SUPPLEMENTAL MATERIALS

Adipocyte-derived serum amyloid A promotes angiotensin II-induced abdominal aortic aneurysms in obese C57BL/6J mice

Preetha Shridas, Ailing Ji, Andrea C Trumbauer, Victoria P Noffsinger, Steve W Leung, Adam J. Dugan, Sean E Thatcher, Lisa A Cassis, Frederick C De Beer, Nancy R Webb, Lisa R Tannock

Affiliations

The Departments of Internal Medicine (PS,AJ,VPN,SWL,FCDB, LRT), Pharmacology and Nutritional Sciences (LAC, NRW), Biostatistics (AJD), Saha Cardiovascular Research Center (PS,ACT, SWL,FCDB, NRW, LRT), Barnstable Brown Diabetes Center (PS, FCDB, NRW, LRT) University of Kentucky, Lexington, Kentucky 40536, Department of Veterans Affairs (LRT), Lexington, KY and the Department of Pharmacology, Temple University, Philadelphia, PA (SET).

Figure S1

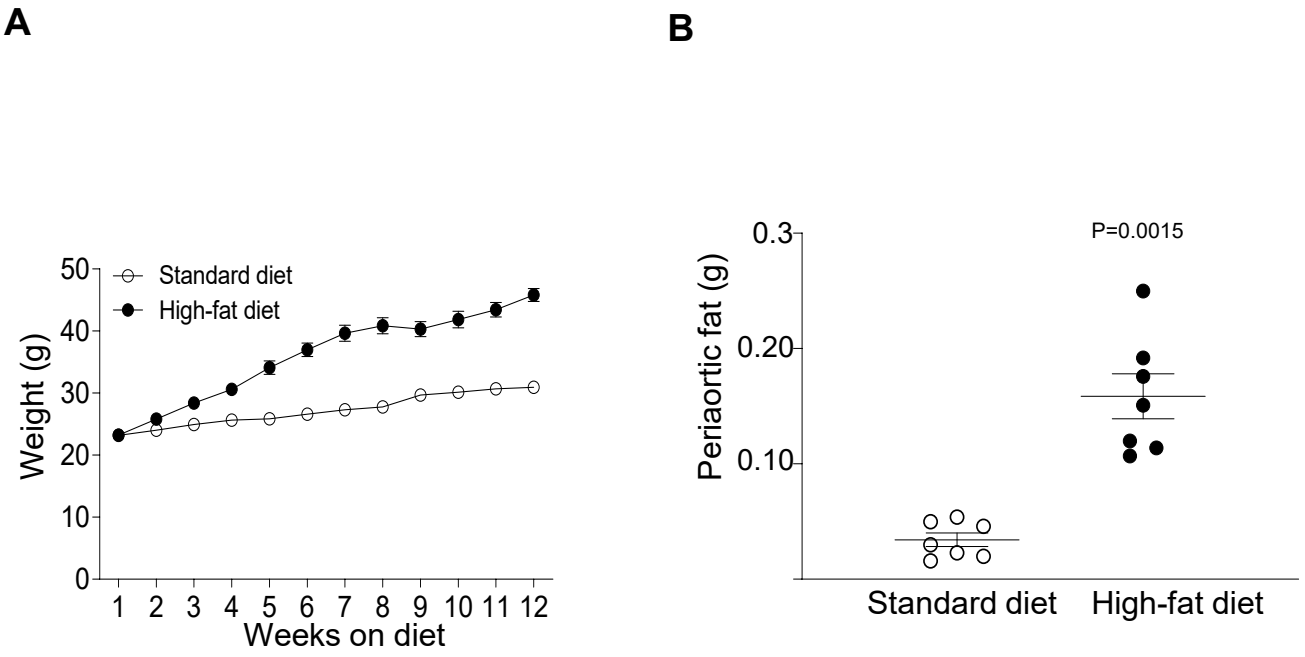


Figure S1. (A) Body weight changes in mice fed a standard diet or high-fat diet for 12 weeks. (B) Periaortic fat weight (mean ±SEM) in mice fed standard versus HF diet for 12 weeks (n=7).

Figure S2

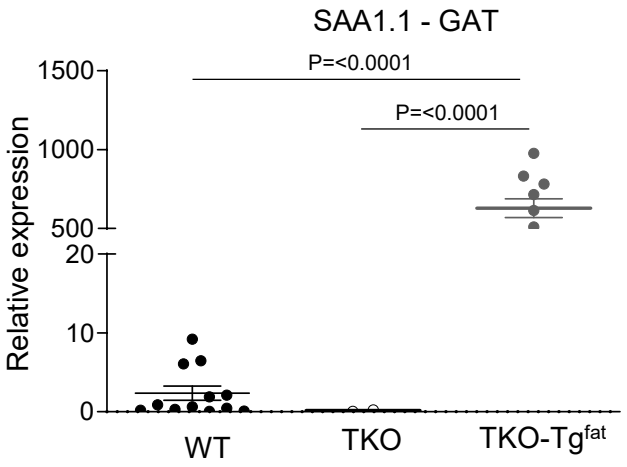
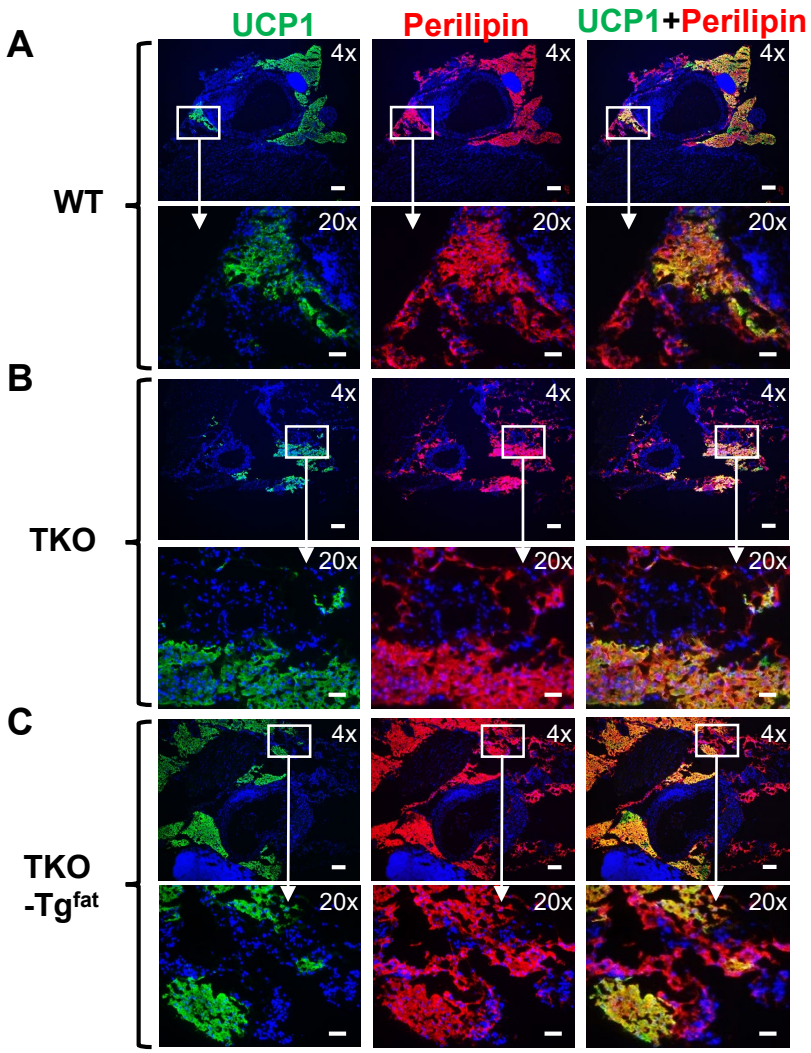


Figure S2. SAA1.1 mRNA expression in gonadal adipose tissues from AngII-infused obese WT, TKO and TKO-Tg^{fat} mice. Data are mean ±SEM.

Figure S3



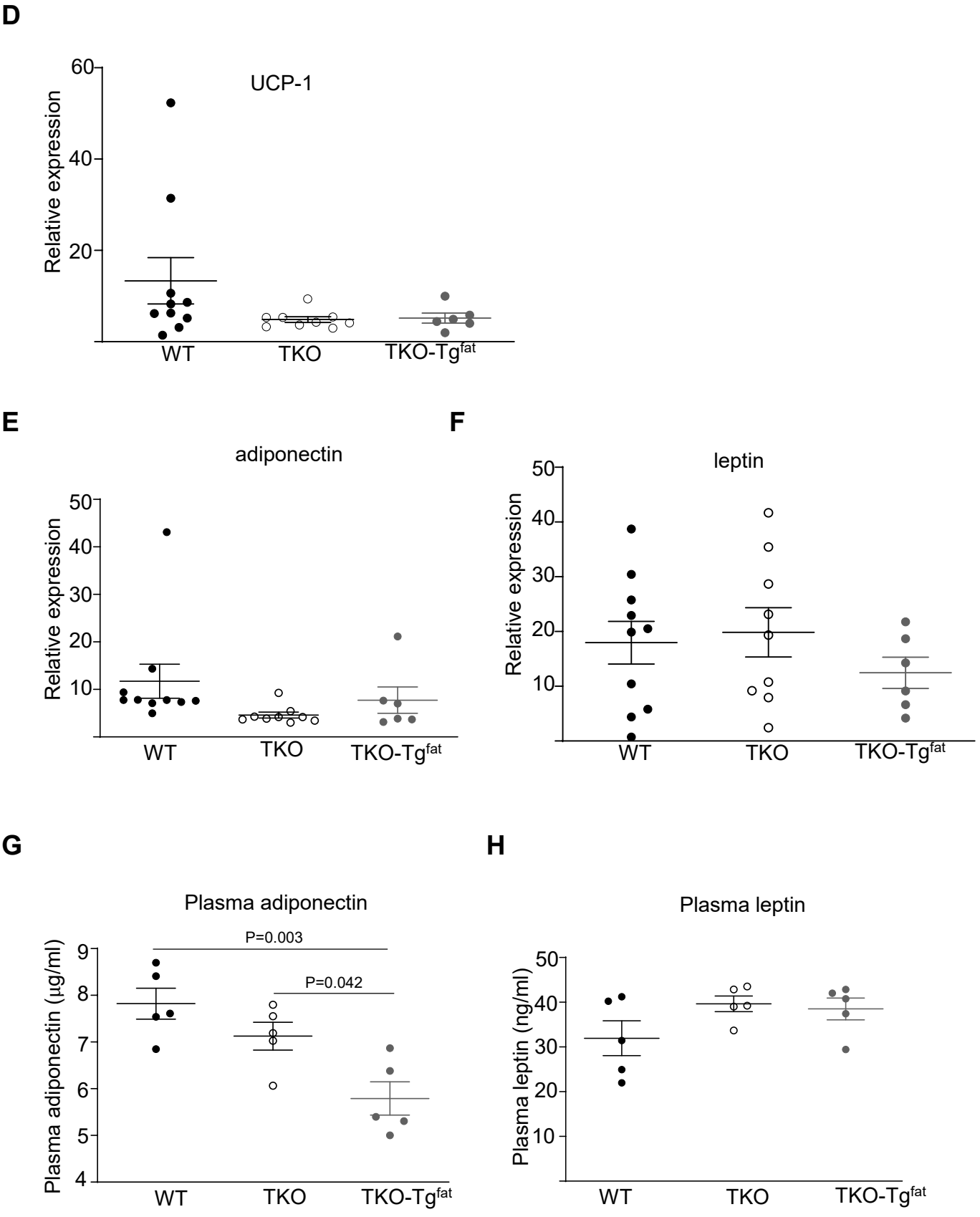

D
UCP-1
Relative expression
60
40
20
WT
TKO
TKO-Tg^fat
E
adiponectin
Relative expression
50
40
30
20
10
WT
TKO
TKO-Tg^fat
F
leptin
Relative expression
50
40
30
20
10
WT
TKO
TKO-Tg^fat
G
Plasma adiponectin
P=0.003
P=0.042
Plasma adiponectin (µg/ml)
9
8
7
6
5
4
WT
TKO
TKO-Tg^fat
H
Plasma leptin
Plasma leptin (ng/ml)
50
40
30
20
10
WT
TKO
TKO-Tg^fat

Figure S3. The expression of SAA in adipocytes has no effect on the presence of UCP1 positive cells. Sections showing the surrounding perivascular fat (PVAT) from WT (A), TKO (B) and TKO-Tg^{fat} (C) mice were processed as described in Methods to detect UCP1 (green fluorescence) and perilipin (red fluorescence), as indicated. Nuclei were identified using DAPI (blue fluorescence). Images photographed under 4× and 20× objective magnification are shown; scale bar in 4x image is 200 μm, scale bar in 20x image is 50 μm. Expression of UCP-1 (D), adiponectin (E), leptin (F) mRNA in PVAT of WT, TKO and TKO-Tg^{fat}. Plasma adiponectin (G) and leptin (H) levels in WT, TKO and TKO-Tg^{fat}.

Figure S4

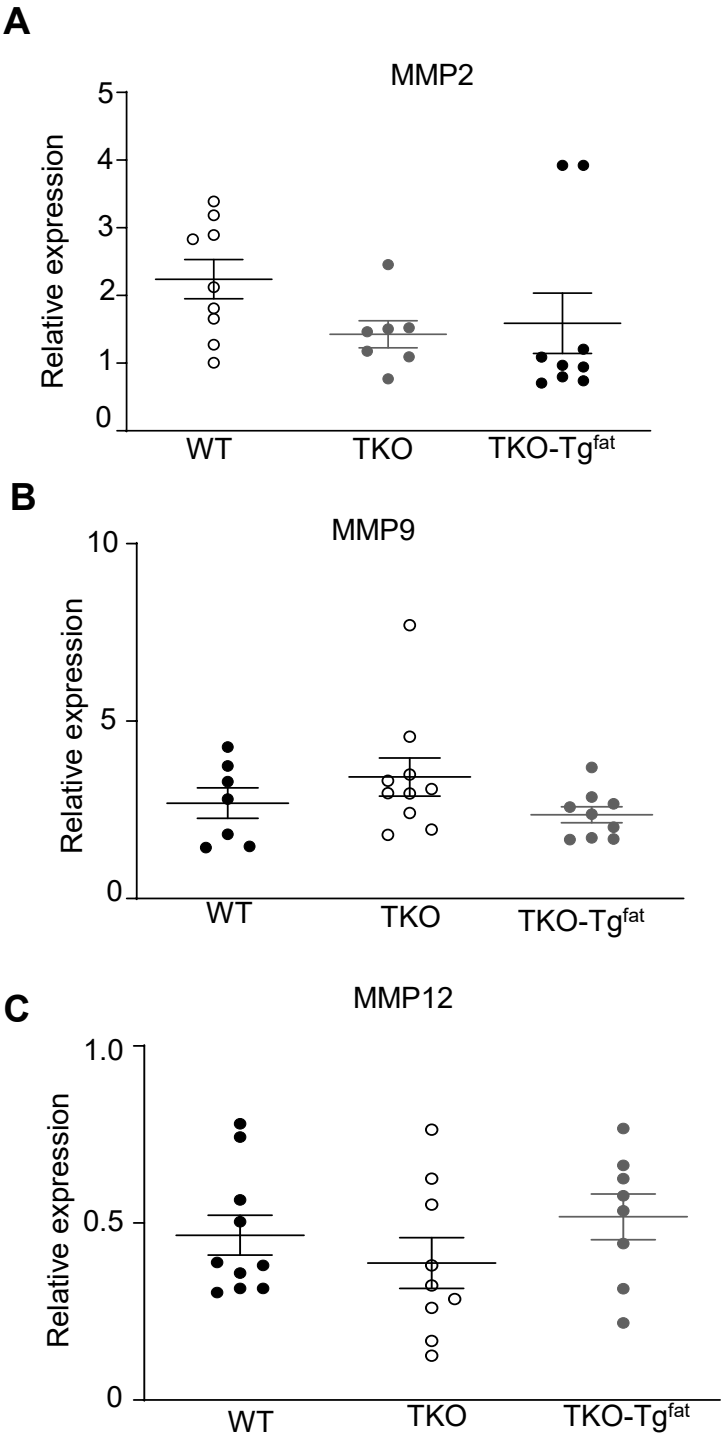
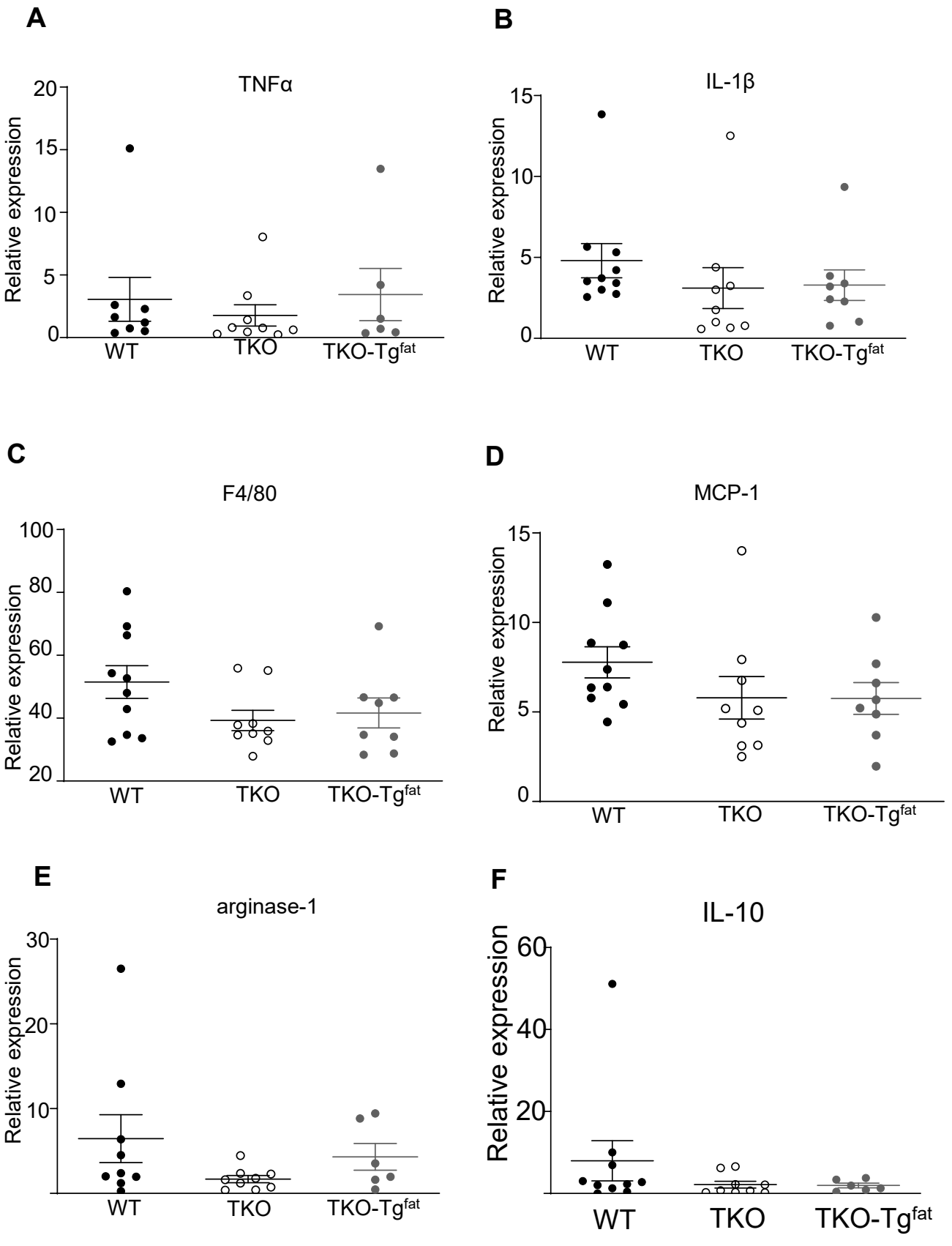


Figure S4. Expression of MMP2 (A), MMP9 (B), MMP12 (C) mRNA in periaortic adipose tissues of WT, TKO and TKO-Tg^{fat}.

Figure S5


A
TNFα
Relative expression
20
15
10
5
0
WT
TKO
TKO-Tg^fat
B
IL-1β
Relative expression
15
10
5
0
WT
TKO
TKO-Tg^fat
C
F4/80
Relative expression
100
80
60
40
20
WT
TKO
TKO-Tg^fat
D
MCP-1
Relative expression
15
10
5
0
WT
TKO
TKO-Tg^fat
E
arginase-1
Relative expression
30
20
10
WT
TKO
TKO-Tg^fat
F
IL-10
Relative expression
60
40
20
WT
TKO
TKO-Tg^fat

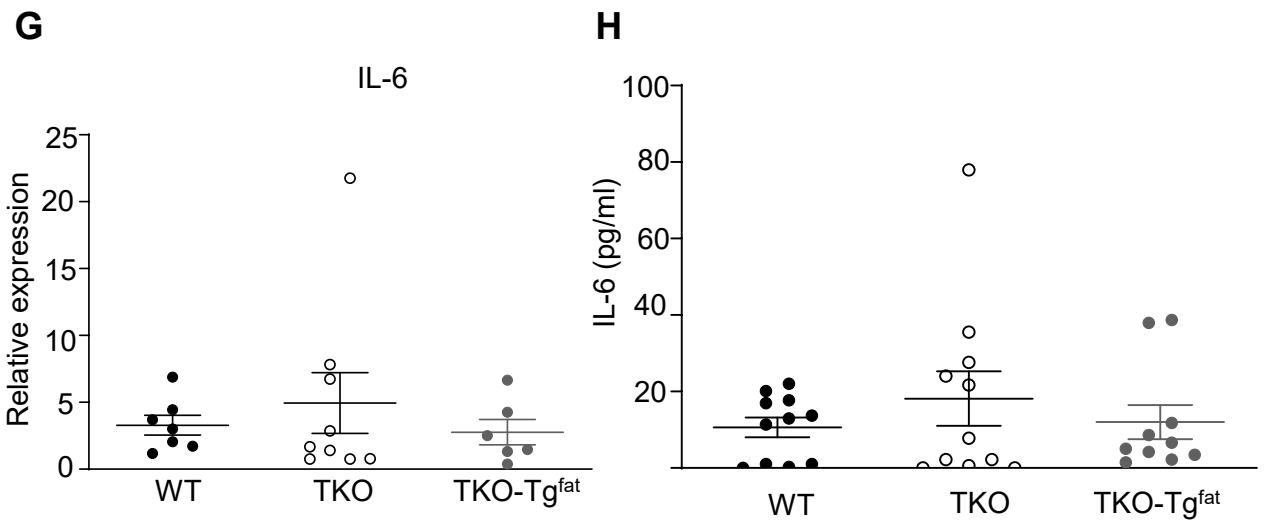


Figure S5. Expression of TNFα (A), IL-1β (B), F4/80 (C), MCP-1 (D), arginase-1 (E), IL-10 (F) and IL-6 (G) mRNAs in periaortic adipose tissues of WT, TKO and TKO-Tg^{fat}. Plasma IL-6 levels in WT, TKO and TKO-Tg^{fat} (H).

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
C57Bl/6J	Generated in-house	C57Bl/6J	M	
TKO (Serum amyloid A 1.1,2.1 and 3 deficient)	Drs. June-Yong Lee and Dan Littman, New York University	C57Bl/6J	M	
Transgenic, expressing Serum amyloid A 1.1 regulated by a tetracycline-responsive promoter	Dr. Paul Simon, University College London, UK	C57Bl/6J	M	
Transgenic expressing reverse tetracycline-controlled transactivator (rtTA) under control of the adipocyte-specific adiponectin promoter	Dr. Philip Scherer, University of Texas Southwestern Medical Center	C57Bl/6J	M	
TKO-Tg^{Fat} with inducible serum amyloid A expression only in adipose tissues	Generated in-house	C57Bl/6J	M	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Application
Mouse SAA	De Beer laboratory	-	1:500 dilution of the stock		IHC
Mouse SAA	Abcam	Ab199030	1.1 µg/ml		IHC
Mouse CD36	Abcam	Ab53444	5.0 µg/ml		IHC
Alexa-fluor 568-labeled goat anti-rabbit IgG	ThermoFisher Scientific	A-11011	10.0 µg/ml		IFC
Alexa-fluor 488-labeled goat anti-rat IgG	ThermoFisher Scientific	A-11006	10.0 µg/ml		IFC
Mouse SAA	Abcam	Ab199030	0.22 µg/ml		WB
Anti-rabbit antibody	Abcam	Ab205718	0.2 µg/ml		WB
Anti-β-actin	Sigma	A5441	~ 1ug/ml		WB
Anti-mouse antibody	Sigma	A4416	1:10,000 dilution of the stock		WB

DOI [to be added]