

Early expression of CD94 and loss of CD96 on CD8+ T cells after allogeneic stem cell transplantation is predictive of subsequent relapse and survival

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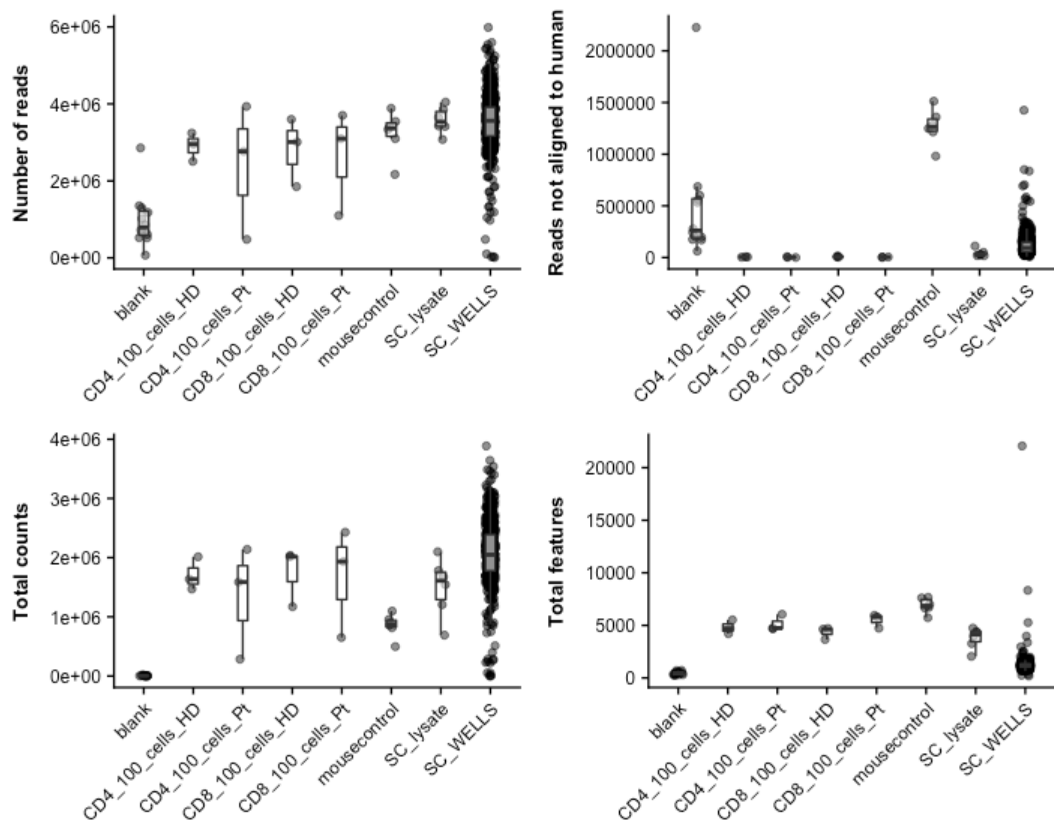
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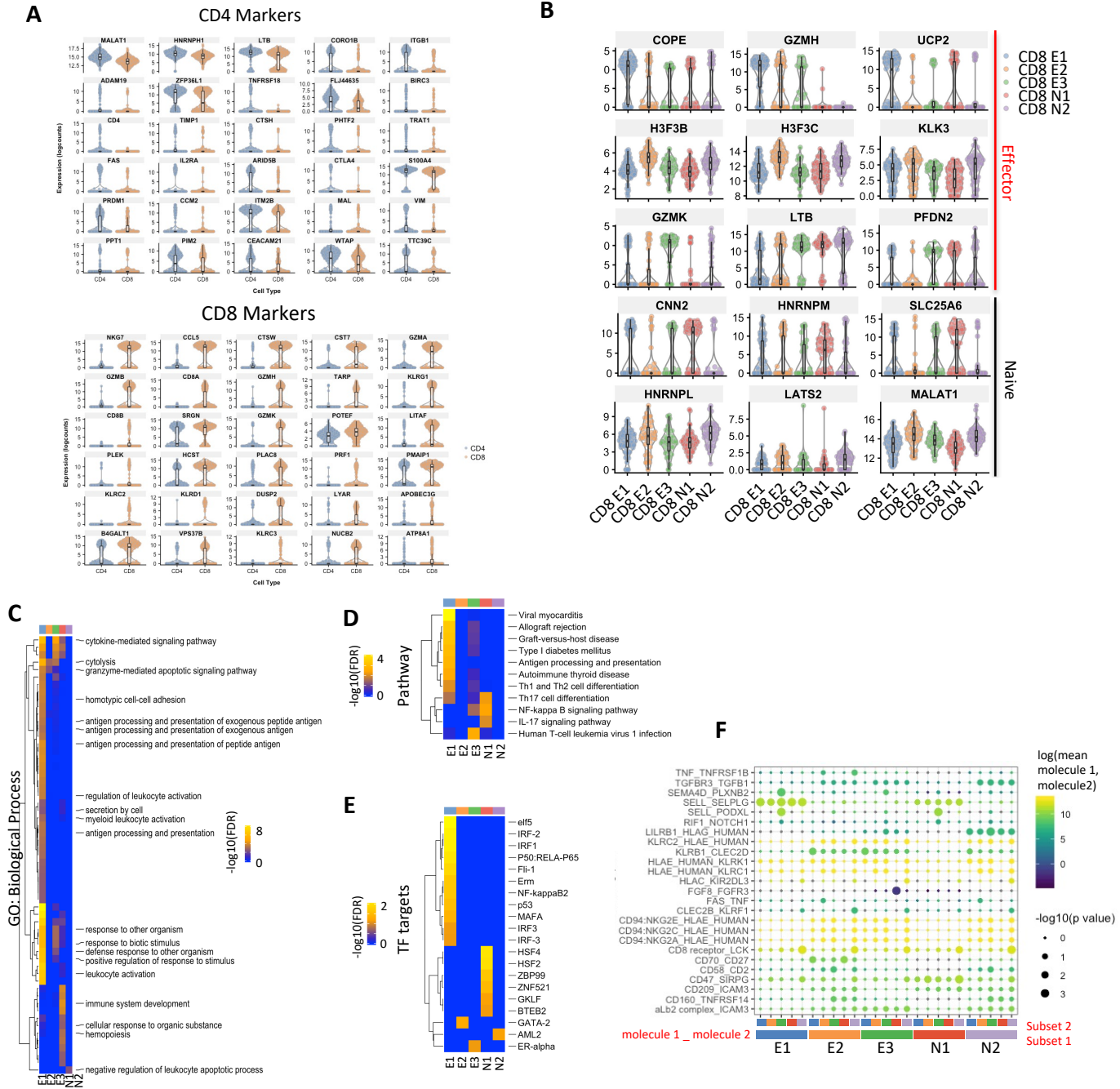
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Supplemental Data

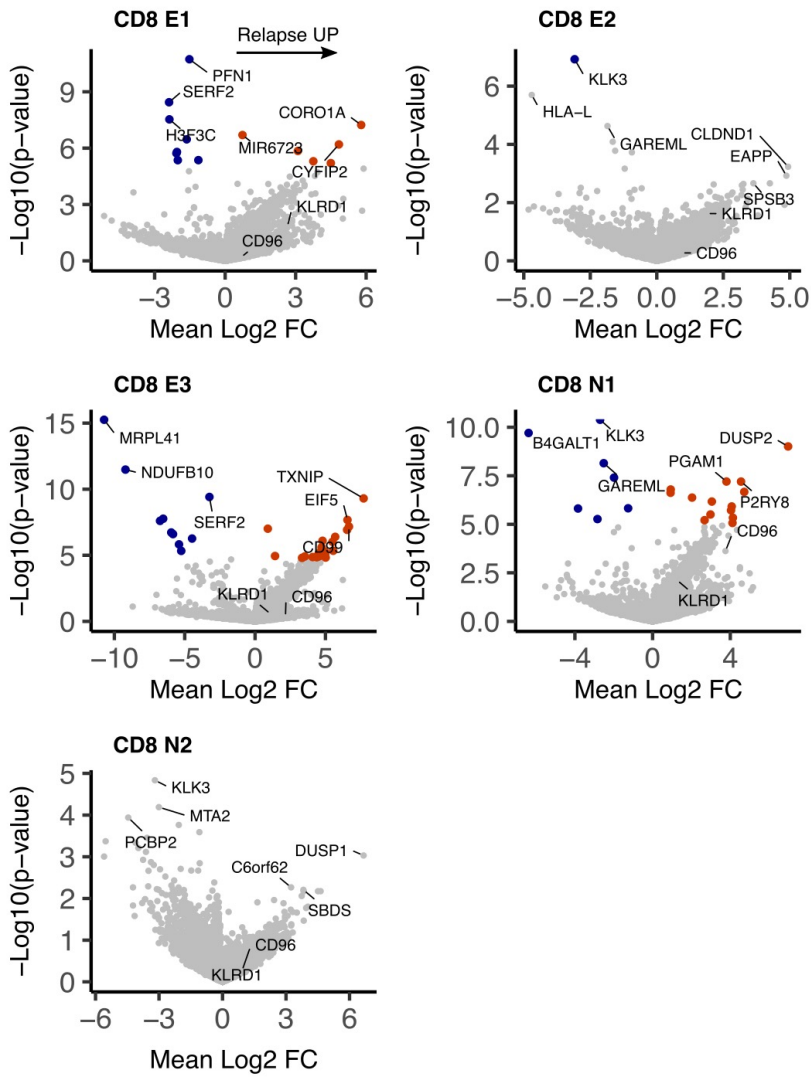
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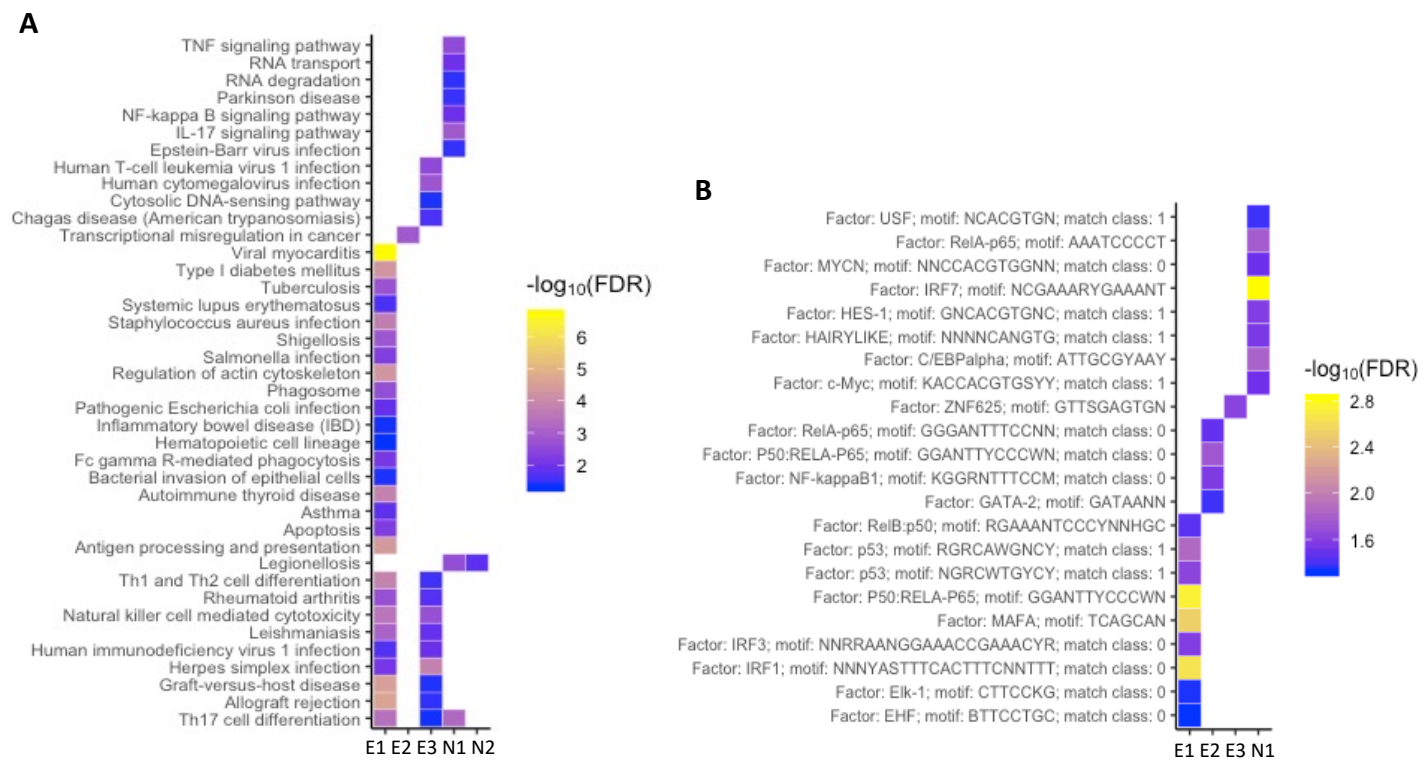
Supplementary Figure S1. Read and Feature count overview from scRNAseq. Quality control metrics from the smart-seq2 sequencing run. Plots show the number of reads, reads not aligning to the human genome, total counts and total features for defined wells from the sequencing experiment. Blank = wells not containing a cell; CD4_100_cells_Pt/HD = CD4 T cells from patient/HD samples (100 cells randomly selected); CD8_100_cells_Pt/HD = CD8 T cells from patient/HD samples (100 cells randomly selected); mousecontrol = control wells containing mouse cells; SC_lystate = single cell lysate from non-control wells; SC_WELLS = all non-control wells.



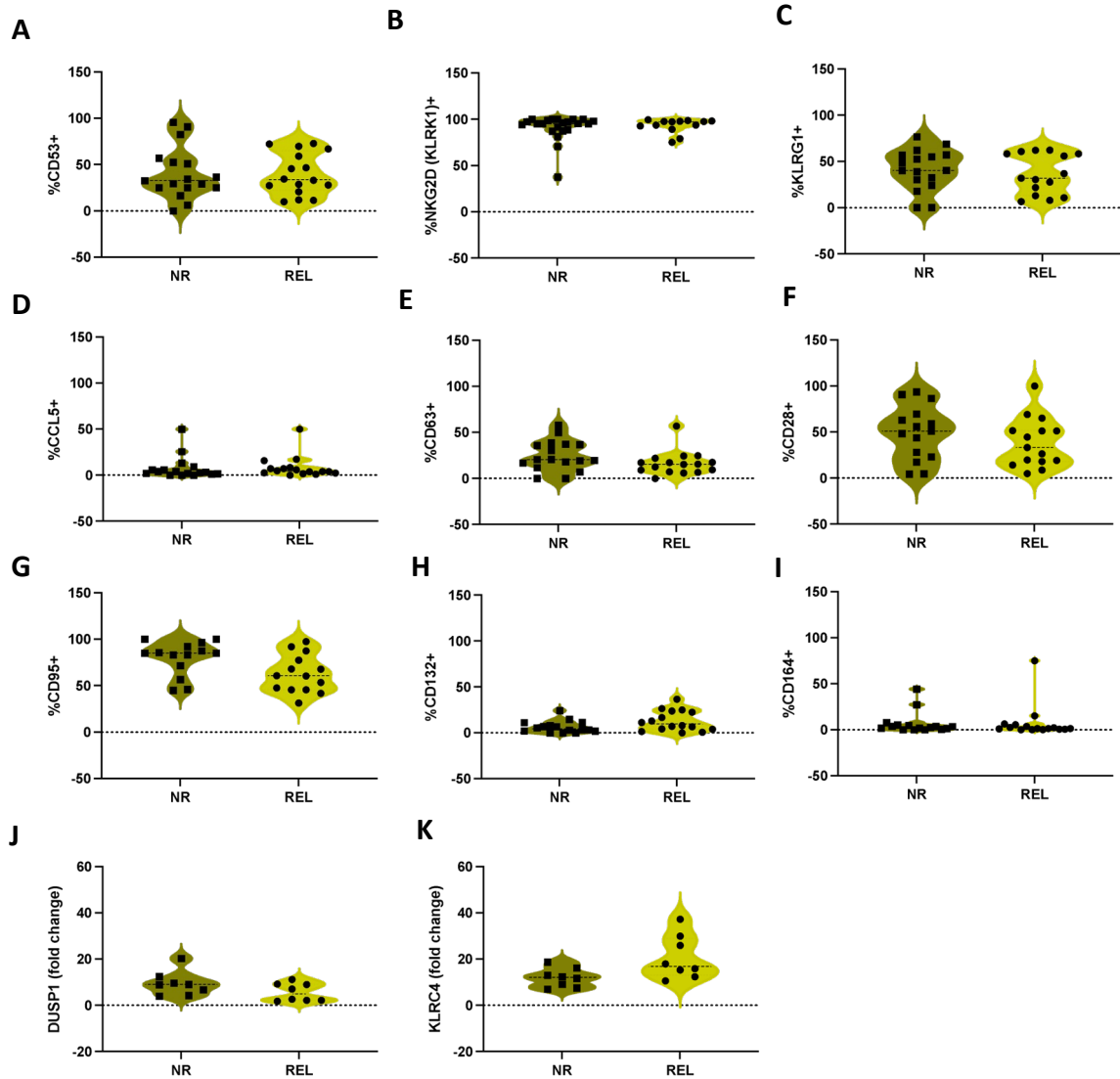
Supplementary Figure S2. Functional enrichments and interactions in T cells at WK2 post transplant. (A) Top 30 significant CD4 Marker genes and CD8 marker genes (FDR<0.05; logFC>1); (B) Selected cluster identifying genes within CD8+ T cells (C) GO term: Biological Process (D) KEGG Pathway; and (E), Transcription factor (TF) targets) within marker genes overexpressed in WK2 CD8+ T cell Effector (E) and Naïve (N) cell populations. (F) Significant estimated interactions between T cell subsets at WK2. Colour scale indicates the means of the average expression of interacting molecule 1 in subset 1 and interacting molecule 2 in subset 2.



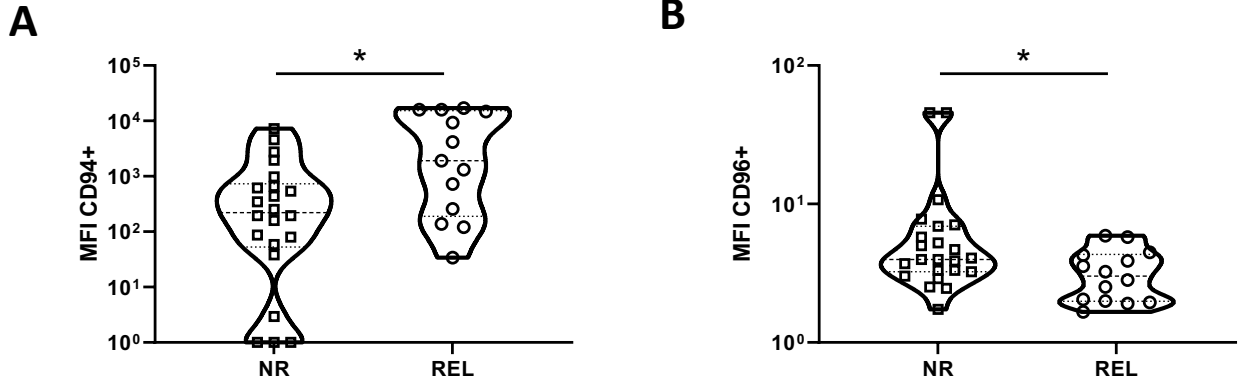
Supplementary Figure S3. CD8+ T cell within-cluster Relapse vs No Relapse differential expression. Within-cluster Relapse vs No Relapse differentially expressed genes (Coloured points, $\text{FDR} < 0.01$ & absolute $\text{logFC} > 0.5$).



Supplementary Figure S4. Functional enrichments within Relapse-expressed genes in CD8+ T cells at 2 weeks post transplant. Selected enrichments of KEGG Pathway (A) and Transcription factor targets (B) within Relapse-overexpressed genes identified from within-cluster differential expression analysis.



Supplementary Figure S6. Validation of selected genes by flow cytometry or qPCR in patients with disease Relapse (REL) or No relapse (NR) post allogeneic SCT. (A-K) Percentage of CD53+, NKG2D+, KLRG1+, CCL5+, CD63+, CD28+, CD95+, CD132+, CD164+ cells within the CD8+ T cell subset is depicted in REL patients (n=16) compared to NR patients (n=18), using flow cytometry. qPCR was used to determine relative expression of the phosphatase DUSP1 as well as KLRK4 which encodes the intracellular NKG2F. Mann Whitney-U test was performed for statistical analysis, * depicts p value <0.05; ** depicts p value <0.001.**



Supplementary Figure S7. Violin plot depicting Mean Fluorescence Intensity (MFI) values for (A) CD94 and (B) CD96+ cells within the CD8+ T cell population in the Relapse (REL) and Non-relapse (NR) group of patients in the validation cohort. * depicts statistical significance of $p < 0.05$.

Relapse

Term	HR	CI	P.val	Adj.HR	Adj.CI	Adj.P.val
Age at transplant	1.01	(0.97 – 1.04)	0.75410	0.95	(0.9 – 1.01)	0.08044
CD94 CD94+ > 15%	4.71	(1.5 – 14.75)	0.00786	10.64	(1.88 – 60.28)	0.00754
CD96 CD96+ > 95%	0.27	(0.08 – 0.85)	0.02602	0.29	(0.07 – 1.26)	0.09886
CMV status Pos	1.58	(0.58 – 4.3)	0.37346	2.25	(0.58 – 8.71)	0.23982
Disease Risk High	0.48	(0.18 – 1.3)	0.14737	1.06	(0.25 – 4.5)	0.93617
Disease subgroup CR1_AML_or_MDS	1.57	(0.56 – 4.37)	0.38864	1.73	(0.26 – 11.65)	0.57135
MRD Pos	0.82	(0.24 – 2.86)	0.76154	0.61	(0.11 – 3.31)	0.56713
MRD Unknown	1.33	(0.4 – 4.4)	0.63960	1.34	(0.18 – 10.1)	0.77748
PSex M	2.64	(0.93 – 7.48)	0.06711	0.95	(0.13 – 6.98)	0.95705
Sex match Mismatch	0.34	(0.11 – 1.07)	0.06615	0.2	(0.02 – 1.56)	0.12447

Overall Death

Term	HR	CI	P.val	Adj.HR	Adj.CI	Adj.P.val
Age at transplant	1.02	(0.98 – 1.06)	0.3079	0.98	(0.93 – 1.04)	0.57432
CD94 CD94+ > 15%	5.3	(1.46 – 19.2)	0.0111	16.52	(2.06 – 132.65)	0.00834
CD96 CD96+ > 95%	0.23	(0.06 – 0.84)	0.0261	0.19	(0.03 – 1.12)	0.0668
CMV status Pos	2.9	(0.94 – 8.92)	0.0628	4.27	(0.91 – 19.98)	0.06518
Disease Risk High	0.48	(0.16 – 1.39)	0.1750	1.26	(0.21 – 7.36)	0.80031
Disease subgroup CR1_AML_or_MDS	1.4	(0.47 – 4.21)	0.5483	0.8	(0.13 – 4.89)	0.8075
MRD Pos	0.83	(0.21 – 3.32)	0.7875	0.26	(0.03 – 2.09)	0.20325
MRD Unknown	1.75	(0.49 – 6.23)	0.3848	1.06	(0.13 – 8.45)	0.95476
PSex M	2.49	(0.82 – 7.55)	0.1057	0.62	(0.06 – 6.99)	0.69929
Sex match Mismatch	0.46	(0.14 – 1.47)	0.1906	0.32	(0.03 – 3.12)	0.32547

Supplementary Figure S8. Relapse and Overall Death covariate associations. Univariate p values and hazard ratios (P.val and HR) and multivariate p values and hazard ratios (Adj.P.val and Adj.HR) based on CoxPH model of Relapse and Overall Death with a 95% CI and adjusted regression coefficients.

Supplementary Table 1. Patient characteristics and percentage CD94 high/low and CD96 high/low expression on T cells in each group of patients.

		ScRNA-seq Validation			<15% CD94+		>15% CD94+		<95% CD96+		>95% CD96+	
		n (N=3)	n (N=34)	%	n (N=18)	%	n (N=16)	%	n (N=17)	%	n (N=17)	%
Gender	Male	1	19	55,9	10	55,6	9	56,3	9	52,9	10	58,8
	Female	2	15	44,1	8	44,4	7	43,8	8	47,1	7	41,2
Disease	AML	3	15	44,1	8	44,4	7	43,8	7	41,2	8	47,1
	CML	0	4	11,8	2	11,1	2	12,5	3	17,6	1	5,9
	MDS	0	5	14,7	2	11,1	3	18,8	2	11,8	3	17,6
	ALL	0	5	14,7	2	11,1	3	18,8	3	17,6	2	11,8
	CLL	0	1	2,9	1	5,6	0	0,0	0	0,0	1	5,9
	HL	0	2	5,9	1	5,6	1	6,3	2	11,8	0	0,0
	NHL	0	1	2,9	1	5,6	0	0,0	0	0,0	1	5,9
	Myelofibrosis	0	1	2,9	1	5,6	0	0,0	0	0,0	1	5,9
Age		49 (22-73)			49(24-64)		50(22-73)		48(24-67)		50 (22-73)	
Source of graft	PBSC	3	32	94,1	18	100,0	14	87,5	15	88,2	17	100,0
	Cord	0	1	2,9	0	0,0	1	6,3	1	5,9	0	0,0
	BM	0	1	2,9	0	0,0	1	6,3	1	5,9	0	0,0
Type	MUD	3	26	76,5	13	72,2	13	81,3	13	76,5	13	76,5
	MRD	0	7	20,6	5	27,8	2	12,5	3	17,6	4	23,5
	Cord	0	1	2,9	0	0,0	1	6,3	1	5,9	0	0,0
Conditioning	FLU/BU	3	9	26,5	6	33,3	3	18,8	6	35,3	3	17,6
	FLAMSA/BU	0	5	14,7	2	11,1	3	18,8	2	11,8	3	17,6
	CYCLO/TBI	0	3	8,8	1	5,6	2	12,5	0	0,0	3	17,6
	CYCLO/FLU	0	2	5,9	1	5,6	1	6,3	1	5,9	1	5,9
	FLU/MELPH	0	12	35,3	6	33,3	6	37,5	6	35,3	6	35,3
	BEAM	0	3	8,8	2	11,1	1	6,3	2	11,8	1	5,9
										0,0		0,0
		ATG	3	14	41,2	7	38,9	7	43,8	7	41,2	7
	CAMPATH	0	17	50,0	9	50,0	8	50,0	9	52,9	8	47,1
CMV	R+/D+	0	11	32,4	5	27,8	6	37,5	6	35,3	5	29,4
	R+/D-	1	6	17,6	5	27,8	1	6,3	3	17,6	3	17,6
	R-/D+	0	1	2,9	1	5,6	0	0,0	0	0,0	1	5,9
	R-/D-	2	16	47,1	7	38,9	9	56,3	8	47,1	8	47,1
Viral reactivation	CMV	2	3	8,8	2	11,1	1	6,3	3	17,6	0	0,0
	EBV	0	1	2,9	0	0,0	1	6,3	1	5,9	0	0,0
Relapse		2	16	47,1	3	16,7	13	81,3	11	64,7	5	29,4
Median days to relapse		47	250		211		242					
GvHD	acute	0	12	35,3	6	33,3	6	37,5	4	23,5	8	47,1
	chronic	0	3	8,8	1	5,6	2	12,5	0	0,0	3	17,6
Survival		1	20	58,8	15	83,3	5	31,3	7	41,2	13	76,5
Median days to death		79	377		449		361					

Supplementary Table 2 Univariate CoxPH results summarising effect of covariates on Overall Death and Relapse.

Overall Death outcome covariates	coef	exp(coef)	se(coef)	z	Pr(> z)	lower	upper	cox.zph.p
CD94 CD94+ > 15%	1.67	5.30	0.66	2.54	0.01	1.46	19.20	0.92
CD96 CD96+ > 95%	-1.48	0.23	0.66	-2.23	0.03	0.06	0.84	0.56
Psex M	0.91	2.49	0.57	1.62	0.11	0.82	7.55	0.45
Age at transplant	0.02	1.02	0.02	1.02	0.31	0.98	1.06	0.87
Dsex M	-0.56	0.57	0.65	-0.86	0.39	0.16	2.05	0.16
Disease risk High	-0.74	0.48	0.54	-1.36	0.18	0.16	1.39	0.78
Disease Subgroup CR1_AML_or_MDS	0.34	1.40	0.56	0.60	0.55	0.47	4.21	0.01
HCT-CI 1	-0.31	0.73	1.07	-0.29	0.77	0.09	5.94	0.53
HCT-CI 2	-0.59	0.55	0.79	-0.75	0.45	0.12	2.61	0.53
HCT-CI 3+	0.42	1.52	0.69	0.60	0.55	0.39	5.91	0.53
MRD UNKNOWN	0.56	1.75	0.65	0.87	0.38	0.49	6.23	0.86
MRD POS	-0.19	0.83	0.71	-0.27	0.79	0.21	3.32	0.86
CR CR1	0.22	1.24	0.62	0.35	0.72	0.37	4.15	0.49
CR CR2	-0.47	0.63	0.87	-0.54	0.59	0.11	3.44	0.49
SexMatch MISMATCH	-0.78	0.46	0.60	-1.31	0.19	0.14	1.47	0.81
TBI YES	0.24	1.27	0.76	0.31	0.76	0.28	5.67	0.12
CMVStatus POS	1.07	2.90	0.57	1.86	0.06	0.94	8.92	0.68
Campath NO	-0.61	0.54	1.08	-0.56	0.57	0.07	4.52	0.04
Campath YES	-0.23	0.80	0.56	-0.41	0.68	0.27	2.38	0.04
GvHDProphylaxis COMBINATION	0.40	1.49	0.56	0.71	0.48	0.49	4.46	0.74
Relapse outcome covariates	coef	exp(coef)	se(coef)	z	Pr(> z)	lower	upper	cox.zph.p
CD94 CD94+ > 15%	1.55	4.71	0.58	2.66	0.01	1.50	14.75	0.99
CD96 CD96+ > 95%	-1.32	0.27	0.59	-2.23	0.03	0.08	0.85	0.39
Psex M	0.97	2.64	0.53	1.83	0.07	0.93	7.48	0.11
Age at transplant	0.01	1.01	0.02	0.31	0.75	0.97	1.04	0.57
Dsex M	-0.71	0.49	0.58	-1.22	0.22	0.16	1.54	0.74
Disease risk High	-0.74	0.48	0.51	-1.45	0.15	0.18	1.30	0.43
Disease Subgroup CR1_AML_or_MDS	0.45	1.57	0.52	0.86	0.39	0.56	4.37	0.13
HCT-CI 1	0.68	1.97	0.80	0.85	0.40	0.41	9.49	0.26
HCT-CI 2	-0.67	0.51	0.79	-0.85	0.40	0.11	2.43	0.26
HCT-CI 3+	0.69	2.00	0.64	1.08	0.28	0.57	6.98	0.26
MRD UNKNOWN	0.29	1.33	0.61	0.47	0.64	0.40	4.40	0.52
MRD POS	-0.19	0.82	0.64	-0.30	0.76	0.24	2.86	0.52
CR CR1	0.29	1.33	0.60	0.48	0.63	0.41	4.35	0.58
CR CR2	-0.11	0.89	0.77	-0.15	0.88	0.20	4.01	0.58
SexMatch MISMATCH	-1.08	0.34	0.59	-1.84	0.07	0.11	1.07	0.16
TBI YES	0.10	1.11	0.76	0.13	0.89	0.25	4.89	0.09
CMV Status POS	0.46	1.58	0.51	0.89	0.37	0.58	4.30	0.89
Campath NO	-1.24	0.29	1.07	-1.15	0.25	0.04	2.37	0.06
Campath YES	-0.99	0.37	0.55	-1.80	0.07	0.13	1.09	0.06
GvHD Prophylaxis COMBINATION	0.91	2.47	0.51	1.78	0.07	0.91	6.69	0.24