SUPPLEMENTAL INFORMATION

Supplemental Table 1: Marker genes used to classify individual cells to major cell types.

Major Cell Type	Markers
T cell	CD3E, CD3G, CD3D
NK cell	NCAM1
AML blast	ELANE, PRAME, CSF3R, ARMH1, ITM2C, EGFL7, C1QTNF4, MGST1, MPO, PRSS57, AZU1, CLEC11A
Normal myeloid cell	LYZ, AIF1, TYROBP
B/Plasma cell	MS4A1, CD79A, CD79B, IGHM, BANK1, CD37

Supplemental Table 2: Clinical information for patients 1-4.

Patient ID	Age at Initial Diagnosis (In Years)	Gender	Vital Status	Cytogenetics & Molecular Genetics Results at Diagnosis	Active Issues at Time of Diagnosis	Type of HCT Received	Conditioning Regimen	Time from Transplant to Relapse (In Months)	Chimerism at Time of Relapse (Donor %)	Immuno-suppression at Time of Relapse	Cytogenetics & Molecular Genetics Results at Relapse	Active Issues at Time of Relapse	Second HCT Information	Host CMV IgG Status	Donor CMV IgG Status
UPN1	17.2	Female	Alive	FLT3 ITD, NRAS (NM_002524) exon2 p.G12A (c.35G>C)	Axillary abscess (on clindamycin)	Unmodified 10/10 HLA-identical sister in CR1	Clofarabine, fludarabine, busulfan	7	BM: 29% bulk BM PBL: 87% total circ lymphs, 98% myeloid/neutrophil, 89% T, 98% B, 95% NK	Tacrolimus	CEBPA (NM_004364) exon1 p.K304Sfs*14 (c.911delA), TP53 (NM_000546) exon5 p.R181H (c.542G>A)	No active issues; no hx GVHD	Unmodified 4/6 HLA-matched UCBT, conditioning w/ cyclophosphamide, fludarabine, thiotepa, TBI in CR2	Negative	Negative
UPN2	14.5	Female	Alive	FLT3 ITD	Parainfluenza, CMV IgM positive	Unmodified 10/10 HLA-identical brother BMT in CR1	Busulfan, melphalan	30	BM: 35% bulk BM	None	TET2 (NM_001127208) exon4 p.E1144K (c.3430G>A)	Coronavirus; hx duodenal GVHD (resolved; off tx)	Unmodified 4/6 HLA-matched double UCBT, conditioning w/ clofarabine, melphalan, thiotepa in CR2	Positive	Positive
UPN3	6.0	Male	Dead	RUNX1-RUNX1T1 (AML1-ETO), IDH1 exon 4 p.R132C, KIT exon17 p.N822K	No active issues/ infections	Unmodified 5/6 HLA-matched single female UCBT in CR2	Clofarabine, melphalan, thiotepa	7	PBL: 85% total circ lymphs, 98% myeloid/neutrophil, 100% T, 100% B, 26% NK	MMF, CSA, prednisolone	RUNX1-RUNX1T1 (AML1-ETO), ASXL1 exon12 p.W898*, CEBPA exon1 pH195_P196dup, IDH1 exon4 p.R132C, KIT exon17 p.N822K	GI GVHD flare 4 months prior, actively tapering steroids; stool adeno +; BK virus +	N/A	Negative	Positive
UPN4	20.3	Female	Dead	MLL rearranged, FLT3-ITD, 5q-	No active issues/ infections	Double 4/6 UCBT + CliniMACS CD34+PBSCT from haplo mismatched father in CR1	Fludarabine, thiotepa, cyclophosphamide, TBI	7	BM: 88% bulk BM	Prednisone	FLT3-ITD negative, MLL rearranged	Active C. diff infection; leukemia cutis; breast chloromas; no hx GVHD	N/A	Positive	CBU1: Positive, CBU2: Negative Haplo Donor: Positive

BM: bone marrow, BMT: bone marrow transplant, CBU: cord blood unit, CMV: cytomegalovirus, CR1: complete remission 1, CR2: complete remission 2, CSA: cyclosporine, GI: gastrointestinal, GVHD: graft-versus-host disease, hx: history, MMF: mycophenolate mofetil, PBL: peripheral blood leukocytes (bulk), PBSCT: peripheral blood stem cell transplant, TBI: total body irradiation, tx: treatment, UCBT: umbilical cord blood transplant

Supplemental Table 3: Change in each AML blast subtype per patient from diagnosis to post-transplant relapse evaluated by Fisher's exact test.

	Patient	Blast Subtype	Cells Diagnosis	Cells Relapse	Blasts Diagnosis	Blasts Relapse	P-Value
0	UPN4	GMP-like	4224	655	1008	105	5.01912683501101E-06
1	UPN4	ProMono-like	4224	655	1044	327	3.36885562127375E-37
2	UPN4	cDC-like	4224	655	759	15	2.13621714716878E-33
3	UPN4	Prog-like	4224	655	536	179	6.26327408678716E-20
4	UPN4	Mono-like	4224	655	809	19	4.10145878418932E-33
5	UPN4	HSC-like	4224	655	68	10	1
6	UPN2	GMP-like	834	1279	79	105	0.343585285
7	UPN2	ProMono-like	834	1279	200	102	3.54286974318953E-24
8	UPN2	cDC-like	834	1279	15	87	3.46006135720216E-08
9	UPN2	Prog-like	834	1279	432	314	3.19566565747533E-37
10	UPN2	Mono-like	834	1279	77	524	3.58598835955242E-62
11	UPN2	HSC-like	834	1279	31	147	6.76707780748383E-11
12	UPN3	GMP-like	3	218	0	69	0.553797693
13	UPN3	ProMono-like	3	218	0	33	1
14	UPN3	cDC-like	3	218	0	3	1
15	UPN3	Prog-like	3	218	2	76	0.284850927
16	UPN3	Mono-like	3	218	1	33	0.395679663
17	UPN3	HSC-like	3	218	0	4	1
18	UPN1	GMP-like	2244	274	399	57	0.213806602
19	UPN1	ProMono-like	2244	274	603	68	0.514871973
20	UPN1	cDC-like	2244	274	46	4	0.649592806

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21	UPN1	Prog-like	2244	274	1128	120	0.047179394
22	UPN1	Mono-like	2244	274	43	10	0.071554087
23	UPN1	HSC-like	2244	274	25	15	7.888840239899 91E-06

Supplemental Table 4: Gene markers used to define T and NK cell subtypes.

Cell Type	Gene Markers
NK Activated	NKG7, TNFSF10, FASLG, TNF, IFNG, GZMB, PRF1
NK Exhausted	PDCD1, HAVCR2, TIGIT
Naive T	CCR7, LEF1, SELL, TCF7
Activated CD8 (ISG)	CD8B, LAMP3, CMPK2, IFIT3, MX1, RSAD2, IFI44L, IFIT1
CD8 Memory	CRTAM, CD8B, CD8A
CD8 Exhausted (MHCII)	PDCD1, DTHD1, HLA-DRB1, IFNG, HLA-DMA, ARHGAP18, GZMK, TIGIT, HLA-DQA1, TOX, HLA-DRA, HLA-DQB1, CCL3, F2R
Activated CD8 (MHCII)	EFHD2, ARAP2, HLA-DRB5, ZBP1, SYTL2, MCOLN2, ENC1, DNAJC1, VAV3, OASL, KLRD1, PTMS, HOPX, GBP5, TBX21, TGFBR3, FOSL2, LAG3, ACTN4, HLA-DPA1, HLA-DPB1, MYO1F, STOM, PTPRE, PRF1, CYTOR, GZMA
T Regulatory	FOXP3, CTLA4, IL2RA, FANK1
T Helper	KLRB1, CTSH, IL26, IL23R, RORC, CD200, PTPN13, BTLA, CXCL13, BCL6, CXCR5

Supplemental Table 5: Amino acid sequences of TCB clonotypes present in UPN 4.

| Amino Acid Sequence	Clone Number
CASSKTADTEAFF	Clonotype 1
CASSFGAGGSNQPQHF	Clonotype 2
CASSLTGGGLYEQYF	Clonotype 3
CASSGVLADYEQYF	Clonotype 4
CASSTRYGPPYEQYF	Clonotype 5
CASSVARLTGGNWGQPQHF	Clonotype 6
CASSLAGYSNQPQHF	Clonotype 7
CASSLHTDSYGYTF	Clonotype 8
CASSLASGAYGYTF	Clonotype 9
CASSQDSGGPDTQYF	Clonotype 10
CAISGTRYNEQFF	Clonotype 11
CAISESGTGRSYEQYF	Clonotype 12
CASSPGTSGGVDNEQFF	Clonotype 13
CSARDRGRTEAFF	Clonotype 14
CASSLGTNSPLHF	Clonotype 15
CASRDRGRLYGYTF	Clonotype 16
CASSSGHYEQYF	Clonotype 17

**Supplemental Figure 1: Experimental schema.**
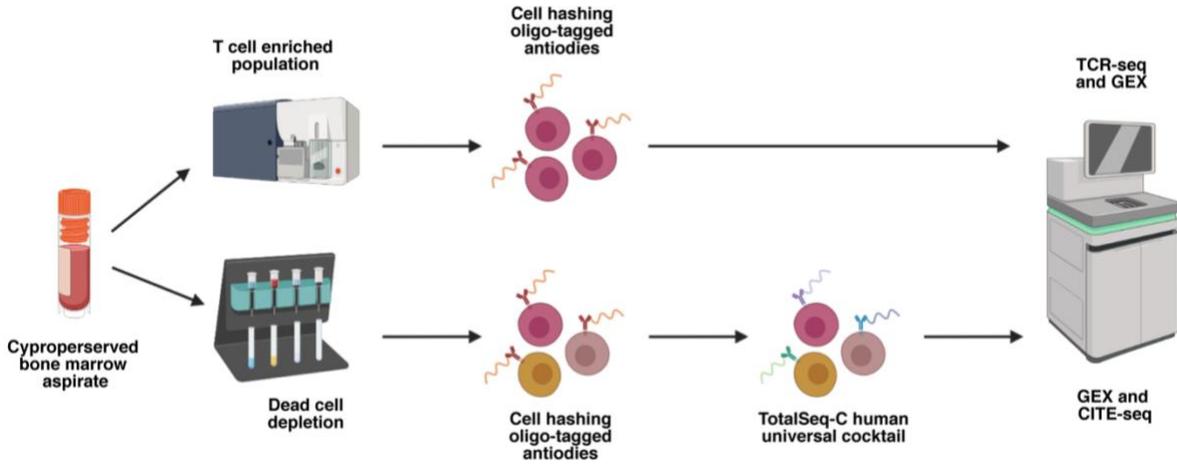
Supplemental Figure 2: Sequencing modalities obtained for individual patient samples.
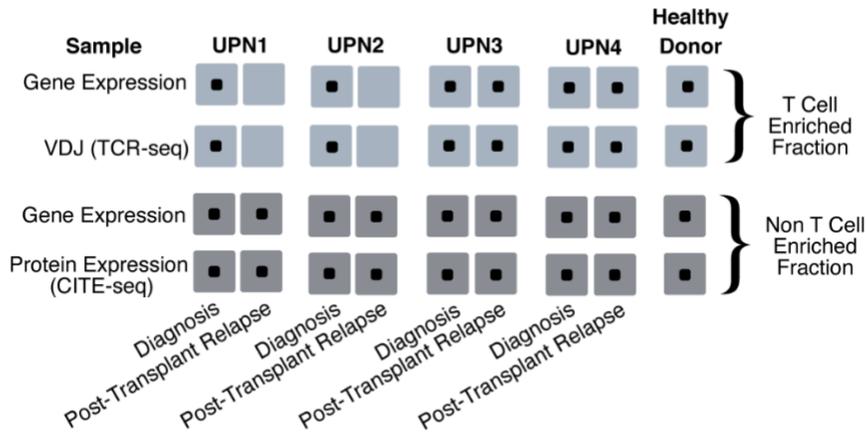
Supplemental Figure 3: Heatmap showing manual validation of 5 transcriptionally distinct cell clusters/broad hematopoietic cell types using canonical gene (A) and surface (B) markers obtained by CITEseq analysis.
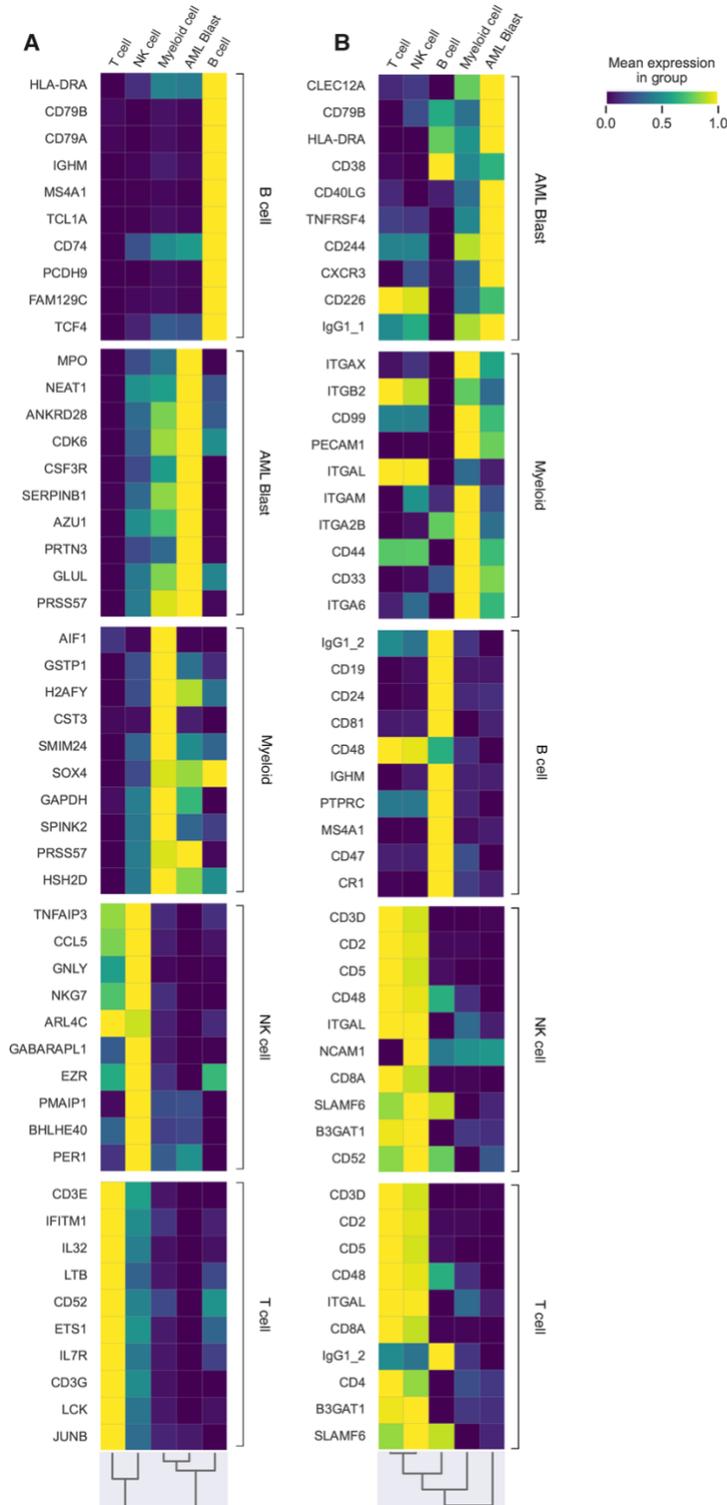
Supplemental Figure 4: Probabilistic cell type assignment reclassified per patient (A) and timepoint (B) showing that each myeloid cluster 1-4 (C) contains known myeloid/AML markers (D-E) and appear to be patient-specific.

A.

B.

C.

leiden
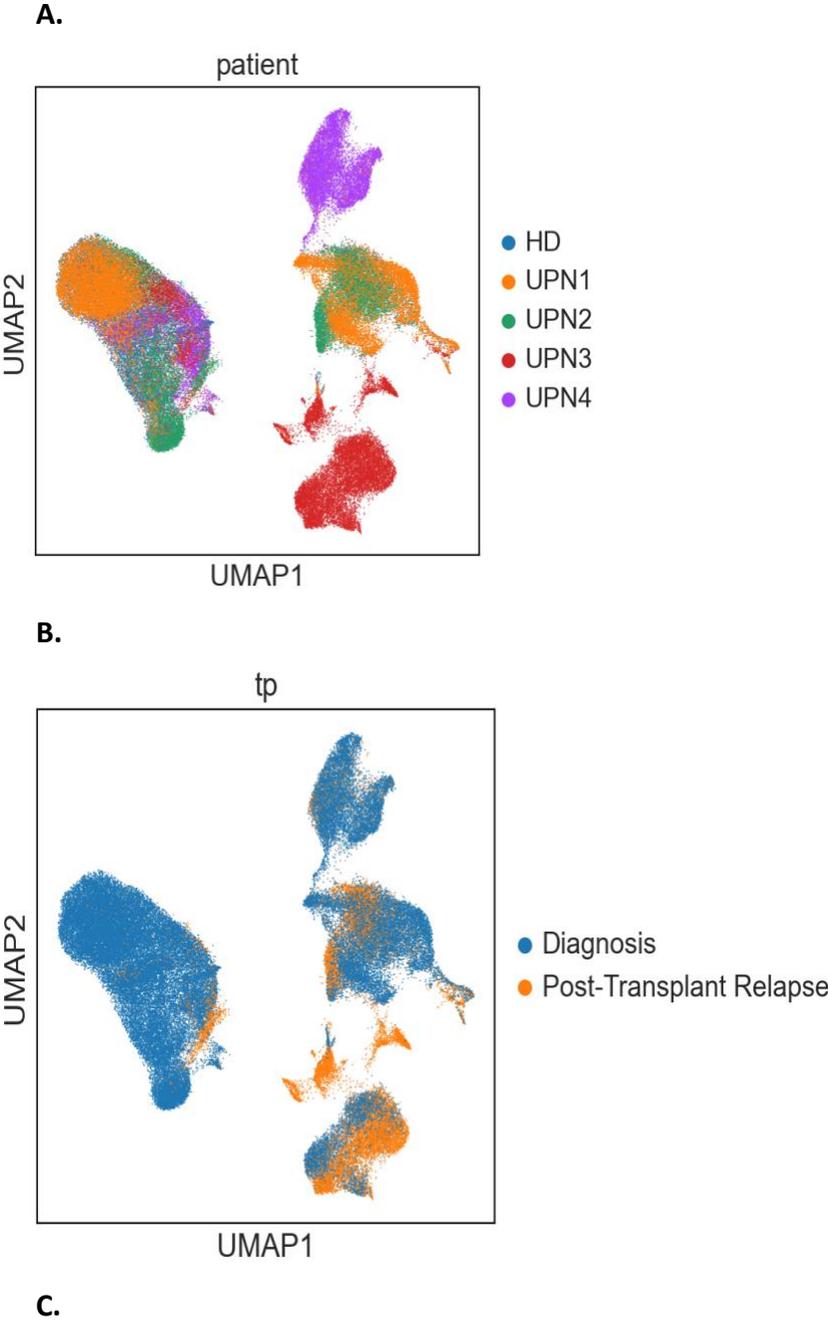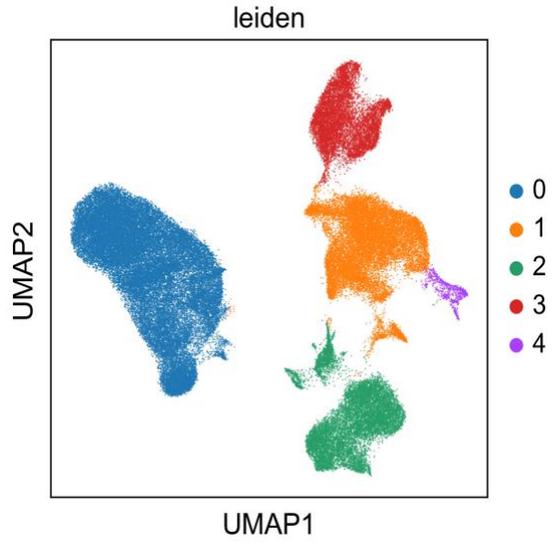
D.

E.
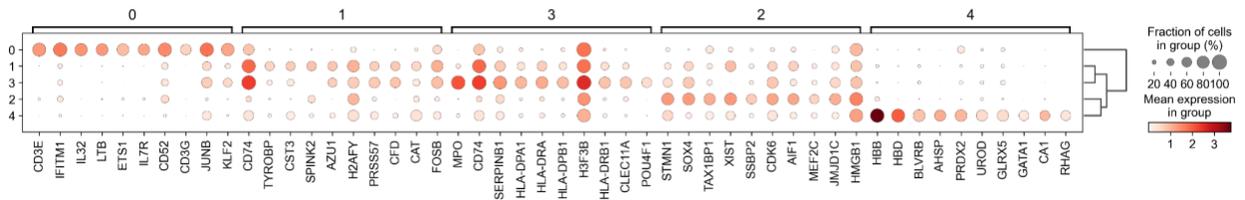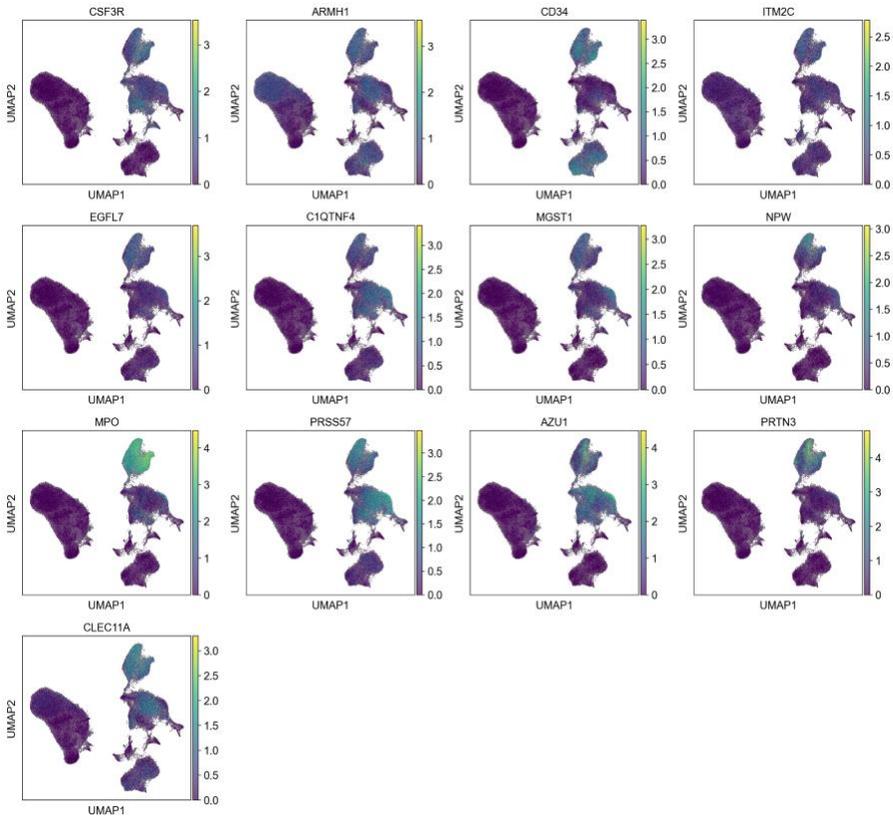
Supplemental Figure 5: Heatmap showing MHC class I and II gene expression changes in AML blasts per patient between diagnosis and post-transplant relapse using CITEseq.
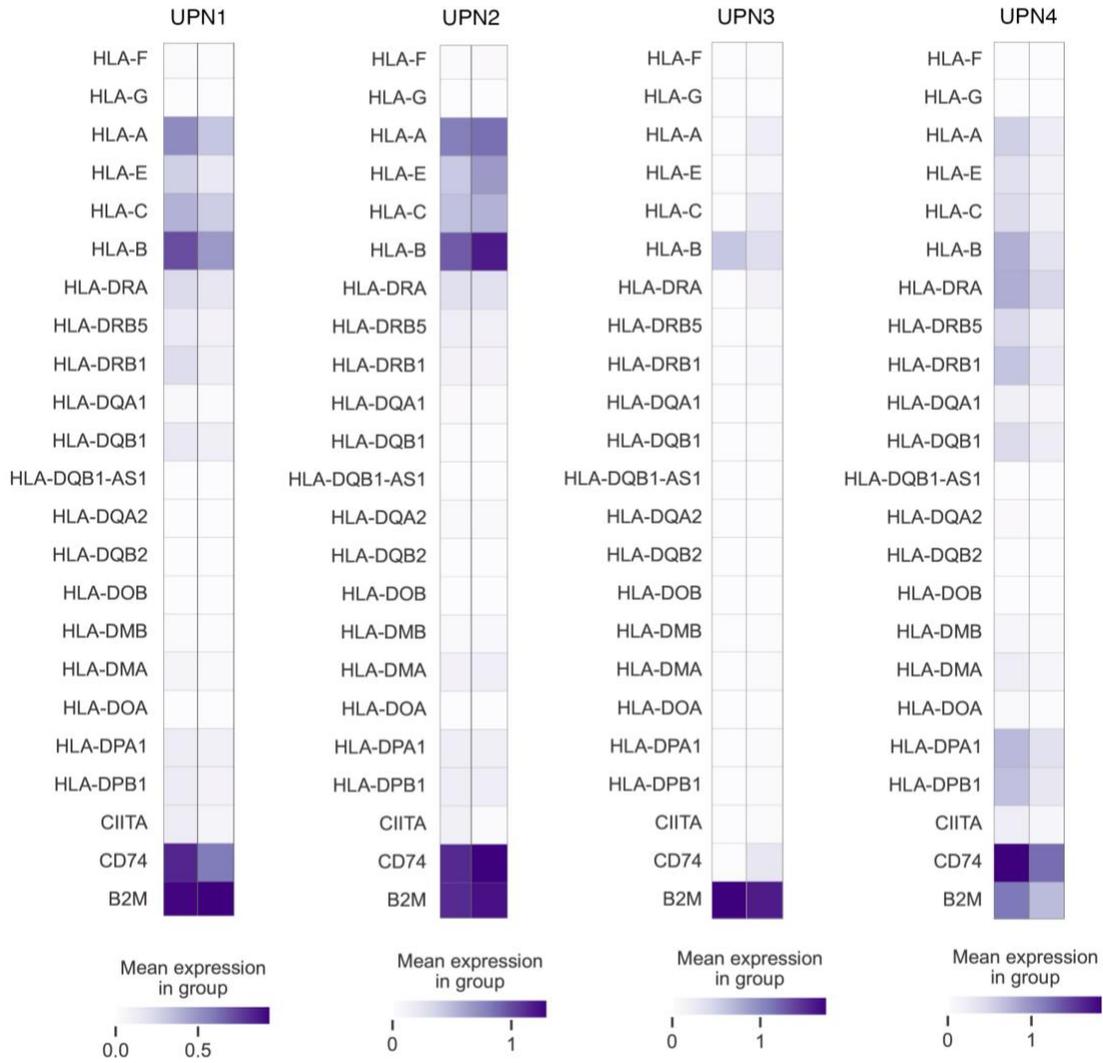
Supplemental Figure 6: Heatmap showing MHC class I and II protein expression changes using CITE-seq between pediatric AML diagnosis and post-transplant relapse for UPN1-4.
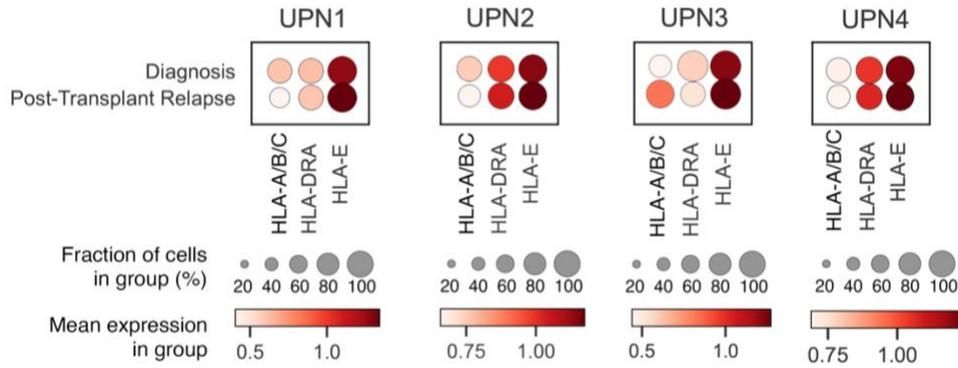

Supplemental Figure 7: Clinical flow cytometric data of HLA-DR expression for UPN1 at diagnosis (A) and post-transplant relapse (B).
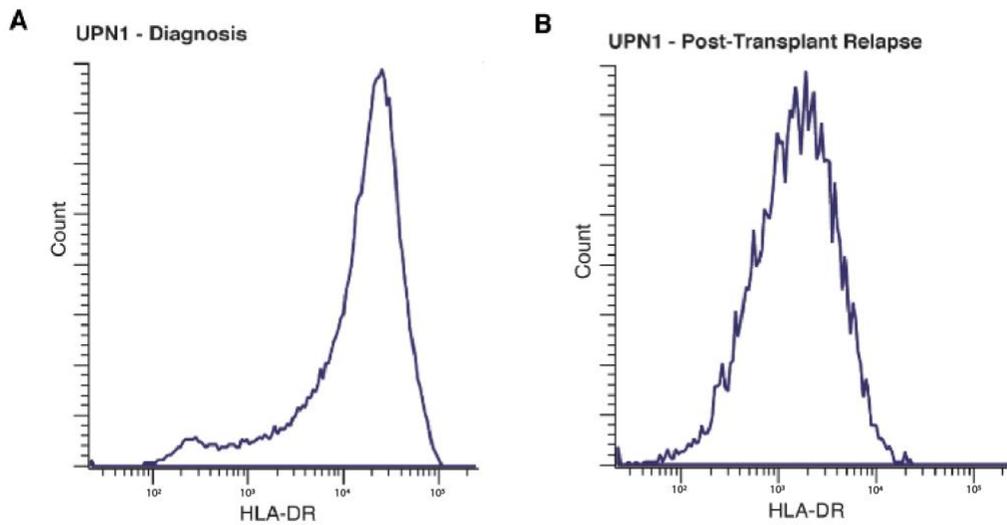
Supplemental Figure 8: Heatmap showing NKG2D ligand gene expression changes between pediatric AML diagnosis and post-transplant relapse aggregated for UPN1-4. * indicates P-value <0.05.
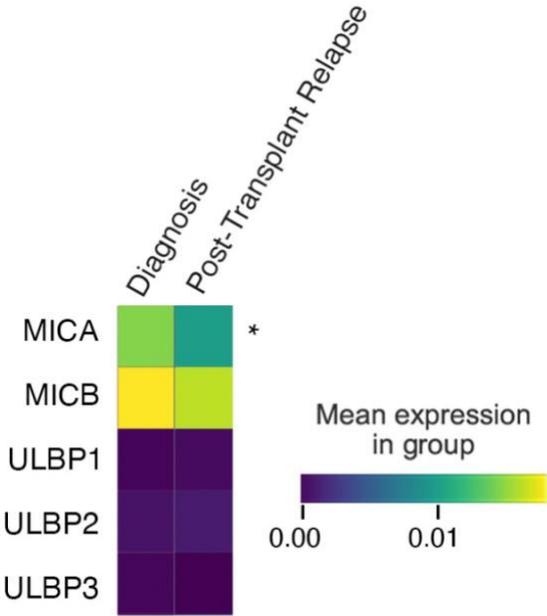

Supplemental Figure 9: Number of AML blasts in bone marrow aspirate samples per patient.
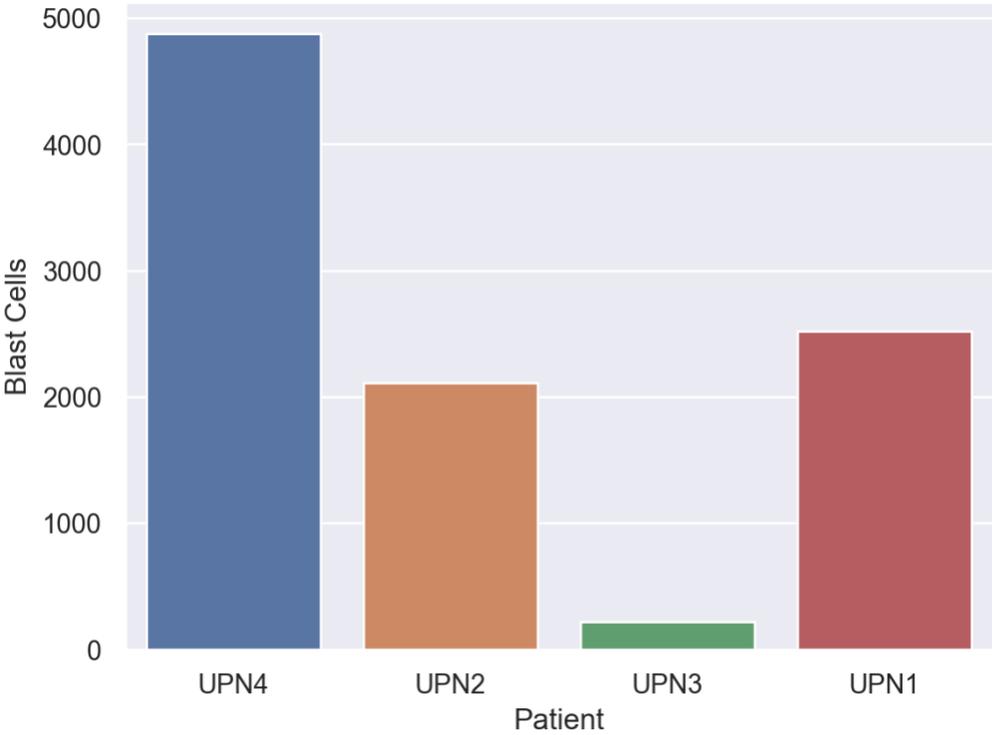
**Supplemental Figure 10: ELANE, MPO, and AZU1 gene expression in AML blasts as compared to healthy myeloid cells. *** indicates P-value <0.001.**
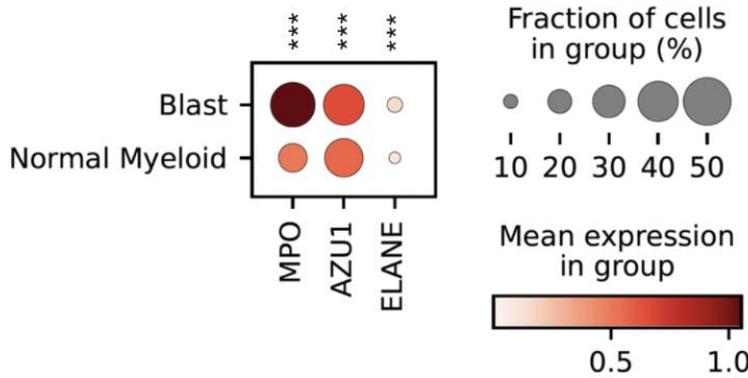

Supplemental Figure 11: Heatmap showing gene expression level for MHC-II, upstream MHC-II activators, and upstream MHC-II repressors for each category of AML blast subtype (HSC-like, progenitor-like, GMP-like, promonocyte-like, monocyte-like, and cDC-like).
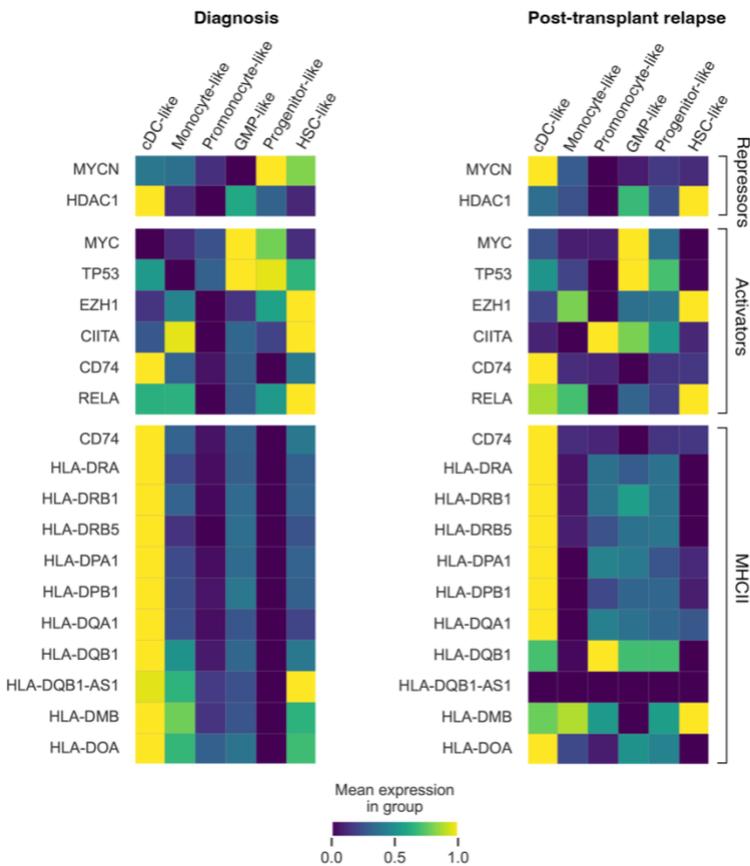
Supplemental Figure 12: Single-cell bone marrow immune microenvironment of healthy donor.
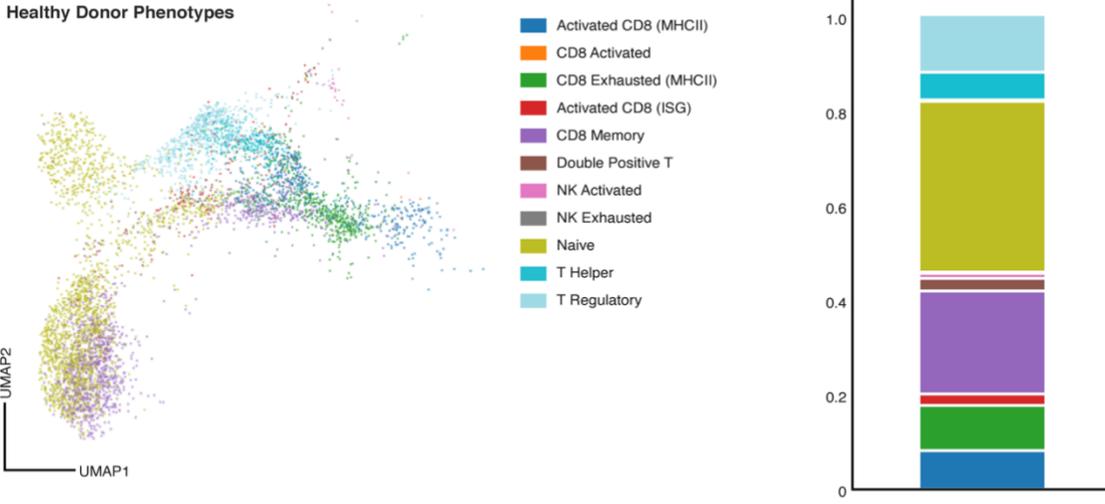
Supplemental Figure 13: Single-cell bone marrow immune microenvironment of UPN1-4.
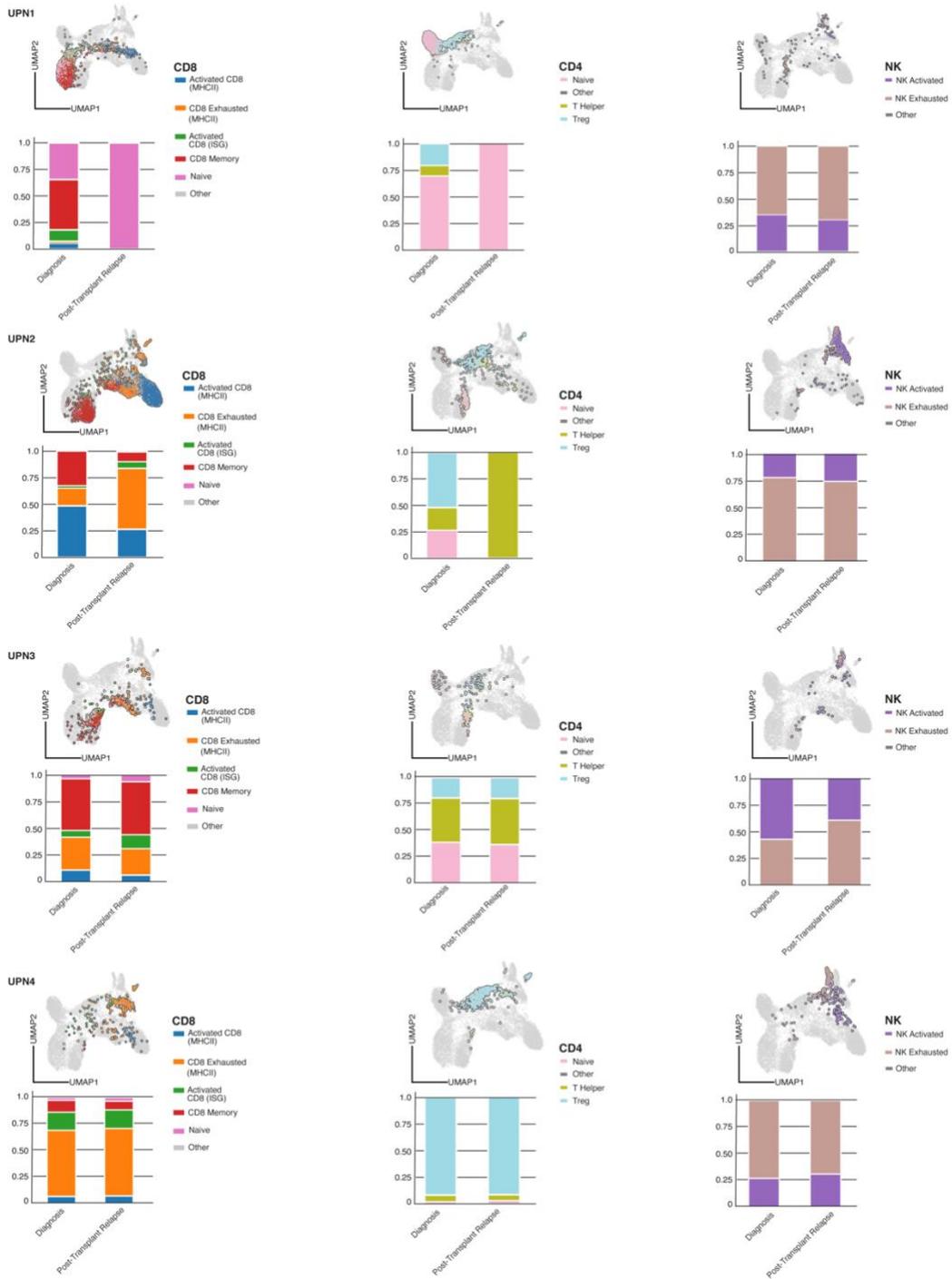
Supplemental Figure 14: Histogram of generation probabilities of public TCR clones.