Supplemental Fig. 1. Three commercially available BSAs contained contaminant(s) activating proinflammatory pathways. BSA-1 (Sigma Catalog #: A8806, Lot 118K7405) (**A, B**), BSA-2 (Sigma Catalog #: A8806, Lot 040M7715V) (**C, D**) and BSA-3 (Sigma Catalog #: A8806, Lot 034K7605) (**E, F**) contained contaminant(s) that induce COX-2 expression in RAW 264.7 macrophages. COX-2 expression induced by these BSAs was not affected by the treatment of LPS sequester polymixin B in RAW 264.7 macrophages suggesting that the contaminant-induced COX-2 expression is mediated through TLR4 independent pathways (**A, C, E**). COX-2 expression induced by these BSAs was abolished in MyD88-/- macrophages suggesting that the contaminants in these BSAs can activate MyD88-dependent TLR or IL-1 receptor-mediated signaling pathways (**B, D, F**). RAW 264.7 (**A, C, E**) or MyD88-/- (**B, D, F**) macrophages were serum starved in 0.25% FBS/DMEM medium for 6 hr and then treated in the same serum poor medium with indicated reagents for 18 hr. For polymixin B experiments, RAW 264.7 cells were pretreated with 7.5 ug/ml polymixin B for 1 hr and then coincubated with the indicated reagents for 18 hr. The protein lysates were probed for COX-2 and β-actin by immunobloting. LPS, 0.2 ng/ml for RAW 264.7 macrophages (**A, C, E**) and 5 ng/ml for MyD88-/- macrophages (**B, D, F**). 50 ng/ml PamCSK4 (Pam), 200 μM MDP, 5 μg/ml poly I:C for MyD88-/- macrophages (**B, D, F**).

Supplemental Fig. 2. Responsiveness of cells to C12:0 or LPS varied significantly with the source of FBS used in the culture medium. RAW 264.7 macrophage cells were serum starved in 0.25% FBS/DMEM for 6 hr and then treated with vehicle (Veh), 0.2 ng/ml LPS or 100 μM lauric acid (C12:0, sodium salt) for 16 hr using FBS from five different sources: Atlanta 1 (premium select FBS, Catalog #: S11595, Lot #: K0109) and Atlanta 2 (Optima FBS, Catalog #:

S12495, Lot #: L0019) from Atlanta Biologicals, Hyclone (Catalog #: SH30071, Lot #: ATD31956) from HyClone, Gibco (Catalog #: 10082, Lot #: 544122) from Invitrogen, and Atlas (Catalog #: F-0500-A, Lot #: 80814) from Atlas Biologicals. The protein lysates were probed for COX-2 and β–actin by immunobloting.

Supplemental Fig. 3. C16:0-BSA induced JNK phosphorylation and IL-8 expression in THP-1 cells. The C16:0-BSA-induced IL-8 expression was inhibited by DHA but was not affected by polymixin B treatment. A. THP-1 cells were serum starved in 0.25% FBS/RPMI-1640 medium for 12 hr and then treated with 500 μM C16:0-BSA or equivalent concentration (50 μM) of BSA in the same low serum medium for 0-180 min. The resulting cell lysates were probed for phospho-JNK (JNK-p), JNK, and β-actin by immunoblotting. **B, C.** THP-1 cells were serum starved as in **A** and then pretreated with or without 1 ug/ml polymixin B (PM) for 1 hr followed by treatment with 100 ng/ml LPS, 100-500 μM C16:0-BSA or 50 μM BSA in the same low serum medium for 24 hr (**B**) or pretreated with or without indicated concentrations of DHA for 1 hr and then coincubated with 500 μM C16:0-BSA for 24 hr (**C**). The resulting culture medium supernatants were assayed for IL-8 by ELISA. *$p<0.05$ and ** $p<0.01$: significantly different from 50 μM BSA control (**B**), or from no DHA control (**C**).

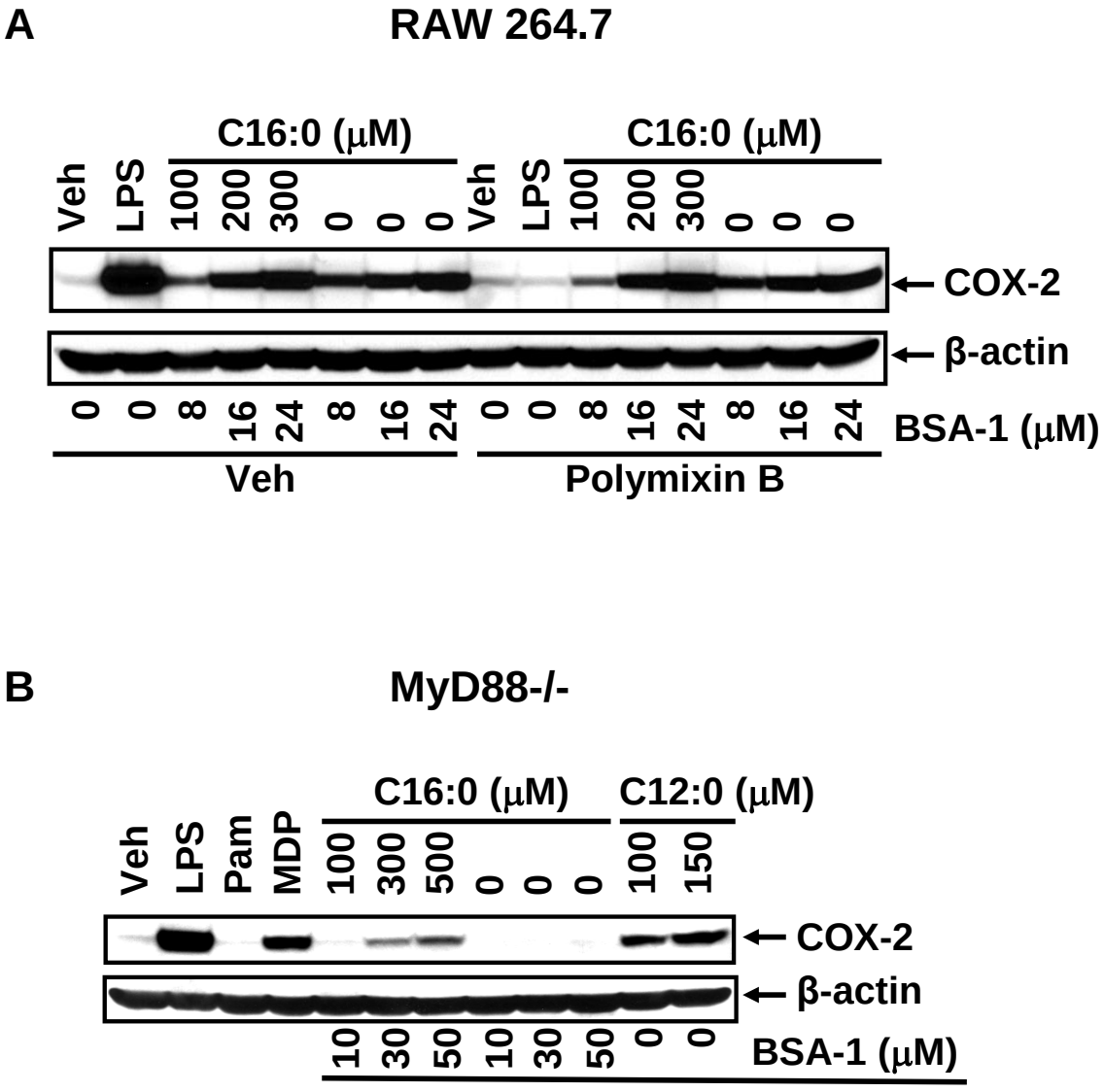
Supplemental Fig. 4. LPS endotoxin titration. The endotoxin levels of LPS (10-200 pg/ml) was measured by LAL assays using the recombinant factor C endotoxin detection system as described in the Methods. The endotoxin level (EU/ml) is plotted against the LPS concentrations (pg/ml). The endotoxin levels of sodium salt C16:0 (75-1200 μM) and free fatty acid C16:0 (250-1000 μM) are included. The correlation coefficient is indicated for the LPS titration curve.

Supplemental Fig. 5. LPS at 20 pg/ml or lower activated much lower levels of COX-2 and TNF-α expression in RAW 264.7 cells (A, B) or IL-8 expression in THP-1 cells (C) compared to sodium salt C12:0 and C16:0-BSA (A, B) or sodium salt C16:0 (C). A. RAW 264.7 cells were serum starved in 0.25% FBS/DMEM for 6 hr then treated with 1-200 pg/ml LPS, 150 μM C12:0, 500 μM C16:0-BSA or 50 μM BSA for 16 hr. The resulting cell lysates were probed for COX-2 and β-actin by immunoblotting. **B.** RAW 264.7 cells were serum starved for 6 hr then treated with 1-200 pg/ml LPS, 150 μM C12:0, 500 μM C16:0-BSA or 50 μM BSA for 16 hr. The expression levels of TNF-α were measured from the resulting medium supernatants by ELISA. **C.** THP-1 cells were serum starved in 0.25% FBS/RPMI for 12 hr then treated with vehicle, 150 μM sodium salt C16:0, or 0.01-10 ng/ml LPS for 24 hr. The resulting medium supernatants were assayed for IL-8 levels by ELISA. *$p<0.05$ and ** $p<0.01$: significantly different between vehicle control and LPS, C12:0 or C16:0-BSA (**B**), or between vehicle control and LPS or sodium salt C16:0 (**C**).

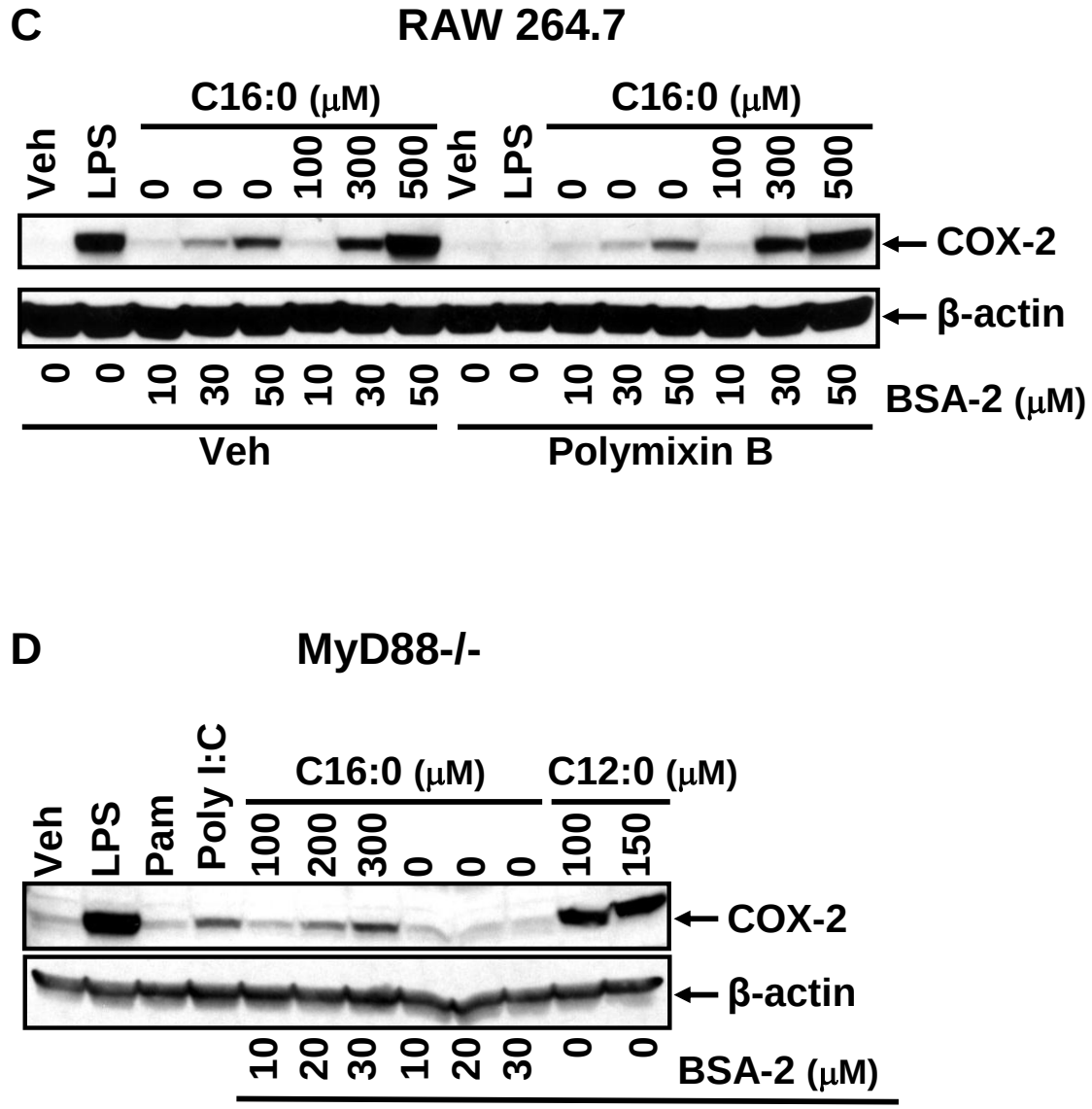
Supplemental Fig. 6. DHA suppressed LPS-induced COX-2 and TNF-α expression. RAW 264.7 cells were serum starved in 0.25% FBS/DMEM for 6 hr. The cells were then pretreated with indicated concentrations of DHA for 1 hr followed by coincubation with 0.2 ng/ml LPS for 16 hr. The protein lysates were probed for COX-2 and β-acin by immunobloting (**A**) and the culture medium supernatants assayed by ELISA (**B**). *$p<0.05$ and ** $p<0.01$: significantly different from no DHA control.

Supplemental Fig. 7. TLR4 signaling inhibitor TAK-242 suppressed LPS- and C12:0-induced activation of COX-2 and TNF-α expression. RAW 264.7 cells were serum starved in 0.25% FBS/DMEM for 6 hr. The cells were then pretreated with indicated concentrations of TAK-242 for 1 hr followed by coincubation with 0.2 ng/ml LPS, 2 ng/ml PamCSK4, or 100 μM C12:0 for 16 hr. The protein lysates were probed for COX-2 and β-acin by immunobloting (**A**) and the culture medium supernatants assayed for TNF-α by ELISA (**B**). *$p<0.05$ and ** $p<0.01$: significantly different from no TAK-242 control.

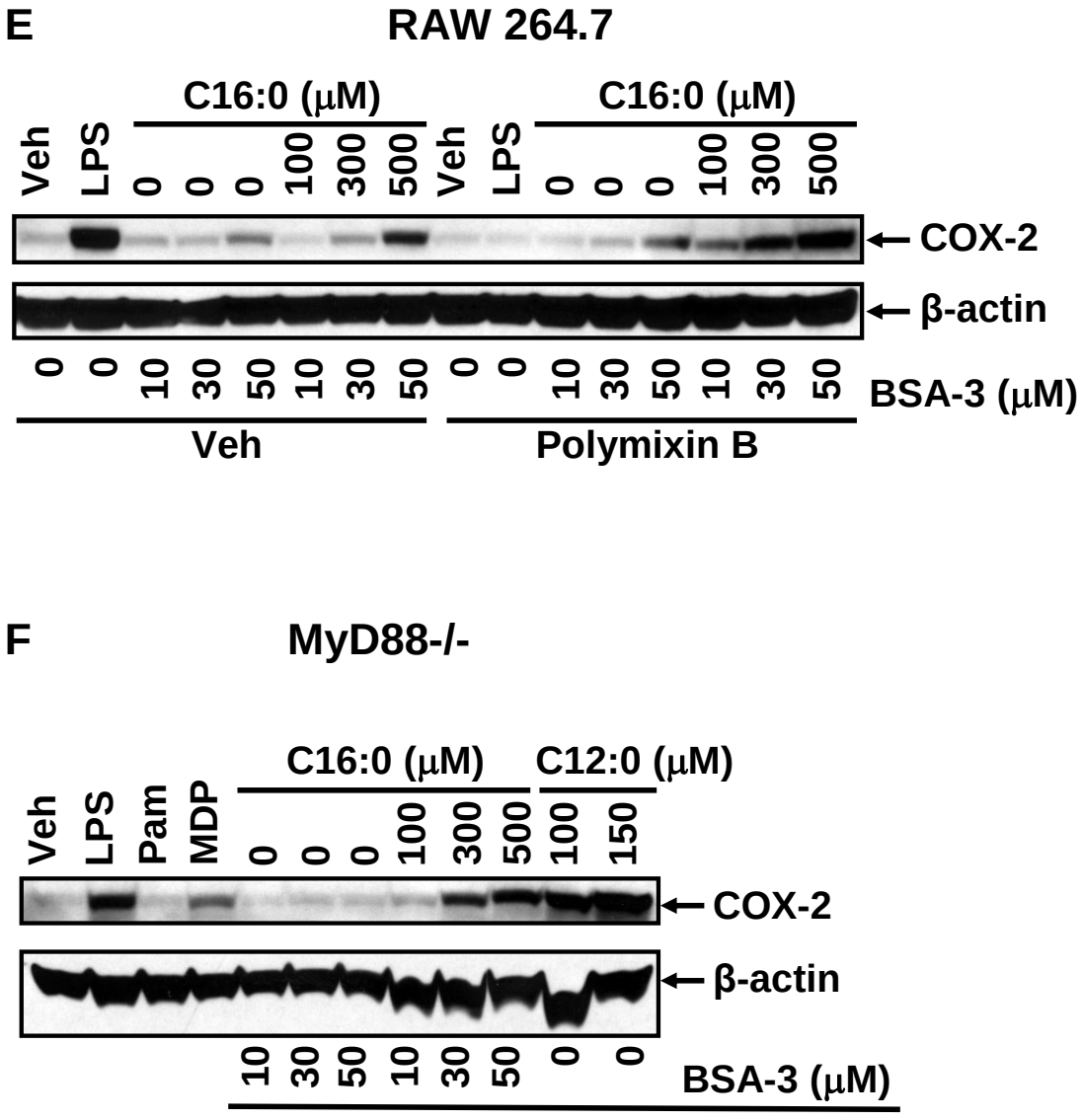
Supplemental Fig. 1A, B



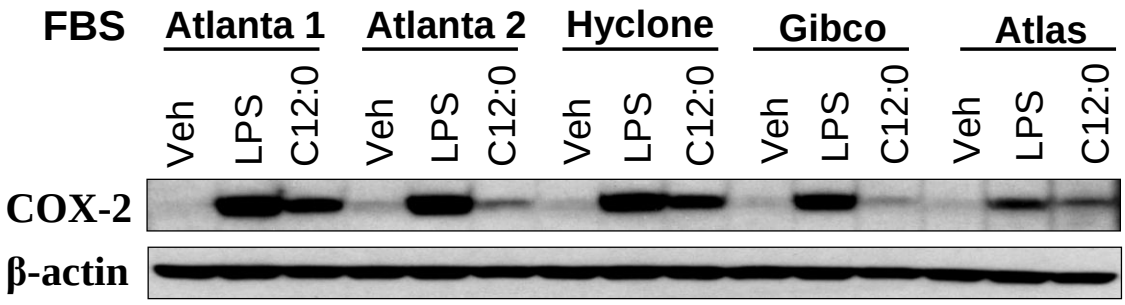
Supplemental Fig. 1C, D



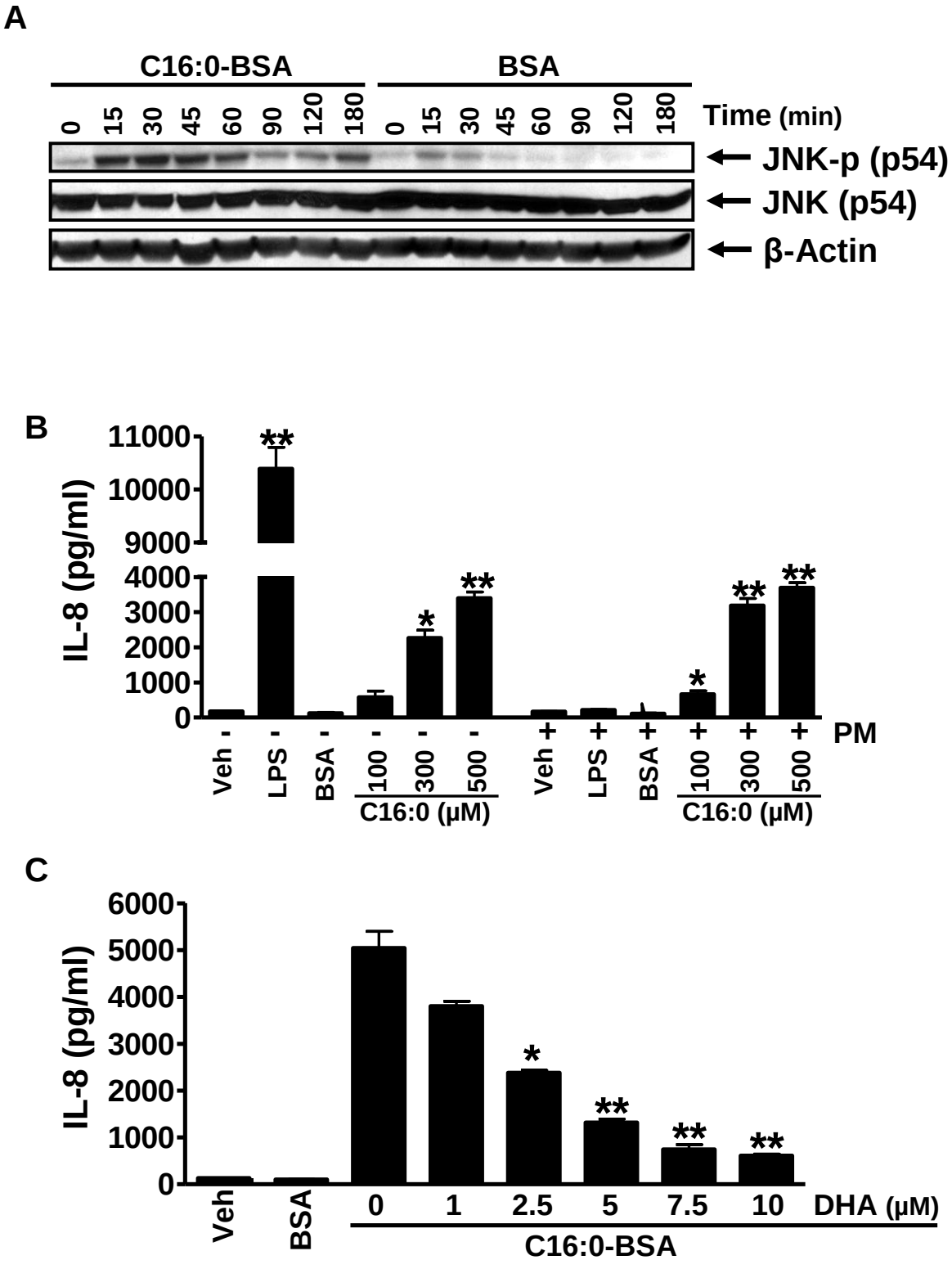
Supplemental Fig. 1E, F



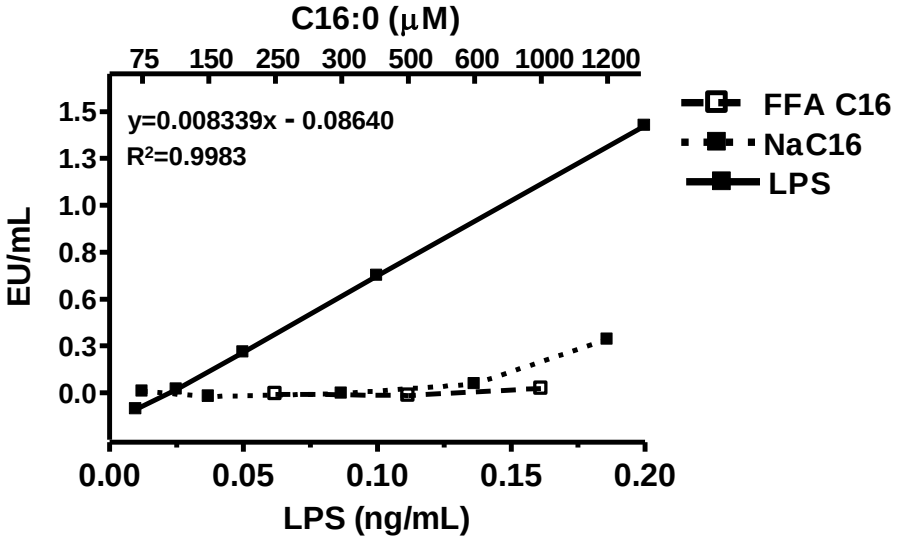
Supplemental Fig. 2



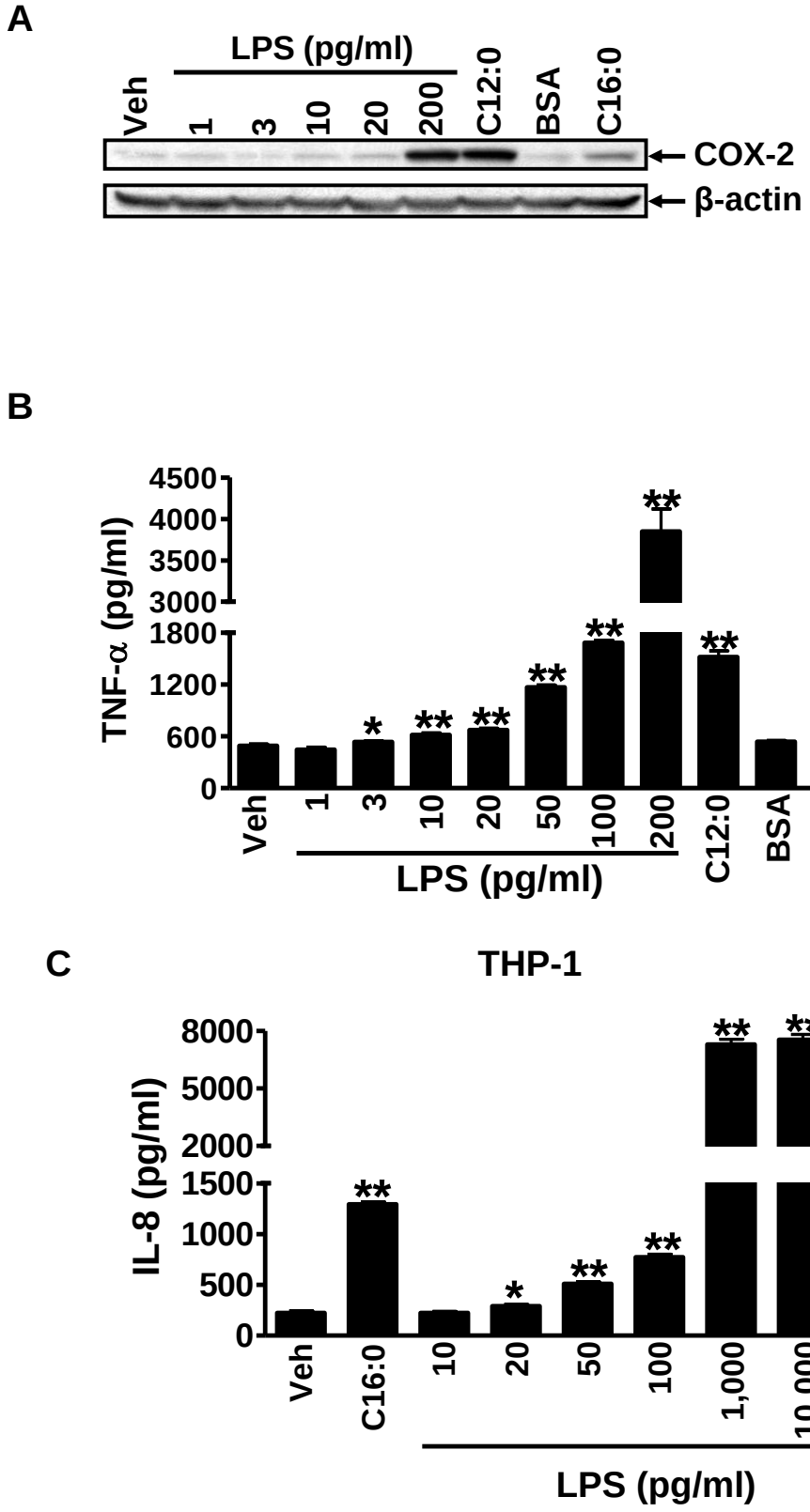
Supplemental Fig. 3A-C



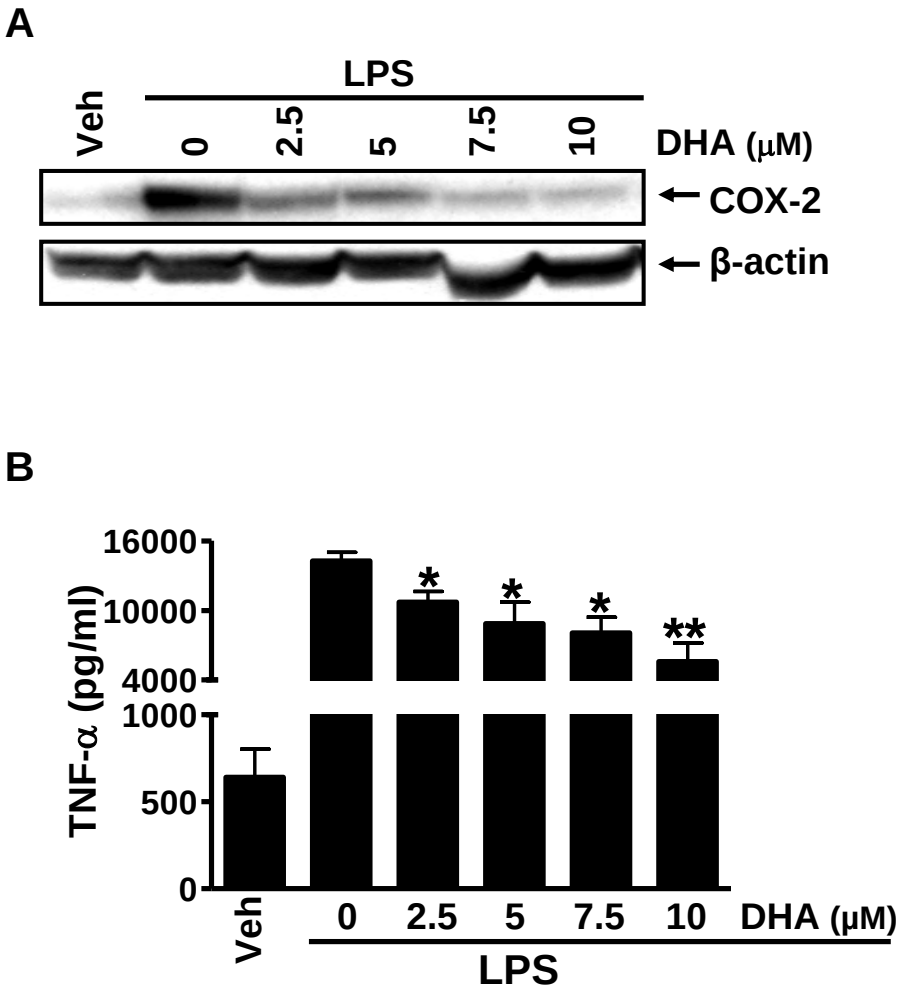
Supplemental Fig. 4



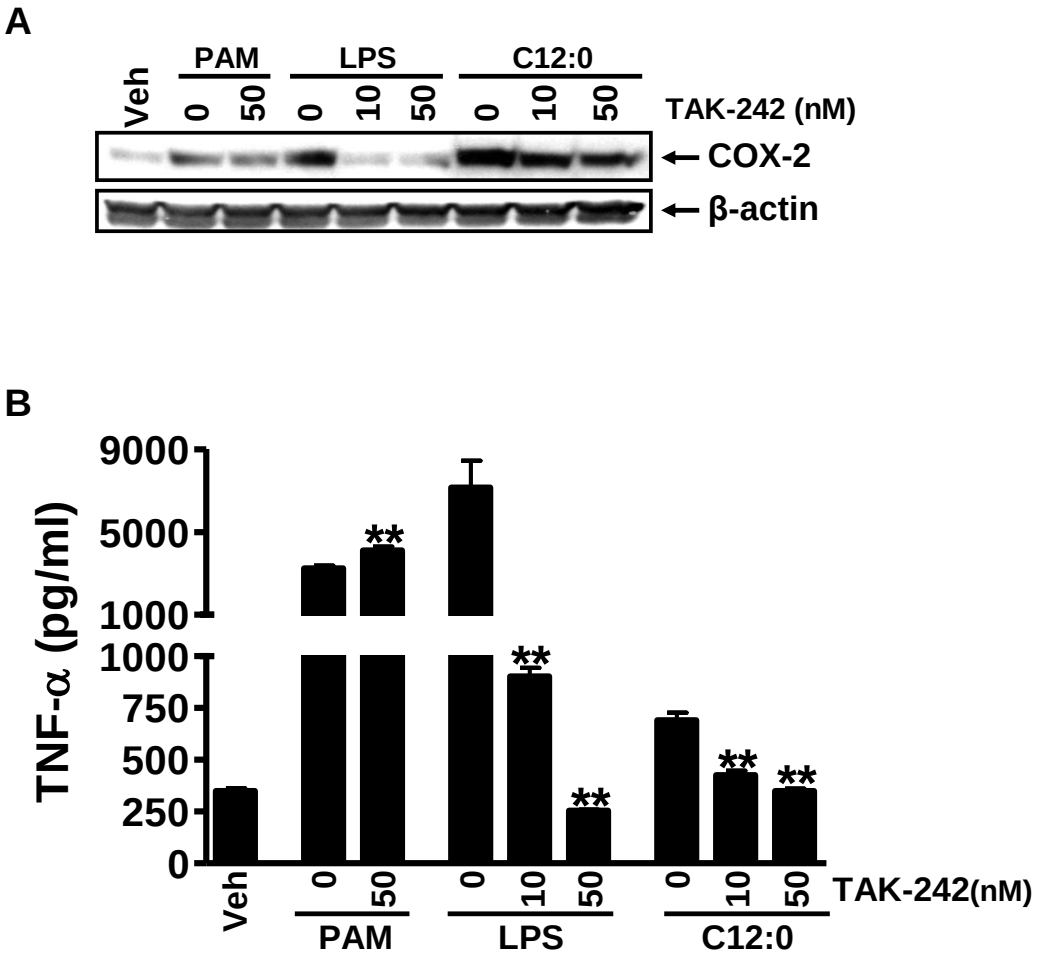
Supplemental Fig. 5A-C



Supplemental Fig. 6A, B



Supplemental Fig. 7A, B



Suppplemental Table 1. LAL assays. The assays for FBS were done using the ToxinSensor chromogenic LAL endotoxin assay kit (Cat #: L00350, GenScript, Piscataway, NJ) following the supplier's procedures. The assays for all other reagents were done using the recombinant factor C endotoxin detection system (Cat #: 50-658U, Lonza, Walkersville, MD) following the supplier's procedures.

Reagent	**Endotoxin (EU/ml)**	**Additional Information**
BSA		
Sigma BSA 1 50 µM	2.196	Sigma Cat #: A8806 Lot 118K7405
Sigma BSA 2 50 µM	1.124	Sigma Cat #: A8806 Lot 040M7715V
Sigma BSA 3 50 µM	1.057	Sigma Cat #: A8806 Lot 034K7605
Fitzgerald BSA 50 µM	UD	Fitzgerald Cat #: 30-AB79 Lot A10072001
Fatty Acid		
Na-C12:0 300 µM	UD	Nu-Chek Cat #: S-1105 Lot S24-N
Na-C16:0 150 µM	0.064	Sigma Cat #: P9767 Lot 079K1444
FFA C16:0 500 µM	0.046	Sigma Cat #: P5585 Lot 020M15811
DHA 20 µM	UD	Sigma Cat #: D8768 Lot 077K5215
Plamid DNA		
pCDNA	UD	1 µg/ml
pDisplay, NFκB-Luc, β-gal	UD	1 ug/ml (0.5 µg/ml pDisplay, 0.25 ug/ml NFκB-Luc, 0.25 ug/ml β-gal)
TLR2, TLR1, NFκB-Luc, β-gal	UD	1 µg/ml (0.25 µg/ml each)
TLR2, TLR6, NFκB-Luc, β-gal	UD	1 µg/ml (0.25 µg/ml each)

TLR4, MD2, NFκB-Luc, β-gal	UD	1 μg/ml (0.25 μg/ml each)
FBS		
FBS (Atlanta Premium Select)	UD	Cat #: S11550 Lot K0109
FBS (Atlanta Optima)	UD	Cat #: S12495 Lot L0019
FBS (Hyclone)	UD	Cat #: SH30071 Lot ATD31956
FBS (Atlas)	UD	Cat #: F-0500-A Lot 80814
FBS (Gibco)	UD	Cat #: 10082 Lot 544122
Medium		
DMEM medium	UD	Gibco Cat #: 31053 Lot 979502
RPMI Medium	UD	Gibco Cat #: 11835 Lot 1007553

FFA, free fatty acid; NFκB-Luc, NFκB-Luciferase; β-gal, β-galactosidase; UD, undetectable.