ANO1 in urethral SMCs contributes to sex differences in urethral spontaneous tone

Defang Chen[3], Wen Meng[4], Ling Shu[5], Shuang Liu[1], Yongzhong Gu[6], Xiaoyan Wang[7], Mei Feng [1,2]*

*Correspondence:

Mei Feng, Central Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324 Jingwu Road, Jinan 250021, Shandong, China. Email: meifeng@sdu.edu.cn

1 Central Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

2 Central Laboratory, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China

3 Outpatient Department, Jinan Central Hospital affiliated to Shandong University, Jinan, China.

4 Pharmacy Intravenous Admixture Services，Jinan Central Hospital affiliated to Shandong University, Jinan, China.

5 Operating Room，Jinan Central Hospital affiliated to Shandong University, Jinan, China.

6 Department of Obstetrics and Gynecology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China.

7 General Practice Department, College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China

Supplemental Figure

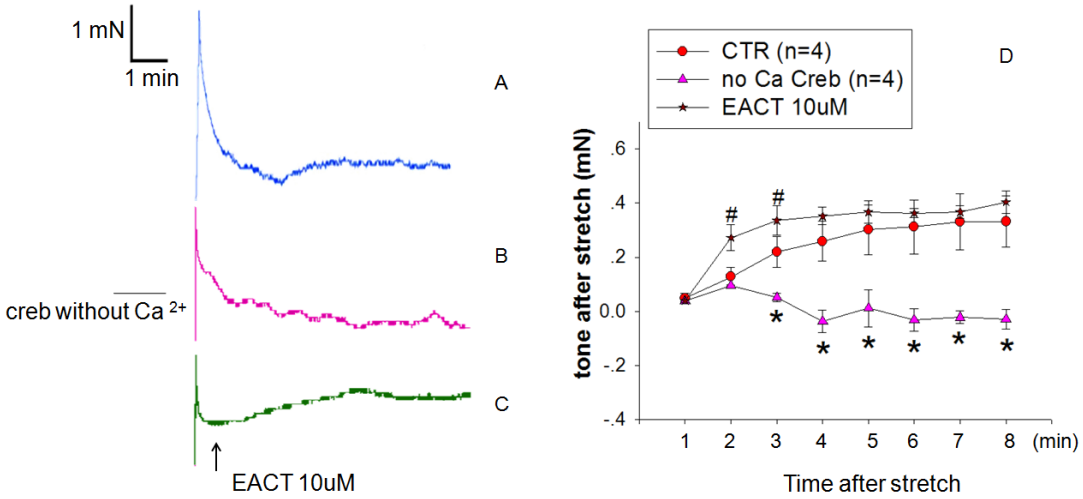

Fig S1. Effects of different treatments on urethral spontaneous tone in female mouse. Normal control (A) was the same as Fig 1D. No spontaneous tone was found in urethrae when Creb solution with 0 mM Ca^{2+} was applied (B). C: EACT 10 μ M was added in bath when spontaneous tone dropped to the lowest point (pointed by arrow). EACT increased the amplitude of spontaneous tone. Significant differences were found between CTR group and no Ca^{2+} group from 3 to 8 minutes (#). Significant differences were found between CTR group and EACT treatment group from 2 to 3 minutes (*).

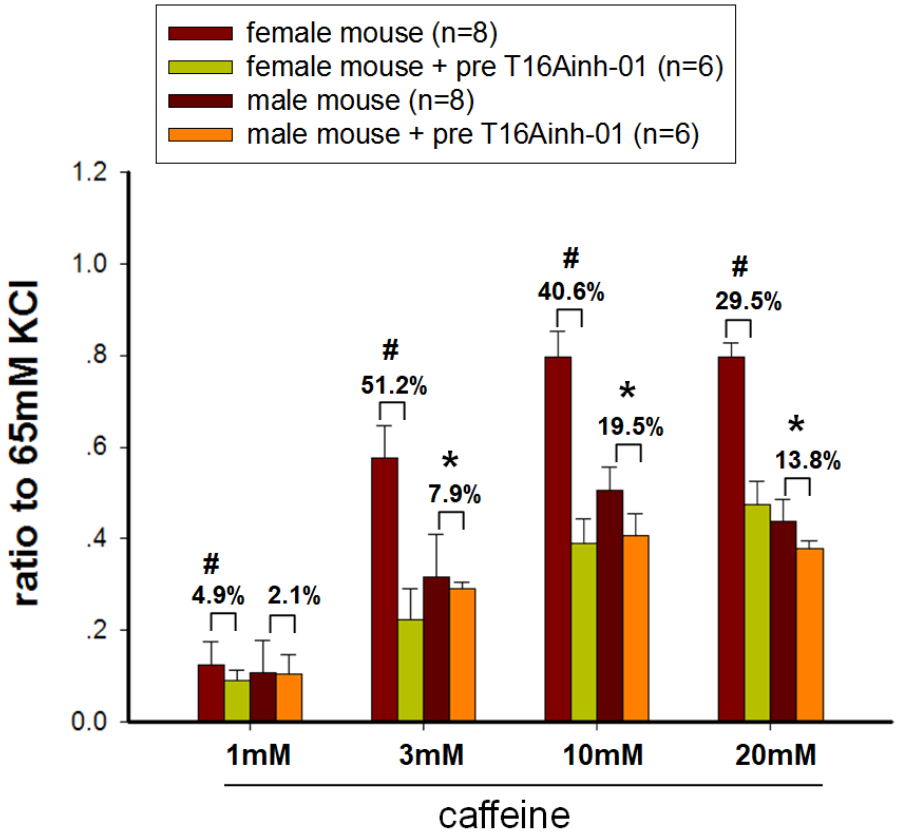

Fig S2. T16Ainh-01 10 μM inhibited caffeine-induced urethral contractions in female (the 2 th column) and male mouse (the 4th column) significantly. Normal control was the same as Fig 2 B. After the urethral contraction was stable, T16Ainh-01 was added to the bath followed by a series of caffeine. As shown in the figure, there is a gender difference in the inhibition rate of T16Ainh-01 at four doses (females: 4.9%, 51.2%, 40.6%, 29.5%; males: 2.1%, 7.9%, 19.5%, 13.8%). Obviously, T16Ainh-01 has a greater inhibitory effect on female mice than male mice.# $p < 0.05$ between the female groups. * $p < 0.05$ between the male groups.

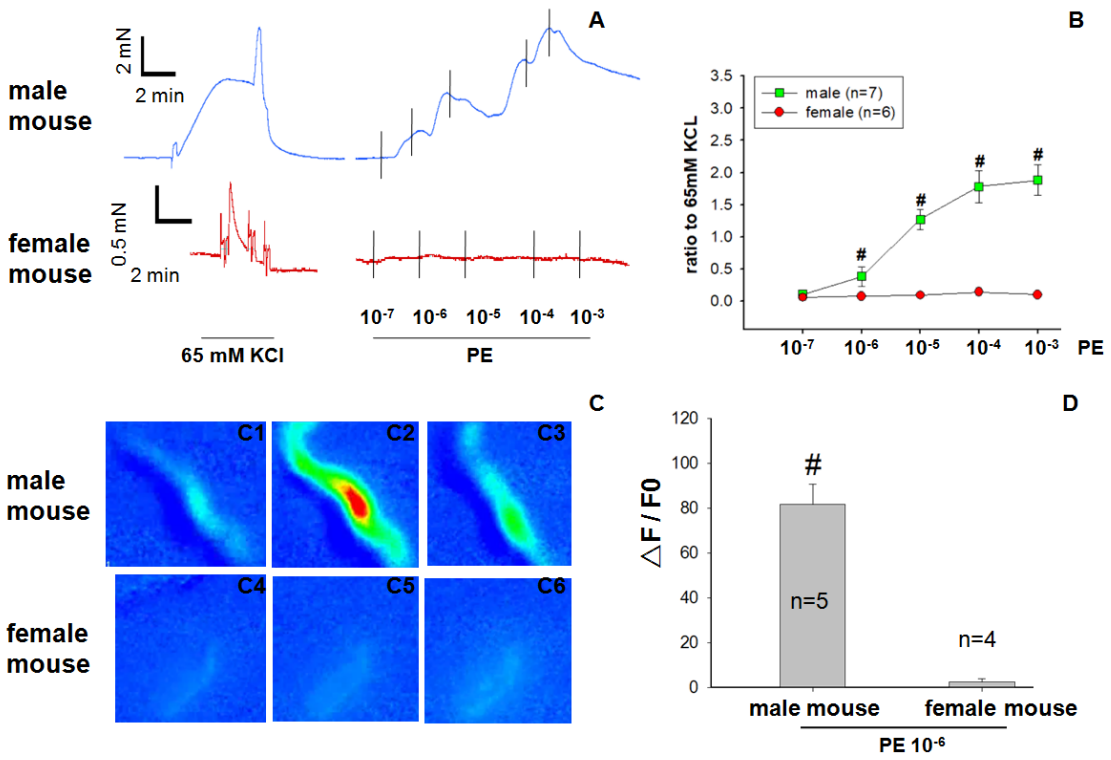

Fig S3. Differences in USM motility and fluctuations in $[Ca^{2+}]_i$ induced by PE were found in female and male mice. (A) A representative recording of urethral contraction in male mice. (B) Dose response curve of the effects of PE on urethral contraction (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}). As shown by the results, PE induced stronger contractions in males than in females. A series of images shows the time course in cells from male mice (C1, C2, C3) and female mice (C4, C5, C6). The procedure can be divided into two main steps: administration of 65 mM KCl for 20 s followed by PE (10^{-6}) for 20 s. The ratio of the fluorescence value of PE to that of KCl (ΔF/F0) was used as a statistical indicator. The statistical results showed that male mice had a substantially higher $[Ca^{2+}]_i$ level than female mice when exposed to PE (D). # $p < 0.05$ between the female and male groups (10 female mice and 12 male mice).