SUPPORTING INFORMATION

Effects of Toxicologically Relevant Xenobiotics and the Lipid-Derived Electrophile 4-Hydroxynonenal on Macrophage Cholesterol Efflux: Silencing Carboxylesterase 1 Has Paradoxical Effects on Cholesterol Uptake and Efflux

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SUPPLEMENTARY INFORMATION FIGURE LEGENDS

Figure S1. Cholesteryl ester (CE) mass in THP-1 macrophages incubated in culture medium containing acLDL (50 μg/mL) for 24 h, followed by overnight incubation in equilibration medium (i.e., no cholesterol acceptors present in media). The end of the cholesterol-loading phase is defined as time 0. CE mass was also measured after a 24-h efflux phase in which foam cells were incubated in culture medium containing the universal cholesterol acceptor, 10% FBS. Data represent the mean ± SD of 3 dishes per group; * $p<0.05$, relative to non-acLDL loaded cells, Student's *t*-test; # $p<0.05$, relative to acLDL-loaded cells at time 0, Student's *t*-test.

Figure S2. Cholesterol mass (CE, FC, and TC) in THP-1 macrophage foam cells (loaded with 50 μg/mL acLDL for 24 h, followed by overnight incubation in equilibration medium) after 24-h incubation in FBS-free medium containing either vehicle (-) or ACATi (+). CE, cholesteryl esters; FC, free cholesterol; TC, total cholesterol. Data represent the mean ± SD of 2 dishes per group.

Figure S3. Evidence for the knockdown of CES1 expression in THP-1 cells. (**A**) Immunoblot of THP-1 macrophage lysates, Control (scrambled shRNA) and CES1 KD (CES1 shRNA) cells. rCES1 indicates recombinant human CES1 protein used as a marker. CES1 and β-actin proteins were detected with antibodies specific for each protein. (**B**) Activity-based protein profiling blot of Control and CES1 KD THP-1 monocytes. Note the absence of a CES1 band in the CES1 KD cells around 60 kDa. Cathepsin (Cath) G and PPT1 designations are based on Wang et al. (2013).[1] rCES1 indicates recombinant human CES1 protein. (**C**) Esterase activities (mAbsorbance/min) of Control and CES1 KD THP-1 monocytes following treatment with increasing amounts of paraoxon. Equivalent amounts of cell lysate protein were treated with indicated amount of paraoxon or vehicle (ethanol, 0.1% v/v) for 30 min, followed by

determination of the p-nitrophenyl valerate (pNPV) hydrolysis activity.[2] Data represent the mean ± SD of 3 determinations.

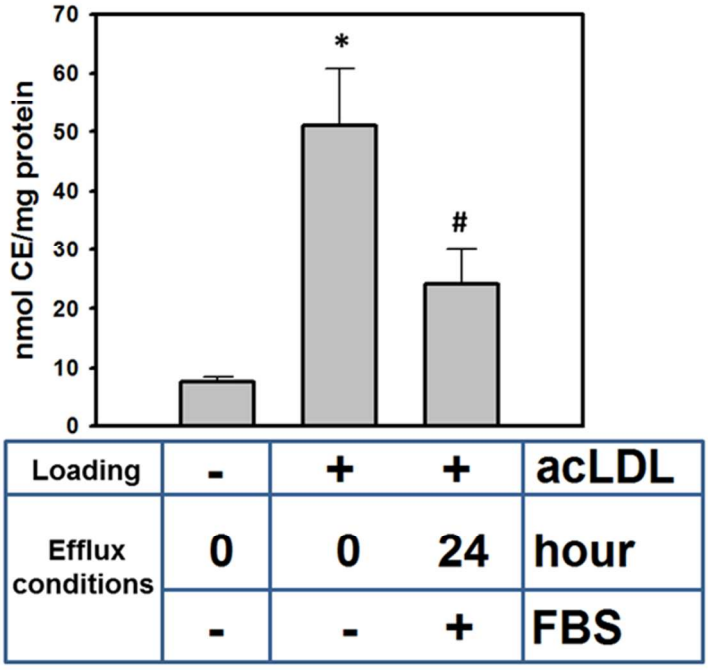
REFERENCES

1) Wang, R., Borazjani, A., Matthews, A. T., Mangum, L. C., Edelmann, M. J., and Ross, M. K. (2013) Identification of palmitoyl protein thioesterase 1 in human THP1 monocytes and macrophages and characterization of unique biochemical activities for this enzyme. *Biochemistry 52*, 7559-7574.

2) Crow, J. A., Middleton, B. L., Borazjani, A., Hatfield, M. J., Potter, P. M., and Ross, M. K. (2008) Inhibition of carboxylesterase 1 is associated with cholesteryl ester retention in human THP-1 monocyte/macrophages. *Biochim. Biophys. Acta 1781*, 643-654.

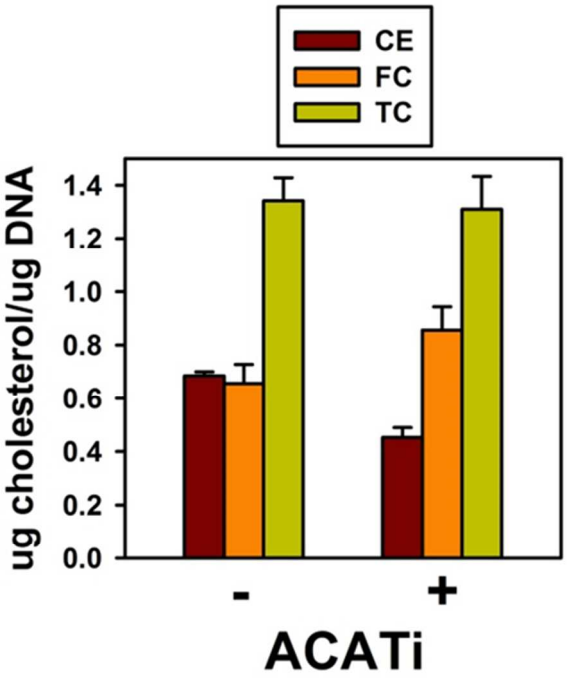
SUPPLEMENTARY INFORMATION FIGURES

Supplementary Figure 1



SUPPLEMENTARY INFORMATION FIGURES

Supplementary Figure 2



SUPPLEMENTARY INFORMATION FIGURES

Supplementary Figure 3