excess of cold probe (lane 7). C, binding of nuclear protein isolated from the adipocyte or stromal-vascular (SVF) fractions of the mammary gland of 5 mice in the High Fat + OVX group. D, binding of nuclear protein from SVF isolated from a mammary gland in the High Fat + OVX group. Lane 1, binding of nuclear protein to labeled oligonucleotide; lane 2, nuclear protein was incubated with labeled oligonucleotide and a 50X excess of cold probe; lanes 3 and 4, nuclear protein incubated with normal IgG (lane 3) or 2μL of phospho-p65 antibody (lane 4). In A-D, the protein-DNA complexes that formed were separated on a 4% polyacrylamide gel.

Fig. 11. Paracrine interactions between adipocytes and macrophages can explain the elevated levels of aromatase in the mammary gland and visceral fat of obese mice. In obesity, lipolysis is increased resulting in increased concentrations of free fatty acids. Saturated fatty acids trigger the activation of NF-κB in macrophages resulting in enhanced production of pro-inflammatory mediators (PGE_2, TNF-α, IL-1β). Each of these pro-inflammatory mediators contributes to the induction of aromatase in preadipocytes and adipocytes.

Legends to Supplemental Figures

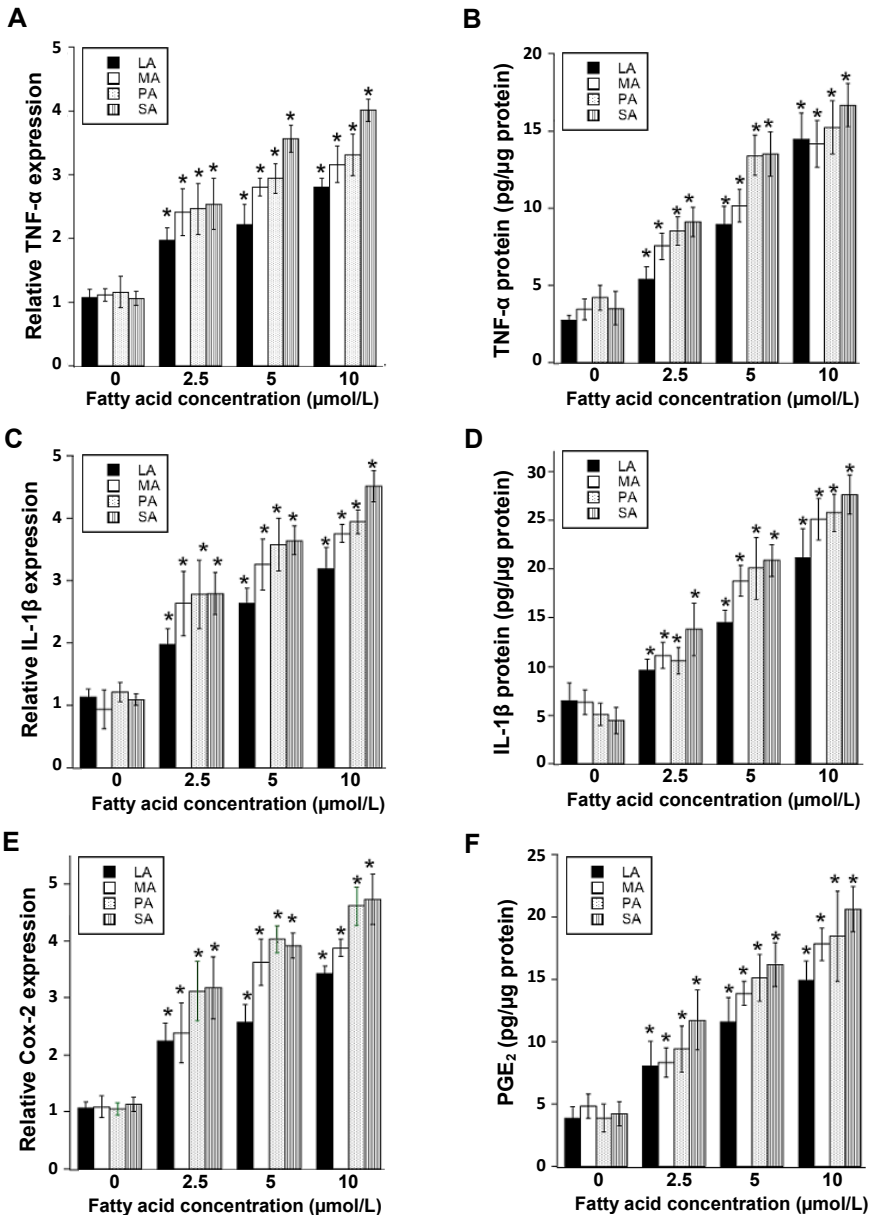
Fig. S1. Treatment of human blood monocyte-derived macrophages with saturated fatty acids causes dose-dependent induction of pro-inflammatory mediators. Human blood monocyte-derived macrophages were treated with the indicated concentration of saturated fatty acid (LA, lauric acid; MA, myristic acid; PA, palmitic acid; SA, stearic acid) for 24 hours. Real-time PCR was then used to quantify TNF-α, IL-1β and Cox-2 mRNAs (A, C, E). Levels of TNF-α protein, IL-1β protein and PGE_2 in the conditioned medium were determined by enzyme immunoassay (B, D, F). Columns, means (n=6); bars, SD. *$P < 0.05$.

Fig. S2. Silencing of pro-inflammatory mediators in stearic acid-treated macrophages attenuates the ability of conditioned medium to induce aromatase. In A, D, G and H, THP-1 cells

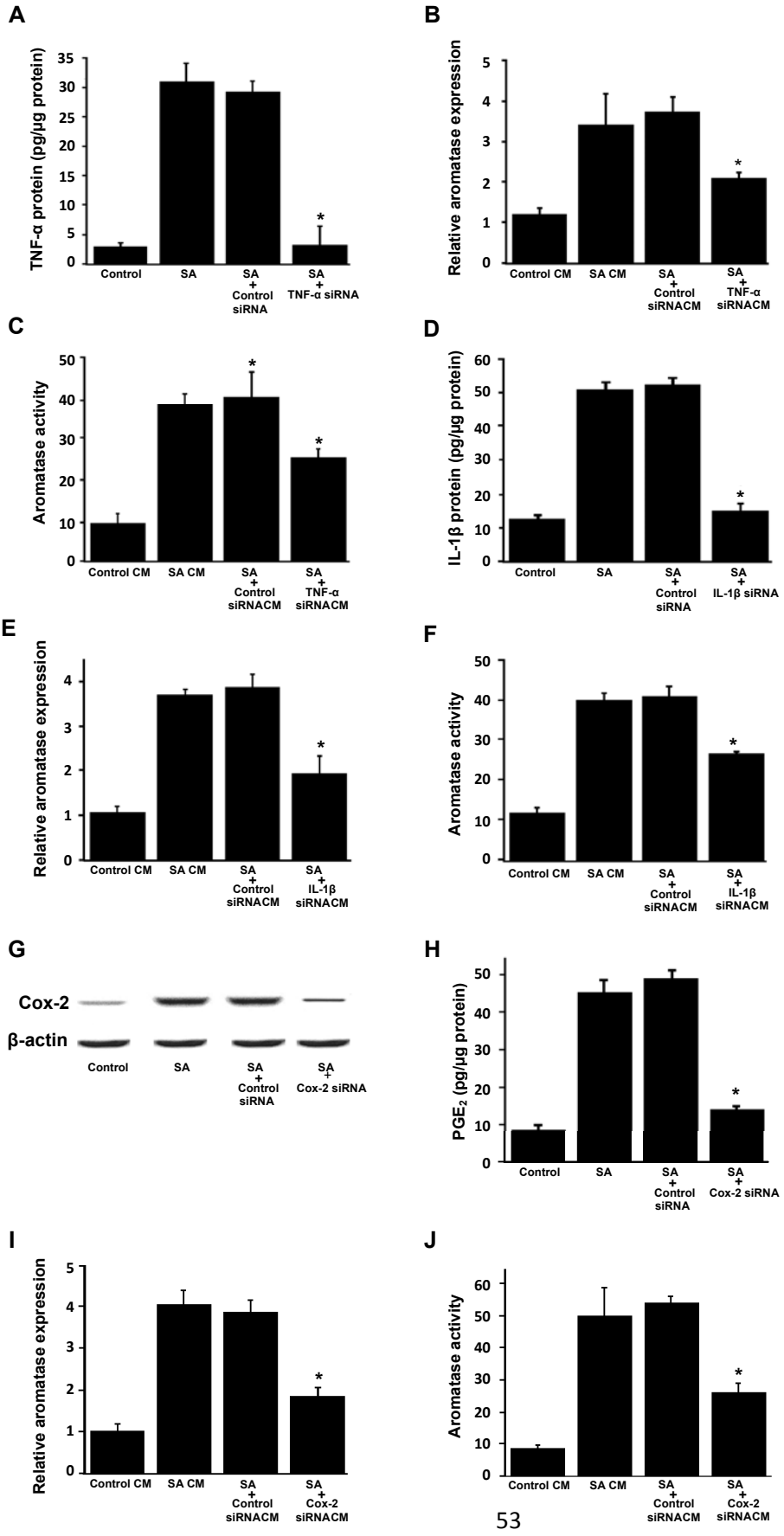
were transfected as indicated with control siRNA or TNF-α siRNA (A), IL-1β siRNA (D) or Cox-2 siRNA (G and H) for 36 hours. As indicated, cells were then treated with vehicle (Control) or 10 μmol/L stearic acid (SA) as described in the Materials and Methods. In A, D and H, levels of TNFα, IL-1β and PGE_2 were measured by enzyme immunoassay in the conditioned medium (CM). In G, Cox-2 protein abundance was determined by immunoblotting of whole cell lysates. β-actin was used as a loading control. In B, C, E, F, I and J, preadipocytes were treated with THP-1 cell-derived CM for 24 hours prior to measurements of aromatase mRNA and activity. Aromatase mRNA levels (B, E, I) and activity (C, F, J) were determined in preadipocytes. Activity is expressed as femtomoles/μg protein/minute. Columns, means (n=6); bars, SD. *P < 0.05.

Fig. S3. Saturated fatty acid-mediated activation of NF-κB in macrophages is important for induction of aromatase in preadipocytes. In A, THP-1 cells were transiently transfected with NF-κB-luciferase and pSV-βgal constructs. Subsequently, cells were treated with vehicle, 10 μmol/L stearic acid (SA) or SA plus the indicated concentrations of BAY11-7082, an inhibitor of NF-κB, for 12 hours. NF-κB luciferase activity represents data that have been normalized to β-galactosidase activity. B and C, THP-1 cells were treated with vehicle (control), 10 μmol/L SA or SA plus BAY11-7082 for 24 hours. Levels of TNF-α protein (B), IL-1β protein (B) and PGE_2 (C) in the conditioned medium (CM) were determined by enzyme immunoassay. In D and E, preadipocytes were treated with THP-1 cell-derived CM for 24 hours prior to measurements of aromatase mRNA and activity. Aromatase mRNA (D) and activity levels (E) were determined in preadipocytes. Activity is expressed as femtomoles/μg protein/minute. A-E, columns, means (n=6); bars, SD. *P < 0.05.

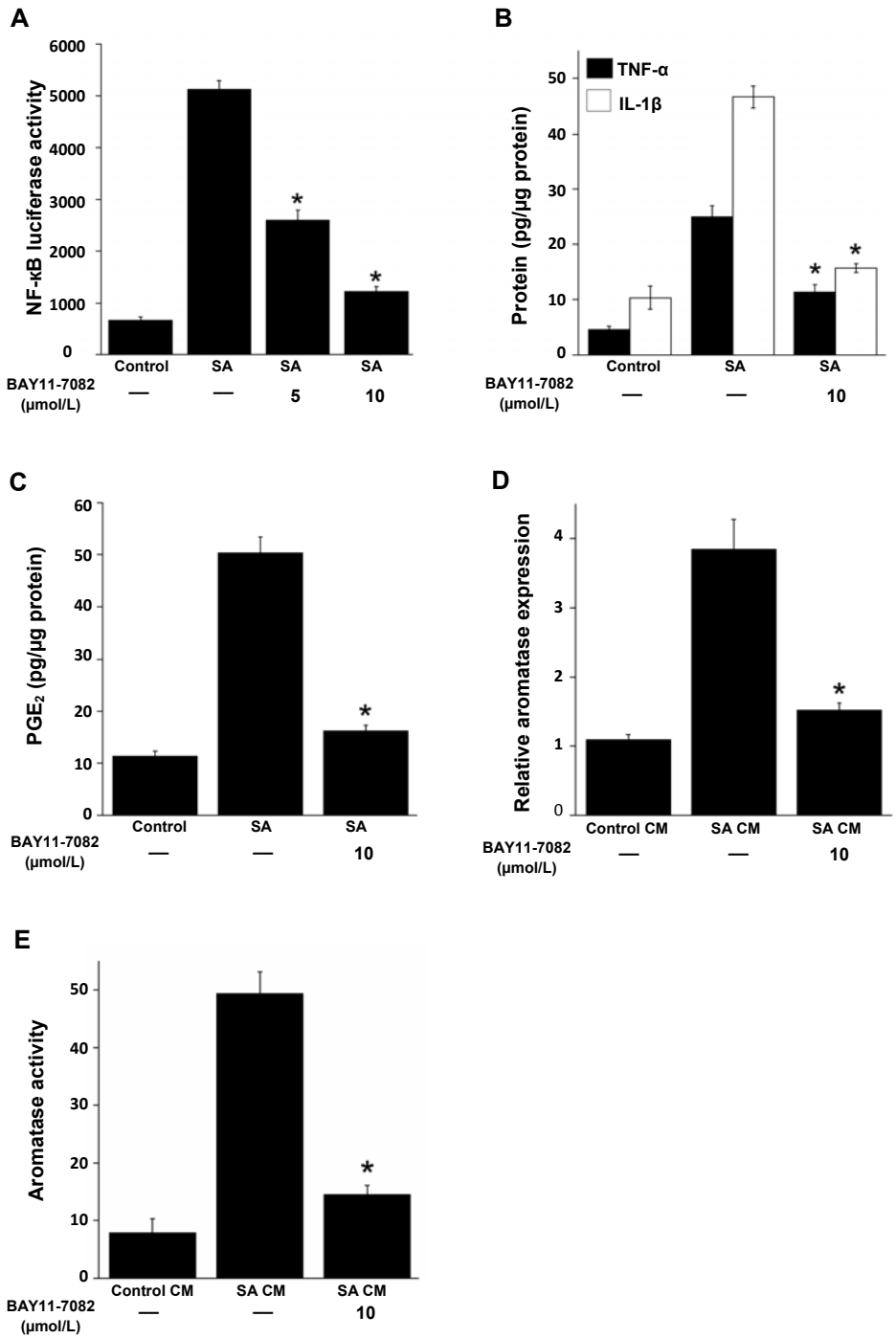
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table S1

Mouse Primers

Cox-2	Forward: 5'-ATTCTTTGCCCAGCACTTCA-3' Reverse: 5'-GGGATACACCTCTCCACCAA-3'
TNF-α	Forward: 5'-CCAGACCCTCACACTCAGATC-3' Reverse: 5'-CACTTGGTGGTTTGCTACGAC-3'
IL-1β	Forward: 5'-TGGGCCTCAAAGGAAAGAAT-3' Reverse: 5'-CAGGCTTGTGCTCTGCTTGT-3'
Aromatase	Forward: 5'-AAGCTCTGACGGGCCCTGGT-3' Reverse: 5'-ACGTAGCCCGAGGTGTCGGT-3'
PR	Forward: 5'-GGTGGGCCTTCCTAACGAG-3' Reverse: 5'-GACCACATCAGGCTCAATGCT-3'
pS2	Forward: 5'-CTGCCCAGGAGAGAAATGAG-3' Reverse: 5'-CAGGGTATGAGGGTTCTCCA-3'
GAPDH	Forward: 5'-AATGTGTCCGTCGTGGATCT-3' Reverse: 5'-CATCGAAGGTGGAAGAGTGG-3'

Human Primers

Cox-2	Forward: 5'-CCCTTGGGTGTCAAAGGTAA-3' Reverse 5'-GCCCTCGCTTATGATCTGTC-3'
TNF-α	Forward: 5'-CTGCTGCACTTTGGAGTGAT-3' Reverse: 5'-AGATGATCTGACTGCCTGGG-3'
IL-1β	Forward: 5'-GGACAAGCTGAGGAAGATGC-3' Reverse: 5'-TCGTTATCCCATGTGTCGAA-3'
Aromatase	Forward: 5'-CACATCCTCAATACCAGGTCC-3' Reverse: 5'-CAGAGATCCAGACTCGCATG-3'
β-*actin*	Forward: 5'- AGAAAATCTGGCACCACACC-3' Reverse: 5'-AGAGGCGTACAGGGATAGCA-3'