SUPPLEMENTARY FIGURE LEGENDS

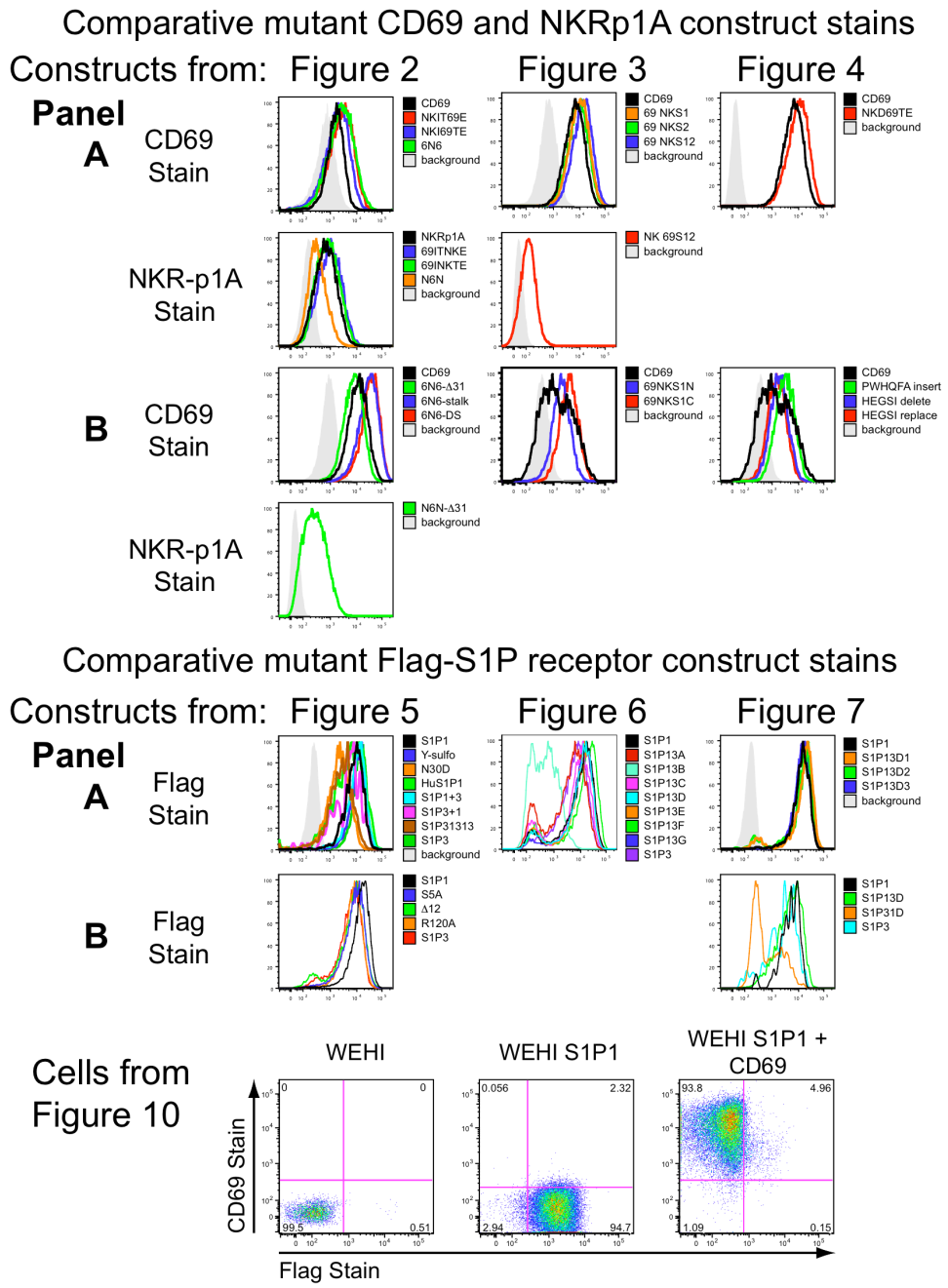
Supplementary Fig. 1. Flow cytometric analysis of wildtype, mutant and chimeric protein surface expression. Cells transduced with the individual constructs indicated were stained for CD69 or NKRp1A ectodomains, or Flag in the case of S1P-receptor constructs. Histogram overlays group constructs in accord with their appearance in Western blots in the designated figure. Dot plots show flow cytometric analysis of Flag-$S1P_1$ and CD69 on the indicated WEHI231 cell lines that correspond to the cells used for the binding studies in Figure 10.

Supplementary Fig. 2. Point mutations of all residues in the HEGSI motif of CD69 to alanine. Non-specific bands are indicated with an asterisk.

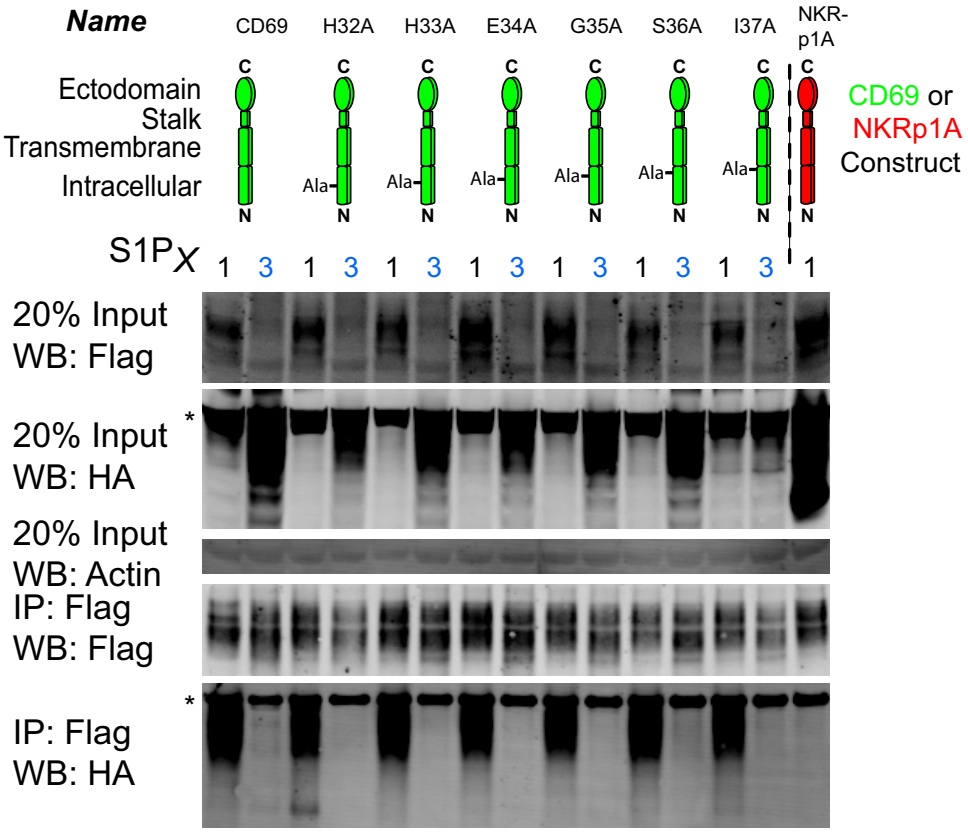
Supplementary Fig. 3. *A*. Flow cytometric analysis of $S1P_1$ mutants with point mutations in helical segments co-transduced with CD69 and hNKRp1A in WEHI231 cells. The cells are costained for CD69 and NKRp1A ectodomains and the Flag-S1P receptors and mutants indicated. *B*. Co-IP of the mutants indicated. No mutant (P1 through P8) exhibited a more than 1.5 fold difference in HA/Flag signal by densitometry. The result is representative of two experiments. Non-specific bands are indicated with an asterisk.

Supplementary Fig. 4. Test of electrostatics of $S1P_1$ and $S1P_3$. Structure was generated with modeller using the most homologous GPCR structure (β1-adrenergic receptor, pdb ID 2VT4). The region of the GPCR represented by helix 4 is highlighted by a black oval. Other helices on this surface are annotated.

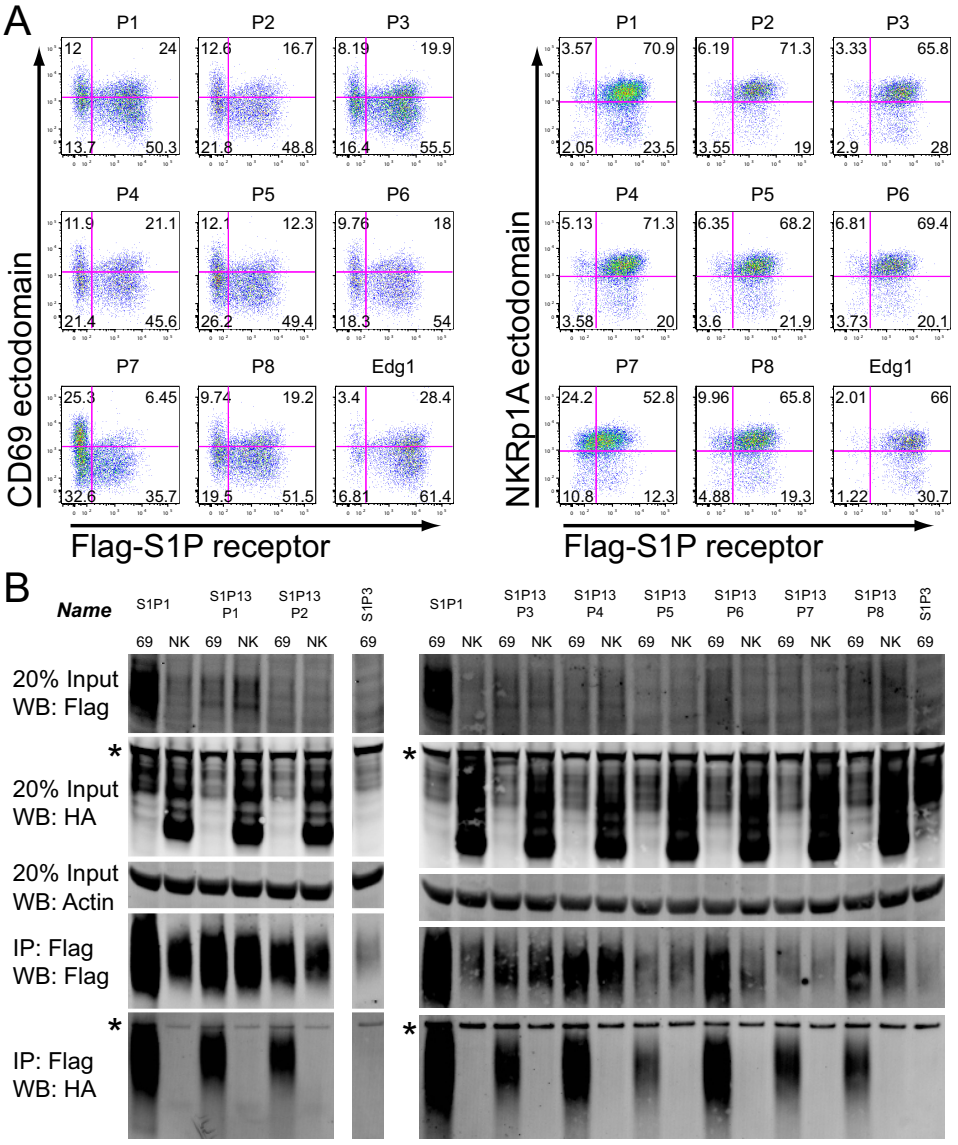
Supplementary Figure 1



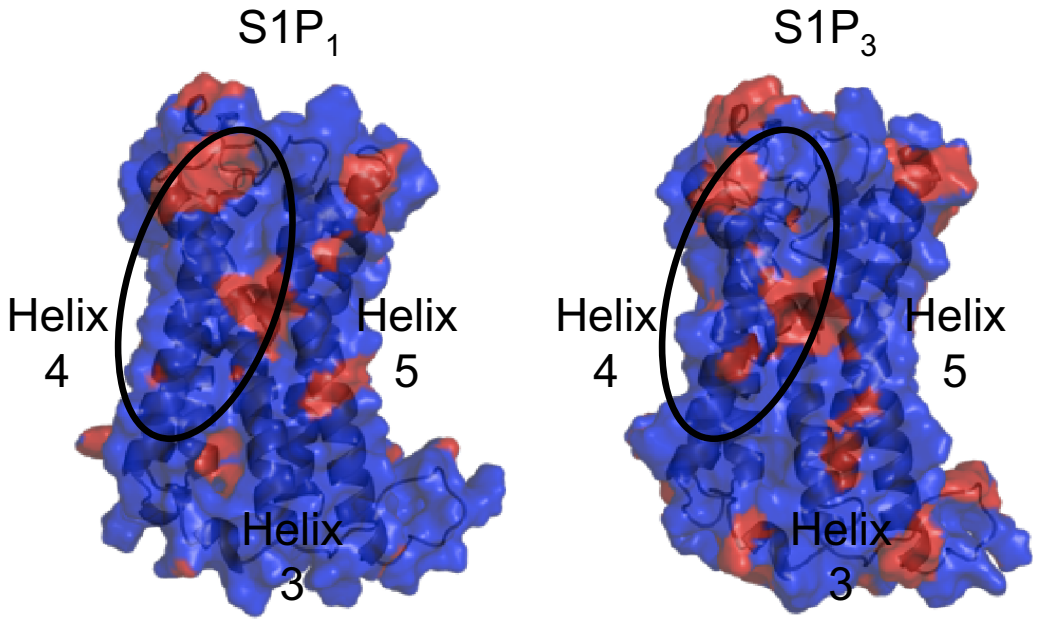
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Protein sequence of constructs used in this paper:

S1P receptor constructs

S1P1 (NCBI Reference Sequence: NP_031927.2)

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ERYITMLKMKLHNGSNSSRSFLLISACWVISLILGGLPIMGWNCISSLSSCSTVLPLYHKHYILFCTTVF
TLLLLSIVILYCRIYSLVRTRSRRLTFRKNISKASRSSEKSLALLKTVIIVLSVFIACWAPLFILLLLDV
GCKAKTCDILYKAEYFLVLAVLNSGTNPIIYTLTNKEMRRAFIRIVSCCKCPNGDSAGKFKRPIIPGMEF
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S1P3 (NCBI Reference Sequence: NP_034231.1)

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MIKMRPYDANKKHRVFLLIGMCWLIAFSLGALPILGWNCLENFPDCSTILPLYSKKYIAFLISIFTAILV
TIVILYARIYCLVKSSSRRVANHNSERSMALLRTVVIVVSVFIACWSPLFILFLIDVACRAKECSILFKS
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Δ12 (2)

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R120A (3)

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Y-sulfo (4)

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GCKAKTCDILYKAEYFLVLAVLNSGTNPIIYTLTNKEMRRAFIRIVSCCKCPNGDSAGKFKRPIIPGMEF
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N30D (5)

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S1P1+3

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S1P3+1

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S1P31313

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R142A

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R142N

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S1P13D

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S1P13E

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S1P13D2

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S1P31D

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S1P13 P1

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S1P13 P2

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S1P13 P3

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S1P13 P4

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S1P13 P5

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S1P13 P6

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S1P13 P7

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S1P13 P8

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MVSTSIPEVKALRSSVSDYGNYDIIVRHYNYTGKLNIGAEKDHGIKLTSVVFILICCFIILENIFVLLTI
WKTKKFHRPMYYFIGNLALSDLLAGVAYTANLLLSGATTYKLTPAQWFLREGSMFVALSASVFSLLAIAI
ERYITMLKMKLHNGSNSSRSFLLISACWVISLILGGLPILGWNCISSLSSCSTVLPLYHKHYILFCTTVF
TLLLLSIVILYCRIYSLVRTRSRRLTFRKNISKASRSSEKSLALLKTVIIVLSVFIACWAPLFILLLLDV
GCKAKTCDILYKAEYFLVLAVLNSGTNPIIYTLTNKEMRRAFIRIVSCCKCPNGDSAGKFKRPIIPGMEF
SRSKSDNSSHPQKDDGDNPETIMSSGNVNSSS
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C-type lectin chimeric constructs

CD69 (NCBI Reference Sequence: NP_001028294.1)

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MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR
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hNKRp1A (NCBI Reference Sequence: NP_002249.1)
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSPWHQFALKLSCAGIILLVLVVTGLSVSVTSLIQKSSIEKCSVD
IQQSRNKTTERPGLLNCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWI
GLNFSLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

69IT-NKE
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCSVDIQQ
SRNKTTERPGLLNCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWIGLN
FSLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

NKIT-69E
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LESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQ
TWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69I-NKTE
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQLSCAGIILLVLVVTGLSVSVTSLIQKSSIEKCSVDIQQS
RNKTTERPGLLNCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWIGLNF
SLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

NKI-69TE
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSPWHQFALKVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYE
KLESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEAN
QTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

6N6
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQLSCAGIILLVLVVTGLSVSVTSLIGKYNCPGLYEKLESS
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ANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

N6N
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSPWHQFALKVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCSV
DIQQSRNKTTERPGLLNCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFW
IGLNFSLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

6N6-Δ31
MDSENCSITENSSSHLERGQKDHGTSIHFEKPWHQFALKLSCAGIILLVLVVTGLSVSVTSLIGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

6N6-stalk
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQLSCAGIILLVLVVTGLSVSVTSLISSIEKCSVDIQQSRN
KTTERPGLLNCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQ
TWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

6N6-DS
MDSENCSITENSSSHLERGQKDHGTSIHFEKPWHQFALKLSCAGIILLVLVVTGLSVSVTSLISSIEKCSVDIQQSR
NKTTERPGLLNCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEAN
QTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

N6N-Δ31
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCSVD
IQQSRNKTTERPGLLNCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWI
GLNFSLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

N6N-stalk
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKGKYNCPGLY
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SLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

N6N-DS
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSPWHQFALKVSIPWAVLIVVLITSLIIALIALNVQKGKYNCPGL
YEKLESSDHHVATCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWIGLN
FSLSEKNWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

69-NKS1
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCSVDIQQ
SKLESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEA
NQTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69-NKS2
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYERNKT
TERPGLLNCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTW
KWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69-NKS12
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCSVDIQQ
SRNKTTERPGLLNCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNE
ANQTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69-NKS1N
MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIQVSIPWAVLIVVLITSLIIALIALNVQKSSIEKCPGLYEK
LESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQ
TWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69-NKS1C
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ESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQT
WKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

69IS-NKE
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SDHHVATCPIYWQQLREKCLLFSHTVNPWNNSLADCSTKESSLLLIRDKDELIHTQNLIRDKAILFWIGLNFSLSEK
NWKWINGSFLNSNDLEIRGDAKENSCISISQTSVYSEYCSTEIRWICQKELTPVRNKVYPDS

NKD-69TE
MDQQAIYAELNLPTDSGPESSSPSSLPRDVCQGSHHEGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEK
LESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQ
TWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

PWHQFA-Insert

MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSIPWHQFAQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGL
YEKLESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNE
ANQTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

HEGSI-delete

MDSENCSITENSSSHLERGQKDHGTSIHFEKH-
QVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLESSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCS
EDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWKWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFH
WVCSKPSR

HESGI-replace

MDSENCSITENSSSHLERGQKDHGTSIHFEKHPWHQFAQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLE
SSDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTW
KWANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

H32A

MDSENCSITENSSSHLERGQKDHGTSIHFEKAHEGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
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WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

H33A

MDSENCSITENSSSHLERGQKDHGTSIHFEKHAEGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

E34A

MDSENCSITENSSSHLERGQKDHGTSIHFEKHHAGSIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
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WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

G35A

MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEASIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

S36A

MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGAIQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

I37A

MDSENCSITENSSSHLERGQKDHGTSIHFEKHHEGSAQVSIPWAVLIVVLITSLIIALIALNVGKYNCPGLYEKLES
SDHHVATCKNEWISYKRTCYFFSTTTKSWALAQRSCSEDAATLAVIDSEKDMTFLKRYSGELEHWIGLKNEANQTWK
WANGKEFNSWFNLTGSGRCVSVNHKNVTAVDCEANFHWVCSKPSR

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