SUPPLEMENTAL DATA

<u>Fig. S1.</u> 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 μ M of each protein in HBS-EP buffer, using a 1 mm cell and a bandwidth of 1 nm. Spectra were accumulated four times.

<u>Fig. S2.</u> 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.

<u>Fig. S4.</u> 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 μ l/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.





		100	11	.0	120	130	14	10	150	160)
hCD161	92	LNCPIYWQQLR	EKCLLFSH	ITVNP <mark>W</mark> NN <mark>S</mark>	SLADCS	TKESSLL	RDKDELI	HTQNLI-	RDKAILFW	GLNFSL	160
hLLT1	73	AACPESWIGFQ	R K C F Y F S D	DTKNWTS	SQRFCD	SQDADLAQ	VESFQELN	LLR···	YKGPSDH <mark>w</mark> i	GLSRE-	138
hNKp65	76	YLCPNDWLLNE	G K C YWF S T	SFKT <mark>W</mark> KE	SQRDCT	QLQAHLLV	IQNLDELE	IQNS	LKPGHFG <mark>W</mark>	GLYVTF	143
hNKp80	112	V L C Q S E W L K Y Q	G K C Y W F S N	IEMKSWSD	SYVYCL	ERKSHLLI	HDQLEMA	IQKN	LRQLNYV <mark>WI</mark>	GLNFTS	179
hPILAR	56	VA <mark>C</mark> SGD <mark>W</mark> LGVR	DKCFYFSD	DTRNWTA	SKIFCS	LQKAELAQ	DTQEDME	LKR···	YAG⊤DMH <mark>W</mark> ∣	GLSRK-	121
h AICL	33	SL <mark>C</mark> PYD <mark>W</mark> IGFQ	NKCYYFSK	E E G D <mark>W</mark> N S S	SKYNCS	TQHADLTI	IDNIEEMN	LRR	YKCSSDH <mark>w</mark> i	GLKMA -	98
mDectin-1	117	Q S <mark>C</mark> L P N <mark>W</mark> I M H G	K S <mark>C Y L F S</mark> F	SGNS <mark>W</mark> YG	SKRHCS	QLGAH <mark>LL</mark> K	IDNSK <mark>e</mark> fef	IESQTS	SHRINAFWI	GLSRNQ	186
hCD69	83	S S <mark>C</mark> S E D <mark>W</mark> V G Y Q	R <mark>k c</mark> y f i s t	VKRS <mark>w</mark> TS/	A Q N A <mark>C</mark> S	EHGATLAV	IDSEKDMN	LKR···	YAGREEH <mark>W</mark> ∖	/ <mark>gl</mark> kke-	148
hKLRG1	73	P S <mark>C P D R W</mark> MKYG	NHCYYFSV	E E K D <mark>W</mark> N S S	S L E F <mark>C</mark> L .	ARDSH <mark>ll</mark> v	ITDNQ <mark>E</mark> MSI	LQVFL	- SEAFCW	GLRNN-	138
hNKG2A	117	G H <mark>C P</mark> E E <mark>W</mark> I T Y S	NS <mark>CYY</mark> I <u>G</u> K	ERRTWEE	SLLACT	SKNSS <mark>ll</mark> s	IDNEEEMK	LSIIS-	• • • • PSS <mark>W</mark>	GVFRNS	181
hCD94	59	C S <mark>C</mark> Q E K <mark>W</mark> V G Y R	C N C Y F I <mark>S</mark> S	EQKTWNE	SRHLCA	SQKSS <mark>LL</mark> QI	LQNTDELD	MSSSQ.	QFY <mark>W</mark>	GLSYSE	123
mLy49A	137	RGDKVY <mark>w</mark> fC <mark>y</mark> G	MKCYYFVN	1 D R <mark>K</mark> T <mark>W</mark> S G (C K Q A <mark>C</mark> Q	SSSLS <mark>LL</mark> K	IDDED <mark>E</mark> lk <mark>i</mark>	LQLVV	• • P S D S C W	/ <mark>gl</mark> sydn	203
		170	2	180		190	200	21	0 220	1	
hCD161	161	170 Seknwkwings	Í Flnsnd <u>le</u>	180 I Irgdak-	ENS	190 I I CISISQTS	200 I Vyseycs		0 220 I QKELTPVRM	NKVYPDS	225
hCD161 hLLT1	161 139	170 I SEKNWKWINGS QGQPWKWINGT	FLNSND <u>L</u> E Ewtrqfpi	180 rgdak	•••ENS	190 ISIS <u>QTS</u> CAYLNDKG	200 I Vyseycs Assarhy	21 * I TEIRWIC TERKWIC	0 220 I QKELTPVRM SKSDIHV	NKVYPDS	225 191
hCD161 hLLT1 hNKp65	161 139 144	170 I Seknwkwings QgQpwkwingt Qgnlwmwideh	FLNSNDLE EWTRQFPI FLVPELFS	180 	ENS AGE DRS	190 LISISQTS CAYLNDKG CAVITGNW	200 - Vyseycs - Assarhy - Vysedcs	21 * TEIRWIC TERKWIC STFKGIC	0 220 QKELTPVRI SKSDIHV QRDAILTHI	NKVYPDS	225 191 207
h CD 161 hLL T1 hNKp 65 hNKp 80	161 139 144 180	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF	180 IRGDAK LG VIGPTD IKGPAK	ENS AGE DRS ENS	190 CISISQTS CAYLNDKG CAVITGNW CAAIKESK	200 I ASSARHY VYSEDCSS IFSETCSS	21 * TEIRWIC TERKWIC STFKGIC SVFKWIC	0 220 QKELTPVRI SKSDIHV QRDAILTHI QY	NKVYPDS Ngtsgv	225 191 207 231
hCD161 hLLT1 hNKp65 hNKp80 hPILAR	161 139 144 180 122	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI	180 IRGDAK- LG VIGPTD- IKGPAK- IG	ENS AGE DRS ENS NGS	190 CISISQTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF	21 E I RWIC TE I RWIC STFKGIC SVFKWIC I D I KWIC	0 220 A QKELTPVRI SKSDIHV QRDAILTHI QY SKPKYFL	NKVYPDS NGTSGV-	225 191 207 231 174
hCD161 hLLT1 hNKp65 hNKp80 hPILAR hAICL	161 139 144 180 122 99	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI TFTKSFGM	180 I LG VIGPTD IKGPAK IG	ENS AGE DRS ENS NGS SEG	190 ISISQTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY	211 E I RWIC E RKWIC STFKGIC SVFKWIC I D I KWIC F E RKWIC	0 220 I QKELTPVRI SKSDIHV QRDAILTHI QY SKPKYFL RKRIH	NKVYPDS NGTSGV-	225 191 207 231 174 149
h CD 161 hLL T1 hNKp 65 hNKp 80 hPILAR hAICL mDectin-1	161 139 144 180 122 99 187	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA SEGPWFWEDGS	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI TFTKSFGM AFFPNSFQ	180 I LG I KG P T D I KG P A K I G I RG I RG	ENS AGE DRS ENS SEG ESLLHN	190 ISISQTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG CWVIHGSE	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY	21 E I RWIC TERKWIC STFKGIC SVFKWIC I D I KWIC TERKWIC TSSYSIC	0 220 I QKELTPVRI SKSDIHV QRDAILTHI QY SKPKYFL RKRIH EKEL	NKVYPDS NGTSGV-	225 191 207 231 174 149 244
h CD 161 hLLT1 hNKp 65 hNKp 80 hPILAR hAICL mDectin-1 h CD69	161 139 144 180 122 99 187 149	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA SEGPWFWEDGS PGHPWKWSNGK	F L N S N D LE EWT R Q F P I F L V P E L F S P I D S K I F F T F N G W F E I T F T K S F G M A F F P N S F Q E F N N W F N V	180 I LG VIGPTD IKGPAK IG IRG VRNTVPQI (TG	ENS AGE DRS ENS SEG ESLLHN SDK	190 ISIS <u>QTS</u> CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG CVWIHGSE CVVIHGSE	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY VYNQICN VSSMECE	210 ERKWIC ERKWIC STFKGIC SVFKWIC IDIKWIC FERKWIC SSYSIC (NLYWIC	0 220 Q K E L T P V R I S K S D I H V · · Q R D A I L T H I Q Y · · · · · · S K P K Y F L · · R K R I H · · · · E K E L · · · · · N K P Y K · · · ·	NKVYPDS NGTSGV-	225 191 207 231 174 149 244 199
hCD161 hLLT1 hNKp65 hNKp80 hPILAR hAICL mDectin-1 hCD69 hKLRG1	161 139 144 180 122 99 187 149 139	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA SEGPWFWEDGS PGHPWKWSNGK SGWRWEDGS	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI TFTKSFGM AFFPNSFQ EFNNWFNV PLNFSRIS	180 I LG VIGPTD IKGPAK IG IRG VRNTVPQI (TG		190 ISISOTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG CWIHGSE CVFLKNTE	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY VYNQICN VSSMECEI LQASSCE	21: ERKWIC ERKWIC STFKGIC SVFKWIC IDIKWIC TERKWIC TSSYSIC (NLYWIC /PLWVC	0 220 Q K E L T P V R I S K S D I H V · · Q R D A I L T H I Q Y · · · · · · S K P K Y F L · · R K R I H · · · · E K E L · · · · · N K P Y K · · · · K K C P F A D Q A	NKVYPDS NGTSGV-	225 191 207 231 174 149 244 199 195
hCD161 hLLT1 hNKp65 hNKp80 hPILAR hAICL mDectin-1 hCD69 hKLRG1 hNKG2A	161 139 144 180 122 99 187 149 139 182	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA SEGPWFWEDGS PGHPWKWSNGK SGWRWEDGS SHHPWVTMNGL	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI TFTKSFGM AFFPNSFQ EFNNWFNV PLNFSRIS AFKHEIKD	180 I I RG DAK I G P T D I KG P AK I G P AK I RG P AK I		190 ISISOTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG CWIHGSE CVFLKNTE GAINKNG	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY VYNQICN VSSMECEI LQASSCE	21: ERKWIC ERKWIC STFKGIC SVFKWIC IDIKWIC FERKWIC SSYSIC (NLYWIC (PLUWVC SSILYHC	0 220 Q K E L T P V R I S K S D I H V · · Q R D A I L T H I Q Y · · · · · · S K P K Y F L · · R K R I H · · · · E K E L · · · · · N K P Y K · · · · K K C P F A D Q A K H K L · · · · ·	NKVYPDS NGTSGV-	225 191 207 231 174 149 244 199 195 233
hCD161 hLLT1 hNKp65 hNKp80 hPILAR hAICL mDectin-1 hCD69 hKLRG1 hNKG2A hCD94	161 139 144 180 122 99 187 149 139 182 124	170 SEKNWKWINGS QGQPWKWINGT QGNLWMWIDEH LKMTWTWVDGS QGDSWKWTNGT KNRTGQWVDGA SEGPWFWEDGS PGHPWKWSNGK SGWRWEDGS SHHPWVTMNGL EHTAWLWENGS	FLNSNDLE EWTRQFPI FLVPELFS PIDSKIFF TFNGWFEI TFTKSFGM AFFPNSFQ EFNNWFNV PLNFSRIS AFKHEIKD ALSQYLFP	180 I I RG DAK I G P T D I KG P AK I G P AK I RG P AK I		190 ISISOTS CAYLNDKG CAVITGNW CAAIKESK FAFLSADG CAYLSDDG CWIHGSE CVFLKNTE GAINKNG CAVLOVNR	200 I ASSARHY VYSEDCS IFSETCS VHSSRGF AATARCY VYNQICN VSSMECEI LQASSCE LQASSCE NALDESCEI	210 ERKWIC STFKGIC SVFKWIC IDIKWIC FERKWIC SSYSIC (NLYWIC (PLWVC SSIYHC DKNRYIC	0 220 Q K E L T P V R I S K S D I H V · · Q R D A I L T H I Q Y · · · · · · S K P K Y F L · · R K R I H · · · · E K E L · · · · · K K C P F A D Q A K H K L · · · · · K Q Q L I · · · ·	NKVYPDS NGTSGV-	225 191 207 231 174 149 244 199 195 233 179





Analyte	Immobilized	k _{on}	k _{off}	K _d	References ^c	
		$x10^{5} (M^{-1}s^{-1})$	(s^{-1})	(µM)		
	CD161 ^a	1 1 1 0 1	5 2 1 0 55	40 5 17 5	This stalls	
LLII HI/6C	(430 RU) ^b	1.1±0.1	5.3±0.55	48.3±7.3	This study	
Other protein-p	orotein 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)	
CD8aa	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)	
FcyRIIa,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)	

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

^aThe values are means \pm range of 2 experiments.

^bRU, response unit(s)

^cReferences

- Wild, M. K., Huang, M. C., Schulze-Horsel, U., van der Merwe, P. A., and Vestweber, D. (2001) J Biol Chem 276, 31602-31612
- Nicholson, M. W., Barclay, A. N., Singer, M. S., Rosen, S. D., and van der Merwe, P. A. (1998) *J Biol Chem* 273, 763-770
- 3. Mehta, P., Cummings, R. D., and McEver, R. P. (1998) J Biol Chem 273, 32506-32513
- 4. Chapman, T. L., Heikeman, A. P., and Bjorkman, P. J. (1999) *Immunity* 11, 603-613
- Maenaka, K., Juji, T., Nakayama, T., Wyer, J. R., Gao, G. F., Maenaka, T., Zaccai, N. R., Kikuchi, A., Yabe, T., Tokunaga, K., Tadokoro, K., Stuart, D. I., Jones, E. Y., and van der Merwe, P. A. (1999) J Biol Chem 274, 28329-28334
- Gao, G. F., Willcox, B. E., Wyer, J. R., Boulter, J. M., O'Callaghan, C. A., Maenaka, K., Stuart, D. I., Jones, E. Y., Van Der Merwe, P. A., Bell, J. I., and Jakobsen, B. K. (2000) J Biol Chem 275, 15232-15238

- 7. Bakker, T. R., Piperi, C., Davies, E. A., and Merwe, P. A. (2002) *Eur J Immunol* **32**, 1924-1932
- van der Merwe, P. A., Bodian, D. L., Daenke, S., Linsley, P., and Davis, S. J. (1997) *J Exp Med* 185, 393-403
- Maenaka, K., van der Merwe, P. A., Stuart, D. I., Jones, E. Y., and Sondermann, P. (2001) J Biol Chem 276, 44898-44904
- Willcox, B. E., Gao, G. F., Wyer, J. R., Ladbury, J. E., Bell, J. I., Jakobsen, B. K., and van der Merwe, P. A. (1999) *Immunity* 10, 357-365
- Boniface, J. J., Reich, Z., Lyons, D. S., and Davis, M. M. (1999) *Proc Natl Acad Sci U S A* 96, 11446-11451

Analyta	Immobilized	ΔG	ΔH	- ΤΔS	ΔCp	
Analyte			$(\text{kcal} \cdot \text{mol}^{-1})$		$(\text{kcal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1})$	
LLT1	$CD161^{a}$	5 0+0 02	2 2+0 12	2 7 ⊥0 11	0.41+0.01	
H176C	CD101	- <i>3.9</i> ±0.02	- 3.2±0.15	- 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	

Table SII. Thermodynamic parameters of the interactions at $25^{\circ}C$

^aThe values are means \pm standard error of 3 experiments.