The early expansion of anergic NKG2A^{pos}/CD56^{dim}/CD16^{neg} natural killer represents a therapeutic target in haploidentical hematopoietic stem cell transplantation

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 doi:10.3324/haematol.2017.186619

Received: December 16, 2017.

Accepted: April 23, 2018.

Pre-published: April 26, 2018.

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The early expansion of anergic NKG2A^{pos}/CD56^{dim}/CD16^{neg} natural killer cells represents a therapeutic target in haploidentical haematopoietic stem cell transplantation

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Supplementary Methods

NK cell culture and functional assays

FACS-sorted NK cell subsets were seeded at 0.1 x 10^6 cells/ml and cultured in presence of 30 U/ml rhIL-15 (Miltenyi) and 100 ng/ml rhIL-18 (MBL International Corporation) for 14 days. NK cell proliferation was determined by the analysis of 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; Life Technologies) dilution. Before stimulation, cells were stained with 2 μM CFSE for 7 min at 37°C and then washed in FBS and medium. Cells were cultured at 37°C in 5% CO_2 humidified atmosphere with one-half medium exchange every 3 days. Proliferation index was assessed as previously described(1).

For degranulation assays, PBMCs were thawed and left 18 hour in medium at 37°C in 5% CO_2, then re-suspended at 1x10^6 cells/ml and cultured with K562 (human immortalized chronic myelogenous leukemia) cell for 4 hours at PBMCs/target ratio 1:1 in the presence of anti-CD107a antibody (BD Bioscience). For masking experiments, PBMCs were left 1 hour in medium, then re-suspended at 4x10^6 cells/ml in the presence of Y9 mAb (IgM, anti-CD94) for 30 minutes at 37°C in 5% CO_2 humidified atmosphere. Subsequently, PBMCs were seeded at 1x10^6 cells/ml with 200 U/ml rhIL-2 in the presence of anti-CD107a mAb and HLA-E^{pos} 721.221G target cell lines at an

PBMCs/target ratio of 5:1(2). After 4 hours, degranulation capacity was assessed measuring the CD107a surface expression cells by flow cytometry staining. Y9 (IgM, anti-CD94) mAb were kindly provided by A. Moretta(3).

RNA isolation and microarray hybridization

RNA samples were obtained with the microRNeasy kit (Qiagen), quality and quantity of the nucleic acid samples were established using Bioanalyzer RNA Nano kit (Agilent Technologies) and Nanodrop 1000 (ThermoFisher Scientific). The Ovation Pico WTA System V2 (NuGen Technologies) allowed a single tube amplification protocol from as less as 5 nanograms of total RNA per sample, providing sufficient amplified cDNA that can be fragmented and labeled using the Encore Biotin Module (NuGen Technologies) and therefore hybridized onto GeneChip® Human Transcriptome Arrays 2.0 (Affymetrix, ThermoFisher Scientific), following the manufacturer's indications. To avoid batch effect among samples, they were randomized during RNA isolation, sample preparation and hybridization/scanning working sessions. All samples were processed with the same reagents lot number, when available. Sample and hybridization quality controls were carried out with Affymetrix Expression Console software.

Microarray data analysis

Microarray probe fluorescence signals were converted to expression values using robust multiarray average procedure RMA(4) of Bioconductor affy package. Specifically, fluorescence intensities have been background adjusted, normalized using quantile normalization, and $\log_2$ expression values for a total of 32500 custom probe sets calculated using median polish summarization and custom chip definition files for Affymetrix Human Transcriptome Array 2.0 based on Entrez genes (hta20_Hs_ENTREZG version 21.0.0)(5). All data analyses were performed in R version 3.3.3. Genes with statistically significant differential expression between $cCD56^{bright}$, $uCD56^{dim}$ and $cCD56^{dim}$ NK cells from healthy donors were identified using one-way analysis of variance (ANOVA, $p<0.05$). Global clustering was performed using the list of 3072 differentially expressed genes and the function hclust of R stats package with Pearson correlation as distance metric and average agglomeration method. To confirm the relationship between sample clusters, we used Principal Component Analysis (PCA) coded by the prcomp function of the R stats package. Raw data are available at Gene Expression Omnibus (GEO) GSE107021. Over-representation analysis was performed using the Gene Set Enrichment Analysis (GSEA) software(6) (http://www.broadinstitute.org/gsea/msigdg/gsea/msigd). GSEA was applied on $\log_2$ expression data of the entire dataset. To determine which set of genes shows statistically significant,

concordant differences between healthy and transplanted subjects, we performed GSEA using Signal2Noise as metric and 1,000 permutations of gene set.

High dimensional single cell analysis of flow cytometry data

Flow Cytometry Standard (FCS) 3.0 files were were analyzd with FlowJo software version 9. Flow cytometry data were compensated in FlowJo by using experiment-specific single-stained controls (BD Compbeads incubated with fluorescently-conjugated antibodies), as previously described(7). A unique computational barcode was assigned to single samples that were subsequently concatenated (~4,800 PBMCs per sample; 293,000 cells in total) and visualized with t-distributed stochastic neighbour embedding (t-SNE; Barnes-Hut implementation; iterations, 1000; perplexity, 40; initialization, deterministic; theta, 0.5; eta: 200) and taking into consideration the expression of the following markers: Perforin, CD16, CD20, NKG2D, NKG2A, Granzyme B, CD3, NKp46, CD56 and CD8 (Supplementary Figure 3).

Statistical analysis

Analysis was performed using GraphPad PRISM (6.0b) and SPICE 5.22 software. Paired Student's t-test, non-parametric paired Wilcoxon rank test and unpaired Mann-Whitney test were used to compare the different variables. One-way Anova with Bonferroni's correction test was used to analyze the kinetics of NK cell subset proliferation and differentiation. P values are two-sided and were considered significant when ≤ 0.05. The abbreviation n.s. is referred to not statistically significant comparisons.

Supplementary References

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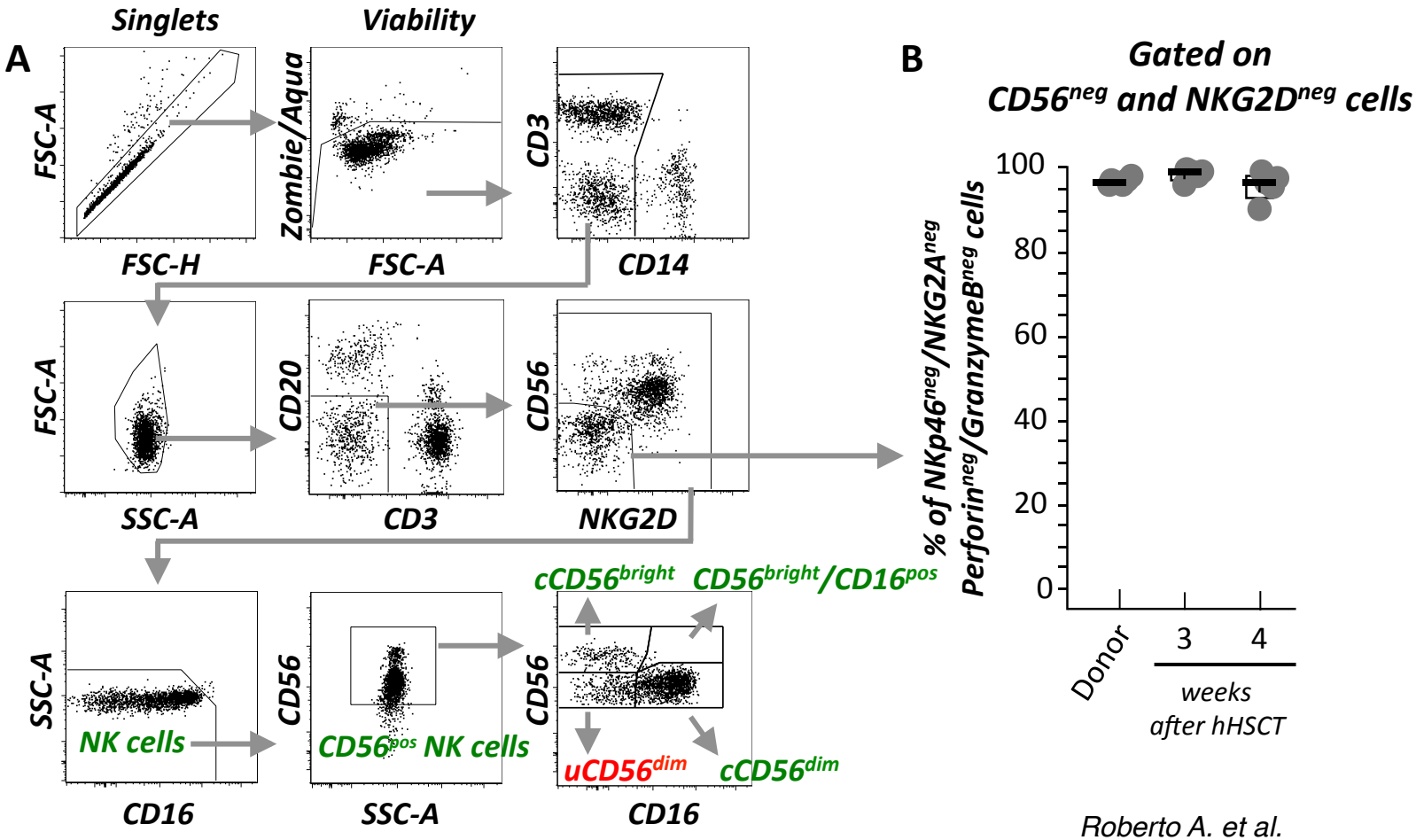

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Figure S1

Supplementary Figure S1.
NK cell gating strategy

(A) *Representative example of polychromatic flow cytometry dot plots from an healthy donor showing the gating strategy used to identify the following four CD56^{pos} NK cell subsets: 1) conventional CD56^{bright}/CD16^{neg} (cCD56^{bright}) NK cells; 2) CD56^{bright}/CD16^{pos} NK cells; 3) conventional CD56^{dim}/CD16^{pos} (cCD56^{dim}) NK cells; 4) unconventional CD56^{dim}/CD16^{neg} (uCD56^{dim}) NK cells (labelled in red). Dead cells, CD14^{pos} monocytes, CD3^{pos}T cells, CD20^{pos} B cells and 'non classical' SSC^{hi}/CD14^{low}/CD16^{bright} monocytes were excluded from the analysis. NKG2D^{pos} NK cell subsets were defined on the basis of CD56 and CD16 expression.* ***(B)*** *Summary graph showing the frequency (median ± SEM) of CD56^{neg}/NKG2D^{neg} lacking the expression of the NK cell surface markers NKp46, NKG2A, Perforin and Granzyme (i.e. not NK cells) from 6 healthy donors and 5 haplo-HSCT (H-HSCT) recipients at 3 and 4 weeks after transplant.*

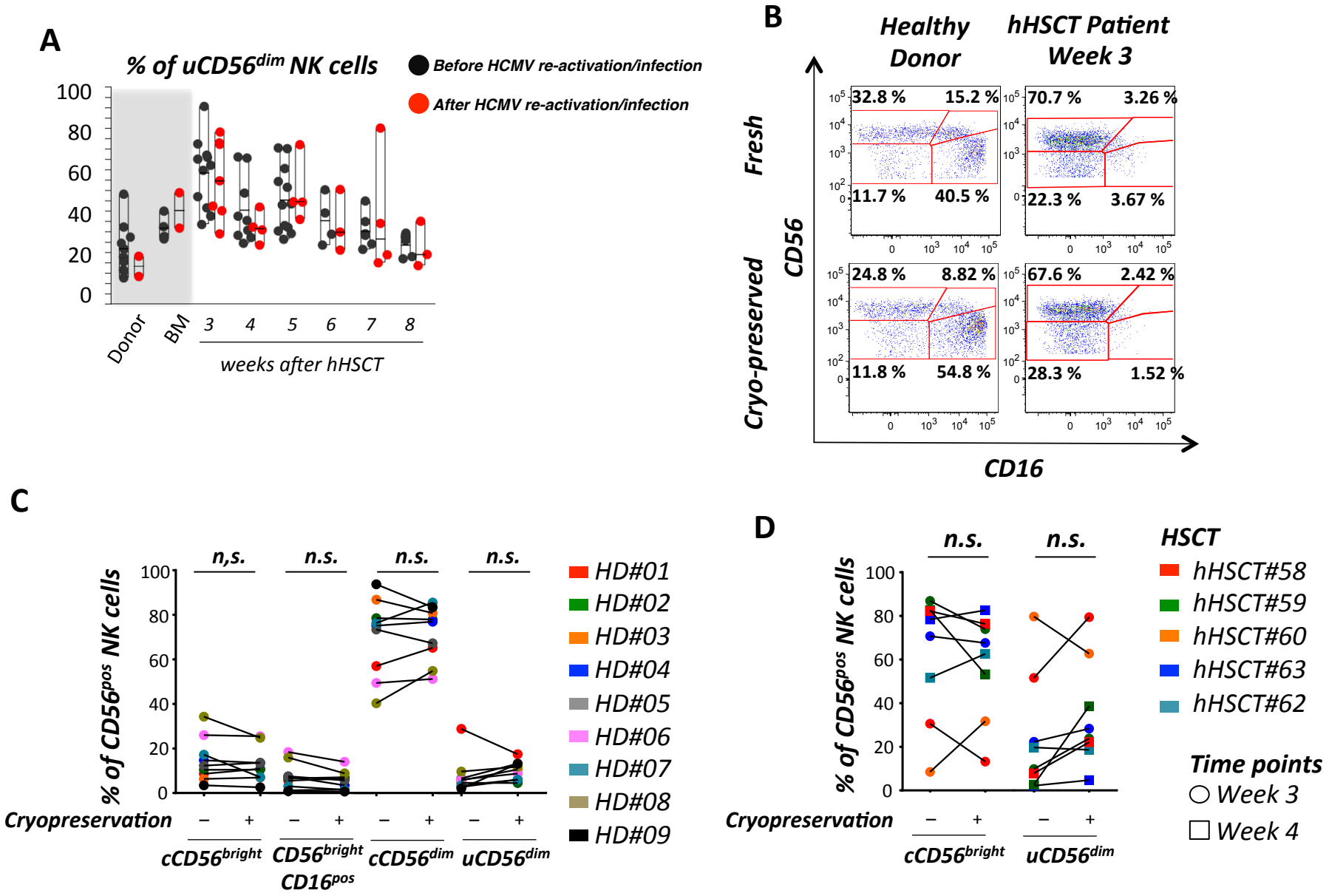

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Figure S2

Supplementary Figure S2.

Effect on HCMV infection/re-activation and cryopreservation on the frequencies of uCD56^{dim} NK cell

(A) *Summary statistical graph showing the frequency (median ± SEM) of unconventional CD56^{dim}/CD16^{neg} (uCD56^{dim}) NK cell subsets in the peripheral blood (PB) and bone marrow (BM) of hematopoietic stem cell healthy donors (HDs) compared to their counterparts in the blood of the related recipients up to 8 weeks after hHSCT either in the presence or in absence of human cytomegalovirus (HCMV) re-activation/ infection.* ***(B)*** *Representative example of flow cytometry dot plots from an healthy donor and a patient after 3 weeks from haplo-identical HSCT (hHSCT) showing the percentages of conventional CD56^{bright}/CD16^{neg} (cCD56^{bright}), CD56^{bright}/CD16^{pos}, conventional CD56^{dim}/ CD16^{pos} (cCD56^{dim}) and unconventional CD56^{dim}/CD16^{neg} (uCD56^{dim}) NK cell subsets within the same freshly purified (upper line) and cryopreserved (lower line) PBMCs.* ***(C-D)*** *Statistical summary graphs showing the frequencies of the above-mentioned CD56^{pos} NK cell subsets within the same freshly purified (-) and cryopreserved (+) PBMCs from 9 healthy donors (HD) (**B**) and from 5 hHSCT patients at 3 and 4 weeks after the transplant.*

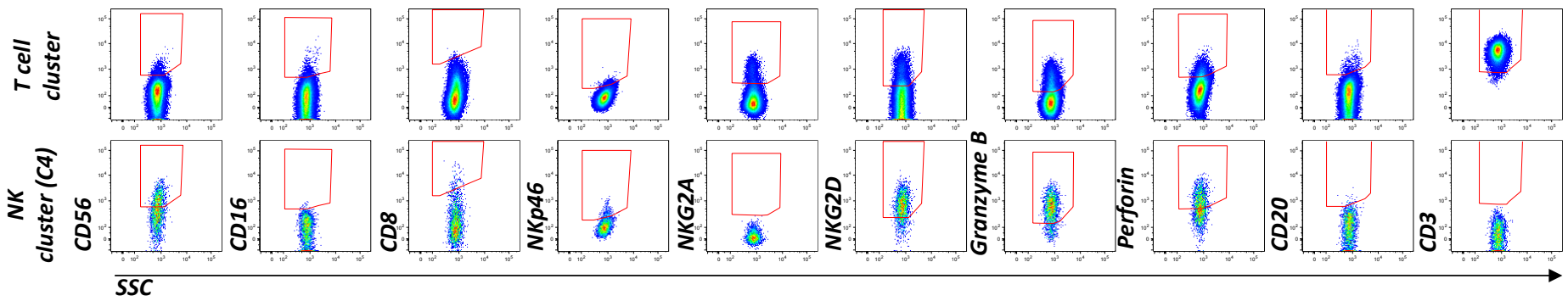

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Figure S3

Supplementary Figure S3.
Representative gating strategy defining the identity of the t-SNE clusters

Representative example of arbitrary defined clusters of Natural Killer cells (shown is NK cell cluster C4, Figure 2A) following t-SNE analysis. NK cells were investigated for the expression of surface and intracellular markers used for the t-SNE classification. The T cell cluster identified by $CD3^{pos}$ expression is shown as a control.

No.	DISEASE	DONOR	AGE D/R	CMV D/R	DISEASE STATUS BEFORE TRANSPLANT	INDICATION FOR TRANSPLANT	aGvHD (grade, localization)	aGvHD (therapy)	cGvHD (grade, localization)	cGvHD (therapy)	INFECTIONS/VIRUS REACTIVATION	FOLLOW-UP (weeks)	REASON FOR STOPPING FOLLOW UP	DISEASE STATUS	Survival
1	HL	Mother	51/20	-/-	CR	RAA	-	-	-	-	FUO	53	End of the study	CR	A, > 5 years
2	HL	Father	52/22	-/+	CR	RAA	II, Skin	ECP	-	-	Pneumonia of unknown origin, CMV reactivation	53	End of the study	CR	A, > 5 years
3	NHL	Sister	47/44	+/+	PR	RAA	-	-	-	-	JC	10	JC encephalytis	CR	D, 10 weeks
4	HL	Brother	47/45	-/+	CR	RAA	II, Skin	ECP	Mild	Steroid	BK, EBV reactivation	53	End of the study	CR	A, > 4 years
5	HL	Mather	61/33	+/+	CR	RAA	-	-	-	-	BK	53	End of the study	CR	A, > 4 years
6	NHL-T	Brother	47/45	+/+	CR	HR	-	-	-	-	ESBL E. coli, CMV reactivation	9	Liver failure	-	D, 9 weeks
7	NHL	Brother	62/57	+/-	CR	RAA	II, Skin	ECP+steroid	-	-	-	40	PD	PD	D, 40 weeks
8	HL	Brother	29/34	-/-	CR	Ref	II, Skin	ECP+steroid	-	-	FUO, EBV reactivation	53	End of the study	PD	D, > 3 years
9	NHL	Son	23/44	+/+	CR	Ref	-	-	-	-	E. foecalis, A. fumigatus	7	Lung Aspergillosis	-	D, 7 weeks
10	NHL	Brother	46/54	+/+	PR	Ref	I, Skin	-	-	-	ESBL E. coli, CMV reactivation	53	End of the study	CR	A, > 4 years
11	HL	Son	24/57	-/+	CR	Ref	-	-	-	-	A. fumigatus, E.coli, HHV6	53	End of the study	CR	A, > 4 years
12	NHL	Brother	62/57	+/+	CR	RAA	II, Skin	ECP	-	-	C. difficile, CMV reactivation	22	Heart failure	CR	D, 22 weeks
13	HL	Mother	60/24	+/+	CR	Ref	-	-	-	-	RSV	5	-	-	D, 5 weeks
14	HL	Mother	45/24	+/+	PD	RAA	-	-	-	-	Liver Mycosis, CMV	53	End of the study	PD	D, = 2 years
15	NHL-T	Father	51/25	+/+	CR	HR	I, Skin	-	-	-	FUO	53	End of the study	CR	A, > 4 years
16	HL	Sister	38/47	+/+	CR	RAA	-	-	Moderate	Steroid	-	53	End of the study	CR	A, > 4 years
17	NHL	Son	35/62	+/-	PR	RAA	III, Skin	ECP	-	-	S. Maltophilia, CMV	53	End of the study	CR	A, > 3 years
18	NHL	Sister	49/42	+/+	PR	RAA	I, Skin	-	-	-	FUO	53	End of the study	CR	A, > 3 years
19	HL	Cousin	48/40	+/+	PR	RAA	-	-	-	-	-	50	PD	PD	D, 50 weeks
20	HL	Brother	47/37	-/-	SD	RAA	-	-	-	-	E coli, A. fumigatus, HHV6 reactivation	16	PD	PD	D, 16 weeks
21	AML	Mother	63/33	+/+	CR	Ref	-	-	-	-	C. difficile, CMV reactivation	53	End of the study	CR	A, > 2 years
22	HL	Brother	22/30	+/+	CR	RAA	-	-	Mild	Steroid	Pneumonia of unknown origin, CMV reactivation	53	End of the study	CR	A, > 2 years
23	NHL-T	Cousin	27/44	+/+	CR	HR	-	-	-	-	HHV6, CMV	44	PD	PD	D, 44 weeks
24	ALL	Brother	35/46	-/+	CR	HR	II, Skin	ECP+steroid	-	-	FUO	53	End of the study	CR	A, > 2 years
25	AML	Brother	59/70	+/+	CR	HR	II, Skin	ECP+steroid	-	-	-	15	CNS haemorrage	CR	D, 15 weeks
26	MDS	Son	32/70	+/+	PD	HR	-	-	-	-	A. fumigatus, CMV reactivation	53	End of the study	CR	A, > 1 year
27	HL	Father	47/20	-/+	CR	Ref	II, Skin	ECP+steroid	-	-	E coli, CMV	34	Ongoing	CR	A, 34 weeks
28	NHL	Son	21/59	-/+	CR	RAA	-	-	-	-	CMV reactivation	53	End of the study	CR	A, > 1 year
29	HL	Brother	28/25	+/-	CR	HR	-	-	-	-	CMV reactivation	53	End of the study	CR	A, > 1 year
30	HL	Father	61/26	+/-	CR	RAA	I, Skin	-	-	-	HZV	53	End of the study	CR	A, > 1 year

Supplementary Table S1.
Clinical and epidemiological features of patients and donors enrolled in the study

Abbreviations:

HL: Hodgkin lymphoma; NHL: non-Hodgkin lymphoma; NHL-T: T-cells non-Hodgkin lymphoma; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; MDS: Myelodysplastic Syndrome; D/R: donor/recipient; CMV: Cytomegalovirus; CR: complete response; PR: partial response; PD: progressive disease; SD: stable disease; RAA: Relapse after autologous; HR: High-risk; Ref, Refractory; -: not applicable; ECP: Extracorporeal photochemotherapy; FUO: Fever of Unknown Origin; JC: John Cunningham polyomavirus; BK: BK polyomavirus; EBV: Epstein-Barr virus; E. foecalis: Enterococcus faecalis; A. fumigatus: Aspergillus Fumigatus; E. coli: Escherichia Coli; ESBL E. coli: Extended-spectrum beta-lactamase-producing Escherichia Coli; HHV6: Human Herpes Virus 6; C. difficile: Clostridium Difficile; RSV: Respiratory Syncytial Virus;S. Maltophilia: Stenotrophomo-s Maltophilia; HZV: Herpes Zoster; A: Alive; D: Deceased

Supplementary Table S2.

Clones of monoclonal antibodies (mAbs) used in flow cytometry.

mAbs	**Clone**
Anti-human CD107a	H4A3
Anti-human CD117	104D2D1
Anti-human CD127	A019D5
Anti-human CD14	M5E2 or TüK4
Anti-human CD159a (NKG2A)	REA110 or Z199
Anti-human CD16	*3G8*
Anti-human CD19	LT19 or SJ25-C1
Anti-human CD20	2H7
Anti-human CD3	OKT3, SK7 or SP34-2
Anti-human CD314 (NKG2D)	1D11
Anti-human CD335 (NKp46)	*9E2*
Anti-human CD337 (NKp30)	*P30-15, Z2A*
Anti-human CD34	581
Anti-human CD56	B159, HCD56 or NCAM16.2
Anti-human CD8	*3B5*
Anti-human Granzyme B	*GB11*
Anti-human HLA -A2	BB7.2
Anti-human Ki-67	*B56*
Anti-human Perforin	*δG9*

Supplementary Table S3: Gene sets significantly enriched (FDR<0.00001) in NK cell subsets from HSCT patients compared to healthy donors	NES	FDR q-val
REACTOME_CELL_CYCLE	4,14	0
REACTOME_CELL_CYCLE_MITOTIC	4,05	0
REACTOME_DNA_REPLICATION	3,92	0
REACTOME_MITOTIC_M_M_G1_PHASES	3,90	0
REACTOME_CELL_CYCLE_CHECKPOINTS	3,77	0
REACTOME_MITOTIC_G1_G1_S_PHASES	3,71	0
REACTOME_G1_S_TRANSITION	3,67	0
REACTOME_S_PHASE	3,62	0
REACTOME_SYNTHESIS_OF_DNA	3,61	0
REACTOME_M_G1_TRANSITION	3,47	0
REACTOME_MITOTIC_PROMETAPHASE	3,43	0
REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE	3,43	0
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_MITOTIC_PROTEINS	3,42	0
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	3,39	0
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS	3,36	0
REACTOME_MEIOTIC_RECOMBINATION	3,35	0
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX	3,35	0
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	3,34	0
REACTOME_CHROMOSOME_MAINTENANCE	3,33	0
REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	3,30	0
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC_C	3,30	0
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	3,29	0
REACTOME_TRANSCRIPTION	3,26	0
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	3,26	0
REACTOME_RNA_POL_I_RNA_POL_III_AND_MITOCHONDRIAL_TRANSCRIPTION	3,22	0
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION	3,21	0
REACTOME_MEIOSIS	3,21	0
REACTOME_ER_PHAGOSOME_PATHWAY	3,21	0
REACTOME_SCFSKP2_MEDIATED_DEGRADATION_OF_P27_P21	3,20	0
REACTOME_DNA_REPAIR	3,19	0
REACTOME_RNA_POL_I_TRANSCRIPTION	3,18	0
REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION	3,18	0
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	3,18	0
REACTOME_TELOMERE_MAINTENANCE	3,17	0
REACTOME_HIV_INFECTION	3,17	0
REACTOME_CDK_MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6	3,13	0
REACTOME_P53_INDEPENDENT_G1_S_DNA_DAMAGE_CHECKPOINT	3,13	0
REACTOME_DNA_STRAND_ELONGATION	3,12	0
REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE	3,11	0
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	3,10	0
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	3,10	0
REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU_RICH_ELEMENTS	3,10	0
REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES	3,09	0
REACTOME_G2_M_CHECKPOINTS	3,08	0
REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	3,07	0
REACTOME_METABOLISM_OF_RNA	3,06	0
REACTOME_CLASS_I_MHC_MEDIATED_ANTIGEN_PROCESSING_PRESENTATION	3,06	0
REACTOME_APOPTOSIS	3,05	0

REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COP1	3,05	0
REACTOME_MITOTIC_G2_G2_M_PHASES	3,04	0
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE_ODC	3,03	0
REACTOME_RNA_POL_I_PROMOTER_OPENING	3,00	0
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	3,00	0
REACTOME_REGULATION_OF_APOPTOSIS	2,99	0
REACTOME_ANTIGEN_PROCESSING_UBIQUITINATION_PROTEASOME_DEGRADATION	2,99	0
REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0	2,98	0
REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	2,97	0
REACTOME_SIGNALING_BY_WNT	2,97	0
REACTOME_FACTORS_INVOLVED_IN_MEGAKARYOCYTE_DEVELOPMENT_AND_PLATELET_PRODUCTION	2,96	0
REACTOME_MRNA_PROCESSING	2,94	0
REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	2,92	0
REACTOME_TRNA_AMINOACYLATION	2,91	0
REACTOME_ACTIVATION_OF_NF_KAPPAB_IN_B_CELLS	2,88	0
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	2,87	0
REACTOME_MRNA_SPLICING	2,85	0
REACTOME_EXTENSION_OF_TELOMERES	2,83	0
REACTOME_NUCLEOTIDE_EXCISION_REPAIR	2,82	0
REACTOME_RECRUITMENT_OF_MITOTIC_CENTROSOME_PROTEINS_AND_COMPLEXES	2,82	0
REACTOME_AMYLOIDS	2,82	0
REACTOME_METABOLISM_OF_MRNA	2,80	0
REACTOME_TRANSCRIPTION_COUPLED_NER_TC_NER	2,79	0
REACTOME_HIV_LIFE_CYCLE	2,78	0
REACTOME_PACKAGING_OF_TELOMERE_ENDS	2,77	0
REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	2,76	0
REACTOME_METABOLISM_OF_NON_CODING_RNA	2,75	0
REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	2,73	0
REACTOME_G0_AND_EARLY_G1	2,72	0
REACTOME_LOSS_OF_NLP_FROM_MITOTIC_CENTROSOMES	2,71	0
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	2,69	0
REACTOME_RNA_POL_II_TRANSCRIPTION	2,68	0
REACTOME_LATE_PHASE_OF_HIV_LIFE_CYCLE	2,66	0
REACTOME_METABOLISM_OF_NUCLEOTIDES	2,65	0
REACTOME_LAGGING_STRAND_SYNTHESIS	2,65	0
REACTOME_APC_CDC20_MEDIATED_DEGRADATION_OF_NEK2A	2,62	0
REACTOME_REPAIR_SYNTHESIS_FOR_GAP_FILLING_BY_DNA_POL_IN_TC_NER	2,61	0
REACTOME_MITOCHONDRIAL_PROTEIN_IMPORT	2,61	0
REACTOME_INTERFERON_SIGNALING	2,61	0
REACTOME_KINESINS	2,60	0
REACTOME_RNA_POL_II_TRANSCRIPTION_PRE_INITIATION_AND_PROMOTER_OPENING	2,60	0
REACTOME_MRNA_SPLICING_MINOR_PATHWAY	2,60	0
REACTOME_GLOBAL_GENOMIC_NER_GG_NER	2,59	0
REACTOME_CITRIC_ACID_CYCLE_TCA_CYCLE	2,56	0
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_CYCLIN_B	2,55	0
REACTOME_POL_SWITCHING	2,55	0
REACTOME_UNWINDING_OF_DNA	2,54	0
REACTOME_DOUBLE_STRAND_BREAK_REPAIR	2,54	0
REACTOME_PEROXISOMAL_LIPID_METABOLISM	2,53	0

REACTOME_INHIBITION_OF_THE_PROTEOLYTIC_ACTIVITY_OF_APC_C_REQUIRED_FOR_THE_ONSET_OF_ANAPHASE_BY_MITOTIC_SPINDLE_CHECKPOINT_COMPONENTS	2,52	0
REACTOME_MEIOTIC_SYNAPSIS	2,51	0
REACTOME_FORMATION_OF_ATP_BY_CHEMIOSMOTIC_COUPLING	2,48	0
REACTOME_PHOSPHORYLATION_OF_THE_APC_C	2,48	0
REACTOME_PYRUVATE_METABOLISM_AND_CITRIC_ACID_TCA_CYCLE	2,46	0
REACTOME_DOWNSTREAM_SIGNALING_EVENTS_OF_B_CELL_RECEPTOR_BCR	2,46	0
REACTOME_DEADENYLATION_DEPENDENT_MRNA_DECAY	2,46	0
REACTOME_INTERFERON_GAMMA_SIGNALING	2,45	0
REACTOME_PROCESSIVE_SYNTHESIS_ON_THE_LAGGING_STRAND	2,44	0
REACTOME_PROCESSING_OF_CAPPED_INTRONLESS_PRE_MRNA	2,44	0
REACTOME_CYCLIN_A_B1_ASSOCIATED_EVENTS_DURING_G2_M_TRANSITION	2,43	0
REACTOME_RNA_POL_II_PRE_TRANSCRIPTION_EVENTS	2,43	0

Supplementary Table S4.

List of genes of uCD56^{dim} NK cells from HSCT patients similarly expressed in cCD56^{bright} NK cells from healthy donors [FC_x (log_2) < |1|] but differently modulated in cCD56^{dim} NK cells from healthy donors (FC_y (log_2) > |1|).

Gene equally expressed in uCD56^{dim} NK cells from HSCT patients compared to cCD56^{bright} NK cells from healthy donors.	
Gene up-regulated in uCD56^{dim} NK cells from HSCT patients compared to cCD56^{dim} NK cells from healthy donors.	**Gene down-regulated in uCD56^{dim} NK cells from HSCT patients compared to cCD56^{dim} NK cells from healthy donors.**
GZMK	**GZM-M**
IL3	**CD160**
IL18R1	**CXCR2 (IL8R)**
CCR2	**TIGIT**