Supporting Information

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SI Materials and Methods

Mice and Cell Lines. All mice were bred and maintained at the CIML or at Innate Pharma animal facilities in specific pathogen-free conditions. C57BL/6J (B6) mice were purchased from Charles River. KbDbKO mice were kindly provided by F. Lemonier (Pasteur Institute, Paris). C57BL/6-HLA-Cw3 transgenic (TgHLA) mice were previously described (1). TgKIR mice were previously generated in the laboratory and were back-crossed on B6 background (2). These 3 strains were crossed to obtained KbDbKO-TgKIR, KbDbKO-TgHLA and KbDbKO-TgKIR/HLA. YAC-1 is a Moloney virus-induced lymphoma of the A/Sn strain that down-regulates its MHC class I expression upon prolonged in vitro culture. RMA (H-2b) is a sub-line of RBL-5, originating from the EL-4 T cell lymphoma, induced in a B6 mouse.

Receptor Saturation Assay. Blood or spleen lymphocytes from mice treated or not with anti-KIR mAb were purified, washed 2 times, and stained with PE-labeled GL183 mAb for detection of free KIR2DL3 receptors. For each mouse, the mean fluorescence intensity (MFI) of PE-GL183 before dosing was measured by FACS and compared with the MFI of GL183-PE staining obtained at each time point. Acquisition of the samples was standardized to allow the comparison of MFI values across several time points. Percentages of KIR2DL3 receptor occupancy were calculated for each mouse using the following formula: %KIR2DL3 receptor occupancy = $100 - [100\times(\text{MFI time X/MFI PD})]$, where MFI PD is the mean fluorescence intensity of PE-GL183 at predose and MFI time X is the MFI of PE-GL183 obtained at each time point.

Antibodies and FACS Analysis. MAbs used for cytometry were: anti-NK1.1 (PK136)-APC, anti-CD3 (145–2C11)-PerCP-Cy5.5, anti-IFN-γ (XMG1.2)-PE, anti-CD107a (1B4B)-FITC (BD Biosciences), anti-NKp46 (29A1.4)-Alexa 647 (3). Fc receptors were blocked by incubation with anti-FcγRII/III (2.4G2) during staining. Results were acquired with a FACSCanto or a FACSDiva (BD Biosciences) and analyzed using the flowJo software (Three Star).

In Vitro Stimulation Assay. Splenocytes (5×10^5) from naïve mice were either co-cultured with YAC-1 cells (2×10^5) or added in plates precoated (overnight at 4 °C) with 25 μg/mL purified anti-NK1.1 mAb (PK136) or isotype control. For negative and positive controls, cells were incubated in culture medium alone or were stimulated with a mix of IL-12 (25 ng/mL, Cliniscience) and IL-18 (20 ng/mL, MDL) or with a mix of PMA (200 ng/mL) and ionomycin (5 μg/mL). Plates were incubated at 37 °C for 4 h followed by cell surface staining as described above. For intracellular IFN-γ detection, cells were fixed and permeabilized using the Cytofix/cytoperm kit (BD Pharmingen) followed by intracellular staining using Perm/wash (BD Pharmingen).

Monitoring of Blood Parameters. A fully automated hematology analyzer, the ABX Pentra 60 C + (HORIBA MEDICAL Corporate) was used for in vitro diagnostic of whole blood specimen after calibration to mouse cells.

Statistical Analysis. Statistical analyses were performed using the Prism software. Data obtained in different groups of mice were compared using a nonparametric, one-tailed Mann–Whitney (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

1. Dill O, Kievits F, Koch S, Ivanyi P, Hämmerling GJ (1988) Immunological function of HLA-C antigens in HLA-Cw3 transgenic mice. *Proc Natl Acad Sci USA* 85:5664–5668.
2. Cambiaggi A, et al. (1997) Natural killer cell acceptance of H-2 mismatch bone marrow grafts in transgenic mice expressing HLA-Cw3 specific killer cell inhibitory receptor. *Proc Natl Acad Sci USA* 94:8088–8092.
3. Walzer T, et al. (2007) Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46. *Proc Natl Acad Sci USA* 104:3384–3389.

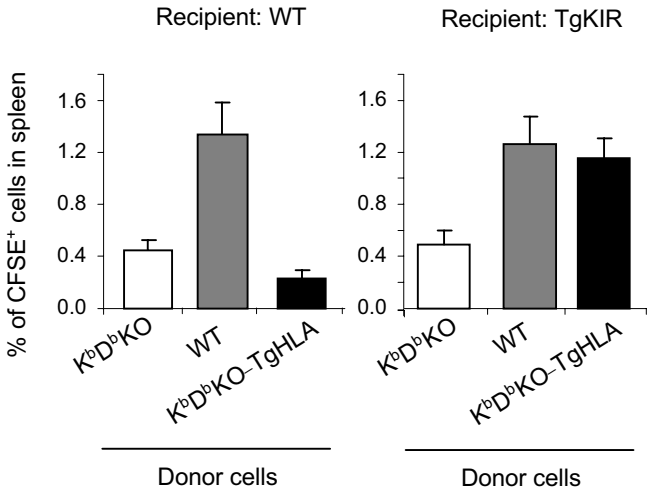

Fig. S1. Engagement of KIR2DL3 by its ligand HLA-Cw3 protects target cells from lysis in vivo. WT, K^bD^bKO, and K^bD^bKO-TgHLA were stained with the fluorescent dye CFSE, mixed at an equal ratio, and transferred into WT or TgKIR recipient. Mice were killed 40 h after injection, and the percentage of CFSE-labeled cells in spleen cells was assessed by flow cytometry, 3 mice per group ($n = 4$).

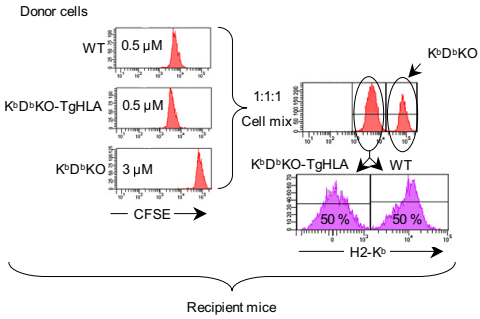

Fig. S2. Identification of 3 populations of CFSE labeled splenocytes after transfer in recipient mice. WT and K^bD^bKO-TgHLA splenocytes were labeled with CFSE (0.5 μM) and subsequently differentiated by staining with an anti-H-2K^b/D^b mAb by FACS analysis. K^bD^bKO cells were labeled with CFSE (3.0 μM). The 3 subsets were mixed at a ratio 1:1:1 before transfer to recipient mice.

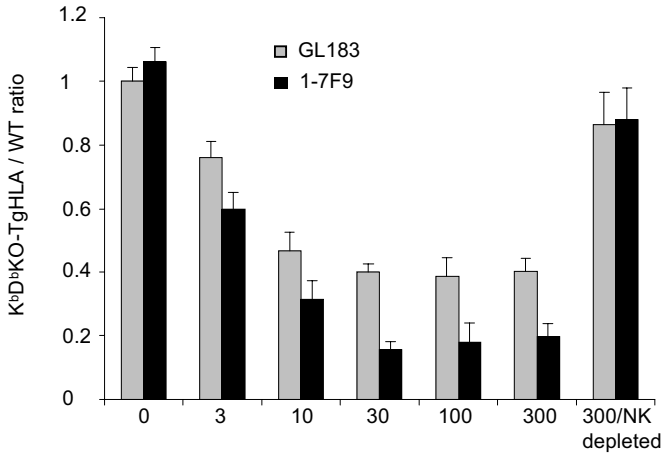

Fig. S3. 1–7F9 and GL183 anti-KIR mAbs induce the in vivo elimination of HLA$^+$ cells in TgKIR mice. WT and K^{b}D^{b}KO-TgHLA splenocytes were stained with CFSE, mixed at an equal ratio, and transferred into RagKO-TgKIR recipients which were pretreated or not with increasing doses of the anti-KIR mAbs 1–7F9 or GL183 (from 3 to 300 μg per mouse). For a group of mice, treated with the highest dose of mAbs (300 μg per mouse), NK cells were depleted with anti-NK1.1 mAbs a day before cell transfer. Recipient mice were killed 20 h after injection, and the percentage of each cell type among CFSE-labeled cells was assessed in peripheral blood lymphocytes of the recipients by flow cytometry. The mean (+/− SD) of the ratio between K^{b}D^{b}KO-TgHLA and WT CFSE+ cells is shown (3 mice per group).

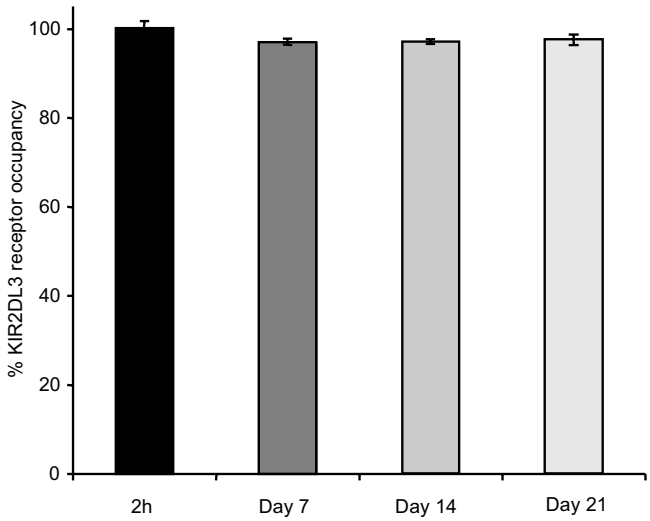

Fig. S4. KIR2DL3 receptor saturation during the course of long-term treatment with GL183 mAb. GL183 mAb (400 μg) was injected in K^bD^bKO-TgKIR/HLA mice every 4 days starting at day 0. KIR2DL3 receptor saturation was assessed on peripheral NK cells by staining peripheral blood mononuclear cell with PE-labeled-GL183 mAb. Percentages of KIR2DL3 receptor occupancy were calculated for each mouse at each time point: 2h (n = 12), 7 days (n = 4), 14 days (n = 4), or 21 days (n = 4) after the first injection.

Table S1. Blood parameters before and after treatment with anti-KIR mAb

	WBC, 10^3/ml	RBC, 10^6/ml	Hgb, g/dL	Hct, %	MCV, μm^3	RDW, %	Plt, 10^3/ml	MPV, μm^3	Mouse weight, g
Anti-KIR mAb									
Day 0 (n = 12)	13.9 (3.9)	9.1 (0.7)	13.3 (1.7)	41.0 (5.9)	44.7 (4.4)	13.5 (1.8)	1076.9 (240.6)	6.0 (0.5)	24.4 (4.0)
Day 7 (n = 12)	12.8 (5.2)	9.1 (0.8)	13.3 (2.4)	41.7 (7.0)	45.5 (4.7)	14.1 (1.7)	1198.8 (346.7)	6.0 (0.4)	24.9 (3.7)
Day 14 (n = 8)	11.1 (3.1)	9.3 (0.9)	13.3 (2.7)	41.8 (7.5)	44.5 (4.8)	13.8 (1.0)	1134.1 (440.6)	6.1 (0.4)	25.8 (3.5)
Day 21 (n = 4)	10.1 (2.2)	9.1 (1.1)	12.9 (2.9)	40.6 (8.4)	44.2 (5.0)	14.7 (0.7)	1026.0 (507.5)	6.9 (0.6)	24.1 (4.3)
Mock-injected									
Day 0 (n = 6)	16.3 (4.9)	9.2 (1.4)	13.2 (2.8)	40.0 (8.3)	43.6 (5.1)	14.8 (3.0)	1185.2 (425.5)	6.5 (1.0)	24.5 (5.1)
Day 7 (n = 6)	13.9 (5.3)	8.6 (1.2)	11.6 (2.9)	35.6 (7.6)	41.5 (6.3)	13.7 (1.8)	1330.3 (601.6)	6.9 (0.8)	24.8 (5.5)
Day 14 (n = 4)	12.9 (2.9)	9.7 (1.5)	13.5 (3.7)	42.2 (10.9)	43.1 (5.1)	14.0 (3.1)	922.3 (560.9)	6.5 (0.7)	23.6 (3.6)
Day 21 (n = 2)	11.7	10.2	15.4	48.0	47.0	14.2	811.5	6.1	22.7

K^bD^bKO-TgKIR/HLA mice were treated every 4 days by 400 μg of anti-KIR mAb GL183 starting at day 0 or with PBS in mock-injected mice. Blood parameters were measured at day 0, 7, 14, and 21 using the hematology analyzer ABX Pentra 60 C+ (HORIBA ABX Diagnostic), which has been calibrated for mouse cells. Multiple parameters were measured, including white blood cells (WBC), red blood cells (RBC) or platelet (Plt) counts, hemoglobinemia (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), red cell distribution width (RDW), mean platelet volume (MPV), and mouse weight. Numbers represent means (SD). The number of mice tested at each time point is indicated.

Table S2. Leukocyte subsets before and after treatment with anti-KIR mAb

%	% Lymphocytes	% Monocytes	% Neutrophils	% Eosinophils	% Basophils
Anti-KIR mAb					
Day 0 ($n = 12$)	72.7 (5.7)	10.0 (3.5)	14.7 (2.3)	2.3 (1.8)	0.3 (0.1)
Day 7 ($n = 12$)	72.3 (4.5)	10.2 (2.8)	14.9 (2.6)	2.3 (1.0)	0.2 (0.1)
Day 14 ($n = 8$)	74.2 (3.4)	8.9 (2.0)	14.7 (3.2)	2.0 (1.1)	0.2 (0.2)
Day 21 ($n = 4$)	74.3 (3.8)	15.0 (7.0)	9.6 (9.1)	0.9 (1.2)	0.3 (0.2)
Mock-injected					
Day 0 ($n = 6$)	64.1 (11.3)	11.2 (2.2)	20.1 (8.3)	2.0 (0.7)	0.2 (0.1)
Day 7 ($n = 6$)	63.7 (6.9)	12.3 (1.7)	20.3 (6.4)	2.3 (1.2)	1.4 (1.7)
Day 14 ($n = 4$)	62.8 (15.3)	10.2 (1.3)	16.2 (3.2)	2.5 (1.0)	0.3 (0.1)
Day 21 ($n = 2$)	74.0	17.0	7.4	1.4	0.3

K^bD^bKO-TgKIR/HLA mice were treated every 4 days by 400 μg of anti-KIR mAb GL183 starting at day 0 or with PBS in mock-injected mice. Blood parameters were measured at day 0, 7, 14, and 21. Among leukocytes the percentage of lymphocytes, monocytes, neutrophils, eosinophils, and basophils was assessed by using the ABX Pentra 60 C+. Numbers represent means (SD). The number of mice tested at each time point is indicated.