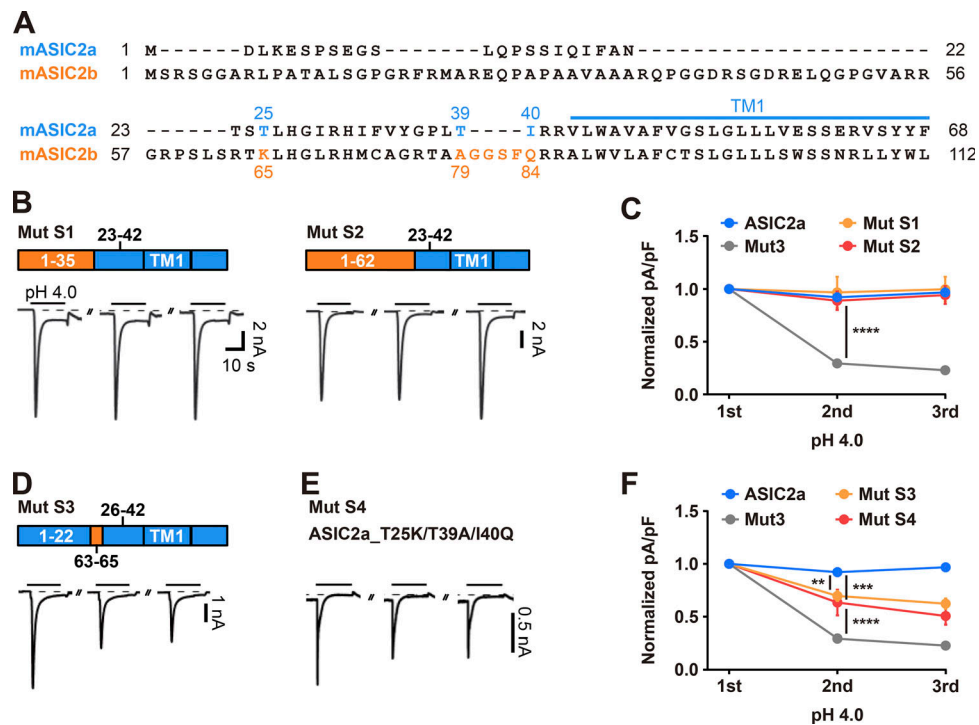# Supplemental material

Figure S1. **The responses of ASIC2a mutants to three consecutive pH 4.0 treatments. (A)** Alignment of amino acid sequences of ASIC2a (1–68) and ASIC2b (1–112). **(B, D, and E)** Currents elicited by three consecutive treatments of pH 4.0 with a 2-min interval in HEK293T cells expressing the indicated mutant. Acidic stimuli were delivered by switching the bath solution from pH 7.4 to pH 4.0. **(C and F)** Peak current densities elicited by three successive acidic stimuli, normalized to those evoked by the first stimulus. $n = 5$ for each group. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$, two-way ANOVA followed by Tukey's multiple comparisons corrections.

A

```
mASIC2a   1  M------DLKESPSEGS-------LQPSSIQIFAN--------------------  22
mASIC2b   1  MSRSGGARLPATALSGPGRFRMAREQPAPAAVAAARQPGGDRSGDRELQGPGVARR  56

                            25          39   40                TM1
mASIC2a  23  ------TSTLHGIRHIFVYGPLT----IRRVLWAVAFVGSLGLLLVESSERVSYYF  68
mASIC2b  57  GRPSLSRTKLHGLRHMCAGRTAAGGSFQRRALWVLAFCTSLGLLLSWSSNRLLYWL  112
                          65                 79   84
```

B

Mut4
```
                        1-22  63-84  TM1
ASIC2a  21  ANTSTLHGIRHIFVYGPLT----IRR  42
Mut4    21  ANRTKLHGLRHMCAGRTAAGGSFQRR  46
```

Mut6
```
                        26-38  79-84
                           K
                       1-24        TM1
ASIC2a  23  TSTLHGIRHIFVYGPLT----IRR  42
Mut6    23  TSKLHGIRHIFVYGPLAGGSFQRR  46
```

Mut8
```
                        66-78
                        T  T/I
                       1-64   TM1
ASIC2b  63  RTKLHGLRHMCAGRTAAGGSFQRR  86
Mut8    63  RTTLHGLRHMCAGRTAT----IRR  82
```

Mut S3
```
                        63-65
                        26-42
                       1-22     TM1
ASIC2a  21  ANTSTLHGIRHIFVYGPLT----IRR  42
Mut S3  21  ANRTKLHGIRHIFVYGPLT----IRR  42
```
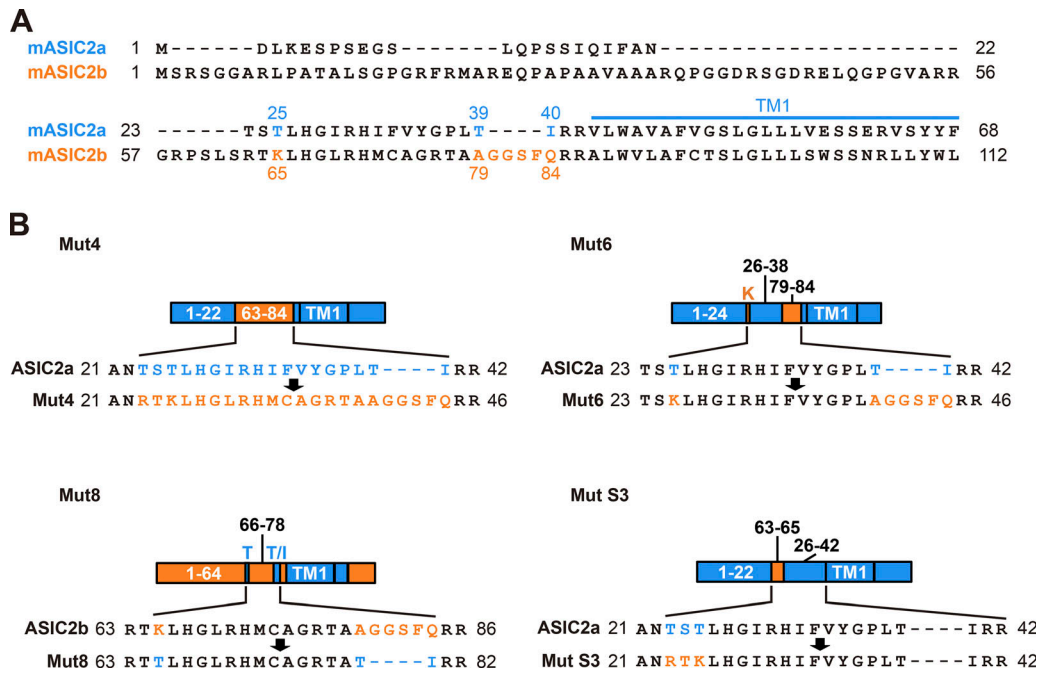
Figure S2.   **The schematics and amino acid sequences of ASIC2a mutants. (A)** Alignment of amino acid sequences of ASIC2a (1–68) and ASIC2b (1–112). **(B)** Schematic and amino acid sequences of the indicated mutants.

Table S1.   **Primers and templates used for chimera construction**

Name	Fragment 1		Fragment 2		Template	
	Forward primer (5′–3′)	Reverse primer (5′–3′)	Forward primer (5′–3′)	Reverse primer (5′–3′)	Fragment 1	Fragment 2
Mut4	GAATTCTATGGACCTCAAGGAGAGC	CCCGTGCAATTTAGTGCGGTTGGCGAAGATCTGGET	ATCCAGATCTTCGCCAACCGCACTAAATTGCACGGG	GGATCCTCAGCAGGCAATCTCCTC	ASIC2a	Mut3
Mut for Mut6 (39TI40 in ASIC2a to 79AGGSFQ84 in ASIC2b)	GAATTCTATGGACCTCAAGGAGAGC	CTGGAAAGAGCCTCCCGCCAGCGGCCCATACACGAA	TTCGTGTATGGGCCGCTGGCGGGAGGCTCTTTCCAG	GGATCCTCAGCAGGCAATCTCCTC	ASIC2a	Mut3
Mut S1	ATGGTACCATGAGCCGGAGCGGCGGAGCCCGGCTG	GCCATGGAGAGTAGAGGTAGCTGCCGCCACCGCCGC	GCGGCGGTGGCGGCAGCTACCTCTACTCTCCATGGC	ATTGGATCCTCAGCAGGCAATCTCCTCCAG	ASIC2b	ASIC2a
Mut S2	ATGGTACCATGAGCCGGAGCGGCGGAGCCCGGCTG	GCCATGGAGAGTAGAGGTACTCAGGGACGGCCGCCC	GGGCGGCCGTCCCTGAGTACCTCTACTCTCATGGC	ATTGGATCCTCAGCAGGCAATCTCCTCCAG	ASIC2b	ASIC2a

Table S2. **Primers and templates used for mutagenesis**

Name	Sense (5′–3′)	Antisense (5′–3′)	Template
Mut5	ACATCTTCGTGTATGGGCCGCTGACCATCCG	GGCGGATGCCATGGAGTTTAGAGGTGTTGGCGAA	ASIC2a
Mut6	TTCGCCAACACCTCTAAACTCCATGGC	GATCTGGATGDGGAAGGTTGCAGGC	Mut for Mut6
Mut7			
T25A	ATCCGCCACATCTTCGTGLATGGGCCG	GCCATGGAGAGCAGAGGLGTTGGCGAAGAT	ASIC2a
T39A, I40A	TATGGGCCGCTGGCCGCCCGGCGTGTGCTT	ACACGAAGATGTGGCGGATGCCATGG	ASIC2a T25A
Mut8			
K65T	GCGGCACATGTGCGCGG	AGCCCGTGCAATGTAGTGCGACTC	Mut2
80-83 del.	TGGGCAGTGGCCFLCGLGGGATCTCTGG	AAGCACCCGOCGCTGCGCCGCCGLG	Mut2 K65T
A79T, Q84I	GGCCTTCGTGGGATCTCTGGGCCTGCTGCT	ACTGCCCAAAGCACCCGCCGGATCGTCGCCGTGCGC	Mut2 K65T, 80-83 del.
Mut9	TGAGCGGCTGACTATCAAGCGGGCACT	TAGGAGAAGATGTGGGCAAGACCGTGCAGC	ASIC1a
Mut 10	GGTCTTGCCCACATCTTCTCCTATGAGC	GTGCAGCGTGCAGCTGTTGGC	ASIC1a S23N
Mut S3	TGGCATCCGCCACATCTTCGTGTATG	TGGAGTTTAGTGCGGTTGGCGAAGATC	Mut5
Mut S4	CAGCGGCGGGTGCTTTGG	CGCCAGCGGCCCATACAC	Mut6

Table S3. **Primers and templates used for ASIC2a-Mut3-ASIC2a concatemer**

Name	Sequence (5′–3′)	Template
Mut 3- ASIC2a insertion		
Fragment 1		Mut3
Forward primer	ATGGTACCATGAGCCGGAGCGGCGGAGCCCGGCTG	
Reverse primer	CTGTTGTTGCTGCTGTTGCTGATTGCAGGCAATCTCCTCCAGGGTGCC	
Fragment 2		ASIC2a
Forward primer	AATCAGCAACAGCAGCAACAACAGATGGACCTCAAGGAGAGCCCCAGC	
Reverse primer	ATTGGATCCTCAGCAGGCAATCTCCTCCAG	
ASIC2a insertion		ASIC2a
Sense	ATTACTCGAGATGGACCTCAAGGAGAGCCCCAGCGAGGGCAGC	
Antisense	ATGGTACCCTGTTGTTGCTGCTGTTGCTGATTTCAGCAGGCAATCTCCTCCAGGG	