Supporting Information

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SI Materials and Methods

Mice. C57BL/6 mice were obtained from CLEA Japan. MHCII *H2-Ab1*–deficient mice (1) were kindly provided by D. Mathis (Harvard Medical School, Boston, MA). C57BL/6 mice deficient in *perforin*, *IFN-γ*, or *β2-microglobulin* were obtained from The Jackson Laboratory. OT-II transgenic/rag-1 knockout mice were obtained from Taconic. DAP12 knockout mice and DAP10/12 double knockout mice were generated as described previously (2).

Adoptive Natural Killer Cell Transfer Assay. Natural killer (NK) cells were prepared from WT B6 mice or *H2-Ab1* knockout mice as described previously (3). In brief, spleen cells were incubated with anti-CD4 and anti-CD8 mAbs, followed by magnetic beads coated with goat anti-mouse Ig and goat anti-rat Ig Abs (Advanced Magnetics). After removing CD4, CD8, and surface Ig-positive cells by magnetic cell sorting, NK cells were stained with phycoerythrin (PE)-labeled DX5 mAb (BioLegend) and purified using anti-PE MACS beads (Miltenyi Biotec). Purified NK cells were cultured with rhIL-2 (1,000 U/mL; Wako) for 5 d. These activated NK cells (5×10^6 per mouse) were labeled with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) (2 μM; Invitrogen) and transferred i.v. into WT B6 mice or *H2-Ab1* knockout mice. The following day, mice were killed, and splenocytes were stained with PE–anti-NK1.1 and antigen-presenting cell (APC)–anti–I-A^b mAbs (BioLegend). Expression of I-A^b and NK1.1 on $CFSE^+$ cells was analyzed on a FACSCanto II (BD Biosciences).

RT-PCR. Splenic $NK1.1^+$ cells were purified from polyI:C (100 μg per mouse; InvivoGen)-treated mice or naive mice as described above. Splenic $CD11c^+$ dendritic cells (DCs) were purified as described previously (4). In brief, spleens were digested with 400 U/mL collagenase (Wako) in the presence of 5 mM EDTA and separated into low- and high-density fractions on an OptiPrep gradient (Axis-Shield). Low-density cells were purified using anti-CD11c MACS beads (Miltenyi Biotec). Then I-Ab^+ $NK1.1^+$ cells or $NK1.1^-$ $CD11c^+$ cells were further purified on an Epics ALTRA cell sorter (Beckman Coulter) (>98% I-Ab^+ $NK1.1^+$ cells; >96% $NK1.1^-$ $CD11c^+$ cells). Total RNA was purified from these purified cells using Sepasol (Nacalai). Complementary DNAs were synthesized from total RNAs by using oligo(dT) primer (Invitrogen). PCR was performed by using 10- or 100-fold serially diluted cDNA templates and AmpliTaq poly (Applied Biosystems). Primers used in this study were as follows: Klrk1-F, 5′-ATG GCA TTG ATT CGT GAT CGA AAG-3′; Klrk1-R, 5′-TTA CAC CGC CCT TTT CAT GCA GAT-3′; H2-Ab1-F, 5′-CGC ATA CGA TAT GTG ACC AGA TAC-3′; H2-Ab1-R, 5′-CAA CAT CAT TTT GCT CCA GGC AGA CTC-3′; class II transactivator (Ciita)-F, 5′-ATG CGC TGC CTG GTT CCT GGC CCT TCT-3′; Ciita-R, 5′-TCC CTG GGG CAG AGT GGT GAA GAT-3′; Actb-F, 5′-ATG GAT GAC GAT ATC GCT GCG CTG-3′; Actb-R, 5′-GTA GCC ACG CTC GGT CAG GAT CTT-3′.

ELISA for DC-Derived Exosomes. Anti-mouse CD9 mAb (10 μg/mL; BioLegend) was precoated on a 96-well EIA/RIA plate (Costar). After blocking with 5% FCS/PBS at room temperature for 1 h, the culture supernatants were added to the wells and incubated at room temperature for 2 h. After washing with PBS containing 0.05% Tween 20, exosomes were detected by biotinylated anti–I-A^b mAb (BioLegend) and streptavidin-HRP (eBioscience).

NK–DC Interaction. CFSE (0.5 μM)-labeled IL-2 (1,000 U/mL)-activated NK cells (5×10^5 per well) and splenic DCs (5×10^5 per well) were cocultured in a 96-well flat-bottom plate for the indicated periods at 37 °C. For the transwell assay, NK cells (3×10^6 per well) were cultured in the upper chamber of a 12-well plate and separated from DCs (3×10^6 per well) by a 0.4 μm-pore semipermeable membrane (Greiner Bio-One). As controls, NK cells and DCs (3×10^6 per well) were cultured together in a 12-well plate. For the inhibition assay, NK cells or DCs were pretreated for 1 h with the following reagents: piceatannol (100 μM), LY294002 (100 μM), cytochalasin D (20 μM), PP2 (50 μM), paraformaldehyde (4%; Wako), polyI (10 μg/mL), dextran sulfate (10 μg/mL; Sigma), RGDS peptides (10 mM; Peptide Institute), NaN_3 (50 mM; Nacalai), and mAbs against FcγRIIb/III (2.4G2, 10 μg/mL; BD Biosciences), FasL (K10, 10 μg/mL), or TRAIL (N2B2, 10 μg/mL; BioLegend). After coculture for the indicated times, cells pretreated with 2.4G2 mAb were stained with the following mAbs: APC-labeled anti–I-A^b, anti-CD80, anti-CD86, or anti-CD11c (BioLegend). In some assays, NK cells were then purified by cell sorting and analyzed via flow cytometry.

In Vitro Antigen Presentation Assay. The antigen presentation assay was performed as described previously (4) with some modifications. Bone marrow-derived DCs (BMDCs) were generated by rmGM-CSF (40 ng/mL; PeproTech). CFSE-labeled NK cells (1×10^7 per well) and BMDCs (5×10^6 per well) were cocultured in a 6-well plate for 1 h. Then these cells were stained with APC-anti–I-A^b mAb, followed by sorting into I-A^{b+} and I-A^{b-} NK subsets on an Epics ALTRA cell sorter (Beckman Coulter) (>98% I-A^{b+} $CFSE^+$; >99% I-A^{b-} $CFSE^+$). BMDCs (5×10^3 per well), and/or sorted NK cells (5×10^3 per well) were cocultured with CFSE (10 μM)-labeled OT-II $CD4^+$ T cells (5×10^4 per well) in a 96-well U-bottom plate for 3 d in the presence of $OVA_{323–339}$ peptides (10 ng/mL; Abgent). CFSE fluorescence intensity of OT-II $CD4^+$ T cells was analyzed by flow cytometry. Production of IL-2 in the culture supernatant at 48 h post-addition of OT-II $CD4^+$ T cells was measured by ELISA (eBioscience). For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

In Vivo OT-II $CD4^+$ Cell Proliferation Assay. OT-II $CD4^+$ cell proliferation in spleen was analyzed as described previously (4) with some modifications. CFSE-labeled naive OT-II $CD4^+$ T cells (3×10^6 per mouse) were adoptively transferred into B6 mice. The next day, mice were i.v. injected with $OVA_{323–339}$ (1 μg/mL)-loaded splenic DCs (3×10^6 per mouse) or a mixture of these DCs (3×10^6 per mouse) and NK cells cocultured with these DCs together (I-A^{b+} NK cells; 3×10^6 per mouse) or separately (I-A^{b-} NK cells; 3×10^6 per mouse). Two days later, mice were killed, and CFSE dilution of $CD4^+$ $V\alpha2^+$ splenocytes was analyzed by flow cytometry. For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

Dermal Delayed-Type Hypersensitivity. A dermal delayed-type hypersensitivity study was performed as described previously (5) with some modifications. OT-II $CD4^+$ T cells were stimulated with $OVA_{323–339}$ (100 ng/mL) for 3 d, and then cells were maintained in IL-2 (10 U/mL)-containing medium without $OVA_{323–339}$ for another 3 d. These cells (6×10^6 per mouse) were transferred i.v. into B6 mice. The next day, recipient mice

were s.c. injected with 50 μL of $OVA_{323–339}$-loaded APCs (collagenase-digested whole splenocytes or BMDCs; 2×10^7 each per mouse) or a mixture of these APCs (2×10^7 per mouse) and NK cells cocultured with these DCs together (I-A^{b+} NK cells; 1×10^7 per mouse) or separately (I-A^{b-} NK cells; 1×10^7 per mouse) into the right footpad. The left footpads were injected with the same cell population without $OVA_{323–339}$ as controls. The following day, Δfootpad thickness was calculated by subtracting the left hind footpad thickness from the right. Three days after the s.c. injection, the OT-II $CD4^+$ $V\alpha2^+$ $V\beta5^+$ cell population migrating into popliteal lymph nodes was measured by flow cytometry. For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

1. Cosgrove D, et al. (1991) Mice lacking MHC class II molecules. *Cell* 66:1051–1066.
2. Inui M, et al. (2009) Signal adaptor DAP10 associates with MDL-1 and triggers osteoclastogenesis in cooperation with DAP12. *Proc Natl Acad Sci USA* 106:4816–4821.
3. Ogasawara K, et al. (2003) Impairment of NK cell function by NKG2D modulation in NOD mice. *Immunity* 18(1):41–51.
4. Nakayama M, et al. (2009) Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood* 113:3821–3830.
5. Ruth AJ, et al. (2004) An IL-12-independent role for CD40-CD154 in mediating effector responses: Studies in cell-mediated glomerulonephritis and dermal delayed-type hypersensitivity. *J Immunol* 173(1):136–144.

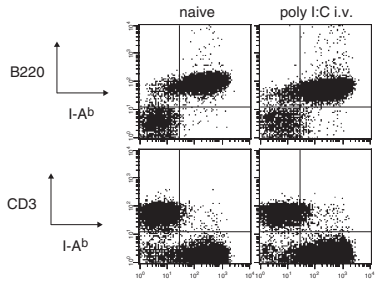

Fig. S1. Expression of MHCII on B and T cells. C57BL/6 mice were i.v. injected with 100 μg of polyI:C. The following day, collagenase-digested splenocytes from these mice or naive C57BL/6 mice were stained with anti-CD3, anti-B220, and anti–I-A^b mAbs. Similar results were obtained in three independent experiments.

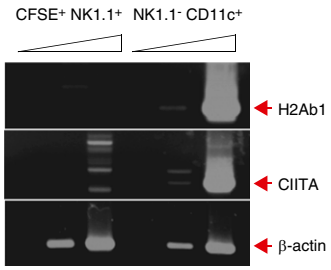

Fig. S2. Expression of H2-Ab1 and CIITA transcripts in purified $CFSE^+$ I-Ab^+ $NK1.1^+$ cells or $NK1.1^-$ $CD11c^+$ cells. IL-2-activated CFSE-labeled NK cells (5×10^6 per mouse) were transferred into mice. The following day, $CFSE^+$ $NK1.1^+$ cells in spleen were purified by cell sorting. $CD11c^+$ cells were purified as described in *SI Materials and Methods*. Expression of the indicated transcripts in purified cells was analyzed by semiquantitative RT-PCR using 100-fold serially diluted cDNA templates. Similar results were obtained in two independent experiments.

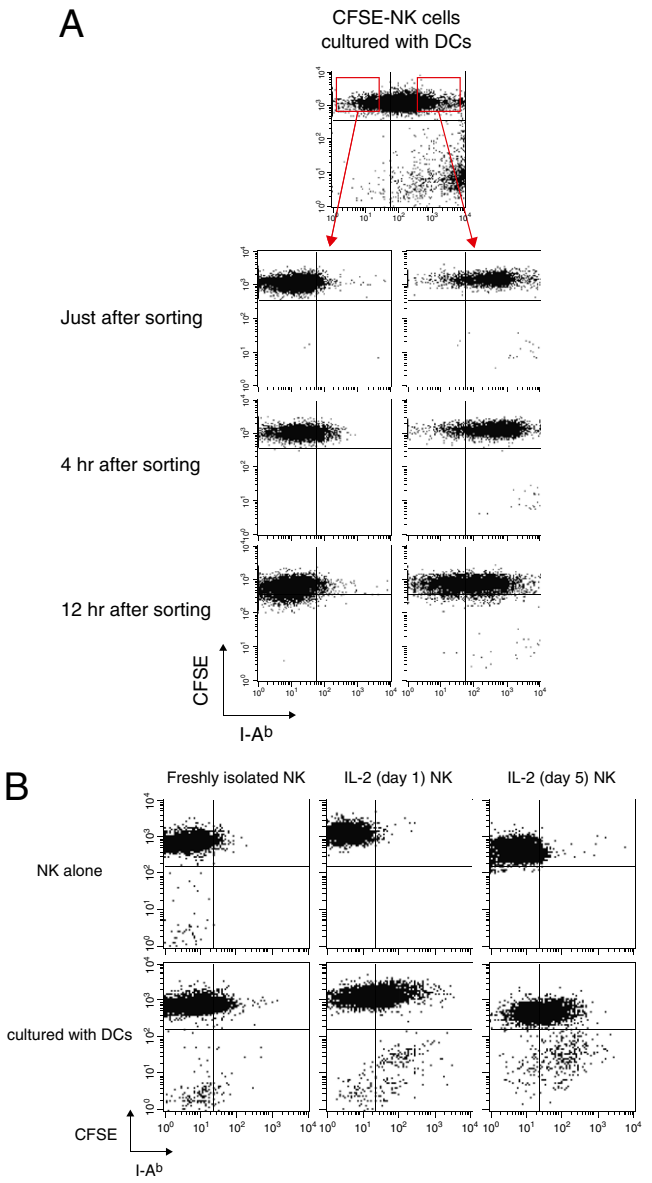

Fig. S3. Activated NK cells acquire MHCII. (*A*) Stability of MHCII on NK cells. I-Ab^{-} and I-Ab^{+} NK cells were purified by cell sorting after coculture with WT DCs. After the indicated times, cells were stained with anti–I-A^{b} mAb. Similar results were obtained in three independent experiments. (*B*) Freshly isolated NK cells do not acquire MHCII. Freshly isolated splenic NK cells or IL-2 (1,000 U/mL)-activated NK cells were labeled with CFSE and then cocultured with splenic CD11c^{+} cells at a 1:1 ratio for 1 h. I-A^{b} expression on NK1.1^{+} cells was analyzed. Similar results were obtained in two independent experiments.

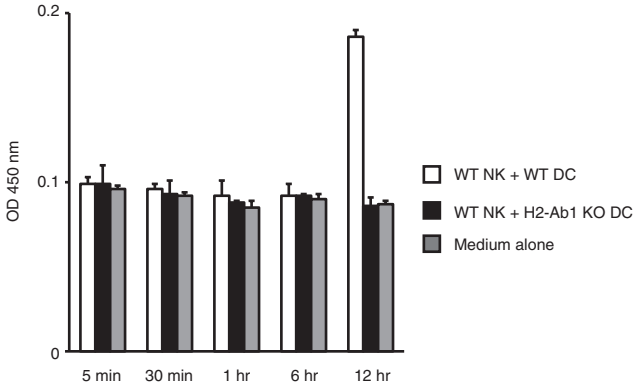

Fig. S4. Exosomes in NK/DC coculture supernatants. IL-2-activated WT NK cells were cocultured with WT or H2-Ab1 knockout splenic DCs at a 1:1 ratio for the indicated times. Exosomes in culture supernatants were detected by sandwich ELISA. Similar results were obtained in two independent experiments.

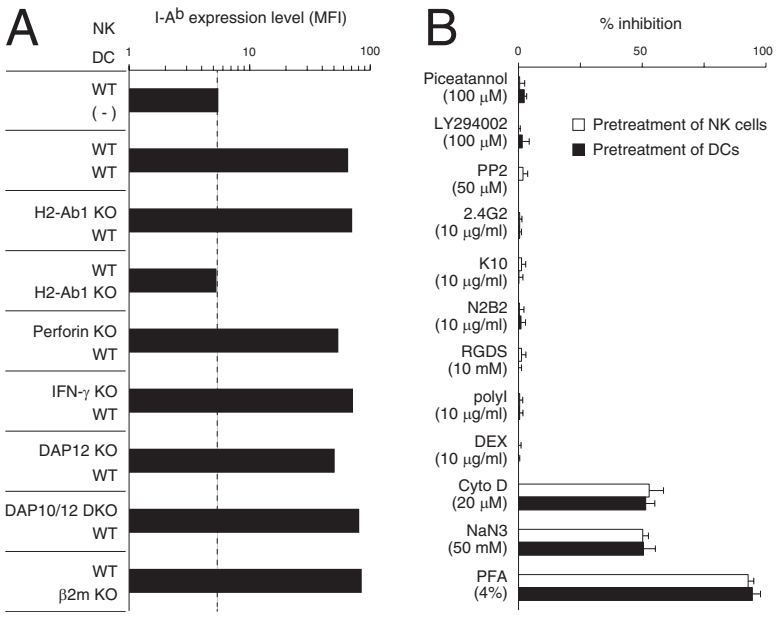

Fig. S5. Intercellular MHCII transfer is NK cell receptor-independent. (*A*) CFSE-labeled NK cells and splenic DCs from the indicated mice were cocultured for 30 min. Surface expression of I-A^b on NK cells was analyzed by flow cytometry, and the mean fluorescence intensity (MFI) is indicated. (*B*) NK cells (white columns) or DCs (black columns) were pretreated with the following inhibitors 1 h before coculture: piceatannol (a Syk family kinase inhibitor), LY294002 (a specific inhibitor of PI3K), PP2 (a Src kinase inhibitor), 2.4G2 (an anti-FcγRIIb/III–blocking mAb), K10 (an anti-FasL–blocking mAb), N2B2 (an anti-TRAIL–blocking mAb), RGDS (an integrin-antagonizing peptide), polyI (polyinosinic acid; a negatively charged reagent), DEX (dextran sulfate; a negatively charged reagent), cytochalasin D (an inhibitor of actin polymerization), NaN_3 (an ATP-depleting reagent), and PFA (paraformaldehyde; a cross-linking reagent). The expression level of I-A^b on NK cells was analyzed by flow cytometry. Percent inhibition was calculated by comparing the MFI in the presence of an inhibitor with the MFI in the absence of inhibitor. Data represent means + SD of triplicates. Similar results were obtained in three independent experiments.

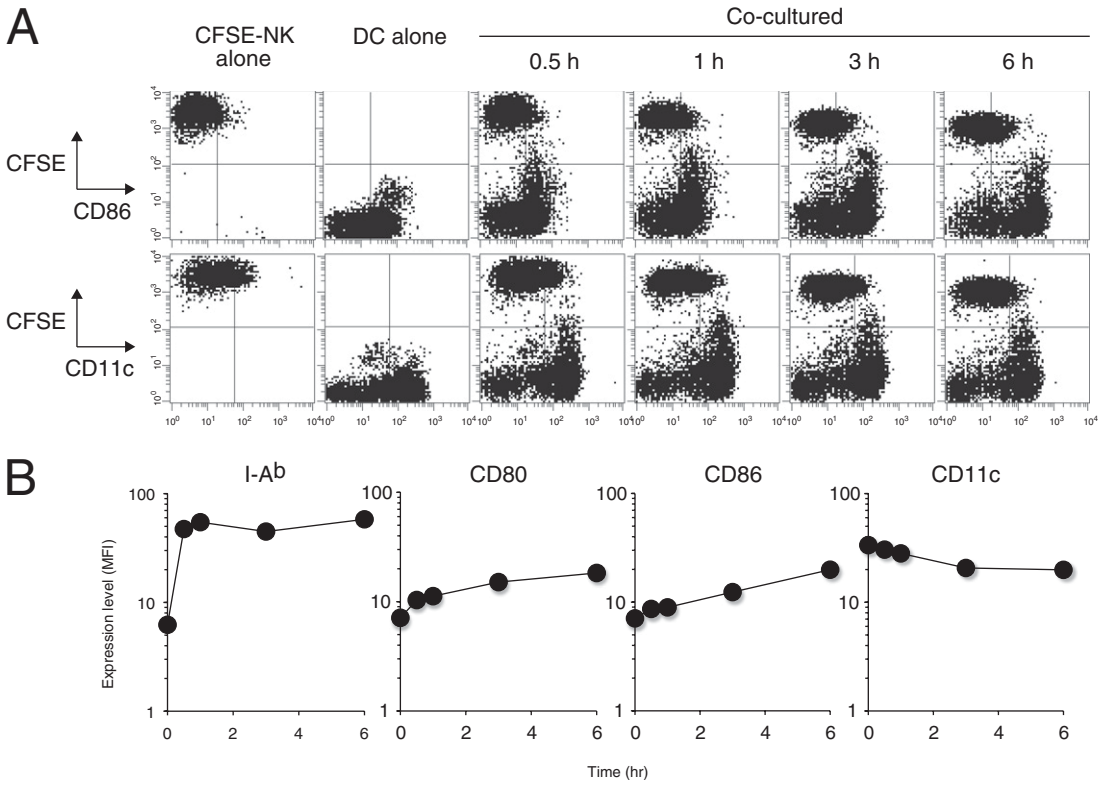

Fig. S6. Kinetic analysis of intercellular transfer of MHCII, CD80, CD86, and CD11c. (*A*) CFSE-labeled NK cells were cocultured with DCs at a 1:1 ratio. After the indicated periods, cells were stained with anti-CD86 and anti-CD11c mAbs. (*B*) The MFI of I-A^b, CD80, CD86, and CD11c expression on NK cells cocultured with DCs was analyzed by flow cytometry. Data represent the mean of triplicates. Similar results were obtained in two independent experiments.

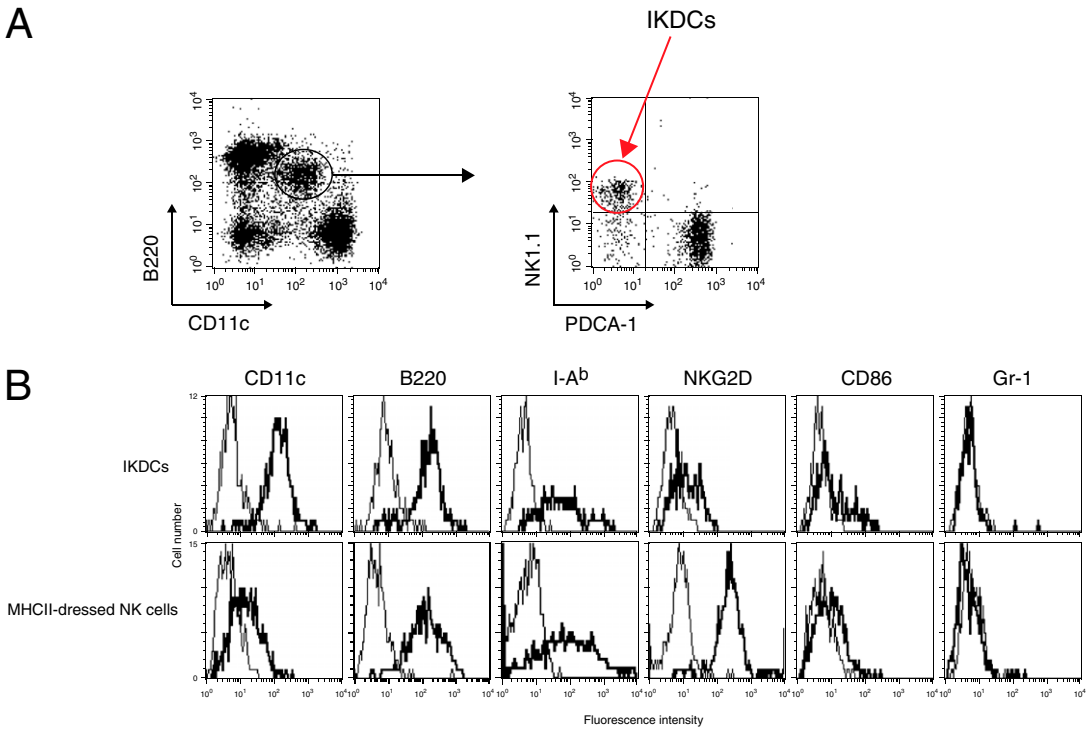

Fig. S7. Antigenic phenotypes of IFN-producing killer DCs (IKDCs) and MHCII-dressed NK cells. (*A*) Collagenase-digested low-density spleen cells were stained with anti-B220, anti-CD11c, anti-NK1.1, and anti–PDCA-1 mAbs. The putative IKDC population is indicated by a red arrow. (*B*) Putative IKDCs and MHCII-dressed NK cells were stained with the indicated mAb (thick histograms) or respective isotype control mAb (thin histograms). Similar results were obtained in three independent experiments.

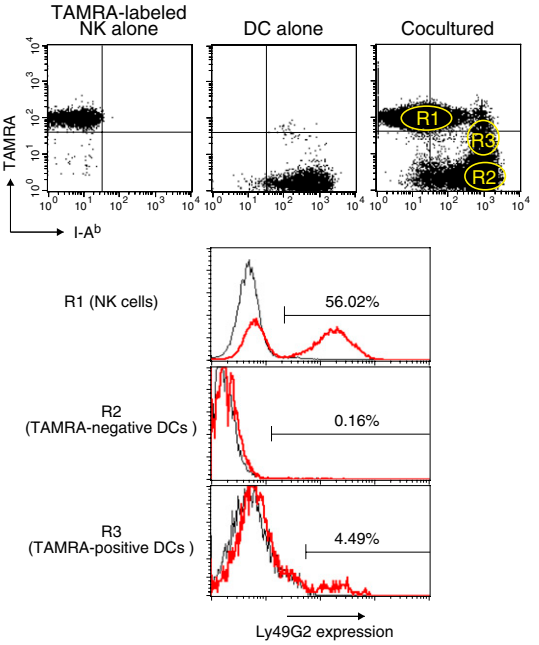

Fig. S8. DC acquisition of Ly49G2 from NK cells. TAMRA [5- (and 6-) carboxytetramethylrhodamine succinimidyl ester]-labeled activated NK cells were cocultured with splenic DCs at a 1:1 ratio for 1 h. Then cells were stained with anti–I-A^b and anti-Ly49G2 mAbs or control rat IgG_{2a}. In histograms, black and red lines indicate control $rIgG_{2a}$ and anti-Ly49G2 mAb staining, respectively. The percentage of Ly49G2-positive cells in R1, R2, and R3 is indicated. Similar results were obtained in two independent experiments.

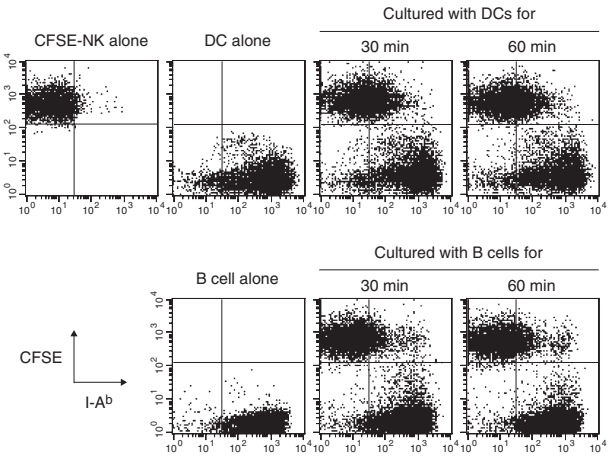

Fig. S9. NK acquisition of MHCII from B cells. CFSE-labeled activated NK cells were cocultured with splenic DCs or B cells at a 1:1 ratio for the indicated periods of time. Then cells were stained with anti–I-A^b mAb. Similar results were obtained in two independent experiments.