Supplemental Figure Legends

Supplemental Figure S1. Effect of extracellular and intracellular Ca^{2+} ion on CCK-induced $[Ca^{2+}]_i$. (A) Representative traces of CCK-induced $[Ca^{2+}]_i$ elevation with and without pre-incubation with BAPTA-AM. (B) Isolated mouse pancreatic acini were incubated with CCK (1 nM) in the presence of bath calcium (2 mM) or absence of extracellular calcium, and some cells were pre-incubated 30 minutes with BAPTA-AM (20 μM) before CCK application. The CCK-induced peak $[Ca^{2+}]_i$ is expressed as the ratio of peak intensity (F_{max})/baseline intensity (F_0) from 35-37 cells. Statistical analyses were performed using Student's *t*-test. $^{**}P \leq 0.01$; $^{****}P \leq 0.0001$. Results are expressed as the mean ± SEM.

Supplemental Figure S2. The CRAC blocker CM4620 inhibits CCK- but not Yoda1-induced elevations in $[Ca^{2+}]_i$ in pancreatic acini. (A) Representative traces show the $[Ca^{2+}]_i$ elevation following incubation with CCK (100 pM) or CCK (100 pM) + CM4620. Mouse pancreatic acini were treated with CCK (100 pM) in the absence or presence of CM4620. CM4620 was added 1 hr before CCK. (B) Peak $[Ca^{2+}]_i$ responses are shown from acini treated with CCK (100 pM) or CCK (100 pM) + CM4620, (n=4 experiments with 47-68 cells). (C) Peak $[Ca^{2+}]_i$ responses are shown from acini treated with CCK (1 nM) or CCK (1 nM) + CM4620, (n=3 experiments with 34-36 cells). (D) A representative experiment shows the effects of CM4620 (5 μM) on Yoda1 (25 μM) -induced $[Ca^{2+}]_i$ in pancreatic acini. Acini were pre-incubated with CM4620 or vehicle for 1 hour. (E) The effects of CM4620 on Yoda1-induced $[Ca^{2+}]_i$ are shown at two time periods (1-2 minutes and 4-5 minutes) (n = 3 experiments with 30-32 cells). Statistical analyses were performed using Student's *t*-test. $^{***}P \leq 0.001$; $^{****}P \leq 0.0001$. Values represent the mean ± SEM.

Supplemental Figure S3. Activation of Piezo1 does not alter CCK sensitivity in pancreatic acini. (A-C) CCK-stimulated live cell calcium imaging in pancreatic acini from wild type (WT) and Piezo1^{aci} KO mice loaded with Calcium 6-QF dye. (A) The traces represent the live cell calcium imaging with CCK (1 nM) from three experiments. (B) The maximum peak $[Ca^{2+}]_i$ is expressed as the ratio of maximum peak intensity (F_{max})/baseline intensity (F_0), and (C) the sustained calcium $[Ca^{2+}]_i$ rise is expressed as the ratio of peak $[Ca^{2+}]_i$ at 9 minutes (F_9)/base line intensity (F_0) from 30-32 cells. (D) CCK responsivity is not altered in Piezo1^{aci} KO mice. The maximum CCK (20 pM) - stimulated $[Ca^{2+}]_i$ elevation is shown in acini from wild type or Piezo1^{aci} KO mice (from 33 cells). (E) The effects of acinar cell pushing with a micropipette and physiological concentrations of CCK (20 pM) on live acinar cell $[Ca^{2+}]_i$ are shown. A representative image from 5 experiments is shown. (F) Peak $[Ca^{2+}]_i$ levels are shown from 5 experiments with a total of 18-19 cells. (G and H) The viability of pancreatic acini from WT mice was measured following stimulation with CCK (1 nM), Yoda1 (50 μM) and CCK (1 nM) + Yoda1 (50 μM) using Propidium Iodide and Calcein AM dyes. Dead cells were marked by Propidium Iodide uptake (red) and live cells were labeled with Calcein AM (green) (scale bar = 100 μm). Quantitative values for pancreatic acinar cell viability from 3 experiments are shown in (H). Statistical analyses were performed using Student's *t*-test (B, C, D, F) and one-way ANOVA with the Tukey's multiple comparison (H). $*P \leq 0.05$, $**P \leq 0.01$; $****P \leq 0.0001$. Results are expressed as the mean ± SEM.

Supplemental Figure S4. Mechanically-induced mitochondrial depolarization and trypsinogen activation. (A) Change in TMRE intensity of pancreatic acini upon 5 μm mechanical pushing for 1 second. F_{low} is the lowest fluorescence intensity over 12 minutes after pushing and F_0 is the basal fluorescence intensity before pushing. 48-53

cells were used in the analysis from 3 independent experiments. (B) Increase in BZiPAR intensity of pancreatic acini upon 5 μm mechanical pushing for 1 second. Peak BZiPAR fluorescence intensity over 50 minutes from 20-26 cells. Statistical analyses were performed using Student's *t*-test.

Supplemental Figure S5. Immunostaining of TRPV4 in mouse and human pancreatic acini and Piezo1 in human pancreatic acini. (A) Left panel - Mouse pancreatic acini were immunostained with TRPV4 antibody (red) and nuclei were identified with Nunc blue. Right panel - Pancreatic acini were immunostained with TRPV4 antibody pre-absorbed with immunogenic peptide to eliminate specific, saturable TRPV4 staining. (B) Human pancreatic acini were immunostained with Piezo1 antibody (green) and nuclei were identified with Nunc blue. (C) Human pancreatic acini were immunostained with TRPV4 antibody (red) and nuclei were identified with Nunc blue. Scale bar = 10 μm.

Supplemental Figure S6. Protein kinase A and C blockers did not significantly affect the Yoda1-induced sustained calcium elevation. Yoda1-induced peak calcium rise with or without protein kinase A blocker, KT5720 (5 μM) or protein kinase C blocker, GF109203X (20 μM). The transient calcium peaks were measured from signals between 1 to 3 minutes and sustained calcium peaks were measured from signals between 5 to 6 minute intervals. A total of 30 cells were used in the analysis. Statistical analyses were performed using Student's *t*-test.

Supplemental Figure S7. Caerulein-induced pancreatitis is independent of TRPV4 channel activation. (A) Live-cell imaging of pancreatic acini loaded with Calcium 6-QF. Pancreatic acini from wild type mice were incubated with CCK (1 nM) with or without HC067 (1 μM). (B) The maximum CCK-induced peak $[Ca^{2+}]_i$ is expressed as the

ratio of maximum peak intensity (F_{max})/baseline intensity (F_0), and (C) the sustained $[Ca^{2+}]_i$ is expressed as the ratio of peak $[Ca^{2+}]_i$ at 9 minutes (F_9)/baseline intensity (F_0) from 33 cells. (D-E) Caerulein-induced pancreatitis in wild type and TRPV4 KO mice. The pancreatitis parameters presented are edema (D), serum amylase (E), tissue MPO (F), and pancreatic histology score (G) in vehicle- and CCK-treated wild type (WT) and TRPV4 KO mice (n = 3-5). (H) Representative H&E stained images of the mid-region of pancreas are shown (scale bar = 100 μm). Statistical analyses were performed using Student's *t*-test (B, C) and one-way ANOVA with the Tukey's multiple comparison (D, E, F and G). Values represent the mean ± SEM; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Supplemental Movie Legends

Movie 1: Yoda1 (25 μM) induced intracellular calcium elevation in mouse (wild type) pancreatic acini. Time (min: sec).

Movie 2: Yoda1 (50 μM) caused morphological changes in mouse (wild type) pancreatic acini. Time (min: sec).

Movie 3: Yoda1 (50 μM) did not affect the morphology of pancreatic acini isolated from Piezo1^{aci} KO mouse. Time (min: sec).

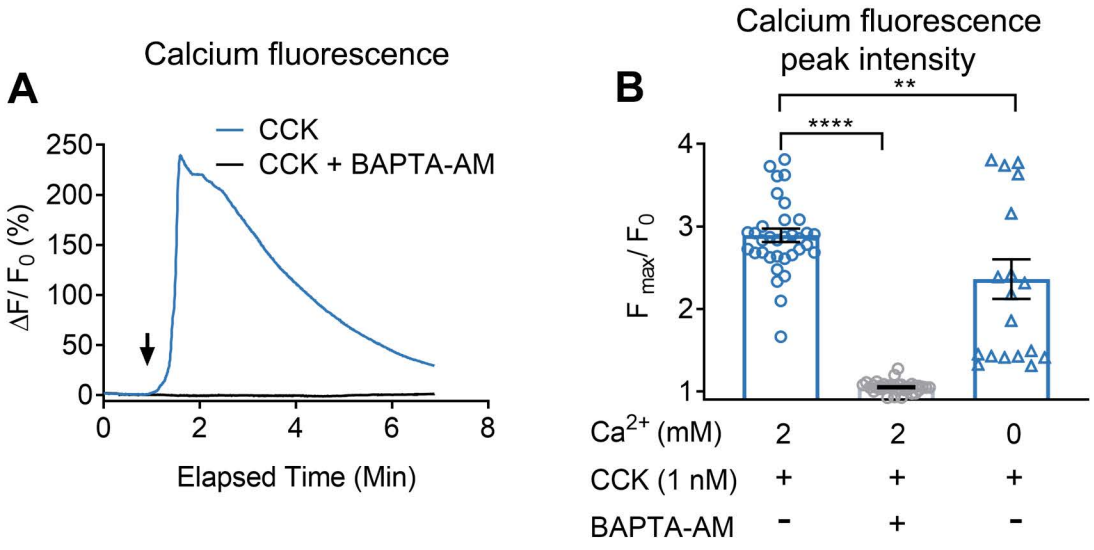
Movie 4: Yoda1 (50 μM) and CCK (10 nM) induced trypsinogen activation in wild type pancreatic acini. Time (min).

Movie 5: Mechanical pushing a wild type acinar cell to a depth of 5 μm for 1 sec increased intracellular calcium. Time (min: sec).

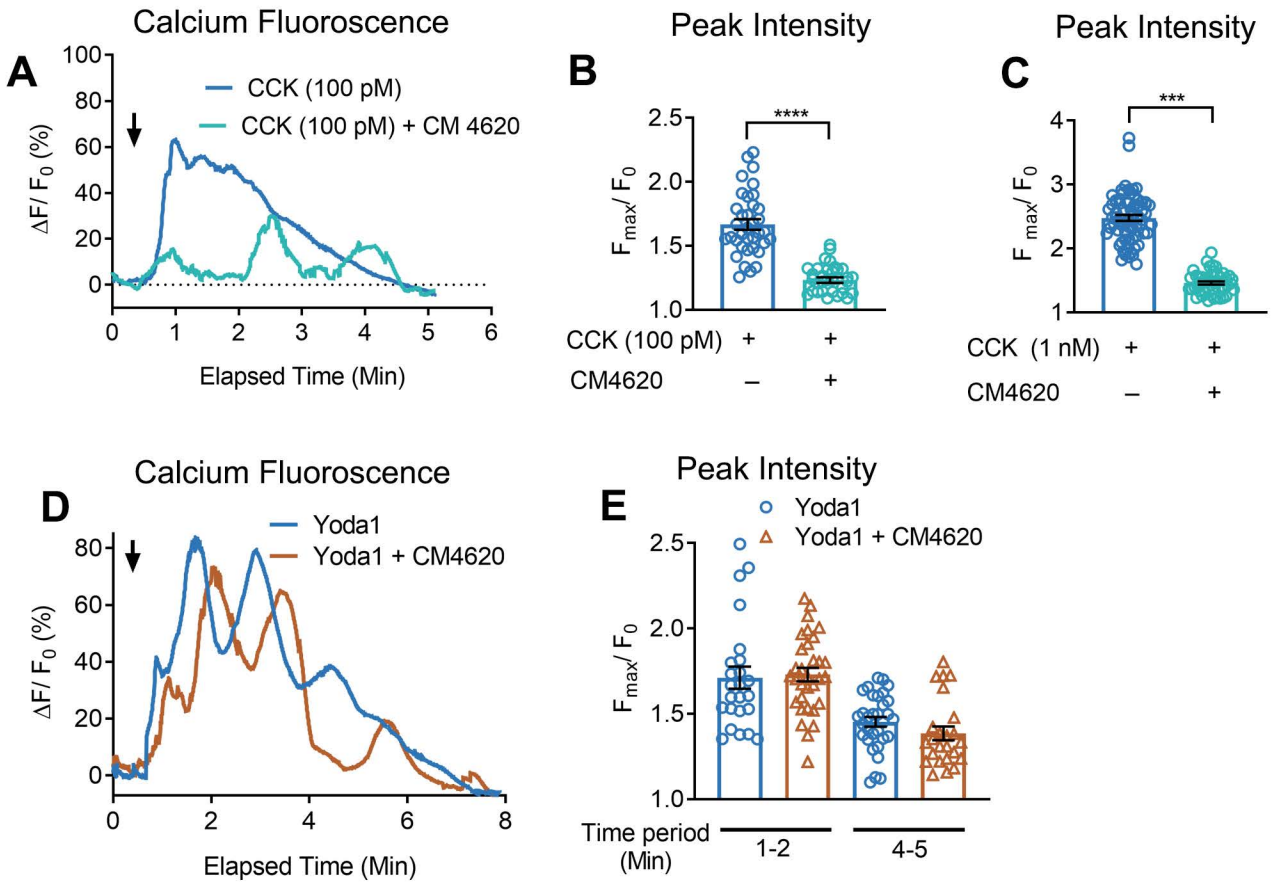
Movie 6: Mechanical pushing did not increase intracellular calcium in pancreatic acini from a Piezo1^{aci} KO mouse. Time (min: sec).

Movie 7: Fluid shear stress (12 dyne/ cm^2 for 30 sec) induced mitochondrial depolarization in pancreatic acini from wild type and Piezo1^{aci} KO mice. Time (min: sec).

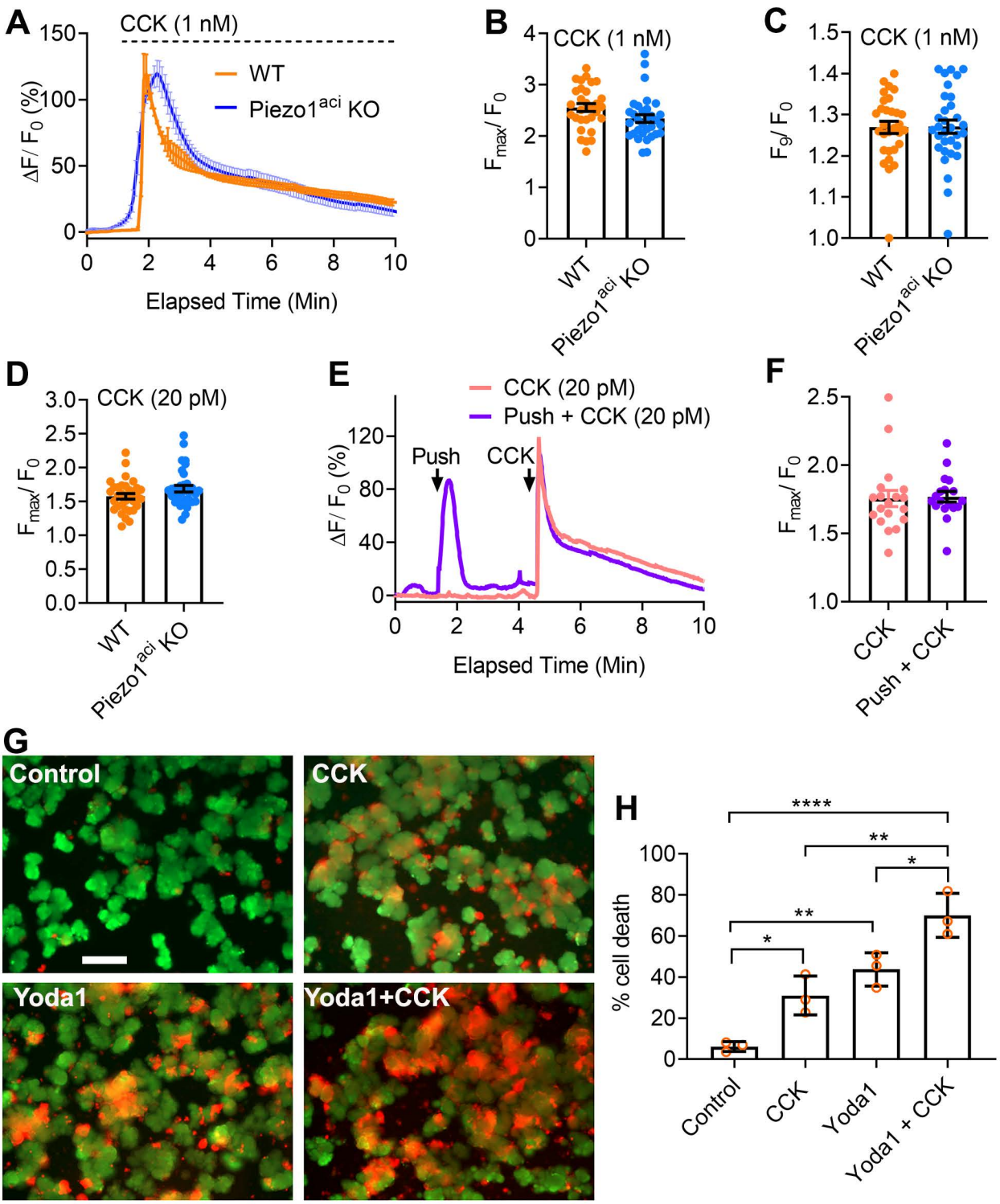
Movie 8: Yoda1 (25 μM) induced PLA2 activation in wild type mouse pancreatic acini. Time (min: sec).



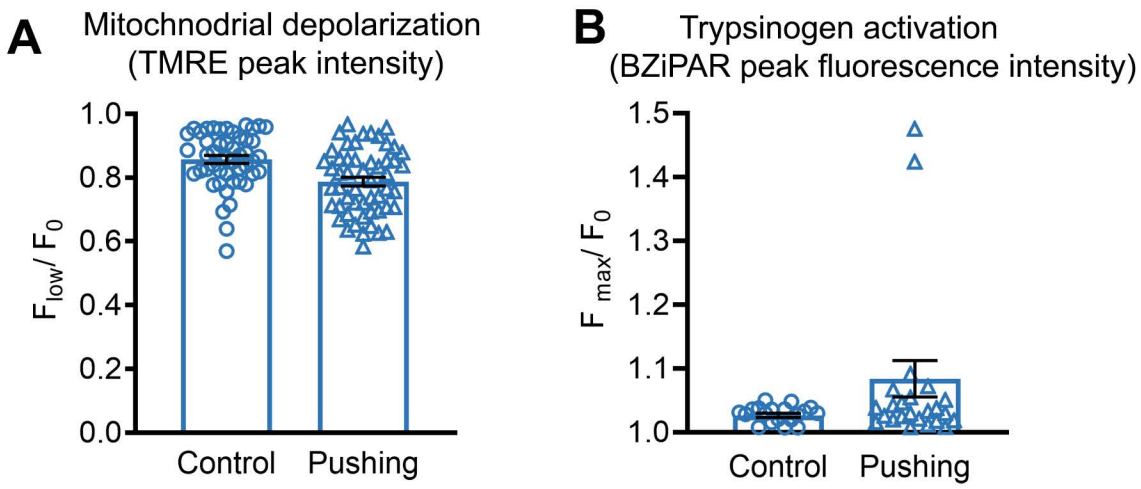
Supplemental Figure S1



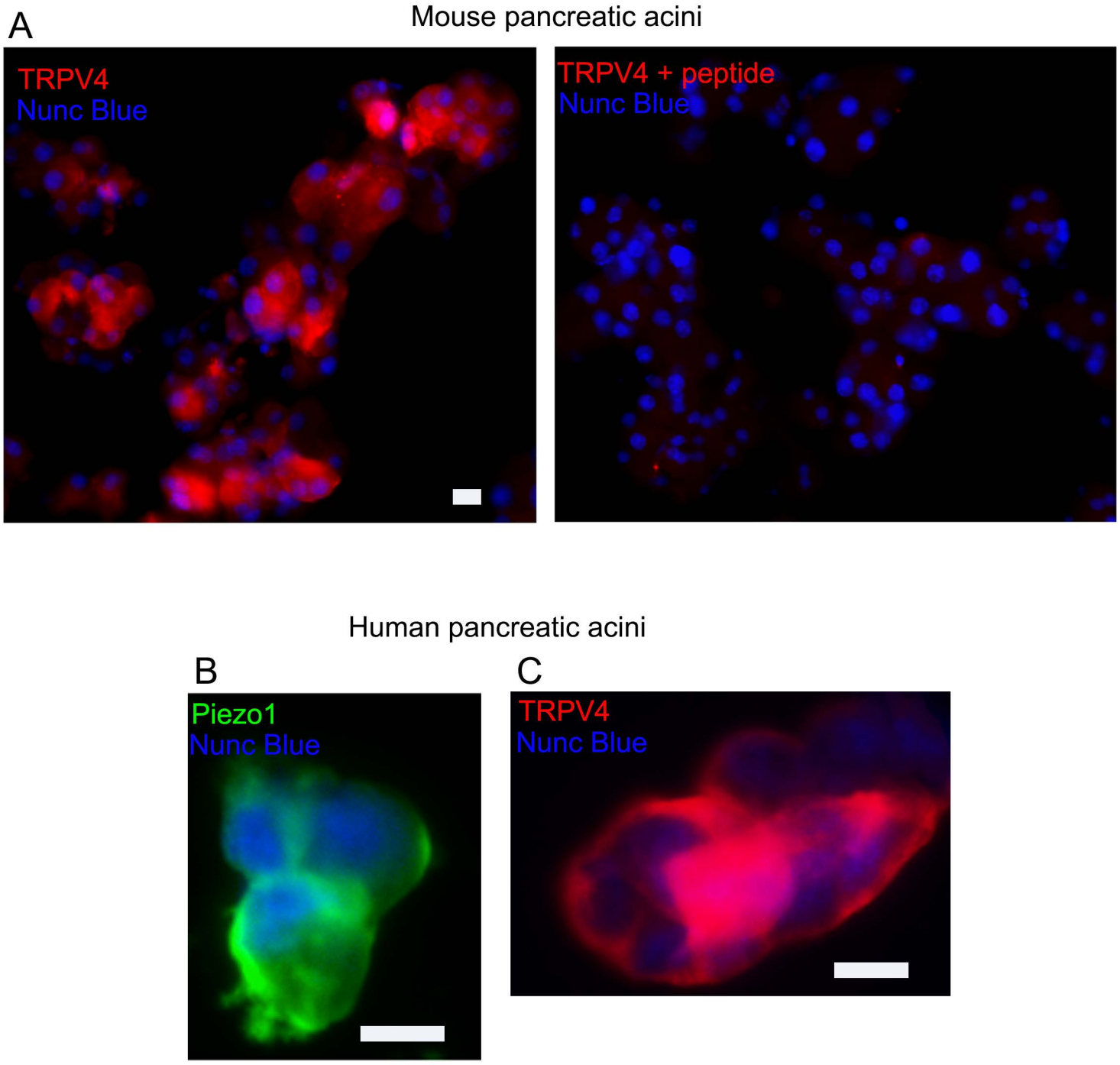
Supplemental Figure S2



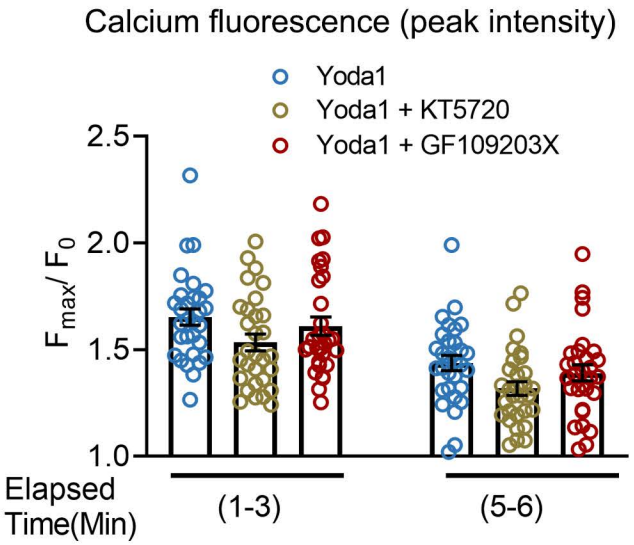
Supplemental Figure S3



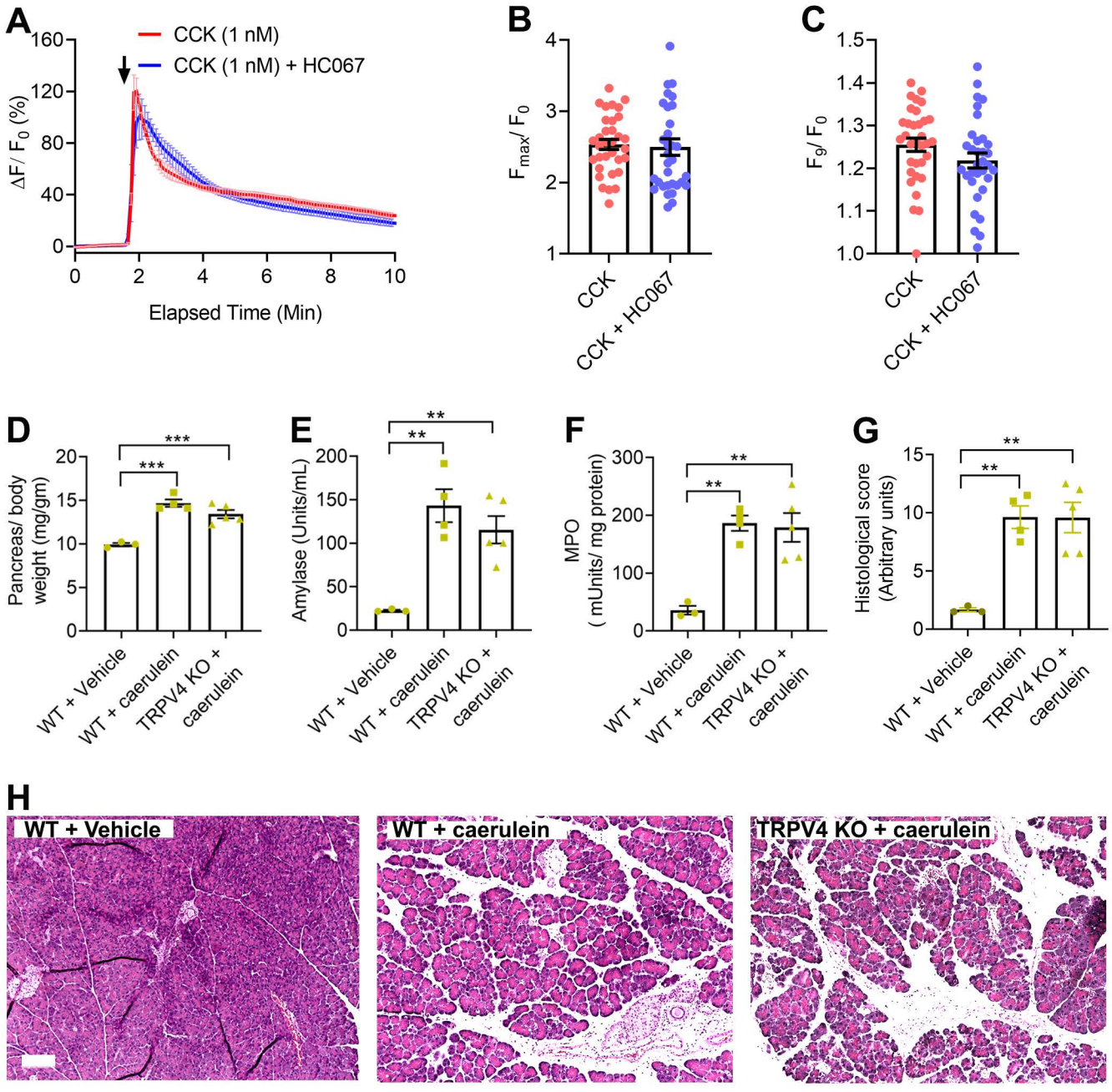
Supplemental Figure S4



Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7