

Deciphering engraftment of bone marrow human single cells after allogeneic stem cell transplantation

Supplementary Figures & Tables

As appear in the main text

Table S1: Patient-, disease- and transplant-characteristics; Overall cohort

Parameter	N = 42
Age (years; range)	52 (24-69)
Gender (Male/Female; N (%))	26 (62%) /16 (38%)
Conditioning regimen (RIC/MAC; N (%))	30 (71%) /12 (29%)
Control type	
HLA-identical sibling (N (%))	15(36%)
Haploidentical (N (%))	12 (28%)
Matched Unrelated (N (%))	15 (36%)
Diagnosis (N (%))	
Myelodysplastic syndrome	18 (43%)
Acute myelogenous leukemia	15 (36%)
Acute bi phenotypic leukemia	5 (12%)
Other	4 (9%)

RIC: reduced intensity conditioning; MAC: myeloablative conditioning

Table S2: Population of patients with bone marrow analyses; Patient-, disease-, and transplant-characteristics.

	Global (N=26)	Recipient cluster 1 (N=14)	Recipient cluster 2 (N=12)
Gender			
Age (years; range)	51 (29-68)	50 (29-65)	51 (27-68)
Diagnosis			
Acute myeloid leukemia (N, %)	11/26 (42%)	6/14 (42.8%)	5/12 (41.7%)
Myelodysplastic syndrome (N, %)	12/26 (46%)	7/14 (50%)	5/12 (41.7%)
Acute bi phenotypic leukemia (N, %)	3 (12%)	1/14 (7.2%)	2/12 (16.7%)
Control type (N; %)			
HLA identical sibling	4 (15%)	2 (14%)	2 (16%)
Haploidentical	7 (27%)	2 (14%)	5 (42%)
Matched unrelated	15 (58%)	10 (72%)	5 (42%)
Conditioning regimen*			
Reduced intensity.	14 (54%)	8 (57%)	6 (50%)
Myeloablative	12 (46%)	6 (43%)	6 (50%)
GVHD prophylaxis			
CSA / MMF	12 (46%)	9 (64%)	3 (25%)
CSA / MTX	6 (23%)	3 (21%)	3 (25%)
PTCy / CSA /MMF	8 (31%)	2 (15%)	6 (50%)
Acute GVHD Grade (Before sampling)			
0	14	11	3
I-IV	13 (50%)	4 (28%)	9 (75%)
≥ II	11 (42%)	4 (28%)	7 (58%)
Immunosuppressive drugs at sampling			
CSA	16	12	4
CSA + prednisone	10/26 (38.5%)	2/14 (14%)	8/12 (67%)
CMV serological status			
D-/R+	3 (11.5%)	2 (14.5%)	1 (8%)
D-/R-	8 (31%)	4 (28.5%)	4 (34%)
D+/R-	4 (15%)	4 (28.5%)	0
D+R+	11 (42.5%)	4 (28.5%)	7 (58%)
Alive /Dead	21/5	14/3	12/2
Cause of death		Relapse, COVID (2)	GVHD, relapse

Table S3: Bone marrow aspiration cytology

	All recipients N = 26	Recipient Cluster 1 N = 14	Recipient Cluster 2 N = 12
Cellularity, n(%)			
Rich	3 (12)	3 (21)	0 (0)
Normal	13 (50)	5 (36)	8 (57)
Average	7 (27)	4 (29)	3 (25)
Poor	4 (15)	2 (14)	2 (17)
Granulocytic Lineage (expected range;50-75%)	52	50	54
	0.83	0.84	0.82
Myeloblast	5.1	4.1	6.3
Promyelocyte	10.0	8.8	11.4
Myelocyte	12.1	11.3	13.0
Metamyelocyte	21.4	21.9	20.9
Neutrophil			
Erythroblasts (expected range;10-30%)	33.3	30.8	36.2
Lymphocytes* (expected range;5-15%)	10.3	13.8	6.2
Monocytes (expected range;0-3%)	3.2	3.3	3
Megakaryocytes (cellularity)			
Normal	12	8	4
Average	8	4	4
Poor	6	2	4

* 4 patients had detectable hematogones (3 in cluster 1, 1 in cluster 2)

Table S4A: Panel of antibodies used for mass cytometry analyses of bone marrow cell population (according to (19))

Metal	Target	Clone	Reference	Staining temp.	Dilution (μl/100μl)	Panel BMMNC	Panel PBMC	Inhouse labeling*
148Nd	CD34	581	3148001B	4°C	1	■	■	No
174Yb	CD38	HIT2	303535	4°C	0,5	■	■	Yes
172Yb	IgM	MHM-88	3172004B	4°C	0,5	■	■	No
145Nd	CD45RB	MEM-55	3145009B	4°C	0,5	■	■	No
153Eu	CD62L (L-selectin)	DREG-56	3153004B	4°C	0,5	■	■	No
144Nd	CD15 (SSEA-1)	W6D3	3144019B	4°C	1	■	■	No
176Yb	CD123 (IL-3R)	6H6	306027	4°C	0,25	■	■	Yes
173Yb	CD117 (c.kit)	104D2	313223	4°C	0,5	■		Yes
175Lu	CD71	OKT-9	3175011B	4°C	0,5	■		No
89Y	CD45	HI30	3089003B	4°C	0,5	■	■	No
143Nd	CD5	UCHT2	3143007B	4°C	0,5	■	■	No
147Sm	CD20	2H7	3147001B	4°C	0,5	■	■	No
169Tm	CD24	ML5	3169004B	4°C	1	■	■	No
167Er	CD27	L128	3167006B	4°C	0,5	■	■	No
160Gd	Ig kappa (light chain)	MHK-49	3160005B	4°C	0,5	■	■	No
151Eu	Ig lambda (light chain)	MHL-38	3151004B	4°C	0,75	■	■	No
146Nd	IgD	IA6-2	3146005B	4°C	0,25	■	■	No
170Er	CD45RA	HI100	3170010B	37°C	0,25	■	■	No
164Dy	TdT	E17-1519	3164015B	4°C	0,5	■	■	No
165Ho	CD127 (IL-7Ra)	A019D5	3165008B	4°C	0,5	■	■	No
150Nd	CD43	84-3C1	3150006B	4°C	0,5	■	■	No
154Sm	Pax5	1H9	14-9918-82	4°C	0,5	■	■	No
161Dy	CD9	HI9a	312102	4°C	0,25	■	■	No
171Yb	RAG1	1D9	MA517163	4°C	1	■	■	No
155Gd	CD23	EBVCS-5	338502	4°C	0,5	■	■	Yes
163Dy	CD72	3F3	316202	4°C	0,25	■	■	Yes
152Sm	CD21	BL13	3152010B	4°C	0,5	■	■	No
159Tb	CD22	HIB22	3159005B	4°C	0,75	■	■	No
142Nd	CD40	5C3	3142010B	4°C	1	■	■	No
158Gd	CD179b	HSL11	3158016B	4°C	0,25	■		No
149Sm	CD179a	HSL96	3149012B	4°C	0,5	■		No
162Dy	Integrin b7	FIB504	3162026B	37°C	0,5	■	■	No
141Pr	CD79B	CB3-1	14-0793-82	4°C	0,5	■	■	No
168Er	CD138	DL-101	3168009B	4°C	0,5	■		No
168Er	CD73	AD2	3168015B	4°C	0,5		■	No
156Gd	CD10	HI10a	3156001B	4°C	0,5	■	■	No
166Er	CD7	M-T701	3166027B	4°C	0,25	■		No
209Bi	CD11b	ICRF44	3209003B	4°C	0,25	■		No
Cd106	CD19	HIB19	302247	4°C	0,5	■	■	Yes
Cd110	IgG	HP6017	409302	4°C	0,5	■	■	Yes
Cd113	HLA-DR	L243	307651	4°C	0,5	■	■	Yes
Cd116	CD79a	HM47	333502	4°C	0,5	■	■	Yes

Temp: Temperature. *In house labeling was performed with MaxPar X8 and MCP9 reagents kit following manufacturer instructions. At the end of the procedure, recovered labelled antibodies were diluted to 1 mg/mL

Table S4B: Phenotypic definitions of bone marrow cell populations (according to (19))

Progenitor cells	
Cell population	Phenotype
Hematopoietic stem cell (HSC)	CD34 ⁺ CD38 ⁻ CD45 ⁻ Lin ⁻
Lymphoid-primed multipotent progenitor (LMPP)	CD34 ⁺ CD38 ⁺ CD117 ⁺ CD127 ⁻
Common lymphoid progenitor (CLP)	CD34 ⁺ CD38 ⁺ CD117 ⁻
Common myeloid progenitor (CMP)	CD34 ⁺ CD38 ⁺ CD123 ⁺ CD45RA ⁻

B cells	
Cell population	Phenotype
Pre-pro-B	CD34 ⁺ CD38 ⁺ CD127 ⁺ CD10 ⁺
Pro-B	CD34 ⁺ CD38 ⁺ CD10 ⁺ CD24 ⁺ CD19 ^{low}
Pre-B	CD34 ^{low} CD38 ⁺ CD10 ^{int} CD24 ⁺ CD19 ⁺
Immature B	CD38 ⁺ CD10 ^{int/low} CD24 ⁺ CD9 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD79b ⁺ IgK ^{+/-} Igλ ^{+/-}
Transitional B	CD38 ⁺ CD24 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD22 ⁺ CD40 ⁺ CD79b ⁺ IgD ^{+/-} CD21 ⁺ IgK ^{+/-} Igλ ^{+/-}
Naive B	CD38 ⁻ CD24 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD22 ⁺ CD40 ⁺ CD79b ⁺ IgD ⁺ CD21 ⁺ CD27 ⁺ IgK ^{+/-} Igλ ^{+/-}
Plasma cells	CD38 ⁺ CD19 ^{low} CD138 ⁺ CD27 ⁺

T cells	
Cell population	Phenotype
TEM cells (effector memory)	CD45 ⁺ CD5 ⁺ CD45RA ⁻ CD27 ⁻
TEMRA cells	CD45 ⁺ CD5 ⁺ CD45RA ⁺ CD27 ⁺
Naive T cells	CD45 ⁺ CD5 ⁺ CD45RB ⁺ CD45RA ⁺ CD127 ⁺ CD27 ⁺
T-cell Precursors	CD45 ⁺ CD38 ⁺ CD7 ⁺ CD5 ⁻
T cells	CD45 ⁺ CD7 ⁺ CD5 ⁺ CD127 ⁺ CD27 ⁺

Myeloid cells	
Cell population	Phenotype
Myeloid progenitors	CD45 ⁺ CD38 ^{low} CD10 ⁺ CD66b ⁺ CD11b ⁺
Monocytes	CD45 ⁺ CD66b ⁺ CD11b ⁺

Others cells	
Cell population	Phenotype
Granulocytes	CD38 ⁺ CD66b ⁺
Plasmacytoid DC	CD38 ⁺ CD62L ⁺ CD123 ⁺
Erythroid precursors	CD38 ^{low} CD71 ⁺

Table S5A: Panel of antibodies used to study bone marrow T-cell populations.

Metal	Target	Clone	Reference	Staining temp.	Dilution ($\mu\text{l}/100\mu\text{l}$)
116Cd	CD3	UCHT1	300443	4°C	1
11Cd	CD4	SK3	344625	4°C	0,13
141Pr	CD5	UCHT2	300627	4°C	0,25
113Cd	CD8	SK1	344727	4°C	0,25
112Cd	CD11b	ICRF44	301337	4°C	0,4
147Sm	CD11c	Bu15	3147008B	4°C	0,5
106Cd	CD14	M5E2	301843	4°C	0,75
144Nd	CD15	W6D3	3144019B	4°C	0,5
110Cd	CD19	HIB19	302247	4°C	0,5
169Tm	CD25	2A3	3169003B	4°C	0,5
158Gd	CD33	WM53	3158001B	4°C	0,25
149Sm	CD34	581	3149013B	4°C	1
160Gd	CD39	A1	3160004B	4°C	0,4
89Y	CD45	HI30	3089003B	4°C	0,5
176Yb	CD56	NCAM16.2	3176008B	4°C	0,25
168Er	CD73	AD2	3168015B	4°C	0,5
143Nd	CD117	104D2	3143001B	4°C	0,75
165Ho	CD127 (IL-7Ra)	A019D5	3165008B	4°C	1
175Lu	CD223/LAG-3	11C3C65	3175033B	4°C	1
164Dy	CD272 (BTLA)	MIH26	344502	4°C	0,5
174Yb	CD279 (PD-1)	EH12.2H7	3174020B	4°C	1
142Nd	TGIT	MBSA43	372702	4°C	1
154Sm	TIM-3	F38-2E2	3154010B	4°C	1,5
167Er	CD197 (CCR7)	G043H7	3167009A	37°C	0,5
155Gd	CD45RA	HI100	3155011B	37°C	0,5
173Yb	CD194 (CCR4)	L291H4	359402	37°C	0,3
162Dy	FoxP3	259D/C7	3162024A	4°C	1,25
171Yb	Granzyme B	GB11	3171002B	4°C	0,5

Table S5B: Phenotypic definitions of bone marrow T-cell populations in CyTOF analyses

Progenitor cells	
Cell population	Phenotype
Hematopoietic stem and progenitor cells (HSPC)	CD34 ⁺ CD45 ^{low}

T cells	
Cell population	Phenotype
CD4 ⁺ TEM cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ⁻ CD127 ^{+/-} CD197 ⁻
CD4 ⁺ TCM cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ⁻ CD127 ^{+/-} CD197 ⁺
CD4 ⁺ Treg cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ^{+/-} CD127 ^{low} CD25 ⁺
CD4 ⁺ CCR4 ⁺ T _{EM/CM} cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ^{+/-} CD127 ^{+/-} CD197 ^{+/-} CD194 ⁺
CD4 ⁺ Naive T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ⁺ CD127 ^{+/-} CD197 ⁺
Innate-like CD4 ⁺ T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ⁻ CD127 ^{+/-} CD56 ⁺
DN T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁻ CD8 ⁻ CD127 ^{+/-} CD45RA ^{+/-}
CD8 ⁺ TEM cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁻ CD127 ^{+/-} CD197 ⁻
CD8 ⁺ TEM/CM cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁻ CD127 ⁺
CD8 ⁺ TEMRA cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁺ CD127 ^{+/-} CD197 ^{+/-} CD194 ⁺
CD8 ⁺ Naive T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁺ CD127 ^{+/-} CD197 ⁺
Innate-like CD8 ⁺ T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁻ CD127 ^{+/-} CD56 ⁺

Natural killer cells	
Cell population	Phenotype
NK	CD56 ⁺
NK 56 ^{bright}	CD56 ^{bright/high}

Myeloid cells	
Cell population	Phenotype
Precursors Myeloid	CD45 ⁺ CD33 ⁺ CD11b ⁻ CD14 ⁻
Monocytes	CD45 ⁺ CD33 ⁺ CD11b ⁺ CD14 ^{low}

Other cells	
Cell population	Phenotype
B cells	CD45 ⁺ CD19 ⁺
Non-immune cells	CD45 ⁻
T-mono complex	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD8 ⁺ CD45RA ⁻ CD127 ^{+/-} CD11b ⁺ CD14 ⁺

Table S6A: TotalSeq™-C Human Universal antibody used in CITEseq experiments.

Description	Clone	Barcode	Name
anti-human CD86	IT2.2	GTCTTTGTCAGTGCA	CD86
anti-human CD274 (B7-H1, PD-L1)	29E.2A3	GTTGTCCGACAATAC	CD274
anti-human CD270 (HVEM, TR2)	122	TGATAGAAACAGACC	TNFRSF14
anti-human CD155 (PVR)	SKII.4	ATCACATCGTTGCCA	PVR
anti-human CD112 (Nectin-2)	TX31	AACCTTCCGTCTAAG	NECTIN2
anti-human CD47	CC2C6	GCATTCTGTCACCTA	CD47
anti-human CD48	BJ40	CTACGACGTAGAAGA	CD48
anti-human CD40	5C3	CTCAGATGGAGTATG	CD40
anti-human CD154	24-31	GCTAGATAGATGCAA	CD40LG
anti-human CD52	HI186	CTTTGTACGAGCAAA	CD52
anti-human CD3	UCHT1	CTCATTGTAACCTCT	CD3D
anti-human CD8	SK1	GCGCAACTTGATGAT	CD8A
anti-human CD56	5.1H11	TCCTTTCTGATAGG	NCAM1
anti-human CD19	HIB19	CTGGGCAATTACTCG	CD19
anti-human CD33	P67.6	TAACCTCAGGGCCTAT	CD33
anti-human CD11c	S-HCL-3	TACGCCTATAACTTG	ITGAX
anti-human HLA-A,B,C	W6/32	TATGCGAGGCTTATC	HLA-A
anti-human CD45RA	HI100	TCAATCCTTCCGCTT	PTPRC
anti-human CD123	6H6	CTTCACTCTGTCAGG	IL3RA
anti-human CD7	CD7-6B7	TGGATTCCCGGACTT	CD7
anti-human CD105	43A3	ATCGTCGAGAGCTAG	ENG
anti-human/mouse CD49f	GoH3	TTCCGAGGATGATCT	ITGA6
anti-human CD194 (CCR4)	L291H4	AGCTTACCTGCACGA	CCR4
anti-human CD4	RPA-T4	TGTTCCCGCTCAACT	CD4
anti-mouse/human CD44	IM7	TGGCTTCAGGTCCTA	CD44
anti-human CD14	M5E2	TCTCAGACCTCCGTA	CD14
anti-human CD16	3G8	AAGTTCACCTTTTGC	FCGR3A
anti-human CD25	BC96	TTTGTCTGTACGCC	IL2RA
anti-human CD45RO	UCHL1	CTCCGAATCATGTTG	PTPRC
anti-human CD279	EH12.2H7	ACAGCGCCGTATTTA	PDCD1
anti-human TIGIT (VSTM3)	A15153G	TTGCTTACCGCCAGA	TIGIT
Mouse IgG1, κ isotype Ctrl	MOPC-21	GCCGGACGACATTAA	
Mouse IgG2a, κ isotype Ctrl	MOPC-173	CTCCTACCTAAACTG	
Mouse IgG2b, κ isotype Ctrl	MPC-11	ATATGTATCACGCGA	
Rat IgG2b, κ Isotype Ctrl	RTK4530	GATTCTTGACGACCT	
anti-human CD20	2H7	TTCTGGGTCCCTAGA	MS4A1
anti-human CD335 (NKp46)	9E2	ACAATTTGAACAGCG	NCR1
anti-human CD31	WM59	ACCTTTATGCCACGG	PECAM1
anti-human CD146	P1H12	CCTTGGATAACATCA	MCAM
anti-human IgM	MHM-88	TAGCGAGCCCGTATA	IGHM
anti-human CD5	UCHT2	CATTAACGGGATGCC	CD5
anti-human CD183 (CXCR3)	G025H7	GCGATGGTAGATTAT	CXCR3

anti-human CD195 (CCR5)	J418F1	CCAAAGTAAGAGCCA	CCR5
anti-human CD32	FUN-2	GCTTCCGAATTACCG	FCGR2A
anti-human CD196 (CCR6)	G034E3	GATCCCTTTGTCACT	CCR6
anti-human CD185 (CXCR5)	J252D4	AATTCAACCGTCGCC	CXCR5
anti-human CD103 (Integrin αE)	Ber-ACT8	GACCTCATTGTGAAT	ITGAE
anti-human CD69	FN50	GTCTCTTGGCTTAAA	CD69
anti-human CD62L	DREG-56	GTCCCTGCAACTTGA	SELL
anti-human CD161	HP-3G10	GTACGCAGTCCTTCT	KLRB1
anti-human CD152 (CTLA-4)	BNI3	ATGGTTCACGTAATC	CTLA4
anti-human CD223 (LAG-3)	11C3C65	CATTTGTCTGCCGGT	LAG3
anti-human KLRG1 (MAFA)	SA231A2	CTTATTTCTGCCCT	KLRG1
anti-human CD27	O323	GCACTCCTGCATGTA	CD27
anti-human CD107a (LAMP-1)	H4A3	CAGCCCACTGCAATA	LAMP1
anti-human CD95 (Fas)	DX2	CCAGCTCATTAGAGC	FAS
anti-human CD134 (OX40)	Ber- ACT35 (ACT35)	AACCCACCGTTGTTA	TNFRSF4
anti-human HLA-DR	L243	AATAGCGAGCAAGTA	HLA-DRA
anti-human CD1c	L161	GAGCTACTTCACTCG	CD1C
anti-human CD11b	ICRF44	GACAAGTGATCTGCA	ITGAM
anti-human CD64	10,1	AAGTATGCCCTACGA	FCGR1A
anti-human CD141 (Thrombomodulin)	M80	GGATAACCGCGCTTT	THBD
anti-human CD1d	51,1	TCGAGTCGCTTATCA	CD1D
anti-human CD314 (NKG2D)	1D11	CGTGTTTGTTCCTCA	KLRK1
anti-human CD35	E11	ACTTCCGTCGATCTT	CR1
anti-human CD57 Recombinant	QA17A04	AACTCCCTATGGAGG	B3GAT1
anti-human CD272 (BTLA)	MIH26	GTTATTGGACTAAGG	BTLA
anti-human/mouse/rat CD278 (ICOS)	C398.4A	CGCGCACCCATTAAA	ICOS
anti-human CD58 (LFA-3)	TS2/9	GTTCTATGGACGAC	CD58
anti-human CD39	A1	TTACCTGGTATCCGT	ENTPD1
anti-human CX3CR1	K0124E1	AGTATCGTCTCTGGG	CX3CR1
anti-human CD24	ML5	AGATTCCTTCGTGTT	CD24
anti-human CD21	Bu32	AACCTAGTAGTTCGG	CR2
anti-human CD11a	TS2/4	TATATCCTTGTGAGC	ITGAL
anti-human CD79b (Igβ)	CB3-1	ATTCTTCAACCGAAG	CD79B
anti-human CD244 (2B4)	C1.7	TCGCTTGGATGGTAG	CD244
anti-human CD169 (Sialo adhesin, Siglec-1)	7-239	TACTCAGCGTGTGTTG	SIGLEC1
anti-human/mouse integrin β7	FIB504	TCCTTGGATGTACCG	ITGB7
anti-human CD268 (BAFF-R)	11C1	CGAAGTCGATCCGTA	TNFRSF13C
anti-human CD42b	HIP1	TCCTAGTACCGAAGT	GP1BB
anti-human CD54	HA58	CTGATAGACTTGAGT	ICAM1
anti-human CD62P (P-Selectin)	AK4	CCTCCGTATCCCTT	SELP
anti-human CD119 (IFN-γ R α chain)	GIR-208	TGTGTATTCCCTTGT	IFNGR1

anti-human TCR α/β	IP26	CGTAACGTAGAGCGA	
Rat IgG1, κ isotype Ctrl	RTK2071	ATCAGATGCCCTCAT	
Rat IgG2a, κ Isotype Ctrl	RTK2758	AAGTCAGGTTCGTTT	
Armenian Hamster IgG Isotype Ctrl	HTK888	CCTGTCATTAAGACT	
anti-human CD122 (IL-2Rβ)	TU27	TCATTTCTCCGATT	IL2RB
anti-human CD267 (TACI)	1A1	AGTGATGGAGCGAAC	TNFRSF13B
anti-human FcϵRIα	AER-37 (CRA-1)	CTCGTTTCCGTATCG	FCER1A
anti-human CD41	HIP8	ACGTTGTGGCCTTGT	ITGA2B
anti-human CD137 (4-1BB)	4B4-1	CAGTAAGTTCGGGAC	TNFRSF9
anti-human CD163	GHI/61	GCTTCTCCTTCCTTA	CD163
anti-human CD83	HB15e	CCACTCATTTCCGGT	CD83
anti-human CD124 (IL-4Rα)	G077F6	CCGTCCTGATAGATG	IL4R
anti-human CD13	WM15	TTCAACGCCCTTTC	ANPEP
anti-human CD2	TS1/8	TACGATTTGTCAGGG	CD2
anti-human CD226 (DNAM-1)	11A8	TCTCAGTGTGTGG	CD226
anti-human CD29	TS2/16	GTATCCCTCAGTCA	ITGB1
anti-human CD303 (BDCA-2)	201A	GAGATGTCCGAATTT	CLEC4C
anti-human CD49b	P1E6-C5	GCTTTCTTCAGTATG	ITGA2
anti-human CD81 (TAPA-1)	5A6	GTATCCTTCCTTGGC	CD81
anti-human IgD	IA6-2	CAGTCTCCGTAGAGT	IGHD
anti-human CD18	TS1/18	TATTGGGACACTTCT	ITGB2
anti-human CD28	CD28.2	TGAGAACGACCCTAA	CD28
anti-human CD38	HIT2	TGTACCCGCTTGTGA	CD38
anti-human CD127 (IL-7Rα)	A019D5	GTGTGTTGTCCATATG	IL7R
anti-human CD45	HI30	TGCAATTACCCGGAT	PTPRC
anti-human CD22	S-HCL-1	GGGTTGTTGTCTTTG	CD22
anti-human CD71	CY1G4	CCGTGTTCTCATTAA	TFRC
anti-human CD26	BA5b	GGTGGCTAGATAATG	DPP4
anti-human CD36	5-271	TTCTTTGCCTTGCCA	CD36
anti-human CD158 (KIR2DL1/S1/S3/S5)	HP-MA4	TATCAACCAACGCTT	KIR2DL1
anti-human CD49a	TS2/7	ACTGATGGACTCAGA	ITGA1
anti-human CD49d	9F10	CCATTCAACTTCCGG	ITGA4
anti-human CD73 (Ecto-5'-nucleotidase)	AD2	CAGTTCCTCAGTTCG	NT5E
anti-human TCR Vα7.2	3C10	TACGAGCAGTATTCA	
anti-human TCR Vδ2	B6	TCAGTCAGATGGTAT	
anti-human LOX-1	15C4	ACCCTTTACCGAATA	OLR1
anti-human CD158b (KIR2DL2/L3, NKAT2)	DX27	GACCCGTAGTTTGAT	KIR2DL3
anti-human CD158e1 (KIR3DL1, NKB1)	DX9	GGACGCTTTCCTTGA	KIR3DL1
anti-human CD319 (CRACC)	162,1	AGTATGCCATGTCTT	SLAMF7
anti-human CD99	3B2/TA8	ACCCGTCCCTAAGAA	CD99
anti-human CLEC12A	50C1	CATTAGAGTCTGCCA	CLEC12A

anti-human CD352 (NTB-A)	NT-7	AGTTTCCACTCAGGC	SLAMF6
anti-human CD94	DX22	CTTTCCGGTCCTACA	KLRD1
anti-human Ig light chain κ	MHK-49	AGCTCAGCCAGTATG	IGKC
anti-human CD85j (ILT2)	GHI/75	CCTTGTGAGGCTATG	LILRB1
anti-human CD23	EBVCS-5	TCTGTATAACCGTCT	FCER2
anti-human Ig light chain λ	MHL-38	CAGCCAGTAAGTCAC	
anti-human CD328 (Siglec-7)	6-434	CTTAGCATTTCCTG	SIGLEC7
anti-human GPR56	CG4	GCCTAGTTTCCGTTT	ADGRG1
anti-human HLA-E	3D12	GAGTCGAGAAATCAT	HLA-E
anti-human CD82	ASL-24	TCCCACTTCCGCTTT	CD82
anti-human CD101 (BB27)	BB27	CTACTTCCCTGTCAA	CD101
anti-human CD88 (C5aR)	S5/1	GCCGCATGAGAAACA	C5AR1
anti-human CD224	KF29	CTGATGAGATGTCAG	GGT1

TotalSeq™-C Human Universal Cocktail, V1.0; BioLegend; cat# 399905

Table S6B: Antibodies added to the TotalSeq™-C Human Universal Cocktail for Cite-seq analyses.

Description	Clone	Barcode	Gene Name	Reference
Anti-human CD9	HI9a	GAGTCACCAATCTG C	5H9	312121
Anti-human CD10	HI10a	CAGCCATTTCATTAG G		312233
Anti-human CD117 (c-kit)	104D2	AGACTAATAGCTGA C		313243
Anti-human CD135 (Flt-3/Flk-2)	BV10A4H 2	CAGTAGATGGAGCA T		313323
Anti-human CD34	581	GCAGAAATCTCCCT T		343541
Anti-human CD197	G043H7	AGTTCAGTCAACCG A		353251
Anti-human CD138	MI15	ACTCTTTTCGTTTAC G		356539

Table S6C: Phenotypic definitions used to define cell populations in CITEseq experiments.

Progenitors cells	
Cell population	Phenotype
Lymphoid-primed multipotent progenitor (LMPP)	CD34 ⁺ CD38 ⁺ CD117 ⁺ CD127 ⁻
Common lymphoid progenitor (CLP)	CD34 ⁺ CD38 ⁺ CD117 ⁻

Myeloid cells	
Cell population	Phenotype
Common myeloid progenitor (CMP)	CD34 ⁺ CD38 ⁺ CD123 ⁺ CD45RA ⁻
Granulocyte and monocyte progenitor (GMP)	CD34 ⁺ CD38 ⁺ CD123 ⁺ CD45RA ⁺
Committed myeloid (MPP)	CD34 ^{low} CD38 ^{high} CD11b ⁺ CD33 ⁺ CD16 ⁻
Dendritic cells	CD38 ⁺ CD11c ⁺ CD123 ^{low} CD11b ⁺
Classical monocyte	CD14 ⁺⁺ CD16 ⁻ CD11b ⁺ CD33 ⁺
Intermediate monocyte	CD14 ^{+/++} CD16 ⁺ CD11b ⁺ CD33 ⁺
Non classical monocytes	CD14 ^{low} CD16 ⁺⁺ CD11b ⁺ CD33 ⁺

B cells	
Cell population	Phenotype
Pre-pro-B	CD34 ⁺ CD38 ⁺ CD127 ⁺ CD10 ⁺
Pro-B	CD34 ⁺ CD38 ⁺ CD10 ⁺ CD24 ⁺ CD19 ^{low}
Pre-B	CD34 ^{low} CD38 ⁺ CD10 ^{int} CD24 ⁺ CD19 ⁺
Immature B	CD38 ⁺ CD10 ^{int/low} CD24 ⁺ CD9 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD79b ⁺
Transitional B	CD38 ⁺ CD24 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD22 ⁺ CD40 ⁺ CD79b ⁺ IgD ^{+/-} CD21 ⁺
Naive B	CD38 ⁻ CD24 ⁺ CD19 ⁺ CD20 ⁺ IgM ⁺ CD22 ⁺ CD40 ⁺ CD79b ⁺ IgD ⁺ CD21 ⁺ CD27 ⁻
Plasma cells	CD38 ⁺ CD19 ^{low} CD138 ⁺ CD27 ⁺

T cells	
Cell population	Phenotype
CD4 ⁺ T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ^{+/-} CD127 ^{+/-} CD197 ⁻
CD4 ⁺ TSCM cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RO ⁺ CD45RA ⁺ CD95 ⁺
Treg exhausted cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ^{+/-} CD127 ^{low} CD25 ⁺ CD73 ⁺
CD279 ⁺ TIGIT ⁺ T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ^{+/-} CD8 ^{+/-} CD45RA ⁻ CD127 ^{+/-} CD197 ⁻ CD279 ⁺ TIGIT ⁺
CD4 ⁺ Naive T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ⁺ CD45RA ⁺ CD127 ^{+/-} CD197 ⁺
NK T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD4 ^{+/-} CD8 ^{+/-} CD45RA ^{+/-} CD127 ^{+/-} CD56 ⁺
CD8 ⁺ T cells	CD45 ⁺ CD3 ⁺ CD5 ⁺ CD8 ⁺ CD45RA ⁻ CD127 ^{+/-} CD197 ⁻

Natural killer cells	
Cell population	Phenotype
NK cells	CD56 ⁺ CD16 ⁺
CD56 ^{bright} NK cells	CD56 ^{bright/high} CD16 ⁻

Erythroblast cells	
CD34 ⁺ ERP early	CD34 ⁺ CD38 ⁺ CD36 ⁺ CD105 ⁺ CD10 ⁻ CD123 ⁻ CD45RA ⁻ CD71 ⁺
Megakaryocyte erythroid progenitor (MEP)	CD34 ^{low} CD38 ^{low} CD36 ⁺ CD105 ⁺ CD10 ⁻ CD123 ⁻ CD45RA ⁻ CD71 ⁺
Erythroblast (1)	CD34 ⁻ CD36 ⁺ CD105 ^{low}
Erythroblast (2)	CD34 ⁻ CD36 ^{int} CD105 ^{low}

Others cells	
Cell population	Phenotype
Mesenchymal stem cells (MSC)	CD34 ^{-/low} CD45 ⁻ CD105 ⁺ CD90 ⁺ CD73 ⁺
Eosinophil	CD45 ⁺ CD11b ⁺ CD46d ⁺ CD14 ^{low} (based on expression of RNA)
CD45 ^{neg} cells	CD45 ^{neg}

Figure S1: Overall cohort (42 patients) ; samples repartition: In 16 patients only PBMC were studied. were studied (* Some patients had missing samples at 3 or 6 months: thus, numbers do not add to n=16). In 26 patients bone marrow cells were analyzed with 22/26 being also studied on PBMC

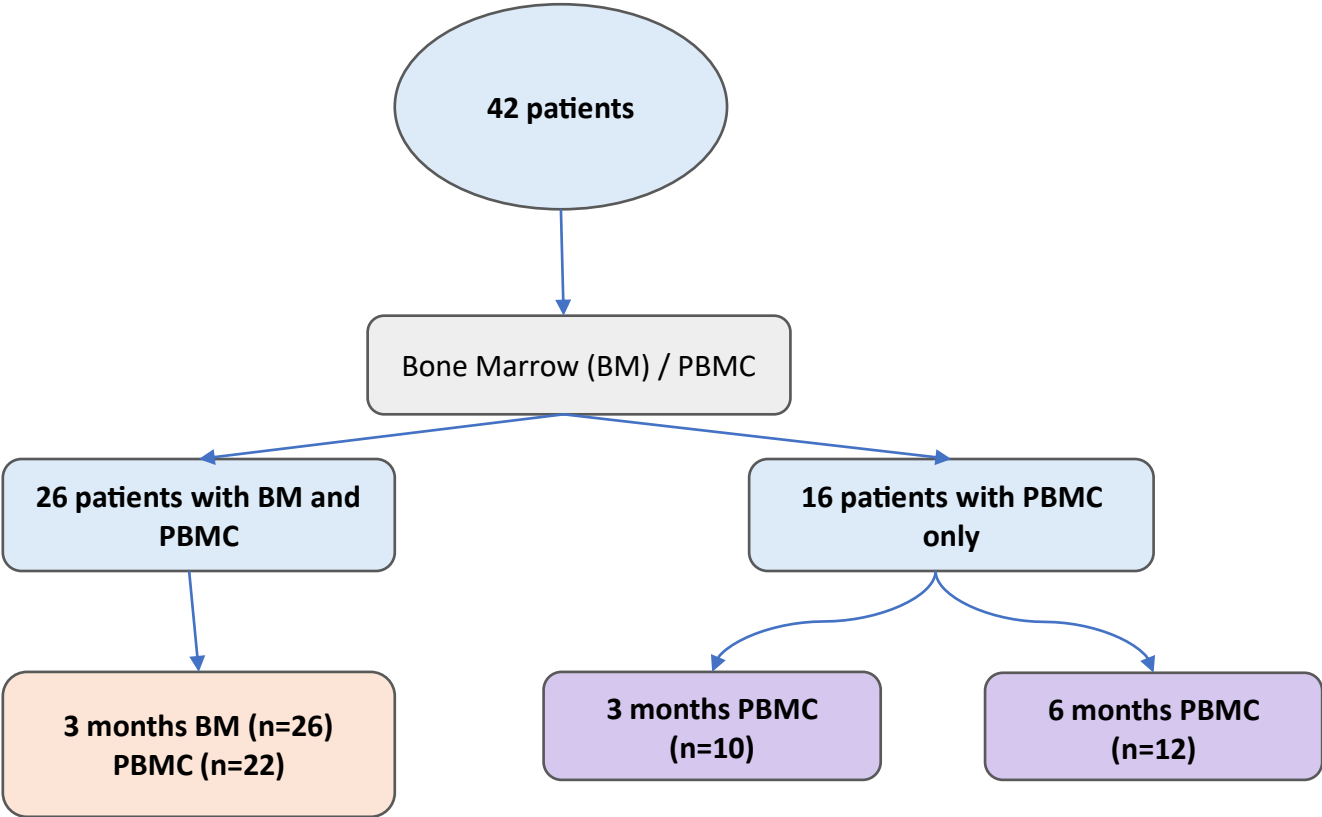
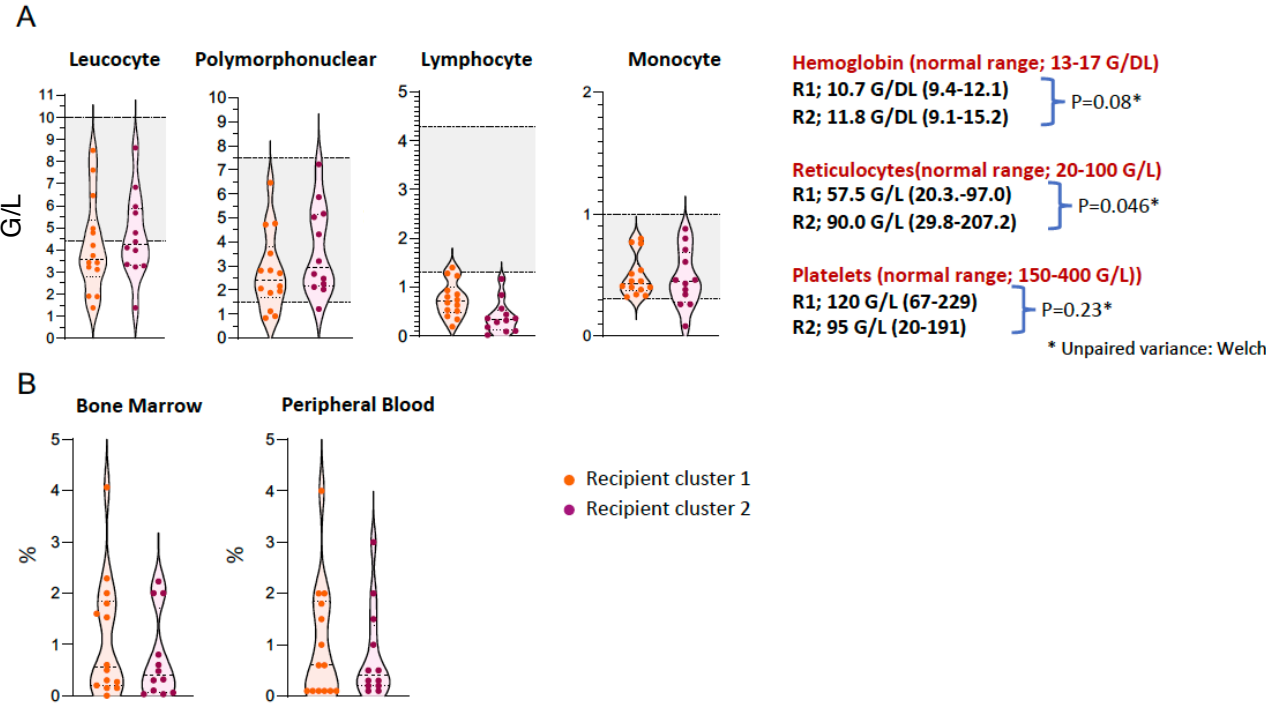


Figure S2: Peripheral Blood; parameters (A) and chimerism analyses (B)



(A) Numeration of leucocyte from peripheral blood in recipient of cluster 1 (n=14) and recipient of cluster 2 (n=12). The normal numeration from controls is represented by gray square. **(B)** Chimerism analyses (as expressed by % of cells of recipient origin) from bone marrow and peripheral blood (using KMR kit, from GenDX ®). As detailed infra, these patients were segregated into two groups [Recipients 1 (R1) and recipients 2 (R2)], but except for reticulocyte counts R1 and R2 do not differ significantly for peripheral blood parameters.

Figure S3: Peripheral Blood mass cytometry analyses of B cell subsets

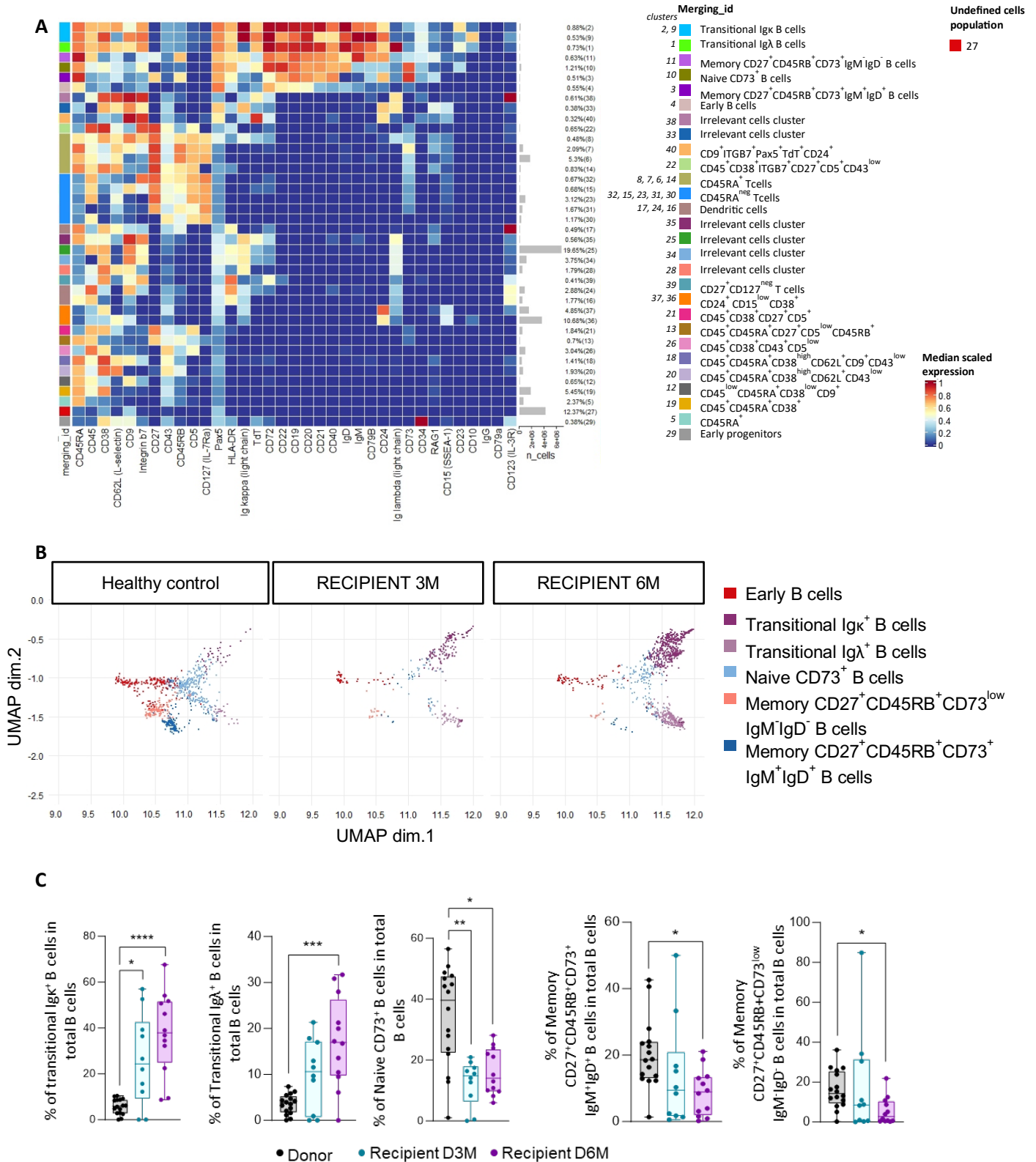


Figure S4: Staining panel design of human bone marrow data set

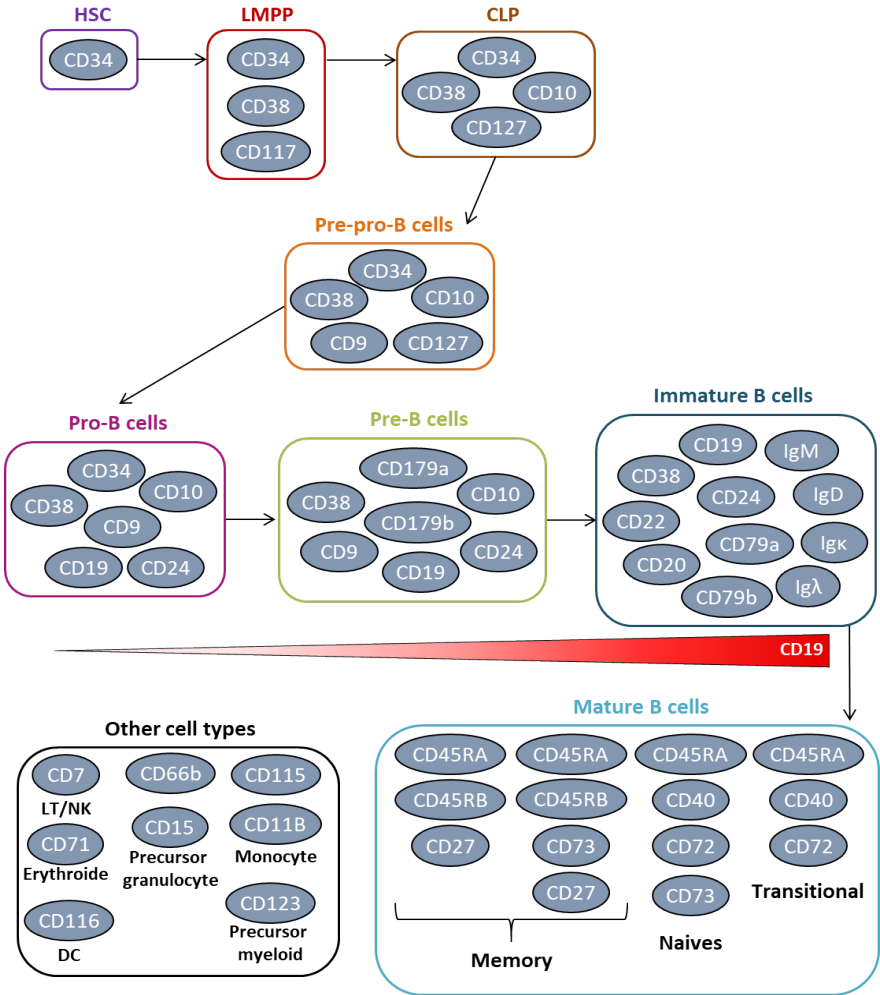
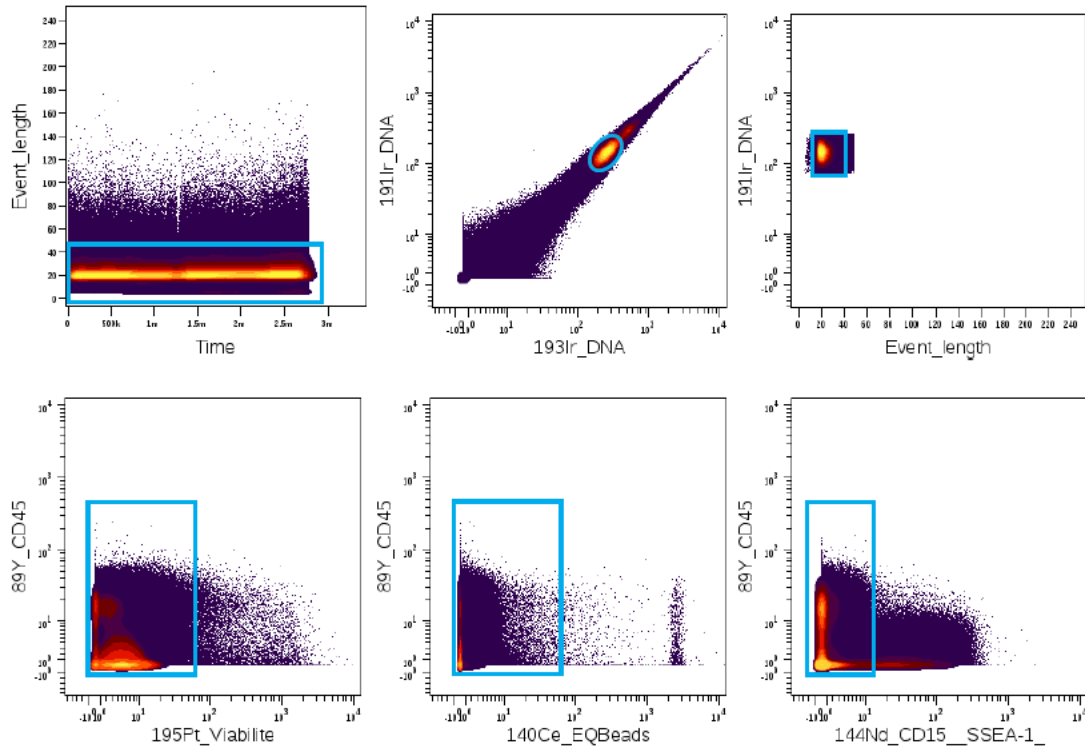


Figure S5:

A. Gating strategy for mass cytometry analyses of CD15 negative cells.



B. Gating strategy of early progenitors for mass cytometry analyses in CD15 negative cells.

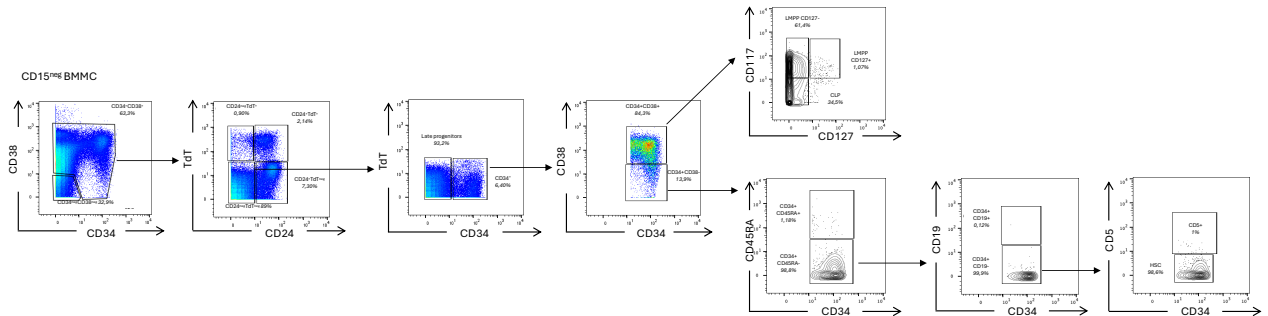
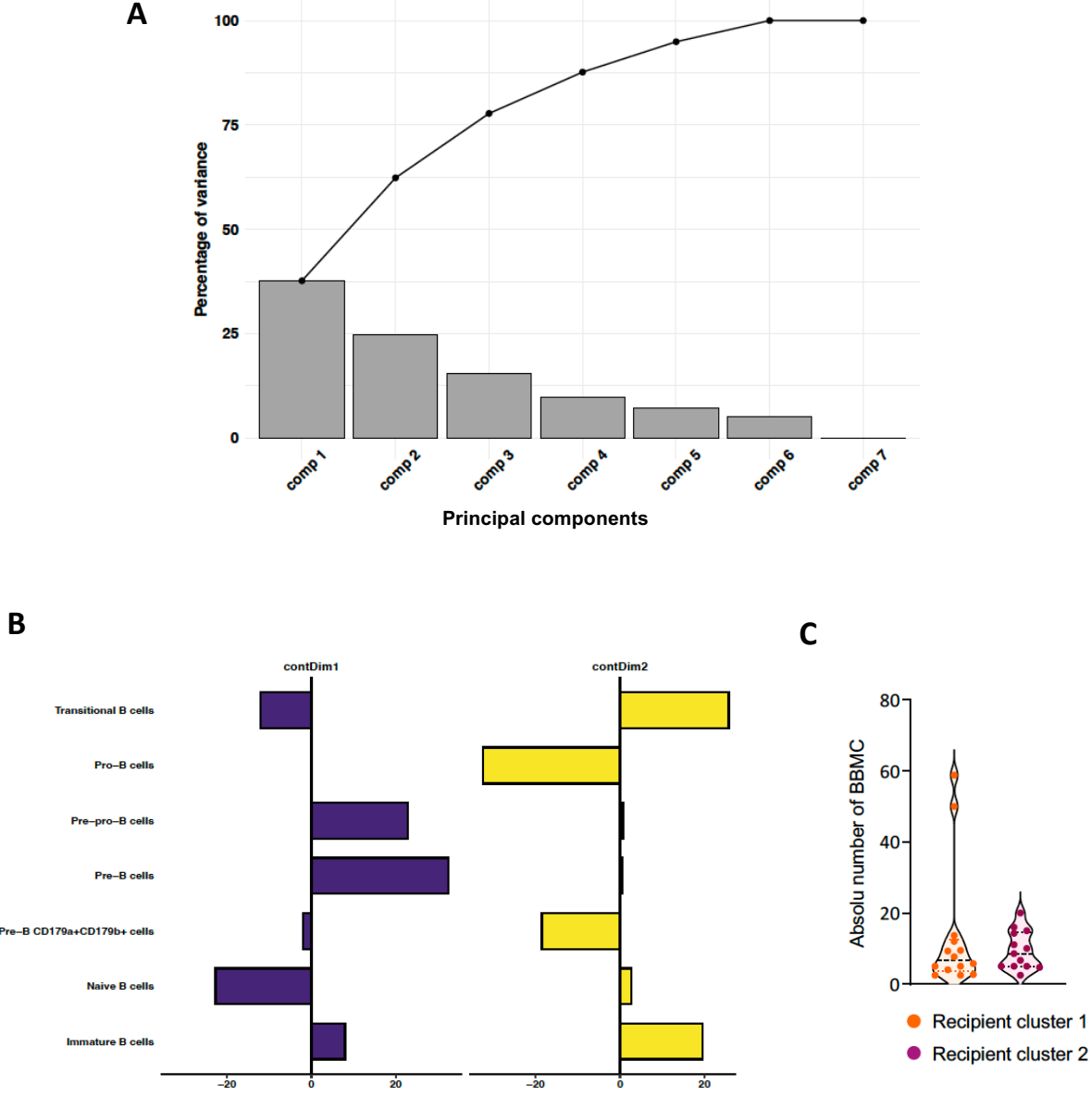
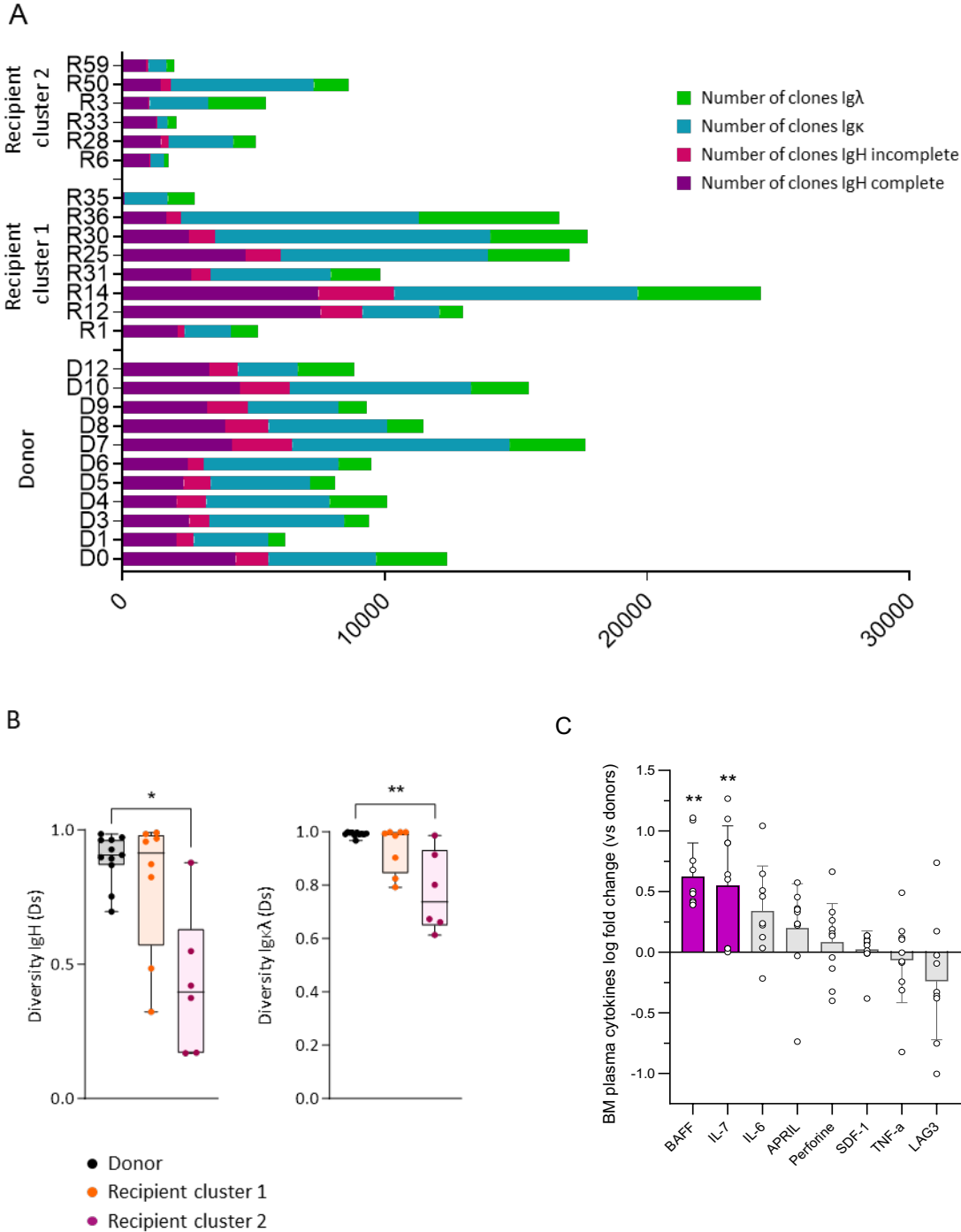


Figure S6: Key parameters associated with the PCA (Figure 2D)



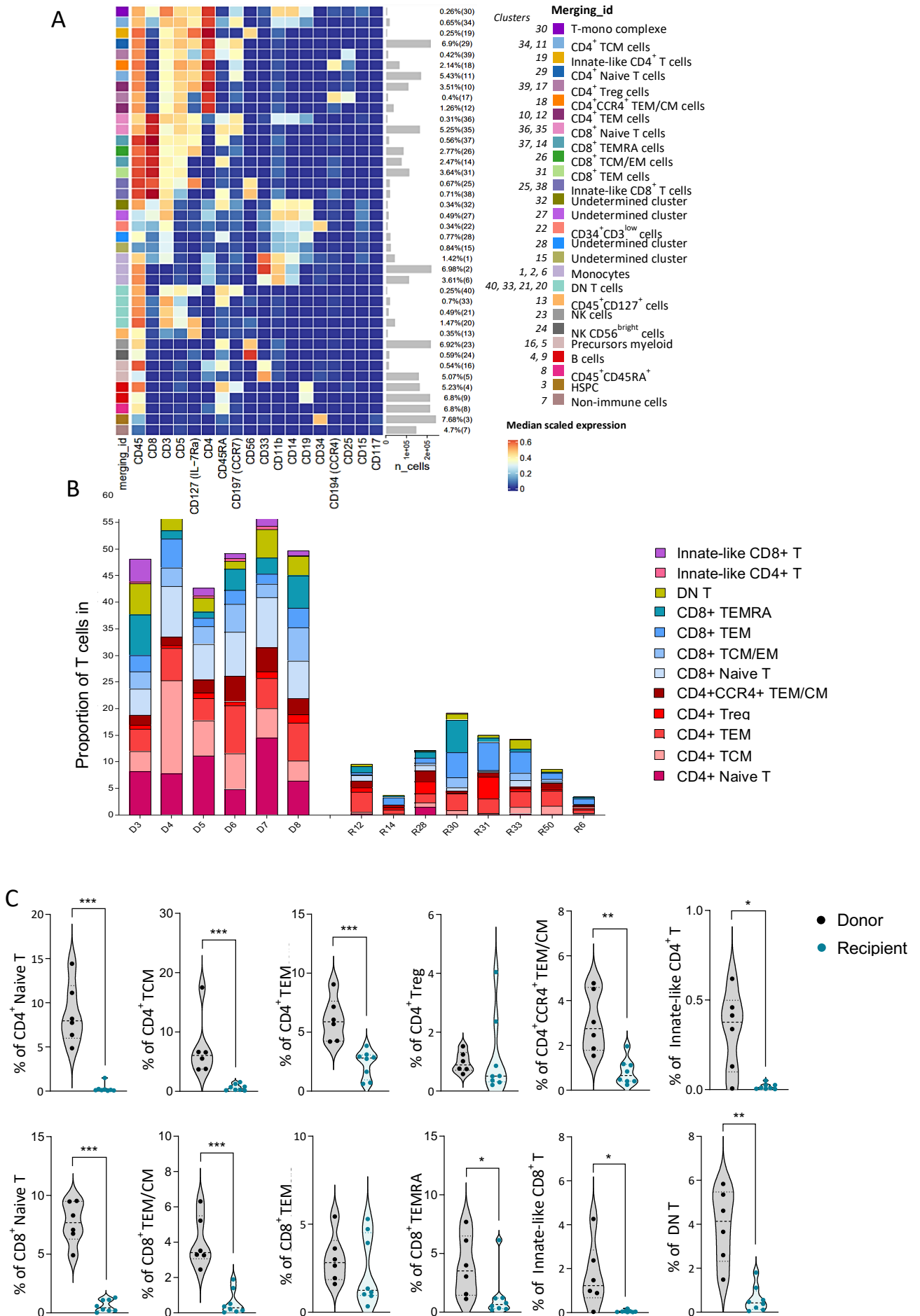
(A) Percentage of variance for each principal component. **(B)** Contribution of each B-cell subset to components 1 and 2. **(C)** Absolute bone marrow cell numbers in recipient of cluster 1 and recipient of cluster 2 (non-significant; Mann-Whitney test)

Figure S7: Clonal analyses of bone marrow B cells.



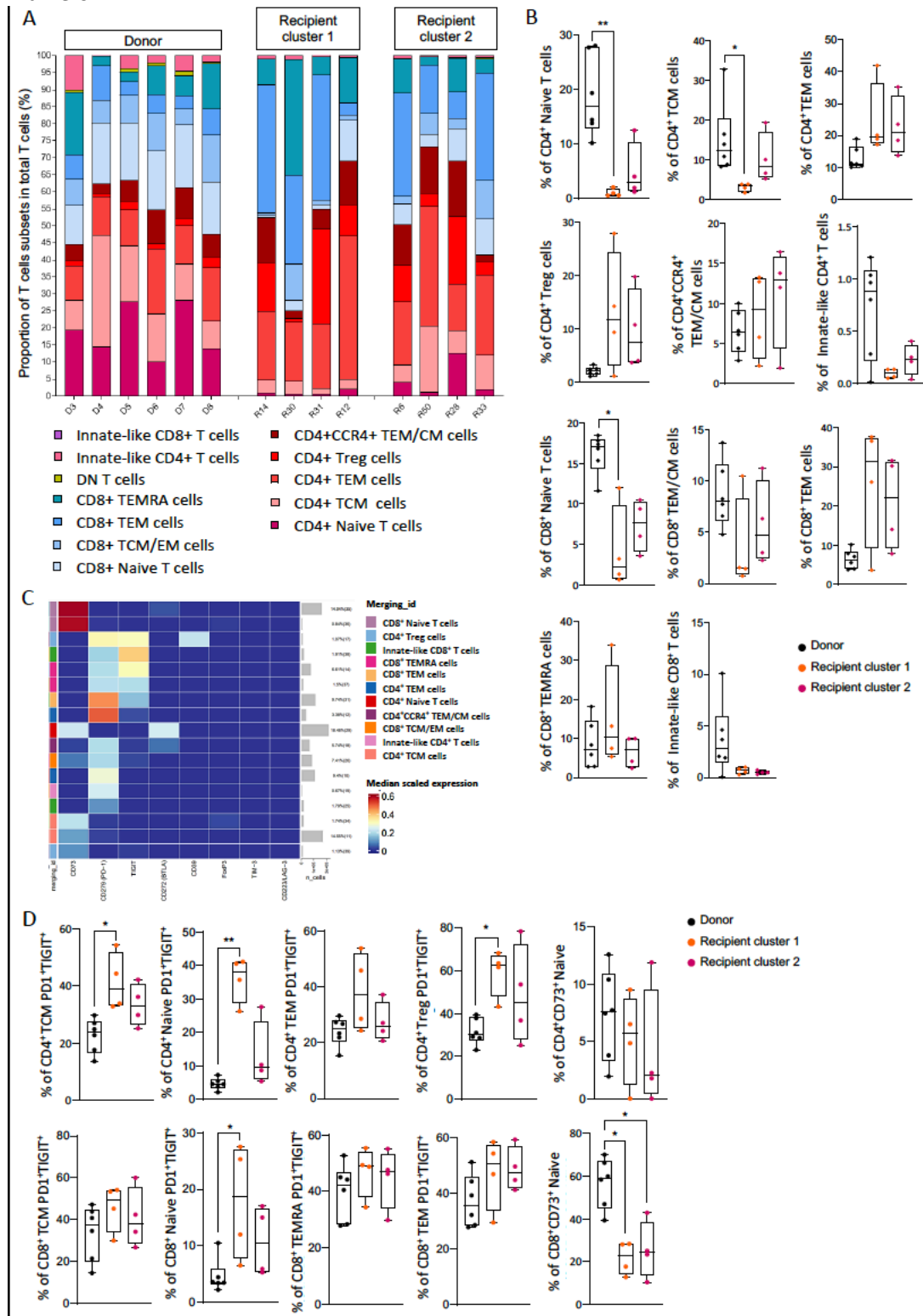
(A) Using next-generation sequencing (NGS) technology to quantify the number of clones of immunoglobulin light chains kappa (blue), immunoglobulin light chains lambda (green) and immunoglobulin heavy chains complete (purple) and incomplete (pink) per individual control (n=11), recipient cluster 1 (n=6) and recipient cluster 2 (n=8) patients. **(B)** Data showing the diversity (as assessed by the relative abundance of the clonotypes) of immunoglobulin heavy chains (IgH) and immunoglobulin light chains kappa/lambda (Igκλ) in controls, recipient cluster 1 and recipient cluster 2 patients. **(C)** Cytokines levels in bone marrow plasma studied by ELISA and Luminex from recipients (n=9) compared with controls (n=6). P-values correspond to Mann-Whitney-Wilcoxon test: *p < 0.05, **p < 0.01. Donor (D) refers to healthy control

Figure S8: Mass cytometry analyses of bone marrow T cells.



(A) Heatmap showing median expression intensities of each protein marker (columns), for each detected cluster (rows) using FlowSOM algorithm, with 41 indicated relevant metaclusters. **(B)** Data showing the proportion of T cells for all subsets per individual in controls (n=6) and recipients (n=8) patients. **(C)** Quantification of percentage of T cells for all subsets from bone marrow controls and recipients. Mann-Whitney tests: *p <0.05; **p<0.01; ***p<0.001. Donor (D) refers to healthy control

Figure S9: Mass cytometry analyses of bone marrow T cells in R1 and R2 and analyses of functional markers.



(A) Data showing the proportion of T cells for all subsets per individual in controls (n=6), recipients of cluster 1 (n=4) and recipients of cluster 2 (n=4) patients. **(B)** Quantification of percentage of T cells for all subsets from bone marrow controls, recipient of cluster 1 and recipient of cluster 2 patients. **(C)** Heatmap depicting scaled cell surface antigen expression in T-cell subsets. **(D)** Quantification of percentage of PD1⁺TIGIT⁺ and CD73⁺ in T-cells subsets. P-values correspond to Mann-Whitney-Wilcoxon test: *p<0.05, **p<0.01. Donor (D) refers to healthy control

Figure S10: Representative B cell progenitors' associated TF expression projected on phenotypically defined cellular population (refer to TF not disclosed in Figure 5 & 6).

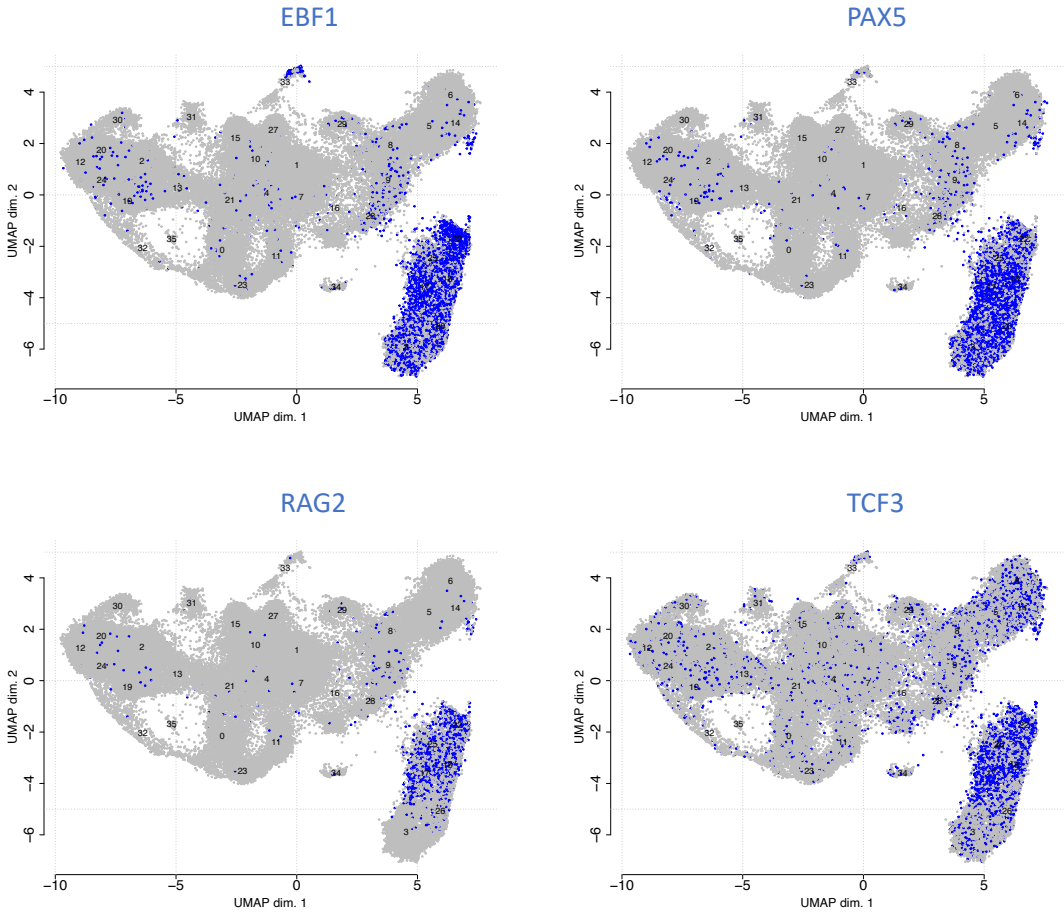
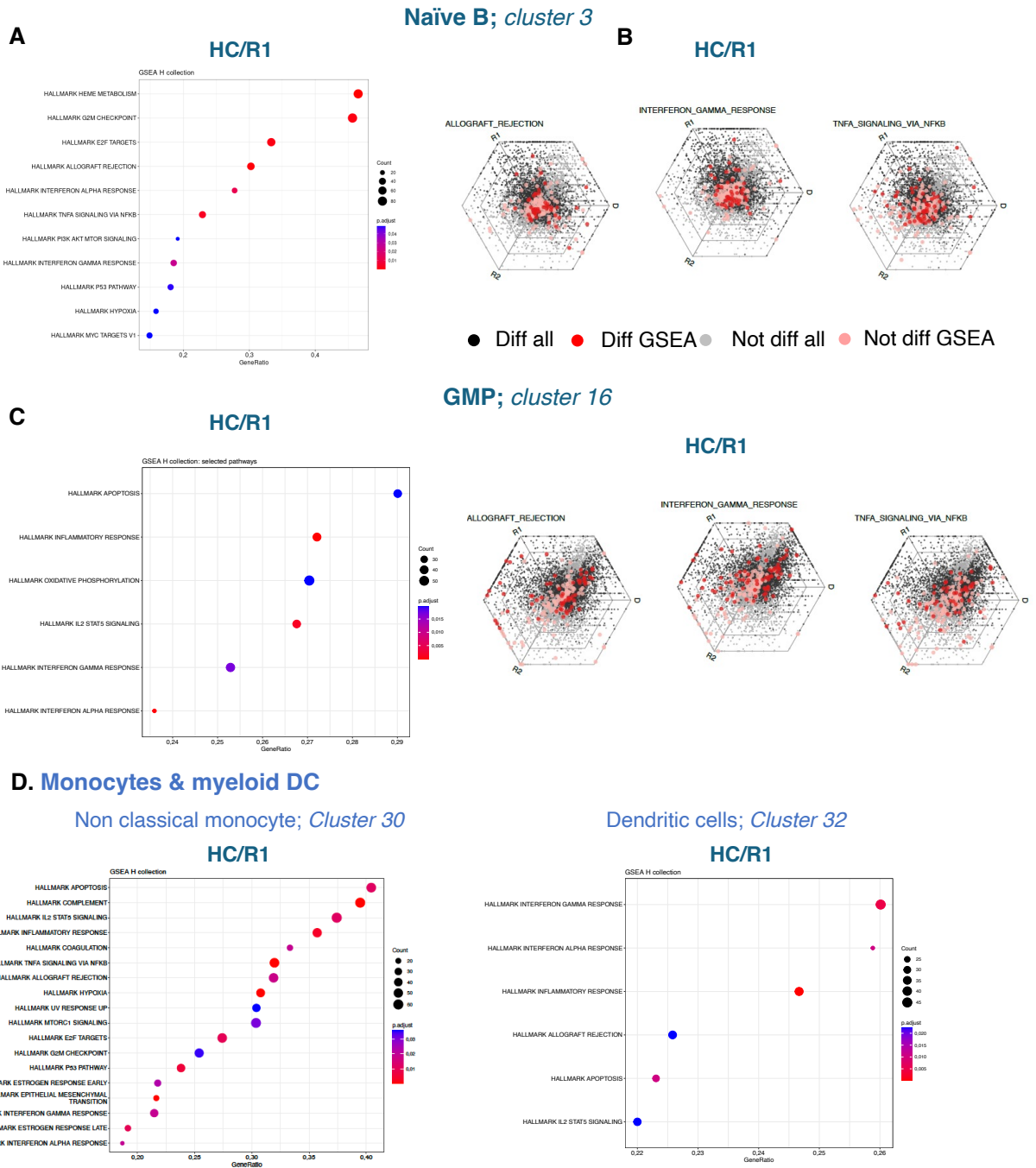


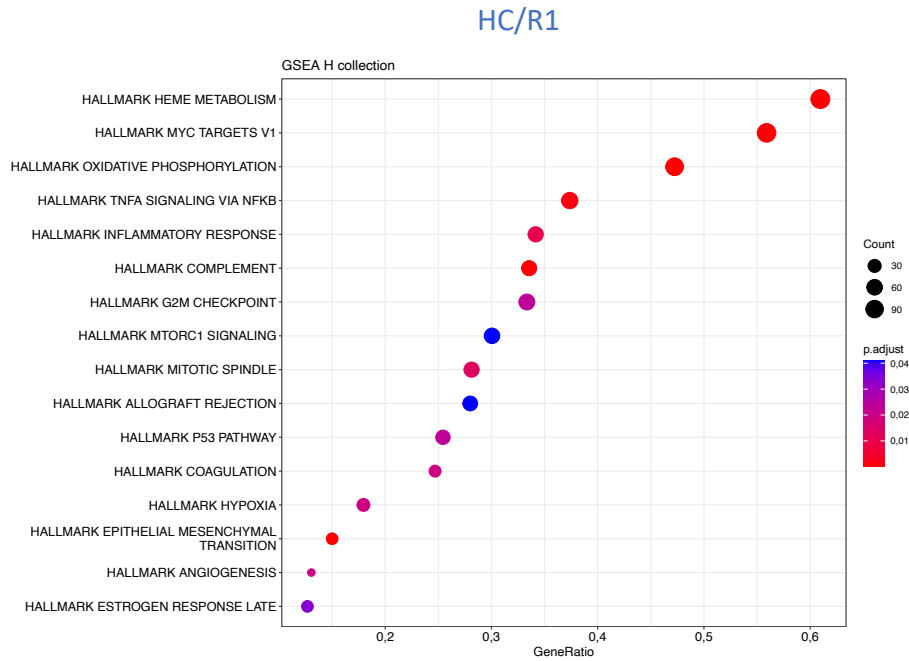
Figure S11: Bone marrow B-cells and myeloid progenitors.



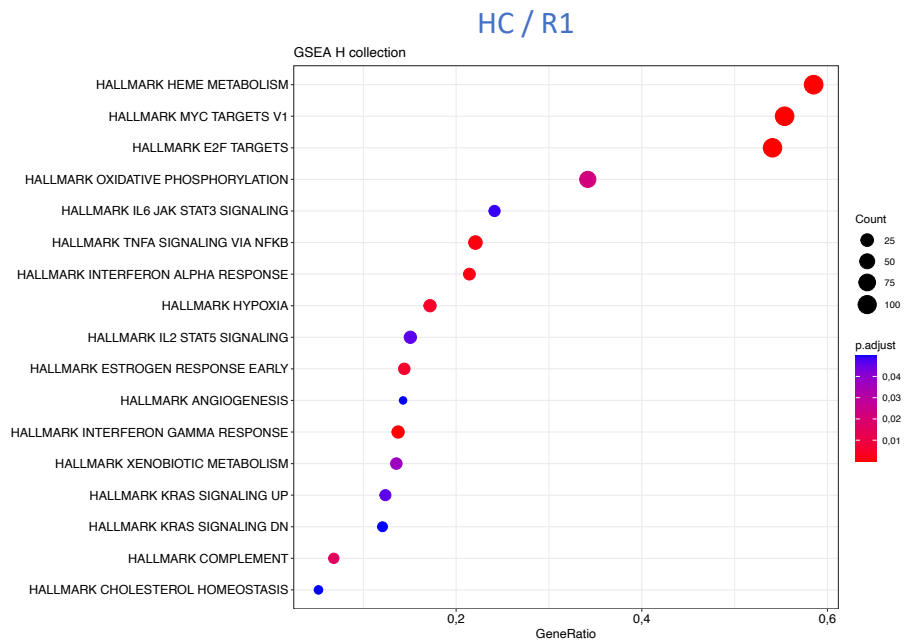
Legend to Supp Figure S11. (A) Functional enrichment analysis of annotated genes using hallmark collection. GSEA analysis based on logFC from limma-trend analysis on scran normalized data. Figures show significant (Benjamini-Hochberg, adjusted p-values <5%) functional enrichment in biological states or processes analysis in naïve B-cell (A), granulo-monocytic progenitors (GMP) (C) and non-classical monocytes & myeloid DC2 (D). (B) Hexagonal 'Triwise' plots displaying all arrayed genes for control (HC), recipient of group 1 (R1) with differentially expressed genes in HC/R1 (BJH adj. p-value < 0.05), depicted as a bigger red bullet for genes from GSEA Hallmarks sets (left: Allograft Rejection, middle: Interferon Gamma Response, right: TNFA Signaling via NFKB) and as a black smaller bullet for others. Non-differentially expressed genes are displayed with lower color saturation. Each grid line represents expression level on the log2 scale. HC/R1 hexagonal plots are displayed in naïve B-cells and GMP.

Figure S12: Bone marrow Erythroid and megakaryocytic progenitors.

CD34+ early ERP, Cluster 8



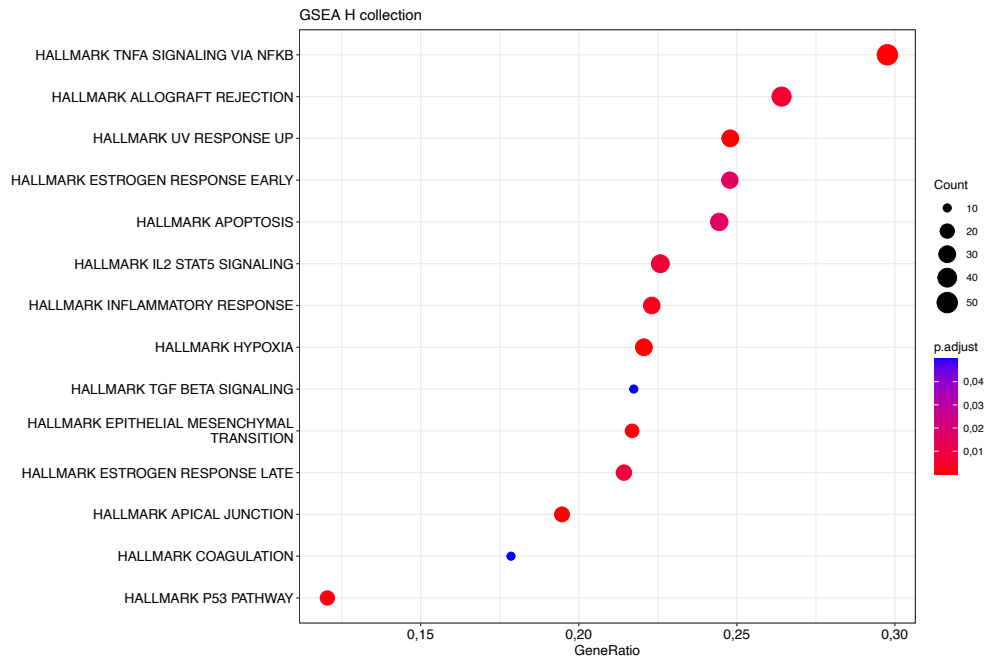
Erythroblast, cluster 14



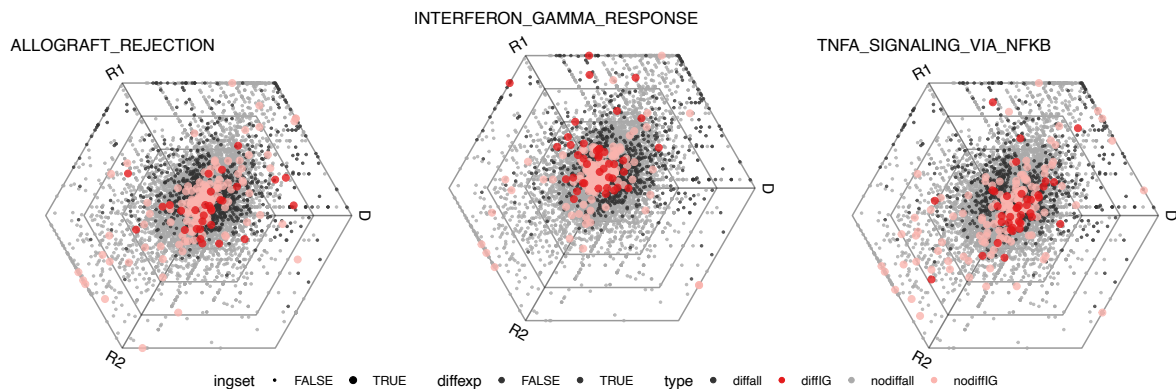
Legend to Supp Figure S12. Functional enrichment analysis of annotated genes using hallmark collection. GSEA analysis based on logFC from limma-trend analysis on scran normalized data. Figures show significant (Benjamini-Hochberg, adjusted p-values <0.05) functional enrichment in biological states or processes analysis in Erythroid and megakaryocytic progenitors (ERP) and in erythroblast between healthy control (HC) and recipient of group 1 (R1) patients.

Figure S13: Bone marrow NK-cells.

NKT, Cluster 11



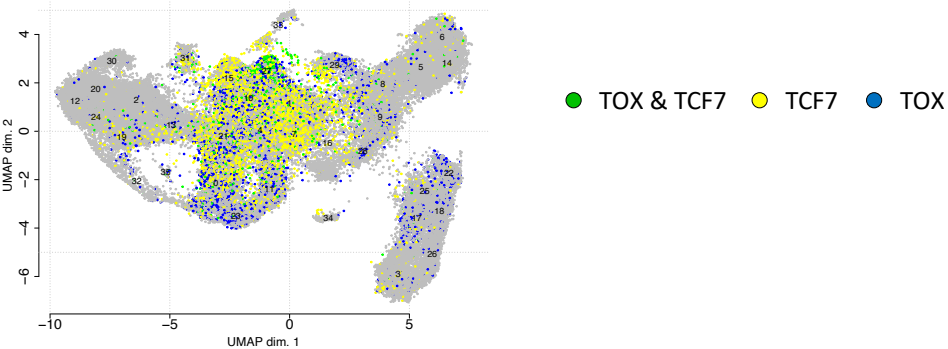
NK, Cluster 0



Legend to Supp Figure S13. Functional enrichment analysis of annotated genes using hallmark collection. GSEA analysis based on log-FC from limma-trend analysis on scrn normalized data. Figures show significant (Benjamini-Hochberg, adjusted p-values <0.05) functional enrichment in biological states or processes analysis in NKT cells clusters between control and recipient of group 1 (R1) patients. Hexagonal 'Triwise' plots displaying all arrayed genes for control (HC), recipient of group 1 (R1) with differentially expressed genes in HC/R1 (BJH adj. p-value < 0.05), depicted as a bigger red bullet for genes form GSEA Hallmarks sets (left: Allograft Rejection, middle: Interferon Gamma Response, right: TNFA Signaling via NFKB) and as a black smaller bullet for others. Non-differentially expressed genes are displayed with lower color saturation. Each grid line represents expression level on the log2 scale. HC/R1 hexagonal plot is displayed in NK cells (cluster 0).

Figure S14: Exhaustion and Cytokines.

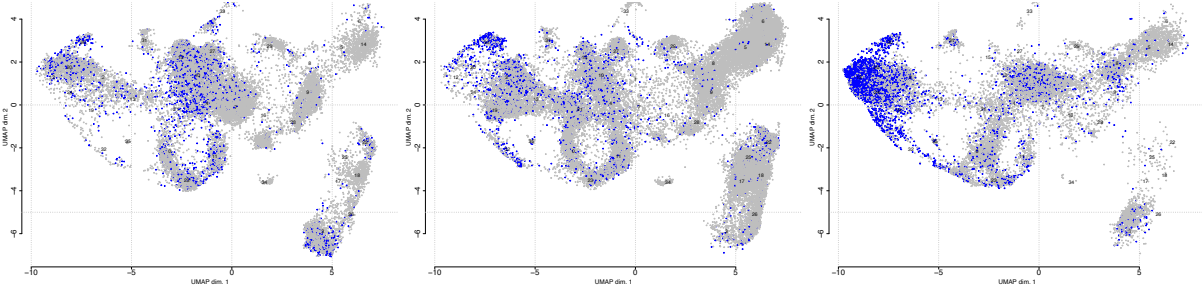
A



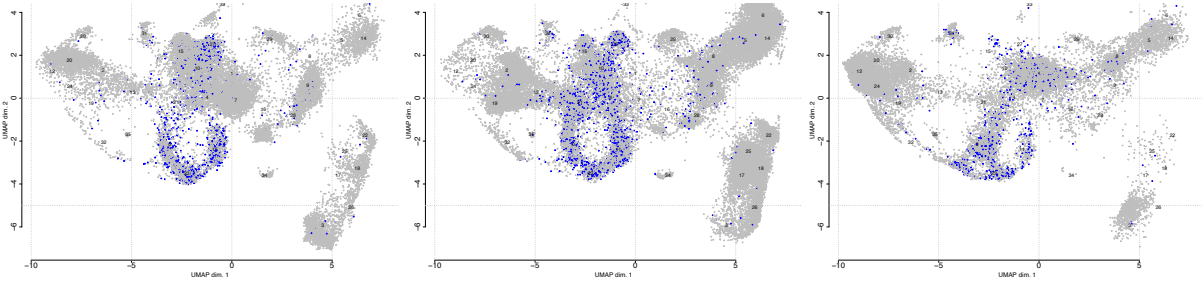
B

Healthy control R1 R2

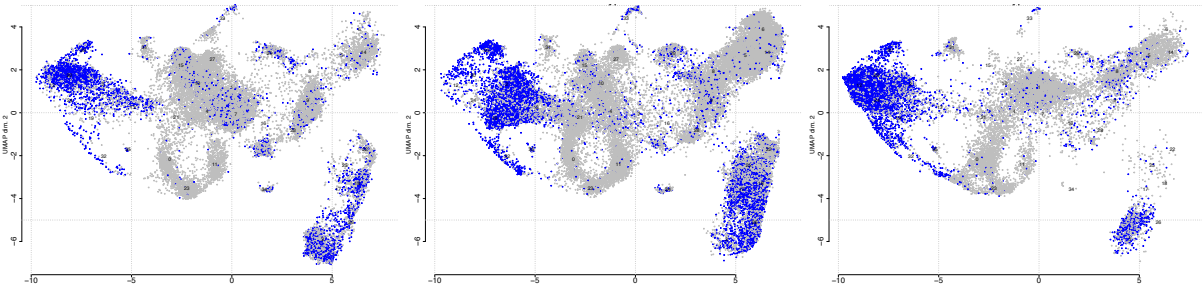
TNF- α



Interferon- γ



Interferon- γ -R2



A. Expression of the TF TOX (blue), TC7 (yellow) or both (green) projected on the original UMAP.
B. Expression of selected mRNAs highlighted in blue on the UMAP from Fig.5A between healthy control, recipient of group 1 (R1) and recipient of group 2 (R2) patients.

Figure S15: GSEA hallmarks' dot plot summary comparing healthy controls to recipients R1.

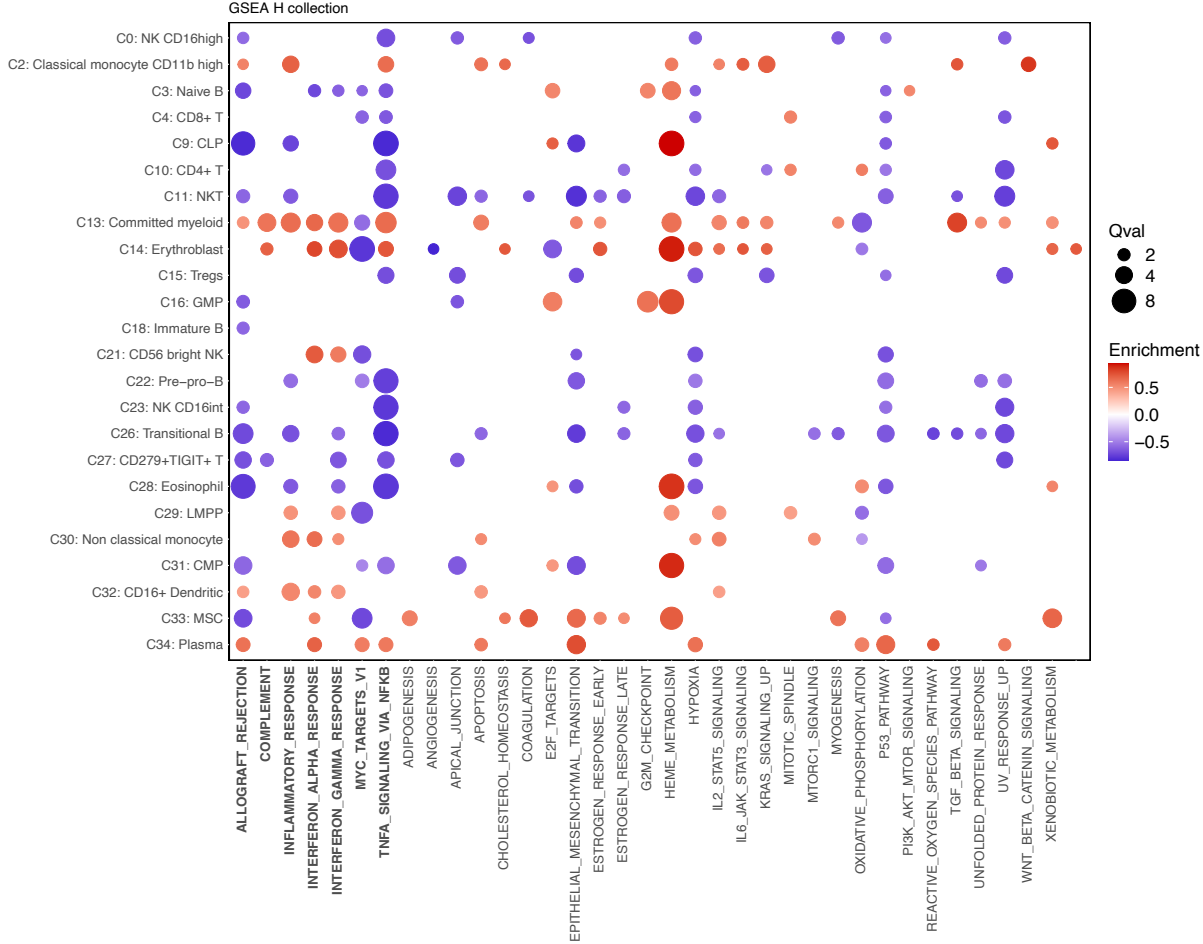
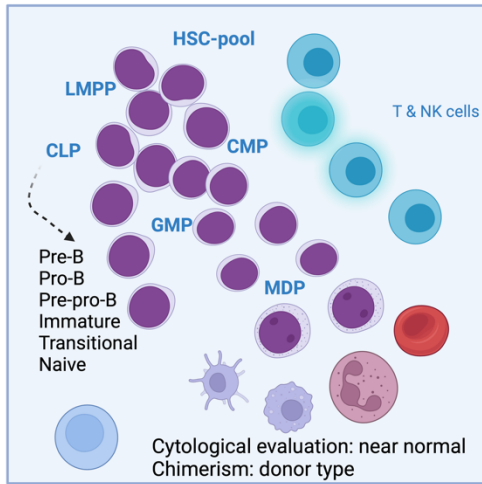


Figure S16: Overall summary of experimental results.

Bone marrow evaluation; 3 months post-HSCT



CITEseq phenotyping

Early Lymphoid & Myeloid progenitors

- Strongly decreased in R2
- Early B-cell progenitors
 - CLP, LMPP & GMP

T, NK & NKT

activated & inflammatory profiles

CYTOF

B-cell progenitors

- Decreased pre-pro B
- Decreased pre-B
- Decreased immature
- Normal BCR rearrangement but oligoclonality

Myeloid progenitors (in CD15- BMNC)

- Decreased HSC-progenitors
- Decreased LMPP
- Decreased CMP
- Decreased GMP

Bone marrow T-cell subsets

- Decreased T-cells
- Strongest decrease in naïve and memory CD4 subsets & naïve CD8
- Exhausted phenotype in naïve and TCM CD4

Variations relative to healthy controls

Normal mature CD11b+CD15+

CITEseq

35 Clusters encompassing all bone marrow cellular subsets
Cluster numbers & abundance were significantly different in R2, R1, & HC

CITEseq transcriptomic aspects (hallmarks)

	HC/R1			HC/R2			R1/R2		
	CD4	CD8	NKcd56H	DC2	CMP	Mono	DCs	CMP	CD8
Allograft rejection	X			X	X	X	X	X	X
Complement	X			X	X	X	X	X	X
Inflammation				X	X	X	X	X	X
INFa resp			X	X	X	X		X	X
INFg resp	X		X		X	X	X	X	X
Myc targets	X	X			X	X		X	X
TNFa signaling	X	X		X	X	X	X	X	X