Supplemental Table S1

Crystallographic data of mutant Fv–HEL complexes

	L-N31D	L-N32D	L-N92D	Wild-type [a]
Space group,	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Unit cell dimensions	$a = b = 56.58$ Å	$a = b = 56.56$ Å	$a = b = 56.57$ Å	$a = b = 56.44$ Å
	$c = 234.43$ Å	$c = 233.55$ Å	$c = 234.68$ Å	$c = 234.49$ Å
Wavelength (Å)	1.000	1.000	1.000	1.000
Unique reflections	36648	36331	34947	36103
Resolution (Å)	1.8	1.8	1.8	1.8
R_{merge} [b]	0.062 (0.200)	0.069 (0.258)	0.078 (0.241)	0.064 (0.181)
Completeness (%)	100 (100)	99.8 (99.9)	96.6 (89.7)	99.4 (98.8)
Multiplicity	13.9	14.1	8.0	7.0
Resolution range (Å)	8.0 – 1.8	8.0 – 1.8	8.0 – 1.8	8.0 – 1.8
Total reflections used	36059	35777	34484	35592
R-factor [c] (%)	18.9	19.4	18.6	19.4
Free R-factor (%)	22.5	22.2	21.6	21.9
RMSD bond length (Å)	0.005	0.005	0.004	0.004
RMSD bond angle (°)	1.30	1.30	1.30	1.30
Water molecules	376	386	393	330

[a] Data of 2DQJ (55)

[b] $$R_{\text{merge}} = \frac{\sum_{hkl} | \text{I} - < \text{I} > |}{\sum_{hkl} < \text{I} >}$$

[c] $$R\text{-factor} = \frac{\sum || \text{F}_{\text{obs}} | - | \text{F}_{\text{calc}} ||}{\sum | \text{F}_{\text{obs}} |}$$

Supplemental Table S2

Hydrogen bonds via interfacial water molecules (No.1)

WT[a]			LN31D			LN32D			LN92D		
W2	YTyr20	Oη	W18	YTyr20	Oη	W242	YTyr20	Oη	W2	YTyr20	Oη
	LTyr50	Oη	(W2)[b]	LTyr50	Oη	(W2)	LTyr50	Oη	(W2)	LTyr50	Oη
	LSer91	Oγ		LSer91	Oγ		LSer91	Oγ		LSer91	Oγ
W5	LTyr50	Oη	W3	LTyr50	Oη	W10	LTyr50	Oη	W6	LTyr50	Oη
	YAsn93	O	(W5)	YAsn93	O	(W5)	YAsn93	O	(W5)	YAsn93	O
W8	LGln53	Nε2				W4	LGln53	Nε2	W1	LGln53	Nε2
	YHis15	Nδ1				(W8)	YHis15	Nδ1	(W8)	YHis15	Nδ1
	YThr89	O					YThr89	O		YThr89	O
	YAsn93	Oδ1					YAsn93	Oδ1		YAsn93	Oδ1
W10	LSer91	O	W2	LSer91	O	W8	LSer91	O	W8	LSer91	O
	YTyr20	Oη	(W10)	YTyr20	Oη	(W10)	YTyr20	Oη	(W10)	YTyr20	Oη
	LTyr96	Oη		LTyr96	Oη		LTyr96	Oη		LTyr96	Oη
	YArg21	Nη1		YArg21	Nη1		YArg21	Nη1		YArg21	Nη1
	YSer100	Oγ		YSer100	Oγ		YSer100	Oγ		YSer100	Oγ
W13	LAsn31	Nδ2	W19	LAsp31	O δ2	W16	LAsn31	Nδ2	W14	LAsn31	Nδ2
	YAsn93	Oδ1	(W13)	YAsn93	Oδ1	(W13)	YAsn93	Oδ1	(W13)	YAsn93	Oδ1
	YLys96	Nζ		YLys96	Nζ		YLys96	Nζ		YLys96	Nζ
W53	LTrp94	N	W49	LTrp94	N						
	YArg21	Nε	(W53)	YArg21	Nε						
W81	HTyr58	Oη	W82	HTyr58	Oη	W109	HTyr58	Oη	W97	HTyr58	Oη
	YVal99	O	(W81)	YVal99	O	(W81)	YVal99	O	(W81)	YVal99	O
				YAsp101	O		YAsp101	O		YAsp101	O
W86	LSer93	Oγ	W45	LSer93	Oγ	W89			W29	LSer93	Oγ
			(W86)			(W86)	LSer92	O	(W86)		
	YAsn19	O		YAsn19	O						
	YArg21	N		YArg21	N		YArg21	N			
	YGly22	N		YGly22	N		YGly22	N		YGly22	N
W178	LSer28	Oγ									
	LGly30	N									
	YAsp18	O									
W200			W50			W34	LIle29	O	W95		
			(W200)			(W200)	LAsp32	Oδ2	(W200)	LAsn32	Nδ2
	LAsn92	Oδ1		LAsn92	Oδ1		LAsn92	Oδ1		LAsp92	Oδ1
	YGly16	O		YGly16	O		YGly16	O		YGly16	O
	YAsp18	O									
				YAsn19	N						
W255	HAsp99	Oδ2	W159	HAsp99	Oδ2	W353	HAsp99	Oδ2			
	YAsn77	Nδ2	(W255)	YAsn77	Nδ2	(W255)	YAsn77	Nδ2			
	HAsp101	Oδ2		HAsp101	Oδ2		HAsp101	Oδ2			
				HAsn97	OD1		HAsn97	OD1			

Supplemental Table S2
Hydrogen bonds via interfacial water molecules (No.2)

WT			LN31D			LN32D			LN92D		
W255	HAsp99	Oδ2	W159	HAsp99	Oδ2	W353	HAsp99	Oδ2			
	YAsn77	Nδ2	(W255)	YAsn77	Nδ2	(W255)	YAsn77	Nδ2			
	HAsp101	Oδ2		HAsp101	Oδ2		HAsp101	Oδ2			
				HAsn97	OD1		HAsn97	OD1			
W295	HSer31	O	W267	HSer31	O	W377	HSer31	O	W360	HSer31	O
	YAsn74	O	(W295)	YAsn74	O	(W295)	YAsn74	O	(W295)	YAsn74	O
	YAsn77	Oδ1		YAsn77	Oδ1		YAsn77	Oδ1		YAsn77	Oδ1
			W366	HAsp27	Oδ2	W138	HAsp27	Oδ2	W176	HAsp27	Oδ1
				YArg73	O		YArg73	O		YArg73	O
						W362	HThr30	O	W382	HThr30	O
							YArg73	Nη1		YArg73	Nη1
						W149	HAsp32	Oδ2	W57	LSer93	Oγ
							HSer31	O		LAsp92	Oδ2
							YAsn77	Oδ1		YAsn19	O
						W382	LAsn92	Nδ2	W296	LAsp92	Oδ1
							YAsn19	O		YAsp18	O

[a] Data of 2DQJ (55)
[b] Parentheses indicate corresponding water molecules in the wild-type complex.

Supplemental Table S3
Interfacial areas of the mutant Fv–HEL complexes (Å^2).

Complex	VL	VH	HEL	Total
Wild type	361	529	990	1880
LN31D	357 (-4)	538 (+9)	977 (-13)	1872 (-8)
LN32D	311 (-50)	530 (+1)	923 (-67)	1764 (-116)
LN92D	330 (-31)	527 (-2)	940 (-50)	1797 (-83)

Interfacial areas were calculated with the program NACCESS. The default values were probe radius; 1.4Å, z-slice; 0.05Å, and van der Waals radii. Values in parentheses are differences from those of the wild-type complex.

Supplemental Table S4
Interactions between each mutant Fv and HEL in the complexes.

VL (No.1)

		WT [a]		LN31D		LN32D		LN92D
LAsn30	Cα		Cα	Gly16 Cα				
LAsn31	Cγ	His15 C	Cγ	His15 C	Cγ	His15 C	Cγ	His15 C
(Ala)		His15 O				His15 O		His15 O
(Asp)		Gly16 N		Gly16 N		(でも 3.95)		
		Gly16 Cα		Gly16 Cα		Gly16 Cα		Gly16 Cα
		Lys96 Nζ		Lys96 Nζ		Lys96 Nζ		Lys96 Nζ
	Oδ1	His15 C	Oδ1	His15 C	Oδ1	His15 C	Oδ1	Gly16 Cα
		Gly16 Cα		Gly16 Cα		His15 O		His15 O
						Gly16 Cα		His15 C
		Lys96 Nζ		*Lys96 Nζ*		Lys96 Nζ		Lys96 Nζ
	Nδ2	His15 C	Oδ2	His15 C	Nδ2	His15 C	Nδ2	His15 C
		His15 O				His15 O		His15 O
LAsn32	Cγ	Tyr20 Cδ1	Cγ	Tyr20 Cδ1	Cγ	Tyr20 Cδ1	Cγ	Tyr20 Cδ1
(Ala)		Tyr20 Cε1		Tyr20 Cε1		Tyr20 Cε1		Tyr20 Cε1
(Asp)		Lys96 Nζ		Lys96 Nζ		Lys96 Nζ		Lys96 Nζ
	Oδ1	Tyr20 Cδ1	Oδ1	Tyr20 Cδ1	Oδ1		Oδ1	Tyr20 Cδ1
		Tyr20 Cε1		Tyr20 Cε1		Tyr20 Cε1		Tyr20 Cε1
		Lys96 Cδ		Lys96 Cδ		Lys96 Cδ		Lys96 Cδ
		Lys96 Cε		Lys96 Cε		Lys96 Cε		Lys96 Cε
		Lys96 Nζ		Lys96 Nζ		*Lys96 Nζ*		Lys96 Nζ
	Nδ2	Gly16 O	Nδ2	Gly16 O	Oδ2	Tyr20 Cδ1	Nδ2	Gly16 O
		Tyr20 Cδ1		Tyr20 Cδ1		Tyr20 Cε1		Tyr20 Cδ1
LTyr50	Cδ1	Lys96 Nζ	Cδ1	Lys96 Nζ	Cδ1	Lys96 Nζ	Cδ1	Lys96 Nζ
				Lys96 Cδ				
	Cε1	Lys96 Nζ	Cε1	Lys96 Nζ	Cε1	Lys96 Nζ	Cε1	Lys96 Nζ
		Lys96 Cδ		Lys96 Cδ		Lys96 Cδ		Lys96 Cδ
	Cδ2	Asn93 Cγ	Cδ2	Asn93 Cγ	Cδ2	Asn93 Cγ	Cδ2	Asn93 Cγ
		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1
	Cε2	Asn93 Cγ	Cε2	Asn93 Cγ	Cε2	Asn93 Cγ	Cε2	Asn93 Cγ
		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1
	Cζ	Lys96 Cδ	Cζ	Lys96 Cδ	Cζ	Lys96 Cδ	Cζ	Lys96 Cδ
	Oη	Lys96 Cδ	Oη	Lys96 Cδ	Oη		Oη	Lys96 Cδ
LGln53	Cδ	Thr89 Cγ2	Cδ	Thr89 Cγ2	Cδ	Thr89 Cγ2	Cδ	Thr89 Cγ2
		Asn93 Nδ2		Asn93 Nδ2		Asn93 Nδ2		Asn93 Nδ2
	Oε1	Thr89 Cγ2	Oε1	Thr89 Cγ2	Oε1	Thr89 Cγ2	Oε1	Thr89 Cγ2
		Asn93 Cγ		Gly16 Cα		Asn93 Cγ		Asn93 Cγ
		Asn93 Nδ2		Asn93 Nδ2		Asn93 Nδ2		Asn93 Nδ2
	Nε2	Thr89 Cγ2	Nε2	Thr89 Cγ2	Nε2	Thr89 Cγ2	Nε2	Thr89 Cγ2
		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1		Asn93 Oδ1

VL (No.2)

		WT		LN31D		LN32D		LN92D
LSer91	O	Tyr20 Cε1	O	Tyr20 Cε1	O		O	Tyr20 Cε1
LAsn92	Nδ2	Asn19 O	Nδ2	Asn19 O	Nδ2			
(Ala)	C	Arg21 Cδ	C	Arg21 Cδ	C		C	
(Asp)	O	Arg21 N	O	Arg21 N	O	Arg21 N	O	Arg21 N
		Arg21 Cα		Arg21 Cα				Arg21 Cα
		Arg21 Cδ		Arg21 Cδ		Arg21 Cδ		Arg21 Cδ
					Oδ1	Asn19 O	Oδ2	
LSer93	Cα	Arg21 Cδ	Cα	Arg21 Cδ	Cα		Cα	
LTrp94	Cβ	Arg21 Cζ	Cβ	Arg21 Cζ	Cβ	Arg21 Cζ	Cβ	Arg21 Cζ
								Arg21 Nη2
LTyr96	Cε1	Arg21 Nη1	Cε1	Arg21 Nη1	Cε1	but 3.85	Cε1	Arg21 Nη1
			Cζ	Arg21 Nη1			Cζ	Arg21 Nη1
	Oη	Arg21 Nη1	Oη	Arg21 Nη1	Oη	Arg21 Nη1	Oη	Arg21 Nη1

VH (No.1)

		WT		LN31D		LN32D		LN92D
HThr30	O	Arg73 Cδ	O	Arg73 Cδ	O	Arg73 Cδ Leu75 Cδ2	O	Leu75 Cδ2
HSer31	Cα	Leu75 Cδ2	Cα	Leu75 Cδ2	Cα	Leu75 Cδ2 Arg73 O	Cα	Leu75 Cδ2 Arg73 O
	Cβ	Arg73 O	Cβ	Arg73 O	Cβ	Arg73 O	Cβ	Arg73 O
					Oγ	Arg73 O	Oγ	Arg73 O
	C	Leu75 Cδ2	C	Leu75 Cδ2	C	Leu75 Cδ2	C	Leu75 Cδ2
	O	Leu75 Cδ2	O	Leu75 Cδ2	O	Leu75 Cδ2 Leu75 Cα	O	Leu75 Cδ2
HAsp32	N	Leu75 Cδ2	N	Leu75 Cδ2	N	Leu75 Cδ2	N	Leu75 Cδ2
	Cα	Leu75 Cδ2	Cα	Leu75 Cδ2	Cα	Leu75 Cδ2	Cα	Leu75 Cδ2
	Cγ	Lys97 Nζ	Cγ	Lys97 Nζ	Cγ	Lys97 Nζ Asn77 Nδ2	Cγ	Lys97 Nζ
	Oδ1	Lys97 Cε *Lys97 Nζ*	Oδ1	Lys97 Cε *Lys97 Nζ*	Oδ1	Lys97 Cε *Lys97 Nζ*	Oδ1	Lys97 Cε *Lys97 Nζ*
					Oδ2	Asn77 Nδ2	Oδ2	Asn77 Nδ2
	C	Leu75 Cδ2						
HTyr33	Cγ	Lys97 Cε	Cγ	Lys97 Cε	Cγ	Lys97 Cε	Cγ	Lys97 Cε
	Cδ1	Lys97 Cε	Cδ1	Lys97 Cε			Cδ1	Lys97 Cε
			Cδ2	Lys97 Cγ	Cδ2	Lys97 Cγ		
	Cε2	Lys97 Cγ Lys97 O	Cε2	Lys97 Cγ Lys97 O	Cε2	Lys97 Cγ Lys97 O Lys97 C	Cε2	Lys97 Cγ Lys97 O Lys97 C
	Cζ	Lys97 Cγ Lys97 O	Cζ	Lys97 Cγ Lys97 O	Cζ	Lys97 Cγ Lys97 O	Cζ	Lys97 Cγ Lys97 O Asp101 Cβ
	Oη	Trp63 Cζ3 Trp63 Cη2 Lys97 C Lys97 O Asp101 Cβ	Oη	Trp63 Cζ3 Trp63 Cη2 Lys97 C Lys97 O Asp101 Cβ	Oη	Trp63 Cζ3 Trp63 Cη2 Lys97 C Lys97 O Asp101 Cβ	Oη	Trp63 Cζ3 Trp63 Cη2 Lys97 C Lys97 O Asp101 Cβ
HTyr50	Cε2	Ser100 O	Cε2	Ser100 O	Cε2	Ser100 O	Cε2	Ser100 O
	Cζ	Ser100 O	Cζ	Ser100 O	Cζ	Ser100 O	Cζ	Ser100 O
	Oη	Arg21 Cz Arg21 Nη1 Arg21 Nη2 Ser100 Cβ Ser100 C Ser100 O	Oη	Arg21 Cζ Arg21 Nη1 Arg21 Nη2 Ser100 Cβ Ser100 C Ser100 O	Oη	Arg21 Cζ Arg21 Nη1 Arg21 Nη2 Ser100 Cβ Ser100 C Ser100 O	Oη	Arg21 Cζ Arg21 Nη1 Arg21 Nη2 Ser100 Cβ Ser100 C Ser100 O

VH (No.2)

		WT		LN31D		LN32D		LN92D
HSer52	Cβ	Asp101 Cα	Cβ	Asp101 Cα	Cβ	Asp101 Cα	Cβ	Asp101 Cα
						Asp101 Cβ		Asp101 Cβ
				Asp101 Cγ		Asp101 Cγ		Asp101 Cγ
		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1
	Oγ	Asp101 Cα	Oγ	Asp101 Cα	Oγ	Asp101 Cα	Oγ	Asp101 Cα
						Asp101 Cβ		
		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ
		Asp101 Oδ1		Asp101 Cδ1		Asp101 Cδ1		Asp101 Cδ1
HTyr53	Cβ	Leu75 Cδ1	Cβ	Leu75 Cδ1	Cβ	Leu75 Cδ1	Cβ	Leu75 Cδ1
		Leu75 Cδ2		Leu75 Cδ2		Leu75 Cδ2		Leu75 Cδ2
	Cγ	Leu75 Cδ1	Cγ	Leu75 Cδ1	Cγ	Leu75 Cδ1	Cγ	Leu75 Cδ1
	Cδ1	Trp63 Cη2	Cδ1	Trp63 Cη2	Cδ1	Trp63 Cη2	Cδ1	Trp63 Cη2
						Leu75 Cδ1		Leu75 Cδ1
		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ
		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ
		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1
	Cε1	Trp63 Cζ2	Cε1	Trp63 Cζ2	Cε1	Trp63 Cζ2	Cε1	Trp63 Cζ2
		Trp63 Cη2		Trp63 Cη2		Trp63 Cη2		Trp63 Cη2
		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ
		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ		Asp101 Cγ
		Asp101 Cδ1		Asp101 Cδ1		Asp101 Cδ1		Asp101 Oδ1
		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2
	Cδ2	Trp62 Cε3	Cδ2	Trp62 Cε3	Cδ2	Trp62 Cε3	Cδ2	Trp62 Cε3
		Trp62 Cζ3		Trp63 Cζ3		Trp62 Cζ3		Trp62 Cζ3
		Leu75 Cδ1		Leu75 Cδ1		Leu75 Cδ1		Leu75 Cδ1
	Cε2	Trp62 Cζ3	Cε2	Trp62 Cε3	Cε2	Trp62 Cε3	Cε2	Trp62 Cε3
		Trp62 Cη3		Trp62 Cζ3		Trp62 Cζ3		Trp62 Cζ3
			Cζ	Trp63 Cζ2				
					Oη	Asp103 Nδ2	Oη	Asp103 Nδ2
HSer54	N	Asp101 Oδ1	N	Asp101 Oδ1	N	Asp101 Oδ1	N	Asp101 Oδ1
	Cα	Asp101 Oδ1	Cα		Cα	Asp101 Oδ1	Cα	Asp101 Oδ1
	Cβ	Asp101 Cγ	Cβ	Asp101 Cγ	Cβ	Asp101 Cγ	Cβ	Asp101 Cγ
		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1
		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2
		Asn103 Nδ2		Asn103 Nδ2		Asn103 Nδ2		Asn103 Nδ2
	Oγ	Asp101 Cγ	Oγ	Asp101 Cγ	Oγ	Asp101 Cγ	Oγ	Asp101 Cγ
		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1
		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2		Asp101 Oδ2

VH (No.3)

		WT		LN31D		LN32D		LN92D
HSer56	Cβ	Gly102 N	Cβ	Gly102 N	Cβ	Gly102 N	Cβ	Gly102 N
		Gly102 Cα		Gly102 Cα		Gly102 Cα		Gly102 Cα
	Oγ	Gly102 N	Oγ	Gly102 N	Oγ	Gly102 N	Oγ	Gly102 N
		Gly102 Cα		Gly102 Cα		Gly102 Cα		Gly102 Cα
		Asp101 Oδ1		Asp101 Oδ1		Asp101 Oδ1		Asp101 Cδ1
HTyr58	Cγ	Arg21 Nη2	Cγ	Arg21 Nη2	Cγ		Cγ	
	Cδ1	Arg21 Nη2	Cδ1	Arg21 Nη2	Cδ1	Arg21 Nη2	Cδ1	Arg21 Nη2
	Cε1	Arg21 Nη2	Cε1	Arg21 Nη2	Cε1	Arg21 Nη2	Cε1	Arg21 Nη2
	Cε2	Ser100 O	Cε2	Ser100 O	Cε2	Ser100 O	Cε2	Ser100 O
	Cζ	Arg21 Nη2	Cζ	Arg21 Nη2	Cζ	Arg21 Nη2	Cζ	Arg21 Nη2
						Ser100 O		Ser100 O
	Oη		Oη		Oη	Asp101 C	Oη	Asp101 C
						Gly102 N		Gly102 N
		Gly102 Cα		Gly102 Cα		Gly102 Cα		Gly102 Cα
HTrp98	Cε3	Lys97 Cγ	Cε3	Lys97 Cγ	Cε3	Lys97 Cγ	Cε3	Lys97 Cγ
		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ
		Lys97 Cε		Lys97 Cε		Lys97 Cε		Lys97 Cε
	Cζ2	Ser100 Cβ	Cζ2	Ser100 Cβ	Cζ2	Ser100 Cβ	Cζ2	Ser100 Cβ
		Ser100 Oγ		Ser100 Oγ		Ser100 Oγ		Ser100 Oγ
	Cζ3	Lys97 Cα	Cζ3	Lys97 Cα	Cζ3	Lys97 Cα	Cζ3	Lys97 Cα
		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ
		Lys97 Cγ		Lys97 Cγ		Lys97 Cγ		Lys97 Cγ
		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ
	Cη2	Lys97 Cα	Cη2	Lys97 Cα	Cη2	Lys97 Cα	Cη2	Lys97 Cα
		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ
				Lys97 Cγ				Lys97 Cγ
HAsp99	Cγ		Cγ		Cγ	Asp77 Nδ2	Cγ	Asp77 Nδ2
		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ
	Oδ1		Oδ1		Oδ1	Lys97 Cδ	Oδ1	Lys97 Cδ
						Lys97 Cε		Lys97 Cε
				Lys97 Nζ		*Lys97 Nζ*		*Lys97 Nζ*
	Oδ2	Asn77 Nδ2	Oδ2	Asp77 Nδ2	Oδ2	Asp77 Nδ2	Oδ2	Asp77 Nδ2
						Asn77 Cγ		
		Lys97 Nζ		*Lys97 Nζ*		*Lys97 Nζ*		*Lys97 Nζ*

Contacting atoms in HEL in the mutant Fv–HEL complexes are listed. The distances between each pair of atoms involved in van der Waals interactions and in hydrogen bonds are as follows; C–C; 4.1Å, C–N; 3.8Å, C–O; 3.7Å, O–O; 3.3Å, O–N; 3.4Å, N–N; 3.4Å. The atoms involved in hydrogen bonds and salt bridges are shown in red and in red italics, respectively. Calculations were performed with CONTACT in the CCP4 program suite.

[a] Data of 2DQJ (55)

Supplemental Table S5

The number of interactions between each mutant Fv and HEL in the complexes compared to those in the wild-type Fv and HEL complex

Complex		Total	Conserved	Gain	Loss
Wild type	(VH)	86			
	(VL)	46			
LN31D	(VH)	88 (+2)	84	4	2
	(VL)	46 (0)	44	2	2
LN32D	(VH)	104 (+18)	83	21	3
	(VL)	40 (-6)	38	2	8
LN92D	(VH)	101 (+15)	84	17	2
	(VL)	45 (-1)	41	4	5

The results presented in this table are a summary of results listed in Supplemental Table S4.

Supplemental Table S6 Thermodynamic parameters of mutant Fv–HEL interactions at 30 °C and pH 7.2 in phosphate buffer

Experimental protocols are described in the main text. Data represent the averages of at least three independent measurements. Errors of all values are within 5% for several experiments. The abbreviations used are as follows: n, stoichiometry; K_a, binding constant; ΔG, ΔH, ΔS, and ΔC_p, changes in Gibbs energy, binding enthalpy, entropy, and heat capacity, respectively.

Mutant	n	K_a [$\times 10^7$ M^{-1}]	ΔG [kJ mol^{-1}]	$\Delta\Delta G^a$	ΔH [kJ mol^{-1}]	$\Delta\Delta H^a$	$T\Delta S$ [kJ mol^{-1}]	$T\Delta\Delta S^a$	ΔS [kJ mol^{-1} K^{-1}]	$\Delta\Delta S^a$	$\Delta C_p{}^b$ [kJ mol^{-1} K^{-1}]	$\Delta\Delta C_p{}^a$
Wild type	1.05	82.1	-51.7	0	-99.7	0	-48.0	0	-0.158	0	−1.53	0
HS52A	0.95	130	-52.9	-0.3	-88.8	10.9	-35.9	12.1	-0.118	0.040	-0.98	0.55
HS54A	0.96	184	-53.7	-2.1	-95.1	4.6	-41.4	6.6	-0.137	0.021	-0.77	0.76
HS56A	0.88	131	-52.9	3.7	-86.5	13.2	-37.6	10.4	-0.124	0.034	-2.08	-0.55
LY50F[c]	1.05	11.2	-46.7	5.0	-74.8	24.9	-28.2	19.8	-0.093	0.065	-1.77	-0.24
LS91A[c]	1.04	36.3	-49.7	2.0	-88.7	11	-39.1	8.9	-0.129	0.029	-1.62	-0.09
LS93A[c]	1.07	74.1	-51.4	0.3	-91.2	8.5	-39.7	8.3	-0.131	0.027	-1.86	-0.33

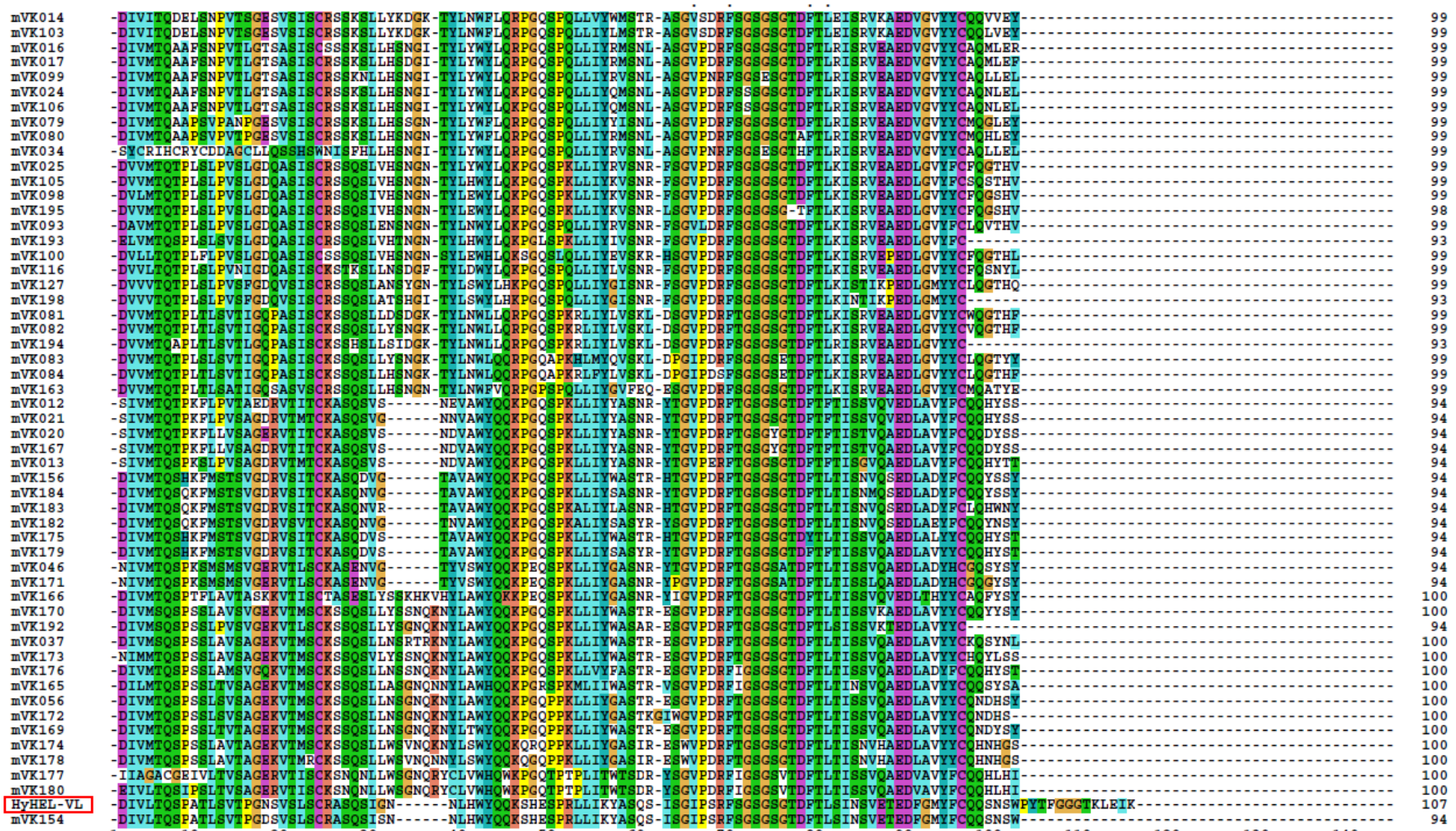
[a] $\Delta\Delta H$, $T\Delta\Delta S$, $\Delta\Delta S$ and $\Delta\Delta C_p$ are the differences in binding enthalpy, entropy and heat capacity between mutant and wild type.

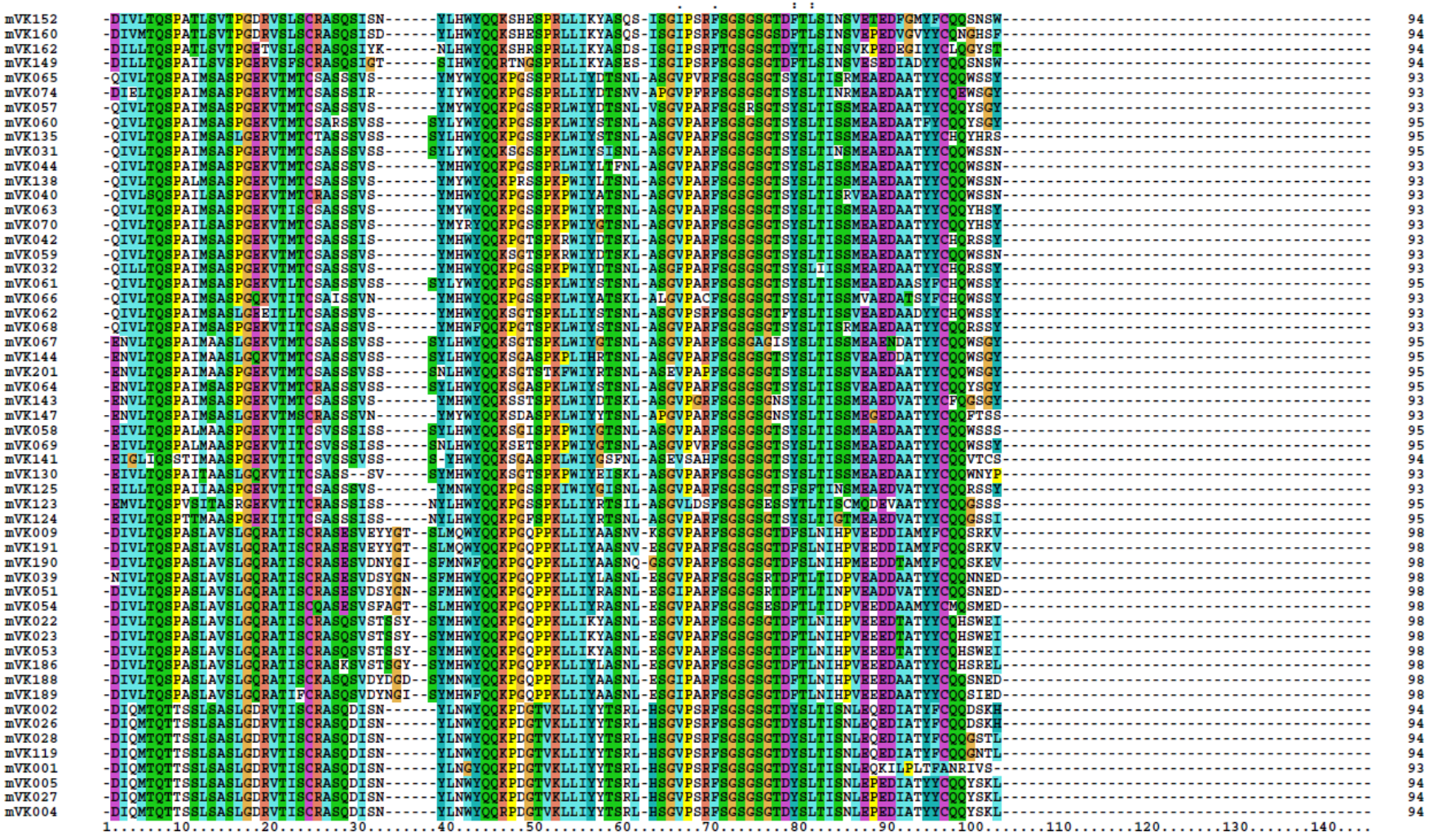
[b] The change in heat capacity were calculated by performing measurements at four temperatures, 25°C , 30°C, 35°C and 40 °C.

[c] Data are from a previous work, Yokota *et al*., 2003 (reference (48) in the text).

Supplemental Fig. S1

Multiple sequence alignment for germline variable genes from the immunoglobulin loci of mouse

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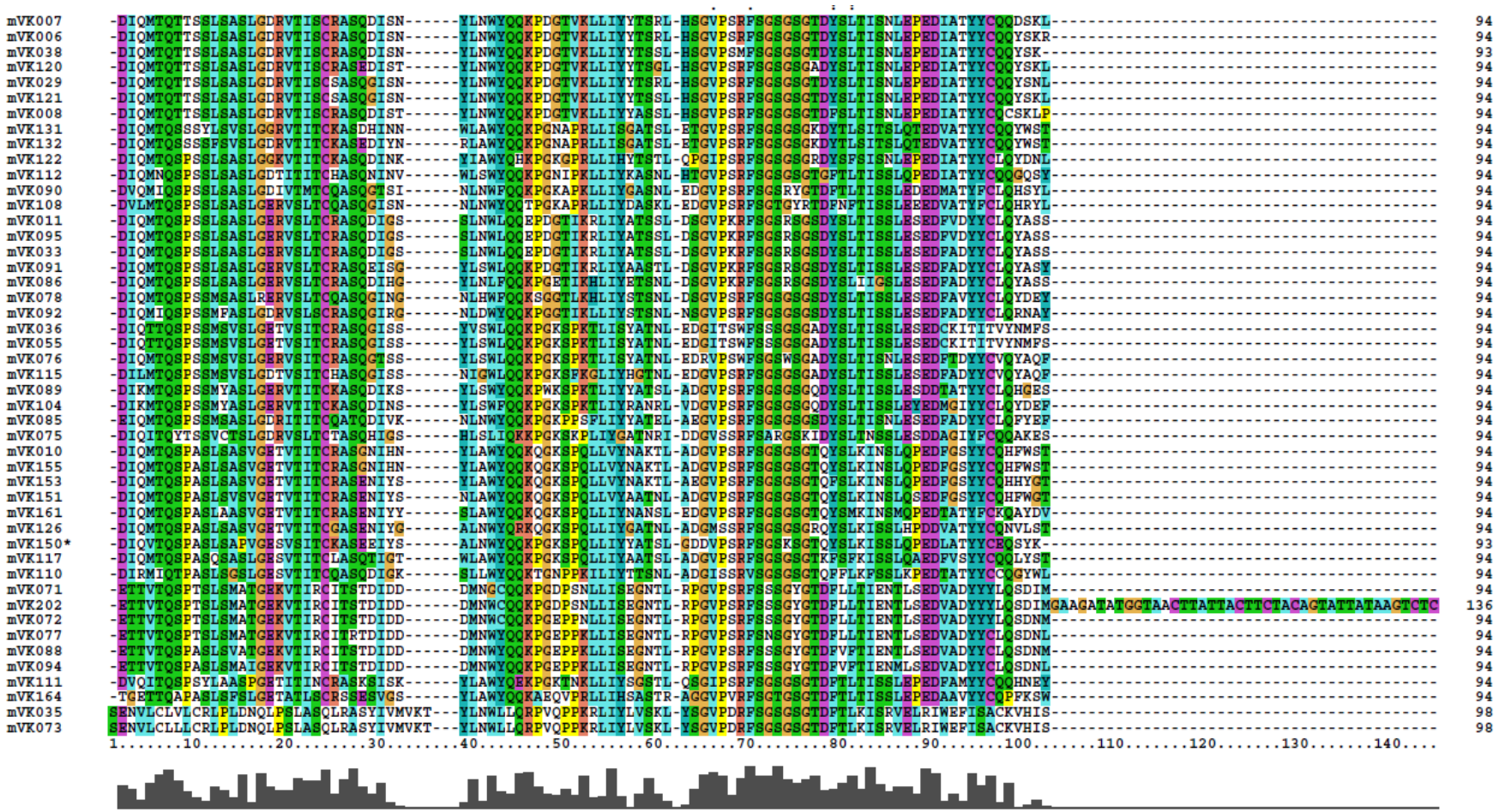
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The light chain of HyHEL-10 (enclosed in red square) aligned with other sequences of the immunoglobulin kappa variable group (IGVK) of mouse from an integrative database of germline variable genes, VBASE2 (http://www.vbase2.org/vbase2.php) (Retter, *et. al.* (2005)*) using ClustalX (2.0.11) (http://bips.u-strasbg.fr/fr/Documentation/ClustalX/) multiple sequence alignment tool. In the resultant alignment, sequence residues at conserved positions are colored automatically according to the physiochemical characteristics of the amino acids as follows: Gly, in *orange*; Cys, in *salmon*; Arg and Lys, in *red*; Asp and Glu, in *purple*; Ala, Ile, Lue, Met, Phe, Trp and Val, in *blue*; His and Tyr, in *sea green*; Gln, Ser and Thr, in *lime green*; Pro, in *green yellow*.

* Retter I, Althaus HH, Münch R, Müller W: VBASE2, an integrative V gene database. *Nucleic Acids Res*. 2005 Jan 1; 33 (Database issue):D671-4.

Supplemental Fig. S2

Temperature dependence of the enthalpy change of the interaction between HyHEL-10 Fv and HEL.

Experimental conditions are provided in the main text. Symbols used: *solid squares* (−■−), wild type; *open circles* (−○−), L-N31A; *solid circles* (−●−), L-N31D; *solid triangles* (−▲−), L-N32D; *solid crosses* (−×−), L-N92D.

There is no data of L-N32A because the measurement for the association between the LN32A and HEL was performed only at one temperature (30 °C) due to lack of its mutant sample.