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Supporting Information for

Ca^{2+} permeation through C-terminal cleaved, but not full-length human Pannexin1 hemichannels, mediates cell death.

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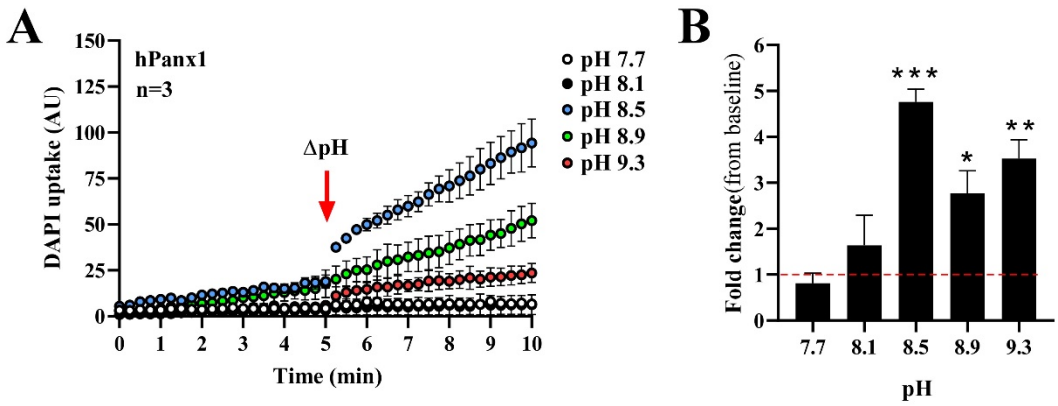

Figure S1. hPanx1 HCs present maximal activity upon exposure to pH 8.5. A. DAPI uptake recording in hPanx1-transfectant cells upon application of different mild alkaline buffers (pH 7.7 – 9.3), after basal incubation with Krebs-Ringer solution pH 7.4 (0-5 min). **B**. Fold change of DAPI uptake rate in response to alkaline buffer compared to basal condition (pH 7.4). n=3. Data represent mean ± SEM. * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$.

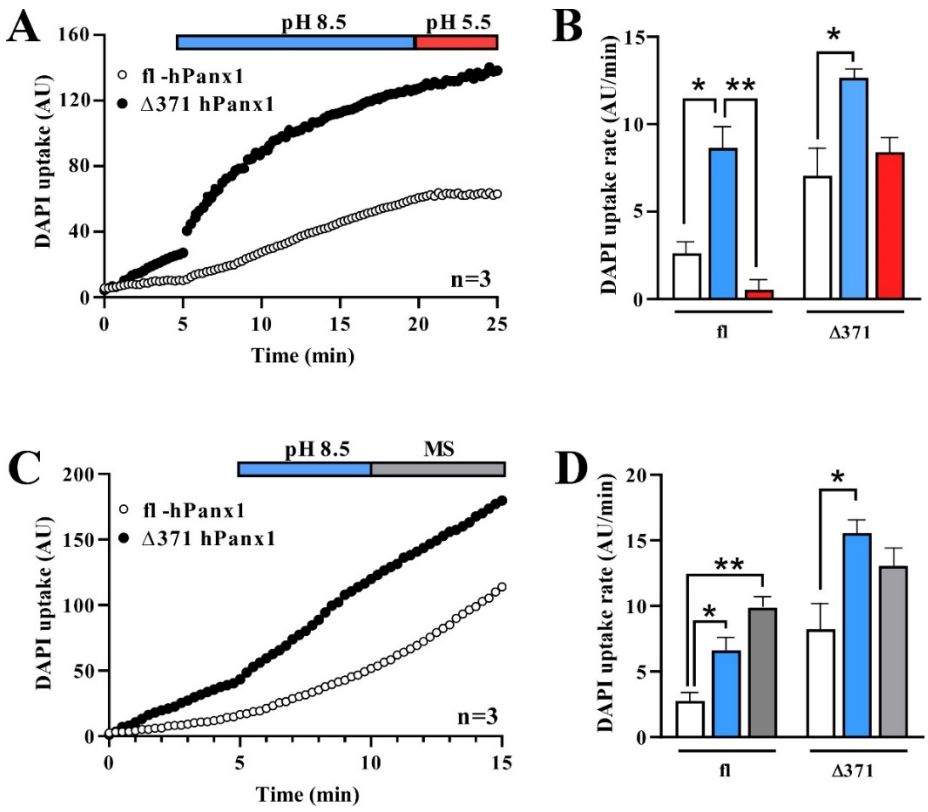

Figure S2. Δ371hPanx1 HCs lack the acidic and stretch sensitivity present in fl-hPanx1 HCs. **A-B**. While the activity of hPanx1 is oppositely modulated by alkaline or acidic pH, truncated hPanx1 does not significantly respond to acidic pH. n=3. **C-D**. In fl-hPanx1 a significant increase in DAPI uptake rate was observed when mechanical stretch was applied after alkaline pH, whereas no changes were observed in Δ371hPanx1. n=3. Data represent mean ± SEM. * $p \leq 0.05$; ** $p \leq 0.01$.

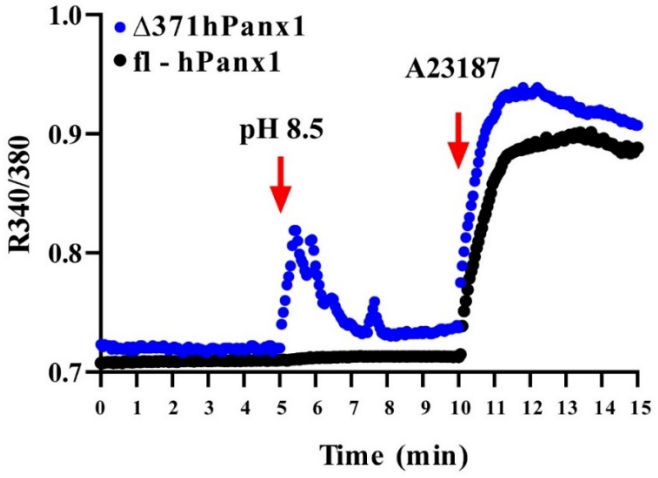

Figure S3. Δ371hPanx1 expression allows a rapid but transient increase in Fura-2 signal upon exposure to pH 8.5. Cx45$^{-/-}$/Panx1$^{-/-}$ HeLa parental cells were transfected with fl-hPanx1 or Δ371hPanx1 vector and then assayed for cytoplasmic Ca^{2+} signal in Fura-2 loaded cells. Only in Δ371hPanx1 transfectants, alkaline pH evokes a transient increase in Fura-2 signal, whereas both transfectants respond to calcium ionophore application. N=3.

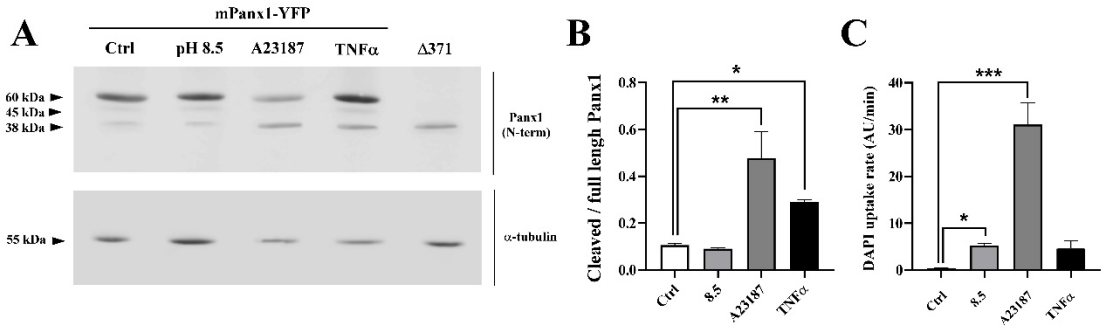

Figure S4. mPanx1 hemichannels show a similar cleavage pattern as compared to hPanx1 hemichannels. A. HeLa cells stably expressing mPanx1 were incubated 4 h with Krebs pH 8.5 (lane 2), 5 µM A23187 (lane 3) or 50 µg/mL TNF-α (lane 4). Other cells were 48 h-transfected with Δ371hPanx1 vector, and proteins were extracted and loaded for Western blot analysis (lane 5). **B**. Densitometric analysis was performed and truncated/full-length mPanx1 was plotted for each condition. n=3. **C**. DAPI uptake rates under each treatment. n=3. Data represent mean ± SEM.* $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$.

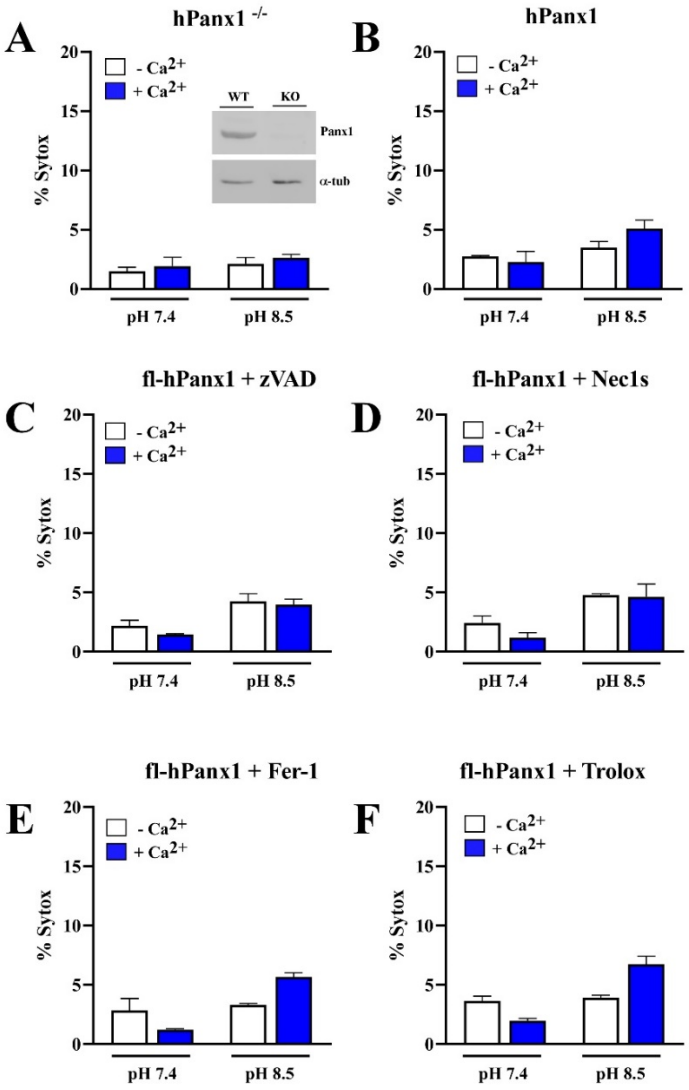

Figure S5. Viability was not affected in fl-hPanx1 HeLa cells upon treatment with cell death inhibitors. Cell death was assayed by Sytox staining in hPanx1$^{-/-}$ (**A**) and hPanx1 HeLa cells (**B-F**) upon exposure to saline solution pH 7.4 or 8.5 and in the presence or absence (**B**) of different inhibitors, such as zVAD-fmk (**C**), Nec1s (**D**), Fer-1 (**E**) or Trolox (**F**). n=3. Data represent mean ± SEM. No significant differences were obtained between pH or Ca^{2+} changes for each treatment.

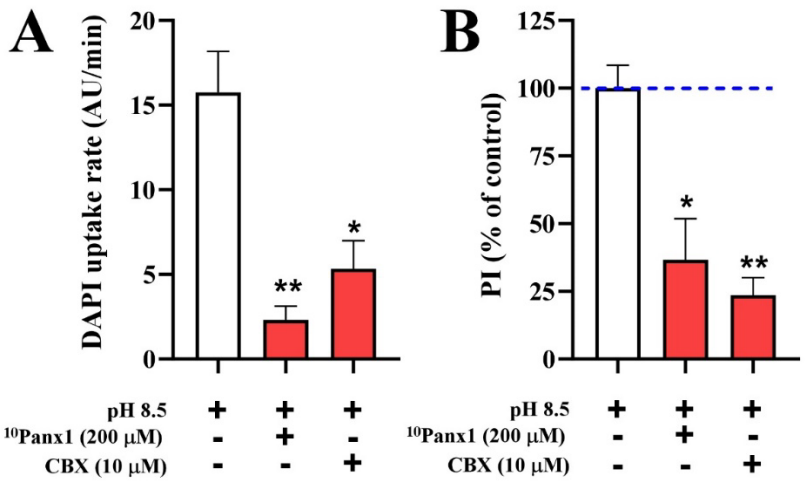

Figure S6. Cell death was dramatically inhibited upon hemichannel inhibition. A. DAPI uptake rate from HeLa-Δ371hPanx1 cells stimulated with alkaline pH (8.5) and then assayed with hPanx1 HC inhibitors, carbenoxolone (CBX) or ^{10}Panx1. n=3. **B.** PI staining (% of control) in Δ371hPanx1 transfectant cells upon 4 h alkaline pH application in the presence of Panx1 HC inhibitors. n=3. Data represent mean ± SEM. * $p \leq 0.05$; ** $p \leq 0.01$.

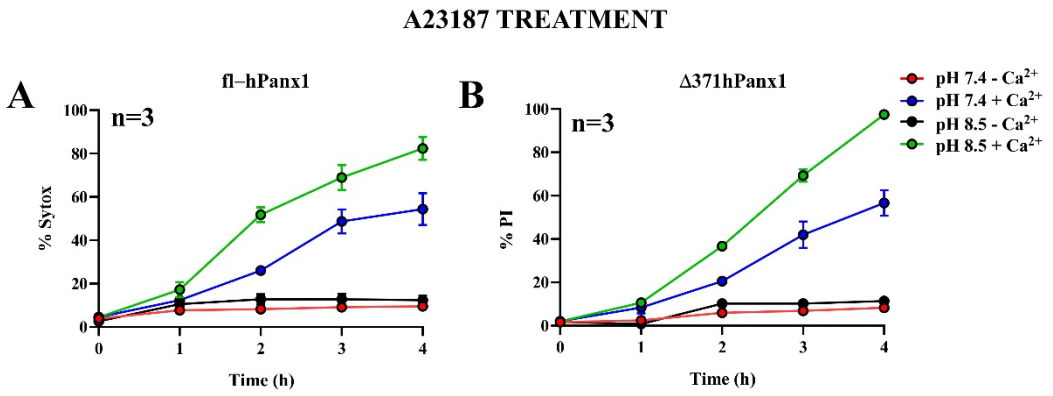

Figure S7. Ca^{2+} ionophore application causes cell death bypassing Panx1 hemichannels. A. HeLa cells transfected with fl-hPanx1 were incubated with 5 µM A23187 Ca^{2+} ionophore and Sytox staining was increased only in the presence of extracellular Ca^{2+}. **B**. Similarly, the application of A23187 only caused a significant increase in PI staining in cells incubated in a solution containing 3 mM Ca^{2+}, regardless of the extracellular pH. n=3. Data represent mean ± SEM.

Supporting Text: Extended Methods

Reagents

Ethidium (Etd^+) bromide, propidium iodide, sodium orthovanadate, TNF-α, xestospongin C and carbenoxolone (CBX) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Turbofect, 4',6-diamidino-2-phenylindole (DAPI), and FURA-2 AM were obtained from Invitrogen / Thermo Fisher Scientific (Waltham, Massachusetts, USA). Sytox green, CellRox deep red, Ferrostatin, Nec1s, Trolox, and zVAD-fmk were from MedChemExpress (Deerpark, NJ, USA).