SUPPLEMENTAL DATA

Fig. S1. Circular dichroism (CD) analysis of wild-type and mutant LLT1 proteins. CD spectra were recorded on a JASCO model J-805 CD spectrometer. Far-UV CD measurements were performed with 20 μM of each protein in HBS-EP buffer, using a 1 mm cell and a bandwidth of 1 nm. Spectra were accumulated four times.

Fig. S2. Equilibrium binding analysis of LLT1s to immobilized CD161. Wild-type LLT1 (A, red line), C163S LLT1 (B, green line), and H176C LLT1 (C, blue line) were injected at the indicated concentrations through flow cells with CD161. Black lines show the responses to the control protein (BSA).

Fig. S3. Amino acid sequence alignment of the CTLDs of CD161 with LLT1, NKp65, NKp80, PILAR, AICL, mDectin-1, hCD69, hKLRG1, hNKG2A, hCD94, and mLy49A. Red lines indicate putative binding regions revealed in this study. Red dashed lines indicate the ligand binding regions of other KLR family members. Magenta triangle and asterisk indicate the pair of residues that showed detrimental effects when mutated independently, but restored the binding when mutated simultaneously.

Fig. S4. SPR measurements of monoclonal antibody (mAb) B199.2 binding to CD161 proteins. MAb B199.2 was injected (solid bar) over the immobilized CD161 proteins at a flow rate of 5 μl/min. The black line shows the response to the control protein (BSA).

Fig. S5. The model structure of the complex between CD161 dimer (green) and LLT1 dimer (dark blue), with the same coloring as in Figs. 3C and 4B.

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Figure S1

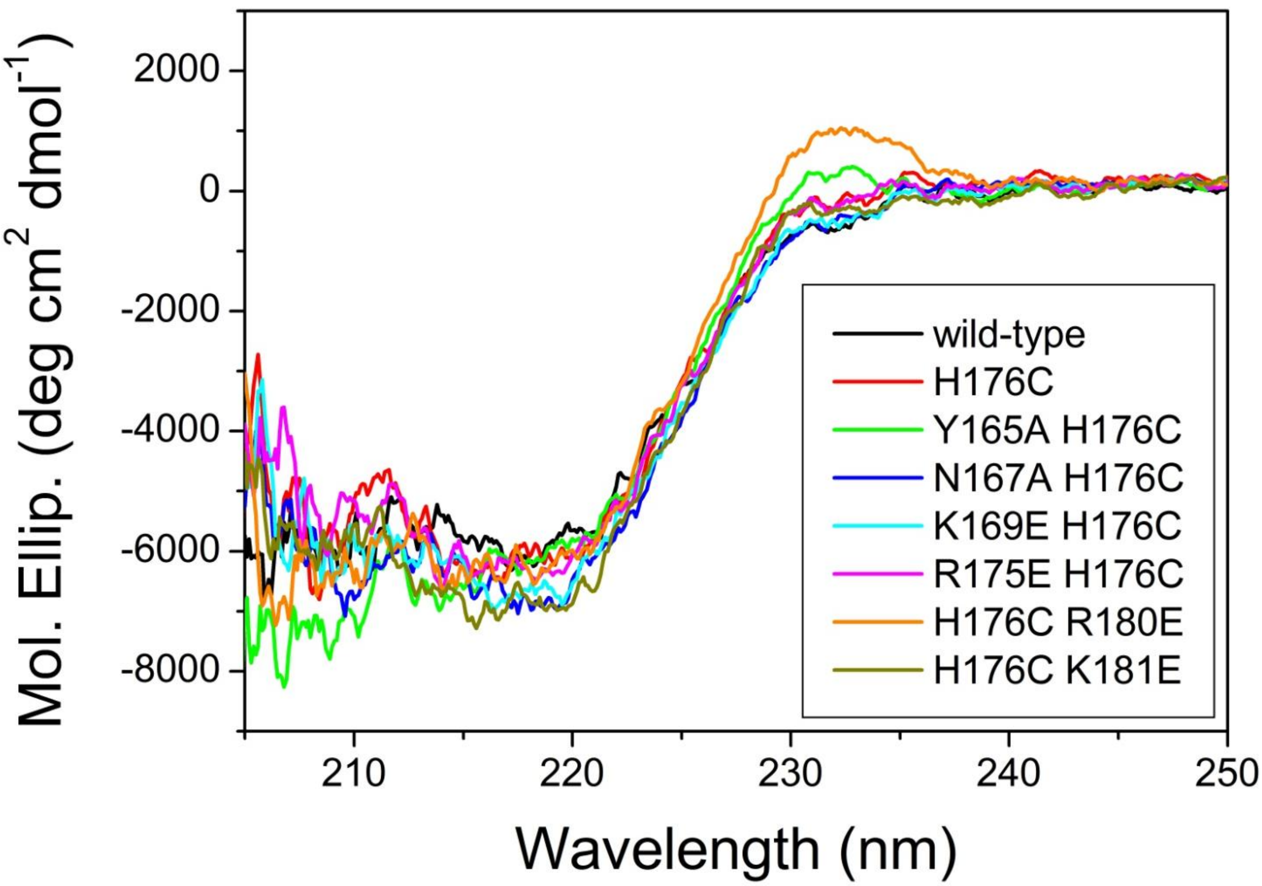

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Figure S2

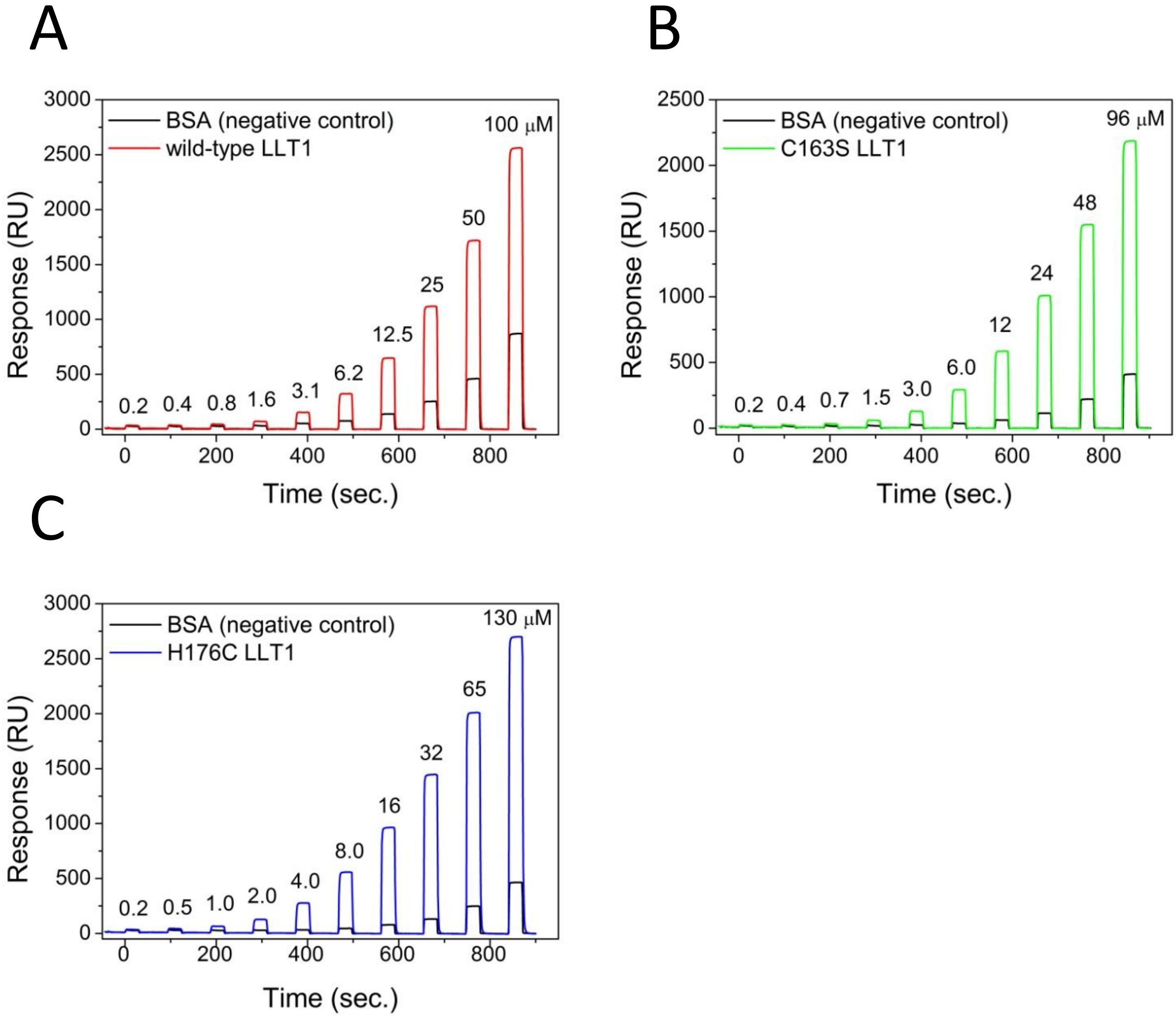

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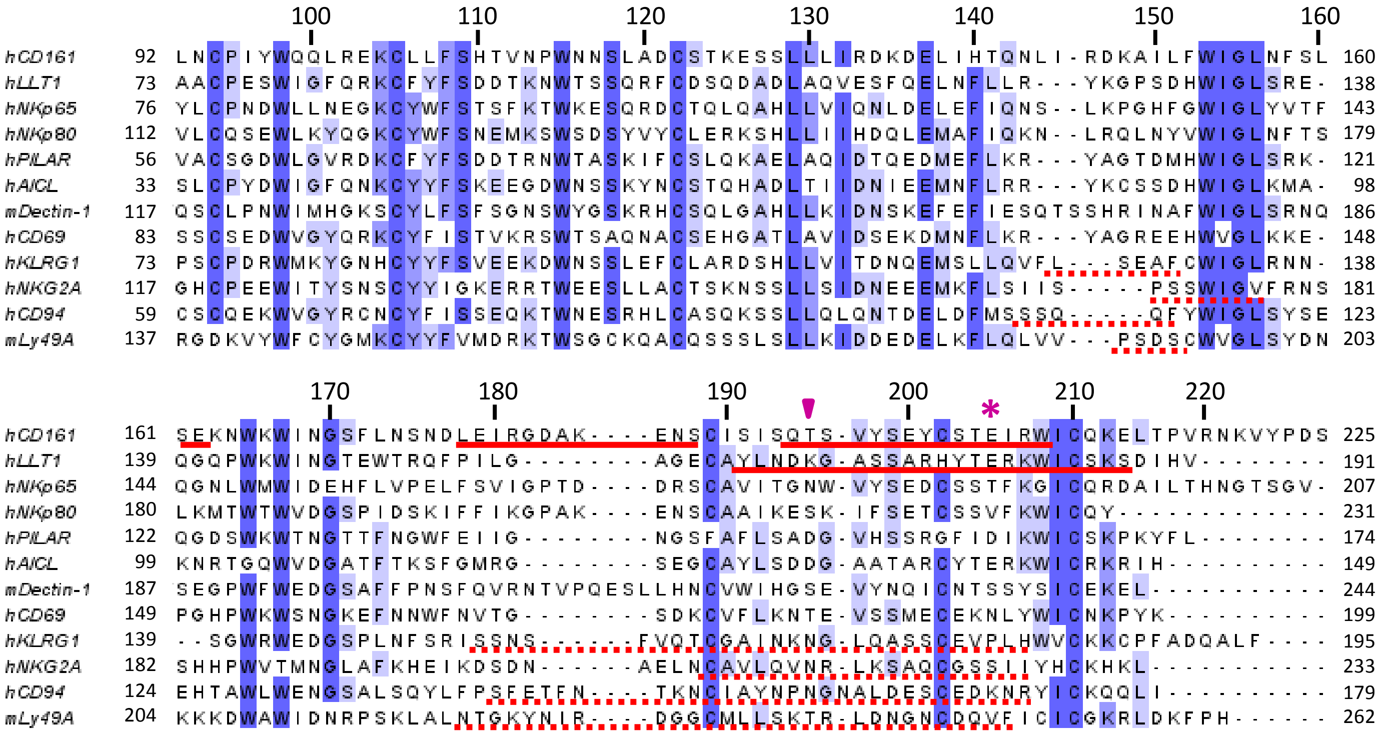
Figure S3



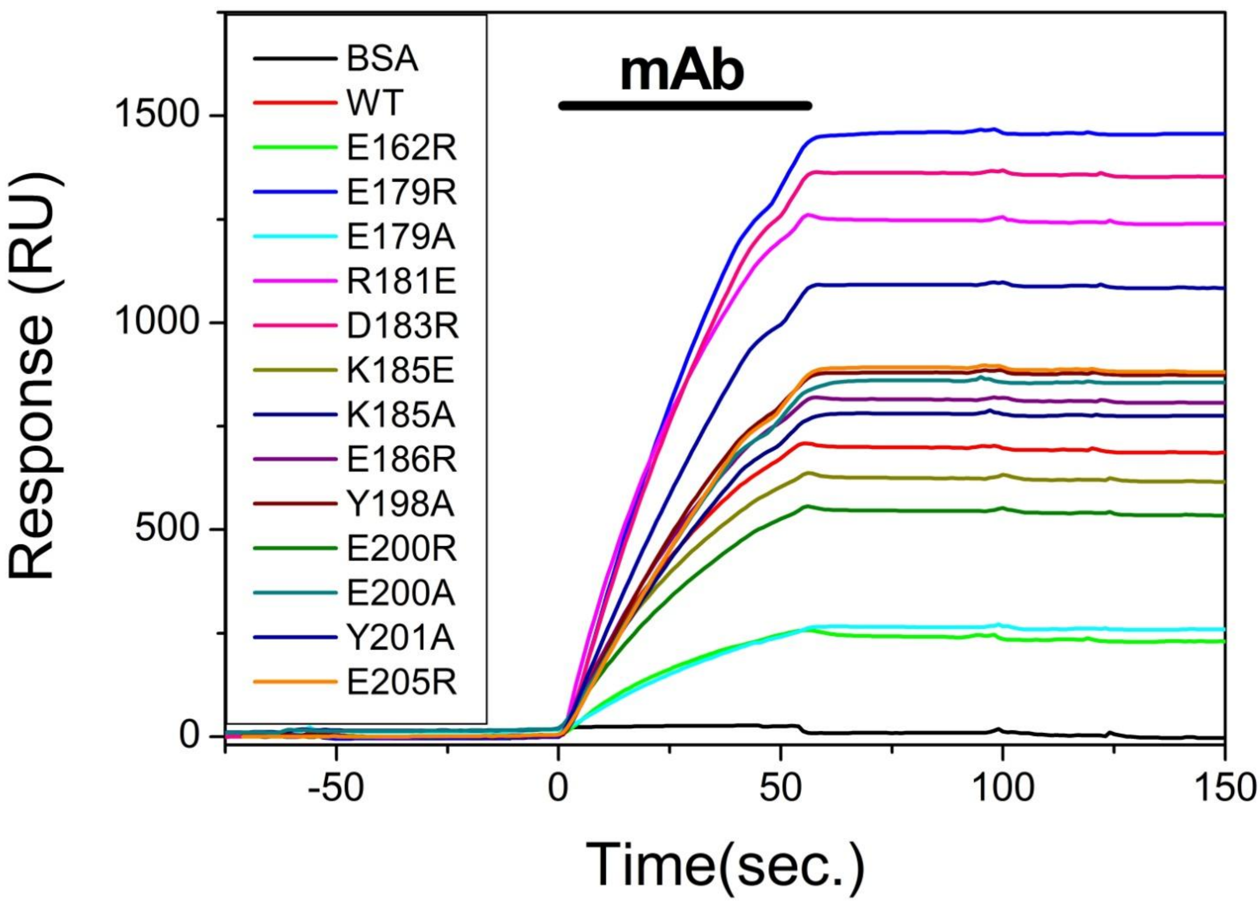

mAb
BSA
WT
E162R
E179R
E179A
R181E
D183R
K185E
K185A
E186R
Y198A
E200R
E200A
Y201A
E205R
Response (RU)
1500
1000
500
0
-50
0
50
100
150
Time(sec.)

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Figure S5

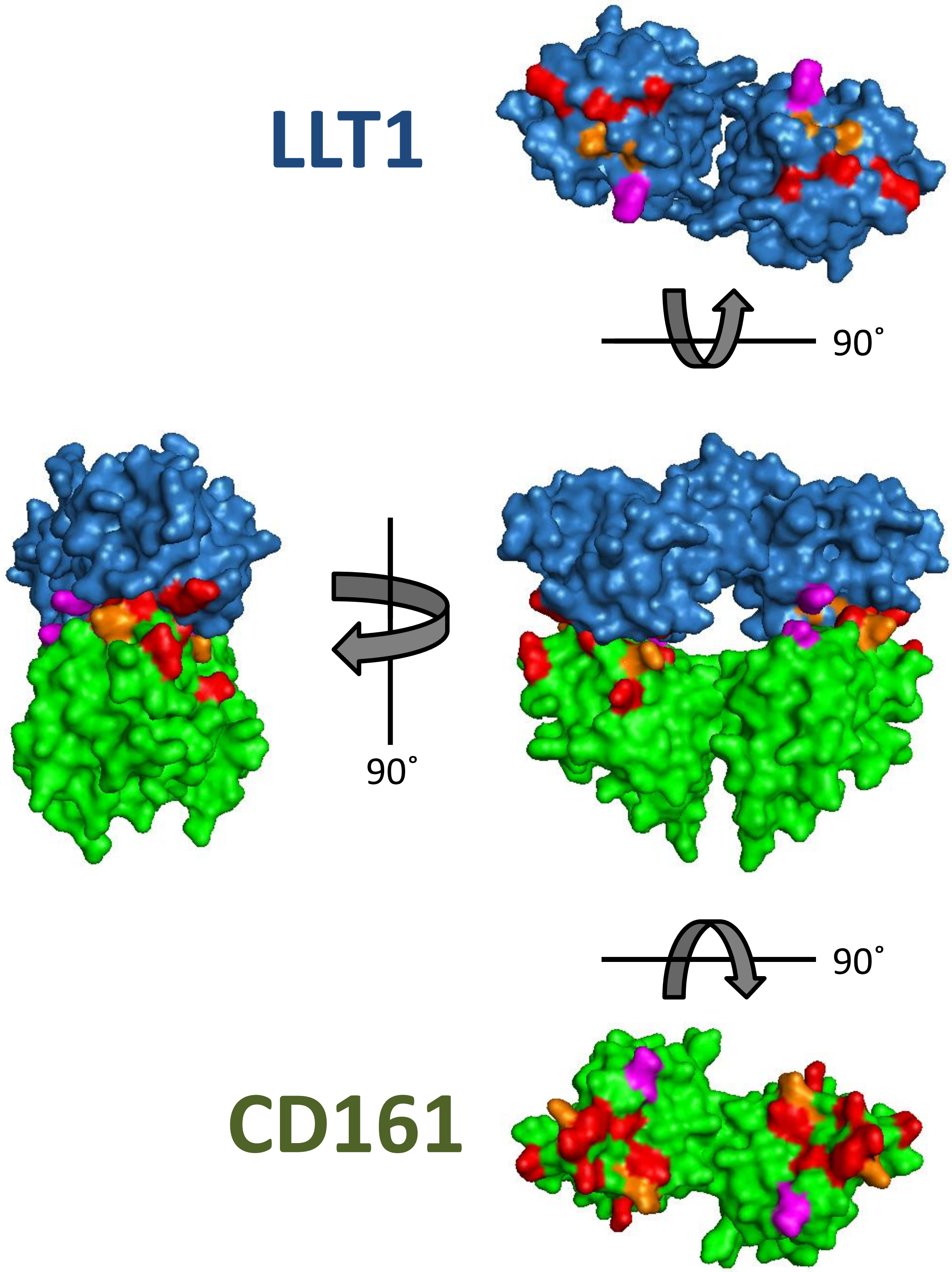

Table SI. Kinetic parameters of the interactions between LLT1 H176C and CD161 at 25˚C

Analyte	Immobilized	k_{on}	k_{off}	K_d	References[c]
		x10^5 ($M^{-1}s^{-1}$)	(s^{-1})	(μM)	
LLT1 H176C	CD161[a] (430 RU)[b]	1.1±0.1	5.3±0.55	48.5±7.5	This study
Other protein-protein interactions					
E-selectin	ESL	0.48	2.7	56	(1)
L-selectin	GlyCAM-1	>1	>10	108	(2)
P-selectin	PSGL-1	44	1.4	0.32	(3)
LILRB1D1D2	UL18	1.4	0.0028	0.0021	(4)
KIR2DL3	HLA-Cw7/DS11	2.1	1.1	5.2	(5)
CD8αα	MHC class I	≥1.0	≥18	~200	(6)
CD22	CD45	≥1.5	≥18	117	(7)
CD80	CTLA-4	9.4	0.43	0.46	(8)
CD80	CD28	6.6	1.6	2.4	(8)
FcγRIIa,IIb,III	hFc1	3.8−4.4	0.31−0.69	0.72−1.9	(9)
TCR	Peptide-MHC	0.009−0.2	0.01−0.1	1−90	(10), (11)

[a]The values are means ± range of 2 experiments.

[b]RU, response unit(s)

[c]References

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Table SII. Thermodynamic parameters of the interactions at 25˚C

Analyte	Immobilized	ΔG	ΔH (kcal•mol^{-1})	- TΔS	ΔCp (kcal•mol^{-1}•K^{-1})
LLT1 H176C	CD161[a]	- 5.9±0.02	- 3.2±0.13	- 2.7±0.11	- 0.41±0.01
TCR	MHC	- 7.1±0.6	- 14.6±5.4	7.1±5.7	- 0.62±0.37
KIR2DL3	HLA-Cw7	- 7.2	- 4.1	- 3.1	- 0.1

[a]The values are means ± standard error of 3 experiments.