

Supplementary Information

Figure S1

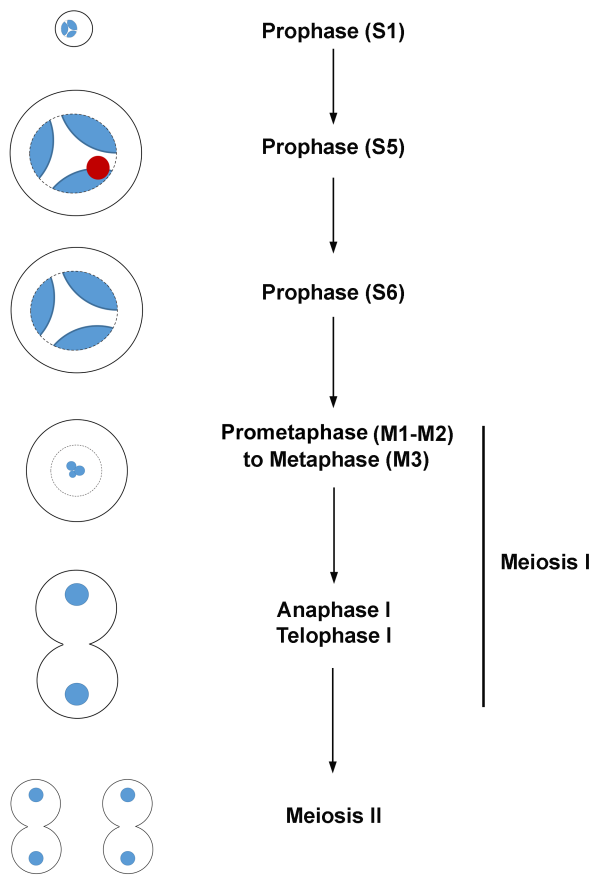


Figure S1. Stages of *Drosophila* male meiosis during spermatocyte prophase and meiotic division. Schematic showing the stages of *Drosophila* male meiosis. The characteristic chromatin features are depicted in blue (based on Cenci et al. 1994). The red circle represents the nucleolus in spermatocytes at Stage S5.

Figure S2

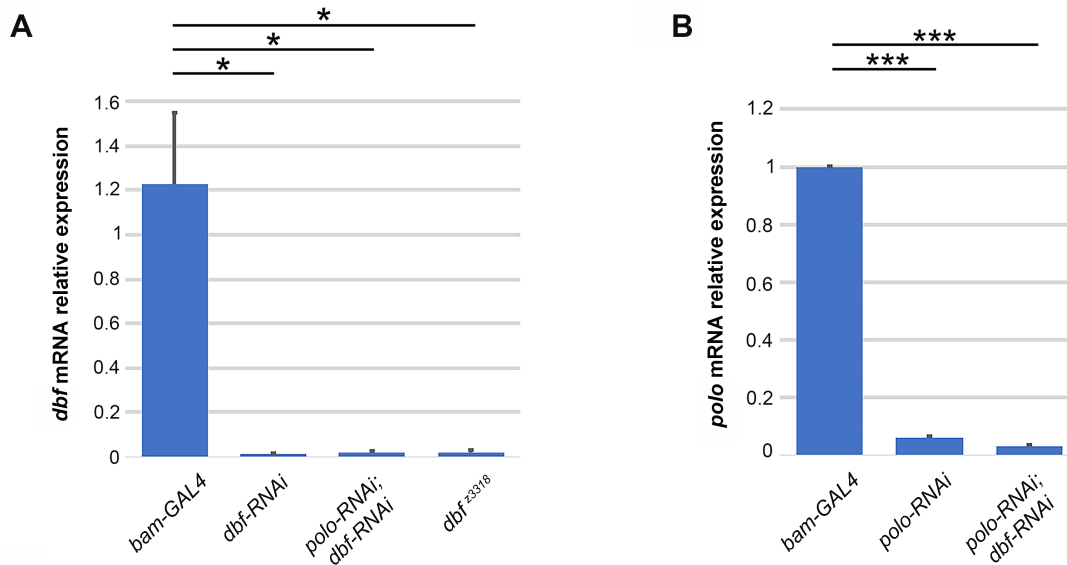


Figure S2. RT-qPCR quantification of the effects of RNAi on *dbf* and *polo* mRNA. (A,B) Quantification of *dbf* and *polo* mRNA expression by RT-qPCR, in testes from males of the indicated genotypes. Relative expression levels were normalized to actin and to control siblings (*bamGAL4*). Mean values of three independent experiments. Error bars indicate SD. Statistically significant differences, * $p < 0.05$; *** $p < 0.0001$ (unpaired Student's *t*-test).

Figure S3

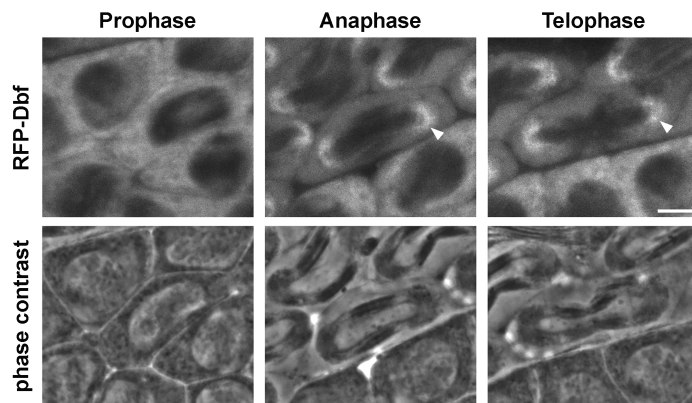


Figure S3. Live imaging of spermatocytes expressing RFP-Dbf. Fluorescence and corresponding phase contrast micrograph of live wild-type spermatocytes expressing RFP-Dbf during prophase, anaphase and telophase. Arrowheads point to the enrichment of RFP-Dbf at the spindle poles. Scale bar, 10 μ m.

Figure S4

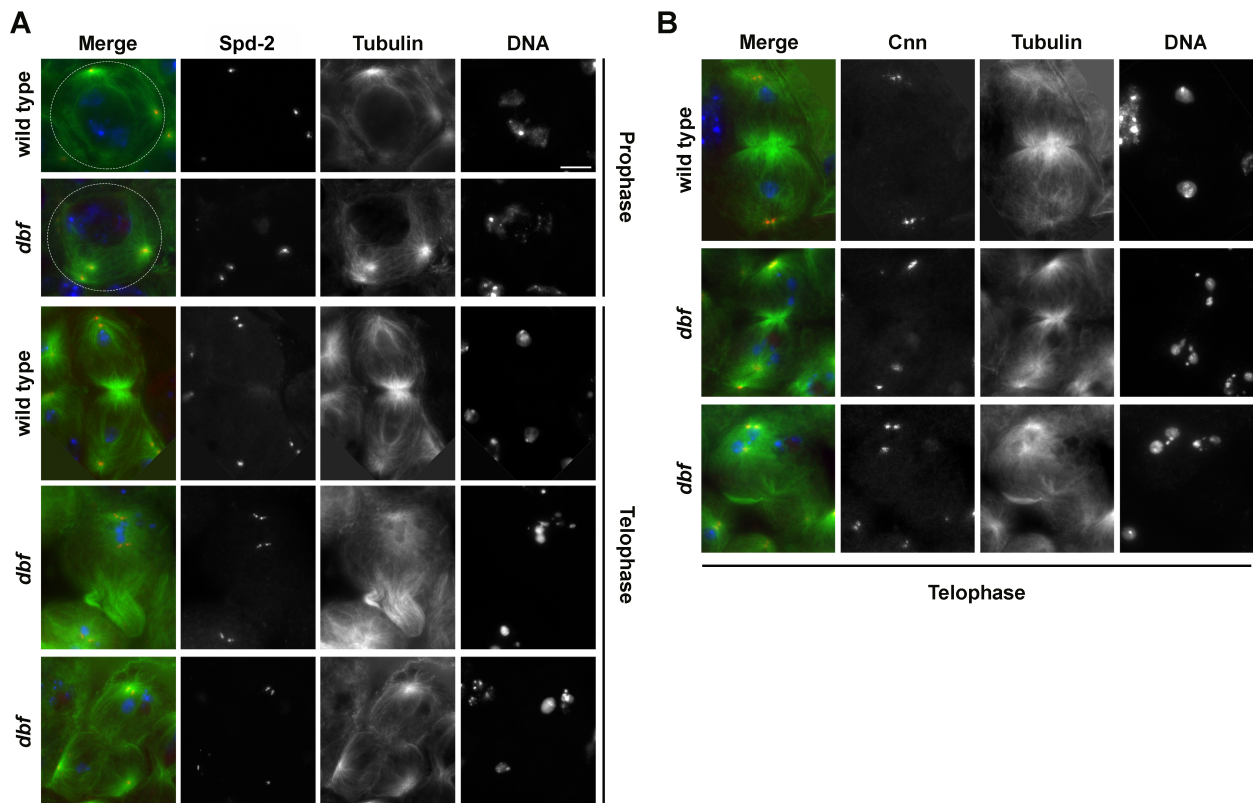


Figure S4. *dbf* mutant spermatocytes display a variable number of centrosomal foci. (A) Wild-type and *dbf³³¹⁸/Df(2L)Exel802* (*dbf*) mutant spermatocytes stained for Spd2 (red), α -tubulin (green) and DNA (blue). N=35 wild-type and N=36 *dbf* mutant spermatocytes at Prophase (Stage S6); N=39 wild-type telophase and N=41 *dbf* telophase spermatocytes. Cells were randomly selected from images taken in 5 independent experiments. (B) Wild-type and *dbf³³¹⁸/Df(2L)Exel802* (*dbf*) mutant spermatocytes stained for Cnn (red), α -tubulin (green) and DNA (blue). N=48 wild-type telophase spermatocytes; N=44 *dbf³³¹⁸/Df(2L)Exel802* mutant telophase spermatocytes randomly selected from images taken in 5 independent experiments. Scale bars, 10 μ m.

Figure S5

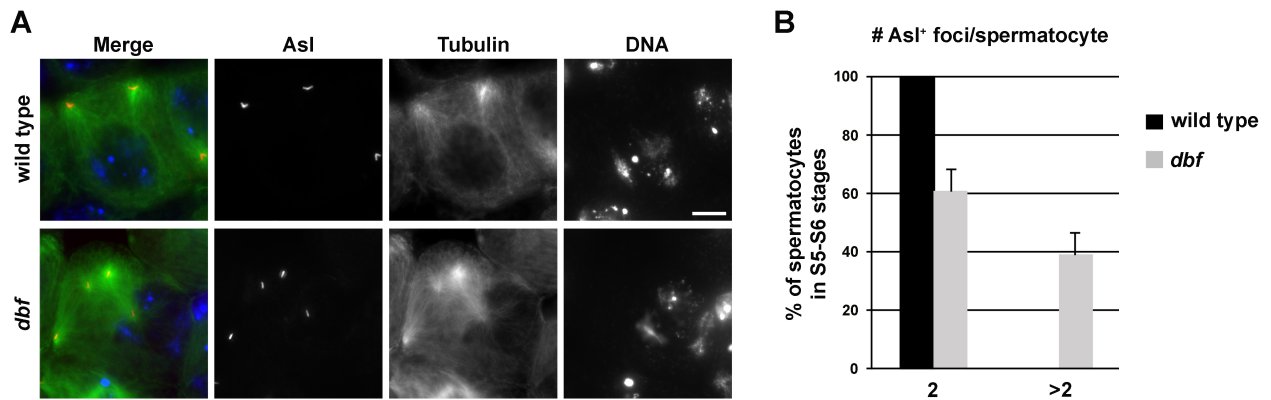


Figure S5. Mutant spermatocytes in *dbf* display premature centriole disengagement. (A) Wild-type and *dbf*³³¹⁸/*Df(2L)Exel802* (*dbf*) mutant spermatocytes at prophase (stage S6), stained for the centriolar protein Asterless (Asl, red), α -tubulin (green) and DNA (blue). Scale bar, 10 μ m. (B) Graph of percentage of stage S5-S6 spermatocytes containing two or more Asl-labeled foci. N=41 *dbf* mutant spermatocytes and N=50 wild-type spermatocytes at stages S5-S6, randomly selected from images taken in 6 experiments.

Figure S6

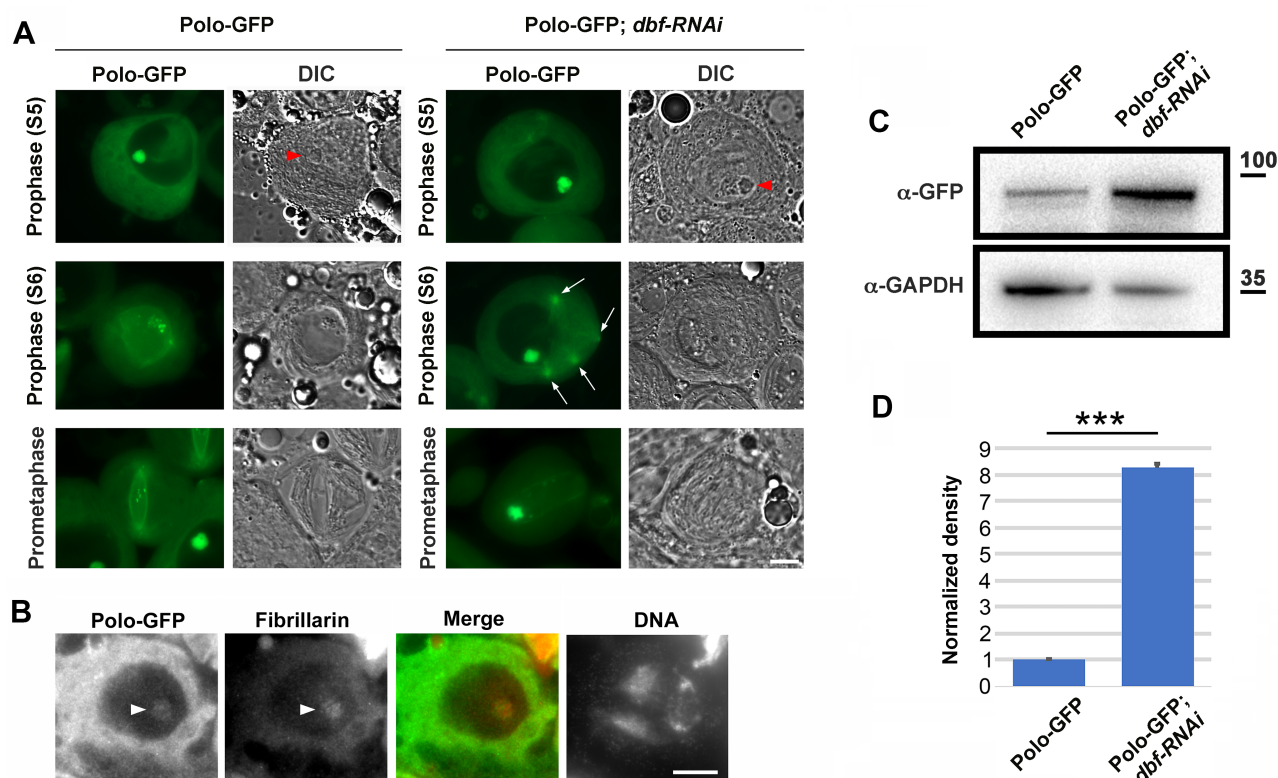


Figure S6. Polo-GFP localization in wild-type and *dbf* mutant spermatocytes. (A) Differential interference contrast (DIC) and corresponding fluorescence micrographs of live squashed spermatocytes expressing Polo-GFP. Spermatocytes depleted of *Dbf*: *Polo-GFP/+; UAS::dbf-RNAi/ bam-GAL4* (*Polo-GFP; dbf-RNAi*) and control spermatocytes, *Polo-GFP/+; bam-GAL4/+* (*Polo-GFP*) were imaged at the same exposure time. (Arrows) Polo-GFP signal at the centrosomes, (Red Arrowheads) nucleolus. Scale bar, 10 μ m. (B) Spermatocytes expressing Polo-GFP at stage S5, immunostained for GFP (green), Fibrillarlin (red) and DNA. (White Arrowheads) Polo-GFP and Fibrillarlin signals at the nucleolus. Scale bar, 10 μ m. (C) Western blot of testis extracts from males expressing Polo-GFP of the genotypes described in A. Molecular masses in kilodaltons. GAPDH was used as a loading control. (D) Quantification of Polo-GFP levels in Western blots from adult testes of males expressing Polo-GFP, of the genotypes described in A. Protein band intensities obtained from three independent experiments. Error bars indicate SD. Statistically significant difference is *** $p < 0.0001$ (unpaired Student's *t*-test).

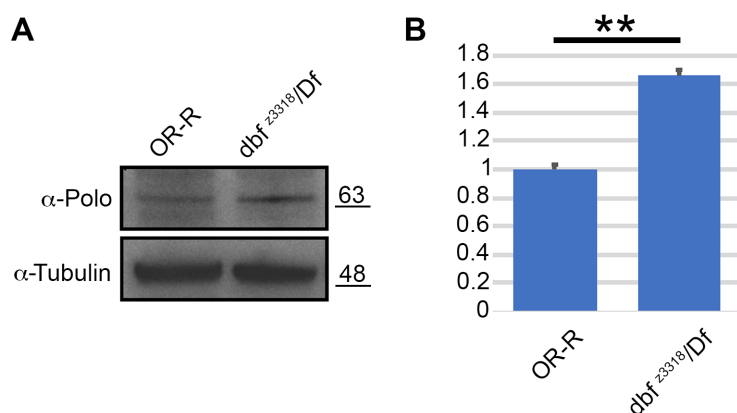
Figure S7

Figure S7. Polo protein levels were reduced in *dbf* mutant testes. (A) Western blot of testis extracts from wild-type and $dbf^{3318}/Df(2L)Exel802$ (*dbf*) mutant males. Molecular masses in kilodaltons. α -Tubulin was used as a loading control. (B) Quantification of Polo protein levels in Western blots from adult testes of males of the genotypes described in A. Protein band intensities obtained from three independent experiments. Error bars indicate SD. Statistically significant difference, ** $p < 0,01$ (unpaired Student's *t*-test).