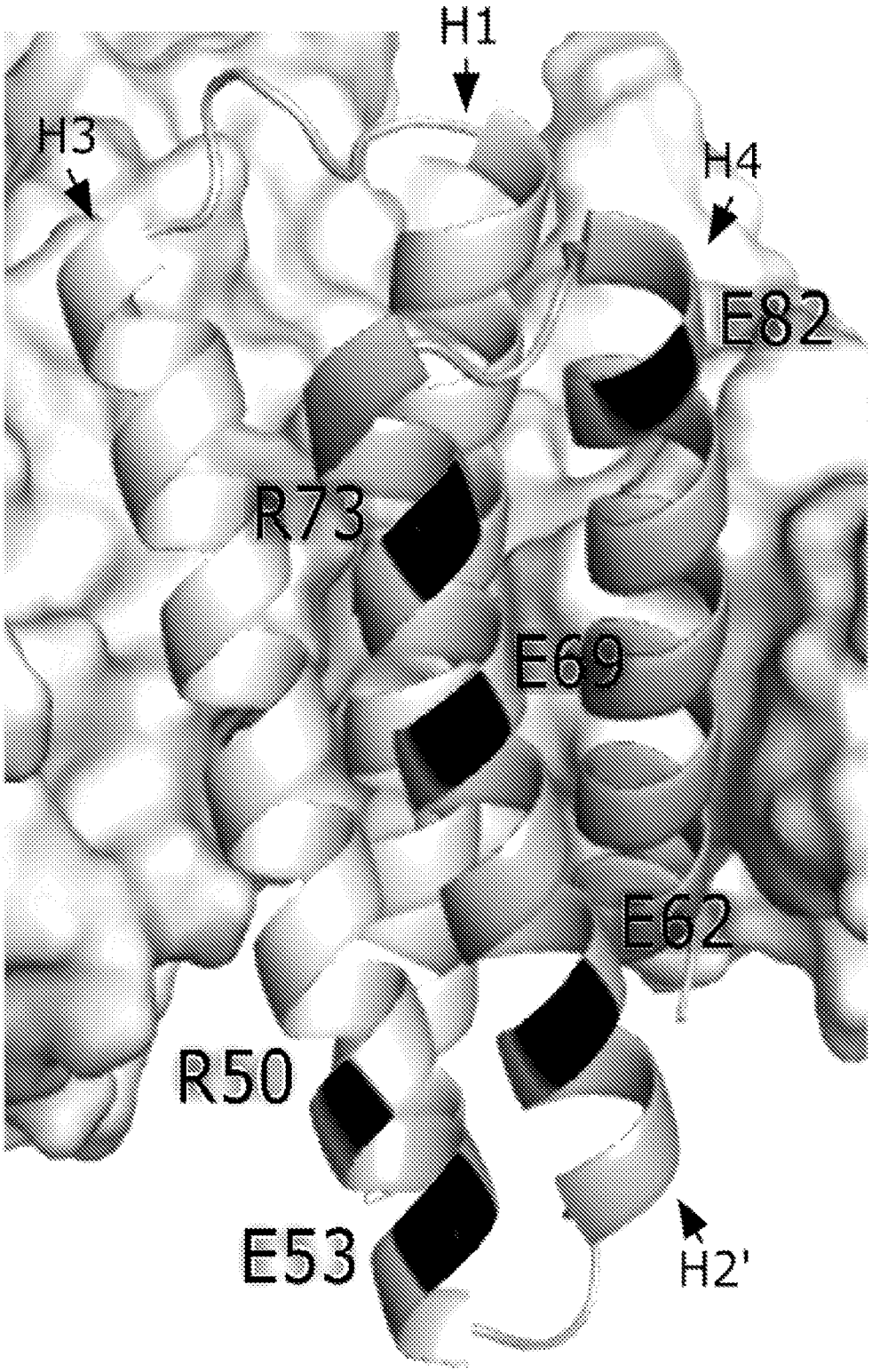


FIG. 19B

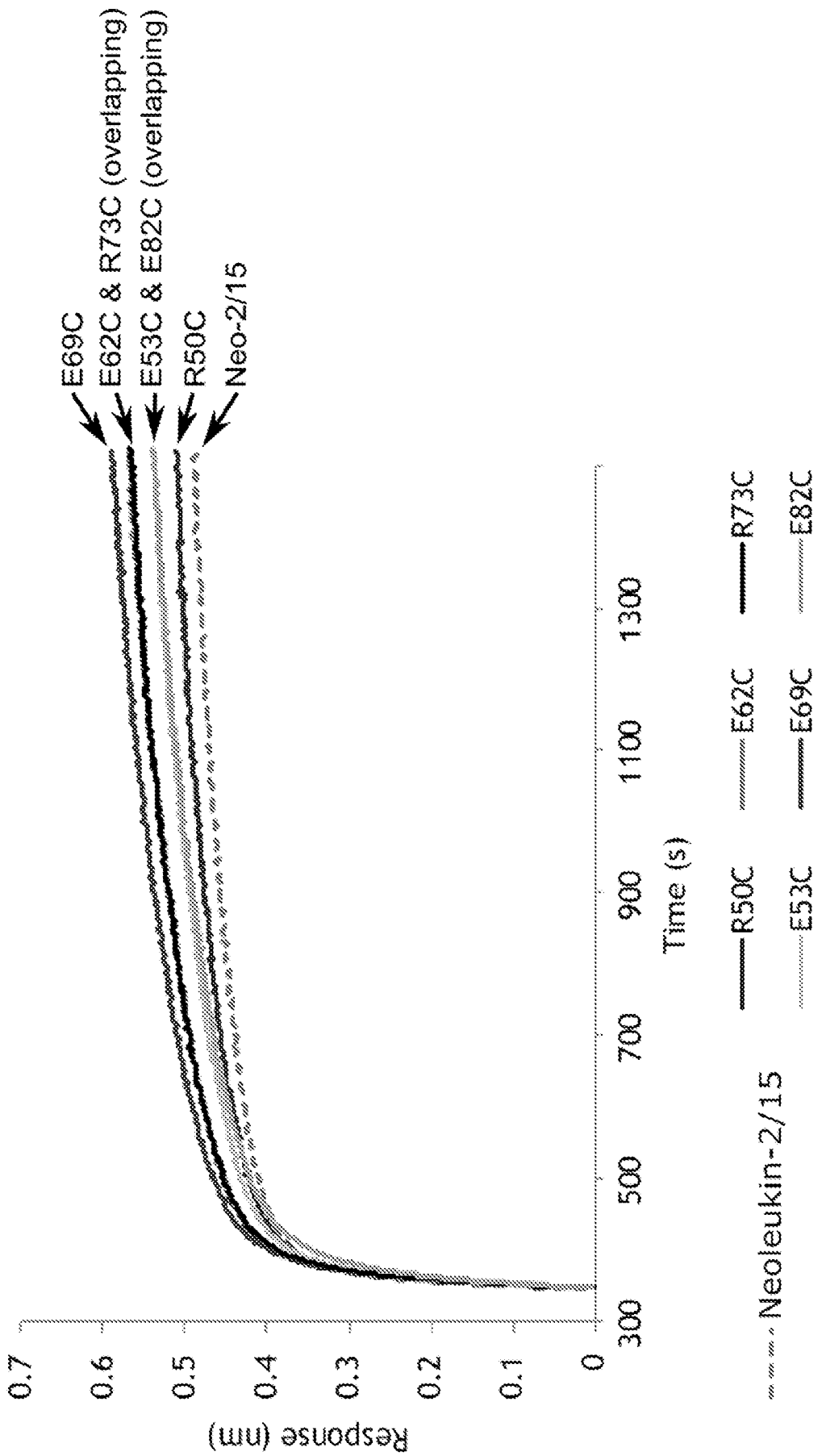

E69C
E62C & R73C (overlapping)
E53C & E82C (overlapping)
R50C
Neo-2/15
Response (nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
300
500
700
900
1100
1300
Time (s)
Neoleukin-2/15
R50C
E62C
R73C
E53C
E69C
E82C

FIG. 20A

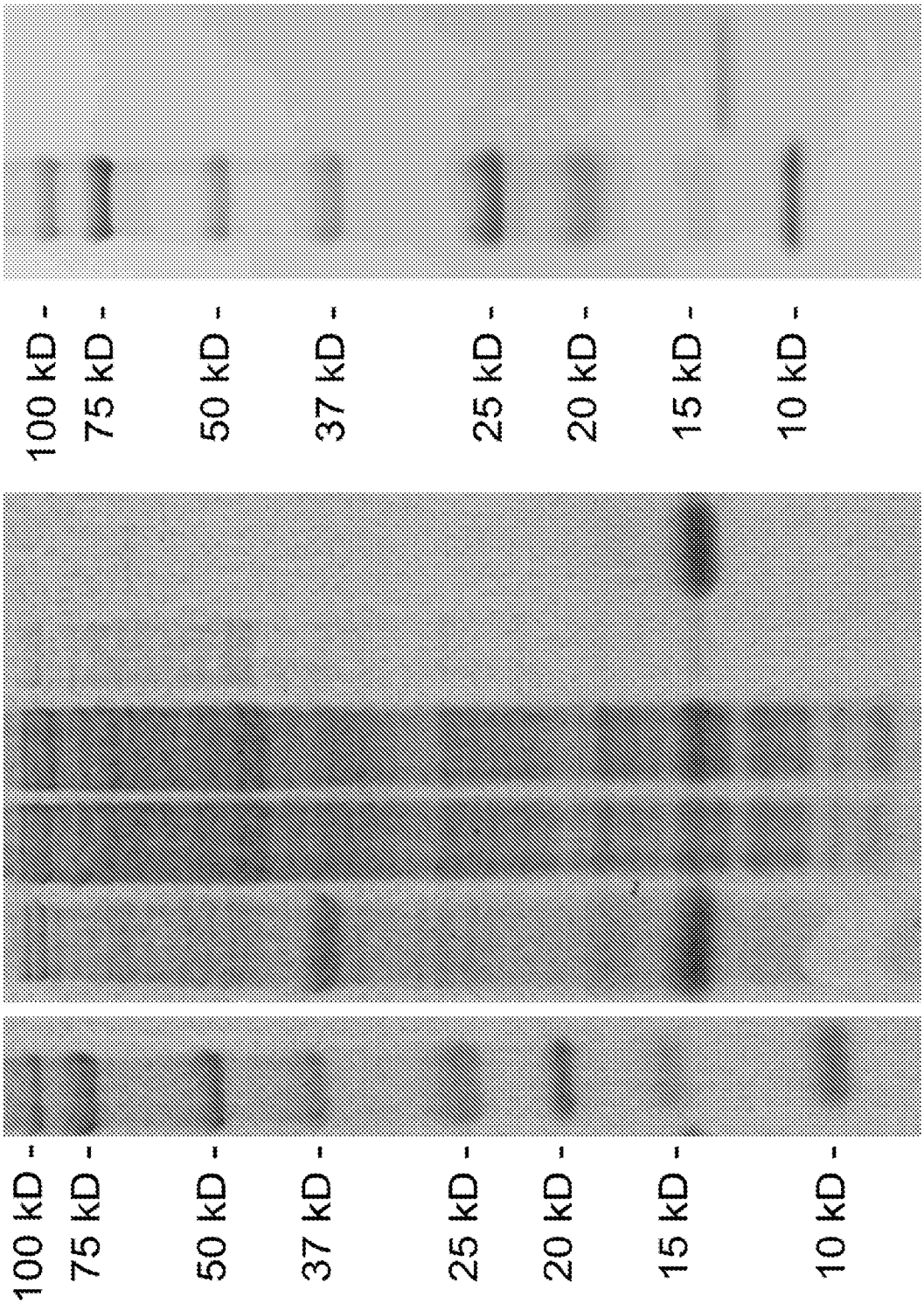

Ladder
Insoluble
Soluble
Flowthrough
Wash
Elution
Ladder
Neo-4
100 kD -
75 kD -
50 kD -
37 kD -
25 kD -
20 kD -
15 kD -
10 kD -

FIG. 20B

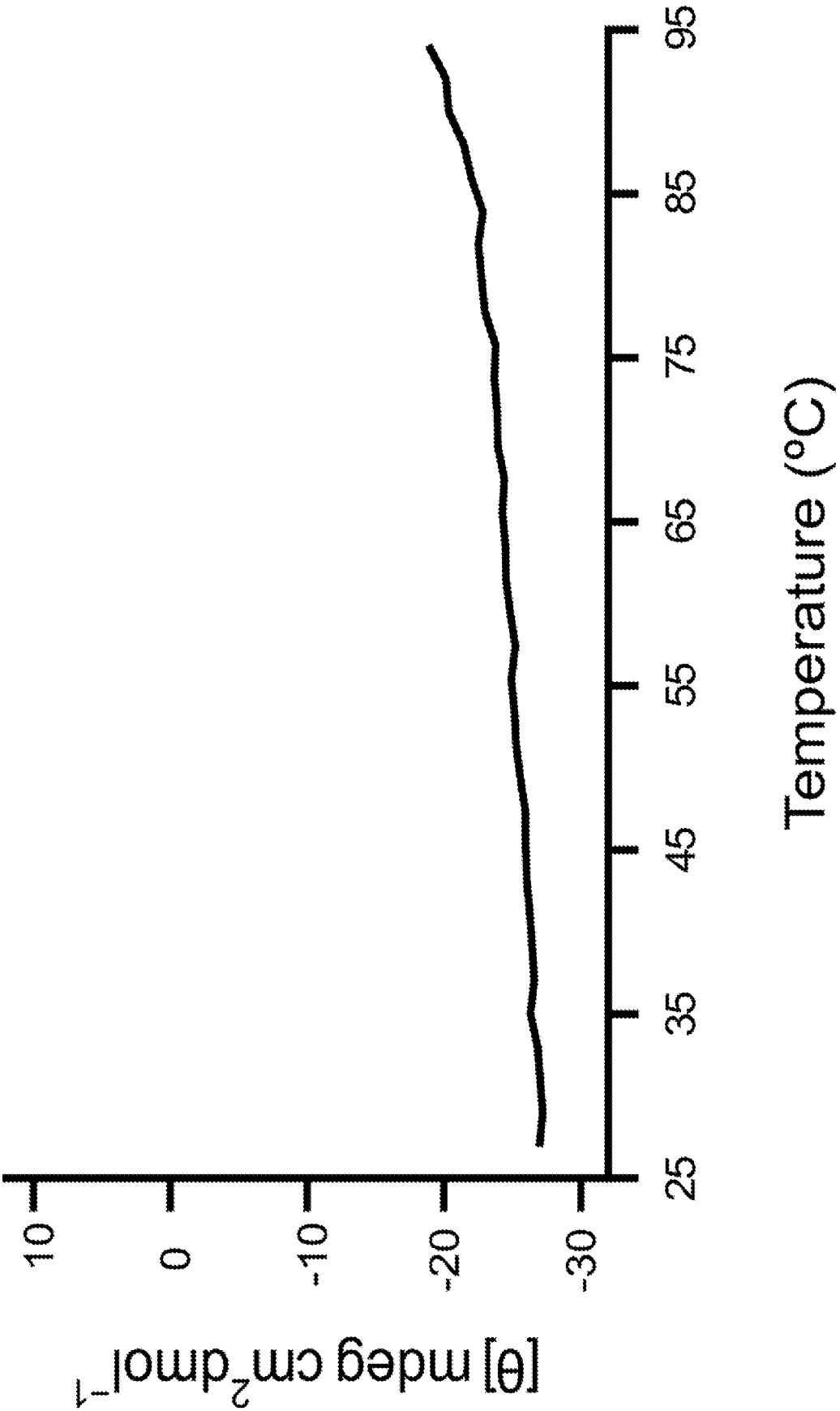

[θ] mdeg cm²dmol⁻¹
10
0
-10
-20
-30
25
35
45
55
65
75
85
95
Temperature (°C)

FIG. 20C

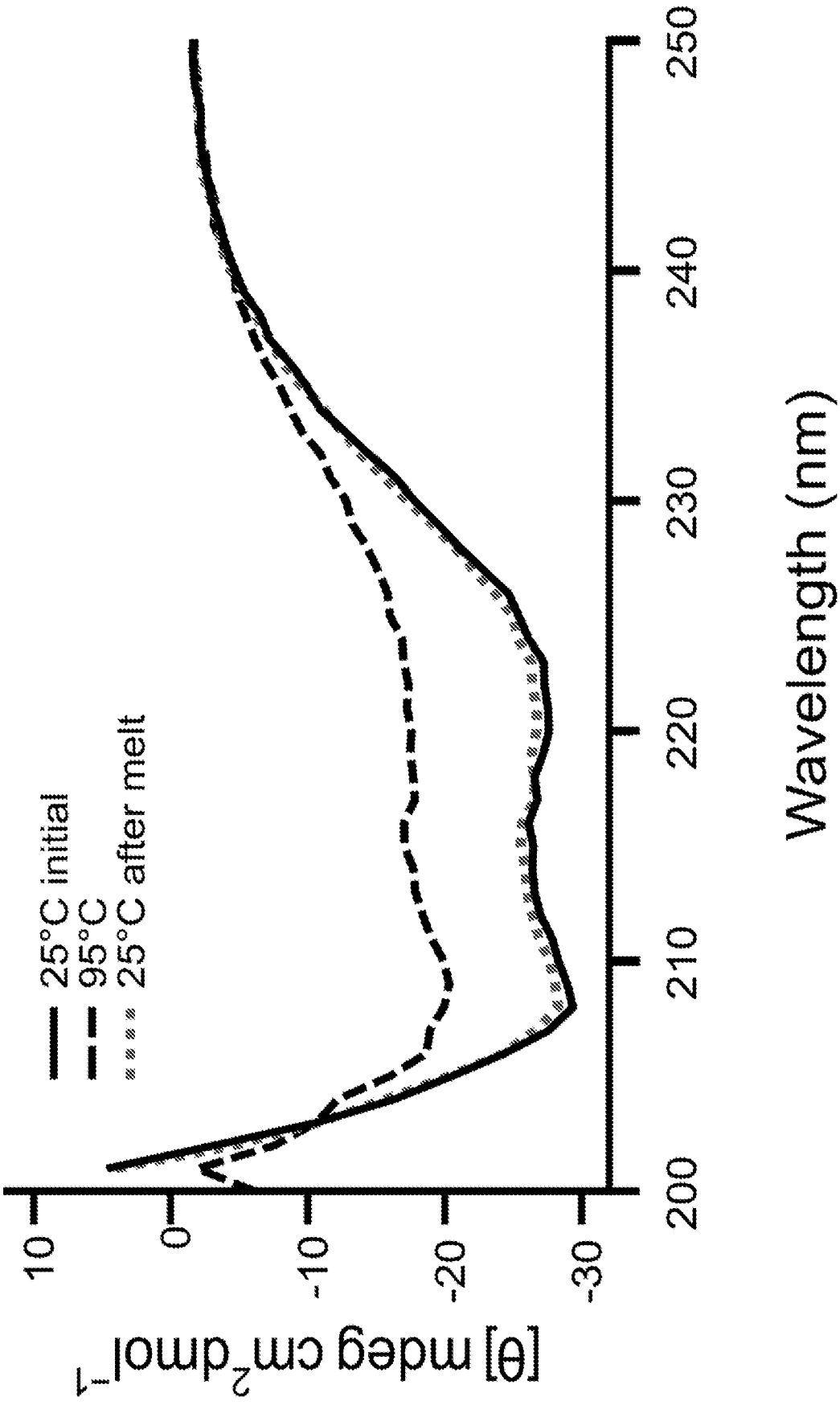

25°C initial
95°C
25°C after melt
[θ] mdeg cm²dmol⁻¹
10
0
-10
-20
-30
200
210
220
230
240
250
Wavelength (nm)

FIG. 21A

IL-10; Neo-2/15

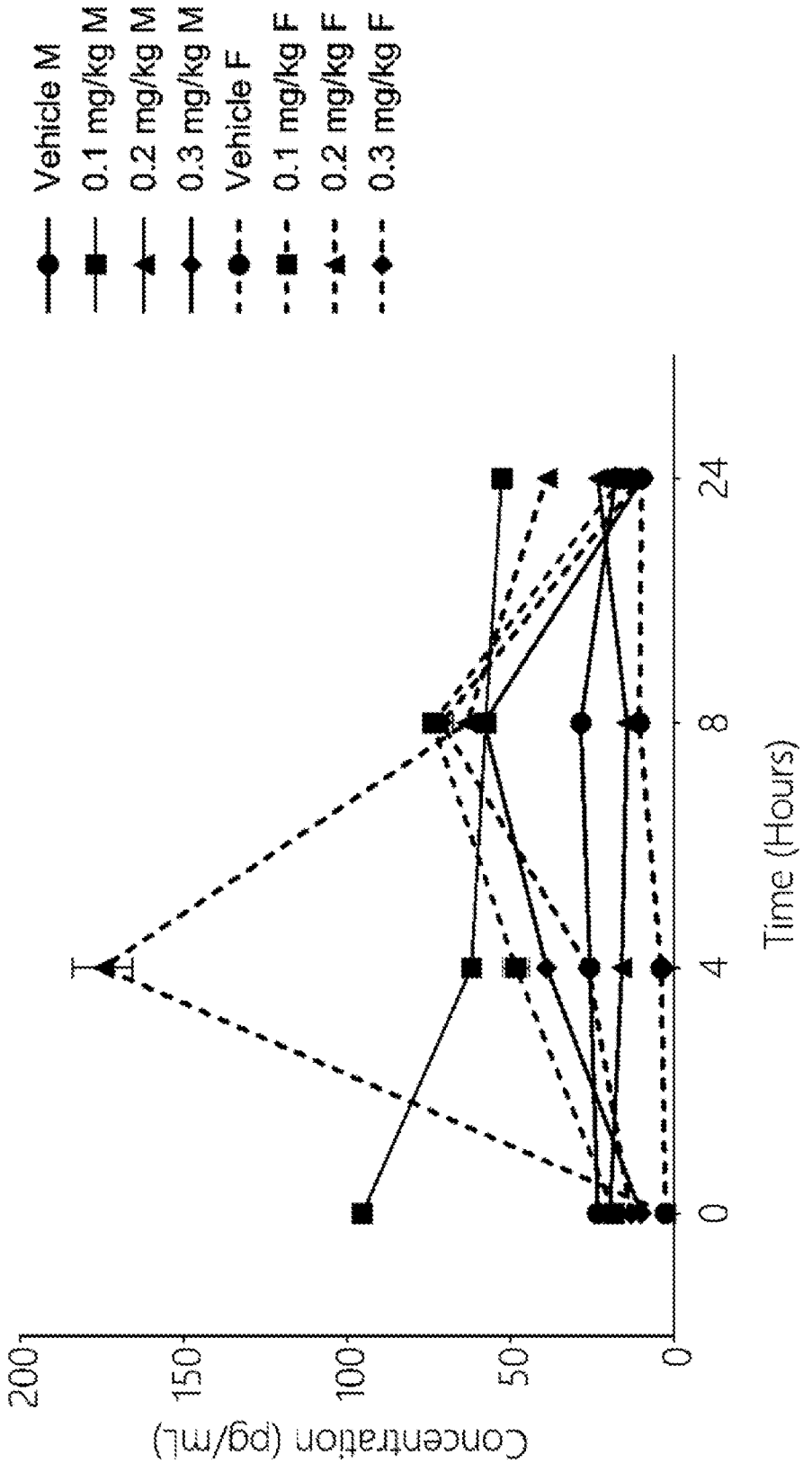


FIG. 21B

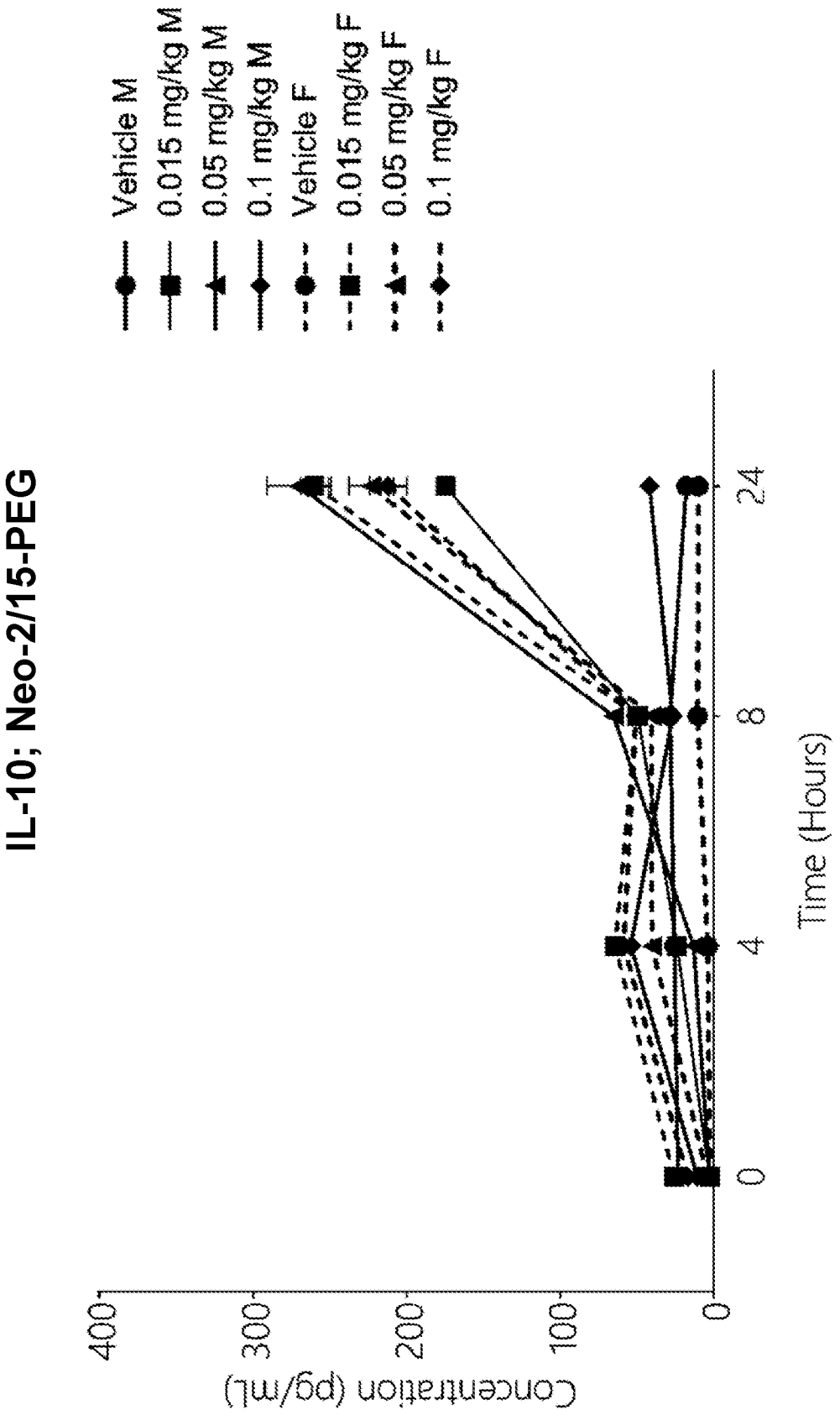

IL-10; Neo-2/15-PEG
Vehicle M
0.015 mg/kg M
0.05 mg/kg M
0.1 mg/kg M
Vehicle F
0.015 mg/kg F
0.05 mg/kg F
0.1 mg/kg F
Concentration (pg/mL)
400
300
200
100
0
0
4
8
24
Time (Hours)

FIG. 21C

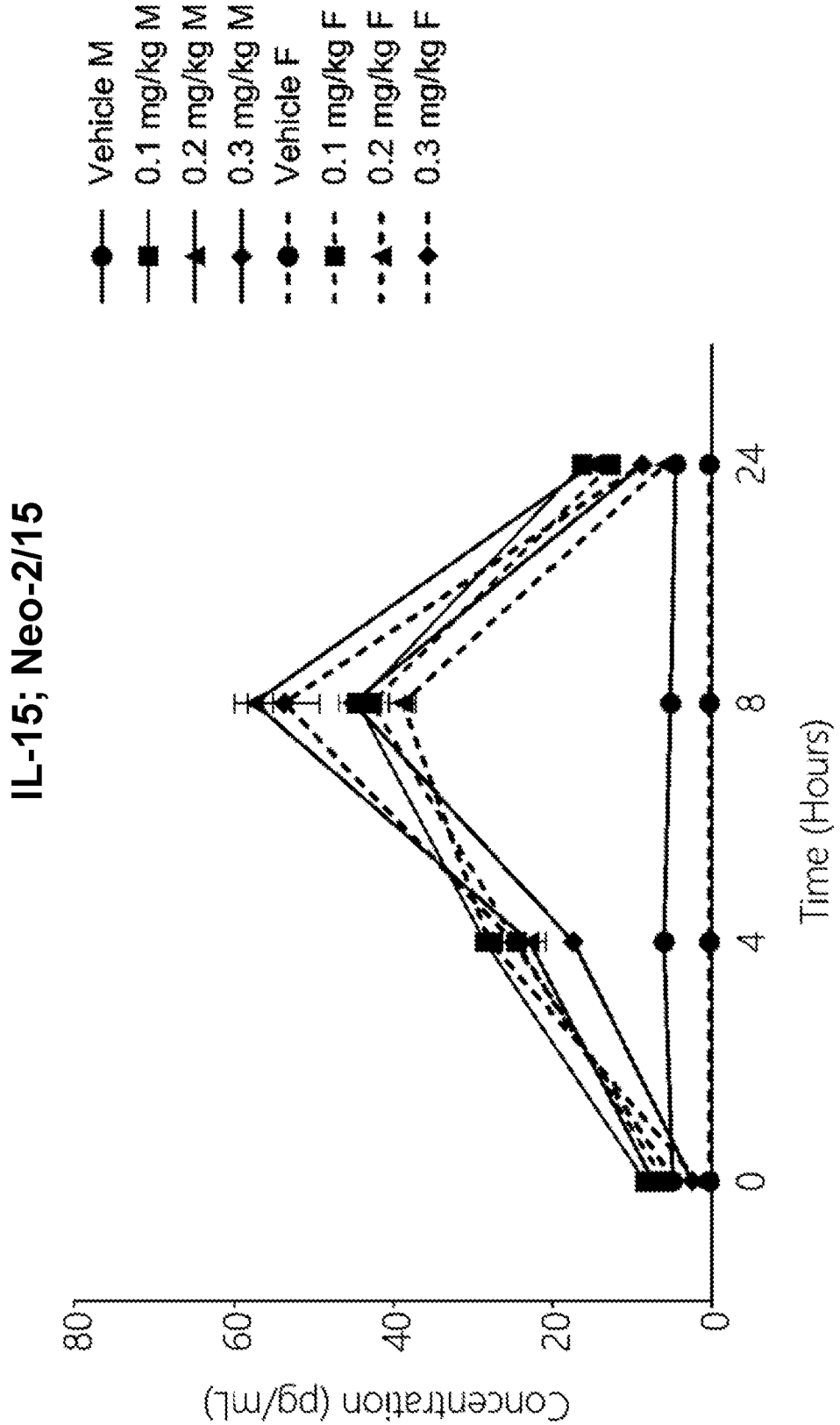
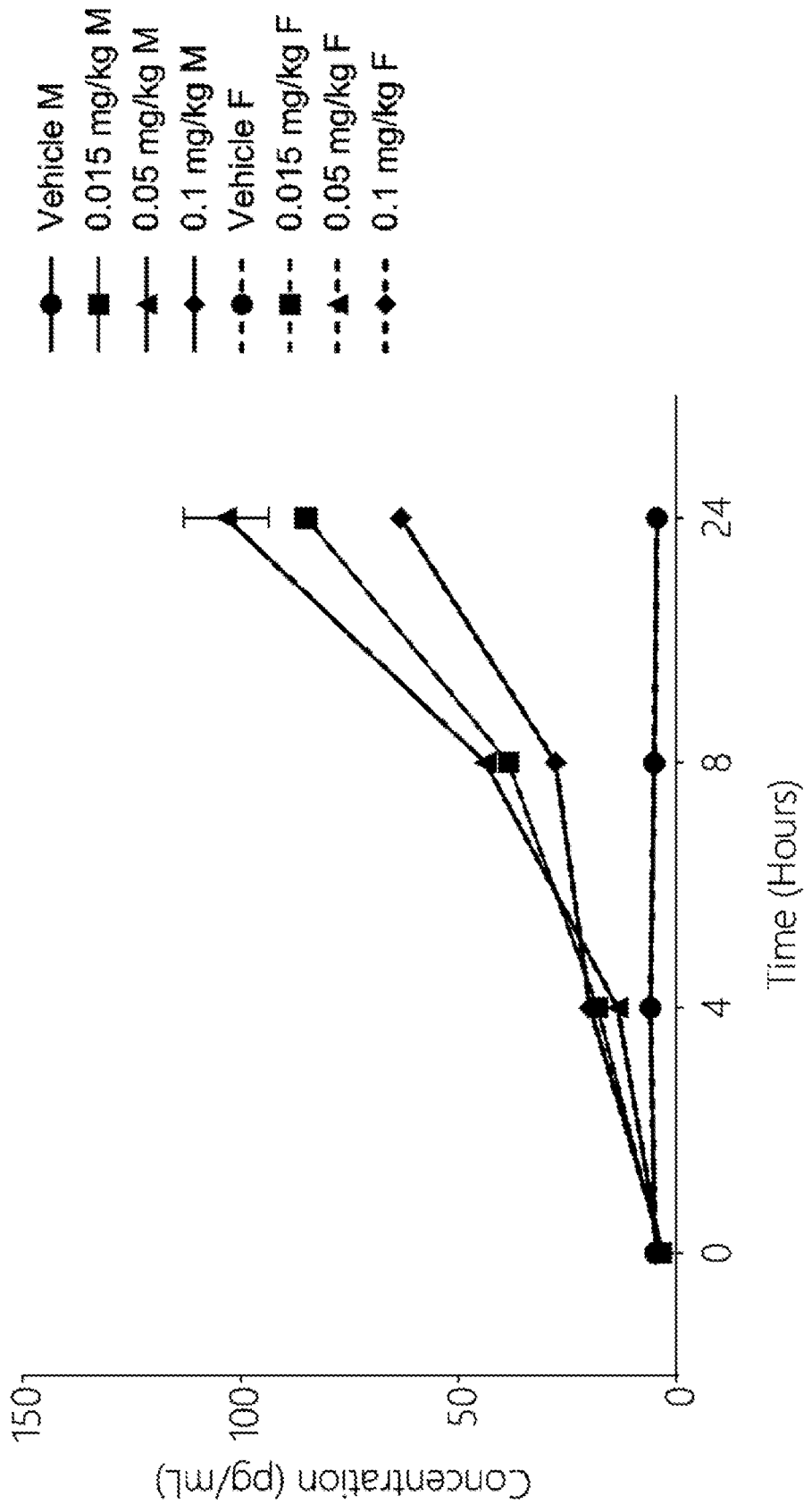

IL-15; Neo-2/15
Vehicle M
0.1 mg/kg M
0.2 mg/kg M
0.3 mg/kg M
Vehicle F
0.1 mg/kg F
0.2 mg/kg F
0.3 mg/kg F
Concentration (pg/mL)
0
20
40
60
80
0
4
8
24
Time (Hours)

FIG. 21D

IL-15; Neo-2/15-PEG



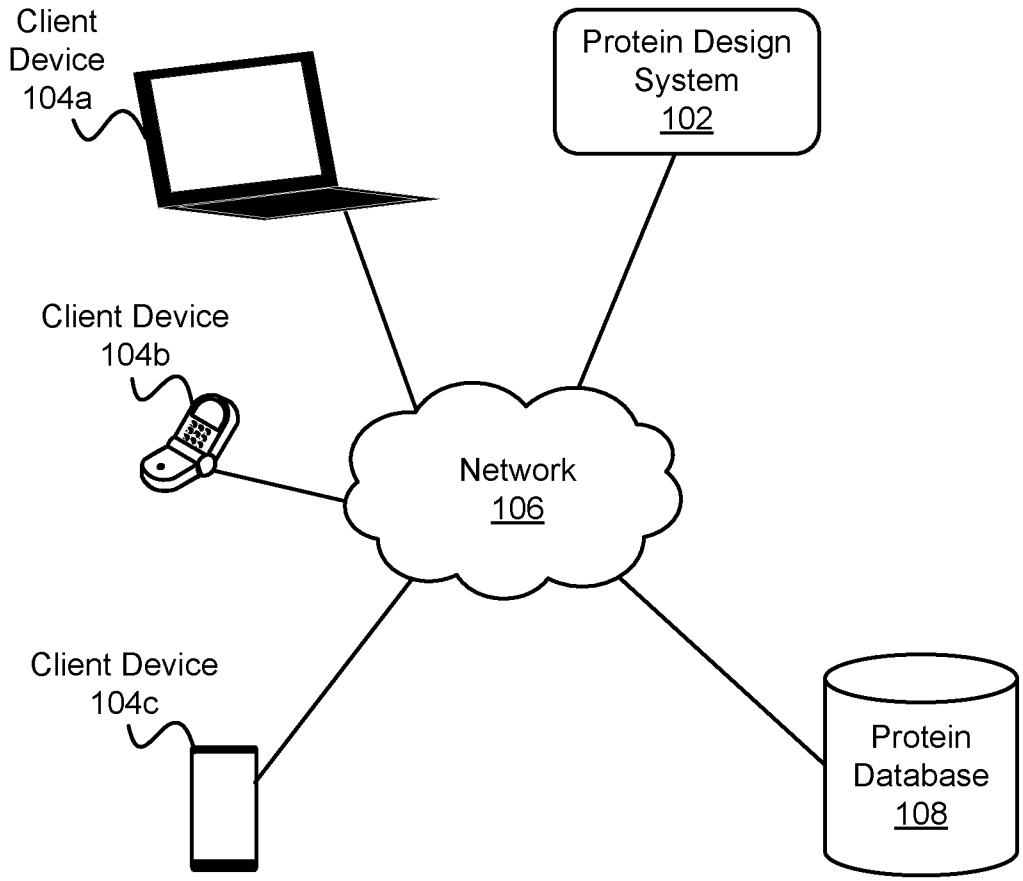

Client
Device
104a
Protein Design
System
102
Client Device
104b
Network
106
Client Device
104c
Protein
Database
108

FIG. 22

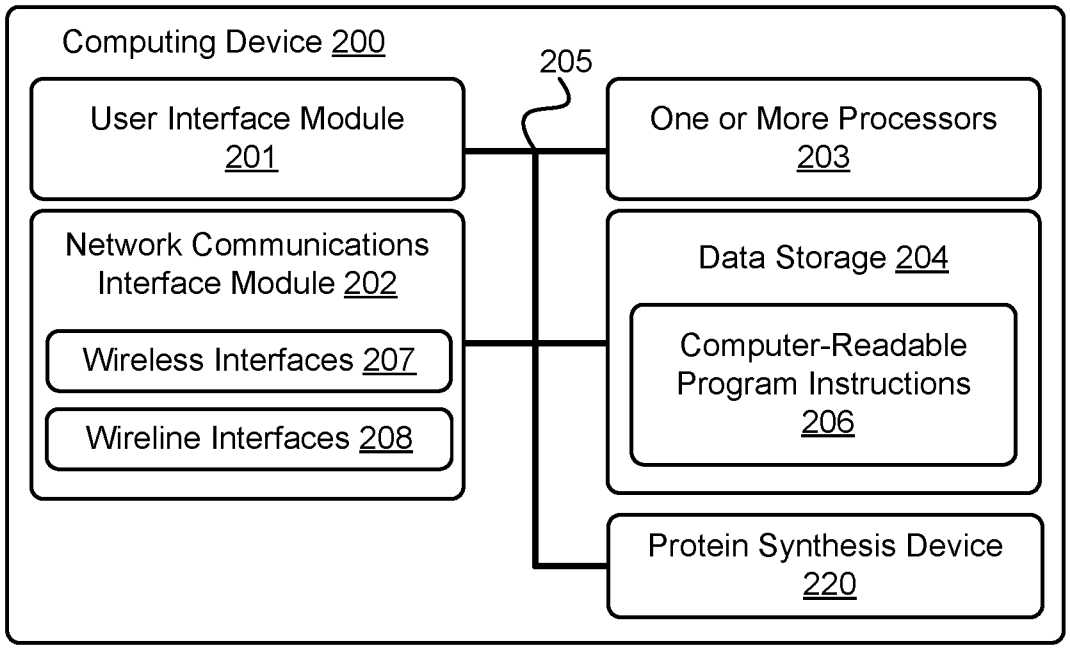


FIG. 23A

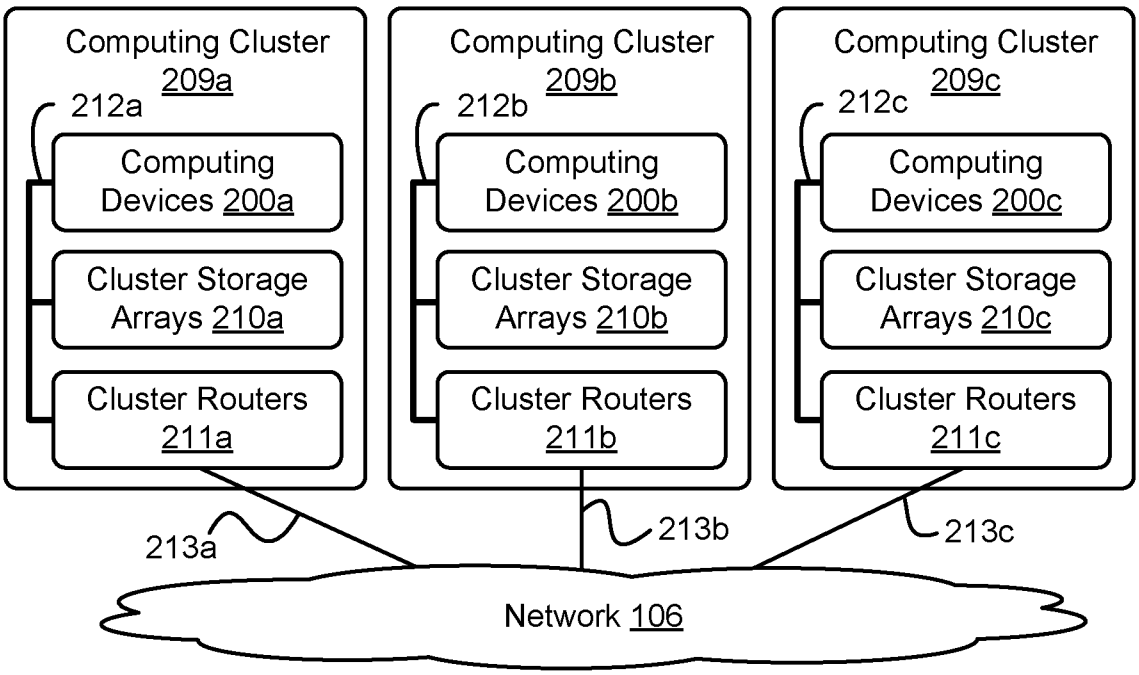


FIG. 23B

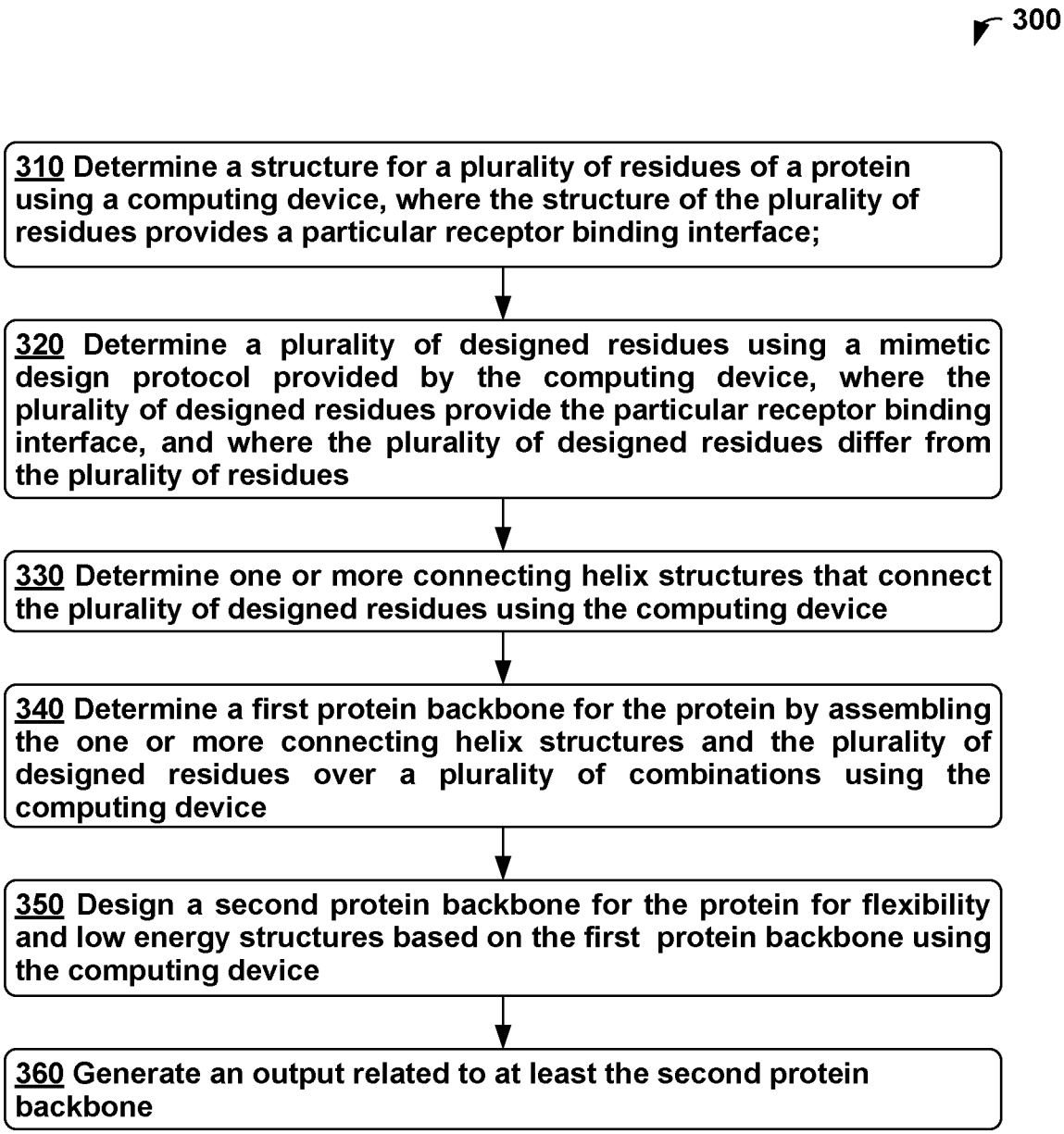

300
310 Determine a structure for a plurality of residues of a protein using a computing device, where the structure of the plurality of residues provides a particular receptor binding interface;
320 Determine a plurality of designed residues using a mimetic design protocol provided by the computing device, where the plurality of designed residues provide the particular receptor binding interface, and where the plurality of designed residues differ from the plurality of residues
330 Determine one or more connecting helix structures that connect the plurality of designed residues using the computing device
340 Determine a first protein backbone for the protein by assembling the one or more connecting helix structures and the plurality of designed residues over a plurality of combinations using the computing device
350 Design a second protein backbone for the protein for flexibility and low energy structures based on the first protein backbone using the computing device
360 Generate an output related to at least the second protein backbone

FIG. 24