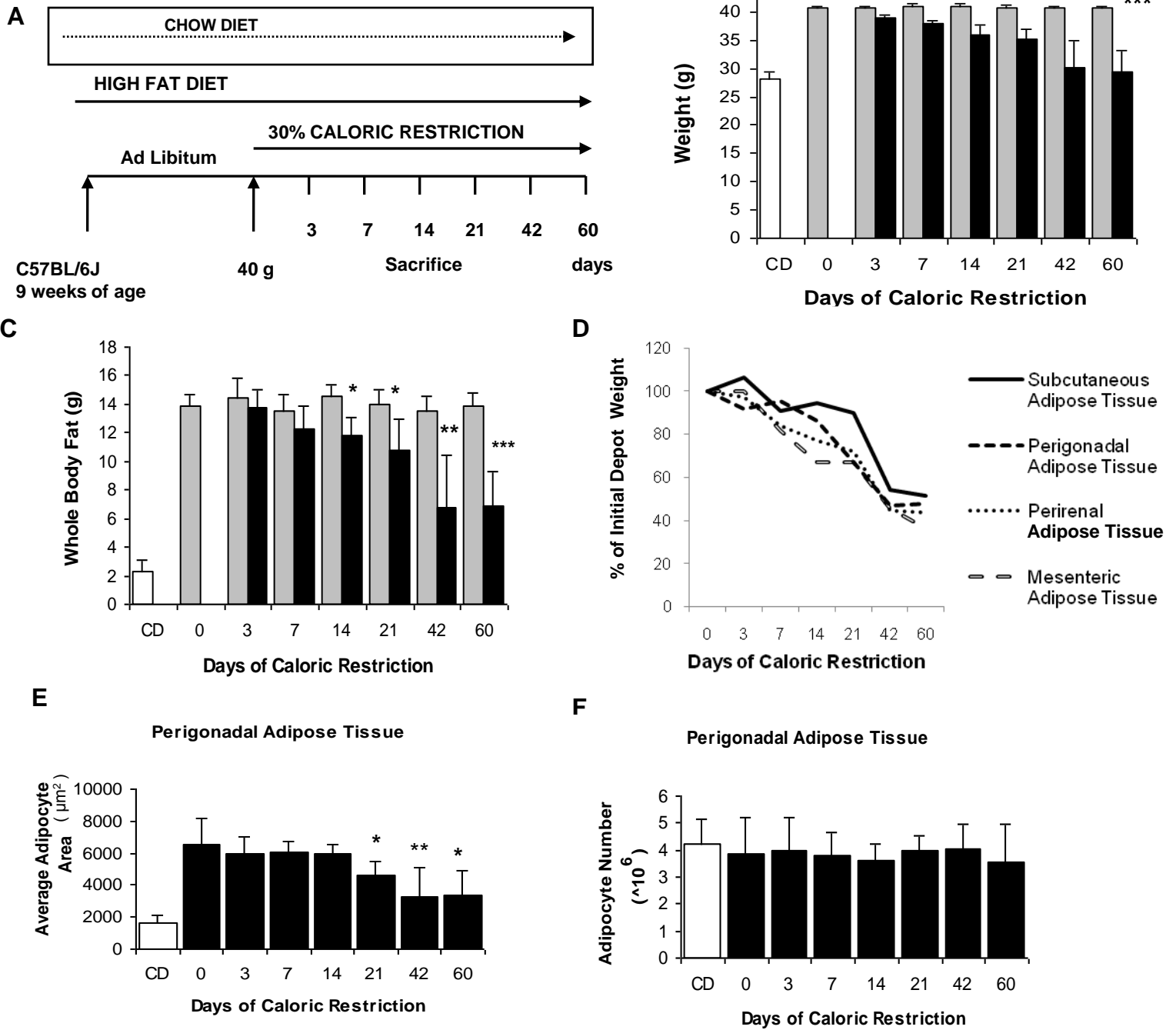


## **Supplemental Figures and Tables**

### **An Immune Response to Weight Loss and Lipolysis in Mice**

Aliki Kosteli, Eiji Sugaru, Guenter Haemmerle, Jayne F. Martin, Jason Lei, Rudolf Zechner and Anthony W. Ferrante Jr.

**Figure S1****Figure S1**

Weight and fat mass loss during weight loss. (A) C57BL/6J mice were placed on a high fat diet and fed ad libitum until they weighed 40 grams, after which they were subjected to a 30% reduction in food intake. Groups of mice were sacrificed on days: 0, 3, 7, 14, 21, 42, and 60 of caloric restriction ( $n = 5-6$  mice/group). A lean age-matched low fat chow diet-fed group was studied as a control. (B) Body Weight of each group before (grey bars) and after (black bars) caloric restriction. White bar represents age-matched lean mice on chow diet (CD), ( $n = 5-6$  mice/group), (\*\* p-value  $< 0.01$ , \*\*\* p-value  $< 0.001$ , initial vs. final weight). (C) Fat Mass of each group before (grey bars) and after (black bars) caloric restriction, ( $n = 5-6$  mice/group) (\* p-value  $< 0.05$ , \*\* p-value  $< 0.01$ , \*\*\* p-value  $< 0.001$ , initial vs. final fat mass). (D) Rate of decrease in fat mass for different fat depots ( $n = 5-6$  mice/group). (E) Mean adipocyte cross-sectional area during caloric restriction. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for the indicated number of days. The white bar represents control lean mice that were fed a chow diet (CD) and did not undergo caloric restriction, ( $n = 5-6$  mice/group), (\* p-value  $< 0.05$ , \*\* p-value  $< 0.01$ , vs. day 0). (F) Average adipocyte number per perigonadal adipose tissue depot, ( $n = 5-6$  mice/group). All data are represented as mean  $\pm$  SD.

Figure S2

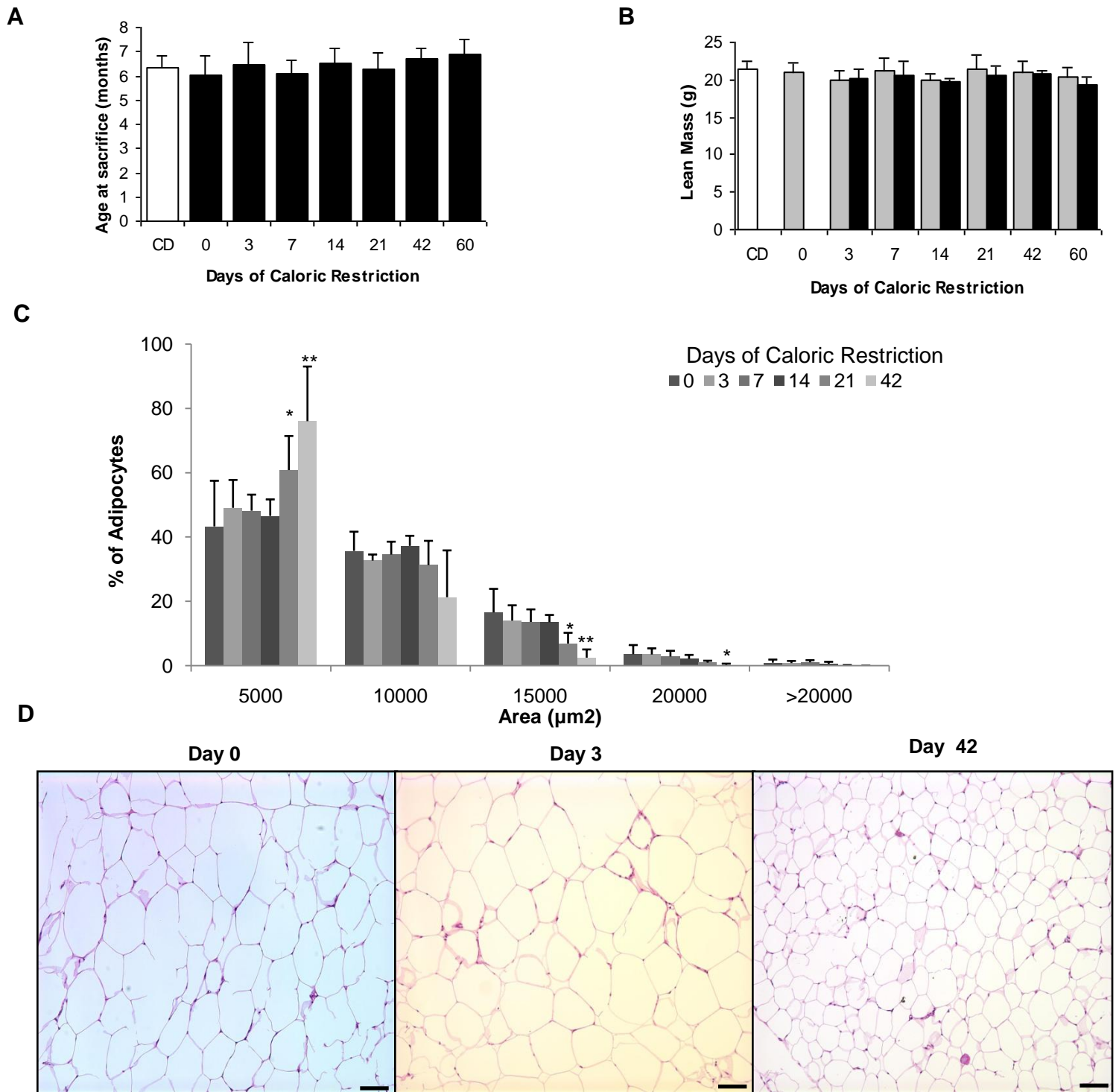


Figure S2

Lean mass and morphometric analysis of adipose tissue during weight loss (A) Age at sacrifice for each group. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for different time intervals. The white bar represents control lean mice that were fed a chow diet (CD) and did not undergo caloric restriction. (B) NMR measurements for lean mass of each group before (grey bars) and after (black bars) caloric restriction. White bar represents age-matched lean mice on chow diet (CD). (C) Histogram of the average distribution of adipocyte area in perigonadal adipose tissue of high fat diet-induced obese ad libitum fed control mice (day 0) and high fat diet-induced obese mice undergoing caloric restriction for different time intervals (\* p-value < 0.05, \*\*p-value < 0.01, vs. day 0). Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group). (D) Hematoxylin and eosin staining of perigonadal adipose tissue sections from high fat diet-induced obese ad libitum fed control mice (day 0) and high fat diet-induced obese mice that underwent caloric restriction for indicated number of days. Calibration mark = 100  $\mu$ m.

Figure S3

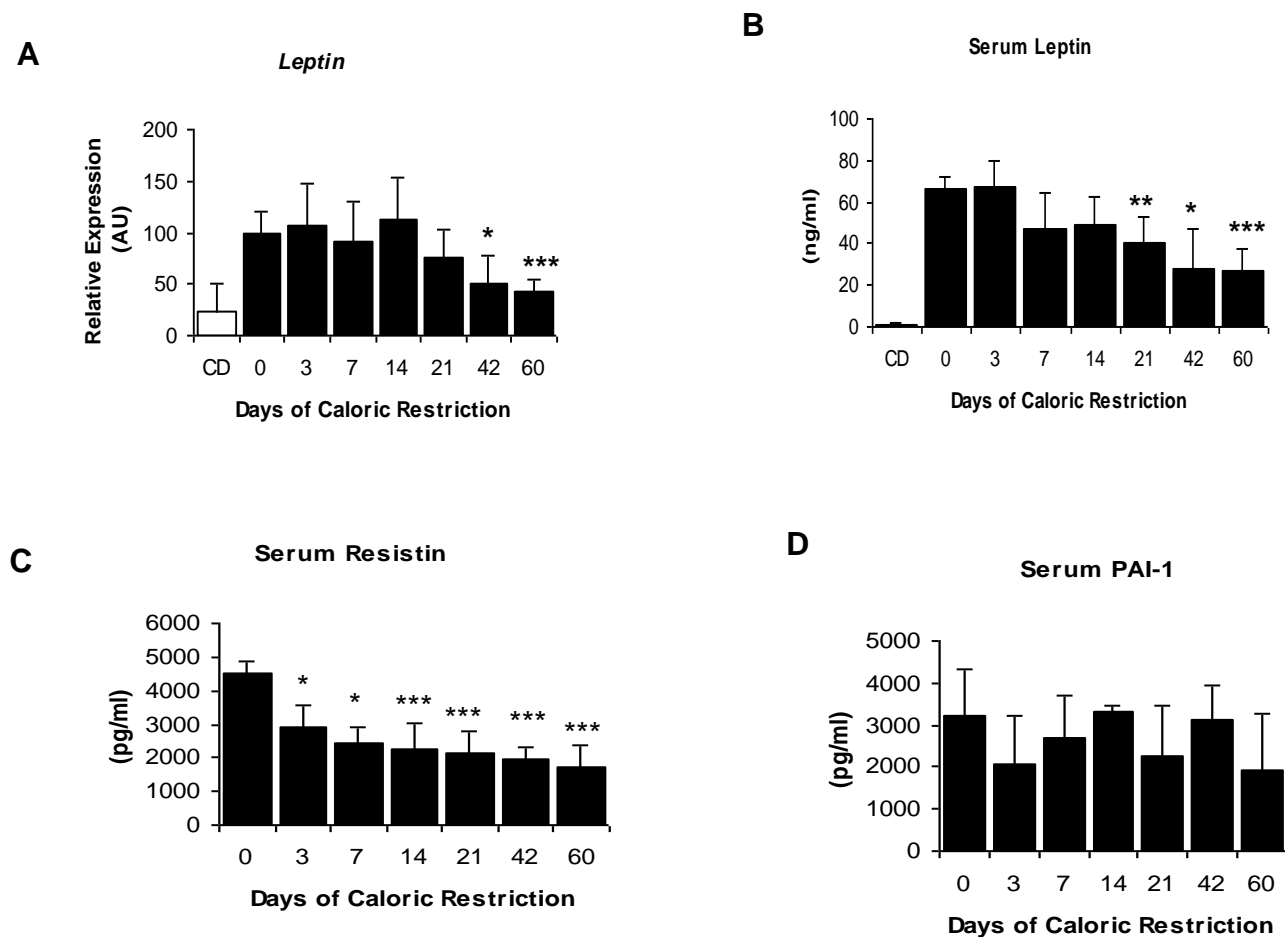
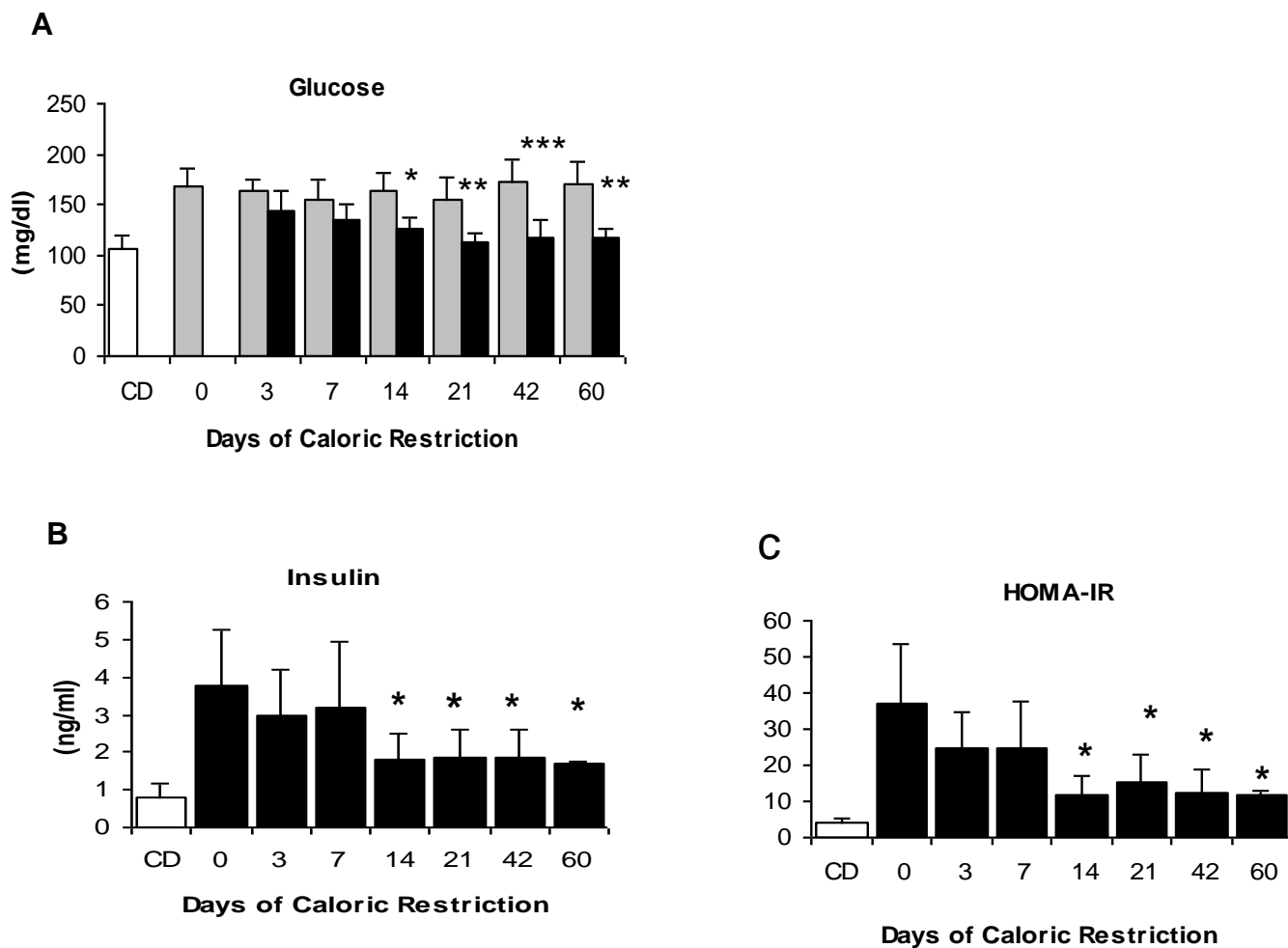


Figure S3

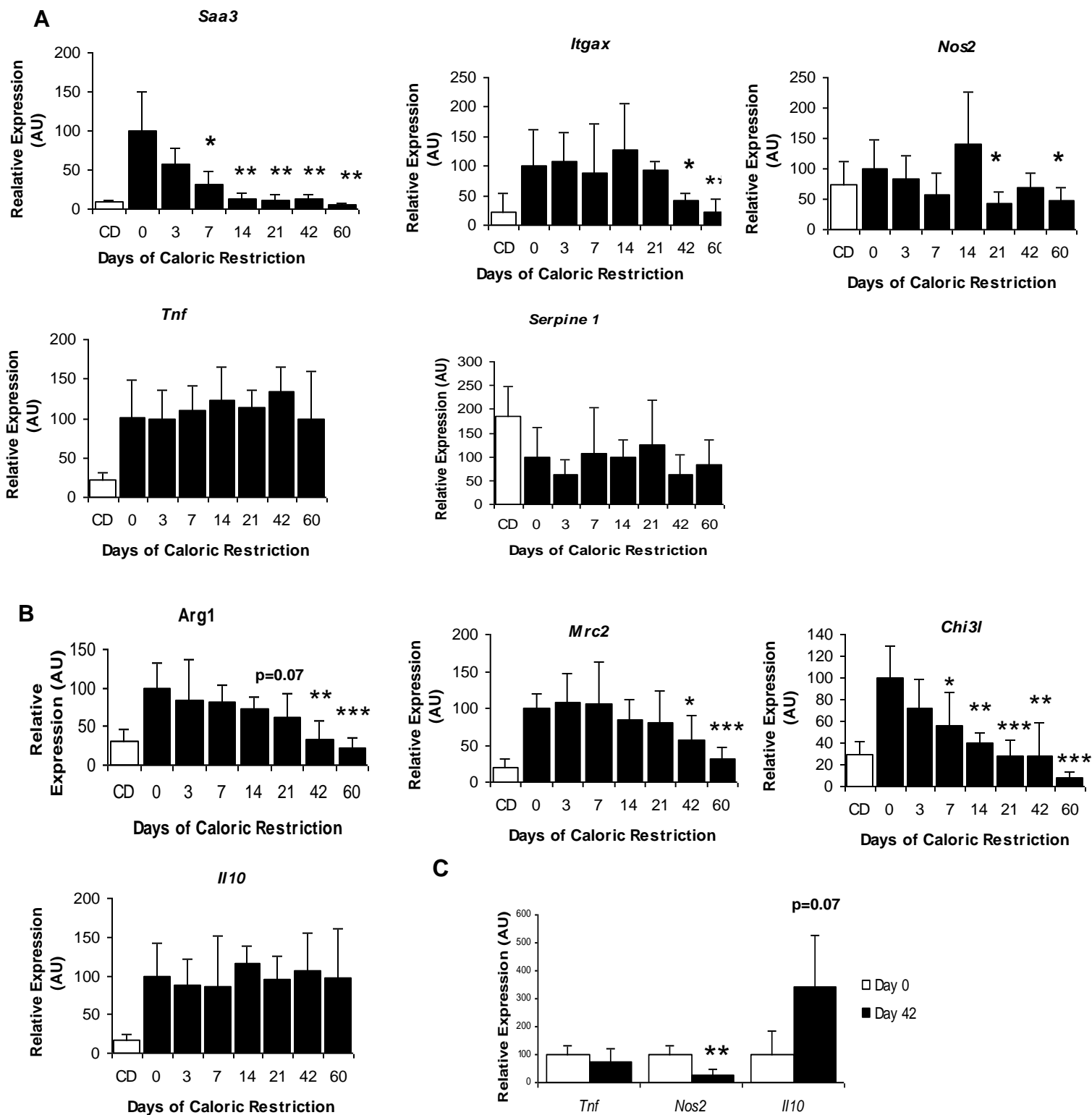
Adipokines during weight loss (A) *Leptin* gene expression in perigonadal adipose tissue of mice during caloric restriction. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for indicated number of days. The white bar represents control lean mice that were fed a chow diet (CD) and did not undergo caloric restriction. (B) Serum leptin concentrations in mice during caloric restriction. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for different time intervals. The white bar represents control lean mice that were fed a chow diet (CD) and did not undergo caloric restriction. Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group). (C) Serum Resistin and (D) PAI-1 measurements in high fat diet-induced obese ad libitum fed control mice (day 0) and high fat diet-induced obese mice subjected to caloric restriction for indicated number of days (\* p-value < 0.05, \*\*p-value < 0.01, \*\*\*p-value < 0.001, vs. day 0). Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group).

Figure S4



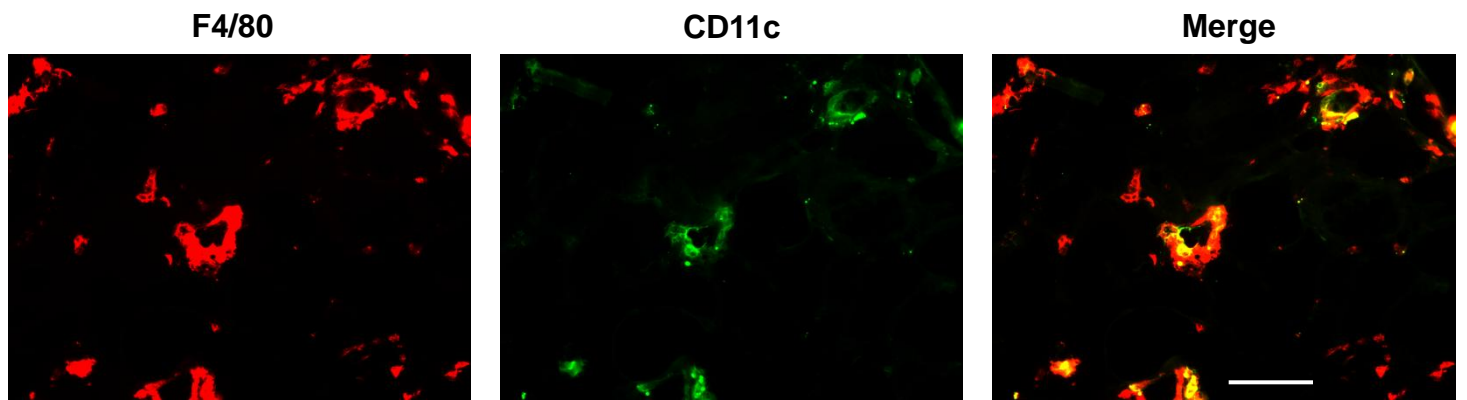
**Figure S4**

Glucose homeostasis during weight loss (A) Blood glucose concentrations for each group of mice before (grey bars) and after (black bars) caloric restriction. White bar represents age-matched lean mice on chow diet (CD). Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group), (\*p-value < 0.05, \*\* p-value < 0.01, \*\*\* p-value < 0.001, initial vs. final blood glucose). (B) Fasting serum insulin concentrations and (C) homeostasis model assessment of insulin resistance (HOMA-IR) . Black bars represent high fat diet-induced obese mice that underwent caloric restriction for different time intervals. The white bar represents control lean mice that were fed a chow diet (CD) and did not undergo caloric restriction. Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group) (\* p-value < 0.05, vs. day 0).

**Figure S5****Figure S5**

Inflammatory gene expression during weight loss (A) Perigonadal adipose tissue expression of genes encoding inflammatory proteins. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for indicated number of days. The white bar represents control lean mice that were fed a low fat chow diet (CD) and did not undergo caloric restriction. Data are represented as mean  $\pm$  SD, ( $n = 5-6$  mice/group) (\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$ , vs. day 0). (B) Perigonadal adipose tissue expression of genes encoding alternative activation and anti-inflammatory proteins. Black bars represent high fat diet-induced obese mice that underwent caloric restriction for indicated number of days. The white bar represents control lean mice that were fed a low fat chow diet (CD) and did not undergo caloric restriction. Data are represented as mean  $\pm$  SD, ( $n = 5-6$  mice/group) (\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$ , \*\*\*  $p$ -value  $< 0.001$  vs. day 0). (C) Expression of representative inflammatory and anti-inflammatory genes in subcutaneous adipose tissue during caloric restriction-induced weight loss (\*\*  $p$ -value  $< 0.01$ , vs. day 0,  $n = 6-12$  per group).

Figure S6

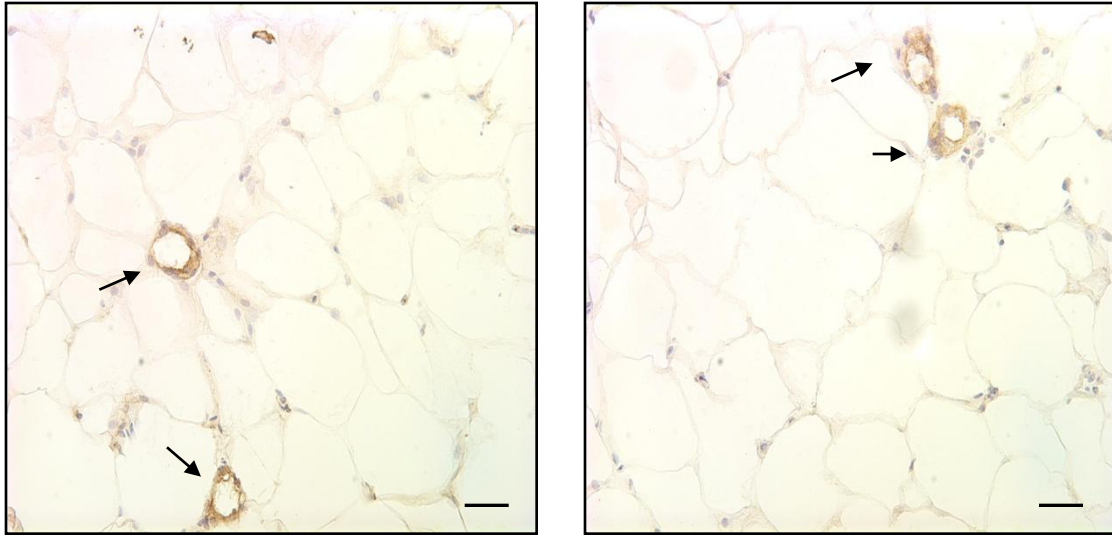


**Figure S6**

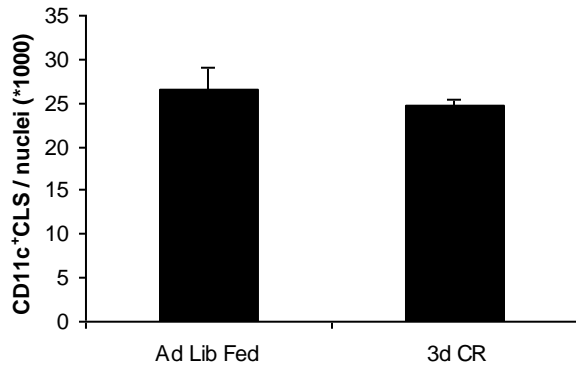
Two populations of ATMs in perigonadal adipose tissue of mice following 3 days of caloric restriction. Immunofluorescence staining for the pan-macrophage antigen F4/80 (left) and the more restricted macrophage-dendritic cell antigen CD11c (middle panel). The merged imaged (right panel) identifies two macrophages populations: 1) F4/80+, CD11c- (red only in merged panel) and 2) F4/80+/CD11c+ (yellow-orange in merged panel). There are few F4/80- / CD11c+ (green in merged panel). Calibration mark = 100  $\mu$ m.

**Figure S7**

**A**



**B**

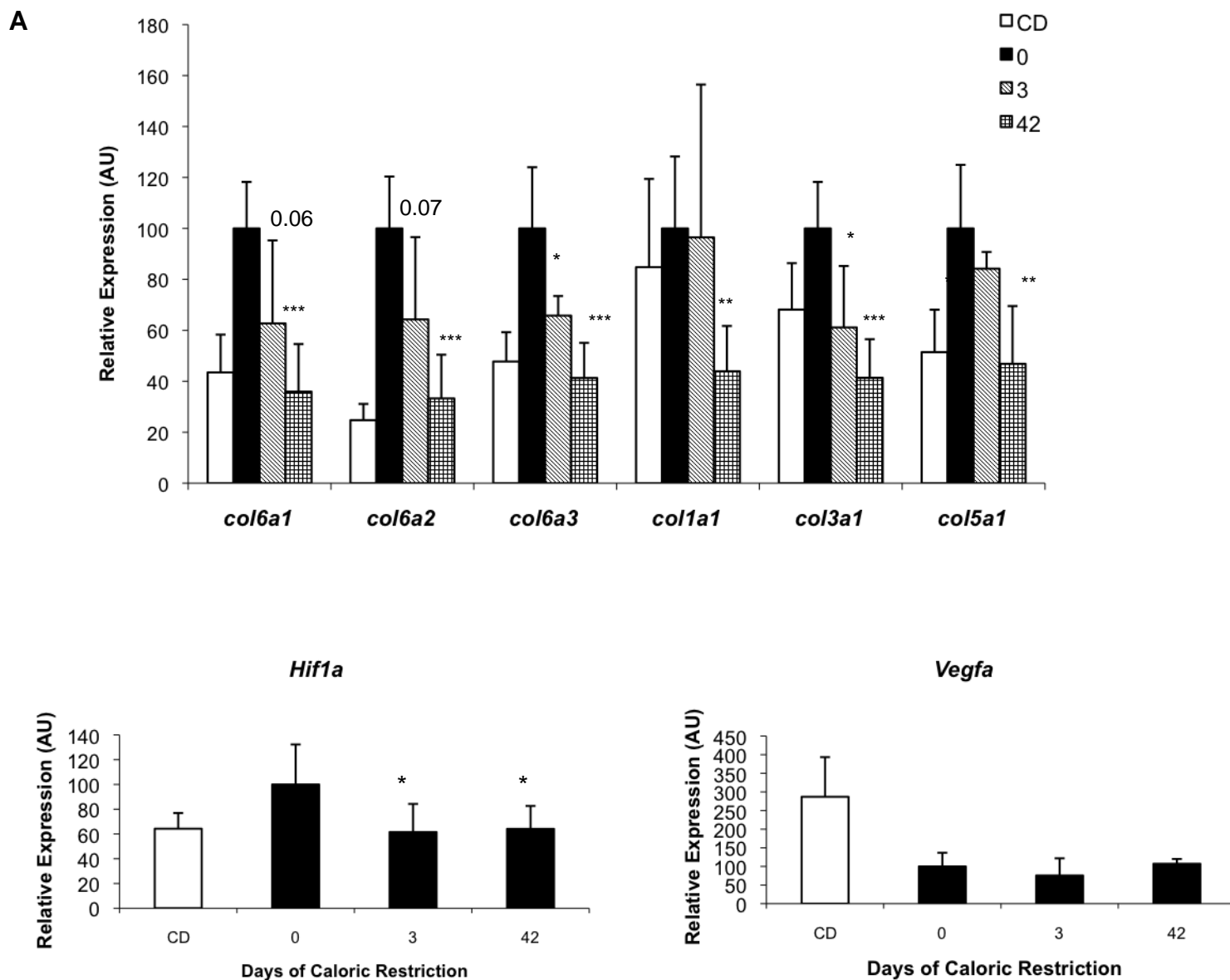


**Figure S7**

Crown-like structures in adipose tissue following 3 days of caloric restriction (A) Immunohistochemical staining of CD11c (MAC-2) expressing macrophages in perigonadal adipose tissue sections from high fat diet-induced obese mice that were either ad libitum fed (left panel) or undergoing 3 days of caloric restriction (right panel). Arrows indicate CD11c+ crown-like structures. Calibration mark = 50  $\mu$ m. (B) The number of CD11c+ crown-like structures (CLS) was no different between ad libitum fed and mice calorically restricted to 70% of their ad libitum food intake (3d CR). Data are represented as mean  $\pm$  SD, (n = 5 mice/group).

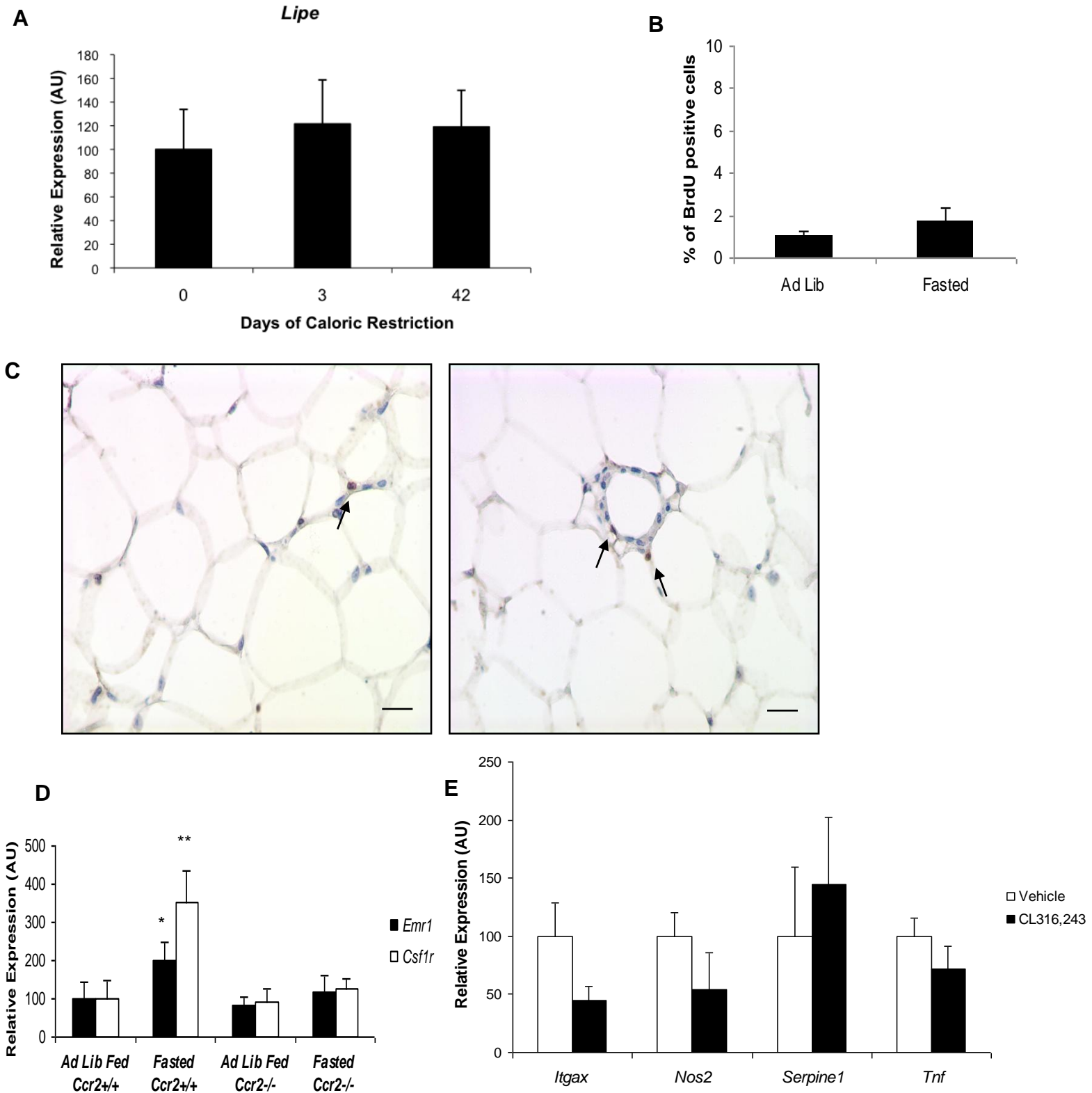


Figure S8

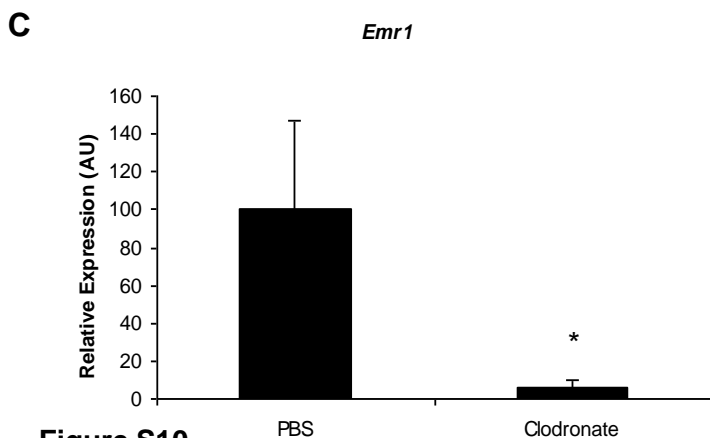
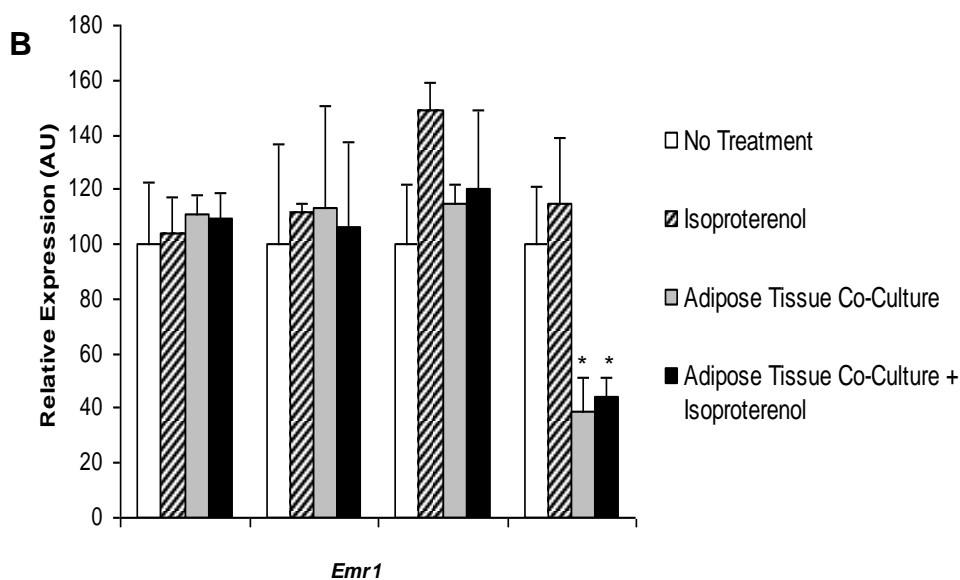
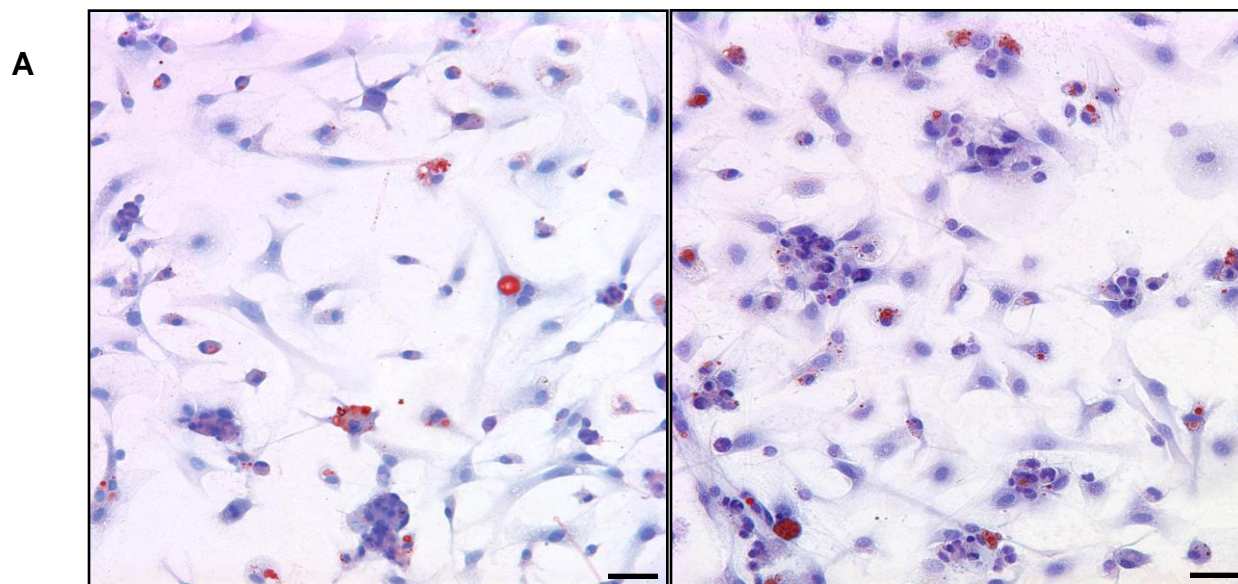


**Figure S8**

Remodeling gene expression during weight loss (A) Expression of genes encoding proteins involved in tissue remodeling. The black bars and the bars with a hatch and grid pattern represent high fat diet-induced obese mice that underwent caloric restriction for indicated number of days. The white bar represents control lean mice that were fed a low fat chow diet (CD) and did not undergo caloric restriction. Data are represented as mean  $\pm$  SD, (n = 5-6 mice/group) (\* p-value < 0.05, \*\* p-value < 0.01, \*\*\* p-value < 0.001 vs. day 0).

**Figure S9****Figure S9**

(A) Perigonadal adipose tissue expression of the gene encoding the lipase HSL (*Lipe*) in high fat diet-induced obese ad libitum fed control mice (day 0) and high fat diet-induced obese mice undergoing caloric restriction for 3 and 42 days. (B) The fraction of BrdU+ nuclei in lean ad libitum and 24-hr fasted mice. Data are represented as mean  $\pm$  SD, (n = 5-6 per group). (C) Sections from perigonadal adipose tissue from lean ad libitum fed (left panel) and 24-hr fasted mice (right panel) Arrows indicate BrdU positive cells. Calibration mark = 100  $\mu$ m. (D) Expression of macrophage-specific genes in perigonadal adipose tissue of lean *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> ad libitum fed and 24-hr fasted mice. (E) Expression of genes encoding inflammatory markers in perigonadal adipose tissue of lean mice treated with vehicle or with CL316,243. (n = 5 mice/group). All data are represented as mean  $\pm$  SD. \* p-value < 0.05, \*\* p-value < 0.01 vs ad libitum fed.

**Figure S10****Figure S10**

Effect of adipose tissue on stromal vascular cells and the depletion of ATMs in clodronate treated mice (A) Stromal vascular cells isolated from perigonadal adipose tissue of high fat diet-induced obese mice were cultured either alone or with perigonadal adipose tissue pieces (harvested from lean animals). The panel shows Oil Red O staining of lipid droplets in stromal vascular cells. Stromal vascular cells cultured alone had fewer lipid filled droplets (left panel) compared to those co-cultured with adipose tissue pieces (right panel). Calibration mark = 50  $\mu$ m. (B) Stromal vascular cells isolated from perigonadal adipose tissue of high fat diet-induced obese mice were cultured either alone or with perigonadal adipose tissue pieces (harvested from lean animals) with or without isoproterenol treatment (10 $\mu$ M) to induce lipolysis in the adipose tissue fraction. The gene expression of *Adipoq*, *Leptin*, *Dlk1* (pref-1) and *Pparg* in stromal vascular cells was measured (\* p-value <0.05; n = 5 per group). (C) Expression of the macrophage marker *Emr1* (F4/80) in the perigonadal adipose tissue of lean C57BL/6J mice treated with clodronate- or PBS- encapsulated liposomes, 4 days post-injection (n= 8). (\* p-value <0.05). All data are represented as mean  $\pm$  SD.

**Table S1**

	<b>High Fat Diet</b>	<b>High Carbohydrate Diet</b>
<b>Source</b>	<b>Kcal %</b>	<b>Kcal %</b>
<b>Fat</b>	60	10
<b>Carbohydrate</b>	20	70
<b>Protein</b>	20	20
<b>Total</b>	100	100

**Table S1**

Macronutrient composition of the high fat and high carbohydrate diets administered to the mice during caloric restriction.

**Table S2**

<b>Baseline Measurements</b>		
<b>Body Weight</b>	g	40.8 ± 0.3
<b>Fat Mass</b>	g	13.9 ± 1.0
<b>Lean Mass</b>	g	20.8 ± 1.4
<b>Fasting Glucose</b>	mg/dL	164.9 ± 19.0

**Table S2**

Measurements before caloric restriction

## Table S3

### Primers

<b>Saa3</b>	Forward: 5'-AGCGATGCCAGAGAGGCTGTTC-3' Reverse: 5'-AGCAGGTCGGAAGTGTTGG-3'
<b>Itgax</b>	Forward: 5'-CCTACTTTGGGGCATCTCTTTG-3' Reverse: 5'-GCACCTCTGTTCTCCTCCTCTC-3'
<b>Nos2</b>	Forward: 5'-AATCTTGGAGCGAGTTGTGG-3' Reverse: 5-CAGGAAGTAGGTGAGGGCTTG-3'
<b>Tnf</b>	Forward: 5'-CCAGACCCTCACTAGATCA-3' Reverse: 5'-CACTTGGTGGTTTGCTACGAC-3'
<b>Serpine1</b>	Forward: 5'-TCCTCATCCTGCCTAAGTTCTC-3' Reverse: 5'-GTGCCGCDCTCGTTTACCTC-3'
<b>Arg1</b>	Forward: 5'-CTCCAAGCCAAAGTCCTTAGAG-3' Reverse: 5'-AGGAGCTGTCATTAGGGACATC-3'
<b>Mrc2</b>	Forward: 5'-TACAGCTCCACGCTATGGATT-3' Reverse: 5'-CACTCTCCCAGTTGAGGTACT-3'
<b>Chi3l3</b>	Forward: 5'-TCTGGGTACAAGATCCCTGAA-3' Reverse: 5'-TTTCTCCAGTGTAGCCATCCTT-3'
<b>Il10</b>	Forward: 5'-GCTCTTACTGACTGGCATGAG-3' Reverse: 5'-CGCAGCTCTAGGAGCATGTG-3'
<b>Emr1</b>	Forward: 5'-CTTTGGCTATGGGCTTCCAGTC-3' Reverse: 5'-GCAAGGAGGACAGAGTTTATCGTG-3'
<b>Cd68</b>	Forward: 5'-CTTCCCACAGGCAGCACAG-3' Reverse: 5'-AATGATGAGAGGCAGCAAGAGG-3'
<b>Csf1r</b>	Forward: 5'-CATACAGCATTACAACCTGGACCTACC-3' Reverse: 5'-CAGGACATCAGAGCCATTACAG-3'
<b>Pnpla2</b>	Forward: 5'-CAGCACATTTATCCCGGTGTAC-3' Reverse: 5'- AAATGCCGCCATCCACATAG-3'
<b>Lipe</b>	Forward: 5'-CACCCATAGTCAAGAACCCTTC-3' Reverse: 5'-TCTACCACTTTCAGCGTCACC-3'
<b>Cd36</b>	Forward: 5'-GCGACATGATTAATGGCACA-3' Reverse: 5'-CCTGCAAATGTCAGAGGAAA-3'
<b>Msr1</b>	Forward: 5'-AGAGGGCTTACTGGACAACTG-3' Reverse: 5'-GGCTTTCCTGGTGCTCCTG-3'
<b>Ccr2</b>	Forward: 5'-TGCCATCATAAAGGAGCCA-3' Reverse: 5'-AGCACATGTGGTGAATCCAA-3'
<b>Adfp</b>	Forward: 5'-CCCGTATTTGAGATCCGTGTT-3' Reverse: 5'-TAGGTATTGGCAACCGCAAT-3'
<b>Ppib</b>	Forward: 5'-CAGCAAGTTCCATCGTGTATC-3' Reverse: 5'-CTCTTTCCTCCTGTGCCATCTC-3'
<b>Dlk-1</b>	Forward: 5'-CGGGAAATTCTGCGAAATAG-3' Reverse: 5'-TGTGCAGGAGCATTCTGTA CT-3
<b>Adipoq</b>	Forward: 5'-GCTCCTGCTTTGGTCCCTCCAC-3' Reverse: 5'-GCCCTTCAGCTCCTGTCAATCC-3'
<b>Pparg</b>	Forward: 5'-GCCCTTGGTGACTTTATGGAG -3' Reverse: 5'-GCAGCAGTTGTCTTGGATG-3'

**Table S3 (continued)**

## Primers

<b><i>Vegfa</i></b>	Forward: 5'-GGAGATCCTTCGAGGAGCACTT -3' Reverse: 5'-GGCGATTTAGCAGCAGATATAAGAA -3'
<b><i>Hif1a</i></b>	Forward: 5'-CAAGATCTCGGCGAAGCAA -3' Reverse: 5- GGTGAGCCTCATAACAGAAGCTTT-3'
<b><i>Col6a1</i></b>	Forward: 5'-GATGAGGGTGAAGTGGGAGA -3' Reverse: 5'-CAGCACGAAGAGGATGTCAA -3'
<b><i>Col6a2</i></b>	Forward: 5'- ATGTGAGGGAGACCTGTGGA -3' Reverse: 5'-TGT GCC TGT TTC TGA CTT GG -3'
<b><i>Col6a3</i></b>	Forward: 5'-CAG AAC CAT TGT TTC TCA CT -3' Reverse: 5'- AGGACT ACA CAT CTT TTC AC -3'
<b><i>Col1a1</i></b>	Forward: 5'-GTGCTCCTGGTATTGCTGGT-3' Reverse: 5'-GGCTCCTCGTTTTCTTCTT -3'
<b><i>Col3a1</i></b>	Forward: 5'- GGGTTTCCCTGGTCCTAAAG -3' Reverse: 5'-CCTGGTTTCCCATTTTCTCC -3'
<b><i>Col5a1</i></b>	Forward: 5'-CTCCAACACCTCCAATCCAG -3' Reverse: 5'-GTCCTCCAATCCCCTCAAAG -3'
<b><i>Acadm</i></b>	Forward: 5'- AGCTCTAGACGAAGCCACGA -3' Reverse: 5'-GCGAGCAGAAATGAACTCC -3'
<b><i>Acadl</i></b>	Forward: 5'-GGTACATGTGGGAGTACCCG -3' Reverse: 5'-TCTTGCATCAGCTCTTTCA -3'
<b><i>Dgat1</i></b>	Forward: 5'-TTCCGCCTCTGGGCATT-3' Reverse: 5'-AGAATCGGCCCAATCCA-3'
<b><i>Lpl</i></b>	Forward: 5'- TTTGGCTCCAGAGTTTGACC-3' Reverse: 5'- TGTGTCTTCAGGGTCCCTTAG-3'
<b><i>Cpt1a</i></b>	Forward: 5'-TGC ACT ACG GAG TCC TGC AA -3' Reverse: 5'-GGA CAA CCT CCA TGG CTC AG-3'
<b><i>ApoE</i></b>	Forward: 5'-CTG ACA GGA TGC CTA GCC G-3' Reverse: 5'- CGC AGG TAA TCC CAG AAG C-3'
<b><i>Abca1</i></b>	Forward: 5'- TGCACAAGGTCCTGAGAATG-3' Reverse: 5'- GGAAGGGACAAATTGTGCTG-3'
<b><i>aP2</i></b>	Forward: 5'-CAGAAGTGGGATGGAAAGTCG-3' Reverse: 5'- CGACTGACTATTGTAGTGTGTTGA- 3'