Supplemental Figures and Figure Legends

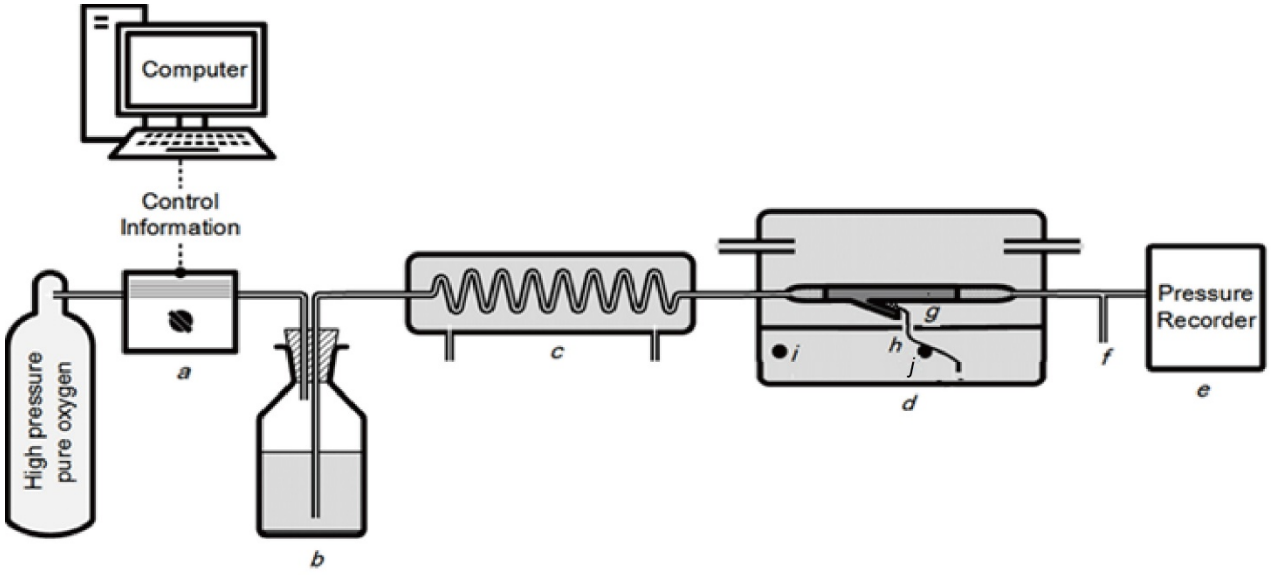


Fig. S1 The custom-made pressure-servo system. a, proportional valve; b, perfusion bottle; c, glass heat exchanger; d, perfusion bath; e, pressure transducer; f, outlet control valve; g, aortic arch blood vessel preparation; h, aortic nerve; i, reference electrode; j, recording electrode.

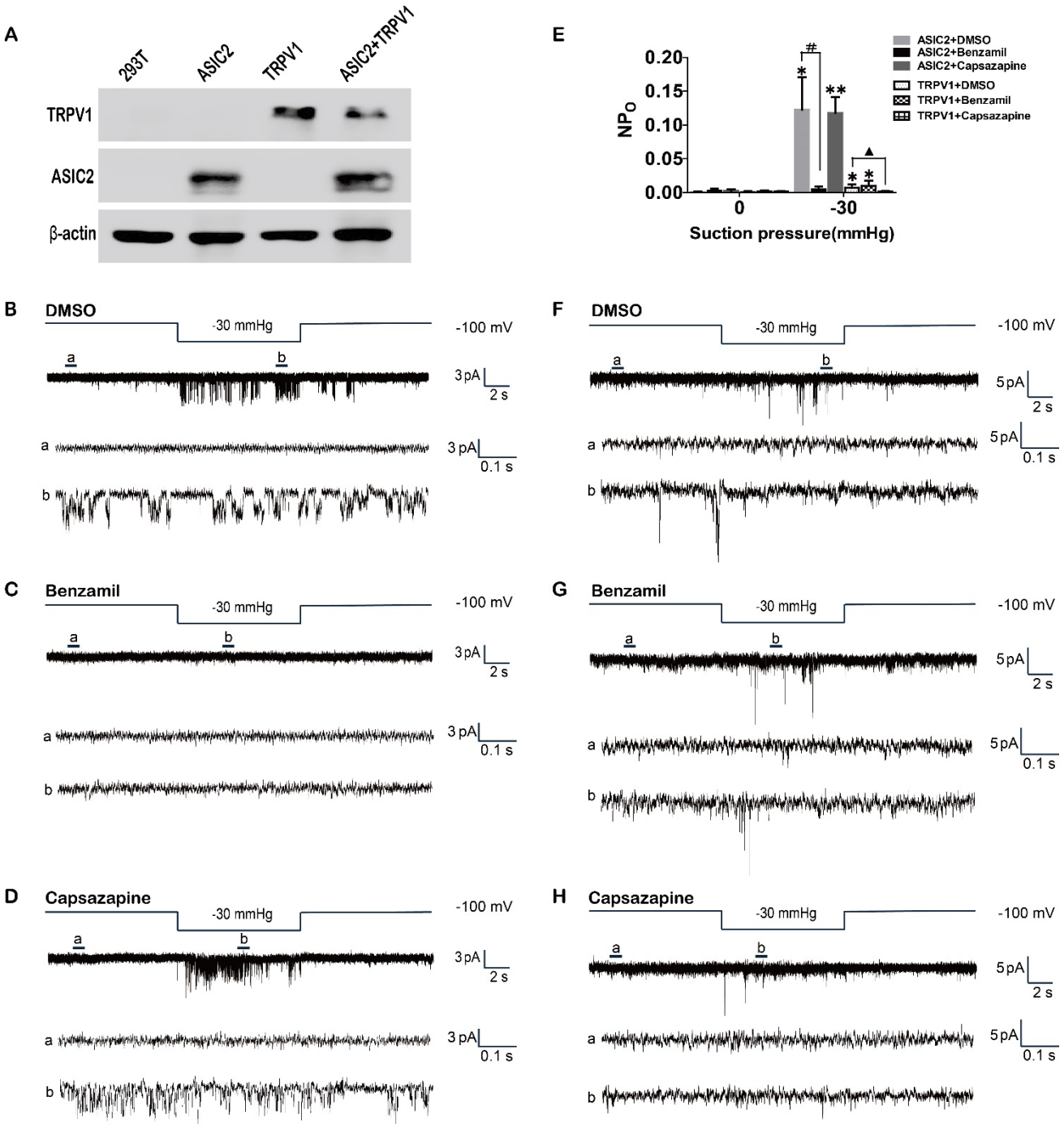


Fig. S2 Effects of benzamil and capsazapine on the activation of ASIC2 or TRPV1 channels in cell-attached patches. **A** Western blotting showing ASIC2- or TRPV1-expressing plasmids successfully transfected into HEK293T cells and proteins expressed. **B** Effects of negative pressure on currents from HEK293T cells expressing ASIC2 only (DMSO control group). **C** Benzamil effectively blocks the activation of ASIC2 under negative pressure stimulation. **D** Capsazapine has no significant effect

on the activation of ASIC2 under negative pressure stimulation. **E** Quantification of *NPo* challenged by negative pressure before and after treatment with 100 μmol/L benzamil and 20 μmol/L capsazapine in cells expressing ASIC2 or TRPV1 alone. For each channel, *NPo* was calculated from 0 – 30 mmHg (*P <0.05, **P <0.01, *vs* 0 mmHg (paired *t*-test), $^{\#}P$ = 0.0001, $^{\blacktriangle}P$ = 0.0268; NP_O, total single-channel open probability). **F** Effects of negative pressure on currents from HEK293T cells expressing TRPV1 alone (DMSO control group). **G** Effect of benzamil on the activation of TRPV1 under negative pressure stimulation. **H** Effect of capsazapine on the activation of TRPV1 under negative pressure stimulation. In **B–D** and **F–H**, lower traces show expanded 1-s segments from 0 (a) and –30 mmHg (b).

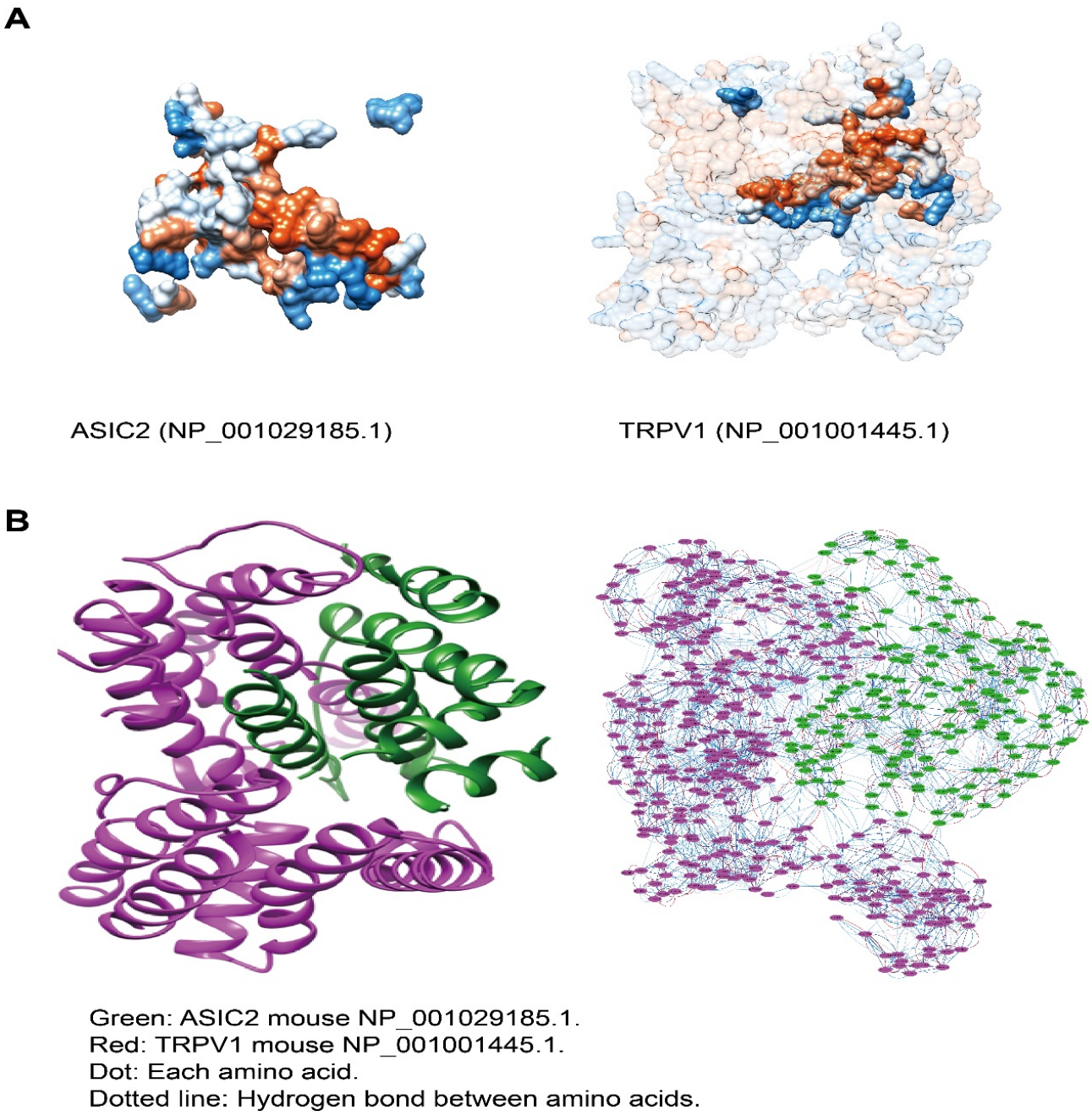
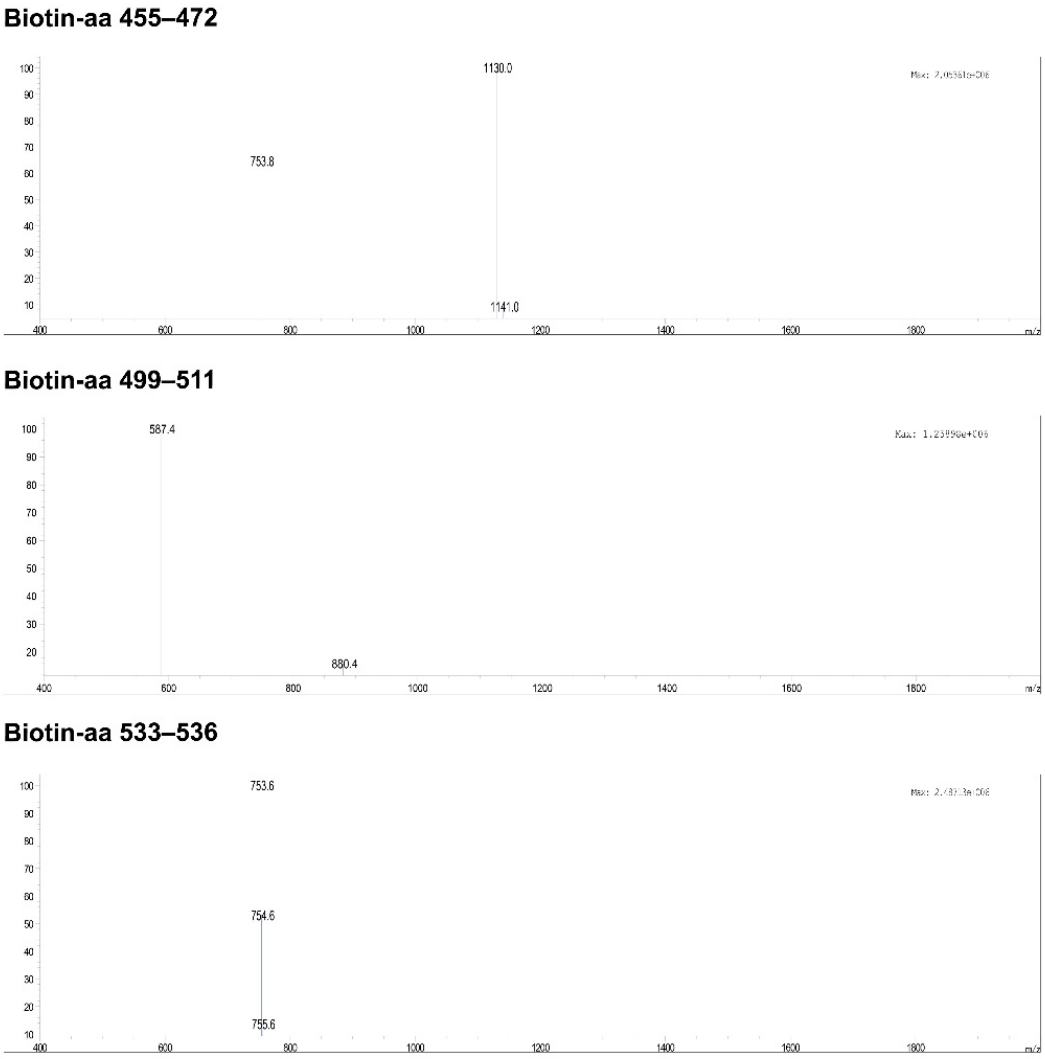


Fig. S3 Interactions between ASIC2 and TRPV1 are predicted by molecular dynamics modeling and protein analysis. **A** Surface map of hydrophobic amino-acids in the range of 5 Å of direct contact between ASIC2 and TRPV1. **B** Left, structural model of the interaction fragments between ASIC2 and TRPV1. Right, hydrogen (H) bond network of hydrophobic amino-acids in ASIC2 and TRPV1.

A



B

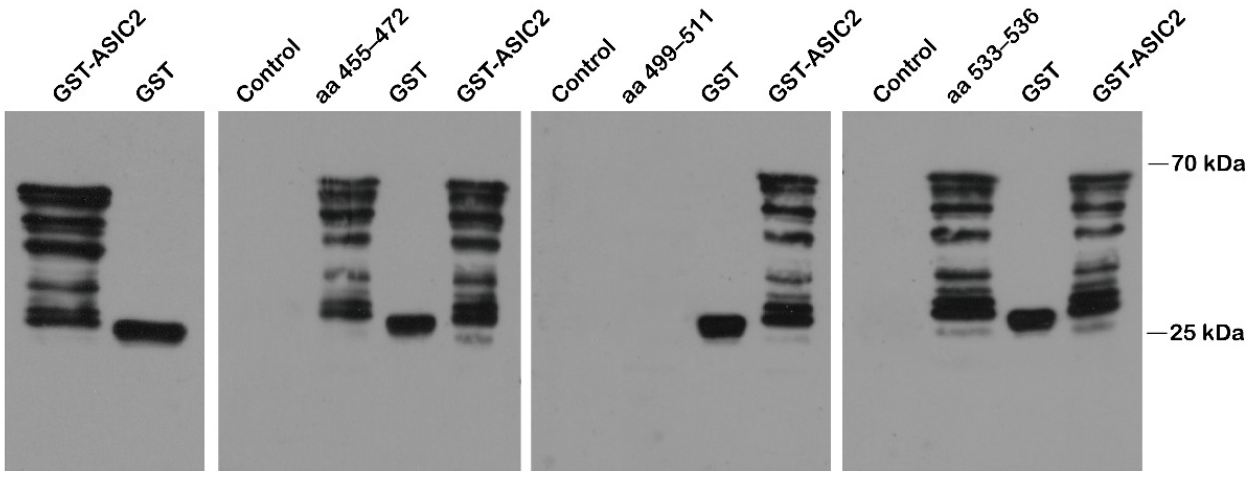


Fig. S4 Verification of direct binding sites between ASIC2 and TRPV1. **A** Mass spectra of biotin-aa 455–472, biotin-aa 499–511, and biotin-aa 533–536 sequences. **B** Direct interaction between ASIC2

extracellular protein (aa 59–427) and TRPV1 extracellular segments (aa 455–472 and aa 533–536) shown by biotin pull-down assays.