Stk31 is Dispensable for Embryonic Development and Spermatogenesis in Mice

Increasing lines of evidence suggest that members of the Tudor domain-containing protein (TDRD) family are critical regulators of germinal granule assembly and transposon control (Pek et al., 2012). Our previous work has shown that serine-threonine kinase 31 (STK31), also called TDRD8 due to the presence of the typical Tudor domain, is exclusively expressed in spermatogenic cells in mice (Bao et al., 2012). STK31 is localized to the cytoplasm of spermatocytes enriched in germinal granules, also called nuage or intermitochondrial cement, and STK31 physically interacts in vivo with MIWI, the murine ortholog of PIWI, (Bao et al., 2012).

To define the physiological role of *Stk31*, we generated *Stk31* global knockout (KO) mice (*Stk31*$^{-/-}$) using a targeted embryonic stem cell clone (EPD0641_1_C02) obtained from the KOMP Repository, in which a "knockout first" (FRT-LacZ-Neo-FRT) cassette was inserted in between exons 6 and 7 of the *Stk31* gene (Fig. 1A). F5 offspring in the C57BL/6J background were inter-crossed for subsequent KO phenotype characterization. Genotyping and quantitative PCR (qPCR) analyses demonstrated that *Stk31* was successfully inactivated (Fig. 1B,D). STK31 protein was not detected in the testes of *Stk31*$^{-/-}$ male mice using Western blots or immunofluorescent staining (Fig. 1C,G), further confirming that the homozygous mice are truly *Stk31*-null. Both female and male *Stk31*-null mice developed normally, with no discernable differences in growth or behavior compared to their wild-type or heterozygous littermates. These data suggest that *Stk31* is dispensable for embryonic and postnatal development.

A 4-month-long fertility test using *Stk31*-null male mice bred with wild-type female mice revealed no significant difference in either litter size or litter interval compared to wild-type breeding pairs (data not shown), suggesting that *Stk31*-null males are completely fertile. Consistent with their normal fertility, testis size and weight of adult *Stk31*-null males were similar to those of wild-type males, and histological analyses of *Stk31*-null testes demonstrated normal seminiferous epithelial structure and full spermatogenesis (Fig. 1E). Taken together, our data indicate that *Stk31* is not essential for male germ-line development and spermatogenesis in mice.

Given that normal testicular morphology and histology may not necessarily correlate with normal cellular function in the testis, we also examined localization patterns of MIWI (an interacting partner of STK31) (Fig. 1I); TDRD1 (a marker protein for nuage) (Fig. 1H); DDX4 (also called MVH for mouse Vasa homolog, a germ cell marker enriched in the nuage of spermatocytes and the chromatoid body of round spermatids) (Fig. 1J); and Y-H2AX (a marker for DNA double strand breaks and normal meiotic sex chromosome inactivation) (Fig. 1K) (Yan and McCarrey, 2009) in *Stk31*-null testes. All of these protein markers showed the characteristic localization pattern observed in wild-type testes.

In summary, our data demonstrate that *Stk31* is dispensable for both embryonic development and spermatogenesis in mice. This notion by no means implies that STK31 does not have an important role in wild-type mice; however, its loss could have been compensated for by other proteins of the TDRD family, which consists of at least 10 members that are mostly expressed in the testis in mice (Pek et al., 2012).

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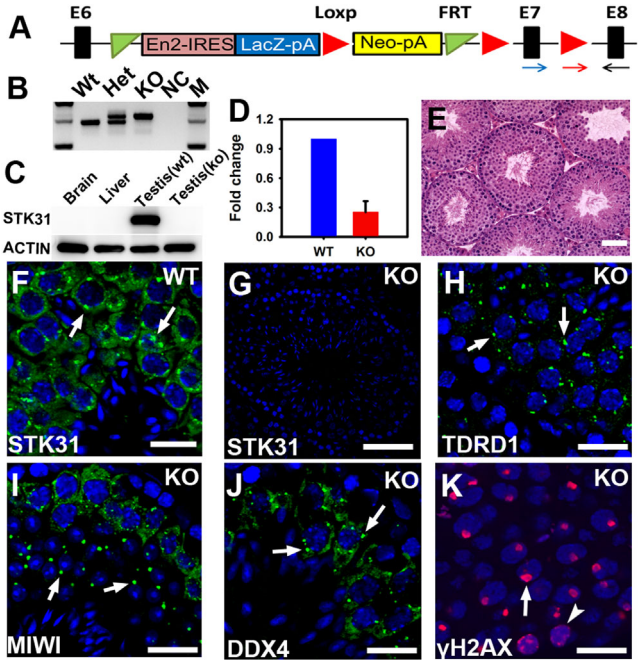

Figure 1. *Stk31* is dispensable for spermatogenesis. **A**: Targeting of the *Stk31* allele for complete inactivation of *Stk31*. **B**: A representative genotyping PCR result. **C**: A representative Western blot showing the complete absence of STK31 in *Stk31* KO testes. **D**: qPCR assays showing drastically decreased level of *Stk31* mRNA in *Stk31* KO testes. **E**: Normal histology in the *Stk31* KO testes. (**F**–**K**): Immunofluorescence staining of various marker proteins, as indicated. Scale bars, 50 μm (E and G) or 20 μm (F, H, I, J, and K).

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