Expanded View Figures

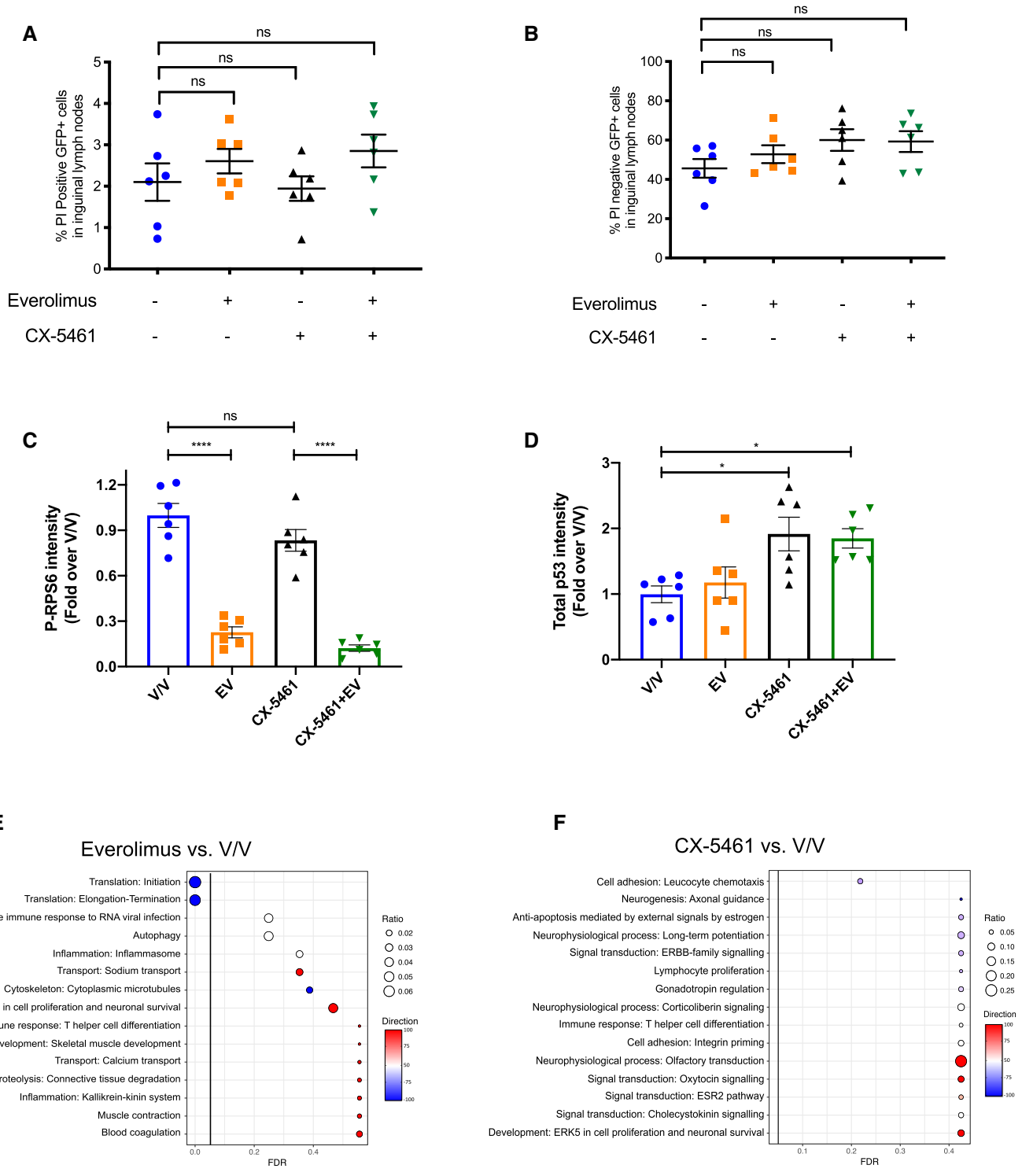

Figure EV1.

Figure EV1. *In vivo* transcriptome-wide analysis of cellular response to ribosome-targeting therapy.

A, B Flow cytometry analysis of inguinal lymph node cells that are (A) GFP-positive and propidium iodide-positive and (B) GFP-positive and propidium iodide-negative isolated from C57BL/6 mice with transplanted Eμ-*Myc* lymphoma cells treated as indicated for 2 h. 10,000 viable cells of the correct morphology were collected for each sample. Graphs represent mean ± SEM of $n = 6$.

C, D Quantitation of (C) phosphorylated RPS6 levels normalized to total RPS6 and (D) p53 protein levels normalized to actin in Fig 1A. Graphs represent mean ± SEM of $n = 6$.

E, F Enrichment analysis by MetaCore® GeneGO of genes in "translation up" and "translation down" categories identified by anota2seq analysis comparing lymph node cells isolated from mice in (E) everolimus single-agent treatment group or (F) CX-5461 single-agent treatment group with those isolated from mice in vehicle group ($n = 6$). "Ratio" is the value obtained by dividing the number of genes in an indicated molecular process that is found in our data with the number of genes curated in MetaCore®'s database. "Direction" visualizes the percentage of genes associated with indicated pathways that are up (red) or down (blue).

Data information: (A–D) Data were analyzed by one-way ANOVA. ns, not significant, $P \geq 0.05$; *$P \leq 0.05$; ****$P \leq 0.0001$.

Source data are available online for this figure.

Figure EV2. Metformin improves the efficacy of CX-5461 as a single agent or in combination with Everolimus *in vitro*.

A Propidium iodide (PI) exclusion analysis of Eμ-*Myc* lymphoma cells treated with CX-5461 in the presence and absence of metformin for 48 h. Graphs represent mean ± SEM of $n = 3$ experiments, and data were analyzed by one-way ANOVA.

B PI exclusion analysis of Eμ-*Myc* lymphoma cells treated with CX-5461 + EV in the presence and absence of metformin as indicated for 48 h. Graphs represent mean ± SEM of $n = 3$ experiments, and data were analyzed by Student's *t*-test.

C–F PI exclusion analysis of (C) MV4-11, (D) SHI-1, (E) SKM-1, and (F) THP-1 human acute myeloid leukemia cell lines in response to treatments with CX-5461, everolimus (EV), and metformin as indicated for 72 h. Graphs represent mean ± SEM of $n = 4$–6, and data were analyzed by two-way ANOVA.

Data information: ns, not significant, $P \geq 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

Source data are available online for this figure.

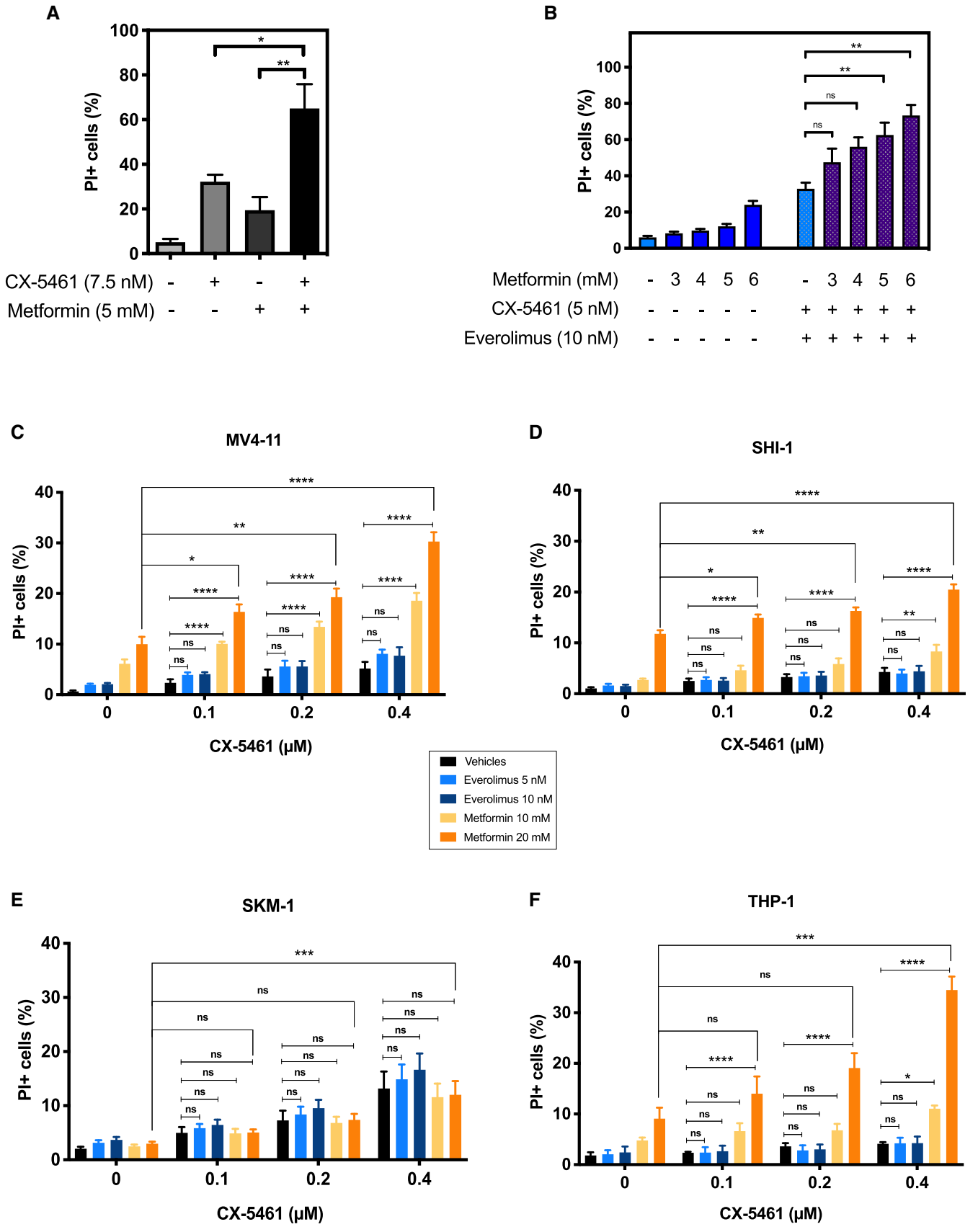

Figure EV2.

Figure EV3. *In vitro* and *in vivo* characterization of the early passage cell lines.

A Kaplan–Meier curve of C57BL/6 mice transplanted with the CX-5461-resistant CX #39 early passage Eμ-*Myc* lymphoma cells treated with CX-5461 (35 mg/kg every 3 days; $n = 5–6$). Survival curves of drug-naïve cells were actual data reproduced from Devlin *et al*, 2016.

B Kaplan–Meier curve of C57BL/6 mice transplanted with CX-5461 + EV-resistant CMB #9 early passage cells treated with CX-5461 (35 mg/kg every 3 days) and everolimus (5 mg/kg daily; $n = 5–6$). Survival curves of drug-naïve cells were actual data reproduced from Devlin *et al*, 2016.

C Western blot analysis of markers for the on-target effect of 20 nM everolimus treatment for 3 h, specifically phosphorylation of RPS6 at Serine 240/244 residues and total RPS6 ($n = 3$). Actin was used as a loading control.

D–J Synthesis rates of 47S pre-rRNAs in (D) drug-naïve (CTRL), (E) everolimus-resistant (EV), (F) CX-5461-resistant (CX), and (G) CX-5461 + EV-resistant (CMB) cell lines in response to 3-h 50 nM CX-5461 treatment were determined by ^{32}P-orthophosphate "pulse" labeling. Synthesis rate of 47S pre-rRNAs in (H) CX and (I) CMB cell lines in response to 10-h 50 nM CX-5461 was determined by ^{32}P-orthophosphate "pulse" labeling and (J) its quantitation ($n = 3$). (D–I) 28S rRNA abundance was used as a loading control. EtBr: ethidium bromide. Data were analyzed by Student's *t*-test. Graphs represent mean ± SEM of $n = 3$. *$P \leq 0.05$. ***$P \leq 0.001$.

K Western blot analysis for p53 abundance in response to 50 nM CX-5461 treatment for 3 h. Actin was used as a loading control ($n = 2–3$).

Source data are available online for this figure.

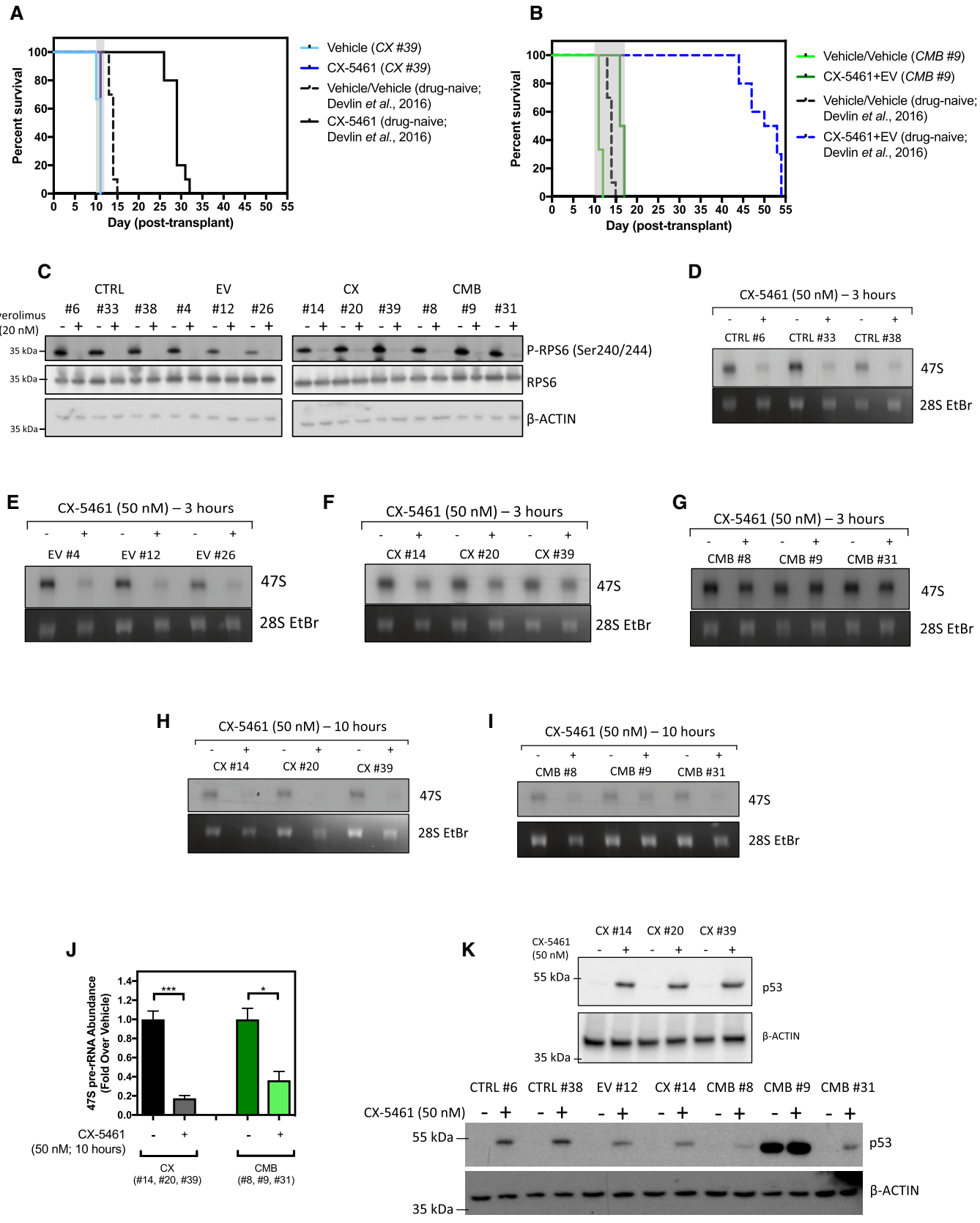

Figure EV3.

Figure EV4. Polysome profiling analysis of the early passage Eμ-*Myc* lymphoma "CX" cell lines and investigating potential roles of *RAPGEF3* (EPAC1) and *RAPGEF4* (EPAC2) in human blood cancers.

A Enrichment analysis by GeneGO MetaCore® of the polysomal RNA-seq data comparing CX-5461-resistant (CX) and drug-naïve cells (CTRL; $n = 3$; false discovery rate (FDR) ≤ 0.05; fold change (FC) ≥ 1.5 or ≤ -1.5).

B, C Median mRNA expression levels of (B) *RAPGEF3* (mRNA encoding EPAC1 protein) and (C) *RAPGEF4* (mRNA encoding EPAC2 protein) generated by gene expression profiling interactive analysis (GEPIA) bioinformatics web tool based on The Cancer Genome Atlas (TCGA) database. LAML: acute myeloid leukemia (tumor, $n = 173$; normal, $n = 70$). DLBC: diffuse large B-cell lymphoma (tumor, $n = 47$; normal, $n = 337$). Red: tumor, black: normal. The central band corresponds to the median. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper and lower whiskers extend from the hinges to the largest and smallest values no further than 1.5 * interquartile range from the hinges, respectively. Data points are plotted individually. Data were analyzed by one-way ANOVA; $*P < 0.05$. tpm, transcript count per million.

D Western analysis of EPAC1 and EPAC2 abundance in white blood cell samples (isolated from healthy donors #PMC-15 and #PMC-16) versus MV4-11, SHI-1, SKM1, and THP1 human AML cell lines (representative of $n = 2$).

E Survival curves of AML patients with low and high EPAC1 (RAPGEF3; $n = 27$ each; cut-off: 50%) generated by GEPIA based on TCGA database.

Source data are available online for this figure.

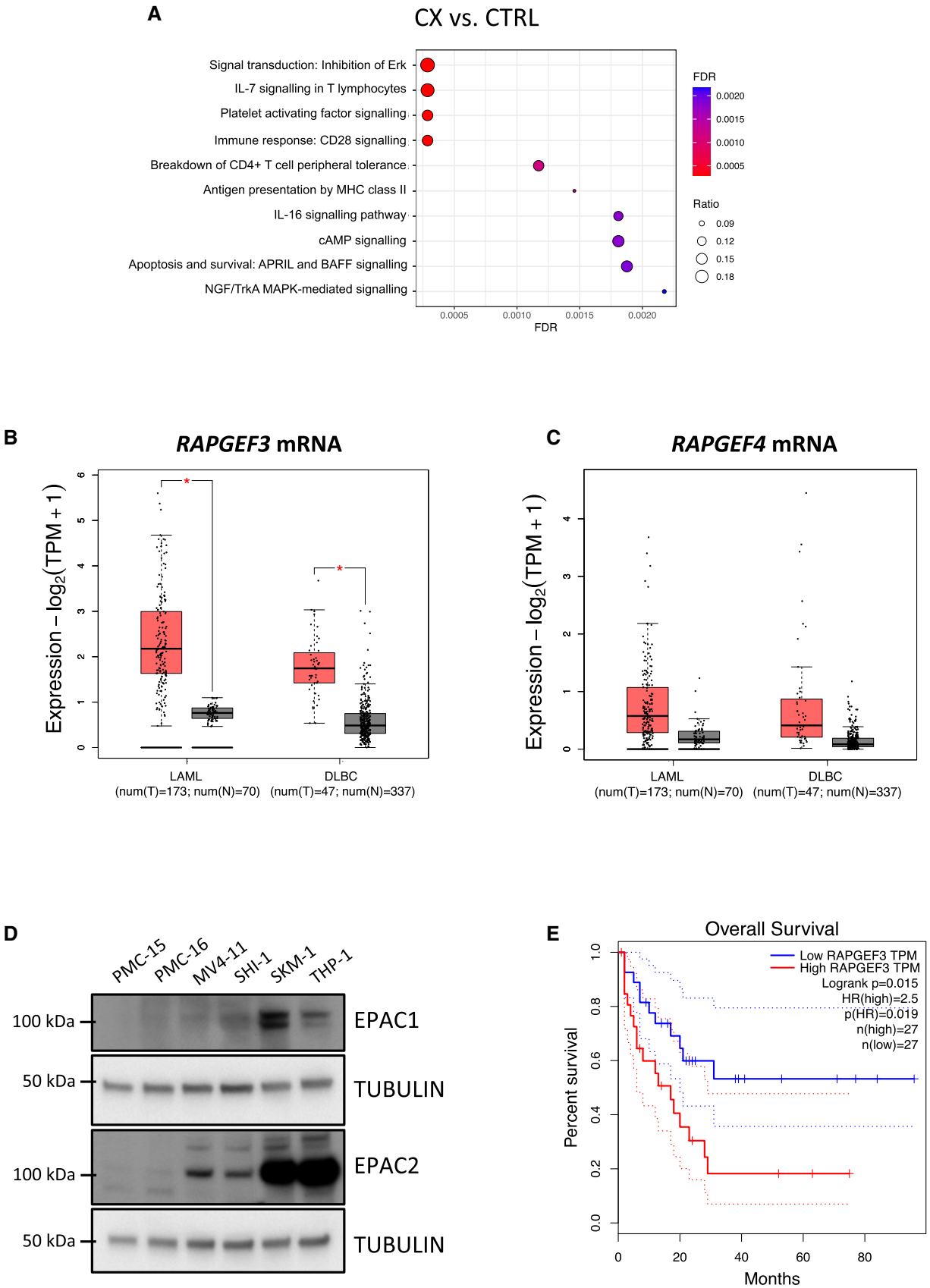

Figure EV4.

Figure EV5. Inhibiting EPAC1/2 in human acute myeloid leukemia (AML) cell lines and *in vivo*-derived Eμ-*Myc* lymphoma cell lines.

A Western analysis of the activated, GTP-bound RAP1 of the indicated human AML cell lines following treatments with the EPAC1 inhibitor CE3F4 and EPAC2 inhibitor ESI-05 for 72 h (representative of $n = 2$).

B CellTiterGLO®-based viability assay of MV4-11, SHI-1, SKM-1, and THP-1 human AML cells treated as indicated for 72 h. Graphs represent mean ± SEM of $n = 3$. Data were analyzed by one-way ANOVA.

C, D Western analysis of PKA activity using the phosphorylated PKA substrate antibody on whole cell extracts isolated from the indicated early passage cell lines treated for 24 h with (C) H89 ($n = 3$) or (D) 6-BNZ-cAMP (representatives of $n = 3$). Actin was used as a loading control.

E Western analysis of the activated, GTP-bound RAP1 of the early passage CX-5461 + EV-resistant (CMB) cell extracts isolated following treatments with indicated EPAC inhibitors for 24 h (representative of $n = 2$).

F Western analysis of activated, GTP-bound RAP1 using early passage drug-naive (CTRL) Eμ-*Myc* lymphoma cell extracts isolated following treatments with the selective EPAC activator 8-pCPT-2-O-Me-cAMP for 24 h ($n = 1$).

G Western analysis of the activated, GTP-bound RAP1 of the early passage CX-5461-resistant (CX) cell extracts isolated following treatments with indicated EPAC inhibitors for 24 h ($n = 1$).

H PI exclusion analysis of the CX cells treated with CX-5461 as indicated in the presence or absence of EPAC1 inhibitor CE3F4 or EPAC2 inhibitor ESI-05 for 48 h. Data were analyzed by paired one-way ANOVA. Graphs represent mean ± SEM of $n = 3$. Black triangle: CX-5461-resistant (CX) cells clone #14, black square: CX cells clone #20, black circle: CX cells clone #39.

Data information: ns, not significant, $P \geq 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

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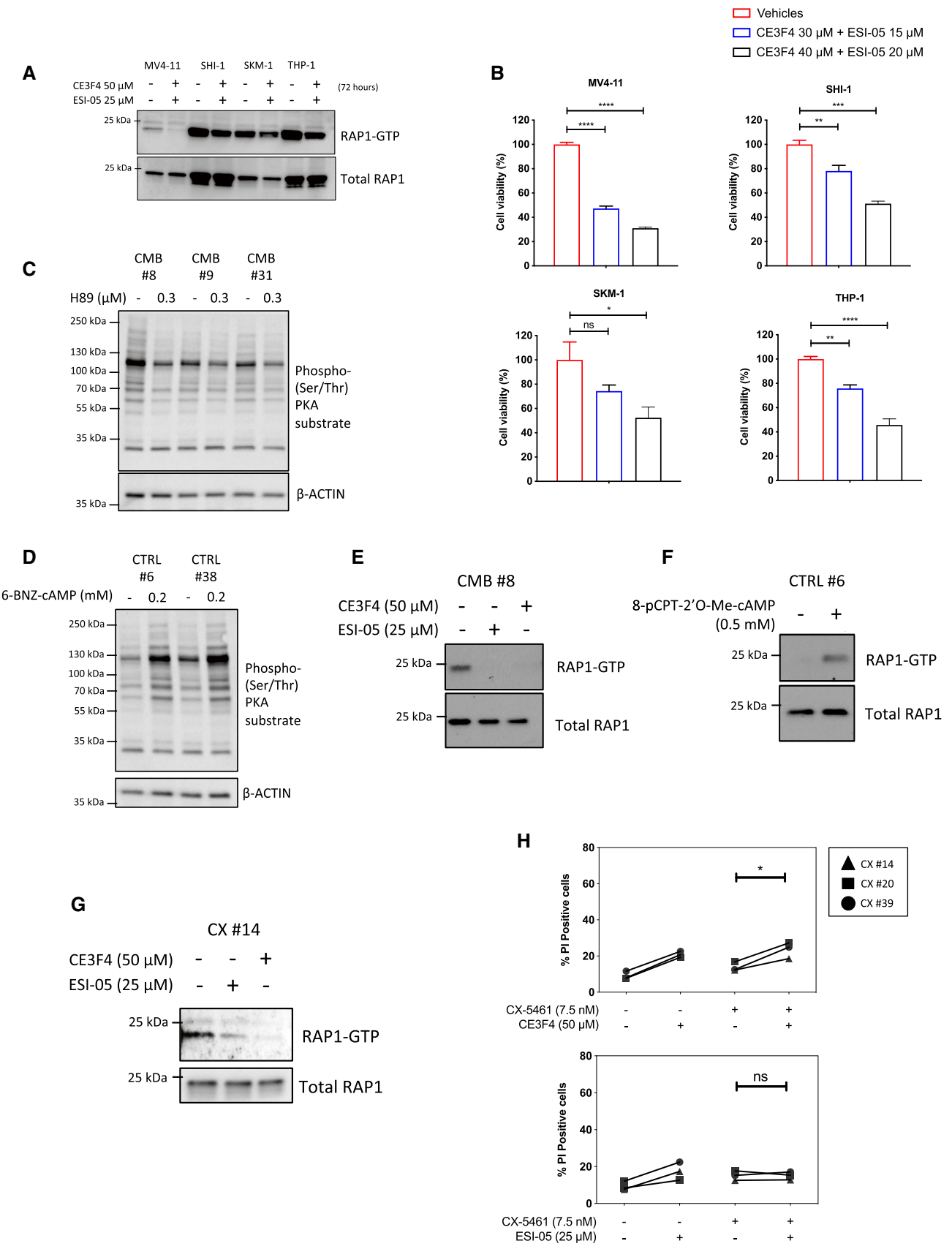

Figure EV5.