Supplemental material

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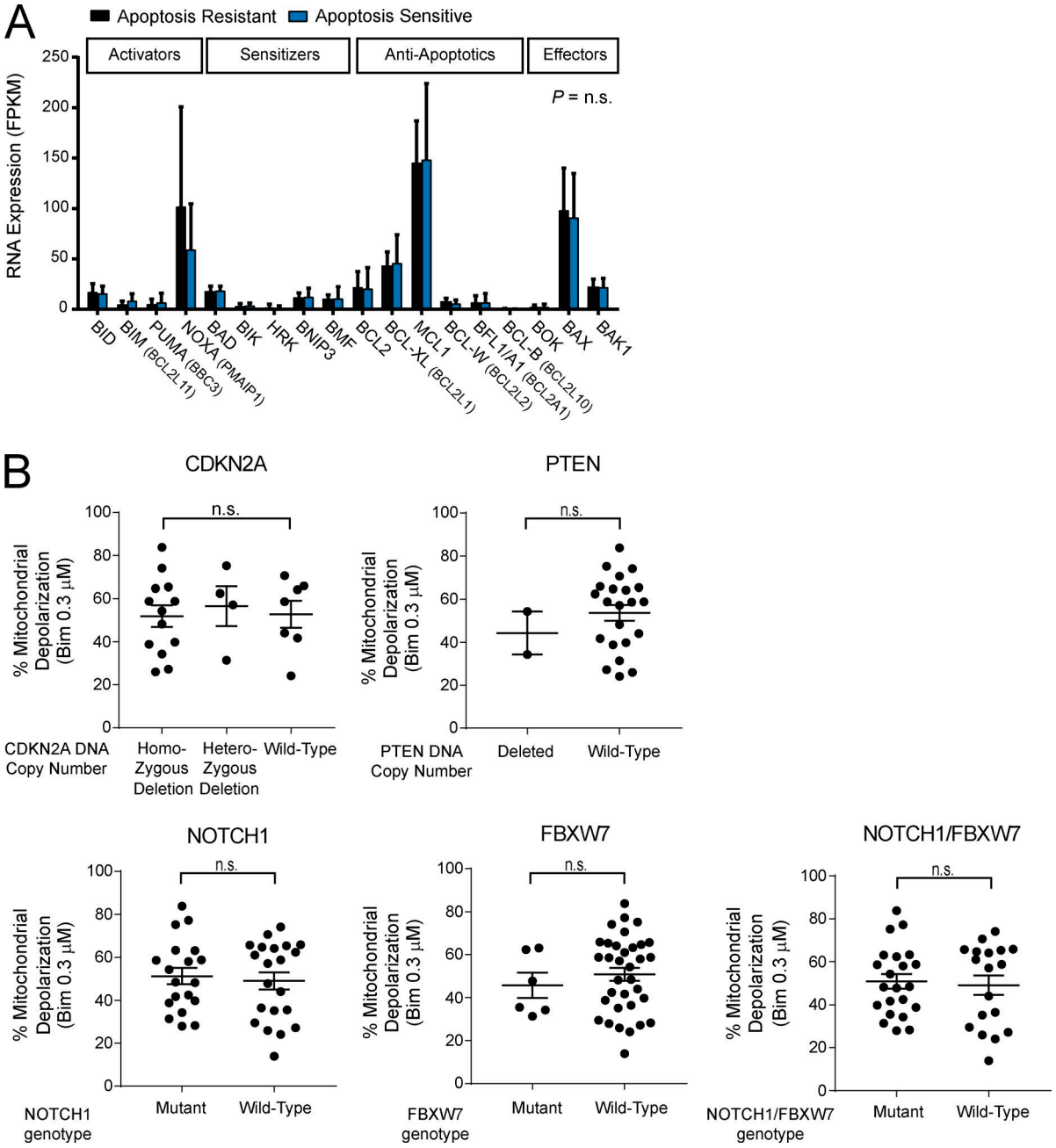

Figure S1. **Lack of association of mitochondrial apoptosis resistance in primary T-ALL patient samples with expression of BCL2 family genes or oncogenic mutations previously implicated in T-ALL treatment response. (A)** RNA expression of pro- and antiapoptotic BCL2 family members in apoptosis-sensitive versus -resistant primary T-ALL patient samples, shown for the 24 T-ALL patient samples on which sufficient material for RNA-seq was available. Samples were classified into apoptosis-sensitive or resistant groups based on whether they were above or below mean mitochondrial depolarization by BH3 profiling. Data shown as mean ± SEM of $n = 12$ samples per group. No differences were statistically significant based on $P < 0.05$ by Welch t test. **(B)** Comparison of mitochondrial apoptotic priming of primary T-ALL cells, as assessed by BH3 profiling analysis for mitochondrial depolarization in response to 0.3 µM BIM peptide, are shown based on T-ALL sample genotype. Effects of *CDKN2A or PTEN* deletions were assessed on the 24 T-ALL samples analyzed by both array CGH and BH3 profiling. Effects of *NOTCH1* or *FBXW7* mutations were assessed in the 40 T-ALL samples analyzed by targeted exome sequencing analysis and BH3 profiling analysis. Data are shown as mean ± SEM. Number of samples per group: CDKN2A: homozygous deletion, $n = 13$; heterozygous deletion, $n = 4$; WT, $n = 7$ ($P = 0.91$ by Welch t test). PTEN: deleted, $n = 2$; WT, $n = 22$ ($P = 0.51$ by Welch t test). NOTCH1: mutant, $n = 19$; WT, $n = 21$ ($P = 0.69$ by Welch t test). FBXW7: mutant, $n = 5$; WT, $n = 35$ ($P = 0.46$ by Welch t test). NOTCH1/FBXW7: mutant, $n = 22$; WT, $n = 18$ ($P = 0.76$ by Welch t test). Each data point represents percent mitochondrial depolarization in an independent patient sample.

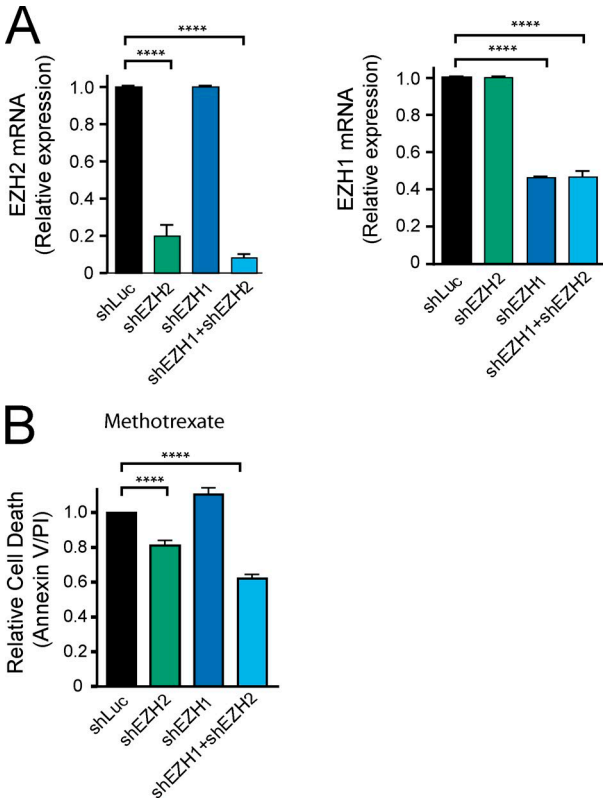

Figure S2. **EZH1 is not required for apoptosis resistance upon EZH2 depletion. (A)** *EZH2* and *EZH1* were down-regulated in CCRF-CEM cells using neomycin-resistant shEZH2 no. 1 and puromycin-resistant shEZH1, alone or in combination, and qRT-PCR for the indicated genes was performed to test knockdown efficacy. Results were normalized to shLuc cells. Significance was assessed by two-way ANOVA with Tukey adjustment for multiple comparisons. $P < 0.0001$ for all indicated comparisons. **(B)** Relative cell death after treatment with 10 µM methotrexate for 48 h was assessed using Annexin V/propidium iodide analysis. Significance was assessed by two-way ANOVA with Tukey adjustment for multiple comparisons. $P < 0.0001$ for all indicated comparisons. All results are shown as mean ± SEM of $n = 3$ biological replicates. ****, $P \leq 0.0001$; n.s., $P > 0.05$.

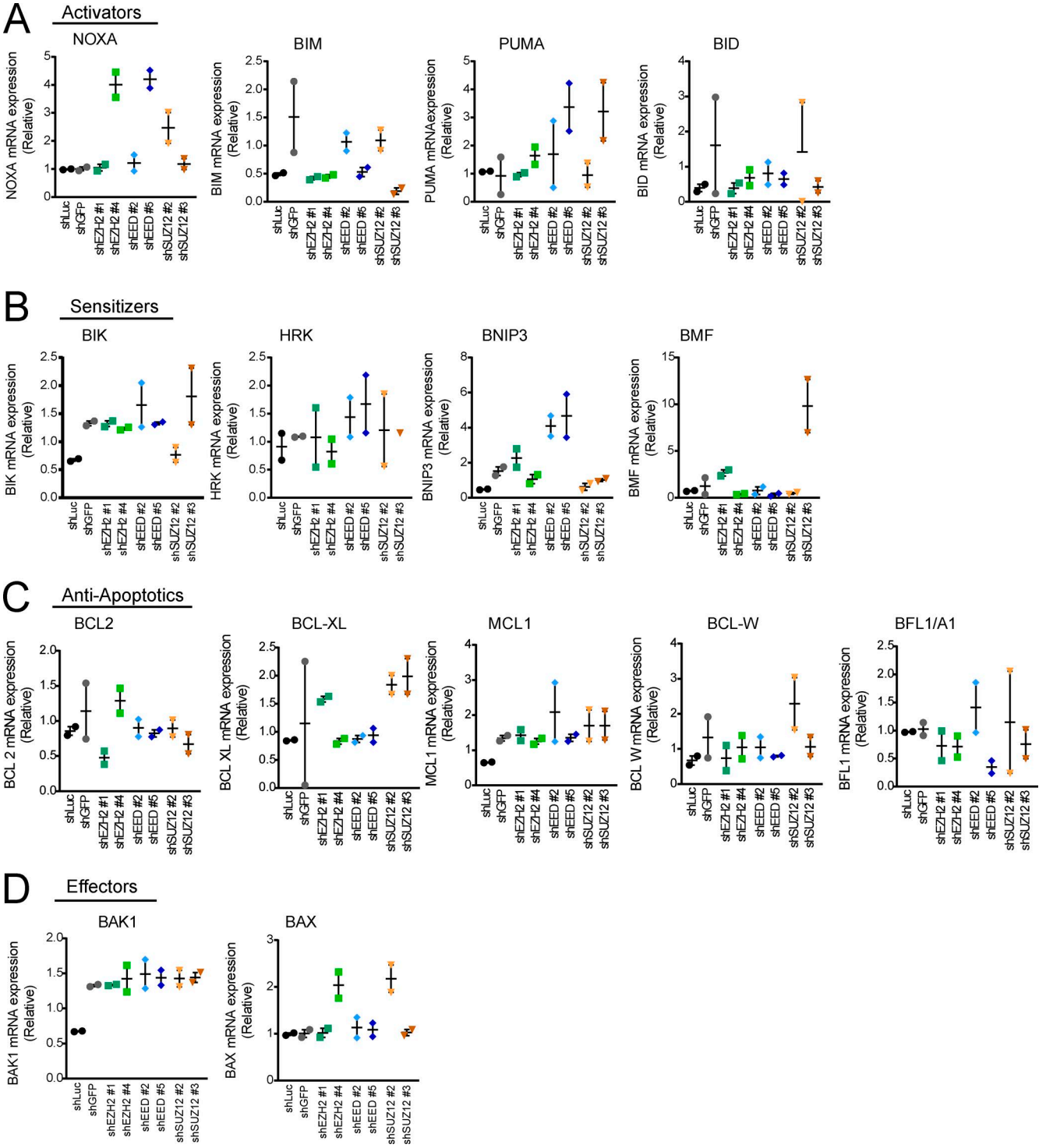

Figure S3. **Independent shRNAs targeting core PRC2 components do not consistently regulate mRNA expression of pro- or antiapoptotic BCL2 family members.** qRT-PCR analysis of CCRF-CEM cells was performed 7 d after transduction with two independent shRNAs targeting each indicated gene (*EZH2, EED*, and *SUZ12*) or control (shLuc and shGFP). Results are normalized to the average of control shRNAs. **(A–D)** Plots indicate the mRNA expression of each gene, relative to the average expression in control (shGFP and shLuc) transduced cells, for expression of BCL2 family members classified as apoptosis activators (A), sensitizers (B), antiapoptotics (C), and effectors (D). Data points shown are from biological duplicates. Note that BAD, BCL-B, and BOK are not shown because they were not detected in any condition by qRT-PCR analysis.

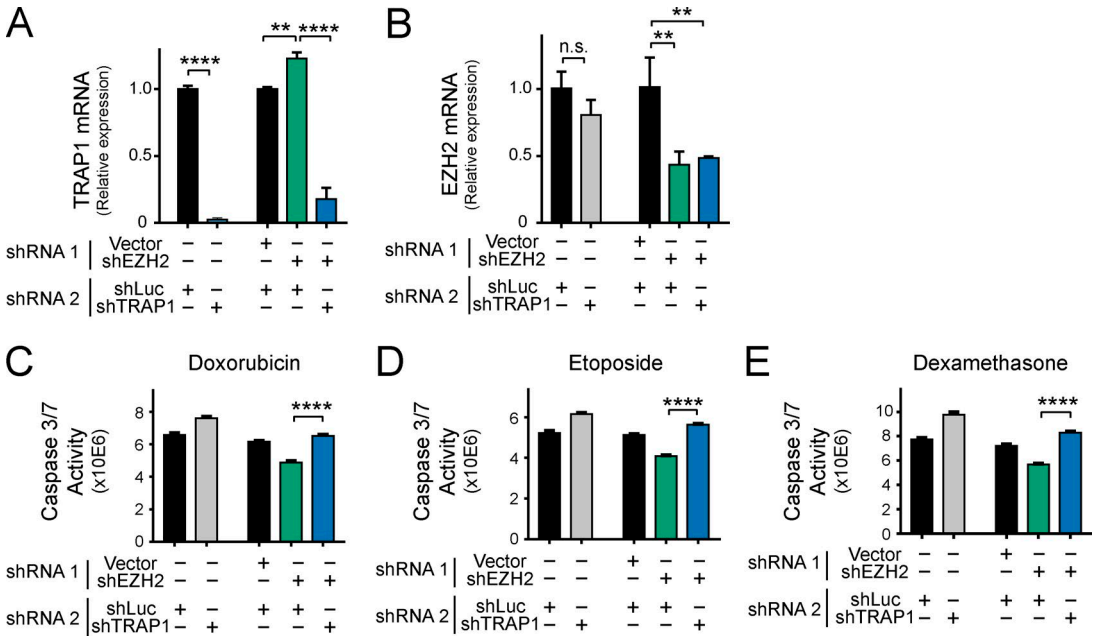

Figure S4. **TRAP1 is required for induction of apoptosis resistance upon *EZH2* depletion.** CCRF-CEM cells were transduced with a neomycin-resistant "shRNA 1" targeting EZH2 or empty vector control and subsequently transduced with a puromycin-resistant "shRNA 2" targeting either TRAP1 or Luciferase control. **(A and B)** Knockdown efficacy was assessed by qRT-PCR of the indicated genes, with β-actin used as control. Results were normalized to the shLuc-alone condition. All results indicate the mean ± SEM from $n = 3$ biological replicates. Significance was assessed by two-way ANOVA, with Tukey correction for multiple hypothesis testing. For TRAP1 qRT-PCR, $P < 0.0001$ for shLuc versus shTRAP1 transduced cells, $P = 0.0022$ for vector-shLuc versus shEZH2-shLuc, and $P < 0.0001$ for shEZH2-shLuc versus shEZH2-shTRAP1. For EZH2 qRT-PCR, $P = 0.17$ for shLuc versus shTRAP1, $P = 0.0012$ for vector-shLuc versus shEZH2-shLuc, and $P = 0.0039$ for vector-shLuc versus shEZH2-shTRAP1. **(C–E)** Cells were treated with the indicated chemotherapeutics for 48 h, and apoptosis induction was assessed using a caspase 3/7 activity assay. All results indicate the mean ± SEM from $n = 3$ biological replicates. Representative data are shown from a representative experiment, which was repeated independently. Significance was assessed by two-way ANOVA, with Tukey correction for multiple hypothesis testing. For the comparison of shEZH2-shLuc versus shEZH2-shTRAP1 transduced cells, $P < 0.0001$ for cells treated with doxorubicin, etoposide, or dexamethasone. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$; n.s., $P > 0.05$.

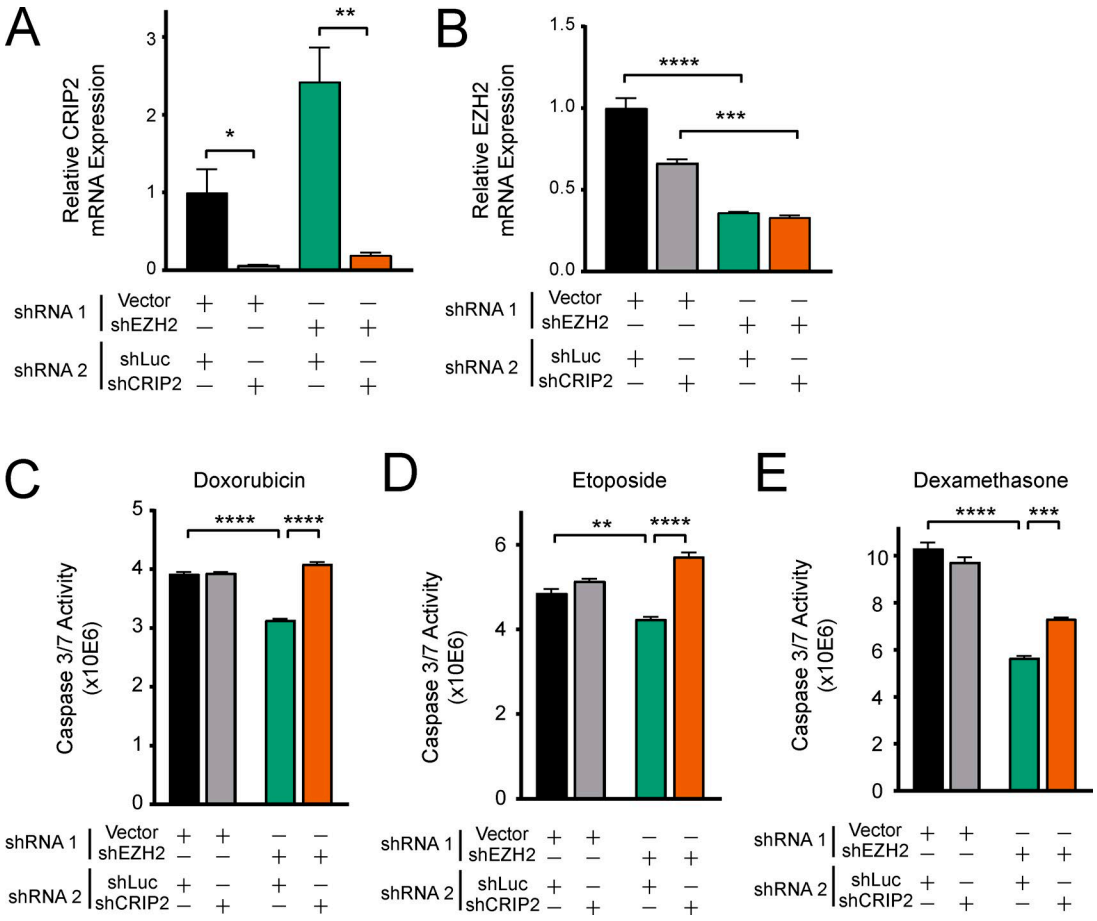

Figure S5. **CRIP2 is required for induction of apoptosis resistance upon *EZH2* depletion.** CCRF-CEM cells were transduced with a neomycin-resistant "shRNA 1" targeting EZH2 or empty vector control and subsequently transduced with a puromycin-resistant "shRNA 2" targeting either CRIP2 or Luciferase control. **(A and B)** Knockdown efficacy was assessed by qRT-PCR of the indicated genes, with β-actin used as control. Results were normalized to the shLuc-alone condition. All results indicate the mean ± SEM of $n = 3$ biological replicates from one representative experiment, which was repeated independently. Significance was assessed by two-way ANOVA, with Tukey correction for multiple hypothesis testing. For CRIP2 mRNA expression, P = 0.023 for vector-shLuc versus vector shCRIP2-transduced cells, and P = 0.0016 for shEZH2-shLuc versus shEZH2-shCRIP2. For EZH2 mRNA expression, P < 0.0001 for vector-shLuc versus shEZH2-shLuc, and P = 0.0006 for shEZH2-shLuc versus shEZH2-shCRIP2. **(C–E)** Cells were treated with the indicated chemotherapeutics for 48 h, and apoptosis induction was assessed using a caspase 3/7 activity assay. All results indicate the mean ± SEM from $n = 3$ biological replicates. Representative data from one experiment are shown, which was repeated independently. Significance was assessed by two-way ANOVA, with Tukey correction for multiple hypothesis testing. P < 0.0001 for all comparisons shown, except P = 0.0029 for vector-shLuc versus shEZH2-shLuc in etoposide-treated cells, and P = 0.0007 for shEZH2-shLuc versus shEZH2-shCRIP2 in dexamethasone-treated cells. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001; ****, P ≤ 0.0001; n.s., P > 0.05.

Tables S1–S7 are included as separate Microsoft Excel files, and Tables S8 and S9 are included as separate text files. Table S1 shows a summary of clinical features and genotyping of the primary T-ALL patient samples analyzed. Table S2 shows association of clinical features with mitochondrial apoptotic priming. Table S3 shows the genes whose protein-coding exons were sequenced by targeted exome sequencing. Table S4 shows results of targeted exome sequencing. Table S5 shows results of array CGH analysis. Table S6 shows results of RNA-seq of primary T-ALL patient samples. Table S7 shows the genotype of EZH2-mutant CCRF-CEM clone E cells. Table S8 shows RNA-seq of CCRF-CEM cells transduced with control or PRC2-targeting shRNAs. Table S9 shows result of H3K27me3 ChIP-seq in CCRF-CEM cells.