Supplementary information

Tumor Necrosis Factor-Inducible Gene 6 Protein Ameliorates Chronic Liver Damage by Promoting Autophagy Formation in Mice

Supplementary Table 1. Sequences of primers used for qRT-PCR

Supplementary Figure 1. TSG-6 decreases inflammation in the liver of MCDE-treated mice. qRT-PCR analysis of murine livers for tnfα, Il-1β, cxcl1 and cxcl2. Mean±SD results are plotted ($n \geq 4$ mice / group) (*$p<0.05$)

Supplementary Figure 2. TSG-6 protects hepatocytes from PA-induced cell death by activating autophagy processes.

(a) Cell viability of AML12 cells treated with TSG-6 was analyzed using an MTS assay. After being exposed to palmitic acid (PA) (400 μM) for 24 hours, AML12 cells were treated with vehicle (PA), 3-MA (PA+3-MA) or TSG-6 with (PA+TSG-6+3-MA) or without 3-MA (PA+TSG-6) for 24 and 48 hours. As a control, AML12 cells were treated with equal volumes of vehicle without PA. The mean±SEM results obtained from three identical experiments are plotted. **(b)** Western blot assay and **(c)** cumulative densitometry analyses for LC3-II in these cell groups. GAPDH was used as an internal control. Data shown represent one of three experiments with similar results and the mean±SEM results obtained from three identical experiments are plotted (*$p<0.05$ vs PA, #$p<0.05$ vs PA+TSG-6 with 3-MA, &$p<0.05$ vs PA+3-MA, \$$p<0.05$ vs control).

Supplementary Figure 3. TSG-6 is involved in activating CMA in ER stress-induced hepatocytes, increasing hepatocyte viability.

(a) qRT-PCR and **(b)** western blot assays of LAMP2A in the AML12 cells treated with either

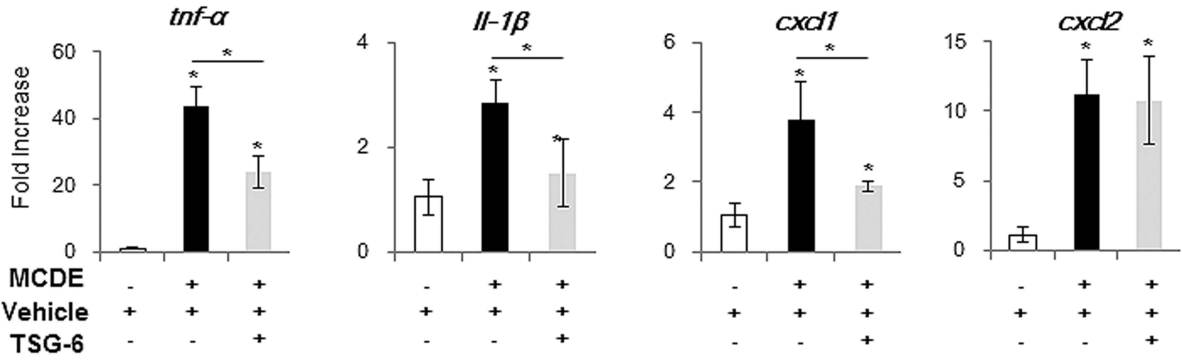
scramble (scr) or *lamp2a* siRNA (si). GAPDH was used as an internal control. Immunoblots shown represent one of three experiments with similar results. Mean±SD results obtained from three identical experiments are plotted (*p<0.05 vs scr). **(c)** Either scr or *lamp2a* siRNA-transfected AML12 cells were exposed to either tunicamycin (TM) (5 ng/ml) or palmitic acid (PA) (400 μM) for 24 hours, and then were treated with either vehicle or TSG-6 for 24 and 48 hours. Cell viability of these cells was assessed using an MTS assay. The mean±SEM results obtained from three identical experiments are plotted. **(d)** qRT-PCR analysis of *atg3, atg7, lc3,* and *lamp2a* expression in these cells damaged by either TM **(d)** or **(e)** PA. Data represent the mean±SEM of three independent experiments ($p<0.05 vs Control (scr-transfected cells with vehicle), *p<0.05 vs TM or PA with scramble RNA, #p<0.05 vs TM or PA+TSG-6 with *lamp2a* siRNA, &<0.05 vs TM or PA with *lamp2a* siRNA).

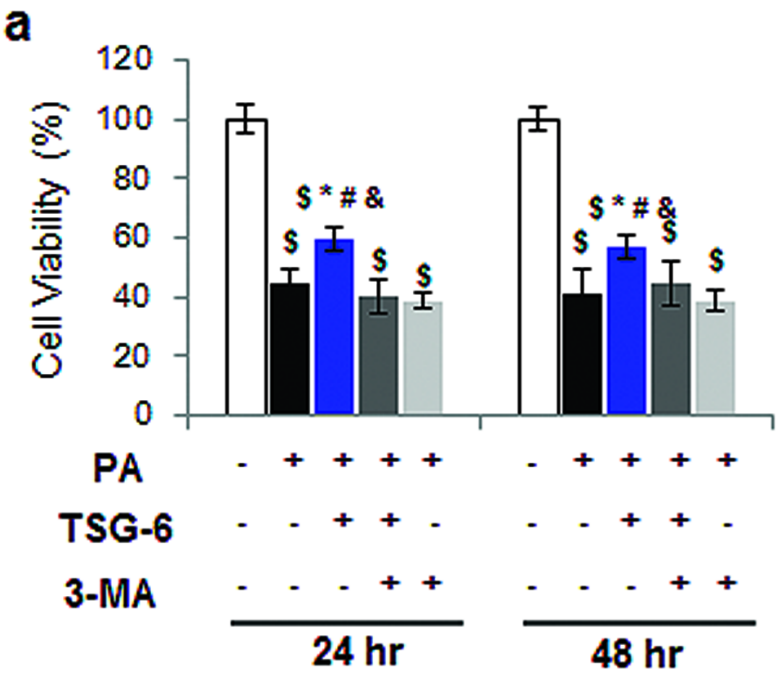
Supplementary Figure 4. 3-MA, an autophagy inhibitor, rarely affects the healthy liver.

(a) H&E-stained liver sections from representative normal mice with (normal+3-MA) or without 3-MA (normal) treatment (X40). **(b)** The ratios of liver to body weight (LW/BW) of these mice. **(c)** qRT-PCR analysis for *atg3*, *atg7* and *lc3* in livers from control mice treated with vehicle or 3-MA. The results are displayed as the mean±SEM ($n \geq 3$ mice / group). **(d)** Western blot analysis for LC3 in these groups. GAPDH was used as an internal control. Data shown represent one of three experiments with similar results.

S1 Table. Sequences of primers used for qRT-PCR

Mouse		
Gene	Forward sequence	Reverse sequence
atg3	GAGGCTACCCTAGACACAAGG	GGGTGCCGTTGCTCATCATA
atg7	CTTCGCCCCCTTTAATAGTGC	TGAACTCCAACGTCAAGCGG
lc3	TTATAGAGCGATACAAGGGGGAG	CGCCGTCTGATTATCTTGATGAG
lamp2a	TTGTGGCAGGGTTGATGTTA	CACCCACTCCAACTCCAACT
tnf-α	TCGTAGCAAACCACCAAGTG	ATATAGCAAATCGGCTGACG
il-1β	ACTCCTTAGTCCTCGGCCA	TGGTTTCTTGTGACCCTGAGC
cxcl1	CCCAAACCGAAGTCATAGCC	TCAGAAGCCAGCGTTCACC
cxcl2	GCCCAGACAGAAGTCATAGCC	TTCTCTTTGGTTCTTCCGTTGA
9 s	GACTCCGGAACAAACGTGAGG	CTTCATCTTGCCCTCGTCCA


tnf-α
Il-1β
cxcl1
cxcl2
Fold Increase
60
40
20
0
4
2
0
6
4
2
0
15
10
5
0
*
MCDE - + +
Vehicle + + +
TSG-6 - - +

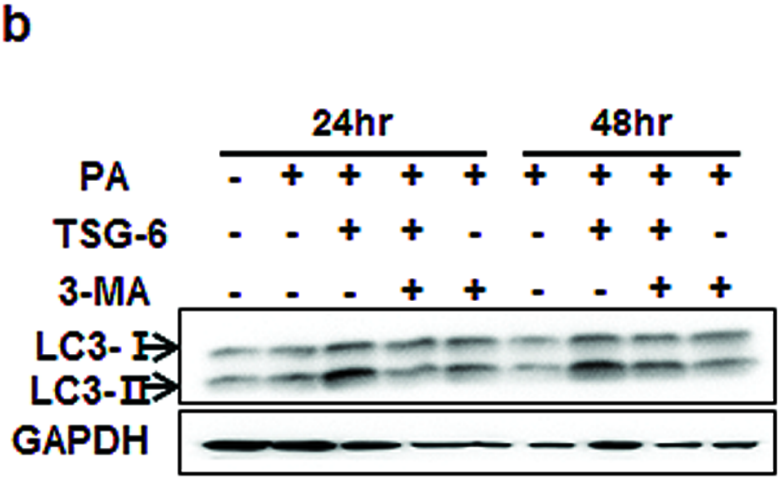


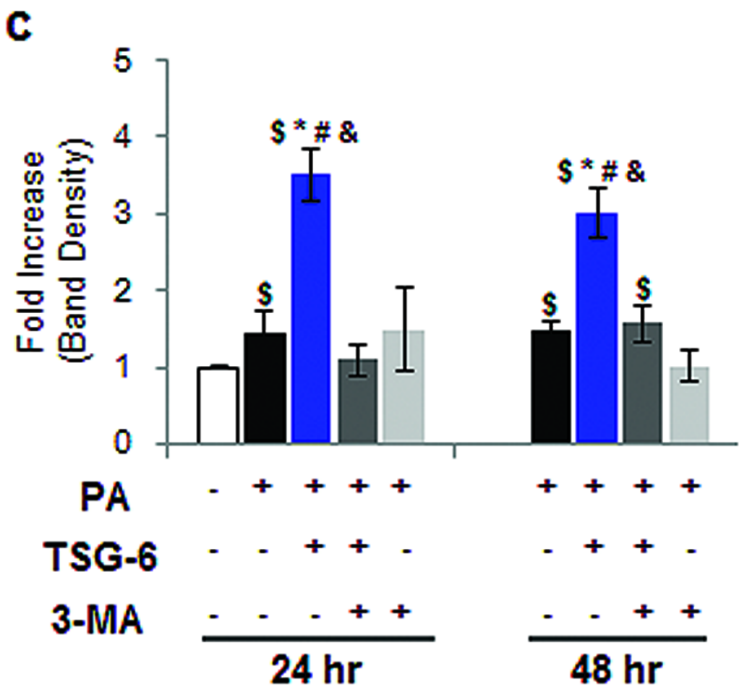
$: vs Control (no treatment)

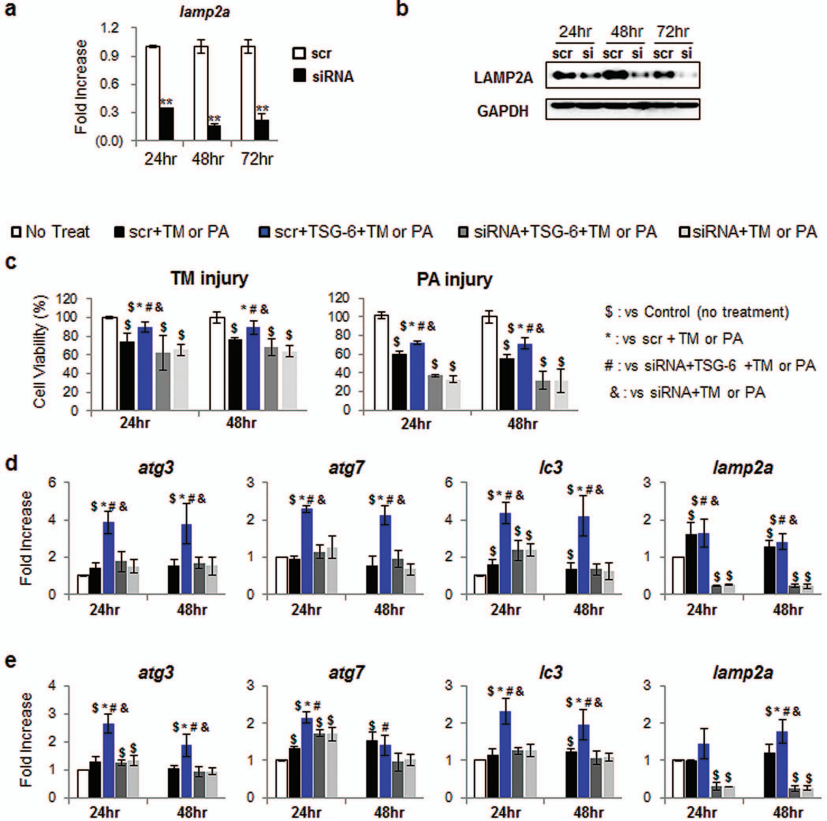
* : vs PA + vehicle

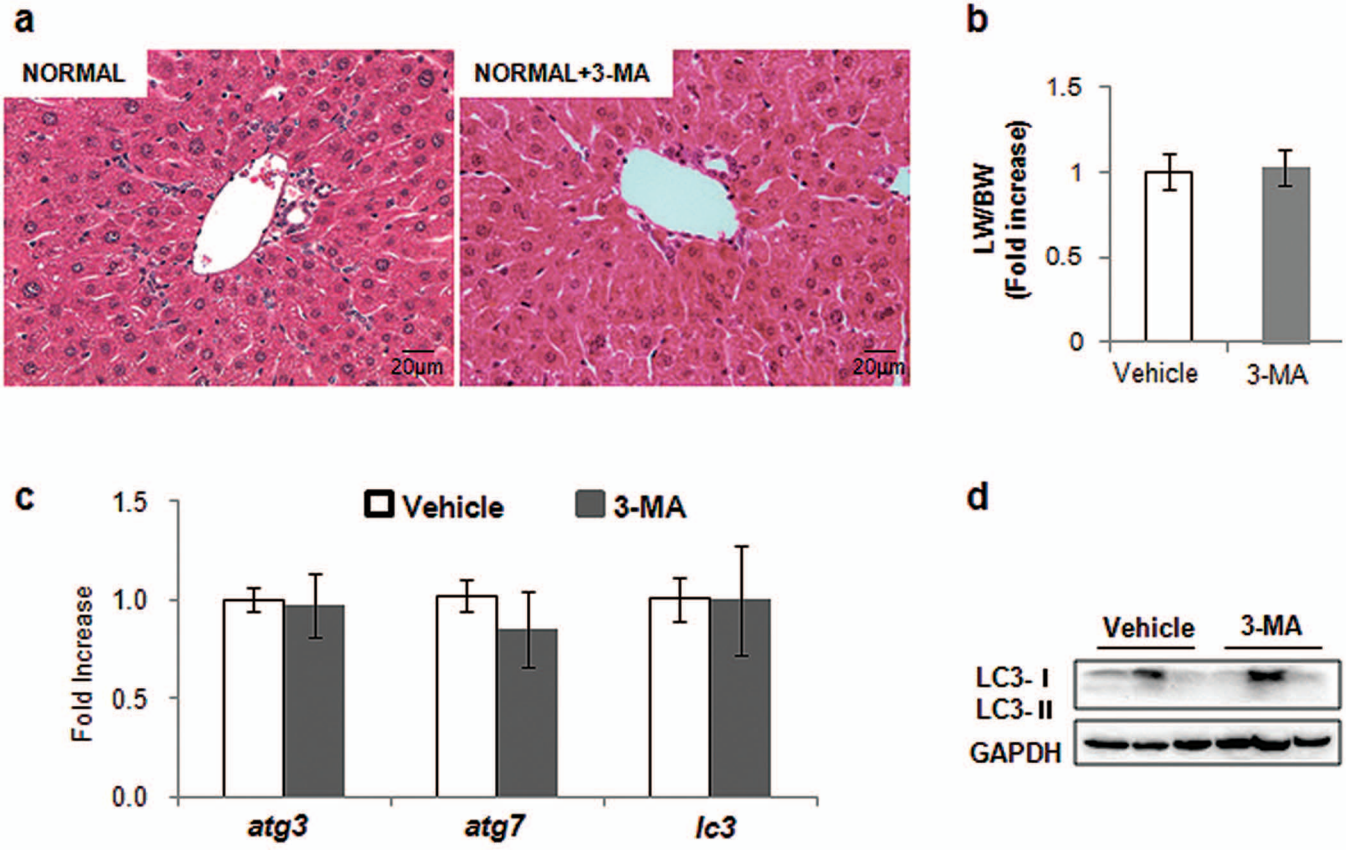
: vs PA+TSG-6 +3-MA

& : vs PA+3-MA






a
lamp2a
Fold Increase
1.2
0.9
0.6
0.3
(0.0)
scr
siRNA
**
24hr 48hr 72hr
b
24hr 48hr 72hr
scr si scr si scr si
LAMP2A
GAPDH
No Treat
scr+TM or PA
scr+TSG-6+TM or PA
siRNA+TSG-6+TM or PA
siRNA+TM or PA
c
TM injury
PA injury
Cell Viability (%)
$: vs Control (no treatment)
* : vs scr + TM or PA
# : vs siRNA+TSG-6 +TM or PA
& : vs siRNA+TM or PA
24hr 48hr
d
atg3
atg7
lc3
lamp2a
Fold Increase
24hr 48hr
e
atg3
atg7
lc3
lamp2a
Fold Increase
24hr 48hr


a
NORMAL
NORMAL+3-MA
20μm
20μm
b
LW/BW
(Fold increase)
1.5
1
0.5
0
Vehicle
3-MA
c
Vehicle
3-MA
Fold Increase
1.5
1.0
0.5
0.0
atg3
atg7
lc3
d
Vehicle
3-MA
LC3- I
LC3- II
GAPDH