Supplemental Figure and Table Legends

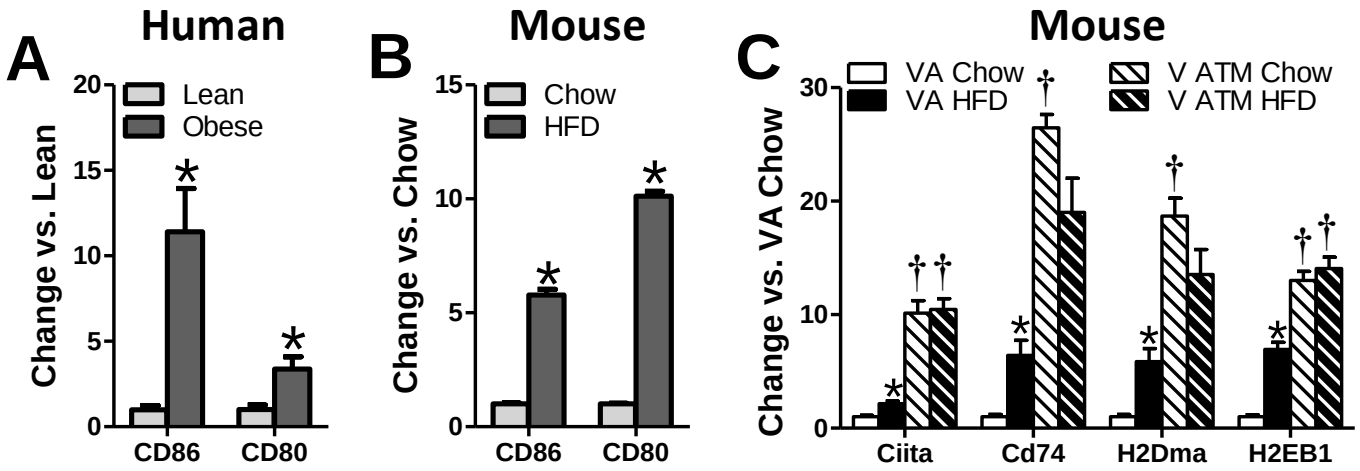
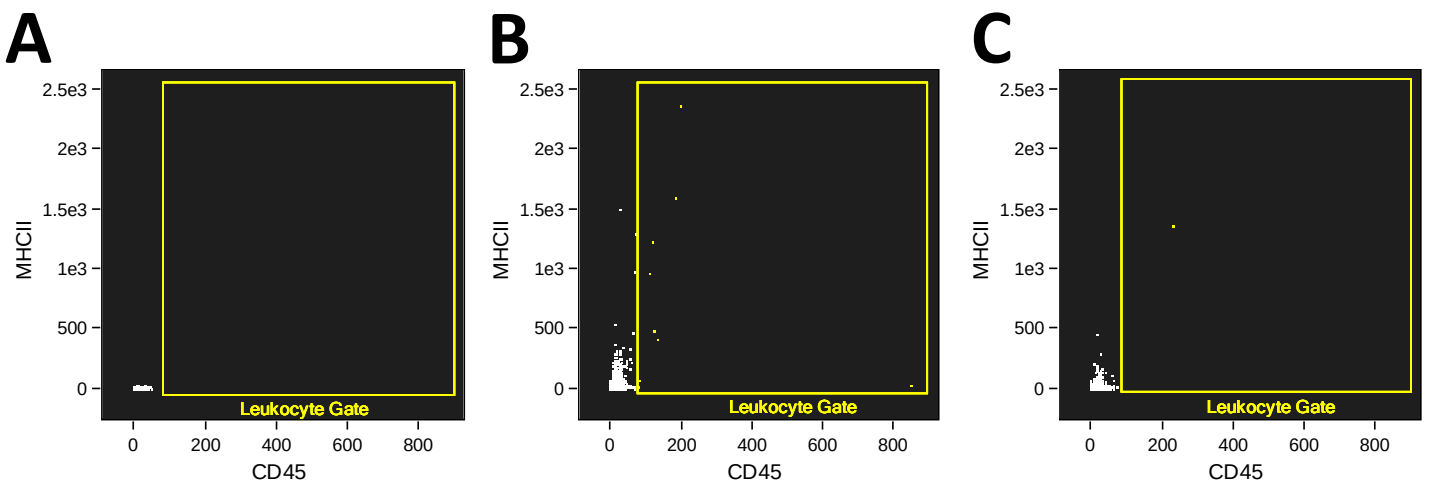


Fig. S1. Adipocyte T-cell co-stimulatory molecule expression and adipocyte and ATM MHCII expression. A-B) RT-PCR analysis of CD80 and CD86 gene expression in adipocytes of **A)** lean and obese women (9-12/group) and **B)** C57BL/6J mice fed 3 months of chow or HFD (N=4/group; 4 mice/sample; *p<0.05 vs. matching lean control by T-test). **C)** RT-PCR analysis of MHCII family gene expression in visceral adipocytes (VA) and visceral ATMs (V ATM) of chow- and HFD-fed $LDLR^{-/-}$ mice. (N=3/group; p<0.05 vs. chow-fed (*) or diet-matched VA (†) samples by T-test). Expression is normalized against Ppia reference gene expression. Data represent means±SEM.



CD45, MHCII

Population	Count	%Gated
All	985	100
Leukocyte Gate	0	0

CD45, MHCII

Population	Count	%Gated
All	15776	100
Leukocyte Gate	9	0.06

CD45, MHCII

Population	Count	%Gated
All	15325	100
Leukocyte Gate	1	0.01

Fig. S2. Isolated adipocytes demonstrate very low contamination with leukocytes. CD45-depleted human adipocytes incubated with CD45-PE and HLA-DR-APC-eF780 antibodies were analyzed by low pressure flow cytometry on an Amnis Imagestream^{x} system to visualize leukocyte contamination (CD45+ cells). **A)** unstained adipocytes and CD45-/HLA-DR-stained **B**) SQ adipocytes and **C)** V adipocytes.

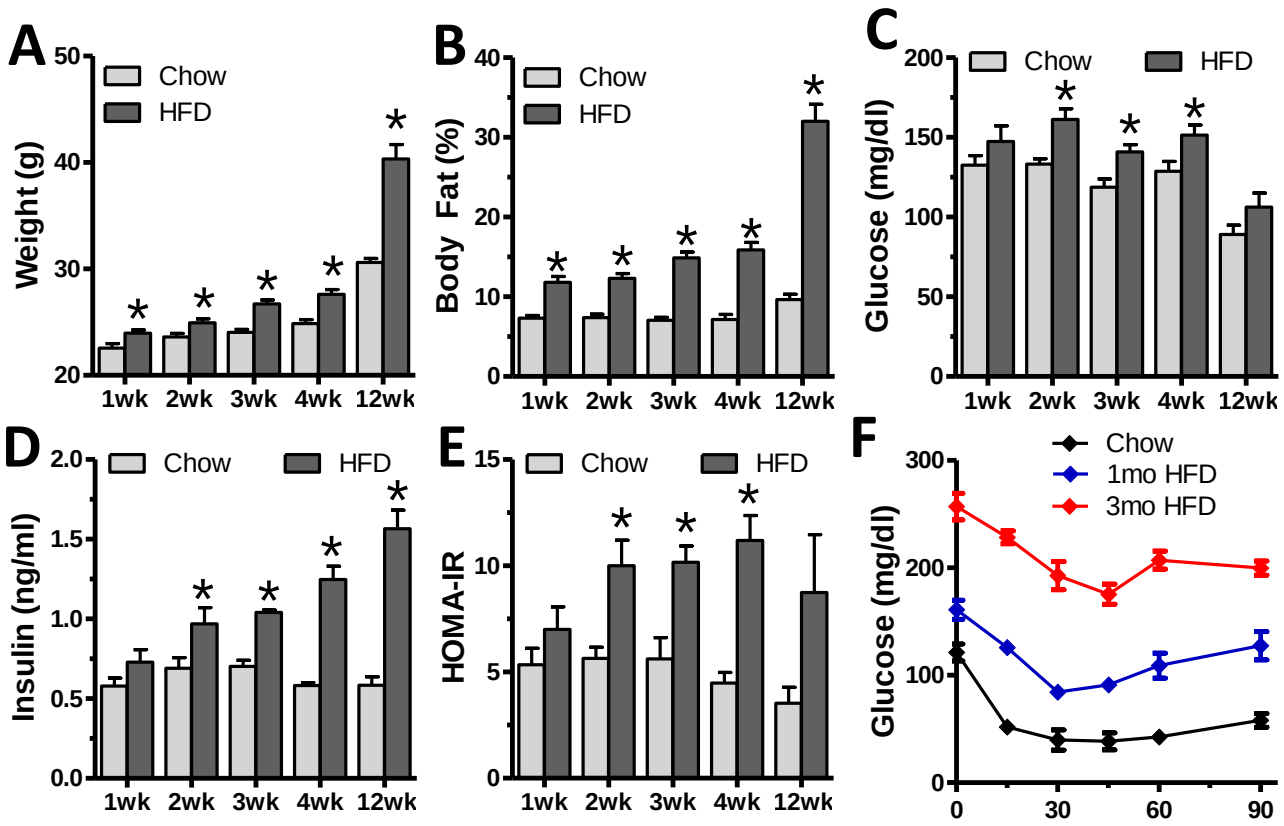


Fig. S3. HFD challenge induces rapid physical and metabolic changes in C57BL/6 mice. A) Body weight, **B)** NMR-determined body fat, **C**) fasting plasma glucose, **D)** insulin, **E)** HOMA-IR and **F)** intraperitoneal insulin tolerance test in 8-week-old male C57BL/6 mice fed 1 to 12 weeks of chow or HFD. (N=9-36/group; *p<0.05 vs. chow-fed mice by T-test). Data represent means±SEM.

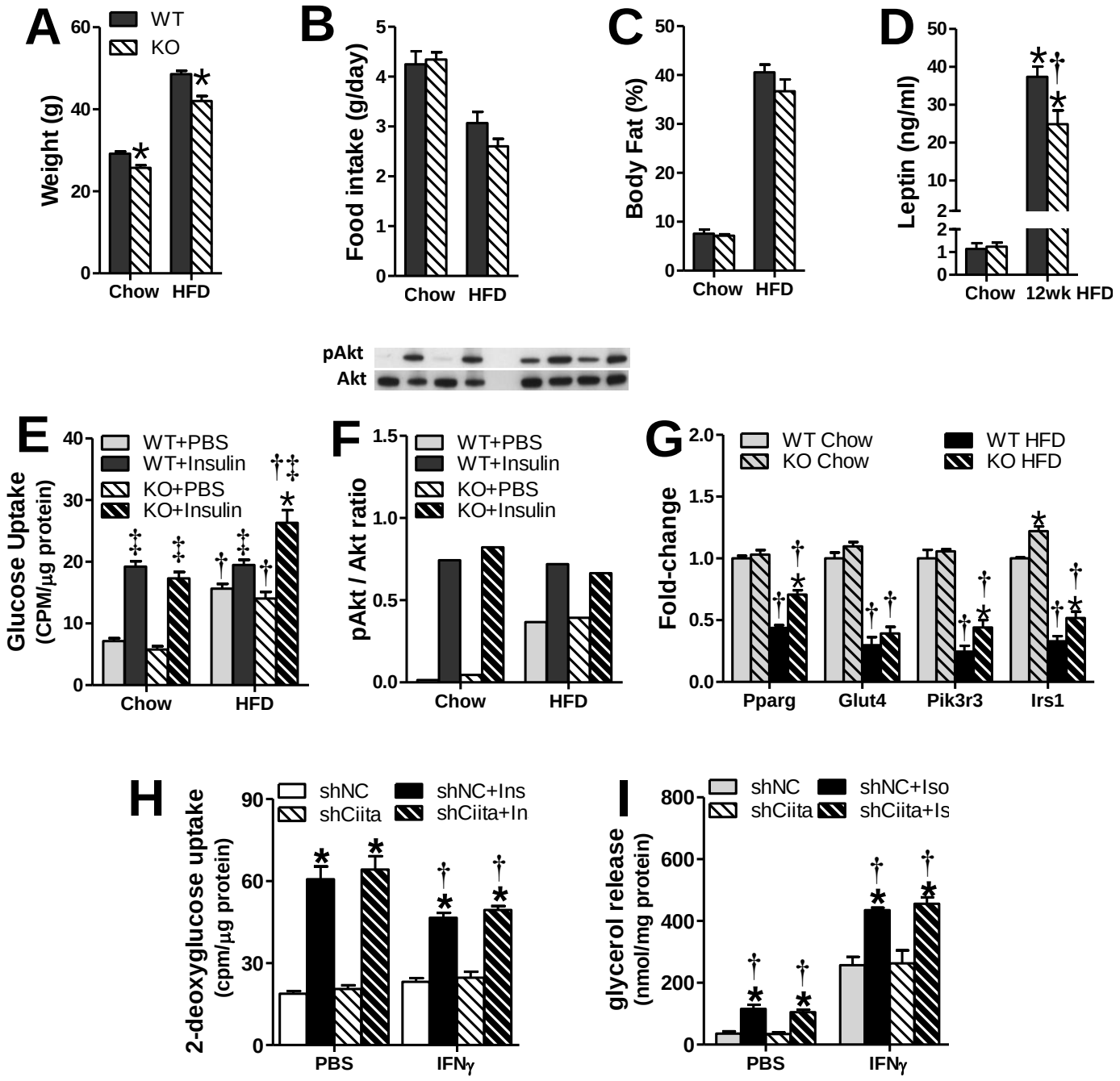


Fig. S4. Weight gain, food intake and body fat and adipocyte function of H2A$^{-/-}$ and WT mice fed chow or HFD. A) Body weight, **B)** food intake and **C)** NMR-determined body fat and **D)** plasma leptin concentration in male H2A$^{-/-}$ (KO) and WT mice fed 3 months of chow or HFD. (N= N=6-12/group; $p<0.05$ vs. matching genotype (*) or diet (†) by T-test). Basal (PBS-treatment) and insulin-stimulated **E)** 2-deoxyglucose uptake and **F)** pAkt/Akt protein ratios and **G)** basal metabolic gene expression in isolated visceral adipocytes of WT and KO mice fed 3 months of chow or HFD. (**E:** N=3/ group, $p<0.05$ vs. KO+PBS (*), genotype- and treatment-matched chow (†) or non-insulin-treated, genotype- and diet-matched (‡) group by T-test; G: N=4/group, $p<0.05$ vs. WT HFD (*) or genotype-matched chow (†) group by T-test). **H)** Insulin-stimulated 2-deoxyglucose uptake or **I)** isoproternol-induced lipolysis in non-induced (PBS-treated) or induced (insulin (Ins) or isoproterenol (Iso)) 3T3-L1 adipocytes expressing negative control (shNC) or Ciita (shCiita) shRNAs previously incubated 24 hours with PBS (negative control) or with INFγ to induce MHCII expression. (N=4/group, $p<0.05$ vs. shRNA-matched sample not treated with insulin or isoproterentol (*) or shRNA-matched and treatment-matched PBS-treated sample (†) by T-test.) Data represent means±SEM.

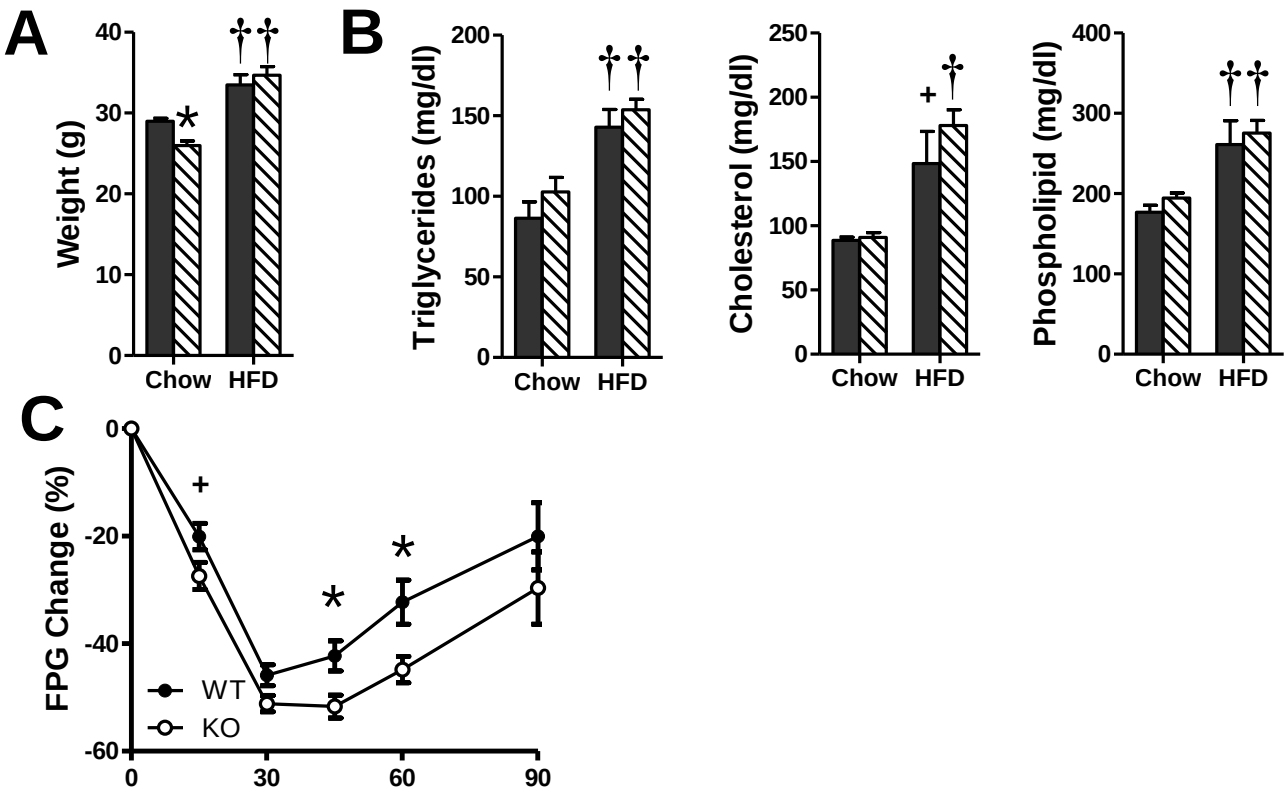


Fig. S5. Weight gain, food intake and body fat in H2A$^{-/-}$ and WT mice fed 1month of chow or HFD. A) Body weight, **B)** plasma lipids and **C)** intraperitoneal insulin tolerance test of H2A$^{-/-}$ and WT mice fed 1 month of chow or HFD. (N=6-12/group; $p<0.05$ vs. matching diet (*) or genotype-matched chow (†) or $p<0.1$ vs. genotype-matched chow (+) mice by T-test). Data represent means±SEM.

		Subject population (N=44)		Microarray subjects		
				Lean (N=7)	Obese (N=7)	
		Mean ± SE	(Quartile Range)	Mean ± SE	Mean ± SE	
	Age (years)	57.6± 1.5	(51.0 - 64.8)	57.7± 2.8	60.7± 2.5	
	BMI (kg/m^2)	31.7± 1.3	(24.7 - 36.1)	20.8± 1	32.8± 0.9	*
MS factors	Waist (in)	39.3 ± 1.2	(34.6 – 46.5)	29.9 ± 1.4	40.8 ± 1.6	*
MS factors	FPG (mg/dL)	87.9± 3.6	(75.0 - 93.5)	74.9± 2.7	85.0± 7.5	
MS factors	TG (mg/dL)	126.5± 10.5	(74 - 158)	90.4± 19.2	107.3± 15.4	
MS factors	HDL (mg/dL)	54.5± 2.0	(46 - 61)	60.7± 6.9	55.3± 3.1	
MS factors	SBP (mmHg)	130.2± 3.1	(113 - 146)	121.6± 8.6	131.7± 6.9	
MS factors	DBP (mmHg)	70.2± 2.4	(58 - 82)	65.1± 5.5	69.7± 5.6	
	FPI (mU/mL)	10.4± 1.4	(4.0 – 12.8)	3.4± 0.6	10.1± 2.4	*
	HOMA-IR	2.5± 0.4	(0.8 – 3.2)	0.6± 0.1	2.1± 0.5	*
	hsCRP (mg/L)	5.1± 0.8	(1.5 - 9.3)	2.2± 0.7	4.2± 1.4	

Table S1. Phenotypic parameters of the total subject population and the lean (BMI <25 kg/m^2) and obese (BMI >30 kg/m^2) post-menopausal women selected for microarray analysis. Asterisks indicate significant differences (p-value <0.05 by T-test) between lean and obese microarray subjects. Phenotype abbreviations: body mass index (BMI), Fasting plasma glucose (FPG) and insulin (FPI), triglycerides (TG), high-density lipoprotein cholesterol (HDL), systolic and diastolic blood pressure (SBP and DBP), homeostatic model assessment of insulin resistance (HOMA-IR) and high-sensitivity C-reactive protein (hsCRP). Data represent Means±SEM

Rank	Name	Size	NES	GSEA p-value	FDR q-value	Sigmif. genes	MHCII family
1	ALLOGRAFT_REJECTION	29	2.85	0	0	13	*
2	ASTHMA	22	2.84	0	0	15	*
3	LEISHMANIA_INFECTION	62	2.82	0	0	26	*
4	GRAFT_VERSUS_HOST_DISEASE	31	2.60	0	0	14	*
5	HEMATOPOIETIC_CELL_LINEAGE	65	2.52	0	0	26	*
6	LYSOSOME	113	2.51	0	0	21	
7	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	38	2.47	0	0	16	*
8	VIRAL_MYOCARDITIS	61	2.46	0	0	18	*
9	TYPE_I_DIABETES_MELLITUS	35	2.46	0	0	14	*
10	AUTOIMMUNE_THYROID_DISEASE	32	2.44	0	0	13	*
11	NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	93	2.39	0	0	30	
12	CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	181	2.38	0	0	50	
13	COMPLEMENT_AND_COAGULATION_CASCADES	54	2.36	0	0	19	
14	FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	89	2.33	0	0	21	
15	ECM_RECEPTOR_INTERACTION	71	2.23	0	0	16	
16	CELL_ADHESION_MOLECULES_CAMS	102	2.18	0	0	20	*
17	NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	57	2.18	0	0	15	
18	LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	95	2.17	0	0	20	
19	SYSTEMIC_LUPUS_ERYTHEMATOSUS	114	2.17	0	0	28	*
20	CHEMOKINE_SIGNALING_PATHWAY	152	2.16	0	0	32	
21	FOCAL_ADHESION	178	2.13	0	0	36	
22	ANTIGEN_PROCESSING_AND_PRESENTATION	64	2.02	0	0.001	16	*
23	DORSO_VENTRAL_AXIS_FORMATION	19	2.00	0.003	0.002	6	
24	B_CELL_RECEPTOR_SIGNALING_PATHWAY	68	1.94	0	0.003	19	
25	FC_EPSILON_RI_SIGNALING_PATHWAY	65	1.91	0	0.005	19	
26	PRION_DISEASES	29	1.89	0.003	0.006	6	
27	PATHOGENIC_ESCHERICHIA_COLI_INFECTION	47	1.84	0	0.009	5	
1	OLFACTORY_TRANSDUCTION	61	-2.25	0	0	21	
2	RIBOSOME	85	-2.12	0	0	13	

Table S2. KEGG pathway gene sets detected by GSEA. Pathways are sorted by GSEA FDR q-value rank (GSEA rank), and annotated with the number of pathway genes detected on the microarray (Size), GSEA nominal enrichment score (NES, pathways up-regulated in obesity have positive NES scores), GSEA NES p-values and FDR q-values, and the number of significant differentially-expressed genes. Gene sets containing differentially-expressed MHCII family gene are indicated by asterisks.

Supplemental Procedures

Study subjects SQ adipose biopsies were obtained from 44 female patients (34 to 78 years-of-age, BMI 16.3 to 48.5 kg/m^2, 35 Caucasian, 6 Hispanic, and 3 African-American) undergoing elective abdominal surgery at The Methodist Hospital (Table 1). Exclusion criteria included fever or infection, renal or neoplastic disease, organ transplantation, steroid use, estrogen replacement, AIDS, >10% body weight change within the prior 3 months, or previous diagnosis of type-1 diabetes, hemochromatosis, or lipodystrophy. Subjects were phenotyped for blood pressure, body mass index (BMI), waist circumference, fasting plasma glucose, triglycerides, high-density lipoprotein cholesterol (HDL), high-sensitivity C-reactive protein (hsCRP) and insulin at The Methodist Hospital Clinical Laboratory and plasma TNFα, IL-6, CCL2, and leptin (Millipore MADPK-71K). Fasting blood samples were obtained on the day of surgery prior to the procedure. SQ abdominal and visceral adipose was biopsied at the incision site near the umbilicus, immediately transferred to ice-cold saline and rapidly processed for adipocyte isolation. This study was approved by the Institutional Review Board of The Methodist Hospital. All subjects gave written informed consent prior to participation.

Microarray analyses. SQ adipocyte RNA isolated from 7 lean (BMI<25 kg/m^2) and 7 obese (BMI>30 kg/m^2) women closely matched for age and NCEP III metabolic syndrome parameters exclusive of waist circumference, (Table 1) was chosen for microarray analysis. Samples were selected from postmenopausal women to reduce hormone-mediated effects on gene expression, and from patients who undergoing similar surgical procedures, with similar histories of hypertension and anti-lipid, anti-hypertensive, and anti-inflammatory medication usage.

RNA quantity and quality was analyzed by UV spectrophotometry and electrophoresis on an Agilent 2100 Bioanalyzer. Genome-wide transcriptome analyses were performed by MOgene, LC (St. Louis, MO) using Agilent Human Whole Genome 4x44K arrays. Chips were scanned using the Agilent Technologies G2505C Microarray Scanner System with the extended dynamic range option. Image analysis and data collection were carried out using the Agilent Feature Extraction (AFE) 10.7.3.1. Background correction, normalization, filtering according to quantification flags, summarization and compiling of annotation information were performed with the R-2.13.2 platform using the Bioconductor Agi4x44Preprocess package under default settings (Lopez-Romero).

All qualified probes corresponded to transcripts with valid/Entrez identifiers and multiple probes corresponding to a same gene (Entrez ID) were consolidated using the GSEA platform CollapseDataset tool with default settings (Subramanian et al., 2005). Genes showing significant differential expression between obese and lean adipocytes were identified using SAM (Significance Analysis of Microarrays) (Tusher et al., 2001).

Raw microarray data sets have been deposited in the NCBI Gene Expression Omnibus (GEO) database under accession number GSE44000.

Cell culture. Post-confluent 3T3-L1 cells were induced to differentiate in DMEM with 10% FBS, 1μM dexamethasone, 0.5 mM IBMX, and 10 μg/ml insulin for 2 days, then maintained in DMEM with 10% FBS and insulin until harvest 8 days post-induction. Primary mouse adipocytes were cultured in DME/F-12 (1:1) with 10% FBS. Mouse spleen T cells were isolated with a Pan T Cell isolation Kit from Miltenyi Biotec (Germany), and cultured in RPMI 1640 with 10% FBS and 55μM 2-mercaptoethanol (Invitrogen). Human adipocytes and mouse 3T3-L1 preadipocyte and adipocytes were stimulated for 24hrs with PBS, 2ng/ml IFNγ, 5ng/ml TNFα, 10ng/ml IL-1β or 10ng/ml IL-6 prior to RT-PCR analsysis of MHCII family gene expression. IFNγ, IFNα, IFNβ, TNFα, IL-1β, IL-10, and IL-6 were obtained from R&D Systems (Minneapolis, MN). Ovalbumin (OVA), and polybrene were purchased from Sigma (Saint Louis, MO). Peritoneal macrophages, derived as previously described (Li et al., 1997), and 3T3-L1 adipocytes were treated 24hrs with PBS or 100pg/ml IFNγ ± 4hrs pre-treatment with 20ng/ml IL-10. C57BL6 mouse splenic T-cells were isolated using a CD4+ T Cell isolation Kit (Miltenyi Biotec, Cambridge, MA) and incubated with PBS or 1μg/ml leptin ± 5μg/ml IFNγ-neutralizing antibody (eBioscience).

Adipocyte/T-cell co-cultures CD45-depeleted adipocytes (0.2x10^6 cells/well) of chow and HFD C57BL/6J mice and T-cells of C57BL/6-Tg(TcraTcrb) mice (2x10^6 cells/well) were incubated with or with 500 μg/ml OVA for 48 hours in 12 well plates, as single cultures or co-cultures in direct contact or with T-cells seeded in

the lower chamber of a transwell plate (Corning Inc., Corning, NY). Cultured cells were continuously shaken to ensure mixing of T-cells and floating adipocytes, and supernatant IL-2 and IFNγ concentrations were analyzed by ELISA (R&D Systems, Minneapolis, MN) after 48 hours incubation. Replicate cultures were supplemented with 1 μCi/well ^{3}H-thymidine after 48 hours culture with or without 500 μg/ml OVA and assessed for ^{3}H-thymidine uptake as a surrogate marker for DNA replication, as previously described (Levings et al., 2002). Mouse primary adipocytes and BMDMs were isolated, cultured and differentiated as previously described (Deng et al., 2011; Nguyen et al., 2007), seeded in 12 well plates at a density of $2x10^{6}$ cells/wells, then incubated with 500 μg/ml OVA for 48 hours and assayed for supernatant IL-2 and IFNγ concentrations by ELISA (R&D Systems).

DBA/1J mice matching the 3T3-L1 $H2^{q}$ haplotype were intraperitoneally-injected with 2 mg Imject Alum (Thermo Scientific) and 200 μg OVA to induce OVA-specific T-cells, reinjected with alum-OVA 5 days later, and sacrificed 14 days after first injection. Splenic T-cells were isolated using CD4+ T Cell isolation Kits (Miltenyi Biotec). Confluent wild-type and Ciita knockdown 3T3-L1 adipocyte 12 well cultures were incubated 24 hours with or without 2ng/ml IFNγ to induce MHCII expression, then repeatedly PBS-washed and co-cultured with T-cells isolated from OVA-immunized DBA/1J mice ($2x10^{6}$ cells/well) in the presence or absence of 500 μg/ml OVA. After 48 hours incubation in the presence or absence of OVA, IL-2 and IFNγ concentrations in cell supernatants were analyzed by ELISA (R&D Systems).

Glucose uptake and lipolysis 3T3-L1 adipocytes expressing Ciita-shRNA or negative control (NC)-shRNA constructs were induced to differentiate for 8 days in 6-well and 96-well plates. Adipocytes in 6-well plates were assayed for insulin-induced 3H-deoxyglucose uptake as previously described (Deng et al., 2011), while adipocytes in 96-well cultures were treated with or without 1 mM isoproterenol for 3 h, and basal and hormone-induced lipolysis was measured by using the Adipocyte Lipolysis Assay Kit from Zen-bio (Research Triangle Park, NC).

Lentiviral constructs Lentiviral plasmids pLKO.1, psPAX2 and pMD2.G were purchased from Addgene (Cambridge MA). The mouse shCiita sequence (5'-CCGGCAAGACTTACATGAGGCACTACTCGAGTAGT-GCCTCATGTAAGTCTTGTTTTTG-3') was cloned into pLKO.1, and HEK293T cells were transfected with pLKO.1 or pLKO.1-shCIITA plasmid and psPAX2 and pMD2.G packaging plasmids using Fugene 6 (Roche, Mannheim, Germany). 3T3-L1 preadipocytes were incubated with viral supernatant supplemented with 8 μg/ml polybrene for 2 days, then cultured in 2 μg/ml puromycin for 2 weeks to generate stable integrants for future experiments.

REFERENCES

Deng, T., Sieglaff, D.H., Zhang, A., Lyon, C.J., Ayers, S.D., Cvoro, A., Gupte, A.A., Xia, X., Baxter, J.D., Webb, P., et al. (2011). A Peroxisome Proliferator-activated Receptor {gamma} (PPAR{gamma})/PPAR{gamma} Coactivator 1{beta} Autoregulatory Loop in Adipocyte Mitochondrial Function. The Journal of biological chemistry 286, 30723-30731.

Levings, M.K., Sangregorio, R., Sartirana, C., Moschin, A.L., Battaglia, M., Orban, P.C., and Roncarolo, M.G. (2002). Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. The Journal of experimental medicine 196, 1335-1346.

Li, Y.M., Baviello, G., Vlassara, H., and Mitsuhashi, T. (1997). Glycation products in aged thioglycollate medium enhance the elicitation of peritoneal macrophages. Journal of immunological methods 201, 183-188.

Lopez-Romero, P. Agi4x44PreProcess, PreProcessing of Agilent 4x44 array data. http://www.bioconductor.org/packages/2.3/bioc/html/Agi4x44PreProcess.html, ed.

Nguyen, M.T., Favelyukis, S., Nguyen, A.K., Reichart, D., Scott, P.A., Jenn, A., Liu-Bryan, R., Glass, C.K., Neels, J.G., and Olefsky, J.M. (2007). A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. The Journal of biological chemistry 282, 35279-35292.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences of the United States of America 102, 15545-15550.
Tusher, V.G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. Proceedings of the National Academy of Sciences of the United States of America 98, 5116-5121.