

# **Inhalation Exposure to PM<sub>2.5</sub> Counteracts Hepatic Steatosis in Mice Fed High-fat Diet by Stimulating Hepatic Autophagy**

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Running title: PM<sub>2.5</sub> exposure counteracts hepatic steatosis in obesity

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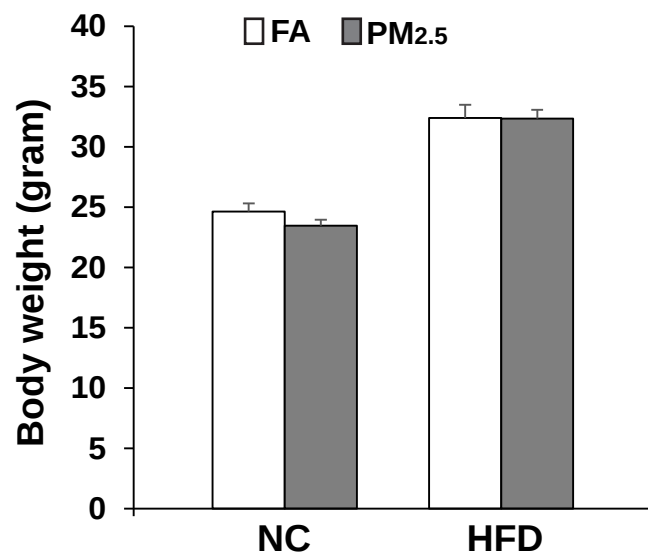
### Supplemental figure legends

**S-figure 1.** Body weights of the normal chow- or HF- fed mice exposed to PM<sub>2.5</sub> or FA for 10 weeks. N = 6 mice for PM<sub>2.5</sub>- or FA- exposed group under the HF diet; or 4 mice for PM<sub>2.5</sub>- or FA- exposed group under the normal chow diet. Each bar denotes mean  $\pm$  SEM.

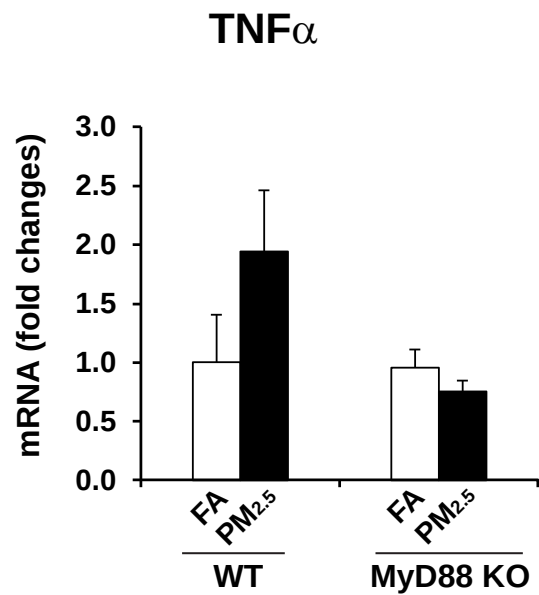
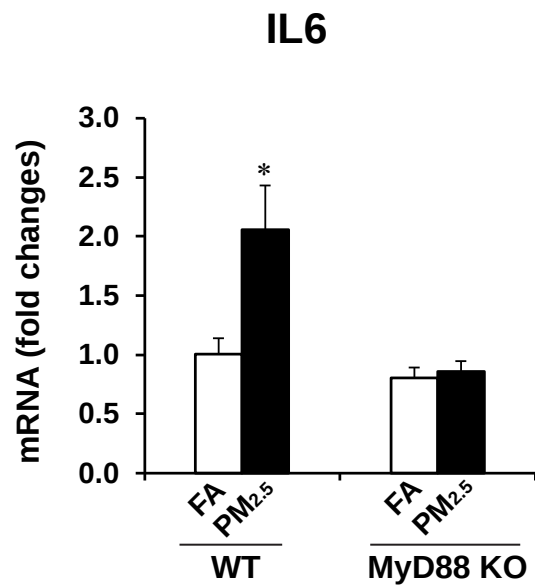
**S-figure 2.** Expression levels of the *IL6* and *TNF $\alpha$*  mRNAs in the livers of the PM<sub>2.5</sub>- or FA- exposed WT and MyD88 KO mice. Expression levels of mRNAs were determined by qPCR. Fold changes of mRNA levels are shown by comparing to that of one of NC-fed mice under FA exposure. Each bar denotes mean  $\pm$  SEM (n = 4 mice per group). \* p < 0.05.

**S-figure 3.** Western blot analyses of levels of LC3 protein in Huh7 cells cultured in the conditioned medium from RAW264.7 cells exposed to PM<sub>2.5</sub> (5 $\mu$ g/mL), PM<sub>2.5</sub> plus 3-MA (2mM), or vehicle control PBS (Ctl) for 28 h. Huh7 cells were incubated with the conditioned medium for 36 h before subjected to Western blot analysis. Levels of GAPDH were determined as loading controls. The graph beside the images shows the quantitative analyses of fold changes of ratios of LC3-II vs LC3-I protein levels, as determined by Western blot densitometry, in Huh7 cells exposed to PM<sub>2.5</sub>, PM<sub>2.5</sub> plus 3-MA, or control medium. The protein level was normalized to that of GAPDH before fold change calculations.

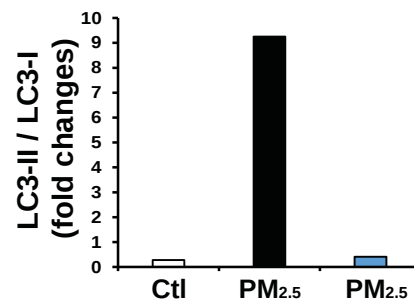
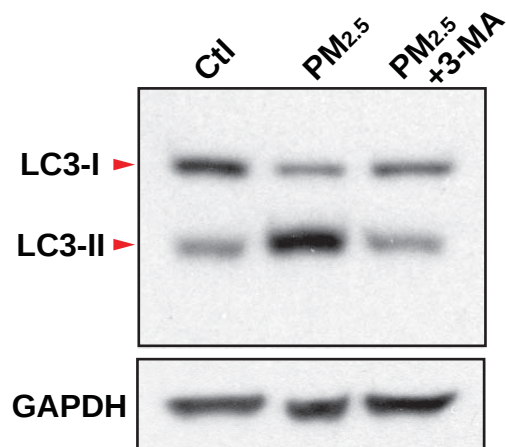
**S-table 1.** Primer sequence information for the real-time PCR analysis in this study.



Supplemental figure 1



Supplemental figure 2



Supplemental figure 3

**S-table 1**

Sequence information for the quantitative real-time RT-PCR analysis in this study

| <b>Gene name</b> | <b>Forward primer</b>         | <b>Reverse primer</b>         |
|------------------|-------------------------------|-------------------------------|
| Lc3b             | GCT CTT TGT TGG TGT GTA       | TCT TCT GTT GCT GTT GTC       |
| Pdgf             | TCA AGG TGG CCA AAG TGG AG    | CTG GGT TCA GGT TGG AGG TC    |
| Apoa4            | GCA TCT AGC CCA GGA AAC TG    | ATG TAT GGG GTC AGC TGG AG    |
| Tfeb             | CAG AAG CGA GAG CTA ACA GAT   | TGT GAT TGT CTT TCT TCT GCC G |
| Ctsa             | GCT ACC TCA GAG CAT CGG AC    | GTT AAG CCA AAG CAC CAC GG    |
| Gba              | GCC TCC CAG AAG AAG ACA CC    | ATA TCC CCT GGC TGA CCC TT    |
| P62              | GCT GAA GGA AGC TGC CCT AT    | GCC TTC ATC CGA GAA ACC CA    |
| Ppara $\alpha$   | GGGAACCTTAGAGGAGAGCCAAG       | CCATGTTGGATGGATGTGGC          |
| Sirt1            | GCC GCG GAT AGG TCC ATA TAC T | GCC ACA GCG TCA TAT CAT CCA   |
| Ppar $\gamma$ 1  | GGT GTG ATC TTA ACT GCC GGA   | ACC TGA TGG CAT TGT GAG ACA   |
| Fasn             | GGAGGTGGTGATAGCCGGTAT         | TGGGTAATCCATAGAGCCCAG         |