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Supplemental information

Pervasive SUMOylation of heterochromatin and piRNA pathway proteins

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Supplemental Figure 1, related to Figure 2

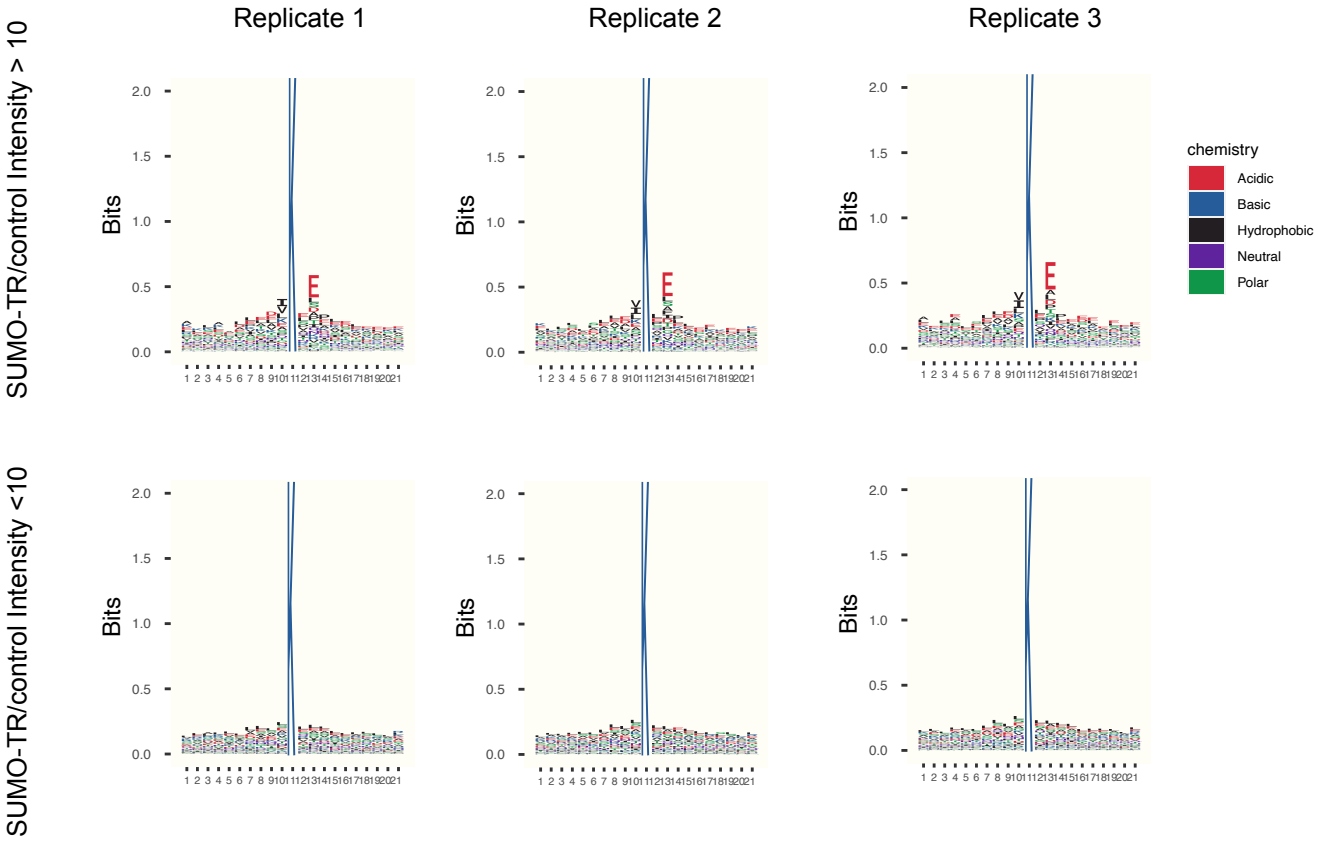

Figure S1. Amino acid frequencies flanking predicted K-ε-GG for sites with high and low SUMO-TR:control intensity ratios in each replicate, related to Figure 2.

Supplemental Figure 2, related to Figure 2

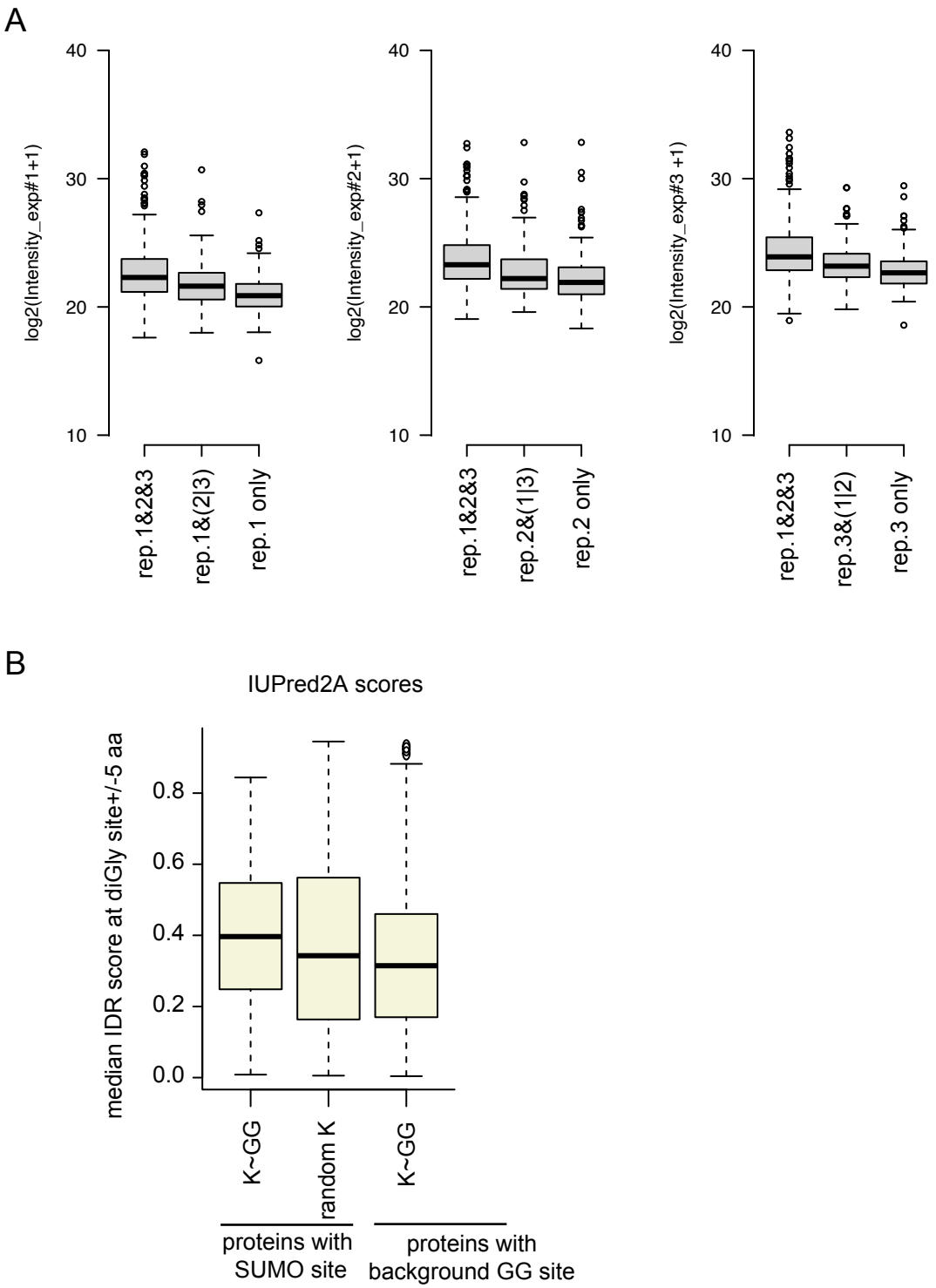

Figure S2. Analysis of diGly peptides intensities and modified lysine position within IDRs, related to Figure 2. A) Relationship of diGly site intensity and reproducibility of detection. Boxplots show the distributions of intensities for all diGly sites detected in each replicate, divided into categories depending on their detectability in other replicates. B) Localization of SUMOylation sites to IUPred2A-perdicteed protein IDRs. Boxplots show the median IDR score for 10-aminoacid region flanking the central diGly modified lysine for bona fide SUMO sites, compared to randomly chosen lysine in the same protein and to background diGly sites.

Supplemental Figure 3, related to Figure 3

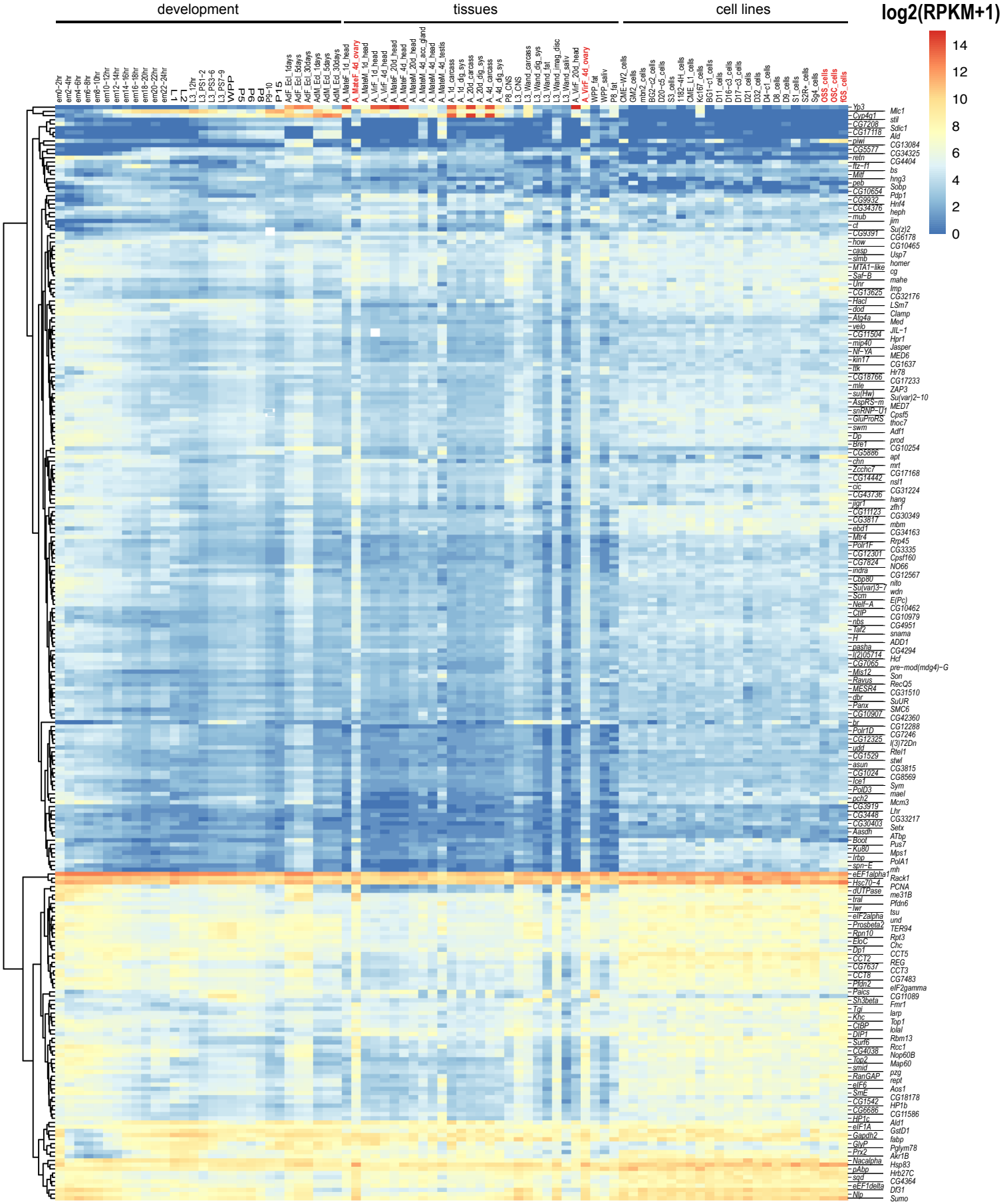

Figure S3. Heatmap of RPKM-normalized gene expression values for proteins with bona fide SUMO sites, related to Figure 3. Data is retrieved from indicated modEncode RNA-seq datasets available on FlyBase; ribosomal proteins were excluded.

Supplemental Figure 4, related to Figures 4&5

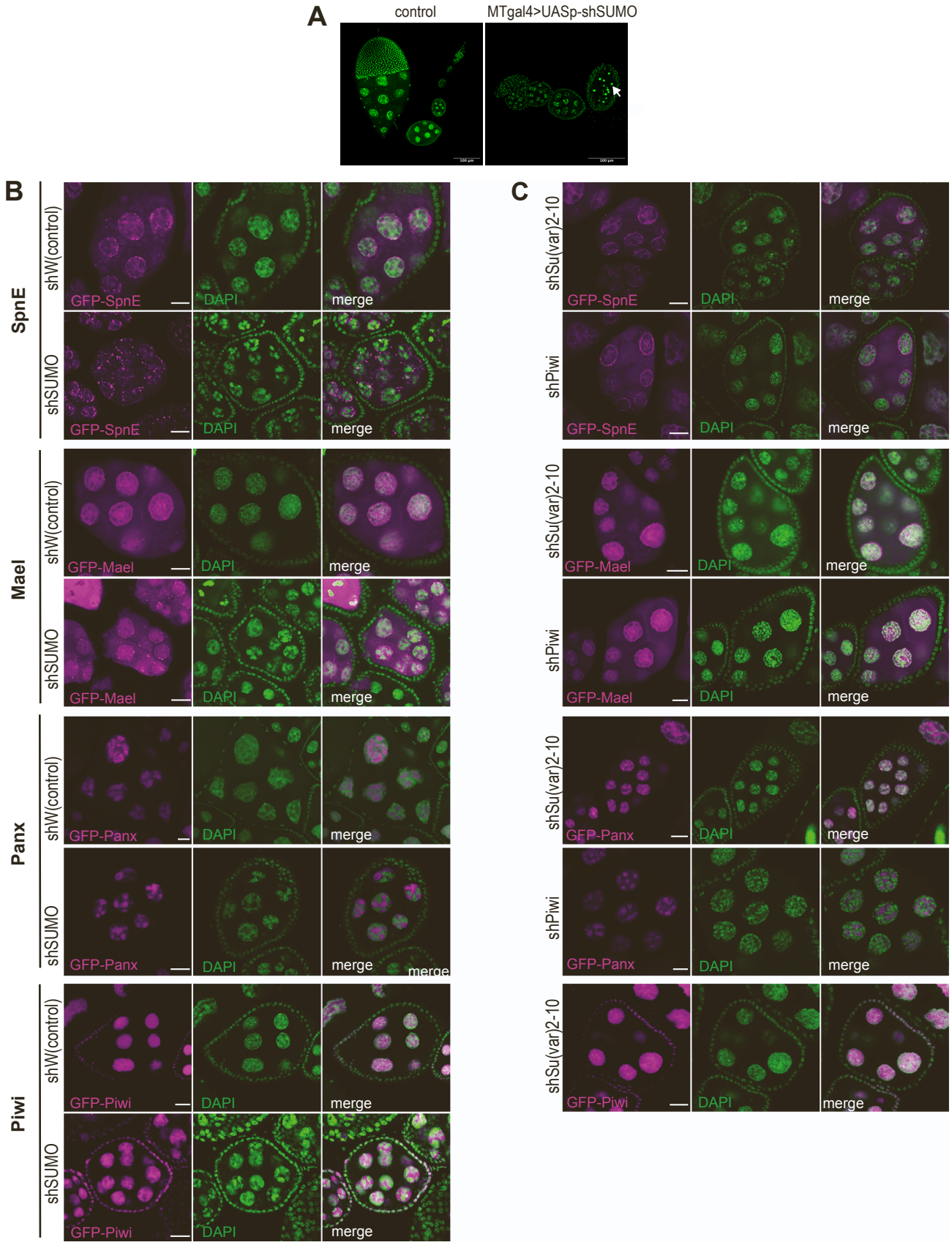

Figure S4. Effects of SUMO germline knockdown on ovarian morphology and piRNA pathway proteins localization, related to Figure 4,5. A) Confocal images of ovarioles from control and SUMO knockdown via MT-gal4 driven shRNA ovaries stained for DAPI. The arrowhead indicates collapsed nuclei in mid-stage egg chambers in SUMO knockdown ovaries. B,C) Confocal images of ovarioles from flies expressing indicated GFP-tagged factors in control and indicated MT-gal4-driven shRNAs. Scale bar=20 μm.

Supplemental Figure 5, related to Figure 5

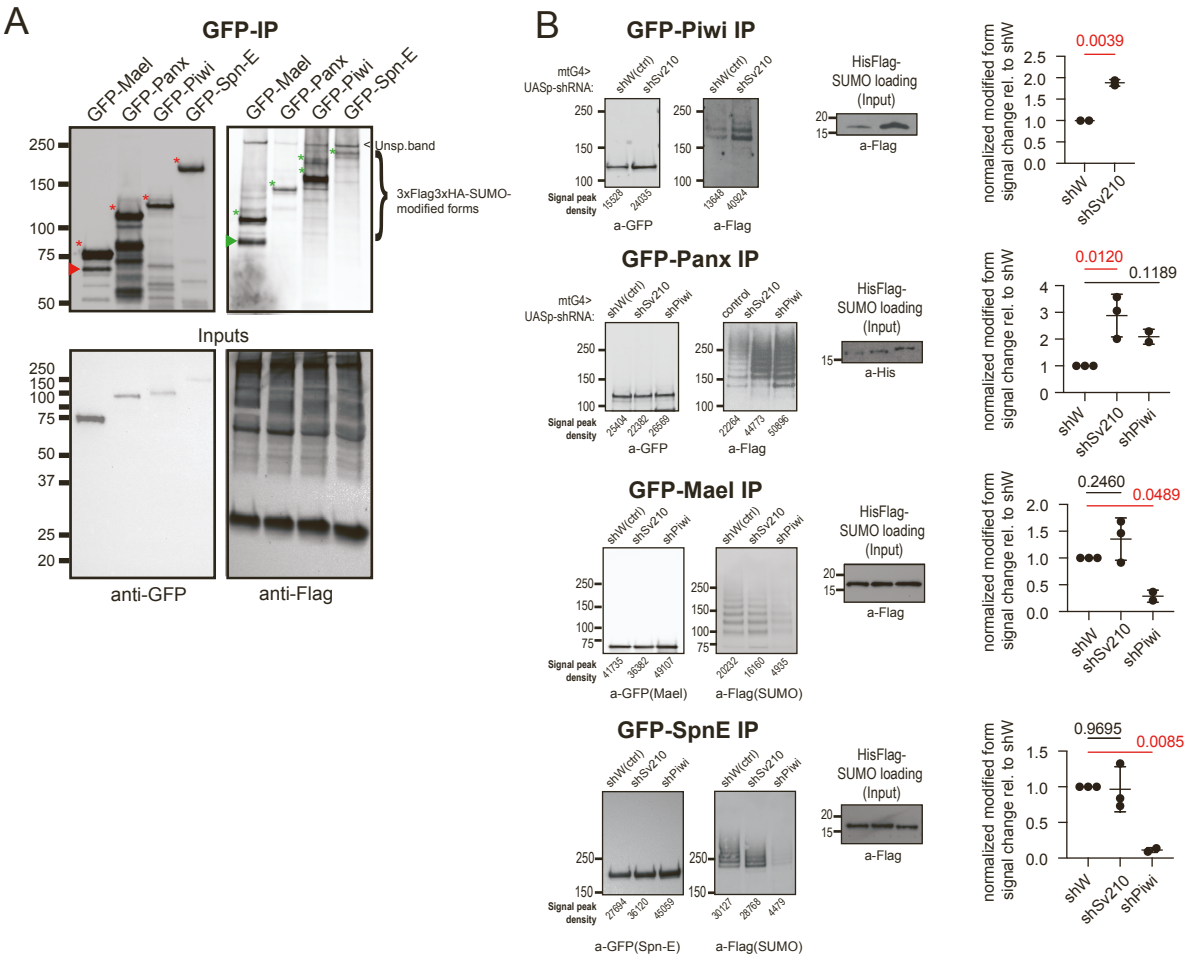

Figure S5. Piwi, Panx, Spn-E and Mael SUMOylation in S2 cells and female germ cells, related to Figure 5. A) Western blot analysis (WB) protein SUMOylation in Drosophila S2 cells. Indicated GFP-tagged proteins were overexpressed in S2 cells alongside 3xFlag3xHA tagged SUMO, and cell lysates were subjected to GFP immunoprecipitation followed by WB detection first with anti-Flag antibody, and after stripping, anti-GFP antibody. Asterisks indicate the full length unmodified GFP-tagged protein form (red) or putative SUMO-modified form (green). Arrows indicate putative degradation products of unmodified (red) and SUMO-modified (green) GFP-tagged protein. Images are representative from two independent biological replicates. B) (Left) Additional biological replicates as described in Fig 5.A,C. (Right) Quantification of Western Blot signal of combined data from panels 5A, 5C, showing modified protein abundance change relative to control ovaries (shW). Numbers indicate p-values obtained by t-test (for Piwi) or 1-way ANOVA followed by Dunnett's multiple comparisons test; error bars reflect SD.