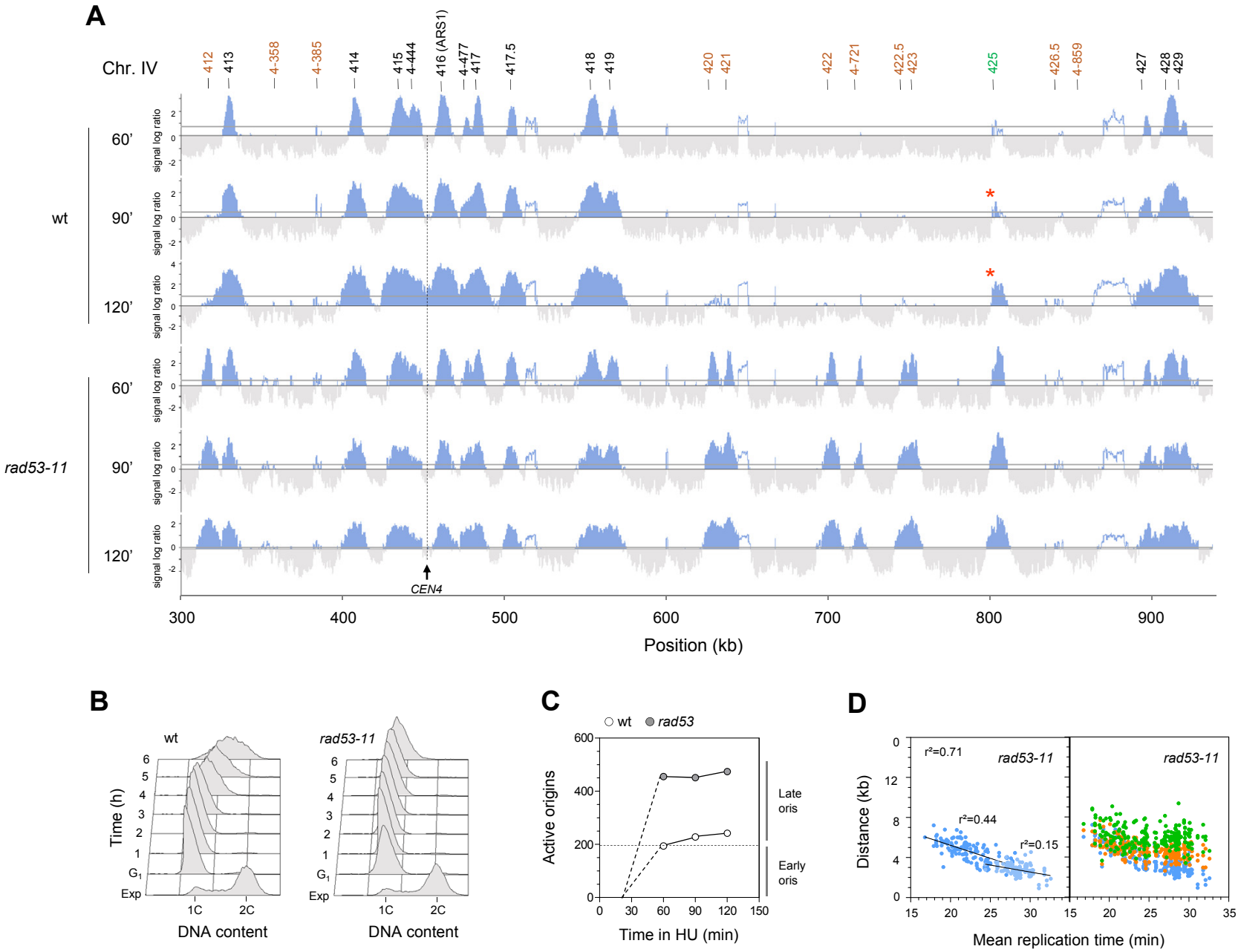
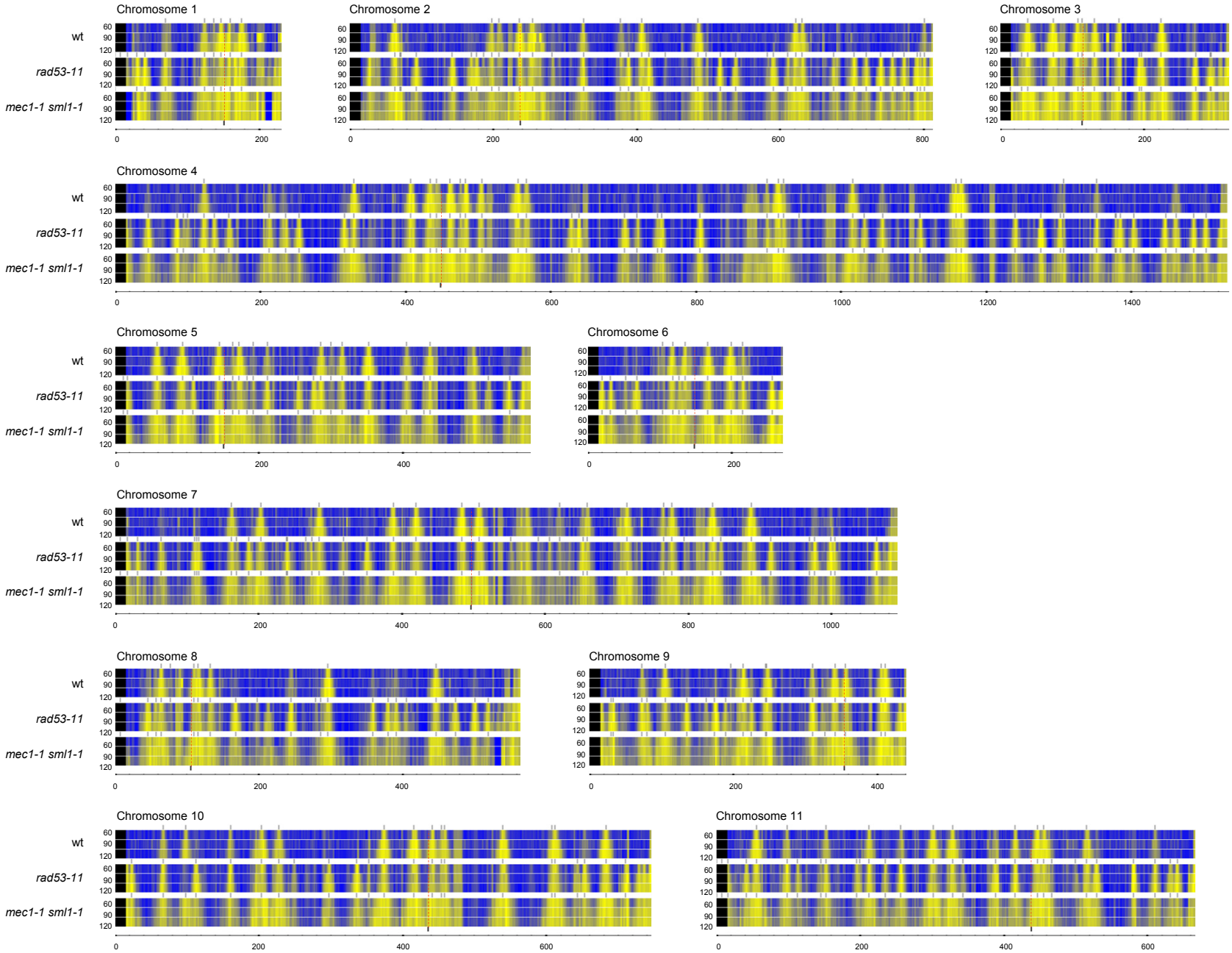
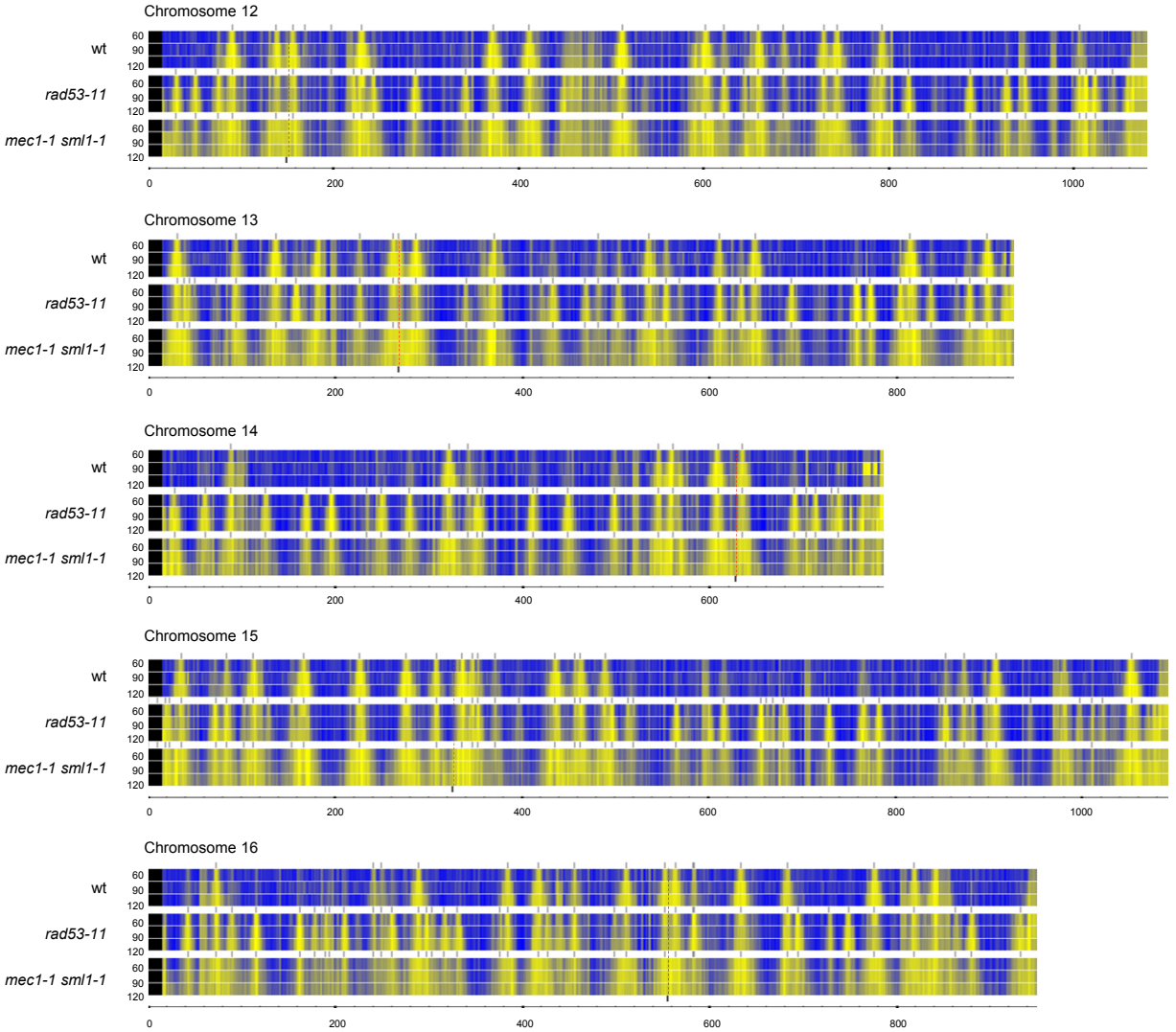


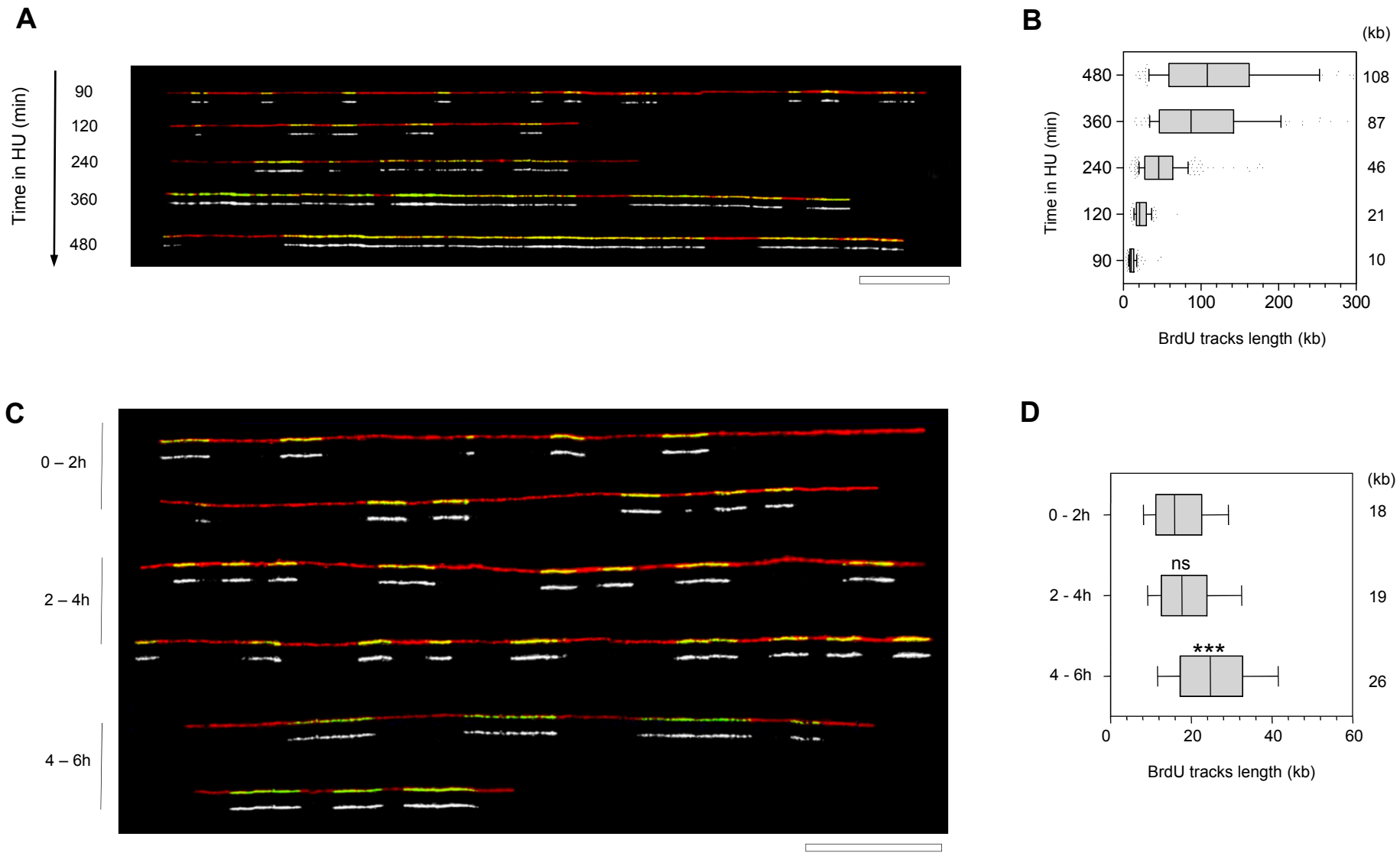
Supplementary Fig. 1



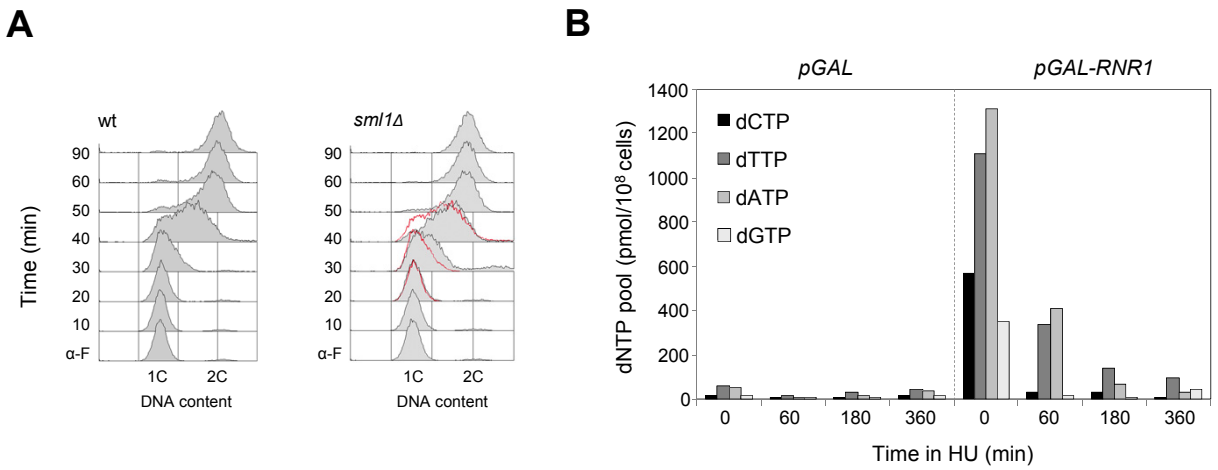
Supplementary Fig. 2



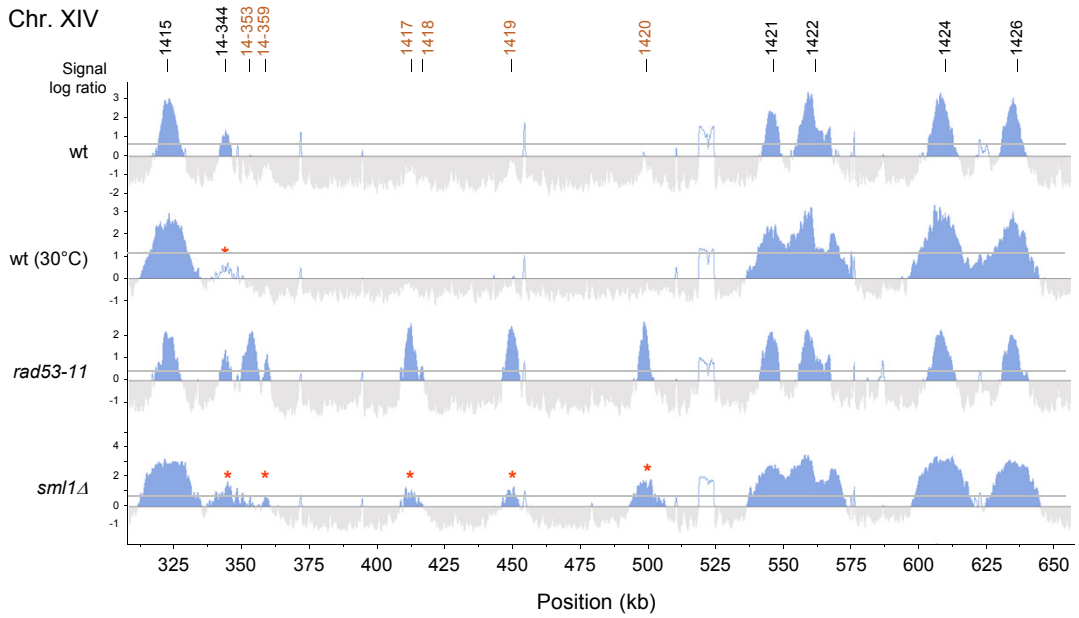
Supplementary Fig. 2



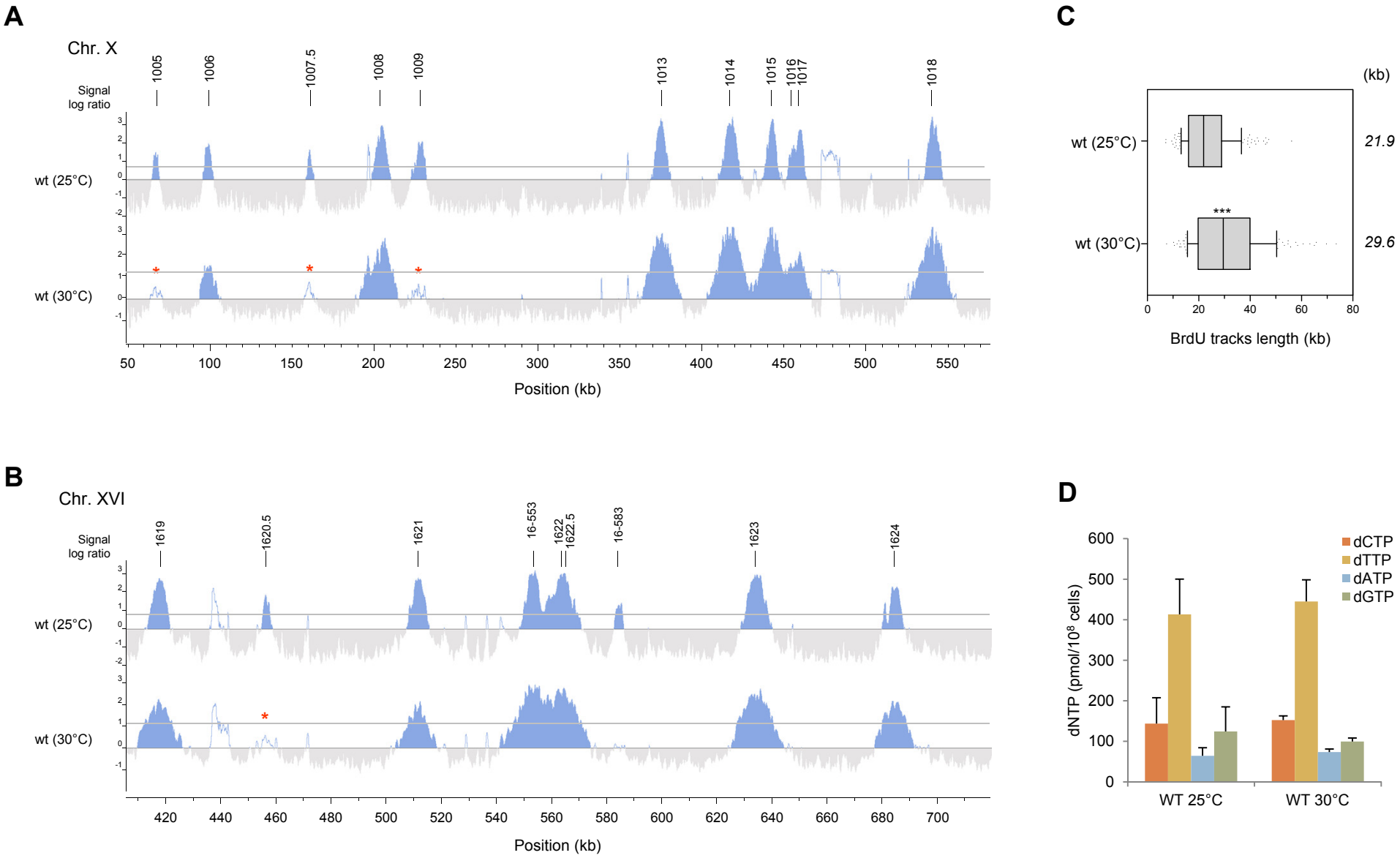
Supplementary Fig. 3



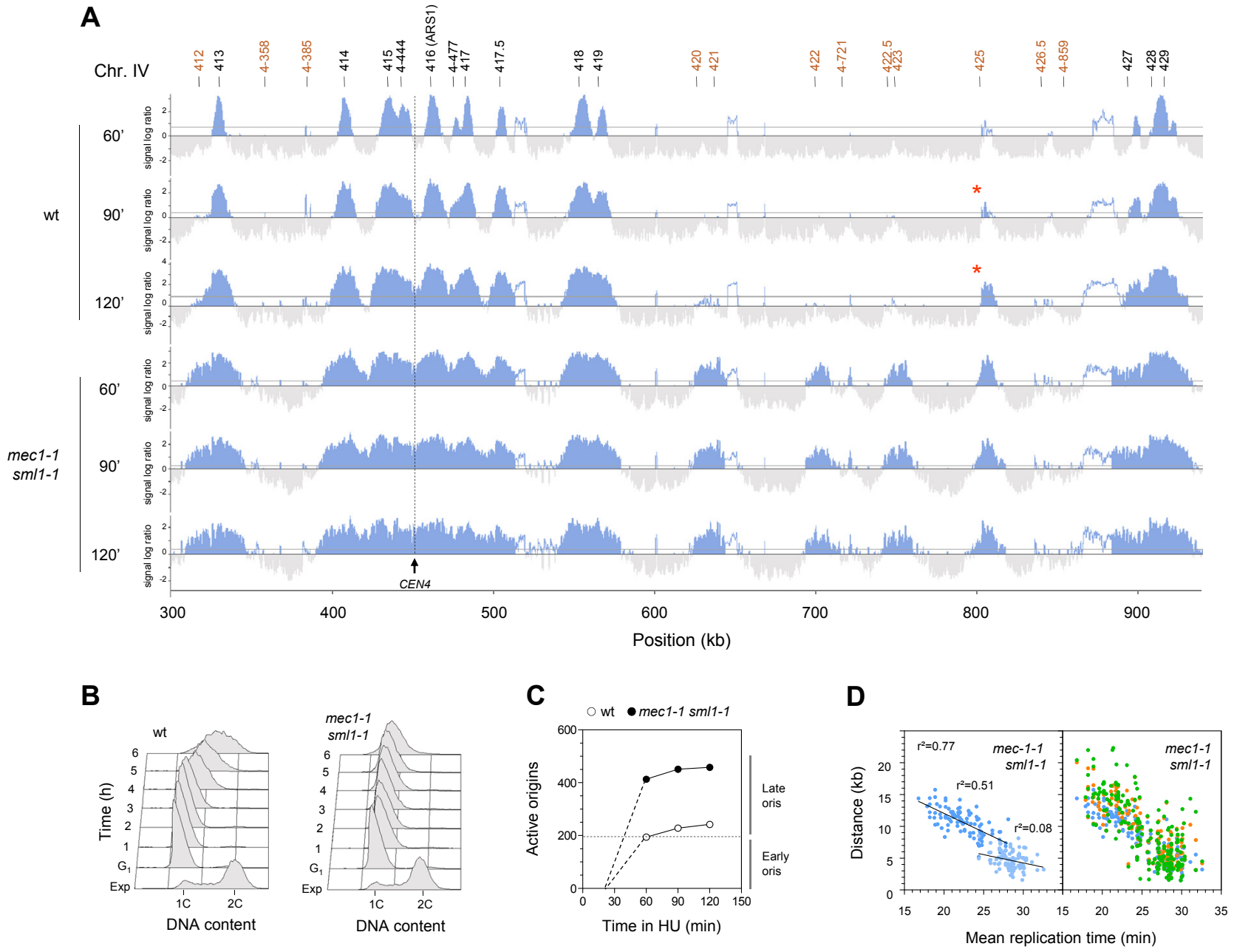
Supplementary Fig.4



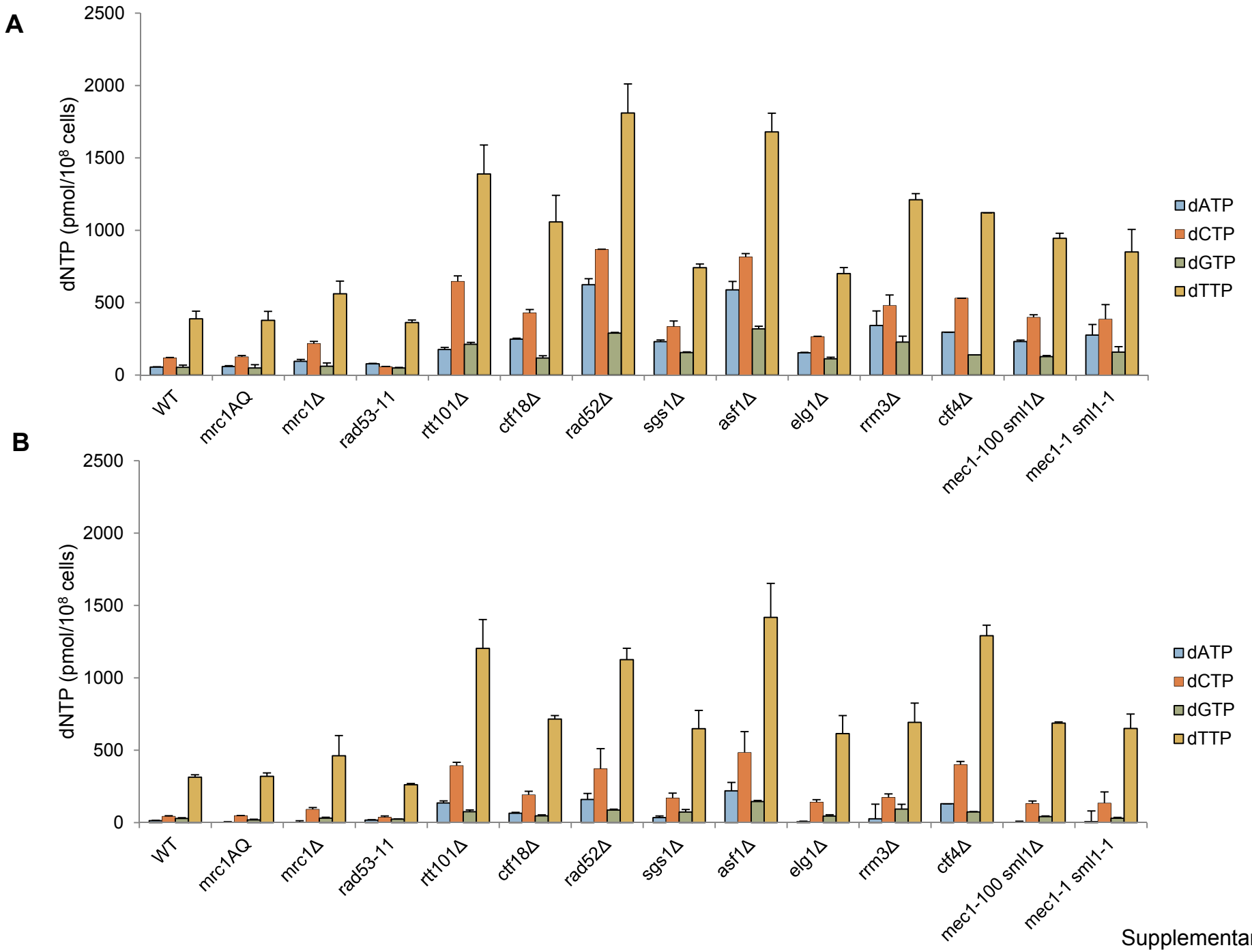
Supplementary Fig. 5



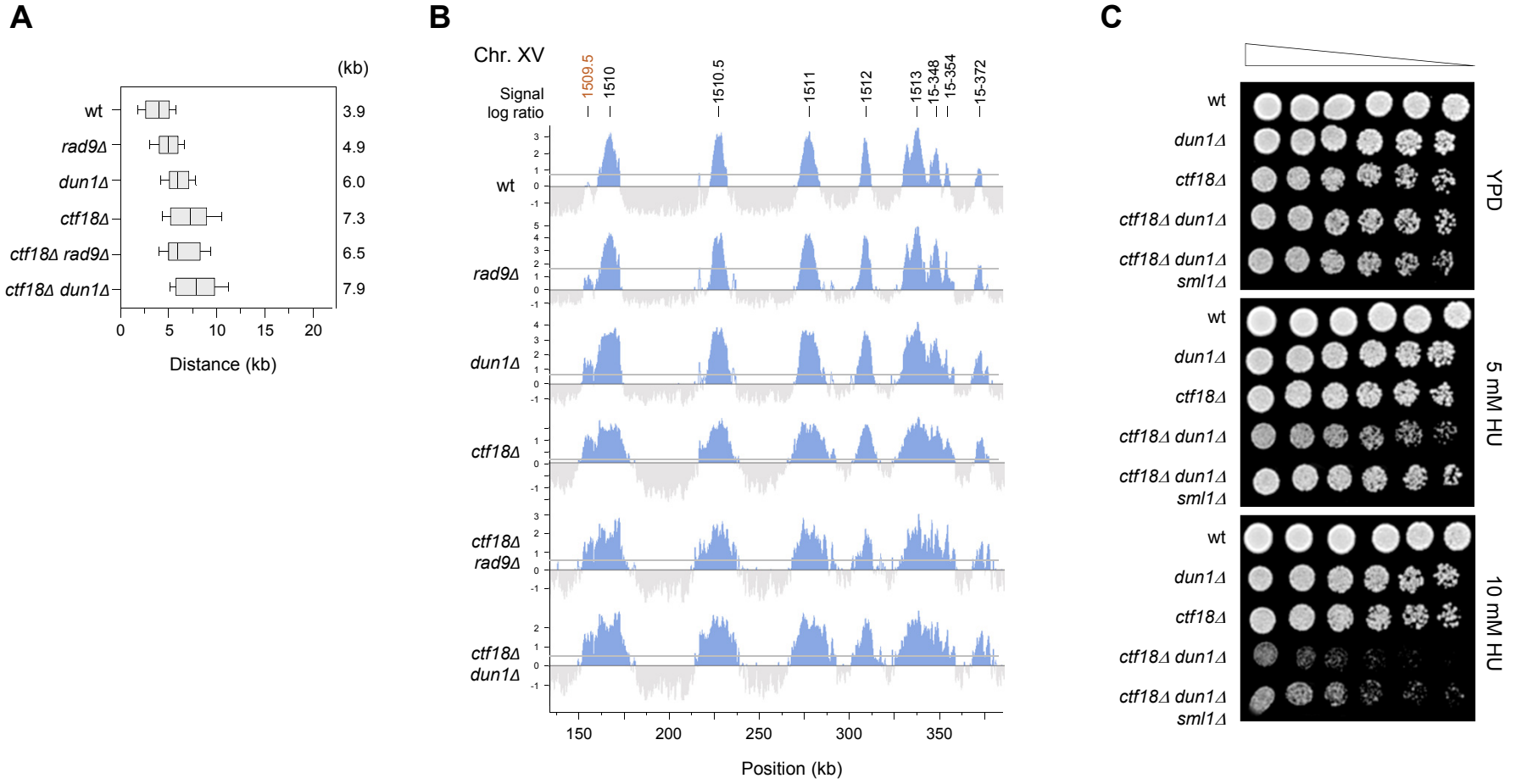
Supplementary Fig. 6



Supplementary Fig. 7



Supplementary Fig. 8



Supplementary Fig. 9

Supplementary Figures Legend

Supplementary Figure 1: Dynamics of origin usage and fork progression in wild type and *rad53-11* cells exposed to HU. Representative maps of a 650 kb region of chromosome IV. Horizontal grey lines indicate the threshold (50% of signal range) used to identify active origins (filled blue areas). Empty areas correspond to non-significant peaks or repeated sequences. Black and red numbers indicate active early and late origins, respectively. The red asterisks indicate the position of *ARS425* (in green) activated at 90 minutes but not at 60 minutes in wild type cells. The vertical black dotted line indicates the position of *CEN4*. (**B**) Flow cytometry analysis of DNA content in wild type and *rad53-11* cells released from G_1 into 200 mM HU for 1 to 6 hours. (**C**) Number of activated origins over time in hydroxyurea - treated wild type and *rad53-11* cells. (**D**) Scatter plot of the distance covered by replication forks versus the mean replication time in a normal S phase. Early and late origins are plotted as dark blue and light blue dots, respectively, for the 60 minutes time point. The distance covered by individual forks after 60, 90 and 120 min in HU is shown as blue, red and green dots, respectively.

Supplementary Figure 2: Kinetics of fork progression in wild type (PP872), *rad53-11* (PP37) and *mec1-1 sml1-1* (PP276) cells. Heat maps of the kinetics of BrdU incorporation at 60, 90 and 120 minutes in HU. Low (blue) to high intensity (yellow) of BrdU incorporation is shown for the 16 yeast chromosomes. Origins active at 60 minutes are indicated with vertical bars. Red dotted lines show the position of centromeres. Note that most of the centromeres are replicated in *mec1-1 sml1-1* mutants, but not in wild type and *rad53-11* cells, as suggested by a recent study (Feng et al., 2009).

Supplementary Figure 3: Replication forks move at a slow but constant rate in hydroxyurea. (**A**) Wild-type (PP872) cells were released from α-factor synchronization into 200 mM HU and pulse-labeled with 400 μg/ml BrdU for the indicated time. Replication was monitored at the single-molecule level by DNA combing. Representative images of DNA fibers are presented. Green: BrdU, red: DNA. Bar, 50 kb. (**B**) The graph depicts the distribution of BrdU track length during the time course. Box and whiskers indicate 25-75 and 10-90 percentiles respectively. The median of BrdU tracks length is indicated in kb. (**C**) Wild-type (PP872) cells were released from α-factor synchronization into 200 mM HU, we performed 2h BrdU pulses (400 μg/ml BrdU) every 2h for 6h. **(D**) The graph depicts the distribution of BrdU track length during the time course. Box and whiskers indicate 25-75 and 10-90 percentiles respectively. The mean of BrdU tracks length is indicated in kb.

Supplementary Figure 4: S-phase progression in wild-type (PP872) and *sml1Δ* (PP924) and dNTP levels in HU-time-course. (**A**) Flow cytometry profiles of wild type (PP872) and *sml1Δ* (PP924) cells collected at t_0 (αF) and at the indicated time in S phase. Red lines represent the corresponding DNA content of wild type cells overlaid on *sml1Δ* profile. (**B**) Exponentially growing *pGAL* (PP917) and *pGAL-RNR1* (PP918) cells were cultured for 2 hours with 2% galactose before addition of 100 mM HU. dNTP concentration was measured during the time course.

Supplementary Figure 5: Pattern of origin usage in wild-type cells, *rad53-11* and *sml1* mutants. (A) G_1-arrested wild-type (PP872), *rad53-11* (PP37) and *sml1Δ* (PP924) cells were released into YPD with 200 mM hydroxyurea and BrdU for 60 minutes at 25°C or 30°C. Representative replication profiles are shown on a portion of chromosome XIV. Early and late origins are depicted as in **Figure 1**. Significant peaks are filled in blue, horizontal grey lines

indicate the threshold used for peak calling (50% of signal range). Empty areas correspond to non-significant peaks or repeated sequences. Black and red numbers indicate active early and late origins, respectively. Asterisks indicate late origins fired in *sml1Δ* cells and an early origin repressed in wild-type cells grown at 30°C.

Supplementary Figure 6: Effect of temperature on wild-type cells replication (**A-B**) Replication profiles in wild type cells (PP872) cultured at 25°C or 30°C**.** G1 arrested cells were released into S phase for 60 minutes in the presence of HU and BrdU. Representative maps of a 500 kb region of chromosome X (**A**) and of a 320 kb region of chromosome XVI (**B**). Empty areas correspond to non-significant peaks or repeated sequences. Significant peaks are filled in blue, horizontal grey lines indicate the threshold used for peak calling (50% of signal range). The red asterisks indicate the position of early origins which are repressed at 30°C but not at 25°C after 60 min in HU. (**C**) DNA replication was monitored at the single-molecule level by DNA combing on asynchronous cells after a 30 min BrdU pulse. The graph depicts the distribution of BrdU track length during the time course. Box and whiskers indicate 25-75 and 10-90 percentiles respectively. The median of BrdU tracks length is indicated in kb. Asterisks indicate the p-value of the statistical test (Mann-Whitney rank sum t-test, *** pvalue < 0.0001) (**D**) dNTP concentration in asynchronous wild-type cells grown either at 25 or 30°C.

Supplementary Figure 7: Dynamics of origin usage and fork progression in wild type and *mec1-1 sml1-1* cells exposed to HU. (**A**) Representative maps of a 650 kb region of chromosome IV. Horizontal grey lines indicate the threshold (50% of signal range) used to identify active origins (filled blue areas). Empty areas correspond to non-significant peaks or repeated sequences. The red asterisks indicate the position of *ARS425* activated at 90 minutes

but not at 60 minutes in wild type cells. The vertical black dotted line indicates the position of *CEN4*. (**B**) Flow cytometry analysis of DNA content in wild type and *mec1-1 sml1-1* cells released from G_1 into 200 mM HU for 1 to 6 hours. (**C**) Number of activated origins over time in hydroxyurea - treated wild type and *mec1-1 sml1-1* cells. (**D**) Scatter plot of the distance covered by replication forks versus the mean replication time in a normal S phase. Early and late origins are plotted as dark blue and light blue dots, respectively, for the 60 minutes time point. The distance covered by individual forks after 60, 90 and 120 min in HU is shown as blue, red and green dots, respectively.

Supplementary Figure 8: dNTPs pools are spontaneously increased in a series of mutants. G_1-arrested wild-type (PP872), *mrc1*^{AQ} (PP679), *mrc1Δ* (PP913), *rad53-11* (PP37), *rtt101Δ* (PP285), *ctf18Δ* (PP907), *rad52Δ* (PP501), *sgs1Δ* (PP35), *asf1Δ* (PP889), *elg1Δ* (PP1013), *rrm3Δ* (PP1039), *ctf4Δ* (PP908), *mec1-100 sml1Δ* (PP685) and *mec1-1 sml1-1* (PP276) cells were released into YPD with 200 mM hydroxyurea. Each dNTP concentration was measured in these strains in G_1 and after 60 min in HU.

Supplementary Figure 9: Elevated pools improve replication stress tolerance in *ctf18Δ* cells. (**A, B**) Wild type (PP872), *rad9Δ* (PP914), *dun1Δ* (PP1387), *ctf18Δ* (PP907), *ctf18Δ rad9Δ* (PP848) and *ctf18Δ dun1Δ* (PP1321) strains cells were synchronized in G_1 with α-factor, and released into medium containing 200 mM hydroxyurea and 400 μg/ml BrdU for 60 minutes. (**A**) The graph depicts the distribution of distances covered by individual replication forks in the indicated strains. Distances were measured on the whole genome BrdU IP-chip replication profiles. Box and whiskers indicate 25-75 and 10-90 percentiles respectively. Mean BrdU tracks length is indicated in kb. (**B**) Replication profiles of a region of chromosome 15. Early and late are depicted in back and red respectively. Significant peaks

are filled in blue, horizontal grey lines indicate the threshold used for peak calling (50% of signal range). (**C**) Exponential YPD cultures of wt (PP872), *dun1Δ* (PP1387), *ctf18Δ* (PP907), *ctf18Δ dun1Δ* (PP1321) and *ctf18Δ dun1Δ sml1Δ* (PP1434) were diluted to $2.5.10^5$ cells/ml and a defined number of cells (5-fold dilutions) were spotted on YPD or YPD containing either 5 or 10 mM HU. Growth of *ctf18Δ* cells in the presence of HU is dependent on Dun1. The growth defect of *ctf18Δ dun1Δ* cells can be partly restored by deleting *SML1*.

Strain	Full genotype
PP872	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5, ura3::URA3/GPD-TK* $_7$
PP35	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, sgs1*::LEU2*
PP37	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5, ura3* ::URA3/GPD-TK$_7$, *rad53-11*
PP276	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5, ura3* ::URA3/GPD-TK$_7$, *mec1-1, sml1-1*
PP285	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, rtt101*::nat*
PP501	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, rad52*::TRP1*
PP679	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5 , ura3::URA3/GPD-TK* $_7$, *mrc1::HIS5, mrc1aq::LEU2*
PP685	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *mec1-100, sml1::HIS3*
PP848	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, ctf18::*TRP1, rad9::HIS3*
PP851	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, ctf4::*TRP1, rad9::HIS3*
PP889	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, asf1*::TRP1*
PP907	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *ctf18::HIS3*
PP908	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *ctf4::HIS3*
PP913	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *mrc1::HIS*
PP914	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *rad9::HIS*
PP917	*MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, RAD5, leu2-3::LEU2/TK-hENT1, ura3::URA3-pGAL*
PP918	*MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, RAD5, leu2-3::LEU2/TK-hENT1, ura3::URA3-pGAL-rnr1*
PP919	*MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, RAD5, leu2-3::LEU2/TK-hENT1, ura3::URA3-pGAL-rnr1-D57N*
PP924	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *sml1::HIS*
PP944	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *sml1::HIS, rad53::KanMX2*
PP1012	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *rad24::TRP1*
PP1013	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, elg*1::TRP1*
PP1039	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, rrm3*::nat*
PP1172	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *ddc1::KanMX4*
PP1321	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, ctf18::*TRP1, dun1::TRP1*
PP1322	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, ctf4::*TRP1, dun1::TRP1*
PP1325	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *tel1::kanMX, mec1::TRP1, sml1::HIS*
PP1387	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *dun1::TRP1*
PP1434	MAT*a, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3,* GAL*, RAD5,* ura3::URA3/GPD-TK$_7$, *ctf18::TRP1, dun1::TRP1, sml1::HIS*
AC402	*MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, RAD5*
AC438	*MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, RAD5, ura3::URA3-pGAL-rnr1*