# Analysis of memory-like natural killer cells in human cytomegalovirus-infected children undergoing αβ+T and B cell-depleted hematopoietic stem cell transplantation for hematological malignancies

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**Supplemental material**

**Material and methods**

**HCMV serology and therapy**

HCMV serology was assessed prior to transplantation using enzyme-linked immunoassay for virus-specific immunoglobulin IgM and IgG. Patients were monitored for HCMV infection in blood by determination of DNAemia twice a week from day 0 until discharge from the hospital, and then once a week for the first three months after transplantation. Subsequently, patients were monitored for HCMV at time of control medical visits or in the presence of clinical symptoms suggestive of HCMV infection. Pre-emptive therapy was administered when the viral load was greater than 5.000 DNA copies/mL and was based on administration of i.v. ganciclovir (5 mg/kg twice a day), replaced by foscarnet (90 mg/kg twice a day) in case of ganciclovir-induced neutropenia (less than $0.5x10^9$ neutrophils/l) or sustained increase of HCMV levels in blood during therapy with ganciclovir. Anti-viral treatment was discontinued after virus clearance from blood, defined as two consecutive negative results. Episodes of HCMV relapse were treated similarly.

**Monoclonal antibodies and flow cytometry**

The following mAbs, all produced in our lab, were used in this study: c127, (IgG1,anti-CD16), AZ20 (IgG1, anti-NKp30), BAB281 (IgG1, anti-NKp46), z231 (IgG1, anti-NKp44), BAT221 (IgG1, anti-NKG2D), KRA236 (IgG1 anti-CD226), PP35 (IgG1, anti-CD244), c218 and FS280 (IgG1 and IgG2a, respectively, anti-CD56), QA79 and Z176 (IgG1 and IgG2b, respectively, anti-siglec-7 or p75/AIRM1), 11PB6 (IgG1, anti-KIR2DL1 and KIR2DS1), GL183 (IgG1, anti-KIR2DL2/L3/S2), FES172 (IgG2a, anti-KIR2DS4), Z27 (IgG1, anti-KIR3DL1/S1), DF200 (IgG1 anti-KIR2DL1/S1/L2/L3/S2/S5), AZZ158 (IgG2a, anti-KIR3DL1/S1/L2), ECM41 (IgM, anti-KIR2DL3), Z199 and Z270 (IgG2b and IgG1 respectively, anti-NKG2A), F278 (IgG1, anti-LIR-1).

Anti-KIR2DL1-PE, -APC or non-conjugated mAb (clone 143211) and anti-NKG2C (mouse IgG2b) were purchased from R&D Systems Inc (Abingdon, United Kingdom). Anti-CD56-PC7 (C218 clone), anti-CD3-Pacific Blue (UCHT1 clone), anti-CD19-Pacific Blue (J3-119 clone), anti-NKG2A-APC (Z199 clone) were purchased from Beckman Coulter, Immunotech (Marseille, France).

Anti-CD16-PerCPCy5.5, anti-CD16-APC-Cy7 (clone 3G8), anti-KIR2DL2/L3-S2-FITC (CH-L clone), anti-CD107a-PE (anti-LAMP1), anti-IFN-γ-PE and anti-IL12R-PE were obtained

from BD Bioscience Pharmingen (San Diego, CA). Anti-CD3-VioGreen, anti-CD20-VioGreen, anti-CD14-VioGreen, anti-CD57-Vioblue or purified, biotin-conjugated anti-NKG2A, anti-KIR3DL1-FITC (DX9 clone), anti-KIR2DL2/L3-S2-APC (DX27 clone), biotin-conjugated anti-KIR2DL1-S1 (11PB6 clone), anti-biotin Vioblue and anti-biotin-PerCPVio700 mAbs were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Anti-IL18Rα (mouse IgG1, clone B-E43) was purchased from ABCAM (Cambridge, UK). Goat anti-mouse isotype-specific secondary reagent was purchased from Southern Biotech (Birmingham, AL) and Jackson ImmunoResearch Laboratories (Suffolk, UK).

NK cell phenotype and effector functions were analyzed on either freshly derived PBMC or thawed PBMC where indicated, gating NK cells by physical parameters and by the combined use of anti-CD56, anti-CD3 and anti-CD19 mAbs. The $CD56^{dim}$ NK cell subset was evaluated whenever indicated.

Cytofluorimetric analyses were performed on FACSVerse (Becton Dickinson & Co, Mountain View, CA) and data were analyzed by FacsSuite software version 1.0.5.

Single fluorescence cytofluorimetric analyses were performed on FACSCalibur (Becton Dickinson) and analyzed by CellQuestpro.

**CD107a degranulation and IFN-γ production**

The medium used throughout the experiments was RPMI-1640 medium supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin-neomycin mixture and 10% heat-inactivated FCS(1).

Whenever indicated, PBMC were cultured either in the presence or in the absence of rhIL-15 or rhIL-12 plus rhIL-18 (Peprotech, London, UK) at the final concentration of 10 ng/ml, 2 ng/ml and 20 ng/ml respectively.

Freshly drawn PBMC from 8 HCMV-reactivating patients at 6 months after HSCT and from 6 HCMV seropositive hd were cultured overnight (18 hours) either in the presence or in the absence of rhIL-15; then PBMC were washed and incubated with the target cells K562 at an effector-to-target cell (E/T) ratio of 1:1 (where effector cells are PB-NK cells) for 3 hours in culture medium supplemented with anti-CD107a-PE mAb. Thereafter, cells were stained with anti-CD56-PC7, anti-CD3 and anti-CD20-Viogreen, anti-CD16-PercpCy5.5, anti-CD57-VioBlue, anti-NKG2C followed by appropriate secondary reagents (anti-mouse IgG2b APC-conjugated, Jackson ImmunoResearch, Suffolk UK), for 35 min on ice. Cells were then washed and analyzed by flow cytometry (FACSCalibur, Becton Dickinson). To detect NK intracellular production of IFN-γ, overnight cultured PBMC were washed and

incubated with K562 or with medium alone, for 4 hours in the presence of GolgiStop (BD Biosciences Pharmingen). PBMCs overnight cultured in the presence of rhIL-12 plus rhIL-18 were analyzed, as well. Thereafter, cells were washed, stained as described above for CD107a assays and then fixed and permeabilized with the BD Cytofix/Cytoperm kit (BD Bioscience Pharmingen). IFN-γ production was detected by subsequent intracellular staining with anti-IFN-γ-PE and cytofluorimetric analysis. The percent of positive cells was calculated subtracting the baseline CD107a or IFN-γ expression in controls cultures without stimuli from targets.

In reverse Ab-dependent cellular cytotoxicity (ADCC) assays, thawed PBMC from 7 HCMV-reactivating patients and from 6 HCMV seropositive hd were cultured overnight with rhIL-15, washed and then incubated with the $Fc\gamma R^+$ p815 murine mastocytoma cell line, either in the presence or in the absence of mAbs specific for the different surface receptors indicated in the text/figures at an effector-to-target cell (E/T) ratio of 1:1 for 1 hour (this time window was chosen so that NKG2C surface expression was detected when NK cells were triggered by anti-NKG2C mAb).

In another set of degranulation assays, thawed PBMC were cultured for 3 days in the presence of rhIL-15, then washed and incubated either in the presence or in the absence of the lymphoblastoid 721.221wt cell line (221wt) or the 721.221.AEH cell line (221.AEH) that has been transfected with a hybrid HLA-E containing the HLA-A2 signal sequence (2), at an effector-to-target cell (E/T) ratio of 1:1 for 3 hours in culture medium supplemented with anti-CD107a-PE mAb. Cells were then stained with anti-CD56-PC7, anti-CD3- and anti-CD20-Viogreen, anti-CD16-APC-Cy7, anti-NKG2A biotin-conjugated plus anti-BiotinPercpvio700, anti-CD57-VioBlue, anti-NKG2C and then analyzed as described above.

**KIR-ligand and KIR gene profile analyses**

DNA of the tested samples was extracted using the QIAamp DNA Blood Mini kit (QIAGEN) according to the manufacture's instruction. KIR ligand and the KIR gene profile analyses were performed using a sequence specific primer-PCR (SSP-PCR) protocol. In particular KIR gene profile, performed using KIR genotype kit (GenoVision, Saltsjoebaden, Sweden), allowed the detection of the presence/absence of all the KIR genes, while, by the use of KIR HLA ligand kit (GenoVision, Saltsjoebaden, Sweden), we typed HLA-C alleles on the basis of the dimorphism present at position 80 (analysis of C1 and C2 epitope), and we

detected the presence of HLA-B and –A alleles characterized by the Bw4 motifs (dividing the HLA-B Bw4$^+$ alleles in two groups according to the residue present at position 80).
NKG2C genotype was analyzed as described elsewhere[29]. All patient samples have been typed as *NKG2C*$^{+/+}$ or *NKG2C*$^{+/-}$, i.e. are characterized by the presence of at least one NKG2C ORF allele[29].

**Statistical analysis**

Wilcoxon-Mann-Whitney non-parametric tests were employed. The statistical significance (p) is indicated (*p<0.05; ** p<0.01; *** p<0.001). Median fluorescence intensity (MFI) values were normalized before calculating statistical significance. Graphic representations and statistical analysis were performed using GraphPad Prism 6 (GraphPad Software, Inc. La Jolla, CA).

**Supplementary figures**

**Figure s1. Analysis of CD56$^-$CD16$^+$ NK cells in patients undergoing HCMV reactivation after receiving αβ$^+$T/B-depleted HSCT or UCBT or purified CD34$^+$ HSCT**

PB NK cells from the various patients were analyzed for the expression of CD56 and CD16. Three representative patients receiving the different type of transplantation indicated (reactivating HCMV after transplantation) are shown at 6 months after HSCT. The percentages of CD56$^-$CD16$^+$ NK cells are indicated in the lower right quadrant.

**Figure s2. Distribution of NK cell subsets identified by NKG2C/CD57 expression in healthy donors and in HSC donors as compared to reactivating HSCT patients**

PB NK cells from healthy donors, from the various patients, and from given HSC donors were analyzed for the expression of NKG2C and CD57. After gating on CD56$^+$CD3$^-$CD19$^-$ lymphocytes, CD56$^{dim}$ NK cells were evaluated. In **A)** the gating strategy is shown for a representative HCMV-reactivating patient and a HCMV$^+$ donor. In **B)** the percentage of the different subsets identified by NKG2C and CD57 on CD56$^{dim}$ NK cells (i.e. NKG2C$^+$CD57$^-$, NKG2C$^+$CD57$^+$, NKG2C$^-$CD57$^-$ and NKG2C$^-$CD57$^+$), in HCMV$^+$ (black bars, n=10) or HCMV$^-$ hd (grey bars, n=7) is reported as 95% CI for the mean. Statistical significance is indicated. In **C)** the percentage of the different NKG2C/CD57 subsets in HCMV$^+$ hd (black bars, n=10) are compared to NK cells from HCMV-reactivating αβ$^+$T/B-depleted haplo-HSCT at 6 months (dark grey bars, n=13) and 12 months (light grey bars, n=10). 95% CI for the mean and statistical significance are reported. In **D**) the percentage of

$CD56^{dim}NKG2C^{+}CD57^{+}$ PB-NK cells isolated from the HSC donors of HCMV-reactivating patients is shown (black bar, n=11) in comparison to PB-NK cells isolated from HCMV-reactivating patients at month 1 (white bar), 3 (light grey bar), 6 (very light grey bar), 12 (dark grey bar) after HSCT. 95% CI for the mean and statistical significance are reported.

**Figure s3. KIR gene and KIR ligand analyses.**

Donor genotypes were analyzed for the presence (grey boxes) or absence (white boxes) of the indicated KIR genes using SSP-PCR approach. Two different sets of primers were used to discriminate between the KIR2DS4 alleles coding for membrane bound receptors (reported as S4) and the alleles coding for putative soluble receptors (reported as d). KIR3DL1*004 indicates the presence of a KIR3DL1 allele coding for intracellularly retained receptor. *004/? indicates the presence of at least one KIR3DL1 allele coding for intracellularly retained receptor. Dark grey boxes indicate educated KIRs.

A/A indicates that the sample is homozygous for group A KIR haplotypes, B/X indicates genotypes containing either one (A/B heterozygous) or two (B/B homozygous) group B haplotypes

**Figure s4. Siglec-7 expression in patients receiving $\alpha\beta^{+}$T/B-depleted haplo-HSCT stratified on HCMV recurrence**

Peripheral blood NK cells from the various patients were analyzed for the expression of Siglec-7 at 1, 3, 6 and 12 months after HSCT. After gating on $CD56^{+}CD3^{-}CD19^{-}$ lymphocytes, $CD56^{dim}$ NK cells were evaluated. The percent of positive $CD56^{dim}$ NK cells in patients either experiencing (empty circles) or not experiencing (filled black squares) HCMV after transplantation are reported at the different time points. 95% CI for the mean and statistical significance are indicated.

**Figure s5. Analysis of the expression of activating receptors by $NKG2C^{+}$ $CD57^{+}$ NK cells from HCMV-reactivating patients and $HCMV^{+}$ hd**

PB NK cells collected at 6 months after HSCT from HCMV reactivating patients and from $HCMV^{+}$ hd were analyzed for the expression of the indicated surface markers, after gating on the different $CD56^{dim}$ NK cell subsets identified by NKG2C and CD57 as shown in fig.4 (i.e. $NKG2C^{+}CD57^{-}$, $NKG2C^{+}CD57^{+}$, $NKG2C^{-}CD57^{-}$ and $NKG2C^{-}CD57^{+}$, indicated for brevity as 2C and 57). 95% CI for the median fluorescence intensity (mfi) is shown for

HCMV reactivating patients at 6m after HSCT (black bars,n=8) and for $HCMV^+$ hd (grey bars, n=7) .

**Figure s6. Comparable levels of degranulation of the different NKG2C/CD57 NK cell subsets exposed to rhIL-12 plus rhIL-18**

Thawed PBMC from HCMV-reactivating patients (n=6), collected at 6 months after HSCT, were cultured overnight in the presence of rhIL-12 plus rhIL-18 or rhIL-15 for comparison. Then PB cells were incubated either with or without K562 cell line for 3 hours. CD107a expression was evaluated in the different NKG2C/CD57 $CD56^{dim}$ NK cell subsets (dark bars: $NKG2C^+CD57^-$, light grey: $NKG2C^+CD57^+$, dark grey: $NKG2C^-CD57^-$, white bars: $NKG2C^-CD57^+$). 95%CI for the mean of CD107a positive NK cells is shown.

**Figure s7. Anti-NKG2C mAbs trigger degranulation of memory-like $NKG2C^+$ $CD57^+$ NK cell subset from $HCMV^+$ hd in reverse ADCC assays**

Thawed PBMC from $HCMV^+$ hd were cultured in the presence of rhIL-15. After overnight culture, cells were incubated for 1h with the $Fc\gamma R^+$ murine cell line p815 in the presence or in the absence of anti-CD16, anti-NKG2C, and anti-KIR specific mAbs alone or in combination. CD107a expression is shown for each NK cell subset as 95%CI summarizing data for n=6 $HCMV^+$ hd (dark bars: $NKG2C^+CD57^-$, light grey: $NKG2C^+CD57^+$, dark grey: $NKG2C^-CD57^-$, white bars: $NKG2C^-CD57^+$). CTR indicates NK cells cultured in the presence of p815, in the absence of mAbs.

**Figure s8. Recognition of HLA-$E^+$ 221 by $NKG2C^+CD57^+$ NK cells from both $HCMV^+$ hd and patients is enhanced by gating on $NKG2C^+CD57^+NKG2A^-$ NK cells**

**A**) 221 wt and 221 AEH were analysed by flow cytometry for the expression of both non-classic HLA-E molecules (3D12 mAb) and HLA-class I (HLA-I) molecules (W63/2 mAb). Empty histograms represent cells incubated with the secondary reagent only. Mean Fluorescence Intensity values are indicated in each histogram plot.

In **B)** and **D)** thawed PBMC were cultured in the presence of rhIL-15. As indicated in figure 6B, after three days of culture, cells were incubated in medium alone (black bars) or with 221 wt (light grey bars) or with 221 expressing HLA-E (221.AEH, dark grey bars). In **B)** CD107a expression is shown for each NK cell subset as 95%CI for the mean summarizing data for n=7 $HCMV^+$ hd. In **C**), the gating strategy to exclude $NKG2A^+$ NK cells before analysing degranulation in the different NKG2C/CD57 NK cell subsets, is shown for a representative hd. In **D)** CD107a expression is shown, after gating on $NKG2A^-$ NK cells, as

95%CI for the mean summarizing data for n=7 $HCMV^+$ donors (left panel) and n=5 $HCMV^-$ reactivating HSCT patients (right panel). Statistical significance is reported.

**Table s1**

| patient # | age | Gender | Diagnosis | Cytogenetic/ molecular abnormalities | Disease stage | Conditioning regimen | HCMV infection/ reactivation | HCMV serostatus R/D | other infections | GVHD, grade | outcome | n°CD34+ cells infused ($10^6$) | n° NK cells infused ($10^6$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11,8 | M | AML | inv 16 | CR2 | TBI+TT+FLU | yes | pos/ pos | aspergillus, VZV | no | alive and well | 15,80 | 33,90 |
| 2 | 6,3 | M | BCP-ALL | normal | CR3 | TT+TREO+FLU | yes | pos/ pos | AdV | acute, 1 | dead due to disease relapse and progression | 11,00 | 52,00 |
| 3 | 5,6 | M | T-ALL | normal | CR1 | TBI+TT+FLU | yes | neg /pos | | no | alive and well | 40,10 | 82,83 |
| 4 | 21,6 | M | BCP-ALL | t(1;19) | CR2 | TBI+TT+L-PAM | yes | pos/ neg | | no | alive and well | 10,22 | 65,95 |
| 5 | 10,3 | F | BCP-ALL | normal | CR2 | TBI+TT+FLU | yes | pos/ pos | VZV | no | alive and well | 10,60 | 34,26 |
| 6 | 7,4 | M | BCP-ALL | normal | CR3 | TBI+TT+FLU | yes | pos/ pos | | no | dead due to disease relapse and progression | 11,28 | 30,93 |
| 7 | 12,4 | F | BCP-ALL | normal | CR3 | TT+TREO+FLU | yes | pos/ pos | | no | alive and well | 12,54 | 39,84 |
| 8 | 6,7 | M | BCP-ALL | normal | CR2 | TBI+TT+FLU | yes | pos/ pos | | acute, 2 | alive and well | 9,63 | 90,26 |
| 9 | 28,6 | F | AML in Fanconi Anemia | compex karyotype | CR1 | TBI+FLU+EDX | yes | pos/ pos | | acute, 2 | dead due to disease relapse and progression | 12,00 | 16,00 |
| 10 | 11 | F | BCP-ALL | normal | CR2 | TBI+TT+FLU | yes | pos/ pos | | no | alive and well | 11,52 | 73,62 |
| 11 | 14,8 | M | AML | FLT3-ITD | CR1 | BUS+EDX+L-PAM | yes | pos/ pos | BK virus | no | alive and well | 7,28 | 69,40 |
| 12 | 9,9 | M | BCP-ALL | t(4;11) | CR1 | TBI+TT+FLU | yes | pos/ pos | | acute 2 | alive and well | 8,29 | 17 |
| 13 | 1,4 | M | T-ALL | normal | CR1 | TBI+TT+FLU | yes | pos/ pos | | no | alive and well | 7,01 | 55 |
| 14 | 14,3 | F | BCP-ALL | normal | CR2 | TBI+TT+L-PAM | no | neg/ neg | | no | dead due to disease relapse and progression | 12,90 | 35,50 |
| 15 | 14,4 | F | BCP-ALL | normal | CR2 | TBI+TT+FLU | no | neg/ pos | | no | alive and well | 11,20 | 53,90 |
| 16 | 11,3 | M | T-ALL | normal | CR2 | TBI+TT+FLU | **no** | pos/ pos | BK virus, AdV | no | alive and well | 13,45 | 86,60 |
| 17 | 21 | M | BCP-ALL | t(9;22) | CR3 | TT+TREO+FLU | no | pos/ pos | | no | alive and well | 8,40 | 42,60 |
| 18 | 14,6 | M | AML | t (7;7) | CR2 | TBI+TT+L-PAM | **no** | neg/ neg | AdV | no | alive and well | 10,50 | 23,00 |
| 19 | 9,7 | M | BCP-ALL | normal | CR2 | TBI+TT+L-PAM | no | neg/ neg | | no | alive and well | 14,29 | 51,58 |
| 20 | 6,9 | M | BCP-ALL | t(12;21) | CR2 | TBI+TT+FLU | no | neg/ pos | | acute, 2 | alive and well | 16,92 | 60,28 |
| 21 | 11 | M | AML | complex karyotype | DISEASE PRESENT | TBI+TT+FLU | no | neg/ neg | | no | alive and well | 12,10 | 73 |
| 22 | 4,4 | M | BCP-ALL | normal | CR2 | TBI+TT+FLU | no | pos/ pos | | no | alive and well | 13,44 | 88 |
| 23 | 8 | F | AML | complex karyotype | CR1 | BUS+EDX+L-PAM | no | pos/ pos | | no | alive and well | 13,52 | 82 |
| 24 | 6,6 | M | BCP-ALL | normal | CR2 | TBI+TT+FLU | no | pos/ pos | | no | alive and well | 11,50 | 37 |
| 25 | 16,9 | F | T-ALL | normal | CR1 | TBI+TT+FLU | no | pos/ pos | | no | alive and well | 10,2 | 13,6 |
| 26 | 11,3 | M | BCP-ALL | t(4;11) | CR2 | TBI+TT+FLU | no | pos/ pos | BK, AdV | no | alive and well | 8,36 | 94,76 |
| 27 | 5,6 | M | BCP-ALL | t(12;21) | CR2 | TBI+TT+FLU | no | pos/ pos | | acute, 1 | alive and well | 14,97 | 97 |

**Table s1 legend**: AML, acute myeloid leukemia; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; CR, complete remission; FLT3-ITD, fms-related tyrosine kinase 3-internal tandem duplications; TBI, Total Body Irradiation; TT, Thiotepa; FLU, Fludarabine; TREO, Treosulfan; BUS, busulfan; L-PAM, melphalan; EDX, endoxan (cyclophosphamide); R/D, recipient/donor; GvHD, Graft-versus-Host Disease; HSCT, hematopoietic stem cell transplantation; HCMV, human cytomegalovirus; AdV, adenovirus; VZV, varicella zoster virus.

**Table s2. HCMV infection/reactivation characteristics**

| HCMV reactivating patient # | Day after HSCT when first HCMV reactivation occurred | Multiple HCMV reactivations (n°) | n°of days with HCMV DNAemia | Duration of anti-viral treatment (days) |
|---|---|---|---|---|
| 1 | 8 | no | 56 | 56 |
| 2 | 10 | yes (2) | 42 | 32 |
| 3 | 30 | no | 21 | 21 |
| 4 | 48 | yes (3) | 13 | 13 |
| 5 | 42 | no | 19 | 9 |
| 6 | 15 | no | 30 | 17 |
| 7 | 53 | no | 13 | 16 |
| 8 | 50 | no | 11 | 8 |
| 9 | 60 | no | 13 | untreated |
| 10 | 50 | no | 6 | 6 |
| 11 | 51 | yes (2) | 18 | 18 |
| 12 | 10 | no | 14 | 14 |
| 13 | 45 | no | 11 | 11 |

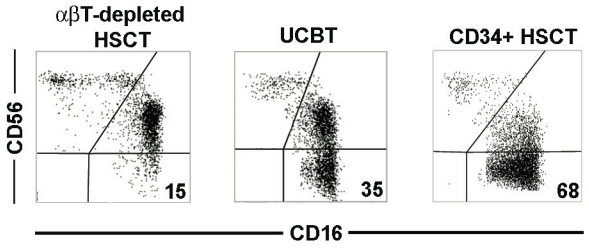
**Table s3. NK cells absolute numbers in patient peripheral blood after HSCT.**

| patient # | 1 m | 3 m | 6 m | 12 m |
|---|---|---|---|---|
| **1** | n.a. | n.a. | n.a. | n.a. |
| **2** | 213 | 479 | 691 | 915 |
| **3** | n.a. | 195 | 272 | 580 |
| **4** | 220 | 1448 | 507 | 240 |
| **5** | 220 | 426 | 293 | 290 |
| **6** | 347 | 280 | 258 | n.a. |
| **7** | 245 | 345 | 559 | 358 |
| **8** | 213 | 1300 | 635 | 354 |
| **9** | 307 | 228 | 45 | 23 |
| **10** | 254 | 734 | 454 | 296 |
| **11** | 156 | 406 | 518 | 250 |
| **12** | 114 | 102 | 167 | n.a. |
| **13** | 384 | 340 | 339 | 465 |
| 14 | 158 | 634 | 729 | n.a. |
| 15 | 85 | 97 | 241 | 204 |
| 16 | 105 | 183 | 224 | 296 |
| 17 | 670 | 324 | 213 | 455 |
| 18 | 304 | 79 | 110 | 108 |
| 19 | 313 | 72 | 54 | 141 |
| 20 | 89 | 88 | 170 | 388 |
| 21 | 760 | 426 | 442 | 441 |
| 22 | 974 | 585 | 723 | 350 |
| 23 | 270 | 77 | 403 | 818 |
| 24 | 449 | 320 | 200 | 79 |
| 25 | 207 | 51 | 78 | 168 |
| 26 | 243 | 476 | 387 | n.a. |
| 27 | 213 | 591 | 678 | 457 |

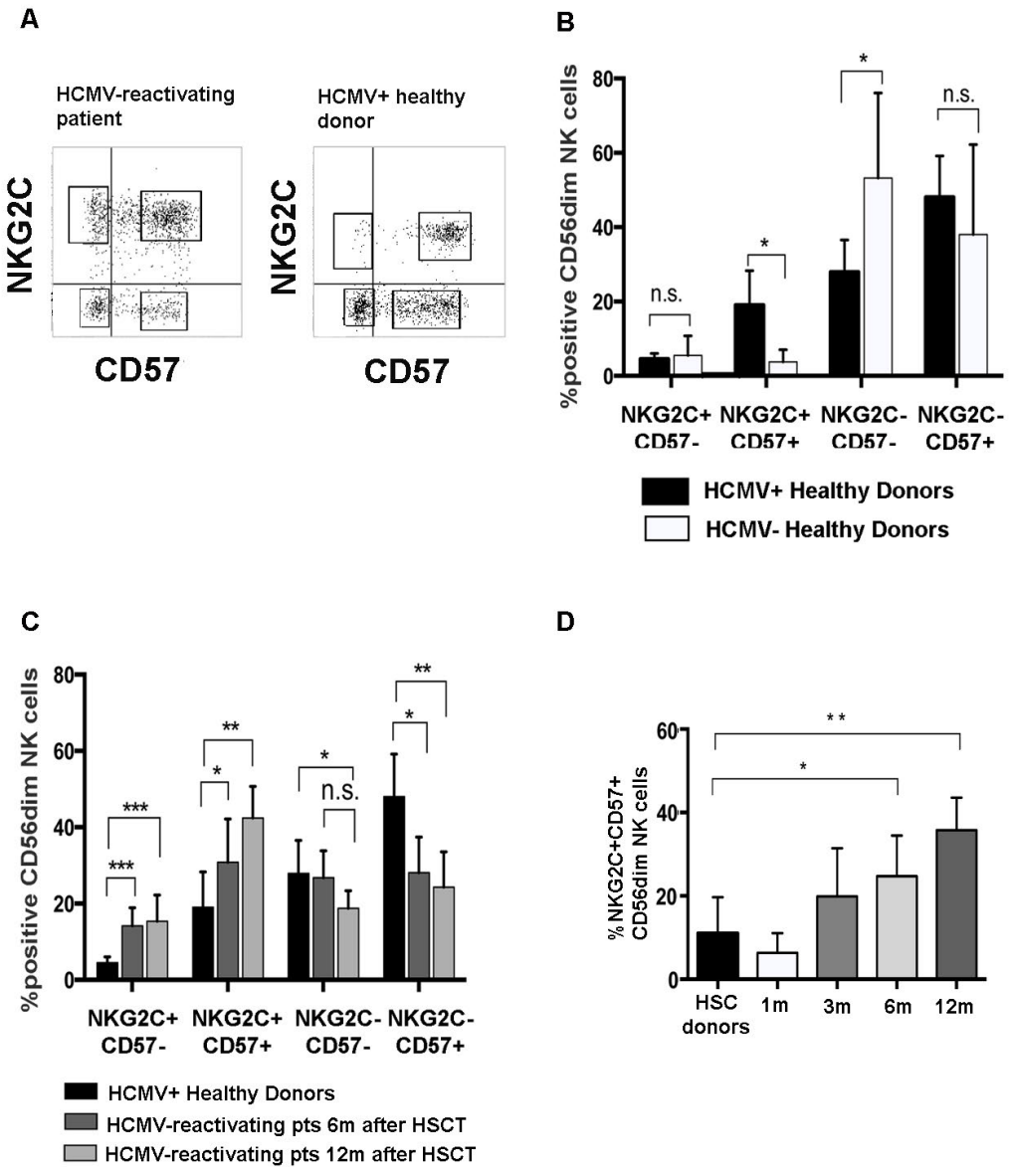
**Table s3 legend: n.a.= not available**
**Patients #1-13 (bold) are those reactivating HCMV after HSCT.**
**The reported values correspond to absolute numbers of NK cells/μl at the different time points after HSCT.**

## Supplemental figures

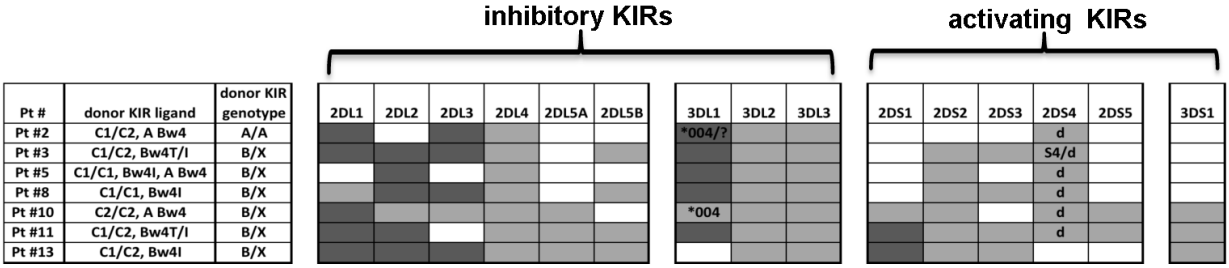
## Figure s1




## Figure s2

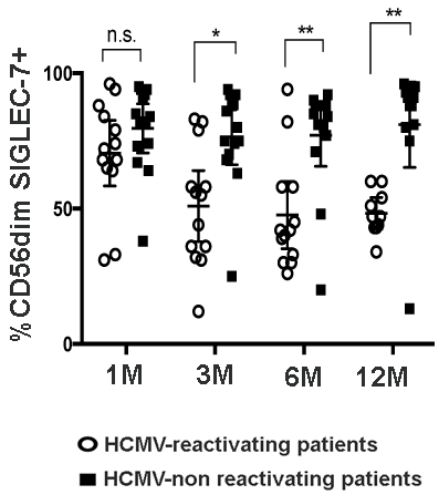


## Figure s3

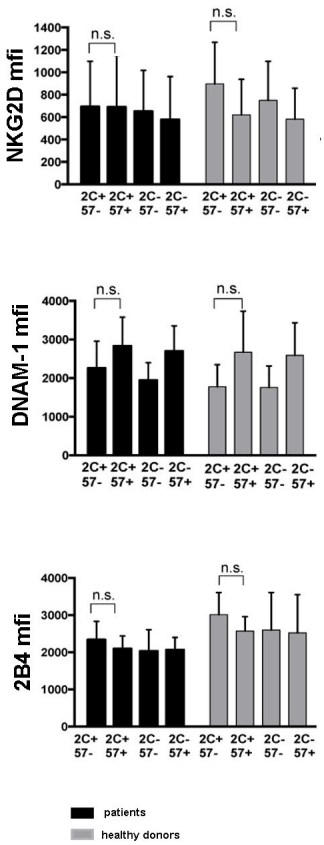



| Pt # | donor KIR ligand | donor KIR genotype | 2DL1 | 2DL2 | 2DL3 | 2DL4 | 2DL5A | 2DL5B | 3DL1 | 3DL2 | 3DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4 | 2DS5 | 3DS1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pt #2 | C1/C2, A Bw4 | A/A | | | | | | | *004/? | | | | | | d | | |
| Pt #3 | C1/C2, Bw4T/I | B/X | | | | | | | | | | | | | S4/d | | |
| Pt #5 | C1/C1, Bw4I, A Bw4 | B/X | | | | | | | | | | | | | d | | |
| Pt #8 | C1/C1, Bw4I | B/X | | | | | | | | | | | | | d | | |
| Pt #10 | C2/C2, A Bw4 | B/X | | | | | | | *004 | | | | | | d | | |
| Pt #11 | C1/C2, Bw4T/I | B/X | | | | | | | | | | | | | d | | |
| Pt #13 | C1/C2, Bw4I | B/X | | | | | | | | | | | | | | | |

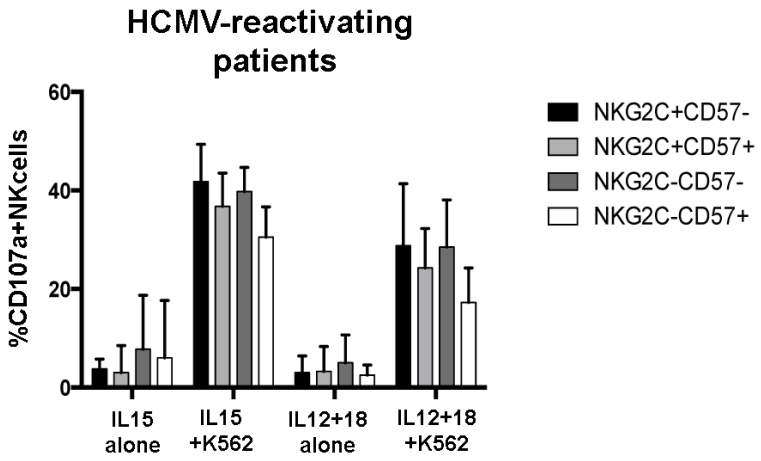
## Figure s4



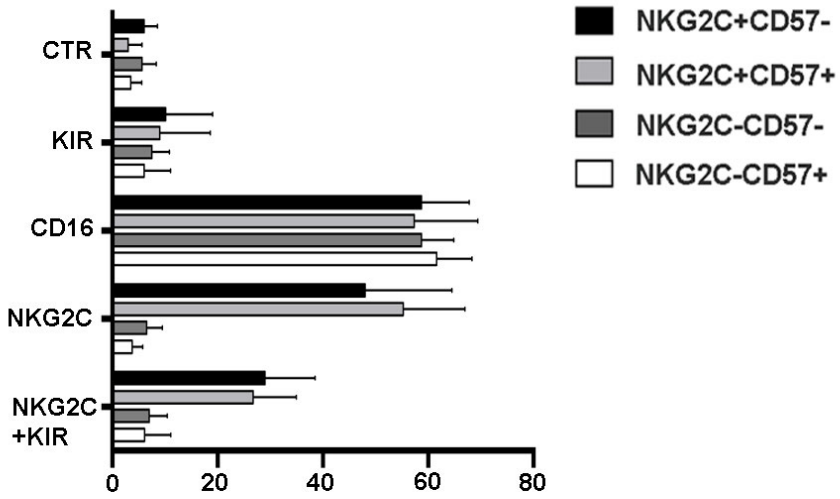
## Figure s5




## Figure s6



## Figure s7




## Figure s8