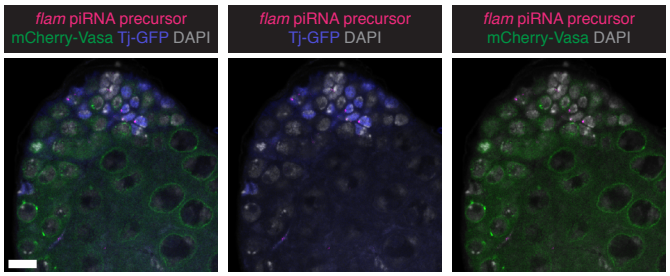

Figure S1 | The *flamenco* piRNA locus is only active in the gonadal soma in testis, related to Figure 1.

RNA *in situ* HCR detecting *flamenco* piRNA precursors in testes expressing mCherry-Vasa and Tj-GFP under endogenous regulatory elements, stained for DAPI. flamenco transcripts are only detected in gonadal somatic cells (including both early cyst cells marked by Tj expression and hub cells marked by the lack of Tj and Vasa expression), but not in germline cells (marked by Vasa expression). Scale bar: 10μm.

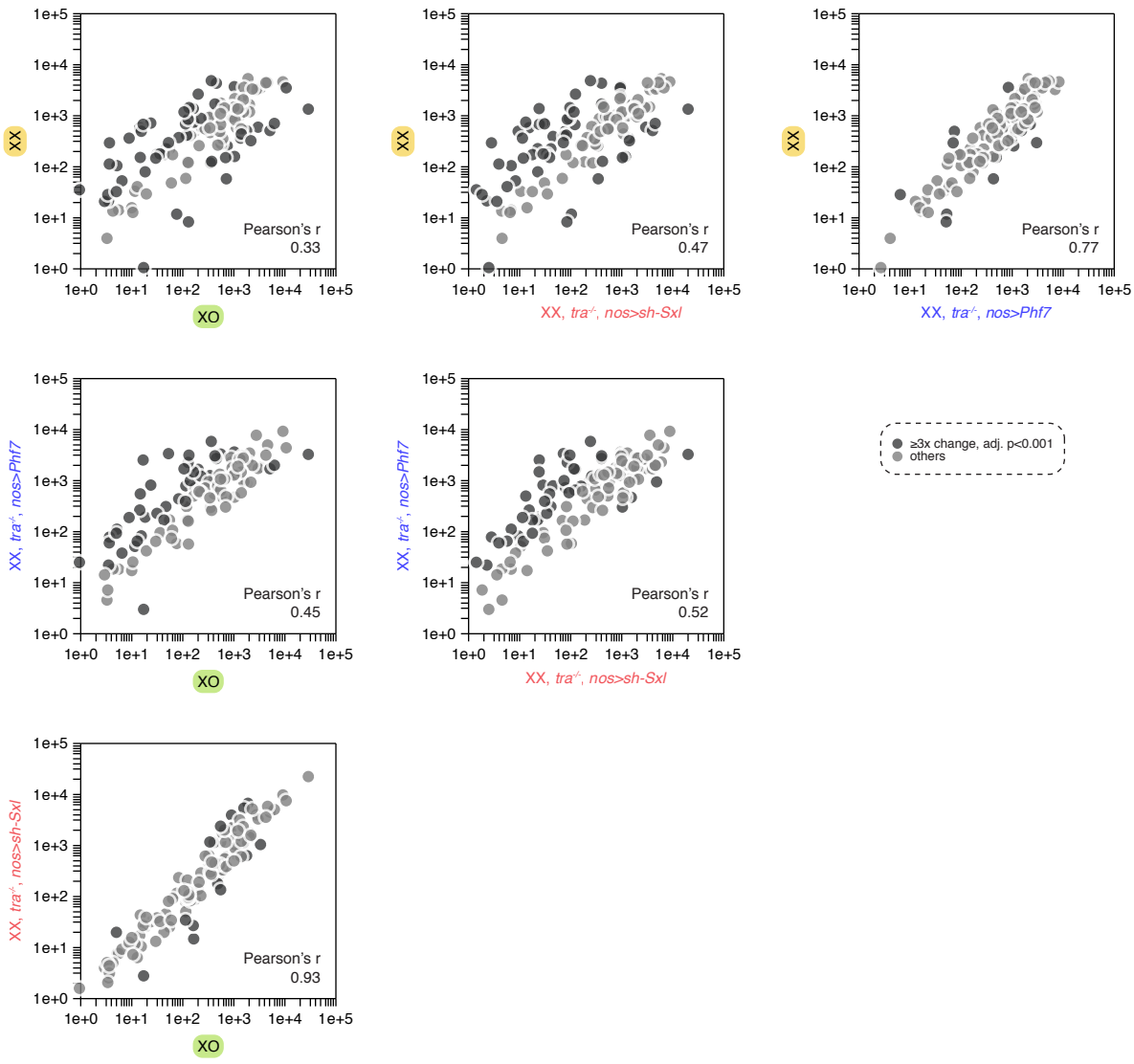

Figure S2 | Pairwise piRNA comparisons of XX female, XO male, and XX masculinized by perturbing either Phf7 or Sxl (in the *tra* mutant background), related to Figure 4.

Pairwise comparison of the abundance of piRNAs against different transposon families in four genotypes: XX female, XO male, XX *tra*$^{-/-}$ with Phf7 ectopic expression, and XX *tra*$^{-/-}$ with Sxl germline knock-down. Sxl perturbation led to a piRNA program that differed from XX female and resembled XO male, more than Phf7 perturbation did.

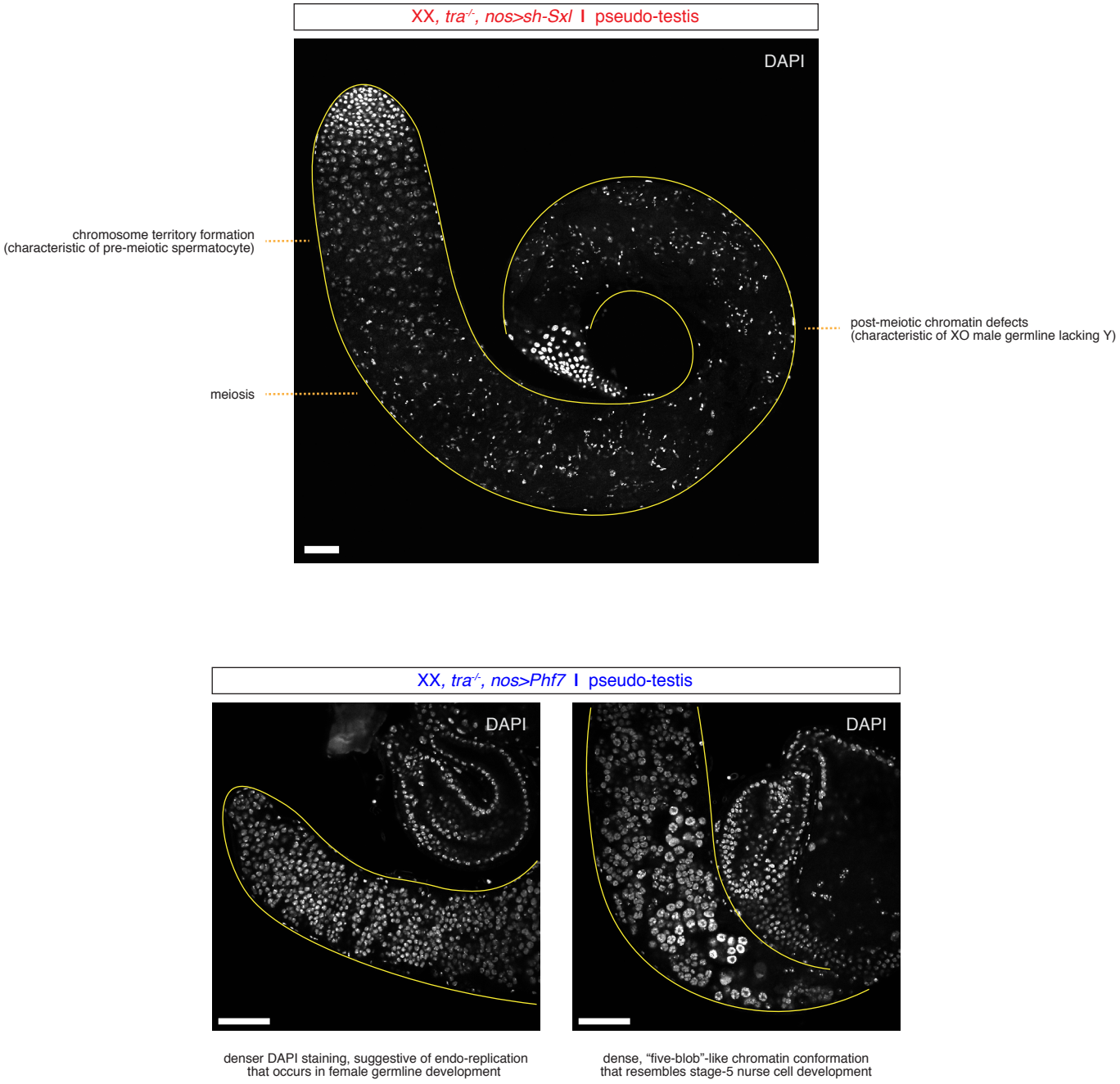

Figure S3 I Germline prevalence and phenotype in masculinized XX, related to Figure 4.

DAPI staining of masculinized XX *tra*$^{-/-}$ germline with either Sxl knock-down (top) or Phf7 ectopic expression (bottom). In both masculinization perturbations, germline is prevalent, but they show distinct phenotypes. Sxl perturbation mimics male germline development (with e.g., chromosome territory formation characteristic of spermatocytes), while Phf7 perturbation resembles female germline development (with e.g., nurse cell-like chromatin conformation), though both surrounded by masculinized gonadal soma. Of note, these germline phenotypes correlate with the "sex" of the piRNA program. Scale bar: 50μm.