Supplemental Figures

Figure S1. Ethanol and rapamycin inhibit mTOR activity.
Mice were given intraperitoneal injection of ethanol (1.2 mg/kg) or rapamycin (2 mg/kg) or both for 16 hours. Liver lysates were analyzed for the level of total and phosphorylated p70 S6K and 4E-BP1. The phosphorylation of both proteins was suppressed by ethanol and rapamycin.

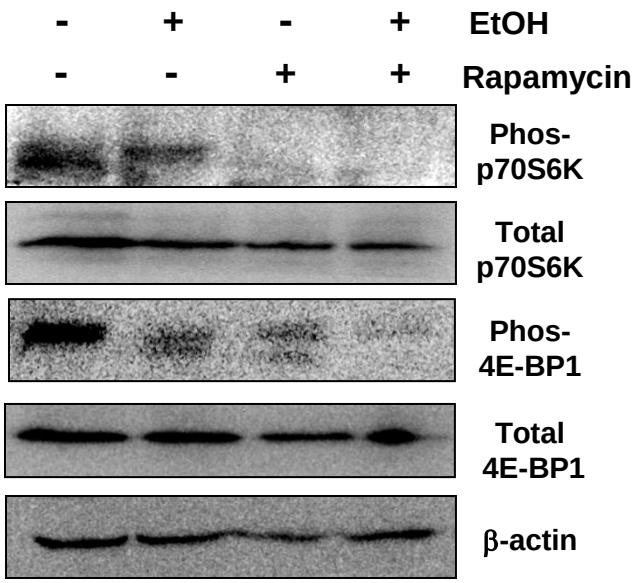
Figure S2. Autophagy remains activated in mice treated with chronic Lieber-DeCarli ethanol diet.
Mice were given control diet (CD) or ethanol diet (ED) that accounts for 29% or 36% of total calorie intake for 4 weeks. Chloroquine (CQ, 60 mg/kg) was given the day before sacrifice. Liver lysates were prepared and the level of p62 was examined (**A**). Each lane represented on mouse sample. Densitometry was conducted and the relatively abundance of p62 (standardized to the load control of beta-actin) was calculated (**B**). Values shown are mean ±-SEM from 2 mice per group. *: $p<0.05$; **: $p<0.01$. *t*-test.

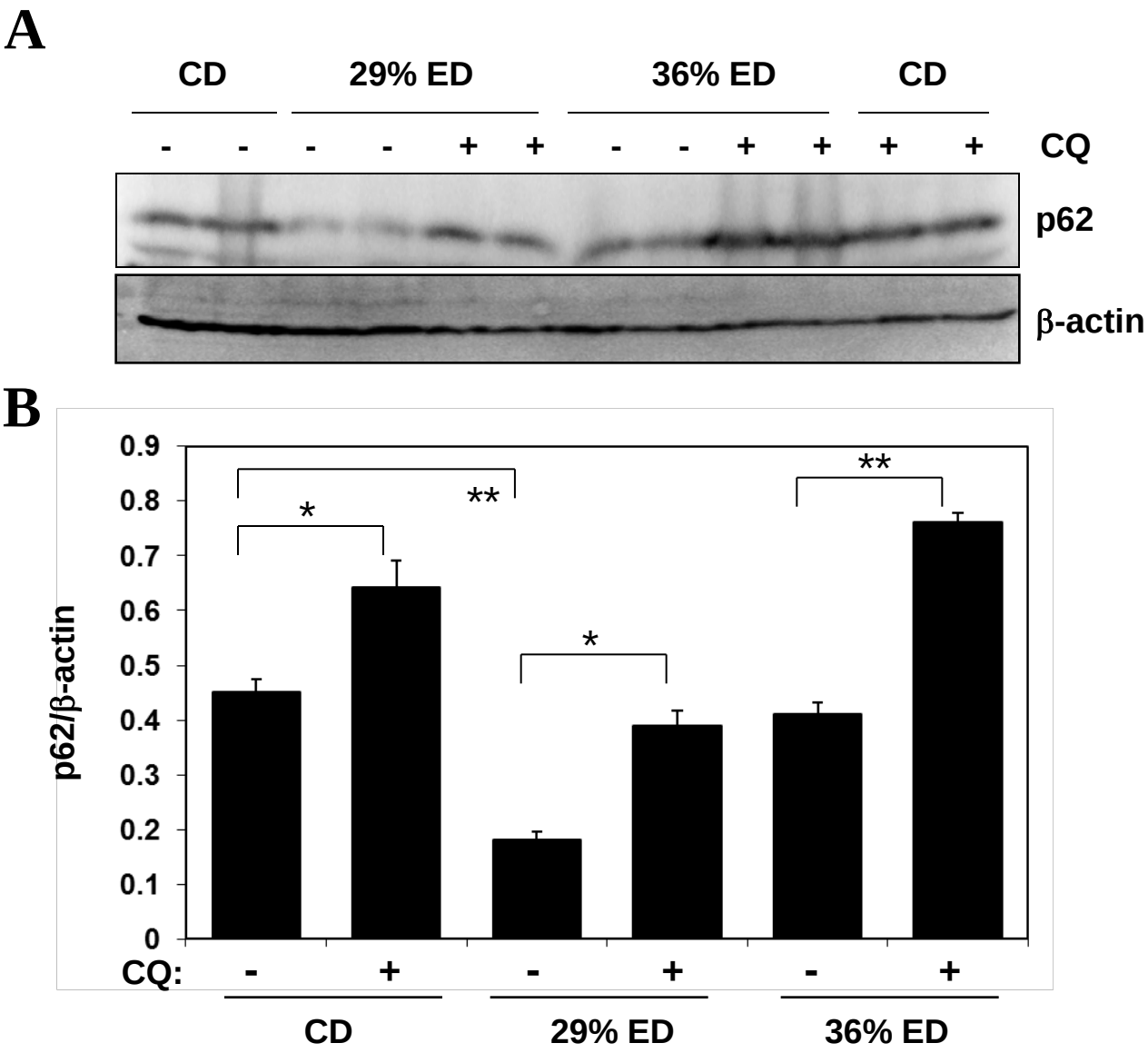
Figure S3. Lysosomal activities in mice treated chronically with ethanol diet.

Mice were given control diet (CD) or ethanol diet (ED) that accounts for 29% or 36% of total calorie intake for 4 weeks. Liver lysates were prepared and analyzed for the activities of cathepsin B (A), cathepsin D/E (B), acidic phosphatase (C) and acid lipase (D) using Z-FR-AMC (Enzo Life Sciences), Mca-GKPILFFRLK (Dnp)-D-R (Enzo Life Sciences), 4-nitrophenyl phosphate (Sigma), and 4-methylumbelliferyl oleate (Sigma) as the substrate, respectively. Cleavage was monitored by fluorescence spectrophotometry (A, B, D) or absorption spectrophotometry (C). *, $p<0.05$; **, $p<0.01$. t-test. RFU: relative fluorescence unit.

Figure S4. Impact of chloroquine on alcoholic liver inflammation.
Livers from mice given control diet (CD), 29% ethanol diet (ED), or 36% ethanol diet (ED) for 4 weeks were fixed by 10% neutral buffered formalin and paraffin-embedded. The sections were stained with an anti-F4/80 antibody (AbD Serotec, MCA497G, Raleigh, NC) that detects the macrophage/Kupffer cell population, which was quantified per high power field (x400). Data shown are mean ± SEM (n= 2-4 per group). $p = 0.164$ among the groups (one-way ANOVA).

Fig. S1


- + - + EtOH
- - + + Rapamycin
Phos-p70S6K
Total p70S6K
Phos-4E-BP1
Total 4E-BP1
β-actin


A
CD
29% ED
36% ED
CD
- - - - + + - - + + + +
CQ
p62
β-actin
B
p62/β-actin
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
**
*
**
CQ: - + - + - +
CD
29% ED
36% ED

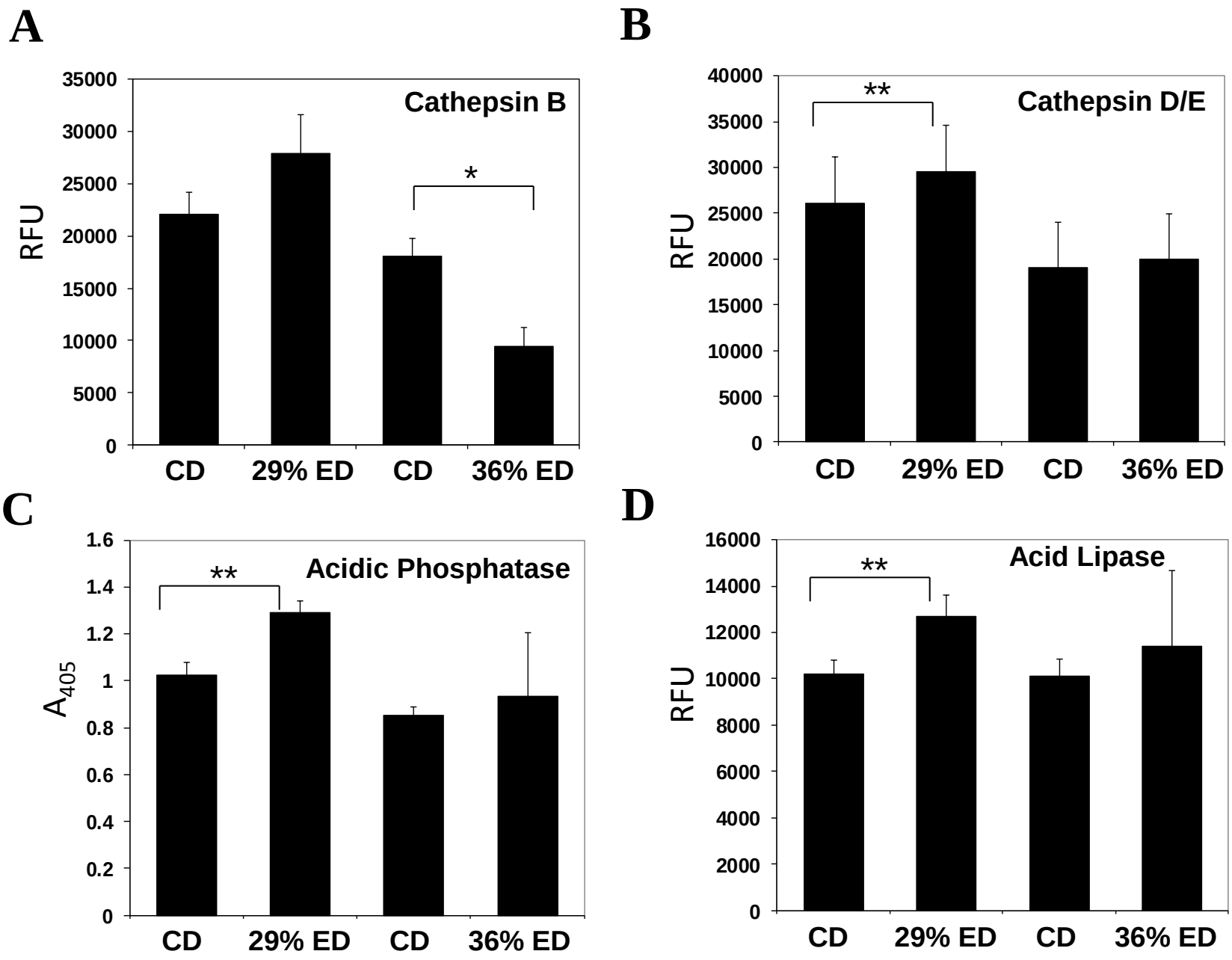
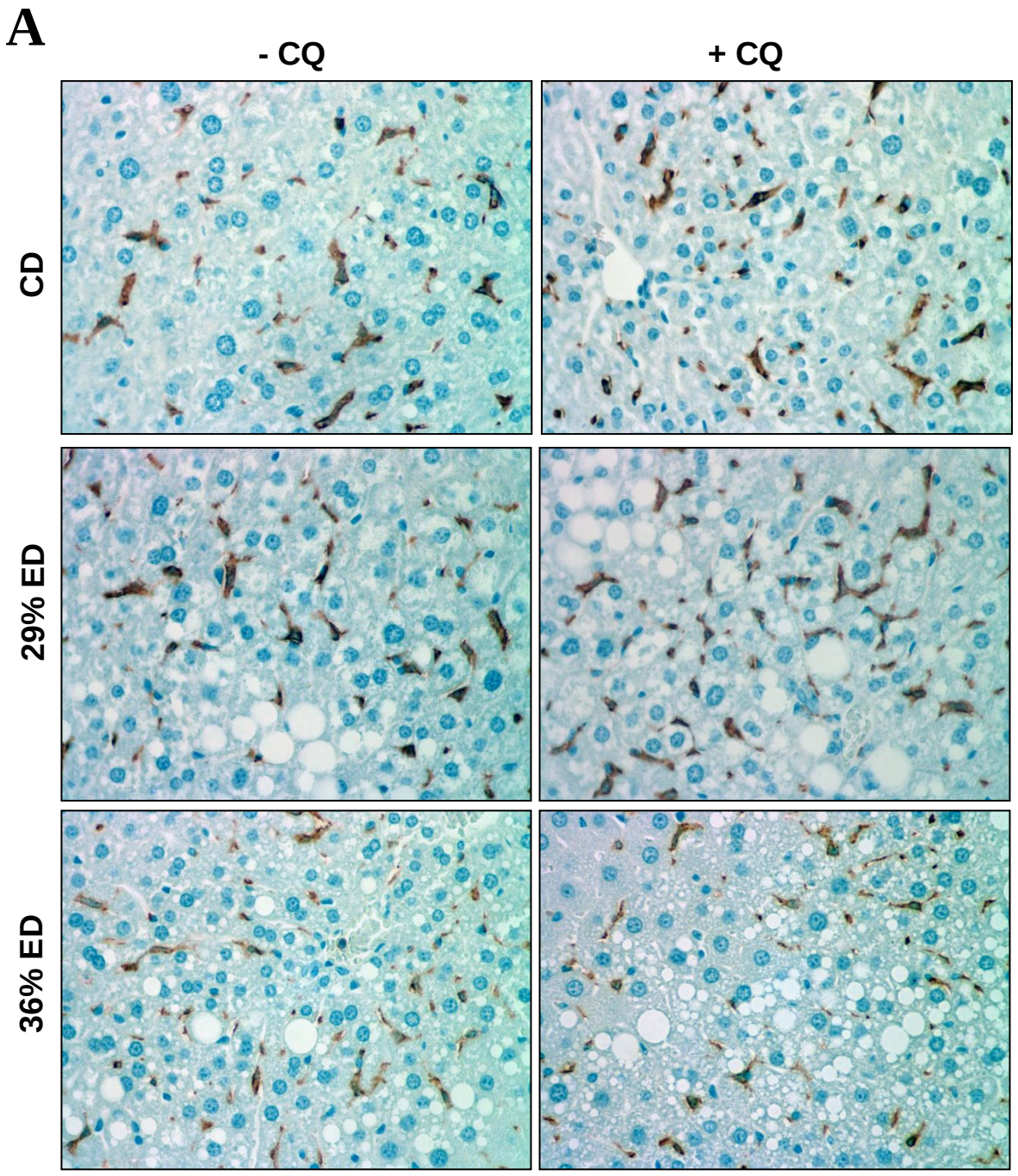
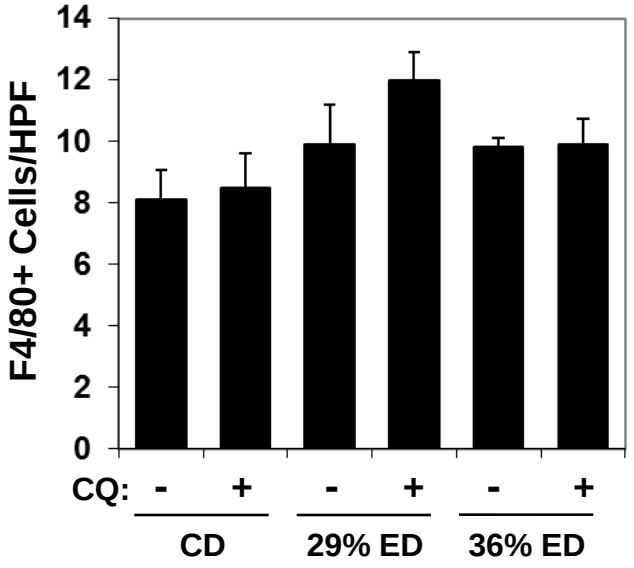

A
Cathepsin B
RFU
35000
30000
25000
20000
15000
10000
5000
0
*
CD
29% ED
CD
36% ED
B
Cathepsin D/E
RFU
40000
35000
30000
25000
20000
15000
10000
5000
0
**
CD
29% ED
CD
36% ED
C
Acidic Phosphatase
A405
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
CD
29% ED
CD
36% ED
D
Acid Lipase
RFU
16000
14000
12000
10000
8000
6000
4000
2000
0
**
CD
29% ED
CD
36% ED

Fig. S4


A
- CQ
+ CQ
CD
29% ED
36% ED


B
F4/80+ Cells/HPF
14
12
10
8
6
4
2
0
CQ: - + - + - +
CD
29% ED
36% ED