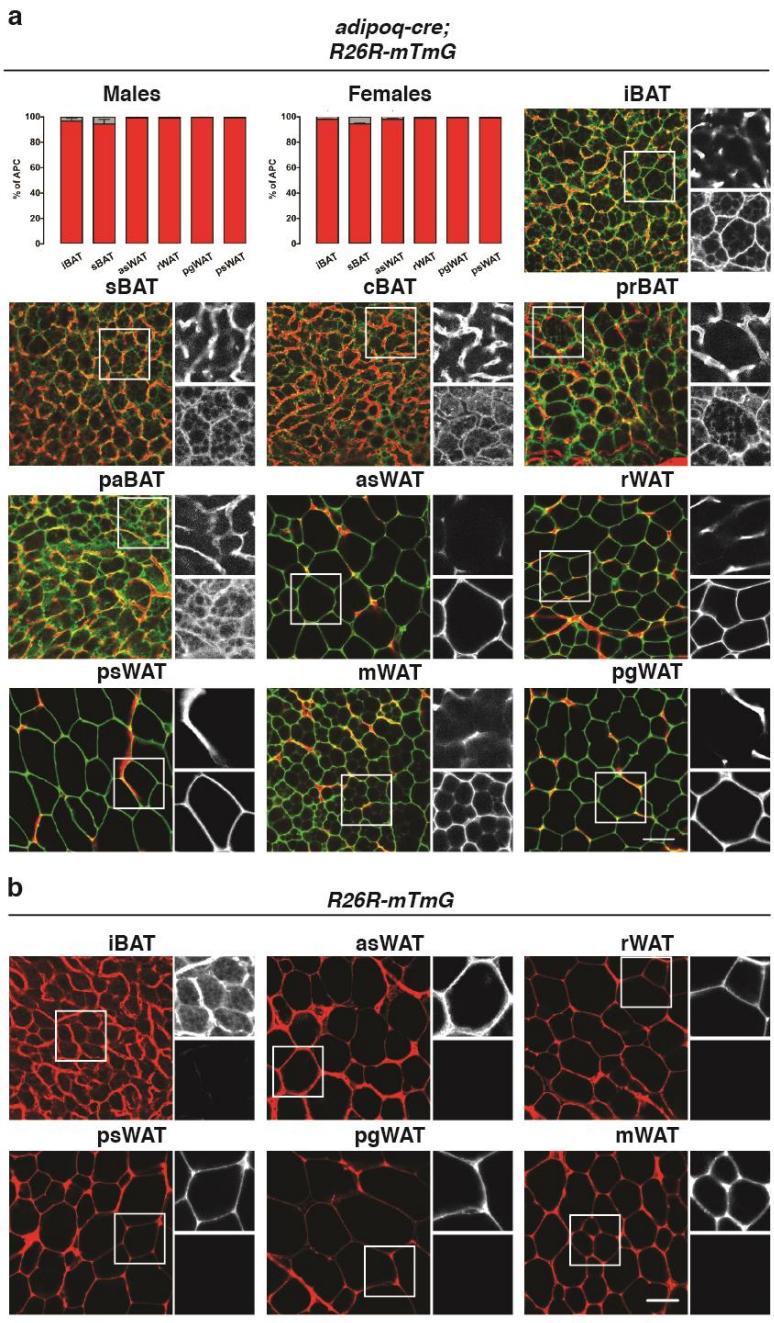
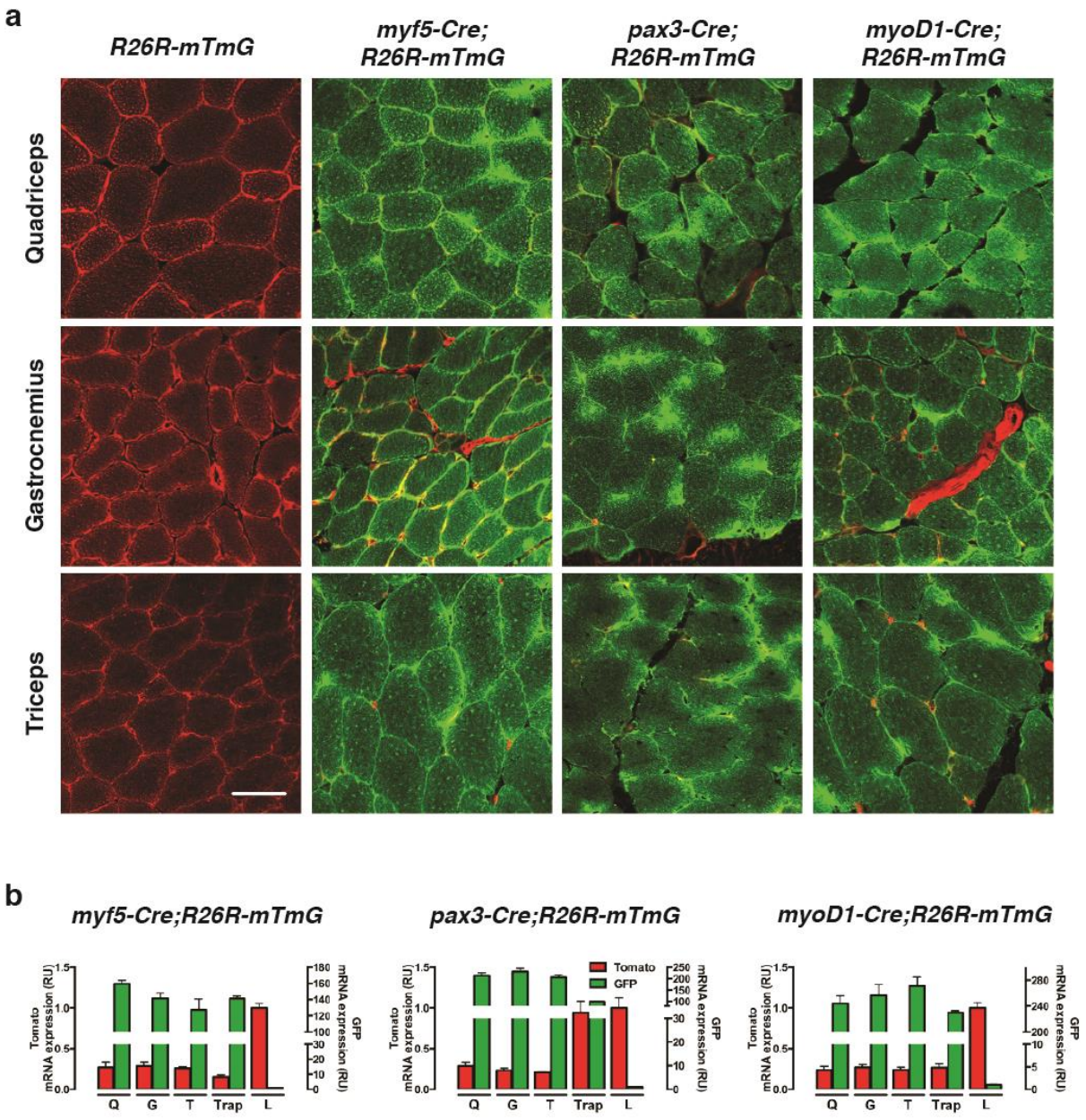


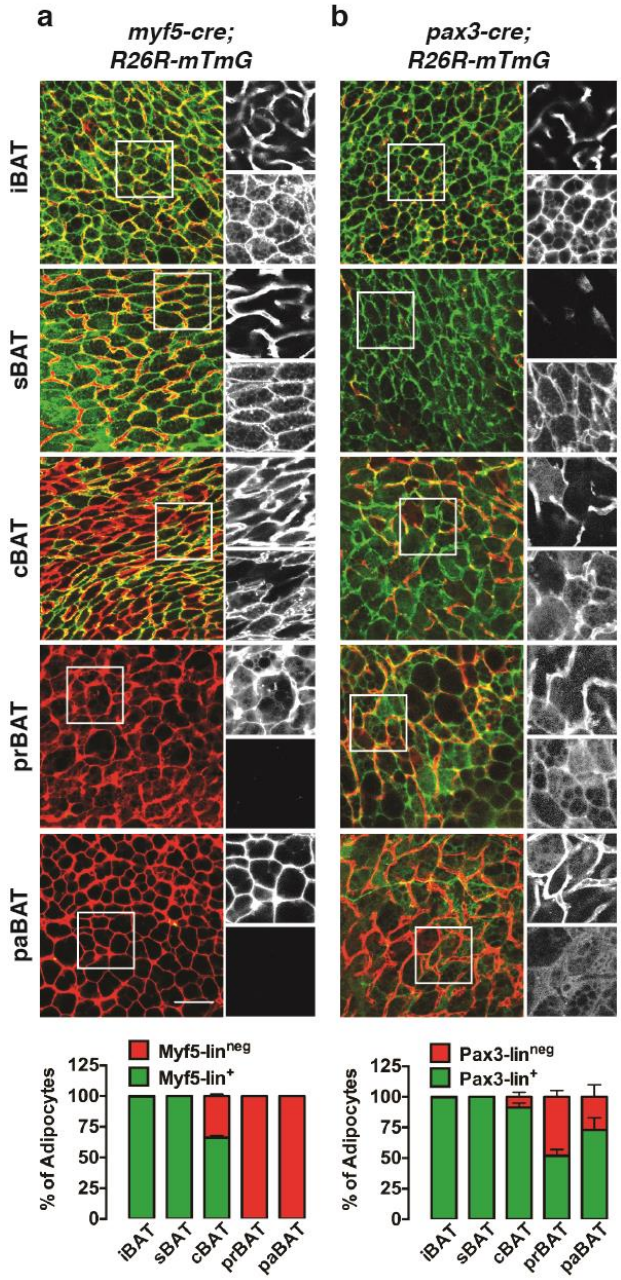
Supplementary Figure 1

(a) (*Top left and to middle panels*) Number of mTFP^{+} and mGFP^{+} APCs isolated from each of the indicated depots of 6 week old *adiponectin-cre;R26R*-mTmG male and female mice (n=5 males; n=3 females; mean+S.E.M). The remaining panels are representative images of whole-mount preparations from the indicated BAT and WAT depots of *adiponectin-cre;R26R-mTmG* mice. **(b)** Representative images of whole-mount preparations from the indicated depots of *R26R-mTmG* males. Scale bar= 50 μm.



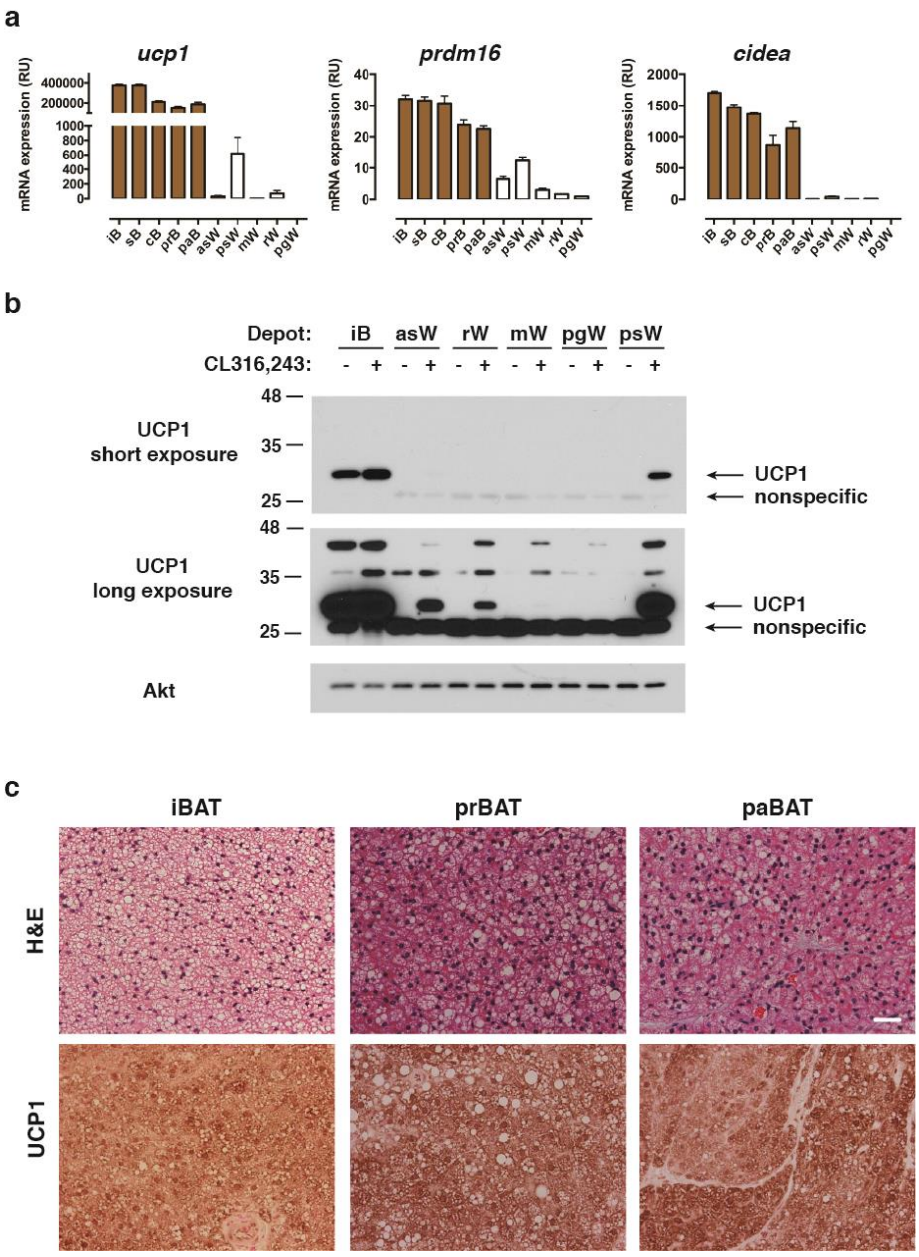
Supplementary Figure 2

(a) Representative confocal microscopy images of frozen sections of the indicated skeletal muscles from *myf5-cre;mTmG*, *pax3-cre;mTmG* and *myod1-cre;mTmG* mice. For all panels, scale bar= 50 µm. **(b)** qRT-PCR analysis of tdTomato and eGFP mRNA expression in quadriceps (Q), gastrocnemious (G), triceps (T),trapezius (Trap) and liver (L) of *myf5-cre;mTmG*, *pax3-cre;mTmG* and *myod1-cre;mTmG* mice. (n=3 for each genotype and tissue; mean+S.E.M).



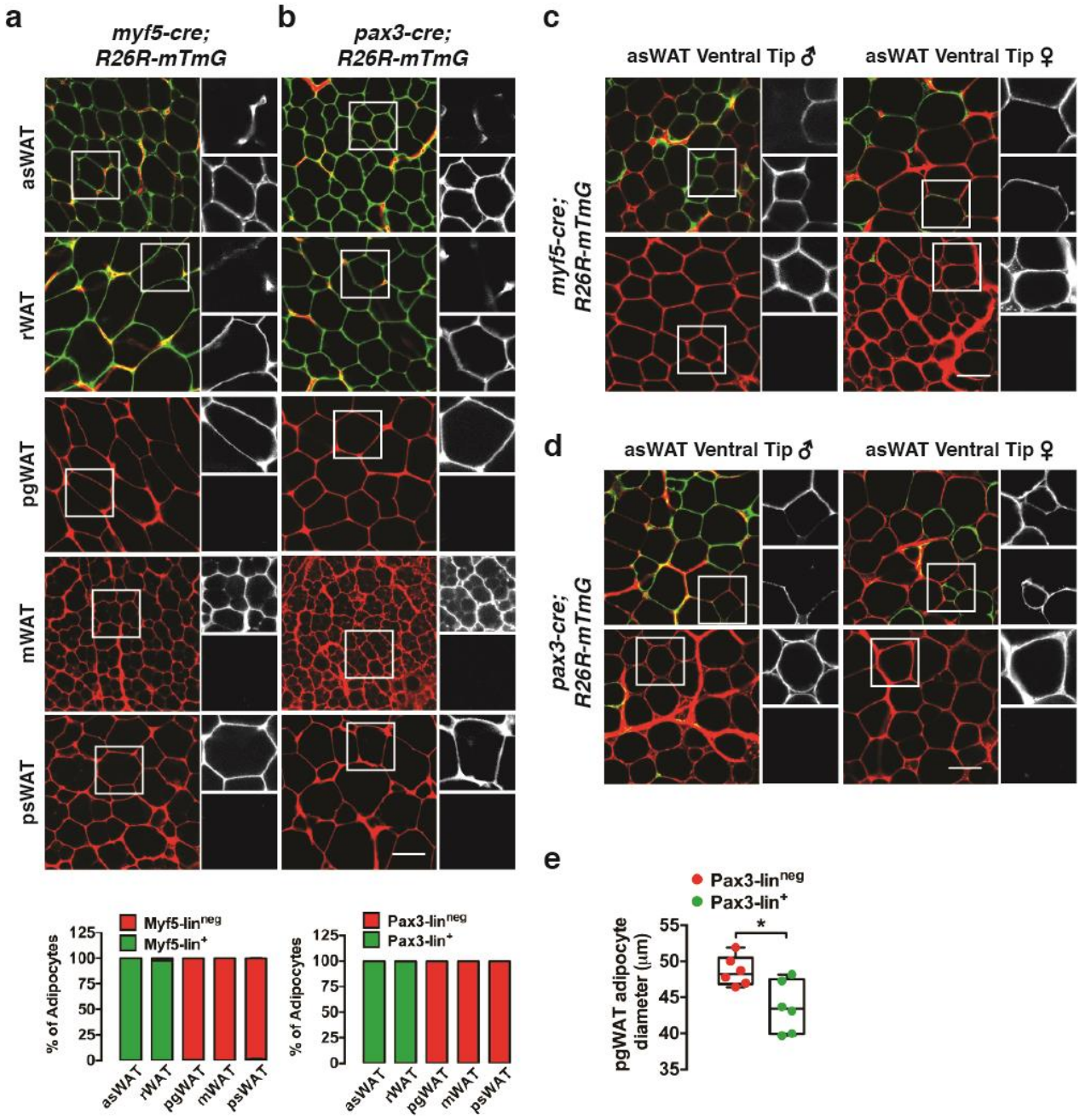
Supplementary Figure 3

(a) Representative images of whole-mount preparations from the indicated depots of *myf5-cre;R26R-mTmG* females. The number of mTFP^{+} and mGFP^{+} mature brown adipocytes is indicated in a graph at the bottom of each column (n=3-6; mean+S.E.M). **(b)** Same as (a) except using *pax3;R26R-mTmG* females. (n=3-9; mean+S.E.M). Scale bar= 50 μm.



Supplementary Figure 4

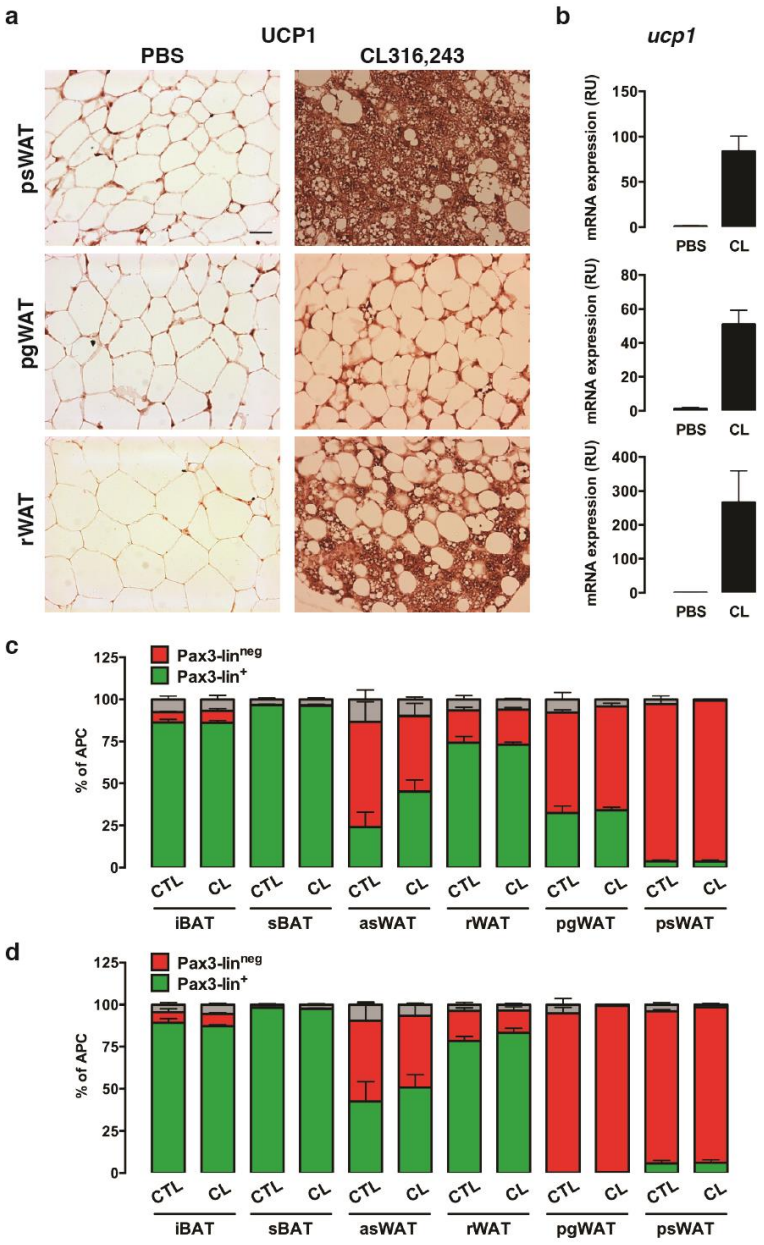
(a) *ucp1*, *prdm16* and *cidea* mRNA expression in the indicated BAT and WAT depots (n=4; mean+S.E.M). **(b)** Representative Western blot for the UCP1 antibody used in this study (Abcam #10983) in mice treated with vehicle or CL316,243 for one week. Note the while a correctly sized UCP1 band is detectable in iBAT and psWAT and in CL-treated asWAT and rWAT, many different sized background bands are also detectable including one particularly strong band around 25kDa. This antibody performed best in our validation studies. Akt is used here as a loading control. **(c)** H&E staining and immunohistochemistry against UCP1 in the indicated BATs using the Abcam antibody. Scale bar= 100 μm.



Supplementary Figure 5

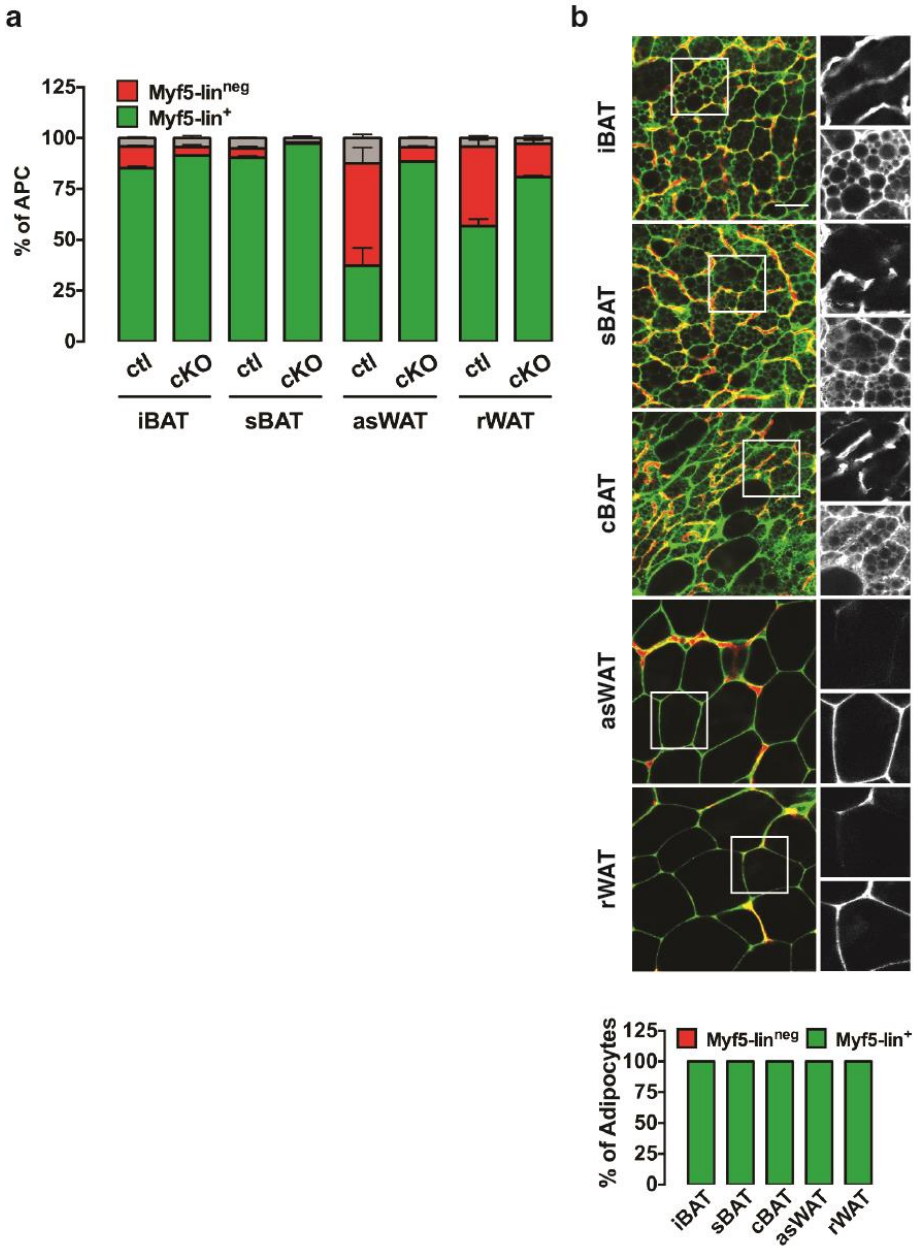
(a-b) Representative images of whole-mount preparations from indicated WATs of 6 weeks old *myf5;R26R-mTmG* females (a) and *pax3-cre;mTmG* (b). (Bottom panel) The number of $mTFP^+$ and $mGFP^+$ mature white adipocytes in the indicated fat depots for *myf5-cre;mTmG*. (n= 3-6 mice; mean+S.E.M). **(b)** Same as in (a) except using *pax3-cre;mTmG* females (n= 3-6 mice; mean+S.E.M).
(c) Representative images of whole-mount preparations from the ventral tip of asWAT in *myf5;R26R-mTmG* males and females. The bottom panel represents the extreme ventral tip, and the top panel represents a gradient that leads into

the Myf5-lin^{neg} extreme ventral tip region. **(d)** Same as (c) except using *pax3;R26R-mTmG* males and females. **(e)** Cell size analysis of the Pax3-lin^{+} and Pax3-lin^{neg} cells from the pgWAT of *pax3-cre;mTmG* males at 6-weeks old. (n=6; boxplot indicating maximum, minimum, media and all data points; *,$p<0.05$; t-test). Scale bars= 50 μm.



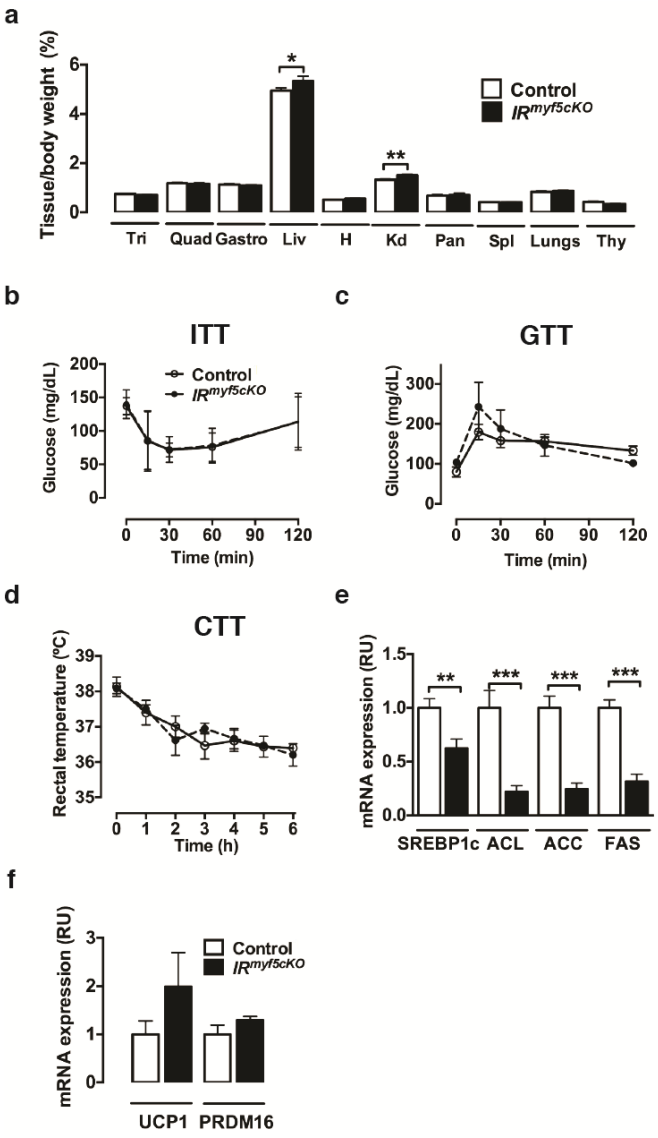
Supplementary Figure 6

(a) UCP1 immunohistochemistry was performed according to a published protocol (see methods) using the Abcam antibody on sections from the indicated depots of mice treated with PBS or Cl316,243 for 1 week. All multilocular cells strongly stain positive for UCP1 although small areas of potentially positive staining is also detectable in the PBS treated cells possibly indicating some level of background. Notably, the morphology of the multilocular cells in whole mount is different from the appearance in histological sections likely due to processing artifacts associated with fixing, embedding, and staining. Scale bar= 100 μm. **(b)** *ucp1* qRT-PCR using mRNA isolated from the indicated fat depots in PBS and CL316,243 treated mice. (n=3; mean+S.E.M).



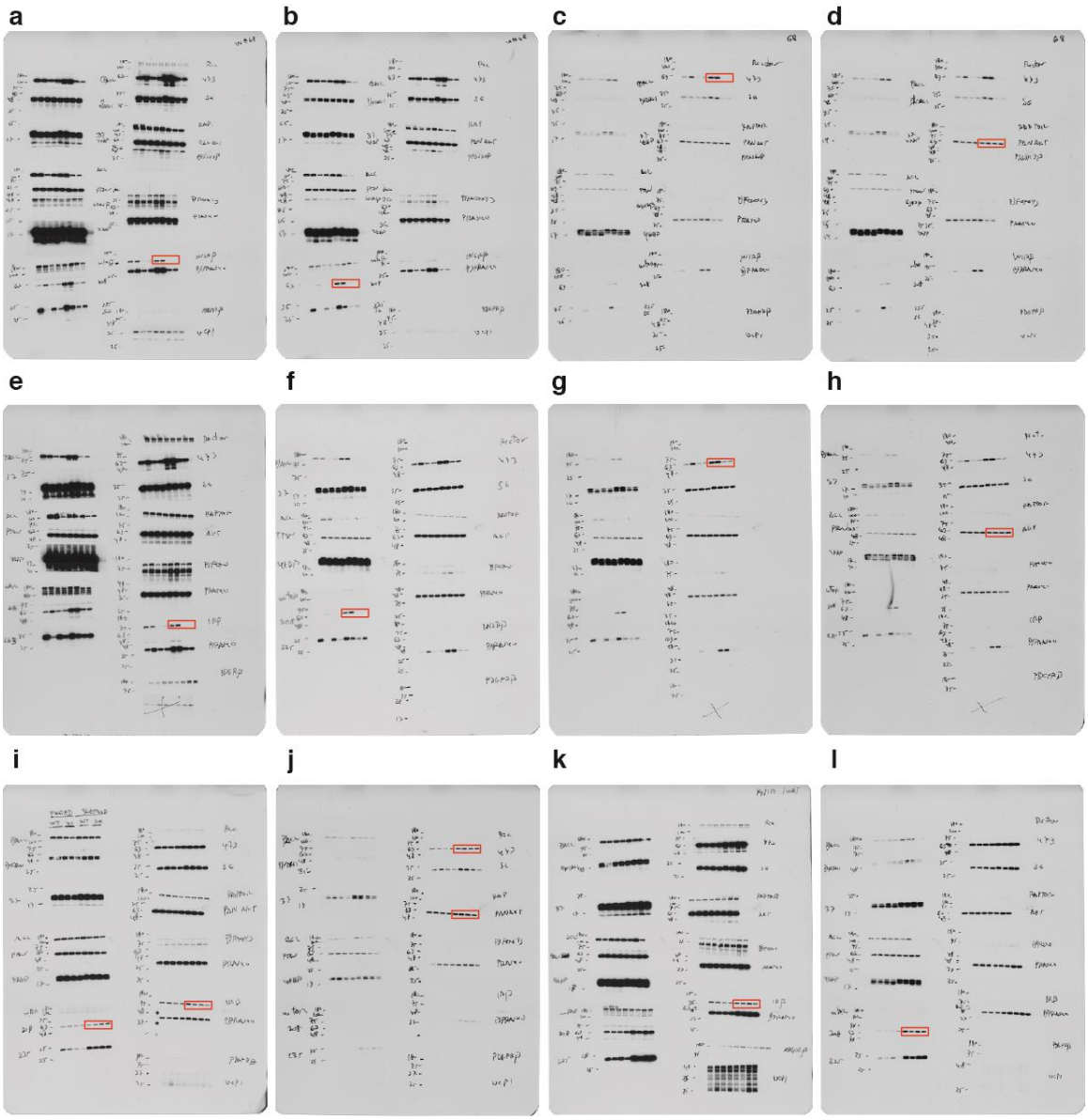
Supplementary Figure 7

(a) The number of mTFP$^+$ and mGFP$^+$ APCs isolated from the indicated depots of 6 week old *PTEN^{myf5cKO};R26R*-mTmG males compared to litter matched controls (n=3; mean+S.E.M). **(b)** Representative confocal images of whole-mount preparations from the indicated BATs and WATs of 6 week old *PTEN^{myf5cKO};R26R-mTmG* males showing that all adipocytes in these mice are mGFP$^+$. The quantification is shown below (n=3; mean+S.E.M). Scale bar= 50 µm.



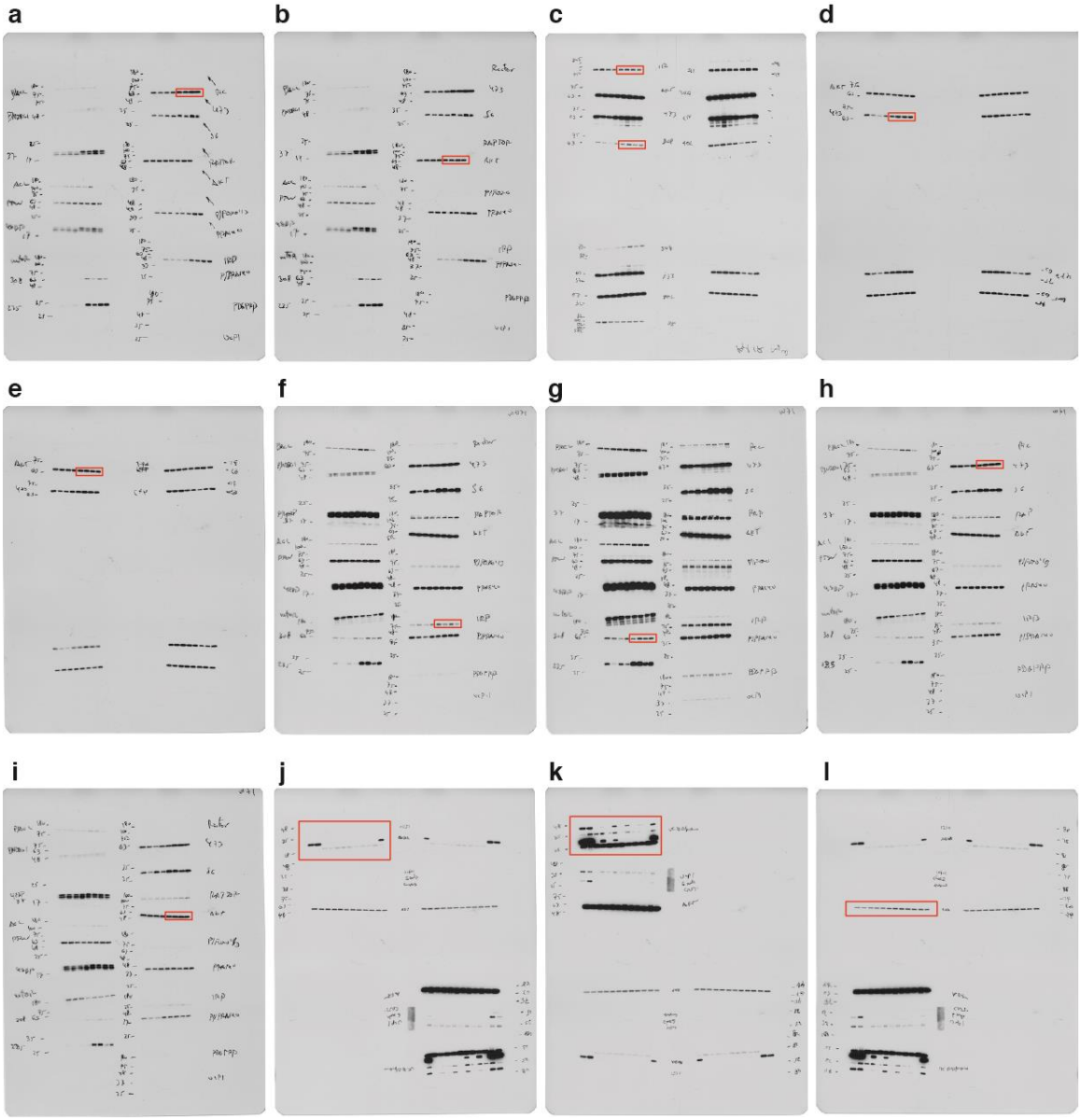
Supplementary Figure 8

(a) Lean tissue mass relative to body weight of the indicated tissues (Tri: triceps, Quad: quadriceps, Gastro: gastrocnemius, Liv: liver, H: heart, Kd: kidneys, Pan: pancreas, Spl: spleen, Thy: thymus). (n=14; mean+S.E.M; *p<0.05; **, p<0.01; t-test). **(b-d)** *IR*^{myf5cKO} and littermate control mice were subjected to insulin tolerance test (n=3 controls and 4 *IR*^{myf5cKO}; mean±S.E.M)(b), glucose tolerance test (n=4 controls and 6 *IR*^{myf5cKO}; mean±S.E.M)(c) and acute cold challenge (n=3 controls and 6 *IR*^{myf5cKO}; mean±S.E.M)(d). **(e)** The mRNA expression of lipogenesis genes in iBAT from *IR*^{myf5cKO} and littermate control mice (n=8 mice; mean+S.E.M; **, p<0.01; ***, p<0.001). **(f)** The mRNA expression of *ucp1* and *prdm16* in the ingWAT of *IR*^{myf5cKO} and littermate control mice (n=8 mice; mean+S.E.M).



Supplementary Figure 9

Original films of the western blot used in Figure 8 (Part 1). In red boxes is noted the bands used. Western blots for lysates of iBAT **(a-d)**, sBAT **(e-h)**, rWAT **(i-j)**, asWAT **(k-l)** are shown.



Supplementary Figure 10

Original films of the western blot used in Figure 8 (Part 2) and in Supplementary Figure 4. In red boxes is noted the bands used. Western blots for lysates of asWAT **(a-b)**, psWAT **(c-e)**, pgWAT **(f-i)** are shown. **(j-l)** Whole blots of various tissue lysates (noted in Supplementary Figure 4) of mice treated with PBS or CL316,243 for a week are shown.