Supplemental Table 1. *S. pombe* strains used in this work. The gene deletion and *mre11* point mutation strains are derivates of KRP3-4. We note that *S. pombe mre11*$^{+}$ and *rad52*$^{+}$ were previously named *rad32*$^{+}$ and *rad22*$^{+}$. *Hermes* insertion sites are noted by the chromosome number and base pair in the *S. pombe* genome sequence (Materials and Methods). Mutants bearing *Hermes* and *kanMX*-tagged mutations were constructed by mating and tetrad dissection as both markers confer G418-resistance.

Strain	Genotype	Source
Wild type and transposon insertion strains		
KRP1	*h- ade6-M216 leu1-32 ura4-D18 his7-366*	This study
KRP3-4	*h- ade6-M216 leu1-32 ura4-D18 his7-366 chr I 1,279,108::Hermes*	This study
KRP3-3	*h- ade6-M216 leu1-32 ura4-D18 his7-366 chr I 5,443,647::Hermes*	This study
KRP201	*h+ ade6-M216 leu1-32 ura4-D18*	This study
P9G2	*h+ ade6-M216 leu1-32 ura4-D18 chr I 633,603::Hermes*	This study
P10G2	*h+ ade6-M216 leu1-32 ura4-D18 chr II 3,487,136::Hermes*	This study
P12G2	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,057,536::Hermes*	This study
P13B2	*h+ ade6-M216 leu1-32 ura4-D18 chr I 2,684,662::Hermes*	This study
P15B11	*h+ ade6-M216 leu1-32 ura4-D18 chr II 147,681::Hermes*	This study
P18G2	*h+ ade6-M216 leu1-32 ura4-D18 chr II 532,055::Hermes*	This study
P24B11	*h+ ade6-M216 leu1-32 ura4-D18 chr II 4,032,934:Hermes*	This study
P22G2	*h+ ade6-M216 leu1-32 ura4-D18 chr II 1,688,066::Hermes*	This study
Strains used to construct mutants		
TN1257	*h+ ade6-M216 leu1-32 ura4-D18 his3-D1 pku70Δ::KanMX*	S Sanders
TN2099	*h+ ade6-M210 leu1-32 ura4-D18 his3-D1 lig4Δ::KanMX*	S Sanders
NR2840	*h+ ade6-M216 leu1-32 ura4-D18 his3-D1 rad50Δ::KanMX*	T Nakamura
TN2389	*h- ade6-M216 leu1-32 ura4-D18 his3-D1 nbs1Δ::KanMX*	T Nakamura
JW4166	*h+ leu1-32 ura4-D18 mre11-13myc:kanMX6*	P Russell
JW4167	*h+ leu1-32 ura4-D18 mre11-L77K-13myc:kanMX6*	P Russell
JW4169	*h+ leu1-32 ura4-D18 mre11-L77K L154D-13myc:kanMX6*	P Russell
JW4170	*h+ leu1-32 ura4-D18 mre11-H68S-13myc:kanMX6*	P Russell
JW4171	*h+ leu1-32 ura4-D18 mre11-H134S-13myc:kanMX6*	P Russell
P34G06	*h+ ade6-M216 leu1-32 ura4-D18 mre11Δ::KanMX4*	Bioneer Version 2
P20C05	*h+ ade6-M216 leu1-32 ura4-D18 ctp1Δ::KanMX4*	Bioneer Version 2
Deletion strains used in Figures 4, 5, S2 and S5		
KRP180	*h- ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes*	This study
KRP175	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes*	This study
KRP176	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes ctp1Δ::KanMX4*	This study
KRP177	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes*	This study

	mre11Δ::KanMX4	
KRP178	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes rad50Δ::KanMX4*	This study
KRP179	*h- ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes nbs1Δ::KanMX4*	This study
KRP181	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes pku70Δ::KanMX4*	This study
KRP183	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes lig4Δ::KanMX4*	This study
KRP184	*h+ ade6-M216 leu1-32 ura4-D18 chr I 1,279,108::Hermes mre11Δ::KanMX4 lig4Δ::KanMX4*	This study
KRP332	*KRP3-4 made ura4$^+$ by transformation (a rad52$^+$ control for KRP341)*	This study
KRP341	*KRP3-4 rad52Δ::ura4$^+$*	This study
mre11 ***mutant strains used in Figures 6, 7, 8 and S7***		
KRP240	*h- ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-13myc:kanMX6*	This study
KRP241	*h- ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-L77K-13myc:kanMX6*	This study
KRP243	*h- ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-L77K L154D-13myc:kanMX6*	This study
KRP245	*h- ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-H134S-13myc:kanMX6*	This study
KRP275	*h- ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-H134S-13myc:kanMX6 ctp1Δ::KanMX4*	This study
KRP263	*h- ade6-M216 ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-13myc:kanMX6*	This study
KRP258	*h- ade6-M216 ura4-D18 leu1-32 chr I 1,279,108::Hermes mre11-H68S-13myc:kanMX6*	This study
KRP333	KRP240 but *ura4$^+$* (a *pso2$^+$* control for KRP330)	This study
KRP330	KRP240 *pso2Δ::ura4$^+$*	This study
KRP331	KRP275 but *ura4$^+$* (a *pso2$^+$* control for KRP334)	This study
KRP334	KRP275 *pso2Δ::ura4$^+$*	This study

Supplemental Table 2. Oligonucleotides used in this work. Gene deletions were constructed by transforming cells with either a PCR product that completely replaces the coding sequences with the *kanMX* gene encoding resistance to G418 (Longtine *et al.* 1998), or by amplifying a validated deletion from purchased deletion library (Kim *et al.* 2010). Overlap PCR products were constructed with ~300 bp of 5' flanking sequence – *kanMX* – 300 bp of 3' flanking sequence and the mutants were verified by using conformation primers in the genome outside of the PCR product and primers that hybridize to the kanMX gene (CPC1-KANC and CPN1-KANB). Conversion of the *ura4-D18* allele was performed by transforming cells with the PCR product amplified from a *ura4*$^{+}$ strain with the ura4-520_S and ura4-250_AS primers. Genbank formatted files of the final deletions with the positions of the primers indicated are available upon request.

Name	Sequence
Primers used to transfer alleles from existing strains	
ura4-250_AS	GAT TCC TCG TAA CAT TGC CAG
ura4-520_S	CGT AAT GAC AAA ACA GCT TGT ATA G
pku70_S	TGC AAC TTA CGA CGG CTT TA
pku70_AS	TGA AGG TTG AAT TGT TTG TCG
lig4_AS	CTT TCA AAG GCA GAC GCT TT
lig4_S	CTT TAA AGCA GGC GAT GAG G
rad32_cp5	GAT CCC TTC TTG GTT CAA AGA AGG A
rad32_cp3	CAA ACT TGC AAA ACT TGA TCT CGT C
ctp1_cp5	TTT ACA AAT GAC GAG TGC TGA CAA A
ctp1_cp3	ATT TCT TGA GCC TAA CTC GAC TTT C
Primers used to create 5' flanking sequence – kanMX – 3' flanking sequence deletion constructs	
rad50_5_S	GTT AAA CCA GTG CCT CCT TTC
kanMX-rad50_AS	CCA GTG TCG AAA ACG AGC TCC ATT TTA AAT TAC TTA AAC TGA ACA CAC TTT C
rad50_3_AS	AGA TCG GAC CTT GCA TTT CTC
kanMX-rad50_S	GAG GCA AGC TAA ACA GAT CTA ATT AAT GAT GAA TAT ATT CGA CCT TAC
kanMX_AS	GAG CTC GTT TTC GAC ACT GG
kanMX_S	AGA TCT GTT TAG CTT GCC TC
pso2-u4_AS	CCA ATC CAC ATT TTC CAA TGA AAG GCG AAA CTT TTT GAC ATC TAA TTT ATT CTG
pso2-u4_S	TTT GCC TTT GTT TAC GTT CAC TGA ATT AAA AAA GTT TGT ATA GAT TAT TTA ATC TAC
psoZ_AS	ACG AAG CTC AAA GAA AGT TTA AAA G

psoY_S (used with pso2W_AS)	CAT TTT AGA CCA GAT CCT GAC C
pso2X_S (used with pso2Z_AS)	CTT TCA TTG GAA AAT GTG GAT TGG
pso2W_AS	TTC AGT GAA CGT AAA CAA AGG CA

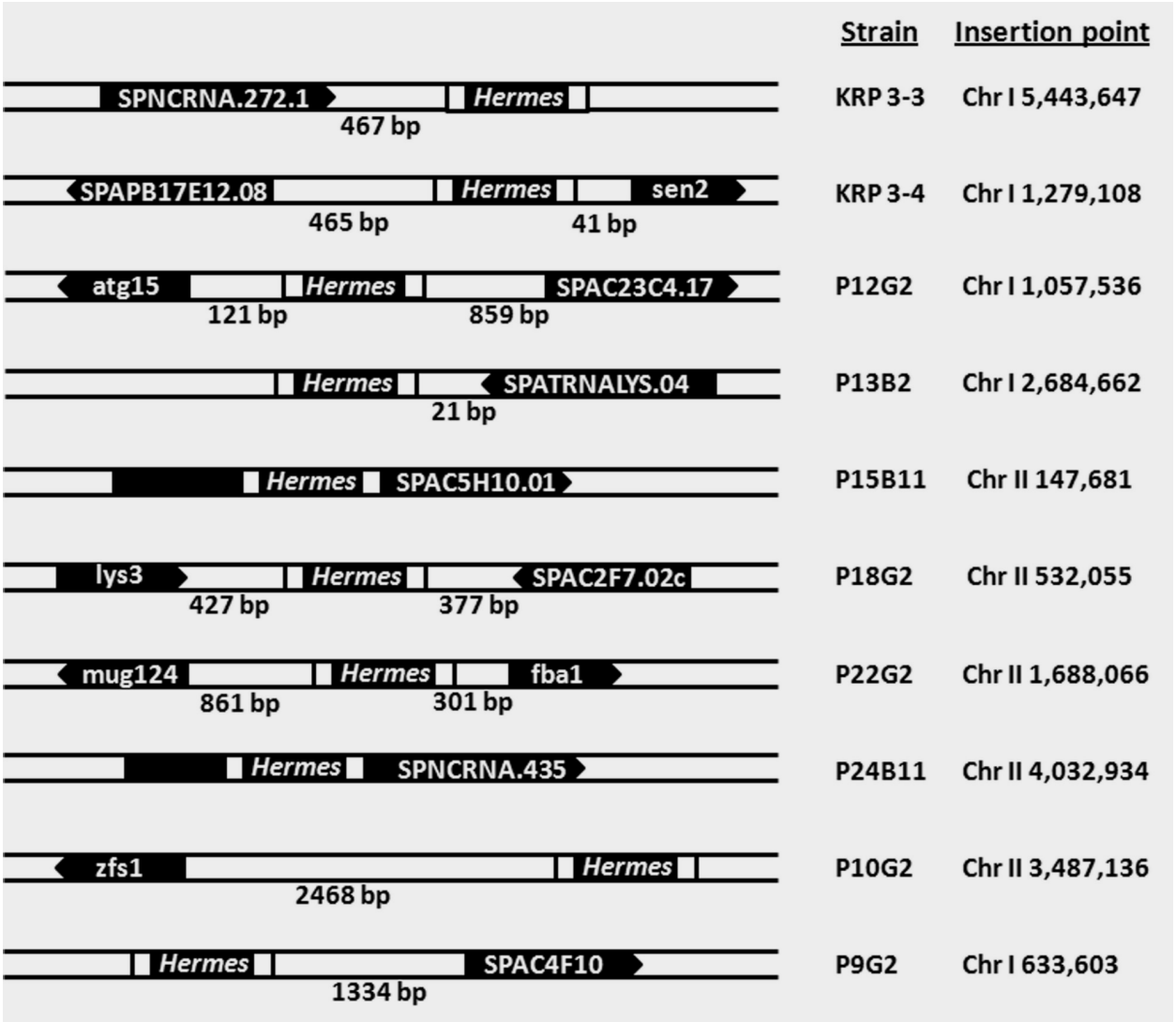
Primers to confirm deletions or transfer of markers by transformation

CPC1-KANC	GGC TGG CCT GTT GAA CAA GTC TGG A
CPN1-KANB	CTG CAG CGA GGA GCC GTA AT
pku70_conf_AS	TGT AGG TGT CCG ATA CCT TC
pku70_conf_S	CCA AAC TGC GTA GCC GAC AC
lig4_Conf_AS	TAG TCT GAC TTG CAG CGC TC
lig4_Conf_S	CTT TCT TTG AGC AGG AGT AAG
lig4_ConfS #1	CTT TCT TTG AGC AGG AGT AAG
lig4_ConfAS#1	TAG TCT GAC TTG CAG CGC TC
rad32_conf_S	TAC ACG GCT TTA AGG TCC TC
rad32_conf_AS	AAA CAT GAT TTG GCA AGA CTC AC
nbs1_conf2_AS	TGC TGA CTC AGG AGG ATT GG
nbs1_conf2_S	CCA GTA GTG CAT ATA CAC GAC
ctp1_conf_AS	AAG GGA CGT GGT CAA ATT TCC
ctp1_conf_S	ATT AGG ATG GTA GTC GCG TC
pso2_conf_AS	CTA AAT GAA CTA ATG TTA AGT ATT C
pso2_conf_S	ACC CTT CCT TAG GTA TAC TTC
ura4g_confAS	GTT ATG TTG CCA AAC ATC CTC C
u4conf_AS	TTT AGA GAA AGA ATG CTG AGT AG
rad52_confS	GCG AAG ATA TGT TTC GCA GTC
rad52_confAS	CTG ATA CAG TAG CGA ATT CAC ATC

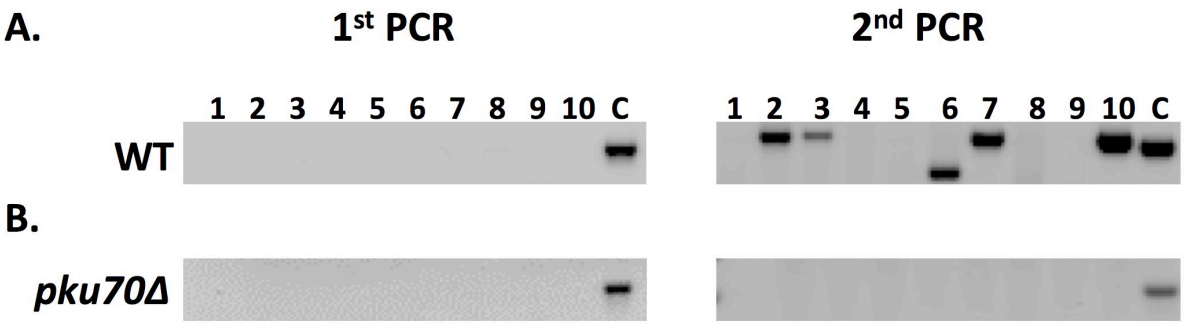
Other primers

Hermes3-4_rightProbe_S	GGC TGA TCC ATT ACT CAA TTG G
Hermes3-4_rightProbe_AS	CTT ACG CAG ACA CTA AAA AAG AAT CC
rad52_5_S	GGG ATT AAT TGA TTA GTA ATT ACG AAA ATC C
ura4-rad52_5_AS	GTA GAT TAA ATA ATC TAT ACA AAC TTT TTT AAG ACA TCC CTT CTA GCT TAT ATG AAG
rad52_3_AS	CCC AAC TAG TGG TAT GAA AGG
ura4-rad52_3_S	CAG AAT AAA TTA GAT GTC AAA AAG TTT CGG GAT AAA AGT AAT GAG GCA AAA TGT GAT G
3-4_S	TCC CGG TAT GTG TAA ATC AAA
3-4_AS	TCG GCT TGC CAT TTT CTA AT
3-4_2S	CCC GGT ATG TGT AAA TCA AAG AAA GC
3-4_2AS	AAT TGC CTC TCA CGA GCA CGA T
3-3_S	CAT GCA ATA ACC CAT TGA AAA A
3-3_AS	GGG GTA TGG AGT GAG AAG GTT3’
M13 forward	TGT AAA ACG ACG GCC AGT
M13 reverse	CAG GAA ACA GCT ATG AC
pHL2577-3633S	CAC AAC TTA ACA ACA ACA GTT GTT TG
pHL2577-3527AS	TTG TGC TTA TCT ATG TGG CTT AC

Hermes_Sau3A I	CAC AAC TTA ACA ACA ACA GTT GTT TG

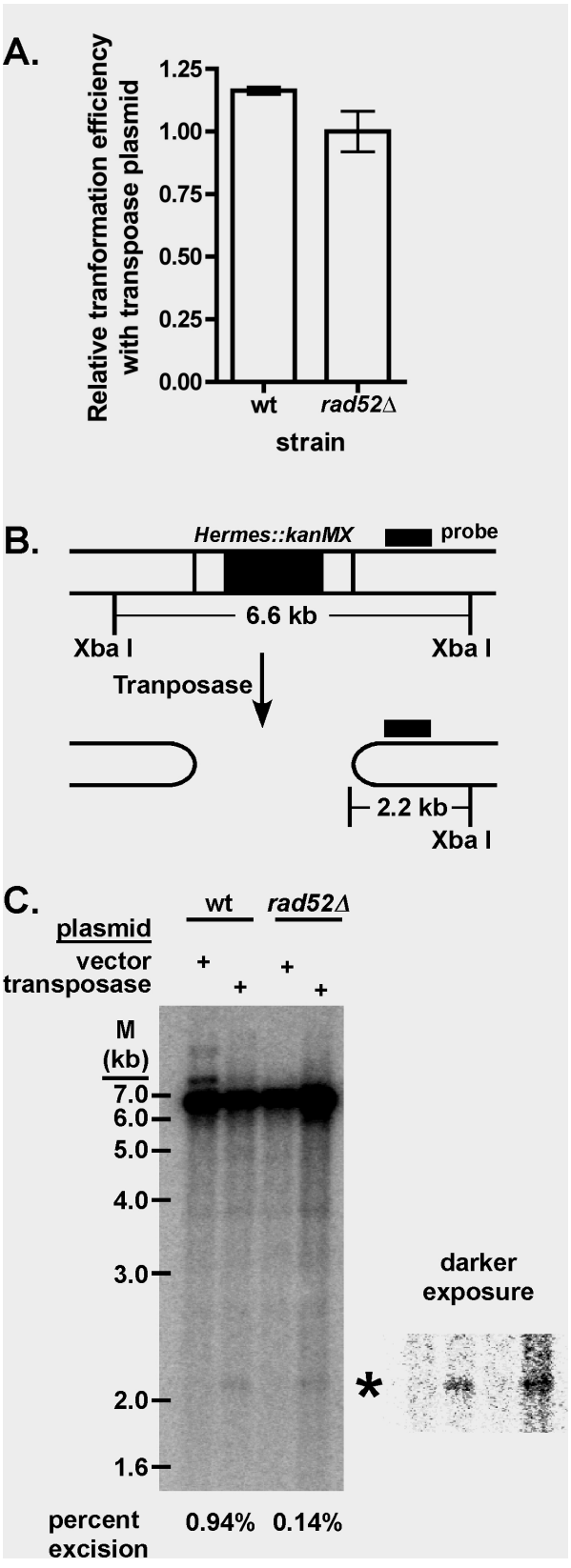


Supplemental Fig. S1. Most *Hermes* transposon insertions from the transient transfection protocol are single events. Transposase producing cells were transformed with the transposon plasmid (as in Fig. 1) and the site of *Hermes* genomic integration was determined by inverse PCR. The transposon is designated as a black box labeled "*Hermes*" bordered by two white boxes. The results from two independent transformations are shown. The top two integrants (KRP3-3 and KRP3-4) are in the strain KRP1 while the remaining integrants are in KRP201. The distance from intergenic *Hermes* insertions to the ATG or stop codon of the nearest ORF is shown. Of the 12 randomly chosen colonies, 10 contained single insertions (above), and two had no insertion but retained the transposon plasmid (pHL2577, Fig. 1).



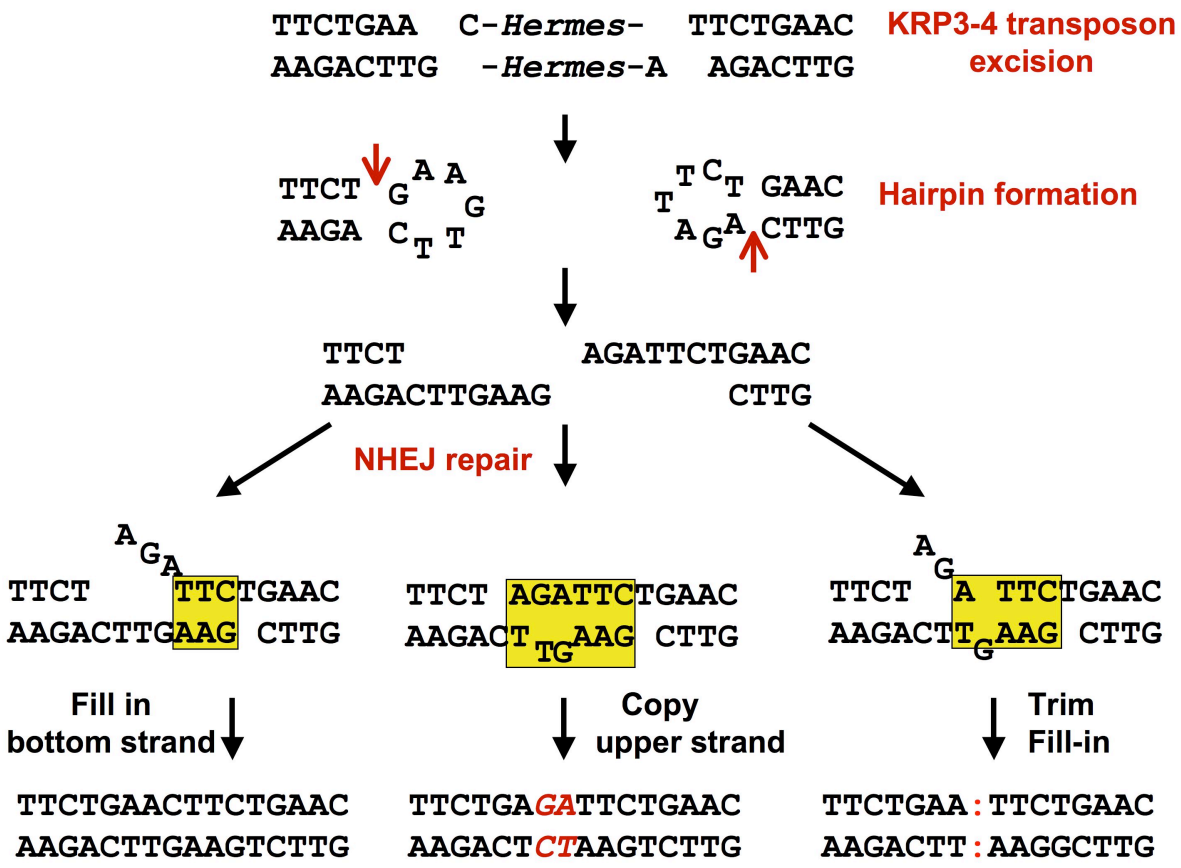
Supplemental Fig. S2. Efficient *Hermes* transposon excision requires transposase expression. A. KRP 3-4 cells lacking the transposase expression plasmid were tested in the transposon excision assay. No products were obtained in the first round PCR, in contrast to cells expressing transposase (Fig. 2B). The low level of correct second round PCR products shows that excision events are rare, and yields a median excision frequency of less than 10^{-7} per cell or 1000-fold less than the excision frequency in transposase-expressing cells (Fig. 5). Thus, transposase is required to produce the excision products shown in Fig. 3A.

B. The assay in part A was performed in cells bearing the *pku70*Δ mutation and lacking the transposase expression plasmid. The results indicate that transposase is required to generate the aberrant excision events in *pku70*Δ mutant cells (Fig. 4).

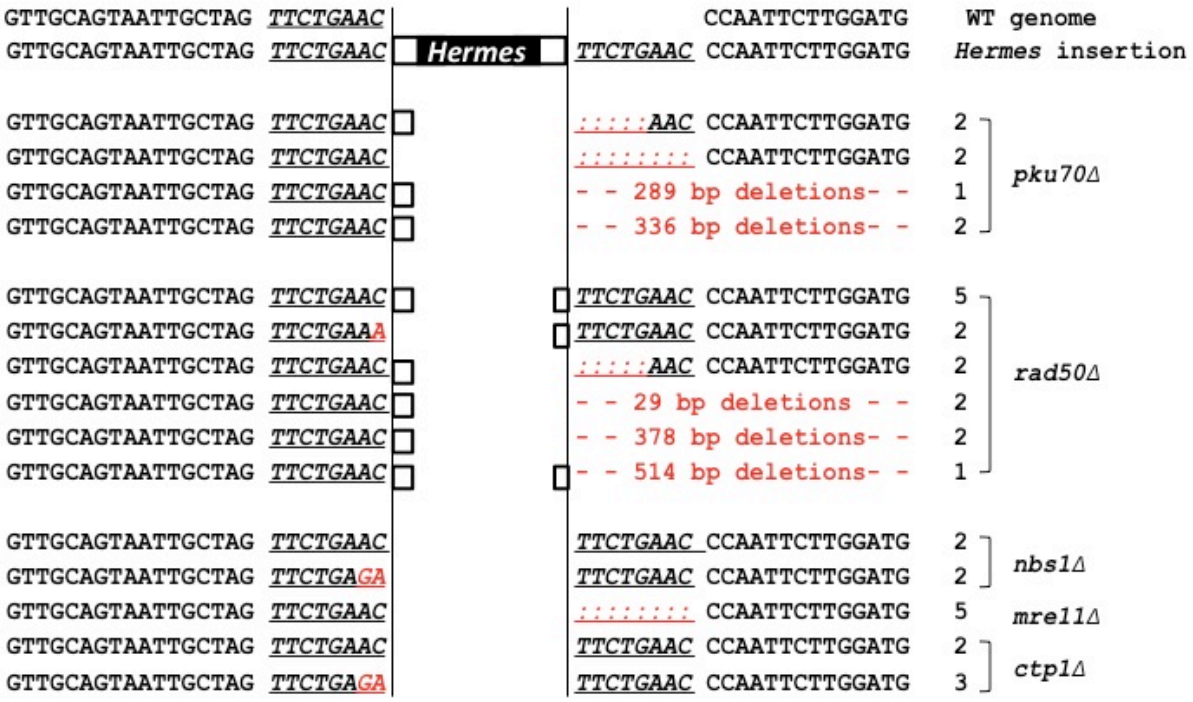


Supplemental Fig. S3. Estimation of *Hermes* excision frequency by quantitative transformation

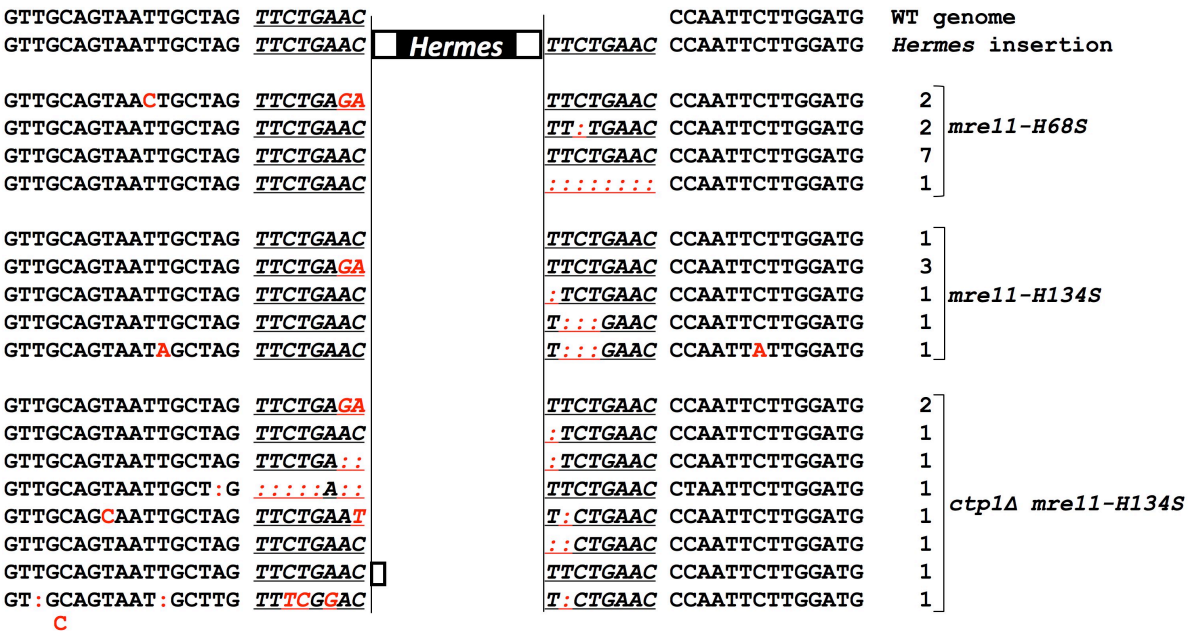
and Southern blotting. A. Wild type and *rad52*Δ cells bearing the *Hermes* insertion (KRP332 and KRP341, Supplemental Table 1) were grown to exponential phase and each strain was transformed with equal molar amounts of either vector (pREP81) or transposase plasmid (pHL2578), and aliquots were plated onto selective medium. The numbers of transposase plasmid transformants normalized to the average number of vector transformants are plotted. While *rad52*Δ strain had a lower efficiency of transformation compared to wild type cells (1.00 versus 1.16), the two values were not significantly different by t-test ($P > 0.11$). Under the hypothesis that excision in a *rad52*Δ strain is lethal, these data indicate that the rate of excision is a few percent or less. B and C. Single wild type and *rad52*Δ transformants from A bearing either the vector (pREP81) or the transposase expression plasmid (pHL2578, (Evertts *et al.* 2007)) were grown in selective, transposase-inducing medium (EMM + ahu + 2% glucose) for ~10 population doublings and genomic DNA was prepared from each culture. DNA (2 µg) was digested with *Xba I* and analyzed by Southern blotting using the probe shown in B. Excision frequency was determined by the ratio of intensities of the excised band (at 2.2 kb, marked by *) to the unexcised band (at 6.6 kb), and is shown at the bottom of the gel in C.



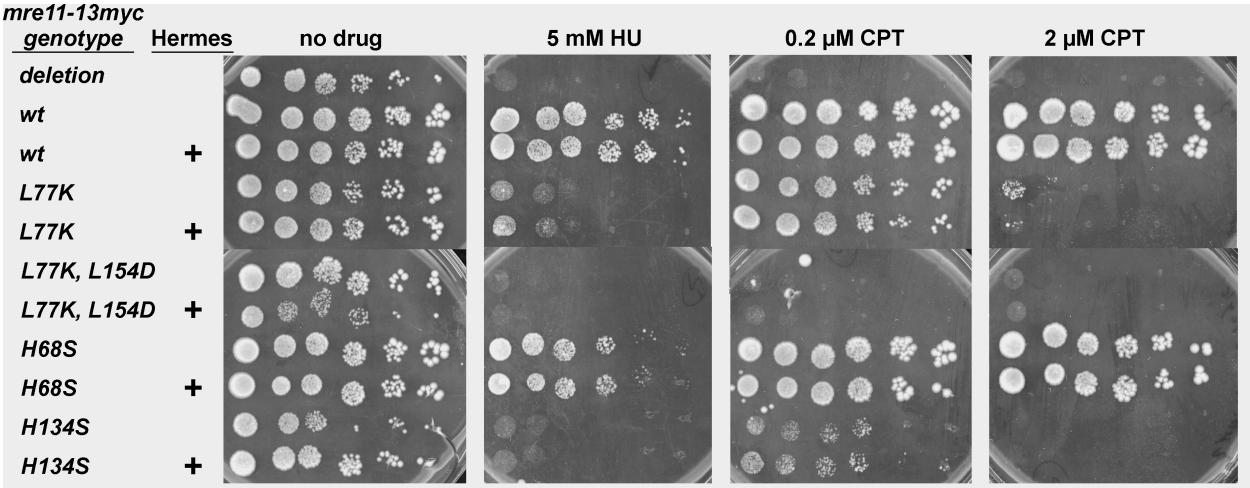
Supplemental Fig. S4. A role for the MRN complex in *S. pombe* NHEJ is consistent with frequently observed repair products. *Hermes* excision produces hairpin ends (Zhou *et al.* 2004) that are cleaved (e.g. at the red arrows), and the structure of Mre11 indicates that it can mediate base pairing of the 5' overhangs (Williams *et al.* 2008). Removal of unpaired bases and/or strand extension similar to the normal processes associated with NHEJ (Daley *et al.* 2005; Mcvey and Lee 2008) could account for 3 of the most frequently observed KRP 3-4 excision footprints (bracketed in Fig. 3A).



Supplemental Fig. S5. *Hermes* excision footprints from different NHEJ mutants. Products from the second round of PCR of mutant cells (Fig. 4) were cloned and sequenced. A red colon signifies a base deletion while the hollow box signifies a portion of the *Hermes* transposon. Sequencing revealed that some mutants (*mre11*Δ, *nbs1*Δ, *ctp1*Δ) gave footprints similar to wild type cells. Other mutants (*pku70*Δ, *rad50*Δ) retained a portion of the *Hermes* transposon and showed larger deletions. These results suggest that an inefficient repair process can heal the hairpin capped DSBs at very low frequencies in the absence of NHEJ.



Supplemental Fig. S6. Sequences of Mre11 nuclease mutant excision events. *Hermes* excision events from sequencing the first round PCR products (Figs. 6, 7) show transposon footprints similar to wild type cells. The 8 bp direct repeats are underlined, and some PCR products contain mutations outside of these repeats. Base changes are shown in red, deletions are indicated by a colon and portions of the *Hermes* transposon are shown by a hollow box. The bottom sequence from *ctp1*Δ *mre11-H134S* cells also contains a "C" insertion that is shown below the sequence.



Supplemental Fig. S7. The *Hermes* insertion does not change the sensitivity of *mre11* mutants to hydroxyurea (HU) or camptothecin (CPT). Cells bearing a deletion of *mre11*$^{+}$ or different alleles in the *mre11*$^{+}$*-13myc* background were tested for drug sensitivity by spotting 5 µl of 5-fold serial dilutions onto YES plates bearing no drug or different concentrations of HU or CPT. The presence of *Hermes* (indicated by a "+") did not cause a significant change in growth on plates bearing the DNA damaging agents. The levels of sensitivity shown here parallel the results originally published for these mutants (Williams *et al.* 2008).

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