Supplemental Materials for

Impaired T and NK Cell Reconstitution after Haploidentical HCT with Post-transplant Cyclophosphamide

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SUPPLEMENTAL METHODS

Patients, Transplantation Procedures and sample collection

This study included 95 patients who underwent allogeneic HCT at the Dana-Farber Cancer Institute and Brigham and Woman's Hospital (Boston, MA) between November 2011 and November 2018. Of these, 60 patients received a haploidentical T cell replete haplo-HCT with PTCy (haplo-HCT group) and 35 underwent a matched (8/8) related (11/35) or unrelated (24/35) donor transplant (MD-HCT group). Both cohorts received reduced intensity conditioning (RIC): cyclophosphamide (29 mg/kg) and fludarabine (120 mg/m^2) and low dose of total body irradiation (TBI, 200 cGy) for haplo-HCT group and intravenous low dose busulfan (260 mg/m^2 or 3.2 mg/Kg) and fludarabine (120 mg/m^2) for MD-HCT group. GVHD prophylaxis for haplo-HCT patients consisted of PTCy (50 mg/kg on days +3 and +4) and tacrolimus (TAC) and mycophenolate mofetil (MMF) starting from day +5. MMF was discontinued at day +28. No systemic corticosteroids were allowed until 24 hours after completion of day +4 cyclophosphamide. GVHD prophylaxis for MD-HCT patients consisted of methotrexate (MTX, 5 mg/m^2) on days +1, +3, +6, +11 and TAC beginning from day –3. Patients received G-CSF (Tbo-Filgrastim) starting from day +5 in the haplo-HCT group and after the last dose of MTX in the MD-HCT group, until ANC was >500 for 2 consecutive days. Patients in both cohorts received acyclovir for viral prophylaxis, with the exception of seven patients in the haplo-HCT group that also received letermovir per institutional policy, after it was approved for cytomegalovirus (CMV) prophylaxis. Any CMV DNA detectable by PCR above the sensitivity threshold after transplant was considered as viral reactivation. Flow cytometry staining for immune reconstitution analysis was performed on fresh whole blood samples. For analysis, both percentages and absolute numbers of the parental cells were considered. Absolute numbers were calculated from the complete blood

cell count (CBC) performed on the same day as flow cytometry analysis. For mass cytometry by time of flight (CyTOF) and NK functional assays, PBMC were isolated from freshly drawn blood samples by density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare) and cryopreserved in RPMI 10% DMSO before being utilized.

Analysis of single cell mass cytometry data for NK cell characterization

NK cells were identified by 2-dimensional gating for the expression of $CD45^{+}CD3^{-}CD19^{-}CD14^{-}CD56^{+}$ markers (supplemental Figure 3). Dimensionality reduction was performed on a concatenated dataset after downsampling NK cells to 5000 events from each sample (approx. 60000 total events for analysis performed at 1 month and 100000 events at 3 months). To interpret high dimensional single-cell data that were produced by mass cytometry, we used a tool based on the viSNE algorithm,[1] which allows visualization of high-dimensional cytometry data on a 2-dimensional map at single-cell resolution and preserve the nonlinearity.[2] In the viSNE map, cell position reflects their proximity in high dimensional space based on the similarity of marker expression. A FlowSOM analysis[3] was then run to identify distinct NK cell meta-clusters. A self-organized map (SOM) is an unsupervised technique for clustering and dimensionality reduction, in which a discretized representation of the input space is trained.[4]

Flow cytometry-based NK functional assay

NK activation after co-culture with target cells was evaluated by measuring the expression of CD107a as a marker of degranulation and by cytokine (IFNγ and TNFα) release. PBMC from patients and HD were thawed using CTL Anti-Aggregate (CTL-AA-001, Cellular Technology

Limited) solution (1:20 dilution with RPMI) and then resuspended in RPMI medium with 10% FBS. After thawing, PBMC were left at 37°C overnight in the presence or absence of IL-15 (5 ng/ml) (Miltenyi Biotec) for NK priming. PBMC were then washed 2 times and cultured for 6 hours at 37 with K562 cells (effector:target ratio 5:1) previously stained with Cell Trace Violet (CTV, Invitrogen C34557) according to the manufacturer's recommended protocol. To detect cytokine release, Golgi Stop (Protein transport inhibitor Monensin BD 554724) and Golgi Plug (Protein transport inhibitor Brefeldin A BD 51-2301KZ) were added after 1 h of co-culture. After co-culture, cells were collected and stained using a panel of surface and intracellular mAbs shown in supplemental Table 4. Zombie Red staining (Biolegend 423109) was used to distinguish live and dead cells. Samples were then resuspended in PBS 2% FBS and acquired on Fortessa LSR flow cytometer (BD). FCS files were analyzed using FlowJo Version 10 software. An example of the gating strategy is shown in supplemental Figure 4.

Statistical analysis

Patient baseline characteristics and immunophenotype data were analyzed primarily descriptively. Overall survival (OS) was defined as the time from stem cell transplant to death from any cause. Progression-free survival (PFS) was defined as the time from stem cell infusion to disease relapse, progression or death from any cause, whichever occurred first. Patients who were alive without disease relapse or progression were censored at the time of the last visit. The Kaplan-Meier method was used to estimate PFS and OS whereas cumulative incidence of non-relapse mortality (NRM), relapse, and GVHD was estimated in the context of a competing risks framework considering relapse for NRM, NRM for relapse, and death or relapse without developing GVHD for GVHD as a competing event. The log-rank and Gray tests[5] were used for

comparing estimates of PFS and OS and estimates of cumulative incidence of NRM and relapse, respectively. Immunophenotype data, CyTOF data and NK functional data were compared using the Wilcoxon rank-sum test for unpaired group comparison and the Wilcoxon signed-rank test for paired comparison. All tests were 2-sided at the significance level of 0.05 and multiple comparisons were not considered. Statistical analyses for immune reconstitution analysis were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC) and R version 3.3.2 (the CRAN project), while functional and CyTOF data were analyze using Prism software (GraphPad). All graphs were made using Prism software (GraphPad). Heatmaps were generated using GENE-E (http://www.broadinstitute.org/ cancer/software/GENE-E).

SUPPLEMENTAL REFERNCES

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Months after HCT	*1*	*2*	*3*	*6*	*9*	*12*	*18*	*24*
Haplo-HCT, n	37	51	46	36	24	25	18	17
MD-HCT, n	21	26	20	14	14	12	10	7

Table S1. Number of samples tested by flow cytometry at each time point after transplant were used for graphs displayed in Figures 1, 2, 3 and 6. Missing data 1 month after HCT was due to technical difficulties drawing samples on inpatient hospital units. This trend was proportionally similar between the two cohorts: 37/60 (62%) and 51/60 (85%) haplo-HCT display a 1 and 2 months sample respectively, versus 21/35 (60%) and 26/35 (74%) for MD-HCT.

TUBE	TARGET	FLUOROCHROME	CLONE	COMPANY	PRODUCT
T cells	CD45RO	FITC	UCHL1	BD	555492
	CD279 PD-1	PE	eBioJ105	eBio	12-2799-42
	CD127	PE-Cy5	eBioRDR5	eBio	15-1278-42
	CD25	PE-Cy7	M-A251	BD	557741
	CD62L	APC	DREG-56	BD	559772
	CD4	APC-H7	RPA-T4	BD	560158
	CD3	Pac Blue (V450)	UCHT1	BD	560365
	CD8	BV510	RPA-T8	BioLegend	301047
B cells	BAFF-R	FITC	8A7	eBio	11-9117-42
	IgD	PE	IA6-2	BD	555779
	CD27	PE-Cy5	O323	eBio	15-0279-42
	CD38	PE-Cy7	HIT2	BD	560677
	CD19	APC	HIB19	BD	555415
	CD20	APC-H7	2H7	BD	560734
	CD5	Pac Blue (V450)	UCHT2	BD	561154
	CD45	BV510	HI30	BD	563204
NK cells	CD16	FITC	3G8	BD	555406
	CD56	PE	B159	BD	555516
	CD8	PE-Cy7	RPA-T8	BD	557746
	NKG2D	APC	1D11	BD	558071
	CD45	APC-H7	2D1	BD	560178
	CD3	Pac Blue (V450)	UCHT1	BD	560365
	CD4	BV510	SK3	BD	562970

Table S2. Conjugated monoclonal antibodies in the flow cytometry immune monitoring panel.

	TARGET	SPECIES	CLONE	ISOTOPE	COMPANY
Surface					
1	CD45	Human	HI30	89Y	Fluidigm
2	HLA-DR	Cross	L243	141Pr	Biolegend
3	CD95	Human	DX2	164Dy	Fluidigm
4	CD3	Human	UCHT1	170Er	Fluidigm
5	CD4	Human	SK3	174Yb	Fluidigm
6	CD8	Human	SK1	168Er	Fluidigm
7	CD25	Human	2A3	149Sm	Fluidigm
8	CD14	Human	M5E2	160Gd	Fluidigm
9	CD19	Human	HIB19	142Nd	Fluidigm
10	CD56	Human	HCD56	145Nd	Biolegend
11	CD16	Human	3G8	209Bi	Fluidigm
12	CD57	Human	HCD57	165Ho	BioLegend
13	CCR7	Human	G043H7	148Nd	BioLegend
14	CD62L	Human	DREG-56	153Eu	Fluidigm
15	CD69	Human	FN50	163Dy	Biolegend
16	TIM-3	Human	F38-2E2	144Nd	BioLegend
17	ICOS(CD278)	Human	DX29	151Eu	Fluidigm
18	ILT1 (CD85h)	Human	24	115In	Biolegend
19	NKp30 (CD337)	Human	Z25	159Tb	Fluidigm
20	NKp44 (CD336)	Human	P44-8	147Sm	Biolegend
21	NKp46 (CD335)	Human	BAB281	162Dy	Fluidigm
22	NKG2D (CD314）	Human	1D11	161Dy	BD Bioscience
23	NKG2C(CD195c)	Human	134591	166Er	R&D
24	DNAM1(CD226)	Human	DX11	152Sm	BD Bioscience
25	NKG2A (CD94a)	Human	Z199	169Tm	Fluidigm
26	ILT2 (CD85j)	Human	GHI/75	156Gd	Fluidigm
27	KIR2DL1/DS1(CD158a,h)	Human	EB6B	158Gd	Milteni
28	KIR2DL2/2DS3(CD158b)	Human	CH-L	172Yb	BD Bioscience
29	KIR3DL1	Human	DX9	167Er	Fluidigm
30	TIGIT	Human	MBSA43	154Sm	Fluidigm
31	TRAIL	Human	RIK-2	150Sm	Biolegend
Intracellular					
32	Ki67	Cross	B56	176Yb	BD Bioscience
33	CTLA-4(CD152)	Human	14D3	146Nd	eBioscience
34	Perforin	Human	B-D48	175Lu	Fluidigm
35	GranzymeB	Human	GB11	173Tb	Fluidigm

Table S3. Antibody-metal conjugate panel used for mass cytometry.

TARGET	FLUOROCHROME	CLONE	COMPANY	PRODUCT
Extracellular				
CD3	AF 700	UCHT1	BD Pharmingen	557943
CD56	BV 510	HCD56 NCAM	Biolegend	318340
CD16	APC/Cy7	3G8	Biolegend	302018
CD107a LAMP-1	APC	H4A3	Biolegend	328620
Isotype mouse IgG1 k	APC	MOPC-21	Biolegend	400122
Intracellular				
IFNγ	PE-Cy7	B27	BD Pharmingen	557643
Isotype mouse IgG1 k	PE-Cy7	MOPC-21	BD Pharmingen	557872
TNFα	FITC	MAb11	BD Pharmingen	552889
Isotype mouse IgG1 k	FITC	MOPC-21	BD Pharmingen	555748

Table S4. Conjugated monoclonal antibodies used in the flow cytometry NK functional assay panel.

UPN	Disease	Sample 1 Mo	Sample 3 Mo	AGE at HCT	SEX	CMV	DAY from HCT to CMV	aGVHD	DAY from HCT to aGVHD	cGVHD	DAY from HCT to cGVHD	Relapse	DAY from HCT to relapse
Haplo_1	NHL	Yes	No	64	F	Yes	31	Yes	31	No		No	
Haplo_2	NHL	Yes	Yes	68	M	Yes	41	No		Yes	202	Yes	614
Haplo_3	MDS	Yes	Yes	68	M	No		Yes	68	Yes	817	No	
Haplo_4	MDS	Yes	Yes	75	M	Yes	35	Yes	163	No		No	
Haplo_5	NHL	Yes	No	49	F	No		No		No		Yes	63
Haplo_6	NHL	Yes	Yes	52	M	Yes	41	Yes	41	No		No	
Haplo_7	NHL	No	Yes	53	M	Yes	41	No		Yes	246	No	
Haplo_8	NHL	No	Yes	56	M	No		Yes	40	No		No	
Haplo_9	CML	No	Yes	20	M	No		No		No		No	
Haplo_10	MDS	No	Yes	73	M	Yes	49	Yes	114	No		No	
MDs_1	HD	Yes	Yes	24	M	No		No		No		Yes	111
MDs_2	ALL	Yes	No	68	F	No		No		Yes	223	Yes	130
MDs_3	HD	Yes	Yes	45	M	Yes	237	Yes	211	No		No	
MDs_4	NHL	Yes	Yes	64	M	No		Yes	27	Yes	197	No	
MD_1	MDS	No	Yes	68	M	No		Yes	55	Yes	302	No	
MD_2	AML	No	Yes	64	M	No		No		Yes	165	No	
MD_3	AML	No	Yes	73	M	No		Yes	42	Yes	488	No	
MD_4	MDS	No	Yes	68	F	No		No		Yes	264	No	
MD_5	HD	No	Yes	19	F	No		No		No		No	
MD_6	PMF	No	Yes	65	M	No		No		Yes	202	No	

Table S5. Clinical characteristics of patients tested in CyTOF assay at 1 and 3 months after HCT. Samples were selected based on the availability of an adequate number of frozen cells. Haploidentical cell transplantation (HAPLO), matched donor cell transplantation with sirolimus/TAC/MTX GVHD prophylaxis (MDs) and matched donor cell transplantation with TAC/MTX GVHD prophylaxis (MD).

UPN	Disease	Age	Sex	CMV	DAY from HCT to CMV	aGVHD	cGVHD	DAY from HCT to cGVHD	Relapse	DAY from HCT to relapse
Haplo_2	NHL	68	M	Yes	41	No	Yes	202	Yes	614
Haplo_5	NHL	49	F	No		No	No		Yes	63
Haplo_11	AML	63	F	No		No	No		Yes	132
Haplo_12	HD	38	F	No		No	No		No	
Haplo_13	ALL	55	F	Yes	26	No	No		Yes	72
Haplo_14	NHL	39	F	Yes	40	No	No		Yes	980
Haplo_15	NHL	69	M	No		No	No		No	
Haplo_16	MDS	73	M	No		No	No		No	
Haplo_17	MDS	47	M	No		No	Yes	145	No	
MD_2	AML	64	M	No		No	Yes	165	No	
MD_4	MDS	68	F	No		No	Yes	264	No	
MD_5	HD	19	F	No		No	No		No	
MD_7	MDS	70	F	No		No	No		No	
MD_8	ALL	74	M	Yes	176	No	Yes	225	No	
MD_9	CMML	67	M	No		No	No		Yes	61
MD_10	AML	62	M	No		No	No		Yes	91
MD_11	AML	66	M	No		No	Yes	120	No	
MD_12	AML	61	F	Yes	51	No	No		Yes	58

Table S6. Clinical characteristics of patients tested in NK functional assays 2 months after HCT. Samples were selected based on the availability of an adequate number of cryopreserved cells.

		MD-HCT				Haplo-HCT				
	Months	**N**	**Med**	**Q1**	**Q3**	**N**	**Med**	**Q1**	**Q3**	**p-value**
ALC	1	21	693	495.9	1084.6	37	210	120	514.08	**0.0002**
	2	26	814.59	700.05	1397	51	699.3	375	1216.8	0.10
	3	20	893.4	645	1378.2	46	658.3	384.2	882	0.07
	6	14	993.3	522.0	1114.1	36	1071.1	531.6	1511.7	0.66
	9	14	1240.7	1039.5	1472.2	24	1360.7	625.4	2368.2	0.80
	12	12	1228.7	840.5	2368.4	25	1026.8	750.0	2494.4	0.66
	18	10	1296.2	851.4	1793.0	18	1674.2	821.3	2723.2	0.28
	24	7	956.8	863.3	1828.3	17	1679.9	871.7	2651.0	0.31
$CD3^+$	1	21	337.5	263.6	458.6	37	65.4	25.4	147.1	**<.0001**
	2	26	420.5	255.8	695.7	51	305.0	113.4	616.9	0.11
	3	20	443.2	257.4	803.8	46	176.4	113.8	404.5	**0.013**
	6	14	526.2	374.3	746.2	36	426.2	184.1	891.9	0.87
	9	14	667.5	501.2	934.9	24	600.7	260.6	1514.5	0.92
	12	12	624.2	465.1	1289.7	25	481.4	318.3	1514.1	0.62
	18	10	829.7	272.6	1228.2	18	881.9	504.7	1607.6	0.40
	24	7	408.3	163.4	1170.1	17	851.7	329.2	1895.5	0.20
$CD19^+$	1	21	11.3	5.4	18.3	37	1.8	0.6	4.0	**<.0001**
	2	26	35.3	17.6	97.3	51	10.4	4.2	47.6	**0.0065**
	3	20	73.0	21.1	120.2	46	35.0	6.5	158.9	0.28
	6	14	49.4	5.7	135.3	36	76.4	21.3	206.4	0.44
	9	14	181.3	69.6	243.0	24	118.4	76.1	211.6	0.58
	12	12	105.7	26.0	160.8	25	114.8	74.3	270.8	0.51
	18	10	123.8	40.4	362.4	18	267.6	167.0	408.0	0.22
	24	7	70.3	54.4	144.6	17	280.0	183.3	323.2	**0.049**
$CD56^+3^-$	1	21	115.3	77.1	221.8	37	52.7	23.9	130.1	**0.03**
	2	26	163.2	95.2	271.9	51	153.1	103.4	272.8	0.70
	3	20	178.8	122.6	226.7	46	156.6	72.9	248.2	0.66
	6	13	109.0	76.6	136.5	36	184.8	102.7	264.4	0.10

	9	14	126.9	80.7	237.0	24	119.9	71.5	416.0	0.75
	12	12	164.6	72.7	205.3	25	113.1	87.8	198.6	0.99
	18	10	172.9	95.3	220.8	18	176.9	110.7	330.9	0.55
	24	7	192.5	65.8	268.3	17	182.8	93.0	238.2	0.95
$CD56^{+}3^{+}$	1	20	15.9	8.7	42.9	36	1.1	0.4	3.1	**<.0001**
	2	26	22.8	11.4	61.7	52	3.7	1.6	8	**<.0001**
	3	20	33.4	11.2	90	49	4.3	1.5	9.7	**<.0001**
	6	13	16.1	8.4	71.3	41	8.2	2.7	14.9	**0.04**
	9	14	33.6	6.7	87.4	28	10.4	3.1	24.2	**0.0217**
	12	11	48.5	4.9	77.6	26	10.8	3.3	23.6	**0.0249**
	18	10	45.2	13.3	65.4	20	14.9	6	26.8	**0.0408**
	24	7	53	18.2	86.7	20	16.2	7	27.3	0.0563

Table S7. The median absolute values (cells/μL) along with corresponding inter-quartile values and p-values referring to Figures 1A and 1C.

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		MD-HCT			Haplo-HCT					
	Months	**N**	**Med**	**Q1**	**Q3**	**N**	**Med**	**Q1**	**Q3**	**p-value**
Treg ($CD4^+25^+127^-$)	1	21	11.6	4.9	14.8	37	2.6	1.2	7.7	**0.0002**
	2	26	12.3	6.9	18.3	51	7.7	4.0	15.4	0.09
	3	20	11.9	5.4	20.7	45	10.4	5.7	16.0	0.59
	6	14	13.5	5.6	30.6	36	14.1	8.1	19.1	0.91
	9	14	15.8	10.2	56.6	24	17.5	10.1	31.9	0.75
	12	12	17.9	10.3	39.9	25	15.8	8.6	23.5	0.47
	18	10	10.9	4.5	25.2	18	21.4	16.7	31.2	0.06
	24	7	8.8	3.5	27.6	17	21.1	16.1	36.5	0.31
Treg EM ($CD25^+127^-45RO^+62L^-$)	1	21	2.4	1.2	5.8	37	0.9	0.4	2.6	**0.01**
	2	26	3.2	2.3	4.8	51	3.2	1.5	7.6	0.68
	3	20	2.9	1.2	5.3	45	3.8	1.3	8.6	0.19
	6	14	3.7	0.5	9.2	36	6.3	3.1	9.7	0.16
	9	14	3.5	1.1	9.9	24	6.4	3.4	17.2	0.20
	12	12	4.6	2.1	9.0	25	4.2	1.4	9.7	0.64
	18	10	3.7	2.7	4.9	18	6.6	3.5	13.1	0.09
	24	7	2.5	1.5	7.1	17	8.6	6.1	10.8	0.09
Treg CM ($CD25^+127^-45RO^+62L^+$)	1	21	5.5	2.6	7.8	37	1.2	0.2	2.9	**0.0003**
	2	26	5.3	2.4	9.5	51	2.6	1.1	5.2	**0.023**
	3	20	8.1	1.5	10.9	45	4.0	1.6	7.3	0.19
	6	14	6.1	4.0	15.5	36	4.5	1.6	7.3	0.16
	9	14	7.5	2.6	22.1	24	6.2	2.8	8.7	0.30
	12	12	11.6	5.0	17.1	25	6.3	2.0	15.0	0.31
	18	10	4.7	1.6	13.3	18	7.6	3.9	16.0	0.46
	24	7	2.1	0.8	6.0	17	11.8	3.0	17.1	0.11
Treg TEMRA ($CD25^+127^-45RO^-62L^-$)	1	21	0.57	0.36	0.80	37	0.05	0.02	0.26	**<.0001**
	2	26	0.49	0.14	1.30	51	0.21	0.09	0.64	0.07
	3	20	0.37	0.06	0.94	45	0.31	0.12	0.72	0.94

	6	14	0.38	0.00	2.07	36	0.69	0.25	1.85	0.31
	9	14	1.77	0.38	3.09	24	0.82	0.22	2.55	0.47
	12	12	0.82	0.33	2.97	25	0.46	0.16	2.13	0.29
	18	10	0.87	0.14	2.59	18	1.55	0.30	3.06	0.72
	24	7	0.71	0.34	4.34	17	1.56	0.67	3.28	0.41
Treg naïve ($CD25^{+}127^{-}$ $45RO^{-}62L^{+}$)	1	21	0.74	0.28	1.39	37	0.03	0.00	0.22	**<.0001**
	2	26	0.60	0.14	2.40	51	0.10	0.02	0.23	**0.0002**
	3	20	0.69	0.18	2.59	45	0.26	0.08	0.56	**0.03**
	6	14	0.48	0.22	3.32	36	0.30	0.08	0.69	0.06
	9	14	1.80	0.49	6.11	24	0.34	0.09	0.57	**0.0027**
	12	12	1.20	0.70	2.79	25	0.45	0.11	1.34	**0.05**
	18	10	0.56	0.21	3.53	18	0.47	0.13	0.92	0.61
	24	7	0.33	0.15	5.75	17	0.80	0.10	1.57	1.00
Tcon	1	21	193.5	119.5	248.7	37	18.7	6.8	35.7	**<.0001**
	2	26	219.0	124.4	298.3	51	102.3	39.7	192.0	**0.0041**
	3	20	220.2	110.6	408.6	45	90.1	51.7	136.6	**0.0038**
	6	14	225.7	141.3	404.5	36	153.0	108.3	226.2	0.11
	9	14	300.4	161.2	348.6	24	208.3	129.1	304.5	0.20
	12	12	293.6	213.9	351.7	25	206.0	119.0	396.8	0.36
	18	10	292.0	133.5	613.9	18	341.1	236.6	448.4	0.79
	24	7	238.5	66.6	432.3	17	298.5	188.4	458.3	0.48
Tcon EM (Tcon $45RO^{+}62L^{-}$)	1	21	51.0	37.0	82.3	37	8.7	2.7	18.5	**<.0001**
	2	26	64.0	25.3	103.9	51	60.8	25.7	123.4	0.80
	3	20	62.1	37.2	87.9	45	57.5	29.8	91.3	0.76
	6	14	52.0	39.3	129.9	36	99.3	58.4	158.4	0.16
	9	14	70.5	33.9	105.1	24	139.6	86.1	196.0	**0.019**
	12	12	76.8	57.4	101.5	25	126.7	53.2	181.6	0.57
	18	10	102.2	61.5	269.3	18	143.0	76.0	264.2	0.38
	24	7	87.1	24.4	218.1	17	134.5	100.3	274.3	0.20
	1	21	60.6	28.4	82.9	37	7.1	0.9	15.6	**<.0001**

Tcon CM (Tcon 45RO$^+$62L$^+$)	2	26	44.7	9.9	125.9	51	9.6	4.4	33.8	**0.0032**
	3	20	62.7	15.8	115.9	45	14.9	7.9	35.8	0.01
	6	14	40.6	19.1	100.5	36	20.9	10.2	37.3	0.04
	9	14	49.9	15.7	80.9	24	25.9	12.3	46.2	0.30
	12	12	56.7	30.0	121.4	25	32.8	18.2	80.6	0.29
	18	10	38.8	4.7	162.7	18	59.6	18.6	122.8	0.58
	24	7	29.9	4.7	95.4	17	72.9	23.9	104.5	0.18
Tcon TEMRA (Tcon 45RO$^-$62L$^-$)	1	21	20.5	8.2	39.0	37	0.3	0.1	1.3	**<.0001**
	2	26	17.4	7.6	57.7	51	3.1	1.2	9.1	**0.0001**
	3	20	22.9	7.0	38.7	45	5.3	2.6	9.3	**0.0033**
	6	14	42.0	1.9	97.4	36	14.4	6.7	27.5	0.54
	9	14	52.2	11.2	79.8	24	15.8	7.7	32.2	0.06
	12	12	60.8	20.0	73.9	25	22.1	5.2	44.1	**0.05**
	18	10	56.5	34.4	127.8	18	27.8	13.8	60.8	0.14
	24	7	50.2	18.2	87.3	17	49.4	16.1	80.0	0.90
Tcon naïve (Tcon 45RO$^-$ 62L$^+$)	1	21	31.8	16.0	63.4	37	0.3	0.1	0.8	**<.0001**
	2	26	26.0	3.0	54.6	51	0.9	0.3	2.7	**<.0001**
	3	20	38.0	6.1	90.0	45	2.3	0.7	6.3	**<.0001**
	6	14	31.8	8.5	84.9	36	4.2	1.3	8.3	**0.0008**
	9	14	34.3	17.4	170.8	24	4.2	1.3	9.3	**<.0001**
	12	12	62.7	15.0	106.3	25	11.1	1.5	18.4	**0.0099**
	18	10	41.6	5.1	83.1	18	8.1	2.8	31.4	0.35
	24	7	18.6	4.8	41.5	17	17.0	5.1	34.9	0.75
CD8$^+$	1	21	88.7	45.3	163.9	37	30.2	8.6	78.5	**0.0066**
	2	26	128.7	59.1	242.8	51	80.3	24.3	334.9	0.26
	3	20	147.8	80.9	289.4	46	64.9	16.2	198.6	0.03
	6	14	202.0	116.7	266.7	36	212.6	69.8	600.5	0.69
	9	14	255.0	141.7	435.0	24	295.0	113.8	1098.4	0.58
	12	12	261.1	156.0	558.4	25	238.3	94.0	1043.3	0.86
	18	10	187.0	108.0	747.7	18	316.0	173.9	982.1	0.30

	24	7	130.7	75.8	631.9	17	321.6	111.3	867.6	0.25
CD8 EM ($CD8^+45RO^+62L^-$)	1	21	23.1	9.7	39.4	37	11.0	4.0	43.3	0.32
	2	26	31.3	14.0	52.4	51	26.7	6.5	151.7	0.90
	3	20	32.3	16.6	72.9	46	14.8	4.7	96.1	0.17
	6	14	42.1	24.3	76.3	36	63.0	30.5	266.8	0.23
	9	14	45.7	12.2	102.8	24	97.2	40.3	427.2	**0.04**
	12	12	55.4	14.2	89.8	25	73.2	32.0	285.4	0.21
	18	10	37.3	25.4	213.9	18	117.5	31.5	467.5	0.13
	24	7	23.0	15.2	410.7	17	104.1	57.2	265.7	0.10
CD8 CM ($CD8^+45RO^+62L^+$)	1	21	5.4	3.6	8.3	37	3.7	0.9	9.3	0.15
	2	26	8.7	2.9	35.2	51	8.6	0.7	17.8	0.26
	3	20	8.9	4.9	24.5	46	3.7	0.7	9.0	**0.0089**
	6	14	8.6	4.0	24.0	36	11.0	3.4	31.3	0.84
	9	14	8.6	4.1	12.7	24	15.7	7.2	28.4	0.06
	12	12	7.3	4.9	11.7	25	18.2	6.9	49.4	**0.03**
	18	10	8.7	3.0	23.8	18	19.4	14.4	51.3	0.12
	24	7	3.0	0.6	14.7	17	20.3	9.7	62.2	**0.02**
CD8 TEMRA ($CD8^+45RO^-62L^-$)	1	21	39.2	17.4	68.0	37	4.6	1.2	17.0	**<.0001**
	2	26	55.1	24.7	81.1	51	28.3	7.7	95.2	0.06
	3	20	51.4	19.9	151.6	46	23.8	7.9	58.8	**0.05**
	6	14	117.2	21.6	156.0	36	72.8	25.7	282.2	0.80
	9	14	119.7	85.6	256.6	24	120.9	31.3	439.2	0.99
	12	12	136.2	69.0	295.5	25	100.2	31.5	566.3	0.78
	18	10	103.2	64.9	403.7	18	146.6	37.1	418.5	0.90
	24	7	79.7	53.7	258.2	17	193.9	58.2	495.4	0.45
CD8 naïve ($CD8^+45RO^-62L^+$)	1	21	18.2	6.3	26.8	37	1.2	0.3	2.9	**<.0001**
	2	26	21.2	5.8	39.3	51	6.9	0.8	15.5	**0.0017**
	3	20	22.2	6.1	62.2	46	6.8	0.8	15.2	**0.0033**
	6	14	27.6	9.4	59.6	36	12.6	3.1	32.4	0.09
	9	14	41.6	15.7	55.6	24	15.3	6.1	52.7	0.09

	12	12	25.3	8.4	97.9	25	18.6	9.1	40.7	0.53
	18	10	24.8	14.7	75.3	18	25.5	6.8	105.2	0.90
	24	7	10.7	2.8	19.7	17	26.2	8.4	71.1	0.16
Treg:Tcon ratio	1	21	0.064	0.043	0.088	37	0.179	0.089	0.342	**0.0001**
	2	26	0.059	0.043	0.087	51	0.099	0.056	0.155	**0.03**
	3	20	0.058	0.052	0.091	45	0.12	0.083	0.178	**0.0005**
	6	14	0.081	0.035	0.125	36	0.093	0.067	0.135	0.42
	9	14	0.078	0.038	0.134	24	0.092	0.062	0.14	0.52
	12	12	0.068	0.035	0.134	25	0.076	0.055	0.096	0.60
	18	10	0.052	0.031	0.076	18	0.082	0.056	0.105	0.12
	24	7	0.07	0.022	0.1	17	0.064	0.05	0.098	0.66
CD4:CD8 ratio	1	21	2.579	1.687	2.863	37	0.671	0.362	1.549	**<.0001**
	2	26	1.741	0.6	2.335	51	1.148	0.351	2.901	0.51
	3	20	1.532	0.893	1.988	46	1.148	0.644	2.984	0.99
	6	14	1.3	0.845	1.66	36	0.837	0.36	2.027	0.16
	9	14	1.191	0.634	2.391	24	0.792	0.334	1.823	0.17
	12	12	1.094	0.635	2.338	25	0.806	0.392	1.806	0.25
	18	10	0.941	0.465	1.331	18	1.127	0.506	1.444	0.90
	24	7	0.931	0.698	2.95	17	0.92	0.57	1.485	0.37

Table S8. The median absolute values along with the corresponding inter-quartile values and p-values referring to Figures 2A, 2B, 2C.

		MD				Haplo				
	Months	**N**	**Median**	**Q1**	**Q3**	**N**	**Median**	**Q1**	**Q3**	**p-value**
NK CD56^{bright} CD16^{-}	1	20	9.6	4	21.8	36	7.7	2.4	41.3	0.91
	2	26	12.7	3.1	28.4	52	23.4	11.7	57.9	**0.0081**
	3	20	10.9	3.9	22.1	49	17.5	11	49.4	**0.0048**
	6	13	8.6	2.4	11.7	41	24.9	12.3	47.2	**0.0017**
	9	14	7.6	5	17.4	28	12.8	5.3	33.9	0.21
	12	11	6	3.6	13.4	26	13	5.6	21.9	0.25
	18	10	5.8	3	7.4	20	10.6	5.8	17.3	0.19
	24	7	3.7	2	9.1	20	9.6	5.8	13.1	0.06
NK CD56^{dim} CD16^{+}	1	20	92.1	53.2	159	36	16.3	4.7	49.2	**0.0006**
	2	26	132.7	76.6	185.1	52	75.7	34.8	134.6	**0.0091**
	3	20	163.3	69.6	217.1	49	81.6	47.3	118.8	**0.011**
	6	13	96.7	56.9	133.1	41	108.6	56.7	162	0.61
	9	14	100.6	66	190.2	28	122.9	48.4	154	0.88
	12	11	97.2	45.9	151.7	26	92.9	49.6	182.4	0.56
	18	10	117.9	47.4	158.7	20	127.1	82.9	258.9	0.55
	24	7	172.1	76.2	284.5	20	111.9	64.2	206.7	0.31
NK CD56^{dim}CD16^{-}	1	20	13.9	6.6	19.9	36	12.7	3.2	26.1	0.73
	2	26	13.3	4.6	23.4	52	15.4	9.3	25.8	0.39
	3	20	9.3	4.5	14.6	49	10.1	5.9	16	0.51
	6	13	6.4	3.9	14.9	41	11.5	7.1	21.5	**0.04**
	9	14	9.1	7	15.6	28	11.7	5.8	24.2	0.62
	12	11	7.2	6.6	14.4	26	10.7	7.5	19	0.50
	18	10	9.3	8.1	10.9	20	12.9	5.6	20.4	0.37
	24	7	6.5	5.7	17.7	20	8.5	4.6	20.2	0.80
NK CD56^{bright}CD16^{+}	1	20	5.6	1.3	10.6	36	3.4	0.8	12.9	0.30
	2	26	8.2	2.6	17.2	52	9.5	3.2	21.1	0.42
	3	20	7.3	2	14.9	49	13.3	7.9	27.8	**0.0035**
	6	13	6.6	3.9	8.6	41	12.5	7.1	16.6	**0.024**

	9	14	5.8	3	10.7	28	8.6	2.6	13.8	0.57
	12	11	4.1	3	9.2	26	5.1	2.8	12.7	0.50
	18	10	5	3.8	5.7	20	5.2	3	11.7	0.71
	24	7	4.6	2	5.7	20	5.7	3.2	9.3	0.16
NK $CD56^{bright}$ $CD16^{-}$: NK $CD56^{dim}$ $CD16^{+}$ ratio	1	21	0.151	0.085	0.228	36	0.443	0.136	1.961	**0.0014**
	2	27	0.092	0.031	0.181	52	0.323	0.101	0.918	**0.0003**
	3	21	0.061	0.026	0.208	49	0.246	0.129	0.661	**<.0001**
	6	13	0.096	0.033	0.152	41	0.263	0.106	0.552	**0.013**
	9	14	0.067	0.037	0.147	28	0.171	0.033	0.36	0.24
	12	12	0.125	0.043	0.218	26	0.147	0.04	0.29	0.66
	18	11	0.098	0.02	0.161	20	0.069	0.024	0.194	0.69
	24	8	0.036	0.016	0.055	20	0.074	0.043	0.202	0.06

Table S9. The median absolute values along with the corresponding inter-quartile values and p-values referring to Figures 3C and 3D.

		No CMV				CMV				
	Months	**N**	**Median**	**Q1**	**Q3**	**N**	**Median**	**Q1**	**Q3**	**p-value**
NK CD16$^-$: CD16$^+$ ratio	1	26	0.83	0.47	1.79	10	2.49	1.23	12.68	**0.0085**
	2	35	0.4	0.22	0.86	17	0.93	0.45	1.73	**0.019**
	3	35	0.35	0.24	0.66	14	0.42	0.27	0.66	0.52
	6	27	0.31	0.13	0.53	14	0.28	0.2	1.03	0.48
	9	17	0.24	0.07	0.32	11	0.28	0.16	1.57	0.3
	12	15	0.28	0.14	0.36	11	0.21	0.08	0.71	0.76

Table S10. The median absolute values along with the corresponding inter-quartile values and p-values referring to Figure 6B.

		No cGVHD				cGVHD				
	Months	**N**	**Median**	**Q1**	**Q3**	**N**	**Median**	**Q1**	**Q3**	**p-value**
NK CD16$^-$: CD16$^+$ ratio	1	28	0.89	0.513	1.882	8	2.959	1.046	12.34	**0.04**
	2	42	0.421	0.241	0.855	10	1.529	1.198	2.728	**0.001**
	3	40	0.34	0.232	0.645	9	0.571	0.364	0.912	0.16
	6	31	0.287	0.148	0.523	10	0.828	0.218	8.355	**0.04**
	9	20	0.207	0.133	0.29	8	0.78	0.361	1.258	0.1
	12	18	0.203	0.093	0.355	8	0.462	0.282	1.53	0.05

Table S11. The median absolute values along with the corresponding inter-quartile values and p-values referring to Figure 6D.

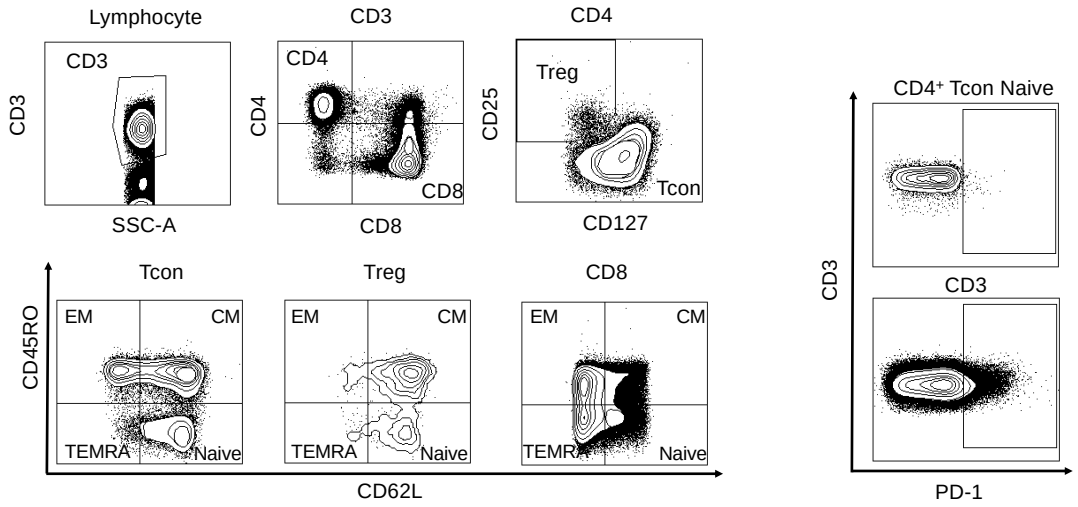

Figure S1. **Gating strategy for T cell subset analysis**. Three major T cell populations, CD4Treg, CD4Tcon, and CD8 T cells, were defined as $CD3^+CD4^+CD8^-CD25^+CD127^-$, $CD3^+CD4^+CD8^-$ non-Treg cells and $CD3^+CD4^-CD8^+$, respectively. Within each T cell population, subsets were defined as follows: naïve ($CD45RO^-CD62L^+$), central memory (CM, $CD45RO^+CD62L^+$), effector memory (EM, $CD45RO^+CD62L^-$), and terminally differentiated effector memory (TEMRA, $CD45RO^-CD62L^-$) T cells. PD-1 expression was monitored on each T cell subset.[6,7]

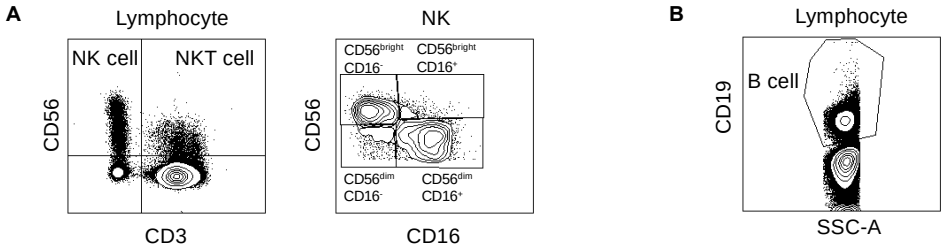

Figure S2. Gating strategy for analyzing NK and B cells. A) NK cells were defined as $CD45^{+}CD3^{-}CD56^{+}$ lymphocytes and divided in 4 subsets based on the expression of CD56 and CD16: $CD56^{bright}CD16^{-}$, $CD56^{dim}CD16^{+}$, $CD56^{dim}CD16^{-}$ and $CD56^{bright}CD16^{+}$ NK cells. NKT cells were defined as $CD45^{+}CD3^{+}CD56^{+}$ lymphocytes. B) B cells were defined as $CD45^{+}CD19^{+}$ lymphocytes.

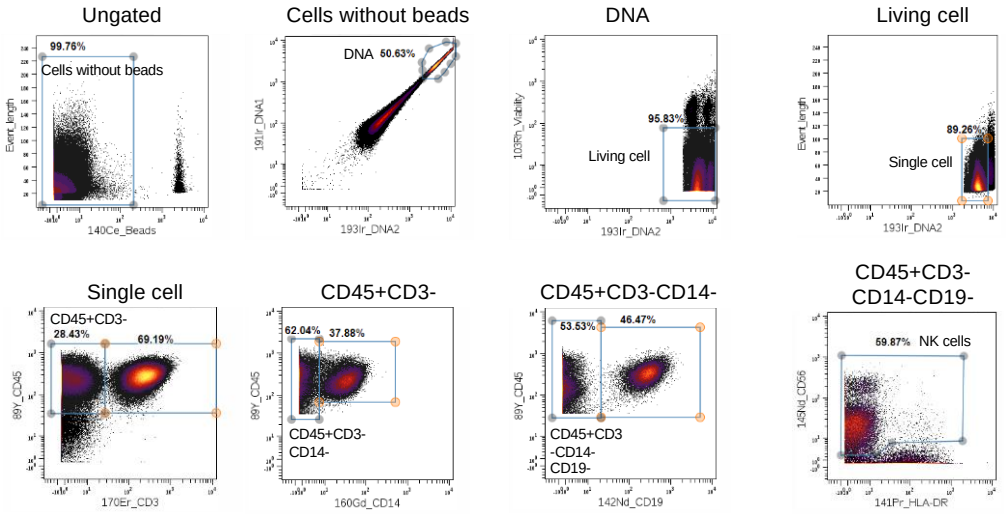

Figure S3. Mass cytometry NK cell gating strategy.

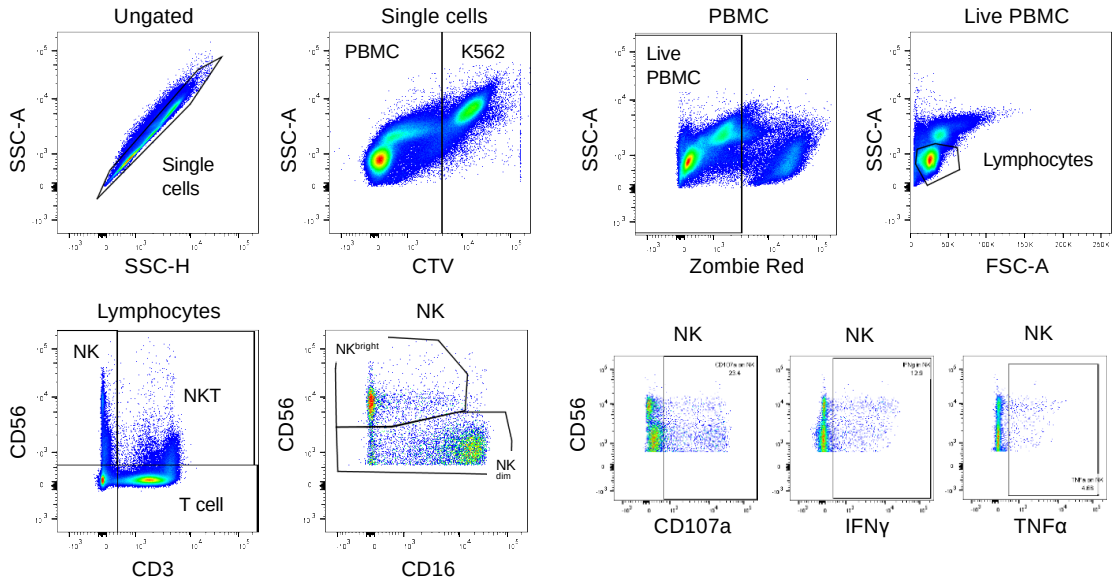

Figure S4. Flow cytometry NK cell gating strategy for functional assays.

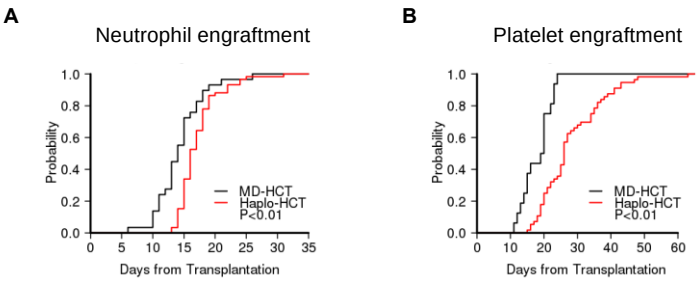

Figure S5. Engraftment after HCT. **A)** Probability of neutrophil engraftment. **B)** Probability platelet engraftment. Values are plotted for haploidentical cohort (haplo-HCT n=60, red line) and matched donor cohort (MD-HCT n=35, black line).

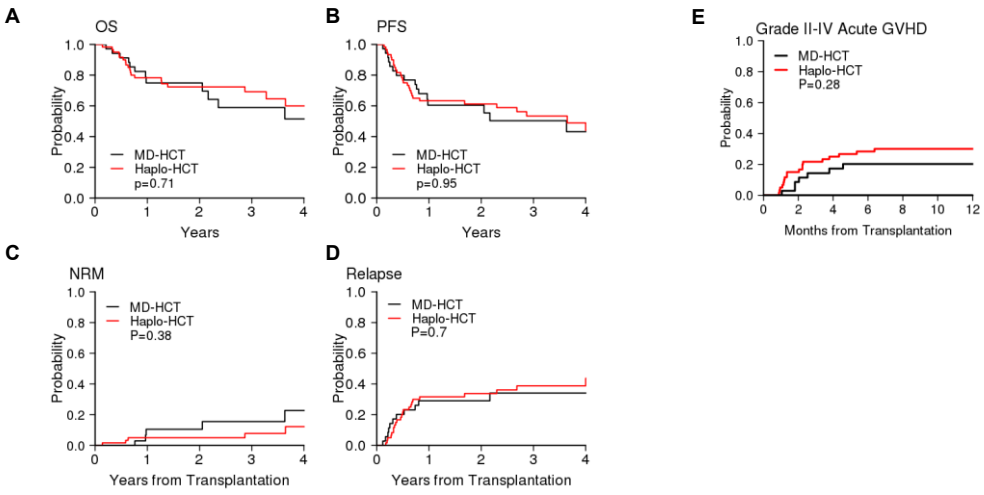

Figure S6. **Clinical outcomes in haplo-HCT and MD-HCT cohorts**. **A)** Probability of overall survival (OS). **B)** Probability of progression free survival (PFS). **C)** Probability of non-relapse mortality (NRM). **D)** Probability of relapse. **E)** Probability of acute graft versus host disease (aGVHD). Values are plotted for haploidentical cohort (haplo-HCT n=60, red line) and matched donor cohort (MD-HCT n=35, black line).

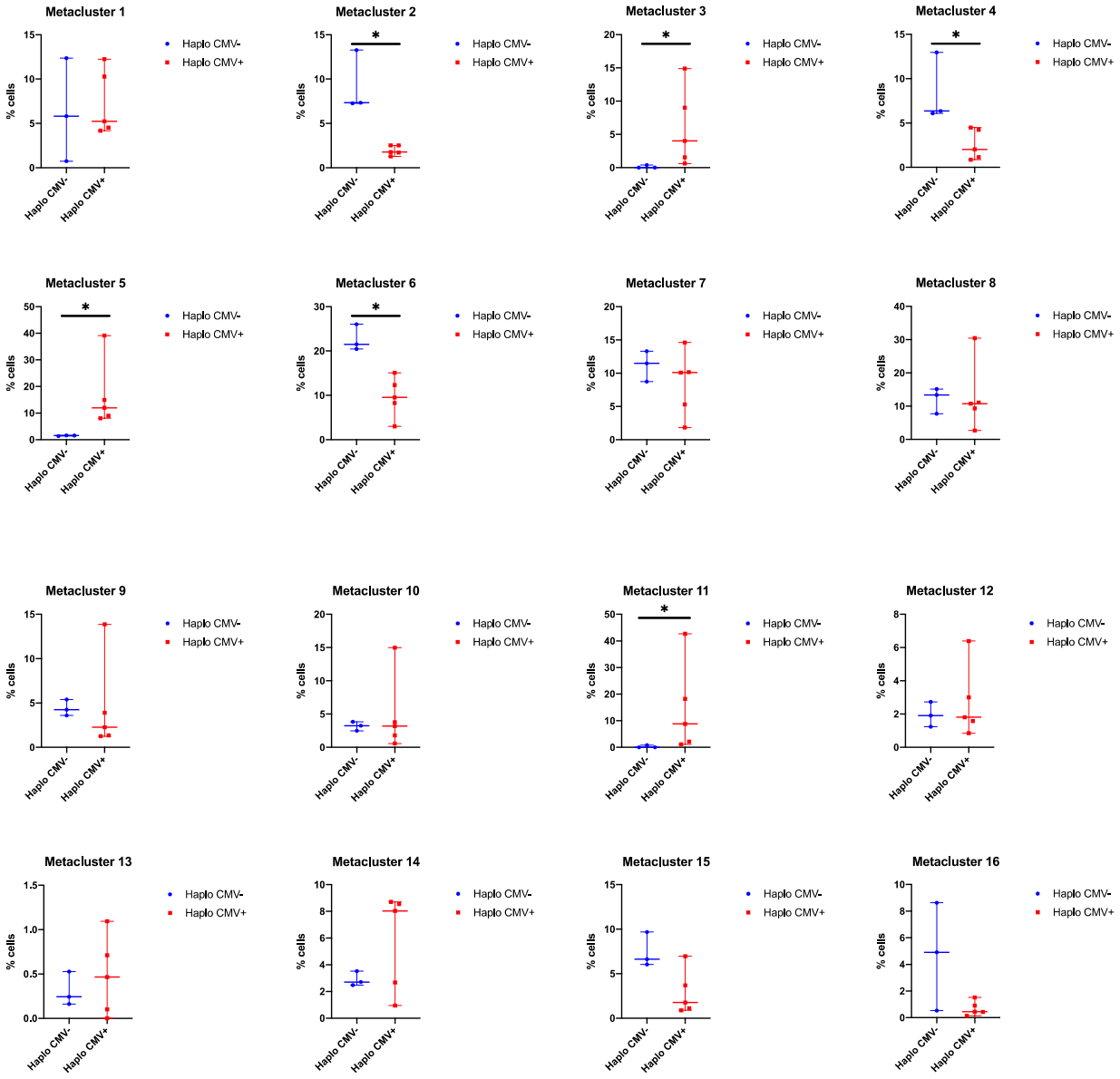

Figure S7. NK cell meta-cluster distribution 3 months after haplo-HCT in patients with and without CMV reactivation. A) Representation of FlowSOM meta-clusters in 8 haplo-HCT samples. **B**) viSNE density map of haplo-HCT patients with CMV reactivation (n=5) and without (n=3). **C**) Percentage distribution of FlowSOM NK cell meta-clusters in patients with CMV reactivation (n=5) compared to patients without (n=3). Data are expressed as median and range and compared using Wilcoxon signed-rank test for comparison, * P< 0.05.

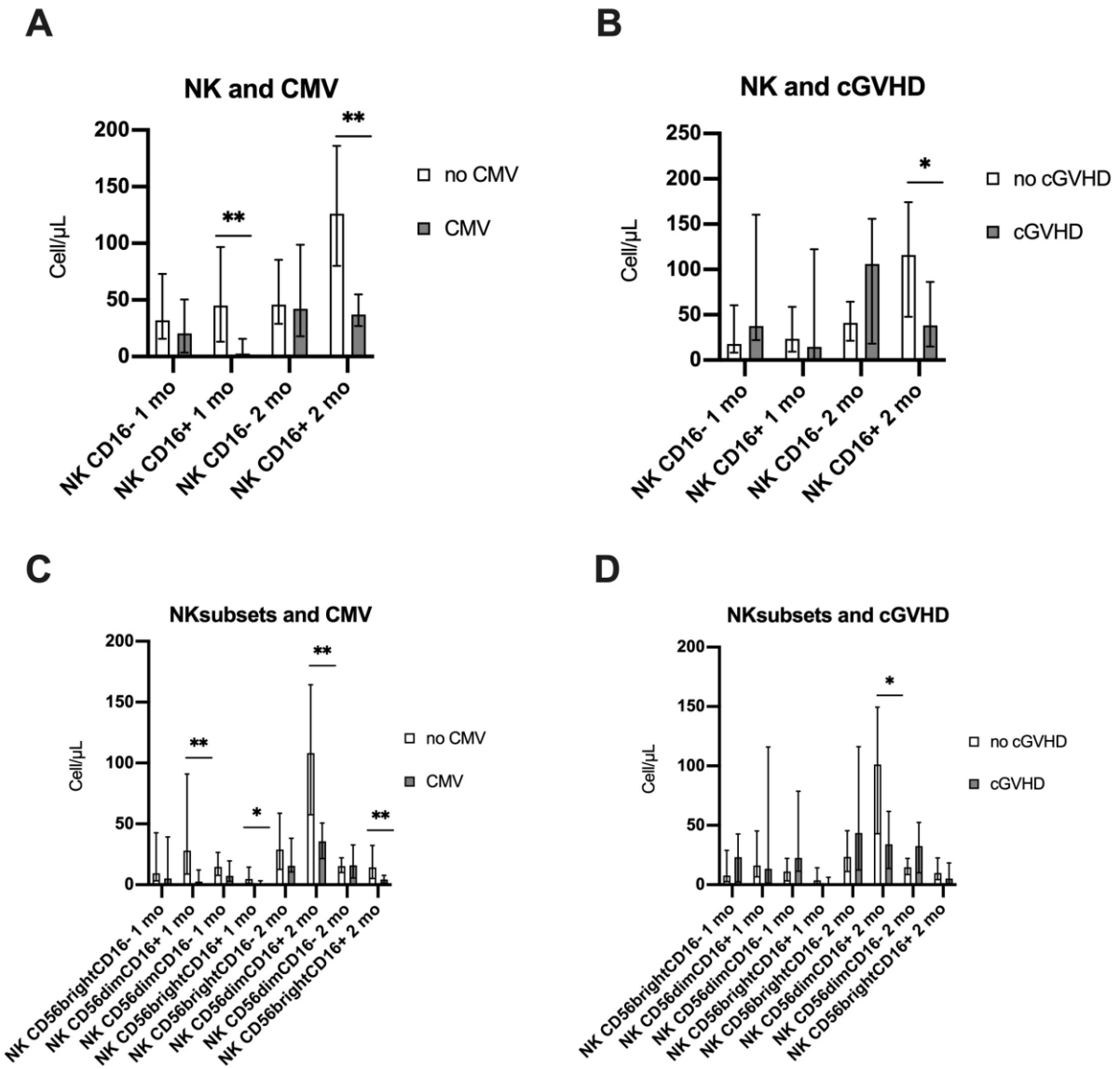

Figure S8. **Absolute numbers of peripheral blood CD16^{+} and CD16^{-} NK cells and NK cell subsets (CD56^{bright}CD16^{-}, CD56^{dim}CD16^{+}, CD56^{bright}CD16^{+} and CD56^{dim}CD16^{-}) 1 and 2 months after haplo-HCT.** **A and C**: Comparison of patients with and without CMV reactivation. **B** and **D**: Comparison of patients who later developed or did not develop cGVHD. Box and whisker plots show medians, along with minimum and maximum values. Groups were compared using Wilcoxon signed-rank test for paired comparison, * P< 0.05 and ** P< 0.01.