

Supporting Information

Receptor Elimination by E3 Ubiquitin Ligase Recruitment (REULR): A Targeted Protein Degradation Toolbox.

Dirk H. Siepe¹, Lora K. Picton¹, K. Christopher Garcia^{1,2,3*}

¹Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305, USA.

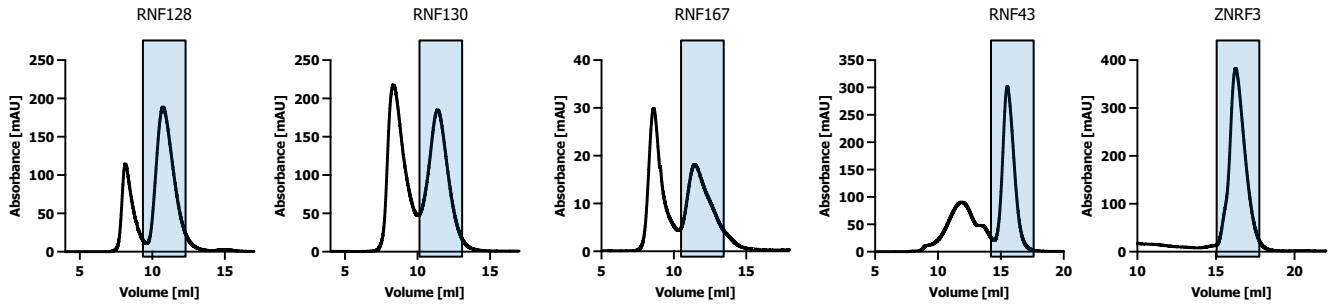
²Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305, USA.

³Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305, USA.

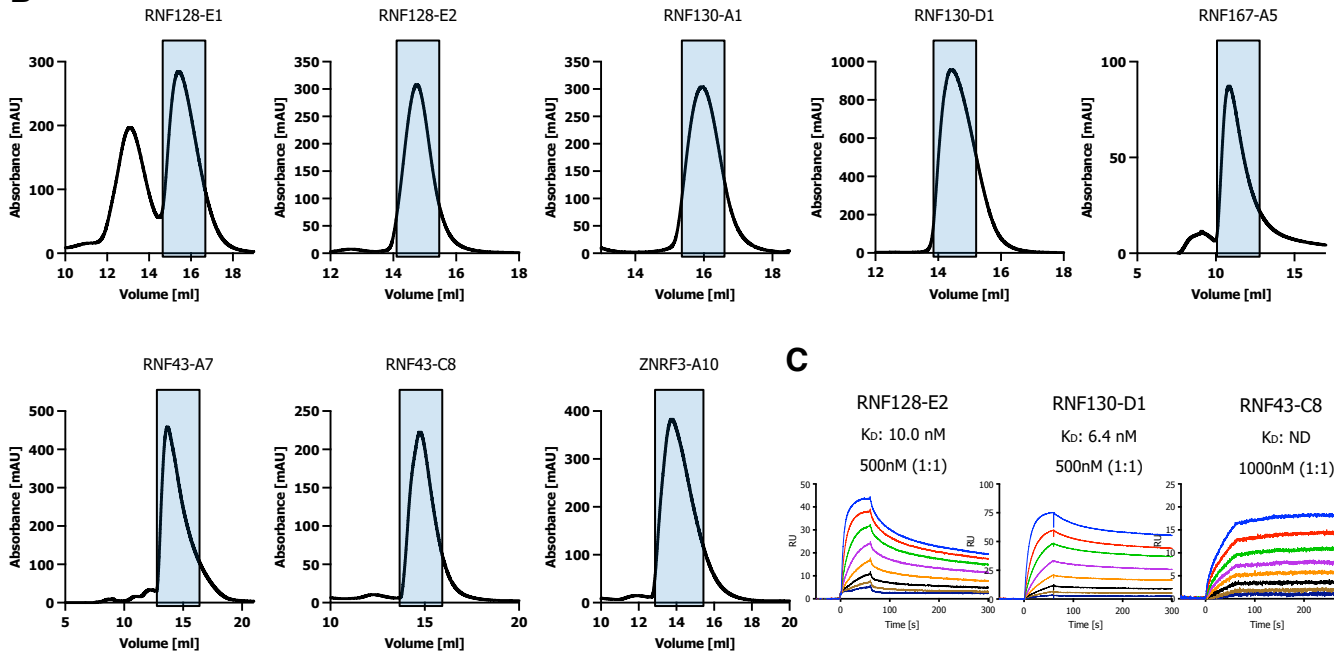
*Lead Contact. kcgarcia@stanford.edu

Figure S1

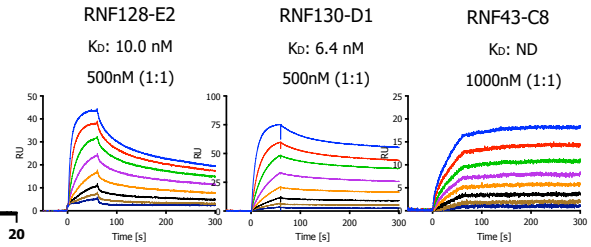
A



B



C



D

Human/Mouse Similarity	Identity	Gaps	
RNF128	97.95	96.15	0.00
RNF130	99.74	98.72	0.00
RNF167	96.3	93.83	0.62
RNF43	95.98	91.38	0.00
ZNRF3	98.78	98.17	0.00
Average [%]	97.75	95.65	0.12

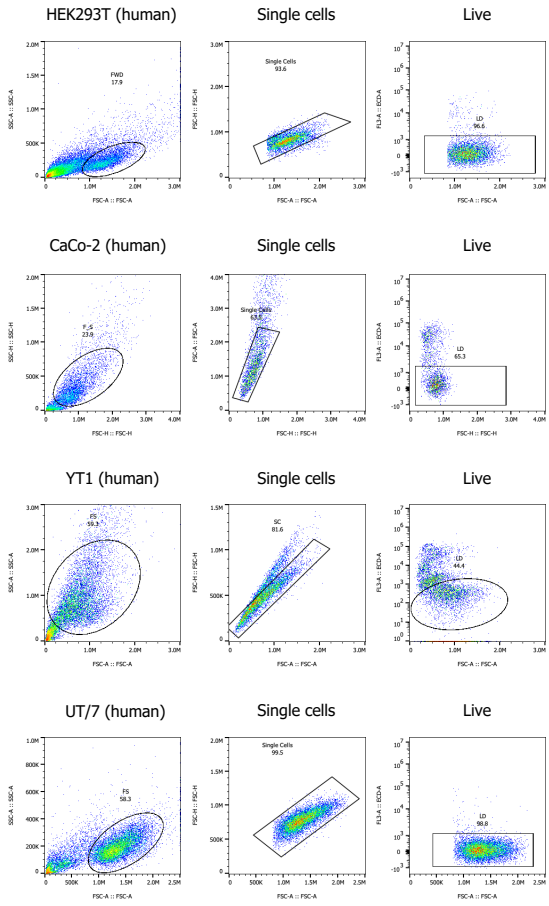
Figure S1

Figure S1. Size-Exclusion Chromatography, SPR sensograms and pairwise sequence alignments.

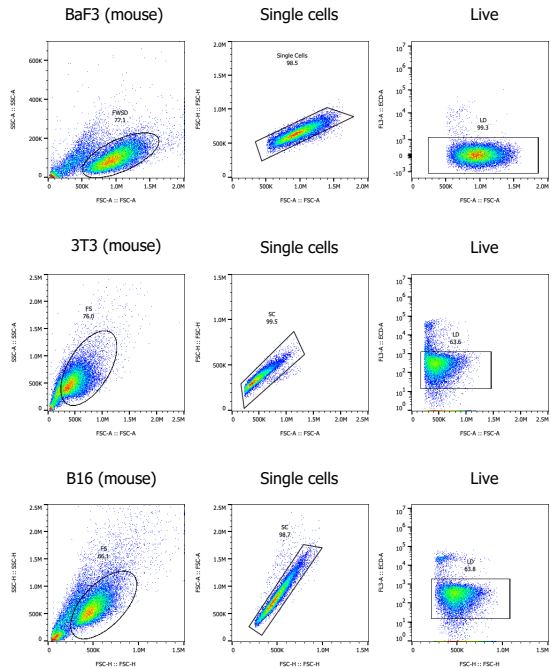
(A, B) Size-Exclusion Chromatography traces of RNF128, RNF130, RNF167, RNF43 and ZNRF3 PA-TM-RING E3 Ligase ECDs used for screening the synthetic nanobody library and SPR validation of PA-TM-RING E3 Ligase VHHs. (C) SPR sensograms and binding affinities of PA-TM-RING ligase selected nanobodies (analytes) for human RNF128, RNF130 and RNF43 ECDs (ligands). (D) Pairwise protein sequence alignments of PA-TM-RING E3 Ligases were performed using Smith-Waterman algorithm to calculate alignments between human and mouse amino acid sequences.

Figure S2

A



B



C

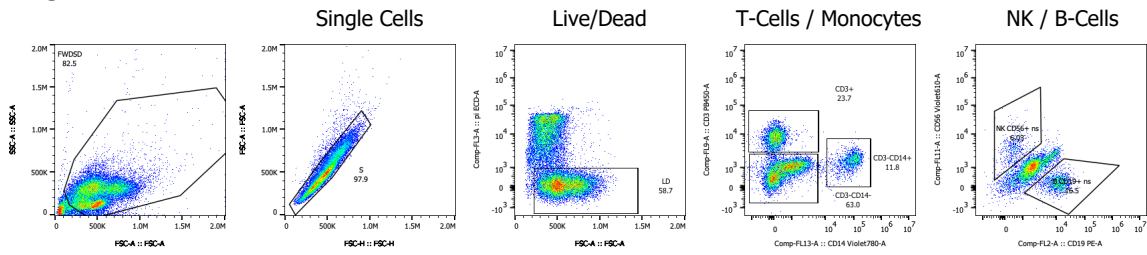


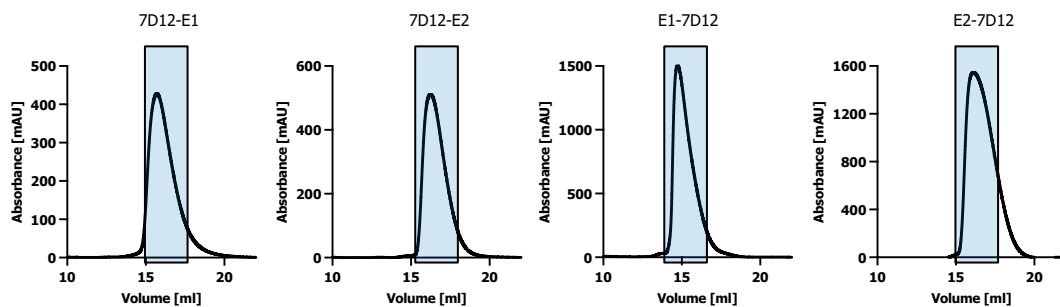
Figure S2

Figure S2. Cell Surface Staining Gating strategies.

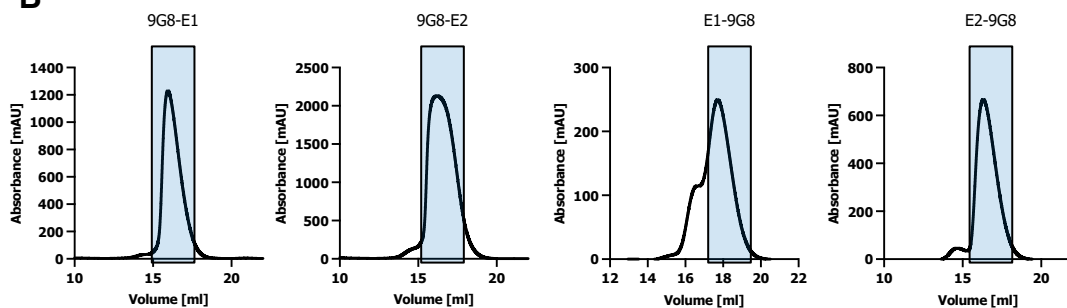
(A) Gating strategy of human HEK293T, CaCo-2, YT1, UT/7 cell lines. (B) Gating strategy of mouse BaF3, 3T3, B16 cell lines. (C) Gating strategy of Primary Peripheral Blood Mononuclear Cells (PBMCs).

Figure S3

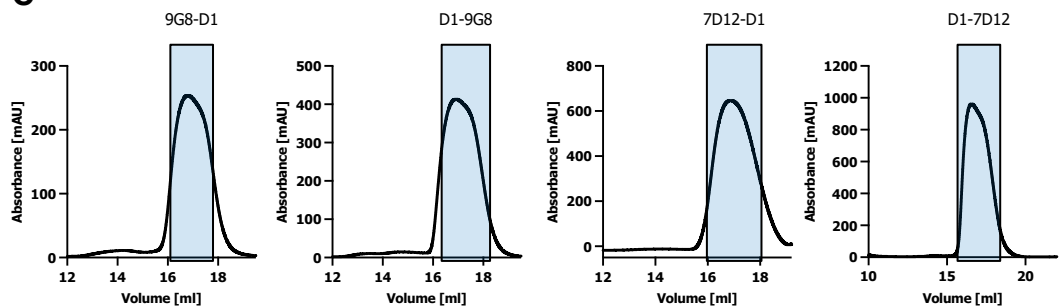
A



B



C



D

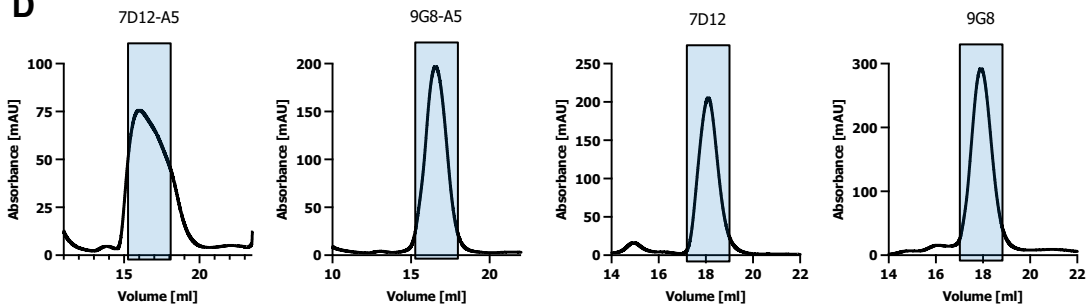


Figure S3

Figure S3. Size-Exclusion Chromatography.

(A-D) Size-Exclusion Chromatography traces of proteins used for EGFR-REULR experiments.

Figure S4

A

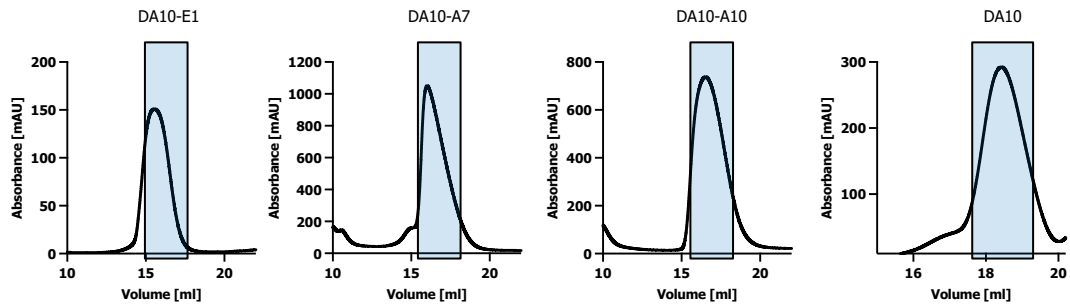


Figure S4

Figure S4. Size-Exclusion Chromatography.

(A) Size-Exclusion Chromatography traces of proteins used for EPOR-REULR experiments.

Figure S5

A

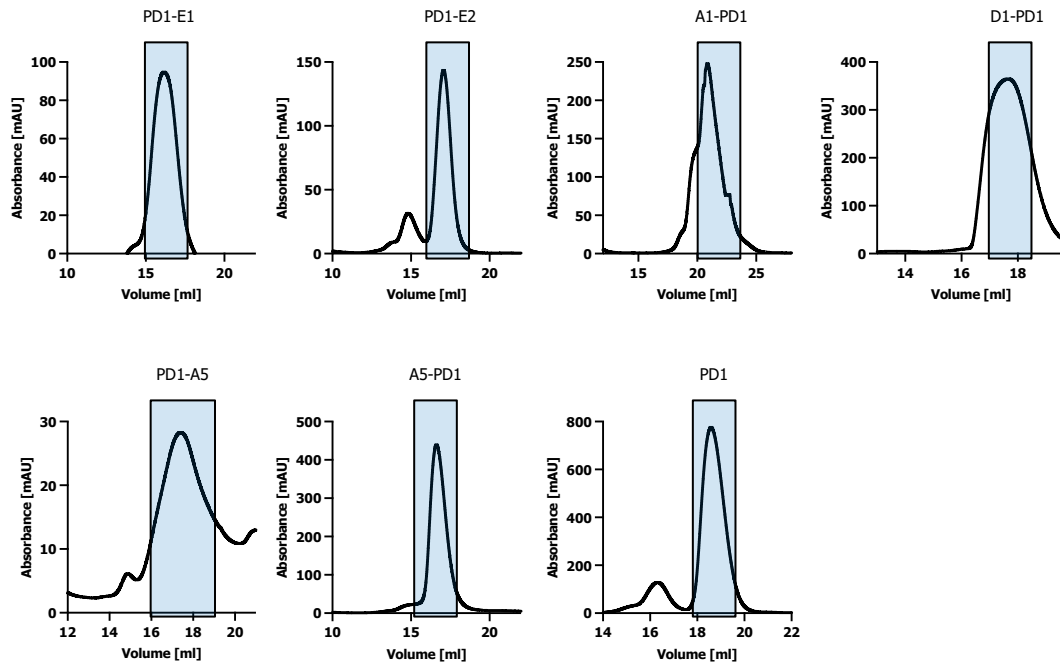


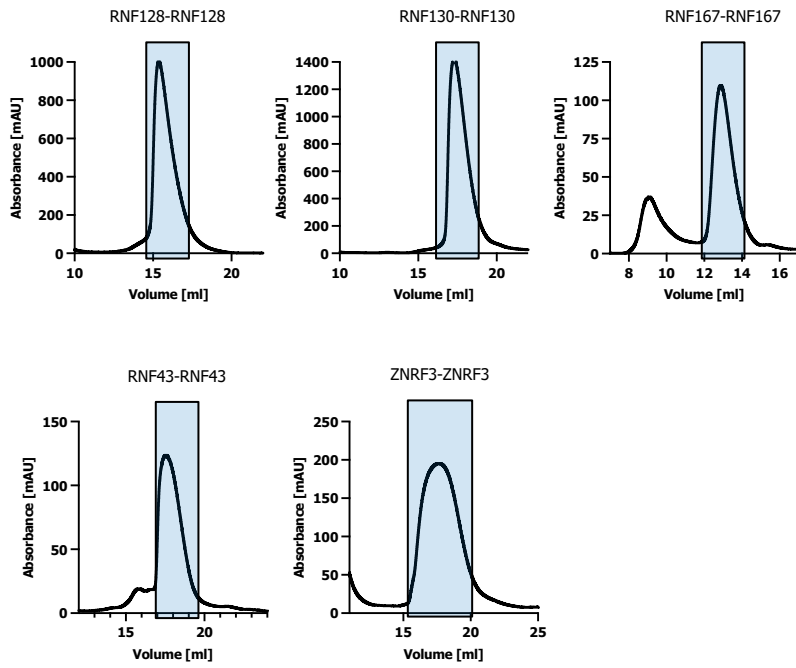
Figure S5

Figure S5. Size-Exclusion Chromatography.

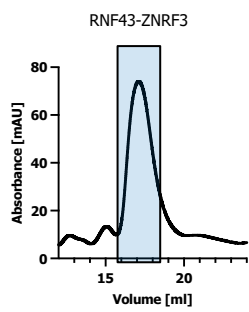
(A) Size-Exclusion Chromatography traces of proteins used for PD1-REULR experiments.

Figure S6

A



B



C

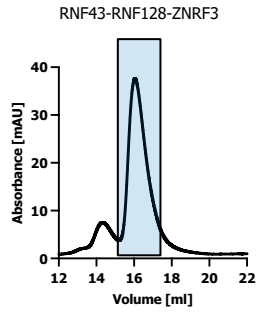


Figure S6

Figure S6. Size-Exclusion Chromatography.

(A-C) Size-Exclusion Chromatography traces of proteins used for Fratricide REULR experiments.

Table S1**A**

Ligand (bio+)	Analyte (bio-)	Max Analyte (nM)	Dilution Series	Injection (sec)	Injection (ul/min)	Dissociation (sec)	Regeneration	Regen (ul/min)
RNF128 ECD	RNF128 E1	500	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF128 ECD	RNF128 E2	500	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF130 ECD	RNF130 A1	500	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF130 ECD	RNF130 D1	500	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF167 ECD	RNF167 A5	1000	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF43 ECD	RNF43 A7	1000	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
RNF43 ECD	RNF43 C8	1000	2	60	30	240	1 x 10 sec Glycin pH 2.5	50
ZNRF3 ECD	ZNRF3 A10	125	2	60	30	240	1 x 10 sec Glycin pH 2.5	50

Table S1

Table S1. SPR Conditions.

(A) Table of SPR conditions for all ligand-analyte pairs tested including ligand RU, maximum analyte concentration, analyte RU at maximum concentration, injection time (seconds), injection rate (ul/minute), dissociation time (seconds) and regeneration conditions.

Table S2

A

NAME	TYPE	FORMAT	DESIGN	AA SEQUENCE	#
RNF128-E1	VHH	Monomer	RNF128	QVQLQESGGGLVQAGGSLRLSCAASGNISVQLDMGWYRQAPGKEREFVAAINQGTITTYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAVYLYDIWNHPYWGQGTQVTVSS	118
RNF128-E2	VHH	Monomer	RNF128	QVQLQESGGGLVQAGGSLRLSCAASGSISGGKGMGWYRQAPGKEREFVAIIGSgaiTYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAVYTTALDEYPYWGQGTQVTVSS	118
RNF130-A1	VHH	Monomer	RNF130	QVQLQESGGGLVQAGGSLRLSCAASGYISGYVYMGWYRQAPGKEREFVASISYGASTYYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAVDFDSNYAHTYWGQGTQVTVSS	118
RNF130-D1	VHH	Monomer	RNF130	QVQLQESGGGLVQAGGSLRLSCAASGTISFIGYMGWYRQAPGKERELVASIASGTSTYYADSVKGRFTISR NAKNTVYLMQNSLKPEDTAVYYCAATQYIQDVHRYWGQGTQVTVSS	118
RNF167-A5	VHH	Monomer	RNF167	QVQLQESGGGLVQAGGSLRLSCAASGSIFRLWYMGWYRQAPGKEREFVASIGIGATTNYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAVFGWAYSGYHDDFLYWGQGTQVTVSS	122
RNF43-A7	VHH	Monomer	RNF43	QVQLQESGGGLVQAGGSLRLSCAASGTIFGLSDMGWYRQAPGKEREFVAAIAGGTITTYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAAYKYDERYHSYWGQGTQVTVSS	118
RNF43-C8	VHH	Monomer	RNF43	QVQLQESGGGLVQAGGSLRLSCAASGSIFWKPVMGWYRQAPGKEREFVAAITSGTNTTYADSVKGRFTISR DNAKNTVYLMQNSLKPEDTAVYYCAVDDYDVVEYPYWGQGTQVTVSS	118
ZNRF-A10	VHH	Monomer	ZNRF3	QVQLQESGGGLVQAGGSLRLSCAASGTISYAHIMGWYRQAPGKERELVAGISQGSITNYADSVKGRFTISR SRDNAKNTVDLQMSLKPEDTAVYYCAVISYDIKSVPFYWGQGTQVTVSS	121
7D12	VHH	Monomer	EGFR	QVKLEESGGGSVQTGGSLRLTCAASGRTRSRYGMWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTI SRDNAKNTVDLQMSLKPEDTAVYYCAAAAGSAWYGTLYEYDYWGQGTQVTVSS	124
9G8	VHH	Monomer	EGFR	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTI SRDNAKNTMYLQMSLKPEDTAVYYCAAGYQINSNGYNFKDYEYDYWGQGTQVTVSS	127
DA10	scFv	Monomer	EPOR	EVQLLESVGGGLVQPGGSLRLSCAASGFTFSSYAMSWFRQAPGKLEWVSAISGGGSGTYADSVKGRFTIS RDNSKNTLYLQMSLRAEDTAVYYCVKDRVAVAGKGSYYFDSWGRGTTVTVSSGGGGGGGGGGGGGS QSVLTQPPSVSEAPGQRVTIACSGSSSNIGNNAVSWYQQLPGKAPTLIIYDNLPSGVSDRFSGSKSGTSA SLAISGLQSEADYYCAAWDDSLNDWVFGGGTKVTVL	249
PD1	VHH	Monomer	PD1	DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLMQNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSS	119

Table S2

B

NAME	TYPE	FORMAT	DESIGN	AA SEQUENCE	#
7D12-E1	VHH-VHH	Dimer	EGFR-RNF128	QVKLEESGGGSVQTGGSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTI SRDNAKNTVDLQMNLSLKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSSLEVLFGQPQVQLQESG GGLVQAGGSLRLSACAASGNISVQLDMGWYRQAPGKEREFVAAINQGGTTTTYYADSVKGRFTISRDNAKNTVY LQMNLSLKPEDTAIYYCAVYLYDIWNHPYWGQGTQVTVSS	250
7D12-E2	VHH-VHH	Dimer	EGFR-RNF128	QVKLEESGGGSVQTGGSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTI SRDNAKNTVDLQMNLSLKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSSLEVLFGQPQVQLQESG GGLVQAGGSLRLSACAASGISGGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISRDNAKNTVY LQMNLSLKPEDTAIYYCAVYTTALDEYPYWGQGTQVTVSS	250
E1-7D12	VHH-VHH	Dimer	EGFR-RNF128	QVQLQESGGGLVQAGGSLRLSACAASGNISVQLDMGWYRQAPGKEREFVAAINQGGTTTTYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAVYLYDIWNHPYWGQGTQVTVSSLEVLFGQPQVQLQESGGGSVQTG GSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMN LKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSS	250
E2-7D12	VHH-VHH	Dimer	EGFR-RNF128	QVQLQESGGGLVQAGGSLRLSACAASGISGGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAVYTTALDEYPYWGQGTQVTVSSLEVLFGQPQVQLQESGGGSVQTG GSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMN LKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSS	250
9G8-E1	VHH-VHH	Dimer	EGFR-RNF128	EVQLVESGGGLVQAGGSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTI SRDNAKNTMYLQMNLSLKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSSLEVLFGQPQVQLQ ESGGGLVQAGGSLRLSACAASGNISVQLDMGWYRQAPGKEREFVAAINQGGTTTTYYADSVKGRFTISRDNAK NTVYLMNLSLKPEDTAIYYCAVYLYDIWNHPYWGQGTQVTVSS	253
9G8-E2	VHH-VHH	Dimer	EGFR-RNF128	EVQLVESGGGLVQAGGSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTI SRDNAKNTMYLQMNLSLKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSSLEVLFGQPQVQLQ ESGGGLVQAGGSLRLSACAASGISGGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISRDNAK NTVYLMNLSLKPEDTAIYYCAVYTTALDEYPYWGQGTQVTVSS	253
E1-9G8	VHH-VHH	Dimer	EGFR-RNF128	QVQLQESGGGLVQAGGSLRLSACAASGNISVQLDMGWYRQAPGKEREFVAAINQGGTTTTYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAVYLYDIWNHPYWGQGTQVTVSSLEVLFGQPQVQLQESGGGLVQAG GSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTISRDNAKNTMYLQMN LKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSS	253
E2-9G8	VHH-VHH	Dimer	EGFR-RNF128	QVQLQESGGGLVQAGGSLRLSACAASGISGGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAVYTTALDEYPYWGQGTQVTVSSLEVLFGQPQVQLQESGGGLVQAG GSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTISRDNAKNTMYLQMN LKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSS	253
9G8-D1	VHH-VHH	Dimer	EGFR-RNF130	EVQLVESGGGLVQAGGSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTI SRDNAKNTMYLQMNLSLKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSSLEVLFGQPQVQLQ ESGGGLVQAGGSLRLSACAASGTISFIGYMGWYRQAPGKERELVASIASGTSTYYADSVKGRFTISRDNAK NTVYLMNLSLKPEDTAIYYCAATQYIQDVHRYWGQGTQVTVSS	253
D1-9G8	VHH-VHH	Dimer	EGFR-RNF130	QVQLQESGGGLVQAGGSLRLSACAASGTISFIGYMGWYRQAPGKERELVASIASGTSTYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAATQYIQDVHRYWGQGTQVTVSSLEVLFGQPQVQLQESGGGLVQAGG SLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTISRDNAKNTMYLQMN LKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSS	253
7D12-D1	VHH-VHH	Dimer	EGFR-RNF130	QVKLEESGGGSVQTGGSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTI SRDNAKNTVDLQMNLSLKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSSLEVLFGQPQVQLQESG GGLVQAGGSLRLSACAASGTISFIGYMGWYRQAPGKERELVASIASGTSTYYADSVKGRFTISRDNAKNTVY LQMNLSLKPEDTAIYYCAATQYIQDVHRYWGQGTQVTVSS	250
D1-7D12	VHH-VHH	Dimer	EGFR-RNF130	QVQLQESGGGLVQAGGSLRLSACAASGTISFIGYMGWYRQAPGKERELVASIASGTSTYYADSVKGRFTISR DNAKNTVYLMNLSLKPEDTAIYYCAATQYIQDVHRYWGQGTQVTVSSLEVLFGQPQVQLQESGGGSVQTG SLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMN LKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSS	250
7D12-A5	VHH-VHH	Dimer	EGFR-RNF167	QVKLEESGGGSVQTGGSLRLTCAASGRTRSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTI SRDNAKNTVDLQMNLSLKPEDTAIYYCAAAGSAWYGTLYEYDYWGQGTQVTVSSLEVLFGQPQVQLQESG GGLVQAGGSLRLSACAASGSIFRLWYMGWYRQAPGKEREFVASIGATTNYADSVKGRFTISRDNAKNTVY LQMNLSLKPEDTAIYYCAVFGWAYSGYHDDFLYWGQGTQVTVSS	254
9G8-A5	VHH-VHH	Dimer	EGFR-RNF167	EVQLVESGGGLVQAGGSLRLSACAASGRTFSSYAMGWFRQAPGKEREFVAINWSSGSTYYADSVKGRFTI SRDNAKNTMYLQMNLSLKPEDTAIYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSSLEVLFGQPQVQLQ ESGGGLVQAGGSLRLSACAASGSIFRLWYMGWYRQAPGKEREFVASIGATTNYADSVKGRFTISRDNAK NTVYLMNLSLKPEDTAIYYCAVFGWAYSGYHDDFLYWGQGTQVTVSS	257

Table S2

C

NAME	TYPE	FORMAT	DESIGN	AA SEQUENCE	#
DA10-E1	scFv-VHH	Dimer	EPOR-RNF128	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKLEWVSAISGGGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCVKDRVAVAGKGSYFDSWGRGTTVTVSSGGGGSGGGGGSGGGGS QSVLTQPPSVSEAPGQRVTIACSGSSNIGNNAVSWYQQLPGKAPTLIIYDNLPSGVSDRFSGSKSGTSA SLAISGLQSEDEADYYCAAWDDSLNDWVFGGGTKVTVLVQVQLQESGGGLVQAGGSLRLSCAASGNISVQL DMGWYRQAPGKEREFVAAINQGGTTTTYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCAVYLDIWI NHPYWGQGTQVTVSS	367
DA10-A7	scFv-VHH	Dimer	EPOR-RNF43	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKLEWVSAISGGGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCVKDRVAVAGKGSYFDSWGRGTTVTVSSGGGGSGGGGGSGGGGS QSVLTQPPSVSEAPGQRVTIACSGSSNIGNNAVSWYQQLPGKAPTLIIYDNLPSGVSDRFSGSKSGTSA SLAISGLQSEDEADYYCAAWDDSLNDWVFGGGTKVTVL	249
DA10-A10	scFv-VHH	Dimer	EPOR-ZNRF3	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKLEWVSAISGGGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCVKDRVAVAGKGSYFDSWGRGTTVTVSSGGGGSGGGGGSGGGGS QSVLTQPPSVSEAPGQRVTIACSGSSNIGNNAVSWYQQLPGKAPTLIIYDNLPSGVSDRFSGSKSGTSA SLAISGLQSEDEADYYCAAWDDSLNDWVFGGGTKVTVL	249
PD1-E1	VHH-VHH	Dimer	PD1-RNF128	DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSSLEVLVQFGPQVQLQESGGGLVQA GGSLRLSCAASGNISVQLDMGWYRQAPGKEREFVAAINQGGTTTTYADSVKGRFTISRDNKNTVYLQMNSL KPEDTAVYYCAVYLDIWIHNHPYWGQGTQVTVSS	245
PD1-E2	VHH-VHH	Dimer	PD1-RNF128	DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSSLEVLVQFGPQVQLQESGGGLVQA GGSLRLSCAASGSIISGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISRDNKNTVYLQMNSL KPEDTAVYYCAVYTTALDEYPYWGQGTQVTVSS	245
A1-PD1	VHH-VHH	Dimer	PD1-RNF130	VQVQLQESGGGLVQAGGSLRLSCAASGYSISGYVMGWYRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLQMNSLRPEDTAVYYCAVDFDSNYAHTYWGQGTQVTVSSLEVLVQFGPQVQLVESGGGVVQPG GSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISRDNKNTVYLQMNSLR PEDTALYYCAGDKHQSSWYDYWGQGTQVTVSS	245
D1-PD1	VHH-VHH	Dimer	PD1-RNF130	VQVQLQESGGGLVQAGGSLRLSCAASGYSISGYVMGWYRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSSLEVLVQFGPQVQLVESGGGLVQA GGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISRDNKNTVYLQMNSLR EDTALYYCAGDKHQSSWYDYWGQGTQVTVSS	245
PD1-A5	VHH-VHH	Dimer	PD1-RNF167	DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISR DNSKNTVYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSSLEVLVQFGPQVQLQESGGGLVQA GGSLRLSCAASGSIIFRLWYMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISRDNKNTVYLQMNSLK PEDTAVYYCAVFGWAYSGYHDDFLYWGQGTQVTVSS	249
A5-PD1	VHH-VHH	Dimer	PD1-RNF167	VQVQLQESGGGLVQAGGSLRLSCAASGSIIFRLWYMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVFGWAYSGYHDDFLYWGQGTQVTVSSLEVLVQFGPQVQLVESGGG VQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSSGITYYADSVKGRFTISRDNKNTVYLQ MNSLRPEDTALYYCAGDKHQSSWYDYWGQGTQVTVSS	249
E2-E2	VHH-VHH	Dimer	RNF128-RNF128	VQVQLQESGGGLVQAGGSLRLSCAASGSIISGKGMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVYTTALDEYPYWGQGTQVTVSSGSLVLFQGGPQVQLQESGGGLV VQAGGSLRLSCAASGSIISGKGMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVYTTALDEYPYWGQGTQVTVSS	248
A1-A1	VHH-VHH	Dimer	RNF130-RNF130	VQVQLQESGGGLVQAGGSLRLSCAASGYSISGYVMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVDFDSNYAHTYWGQGTQVTVSSLEVLVQFGPQVQLQESGGGLV VQAGGSLRLSCAASGSIIFRLWYMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVDFDSNYAHTYWGQGTQVTVSS	248
A5-A5	VHH-VHH	Dimer	RNF167-RNF167	VQVQLQESGGGLVQAGGSLRLSCAASGSIIFRLWYMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVFGWAYSGYHDDFLYWGQGTQVTVSSGSLVLFQGGPQVQLQESGG VQAGGSLRLSCAASGSIIFRLWYMGWYRQAPGKEREFVAVISIGATTNYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVFGWAYSGYHDDFLYWGQGTQVTVSS	256
A7-A7	VHH-VHH	Dimer	RNF43-RNF43	VQVQLQESGGGLVQAGGSLRLSCAASGTFIFGLSDMGWYRQAPGKEREFVAAIAGGTITYYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAAYKYDERYHSYWGQGTQVTVSSGSLVLFQGGPQVQLQESGGGLV QAGGSLRLSCAASGTFIFGLSDMGWYRQAPGKEREFVAAIAGGTITYYADSVKGRFTISRDNKNTVYLQMN LKPEDTAVYYCAAYKYDERYHSYWGQGTQVTVSS	248
A10-A10	VHH-VHH	Dimer	ZNRF3-ZNRF3	VQVQLQESGGGLVQAGGSLRLSCAASGTSIYAHIMGWYRQAPGKERELVAGISQGSITNYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVISYDIKSVPFYRWGQGTQVTVSSGSLVLFQGGPQVQLQESGGG LVQAGGSLRLSCAASGTSIYAHIMGWYRQAPGKERELVAGISQGSITNYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVISYDIKSVPFYRWGQGTQVTVSS	254
C8-A10	VHH-VHH	Dimer	RNF43-ZNRF3	VQVQLQESGGGLVQAGGSLRLSCAASGSIIFWPKVMGWYRQAPGKEREFVAAITSGTNTYYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVDDYDVVEYPYWGQGTQVTVSSGSLVLFQGGPQVQLQESGGG LVQAGGSLRLSCAASGTSIYAHIMGWYRQAPGKERELVAGISQGSITNYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVISYDIKSVPFYRWGQGTQVTVSS	251
C8-E2-A10	VHH-VHH-VHH	Trimer	RNF43-RNF128- ZNRF3	VQVQLQESGGGLVQAGGSLRLSCAASGSIIFWPKVMGWYRQAPGKEREFVAAITSGTNTYYADSVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCAVDDYDVVEYPYWGQGTQVTVSSGSLVLFQGGPQVQLQESGGG LVQAGGSLRLSCAASGSIISGKGMGWYRQAPGKEREFVAAIGSGAITYYADSVKGRFTISRDNKNTVYLQ MNSLRKPEDTAVYYCAVYTTALDEYPYWGQGTQVTVSSGSLVLFQGGPQVQLQESGGGLVQAGGSLRLS CAASGTSIYAHIMGWYRQAPGKERELVAGISQGSITNYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVY YCAVISYDIKSVPFYRWGQGTQVTVSS	381

Table S2

Table S2. REULR Sequences.

(A-C) Table of REULR format, architecture and amino acid sequences.