



**Figure 2-figure supplement 3. AAGAG RNA and not CUCUU RNA is substantially decreased in Bam-GAL4 driven AAGAG RNAi, and AAGAG RNA levels are increased in rescue experiments.** **a**, Although visibly absent in embryos and somatic larval tissues, CUCUU RNA (green) is expressed in adult spermatocytes. Note that CUCUU RNA is localized to the S5 lumen, internal to the chromatin (DAPI), in contrast to the peripheral localization of AAGAG RNA (see Figure 3b); DNA = DAPI (blue). **b**, Projections of AAGAG foci (magenta) in S5 spermatocytes after Bam-GAL4 driven Scrambled control or AAGAG RNAi. Signal was imaged with the same laser intensities for each genotype. **c**, Average median intensities (arbitrary units,  $\pm$  st. dev.) of AAGAG RNA,  $p=2 \times 10^{-5}$  and CUCUU RNA in S5 spermatocytes in AAGAG and Scrambled RNAi testes (not significant). This represents a 72% reduction of AAGAG RNA in S5 spermatocytes after AAGAG RNAi, compared to scrambled controls, with little to no decrease in CUCUU RNA. **d**, Average intensity of AAGAG RNA in S5 spermatocytes after AAGAG RNAi increases significantly ( $p=0.03$ ) upon co-expression of AAGAG(37) RNA (also induced by the Bam-Gal4 driver). two tailed, type three ttest used for all.