Stem Cell Reports

Supplemental Information

Cell-Cycle Control of Bivalent Epigenetic Domains Regulates the Exit from Pluripotency

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Supplementary Figure 1

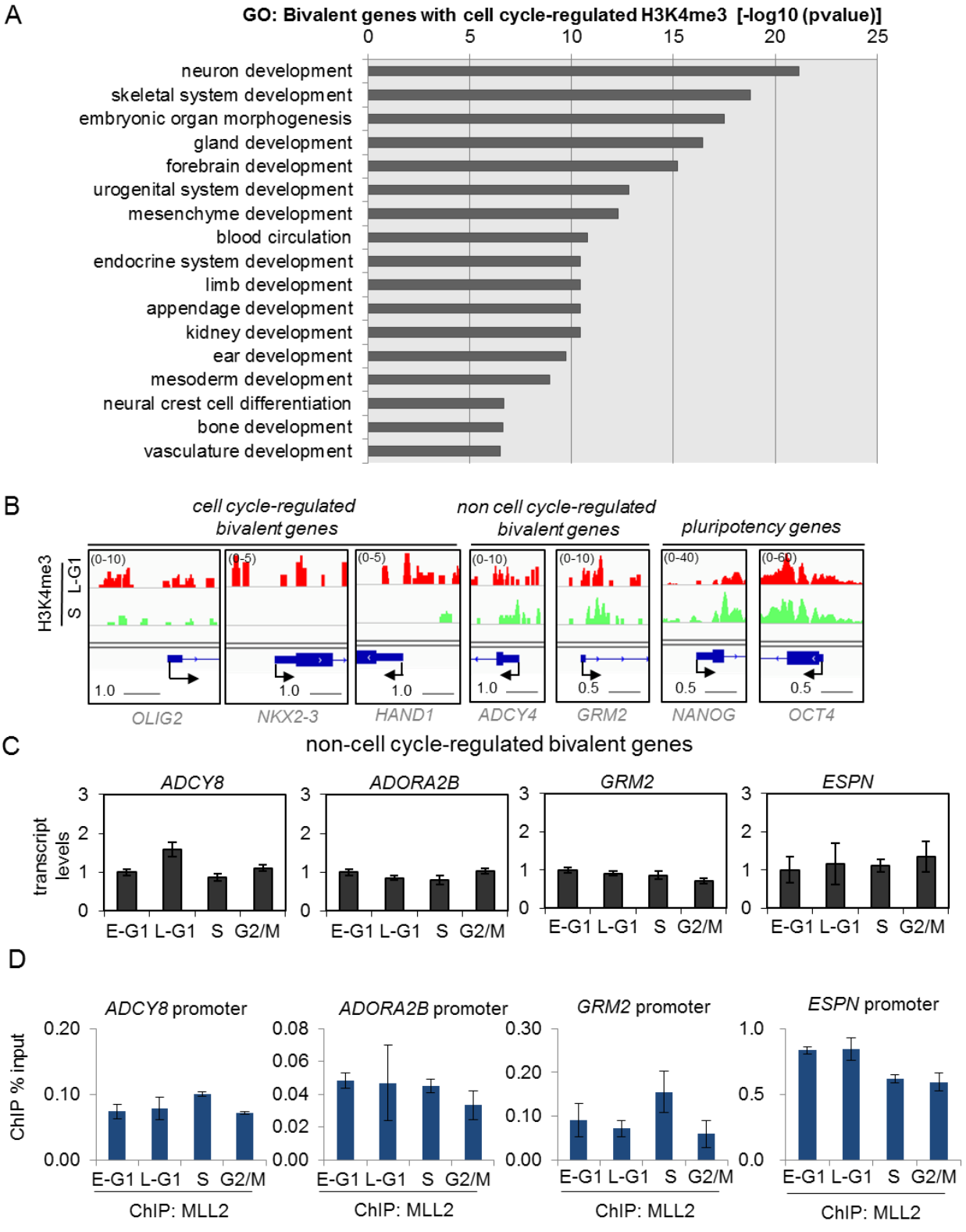

FIGURE S1. Bivalent genes from all germ layers exhibit cell cycle oscillations for H3K4me3. (A) Gene Ontogeny (GO) analysis of bivalent genes with cell cycle regulated H3K4me3. (B) H3K4me3 ChIP-seq profiles of hESC Fucci fractions for selected cell cycle-regulated bivalent genes, non-cell cycle-regulated bivalent genes and pluripotency genes. (C) qRT-PCR transcript analysis of non-cell cycle regulated bivalent genes display no levels of periodicity. Data are the average of three independent replicates. (D) qChIP of MLL2 on non-cell cycle regulated bivalent genes. Data are the average of three independent replicates.

Supplementary Figure 2

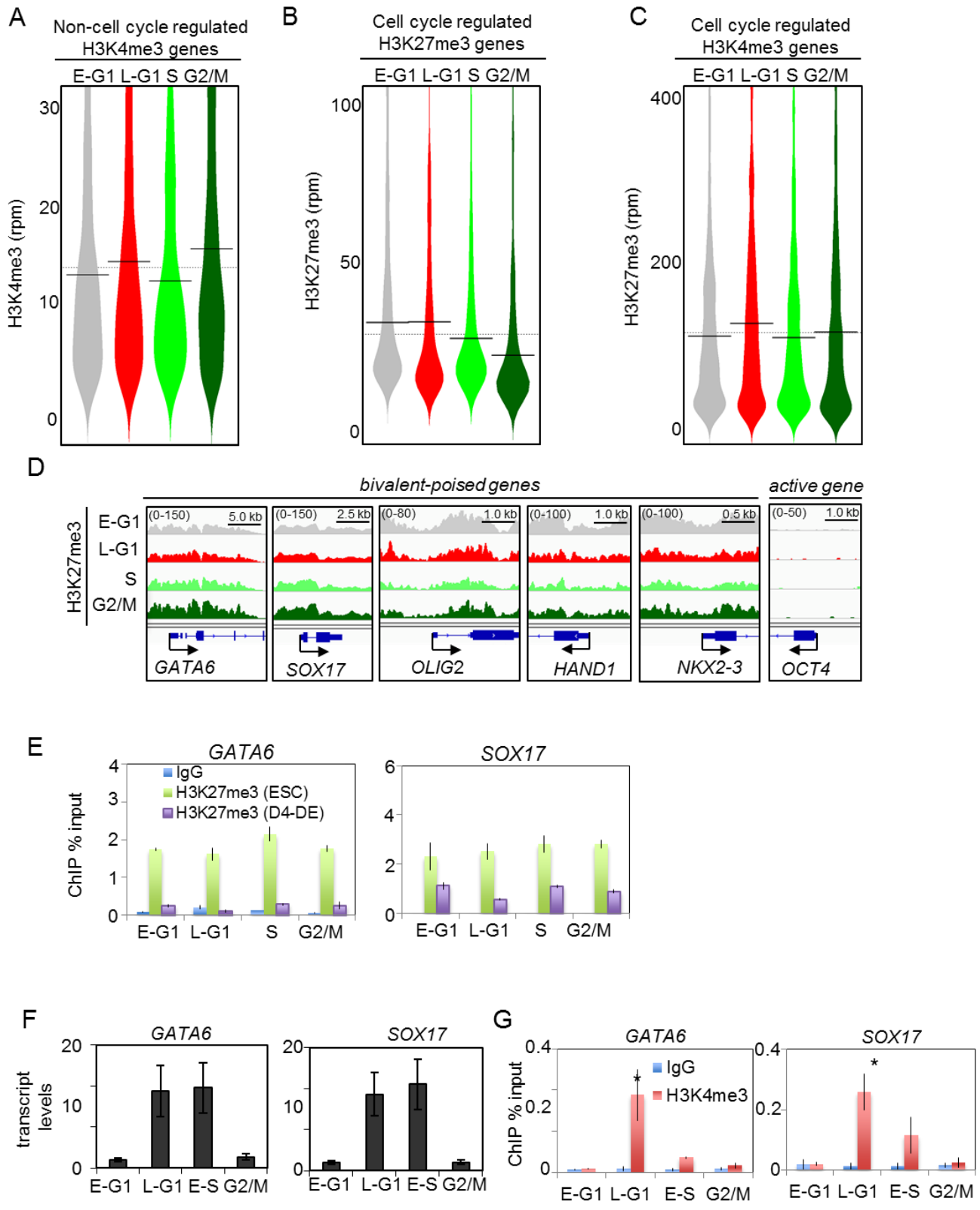

Figure S2. H3K27me3 is not cell cycle regulated. (A, B, C) Bean-plot diagrams showing the distribution of H3K4me3 on non-cell cycle regulated bivalent genes (A), H3K27me3 for cell cycle-regulated H3K27me3 (B) or H3K4me3 (C) genes. Black horizontal line represents median values. (D) ChIP-seq plots of H3K27me3 performed in Fucci cell cycle fractions. Range given in RPM. (E) qChIP of H3K27me3 levels at *GATA6* and *SOX17* promoters in ESCs and DE (day 4 differentiation). Data are the average of three independent replicates. (F) qRT-PCR transcript analysis of Fucci fractions from early-G1 (E-G1, double negative cells), late-G1 (L-G1, KO2+ cells), early-S phase (E-S, KO2+ Az1+ cells), and G2/M (Az1-high cells). Data are the average of three independent replicates. (G) H3K4me3 qChIP using Fucci isolated cell cycle fractions from ESCs as described in (F). Data are the average of three independent replicates. *$p<0.05$.

Supplementary Figure 3

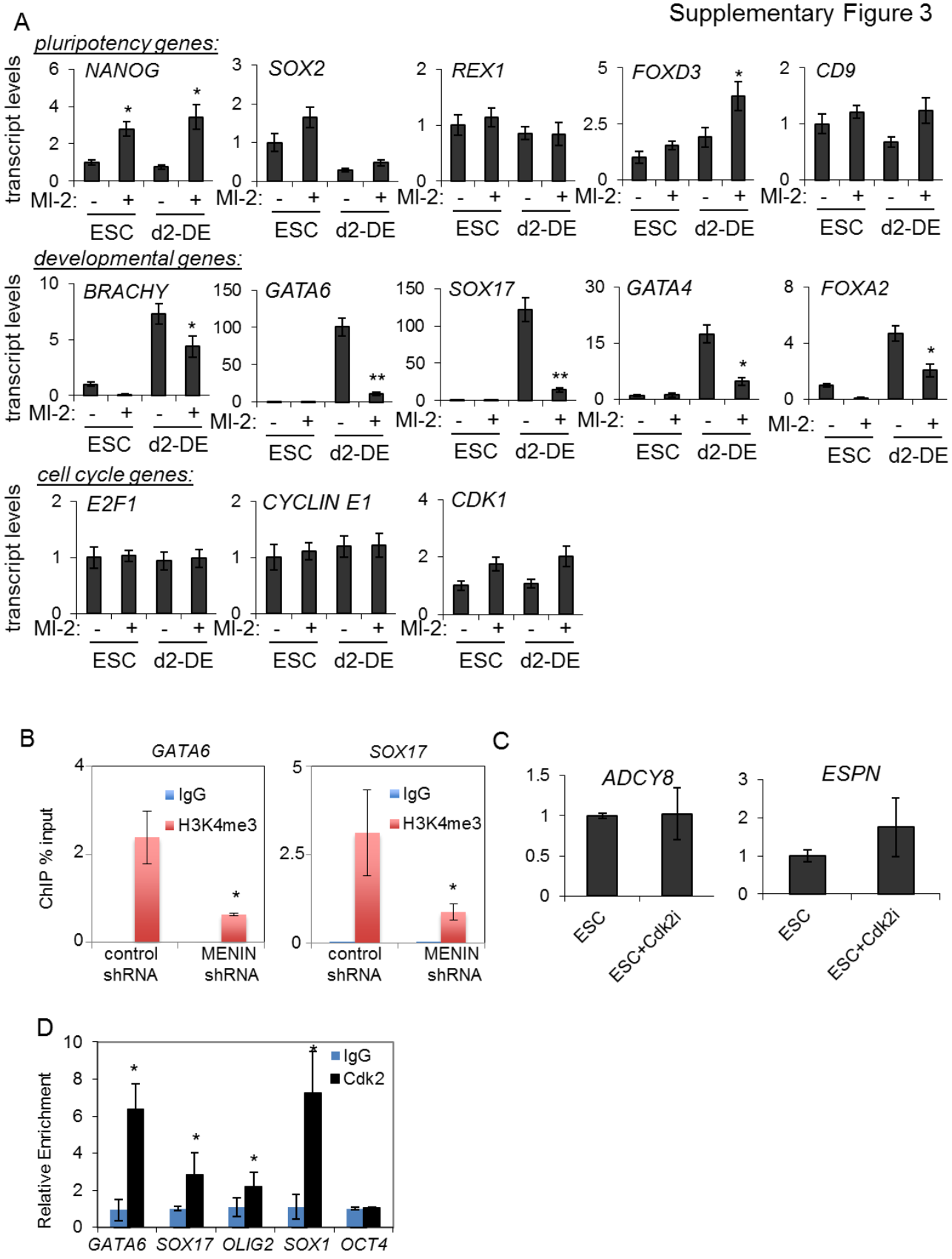

Figure S3. MENIN/MLL2 is required for expression of developmental genes and H3K4me3. (A) qRT-PCR transcript analysis of pluripotency, developmental or cell cycle genes following 24 hours of treatment with MI-2. Data are the average of three independent replicates. (B) H3K4me3 qChIP of *GATA6* and *SOX17* promoters following the infection of GFP-control or *MENIN* shRNA lentivirus in WA09 hESCs after 3 days of puromycin selection. Data are the average of three independent replicates. (C) qRT-PCR transcript analysis of non-cell cycle regulated bivalent genes in the presence or absence of CVT-313 for 4 hours. Data are the average of three independent replicates. (D) CDK2 qChIP on indicated genes in WA09 hESCs. Data are the average of three independent replicates. *$p<0.05$, **$p<0.01$.

Supplementary Figure 4

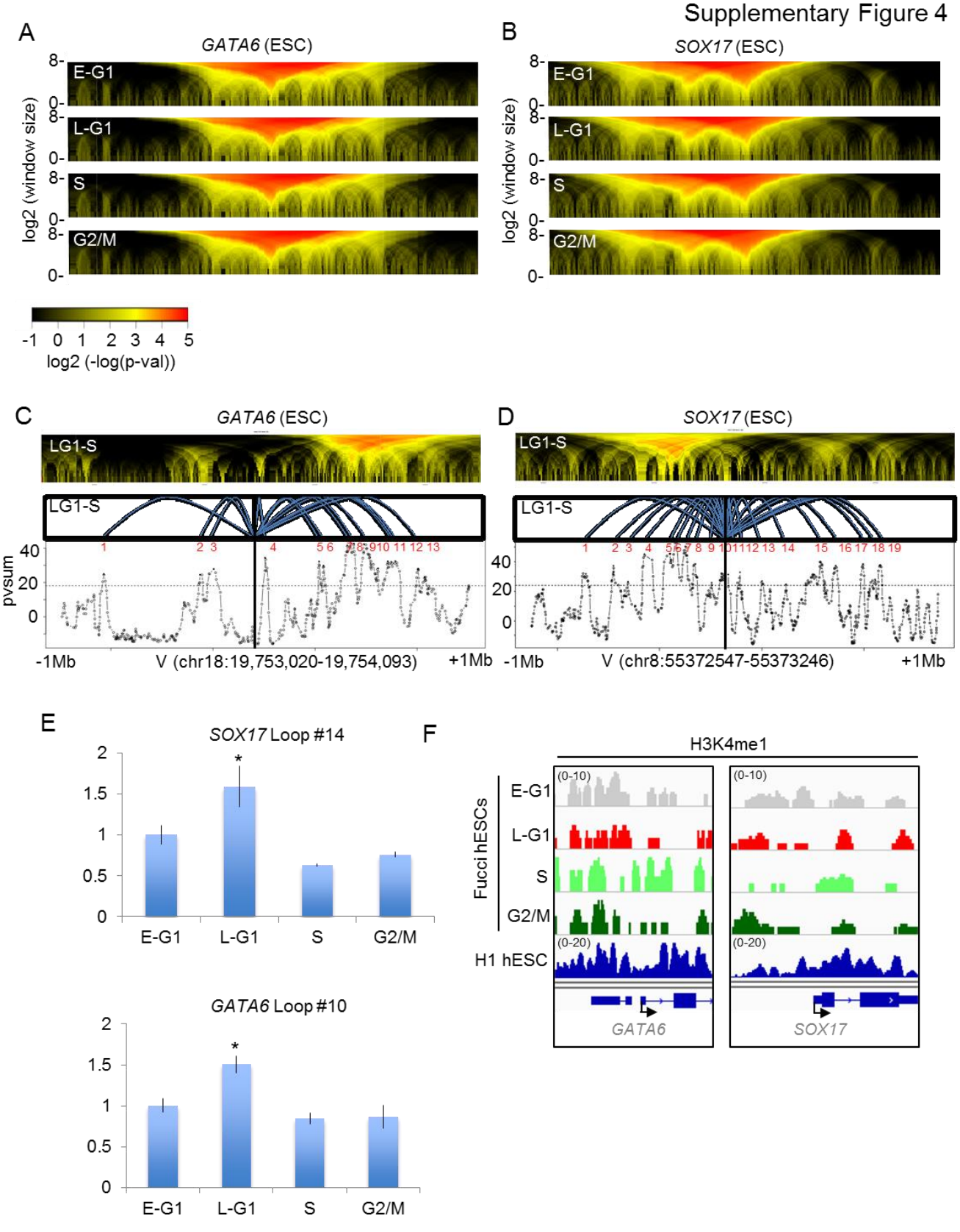

Figure S4. Cell cycle-regulated looping interactions identified by 4C-seq. (A and B) Domainograms from 4C-seq of Fucci fractions at *GATA6* or *SOX17* loci. (C and D) Peak plots with domainograms following subtraction of 4C-seq data (late G1 minus S) identifies numerous cell cycle regulated interactions at the *GATA6* or *SOX17* loci. (E) 3C-looping interactions between indicated loops and the *GATA6* or *SOX17* promoter region. 3C are representative of multiple experiments. Data are the average of three independent replicates. *$p<0.05$. (F) ChIP-seq plots for H3K4me1 at the *GATA6* or *SOX17* promoter show no signs of periodicity. Range is given in RPM.

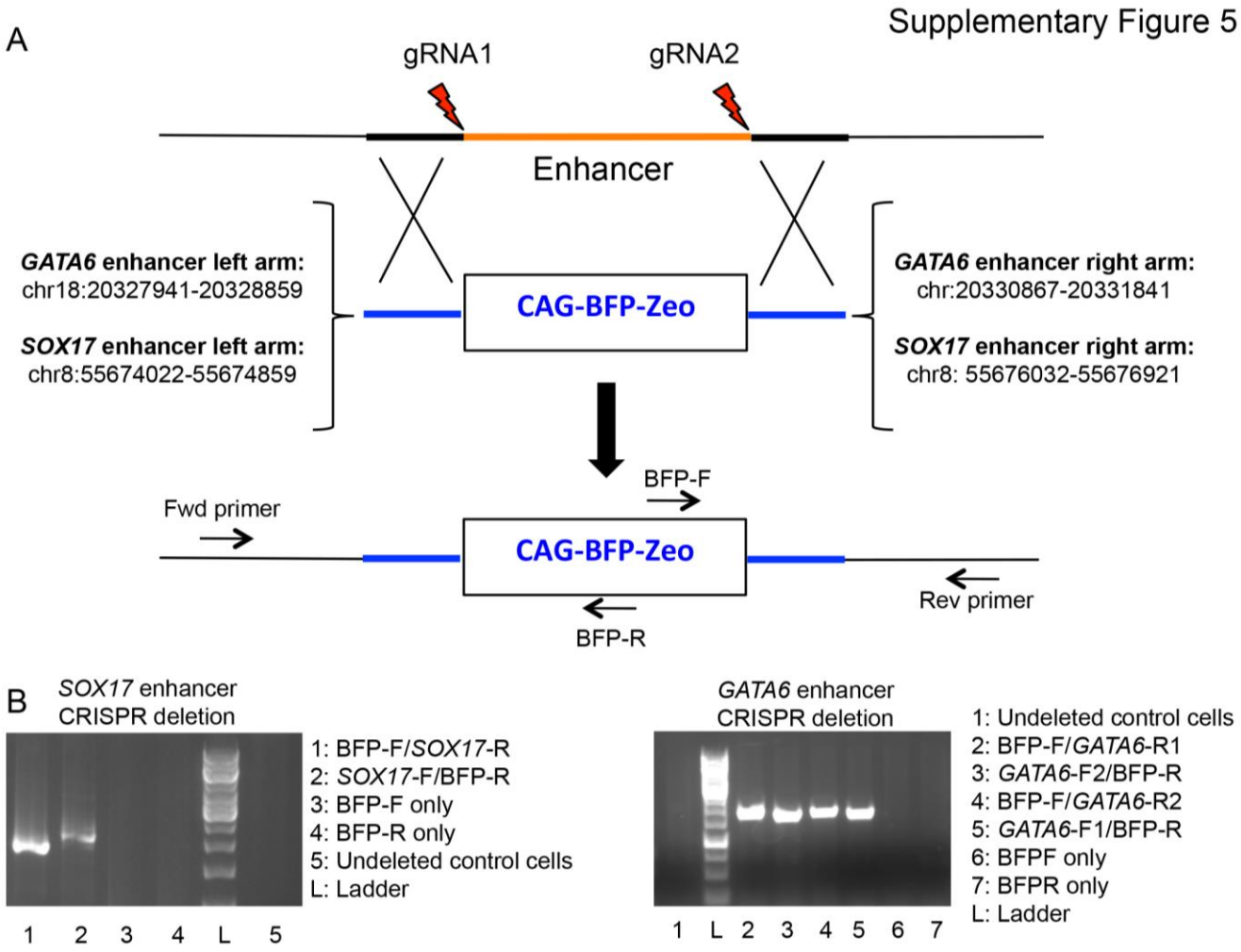

Figure S5. Confirmation of CRISPR-Cas9 directed gene-targeting at *GATA6* and *SOX17* loci. (A) Diagram of the targeting construct used with two guide RNAs (gRNA) in the CRISPR-Cas9 genome-editing system. Genomic coordinates for left and right arms of *GATA6* or *SOX17* targeting construct are indicated. (B) PCR products were resolved by agarose gel electrophoresis then visualized by ethidium bromide staining. Primers used in this analysis are indicated.

Table S1, related to Figure 1. List of bivalent genes that are cell cycle-regulated for H3K4me3. To be included in this list, a >2-fold difference in H3K4me3 levels was required between any two Fucci cell cycle fractions.

Table S2, related to Figure 1. ChIP-seq analysis for H3K4me3 and H3K27me3 on bivalent domains in Fucci cell cycle fractions.

Table S3, related to Figure 4. Chromosome coordinates for cell cycle-regulated interactions from 4C-seq analyses; late G1 minus S.

Table S4, related to Supplemental Experimental Procedures. Primers used in this manuscript.

NAME	SEQUENCE	APPLICATION
BETA-ACTIN-F	GAAGGAAGGTGGGCTCTACA	ChIP-qPCR
BETA-ACTIN -R	TGCCTAGGTCACCCACTAAC	ChIP-qPCR
c-MYC-F	CAGGACAAGGATGCGGTTTG	ChIP-qPCR
c-MYC-R	CTCTCCCTTTCTCTGCTGCT	ChIP-qPCR
GAPDH-F	CTAGGCGCTCACTGTTCTCT	ChIP-qPCR
GAPDH-R	TGACTCCGACCTTCACCTTC	ChIP-qPCR
GATA6-F1	CCAGGGACATCAAAAGTTGG	ChIP-qPCR
GATA6-R1	CGGGAACCTCAAGACAACAT	ChIP-qPCR
GATA6-F2	CGCGGACCAACTTCTAGTCT	ChIP-qPCR
GATA6-R2	TCTCTGCCTGCCTAACTACC	ChIP-qPCR
NANOG-F	CTTCAGGTTCTGTTGCTCGGTTTT	ChIP-qPCR
NANOG-R	TCCCGTCTACCAGTCTCACCA	ChIP-qPCR
OCT4-F	TCAAGCAGGACTAAGGGTGG	ChIP-qPCR
OCT4-R	GGTCACTCATTACTGGCCCA	ChIP-qPCR
OLIG2-F	CGAGCTCCTCAAATCGCATC	ChIP-qPCR
OLIG2-R	CCCCTGTATCGGAGCATTCT	ChIP-qPCR
PAX6-F	CCCTCAGTAACTCGCTTCCA	ChIP-qPCR
PAX6-R	TGCTGTCCCCAAATCAAAGC	ChIP-qPCR
SOX1-F	TATCTACTCCCTCCCCACGT	ChIP-qPCR
SOX1-R	CCGGGCTGCCATTAAATGAG	ChIP-qPCR
SOX17-F	AGGTCACCCACCACTGAAAC	ChIP-qPCR
SOX17-R	GAGACTCGAAAAGCCGTCTG	ChIP-qPCR
GATA6-site-10-F	ACCCGGTTGTTCAAGTCAGA	ChIP-qPCR
GATA6-site-10-R	TGGCAGTGGTGATAGAGAGT	ChIP-qPCR
SOX17-site-14-F	GCCGTTCATAGGGACATTTGT	ChIP-qPCR
SOX17-site-14-R	TGTGTGCATATCTTGGTTACAGT	ChIP-qPCR
GATA6-VIEWPT-F	GAATTTCTTTATCGGGATTTGAGAG	3C-qPCR
GATA6-LOOP2-R	ATCAGAAAATAATCAAAACGGAGTG	3C-qPCR
GATA6-LOOP10-R	AATGGATTAGAGAGTGAGACACAGG	3C-qPCR
SOX17-VIEWPT-1F	AACAAGGTACTACGGGGTTAATTTG	3C-qPCR
SOX17-VIEWPT-2F	GTTTTCTAACTGTTGAATCATAAGC	3C-qPCR
SOX17-LOOP14-R	CCCATGTAAATGATCTTATGACTCC	3C-qPCR
SOX17-LOOP17-R	GTTATTAAAAGATTTGCCAGGGATGG	3C-qPCR
BFP-Crispr-insert-F	CGACCTCCCTAGCAAACTGG	Gel electrophoresis
BFP-Crispr-insert-R	TACAGCTTCATGTGCATGTTCTCC	Gel electrophoresis
GATA6-Crispr10-R1	CATTGGAATTATGTCAGTTTAGC	Gel electrophoresis
GATA6-Crispr10-R2	GTTTCATTAATCCCAAATTACTGG	Gel electrophoresis
SOX17-Crispr14-R1	CTTAAGAACAAGGAAGCACAGGC	Gel electrophoresis
SOX17-Crisprf14-R2	CTTAAGAACAAGGAAGCACAGGC	Gel electrophoresis