

**Table S1 Primer sequences used for PCR amplification and genomic sequencing**

Primer Name	Sequence (5' to 3')
Top2F + 1031 <sup>a</sup>	TAACTGGGTGGGTACTGTAGTCTTCTG
Top2R + 1703	AACGGTGAAGCTGGTGGAGAATATG
Top2F + 1545	TGACCATGCACAAGGAGCAGAAG
Top2R + 2356	ACCTGGAATGGCTTGATGTTGATGC
Top2F + 2145	TCCAACAGGTCTCGTTTGTCAACTC
Top2R + 2924	GCCATCCTGATCCTGATCTGTC
Top2F + 2773	TAATTTCAAGCAGCTTTCGGAGAATG
Top2R + 3483	AAGTGAACATCACCTTCCGCTGAC
Top2F + 3362	ATCACCTATGCAGACTTTATCAATCTGG
Top2R + 4061	TTCACTGATTTCTAAACGGTTTCCG
Top2F + 3959	TACAAGAACTTTTTAGGACGCATGG
Top2R + 4657	TTCTTAGCCTTTGAGCTGACACTGG
Top2F + 4534	ATATCGTCCCGATCCCGTCAAG
Top2R + 5222	ATCAAATTCGTCCACCTCATCG
Top2F + 5035	ACCCGTCGAGTTTAAGATCACCGAAG
Top2R + 5379	CTGAAACACTTACCTTGGCCTTGCTG
Top2F + 5157	AACCGAAGGGCAAGCAGATTAAGC
Top2R + 6249	CCTTTATTTCCCACTTCCGATTTGTG

<sup>a</sup> Primers are relative to the transcription start site (+1).

**Table S2** Complementation tests to define *Top2* alleles  $y^1w^{67c23}$ ; *Top2<sup>m</sup>/CyO*,  $y^+ \times y^1w^{67c23}$ ; *Top2<sup>Df9043</sup>/CyO*,  $y^+$

Allele Name	18°C	25°C	25°C
	Maternally transmitted allele	Maternally transmitted allele	Paternally transmitted allele
	% viability <sup>a</sup>		
<i>Df17</i>	ND	0 <sup>a</sup> (604) <sup>b</sup>	0 (196)
<i>Df35</i>	ND	0 (424)	0 (695)
<b>17-1</b>	<b>1 (710)</b> <sup>c</sup>	<b>0 (359)</b>	<b>0 (412)</b>
<b>17-2</b>	<b>29 (906)</b> <sup>c, d</sup>	<b>0 (193)</b>	<b>0 (170)</b>
<b>17-3</b>	<b>0 (737)</b>	<b>0 (263)</b>	<b>0 (189)</b>
<i>17-4</i>	ND	137 (249)	110 (122)
<b>17-5</b>	<b>0 (895)</b>	<b>0 (430)</b>	<b>0 (287)</b>
<b>17-6</b>	<b>0 (939)</b>	<b>0 (207)</b>	<b>0 (295)</b>
<b>17-7</b>	<b>0 (467)</b>	<b>0 (211)</b>	<b>0 (389)</b>
<b>35-1</b>	<b>0 (535)</b>	<b>0 (300)</b>	<b>0 (99)</b>
<b>35-2</b>	<b>0 (874)</b>	<b>0 (289)</b>	<b>0 (104)</b>
<b>35-3</b>	<b>0 (898)</b>	<b>0 (283)</b>	<b>0 (102)</b>
<i>35-4</i>	ND	25 (438)	87 (453)
<b>35-5</b>	<b>0 (803)</b>	<b>0 (423)</b>	<b>0 (815)</b>
<b>35-6</b>	<b>0 (631)</b>	<b>0 (120)</b>	<b>0 (160)</b>
<i>35-7</i>	ND	127 (229)	159 (208)
<i>35-8</i>	ND	118 (320)	113 (238)
<i>35-9</i>	ND	205 (112)	124 (302)
<b>35-12</b>	<b>0 (703)</b>	<b>0 (69)</b>	<b>0 (207)</b>
<b>35-13</b>	<b>0 (611)</b>	<b>0 (115)</b>	<b>0 (218)</b>
<b>35-14</b>	<b>0 (1065)</b>	<b>0 (226)</b>	<b>0 (162)</b>

<sup>a</sup> Percent viability is the # of  $Cy^+$  flies divided by half the # of  $Cy^-$  flies multiplied by 100.

<sup>b</sup> Total # of  $Cy^-$  flies scored.

<sup>c</sup> *Top2<sup>m</sup>/Top2<sup>Df9043</sup>* flies become stuck in food.

<sup>d</sup> *Top2<sup>17-2</sup>/Top2<sup>Df9043</sup>* males are sterile; females immediately become stuck in food.

ND: Not determined.

Bold indicates the fourteen lethal alleles at 25°C.

**Table S3** Numbers of progeny obtained from rescue crosses using the  $P[Top2-w^+]$  transgene  $y^1w^{67c23}$ ;  $Top2^{Df35}/CyO$ ;  $P[Top2-w^+]/+ \times y^1w^{67c23}/Y$ ;  $Top2^m/CyO, y^+$

Allele transmitted by female				
Allele transmitted by male	$Top2^m/Top2^{Df35}; +/+$	$Top2^m/Top2^{Df35}; P[Top2-w^+]/+$	$Top2^m \text{ or } Df35/CyO \text{ or } CyO, y^+; P[Top2-w^+]/+$	% Viability <sup>a</sup>
<i>Df17</i>	0	32	74	86 <sup>a</sup> (146) <sup>b</sup>
<i>Df35</i>	0	26	82	63 (161)
<i>17-1</i>	0	45	90	100 (185)
<i>17-2</i>	0	67	101	133 (213)
<i>17-3</i>	0	30	56	107 (127)
<i>17-5</i>	0	72	130	111 (256)
<i>17-6</i>	0	44	105	84 (205)
<i>17-7</i>	0	79	130	122 (269)
<i>35-1</i>	0	40	76	105 (157)
<i>35-2</i>	0	53	76	139 (129)
<i>35-3</i>	0	48	68	141 (154)
<i>35-5</i>	0	37	64	116 (131)
<i>35-6</i>	0	35	64	109 (155)
<i>35-12</i>	0	38	69	110 (124)
<i>35-13</i>	0	58	110	105 (210)
<i>35-14</i>	0	78	127	123 (280)

<sup>a</sup>% viability is the # of  $Cy^+$ ,  $P[Top2-w^+]$  flies divided by half the # of  $Cy^-$ ,  $P[Top2, w^+]/+$  flies multiplied by 100.

<sup>b</sup>Total # of  $Cy^-$  flies scored.

Table S4 Percentage of male and female offspring of viable heteroallelic *Top2* genotypes

Allele transmitted by male	Allele transmitted by female					
	17-1 WHD	17-3 TOPRIM	17-6 TOPRIM	35-1 TOPRIM	35-5 ATPase	35-13 WHD
17-1 WHD	NA	<b>F: 9</b> <b>M: 91</b> <b>(23)<sup>a</sup></b>	F: 42 M: 58 (24)	F: 47 M: 53 (139)	F: 50 M: 50 (105)	NA
17-3 TOPRIM	<b>F: 12</b> <b>M: 88</b> <b>(101)</b>	NA	F: 53 M: 47 (97)	F: 57 M: 43 (70)	F: 41 M: 59 (143)	<b>F: 30</b> <b>M: 70</b> <b>(69)</b>
17-6 TOPRIM	F: 60 M: 40 (30)	<b>F: 76</b> <b>M: 24</b> <b>(63)</b>	NA	F: 48 M: 52 (190)	NA	<b>F: 27</b> <b>M: 73</b> <b>(79)</b>
35-1 TOPRIM	F: 44 M: 56 (108)	F: 59 M: 41 (74)	F: 57 M: 43 (205)	NA	F: 51 M: 49 (92)	F: 43 M: 57 (143)
35-5 ATPase	F: 51 M: 49 (94)	F: 45 M: 55 (145)	NA	F: 45 M: 55 (154)	NA	F: 38 M: 62 (125)
35-13 WHD	NA	F: 38 M: 62 (93)	F: 47 M: 53 (73)	F: 46 M: 54 (228)	F: 57 M: 43 (115)	NA

<sup>a</sup>Total number of homozygotes scored.

NA: Not applicable.

Bold: statistically significant ( $p < 0.05$ ).