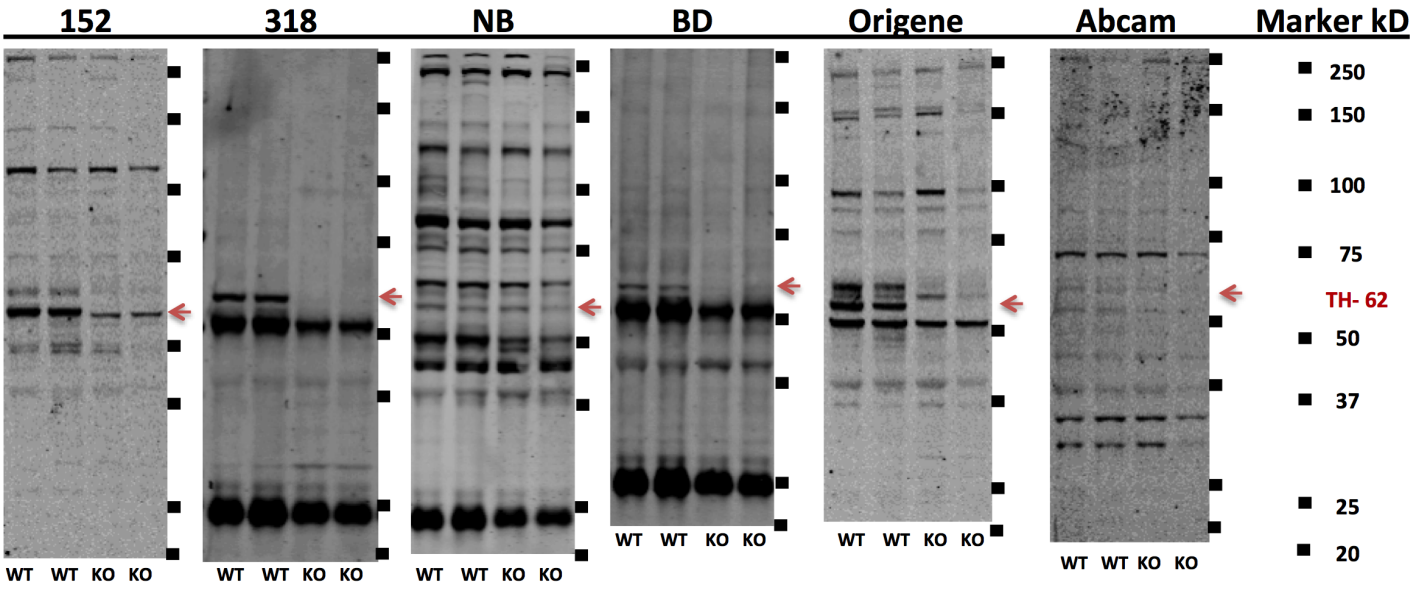
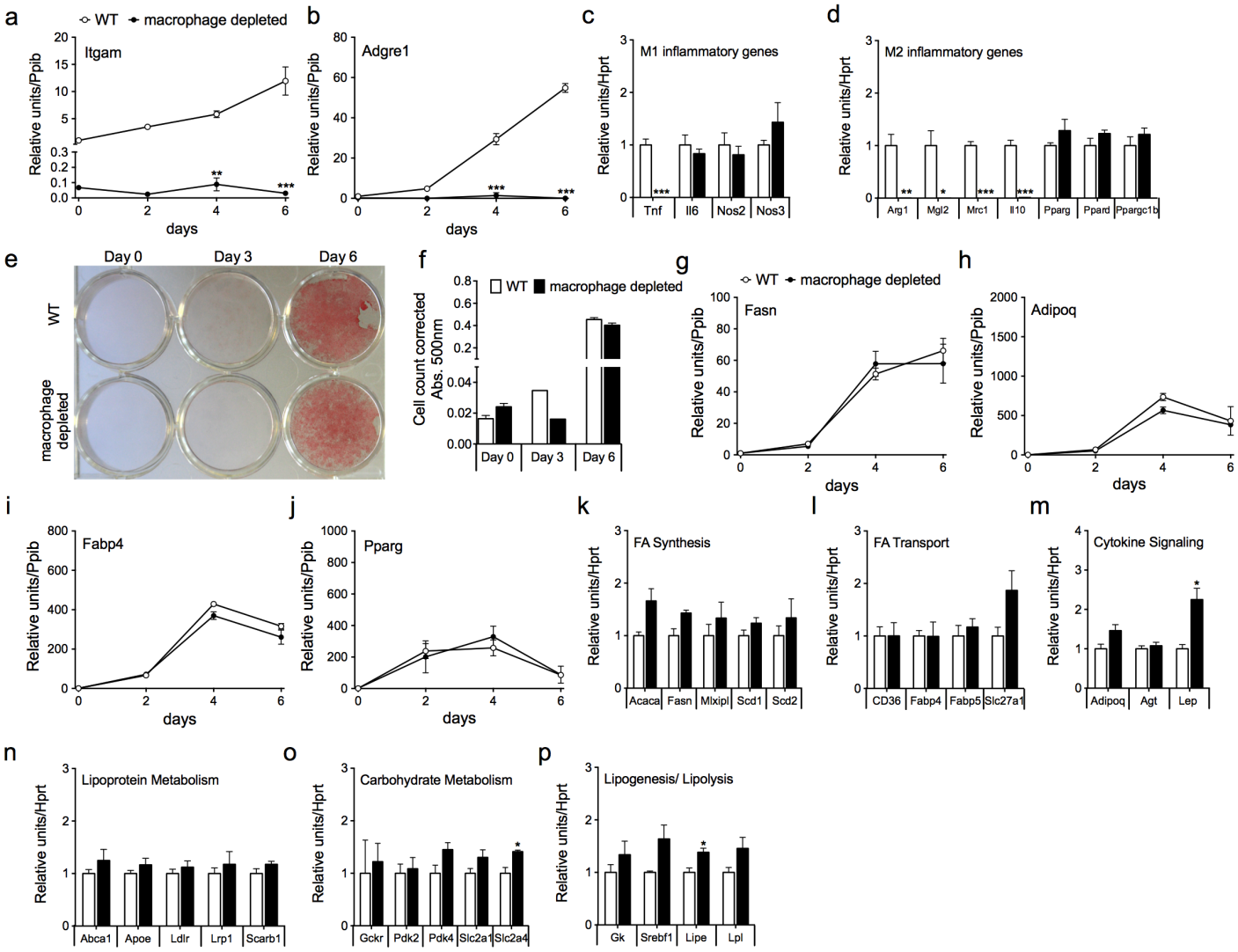
Supplementary Figure 1



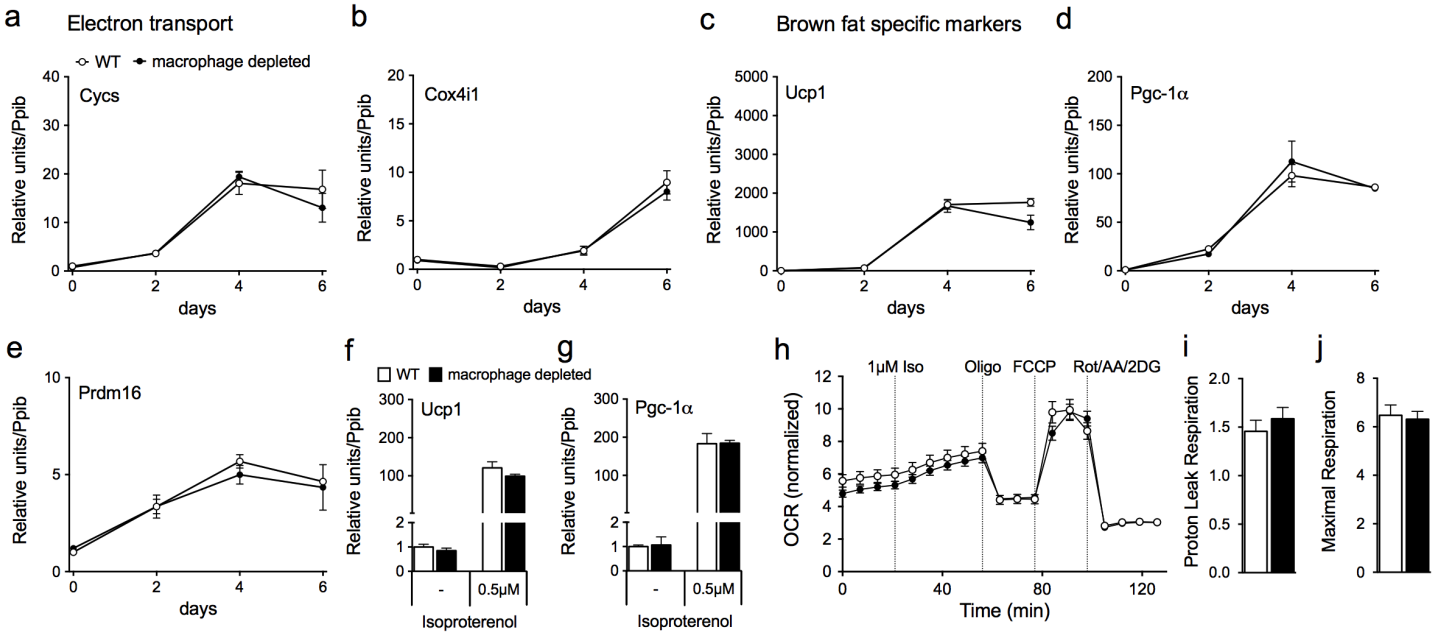
Supplementary Figure 1. Specificity of antibody recognition of TH.
Western blot analysis of the same spleen extracts from WT (*n* = 2) and THΔper mice (KO) (*n* = 2) to evaluate the specificity of the following TH antibodies: 152 (Millipore, Cat#AB152; Lot#2593900); 318 (Millipore, Cat#MAB318; Lot#2523803); NB (Novus Biological, Cat#NBP242212; Lot#A-1); BD (BD Biosciences, Cat#612300; Lot#43397863); Origene (Cat#TA303716; Lot#GR212964); Abcam (Cat#ab41528; Lot#GR226873-1).

Supplementary Figure 2



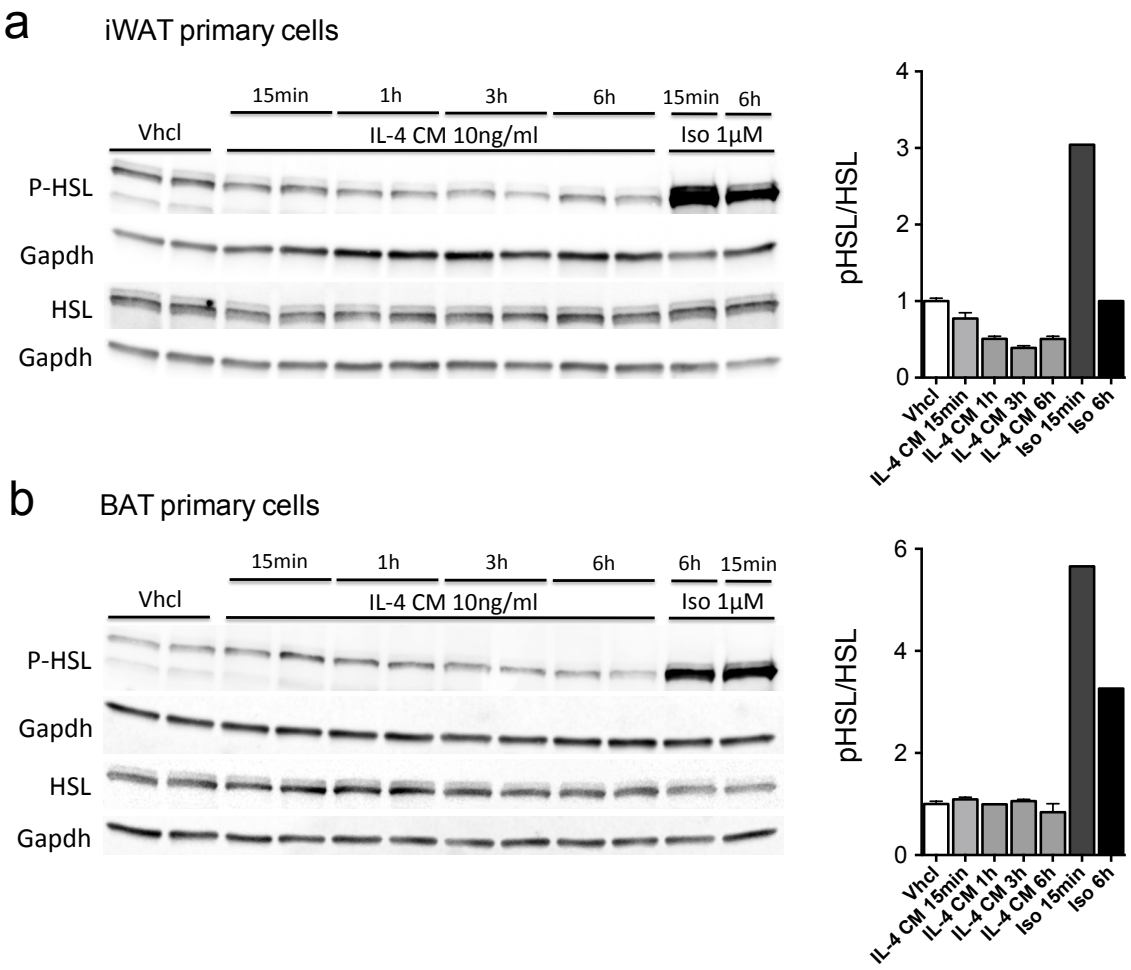
Supplementary Figure 2. Effect of macrophage depletion on lipid accumulation, adipocyte differentiation and key metabolic pathways in primary inguinal white adipocytes.
(**a-d**) Expression of macrophage infiltration marker (*Itgam,* alias *CD11b*) (**a**) and (*Adgre1*) (**b**), genes related to M1 inflammation (*Tnf*, *Il6*, *Nos2*, *Nos3*) (**c**) and M2 inflammation (*Arg1*, *Mgl2*, *Mrc1*, *Il10*, *Pparg*, *Ppard*, *Ppargc1b*) (**d**) in WT or macrophage-depleted iWAT primary cells (n = 3 each group). (**e**) Lipid accumulation measured by Oil Red O staining at different time points during adipocyte differentiation (image is representative for three independent experiments). (**f**) Quantification of cell number corrected (Dapi staining) lipid accumulation (Oil Red O incorporation) after re-elution in isopropanol (n = 2 technical replicates). (**g-j**) Expression profile of markers indicative of white adipocyte differentiation (*Fasn*, *Adipoq*, *Fabp4*, *Pparg*) in WT or macrophage-depleted iWAT primary cells. (**k-p**) Low Density Array analysis of WT and macrophage-depleted iWAT primary cells revealed expression profile of genes related to fatty acid (FA) synthesis (*Acaca*, *Fasn*, *Mlxipl*, *Scd1*, *Scd2*) (**k**), fatty acid transport (*CD36*, *Fabp4*, *Fabp5*, *Slc27a1*) (**l**), cytokine signaling (*Adipoq*, *Agt*, *Lep*) (**m**), lipoprotein metabolism (*Abca1*, *Apoe*, *Ldlr*, *Lrp1*, *Scarb1*) (**n**), carbohydrate metabolism (*Gckr*, *Pdk2*, *Pdk4*, *Slc2a1*, *Slc2a4*) (**o**), lipogenesis (*Gk*, *Screbf1*) and lipolysis (*Lipe*, *Lpl*) (**p**). Gene expression data are normalized to either housekeeping gene *Ppib* (**a,b,g-j**) or *Hprt* (**c,d,k-p**). Data are representative of three independently performed experiments, each performed in technical triplicates.
Data represent means ± s.e.m. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 based on 2-way ANOVA followed by Bonferroni post-hoc comparison of the individual time-points (**a**,**b**) or 2-sided Student's *t*-test (**c**,**d,m,o,p**).

Supplementary Figure 3



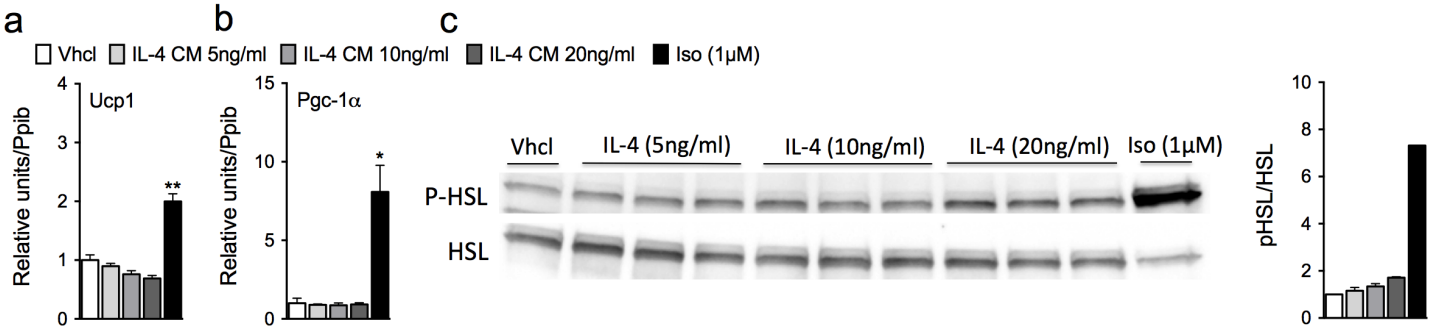
Supplementary Figure 3. Effect of macrophage depletion on browning in primary inguinal white adipocytes. (**a**-**e**) mRNA levels of electron transport markers (*Cycs, Cox4i1*) (**a**-**b**) and brown fat-specific markers (*Ucp1*, *Pgc1α*, *Prdm16*) (**c**-**e**) in iWAT primary cells during adipocyte differentiation. (**f**-**g**) Expression levels of *Ucp1*, *Pgc1α* following stimulation with isoproterenol (0.5 μM) for 6 h in fully differentiated iWAT primary cells. Gene expression data in panel **a**-**g** are normalized to the housekeeping gene *Ppib*. (**h**) Measurement of oxygen consumption rate (OCR), corrected for cell number (Dapi) of inguinal white adipocytes following treatment with isoproterenol (Iso) (1 μM), oligomycin (2 μg/ml), FCCP (1 μM) and rotenone (2.5 μM)/antimycin A (2.5 μM)/2-Deoxyglucose (10 mM). (**i**) Proton Leak Respiration is calculated as the difference between ATP synthesis during blocked respiration (oligomycin) and non-mitochondrial respiration. (**j**) Maximal Respiration is calculated as the difference between FCCP-induced respiration and non-mitochondrial respiration. Data shown in panel **a**-**g** are representative for three independently performed experiments, each performed in triplicates. Displayed results in panel **h**-**j** are representative for two independent experiments (*n* = 23 technical replicates per group). Data represent means ± s.e.m.

Supplementary Figure 4



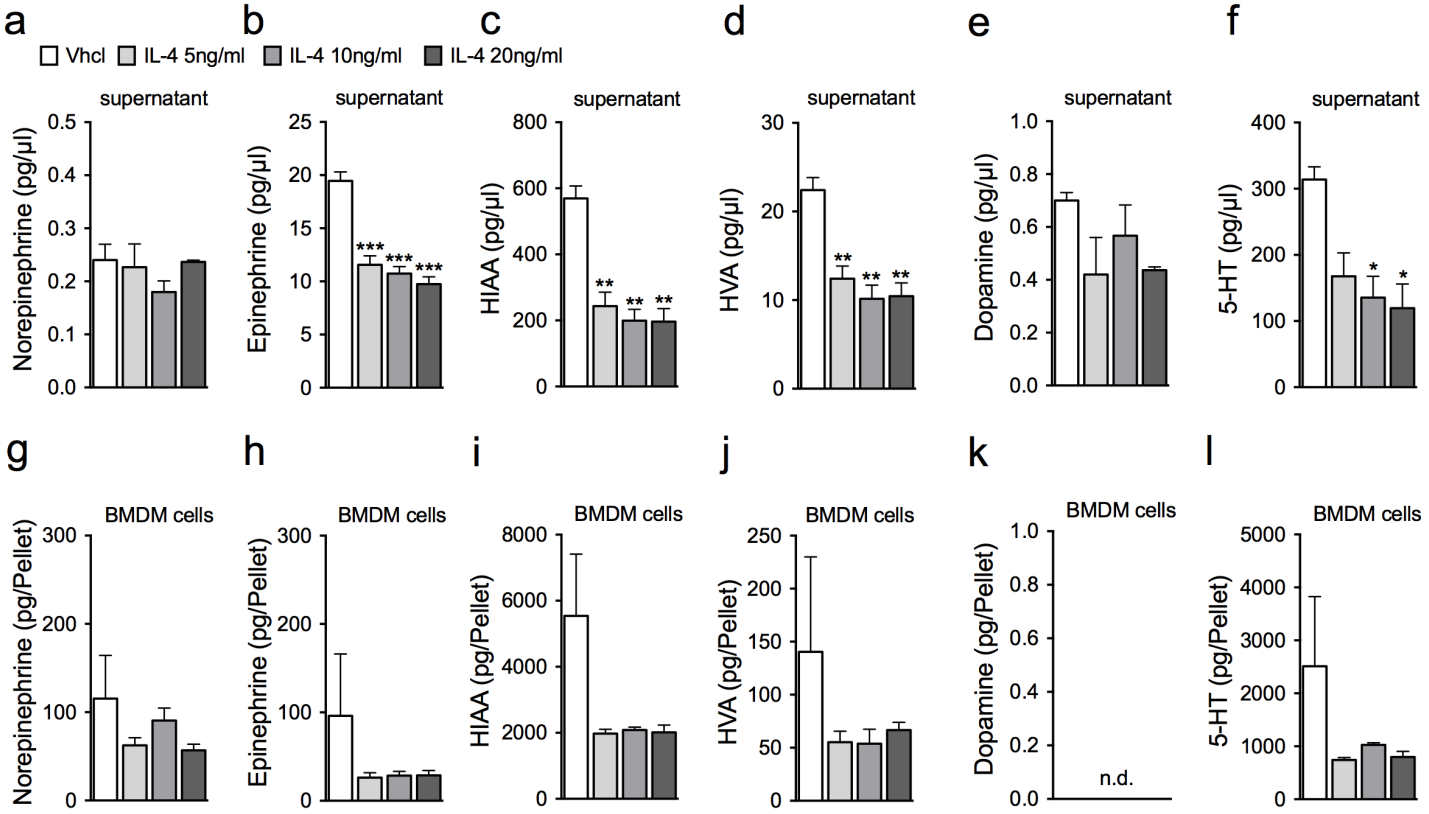
Supplementary Figure 4. Effect of IL-4 on phosphorylation of HSL in BAT and iWAT primary cells.
(**a-b**) Western blot analysis of phosphorylated and total HSL of iWAT (**a**) and BAT (**b**) primary cells treated with conditioned media (CM) of IL-4 treated BMDMs (15 min, 1 h, 3 h, 6 h) or isoproterenol (Iso) (15 min, 6 h, *n* = 2 technical replicates each treatment). Uncropped Western blot images are shown in **Supplementary Figure 12**. Data represent means ± s.e.m.

Supplementary Figure 5



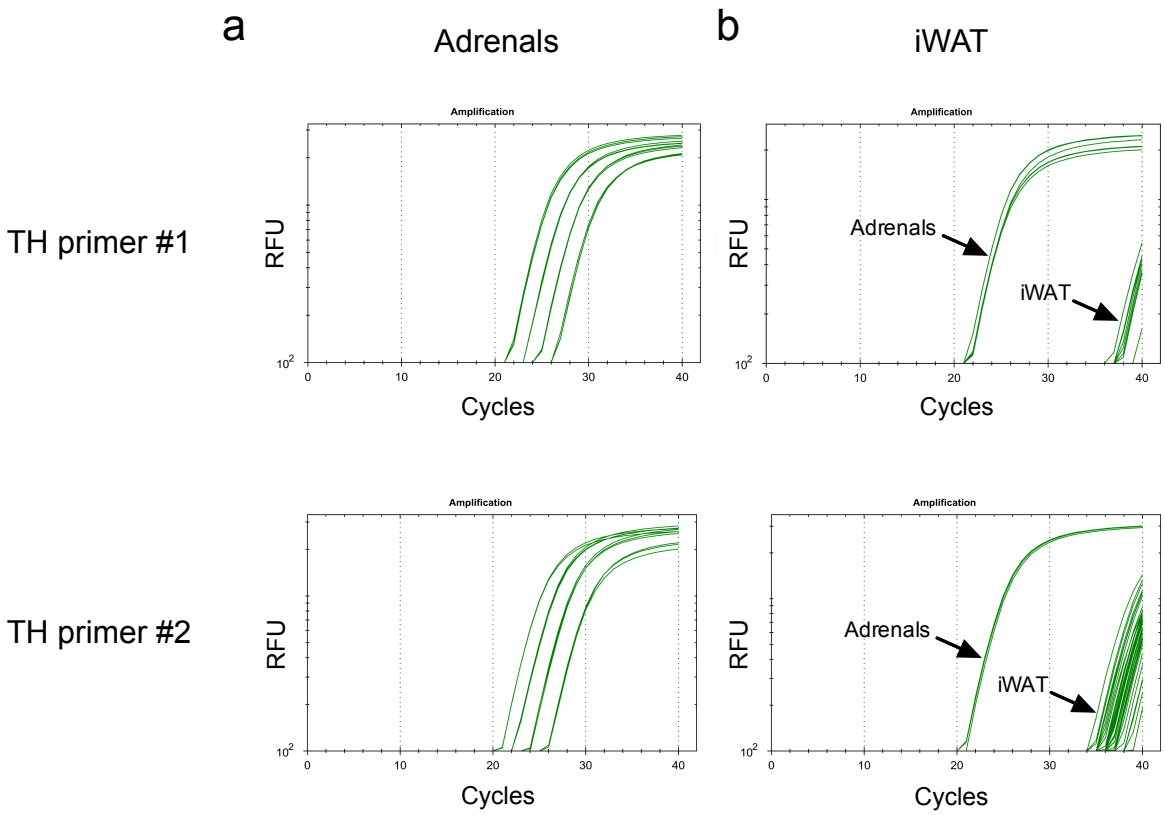
Supplementary Figure 5. Effect of alternatively activated macrophages from the Raw264.7 cell line on thermogenic gene program and HSL activity in primary brown adipocytes.
(**a-b**) Expression profile of brown fat-specific markers (*Ucp1, Pgc-1α*), normalized to *Ppib*, in BAT primary cells treated with conditioned media (CM) from the IL-4 treated Raw264.7 cells or isoproterenol (Iso). (**c**) Western blot displays protein levels of phosphorylated or total HSL and quantification of the protein levels of p-HSL and HSL of 6-d differentiated BAT primary cells treated with CM from IL-4 treated Raw264.7 or Iso (1 μM) for 6 h. Uncropped Western blot images are shown in **Supplementary Figure 12**. Data are representative for three independently performed experiments, each performed with n = 3 technical replicates. Data represent means ± s.e.m. *P < 0.05; **P < 0.01 based on 1-way ANOVA followed by Bonferroni-multiple comparison test.

Supplementary Figure 6



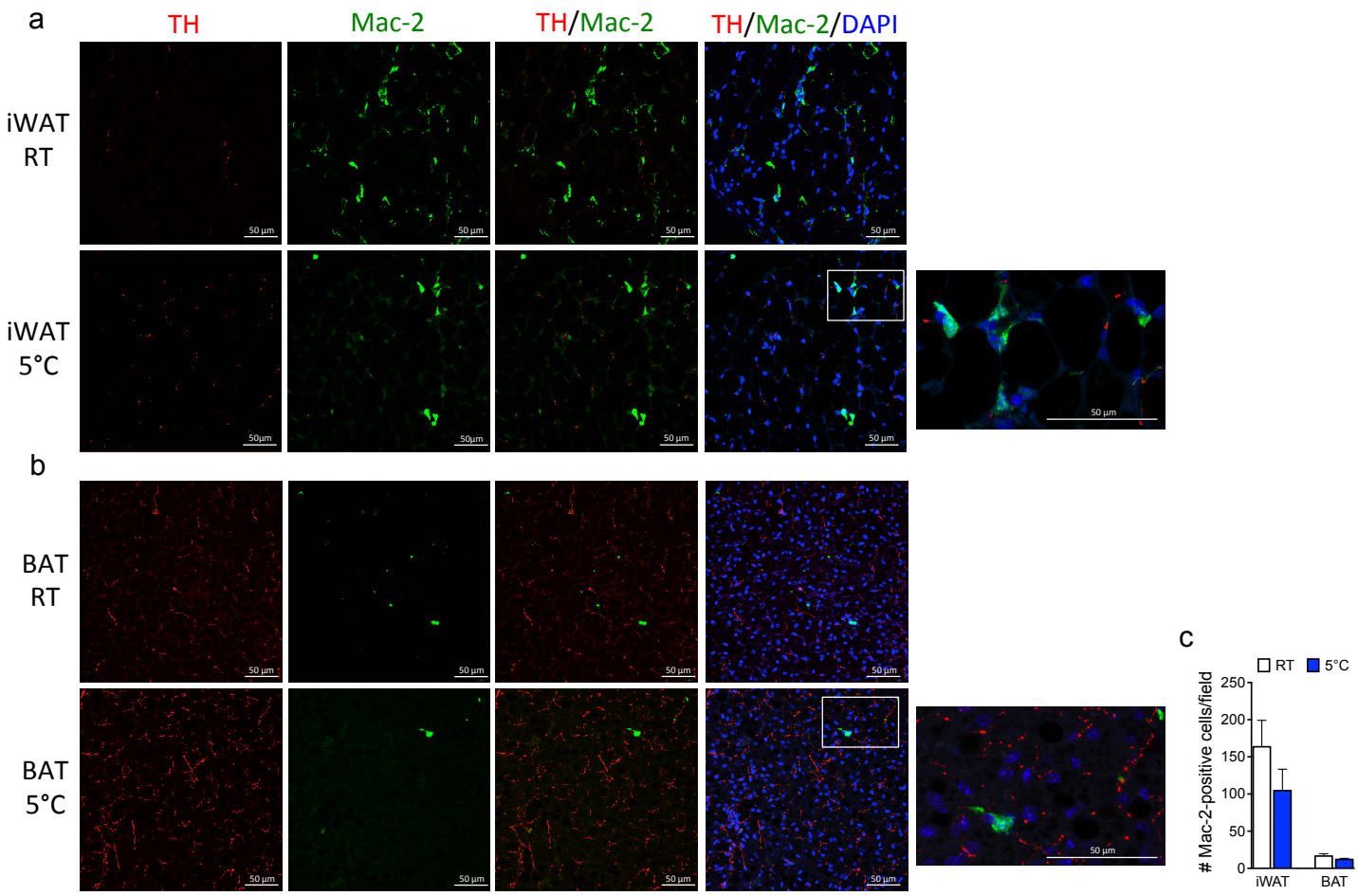
Supplementary Figure 6. Catecholamine production in BMDMs.
(**a-l**) HPLC-based measurement of NE, epinephrine, 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), dopamine and 5-hydroxytryptamin (5-HT) in supernatant (**a**-**f**) or cells (**g**-**l**) of vhcl or IL-4 treated BMDMs (*n* = 3 technical replicates each group). Data represent means ± s.e.m. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 based on 1-way ANOVA followed by Bonferroni-multiple comparison test.

Supplementary Figure 7



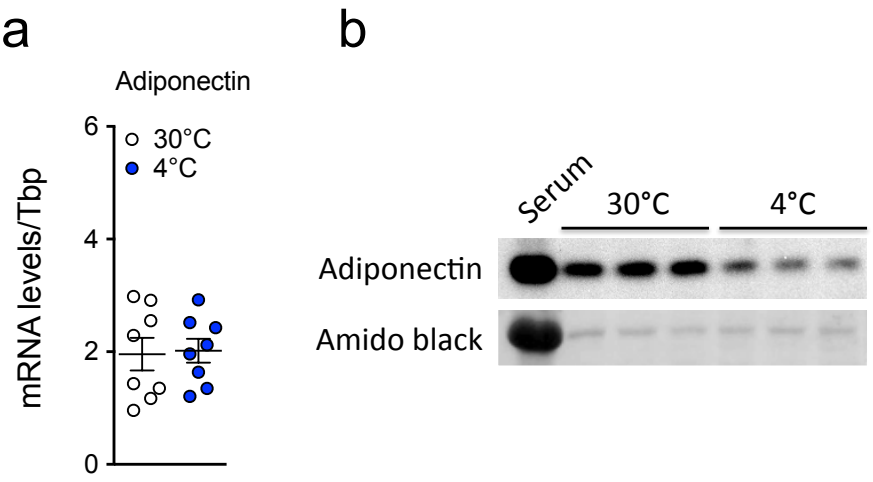
Supplementary Figure 7. TH primer validation
(**a**-**b**) Amplification plots of *TH* gene expression of adrenal (**a**) and iWAT (**b**) extracts from cold-exposed WT mice from 30°C to 4°C. Two different primer pairs (TH primer #1; #2) were validated in *n* = 7 mice. Primer sequences are displayed in **Supplementary Table 1**.

Supplementary Figure 8



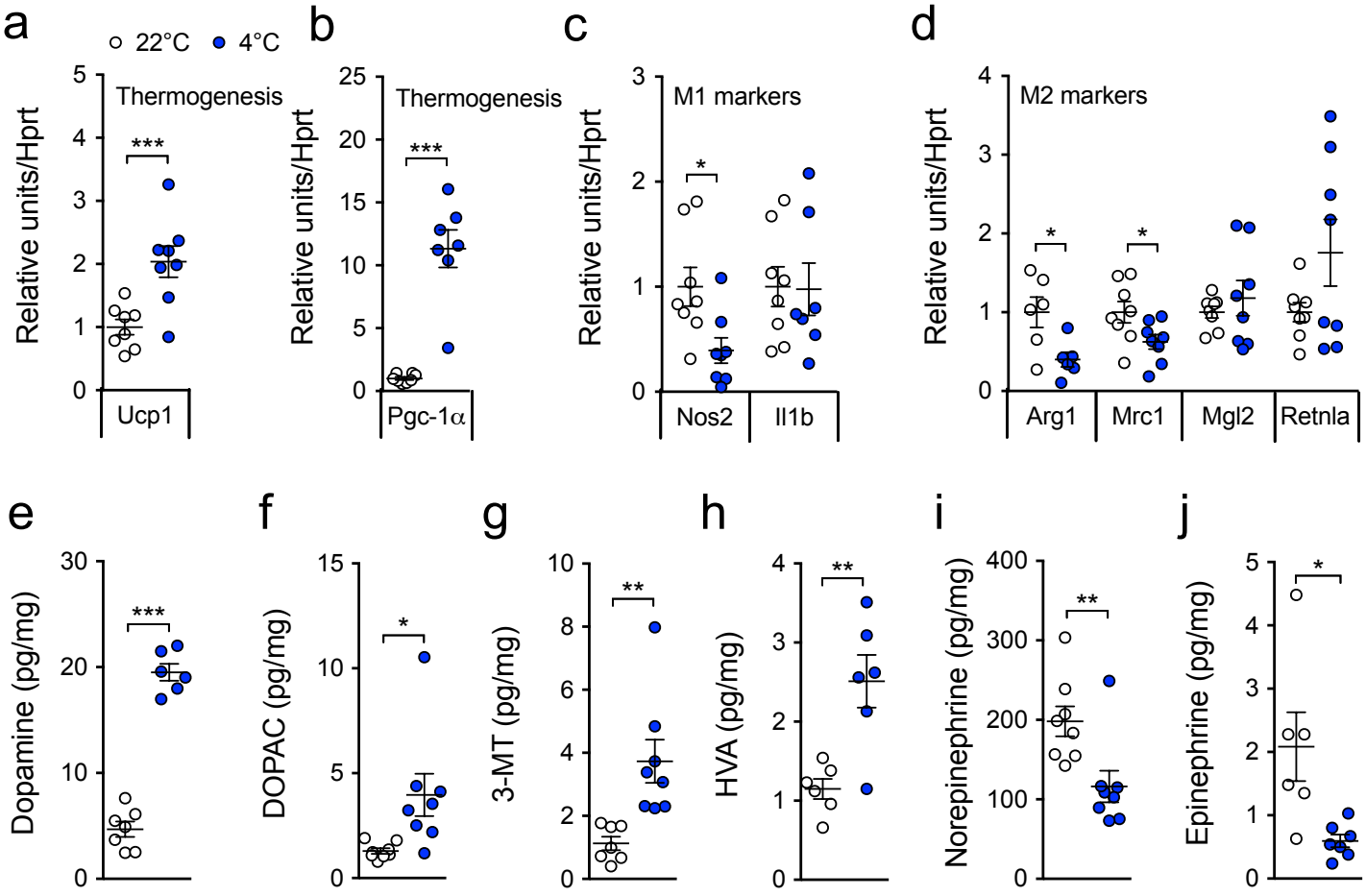
Supplementary Figure 8. TH and Mac-2 co-staining of cold-exposed adipose tissue.
(**a-b**) Immunofluorescence of TH and Mac-2 in iWAT (**a**) and BAT (**b**) of room temperature (RT) (top) or 4-h cold-exposed (5°C) (below) C57Bl/6J mice (*n* = 4 mice each group) (scale bar: 50 µm). (**c**) Quantification of Mac-2-positive cells (number of cells per 20x field) of RT or cold-exposed iWAT and BAT tissues. Displayed fluorescent images are representative images of *n* = 4 mice per tissue and treatment. Rectangles highlight zoomed-in areas that are displayed right next to it. Data represent means ± s.e.m.

Supplementary Figure 9



Supplementary Figure 9. Adiponectin expression in iWAT of long-term cold-exposed C57Bl/6 mice.
(**a-b**) Gene expression (*n* = 8 mice each group), normalized to the housekeeping gene *Tbp*, (**a**) and protein analysis (*n* = 3 mice each group) (**b**) of adiponectin and amido black loading control in iWAT of thermoneutral (30°C) or cold-exposed (4°C) C57Bl/6 mice for 4-5 wks. Uncropped Western blot images are shown in **Supplementary Figure 12**. Data represent means ± s.e.m.

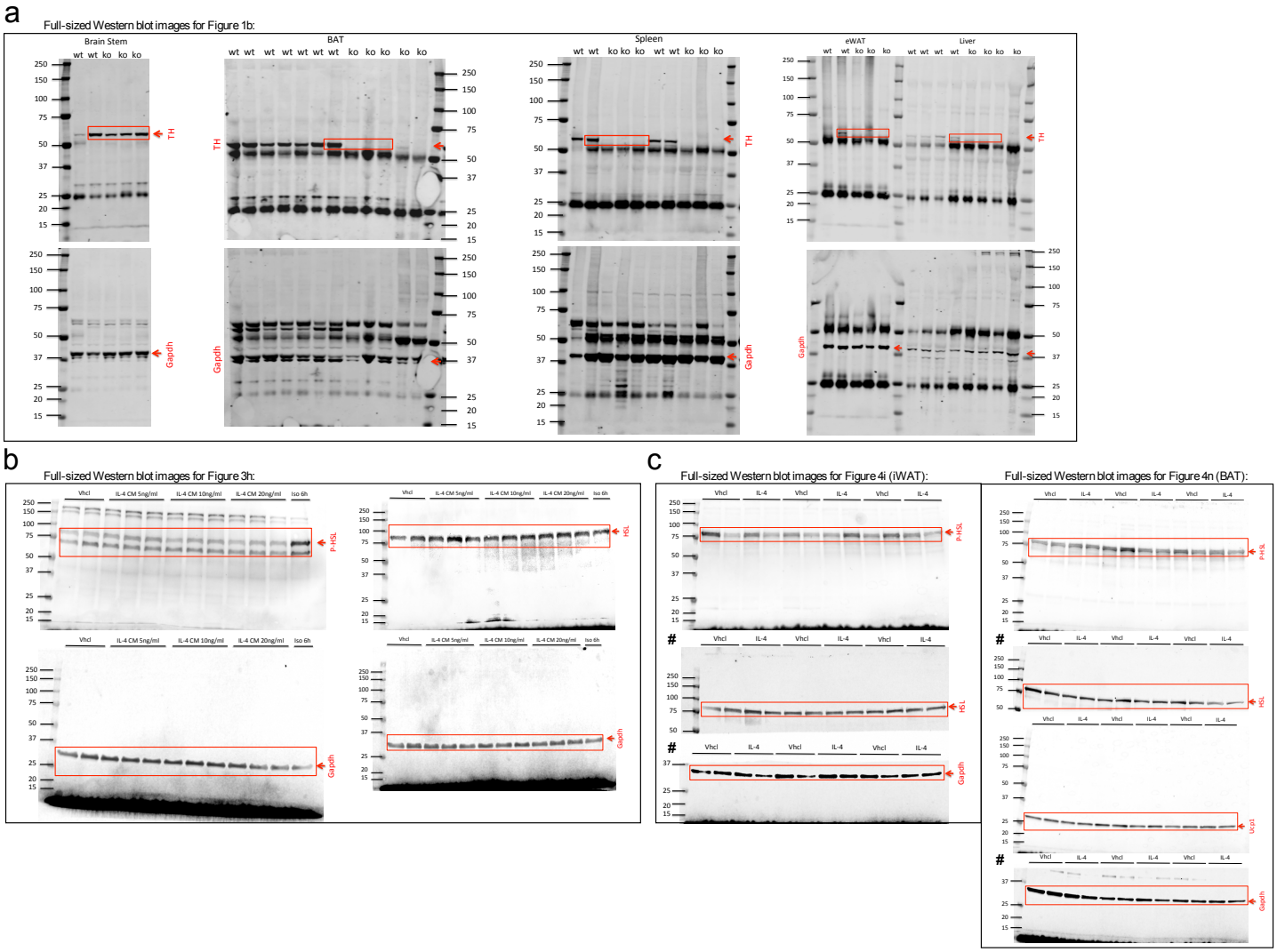
Supplementary Figure 10



Supplementary Figure 10. Effect of 6-h cold exposure on macrophage content and catecholamine turnover in brown fat.

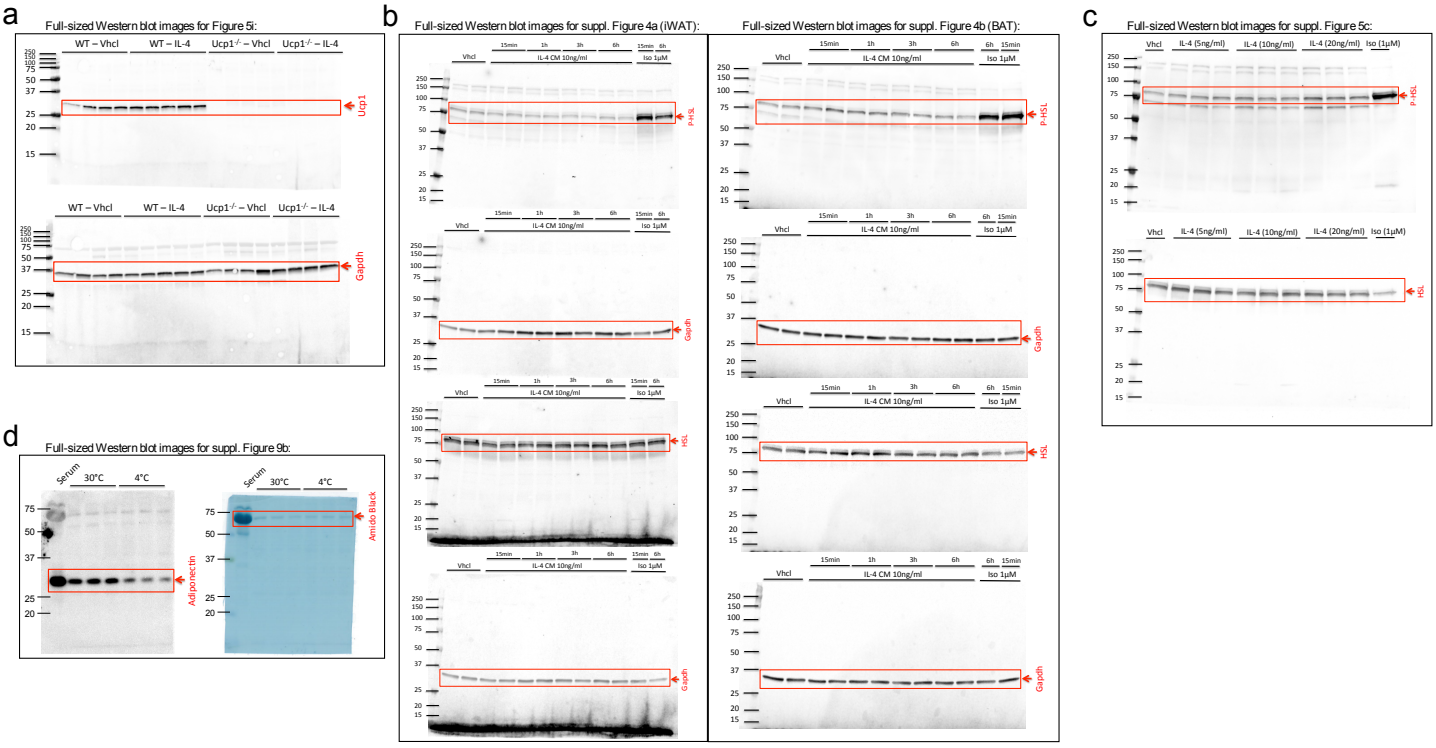
(**a-d**) Gene expression of brown fat-specific markers (*Ucp1, Pgc-1α*) (**a**-**b**), M1 macrophage markers (*Nos1, Il1b*) (**c**) or M2 macrophage markers (*Arg1, Mrc1, Mgl2, Retnla*) (**d**), normalized to the housekeeping gene *Hprt*, in BAT from mice housed at 22°C or 4°C for 6 h (*n* = 8 each group). (**e-j**) Catecholamines and metabolites, namely dopamine (**e**), 3,4-dihydroxyphenylacetic acid (DOPAC) (**f**), 3-methoxytyramine (3-MT) (**g**), homovanillic acid (HVA) (**h**), NE (**i**) and epinephrine (**j**), were measured in BAT from room temperature or cold-exposed mice (*n* = 8 each group). Data represent means ± s.e.m. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 based on 2-sided Student's *t*-test.

Supplementary Figure 11



Supplementary Figure 11. Full-scans of Western blots.
(**a-c**) Full-sized images of Western blot from Figure 1b (**a**), 3h (**b**) and 4i (**c**). Red boxes highlight areas that were cropped and are displayed in the indicated figures. Red arrows indicate location of the detected protein. Western blot membranes of Figure 4 (indicated with #) were cut out at a size of 50 kDa to detect HSL and Gapdh on separated parts of the membranes.

Supplementary Figure 12



Supplementary Figure 12. Full-scans of Western blots.
(**a-d**) Full-sized images of Western blot from Figure 5i (**a**), supplementary Figure 4a,b (**b**), 5c (**c**) and 9b (**d**). Red boxes highlight areas that were cropped and are displayed in the indicated figures. Red arrows indicate location of the detected protein.

Supplementary Table 1

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Dio2	TGCCACCTTCTTGACTTTGC	GGTTCCGGTGCTTCTTAACC
Tnf	AATGGCCTCCCTCTCATCAG	CCCTTGAAGAGAACCTGGGA
Prdm16	CCGCTGTGATGAGTGTGATG	GGACGATCATGTGTTGCTCC
Ppargc1a (Pgc-1α)	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Ucp1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT
Arg1	CTGAGCTTTGATGTCGACGG	TCCTCTGCTGTCTTCCCAAG
Mrc1	TGGATGGATGGGAGCAAAGT	GCTGCTGTTATGTCTCTGGC
Mgl2	TGGAGAGCACAGTGGAGAAG	CGGCAGTACTTGTCAGCTTC
Itgam	TGACCTGGCTTTAGACCCTG	ACCTCTGAGCATCCATAGCC
Adgre1	GAAGCATCCGAGACACACAC	TTGTGGTTCTGAACAGCACG
Cycs	GTTCAGAAGTGTGCCCAGTG	GTCTGCCCTTTCTCCCTTCT
Cox4i1	CTAGAGGGACAGGGACACAC	TGGTTCATCTCTGCGAAGGT
Fasn	AGAGATCCCGAGACGCTTCT	GCTTGGTCCTTTGAAGTCGAAGA
Adipoq	GGTCCTAAGGGTGAGACAGG	AGTCCCGGAATGTTGCAGTA
Fabp4	CAGCGTAAATGGGGATTTGG	CCGCCATCTAGGGTTATGAT
Pparg	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
TH primer 1	GTCTTCCTATGGAGAGCTCCTG	GGCTGGTAGGTTTGATCTTGG
TH primer 2	GCTACCGAGAGGACAGCATT	CACGGGCAGACAGTAGACC
Nos2	CCCCGCTACTACTCCATCAG	CCACTGACACTTCGCACAAA
Il1b	ACTCATTGTGGCTGTGGAGA	TTGTTCATCTCGGAGCCTGT
Retnla	CCCAGGATGCCAACTTTGAA	AGTAGCAGTCATCCCAGCAG
Tbp	GGGAGAATCATGGACCAGAA	GATGGGAATTCCAGGAGTCA
Hprt	AAGCTTGCTGGTGAAAAGGA	TTGCGCTCATCTTAGGCTTT
Ppib	GCATCTATGGTGAGCGCTTC	CTCCACCTTCCGTACCACAT

Supplementary Table 1. List of primer sequences