

Figure S1. Alignment of CT2 regions across the genus *Drosophila*. The latter part of the region has highly conserved patches, while the N-terminal amino acids, which are highly glycine-rich, are more variable in length and composition. Arrows indicate the first and last amino acid in the CT2.

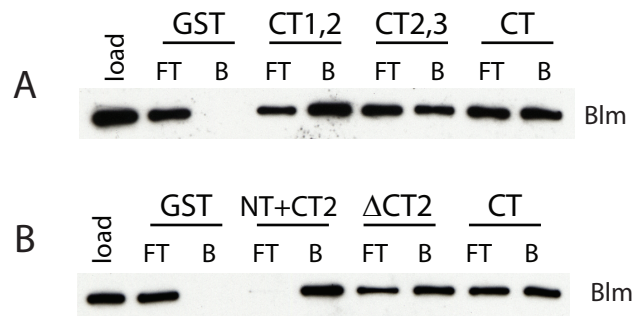


Figure S2. Binding of purified Blm to various Top3α constructs. **A.** Blm binds to both CT1,2 and CT2,3, as expected for constructs containing CT2. **B.** Blm binds NT+CT2 and Top3α ΔCT2, as they contain the core domain plus CT2 or CT1,3.

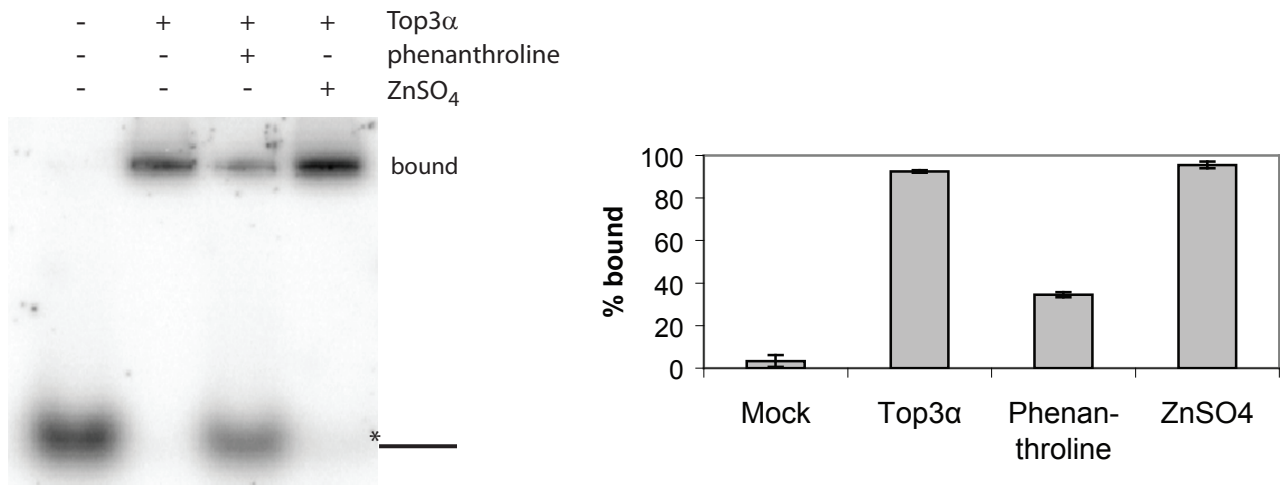


Figure S3. The abrogation of Top3α ssDNA binding by phenanthroline can be restored by adding back zinc. Immobilized Top3α was incubated with 10 mM phenanthroline. An aliquot was incubated with DNA, and the remaining resin was then washed three times with binding buffer before adding back buffer containing 5 μM ZnSO₄. An aliquot was again taken and incubated with labeled ssDNA. By removing the phenanthroline and adding zinc, binding is restored.

	+ / + 0 copies	NT / + 1 copy	NT / NT 2 copies
top3 α ⁵⁴ / Top3 α	85 (1)	202 (2.4)	78 (0.92)
top3 α ⁵⁴ / top3 α ⁵⁴	0 (0)	0 (0)	0 (0)

Table S1. The Top3 α NT truncation mutant cannot rescue viability of the top3 α 54 null flies. The top row lists progeny in the presence of a wildtype copy of Top3 α , while the bottom row lists flies with the null mutant (ie rescues). Number of flies is shown, with the relative ratio underneath in parentheses. Flies containing the null mutant and the Top3 α NT transgene were still inviable, as none were observed.

ID Sequence

7 CGA AGG CCA TGA TTG CGC ACT GAA TAC ATC CTG CCC TGT TAT TAA TTA CGT TAT CTT ACG
8 GTG TTG CCG TCT ACA TGC TTG ATT ATT CTC GAT GTA TTC AGT GCG CAA TCA TGG CCT TCG
9 CGT AAG ATA ACG TAA TTA ATA ACA GGG CAG GAT GTA TTC AGT GCG CAA TCA TGG CCT TCG
11 GCT ACT ACT TCA CTA TCT CGC CGA TCA ACC GAG AAT AAT CAA GCA TGT AGA CGG CAA CAC
12 CGT AAG ATA ACG TAA TTA ATA ACA GGG CAG GGT TGA TCG GCG AGA TAG TGA AGT AGT AGC




Substrate	Description	Components
* 	ssDNA	7
* 	dsDNA	7 & 9
* 	HJ (static-X)	7, 8, 11, & 12

Table S2. Outline of oligonucleotides used to create DNA substrates. Each is 60 nucleotides long; the arms of the static-X are 30 base pairs each. Substrates and their components are diagrammed below.