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Deletion of kallikrein 1b5 (*Klk1b5*) has no impact on fertility in mice

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Kallikreins (KLKs) are a family of serine proteases responsible for many physiological functions in mammals. Of the various family members, KLK1 is well studied and found to be highly conserved among mammalian species. Aberrant gene expression of proteins in the KLK family were linked to asthma, hyperkalemia, artery dysfunction, and many other diseases (Prassas, Eissa, Poda, & Diamandis, 2015). KLK2–15 are known as kallikrein-related peptidases, which are expressed in a wide range of tissues with specialized roles. In rodents, an evolutionary gene duplication resulted in an insertion of 13 additional gene-encoding KLK1-related peptidase subfamily members (*Klk1b1–27*) between *Klk1* and *Klk15* loci (Olsson & Lundwall, 2002).

In the uterus, the expression of *Klk1* and its subfamily members are estrogen (E_2)-dependent. Accordingly, our previous work showed that *Klk1b5* is expressed at the highest level compared to other *Klk* members in the mouse uterus after E_2 treatment (Li, Garcia, Gewiss, & Winuthayanon, 2017). However, the physiological function of *Klk1b5* has never been investigated. As such, we generated a loss-of-function mouse model using CRISPR/Cas9 technology to determine the biological function of *Klk1b5*. A single guide RNA (sgRNA) was generated to target the beginning of the mature peptide coding sequence of exon 2 of the *Klk1b5* gene (Figure 1a). As a result, a 19 bp deletion was introduced to the 5' end of exon 2, causing a frameshift mutation (Figure 1a, bottom sequences). Genotyping of $Klk1b5^{+/+}$ alleles showed the amplicon with the product size of 290 bp, $Klk1b5^{-/-}$ with 271 bp, and $Klk1b5^{+/-}$ with both 290 and 271 bp (Figure. 1b).

Because the antibodies required to differentiate KLK1B5 from other KLK1Bs are not available, quantitative RT-PCR was used to validate the deletion of *Klk1b5* and the expression of other *Klk1b* family genes in highly-expressed tissues such as the

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

submandibular gland. As anticipated, *Klk1b5* mRNA was not detectable in the submandibular glands of *Klk1b5*$^{-/-}$ mice (Figure 1c). *Klk1* was significantly reduced in *Klk1b5*$^{+/-}$ and *Klk1b5*$^{-/-}$ compared to *Klk1b5*$^{+/+}$ mice. Conversely, expression of *Klk1b24* was significantly elevated in *Klk1b5*$^{+/-}$ and *Klk1b5*$^{-/-}$ tissues. There were also no significant differences in growth weight between *Klk1b5*$^{+/-}$, *Klk1b5*$^{-/-}$, and *Klk1b5*$^{+/+}$ male or female mice (Figure 1d). In addition, an evaluation of the histoarchitecture of the submandibular gland and the uterus revealed no morphological differences between *Klk1b5*$^{-/-}$ and *Klk1b5*$^{+/+}$ mice (Figure 1e).

The fecundity of *Klk1b5*$^{-/-}$ and *Klk1b5*$^{+/+}$ mice was also determined using a breeding trial that occurred over the course of 6 consecutive months. No fertility defects in *Klk1b5*$^{-/-}$ male or female mice compared to the *Klk1b5*$^{+/-}$ or *Klk1b5*$^{+/+}$ controls were observed (Figure 1f). Based on these findings, we conclude that the loss of *Klk1b5* did not disrupt male or female fertility. However, the results of our quantitative RT-PCR suggest that this lack of a fertility phenotype in the *Klk1b5*$^{-/-}$ mice may be due to compensation by *Klk1b24. C*ompensation by other *Klk* subfamily members could also have rescued the other physiological abnormalities that may have been present in the *Klk1b5*$^{-/-}$ mice. To test this, we are currently generating a mouse model with a deletion of both *Klk1b5* and *Klk1b24* genes in order to determine if an increase in *Klk1b24* level compensates for uterine function of *Klk1b5*. If this is the case, we would observe a fertility defect in *Klk1b5*$^{-/-}$;*Klk1b24*$^{-/-}$ female mice.

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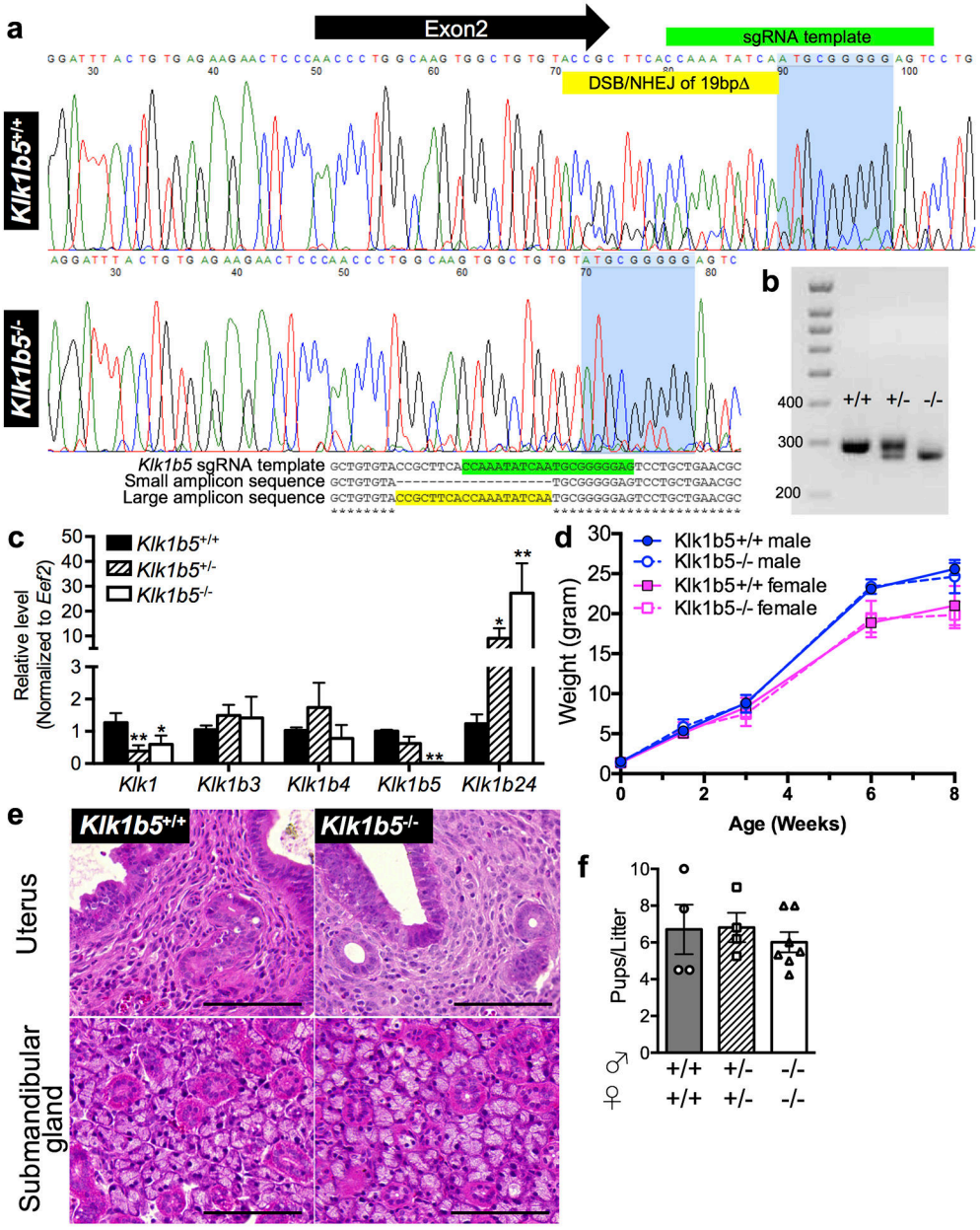

Figure 1.

a. Generation of *Klk1b5*$^{-/-}$ mice. Green highlight is sgRNA target sequence. Yellow highlight is the 19 bp deleted sequence. DBS/NHEJ = double strand break/non-homologous end joining. b. Confirmation of targeted deletion using gel electrophoresis. c. Quantitative RT-PCR of submandibular tissues using specific primers for *Klk1, Klk1b3, Klk1b4, Klk1b5, and Klk1b24. *,**p*<0.05, 0.01; significantly different from *Klk1b5*$^{+/+}$. d. Body weight (grams) of male and female mice at different ages (weeks). e. Hematoxylin and eosin staining of uterine tissues randomly collected at different stage of estrus cycle and submandibular glands from *Klk1b5*$^{+/+}$ and *Klk1b5*$^{-/-}$ mice. Scale bars = 50 μm.

Representative images shown. f. Number of pups per litters from the fertility study over 6 consecutive months. n=3–6 animals/group.