Supplementary Material

Feline Interleukin-31 shares overlapping epitopes with Oncostatin M Receptor and IL-31RA

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Supporting Note S1

>Feline IL-31 DNA Sequence

ATGTCTCACATGGCTCCAGCACATAGATTACAACCATCAGATATTAGAAAGATCATC
TTGGAATTAAGACCAATGTCTAAAGGTTTGTTGCAAGATTATTTGAAGAAAGAAATC
GGTTTACCAGAATCAAACCATTCTTCATTGCCATGTTTATCTTCAGATTCTCAATTGC
CACATATCAACGGTTCAGCAATCTTGCCATACTTTAGAGCTATTAGACCATTGTCAG
ATAAGAACACAATCGATAAGATCATCGAACAACTAGACAAGTTGAAGTTTCAAAGA
GAACCAGAAGCAAAAGTTTCTATGCCAGCTGATAACTTCGAAAGAAAGAATTTCAT
CTTGGCAGTTTTACAACAATTTTCAGCTTGTTTGGAACATGTTTTGCAATCTTTGAAT
TCAGGTCCACAA

>Feline IL-31 Amino Acid Sequence
MSHMAPAHRLQPSDIRKIILELRPMSKGLLQDYLKKEIGLPESNHSSLPCLSSDSQLPHIN
GSAILPYFRAIRPLSDKNTIDKIIEQLDKLKFQREPEAKVSMPADNFERKNFILAVLQQFS
ACLEHVLQSLNSGPQ

>mAb1_VK

EIQMTQSPSSLSASPGDRVTITCRASQGISIWLSWYQQKPGNIPKVLINKASNLHIGVPSRF
SGSGSGTDFTLTISSLEPEDAATYYCLQSQTYPLTFGGGTKLEIK

>mAb1_VH

QVLLVQSGAEVRTPGASVKIFCKASGYSFTSYTIHWLRQAPAQGLEWMGNINPTSGYTE
NNQRFKDRLTLTADTSTNTAYMELSSLRSADTAMYYCARWGFKYDGEWSFDVWGAG
TTVTVSS

Supporting Note S2

Sequence alignment of Feline, Human, Mouse, and Canine IL-31. The Feline sequence does not include the signal peptide. Arrows point to positions identified as belonging to either OSMR or IL-31RA epitopes.

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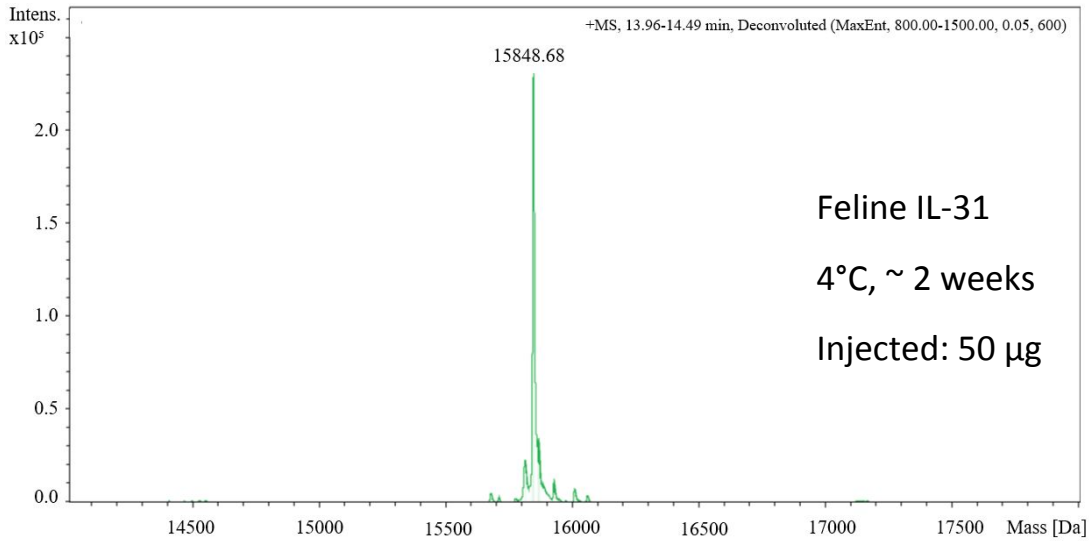
Feline    1  M----------------------SHMAPAHR-LQP-SDIRKIILELRPMSKGLLQDYL-K
Human     1  MASHSGPSTSVLFLFCCLGGWLASHTLPVRL-LRPSDDVQKIVEELQSLSKMLLKDV--E
Mouse     1  MIFHTGTTKPTLVLLCCIGTWLATCSLSFGAPISK-EDLRTTIDLLKQESQDLYNNYSIK
Canine    1  MLSHTGPSRFALFLLCSMETLLSSHMAPTHQ-LPP-SDVRKIILELQPLSRGLLEDYQ-K

Feline   36  KEIGLPESNHSSLPCLSSDSQLPH-INGSAILPYFRAIRPLSDKNTI-DKIIEQLDKLKF
Human    58  EEKGVLVSQNYTLPCLSPDAQPPNNIHSPAIRAYLKTIRQLDNKSVI-DEIIEHLDKLIF
Mouse    60  QASGMSADESIQLPCFSLDREAL--TNISVIIAHLEKVKVLSENTVDTSWVIRWLTNISC
Canine   58  KETGVPESNRTLLLCLTSDSQPPR-LNSSAILPYFRAIRPLSDKNII-DKIIEQLDKLKF

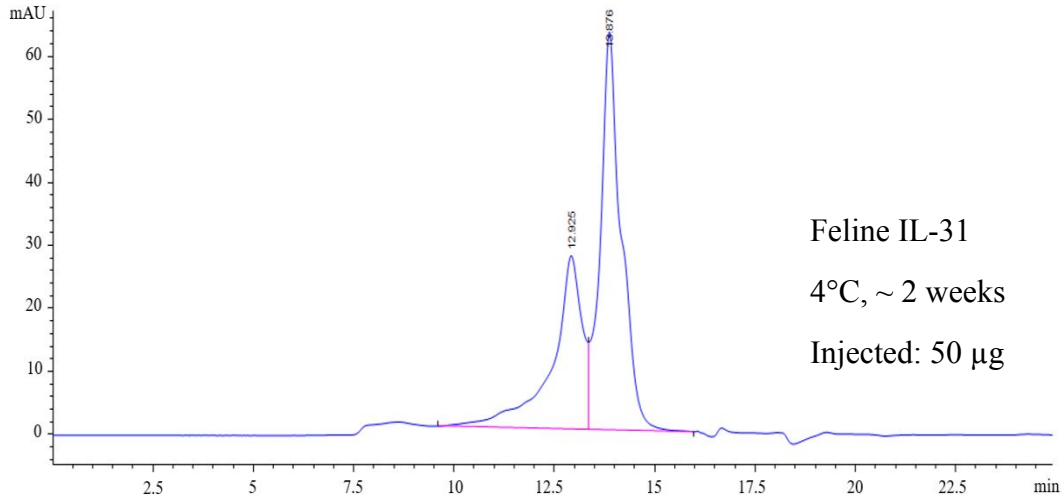
Feline   94  QREPEAKVSMPADN---FERKNFILAVLQQFSACLEHVLQSLNSGP----Q
Human   117  QDAPETNISVPTDT---HECKRFILTISQQFSECMDLALKSLTSGAQQATT
Mouse   118  FNPLNLNISVPGNTDESYDCKVFVLTVLKQFSNCMAELQAKDNTTC-----
Canine  116  QHEPETEISVPADT---FECKSFILTILQQFSACLESVFKSLNSGP----Q
```

Site II
Site III
Shared Site

a.

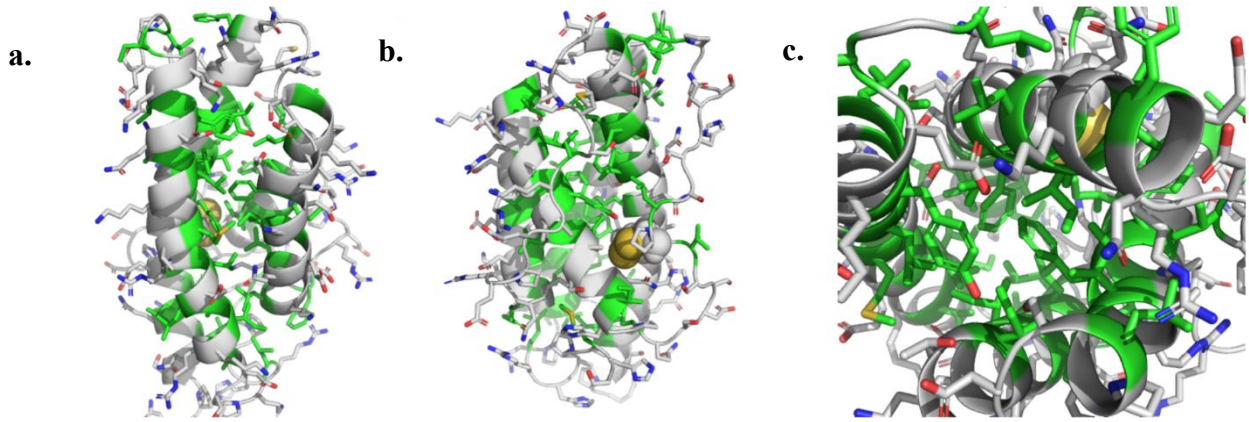


b.



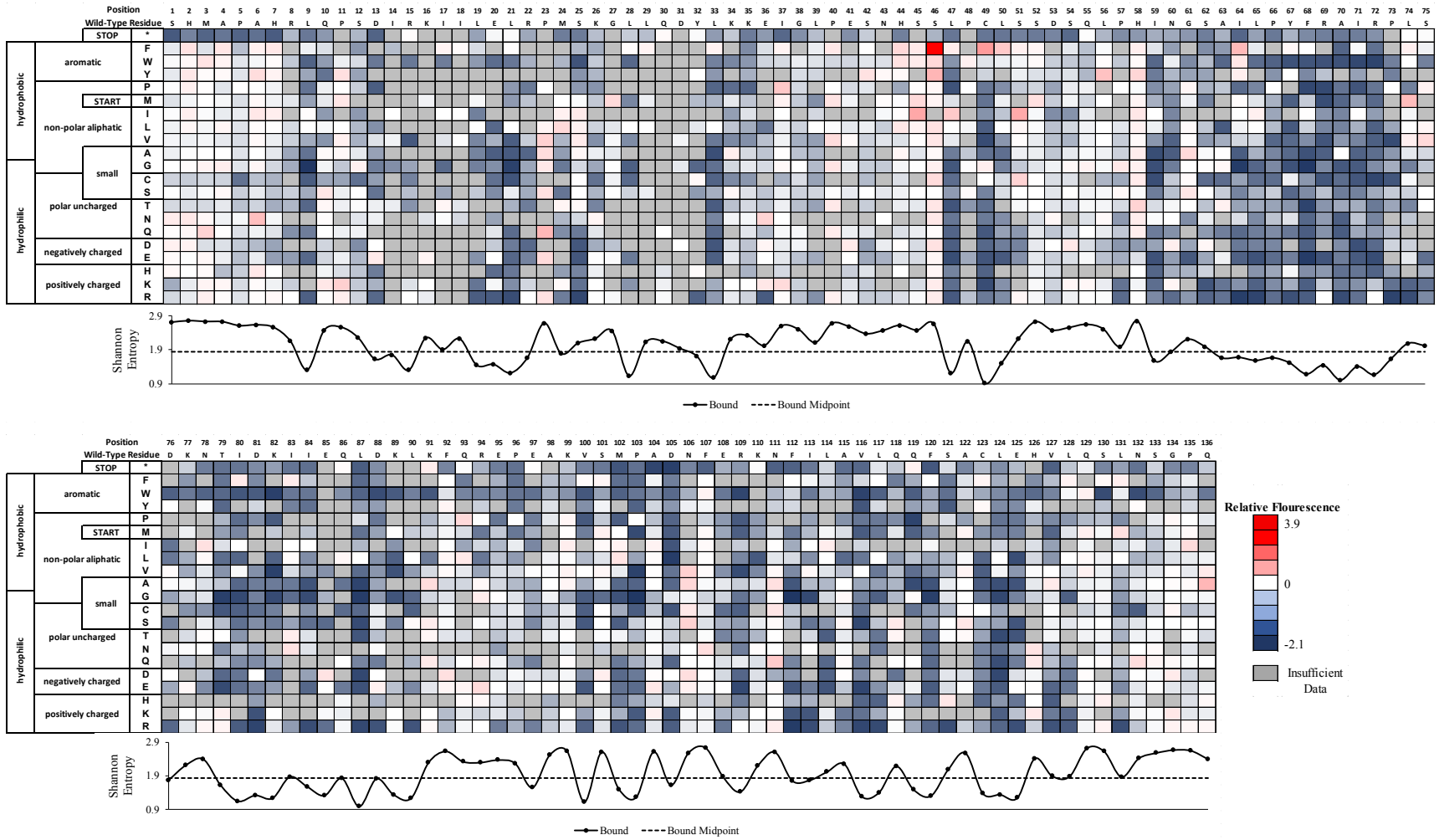
#	Time	Area	Height	Width	Area %	Symmetry
1	12.925	1458.8	27.5	0.7101	38.590	1.991
2	13.876	2321.4	63.2	0.5064	61.410	0.724

Supplementary Figure S1. Mass spectrometry and analytical SEC for feline IL-31 construct. (**a.**) Mass spec analysis of IL-31 after PNGase treatment. Conditions: 50mM Tris, pH 8.0. (**b.**) Chromatographic conditions: TSK SuperSW3000, 4.6 x 30 mm, mobile phase 200 mM sodium phosphate, pH 7.2, flow rate 0.25 mL/min for 25 minutes, injection amount: 50 µg

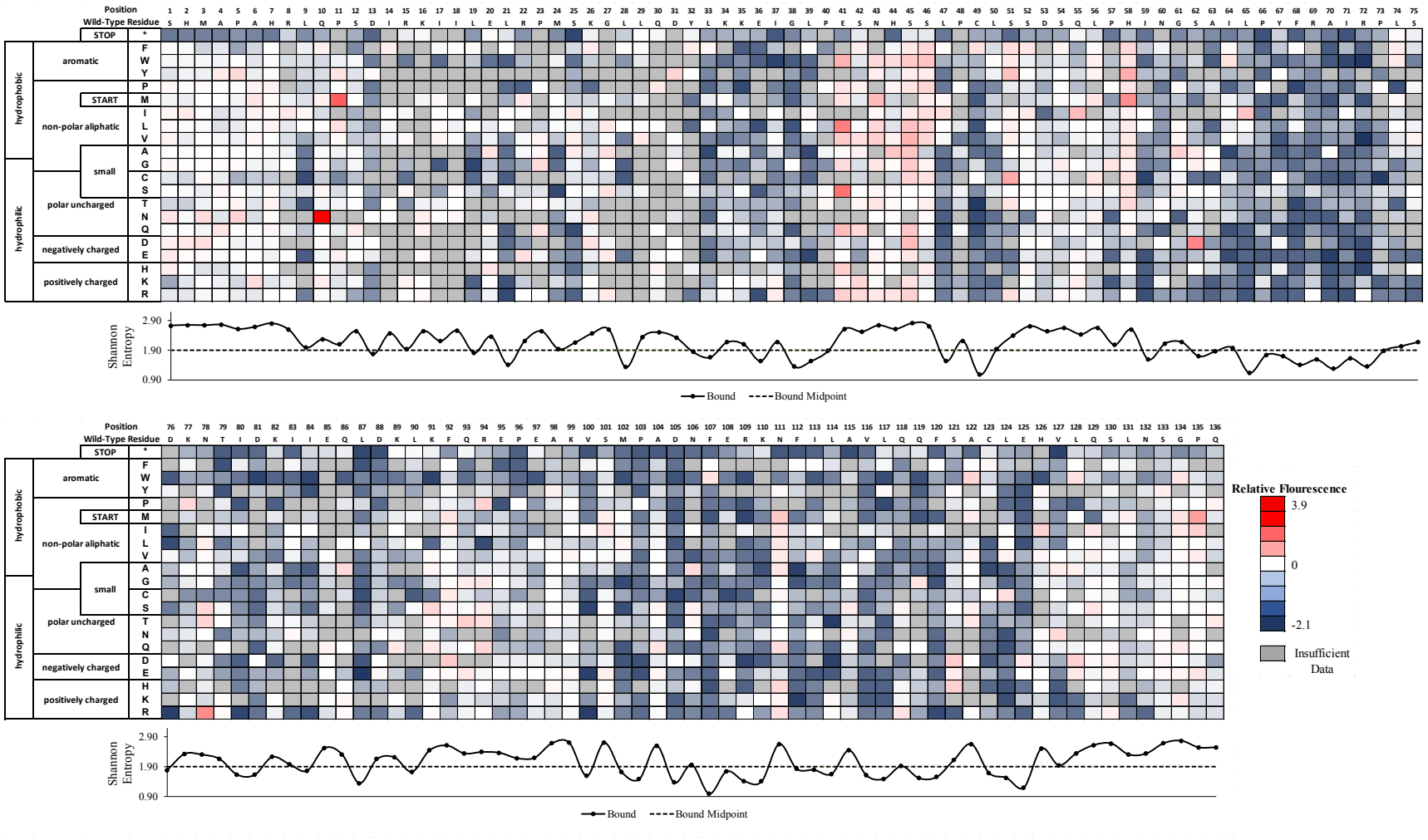


Supplementary Figure S2. Structural model of fIL-31. (**a.-b.)** Two different views showing packing of hydrophobic core. Aliphatic residues Phe, Tyr, Leu, Ile, Val, and Met are colored in

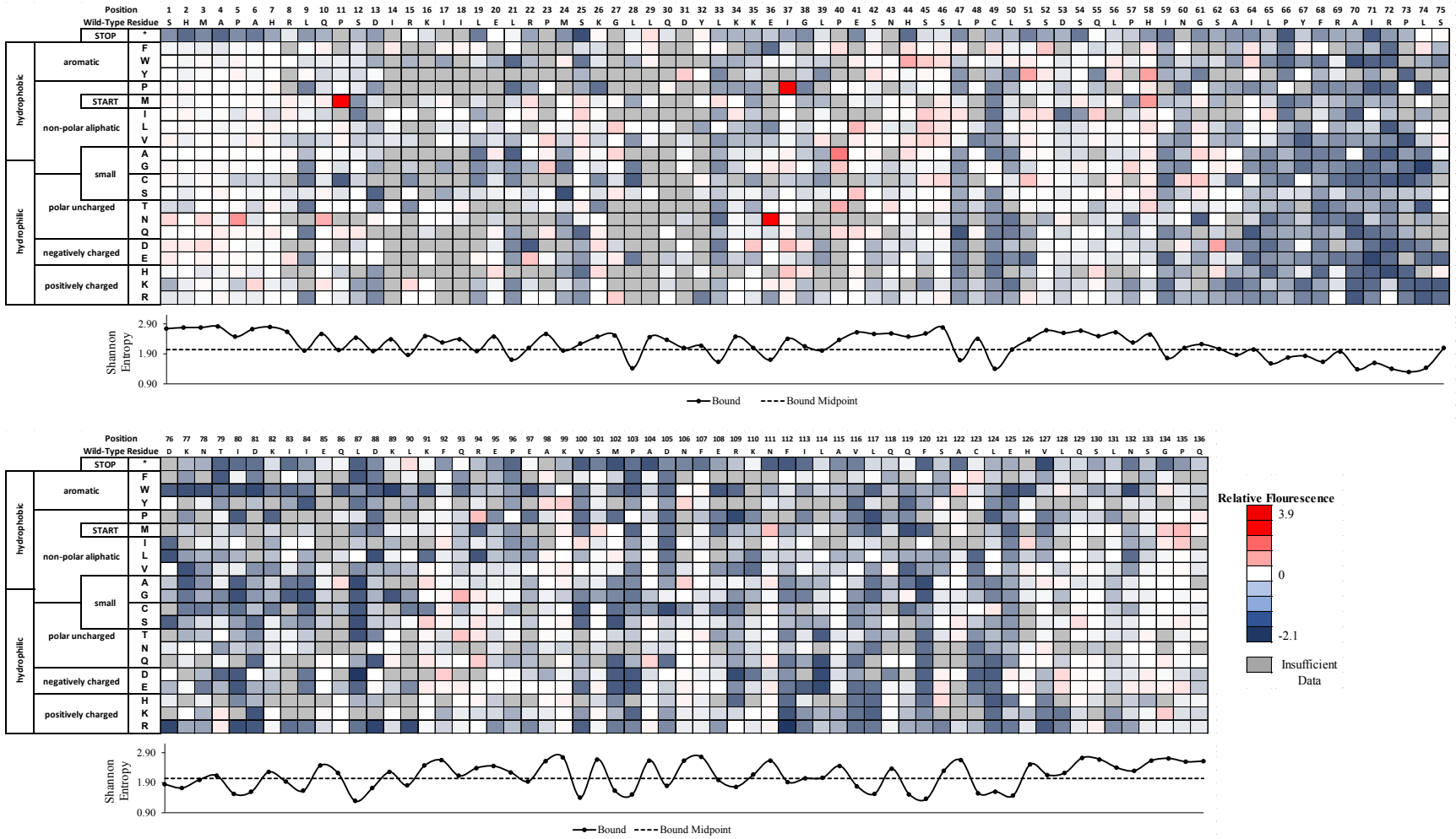
green sticks, and the remaining residues are shown as white sticks. The Cys49-Cys132 disulfide bond is shown as spheres. (**c.)** A closer view of the hydrophobic core of the structural model.



Supplementary Figure S3. Determination of the binding sites for fIL-31/fIL31RA-1FNIII interaction. Fitness Metric heatmap of the top 7% bound population vs the reference population. The Shannon entropy is plotted below with its respective cut-off midpoint (dashed line).



Supplementary Figure S4. Determination of the binding sites for fIL-31/fOSMR-ECD interaction. Fitness Metric heatmap of the top 7% bound population vs the reference population. The Shannon entropy is plotted below with its respective cut-off midpoint (dashed line).



Supplementary Figure S5. Determination of the conformational epitope for fIL-31/mAb#1 interaction. Fitness Metric heatmap of the top 7% bound population vs the reference population. The Shannon entropy is plotted below with its respective cut-off midpoint (dashed line).

Supplementary Table S1. Sorting conditions and FACS collection statistics for fIL-31 libraries.

Antigen	Amount of Collected Cells	Labeling Concentration [nM]	Percent Sorted Library 1	Percent Sorted Library 2
fOSMR-ECD	300,000	5.1	6.5%	6.7%
fIL31RA-1FNIII	300,000	52.8	8.5%	6.0%
mAb #1	300,000	0.13	7.6%	7.8%

Supplementary Table S2. Primers for deep sequencing. L1: Library 1, L2: Library 2, Blue: Illumina Universal Sequence, **NNNNNN**: Indexing Barcode, and Purple: Illumina Adapter, UN: Unselected Population, and CMYC: Displayed Population

Name	***Sequence***
Inner Primer:	
fIL-31_L1_FWD	5' - GTTCAGAGTTCTACAGTCCGACGATCAGGGTCGGCTAGCCATATG - 3'
fIL-31_L1_REV	5' - CCTTGGCACCCGAGAATTCCATCTGACAATGGTCTAATAGCTCT - 3'
fIL-31_L2_FWD	5' - GTTCAGAGTTCTACAGTCCGACGATCTCAGCAATCTTGCCATACTTT - 3'
fIL-31_L2_REV	5' - CCTTGGCACCCGAGAATTCCAATAAGCTTTTGTTCGGATCCG - 3'
Outer Primers:	
Illumina_FWD	5' - AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATC - 3'
Top 5% Displayed Experiment:	
fIL-31_L1_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L1_CMYC_REV	5' - CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_CMYC_REV	5' - CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Labeled with mAb#1 or fOSMR-ECD:	
fIL-31_L1_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L1_mAb#1_REV	5' - CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L1_OSMR_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'

fIL-31_L2_mAb#1_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GGAACT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_OSMR_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GGACGG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Labeled with fIL31RA-1FNIII:	
fIL-31_L1_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**CGAAAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L1_IL31RA-1FNIII_REV	5' - CAAGCAGAAGACGGCATACGAGAT**CCACTC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_UN_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GCTACC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
fIL-31_L2_IL31RA-1FNIII_REV	5' - CAAGCAGAAGACGGCATACGAGAT**GCTCAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'

Supplementary Table S3. fIL-31 library statistics results.

	fOSMR-ECD and mAb#1		***fIL31RA-1FNIII***		***Top 5% Displayed***	
	Library 1	Library 2	Library 1	Library 2	Library 1	Library 2
Percent of possible codon substitutions observed in the unselected population:						
1-base substitution	100%	100.3%	94.4%	95.8%	97.4%	97.4%
2-base substitutions	52.0%	56.4%	39.9%	42.0%	38.5%	42.8%
3-base substitutions	38.3%	41.5%	37.1%	38.0%	37.3%	38.8%
Percent of unselected reads with:						
No nonsynonymous mutations:	41.8%	45.6%	39.1%	45.3%	44.5%	45.9%
One nonsynonymous mutation:	52.6%	50.8%	58.6%	52.3%	48.3%	49.3%
Multiple nonsynonymous mutations:	5.5%	3.5%	2.3%	2.3%	7.2%	4.8%
Coverage of possible single nonsynonymous amino acid mutations:	**77.8%**	**83.2%**	**75.7%**	**80.0%**	**77.9%**	**81.0%**