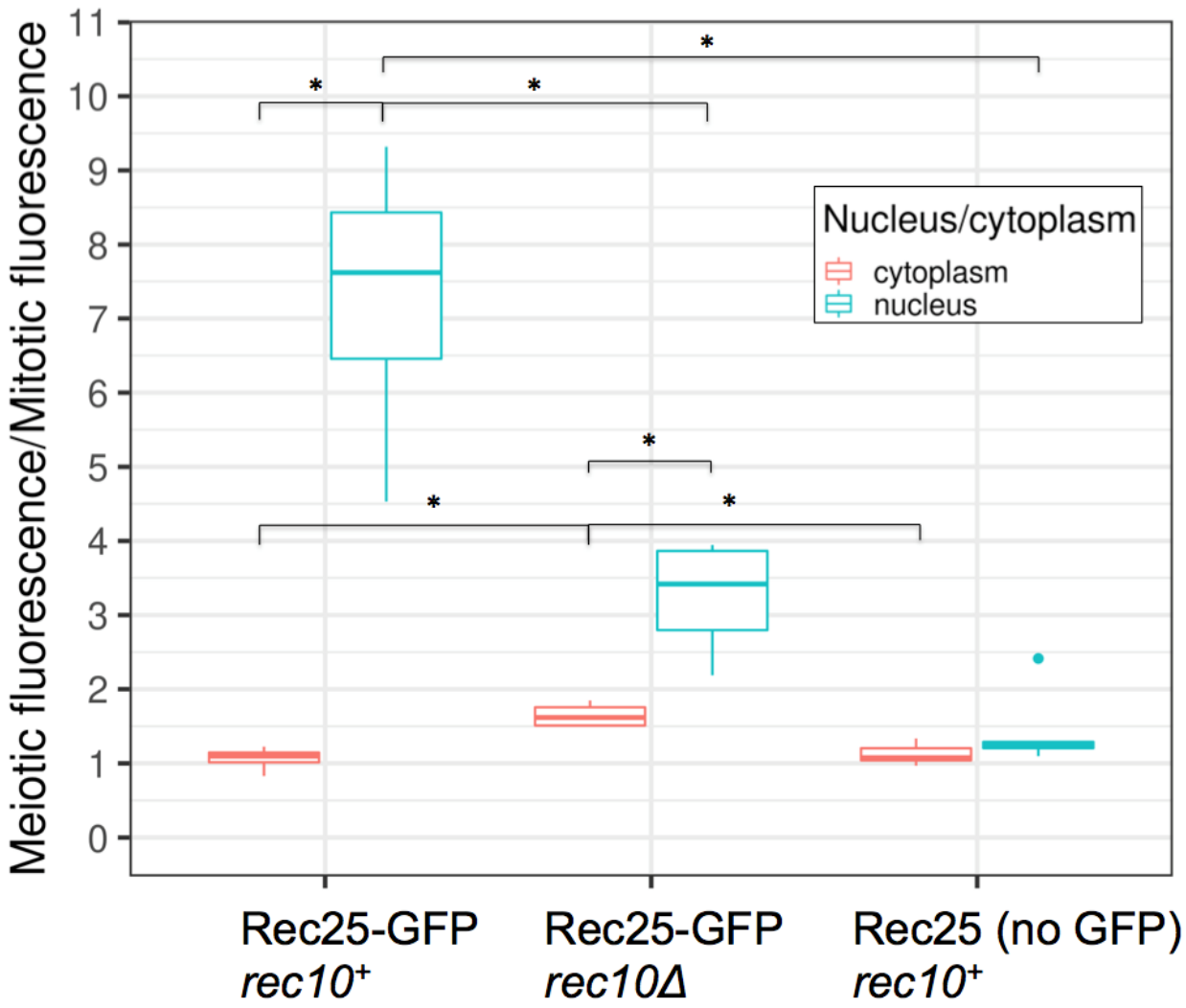

Fig. S1. Rec25 enters the nucleus in *rec10Δ*. Data are quantification of images like those in Fig. 2A showing the ratio of fluorescence in the cytoplasm or in the nucleus, as a ratio of fluorescence in meiotic (horsetail stage) cells to that in mitotic cells. Shown are Rec25-GFP in *rec10*$^{+}$ (left data); Rec25-GFP in *rec10Δ* (middle data); and cells with no *GFP* gene (right data). 8 to 27 cells were examined; boxes show the mean (horizontal line) ± 25% (top and bottom of boxes) and 95% limit (ends of vertical lines); one outlier is visible in the right-most data. Asterisks indicate $p < 0.05$ for the indicated comparisons calculated with a Wilcoxon test corrected for multiple tests. .

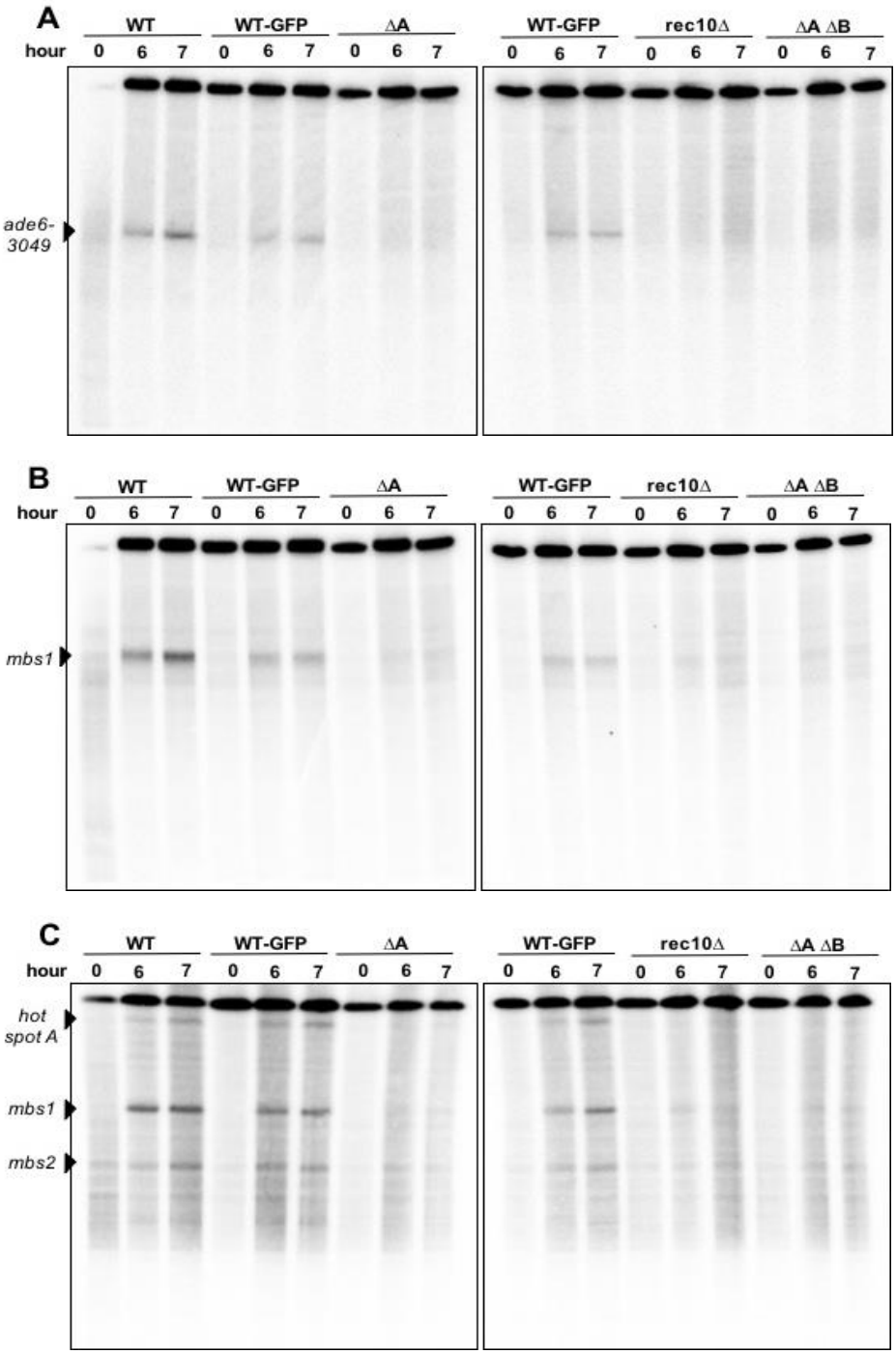

Figure S2. Rec10-NLS mutants have reduced DSBs at hotspots

Strains with the indicated *rec10-GFP* alleles (NLS +, *ΔA*, or *ΔA ΔB),* wild type (*rec10*$^{+}$), or *rec10Δ* (complete coding-sequence deletion) were induced for meiosis and harvested at the indicated times. DNA was digested with *Pme*I (**A** and **B**) or *Not*I (**C**) and analyzed for DSBs at the indicated hotspots as described in Materials and Methods. The fraction of DNA broken at each hotspot is in Table S1.

Table S1. DSB frequencies at DSB hotspots are reduced by *rec10-NLS* mutations

DNA fragment:		*Pme*I fragments[a]		*Not*I fragment J[b]		
DSB hotspot:		*ade6-3049*	*mbs1*	*mbs1*	*mbs2*	A
WT	0 hr	NA[c]	NA	<0.2	3.2[d]	<0.2
	6 hr	5.2	6.7	9.8	1.1	0.9
	7 hr	7.7	8.4	12	5.3	3.4
rec10 nls^{+} GFP	0 hr	<0.2, <0.2, <0.2	<0.2, <0.2, <0.2	<0.2, <0.2	<0.2, <0.2	<0.2, <0.2
	6 hr	2.7, 3.3, 2.5	3.2, 3.3, 3.6	4.4, 4.0	2.3, 1.3	1.5, 2.2
	7 hr	4.0, 3.7, 4.1	3.8, 3.8, 4.2	4.3, 5.7	1.6, 2.0	2.9, 3.6
*rec10*Δ	0 hr	<0.2	<0.2	<0.2	<0.2	0.3
	6 hr	<0.2	1.0	0.9	<0.2	<0.2
	7 hr	<0.2	0.6	<0.2	<0.2	1.5
rec10 nls Δ*A-GFP*	0 hr	<0.2, <0.2	<0.2, <0.2	<0.2	<0.2	<0.2
	6 hr	<0.2, 0.6	1.1, 1.0	<0.2	0.9	0.4
	7 hr	<0.2, 0.8	0.8, 0.7	<0.2	<0.2	1.0
rec10 nls Δ*A* Δ*B-GFP*	0 hr	<0.2, <0.2	<0.2, <0.2	<0.2	<0.2	<0.2
	6 hr	<0.2, <0.2	0.8, 0.4	0.6	0.8	0.3
	7 hr	<0.2, <0.2	0.6, 0.6	0.4	1.0	0.6

DSBs were assayed at the indicated times after induction of strains with the indicated genotypes (see Table S2), as described in Materials and Methods. Where indicated, multiple gels from one induced culture for each strain were analyzed. The lower limit of detection was estimated to be 0.2%.

[a] DNA was digested with *Pme*I. The 74 kb fragment was analyzed for DSBs at *ade6-3049* on Chr. 3, and the 64 kb fragment was analyzed for DSBs at *mbs1* on Chr. 1.

[b] DNA was digested with *Not*I and the 501 kb fragment J was analyzed for *mbs1, mbs2*, and a hotspot, here named A, close to the right end of the fragment.

[c] NA, not available. The agarose plug containing the cells, and thus DNA, for this *Pme*I analysis was degraded (see Figure S2A and B).

[d] This value is questionable, because the band is one of several unexpectedly observed in this 0 hr lane (Figure S2C).

Table S2. *S. pombe* strains[a]

Strain number	Genotype[b]	Use
GP50	*h*^{90}	Microscopy (background control)
GP935	*h- ade6-52 ura4-D18*	Recombination assay, chromosomal *rec10*
GP4414	*h+ ade6-M26 arg1-14 rec10-175::kanMX6*	Recombination assay, chromosomal *rec10*
GP4625	*h*$^{-}$ *ade6-52 rec10-175::kanMX6*	Recombination assay, chromosomal *rec10::GFP*
GP4914	*h*$^{+}$ *ura4-D18 ade6-M26 arg1-14*	Recombination assay, chromosomal *rec10::GFP*; construction of GP9964 – GP9973
GP6994	*h*$^{-}$ *rec10-175::kanMX6 ura4-D18 ade6-52*	Recombination assay, plasmid-borne *rec10*
GP7301	*h*$^{-}$ *ade6-52 ura4-D18 rec10-260::ura4*$^{+}$	Construction of GP9978 – GP9983
GP8762	*h*^{90} *rec10-203::GFP-kanMX6*	Construction of GP9836
GP8766	*h*^{90} *rec25-204::GFP-kanMX6*	Construction of GP9745
GP8819	*h*^{90} *rec27-205-GFP::kanMX6*	Microscopy, Rec27 localization
GP8829	*h*^{90} *mug20-GFP::kanMX6*	Microscopy, Mug20 localization
GP9745	*h*^{90} *rec25-303::GFP-hphMX6*	Microscopy, Rec25 localization; construction of GP9989 – GP9995,
GP9747	*h*^{90} *rec10-301::GFP-natMX6*	Microscopy, Rec10 localization
GP9775	*h*$^{+}$ *rec10-175::kanMX6 ura4-D18 ade6-M26 arg1-14*	Recombination assay, plasmid-borne *rec10*
GP9806	*h*^{90} *rec10-175::kanMX6 rec25-303::GFP-hphMX6 ura4-294*	Microscopy, Rec25 localization, plasmid-borne *rec10*
GP9823	*h*$^{-}$ *rec25-220::tdTomato-hphMX6*	Construction of GP9926 – GP9944
GP9836	*h*^{90} *rec10-301::GFP-natMX6 ura4-D18*	Construction of GP9845
GP9845	*h*^{90} *rec10-306::ura4*$^{+}$*-GFP-natMX6 ura4-D18*	Construction of GP9876 – GP9889
GP9852	*h*$^{+}$ *ade6-M26 ura4-D18 arg1-14 rec10-301::GFP-natMX6*	Recombination assay, chromosomal *rec10::GFP*
GP9859	*h*$^{-}$ *rec10-301::GFP-natMX6 pat1-as1*(L95G)*::kanMX6*	Western blot, Rec10 abundance
GP9860	*h*$^{-}$ *pat1-as1*(L95G)*::kanMX6*	Construction of GP9950 – GP9959
GP9901	*h*$^{-}$ *pat1-as1(L95G)::kanMX6 ade6-3049 rad50S*	Southern blot, DSB abundance

`GP9998	*h⁻ pat1-as1(L95G)::kanMX6 ade6-3049 rad50S rec10-301-GFP::natMX6*	Southern blot, DSB abundance
GP9999	*h⁻ pat1-as1(L95G)::kanMX6 ade6-3049 rad50S rec10-289-GFP::natMX6*	Southern blot, DSB abundance
GR1	*h⁻ pat1-as1(L95G)::kanMX6 ade6-3049 rad50S rec10-175::kanMX6*	Southern blot, DSB abundance
GR2	*h⁻ pat1-as1(L95G)::kanMX6 ade6-3049 rad50S rec10-296-GFP::natMX6*	Southern blot, DSB abundance
GR9	*h^{90} rec27-205-GFP::kanMX6 rec10-296*	Microscopy, Rec27 localization, chromosomal *rec10*
GR10	*h^{90} mug20-GFP::kanMX6 rec10-296*	Microscopy, Mug20 localization, chromosomal *rec10*

[a] Additional strains are in Table S5.

[b] Strains were constructed by standard matings (Smith, 2009) or as described in Methods. Genealogies are available upon request. Sources of alleles, other than commonly used auxotrophies and *mat*, are the following: *mug20-GFP::kanMX6* (Estreicher et al., 2012); *pat1-as1*(L95G)*::kanMX6* (Guerra-Moreno et al., 2012); *rad50S* (Farah et al., 2002); *rec10-175::kanMX6* (Ellermeier and Smith, 2005); *rec10-203::GFP-kanMX6* (Fowler et al., 2013); *rec10-260::ura4*$^{+}$ (Ma et al., 2017); *rec10-301::GFP-natMX6* (Materials and Methods); *rec10-306::ura4*$^{+}$*-GFP-natMX6* (Materials and Methods); *rec25-204::GFP-kanMX6* (Davis et al., 2008); *rec25-220::tdTomato-hphMX6* (Fowler et al., 2013); *rec25-303::GFP-hphMX6* (Materials and Methods); *rec27-205-GFP::kanMX6* (Davis et al., 2008).

Table S3. Plasmids[a]

Plasmid	Genotype	Ref. or origin
pFY20	*ura4*$^{+}$ *ars1 stb amp*	(Li et al., 1997)
pYL176	*rec10*$^{+}$ *URA3*^{b} *ars1 amp*	(Lin and Smith, 1995)
pFA6a-*natMX6*	*natMX6*	(Hentges et al., 2005)
pFA6a-*hphMX6*	*hphMX6*	(Hentges et al., 2005)

[a] Additional plasmids are listed in Table S4.

[b] *S. cerevisiae URA3,* which complements *S. pombe ura4* mutations.

Table S4. Oligonucleotides for *rec10* NLS mutant constructions

Oligo number	Nucleotide sequence (5' → 3')
OL4134	AGATGGAAAGTTTGCAAAATCGACACAAAAATCTTTAAAACCTGATACTG
OL4135	CAGTATCAGGTTTTAAAGATTTTTGTGTCGATTTTGCAAACTTTCCATCT
OL4136	CTGAAAATCAAGAATCTTCGGTGGCGAAATCCAATGTTAATTTGCA
OL4137	TGCAAATTAACATTGGATTTCGCCACCGAAGATTCTTGATTTTCAG
OL4140	GCTGCACAAAAATCTTTAAAACCTGATAC
OL4141	TGCAGCTGTCGATTTTGCAAACTTTC
OL4142	GCTGCAGCGAAATCCAATGTTAATTTG
OL4143	TGCAGCCACCGAAGATTCTTGATTTTC
OL4154	CACTTCCAAGCAAGCATCCCAG (used for PCR amplification of NLS region)
OL4147	CCTGTACTCAAGTTCCTGGCGA (used for PCR amplification of NLS region)
OL4155	GACAAGAGTGTGTGCGACGATG (used for sequencing PCR products)
OL1780	GTAACCGTCACTTATCGATGG (used for PCR analysis of *rec10*$^{+}$ and *rec10-306*)
OL1781	AGCATGGACAGTATTGGCAAC (used for PCR analysis of *rec10*$^{+}$)
OL2124	ATGCTCCTACAACATTACCAC (used for PCR analysis of *rec10-306*)
OL4278	CACGCACAATCAACTGAAAC (*rec10* left forward primer to make *rec10-306*)
OL4279	TTTCGTCAATATCACAAGCTCGGCAGTTCAATTTCTTGC (*rec10* left *ura4* flank reverse primer to make *rec10-306*)
OL4280	GTGGGATTTGTAGCTAAGCTCCTACGATAGCAAACATTGC (*rec10* right *ura4* flank forward primer to make *rec10-306*)
OL4281	TCCTGTACTCAAGTTCCTGG (*rec10* right reverse primer to make *rec10-306*)
OL4282	GCAAGAAATTGAACTGCCGAGCTTGTGATATTGACGAAA (*rec10::ura4*$^{+}$ forward primer to make *rec10-306*)
OL4283	GCAATGTTTGCTATCGTAGGAGCTTAGCTACAAATCCCAC (*rec10::ura4*$^{+}$ reverse primer to make *rec10-306*)

Table S5. *rec10* nuclear localization signal (NLS) mutants

rec10 allele	Alternate designation	Amino acid sequence[a] Site A Site B	Oligos; plasmid recipient	Plasmid isolate	Chromosomal isolate					
					b	c	d	e	f	g
301	+	**KRKK KNKK**			GP9836		GP9747		GP9859	GP9582
289	ΔA	ΔΔΔΔ ----	OL4134, OL4135 pYL176	pMW2	GP9876	GP9982	GP9926	GP9992	GP9950	GP9964
290	ΔB	---- ΔΔΔΔ	OL4136, OL4137 pYL176	pMW3	GP9877	GP9978	GP9928	GP9989	GP9951	GP9965
296	ΔA ΔB	ΔΔΔΔ ΔΔΔΔ	OL4134, OL4135 pMW3	pMW9	GP9886	GP9983	GP9938	GP9993	GP9956	GP9970
292	AlaA	AAAA ----	OL4140, OL4141 pYL176	pMW5	GP9878	GP9979	GP9930	GP9990	GP9952	GP9966
293	AlaB	---- AAAA	OL4142, OL4143 pYL176	pMW6	GP9879	GP9980	GP9932	GP9995	GP9953	GP9967
299	AlaA AlaB	AAAA AAAA	OL4140, OL4141 pMW6	pMW12	GP9889	GP9981	GP9944	GP9991	GP9959	GP9973

[a] Rec10 amino acids from 497 – 519 (**KRKK**QKSLKPDTENQESSV**KNKK)**. The wild-type sequence is given for the + allele (*rec10*$^{+}$ on the plasmid and *rec10*$^{+}$ or *rec10-301::GFP-natMX6* on the chromosome). For other alleles "-" indicates the amino acid is that of wild type, and "Δ" or "A" indicates the amino acid is deleted or changed to Ala, respectively.

[b] Strains with the *rec10-NLS-GFP* fusion alleles, used to make strains in column d. Strains other than GP9836 were derived from 5-fluoro-orotic acid-resistant (FOA^{R}) transformants of strain GP9845.

[c] Strains with the *rec10-NLS* alleles without GFP fusion, used to make strains in column e and for recombination assays. Strains were derived from FOA^{R} transformants of strain GP7301.

[d] Strains with the *rec10-NLS-GFP* fusion alleles, used for microscopy of Rec10-GFP localization. Strains were derived from crosses of GP9823 with strains in column b.

[e] Strains with the *rec10-NLS* alleles without GFP fusion, used for microscopy of Rec25-GFP localization. Strains were derived from crosses of GP9745 with strains in column c.

[f] Strains with the *rec10-NLS-GFP* fusion alleles, used to assay Rec10 abundance by Western blot analyses. Strains were derived from crosses of GP9860 with strains in column b.

[g] Strains with the *rec10-NLS-GFP* alleles, used for recombination assays. Strains were derived from crosses of GP4914 with strains in column b.

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