Supplemental Figure 1: Deglycosylation of IL-1RAcP by Endoglycosidase A and IL-1/IL-33 Cell Signaling Inhibition (A) Lane 1: Bio-Rad unstained protein ladder. Lane 2: Endoglycosidase A (EndoA). Lane 3: Glycosylated IL-1RAcP. Lane 4: Deglycosylated IL-1RAcP. **(B)** Table containing the IC_{50} and R-square values for IL-1 and IL-33 cell signaling assays.

Supplemental Figure 2: Deuterium uptake of IL-1RAcP in apo form and antibody bound form by HDX-MS. (A) Deuterium uptake graphs of CAN03 bound (red), CAN04 bound (blue), and Apo IL-1RAcP (black). This stretch of residues was used in figure 3A for our HDX-MS analysis of CAN03 binding to IL-1RAcP. **(B)** Deuterium uptake graphs of CAN03 bound (red), CAN04 bound (blue), and Apo IL-1RAcP (black). This stretch of residues was used in figure 3B for our HDX-MS analysis of CAN04 binding to IL-1RAcP. **(C)** IL-1RAcP HDX-MS peptide coverage on IL-1RAcP, wherein coverage is colored green and lack of coverage is colored dark grey. **(D)** Peptide coverage map of IL-1RAcP, wherein coverage was 65.2% and redundancy was 2.96.

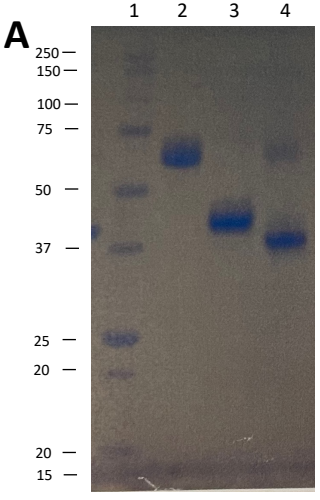
Supplemental Table 2: PISA analysis of IL-1 and IL-33 Signaling Complexes (A) Table of PISA interface of chain C (IL-1RAcP) to chain A (IL-1β) from the crystal structure of the IL-1β signaling complex (pdb: 4DEP). **(B)** Table of PISA interface of chain C (IL-1RAcP) to chain B (IL-1RI) from the crystal structure of the IL-1β signaling complex (pdb: 4DEP). **(C)** Table of PISA interface of chain C (IL-1RAcP) to chain A (IL-33) from the crystal structure of the IL-33 signaling complex (pdb: 5VI4). **(D)** Table of PISA interface of chain C (IL-1RAcP) to chain B (ST2) from the crystal structure of the IL-33 signaling complex (pdb: 5VI4).

Supplemental Figure 3: Sensorgrams from corresponding SPR experiments for Extensive Alanine Scan (A) Sensorgrams of IL-1RAcP alanine scan (Fc-fused IL-1RAcP with alanine mutations) and CAN03 as the analyte with Chi^2 values of fit. **(B)** Sensorgrams of IL-1RAcP alanine scan (Fc-fused IL-1RAcP with alanine mutations) and CAN04 as the analyte with Chi^2 values of fit.

Supplemental Table 3: Kinetic data from Extensive Alanine Scan (A) k_a, k_d, and K_D values of alanine scan SPR experiment, with CAN03 as the analyte. **(B)** k_a, k_d, and K_D values of alanine scan SPR experiment, with CAN04 as the analyte.

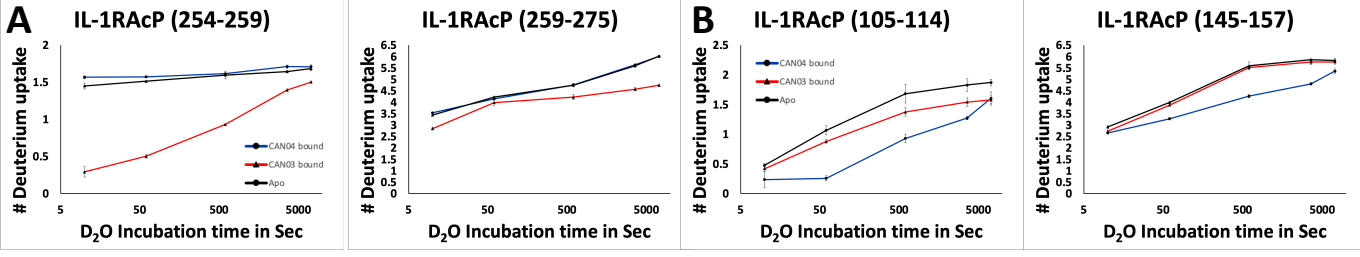
Supplemental Figure 4: CAN03 and CAN04 combination IL-1β Cell Signaling Inhibition Assay (A) A cell signaling assay containing CAN03 (red), CAN04 (blue), and a mix of CAN03/CAN04 (purple) to accompany Figure 7.

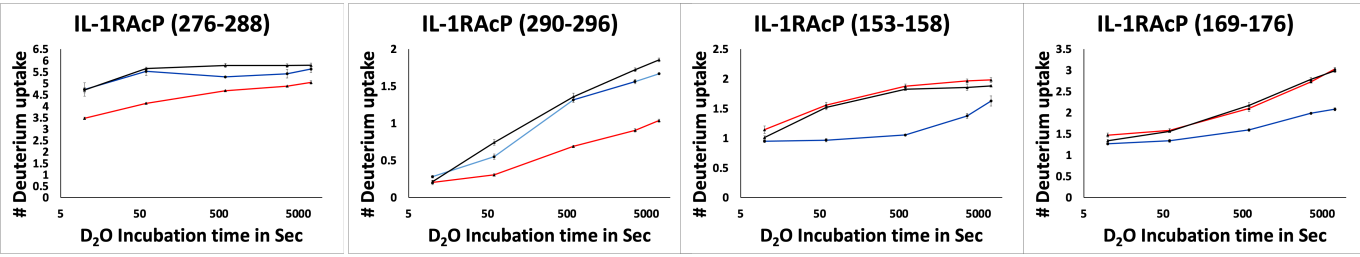
A

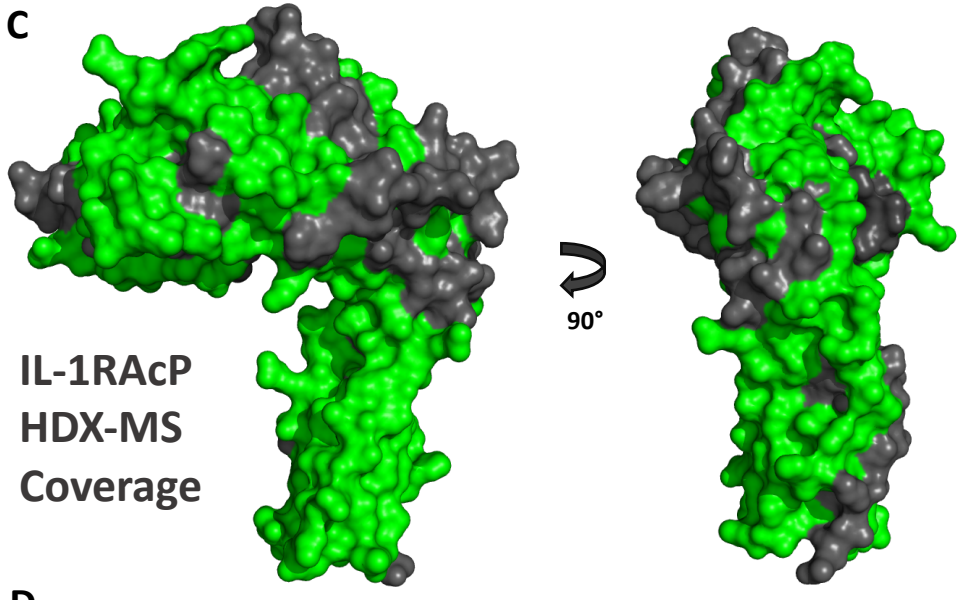


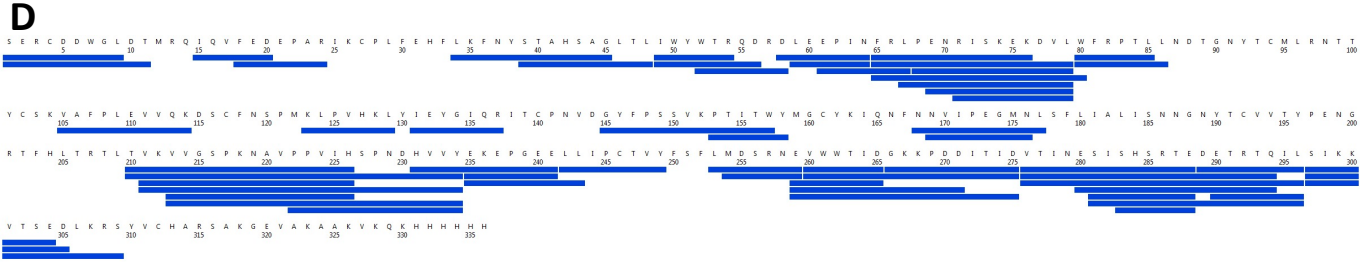
B

Antagonist	IC_{50} of antagonists for IL-1 Signaling Inhibition	R-Square of IL-1 Non-linear fit	IC_{50} of antagonists for IL-33 Signaling Inhibition	R-Square of IL-33 Non-linear fit
Interleukin-1 Receptor Antagonist (IL-1Ra)	$1.64 * 10^{-10}$	0.98	-	-
Soluble ST2	-	-	$1.16 * 10^{-10}$	0.97
CAN03	$4.37 * 10^{-9}$	0.88	$2.20 * 10^{-8}$	0.90
CAN04	$4.87 * 10^{-11}$	0.98	$7.82 * 10^{-10}$	0.90


A
IL-1RAcP (254-259)
IL-1RAcP (259-275)
B
IL-1RAcP (105-114)
IL-1RAcP (145-157)
Deuterium uptake
D2O Incubation time in Sec
CAN04 bound
CAN03 bound
Apo


IL-1RAcP (276-288)
IL-1RAcP (290-296)
IL-1RAcP (153-158)
IL-1RAcP (169-176)
Deuterium uptake
D2O Incubation time in Sec


C
IL-1RAcP
HDX-MS
Coverage
90°


D

A PISA Analysis: IL-1β interface on IL-1RAcP

Region	##	Structure 1	HSDC	ASA	BSA	ΔiG
Domain 2	129	C:ILE 131		35.1	29.76 ||||||||	0.48
Domain 2	130	C:GLU 132	S	105.5	51.52 |||||	-0.25
Domain 2	157	C:MET 159		51.9	23.85 |||||	0.85
Domain 2	160	C:TYR 162		163.9	15.49 |	0.25
Domain 2	163	C:GLN 165	H	66.6	14.10 |||	-0.07
Domain 2	164	C:ASN 166	H	155.7	52.41 ||||	-0.29
Domain 2	165	C:PHE 167		65.6	43.42 ||||||	0.56
Domain 2	166	C:ASN 168	H	140.2	113.28 ||||||||	0.02
Domain 2	167	C:ASN 169		18.8	0.61 |	-0.01
Domain 2	168	C:VAL 170		3.99	2.68 ||||||	0.04
Domain 2	179	C:ILE 181		90.6	1.49 |	0.02
Domain 2	181	C:LEU 183		79.0	64.84 ||||||||	1.04
Domain 2	182	C:ILE 184		75.5	59.26 |||||||	0.82
Domain 2	183	C:SER 185		83.1	79.12 |||||||||	0.71
Domain 2	184	C:ASN 186		8.18	8.18 ||||||||||	-0.09
Domain 2	188	C:TYR 190		1.40	0.49 ||||	-0.01
Linker	217	C:ASN 219		105.8	14.86 ||	-0.17
Domain 3	260	C:ARG 286	HS	183.7	106.51 ||||||	-0.98
Domain 3	261	C:THR 287		29.4	16.16 ||||||	0.23

Region	Domain Location of Interaction
##	Line Number
Structure 1	Chain – Amino Acid - # in PDB file
HSDC	Residues making **H**ydrogen/**D**isulphide bond, **S**alt Bridge, or **C**ovalent Link.
ASA	Accessible Surface Area, Å²
BSA	Buried Surface Area, Å²
Δ^{i}G	Solvation energy effect, kcal/mol
||||	Buried area percentage, one bar per 10%

B PISA Analysis: IL-1RI interface on IL-1RAcP

Region	##	Structure 1	HSDC	ASA	BSA	ΔiG
Domain 2	130	C:GLU 132		105.5	47.49 ‖‖‖‖	-0.49
Domain 2	131	C:TYR 133		76.9	3.29 |	0.05
Domain 2	132	C:GLY 134	H	37.9	36.15 ‖‖‖‖‖‖‖‖	0.38
Domain 2	133	C:ILE 135		106.6	74.39 ‖‖‖‖‖‖	1.14
Domain 2	135	C:ARG 137		125.1	4.96 |	-0.18
Domain 2	164	C:ASN 166		155.7	2.82 |	-0.05
Domain 2	165	C:PHE 167		65.6	4.54 |	-0.05
Domain 2	166	C:ASN 168	H	140.2	26.96 ‖	-0.22
Domain 2	167	C:ASN 169		18.8	1.34 |	0.02
Domain 2	169	C:ILE 171		76.2	44.32 ‖‖‖‖‖	0.71
Domain 2	171	C:GLU 173		74.9	9.46 ‖	-0.16
Domain 2	178	C:LEU 180		63.7	57.32 ‖‖‖‖‖‖‖‖	0.92
Domain 2	179	C:ILE 181		90.6	63.33 ‖‖‖‖‖‖	1.01
Domain 2	182	C:ILE 184		75.5	1.32 |	0.02
Linker	216	C:LYS 218	S	152.4	66.13 ‖‖‖‖	-0.97
Linker	217	C:ASN 219		105.8	55.59 ‖‖‖‖‖	-0.25
Domain 3	224	C:HIS 226	S	169.8	82.39 ‖‖‖‖	0.39
Domain 3	227	C:PRO 245		79.3	7.85 |	0.13
Domain 3	229	C:THR 247		30.0	5.52 ‖	0.09
Domain 3	231	C:TYR 249		45.4	22.25 ‖‖‖‖	0.36
Domain 3	259	C:SER 285		55.1	24.16 ‖‖‖‖	0.39
Domain 3	260	C:ARG 286		183.7	30.90 ‖	0.28
Domain 3	261	C:THR 287		29.4	13.30 ‖‖‖‖	0.03
Domain 3	263	C:ASP 289		47.8	17.60 ‖‖‖	-0.21
Domain 3	265	C:THR 291	H	47.0	27.27 ‖‖‖‖‖	0.11

Region	Domain Location of Interaction
##	Line Number
Structure 1	Chain – Amino Acid - # in PDB file
HSDC	Residues making **H**ydrogen/**D**isulphide bond, **S**alt Bridge, or **C**ovalent Link.
ASA	Accessible Surface Area, Å²
BSA	Buried Surface Area, Å²
Δ^{i}G	Solvation energy effect, kcal/mol
||||	Buried area percentage, one bar per 10%

C PISA Analysis: IL-33 interface on IL-1RAcP

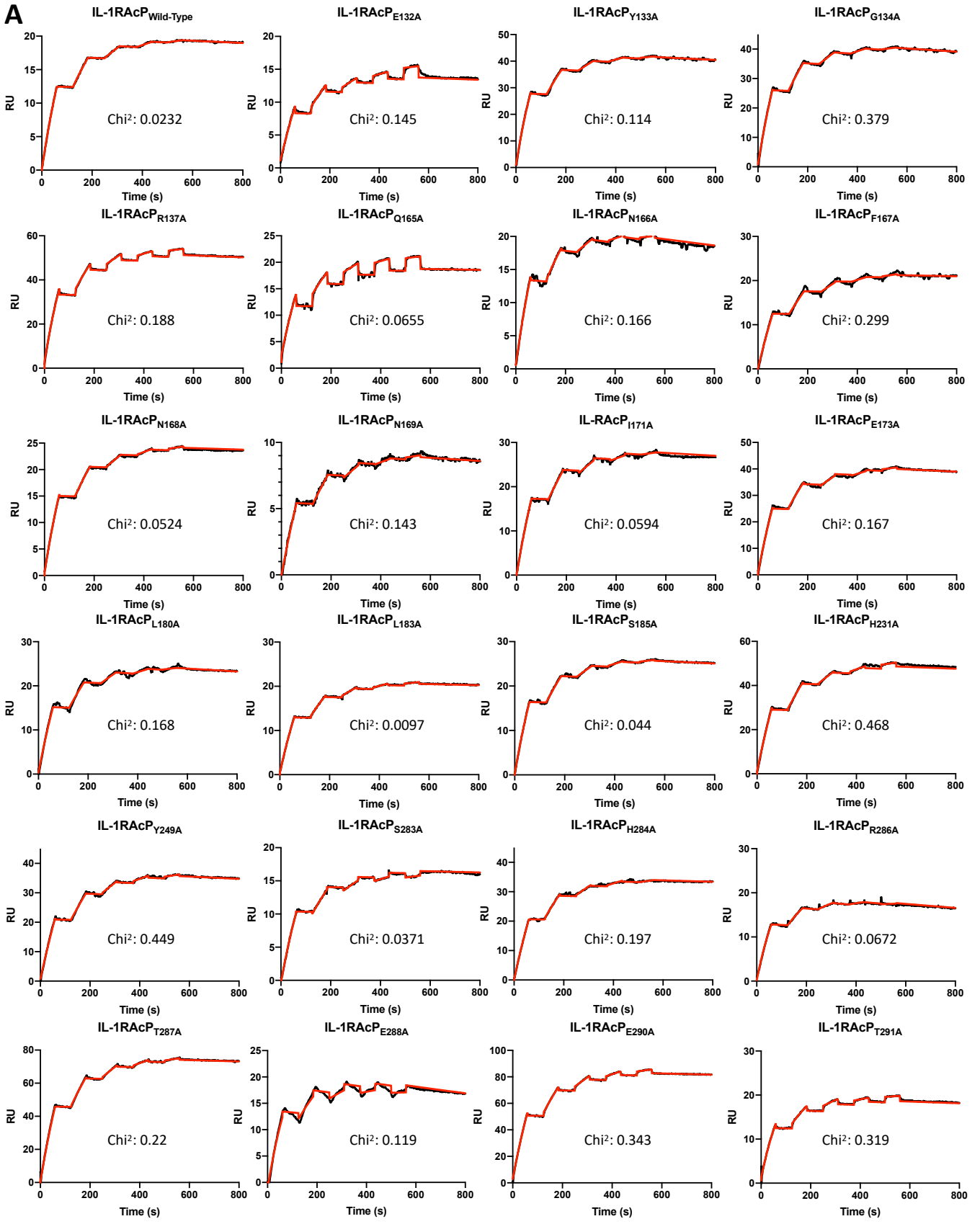
Region	##	Structure 1	HSDC	ASA	BSA	ΔiG
Domain 2	129	C:ILE 151		29.3	1.50 |	0.02
Domain 2	130	C:GLU 152	H	114.1	48.79 |||||	-0.45
Domain 2	157	C:LYS 179	HS	58.3	23.75 |||||	-0.18
Domain 2	164	C:ASP 186		83.2	20.12 |||	-0.23
Domain 2	165	C:PHE 187		72.3	48.31 |||||||	0.77
Domain 2	166	C:HIS 188	H	177.5	112.50 ||||||	-0.06
Domain 2	167	C:ASN 189		16.8	8.25 |||||	0.10
Domain 2	181	C:LEU 203		58.8	51.06 ||||||||	0.82
Domain 2	182	C:VAL 204		63.3	5.84 |	0.09
Domain 2	183	C:SER 205	H	87.0	60.08 |||||||	0.09
Domain 2	184	C:ASN 206		6.30	1.52 |||	-0.02
Domain 3	239	C:LYS 267		91.3	13.16 ||	-0.49
Domain 3	264	C:VAL 302		68.7	6.99 ||	-0.08
Domain 3	265	C:SER 303	H	65.8	49.12 |||||||	0.24
Domain 3	266	C:TYR 304		132.7	17.50 ||	-0.20
Domain 3	267	C:SER 305		45.1	5.74 ||	0.09
Domain 3	268	C:SER 306		71.9	23.60 ||||	0.20

Region	Domain Location of Interaction
##	Line Number
Structure 1	Chain – Amino Acid - # in PDB file
HSDC	Residues making **H**ydrogen/**D**isulphide bond, **S**alt Bridge, or **C**ovalent Link.
ASA	Accessible Surface Area, Å²
BSA	Buried Surface Area, Å²
Δ^{i}G	Solvation energy effect, kcal/mol
||||	Buried area percentage, one bar per 10%

D PISA Analysis: ST2 interface on IL-1RAcP

Region	##	Structure 1	HSDC	ASA	BSA	ΔiG
Domain 2	129	C:ILE 151		29.3	5.90 |||	0.03
Domain 2	130	C:GLU 152		114.1	65.32 ||||||	-0.26
Domain 2	131	C:HIS 153		84.3	1.68 |	0.03
Domain 2	132	C:GLY 154		53.3	38.38 ||||||||	0.36
Domain 2	133	C:ILE 155	H	116.5	90.85 ||||||||	1.16
Domain 2	135	C:LYS 157		101.9	17.42 ||	0.06
Domain 2	164	C:ASP 186	HS	83.2	42.91 ||||||	-0.57
Domain 2	165	C:PHE 187	H	72.3	11.40 ||	-0.01
Domain 2	166	C:HIS 188	H	177.5	65.01 ||||	0.29
Domain 2	167	C:ASN 189		16.8	4.00 |||	0.06
Domain 2	168	C:VAL 190		0.12	0.12 ||||||||||	-0.00
Domain 2	169	C:LEU 191		62.8	21.25 ||||	0.34
Domain 2	171	C:GLU 193	HS	80.3	11.72 ||	-0.16
Domain 2	176	C:SER 198	H	1.54	1.54 |||||||||	0.01
Domain 2	178	C:PHE 200		76.9	76.36 |||||||||	1.15
Domain 2	179	C:ILE 201		101.3	87.43 |||||||||	1.40
Linker	214	C:SER 236		6.47	3.44 ||||||	-0.04
Linker	216	C:LYS 238	HS	171.7	63.95 ||||	-1.06
Linker	217	C:ASP 239	HS	92.5	71.14 ||||||||	-0.15
Domain 3	219	C:LEU 241		87.1	69.44 ||||||||	1.11
Domain 3	220	C:PRO 242		52.1	12.23 |||	0.20
Domain 3	221	C:PRO 243		5.26	1.35 |||	-0.02
Domain 3	222	C:GLN 244	H	68.8	67.95 |||||||||	-0.52
Domain 3	223	C:ILE 245	H	35.5	35.56 |||||||||	-0.21
Domain 3	224	C:TYR 246		116.7	113.59 |||||||||	0.91
Domain 3	225	C:SER 247		26.8	9.37 ||||	0.15
Domain 3	226	C:PRO 248		1.72	1.22 ||||||||	-0.01
Domain 3	227	C:ASN 249	H	62.1	52.72 |||||||||	-0.11
Domain 3	228	C:ASP 250		83.6	16.04 ||	-0.11
Domain 3	229	C:ARG 251		227.9	119.57 ||||||	-0.89
Domain 3	230	C:VAL 252		53.0	30.94 ||||||	0.50
Domain 3	239	C:LYS 267		91.3	15.66 ||	-0.42
Domain 3	241	C:TYR 269		53.4	40.91 ||||||||	0.24
Domain 3	243	C:SER 271		5.37	1.10 |||	-0.01
Domain 3	298	C:ALA 341		23.8	9.37 ||||	0.15
Domain 3	299	C:GLU 342		121.9	10.68 |	-0.12
Domain 3	300	C:GLN 343	H	56.9	30.03 ||||||	-0.27

Region	Domain Location of Interaction
##	Line Number
Structure 1	Chain – Amino Acid - # in PDB file
HSDC	Residues making **H**ydrogen/**D**isulphide bond, **S**alt Bridge, or **C**ovalent Link.
ASA	Accessible Surface Area, Å²
BSA	Buried Surface Area, Å²
ΔiG	Solvation energy effect, kcal/mol
||||	Buried area percentage, one bar per 10%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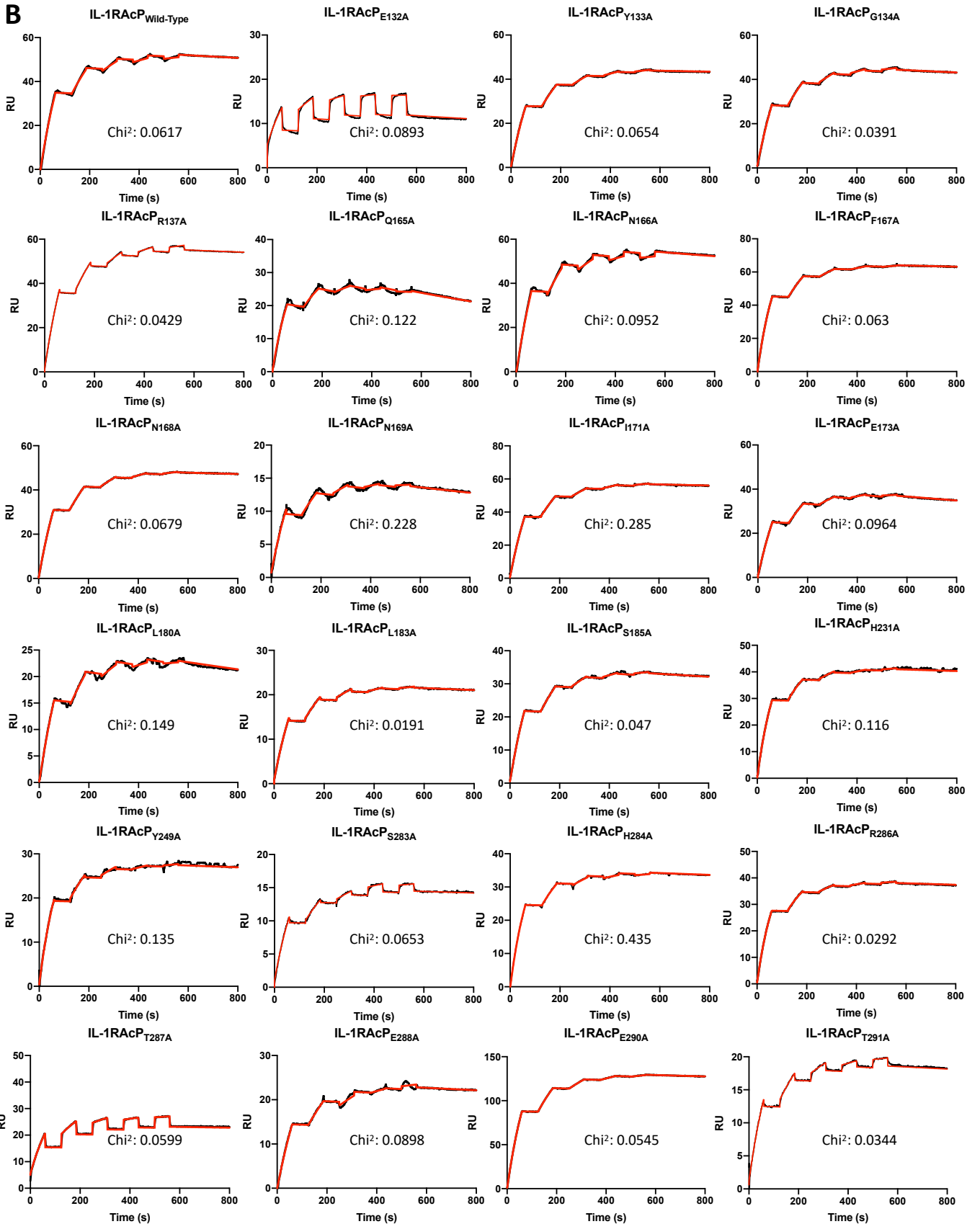
IL-1RAcP Wild-Type
Chi²: 0.0232
IL-1RAcP E132A
Chi²: 0.145
IL-1RAcP Y133A
Chi²: 0.114
IL-1RAcP G134A
Chi²: 0.379
IL-1RAcP R137A
Chi²: 0.188
IL-1RAcP Q165A
Chi²: 0.0655
IL-1RAcP N166A
Chi²: 0.166
IL-1RAcP F167A
Chi²: 0.299
IL-1RAcP N168A
Chi²: 0.0524
IL-1RAcP N169A
Chi²: 0.143
IL-RAcP I171A
Chi²: 0.0594
IL-1RAcP E173A
Chi²: 0.167
IL-1RAcP L180A
Chi²: 0.168
IL-1RAcP L183A
Chi²: 0.0097
IL-1RAcP S185A
Chi²: 0.044
IL-1RAcP H231A
Chi²: 0.468
IL-1RAcP Y249A
Chi²: 0.449
IL-1RAcP S283A
Chi²: 0.0371
IL-1RAcP H284A
Chi²: 0.197
IL-1RAcP R286A
Chi²: 0.0672
IL-1RAcP T287A
Chi²: 0.22
IL-1RAcP E288A
Chi²: 0.119
IL-1RAcP E290A
Chi²: 0.343
IL-1RAcP T291A
Chi²: 0.319
RU
Time (s)


B
IL-1RAcP Wild-Type
Chi²: 0.0617
IL-1RAcP E132A
Chi²: 0.0893
IL-1RAcP Y133A
Chi²: 0.0654
IL-1RAcP G134A
Chi²: 0.0391
IL-1RAcP R137A
Chi²: 0.0429
IL-1RAcP Q165A
Chi²: 0.122
IL-1RAcP N166A
Chi²: 0.0952
IL-1RAcP F167A
Chi²: 0.063
IL-1RAcP N168A
Chi²: 0.0679
IL-1RAcP N169A
Chi²: 0.228
IL-1RAcP I171A
Chi²: 0.285
IL-1RAcP E173A
Chi²: 0.0964
IL-1RAcP L180A
Chi²: 0.149
IL-1RAcP L183A
Chi²: 0.0191
IL-1RAcP S185A
Chi²: 0.047
IL-1RAcP H231A
Chi²: 0.116
IL-1RAcP Y249A
Chi²: 0.135
IL-1RAcP S283A
Chi²: 0.0653
IL-1RAcP H284A
Chi²: 0.435
IL-1RAcP R286A
Chi²: 0.0292
IL-1RAcP T287A
Chi²: 0.0599
IL-1RAcP E288A
Chi²: 0.0898
IL-1RAcP E290A
Chi²: 0.0545
IL-1RAcP T291A
Chi²: 0.0344
RU
Time (s)

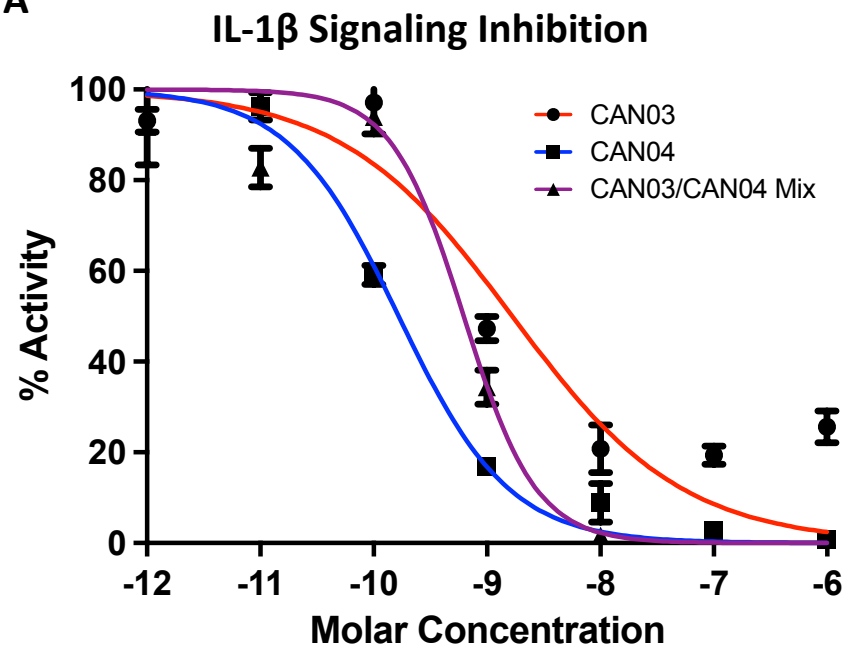
A **Analyte: CAN03 Fab**

Ligand	*k*a (10^5 M^{-1} S^{-1})	*k*d (10^{-5} S^{-1})	K_D (100pM)
Wild-type	3.2 ± 0.6	10.1 ± 3.5	3.1 ± 1.1
E132A	2.0 ± 0.5	10.0 ± 0.5	5.1 ± 0.9
Y133A	3.5 ± 2.4	10.1 ± 2.5	3.5 ± 1.7
G134A	4.3 ± 0.2	13.5 ± 0.0	3.1 ± 0.2
R137A	4.3 ± 0.1	11.7 ± 4.3	2.8 ± 1.1
Q165A	2.7 ± 0.0	14.9 ± 1.3	5.5 ± 0.5
N166A	3.3 ± 0.5	9.5 ± 3.6	2.8 ± 0.7
F167A	2.2 ± 0.1	6.4 ± 1.8	2.6 ± 0.7
N168A	3.5 ± 0.2	7.6 ± 1.8	2.1 ± 0.4
N169A	2.6 ± 0.4	14.7 ± 0.3	5.7 ± 1.1
I171A	3.2 ± 0.3	10.4 ± 4.7	3.2 ± 1.2
E173A	3.3 ± 0.3	9.4 ± 2.7	2.8 ± 0.6
L180A	3.6 ± 0.1	15.7 ± 1.0	4.3 ± 0.2
L183A	3.3 ± 0.7	7.4 ± 1.7	2.3 ± 1.0
S185A	4.7 ± 0.5	11.5 ± 0.3	2.4 ± 0.3
H231A	3.8 ± 0.7	9.0 ± 2.4	2.5 ± 1.1
Y249A	2.5 ± 1.3	12.2 ± 1.8	5.3 ± 2.0
S283A	3.2 ± 0.5	5.5 ± 0.2	1.7 ± 0.3
H284A	3.2 ± 0.1	6.5 ± 1.5	2.0 ± 0.5
R286A	4.9 ± 1.4	23.0 ± 3.0	4.7 ± 2.8
T287A	3.1 ± 1.1	7.1 ± 1.5	2.5 ± 1.4
E288A	3.2 ± 0.0	2.4 ± 0.2	0.8 ± 0.1
E290A	3.8 ± 0.6	6.7 ± 1.8	1.7 ± 0.2
T291A	3.5 ± 0.4	8.6 ± 2.7	2.5 ± 1.0

B **Analyte: CAN04 Fab**

Ligand	*k*a (10^5 M^{-1} S^{-1})	*k*d (10^{-5} S^{-1})	K_D (100pM)
Wild-type	5.6 ± 2.0	9.8 ± 5.0	1.8 ± 0.6
E132A	5.3 ± 0.6	18.7 ± 6.2	3.6 ± 1.6
Y133A	4.0 ± 0.3	6.5 ± 2.3	1.6 ± 0.5
G134A	4.5 ± 0.7	12.1 ± 0.4	2.7 ± 0.4
R137A	5.4 ± 1.6	13.1 ± 6.8	2.3 ± 0.5
Q165A	3.0 ± 2.2	53.2 ± 21.5	20.4 ± 7.6
N166A	4.1 ± 0.4	15.3 ± 1.2	3.7 ± 0.0
F167A	7.3 ± 1.1	6.0 ± 0.5	0.8 ± 0.1
N168A	4.3 ± 0.2	6.3 ± 2.4	1.5 ± 0.5
N169A	4.1 ± 0.1	17.7 ± 0.6	4.3 ± 0.0
I171A	4.7 ± 0.2	4.3 ± 0.1	0.9 ± 0.1
E173A	3.8 ± 0.1	23.9 ± 2.3	6.3 ± 0.4
L180A	3.3 ± 0.4	24.0 ± 9.3	7.4 ± 3.7
L183A	4.5 ± 0.2	9.1 ± 2.2	2.4 ± 0.6
S185A	4.6 ± 0.1	12.3 ± 2.9	2.7 ± 0.6
H231A	5.7 ± 1.5	8.4 ± 2.3	1.6 ± 0.8
Y249A	5.2 ± 1.7	9.6 ± 2.8	2.0 ± 1.2
S283A	5.1 ± 0.3	9.1 ± 1.8	2.0 ± 0.3
H284A	5.5 ± 1.9	8.9 ± 1.0	1.8 ± 0.8
R286A	11.6 ± 1.5	7.7 ± 0.9	1.3 ± 0.5
T287A	5.2 ± 0.1	5.1 ± 1.8	1.0 ± 0.4
E288A	3.5 ± 0.4	10.8 ± 2.1	3.1 ± 1.0
E290A	6.7 ± 1.8	8.4 ± 3.6	1.2 ± 0.2
T291A	4.5 ± 0.3	10.0 ± 0.6	2.2 ± 0.0

A


IL-1β Signaling Inhibition
CAN03
CAN04
CAN03/CAN04 Mix
% Activity
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
Molar Concentration