

**Supplemental Information**

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in endothelial cells stimulates adipose tissue  
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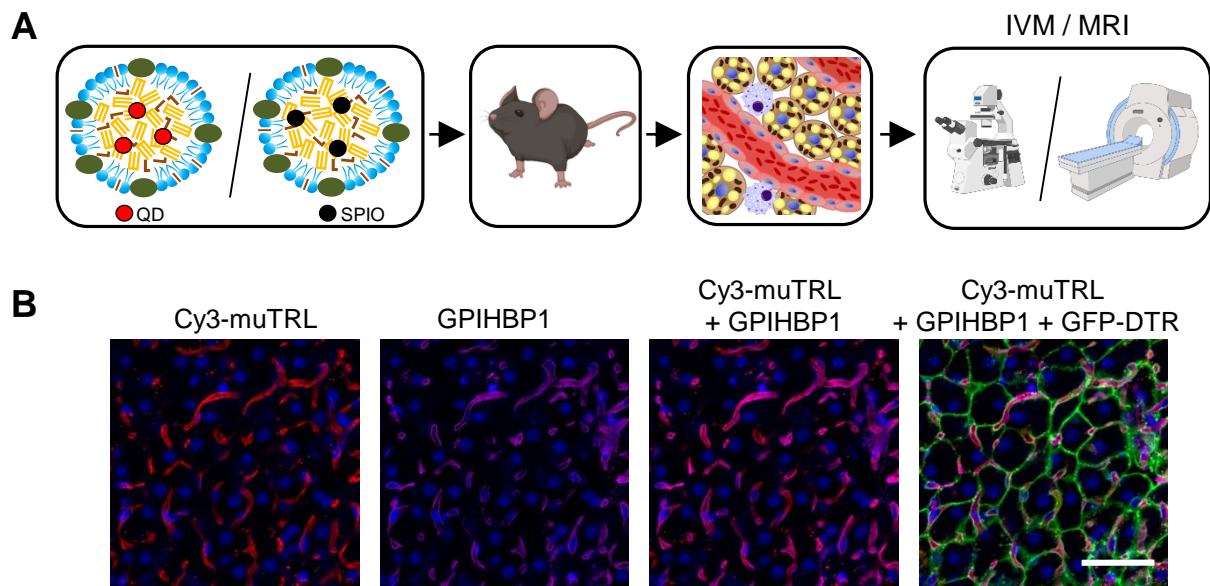
## **Cell Metabolism**

### **Supplemental Information**

#### **Lysosomal lipoprotein processing in endothelial cells stimulates adipose tissue thermogenic adaptation**

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**Figure S1**



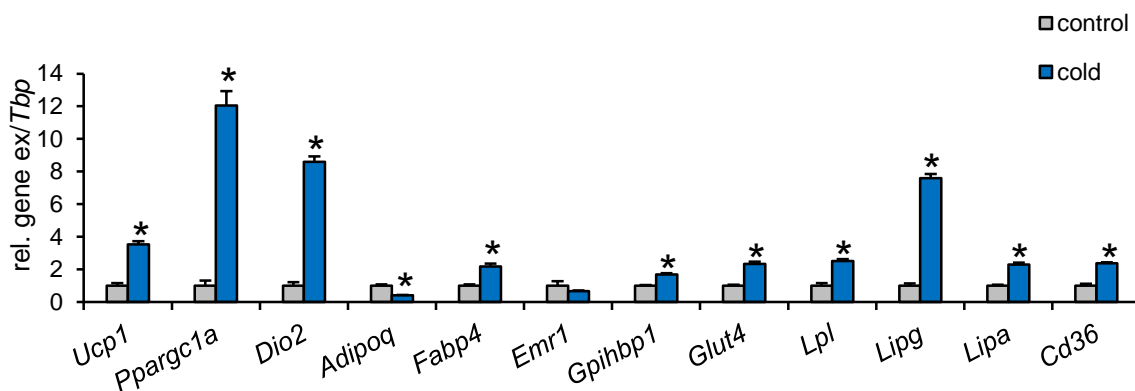
**Figure S1. Related to Figure 1. Visualization of lipoprotein particle processing by endothelial cells of activated BAT *in vivo*.**

(A) Experimental paradigm depicting injection of QD- or SPIO-labelled TRL for visualization of BAT TRL metabolism via intravital microscopy (IVM) or magnetic resonance imaging (MRI).

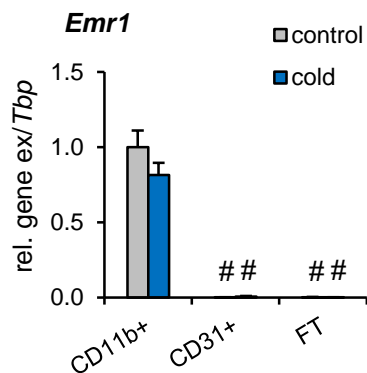
(B) Wild type mice were exposed to 6°C for 24 hours and Cy3-TRL were injected in transgenic mice expressing eGFP-tagged diphtheria toxin receptor (GFP-DTR) in brown adipocytes. Whole mount (immuno)-staining was performed to visualize nuclei (DAPI, blue), Cy3-murine TRL (red), GPIHBP1 (purple) and plasma membrane-associated GFP-DTR (green). Scale: 50  $\mu\text{m}$ .

**Figure S2**

**A**



**B**



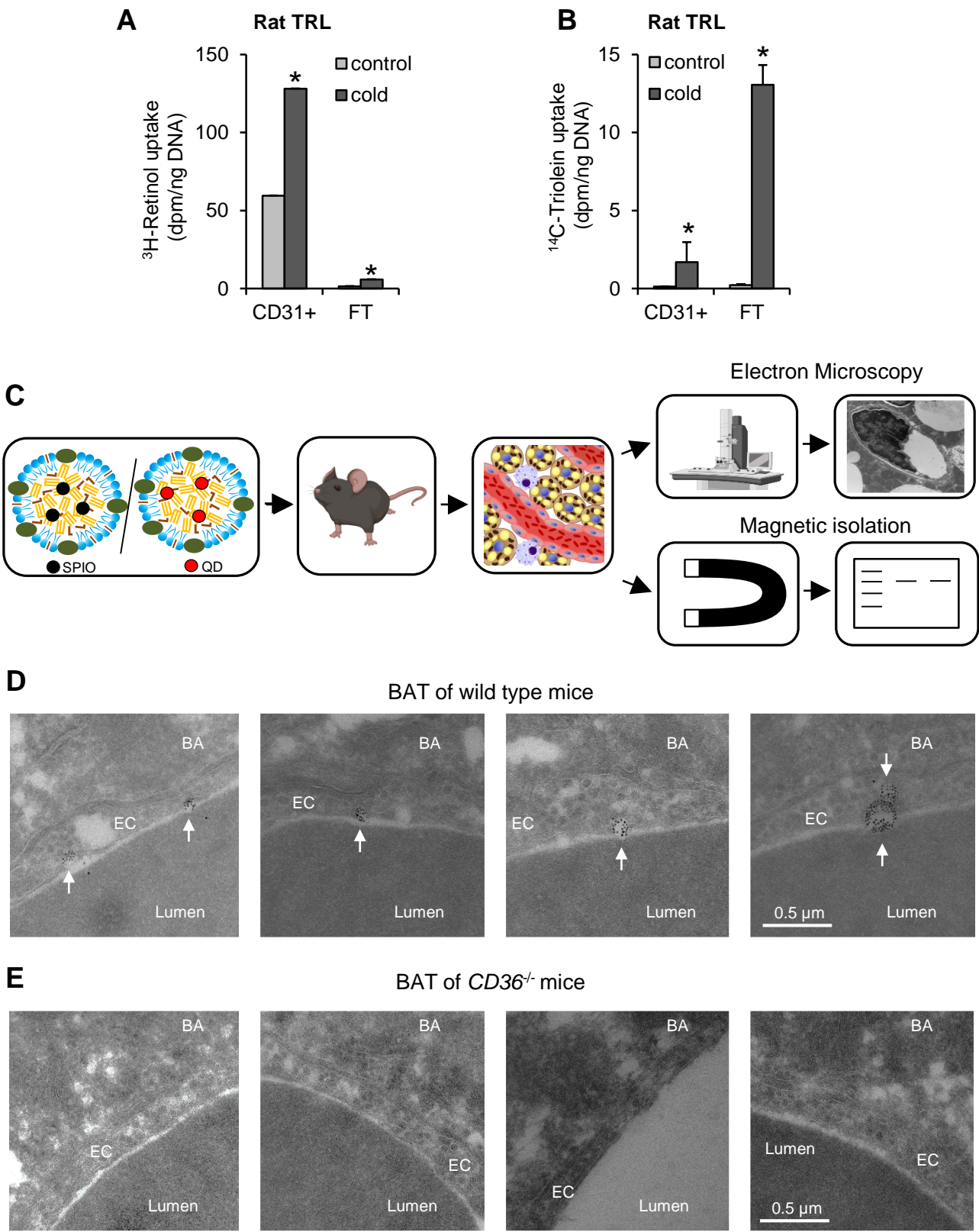
**Figure S2. Related to Figure 2. Cell type-specific expression of lipoprotein handling genes in murine and human BAT.**

(A) Gene expression analysis of BAT from control and 24-hour cold-exposed mice. Markers of thermogenic activation (Ucp1, Ppargc1a, Dio2), adipocyte identity (Adipoq, Fabp4), macrophages (Emr1), endothelial cells (Gpihbp1), glucose metabolism (Glut4), as well as lipid metabolism (Lpl, Lipg, Lipa, Cd36) was measured and normalized to expression of the housekeeper Tbp (n=8).

(B) Gene expression in CD11b+, CD31+ and adipocyte-enriched flow through fraction isolated from BAT of control and cold-exposed mice. Gene expression of Emr1 is presented as mean values  $\pm$  SEM from 12-13 independent MACS® isolations.

Results are presented as mean values  $\pm$  SEM. \*p < 0.05 by Student's t test comparing control vs. cold for (A). ##p < 0.05 by two-way ANOVA comparing cell types (B).

Figure S3



**Figure S3. Related to Figure 3. Endothelial cells of activated BAT internalize and sequester whole TRL particles via CD36.**

(A,B) Wild type mice were housed at 22°C (control) or for acute thermogenic activation exposed to 6°C (cold) for 24 hours. Native rat chylomicrons labelled with  $^3\text{H}$ -retinylester (core label) and  $^{14}\text{C}$ -triolein (fatty acid label) were injected intravenously.

(A) Uptake of  $^3\text{H}$ -retinylester into endothelial cell (CD31+) and flow through (FT) fractions representing brown adipocytes (n=3).

(B) Uptake of  $^{14}\text{C}$ -triolein into endothelial cell (CD31+) and flow through (FT) fractions representing brown adipocytes (n=3).

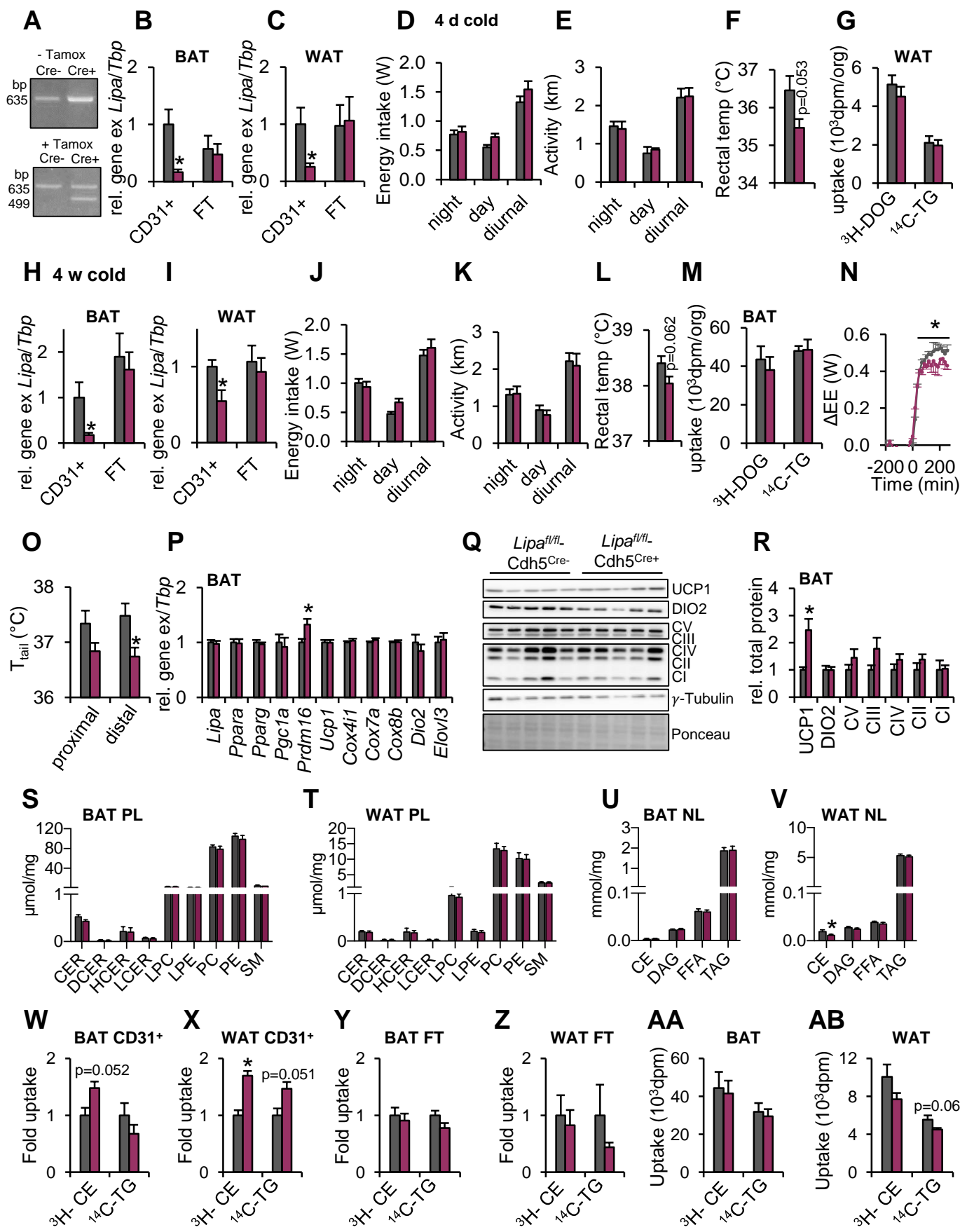
(C) Experimental paradigm for electron microscopy and magnetic organelle isolation to follow the fate of injected TRLs.

(D,E) Transmission electron microscopy analysis was performed 15 min after injection of SPIO-labelled TRLs into (D) wild type littermate controls and (E)  $\text{Cd36}^{-/-}$  mice (n= 4). In this setup, mice were housed at 22°C followed by acute exposure to 6°C for 4 hours. Cold exposure was limited to 4 hours to avoid hypothermia described for CD36-deficient mice (Bartelt et al., 2011). Representative images showing uptake of processed SPIO-TRL (see white arrows) into endosomal compartments of endothelial cells (EC) in wild type but not CD36-deficient mice. Brown adipocyte (BA), scale: 100 nm.

Data in (A) and (B) are presented as mean values  $\pm$  SEM. Statistical significance by Student's t test (\*p < 0.05).

**Figure S4**

For all data in S4:  $Lipa^{fl/fl}$ -Cdh5<sup>Cre-</sup>  $Lipa^{fl/fl}$ -Cdh5<sup>Cre+</sup>



## **Figure S4. Related to Figure 4. Endothelial LAL deletion causes impaired adaptive thermogenesis**

(A) Genomic recombination in BAT of *Cdh5*-Cre negative (Cre-) and positive (Cre+) *Lipa<sup>fl/fl</sup>* mice with and without tamoxifen treatment.

(B) *Lipa* gene expression in MACS®-isolated endothelial cell (CD31+) and adipocyte enriched flow-through (FT) fractions of BAT from mice housed under control conditions (n = 2-3).

(C) *Lipa* gene expression in inguinal WAT cell fractions generated as described in (B).

(D-G). Transgenic mice were treated with tamoxifen to generate mice lacking LAL in endothelial cells (*Lipa<sup>fl/fl</sup>-Cdh5Cre+*) and controls (*Lipa<sup>fl/fl</sup>-Cdh5Cre-*) and fed a cholesterol-rich high fat diet. Mice were acclimated to thermoneutrality (30°C) for two weeks, followed by 3 days at 18°C and 4 days exposure to cold (6°C).

(D) Energy intake during indirect calorimetry experiment (n = 4).

(E) Activity during indirect calorimetry experiment (n = 4).

(F) Rectal temperatures (n = 7-8).

(G) Whole inguinal WAT uptake of <sup>3</sup>H-deoxyglucose (<sup>3</sup>H-DOG) and <sup>14</sup>C-triolein-TRL after a combined oral glucose and fat gavage (n = 7-8).

(H-AB). Mice were housed at room temperature, followed by chronic (4 weeks) acclimation to cold (6°C).

(H) *Lipa* gene expression in MACS®-isolated endothelial cell (CD31+) and adipocyte enriched flow-through (FT) fractions of BAT (n = 6).

(I) *Lipa* gene expression in inguinal WAT cell fractions generated as described in (H).

(J) Energy intake during indirect calorimetry (n = 8).

(K) Activity during indirect calorimetry (n = 8).

(L) Rectal temperatures (n = 10-11).

(M) Whole BAT uptake of <sup>3</sup>H-DOG and <sup>14</sup>C-triolein-TRL after an oral glucose and fat gavage (n=4-9).

(N) Energy expenditure in response to CL316,243 (n = 5-10).

(O) Tail temperatures in cold-acclimated mice following CL-injection, determined by thermal imaging (n = 5) as described (Fischer et al., 2016).

(P) BAT gene expression (n = 5-6).

(Q) BAT protein analysis by Western blot including Ponceau staining.

(R) Quantification per BAT depot and normalization of proteins shown in (Q) (n = 5).

(S) Quantification of phospholipids (PL) in BAT (n = 9).

(T) Quantification of phospholipids (PL) in inguinal WAT (n = 9).

(U) Quantification of neutral lipids (NL) in BAT (n = 9).

(V) Quantification of neutral lipids (NL) in inguinal WAT (n = 9).

(W-AB) Mice (n=10-11) were injected with TRL double-radiolabeled with <sup>3</sup>H-cholesteryl ether (<sup>3</sup>H-CE) and <sup>14</sup>C-triolein. 20 min after injection, organs were harvested and 3-4 adipose tissue depots were pooled for three independent MACS®-based cell isolations.

(W) Uptake into BAT CD31+ cells (n = 3).

(X) Uptake into CD31+ cells from inguinal WAT (n = 3).

(Y) Uptake into BAT FT (n = 3).

(Z) Uptake into inguinal WAT FT (n = 3).

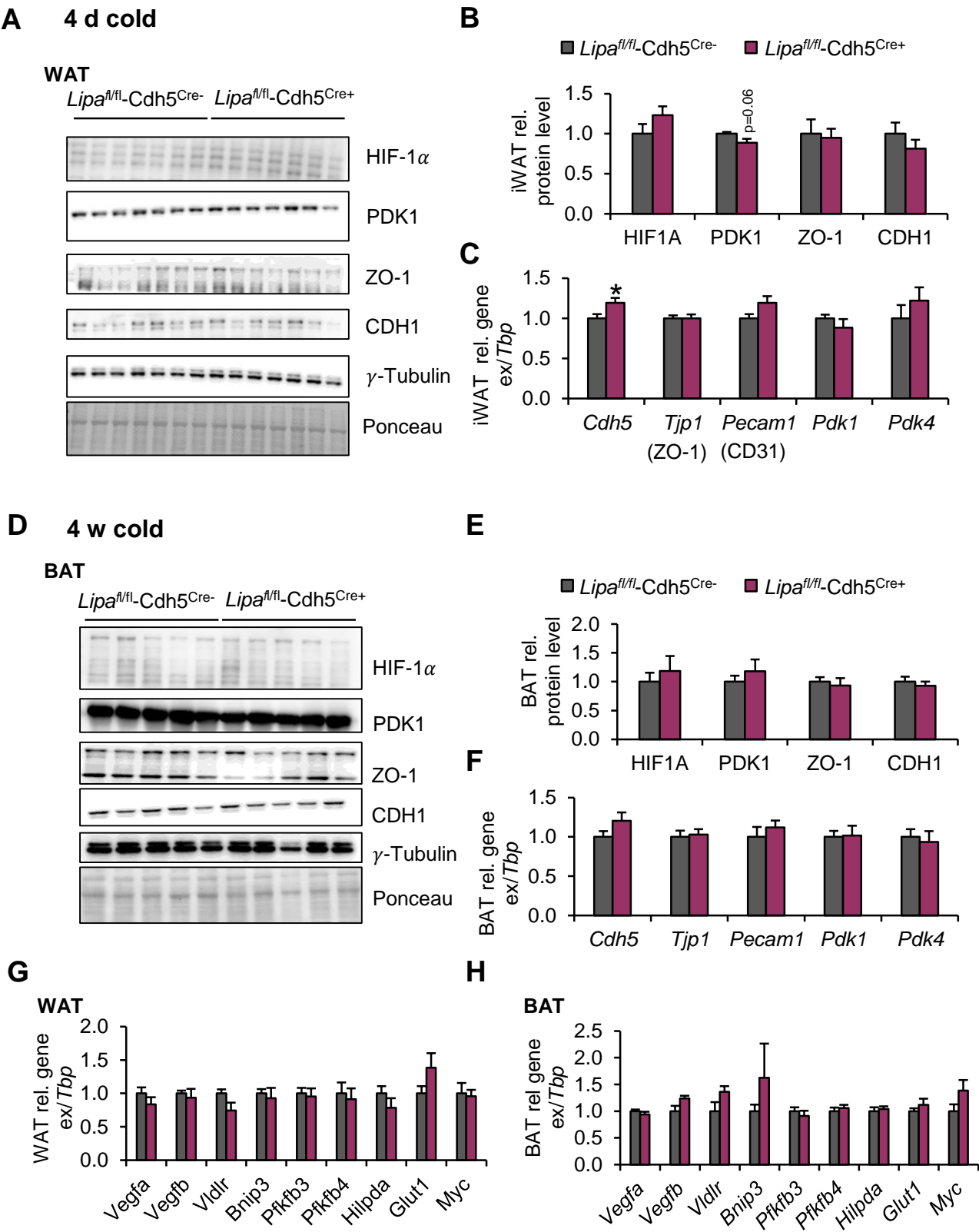
(AA) Whole organ uptake into BAT (n=10-11).

(AB) Whole organ uptake into inguinal WAT (n=10-11).

Data are means ± S.E.M. \*p ≤ 0.05 by student's t test.

CE, cholesterol esters; DAG, diacylglycerols; FFA, free fatty acids; TAG, triacylglycerol, CER, ceramides; DCER, dihydroceramides; HCER, hexosylceramides; LCER, lactosylceramides; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin.

Figure S5



**Figure S5. Related to Figure 5. LAL modulates abundance of HIF1 $\alpha$  and endothelial markers upon thermogenic recruitment.**

Transgenic mice were treated with tamoxifen to generate mice lacking LAL in endothelial cells (Lipa<sup>fl/fl</sup>-Cdh5Cre+) and controls (Lipa<sup>fl/fl</sup>-Cdh5Cre-) and fed a cholesterol-rich high fat diet.

(A-C) Mice were acclimated to thermoneutrality (30°C) for two weeks, followed by 3 days at 18°C and 4 days exposure to cold (6°C).

(A) Western blot analysis of inguinal WAT including Ponceau staining.

(B) Quantification of proteins shown in (A) (n = 7).

(C) Expression of angiogenic genes and Pdk1/4 in inguinal WAT (n = 7-8).

(D-H) Mice were housed at room temperature, followed by chronic (4 weeks) acclimation to cold (6°C).

(D) Western blot analysis of BAT including Ponceau staining.

(E) Quantification of proteins shown in (D) (n = 5).

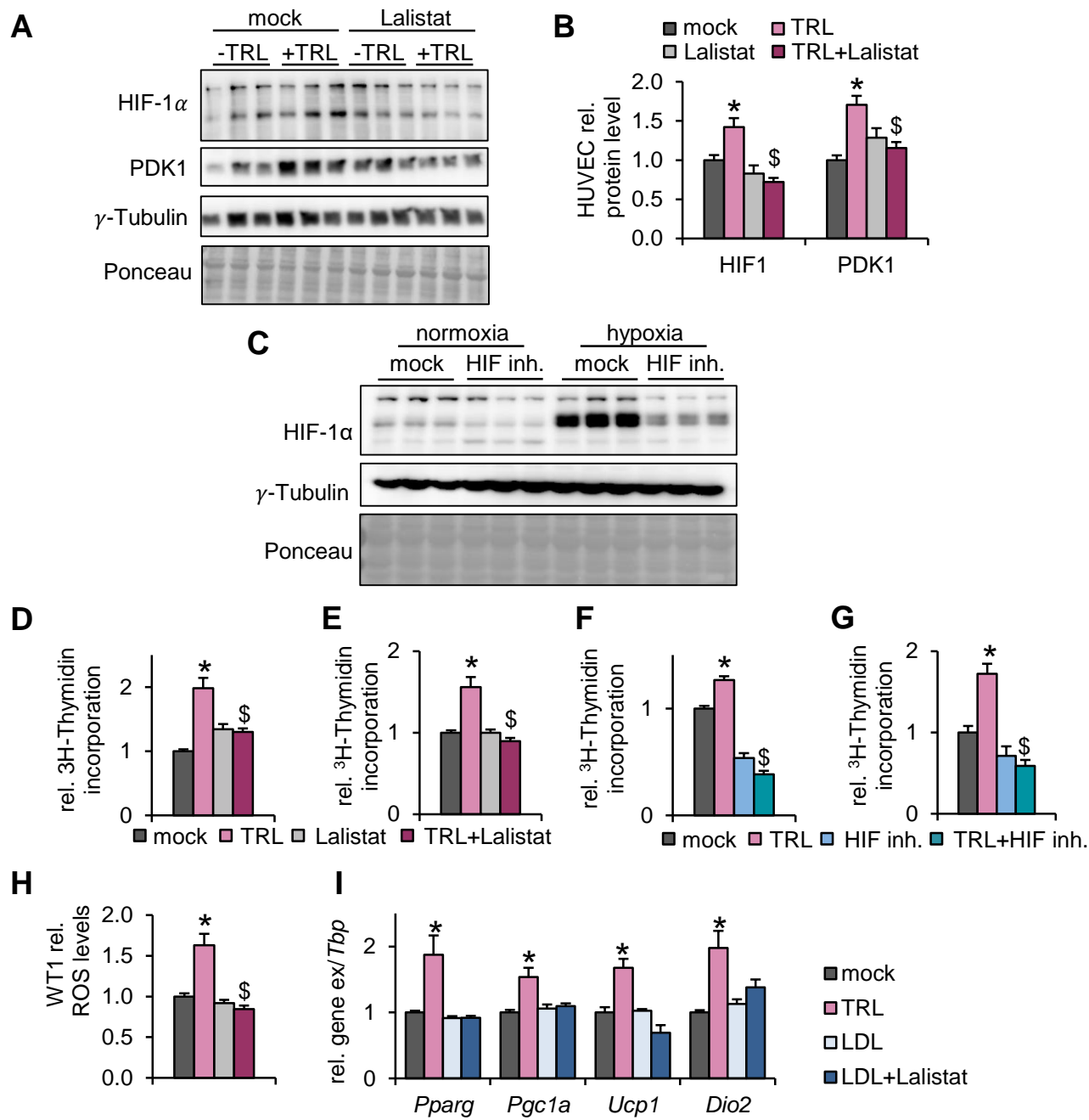
(F) Expression of endothelial marker genes and Pdk1/4 in BAT (n = 6-8).

(G) Expression of HIF1a target genes in inguinal WAT (n = 6-8).

(H) Expression of HIF1a target genes in BAT (n = 6-8).

Data are presented as mean values  $\pm$  SEM, \*p < 0.05 by Student's t test comparing Lipa<sup>fl/fl</sup>-Cdh5Cre+ vs. Lipa<sup>fl/fl</sup>-Cdh5Cre-.

**Figure S6**



**Figure S6. Related to Figure 6. LAL-dependent TRL processing stimulates proliferation and thermogenic differentiation via HIF1 $\alpha$ .**

(A) Effect of TRL and Lalistat on HIF1 $\alpha$  and PDK1 protein levels in HUVEC. Representative Western blot of three independent experiments.

(B) Quantification of proteins described in (A) (n=9).

(C) Western blot analysis of immortalized brown adipocyte precursor cells (WT1) that were cultured under normoxic (20% oxygen) or hypoxic (5% oxygen) conditions for 24 hours in the presence or absence of the HIF1 $\alpha$ -inhibitor.

(D) Proliferation determined by <sup>3</sup>H-thymidine incorporation in response to TRL treatment and LAL inhibition in HUVEC (n = 6).

(E) Proliferation in response to TRL treatment and LAL inhibition in WT1 cells (n = 9).

(F) Proliferation in response to TRL treatment and HIF1 $\alpha$  inhibition in HUVEC (n = 6).

(G) Proliferation in response to TRL treatment and HIF1 $\alpha$  inhibition in WT1 cells (n = 9).

(H) Relative reactive oxygen species (ROS) production in WT1 cells in response to TRL  $\pm$  Lalistat (n = 9, three independent experiments).

(I) Expression of thermogenic adipocyte genes in stromal-vascular cells differentiated for 7 days in the absence or presence of TRL and LDL  $\pm$  Lalistat (n = 7). Note that the “mock” and “TRL” groups are the same as shown in Fig. 5M (shown for direct comparison only). Data are presented as mean values  $\pm$  SEM. p < 0.05 by ANOVA comparing mock vs. TRL(\*) and TRL vs. TRL + inhibitors (\$).