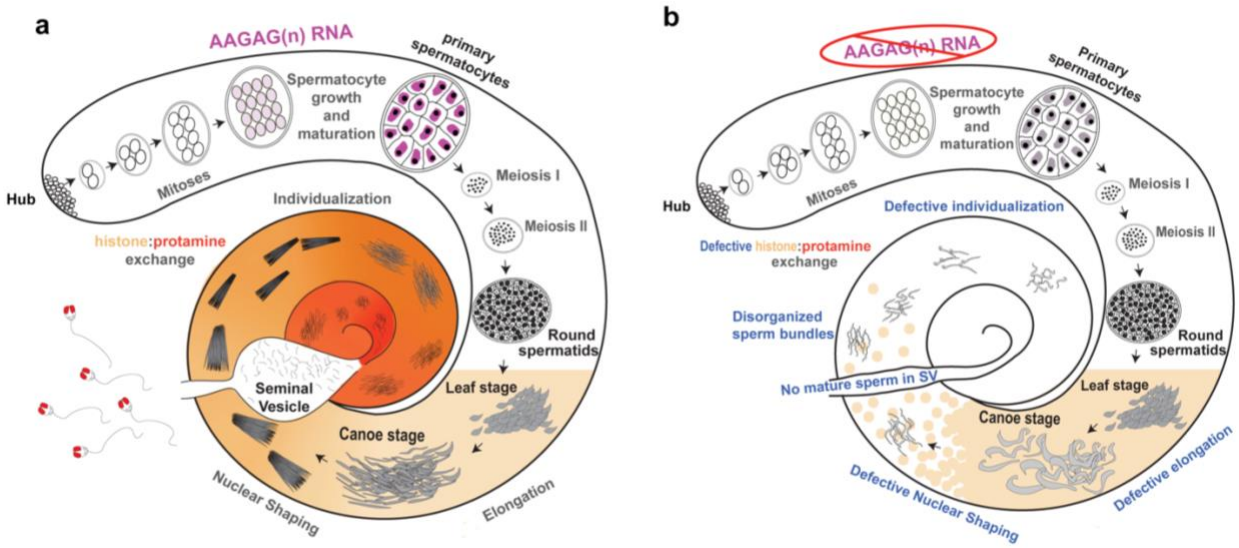

Figure 2-figure supplement 1. Overview of normal spermatogenesis and defects observed after AAGAG RNA depletion. a, Spermatogenesis in *Drosophila melanogaster* initiates at the apical end of the testes (Hub), where GSCs divide asymmetrically, producing gonialblasts (GBs) that begin cell-differentiation. GB cells then undergo four mitotic divisions with incomplete cytokinesis to produce a cyst of 16 primary spermatocytes. Spermatocytes then undergo pre-meiotic S phase, mature during a prolonged G2 phase, and increase substantially in volume. The majority of testes-specific gene expression occurs at the primary spermatocyte stage, while genes not required until later stages are translationally repressed (reviewed in (White-Cooper 2010)). Mature spermatocytes then undergo two rounds of meiosis to produce round spermatids(McKee et al. 2014), which are then processed into independent, condensed sperm nuclei in two stages(Rathke et al. 2014; Eren-Ghiani et al. 2015; Steinhauer 2015). First, round spermatids undergo chromatin compaction, acrosome formation and flagellar elongation(Rathke et al. 2014; Eren-Ghiani et al. 2015). During chromatin compaction, a wave of histone H4 acetylation occurs, followed by deposition of the transition protein Mst77f.(Kost et al. 2015). Next, transition proteins are removed followed by the incorporation of protamines and prtl99c (histone:protamine exchange, indicated by tan to deep orange gradient)(Rathke et al. 2014; Eren-Ghiani et al. 2015). Finally, spermatid individualization involves removal of cytoplasm and tight condensing and coiling of chromatin(Steinhauer 2015). Mature sperm are then stored in the seminal vesicle. **b,** Summary of defects in late stages of spermatogenesis observed after depletion of AAGAG RNA by RNAi, using the Bam-Gal4 driver (data in Figure 3). Although AAGAG RNA is not visible in normal testes after the S6 spermatocyte stage (see **a**), RNAi depletion of AAGAG RNA only produces visible defects after the round spermatid stage. Aberrant elongation, sperm bundles, and defective histone:protamine exchange likely cause the observed complete absence of mature sperm in the SV.