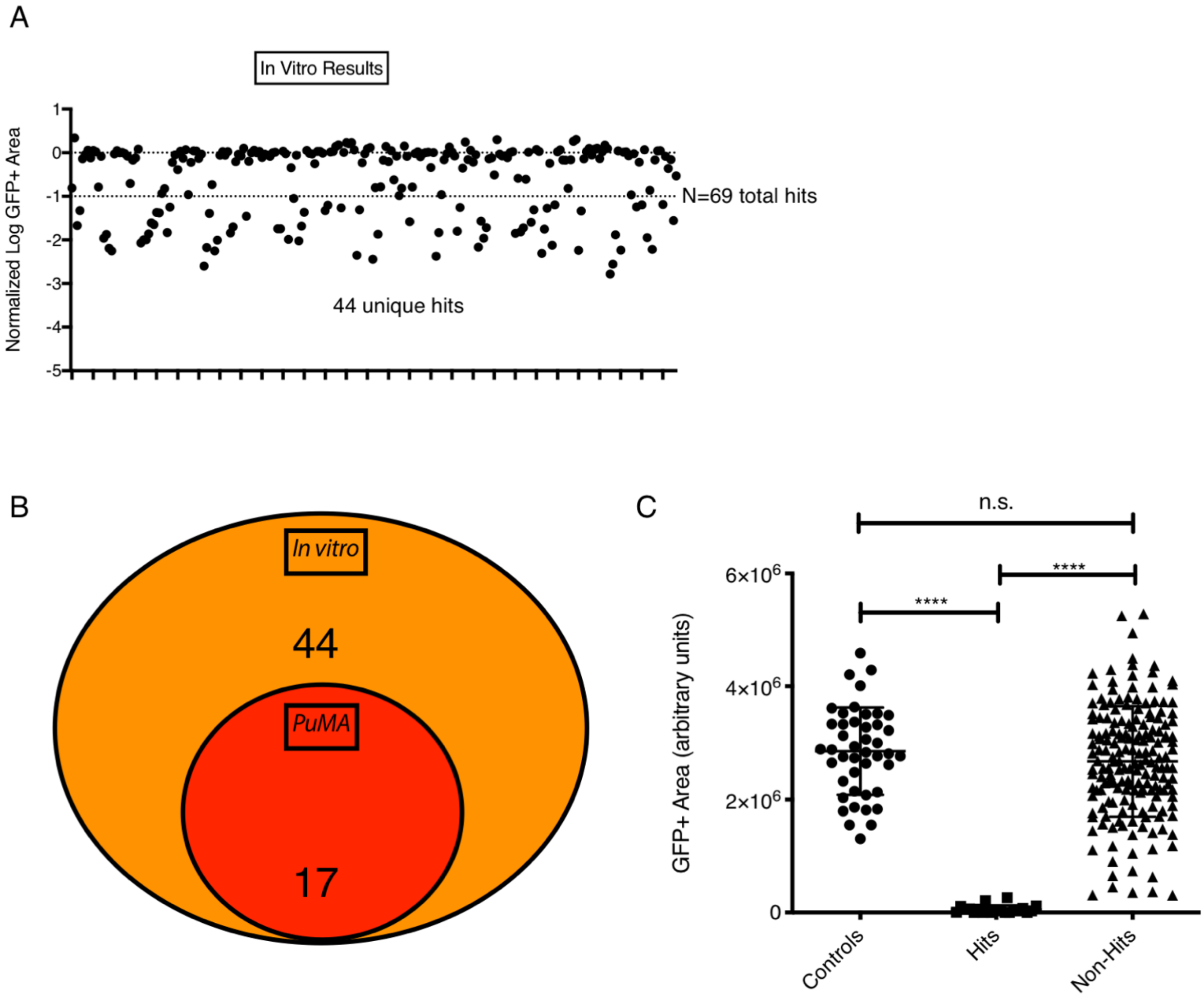
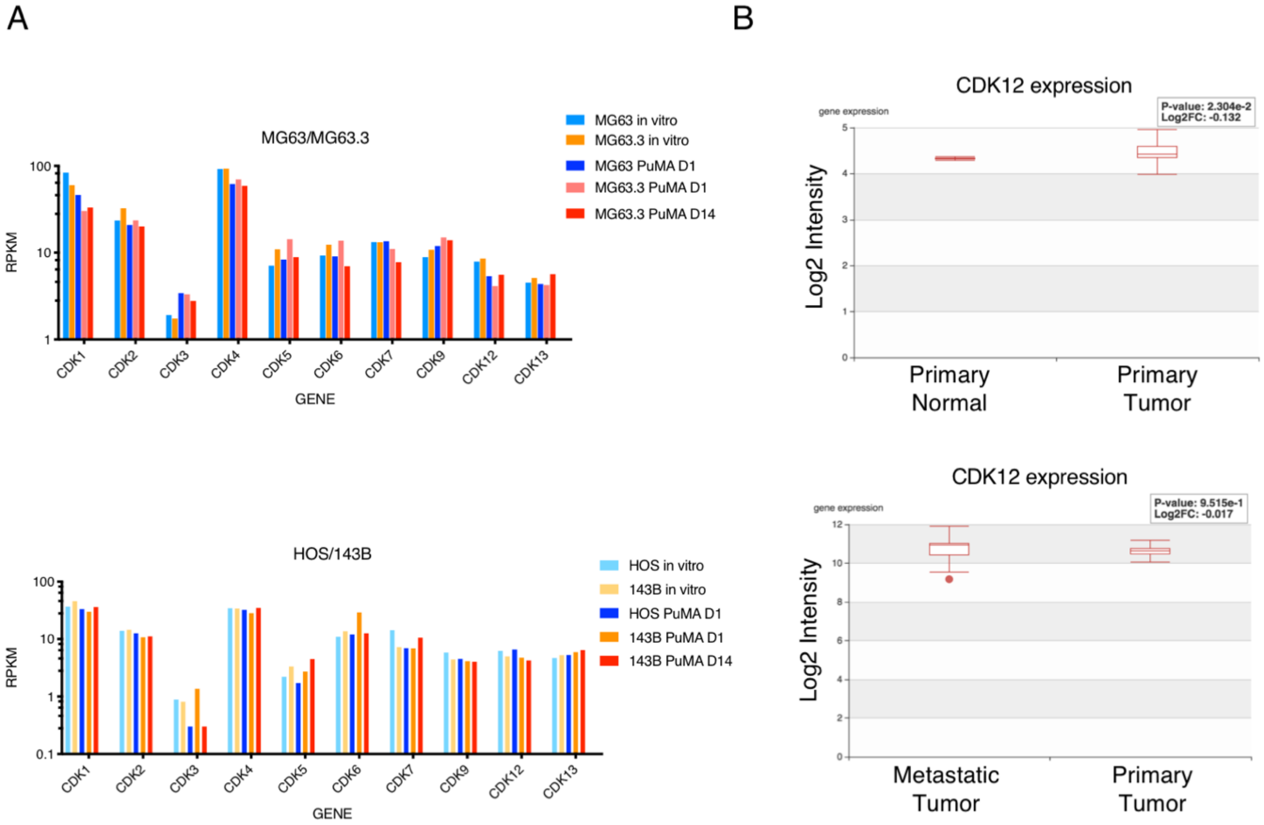
Supplemental Figure 1



Supplemental Figure 1. (A) Scatterplot showing all hits in the parallel in vitro screen in MG63.3 cells. (B) Venn diagram showing overlap of unique hits between the in vitro screen and PuMA screen. (C) Comparison of GFP+ Area for all lung explants of the ex vivo screen. Data are presented as means +/- SD of GFP+ area for the explants. Ordinary one-way ANOVA with Tukey's multiple comparison test used with *** $p < 0.001$ and n.s. meaning "not significant".

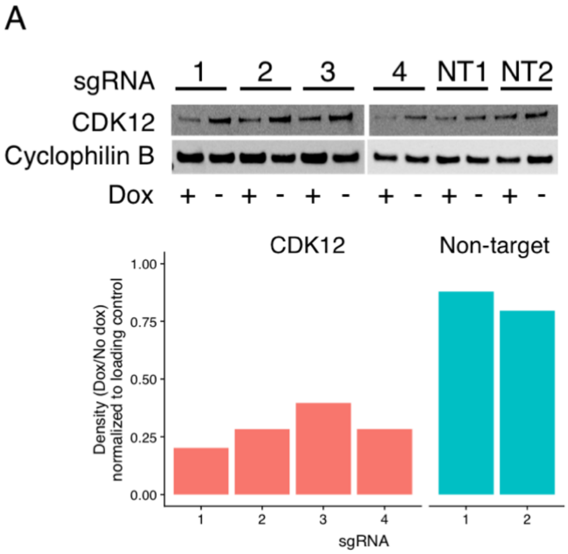
Supplemental Table 1: Full list of compounds used in the PuMA and In vitro screen together with dosages and the percent killing (reduction in GFP+ area compared to control) that was achieved at each dose. Compounds highlighted in yellow were “hits” with at least one dose achieving 90% reduction in the GFP+ area of OS cells.

Supplemental Figure 2



Supplemental Figure 2. (A) RNA-seq data for the indicated CDK gene. Top; MG63 and MG63.3 isogenic cell lines in vitro and in PuMA ex vivo culture (D1 indicates 1 day in the PuMA ex vivo culture and D14 indicates 14 days in the PuMA ex vivo culture). Bottom; HOS and 143B isogenic cell lines in vitro and in PuMA ex vivo culture. (B) Patient data from the Human Cancer Metastasis Database. Top shows the microarray expression data for CDK12 comparing primary normal tissue to primary tumor tissue. Bottom shows microarray expression data for CDK12 comparing metastatic tumor tissue to primary tumor tissue.

Supplemental Figure 3



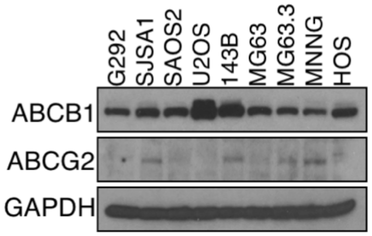
Supplemental Figure 3 (A) Western blot of whole cell isolates of MG63.3 GFP/Cas9/Tet cells with the indicated guides and doxycycline state. Quantification of blot band density normalized to Cyclophilin B loading control shown below the blot.

Supplemental Figure 4

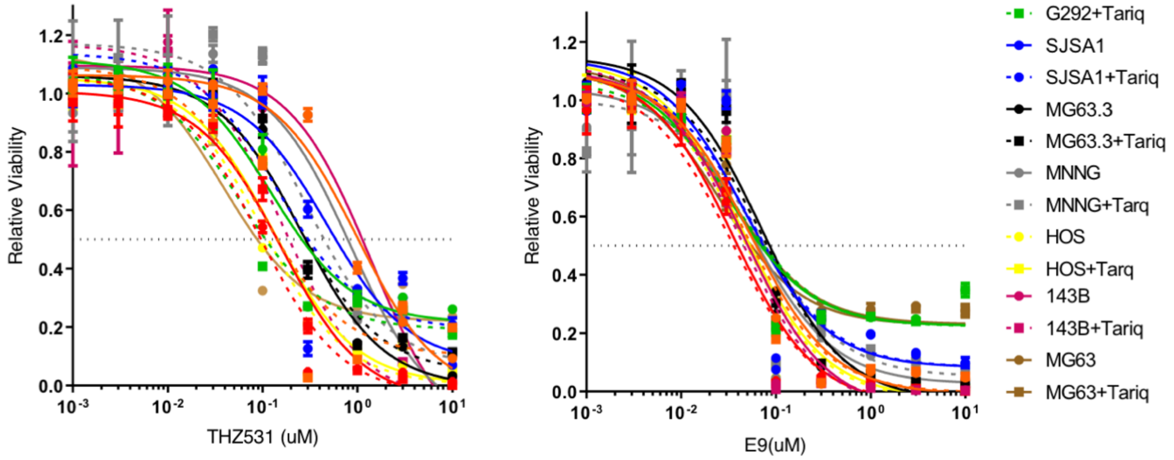
A

IC50 values (nM)	THZ531	THZ531 +Tariq	E9	E9 +Tariq
U2OS	1000	130	60	55
SAOS2	150	100	37	37
G292	350	150	100	100
SJSA1	640	360	50	60
MG63.3	260	270	75	75
MNNG	650	330	80	80
HOS	120	130	50	40
143B	600	150	47	40
MG63	120		90	85

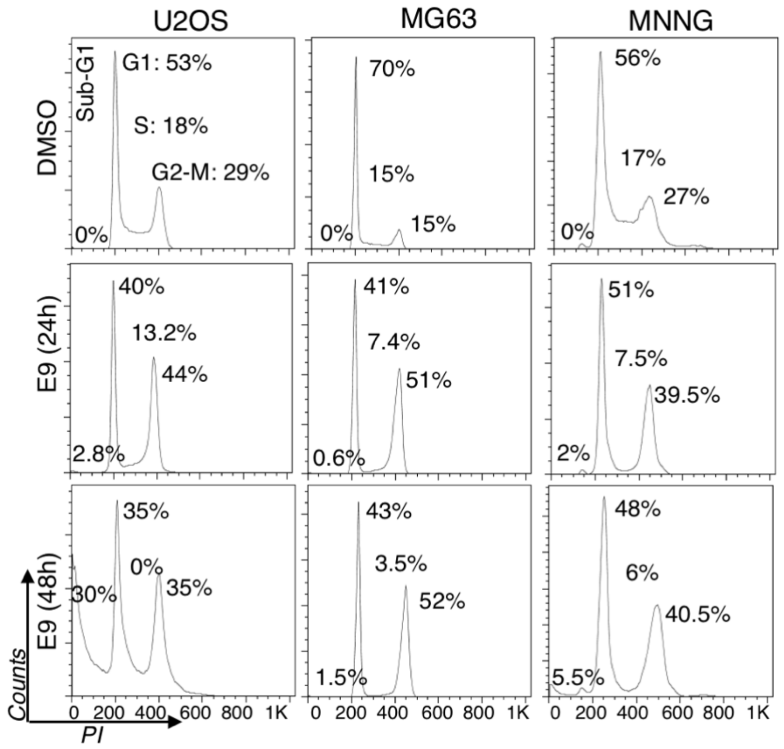
B



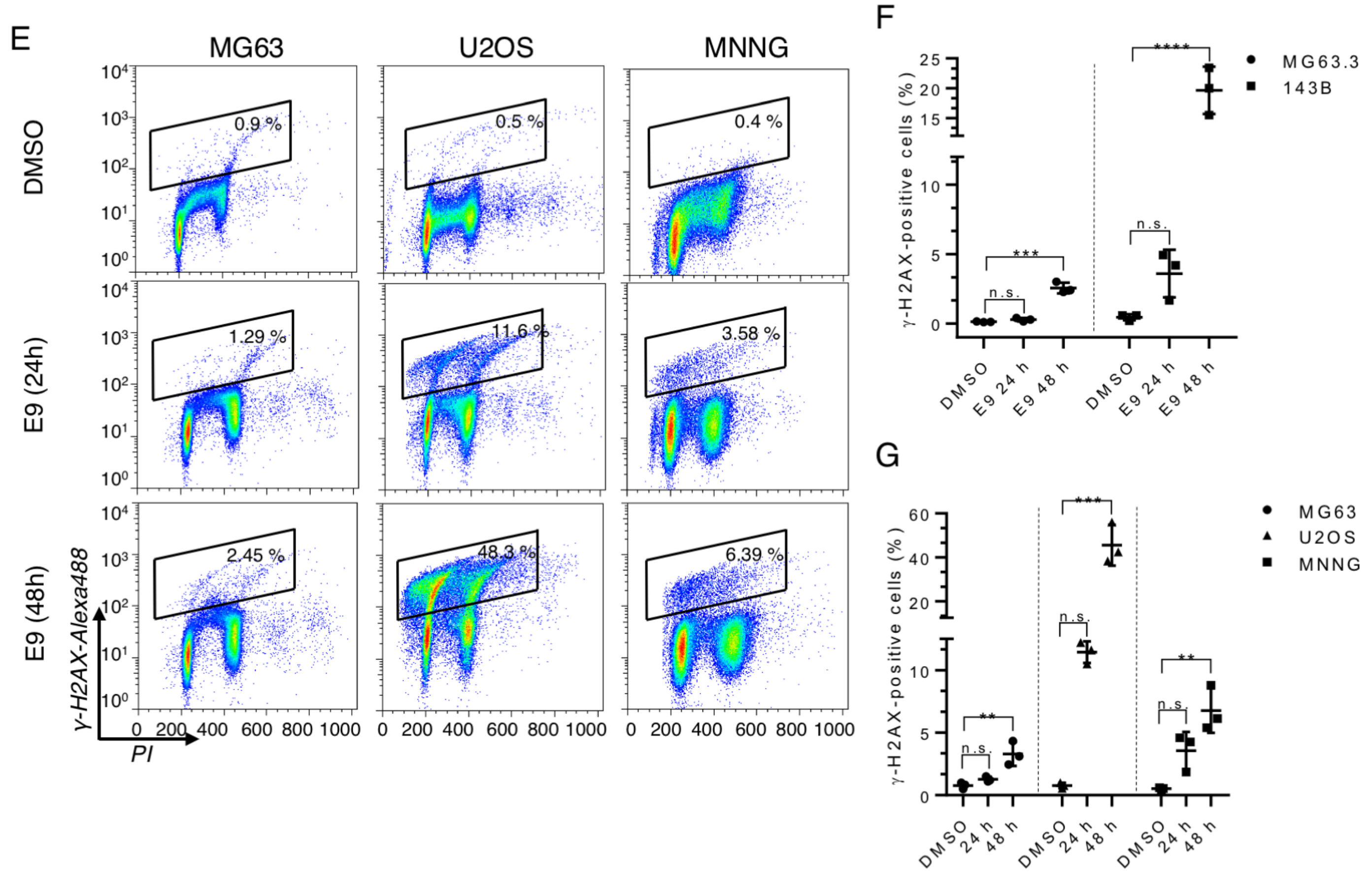
C



D

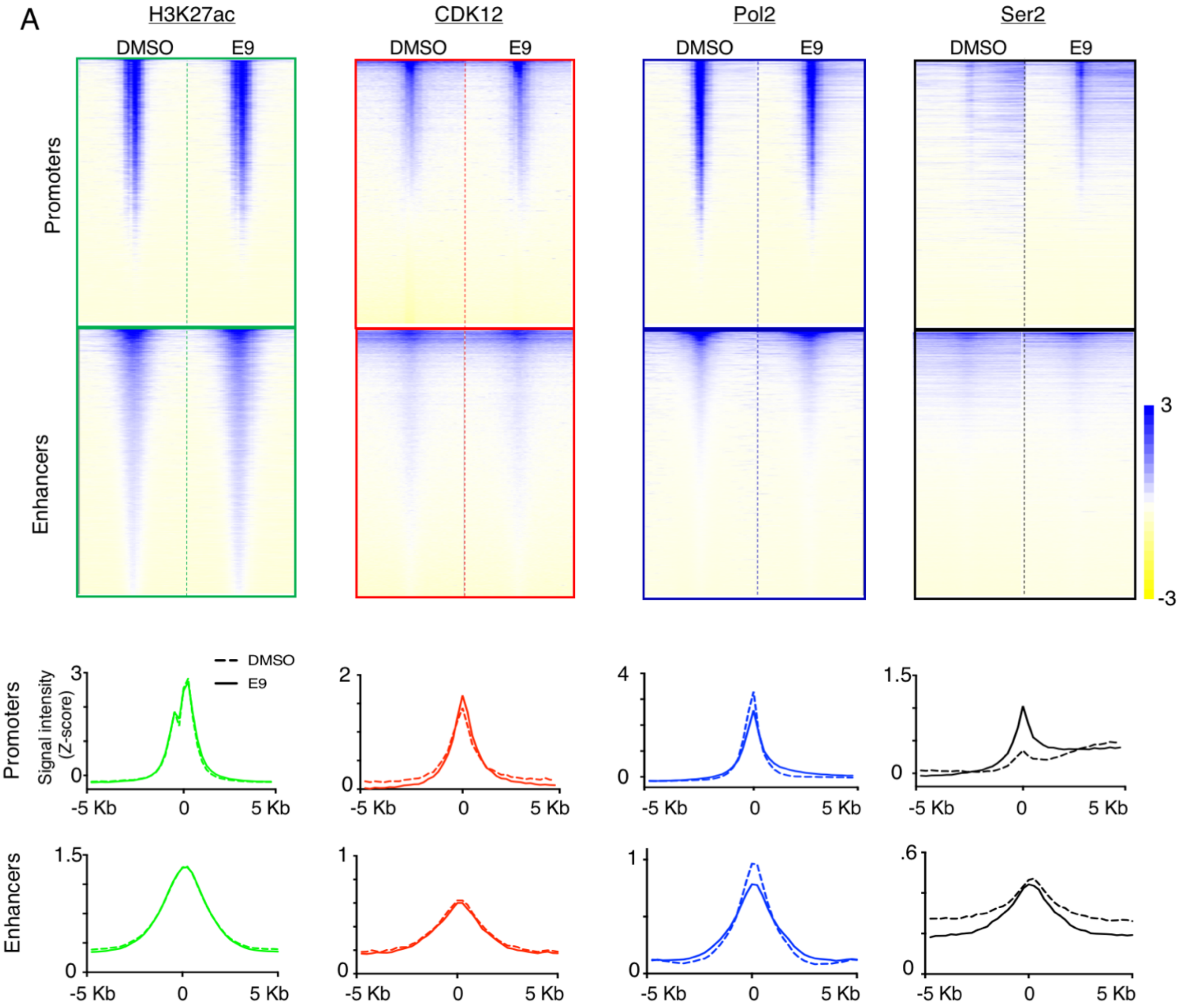


Supplemental Figure 4 cont.

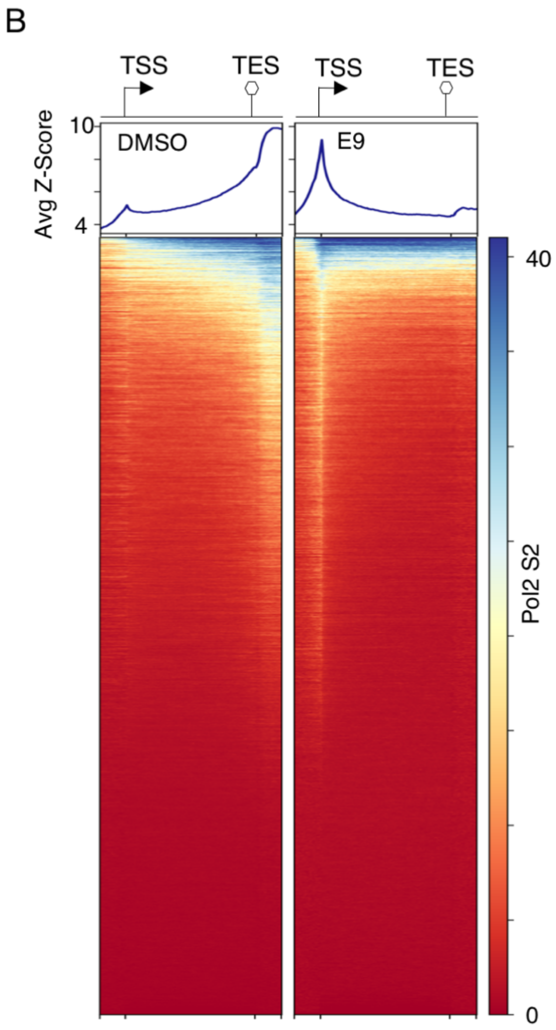


Supplemental Figure 4. (A) IC$_{50}$ values for THZ531 and E9 alone and in combination with tariquidar (125 nM) in OS cells at 72 h. (B) Western blot analysis of ABCB1 and ABCG2 in OS cell lines. GAPDH, loading control. (C) Dose-response curves for OS cells treated with increasing concentrations of THZ531 (*left*) or E9 (*right*) for 72 hr alone and in combination with tariquidar (125 nM). (D) Cell-cycle analysis of OS cell lines exposed to 400 nM of E9 for 24 and 48 hours by flow cytometry with propidium iodide (PI) staining. The scale and axes are indicated in the lower left corner. (E) Flow cytometry analysis of γ-H2AX staining in OS cell lines treated with 400 nM E9 for the indicated time points. Quantification (F and G) of three independent experiments, means ± SD (F and G). **$p < 0.01$, ***$p < 0.001$, **** $p< 0.0001$, and n.s. means “non-significant”. One-way ANOVA with Dunnett’s multiple comparison correction used to analyze the data.

Supplemental Figure 5

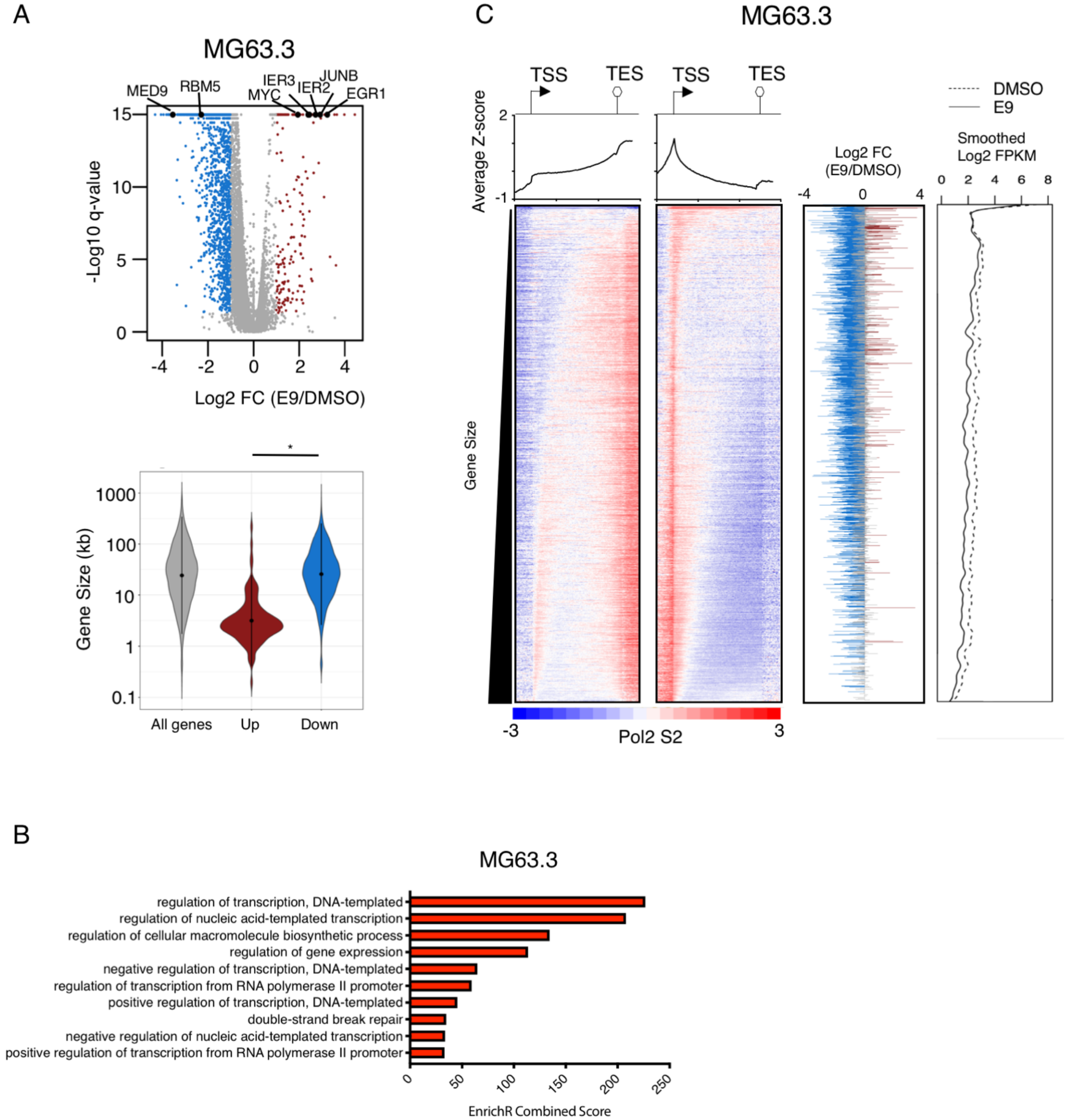


Supplemental Figure 5 Cont.

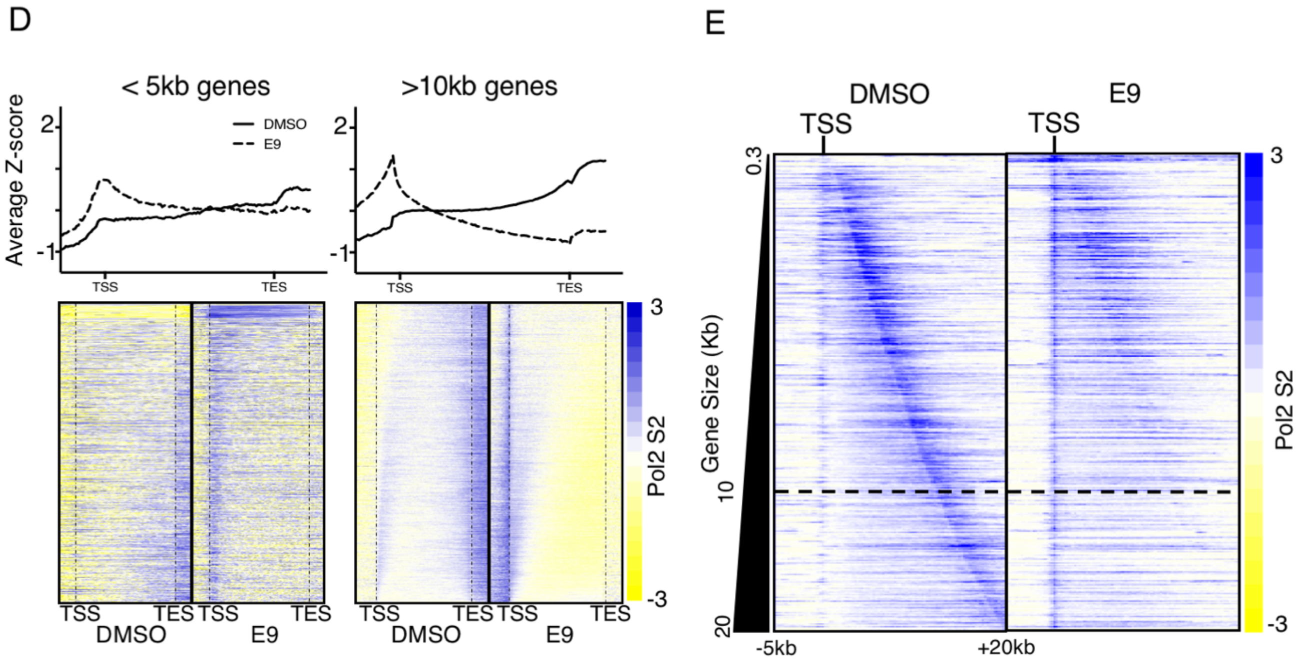


Supplemental Figure 5: (A) Windowed heat maps showing H3K27ac, CDK12, RNA Pol2 and RNA Pol2 Ser2 ChIP-seq signals +/- 5 kb from the TSS in the MG63.3 cells. Below are aggregate plots showing the respective ChIP-seq signals for the above heatmaps. (B) Metagene analysis of Ser2 ChIP-seq signal in MG63.3 cells across all genes sorted in descending order based on the average signal per binned region. Windows encompass 1 kb upstream of the TSS and 1 kb downstream of the TES.

Supplemental Figure 6

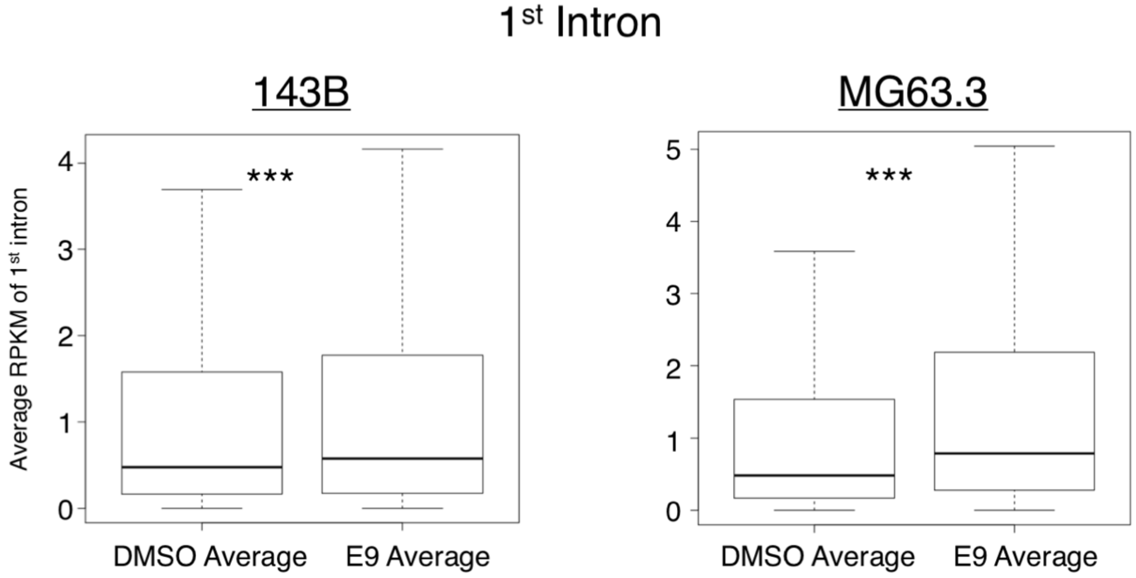


Supplemental Figure 6 Cont

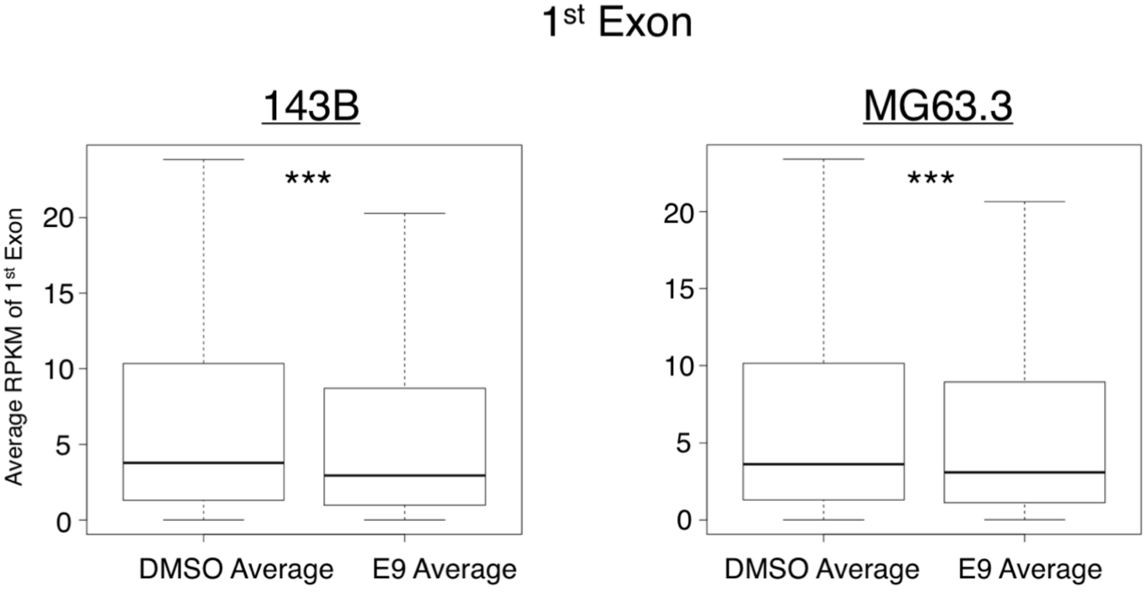


Supplemental Figure 6 Cont

F



G



Supplemental Figure 6:(A) Top; Volcano plot of RNA-seq-based expression differences between E9 versus control-treated MG63.3 cells. 2321 genes changed >2 fold (q value <0.05). RNA-seq for the cell line and condition was performed in triplicate. Bottom; Violin plot of gene sizes for the indicated gene categories in MG63.3. Mann-Whitney U test was used with $*p < 0.001$. (B) Gene ontology scores and associated terms from of all genes downregulated >2 fold in MG63.3 cells using EnrichR. Terms ranked on EnrichR combined scores. (C) Heatmap of Ser2 ChIP-seq signal in MG63.3 cells ranked by increasing gene size. Aggregate plots shown above. Plotted immediate right are corresponding fold changes in transcript levels upon E9 treatment. The smoothed line plot far right denotes baseline transcript levels and E9 treated transcript levels. All genes are ordered the same in all plots. (D) Heatmap of Ser2 ChIP-seq signal in MG63.3 cells in a metagene analysis +/- 1 kb of all active genes of the indicated size, ranked by size. Aggregate plots shown above. (E) Heatmap of Ser2 ChIP-seq signal in MG63.3 cells +5 kb and -20 kb of the TSS for all active genes up to 20 kb in length, sorted by increasing gene length. The dark blue signal that runs diagonally from the top to the bottom of the left heatmap corresponds to TESs. (F) Box and whisker plot of average RPKM signal from the first intron in all active genes comparing DMSO versus E9-treated cells. Each boxplot represents the average RPKM from three independent RNA-seq experiments. *** $p < 0.0001$ by the Mann-Whitney U test. Values of minimum, 1st quartile, median, 3rd quartile, and maximum for each boxplot as follows. 143B DMSO average: 0.000602, 0.165718, 0.476874, 1.577185, 3.693497. 143B E9 Average: 0.00424, 0.174923, 0.577634, 1.771375, 4.162403. MG63.3 DMSO Average: 0.00059, 0.168906, 0.482218, 1.536233, 3.585073. MG63.3 E9 Average: 0.000629, 0.279225, 0.788004, 2.186375, 5.044133. (G) Box and whisker plot of average RPKM signal from first exon in all active genes for DMSO versus E9-treated cells. Each boxplot represents the average RPKM from three independent RNA-seq experiments. *** $p < 0.0001$ by the Mann-Whitney U test. Values of minimum, 1st quartile, median, 3rd quartile, and maximum for each boxplot as follows. 143B DMSO Average: 0.01081103, 1.31023567, 3.77867333, 10.3335, 23.8472333. 143B E9 Average: 0.00933497, 0.988045, 2.94453, 8.71289833, 20.2828667. MG63.3 DMSO Average: 0.01127269, 1.304939167, 3.61973667, 10.1472517, 23.4097667. MG63.3 E9 Average: 0.02033345, 1.1258785, 3.09256333, 8.93956333, 20.6483333.

Supplemental Table 2: Gene set enrichment analysis performed on downregulated genes (>1.5 fold, q<0.01, from Volcano plot) in both the 143B and MG63.3 cell lines after E9 treatment against the C5 GO gene sets. The top 100 significant terms are listed for each line.