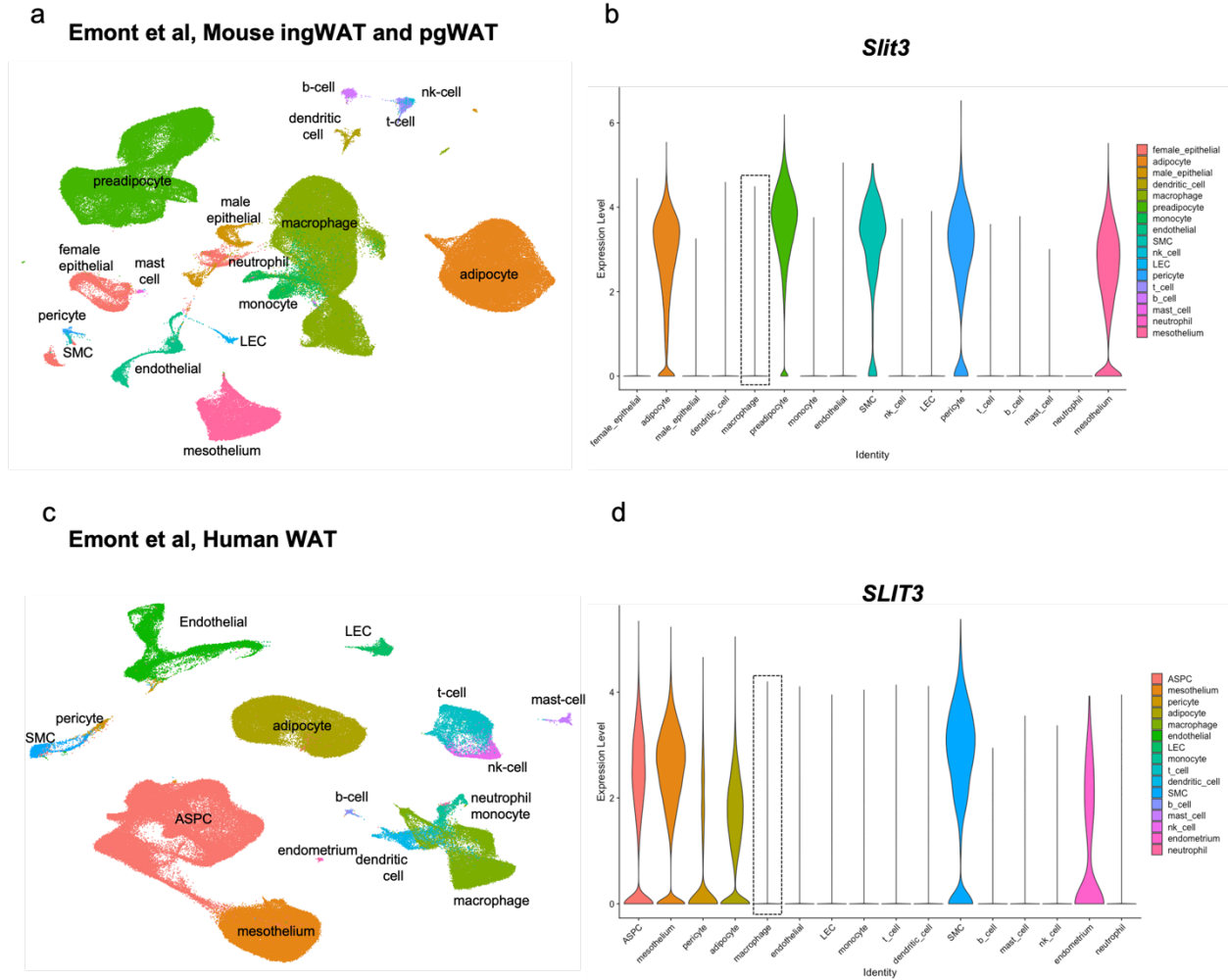
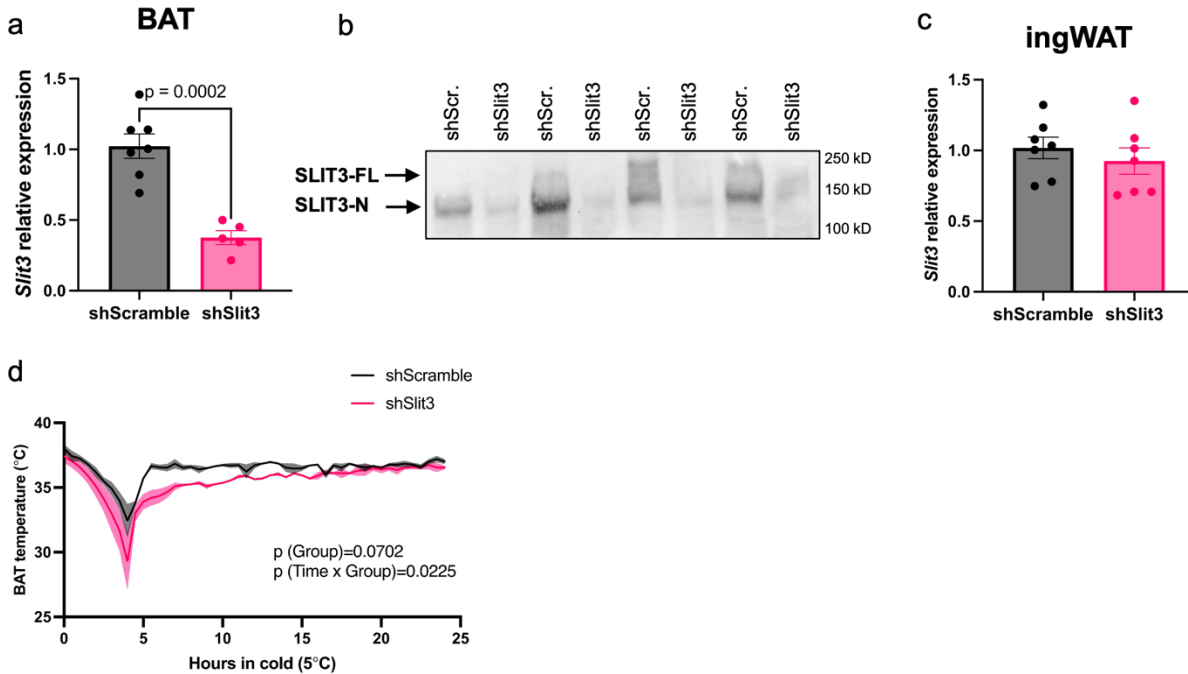


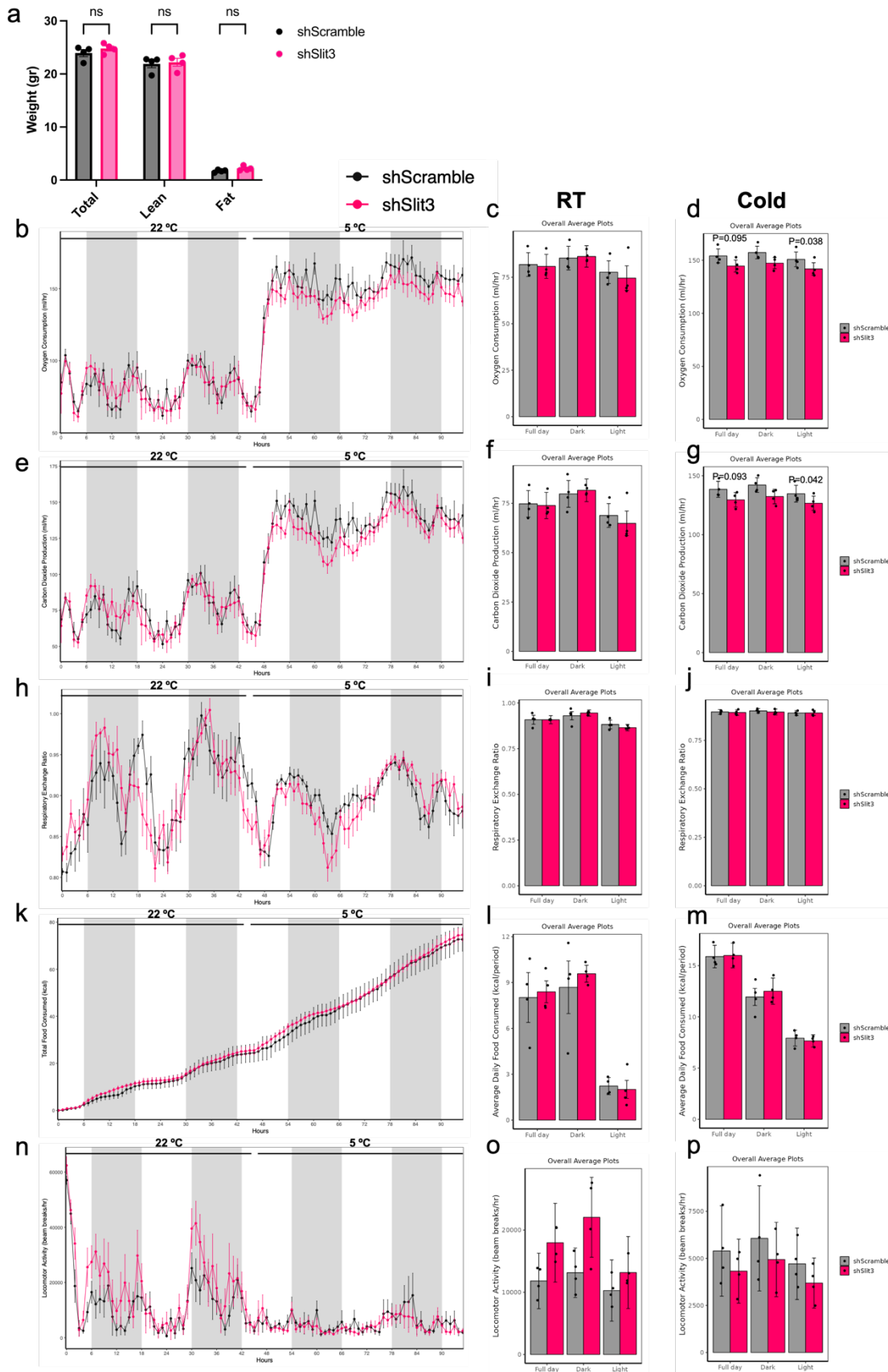
Supplementary Figure 1. Related to Figure 1. Expression of (a) *Pdgfra*, (b) *Adipoq*, (c) *Adgre1* in isolated adipocyte progenitors, macrophages, adipocytes, and the total stromal vascular fraction isolated from mouse BAT. Expression of (a) *Slit3* and (b) *Robo4* in Sca1^{neg}, Sca1^{pos}Cd31^{pos}, and Sca1^{pos}Pdgfra^{pos} cells isolated from BAT of mice housed at TN (30°C) or cold (5°C) for 7 days. N=3-5 per group. Data are presented as means ± SEM and analyzed by unpaired two-sided Student's t-tests.



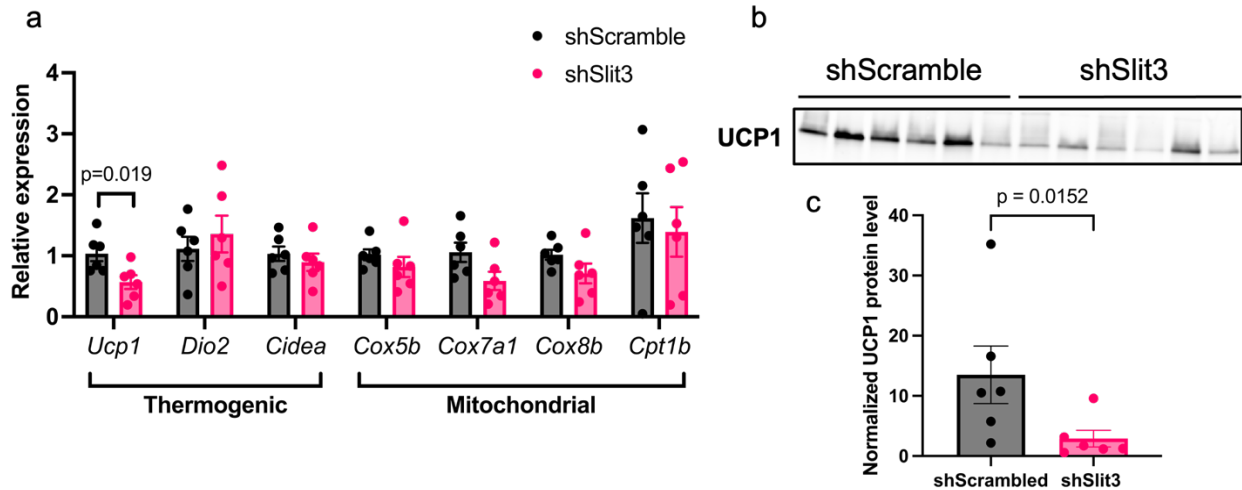
Supplementary Figure 2. Related to Figure 1. (a) Unsupervised clustering of nuclei from mouse ingWAT and pgWAT represented on a UMAP and (b) violin plot showing expression level and distributions of *Slit3* transcript in each cluster. (c) Unsupervised clustering of nuclei from human WAT represented on a UMAP and (d) violin plot showing expression level and distributions of *SLIT3* transcript in each cluster.



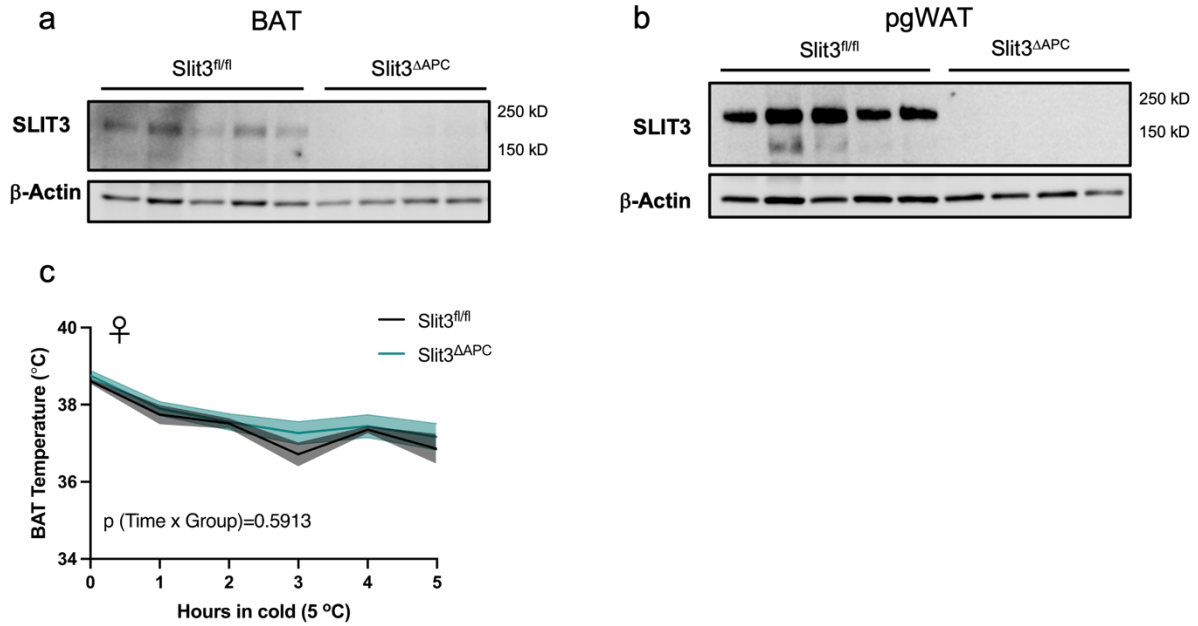
Supplementary Figure 3. Related to Figure 1. Slit3 (a) transcript and (b) protein in BAT, (c) Slit3 transcript in ingWAT, and (d) BAT temperature in mice receiving AAV-shSlit3 or scramble shRNA. N= 5-7 per group. Data are presented as means \pm SEM and analyzed by unpaired two-sided Student's t-tests (a and c) and repeated measures ANOVA (d).



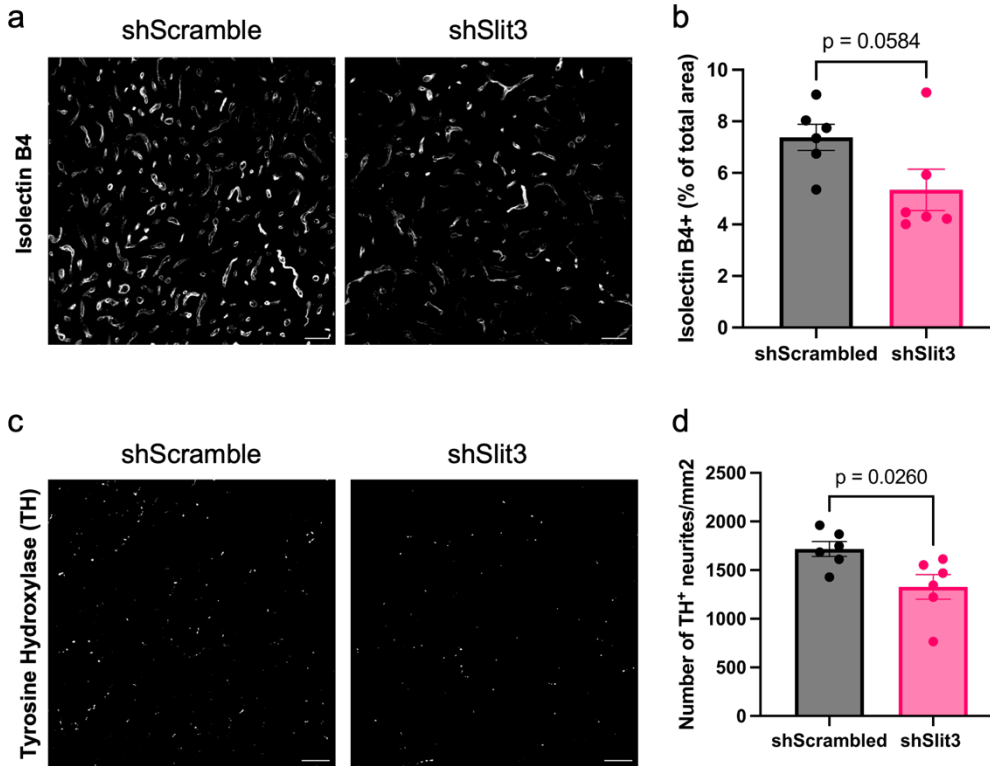
Supplementary Figure 4. Related to Figure 1. (a) Body composition analysis, (b, e, h, k, n) hourly rate, and (c-d, f-g, i-j, l-m, o-p) cumulative indirect calorimetry measurements in mice injected with AAV-shSlit3 or shScramble. N= 4 per group. Data are presented as means \pm SEM and analyzed by unpaired two-sided Student's t-tests (d and g).



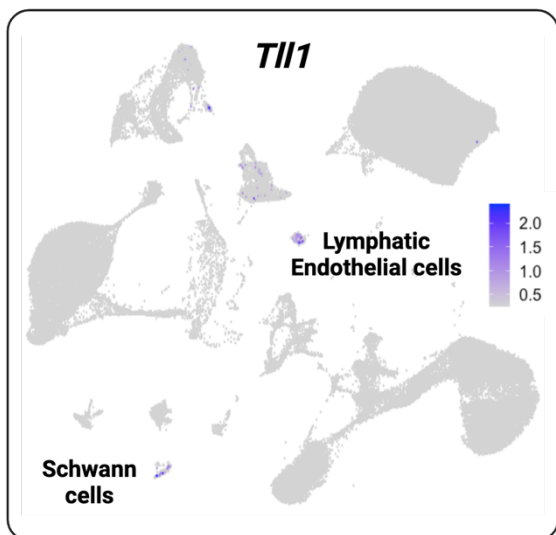
Supplementary Figure 5. Related to Figure 1. (a) expression of thermogenic and mitochondrial genes and (b) UCP1 protein level in BAT of mice receiving AAV-shSlit3 or scramble shRNA and housed at room temperature. N= 6 per group. Data are presented as means \pm SEM and analyzed by unpaired two-sided Student's t-tests.



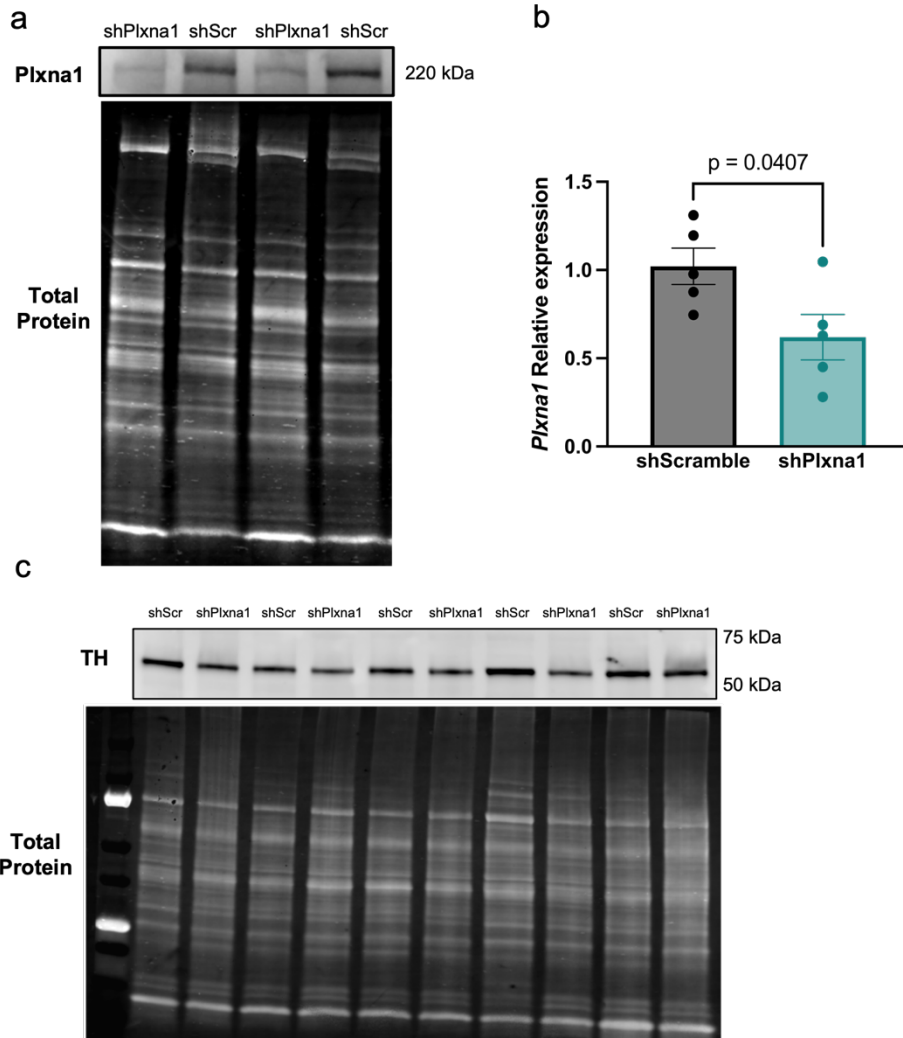
Supplementary Figure 6. Related to Figure 1. Slit3 protein in (a) BAT, (b) pgWAT and (c) BAT temperature in female Slit3^{fl/fl} and Slit3^{ΔAPC} mice. N= 4-5 per group (a-b) and N= 7-9 per group (c). Data are presented as means ± SEM and analyzed by repeated measures ANOVA (c).



Supplementary Figure 7. Related to Figure 2. (a) Representative images of Isolectin B4 staining (scale bar=20 μm), (b) quantification of the percentage of Isolectin B4⁺ area, (d) representative images of staining (scale bar=25μm), and (e) quantification of the number of TH⁺ neurites per area in BAT of mice receiving AAV-shSlit3 or scramble shRNA and housed at room temperature. N= 6 per group. Data are presented as means ± SEM and analyzed by unpaired two-sided Student's t-tests.



Supplementary Figure 8. Related to Figure 3. Expression of *Tll1* in scRNA-seq data of mouse BAT.



Supplementary Figure 9. Related to Figure 5. Plxna1 (a) protein, (b) transcript levels, and (c) TH western blot in BAT of mice treated with AAV-shPlxna1 or scramble shRNA. N = 5 per group. Data are presented as mean \pm SEM and analyzed by unpaired, two-sided Student's t-test (b).