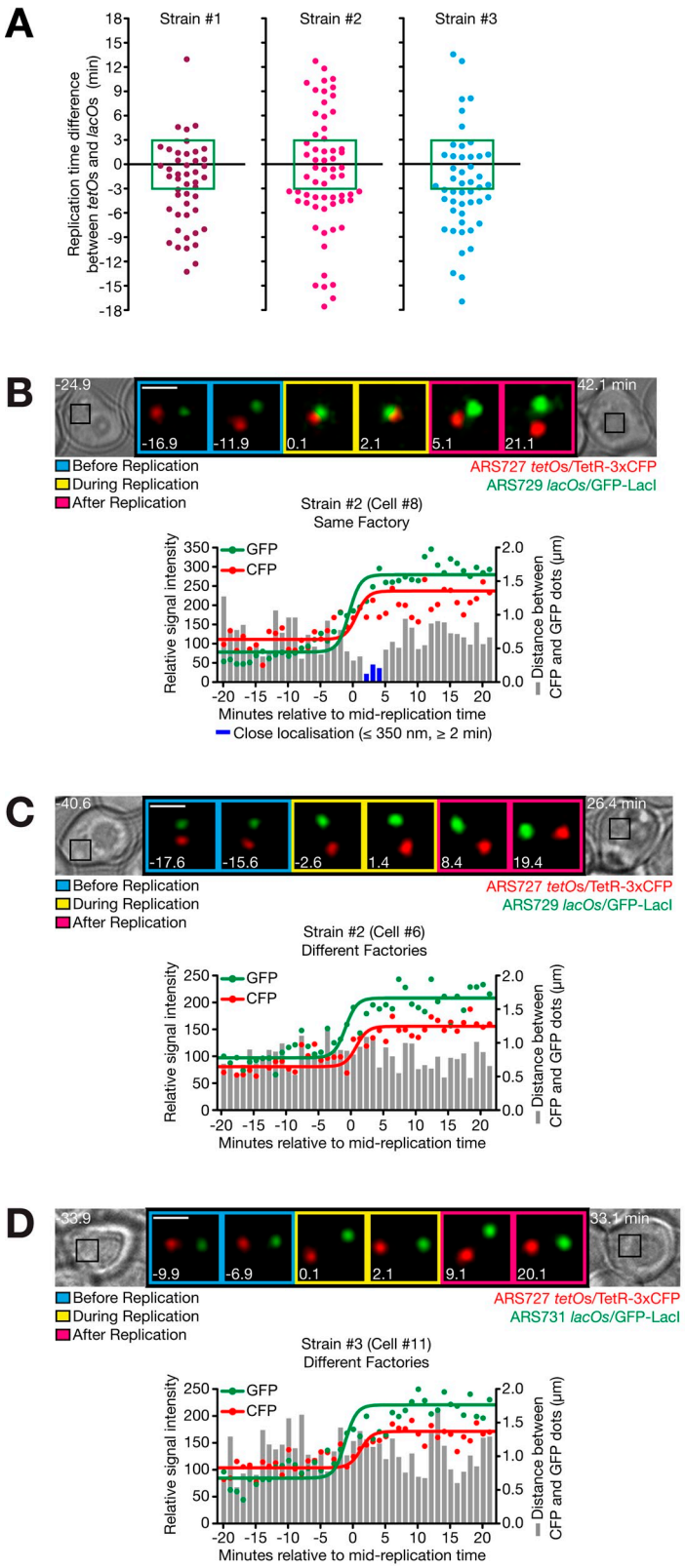
Saner et al., http://www.jcb.org/cgi/content/full/jcb.201306143/DC1

Figure S1. **Results supplemental to Fig. 1.** (A) The difference in replication time between two fluorescent dots ([time for CFP dot] − [time for GFP dot]) in individual cells of strains #1–3 (see Fig. 1 A), n = 46 (strain #1), 62 (strain #2), 48 (strain #3). The cells within the green rectangle, in which replication time difference was within ±3 min, were analyzed further; the numbers of such cells n = 23 (strain #1), 19 (strain #2), 19 (strain #3). (B and C) Representative examples of strain #2 showing replication of CFP and GFP dots at the same factory (B) and at different factories (C). Cells were treated and analyzed as in Fig. 1, C and D. Fitness of signal intensity by a sigmoidal curve: R^2 = 0.73 (B, CFP), 0.90 (B, GFP), 0.81 (C, CFP) and 0.85 (C, GFP). (D) A representative example of strain #3 showing replication of CFP and GFP dots at different factories. Cells were treated and analyzed as in Fig. 1, C and D. R^2 = 0.81 (CFP) and 0.88 (GFP). Bars, 1 µm.



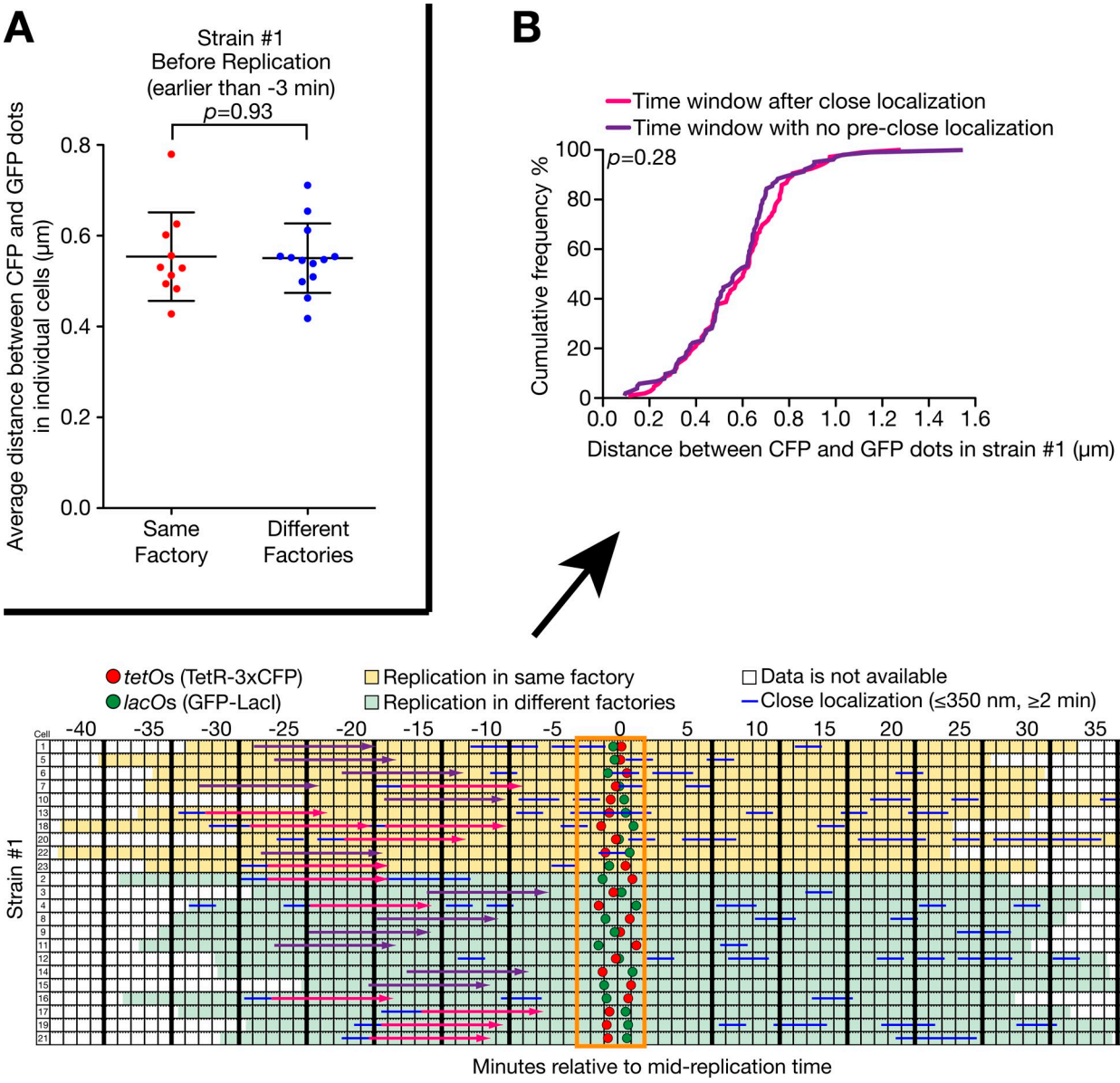


Figure S2. **Results supplemental to Fig. 2.** (A) The shorter physical distance between two fluorescent dots before replication is not correlated with their replication at the same replication factory. The average distance between the dots before replication (earlier than −3 min) in individual cells of strain #1 is plotted separately for replication at the same factory (0.55 ± 0.10 μm, mean ± SD, n = 10) and at different factories (0.55 ± 0.08 μm, n = 13). The middle bars and error bars represent means and SD, respectively. (B) Was the shorter distance between two fluorescent dots, after they were replicated at the same factory (observed in Fig. 2 C), simply due to correlation in distance with earlier time points? For example, if the two dots did not move vigorously after they had localized closely around their replication time (which was evaluated as their replication at the same factory), they would tend to localize closely for a while. To test whether there is such correlation in the distance between earlier and later time points, we analyzed the distances for 9 min, after their accidental close localization in G1 phase (pink arrows, allocated in 11 cells) and after a randomly chosen time point in G1 without such close localization (purple arrows as a control, allocated in 12 cells). We then analyzed the distances from the time points spanned by colored arrows (bottom), separately for pink and purple arrows (12 arrows for each color) but collectively for the arrows of each color; i.e., the distances at 12 × 9 = 108 time points (the distance was measured every min) were analyzed together for each group, represented by the arrow of each color, as follows. The distributions of these distances are shown in cumulative frequency in pink and purple lines (top); each line corresponds to the group of arrows of the same color in the bottom. The distributions did not show any significant difference between the two groups. Thus, it is unlikely that the observation in Fig. 2 C was simply due to correlation in distance between earlier and later time points. In fact, the motion of the dots was relatively rapid (see Materials and methods, Mathematical modeling ii), precluding such correlation.

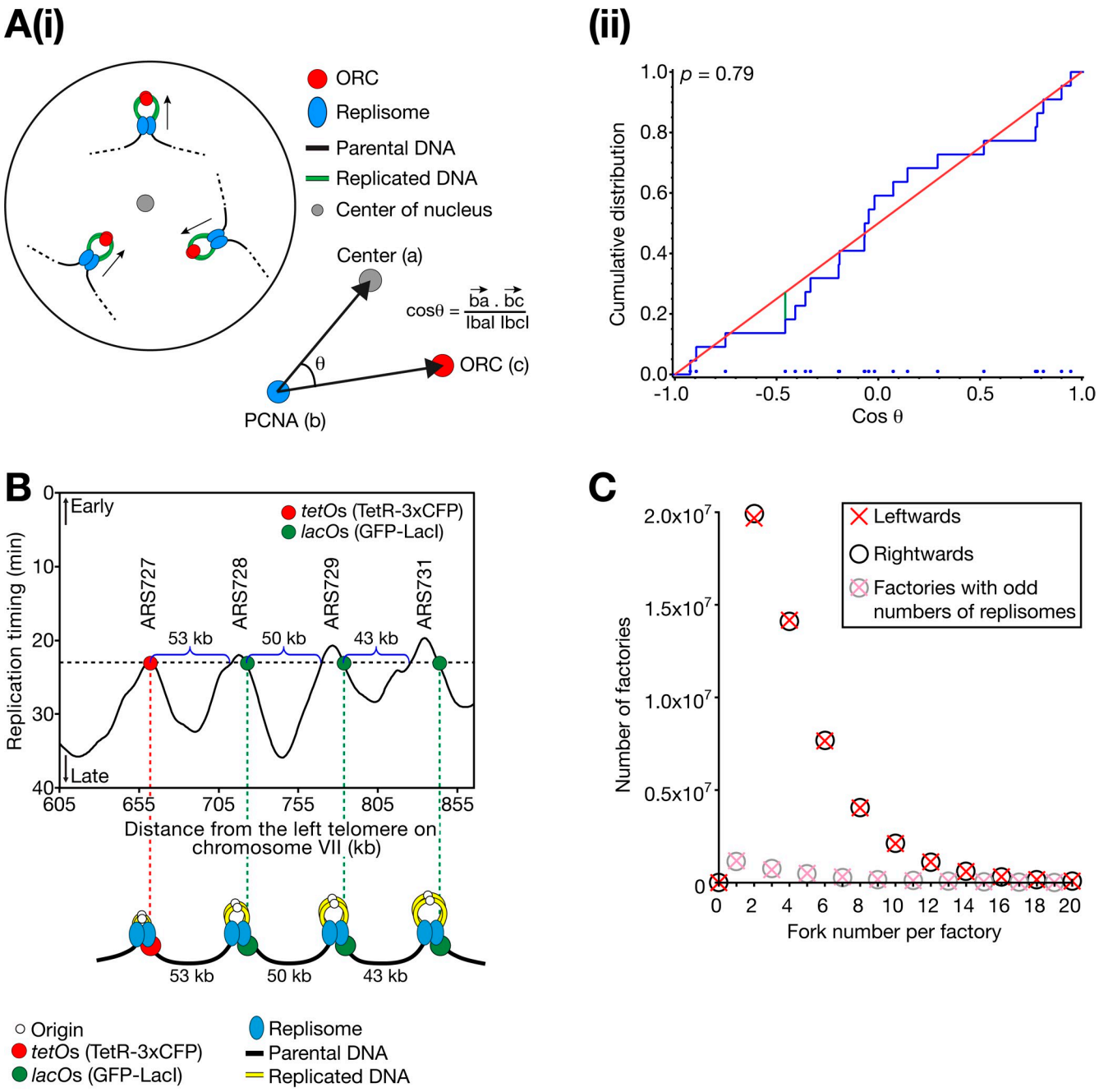


Figure S3. **Results supplemental to Fig. 3 F, Fig. 4 C, and Mathematical modeling iii.** (A) The direction in which replicated DNA was reeled out of a factory was random. (i) The direction was analyzed in images obtained for Fig. 3 F. To define the direction of newly replicated DNA relative to the center of the nucleus, cosθ was defined as illustrated in the three-dimensional space. Assuming that ORC2-bound origins were dispersed in the nuclear volume, the center of the nucleus was estimated from the mean coordinate of ORC2 objects identified in the nucleus using 3D surface rendering. (ii) The cumulative distribution of cosθ (blue) was obtained from 22 samples, where a group of ORC, EdU, and PCNA signals (one signal for each) was isolated from others and their signals localized adjacently in this order (e.g., Fig. 3 F, top). There was no significant deviation from random distribution (red), suggesting that replicated DNA comes out of a replication factory in random direction, relative to the center of the nucleus. D = 0.14 (the maximum difference between the curves, indicated by the green vertical line). (B) To plot the grouping probability of replisome pairs (obtained in Fig. 2 A) in the graph of Fig. 4 C, we needed to estimate the average chromosomal distance between relevant replisome pairs upon replication of fluorescent dots (i.e., *tetO* and *lacO* arrays). We estimated this distance as follows. We assumed, upon replication of fluorescent dots, relevant replication forks were reaching in the middle of *tetO* and *lacO* arrays (11.2 and 10.1 kb in length, respectively). In addition, we considered the following things: (1) In the replication profile (top, copy of a part of Fig. 1 A), the degrees of slopes were similar between right and left sides of the four relevant replication origins (ARS) and between the four relevant replicons; (2) the sizes of *tetO* and *lacO* arrays were similar. Therefore, while a replication fork from ARS727 moved rightward from the left end of *tetO*s to the middle of the array for 5–6 kb, sister replication forks from ARS728 (or ARS729 or ARS731) could also move for 5–6 kb leftward and rightward; the leftward fork movement led to offset the remaining half length of *tetO*s (in terms of chromosomal distance between replisome pairs), while the rightward fork moved from the left end of *lacO*s to the middle of the array (defining its replication). Thus, to obtain chromosomal distance between replisome pairs, we only needed to consider the integration sites of *tetO*s and *lacO*s (top), but not the length of these arrays, as shown at bottom. The replication timing profile (top) was obtained from Yabuki et al. (2002). (C) The number of factories with the indicated number of replication forks, obtained from one million genome-wide simulations. See the detail in Materials and methods, Mathematical modeling iii.

Reference

Yabuki, N., H. Terashima, and K. Kitada. 2002. Mapping of early firing origins on a replication profile of budding yeast. *Genes Cells*. 7:781–789. http://dx.doi.org/10.1046/j.1365-2443.2002.00559.x