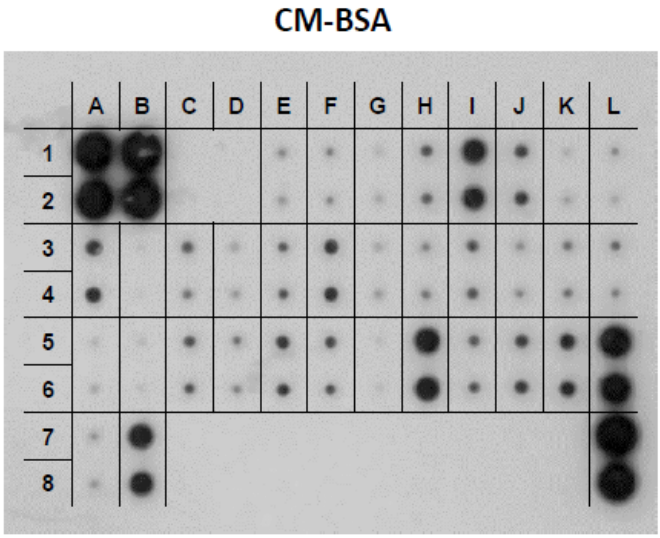
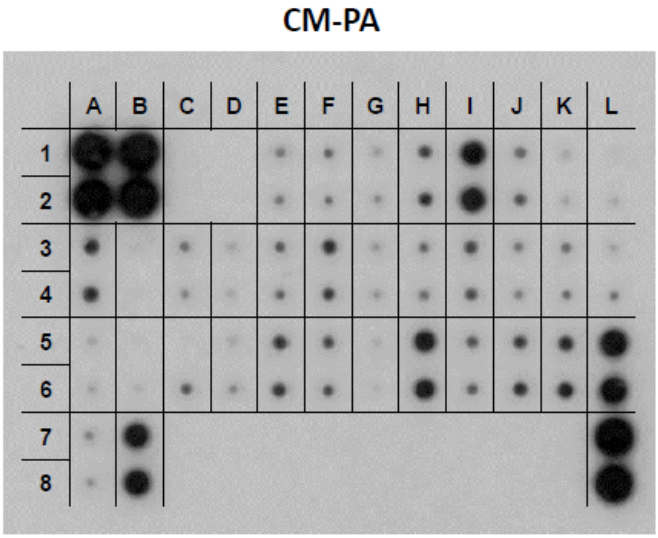
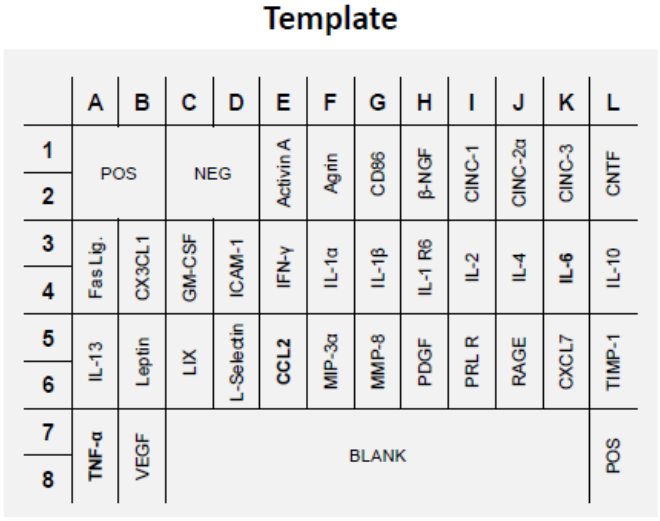
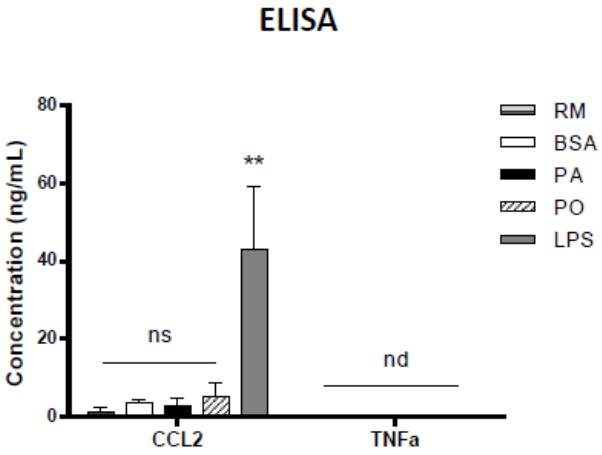
SUPPLEMENTARY DATA

Supplementary Figure 1. Palmitate does not affect the release of cytokines and chemokines from L6 myotubes. L6 myotubes were treated with 0.5mM PA or PO for 18h. Cytokine and chemokine content in myotube conditioned-media was analyzed using a cytokine array (Rat Cytokine Array C2, RayBiotech) and concentration of TNFα and MCP1/CCL2 in supernatant was confirmed using ELISA immunoassays (Rat quantikine, R&D systems), following manufacturer's instructions



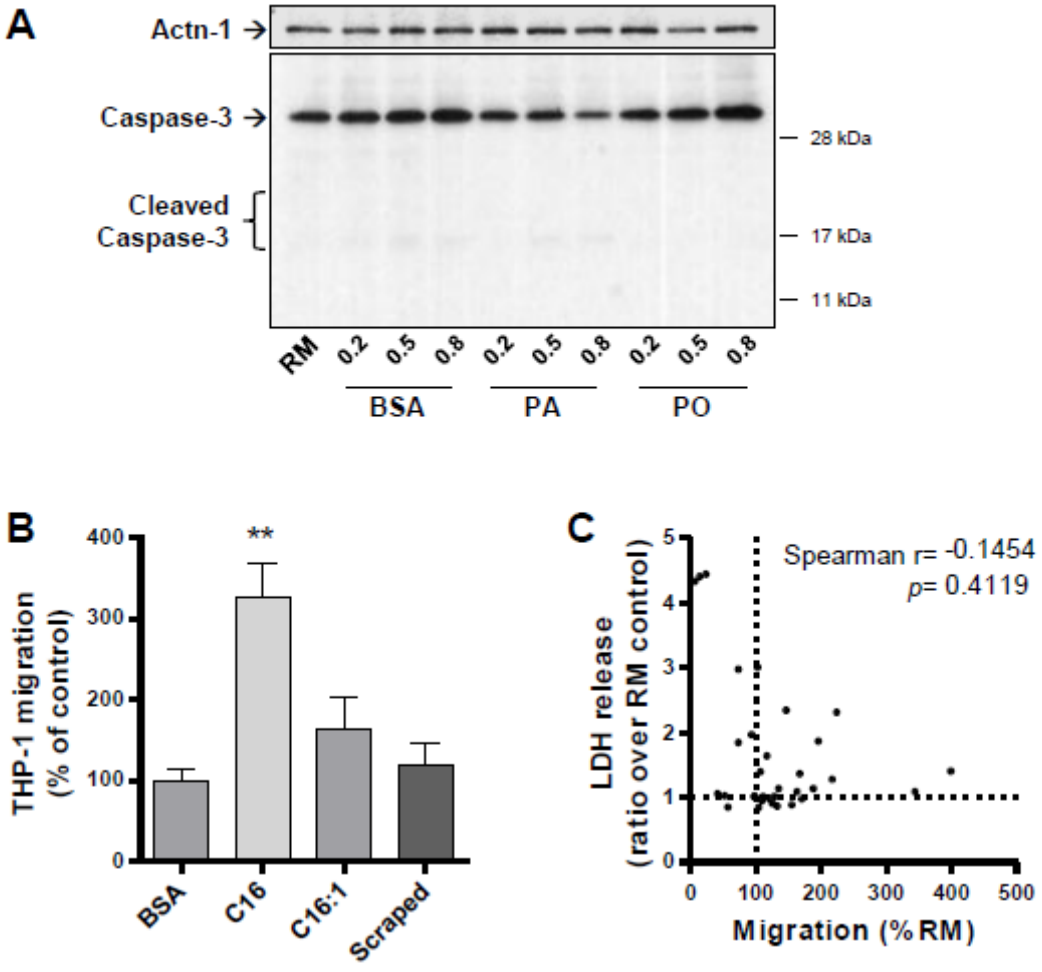






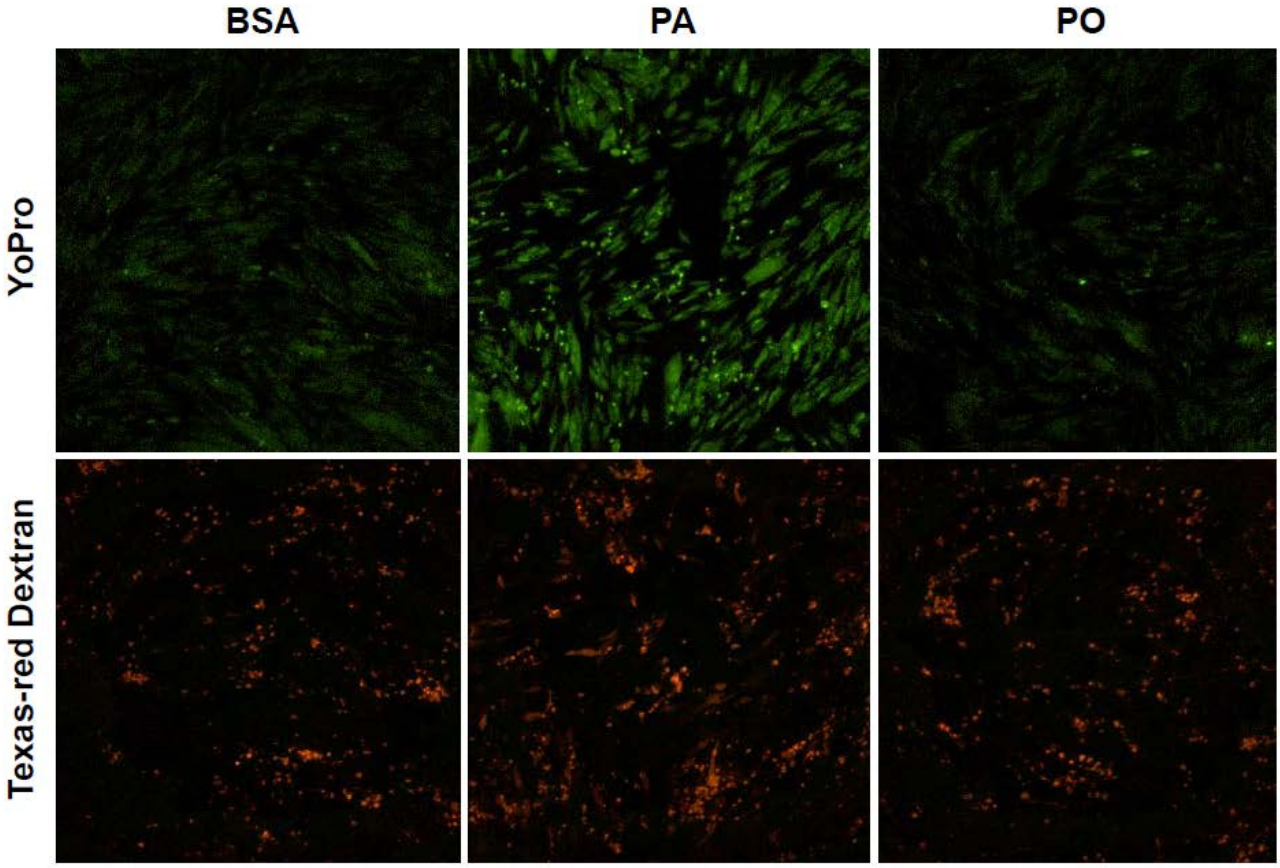
SUPPLEMENTARY DATA

Supplementary Figure 2. Necrosis and apoptosis do not correlate with monocyte migration. A) L6 myotubes were treated with PA, PO or BSA control for 18h. Caspase-3 was then measured in cell lysate using specific antibody recognizing both the proand clived forms of caspase-3. A representative blot is show. B) L6 myotubes were mechanically damaged by scraping and vortexing to induce necrosis. Conditioned media was then collected, centrifuged to pellet debris and tested for monocyte attraction as described in methods. Mean ± SEM, n=4. C) LDH release was plotted against CM-induced monocyte migration. Results are the mean from 18 independent experiments.



SUPPLEMENTARY DATA

Supplementary Figure 3. YoPro uptake into myotubes in response to fatty acids. L6 myotubes differentiated in 6-well plates were treated with 0.5 mM BSA, PA or PO for 18 h as described in Methods. YoPro dye (1 μM) and Texas-red dextran (10 kDa, 0.1 mg/mL) were added for 15 min, then cells were washed 4x in ice-cold PBS, fixed with 3% PFA for 10 min and washed again with PBS. Images were taken with a Leica DMIRE2 fluorescence microscope using the 10X air objective. Total green and red fluorescence were measured using ImageJ software in 15 random fields per condition.



SUPPLEMENTARY DATA

Supplementary Table 1. MRM transitions and ESI polarity mode used for detection of AMP, ADP, UDP, GMP, CMP, UMP, IMP and adenosine.

Component	**Precursor ion (m/z)**	**Product ion (*m/z*)**	**Polarity mode**
AMP	348	136	positive
ADP	426	159	negative
UDP	403	159	negative
CMP	324	112	positive
GMP	364	152	positive
UMP	325	97	positive
IMP	349	137	positive
Adenosine	268	136	positive

Supplementary Table 2. Measured NEFA concentration.

	Initial		**Supernatant**		**Change**		**Filtrate**	
RM	*25*	± 25	29	± 34	*+16*	*%*	<	*10*
0.2 mM								
BSA	15	± 14	24	± 26	*+57*	*%*	<	*10*
PA	197	± 58	90	± 21	*-54*	*%*	<	*10*
PO	252	± 80	112	± 24	*-56*	*%*	<	*10*
0.5mM								
BSA	18	± 17	28	± 26	*+56*	*%*	<	*10*
PA	478	± 73	242	± 36	*-49*	*%*	<	*10*
PO	442	± 119	299	± 44	*-32*	*%*	<	*10*
0.8 mM								
BSA	34	± 35	49	± 34	*+45*	*%*	<	*10*
PA	723	± 136	438	± 58	*-40*	*%*	<	*10*
PO	737	± 222	467	± 72	*-37*	*%*	<	*10*

NEFA concentrations in the initial media, myotube conditioned-media and filtrates (<3000 Da) were measured using the enzymatic method based on Acyl-CoA oxidase described in *Methods*. Results are means ± SD expressed in micromoles/litre from at least 4 independent experiments (n≥4).

SUPPLEMENTARY DATA

Supplementary Table 3. Measured glucose concentration.

	Initial		**Supernatant**		**Change**		**Filtrate**	
RM	6.7	± 0.4	3.9	± 0.6	*-46*	*%*	3.5	± 0.2
BSA	6.6	± 0.2	4.2	± 0.2	*-39*	*%*	4.0	± 0.2
PA	7.0	± 0.1	3.5	± 0.4	*-48*	*%*	3.8	± 0.2
PO	6.4	± 0.6	3.5	± 0.7	*-43*	*%*	4.0	± 0.1

Glucose concentration in the initial media, conditioned-media and filtrates from L6 myotubes treated for 18h with 0.5 mM palmitate, palmitoleate or BSA control. Concentrations were measured using a glucometer. Results are means ± SD, expressed in mM from at least 4 independent experiments (n≥4).

Supplementary Table 4. Viability of L6 myotubes.

LDH release	**0.2 mM**		**0.5 mM**		**0.8 mM**	
BSA	0.97	± 0.08	0.75	± 0.05	0.90	± 0.07
PA	0.95	± 0.03	0.99	± 0.08	1.22	± 0.04*
PO	0.85	± 0.10	0.79	± 0.04	1.05	± 0.10
MTT reduction	**0.2 mM**		**0.5 mM**		**0.8 mM**	
BSA	0.79	± 0.02	0.75	± 0.05	0.73	± 0.05
PA	0.73	± 0.09	0.54	± 0.04	0.45	± 0.07*
PO	0.79	± 0.07	0.70	± 0.05	0.64	± 0.04

The potential cytotoxicity caused by fatty acid treatment was estimated through LDH release and MTT reduction after exposing L6 myotubes to BSA, PA and PO for 18h. Results are normalized to the regular media control and expressed as mean ± SD, n ≥4. * $p < 0.05$ vs BSA control.