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Supplemental Information

Accurate Recycling of Parental Histones Reproduces the Histone Modification Landscape during DNA Replication

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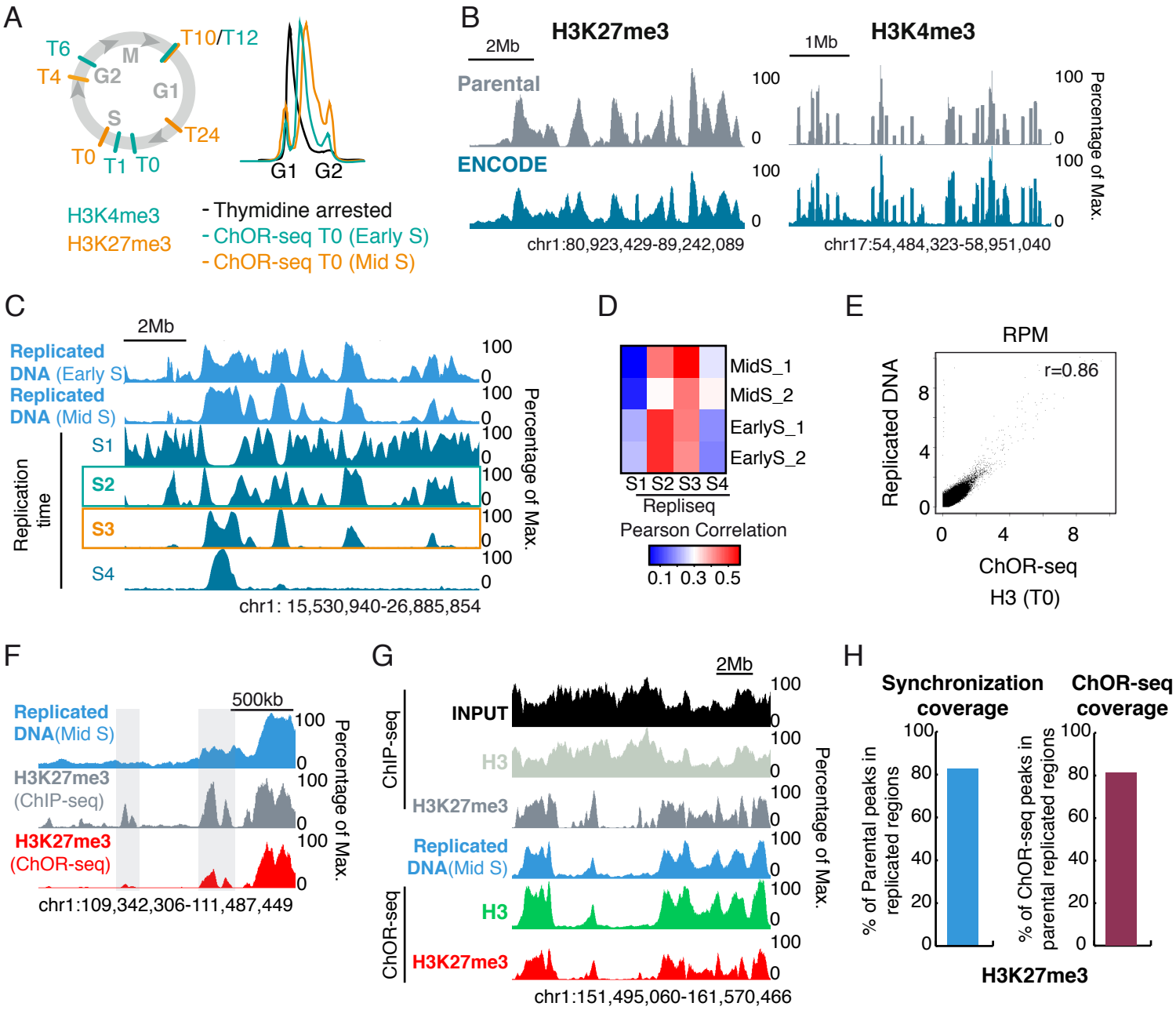

Figure S1. Tracking histone PTM occupancy after DNA replication with ChOR-seq, Related to Figure 1. **(A)** Outline of time course analysis for H3K4me3 and H3K27me3 ChOR-seq. (left) Experimental set-up. For H3K4me3 (green), cells were pulsed with EdU in early S phase and ChOR-seq samples were harvested immediately (T0, nascent) and 1 (T1), 6 (T6) and 12 (T12) hours later. For H3K27me3 (orange), cells were pulsed with EdU in mid S phase and ChOR-seq samples were harvested immediately (T0, nascent) and 4 (T4), 10 (T10) and 24 (T24) hours later. Cell cycle progression as indicated in the experimental set-up was assessed by FACS analysis of DNA content, as illustrated for T0 (right). **(B)** Profiles of parental H3K27me3 (left) and H3K4me3 (right) ChIP-seq in S phase synchronized cultures (gray) compared to ENCODE profiles from asynchronous Hela S3 cells (blue). Signal is scaled as percentage of maximum at the locus depicted. **(C)** Replicated DNA profiles from samples labelled in early or mid S phase compared with replication timing profiles determined by Repli-seq (ENCODE Project Consortium, 2012). Signal is scaled as percentage of maximum at the locus depicted. **(D)** Heat maps showing the global Pearson correlation between replicated DNA profiles of samples labelled in early or mid S and replication timing profiles determined by Repli-seq (ENCODE Project Consortium, 2012). Correlations are calculated from 500 bp non-overlapping windows and RPM. **(E)** Dot plots comparing signal in replicated DNA and pan-H3 nascent ChOR-seq. Dots represent 500 bp non-overlapping windows within replicated regions. R-values shown are Pearson correlations. **(F)** Profiles of replicated DNA (blue), parental H3K27me3 ChIP-seq (gray) and nascent H3K27me3 ChOR-seq (red). The left highlighted region shows a locus with parental signal but no nascent signal, owing to a low degree of replication (low enrichment of replicated DNA). The right highlighted region shows a locus with high parental signal but moderate nascent signal, owing to it being replicated to a moderate degree. The locus also includes a highly replicated region showing a strong agreement of the nascent signal with the parental. Signal is scaled as percentage of maximum at the locus depicted. **(G)** Parental ChIP-seq and nascent ChOR-seq profiles of pan-H3 and H3K27me3 using biotin-dUTP labelling and omitting spike-in chromatin. Replicated DNA profile is shown in blue. Signal was scaled as percentage of maximum at the locus depicted. **(H)** Bar plots showing the synchronization coverage (left) and ChOR-seq coverage (right) in H3K27me3 datasets from (G). Percentage is calculated from peaks subsetted into 500 bp non-overlapping windows.

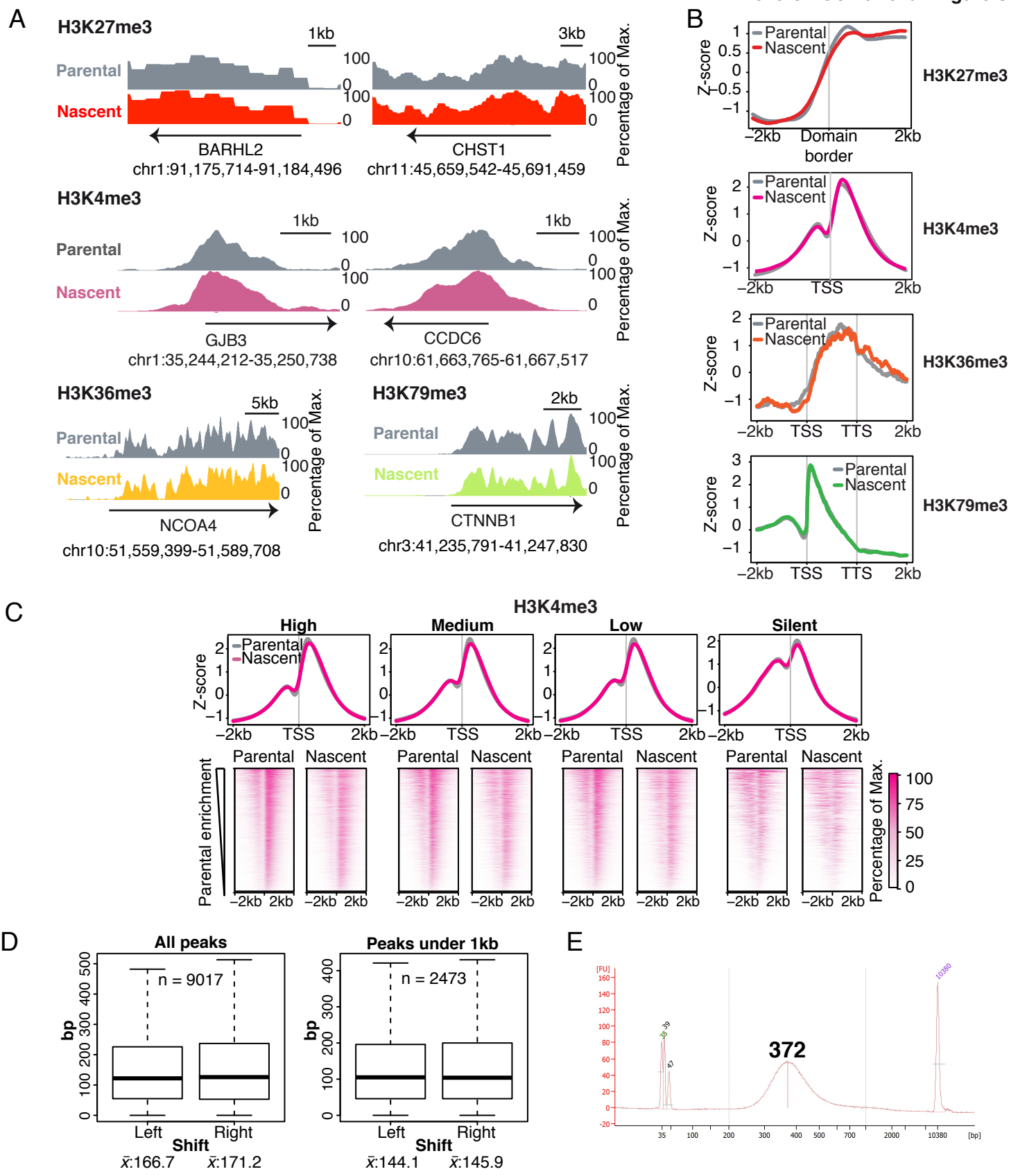

Figure S2. The histone H3 modification landscape is accurately reproduced in active and repressed genomic loci, Related to Figure 2. (A) Profiles of parental ChIP-seqs (gray and nascent H3K27me3 (red, H3K4me3 (pink, H3K36me3 (yellow, and H3K79me3 (green qChOR-seq at high resolution. Signal was scaled as percentage of maximum at the locus depicted. **(B)** Average profiles of parental and nascent H3K27me3, H3K4me3, H3K36me3, and H3K79me3 in replicate 2. H3K27me3 signal is plotted across 4 kb centered on borders of replicated H3K27me3 domains. H3K4me3 signal is plotted across 4 kb centered on replicated TSSs. H3K36me3 and H3K79me3 signals are plotted from 2 kb upstream to 2 kb downstream of replicated open reading frames. All data shown is z-score normalized. **(C)** Average profiles of parental and nascent H3K4me3 at TSSs parsed by expression level. Signal is plotted across 4 kb centered on replicated TSSs. All data shown is z-score normalized. **(D)** Box plots of difference in localization of parental and nascent H3K4me3 peaks (left and right shift) in base pairs for all peaks (left panel) and narrow peaks of less than 1 Kb (right panel). The narrow peaks containing a maximum of 5 nucleosomes provide the best resolution with respect to diffusion. n indicates the number of peaks analyzed. x̄ indicates the mean value of the absolute distance in bp between parental and nascent H3K4me3 peaks, note that the median is shown in the box plot. **(E)** Representative Bioanalyzer profile of a H3K4me3 ChOR-seq library. Taking into account the length of adaptors (approximately 120 bp), the average fragment size is 250 bp.

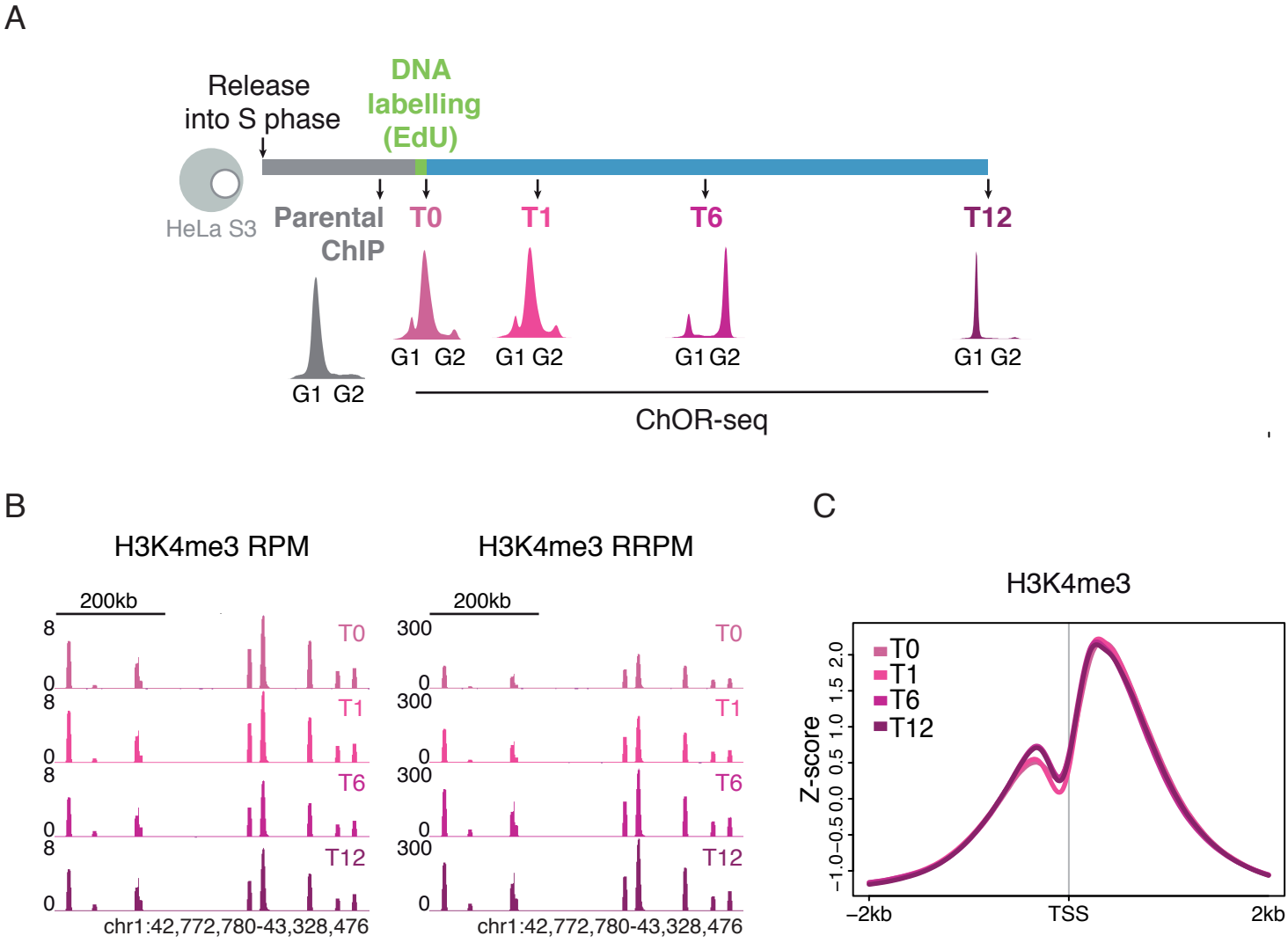

Figure S3. H3K4me3 restoration is complete within 6 hours and fastest in highly expressed promoters, Related to Figure 3. (A) Experimental set-up for H3K4me3 qChOR-seq. Parental chromatin was collected 1 hour before EdU labelling (10 minutes) and samples for qChOR-seq were harvested at the indicated time points post-EdU label. Cell cycle progression was assessed by FACS analysis of DNA content. **(B)** Comparison of H3K4me3 qChOR-seq profiles normalized to reads per million (RPM, left) or reference-adjusted reads per million using exogenous spike-in chromatin (RRPM, right). **(C)** Average profiles of H3K4me3 qChOR-seq. H3K4me3 signal is plotted across 4 kb centered on replicated TSSs. All data shown is z-score normalized.

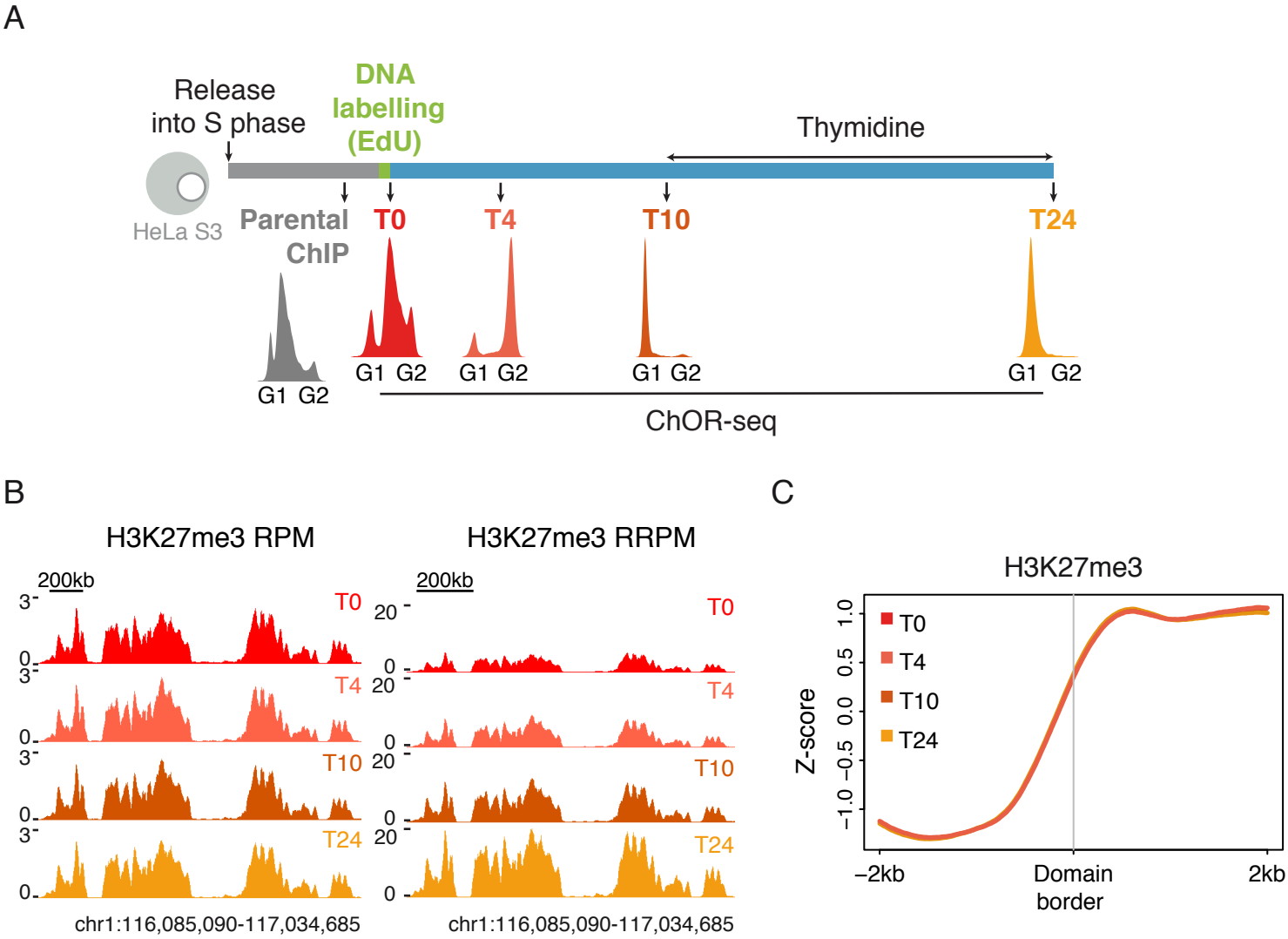

Figure S4. High PRC2 occupancy sites show faster H3K27me3 restoration, Related to Figure 4. (A) Experimental set-up for H3K27me3 qChOR-seq. Parental chromatin was collected 1 hour before EdU labelling (20 minutes) and samples for qChOR-seq were harvested at the indicated time points post-EdU label. Thymidine was added at 12 hours to prevent cells from entering a new round of replication. Cell cycle progression was assessed by FACS analysis of DNA content. **(B)** Comparison of H3K27me3 qChOR-seq profiles normalized to reads per million (RPM, left) or reference-adjusted reads per million using exogenous spike-in chromatin (RRPM, right). **(C)** Average profiles of H3K27me3 qChOR-seq. H3K27me3 signal is plotted across 4 kb centered on borders of replicated H3K27me3 domains. All data shown is z-score normalized.

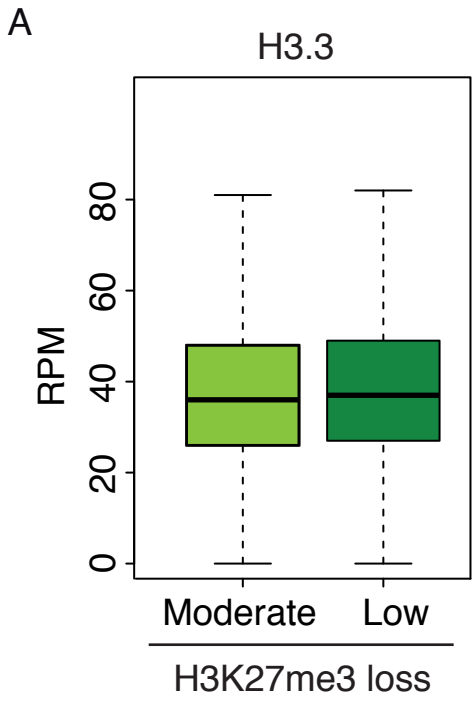

Figure S5. Parental H3K27me3 domains are stable across the cell cycle, Related to Figure 5. (A) Box plots of H3.3 ChIP-seq signal in moderate and low H3K27me3 loss regions. Data is subsetted into 2 kb non-overlapping windows and quantitated using reads per million (RPM). H3.3 data are from (Ray-Gallet et al., 2011).