*Supplementary Material and Figures to*

# A stomatin dimer modulates the activity of acid-sensing ion channels

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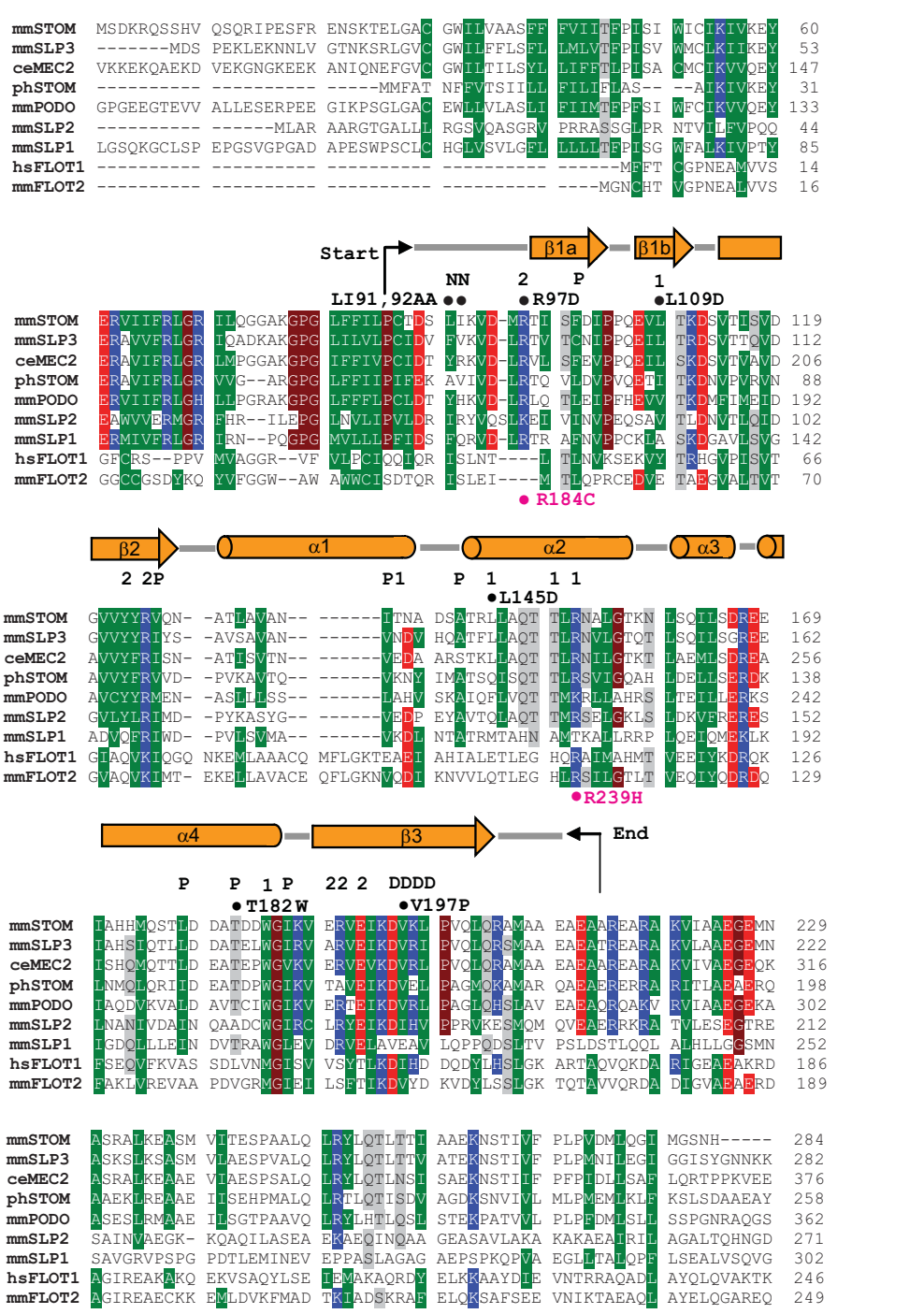
# Supplementary Figure 1

A

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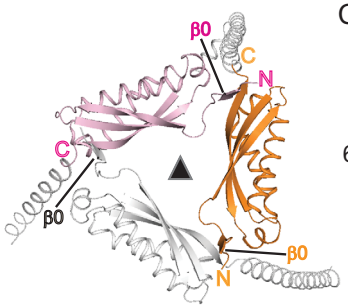


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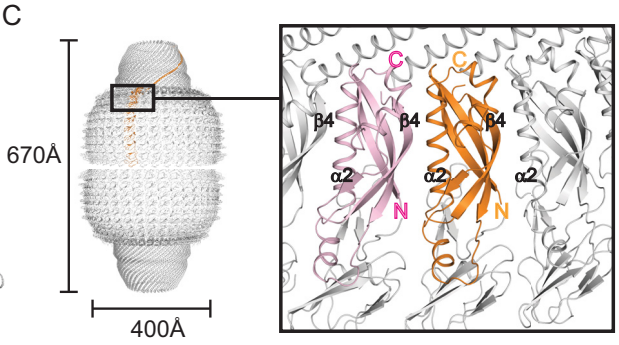
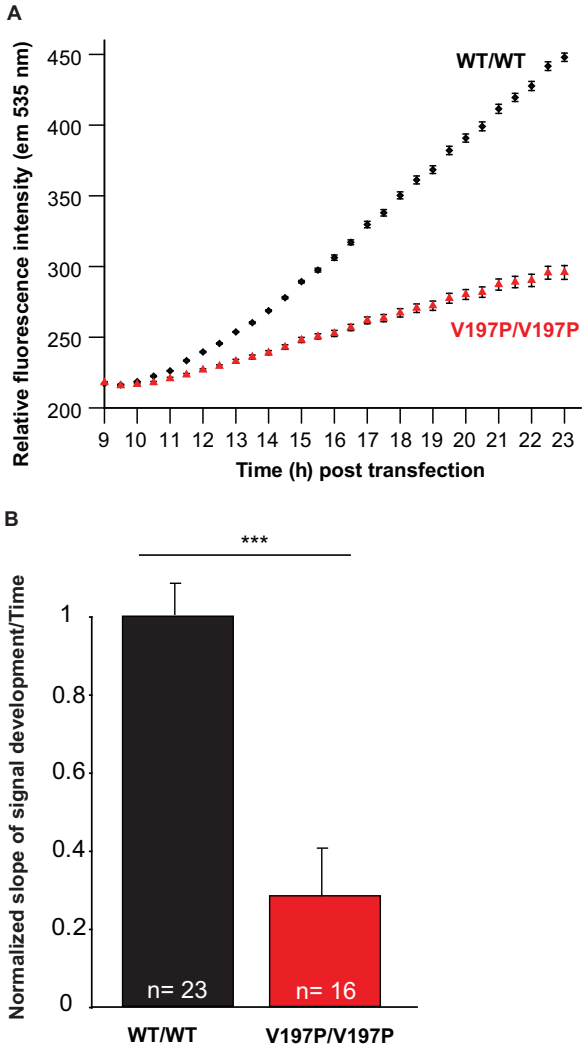


**Suppl. Fig. 1: Sequence alignment and oligomerization of SPFH domain proteins**

A) Sequences of mouse stomatin (Expasy accession number P54116), mouse STOML-1 (Q8CI66), mouse STOML-2 (Q99JB2), mouse STOML-3 (Q6PE84), mouse podocin (Q91X05), human Flotillin 1 (O75955), mouse Flotillin 2 (Q60634) and ph stomatin (O59180) were aligned using ClustalW (Thompson et al., 1994), and the alignment was manually adjusted. Secondary structure elements are shown on top. The following residues are indicated: D - dimerization interface, 1 - oligomerization interface-1, 2 - oligomerization interface-2, P - hydrophobic pocket. Selected mutation in MEC-2 causing touch insensitivity in *C. elegans* (Chalfie and Sulston, 1981; Zhang et al., 2004) are indicated in pink.

B, C) Oligomerization of SPFH domain proteins. (B) For ph stomatin (pdb 3BK6), a trimeric assembly was found where residues N-terminal of the stomatin domain form a β-strand which contacts the C-terminus of the next monomer. (C) The SPFH domains of the major vault protein (MVP, pdb 2ZUO) within the vault are stacked next to each other via lateral contacts of α2 and β4. The N- and C- termini of the MVP SPFH domain are not involved in the assembly of the higher-order oligomers.

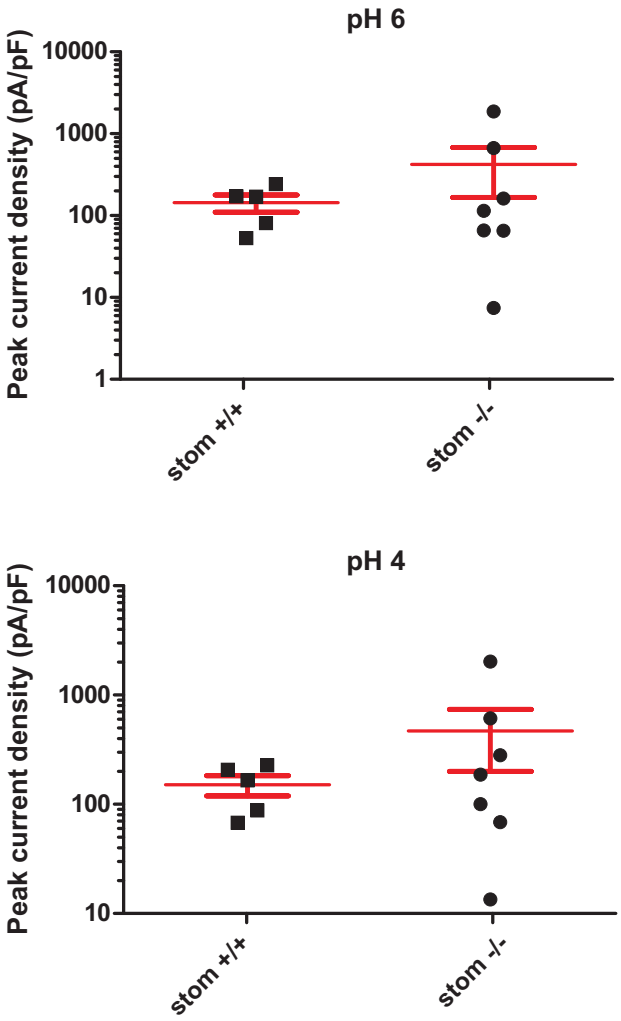
## Supplementary Figure 2




**Suppl. Fig. 2: BiFC time course experiments**

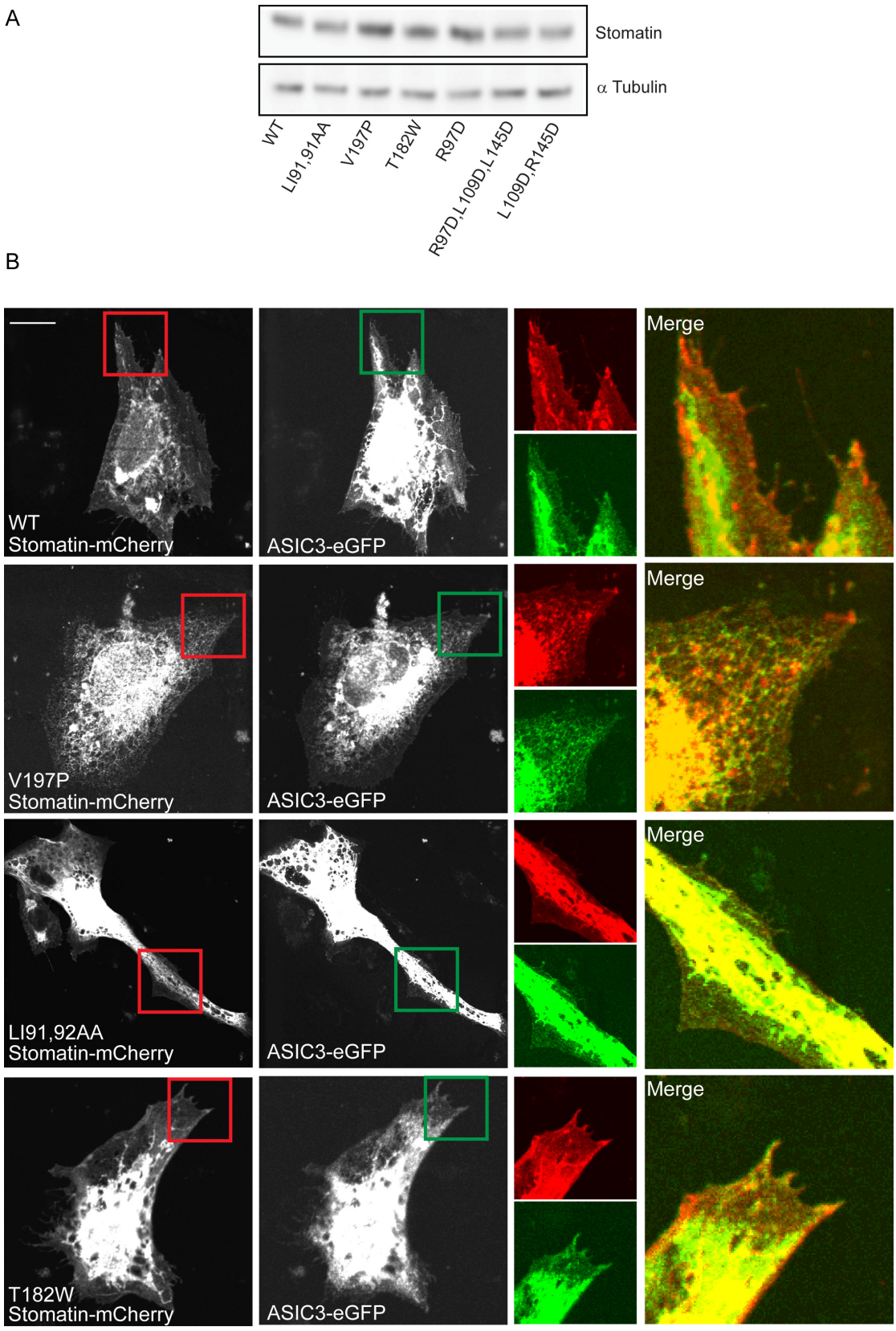
HEK cells were co-transfected with plasmids encoding stomatin fused to N- and C-terminal fragments of YFP. Relative fluorescence intensity was monitored 8 h after transfection over 16 hours. (A) Representative results for stomatin/stomatin (n=3) and the stomatin V197P/V197P mutant (n=5) from parallel experiments. (B) The slope of the increase in relative fluorescence intensity over time was determined by a linear fit. Data of the V197P mutant were normalized to stomatin. n = number of individual transfections. *** $p < 0.001$ according to two tailed T-test.

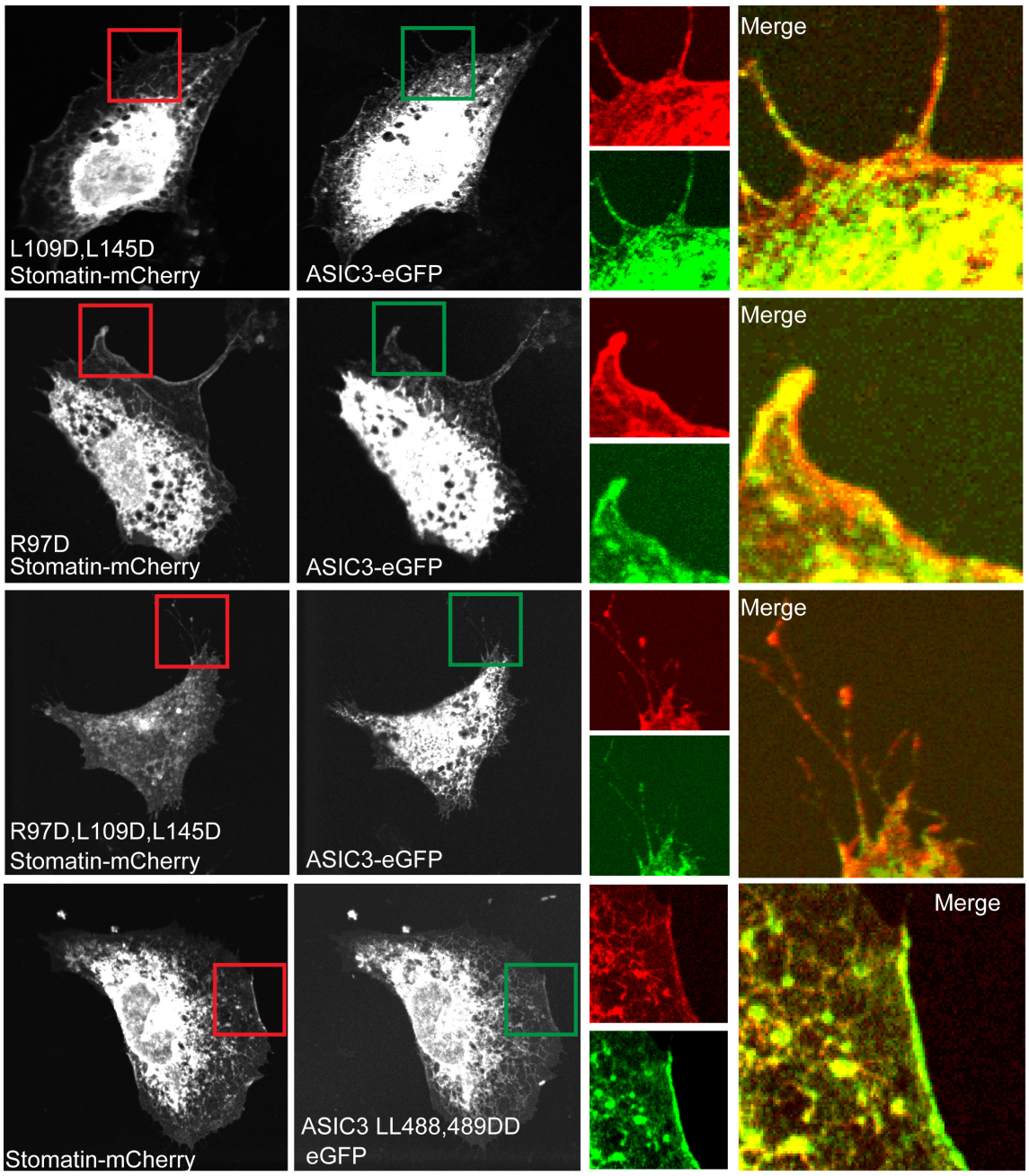
**Supplementary Figure 3**




**Suppl. Fig. 3: ASIC3-mediated currents in adult mouse fibroblasts.**
Tail derived mouse adult fibroblasts from wild type and *stomatin*$^{-/-}$ mice were transfected with an ASIC3 encoding plasmid and an eGFP expressing plasmid. Proton-induced currents were recorded to stimuli of pH 4.0 and pH 6.0 in GFP-expressing cells. Each dot represents one cell measurement and the red lines show mean ± SEM. Fibroblasts were obtained from 2 wild type and 2 *stomatin*$^{-/-}$ mice.
In agreement with our previous results, ASIC3-currents were on average larger in *stomatin*$^{-/-}$ fibroblasts compared to wild type cells, but the difference was not significant, according to Student's T-test. We found that many GFP expressing cells of both genotypes failed to exhibit any ASIC3-like current, perhaps indicating that channel trafficking to the membrane is intrinsically different in primary mouse fibroblasts compared to CHO cells.

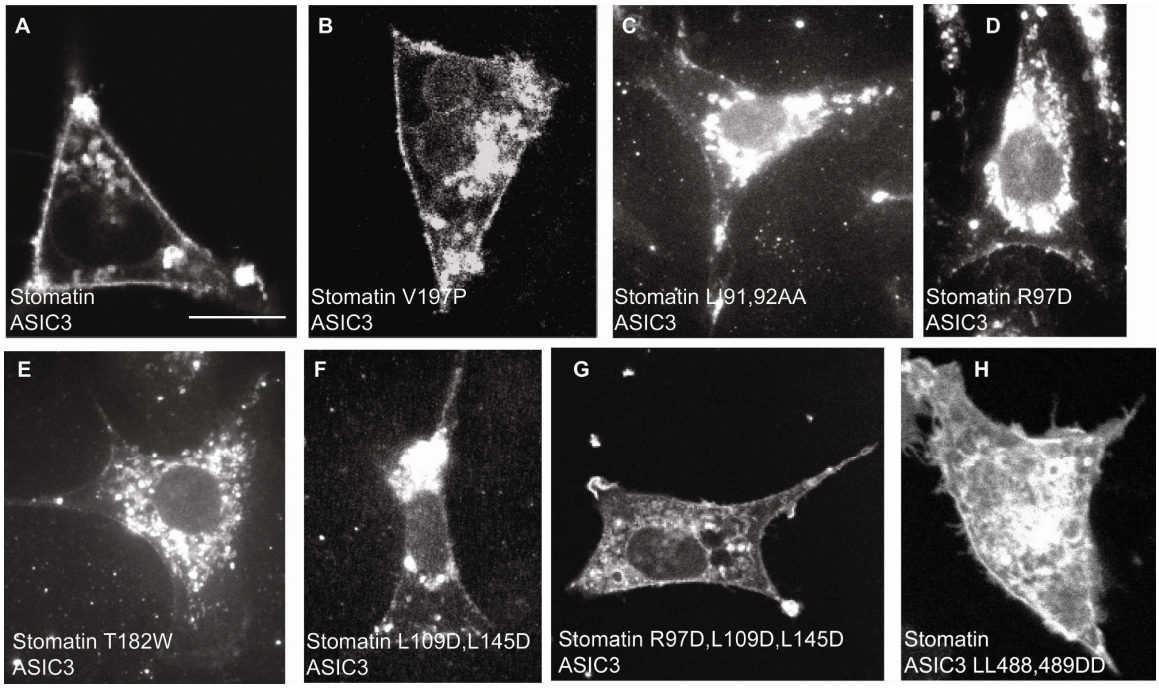
## Supplementary Figure 4





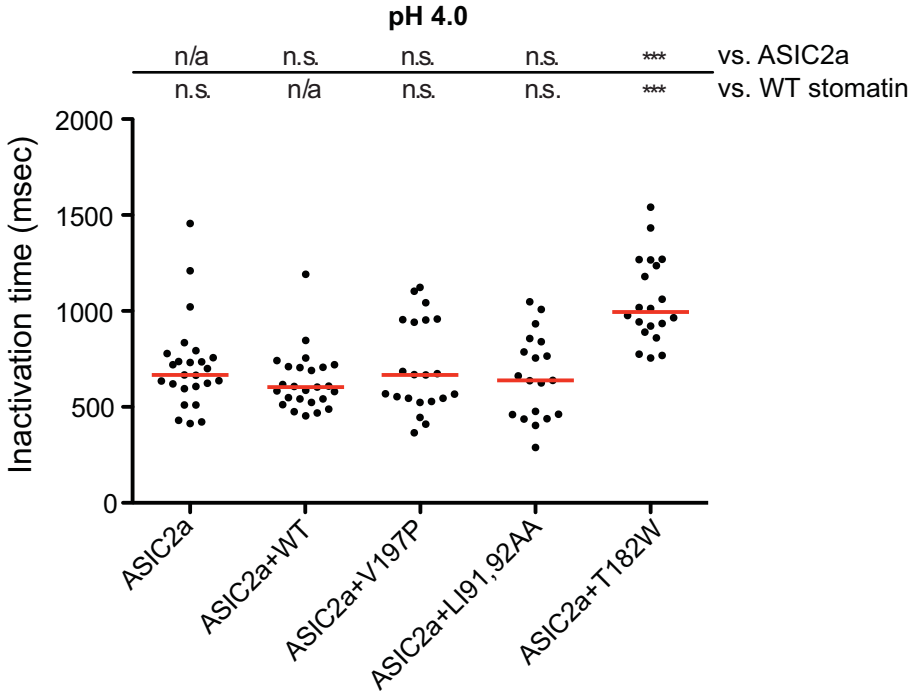

**Suppl. Fig. 4: Cellular localization and expression of stomatin mutants in CHO cells**
A) Western blot analysis of CHO cells transfected with Myc-His stomatin plasmids, using an anti-Myc and anti-tubulin antibody as loading control, showing comparable expression levels for all mutants.
B) CHO cells were grown to a density of 60% before co-transfection with plasmids containing mouse stomatin-mCherry or the indicated stomatin mutants and eGFP-ASIC3 or the indicated mutants. 24 h post transfection, cells were fixed and analyzed by confocal microscopy using the ImageJ software. Representative cells are shown. Sections marked by red or green boxes are merged and shown magnified on the right. The scale bar represents 20 µm.

**Supplementary Figure 5**




**Suppl. Fig. 5: BiFC microscopy of stomatin and ASIC3 in CHO cells.**
CHO cells were co-transfected with plasmids encoding ASIC3 fused to a C-terminal fragment of YFP and stomatin fused to an N-terminal fragment of YFP. 24 h post transfection, cells were subjected to live cell imaging using epifluorescence microscopy. For all stomatin constructs, co-localization with ASIC3 in perinuclear structures and at the plasma membrane was visible (A-G). Also the LL488,489DD mutant of ASIC3 similarly co-localized with stomatin. The white scale bar corresponds to 20 µm.

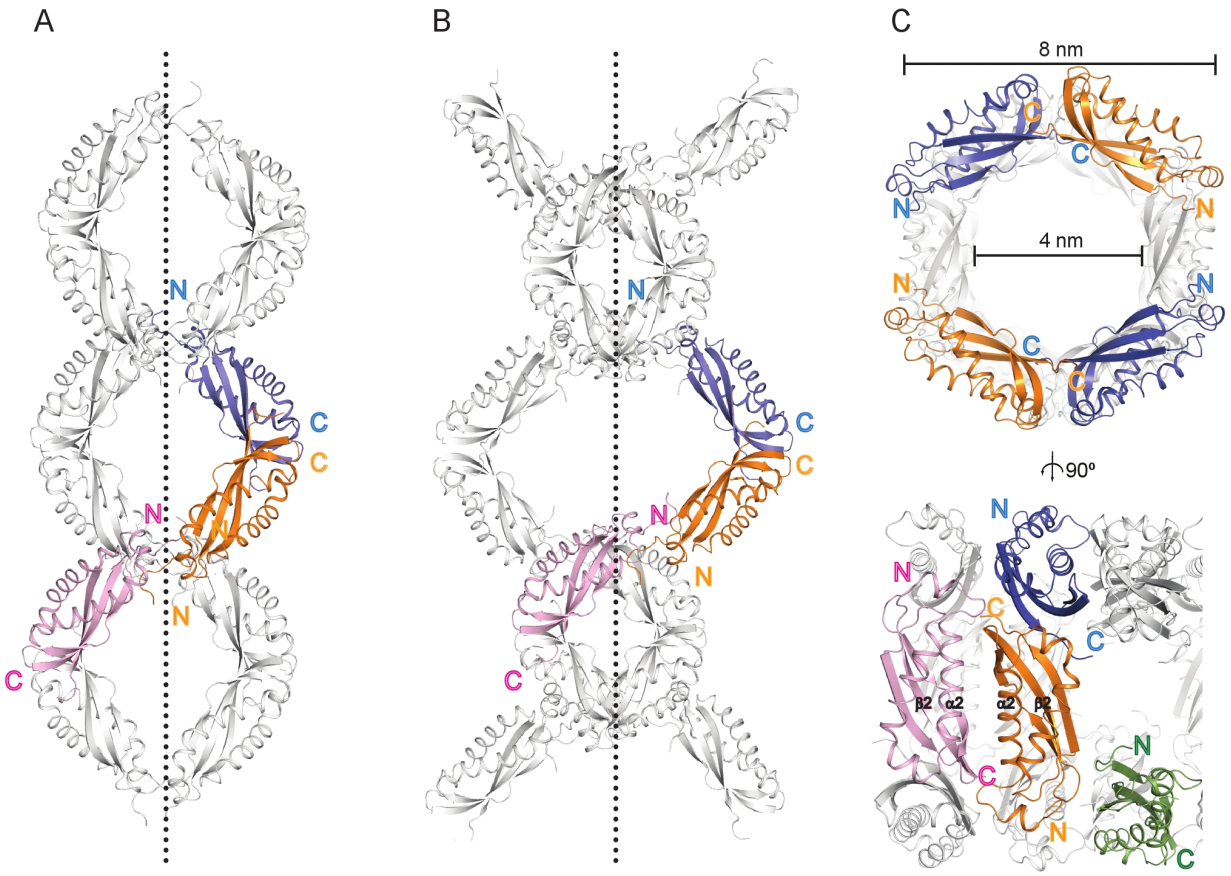
**Supplementary Figure 6**




**Suppl. Fig. 6: ASIC2a inactivation at pH 4.0**

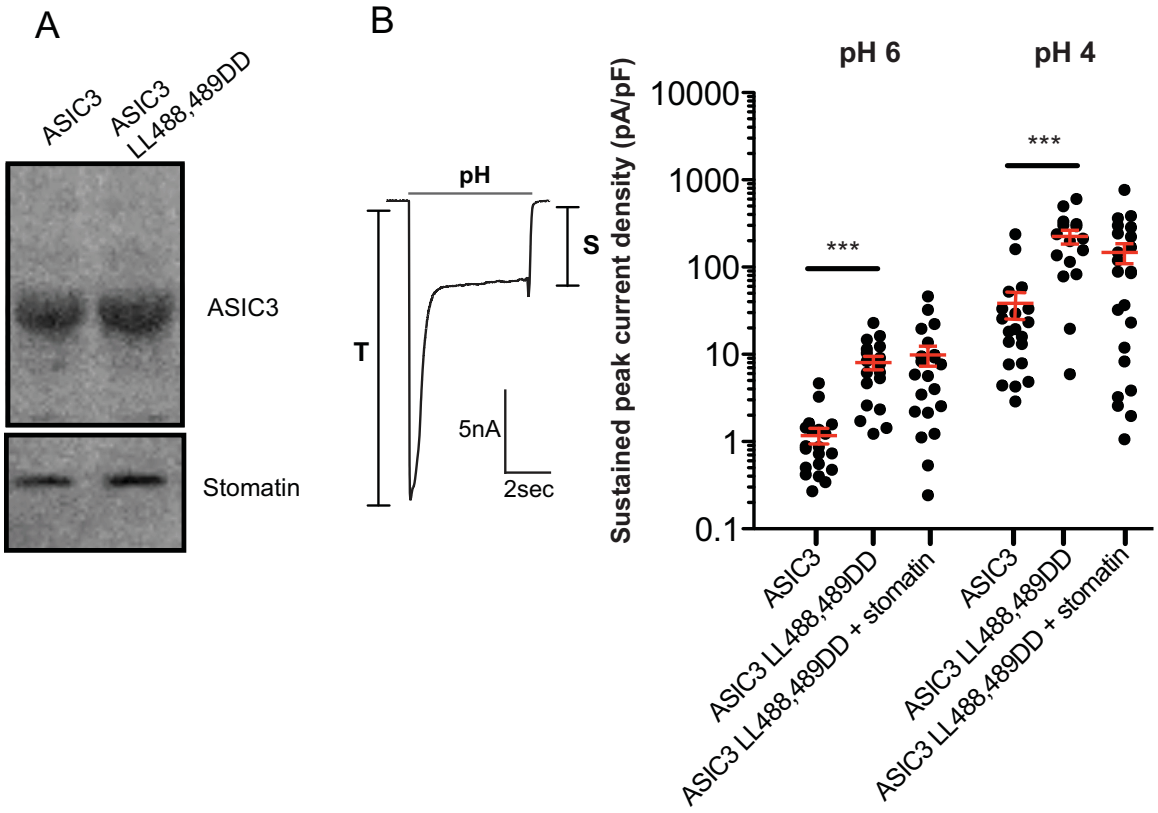
Dot plots of inactivation time of ASIC2a at pH 4.0. Each dot represents one cell measurement, and the red lines show the median for each data set. The Kruskal-Wallis test followed by Dunn's post test and statistical comparisons of inactivation time compared to ASIC2a expressed alone and ASIC2a co-expressed with stomatin or the indicated mutants is shown. n/a, not applicable; n.s., not significant; ***, $p < 0.001$.

**Supplementary Figure 7**




**Suppl. Fig. 7: Crystal packing of the mouse stomatin domain constructs**
In all three crystal forms, a stomatin dimer with a C-terminal dimerization interface was observed. In crystal form 1 (A) and 2 (B), the dimers show an intertwined helical crystal packing, formed by additional contacts of the N-terminal loop preceding the stomatin domain (including Leu91 and Ile92) with the hydrophobic pocket of the adjacent dimer. In (A), the dimers form a homogeneous helix, whereas in (B), the helix is bulky. This difference originates from the flexibility of the N-terminal loops. (C) 3D figure showing the arrangement of stomatin dimers in crystal form 3, as seen in Fig. 6B. For viewing, the latest version of the freely available Adobe Reader 9 should be installed. Move the cursor over the figure and use left button to activate the 3D-mode. Use mouse left button for rotation, left button / shift for zoom function and left button / ctrl for translation. Preset views are available in the Toggle menu tree. To return to Supplementary Figure 3, use right mouse button and "Disable 3D". In crystal form 3, a tubular oligomeric arrangement is established by symmetric contacts of the stomatin domain via interface-1 and 2. Our mutagenesis data and the surface conservation plots might indicate that higher-order stomatin assembly proceeds in a similar fashion as in crystal form 3. It might also involve hetero-oligomerization between different stomatin members (Lapatsina *et al.*, 2012; Mairhofer *et al.*, 2009). Both N- and C-termini of stomatin point towards gaps in the tubular oligomers. From the crystal structure, it is unclear whether the termini would further extend to the exterior or interior of the tubular oligomer.

**Supplementary Figure 8**




**Suppl. Fig. 8: Functional characterization of the ASIC3 LL488,489DD mutant**
(A) Similar levels of ASIC3 and ASIC3 LL488,489DD were co-immunopreciptated by Myc-tagged stomatin.
(B) Overexpressed ASIC3 LL488, 489DD in CHO cells shows increased transient (T) and sustained (S) currents. The maximal sustained current amplitude was determined as indicated on the left. The ASIC3 LL488, 489DD mutant showed increased sustained (S) current amplitudes compared to ASIC3 which were not affected by stomatin co-expression. ***, $p < 0.001$

**References exclusively used in the Supplementary Material**

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22:** 4673-4680

Zhang S, Arnadottir J, Keller C, Caldwell GA, Yao CA, Chalfie M (2004) MEC-2 is recruited to the putative mechanosensory complex in C. elegans touch receptor neurons through its stomatin-like domain. *Curr Biol* **14:** 1888-1896