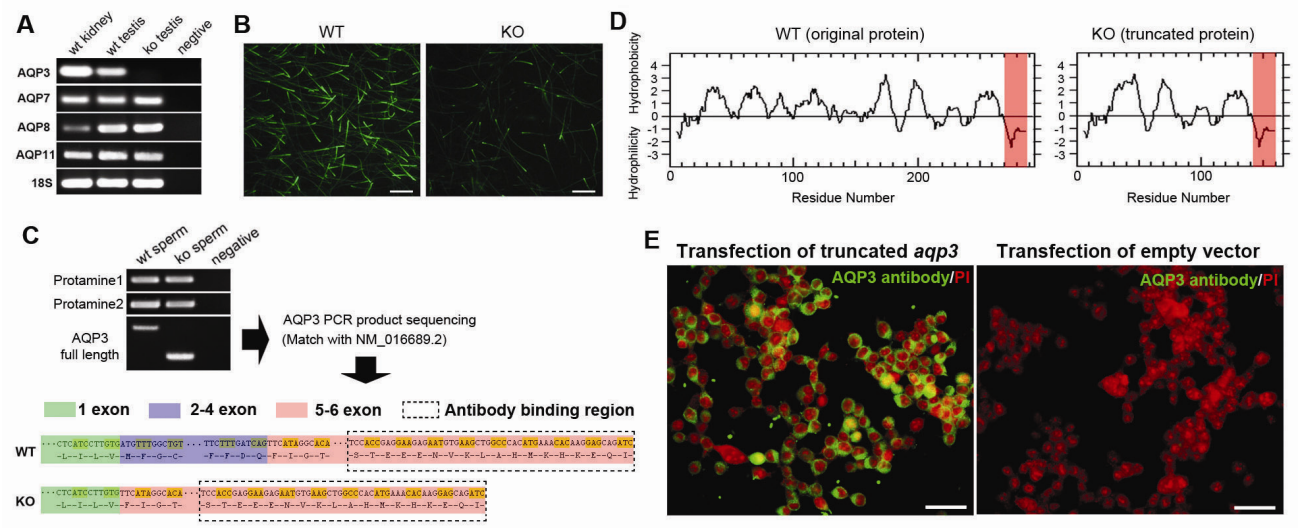
Supplementary information

Manuscript title: “Aquaporin3 is a sperm water channel essential for post-copulatory sperm osmo-adaptation and migration”

Author list: Qi Chen, Hongying Peng, Li Lei, Ying Zhang, Haibin Kuang, Yujing Cao, Qi-xian Shi, Tonghui Ma, Enkui Duan

Supplementary Figure S1



Supplementary Figures S1 AQP3 expression in testis and sperm. **A.** Semiquantitative RT-PCR detection of AQP3, 7, 8, 11 in testis. AQP3 primers designed within exon 3. **B**. Immunofluorescence detection of AQP3 in sperm. Scale bars represent 20 μm. It should be noted that sperm from *Aqp3*$^{-/-}$ mice also showed positive staining, although the intensity is lower. This is because the *Aqp3*$^{-/-}$ sperm could express a truncated aqp3 transcript as shown in panel (**C,D,E**). **C.** Full-length aqp3 PCR detection showed that sperms from both wild-type and *Aqp3*$^{-/-}$ mice express aqp3 mRNA, the *Aqp3*$^{-/-}$ sperm express a truncated aqp3 transcript lacking exons 2-4, retaining a full sequence of exon1 linking with exons 5-6, which could be translated into a nonfunctional peptide containing the antibody detection region (C-terminal). Protamine1 and 2 were used as an internal control for sperm mRNA. **D.** Kyte-Doolittle analyses showed hydrophobic scores of original and truncated AQP3 protein, suggesting that the truncated AQP3 protein is highly hydrophobic with three predicted transmembrane domain, leaving the hydrophilic C-terminal detectable by the antibody. The red shaded region demonstrate antibody detection region. **E**. The KO truncated *aqp3* was obtained and subcloned into the vector pcDNA4/TO/MycHis B (Invitrogen), then transfected into 293T cells using FuGENE (Roche) following manufacturer’s instructions. After Zeocin selection, cells were processed for AQP3 immunofluorence assay and showed cytoplasmic/membrane localization. These results explained the observed positive AQP3 staining in *Aqp3*$^{-/-}$ sperm. scale bar: 50μm. For each data presented, results were repeated in 2-3 independent mice.