**Supplementary Materials**

## METHODS

### Co-culture of THP-1 with HuH-7

THP-1 cells were cultured and differentiated as mentioned above. Co-culture of THP-1 with HuH-7, an HCC cell line, was performed using 6-well Transwell system (#3412, Corning, NY, USA). THP-1 was seeded in 6-well plate at a density of $8 \times 10^5$ per well, and differentiated to macrophage-like cells. HuH-7 was seeded in the upper chamber at a density of $4 \times 10^5$ per well, and grown in RPMI1640 culture medium. After 24 h of incubation, the cells were subjected to co-culture with differentiated THP-1 cells. Either palmitate or vehicle was added to the upper chamber, and 24 h after the co-culture, the whole cell lysate of THP-1 was collected and subjected to western blotting.

### Preparation of palmitate solution

Palmitate was prepared as previously described (1). Briefly, 20% stock solution of fatty acid-free BSA (#017-15141, Fujifilm Wako pure chemical co. Osaka, Japan) was prepared by dissolving 750 mg of BSA in 3.75 mL of PBS. Then, 5.6 mg of sodium palmitate (#25919-62, Nacalai tesque, Kyoto, Japan) was dissolved in 1 mL ethanol, and palmitate-BSA complex was prepared by mixing 1 mL of palmitate solution to 3.3 mL of BSA 20% stock solution. The complex was added to 15.7mL RPMI culture medium at 37 °C. The sterile filtered solution was used as 1 mM palmitate solution.

## REFERENCE

(1) Nissar, AU., L. Sharma, M.A. Mudasir, L.A. Nazir, S.A. Umar, P.R. Sharma, R.A. Vishwakarma, and S.A. Tasduq. 2017. Chemical chaperone 4-phenyl butyric acid (4-PBA) reduces hepatocellular lipid accumulation and lipotoxicity through induction of autophagy. *J Lipid Res*. 58(9):1855-1868.

**Figure Legend**

Supplement. Fig. 1

The influence of palmitate on the expression of MDA5 in THP-1. PMA-differentiated THP-1 cells were incubated with 200 μM of palmitate or vehicle for 4, 24 or 48 h, and total RNA was extracted from cells. Expression of MDA5 mRNA was analyzed by qPCR (a). Cells were stimulated with various concentration of palmitate for 24 h, and the cell lysates were subjected to western blotting for MDA5 and GAPDH (b). Transwell co-culture system was performed using HuH-7 in the upper chamber and THP-1 in the lower chamber. HuH-7 was stimulated with palmitate (c). HuH-7 cells in the upper chamber were incubated with various concentration of palmitate for 24 h. THP-1 cells were then lysed and subjected to western blotting for MDA5 and GAPDH (d).