cells

MDPI

*Supplementary Materials*

# Increased Levels of Phosphorylated ERK Induce CTGF Expression in Autophagy-Deficient Mouse Hepatocytes

## Materials and Methods

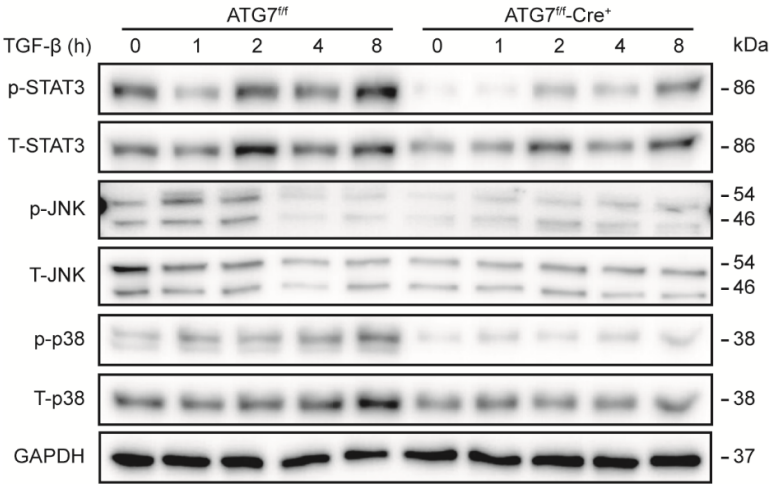
3-Methyladenine (3MA, M9281) was purchased from Sigma-Aldrich. An anti-phospho-STAT3 (Tyr705) (CS9138), anti-STAT3 (CS4904), anti-phospho-JNK (CS9251), anti-JNK (CS3252), anti-phospho-p38 (CS9211), anti-p-38 (CS9212) and anti-Yap (CS4912) antibodies were purchased from Cell Signaling Technology.

## Small Interfering RNA (siRNA)-Mediated Depletion of ATG7

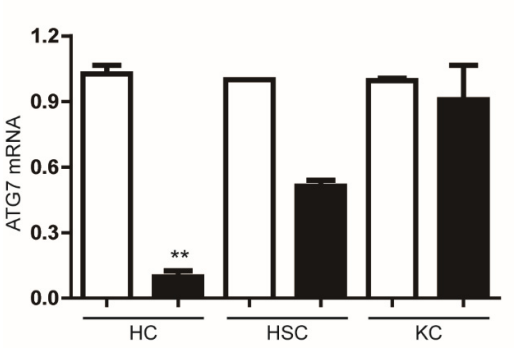
A pre-designed siRNA targeting ATG7 (*siATG7*) (SC41448) and a scrambled control siRNA (*siCon*) (SC37007) were purchased from Santa Cruz Biotechnology. AML12 Cells were transfected with 100 nM siRNA using Lipofectamine RNAiMAX (Thermo Scientific, 13778-075) for 5 h, cultured in medium containing 0.5% FBS, and harvested ~48 h after transfection.

## Lysotracker Staining

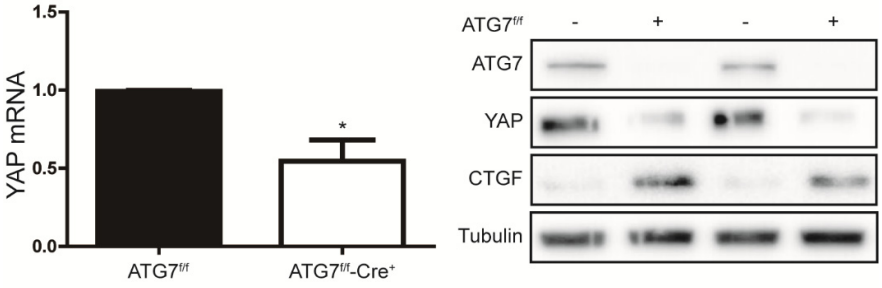
For lysotraker staining, primary hepatocytes from Cre-negative and Cre-positive $Atg7^{flox/flox}$ ($Atg7^{f/f}$ and $Atg7^{f/f}$-$Cre^{+}$) mice were stained with 100 nM Lysotracker Red DND-99 (Invitrogen, L7528) for 10 min at 37°C, and the hepatocytes were imaged using a microscope.



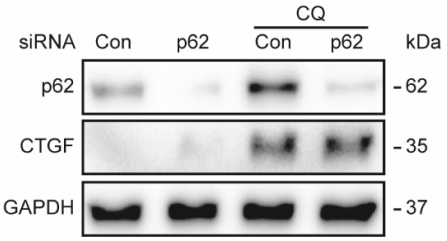

**Supplementary Figure S1.** Western blot analyses of phospho-STAT3 (p-STAT3), total-SMAD3 (T-SMAD3), p-ERK, and total ERK (T-ERK) in primary hepatocytes from $Atg7^{f/f}$ and $Atg7^{f/f}$-$Cre^{+}$ mice after treatment with or without TGF-β (5ng/ml) for the indicated times. The expression of GAPDH was measured as a loading control.



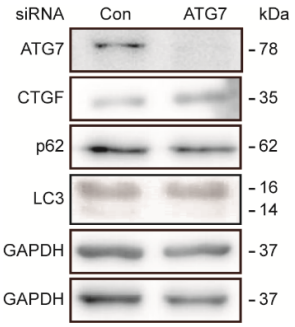

**Supplementary Figure S2.** Real time RT-PCR analyses of ATG7 mRNA level in primary hepatocytes, primary hepatic stellate cell (HSC) and hepatic kupffer cell (KC) from Atg7$^{f/f}$ and Atg7$^{f/f}$-Cre$^{+}$ mice.



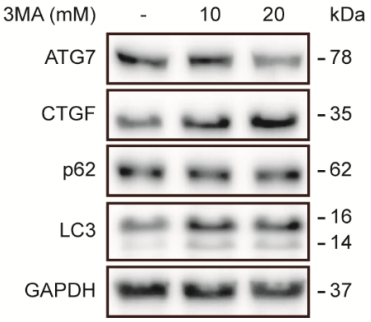

**Supplementary Figure S3.** Real time RT-PCR analyses of YAP mRNA level and western blot analyses of YAP protein expression in primary hepatocytes from Atg7$^{f/f}$ and Atg7$^{f/f}$-Cre$^{+}$ mice.



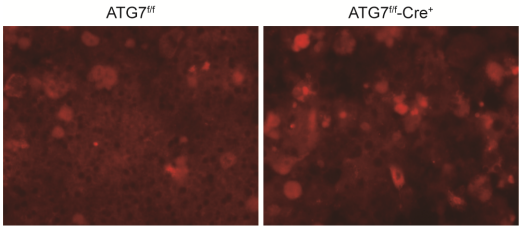

**Supplementary Figure S4.** Western blot analysis showing the effect of small interfering RNA (*siRNA*)-SQSTM1/p62 on CQ-stimulated CTGF protein expression. AML12 cells were transfected with 100nM *siRNA-SQSTM1/p62* or *control siRNA*, and then treated with or without CQ. .




**Supplementary Figure S5.** Western blot analysis showing the effect of small interfering RNA (*siRNA*)-ATG7 on CTGF, p62 and LC3 protein expression in AML12 cells.




**Supplementary Figure S6.** Western blot analyses showing the effects of 3MA on ATG7, CTGF, p62 and LC3 expression in AML12 cells.




**Supplementary Figure S7.** Lysotracker staining was performed in primary hepatocytes from Atg7$^{f/f}$ and Atg7$^{f/f}$-Cre$^{+}$ mice.