



Supplementary Figure 14. Spermatogenesis in *prosa4T1[SK2]* homozygotes. (A-E) In wild-type testes, the nuclei of differentiated spermatids reside in the basal region of the testis, and flagella extend apically. (A) Each nuclear bundle forms an actin-rich individualization complex (IC), and the ICs (red, phalloidin, arrows) move away from the nuclear bundles (blue, DAPI), traversing the sperm tails and eventually forming waste bags in the apical region (arrowheads). (B) Individualizing cysts stain positively for active caspase (green, cleaved Caspase-3) as they enact an apoptosis-like program to degrade extruded cytoplasm. (C) Differentiated spermatid nuclei incorporate protamines (green, protamine-GFP). (D) ICs (red, phalloidin, arrows) are visible forming around bundled basal nuclei (D', arrows). Following individualization, mature sperm are coiled in the base of the testis, and mature sperm nuclei become tightly compacted (D' arrowheads). (E) Progressing ICs are highly organized during their transit. (F-J) In *prosa4T1* mutants, normal Caspase-3 staining is seen in individualizing spermatids (G), protamines are incorporated normally into the maturing spermatid nuclei (H), and ICs are formed around bundled nuclei of individualizing spermatids (I, arrows). However, progressing ICs (J) and nuclei in the coiling region (I', arrowheads) are disorganized. (K) In the germinal proliferation center at the apical tip of the testis, a-spectrin staining reveals the round spectrosomes (green, arrowheads) of the germline stem cells at the hub (asterisk) and of the gonialblasts. Branched fusomes are observed in mitotic spermatogonia (green, arrows). (L) Phase contrast imaging of testis squashes reveals the post-meiotic (pre-individualization) spermatids, each with one phase-dark mitochondrial aggregate per phase-light nucleus. (M) Mature sperm are deposited in the seminal vesicle for storage until mating (DAPI). (N-P) *prosa4T1* mutants show normal a-spectrin staining (N) and normal post-meiotic spermatids (O). However, mutant seminal vesicles are devoid of mature sperm (P). Scale bars are 20 μ m.