Supplemental Information for

The role of Blm helicase in homologous recombination, gene conversion tract length, and recombination between diverged sequences in *Drosophila*

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Supplemental Table S1. DSB repair outcome means for individual experiments

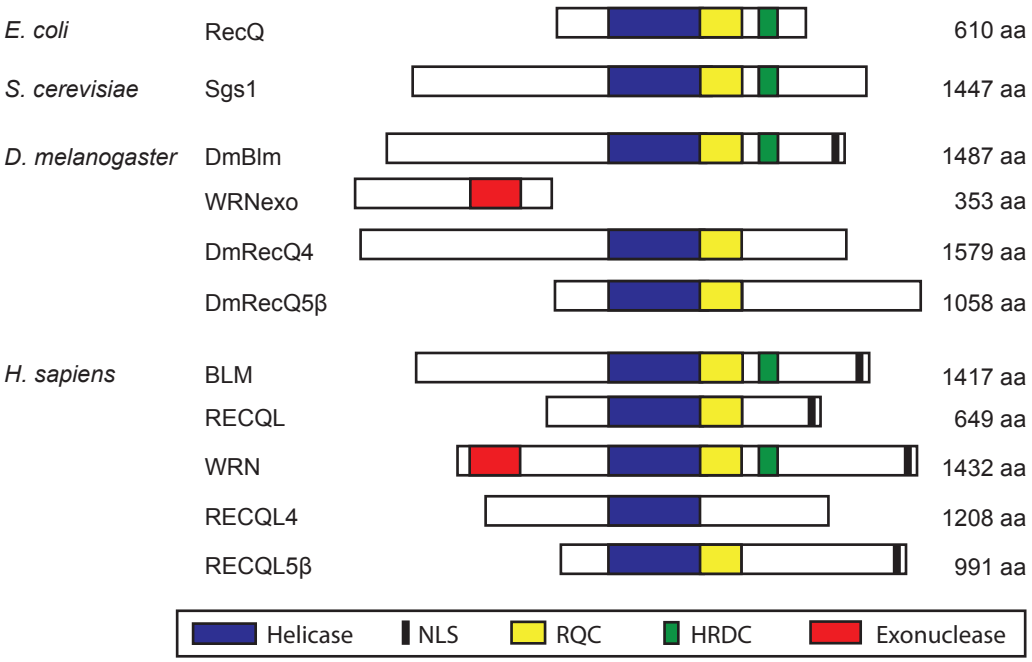
			mean % (± S.E.M.)		
Genotype (I-SceI expression)	**DSB repair assay**	**Experiment # (total # of individual male germlines)**	**$y^+ w^-$ NHEJ, intersister HR, no DSB**	**$y^+ w^+$ intra-chromosomal HR**	**$y^- w^-$ SSA, deletion, mitotic CO**
N1/TM6B (heatshock)	DR-*white*	1[a] (28)	77.3 (1.9)	20.8 (1.8)	1.9 (0.4)
		2[a] (22)	75.8 (1.8)	22.1 (1.7)	2.1 (0.5)
		3 (25)	73.2 (3.1)	22.5 (3.2)	4.2 (0.6)
		4 (22)	70.4 (3.0)	23.3 (2.6)	5.4 (1.0)
		5 (28)	71.4 (2.3)	25.0 (2.2)	3.6 (0.6)
		compiled (125)	**74.3 (1.1)**	**22.6 (1.0)**	**3.1 (0.3)**
	DR-*white.mu*	1[a] (13)	84.9 (2.8)	13.7 (2.5)	1.3 (0.5)
		2[a] (19)	82.6 (2.3)	15.4 (2.2)	2.1 (0.4)
		compiled (32)	**83.5 (1.7)**	**14.7 (1.6)**	**1.8 (0.3)**
N1/D2 (heatshock)	DR-*white*	1[a] (17)	84.5 (1.9)	7.23 (1.5)	8.3 (1.1)
		2[a] (24)	87.7 (1.5)	6.1 (0.8)	6.2 (1.2)
		3 (12)	76.8 (3.6)	13.9 (3.9)	9.3 (1.7)
		4 (14)	77.3 (9.6)	15.3 (5.2)	15.4 (3.4)
		5 (27)	72.6 (1.6)	16.8 (1.8)	11.3 (0.8)
		compiled (94)	**78.3 (1.2)**	**11.6 (1.0)**	**10.1 (0.8)**
	DR-*white.mu*	1[a] (16)	89.9 (1.8)	4.2 (1.0)	6.0 (1.4)
		2[a] (20)	91.1 (1.5)	4.2 (1.2)	4.7 (1.1)
		compiled (36)	**90.6 (1.1)**	**4.2 (0.8)**	**5.3 (0.8)**
D3/TM6B (heatshock)	DR-*white*	1[a] (12)	66.4 (4.2)	29.8 (4.1)	3.9 (1.4)
		2[a] (30)	69.9 (1.9)	27.1 (1.6)	2.9 (0.8)
		3 (17)	77.5 (2.9)	21.7 (2.9)	0.7 (0.3)
		compiled (59)	**71.1 (1.5)**	**26.5 (1.4)**	**2.4 (0.4)**
	DR-*white.mu*	1[a] (15)	75.1 (3.8)	21.9 (3.3)	3.0 (1.0)
		2[a] (33)	75.6 (1.9)	21.1 (1.7)	3.4 (0.5)
		3 (27)	72.9 (2.2)	20.8 (1.9)	6.2 (1.1)
		compiled (75)	**74.8 (1.4)**	**21.4 (1.3)**	**3.9 (0.4)**
D3/D2 (heatshock)	DR-*white*	1[a] (21)	74.3 (3.1)	17.4 (2.3)	8.3 (1.4)
		2[a] (27)	76.4 (1.8)	15.6 (1.2)	7.9 (1.1)
		3 (4)	89.4 (2.6)	9.0 (1.9)	1.6 (0.9)
		compiled (52)	**77.2 (1.5)**	**14.6 (1.1)**	**8.2 (0.8)**
	DR-*white.mu*	1[a] (5)	79.3 (5.9)	13.0 (6.2)	7.7 (0.8)
		2[a] (24)	83.9 (1.9)	8.8 (1.3)	7.3 (1.2)
		3 (11)	78.3 (2.4)	11.5 (1.9)	10.2 (1.4)
		compiled (42)	**82.3 (1.5)**	**9.9 (1.2)**	**7.7 (0.8)**
D2/TM6B (heatshock)	*DR-white.mu*	1 (40)	71.2 (2.2)	19.7 (2.0)	9.1 (0.8)
		compiled (40)	**71.2 (2.2)**	**19.7 (2.0)**	**9.1 (0.8)**
N1 or *D2/TM6B* (constitutive)	DR-*white*	1 (6)	39.9 (1.9)	42.9 (2.6)	17.1 (0.9)
		2 (36)	50.5 (1.9)	41.7 (2.2)	7.8 (0.9)
		3 (9)	51.0 (3.2)	37.8 (4.1)	11.2 (2.9)
		4 (17)	44.6 (2.8)	38.1 (2.2)	19.5 (1.8)
		compiled (68)	**48.2 (1.3)**	**40.4 (1.4)**	**12.1 (1.0)**
N1/D2 (constitutive)	DR-*white*	1 (5)	42.6 (1.7)	30.6 (3.6)	26.7 (3.3)
		2 (8)	57.8 (6.5)	30.7 (7.2)	11.5 (1.9)
		3 (2)	53.0 (20)	31.5 (18.5)	15.5 (2.4)
		4 (16)	54.9 (3.4)	26.6 (3.2)	18.5 (2.1)
		compiled (31)	**51.7 (2.8)**	**30.8 (2.9)**	**17.5 (1.6)**

[a] Experiments performed side-by-side with DR-*white* and DR-*white.mu* assay to calculate suppression of recombination of diverged sequences (i.e., HR frequencies relative to DR-*white*).

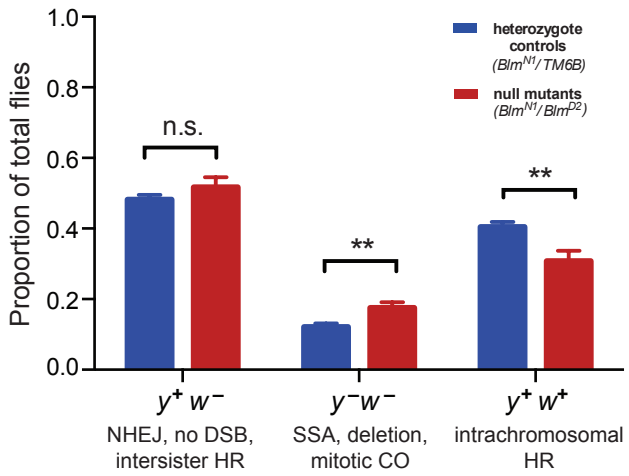
Supplemental Table S2. NHEJ junction analysis by experiment

NHEJ with processing analysis				
Genotype	**I-SceI expression**	**Experiment #**	**# y^+ w^- isolates analyzed**	**# NHEJ w/ processing (%)**
N1/TM6B	heatshock	1	47	8 (17)
		2	20	0 (0)
		3	82	4 (4.9)
		compiled	**149**	**12 (8.1)**
N1/D2	heatshock	1	19	0 (0)
		2	14	2 (14)
		3	52	3 (5.8)
		compiled	**85**	**5 (5.9)**
D3/TM3	heatshock	1	19	1 (5.3)
		3	43	6 (14)
		compiled	**62**	**7 (11)**
D3/D2	heatshock	1	34	2 (5.9)
		3	40	4 (10)
		compiled	**74**	**6 (8.1)**
Microhomology analysis[a]				
Genotype	**I-SceI expression**	**Experiment #**	**# y^+ w^- isolates analyzed**	**# with microhomologies (%)**
N1/TM6B	constitutive	1	11	7 (64)
		2	27	16 (60)
		compiled	**38**	**23 (61)**
N1/D2	constitutive	1	9	2 (22)
		2	25	20 (80)
		compiled	**34**	**22 (65)**

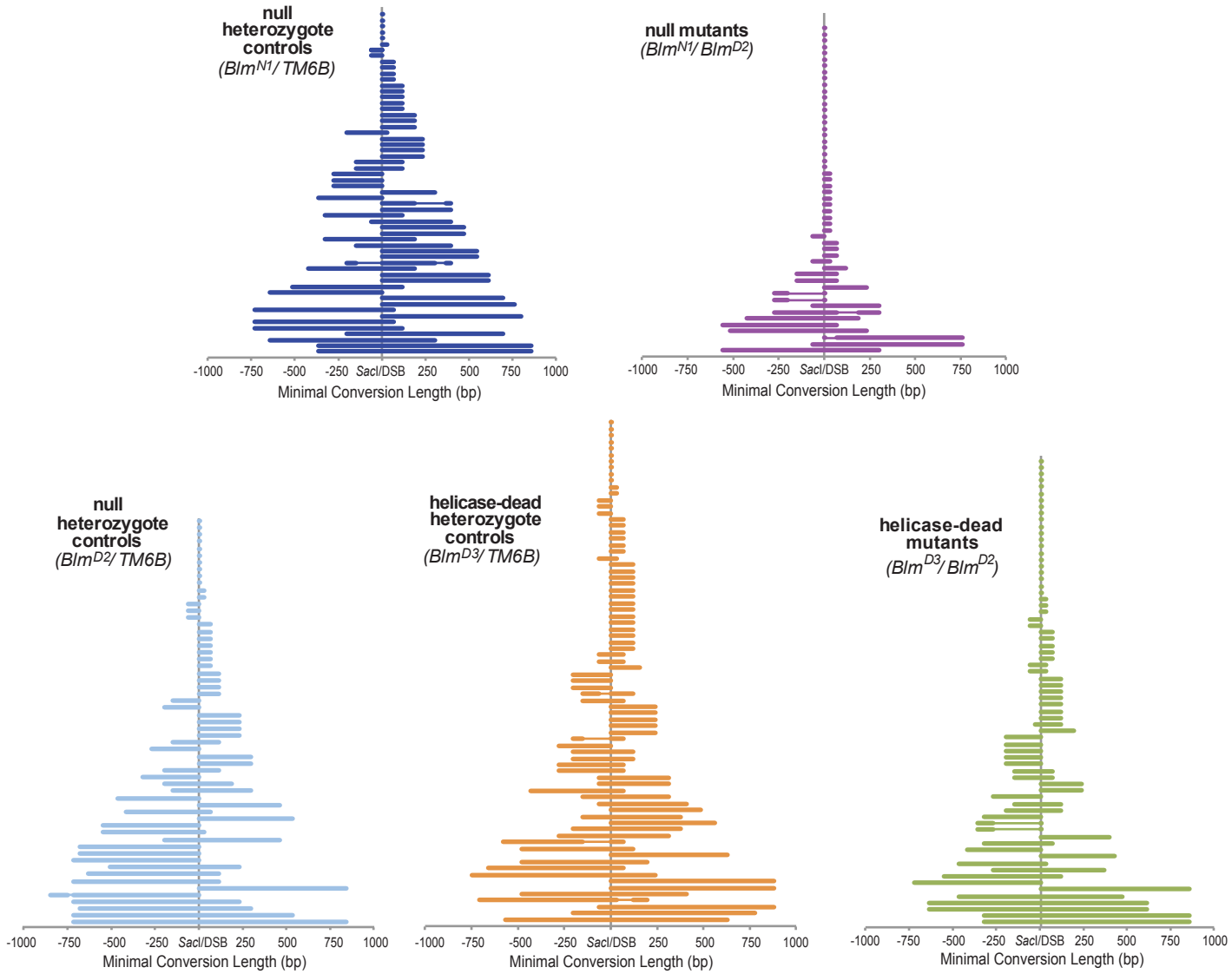
[a] microhomologies were defined by 1+ nucleotides long, as in Do*, et al.* 2014.



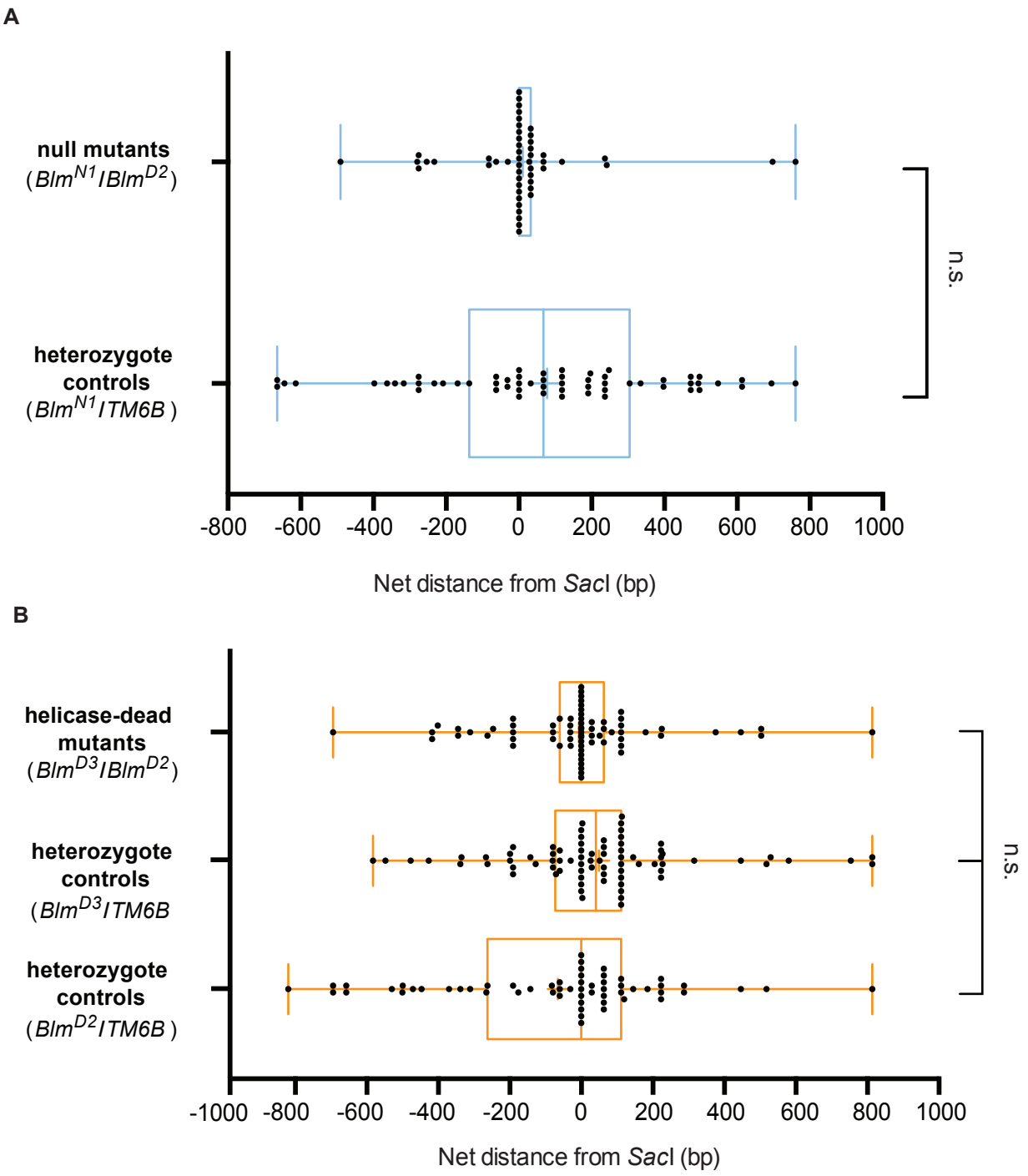
Supplemental Figure S1. RecQ helicase family members within and between species. Schematic representation of the RecQ helicase protein family across multiple species. The highly conserved helicase domains (blue) align all protein schematics, and functionally relevant motifs or stretches of amino acid acids are colored as indicated (not to scale). NLS (black) = Nuclear Localization Signal; RQC (yellow) = Helicase C-terminal; HRDC (green) = RNase C-terminal. Protein lengths (amino acids) are provided to the right.



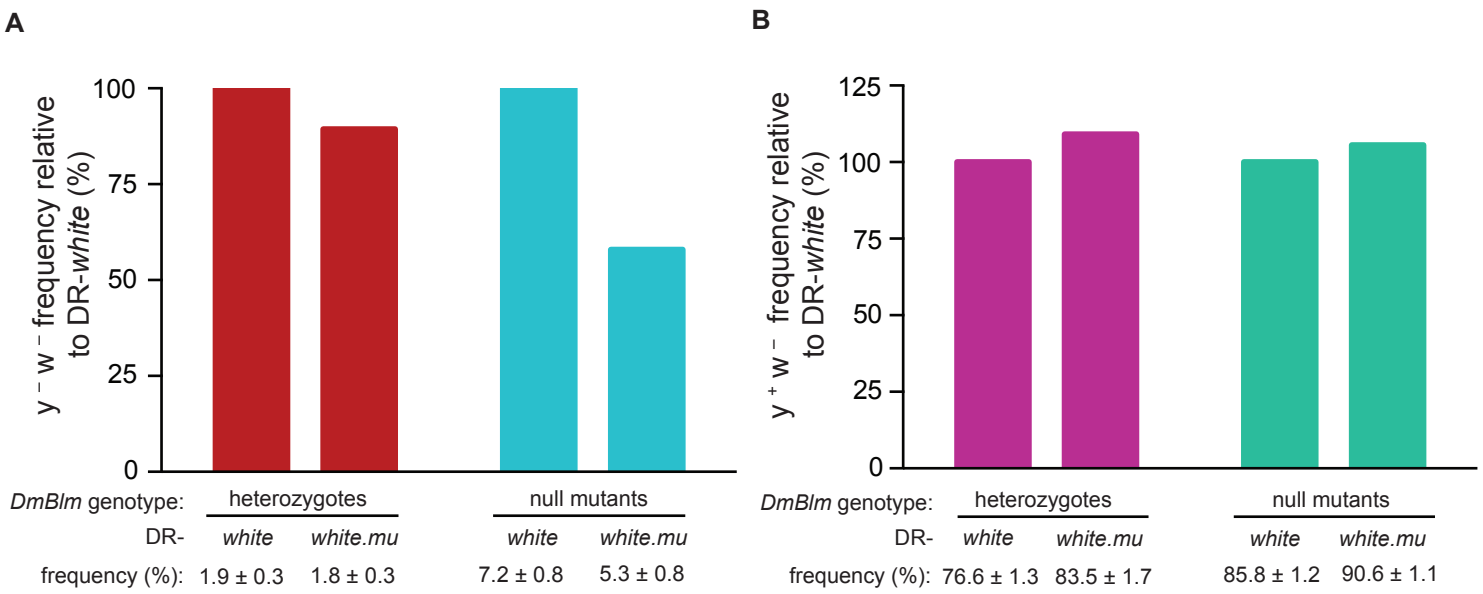
Supplemental Figure S2. DmBlm impacts DSB repair pathway usage with constitutively active I-*Sce*I expression. DSB repair events from constitutively-active I-*Sce*I enzyme in a *DmBlm*$^{N1/D2}$ null mutant background (red; n = 31) compared to *DmBlm*^{N1} heterozygote controls (blue; n = 68). Results shown are averages and S.E.M. of individual male germline events compiled from four independent experiments. **p < 0.01 by unpaired Student's *t* test.



Supplemental Figure S3. Individual DmBlm gene conversion tracts. Depictions of minimal conversion lengths are displayed for *DmBlm*^{N1} null heterozygote control HR events (n = 59; top left), *DmBlm*$^{N1/D2}$ null mutant HR events (n = 51; top right), *DmBlm*^{D2} null heterozygote control HR events (n = 59; bottom left), *DmBlm*^{D3} helicase-dead heterozygote control HR events (n = 78; bottom center), and *DmBlm*$^{D3/D2}$ helicase-dead mutants (n = 71; bottom right), including the last polymorphism converted. Discontinuous conversion is indicated by a thin line. Data sets are same as in Figure 4, and compiled from two (top) and four (bottom) independent experiments.



Supplemental Figure S4. Directionality of DmBlm gene conversion tracts. Directionality of (A) DmBlm^{N1} heterozygote control and DmBlm$^{N1/D2}$ null mutant GCTs and (B) DmBlm^{D3} heterozygote control, DmBlm^{D2} heterozygote control and DmBlm$^{D3/D2}$ helicase-dead mutant GCTs. Net directionality for each class of GCTs was calculated by defining the GCT length to the left of the *Sac*I site/DSB as negative, and then summing this number with the positive GCT length to the right of the *Sac*I site/DSB. Data sets are compiled from two (null) and four (helicase dead) independent experiments. Tested for significance by unpaired Student's *t* test.



Supplemental Figure S5. Relative frequencies between DR-*white* and DR-*white.mu* are consistant within the other phenotypic classes. Relative y-w- (A) and y+w- frequencies (B) between homologous and diverged sequences were determined using DR-*white* and DR-*white.mu*, respectively. Data represent analysis in *DmBlm*^{N1}heterozygote controls and *DmBlm* $^{N1/D2}$ null mutants. Average frequencies (with S.E.M.) of each class were compiled from individual germlines from two independent experiments and are presented below the graphs (and in Supplemental Table S1). Average frequencies of each class relative to DR-*white* are plotted.