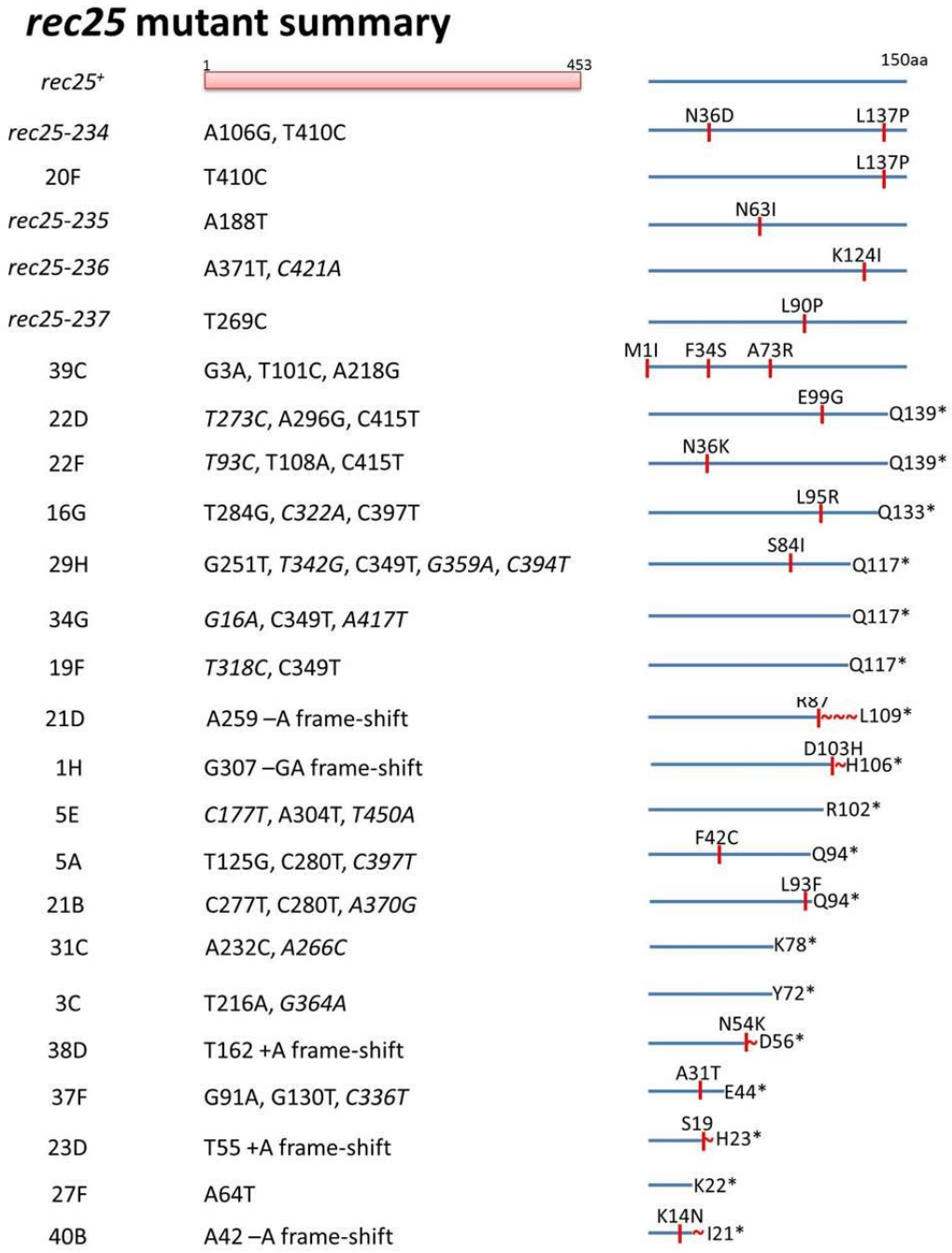
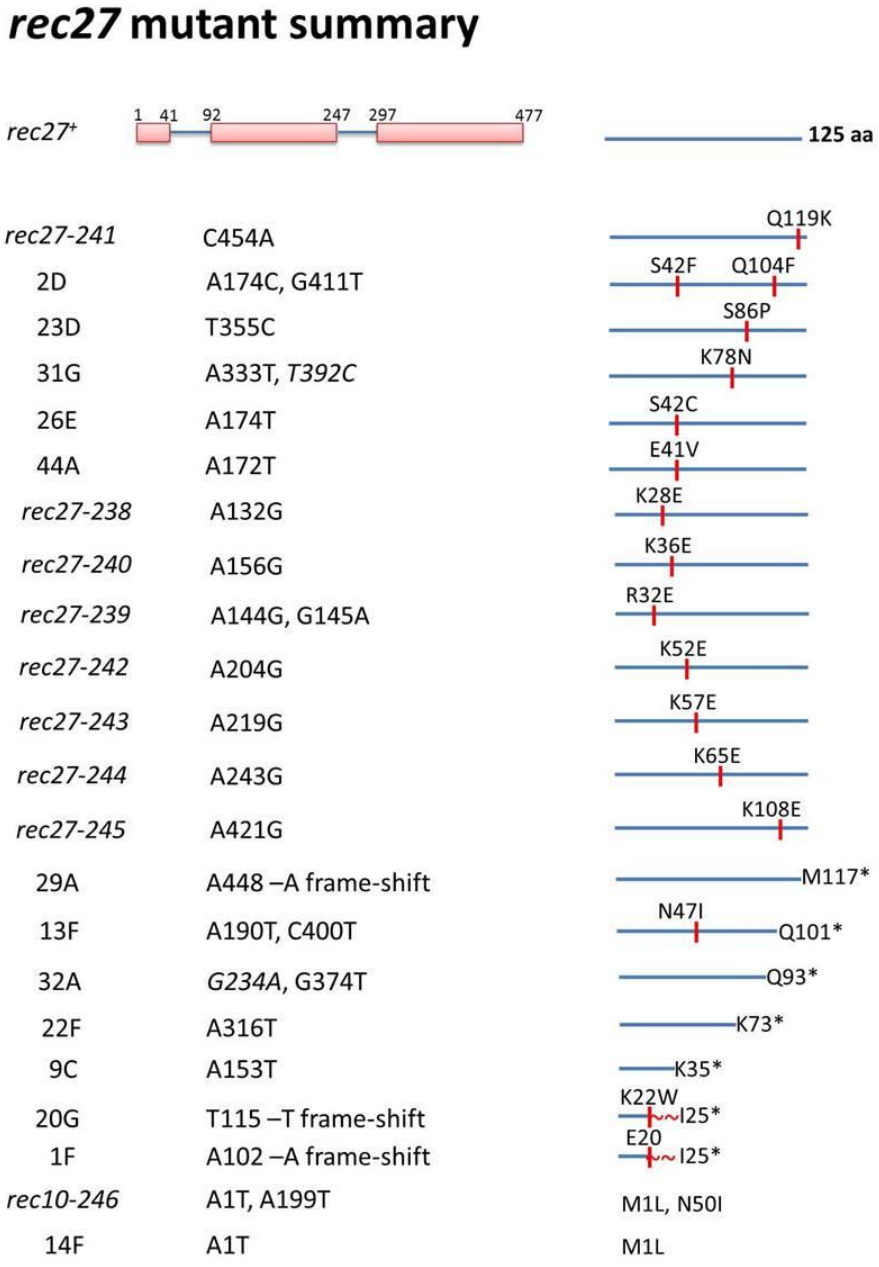
Supplementary Figures and Tables for

Functional organization of protein determinants of meiotic DNA break hotspots

Lijuan Ma[1], Kyle R. Fowler[1,2], Cristina Martín-Castellanos[3] and Gerald R. Smith[1,*]

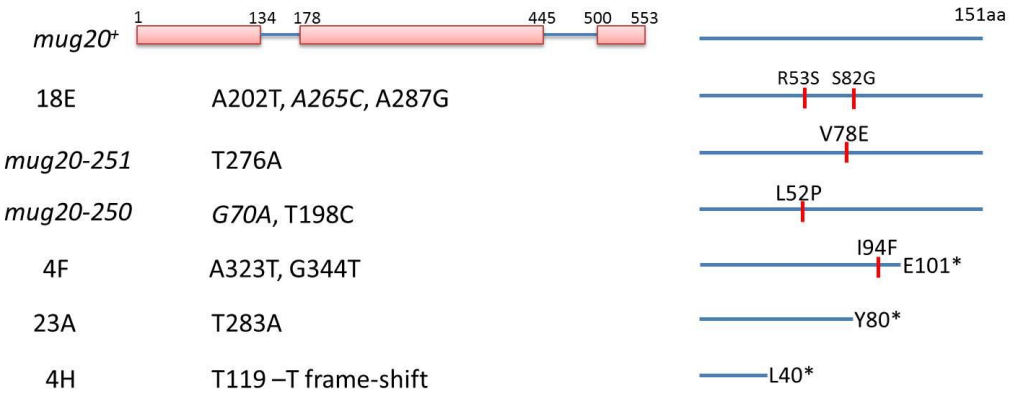


Supplementary Figure S1. Summary of *rec25* mutants. Mutants listed here were from the screen and were recombination-negative in qualitative tests (low frequency of white papillae on red spots (Figure 1). Nucleotide changes in italics do not change protein coding. * Non-sense mutation, ~ frame-shift. The mutants without an assigned allele number were not tested further. See main text for analysis of other mutants. Pink box is the exon.

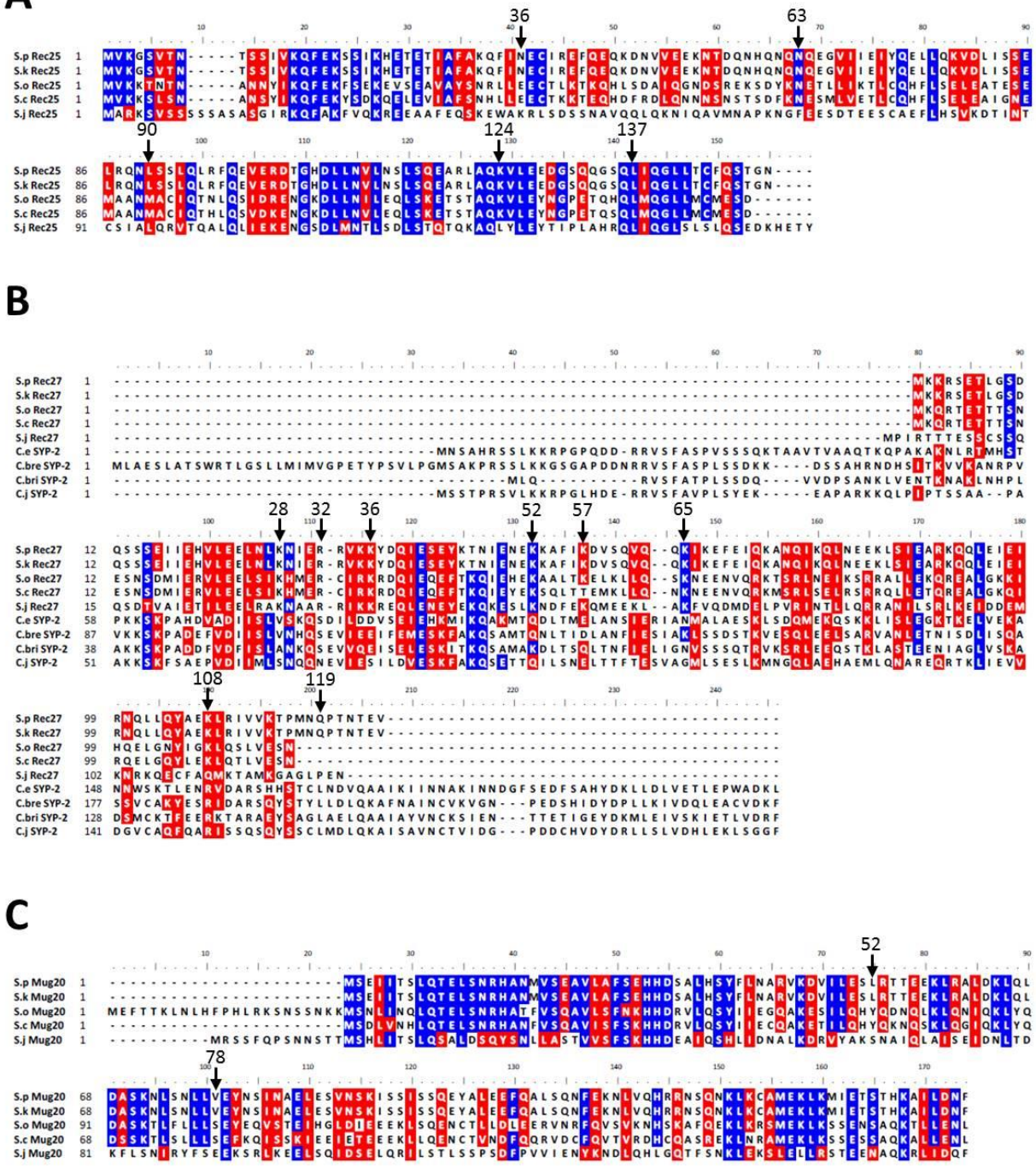


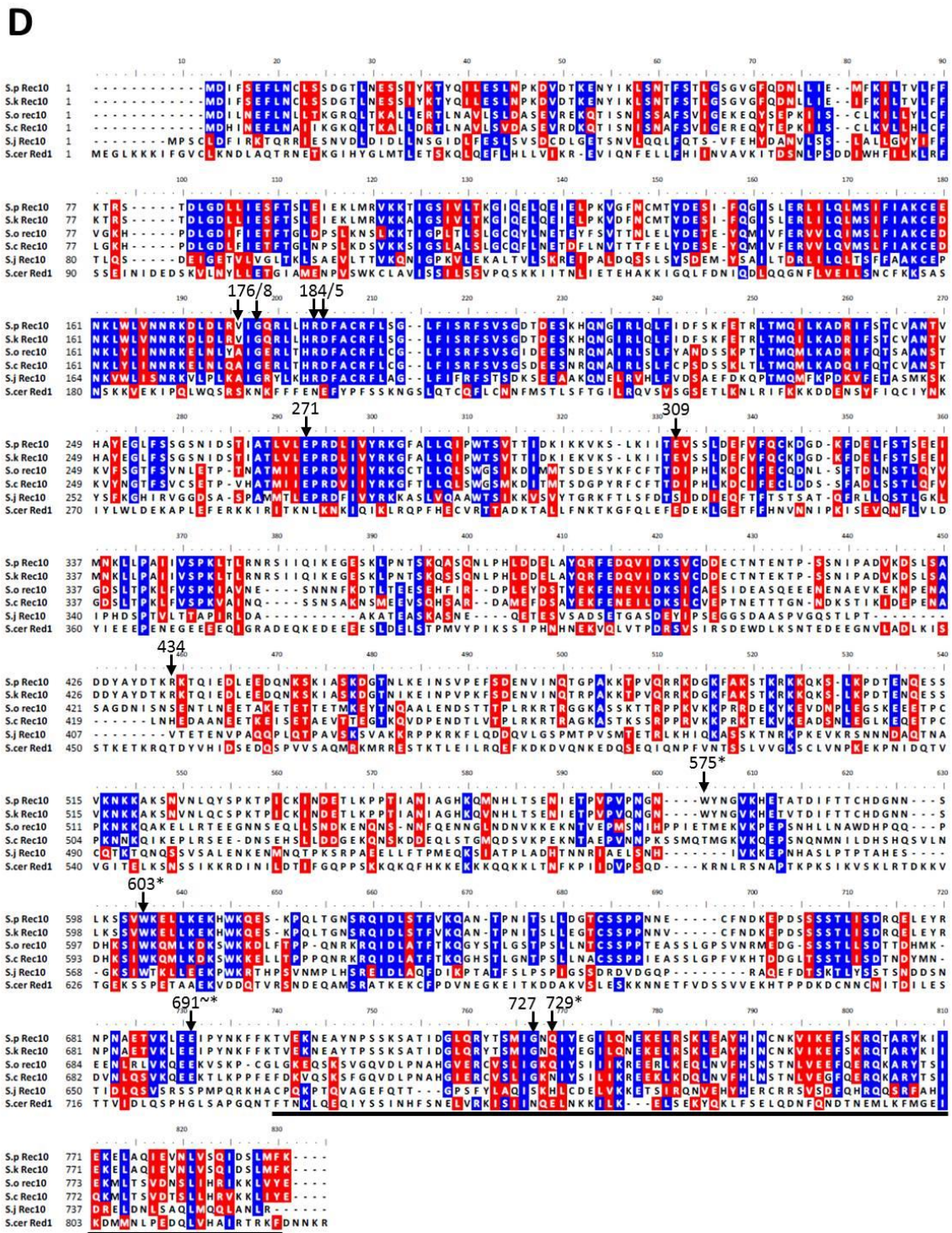
Supplementary Figure S2. Summary of *rec27* mutants. Mutants listed here were from the screen (except for *rec27-239*, *242*, *243*, *244* and *245* which were from site-directed mutagenesis) and were recombination-negative in qualitative tests (low frequency of white papillae on red spots (Figure 1). Nucleotide changes in italics do not change protein coding. * Non-sense mutation, ~ frame-shift. The mutants without an assigned allele number were not tested further. See main text for analysis of other mutants. Pink boxes are exons.

mug20 mutant summary



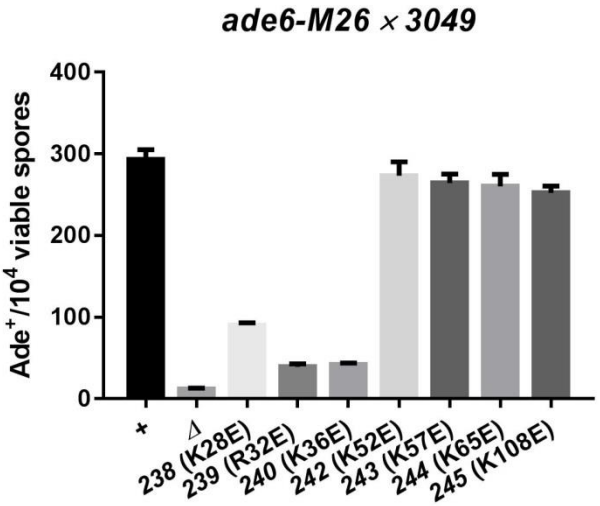
Supplementary Figure S3. Summary of *mug20* mutants. Mutants listed here were from the screen and were recombination-negative in qualitative tests (low frequency of white papillae on red spots (Figure 1). Nucleotide changes in italics do not change protein coding. * Non-sense mutation. The mutants without an assigned allele number were not tested further. See main text for analysis of other mutants. Pink boxes are exons.


A
36
63
90
124
137
S.p Rec25
S.k Rec25
S.o Rec25
S.c Rec25
S.j Rec25
B
28
32
36
52
57
65
108
119
S.p Rec27
S.k Rec27
S.o Rec27
S.c Rec27
S.j Rec27
C.e SYP-2
C.bre SYP-2
C.bri SYP-2
C.j SYP-2
C
52
78
S.p Mug20
S.k Mug20
S.o Mug20
S.c Mug20
S.j Mug20

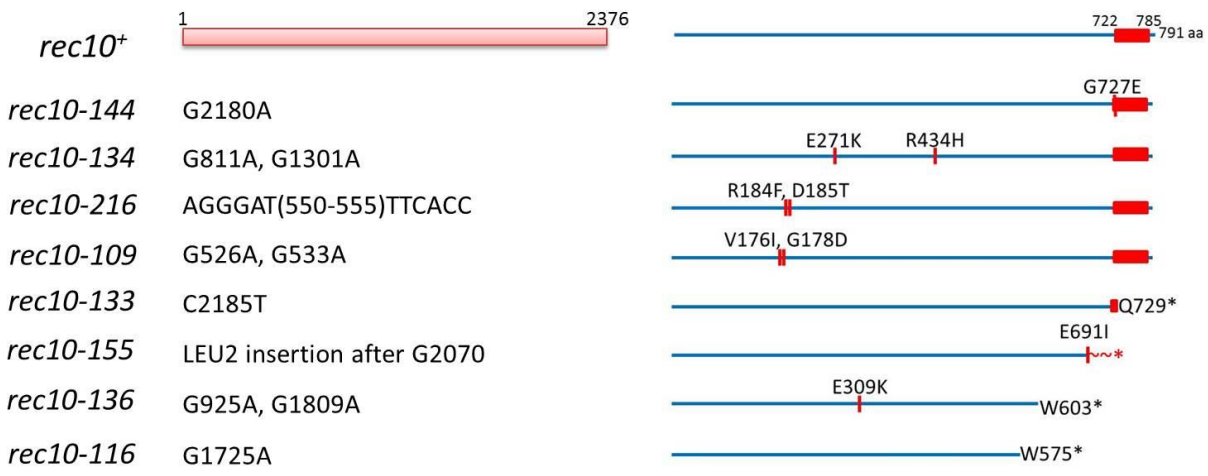


Supplementary Figure S4. Similarity between amino acid sequences of *S. pombe* Rec25, Rec27, Mug20 and Rec10 and proteins from other *Schizosaccharomyces* spp., *Caenorhabditis* spp. and *Saccharomyces cerevisiae.* The amino acid sequence alignment was performed in BioEdit using ClustalW [1]. Amino acids with similar physicochemical properties

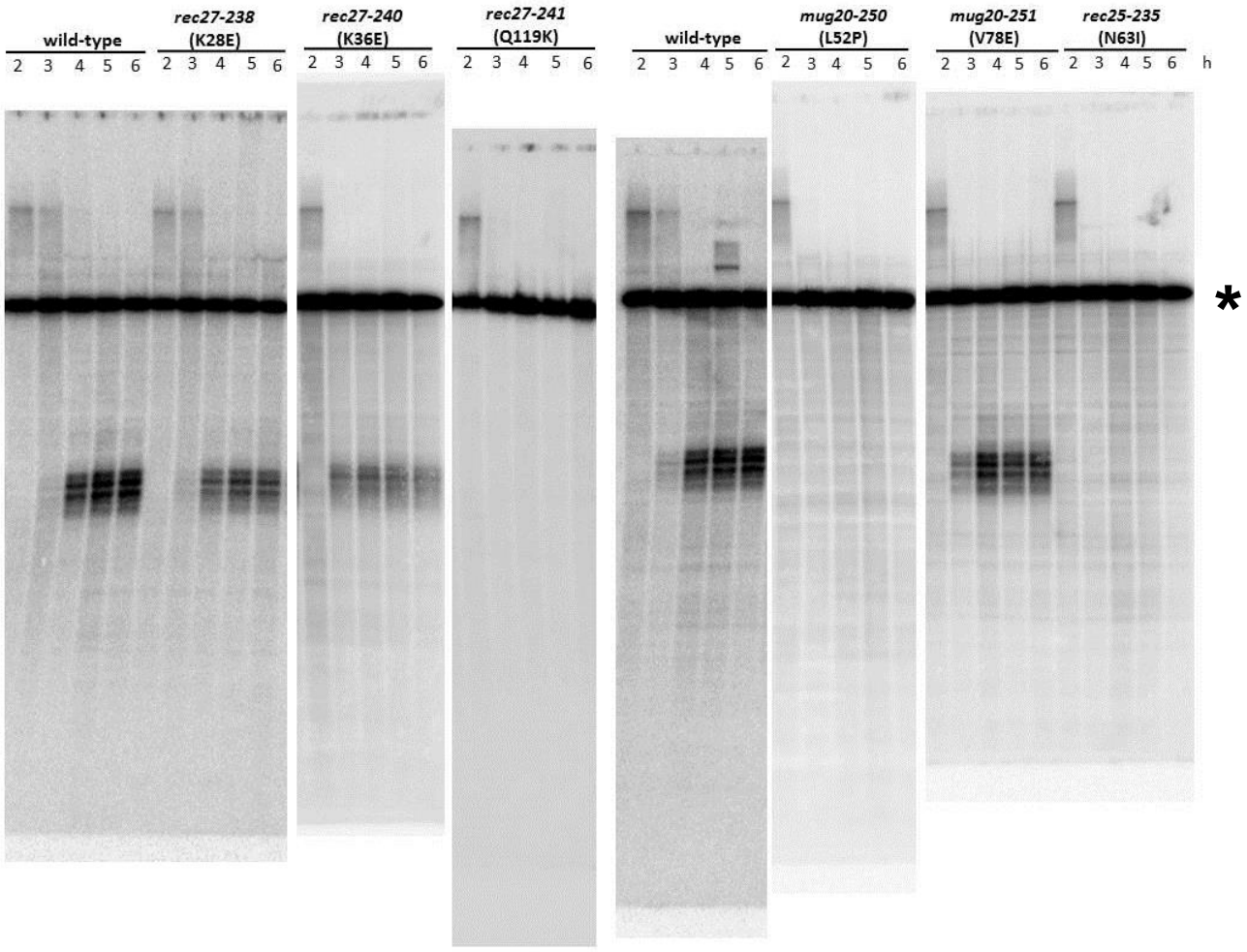
are shaded in red (BLOSUM62 similarity matrix with 66% threshold). Identical amino acids are shaded with blue. Gaps are indicated with hyphens. Positions (numbered as in *S. pombe*) of amino acids changed in the mutants studied here are indicated by vertical arrows, * Non-sense mutation, ~ frame-shift. *Schizosaccharomyces* spp. are abbreviated as follows: S.p, *S. pombe*; S.k, *S. kambucha*; S.c, *S. cryophilus*; S.o, *S. octosporus*; S.j, *S. japonicus*. *Schizosaccharomyces* species are shown in their order of evolutionary distance [2]. *Saccharomyces cerevisiae* is abbreviated as *S.cer*. *Caenorhabditis* spp. are abbreviated as follows: C.e, *C. elegans*; C.bre, *C. brenneri*; C.bri, *C. briggsae*; C.j, *C. japonica*. The region of *Saccharomyces cerevisiae* Red1 with amino acid similarity to Rec10 is underlined in panel D.



Supplementary Figure S5. Clustered *rec27* mutants with conserved positively charged arginine or lysine, changed to negatively charged glutamate, are recombination-deficient. Meiotic recombination between *ade6-M26* and *ade6-3049* was assayed in the indicated mutants. The indicated amino acids are conserved in *Schizosaccharomyces* spp. (*rec27-239* and *rec27-244*) and in *Caenorhabditis* spp. (*rec27-242*, *rec27-243* and *rec27-245*) (Figure S4B). Data (mean ± SEM; n=4 except for *rec27*$^{+}$ and *rec27Δ*, for which n=12) are from Suppl. Table S3; *rec27-238* and *rec27-240* data from Figure 2B are shown for comparison.



Supplementary Figure S6. Summary of *rec10* mutants. *rec10-216* was Isolated by random mutagenesis of two codons (R184 and D185). Other mutants were previously described [3-5]. * Non-sense mutation, ~ frame-shift. Red box indicates the region (722 – 785 aa) of similarity with *S. cerevisiae* Red1 [6]. Pink box is the exon.



Supplementary Figure S7. Meiotic DSBs of LinE mutants. Shown are full-length blots of the gels corresponding to the data in Figure 4. Note that at 2 and 3 h, replication intermediates (Y-shaped molecules, etc.) migrate more slowly than the linear 10.1 kb restriction fragment (*), whose position was determined relative to ethidium bromide-stained markers (not visible in these autoradioagraphs). In the wild-type 5 h lane on the right, low-level partial digestion products also migrate more slowly. The irregular bands near the tops of the gels are the wells into which the DNA was loaded.

Supplementary Table S1. *S. pombe* strains

Strain	Genotype	Used in
GP13	*h⁻ ade6-52*	pLM06, pLM08 and pLM09 construction
GP20	*h⁺ leu1-32*	Wild-type *rec27⁺* sequencing
GP1023	*h⁻ ade6-52 rec10-109 ura1-171*	Table 2
GP4414	*h⁺ ade6-M26 arg1-14 rec10-175::kanMX6*	Table 2
GP4915	*h⁻ ade6-M26 ura4-D18 arg1-14*	Construction of *rec25-256::ura4⁺*, *rec27-257::ura4⁺* and *mug20-258::ura4⁺*
GP5366	*h⁺ ade6-3049 pat1-114 rad50S*	Figures 4 and S7
GP5570	*h⁺ ade6-M26 lys3-37 met5-1*	Table 2
GP6294	*h⁻ ade6-52 ura1-171 rec25-180::kanMX6*	Table 2
GP6295	*h⁺ ade6-52 ura1-171 rec25-180::kanMX6*	Figure 3A, Table 1 and Table S3
GP6297	*h⁻ ade6-52 ura1-171*	Table 2
GP6323	*h⁺ ade6-M26 lys3-37 met5-1 rec25-180::kanMX6*	Table 2
GP6558	*h⁺ ade6-M26 lys3-37 met5-1 rec10-155*	Table 2
GP6912	*h⁺ ade6-M26 lys3-37 met5-1 rec10-109*	Table 2
GP6913	*h⁻ ade6-52 ura1-171 rec10-109*	Table 2
GP6914	*h⁺ ade6-M26 lys3-37 met5-1 rec10-116*	Table 2
GP6915	*h⁻ ade6-52 ura1-171 rec10-116*	Table 2
GP6916	*h⁻ ade6-52 ura1-171 rec10-155*	Table 2
GP6917	*h⁺ ade6-M26 lys3-37 met5-1 rec10-175::kanMX6*	Table 2
GP6918	*h⁻ ade6-52 ura1-171 rec10-175::kanMX6*	Table 2
GP6963	*h⁺ ade6-M26 lys3-37 met5-1 rec10-144*	Table 2
GP6964	*h⁻ ade6-52 ura1-171 rec10-144*	Table 2
GP6993	*h⁺ ade6-M26 rec10-175::kanMX6 ura4-D18 lys3-37 met5-1*	Construction of *rec10-216*
GP6994	*h⁻ ade6-52 ura4-D18 rec10-175::kanMX6*	Construction of *rec10-216 and rec10-260::ura4⁺*
GP6996	*h⁺ ade6-M26 lys3-37 met5-1 rec10-134*	Table 2
GP6997	*h⁻ ade6-52 ura1-171 rec10-134*	Table 2
GP7301	*h⁻ ade6-52 ura4-D18 rec10-260::ura4⁺*	Construction of *rec10-216*
GP7491	*h⁺ ade6-52 mug20::natMX6*	Figure 3C, Table 1 and Table S3
GP7714	*h⁻ ade6-52 ura4-D18 rec10-216*	Table 2
GP7747	*h⁻ ade6-M26 arg1-14*	Figures 2, 3 and S6, Table 1 and Table S3
GP8210	*h⁻ ade6-M26 ura4-D18 arg1-14 rec25-256::ura4⁺*	Figure 3A, Table 1 and Table S3
GP8211	*h⁻ ade6-M26 ura4-D18 arg1-14 mug20-258::ura4⁺*	Figure 3C, Table 1 and Table S3
GP8325	*h⁺ ade6-3049 rec25-180::kanMX6*	Figure 3A, Table 1 and Table S3
GP8365	*h⁺ ade6-52 ura4-D18 rec27::KanMX6 lys4-95*	Figure 3B, Table 1 and Table S3
GP8367	*h⁺/h⁻ ade6-3049/ade6-M26 ura4-D18/ura4-D18 rec27::kanMX6/rec27::kanMX6 lys4-95/+ +/his4-239*	Figure 1
GP8370	*h⁺/h⁻ ade6-3049/ade6-M26 ura4-D18/ura4-D18 rec25::kanMX6/rec25::kanMX6 lys4-95/+ +/his4-239*	Figure 1

GP8484	*h⁺/h⁻ ade6-3049/ade6-M26 ura4-D18/ura4-D18 mug20::natMX6/mug20::natMX6lys4-95/+ +/his4-239*	Figure 1
GP8513	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-257::ura4⁺*	Figures 2A, 3B, Table 1 and Table S3
GP8527	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-247*	Figure 2A, Table 1 and Table S3
GP8528	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-248*	Figure 2A, Table 1 and Table S3
GP8530	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-246*	Figure 2A, Table 1 and Table S3
GP8532	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-238*	Figure 3B, Table 1 and Table S3
GP8533	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-249*	Figure 2A, Table 1 and Table S3
GP8534	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-240*	Figure 3B, Table 1 and Table S3
GP8555	*h⁺ ade6-3049 ura4-D18 rec27::kanMX6 lys4-95*	Figures 2A, 3B and S6, Table 1 and Table S3
GP8587	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-243*	Figure S1, Table 1 and Table S3
GP8588	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-244*	Figure S1, Table 1 and Table S3
GP8589	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-245*	Figure S1, Table 1 and Table S3
GP8623	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-241*	Figure 3B, Table 1 and Table S3
GP8635	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-239*	Figure S1, Table 1 and Table S3
GP8636	*h⁻ ade6-M26 ura4-D18 arg1-14 rec27-242*	Figure S1, Table 1 and Table S3
GP8640	*h⁻ ade6-M26 ura4-D18 arg1-14 rec25-234*	Figure 3A, Table 1 and Table S3
GP8641	*h⁻ ade6-M26 ura4-D18 arg1-14 rec25-235*	Figure 3A, Table 1 and Table S3
GP8642	*h⁻ ade6-M26 ura4-D18 arg1-14 rec25-236*	Figure 3A, Table 1 and Table S3
GP8644	*h⁻ ade6-M26 ura4-D18 arg1-14 rec25-237*	Figure 3A, Table 1 and Table S3
GP8658	*h⁻ ade6-M26 ura4-D18 arg1-14 mug20-251*	Figure 3C, Table 1 and Table S3
GP8659	*h⁻ ade6-M26 ura4-D18 arg1-14 mug20-250*	Figure 3C, Table 1 and Table S3
GP8689	*h⁺ ade6-3049 mug20::natMX6*	Figure 3C, Table 1 and Table S3
GP8914	*h⁺ ade6-3049 pat1-114 rad50S rec27-241*	Figures 4 and S7
GP8970	*h⁺ ade6-3049 pat1-114 rad50S rec27-238*	Figures 4 and S7
GP8971	*h⁺ ade6-3049 pat1-114 rad50S rec27-239*	Figures 4 and S7
GP9024	*h⁺ ade6-3049 pat1-114 rad50S mug20-250*	Figures 4 and S7
GP9025	*h⁺ ade6-3049 pat1-114 rad50S mug20-251*	Figures 4 and S7
GP9026	*h⁺ ade6-3049 pat1-114 rad50S rec25-235*	Figures 4 and S7
GP9295	*h⁺ ade6-M26 rec25-180::kanMX6 rec10-144 lys3-37*	Table 2
GP9253	*h⁻ ade6-52 rec25-180::kanMX6 rec10-144 ura1-171*	Table 2
KF1	*h⁺ ade6-M26 lys3-37 met5-1 rec10-109 rec25-180::kanMX6*	Table 2
KF2	*h⁻ ade6-52 ura1-171 rec10-109 rec25-180::kanMX6*	Table 2
CMC78	*h⁻/h⁻ pat1-114/pat1-114 ade6-M210/ade6-M216 leu1-32/leu1-32 rec25-204::GFP-kanMX6/rec25-204::GFP-kanMX6*	Figure 5
CMC841	*h⁻/h⁻ pat1-114/pat1-114 ade6-52/ade6-M210 leu1-32/leu1-32 rec25-204::GFP-kanMX6/rec25-204::GFP-kanMX6 rec10-109/rec10-109*	Figure 5
CMC844	*h⁻/h⁻ pat1-114/pat1-114 ade6-52/ade6-M210 leu1-32/leu1-32 rec25-204::GFP-kanMX6/rec25-204::GFP-kanMX6 rec10-144/rec10-144*	Figure 5
CMC851	*h⁻/h⁻ pat1-114/pat1-114 ade6-52/ade6-M210 leu1-*	Figure 5

	32/leu1-32 rec25-204::GFP-kanMX6/rec25-204::GFP-kanMX6 rec10-216/rec10-216	

Alleles other than commonly used auxotrophies and mating type are described in the following references: *ade6-3049* [7], *ade6-M26* [8], *mug20::natMX6* [9], *pat1-114* [10], *rad50S* [11], *rec10-109* [4], *rec10-116* [3], *rec10-133* [3], *rec10-134* [3], *rec10-136* [3], *rec10-144* [12], *rec10-175::kanMX6* [13], *rec25-180::kanMX6* [14], *rec25-204::GFP-kanMX6* [5], and *rec27-184::kanMX6* [14]. *mug20-258::ura4*$^{+}$, *rec10-216*, *rec10-260::ura4*$^{+}$, *rec25-256::ura4*$^{+}$, *rec27-257::ura4*$^{+}$, and other *rec25*, *rec27* and *mug20* alleles were isolated in this study.

Supplementary Table S2. Oligonucleotides

Name	Sequence (5'-3')	Used for
OL326	AATTGTTGGCTTTGATGGAAG	*rec25-256::ura4*$^{+}$and *mug20-258::ura4*$^{+}$identification
OL1192	GCAAGAAATTGAACTGCCGAAAGTCGG	*rec10-216* sequencing
OL1778	GTCAAGCTCGAGGAAATACC	*rec10-216* and *rec10-260::ura4*$^{+}$identification
OL1779	TACCCCTGTCTTCTGCATTG	*rec10-216* and *rec10-260::ura4*$^{+}$identification
OL2502	CGAGTAATTGGTCAACGCTTGCTTCACNNNNNNTTCG CATGTCGCTTCTTAAGCGGTCTA	*rec10-216* construction
OL2503	TAGACCGCTTAAGAAGCGACATGCGAANNNNNNGTG AAGCAAGCGTTGACCAATTACTCG	*rec10-216* construction
OL2722	TAACGCTTCCAATGTGAATTGTTTTAAAGTAATATGTC AATATAAGCCAATATCAATAAATTTGATACTATAGGTT CAAGAGCTTGTGATATTGACGAAA	*rec10-260::ura4*$^{+}$ construction
OL2723	ATCAACTGAAACCGTTTTACGTATTTAATCCTATTTATT ATTCCAAAAAAAATCTATTAACACTTAAACGTAACTAA AATAGCTTAGCTACAAATCCCAC	*rec10-260::ura4*$^{+}$ construction
OL3248	GACTGAGGTACCAGAAGGCCACAAATGGTAAGA	pLM08 construction
OL3249	GACTGAGGTACCGAGGAATGCATTGCAGAAAGG	pLM08 construction
OL3250	GACTGACCCGGGTTGACAATGCTTGACTACGAA	pLM06 construction
OL3251	GACTGACCCGGGCCGCTAACCTTTGTGTTGTAC	pLM06 construction
OL3253	GACTGAGGCGCCAACCTCCCCTTGTCACTATTT	pLM09 construction
OL3254	GACTGAGGCGCCAATGATGTCTTCTCCGAAACC	pLM09 construction
OL3255	TACAGGTATCGCATTGTCAAG	*rec27* mutant library construction; *rec27* allele and *rec27-257::ura4*$^{+}$ identification
OL3256	TTTAGAAACGATTAGCCTCAC	*rec27* mutant library construction; *rec27* allele and *rec27-257::ura4*$^{+}$ identification
OL3257	ACCGCGAACATAGTCGGATTT	*rec25* mutant library construction; *rec25* allele identification
OL3258	AATGGGTTTAAAAGAATGCTG	*rec25* mutant library construction; *rec25* allele and *rec25-256::ura4*$^{+}$ identification
OL3259	GACGCGTAGACACTATTAACA	*mug20* mutant library construction; *mug20* allele identification
OL3260	CTTGGTTTGAGGCAGTATGAC	*mug20* mutant library construction; *mug20-*

		258::ura4^{+} identification
OL3266	AATGCGACCCTCATTTTAACTTATTAGTATTTGTAAAC GAATTTCAATCCAACCGCGAACATAGTCGGATTTAAAA ATGGATGCTAGAGTATTTCAAAGC	*rec25-256::ura4*$^{+}$ construction
OL3267	ATTAATAACATTTAGATGAAAAAGTAGTAGAGTTGGA ATAAATTTAGCTTTGAGTTTCAATCGTAATTTAGCTTAT TTAATGCTGAGAAAGTCTTTGCTG	*rec25-256::ura4*$^{+}$ construction
OL3268	AGTACGCTATATATCGCTAGCTTGATGTAAACAAAAG ACGCGTAGACACTATTAACATGTTATCAAGAACCACAA C ATGGATGCTAGAGTATTTCAAAGC	*mug20-258::ura4*$^{+}$ construction
OL3269	CGTGTTTGATGAAAGTTGACTTTCTTCGTTATTATCAA GCTTGGTTTGAGGCAGTATGACATTAGATCTCTGAAAT TTAATGCTGAGAAAGTCTTTGCTG	*mug20-258::ura4*$^{+}$ construction
OL3399	TCATACTTTTTGACGCGTTCTTCAATGTTTTTGAGGTTT AACTCTTCCAAAACA	*rec27-239* site-directed mutagenesis
OL3400	TGTTTTGGAAGAGTTAAACCTCAAAAACATTGAAGAA CGCGTCAAAAAGTATGA	*rec27-239* site-directed mutagenesis
OL3401	GGAAACATCCTTTATAAAAGCCTTTTCCTCATTTTCTAT ATTGGTCTTGTATT	*rec27-242* site-directed mutagenesis
OL3402	AATACAAGACCAATATAGAAAATGAGGAAAAGGCTTT TATAAAGGATGTTTCC	*rec27-242* site-directed mutagenesis
OL3403	ACCTGGGAAACATCCTCTATAAAAGCCTTTTTCTCATTT TCTATATTG	*rec27-243* site-directed mutagenesis
OL3404	CAATATAGAAAATGAGAAAAAGGCTTTTATAGAGGAT GTTTCCCAGGT	*rec27-243* site-directed mutagenesis
OL3405	CCGTTTTATTTTCGTACATTTCCTGTTGAACCTGGGAAA CATC	*rec27-244* site-directed mutagenesis
OL3406	GATGTTTCCCAGGTTCAACAGGAAATGTACGAAAATA AAACGG	*rec27-244* site-directed mutagenesis
OL3407	TTTTAACAACAATTCGCAGTTCCTCGGCATACTGTAAC AACTG	*rec27-245* site-directed mutagenesis
OL3408	CAGTTGTTACAGTATGCCGAGGAACTGCGAATTGTTG TTAAAA	*rec27-245* site-directed mutagenesis
OL3438	ATAGCCAGTGGGATTTGTAGCTAAGCTATGCTAAAAT ATACAACAGA	*rec27-257::ura4*$^{+}$ construction
OL3439	CAAAAAGTTTCGTCAATATCACAAGCTAGTCATTTTAA ACACATTAT	*rec27-257::ura4*$^{+}$ construction
OL3440	GAGATGAAAAGCTGCAGACT	*rec27-257::ura4*$^{+}$ construction
OL3441	GATCAATTCCAGAAGGCCAC	*rec27-257::ura4*$^{+}$ construction

OL3442	CAGACTTTCCTTCGCTCCAG	*rec27-257::ura4*$^{+}$ construction
OL3443	AGAAGGCCACAAATGGTAAGA	*rec27-257::ura4*$^{+}$ construction
OL3693	CAAAGTCCGACTCGTCTTCC	Probe amplification
OL3694	TAGAAGTGTAAGGCGCACGG	Probe amplification

Supplementary Table S3. Meiotic recombination of *rec25*, *rec27* and *mug20* mutants

Mutant	Gene conversion to *ade6*$^{+}$ [a]		Crossing-over (cM)[b] (*ade6 – arg1*)
	ade6-M26 × 3049	*ade6-M26 × 52*	
rec25^{+}	315 ± 20 (3)	18.7 ± 0.96 (4)	62 ± 6.8 (4)
rec25Δ	8.2 ± 1.3 (3)	0.34 ± 0.016 (4)	3.3 ± 0.4 (4)
rec25-234	7 ± 1 (4)	0.35 ± 0.027 (4)	2.2 ± 0.3 (4)
rec25-235	10.6 ± 1 (4)	0.4 ± 0.017 (4)	3.9 ± 0.8 (4)
rec25-236	8.9 ± 0.6 (4)	0.36 ± 0.023 (4)	3.3 ± 0.5 (4)
rec25-237	10.5 ± 0.2 (4)	0.39 ± 0.018 (4)	3.3 ± 0.7 (4)
rec27^{+}	293 ± 12 (12)	17.4 ± 0.8 (8)	55.8 ± 10.5 (4)
rec27Δ	12 ± 1 (12)	0.39 ± 0.02 (4)	2.9 ± 0.57 (4)
rec27-238	90 ± 3 (4)	10 ± 0.7 (5)	38.6 ± 3.8 (5)
rec27-239	39 ± 4 (4)	ND[c]	ND
rec27-240	42 ± 1.7 (4)	3.6 ± 0.2 (5)	20.8 ± 3 (5)
rec27-241	8.6 ± 0.3 (4)	0.33 ± 0.03 (4)	2.5 ± 0.68 (4)
rec27-242	273 ± 17 (4)	ND	ND
rec27-243	264 ± 11 (4)	ND	ND
rec27-244	260 ± 15 (4)	ND	ND
rec27-245	252 ± 8.4 (4)	ND	ND
rec27-246	11.5 ± 0.9 (4)	0.3	3
rec27-247	216 ± 6.6 (4)	12.58	55
rec27-248	183 ± 15 (4)	15.02	77
rec27-249	246 ± 6.4 (4)	16.24	51
mug20^{+}	290 ± 10 (12)	19.2 ± 1.16 (4)	58 ± 4.1 (4)
mug20Δ	10 ± 0.5 (8)	0.22 ± 0.02 (4)	3.8 ± 1.1 (4)
mug20-250	11 ± 1 (4)	0.17 ± 0.03 (4)	2.2 ± 0.7 (4)
mug20-251	130 ± 6.7 (4)	5.4 ± 0.35 (4)	38 ± 2.5 (4)

[a] Ade^{+} spores/10^{4} viable spores, as mean ± SEM from n crosses (in parentheses).

[b] 140 spore colonies from each of the four or five (as shown in parentheses) crosses were tested for *ade6 – arg1* recombinants. Frequencies were converted to cM using Haldane's equation, as mean ± SEM.

[c] ND, not determined.

References

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