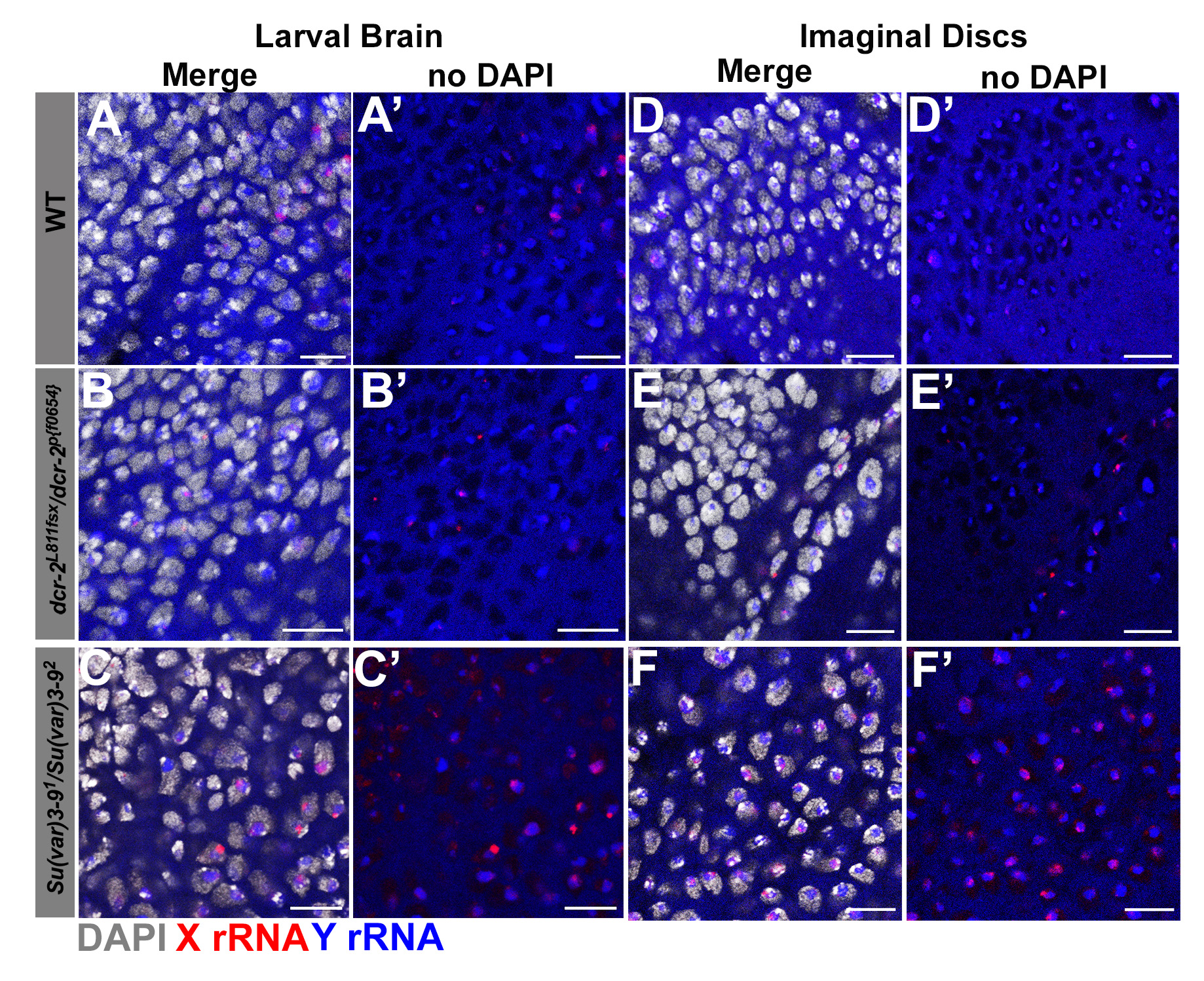
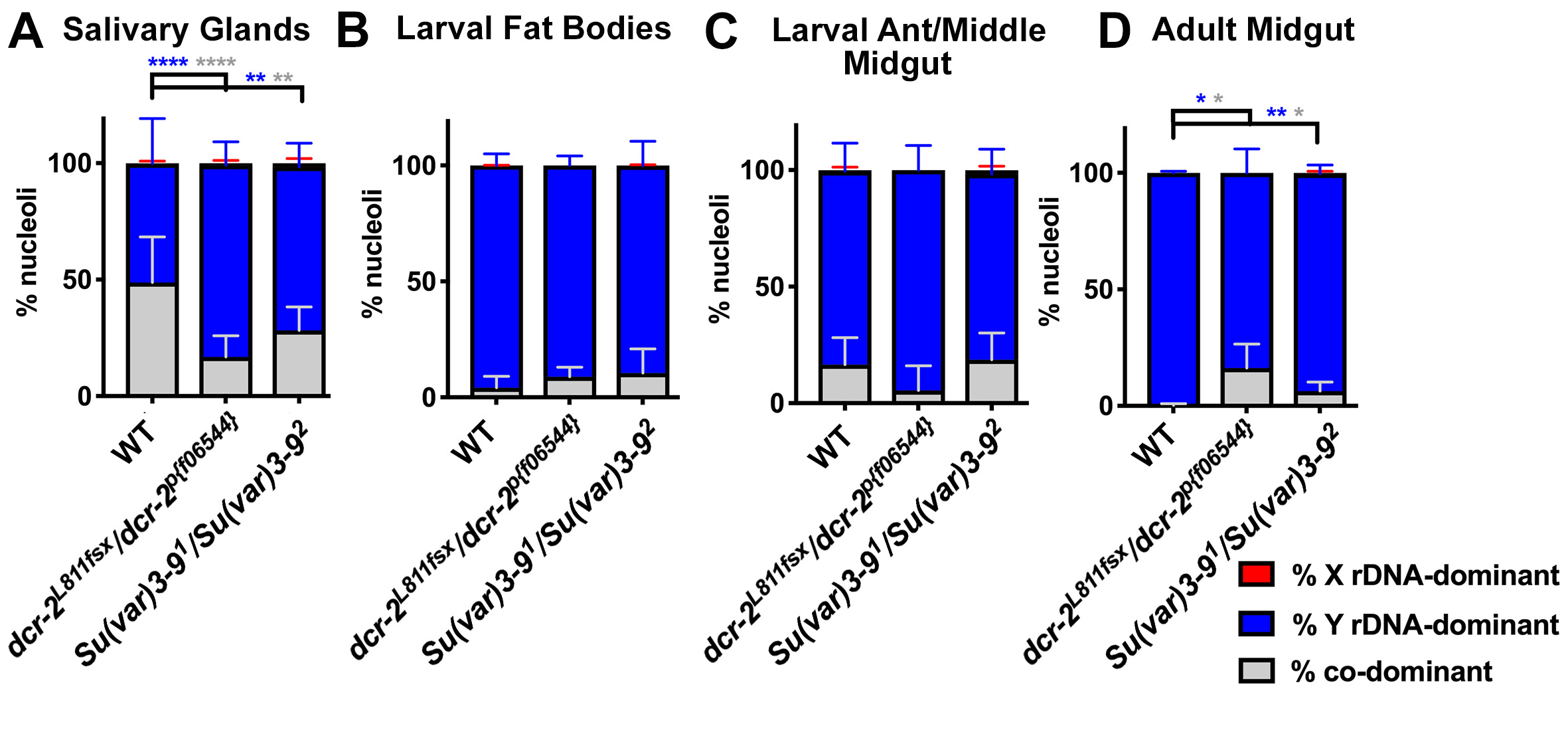
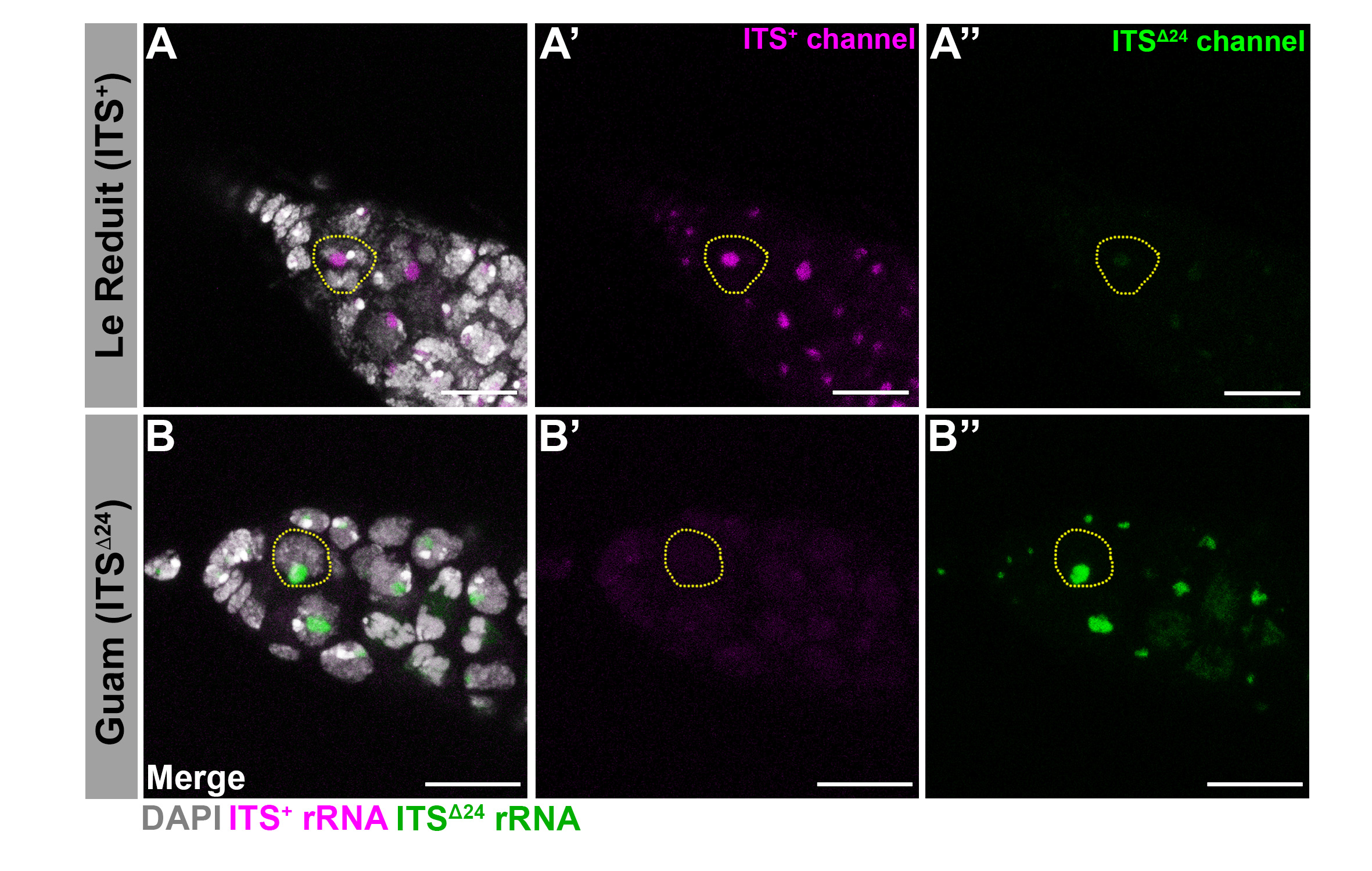
**Supplementary Figures**

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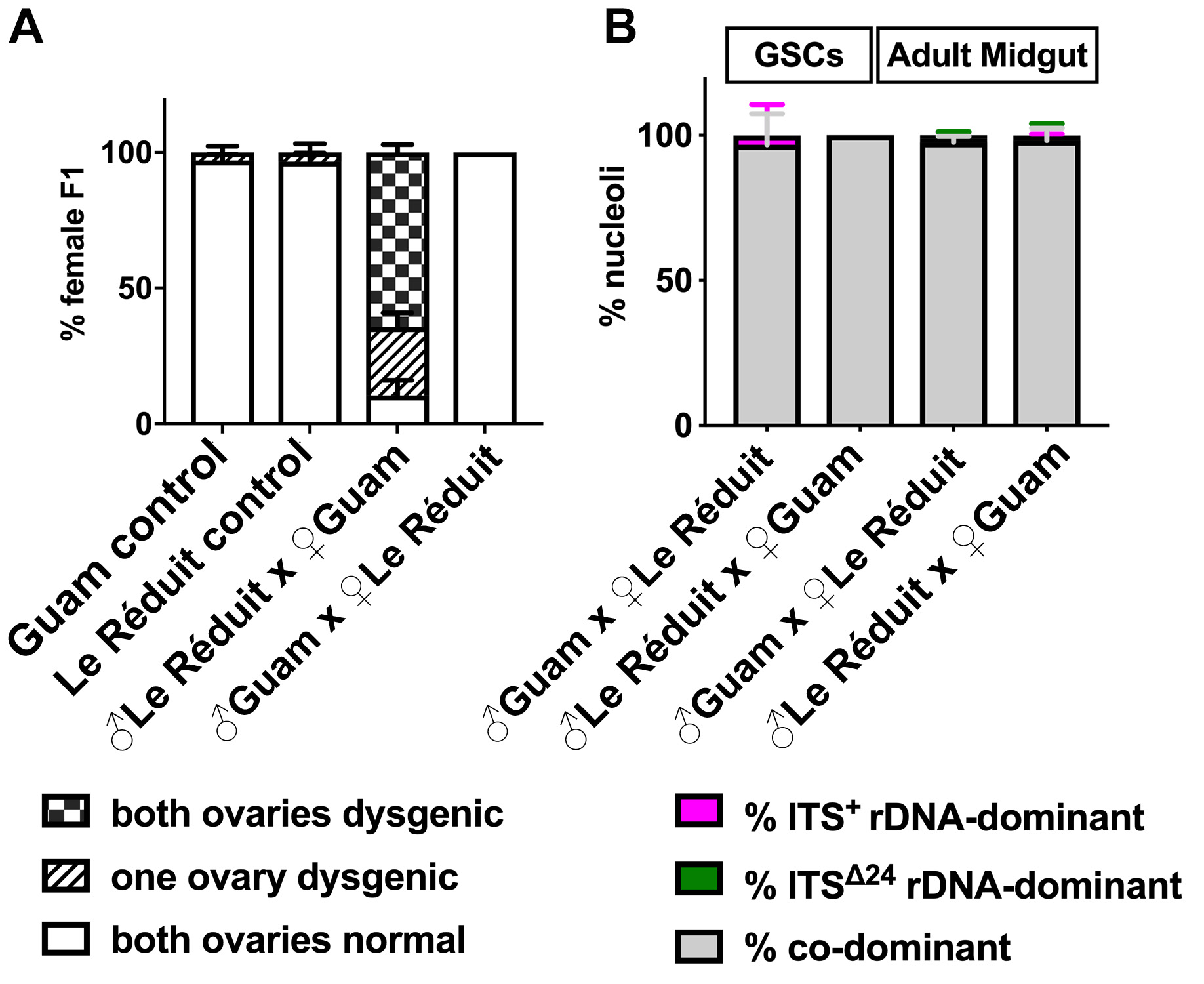
**Figure S1: Heterochromatin formation aids in Y rDNA dominance in males.** Representative images of larval brains from A) wild type (*yw*), B) *dcr-2L811fsx/ dcr-2p[f06544]* mutants, and C) *Su(var)3-91/Su(var)3-92* mutants. Representative images of imaginal discs from D) wild type, E) *dcr-2L811fsx/ dcr-2p[f06544]* mutants, and F) *Su(var)3-91/Su(var)3-92* mutants. Red = X rRNA, blue Y rRNA, white = DAPI. All scale bars = 10μm.



**Figure S2: The state of nucleolar dominance in polyploid tissues in *dcr-2* and *Su(var)3-9* mutants.** Quantification of nucleolar dominance in males in A) salivary glands of wild type (*yw*) (n = 878 cells from 15 salivary glands), *dcr-2L811fsx/ dcr-2p[f06544]*mutants (n = 373 cells from 8 salivary glands), and *Su(var)3-91/Su(var)3-92* mutants (n = 423 cells from 11 salivary glands), B) larval fat bodies of wild type (n = 1575 cells from 17 fat bodies), *dcr-2L811fsx/ dcr-2p[f06544]* mutants (n = 320 cells from 4 fat bodies), and *Su(var)3-91/Su(var)3-92* mutants (n = 351 cells from 7 fat bodies), C) larval anterior/middle midgut enterocytes of wild type (n = 181 cells from 6 guts), *dcr-2L811fsx/ dcr-2p[f06544]*mutants (n = 150 cells from 5 guts), and *Su(var)3-91/Su(var)3-92* mutants (n = 172 cells from 5 guts), and D) adult anterior midgut enterocytes of wild type (n = 922 cells from 7 guts), *dcr-2L811fsx/ dcr-2p[f06544]* mutants (n = 614 cells from 6 guts), and *Su(var)3-91/Su(var)3-92* mutants (n = 476 cells from 6 guts). Red = % X rDNA-dominant, blue = % Y rDNA-dominant, grey = % co-dominant nuclei. p-values calculated using Welch’s unpaired, unequal variances *t-*test using n = number of tissues*.* (no star) = not significant, \* = < 0.05, \*\* = < 0.01, \*\*\*\* = < 0.0001. Colors of asterisks correspond to colors of bars for which p-values were calculated (e.g. blue asterisk for Y rDNA-dominant p-values).



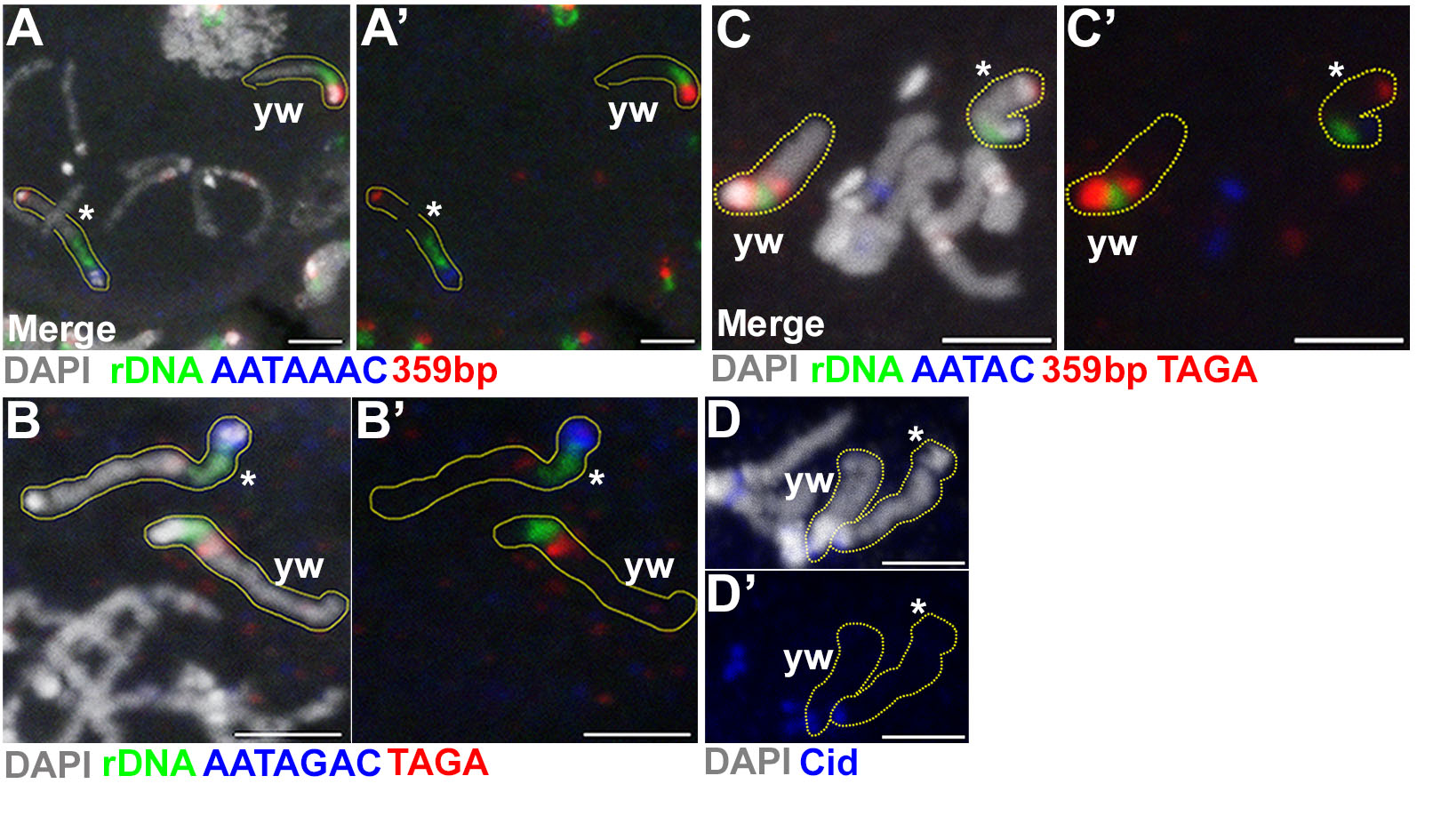
**Figure S3: ITS probes that differentially identify distinct X rDNA variants.** A) Representative images of germarium from Le Réduit*,* A’) ITS+ channel only, A’’) ITSΔ24 channel only, and B) Guam *D. melanogaster* strains, B’) ITS+ channel only, B’’) ITSΔ24 channel only. All scale bars = 10μm. Magenta = ITS+ rDNA transcript, green = ITSΔ24 rDNA transcript, white = DAPI. Yellow circles to denote an example of a germline stem cell (GSC).



**Figure S4: Two X chromosomes (ITS+ vs. ITSΔ24 variants) exhibit co-dominance irrespective of parental origins.** A) Quantification of hybrid dysgenesis in the crosses of ♂Guam x ♀Guam controls (n = 104 females from 4 vials), ♂Le Réduit x ♀Le Réduit controls (n = 185 females from 4 vials), and ♂Le Réduit x ♀Guam (n = 145 females from 3 vials), and ♂Guam x ♀Le Réduit (n = 202 females from 4 vials) performed at 25° (Engels and Preston 1979). B) Quantification of female nucleolar dominance between two X rDNA comparing both cross directions (data from Figure 4 is reproduced to show that the parental origin minimally influence the state of nucleolar dominance). GSCs scored: ♂Guam x ♀Le Réduit (n = 150 cells from 57 germarium),♂Le Réduit x ♀Guam (n = 107 cells from 51 germarium). Adult anterior midgut scored: ♂Guam x ♀Le Réduit (n = 904 cells from 9 guts),♂Le Réduit x ♀Guam (n = 962 cells from 9 guts).

Reference:

Engels, W. R., and C. R. Preston, 1979 Hybrid dysgenesis in Drosophila melanogaster: the biology of female and male sterility.  
Genetics 92: 161–174.

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**Figure S5: Cytological characterization of the Xbb-YS chromosome.** DNA fluorescent *in situ* hybridization (FISH) on mitotic chromosome spreads from larval brains of Xbb-YS/X females with A) AATAAAC (blue) and 359-bp (red), A’) no DAPI. B) AATAGAC (blue) and TAGA (red), B’) no DAPI. C) AATAC (blue), TAGA, and 359-bp, C’) no DAPI. D) Immunofluorescence (IF) of centromeric protein Cid (blue) on mitotic chromosome spreads, D’) no DAPI. Xbb-YS (\*) and wild type X (wt), both outlined in yellow. White = DAPI. All scale bars = 3μm.