

**Supplemental Figure 1.** Genomic structure, functional domains, and evolutionary conservation of RANBP17. **A**) RANBP17 is encoded by a gene (*Ranbp17*) which consists of 28 exons and is located on the reverse strand of chromosome 11. The 1088 a.a. RANBP17 contains two conserved functional domains: importin-β and armadillo (ARD)-type fold in its N-terminus. **B**) The phylogenetic tree of 16 mouse Ran-binding protein paralogs. **C**) Amino acid sequence conservation among 6 mammalian orthologous RANBP17. Humans and mice display ~93.8% sequence identity in this protein.



**Supplemental Figure 2.** Multiple alignment analyses of 6 mammalian RANBP17 orthologs. Conserved residues are shaded in black.



**Supplemental Figure 3.** High-power confocal images (upper 6 panels) showing RANBP17 localization to the XY body in pachytene spermatocytes and to the manchette in step 12 spermatids in adult mouse testes. All upper 6 panels are in the same magnification and the scale bar represents 20µm. The subcellular localization of RANBP17 during spermatogenesis is schematically summarized in the lower panel (painted in green). Roman numerals indicate stages of the seminiferous epithelial cycles. As, single type A spermatogonium; Apr, paired type A spermatogonium; Aal, Aligned type A spermatogonium; A1-4, type A1-A4 spermatogonium, B, type B spermatogonium, In, Intermediate spermatocyte; Di, diplotene spermatocyte; L, leptotene spermatocyte; Z, zygotene spermatocyte; P, pachytene spermatocyte; Di, diplotene spermatocyte; M, meiotically dividing spermatocyte; Arabic numbers indicate steps of developing spermatids.



**Supplemental Figure 4.** Co-localization of RANBP17 and  $\gamma$ H2Ax to the XY body in pachytene spermatocytes. Red immunofluorescence represents the RANBP17 immunoreactivity and green for that of  $\gamma$ H2Ax. The nuclei were counter-stained with DAPI (blue). Arrows indicate the XY body in pachytene spermatocytes and arrowheads point to the manchette-like structure in elongating spermatids. Roman numerals indicate stages of the seminiferous epithelial cycles. Panels in the same row are in the same magnification and the scale bars represent 20 $\mu$ m.



**Supplemental Figure 5.** RANBP17 and  $\beta$ -Tubulin remain localized to the manchette of *Spem1*-null elongating spermatids. Green fluorescence represents the RANBP17 immunoreactivity and red fluorescence indicates the immunoreactivity of  $\beta$ -Tubulin, a marker for the manchette. Cell nuclei were counterstained using DAPI (blue). Note that the patchy and uneven-looking of both RANBP17 and  $\beta$ -Tubulin immunofluorescent signals in *Spem1*-null manchette comparing to their smooth and even staining patterns of the wild-type manchette shown in Figs. 5 and 6. Arabic numbers on the left stand for steps of the developing spermatids. All panels are in the same magnification. Scale bar = 20µm.