SUPPLEMENTAL DATA

FIGURE LEGENDS

Figure S1

(A) Western blot analysis of histone H4 in wild-type and *t::HHF2*, *G::HHF2* and *M::HHF2* cells synchronized in G1 and released into fresh medium for 60 minutes (2N DNA content) under conditions of histone depletion. Pgk1 was used as loading control. The average and SEM of 3 independent experiments is shown. One of them is shown on the right. One asterisk indicates a statistically significant difference according to a paired *t*-test (p-value <0.05).
(B) Cell cycle distribution of wild-type and *t::HHF2* cells growing asynchronously under conditions of histone depletion, based on the nuclear and spindle morphologies by DAPI and immunofluorescence with antibodies against tubulin. The relative amount of cells at the different cell cycle stages was obtained by counting 200 cells for each strain from two independent cultures.

Figure S2

(A) S phase checkpoints are not required for *t::HHF2* viability. Cell growth analyses of wild-type, *t::HHF2*, *mec1*Δ, *rad53*Δ *chk1*Δ, *t::HHF2 mec1*Δ and *t::HHF2 rad53*Δ *chk1*Δ. All strains are *sml1*Δ.
(B) Metaphase arrest by histone loss is independent of histone H2A phosphorylation at Ser129. Flow cytometry analysis of wild-type, *t::HHF2*, *hta1,2S129** and *t::HHF2 hta1,2S129** cells synchronized in G1 and released into fresh medium under conditions of histone depletion.
(C) Metaphase arrest by histone loss depends on Mad2. Cell cycle progression analysis by immunofluorescence with antibodies against tubulin of wild-type, *G::HHF2*, *mad2*Δ, and *G::HHF2 mad2*Δ cells synchronized in G1 and released into fresh medium under conditions of histone depletion.

(D) Cell growth in galactose 2% and glucose 2% containing media (left), and immunofluorescence analysis with antibodies against tubulin (right) of *chr-G::HHF1* and *chr-G::HHF1 mad2*Δ cells synchronized in G1 and released into fresh medium under conditions of histone depletion. *chr-G::HHF1* cells express histone H4 exclusively from the chromosomal locus *HHF1*, where the endogenous *HHF1* promoter was replaced by the *GAL1* promoter.

Figure S3

(A) Histone depletion increases the spindle length. Distribution of cells with two or a single cenIV-GFP focus according to their spindle length, which was determined as the distance between the SPBs (detected as Spc42-Cherry foci) in the metaphase arrested cells from Figure 3C. The average (± SEM) spindle length is also shown. Three asterisks indicate a statistically significant difference according to a one-way Anova test (p-value <0.001).

(B) Distribution of cells with two or a single cenIV-GFP focus according to the symmetry of their centromeres relative to their SPBs, which was determined as the ratio between the short and long distances from the centromere (detected as a cenIV-GFP dot) to each SPB (detected as two Spc42-Cherry dots) in the metaphase arrested cells from Figure 3C.

Figure S4

(A) Western blot analysis of histone H4 in *t::HHF2*, *t::HHF2 smc2-8*, *M::HHF2* and *M::HHF2 top2^{td}* cells synchronized in G1 and released into fresh medium under conditions of histone depletion for 120 (*t::HHF2* strains) and 90 (*M::HHF2* strains) minutes at restrictive conditions for either *smc8-2* or *top2^{td}*. Pgk1 was used as loading control. MW, molecular weight marker.

(B) Cell cycle progression analysis by Flow cytometry of wild-type, *t::HHF2*, *smc2-8* and *t::HHF2 smc2-8* cells synchronized in G1 with α-factor and released into fresh medium at 26^{o}C under conditions of histone depletion.

(C) DNA damage sensitivity of wild-type and *smc2-8* cells at permissive temperature as

determined by plating ten-fold serial dilutions from the same number of mid-log phase cells in SMM plates containing either methyl-methane sulfonate (MMS) or hydroxyurea (HU) at the indicated concentrations.

(D) Cell cycle progression analysis by Flow cytometry of wild-type and *smc2-8* cells synchronized in G1 with α-factor and released in the presence of 50 mM HU for different times at 26^{o}C.

Figure S5

S phase checkpoints are not required for *t::HHF2* viability in the absence of Mad2. Cell growth analyses of wild-type, *mec1*Δ, *mad2*Δ, *t::HHF2*, *t::HHF2 mec1*Δ, *t::HHF2 mad2*Δ, *t::HHF2 mec1*Δ *mad2*Δ **(A)**, *mad2*Δ, *rad53*Δ *chk1*Δ, *mad2*Δ *rad53*Δ *chk1*Δ, *t::HHF2 mad2*Δ, *t::HHF2 rad53*Δ *chk1*Δ, and *t::HHF2 mad2*Δ *rad53*Δ *chk1*Δ **(B)**. All strains are *sml1*Δ.

Figure S6

Southern analysis of intermediates of the centromeric plasmid pRS416 in *top2^{td}* transformants synchronized in G1 and released into fresh medium at restrictive conditions for Top2 expression. CI, CII and CIII show different catenated intermediates as inferred by their accumulation in the absence of Top2; rM, relaxed monomers; SC(-)M, negatively supercoiled monomers. *t::HHF2* (times 90 and 120 min) are samples from Figure 7C. The asterisk marks an unspecific signal.

Table S1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Ref.	Figure
w303-1aR5	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5*	(1)	1A-C; 3A; 4D; 5A; 6A-C; S2B
YK402-2	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 bar1Δ::hisG*	(2)	1E; 2A; 4A,C,E-H; 5C; 7A,C; S1A-B; S4B-D
wtH4-9	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4)*	(3)	1A-C; 3A; 6A-C; S2B
wtH4-9b	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) bar1Δ::NatMX4*	This work	1E; 2A; 4C,E-F,H; 5C; 7A,C; S1A-B; S4A-B; S6
w303s1r53c1-1B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 sml1Δ::URA3 rad53Δ::LEU2 chk1Δ::NatMX4*	(1)	1A-B; S2A; S5B
wtH4r53s1c1-1D	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) sml1Δ::URA3 rad53Δ::LEU2 chk1Δ::NatMX4*	This work	1A-B; S2A; S5B
wm2-2	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 bar1Δ::hisG mad2Δ::LEU2*	This work	1A-B; 6A; 7B
wtH4bm2-1	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) bar1Δ::NatMX4 mad2Δ::LEU2*	This work	1A-B; 6A; 7B
wm1-1	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 mad1Δ::NatMX4*	This work	6A
wtH4m1-17B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) mad1Δ::NatMX4*	This work	6A
whta	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hta1-S129**	This work	S2B
wtH4hta-2B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) hta1-S129**	This work	S2B
wndc10-1 20C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 ndc10-1*	This work	1C; 6B
wtH4ndc10-1 1D	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) ndc10-1*	This work	1C; 6B
BYPds1HAb-1	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 trp1Δ::KANMX4 bar1Δ::HYGMX4 pds1::PDS1-3HA::TRP1*	This work	1D
BYtH4Pds1HAb-12A	*MATa his3Δ1 leu2Δ0 ura3Δ0 hhf1Δ::KANMX4 hhf2Δ::KANMX4 (p413TARtetH4) bar1Δ::HYGMX4 pds1::PDS1-3HA::TRP1*	This work	1D
KR3011	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 ade3 can1-100 his3-11,15 rad5-135 bar1Δ pds1Δ::LEU2*	(4)	1E
wtH4pds1b-1B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) bar1Δ::NatMX4 pds1Δ::LEU2*	This work	1E
wm2GFP-20C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 mad2::MAD2-eGFP::HIS3*	This work	2B
wtH4m2GFP-4B	*MATalpha leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) mad2::MAD2-eGFP::HIS3*	This work	2B
wtH4m2GFP-9A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) mad2::MAD2-eGFP::HIS3*	This work	2B
F267	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15*	(5)	3A; 6C

	rad5-535 ipl1-321		
wtH4ipl1-321-1B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) ipl1-321*	This work	3A; 6C
wcIVM20-7C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3 CDC20::MetCDC20::URA3*	This work	3B
wGH4cIVM20-10C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (pUK421) pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3 CDC20::MetCDC20::URA3*	This work	3B
wcIVM20Spc-1D	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3 CDC20::MetCDC20::URA3 Spc42::Spc42-mCherry::KANMX4*	This work	3C; S3A-B
wGH4cIVM20Spc-18A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (pUK421) pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3 CDC20::MetCDC20::URA3 Spc42::Spc42-mCherry::KANMX4*	This work	3C; S3A-B
Y2200	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 Brn1-Pk9::HIS3*	(6)	4D; 5A-B
GH4BPk9-5D	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (pUK421) Brn1-Pk9::HIS3*	This work	4D; 5A-B
wtop2tdb	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 bar1ΔNatMX4 UBR1::Gal-Ubiquitin-M-LacIfragment-Myc-UBR1::HIS3 leu2-3,112::pCM244(CMVp-tetR'-SSN6,LEU2)x3 top2td TOP5' upstream-100 to -1 replaced with KANMX-ttA(tetR-VP16)tetO$_2$-Ub-DHFRts-Myc*	This work	4A,B,G; S6
MetH4-8Cb	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 bar1ΔNatMX4 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p416MetH4)*	This work	4A,G; S1A; S4A
MetH4top2td-2Bb	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 bar1ΔNatMX4 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p416MetH4) UBR1::Gal-Ubiquitin-M-LacIfragment-Myc-UBR1::HIS3 leu2-3,112::pCM244(CMVp-tetR'-SSN6,LEU2)x3 top2td TOP5' upstream-100 to -1 replaced with KANMX-ttA(tetR-VP16)tetO2-Ub-DHFRts-Myc*	This work	4A,B,G; S4A
wsmc2-8b-10B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 smc2-8::TRP1 bar1ΔNatMX4*	This work	4C,E,H; 5C; 7C; S4B-D
wtH4smc2-8b-9B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) smc2-8::TRP1 bar1ΔNatMX4*	This work	4C,E,H; 5C; 7C; S4A-B
w303cIVGFP-1C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3*	This work	6D; S1A; S2C
wGH4cIVGFP-13A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (pUK421) pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3*	This work	6D; S1A; S2C
wm2cIVGFP-47C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 mad2Δ::KANMX4 pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3*	This work	6D; S2C
wm2GH4cIVGFP-19B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 rad5-535 mad2Δ::KANMX4 hhf1Δ::HYGMX4 hhf2Δ::KANMX4(pUK421) pURA3::tetRGFP::LEU2 cenIV::tetOx448::URA3*	This work	6D; S2C
w303sml1-10B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 sml1Δ::URA3*	(1)	S2A; S5A
w303s1m1-12A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 sml1Δ::URA3 mec1Δ::LEU2*	(1)	S2A; S5A
wtH4s1	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4)*	This work	S2A; S5A

	sml1Δ::URA3		
wtH4m1s1-4B	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) sml1Δ::URA3 mec1Δ::LEU2*	This work	S2A; S5A
ws1m2-2A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 sml1Δ::URA3 mad2Δ::KANMX4*	This work	S5A-B
wtH4s1m2-2C	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) sml1Δ::URA3 mad2Δ::KANMX4*	This work	S5A-B
wtH4m1s1m2	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) sml1Δ::URA3 mec1Δ::LEU2 mad2Δ::KANMX4*	This work	S5A
wr53s1c1m2-2A	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 sml1Δ::URA3 rad53Δ::LEU2 chk1Δ::NatMX4 mad2Δ::KANMX4*	This work	S5B
wtH4r53s1c1m2-10D	*MATa leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100 his3-11,15 RAD5 hhf1Δ::HYGMX4 hhf2Δ::KANMX4 (p413TARtetH4) sml1Δ::URA3 rad53Δ::LEU2 chk1Δ::NatMX4 mad2Δ::KANMX4*	This work	S5B
BYh2cGh1-5	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hhf2Δ::KANMX4 hhf1::GALp-hhf1::URA3*	This work	S2D
BYh2cGh1m2-3A	*MATa his3Δ1 leu2Δ0 ura3Δ0 hhf2Δ::KANMX4 hhf1::GALp-hhf1::URA3 mad2Δ::KANMX4*	This work	S2D

Table S2. Oligos used for real-time PCR amplifications

Oligo	Sequence
CEN3-1	GTTGAGCATCCCATCCAGTT
	GGGTAATGGCAAATCTGCTT
CEN3-2	TGAAGGGAAGAGGCTCATTT
	CAATTGGAGGCATCTCAAGC
CEN4	TGGTGTGGAAGTCCTAATATCG
	TGCATGATCAAAAGGCTCAA
CEN5	GTTGCATTTGCCTTTGGACT
	CCCAATTTTAAACGCTCCAA
rDNA	TTTCTGCCTTTTTCGGTGAC
	TGGCATGGATTTCCCTTTAG

SUPPLEMENTAL REFERENCES

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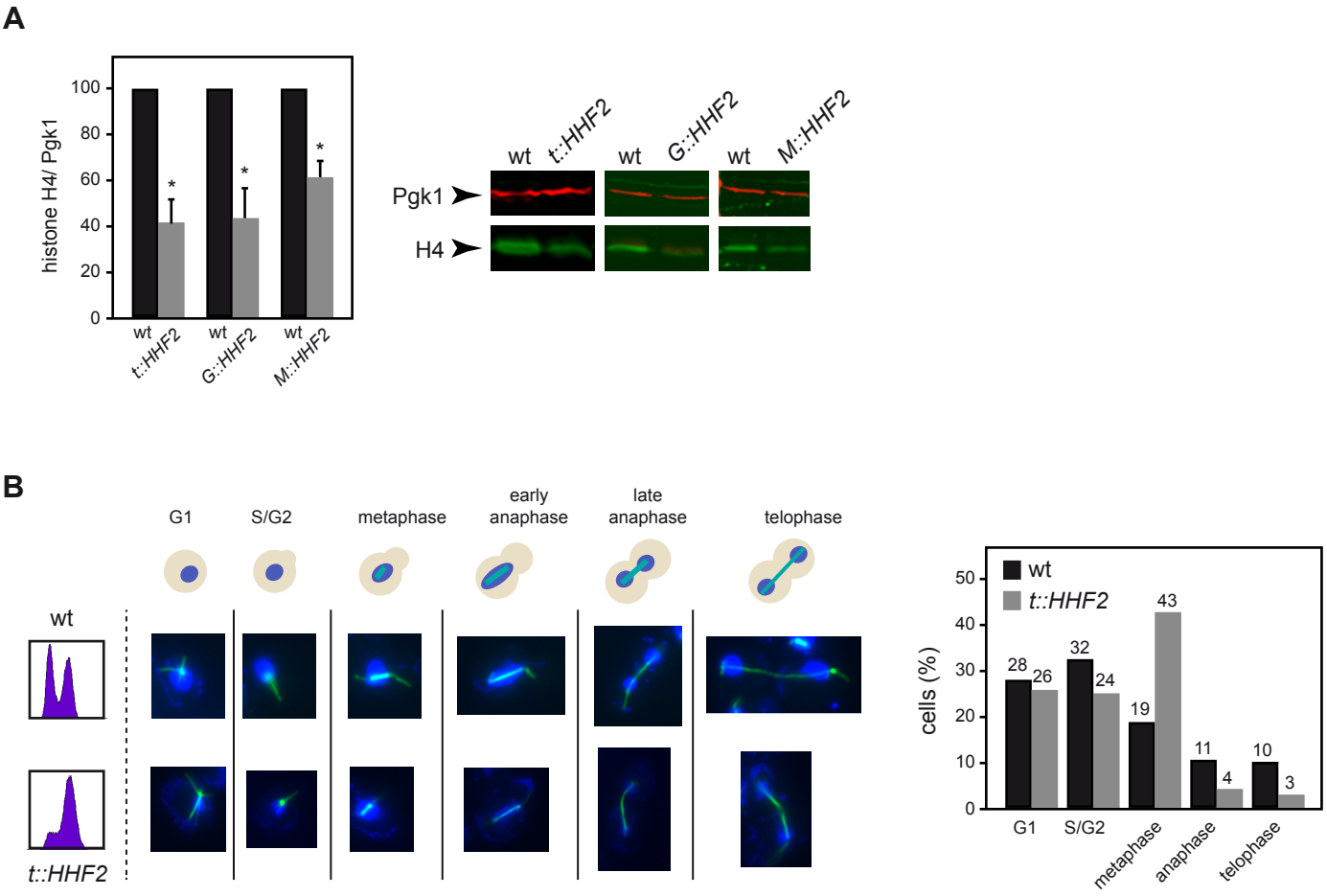
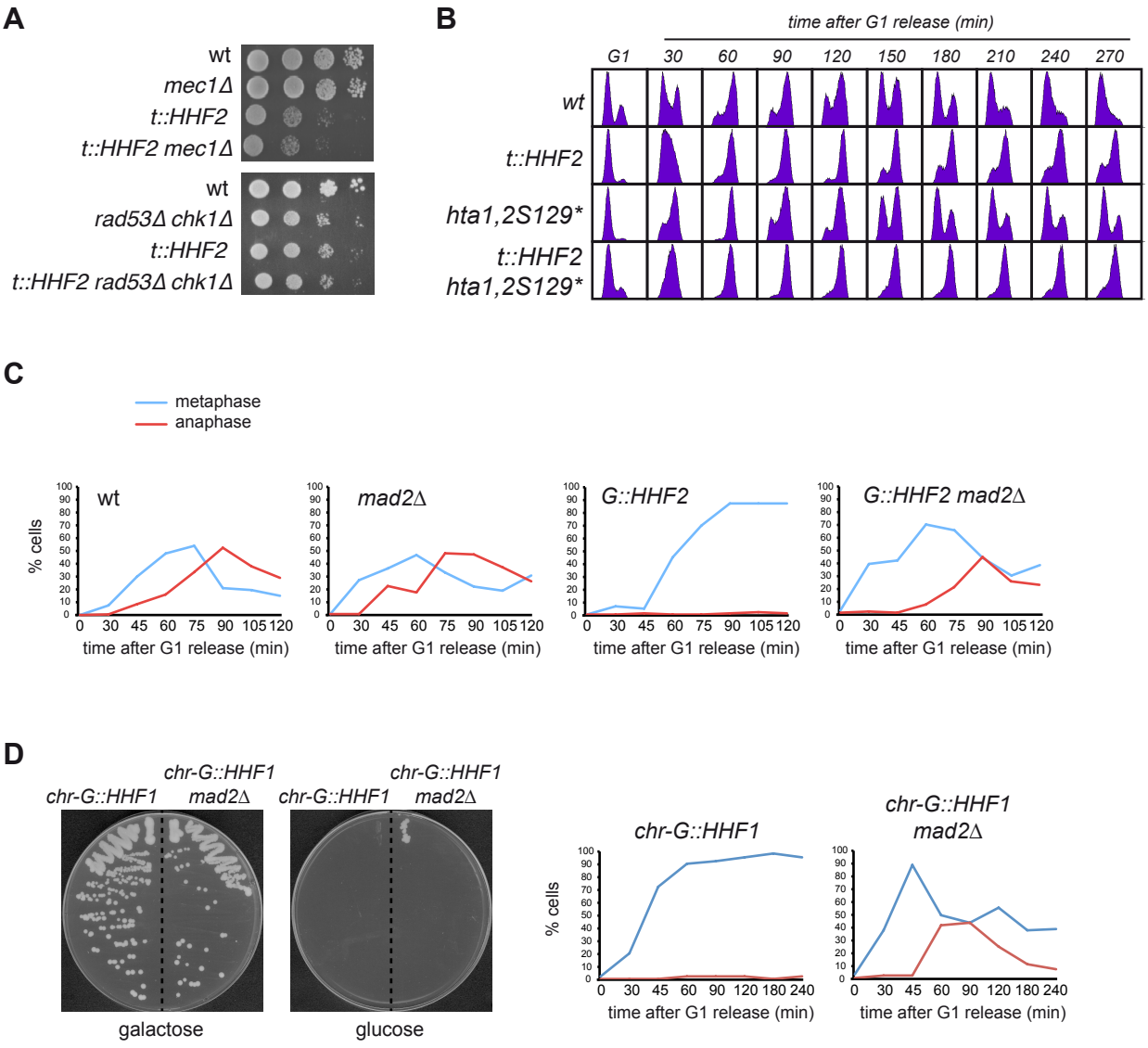
6. D'Ambrosio, C., Schmidt, C.K., Katou, Y., Kelly, G., Itoh, T., Shirahige, K. and Uhlmann, F. (2008) Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. *Genes Dev.*, **22**, 2215–2227.

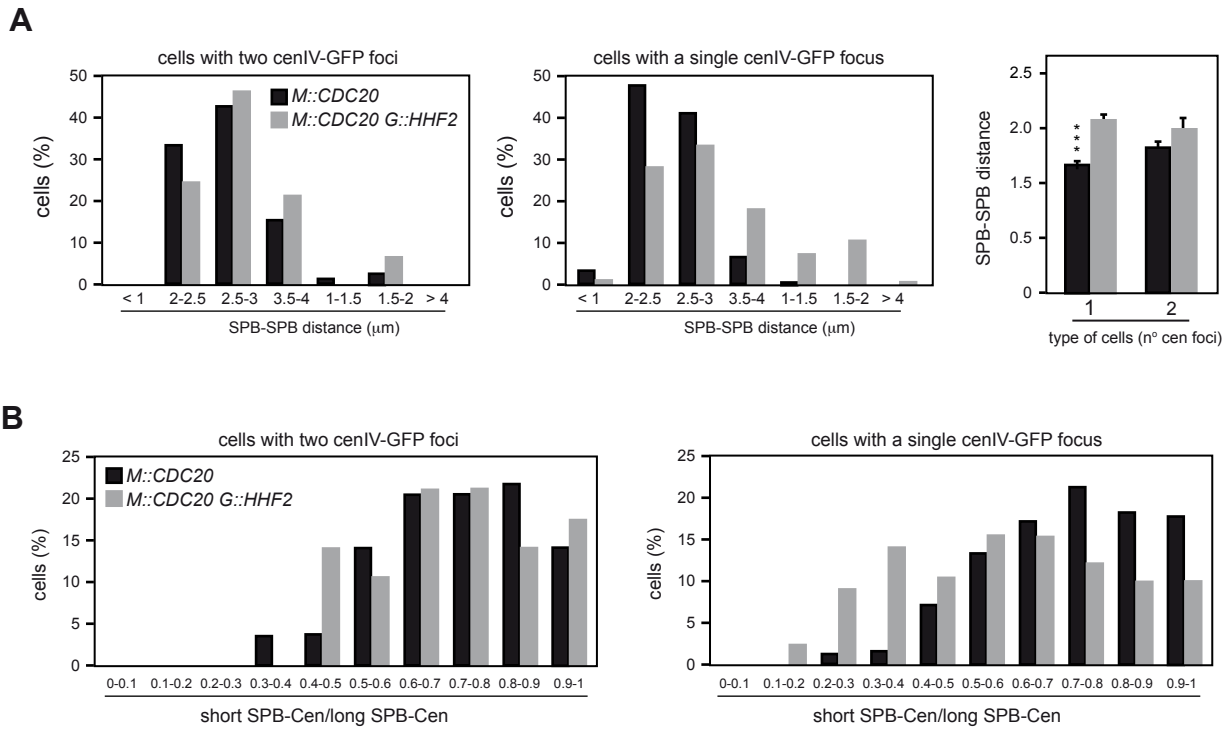
Murillo_Figure S1



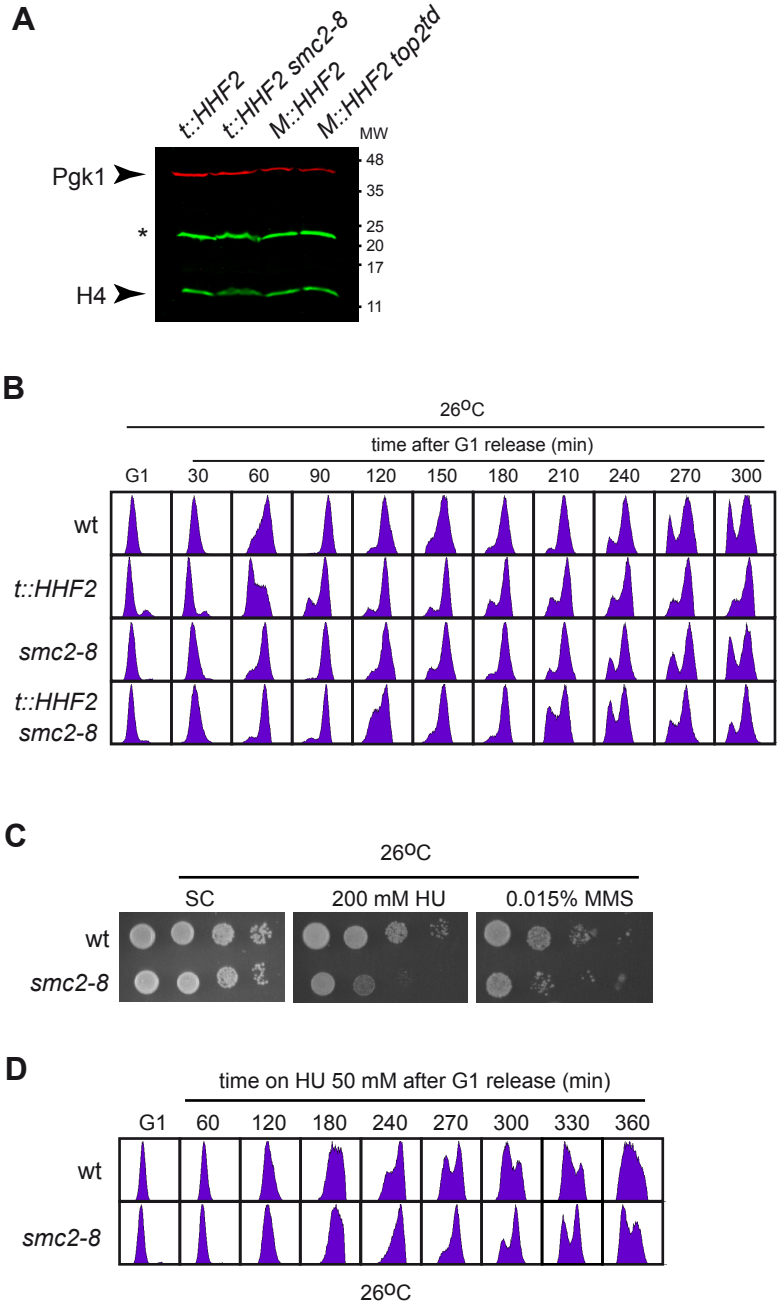
Murillo_Figure S2



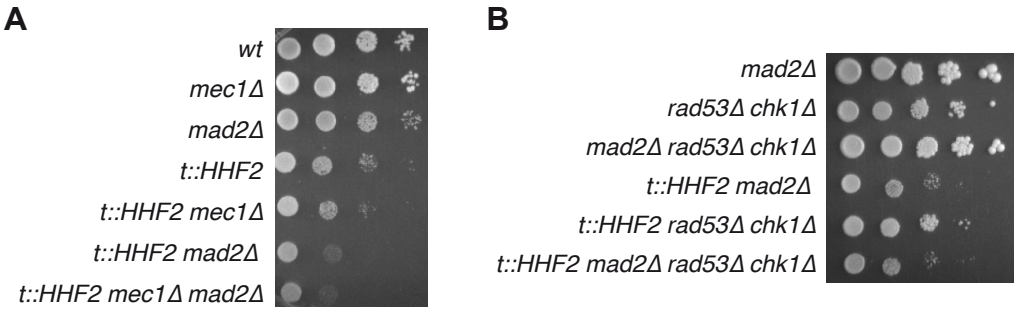
Murillo_Figure S3



Murillo_Figure S4



Murillo_Figure S5



Murillo_Figure S6