Supplementary information for

The nonproton ligand sensing domain is required for paradoxical stimulation of ASIC3 channels by amiloride

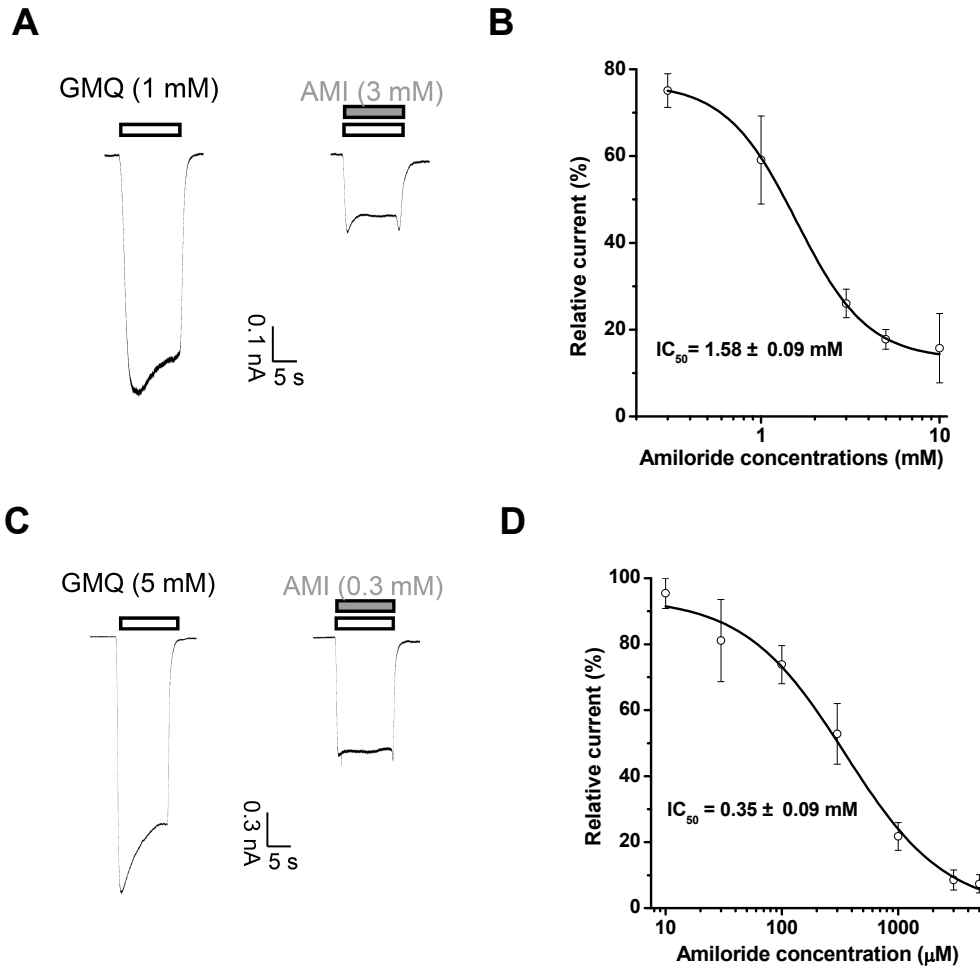**Wei-Guang Li[1,3]*, Ye Yu[1,2]*, Chen Huang[3], Hui Cao[1,3], Tian-Le Xu[1,3]**

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This file includes:

Supplementary Figures 1 to 3 and their legends

Supplementary Table 1
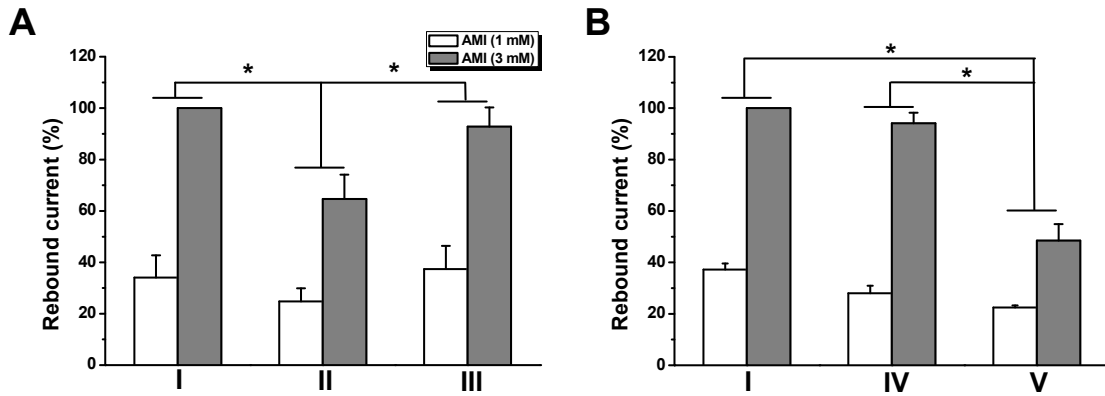
A

GMQ (1 mM)　　　AMI (3 mM)

0.1 nA | 5 s

B

$IC_{50} = 1.58 \pm 0.09$ mM

Relative current (%)

Amiloride concentrations (mM)

C

GMQ (5 mM)　　　AMI (0.3 mM)

0.3 nA | 5 s

D

$IC_{50} = 0.35 \pm 0.09$ mM

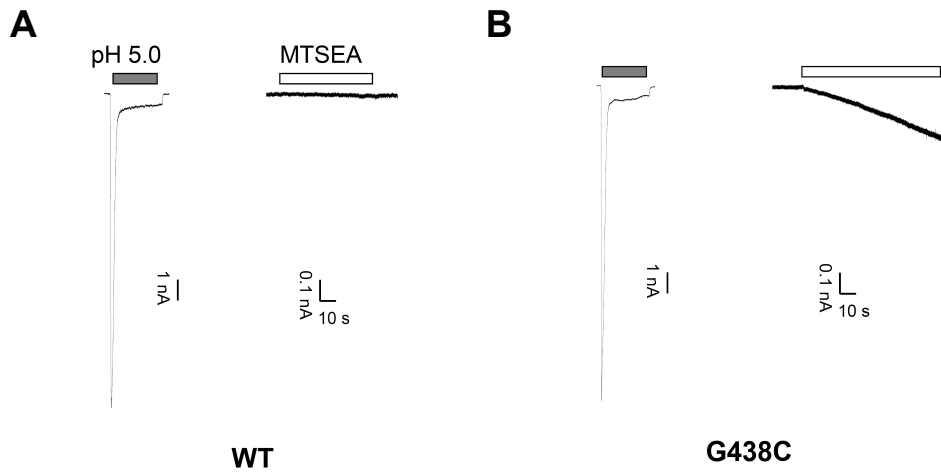Relative current (%)

Amiloride concentration (μM)

Supplementary Figure 1. Effects of amiloride (AMI) on the GMQ-mediated ASIC3 activation. *A,C*

Typical recording for the effects of amiloride (3 mM, *A*; 0.3 mM, *C*) on the GMQ (1 mM, *A*; 5 mM,

C)-induced currents in ASIC3-expressing CHO cells. *B,D* Dose-dependent inhibitory effects of amiloride

on GMQ (1 mM, *B*; 5 mM, *D*)-evoked currents in ASIC3-expressing CHO cells. Data points are means ±

S.E.M. of four to five measurements and the solid lines are the fit to the Hill equation.

Supplementary Figure 2. Statistical analysis of effects of DTDP on the peak currents of ASIC3^{E79C} channels upon washout of AMI at 1 and 3 mM. *A,B* Pooled data from experiments in Fig. *6C* and *6D,* respectively. Data points are means ± S.E.M. of six measurements of the peak currents induced after AMI washout, normalized to the peak currents upon washout of 3 mM AMI before DTDP treatment. **p* < 0.05, different between groups as indicated.

Supplementary Figure 3. Typical recordings showing the effect of MTSEA on WT (*A*) or G438C (*B*)

mutant ASIC3 channel activation.

Supplementary Table 1. Current amplitude of ASIC3 mutants (data from Figure 3).

Mutants	pH 5.0 (pA)	AMI-1 mM (pA)	AMI-3 mM (pA)
WT	11744 ± 1468	1579 ± 433	2002 ± 607
E79A	1618 ± 519	5.1 ± 1.8	41.4 ±15.8
E79C	3606 ± 705	32 ± 8.5	133.2 ± 47.6
E79D	3298 ± 533	72.7 ± 16.5	138.8 ± 18.4
E79L	6255 ± 2620	398.0 ± 86.4	696.7 ± 140.1
E79Q	10082 ± 2173	184.3 ± 42.6	452.7 ± 137.1
E79R	4884 ± 875	59.4 ± 17.2	229.3 ± 53.8
E79S	6129 ± 529	150.4 ± 21.9	865.4 ± 85.8
E423A	9023 ± 913	14.8 ± 9.1	44.9 ± 16.4
E423C	4928 ± 1987	4.3 ± 1.3	11.0 ± 3.1
E423D	744.3 ± 195.4	1.5 ± 0.6	4.4 ± 1.6
E423L	12152 ± 648	9.3 ± 4.8	36.3 ± 21.1
E423Q	8675 ± 588	10.6 ± 2.5	72.7 ± 20.2
E423R	3754 ± 814	8.7 ± 1.9	15.7 ± 1.0
E423S	1254 ± 245	6.7 ± 0.7	16.4 ± 1.9