

Figure S1. Repeated cold exposure induces uncoupling protein 1 (UCP1) and transmembrane protein 26 (TMEM26) in human abdomen subcutaneous white adipose tissue (SC WAT). An ice pack was applied to the abdomen for 30 min each day for 10 consecutive days. SC WAT was isolated and subjected UCP1 and TMEM26 immunohistochemistry as described in Methods. A and B) UCP1 and TMEM26 expression was determined as described in Methods. The data are expressed as area of UCP1 (A) or TMEM26 (B) staining (μm^2) per adipocyte number. The data were analyzed by a repeated measures MANOVA as described in Methods. Data represent means \pm SEM (n=11); **P<0.01; ***P<0.001.

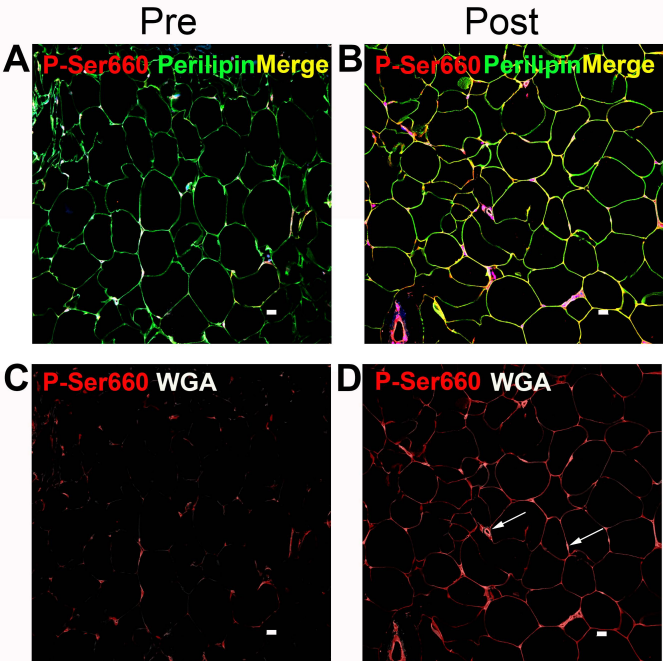
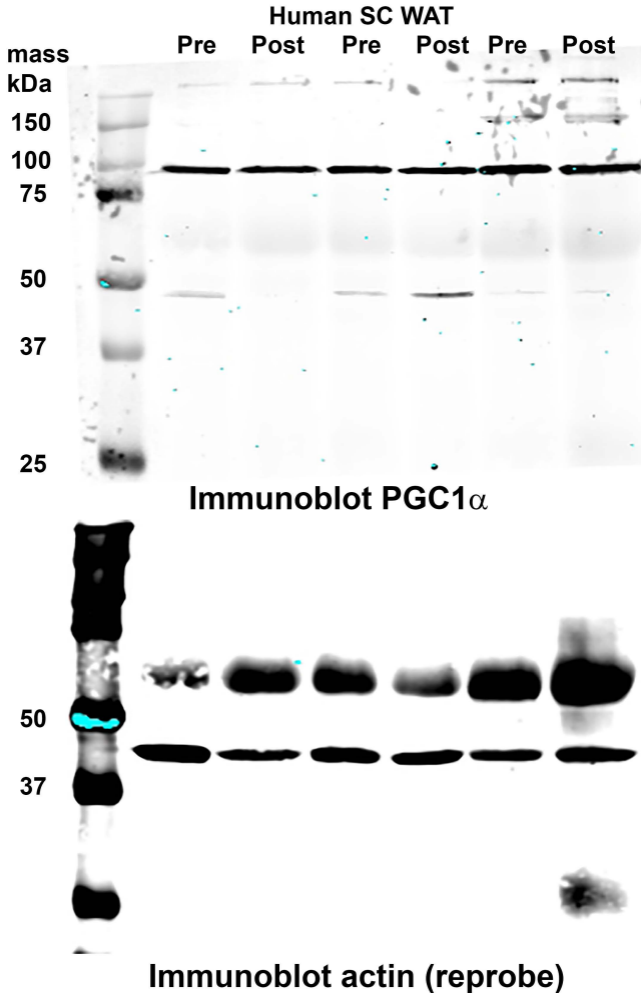


Figure S2. Phospho hormone sensitive lipase (HSL) serine⁶⁶⁰ (P-Ser660) staining colocalizes with perilipin and endothelial cells. An overlay of phospho-HSL serine⁶⁶⁰ staining (red) from Figure 8 with perilipin (green) and wheat germ agglutinin (WGA; white) is shown (scale bar: 10 μ m). A and B) phospho-HSL serine⁶⁶⁰ and perilipin (yellow indicates overlapping staining). C and D) An overlay of phospho-HSL serine⁶⁶⁰ staining (red) with WGA (white) from A and B is shown. Arrows point to examples of overlap of phospho-HSL serine⁶⁶⁰ staining with endothelial cells in capillaries.



Immunoblot actin (reprobe)

Figure S3. Uncropped immunoblots of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1 α) and actin. The uncropped immunoblots of human subcutaneous white adipose tissue before and after mirabegron treatment from the Figure 8E inset are shown. Reprobing with actin antibody resulted in a non specific band and a band at 45 KDa, the correct molecular mass of actin.

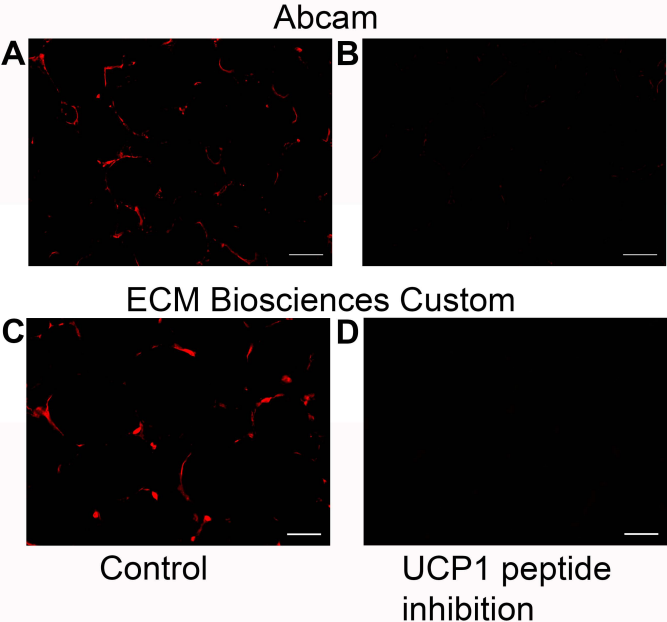


Figure S4. The reactivity of the Abcam and ECM biosciences uncoupling protein 1 (UCP1) antibodies is inhibited by UCP1 peptide. Thigh subcutaneous white adipose tissue from the cold treated leg of a lean research participant was stained with the indicated antibody with and without pre incubation with free UCP1 peptide (scale bar: 50 μ m).