Supplemental file:

Mutational analysis of Mei5, a subunit of Mei5-Sae3 complex, in Dmc1-mediated recombination during yeast meiosis

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2 Tables; 7 Figures

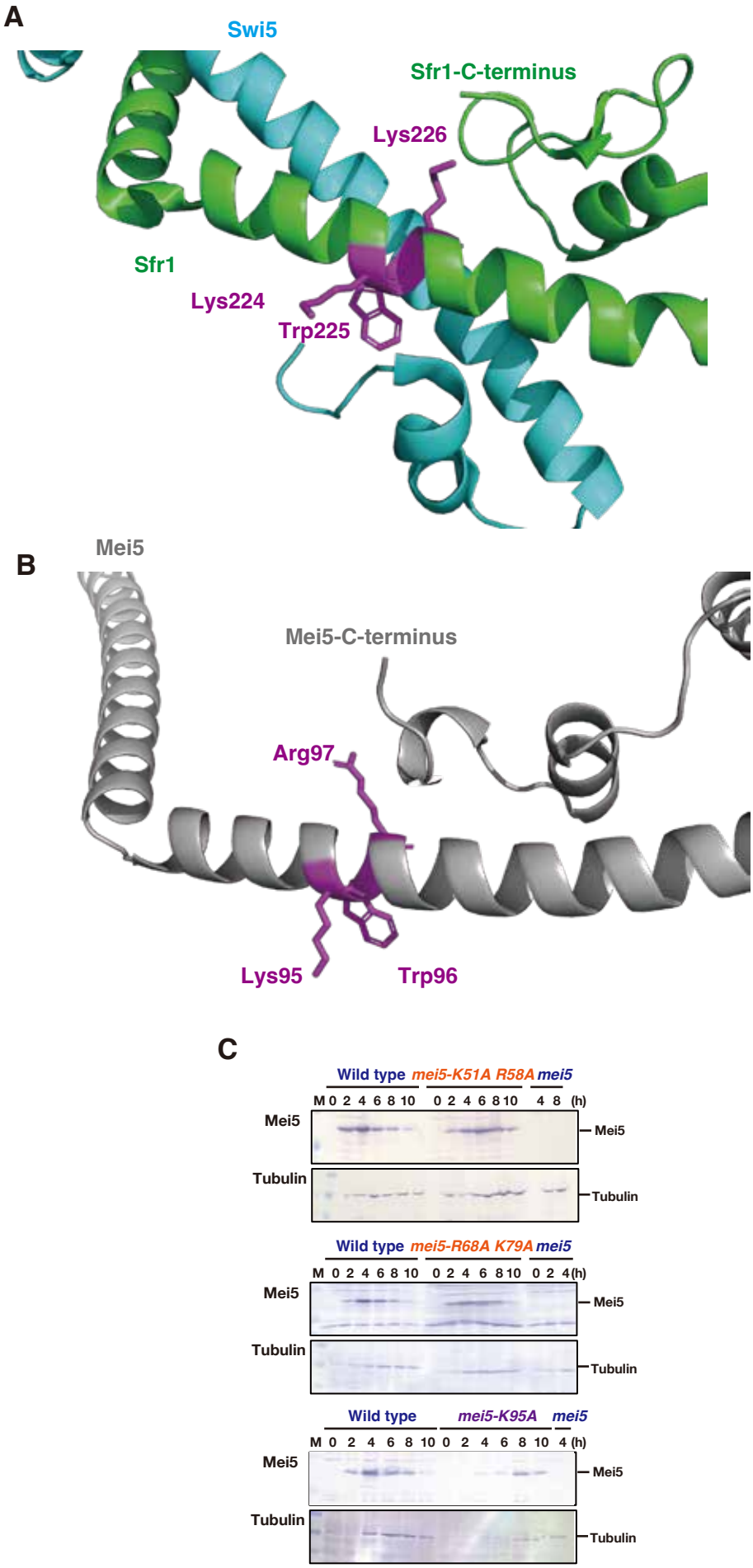
Table S1. Strain List

Strain number	Genotypes
MSY832/833	*MAT**a**/α, ho::LYS2/", lys2/", ura3/", leu2::hisG/", trp1::hisG/"*
NKY1551	*MAT**a**/α, ho::LYS2/", lys2/", ura3/", leu2::hisG/", his4X-LEU2(BamHI)-URA3/his4B-LEU2(MluI), arg4-bgl/arg4-nsp*
PSY64/65	MSY832/833 with *mei5::URA3/"*
PSY33/34	MSY832/833 with *mei5-K51A, R58A/"*
PSY23/24	MSY832/833 with *mei5-R68A, K79A/"*
PSY11/12	MSY832/833 with *mei5-K95A/"*
PSY123/124	MSY832/833 with *mei5-K95L/"*
PSY15/16	MSY832/833 with *mei5-R97A/"*
PSY13/14	MSY832/833 with *mei5-R97L/"*
PSY33/34	MSY832/833 with *mei5-K114A/"*
PSY78/79	MSY832/833 with *mei5-R117A/"*
PYS5/6	NKY1551with *mei5::URA3/"*
PSY133/137	NKY1551with *mei5-K95L/"*
PSY86/90	NKY1551with *mei5-R97A/"*
PSY131/141	NKY1551with *mei5-R97L/"*
PSY31/32	NKY1551with *SAE3-Flag::KamMX4/"*
PSY166/167	NKY1551with *mei5::URA3 SAE3-Flag::KamMX4/"*
PSY144/148	NKY1551with *mei5-K95L SAE3-Flag::KamMX4/"*
PSY157/158	NKY1551with *mei5-R97L SAE3-Flag::KamMX4/"*
SMY199/202	NKY1551with *mei5-R117A SAE3-Flag::KamMX4/"*
SMY209/212	NKY1551with *MEI5-Flag::KamMX4/"*
SMY219/222	NKY1551with *mei5-R117A-Flag::KamMX4/"*
SMY192/195	NKY1551with *mei5-R117A/"*
SMY249/251	NKY1551with *mei5-R117K/"*
SMY253/255	NKY1551with *mei5-R117E/"*
SMY258/259	NKY1551with *mei5-K133A/"*
SMY280/281	NKY1551with *mei5-R134A/"*
SMY343/345	NKY1551with *mei5-d(190-221)::KamMX4/"*
SMY270/272	NKY1551with *mei5-d(197-221)::KamMX4/"*
SMY261/263	NKY1551with *sae3::KamMX4/"*
SMY265/267	NKY1551with *mei5-R117A/" sae3::KamMX4/"*
SMY291/292	MSY832/833 with YES2-*GAL1/10-MEI5::URA3*
SMY322/323	MSY832/833 with YES2-*GAL1/10-MEI5-R117A::URA3*

Table S2. Primer List

Primer name	Sequence
MEI5-Seq-F	5’-GCCGCGGAAAAAGTATTAGC
MEI5-Seq-R	5’-TGGCTTTTAATGCTAAGTTA
MEI5-K95L-F	5’-ATAGAACTTTGGAGGACCATTTGTGAG
MEI5-K95L-R	5’-GGATTTTCTGTTCTCAATTATCTTGTT
MEI5-R97L-F	5’-AAA TGG TTG ACA ATA TGT GAG ATG GAA
MEI5-R97L-R	5’-TTC GAT CGA TTT TCT GTT CTC AAT GAT
MEI5-R117A-F	5’-AAAATCAATGCTATGGGCGGCTATAAAG
MEI5- R117A/K/E-R	5’-GATTAAAGTGGAATTCAAAATAAAAGAC
MEI5-R117K-F	5’-AAAATCAATAAAATGGGCGGCTATAAAG
MEI5-R117E-F	5’-AAAATCAATGAAATGGGCGGCTATAAAG
MEI5-K133A/R134A-F	5’-GGCGGCTATAAGGATTTCCTAGAAAAA
MEI5-K133A-R	5’-CAATCTAGCCTTTTTAGCCTCCATTTC
MEI5-R134A-R	5’-CAATCTTCTAGCTTTAGCCTCCATTTC
MEI5-d(190-221)-F	5’- ACAGCTGAAAGAGTTGGAAAAAAAAAAAATAGC GGAGCTGGAAAAATGAcccaccaccatcatcatca
MEI5-d(197-221)-F	5’- AAAAAAATAGCGGAGCTGGAAAAATTGAATAAG GTGCTGCATGATTGAtcccaccaccatcatcatc
MEI5- deletion-R	5’-CATCGATTTTATAGGCTTTGCCAAC

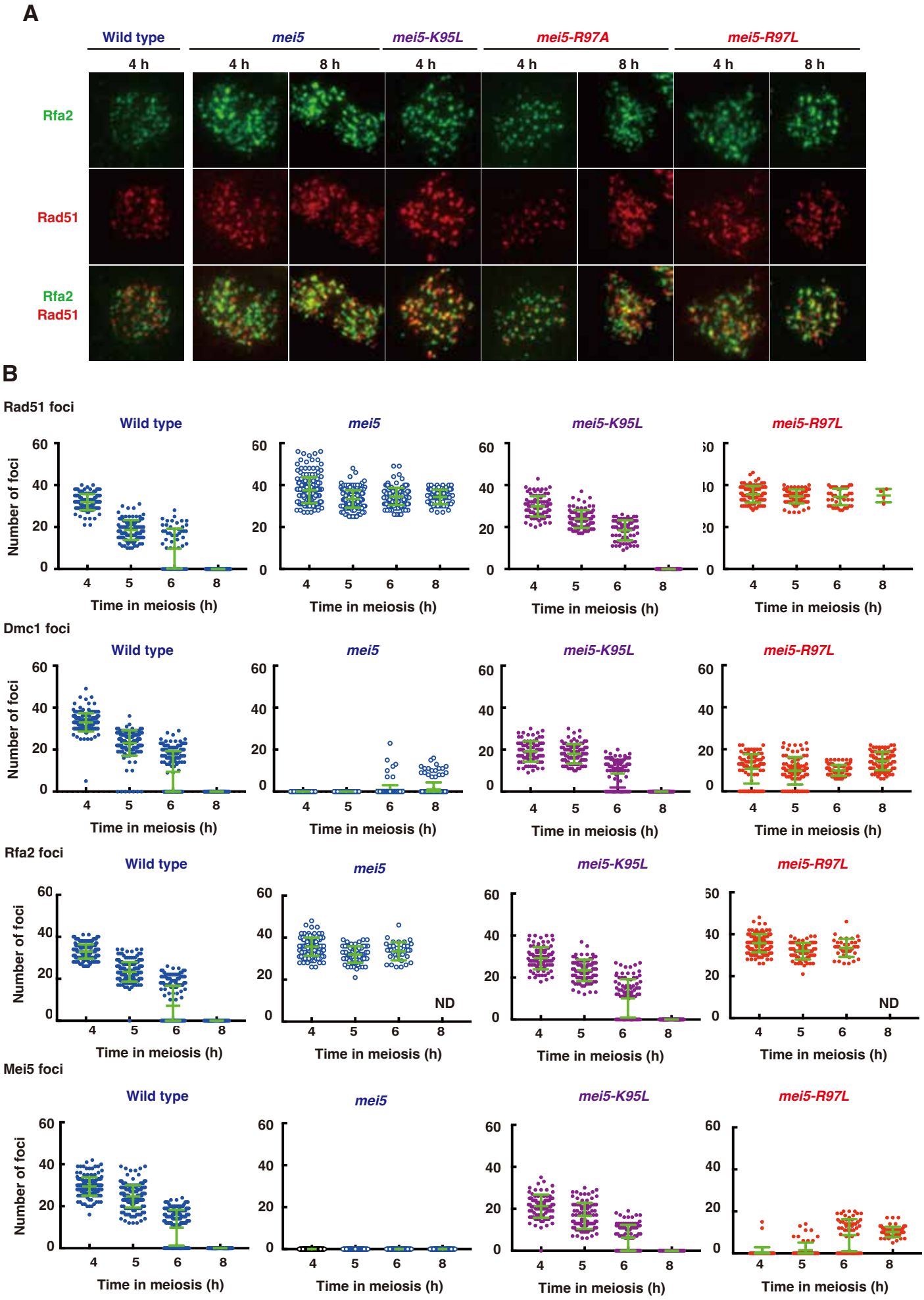
Supplemental Figure S1. Mwaniki et al.



Supplemental Figure S1.

A. Crystal structure of fission yeast Sfr1-Swi5. An enlarged portion of Sfr1 (green) and Swi5 (pale blue) are shown. Conserved KWK/R residues are shown in the stick model (purple).
B. AlphaFold2-predicted Mei5 structure. Conserved KWK/R residues; Lys95, Trp96, and Arg97, are shown in the stick model (purple).
C. Expression of various mutant Mei5 proteins in meiosis. Lysates obtained from the cells at various time points during meiosis were analyzed by western blotting using anti-Mei5 (upper) or anti-tubulin (lower) antibodies.

Supplemental Figure S2. Mwaniki et al.

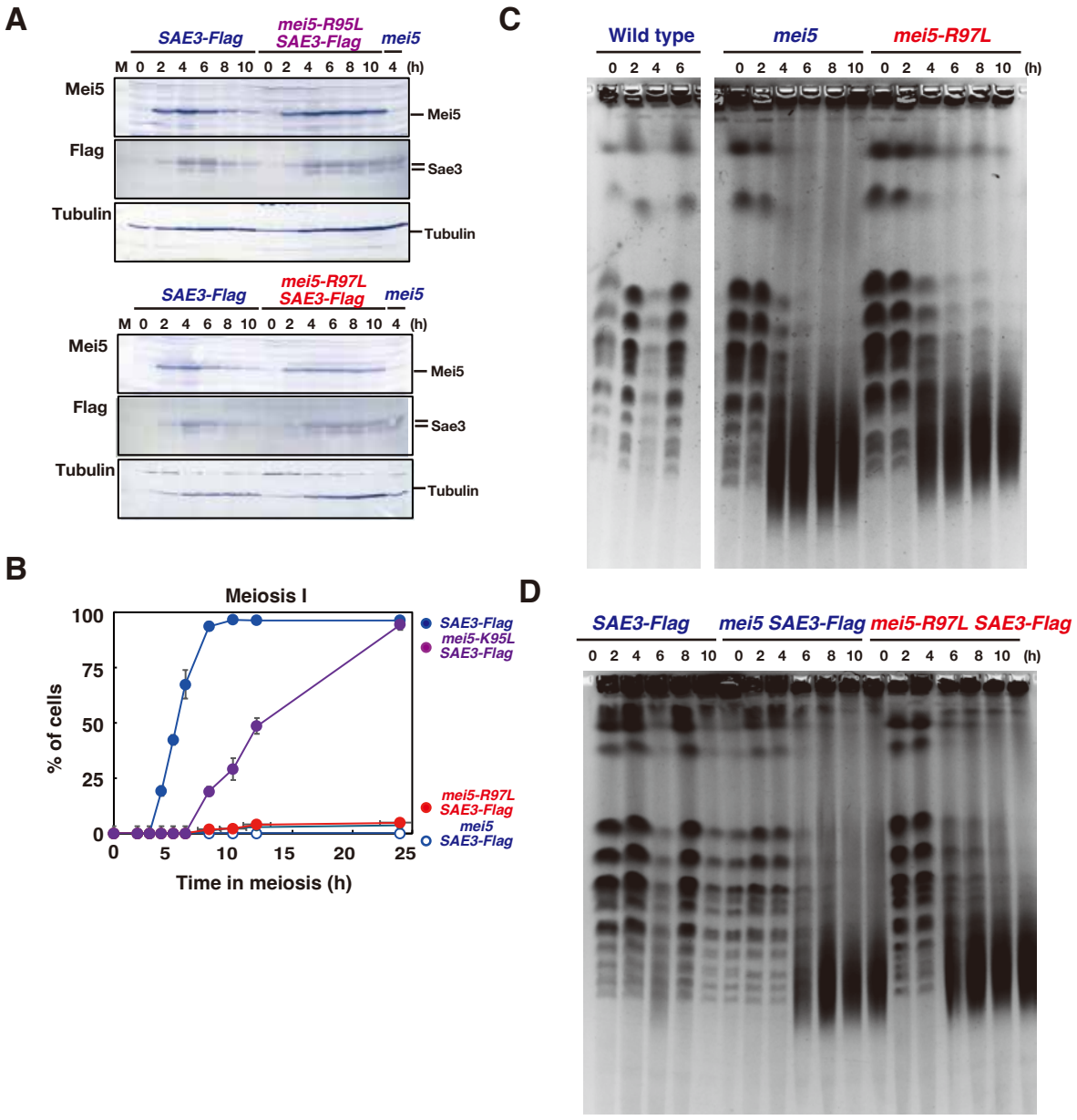


Supplemental Figure S2.

A. Rad51 and Rfa2 staining. Nuclear spreads were stained with anti-Rad51 (red) and anti-Rfa2 (green). Representative images at each time point under the two conditions are shown. Bar = 2 μm.

B. Focus number at different time points: At each time point, foci were manually counted. The graphs show the focus number combined from three independent time courses. Error bars (green) is a mean with standard deviation.

Supplemental Figure S3. Mwaniki et al.



Supplemental Figure S3.

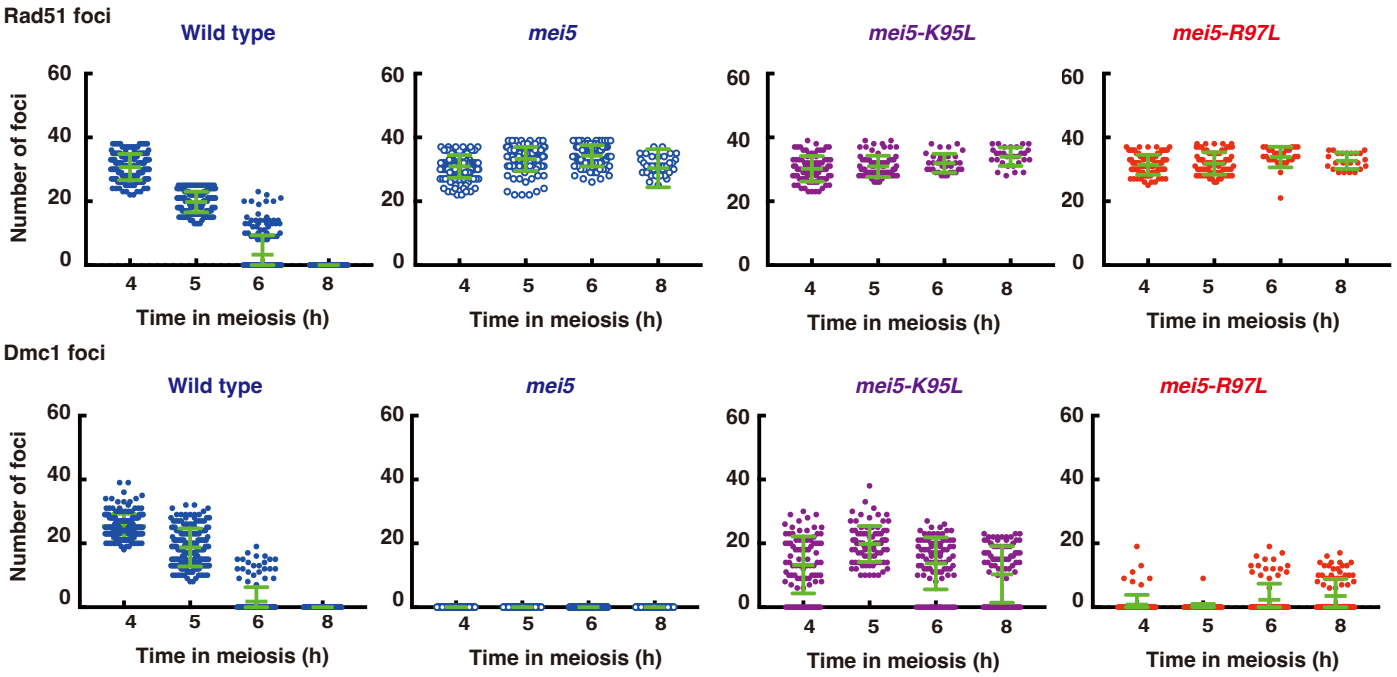
A. Expression of various mutant Mei5 and Sae3-Flag proteins in meiosis. Lysates obtained from the cells at various time points during meiosis were analyzed by western blotting using anti-Mei5 (upper), anti-Flag (middle) or anti-tubulin (lower) antibodies.

B. The entry into meiosis I in various strains was analyzed by DAPI staining. The number of DAPI bodies in a cell was counted. A cell with 2, 3, and 4 DAPI bodies was defined as a cell that passed through meiosis I. The graph shows the percentages of cells that completed MI or MII at the indicated time points.

C. CHEF analysis of meiotic DSB repair. Chromosomal DNAs from Wild-type (NKY1551), *mei5*(PSY5/6), and *mei5-R97L* (PSY131/141) cells were analyzed by CHEF electrophoresis.

D. CHEF analysis of meiotic DSB repair. Chromosomal DNAs from *SAE3-Flag* (PSY31/32), *mei5 SAE3-Flag* (PSY166/167), and *mei5-R97L SAE3-Flag* (PSY157/158) cells were studied by CHEF electrophoresis.

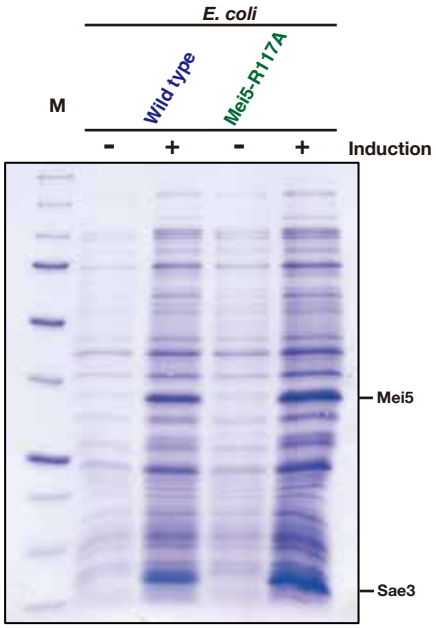
Supplemental Figure S4. Mwaniki et al.



Supplemental Figure S4.

Focus number at different time points: At each time point, foci were manually counted. The graphs show the focus number combined from three independent time courses. Error bars (green) is a mean with standard deviation.

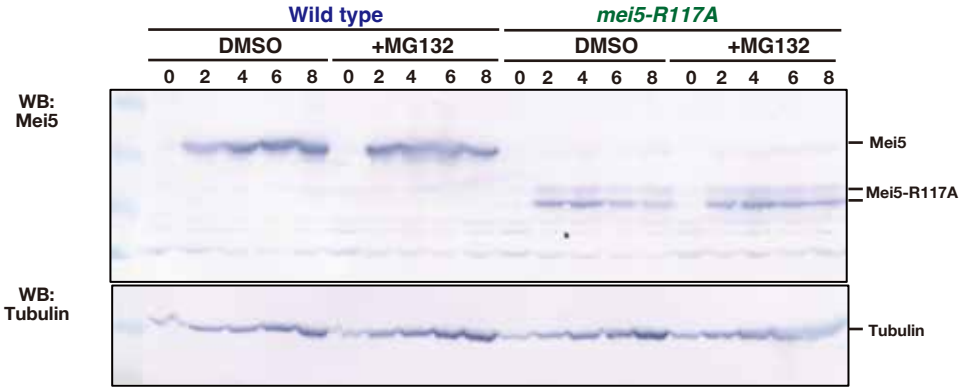
Supplemental Figure S5. Mwaniki et al.



Supplemental Figure S5.

E. coli cell lysates were stained with Coomassie Brilliant blue. BL21(DE3) cells with various vectors were cultured with or without IPTG for 3 hours.

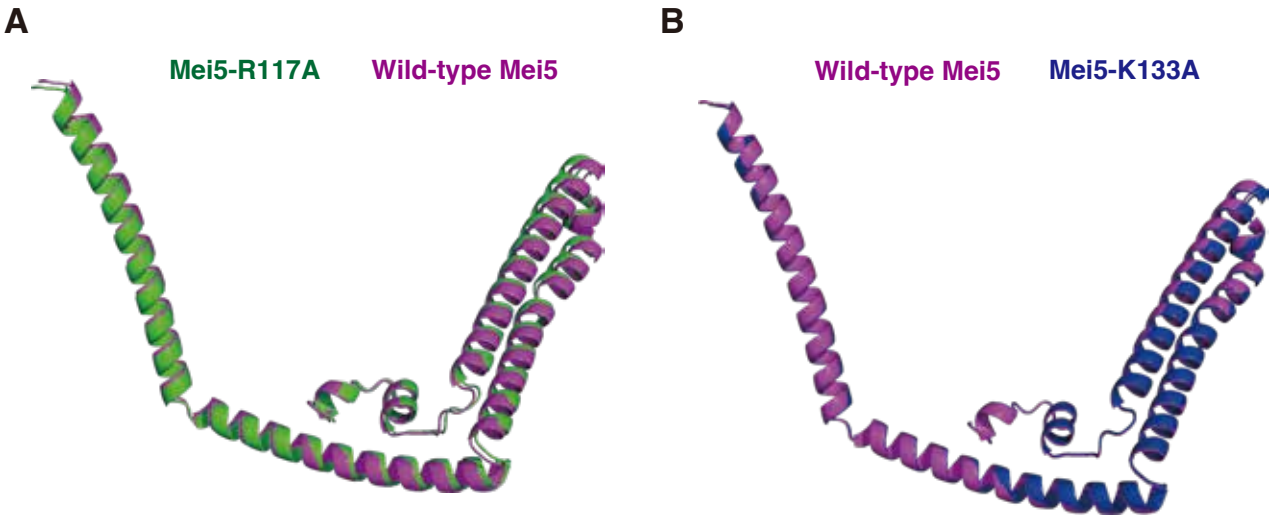
Supplemental Figure S6. Mwaniki et al.



Supplemental Figure S6.

Expression of Mei5-R117A. Meiosis was induced in the presence or the absence of a proteasome inhibitor, MG132. Cell lysates at each time were verified by western blotting with anti-Mei5 and anti-tubulin (control). MG132 was added at 0 h at a concentration of 50 μM. Strains used are as follows: Wild-type, NKY1551; *mei5-R117A*, SMY192/195.

Supplemental Figure S7. Mwaniki et al.



Supplemental Figure S7.

Comparison of the structure of putative Mei5 mutant proteins. Wild-type Mei5 (magenta), Mei5-R117A (green), and Mei5-K133A (purple) were predicted by Alphafold2 and overlaid using the PyMol “super” command. RSMD values between wild-type and Mei5-R117A or Mei5-K133A were 0.079 or 0.416, respectively. The disordered N-terminal region (1-45) of Mei5 is deleted in the schematic model.