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Supplemental Fig. S1

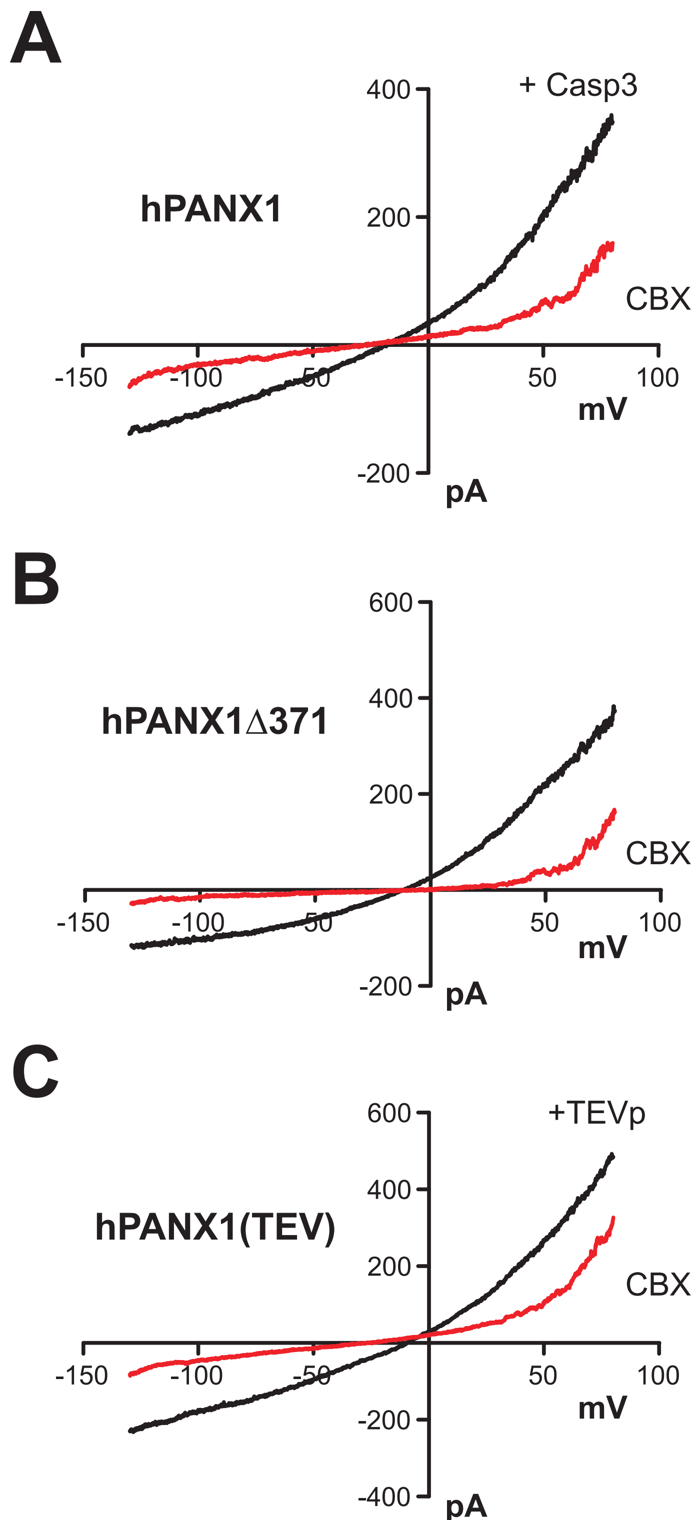

Figure S1. ***I-V properties of truncated and protease-activated hPANX1 channel currents in inside-out patches.***

Inside-out patch currents were recorded from HEK293T cells expressing: wild type hPANX1, after treatment with purified, active Caspase **(A)**; truncated, constitutively active hPANX1Δ371 **(B)**; and hPANX1(TEV), after treatment with purified, active TEVp **(C)**.

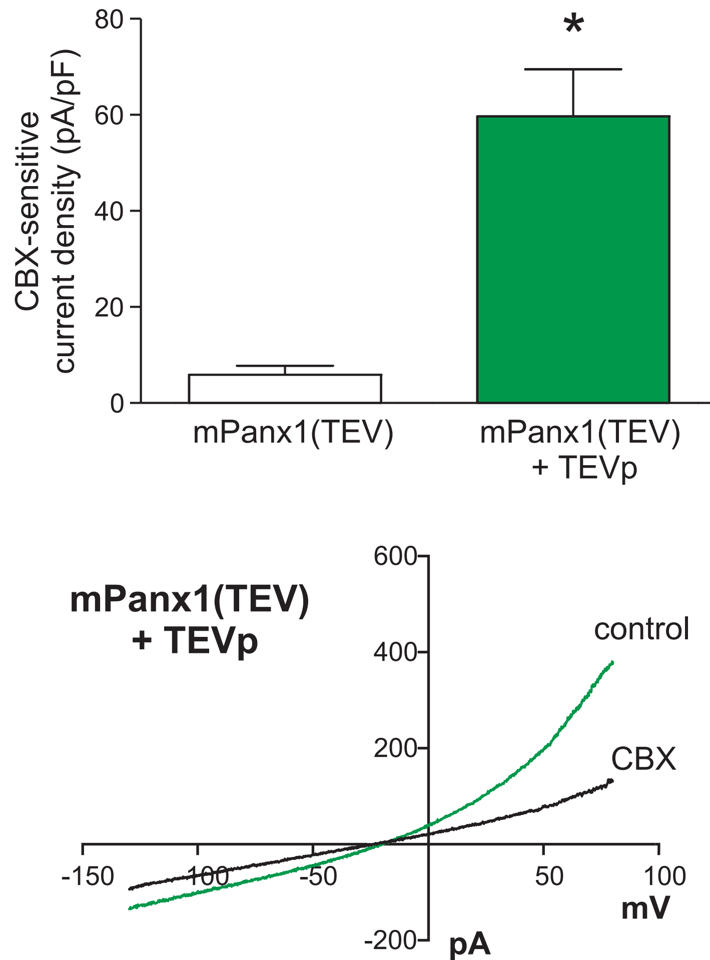

Figure S2. ***Mouse Panx1 is activated by C terminal cleavage.***

Whole cell recordings were obtained from cells expressing mPanx1(TEV), either alone or co-expressed with TEV protease. *Upper*: Averaged peak carbenoxolone (CBX)-sensitive current density from mPanx1(TEV) was markedly enhanced in TEVp-expressing cells; the current magnitude was similar to that seen in cells expressing hPANX1(TEV) + TEVp. *, $P<0.0001$, by unpaired t-test. *Lower*: The *I*-V relationship from the TEVp-activated mPanx1(TEV) is similar to that reported for caspase-activated and TEVp-activated hPANX1 channels (*cf*. Fig. 1A, 1B & 1C; main text).

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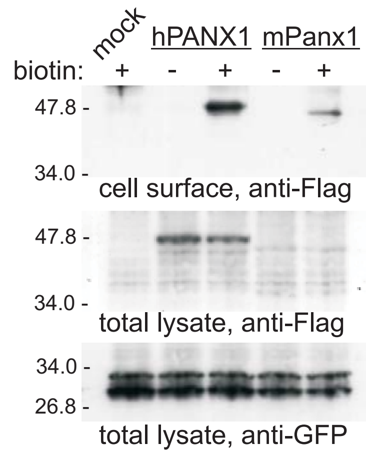
Supplemental Fig. S3

A



B

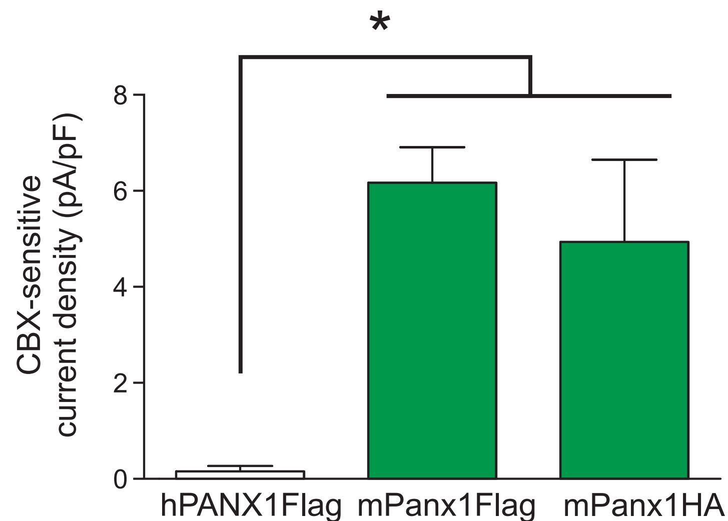


Figure S3. ***Currents from mPanx1 are greater than from hPANX1, despite lower expression levels.***

A. Whole cell lysate and avidin pull-downs of surface biotinylated, Flag-tagged hPANX1 and mPanx1 in HEK293T cells. Note that mPanx1 is expressed at lower levels than hPANX1, which is also reflected in decreased cell surface localization. Co-expressed GFP was used as a loading control; the antibody consistently recognizes two bands in our expression system. The blot is representative of 3 independent experiments. **B.** Whole cell recordings were obtained from cells expressing mPanx1 and hPANX1 constructs. CBX-sensitive currents from hPANX1-Flag were virtually undetectable whereas mPanx1 constructs generated substantial current, with either a Flag or HA epitope tag. (*, $P<0.01$, by ANOVA).

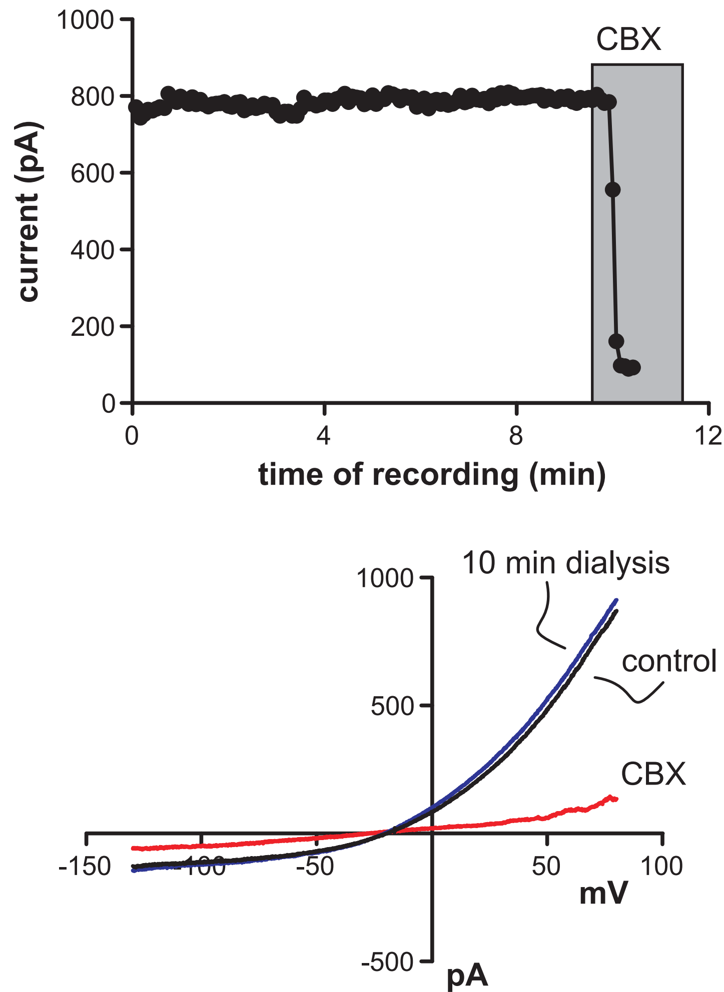


Figure S4. ***A short C terminal peptide from the region downstream of the caspase cleavage site does not inhibit truncated hPANX1 channels.***

Whole cell recordings were obtained from TEVp-activated hPANX1(TEV) channels using pipettes that contained a 12 amino acid peptide from the C terminal region immediately distal to the caspase site (GKTPMSAEMREE). The peptide had no effect on hPANX1 channel currents (n=5 cells).

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Supplemental Fig. S5

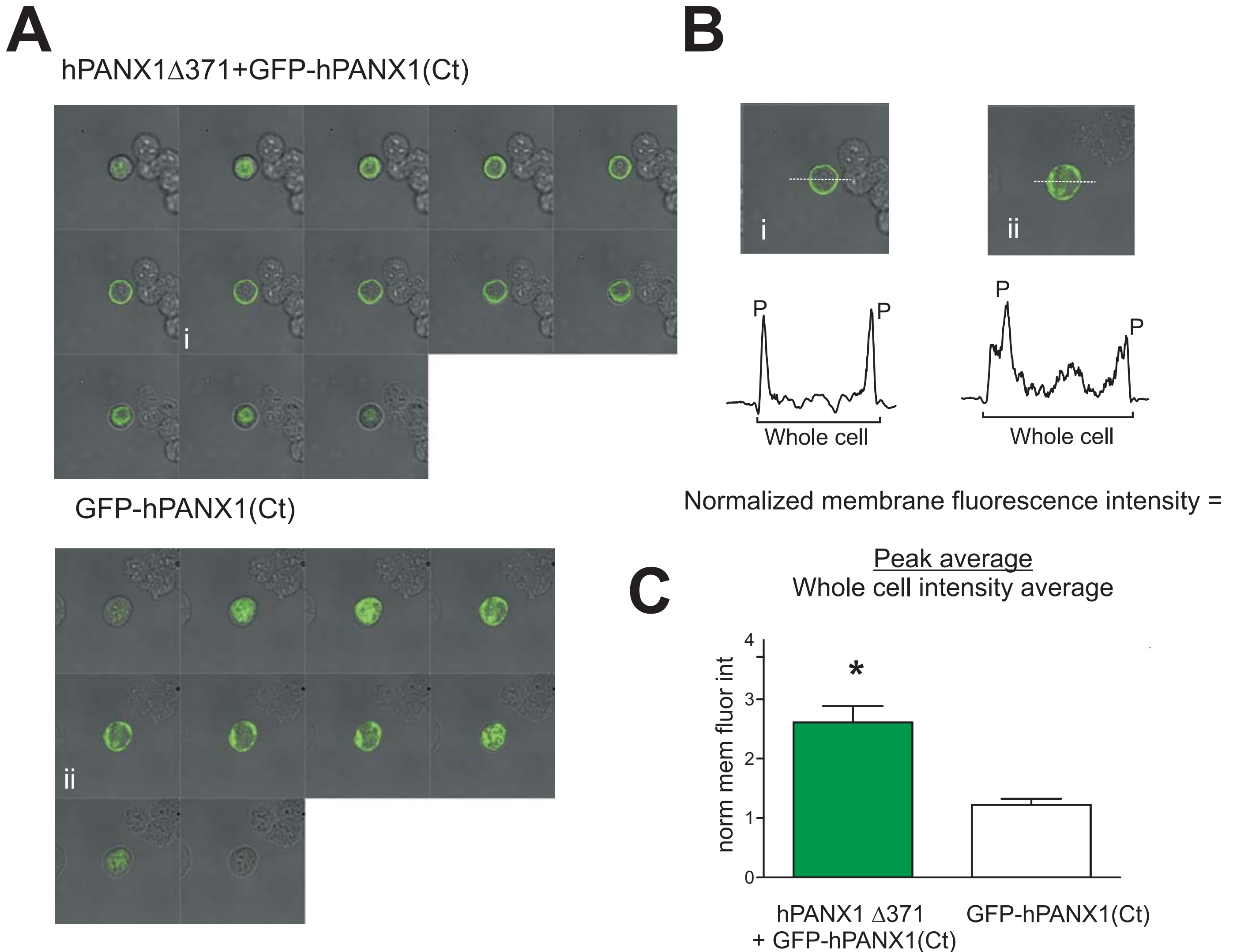

Figure S5. ***An hPANX1 C terminal construct is localized to the plasma membrane in the presence of hPANX1Δ371.***

A. Z-stacks from HEK 293T cells taken at 1 μm intervals. Membrane localization of N-terminally GFP-tagged hPANX1(Ct) was enhanced when co-expressed with constitutively active hPANX1Δ371. **B.** Normalized membrane fluorescence intensity was calculated by measuring GFP fluorescence intensity across a line bisecting the cell, and dividing the average of the membrane peak intensity (P) by the average intensity of the entire cross section (whole cell). **C.** Normalized membrane fluorescence intensity of hPANX1(Ct) was greater in the presence of hPANX1Δ371. *, $P<0.01$, by unpaired t-test.