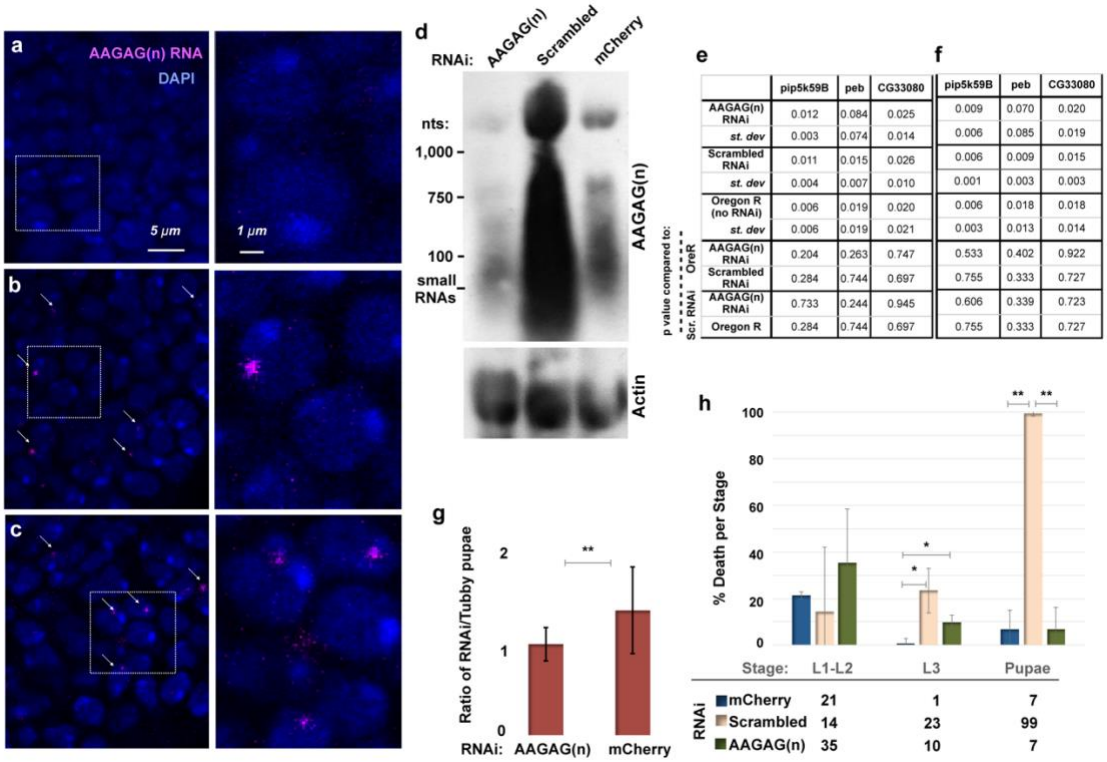

	pip5k59B	peb	CG33080	pip5k59B	peb	CG33080
AAGAG(n) RNAi	0.012	0.084	0.025	0.009	0.070	0.020
st. dev	0.003	0.074	0.014	0.006	0.085	0.019
Scrambled RNAi	0.011	0.015	0.026	0.006	0.009	0.015
st. dev	0.004	0.007	0.010	0.001	0.003	0.003
Oregon R (no RNAi)	0.006	0.019	0.020	0.006	0.018	0.018
st. dev	0.006	0.019	0.021	0.003	0.013	0.014
AAGAG(n) RNAi	0.204	0.263	0.747	0.533	0.402	0.922
Scrambled RNAi	0.284	0.744	0.697	0.755	0.333	0.727
AAGAG(n) RNAi	0.733	0.244	0.945	0.606	0.339	0.723
Oregon R	0.284	0.744	0.697	0.755	0.333	0.727

Figure 1-figure supplement 6. AAGAG RNA is decreased and foci abolished in L3 with actin-GAL4 driven RNAi to AAGAG, without affecting levels of genes whose mRNAs contain short runs of AAGAG. Also, AAGAG RNAi results in lethality. a-c Left column- section of brain lobes stained with DAPI (blue) and RNA-FISH to AAGAG RNA (magenta) imaged with the same intensity. Arrows point to AAGAG RNA foci. Right column- expanded images of left column indicated by dashed square. **a,** Brain lobe from AAGAG RNAi **b,** Brain lobe from scrambled RNAi. **c**, Brain lobe from mCherry RNAi **d**, Northern blot with probes to AAGAG RNA or actin-5c in L3 RNAi. The AAGAG RNAi L3 AAGAG RNA top band signal is approximately 86% and 75% reduced compared to either scrambled or mCherry controls, respectively, when normalized to the actin-5c loading control. **e,** RNA transcript levels (qRT-PCR) for euchromatic genes whose mRNAs contain short runs of AAGAG (pip5k59B, peb, CG33080). Numbers are means (from three biological replicates) ± standard deviation, after normalization to either **e**, actin-5c loading control, or **f**, rpl32 loading control. t-tests were performed in comparison to Oregon R or scrambled RNAi controls. This demonstrates that RNA levels of the few mRNAs containing an AAGAG sequence are not affected by AAGAG RNAi, ruling out the possibility that the observed lethality is due to off-target effects. **g,** Ratio of pupae containing RNAi (driven by ubiquitous actin-GAL4 driver) or Tubby control, demonstrating lethality in AAGAG RNAi prior to the adult stage. **h,** For embryos that hatch, death rates in larval and pupal stages differ after RNAi depletion of AAGAG, scrambled and mCherry controls (driven by ubiquitous actin-GAL4 driver). Note that death rate per stage is a measure of death only for those that survive to the indicated stage. **p≤0.01, *p≤0.05; error bars = SD; two tailed, type three ttest.