Supporting Information

Engineering Encodable Lanthanide Binding Tags (LBTs) into Loop Regions of Proteins

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Primer sequence for cloning of IL1β-L2

For_IL1b(Bam,TEV): 5'-CGGGATCCGAGAATTTGTATTTTCAGG-3’

Rev_IL1b(loop2): 5’-CCTTCAATCCAGCCATCGTTGTTGGTATCAATATAACCTTTCAACACG CAGGACAGGTAC-3’

For_IL1b(loop2): 5’-CCAACAACGATGGCTGGATTGAAGGCGATGAACTGTATAAGCCCACT CTACAGCTGGAGAG-3’

Rev_IL1b(Xho): 5’-CCGCTCGAGTTAGGAAGACACAAATTG-3’

Primer sequence for cloning of IL1β-L1 and IL1β-L3 via Site-Directed Mutagenesis of IL1β-L2

IL1β-L1

IL1bL1_D1: 5’-GTCCTGCGTGTTGaaagatGGTTATATTGATACCAAC-3’

IL1bL1_D1-r: 5’-GTTGGTATCAATATAACCATCTTTCAACACGCAGGAC-3’

IL1bL1_D2: 5’-GAAGGCGATGAACTGTATgataagCCCACTCTACAG-3’

IL1bL1_D2-r: 5’-CTGTAGAGTGGGCTTATCATACAGTTCATCGCCTTC-3’

IL1β-L3

IL1bL1_K1: 5’-CTGTCCTGCGTGTTGGGTTATATTGATACCAACAAC-3’

Il1bL1_K1-r: 5’-GTTGTTGGTATCAATATAACCCAACACGCAGGACAG-3’

IL1bL1_K2: 5’-GAAGGCGATGAACTGTATCCCACTCTACAGCTGG-3’

IL1bL1_K2-r: 5’-CCAGCTGTAGAGTGGGATACAGTTCATCGCCTTC-3’

Primer sequence for cloning of IL1β-R2

For_IL1b(Bam,TEV): 5'-CGGGATCCGAGAATTTGTATTTTCAGG-3’

Rev_IL1b(loop3): 5’-CCTTCAATCCAGCCATCGTTGTTGGTATCAATATAACCTTTGGTCCCTCCCAGGAAGAC-3’

For_IL1b(loop3): 5’-CCAACAACGATGGCTGGATTGAAGGCGATGAACTGTATCAGGATATAACTGACTTCACC -3’

Rev_IL1b(Xho): 5’-CCGCTCGAGTTAGGAAGACACAAATTG-3’

Primer sequence for cloning of IL1β-R1 and IL1β-R3 via Site-Directed Mutagenesis of IL1β-R2

IL1β-R1

For_IL1b(Gly1): 5’-CTGGGAGGGACCAAAGGCGGTTATATTGATACC-3’

Rev_IL1b(Gly1): 5’-GGTATCAATATAACCGCCTTTGGTCCCTCCCAG-3’

For_IL1b(Gly2): 5’-GATGAACTGTATGGCCAGGATATAACTGACTTCAC-3’

Rev_IL1b(Gly2): 5’-GTGAAGTCAGTTATATCCTGGCCATACAGTTCATC-3’

IL1β -R3

For_IL1b(Lys): 5’-GTCTTCCTGGGAGGGACCGGTTATATTGATACC-3’

Rev_IL1b(Lys): 5’-GGTATCAATATAACCGGTCCCTCCCAGGAAGAC-3’

For_IL1b(Gln): 5’-GGCGATGAACTGTATGATATAACTGACTTCACC-3’

Rev_IL1b(Gln): 5’-GGTGAAGTCAGTTATATCATACAGTTCATCGCC-3’

Primer sequence for site-directed mutagenesis of all IL1β-LBT proteins to remove Gly from TEV site

For_IL1b(Met): 5'-GAGAATTTGTATTTTCAGATGGCACCTGTACGATCGC-3'

Rev_IL1b(Met): 5'-GCGATCGTACAGGTGCCATCTGAAAATACAAATTCTC-3'

Primer sequence for cloning of IL1β-S1, -S2 and –S3

For_IL1b(Bam, TEV): 5'-CGGGATCCGAGAATTTGTATTTTCAGGGCATGGCACCTGTACGATCGCTGAAC-3'

Rev_IL1b(Xho): 5'- CCGCTCGAGTTAGGAAGACACAAATTGCATGGT-3'

Expression of GST-TEV-IL1β-LBT by Auto-induction

Starting from an overnight culture in 2.5 mL minimal non-inducing media, BL21-CodonPlus(DE3)-RIL expressing the desired GST-fusion protein were grown in 500 mL complex auto-inducing media (ZYM-5052) and shaken for 24 h at 37 °C. The cells were harvested by centrifugation and the final pellets were stored at -80°C until needed.[1]

Expression and purification of ^{15}N-GST-TEV-IL1β-LBT by Auto-induction.

Starting from an overnight culture in 2.5 mL minimal non-inducing media, BL21-CodonPlus(DE3)-RIL expressing the desired GST-fusion protein were grown in 500 mL minimal autoinducing media (P-5052) and shaken for 24 h at 37 °C. The cells were harvested by centrifugation and the final pellets were stored at -80°C until needed.[1]

All purification was performed at 4 °C. The cell pellet from the 500 mL growth was thawed and resuspended in a lysis buffer (1 x PBS, 100 mM EDTA, 10% Glycerol, 1% Triton X-100, 5 mM β-mercaptoethanol (β-ME), 1 mg/mL Hen egg white lysozyme, 1000 x dilution of Protease Inhibitor Cocktail III (Calbiochem)) and incubated for 20 minutes. Cells were lysed by sonication, and cellular

debris was pelleted by centrifugation. Supernatant was incubated over night with Glutathione-sepharose resin (Amersham Biosciences), washed extensively with PBS, and the GST-construct was then eluted using a 10 mM glutathione solution in 50 mM Tris (pH 8.0) buffer containing the same protease inhibitor cocktail. Elution fractions were analyzed by 12% SDS-PAGE. Purified protein was stored at 4°C until cleavage by mTEV protease.

Cleavage by mTEV protease and purification of GST-TEV-IL1β-LBT proteins.

The cleavage reactions were conducted in50 mM Tris, 150 mM NaCl, 0.5 mM EDTA and 10mM β-ME using 20 µL recombinant mTEV protease per mg protein. The reaction mixtures were incubated overnight at room temperature and analyzed on 12%-SDS-Page gels for completeness.

The cleaved proteins were purified using size exclusion chromatography. A HiLoad 16/60 Superdex-75 column was equilibrated with 10 mM HEPES, 100 mM NaCl, 5 mM β-ME at pH 7. The protein was loaded onto the column and after a 50-minute delay, 1mL fractions were collected for 40 minutes at a flow rate of 1 mL/min. The absorbance at 280 nm was monitored and fractions corresponding to the two peaks were collected and analyzed on 12% SDS-Page gels (Figure S1).

Construct	LBT between loop residues	Expression yields out of 500 mL ^{15}N-AI minimum culture
Il1β-S1	53-54	5 mg
Il1β-S2	52-55	3 mg
Il1β-S3	51-56	4 mg
Il1β-L1	75-76	2 mg
Il1β-L2	74-77	3 mg
Il1β-L3	73-78	1 mg
Il1β-R1	139-140	2 mg
Il1β-R2	138-141	8 mg
Il1β-R3	137-142	3 mg

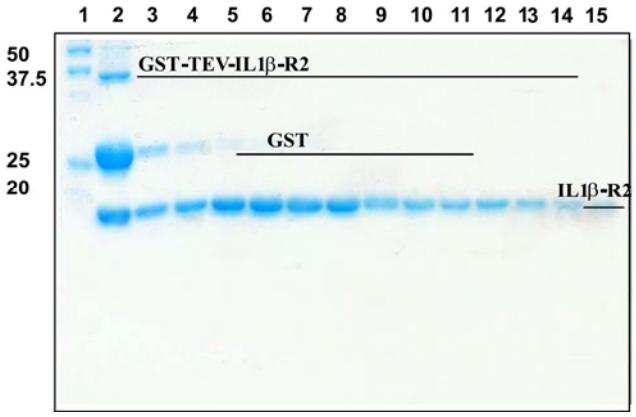

Figure S1 Expression yields of IL1β- S, -L and –R series (left) and a representative 12 % SDS page gel of IL1β-R2 stained with Coomassie brilliant blue (right). Lane 1: protein mass ladder; lane 2: GST-TEV- IL1β-R2; lane 3 to 15: Fractions of purified IL1β-R2 by Size exclusion chromatography.

Expression of IL1β-S1 for crystallization

For expression of the IL1β-S1 constructs, a single colony was transferred to 2mL of ZYP-0.8g media containing 50 μg/ml of ampicillin and grown at 37 °C until turbid. The culture (200μl) was then transferred to 400 ml of ZYM5052 containing 50 μg/ml of ampicillin and grown at 25 °C overnight until the OD600 reached a plateau (typically OD600 = 7-9). Cells were harvested by centrifugation at 8000 x g for 20 min, the pellet resuspended in 50ml 15 mM Tris pH 8.0, centrifuged at 4000 x g, and the supernatant removed and the pellet frozen overnight at -80 °C.

The frozen cell pellet was resuspended in 50 mL lysis buffer (50 mM Tris pH 8.0, 1 mM EDTA, 1 mM PMSF, 1 mM BME) and stirred on ice. A spatula tip of DNAse I and lysozyme were added and stirring continued for 45 min. Cells were sonicated 3 x 30 seconds and the suspension centrifuged 145 x kg for thirty min. and the supernatant retained. Ammonium sulfate cuts of the supernatant were made and protein that precipitated between 40-75% ammonium sulfate was pooled and dialyzed against 1M NH_4SO_4 in 50 mM Tris pH 7.5, 1mM BME (Buffer A), loaded on a HR 16/10 butyl sepharose column and eluted with a 15 column volume gradient from 1M to 0 M of NH_4SO_4 in Buffer A. Fractions containing protein were pooled and precipitated in 75% ammonium sulfate to reduce volume. The precipitate was resuspended in 5 ml Buffer A and dialyzed against the same buffer. The sample was loaded on a 5 ml HiTrap Q HP 5ml column and eluted with a 20 column volume gradient from 0 M to 1 M sodium chloride in Buffer A. Fractions containing IL1β-Sl (approximately 95% pure) were pooled, dialyzed against 50mM Hepes pH 7.5, 150mM NaCl, concentrated in an Amicon concentrator to 0.5 ml and loaded on a HiPrep 26/60 Sephacryl S-200 column and eluted at 1.5ml/min with 50mM Hepes pH 7.5, 150 mM NaCl. Eluted protein was a single band on an SDS-PAGE. Protein fractions were pooled and dialyzed against 20 mM Hepes pH 7.3 and stored at 4 °C.

Receptor-binding assay.

In a microspin column (Biorad) 50 µL glutathione sepharose beads (GE Healthcare Biosciences, max binding capacity = 500 µg) were loaded with 1.0 mg of the appropriate GST fusion protein and incubated with the resin with gentle agitation at 4°C for 2 hours. 125 µL (200µg/mL) lyophilized s-IL-$1R_1$ (R&D Systems) was then added to the beads along with 375 µL PBS+0.1% BSA, incubated overnight at 4°C (Figure S2).

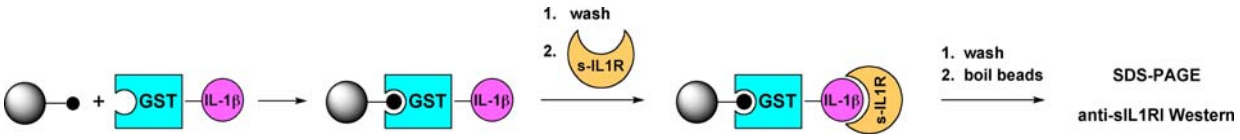

Figure S2 GST pull-down assay for s-IL-$1R_1$ receptor binding.

After extensive washing, receptor capture by the GST-tagged IL1β-LBT was analyzed by SDS-PAGE and Western blot. Control experiments were performed to ensure that the receptor would bind to GST-tagged IL1β without an LBT. Two negative controls were also performed - one in which the beads were incubated only with PBS buffer prior to addition of the receptor, and one in which the beads were incubated with GST prior to receptor-binding. These experiments established that there was no nonspecific binding of the receptor to either beads or GST. The results for receptor-binding assays for the three loop series confirm that the receptor does bind to all of the constructs as analyzed by SDS-PAGE and an anti-s-IL-$1R_1$ Western blot (Figure S3)

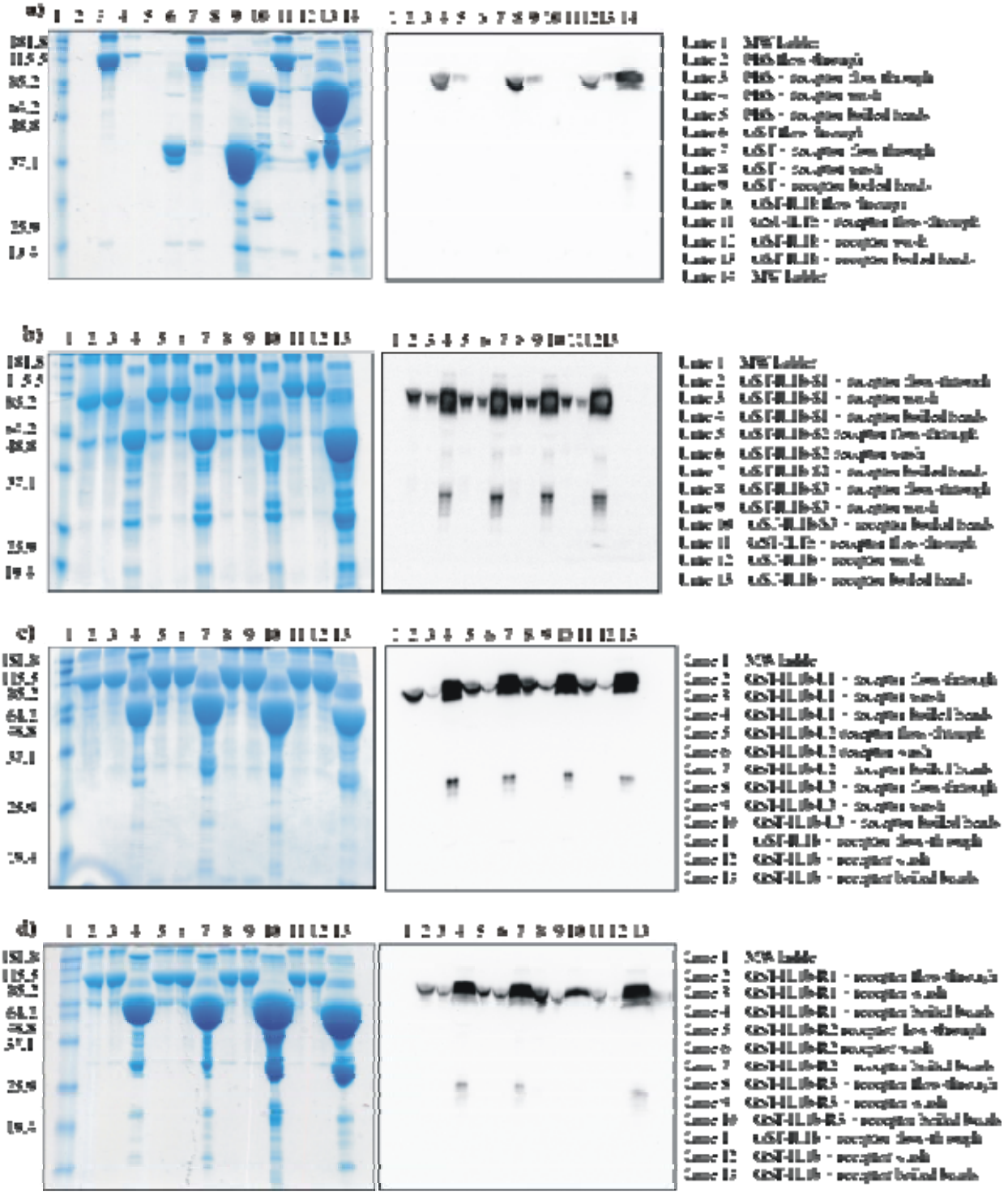

Figure S3 Receptor-binding assay results, with 12% SDS-Page gels (left) and anti-s-IL-1R_1 Western blots (right). (a) Control binding studies. Nonspecific binding was not observed. Assay with (b) GST-IL1β-S1, -S2 and S3, (c) GST- IL1β-L1, -L2 and L3 and (d) GST- IL1β-R1, -R2 and -R3.

Number of bound water molecules in the coordination sphere of terbium.

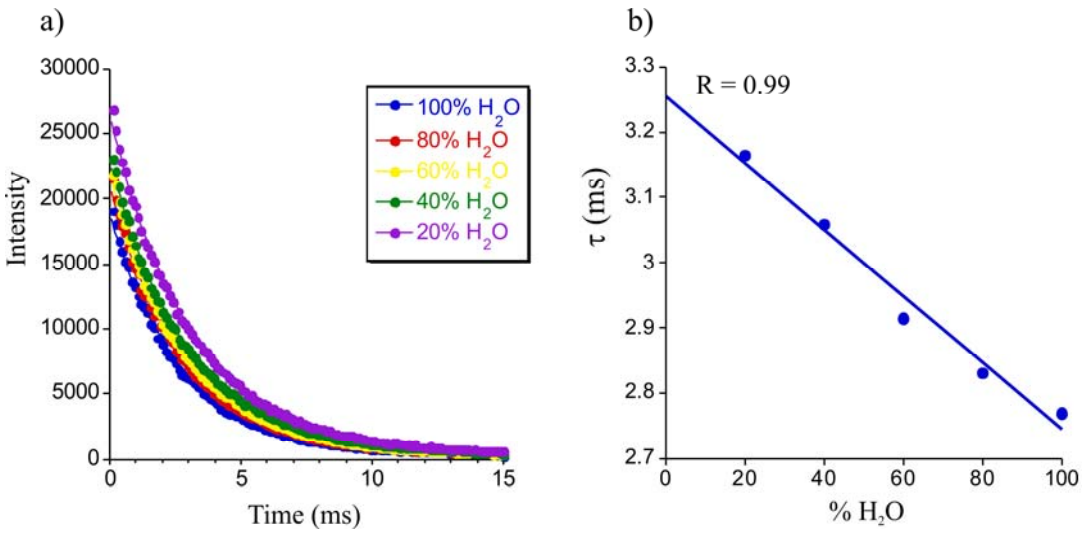

Figure S4 (a) Luminescence decay measurements in varying concentrations of H_2O and D_2O. Representative data shown for IL1β-L1. (b) Values of τ (determined from fits of data in (a)) plotted against the percentage of H_2O, allowing extrapolation to 100% D_2O.

Table S1. ^{1}H and ^{15}N Chemical Shift values for ^{15}N-IL1β– R2 at pH 7 and 20 °C, 10 mM HEPES, 100 mM NaCl, 10 µM DSS.

	Residue		^{1}H [ppm]	^{15}N [ppm]		Residue		^{1}H [ppm]	^{15}N [ppm]
	A Ala	1				S Ser	43	9,23	115,64
	P Pro	2				M Met	44	9,69	132,12
	V Val	3	8,15	119,44		S Ser	45	8,95	121,24
	R Arg	4	9,02	128,30		F Phe	46	8,53	123,53
	S Ser	5	8,42	118,91		V Val	47	7,42	118,23
	L Leu	6	9,14	122,76		Q Gln	48	8,61	120,22
	N Asn	7	8,89	121,00		G Gly	49	8,44	112,29
	C Cys	8	9,68	118,10		E Glu	50	8,58	121,76
	T Thr	9	8,99	109,67		E Glu	51	8,62	123,15
	L Leu	10	9,50	120,83		S Ser	52	8,49	117,28
	R Arg	11	8,68	119,78	a)	N Asn	53		
	D Asp	12	8,51	120,43		D Asp	54	8,57	116,06
	S Ser	13	7,55	114,85		K Lys	55	7,63	119,03
	Q Gln	14	8,09	120,77	a)	I Ile	56		
	Q Gln	15	8,48	111,25		P Pro	57		
a)	K Lys	16				V Val	58	10,10	118,10
a)	S Ser	17				A Ala	59	8,73	120,83
	L Leu	18	8,90	122,40		L Leu	60	10,61	124,40
	V Val	19	8,85	115,55		G Gly	61	8,33	110,19
	M Met	20	8,77	120,71		L Leu	62	8,29	121,76
	S Ser	21	8,71	120,21		K Lys	63		
	G Gly	22	8,20	112,08		E Glu	64	9,01	118,98
	P Pro	23				K Lys	65	7,62	115,40
	Y Tyr	24	8,11	110,63	a)	N Asn	66		
	E Glu	25	7,24	119,80		L Leu	67	6,89	115,79
	L Leu	26	8,70	123,13		Y Tyr	68	9,23	121,61
	K Lys	27	9,25	119,52		L Leu	69	8,54	122,02
	A Ala	28	7,67	119,02		S Ser	70	9,22	116,09
	L Leu	29	9,49	126,03		C Cys	71	8,48	119,76
	H His	30	10,22	122,28		V Val	72	8,88	117,36
	L Leu	31	8,50	126,39		L Leu	73	8,63	123,92
	Q Gln	32	9,00	120,32		K Lys	74	8,28	126,51
	G Gly	33	8,85	109,82		D Asp	75	9,37	128,30
	Q Gln	34	8,98	122,24		D Asp	76	8,57	109,84
	D Asp	35	8,00	119,18		K Lys	77	7,76	120,40
	M Met	36	7,69	118,44		P Pro	78		
	E Glu	37	8,16	117,67		T Thr	79	8,84	120,09
	Q Gln	38	8,00	117,24		L Leu	80	8,97	127,17
	Q Gln	39	7,57	117,32		Q Gln	81	9,78	126,66
	V Val	40	8,40	123,06		L Leu	82	8,57	122,45
	V Val	41	7,80	123,97		E Glu	83	9,23	124,07
	F Phe	42	9,68	127,64		S Ser	84	8,83	121,73

Table S1. (continued)

	Residue			^{1}H [ppm]	^{15}N [ppm]		Residue			^{1}H [ppm]	^{15}N [ppm]
a)	V	Val	85			A	Ala	127	8,24	121,18
	D	Asp	86	8,21	122,14	E	Glu	128	8,57	117,44
	P	Pro	87			N	Asn	129	8,13	114,04
	K	Lys	88	8,48	117,31	M	Met	130	8,98	118,16
	N	Asn	89	7,80	114,86	P	Pro	131		
	Y	Tyr	90	7,29	116,49	V	Val	132	8,08	125,86
	P	Pro	91			F	Phe	133	8,81	125,25
	K	Lys	92	7,27	116,54	L	Leu	134	9,03	122,55
	K	Lys	93	8,37	119,69	G	Gly	135	9,40	115,44
	K	Lys	94	7,93	118,23	G	Gly	136	8,77	110,73
	M	Met	95	7,69	122,70	T	Thr	137	7,76	114,06
	E	Glu	96	9,23	122,87	K	Lys	138	8,33	124,14
	K	Lys	97	8,29	121,91	G	**Gly**	139	8,09	109,69
	R	Arg	98	7,99	114,41	Y	**Tyr**	140	8,07	118,91
	F	Phe	99	7,92	116,70	I	**Ile**	141	9,00	123,15
	V	Val	100	7,50	117,99	D	**Asp**	142	8,73	124,71
	F	Phe	101	9,90	127,36	T	**Thr**	143	8,14	120,92
	N	Asn	102	10,47	121,32	N	**Asn**	144	8,45	115,75
	K	Lys	103	9,41	127,84	N	**Asn**	145	8,14	115,51
	I	Ile	104	9,35	134,28	D	**Asp**	146	8,09	115,38
	E	Glu	105	8,56	127,48	G	**Gly**	147	9,77	111,24
	I	Ile	106	8,62	125,96	W	**Trp**	148	7,98	120,03
	N	Asn	107	9,42	126,43	I	**Ile**	149	9,27	131,40
	N	Asn	108	8,92	110,35	E	**Glu**	150	8,85	109,12
	K	Lys	109	8,05	120,49	G	**Gly**	151	9,29	127,24
	L	Leu	110	9,40	122,31	D	**Asp**	152	8,32	118,35
	E	Glu	111	8,74	118,58	E	**Glu**	153	8,65	127,27
	F	Phe	112	10,46	119,72	L	**Leu**	154	7,20	115,57
	E	Glu	113	8,82	126,28	Y	**Tyr**	155	7,34	119,58
	S	Ser	114	9,24	122,11	Q	Gln	156	8,51	122,97
	A	Ala	115	8,18	128,62	D	Asp	157	7,91	122,09
	Q	Gln	116	8,00	117,24	I	Ile	158	8,34	122,31
	F	Phe	117	7,44	117,17	a) T	Thr	159		
	P	Pro	118			a) D	Asp	160		
	N	Asn	119	10,40	114,33	F	Phe	161	9,44	117,54
	W	Trp	120	8,19	120,46	T	Thr	162	9,64	110,00
	Y	Tyr	121	9,49	119,73	M	Met	163	8,78	121,57
a)	I	Ile	122			Q	Gln	164	8,49	123,96
a)	S	Ser	123			F	Phe	164	8,85	123,75
	T	Thr	124	9,11	110,25	V	Val	166	8,12	123,34
	S	Ser	125	9,62	114,01	S	Ser	167	8,52	119,94
	Q	Gln	126	8,88	122,26	S	Ser	168	8,12	122,98

a) The amino acid backbone resonances of these amino acids could not be assigned in the ^{1}H-^{15}N-HSQC spectra.

Comparison of IL1β-R2 with wt-IL1β

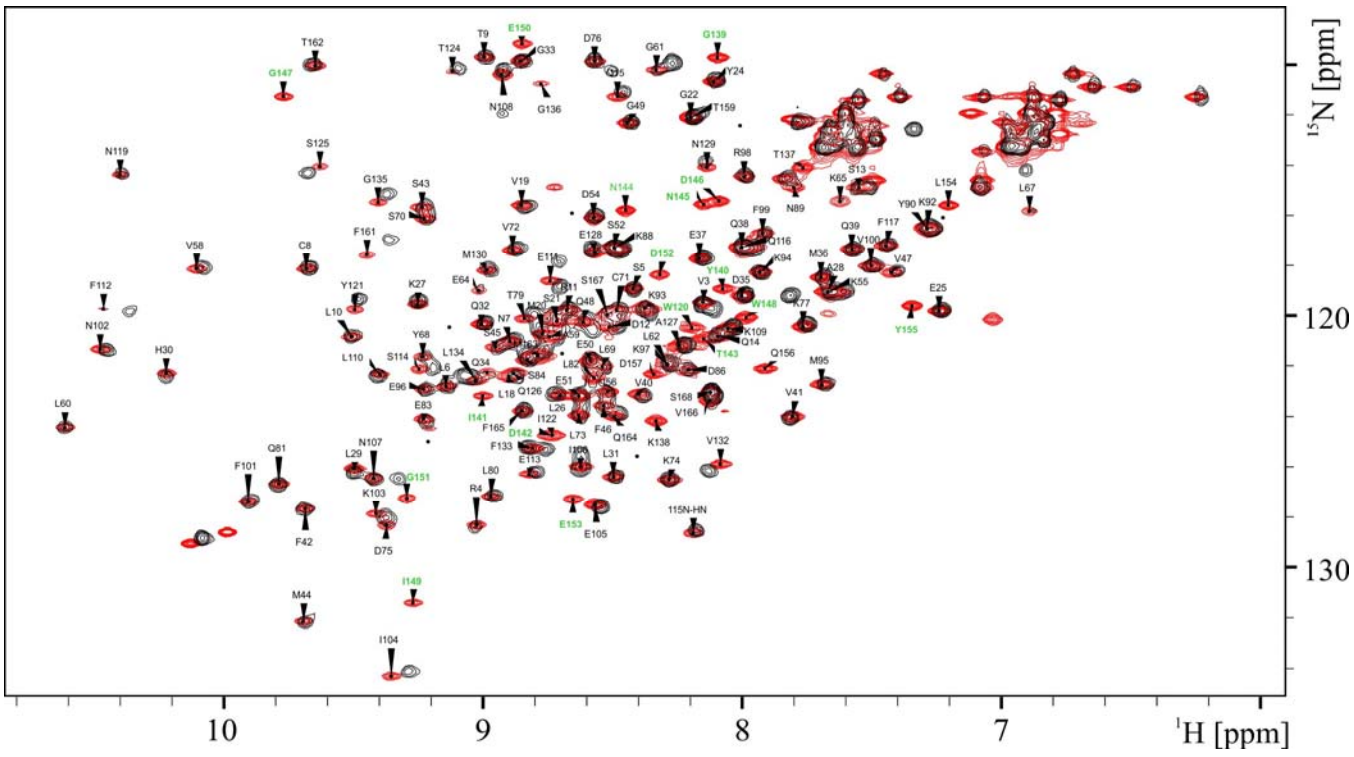

Figure S5. Overlay of the 2D-^{1}H- ^{15}N HSQC spectra of IL1β-R2 (red) and wt-IL1β (black) at pH 7 and 20 °C, 10 mM HEPES, 100 mM NaCl, 10 µM DSS. Peaks originated from the LBT are shown in green.

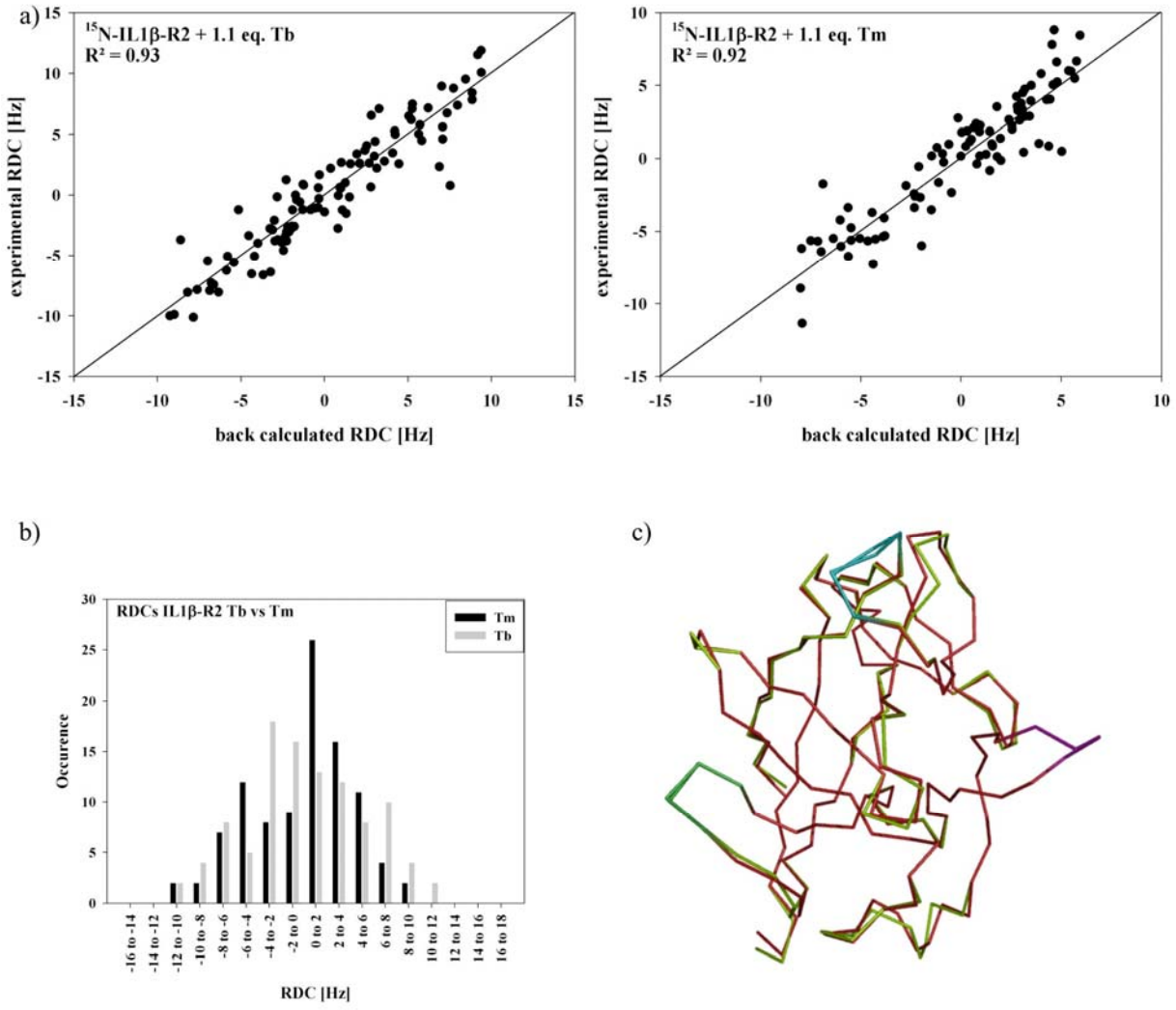

Figure S6. (a) Scatter plot showing the correlation between observed $^{1}D_{HN}$ dipolar shifts [Hz] and those back-calculated with the program PALES[2] based on the crystal structure of IL1β (PDB entry 9ILB) for ^{15}N-IL1β-R2 using Tb^{3+} or Tm^{3+} as paramagnetic lanthanide ion. The correlation coefficient is 0.93 or Tb^{3+} and 0.92 for Tm^{3+}. The protein concentration was 0.5 mM. NMR buffer composition was 10 mM HEPES (pH 7), 100 mM NaCl at 36 °C. (b) RDC histogram for ^{15}N-IL1β-R2 loaded with Tb^{3+} compared with that for ^{15}N-IL1β-R2 loaded with Tm^{3+}. (c) Overlay of the refined model of IL1β-R2 (green) with the reference crystal structure 9ILB (red). The RMSD of both structures is 0.398 Å. The modelling was performed using the crystal structure of IL1β (PDB entry 9ILB) and the previously optimized Δχ-tensor parameters.

Table S2. PCSs of backbone ^{1}H and ^{15}N of IL1β-R2.

R2		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)			PCS [ppm] Tb (III)	PCS [ppm] Tm (III)		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)
A1							K55	HN		-0.294	N		-0.256
P2							I56	HN	0.320		N	0.317	
V3	HN	0.088	-0.059	N	0.126	-0.129	P57						
R4	HN	0.102	-0.079	N	0.107	-0.114	V58	HN	0.267	-0.211	N	0.196	-0.210
S5	HN	0.104	-0.059	N	0.170	-0.105	A59	HN	0.194	-0.134	N	0.199	-0.168
L6	HN	0.121	-0.081	N	0.098	-0.063	L60	HN	0.200	-0.146	N	0.174	-0.110
N7	HN		-0.050	N		-0.053	G61	HN	0.136	-0.093	N	0.166	-0.099
C8	HN	0.081	-0.061	N	0.125	-0.047	L62	HN	0.104	-0.065	N	0.105	0.006
T9	HN	0.064	-0.038	N	0.083	-0.034	K63						
L10	HN	0.071	-0.054	N	0.152	-0.054	E64	HN	0.051	-0.023	N	0.058	-0.014
R11	HN	0.110	-0.081	N	0.092	-0.106	K65	HN	0.053		N	0.076	
D12	HN		0.043	N		0.055	N66						
S13							L67	HN	0.080		N	0.107	
Q14							Y68	HN	0.119	-0.075	N	0.129	-0.078
Q15	HN	-0.020		N	-0.086		L69	HN	0.169	-0.116	N	0.197	-0.114
K16							S70	HN	0.204	-0.140	N	0.195	-0.181
S17							C71						
L18							V72	HN	0.284	-0.199	N	0.232	-0.223
V19	HN	-0.013	0.028	N	0.015	0.019	L73	HN		-0.155	N		-0.219
M20							K74	HN	0.254		N	0.223	
S21	HN	0.001		N	0.026		D75	HN	0.122	-0.047	N	0.145	-0.099
G22	HN	-0.009	0.045	N	0.022	0.044	D76	HN	0.157	-0.086	N	0.091	-0.100
P23							K77	HN	0.270	-0.175	N	0.351	-0.248
Y24	HN	0.031	0.015	N	0.004	-0.009	P78						
E25	HN	0.043	0.008	N	0.097	0.017	T79	HN		-0.247	N		-0.240
L26	HN	0.083	-0.034	N	0.092	0.001	L80	HN	0.340	-0.236	N	0.312	-0.271
K27	HN	0.003	0.019	N	0.066	0.055	Q81	HN	0.225	-0.157	N	0.142	-0.173
A28	HN	-0.034	0.130	N	-0.067	0.039	L82	HN	0.126	-0.071	N	0.141	-0.132
L29	HN	-0.130		N	-0.192		E83	HN	0.119	-0.075	N	0.108	-0.070
H30	HN	-0.313	0.246	N	-0.328	0.104	S84			-0.045	N		-0.021
L31	HN	-0.186	0.143	N	-0.259	0.109	V85						
Q32	HN	-0.192	0.152	N	-0.226	0.143	D86	HN	0.079	-0.048	N	0.121	-0.021
G33	HN	-0.139	0.102	N	-0.142	0.078	P87						
Q34							K88	HN	0.053	-0.020	N	0.054	-0.114
D35	HN	-0.124	0.094	N	-0.142	0.080	N89	HN	0.064		N	0.031	
M36	HN	-0.098	0.083	N	-0.071	0.079	Y90	HN	0.073		N	0.133	
E37	HN	-0.085	0.063	N	-0.040	0.019	P91						
Q38	HN	-0.074	0.052	N	-0.089	0.064	K92						
Q39	HN	-0.038	0.040	N	-0.013	0.055	K93	HN	0.088	-0.075	N	0.089	-0.049
V40	HN		0.004	N		-0.053	K94	HN	0.106	-0.085	N	0.096	-0.098
V41	HN	0.031	-0.014	N	0.027	-0.008	M95	HN	0.140	-0.105	N	0.127	-0.082
F42	HN	0.080	-0.056	N	0.144	-0.060	E96	HN	0.144	-0.106	N	0.168	-0.106
S43	HN	0.113	-0.074	N	0.162	-0.058	K97	HN	0.166	-0.132	N	0.220	-0.192
M44	HN	0.127	-0.094	N	0.161	-0.076	R98	HN		-0.152	N		-0.199
S45	HN		-0.108	N		-0.149	F99	HN	0.201	-0.152	N	0.153	-0.177
F46	HN		-0.098	N		-0.125	V100	HN	0.249	-0.191	N	0.306	-0.157
V47	HN		-0.137	N		-0.160	F101	HN	0.281	-0.217	N	0.312	-0.282
Q48	HN	0.149		N	0.170		N102	HN	0.426	-0.343	N	0.369	-0.339
G49	HN	0.175	-0.139	N	0.188	-0.161	K103	HN	0.342	-0.269	N	0.340	-0.362
E50	HN	0.215	-0.175	N	0.213	-0.184	I104	HN	0.469	-0.361	N	0.414	-0.368
E51	HN	0.208	-0.207	N	0.184	-0.567	E105	HN	0.417	-0.316	N	0.353	-0.457
S52	HN	0.238	-0.203	N	0.287	-0.190	I106	HN	0.478	-0.383	N	0.436	-0.453
N53							N107						
D54	HN	0.290	-0.242	N	0.326	-0.222	N108	HN	0.299		N	0.287	

Table S2 (continued). PCSs of backbone ^{1}H and ^{15}N of IL1β-R2.

R2		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)			PCS [ppm] Tb (III)	PCS [ppm] Tm (III)		PCS [ppm] Tb (III)	PCS [ppm] Tm (III)
N108	HN	0.299		N	0.287		D145						
K109	HN	0.390	-0.321	N	0.383	-0.320	F146	HN	0.471	-0.335	N	0.405	-0.271
L110	HN		-0.349	N		-0.321	T147	HN	0.178	-0.170	N	0.191	-0.229
E111							M148	HN	0.144		N	0.102	
F112	HN	0.578		N	0.479		Q149	HN	0.077	-0.042	N	0.066	-0.017
E113	HN	0.550	-0.421	N	0.550	-0.439	F150	HN	0.028		N	0.079	
S114							V151	HN	0.085	-0.036	N	0.118	-0.031
A115	HN	0.392	-0.318	N	0.397	-0.311	S152						
Q116	HN	0.416		N	0.391		S153	HN		-0.050	N		-0.089
F117	HN	0.478	-0.381	N	0.511	-0.373							
P118													
N119	HN	0.811	-0.695	N	0.875	-0.773							
W120	HN	0.856		N	0.937								
Y121	HN	0.983	-0.811	N	0.804	-0.729							
I122	HN	0.599	-0.505	N	0.621	-0.534							
S123													
T124													
S125	HN	0.010	-0.025	N	0.101	-0.109							
Q126													
A127	HN		0.554	N		0.576							
E128	HN		0.534	N		0.365							
N129	HN	-0.313		N	-0.341								
M130	HN	-0.245	0.208	N	-0.204	0.210							
P131													
V132	HN	0.098	-0.039	N	0.102	-0.085							
F133	HN	0.357	-0.259	N	0.332	-0.242							
L134	HN	0.537	-0.415	N	0.571	-0.425							
G135	HN	0.866	-0.695	N	0.888	-0.767							
G136													
T137													
K138													
G													
Y													
I													
D													
T													
N													
N													
D													
G													
W													
I													
E													
G													
D													
E													
L													
Y													
G139													
G140													
Q141													
D142													
I143													
T144													

2D ^{1}H-^{15}N-HSQCs of IL1β-LBT

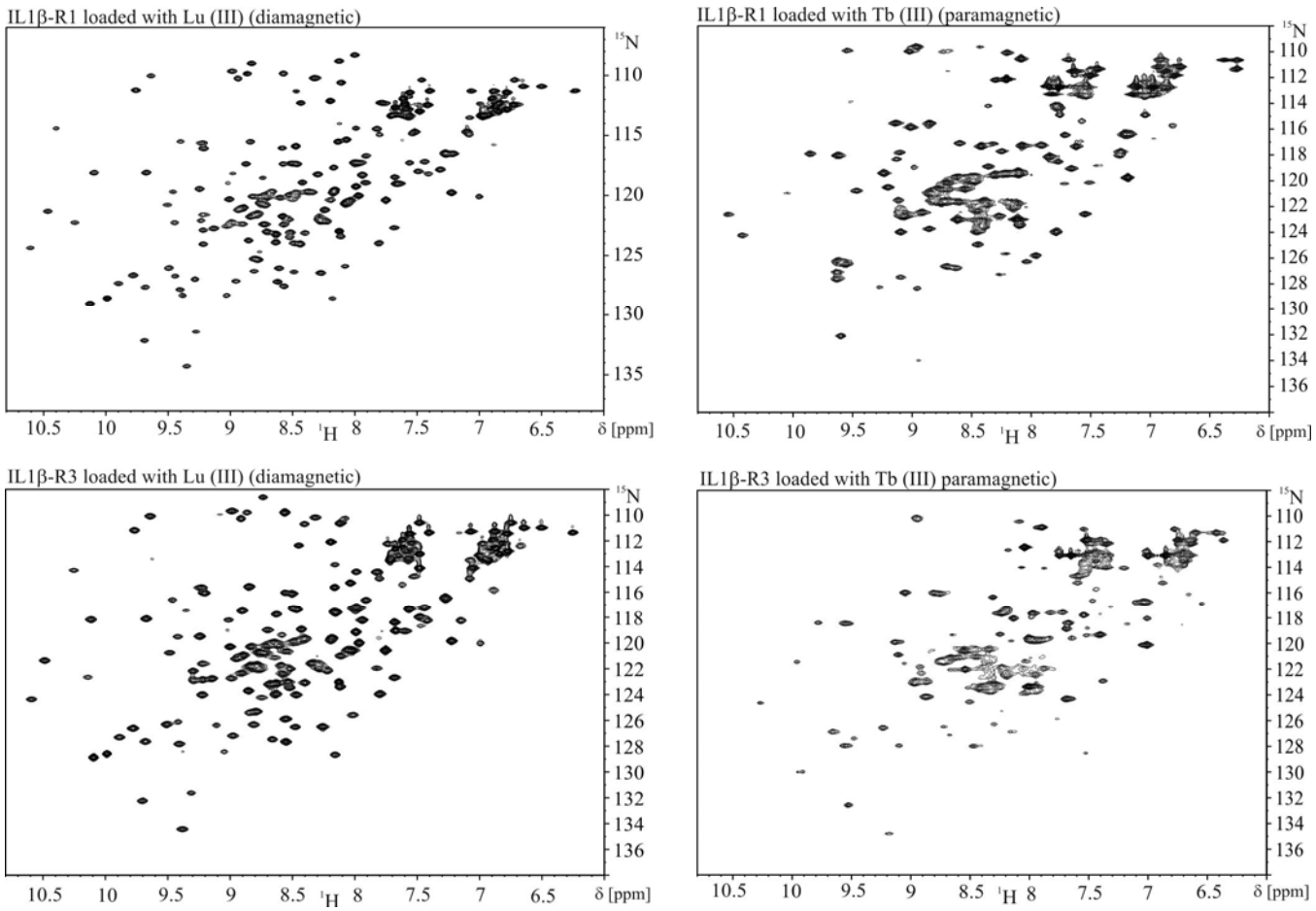

Figure S7. 2D-^{1}H-^{15}N-HSQC spectra comparing (left) ^{15}N-IL1β-R1 and R3 loaded with diamagnetic Lu (III) with (right) ^{15}N-IL1β-R1 and R3 loaded with paramagnetic Tb (III). Spectra for IL1β-R1 were recorded at pH 7 and 20 °C in 10 mM HEPES, 100 mM NaCl and 10 μM DSS. Spectra for IL1β-R3 were recorded at pH 7 and 20 °C in 10 mM Tris, 100 mM NaCl and 10 μM DSS due to precipitation of IL1β-R3 in HEPES buffer.

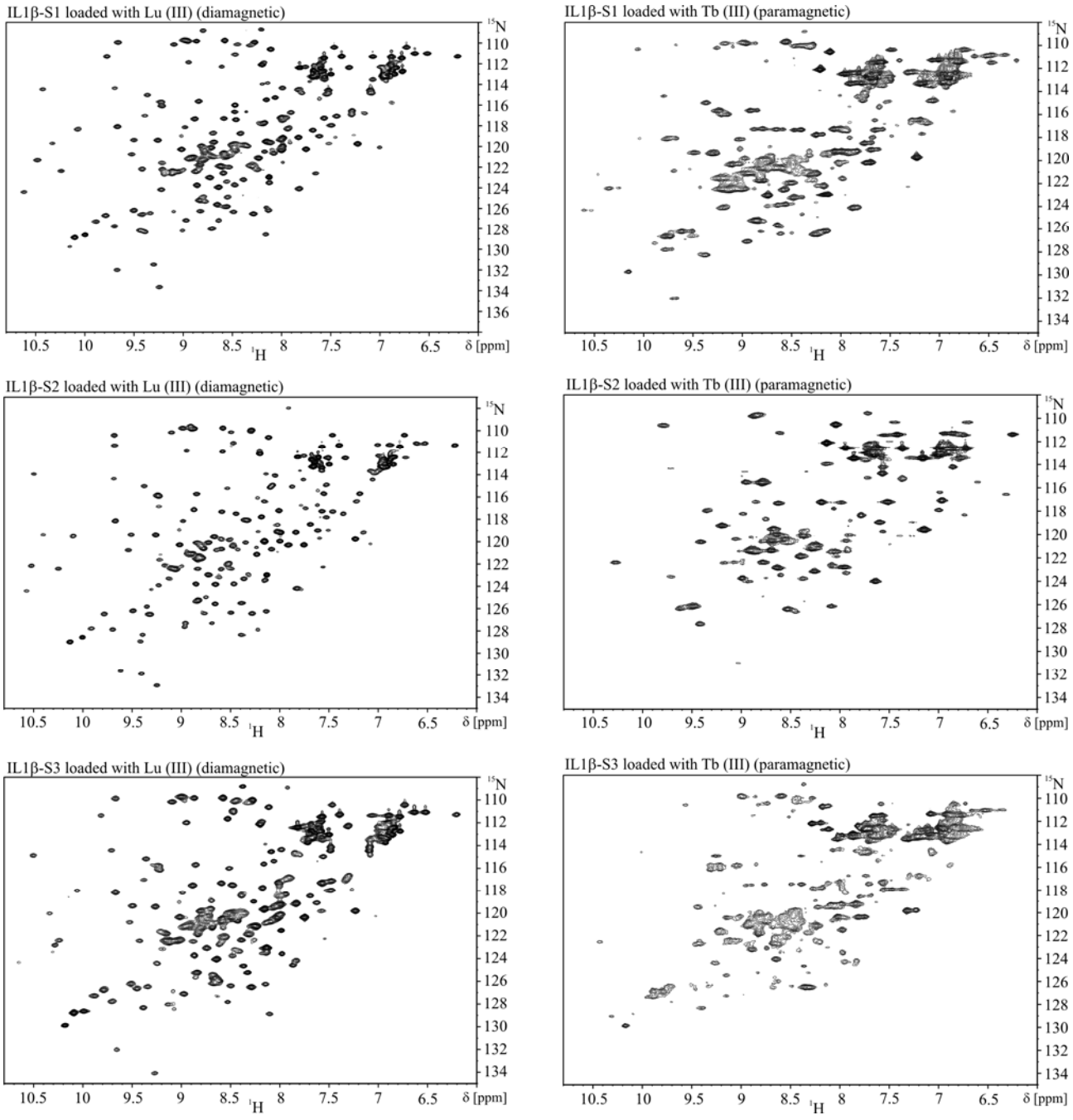

Figure S8. 2D-^{1}H,-^{15}N- HSQC spectra comparing (left) IL1β-S1, -S2 and -S3 loaded with diamagnetic Lu (III) with (right) IL1β-S1, -S2 and -S3 loaded with paramagnetic Tb (III). Spectra were recorded at pH 7 and 20 °C in 10 mM HEPES, 100 mM NaCl and 10 µM DSS.

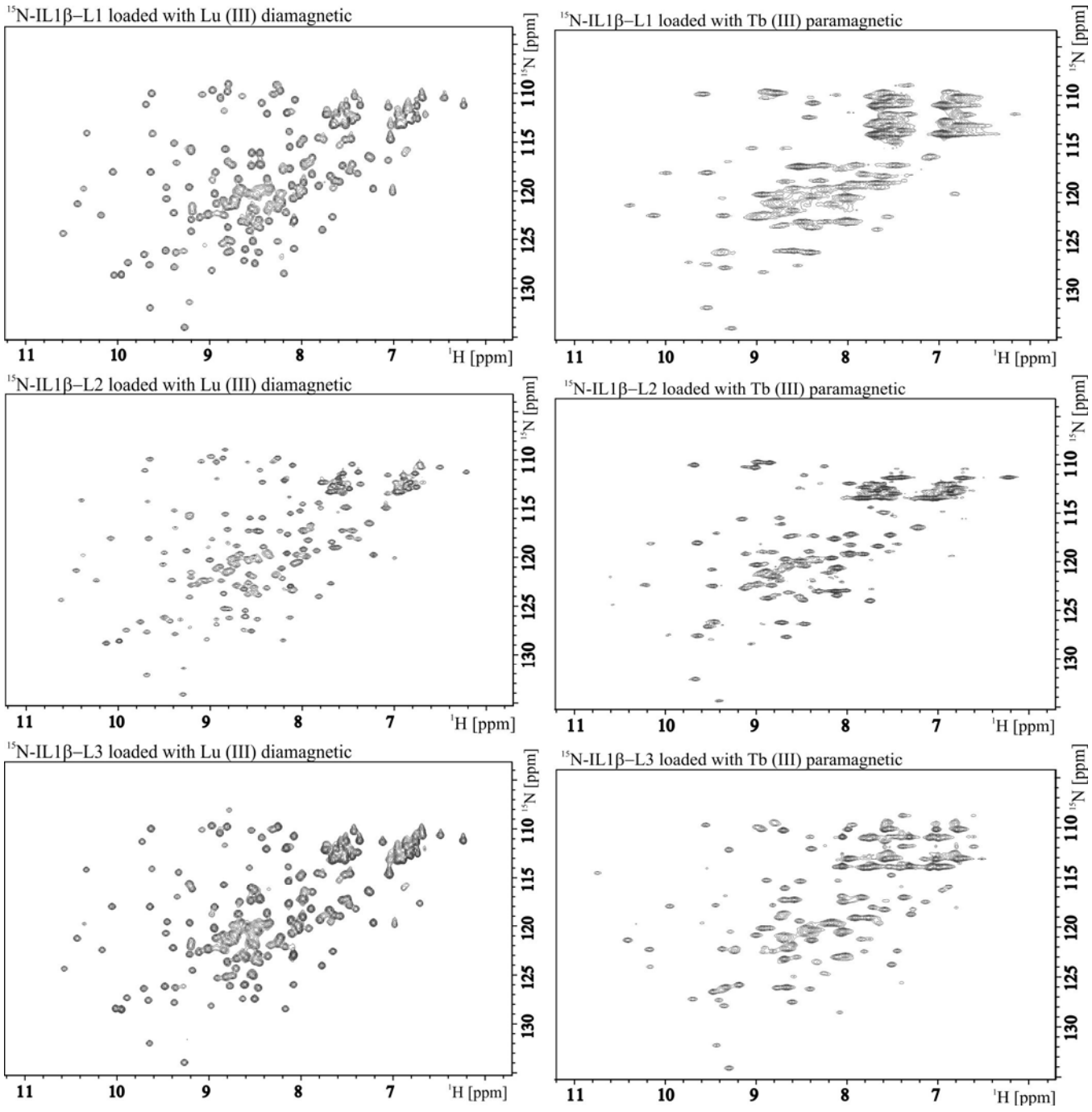

Figure S9. 2D-^{1}H-^{15}N- HSQC spectra comparing (left) IL1β-L1, -L2 and -L3 loaded with diamagnetic Lu (III) with (right) IL1β-S1, -S2 and -S3 loaded with paramagnetic Tb (III). Spectra were recorded at pH 7 and 20 °C in 10 mM HEPES, 100 mM NaCl and 10 µM DSS.

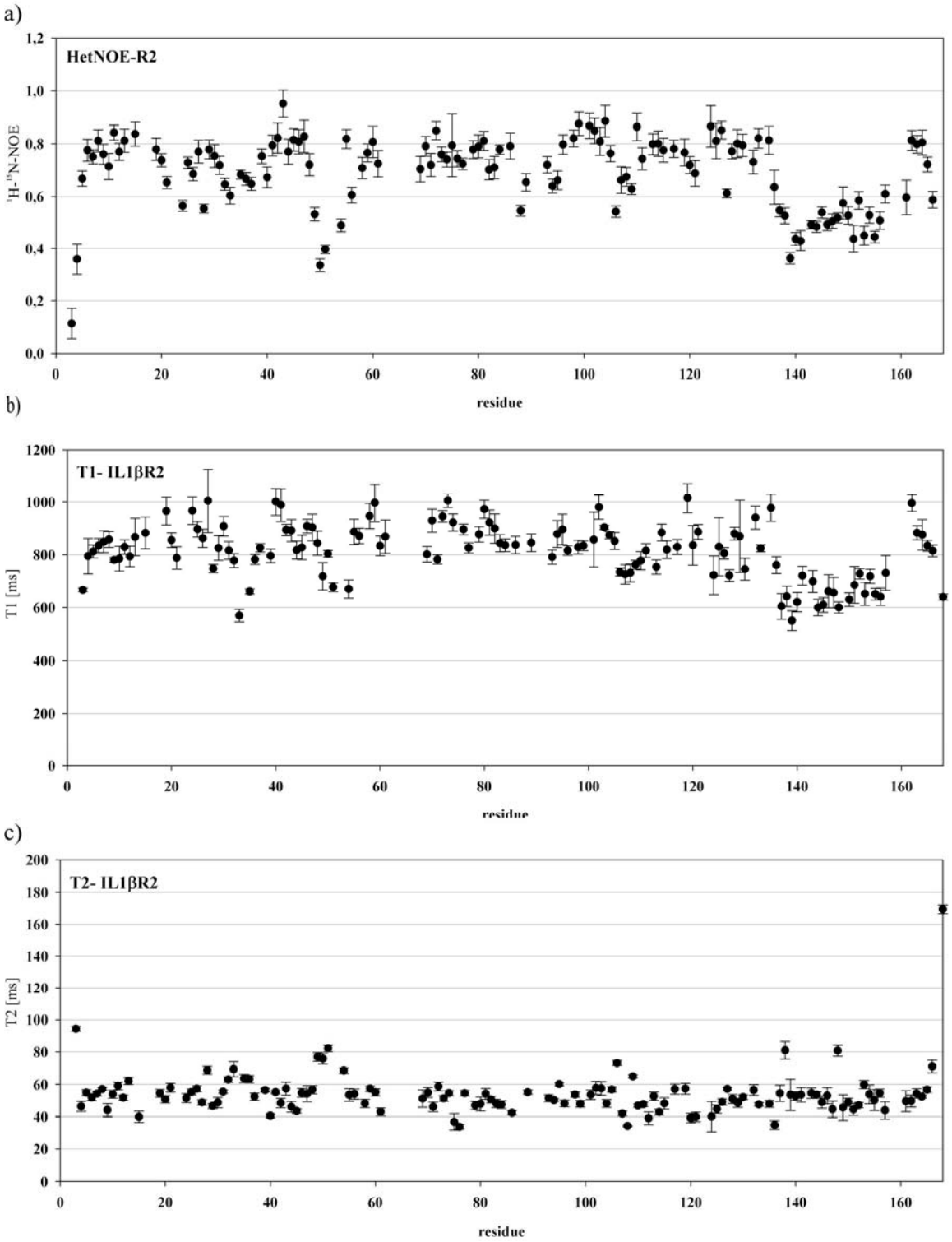

Figure S10. ^{15}N- relaxation data of IL1β-R2. (a) {^{1}H}-^{15}N- HetNOE, (b) Spin-lattice (longitudinal) relaxation time T_1 and (c) spin-spin (transverse) relaxation time T_2.

Table S3. Relaxation data and order parameters for IL1β-R2

R2	HetNOE	ΔHetNOE	T1	ΔT1	T2	ΔT2	S2	ΔS2
A1								
P2								
V3	0,11	0,06	667	7	94,3	1,5		
R4	0,36	0,06	794	68	46,3	3,2	0,84	0,03
S5	0,67	0,03	812	25	54,8	1,9	0,89	0,01
L6	0,78	0,04	834	27	51,9	1,9	0,97	0,01
N7	0,75	0,03	849	41	54,2	1,3	0,95	0,01
C8	0,81	0,04	858	30	57,0	0,9	0,99	0,01
T9	0,76	0,04	780	9	44,0	4,1	0,92	0,01
L10	0,71	0,05	785	47	53,8	2,5	0,92	0,02
R11	0,84	0,03	829	27	59,0	2,4	0,98	0,01
D12	0,77	0,03	793	40	51,7	1,7	1,00	0,02
S13	0,81	0,04	867	71	62,1	2,2	0,98	0,01
Q14								
Q15	0,84	0,05	882	61	39,8	3,4	0,93	0,03
K16								
S17								
L18								
V19	0,78	0,04	965	53	54,4	2,4	0,99	0,01
M20	0,74	0,02	855	28	50,7	2,3	0,95	0,01
S21	0,65	0,02	788	43	57,9	2,7	0,94	0,02
G22								
P23								
Y24	0,56	0,02	966	52	51,4	2,8	0,83	0,02
E25	0,73	0,02	896	29	55,2	1,7	0,89	0,02
L26	0,69	0,02	862	35	57,2	1,8	0,91	0,02
K27	0,77	0,04	1004	121	48,7	1,7	0,86	0,06
A28	0,55	0,02	747	16	68,6	2,4	0,78	0,01
L29	0,78	0,04	825	46	46,5	1,5	0,92	0,03
H30	0,75	0,04	908	37	48,7	3,2	0,99	0,01
L31	0,72	0,03	816	34	55,4	0,9	0,94	0,02
Q32	0,65	0,02	778	27	62,9	1,8	0,92	0,01
G33	0,60	0,03	570	23	69,3	4,6	0,60	0,03
Q34								
D35	0,68	0,02	661	10	63,6	2,5	0,77	0,01
M36	0,67	0,02	782	19	63,2	2,3	0,94	0,01
E37	0,65	0,02	826	16	52,4	2,2	0,90	0,01
Q38								
Q39	0,75	0,03	796	26	56,4	1,1	0,95	0,01
V40	0,67	0,04	1001	52	40,5	1,1	0,87	0,02
V41	0,79	0,04	989	64	55,2	0,9	0,83	0,03
F42	0,82	0,06	894	21	48,3	2,9	0,94	0,01
S43	0,95	0,05	891	42	57,3	4,1	0,98	0,02
M44	0,77	0,05	817	34	46,0	3,4	0,98	0,02
S45	0,82	0,04	827	47	43,4	1,4	0,97	0,03
F46	0,81	0,05	908	26	54,7	2,7	0,99	0,01
V47	0,83	0,06	903	50	54,2	4,9	0,99	0,02
Q48	0,72	0,04	843	46	56,7	2,5	0,98	0,02
G49	0,53	0,03	718	52	76,9	2,7	0,76	0,02
E50	0,34	0,02	803	13	75,7	3,2	0,82	0,01
E51	0,40	0,02	677	17	82,2	2,0	0,71	0,01
S52								
N53								
D54	0,49	0,02	670	35	68,4	1,7	0,71	0,03
K55	0,82	0,04	886	48	53,4	3,9	0,95	0,02
I56	0,61	0,03	871	29	53,9	3,1	0,90	0,02
P57								
V58	0,71	0,04	946	48	48,1	2,5	0,94	0,02
A59	0,77	0,03	997	72	57,2	1,6	0,97	0,01
L60	0,81	0,06	833	37	55,0	2,1	1,00	0,01
G61	0,72	0,05	868	63	42,9	2,3	1,00	0,03
L62								
K63								
E64								
K65								
N66								
L67								
Y68								
L69	0,70	0,05	801	29	51,1	5,4	0,91	0,02
S70	0,79	0,04	929	43	54,8	3,1	0,89	0,02
C71	0,72	0,04	782	18	46,0	3,0	0,90	0,01
V72	0,85	0,03	945	22	58,6	2,8	0,86	0,01
L73	0,76	0,03	1005	26	51,2	1,7	0,83	0,01
K74	0,74	0,03	922	32	54,5	1,5	0,89	0,02
D75	0,79	0,12			36,8	5,1		
D76	0,74	0,03	896	21	33,7	1,5	0,94	0,01
K77	0,72	0,02	826	20	54,4	1,7	0,94	0,01
P78								
T79	0,78	0,03	877	29	46,9	2,6	0,95	0,02
L80	0,79	0,04	972	35	47,8	4,4	0,89	0,02
Q81	0,81	0,04	921	48	54,1	3,4	0,92	0,02
L82	0,70	0,04	899	55	50,6	2,0	0,93	0,02
E83	0,71	0,04	843	32	48,2	2,5	0,96	0,01
S84	0,78	0,02	836	24	47,4	2,4	0,95	0,01
V85								
D86	0,79	0,05	837	32	42,3	1,4	0,93	0,02

R2	HetNOE	ΔHetNOE	T1	ΔT1	T2	ΔT2	S2	ΔS2
P87								
K88	0,55	0,02						
N89	0,65	0,03	845	33	55,1	1,3	0,91	0,02
Y90								
P91								
K92								
K93	0,72	0,03	791	27	51,4	2,1	0,91	0,01
K94	0,64	0,03	878	51	50,2	0,9	0,92	0,02
M95	0,66	0,04	895	58	60,1	1,2	0,91	0,02
E96	0,80	0,04	816	18	48,1	2,1	0,96	0,01
K97								
R98	0,82	0,03	829	26	53,5	1,6	0,99	0,01
F99	0,88	0,04	834	19	47,9	2,1	0,98	0,01
V100								
F101	0,87	0,05	857	104	53,5	3,0	1,00	0,02
N102	0,85	0,05	980	46	57,7	4,0	0,94	0,02
K103	0,81	0,05	903	8	57,5	4,2	0,98	0,00
I104	0,89	0,06	874	13	48,1	2,4	0,98	0,01
E105	0,76	0,03	852	32	56,8	1,7	0,94	0,02
I106	0,54	0,02	734	15	73,0	1,4	0,83	0,01
N107	0,66	0,05	726	37	41,9	1,4	0,85	0,03
N108	0,67	0,04	731	34	34,2	0,4	0,83	0,02
K109	0,63	0,02	762	17	64,9	1,0	0,90	0,01
L110	0,86	0,05	777	34	46,8	1,1	0,96	0,02
E111	0,74	0,04	815	27	47,6	1,5	0,98	0,01
F112					39,0	3,9		
E113	0,80	0,04	753	27	52,6	2,5	0,87	0,03
S114	0,80	0,05	883	32	42,8	1,8	1,00	0,01
A115	0,78	0,05	819	34	48,1	3,5	0,99	0,01
Q116								
F117	0,78	0,03	829	28	57,0	3,1	0,98	0,01
P118								
N119	0,77	0,05	1015	57	57,3	3,4	0,93	0,02
W120	0,72	0,03	836	75	39,1	3,0	0,96	0,03
Y121	0,69	0,05	886	28	39,8	2,9	0,94	0,01
I122								
S123								
T124	0,87	0,08	722	73	40,0	9,3	1,00	0,04
S125	0,81	0,07	830	110	44,6	2,6	0,99	0,05
Q126	0,85	0,03	805	22	49,0	1,9	0,97	0,01
A127	0,61	0,02	721	22	57,1	0,8	0,84	0,02
E128	0,77	0,03	879	24	50,9	2,8	0,94	0,01
N129	0,80	0,05	869	137	48,4	2,7	1,00	0,02
M130	0,79	0,04	745	41	52,0	1,6	0,91	0,04
P131								
V132	0,73	0,05	940	44	56,4	3,3	0,90	0,02
F133	0,82	0,04	824	14	47,5	1,1	0,98	0,01
L134								
G135	0,81	0,05	977	51	47,9	2,2	0,92	0,03
G136	0,64	0,06	761	32	34,8	2,7	0,84	0,02
T137	0,55	0,02	605	48	54,4	5,1	0,62	0,04
K138	0,53	0,03	642	39	80,9	5,4	0,68	0,02
G	0,36	0,02	551	36	53,4	9,7	0,43	0,05
Y	0,44	0,02	621	37	52,6	2,6	0,61	0,04
I	0,43	0,04	720	35	53,5	4,4	0,74	0,03
D								
T	0,49	0,02	698	42	54,5	3,3	0,73	0,03
N	0,48	0,02	600	31	53,4	1,8	0,60	0,03
N	0,54	0,02	610	28	48,9	3,9	0,63	0,03
D	0,49	0,02	661	63	52,9	5,0	0,68	0,05
G	0,51	0,03	656	58	44,5	5,1	0,67	0,05
W	0,52	0,02	600	21	80,7	3,5	0,65	0,02
I	0,58	0,06			45,5	8,0		
E	0,53	0,03	630	26	48,9	2,6	0,67	0,02
G	0,44	0,05	685	70	44,4	3,7	0,69	0,06
D	0,58	0,03	728	20	47,1	1,6	0,78	0,01
E	0,45	0,04	652	44	59,8	2,5	0,66	0,04
L	0,53	0,03	718	27	53,8	5,9	0,78	0,02
Y	0,45	0,02	651	22	50,2	6,5	0,63	0,02
G139								
G140								
Q141	0,51	0,03	641	33	54,5	2,5	0,67	0,03
D142	0,61	0,03	731	66	43,9	5,3	0,84	0,04
I143								
T144								
D145								
F146	0,60	0,07			49,5	6,5		
T147	0,81	0,04	995	32	49,6	3,8	0,82	0,02
M148	0,80	0,03	883	27	54,2	3,0	0,94	0,02
Q149	0,80	0,05	875	58	52,3	0,9	0,97	0,03
F150	0,72	0,03	834	21	56,7	1,7	0,96	0,01
V151	0,59	0,03	815	22	71,0	3,9	0,90	0,01
S152								
S153	-0,12	-0,01	640	12	169,4	2,8		

Crystallization and Structure determination of IL1β-L3

For crystallization protein was loaded with $TbCl_3$ as described for IL1β-S1 and concentrated to 15 mg/ml. Initial screening used the Hampton Index screen. IL1β-L3 crystallized readily from 0.2 M ammonium sulfate, 0.1 M Bis-Tris pH 6.5, 25% w/v PEG 3,350 (condition 67, Hampton Index Screen).

Data were collected at beamline X12C at the National Synchrotron Light Source. IL1β-L3 crystals were cryoprotected by soaking the crystals in 15% glucose in mother liquid and then transferred to 30% glucose solution plus mother liquor. Crystals were flash frozen in the gaseous cryogenic N_2 stream. Data were collected at beamline X12C at the National Synchrotron Light Source using a wavelength of 0.95 Å and processed with DENZO/SCALEPACK[3]. Crystals diffracted to 1.7 Å and belong to space group P4(1). Data collection and refinement statistics are presented in Table S4.

The structure solution was carried out via molecular replacement with the program Phenix[4] using the structure of wild-type IL1β as the model (PDB 1T4Q) with residues 74-77 and all solvent molecules removed. Manual protein rebuilding was performed in COOT[5] with alternating rounds of refinement carried out in Phenix. The final model contains the entire IL1β molecule (excluding residues 80-86) including 7 of 17 residues of the LBT and 123 waters.

Table S4 Data collection, Structure Determination and Refinement Statistics of IL1β-L3

Data Collection – IL1β-L3 Mutant	
Space group and unit cell	$P4_1$; $a = b = 42.6$ Å, $c = 88.0$ Å
Wavelength (Å)	1.54
Resolution limits (Å) (highest resolution shell)	19.17-1.70 (1.76-1.70)
no. of reflections	
Measured	298,082
Unique	33,108
Completeness (%)	
All data (highest resolution shell)	97.4 (91.0)
R_{sym}[a] (on I) (highest resolution shell)	0.039 (0.48)
$[I/\sigma(I)]$	
all data (highest resolution shell)	33.8 (4.77)
Refinement	
Resolution (Å)	19.2-1.70
R factor	0.174
R free	0.213
Reflections in test set	3,355
non-hydrogen atoms	1,429
RMS deviations	
Bond lengths (Å)	0.007
Angles (°)	1.13
Average B factor (Å^2) (all atoms)	39.1

$R_{sym} = \sum|I_{obs} - \langle I \rangle|/\sum I_{obs}$

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