Major role of adipocyte prostaglandin E_2 in lipolysis-induced macrophage recruitment

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Supplement

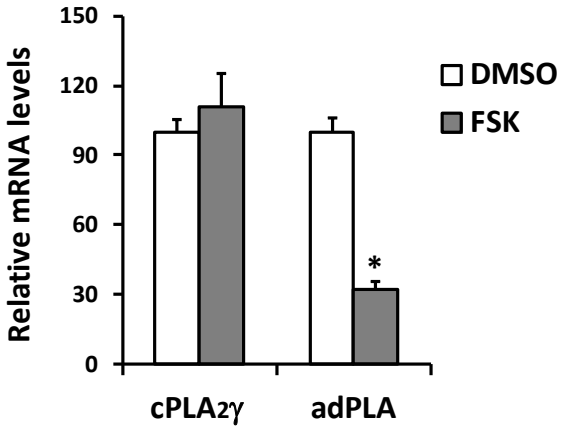
Supplementary Figure S1. Regulation of adipocyte cPLA$_2\gamma$ and adPLA mRNA levels by forskolin.

Supplementary Figure S2. Isoproterenol enhances phosphorylation of cPLA$_2\alpha$ and ERK in adipocytes.

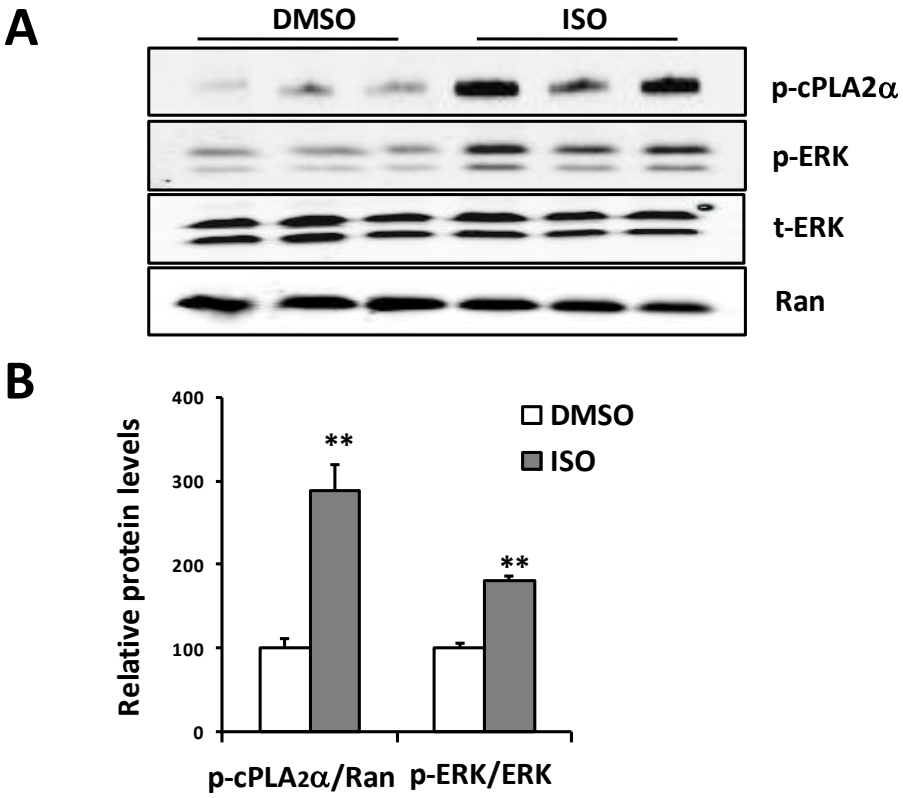
Supplementary Table S1. Primers used for real-time qPCR analysis.

Supplementary Table S2. FFA concentrations in adipocyte conditioned media.

Supplementary Figure S1. Regulation of cPLA$_2\gamma$ and adPLA mRNA levels in adipocytes by forskolin. mRNA samples of vehicle (DMSO) or forskolin (FSK) treated adipocytes were prepared and analyzed by RT-PCR. Data shown are averages of triplicates from two separate experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.



Supplementary Figure S2. Isoproterenol enhances phosphorylation of cPLA$_2\alpha$ and ERK in adipocytes. Whole-cell lysates of adipocytes pretreated with vehicle (DMSO) or isoproterenol (ISO) for 30 min were prepared and analyzed by Western blot for phospho-cPLA$_2\alpha$ (S505) (p-cPLA$_2\alpha$), phospho-ERK1/2 (T202, Y204) (p-ERK), total ERK1/2 (t-ERK) and Ran. Protein levels were quantified using Li-Cor technology. Data are from two experiments conducted in triplicates. p-cPLA$_2\alpha$ adjusted to the control Ran and p-ERK to t-ERK are presented relative to vehicle treated cells and are plotted as means ± SE. $*P < 0.05$, $**P < 0.01$.



Supplementary Table S1. Primers used for real-time qPCR analysis.

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
cPLA$_2\alpha$	CCT TTG AGT TCA TTT TGG ATC CTA A	TGT AGC TGT GCC TAG GGT TTC AT
cPLA$_2\gamma$	GCT GAA AGA ACT TGG CCT GTT G	CAG CTC CTC TTC TAT CCC TTC CA
adPLA	ACT CTG CGG CCA TTA AAC CA	AGG CTT GGG TTC TGG TAT GG
COX1	AGG TTG GTT CCT CGA ATG TG	CCT TCT CCT TCT CCT TCA GC
COX2	CTC ACG AAG GAA CTC AGC ACT	TAG AAT CCA GTC CGG GTA CAG T
F4/80	CCT GAT GGT GAG AAA CCT GA	CCC CAG GAA ACT CCA GAT AA
CD11b	CAT GGC TTC AAT CTG GAC AC	TGC TGT AGT CAC ACT GGT AGA GG
36B4	GCA GAC AAC GTG GGC TCC AAG CAG AT	GGT CCT CCT TGG TGA ACA CGA AGC CC

Supplementary Table S2. FFA concentrations in adipocyte conditioned media. Data are means of triplicates from three separate experiments and presented as μM.

FFA	C14:0	C15:0	C16:0	C16:1	C17:1	C17:0	C18:1	C18:2	C20:4
DMSO-CM	2.0	-	4.8	4.3	-	-	1.6	3.6	1.4
FSK-CM	2.6	2.0	31.1	18.8	4.3	1.6	20.3	7.1	3.5