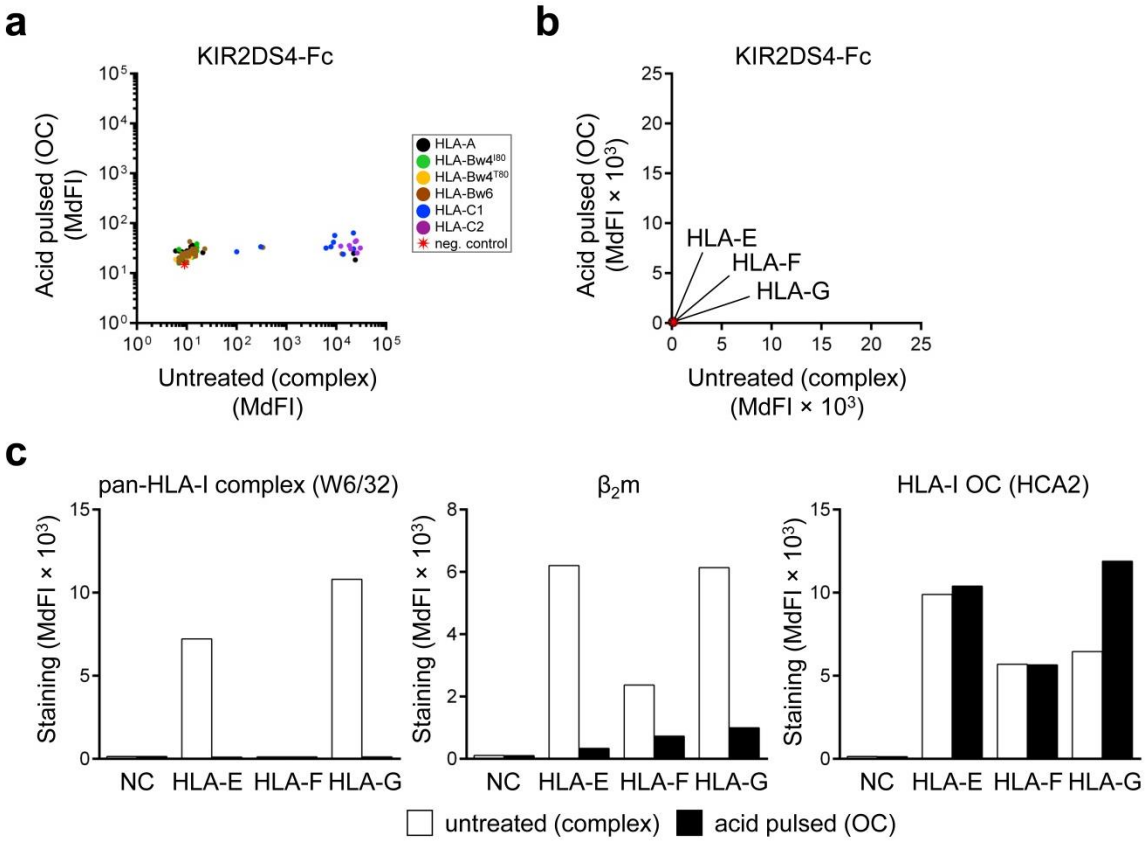
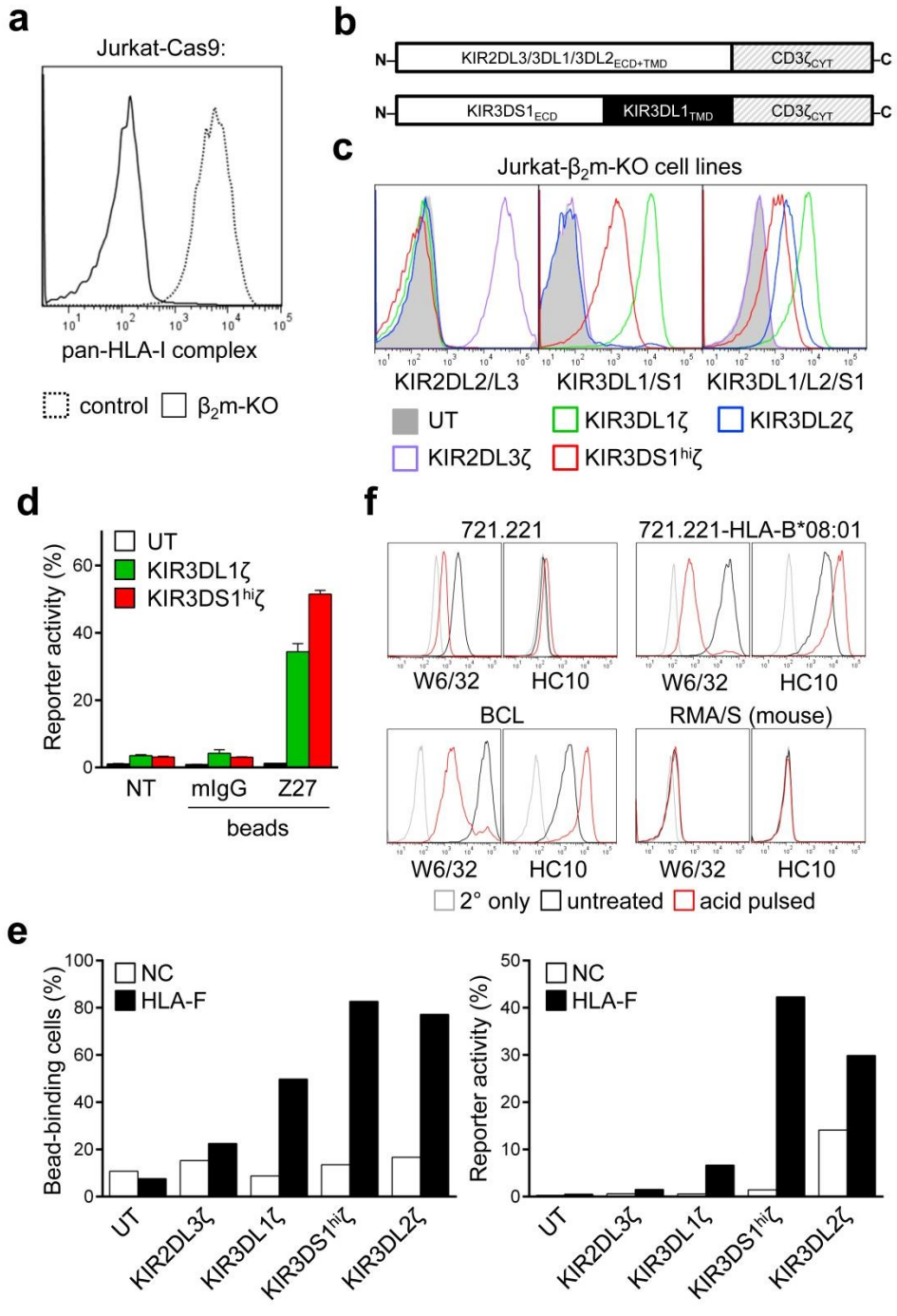


Supplementary Figure 1

KIR2DS4-Fc and antibody staining of beads coated with HLA class I.

(**a**, **b**) Binding of KIR2DS4-Fc to untreated (complex) versus acid-pulsed (open conformer; OC) beads coated with classical HLA class I allotypes (in **a**); HLA-E, -F, or -G (in **b**); or nothing (negative control beads; red star) was measured and reported as median fluorescence intensity (MdFI). Each dot indicates a single HLA class I allotype, and color coding is used in **a** to indicate HLA class I allotype grouping (see legend and On-line Methods for further details). (**c**) HLA-E, -F-, and -G-coated beads and negative control beads (NC) were untreated (complex) or acid pulsed (OC) and stained with anti–pan-HLA class I complex antibody (clone: W6/32) (left panel) to assess for complex conformation on beads, anti-β_2m antibody (middle panel) to quantify β_2m content, and an antibody to HLA class I OCs (clone: HCA2) (right panel) to assess HLA-F reactivity; median fluorescence intensity (MdFI) is reported.

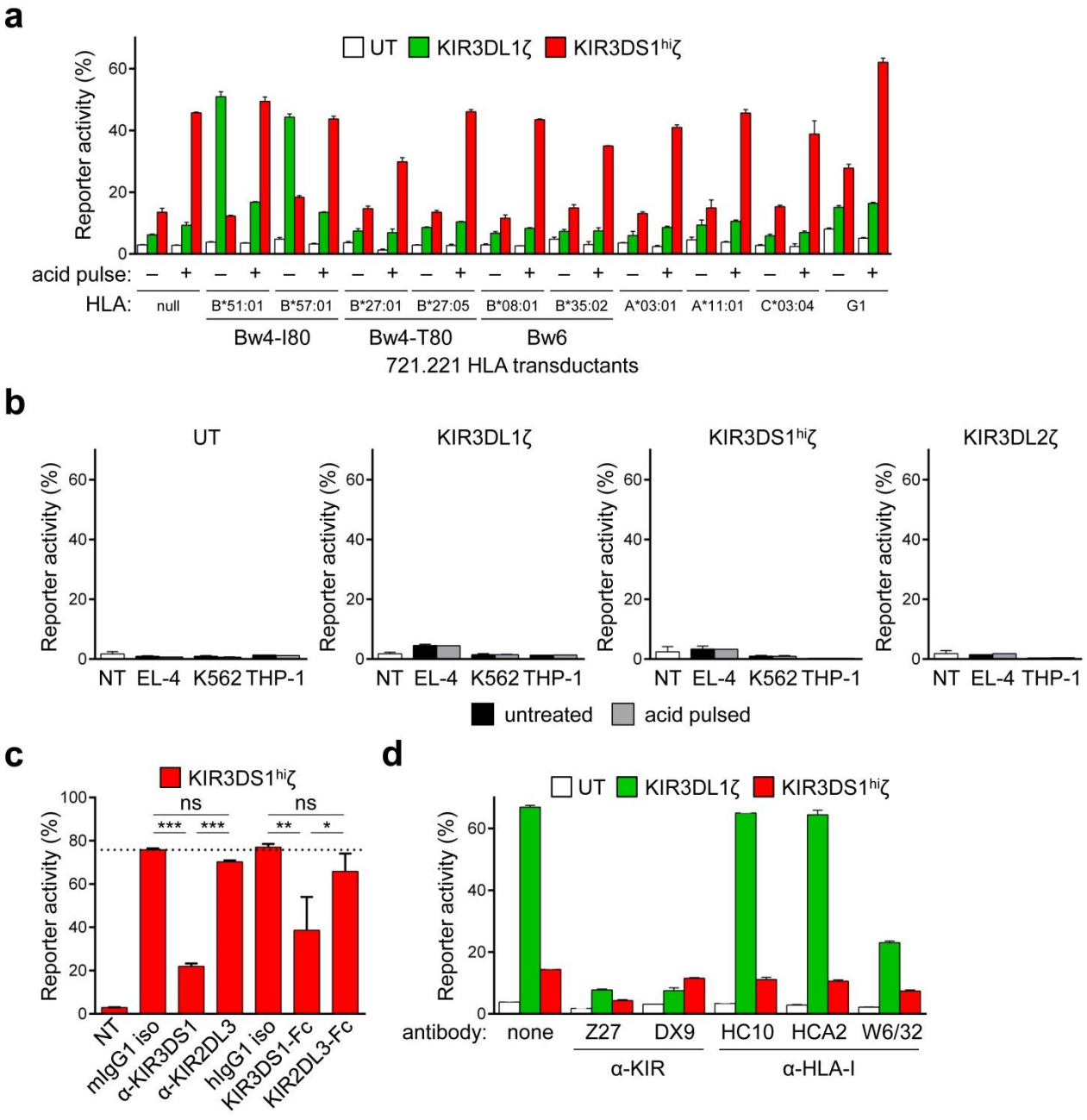


Supplementary Figure 2

Generation and testing of KIRζ^{+} Jurkat reporter cell lines and assessment of HLA class I OCs on target cells.

(**a**) Staining of anti–pan-HLA class I complex was assessed in Jurkat cells that underwent CRISPR/Cas9-mediated β_2m knockout (KO). (**b**) KIRζ chimeric receptors for inhibitory and activating KIRs were designed by fusing the extracellular domain (ECD) and transmembrane domain (TMD) of the indicated KIRs with the triple immunoreceptor tyrosine-based activating motif (ITAM)-containing cytoplasmic tail (CYT) of CD3ζ. (**c**) KIR expression was assessed in Jurkat-β_2m-KO cells stably transduced with the indicated KIRζ chimeric receptors via staining with anti–KIR2DL2-KIR2DL3, anti–KIR3DS1-KIR3DL1, and anti–KIR3DL1-KIR3DL2-KIR3DS1; UT signifies untransduced. (**d**) Reporter activity of untransduced (UT), KIR3DL1ζ^{+} and KIR3DS1^{hi}ζ^{+} Jurkat cells co-incubated with cell-sized beads covalently conjugated to irrelevant mouse IgG (mIgG) or anti–KIR3DS1-KIR3DL1 antibody (Z27) was measured as percentage

of CD69^{hi} Jurkat cells. (**e**) Untransduced (UT), KIR2DL3ζ^{+}, KIR3DL1ζ^{+}, KIR3DS1^{hi}ζ^{+} and KIR3DL2ζ^{+} Jurkat cells were co-incubated with streptavidin beads coated with free biotin (negative control; NC) or biotinylated HLA-F monomers (HLA-F) and the percentage of bead-binding cells (left panel) as well as reporter activity as a percentage of CD69^{hi} cells (right panel) was measured. (**f**) Staining of anti–pan-HLA class I complex (clone: W6/32) and an antibody to HLA class I OCs (clone: HC10) antibodies was measured on untreated and acid-pulsed cell lines to confirm OC generation; 2° only signifies secondary only staining. Data in **d** is representative of three experiments and shows three technical replicates; **a**, **c**, **e**, and **f** show one experiment; **d** shows mean + s.d.

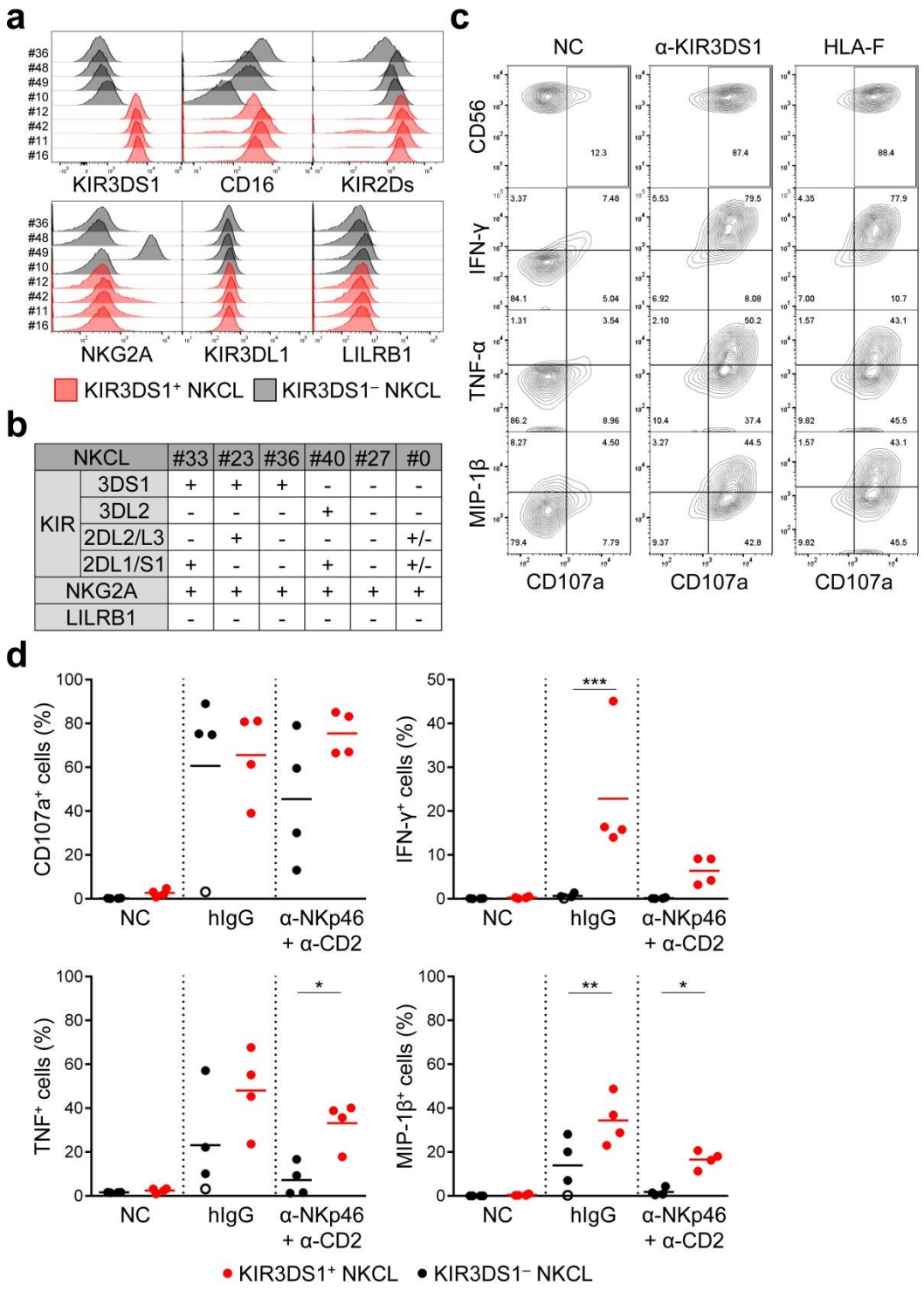


Supplementary Figure 3

Triggering of KIRζ^{+} Jurkat reporter cells by various cell lines.

(**a**) Reporter activity of untransduced (UT), KIR3DL1ζ^{+} and KIR3DS1^{hi}ζ^{+} Jurkat cells co-incubated with untreated (–) or acid pulsed (+) 721.221 cells expressing the indicated HLA class I allotypes was measured as percentage of CD69^{hi} Jurkat cells. (**b**) Reporter activity of untransduced (UT), KIR3DL1ζ^{+}, KIR3DS1^{hi}ζ^{+} and KIR3DL2ζ^{+} Jurkat cells co-incubated with untreated (black bars) or acid-pulsed (gray bars) cell lines was measured as percentage of CD69^{hi} Jurkat cells. Cell lines used were EL-4 cells (mouse T-lymphoblastoid cell line), K562 cells (human chronic myelogenous leukemia line), and THP-1 cells (human acute monocytic leukemia line), all of which do not express HLA-F; NT signifies no target cells. (**c**) Reporter activity of KIR3DS1^{hi}ζ Jurkat cells co-incubated with acid-pulsed HLA-Bw4$^{–}$ BCL in the presence of the indicated antibodies or KIR-Fc constructs (50 μg/mL each) was measured as percentage of CD69^{hi} Jurkat cells. The labels indicate the following: NT, no target cells; mIgG1 iso, mouse IgG1 isotype control; α-KIR3DS1, anti–KIR3DS1-KIR3DL1; α-KIR2DL3, anti–KIR2DL2-KIR2DL3; hIgG1 iso, human IgG1 isotype control antibody; KIR3DS1-Fc, KIR3DS1-Fc fusion construct with Fc region of hIgG1; KIR2DL3-Fc, KIR2DL3-Fc fusion construct with Fc region of hIgG1. (**d**) Reporter activity of

untransduced (UT), KIR3DL1ζ^+ and KIR3DS1$^{hi}\zeta^+$ Jurkat cells co-incubated with untreated HLA-Bw4$^+$ BCLs in the presence of the indicated antibodies to KIR or HLA class I (25 μg/mL each) was measured as percentage of CD69^{hi} Jurkat cells. Blocking antibodies used (clone and antigen) are the following: Z27, anti–KIR3DS1-KIR3DL1 antibody; DX9, anti-KIR3DL1; HC10, anti-HLA class I OC; HCA2, anti-HLA class I OC; W6/32, anti–pan-HLA class I complex. Data in **a**, **c**, and **d** show three technical replicates from one experiment; **b** shows pool data from two independent experiments; **a**, **b**, **c**, and **d** show mean + s.d. In **c**, a one-way ANOVA with Tukey multiple comparisons test comparing all columns was performed; only some statistics are shown; *P < 0.01, **P < 0.001 and ***P < 0.0001.



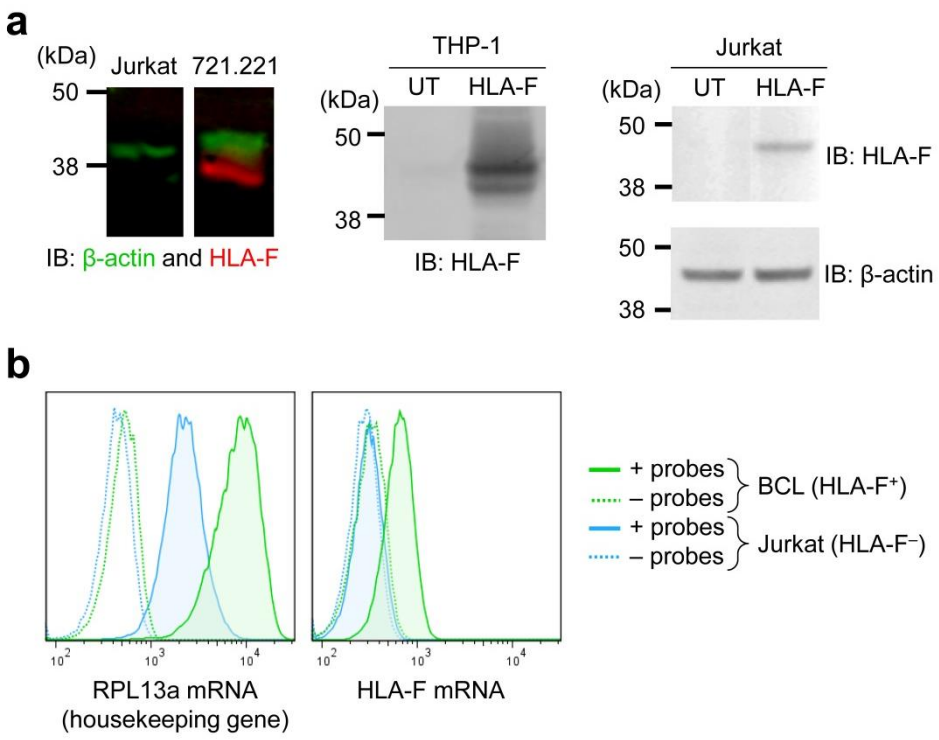
NKCL		#33	#23	#36	#40	#27	#0
KIR	3DS1	+	+	+	-	-	-
	3DL2	-	-	-	+	-	-
	2DL2/L3	-	+	-	-	-	+/-
	2DL1/S1	+	-	-	+	-	+/-
NKG2A		+	+	+	+	+	+
LILRB1		-	-	-	-	-	-

Supplementary Figure 4

NKCL phenotypes and functional responses.

(**a**, **b**) NK-cell lines used for plate-bound ligand assays (in **a**) and clones used for HIV-1 replication inhibition assays (in **b**) were stained for various NK-cell receptors, as indicated; KIR2Ds in **a** was measured by staining with anti–KIR2DL2-KIR2DL3 and anti–KIR2DL1-KIR2DS1 having the same fluorophore. In **b**, '+' denotes >90% expression of the indicated receptor, '–' denotes a frequency of <5%, and '+/–' denotes a frequency >5% (in both cases for clone #0, ~10%). (**c**) Representative flow plots of degranulation (i.e. CD107a

expression) and production of IFN-γ, TNF-α, and MIP-1β by one KIR3DS1^{+} NK-cell line seeded onto well plates coated with irrelevant protein (negative control; NC), anti–KIR3DS1-KIR3DL1 (plate-bound α-KIR3DS1), or HLA-F monomers (plate-bound HLA-F) are shown. (**d**) Degranulation and production of IFN-γ, TNF-α, and MIP-1β by KIR3DS1^{+} (red dots) and KIR3DS1^{-} (black dots) NKCLs seeded onto well plates coated with irrelevant protein (negative control; NC), human IgG (hIgG), or anti-NKp46 and anti-CD2 (α-NKp46 + α-CD2) were measured. The percentages of CD107a^{+} (top left panel), IFN-γ^{+} (top right panel), TNF^{+} (bottom left panel), and MIP-1β^{+} (bottom right panel) NKCLs are presented. For **d**, which shows mean, one-way ANOVA with Sidak multiple comparisons test comparing select columns was performed, and all statistically significant differences are presented; *P < 0.05, **P < 0.01 and ***P < 0.001.



Supplementary Figure 5

Quality-control testing of the antibody to HLA-F used for immunoblot analysis and HLA-F mRNA probes for fluorescence *in situ* hybridization.

(**a**) Cell lysates (non-nuclear) from cell lines were run on reducing/denaturing SDS-PAGE, followed by immunoblotting (IB) for HLA-F and β-actin (loading control). Left image shows Jurkat cells (HLA-F$^-$) and 721.221 cells (HLA-F$^+$). Middle image shows THP-1 cells (HLA-F-deficient) and THP-1 cells transduced with N-terminally FLAG-tagged HLA-F. Right image shows Jurkat cells and Jurkat cells transduced with N-terminally FLAG-tagged HLA-F. (**b**) Fluorescent *in situ* hybridization and flow cytometry was done on an HLA-F$^+$ cell line (BCL) and an HLA-F$^-$ cell line (Jurkat cells) with probes for HLA-F mRNA and also RPL13a mRNA (a housekeeping gene). Dotted histograms indicate staining without probes ('– probe'; negative control) and solid-line, filled histograms are staining with probe ('+ probe').

SUPPLEMENTARY TABLES

Supplementary Table 1 Antibodies used in this study.

Antibody	Clone (Cat#)	Fluorophore	Manufacturer	Dilution	Application
anti-KIR3DL1/S1	Z27.3.7	unconjugated, PE	Beckman Coulter	1:5	Flow cytometry, blocking assays
anti-KIR3DL1	DX9	unconjugated, PE, BV421	BioLegend	1:25	Flow cytometry, blocking assays
anti-KIR3DL1/L2	5.133	PE	Miltenyi	1:5	Flow cytometry
anti-KIR3DL2	539304	PE	R&D	1:5	Flow cytometry
anti-KIR2DL2/L3	DX27	PE, PE-Cy7	Miltenyi	1:50	Flow cytometry
anti-pan-HLA-I complex	W6/32	unconjugated, BV510, PE, APC	BioLegend	1:20	Flow cytometry, blocking assays
anti-HLA-I OC (HC10)	HC10	unconjugated	tebu-bio	1:10	Flow cytometry, blocking assays
anti-HLA-I OC (HCA2)	HCA2	unconjugated	tebu-bio	1:10	Flow cytometry, blocking assays
anti-CD3 (OKT3)	OKT3	unconjugated	BioLegend	(see text)	plate coating
anti-CD28	CD28.2	unconjugated	BioLegend	(see text)	plate coating
anti-CD3	HIT3a	PE, FITC, AF700, PerCP-Cy5.5	BioLegend	1:50	Flow cytometry
anti-CD4	RPA-T4	PE, Pacific Blue	BioLegend	1:50	Flow cytometry
anti-CD69	FN50	BV421	BioLegend	1:50	Flow cytometry
anti-tetherin	RS38E	APC	BioLegend	1:20	Flow cytometry
anti-HIV-1 p24	KC57	FITC	BD Biosciences	1:100	Flow cytometry
anti-human IgG(Fc) $F(ab')_2$	(Cat#: H10104)	PE	Thermo Fisher	1:50	Flow cytometry (secondary stain)
mouse anti-β-actin	mAbcam 8224	unconjugated	Abcam	1:5,000	Immunoblotting (primary stain)
rabbit anti-HLA-F	EPR6803	unconjugated	Abcam	1:1,000	Immunoblotting (primary stain)

Antibody	Clone (Cat#)	Fluorophore	Manufacturer	Dilution	Application
goat anti-rabbit IgG(H+L)	(Cat#: 926-68071)	IRDye 680RD	LI-COR	1:10,000	Immunoblotting (secondary stain)
anti-CD56	HCD56	BV421	BioLegend	1:50	Flow cytometry
anti-CD16	3G8	BV785	BioLegend	1:50	Flow cytometry
anti-NKG2A	Z199	APC	Beckman Coulter	1:25	Flow cytometry
anti-LILRB1	GHI/75	PE-Cy5	BD Biosciences	1:10	Flow cytometry
anti-KIR2DL1/S1	HP-MA4	PE, PerCP-Cy5.5	BioLegend	1:50	Flow cytometry
mIgG1 isotype control	MG1-45	unconjugated	BioLegend	(see text)	Flow cytometry, blocking assays
anti-CD107a	H4A3	PE-Cy7	BioLegend	1:66.7	Flow cytometry
anti-IFN-γ	4S.B3	AF647	BioLegend	1:20	Flow cytometry
anti-TNF-α	MAb11	BV650	BioLegend	1:20	Flow cytometry
anti-MIP-1β	FL34Z3L	PerCP-eFluor710	eBioscience	1:20	Flow cytometry