Supporting Information

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SI Materials and Methods

Cells. The human embryonic kidney cell line 293T was kindly provided by Dr. W. Uckert (MDC, Berlin) and was used as a packaging cell line for retroviral vectors as earlier described (1, 2). It was grown in DMEM supplemented with 10% (vol/vol) heat-inactivated FBS. PBMCs were collected from the blood of anonymous healthy donors and were handled as per institutional and local regulations. Mononuclear cells collected from the samples by centrifugation on a Lymphoprep density step (Bicoll, Biochrom AG) were washed in PBS and were frozen to maintain standard conditions. C1R/A2 and its transfectant C1R/A2HER2, which expresses HER-2, were described elsewhere (3). These cell lines were kept in RPMI medium 1640 with 10% (vol/vol) FBS and 200 U/ml Hygromycin B (Calbiochem, EMD Biosciences). Ovarian tumor cells were isolated from the ascitic fluid of patients with advanced epithelial ovarian cancer, the erythromyeloid leukemia K562, the ovarian carcinoma SKOV3 and the breast carcinoma MCF-7 cell lines were obtained as earlier described (3, 4). Control or HER-2 extracellular domain expressing lymphoblastoid cell lines (control mini LCL and HER-2 mini LCL, respectively) were produced by transforming B cells by control or HER-2 extracellular domain expressing miniEBV constructs (5).

The breast carcinoma cell lines were kindly provided by the following investigators from MDC: MDA MB 468 and MDA MB 453 (Dr. W. Uckert), HBL 100 and Cal 51 (Dr. W. Birchmeier, MDC) and MDA-N, ZR-75–1, and MDA MB 435 (Dr. S. Scherneck, MDC), all cultured in RPMI medium 1640, 10% (vol/vol) FBS, except Cal 51, which was cultured in DMEM, 10% (vol/vol) FBS, and ZR-75–1 which was cultured in RPMI medium 1640, 20% (vol/vol) FBS. All tissue culture media were purchased from GIBCO (Invitrogen).

Flow Cytometry and T cell Depletion. T cells and NKT cells were depleted using the "Dynabeads CD3 negative selection protocol" (Invitrogen). The following antibodies were used for immunophenotypic characterization of expanded and transduced cells: anti-CD3 conjugated to FITC or Cy5; anti-CD56 conjugated to PE or APC. Surface expression of the CR was determined using the $F(ab)_2$ fragment from goat anti-human Ig polyclonal Ab conjugated to PE or APC (Southern Biotech via Biozol). Surface expression of MHC class I molecules on tumor cell lines was determined using an anti HLA-ABC antibody conjugated to FITC. Surface expression of HER-2 on transformed LCL cells was determined using an anti HER-2 antibody conjugated to PE (antibodies from BD biosciences). Data acquisition and analysis was performed on a FACSCalibur (BD Biosiences) using CellQuest Pro (BD Biosciences) and FlowJo (Tree star) softwares.

^{51}Cr Release Assay. For direct detection of NK cell mediated lysis, the SKOV3 cell line was labeled for 1 h with 50 μCi sodium 51chromate (PerkinElmer), washed three times and adjusted to 5,000 cells per well in 100 μL volume of a 96-well V-bottom microtiter plate. Mock-transduced or CR-NK cells from 3 donors were added in 100-μl volume at various effector to target (E:T) ratios in triplicates, respectively. After 4 hours of incubation at 37 °C, 50 μL of supernatant from each well were harvested, transferred to a solid scintillator coated LumaPlate (PerkinElmer), dried over night and measured on a TopCount counter (Canberra Packard). Maximal ^{51}Cr release was determined by mixing wells and transferring supernatant containing SKOV3 cells. Percentage of specific lysis was calculated using the standard formula: 100 × (cpm experimental release−cpm spontaneous release) / (cpm maximum release−cpm spontaneous release).

IL-2 Secretion Assay. For detection of IL-2 secretion on a single-cell level, 5×10^5 mock or CR-transduced NK cells were cultured either alone or with equal number of SKOV3 target cells for 5 h. The assay was performed using the MACS IL-2 secretion Kit (Miltenyi Biotec) according to the manufacturer's protocol. Cells were additionally stained for CD3 and CD56 and analyzed on a FACSCalibur (BD Biosciences) using CellQuest Pro (BD Biosciences) and FlowJo (Tree star) software.

Cytokine Release Assay. Target cells (5×10^4) were cocultured with an equal number of effector cells in 96-well flat bottom plates for 24 h. The supernatant was assayed for IFN-γ and IL-2 by ELISA (Mabtech or BD Biosciences) according to the manufacturer's protocol. The data represents mean values of triplicates, derived from 1 representative experiment. Experiments were performed at least twice with similar results. The detection limit of the ELISA is 100 pg/ml IFN-γ or 20 pg/ml IL-2. Statistcal analysis was performed using SigmaStat 3.5 software (Statcon). *P* values were calculated using the Wilcoxon–Mann–Whitney test. Correlation between cytokines production and HER-2 expression level was calculated using Microsoft Excel 2007, (Microsoft Deutschland GmbH).

CD107a Degranulation Assay. The degranulation assay was performed as earlier described (6) with minor modification. Target cells (5×10^5) were cocultured with an equal number of effector cells in 1 mL per well in a 48-well plate. In some cases, labeling of effector cells with 0.4 μM CFSE was performed prior to coculture by using the CellTrace Kit (Invitrogen) according to the manufacturer's protocol. Control wells contained either NK cells alone or NK cells stimulated with PMA (2.5 μg/ml) and ionomycin (0.5 μg/ml). Anti-CD107a Ab (10 μL per well) conjugated to either PE or to PE-Cy5 (BD Biosciences) was added and incubated for 1 h at 37 °C. Golgi Stop (BD Biosciences) was added to a final concentration of 6 μg/mL and further incubated for 4 more hours. Cells were washed 2 times with PBS, optionally stained for CD56 and analyzed on a FACSCalibur.

1. Charo J, *et al.* (2005) Bcl-2 overexpression enhances tumor-specific T-cell survival. *Cancer Res* 65:2001–2008.
2. Sommermeyer D, *et al.* (2006) Designer T cells by T cell receptor replacement. *Eur J Immunol* 36:3052–3059.
3. Rongcun Y, *et al.* (1999) Identification of new HER2/neu-derived peptide epitopes that can elicit specific CTL against autologous and allogeneic carcinomas and melanomas. *J Immunol* 163:1037–1044.
4. Choudhury A, *et al.* (2004) Small interfering RNA (siRNA) inhibits the expression of the Her2/neu gene, upregulates HLA class I and induces apoptosis of Her2/neu positive tumor cell lines. *Int J Cancer* 108:71–77.
5. Moosmann A, *et al.* (2002) B cells immortalized by a mini-Epstein–Barr virus encoding a foreign antigen efficiently reactivate specific cytotoxic T cells. *Blood* 100:1755–1764.
6. Alter G, Malenfant JM, Altfeld M (2004) CD107a as a functional marker for the identification of natural killer cell activity. *J Immunol Methods* 294:15–22.

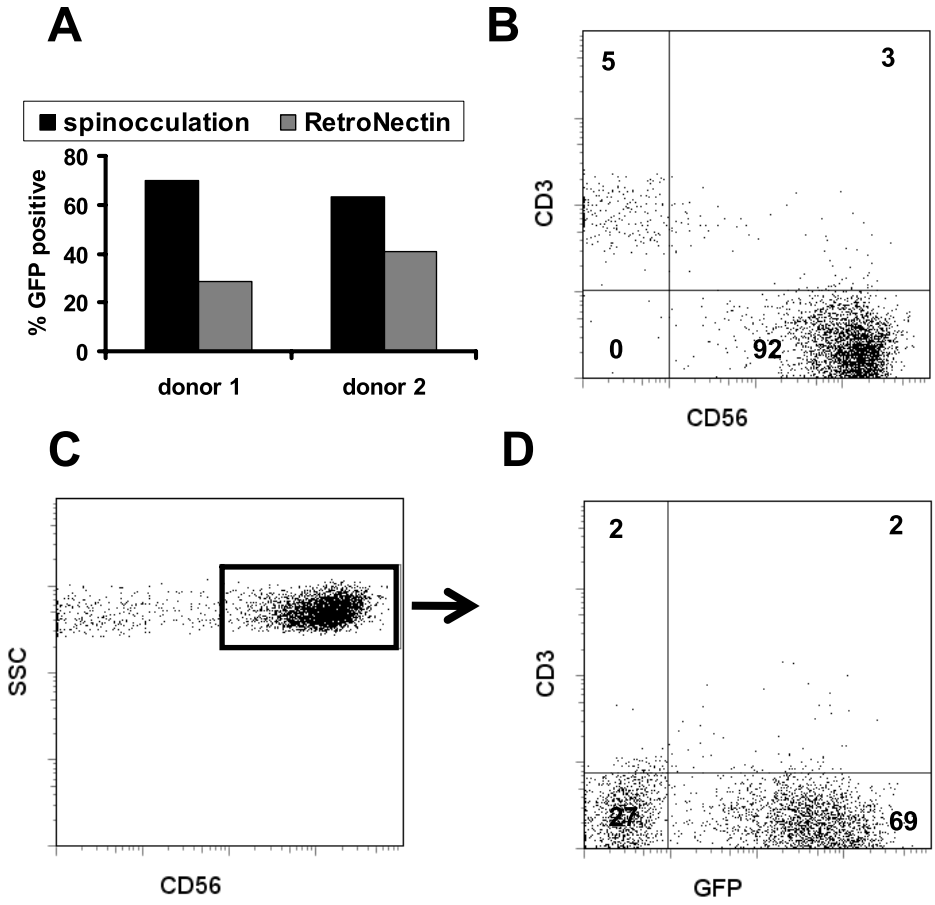

Fig. S1. High levels of NK transduction can be achieved by spinoculation. (*A*) NK cells from 2 donors were transduced with pMIG using either the spinoculation protocol or RetroNectin-coated plates and cells were then analyzed for GFP expression by FACS. (*B–D*) FACS analysis of CD3 and CD56 staining and GFP expression of transduced PBLs using the spinoculation protocol. Cells were gated on the $CD56^+$ population (*C*) and were then analyzed for CD3 and GFP expression (*D*).

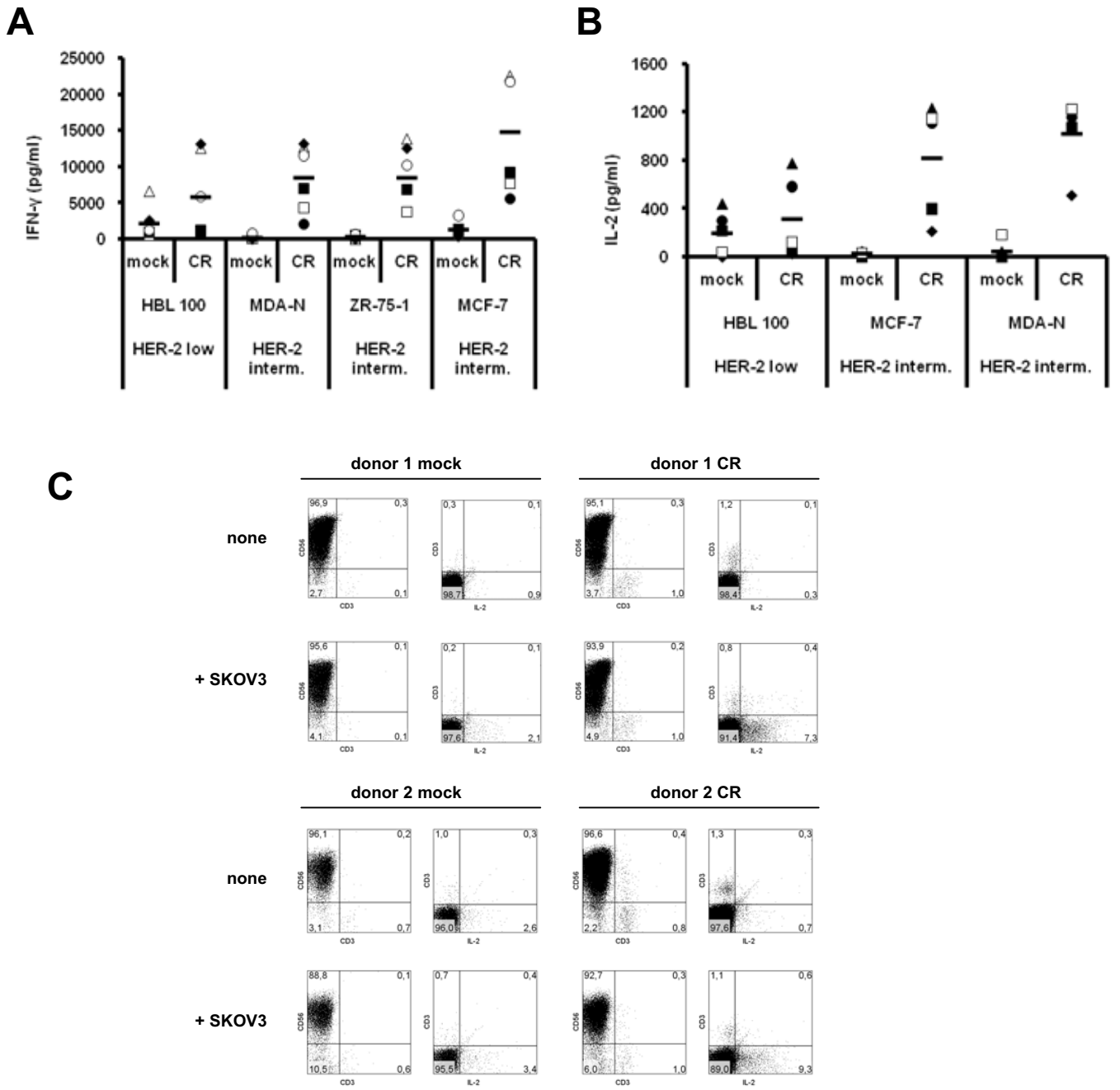

Fig. S2. CR-NK cells recognize HER-2$^+$ carcinoma. Effector cells were pMIG (mock)-transduced or CR-transduced NK cells. They were cocultured with equal numbers of the indicated carcinoma cell line. (*A* and *B*) Supernatants were harvested and measured for IFN-γ or IL-2 using specific ELISA. (A) IFN-γ production by NK cells derived from 6 donors in response to stimulation by 4 HER-2$^+$ carcinoma cell lines. (*B*) IL-2 production by NK cells derived from 5 donors in response to stimulation by 3 HER-2$^+$ carcinoma cell lines. (*C*) Mock- or CR-NK cells were cocultured alone or with SKOV3 as target and were measured for IL-2 production by IL-2 secretion assay.

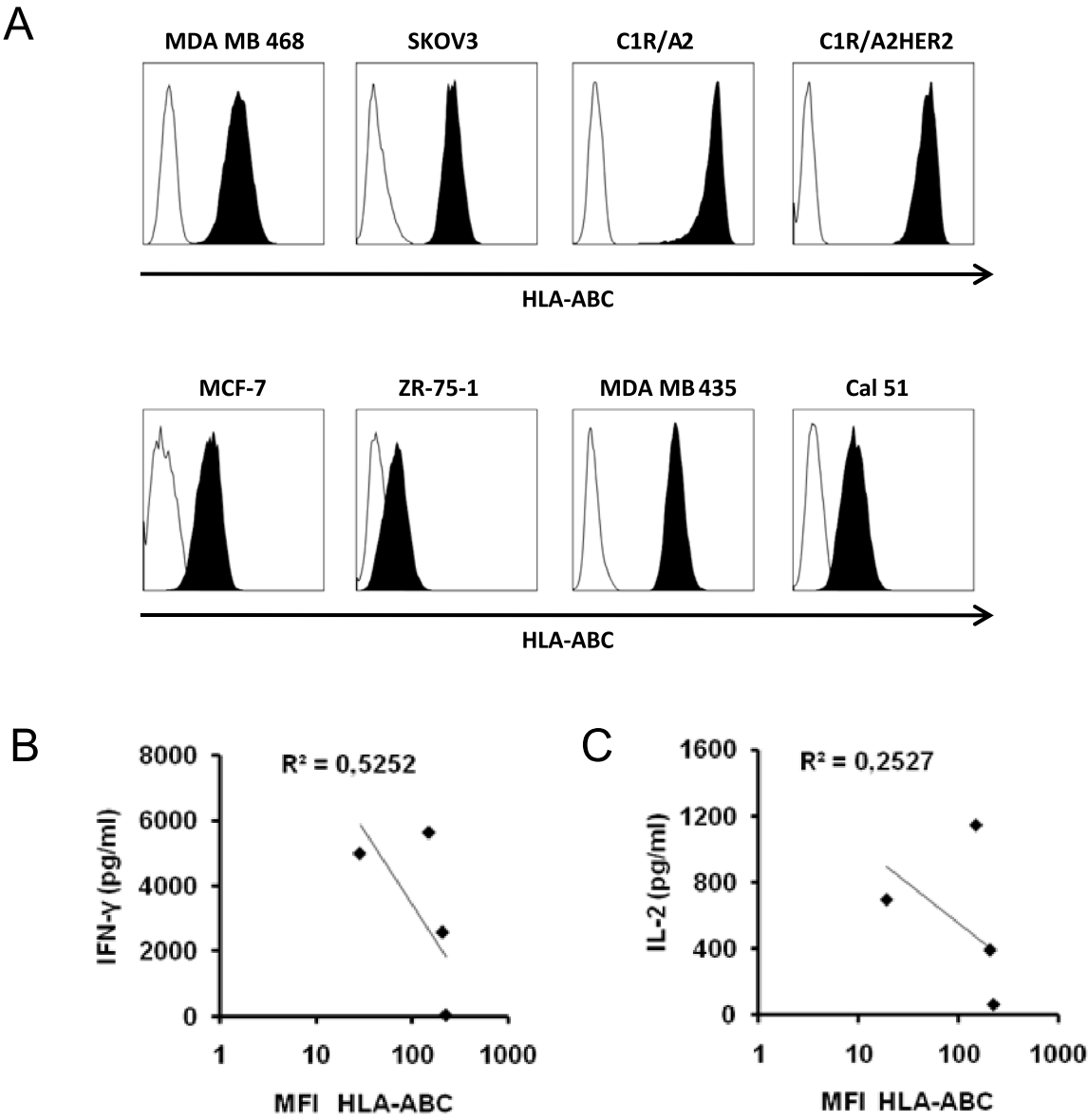

Fig. S3. No correlation of MHC Class I expression on tumor cell lines and cytokine production. (*A*) Various tumor lines were stained with an anti HLA-ABC antibody conjugated to FITC and analyzed for MHC Class I expression by flow cytometry. Open histograms represent the isotype control; filled histograms represent HLA-ABC expression in the respective overlay of each tumor cell line. Correlation between HLA-ABC expression levels on the different carcinoma lines and IFN-γ (*B*) or IL-2 (*C*) production levels by CR-NK cells in response to stimulation by the same tumor cell lines as depicted in Fig. 3 *C* and *E*.

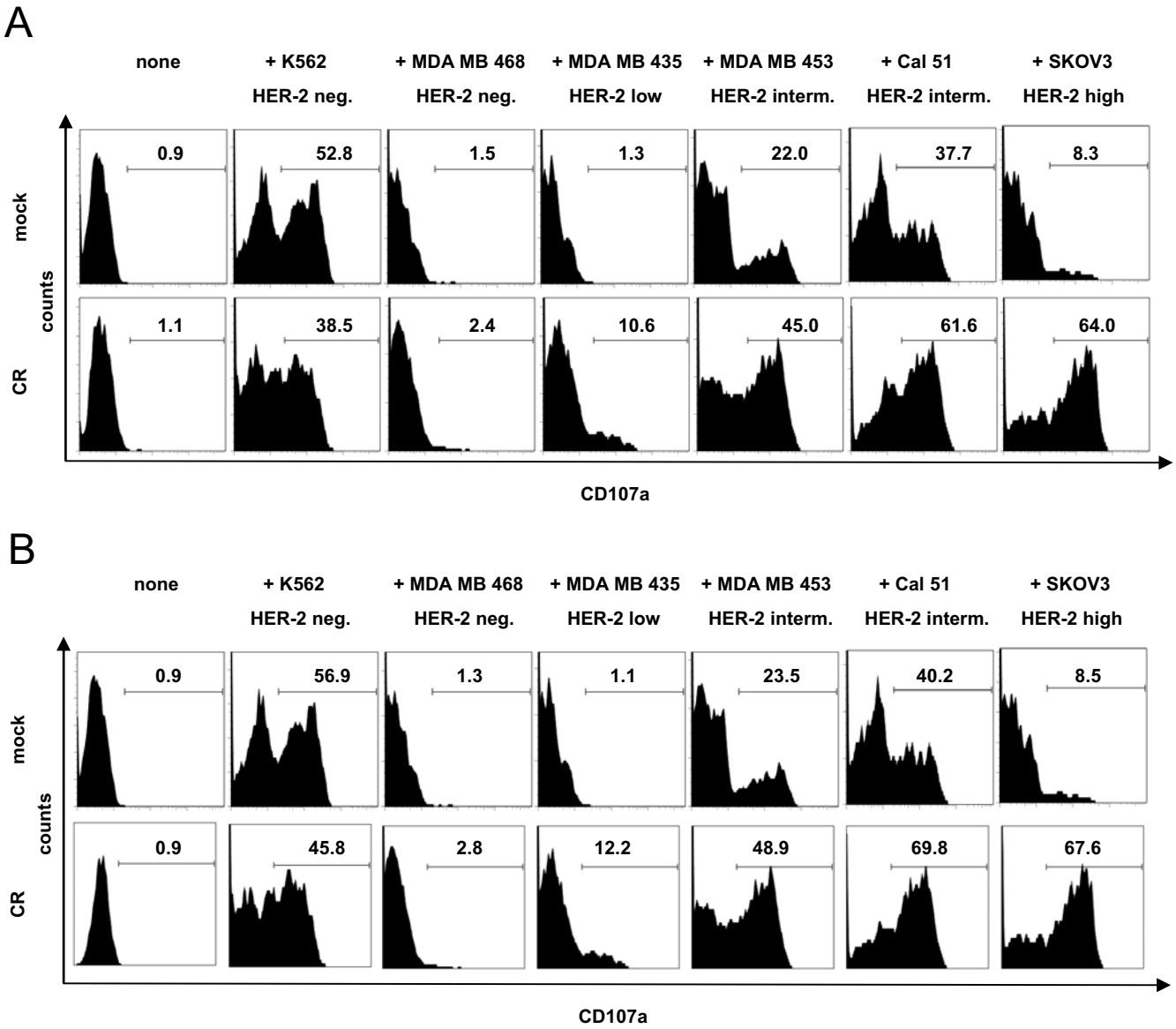

Fig. S4. CR-NK degranulate in response to HER-2 specific stimulation. (*A*) Effector cells gated on size and granularity were pMIG (mock)-transduced or CR-transduced NK cells from 1 donor different from the donor in Fig. 4*A*. They were either cultured without target cells (none), or cocultured with the indicated cell line for 5 h while being stained for CD107a conjugated with PE-Cy5. One representative experiment out of five performed is depicted. (*B*) The same NK cells as shown in *A* were gated based on $CD56^{+}$ staining and analyzed for CD107a staining.

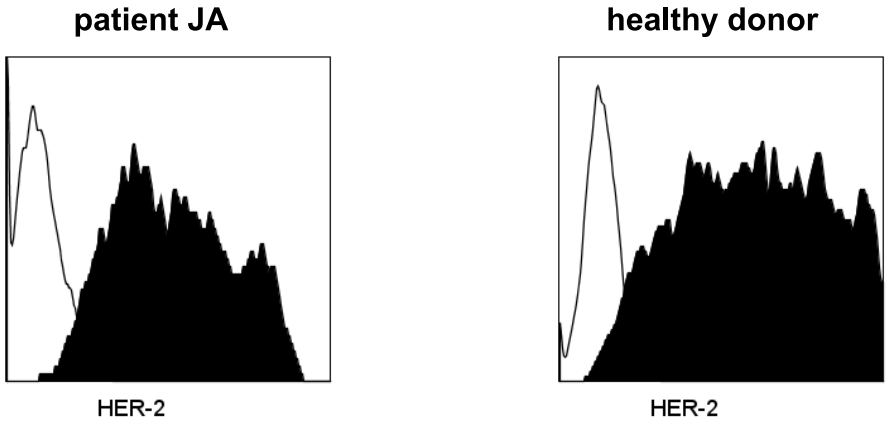

Fig. S5. HER-2 expression on mini LCLs. Mini LCLs from 2 donors were stained with an anti HER-2 antibody conjugated to PE and analyzed by flow cytometry. Open histograms represent control mini LCLs, filled histograms represent HER-2 mini LCLs in the respective overlay.

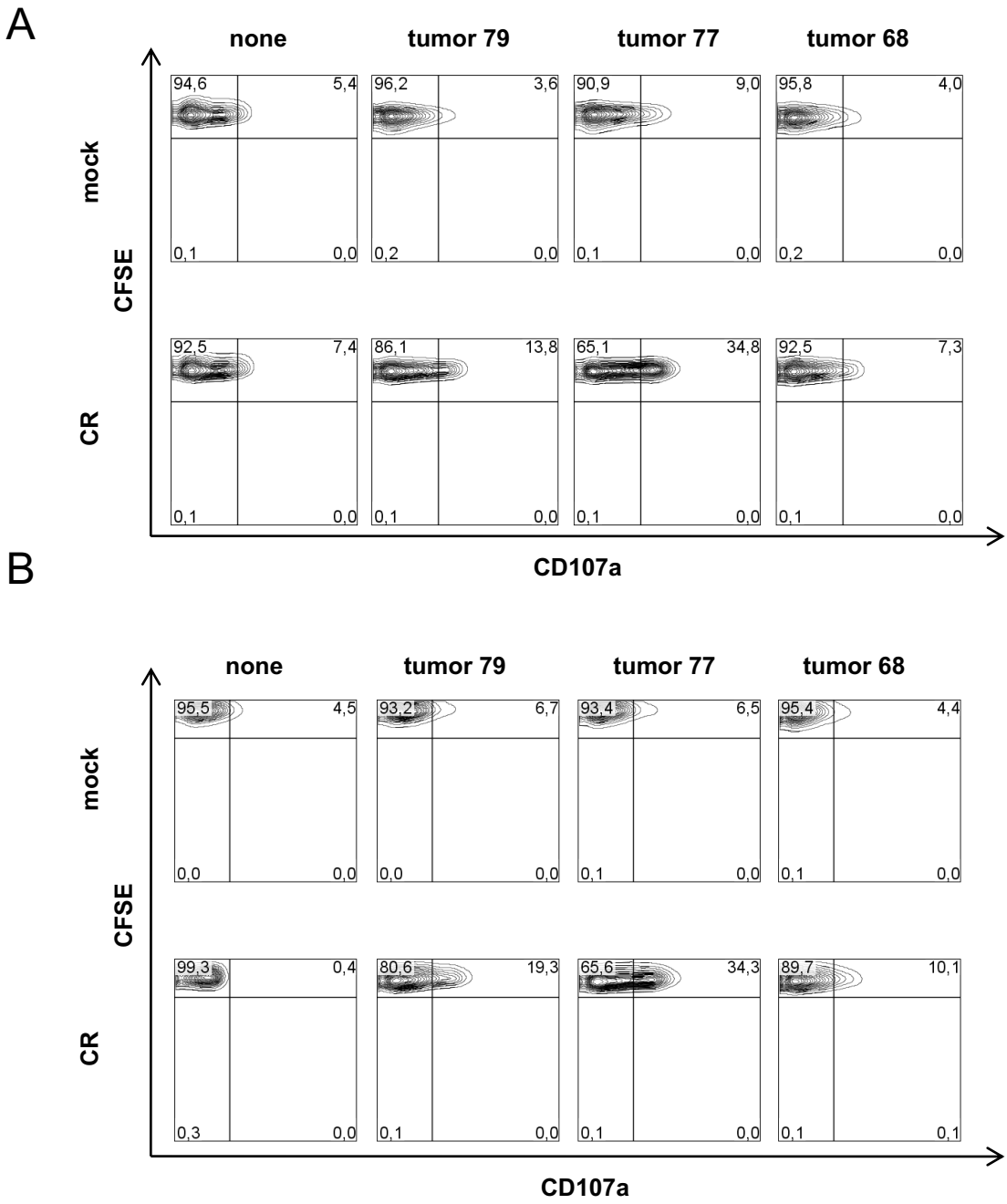

Fig. S6. CR-NK cells degranulate in response to HER-2-positive freshly isolated tumor cells. Effector cells gated on CFSE were pMIG (mock)-transduced or CR-transduced NK cells from patient 79 (*A*) or from a healthy donor (*B*). They were either cultured without target cells (none), or cocultured with the indicated tumor cells for 5 h while being stained for CD107a conjugated with PE-Cy5.

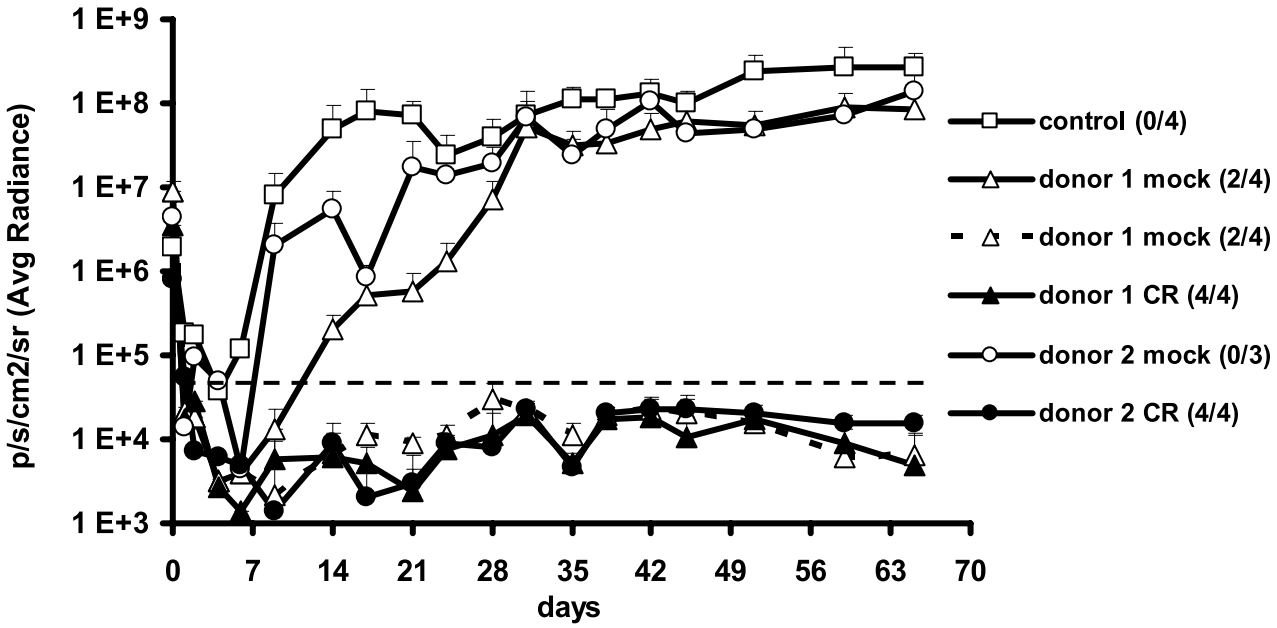

Fig. S7. Kinetic of light signal intensity from tumor region. *RAG2*$^{-/-}$ mice were inoculated s.c. with 5×10^6 SKOV/CBG cells alone or together with the same number of CR$^+$-NK cells or mock-NK cells from 2 different donors. In vivo imaging was performed to visualize tumor cell outgrowth or death. Background signal as measured for the imaging platform is indicated by the dashed line. Open squares represent 4 control mice, open triangles represent 4 mice that received mock-NK cells from donor 1, tumor grew in 2 of these mice (continuous line) but not in the other 2 mice (dashed line), filled triangles represent 4 mice that received CR-NK cells from donor 1, open circles represent 3 mice that received mock-NK cells from donor 2, and filled circles represent 4 mice that received CR-NK cells from donor 2. Error bars represent standard deviations.