

Legends to Supplemental Figures

Figure S1. COX-2 in stearic acid (SA)-activated macrophages plays a role in stimulating PKA-mediated induction of aromatase in preadipocytes. THP-1 cells were treated with vehicle or 10 μ M SA as detailed in the Methods Section to generate conditioned medium (CM). CM derived from vehicle treated THP-1 cells is referred to as CM; CM derived from SA-treated THP-1 cells is referred to as SA CM. A, preadipocytes were treated with CM, SA CM or SA CM plus 10 μ M H89 for 24 hours. B, preadipocytes or preadipocytes that expressed wild type PKA or dominant negative PKA (PKA DN) were treated for 24 hours with CM or SA CM from THP-1 cells. Aromatase activity was determined in preadipocytes and expressed as femtomoles/ μ g protein/minute. C, THP-1 cells were untreated or treated with control siRNA or siRNA to COX-2. Conditioned medium was prepared and preadipocytes were then treated with CM or SA CM as indicated for 24 hours prior to measurements of levels of PR mRNA. Columns, means (n=6); bars, SD. *, P<0.05.

Figure S2. PGE₂ from stearic acid (SA)-activated blood monocyte-derived macrophages stimulates the cAMP→PKA pathway leading to increased *aromatase* transcription in preadipocytes. A, blood monocyte-derived macrophages were untreated or treated with control siRNA or siRNA to COX-2. Subsequently, the macrophages were treated with vehicle (Control) or 10 μ M SA for 24 hours. The abundance of COX-2 protein in cell lysates (inset) was determined by immunoblotting. β -actin was used as a loading control. Levels of PGE₂ in the medium were determined by enzyme immunoassay. B-H, preadipocytes were treated with macrophage-derived CM for 24 hours prior to measurements of cAMP (B), PKA activity (C), relative aromatase expression (D), aromatase activity expressed as femtomoles/ μ g protein/minute (E), aromatase mRNA derived from promoters I.3 (F) and II (G) and PR protein levels (H). In I and J, the effect of inhibiting PKA activity with 10 μ M H89 for 24 hours or

overexpressing a dominant negative form of PKA (PKA DN) on SA CM induced aromatase activity in preadipocytes was investigated. Columns, means ($n=6$); bars, SD. *, $P < 0.05$.