

**Manuscript Title:**

SREBP-2/PNPLA8 axis improves non-alcoholic fatty liver disease through activation of autophagy

**Author List:**

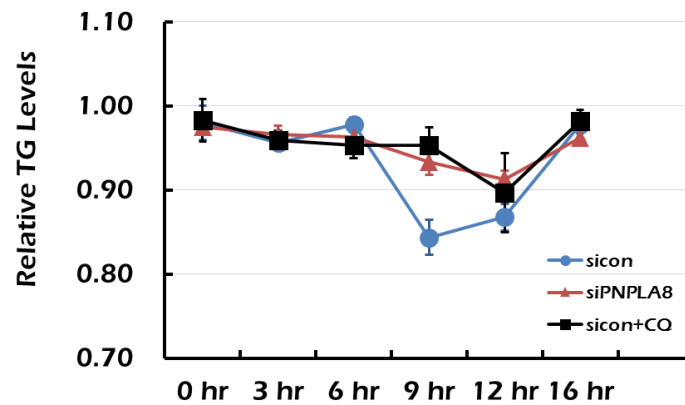
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**Supplementary Table S1; Primers and Oligonucleotides used.**

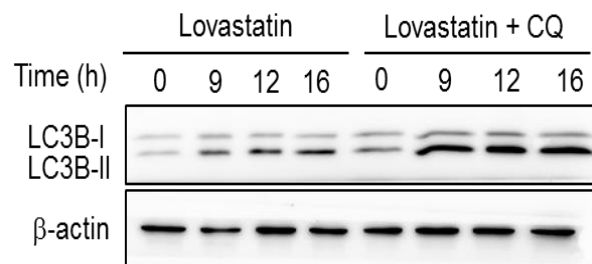
| <b>qPCR primers for mRNA identification</b> |                                                                                           |                           |
|---------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------|
|                                             | <b>Forward</b>                                                                            | <b>Reverse</b>            |
| <b>Pnpla1</b>                               | ACCCAGATACCCCTCATTCC                                                                      | CAGGCCCATATTCAGCACTC      |
| <b>Pnpla2</b>                               | TGTGGCCTCATTCTCTAC                                                                        | GATGTTGGTGGAGCTGTCCT      |
| <b>Pnpla3</b>                               | GGGCTACGCTATGTCTGAGC                                                                      | ATGTTGAAGAACGGGTGGAG      |
| <b>Pnpla4</b>                               | TAACGCCCGGTTATGACTTC                                                                      | TGCTGGCTAGGAGGACCTTA      |
| <b>Pnpla5</b>                               | TGCTCCAATCTCCTGGACTT                                                                      | GAGTGGCAAAATCGGTGACT      |
| <b>Pnpla6</b>                               | TTATGCGGAAGGTGTCACAG                                                                      | AGAGGGTAGGTGGGAATTGG      |
| <b>Pnpla7</b>                               | CCTCCTTACCCTAGCCTTCG                                                                      | GCAGAGGTATTCCCAACCAA      |
| <b>Pnpla8</b>                               | TTCTGCTCCCAAGGGACTTA                                                                      | GACTCGACGGCTTGCTTAAC      |
| <b>Srebp-2</b>                              | TGGGAGAGTTCCCTGATTTG                                                                      | GATAATGGGACCTGGCTGAA      |
| <b>Srebp-1c</b>                             | CCGGGGAACCTTTTCCTTAAC                                                                     | GTTGTTGATGAGCTGGAGCA      |
| <b>Hmgcr</b>                                | GCCAGCAATACCCAGAATGT                                                                      | CAACAAGATCTGTGGCTGGA      |
| <b>Ldlr</b>                                 | TCCTGGAGATGTGATGGACA                                                                      | GAGCCATCTAGGCAATCTCG      |
| <b>qPCR primers for manual ChIP</b>         |                                                                                           |                           |
| <b>Pnpla8</b>                               | ACCAAATCCACCTCCGAT<br>G                                                                   | GATGGGGCCATTAAACATG<br>AC |
| <b>LDLr</b>                                 | GAAGTTCCCACTGCTGC                                                                         | CACGCCCAGAG TCATTC        |
| <b>siRNA Sequences</b>                      |                                                                                           |                           |
| <b>PNPLA8</b>                               | 1.GGUUGAAAUACAUAGAAAGAAAUGA<br>2.AACAUGCAACUAAGAACAACUUGTA<br>3.GUCCUAAACUGAUGUAGUUCAUCTG |                           |

**Supplementary Figure S1. Tracking TG in HepG2 cells.**

**a**

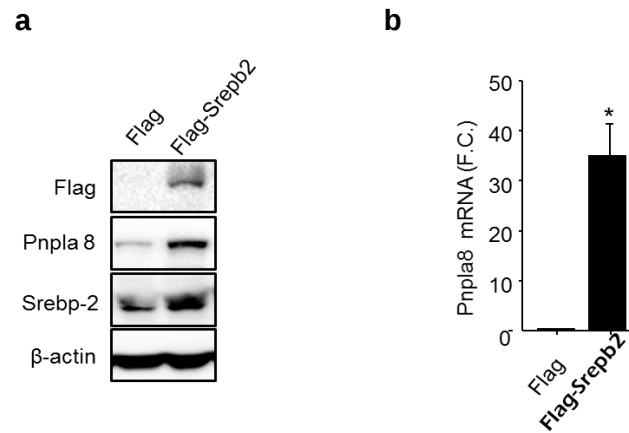


**b**



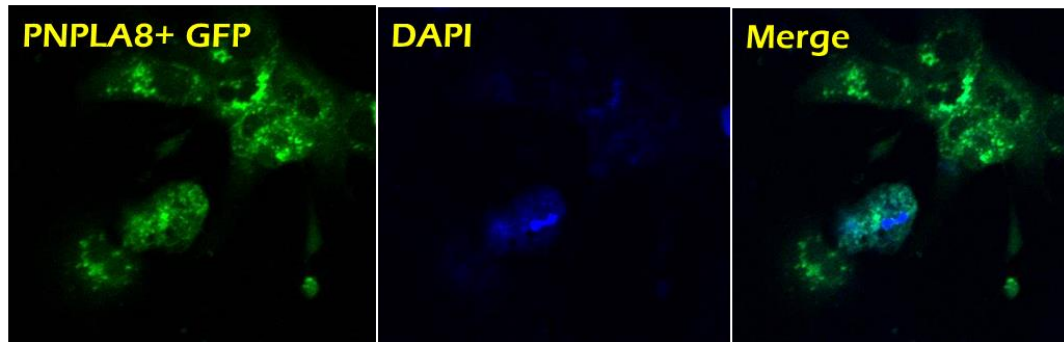
**Supplementary Figure S1.** After 24 hr of palmitic acid (400  $\mu$ M, Sigma-Aldrich) treatment, HepG2 cells were treated with lovastatin (5  $\mu$ M) or chloroquine (50  $\mu$ M) treatment. Endogenous triglyceride levels from si PNPLA8, si control, and si control + chloroquine treated HepG2 cells were measured over the time points of 0 to 16 hr (**a**). Consistent with TG clearance by autophagy formation, LC3IIB levels were increased over the time points in chloroquine treatment (**b**).

**Supplementary Figure S2. SREBP-2 upregulates PNPLA8 expression in mouse liver.**



**Supplementary Figure S2.** A control vector (Flag) or a Flag-SREBP-2 expression vector were transfected into HEK293 cells and SREBP-2 and PNPLA8 protein (**a**) and PNPLA8 RNA (**b**) levels were measured (F.C.; fold change). All data are representative of three independent experiments. Data are shown as means  $\pm$  standard deviations. \* $p < 0.01$ .

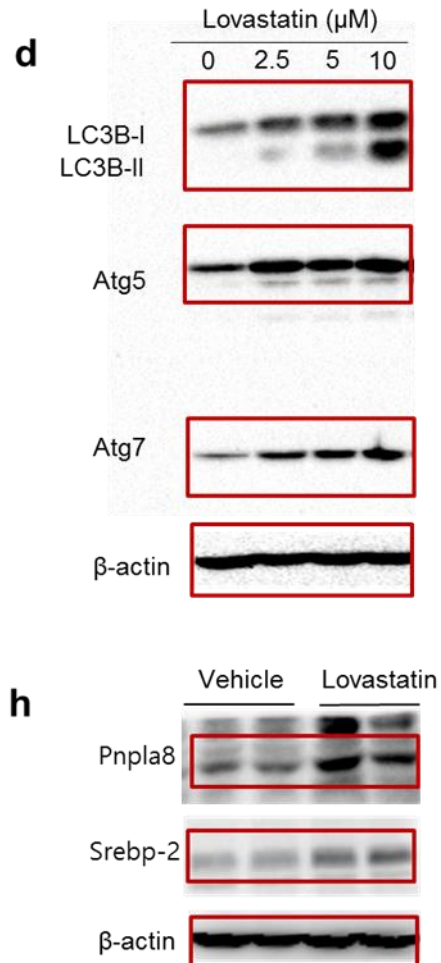
**Supplementary Figure S3.** Tracking of the functional properties of Pnpla8 *in vivo*.



**Supplementary Figure S3.** The GFP-PNPLA8 expressing plasmid were injected into the tail veins of 12 weeks-HFD mice. Mice were sacrificed and GFP expression was analyzed from the primary hepatocytes.

**Supplementary Figure S4. Full unedited blots.**

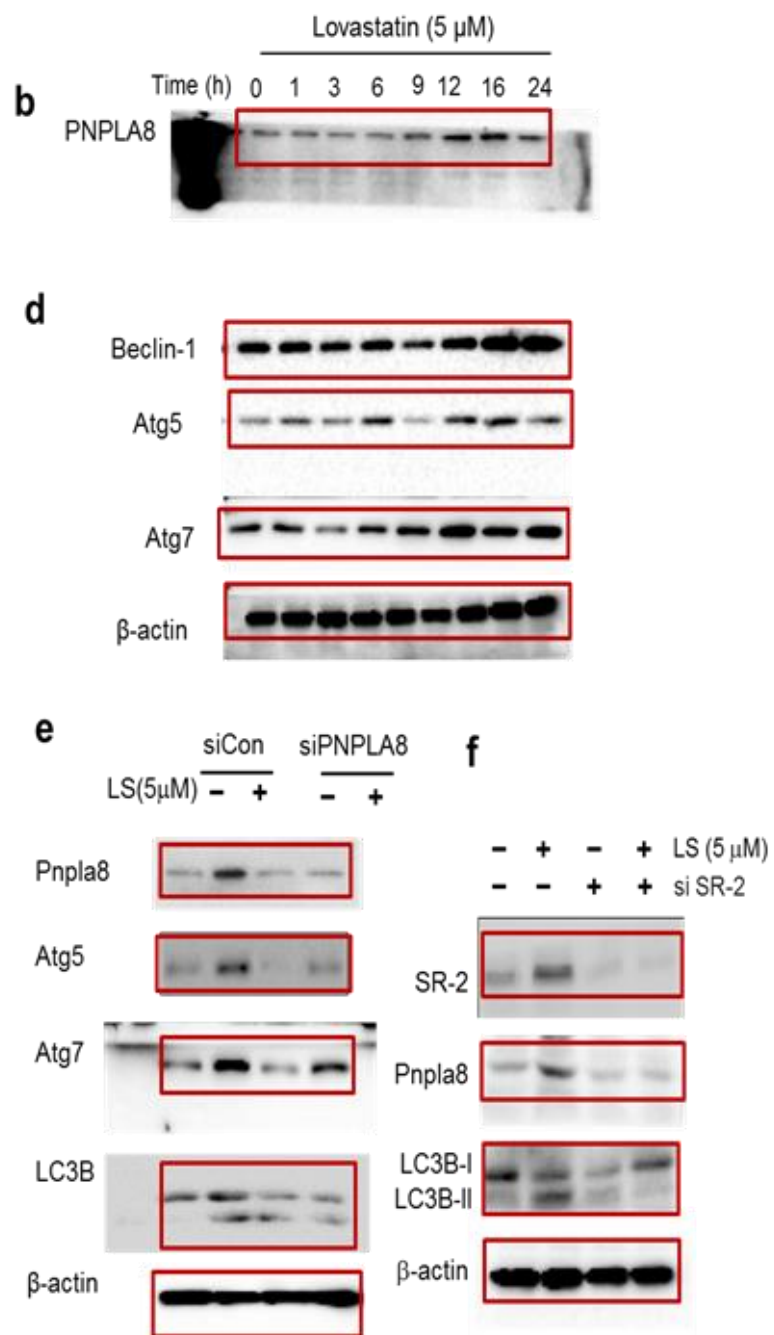
**Figure 2.**



**Supplementary Figure S4.** (Figure 2d h, Figure 3b d e f, Figure 5f, Figure 7a, Supplementary Figure S1) Red boxes indicate images shown in the corresponding Figures.

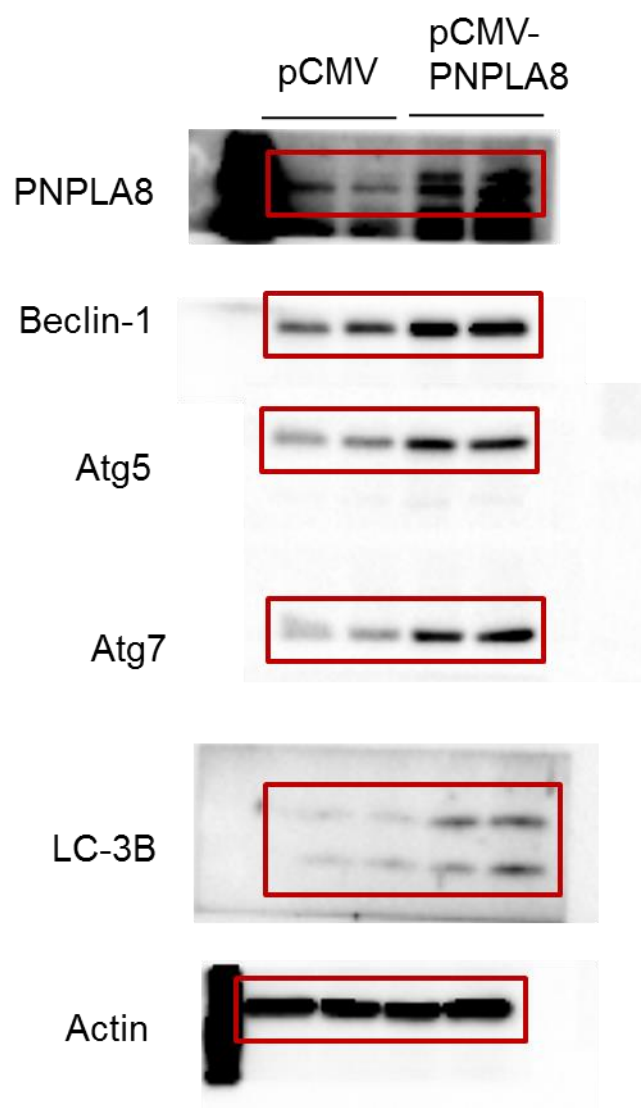
Supplementary Figure S4 continued.

Figure 3.



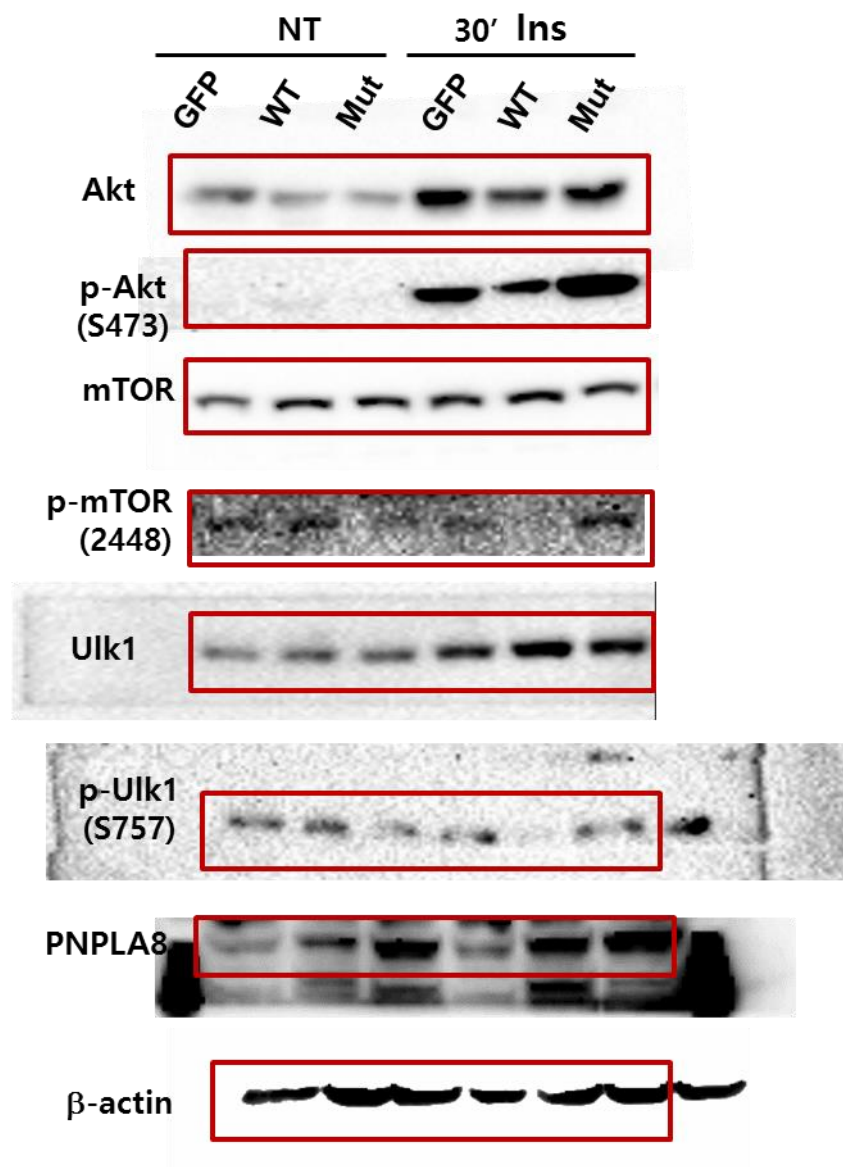
Supplementary Figure S4 continued.

Figure 5f.



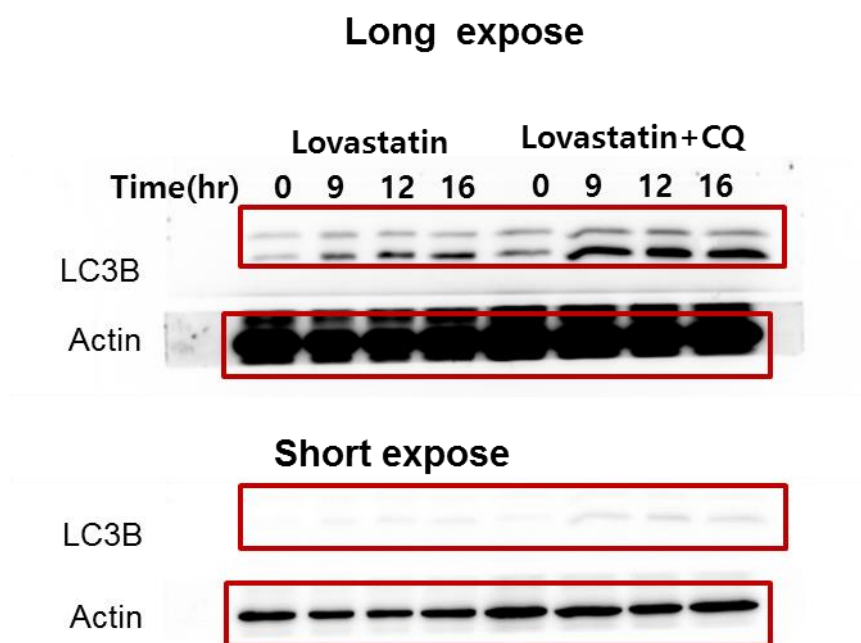
Supplementary Figure S4 continued.

Figure 7a.



Supplementary Figure S4 continued.

Supplementary Figure S1.



**Supplementary Video S1. Dynamic interactions between PNPLA8 and LC3.**

**a** Uploaded as separate video file.

**b** Uploaded as separate video file.

**Supplementary Video S1.** (a) PC-3 cells were transfected with pCMV+PNPLA8-GFP (green) or pCMV+LC3B (red) and imaged by confocal microscopy (LSM780NLO' Carl Zeiss, Germany) in time lapse. (b) pCMV+GFP or pCMV+LC3B as a control movies were imaged identically. Images at 3 frames per second are shown. Scale bar, 10  $\mu$ m.