SUPPLEMENTAL MATERIAL FOR

THE CONTACT REGION BETWEEN THREE DOMAINS OF THE EXTRACELLULAR LOOP OF ASIC1A IS CRITICAL FOR CHANNEL FUNCTION

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell surface protein biotinylation and western blot analysis – The experiments described in Fig. S2 were carried out exactly as described in "*Experimental procedures*" except for the number of oocytes per condition which was 40.

Electrophysiological analysis of MTSET kinetics experiments – The stimulation solution of pH 5.5 was applied once every minute during 5 s. Between acidic stimulations, the recording chamber was perfused with the conditioning solution at pH 7.4, which was changed after 4-5 acidic stimulations to one containing MTSET at a concentration that allowed resolution of the kinetics of current decrease, as indicated for each of the three mutants shown in Fig. S3B. Immediately before each acidic stimulation the conditioning solution with MTSET was changed for 5 s to one containing no MTSET. Kaleidagraph (Synergy software) was used to fit the kinetics of the current decrease of the normalized data, by using the equation: $I = a + (1- a)\cdot\exp^{-kt}$, where I is the peak current amplitude normalized to that before MTSET addition, a, is the fraction of normalized current that is not inhibited by MTSET, k is the rate constant in min^{-1} and t is the time after start of MTSET exposure in min.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Structure of the finger-ball interaction zone in ASIC1a in Stereo view. A, Structure of hASIC1a established by homology modeling based on cASIC1 (PDB ID 2QTS). One of the three ASIC subunits is shown in color, identifying different parts of ASIC subunits (red: transmembrane segments, yellow: palm, orange: β-ball, green: thumb, purple: finger, turquoise: knuckle. B, View of the finger-ball interaction zone with loop 1 in black, loop 2 in grey and loop 3 in brown. The side chains of the residues mutated in this study are represented as "ball-and-stick" (Category I residues, see text) and as "stick" (Category II residues). Hydrogen bonds predicted in Chimera (1) are indicated as blue solid lines.

Figure S2. ASIC protein and current expression. A, Representative Western blots of the cell surface expression of ASIC1a wt and ASIC1a H163C fused or not to UTR of *Xenopus* β-globin (see *Experimental Procedures*) as indicated. Cell-surface proteins were isolated by biotinylation, as described under "*Experimental Procedures*" from 40 ASIC1a-expressing oocytes 1 day after injection of 10 ng of cRNA. ASIC1a (~70 kDa) was visualized by a specific antibody (MTY19, (2)) that recognizes a C-terminal epitope of hASIC1. Representative blots of the unbound and the bound fraction from one out of 3 similar experiments per channel type are shown. The bands in the unbound fraction of non-injected oocytes are from a contamination of neighboring lanes. **B**, Peak current amplitude of ASIC1a wt and ASIC1a H163C fused or not to UTR of *Xenopus* β-globin induced by acidification to pH 4.5 at a holding potential of -60 mV, 1-2 days after injection of 10 ng (wt) or 10-50 ng cRNA per oocyte (mutants; n = 15-86 per condition, presented as mean ± SEM). *, different, $p < 0.05$ (ANOVA and Dunnet post hoc test). The pH50 of activation of ASIC1a wt (6.1 ± 0.0, n=3) and H163C (6.0 ± 0.0, n=4) are not modified by the UTR *Xenopus* β-globin fusion (6.1 ± 0.0, n=3 and 6.0 ± 0.0, n=4 for ASIC wt and H163C, respectively; data not shown).

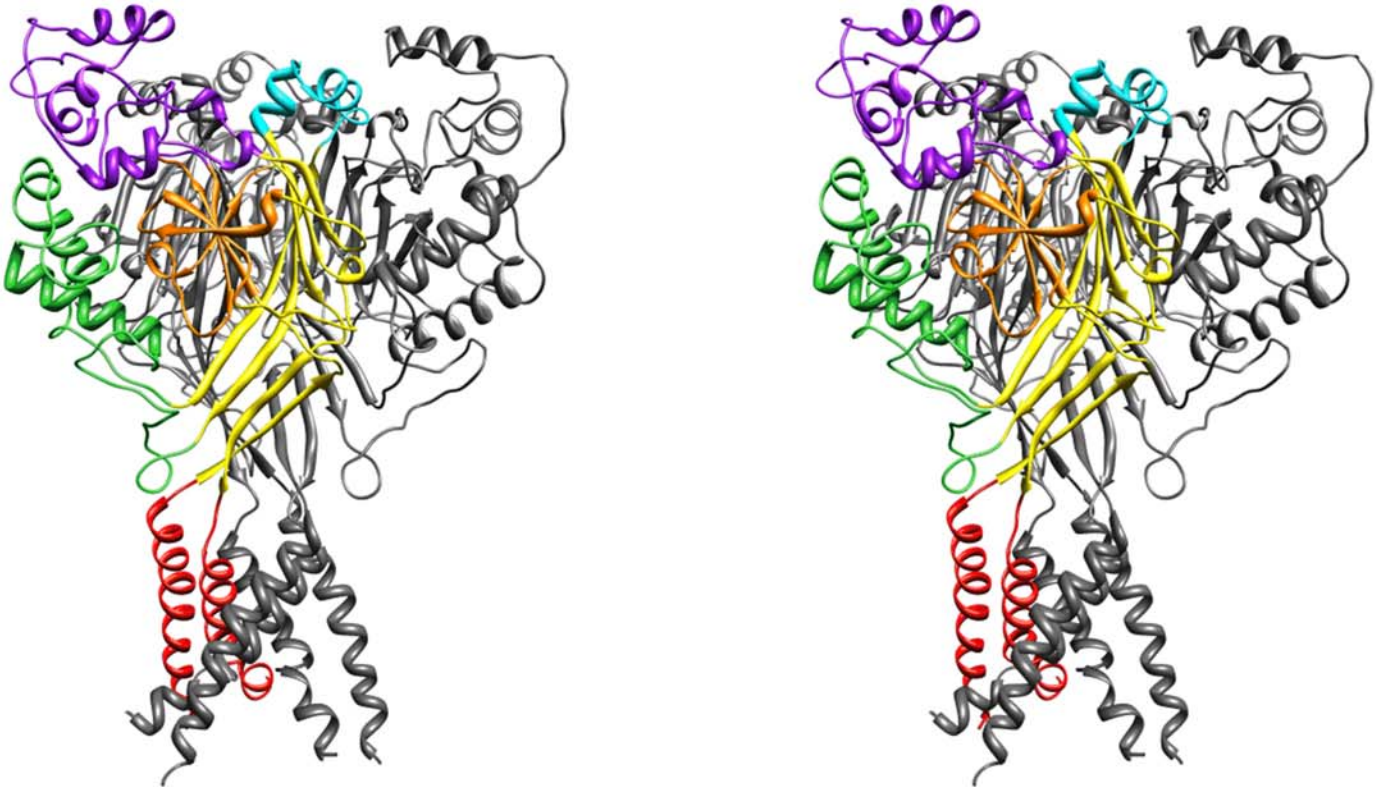
Figure S3. Kinetics of peak current inhibition of selected ASIC1a mutants by extracellular MTSET. A, Representative current traces of oocytes expressing ASIC1a Q225C obtained by 5-s acidification to the indicated pH values at a holding potential of -60 mV, before and during incubation with 0.15 mM MTSET. **B**, pH 5.5-induced peak current amplitude, normalized to the amplitude before application of the reagent, shown before and during exposure to MTSET for the ASIC1a mutants N96C (▼, MTSET 0.01mM), H163C (●, MTSET 0.25 mM) and Q225C (▲, MTSET 0.15 mM) (n = 4-6). Data are presented as mean ± SEM. The lines represent fits to single exponential (see *Supplemental Experimental Procedures*) yielding a rate constant k (min^{-1}) of current decrease. **C**, Rate constant (k, min^{-1} M^{-1}) of MTSET inhibition of peak currents of mutant channels of category I, calculated from the rate of current decrease (in min^{-1}) divided by the concentration of MTSET used (in M), mean ± SEM . *, Kinetic constant different from other mutant channels, $p < 0.05$ (ANOVA and Dunnet post hoc test).

Figure S4. Time constant of open channel inactivation of ASIC1a wt and mutants. ASIC currents were induced by acidification to pH 6.0 or 4.5 before and after a 5-min incubation with 1 mM MTSET and measured at a holding potential of -60 mV. The time course of open-channel inactivation of the traces was fitted to a single exponential by using Clampfit (Molecular Devices), yielding the time constants presented. *, different from wt (same condition), $p < 0.05$ (ANOVA and Dunnet post hoc test).

REFERENCES FOR SUPPLEMENTAL DATA

1. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) *J Comput Chem* **25**, 1605-1612
2. Wemmie, J. A., Askwith, C. C., Lamani, E., Cassell, M. D., Freeman, J. H., and Welsh, M. J. (2003) *J. Neurosci.* **23**, 5496-5502

A



B

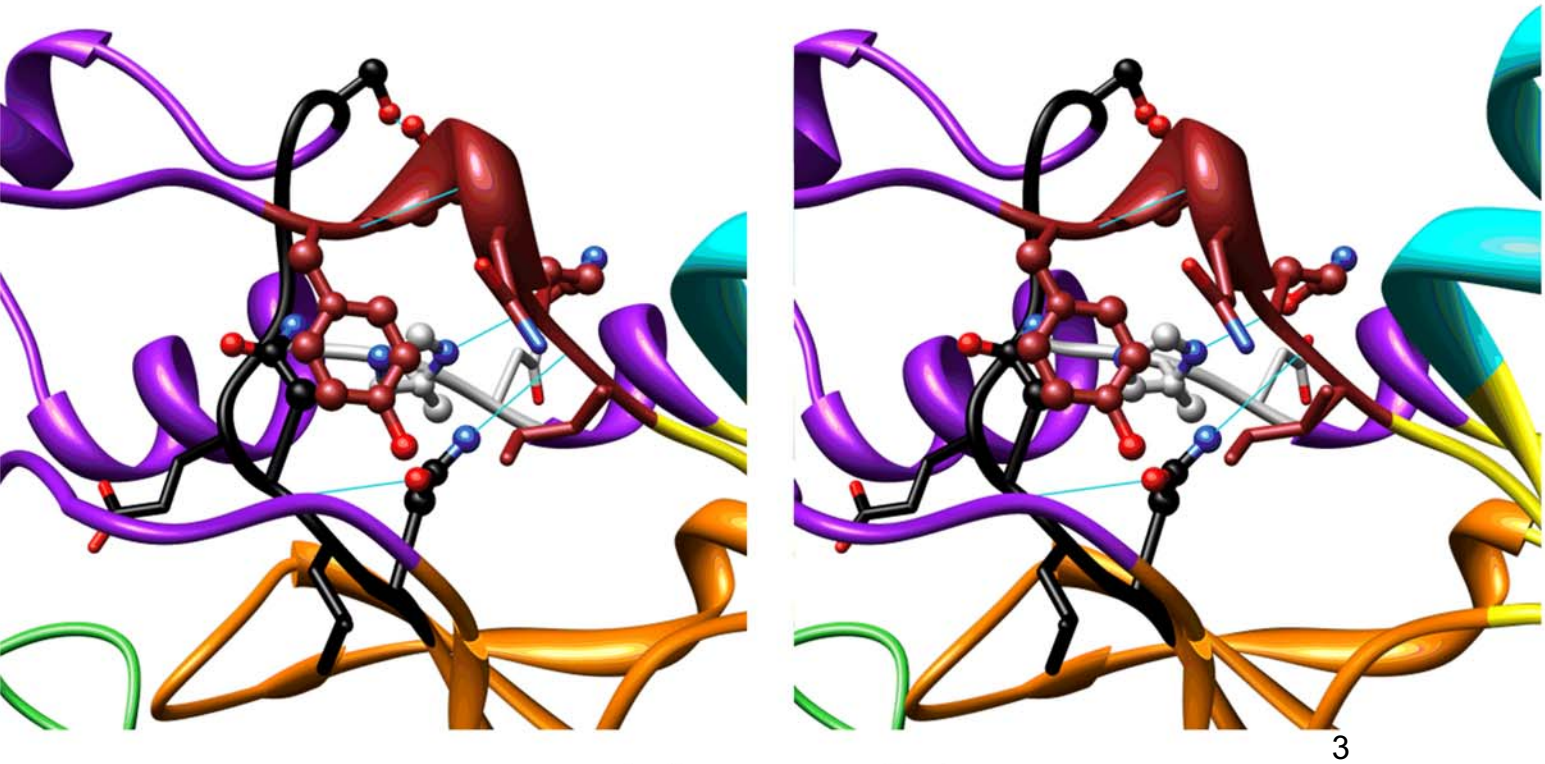
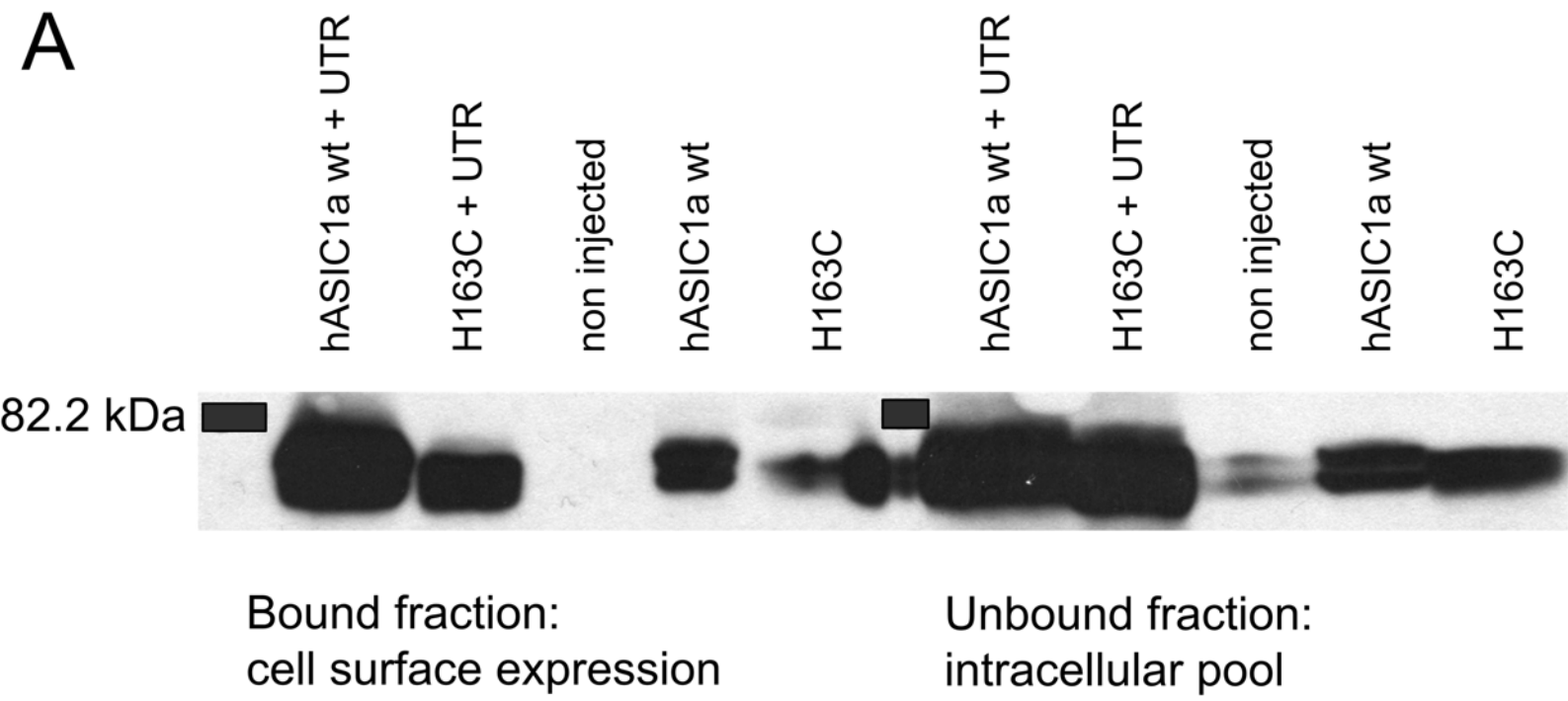


Figure S1


A
hASIC1a wt + UTR
H163C + UTR
non injected
hASIC1a wt
H163C
hASIC1a wt + UTR
H163C + UTR
non injected
hASIC1a wt
H163C
82.2 kDa
Bound fraction:
cell surface expression
Unbound fraction:
intracellular pool

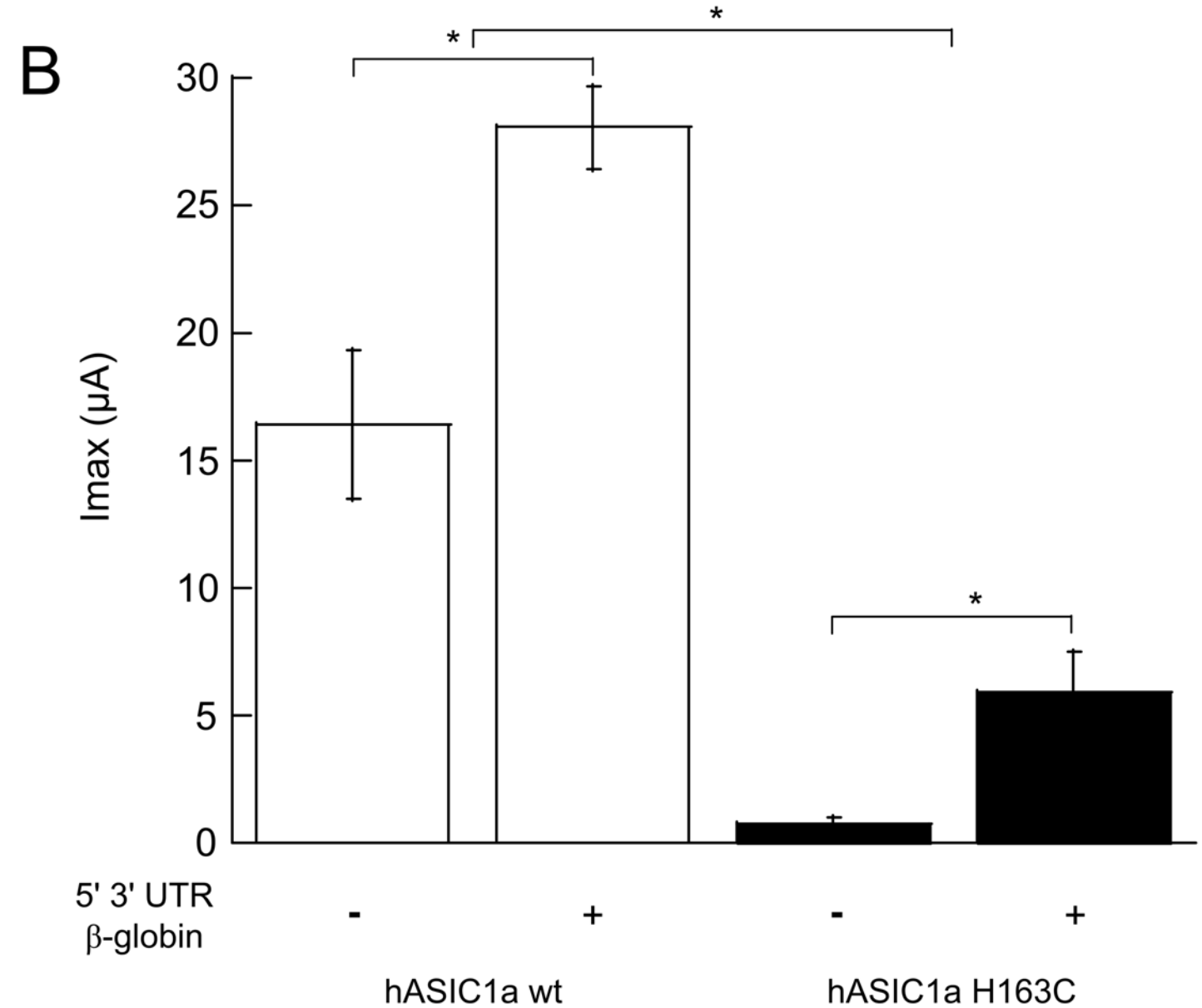
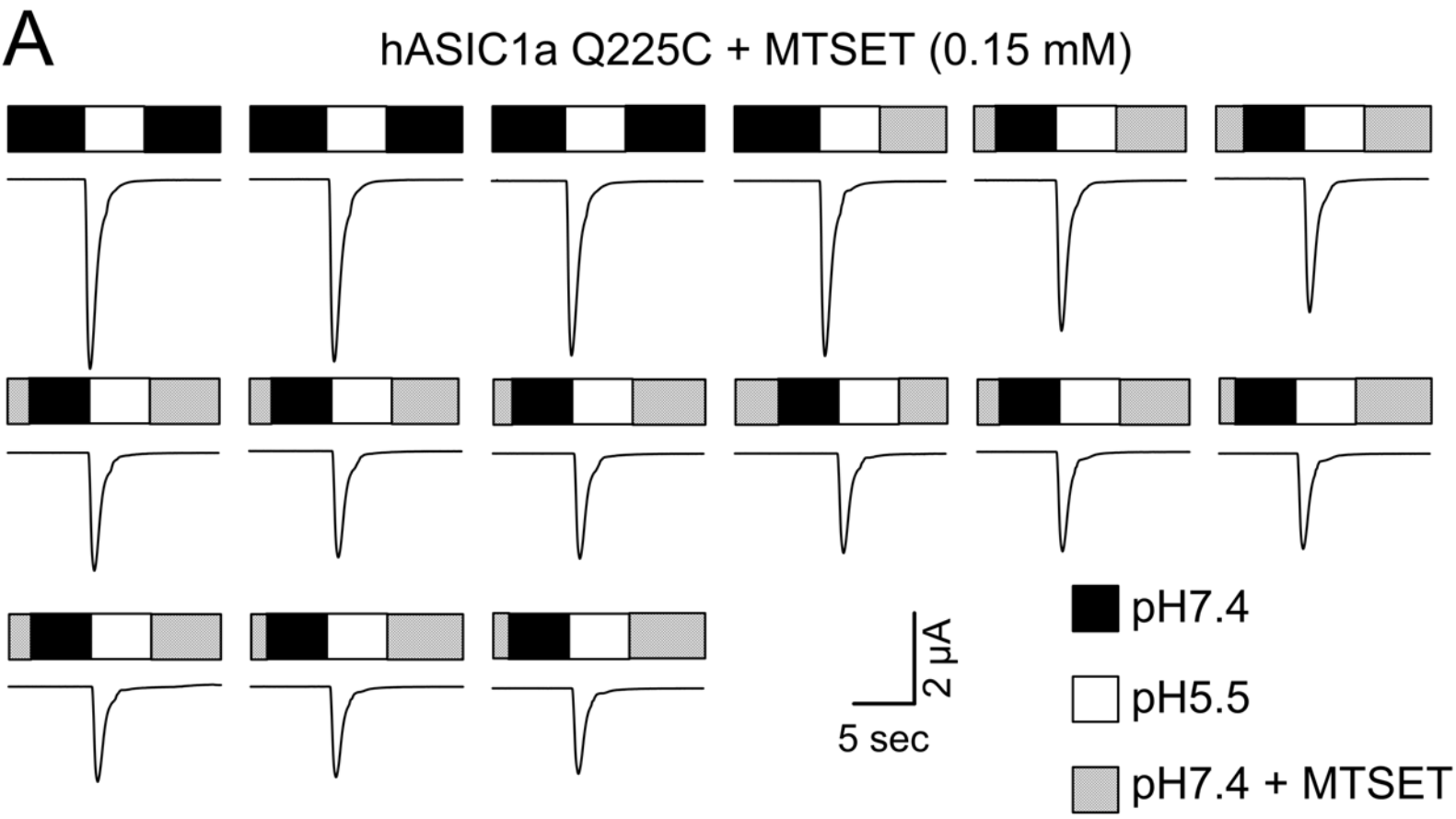
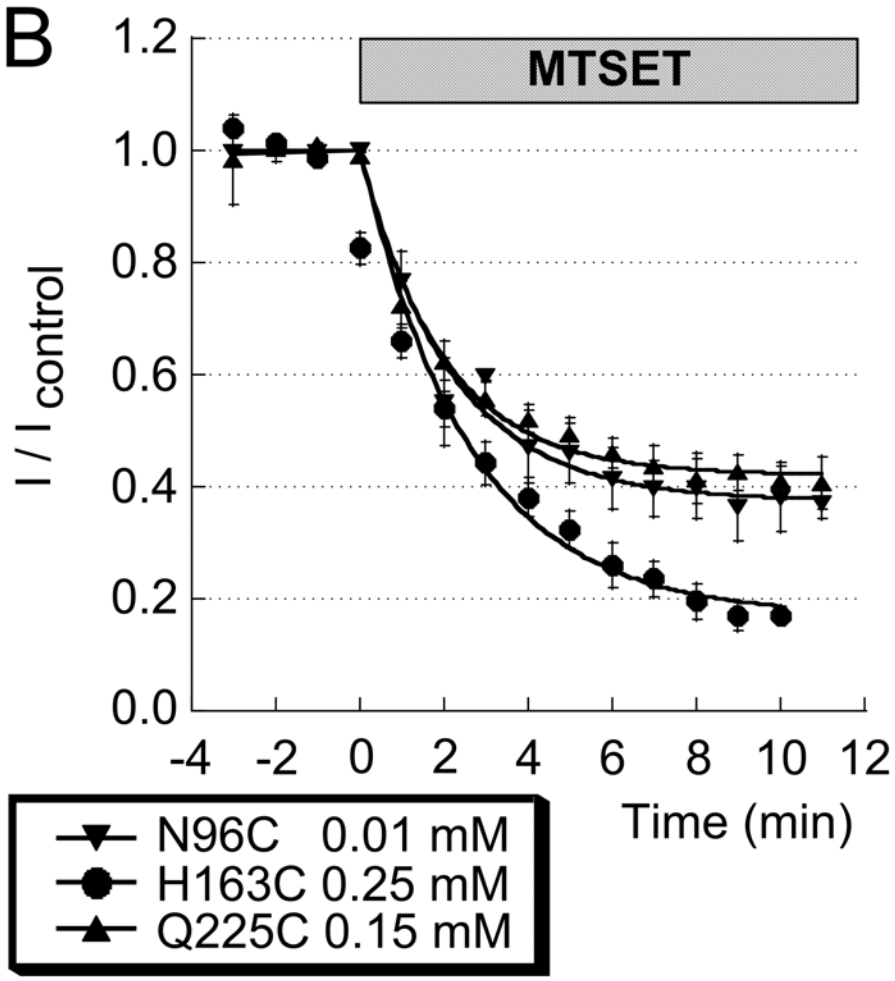

B
*
*
*
30
25
20
15
10
5
0
Imax (μA)
5' 3' UTR
β-globin
-
+
-
+
hASIC1a wt
hASIC1a H163C

Figure S2





C Rate constant of MTSET-induced current decrease

hA1a	k ($M^{-1}min^{-1}$)
N94C	2696 ± 1091
N96C*	26130 ± 4869
S101C	1677 ± 528
G162C	1739± 591
H163C	1540 ± 154
Q225C	3055 ± 383
E228C	6861 ± 1175

Figure S3

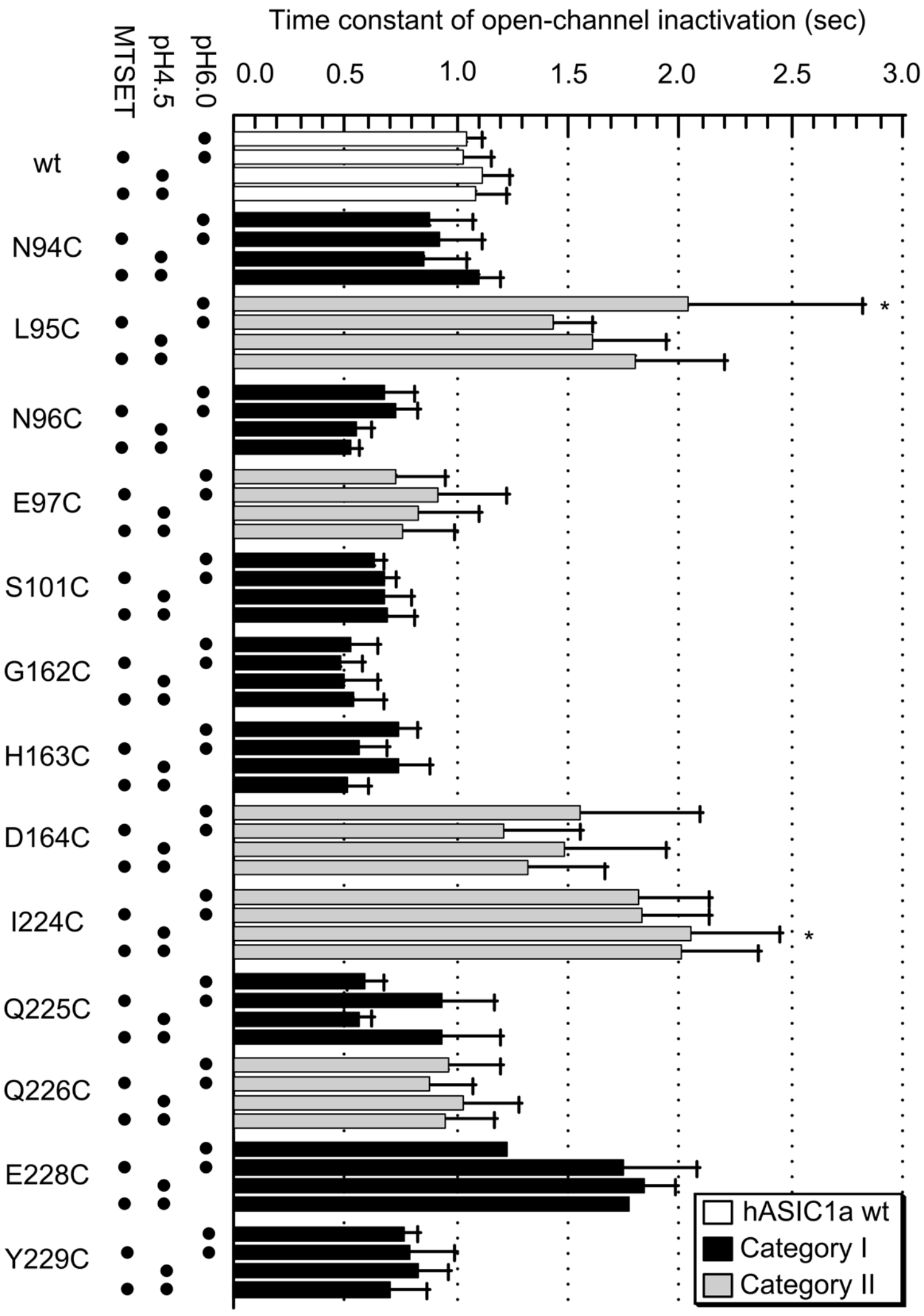

Time constant of open-channel inactivation (sec)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
MTSET
pH4.5
pH6.0
wt
N94C
L95C
N96C
E97C
S101C
G162C
H163C
D164C
I224C
Q225C
Q226C
E228C
Y229C
*
*
hASIC1a wt
Category I
Category II

Figure S4