

Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis.

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Running title: Autophagic flux is impaired in acute pancreatitis.

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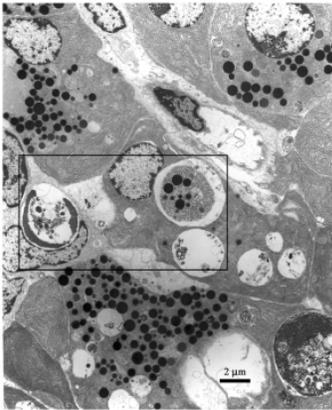
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The authors declare that no conflict of interest exists

SUPPLEMENTAL FIGURES

Figure S1 CR (a)



CR (b)

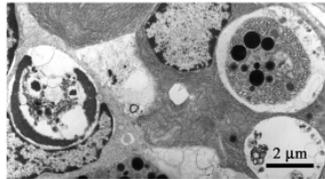


Figure S1 L-Arg (a)

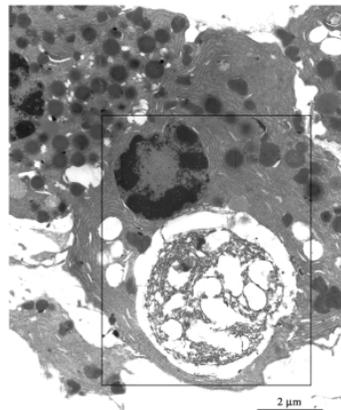
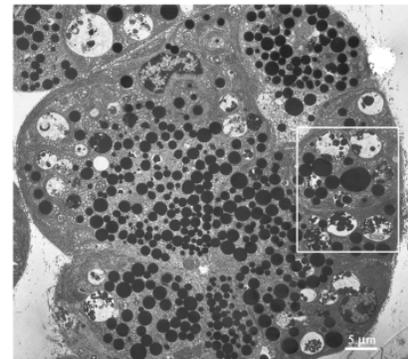


Figure S1 CDE (a)



CDE (b)

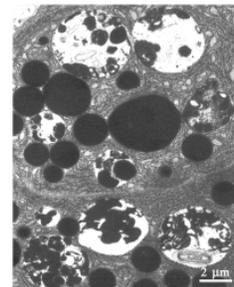
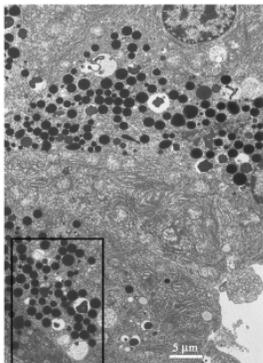


Figure S1 CCK, cells (a)



CCK, cells (b)

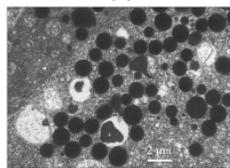


Figure S1 human (a)

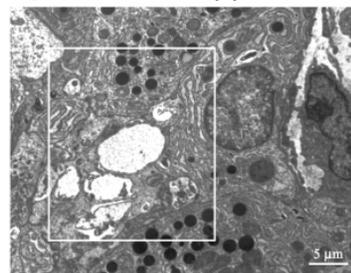


Figure S1

Supplemental Figure S1. Electron micrographs of pancreatic tissue (or isolated acinar cells) from experimental models of pancreatitis (see *Methods*) and from a patient with acute pancreatitis. Shown are pancreatitis induced in rats by i.p. injections of cerulein (CR) or L-arginine (L-Arg); in mice, by choline-deficient ethionine supplemented diet (CDE); and the in vitro model of rat pancreatic acinar cells stimulated with supramaximal CCK-8 (CCK, cells). Panels (a) show larger fields; panels (b) – the boxed areas, which are presented in smaller size in Figure 1A of the main text.

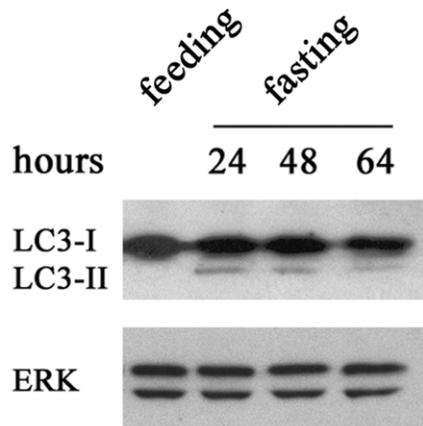


Figure S2

Supplemental Figure S2. Conversion of LC3-I to LC3-II was measured by immunoblot in pancreatic tissue of rats either normally fed or fasted for indicated times. ERK1/2 served as loading control.

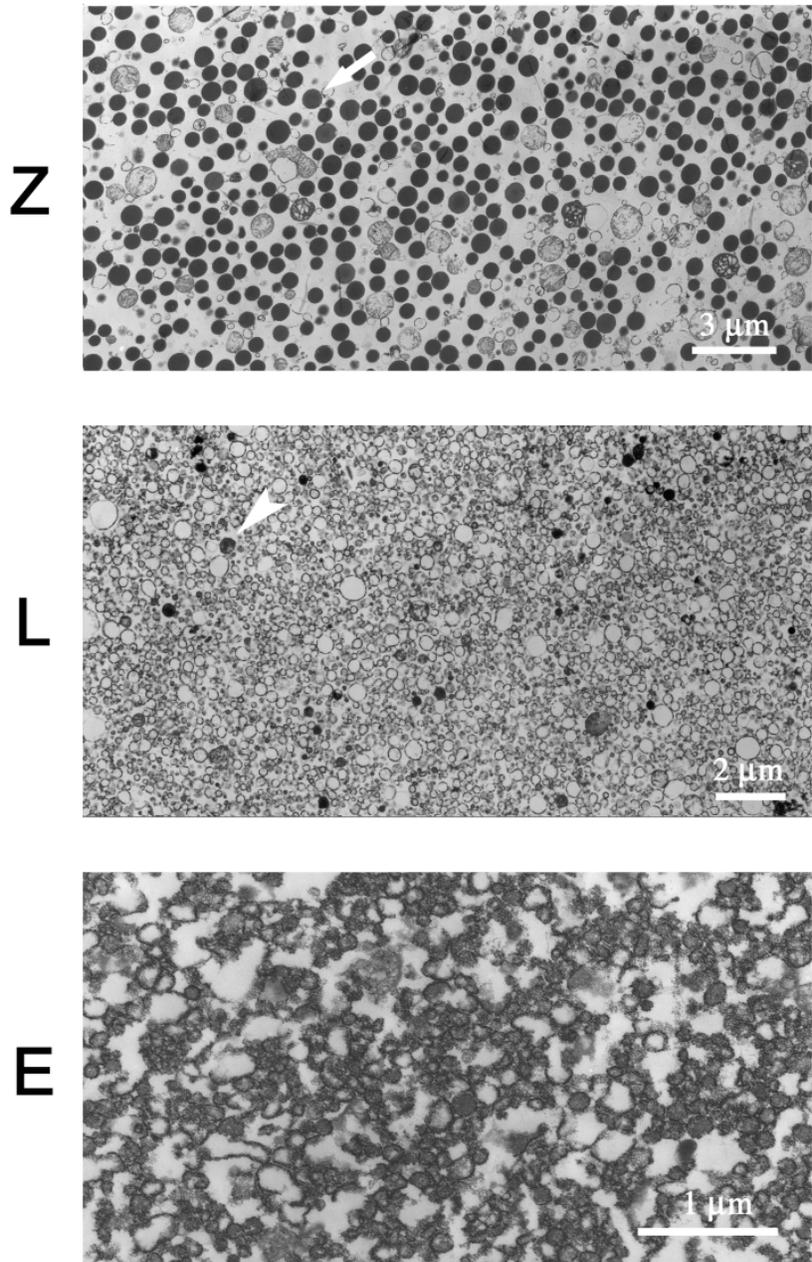


Figure S3

Supplemental Figure S3. Electron micrographs of pancreatic tissue subcellular fractions from normally fed rat. Pancreas homogenate was fractionated by differential centrifugation, as described in *Methods*, to obtain 1,300xg pellet enriched in zymogen granules (*fraction Z*); 12,000xg pellet enriched in lysosomes (*fraction L*); and 12,000xg supernatant containing early endosomes and cytosolic proteins (*fraction E*). Fractions Z and L were directly analyzed under electron microscope; fraction E was centrifuged at 2,000xg for 1 h and the pellet collected for electron microscopy examination. *Arrow* indicates a zymogen granule; *arrowhead*, a lysosome.

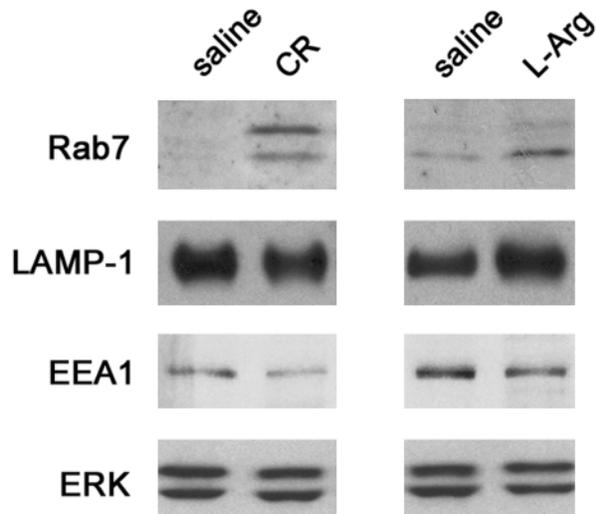


Figure S4

Supplemental Figure S4. Effects of cerulein- (CR) or L-Arg induced pancreatitis on pancreatic levels of endo/lysosomal markers measured by immunoblot. ERK1/2 served as loading control. EEA1, early endosomal antigen.

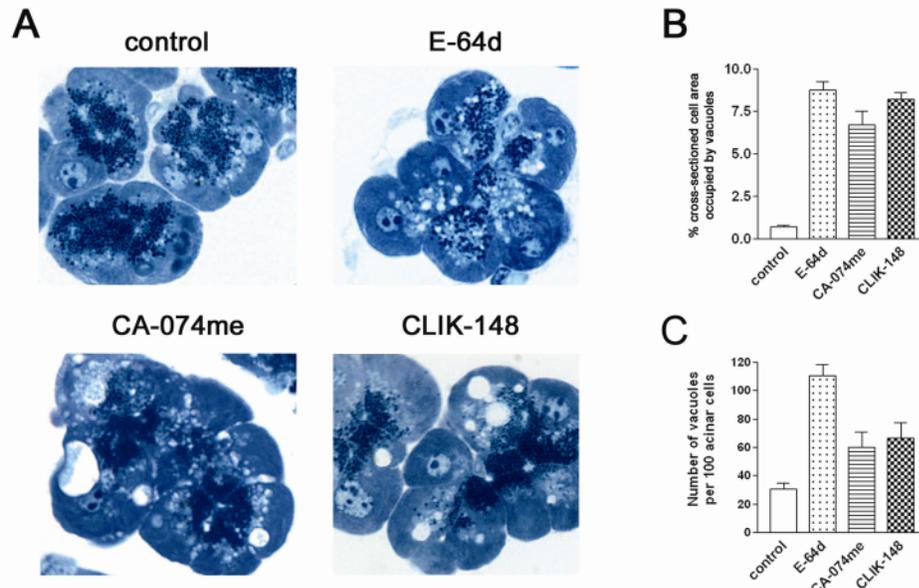


Figure S5

Supplemental Figure S5. Freshly isolated rat pancreatic acinar cells were incubated for 30 min without (control) and with cathepsin inhibitors: E-64d (50 μ M), an inhibitor of both CatB and CatL; CA-074me (50 μ M), a specific CatB inhibitor; or CLIK-148 (20 μ M), a specific CatL inhibitor. Cells were stained with toluidine blue (**A**), and the fraction of cross-sectioned cell area occupied by vacuoles (**B**) and the number of vacuoles (**C**) were quantified under light microscope with the use of ImageJ software. Values are means \pm SE from at least 150 cells for each condition, obtained on 3 separate acinar cell preparations.

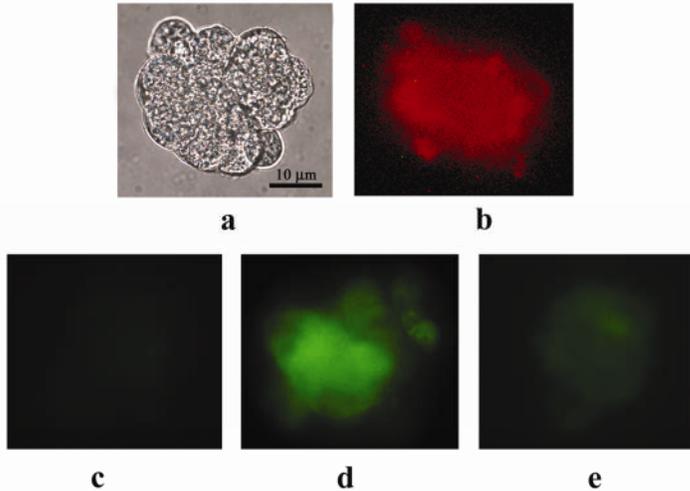


Figure S6

Supplemental Figure S6. Mouse pancreatic acinar cells were transfected with Atg5 siRNA or control non-targeting siRNA (all from Dharmacon) as described in *Methods*. Trypsin activity was measured in live cells loaded with a fluorogenic trypsin substrate BZiPAR [rhodamine 110 bis-(CBZ-L-isoleucyl-L-prolyl-Arginine amide) dihydrochloride] and incubated for 30 min without and with 100 nM CCK-8. Alternatively, cells were transfected with siGLO Cyclophilin B siRNA labeled with fluorescent DY547 (Dharmacon) to assess the efficiency of transfection. (a, b). A group of acinar cells transfected with siGLO Cyclophilin B siRNA was examined under light and fluorescence microscope. (c-e). Trypsin activity was measured using BZiPAR in cells transfected with control siRNA and incubated (c) without CCK or (d) with 100 nM CCK; or (e) cells transfected with Atg5 siRNA and incubated with 100 nM CCK. The scale is the same for all images.

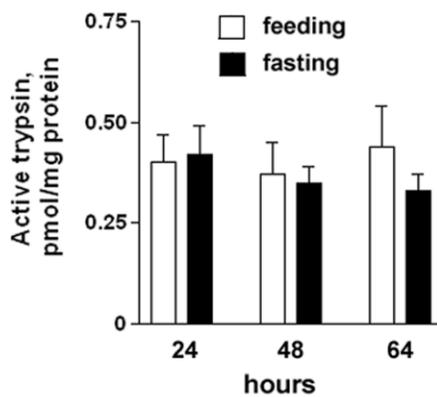


Figure S7

Supplemental Figure S7. Rats were normally fed or fasted for indicated times, and trypsin activity in pancreatic tissue was measured by a fluorogenic assay using Boc-Gln-Ala-Arg-AMC as a substrate. Values are means \pm SE (n=3).

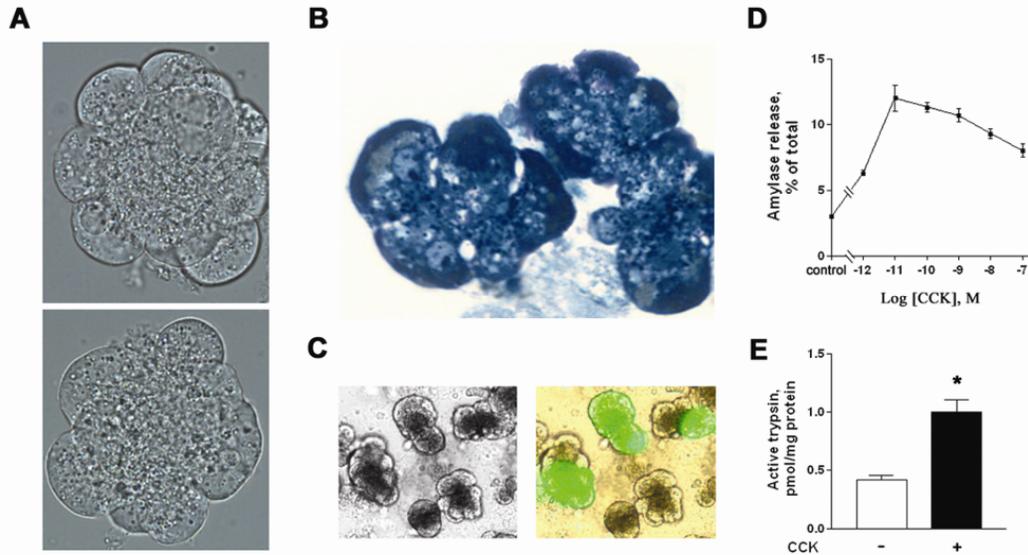


Figure S8

Supplemental Figure S8. Pancreatic acinar cells retain their characteristics in prolonged culture. Upon isolation, mouse pancreatic acinar cells were cultured for 24 h as described in *Methods*, and examined (**A-C**) under light and fluorescence microscope. Panel (**A**) shows non-transfected acinar cells; (**B**) – cells transfected with non-targeting (negative control) siRNA (see *Methods*) and stained with toluidine blue; (**C**) – cells transfected with siGLO Cyclophilin B siRNA (positive control for transfection efficiency). Original magnification: x400 in (A, B), x100 in (C). (**D, E**). Non-transfected acinar cells after 24-h culture were re-suspended in 199 medium and incubated for 30 min with and without CCK-8 at indicated concentrations (**D**) or at 100 nM (**E**). Amylase release (**D**) and trypsin activity (**E**) were measured as described in *Methods*. Values are means \pm SE from 3 separate cell preparations. *p < 0.05 vs. control (i.e., no CCK).