Supplementary Figures

Fig. S1

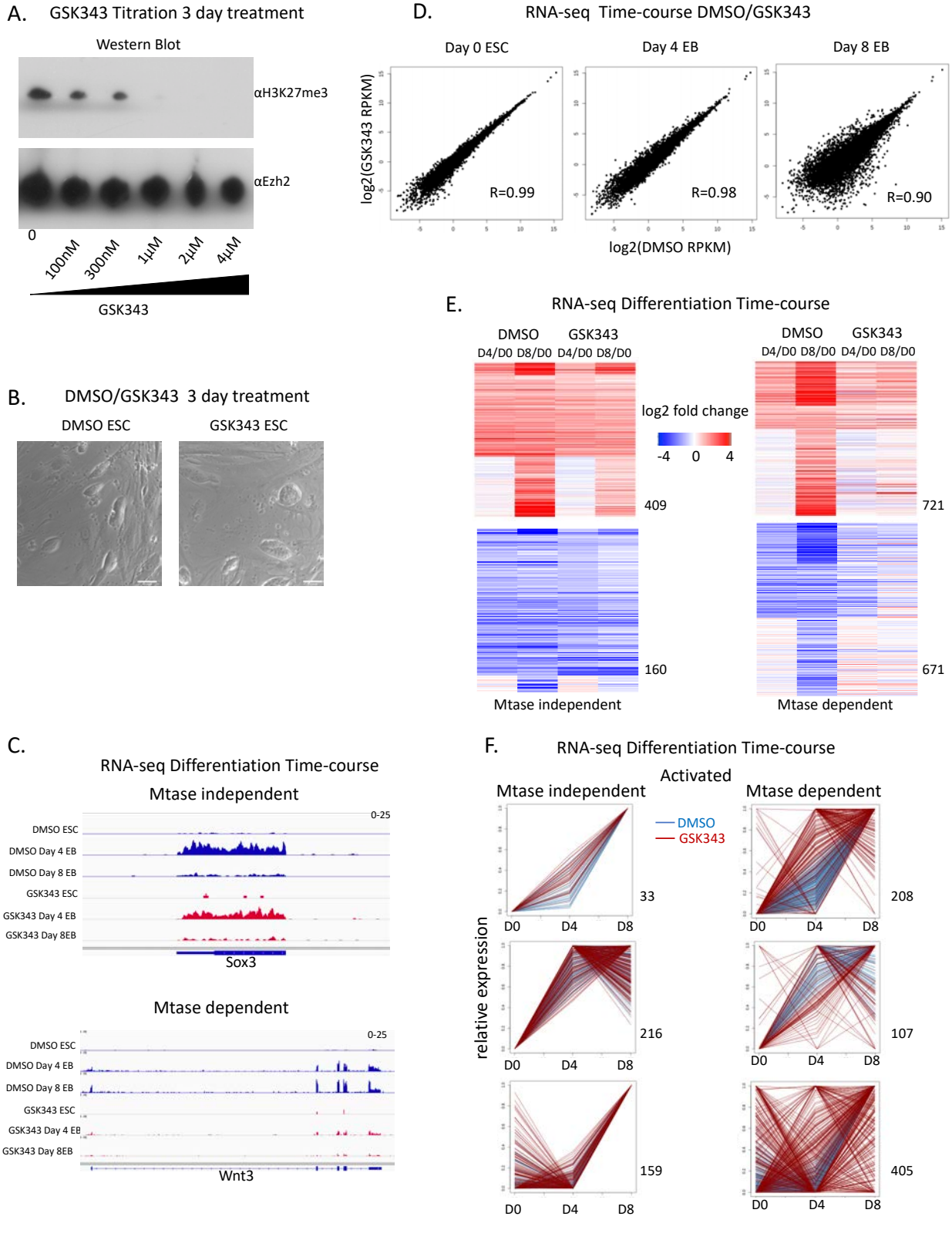


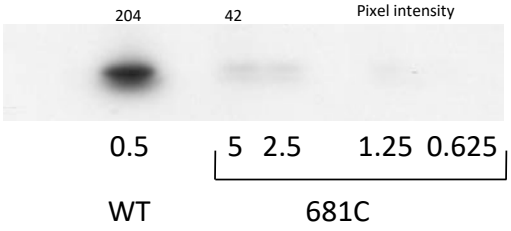
Fig. S1

(A) Western blot of WT CJ7 cells treated for three days with an increasing concentration of GSK343 probed with antibodies to H3K27me3 and EZH2. The 4μM concentration was non-toxic to cells and yielded a loss of H3K27me3 signal and so this concentration was used for all experiments. (B) 54x magnification of ES cells treated for three days with DMSO or GSK343. ES colonies show characteristic halo and are indistinguishable between treatments. (C) Screen shots from RNA-seq over the EB differentiation time-course from Mtase independent (Sox3) and Mtase dependent (Wnt3) genes from cells treated with DMSO (blue) or GSK343 (red). (D) Comparisons of log2 (RPKM) from cells treated with DMSO or GSK343 and differentiated as embryoid bodies. At the genome level there are many genes that have the same expression pattern across the two treatments. All expressed genes (14,608) are shown and Pearson correlation coefficient is calculates as the R value. (E) Heatmaps showing the log2 fold change at PRC2 target genes over EB differentiation time-course separated into Mtase dependent and independent genes as in Fig. 1E. (F) Line diagrams showing relative gene expression of activated PRC2 target genes from (Fig. 1E) separated by Mtase dependent and independent genes and expression pattern in DMSO treated cells.

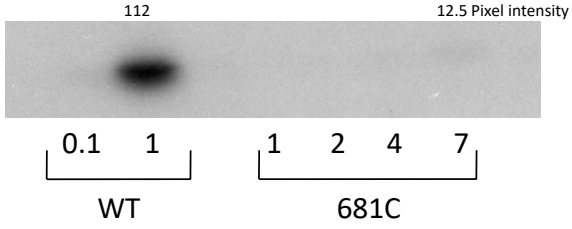
Fig. S2

A.

In vitro methyltransferase assay

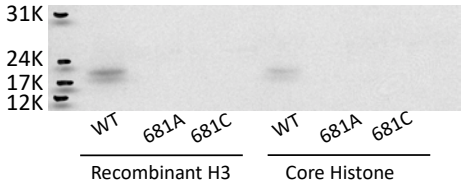


B. In vitro methyltransferase assay

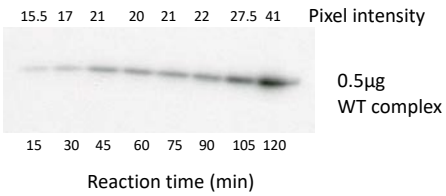


C.

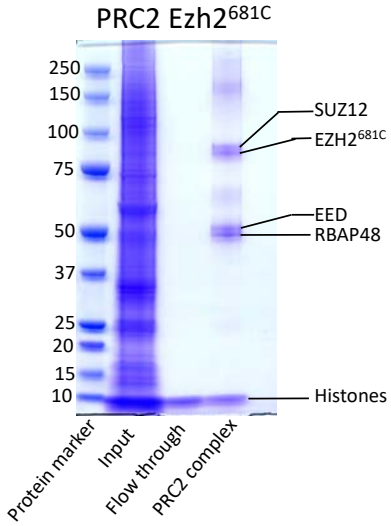
In vitro Methyltransferase Assay



D. In vitro Methyltransferase Assay



E. Protein purification



F.

Cell Images 54X magnification

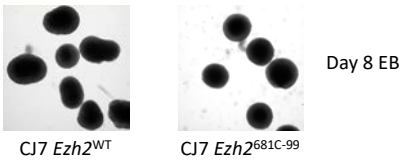


Fig. S2
(A,B) In vitro methyltransferase assay comparing EZH2-WT and EZH2-681C. Concentration of the PRC2 complex is indicated below the image with pixel intensity indicated above. (C) In vitro methyltransferase assay with WT, 681A and 681C complexes with both recombinant histones that do not have any covalent modifications and core histones that do as the substrate. With both substrates, the mutant complexes did not show signal. (D) In vitro methyltransferase assay with WT complex over time. All other methyltransferase assays shown here were done for 60 minutes which is well within the linear range. (E) Coomassie stain of mutant PRC2 complex. Complex was purified from Sf9 cells by using a Flag-tagged SUZ12 and purifying over flag beads. (F) 54x magnification of embryoid bodies that have differentiated for 8 days from WT and 681C-99 cells.

Fig. S3

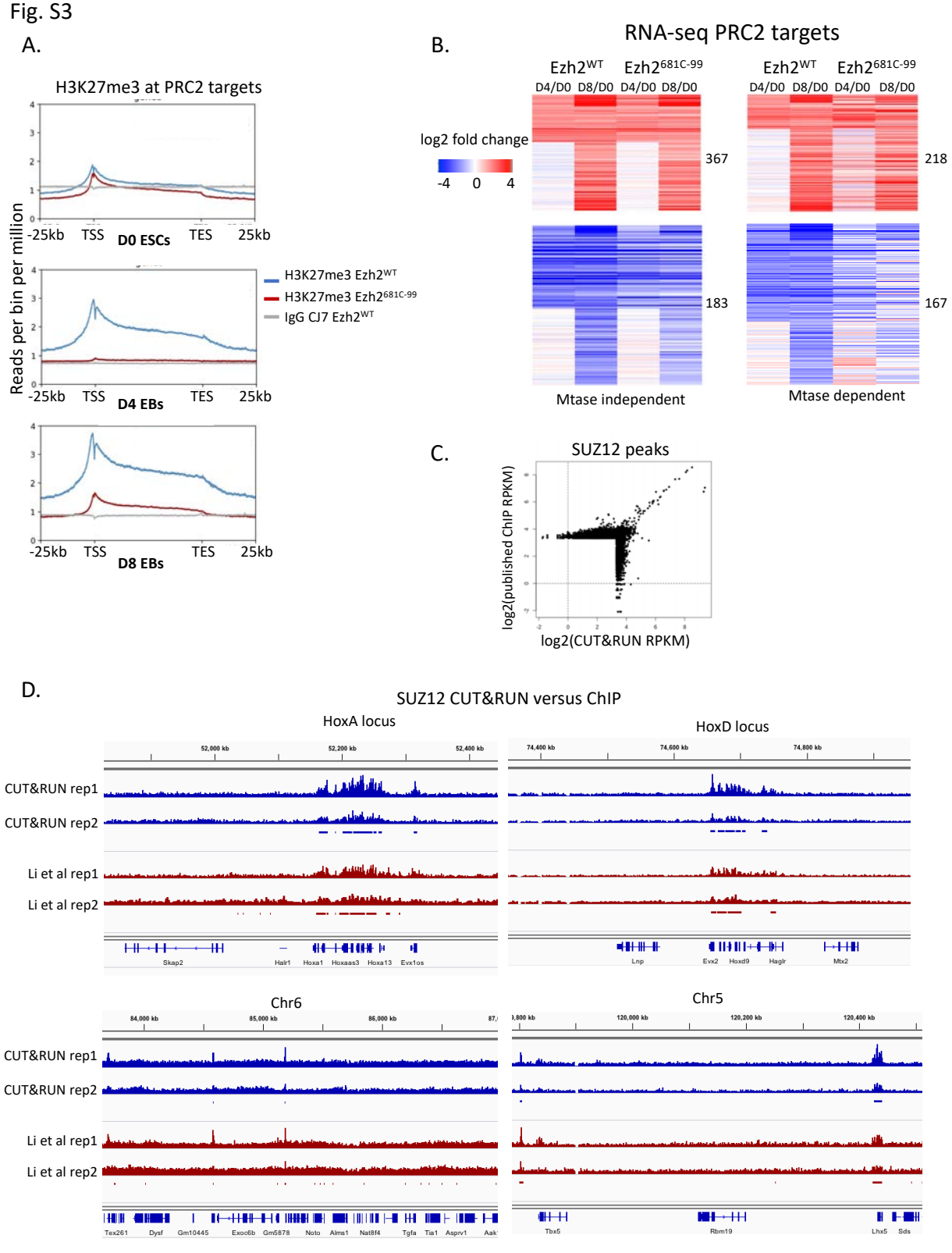


Fig. S3
(A) Average metagene profiles for H3K27me3 or IgG CUT&RUN from WT or 681C-99 mutant cells over the differentiation time-course at 7190 PRC2 target genes. (B) Heatmaps showing the log2 fold change of RNA-seq data at PRC2 target genes over EB differentiation time-course separated into Mtase dependent and independent genes from WT and 681C-99 cells. (C) Comparison of SUZ12 peaks called by Li et al 2017 or the CUT&RUN experiment in log2 RPKM. 72598 peaks, merged from both experiments were compared. (D) Screen shots showing the two replicates of CUT&RUN SUZ12 (blue) or Li et al ChIP-seq (red) at the HoxA locus, HoxD locus, Chromosome 6 around Gm5878 and Chromosome 5 around Tbx5 and Lhx5.

Fig. S4

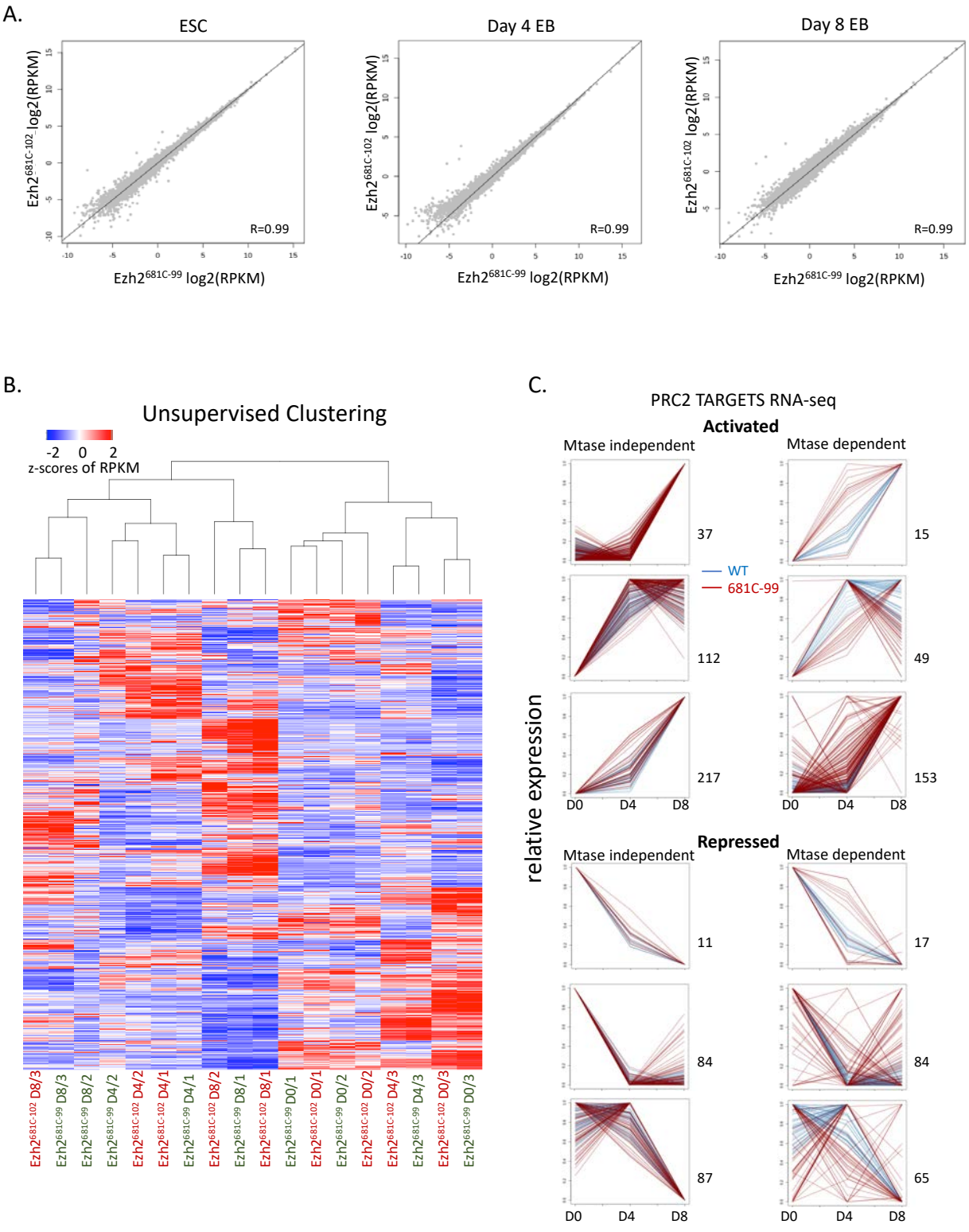


Fig. S4

(A) Comparisons between Ezh2$^{618C-99}$ and Ezh2$^{681C-102}$ RNA-seq over the differentiation time-course. The two mutants are nearly identical at all time points. All expressed genes (15,108) were used for the comparisons. Pearson correlation coefficient was calculated for R value. (B) Unsupervised clustering of Ezh2$^{618C-99}$ and Ezh2$^{681C-102}$ RNA-seq showing that the day of differentiation is more relevant predictor of similarity rather than the mutant strain. All expressed genes are shown (15,108). (C) Line diagrams showing relative gene expression of PRC2 target genes from WT and 681C-99 samples separated by Mtase dependent and independent genes and expression pattern in WT treated cells.

Fig. S5

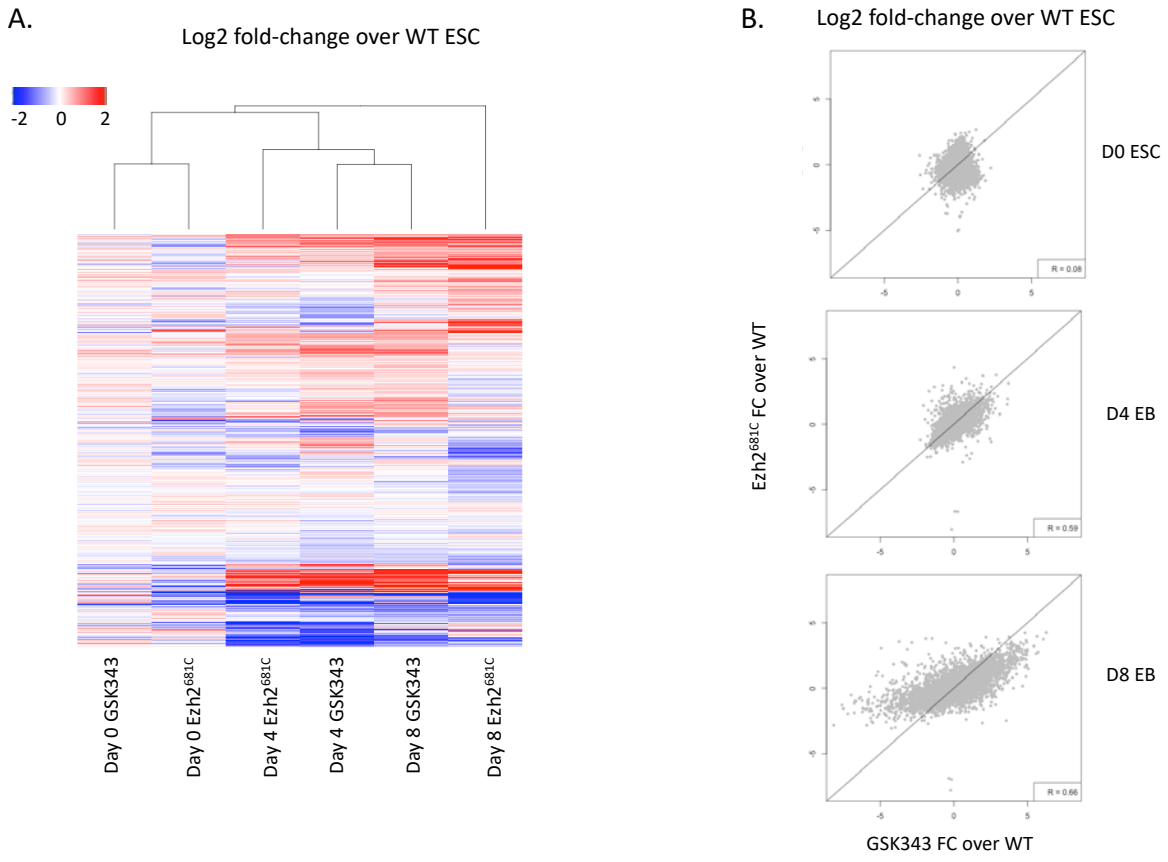


Fig. S5
(A) Unsupervised clustering showing the fold change over WT ESC of GSK343 treated or Ezh2$^{681C-99}$ cells. The day of differentiation is the stronger predictor of clustering. All expressed genes (14,387) are shown.
(B) Direct comparison of mutant and drug treated cells RNA-seq fold change over WT gene expression. All expressed genes (14,387) are shown. Pearson correlation coefficient is shown as R value.

Fig. S6

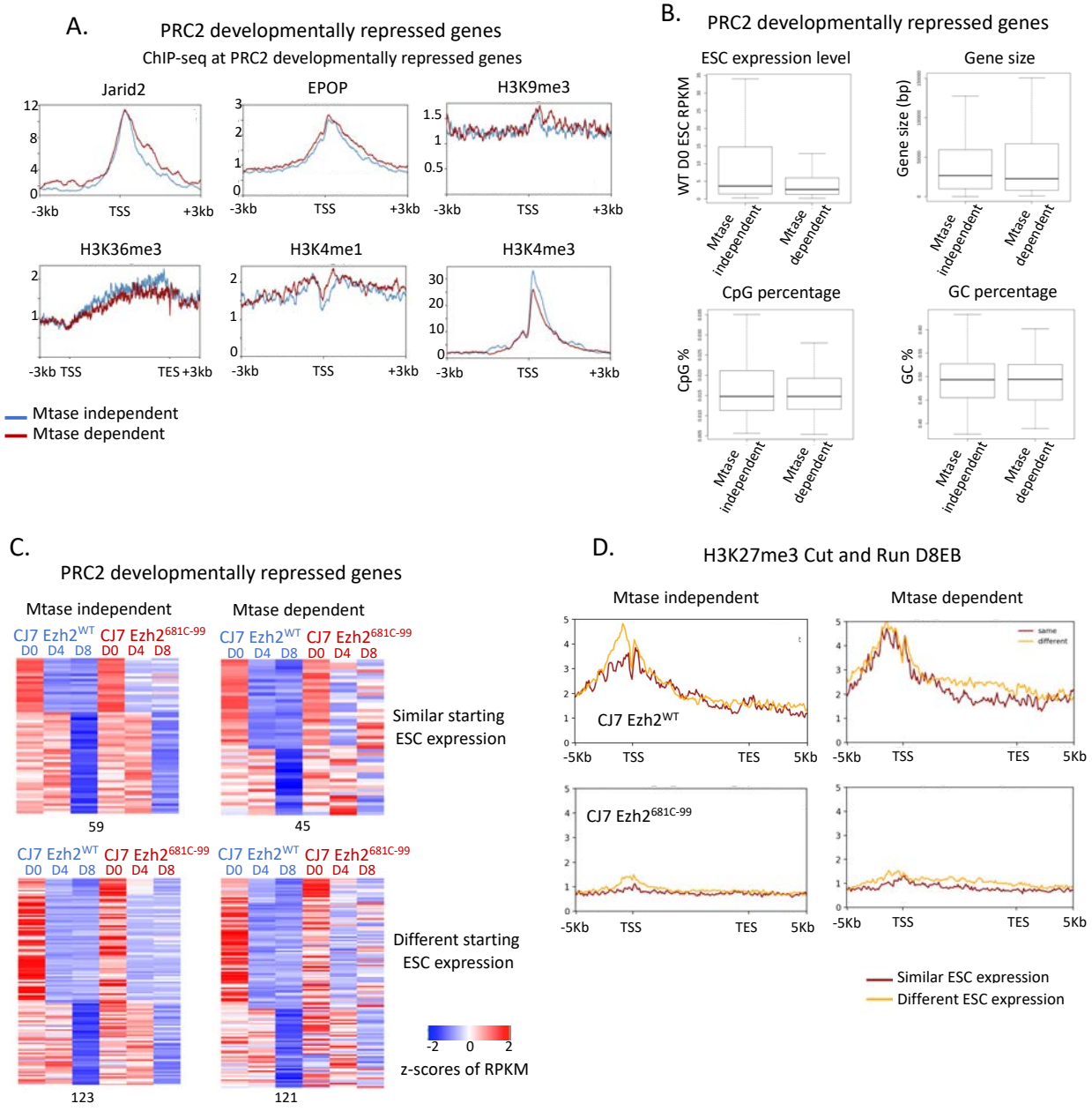
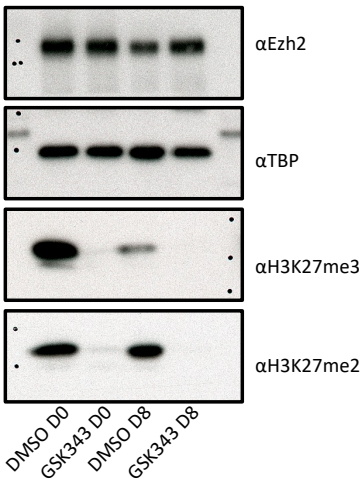


Fig. S6

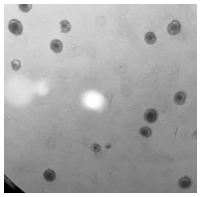
(A) Average profiles showing binding of other PRC2 components and histone marks in wild-type cells at Mtase dependent (167) or independent (183) and developmentally repressed PRC2 target genes over the time course. (B) Boxplots showing comparisons of different characteristics of Mtase dependent (167) and independent (183), developmentally repressed PRC2 target genes in wild-type and mutant cells. (C) Heatmaps showing developmentally repressed genes from Fig. 3D, separated based on expression levels of wild-type and mutant ES cells at Day 0 of the time-course. Genes with similar expression levels at Day 0 in wild-type and mutant (within 1.2 fold) are on the top and those with different starting expression levels (more than 1.2 fold) are at the bottom. (D) Average profiles showing H3K27me3 binding in wild-type and mutant cells by CUT&RUN at Mtase dependent or independent and developmentally repressed PRC2 target genes that have either different (more than 1.2 fold) or similar (within 1.2 fold) expression over the time course.

Fig. S7

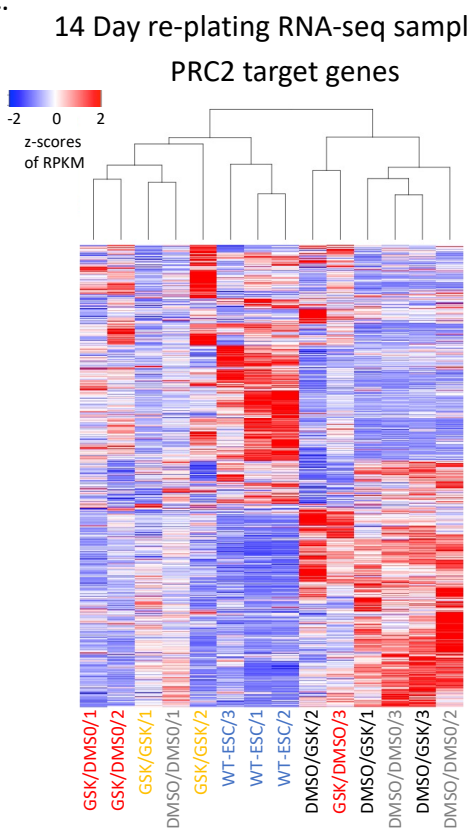
A.



B.



C.



D.

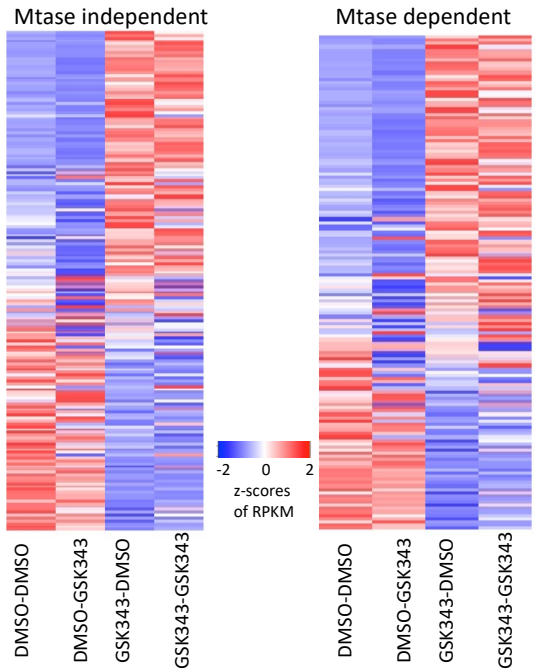


Fig. S7
(A) Western blot of CJ7 cells treated with DMSO or GSK343 showing the (levels of EZH2, H3K27me3, H3K27me2 and TBP control at Day 0 and Day 8 or EB formation. (B) 10x magnification of embryoid bodies that have differentiated for 4 days from 681C-99 cells that had previously been differentiated as EBs for 14 days then replated into ES medium to for 5 days. (C) Unsupervised clustering of PRC2 target genes in re-plated cells and wild-type ES-cells. Untreated or drug treated cells were replated at 14 days in DMSO or GSK343. Z-scores of average RPKMs of triplicates (duplicates for GSK-GSK) are shown for each gene across all samples. All expressed genes (14,834) are shown. (D) Heatmaps showing comparison of treated and untreated cells, re-plated in DMSO or GSK343 for previously identified Mtase dependent (167) and independent genes (183). Z-scores of average RPKMs of triplicates (duplicates for GSK-GSK) are shown for each gene across all samples.