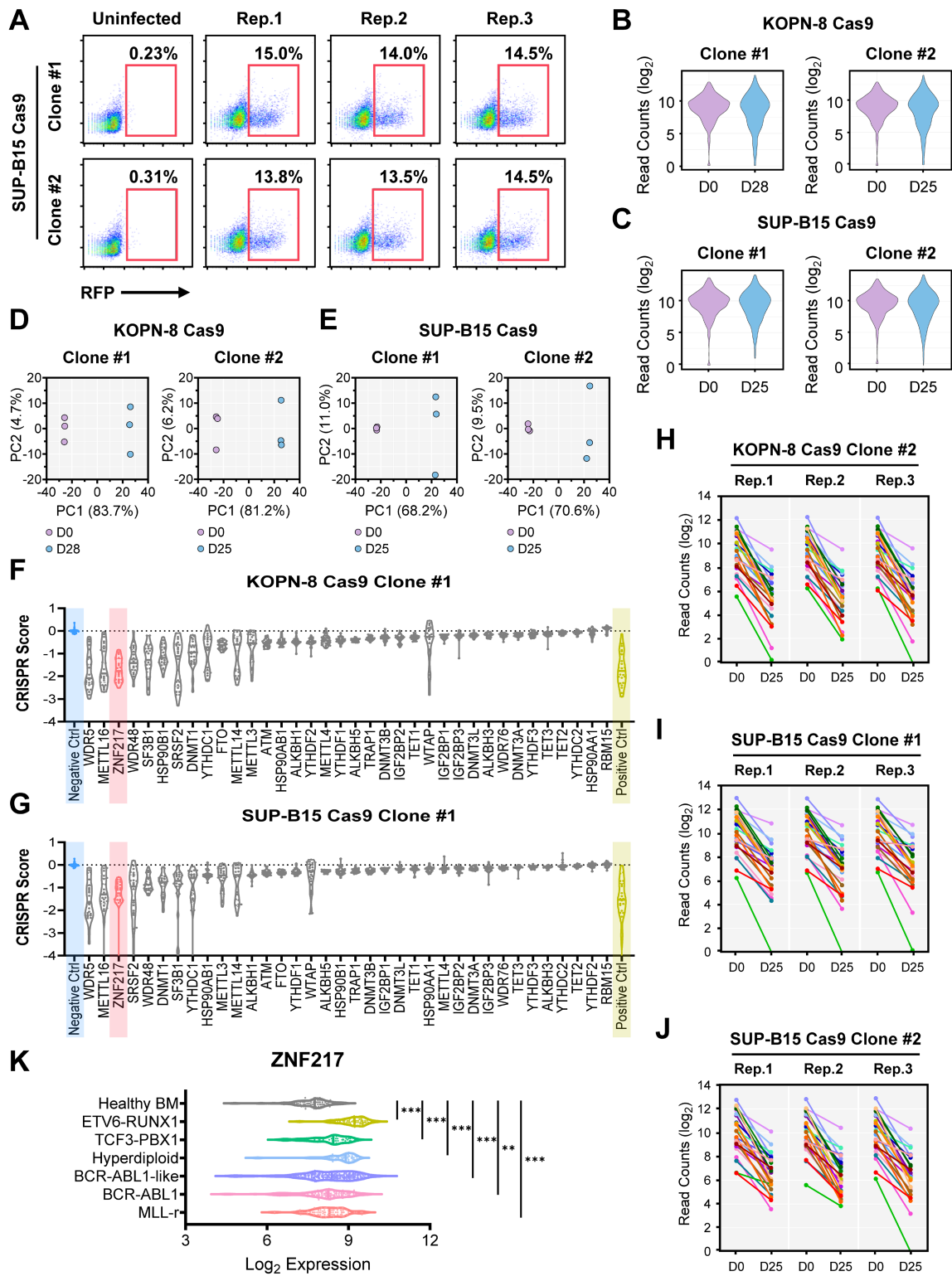


Figure S1



**Figure S1. CRISPR screen identifies essential genes for the survival of B-ALL cells.  
(Related to Figure 1)**

**(A)** The lentiviral transduction efficiency of sgRNA library in CRISPR screen using SUP-B15 Cas9 single clones.

**(B)** The distribution of sgRNA read counts in the NGS samples collected at the “initial” and “final” points of CRISPR screen using KOPN-8 Cas9 single clones.

**(C)** The distribution of sgRNA read counts in the NGS samples collected at the “initial” and “final” points of CRISPR screen using SUP-B15 Cas9 single clones.

**(D)** PCA analysis of NGS samples collected at the “initial” and “final” points of CRISPR screen using KOPN-8 Cas9 single clones.

**(E)** PCA analysis of NGS samples collected at the “initial” and “final” points of CRISPR screen using SUP-B15 Cas9 single clones.

**(F)** The CRISPR scores of sgRNAs targeting the 36 RNA and DNA methylation machinery-associated genes in CRISPR screen using KOPN-8 Cas9 single clones. The CRISPR scores have been normalized against the mean score of the negative control sgRNAs (set at 0.0).

**(G)** The CRISPR scores of sgRNAs targeting the 36 RNA and DNA methylation machinery-associated genes in CRISPR screen using SUP-B15 Cas9 single clones. The CRISPR scores have been normalized against the mean score of the negative control sgRNAs (set at 0.0).

**(H)** Read counts of the 25 sgRNAs targeting *ZNF217* in KOPN-8 Cas9 Clone #2.

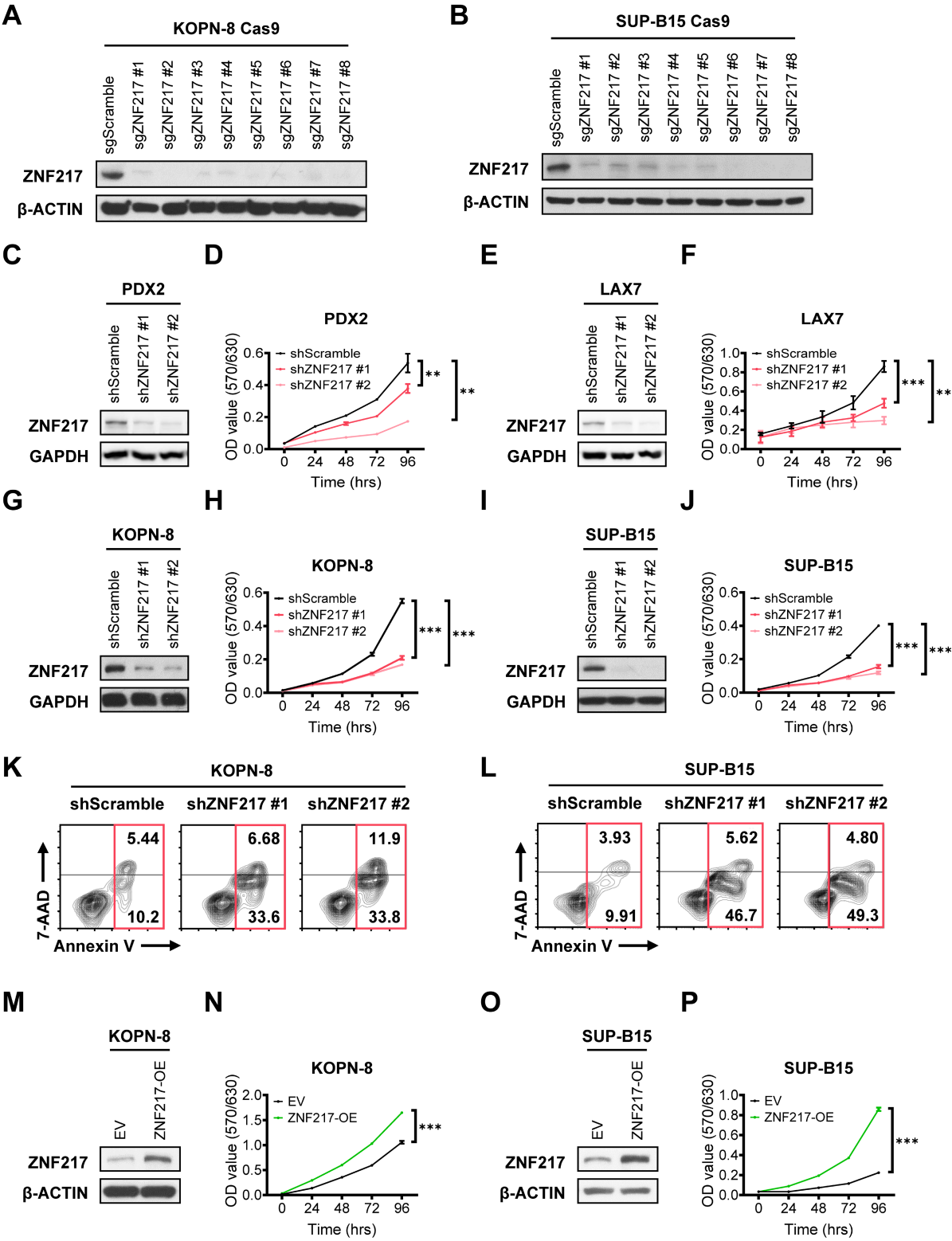
**(I)** Read counts of the 25 sgRNAs targeting *ZNF217* in SUP-B15 Cas9 Clone #1.

**(J)** Read counts of the 25 sgRNAs targeting *ZNF217* in SUP-B15 Cas9 Clone #2.

**(K)** Expression of *ZNF217* across different B-ALL subtypes and in healthy bone marrow. *ZNF217* expression data were sourced from the Microarray Innovations in Leukemia (MILE) study (accession GSE13159). n = 70 for MLL-r; n = 122 for BCR-ABL1; n = 237 for BCR-ABL1-like; n = 40 for hyperdiploid; n = 36 for TCF3-PBX1; n = 58 for ETV6-RUNX1; n = 73 for healthy BM.

The p values were calculated using a two-tailed *t*-test. \*\* p < 0.01; \*\*\* p < 0.001.

Figure S2



**Figure S2. *ZNF217* KO efficacy in B-ALL cells, and effects of *ZNF217* KD and OE on B-ALL cell growth and/or apoptosis. (Related to Figure 2)**

**(A)** KO efficacy of the 8 *ZNF217*-targeting sgRNAs, which were used in the growth competition assay, in KOPN-8 Cas9 cells.

**(B)** KO efficacy of the 8 *ZNF217*-targeting sgRNAs, which were used in the growth competition assay, in SUP-B15 Cas9 cells.

**(C)** *ZNF217* KD efficacy in PDX2 B-ALL PDX cells.

**(D)** Effect of *ZNF217* KD on the growth of PDX2 B-ALL PDX cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

**(E)** *ZNF217* KD efficacy in LAX7 B-ALL PDX cells.

**(F)** Effect of *ZNF217* KD on the growth of LAX7 B-ALL PDX cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

**(G)** *ZNF217* KD efficacy in KOPN-8 B-ALL cells.

**(H)** Effect of *ZNF217* KD on the growth of KOPN-8 B-ALL cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

**(I)** *ZNF217* KD efficacy in SUP-B15 B-ALL cells.

**(J)** Effect of *ZNF217* KD on the growth of SUP-B15 B-ALL cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

**(K)** Effect of *ZNF217* KD on the apoptosis of KOPN-8 B-ALL cells.

**(L)** Effect of *ZNF217* KD on the apoptosis of SUP-B15 B-ALL cells.

**(M)** *ZNF217* OE efficacy in KOPN-8 B-ALL cells.

**(N)** Effect of *ZNF217* OE on the growth of KOPN-8 B-ALL cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

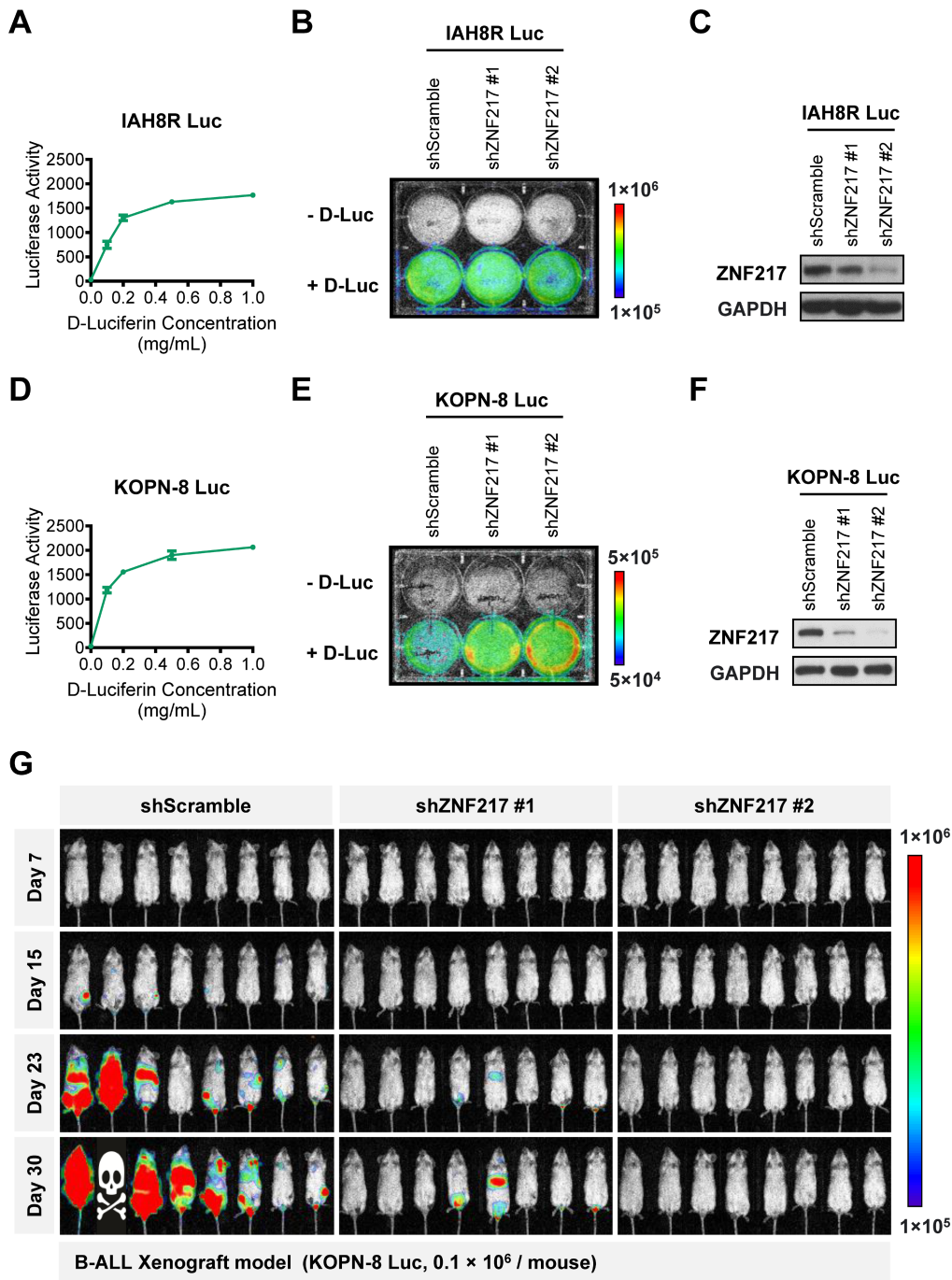
**(O)** *ZNF217* OE efficacy in SUP-B15 B-ALL cells.

**(P)** Effect of *ZNF217* OE on the growth of SUP-B15 B-ALL cells as determined by MTT assay. Data was presented as mean  $\pm$  SD (n = 4 biological replicates).

The p values were calculated using a two-tailed *t*-test. \*\* p < 0.01; \*\*\* p < 0.001.



Figure S3



**Figure S3. The firefly luciferase efficacy in the donor cells of B-ALL PDX and xenograft models, and the effect of *ZNF217* depletion on *in vivo* B-ALL progression. (Related to Figure 3)**

**(A)** The efficacy of firefly luciferase in IAH8R Luc cells. Data was presented as mean  $\pm$  SD (n = 4 technical replicates).

**(B)** The efficacy of firefly luciferase in the donor cells of B-ALL PDX model.

**(C)** *ZNF217* KD efficacy in IAH8R Luc cells.

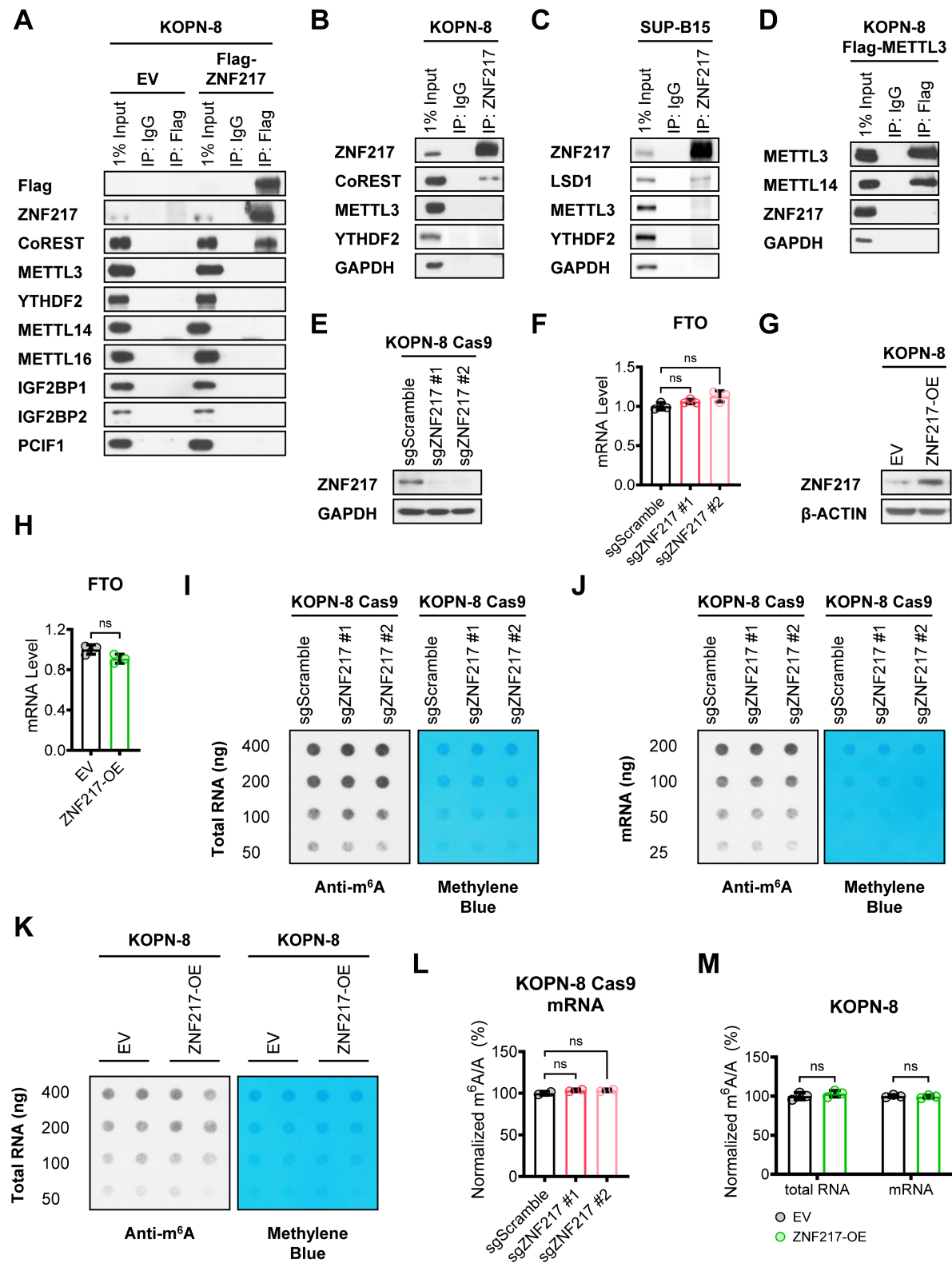
**(D)** The efficacy of firefly luciferase in KOPN-8 Luc cells. Data was presented as mean  $\pm$  SD (n = 4 technical replicates).

**(E)** The efficacy of firefly luciferase in the donor cells of B-ALL xenograft model.

**(F)** *ZNF217* KD efficacy in KOPN-8 Luc cells.

**(G)** Effect of *ZNF217* KD on leukemia burden in B-ALL xenograft model as determined by bioluminescence imaging.

Figure S4



**Figure S4. The oncogenic role of ZNF217 in B-ALL is unlikely to be mediated through m<sup>6</sup>A-associated mechanisms.**

**(A)** Exogenous Co-IP assay using KOPN-8 cells expressing Flag-tagged ZNF217 or an empty vector.

**(B)** Endogenous Co-IP assay using KOPN-8 cells.

**(C)** Endogenous Co-IP assay using SUP-B15 cells.

**(D)** Exogenous Co-IP assay using KOPN-8 cells expressing Flag-tagged METTL3 or an empty vector.

**(E)** *ZNF217* KO efficacy in KOPN-8 Cas9 cells.

**(F)** Effect of *ZNF217* KO on *FTO* transcription in KOPN-8 cell, as determined by RT-qPCR. Data was presented as mean  $\pm$  SD (n = 3 technical replicates).

**(G)** *ZNF217* OE efficacy in KOPN-8 Cas9 cells.

**(H)** Effect of *ZNF217* OE on *FTO* transcription in KOPN-8 cell, as determined by RT-qPCR. Data was presented as mean  $\pm$  SD (n = 3 technical replicates).

**(I)** Effect of *ZNF217* KO on the global m<sup>6</sup>A level in the total RNA in KOPN-8 cells, as determined by dot blot assay.

**(J)** Effect of *ZNF217* KO on the global m<sup>6</sup>A level in the mRNA in KOPN-8 cells, as determined by dot blot assay.

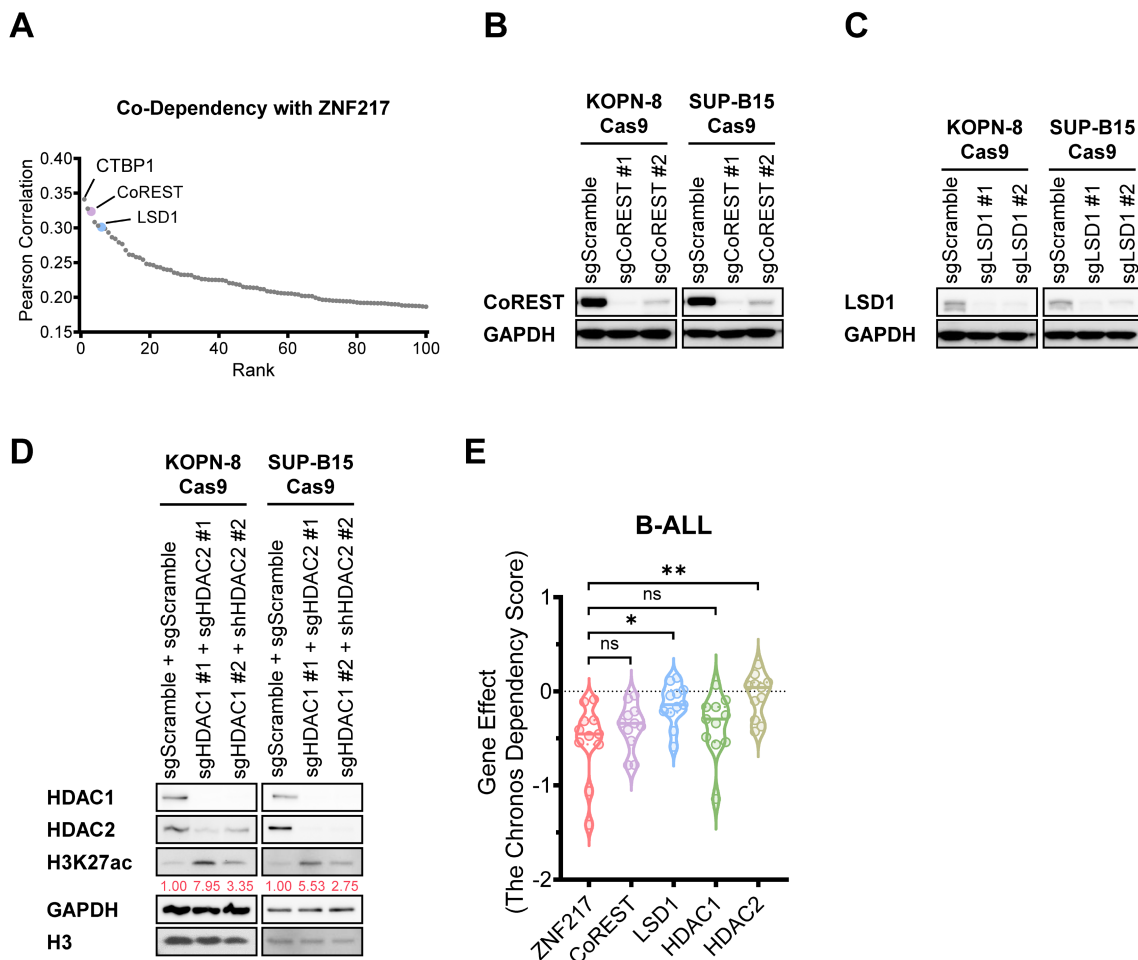
**(K)** Effect of *ZNF217* OE on the global m<sup>6</sup>A level in the total RNA in KOPN-8 cells, as determined by dot blot assay.

**(L)** Effect of *ZNF217* KO on the global m<sup>6</sup>A level in the mRNA in KOPN-8 cells, as determined by UHPLC-QQQ-MS/MS assay. Data was presented as mean  $\pm$  SD (n = 2 biological replicates).

**(M)** Effect of *ZNF217* OE on the global m<sup>6</sup>A levels in the total RNA and mRNA in KOPN-8 cells, as determined by UHPLC-QQQ-MS/MS assay. Data was presented as mean  $\pm$  SD (n = 3 biological replicates).

The p values were calculated using a two-tailed *t*-test. ns, not significant.

**Figure S5**



**Figure S5. ZNF217 collaborates with the CoREST complex to regulate histone modifications. (Related to Figure 4)**

**(A)** The top 100 genes exhibiting co-dependency with *ZNF217* across 1,139 cancer cell lines. Data was retrieved from the DepMap portal. Co-dependency rankings are based on Pearson correlation coefficients between the genes' corresponding dependency scores.

**(B)** *CoREST* KO efficiency in KOPN-8 Cas9 and SUP-B15 Cas9 cells as determined by western blotting.

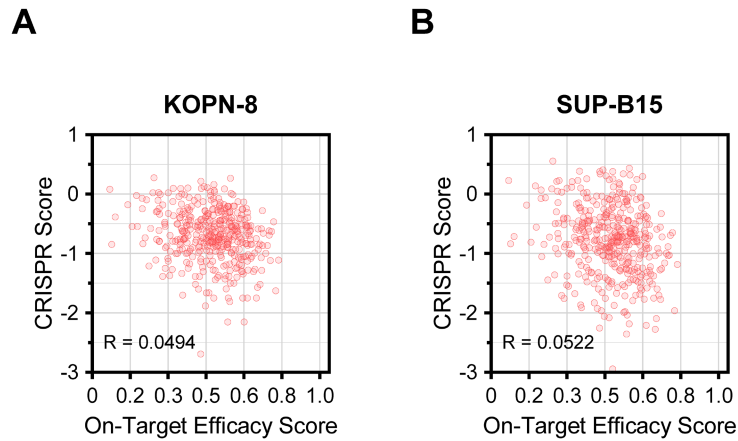
**(C)** *LSD1* KO efficiency in KOPN-8 Cas9 and SUP-B15 Cas9 cells as determined by western blotting.

**(D)** *HDAC1/HDAC2* KO efficiency in KOPN-8 Cas9 and the effect of *HDAC1/HDAC2* KO on H3K27ac deacetylation as determined by Western blotting. Quantification of H3K27ac levels is shown in red beneath the corresponding Western blot bands.

**(E)** The Chronos Dependency Scores of *ZNF217*, *CoREST*, *LSD1*, *HDAC1*, and *HDAC2* in B-ALL cell lines. All the Chronos Dependency Scores were derived from the DepMap portal (<https://depmap.org/portal/>). n = 11 for each gene.

The p values were calculated using a two-tailed t-test. ns, not significant; \* p < 0.05; \*\* p < 0.01.

**Figure S6**



**Figure S6. Correlations between the CRISPR scores of ZNF217 sgRNAs and their on-target efficacy scores. (Related to Figure 5)**

**(A)** Correlation between the CRISPR scores of 416 *ZNF217* sgRNAs in KOPN-8 Cas9 single clones and their estimated on-target efficacy scores.

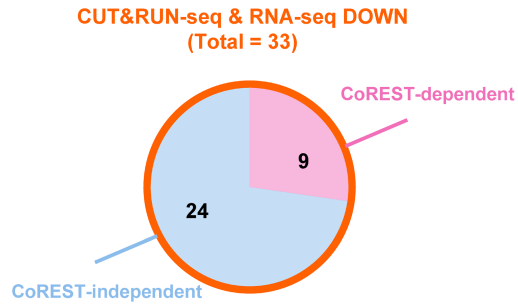
**(B)** Correlation between the CRISPR scores of 416 *ZNF217* sgRNAs in SUP-B15 Cas9 single clones and their estimated on-target efficacy scores.

On-target efficacy scores were predicted using the Genetic Perturbation Platform

(<https://portals.broadinstitute.org/gpp/public/>).

**Figure S7**

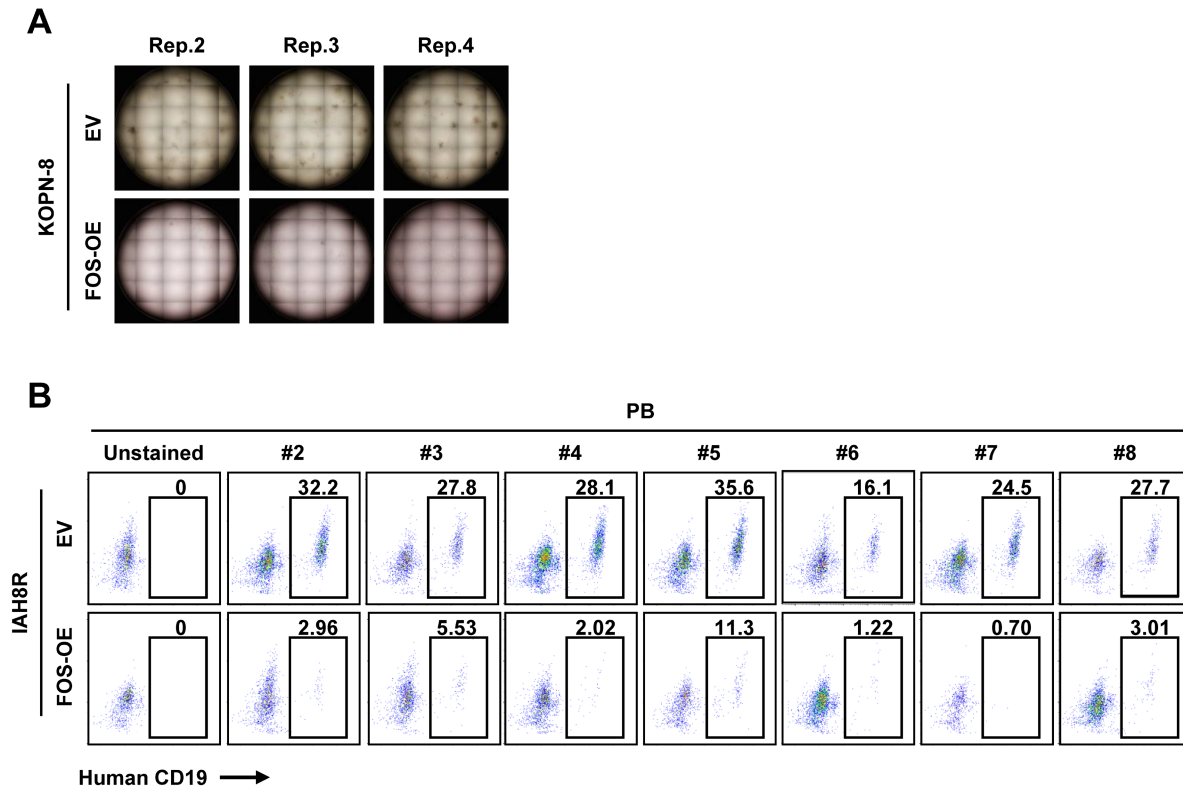
**A**



**Figure S7. Multiple-omics analysis of ZNF217 downstream targets in B-ALL cells (Related to Figure 6)**

**(A)** The overlap of downregulated genes identified by RNA-seq and ZNF217-bound genes identified by CUT&RUN-seq.

**Figure S8**



**Figure S8. FOS plays a tumor suppressor role in B-ALL. (Related to Figure 7)**

**(A)** Effect of *FOS* OE on the colony-forming ability of KOPN-8.

**(B)** Effect of *FOS* OE on leukemia burden in B-ALL PDX recipient (NSG) mice, as determined by flow cytometry measuring the percentage of human CD19<sup>+</sup> cells in the peripheral blood of recipient mice.