

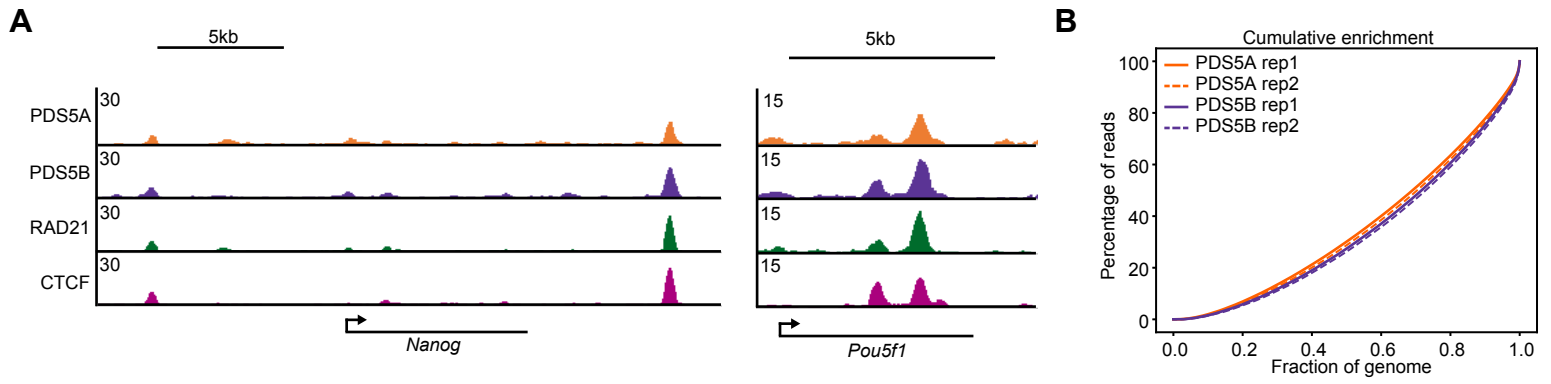


Figure S1. PDS5A and PDS5B occupy the same sites across the genome

- A. Genome browser tracks of PDS5A, PDS5B, RAD21, and CTCF ChIP-seq signal near the *Nanog* and *Pou5f1*(OCT4) genes in WT mESCs (Z-score normalized).
- B. Fingerprint plot showing cumulative enrichment of reads per fraction of the genome for each biological replicate of PDS5A and PDS5B ChIP-seq data.

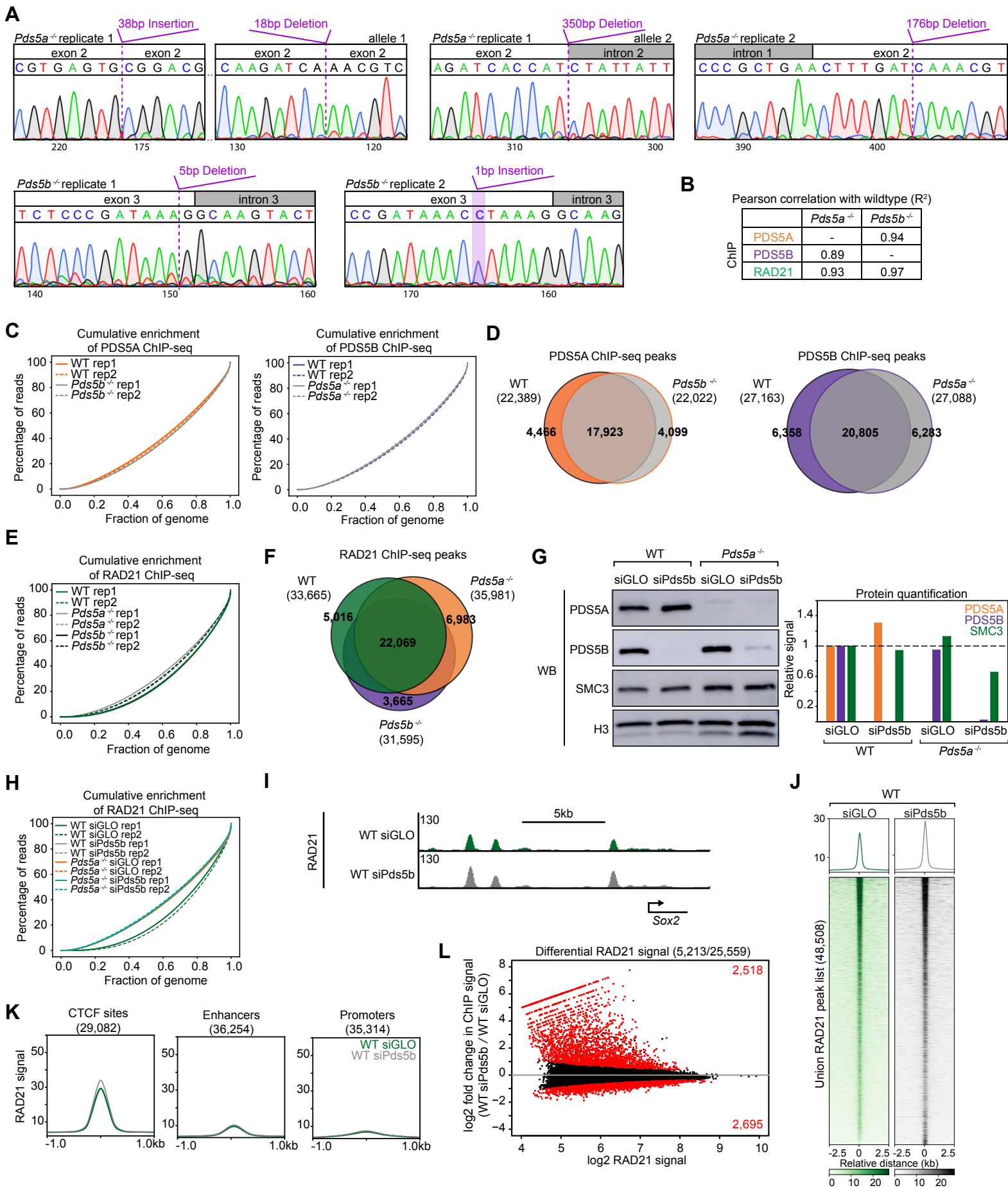


Figure S2. Cohesin localization is largely independent of PDS5 proteins

- A. Sequencing chromatograms for *Pds5a*^{-/-} and *Pds5b*^{-/-} mESCs. Purple dashed lines indicate edit sites.
- B. Pearson correlation (R^2) values for PDS5A, PDS5B, and RAD21 ChIP-seq signal between WT and knockout mESCs.
- C. Fingerprint plots showing cumulative enrichment of reads per fraction of the genome for each biological replicate of PDS5A and PDS5B ChIP-seq data in WT and knockout mESCs.
- D. Overlap of PDS5A ChIP-seq peaks in WT and *Pds5b*^{-/-} mESCs as well as overlap of PDS5B ChIP-seq peaks in WT and *Pds5a*^{-/-} mESCs.
- E. Fingerprint plot showing cumulative enrichment of reads per fraction of the genome for each biological replicate of RAD21 ChIP-seq in WT, *Pds5a*^{-/-}, and *Pds5b*^{-/-} mESCs.
- F. Overlap of RAD21 ChIP-seq peaks in WT, *Pds5a*^{-/-}, and *Pds5b*^{-/-} mESCs.
- G. Western blot analysis and quantification of nuclear lysates of WT siGLO, WT siPds5b, *Pds5a*^{-/-} siGLO, and *Pds5a*^{-/-} siPds5b mESCs. Quantifications are relative to H3 loading control.
- H. Fingerprint plot showing cumulative enrichment of reads per fraction of the genome for each biological replicate of RAD21 ChIP-seq in WT siGLO, WT siPds5b, *Pds5a*^{-/-} siGLO, and *Pds5a*^{-/-} siPds5b mESCs.
- I. Genome browser tracks of RAD21 ChIP-seq signal in WT siGLO and WT siPds5b mESCs at the *Sox2* gene locus.
- J. Heatmaps of RAD21 ChIP-seq signal in WT siGLO and WT siPds5b mESCs at a union peak list of all RAD21 peaks in both conditions.
- K. Average signal plots of RAD21 ChIP-seq in WT siGLO and WT siPds5b mESCs at CTCF sites, enhancers, and promoters.
- L. MA plot showing differential RAD21 ChIP-seq signal between WT siPds5b and WT siGLO mESCs at conserved binding sites.

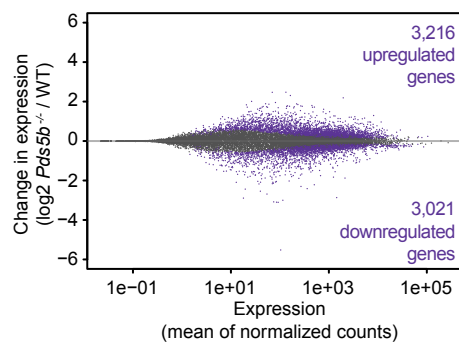
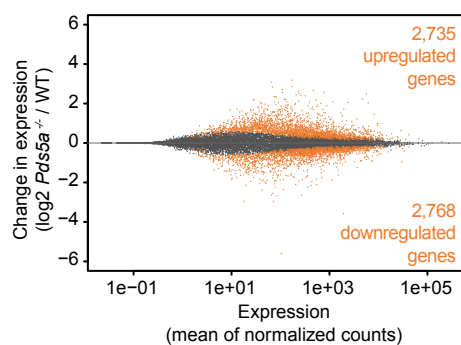
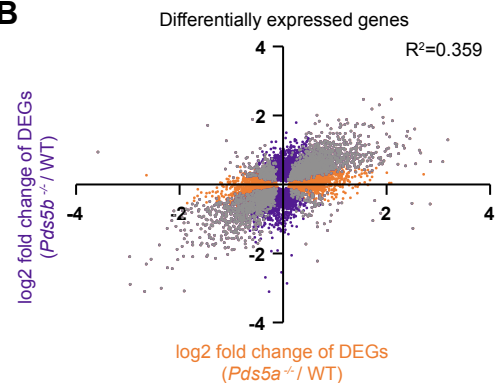
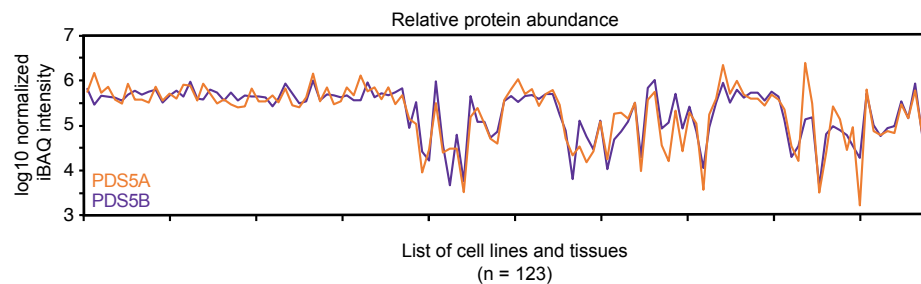
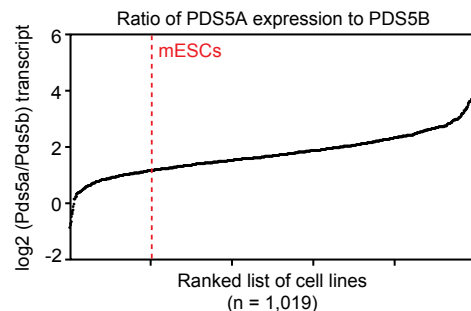
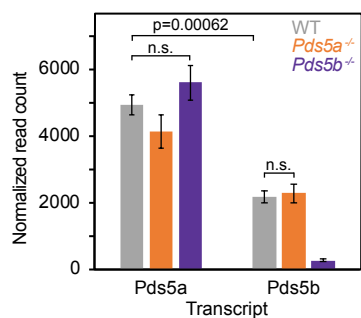
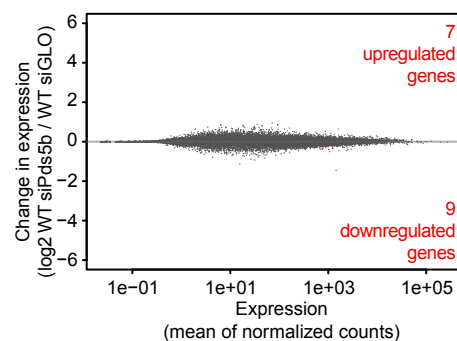
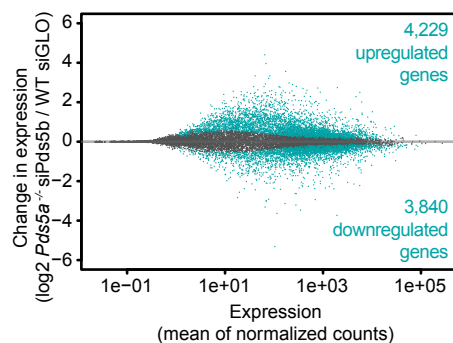
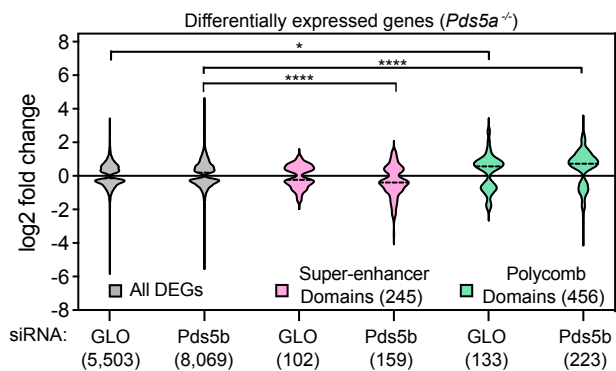
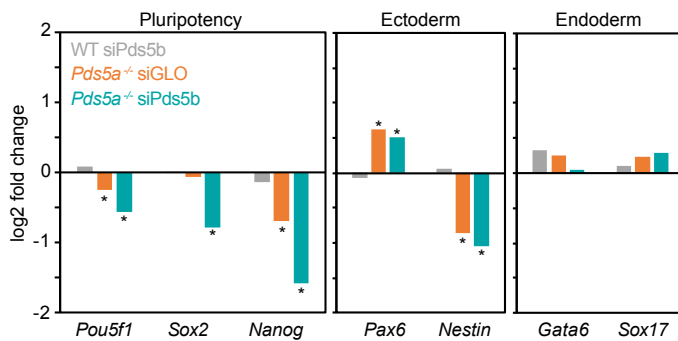
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Figure S3. Role of PDS5A and PDS5B in gene expression

- A. MA plots showing DEGs in *Pds5a*^{-/-} or *Pds5b*^{-/-} mESCs relative to WT mESCs. All cells were treated with siGLO as a transfection control.
- B. Correlation plot of log2 fold changes of all DEGs in *Pds5a*^{-/-} and *Pds5b*^{-/-} mESCs. The DEGs commonly misexpressed in both conditions are in gray while those specific to *Pds5a*^{-/-} mESCs or *Pds5b*^{-/-} mESCs are in orange and purple, respectively.
- C. Relative abundance of PDS5A and PDS5B (log10 normalized iBAQ intensity) in 123 cell and tissue types from ProteomicsDB (Lautenbacher et al. 2022).
- D. Relative transcript levels (log2 *Pds5a*/*Pds5b*) in our mESCs (raw ratio value = 2.27) and 1,019 cell lines from the Cancer Cell Line Encyclopedia.
- E. Normalized read counts for *Pds5a* and *Pds5b* transcripts in WT, *Pds5a*^{-/-} and *Pds5b*^{-/-} mESCs. Data represented as the average ± standard deviation across three biological replicates. A 2-tailed unpaired t-test was used to determine significance between groups with n.s. representing no significance.
- F. MA plots showing DEGs in *Pds5a*^{-/-} siPds5b or WT siPds5b mESCs relative to WT siGLO mESCs.
- G. Violin plot of log2 fold changes for all DEGs, those within Super-enhancer Domains, and those within Polycomb Domains for *Pds5a*^{-/-} siGLO, and *Pds5a*^{-/-} siPds5b mESCs. Significance was determined using a Kruskal-Wallis test followed by Dunn's multiple comparisons test. Asterisks indicate significant differences between groups (* $p < 0.05$, **** $p < 0.0001$).
- H. Bar graphs of log2 fold change in expression of pluripotency genes (*Pou5f1*, *Sox2*, *Nanog*), ectodermal lineage genes (*Pax6* and *Nestin*), and endodermal lineage genes (*Gata6* and *Sox17*) in WT siPds5b, *Pds5a*^{-/-} siGLO, and *Pds5a*^{-/-} siPds5b mESCs. Asterisks indicate significant differences from WT siGLO mESCs determined using DESeq2 (padj < 0.01).

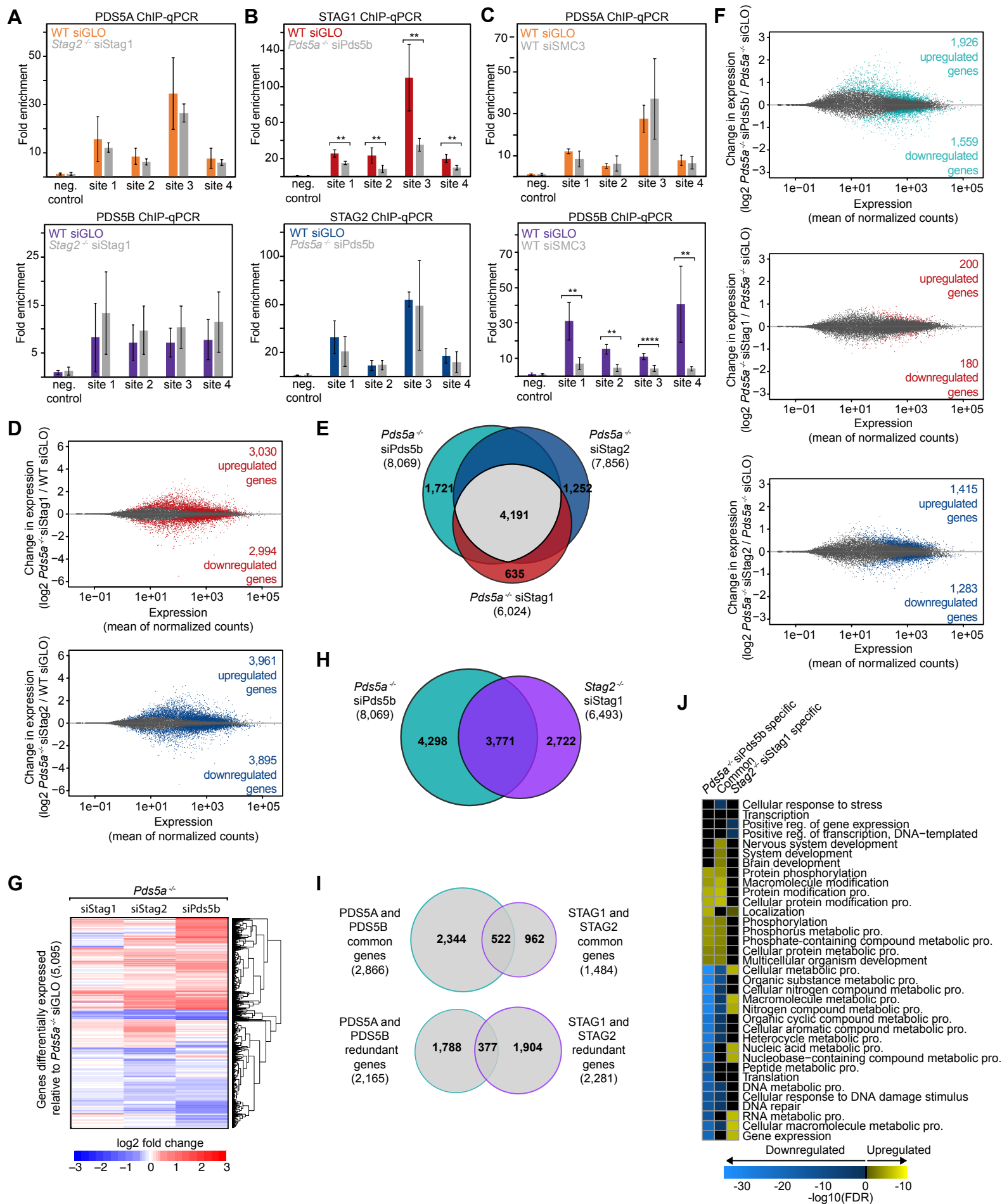


Figure S4. Knockdown of STAG1 and STAG2 in cells lacking PDS5A causes changes similar to loss of PDS5B in *Pds5a*^{-/-} mESCs

- A. ChIP-qPCR for PDS5A and PDS5B in WT siGLO and *Stag2*^{-/-} siStag1 mESCs. Fold enrichment at four different CTCF sites, relative to 5% input material and the negative control region is depicted. Data represented as mean $\pm$ standard deviation across two biological replicates, each with three technical replicates. A 2-tailed unpaired t-test was used to determine significance between WT and dual depletion lines for every primer set. No significance was found for any of the primer sets.
- B. ChIP-qPCR for STAG1 and STAG2 in WT siGLO and *Pds5a*^{-/-} siPds5b mESCs. Fold enrichment at four different CTCF sites, relative to 5% input material and the negative control region is depicted. Data represented as mean $\pm$ standard deviation across two biological replicates, each with three technical replicates. A 2-tailed unpaired t-test was used to determine significance between WT and dual depletion lines for every primer set. Asterisks indicate significant differences between groups (** $p < 0.01$), otherwise, no significance was found.
- C. ChIP-qPCR for PDS5A and PDS5B in WT siGLO and WT siSMC3 mESCs. Fold enrichment at four different CTCF sites, relative to 5% input material and the negative control region is depicted. Data represented as mean $\pm$ standard deviation across two biological replicates, each with three technical replicates. A 2-tailed unpaired t-test was used to determine significance between WT and depletion lines for every primer set. Asterisks indicate significant differences between groups (**** $p < 0.0001$, ** $p < 0.01$), otherwise, no significance was found.
- D. MA plots showing DEGs in *Pds5a*^{-/-} siStag1 or *Pds5a*^{-/-} siStag2 mESCs relative to WT siGLO mESCs.
- E. Overlap of differentially expressed genes (DEGs) in *Pds5a*^{-/-} siPds5b, *Pds5a*^{-/-} siStag1, and *Pds5a*^{-/-} siStag2 mESCs relative to WT siGLO mESCs.
- F. MA plots showing DEGs in *Pds5a*^{-/-} siStag1, *Pds5a*^{-/-} siStag2, and *Pds5a*^{-/-} siPds5b mESCs relative to *Pds5a*^{-/-} siGLO mESCs.
- G. Clustered heatmap of log2 fold changes for a combined list of DEGs in *Pds5a*^{-/-} siStag1, *Pds5a*^{-/-} siStag2, and *Pds5a*^{-/-} siPds5b mESCs relative to *Pds5a*^{-/-} siGLO mESCs.
- H. Overlap of DEGs in *Pds5a*^{-/-} siPds5b and *Stag2*^{-/-} siStag1 mESCs relative to WT siGLO mESCs (Arruda et al., 2020).
- I. Overlaps of DEGs of Common and Redundant genes respectively from *Pds5a*^{-/-} siPds5b and *Stag2*^{-/-} siStag1 mESCs relative to WT siGLO mESCs (Arruda et al., 2020).
- J. Gene Ontology (GO) terms for biological processes that are *Pds5a*^{-/-} siPds5b specific, *Stag2*^{-/-} siStag1 specific, and common to both conditions (from overlap in S4H) (Arruda et al., 2020).

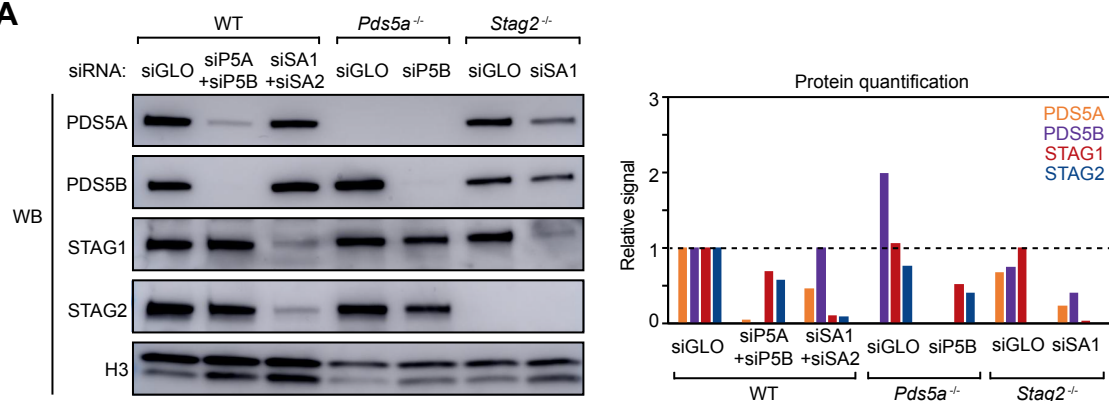
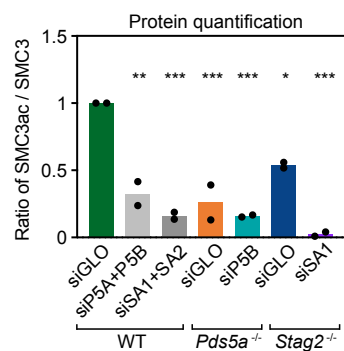
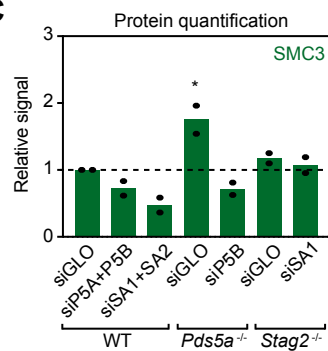
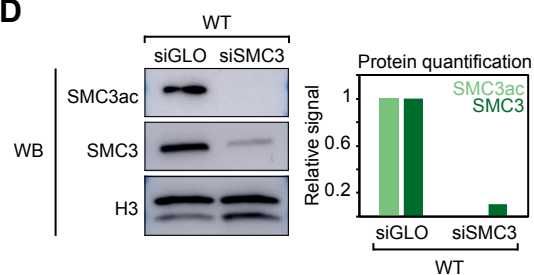
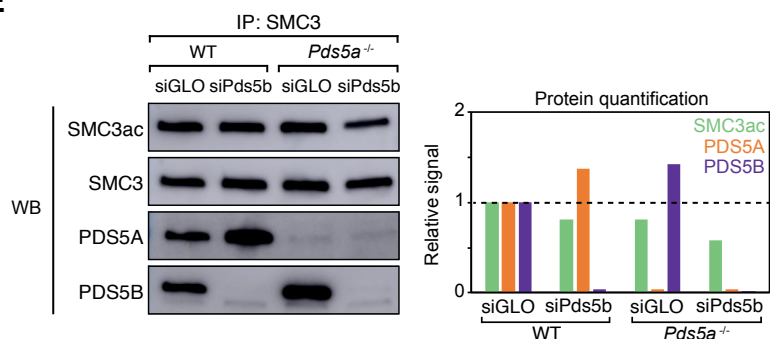
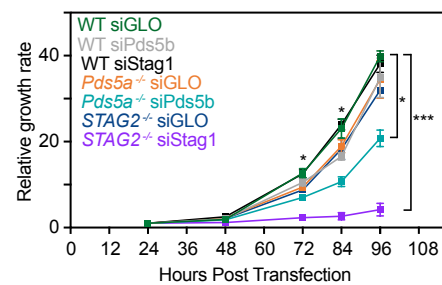
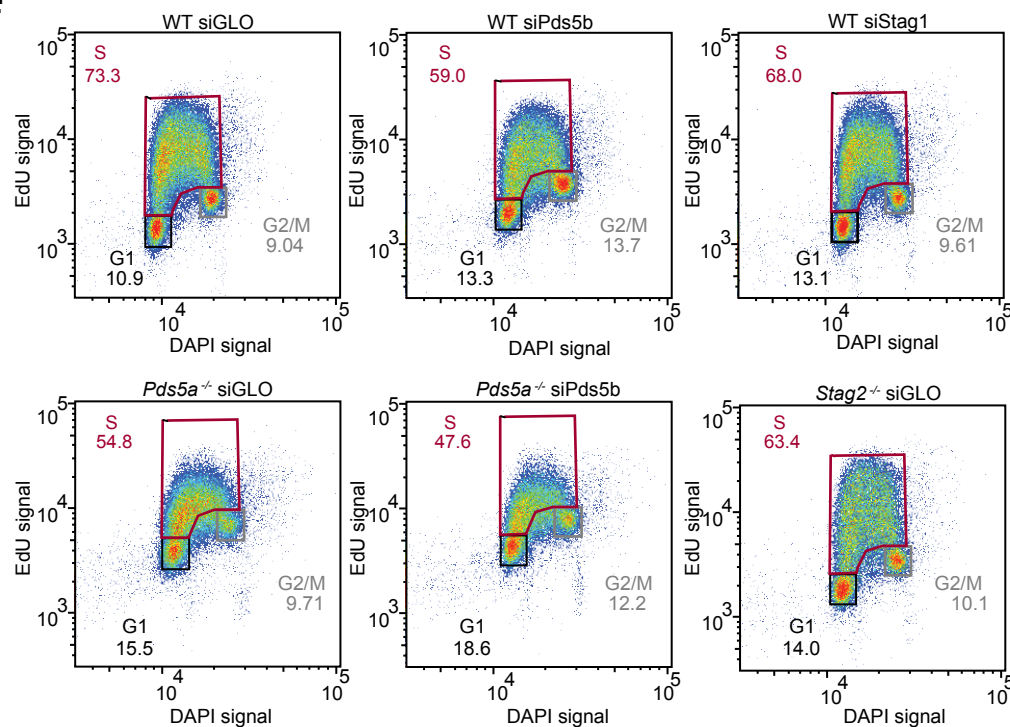
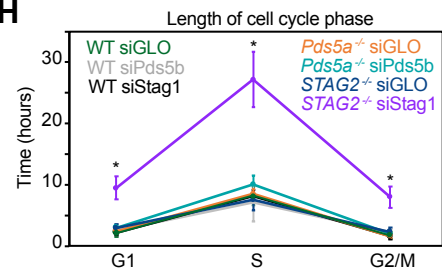
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Figure S5. Acute and chronic loss of cohesin accessory proteins reduces SMC3ac levels

- A. Western blot analysis and quantification of nuclear lysates showing knockdown efficiencies in the seven siRNA conditions. Quantifications are relative to the average of SMC3 and two H3 loading controls.
- B. Quantification of Figure 5A depicted as the ratio of SMC3ac signal over SMC3 signal for each condition from two biological replicates. Significance was determined using a one-way ANOVA followed by Tukey's multiple comparisons test. Asterisks indicate significant differences from WT siGLO mESCs ($***p < 0.001$, $**p < 0.01$, $*p < 0.05$).
- C. Quantification of Figure 5A showing total SMC3 signal from two biological replicates. Significance was determined using a one-way ANOVA followed by Tukey's multiple comparisons test. Asterisk indicates significant differences from WT siGLO mESCs ($*p = 0.026$). Quantifications are relative to H3 loading control.
- D. Western blot analysis and quantification of SMC3ac and SMC3 in WT siGLO and WT siSMC3 mESCs. Quantifications are relative to H3 loading control.
- E. Western blot analysis and quantification following co-immunoprecipitation of SMC3 done in the presence of 5mM sodium butyrate, in WT siGLO, WT siPds5b, *Pds5a*^{-/-} siGLO, and *Pds5a*^{-/-} siPds5b mESCs. Quantifications are relative to SMC3 signal.
- F. Scatterplots showing flow cytometry results for one of three biological replicates for all seven conditions quantified in Figure 5B.
- G. Proliferation rate of mESC lines represented relative to the original plating density. Data represented as the average of three biological replicates $\pm$ standard deviation. A two-way ANOVA followed by Tukey's multiple comparisons test was used to determine significance. Asterisks indicate significant differences from WT siGLO mESCs ($*p < 0.05$, $***p < 0.001$). Significance at 72 and 84hr is for *Stag2*^{-/-} siStag1 relative to WT siGLO mESCs.
- H. Length of cell cycle phases calculated from the population doubling time (the 72 and 84hr timepoints in the proliferation assay from Figure S5G) and the percentage of cells in each cell cycle phase from the flow cytometry analysis. A 2-tailed unpaired t-test was used to determine significance. Asterisks indicate significant differences from WT siGLO mESCs ($*p < 0.05$).

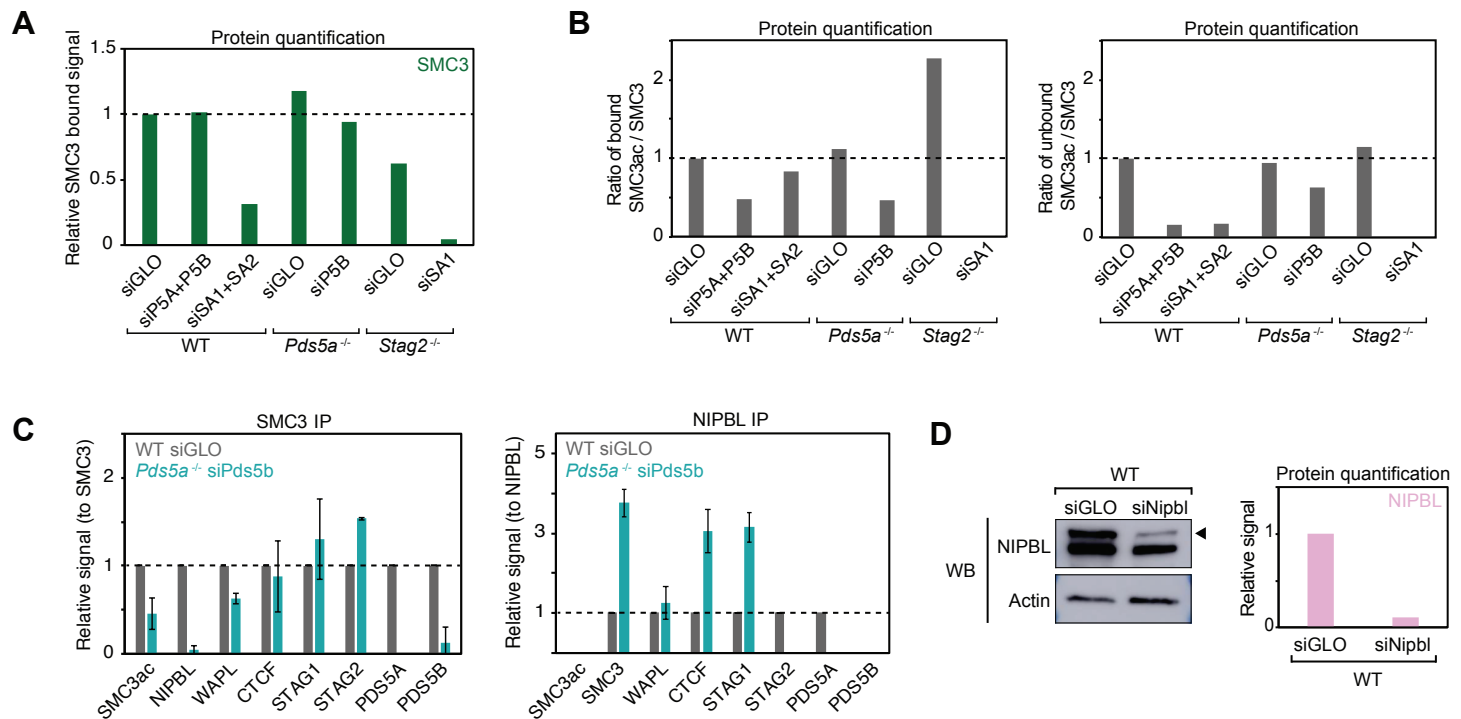


Figure S6. Loss of cohesin accessory proteins differentially affect bound cohesin

- A. Quantification of Figure 5B showing chromatin-bound SMC3 signal in all seven siRNA conditions all relative to WT siGLO. Quantifications are relative to H3 loading control.
- B. Quantification of Figure 5B depicted as the ratio of SMC3ac signal over SMC3 signal for both chromatin-bound and unbound fractions in all seven siRNA conditions all relative to WT siGLO.
- C. Quantification of Figure 5C for both the SMC3 IP relative to SMC3 signal, and the NIPBL IP relative to NIPBL signal. Data represented as mean $\pm$ standard deviation across two biological replicates.
- D. Western blot analysis and quantification of NIPBL in WT siGLO and WT siNipbl mESCs to test antibody specificity. Black triangle in NIPBL blot points to the specific band (top). Quantifications are relative to Actin loading control.