Loss of cAMP signaling in CD11c immune cells protects against diet-induced obesity

Liping Zeng, D. Scott Herdman, Sung Min Lee, Ailin Tao, Manasi Das, Samuel Bertin, Lars Eckmann, Sushil Mahata, Panyisha Wu, Miki Hara, Ji-Won Byun, Shwetha Devulapalli, Hemal H. Patel, Anthony J.A. Molina, Olivia Osborn, Maripat Corr, Eyal Raz, and Nicholas J.G. Webster**

Detailed Research Methods

Mouse studies: C57BL/6 (B6) mice were purchased from Jackson Laboratories. *Gnas*$^{\Delta CD11c}$ (*Gnas*$^{tm5.1Lsw/tm5.1Lsw}$*/Tg(Itgax-cre)1-1Reiz*) mice and *Gnas*$^{\Delta LysM}$ (*Gnas*$^{tm5.1Lsw/tm5.1Lsw}$*/Lyz2*$^{tm1(cre)Ifo}$) mice were generated as previously described(Lee *et al.*, 2015). Briefly, to generate *Gnas*$^{\Delta CD11c}$ and *Gnas*$^{\Delta LysM}$ mice, floxed *Gnas*$^{fl/fl}$ (*Gnas*$^{tm5.1Lsw/tm5.1Lsw}$) mice were crossed with *CD11c-Cre* and *LysM-Cre (Lyz2-cre)* mice, respectively. Floxed mice were used as littermate controls. *Rag1-/-/Gnas*$^{\Delta CD11c}$ (Rag.ΔCD11c) mice were generated by crossing *Rag1-/-* mice with *Gnas*$^{\Delta CD11c}$ mice, and *Rag1-/-/Gnas*$^{fl/fl}$ (Rag.Flox) mice were used as their littermate controls. In metabolic balance studies, mice were placed in metabolic cages (Columbus Instruments) to assess their food intake, drinking, energy expenditure, O_2 consumption, CO_2 production and physical activity. Energy expenditure was calculated from VO_2 and RER using the Lusk equation, EE in Kcal/hr = (3.815 + 1.232 X RER) X VO_2 in ml/min. In some experiments, mice were fed high fat diet (HFD, D12492, 60% kcal from fat; 5.24 kcal/g, Research Diets Inc, New Brunswick, NJ) for 8-10 weeks to cause obesity, or subjected to cold room (4 ℃) stress for indicated time. Control mice were fed normal chow diet (NCD, rodent 5001). For the diet switch study, mice were fed with normal chow diet for 3 days and then switched to and fed with HFD for another 3 days. Mice used in this study were aged between 2 and 4 months unless otherwise stated. All mice were housed at 24 ± 2 ℃ on a 12 h light/12 h dark cycle (light on at 6 am) and the animals had free access to food and water prior to being placed in study groups. All procedures were approved by the Institutional Animal Care and Use Committee of University of California San Diego, School of Medicine.

Fecal microbiota transplantation: C57BL/6 germ-free female mice were obtained from UCSD Animal care program and maintained in flexible film isolators. Experimental mice were housed in Sentry SPP System (Allentown) on a 12 h light–dark cycle. Mice were fed with irradiated HFD or autoclaved 2019S (Envigo) and autoclaved distilled water ad libitum two weeks in advance fecal transplantation. Stool samples were collected freshly from HFD-fed KO and Flox female mice and suspended in PBS. Mice were gavaged with 100

µl of stool suspensions (0.1 g/mL) every week for 3 times. Body weight was measured every week using autoclaved beakers.

Glucose and insulin tolerance tests: For glucose tolerance test (GTT), mice were fasted for 8 h and injected with D-glucose (2 g/kg) intraperitoneally. Blood glucose was measured by tail bleed at 0 min and monitored at intervals up to 120 min using a glucose meter (Easy Step Blood Glucose Monitoring System, Home Aide Diagnostics, Inc., Deerfield Beach, FL). For insulin tolerance test (ITT), mice were fasted for 6 h and injected i.p. with recombinant human insulin (0.4 U/kg). Blood glucose was measured at intervals up to 120 min. Terminal fasting glucose was also measured following 6 h fasting. Terminal fasting plasma insulin was measured using the mouse insulin kit (Meso Scale Diagnostics, Rockville, MD) and the homeostatic model of insulin resistance (HOMA-IR) was calculated as follows: (fasting serum insulin concentration (mU/ml)) x (fasting blood glucose levels (mg/dl))/(405)

Core body temperature and infrared thermography: Implantable radio transmitters (Mini Mitter Respironics) were used to determine core body temperature and locomotor activity of freely moving Flox and KO mice. The mice were anesthetized using isoflurane, and transmitters were implanted into the peritoneal cavity. The animals were allowed to recover for 1 wk before baseline set recording for several days, and then core body temperature and locomotor activity were recorded over a 24 h period. For the cold challenge, cages were placed in the cold room at 4˚C for 4.5 h and body temperature recorded. Cages were then returned to room temperature for 1 h. Temperature was measured using an infrared camera (FLIR Systems). The mice were anesthetized using isoflurane and placed in dorsal or ventral positions to acquire static thermal images at a focal length of 40 cm. Thermal images were acquired by infrared camera mounted on top of a tripod during the light phase. The maximum temperature in the interscapular area of mice was calculated using FLIR Tool software.

Lipid absorption studies, lipid profiling and lipidomics: Mice were fasted overnight (≥16 hours) before lipid absorption experiments. sesame oil (400 µL/mouse) was

administered by oral gavage, using a blunt ball-tipped syringe. Blood was collected by tail bleed at indicated time points, and plasma triglyceride was measured using triglyceride quantification colorimetric/fluorometric Kit (Sigma, MAK266). For blood lipid profiling, 40 µL mouse blood samples collected from tail bleed were placed into the cholestech LDX cassettes within 8 minutes after collection and analysed using the cholestech LDX™ system for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TRG) according to the manufacturer's instructions. To assess in vivo lipolysis, serum glycerol was measured in blood obtained from non-fasted mice before and 15 min after i.p. injection of CL-316,243 (1 mg/kg). Serum glycerol were measured using glycerol assay kit (Sigma, MAK117) and normalized to the weight of body fat.

Morphological studies: Mouse tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. For histopathological evaluation, sections of 5-µm-thick were stained with haematoxylin and eosin according to standard protocols. For adipose tissue, pictures of representative areas from each section in ×100 magnification were taken, and Adiposoft software was used to calculate cell size of 3 images/section/mouse. Minimal 20 µm and maximum 100 µm thresholds were set for automated measurement of adipocyte diameter followed by manual correction. A frequency distribution was calculated for each group. Total adipocyte number within the distribution was subsequently calculated, and the frequency was converted to a percentage of total adipocytes counted.

RNA isolation and real-time PCR: Total RNA was extracted from cells or tissues using an PureLink™ RNA Mini Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. 0.5-1 µg RNA was transcribed to complementary DNA with the reverse transcription system (Invitrogen). Real-time PCR was performed on a Bio-Rad CFX384™ real-time PCR detection system using iTaq™ Universal SYBR® Green Super mix (BioRad). Primers used in this study were provided in **Supplementary Table 1**. Data were normalized to m36b4 and analyzed using the $^{\Delta\Delta}$CT method.

Protein preparation and western blotting: Proteins from cells or tissues were prepared using RIPA buffer (ThermoFisher Scientific) containing protease and phosphatase

inhibitors. Lysates were incubated for 30 min on ice and centrifuged for 15 min at 10,000 rpm at 4°C. Protein lysates were quantified using the micro-BCA protein assay kit (23225, ThermoFisher Scientific, New York, NY) using BSA as standard. Total protein lysates (10-20 µg) were separated on 10-15% polyacrylamide gels and transferred onto Polyvinylidene difluoride (PVDF) membrane (IPVH00010, Millipore Sigma). Membranes were blocked in 5% non-fat milk or BSA in PBS with 0.1% Tween 20 (PBST) for 1 h at RT and then incubated at 4˚C overnight with the primary antibody at indicated concentration. Next the membranes were washed for 5 min X 4 times with PBST and incubated with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, SantaCruz, CA) for 1 hour. The membranes were visualized on X-ray films or ChemiDoc Imaging System (BioRad, Hercules, CA) following the reaction with the enhanced chemiluminescence substrate (SuperSignal™ West Pico PLUS Chemiluminescent Substrate, ThermoFisher Scientific). Actin, HSP90 or a-tubulin were used as the internal controls. The representative blotting bands were repeated in at least three mice. For western blotting: anti-phospho-PKA substrates (4056), anti-phospho-CREB (Ser133, 9198), anti-CREB (9104), anti-phospho-p70 S6 Kinase (Thr389, 9206), anti-p70 S6 (9202), anti-β actin (3700), anti-NLRP3 (15101), anti-IL1b (12426), anti-PPARG (2435), anti-phospho-JNK (Thr183/Tyr185, 9255), anti-JNK (9258), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204,4370), anti-p44/42 MAPK (Erk1/2, 9102), anti-phospho-p38 MAPK (Thr180/Tyr182, 9215), anti-p38 MAPK (9228), anti-phospho-NFκB/p65 (Ser536, 3033) and anti-NFκB p65 (3034) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Antibodies to IκB were purchased from IMGENEX (IMG-127A). Antibodies to UCP1 (ab10983), PGC1α (ab188102) were purchased from Abcam (Cambridge, MA). Antibodies were used according to manufacturer's recommendation.

Cell culture: The 3T3-L1 cell line was obtained from ATCC and maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in an atmosphere of 5% CO_2. Unless otherwise described, chemicals used in this study were all obtained from Sigma and dissolved in appropriate solvents. 3T3-L1 preadipocytes were maintained in DMEM containing 100 U/mL penicillin, 100 µg/mL streptomycin and 10% calf serum and allowed to reach 100% confluence.

Cells were differentiated 2 days after confluency (designated as day 0) by the addition of DMEM with 10% FBS supplemented with 1 μg/mL insulin (Sigma, I6634), 0.25 μM dexamethasone (Sigma, D4902), and 0.5 μM 3-isobutyl-1-methylxanthine (IBMX, Sigma, I5879). After 3 days, the media were replaced with DMEM containing 10% FBS and 1 μg/mL insulin and were refreshed on day 6. On day 9, media were changed into DMEM with 10% FBS. 3T3-L1 adipocytes cells were ready for coculture experiments on day 10-14.

Bone marrow cells from mice were isolated by flushing femurs and tibias with PBS, supplemented with 2% FBS. The cells were centrifuged and then filtered through a 70 μm cell strainer before being re-suspended in RPMI-1640 containing 100 U/mL penicillin, and 100 μg/mL streptomycin and supplemented with 10% FBS and differentiated for 7 days in the presence of 10 ng/mL mouse recombinant GM-CSF (BioLegend; 576302). On day 3, an additional 50% of culture medium with 20 ng/ml GM-CSF was added, and on day 6, half of the medium was removed and supplemented with 10 ng/ml GM-CSF culture medium. Adherent cells were collected on day 7 and CD11c+ BMDM were selected by anti-CD11c linked magnetic beads (Stemcell; 18780). CD11c+BMDM Cells were primed by 3 h treatment with ultrapure LPS (100 ng/ml; Invivogen) alone; inflammasome stimulation was provided by treatment with ATP (5 mM; 1h).

Coculture of adipocytes and macrophages or splenocytes was performed in 2 different ways as follows, in the contact system, differentiated 3T3-L1 adipocytes was cultured in 24-well plates. CD11c+ BMDM or CD11c+ splenocytes ($1X10^5$/well) were harvested by CD11c positive selection magnetic beads (Stemcell) and then were plated onto 3T3-L1 adipocytes. Cells were stimulated with vehicle or CL-316,243 (1 μM) for 3 hours after cultured for 24 hours with contact to each other. Supernatants were collected for glycerol measurement (Sigma, MAK117). And cells were lysed and collected for protein quantification. In the transwell system, cells were cocultured by using transwell inserts with a 0.4-μm porous membrane (Corning) to separate adipocytes from BMDM or splenocytes. Differentiated 3T3-L1 adipocytes were seeded at the bottom of the well, whereas BMDM ($1x10^5$/well) were seeded on the membrane insert. After incubation for 24 hours, the inserts were removed and the cells in the lower well were stimulated with

vehicle or CL-316,243 (1 μM) for 3 hours. Supernatants were collected for glycerol measurement and cells were lysed and collected for protein quantification.

Hormones, cytokine measurements and ELISAs: Serum insulin was determined using a rat/mouse insulin ELISA kit (Millipore, Billerica, Merck KGaA, USA) as instructed. Serum adipsin (ab170244) and adiponectin (ab108785) were determined using a mouse adipsin/adiponectin ELISA kit (abcam). Cytokine/chemokine measurements in sera were performed using U-PLEX Adipokine assays (Meso Scale Discovery, Millipore). For measurement of cAMP levels, 3T3-L1 cells were serum-starved overnight and then treated with vehicle, forskolin (10 μM), 3% serum for 10 min at 37˚C, in the presence or absence of the PDE inhibitor isobutylmethylxanthine (IBMX, 200 μM) and/or non-selective beta-adrenergic receptor blocker propranolol (50 μM). Cyclic AMP level was determined by cyclic AMP ELISA kit (Cayman, 581001) according to the manufacturer's protocol.

Flow cytometry: Epididymal fat pads were weighed, rinsed three times in PBS, and then minced and digested with 1 mg/ml collagenase type II (Worthington) in PBS supplemented with 1% BSA for 30 min at 37˚C with shaking, followed by quenching with 10% FBS DMEM (Invitrogen). Cell suspensions were filtered through a 100-μm filter and centrifuged at 2000 g for 10 minutes. Stromal vascular cell (SVCs) pellets were then incubated with RBC Lysis Buffer (eBioscience) for 5 minutes prior to centrifugation (500 g for 5 minutes). Cells were then stained for viability with Aqua amine-reactive dye (Biolegend) for 10 min in the dark at room temperature. SVCs were resuspended in FACS buffer (PBS containing 2% FBS). SVCs were washed with FACS buffer and incubated with Fc Block for 20 minutes prior to staining with indicated fluorescently labeled primary antibodies or control IgGs, for 30 minutes on ice. After two times of wash, cells were used for analysis. Antibodies used for cell labeling were purchased from BD Pharmingen and eBiosciences. The data were acquired by a Cytoflex flow cytometer (Beckman Coulter) and analyzed by FlowJo Software.

Bone Marrow Transplantation: To generate chimeric mice, the CD45.1 lean host mice were lethally irradiated (950 Rads from a cesium source), followed by i.v. injection with 10^7

bone marrow (BM) cells harvested from CD45.2 GnasΔCD11c or Gnasfl/fl donor mice. All the recipients received sulfamethoxazole and trimethoprim in drinking water ad libitum for two weeks after irradiation to prevent opportunistic infections. The recipients were kept on HFD for 8 weeks and the body weight and food intake were recorded weekly. Assessment of hematopoietic reconstitution in the host was carried out by flow cytometric analysis of the percentage of CD45.2+ donor cells in the peripheral blood at sacrifice.

In a second experiment, $6x10^6$ bone marrow cells isolated from femur bones of GnasΔCD11c or Gnasfl/fl donor mice were i.v. injected into HFD-indued obese mice after irradiation at 950 Rads. Eight weeks after irradiation, chimerism was confirmed by qPCR analysis of Cre genomic DNA expression in peripheral blood cells as we previously described (de Jong et al., 2015; Lee et al., 2010). The mice were kept on HFD for another 13 weeks and the body weights were recorded weekly. GTTs and ITTs were carried out 7 weeks after BMT.

Oxygen consumption rate measurements: For the Seahorse study, FACS-sorted splenic cDC2 ($0.5x10^6$/well) of HFD-fed KO and flox mice were seeded into poly-D-lysine-coated XF96 plate and oxygen consumption rates (OCRs) were determined under basal conditions followed by the sequential addition of oligomycin (1 μM), FCCP (2 μM), and antimycin/rotenone (1 μM).

Oxygen consumption rates in fresh liver, soleus and gastrocnemius muscles, eWAT, iWAT, BAT were measured using the Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria). Approximately 50-100 mg was transferred into ice-cold preservation medium (BIOPS solution). O_2 fluxes in the indicated tissues were obtained upon CI substrates (glutamate/malate, Leak), followed by addition of ADP (Basal Oxphos), followed by addition of CI substrate pyruvate (CI max), then CII substrate succinate (CI & CII max), then FCCP to uncouple mitochondria (max UC), then addition of rotenone to inhibit CI to assess CII uncoupled (CII max uncoupled), and malonate/antimycin to inhibit CII and CIII activity to assess residual oxygen consumption (ROX).

In vitro and in vivo Macrophage chemotaxis assays: In vivo macrophage tracking experiments were performed as described previously, with minor modifications

(PubMed:22190646). CD11c+ BMDM were labeled with a fluorescent dye PKH26 following the protocol. After labeling, $2x10^6$ cells were resuspended in 200 µl PBS and then injected into obese WT mice via tain veil. 3 days after injection, the percentage of PKH26+ cells out of total ATM in eWAT was analyzed by flow cytometry.

In vitro macrophage migration assay was performed by using transwell inserts (Sigma, CLS3422). Briefly, CD11c+ BMDM (10^5/insert) were seeded in top chambers of transwell plates in 200ul of DMEM media with membrane inserts. To the well of the lower compartment, either fully differentiated 3T3L1 adipocytes or 10% FBS in 0.6 ml DMEM were added as attractant. 24 h after incubation, the cells that migrate to the lower surface of the membrane are fixed with 2% paraformaldehyde (Thermofisher, 15710-S) for 15 min at room temperature, followed by staining with 1% crystal violet for addition 20 min. The inserts were washed with PBS for several seconds to remove excess dye. Cells in the upper compartment of the insert were removed by gently wiping the upper side of the membrane with a cotton swab. The number of the cells on the lower side of the insert membrane were counted under microscope.

Determination of catecholamine contents by ultra-performance liquid chromatography (UPLC): Catecholamine amounts were measured on a UPLC (Waters). The snap-frozen adrenal glands, eWAT, iWAT samples were thawed and homogenized in 300 mL of cold PBS and centrifuged at 4˚C for 15 min at 15,000 x g. Plasma samples (200 µL) were thawed on ice and add 2 ng DHBA. Plasma, supernatants cleaned from residue, were collected and used for HPLC measurements. DA, NE and EPI concentrations were assayed using alumina extraction followed by separation and analysis with HPLC. Dihydroxybenzylamine (Sigma, 858781) was used as an internal standard in each tissue sample. The data were analyzed with Empower software (Waters).

Statistics: Data were analyzed using Prism (Graphpad) and are presented as mean ± SEM or mean ± SD. Statistical significance was determined using the unpaired two-tailed Student's t-test or 1-way or 2-way ANOVA followed by Tukey post-tests for multiple variables. A p value of < 0.05 was considered significant and is presented as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, or $****p < 0.0001$. Calorimetric data were analyzed by general

linear regression models using body mass or activity as covariates using the CalR(Mina et al., 2018) or MMPC Energy Expenditure Analysis software.

Supplementary Table 1. List of Antibodies for Western blot and Flow cytometry

Antibody	Vendor	Catlog#	Clone
Anti-mouse CD16/CD32	Biolegend	101320	93
Anti-mouse CD45	Biolegend	103132	30-F11
Anti-mouse CD4	Biolegend	100548	RM4-5
Anti-mouse CD8	Biolegend	100750	53-6.7
Anti-mouse CD11c	Biolegend	117306/117308	N418
Anti-mouse CD11b	Biolegend	101216	M1/70
Anti-mouse F4/80	Biolegend	123116/123112	BM8
Anti-mouse Ly-6G	Biolegend	127641	1A8
Anti-mouse Siglec-F	Biolegend	155506	S17007L
Anti-mouse CD45.1	Invitrogen	11-0453-82	A20
Anti-mouse CD45.2	Invitrogen	17-0454-81	104
Anti-mouse CD45.1	Invitrogen	11-0453-82	
Anti-mouse CD45.2	Invitrogen	17-0454-81	
phospho-PKA substrates	Cell Signaling Technology	4056	
phospho-CREB (Ser133)	Cell Signaling Technology	9198	
CREB	Cell Signaling Technology	9104	
Phospho-p70 S6 (Thr389)	Cell Signaling Technology	9206	
p70 S6	Cell Signaling Technology	9202	
HSP90	Cell Signaling Technology	4874	
β actin	Cell Signaling Technology	3700	
NLRP3	Cell Signaling Technology	15101	
IL1β	Cell Signaling Technology	12426	
PPARG	Cell Signaling Technology	2435	
α-Tubulin	Cell Signaling Technology	3873	
Phospho-JNK (Thr183/Tyr185)	Cell Signaling Technology	9255	
JNK	Cell Signaling Technology	9258	
phospho-p44/42 MAPK (Thr202/Tyr204)	Cell Signaling Technology	4370	
p44/42 MAPK	Cell Signaling Technology	9102	
phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling Technology	9215	
p38 MAPK	Cell Signaling Technology	9228	
phospho-NFκB/p65 (Ser536)	Cell Signaling Technology	3033	
NFκB/p65	Cell Signaling Technology	3034	
Phospho-HSL (Ser563)	Cell Signaling Technology	4139	
HSL	Cell Signaling Technology	18381	
ATGL	Cell Signaling Technology	2439	
Phospho-Perilipin 1 (Ser 522)	Valasciences	4856	
Perilipin 1	Cell Signaling Technology	9349	
UCP1	Abcam	10983	
PGC1α	Abcam	188102	
IκB	IMGENEX	IMG-127A	

Supplementary Table 2. Primer sequences for quantitative PCR

Gene	Sequence	Gene	Sequence
Adrb1	GCCCTTTCGCTACCAGAGTT	*Ckmtb1*	TGAGGAGACCTATGAGGTATTTGC
	ACTTGGGGTCGTTGTAGCAG		TCATCAAAGTAGCCAGAACGGA
Adrb2	AATAGCAACGGCAGAACGGA	*Serca2b*	ACCTTTGCCGCTCATTTTCCAG
	CTTCCTTGGGAGTCAACGCT		AGGCTGCACACACTCTTTACC
Adrb3	CACCGCTCAACAGGTTTGATG	*Adipsin*	TCGAAGGTGTGGTTACGTGG
	TCTTGGGGCAACCAGTCAAG		TGTTTTCGATCCACATCCGGT
36B4	AGGCGTCCTCGTTGGAGTG	*Adiponectin*	GCCAAACACCGATTGGGGT
	AGAGCTGGGTTGTTCTCCAG		GGCTCCAAATCTCCTTGGTAGTT
Cidea	ATCACAACTGGCCTGGTTACG	*Itgax*	GACCTAGCACACGGTTCTCC
	TACTACCCGGTGTCCATTTCT		GAGCTCCACTTTGGGTGGTG
Ucp1	CACCTTCCCGCTGGACACT	*Itgam*	CTTCTGGTCACAGCCCTAGC
	CCCTAGGACACCTTTATACCTAATGG		GGGGGACAGTAGAAACAGCC
Pgc1α	AGCCGTGACCACTGACAACGA	*Emr1*	CTTTGGCTATGGGCTTCCAGTC
	GCTGCATGGTTCTGAGTGCT		GCAAGGAGGACAGAGTTTATCGTG
Pparg	TGAAAGAAGCGGTGAACCACTG	*Ccl2*	CACTCACCTGCTGCTACTCA
	TGGCATCTCTGTGTCAACCATG		GCTTGGTGACAAAAACTACAGC
Oplah	ACACAAGCAAGACTAGAGCGG	*IL6*	ATCCAGTTGCCTTCTTGGGACTGA
	CCGGGTTCTCCACGATACAG		TAAGCCTCCGACTTGTGAAGTGGT
Pdk4	CTACGGATCCTAACCACCGC	*IL10*	GGTTGCCAAGCCTTATCGGA
	TGCCTTGAGCCATTGTAGGG		CACCTTGGTCTTGGAGCTTATT
Fgf21	GTGTCAAAGCCTCTAGGTTTCTT	*Slc6a2*	GTGTGGCGGTTCCCTTATCT
	GGTACACATTGTAACCGTCCTC		CACAGCATAGCCCACTCCTTT
Maoa	TTCCAATGGGGGCTGTCATC	*Slc6a3*	CCTGTCCTGAAAGGTGTGGG
	GGGCAAGTATGAAGCCCATGA		TCCCAAAGGTGTCGTTGAGG
Maob	GGAAGGGTTCTACGTCAGCC	*Slc22a3*	GAAGGGATACCGTGGCTGAG
	TCGTGCAGGGACATCCAAAG		GACGCCAGGATCCCAAAGAT
Comt	CGAGGGGATGAGAGAGTCCTA	*IL1β*	GAAGAAGAGCCCATCCTCTG
	GCACGAACTCAAACCAACCA		TCATCTCGGAGCCTGTAGTG
Nlrp3	AGGCTGCTATCTGGAGGAACT	*Caspase-1*	AACGCCATGGCTGACAAGA
	GCAACGGACACTCGTCATCT		TGATCACATAGGTCCCGTGC
Crem	TGAGCAAATGTGGCAGGAAA	*Pycard*	TGACAGTGCAACTGCGAGAA
	TGCAATTGTTGCTACCTGAGC		CTGGTCCACAAAGTGTCCTGT

Supplementary Table 3. List of reagents

Reagent	Vendor	Catlog#
8-(4-Chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate sodium salt	Sigma-Aldrich	C3912
Forskolin	Sigma-Aldrich	F6886
3-Isobutyl-1-methylxanthine (IBMX)	Sigma-Aldrich	I5879
Insulin from bovine pancreas	Sigma-Aldrich	I6634
Dexamethasone	Sigma-Aldrich	D4902
Lipopolysaccharides	Sigma-Aldrich	L2630
Formoterol fumarate dihydrate	Sigma-Aldrich	F9552
Propranolol hydrochloride	Tocris	0624
Isoproterenol hydrochloride	Tocris	1747
Dibutyryl-cAMP	Tocris	1141
CL 316243	Tocris	1499
cOmplete™, Mini Protease Inhibitor Cocktail	Sigma-Aldrich	11836153001
Phosphatase Inhibitor Cocktail 3	Sigma-Aldrich	P0044
PKH26 Red Fluorescent Cell Linker Mini Kit for General Cell Membrane Labeling	Sigma-Aldrich	MINI26
Rp-cAMP	BioLog	A002S
Recombinant Mouse GM-CSF	BioLegend	576302
Zombie Aqua™ Fixable Viability Kit	BioLegend	423101
UltraComp eBeads™ Compensation Beads	Thermofisher	01-2222-42
ArC™ Amine Reactive Compensation Bead Kit	Thermofisher	A10628
RIPA Lysis and Extraction Buffer	Thermofisher	89900
qScript cDNA SuperMix	Quantabio	101414-102
RNA STAT-60	amsbio	CS-110
PowerUp™ SYBR™ Green Master Mix	Applied Biosystems™	A25742
Mouse/Rat Insulin Kit	Meso Scale Diagnostics	K152BZC-1
U-PLEX Metabolic Group 1 (ms) Assay	MSD	K15299K
Cyclic AMP ELISA Kit	Cayman	581001
EasySep™ Mouse CD11c Positive Selection Kit II	Stemcell	18780A
SULFATRIM PEDIATRIC SUSPENSION	PAI pharma	00121-0854-16

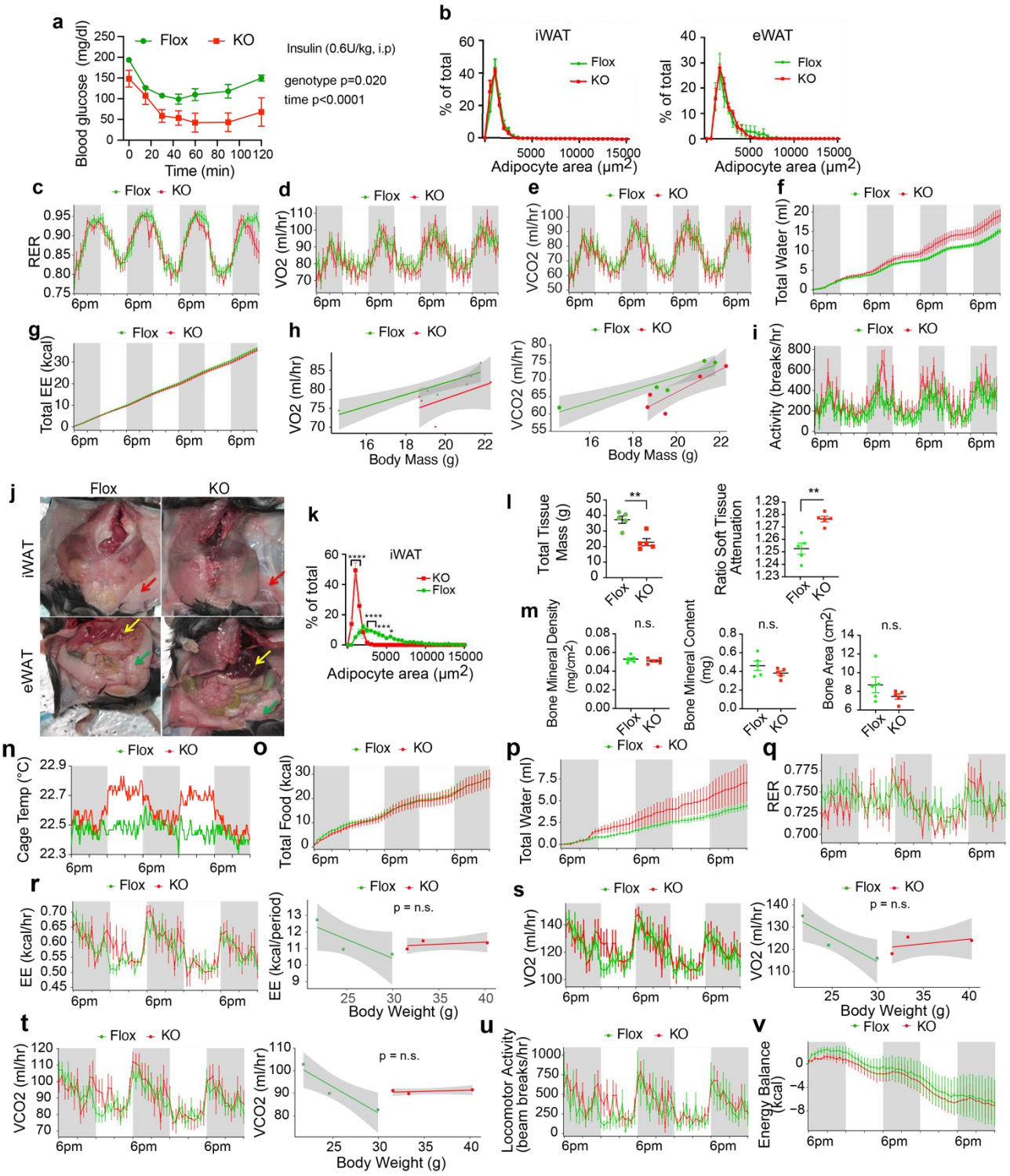

Fig. S1. Metabolic characterization of *Gnas* KO mice. (a) ITT on male mice on normal chow using 0.6 U/kg insulin. 2-way ANOVA indicated significant genotype and time effects. Flox mice are shown in green, KO mice in red. **(b)** Adipocyte area distribution in iWAT and eWAT from 4-month-old male Flox and KO mice on NCD, n=5/group. (**c-i**) Metabolic assessment of NCD-fed Flox and KO mice over 3 days and 4 nights. Gray blocks indicate the dark phase with lights out starting at 6 pm. (**c**) Circadian changes in respiratory exchange ratio (RER) over the course of the study. (**d**) Circadian changes in absolute oxygen consumption rate (VO_2) over the course of the study. (**e**) Circadian changes in absolute carbon dioxide production rate (VCO_2). (**f**) Cumulative water consumption. (**g**) Cumulative energy expenditure (EE). (**h**) Covariate analysis by generalized linear modeling (right) indicating a significant body mass effect (p=0.012) but the genotype effect was not quite significant (p=0.071) for VO_2 and significant body mass and genotype effects (p=0.002 & p=0.05, resp.) for

VCO_2. (**i**) Circadian changes in ambulatory activity over the course of study. (**j**) Representative images of eWAT and iWAT depots in Flox and KO mice on HFD. Red arrows indicate iWAT, green arrows eWAT and yellow arrows liver. (**k**) Adipocyte area distribution for iWAT from mice on HFD. (**l)** Total tissue mass and ratio of soft tissue from DEXA scans. (**m**) Bone mineral density, bone mineral content, bone area from DEXA. Metabolic assessment of HFD-fed Flox and KO mice by CLAMS: (**n**) cage temperature; (**o**) cumulative food intake; (**p**) cumulative water intake; (**q**) circadian changes in RER; (**r**) circadian changes in EE and covariate analysis; (**s**) circadian changes in VO_2 and covariate analysis; (**t**) circadian changes in VCO_2 over time and covariate analysis; (**u**) circadian changes in locomotor activity; and (**v**) cumulative energy balance. Data are represented as mean ± SEM and asterisks indicate statistical significance by 2-way repeated measures ANOVA or unpaired Student's t-test as appropriate; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

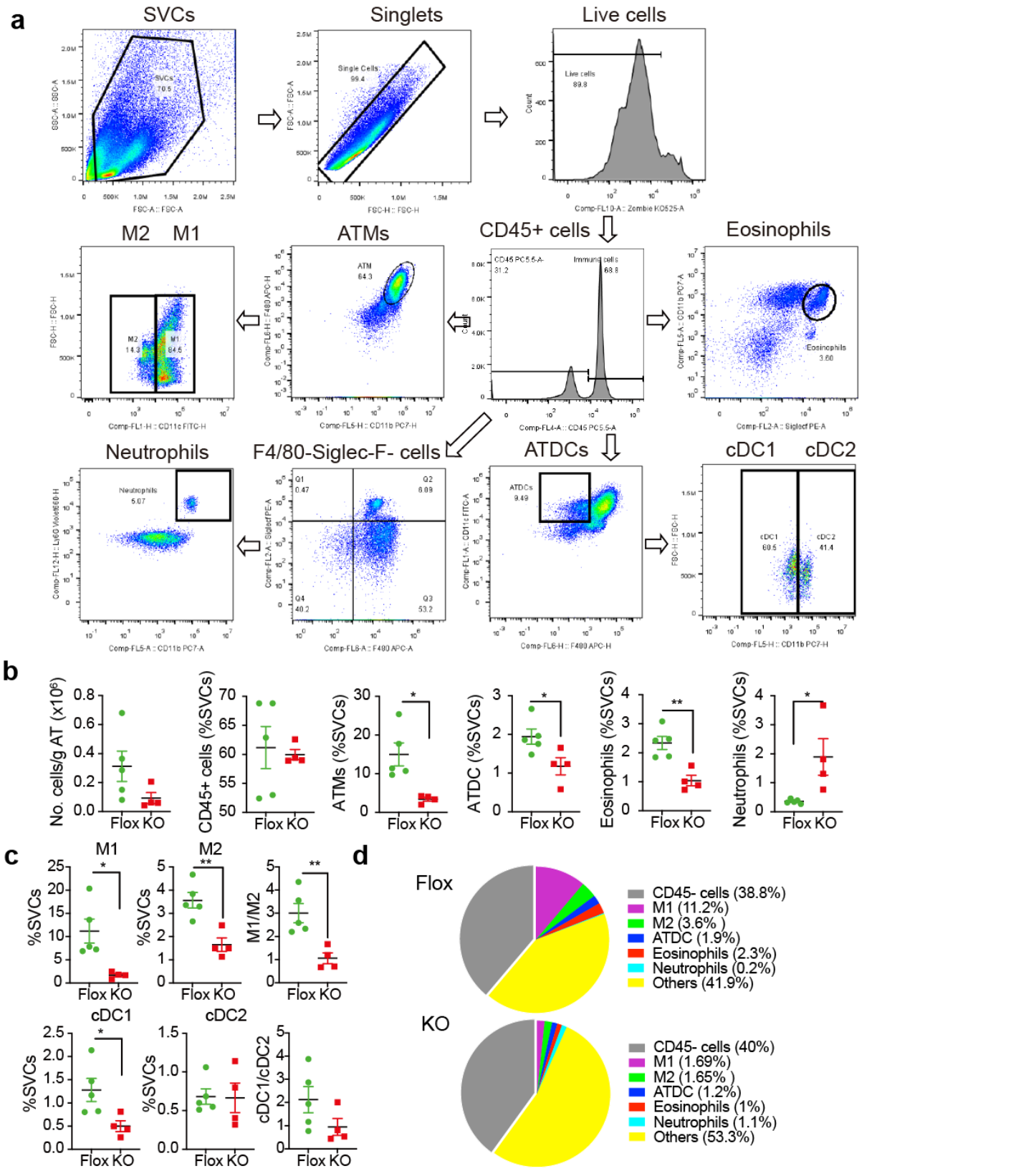

Fig. S2: Flow cytometry analysis of iWAT.

(a) Gating strategy for flow cytometry analysis of stromovascular cells from WAT. **(b)** Total number of stromovascular cells (SVCs) per gram tissue, % CD45 +ve cells in SVCs, % adipose tissue macrophages (ATMs), % adipose tissue dendritic cells (ATDCs), % eosinophils and % neutrophils. Data are presented as % of SVCs. Flox mice are shown in green, KO in red. **(c)** Numbers of CD11c+ve M1 macrophages and CD11c-ve M2 macrophages and ratio of M1/M2. Numbers of CD11b-ve cDC1 and CD11b+ve cDC2 subsets of dendritic cells. Data are presented as % of SVCs. Flox mice are shown in green, KO in red. **(d)** Pie chart showing relative proportions of cells in iWAT from Flox and KO mice.

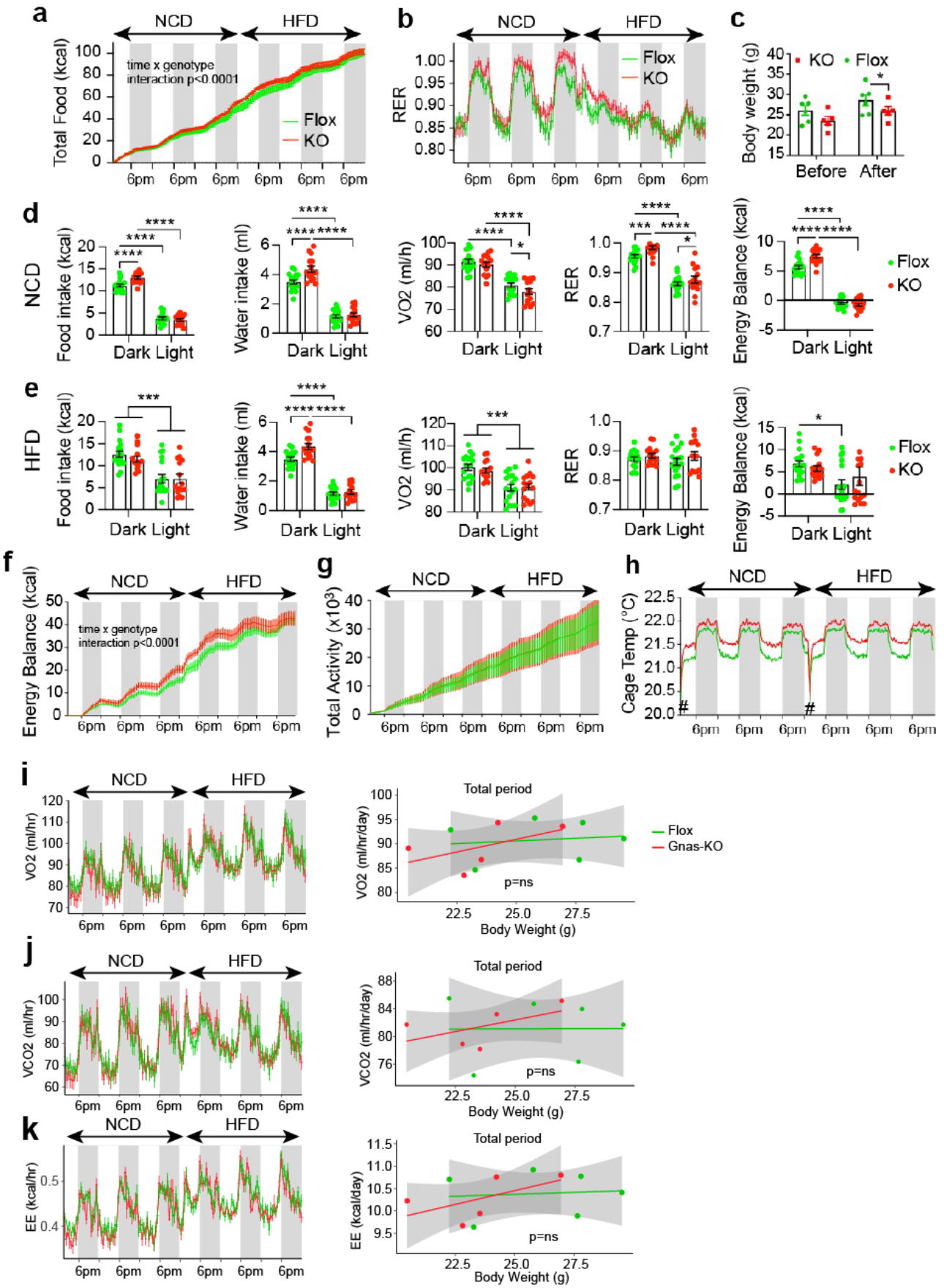

Fig. S3. Diet switch experiment in Flox and KO mice

For all panels *Gnas*$^{fl/fl}$ (Flox) mice are shown in green, *Gnas*$^{\Delta CD11c}$ (KO) mice in red. (**a**) Cumulative food intake in male mice in metabolic cages during three days of NCD feeding or three days of HFD

feeding. Gray shaded regions represent dark phase (lights off at 6 pm). Data are represented as mean ± SEM. 2-way repeated measures ANOVA indicated a time by genotype interaction ($p<0.0001$). (**b**) RER over time during NCD and HFD feeding. (**c**) Body weights before and after study. (**d**) Metabolic cage measurements during dark and light phases of three days of NCD feeding including food and water intake, VO2, RER, and energy balance. (**d**) Metabolic cage measurements during dark and light phases of three days of HFD feeding. (**f**) Cumulative energy balance over the entire course of the study. (**g**) Cumulative locomotor activity over the course of the study. (**h**) Average cage temperature over the course of the study. Hashtags indicate when the cages were opened to change diets. Data are represented as mean ± SEM and asterisks indicate statistical significance by 2-way ANOVA; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ for indicated comparisons. (**i**) VO_2 over time for entire period and covariate analysis for VO_2 and body weight. (**j**) VCO_2 over time for entire period and covariate analysis for VCO_2 and body weight. (**k**) Energy expenditure (EE) and covariate analysis against body weight. Data are represented as mean ± SEM and asterisks indicate Statistical significance by 2-way ANOVA or by covariate analysis; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p<0.0001$.

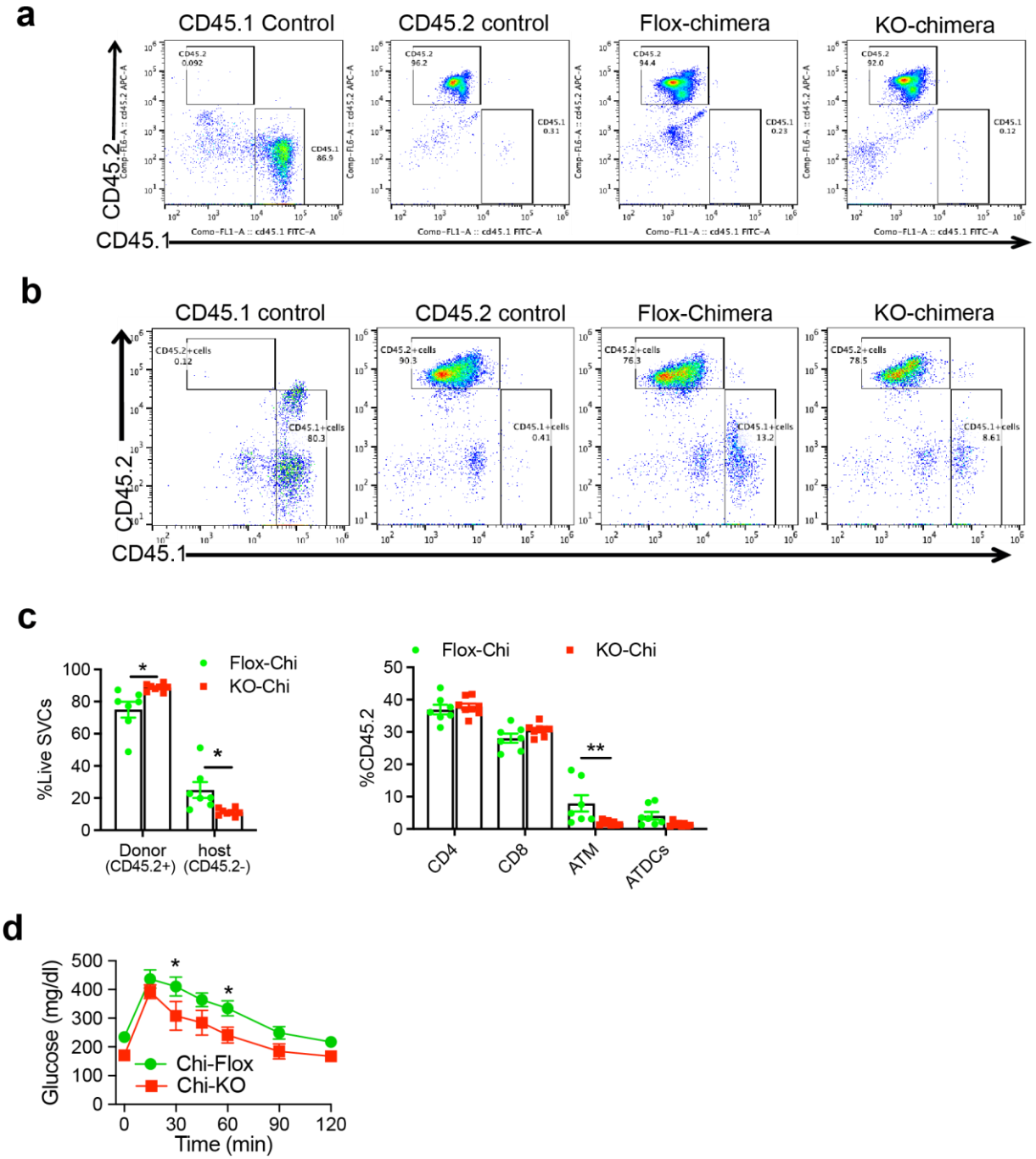

Fig. S4. Flow cytometric analysis of bone marrow engraftment.

(**a**) Analysis of engraftment percentage in peripheral blood mononuclear cells. CD45.2 cells are derived from donor and CD45.1 cells are from host recipient. (**b**) Analysis of engraftment percentage in eWAT. (**c**) Proportions of donor and host cells as % SVC in iWAT. Relative distribution of cell types in SVC from iWAT. Elevated B and T cells indicates contamination from inguinal lymph node. (**d**) GTT on obese chimeric mice after 11 weeks of engraftment.

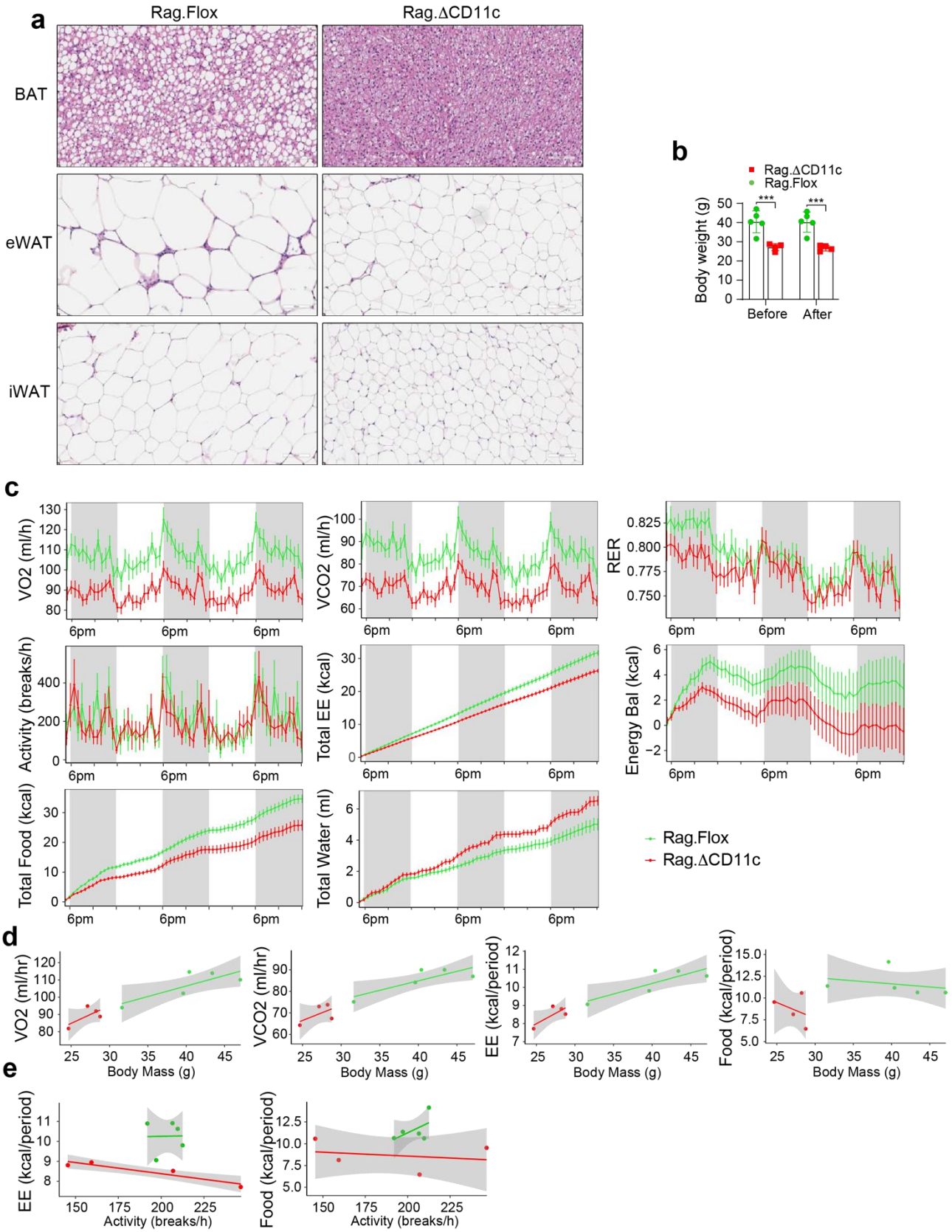

Fig. S5. Metabolic cage study on HFD-fed Rag.ΔCD11c mice

(**a**) Representative sections of BAT, eWAT and iWAT from Rag.Flox and Rag.ΔCD11c mice. (**b**) Body weights (BW) of the mice before and after the metabolic study. Rag.Flox mice shown in green and Rag.ΔCD11c mice in red. (**c**-**e**) Metabolic assessment of HFD-fed Rag.Flox and Rag.ΔCD11c mice: (**c**) Hourly VO2, VCO2, RER, locomotor activity, and cumulative EE, energy balance, food and water consumption over the course of the study. (**d**) Covariate analysis of VO2, VCO2, EE and food intake against body mass. VO2, VCO2 and EE showed a mass effect (p=0.0202, 0.0256 & 0.0303, resp.),

whereas food intake showed a genotype difference (p=0.0372) during the dark phase. (**e**) Covariate analysis of EE and food intake against locomotor activity. Both showed genotype effects (p=0.0063 & 0.0457, resp.). Data are represented as mean ± SEM. n=5 for Rag.Flox group and n=4 for Rag.ΔCD11c group; ***p ≤ 0.001 and ****p ≤ 0.0001 by 2-way ANOVA.

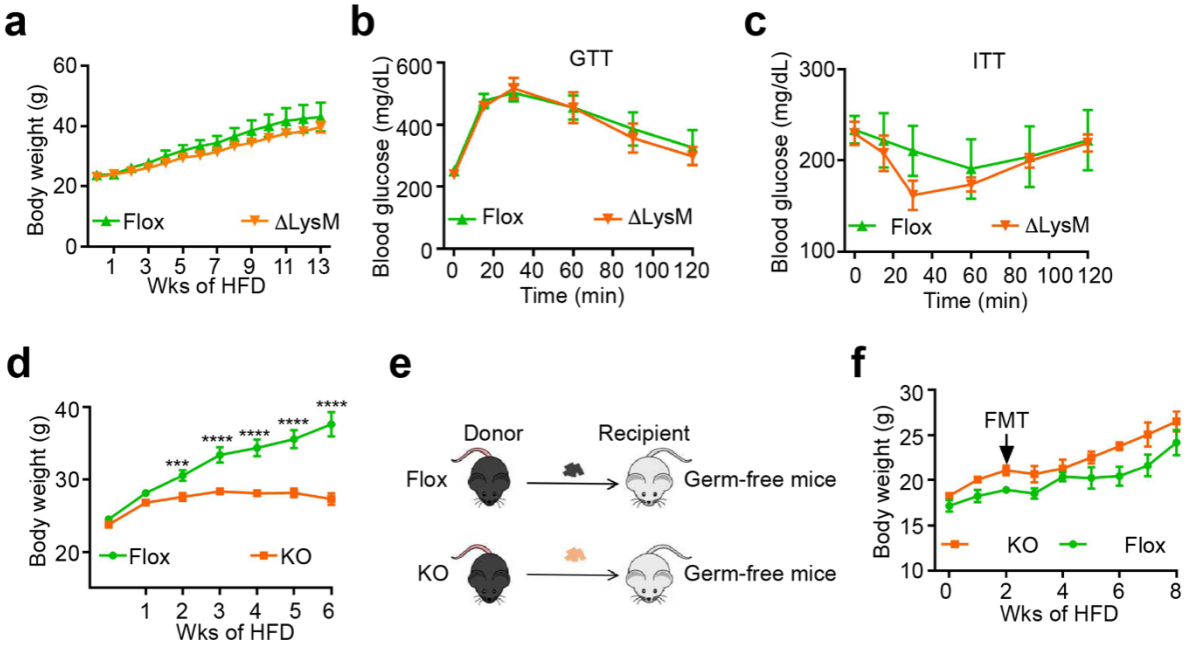

Fig. S6. Deletion of Gnas in macrophages using LysM-cre and effect of microbiota on lean phenotype.

(**a**) Body weights of $Gnas^{\Delta LysM}$ (ΔLysM) and $Gnas^{fl/fl}$ (Flox) male mice on HFD were measured over time for 13 weeks. Glucose tolerance test (**b**) and insulin tolerance test (**c**) in Flox and Flox mice on HFD (n=6 per group). (**d**) Body weight of co-housed $Gnas^{fl/fl}$ (Flox) and $Gnas^{\Delta CD11c}$ (KO) mice on HFD. (**e**) Schematic diagram of fecal microbiota transplantation on germ-free mice. 6 weeks-old germfree mice were first put on 60% HFD for 2 weeks. Thereafter, mice were orally treated with fecal microbiota of HFD-fed $Gnas^{fl/fl}$ and $Gnas^{\Delta CD11c}$ mice weekly for 3 weeks and body weight were followed for another 6 weeks. n=4 per group. (**f**) The recipient mice after fecal transfer from Flox or KO mice were put on HFD and body weights were recorded for 6 weeks. n=14 for Flox transfer group, n=10 for KO transfer group, ***p ≤ 0.001 and ****p ≤ 0.001 by 2-way ANOVA. Results represent mean ± SEM.

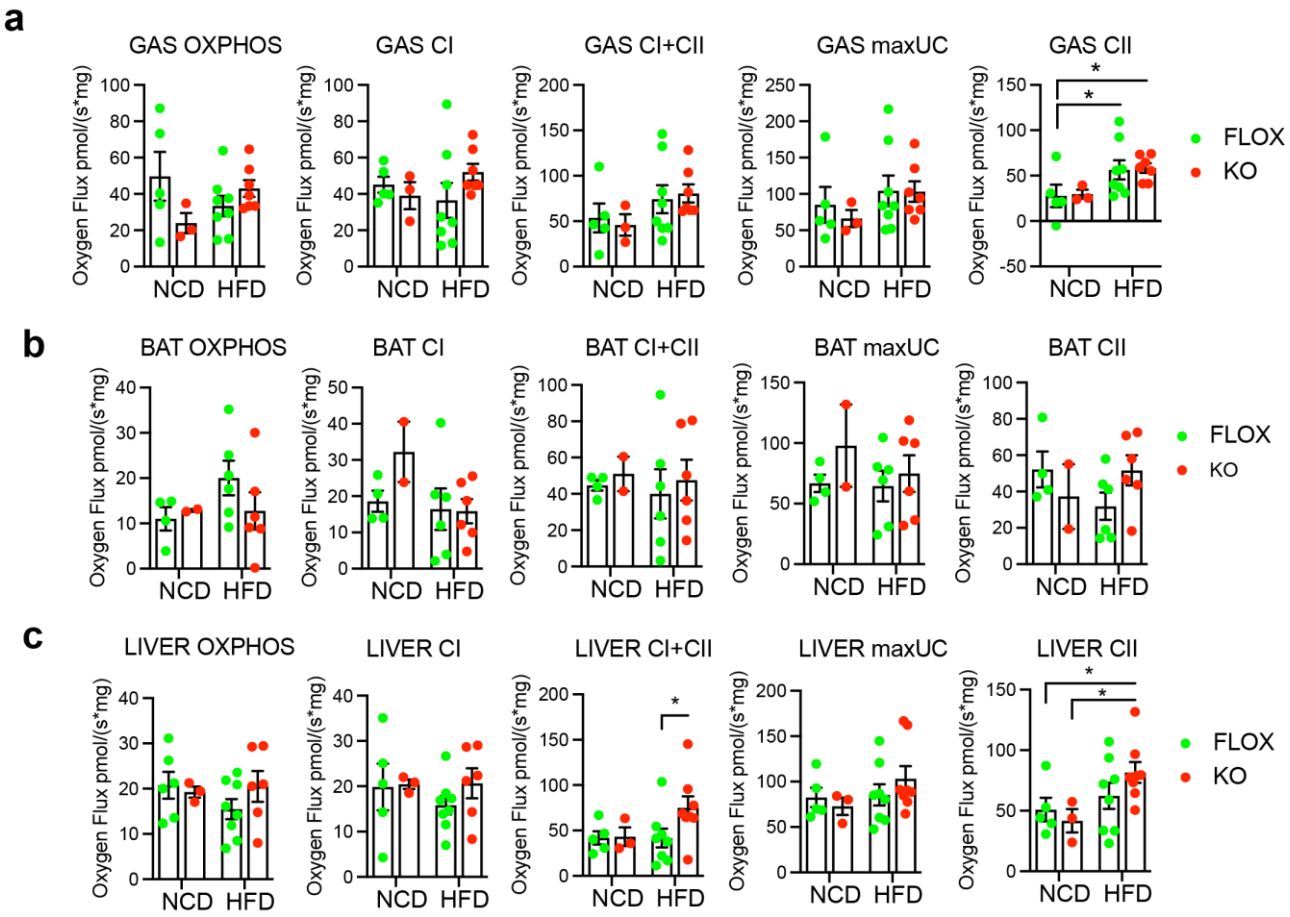

Fig. S7. Tissue oxygen flux by Oroboros.

(**a**) Gastrocnemius muscle (GAS). (**b**) Brown adipose tissue (BAT). (**c**) Liver. O_2 fluxes were obtained by addition of ADP (Basal Oxphos), then the CI substrate pyruvate (CI max), then CII substrate succinate (CI & CII max), then FCCP to uncouple mitochondria (max UC), then addition of rotenone to inhibit CI to assess CII uncoupled (CII max uncoupled).

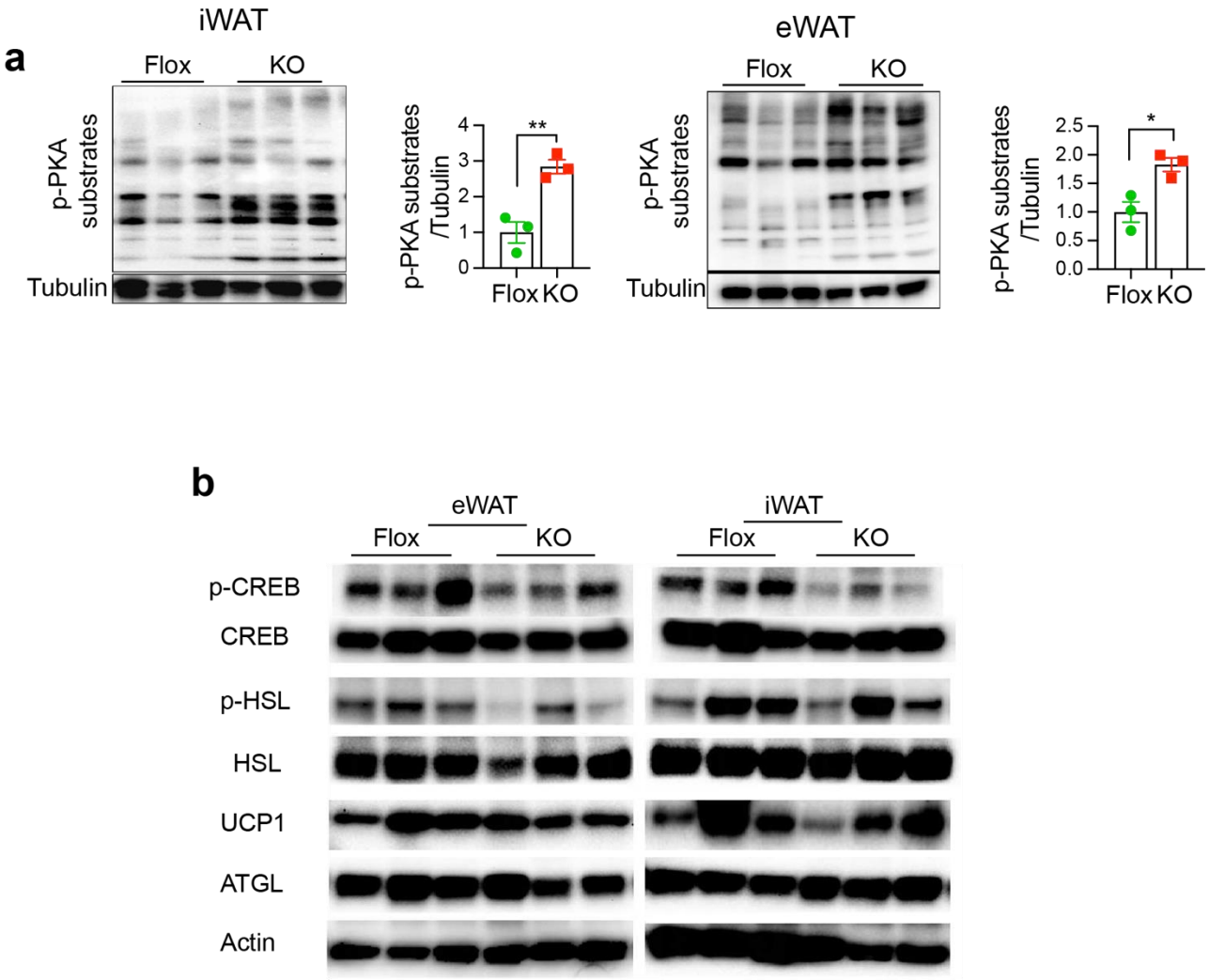

Fig. S8. Elevated PKA signaling
(**a**) Representative Western blot of iWAT (left) and eWAT (right) from Flox and KO mice on NCD with antibodies against phosphor-PKA substrate antibody. n=3 for each group. (**b)** Representative Western blot of eWAT (left) and iWAT (right) from Flox and KO mice on NCD with antibodies against pCREB-S133/CREB, pHSL-S563/HSL, UCP1, ATGL and Actin. n=3 for each group.

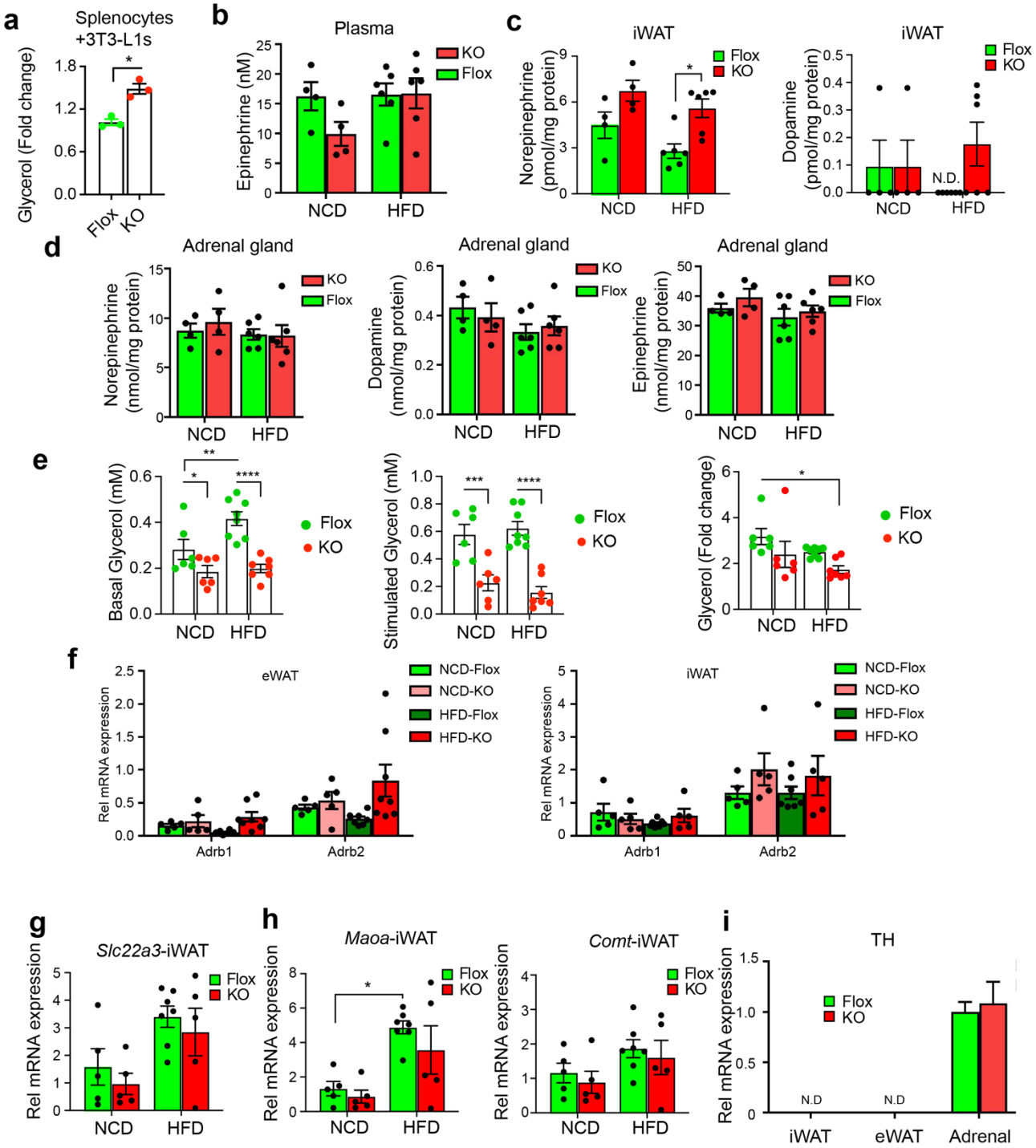

Fig. S9. Catecholamine levels, stimulation of lipolysis, and adrenergic receptor expression
(a) Fold change in glycerol levels in 3T3-L1 conditioned media when co-cultured with splenocytes from Flox or KO mice. (**b**) Plasma epinephrine levels in Flox and KO mice. (**c**) Norepinephrine levels in iWAT by UPLC. Dopamine levels in iWAT. (**d**) Adrenal norepinephrine, epinephrine and dopamine levels. (**e**) Basal serum glycerol levels (mM) in NCD-fed or HFD-fed Flox and KO mice. 2-way ANOVA indicated significant diet and genotype effects (p=0.05 and p<0.0001, resp.). (**d**) The change in serum glycerol levels (mM) 15 min after CL-316,243 injection. 2-way ANOVA indicated significant genotype effect (p<0.0001). (**e**) Fold increase in serum glycerol levels after CL injection in the mice (n=5-8/group). Statistical analysis indicated significant diet and genotype effects (p=0.0423 & p=0.0204, resp.). Relative mRNA levels of beta-adrenergic receptors (Adrb1, 2) in eWATs and iWATs of NCD- or HFD-fed Flox and KO mice, normalized to m36B4 as housekeeping gene (n=5, 5, 8, 8 for eWAT groups, n=5, 5, 7, 5 for iWAT groups). (**g-i**) Expression of *Slc22a3*, *Maoa*, and *Comt* in iWAT by QPCR. (**I**) Expression of tyrosine hydroxylase (TH) mRNA was determined by qPCR normalized to m36B4 in iWAT, eWAT and adrenal glands of NCD- or HFD-fed Flox and KO mice.

Data are represented as mean ± SEM and were analyzed by ANOVA. Asterisks indicate statistical significance; *p≤0.05, **p ≤ 0.01 and ***p ≤ 0.001.

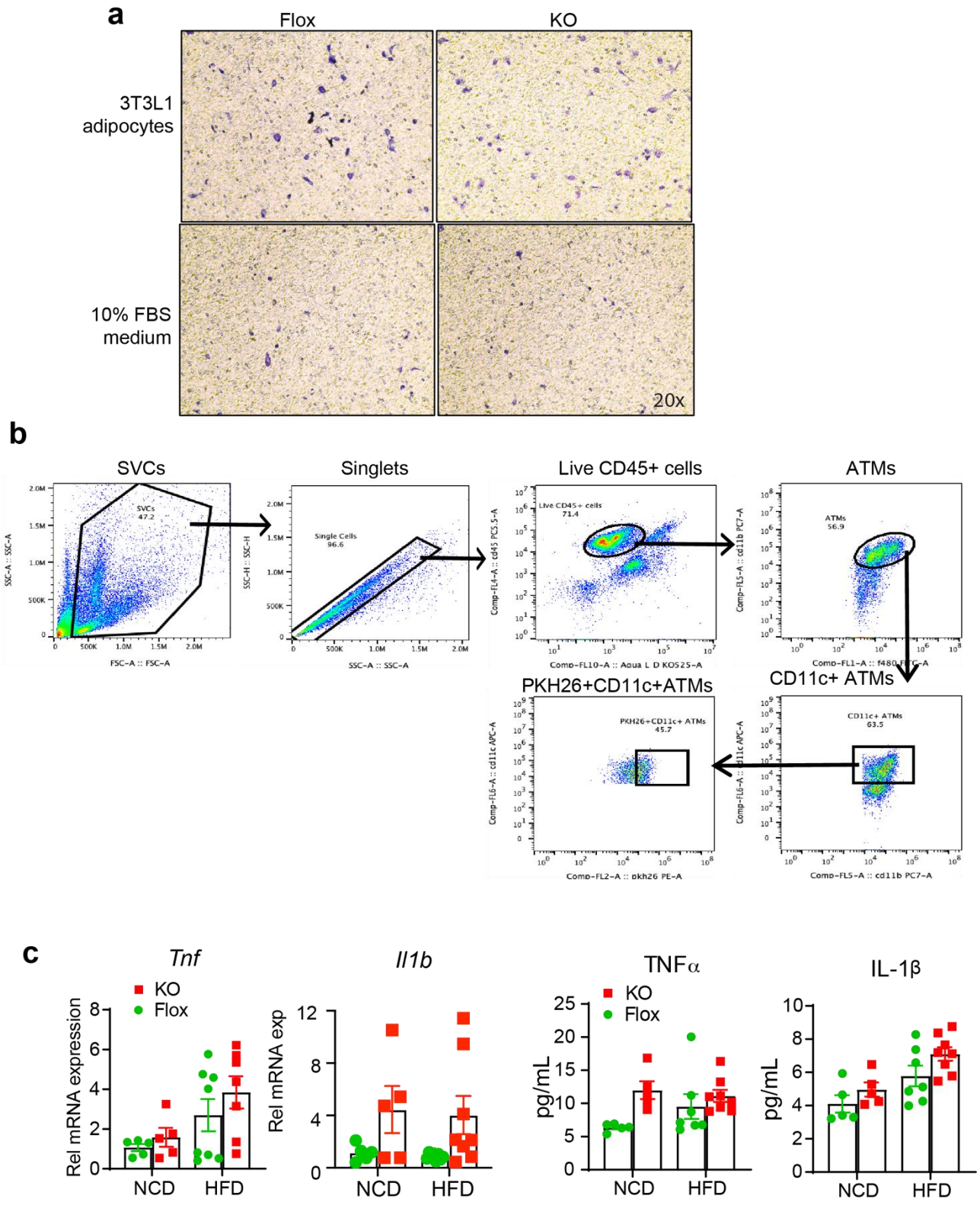

Fig. S10. Macrophage migration and cytokine profiles of KO mice
(**a**) In-vitro migration assay with BMDM from Flox or KO mice in response to 3T3-L1 conditioned media or 10% FCS. Cells migrated through the membrane are stained blue. (**b**) Gating strategy for

measurement of in-vivo migrated PKH26-labeled macrophages from WAT. (**c**) Gene expression of *Tnf* and *Il1b* in eWAT and plasma IL-1β and TNFα from Flox and KO mice on NCD or HFD. n=5-8 for each group. Statistical significance is indicated by asterisks **p<0.01; **p<0.0001 by 2-way ANOVA. Data are represented as mean ± SEM.

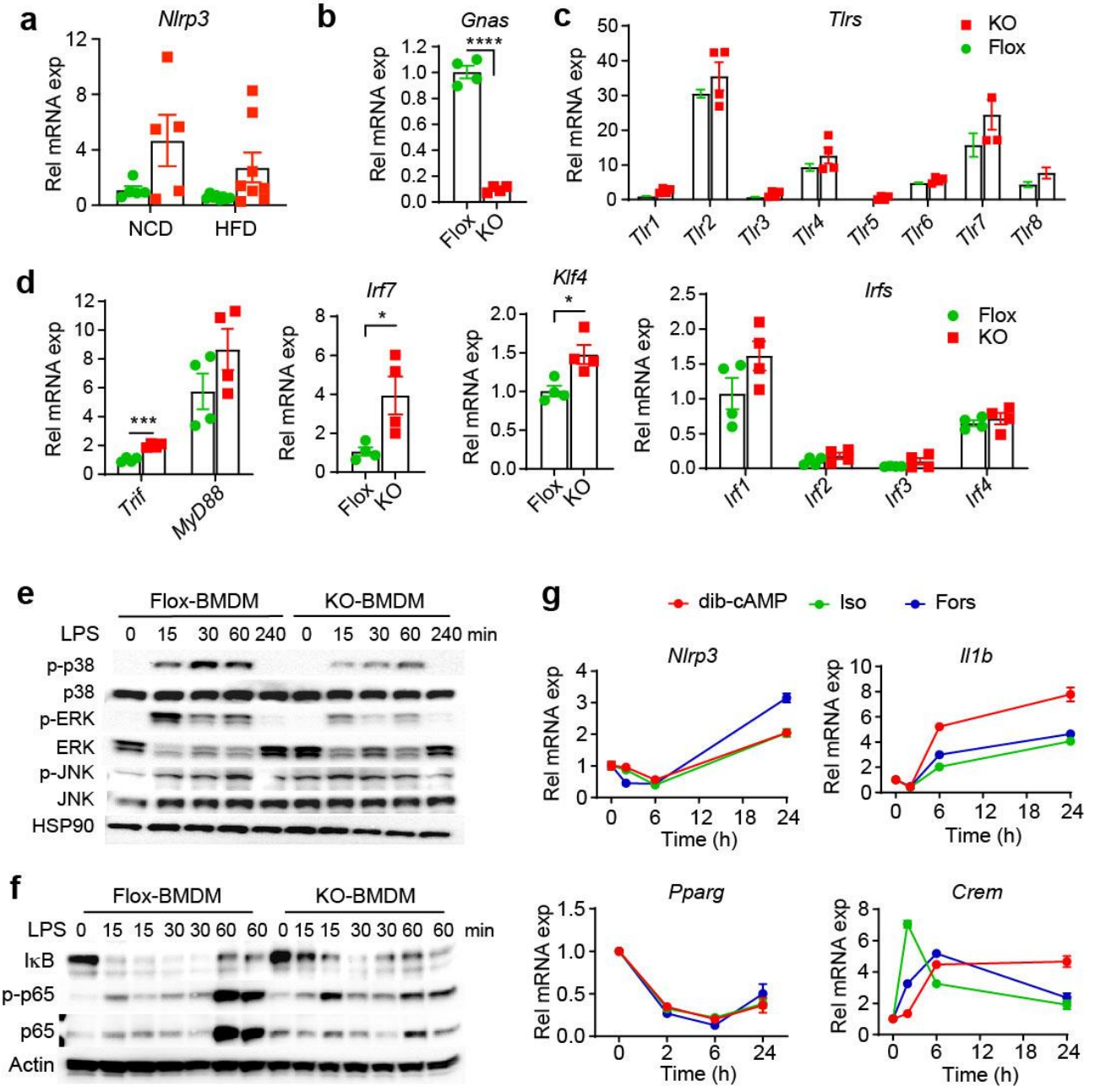

Fig. S11. Inflammatory signaling components in eWAT or BMDM of Flox or KO mice.
(**a**) Gene expression of Nlrp3 in eWAT from Flox or KO mice on NCD or HFD. 2-way ANOVA indicated a significant genotype effect (p=0.0091). (**b**) Expression of *Tlrs 1-8* in BMDM from Flox and KO mice. (**c**) Expression of *Gnas* in BMDM from Flox and KO mice. (**d**) Expression of *Trif, MyD88, Irf7, klf4,* and *Irf1-4* in BMDM from Flox and KO mice. (**e**) Western blot of phospho-p38MAPK, -JNK and -ERK signaling in BMDM from Flox and KO mice treated with LPS (100 ng/ml) for increasing times. (**f**) Western blot of IκB and phospho-p63 RelA (NFκB) signaling in BMDM from Flox and KO mice treated with LPS for increasing times. (**g**) Time course of *Nlrp3, Il1b, Pparg* and *Crem* expression in BMDM from Flox and KO mice treated with dibutyryl-cAMP (dib-cAMP, 50μM), isoproterenol (Iso, 1μM) or Forskolin (Fors, 10μM) for increasing times.