

# **Inducible MLL-AF9 expression drives an AML program during human pluripotent stem cell-derived hematopoietic differentiation**

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Figure S1

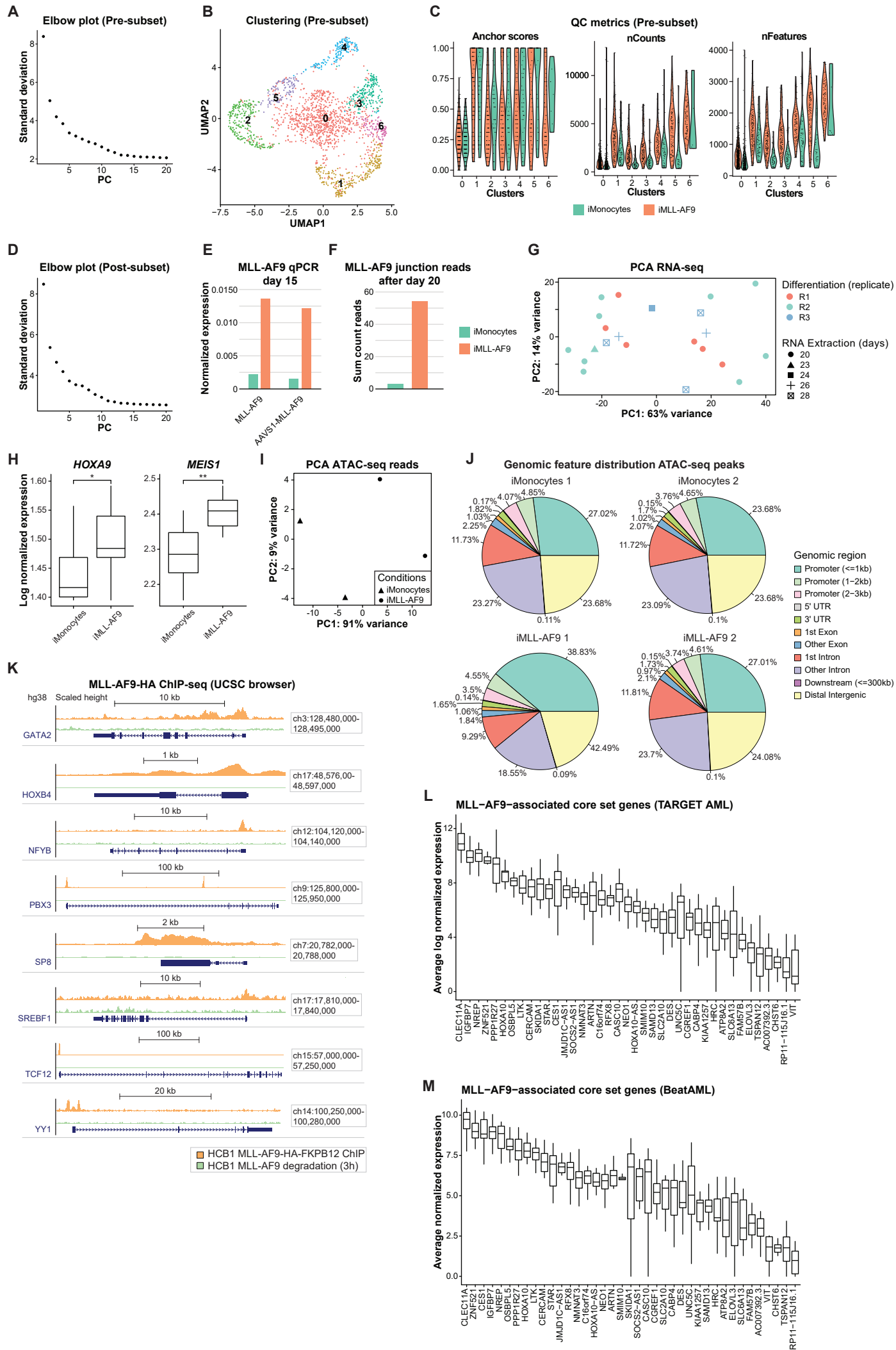
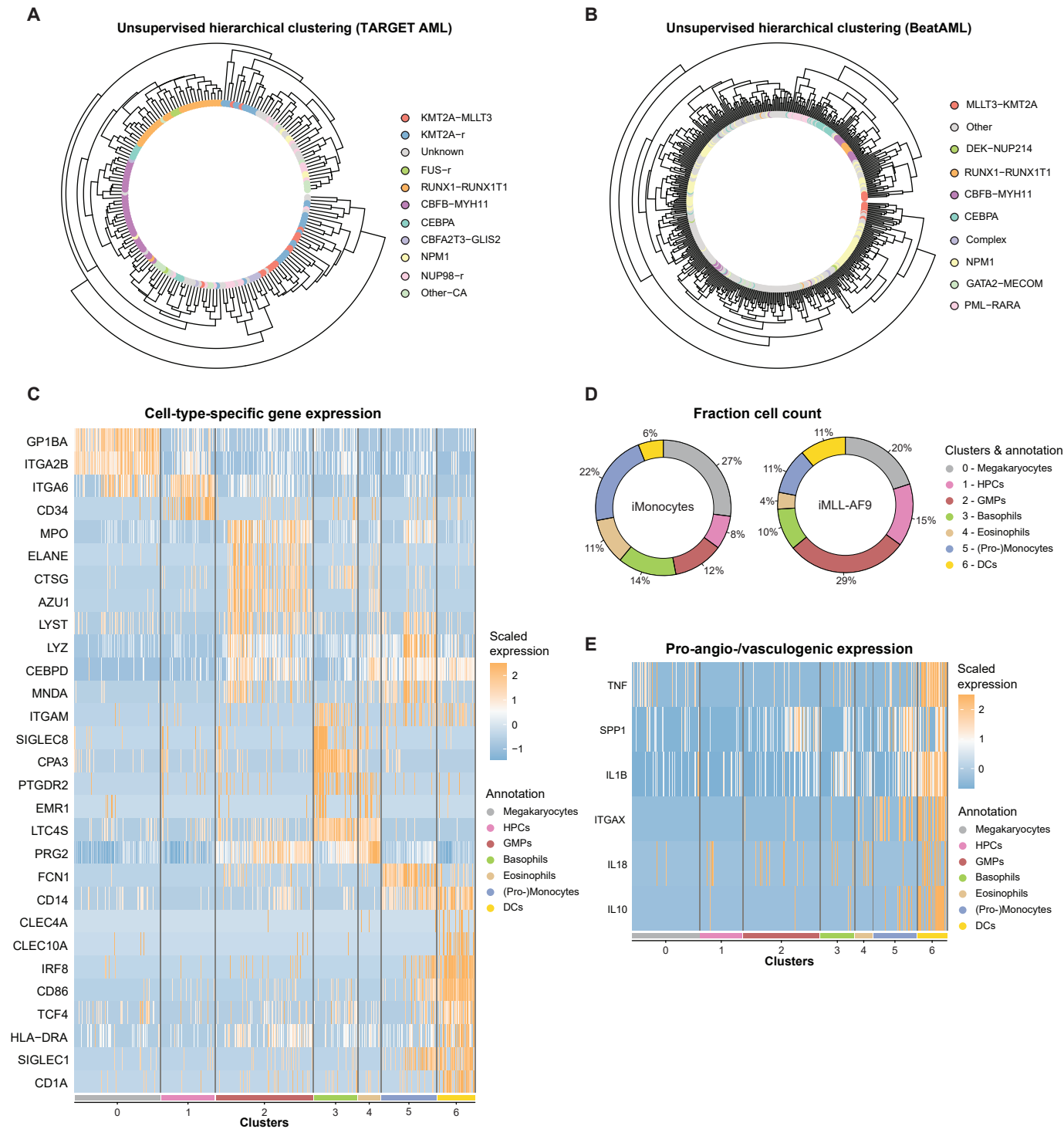


Figure S2



## Supplementary figure legends

### Supplementary Figure S1. Single-cell RNA-seq QC, MLL-AF9-associated gene expression, ATAC-seq QC, UCSC regions of predicted important TFs of iMLL-AF9, and unsupervised hierarchical clustering using only MLL-AF9-associated core set genes.

- (A) Elbow plot visualizing the standard deviation of each PC (pre-subset dataset).
- (B) UMAP visualizing Louvain clusters (pre-subset dataset).
- (C) Violin plots depicting anchors scores, number of features, and number of counts for each cluster. The color represents the conditions.
- (D) Elbow plot visualizing the standard deviation of each PC (post-subset dataset).
- (E) Bar charts representing MLL-AF9 qPCR normalized to GAPDH. Two primer pairs were used for the qPCR (MLL-AF9 and AAVS1-MLL-AF9). Green color depicts iMonocytes and orange the iMLL-AF9 cells.
- (F) Bar charts representing summed junction reads mapped to MLL-AF9 after day 20. Colors represent the doxycycline treatment conditions.
- (G) PCA plot of iMonocytes and iMLL-AF9 cells. RNA-seq samples collected from three independent differentiation experiments, including at least three biological replicates each between day 20 and day 28. Colors represent the three different differentiation experiments that were used for analysis. Shapes depict the days at which RNA was extracted.
- (H) Boxplots depicting log normalized expression of *HOXA9* and *MEIS1*, respectively. The whiskers represent standard deviation, edges depict the inter-quartile ranges, and the black center line the median. A single or double asterisk signifies a p-value < 0.05 or p-value < 0.01, respectively (one-way ANOVA).
- (I) Principal component analysis plot of iMonocytes and iMLL-AF9 cells ATAC-seq reads .
- (J) Pie charts visualizing the fraction of ATAC peaks that reside in a particular genomic region.
- (K) UCSC genome browser illustration of HCB1 MLL-AF9-HA-FKPB12 and MLL-AF9 degraded (3h) ChIP-seq regions of predicted important TFs.
- (L) Boxplots depicting the average log normalized expression of MLL-AF9-associated core set genes in primary MLL-AF9 AML (TARGET AML cohort). The whiskers represent standard deviation, edges depict the inter-quartile ranges, and the black center line the median. A single or double asterisk signifies a p-value < 0.05 or p-value < 0.01, respectively.
- (M) Similar to (L), except using the BeatAML cohort.

### Supplementary Figure S2. Unsupervised clustering AML cohorts using MLL-AF9 core genes, and single-cell cluster annotation.

- (A) Radial dendrogram illustrating unsupervised hierarchical clustering using only expression of MLL-AF9-associated core set genes in primary MLL-AF9 AML (TARGET AML cohort).

- (B) Similar to (A), except using the BeatAML cohort.
- (C) Expression heatmap depicting a number of established myeloid cell type markers.
- (D) Donut charts visualizing the fraction of cells per cluster for iMonocyte and iMLL-AF9 conditions.
- (E) Expression heatmap depicting a number of angio-/vasculogenic markers.