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De novo design of potent and selective mimics of IL-2 and IL-15

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- *Extended acknowledgments*

Supplemental figures

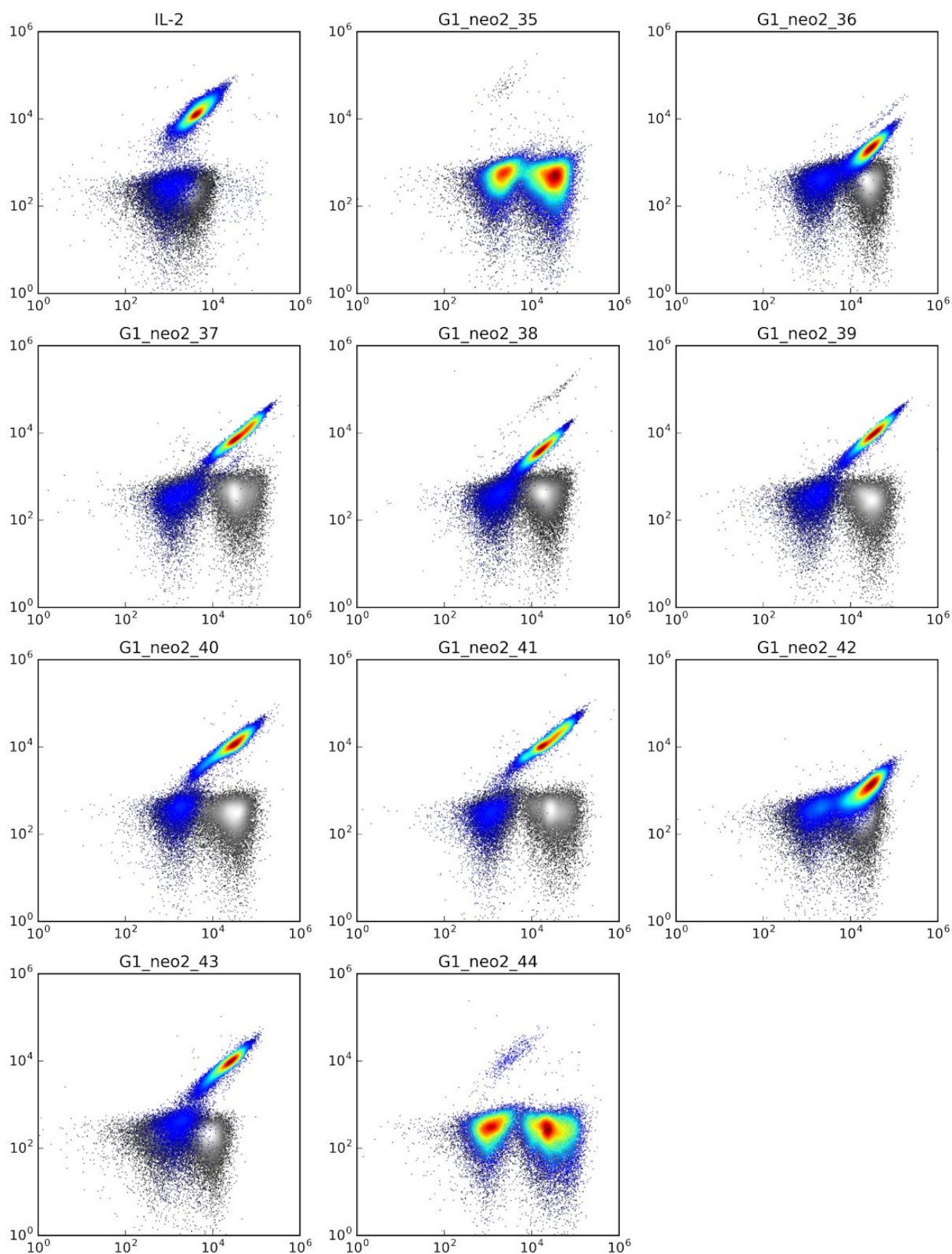


Figure S1. Yeast display for human IL-2 mimetic de novo designs generation-1. Flow cytometry screening of designed first-round protein binders. Designs were expressed on yeast and incubated with 2 nM of heterodimeric 1 human IL-2R $\beta\gamma_c$ receptor (colored) or no receptor (gray) (see Main Text Methods). The experiments for proteins exhibiting binding were performed twice with similar results.

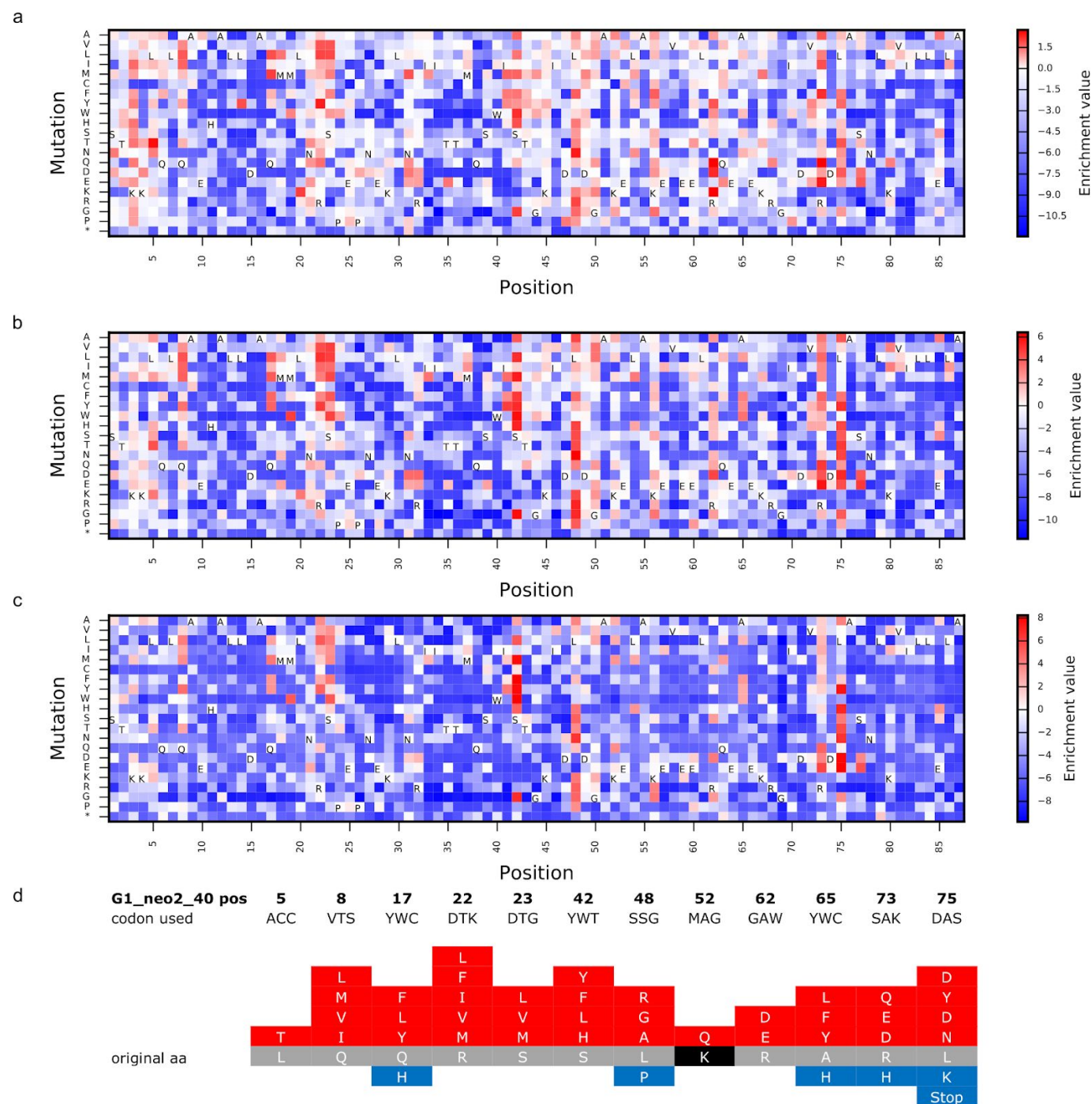


Figure S2. Experimental optimization of G1_neo2_40. a-c) Heatmaps for G1_neo2_40 site saturation mutagenesis library² showing enrichment at specific positions after consecutive rounds of selection with increasingly stringent conditions for binding human IL-2R $\beta\gamma_c$ heterodimeric receptor, at: **a)** 50 nM, **b)** 2 nM, and **c)** 0.2 nM. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 1.5×10^6 . **d)** Amino acid residues available in the initial combinatorial library are depicted with red indicating residues predicted to be advantageous and blue deleterious; black indicates residues in the starting sequence represented in the combinatorial library and gray, residues in the starting sequence not represented in the combinatorial library. The experiments were performed once.

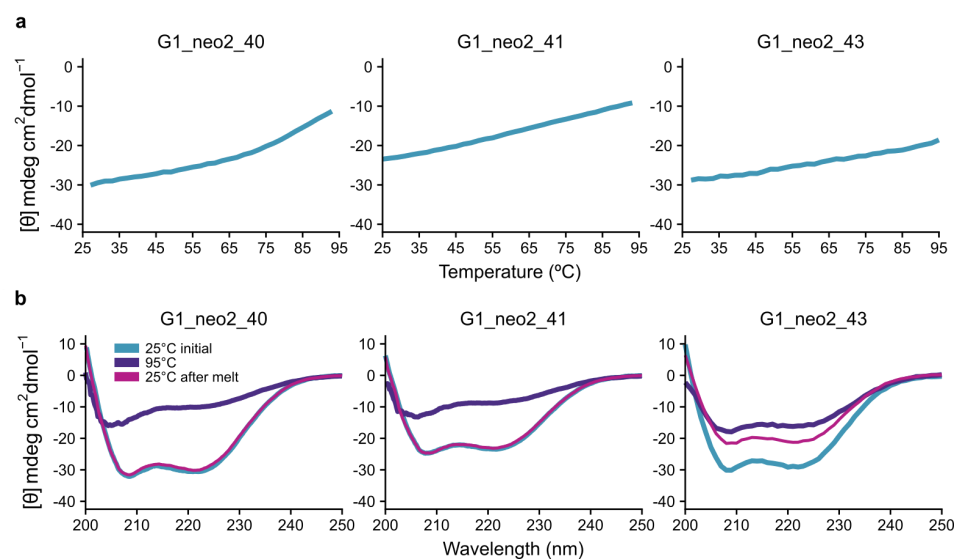


Figure S3. Circular Dichroism (CD) thermal denaturation experiments for multiple IL-2/IL-15 de novo designed mimetics, generation-1.
a) Thermal denaturation curves, and b) wavelength scans (222 nm). The experiments were performed once.

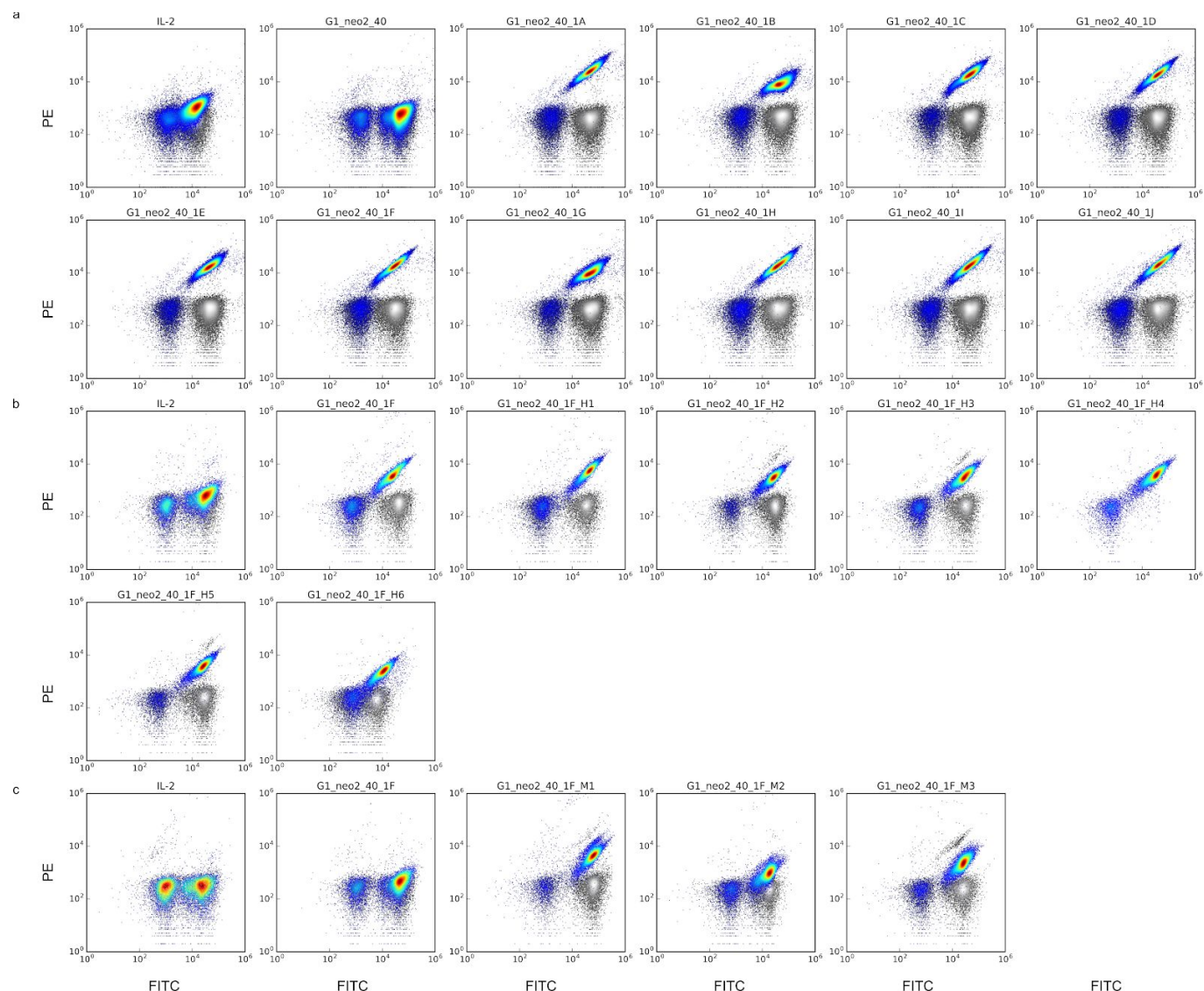


Figure S4. Yeast display for human IL-2/IL-15 mimetic de novo designs, generation-1 experimentally optimized. Flow cytometry screening of designed experimentally optimized first-round protein binders. Designs indicated were expressed on yeast and incubated with 2 nM of heterodimeric ¹ fluorescently labeled human IL-2Rβγ_c (a and b) or mouse IL-2Rβγ_c (c), both denoted in color, or no receptor in gray (see Main Text Methods). The experiments were performed once.

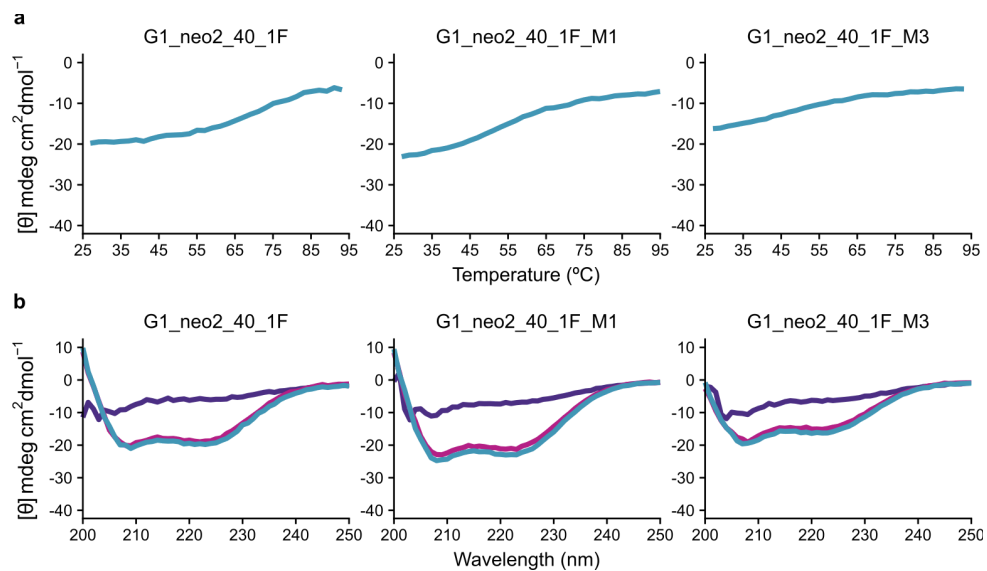


Figure S5. Circular Dichroism (CD) thermal denaturation experiments for multiple IL-2/IL-15 de novo designed mimetics, generation-1 experimentally optimized. a) Thermal denaturation curves, and b) wavelength scans (222 nm). The experiments were performed 2 times with similar results.

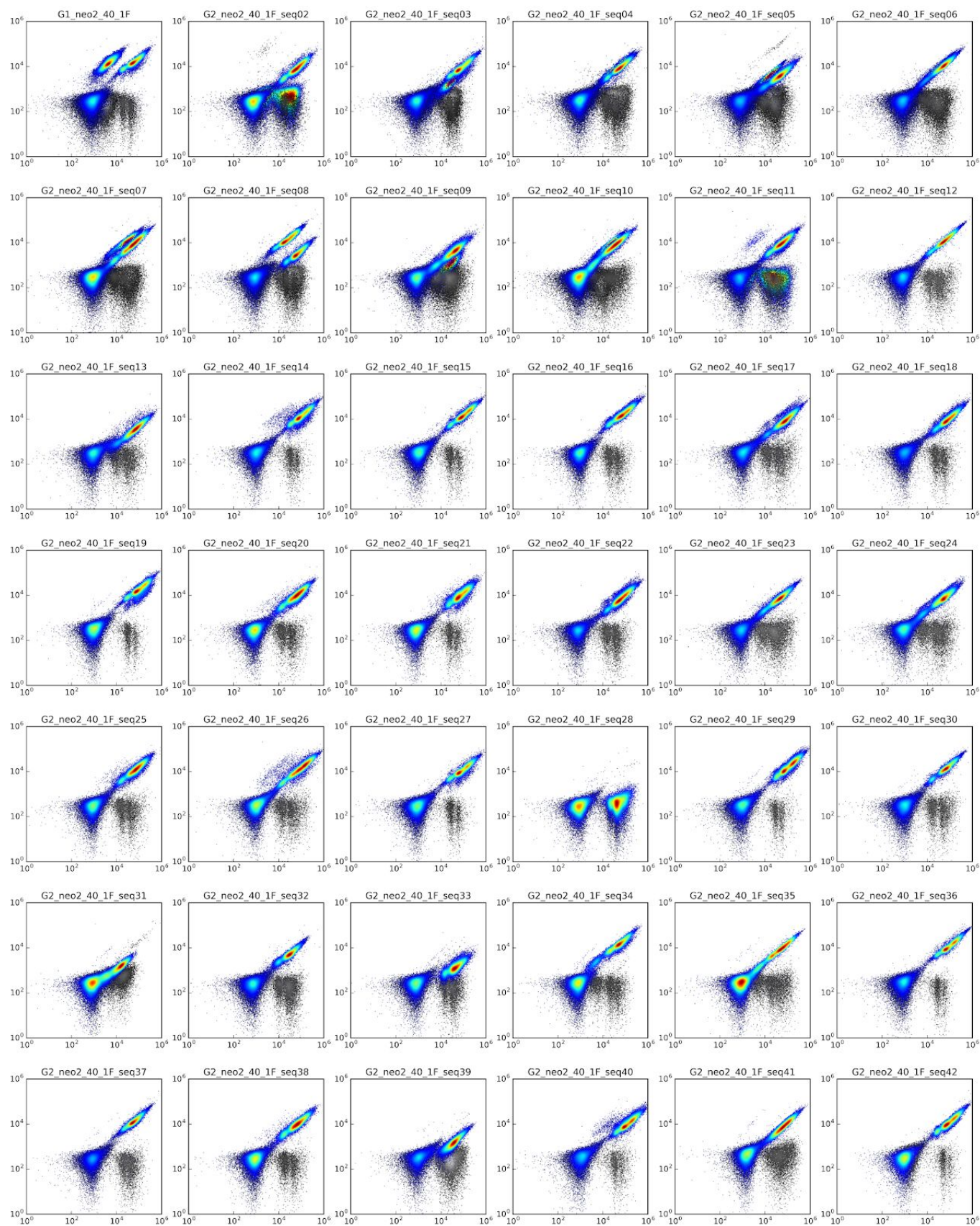


Figure S6. Yeast display for IL-2/15 mimetic de novo designs generation-2. Flow cytometry screening of designed second-round protein binders. Designs indicated were expressed on yeast and incubated with 2 nM of heterodimeric γ_c human IL-2R β receptor (colored) or no receptor (gray) (see Main Text Methods). The experiments were performed 2 times with similar results.

Figure S7. Circular dichroism thermal melts for IL-2/IL-15 mimetic designs generation-2. *a) Thermal denaturation curves and b) wavelength scans.* The experiments were performed once.

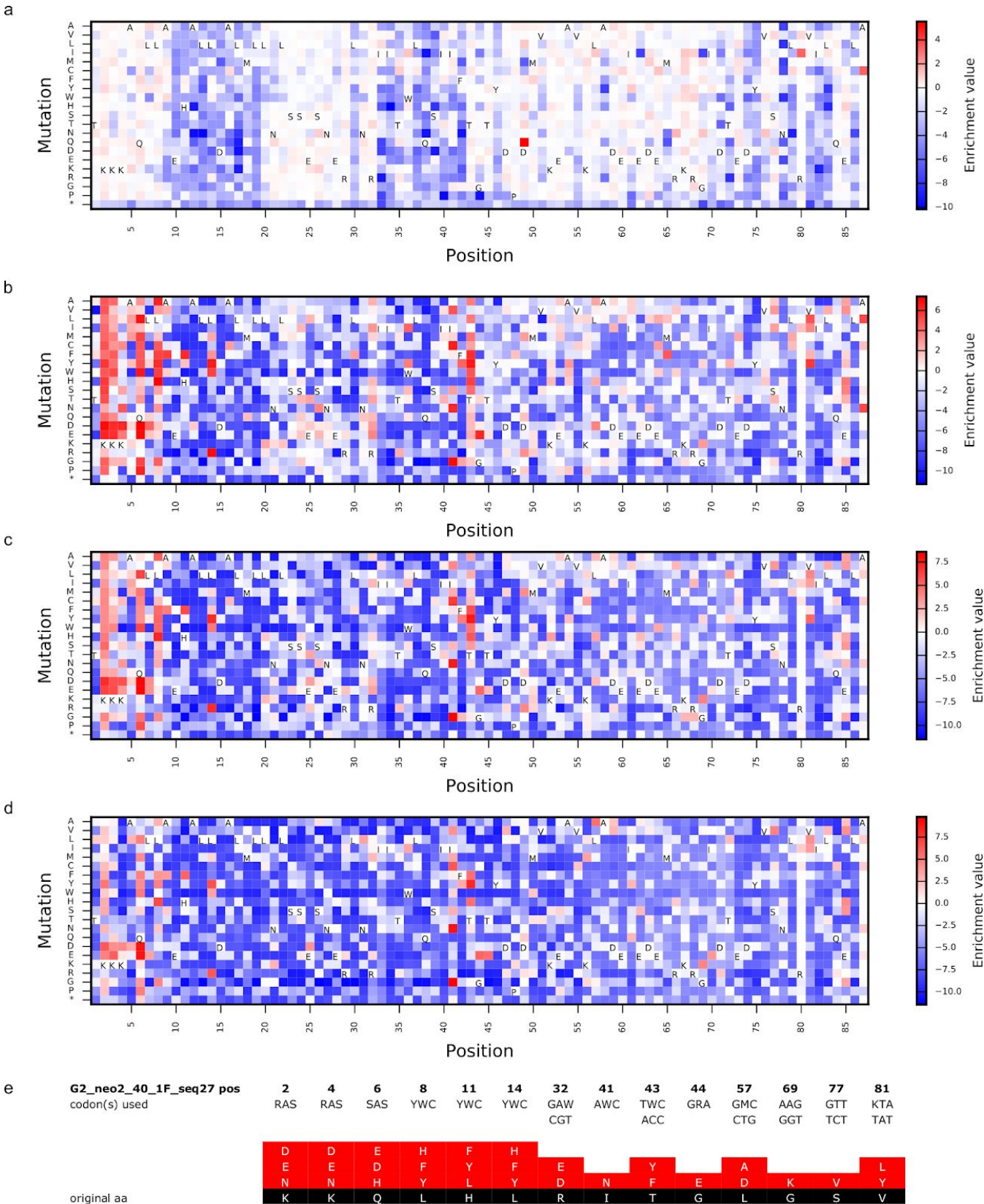


Figure S8. Experimental optimization of G2_neo2_40_1F_seq27. Heatmaps for G2_neo2_40_1F_seq27 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of selection with increasingly stringent conditions for binding mouse IL-2R β γ_c heterodimeric receptor, at: **a**) 10 nM, **b**) 1 nM, **c**) 0.1 nM, and once more at **d**) 0.1 nM mouse IL-2R β γ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 5.3×10^6 ; **e**) Amino acid residues available in the initial combinatorial library are depicted with red indicating residues predicted to be advantageous; black indicates residues in the starting sequence represented in the combinatorial library. The experiments were performed once.

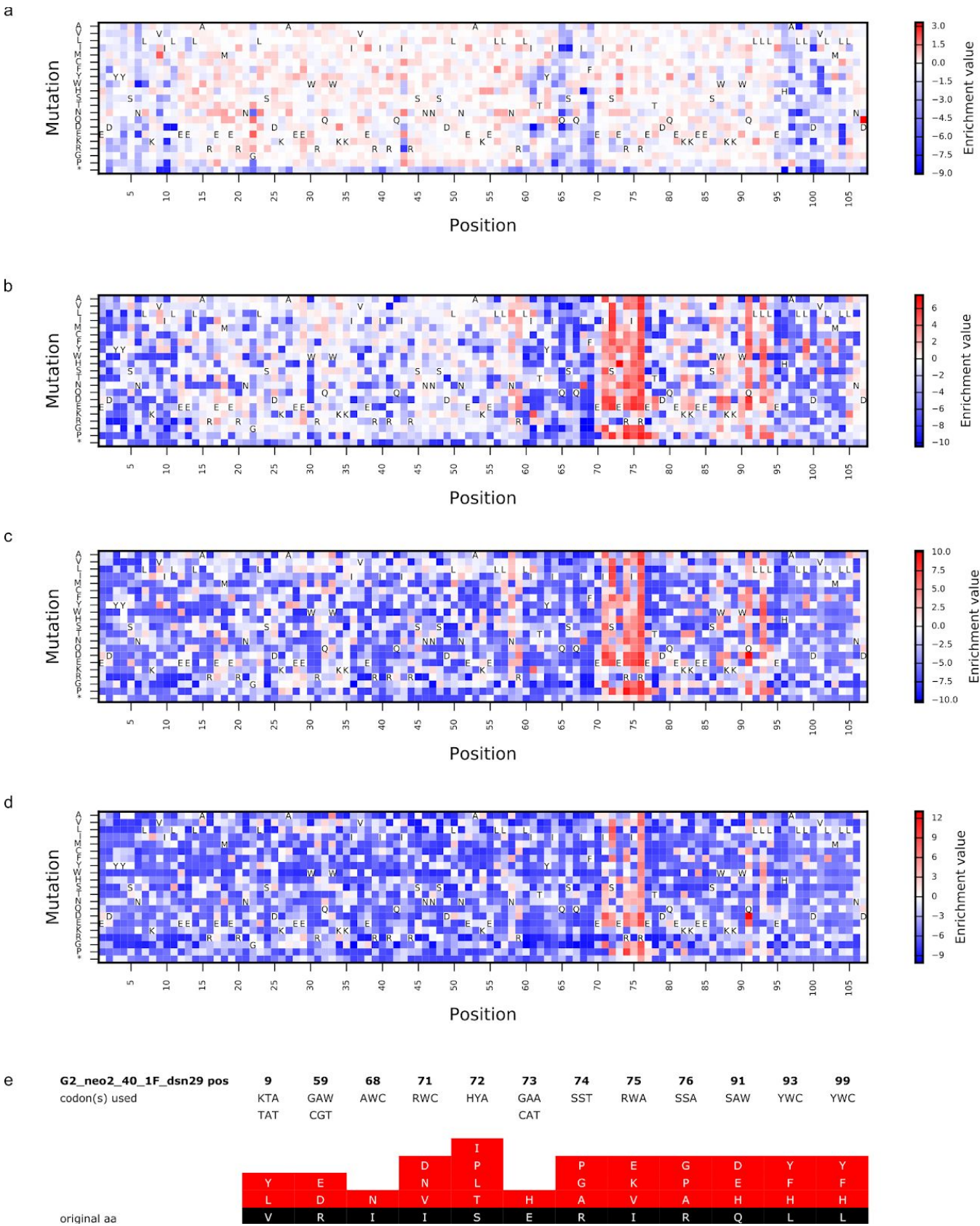


Figure S9. Experimental optimization of G2_neo2_40_1F_seq29. Heatmaps for G2_neo2_40_1F_seq29 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of selection with increasingly stringent conditions for binding mouse IL-2Rβγ_c heterodimeric receptor, at: **a**) 10 nM, **b**) 1 nM, **c**) 0.1 nM, and once more at **d**) 0.1 nM mouse IL-2Rβγ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 2.9×10^6 ; **e**) Amino acid residues available in the initial combinatorial library are depicted with red indicating residues predicted to be advantageous; black indicates residues in the starting sequence represented in the combinatorial library. The experiments were performed once.

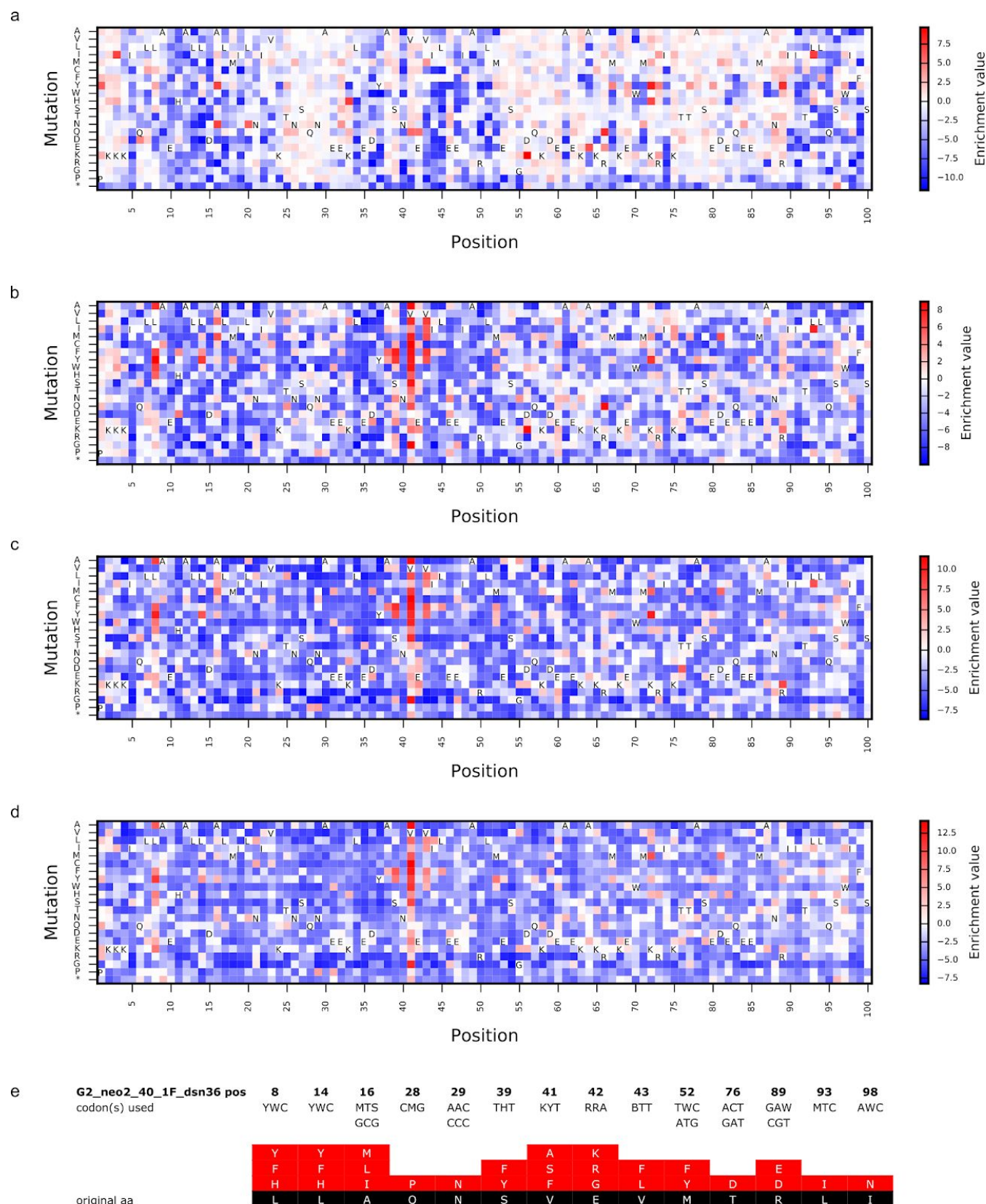


Figure S10. Experimental optimization of G2_neo2_40_1F_seq36. Heatmaps for G2_neo2_40_1F_seq36 single-site mutagenesis library showing enrichment at specific positions after consecutive rounds of selection with increasingly stringent conditions for binding mouse IL-2Rβγ_c heterodimeric receptor, at: **a**) 10 nM, **b**) 1 nM, **c**) 0.1 nM, and once more at **d**) 0.1 nM mouse IL-2Rβγ_c heterodimer. Based on these enrichment data, a combinatorial library was designed with nucleotide diversity 2.7×10^6 ; **e**) Amino acid residues available in the initial combinatorial library are depicted with red indicating residues predicted to be advantageous and blue deleterious; black indicates residues in the starting sequence represented in the combinatorial library. The experiments were performed once.

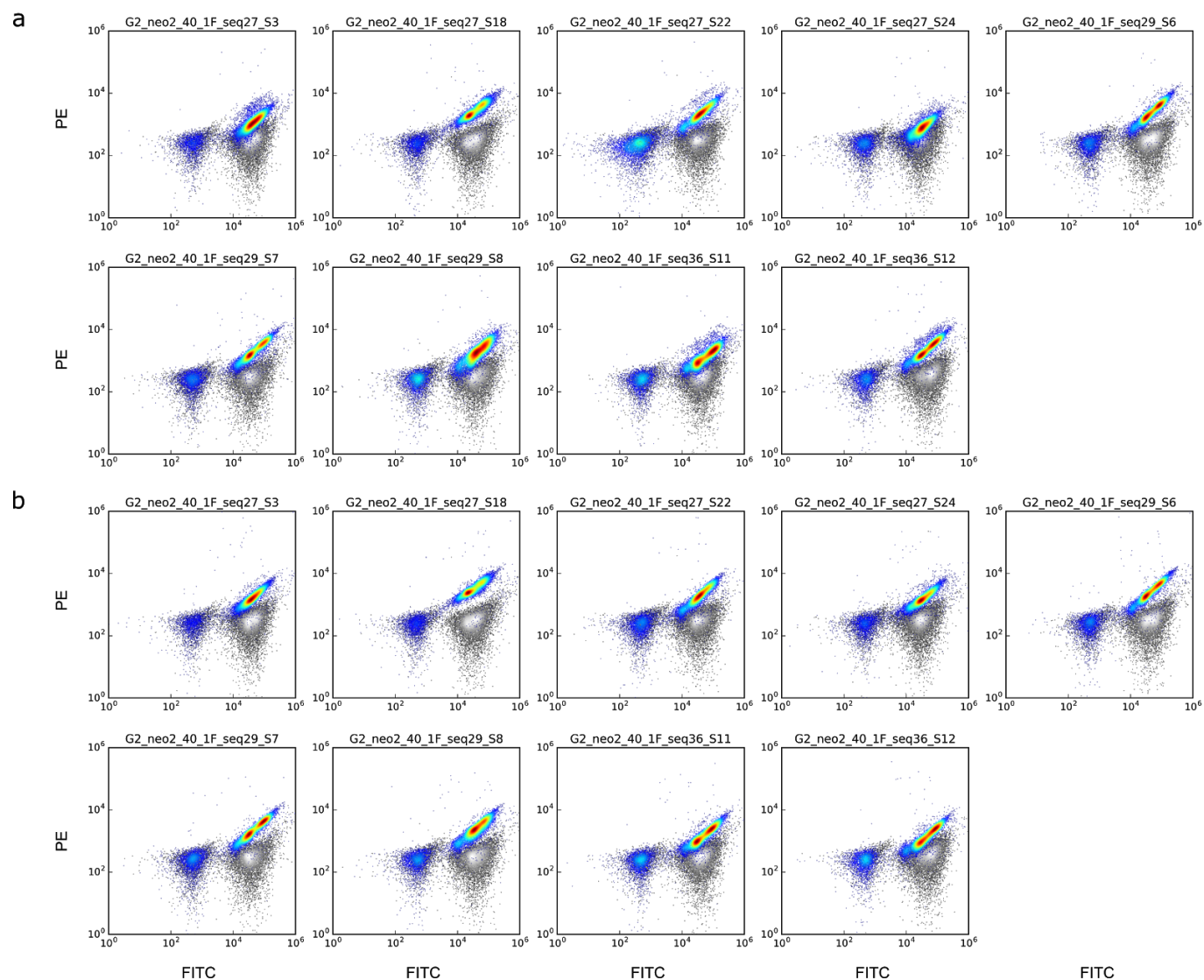


Figure S11. Yeast display for de novo IL-2/IL-15 mimetic designs generation-2, experimentally optimized. Flow cytometry screening of designed experimentally optimized second-round protein binders. Designs indicated were expressed on yeast and incubated with 0.5 nM of heterodimeric γ_c human IL-2R β (a) or mouse IL-2R β (b) receptor, both denoted in color, or no receptor in gray (see Main Text Methods). The experiments were performed once.

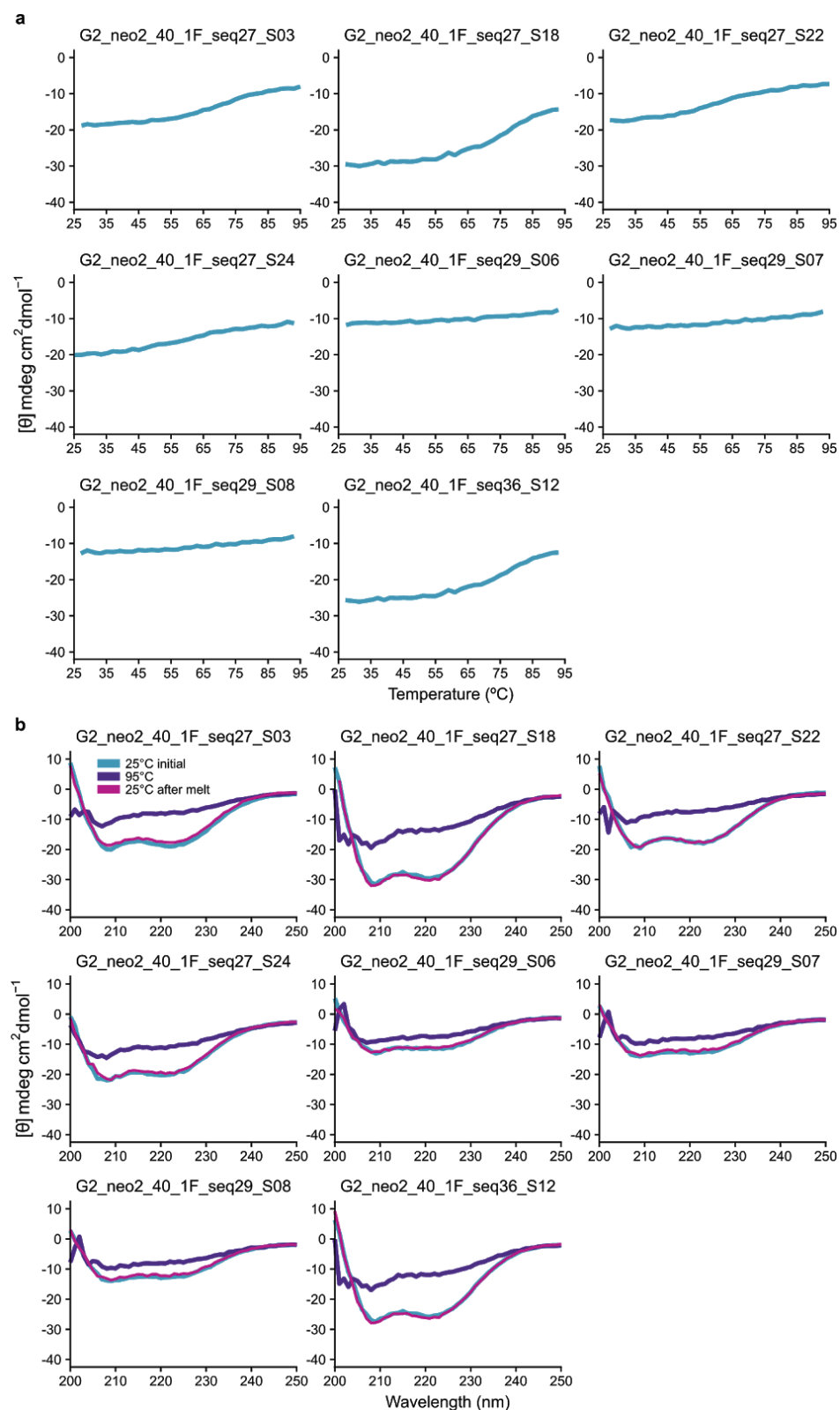


Figure S12. Circular dichroism thermal melts for IL-2/IL-15 mimetic designs generation-2, experimentally optimized. a) Thermal denaturation curves, and b) wavelength scans (222 nm). The experiments were performed once.

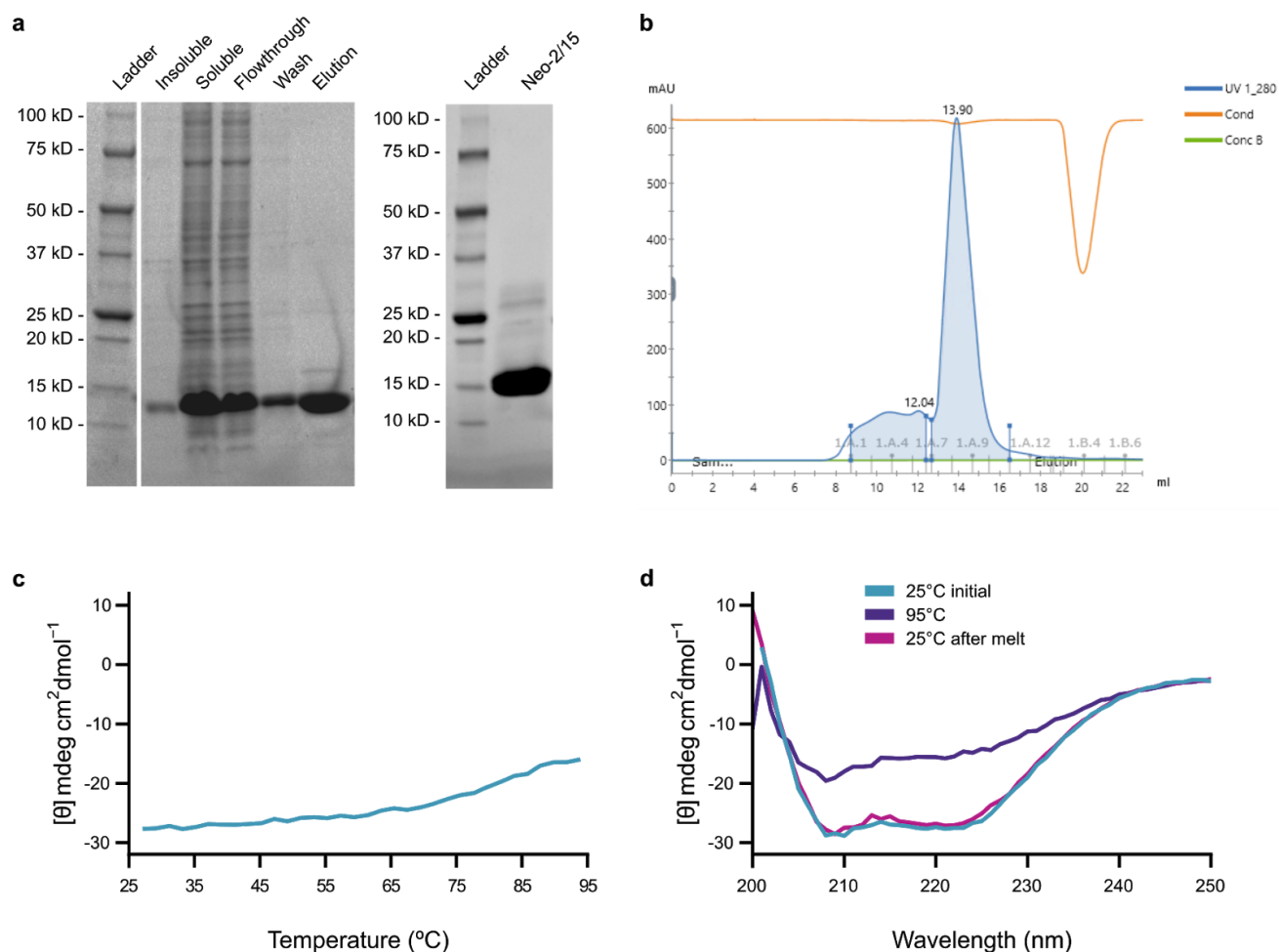


Figure S13. Expression, purification, and thermal denaturation of Neo-2/15. **a)** SDS Tris-Tricine gel electrophoresis showing expression and purification over affinity column (left), as well as size exclusion purified fraction (right); **b)** Size exclusion purification, showing monodisperse peak at expected elution time for Neo-2/15 (Neo-2/15, with a molecular weight = 13.8 kD for the 6x-his N-terminal tagged protein with thrombin restriction site; tag sequence: MGSSHHHHHSSGLVPRGSHM); **c)** Shows thermal scans of Neo-2/15 (followed by its circular dichroism signal at 222 nm) from 25°C to 95°C (heating rate ~2°C/min), showing the robust temperature stability of Neo 2/15. Up to ~65 °C little or not-unfolding is observed, a partial melting transition occurs afterwards (T_m ~80 °C), but ~50% of the ellipticity signal remains at 95°C, suggesting that even at near boiling temperature the protein still remains partially folded; **d)** Circular dichroism wavelength scans (200-250 nm) at 25°C, 95°C and then again 25°C for the thermal unfolding in “c”, showing that Neo-2/15 exhibits fully reversible thermal unfolding. The experiments were performed 3 times with similar results.

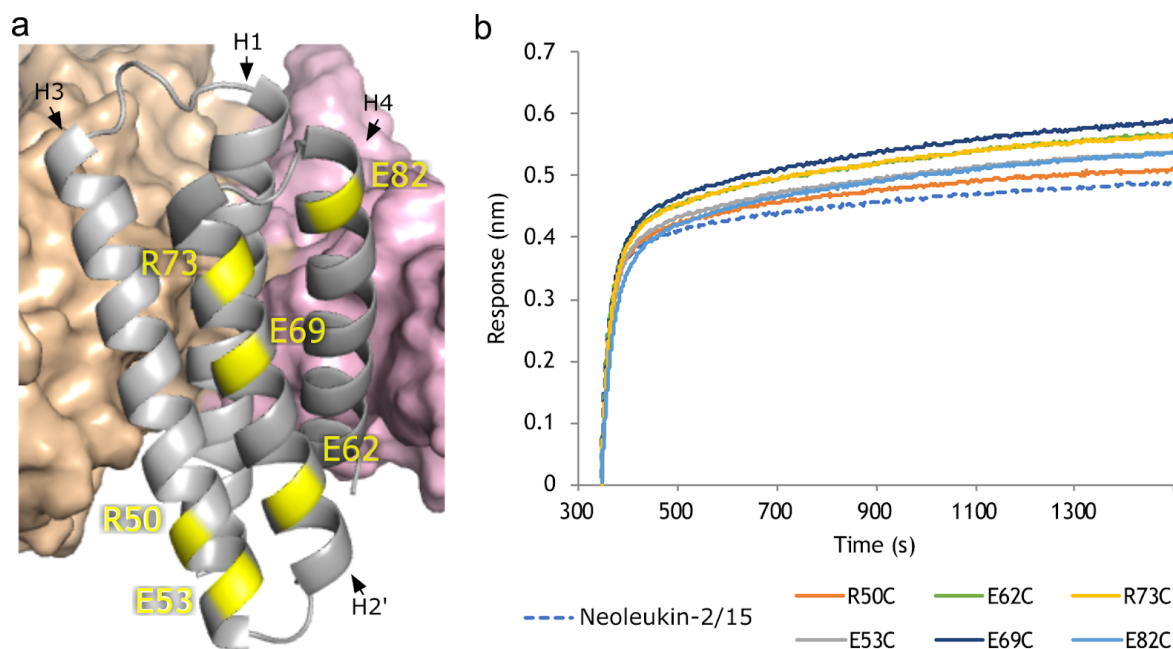


Figure S14. Robustness of Neo-2/15 to single-point cysteine mutants on positions not interfering with the binding interface. **a)** Schematic showing single-point positions in Neo-2/15 that were mutated to Cysteines. All the positions selected are far from the binding site (in order to avoid interference with receptor binding). **b)** The association kinetics of Neo-2/15 cysteine mutants with IL-2R $\beta\gamma_c$ show little or no-effect in binding (OCTET, rate constants were fit using a 1:1 binding model, see main text Methods). Since Neo-2/15 contains no other cysteine on its sequence, these single-cysteine mutations can be useful for further introducing chemical modifications to improve its therapeutic properties, for example, pegylation. The experiments were performed twice times with similar results.

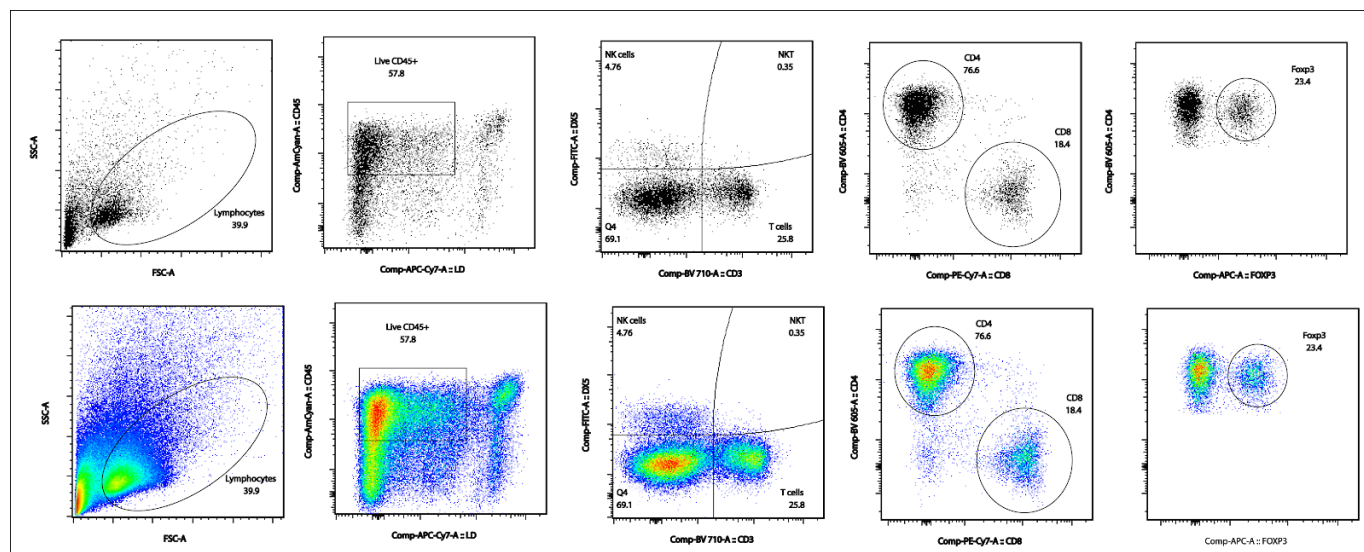


Figure S15. FACS gating strategy for colorectal carcinoma in vivo mice experiments.

Supplemental tables

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 ILR β _y at low nanomolar concentrations (see SI Fig. S1).

Design	Topology	Expr /Bind	Sequence
G1_neo2_33	H1->H4->H2'->H3	No/NA	STKKWQLQAEHALLDVQMALNKSPEPNENLNRAITAAQSWISTGKIDLDKAEDIRRNSDQARREAEKRGIDVRDLISNAQVILLEAR
G1_neo2_34	H1->H4->H2'->H3	No/NA	STKKWQLQAEHALLDVQMALNKSPEPNENLNRAITAAQSCISTGKCDLKAEDIRRNSDQARREAEKRGIDVRDLISNAQVILLEAR
G1_neo2_35	H1->H4->H2'->H3	Yes/No	STKKWQLQAEHALLDVQMALNKSPEPNENLNRAITAAQSWISTGKIDCDKAEDIRRNSDQARREAEKRGIDVRDLISNAQVILLEAC
G1_neo2_36	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELARNLEKVRDEALKRGIDVRDLVSNKIVIALELK
G1_neo2_37	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHFLLDVQMILNESPEPNEELNRCITDAQSWISTGKIDLDRAEECARNLEKVRDEALKRGIDVRDLVSNKIVIALELK
G1_neo2_38	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSCISTGKCDLDRAEELARNLEKVRDEALKRGIDVRDLVSNKIVIALELK
G1_neo2_39	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHFLLDVQMILNESPEPNEELNRAITDAQSWISTGKIDLDRAEELCRNLEKVRDEALKRGIDVRDLVSNKIVIALELK
G1_neo2_40	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHALDQMMNLNRSPEPNEELNRIITTMQSWISTGKIDLDGAKELAKEVEELRQAEKRGIDVRDLASNLKVILLELA
G1_neo2_41	H1->H4->H2'->H3	Yes/Yes	STKKLQLQAEHALDQMMNLNRSPEPNEELNRIITTMQSWISTGKCDLDGAKELAKEVEELRQAEKRGIDVRDLASNLKVILLELA
G1_neo2_42	H1->H4->H2'->H3	Yes/Yes	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMAKEAEKIRKEMEKGIDVRDLISNIIVILLELS
G1_neo2_43	H1->H4->H2'->H3	Yes/Yes	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKCDLDNAQEMAKEAEKIRKEMEKGIDVRDLISNIIVILLELS
G1_neo2_44	H1->H4->H2'->H3	Yes/No	STKKIQLQLEHALLDVQMALNRSPEPNESLNRMITWLQSWISTGKIDLDNAQEMCKEAEKIRKEMEKGIDVRDLISNICVILLELS

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

Design	Topology	Expr /Bind	Sequence
G1_neo2_40_1A	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAFMMLNVVPEPNEKLNRIITTMQSWIYTGKIDADGAKELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1B	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAHMMMLNMLPEPNEKLNRIITTMQSWIHTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1C	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAFMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1D	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDALMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1E	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAHMMMLNVVPEPNEKLNRIITTMQSWIYTGKIDRDGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDALMMLNLLPEPNEKLNRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1G	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAYMMLNMVPEPNEKLNRIITTMQSWILTGKIDSDGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1H	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAYMMLNMVPEPNEKLNRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1I	H1->H4->H2'->H3	Yes/Yes	STKKTQLLAEHALLDAYMMLNVPEPNEKLNRIITTMQSWIFTGKIDADGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1J	H1->H4->H2'->H3	Yes/Yes	STKKTQLMAEHALLDAFMMLNVLPPEPNEKLNRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H1	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALMSRNLPEPNEKLSRIITTMQSWIFTGKIDGGAQQLAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H2	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALHMRNLPEPNEKLSRIITTMQSWIFTGKIDGGAQELAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H3	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALMRKKLPPEPNEKLSRIITTMQSWIFTGKIDGGAQQLAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H4	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALHMSRELPEPNEKLNRIITTMQSWIFTGKIDGGAQDLAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H5	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALHMSRKLPEPNEKLSRIITTMQSWIFTGKIDGGAQHLAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_H6	H1->H4->H2'->H3	Yes/Yes	STKKTQLLIEHALLDALHMKRKLPEPNEKLNRIITTMQSWIFTEKIDGGAQDLAKEVEELEQEYEKRGIDVEDDASNLKVILLELA
G1_neo2_40_1F_M1	H1->H4->H2'->H3	Yes/Yes	STEKTQLAAEHALLDALMLKHLNPEPNEKLARIITTMQSWQFTGKIDGGAQELAKEVEELEQQEHEVRGIDVEDDASNLKVILLHLA
G1_neo2_40_1F_M2	H1->H4->H2'->H3	Yes/Yes	STKNTQLAAEDALLDALMLRNLNPEPNEKLARIITTMQSWQFTEKIDGGAQELAKEVEELEQQEHEVRGIDVEDDASNLKVILLQLA
G1_neo2_40_1F_M3	H1->H4->H2'->H3	Yes/Yes	STEKTQHAEDALLDALMLRNLNPEPNEKLARIITTMQSWQFTEKIDGGAQELAKEVEELEQQEHEVRGIDVEDDASNLKVILLQLA

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	Topology	Expr /Bind	Sequence
G2_neo2_40_1F_seq02	H1->H4->H2'->H3	Yes/Yes	TQKKQQLLAEHALLDALMILNMLKTSSEAVNRMITIAQSWIFTGTSNPEEAKEMIKMAEQAEAEARREGVDYVSNLKVILKEIA
G2_neo2_40_1F_seq03	H1->H4->H2'->H3	Yes/Yes	TTKKYQLLVEHALLDALMMLNLSSESNEKMNRIITMQSWIFTGTFDPDQAEELAKLVEELREEFRKRGIDTEDYASNLKVILKELS
G2_neo2_40_1F_seq04	H1->H4->H2'->H3	Yes/Yes	TTKKIQLLVEHALLDALMILNLSSESNEKLNRIITTLQSWIFRGEIDPDRAELAKLLEEIREEMRKRGIDTEDYVSNMIVIRELA
G2_neo2_40_1F_seq05	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLAEHVLDDLMLNLSSESNEKMNRIITVQSWIFTGIDPDQAEEMAKWVEELREEFRKRGIDTEDYASNVKVKELKELS
G2_neo2_40_1F_seq06	H1->H4->H2'->H3	Yes/Yes	TKKKYQLLIEHLDDLMLNLSSESNEKLNRIITLQSWIFTGTWDPDLAEEMKLMQIEEELRRRGIDTEDYMSNMRIIVIKELS
G2_neo2_40_1F_seq07	H1->H4->H2'->H3	Yes/Yes	TKKKQLLVEHLDDLMLNLSSESNEKLNRIITELQSWIFRGEIDPDKAEEMKIMEEIEKELRERGITDEDYMSNAKVIKELS
G2_neo2_40_1F_seq08	H1->H4->H2'->H3	Yes/Yes	TSKKQQLLAEHALLDALMILNLSSESSEAVNRAITWLQSWIFKGTVPDQAEEMRKLAQIREEMRKRGIDTEDYVSNLEVIKELS
G2_neo2_40_1F_seq09	H1->H4->H2'->H3	Yes/Yes	TKKKYQLLIEHLDDLMLNLSSESNEKLNRIITWLQSWIFTGTYPDLAEEMKYLEELREEMRERGITDEDYMSNMRIIVIKELS
G2_neo2_40_1F_seq10	H1->H4->H2'->H3	Yes/Yes	TKKKQQLLIEHLDDLMLNLSSESNEKLNRIITWLQSWIFTGTYPDLAEEMKMMDEIEDELREERGIDTEDYMSNAKVIKELS
G2_neo2_40_1F_seq11	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMILNLSSESNEKLNRIITMQSWIFTGTIDPDQAEELSKLVEEIREEMRKRGIDTEDYVSNLKVILDELS
G2_neo2_40_1F_seq12	H1->H4->H2'->H3	Yes/Yes	TEKKQLLVEHALLDALMILNLSSESNEKLNRIITMQSWIFTGRIDPDKAEELAKLVEELREEARERGITDEDYVSNLKVILKELS
G2_neo2_40_1F_seq13	H1->H4->H2'->H3	Yes/Yes	TKKKYQLMEHLDDLMLNLSSESNEKLNRIITIQSWIFTGTWDPDKAEEMAKLKEIEDELREERGIDTEDYMSNMIVIMKELS
G2_neo2_40_1F_seq14	H1->H4->H2'->H3	Yes/Yes	TTKKIQLLVEHALLDALMLNLSSESNEKMNRIITMQSWIFEGRIDPDQAEELAKLVEELREEFRKRGIDTEDYVSNLKVILEELS
G2_neo2_40_1F_seq15	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITMQSWIFTGTIDPDQAEELAKLVRELREEFRKRGIDTEDYASNLEVILRELS
G2_neo2_40_1F_seq16	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMILNLSKSNEKLNRIITMQSWIFNGTIDPDRAELAKLVEEIRDEMEKNGIDTEDYVSNLKVILEELA
G2_neo2_40_1F_seq17	H1->H4->H2'->H3	Yes/Yes	TKKKYQLLIEHVLDDLMLNLSSESNEKMNRIITLQSWIFTGTYPDKAEEMAKLKEELREEFRERGITDEDYISNAIVIKELS
G2_neo2_40_1F_seq18	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITMQSWIFTGTIDPDRAEELAKLVEELREEFRKRGIDTEDYASNLKVILKELS
G2_neo2_40_1F_seq19	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMMLNLSSESNEKLNRIITMQSWIFNGTIDPDQAEELAKLVEELREEFRKRGIDTEDYASNLKVILEELA
G2_neo2_40_1F_seq20	H1->H4->H2'->H3	Yes/Yes	TKKKQLLVEHALLDALMLNLSSESNEKLNRIITMQSWIFTGTVDPDQAEELAKLVEEIREELRKRGIDTEDYVSNLKVILKELS
G2_neo2_40_1F_seq21	H1->H4->H2'->H3	Yes/Yes	TTKKYQLLVEHALLDALMILNLSSESNEKLNRIITMQSWIFTGTFDPDQAEELAKLVREIREEMRKRGIDTEDYVSNLEVILRELS
G2_neo2_40_1F_seq22	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMILNLSSESNEKLNRIITMQSWIFTGTIDPDRAEELAKLVREIREEMRKRGIDTEDYVSNLEVILRELS
G2_neo2_40_1F_seq23	H1->H4->H2'->H3	Yes/Yes	TKKKYQLLIEHLDDLMLNLSSESNEKLNRIITWLQSWIFRGEWDPDKAEWAKLKEIREELRERGITDEDYMSNAIVIMKELS
G2_neo2_40_1F_seq24	H1->H4->H2'->H3	Yes/Yes	TDKKQLLVEHLDDLMLNLSKSNEKMNRIITIAQSWIFTGKVDPLAREMIKLLEETEDENRKNIGIDTEDYVSNARVIAKELE
G2_neo2_40_1F_seq25	H1->H4->H2'->H3	Yes/Yes	TKKKIQLLVEHALLDALMLNLSSESNEKMNRIITMQSWIFTGTIDPDQAEELAKLVEELKEEFKRGIDTEDYVSNLKVILKELS
G2_neo2_40_1F_seq26	H1->H4->H2'->H3	Yes/Yes	TKKKYQLLIEHALLDALMILNLSSESNEKLNRIITMQSWIFTGTYPDKAELEKLAKIEDEARERGITDEDYMSNLRVILKELS
G2_neo2_40_1F_seq27	H1->H4->H2'->H3	Yes/Yes	TKKKAQLLAEHALLDALMLNLSSESNERLNRIITWLQSIIFTGTYPDMVKEAVKLADEIEDEMRKRGIDTEDYVSNLRVILQELA
G2_neo2_40_1F_seq28	H1->H4->H2'->H3	Yes/No	TQKKQLLAEHLDDLMLNLSSESSEVANRIITWAQSWIFEGRVDPNKAEAKLAKKLEEEMRKRGIDMEDYISNMKVIAEEMS
G2_neo2_40_1F_seq29	H3->H2'->H4->H1	Yes/Yes	EDYVSNLKVILEELAREMERNGLSKAEAEWRQWKKIVERIRQIRSNNSDLNEAKELNRLITYIQSQIFEISERIRETDQEKKEESWKKWQLLLEHALLDVLMLLND
G2_neo2_40_1F_seq30	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLEHILLDALMLNLLETNPQNTESKFEDYISNAEVIAEELAKLMESLGLSDEAEKFKKIQWLREVWRIWSSNTWSTLEDKARELNRIITTIQSQIFY
G2_neo2_40_1F_seq31	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLEHILLDLMLNMIETNRENTESMEDYMSNVRLRELARLMEELNYKELSELMEERMRKIVEKIRQIVTNSSLDTAREWLNRLITWISLIIFR
G2_neo2_40_1F_seq32	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHALLDALMLNIIETNSKNTESKMEDYVSNLEVLTEFKKLAELNFSSEAEARMRKWARAYQMMLDLSLDKAKEMLNRIITILQSIIFN
G2_neo2_40_1F_seq33	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHLDDLMLNMGNASLKDYASNAQVIADEFRELARELGLTDEAKKAEKIEALERAREWLNNDKKEKAKALNRAITIAQSWIFN
G2_neo2_40_1F_seq34	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLEHLLDDLMLNMLRTNPKNIESDWEDYMSNIEVIEELRKIMESLGRSEKAKWKRMQWVRRIEIVKNSDLAEAKEWLNRLITIVQSEIFE
G2_neo2_40_1F_seq35	H1->H3->H2'->H4	Yes/Yes	WEKKRQLLEHLLDDLMLNMRWTRNPQNTESLMEDYMSNAKVIVEELARMMSQGLDEKAREWEEMKKRIEIRIQIQNNSSKERAKEELNRLITYVQSEIFR
G2_neo2_40_1F_seq36	H1->H3->H2'->H4	Yes/Yes	PKKKIQLLAEHALLDALMILNIVKTNQNAEELKEDYASNVEVILEEIALRMESGDQKDEAEKAKRMKEWMKRIKTASEDEQEEMANRIITLQSWIFS
G2_neo2_40_1F_seq37	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHALLDALMILNIIQTNPQNAEELKEDYMSNVEVIMEEFARMNRNGRSEEAENAEIRKKWRKASSTASSEEQREMMNRAITLQSWIFE
G2_neo2_40_1F_seq38	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHLDDLMLNMLTTNSKNTEEKLEDYISNMKVIKEMIELMRSLGRLEAEKWKALKAVEKIGSRMDSSETARELANRIITLAQSAIFY
G2_neo2_40_1F_seq39	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHALLDALMFLNLVETNPQAEELKEDYASNLRVIAEELARLFENLGRLEAQKAKDIKELARARSRVSEKRKEAMNRAITLQSMIFR
G2_neo2_40_1F_seq40	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHALLDALMILNIITNSDNTESKLEDYISNLKVILEEIALRMESLGLSDEAEKAKEAMRLADKAGSTASEEKKKEAMNRAITLQSWIFN
G2_neo2_40_1F_seq41	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHALLDALMMLNIIRTNPDAEELKEDYVSNLIVILREIAKLMEGLTDEAEKAKEARWAEARTTASKDQRRELANRIITLQSWIFS
G2_neo2_40_1F_seq42	H1->H3->H2'->H4	Yes/Yes	PEKKRQLLAEHLDDLALMILNIIETNEQNAESKLEDYISNAKVLDEFREMARLDGLDEAKKAEKMKRWLEKMRNSASSDERREWANRMITTAQSWIFN

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

Design	Topology	Expr /Bind	Sequence
G2_neo2_40_1F_seq27_S3	H1->H4->H2'->H3	Yes/Yes	TNKKQLHAFAFALHDALMLNLSSSESNERLNRIITWLQSIIFYGTYPDMVKEAVKDADEIEDEMRRKRGIDTEDYVSNLRILQLA
G2_neo2_40_1F_seq27_S18	H1->H4->H2'->H3	Yes/Yes	TNKEAQLHAFAFYDALMLNLSSSESNERLNRIITWLQSIIFYETYPDMVKEAVKLADEIEDEMRRKRGIDTEDYVSNLRILQLA
G2_neo2_40_1F_seq27_S22	H1->H4->H2'->H3	Yes/Yes	TKKDAELLAFAFYDALMLNLSSSESNERLNEIITWLQSIIFYGTYPDMVKEAVKLADEIEDEMRRKRGIDTEDYVSNLRILQLA
G2_neo2_40_1F_seq27_S24	H1->H4->H2'->H3	Yes/Yes	TNKKQLHAFAFYDALMLNLSSSESNERLNDIITWLQSIIFTGTYPDMVKEAVKLADEIEDEMRRKRGIDTEDYVSNLRILQLA
G2_neo2_40_1F_seq29_S6	H3->H2'->H4->H1	Yes/Yes	EDYYSNLKLIIEELAREMERNGLSKAEQWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYIQSQIFEVLEHGVGETDQEKKEESWKKWDLLEHALLDVLMLND
G2_neo2_40_1F_seq29_S7	H3->H2'->H4->H1	Yes/Yes	EDYYSNLKLIIEELAREMERNGLSKAEQWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYIQSQIFEVIEREGETDQEKKEESWKKWELHLEHALLDVLMLND
G2_neo2_40_1F_seq29_S8	H3->H2'->H4->H1	Yes/Yes	EDYYSNLKLIIEELAREMERNGLSKAEQWRQWKKIVERIRQIRSNNSDLNEAKELLNRLITYIQSQIFEVLEGVGETDQEKKEESWKKWELHLEHALLDVLMLND
Neoleukin-2/15 (Neo-2/15) (i.e. G2_neo2_40_1F_seq36_S11)	H1->H3->H2'->H4	Yes/Yes	PKKKIQLHAFAFYDALMILNIVKTNPPAEKLEDYAFNLFELILEETARLFESGDQKDEAEKAKRMKEWMKRIKTASEDEQEEMANAIIITILQSWIFS
G2_neo2_40_1F_seq36_S12	H1->H3->H2'->H4	Yes/Yes	PKKKIQLHAFAFDLMLNIVKTNQNAEEKLEDYAYNAGVILEETARLFESGDQKDEAEKAKRMKEWMKRIKDTASEDEQEEMANEIITILQSWNFS

Table S5. List of primers used in the creation of SSM library for G1_neo2_40.

Primer	Sequence
G1_neo2_40_01F	5'-AGGGTCGGCTAGCCATATGNNKACCAAAAACTCCAGCTGCA-3'
G1_neo2_40_01R	5'-CATATGGCTAGCCGACCC-3'
G1_neo2_40_02F	5'-GGGTCGGCTAGCCATATGTCTNNKAAAAAACTCCAGCTGCAGGC-3'
G1_neo2_40_02R	5'-AGACATATGGCTAGCCGACCC-3'
G1_neo2_40_03F	5'-TCGGCTAGCCATATGTCTACCNKAAAACTCCAGCTGCAGGC-3'
G1_neo2_40_03R	5'-GGTAGACATATGGCTAGCCGA-3'
G1_neo2_40_04F	5'-CGGCTAGCCATATGTCTACCAAAANNKCTCCAGCTGCAGGCG-3'
G1_neo2_40_04R	5'-TTTGGTAGACATATGGCTAGCCG-3'
G1_neo2_40_05F	5'-GGCTAGCCATATGTCTACCAAAAAANNKAGCTGCAGGCGGAAC-3'
G1_neo2_40_05R	5'-TTTTTTGGTAGACATATGGCTAGCC-3'
G1_neo2_40_06F	5'-TAGCCATATGTCTACCAAAAACTCNNKCTGCAGGCGGAACACG-3'
G1_neo2_40_06R	5'-GAGTTTTTTGGTAGACATATGGCTA-3'
G1_neo2_40_07F	5'-GCCATATGTCTACCAAAAACTCCAGNNKAGGCGGAACACGCG-3'
G1_neo2_40_07R	5'-CTGGAGTTTTTTGGTAGACATATGGC-3'
G1_neo2_40_08F	5'-TGCTACCAAAAACTCCAGCTGNNKGGCGGAACACGCGCT-3'
G1_neo2_40_08R	5'-CAGCTGGAGTTTTTTGGTAGACA-3'
G1_neo2_40_09F	5'-ACCAAAAACTCCAGCTGCAGNNKGAACACGCGCTCCTGG-3'
G1_neo2_40_09R	5'-CTGCAGCTGGAGTTTTTTGGT-3'
G1_neo2_40_10F	5'-AACTCCAGCTGCAGGCGNNKACGCGCTCCTGGAC-3'
G1_neo2_40_10R	5'-CGCCTGCAGCTGGAGTTT-3'
G1_neo2_40_11F	5'-CTCCAGCTGCAGGCGGAANNKGGCGCTCCTGGACGC-3'
G1_neo2_40_11R	5'-TTCCGCTGCAGCTGGAG-3'
G1_neo2_40_12F	5'-CAGCTGCAGGCGGAACANNKCTCCTGGACGCGCAG-3'
G1_neo2_40_12R	5'-GTGTTCCGCTGCAGCTG-3'
G1_neo2_40_13F	5'-CTGCAGGCGGAACACGCGNNKCTGGACGCGCAGATGAT-3'
G1_neo2_40_13R	5'-CGCGTGTTCGCGCTGCAG-3'
G1_neo2_40_14F	5'-CAGGCGGAACACGCGCTCNNKAGCGCGCAGATGATGCT-3'
G1_neo2_40_14R	5'-GAGCGCGTGTTCGCGCTG-3'
G1_neo2_40_15F	5'-GCGGAACACGCGCTCCTGNNKGGCGCAGATGATGCTCAAC-3'
G1_neo2_40_15R	5'-CAGGAGCGCGTGTTCGCG-3'
G1_neo2_40_16F	5'-GAACACGCGCTCCTGGACNNKAGATGATGCTCAACCGTTC-3'
G1_neo2_40_16R	5'-GTCAGGAGCGCGTGTTC-3'
G1_neo2_40_17F	5'-CACGCGCTCCTGGACGCGNNKATGATGCTCAACCGTTCTCC-3'
G1_neo2_40_17R	5'-CGGTCACGAGGCGCGTG-3'
G1_neo2_40_18F	5'-GCGCTCCTGGACGCGCAGNNKATGCTCAACCGTTCTCCG-3'
G1_neo2_40_18R	5'-CTGCGCTCCAGGAGCGC-3'
G1_neo2_40_19F	5'-CTCCTGGACGCGCAGATGNNKCTCAACCGTTCTCCGGAAC-3'
G1_neo2_40_19R	5'-CATCTGCGCGTCCAGGAG-3'
G1_neo2_40_20F	5'-CTGGACGCGCAGATGATGNNKAACCGTTCTCCGGAACCG-3'
G1_neo2_40_20R	5'-CATCATCTGCGCGTCCAG-3'
G1_neo2_40_21F	5'-GACGCGCAGATGATGCTCNNKCGTTCTCCGGAACCGAAC-3'
G1_neo2_40_21R	5'-GAGCATCATCTGCGCGTC-3'
G1_neo2_40_22F	5'-CGGCGCAGATGATGCTCAACNNKCTCCGGAACCGAACGA-3'
G1_neo2_40_22R	5'-GTTGAGCATCATCTGCGCG-3'
G1_neo2_40_23F	5'-CGCAGATGATGCTCAACCGTTNNKCCGGAACCGAACGAAAAAC-3'
G1_neo2_40_23R	5'-ACGGTTGAGCATCATCTGCG-3'
G1_neo2_40_24F	5'-CAGATGATGCTCAACCGTTCTNNKGAACCGAACGAAAACTGAACC-3'
G1_neo2_40_24R	5'-AGAACGGTTGAGCATCATCTG-3'
G1_neo2_40_25F	5'-ATGCTCAACCGTTCTCCGNNKCCGGAACGAAAACTGAACCG-3'
G1_neo2_40_25R	5'-CGGAGAACGGTTGAGCAT-3'
G1_neo2_40_26F	5'-TGCTCAACCGTTCTCCGGAANNKACGAAAACTGAACCGTATCAT-3'
G1_neo2_40_26R	5'-TTCCGGAGAACGGTTGAGCA-3'
G1_neo2_40_27F	5'-AACCGTTCTCCGGAACCGNNKGAACGAAAACTGAACCGTATCATCACC-3'
G1_neo2_40_27R	5'-CGGTTCCGGAGAACGGTT-3'
G1_neo2_40_28F	5'-CCGTTCTCCGGAACCGAACNNKAACTGAACCGTATCATCACCA-3'
G1_neo2_40_28R	5'-GTTCCGTTCCGGAGAACGG-3'
G1_neo2_40_29F	5'-TTCTCCGGAACCGAACGAANNKCTGAACCGTATCATCACCACC-3'
G1_neo2_40_29R	5'-TTCGTTCCGTTCCGGAGAA-3'
G1_neo2_40_30F	5'-CTCCGGAACCGAACGAAAAANNKAACTGATCATCACCACCATG-3'
G1_neo2_40_30R	5'-TTTTTCGTTCCGTTCCGGAG-3'
G1_neo2_40_31F	5'-CCGGAACCGAACGAAAACTGNNKCGTATCATCACCACCATGCA-3'
G1_neo2_40_31R	5'-CAGTTTTTCGTTCCGTTCCGG-3'
G1_neo2_40_32F	5'-CGGAACCGAACGAAAACTGAACNNKATCATCACCACCATGCAGTC-3'
G1_neo2_40_32R	5'-GTTGAGTTTTTCGTTCCGTTCCGG-3'
G1_neo2_40_33F	5'-CCGAACGAAAACTGAACCGTNNKATCACCACCATGCAGTCTTG-3'
G1_neo2_40_33R	5'-ACGGTTCAGTTTTTCGTTCCGG-3'

G1_neo2_40_34F	5'-CCGAACGAAAACTGAACCGTATCANNKACCACCATGCAGTCTTGG-3'
G1_neo2_40_34R	5'-GATACGGTTCAGTTTTTCGTTTCGG-3'
G1_neo2_40_35F	5'-AACGAAAACTGAACCGTATCATCANNKACCATGCAGTCTTGGATCTC-3'
G1_neo2_40_35R	5'-GATGATACGGTTCAGTTTTTCGTT-3'
G1_neo2_40_36F	5'-GAAAACTGAACCGTATCATCANNKATGCAGTCTTGGATCTCTACTG-3'
G1_neo2_40_36R	5'-GGTGATGATACGGTTCAGTTTTTC-3'
G1_neo2_40_37F	5'-CTGAACCGTATCATCACCACNNKAGTCTTGGATCTCTACTGGTAAG-3'
G1_neo2_40_37R	5'-GGTGGTGATGATACGGTTCAG-3'
G1_neo2_40_38F	5'-AACCGTATCATCACCACCATGNNKTCTTGGATCTCTACTGGTAAGATCG-3'
G1_neo2_40_38R	5'-CATGGTGGTGATGATACGGTTC-3'
G1_neo2_40_39F	5'-CCGTATCATCACCACCATGCAGNNKTGGATCTCTACTGGTAAGATCGA-3'
G1_neo2_40_39R	5'-CTGCATGGTGGTGATGATACGG-3'
G1_neo2_40_40F	5'-TCATCACCACCATGCAGTCTNNKATCTCTACTGGTAAGATCGACCT-3'
G1_neo2_40_40R	5'-AGACTGCATGGTGGTGATGA-3'
G1_neo2_40_41F	5'-CACCACCATGCAGTCTTGGNNKTCTACTGGTAAGATCGACCTGG-3'
G1_neo2_40_41R	5'-CCAAGACTGCATGGTGGTG-3'
G1_neo2_40_42F	5'-ACCACCATGCAGTCTTGGATCANNKACTGGTAAGATCGACCTGGA-3'
G1_neo2_40_42R	5'-GATCCAAGACTGCATGGTGGT-3'
G1_neo2_40_43F	5'-ACCATGCAGTCTTGGATCTCANNKGGTAAGATCGACCTGGATGG-3'
G1_neo2_40_43R	5'-AGAGATCCAAGACTGCATGGT-3'
G1_neo2_40_44F	5'-CCATGCAGTCTTGGATCTCTACTNNKAGATCGACCTGGATGGTGC-3'
G1_neo2_40_44R	5'-AGTAGAGATCCAAGACTGCATGG-3'
G1_neo2_40_45F	5'-TGCACTCTTGGATCTCTACTGGTNNKATCGACCTGGATGGTGC-3'
G1_neo2_40_45R	5'-ACCAGTAGAGATCCAAGACTGCA-3'
G1_neo2_40_46F	5'-GCAGTCTTGGATCTCTACTGGTAAGNNKAGACCTGGATGGTGCAG-3'
G1_neo2_40_46R	5'-CTTACCAGTAGAGATCCAAGACTGC-3'
G1_neo2_40_47F	5'-AGTCTTGGATCTCTACTGGTAAGATCANNKCTGGATGGTGCAGGAA-3'
G1_neo2_40_47R	5'-GATCTTACCAGTAGAGATCCAAGACT-3'
G1_neo2_40_48F	5'-TGGATCTCTACTGGTAAGATCGACNNKAGTGGTGCAGGAACTCG-3'
G1_neo2_40_48R	5'-GTCGATCTTACCAGTAGAGATCCA-3'
G1_neo2_40_49F	5'-TCTCTACTGGTAAGATCGACCTGNNKGGTGCAGGAACTCGC-3'
G1_neo2_40_49R	5'-CAGGTCGATCTTACCAGTAGAGA-3'
G1_neo2_40_50F	5'-TACTGGTAAGATCGACCTGGATNNKGCAGGAACTCGCGA-3'
G1_neo2_40_50R	5'-ATCCAGGTCGATCTTACCAGTA-3'
G1_neo2_40_51F	5'-TGGAAGATCGACCTGGATGGTNNKAAAGAACTCGCGAAAGAA-3'
G1_neo2_40_51R	5'-ACCATCCAGGTCGATCTTACCA-3'
G1_neo2_40_52F	5'-ATCGACCTGGATGGTGCNNKGAAGTTCGCGAAAGAAAGTTGAA-3'
G1_neo2_40_52R	5'-CGCACCATCCAGGTCGAT-3'
G1_neo2_40_53F	5'-CGACCTGGATGGTGCAGANNKCTCGCGAAAGAAAGTTGAAGAA-3'
G1_neo2_40_53R	5'-CTTCGCACCATCCAGGTCG-3'
G1_neo2_40_54F	5'-CCTGGATGGTGCAGGAANNKGCAGAAAGTTGAAGAACTGC-3'
G1_neo2_40_54R	5'-TTCTTCGCGACCATCCAGG-3'
G1_neo2_40_55F	5'-TGGATGGTGCAGGAACTCANNKAAAGAAAGTTGAAGAACTGCGTC-3'
G1_neo2_40_55R	5'-GAGTTCCCTTCGCACCATCCA-3'
G1_neo2_40_56F	5'-GGTGCAGGAACTCGCGNNKGAAGTTGAAGAACTGCGTCAG-3'
G1_neo2_40_56R	5'-CGCGAGTTCCCTTCGCACC-3'
G1_neo2_40_57F	5'-TGCGAAGGAACTCGCGAANNKGTGAAGAACTGCGTCAGGA-3'
G1_neo2_40_57R	5'-TTTCGCGAGTTCCCTTCGCA-3'
G1_neo2_40_58F	5'-GCGAAGGAACTCGCGAAGANNKGAAGAACTGCGTCAGGAAGC-3'
G1_neo2_40_58R	5'-TTCTTTCGCGAGTTCCCTTCGC-3'
G1_neo2_40_59F	5'-AAGGAACTCGCGAAGAAAGTTNNKGAAGTTCGTCAGGAAGCG-3'
G1_neo2_40_59R	5'-AACTTCTTTCGCGAGTTCCCTT-3'
G1_neo2_40_60F	5'-GGAAGTTCGCGAAGAAAGTTGAANNKCTGCGTCAGGAAGCGG-3'
G1_neo2_40_60R	5'-TTCAACTTCTTTCGCGAGTTCC-3'
G1_neo2_40_61F	5'-ACTCGCGAAGAAAGTTGAAGANNKCGTCAGGAAGCGGAAAAAC-3'
G1_neo2_40_61R	5'-TTCTTCAACTTCTTTCGCGAGT-3'
G1_neo2_40_62F	5'-TCGCGAAGAAAGTTGAAGAACTGNNKAGGAAGCGGAAAAACGTG-3'
G1_neo2_40_62R	5'-CAGTTCTTCAACTTCTTTCGCGA-3'
G1_neo2_40_63F	5'-CGAAGAAAGTTGAAGAACTGCGTNNKGAAGCGGAAAAACGTGGTAT-3'
G1_neo2_40_63R	5'-ACGCAGTTCTTCAACTTCTTTCG-3'
G1_neo2_40_64F	5'-AGAAGTTGAAGAACTGCGTCAGNNKCGCGGAAAAACGTGGTATCG-3'
G1_neo2_40_64R	5'-CTGACGCAGTTCTTCAACTTCT-3'
G1_neo2_40_65F	5'-GTTGAAGAACTGCGTCAGGAANNKGAAGAACTGCGTACGAGTT-3'
G1_neo2_40_65R	5'-TTCTGACGCAGTTCTTCAAC-3'
G1_neo2_40_66F	5'-GAAGTTCGTCAGGAAGCGNNKAAAGTGGTATCGACGTTTCG-3'
G1_neo2_40_66R	5'-CGCTTCTGACGCAGTTTC-3'
G1_neo2_40_67F	5'-CTGCGTCAGGAAGCGAANNKCGTGGTATCGACGTTTCG-3'
G1_neo2_40_67R	5'-TTCCGCTTCTGACGCAG-3'

G1_neo2_40_68F	5'-TGCCTCAGGAAGCGGAAAAANNKGGTATCGACGTTCTGTGACC-3'
G1_neo2_40_68R	5'-TTTTTCCGCTTCCTGACGCA-3'
G1_neo2_40_69F	5'-GTCAGGAAGCGGAAAAACGTNNKATCGACGTTCTGTGACCTG-3'
G1_neo2_40_69R	5'-ACGTTTTTCCGCTTCCTGAC-3'
G1_neo2_40_70F	5'-AGGAAGCGGAAAAACGTGGTNNKGACGTTCTGTGACCTGGC-3'
G1_neo2_40_70R	5'-ACCACGTTTTTCCGCTTCCT-3'
G1_neo2_40_71F	5'-GAAGCGGAAAAACGTGGTATCNNGTTCGTGACCTGGCGT-3'
G1_neo2_40_71R	5'-GATACCACGTTTTTCCGCTTC-3'
G1_neo2_40_72F	5'-CGCGAAAAACGTGGTATCGACNNKCGTGACCTGGCGTCTAAC-3'
G1_neo2_40_72R	5'-GTCGATACCACGTTTTTCCGC-3'
G1_neo2_40_73F	5'-GGAAAAACGTGGTATCGACGTTNNKGACCTGGCGTCTAACCTG-3'
G1_neo2_40_73R	5'-AACGTCGATACCACGTTTTTCC-3'
G1_neo2_40_74F	5'-ACGTGGTATCGACGTTCTGNNKCTGGCGTCTAACCTGAAAGT-3'
G1_neo2_40_74R	5'-ACGAACGTCGATACCACGT-3'
G1_neo2_40_75F	5'-GTGGTATCGACGTTCTGACNNKCGCTCTAACCTGAAAGTTATCC-3'
G1_neo2_40_75R	5'-GTCACGAACGTCGATACCAC-3'
G1_neo2_40_76F	5'-ATCGACGTTCTGTGACCTGNNKCTAACCTGAAAGTTATCCTGCTG-3'
G1_neo2_40_76R	5'-CAGGTCACGAACGTCGAT-3'
G1_neo2_40_77F	5'-GACGTTCTGTGACCTGGCGNNKAACCTGAAAGTTATCCTGCTGG-3'
G1_neo2_40_77R	5'-CGCGAGGTCACGAACGTC-3'
G1_neo2_40_78F	5'-GTTCTGTGACCTGGCGTCTNNKCTGAAAGTTATCCTGCTGGAGC-3'
G1_neo2_40_78R	5'-AGACGCCAGGTCACGAAC-3'
G1_neo2_40_79F	5'-TCGTGACCTGGCGTCTAACNNKAAAGTTATCCTGCTGGAGCTG-3'
G1_neo2_40_79R	5'-GTTAGACGCCAGGTCACGA-3'
G1_neo2_40_80F	5'-TGACCTGGCGTCTAACCTGNNKGTATCCTGCTGGAGCTGG-3'
G1_neo2_40_80R	5'-CAGGTTAGACGCCAGGTCA-3'
G1_neo2_40_81F	5'-ACCTGGCGTCTAACCTGAAANNKATCCTGCTGGAGCTGGC-3'
G1_neo2_40_81R	5'-TTTCAGGTTAGACGCCAGGT-3'
G1_neo2_40_82F	5'-CTGGCGTCTAACCTGAAAGTTNNKCTGCTGGAGCTGGCTC-3'
G1_neo2_40_82R	5'-AACTTTCAGGTTAGACGCCAG-3'
G1_neo2_40_83F	5'-TGGCGTCTAACCTGAAAGTTATCNNGTGGAGCTGGCTCTCGA-3'
G1_neo2_40_83R	5'-GATAACTTTCAGGTTAGACGCCA-3'
G1_neo2_40_84F	5'-GGCGTCTAACCTGAAAGTTATCCTGNNKGAGCTGGCTCTCGAGGG-3'
G1_neo2_40_84R	5'-CAGGATAACTTTCAGGTTAGACGCC-3'
G1_neo2_40_85F	5'-TCTAACCTGAAAGTTATCCTGCTGNNKCTGGCTCTCGAGGGAGG-3'
G1_neo2_40_85R	5'-CAGCAGGATAACTTTCAGGTTAGA-3'
G1_neo2_40_86F	5'-ACCTGAAAGTTATCCTGCTGGAGNNKCTCTCGAGGGAGGCG-3'
G1_neo2_40_86R	5'-CTCCAGCAGGATAACTTTCAGGT-3'
G1_neo2_40_87F	5'-AAAGTTATCCTGCTGGAGCTGNNKCTCGAGGGAGGCGGAT-3'
G1_neo2_40_87R	5'-CAGTCCAGCAGGATAACTTT-3'
COF	5'-TGACAACTATATGCGAGCAATCCCTCAC-3'
COR	5'-AACTTTTTCGCAGTTCGCGA-3'

Table S6. List of primers used in the creation of SSM libraries for G2_neo2_40_1_seq27, G2_neo2_40_1_dsn29, and G2_neo2_40_1_dsn36.

Primer	Sequence
G2_neo2_40_1F_seq27_01	5'-CATATGCGAAGCCGACCT-3'
G2_neo2_40_1F_seq27_01R	5'-AGGGTCGGCTTCGCATATGNNKAAAGAAAAAGCGCAGCTCC-3'
G2_neo2_40_1F_seq27_02	5'-CGTCATATGCGAAGCCGAC-3'
G2_neo2_40_1F_seq27_02R	5'-GTCGGCTTCGCATATGACGNKAAAAAGCGCAGCTCCTG-3'
G2_neo2_40_1F_seq27_03	5'-CTTCGTCATATGCGAAGCCG-3'
G2_neo2_40_1F_seq27_03R	5'-CGGCTTCGCATATGACGAAGNNKAAAGCGCAGCTCCTGG-3'
G2_neo2_40_1F_seq27_04	5'-TTTCTTCGTCATATGCGAAGCC-3'
G2_neo2_40_1F_seq27_04R	5'-GGCTTCGCATATGACGAAGAAANNKGCAGCTCCTGGC-3'
G2_neo2_40_1F_seq27_05	5'-TTTTTTCTTCGTCATATGCGAAGCC-3'
G2_neo2_40_1F_seq27_05R	5'-GGCTTCGCATATGACGAAGAAAAANNKAGCTCCTGGCCGAGC-3'
G2_neo2_40_1F_seq27_06	5'-CGCTTTTTTCTTCGTCATATGCG-3'
G2_neo2_40_1F_seq27_06R	5'-CGCATATGACGAAGAAAAAGCGNNKCTCCTGGCCGAGCAGC-3'
G2_neo2_40_1F_seq27_07	5'-CTGCGCTTTTTTCTTCGTCAT-3'
G2_neo2_40_1F_seq27_07R	5'-ATGACGAAGAAAAAGCGCAGNNKCTGGCCGAGCAGC-3'
G2_neo2_40_1F_seq27_08	5'-GAGCTGCGCTTTTTTCTTCGTCAT-3'
G2_neo2_40_1F_seq27_08R	5'-ACGAAGAAAAAGCGCAGCTCANNKCCGAGCAGCAGC-3'
G2_neo2_40_1F_seq27_09	5'-CAGGAGCTGCGCTTTTTTCTTC-3'
G2_neo2_40_1F_seq27_09R	5'-GAAAAAGCGCAGCTCCTGNNKAGCAGCAGCTGCT-3'
G2_neo2_40_1F_seq27_10	5'-GGCCAGGAGCTGCGCTTT-3'
G2_neo2_40_1F_seq27_10R	5'-AAAGCGCAGCTCCTGGCCNNKACGCGCTGCTGGAC-3'
G2_neo2_40_1F_seq27_11	5'-CTCGGCCAGGAGCTGCGC-3'
G2_neo2_40_1F_seq27_11R	5'-GCGCAGCTCCTGGCCGAGNNKCGCTGCTGGACGC-3'
G2_neo2_40_1F_seq27_12	5'-GTGCTCGGCCAGGAGCTG-3'
G2_neo2_40_1F_seq27_12R	5'-CAGCTCCTGGCCGAGCANNKCTGCTGACGCGCTC-3'
G2_neo2_40_1F_seq27_13	5'-CGCGTGCTCGGCCAGGAG-3'
G2_neo2_40_1F_seq27_13R	5'-CTCCTGGCCGAGCAGCGNNKCTGGACGCGCTCATGC-3'
G2_neo2_40_1F_seq27_14	5'-CAGCGCGTGCTCGGCCAG-3'
G2_neo2_40_1F_seq27_14R	5'-CTGGCCGAGCAGCGCTGNNKAGCAGCGCTCATGCTGC-3'
G2_neo2_40_1F_seq27_15	5'-CAGCAGCGCGTGCTCGGC-3'
G2_neo2_40_1F_seq27_15R	5'-GCCGAGCAGCGCTGCTGNNKCGCTCATGCTGCTGA-3'
G2_neo2_40_1F_seq27_16	5'-GTCCAGCAGCGCGTGCTC-3'
G2_neo2_40_1F_seq27_16R	5'-GAGCAGCGCTGCTGGACNNKCTCATGCTGCTGAACCTGT-3'
G2_neo2_40_1F_seq27_17	5'-CGCGTCCAGCAGCGCTG-3'
G2_neo2_40_1F_seq27_17R	5'-CACGCGTGCTGGACGCGNNKATGCTGCTGAACCTGTCTT-3'
G2_neo2_40_1F_seq27_18	5'-GAGCGCGTCCAGCAGCGC-3'
G2_neo2_40_1F_seq27_18R	5'-GCGCTGCTGGACGCGCTGNNKCTGCTGAACCTGTCTTCTGA-3'
G2_neo2_40_1F_seq27_19	5'-CATGAGCGCGTCCAGCAG-3'
G2_neo2_40_1F_seq27_19R	5'-CTGCTGGACGCGCTCATGNNKCTGAACCTGTCTTCTGAATCTAAC-3'
G2_neo2_40_1F_seq27_20	5'-CAGCATGAGCGCGTCCAG-3'
G2_neo2_40_1F_seq27_20R	5'-CTGGACGCGCTCATGCTGNNKAACTGTCTTCTGAATCTAACGA-3'
G2_neo2_40_1F_seq27_21	5'-CAGCAGCATGAGCGCTC-3'
G2_neo2_40_1F_seq27_21R	5'-GACGCGCTCATGCTGCTGNNKCTGCTTCTGAATCTAACGAACG-3'
G2_neo2_40_1F_seq27_22	5'-GTTACAGCATGAGCGCG-3'
G2_neo2_40_1F_seq27_22R	5'-CGCGCTCATGCTGCTGAACNNKCTTCTGAATCTAACGAACGCT-3'
G2_neo2_40_1F_seq27_23	5'-CAGGTTACAGCATGAGCG-3'
G2_neo2_40_1F_seq27_23R	5'-CGCTCATGCTGCTGAACCTGNNKCTGAATCTAACGAACGCTGA-3'
G2_neo2_40_1F_seq27_24	5'-AGACAGGTTACAGCATGA-3'
G2_neo2_40_1F_seq27_24R	5'-TCATGCTGCTGAACCTGCTNNKGAATCTAACGAACGCTGAACC-3'
G2_neo2_40_1F_seq27_25	5'-AGAAGACAGGTTACAGCAGCA-3'
G2_neo2_40_1F_seq27_25R	5'-TGCTGCTGAACCTGTCTTCTNNKCTAACGAACGCTGAACCG-3'
G2_neo2_40_1F_seq27_26	5'-TTCAGAAGACAGGTTACAGCAGC-3'
G2_neo2_40_1F_seq27_26R	5'-GCTGCTGAACCTGTCTTCTGAANNKAAACGAGCTGCTGAACCGT-3'
G2_neo2_40_1F_seq27_27	5'-AGATTACAGAAGACAGGTTACAGCA-3'
G2_neo2_40_1F_seq27_27R	5'-TGCTGAACCTGTCTTCTGAATCTNNKGAACGCTGAACCGTATCATCA-3'
G2_neo2_40_1F_seq27_28	5'-GTTAGATTACAGAAGACAGGTTACAGC-3'
G2_neo2_40_1F_seq27_28R	5'-GCTGAACCTGTCTTCTGAATCTAACNNKCGTCTGAACCGTATCATCACT-3'
G2_neo2_40_1F_seq27_29	5'-TTCGTTAGATTACAGAAGACAGGTT-3'
G2_neo2_40_1F_seq27_29R	5'-AACCTGTCTTCTGAATCTAACGAANNKCTGAACCGTATCATCACTTGGC-3'
G2_neo2_40_1F_seq27_30	5'-ACGTTCTGTTAGATTACAGAAGACAG-3'
G2_neo2_40_1F_seq27_30R	5'-CTGTCTTCTGAATCTAACGAACGNNKAAACCGTATCATCACTTGGCT-3'
G2_neo2_40_1F_seq27_31	5'-CAGACGTTCTGTTAGATTACAGAAGA-3'
G2_neo2_40_1F_seq27_31R	5'-TCTTCTGAATCTAACGAACGCTGNNKCGTATCATCACTTGGCTGCA-3'
G2_neo2_40_1F_seq27_32	5'-GTTACAGCGTTCTGTTAGATTACAGA-3'
G2_neo2_40_1F_seq27_32R	5'-TCTGAATCTAACGAACGCTGAACNNKATCATCACTTGGCTGCAGT-3'

G2_neo2_40_1F_seq27_33	5'-ACGGTTCAGACGTTTCGTTA-3'
G2_neo2_40_1F_seq27_33R	5'-TAACGAACGCTCTGAACCGTNNKATCACTTGGCTGCAGTCTATC-3'
G2_neo2_40_1F_seq27_34	5'-GATACGGTTCAGACGTTTCGTT-3'
G2_neo2_40_1F_seq27_34R	5'-AACGAACGCTCTGAACCGTATCNNKACTTGGCTGCAGTCTATCATC-3'
G2_neo2_40_1F_seq27_35	5'-GATGATACGGTTCAGACGTTTCGT-3'
G2_neo2_40_1F_seq27_35R	5'-ACGAACGCTCTGAACCGTATCATCNNKTGGCTGCAGTCTATCATCTTC-3'
G2_neo2_40_1F_seq27_36	5'-AGTGATGATACGGTTCAGACGT-3'
G2_neo2_40_1F_seq27_36R	5'-ACGTCTGAACCGTATCATCACTNNKCTGCAGTCTATCATCTTCACCG-3'
G2_neo2_40_1F_seq27_37	5'-CCAAGTATGATACGGTTCAGAC-3'
G2_neo2_40_1F_seq27_37R	5'-GTCTGAACCGTATCATCACTTGGNNKCACTATCATCTTCACCGGT-3'
G2_neo2_40_1F_seq27_38	5'-CAGCCAAGTATGATACGGTT-3'
G2_neo2_40_1F_seq27_38R	5'-AACCGTATCATCACTTGGCTGNNKCTATCATCTTCACCGGTACCT-3'
G2_neo2_40_1F_seq27_39	5'-CTGCAGCCAAGTATGATACGG-3'
G2_neo2_40_1F_seq27_39R	5'-CCGTATCATCACTTGGCTGCAGNNKATCATCTTCACCGGTACCTACG-3'
G2_neo2_40_1F_seq27_40	5'-AGACTGCAGCCAAGTATGA-3'
G2_neo2_40_1F_seq27_40R	5'-TCATCACTTGGCTGCAGTCTNNKATCTTCACCGGTACCTACGA-3'
G2_neo2_40_1F_seq27_41	5'-GATAGACTGCAGCCAAGTAT-3'
G2_neo2_40_1F_seq27_41R	5'-ATCACTTGGCTGCAGTCTATCNNKTTACCGGTACCTACGACC-3'
G2_neo2_40_1F_seq27_42	5'-GATGATAGACTGCAGCCAAGTG-3'
G2_neo2_40_1F_seq27_42R	5'-CACTTGGCTGCAGTCTATCATCNNKACCGGTACCTACGACCC-3'
G2_neo2_40_1F_seq27_43	5'-GAAGATGATAGACTGCAGCCAA-3'
G2_neo2_40_1F_seq27_43R	5'-TTGGCTGCAGTCTATCATCTTCNNKGGTACCTACGACCCGGA-3'
G2_neo2_40_1F_seq27_44	5'-GGTGAAGATGATAGACTGCAGCC-3'
G2_neo2_40_1F_seq27_44R	5'-GGCTGCAGTCTATCATCTTCACCNKACCTACGACCCGGACATG-3'
G2_neo2_40_1F_seq27_45	5'-ACCGGTGAAGATGATAGACTGC-3'
G2_neo2_40_1F_seq27_45R	5'-GCAGTCTATCATCTTCACCGTNNKTAGCACC GGACATGGT-3'
G2_neo2_40_1F_seq27_46	5'-GGTACCGGTGAAGATGATAGACT-3'
G2_neo2_40_1F_seq27_46R	5'-AGTCTATCATCTTCACCGGTACCNKAGACCCGGACATGGTTAAAGA-3'
G2_neo2_40_1F_seq27_47	5'-GTAGGTACCGGTGAAGATGATAGA-3'
G2_neo2_40_1F_seq27_47R	5'-TCTATCATCTTCACCGGTACCTACNNKCCGGACATGGTTAAAGAAGC-3'
G2_neo2_40_1F_seq27_48	5'-GTCGTAGGTACCGGTGAAGA-3'
G2_neo2_40_1F_seq27_48R	5'-TCTTCACCGGTACCTACGACNNKAGACATGGTTAAAGAAGCGGTT-3'
G2_neo2_40_1F_seq27_49	5'-CGGGTCGTAGGTACCGGT-3'
G2_neo2_40_1F_seq27_49R	5'-ACCGGTACCTACGACCCGNNKATGGTTAAAGAAGCGGTTAAACTG-3'
G2_neo2_40_1F_seq27_50	5'-GTCCGGGTCTAGGTACCG-3'
G2_neo2_40_1F_seq27_50R	5'-CGGTACCTACGACCCGGACNNKGTAAAGAAGCGGTTAAACTGGC-3'
G2_neo2_40_1F_seq27_51	5'-CATGTCCGGTCTAGGTA-3'
G2_neo2_40_1F_seq27_51R	5'-TACCTACGACCCGGACATGNKAAAGAAGCGGTTAAACTGGC-3'
G2_neo2_40_1F_seq27_52	5'-AACCATGTCCGGTCTGA-3'
G2_neo2_40_1F_seq27_52R	5'-TACGACCCGGACATGGTTNNKGAAGCGGTTAAACTGGCTG-3'
G2_neo2_40_1F_seq27_53	5'-TTTAACCATGTCCGGTCTGT-3'
G2_neo2_40_1F_seq27_53R	5'-ACGACCCGGACATGGTTAAANNKCGGTTAAACTGGCTGACG-3'
G2_neo2_40_1F_seq27_54	5'-TTCCTTAACCATGTCCGGTCTG-3'
G2_neo2_40_1F_seq27_54R	5'-CGACCCGGACATGGTTAAAGAANNKGTAAACTGGCTGACGAAATTGA-3'
G2_neo2_40_1F_seq27_55	5'-CGCTTCTTTAACCATGTCCGG-3'
G2_neo2_40_1F_seq27_55R	5'-CCGGACATGGTTAAAGAAGCGNNKAAACTGGCTGACGAAATTGAG-3'
G2_neo2_40_1F_seq27_56	5'-AACCGCTTCTTTAACCATGTCC-3'
G2_neo2_40_1F_seq27_56R	5'-GGACATGGTTAAAGAAGCGGTTNNKCTGGCTGACGAAATTGAGGA-3'
G2_neo2_40_1F_seq27_57	5'-TTTAACCGCTTCTTTAACCATGTC-3'
G2_neo2_40_1F_seq27_57R	5'-GACATGGTTAAAGAAGCGGTTAAANNKGTGACGAAATTGAGGATGAAA-3'
G2_neo2_40_1F_seq27_58	5'-CAGTTTAACCGCTTCTTTAACCAT-3'
G2_neo2_40_1F_seq27_58R	5'-ATGGTTAAAGAAGCGGTTAAACTGNNKAGACGAAATTGAGGATGAAATGCG-3'
G2_neo2_40_1F_seq27_59	5'-AGCCAGTTTAACCGCTTCTT-3'
G2_neo2_40_1F_seq27_59R	5'-AAGAAGCGGTTAAACTGGCTNNKGAATGAGGATGAAATGCGTAAG-3'
G2_neo2_40_1F_seq27_60	5'-GTCAGCCAGTTTAACCGCTT-3'
G2_neo2_40_1F_seq27_60R	5'-AAGCGGTTAAACTGGCTGACNNKATTGAGGATGAAATGCGTAAGC-3'
G2_neo2_40_1F_seq27_61	5'-TTCGTGAGCCAGTTTAACCGC-3'
G2_neo2_40_1F_seq27_61R	5'-GCGGTTAAACTGGCTGACGAANNKAGGATGAAATGCGTAAGCG-3'
G2_neo2_40_1F_seq27_62	5'-AATTTTCGTGAGCCAGTTTAACCG-3'
G2_neo2_40_1F_seq27_62R	5'-CGGTTAAACTGGCTGACGAAATTNNKATGAAATGCGTAAGCGTGG-3'
G2_neo2_40_1F_seq27_63	5'-CTCAATTTTCGTGAGCCAGTTTA-3'
G2_neo2_40_1F_seq27_63R	5'-TAAACTGGCTGACGAAATTGAGNNKGAATGCGTAAGCGTGGTATC-3'
G2_neo2_40_1F_seq27_64	5'-ATCCTCAATTTTCGTGAGCCAG-3'
G2_neo2_40_1F_seq27_64R	5'-CTGGCTGACGAAATTGAGGATNNKATGCGTAAGCGTGGTATCG-3'
G2_neo2_40_1F_seq27_65	5'-TTCATCTCAATTTTCGTGAGCCA-3'
G2_neo2_40_1F_seq27_65R	5'-TGGCTGACGAAATTGAGGATGAANNKCGTAAGCGTGGTATCGACA-3'
G2_neo2_40_1F_seq27_66	5'-CATTTTCATCTCAATTTTCGTGAGCC-3'
G2_neo2_40_1F_seq27_66R	5'-GGCTGACGAAATTGAGGATGAAATGNNKAGCGTGGTATCGACACC-3'

G2_neo2_40_1F_seq27_67	5'-ACGCATTTCATCCTCAATTCGT-3'
G2_neo2_40_1F_seq27_67R	5'-ACGAAATTGAGGATGAAATGCGTNNKCGTGGTATCGACACCGAA-3'
G2_neo2_40_1F_seq27_68	5'-CTTACGCATTTCATCCTCAATTCGT-3'
G2_neo2_40_1F_seq27_68R	5'-CGAAATTGAGGATGAAATGCGTAAGNNKGGTATCGACACCGAAGATTACG-3'
G2_neo2_40_1F_seq27_69	5'-ACGCTTACGCATTTCATCCTC-3'
G2_neo2_40_1F_seq27_69R	5'-GAGGATGAAATGCGTAAGCGTNNKATCGACACCGAAGATTACGT-3'
G2_neo2_40_1F_seq27_70	5'-ACCACGCTTACGCATTTCAT-3'
G2_neo2_40_1F_seq27_70R	5'-ATGAAATGCGTAAGCGTGGTNNKGACACCGAAGATTACGTTTCTAAC-3'
G2_neo2_40_1F_seq27_71	5'-GATACCACGCTTACGCATTTC-3'
G2_neo2_40_1F_seq27_71R	5'-TGAAATGCGTAAGCGTGGTATCANNKACCGAAGATTACGTTTCTAACCT-3'
G2_neo2_40_1F_seq27_72	5'-GTCGATACACGCTTACGCA-3'
G2_neo2_40_1F_seq27_72R	5'-TGCCTAAGCGTGGTATCGACNNKGAAGATTACGTTTCTAACCTGCG-3'
G2_neo2_40_1F_seq27_73	5'-GGTGTGATACACGCTTA-3'
G2_neo2_40_1F_seq27_73R	5'-TAAGCGTGGTATCGACACNNKGAATTACGTTTCTAACCTGCGTG-3'
G2_neo2_40_1F_seq27_74	5'-TTCGGTGTGATACACGCG-3'
G2_neo2_40_1F_seq27_74R	5'-GCGTGGTATCGACACCGAANNKTAACGTTTCTAACCTGCGTGT-3'
G2_neo2_40_1F_seq27_75	5'-ATCTTCGGTGTGATACACG-3'
G2_neo2_40_1F_seq27_75R	5'-CGTGGTATCGACACCGAAGATNNKGTTCCTAACCTGCGTGTATCC-3'
G2_neo2_40_1F_seq27_76	5'-GTAATCTTCGGTGTGATACAC-3'
G2_neo2_40_1F_seq27_76R	5'-GTGGTATCGACACCGAAGATTACNNKCTAACCTGCGGTATATCCTG-3'
G2_neo2_40_1F_seq27_77	5'-AACGTAATCTTCGGTGTGAT-3'
G2_neo2_40_1F_seq27_77R	5'-ATCGACACCGAAGATTACGTTNNKAACCTGCGGTATATCCTGC-3'
G2_neo2_40_1F_seq27_78	5'-AGAAACGTAATCTTCGGTGTGCA-3'
G2_neo2_40_1F_seq27_78R	5'-TCGACACCGAAGATTACGTTTCTNNKTCGCGTGTATCCTGCAAG-3'
G2_neo2_40_1F_seq27_79	5'-GTTAGAAACGTAATCTTCGGTGTG-3'
G2_neo2_40_1F_seq27_79R	5'-GACACCGAAGATTACGTTTCTAACNNKCGTGTATCCTGCAAGAACTG-3'
G2_neo2_40_1F_seq27_80	5'-CAGGTTAGAAACGTAATCTTCGGT-3'
G2_neo2_40_1F_seq27_80R	5'-ACCGAAGATTACGTTTCTAACCTGNNKGTATCCTGCAAGAACTGGC-3'
G2_neo2_40_1F_seq27_81	5'-ACGCAGGTTAGAAACGTAATCTT-3'
G2_neo2_40_1F_seq27_81R	5'-AAGATTACGTTTCTAACCTGCGTNNKATCCTGCAAGAACTGGCC-3'
G2_neo2_40_1F_seq27_82	5'-AACACGAGGTTAGAAACGTA-3'
G2_neo2_40_1F_seq27_82R	5'-TACGTTTCTAACCTGCGTGTNNKCTGCAAGAACTGGCCCTC-3'
G2_neo2_40_1F_seq27_83	5'-GATAACACGAGGTTAGAAACGT-3'
G2_neo2_40_1F_seq27_83R	5'-ACGTTTCTAACCTGCGTGTATCANNKCAAGAACTGGCCCTCGAG-3'
G2_neo2_40_1F_seq27_84	5'-CAGGATAACACGAGGTTAGAA-3'
G2_neo2_40_1F_seq27_84R	5'-TTCTAACCTGCGTGTATCCTGNNKGAAGTGGCCCTCGAGGG-3'
G2_neo2_40_1F_seq27_85	5'-TTGCAGGATAACACGAGGT-3'
G2_neo2_40_1F_seq27_85R	5'-ACCTGCGTGTATCCTGCAANNKCTGGCCCTCGAGGGTG-3'
G2_neo2_40_1F_seq27_86	5'-TTCTTGAGGATAACACGAG-3'
G2_neo2_40_1F_seq27_86R	5'-CTGCGTGTATCCTGCAAGAANNKGCCTCGAGGGTGAG-3'
G2_neo2_40_1F_seq29_001	5'-CATATGCGAAGCCGACCCT-3'
G2_neo2_40_1F_seq29_01R	5'-AGGGTCGGCTTCGCATATGNNKGACTACTACTCTAACCTCAAAGTTATCC-3'
G2_neo2_40_1F_seq29_002	5'-CTCCATATGCGAAGCCGAC-3'
G2_neo2_40_1F_seq29_02R	5'-GTCGGCTTCGCATATGGAGNNKTACTACTCTAACCTCAAAGTTATCCTG-3'
G2_neo2_40_1F_seq29_003	5'-GTCCTCCATATGCGAAGCCG-3'
G2_neo2_40_1F_seq29_03R	5'-CGGCTTCGCATATGGAGGACNNKTACTCTAACCTCAAAGTTATCCTGG-3'
G2_neo2_40_1F_seq29_004	5'-GTAGTCCTCATATGCGAAGCC-3'
G2_neo2_40_1F_seq29_04R	5'-GGCTTCGCATATGGAGGACTACNNKCTAACCTCAAAGTTATCCTGGAA-3'
G2_neo2_40_1F_seq29_005	5'-GTAGTAGTCTCCATATGCGAAG-3'
G2_neo2_40_1F_seq29_05R	5'-CTTCGCATATGGAGGACTACTACNNKAACCTCAAAGTTATCCTGGAAGA-3'
G2_neo2_40_1F_seq29_006	5'-AGAGTAGTAGTCTCCATATGCGA-3'
G2_neo2_40_1F_seq29_06R	5'-TCGCATATGGAGGACTACTACTCTNNKCTCAAAGTTATCCTGGAAGAGCT-3'
G2_neo2_40_1F_seq29_007	5'-GTTAGAGTAGTAGTCTCCATATGCG-3'
G2_neo2_40_1F_seq29_07R	5'-CGCATATGGAGGACTACTACTCTAACNNKAAAGTTATCCTGGAAGAGCTGG-3'
G2_neo2_40_1F_seq29_008	5'-GAGGTTAGAGTAGTAGTCTCCAT-3'
G2_neo2_40_1F_seq29_08R	5'-ATGGAGGACTACTACTCTAACCTCANNKGTATCCTGGAAGAGCTGGC-3'
G2_neo2_40_1F_seq29_009	5'-TTTGAGGTTAGAGTAGTAGTCTCCA-3'
G2_neo2_40_1F_seq29_09R	5'-TGGAGGACTACTACTCTAACCTCAAANNKATCCTGGAAGAGCTGGCA-3'
G2_neo2_40_1F_seq29_010	5'-AACTTTGAGGTTAGAGTAGTAGTCTCT-3'
G2_neo2_40_1F_seq29_10R	5'-AGGACTACTACTCTAACCTCAAAGTTNNKCTGGAAGAGCTGGCACG-3'
G2_neo2_40_1F_seq29_011	5'-GATAACTTTGAGGTTAGAGTAGTAGTCTCT-3'
G2_neo2_40_1F_seq29_11R	5'-AGGACTACTACTCTAACCTCAAAGTTATCANNKGAAGAGCTGGCACGTGA-3'
G2_neo2_40_1F_seq29_012	5'-CAGGATAACTTTGAGGTTAGAGTAGTA-3'
G2_neo2_40_1F_seq29_12R	5'-TACTACTCTAACCTCAAAGTTATCCTGNNKGAGCTGGCACGTGAAATG-3'
G2_neo2_40_1F_seq29_013	5'-TCCAGGATAACTTTGAGGTTAGAG-3'
G2_neo2_40_1F_seq29_13R	5'-CTCTAACCTCAAAGTTATCCTGGAANNKCTGGCACGTGAAATGGAGC-3'
G2_neo2_40_1F_seq29_014	5'-CTCTCCAGGATAACTTTGAGGTT-3'
G2_neo2_40_1F_seq29_14R	5'-AACCTCAAAGTTATCCTGGAAGAGNNKGCACGTGAAATGGAGCG-3'

G2_neo2_40_1F_seq29_015	5'-CAGCTCTTCCAGGATAACTTTGAG-3'
G2_neo2_40_1F_seq29_15R	5'-CTCAAAGTTATCCTGGAAGAGCTGNNKCGTGAAATGGAGCGTAATGG-3'
G2_neo2_40_1F_seq29_016	5'-TGCCAGCTCTTCCAGGAT-3'
G2_neo2_40_1F_seq29_16R	5'-ATCCTGGAAGAGCTGGCANNKGAATGGAGCGTAATGGTCTG-3'
G2_neo2_40_1F_seq29_017	5'-ACGTGCCAGCTCTTCCAG-3'
G2_neo2_40_1F_seq29_17R	5'-CTGGAAGAGCTGGCAGCTNNKCTGAGCGTAATGGTCTGTC-3'
G2_neo2_40_1F_seq29_018	5'-TTCACGTGCCAGCTCTTCC-3'
G2_neo2_40_1F_seq29_18R	5'-GGAAGAGCTGGCAGCTGAANNKGAGCGTAATGGTCTGTCTGA-3'
G2_neo2_40_1F_seq29_019	5'-CATTTACGTGCCAGCTCT-3'
G2_neo2_40_1F_seq29_19R	5'-AGAGCTGGCAGCTGAAATGNNKCGTAATGGTCTGTCTGACAAAG-3'
G2_neo2_40_1F_seq29_020	5'-CTCCATTTCAGTGCCAGCT-3'
G2_neo2_40_1F_seq29_20R	5'-AGCTGGCAGCTGAAATGGAGNNKAAATGGTCTGTCTGACAAAGCG-3'
G2_neo2_40_1F_seq29_021	5'-ACGCTCCATTTCACGTGCC-3'
G2_neo2_40_1F_seq29_21R	5'-GGCAGTGAAATGGAGCGTNNKGGTCTGTCTGACAAAGCGG-3'
G2_neo2_40_1F_seq29_022	5'-ATTACGCTCCATTTCACGTGCC-3'
G2_neo2_40_1F_seq29_22R	5'-GGCAGTGAAATGGAGCGTAATNNKCTGTCTGACAAAGCGGAAGA-3'
G2_neo2_40_1F_seq29_023	5'-ACCATTACGCTCCATTTCACGT-3'
G2_neo2_40_1F_seq29_23R	5'-ACGTGAAATGGAGCGTAATGGTNNKCTGACAAAGCGGAAGAATGG-3'
G2_neo2_40_1F_seq29_024	5'-CAGACCATTACGCTCCATTCA-3'
G2_neo2_40_1F_seq29_24R	5'-TGAAATGGAGCGTAATGGTCTGNNKGACAAAGCGGAAGAATGGC-3'
G2_neo2_40_1F_seq29_025	5'-AGACAGACCATTACGCTCCAT-3'
G2_neo2_40_1F_seq29_25R	5'-ATGGAGCGTAATGGTCTGTCTNNKAAAGCGGAAGAATGGCGT-3'
G2_neo2_40_1F_seq29_026	5'-GTCAGACAGACCATTACGCTC-3'
G2_neo2_40_1F_seq29_26R	5'-GAGCGTAATGGTCTGTCTGACNNKGGCGGAAGAATGGCGTCA-3'
G2_neo2_40_1F_seq29_027	5'-TTTGTGACAGACCATTACGCT-3'
G2_neo2_40_1F_seq29_27R	5'-AGCGTAATGGTCTGTCTGACAAANNKGAAGAATGGCGTCAGTGGA-3'
G2_neo2_40_1F_seq29_028	5'-CGCTTTGTGACAGACCAT-3'
G2_neo2_40_1F_seq29_28R	5'-ATGGTCTGTCTGACAAAGCGNNKGAATGGCGTCAGTGAAAAA-3'
G2_neo2_40_1F_seq29_029	5'-TTCCGCTTTGTGACAGACCC-3'
G2_neo2_40_1F_seq29_29R	5'-GGTCTGTCTGACAAAGCGGAANNKTTGGCGTCAGTGAAAAAGA-3'
G2_neo2_40_1F_seq29_030	5'-TTCTTCCGCTTTGTGACAGC-3'
G2_neo2_40_1F_seq29_30R	5'-CTGTCTGACAAAGCGGAANNKCGTCAGTGAAAAAGATTGTAGA-3'
G2_neo2_40_1F_seq29_031	5'-CCATTCTTCCGCTTTGTGACA-3'
G2_neo2_40_1F_seq29_31R	5'-TCTGACAAAGCGGAAGAATGGNNKAGTGGAAAAAGATTGTAGAACGC-3'
G2_neo2_40_1F_seq29_032	5'-ACGCCATTCTTCCGCTTT-3'
G2_neo2_40_1F_seq29_32R	5'-AAAGCGGAAGAATGGCGTNNKGGAAAAAGATTGTAGAACGCAT-3'
G2_neo2_40_1F_seq29_033	5'-CTGACGCCATTCTTCCGCT-3'
G2_neo2_40_1F_seq29_33R	5'-AGCGGAAGAATGGCGTCAGNNKAAAAAGATTGTAGAACGCATTCGT-3'
G2_neo2_40_1F_seq29_034	5'-CCACTGACGCCATTCTTCCG-3'
G2_neo2_40_1F_seq29_34R	5'-CGGAAGAATGGCGTCAGTGGNNKAAAGATTGTAGAACGCATTCGTC-3'
G2_neo2_40_1F_seq29_035	5'-TTTCCACTGACGCCATTCTT-3'
G2_neo2_40_1F_seq29_35R	5'-AAGAATGGCGTCAGTGGAANNKATTGTAGAACGCATTCGTGAG-3'
G2_neo2_40_1F_seq29_036	5'-CTTTTCCACTGACGCCATTC-3'
G2_neo2_40_1F_seq29_36R	5'-GAATGGCGTCAGTGGAANNKGTAGAACGCATTCGTGAGATTC-3'
G2_neo2_40_1F_seq29_037	5'-AATCTTTTCCACTGACGCCAT-3'
G2_neo2_40_1F_seq29_37R	5'-ATGGCGTCAGTGGAAGAAGATTNNKGAACGCATTCGTGAGATTCG-3'
G2_neo2_40_1F_seq29_038	5'-TACAATCTTTTCCACTGACGCC-3'
G2_neo2_40_1F_seq29_38R	5'-GGCGTCAGTGGAAGAAGATTGANNKCGCATTCGTGAGATTCGTTTC-3'
G2_neo2_40_1F_seq29_039	5'-TTCTACAATCTTTTCCACTGACGC-3'
G2_neo2_40_1F_seq29_39R	5'-GCGTCAGTGGAAGAAGATTGTAGAANNKATTCGTGAGATTCGTTCTAACAAC-3'
G2_neo2_40_1F_seq29_040	5'-GCGTTCTACAATCTTTTCCACTG-3'
G2_neo2_40_1F_seq29_40R	5'-CAGTGGAAGAAGATTGTAGAACGCNNKCGTCAGATTCGTTCTAACAATC-3'
G2_neo2_40_1F_seq29_041	5'-AATGCGTTCTACAATCTTTTCCAC-3'
G2_neo2_40_1F_seq29_41R	5'-GTGGAAAAAGATTGTAGAACGCATTNNKAGATTCGTTCTAACAATCTGAC-3'
G2_neo2_40_1F_seq29_042	5'-ACGAATGCGTTCTACAATCTTTT-3'
G2_neo2_40_1F_seq29_42R	5'-AAAAAGATTGTAGAACGCATTCGTNNKATTCGTTCTAACAACCTTGACCT-3'
G2_neo2_40_1F_seq29_043	5'-CTGACGAATGCGTTCTACAATC-3'
G2_neo2_40_1F_seq29_43R	5'-GATTGTAGAACGCATTCGTGAGNNKCGTTCTAACAACCTTGACCTCA-3'
G2_neo2_40_1F_seq29_044	5'-AATCTGACGAATGCGTTCTACAA-3'
G2_neo2_40_1F_seq29_44R	5'-TTGTAGAACGCATTCGTGAGATTNNKCTAACAACCTTGACCTCAATGAG-3'
G2_neo2_40_1F_seq29_045	5'-ACGAATCTGACGAATGCGTT-3'
G2_neo2_40_1F_seq29_45R	5'-AACGCATTCGTGAGATTCGTNNKAAACAACCTTGACCTCAATGAGG-3'
G2_neo2_40_1F_seq29_046	5'-AGAACGAATCTGACGAATGCGT-3'
G2_neo2_40_1F_seq29_46R	5'-ACGCATTCGTGAGATTCGTTCTNNKAACTCTGACCTCAATGAGGC-3'
G2_neo2_40_1F_seq29_047	5'-GTTAGAACGAATCTGACGAATGCG-3'
G2_neo2_40_1F_seq29_47R	5'-CGCATTCGTGAGATTCGTTCTAACNNKCTGACCTCAATGAGGCGA-3'
G2_neo2_40_1F_seq29_048	5'-GTTGTTAGAACGAATCTGACGAAT-3'
G2_neo2_40_1F_seq29_48R	5'-ATTGTCGAGATTCGTTCTAACAACNNKAGCTCAATGAGGCGAAGG-3'

G2_neo2_40_1F_seq29_049	5'-AGAGTTGTTAGAACGAATCTGACGA-3'
G2_neo2_40_1F_seq29_49R	5'-TCGTCAGATTCTGTTCTAACAACTCTNNKCTCAATGAGGCGAAGGAGC-3'
G2_neo2_40_1F_seq29_050	5'-GTCAGAGTTGTTAGAACGAATCTGA-3'
G2_neo2_40_1F_seq29_50R	5'-TCAGATTCTGTTCTAACAACTCTGACNNKAATGAGGCGAAGGAGCTG-3'
G2_neo2_40_1F_seq29_051	5'-GAGGTCAGAGTTGTTAGAACGAA-3'
G2_neo2_40_1F_seq29_51R	5'-TTCGTTCTAACAACTCTGACCTNNKGAGGCGAAGGAGCTGC-3'
G2_neo2_40_1F_seq29_052	5'-ATTGAGGTCAGAGTTGTTAGAACGA-3'
G2_neo2_40_1F_seq29_52R	5'-TCGTTCTAACAACTCTGACCTCAATNNKCGAAGGAGCTGCTCAA-3'
G2_neo2_40_1F_seq29_053	5'-CTCATTGAGGTCAGAGTTGTTAGAA-3'
G2_neo2_40_1F_seq29_53R	5'-TTCTAACAACTCTGACCTCAATGAGNNKAAGGAGCTGCTCAACCG-3'
G2_neo2_40_1F_seq29_054	5'-CGCCTCATGAGGTCAGAGT-3'
G2_neo2_40_1F_seq29_54R	5'-ACTCTGACCTCAATGAGGCGNNKGAGCTGCTCAACCGTCTG-3'
G2_neo2_40_1F_seq29_055	5'-CTTCGCCTCATGAGGTCAG-3'
G2_neo2_40_1F_seq29_55R	5'-CTGACCTCAATGAGGCGAAGNNKCTGCTCAACCGTCTGATCA-3'
G2_neo2_40_1F_seq29_056	5'-CTCCTTCGCCTCATGAGT-3'
G2_neo2_40_1F_seq29_56R	5'-ACCTCAATGAGGCGAAGGAGNNKCTCAACCGTCTGATCACCTAC-3'
G2_neo2_40_1F_seq29_057	5'-CAGCTCCTTCGCCTCATG-3'
G2_neo2_40_1F_seq29_57R	5'-CAATGAGGCGAAGGAGCTGNNKAACCGTCTGATCACCTACATC-3'
G2_neo2_40_1F_seq29_058	5'-GAGCAGCTCCTTCGCCTC-3'
G2_neo2_40_1F_seq29_58R	5'-GAGGCGAAGGAGCTGCTNNKCGTCTGATCACCTACATCCAG-3'
G2_neo2_40_1F_seq29_059	5'-GTTGAGCAGCTCCTTCGCC-3'
G2_neo2_40_1F_seq29_59R	5'-GGCGAAGGAGCTGCTCAACNNKCTGATCACCTACATCCAGTCTC-3'
G2_neo2_40_1F_seq29_060	5'-ACGGTTGAGCAGCTCCTT-3'
G2_neo2_40_1F_seq29_60R	5'-AAGGAGCTGCTCAACCGTNNKATCACCTACATCCAGTCTCAAAT-3'
G2_neo2_40_1F_seq29_061	5'-CAGACGGTTGAGCAGCTC-3'
G2_neo2_40_1F_seq29_61R	5'-GAGCTGCTCAACCGTCTGNNKACCTACATCCAGTCTCAAATCTTC-3'
G2_neo2_40_1F_seq29_062	5'-GATCAGACGGTTGAGCAGCT-3'
G2_neo2_40_1F_seq29_62R	5'-AGCTGCTCAACCGTCTGATCCTNNKATACATCCAGTCTCAAATCTTCGA-3'
G2_neo2_40_1F_seq29_063	5'-GGTGATCAGACGGTTGAGCA-3'
G2_neo2_40_1F_seq29_63R	5'-TGCTCAACCGTCTGATCACCNNKATCCAGTCTCAAATCTTCGAAATC-3'
G2_neo2_40_1F_seq29_064	5'-GTAGGTGATCAGACGGTTGAGC-3'
G2_neo2_40_1F_seq29_64R	5'-GCTCAACCGTCTGATCACCTACNNKAGTCTCAAATCTTCGAAATCTCC-3'
G2_neo2_40_1F_seq29_065	5'-GATGTAGGTGATCAGACGGTTG-3'
G2_neo2_40_1F_seq29_65R	5'-CAACCGTCTGATCACCTACATCCTNNKCTCAAATCTTCGAAATCTCCGA-3'
G2_neo2_40_1F_seq29_066	5'-CTGGATGTAGGTGATCAGACGG-3'
G2_neo2_40_1F_seq29_66R	5'-CCGTCTGATCACCTACATCCAGNNKCAAATCTTCGAAATCTCCGAACG-3'
G2_neo2_40_1F_seq29_067	5'-AGACTGGATGTAGGTGATCAGAC-3'
G2_neo2_40_1F_seq29_67R	5'-GTCTGATCACCTACATCCAGTCTNNKATCTTCGAAATCTCCGAACGT-3'
G2_neo2_40_1F_seq29_068	5'-TTGAGACTGGATGTAGGTGATCA-3'
G2_neo2_40_1F_seq29_68R	5'-TGATCACCTACATCCAGTCTCAANNKTTGAAATCTCCGAACGTATCC-3'
G2_neo2_40_1F_seq29_069	5'-GATTTGAGACTGGATGTAGGTGAT-3'
G2_neo2_40_1F_seq29_69R	5'-ATCACCTACATCCAGTCTCAAATCCTNNKGAATCTCCGAACGTATCCGT-3'
G2_neo2_40_1F_seq29_070	5'-GAAGATTTGAGACTGGATGTAGGTG-3'
G2_neo2_40_1F_seq29_70R	5'-CACCTACATCCAGTCTCAAATCTTCNNKATCTCCGAACGTATCCGTG-3'
G2_neo2_40_1F_seq29_071	5'-TTCGAAGATTTGAGACTGGATGTA-3'
G2_neo2_40_1F_seq29_71R	5'-TACATCCAGTCTCAAATCTTCGAANNKTCGAAACGTATCCGTGAAAC-3'
G2_neo2_40_1F_seq29_072	5'-GATTTGGAAGATTTGAGACTGGATG-3'
G2_neo2_40_1F_seq29_72R	5'-CATCCAGTCTCAAATCTTCGAAATCCTNNKGAACGTATCCGTGAAACTGAC-3'
G2_neo2_40_1F_seq29_073	5'-GGAGATTTGGAAGATTTGAGACTGG-3'
G2_neo2_40_1F_seq29_73R	5'-CCAGTCTCAAATCTTCGAAATCTCCTNNKGTATCCGTGAAACTGACCA-3'
G2_neo2_40_1F_seq29_074	5'-TTCGGAGATTTGGAAGATTTGAGA-3'
G2_neo2_40_1F_seq29_74R	5'-TCTCAAATCTTCGAAATCTCCGAANNKATCCGTGAAACTGACCAGG-3'
G2_neo2_40_1F_seq29_075	5'-ACGTTTCGGAGATTTGGAAGAT-3'
G2_neo2_40_1F_seq29_75R	5'-ATCTTCGAAATCTCCGAACGTNNKCGTGAAACTGACCAGGAGAA-3'
G2_neo2_40_1F_seq29_076	5'-GATACGTTTCGGAGATTTGGAAGA-3'
G2_neo2_40_1F_seq29_76R	5'-TCTTCGAAATCTCCGAACGTATCCTNNKGAACGTGACCAGGAGAAAAAGG-3'
G2_neo2_40_1F_seq29_077	5'-ACGGATACGTTTCGGAGATTTTC-3'
G2_neo2_40_1F_seq29_77R	5'-GAAATCTCCGAACGTATCCGTNNKACTGACCAGGAGAAAAAGGAG-3'
G2_neo2_40_1F_seq29_078	5'-TTCACGGATACGTTTCGGAGA-3'
G2_neo2_40_1F_seq29_78R	5'-TCTCCGAACGTATCCGTGAANNKGAACGAGGAGAAAAAGGAGGAA-3'
G2_neo2_40_1F_seq29_079	5'-AGTTTCACGGATACGTTTCGGA-3'
G2_neo2_40_1F_seq29_79R	5'-TCCGAACGTATCCGTGAAACTNNKAGGAGAGAAAAAGGAGGAATCTTG-3'
G2_neo2_40_1F_seq29_080	5'-GTCAGTTTCACGGATACGTTTCG-3'
G2_neo2_40_1F_seq29_80R	5'-CGAACGTATCCGTGAAACTGACNNKAGGAGAAAAAGGAGGAATCTTGGAAG-3'
G2_neo2_40_1F_seq29_081	5'-CTGGTCAGTTTCACGGATACGT-3'
G2_neo2_40_1F_seq29_81R	5'-ACGTATCCGTGAAACTGACGAGNNKAAAAAGGAGGAATCTTGGAAGAAG-3'
G2_neo2_40_1F_seq29_082	5'-CTCCTGGTCAGTTTCACGGA-3'
G2_neo2_40_1F_seq29_82R	5'-TCCGTGAAACTGACCAGGAGNNKAAGGAGGAATCTTGGAAGAAGTG-3'

G2_neo2_40_1F_seq29_083	5'-TTTCTCCTGGTCAGTTTCACGG-3'
G2_neo2_40_1F_seq29_83R	5'-CCGTGAAACTGACCAGGAGAAANNKGAGGAATCTTGAAGAAGTGGC-3'
G2_neo2_40_1F_seq29_084	5'-CTTTTCTCCTGGTCAGTTTCACG-3'
G2_neo2_40_1F_seq29_84R	5'-CGTGAAACTGACCAGGAGAAAAAGNNKGAAATCTTGAAGAAGTGGCAAC-3'
G2_neo2_40_1F_seq29_085	5'-CTCCTTTTCTCCTGGTCAGTT-3'
G2_neo2_40_1F_seq29_85R	5'-AACTGACCAGGAGAAAAAGGAGNNKCTTGAAGAAGTGGCAACT-3'
G2_neo2_40_1F_seq29_086	5'-TTCCTCCTTTTCTCCTGGTCA-3'
G2_neo2_40_1F_seq29_86R	5'-TGACCAGGAGAAAAAGGAGGAANNKTGGAAGAAGTGGCAACTCC-3'
G2_neo2_40_1F_seq29_087	5'-AGATTCTCCTTTTCTCCTGGT-3'
G2_neo2_40_1F_seq29_87R	5'-ACCAGGAGAAAAAGGAGGAATCTNNKAAGAAGTGGCAACTCCTCC-3'
G2_neo2_40_1F_seq29_088	5'-CCAAGATTCTCCTTTTCTCCTG-3'
G2_neo2_40_1F_seq29_88R	5'-CAGGAGAAAAAGGAGGAATCTTGGNNKAAGTGGCAACTCCTCCTCG-3'
G2_neo2_40_1F_seq29_089	5'-CTTCCAAGATTCTCCTTTTCTCC-3'
G2_neo2_40_1F_seq29_89R	5'-GGAGAAAAAGGAGGAATCTTGAAGNNKTGGCAACTCCTCCTCGA-3'
G2_neo2_40_1F_seq29_090	5'-CTTCTTCCAAGATTCTCCTTTTTC-3'
G2_neo2_40_1F_seq29_90R	5'-GAAAAAGGAGGAATCTTGAAGAAGNNKCAACTCCTCCTCGAACACG-3'
G2_neo2_40_1F_seq29_091	5'-CCACTTCTTCCAAGATTCTCCT-3'
G2_neo2_40_1F_seq29_91R	5'-AGGAGGAATCTTGAAGAAGTGGNNKCTCCTCCTCGAACACGC-3'
G2_neo2_40_1F_seq29_092	5'-TTGCCACTTCTTCCAAGATTCT-3'
G2_neo2_40_1F_seq29_92R	5'-AGGAATCTTGAAGAAGTGGCAANNKCTCCTCGAACACGCGC-3'
G2_neo2_40_1F_seq29_093	5'-GAGTTGCCACTTCTTCCAAGAT-3'
G2_neo2_40_1F_seq29_93R	5'-ATCTTGAAGAAGTGGCAACTCANNKCTCGAACACGCGCTCC-3'
G2_neo2_40_1F_seq29_094	5'-GAGGAGTGGCACTTCTTCCA-3'
G2_neo2_40_1F_seq29_94R	5'-TGAAGAAGTGGCAACTCCTCANNKGAACACGCGTCTCTGG-3'
G2_neo2_40_1F_seq29_095	5'-GAGGAGGAGTTGCCACTTCT-3'
G2_neo2_40_1F_seq29_95R	5'-AGAAAGTGGCAACTCCTCCTCANNKACGCGCTCCTGGAC-3'
G2_neo2_40_1F_seq29_096	5'-TTCGAGGAGGAGTTGCCAC-3'
G2_neo2_40_1F_seq29_96R	5'-GTGGCAACTCCTCCTCGAANNKGGCTCCTGGACGTG-3'
G2_neo2_40_1F_seq29_097	5'-GTGTTTCGAGGAGGAGTTGCC-3'
G2_neo2_40_1F_seq29_97R	5'-GGCAACTCCTCCTCGAACACNNKCTCTGGACGTGCTGATG-3'
G2_neo2_40_1F_seq29_098	5'-CGCGTGTTCGAGGAGGAG-3'
G2_neo2_40_1F_seq29_98R	5'-CTCCTCCTCGAACACGCGNNKCTGGACGTGCTGATGCT-3'
G2_neo2_40_1F_seq29_099	5'-GAGCGCGTGTTCGAGGAG-3'
G2_neo2_40_1F_seq29_99R	5'-CTCCTCGAACACGCGCTCANNKACGTGCTGATGCTGCT-3'
G2_neo2_40_1F_seq29_100	5'-CAGGAGCGCGTGTTCGAG-3'
G2_neo2_40_1F_seq29_100R	5'-CTCGAACACGCGCTCCTGNNKGTGCTGATGCTGCTGAATG-3'
G2_neo2_40_1F_seq29_101	5'-GTCCAGGAGCGCGTGTTC-3'
G2_neo2_40_1F_seq29_101R	5'-GAACACGCGCTCCTCGAACNNKCTGATGCTGCTGAATGATCTC-3'
G2_neo2_40_1F_seq29_102	5'-CACGTCCAGGAGCGCGT-3'
G2_neo2_40_1F_seq29_102R	5'-CACGCGCTCCTGGACGTGNNKATGCTGCTGAATGATCTCGA-3'
G2_neo2_40_1F_seq29_103	5'-CAGCACGTCCAGGAGCGC-3'
G2_neo2_40_1F_seq29_103R	5'-GCGCTCCTGGACGTGCTGNNKCTGCTGAATGATCTCGAGGG-3'
G2_neo2_40_1F_seq29_104	5'-CATCAGCACGTCCAGGAGC-3'
G2_neo2_40_1F_seq29_104R	5'-GCTCCTGGACGTGCTGATGNNKCTGAATGATCTCGAGGGTGG-3'
G2_neo2_40_1F_seq29_105	5'-CAGCATCAGCACGTCCAG-3'
G2_neo2_40_1F_seq29_105R	5'-CTGGACGTGCTGATGCTGNNKATGATCTCGAGGGTGGAGG-3'
G2_neo2_40_1F_seq29_106	5'-CAGCAGCATCAGCACGTC-3'
G2_neo2_40_1F_seq29_106R	5'-GACGTGCTGATGCTGCTGNNKATCTCGAGGGTGGAGGT-3'
G2_neo2_40_1F_seq36_01	5'-CATATGCGAAGCCGACCCT-3'
G2_neo2_40_1F_seq36_01R	5'-AGGGTCGGCTTCGCATATGNNKAAAAAGAAATTCAGCTGCTCGC-3'
G2_neo2_40_1F_seq36_02	5'-AGGCATATGCGAAGCCGA-3'
G2_neo2_40_1F_seq36_02R	5'-TCGGCTTCGCATATGCCTNNKAAGAAATTCAGCTGCTCGC-3'
G2_neo2_40_1F_seq36_03	5'-TTTAGGCATATGCGAAGCCGA-3'
G2_neo2_40_1F_seq36_03R	5'-TCGGCTTCGCATATGCCTAAANNKAAATTCAGCTGCTCGC-3'
G2_neo2_40_1F_seq36_04	5'-CTTTTATAGCATATGCGAAGCCG-3'
G2_neo2_40_1F_seq36_04R	5'-CGGCTTCGCATATGCCTAAAAAGNNKATTCAGCTGCTCGCGG-3'
G2_neo2_40_1F_seq36_05	5'-TTTCTTTTATAGGCATATGCGAAGCC-3'
G2_neo2_40_1F_seq36_05R	5'-GGCTTCGCATATGCCTAAAAAGAAANNKAGCTGCTCGCGGAA-3'
G2_neo2_40_1F_seq36_06	5'-AATTTTCTTTTATAGGCATATGCGAAGC-3'
G2_neo2_40_1F_seq36_06R	5'-GCTTCGCATATGCCTAAAAAGAAATNNKCTGCTCGCGGAACACG-3'
G2_neo2_40_1F_seq36_07	5'-CTGAATTTTCTTTTATAGGCATATGCGA-3'
G2_neo2_40_1F_seq36_07R	5'-TCGCATATGCCTAAAAAGAAATTCAGNNKCTCGCGGAACACGCG-3'
G2_neo2_40_1F_seq36_08	5'-CAGCTGAATTTTCTTTTATAGGCATATG-3'
G2_neo2_40_1F_seq36_08R	5'-CATATGCCTAAAAAGAAATTCAGCTGNNKCGGAACACGCGCT-3'
G2_neo2_40_1F_seq36_09	5'-GAGCAGCTGAATTTTCTTTTATAGGC-3'
G2_neo2_40_1F_seq36_09R	5'-GCCTAAAAAGAAATTCAGCTGCTCANNKGAACACGCGCTCCTGG-3'
G2_neo2_40_1F_seq36_10	5'-CGCGAGCAGCTGAATTTTC-3'
G2_neo2_40_1F_seq36_10R	5'-GAAATTCAGCTGCTCGCGNNKACGCGCTCCTGGAC-3'

G2_neo2_40_1F_seq36_11	5'-TTCCGCGAGCAGCTGAAT-3'
G2_neo2_40_1F_seq36_11R	5'-ATTGAGCTGCTCGCGGAANNKCGCTCCTGGACGC-3'
G2_neo2_40_1F_seq36_12	5'-GTGTTCCGCGAGCAGTG-3'
G2_neo2_40_1F_seq36_12R	5'-CAGCTGCTCGCGGAACACNNKCTCCTGGACGCGTG-3'
G2_neo2_40_1F_seq36_13	5'-CGCGTGTTCGCGAGCAG-3'
G2_neo2_40_1F_seq36_13R	5'-CTGCTCGCGGAACACGCGNNKCTGGACGCGTGATGAT-3'
G2_neo2_40_1F_seq36_14	5'-GAGCGCGTGTTCGCGAG-3'
G2_neo2_40_1F_seq36_14R	5'-CTCGCGGAACACGCGCTCANNKACGCGTGATGATCCT-3'
G2_neo2_40_1F_seq36_15	5'-CAGGAGCGCGTGTTCGC-3'
G2_neo2_40_1F_seq36_15R	5'-GCGGAACACGCGCTCCTGNNKCGCTGATGATCCTGAACA-3'
G2_neo2_40_1F_seq36_16	5'-GTCCAGGAGCGCGTGTTC-3'
G2_neo2_40_1F_seq36_16R	5'-GAACACGCGCTCCTGGACNNKCTGATGATCCTGAACATCGTTAAA-3'
G2_neo2_40_1F_seq36_17	5'-CGCGTCCAGGAGCGCGTG-3'
G2_neo2_40_1F_seq36_17R	5'-CACGCGCTCCTGGACGCGNNKATGATCCTGAACATCGTTAAACG-3'
G2_neo2_40_1F_seq36_18	5'-CAGCGCGTCCAGGAGCGC-3'
G2_neo2_40_1F_seq36_18R	5'-GCGCTCCTGGACGCGTGNKATCCTGAACATCGTTAAACGAAC-3'
G2_neo2_40_1F_seq36_19	5'-CATCAGCGCTCCAGGAG-3'
G2_neo2_40_1F_seq36_19R	5'-CTCCTGGACGCGTGATGNNKCTGAACATCGTTAAACGAACCTCT-3'
G2_neo2_40_1F_seq36_20	5'-GATCATCAGCGCGTCCAG-3'
G2_neo2_40_1F_seq36_20R	5'-CTGGACGCGTGATGATCANNKACATCGTTAAACGAACCTCTCAG-3'
G2_neo2_40_1F_seq36_21	5'-CAGGATCATCAGCGCGTC-3'
G2_neo2_40_1F_seq36_21R	5'-GACGCGTGATGATCCTGNNKATCGTTAAACGAACCTCTCAGAAC-3'
G2_neo2_40_1F_seq36_22	5'-GTTCCAGGATCATCAGCGCGT-3'
G2_neo2_40_1F_seq36_22R	5'-ACGCGTGATGATCCTGAACNNKGTAAACGAACCTCTCAGAACGC-3'
G2_neo2_40_1F_seq36_23	5'-GATGTTCCAGGATCATCAGCGCG-3'
G2_neo2_40_1F_seq36_23R	5'-CGCGTGATGATCCTGAACATCANNKAAACGAACCTCTCAGAACGC-3'
G2_neo2_40_1F_seq36_24	5'-AACGATGTTCCAGGATCATCAGCG-3'
G2_neo2_40_1F_seq36_24R	5'-CGTGATGATCCTGAACATCGTTNNKACGAACCTCTCAGAACGC-3'
G2_neo2_40_1F_seq36_25	5'-TTTAACGATGTTCCAGGATCATCAGC-3'
G2_neo2_40_1F_seq36_25R	5'-GCTGATGATCCTGAACATCGTTAAANNKAACTCTCAGAACGCGGAAG-3'
G2_neo2_40_1F_seq36_26	5'-CGTTTTAACGATGTTCCAGGATCAT-3'
G2_neo2_40_1F_seq36_26R	5'-ATGATCCTGAACATCGTTAAACGNNKCTCTCAGAACGCGGAAGAAA-3'
G2_neo2_40_1F_seq36_27	5'-GTTTCGTTTTAACGATGTTCCAGGAT-3'
G2_neo2_40_1F_seq36_27R	5'-ATCCTGAACATCGTTAAACGAACNNKAGAACGCGGAAGAAAACT-3'
G2_neo2_40_1F_seq36_28	5'-AGAGTTCGTTTTAACGATGTTCCAGG-3'
G2_neo2_40_1F_seq36_28R	5'-CCTGAACATCGTTAAACGAACCTCTNNKACGCGGAAGAAAACTGG-3'
G2_neo2_40_1F_seq36_29	5'-CTGAGAGTTCGTTTTAACGATGTT-3'
G2_neo2_40_1F_seq36_29R	5'-AACATCGTTAAACGAACCTCTCAGNNKCGGAAGAAAACTGGAGGA-3'
G2_neo2_40_1F_seq36_30	5'-GTTCTGAGAGTTCGTTTTAACGAT-3'
G2_neo2_40_1F_seq36_30R	5'-ATCGTTAAACGAACCTCTCAGAACNNKGAAGAAAACTGGAGGACTATGC-3'
G2_neo2_40_1F_seq36_31	5'-CGCGTCTCAGAGTTCGTT-3'
G2_neo2_40_1F_seq36_31R	5'-AACGAACCTCTCAGAACGCGNNKGAAGAACTGGAGGACTATGCG-3'
G2_neo2_40_1F_seq36_32	5'-TTCCGCGTCTCAGAGTTCG-3'
G2_neo2_40_1F_seq36_32R	5'-CGAAGCTCTCAGAACGCGGAANNKAACTGGAGGACTATGCGTC-3'
G2_neo2_40_1F_seq36_33	5'-TTCTTCCGCGTCTCAGAGT-3'
G2_neo2_40_1F_seq36_33R	5'-ACTCTCAGAACGCGGAAGAAANNKCTGGAGGACTATGCGTCTAAC-3'
G2_neo2_40_1F_seq36_34	5'-TTTTTCTTCCGCGTCTGAGA-3'
G2_neo2_40_1F_seq36_34R	5'-TCTCAGAACGCGGAAGAAAAANNKAGGAGGACTATGCGTCTAACGT-3'
G2_neo2_40_1F_seq36_35	5'-CAGTTTTTCTTCCGCGTCTG-3'
G2_neo2_40_1F_seq36_35R	5'-CAGAACGCGGAAGAAAACTGNNKAGCTATGCGTCTAACGTTGAAG-3'
G2_neo2_40_1F_seq36_36	5'-CTCCAGTTTTCTTCCGCGT-3'
G2_neo2_40_1F_seq36_36R	5'-ACGCGGAAGAAAACTGGAGNNKATGCGTCTAACGTTGAAGTTATC-3'
G2_neo2_40_1F_seq36_37	5'-GTCTCCAGTTTTCTTCCGCG-3'
G2_neo2_40_1F_seq36_37R	5'-CGCGGAAGAAAACTGGAGGACNNKCGCTCTAACGTTGAAGTTATCC-3'
G2_neo2_40_1F_seq36_38	5'-ATAGTCTCCAGTTTTCTTCCGCG-3'
G2_neo2_40_1F_seq36_38R	5'-GCGGAAGAAAACTGGAGGACTATNNKCTAACGTTGAAGTTATCCTGGA-3'
G2_neo2_40_1F_seq36_39	5'-CGCATAGTCTCCAGTTTTTCT-3'
G2_neo2_40_1F_seq36_39R	5'-AGAAAACTGGAGGACTATGCGNNKACGTTGAAGTTATCCTGGAAGA-3'
G2_neo2_40_1F_seq36_40	5'-AGACGCATAGTCTCCAGTT-3'
G2_neo2_40_1F_seq36_40R	5'-AAGTGGAGGACTATGCGTCTNNKGTGAAGTTATCCTGGAAGAGATCG-3'
G2_neo2_40_1F_seq36_41	5'-GTTAGACGCATAGTCTCCAGT-3'
G2_neo2_40_1F_seq36_41R	5'-ACTGGAGGACTATGCGTCTAANNKGAAGTTATCCTGGAAGAGATCGC-3'
G2_neo2_40_1F_seq36_42	5'-AACGTTAGACGCATAGTCTCC-3'
G2_neo2_40_1F_seq36_42R	5'-GGAGGACTATGCGTCTAACGTTNNKGTATCCTGGAAGAGATCGCC-3'
G2_neo2_40_1F_seq36_43	5'-TTCAACGTTAGACGCATAGTCT-3'
G2_neo2_40_1F_seq36_43R	5'-AGGACTATGCGTCTAACGTTGAANNKATCCTGGAAGAGATCGCCC-3'
G2_neo2_40_1F_seq36_44	5'-AACTTCAACGTTAGACGCATAGT-3'
G2_neo2_40_1F_seq36_44R	5'-ACTATGCGTCTAACGTTGAAGTTNNKCTGGAAGAGATCGCCCC-3'

G2_neo2_40_1F_seq36_45	5'-GATAACTTCAACGTTAGACGCAT-3'
G2_neo2_40_1F_seq36_45R	5'-ATGCGTCTAACGTTGAAGTTATCANNKGAAGAGATCGCCCGTCTG-3'
G2_neo2_40_1F_seq36_46	5'-CAGGATAACTTCAACGTTAGACGC-3'
G2_neo2_40_1F_seq36_46R	5'-GCGTCTAACGTTGAAGTTATCCTGNNKGAGATCGCCCGTCTGATG-3'
G2_neo2_40_1F_seq36_47	5'-TTCCAGGATAACTTCAACGTTAGAC-3'
G2_neo2_40_1F_seq36_47R	5'-GTCTAACGTTGAAGTTATCCTGGAANNKATCGCCCGTCTGATGGA-3'
G2_neo2_40_1F_seq36_48	5'-CTCTTCCAGGATAACTTCAACGTT-3'
G2_neo2_40_1F_seq36_48R	5'-AACGTTGAAGTTATCCTGGAAGAGNNKCGCCGCTGATGGAATCTG-3'
G2_neo2_40_1F_seq36_49	5'-GATCTCTTCCAGGATAACTTCAACGT-3'
G2_neo2_40_1F_seq36_49R	5'-ACGTTGAAGTTATCCTGGAAGAGATCANNKCGTCTGATGGAATCTGGTGA-3'
G2_neo2_40_1F_seq36_50	5'-GGCGATCTCTTCCAGGATAACT-3'
G2_neo2_40_1F_seq36_50R	5'-AGTTATCTCTGGAAGAGATCGCCNNKCTGATGGAATCTGGTGACCA-3'
G2_neo2_40_1F_seq36_51	5'-ACGGGCGATCTCTTCCAG-3'
G2_neo2_40_1F_seq36_51R	5'-CTGGAAGAGATCGCCCGTNNKATGGAATCTGGTGACCAGAAAG-3'
G2_neo2_40_1F_seq36_52	5'-CAGACGGGCGATCTCTTCC-3'
G2_neo2_40_1F_seq36_52R	5'-GGAAGAGATCGCCCGTCTGNNKGAATCTGGTGACCAGAAAGACG-3'
G2_neo2_40_1F_seq36_53	5'-CATCAGACGGGCGATCTCT-3'
G2_neo2_40_1F_seq36_53R	5'-AGAGATCGCCCGTCTGATGNNKCTTGGTGACCAGAAAGACGA-3'
G2_neo2_40_1F_seq36_54	5'-TTCCATCAGACGGGCGAT-3'
G2_neo2_40_1F_seq36_54R	5'-ATCGCCCGTCTGATGGAANNKGGTGACCAGAAAGACGAAGC-3'
G2_neo2_40_1F_seq36_55	5'-AGATTCCATCAGACGGGCGA-3'
G2_neo2_40_1F_seq36_55R	5'-TCGCCCGTCTGATGGAATCTNNKGACCAGAAAGACGAAGCGG-3'
G2_neo2_40_1F_seq36_56	5'-ACCAGATTCCATCAGACGGGCG-3'
G2_neo2_40_1F_seq36_56R	5'-GCCCGTCTGATGGAATCTGGTNNKAGAAAGACGAAGCGGAAAA-3'
G2_neo2_40_1F_seq36_57	5'-GTCACCAGATTCCATCAGACGG-3'
G2_neo2_40_1F_seq36_57R	5'-CCGTCTGATGGAATCTGGTGACNNKAAAGACGAAGCGGAAAGGC-3'
G2_neo2_40_1F_seq36_58	5'-CTGGTCACCAGATTCCATCAG-3'
G2_neo2_40_1F_seq36_58R	5'-CTGATGGAATCTGGTGACCAGNNKAGCAGCGGAAAGGCC-3'
G2_neo2_40_1F_seq36_59	5'-TTTCTGGTCACCAGATTCCATCA-3'
G2_neo2_40_1F_seq36_59R	5'-TGATGGAATCTGGTGACCAGAAANNKGAAGCGGAAAGGCCAAG-3'
G2_neo2_40_1F_seq36_60	5'-GTCTTTCTGGTCACCAGATTCCA-3'
G2_neo2_40_1F_seq36_60R	5'-TGGAATCTGGTGACCAGAAAGACNNKCGGAAAGGCCAAGCG-3'
G2_neo2_40_1F_seq36_61	5'-TTCGTCTTTCTGGTCACCAGA-3'
G2_neo2_40_1F_seq36_61R	5'-TCTGGTGACCAGAAAGACGAANNKGAAGGCCAAGCGCATG-3'
G2_neo2_40_1F_seq36_62	5'-CGCTTCGTCTTTCTGGTCAC-3'
G2_neo2_40_1F_seq36_62R	5'-GTGACCAGAAAGACGAAGCGNNKAAAGGCCAAGCGCATGAA-3'
G2_neo2_40_1F_seq36_63	5'-TTCCGCTTCGTCTTTCTGGT-3'
G2_neo2_40_1F_seq36_63R	5'-ACCAGAAAGACGAAGCGGAANNKCGCAAGCGCATGAAAGAA-3'
G2_neo2_40_1F_seq36_64	5'-CTTTTCCGCTTCGTCTTTCTGG-3'
G2_neo2_40_1F_seq36_64R	5'-CCAGAAAGACGAAGCGGAAAGNNKAAAGCGCATGAAAGAATGGATG-3'
G2_neo2_40_1F_seq36_65	5'-GGCCTTTTCCGCTTCGTC-3'
G2_neo2_40_1F_seq36_65R	5'-GACGAAGCGGAAAGGCCNNKCGCATGAAAGAATGGATGAAACG-3'
G2_neo2_40_1F_seq36_66	5'-CTTGCCCTTTTCCGCTTCG-3'
G2_neo2_40_1F_seq36_66R	5'-CGAAGCGGAAAGGCCAAGNNKATGAAAGAATGGATGAAACGTATCA-3'
G2_neo2_40_1F_seq36_67	5'-GCGCTTGCCCTTTTCCGC-3'
G2_neo2_40_1F_seq36_67R	5'-GCGGAAAGGCCAAGCGCANNKAAAGAATGGATGAAACGTATCAAGA-3'
G2_neo2_40_1F_seq36_68	5'-CATGCGCTTGCCCTTTTCC-3'
G2_neo2_40_1F_seq36_68R	5'-GGAAAGGCCAAGCGCATGNNKGAATGGATGAAACGTATCAAGACT-3'
G2_neo2_40_1F_seq36_69	5'-TTTCATGCGCTTGCCCTT-3'
G2_neo2_40_1F_seq36_69R	5'-AAGGCCAAGCGCATGAAANNKTTGGATGAAACGTATCAAGACTACC-3'
G2_neo2_40_1F_seq36_70	5'-TTCTTTCATGCGCTTGCC-3'
G2_neo2_40_1F_seq36_70R	5'-GGCCAAGCGCATGAAAGAANNKATGAAACGTATCAAGACTACCGC-3'
G2_neo2_40_1F_seq36_71	5'-CCATTCTTTCATGCGCTTGCC-3'
G2_neo2_40_1F_seq36_71R	5'-GCCAAGCGCATGAAAGAATGNNKAAACGTATCAAGACTACCGCT-3'
G2_neo2_40_1F_seq36_72	5'-CATCCATTCTTTCATGCGCTT-3'
G2_neo2_40_1F_seq36_72R	5'-AAGCGCATGAAAGAATGGATGNNKCGTATCAAGACTACCGCTTCC-3'
G2_neo2_40_1F_seq36_73	5'-TTTCATCCATTCTTTCATGCGCT-3'
G2_neo2_40_1F_seq36_73R	5'-AGCGCATGAAAGAATGGATGAAANNKATCAAGACTACCGCTTCCG-3'
G2_neo2_40_1F_seq36_74	5'-ACGTTTCATCCATTCTTTCATGCG-3'
G2_neo2_40_1F_seq36_74R	5'-CGCATGAAAGAATGGATGAAACGTNNKAAAGACTACCGCTTCCGAGG-3'
G2_neo2_40_1F_seq36_75	5'-GATACGTTTCATCCATTCTTTCATGC-3'
G2_neo2_40_1F_seq36_75R	5'-GCATGAAAGAATGGATGAAACGTATCANNKACTACCGCTTCCGAGGAC-3'
G2_neo2_40_1F_seq36_76	5'-CTTGATACGTTTCATCCATTCTTCA-3'
G2_neo2_40_1F_seq36_76R	5'-TGAAAGAATGGATGAAACGTATCAAGNNKACCGCTTCCGAGGACG-3'
G2_neo2_40_1F_seq36_77	5'-AGTCTTGATACGTTTCATCCATTCT-3'
G2_neo2_40_1F_seq36_77R	5'-AGAAATGGATGAAACGTATCAAGACTNNKGTCTCCGAGGACGAGC-3'
G2_neo2_40_1F_seq36_78	5'-GGTAGTCTTGATACGTTTCATCCA-3'
G2_neo2_40_1F_seq36_78R	5'-TGGATGAAACGTATCAAGACTACNNKTCGAGGACGAGCAAGA-3'

G2_neo2_40_1F_seq36_79	5'-AGCGGTAGTCTTGATACGTTTC-3'
G2_neo2_40_1F_seq36_79R	5'-GAAACGTATCAAGACTACCGCTNNKGAGGACGAGCAAGAGGAGA-3'
G2_neo2_40_1F_seq36_80	5'-GGAAGCGGTAGTCTTGATACGT-3'
G2_neo2_40_1F_seq36_80R	5'-ACGTATCAAGACTACCGCTTCCNNKGACGAGCAAGAGGAGATGG-3'
G2_neo2_40_1F_seq36_81	5'-CTCGGAAGCGGTAGTCTTGA-3'
G2_neo2_40_1F_seq36_81R	5'-TCAAGACTACCGCTTCCGAGNNKGAGCAAGAGGAGATGGCC-3'
G2_neo2_40_1F_seq36_82	5'-GTCCTCGGAAGCGGTAGTC-3'
G2_neo2_40_1F_seq36_82R	5'-GACTACCGCTTCCGAGGACNNKCAAGAGGAGATGGCCAACC-3'
G2_neo2_40_1F_seq36_83	5'-CTCGTCTCGGAAGCGGT-3'
G2_neo2_40_1F_seq36_83R	5'-ACCGCTTCCGAGGACGAGNNKGAGGAGATGGCCAACCG-3'
G2_neo2_40_1F_seq36_84	5'-TTGCTCGTCTCGGAAGCG-3'
G2_neo2_40_1F_seq36_84R	5'-CGCTTCCGAGGACGAGCAANNKGAGATGGCCAACCGCA-3'
G2_neo2_40_1F_seq36_85	5'-CTCTTGCTCGTCTCGGA-3'
G2_neo2_40_1F_seq36_85R	5'-TCCGAGGACGAGCAAGAGNNKATGGCCAACCGCATCAT-3'
G2_neo2_40_1F_seq36_86	5'-CTCCTCTTGCTCGTCTCGG-3'
G2_neo2_40_1F_seq36_86R	5'-CCGAGGACGAGCAAGAGGAGNNKGCCAACCGCATCATCACC-3'
G2_neo2_40_1F_seq36_87	5'-CATCTCCTCTTGCTCGTCTCT-3'
G2_neo2_40_1F_seq36_87R	5'-AGGACGAGCAAGAGGAGATGNNKAACCGCATCATCACCCTG-3'
G2_neo2_40_1F_seq36_88	5'-GGCCATCTCTCTTGCTCG-3'
G2_neo2_40_1F_seq36_88R	5'-CGAGCAAGAGGAGATGGCCNNKCGCATCATCACCCTGCT-3'
G2_neo2_40_1F_seq36_89	5'-GTTGGCCATCTCTCTTGCT-3'
G2_neo2_40_1F_seq36_89R	5'-AGCAAGAGGAGATGGCCAACNNKATCATCACCCTGCTGCAG-3'
G2_neo2_40_1F_seq36_90	5'-GCGGTGGCCATCTCTCT-3'
G2_neo2_40_1F_seq36_90R	5'-GAGGAGATGGCCAACCGNNKATCACCCTGCTGCAGTCT-3'
G2_neo2_40_1F_seq36_91	5'-GATGCGGTTGGCCATCTC-3'
G2_neo2_40_1F_seq36_91R	5'-GAGATGGCCAACCGCATCNNAACCTGCTGCAGTCTTGG-3'
G2_neo2_40_1F_seq36_92	5'-GATGATGCGGTTGGCCAT-3'
G2_neo2_40_1F_seq36_92R	5'-ATGGCCAACCGCATCATCNKCTGCTGCAGTCTTGGATCT-3'
G2_neo2_40_1F_seq36_93	5'-GGTGATGATGCGGTTGGCC-3'
G2_neo2_40_1F_seq36_93R	5'-GGCCAACCGCATCATCACNNKCTGCAGTCTTGGATCTTTTCTC-3'
G2_neo2_40_1F_seq36_94	5'-CAGGGTGATGATGCGGTTG-3'
G2_neo2_40_1F_seq36_94R	5'-CAACCGCATCATCACCCTGNKAGTCTTGGATCTTTTCTCTCGA-3'
G2_neo2_40_1F_seq36_95	5'-CAGCAGGGTGATGATGCGG-3'
G2_neo2_40_1F_seq36_95R	5'-CCGCATCATCACCCTGCTGNKCTTGGATCTTTTCTCTCGAGG-3'
G2_neo2_40_1F_seq36_96	5'-CTGCAGCAGGGTGATGATG-3'
G2_neo2_40_1F_seq36_96R	5'-CATCATCACCCTGCTGCAGNNKGGATCTTTTCTCTCGAGGGT-3'
G2_neo2_40_1F_seq36_97	5'-AGACTGCAGCAGGGTGAT-3'
G2_neo2_40_1F_seq36_97R	5'-ATCACCCTGCTGCAGTCTNNKATCTTTCTCTCGAGGGTGGA-3'
G2_neo2_40_1F_seq36_98	5'-CCAAGACTGCAGCAGGGT-3'
G2_neo2_40_1F_seq36_98R	5'-ACCCTGCTGCAGTCTTGGNNKTTTCTCTCGAGGGTGAGG-3'
G2_neo2_40_1F_seq36_99	5'-GATCCAAGACTGCAGCAGGG-3'
G2_neo2_40_1F_seq36_99R	5'-CCCTGCTGCAGTCTTGGATCNKCTCTCTCGAGGGTGAGGT-3'
COF	5'-TGACAACTATATGCGAGCAATCCCCTCAC-3'
COR	5'-AACTTTTTCGCAGTTCGCGA-3'

Table S7. List of primers used in the creation of combinatorial library for G1_neo2_40.

Oligonucleotide	Sequence
G1_neo2_40_combinatorial_f1	5' -AGCGGAGGCGGAGGGTCGGCTAGCCATATGTCTACCAAAAAACCCAGCTG-3'
G1_neo2_40_combinatorial_r2	5' -GCGTCCAGGAGCGCGTGTCCGCSABCAGCTGGGTTTTTTTGGTAG-3'
G1_neo2_40_combinatorial_f3	5' -GAACACGCGCTCCTGGACGCGYWCATGATGCTCAACDTKDTGCCGGAACCGAACGAAAAA-3'
G1_neo2_40_combinatorial_r4	5' -GATCCAAGACTGCATGGTGGTGATGATACGGTTCAGTTTTTCGTTCGGTTCGGCA-3'
G1_neo2_40_combinatorial_f5	5' -CCACCATGCAGTCTTGGATCYWTACTGGTAAGATCGACSSGGATGGTGCGMAGGAACTCGCGAAAGAAGTTG-3'
G1_neo2_40_combinatorial_r6	5' -ACGTCGATACCACGTTTTTCGWRTTCCTGWTCCAGTTCTTCAACTTCTTCGCGAGTTCC-3'
G1_neo2_40_combinatorial_f7	5' -GAAAAACGTGGTATCGACGTTSAKGACDASGCGTCTAACCTGAAAGTTATCCTGCT-3'
G1_neo2_40_combinatorial_r8	5' -AAGCTTTTGTTCGGATCCGCCTCCCTCGAGAGCCAGCTCCAGCAGGATAACTTTCAGGTT-3'

Table S8. List of primers used in the creation of a combinatorial libraries for for G2_neo2_40_1_seq27, G2_neo2_40_1_dsn29, and G2_neo2_40_1_dsn36.

Oligonucleotide	Sequence
G2_neo2_40_1F_seq27_combo_f01	5'-GAGGCGGAGGGTCGGCTTCGCATATGACGRASAAAASGCGSASCTCYWCGCCGAGYWC GCGCTGYWCGACGCGCTCATGCTGCTGA-3'
G2_neo2_40_1F_seq27_combo_f01.0	5'-GAGGCGGAGGGTCGGCTTCGCATATGACG-3'
G2_neo2_40_1F_seq27_combo_r02	5'-CGTTAGATTTCAGAACAGGTTACGACGATGAGCGCTCG-3'
G2_neo2_40_1F_seq27_combo_f03.1	5'-CCTGTCTCTGAAATCTAACGAACGTCGTAACGAWATCATCACTTGGCTGCAGTCTATCA-3'
G2_neo2_40_1F_seq27_combo_f03.2	5'-CCTGTCTCTGAAATCTAACGAACGTCGTAACCGTATCATCACTTGGCTGCAGTCTATCA-3'
G2_neo2_40_1F_seq27_combo_r04.1	5'-GCTTCTTTAACCATGTCGGGTCGTAGGTTTCGAGAGAGTGTATAGACTGCAGCCAAAGTG-3'
G2_neo2_40_1F_seq27_combo_r04.2	5'-GCTTCTTTAACCATGTCGGGTCGTAGGTTTCGAGAGAGTGTATAGACTGCAGCCAAAGTG-3'
G2_neo2_40_1F_seq27_combo_f05.1	5'-CCGACATGGTTAAAGAAGCGGTTAAAGMCGCTGACGAAATTGAGGATGA-3'
G2_neo2_40_1F_seq27_combo_f05.2	5'-CCGACATGGTTAAAGAAGCGGTTAAACTGGCTGACGAAATTGAGGATGA-3'
G2_neo2_40_1F_seq27_combo_r06.1	5'-AACGTAATCTTCGGTTCGATCTTACGCTTACGCATTTCATCCTCAATTTTCGTCAGC-3'
G2_neo2_40_1F_seq27_combo_r06.2	5'-AACGTAATCTTCGGTTCGATACACGCTTACGCATTTCATCCTCAATTTTCGTCAGC-3'
G2_neo2_40_1F_seq27_combo_f07.1	5'-TCGACACCGAAGATTACGTTGTAACTGCGTKTAATCCTGCAAGAAGTGGCCCT-3'
G2_neo2_40_1F_seq27_combo_f07.2	5'-TCGACACCGAAGATTACGTTGTAACTGCGTKTAATCCTGCAAGAAGTGGCCCT-3'
G2_neo2_40_1F_seq27_combo_f07.3	5'-TCGACACCGAAGATTACGTTTCTAACCTGCGTKTAATCCTGCAAGAAGTGGCCCT-3'
G2_neo2_40_1F_seq27_combo_f07.4	5'-TCGACACCGAAGATTACGTTTCTAACCTGCGTKTAATCCTGCAAGAAGTGGCCCT-3'
G2_neo2_40_1F_seq27_combo_r08	5'-TTTTGTTCCGAACCTCCACCTCGAGGCGCAGTTCTTCAGGAT-3'
G2_neo2_40_1F_seq29_combo_f01	5'-GAGGCGGAGGGTCGGCTTCGCATATGGAGGACTACTACTCTAACCTCAA-3'
G2_neo2_40_1F_seq29_combo_r02.1	5'-CATTACGCTCCATTTACGTCGCAGCTCTTCCAGGATTAMTTGAGGTTAGAGTAGTAGT-3'
G2_neo2_40_1F_seq29_combo_r02.2	5'-CATTACGCTCCATTTACGTCGCAGCTCTTCCAGGATATATTTGAGGTTAGAGTAGTAGT-3'
G2_neo2_40_1F_seq29_combo_r03	5'-ACGTGAAATGGAGCGTAATGGTCTGTCTGACAAAGCGGAAGAATGGCGTCAGTGGAAAAA-3'
G2_neo2_40_1F_seq29_combo_r04	5'-AGAGTTGTTAGAACGAATCTGACGAATGCGTTCTACAATCTTTTCCACTGACGCCATT-3'
G2_neo2_40_1F_seq29_combo_f05	5'-AGATTTCGTTCTAACAACTCTGACCTCAATGAGGCGAAGGAGCTGCTCAAC-3'
G2_neo2_40_1F_seq29_combo_r06.1	5'-TTTGAGACTGGATGTAGGTGATCAGWTCGTTGAGCAGCTCCTTCGCCT-3'
G2_neo2_40_1F_seq29_combo_r06.2	5'-TTTGAGACTGGATGTAGGTGATCAGCAGGTTGAGCAGCTCCTTCGCCT-3'
G2_neo2_40_1F_seq29_combo_f07.1	5'-CACCTACATCCAGTCTCAAATCTTCGAARWCHYAGASSTRWASSAGAACTGACCAGGAGAAAA-3'
G2_neo2_40_1F_seq29_combo_f07.2	5'-CACCTACATCCAGTCTCAAATCTTCGAARWCHYACATSSSTRWASSAGAACTGACCAGGAGAAAA-3'
G2_neo2_40_1F_seq29_combo_r08	5'-CCACTTCTTCCAAGATTCCTCCTTTTCTCCTGGTCAGTTTCT-3'
G2_neo2_40_1F_seq29_combo_f09	5'-AGGAATCTTGGAGAAGTGGSAWCTCYWCCTCGAACACGCGCTCYWCGACGTGCTGATGCTGCTGA-3'
G2_neo2_40_1F_seq29_combo_r10	5'-TTTTGTTCCGAACCTCCACCTCGAGATCATTACGACGATCAGCAGCTCG-3'
G2_neo2_40_1F_seq36_combo_f01	5'-GAGGCGGAGGGTCGGCTTCGCATATGCTCTAAAAAGAAAATTCAGCTG-3'
G2_neo2_40_1F_seq36_combo_r02.1	5'-GAGAGTTCGTTTAAACGATGTTACGATCATCAGSAKGTGWRGAGCGCTGTTCCGCGWRACGTGAATTTCTTTTAG-3'
G2_neo2_40_1F_seq36_combo_r02.2	5'-GAGAGTTCGTTTAAACGATGTTACGATCATCAGCGCTGWRGAGCGCTGTTCCGCGWRACGTGAATTTCTTTTAG-3'
G2_neo2_40_1F_seq36_combo_f03.1	5'-CATCGTTAAACGAACCTCTCMGAACGCGGAAGAAAACTGGAGGACTATGCGT-3'
G2_neo2_40_1F_seq36_combo_f03.2	5'-CATCGTTAAACGAACCTCTCMGCCGCGGAAGAAAACTGGAGGACTATGCGT-3'
G2_neo2_40_1F_seq36_combo_r04	5'-CAGACGGGCGATCTCTCCAGGATAAVYYARMGTTADACGCATAGTCTCCAGTTT-3'
G2_neo2_40_1F_seq36_combo_f05.1	5'-TGGAAGAGATCGCCGCTGTGTCGAATCTGGTGACCAAGAACGAGCGAAAGGCCA-3'
G2_neo2_40_1F_seq36_combo_f05.2	5'-TGGAAGAGATCGCCGCTGTGTCGAATCTGGTGACCAAGAACGAGCGAAAGGCCA-3'
G2_neo2_40_1F_seq36_combo_r06	5'-CTTGATACGTTTCATCCATTCTTTCATGCGCTTGGCCTTTCCGCTTCGTCT-3'
G2_neo2_40_1F_seq36_combo_f07.1	5'-AATGGATGAACGATATCAAGACTACCGCTTCCAGGACGAGCAAGAGGAGATGGCCAAC-3'
G2_neo2_40_1F_seq36_combo_f07.2	5'-AATGGATGAACGATATCAAGATACCGCTTCCAGGACGAGCAAGAGGAGATGGCCAAC-3'
G2_neo2_40_1F_seq36_combo_r08.0	5'-TTTTGTTCCGAACCTCCACCTCGAGAGAAAAG-3'
G2_neo2_40_1F_seq36_combo_r08.1	5'-TTTTGTTCCGAACCTCCACCTCGAGAGAAAAGWTCCAAGACTGCAGGAKGGTGATGATWTCGTTGCCATCTCCTTTGCT-3'
G2_neo2_40_1F_seq36_combo_r08.2	5'-TTTTGTTCCGAACCTCCACCTCGAGAGAAAAGWTCCAAGACTGCAGGAKGGTGATGATAGCGTTGCCATCTCCTTTGCT-3'

Table S9. *In vitro* characterization of Neo-2/15 phospho-STAT5 signaling activation (tabulation of fits from Figure 2b). EC₅₀ value differences are marked as significant if their respective 95% confidence intervals do not overlap. “S” stands for significant and “NS” for nonsignificant.

Construct	IL-2Rα ⁺ YT-1 Human NK Cell EC ₅₀ (nM)			
Neo-2/15	0.073 ± 0.023	] NS]	] S]	] S]
hIL-2	0.056 ± 0.020			
Super-2	0.034 ± 0.0094			
mIL-2	1.3 ± 0.43			

Construct	IL-2Rα ⁻ YT-1 Human NK Cell EC ₅₀ (nM)			
Neo-2/15	0.049 ± 0.015	] S]	] NS]	] S]
hIL-2	0.41 ± 0.011			
Super-2	0.066 ± 0.017			
mIL-2	39 ± 7.5			

Construct	IL-2Rα ⁺ Mouse Primary T Cell EC ₅₀ (nM)		
Neo-2/15	0.024 ± 0.015	] S]	] S]
Super-2	0.0012 ± 0.00051		
mIL-2	0.00020 ± 0.00014		

Construct	IL-2Rα ⁻ Mouse Primary T Cell EC ₅₀ (nM)		
Neo-2/15	0.13 ± 0.086	] S]	] S]
Super-2	0.66 ± 0.040		
mIL-2	30 ± 10		

Appendices

Appendix A. RosettaScripts XML protocol for sequence design of mimetics generation-1. This script is provided solely as a guideline since it has not been modernised for compatibility with the most recent versions of RosettaScripts.

```
<ROSETTASCRIPITS>
  <SCOREFXNS>
    <SFXN6 weights=talaris2013.wts />
    <SFXN6dA weights=talaris2013_downA1a.wts />
  </SCOREFXNS>

  <FILTERS>
    <SSPrediction name="sspred" cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single" use_probability="0" use_svm="0"
threshold=0.80 confidence="1"/>
    <ScoreType name="rama" scorefxn="SFXN6" score_type="rama" threshold=0.0 confidence="0" />
    <PackStat name=pack threshold=0.63 confidence=1/>
    <Holes name=holes threshold=1.2 confidence=0/>
    <ScoreType name="score" scorefxn="SFXN6" score_type="total_score" threshold=0.0 confidence="0" />
    <ResidueCount name="nres" confidence="0" />
    <CalculatorFilter name="score_res" equation="SCORE/NRES" threshold="-1.7" confidence="1">
      <SCORE name="SCORE" filter_name="score" />
      <NRES name="NRES" filter_name="nres" />
    </CalculatorFilter>
    <CompoundStatement name=filt >
      <AND filter_name=sspred />
      <AND filter_name=rama />
      <AND filter_name=score_res />
      <AND filter_name=pack />
    </CompoundStatement>
  </FILTERS>

  <TASKOPERATIONS>
    <InitializeFromCommandline name="init"/>
    <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>
</ROSETTASCRIPITS>
```

Appendix B. RosettaScripts XML protocol for sequence redesigns of G1_Neo2_40_1F (i.e. generation-2 sequence redesigns based on G1_Neo2_40_1F as scaffold). This script is provided solely as a guideline since it has not been modernised for compatibility with the most recent versions of RosettaScripts.

```
<ROSETTASCRIPITS>
  <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,not_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_pspred="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"
        boltz_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). This script is provided solely as a guideline since it has not been modernised for compatibility with the most recent versions of RosettaScripts.

```
<ROSETTASCRIPITS>
  <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,not_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_pspred="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"
        boltz_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 D. Modernised RosettaScripts XML protocol for sequence design of new mimetics generation-2 (i.e. mimetics based on G1_Neo2_40_1F as template). The script has been modernised and adapted to be compatible with the most recent RosettaScripts versions and the latest beta energy function (as of the main article publication date).

```
<ROSETTASCRIPTS>
  <SCOREFXNS>
    <ScoreFunction name="SFXN6_vanilla" weights="beta" symmetric="0" />
    <ScoreFunction name="SFXN6_vanilla_cst" weights="beta" symmetric="0" >
      <Reweight scoretype="coordinate_constraint" weight="1.0"/>
    </ScoreFunction >
    <ScoreFunction name="SFXN6dA_norep_elect" weights="beta" symmetric="0" >
      <Reweight scoretype="fa_rep" weight="0.05" />
      <Reweight scoretype="fa_elec" weight="1.0" />
    </ScoreFunction >
    <ScoreFunction name="SFXN6dA_elect" weights="beta" symmetric="0" >
      <Reweight scoretype="fa_elec" weight="2.0" />
    </ScoreFunction >
  </SCOREFXNS>

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

  <FILTERS>
    <!--Generic-->
    <ScoreType name="score_disabled" scorefxn="SFXN6_vanilla" score_type="total_score" threshold="0.0" confidence="0" />
    <ResidueCount name="nres_disabled" confidence="0" />
    <!--Not Enabled-->
    <PackStat name="packstat_disabled" threshold="0.63" repeats="5" confidence="0" />
    <SSPrediction name="sspred_disabled" cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single" use_probability="0" use_svm="0"
threshold="0.80" confidence="0"/>
    <BuriedUnsatHBonds name="unsat_core_disabled" residue_selector="coreLayerSelector" report_sc_heavy_atom_unsats="true"
scorefxn="SFXN6_vanilla" cutoff="2" residue_surface_cutoff="20.0" ignore_surface_res="true" print_out_info_to_pdb="true"
confidence="0"/>
    <RmsdSimple name="rmsd_chainA_not_align_disabled" reference_name="reference_conformation" chain="1" align="0"
threshold="2.7" confidence="0"/>
    <RmsdSimple name="rmsd_chainA_align_disabled" reference_name="reference_conformation" chain="1" align="1"
threshold="2.2" confidence="0"/>
    <CalculatorFilter name="score_res_disabled" equation="SCORE/NRES" threshold="-2.1" confidence="0">
      <Var name="SCORE" filter_name="score_disabled" />
      <Var name="NRES" filter_name="nres_disabled" />
    </CalculatorFilter>

    <!--Enabled-->
    <PackStat name="packstat_enabled" threshold="0.63" repeats="5" confidence="1" />
    <SSPrediction name="sspred_enabled" cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single" use_probability="0" use_svm="0"
threshold="0.80" confidence="1"/>
    <CavityVolume name="cav_vol_disabled" />
    <BuriedUnsatHBonds name="unsat_core_enabled" residue_selector="coreLayerSelector" report_sc_heavy_atom_unsats="true"
scorefxn="SFXN6_vanilla" cutoff="2" residue_surface_cutoff="20.0" ignore_surface_res="true" print_out_info_to_pdb="true"
confidence="1" />
    <RmsdSimple name="rmsd_chainA_not_align_enabled" reference_name="reference_conformation" chain="1" align="0" threshold="2.7"
confidence="1"/>
    <RmsdSimple name="rmsd_chainA_align_enabled" reference_name="reference_conformation" chain="1" align="1" threshold="2.2"
confidence="1"/>
    <CalculatorFilter name="score_res_enabled" equation="SCORE/NRES" threshold="-2.1" confidence="1">
      <Var name="SCORE" filter_name="score_disabled" />
      <Var name="NRES" filter_name="nres_disabled" />
    </CalculatorFilter>
    <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"/>
  </FILTERS>
```

```

<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="HOTSPOT" />
  <Layer name="surfaceLayerSelector" select_core="0" select_boundary="0" select_surface="1"
    ball_radius="2.0" use_sidechain_neighbors="1"
    sc_neighbor_dist_exponent="1.0" sc_neighbor_dist_midpoint="9.0"
    sc_neighbor_denominator="1.0" sc_neighbor_angle_shift_factor="0.5"
    sc_neighbor_angle_exponent="2.0"
    core_cutoff="5.2" surface_cutoff="1.0"
  />
  <Layer name="coreLayerSelector" select_core="1" select_boundary="0" select_surface="0"
    ball_radius="2.0" use_sidechain_neighbors="1"
    sc_neighbor_dist_exponent="1.0" sc_neighbor_dist_midpoint="9.0"
    sc_neighbor_denominator="1.0" sc_neighbor_angle_shift_factor="0.5"
    sc_neighbor_angle_exponent="2.0"
    core_cutoff="5.2" surface_cutoff="1.0"
  />
</RESIDUE_SELECTORS>

<TASKOPERATIONS>
  <InitializeFromCommandline name="init"/>
  <IncludeCurrent name="inclcur"/>
  <LimitAromaChi2 name="limitchi2" />
  <DisallowIfNonnative name="only_native_H" disallow_aas="H"/>
  <ReadResfile name="resfile" filename="%%resfile_name%%" />
  <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>
  <!--Helps to correct/remove TRP in the surface-->
  <OperateOnResidueSubset name="surfaceWcorrector" selector="surfaceLayerSelector" >
    <RestrictAbsentCanonicalAASRLT aas="ACDEFGHIKLMNPQRSTVY"/>
  </OperateOnResidueSubset>
</TASKOPERATIONS>

<MOVERS>
  <SavePoseMover name="save_RMSDreference_conformation" reference_name="reference_conformation"/>
  <SavePDBInfoMover name="saveInitPDBInfo" reference_info_name="initPDBInfo" restore_info="0" />
  <SavePDBInfoMover name="reloadInitPDBInfo" reference_info_name="initPDBInfo" restore_info="1" />
  <AddConstraintsToCurrentConformationMover name="constrainCA" task_operations="init" CA_only="1" cst_weight="1.0" />
  <ClearConstraintsMover name="clearAllConstraints" />
  <PackRotamersMover name="design_all_norep" scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,not_chain_B,not_chain_C" />
  <PackRotamersMover name="design_onlyCore_norep" scorefxn="SFXN6dA_norep_elect"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,only_core_residues,not_chain_B,not_chain_C" />
  <TaskAwareMinMover name="min_vanilla_SC" scorefxn="SFXN6_vanilla" bb="0" chi="1" jump="0"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,not_chain_B,not_chain_C" />
  <TaskAwareMinMover name="min_vanilla_BBSC" scorefxn="SFXN6_vanilla_cst" bb="1" chi="1" jump="0"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,not_chain_B,not_chain_C" />
  <FastDesign name="fdesign_vanilla" scorefxn="SFXN6_vanilla"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,not_chain_B,not_chain_C"
repeats="3" clear_designable_residues="0" >
    <MoveMap name="mappyfd">
      <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>
  </FastDesign>
</MOVERS>

```

```

    </MoveMap>
  </FastDesign>
  <FastRelax name="fast_relax_vanilla" scorefxn="SFXN6_vanilla"
task_operations="init,resfile,inclcur,limitchi2,surfaceWcorrector,only_native_H,hotspot_onlyrepack,not_chain_B,not_chain_C"
repeats="3" >
    <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="reloadHS_and_design_onlyCore_norep" >
    <Add mover_name="reloadInitPDBinfo" />
    <Add mover_name="design_onlyCore_norep" />
  </ParsedProtocol>
  <ParsedProtocol name="reloadHS_and_quickdesign_all_w_minimize_vanilla" >
    <Add mover_name="reloadInitPDBinfo" />
    <Add mover_name="design_all_norep" />
    <Add mover_name="min_vanilla_SC" />
    <Add mover_name="constrainCA" /> <!-- START CA-constraints -->
    <Add mover_name="min_vanilla_BBSC" />
    <Add mover_name="clearAllConstraints" /> <!-- END CA-constraints -->
  </ParsedProtocol>
  <ParsedProtocol name="reloadHS_and_fastDesign" >
    <Add mover_name="reloadInitPDBinfo" />
    <Add mover_name="fdesign_vanilla" />
  </ParsedProtocol>
  <GenericSimulatedAnnealer name="SA_DesignProtein"
mover_name="reloadHS_and_design_onlyCore_norep" trials="100"
periodic_mover="reloadHS_and_quickdesign_all_w_minimize_vanilla" eval_period="20" history="10"
boltz_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>
  <!-- FastDesign GMC, increase trials if you wanna more through designs, but it will take much longer -->
  <GenericMonteCarlo name="MC_FastDesignProtein"
mover_name="reloadHS_and_fastDesign"
filter_name="cav_vol_chainA_disabled"
sample_type="low"
trials="2" 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="saveInitPDBinfo" />
  <Add mover_name="save_RMSDreference_conformation" />
  <Add mover_name="SA_DesignProtein" />
  <Add filter_name="rmsd_chainA_not_align_enabled" />
  <Add filter_name="rmsd_chainA_align_enabled" />
  <Add mover_name="MC_FastDesignProtein" />
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  <Add filter_name="rmsd_chainA_align_enabled" />
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  <Add filter_name="rmsd_chainA_align_enabled" />
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  <Add filter_name="rmsd_chainA_not_align_enabled" />
  <Add filter_name="rmsd_chainA_align_enabled" />

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<Add mover_name="fast_relax_vanilla" />
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<Add filter_name="rmsd_chainA_align_enabled" />
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<Add filter_name="unsat_core_chainA_enabled" />
<Add filter_name="cav_vol_chainA_disabled" />
</PROTOCOLS>
</ROSETTASCRIPTS>
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