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Supplementary Materials for

Single-cell genotypic and phenotypic analysis of measurable residual disease in acute myeloid leukemia

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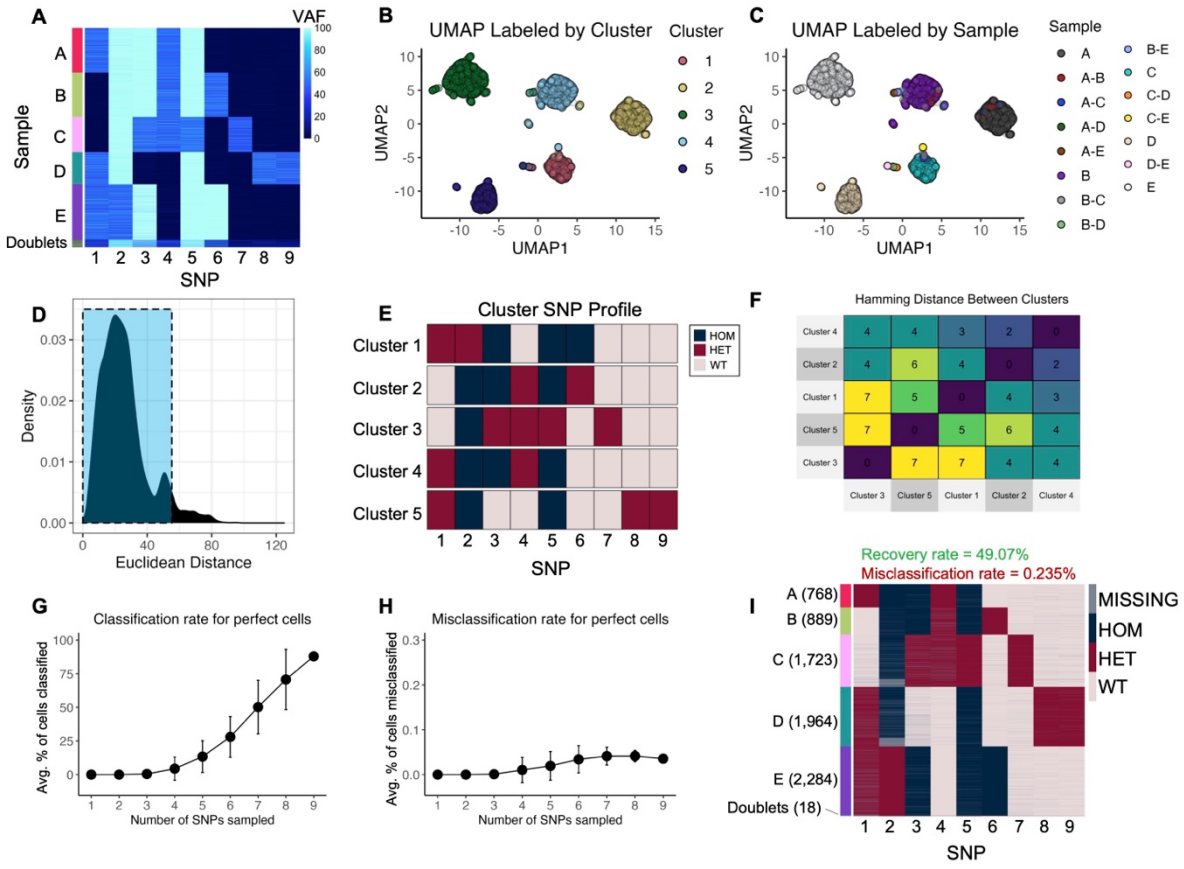
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

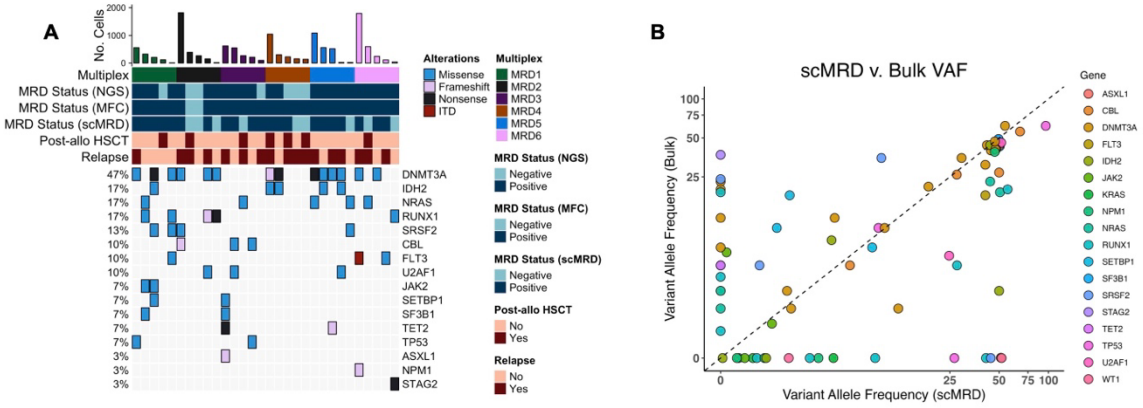
Supplementary Figures

Figure S1



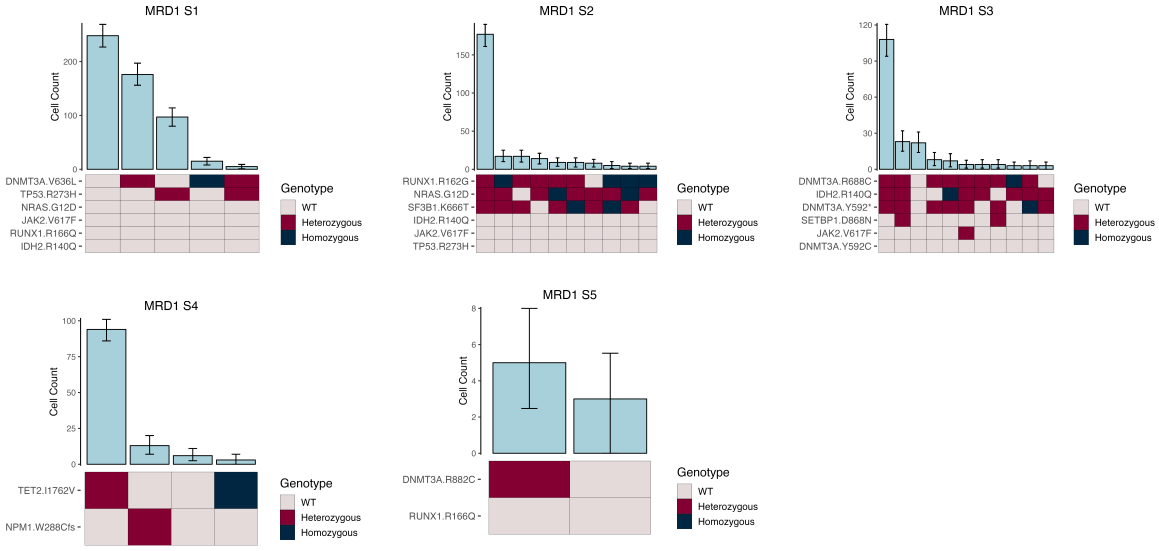
Supplementary Figure S1. Synthetic multiplexing of patient AML samples. **A.** Heatmap of SNP allele frequencies in a subset of cells with complete genotyping information for the top 9 SNPs used for demultiplexing. **B.** UMAP plot showing the results of K-means clustering on SNP allele frequencies with k=5. **C**. UMAP plot labeled by sample, showing the partitioning of most singlets and doublets into clusters in UMAP space. **D**. Distribution of Euclidean distances from cells to their respective cluster centers. **E**. Most common SNP profile for each of the 5 clusters after doublet exclusion. **F**. Heatmap showing Hamming distances between each cluster SNP profile within the synthetic mixing dataset. **G**. Classification rate and misclassification rate (**H**) when 1-9 SNPs are selected at random from cells with complete genotyping. After excluding repeat samples, the following number of sample replicates remained (n = 1 (9), n = 2 (26), n = 3 (39), n =4 (36), n=5 (42), n=6 (37), n=7(28), n=8 (9), n=9 (1). **I.** Heatmap showing cells classified for each sample in the synthetic mixing experiment.

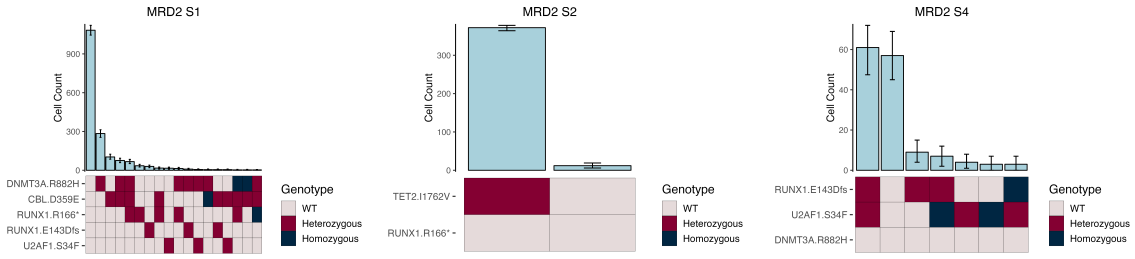
Figure S2



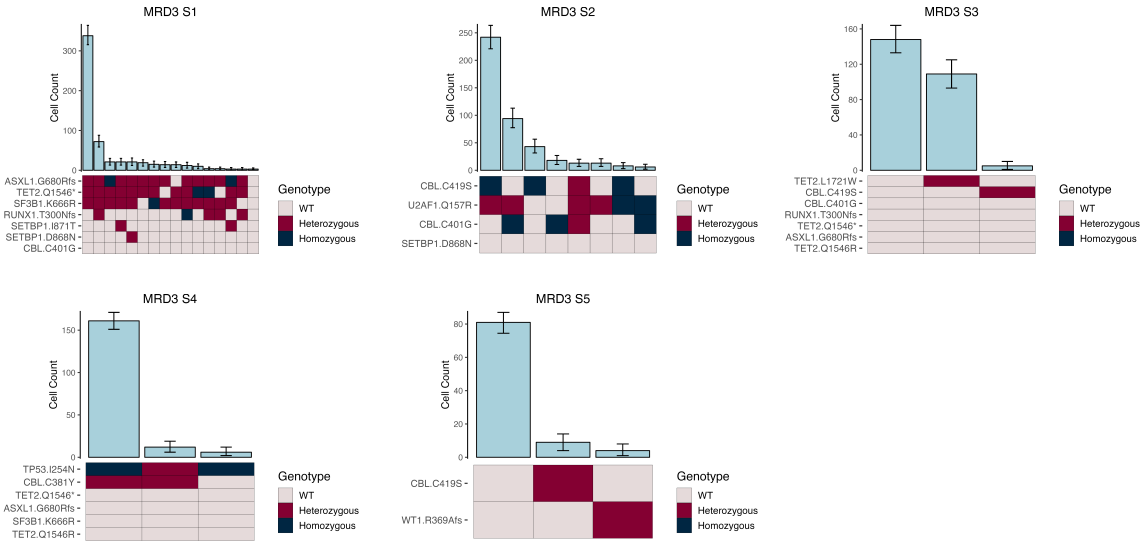
Supplementary Figure S2. Cohort overview and comparison of variant allele frequency (VAF) between sequencing methods. **A.** Oncoprint showing concordance of MRD detection by bulk NGS assay (sensitivity 2%), scMRD, and MFC (sensitivity 0.1%). Bar plot (top) represents the number of cells recovered after computational demultiplexing. Mutations represent those that were detected by bulk NGS at the remission timepoint and are covered by the custom scDNA panel. Post allo-HSCT represents the time of MRD assessment. Relapse represents patient outcomes after MRD assessment. **B.** Scatter plot showing mutation VAF as detected by scMRD (x-axis) or bulk NGS (y axis).

Figure S3


MRD1 S1
Cell Count
0
100
200
DNMT3A.V636L
TP53.R273H
NRAS.G12D
JAK2.V617F
RUNX1.R166Q
IDH2.R140Q
Genotype
WT
Heterozygous
Homozygous
MRD1 S2
Cell Count
0
50
100
150
RUNX1.R162G
NRAS.G12D
SF3B1.K666T
IDH2.R140Q
JAK2.V617F
TP53.R273H
Genotype
WT
Heterozygous
Homozygous
MRD1 S3
Cell Count
0
30
60
90
120
DNMT3A.R688C
IDH2.R140Q
DNMT3A.Y592*
SETBP1.D868N
JAK2.V617F
DNMT3A.Y592C
Genotype
WT
Heterozygous
Homozygous
MRD1 S4
Cell Count
0
25
50
75
100
TET2.I1762V
NPM1.W288Cfs
Genotype
WT
Heterozygous
Homozygous
MRD1 S5
Cell Count
0
2
4
6
8
DNMT3A.R882C
RUNX1.R166Q
Genotype
WT
Heterozygous
Homozygous



MRD2 S3 and S5 are MRD- by scMRD: can’t determine clonality


MRD3 S1
Cell Count
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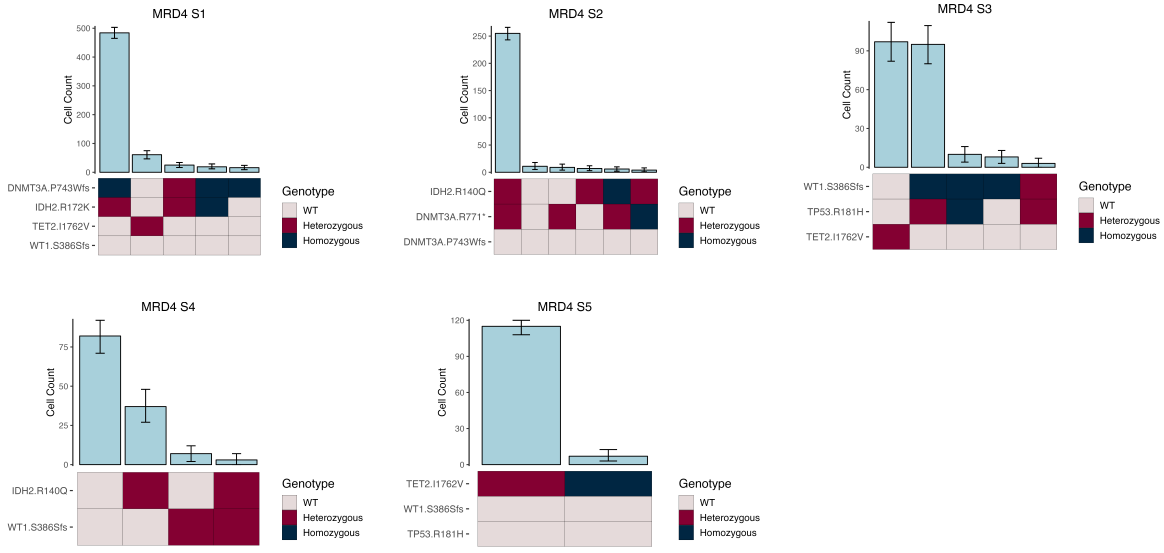
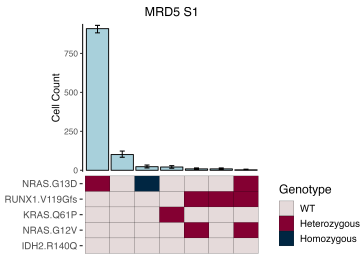
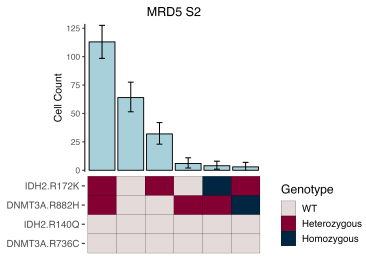
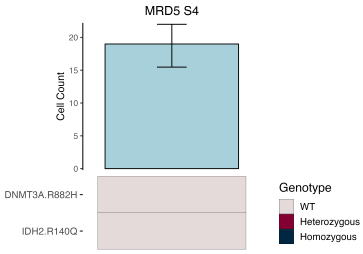
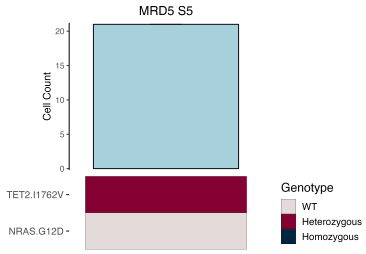
TET2.Q1546*
SF3B1.K666R
RUNX1.T300Nfs
SETBP1.I871T
SETBP1.D868N
CBL.C401G
Genotype
WT
Heterozygous
Homozygous
MRD3 S2
Cell Count
CBL.C419S
U2AF1.Q157R
CBL.C401G
SETBP1.D868N
Genotype
WT
Heterozygous
Homozygous
MRD3 S3
Cell Count
TET2.L1721W
CBL.C419S
CBL.C401G
RUNX1.T300Nfs
TET2.Q1546*
ASXL1.G680Rfs
TET2.Q1546R
Genotype
WT
Heterozygous
Homozygous
MRD3 S4
Cell Count
TP53.I254N
CBL.C381Y
TET2.Q1546*
ASXL1.G680Rfs
SF3B1.K666R
TET2.Q1546R
Genotype
WT
Heterozygous
Homozygous
MRD3 S5
Cell Count
CBL.C419S
WT1.R369Afs
Genotype
WT
Heterozygous
Homozygous

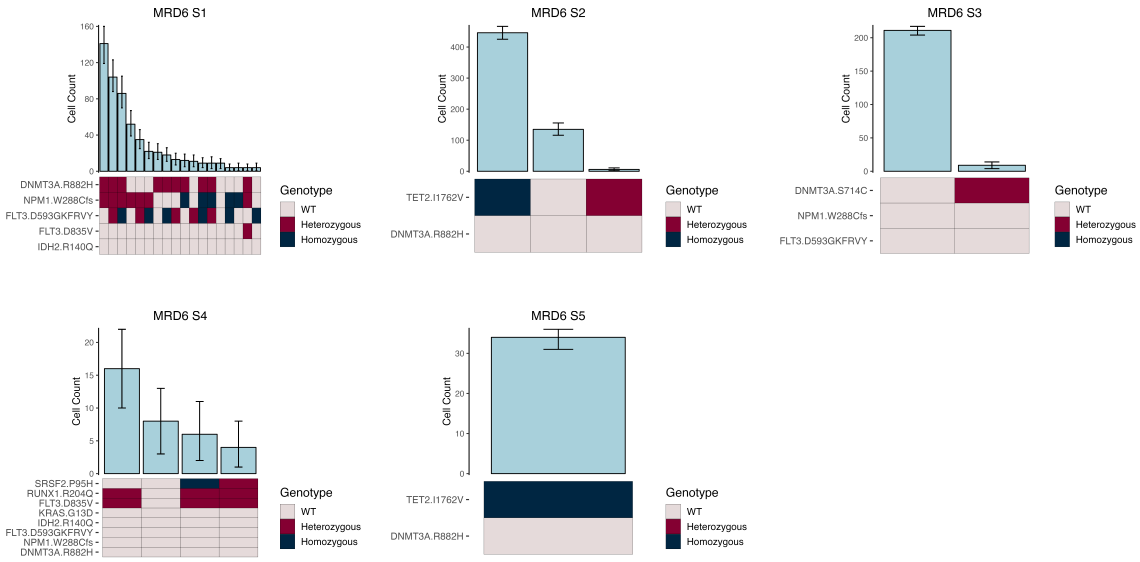

MRD4 S1
Cell Count
500
400
300
200
100
0
DNMT3A.P743Wfs
IDH2.R172K
TET2.I1762V
WT1.S386Sfs
Genotype
WT
Heterozygous
Homozygous
MRD4 S2
Cell Count
250
200
150
100
50
0
IDH2.R140Q
DNMT3A.R771*
DNMT3A.P743Wfs
Genotype
WT
Heterozygous
Homozygous
MRD4 S3
Cell Count
90
60
30
0
WT1.S386Sfs
TP53.R181H
TET2.I1762V
Genotype
WT
Heterozygous
Homozygous
MRD4 S4
Cell Count
75
50
25
0
IDH2.R140Q
WT1.S386Sfs
Genotype
WT
Heterozygous
Homozygous
MRD4 S5
Cell Count
120
90
60
30
0
TET2.I1762V
WT1.S386Sfs
TP53.R181H
Genotype
WT
Heterozygous
Homozygous


MRD5 S1
Cell Count
750
500
250
0
NRAS.G13D
RUNX1.V119Gfs
KRAS.Q61P
NRAS.G12V
IDH2.R140Q
Genotype
WT
Heterozygous
Homozygous


MRD5 S2
Cell Count
125
100
75
50
25
0
IDH2.R172K
DNMT3A.R882H
IDH2.R140Q
DNMT3A.R736C
Genotype
WT
Heterozygous
Homozygous

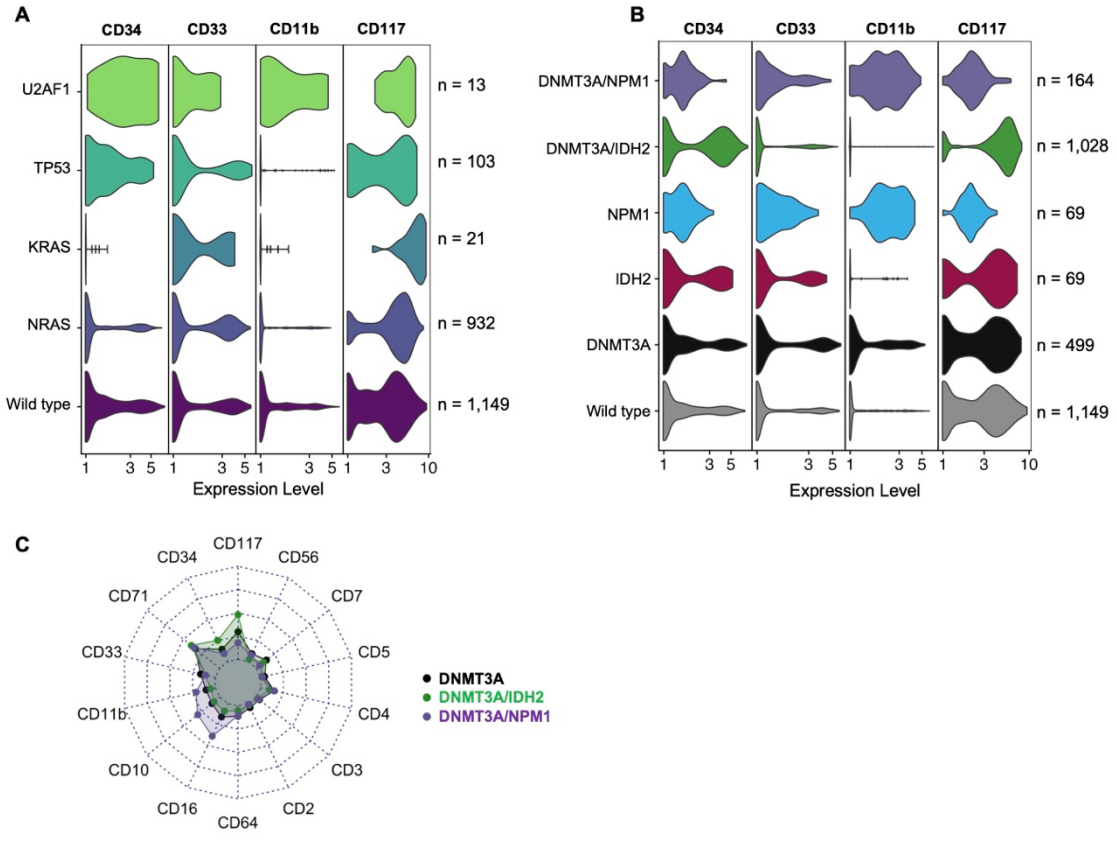

MRD5 S4
Cell Count
20
15
10
5
0
DNMT3A.R882H
IDH2.R140Q
Genotype
WT
Heterozygous
Homozygous


MRD5 S5
Cell Count
20
15
10
5
0
TET2.I1762V
NRAS.G12D
Genotype
WT
Heterozygous
Homozygous



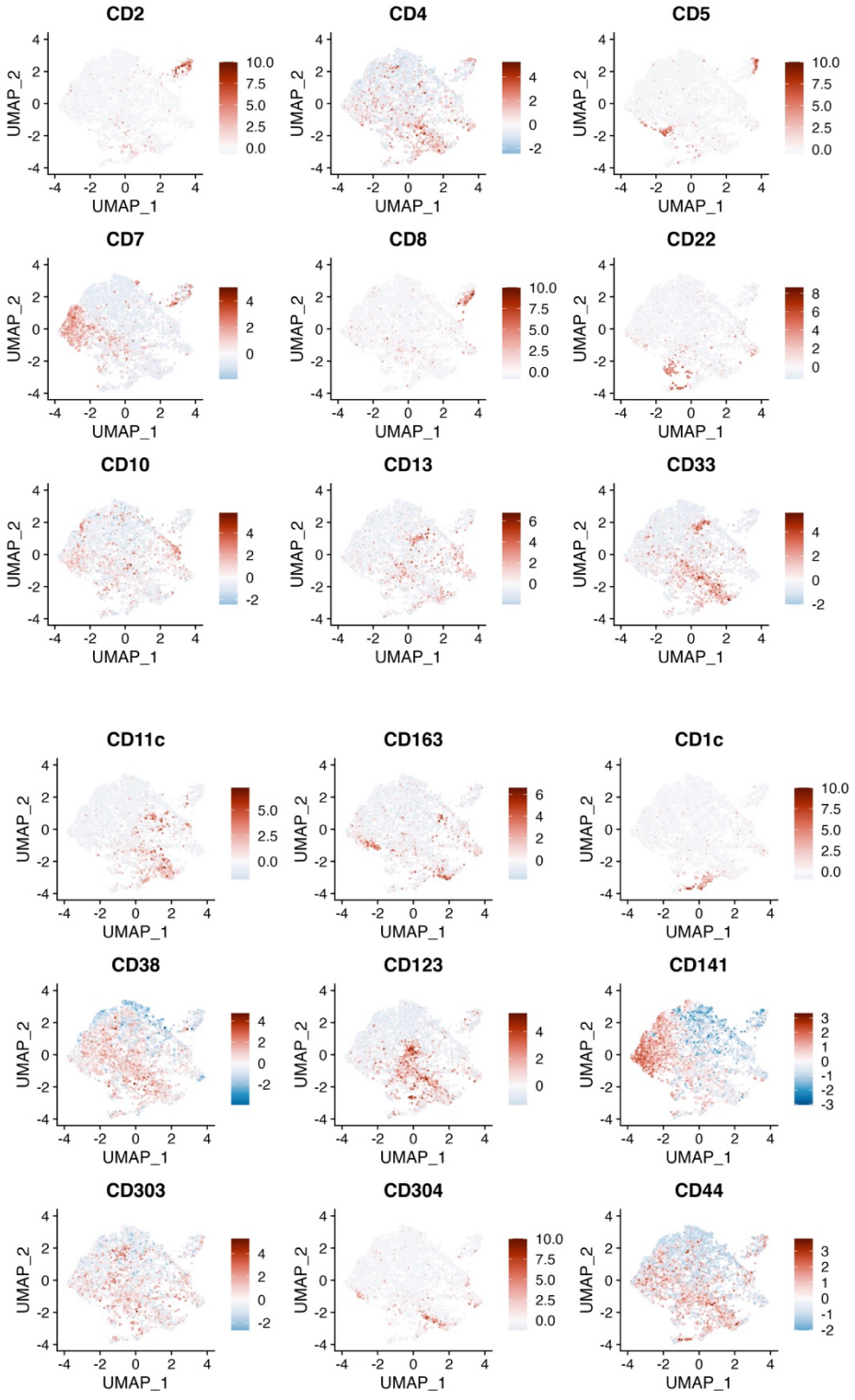
Supplementary Figure S3. Clonal bar plots for all samples included in the study, except for MRD2-S3 and MRD2-S5, which were MRD- by scMRD and uninformative for clonal architecture analysis. Besides *TET2* p.I762V and *TET2* p. L1721W, which are two germline SNPs included here to differentiate between donor and host cells, all other y-axis labels denote pathogenic mutations. Mutations shown in clonal bar plots correspond to all mutations detected at any VAF within the sample (Fig 3). Some mutations are missing here due to not passing the clone cutoff of ≥3 cells.

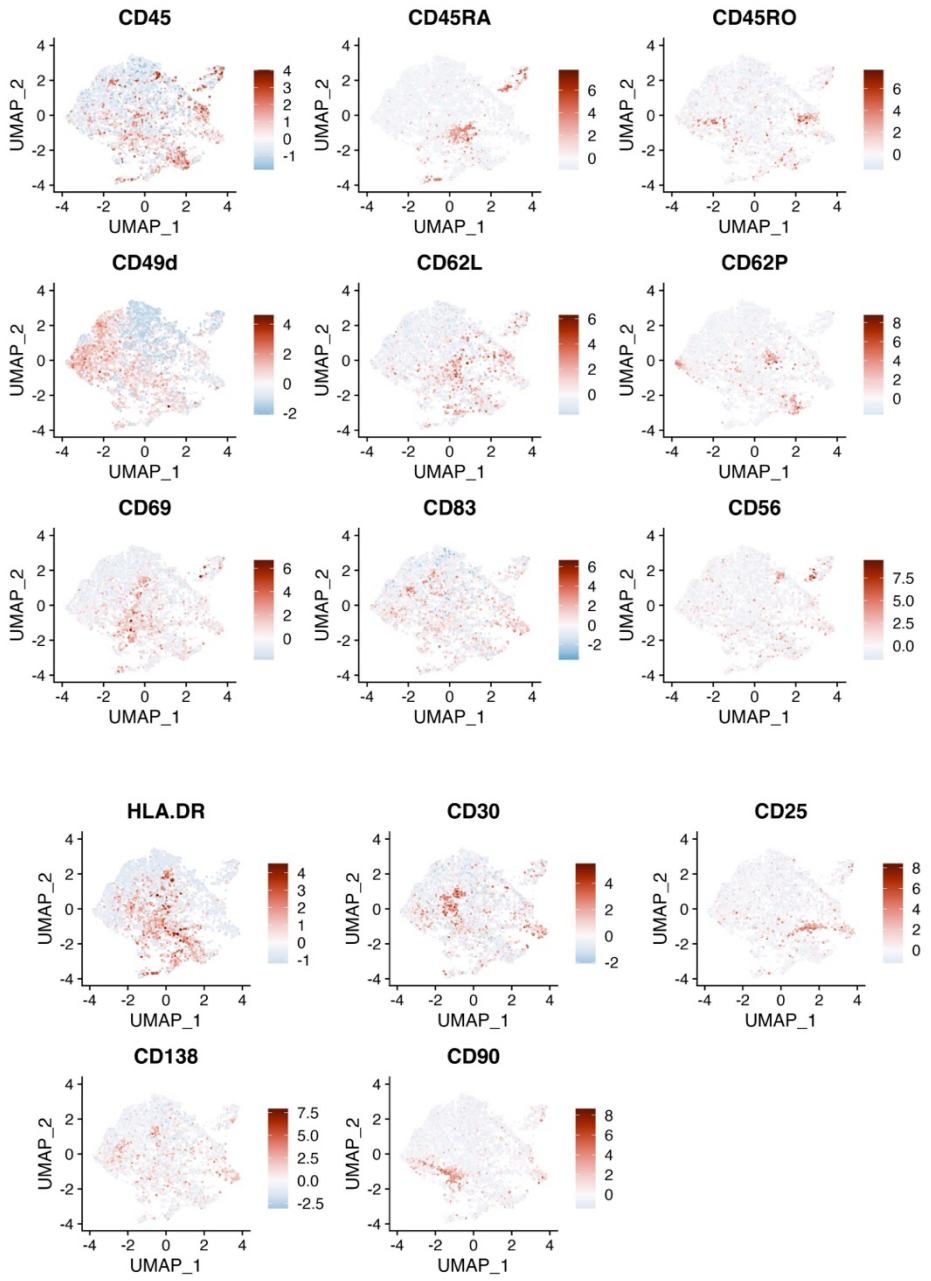
Figure S4



Supplementary Figure S4. Analysis of protein sequencing data of MRD clones. **A**. Violin plots showing CLR-normalized differential surface marker expression of various MRD clones. **B**. Violin plots showing differential surface marker expression of CH/preleukemic vs leukemic clones. **C**. Radar plot showing differential surface marker expression of CH/preleukemic (*DNMT3A*) vs leukemic (*DNMT3A/NPM1*, *DNMT3A/IDH2*) clones. Each marker is scaled relative to the maximum and minimum expression values for all cells with *DNMT3A*, *DNMT3A/NPM1*, or *DNMT3A/IDH2* mutations.

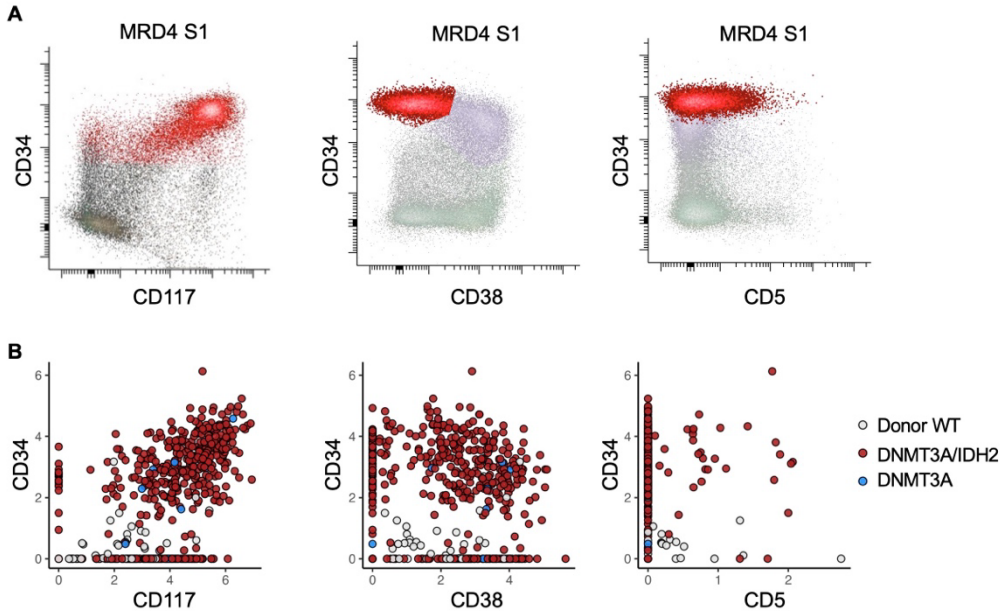
Figure S5


CD2
CD4
CD5
CD7
CD8
CD22
CD10
CD13
CD33
CD11c
CD163
CD1c
CD38
CD123
CD141
CD303
CD304
CD44
UMAP_1
UMAP_2



Supplementary Figure S5. UMAP plots showing protein expression of 32 cell surface markers across the aggregated dataset.

Figure S6



Supplementary Figure S6. Concordance of immunophenotype between MFC and scMRD assay from a representative patient (MRD4-S1). **A**. Flow plots showing abnormal expression of bright CD117, dim to negative CD38 and partial CD5 on CD34 positive myeloblasts. **B**. scMRD protein analysis shows similar immunophenotype to that characterized by clinical MFC.