Supplemental Table S1 Crystallographic data of mutant Fv–HEL complexes

	L-N31D	L-N32D	L-N92D	Wild-type ^a
Space group,	$P4_{1}2_{1}2$	$P4_{1}2_{1}2$	$P4_{1}2_{1}2$	P41212
Unit cell dimensions	<i>a</i> = <i>b</i> = 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
$R_{\rm merge}^{\ \ b}$	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
<i>R</i> -factor c (%)	18.9	19.4	18.6	19.4
Free <i>R</i> -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
$$R_{\text{merge}} = \frac{\sum_{hkl} |I - \langle I \rangle|}{\sum_{hkl} \langle I \rangle}$$

^c R -factor $= \frac{\sum ||F_{\text{obs}}| - |F_{\text{calc}}||}{\sum |F_{\text{obs}}|}$

Supplemental Table S2		
Hydrogen bonds via int	erfacial water molecules	(No.1)

WT^{a}			LN31D			LN32D			LN92D		
W2	YTyr20	Οη	W18	YTyr20	Οη	W242	YTyr20	Οη	W2	YTyr20	Οη
	LTyr50	Οη	(W2) ^b	LTyr50	Οη	(W2)	LTyr50	Οη	(W2)	LTyr50	Οη
	LSer91	Ογ		LSer91	Ογ		LSer91	Ογ		LSer91	Ογ
W5	LTyr50	Οη	W3	LTyr50	Οη	W10	LTyr50	Οη	W6	LTyr50	Οη
	YAsn93	0	(W5)	YAsn93	0	(W5)	YAsn93	0	(W5)	YAsn93	0
W8	LGIn53	Νε2				W4	LGIn53	Ne2	W1	LGIn53	Ne2
	YHis15	Νδ1				(W8)	YHis15	Νδ1	(W8)	YHis15	Νδ1
	YThr89	0					YThr89	0		YThr89	0
	YAsn93	Οδ1					YAsn93	Οδ1		YAsn93	Οδ1
W10	LSer91	0	W2	LSer91	0	W8	LSer91	0	W8	LSer91	0
	YTyr20	Οη	(W10)	YTyr20	Οη	(W10)	YTyr20	Οη	(W10)	YTyr20	Οη
	LTyr96	Οη		LTyr96	Οη		LTyr96	Οη		LTyr96	Οη
	YArg21	Nŋ1		YArg21	Nŋ1		YArg21	Nŋ1		YArg21	Nŋ1
	YSer100	Ογ		YSer100	Ογ		YSer100	Ογ		YSer100	Ογ
W13	LAsn31	Νδ2	W19	LAsp31	Ο δ2	W16	LAsn31	Νδ2	W14	LAsn31	Νδ2
	YAsn93	Οδ1	(W13)	YAsn93	Οδ1	(W13)	YAsn93	Οδ1	(W13)	YAsn93	Οδ1
	YLys96	Nζ		YLys96	Nζ		YLys96	Nζ		YLys96	Nζ
W53	LTrp94	Ν	W49	LTrp94	Ν						
	YArg21	Νε	(W53)	YArg21	Νε						
W81	HTyr58	Οη	W82	HTyr58	Οη	W109	HTyr58	Οη	W97	HTyr58	Οη
	YVal99	0	(W81)	YVal99	0	(W81)	YVal99	0	(W81)	YVal99	0
				YAsp101	0		YAsp101	0		YAsp101	0
W86	LSer93	Ογ	W45	LSer93	Ογ	W89			W29	LSer93	Ογ
			(W86)			(W86)	LSer92	0	(W86)		
	YAsn19	0		YAsn19	0						
	YArg21	Ν		YArg21	Ν		YArg21	Ν			
	YGly22	Ν		YGly22	Ν		YGly22	Ν		YGly22	Ν
W178	LSer28	Ογ									
	LGly30	Ν									
	YAsp18	0									
W200			W50			W34	LIle29	0	W95		
			(W200)			(W200)	LAsp32	Οδ2	(W200)	LAsn32	Νδ2
	LAsn92	Οδ1		LAsn92	Οδ1		LAsn92	Οδ1		LAsp92	Οδ1
	YGly16	0		YGly16	0		YGly16	0		YGly16	0
	YAsp18	0									
				YAsn19	Ν						
W255	HAsp99	Οδ2	W159	HAsp99	Οδ2	W353	HAsp99	Οδ2			
	YAsn77	Νδ2	(W255)	YAsn77	Νδ2	(W255)	YAsn77	Νδ2			
	HAsp101	Οδ2		HAsp101	Οδ2		HAsp101	Οδ2			
				HAsn97	OD1		HAsn97	OD1			

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

WT			LN31D			LN32D			LN92D		
W255	HAsp99	Об2	W159	HAsp99	Об2	W353	HAsp99	Οδ2			
	YAsn77	Νδ2	(W255)	YAsn77	Νδ2	(W255)	YAsn77	Νδ2			
	HAsp101	Об2		HAsp101	Οδ2		HAsp101	Οδ2			
				HAsn97	OD1		HAsn97	OD1			
W295	HSer31	0	W267	HSer31	0	W377	HSer31	0	W360	HSer31	0
	YAsn74	0	(W295)	YAsn74	0	(W295)	YAsn74	0	(W295)	YAsn74	0
	YAsn77	Οδ1		YAsn77	Οδ1		YAsn77	Οδ1		YAsn77	Οδ1
			W366	HAsp27	Οδ2	W138	HAsp27	Οδ2	W176	HAsp27	Οδ1
				YArg73	0		YArg73	0		YArg73	0
						W362	HThr30	0	W382	HThr30	0
							XX4 50			XX4 50	
							YArg73	Nηl		YArg73	Nηl
						W149	HAsp32	Οδ2	W57	LSer93	Ογ
							HSer31	0		LAsp92	Οδ2
							YAsn77	Οδ1		YAsn19	0
						W382	LAsn92	Νδ2	W296	LAsp92	Οδ1
							YAsn19	0		YAsp18	0

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

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)

Supplemental Table S3 Interfacial areas of the mutant Fv–HEL complexes (Å²).

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 Gly16 Ca Cα Cα LAsn31 His15 C His15 C Сү His15 C Сγ His15 C Сγ Сγ (Ala) His15 O His15 O His15 O Gly16 N (Asp) Gly16 N (でも 3.95) Gly16 Ca Gly16 Ca Gly16 Ca Gly16 Ca Lys96 Nζ Lys96 Nζ Lys96 Nζ Lys96 Nζ Οδ1 His15 C Οδ1 His15 C Oδ1 His15 C Οδ1 Gly16 Ca Gly16 Ca Gly16 Ca His15 O His15 O Gly16 Ca His15 C Lvs96 NC Lvs96 NZ Lys96 Nζ Lys96 Nζ Νδ2 His15 C Οδ2 His15 C N₈₂ His15 C N₈₂ His15 C His15 O His15 O His15 O LAsn32 Cγ Tyr20 Cδ1 Сγ Tyr20 Cδ1 Tyr20 Cδ1 Tyr20 Cδ1 Сγ Сγ (Ala) Tyr20 Ce1 Tyr20 Ce1 Tyr20 Ce1 Tyr20 Cɛ1 (Asp) Lys96 Nζ Lys96 Nζ Lys96 Nζ Lys96 Nζ Tyr20 Cδ1 Tyr20 Cδ1 Οδ1 Οδ1 Οδ1 Οδ1 Tyr20 Cδ1 Tyr20 Cɛ1 Tyr20 Cɛ1 Tyr20 Ce1 Tyr20 Cɛ1 Lys96 Cð Lys96 Co Lys96 Cδ Lys96 Cδ Lys96 Ce Lys96 Ce Lys96 Ce Lys96 Ce Lys96 Nζ Lys96 Nζ Lys96 Nζ Lys96 Nζ Νδ2 Gly16 O Νδ2 Gly16 O Οδ2 Tyr20 Cδ1 Nδ2 Gly16 O Tyr20 Cδ1 Tyr20 Cδ1 Tyr20 Cɛ1 Tyr20 Cδ1 LTyr50 Cδ1 Cδ1 Cδ1 Cδ1 Lys96 Nζ Lys96 Nζ Lys96 Nζ Lys96 Nζ Lys96 Co Ce1 Lys96 Nζ Ce1 Lys96 Nζ Ce1 Lys96 Nζ Cɛ1 Lys96 Nζ Lys96 Cð Lys96 Cð Lys96 Cδ Lys96 Cδ Сδ2 Asn93 Cy Cδ2 Asn93 Cy Сδ2 Asn93 Cy Cδ2 Asn93 Cy Asn93 Oõ1 Asn93 Oõ1 Asn93 Oõ1 Asn93 O₀1 Ce2 Asn93 Cy Asn93 Cy Ce2 Ce2 Asn93 Cy Ce2 Asn93 Cy 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 Οη Οη Lys96 Cδ LGln53 Сδ Thr89 Cy2 Сδ Thr89 Cy2 Thr89 Cy2 Сδ Thr89 Cy2 Сδ Asn93 Nδ2 Asn93 Nδ2 Asn93 Nδ2 Asn93 Nδ2 Οε1 Thr89 Cy2 Thr89 Cy2 Thr89 Cy2 Thr89 Cy2 0ε1 Oε1 0ε1 Asn93 Cy Gly16 Ca Asn93 Cy Asn93 Cy Asn93 Nδ2 Asn93 Nδ2 Asn93 N₈₂ Asn93 Nδ2 Thr89 Cy2 Thr89 Cy2 Thr89 Cy2 Thr89 Cy2 Ne2 Ne2 Ne2 Ne2 Asn93 Oδ1 Asn93 Oõ1 Asn93 Oδ1 Asn93 Oδ1

VL (No.2)

		WT		LN31D		LN32D		LN92D
LSer91	0	Tyr20 Cε1	0	Tyr20 Cε1	0		0	Tyr20 Cɛ1
LAsn92	Νδ2	Asn19 O	Νδ2	Asn19 O	Νδ2			
(Ala)	С	Arg21 Cδ	С	Arg21 Cδ	С		С	
(Asp)	0	Arg21 N						
		Arg21 Ca		Arg21 Ca				Arg21 Ca
		Arg21 Cδ		Arg21 Cδ		Arg21 Cδ		Arg21 Cδ
					Οδ1	Asn19 O	Οδ2	
LSer93	Cα	Arg21 Cδ	Сα	Arg21 Cδ	Сα		Сα	
LTrp94	Сβ	Arg21 Cζ						
								Arg21 Nŋ2
LTyr96	Cɛ1	Arg21 Nŋ1	Ce1	Arg21 Nη1	Ce1	but 3.85	Ce1	Arg21 Nŋ1
			Сζ	Arg21 Nη1			Сζ	Arg21 Nŋ1
	Οη	Arg21 Nŋ1	Οη	Arg21 Nη1	Οη	Arg21 Nη1	Οη	Arg21 Nŋ1

VH (No.1)

		WT		LN31D		LN32D		LN92D
HThr30	0	Arg73 Cδ	0	Arg73 Cδ	0	Arg73 Cδ	0	Leu75 C _{δ2}
						Leu75 C _{δ2}		
HSer31	Сα	Leu75 Co2	Сα	Leu75 Cô2	Cα	Leu75 C ₈₂	Сα	Leu75 C ₈₂
						Arg73 O		Arg73 O
	Сβ	Arg73 O	Сβ	Arg73 O	Сβ	Arg73 O	Сβ	Arg73 O
					Ογ	Arg73 O	Ογ	Arg73 O
	С	Leu75 Co2	С	Leu75 Cô2	С	Leu75 Co2	С	Leu75 C ₈₂
	0	Leu75 C ₈₂	0	Leu75 Co2	0	Leu75 C _{δ2}	0	Leu75 C ₈₂
						Leu75 Ca		
HAsp32	Ν	Leu75 C ₈₂	Ν	Leu75 Cô2	Ν	Leu75 C ₈₂	Ν	Leu75 C ₈₂
	Сα	Leu75 Co2	Сα	Leu75 Co2	Сα	Leu75 Cô2	Сα	Leu75 Co2
	Сү	Lys97 Nζ	Сү	Lys97 Nζ	Сү	Lys97 Nζ	Сү	Lys97 Nζ
						Asn77 Nδ2		
	Οδ1	Lys97 Ce	Οδ1	Lys97 Ce	Οδ1	Lys97 Ce	Οδ1	Lys97 Cε
		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ
					Οδ2	Asn77 Nδ2	Οδ2	Asn77 Nδ2
	С	Leu75 Cõ2						
HTyr33	Сү	Lys97 Ce	Сү	Lys97 Ce	Сү	Lys97 Ce	Сү	Lys97 Ce
	Cδ1	Lys97 Ce	Cδ1	Lys97 Cε			Cδ1	Lys97 Ce
			Сδ2	Lys97 Cy	Сδ2	Lys97 Cy		
	Ce2	Lys97 Cy	Ce2	Lys97 Cy	Ce2	Lys97 Cy	Ce2	Lys97 Cγ
		Lys97 O		Lys97 O		Lys97 O		Lys97 O
						Lys97 C		Lys97 C
	Cζ	Lys97 Cy	Cζ	Lys97 Cy	Cζ	Lys97 Cy	Сζ	Lys97 Cγ
		Lys97 O		Lys97 O		Lys97 O		Lys97 O
								Asp101 Cβ
	Οη	Trp63 Cζ3	Οη	Trp63 Cζ3	Οη	Trp63 Cζ3	Οη	Trp63 Cζ3
		Тгр63 Сղ2		Trp63 Cŋ2		Trp63 Cŋ2		Trp63 Cŋ2
		Lys97 C		Lys97 C		Lys97 C		Lys97 C
		Lys97 O		Lys97 O		Lys97 O		Lys97 O
		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ
HTyr50	Ce2	Ser100 O	Ce2	Ser100 O	Ce2	Ser100 O	Ce2	Ser100 O
	Cζ	Ser100 O	Сζ	Ser100 O	Сζ	Ser100 O	Сζ	Ser100 O
	Οη	Arg21 Cz	Οη	Arg21 Cζ	Οη	Arg21 Cζ	Οη	Arg21 Cζ
		Arg21 Nη1		Arg21 Nη1		Arg21 Nη1		Arg21 Nη1
		Arg21 Nη2		Arg21 Nη2		Arg21 Nη2		Arg21 Nη2
		Ser100 CB		Ser100 CB		Ser100 CB		Ser100 CB
		Ser100 C		Ser100 C		Ser100 C		Ser100 C
		Ser100 O		Ser100 O		Ser100 O		Ser100 O

VH (No.2)

		WT		LN31D		LN32D		LN92D
HSer52	Сβ	Asp101 Ca	Сβ	Asp101 Ca	Сβ	Asp101 Ca	Сβ	Asp101 Ca
						Asp101 Cβ		Asp101 Cβ
				Asp101 Cy		Asp101 Cy		Asp101 Cy
		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1
	Ογ	Asp101 Ca	Ογ	Asp101 Ca	Ογ	Asp101 Ca	Ογ	Asp101 Ca
						Asp101 Cβ		
		Asp101 Cy		Asp101 Cy		Asp101 Cy		Asp101 Cy
		Asp101 Οδ1		Asp101 Cδ1		Asp101 Cδ1		Asp101 Cδ1
HTyr53	Сβ	Leu75 Cõl	Сβ	Leu75 Cõ1	Сβ	Leu75 Cõ1	Сβ	Leu75 Cõ1
		Leu75 C ₈₂		Leu75 Co2		Leu75 Co2		Leu75 C ₈₂
	Сү	Leu75 Cõ1	Сү	Leu75 Cõ1	Сү	Leu75 Cõ1	Сү	Leu75 Cõ1
	Cδ1	Trp63 Cy2	Cδ1	Trp63 Cy2	Cδ1	Trp63 Cy2	Cδ1	Trp63 C ₁ 2
						Leu75 Cõ1		Leu75 Cõ1
		Asp101 CB		Asp101 Cβ		Asp101 Cβ		Asp101 CB
		Asp101 Cy		Asp101 Cy		Asp101 Cy		Asp101 Cy
		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1
	Cɛ1	Trp63 Cζ2	Cɛ1	Trp63 Cζ2	Cɛ1	Trp63 Cζ2	Cɛ1	Trp63 Cζ2
		Trp63 Cŋ2		Trp63 Cŋ2		Trp63 Cy2		Тгр63 Сη2
		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ		Asp101 Cβ
		Asp101 Cy		Asp101 Cy		Asp101 Cy		Asp101 Cy
		Asp101 Cδ1		Asp101 Cõ1		Asp101 Cõ1		Asp101 Οδ1
		Asp101 Οδ2		Asp101 Οδ2		Asp101 Οδ2		Asp101 Οδ2
	Сδ2	Trp62 Ce3	Сδ2	Trp62 Ce3	Сδ2	Trp62 Ce3	Сδ2	Trp62 Ce3
		Trp62 Cζ3		Trp63 Cζ3		Trp62 Cζ3		Trp62 Cζ3
		Leu75 Cõ1		Leu75 Cõ1		Leu75 Cõ1		Leu75 Cõ1
	Ce2	Trp62 Cζ3	Ce2	Trp62 Ce3	Cε2	Trp62 Ce3	Ce2	Trp62 Ce3
		Trp62 Cŋ3		Trp62 Cζ3		Trp62 Cζ3		Trp62 Cζ3
			Сζ	Trp63 Cζ2				
					Οη	Asp103 Nδ2	Οη	Asp103 Nδ2
HSer54	Ν	Asp101 Οδ1	Ν	Asp101 Οδ1	Ν	Asp101 Οδ1	Ν	Asp101 Οδ1
	Сα	Asp101 Οδ1	Сα		Сα	Asp101 Οδ1	Сα	Asp101 Οδ1
	Сβ	Asp101 Cy	Cβ	Asp101 Cy	Cβ	Asp101 Cy	Сβ	Asp101 Cy
		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1
		Asp101 Oδ2		Asp101 Οδ2		Asp101 Οδ2		Asp101 Οδ2
		Asn103 Nδ2		Asn103 Nδ2		Asn103 Nδ2		Asn103 Nδ2
	Ογ	Asp101 Cy	Ογ	Asp101 Cy	Ογ	Asp101 Cy	Ογ	Asp101 Cy
		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1
		Asp101 Οδ2		Asp101 Οδ2		Asp101 Οδ2		Asp101 Οδ2

VH (No.3)

		WT		LN31D		LN32D		LN92D
HSer56	Сβ	Gly102 N	Сβ	Gly102 N	Сβ	Gly102 N	Сβ	Gly102 N
		Gly102 Ca		Gly102 Ca		Gly102 Ca		Gly102 Ca
	Ογ	Gly102 N	Ογ	Gly102 N	Ογ	Gly102 N	Ογ	Gly102 N
		Gly102 Ca		Gly102 Ca		Gly102 Ca		Gly102 Ca
		Asp101 Οδ1		Asp101 Οδ1		Asp101 Οδ1		Asp101 Cδ1
HTyr58	Сү	Arg21 Nη2	Сү	Arg21 Nŋ2	Сү		Сү	
	Cδ1	Arg21 Nη2	Cδ1	Arg21 Nη2	Cδ1	Arg21 Nη2	Cδ1	Arg21 N ₁ 2
	Ce1	Arg21 Nη2	Cɛ1	Arg21 Nŋ2	Cel	Arg21 Ny2	Cɛ1	Arg21 Nŋ2
	Ce2	Ser100 O	Ce2	Ser100 O	Ce2	Ser100 O	Ce2	Ser100 O
	Cζ	Arg21 Nη2	Сζ	Arg21 Nŋ2	Сζ	Arg21 Ny2	Сζ	Arg21 N ₁ 2
						Ser100 O		Ser100 O
	Οη		Οη		Οη	Asp101 C	Οη	Asp101 C
						Gly102 N		Gly102 N
		Gly102 Ca		Gly102 Ca		Gly102 Ca		Gly102 Ca
HTrp98	Ce3	Lys97 Cγ	Ce3	Lys97 Cγ	Ce3	Lys97 Cγ	Ce3	Lys97 Cγ
		Lys97 Cδ		Lys97 Cδ		Lys97 Cð		Lys97 Cδ
		Lys97 Cε		Lys97 Cε		Lys97 Cε		Lys97 Ce
	Сζ2	Ser100 CB	Сζ2	Ser100 CB	Сζ2	Ser100 CB	Сζ2	Ser100 CB
		Ser100 Oy		Ser100 Oy		Ser100 Oy		Ser100 Oy
	Сζ3	Lys97 Ca	Сζ3	Lys97 Ca	Сζ3	Lys97 Ca	Сζ3	Lys97 Ca
		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ
		Lys97 Cγ		Lys97 Cy		Lys97 Cy		Lys97 Cγ
		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ		Lys97 Cδ
	Cη2	Lys97 Ca	Cη2	Lys97 Ca	Cη2	Lys97 Ca	Cη2	Lys97 Ca
		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ		Lys97 Cβ
				Lys97 Cγ				Lys97 Cγ
HAsp99	Сү		Сү		Сү	Asp77 Nδ2	Сү	Asp77 Nδ2
		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ		Lys97 Nζ
	Οδ1		Οδ1		Οδ1	Lys97 Cð	Οδ1	Lys97 Cδ
						Lys97 Cɛ		Lys97 Ce
				Lys97 Νζ		Lys97 Nζ		Lys97 Nζ
	Οδ2	Asn77 Nδ2	Οδ2	Asp77 Nδ2	Οδ2	Asp77 Nδ2	Οδ2	Asp77 Νδ2
						Asn77 Cy		
		Lys97 N ζ		Lys97 Νζ		Lys97 Nζ		Lys97 Νζ

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.

Mastant		K_{a}	ΔG	$\Delta\Delta G^{a}$	ΔH	$\Delta\Delta H^{a}$	$T\Delta S$	$T\Delta\Delta S^{a}$	ΔS	$\Delta\Delta S^{a}$	$\Delta C_{ m p}^{\ m b}$	$\Delta\Delta C_{ m p}{}^{ m a}$
Mutant	n	$[\times 10^7 \text{ M}^{-1}]$	[kJ n	nol ⁻¹]	[kJ n	nol ⁻¹]	[kJ n	nol ⁻¹]	[kJ mo	$I^{-1} K^{-1}$]	[kJ mo	$l^{-1} K^{-1}$]
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

mVK014	-DIVITODELSNPVTSCESVSISCRSSKSLLYKDCK	<pre>K-TYLNWFLQRPGQSPQLLVYWMSTR-ASGVSDRFSGSGSGTDFTLEISRVKAEDVGVYYCQQVVEY</pre>	- 99
mVK103	-DIVITODELSNPVTSCESVSISCRSSKSLLYKDCK	(-TYLNWFLQRPGQSPQLLIYIMSTR-ASGVSDRFSGSGSGTDFTLEISRVKAEDVGVYYCQQLVEY	- 99
mVK016	-DIVMTQAAFSNPVTLGTSASISCSSSKSLLHSNGI	I- TYLYWYLQRPGQ<mark>SPQ</mark>LLIYRMSNL-ASGVPDRFSGSGSGTDFTL RISR VEAED VGVYYCAQMLER	- 99
mVK017	-DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSDGI	I-TYLYWYLQRPGQSPQLLIYRMSNL-ASGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCAQMLEF	- 99
mVK099	-DIVMTQAAFSNPVTLGTSASISCRSSKNLLHSNGI	I-TYLYWYLQRPGQSPQLLIYRVSNL-ASGVPNRFSGSESGTDFTLRISRVEAEDVGVYYCAQLLEL	- 99
mVK024	-DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGI	I-TYLYWYLQKPGQSPQLLIYQMSNL-ASGVPDRFSSSGCGCTDFTLRISRVEAEDVGVYYCAQNLEL	- 99
mVK106	-DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGI	I-TYLYWYLQKPGQSPQLLIYQMSNL-ASGVPDRFSSSGCGCTDFTLRISRVEAEDVGVYYCAQNLEL	- 99
mVK079	-DIVMTQAAPSVPANPGESVSISCRSSKSLLHSSGN	1-TYLYWFLQRPGQSPQLLIYYISNL-ASCVPDRFSGSCSCTDFTLRISRVEAEDVCVYYCMQCLEY	- 99
mVK080	-DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGN	N-TYLYWFLQRFGQSPQLLIYRMSNL-ASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEY	- 99
mVK034	-SYCRIHCRYCDDAGCLLQSSHSWNISFHLLHSNGI	I-TYLYWYLQRPGQSPQLLIYRVSNL-ASGVPNPPSGSESCTHPTLRISRVEAEDVGVYYCAQLLEL	- 99
mVK025	- DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGN	-TYLYWYLQKPGQSPKLLIYRVSNR-PSGVPDRFSGSGSGTDFTLKISRVEARDLGVYFCFQGTHV	- 99
mVK105	-DVVMTQTPLSLPVSLCDQASISCRSSQSLVHSNCN	-TYLHWYLOKPGQSPRILIYKVSNR-PSGVPDRPSGSGSGTDFTLKISRVEARDLGVYFCSQSTHV	- 99
mVK098	-DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGN	-TYLEWILGKPGOSPKLLIYKVSNR-FSGVPDRFSGSGSGTDFTLKISKVEAEDLGVYYCFOGSEV	- 99
mVK195	-DVVMTQTPLSLPVSLGDQASISCRSSQSIVHSNGN	-TYLEWIDGKPGOSPKLLIYKVSNR-LSGVPDRFSGSGSG-TFTLKISKVEAEDLGVYYCFOGSEV	- 98
mVK093	-DAVMTOTPLSLPVSLGDQASISCRSSQSLENSNGN	- TILNWILGRPGOSPOLLITRVSNR - FSGVLDRFSGSGSGTDFILKISRVEAEDLGVIFCLVTHV	- 99
mVK193	- HLVMTQSPLSLSVSLGDQASISCRSSQSLVHINGN	- TILHWILDKPGLSPKLLITIVSNR - FSGVPDRFSGSGSGTDFTLKISKVEAEDLGVIFC	- 93
mVK100			- 99
mVK110	DUNUTOTIL EL DUERCIOUETECH COOL ANEVON		- 99
mVK127	DUVUTOTIL OL DUPPODOUCTOOD COOL ATOUCT		- 99
mVK198			- 93
mVK081	-DVVMTOTPLTLSVTTCOPASTSCKSSQSLLDSDGK	- TILNMILLYRG OFFALLILIYDAU - DOGYD AF IGOGGIDFILLRY FALLGY'L IWGLAF	- 99
mVK194	-DVVMTOADLTLSVTLCODASTSCKSSUSLLSTDCK		- 03
mVK093	-DVVMTOTDLSLSVTTCODASTSCKSSOSLLVSNCK		_ 00
mVK094	DVVMTOTDLTLSVTTCODASTSCKSSOSTLUSNCK		_ 00
mVK163	-DVVMTOTPLTLSATICOSASVSCPSSOSLLHSNCN		- 99
mVK012	-SIVMTOTPKELPVTARDRVTITCKASOSVS	- NEVAWYOOK PGOSPELLIVYASNE - YTGY PDEFTGSGSGTDFFFTTSSVOVEDLAVYFCOOHYSS	- 94
mVK021	-SIVMTOTPKELPVSAGDRVTMTCKASOSVG		- 94
mVK020	-SIVMTOTPKFLLVSAGERVTITCKASOSVS	- NDVAWYOOKPGOSPKILIYYASNE-YTGYPDEFTGSGYCTDFTFTISTYOARDLAVYFCOODYSS	- 94
mVK167	-SIVMTOTPKFLLVSAGDRVTITCKASOSVS	- NDVAWYOOKPGOSPKILIYYASNE-YTGYPDEFTGSGYCTDFTFTISTYOARDLAVYFCOODYSS	- 94
mVK013	-SIVMTOSPKSLPVSAGDRVTMTCKASOSVS	NDVAWYOOKPGOSPKLLIYYASNR-YTGVPERFTGSGSGTDFTFTISGVOABDLAVYFCOOHYTT	- 94
mVK156	-DIVMTOSHKFMSTSVGDRVSITCKASODVG	-TAVAWYQCKPGOSPKLLIYWASTR-HTGVPDRFTGSGSGTDFTLTISNVOSEDLADYFCQQYSSY	- 94
mVK184	-DIVMTOSOKFMSTSVGDRVSITCKASONVG	-TAVAWYQQKPGQSPKLLIYSASNR-YTGVPDRFTGSGSGTDFTLTISNMQSEDLADYFCQQYSSY	- 94
mVK183	-DIVMTQSQKFMSTSVGDRVSITCKASQNVR	-TAVAWYOCKPGOSPKALIYLASNR-HTGVPDRFTGSCSCTDFTLTISNVOSEDLADYFCLOHWNY	- 94
mVK182	-DIVMTQSQKFMSTSVGDRVSVTCKASQNVG	TNVAWYQQKPGQSPKALIYSASYR-YSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSY	- 94
mVK175	-DIVMTQSHKFMSTSVGDRVSITCKASQDVS	TAVAWYQQKPGQ <mark>8PKLLIYWASTR-HTGVPDRFTGSGSGTDYTLTISSVQAEDLALYYCQQ</mark> HYST	- 94
mVK179	-DIVMTQSHKFMSTSVGDRVSITCKASQDVS	TAVAWYQQKPGQ <mark>8PKLLIY</mark> SASYR-YTGVPDRFTGSCSGTDFTFTISSVQAEDLAVYYCQQHYST	- 94
mVK046	-NIVMTQSPKSMSMSVGERVTLSCKASENVG	- TYVSWYQQKP EQ SPKLLIYGAS NR- YTGVPDRFTGSGSATDFTLTISSVQAEDLA D YHCGQ SYS Y	- 94
mVK171	-NIVMTQSPKSMSMSVGERVTLSCKASENVG	TYVSWYQQKPEQSPKLLIYGASNR-YPGVPDRFTGSGSATDFTLTISSLQAEDLADYHCGQGYSY	- 94
mVK166	-DIVMTQSPTFLAVTASKKVTISCTASESLYSSKHK	VHYLAWYQKKPEQ <mark>SPELLIYGAS</mark> NR-YIGVPDEFTGSCSCTDFTLTISSVQVEDLTHYYCAQFYSY	- 100
mVK170	-DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQ	XNYLAWYQQKPGQSPELLIYWASTR-ESGVPDFFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYSY	- 100
mVK192	-DIVMSQSPSSLPVSVGEKVTLSCKSSQSLLYSGNQ	XNYLAWYQQXPGQSPKLLIYWASAR-ESGVPDRFTGSGSGTDFTLSISSVKTEDLAVYYC-	- 94
mVK037	-DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTR	KNYLAWYQQKPGQSPELLIYWASTR-ESGVPDFFTGSCSCTDFTLTISSVQAEDLAVYYCKQSYNL	- 100
mVK173	-NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQ	KNYLAWYOOKPGQSPELLIYWASTE-ESGVPDFFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSS	- 100
mVK176	-DIVMTQSPSSLAMSVCQKVTMSCKSSQSLLNSSNQ	IKNYLAWYQQKPGQSPKILVYFASTR-ESGVPDRFIGSGSGTDFTLTISSVQARDLADYFCQQHYST	- 100
mVK165	-DILMTQSPSSLTVSAGEKVTMSCKSSQSLLASGNQ	NNYLAWHQQKPGRSPKMLIIWASTR VSGVPDRFIGSCSGTDFTLTINSVQAEDLAVYYCQQSYSA	- 100
mVK056	-DIVMTQSPSSLSVSAGEKVTMSCKSSQSLLNSGNQ	KNYLAWYQQKPGQPPKLLIYGASTR-ESGVPDRFTGSGSGTDFTLFISSVQAEDLAVYYCUNDES	- 100
mVK172	-DIVMTOSPSSLSVSAGEKVTMSCKSSOSLLNSGNQ	KNYLAWYOKPGOPPKLLIYGABTKGIWGVPDRFTGSGSGTDFTLISSVOARBDLAVYYCONDHS-	- 100
mVK109	DTUNTORDORD OF AUTA OF UTAGEN VINSON OF CONTACT OF AUTA		- 100
mVE179			- 100
mVE177	TINGS COPTULTURS COPUTIECT SNOW INCOME	INTI JOHTAVAVAV FRANLITSARIA ZANVENKI UGOSUFITI LI JAVAABUAVI LUANNSO	- 100
mVII 100			- 100
HVHRL-VI	-DIVLTOSPATISVTPCNSVSLSCRASOSTCN	NI, HWOOK SHEAPDLI, TY ASOS - IS CTOSPECE COST FTLATING WERT RDF CMY PCOG SNSWPY FCGCTKLETK	- 107
mVK154	-DIVLTOSPATLSVTPGDSVSLSCRASOSISN		- 94
	1	40	

mVK152	-DIVLTQSPATLSVTPGDRVSLSCRASQSISN	YLHWYQQKSHE <mark>SPRLLIKYAS</mark> QS-I <mark>SGIPSRFSGSGSGTDFTLSINSVETED</mark> FCMYFCQQSNSW	94
mVK160	-DIVMTQSPATLSVTPGDRVSLSCRASQSISD	YLHWYQQKSHE <mark>SPR</mark> LLIKYASQS-ISGIPSRFSGSGSGSDFTLSINSVEPEDVGVYYCQNGHSF	94
mVK162	-DILLTQSPATLSVTPGETVSLSCRASQSIYK	NLHWYQQKSHR <mark>SPR</mark> LLIKYASDS-ISGIPSRFTGSCSCTDYTLSINSVKPEDECIYYCLQCYST	94
mVK149	-DILLTQSPAILSVSPGERVSFSCRASQSIGT	SIHWYQQRTNGSPRLLIKYASES-I <mark>SGIPSRFSGSGTGTDFTLS</mark> INSVESEDIADYYCQQSNSW	9
mVK065	-QIVLTQSPAIMSASPGEKVTMTCSASSVS	YMYWYQQKPGS <mark>SPRLLIY</mark> DTSNL-A <mark>SCVPVRFSCSCSCT</mark> SYSLTISRMBAEDAATYYCQQWSSY	9:
mVK074	-DIELTQSPAIMSASPGERVTMTCSASSSIR	YIYWYQQKPGS <mark>SPRLLIYDTSNV-APGVPFRFSGSGSGT</mark> SYSLTINRMEAEDAATYYCQEwsCY	9:
mVK057	-QIVLTQSPAIMSASPGEKVTMTCSASSVS	YMYWYQQKPGS <mark>SPRLWIY</mark> DTSNL-V <mark>SCVPARFSCSRSCTSYSLTISSMEAED</mark> AATYYCQQYS <mark>CY</mark>	9:
mVK060	-QIVLTQSPAIMSASPGEKVTMTCSARSSVSS	YLYWYQQKPGS <mark>8PK</mark> LWIYSTSNL-ASGVPARFSGSGSGTSYSLTISSMEAEDAATFYCQQYS <mark>GY</mark>	9
mVK135	-QIVLTQSPAIMSASLGERVTMTCTASSSVSS	YLHWYQQKPGS <mark>SPKLWIYSTSNL-ASGVPARPSGSCSGTSYSLTISSMEAED</mark> AATYYCH <u>Q</u> YHRS	9
mVK031	-QIVLTQSPAIMSASPGERVTMTCSASSSVSS	YLYWYQQKSGS <mark>SPKLWIYSIENL-ASGVPARPSGSGSGTSYSLTINSMEAED</mark> AATYYCQQWSSN	9
mVK044	-QIVLTQSPAIMSASPGEKVTMTCSASSVS	YMHWYQQKPGS <mark>SPRLWIYLTFNL-ASGVPARFSGSGSGTSYSLSISSMEAEDAATYYCQQ</mark> WSSN	9:
mVK138	-QIVLTQSPALMSASPGEKVTMTCSASSSVS	YMYWYQQKPRSSPKPWIYLTSNL-ASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSN	9:
mVK040	-QIVLSQSPAILSASPGEKVTMTCRASSSVS	YMHWYQQKPGSSPKPMIYATSNL-ASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSSN	9
mVK063	-QIVLTQSPAIMSASPGEKVTISCSASSSVS	YMYWYQQKPGSSPRPWIYRTSNL-ASGVPARPSGSGSCTSYSLTISSMEAEDAATYYCQQYHSY	9
mVK070	-QIVLTQSPAILSASPGEKVTMTCSASSSVS	YMYRYQQKPGSSPKPMIYGTSNL-ASGVPARFSGSGSGTSYSLTISSMEARDAATYYCQQYESY	9
mVK042	-QIVLTQSPAIMSASPGEKVTMTCSASSSIS	YMHWYQQKPGTSPRRWIYDTSKL-ASGVPARPSGSGSCTSYSLTISSMEAEDAATYYCHQRSSY	9
mVK059	-QIVLTQSPAIMSASPGEKVTMTCSASSSVS	YMHWYQQKSGTSPKRMIYDTSKL-ASGVPARFSGSGSGTSYSLTISSMEARDAATYYCOOWSSN-	9
mVK032	-QILLTQSPAIMSASPGEKVTMTCSASSSVS	YMHWYQQKPGSBFRPWIYDTSNL-ASGPPARFSGSGSGTSYSLIISSMEAEDAATYYCHORSSY	9
mVK061	-QIVLTQSPAIMSASPGEKVTLTCSASSSVSS	YLYWYQQKPGSSPKLMIYSTSNL-ABGVPARPSGSGSGTSYSLTISSMEARDAASYPCHOWSSY	9
mVK066	-QIVLTQSPAIMSASPGQKVTITCSAISSVN	YMHWYQQKPGSSPKLMIYATSKL-ALGVPACFSGSGSGTSYSLTISSMVARDATSYPCHOWSSY	9
mVK062	-QIVLTQSPAIMSASLGEEITLTCSASSSVS	YMHWYQQKSGTSPKLLIYSTSNL-ASGVPSRFSGSGSGTFYSLTISSVEARDAADYYCHOWSSY	9
mVK068	-QIVLTQSPAIMSASPGEKVTITCSASSSVS	IMHNYOUKPGTBYRLWIISTENL ASGVPARPSGSGSGTSIISLIISTENLASGVPARPSGSGGGTSIISLIISTENLASGVPARPSGSGGGTSI	9.
mVK067	-ENVLTOSPAIMAASLGEKVIMICSASSSVSS	YLHWYQCKSCTBFRLWIYGTSNL-ASGVPARPSGSGAGISYSLTISSMEAENDATYYCQUWSGY	9
mVK144	- KNVLTUSPAIMAASLGUKVTMTUSASSSVSS		9:
mVK201	- ENVLTOSPAIMAASPGEKVIMICSASSSVSS	NLHWIQQKSGISIKKWIIKTSNL-ASEVPAPPSGGGGGISISLTISSVEAEDAATYICQWSGI	9
mVK064	- ENVLTOSPAIMSASPGEKVTMTCRASSSVSS	ILHWIQQKSGASPKLMIYSTSNL-ASGVPARPSGSGSGTSISLTISSVEAEDAATYYCQTSGY	9
mVK143	-KNVLTQSPAIMSASPGEKVTMTCSASSSVS		9.
mVK147	- KNVLTUSPAIMSASLGEKVTMSCRASSSVN		9.
mVK058	-KIVLIQSPALMAASPGERVIIICSVSSSISS		9:
mVK069	PTOL TOPOTTMA ASPGER VIIICSVSSSISS		9
mVK141			
mVK130	PTLLTOCDATIA COOPYTTE CACCEUS		0
mWK123	DWULTOGDUGTTA CDODUUTTCDA CCTCC		0
mWK123	DTUL TOODTTWAN ODODVTTTTOCA COTCO		9:
mVK124	TULTOGDAGLAVGLOODATTGCDAGDGUDVVCT-		0
mVI 101	DIVITOGDAGI AVGLOODATIGCDAGDGUDVOT		0
mVX191	DIVIJOSPASLAVSLOOPATISCASESVDNVCT		0
mVEO20	NTVLTOGDAGLAVELOODATTGCDAGDGVDGVON-		6
mVK051	-DIVLTOSPASLAVSLCOPATISCRASPSVDSVCN		9
mVK054	-DIVITOSPASLAVSLOOPATISCOASESVSFACT	IMHWYOOK PCOPPKILTYPASNIL FSCVPARFSCSCSFSDFTLTDDVFFDDAAMYYCMOSMED	9
mVK022	-DIVLTOSPASLAVSLOORATISCRASOSVSTSSY	YMHWYOCKPGOPPELLIKYASNI-ESCUPAPPSCSCSCTOPTINTHPVEERDTATYYCOHSWET-	9
mVK023	-DIVLTOSPASLAVSLOOPATISCRASOSVSTSSY	YMHWYOOK PGOPPELLIKYASNI-ESQUPAPPSGSGSGTDFTINTHPVEERDTATYYCOHSWET-	9
mVK053	-DIVLTOSPASLAVSLOOPATISCRASOSVSTSSY	YMHWYOOK PCOPPKLLTKYASNL-PSCVPAR FSCSCSCTPTINTHPVFFFTTATYYCOHSWFT	9
mVK186	-DIVLTOSPASLAVSLGORATISCRASKSVSTSGY	YMHWYOOKPGOPPKLLIYLASNL-ESGYPARFSGSGSGTDFTLNIHPVEEBDAATYYCOHSREL	9
mVK188	-DIVLTOSPASLAVSLGORATISCKASOSVDYDGD	YMNWYOOKPGOPPKLLIYAASNL-ESGIPARFSGSGSCTDFTLNIHPVEERDAATYYCOGSNED	9
mVK189	-DIVLTOSPASLAVSLOORATIFCRASOSVDYNGI	YMHWFOOKPGOPPKLLTYAASNL-ESCIPARFSCSCSCTOFTLNIHPVEERDAATYYCOOSIED	9
mVK002	-DIOMTOTTSSLSASLGDRVTISCRASODISN	YLNWYOOKPDCTVKLLIYYTSRL-HSCVPSRPSCSCSCTDYSLTISNLEOEDIATYFCOODSKH	9
mVK026	-DIOMTOTTSSLSASLCDRVTISCRASODISN	YLNWYQQKPDCTVKLLIYYTSRL-HSCVPSRPSCSCSCTDYSLTISNLEQEDIATYFCQQDSKH	94
mVK028	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNWYQCKPDCTVKLLIYYTSRL-HSCVPSRFSCSCSCTDYSLTISNLEOEDIATYFCQCCSTL	94
mVK119	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNWYQQKPDCTVKLLIYYTSRL-HSCVPSRFSCSGSCTDYSLTISNLEOEDIATYFCQQCNTL	94
mVK001	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNGYQQKPDCTVKLLIYYTSRL-HSGVPSRFSCSCSCTDYSLTISNLEQKILPLTFANRIVS-	93
mVK005	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNWYQCKPDCTVKLLIYYTSRL-HSGVPSRFSCSCTDYSLTISNLEPEDIATYYCCQYSKL	94
mVK027	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNWYQQKPDCTVKLLIYYTSRL-HSCVPSRFSCSCSCTDYSLTISNLEPEDIATYYCQQYSKL	94
mVK004	-DIQMTQTTSSLSASLCDRVTISCRASODISN	YLNWYQQRPDCTVKLLIYYTSRL-HSGVPSRFSCSCTDYSLTISNLRPRDIATYYCQQYSKL	94
	1	4050	

mVK007	-DIQMTQTTSSLSASLCDRVTISCRASQDISN	LNWYQQKPDGTVKLLIYYTSRL-HSGVPSRF8GSGSGTDYSLTISNLEPEDIATYYCQQDSKL	94
mVK006	-DIQMTQTTSSLSASLGDRVTISCRASQDISN	LNWYQQKPDGTVKLLIYYTSRL-HSGVPSRFSGSGGTDYSLTISNLEPEDIATYYCQQYSKR	94
mVK038	-DIQMTQTTSSLSASLGDRVTISCRASQDISN	LNWYQQKPDGTVKLLIYYTSSL-HSGVPSMF <mark>SGSGGSTDYSLTIS</mark> NLEPEDIATYYCQQYSK	93
mVK120	-DIQMTQTTSSLSASLGDRVTISCRASEDIST	LNWYQQKPDGTVKLLIYYTSGL-HSGVPSRFSGSGADYSLTISNLEPEDIATYYCQQYSKL	94
mVK029	-DIQMTQTTSSLSASLGDRVTISCSASQGISN	LNWYQQKPDGTVKLLIYYTSRL-HSGVPSRFSCSCTDYSLTISNLEPEDIATYYCQQYSNL	94
mVK121	-DIQMTQTTSSLSASLGDRVTISCSASQGISN	LNWYQQKPDGTVKLLIYYTSSI-HSGVPSRFSCSCTDYSLTISNLEPEDIATYYCQQYSKL	94
mVK008	-DIQMTQTTSSLSASLGDRVTISCRASQDIST	LNWYQQKPDGTVKLLIYYASSI-HSGVPSRFSCSCGTDFSLTISNLEPEDIATYYCQCSKLP	94
mVK131	-DIQMTQSSYLSVSLGGRVTITCKASDHINN	ILAWYQQKPCNAPRLLISGATSI-ETGVPSRFSCSCSCKDYTLSITSLQTEDVATYYCQQYWST	94
mVK132	-DIQMTQSSSFSVSLGDRVTITCKASEDIYN	LAWYQQKPCNAPRLLISGATSL-ETGVPSRFSCSCKDYTLSITSLQTEDVATYYCQQYWST	94
mVK122	-DIQMTQSPSSLSASLGGKVTITCKASQDINK	IAWYQHKPCKCPRLLIHYTSTL-QPCIPSRFSCSCSCRDYSFSISNLEPEDIATYYCLQYDNL	94
mVK112	-DIQMNQSPSSLSASLGDTITITCHASQNINV	ILSWYQQKPCNIPKLLIYKASNI-HTGVPSRFSCSCTGFTLTISSLQPEDIATYYCQQGQSY	94
mVK090	-DVQMIQSPSSLSASLGDIVTMTCQASQGTSI	ILNWFQQKPCKAPKLLIYGASNI-EDCVPSRFSCSRYCTDFTLTISSLEDEDMATYFCLQHSYL	94
mVK108	-DVLMTQSPSSLSASLGERVSLTCQASQGISN	ILNWYQQTPGKAPRLLIYDASKL-EDGVPSRFSGTGYRTDFNFTISSLEEEDVATYFCLQHRYL	94
mVK011	-DIQMTQSPSSLSASLGERVSLTCRASQDIGS	LNWLQQEPD <mark>GTIK</mark> RLIYATSSL-D <mark>SGVPKRFSGS</mark> R <mark>SGSDYSLTISSLE</mark> SEDFVDYYCLQYASS	94
mVK095	-DIQMTQSPSSLSASLGERVSLTCRASQDIGS	LNWLQQEPD <mark>GTIK</mark> RLIYATSSL-D <mark>SGVPKRFSGS</mark> R <mark>SGSDYSLTISSLE</mark> SEDFVDYYCLQYASS	94
mVK033	-DIQMTQSPSSLSASLGERVSLTCRASQDIGS	LNWLQQEPD <mark>GTIK</mark> RLIYATSSL-D <mark>SGVPKRFSGSRSGSDYSLTISSLE</mark> SEDFADYYCLQYASS	94
mVK091	-DIQMTQSPSSLSASLGERVSLTCRASQEISG	LSWLQQKPD <mark>GTIK</mark> RLIYAASTL-D <mark>SGVPKRFSGSRSGSDYSLTISSLE</mark> SEDFADYYCLQYAS <mark>Y</mark>	94
mVK086	-DIQMTQSPSSLSASLGERVSLTCRASQDIHG	LNLFQQKPGETIKHLIYETSNL-D <mark>SGVP</mark> KRF <mark>SGSRSGSDYS</mark> LII <mark>GSLE</mark> SEDFADYYCLQYASS	94
mVK078	-DIQMTQSPSSMSASLRERVSLTCQASQGING	ILHWFQQKSCGTLKHLIYSTSNL-DEGVPSRFECEGESGSDYSLTISSLESEDFAVYYCLQYDEY	94
mVK092	-DIQMIQSPSSMFASLGDRVSLSCRASQGIRG	ILDWYQQKPGGTIKLLIYSTSNL-NSGVPSRFSCSCSGYSLTISSLESEDFADYYCLQRNAY	94
mVK036	-DIQTTQSPSSMSVSLGETVSITCRASQGISS	VSWLQQKPCKSPKTLISYATNL-EDGITSWFSSSCGADYSLTISSLESECKITITVYNMFS	94
mVK055	-DIQTTQSPSSMSVSLGETVSITCRASQGISS	LSWLQQKPGKSPKTLISYATNL-EDGITSWFSS <mark>SGSGADYSLTISSLE</mark> SEDCKITITVYNMFS	94
mVK076	-DIQMTQSPSSMSVSLGERVSITCRASQGTSS	LSWLQQKPGKSPKTLISYATNL-EDRVPSWFSGSWSGADYSLTISNLESEDFTDYYGVQYAQF	94
mVK115	-DILMTQSPSSMSVSLGDTVSITCHASQGISS	IIGWLQQKPGKSFKGLIYHGTNL-EDGVPSRFSGSGADYSLTISSLESEDFADYYGVQYAQE	94
mVK089	-DIKMTQSPSSMYASLGERVTITCKASQDIKS	LSWYQQKPWKEPKTLIYYATSI-ADGVPSRFSCSCQQYSLTISSLESDDTATYYCLQHGES	94
mVK104	-DIKMTQSPSSMYASLGERVTITCKASQDINS	LSWFQQKPQKBPKTLIYRANRI-VDGVPSRFSCSCQQDYSLTISSLEYEDMGIYYCLQYDEF	94
mVK085	-EIQMTQSPSSMSASLGDRITITCQATQDIVK	ILNWYQQKPGKPPSFLIYYATEL-AEGVPSRF <mark>SGSGSGYSLTIS</mark> NLES <mark>ED</mark> FADYYCLQFYEF	94
mVK075	-DIQITQYTSSVCTSLGDRVSLTCTASQHIGS	LLSLI <mark>QKKPC</mark> KSKPLIYCATNRI-DDCVSSRFSARCSKIDYSLTNSSLESDDAGIYFCQQAKES	94
mVK010	-DIQMTQSPASLSASVGETVTITCRASGNIHN	LAWYQQKQCKSPQLLVYNAKTL-ADCVPSRFSCSCSCTQYSLKINSLQPEDFCSYYCQHFWST	94
mVK155	-DIQMTQSPASLSASVGETVTITCRASGNIHN	LAWYQQKQCKSPQLLVYNAKTL-ADCVPSRFSCSCSCTQYSLKINSLQPEDFCSYYCQHFWST	94
mVK153	-DIQMTQSPASLSASVGETVTITCRASENIYS	LLAWYQQKQCKSPQLLVYNAKTL-AEGVPSRFSCSCSCTOFSLKINSLQPEDFCSYYCQHHYGT	94
mVK151	-DIQMTQSPASLSVSVGETVTITCRASENIYS	LLAWYQQKQCKSPQLLVYAATNL-ADGVPSRFSCSCSCTQYSLKINSLQSEDFGSYYCQHFWGT	94
mVK161	-DIQMTQSPASLAASVGETVTITCRASENIYY	LAWYQQKQCKSPQLLIYNANSL-EDCVPSRFSCSCSCTQYSMKINSMQPEDTATYFCKQAYDV	94
mVK126	-DIQMTQSPASLSASVGETVTITCGASENIYG	LLNWYQRKQCKSPQLLIYGATNL-ADGMSSRF <mark>SGSGSG</mark> RQYSLKISSLHPDDVATYYCQNVLST	94
mVK150*	-DIQVTQSPASLSAPVGESVSITCKASEEIYS	LINWYQCKPCKSPCLLIYYATSL-CDDVPSRFSCSKSCTQYSLKISSLQPEDLATYYCEQSYK	93
mVK117	-DIQMTQSPASQSASLGESVTITCLASQTIGT	LLAWYQQKPQKSPQLLIYAATSL-ADGVPSRFSGSGSGTKFSFKISSLQAEDFVSYYCQQLYST	94
mVK110	-DIRMIQTPASLSCSLCBSVTITCQASQDICK	LLWYQCKTGNPPKILIYTTSNL-ADGISSRVSCSGSGTQFFLKFSSLKPEDTATYYCCQGYWL	94
mVK071	-BTTVTQSPTSLSMATGEKVTIRCITSTDIDDI	MNGCQCKPCDPSNLLISECNTL-RPCVPSRFSSCYCTDFLLTIENTLSEDVADYYYLQSDIM	94
mVK202	-ETTVTQSPTSLSMATGEKVTIRCITSTDIDDI	MNWCOCKPGDPSNLLISEGNTL-RPGVPSRFSSSGYGTDFLLTIENTLSEDVADYYYLGSDIMGAAGATATGGTAACTTATTACTTCTACAGTATTATAAGTCTC	136
mVK072	-ETTVTQSPTSLSMATGEKVTIRCITSTDIDDI	MNWCOOKPGEPPNLLISEGNIT-RPGVPSRPSSGYGTDFLLTINTLSEDVADYYYLOSDNM	94
mVK077	-ETTVTQSPTSLSMATGEKVTIRCITRTDIDDI	MNWYQQKPGEPPKLLISEGNIT-RPGVPSRFSNSGYGTDFLLTIENTLSEDVADYYCLQSDNL	94
mVK088	-ETTVTQSPASLSVATGEKVTIRCITSTDIDDI	MNWTQVKPGEPPKLLIBEGNIL-KPGVPSRPSS69GTDFVFIENLSEDVADYYCLOSDM	94
mvk094	-ETTVTQSPASLSMAIGEKVTIRCITSTDIDDI	MNWIQKPGEPPKLLIBEGNIL-RPGVPSRPSSGYGTDFVFILEMLSEDVADYYCLSDNL	94
mVK111	-DVQ1TQSPSYLAASPGETITINCRASKSISK		94
mVK164	- IGETIGAPASLSPSLGETATLSCKSSESVGS		94
mvk035	SERVICEVICELPIDNOLPSIASOLRASYIVMVKT		98
mvk073	BRUATCTTTCKTLTDUŐTLSTVBŐTKVST.	LINWLLQKFYQFFKLLILLVBKL-IBGVFDKFBGBGBGTDFILKLBKVKLKIWFFIBACKVHIS-	98
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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 (<u>http://www.vbase2.org/vbase2.php</u>) (Retter, *et. al.* (2005)*) using ClustalX (2.0.11) (<u>http://bips.u-strasbg.fr/fr/Documentation/ClustalX/</u>) 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 $^{\circ}$ C) due to lack of its mutant sample.

