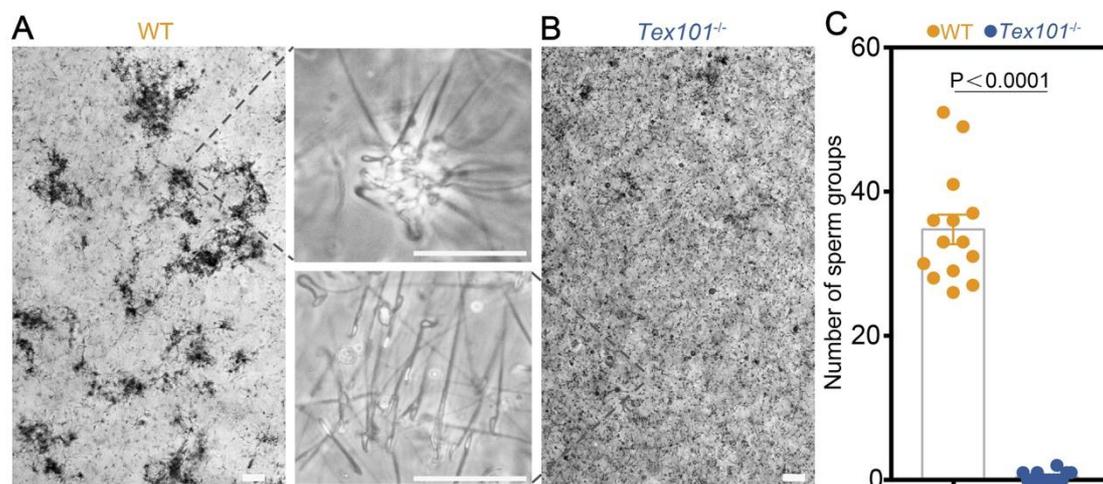
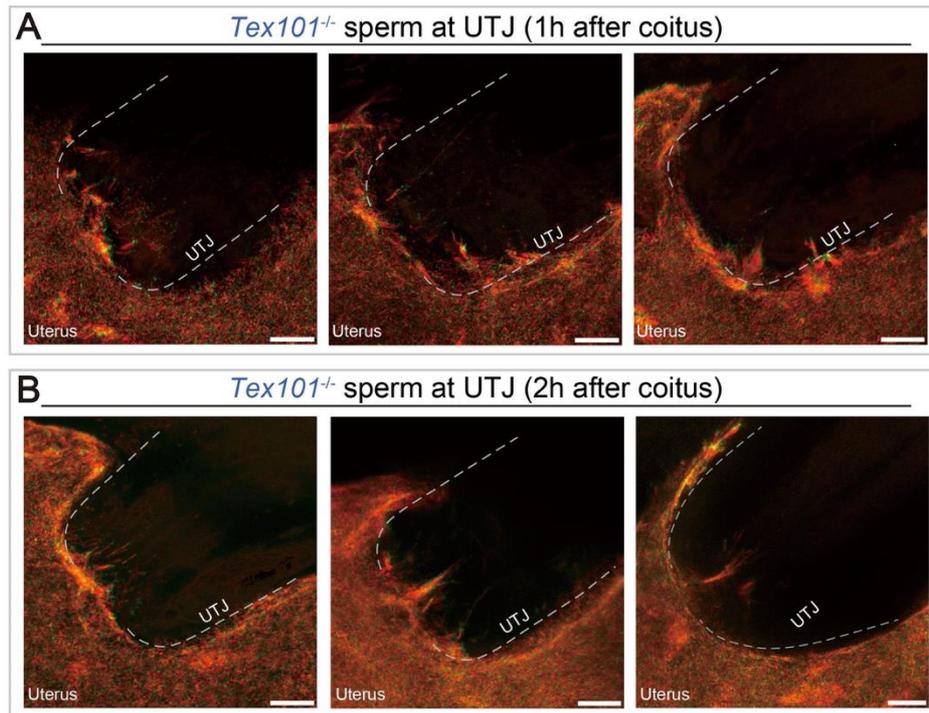


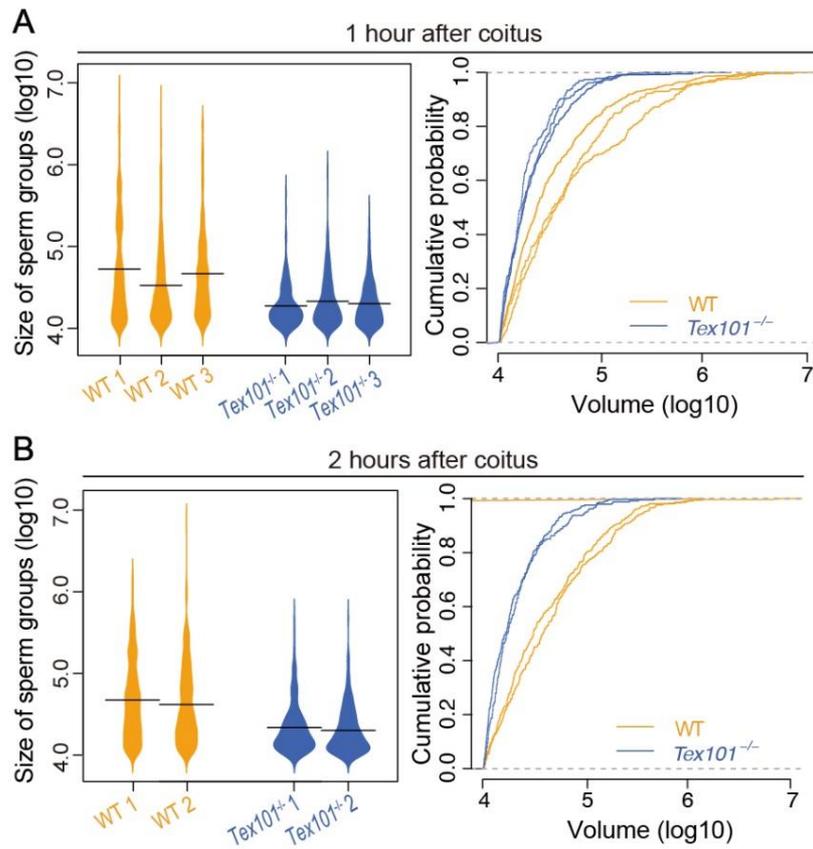
**Figure S1. WT sperm behavior at UTJ.** (A) WT sperm behavior at UTJ 1h after coitus. (B) WT sperm behavior at UTJ 2h after coitus. scale bars, 200µm.



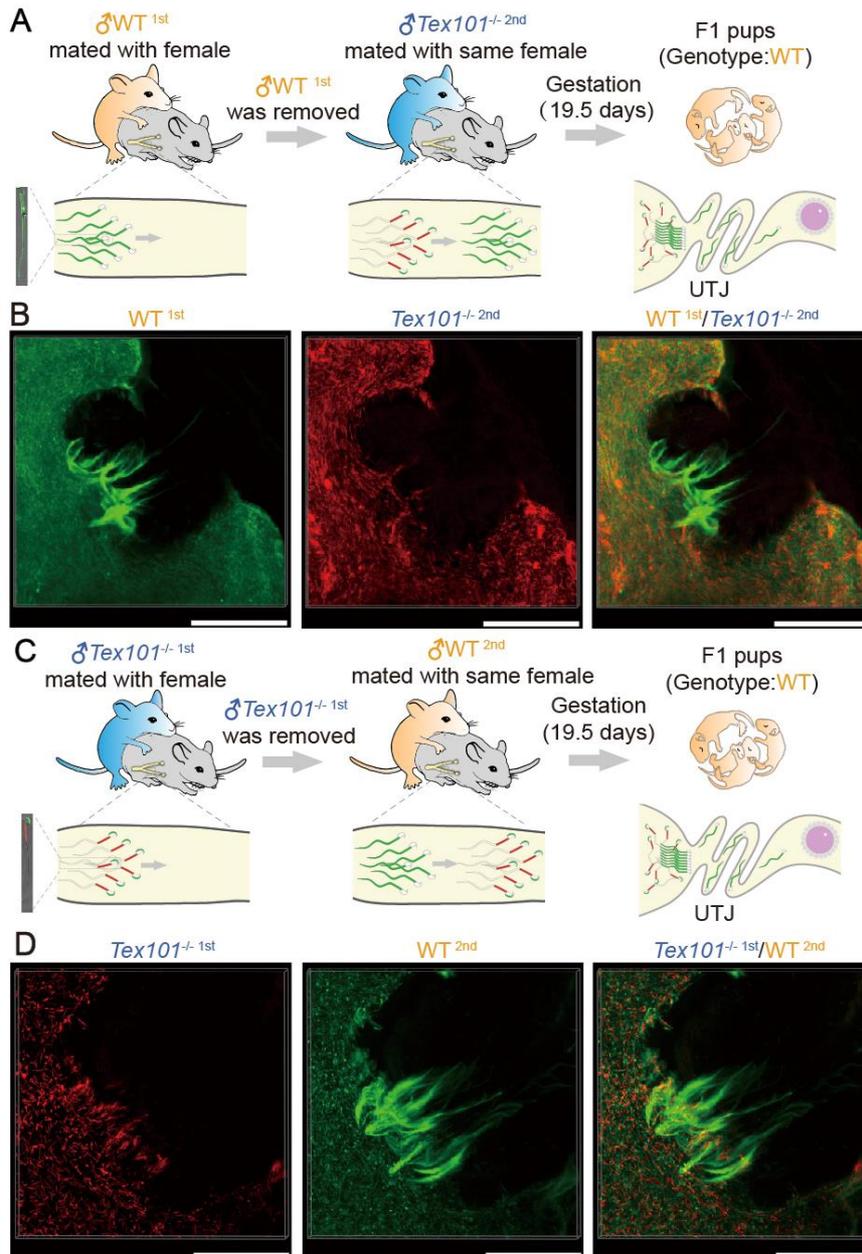
**Figure S2. Comparison of WT and *Tex101<sup>-/-</sup>* aggregation ability *in vitro*.** (A) WT sperm aggregate during *in vitro* culture. (B) *Tex101<sup>-/-</sup>* sperm show impaired aggregation. (C) Quantification of aggregated sperm groups (> 10 sperm per group) for WT and *Tex101<sup>-/-</sup>* mice. The results are shown as the mean  $\pm$  SEM, n = 14 different visual fields from 4 male mice each. Scale bars, 50µm.



**Figure S3.** *Tex101*<sup>-/-</sup> sperm behavior at UTJ. (A) *Tex101*<sup>-/-</sup> sperm behavior at UTJ 1h after coitus. (B) *Tex101*<sup>-/-</sup> sperm behavior at UTJ 2h after coitus. Scale bars, 200 $\mu$ m.



**Figure S4. Quantitative comparison of the size of sperm groups (volumetric analysis of the sperm fluorescent signal) in uterus.** WT sperm aggregated into sperm clusters and scattered *Tex101<sup>-/-</sup>* sperm at one (three WT and three *Tex101<sup>-/-</sup>* replicates) (A) and two hours (two WT and two *Tex101<sup>-/-</sup>* replicates) (B) after coitus, respectively. Left panel is a violin plot and the right panel is the cumulative distribution. Results are shown as the mean  $\pm$  SEM,  $n=3$  male mice in (A),  $n=2$  male mice in (B).



**Figure S5. Sequential mating experiments showing that sperm clustering is necessary for passing through the UTJ.** (A, C) Schematic diagrams of sequential mating experiment. In (A) the female mated first with the WT male and then with the *Tex101*<sup>-/-</sup> male (WT1st, *Tex101*<sup>-/-</sup>2nd). In (C) the female mated first with the *Tex101*<sup>-/-</sup> male and then with the WT male (*Tex101*<sup>-/-</sup>1st, WT2nd). (B, D) 3D visualization of fluorescently labeled WT and *Tex101*<sup>-/-</sup> sperm behavior at the UTJ after sequential mating. In (B) the female mated first with the WT male and then with the *Tex101*<sup>-/-</sup> male (WT1st, *Tex101*<sup>-/-</sup>2nd). In (D) the female mated first with the *Tex101*<sup>-/-</sup> male and then with the WT male (*Tex101*<sup>-/-</sup>1st, WT2nd). Scale bars, 200µm.