SUPPLEMENTARY MATERIALS

I. WC currents in Panx1-expressing cells.

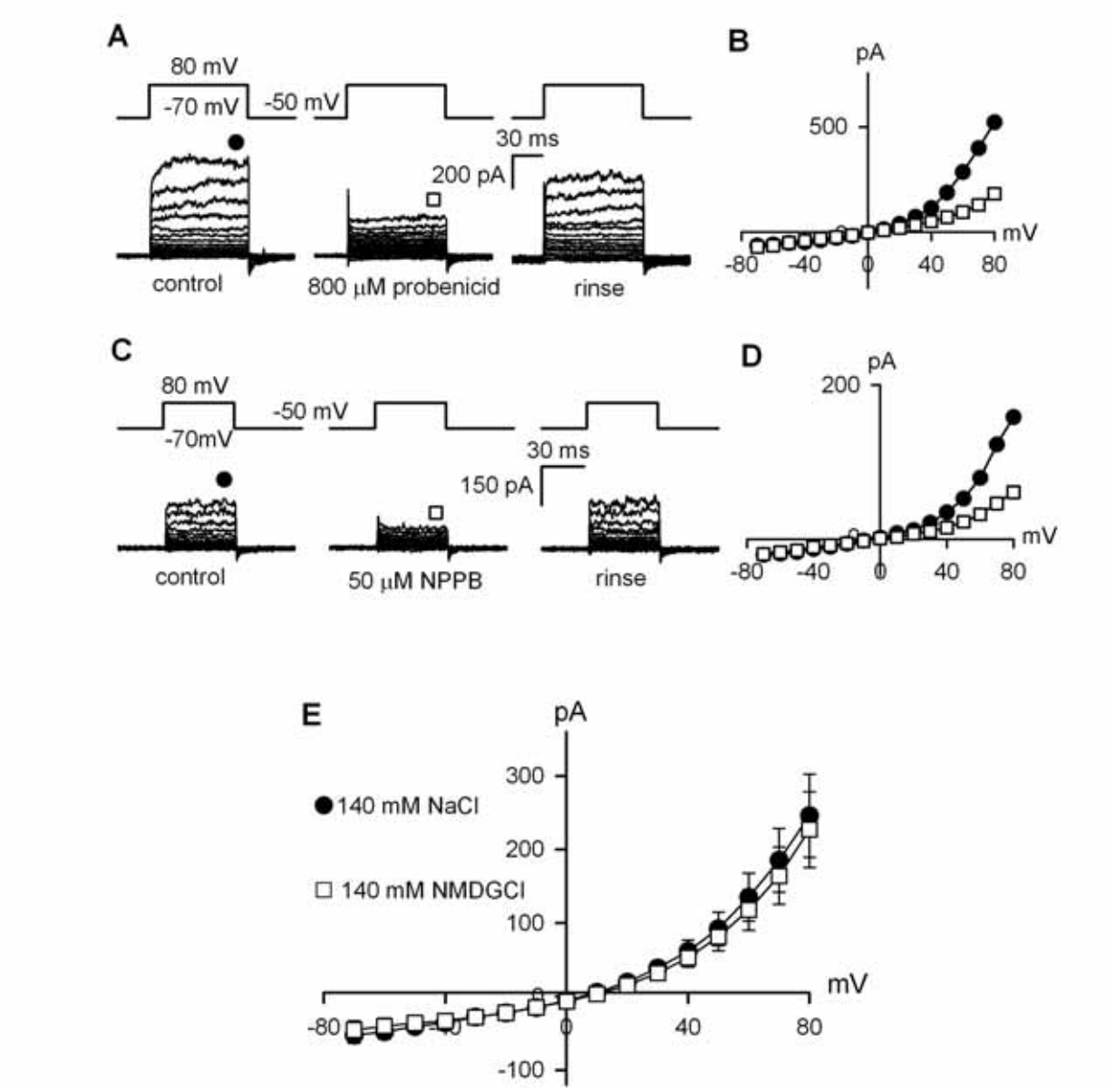

Figure S1. WC-currents in Panx1-positive HEK-293 observed under different recording conditions. (**A, C**) Probenicid (**A**) and NPPB (**B**) inhibited WC-currents reversibly. Cells were held at –50 mV and polarized by 100-ms (**A**) or 50-ms (**B**) voltage pulses between -70 and 80 mV. (**B, D**) I-V curves of the WC currents shown in (**A**, **C**); the currents were measured at the moments marked by the corresponding symbols. (**E**) Kinetics of WC currents and their I-V curves (n=4) varied insignificantly when 140 mM NaCl + 10 mM HEPES-NaOH in the bath was substituted for 140 mM NMDG-Cl + 10 mM HEPES-NMDG-OH. In all cases, cells were perfused with 140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES-NaOH; the patch pipette contains 100 mM CsCl, 0.5 mM $MgCl_2$, 10 mM BAPTA-40 CsOH, 10 mM HEPES-CsOH.

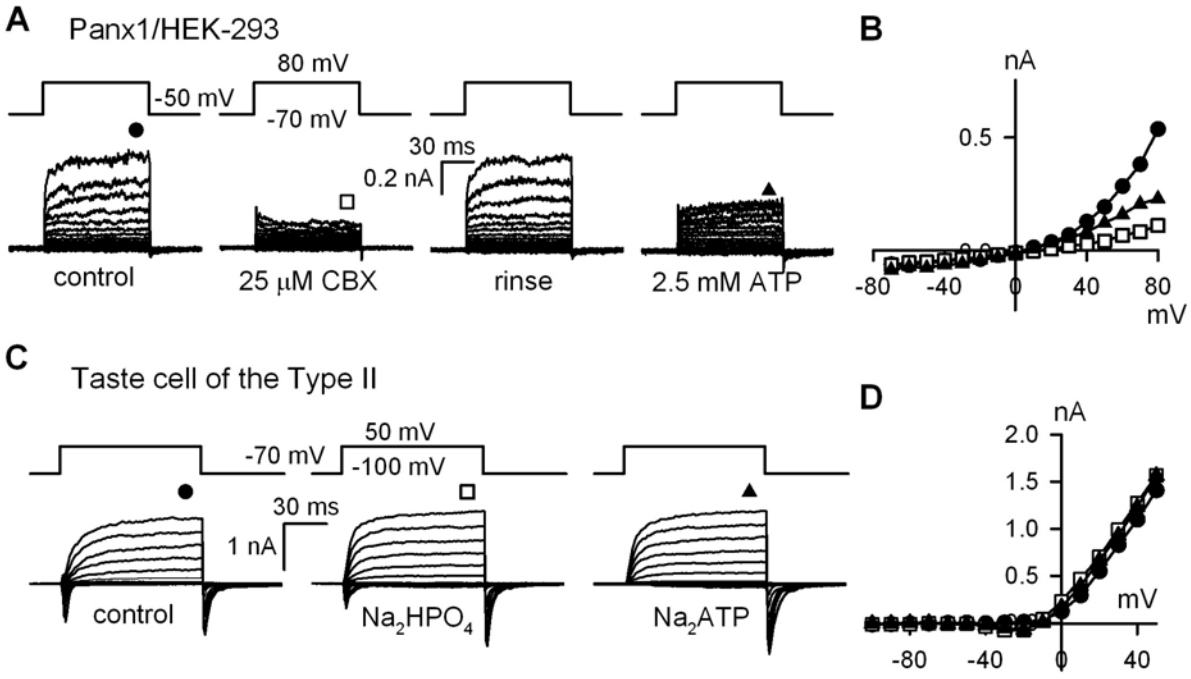

Figure S2. ATP sensitivity of Panx1-currents. (**A**) Representative recording (n=5) of WC currents from a Panx1-positive HEK-293 cell in control and in the presence of 25 μM CBX or 2.5 mM ATP. The recording conditions were as in the Supplementary Fig.**1**. (**B**) I-V curves of the WC currents measured at the moments indicated by the symbols in (**A**). (**C**) Unlike Panx1-positive HEK-293 cells, bath ATP did not affect WC currents in taste cells of the Type II. In the middle and right panels, 140 mM NaCl in the bath was substituted for 40 mM NaCl + 50 мM Na_2HPO_4 or for 40 mM NaCl + 50 мM Na_2ATP. In (**C**), currents were recorded using the perforated-patch clamp approach with the pipette containing (mM): 140 CsCl, 0.6 $MgCl_2$, 1 EGTA, 0.1 EDTA, 10 HEPES-CsOH, 400 μg/ml nistatine. (**D**) I-V curves of the currents measured at the moments indicated by the symbols in (**C**).

II. Depolarization of SK-N-SH and CHO cells expressing Panx1 does not trigger ATP release.

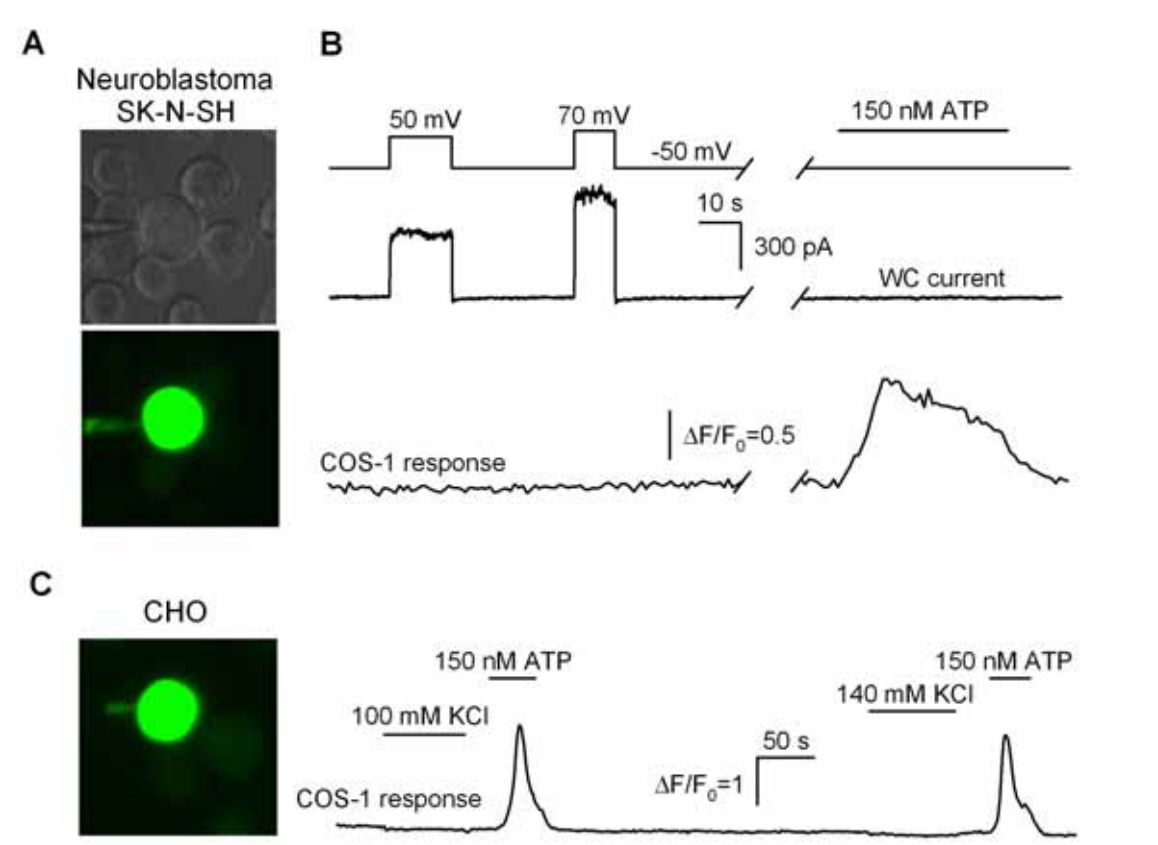

Figure S3. Panx1-positive SK-N-SH (**A**) and CHO (**C**) cells show undetectable ATP release on depolarization. (**A)** Images of a SK-N-SH cell transfected with the pIRES2-EGFP/Panx1 construct and nearby COS-1 cells loaded with Fluo-4 in transparent light (upper panel) and their fluorescent image (bottom panel. (**B**) Representative concurrent recordings of a WC current (middle panel) from the SK-N-SH cell in (A) (bright green cell) and fluorescence from a nearby COS-1 cell (bottom panel). The upper panel illustrates the time course of membrane voltage held in the SK-N-SH cell. Depolarization elicited a strong inward current in the SK-N-SH cell that was not accompanied by meaning response of the ATP-biosensor, the addition of 150 nM ATP to the bath stimulated strong sensor response (bottom panel). (**C**) Left panel, fluorescent imaging of a CHO cell transfected with pIRES2-EGFP/Panx1. In the right panel, depolarization of this cell with 100 mM KCl or 140 mM KCl resulted in negligible ATP release, as indicated by insignificant ATP-biosensor responses. The strong responses of the ATP-sensor to 150 nM ATP indicate that its sensitivity to the nucleotide was high enough. In all cases, cells were perfused with 140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES-NaOH; the patch pipette in (**A**) contains 100 mM CsCl, 2 mM MgATP, 10 mM BAPTA-40 CsOH, 10 mM HEPES-CsOH. In (**C**), the pipette filled with the

bath solution was used solely to move the assayed CHO cell that was not sucked to achieve gigaseal.

III. Analysis of pannexin transcripts in CV papilae from WT and Panx1 KO mice.

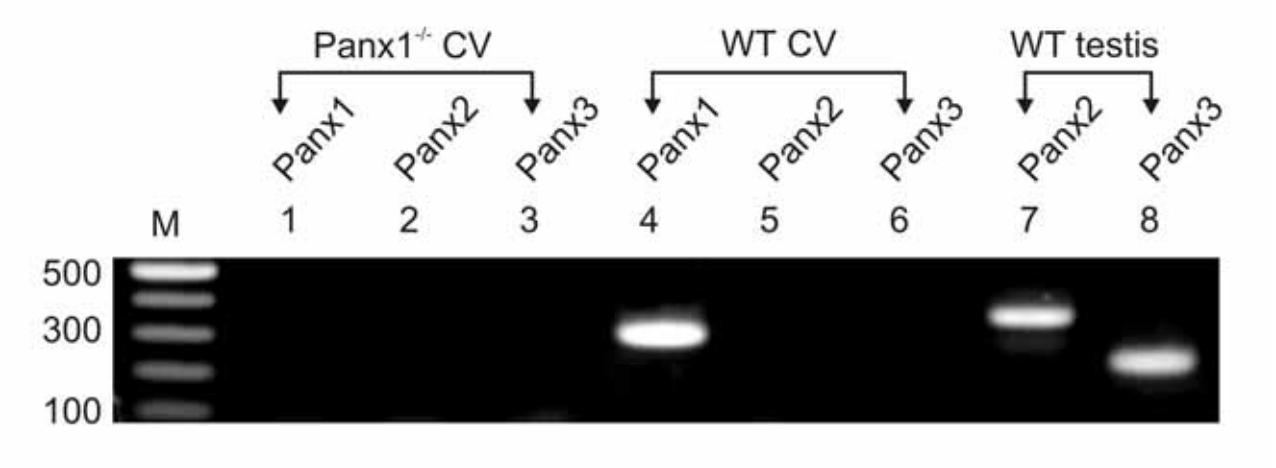

Figure S4. Detection of Panx transcripts in CV papillae. The representative RT-PCR analysis (n=4) of Panx1, Panx2, and Panx3 transcripts using RNA isolated from an individual CV papilla of Panx1$^{-/-}$ (lanes 1-3) and WT (lanes 4-6) mice and from WT testis (lanes 7-8) (positive control). The molecular weight markers (M) are GeneRuler 100 bp DNA Ladder (Fermentas). Their sizes (bp) are indicated by the digits on the left of the 1,2% agarose gel stained with ethidium bromide. The following primer pairs were used:

5'-GCTGCTCAGCCTCATTAACC-3' and 5'-GCCCAGGTTTGTCAGGAGTA-3' for Panx1; 5'-GCAGCACCCACCAAAGATG-3' and 5'-ACAACTGTTCTCGGCTCCT-3' for Panx2, and 5'-GGGATGAGCTAGAGAAGGC-3' and 5'-TAGCAGCCCTGTCTTGATG-3' for Panx3. The exon4-specific amplicon of Panx1 is of 292 bp, Panx2 amplicon is of 346 bp, and Panx3 amplicon is of 224 bp.

IV. Panx1 channels are not gated by intracellular Ca^{2+}.

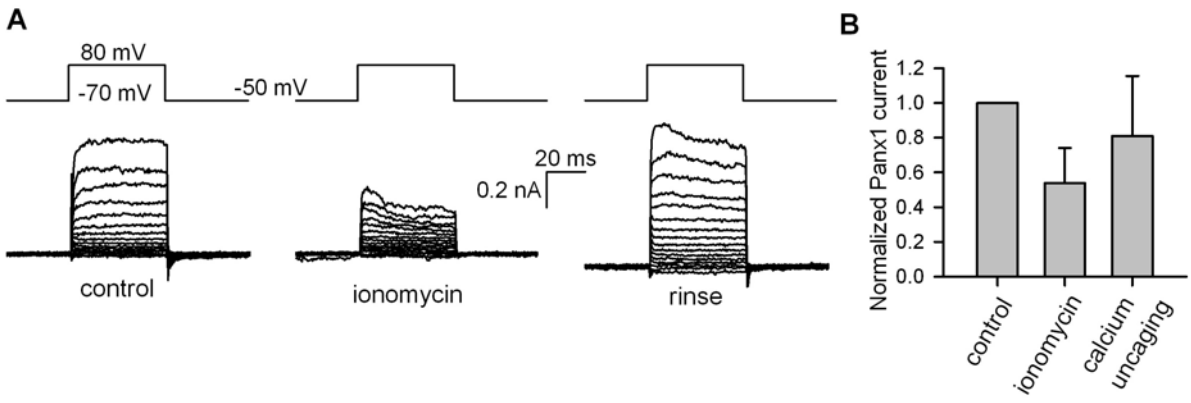

Figure S5. Increase in intracellular Ca^{2+} does not stimulate Panx1 currents in HEK-293 cells. (**A**) Representative WC-currents in control and with 5 μM ionomycin in the bath. (**B**) Normalized Panx1 currents in control, in the presence of 5 μM ionomycin, or immediately after Ca^{2+} uncaging due to photodistraction of NP-EGTA (4 μM) with a 2 s-flash at 355 nm. While ionomycin markedly inhibited WC Panx1-current ($p<0.01$), Ca^{2+} uncaging led to an insignificant decrease in Panx currents. The data in (**B**) are presented as a mean ± SD (n=7 for ionomycin and n=8 for NP-EGTA). WC currents were recorded with the perforated patch approach under the conditions as in Fig.2B.

V. Genetic ablation of Panx1 affects insignificantly specific uptake of carboxyfluorescein in taste cells.

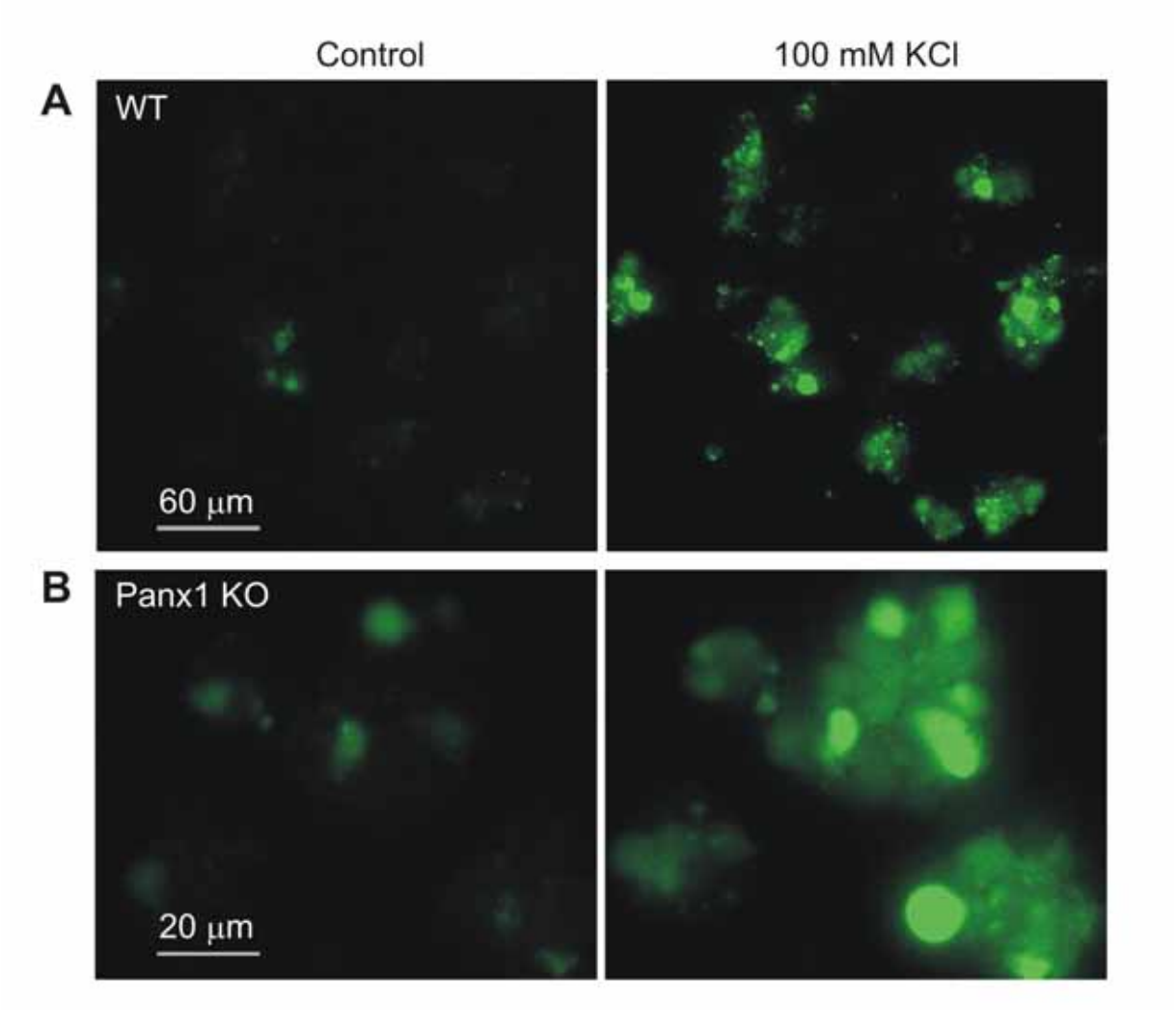

Figure S6. Loading of taste cells with carboxyfluorescein. (**A**) Left panel, representative fluorescent imaging of undissociated taste buds isolated from a CV papilla of a WT mouse (n=5). In control, taste buds were subjected to 2 min staining with carboxyfluorescein (250 mg/l) dissolved in the bath solution containing 140 mM

NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES-NaOH. Thereafter, the buds were rinsed with the bath solution without carboxyfluorescein, and their fluorescent image was captured. As indicated, very few taste cells took up the dye at rest. Next, the same buds were again stained with carboxyfluorescein for 2 min in the bath solution, wherein 140 mM NaCl was substituted for 40 mM NaCl + 98 mM KCl. After careful rinse of the taste buds with the normal bath solution, their fluorescence was monitored. As illustrated in the right panel, carboxyfluorescein loading into taste cells was markedly enhanced under the depolarizing conditions. (**B**) Similar experiments were carried out with taste buds from Panx1-null CV papillae (n=3). Qualitatively, no difference was revealed in staining patterns between WT and Panx1 KO mice. As was the case with normal taste cells, bath application of 100 mM KCl dramatically increased carboxyfluorescein loading, indicating that responsible voltage-gated pathways did not collapse due to the genetic ablation of Panx1. In (**A**) and (**B**), fluorescence was excited at 480±10 nm and captured at 535±25 nm with a fluorescent Axioscope-2 microscope and an EMCCD Andor iXON camera.

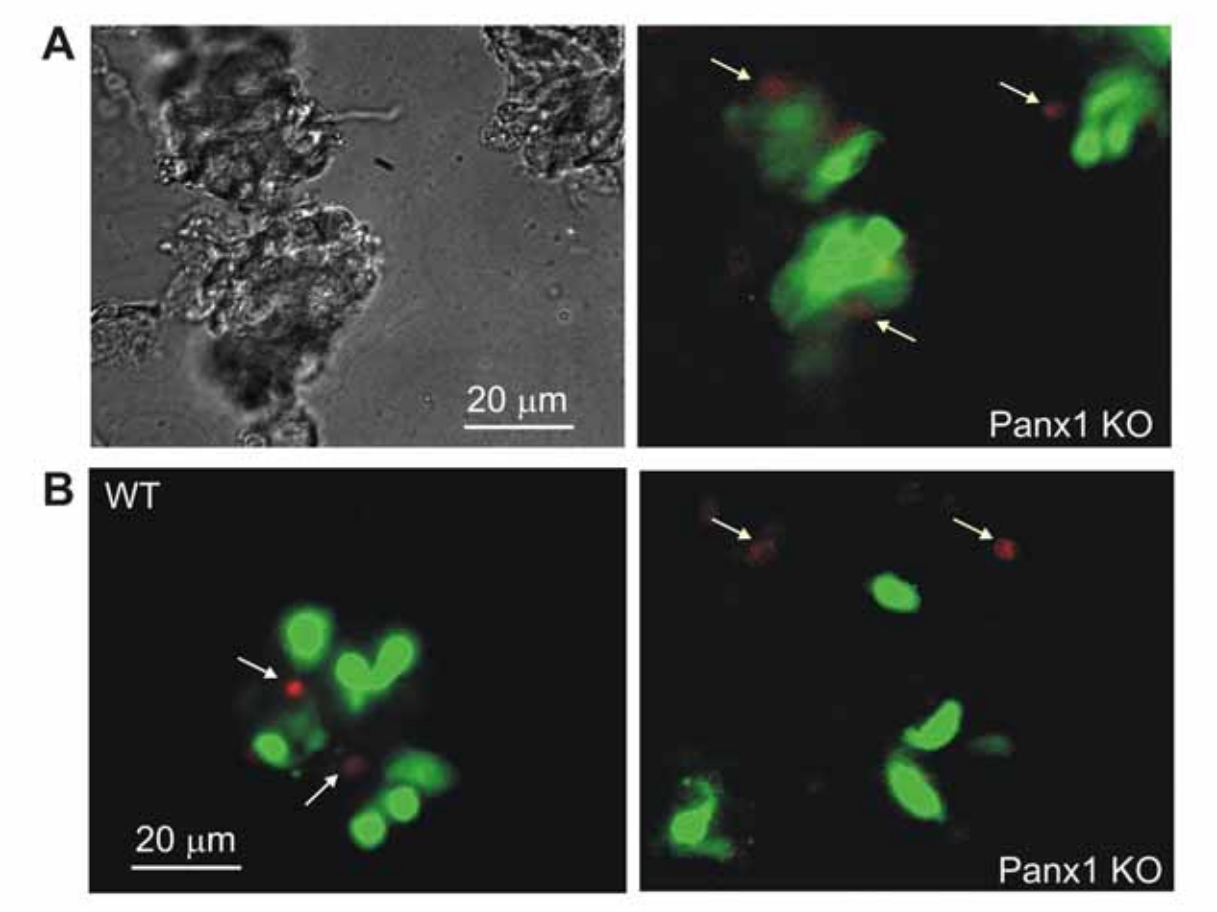

Figure S7. Accumulation of carboxyfluorescein and ethidium bromide by taste cells. (**A**) Left panel, transparent light imaging of undissociated taste buds isolated from a CV papilla of a Panx1 KO mouse (n=3). The taste buds were subjected to 2 min staining with ethidium bromide (20 mg/l) in the normal bath solution, next they were washed out and incubated in the depolarizing bath solution (see Fig.6 legend) containing carboxyfluorescein (100 mg/l) for 2 min. Thereafter, the buds were rinsed with the normal bath solution, and their fluorescent image was captured. As indicated, ethidium bromide stained a nucleus (arrows) in few cells, pointing out that they were dead. Meanwhile, many but not all cells in a taste bud took up carboxyfluorescein in response to depolarization with 100 mM KCl (right panel). Cell staining in control (not presented) was similar to that shown in Fig.6B, left panel. The overall data on dye loading implicate a specific voltage-gated pathway for carboxyfluorescein accumulation. (**B**) Representative loading experiments with individual taste cells isolated from CV papillae of WT (n=3) (left panel) and Panx1 KO (n=3) (right panel) mice. Cells were stained as described in (**A**). Qualitatively, taste cells from Panx1 KO and WT mice exhibited similar loading patterns. In particular, carboxyfluorescein was effectively accumulated only upon depolarization with 100 mM KCl. The arrows indicate dead cells with nuclei stained with ethidium bromide. In (**A**) and (**B**), the images were obtained using the "Leave-Dead" optical filter set (Chroma).