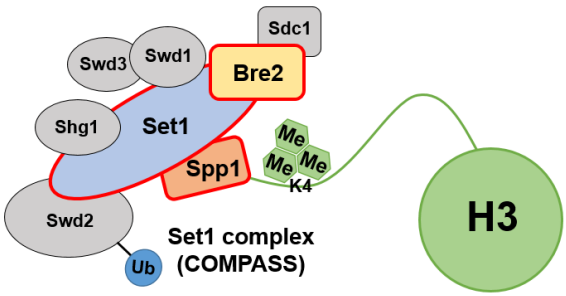
Supplementary Information

H3K4 methylation at active genes mitigates transcription-replication conflicts during replication stress

By Chong *et al.*

Supplementary Figure 1

a



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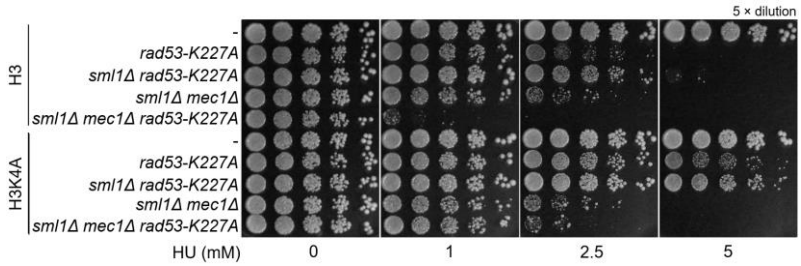
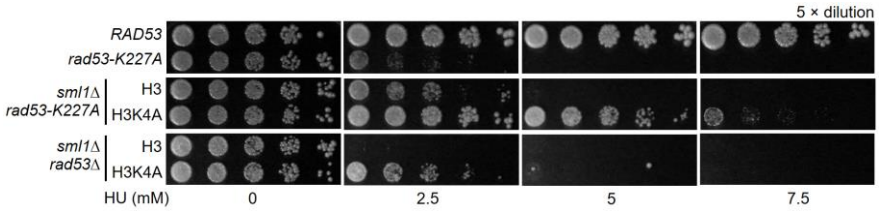
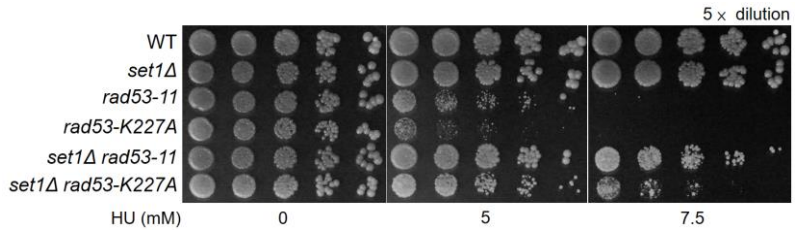


c



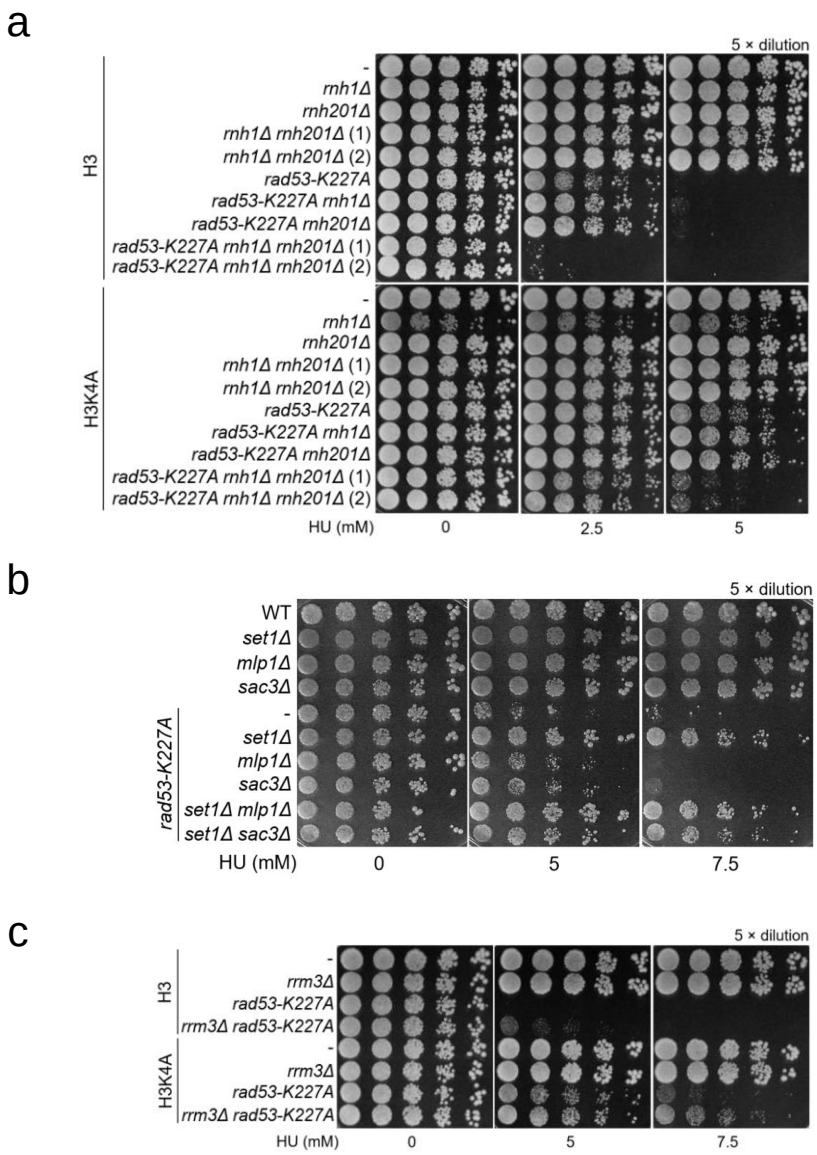
d



Supplementary Figure 1. *rad53* HU sensitivity is negatively correlated with H3K4 methylation levels and independent of Mec1 signaling.

a Schematic representation of the Set1 Complex. HU sensitivity was determined with **b** WT (*MEC1 RAD53*), *sml1Δ mec1Δ*, *rad53-K227A* and *sml1Δ mec1Δ rad53-K227A* backgrounds; **c** *sml1Δ rad53Δ and sml1Δ rad53-K227A* with either H3 or H3K4A mutation; **d** *set1Δ* and kinase-dead *rad53* mutant with mutation of K227A or G653E (*rad53-11*).

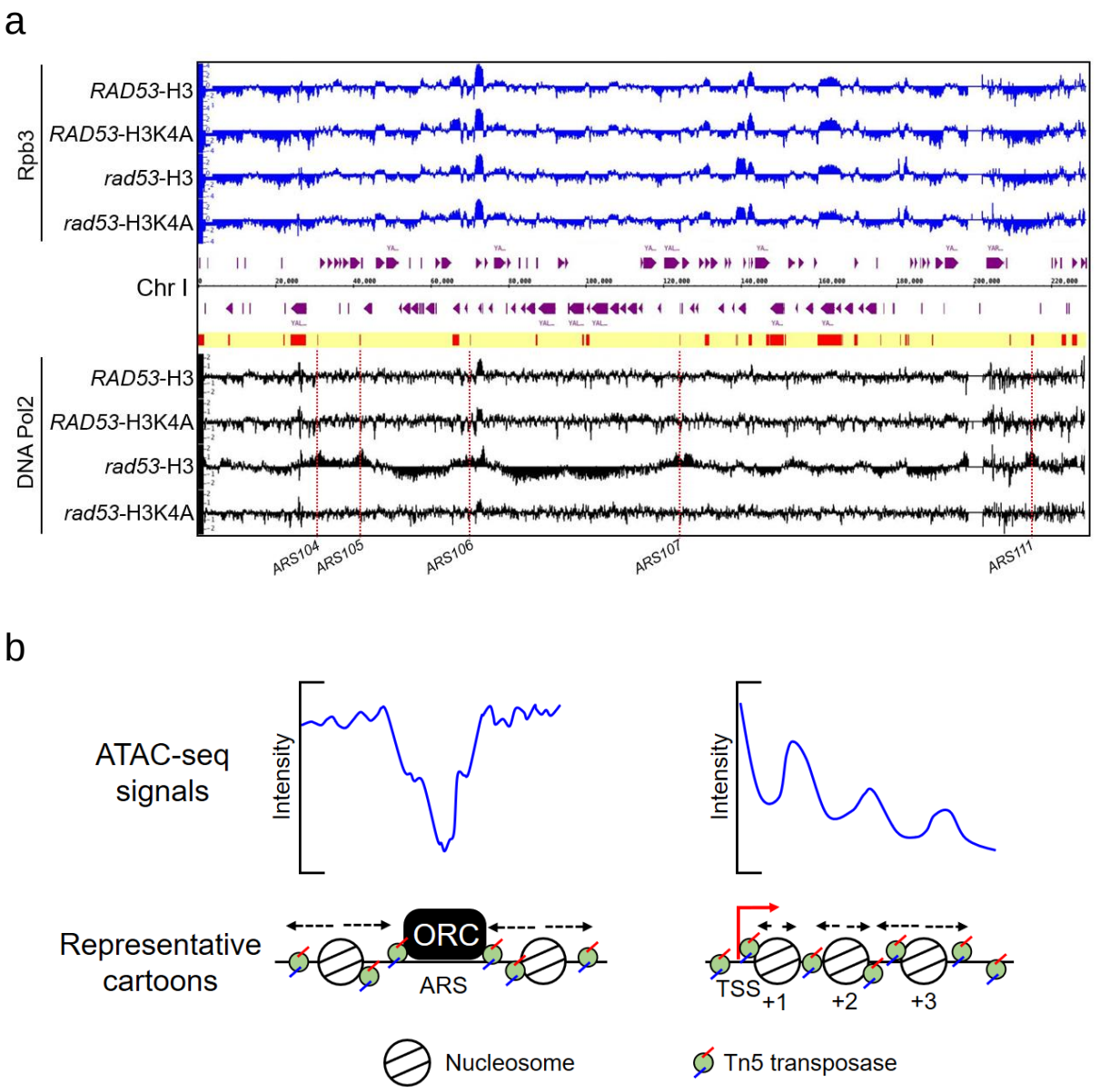
Supplementary Figure 2



Supplementary Figure 2. The interaction between ablation of H3K4 methylation and *rad53* mutation is independent of the R-loop and known *rad53* HU-sensitivity suppressors.

a-c HU sensitivity was determined with **a**, RNase H mutation(s); **b** gene gating gene mutation and **c** Rad53-suppressed helicase mutation in *RAD53* and *rad53-K227A* backgrounds with normal (H3) or ablated (H3K4A or with *set1Δ*) H3K4 methylation.

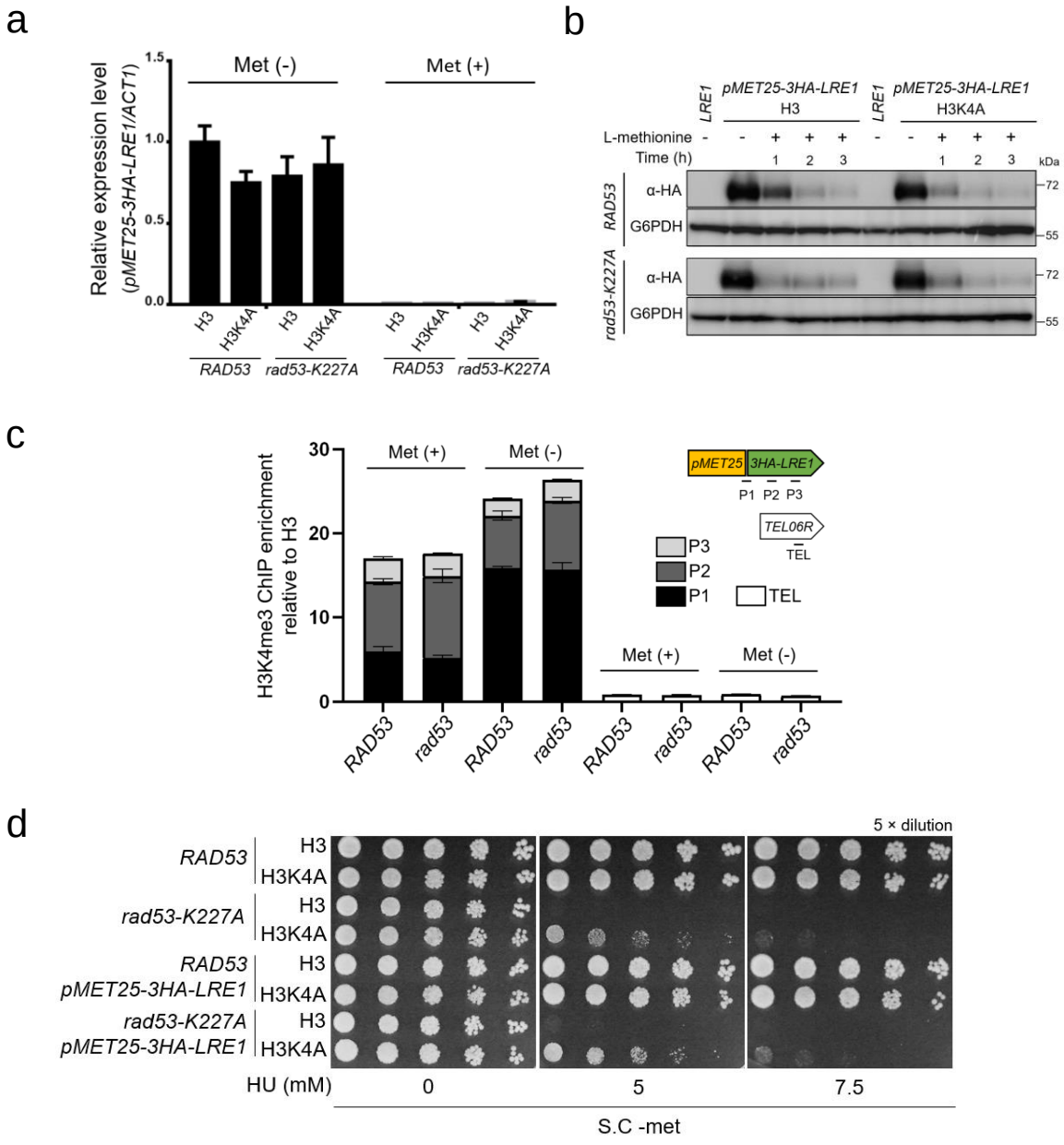
Supplementary Figure 3



Supplementary Figure 3. The restored-replication progression of *rad53* mutants in HU by losing H3K4me is unconcerned with alterations in chromatin structure.

a Rbp3 and DNA Pol2 ChIP-seq tracks of *RAD53*-H3, *RAD53*-H3K4A, *rad53*-H3 and *rad53*-H3K4A strains on chromosome I (Chr I). *rad53* refers to *rad53-K227A* mutant. Red dashed lines indicate ARSs with stalled DNA Pol2 in *rad53* cells. **b** Representative cartoons to illustrate ATAC-seq signal found on ORC-bound origins and TSS regions; with longer arrows on nucleosomes representing nucleosomes that are less consistently positioned.

Supplementary Figure 4



Supplementary Figure 4. Evaluation of the *pMET25-3HA-LRE1-ARS305* TRC system.

a The gene expression levels of *pMET25-3HA-LRE1* of *RAD53* and *rad53-K227A* cells with either normal H3 or H3K4A mutation after incubation in S.C.-met medium (Met (-), activation) or S.C. medium (Met (+), suppression) **b** Protein levels resulting from activated and suppressed *pMET25-3HA-LRE1* gene in strains similar to **a**, cells were initially cultured in S.C.-met medium with addition of L-methionine (0.005%, w/v) into medium for the indicated time. **c** H3K4me3 deposition on *pMET25-3HA-LRE1* on indication regions under suppressed (met+) and activated (met-) conditions **d** Induction of additional HO-oriented TRCs between *pMET25-3HA-LRE1* transcription and replication fork from *ARS305* do not affect cell survival upon HU treatment. Error bars represent ± SEM (*n* = 3 technical replicates).

Supplementary Table 1: Strains used in this study

Strains	Number	Genotype	Reference/source
MSY421 (wild-type)	CSY0016	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS329-*copy I [URA3, HHT1-HHF1]*	David Allis's lab[1]
RAD53-H3	CSY0019	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1a, S1b-c, 7a, S2a)
RAD53-H3K4A	CSY0020	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 1a, 7a, S1b, S2a)
RAD53-H3R2A	CSY0024	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 hht1-H3R2A-HHF1]*	This study (Fig. 1a, 7a)
RAD53-H3K36A	CSY0022	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K36A-HHF1]*	This study (Fig. 1a, 7a)
RAD53-H3K79A	CSY0023	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K79A-HHF1]*	This study (Fig. 1a, 7a)
rad53-K227A-H3	CSY0025	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1a, S1b-c, S2A)
rad53-K227A-H3K4A	CSY0026	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A pMS337 [CEN4 ARS1 LEU2 HHT1-H3K4A-HHF1]*	This study (Fig. 1a, S1b, S2a)
rad53-K227A-H3R2A	CSY0030	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A pMS337 [CEN4 ARS1 LEU2 HHT1-H3R2A-HHF1]*	This study (Fig. 1a)
rad53-K227A-H3K79A	CSY0028	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A pMS337 [CEN4 ARS1 LEU2 HHT1-H3K36A-HHF1]*	This study (Fig. 1a)
rad53-K227A-H3K79A	CSY0029	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A pMS337 [CEN4 ARS1 LEU2 HHT1-H3K79A-HHF1]*	This study (Fig. 1a)
H3	CSY0252	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1b-d, 4e, 5b-c, 6b-c, 7c-d, 7f, S2c, S4a-d)
rad53-K227A-H3	CSY0186	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1b-e, 3a, 4h, 5b-c, S2c, S4a, S4c-d)
rad53-K227A set1Δ-H3	CSY0302	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 set1::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1b-e, 3a, 4h)
rad53-K227A bre2Δ-H3	CSY0303	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 bre2::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1b-e, 3a, 4h)
rad53-K227A spp1Δ-H3	CSY0304	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 spp1::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1b-e, 3a, 4h)
set1Δ-H3	CSY0299	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 set1::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1c, 5c, 7a)
bre2Δ-H3	CSY0300	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 bre2::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1c, 7a)

spp1Δ-H3	CSY0301	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 spp1::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1c, 7a)
H3K4A	CSY0253	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 1d, 4e, 5b, 6b-c, 7c-d, 7f, S2c, S4a-b, S4d)
rad53-K227A-H3K4A	CSY0187	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 1d, 3a, 4f, 5b, 6b, S2c, S4a, S4c-d)
RAD53-H3 (DNA Pol2-3×FLAG)	CSY0489	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] POL2-3×FLAG-kanMX4* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 2, 3b, S3a)
RAD53-H3K4A (DNA Pol2-3×FLAG)	CSY0490	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] POL2-3×FLAG-kanMX4* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 2, 3b, S3a)
rad53-K227A-H3 (DNA Pol2-3×FLAG)	CSY0491	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A POL2-3×FLAG-kanMX4 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 2, 3b, S3a)
rad53-K227A-H3K4A (DNA Pol2-3×FLAG)	CSY0492	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A POL2-3×FLAG-kanMX4 pMS337 [CEN4 ARS1 LEU2 HHT1-H3K4A-HHF1]*	This study (Fig. 2, 3b, S3a)
RAD53-H3	CSY0095	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 3c-f, 4c, S4d)
RAD53-H3K4A	CSY0096	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 3c-f, 4c, S4d)
rad53-K227A-H3	CSY0101	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 3c-f, 4c, S4d)
rad53-K227A-H3K4A	CSY0102	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 pMS337 [CEN4 ARS1 LEU2 HHT1-H3K4A-HHF1]*	This study (Fig. 3c-f, 4c, S4d)
rad53-K227A HO-pMET25-3HA-LRE1-H3	CSY0457	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 HO-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 4g)
rad53-K227A HO-pMET25-3HA-LRE1-H3K4A	CSY0458	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 HO-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 4g)
rad53-K227A CD-pMET25-3HA-LRE1-H3	CSY0459	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 CD-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 4g)
rad53-K227A CD-pMET25-3HA-LRE1-H3K4A	CSY0460	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 CD-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 4g)
jhd2Δ-H3	CSY0256	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 jhd2::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (5b-c)
jhd2Δ-H3K4A	CSY0257	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 jhd2::HPH* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (5b)

rad53-K227A jhd2Δ-H3	CSY0258	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 jhd2::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (5b-f)
rad53-K227A jhd2Δ-H3K4A	CSY0259	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 jhd2::HPH* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (5b, 5d, 5f)
rad53-K227A-H3	CSY0416	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 6b-c, 7d)
rad53-K227A-H3K4A	CSY0417	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 6b-c, 7d)
RAD53-H3K4R	CSY0021	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2]* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4R-HHF1]*	This study (Fig. 7a)
RAD53 pTEF-CAN1-H3	CSY0545	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 kanMX4-pTEF-CAN1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 7c-f)
RAD53 pTEF-CAN1-H3K4A	CSY0546	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 kanMX4-pTEF-CAN1* pMS337 *[CEN4 ARS1 LEU2 hht1*-H3K4A-*HHF1]*	This study (Fig. 7d-f)
rad53-K227A pTEF-CAN1-H3	CSY0547	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 kanMX4-pTEF-CAN1* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. 7c-d)
rad53-K227A pTEF-CAN1-H3K4A	CSY0548	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 kanMX4-pTEF-CAN1* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. 7d-e)
sml1Δ rad53-K227A-H3	CSY0112	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A sml1::HPH pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S1b-c)
sml1Δ rad53-K227A-H3K4A	CSY0113	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A sml1::HPH pMS337 [CEN4 ARS1 LEU2 hht1*-H3K4A-*HHF1]*	This study (Fig. S1b-c)
sml1Δ mec1Δ-H3	CSY0463	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] sml1::HPH mec1::kanMX4* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S1b)
sml1Δ mec1Δ-H3K4A	CSY0464	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] sml1::HPH mec1::kanMX4* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S1b)
sml1Δ mec1Δ rad53-K227A-H3	CSY0465	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A sml1::HPH mec1::kanMX4 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S1b)
sml1Δ mec1Δ rad53-K227A-H3K4A	CSY0466	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A sml1::HPH mec1::kanMX4 pMS337 [CEN4 ARS1 LEU2 hht1*-H3K4A-*HHF1]*	This study (Fig. S1b)
sml1Δ rad53Δ-H3	CSY0116	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] sml1::HPH rad53::HIS5 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S1c)
sml1Δ rad53Δ-H3K4A	CSY0117	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] sml1::HPH rad53::HIS5 pMS337 [CEN4 ARS1 LEU2 hht1*-H3K4A-*HHF1]*	This study (Fig. S1c)

WT	CSY0001	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	W303, This study (Fig. S1d, S2b)
set1Δ	CSY0002	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 set1::kanMX4*	This study (Fig. S1d)
rad53-11	CSY0003	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 TRP1-rad53-11*	This study (Fig. S1d)
rad53-K227A	CSY0004	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A*	This study (Fig. S1d, S2b)
rad53-11 set1Δ	CSY0005	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 TRP1-rad53-11 set1::kanMX4*	This study (Fig. S1d)
rad53-K227A set1Δ	CSY0006	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 atNT2-rad53-K227A set::kanMX4*	This study (Fig. S1d)
rnh1Δ-H3	CSY0220	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh1::kanMX4* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rnh201Δ-H3	CSY0221	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh201::kanMX4* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rnh1Δ rnh201Δ-H3	CSY0222	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh1::kanMX4 rnh201::HIS5* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh1Δ-H3	CSY0224	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh1::kanMX4 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh201Δ-H3	CSY0225	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh201::kanMX4 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh1Δ rnh201Δ-H3	CSY0226	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh1::kanMX4 rnh201::HIS5 pMS337 [CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2a)
rnh1Δ-H3K4A	CSY0228	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh1::kanMX4* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
rnh201Δ-H3K4A	CSY0229	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh201::kanMX4* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
rnh1Δ rnh201Δ-H3K4A	CSY0230	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] rnh1::kanMX4 rnh201::HIS5* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh1Δ-H3K4A	CSY0232	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh1::kanMX4 pMS337 [CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh201Δ-H3K4A	CSY0233	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh201::kanMX4 pMS337 [CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
rad53-K227A rnh1Δ rnh201Δ-H3K4A	CSY0234	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] natNT2-rad53-K227A rnh1::kanMX4 rnh201::HIS5 pMS337 [CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2a)
mlp1Δ	CSY0572	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 mlp1::kanMX4*	This study (Fig. S2b)
sac3Δ	CSY0573	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sac3::kanMX4*	This study (Fig. S2b)
set1Δ	CSY0049	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 set1::HIS5*	This study (Fig. S2b)
rad53-K227A set1Δ	CSY0050	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A set1::HIS5*	This study (Fig. S2b)
rad53-K227A mlp1Δ	CSY0554	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A mlp1::kanMX4*	This study (Fig. S2b)
rad53-K227A sac1Δ	CSY0555	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A sac3::kanMX4*	This study (Fig. S2b)

rad53-K227A set1Δ mlp1Δ	CSY0053	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A set1::HIS5 mlp1::kanMX4*	This study (Fig. S2b)
rad53-K227A set1Δ sac1Δ	CSY0054	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 natNT2-rad53-K227A set1::HIS5 sac3::kanMX4*	This study (Fig. S2b)
rrm3Δ-H3	CSY0318	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 rrm3::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2c)
rrm3Δ-H3K4A	CSY0319	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] bar1::HIS3 natNT2-pMET25-3HA-LRE1 rrm3::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2c)
rad53-K227A rrm3Δ-H3	CSY0320	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 rrm3::HPH* pMS337 *[CEN4 ARS1 LEU2 HHT1-HHF1]*	This study (Fig. S2c)
rad53-K227A rrm3Δ-H3K4A	CSY0321	*MATa ura3-52 leu2-3,112 trp1 his3 Δ[HHT1-HHF1] Δ[HHT2-HHF2] kanMX4-rad53-K227A bar1::HIS3 natNT2-pMET25-3HA-LRE1 rrm3::HPH* pMS337 *[CEN4 ARS1 LEU2 hht1-H3K4A-HHF1]*	This study (Fig. S2c)

Supplementary Table 2: Primers used in this study

Experiments	Genes/regions	Sequences (5' - 3')	Reference/source/note(s)
Yeast construction	*pMET25-3HA-LRE1*	(F) GAACCCTCGGCAATTATTTCATACTCCTTCTCAGAA TAGGAAAATGCGTACGCTGCAGGTCGAC (R) CAGGATTTGGCTCTGATATTTGCACATGTTGAGTAT GCGTATTCGGCATCGATGAATTCTCTGTCG	To amplify *kanMX4-pMET25-3HA* fragment from pYM-N36[2] for yeast transformation
	lre1:URA3	(F) AACCCTCGGCAATTATTTCATACTCCTTCTCAGAAT AGGAAAATGAGCAGATTGTACTGAGAGTGC (R) CAATGCCGCTAAGGTTACGATTCACCTTTTTATTAC TTATTATTAAGCTCTAATTTGTGAGTTTAG	To amplify *lre1::URA3* cassette from pRS406 for yeast transformation
	HO-pMET25-3HA-LRE1	(F) CTGAACCCTCGGCAATTATTTCATACTCCTTCTCAG AATAGGAAACTTAGCTCTCATTATTTTTTGCTTTTTC (R) CTCCAAGATTACTGAAAAACCTGAA	To amplify marker-free *HO-pMET25-3HA-LRE1* fragment from CSTY0252
	CD-pMET25-3HA-LRE1	(F) CTGAACCCTCGGCAATTATTTCATACTCCTTCTCAG AATAGGAAACGTTAAGGTTACGATTCACCTTTTT (R) TTTATTATTCTACACTTTATTATTAAAACTACATGC AATCAATGCCTTAGCTCTCATTATTTTTTGCTTTTTC	To amplify marker-free *CD-pMET25-3HA-LRE1* fragment from CSTY0252
	pTEF-CAN1	(F) TTCTTAACTCCTGTAAAAACAAAAAAAAAAAAAGGC ATAGCAATGCGTACGCTGCAGGTCGAC (R) GTACATATGCTTCTCCTCTATGTCGGCGTCTTCTTT TGAATTTGTCATCGATGAATTCTCTGTCG	To amplify *kanMX4-pTEF* fragment from pYM-N18[2] for yeast transformation
	kanMX_pRS	(F) GGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGT TTAGTTCGGTGATGACGGTGAAAACCT (R) TTTTCGACACTGGATGGCGGCGTTAGTATCGAATC GACAGCCTGATTTAACAAAAATTTAACGCGA	Universal primers for amplification of any markers from pRS plasmid series for replacing *kanMX4* cassette by indicated marker in any mutants from BY4741 deletion library
	kanMX4_U2D2	(F) CGTACGCTGCAGGTCGAC (R) ATCGATGAATTCGAGCTCG	Universal primers for amplification of *kanmx4*::Marker fragment from maker-transformed BY4741 mutants
	BAR1	(F) CGGTACACATTTGTTGCATTTATTA (R) GTGATAGTTTAATGGTCAGAATGGG	Gene knockout
	SET1	(F) GGCAAAGAACAAAGAAATACACAGT (R) TGTTCGTGTTTTCAGGCATTT	
	BRE2	(F) GCTAGGAAACTCCTGATCACATTTA (R) TGGCATTTTGAAATGTTATGTAATG	
	SPP1	(F) GGCGATATTCGATAGACTTAACAGA (R) TCTTCTGCAATATCTAAAGTGCTCA	
	JHD2	(F) TCGTGATATAGAAGTAACGCATTGA (R) CGATCGTGAATAAAAATGCTACTCT	
	MEC1	(F).ATTCCTTTTCAAGGCTCCATAACTA (R) TTTTCCATATCTTCGAGCTCTTCTA	
	sml1_hphNT1	(F) GGTCTCACTAACCTCTCTTCAACTGCTCAATAATTT	

		CCCGCTATGCGTAC GCTGCAGGTCGAC (R) GTGGGAAATGGAAAGAGAAAAGAAAAGAGTATGAA GGAACTTTAATCGATGAATTCGAGCTCG	
	rad53_pUG73[3]	(F) AGAATAGTGAGAAAAGATAGTGTTACACAACATCAA CTAAAAATGCAGCTGAAGCTTCGTACGC (R) TCTTAAAAAGGGGCAGCATTTTCTATGGGTATTTGT CCTTGGTTAGCATAGGCCACTAGTGGATCTG	
	RNH1	(F) CATGTATAAAGCACACTGCACCTAC (R) GATGAAAAACTCGAAGAAATTGAAA	
	RNH201	(F) AATTGACAGGAAACAAAACTGAGAC (R) ACAAAGGCAGACATAGTACGCTAAT	
	MLP1	(F) ATCTTTGTTTCCTTTCATTCCTCTT (R) GGAACACTAAGAAAATCTGTTTGGA	
	SAC3	(F) AGAATTCGTGATTCAATGTTCATTAT (R) AGCAGTTTATAACTTTTTGGCCTCT	
	RRM3	(F) TATCTTCCCTTACCGGATTTATTTC (R) GGCTAGATCTCCTTTTTCAGTTTCT	
	rad53-K227A		Integration of *Eco*RI-linearized PCH8-*rad53-K227A* plasmid at the *RAD53* locus[4]
Site-directed mutagenesis	H3R2A	ACGCAAACAATGGCCGCAACAAAGCAAACAGCA	To generate H3 mutant plasmids (pMS337) by PCR mutagenesis[1]. Underlined codons represent mutagenesis in indicated plasmids
	H3K4A	ACAATGGCCAGAACAGCGCAAACAGCAAGAAAG	
	H3K4R	ACAATGGCCAGAACAAGGCAAACAGCAAGAAAG	
	H3K36A	TCTACCGGTGGTGGTTGCGAAGCCTCACAGATAT	
	H3K79A	ATCGCTCAAGATTTCGCGACCGACTTGAGATTT	
Gene expression (qPCR)	*pMET25-3HA-LRE1*	(F) GCGGCTGAAAATTGTCCTT (R) TGTAGCGTATTGGCGATAATGT	This study (Fig. 5d, S4a)
	SCR1	(F) CGGCTAGACACGGATTG (R) GAGGAAAGACGGTTGCCA	This study (Fig. 5d)
	ACT1	(F) TCACGCCATTTTGAGAATCG (R) TTCAGCAGTGGTGGAGAAAGAG	This study (Fig.S4a)
ChIP-qPCR	*pMET25-3HA-LRE1* (P1)	(F) TATTACCCCCATCCATACTCTTGAA (R) ATAGGGATAGCCCGCATAGTCAGGA	This study (Fig. 5e, S4c)
	pMET25-3HA-LRE1 (P2)	(F) TTGAAGACAAGGCCTAGGTCATTT (R) ACAATTGCCCTTATCTACCATAACC	
	pMET25-3HA-LRE1 (P3)	(F) CATTAATGCACAGTCCATGCCCTTT (R) TGCATCGTCCGCTCTCGTAATAAC	
	CAN1 (P1)	(F) ATGACAAATTCAAAAGAAGACGCCG (R) TGAAGCTCCAACGTCGTGAAAGAGG	This study (Fig. 7c)
	CAN1 (P2)	(F) CTGGCGGCATGGATTAGTATTTTTT (R) GACCCAGAACTCGAATTCACCGTAA	
	CAN1 (P3)	(F) CCTAAATTCCTGTCAAGGACCACCA (R) CTCCATGTAAGCCAAAGCGCCAAAT	
	TEL06R (TEL)	(F) CGTGTGTAGTGATCCGAACTCAGT	This study (Fig. 7c, S4c)

		(R) GACCAGTCCTCATTTCCATCAATAG	
2D electrophoresis (DNA fragments for probes)	*ARS305*	(F) AGGTTCAGTATTGAAAGTTGGTA (R) ACTATTTACATTATCGTCCTGTCA	This study (Fig 4c, 4e-f, 5f, 6c)
	ARS305 (HO/CD-*LRE1*)	(F) GCGGCTGAAAATTGTCCTT (R) TTACTTCCTTGAAAAAATATGTCTA	This study (Fig 4g)
	ARS1211 (*PDC1*)	(F) CAAATATCGTTTGAATATTTTTCCG (R) TACACTAATGCAGTTTCAGGGTTTT	This study (Fig 4h)
	ARS305L	(F) CATGAACTTTGTATCTGAATTTTTG (R) ATCTAACTACAAACTGAAGCAGAAT	This study (Fig 6c)
	ARS305LL	(F) GTAATAGCTGCTGATTGGTCAGAAT (R) GAATCTTTCGTCTCGATAAACTTCA	
	ARS1211	(F) CATGGATGTACAGTAGAAATTACAT (R) TGATTTCTATAAATTGGATGTCGAT	
	ARS1211R	(F) TTGAATAGAATCCGTCTCTGCATCA (R) AGGGTGCAAATAACATGTATGAACT	
	ARS1211RR	(F) CTTGTCGATTGTTGTCAGATTACAC (R) TCGTATTGCAATTTTTGTTTGTCTA	
	ARS1212	(F) AATAAGAAGGGAGAGGTGGTAGAGA (R) TATATTGCTGTGATCTGAATCTGGA	

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