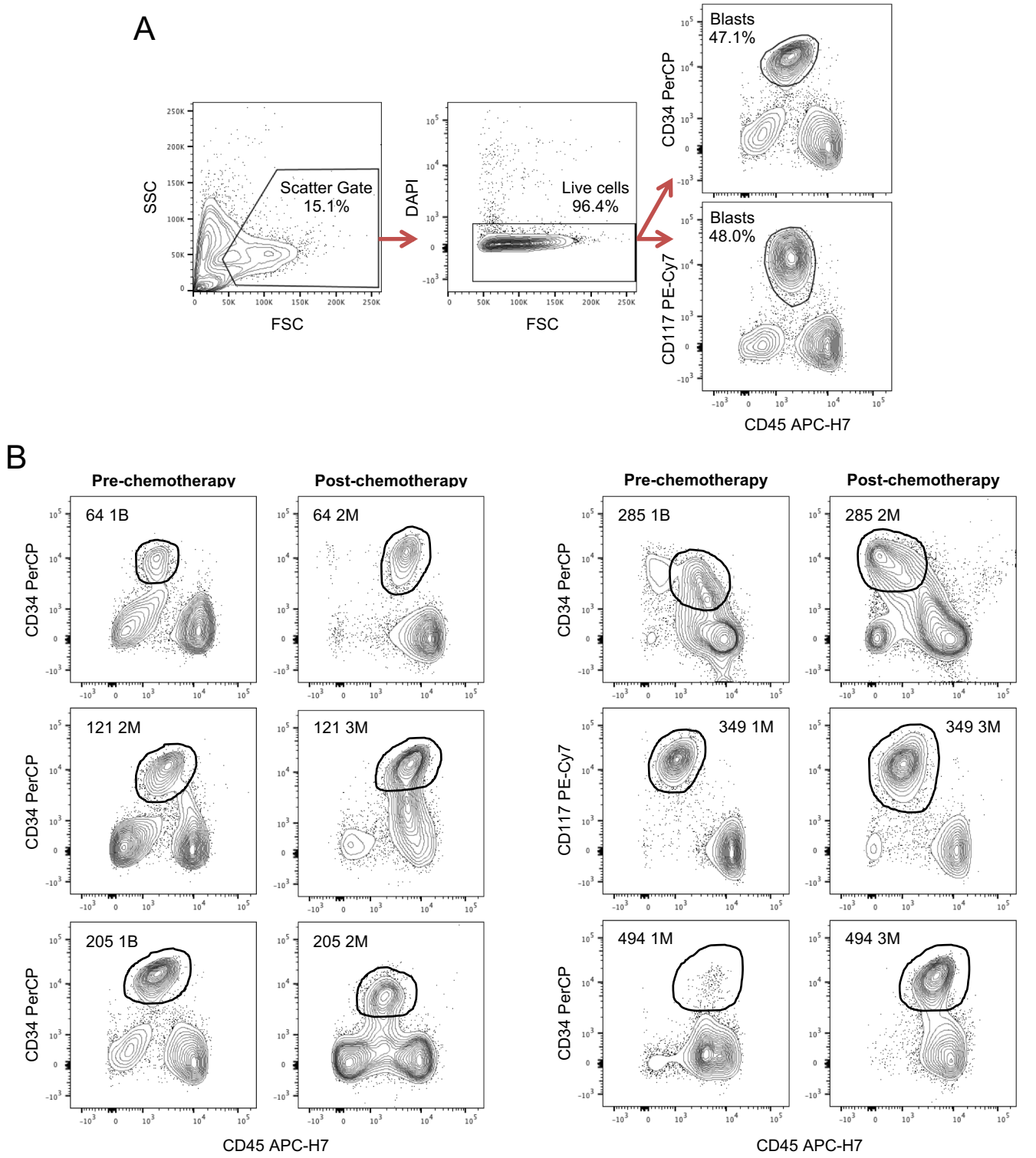
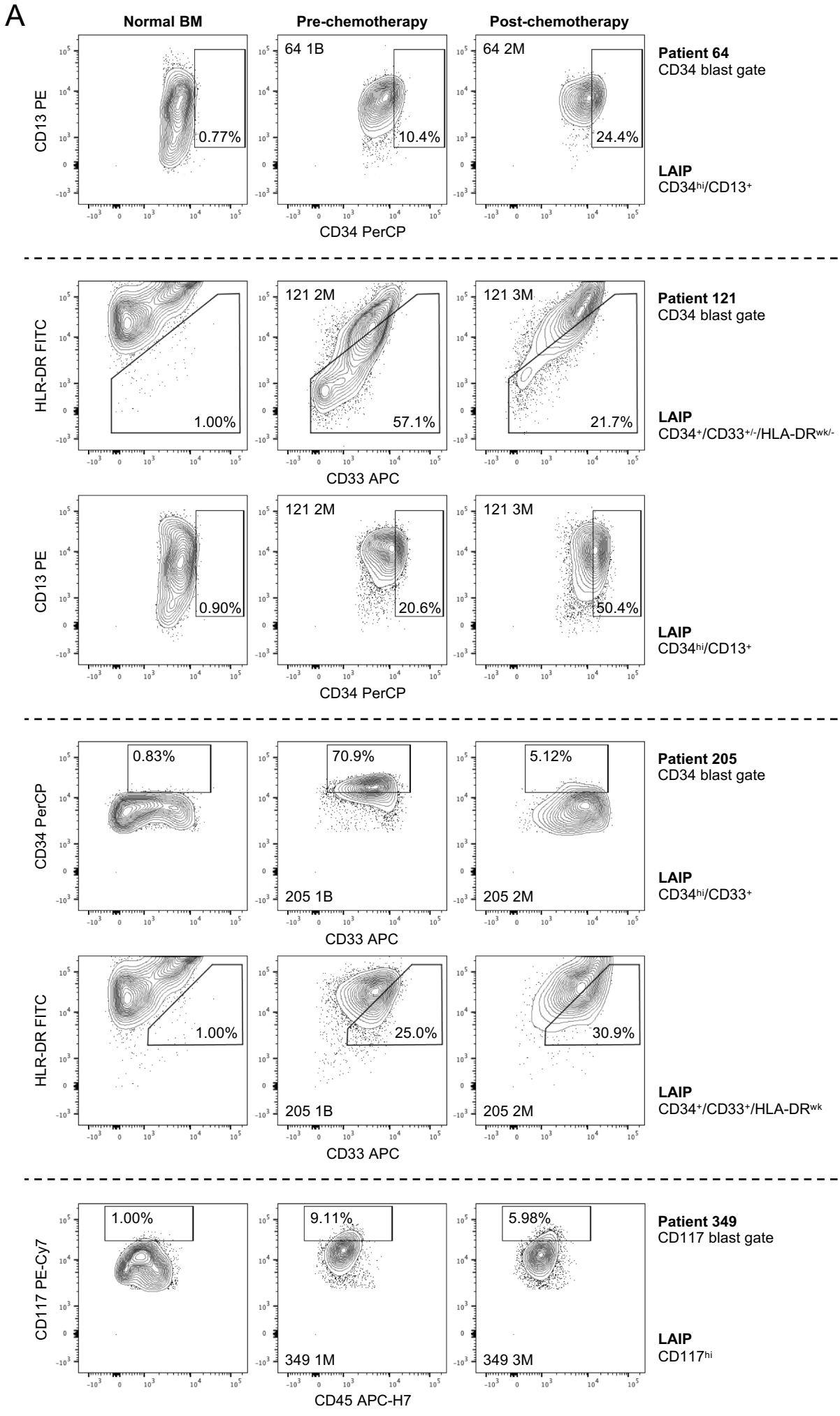


Figure S1. Flow cytometry gating strategy.

(A) Representative flow cytometry scatter plots showing the gating approach used to isolate blasts on the basis of scatter characteristics, DAPI negativity, CD45$^{low/int}$ expression and expression of either CD34 or CD117. (B) Flow cytometry scatter plots show gating strategy for the blast populations isolated from each sample.


A
Normal BM
Pre-chemotherapy
Post-chemotherapy
CD13 PE
0.77%
64 1B
10.4%
64 2M
24.4%
CD34 PerCP
Patient 64
CD34 blast gate
LAIP
CD34hi/CD13+
HLR-DR FITC
1.00%
121 2M
57.1%
121 3M
21.7%
CD33 APC
Patient 121
CD34 blast gate
LAIP
CD34+/CD33+/-/HLA-DRwk/-
CD13 PE
0.90%
121 2M
20.6%
121 3M
50.4%
CD34 PerCP
LAIP
CD34hi/CD13+
CD34 PerCP
0.83%
70.9%
5.12%
205 1B
205 2M
CD33 APC
Patient 205
CD34 blast gate
LAIP
CD34hi/CD33+
HLR-DR FITC
1.00%
25.0%
30.9%
205 1B
205 2M
CD33 APC
LAIP
CD34+/CD33+/HLA-DRwk
CD117 PE-Cy7
1.00%
9.11%
5.98%
349 1M
349 3M
CD45 APC-H7
Patient 349
CD117 blast gate
LAIP
CD117hi

B

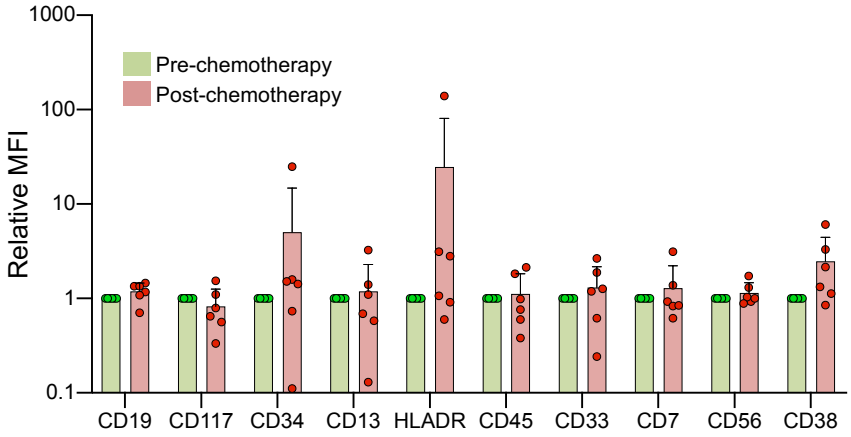


Figure S2. Leukemia associated immunophenotypes (LAIPs).

(A) Flow cytometry scatter plots showing the highest frequency leukemia associated immunophenotypes (LAIPs) identified in the indicated primary AML samples using a difference-from-normal gating strategy. (B) Mean±SD relative median fluorescence intensity (MFI) for the indicated cell surface markers on primary AML blasts pre- and post-chemotherapy (n=6). *P*=not significant by unpaired t-test for all comparisons.

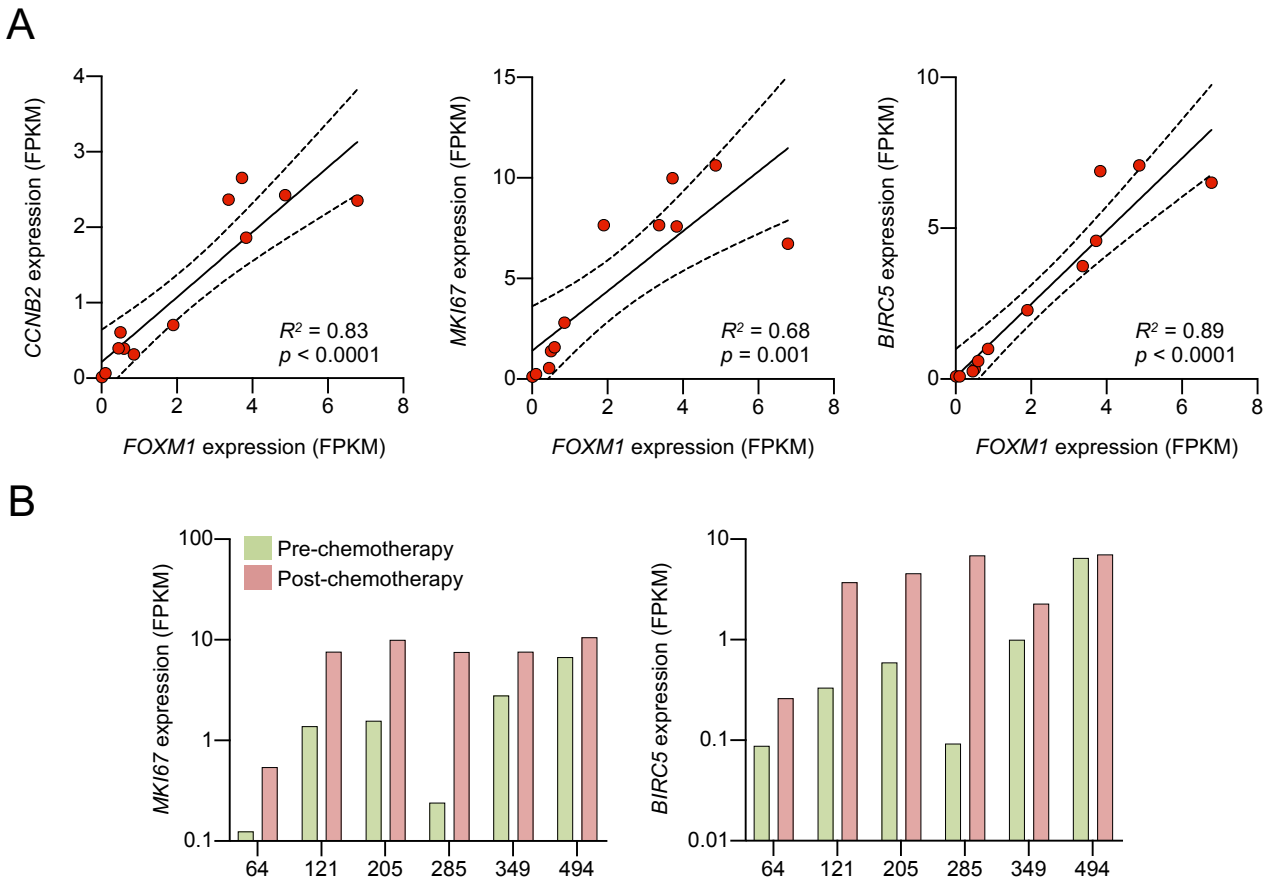


Figure S3. Gene set enrichment analysis (GSEA) and *FOXM1* correlation analyses in post-chemotherapy blasts.

(A) Scatter plots show correlations between *FOXM1* and the indicated cell cycle genes. *R* represents the Pearson product moment correlation coefficient. Dashed lines indicated 95% confidence intervals for the line of best fit. (B) Bar charts show expression of the indicated genes in all samples. FPKM, fragments per kilobase of transcript per million mapped reads.

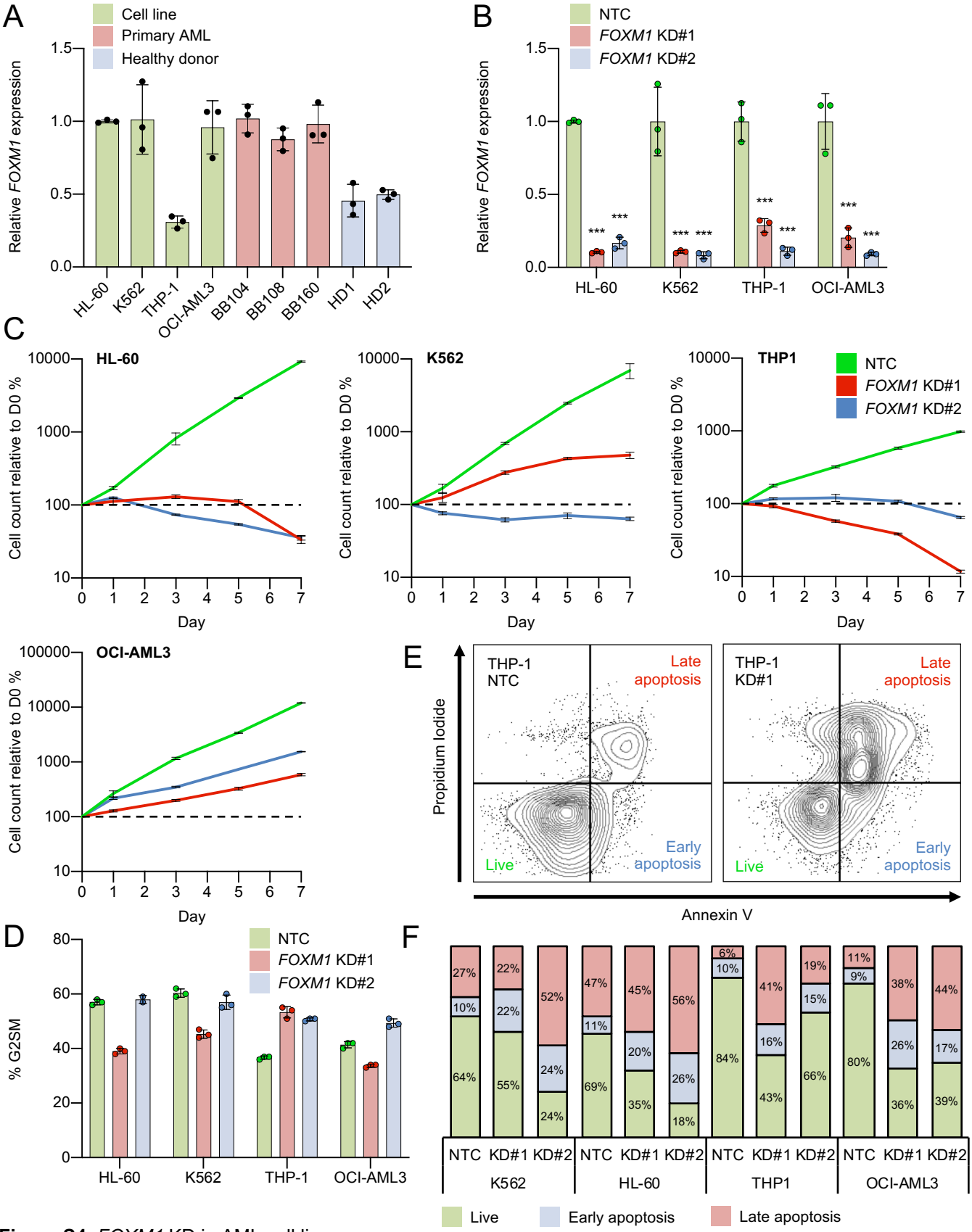


Figure S4. *FOXM1* KD in AML cell lines.

(A) Mean±SD (n=3) *FOXM1* expression relative to HL-60 cells by quantitative PCR in AML cell lines, primary AML and normal CD34⁺ HSPCs from healthy donors. (B) Mean±SD (n=3) *FOXM1* expression in KD versus control conditions for the indicated lines. *** $P<0.001$ (one-way ANOVA with Dunnett's multiple comparison test). (C) Graphs show mean±SD (n=3) cell count relative to Day 0 on the indicated day of culture for the indicated conditions. (D) Mean±SD (n=3) percentage of cells in G2SM in *FOXM1* KD versus control. (E) Representative flow cytometry scatter plots. (F) Proportion of live, early and late apoptotic cells for *FOXM1* KDs versus control for the indicated cell lines (n=3). NTC, non-targeting control; BB numbers indicate Biobank identifier.

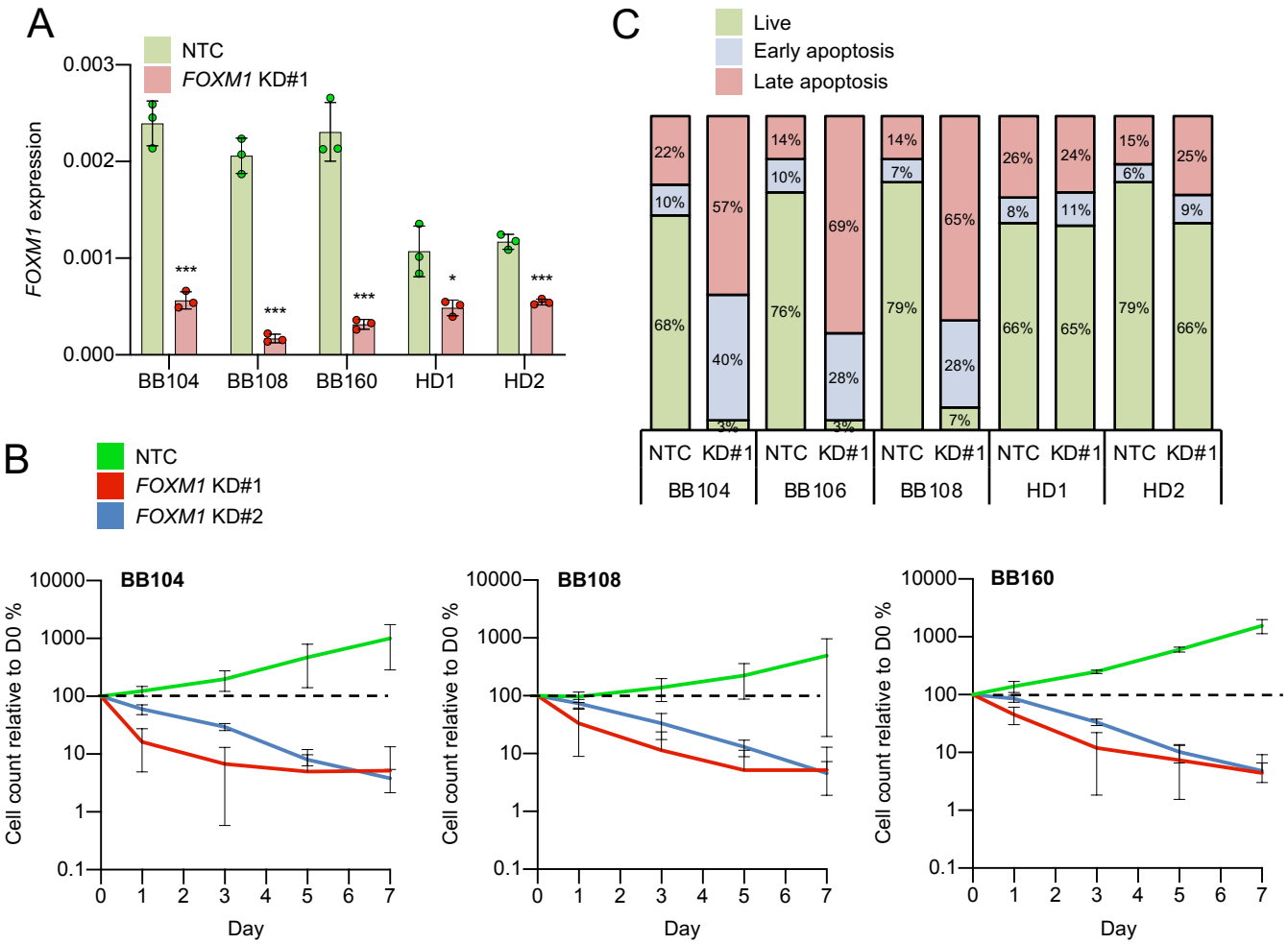
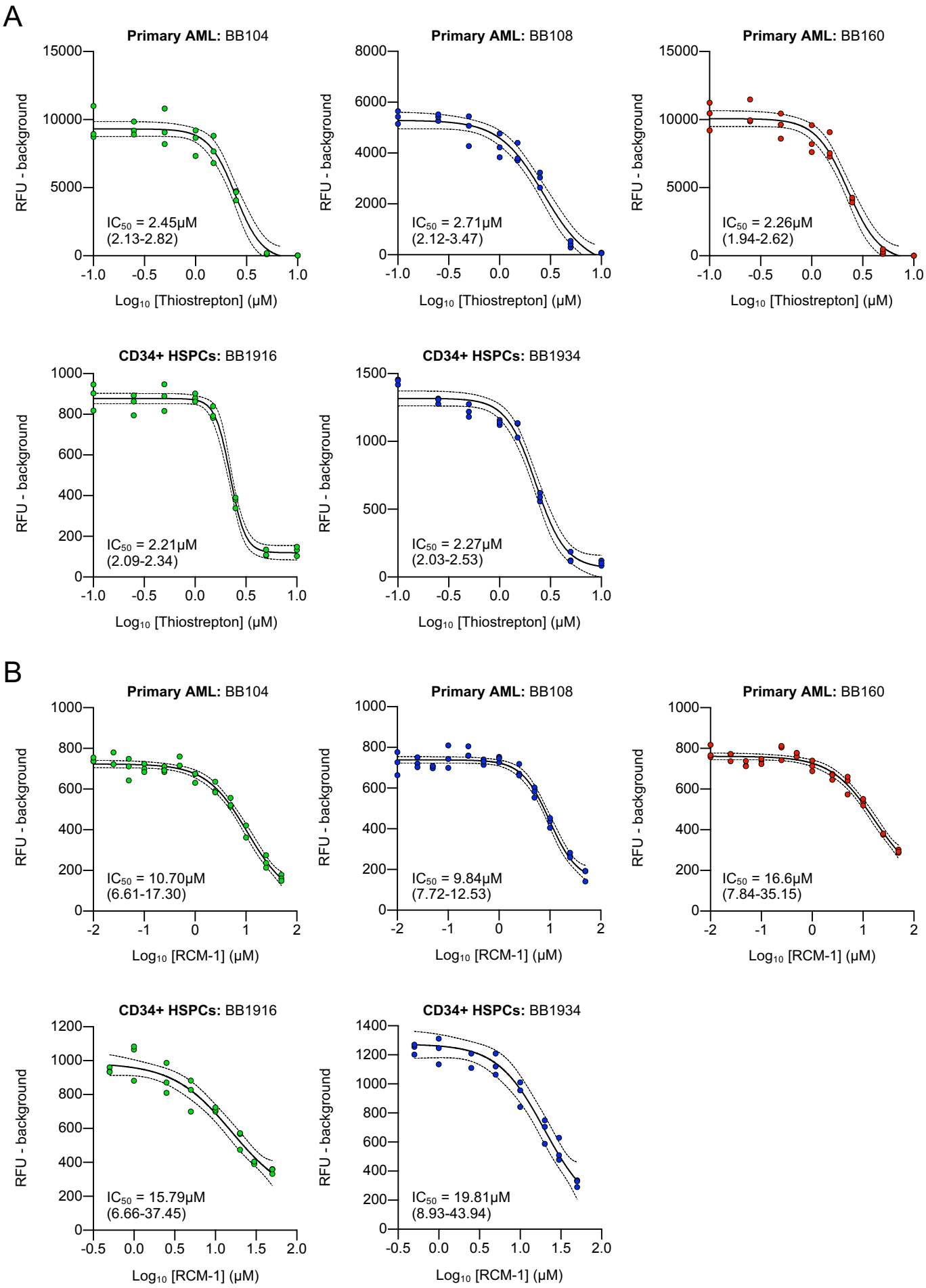


Figure S5. *FOXM1* knockdown in primary AML and normal CD34+ HSPCs..

(A) Mean±SD (n=3) *FOXM1* expression by quantitative PCR in the indicated conditions and the indicated primary AML and normal CD34⁺ HSPC cell types. * $P<0.05$, *** $P<0.001$ by unpaired *t* test. (B) Graphs show mean±SD (n=3) cell count on the indicated day of culture for the indicated primary AML samples. (C) Proportion of live, early and late apoptotic cells for *FOXM1* KD versus control for the indicated primary AML and normal CD34⁺ HSPCs (n=3 for primary AML, n=1 for normal CD34+ HSPCs); see Figure S4E for definitions. NTC, non-targeting control; BB numbers indicate Biobank identifier.


A
Primary AML: BB104
RFU - background
IC50 = 2.45μM
(2.13-2.82)
Log10 [Thiostrepton] (μM)
Primary AML: BB108
IC50 = 2.71μM
(2.12-3.47)
Primary AML: BB160
IC50 = 2.26μM
(1.94-2.62)
CD34+ HSPCs: BB1916
IC50 = 2.21μM
(2.09-2.34)
CD34+ HSPCs: BB1934
IC50 = 2.27μM
(2.03-2.53)
B
IC50 = 10.70μM
(6.61-17.30)
Log10 [RCM-1] (μM)
IC50 = 9.84μM
(7.72-12.53)
IC50 = 16.6μM
(7.84-35.15)
IC50 = 15.79μM
(6.66-37.45)
IC50 = 19.81μM
(8.93-43.94)

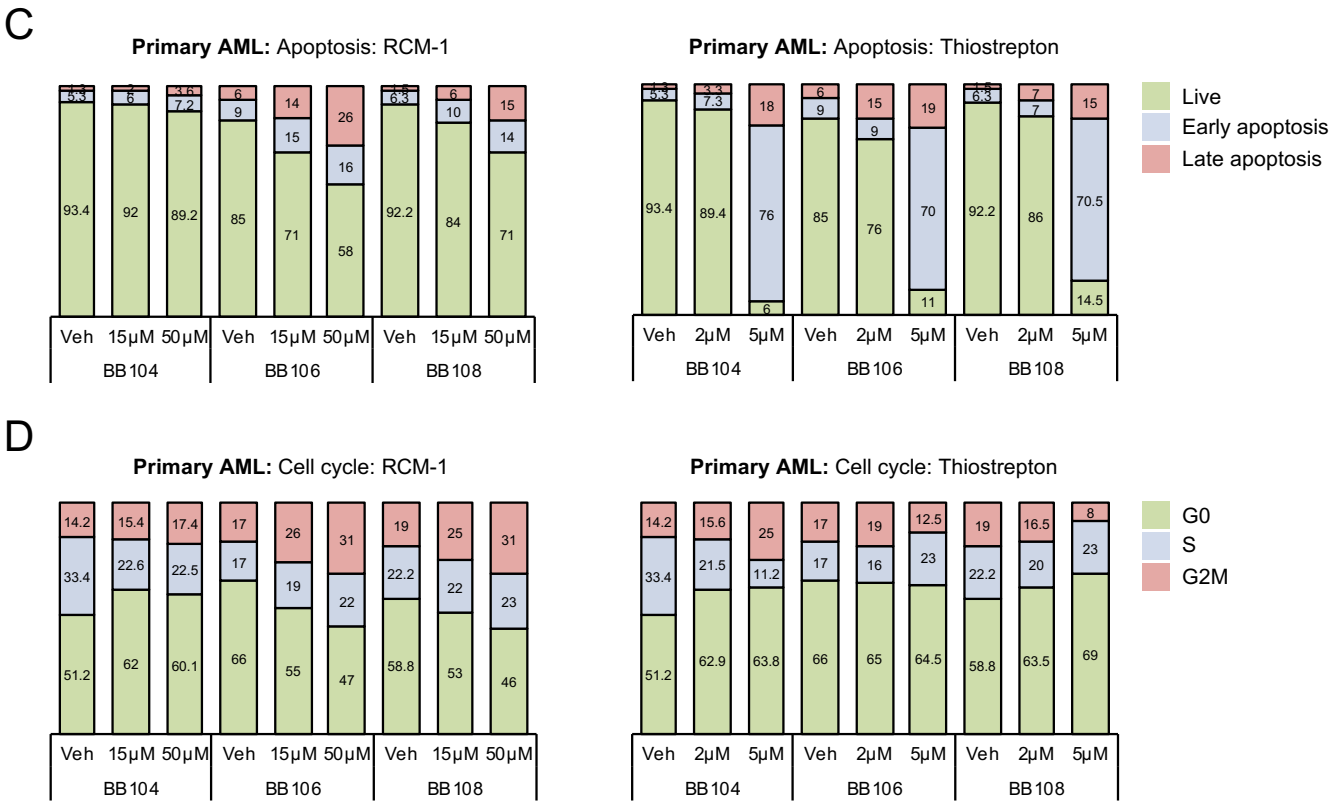


Figure S6. FOXM1 inhibition in primary AML and normal CD34+ HSPCs.

(A-B) Dose response curves for primary AML and normal CD34^{+} HSPCs treated with Thiostrepton (A) or RCM-1 (B). IC_{50} shown with 95% CI in parentheses (n=3). RFU, relative fluorescence units. (C) Proportion of live, early and late apoptotic cells for RCM-1 (left) or Thiostrepton (right) versus vehicle for the indicated primary AML. (D) Proportion of cells in G0, S or G2M phase for RCM-1 (left) or Thiostrepton (right) versus vehicle for the indicated primary AML. BB numbers indicate Biobank identifier.