Supplemental material

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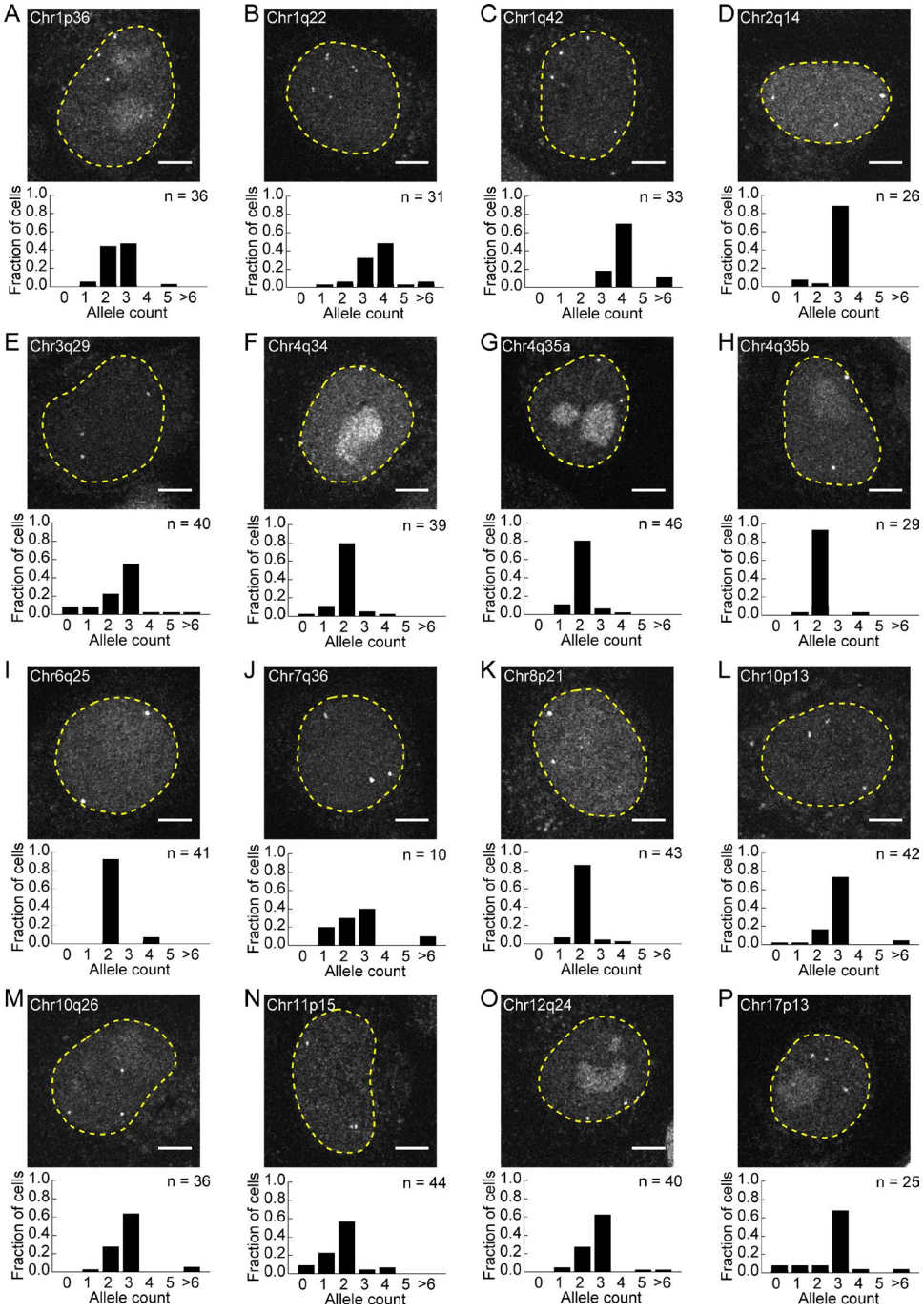

Figure S1. **Allele counts of dCas9-mEGFP–labeled foci per cell. (A–P)** Confocal images of representative live cells for each of the 16 cell lines with dCas9-mEGFP/sgRNA–labeled loci. Images show projections of 21 z sections; dashed lines indicate the nuclear rim. Bar graphs show the distribution of fluorescent dot numbers in each cell line. Fluorescent dots were counted in S or G2 cells, and pairs of foci that were closer than 2 µm were scored as a doublet representing a single replicated locus. n = number of cells. Bars, 5 µm.

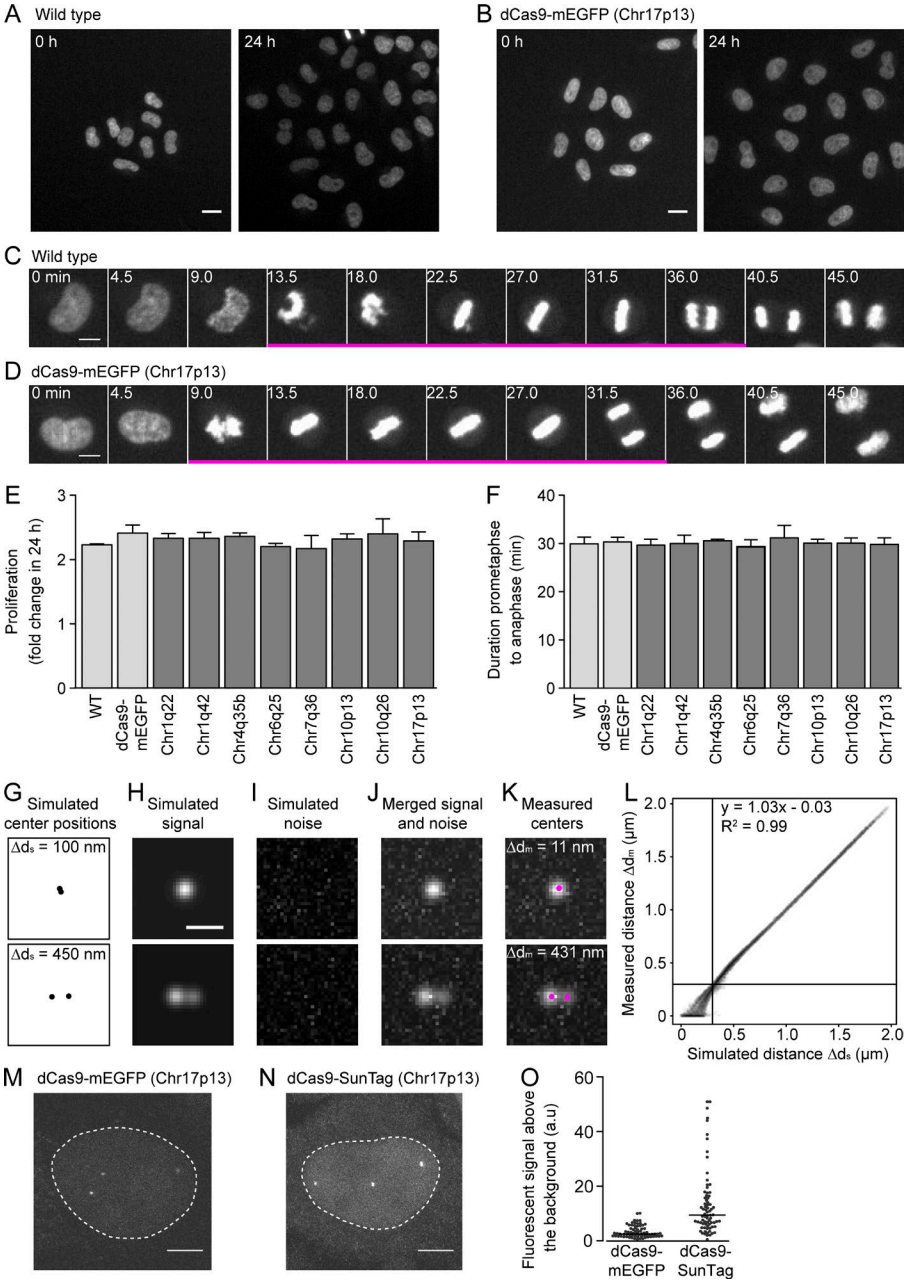

Figure S2. **Characterization of dCas9-mEGFP/sgRNA cell lines and validation of image analysis procedure. (A and B)** Time lapse images of WT cells (A) or cell lines expressing dCas9-mEGFP and sgRNA targeting Chr17p13 (B) were stained with SiR-Hoechst. **(C and D)** Example of a WT cell (C) and Chr17p13-labeled cell (D) progressing through mitosis. Single *z* sections are shown; numbers indicate time (min); the magenta bar indicates prometaphase to anaphase frames. **(E)** Quantification of cell proliferation from images as in A and B for cell lines as indicated. Differences are not significant (P = 0.12 by one-way ANOVA test). *n* = 3 experiments. **(F)** Quantification of mitotic duration (prometaphase until anaphase) in images as in C and D. *n* = 3 experiments, and *n* = 10 cells each. Differences are not significant (P = 0.97 by one way ANOVA test). Error bars indicate means ± SD. **(G–L)**. The accuracy of interchromatid distance measurements by the 2D Gaussian mixture model fitting procedure (Fig. 1, D–H) was estimated from simulated images. **(G)** Two simulated point light sources at random positions with simulated distance Δd_s = 100 nm (top) and Δd_s = 450 nm (bottom). **(H)** Two 2D Gaussian functions approximate signal in the microscopy images around the simulated point light sources in G. Variance and amplitude of the Gaussian functions are randomly sampled from the respective distributions observed in real microscopy images of the Chr3q29 locus. **(I)** Image noise is derived by randomly sampling noise values for each pixel from the distribution of noise intensities observed in real microscopy images of Chr3q29 locus. **(J)** Simulated images resulting from merged signal in G and noise in H. **(K)** Center positions (magenta) estimated in simulated images as in J by fitting mixture of two 2D Gaussian functions as in Fig. 1 (D–H). Δd_m indicates measured distances between measured center positions. **(L)** Relationship between simulated distances Δd_s in simulated images and distances Δd_m measured in these images using a mixture of two 2D Gaussian function–fitting procedures as in Fig. 1 (D–H). Vertical and horizontal lines indicate 300-nm threshold. *n* = 10,800 simulated images. **(M–O)** Comparison of signal intensity of dCas9-mEGFP– and dCas9-SunTag–labeled loci. *n* > 75. **(M and N)** Example cell nuclei (dashed lines) with the Chr17p13 locus labeled using dCas9-mEGFP (M) and dCas9-SunTag (N). **(O)** Signal intensity above the nucleoplasmic background at the Chr17p13 locus labeled with dCas9-mEGFP and dCas9-SunTag in the corresponding stable cell lines (P = 4.2×10^{-10} by unpaired two-sided *t* test). Bars: (A) 20 µm; (C, D, M, and N) 5 µm; (H–K) 1 µm.

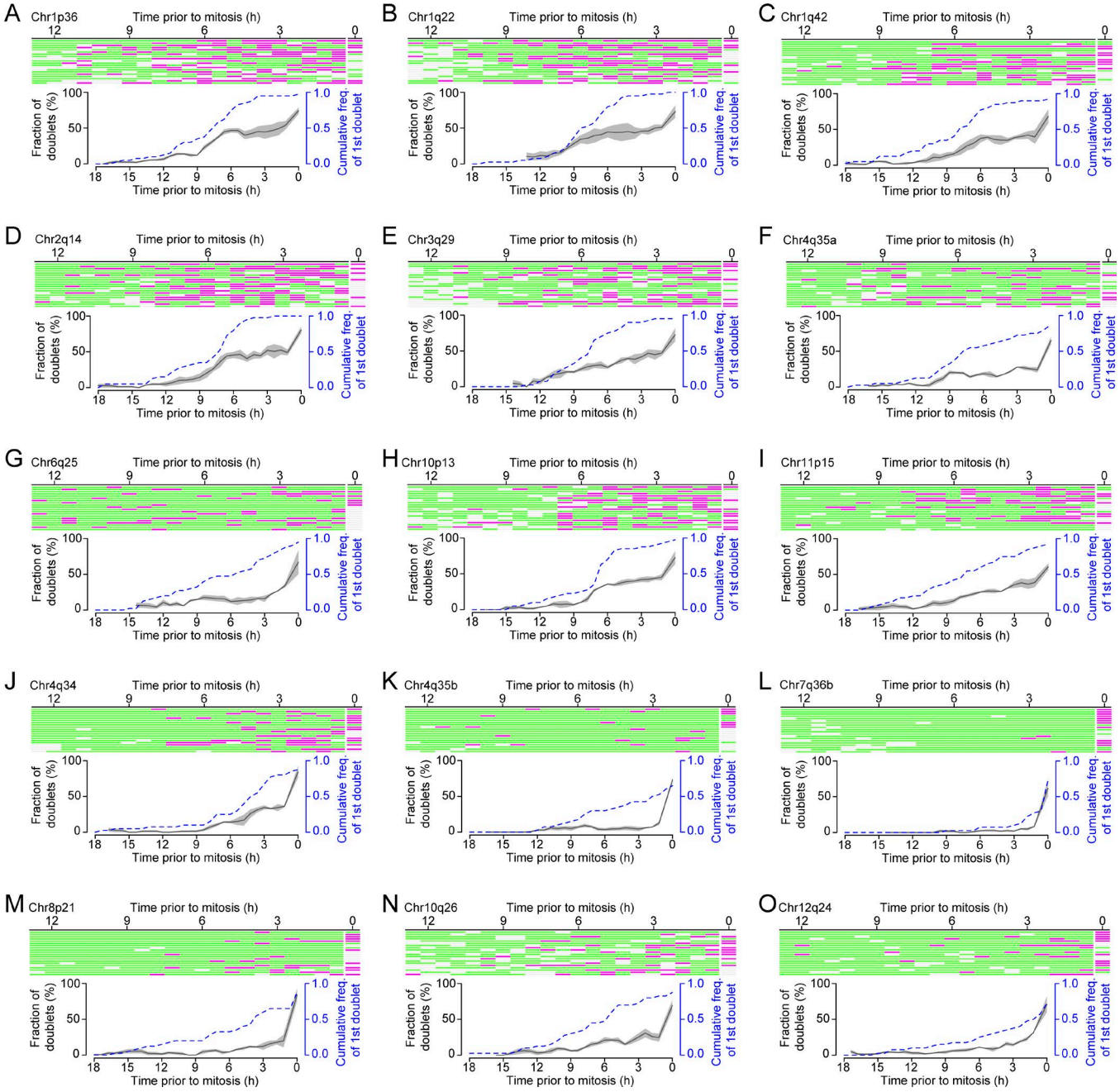

Figure S3. **Kinetics of sister locus resolution from S-phase until mitosis. (A–O)** Full datasets for individual locus trajectories as shown for Chr17p13 in Fig. 6 (B–E) for the other 15 loci. Top panels show individual allele trajectories during cell cycle progression. Doublets (magenta), singlets (green), and missing data points (light gray) were automatically annotated based on Gaussian mixture model fitting as in Fig. 1 (D–H; *n* = 20 trajectories for each genomic site). Bottom panels indicate fractions of doublets. Solid lines and shaded areas indicate means ± SEM, respectively. *n* = 3 experiments. Dashed lines indicate cumulative frequency of the first detected doublet. *n* = 40 trajectories. **(A–I)** Loci replicating during early S phase as shown in Fig. 7 (blue). **(J–O)** Loci replicating during late S phase as shown in Fig. 7 (red).

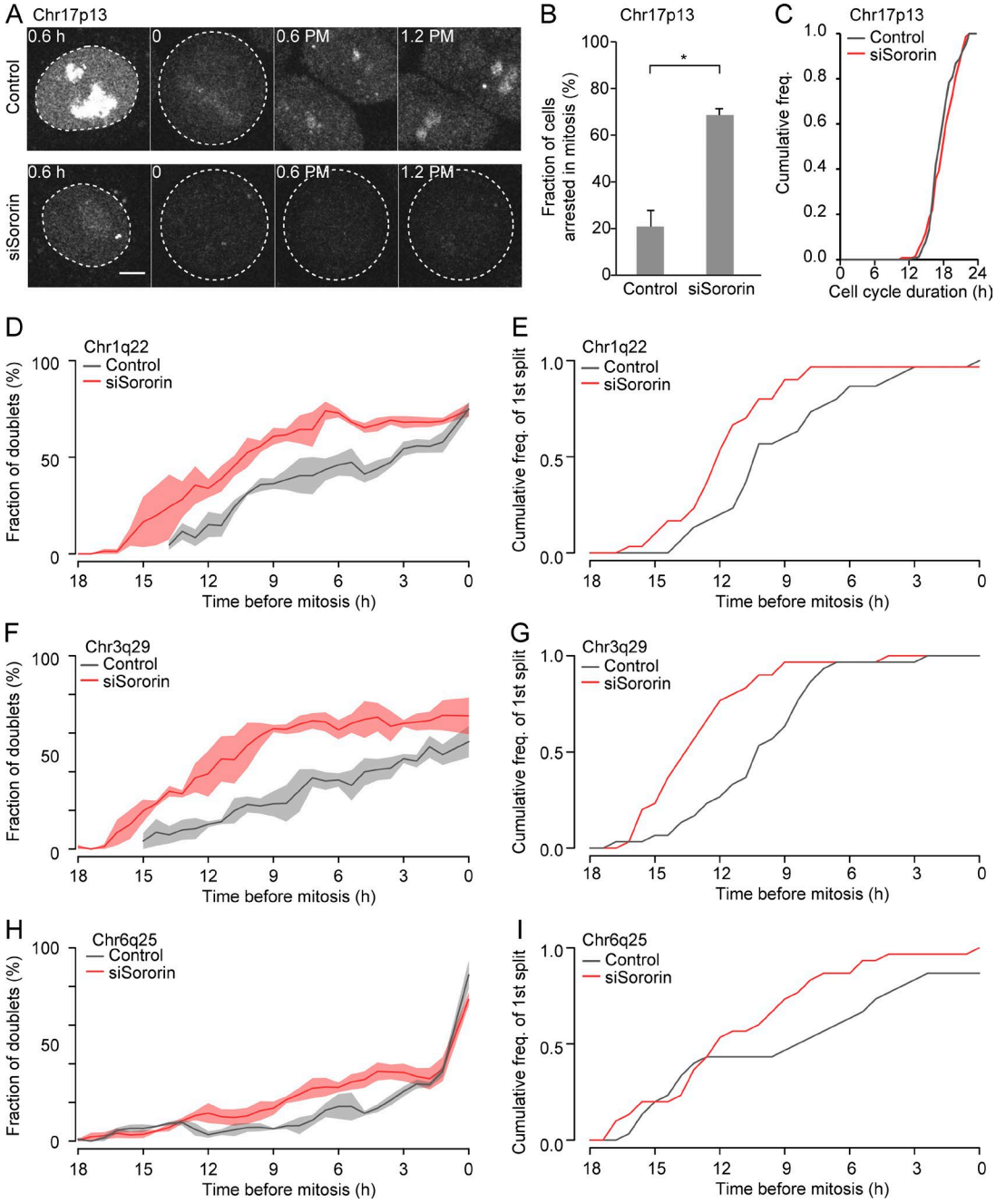

Figure S4. **Local sister chromatid cohesion persists for several hours after DNA replication. (A)** Example images of a control and Sororin-depleted cell entering mitosis. As judged by rounded appearance, the control cell stays in mitosis only for one frame, and thus mitosis takes <72 min. The Sororin-depleted cell, however, arrests in mitosis for more than three frames, and thus mitosis takes >72 min after Sororin depletion. Maximum-intensity projections of z sections 3.5 and 10.5 µm above the cover glass corresponding with middle sections of interphase and mitotic cells, respectively. Bar, 5 µm. **(B)** Cells arrested in mitosis after Sororin depletion as quantified from images in A. Error bars represent means ± SEM. $n = 3$ experiments. *, $P < 0.001$ derived from two-sided unpaired t test. **(C)** Cumulative histogram of cell cycle duration from three experiments of cells with the Chr17p13 locus labeled in control ($n = 98$) and Sororin depletion ($n = 110$) as in Fig. 8 (A–F). Cells were synchronized by mitotic shakeoff and imaged while progressing through cell cycle as in Fig. 1. Cell cycle duration was measured as time from the beginning of imaging to the first frame in mitosis (rounding of the cell as in A). **(D, F, and H)** The fraction of doublets over the cell cycle in control (gray) and Sororin-depleted cells (red) at Chr1q22 (D), Chr3q29 (F), and Chr6q25 (H) loci. Shaded areas indicate means ± SEM. $n = 3$ experiments. **(E, G, and I)** Cumulative frequency of the first observed doublets in control (gray) and Sororin-depleted (red) cells at Chr1q22 (E), Chr3q29 (G), and Chr6q25 (I) loci. $n = 30$ trajectories.

Table S1 is a separate Excel file showing positions and genomic neighborhoods of dCas9-mEGFP/sgRNA–labeled endogenous loci.

Data S1 is a separate Word document containing a FIJI macro for semiautomated tracking of individual alleles.

Data S2 is a separate Word document containing an R script for Gaussian fitting procedure to determine labeled locus positions.