



Figure S1: *SMT3* KO heterokaryons undergo normal DNA elimination events during conjugation. Wild type (wt) and *SMT3* KO heterokaryons (*SMT3*het) were mixed in three matings: wt × wt, wt × *SMT3*het, *SMT3*het × *SMT3*het. Cell lysates for PCR were prepared at mixing (t0). At 2 hours, >500 mated pairs were hand isolated into drops for each mating. After 24 hours (t24) when conjugation was complete the progeny were lysed and used as template in PCR reactions. The assay utilizes oligonucleotide primers that are able to amplify the unrearranged and rearranged forms of two IES elements (M and R). Comparison of PCR-amplified products on ethidium-bromide stained agarose gels, allowed visualization of the IES excision products. Only the longer 593 bp unrearranged form of the M element was found in the cells at t0, but the shorter 279 bp rearranged M element was the major product from progeny cells at t24 hours. This confirms that normal M element rearrangement occurred in progeny of *SMT3* KO heterokaryons. There is only one R element product in the MAC but the amplified products are the same at t0 and t24 as expected in for normal excision. Schematic of IES products following arrangement are shown below.

Table S1. List of oligos used in this study.

Purpose	Name	Sequence (5'>3')
GFP constructs	bzSUMO+1F	CACCAATAAAATGACTGATTAAAACGCTAACGCT
	bzSUMO+930R	GATATCGAAAGAGCCACCAACTTGTTCT
	bzUBA2+1F	CACCAAGGATATGAGTTTAGGAAGAATAAATC
	bzUBA2+2262R	GATATCCACTTTTAACTTTTGTGTCT
Knockout cassette	SMT3 5'flank F	GTCACTCGAGGCAGTTTGTCTTTTATCCATTT
	SMT3 5'flank R	GCCGAGATCTCTTCAAATATTTATTGTTTCGAC
	SMT3 3'flank F	ATCGGGATCCCTTCAAAATTTAGTTGATTGTGATAACA
	SMT3 3'flank R	ATTAGCGGCCGCATCTCAAATAAGTCTAAAT
	UBA2 5'flank F	ATCGCTCGAGAGTACTCGACGGATCTCATAAA
	UBA2 5'flank R	CGGCGGATCCACTCATATCCTTATCAATTAAA
	UBA2 3'flank F	ACGTGGATCCAAAGCTGTAGATTTTAGTTAAA
	UBA2 3'flank R	AACAGCGGCCGCTAGCTTATTAATTCTTCTA
PCR to confirm knockout lines	SMT3WT upstream F	GATTGTTTGATGCTACATTCCTTC
	SMT3WT upstream R	TGCTAAAGACGGTTGGCTCT
	UBA2WT upstream F	TTTGCTTGTTGTTTGGTTTGT
	UBA2WT upstream R	CCAATGCCTCCTACACCAAT
	MTTp 1940R	TTTGCTAACCATAGCCAAAAT
RT-PCR assay of conditional line	SMT3WT 3'UTR F	ACTGATTAAAACGCTAACGCT
	SMT3WT 3'UTR R	ATTAGTTTATAAGCAAGCACATAC
	BTU1 3'UTR R	GTTGGTTTAGCTGACCGATTCT
	NEO3 3' UTR F	ACCGCTATCAGGACATA
	NEO3 3' UTR R	GCAGACAAATTTTAAAGAGC