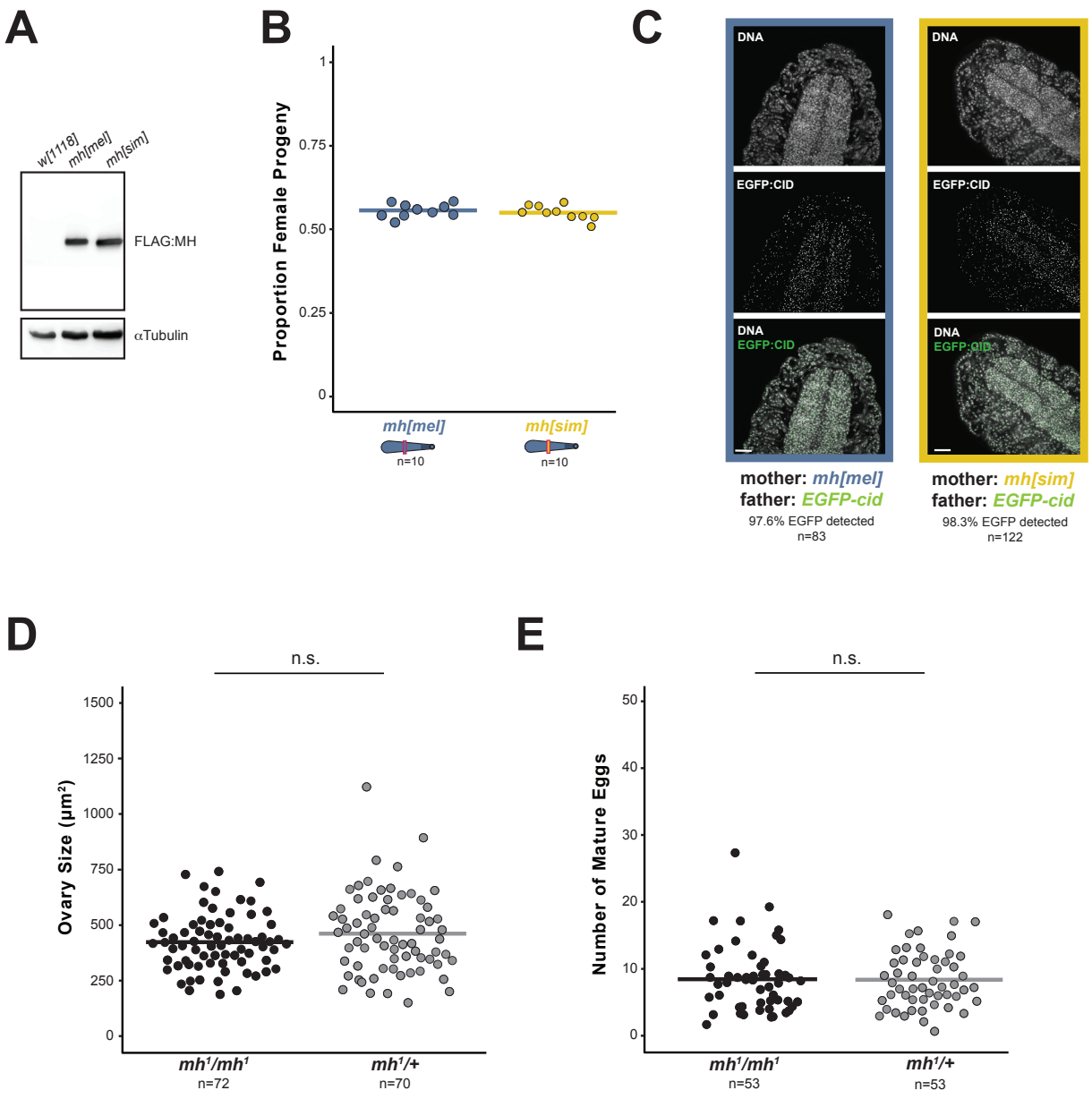


Figure S1. MH[sim] is expressed at comparable levels to MH[mel] and does not phenocopy *mh* null. Related to Figure 1. (A) Western Blot of *mh[mel]* and *mh[sim]* ovaries probed with anti-FLAG and anti-αTubulin. (B) Proportion of female progeny from *mh[mel]* and *mh[sim]* mothers. (C) Representative images of diploid embryos from crosses between *EGFP-cid* fathers and *mh[mel]* or *mh[sim]* mothers stained with anti-GFP. Diploid embryos are GFP-positive and haploid embryos are GFP-negative. (D) Ovary size estimates from mh^1 homozygous and mh^1/+ heterozygous females. (E) Number of mature eggs per ovary pair from mh^1 homozygous and mh^1/+ heterozygous females. (*t*-test: "n.s." $p > 0.05$, scale bar = 25µm)

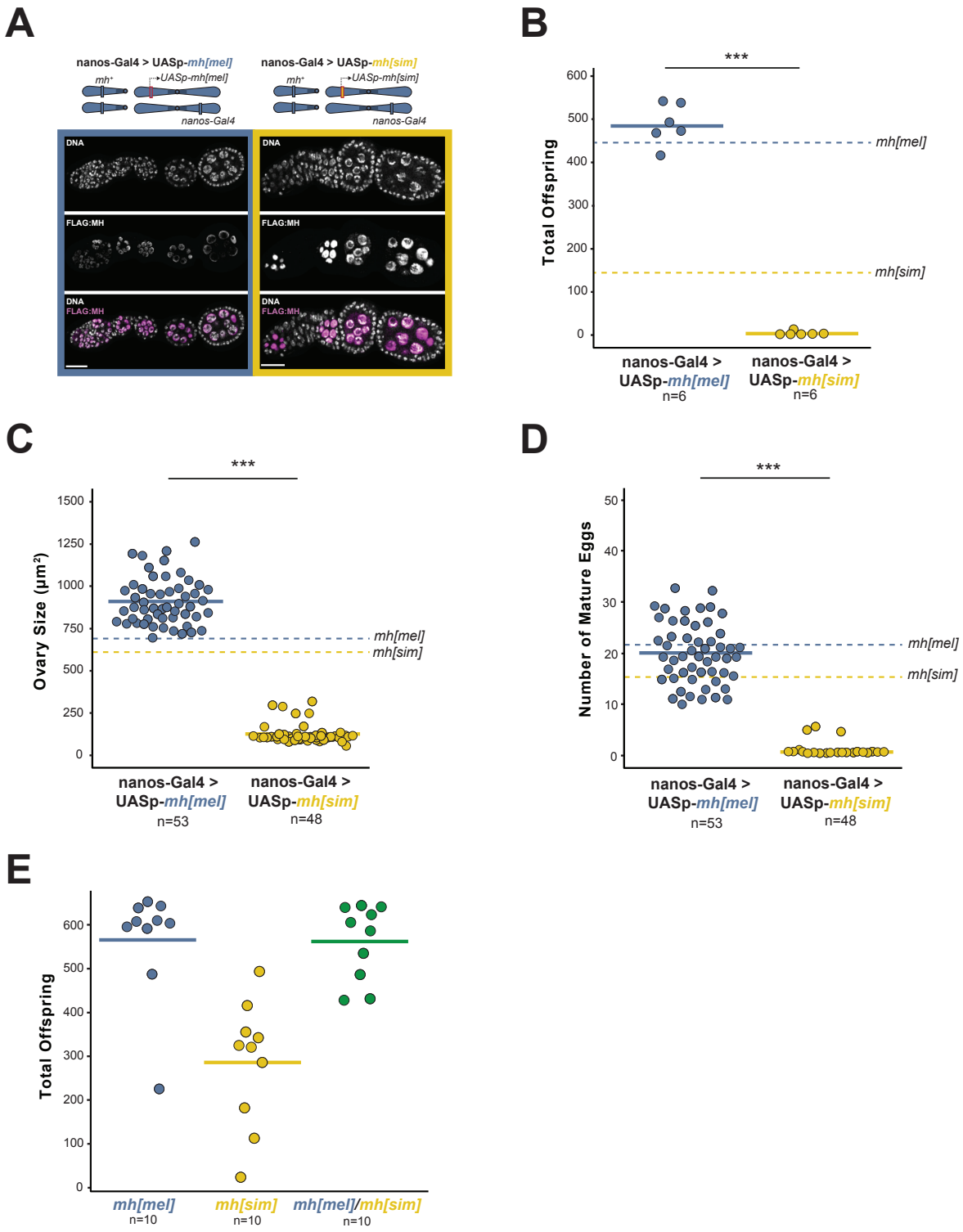

A
nanos-Gal4 > UASp-mh[mel]
nanos-Gal4 > UASp-mh[sim]
mh+
UASp-mh[mel]
UASp-mh[sim]
nanos-Gal4
DNA
FLAG:MH
B
***
Total Offspring
mh[mel]
mh[sim]
nanos-Gal4 > UASp-mh[mel] n=6
nanos-Gal4 > UASp-mh[sim] n=6
C
***
Ovary Size (µm²)
mh[mel]
mh[sim]
nanos-Gal4 > UASp-mh[mel] n=53
nanos-Gal4 > UASp-mh[sim] n=48
D
***
Number of Mature Eggs
mh[mel]
mh[sim]
nanos-Gal4 > UASp-mh[mel] n=53
nanos-Gal4 > UASp-mh[sim] n=48
E
Total Offspring
mh[mel] n=10
mh[sim] n=10
mh[mel]/mh[sim] n=10

Figure S2. MH[sim] toxicity is dose-dependent. Related to Figure 2. (A) Anti-FLAG staining of *mh[mel]* and *mh[sim]* upon overexpression under the UAS-GAL4 system. The driver nos-GAL4-VP16 expresses the *mh* transgene in the germline only. Corresponding chromosome *X* (wildtype) and chromosome *3* are shown in the images above. Note that wildtype *mh* is present in these genotypes. (B) Progeny counts of the nos-Gal4-VP16-driven UASp-*mh[mel]* or UASp-*mh[sim]* females crossed to wildtype (w^{1118}) males. (C) Ovary size estimates of the nos-Gal4-VP16-driven UASp-*mh[mel]* or UASp-*mh[sim]* females. (D) Number of mature eggs per ovary pair from nos-Gal4-VP16-driven UASp-*mh[mel]* or UASp-*mh[sim]* females. (E) Total offspring of *mh[mel], mh[sim],* and *mh[mel]/mh[sim]* heterozygous females crossed to wildtype (w^{1118}) males. In panels B, C, and D, dotted lines correspond to *mh[mel]* and *mh[sim]* averages reported in Figure 1C, 1E, and 1F, respectively. (*t*-test: "***" = $p < 0.001$, scale bar = 25μm)

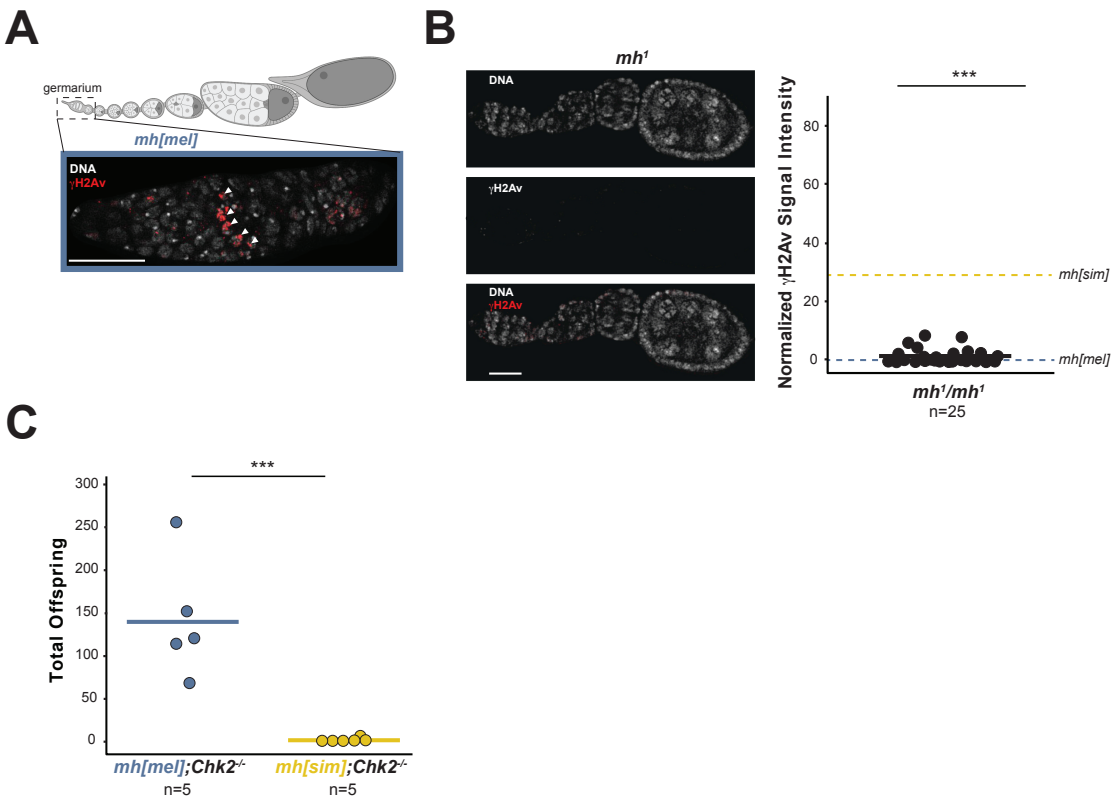
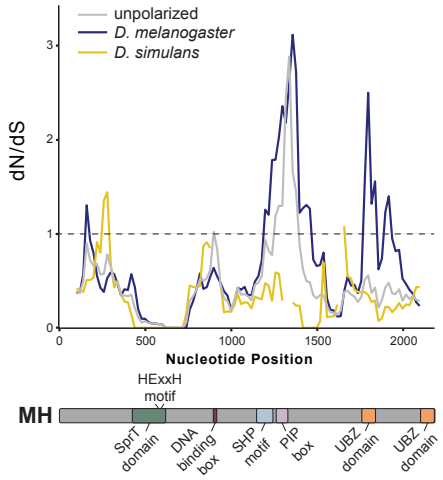


Figure S3. DNA damage signaling further distinguishes MH[sim] from *mh* null. Related to Figure 2. (A) *Drosophila* ovariole (above) showing the germarium where meiotic recombination occurs. Germarium of an *mh[mel]* female (below) showing programmed double-strand breaks (arrowheads) occurring in "region 2A" of the germarium detected under higher laser power relative to images displayed in Figure 2D. These double-strand breaks are repaired via meiotic recombination pathways. (B) γH2Av signal in mh^{1} ovaries and quantification of normalized fluorescent signal intensity. (C) Progeny counts from *mh[mel]; Chk2*$^{-/-}$ and *mh[sim]; Chk2*$^{-/-}$ females crossed to wildtype (w^{1118}) males. Note that *mh[mel]; Chk2*$^{-/-}$ reduced progeny counts are due to the *Chk2*$^{-/-}$ mutation.[S1] In panel B, dotted lines correspond to *mh[mel]* and *mh[sim]* averages reported in Figure 2D. (*t*-test: "***" = $p < 0.001$, scale bar = 25μm)

A



B

	Poly-morphism	Fixation
Syn	**50**	**112**
Non-syn	**8**	**56**

Figure S4. Lineage-specific MH adaptive evolution and Top2 adaptive evolution. Related to Figure 1 and Figure 4. (A) A 200bp sliding window of dN/dS across the *mh* coding region. dN/dS estimates from a comparison of *D. melanogaster* and *D. simulans* are in grey. dN/dS estimates from a comparison of *D. melanogaster and* the *D. melanogaster-D. simulans* reconstructed ancestor are in blue. dN/dS estimates from a comparison of *D. simulans* and the *D. melanogaster-D. simulans* reconstructed ancestor are in yellow. Windows without synonymous sites are represented as gaps. Windows of dN/dS > 1 are significantly enriched in the *D. melanogaster* lineage (Table S2). (B) Counts of synonymous and nonsynonymous polymorphic and fixed sites within and between *D. melanogaster* and *D. simulans;* χ^2 test, p = 0.004.

		Polymorphism	Fixation
Synonymous	*D. melanogaster* lineage	4	36
	D. simulans lineage	12	17
	Unpolarized	21	65
Non-Synonymous	*D. melanogaster* lineage	4	48
	D. simulans lineage	8	20
	Unpolarized	12	83

Table S1. Counts of unpolarized and polarized synonymous and non-synonymous polymorphic and fixed sites in the *mh* coding sequence. Related to Figure 1. Unpolarized MK-test: X^2, p = 0.04. *D. melanogaster* lineage MK-test: X^2, p = 0.70. *D. simulans* lineage MK-test: X^2, p = 0.31.

	dN/dS windows < 1	dN/dS windows > 1
D. melanogaster mh* – ancestor *mh	80	21*
D. simulans mh* – ancestor *mh	93	3

Table S2. Lineage-specific dN/dS analysis of *mh* in 200bp windows. Related to Figure 1 and Figure S4. *Windows with dN/dS >1 are enriched along the *D. melanogaster* lineage (Fisher's Exact Test, p = 0.0001)

Forward	Reverse	Purpose
GATCCAGGAAATACTCGGCC	CCAAAGACTGGTCACACTGC	Primers used to amplify the *mh* ortholog in *D. simulans*
ATCCTGGCCAACAGTGACTC		Sequencing primer for *mh* ortholog in *D. simulans*
	TTTGCCCAAAATATCGAACG	Sequencing primer for *mh* ortholog in *D. simulans*
GCAGAGCAGACGAGTCAGTG		Sequencing primer for *mh* ortholog in *D. simulans*
	TTGACCTTGTGTGAGGACGA	Sequencing primer for *mh* ortholog in *D. simulans*
ACGACGCCTCTTTTTGGTAA		Sequencing primer for *mh* ortholog in *D. simulans*
	ACATACGCAACTGGTTCGAC	Sequencing primer for *mh* ortholog in *D. simulans*
CACCTTGTTGATCGTCTCCA		Sequencing primer for *mh* ortholog in *D. simulans*
	GATCCCACTCCGGACATCTA	Sequencing primer for *mh* ortholog in *D. simulans*
TTCTCCATTTTTGTCTTGTGGA		Sequencing primer for *mh* ortholog in *D. simulans*
AAGTGTCGCGCTATTTCACC	TCACCGTCATGGTCTTTGTAGTCCAT	Reverse primer anneals in FLAG-tag. Amplifies *mh[mel]* and *mh[sim]* CRISPR-introduced transgenes
AATGGATTTCGGCAAATGAG	GTCGTTGTAGGAGCCCATGT	Primers anneal outside homology arms. Amplicon spans entire *mh[mel]* and *mh[sim]* CRISPR-introduced transgenes
ATGCGCAGGCTAAATTCGAT		Sequencing primer for *mh[mel]* and *mh[sim]* region
GATTACCTGTTCGCCCTGAA		Sequencing primer for *mh[mel]* and *mh[sim]* region
ACCAACATCACCGTGTACCA		Sequencing primer for *mh[mel]* and *mh[sim]* region
CATTGAGCACATCGACCTGT		Sequencing primer for *mh[mel]* region
CATCGAGAACATCGATATCT		Sequencing primer for *mh[sim]* region
CTCCCTGACCAGCAAGGAT		Sequencing primer for *mh[mel]* region
CAGCTTCACCTCCAAGGAT		Sequencing primer for *mh[sim]* region
TCACGAATTCCGCCCTCT		Sequencing primer for *mh[mel]* and *mh[sim]* region
GGCCCTGCTCATATCGTATC	AAGAACCTTACTGCGTGCAAC	Primers anneal in *mh* introns. Amplifies endogenous *mh* gene in *D. melanogaster*.
TATTCTTACATCTATGTGACC	GTTTTGAGCAGCTAATTACC	Primers amplify a repeat unit of the *359-bp* satellite array[S2]
	CAGGTTCAGGGCGAACAGGTAATCGGCG	Nested gene specific primer A used for genome walking
	TGATGAACCACCCTTGTCATCGTCATCC	Nested gene specific primer B used for genome walking
	TCACCGTCATGGTCTTTGTAGTCCAT	Nested gene specific primer C used for genome walking
TGGGTACAGTGCGCATTCAT	CGAGCAAGTCCGCAAAAAGT	Primers used to amplify the *Top2* ortholog in *D. simulans*
CAGCTTAAGCACAGCGACAC		Sequencing primer for *Top2* ortholog in *D. simulans*
CCAGCTTCACCGTTGAGACT		Sequencing primer for *Top2* ortholog in *D. simulans*
TAGCTACCTACAAGGGCGGT		Sequencing primer for *Top2* ortholog in *D. simulans*
AGGGAGACTCAGCCAAGTCA		Sequencing primer for *Top2* ortholog in *D. simulans*
TTTTCAAGACATGCAGCGCC		Sequencing primer for *Top2* ortholog in *D. simulans*
GCCAGCGCTCGTTACATTTT		Sequencing primer for *Top2* ortholog in *D. simulans*
CCACCGTTCGCTTTGTGATC		Sequencing primer for *Top2* ortholog in *D. simulans*
CGATCCGGAAAAGGCCTTTA		Sequencing primer for *Top2* ortholog in *D. simulans*
AAGCAGATCCTGATGCCAGC		Sequencing primer for *Top2* ortholog in *D. simulans*

Table S3. Primers used in this study. Related to STAR Methods.

SUPPLEMENTAL REFERENCES

S1. Xu, J., and Du, W. (2003). Drosophila chk2 plays an important role in a mitotic checkpoint in syncytial embryos. FEBS Lett *545*, 209-212. 10.1016/s0014-5793(03)00536-2.

S2. Rosic, S., Kohler, F., and Erhardt, S. (2014). Repetitive centromeric satellite RNA is essential for kinetochore formation and cell division. J Cell Biol *207*, 335-349. 10.1083/jcb.201404097.