# *Ubqln3*, a Testis-Specific Gene, is Dispensable for Embryonic Development and Spermatogenesis in Mice

Increasing lines of evidence suggest that the ubiquitin-proteasome pathway (UPP) plays a key role in spermatogenesis (Hou and Yang, 2013). Ubiquilins are ubiquitin-like proteins, all of which contain an amino-terminal UBL domain and a carboxy-terminal ubiquitin-associated (UBA) domain in its structure. Among all ubiquilin proteins identified so far, Ubiquilin 3 is the only one that has been reported to be testis-specific (Conklin et al., 2000). By examining the expression of *Ubqln3* mRNAs and protein in eight different mouse organs using quantitative PCR (qPCR) and Western blot, we found that *Ubqln3* was indeed expressed exclusively in the testis, with both transcript and protein detectable in the testes starting at postnatal Day 28 (P28); the highest levels were detected in adult testes (Fig. 1A–D).

The onset of testicular *Ubqln3* expression at P28 coincides with the elongation steps during late spermiogenesis, suggesting that *Ubqln3* is mainly expressed in elongating/elongated spermatids. To define its physiological role in the testis, we generated *Ubqln3* global knockout mice (on the C57BL/6J background) using a targeted embryonic stem cell line (cell-line number 11472C-G12) obtained from the Knockout Mouse Project repository; in this case, a gene-trap cassette (LacZ-pA) had recombined with exon 2 of *Ubqln3*, leading to a ''gene trap'' allele (Fig. 1E). Neither *Ubqln3* mRNAs (Fig. 1F) nor UBQLN3 protein (Fig. 1B) was detected in homozygous knockout mice, suggesting that these animals are truly *Ubqln3*-null (herein called *Ubqln3* KO mice). Both male and female *Ubqln3* KO mice were viable and displayed normal development, with no discernible differences in growth compared to wild-type (WT) mice. Together, these data indicate that *Ubqln3* is dispensable for embryonic and postnatal development in mice.

To determine the fertility of *Ubqln3* KO males, we performed a fecundity test using *Ubqln3* KO males bred with fertility-proven adult WT females. Our breeding data showed that no significant difference in either litter size or litter interval compared to those WT breeding pairs over a 6-month period (data not shown), suggesting that *Ubqln3* KO males are fertile. Consistent with their normal fertility, both sperm count and sperm motility are comparable between gametes isolated from *Ubqln3* KO and WT males (Fig. 1G–H) and histological analyses of *Ubqln3* KO testes and sperm demonstrated normal spermatogenesis and sperm morphology (Fig. 1I–J). Taken together, these data indicate that *Ubqln3* is not required for male germ-cell development or spermatogenesis in mice. Interestingly, *Ubqln1*, *Ubqln2*, and *Ubqln4* transcript abundance was increased significantly in *Ubqln3* KO compared to WT testes (Fig. 1F), suggesting that other members of the ubiquilin family may compensate for the loss of *Ubqln3*. Therefore, the testis-specific expression of *Ubqln3* is dispensable for both embryonic development and spermatogenesis in mice.

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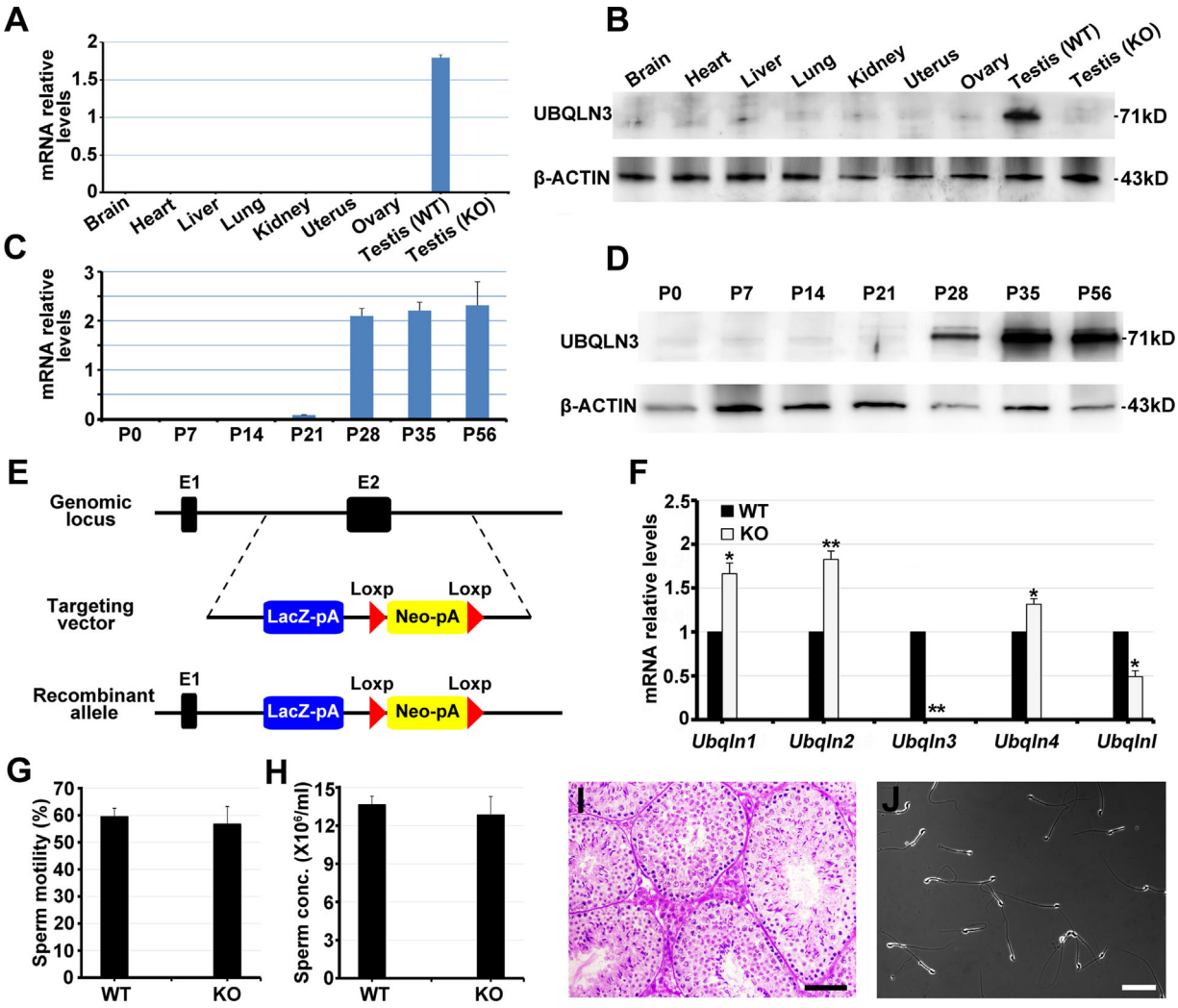


**Figure 1.** *Ubqln3* is a testis-specific gene dispensable for spermatogenesis. **A**: qPCR analyses of *Ubqln3* mRNA levels in eight organs of adult mice. **B**: A representative Western blot showing that UBQLN3 is exclusively detected in wild-type (WT) but not in *Ubqln3* KO testes. (The rabbit polyclonal UBQLN3 antibody (1:1000) was purchased from ProteinTech Group, Inc. [Chicago, IL], catalog number 13568-1AP.) **C**: qPCR analyses of *Ubqln3* mRNA levels in developing testes. Testes at postnatal Day 0 (P0, newborn), P7, P14, P21, P28, P35, and P56 were analyzed. **D**: A representative Western blot showing UBQLN3 expression in developing mouse testes. **E**: Schematic illustration of the targeting strategy for generating a *Ubqln3*-null allele in mouse embryonic stem cells. **F**: qPCR assays showing mRNA levels of five ubiquilin-family genes in *Ubqln3* KO testes. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to WT (n = 3, Student's t-test). **G** and **H:** Normal sperm concentration and sperm motility in *Ubqln3* KO males. **I**: Normal histology in *Ubqln3* KO testes. **J***:* Representative phase-contrast micrograph showing *Ubqln3* KO sperm. Scale bars, 100 μm (I) or 50 μm (J).