SUPPLEMENTAL MATERIAL

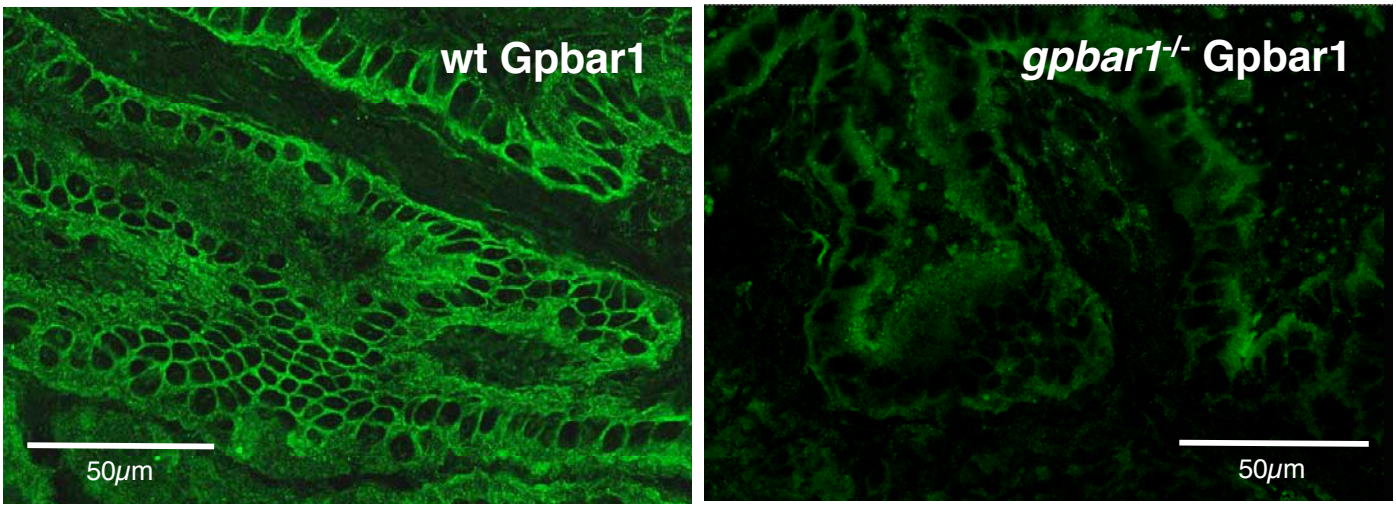
Methods

Tissue preparation for in-vitro studies. In-vitro studies were performed using freshly isolated dispersed pancreatic acini obtained following collagenase digestion of the gland as previously described[23] and, for our studies, we employed two types of dispersed acini. Calcium studies were performed using small acini (defined as 1-10 cells/clump) prepared by filtering the acinar preparation through a 40 μm Nitex mesh (Sefar America). These small acini were found to be most suitable for monitoring calcium changes within individual cells. All of the other in-vitro studies using dispersed acini were performed using standard-sized acini that were larger (i.e. 1-100 cells/clump, obtained by filtration through a 150 μm Nitex mesh) and less susceptible to non-specific injury.

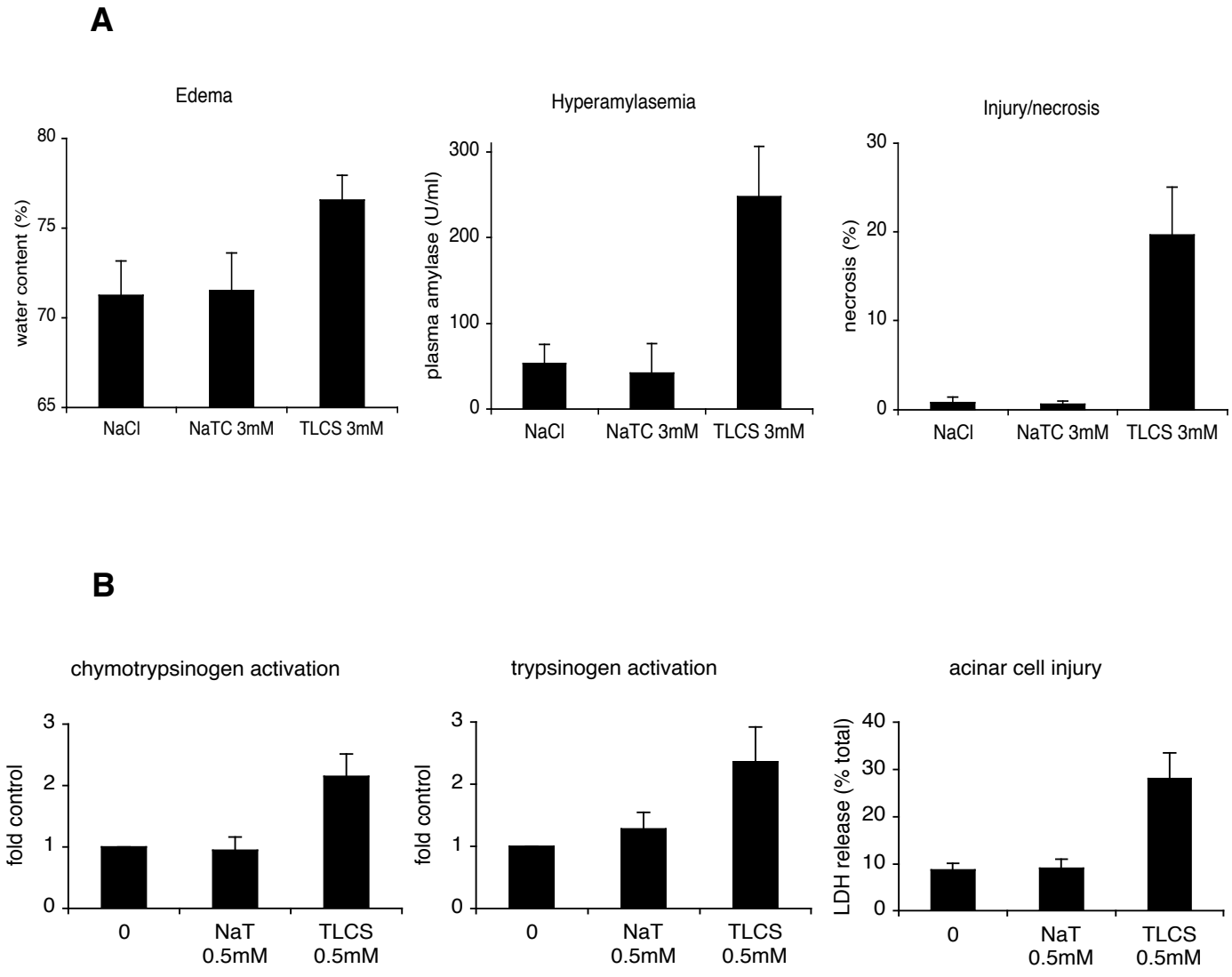
Expression of Gpbar1 in mouse pancreas. Gpbar1 expression in mouse pancreas was evaluated by rt-PCR using acidic ribosomal phosphoprotein (ARP) as the positive control. The primers for ARP ($_{533}$AAGACTGGAGACAAGGTGGGA (forward), TTGGTTACTTTGGCGGGATTA$_{1021}$ (reverse)) were used to generate a 489 bp product while those for Gpbar1 ($_{66}$CCTGGCAAGCCTCATCGTC (forward) and AGCAGCCCGGCTAGTAGTAG$_{185}$ (reverse)) were used to generate a 120bp product. Gpbar1 expression was also evaluated by immunofluorescence microscopy using rabbit antiserum raised to the 24 amino-acid C-terminus of mouse Gpbar1 (KRDNPGPSTAYHTSSQCSIDLDLN). Rabbits were injected subcutaneously with the immunogen conjugated to KLH and resuspended in complete Freund's adjuvant and, 4 weeks later, the rabbits were re-injected with the antigen in incomplete Freund's aduvant. Two weeks later, the animals were bled and antibodies were enriched by ammonium sulfate precipitation. Immunofluorescence microscopic studies were performed using a LEICA TCS SP2 confocal microscope. Eight μm sections were prepared and fixed in acetone (-20 ^{o}C) for 20 minutes. Sections were stored at –70 ^{o}C till used. They were incubated with anti-Gpbar1 rabbit antibodies (1/100) for 1 hr at room temperature followed by incubation with goat anti-rabbit fluorescein-conjugated antibody. Actin staining was achieved by incubation with Alexa-fluor 546 conjugated phalloidin (1U/ml) for 1 hour at room temperature. The sections were extensively washed between each incubation in PBS containing 0.1% bovine serum albumin. Samples were then coverslipped and examined under a Leica TCS SP2 confocal microscope at the Tufts Center for Neuroscience Research (P30 NS047423).

Measurement and analysis of changes in cytosolic calcium levels in single acinar cells. Freshly prepared small pancreatic acini, suspended in Dulbecco's modified Eagle's medium/F-12 that contained 2 mM sodium pyruvate, 0.1% bovine serum albumin, and 1 mM $CaCl_2$ (Buffer A), were pre-loaded by incubation with 1 μM Fura-2/AM in the presence of 0.0005% Pluronic F-127 for 10 min at room temperature followed by extensive washing. They were resuspended in Buffer A and calcium transients in single cells within the acinar cluster were monitored using a Nikon Eclipse TEU 2000 inverted microscope as described previously[24]. Background subtraction was carried out independently at each of the excitation wavelengths. The ratios of fluorescence

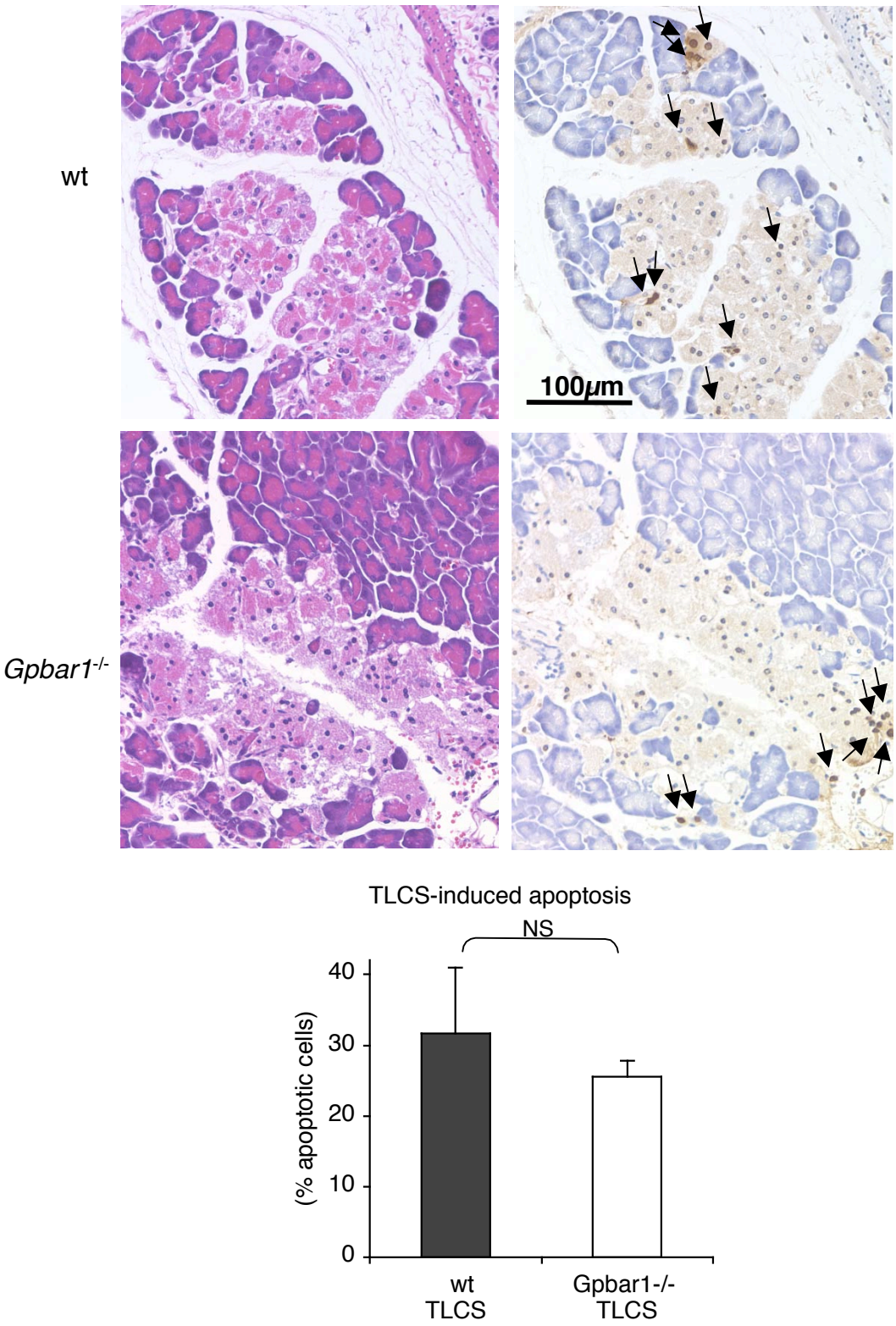
emission (510 nm) at the two excitation wavelengths (340 and 380 nm) were determined and expressed as the ratio of 340/380 fluorescence. As noted previously[23], four patterns of response are observed. Some cells fail to respond ("no response") while others display an oscillatory rise and fall in calcium levels ("oscillations alone"). Still other cells display an oscillatory response superimposed upon a baseline that rises quickly and then falls to a level that remains above that noted in the absence of stimulation ("oscillations + peak/plateau"). The remaining cells respond with only a peak/plateau rise and fall of the baseline value ("peak plateau"). For our studies, 20-25 individual acinar cells, in each group, were monitored in each experiment and 3 or more independent experiments were performed. We determined the percent of monitored cells manifesting each of the four types of response pattern.



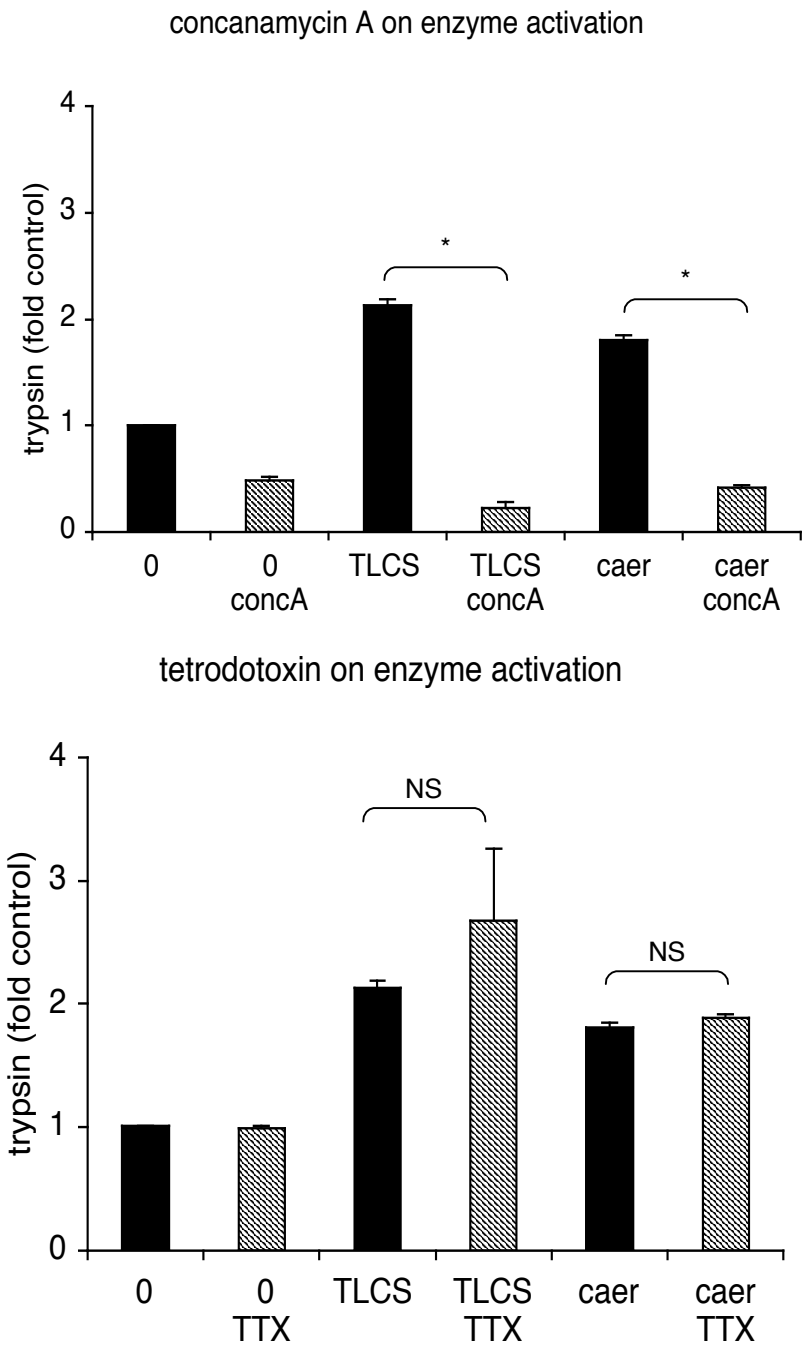
Supplemental Figure 1. Gpbar1 is expressed in the mouse gallbladder. Gpbar1 expression in the gallbladder of wild type and Gpbar1$^{-/-}$ mice was evaluated by immuno-fluorescence microscopy. Note intense anti-Gpbar1 antibody fluorescence of the simple columnar epithelial cells of wild type mice, while only diffuse, presumably non-specific, staining in *gpbar1*$^{-/-}$ mice.



Supplemental Figure 2. In vivo and in vitro effects of Na-taurocholate. Panel A: Mice were retrogradely infused with 3 mM Na-taurocholate or 3 mM TLCS and pancreatitis severity was determined 24 hours later. No pancreatitis was elicited by the infusion of 3mM of Na-taurocholate. Panel B: Freshly prepared standard-sized acini were suspended in buffer alone or in buffer containing either 0.5 mM Na-taurocholate or 0.5 mM TLCS. Zymogen activation and LDH leakage were quantitated as described in the text. No zymogen activation nor LDH leakage was observed in samples from acini treated with 0.5 mM Na-taurocholate.



Supplemental Figure 3. Effects of Gpbar1 deletion on TLCS-induced acinar cell apoptosis. TLCS-induced pancreatitis was elicited in wild type and *Gpbar1*$^{-/-}$ mice (n = 6) by retrograde infusion of 3mM TLCS and mice were sacrificed 6 hours later. The pancreas was removed, embedded in paraffin and 5μm sections were subjected to hematoxylin and eosin and TUNEL staining. Areas within the pancreas containing injured or dead acinar cells were identified. TUNEL-positive cells (arrows) were counted and expressed as a percent of total injured or dead cells. NS = not significant.



Supplemental Figure 4. Effect of concanamycin A and tetrodotoxin on TLCS- and caerulein-induced trypsinogen activation in acini from wild type mice. Freshly prepared standard-sized acini from wild type mice were incubated with buffer containing either concanamycin A (100 nM) or tetrodotoxin (5 μM) with or without TLCS (500 μM) for 15 min and trypsin activity was quantitated as described in the text. Asterisks denote significant differences ($p<0.05$) between bracketed groups and NS = not significant.