

Haerteis et al., <http://www.jgp.org/cgi/content/full/jgp.201110763/DC1>

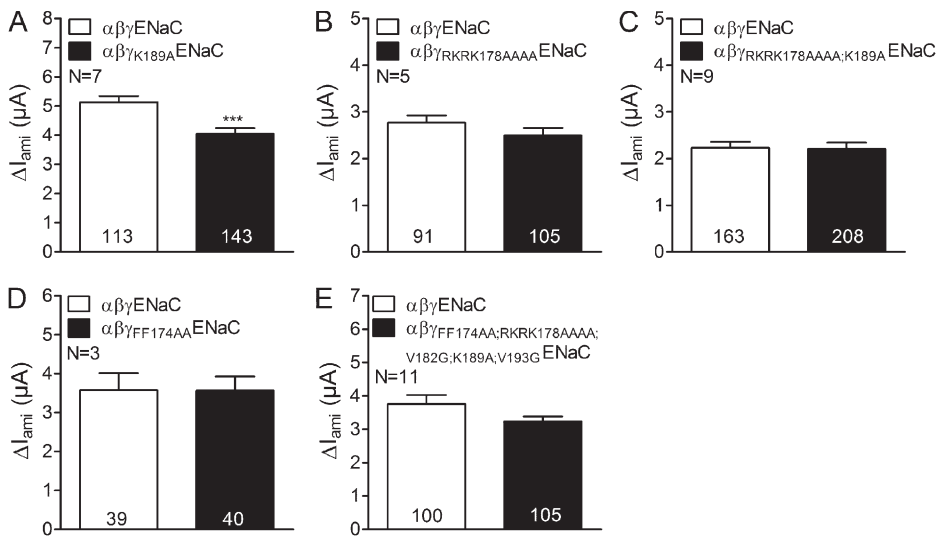


Figure S1. Effect of mutated cleavage sites on baseline ΔI_{ami} . (A–E) Mean baseline ΔI_{ami} from experiments as shown in Figs. 2 B (A), 3 B (B), 4 B (C), 10 A (D), and 11 A (E) before incubation in protease-containing solution. The results indicate that the mutations per se have no major impact on baseline ENaC function. Numbers inside the columns indicate the number of individual oocytes measured. *N* indicates the number of different batches of oocytes. ***, $P < 0.001$; unpaired *t* test.

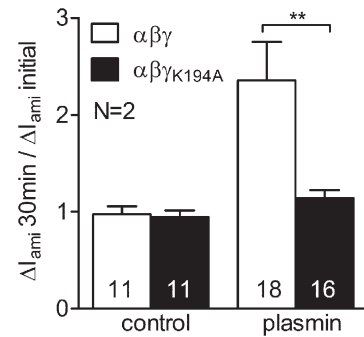


Figure S2. The stimulatory effect of plasmin is abolished in oocytes expressing mouse ENaC with a mutated putative plasmin site (γ_{K194A}). Oocytes expressing mouse $\alpha\beta\gamma$ (open symbols) or $\alpha\beta\gamma_{K194A}$ ENaC (closed symbols) were incubated for 30 min in protease-free solution (control) or in solution containing 10 $\mu g/ml$ plasmin. Amiloride-sensitive whole cell currents (ΔI_{ami}) were detected before (–) and after (+) incubation. Columns represent relative stimulatory effect on ΔI_{ami} calculated as the ratio of ΔI_{ami} measured after a 30-min incubation ($\Delta I_{ami} 30min$) to the initial ΔI_{ami} ($\Delta I_{ami} initial$) measured before incubation. Numbers inside the columns indicate the number of individual oocytes measured. *N* indicates the number of different batches of oocytes. **, $P < 0.01$; unpaired *t* test.

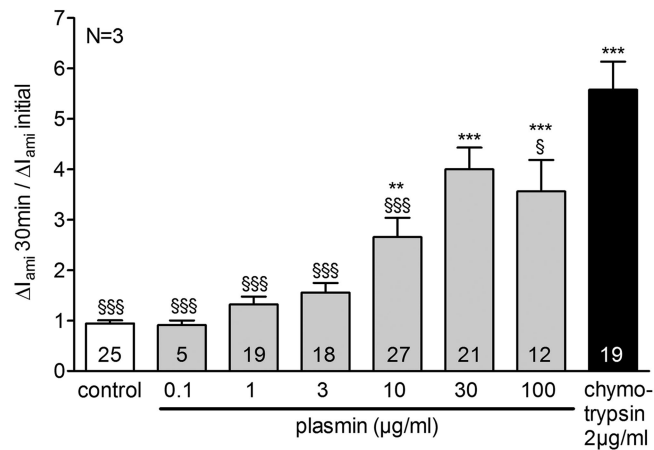


Figure S3. Stimulatory effect of plasmin on human ENaC is concentration dependent. Oocytes expressing human $\alpha\beta\gamma$ ENaC were incubated for 30 min in protease-free solution (control), in solutions containing different concentrations of plasmin (0.1, 1, 3, 10, 30, and 100 $\mu\text{g/ml}$), or in a solution containing 2 $\mu\text{g/ml}$ chymotrypsin. Amiloride-sensitive whole cell currents (ΔI_{ami}) were detected before (ΔI_{ami} initial) and after incubation (ΔI_{ami} 30 min). Columns represent the relative stimulatory effect on ΔI_{ami} calculated as the ratio of ΔI_{ami} 30 min/ ΔI_{ami} initial. Numbers inside the columns indicate the number of individual oocytes measured. N indicates the number of different batches of oocytes. Statistical significance was tested by using one-way ANOVA followed by Tukey's multiple comparison test versus control (**, $P < 0.01$; ***, $P < 0.001$) or chymotrypsin (§, $P < 0.05$; §§§, $P < 0.001$).