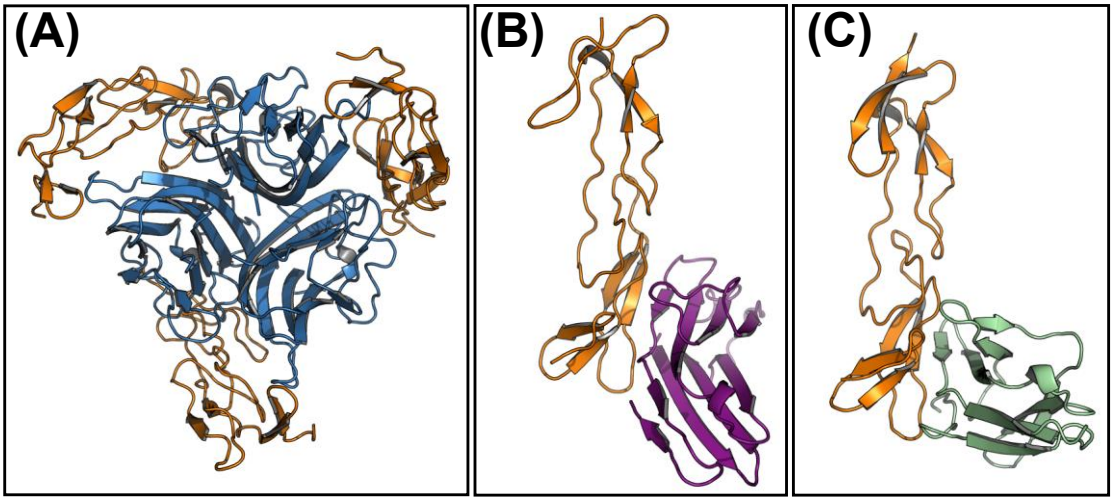
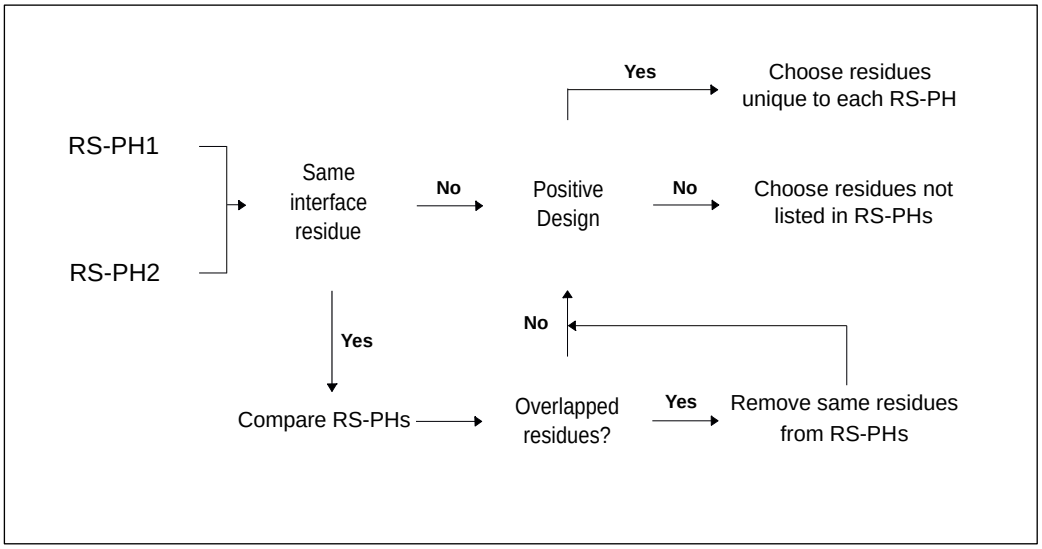
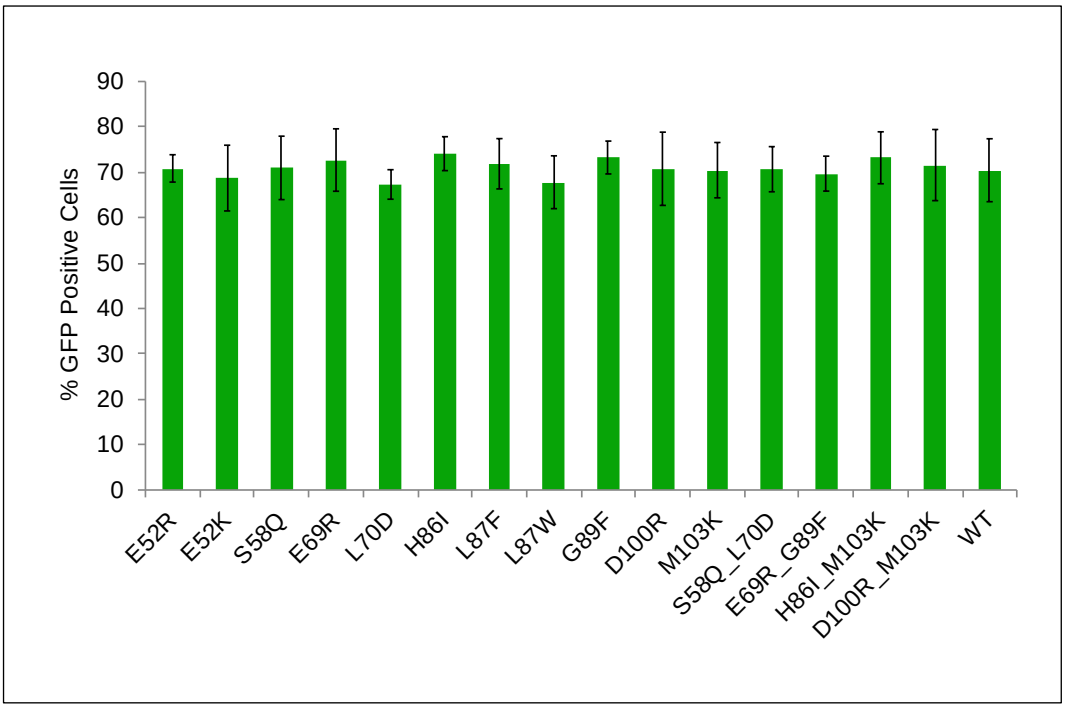
Supplementary Fig. 1:. Cartoon representation of available crystal structures using PyMol program. (A) HVEM:LIGHT (4RSU), (B) HVEM:CD160 (6NG3), and (C) HVEM:BTLA (2AW2). HVEM is colored with brown while LIGHT, CD160, and BTLA are represented using blue, magenta, and green colors respectively. Related to Fig.1.



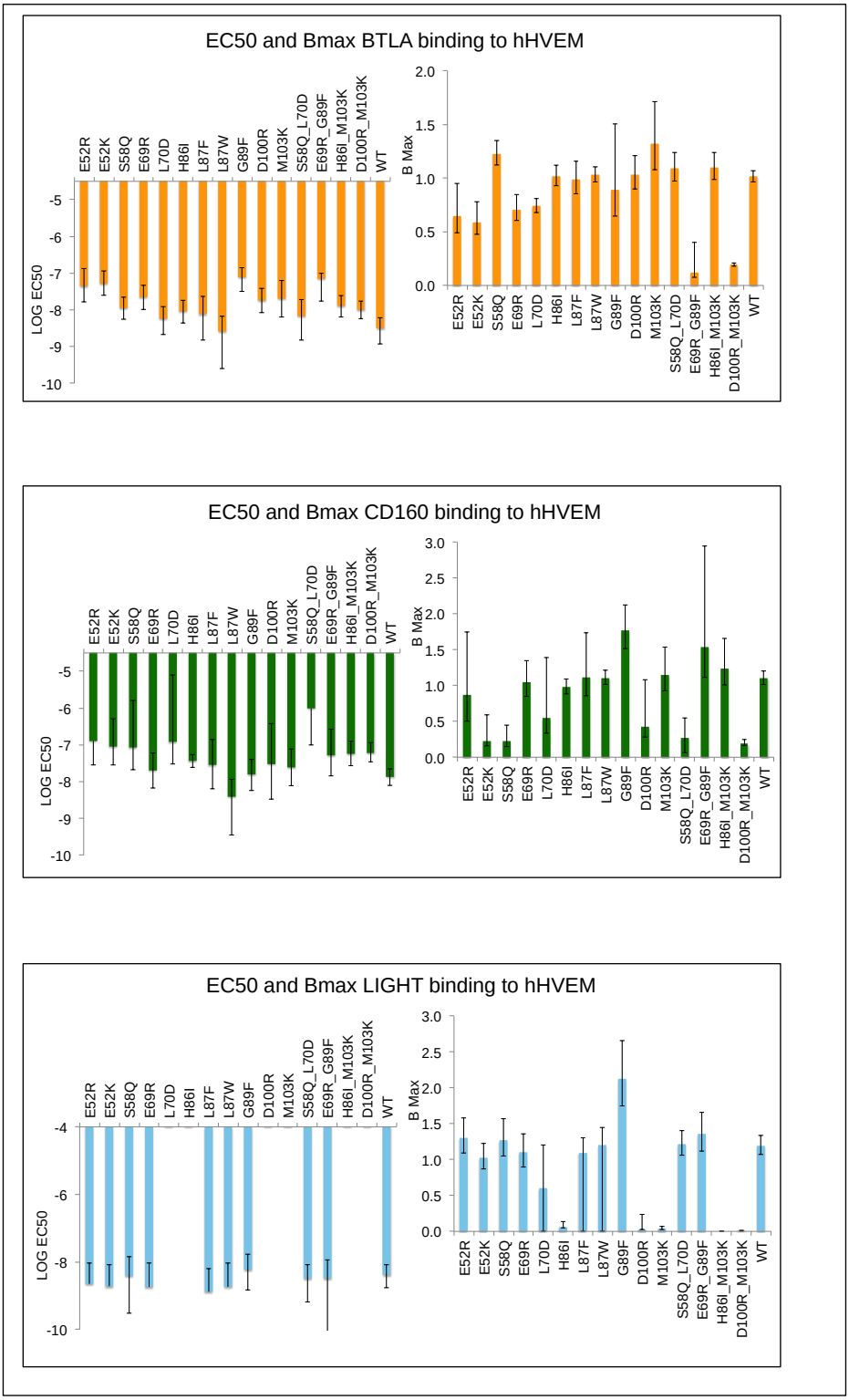
Supplementary Figure 2:. Flowchart of the decision process how to utilize rs-pharmacophores for selecting residues for mutational experiments. RS-PH stands for rs-pharmacophore. Related to Fig. 1.



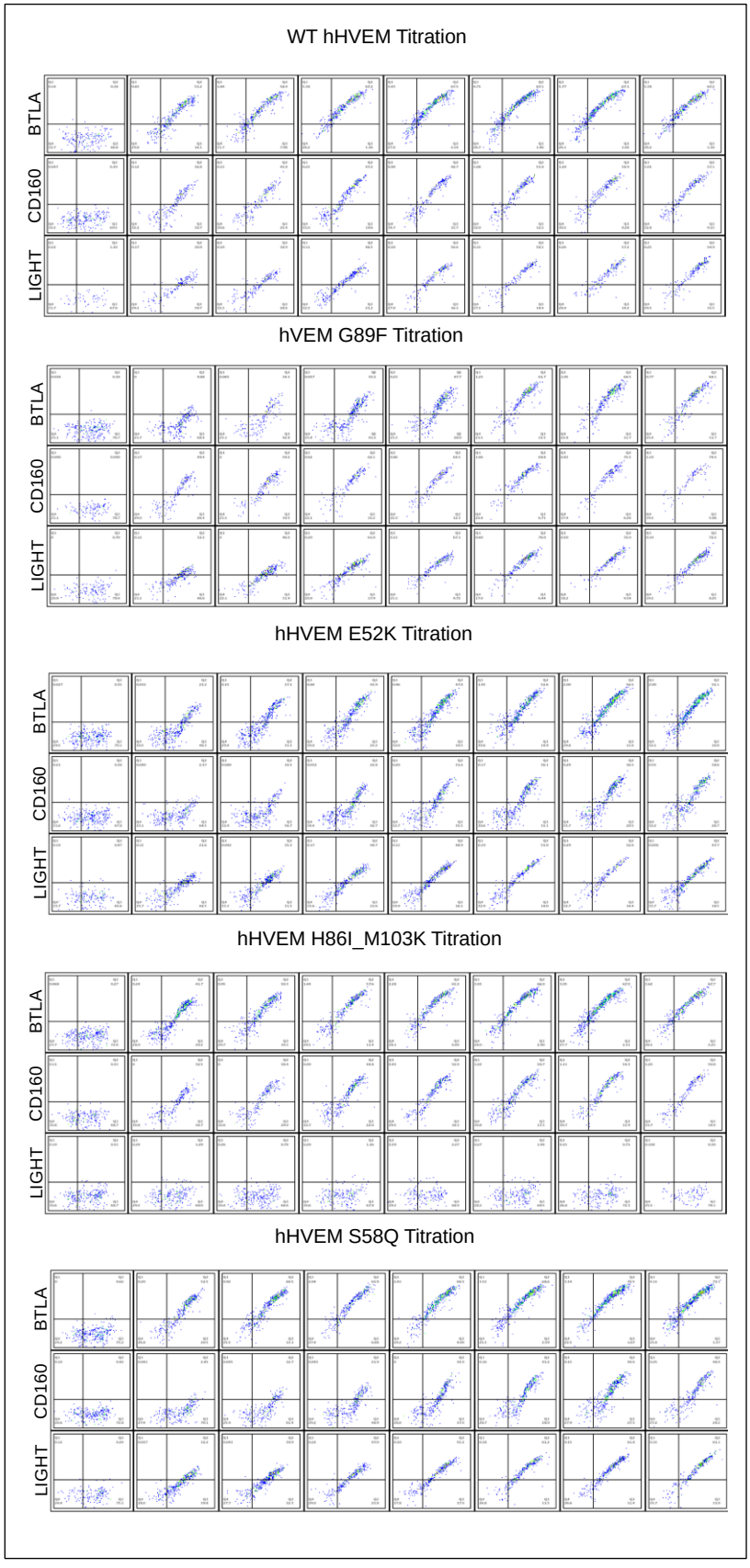
Supplementary Figure 3: hHVEM mutants show expression similar to wild-type protein. All of the HVEM mutants were expressed in the context of the full-length protein with the addition of a C-terminal GFP fusion. The data shows the average percent GFP expression for the subset of hHVEM mutants that were the focus of follow-up experiments. The error bars show the standard deviations. There was no statistical difference (by One-way ANOVA) between WT and any of the mutants suggesting all the mutants express comparable to WT hHVEM. Related to Fig. 2, 3 and 4 .



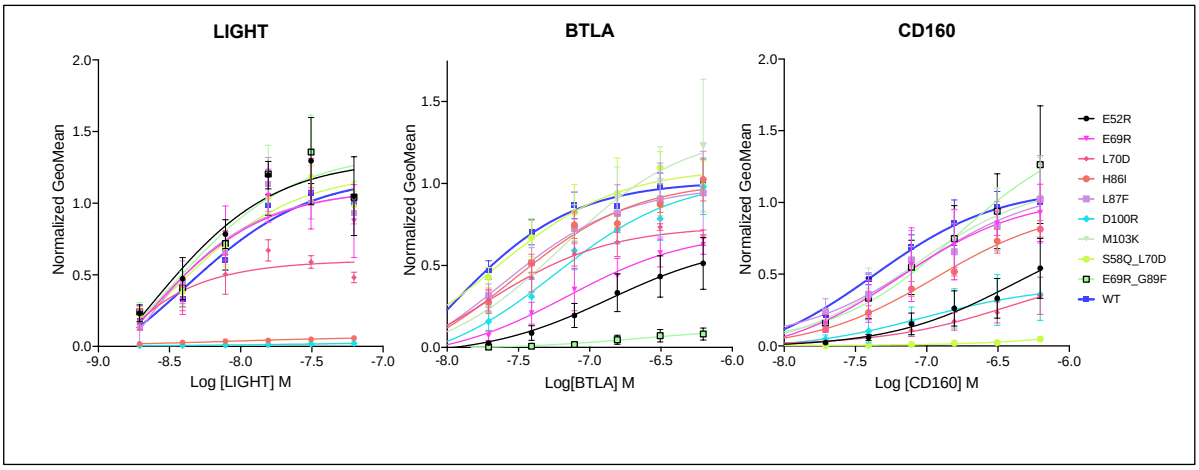
Supplementary Figure 4: Charts showing EC50 and Bmax data obtained from the titration experiments with error bars showing the 95% confidence interval from the fits. Color codes of ligands follow the ones used in other figures. Related to Fig 3 and 4.



Supplementary Figure 5: FACS plots of binding of hHVEM WT and mutants to BTLA, CD160 and LIGHT ligands. These scatter plots show all cells gated for GFP on the X-axis and Anti-HIS on the Y-axis. A select number of plots are shown: for WT, and G89F, E52K, S58Q and H86I_M103K mutant titrations, which are discussed in depth in the manuscript. Related to Fig. 2, 3 and 4 .



Supplementary Figure 6. Geometric mean of percent bound ligand as a function of concentration. Panels from left to right show HVEM binding of ligands LIGHT, BTLA and CD160, respectively. Different colors of lines refer to different mutant variants of HVEM: black circle: E52R, pink triangle: E69R, red diamond: L70D, red circle: H86I, purple square: L87F, light blue diamond: D100R, light green triangle: M103K, green circle: S58Q_L70D, black square: E69R_G89F, blue square: wild type HVEM. Related to Fig. 2, 3 and 4.



Supplementary Table 1: Rs-pharmacophore comparison for BTLA and CD160. The interface residues of HVEM:CD160 are in the first two columns, for BTLA in the las two columns. The corresponding rs-pharmacophore predicted residues for CD160 are shown in the third, for BTLA in the fifth columns. The selected mutations listed in the middle (fourth column). Bold-italic residues in the columns of HVEM represent effective positions for mutations. Sequence position is referenced from the UniProt database. Related to Fig. 1 and Fig 2.

CD160:HVEM			**Selected mutations**	**BTLA:HVEM**		
Interface residues		**RS-pharmacophores**		**RS-pharmacophores**	**Interface residues**	
CD160	**HVEM**				**HVEM**	**BTLA**
N28	*T71*	DENPQSTY		KNPQRSTY	G72, *T71*	Q37
I29	G72, *T71*	KNQRSWY		HKNQW	G72, *T71*	L38
			RK, R	DENPQSTY	C53, ***E52***, ***E69***, G68	R42
G66	***D45***	HST	F	HKNQR	***D45***, E44	G76
				PY	E44	T77
D67	***S58***	KNQRW	LKQR			
				KNQR	K43	S112
			F	DENPQSTY	***D45***, K56	R114
G120	Y61	NQRW		HKNQRY	*Y61*	S121
I121	C75, V74	FWY		HKNPQRTWY	C75, V74	L123
				HKNQRTWY	E76, *K64*	N122
R122	C75, ***S58***	DEKNPQRSTWY	LKQR			
Q124	*K56*, G72, ***S58***, *T73*	DEHKNPQRSTWY	LKQR	KNQRTWY	C57, G72, ***S58***, *T73*	E125
H126	C53, C67, G72, *T71*	DEHKNPQRSTWY				
			R	DEHNPQRSTWY	***E69***, G68, *T71*	H127
F127	***D45***, E46, P55	2	F			

Supplementary Table 2: Summary of rs-pharmacophores suggested for HVEM that are generated on the BTLA and CD160 interfaces. Recapitulation of wild type residues by rs-pharmacophore is

shown in the 3rd and 7th columns for CD160 and BTLA, respectively. Number of interacting residues for each position in HVEM are shown for CD160 (5th column) and BTLA (9th column). The last row, bold and bold-italic numbers represent the sums and averages, respectively. Related to Fig. 1 and Fig 2.

HVEM	CD160				BTLA			
	Rs-pharmacophore	**Wild type match**	**# of variants**	**# of interacting residues**	**Rs-pharmacophore**	**Wild type match**	**# of variants**	**# of interacting residues**
K43					KNQR	Yes	4	1
E44					HKNPQRY	-	7	2
D45	FHIST	-	5	2	DEHKNPQRSTY	Yes	11	2
E46	FI	-	2	1				
E52					DENPQSTY	Yes	8	1
P55	FI	-	2	1				
K56	DEHKNPQRSTWY	Yes	12	1	DENPQSTY	-	8	1
S58	DEHKNPQRSTWY	Yes	12	3	KNQRTWY	-	7	1
Y61	NQRW	-	4	1	HKNQRY	Yes	6	1
K64					HKNQRTWY	Yes	8	1
G68					DEHNPQRSTWY	-	11	2
E69					DEHNPQRSTWY	Yes	11	2
T71	DEHKNPQRSTWY	Yes	12	3	DEHKNPQRSTWY	Yes	12	3
G72	DEHKNPQRSTWY	-	12	3	HKNPQRSTWY	-	10	3
T73	DEHKNPQRSTWY	Yes	12	1	KNQRTWY	1 Yes	7	1
V74	FWY	-	3	1	HKNPQRTWY	-	9	1
E76					HKNQRTWY	-	8	1
		sum: 4	avg. :8	avg. 2		sum:8	avg. 8	avg. 2

Supplementary Table 3: Interface residues of HVEM:LIGHT complex along with predicted rs-pharmacophore (3rd column) and suggested mutations (4th column). HVEM interacts with a dimer of LIGHT therefore asterisk (*) indicates the residues from the other unit. Sequence positions are

numbered according to the UniProt database. Positions selected for mutations are shown in bold-italic. Related to Fig 2.

Interface residues (LIGHT:HVEM)		RS-pharmacophore	Suggested mutations
LIGHT	HVEM		
R172*	N88, ***G89***, S91	DEHKNPQRSTWY	G89IF
Y173*	A85, N88, ***H86***, I84, L90	IKLMNQRV	H86I
P174*	***H86***	F	H86I
E175*	***H86***	KNRWY	H86I
G100	Q95	HKNQRWY	
Q117	K92	KNQRW	
G119	K92	HKNQRSTW	
V196	***V129***	V	V129K
W198	***M103***	LP	M103K
R226	***D100***	DEHNPQSTY	D100R
R228	Q97, M98	DEHKNPQRSTWY	
D229	Q95	HNQRWY	

Supplementary Table 4: HVEM variants suggested by LIGHT-specific rs-pharmacophore. The number of interacting residues in LIGHT are shown for each HVEM residue position (5th column). Corresponding wild type residues to the rs-pharmacophore are in the 3rd column. In the last row summarized the column sums or averges. Related to Fig 2.

HVEM	Rs-pharmacophore	Wild type match	# of variants	# interacting residues
I84	IKLMNQRV	Yes	8	1
A85	IKLMNQRV	-	8	1
H86	FIKLMKNQRVWY	-	12	3
N88	DEHIKLMNPQRSTWYV	Yes	16	2
G89	DEHKNPQRSTWY	-	12	1
L90	IKLMNQRV	Yes	8	1
S91	DEHKNPQRSTWY	Yes	12	1
K92	HKNQRSTW	Yes	8	2
Q95	HKNQRWY	Yes	7	2
Q97	DEHKNPQRSTWY	Yes	12	1
M98	DEHKNPQRSTWY	-	12	1
D100	DEHNPQSTY	Yes	9	1
V129	V	Yes	1	1
M103	LP	-	2	1
		sum: 9	avg.: 9	avg.: 1

Supplementary Table 5: Welch's t-test and p-value of each mutation on normalized percentage bound of HVEM with BTLA, CD160, and LIGHT. The mean and std. deviations were calculated from three independent samples. Values at 95% confidence level are in bold. Related to Table 1.

	BTLA		**CD160**		**LIGHT**		**P-value**		
	Mean	**Std. dev.**	**Mean**	**Std. dev.**	**Mean**	**Std. dev.**	**BTLA: CD160**	**CD160 :LIGHT**	**BTLA: LIGHT**
D45F	1.13E+00	1.36E-01	1.22E+00	1.56E-01	9.43E-01	2.11E-02	4.72E-01	8.83E-02	1.41E-01
E52R	3.13E-01	2.70E-01	2.95E-01	3.07E-02	9.35E-01	6.05E-02	9.19E-01	**5.30E-04**	**5.12E-02**
E52K	4.16E-01	1.12E-01	1.69E-01	1.06E-01	9.68E-01	2.91E-02	4.98E-02	**3.56E-03**	**9.62E-03**
S58L	8.64E-01	1.07E-01	3.25E-01	1.36E-01	1.07E+00	4.65E-02	**6.73E-03**	**6.24E-03**	6.56E-02
S58K	9.15E-01	2.53E-02	4.55E-01	4.85E-01	9.57E-01	3.30E-02	2.42E-01	2.14E-01	1.59E-01
S58Q	1.34E+00	1.67E-01	2.43E-01	6.72E-02	1.06E+00	3.39E-02	**3.17E-03**	**3.63E-04**	9.58E-02
S58R	1.09E+00	2.25E-01	3.64E-01	3.21E-01	1.01E+00	3.27E-02	3.85E-02	7.09E-02	6.31E-01
E69R	5.07E-01	1.21E-01	1.04E+00	1.11E-01	8.64E-01	9.99E-02	**4.90E-03**	1.05E-01	**1.82E-02**
L70D	1.25E+00	1.10E-01	6.67E-01	6.52E-02	1.02E+00	2.41E-02	**3.10E-03**	**5.69E-03**	6.23E-02
H86I	1.02E+00	2.49E-02	8.28E-01	5.87E-02	3.29E-01	1.91E-01	1.79E-02	3.62E-02	2.30E-02
L87F	9.06E-01	5.68E-02	1.47E+00	1.77E-01	1.03E+00	2.35E-02	**2.27E-02**	**4.71E-02**	4.97E-02
L87Y	1.17E+00	1.25E-01	1.27E+00	2.05E-01	1.02E+00	2.41E-02	5.06E-01	1.67E-01	1.74E-01
L87W	1.18E+00	1.48E-01	1.90E+00	3.75E-01	9.81E-01	1.25E-02	**6.58E-02**	**5.15E-02**	1.41E-01
G89I	9.25E-02	8.95E-02	1.80E-01	4.60E-03	3.49E-01	1.00E-01	2.33E-01	9.94E-02	3.03E-02
G89F	8.65E-02	1.26E-01	1.47E+00	3.42E-01	1.08E+00	3.51E-02	**1.17E-02**	1.83E-01	**3.21E-03**
D100R	8.77E-01	3.67E-02	3.83E-01	5.85E-02	1.42E-01	4.14E-02	6.40E-04	5.91E-03	2.39E-05
M103K	7.73E-01	1.27E-01	7.03E-01	2.29E-03	2.57E-01	2.60E-02	4.43E-01	**1.04E-03**	**1.64E-02**
V129K	1.12E+00	5.97E-02	1.01E+00	2.43E-02	9.11E-01	3.16E-02	6.78E-02	1.33E-02	1.17E-02
S58Q:L70D	1.15E+00	1.48E-01	1.69E-01	8.34E-02	1.05E+00	3.21E-02	**1.75E-03**	**1.00E-03**	3.85E-01
E69R:G89F	1.11E-01	6.90E-02	1.11E+00	2.72E-01	9.77E-01	8.56E-02	**1.86E-02**	4.88E-01	**2.19E-04**
H86:G89I	1.62E-01	7.68E-02	3.60E-01	1.32E-01	1.38E-01	8.31E-02	1.04E-01	8.08E-02	7.29E-01
H86I:D100R	6.03E-01	1.09E-01	2.20E-01	7.08E-02	9.49E-02	3.97E-02	**1.05E-02**	7.19E-02	**8.56E-03**
H86I:M103K	1.03E+00	4.82E-02	8.12E-01	1.04E-01	9.66E-02	6.60E-02	4.85E-02	**1.22E-03**	**7.31E-05**
G89I:D100R	7.87E-01	9.49E-02	3.02E-01	5.84E-02	1.01E-01	3.97E-02	3.34E-03	1.08E-02	2.32E-03
D100R:M103K	8.24E-01	9.79E-02	2.27E-01	8.35E-02	8.10E-02	5.84E-02	**1.44E-03**	7.53E-02	**1.00E-03**

Supplementary Table 6: Listing equivalent residue positions among the different PDB files (listed in the first row using 4 letter PDB code and a chain identifier) and UNIPROT, for all the interface residues in HVEM. Related to Fig 1.

UniProt	2AW2.B	4RSU.D	6NG3
K43	5	43	1043
E44	6	44	1044
D45	7	45	1045
E46	8	46	1046
E52	14	52	1052
C53	15	53	1053
P55	17	55	1055
K56	18	56	1056
C57	19	57	1057
S58	20	58	1058
Y61	23	61	1061
K64	26	64	1064
C67	29	67	1067
G68	30	68	1068
E69	31	69	1069
T71	33	71	1071
G72	34	72	1072
T73	35	73	1073
V74	36	74	1074
C75	37	75	1075
E76	38	76	1076