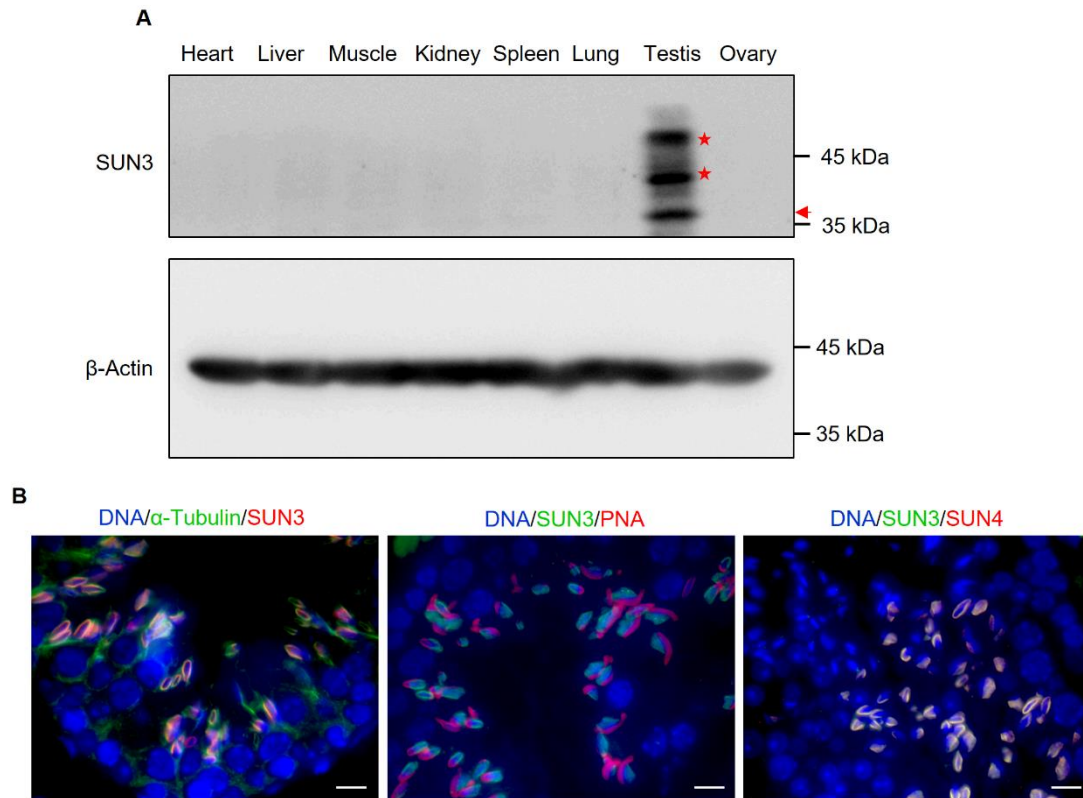
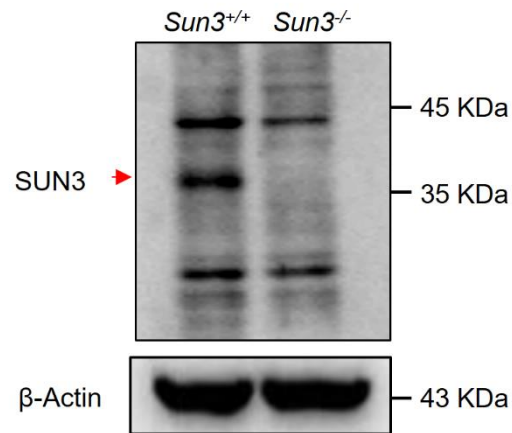


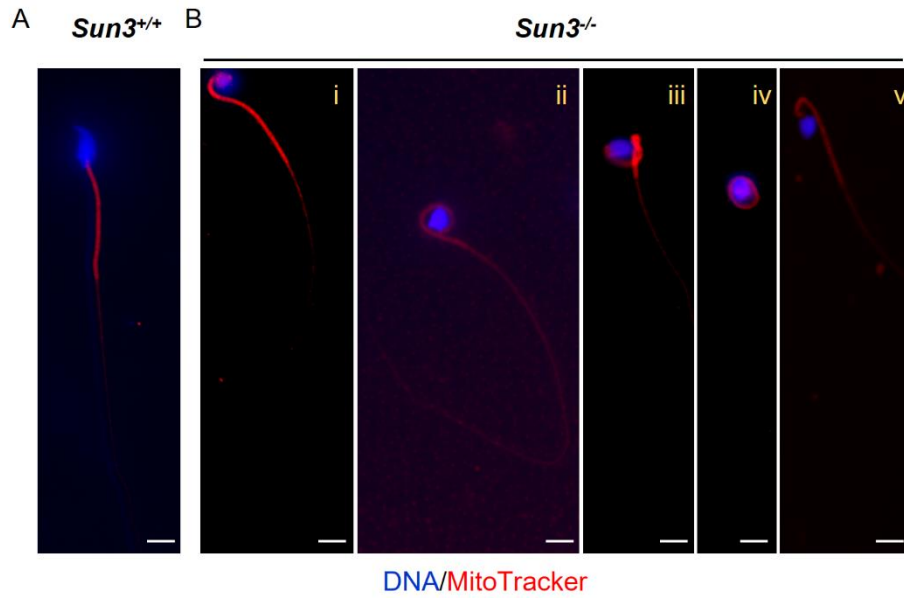
## Supplementary figures



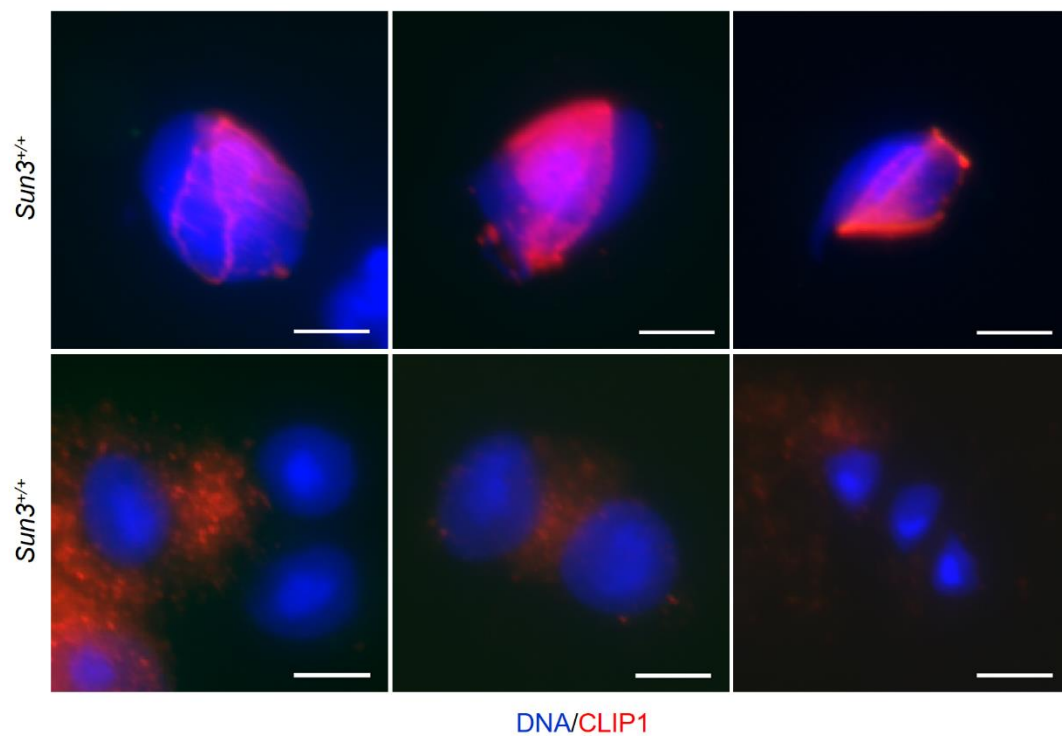
**Fig. S1. SUN3 is testis-specifically expressed and localizes in manchette.** (A) Western blotting using the guinea pig anti-SUN3 antibodies to detect SUN3 expression in different tissues. Arrows, band corresponding to the size of SUN3. Asterisks indicate non-specific bands. (B) Immunofluorescence staining of SUN3 in combination with  $\alpha$ -tubulin, PNA and SUN4 in testis section. Scale bars = 10  $\mu$ m.



**Fig. S2. Deletion of SUN3 proteins in *Sun3*<sup>-/-</sup> mice.** Western blotting from the testis lysates of *Sun3*<sup>+/+</sup> and *Sun3*<sup>-/-</sup> using the rabbit anti-SUN3 antibodies detected a band, indicated by arrow, corresponding to the size of SUN3 (37 kDa), in control mice but not in *Sun3*<sup>-/-</sup> mice.



**Fig. S3. Sperm midpiece defects in *Sun3*<sup>-/-</sup> mice.** (A) Representative image of epididymal sperm of *Sun3*<sup>+/+</sup> mice with a normal morphology of midpiece, as labeled with mitotracker (red). (B) Midpiece, as labeled with mitotracker (red) of epididymal sperm from *Sun3*<sup>-/-</sup> mice displayed various defects, including thickened (i), coiled (ii-v), thinned (iv-v) and cracked (iii). Scale bars = 5  $\mu$ m.



**Fig. S4. Disrupted formation of perinuclear rings in *Sun3*<sup>-/-</sup> mice.** Immunofluorescence staining of testis cell smears from 8-week-old *Sun3*<sup>+/+</sup> and *Sun3*<sup>-/-</sup> mice showing the perinuclear ring was disrupted in spermatids of *Sun3*<sup>-/-</sup> mice. Scale bars = 5  $\mu$ m.