

**Table S4.** Crystallographic data collection and refinement statistics.

Data Collection							
Protein	WT	WT	V132A	V132L	V140A	V140L	H160L
X-ray source	In house <sup>d</sup>	I24 <sup>e</sup>	I04 <sup>e</sup>	SLS	I04 <sup>e</sup>	I24 <sup>e</sup>	I24 <sup>e</sup>
Wavelength (Å)	1.5418	0.9795	0.9687	0.9795	0.9687	0.9795	0.9687
Space group	P1	P12 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub>	I121	P2 <sub>1</sub> 2 <sub>1</sub>	P12 <sub>1</sub>
Cell dimensions							
a, b, c (Å)	45.3, 57.1, 91.4	45.5, 101.9, 53.8	45.9, 93.3, 104.2	50.3, 93.1, 105.4	291.3, 50.2, 116.3	45.4, 99.8, 102.8	56.8, 72.3, 58.4
α, β, γ (°)	91.3, 102.0, 104.2	90.0, 111.1, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 109.0, 90.0	90.0, 90.0, 90.0	90.0, 109.9, 90.0
Resolution (Å)	2.75	1.48	2.20	2.05	1.75	2.30	1.50
R <sub>merge</sub> (%) <sup>†‡</sup>	0.077 (0.40)	0.062 (0.425)	0.084 (0.228)	0.076 (0.524)	0.075 (0.366)	0.116 (0.642)	0.054 (0.451)
I/σ(I) <sup>§</sup>	12.6 (2.0)	8.0 (2.0)	11.5 (4.5)	15.7 (4.3)	12.2 (3.7)	6.3 (2.4)	6.9 (2.3)
Completeness (%) <sup>¶</sup>	91.2	98.8 (99.7)	96.5 (83.0)	99.3 (98.8)	99.6 (98.9)	99.4 (99.1)	99.7 (99.7)
Multiplicity <sup>¶</sup>	3.9	3.0 (3.0)	5.5 (3.4)	7.9 (8.2)	5.4 (5.1)	4.9 (4.7)	3.3 (3.2)
Refinement							
Resolution (Å)	2.75	1.48	2.20	2.05	1.75	2.30	1.50
No. measurements	85778	226403	128654	233799	858471	103642	233544
No. unique	22122	71204	23348	29362	160426	21298	70844
R <sub>work</sub> / R <sub>free</sub>	0.1855 / 0.2659	0.195 / 0.2415	0.1787 / 0.2332	0.2008 / 0.2563	0.1848 / 0.2157	0.195 / 0.2619	0.1803 / 0.2083
No. protein chains	4	2	2	2	6	2	2
No. atoms							
Protein	6012	3124	3165	3165	9507	3109	3196
Ligand/ion	84	109	69	42	189	64	63
Water	112	293	116	124	927	83	331
Average B-factor	31.089	30.662	32.739	43.22	24.386	48.431	23.915
R.m.s. deviations							
Bond lengths (Å)	0.0127	0.0112	0.0189	0.0194	0.0114	0.015	0.0115
Bond angles (°)	0.9048	1.6357	1.0213	1.0192	1.5114	1.9485	1.6006
PDB codes	e	4HZF	4I0A	4I09	4I02	4I01	4I0B

<sup>†</sup> Number in parentheses is for the last shell

<sup>‡</sup>  $R = \sum (|I_i - \langle I \rangle|) / \sum I_i$

<sup>§</sup> Diamond Light Source

<sup>e</sup> Bruker MicroStar

<sup>¶</sup> Isostructural to 2GZW