Supplemental Methods

Isolation and cultivation of MSC

BM samples were treated with red cell lysis buffer (155 mM ammonium chloride, 10mM potassium hydrogencarbonate, 0.1mM $Na_2EDTA \cdot 2H_2O$; obtained from the Hospital Pharmacy, University Clinic Düsseldorf, Germany) at a ratio of 1:2 and incubated for 10 min at room temperature. After washing with HBSS (Lonza), cells were resuspended in complete medium consisting of Dulbecco's Modified Eagle Medium low glucose (Lonza), 2mM Glutamin (Lonza), 1 IE / ml Na-Heparin (Ratiopharm), 5% human fresh frozen plasma, 5% platelet-lysate (both obtained from the Institute of Hemostasis and Transfusion Medicine, University Clinic Düsseldorf, Germany), and transferred at a density of $\leq 10^6$ WBC/0.2ml/cm^2 into T175 flasks (Corning). Cultures were maintained at 37°C under 10 % CO_2 using a humidified incubator (Binder). After 24h, non-adherent cells were transferred into a new T175-flask and the first T175-Flask was received fresh complete medium. After further 24h, the supernatant with non-adherent cells form second Flask was exchanged with complete medium. When the cultures reached approximately 70-80% confluence, adherent cells were detached by treatment with TrypLE (Invitrogen) and re-plated into CellStack®-1 chamber or/and into CellStack®-5 Chamber (all from Corning) at a density of 2000-4000 cells/cm^2. Dependent on proliferation rate of GMP-grade MSC-charge, the range of cells is for splitting 2000-4000 cells/cm^2 for two passages. After three to four weeks from start of culture GMP-Grade MSCs were harvested, washed, resuspended in NaCl 0,9% (Braun) +1 % Human albumin (Octapharma) and cryopreserved 1-2x10^6 MSCs/ml in a final concentration of 10% DMSO (WAK Chemie Medical GmbH) and 5% Human albumin (Octapharma). GMP-Grade-MSCs isolated and expanded under these conditions displayed a characteristic immune phenotype with more than 90% of $CD73^+CD105^+$ and the absence (<1%) of CD3 and CD45 (supplemental Figure 9). MSCs from passage three to five were utilized for the subsequent experiments. For characterization of MSCs the following monoclonal antibodies were used: CD73 (BD Biosciences), CD105 (Ancell), CD45 (Beckmann Coulter), and CD3 (Beckmann Coulter).

MSC/HSPC NK cell differentiation assay

Human $CD34^+Lin^-$ HSCs were enriched from cord blood or bone marrow using a lineage cell depletion kit. Subsequently $CD34^+$ cells were enriched using a CD34 MicroBead kit (Miltenyi Biotec). $CD34^+$ HSPCs were seeded onto MSC layers in 24-well dishes (50.000 MSC/well). The culture medium contained 2/3 DMEM high glucose (Gibco), 1/3 HAM's F12 (Biochrom), 20% human AB serum (Lonza), 1% penicillin/streptomycin (Gibco), 20mg/L ascorbic acid (Sigma), 50µM ethanolamine (Sigma), 50µg/L sodium selenite (Sigma), and 24µM β-mercaptoethanol (Gibco) and the cytokines IL-2 (1000U/ml), IL-3 (5ng/ml), IL-7 (20ng/ml), SCF (20ng/ml), and FLT3-L (10ng/ml) (all from Miltenyi Biotec). At day 7, IL-3 was replaced by IL-15 (10ng/ml).

Antibodies and flow cytometry

Single-cell suspensions of HSPC or *in vitro* generated NK cell populations were stained with the following monoclonal antibodies: CD56-PC5 or -PE, NKG2A-PE, -Alexa Fluor 750, or

-PC7, KIR2DL2/3/S2-PE, -PC7 or PC5.5, KIR2DS4-PE (Beckman Coulter); CD107a-FITC or -APC, IFN-γ-FITC, Granzyme B-FITC, Perforin-PE, CD94-PE, CD45RA-FITC, CD56 PE/Dazzle594, CD16-FITC, -PE or -PC5, CD38-PC5, CD34-PE, KIR3DL1-FITC or Brilliant Violet 421 (BioLegend); KIR2DL1-APC or -FITC (R&D systems).

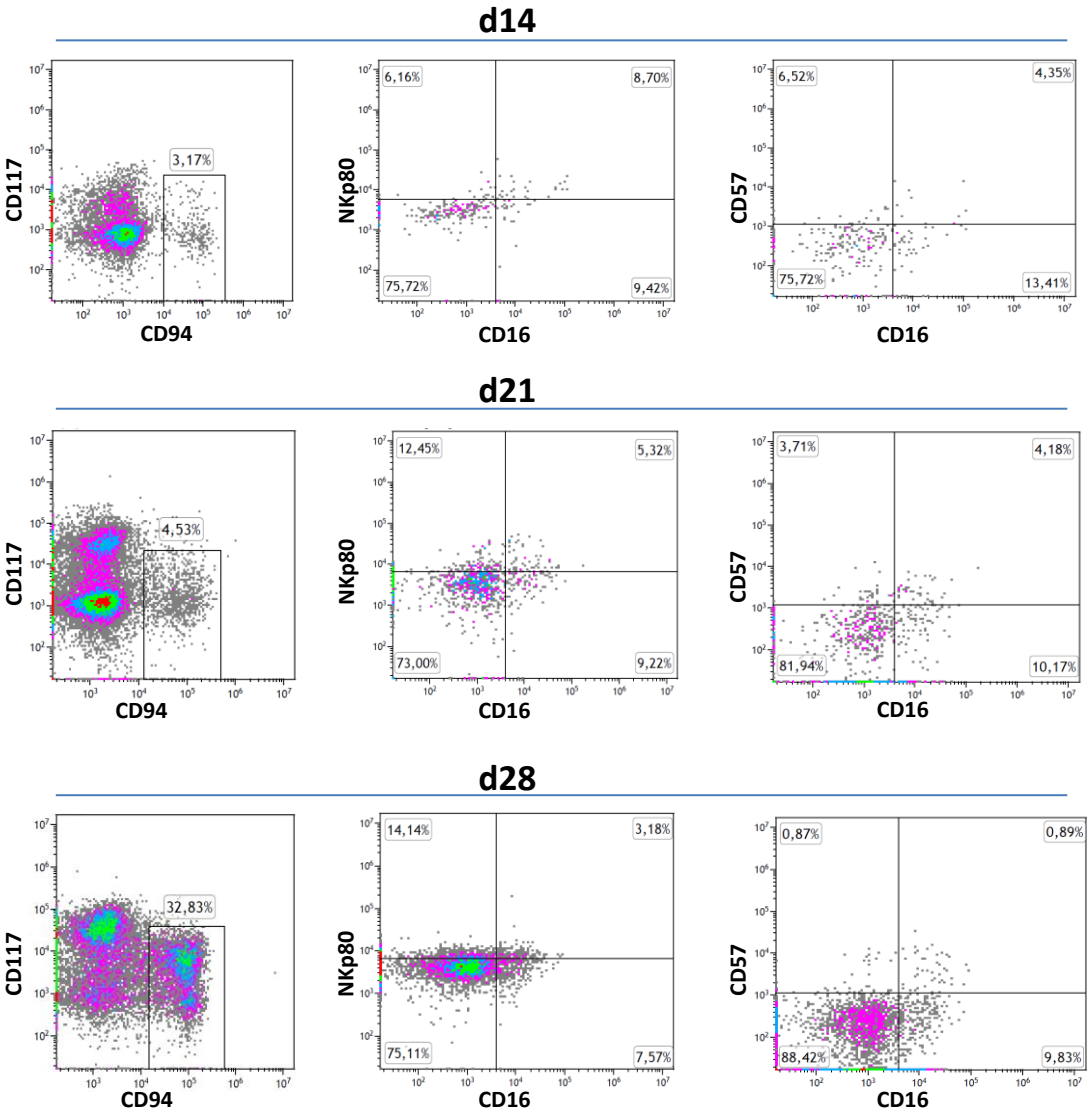
shRNAs and lentiviral infection

shRNAs were ligated into the lentiviral vector PCL2.THPC containing a green fluorescent protein (GFP) reporter. For lentiviral transduction of MSC, tissue culture dishes (diameter: 10cm) were coated with 10µg/ml poly-D-lysine and incubated for ≥ 30 minutes at 37°C and washed once with PBS. $4x10^6$ Hek293T cells/dish were incubated over night in Hek293T medium (DMEM high glucose, 10% FCS, 1% non essential amino acids, 1% sodium-pyruvate, 1% Penicillin/Streptomycin). Shortly before transfection, medium was changed to advanced DMEM medium (Gibco), 2% FCS (ESC-qualified), 1% Penicillin/Streptomycin and 1% L-glutamine (9ml/dish) and incubated for 1h. The transfection reagent was mixed in the following order: H_2O (fill up to 600µl), 18,5µg shRNA-containing vector, 9,25µg helper vector, 9,25µg envelope vector and 61,5µl 2,5 M $CaCl_2$. 600µl HBS-buffer (100 mM Hepes, 280mM NaCl, 1,5mM Na_2HPO_4, pH 6,95-7,05) was added and the mixture incubated at RT for 15 min. Next, chloroquine (final concentration of 25µM) and the final transfection mix was added dropwise to Hek293T cells. After 6h, the medium was changed to advanced DMEM, 5% FCS (ESC-qualified), 1% Penicillin/Streptomycin and 1% L-glutamine (15ml/dish), and incubated over night. At day 3, medium was changed to advanced DMEM with 5% FCS, 1% Penicillin/Streptomycin and 1% L-glutamine (15ml/dish). At day 4 (30h later), virus supernatant was harvested, centrifuged at 1900 rpm for 7 min to pellet debris and filtered through 0,45µm filters (Sarstedt). The filtered supernatant was centrifuged at 15000 rpm, 4°C for 90min and the virus pellet was resuspended in RPMI1640 medium (Lonza) containing 10% FCS and 1% Penicillin/Streptomycin and stored at 4°C. Another 15ml of 5% FCS containing advanced DMEM medium was added to the Hek393T cells and incubated over night for a second harvest. At day 5, the second harvest of virus supernatant was performed as described above. After centrifugation, virus pellet was resuspended with the first harvested virus supernatant. 500µl aliquots were stored at -80°C or directly used. For infection, 100.000 MSC/well were seeded in 24-well tissue culture plates in 1ml culture medium. After 4-5h, 500µl medium was removed, and 500µl virus supernatant was added. Cells were incubated with virus for 24h and supernatant was completely removed from cells. Cells were washed 3x with PBS and cultured with fresh medium for further use in the NK cell differentiation assay. For analysis of β2M expression in the transfectants by real-time PCR, the following primers were used: 5': CTCACGTCATCCAGCAGAGA; 3': CGGCAGGCATACTCATCTTT

Supplemental Table 1. KIR acquisition is independent of the HLA-C ligands on the hematopoietic progenitor cells

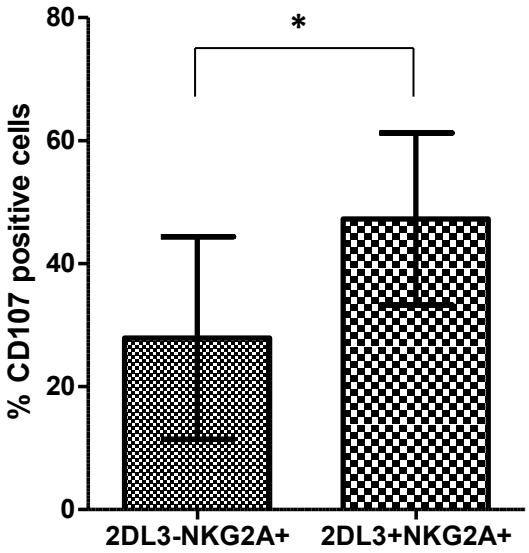
Cordblood	KIR Genotype[1]	HLA-C
1	n.d.	C1/C1
2	1	C1/C1
3	2	C1/C1
4	12	C1/C2
5	2	C1/C2
6	4	C1/C2
7	1	C1/C2
8	1	C1/C2
9	2	C2/C2
10	5	C2/C2
11	1	C2/C2
12	2	C2/C2
13	1	C2/C2

[1]KIR genotype according to Uhrberg et al. 2002,



Supplemental Figure 1. Analysis of NK cell stage-specific surface markers during NK cell differentiation in vitro from cord blood CD34⁺ HSPCs. CD34⁺ HSPC were isolated from cord blood and seeded at $3x10^3$ cells/well on monolayers of MSC. Flow cytometric analysis of cultures at day 14 (upper panel), day 21 (middle panel) and day 28 (lower panel) using CD117, NKp80, CD57 (y-axes) and CD94, CD16 (x-axes) on the indicated dot plots. Middle and right panels are gated on CD94⁺ NK cells as indicated in the left panel.

A



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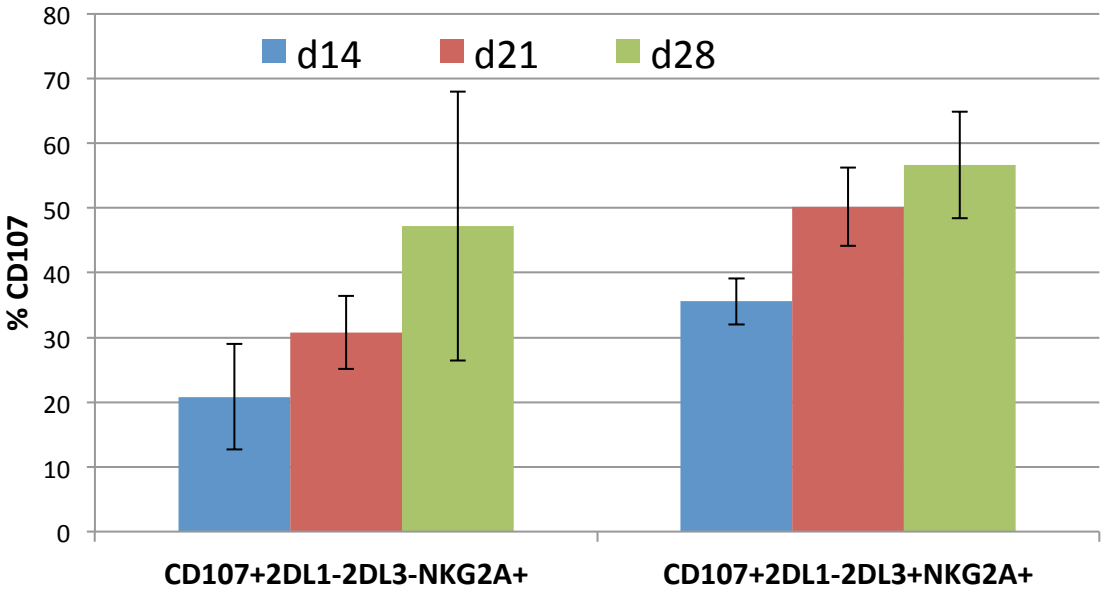
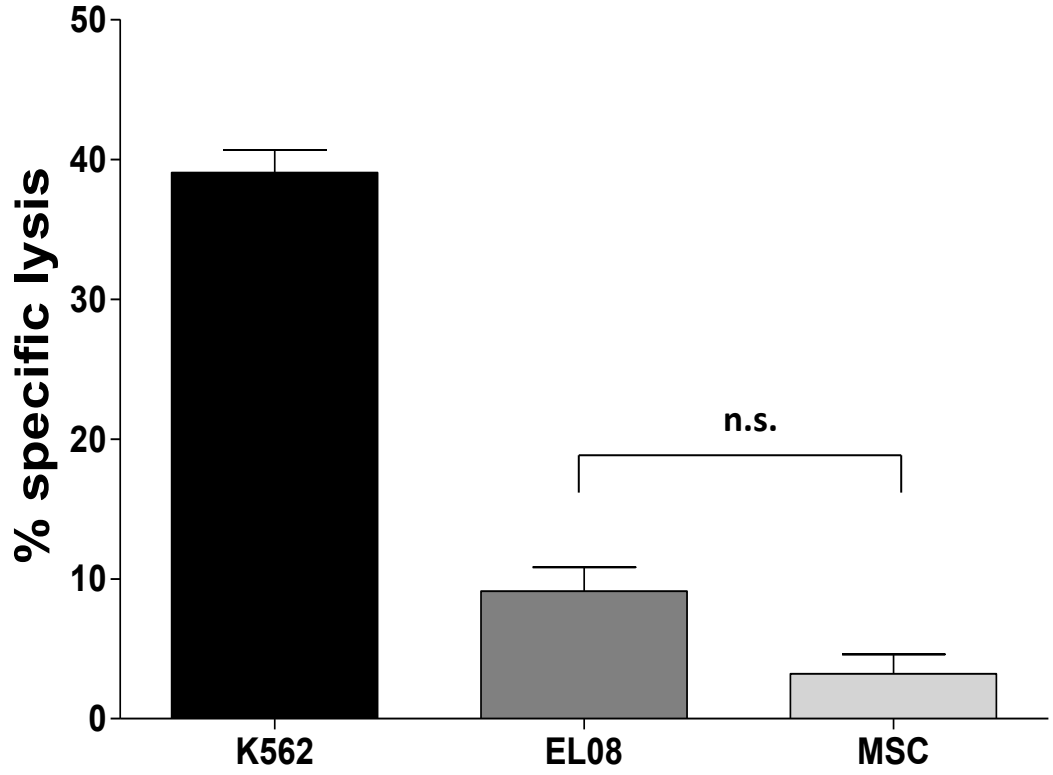
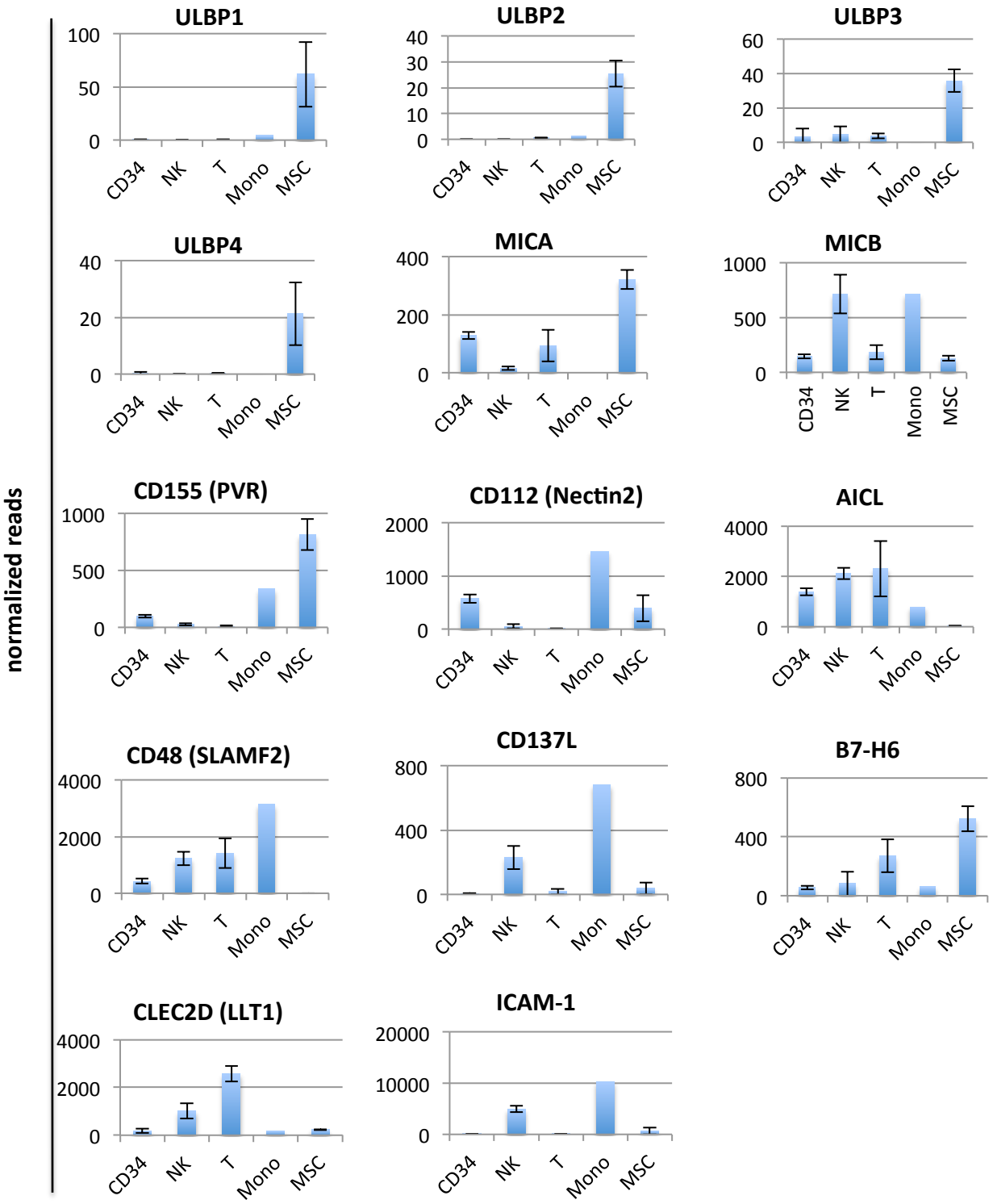


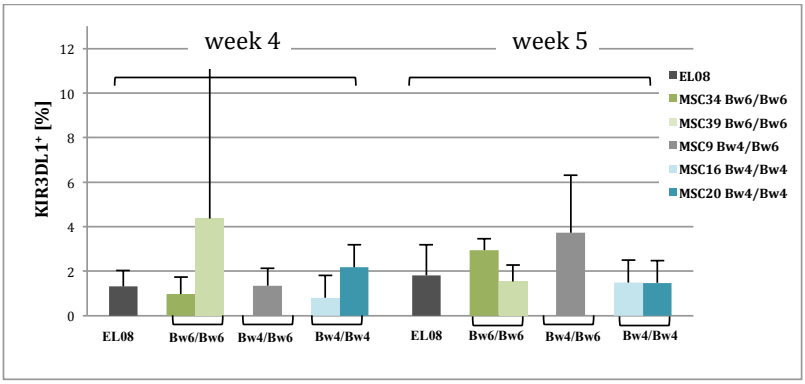
Supplemental Figure 2. Analysis of degranulation activity during NK cell-differentiation in vitro from cord blood CD34⁺ HSPCs. *Frequency of CD107⁺ NK cells against K562 following a 5h co-incubation at an effector/target ratio of 1:1 for the indicated NK cell subsets (n =10) (A) and at the indicated time points for NKG2A⁺ NK cells without (left panel) and with (right panel) co-expression of KIR2DL3 (n=4). The experiments were performed on MSC expressing C1/C1 KIR ligands. Statistical significance was determined using the Wilcoxon test.*



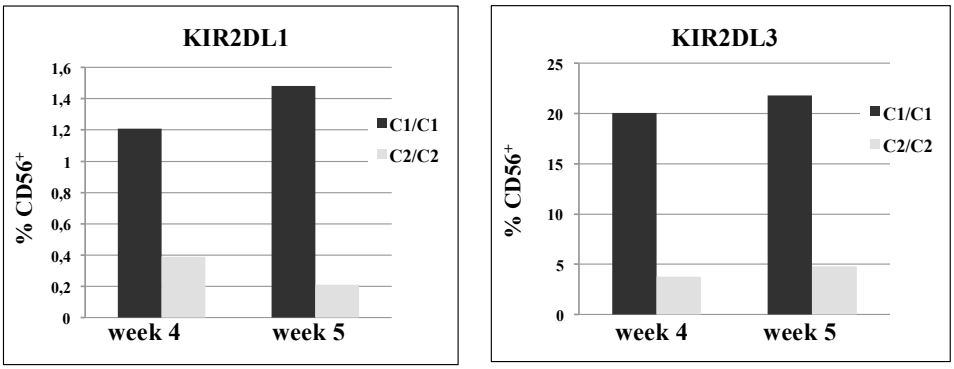
Supplemental Figure 3: Human MSC are not efficiently killed by allogeneic NK cells. *NK cells were freshly isolated from peripheral blood and incubated with the indicated, CFSE-labeled target cells for 5h at an effector/target ratio of 1:1. Cytotoxicity was determined flow cytometrically using propidium iodide-staining. The data represent mean values and standard deviations from three different donors. Statistical significance between EL08 and MSC was determined using the Wilcoxon test.*



Supplemental Figure 4. Expression profiling of NK cell receptor ligands in MSC. *Normalized RNAseq transcription data (Illumina HiSeq) of mesenchymal stem cells (MSC) in comparison to flow cytometrically sorted CD34⁺ hematopoietic progenitor cells (CD34), CD56^{dim} NK cells (NK), CD8⁺ T cells (T), and monocytes (Mono). Mean values and standard deviations were calculated for each cell type from three different donors except monocytes (2 donors).*

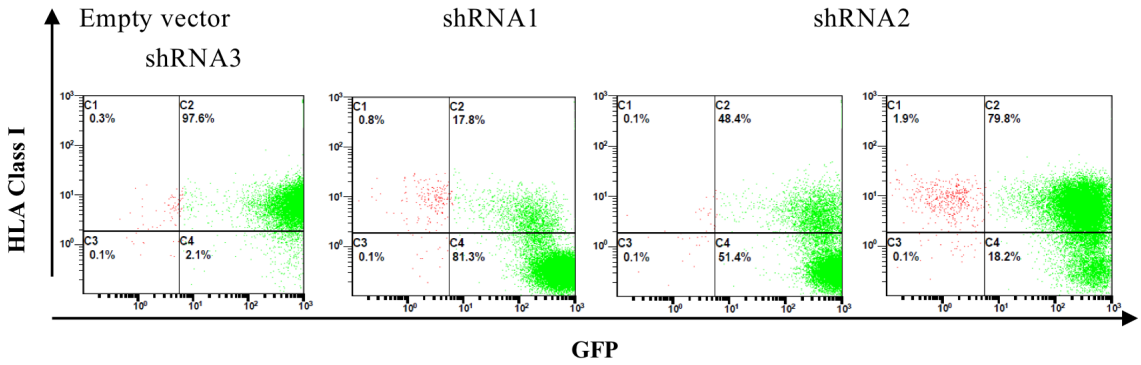


Supplemental Figure 5. Expression of KIR3DL1 during NK cell-differentiation in vitro from cord blood CD34+ HSPCs is independent of the presence of Bw4 epitopes. *Frequency of KIR3DL1-positive NK cells in week 4 and 5 on MSC lines expressing either Bw6/Bw6, Bw4/Bw6 or Bw4/Bw4 KIR ligands as indicated (n=4 (MSC39), other n=3).*



Supplemental Figure 6. NK cell differentiation with change of MSC layers*. HSPC were isolated from CB and cultured for 14 days on MSC39 (C1/C1) and MSC11 (C2/C2), respectively. On day 14, non-adherent cells were harvested and seeded onto fresh MSC layers and analyzed after the indicated time points for expression of KIR2DL1 (left panel) and KIR2DL3 (right panel).*

A



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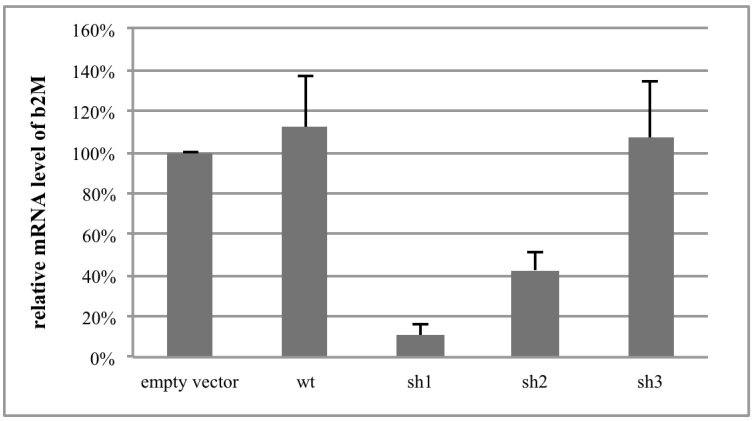
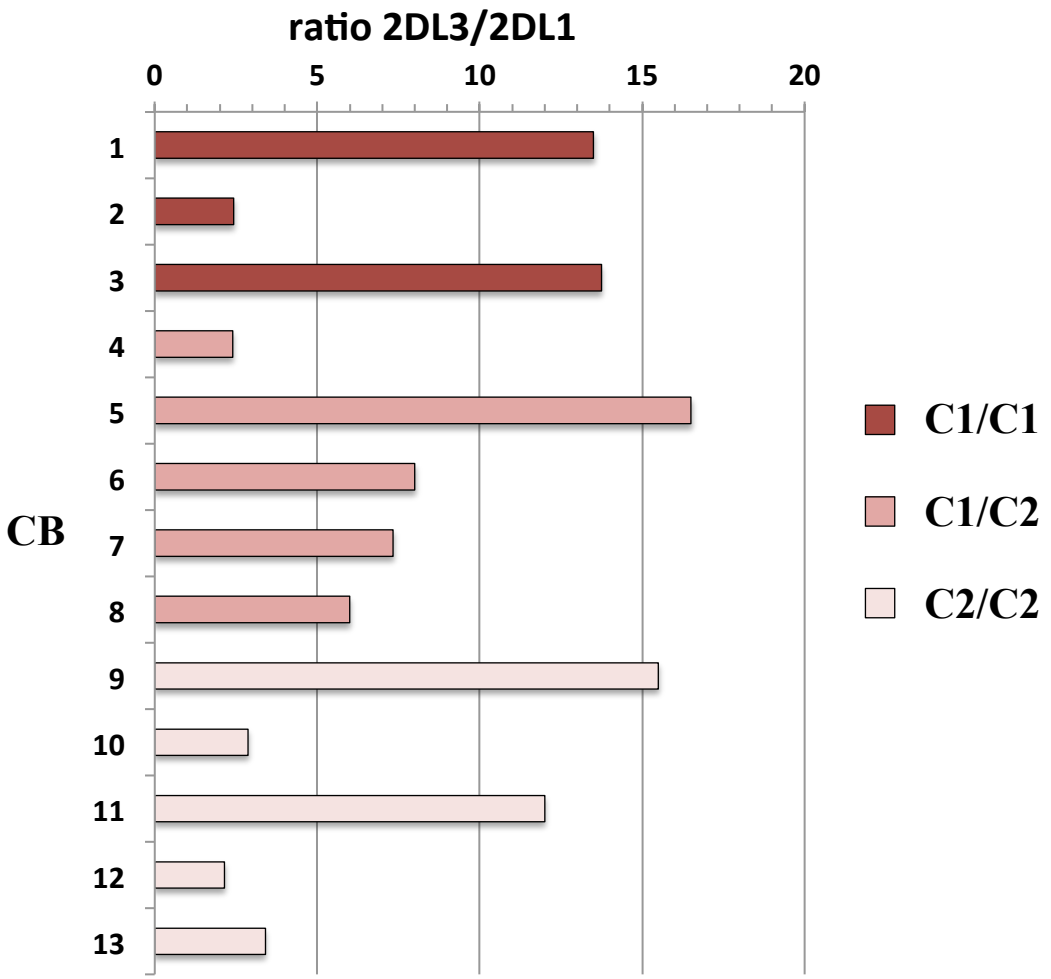
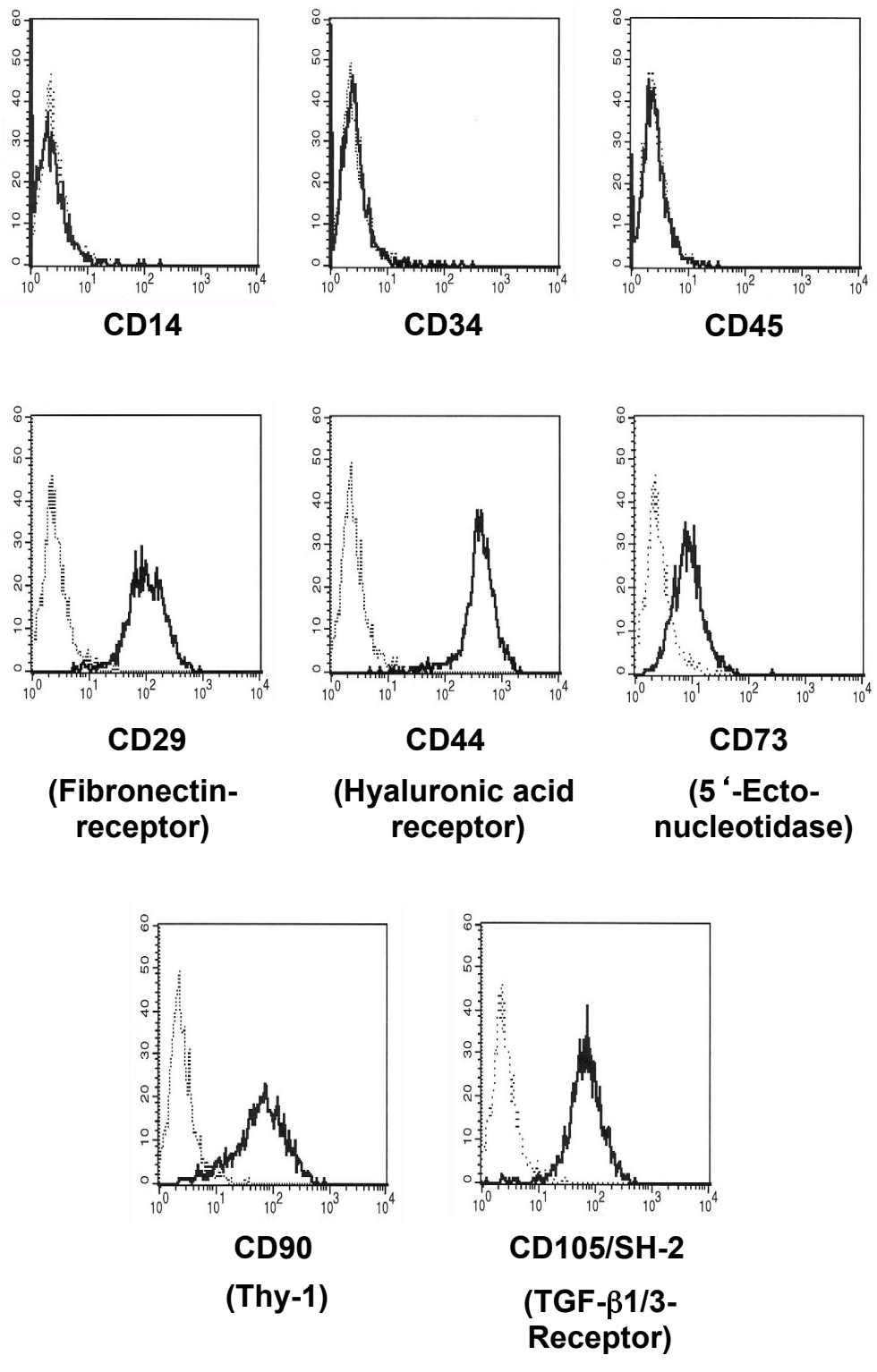


Supplemental Figure 7. Down regulation of HLA class I via shRNA-mediated knockdown of b2M expression. *MSC were lentivirally infected with* β2M*-specific shRNAs in GFP-containing expression vectors or empty vector. (A) Dot plot analysis of HLA class I expression using pan HLA class I-specific W6/32 antibody in MSC infected with the three different* β2M*-specific shRNAs by flow cytometry. (B) One week after the lentiviral infection, the amount of* β2M *on mRNA level was analyzed by real-time RT-PCR and normalized to the empty vector control (n=3).*



Supplemental Figure 8. KIR acquisition is independent of the HLA-C ligands on the hematopoietic progenitor cells. *$CD34^+$ hematopoietic progenitor cells were isolated from 13 different cord bloods (CB) and subjected to NK cell differentiation conditions on EL-08 stroma cells for 28 days. Bars represent the ratio of the frequencies of KIR2DL2/3 and KIR2DL1. KIR genotypes of the CBs are listed in supplemental Table 1. No significant differences were found between the three groups C1/C1, C1/C2 and C2/C2 (Kruskal Wallis statistical test)*



Supplemental Figure 9. Immunophenotype of bone marrow-derived MSC. *Flow cytometric analysis of surface markers associated with MSC identity. Histograms are representative for all MSC lines utilized in this study.*