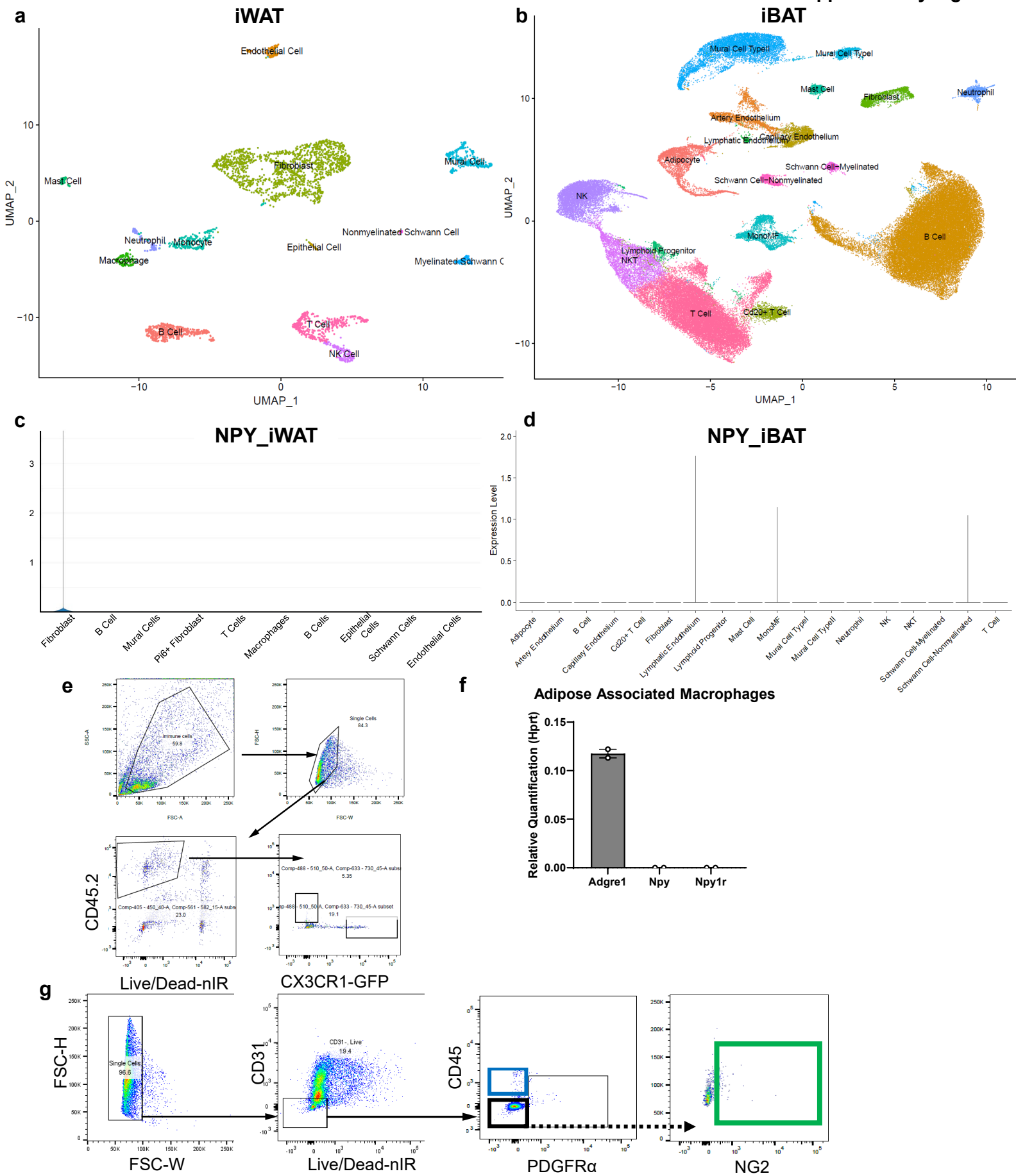

Supplementary information

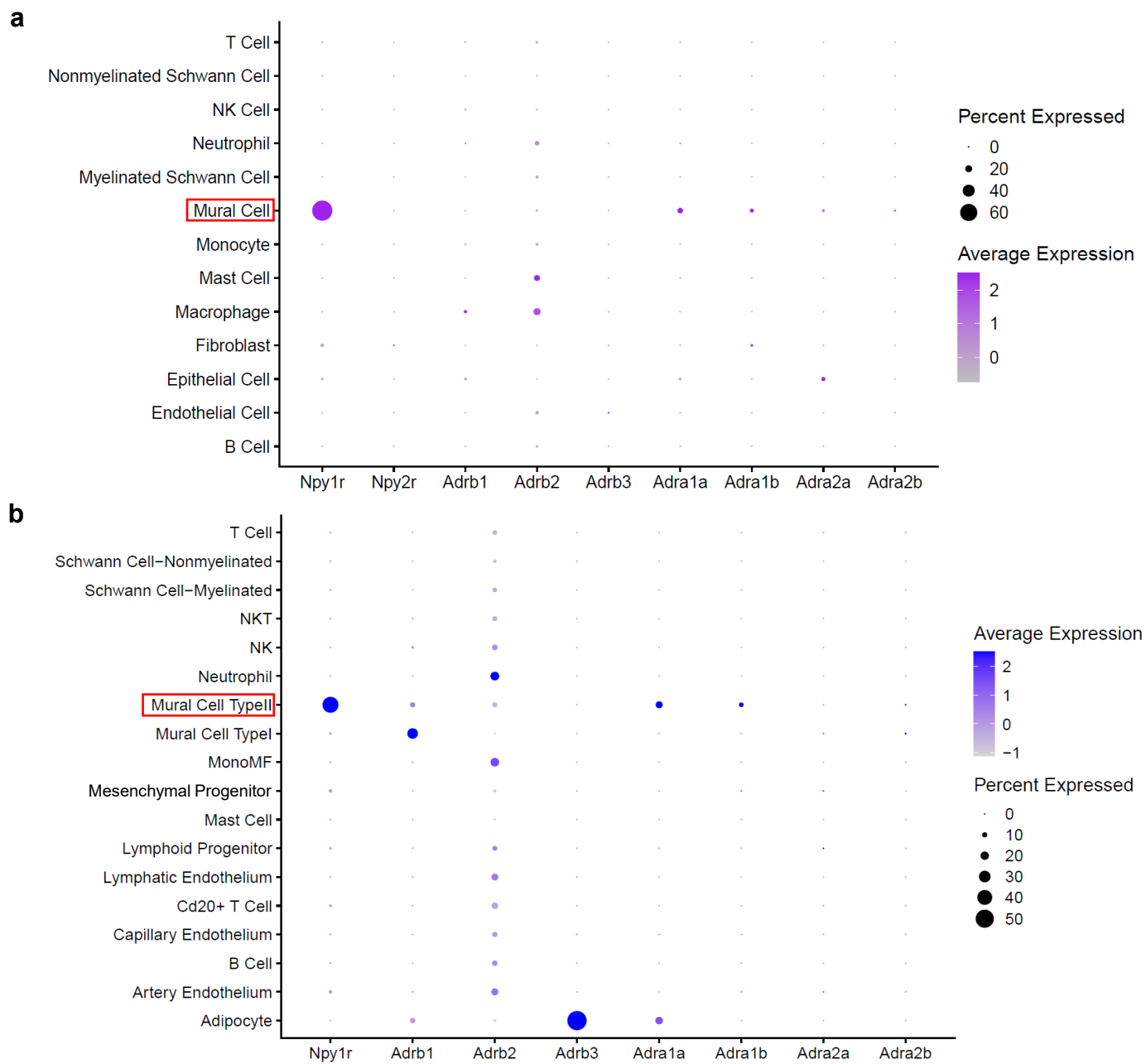
Sympathetic neuropeptide Y protects from obesity by sustaining thermogenic fat

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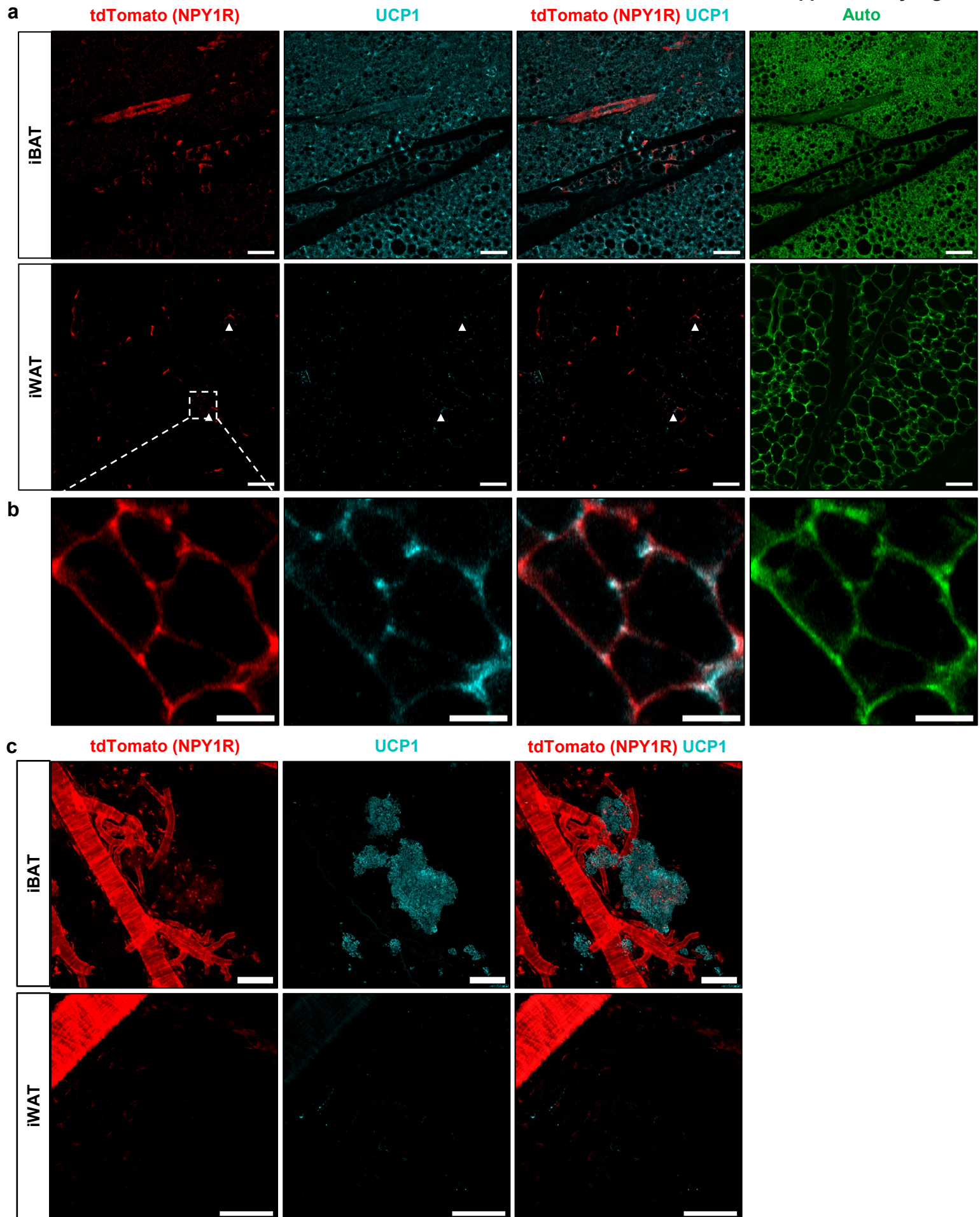
Supplementary Figure 1. *Npy* is not expressed in adipose tissues, and NPY receptors are not expressed in macrophages.

(a-b) Embedding plots showing different clusters of cells in the SVF of murine (a) iWAT¹ and (b) iBAT². (c-d) Violin plot showing the expression of *Npy* in the SVF of murine (c) iWAT and (d) iBAT. (e) Gating strategy of sorting adipose associated macrophages (ATM) from *Cx3cr1*^{GFP/+} reporter mice. ATMs were sorted as live/CD45.2⁺/GFP⁺ cells. (f) The expression of *Adgre1* (F4/80), *Npy*, and *Npy1r* in adipose associated macrophages (ATM) (n=2). (g) Gating strategy for sorting mural cells from the SVF of the iWAT of wild type normal-diet treated mice, used for Figure 2e. Mural cells (green box) are sorted as live/CD31⁺/CD45⁺/PDGFR α ⁺/NG2⁺ cells; immune cells (blue box) are sorted as live, CD31⁺/CD45⁺ cells. The same gating strategy was also used for cell cytometry data shown in Extended Data Fig. 8c-d. All values are expressed as mean \pm SEM.



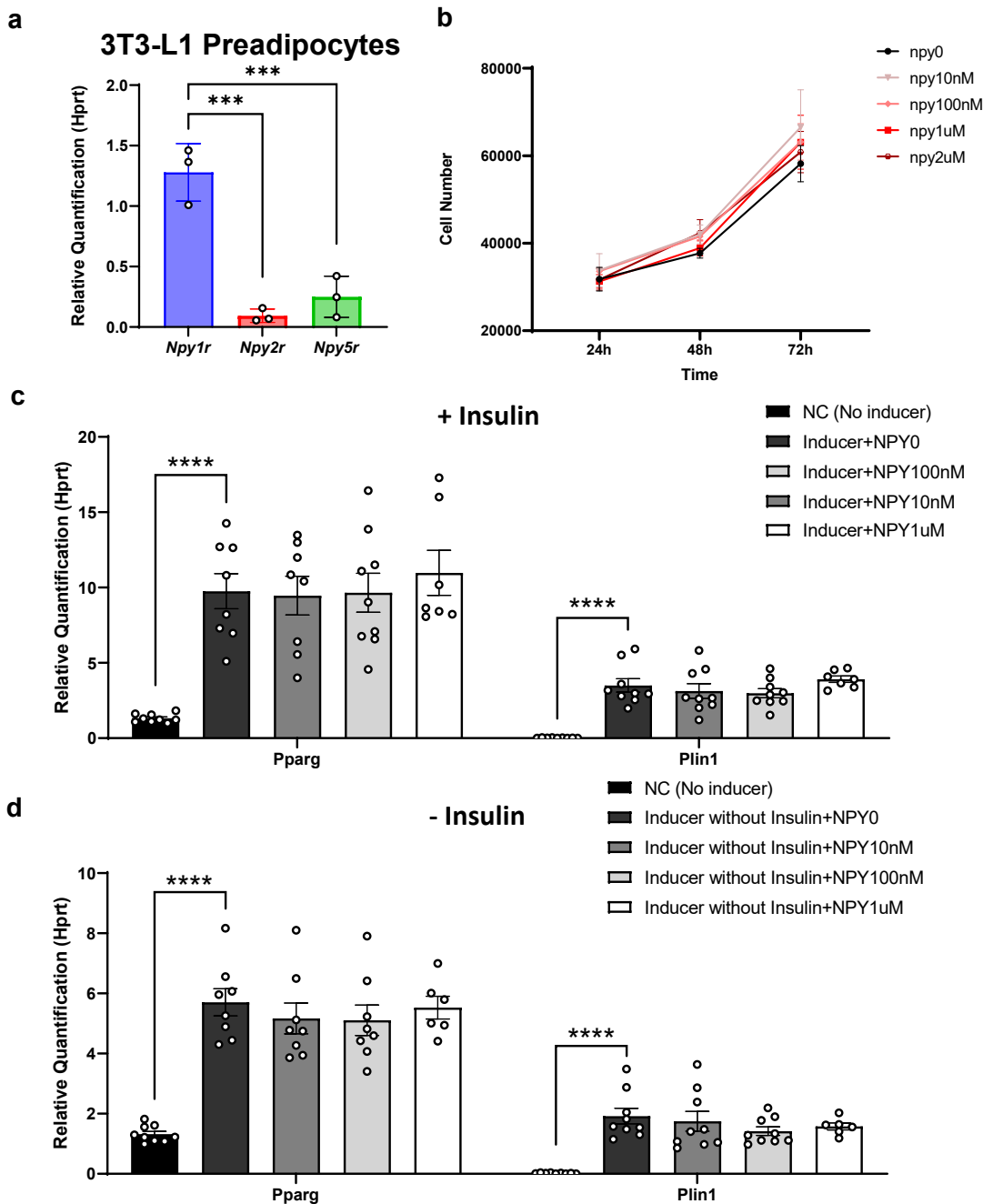
Supplementary Figure 2. The expression levels of *Npy2r* and *Npy5r* in adipose tissues are negligible.

(a) Dot plot showing the expression of *Npy1r*, *Npy2r*, *Adra1a*, *Adra1b*, *Adra2a*, *Adrb1*, *Adrb2*, and *Adrb3* in different cells in the SVF of mice iWAT. **(b)** Dot plot showing the expression of *Npy1r*, *Adrb1*, *Adrb2*, *Adrb3*, *Adra1a*, *Adra1b*, *Adra2a*, and *Adra2b* in different types of cells in mouse iBAT. The size of the dot represents the percentage of cells expressing a certain gene, and the darkness of the dot represents the expression level. Mural cell clusters are highlighted with red rectangles.



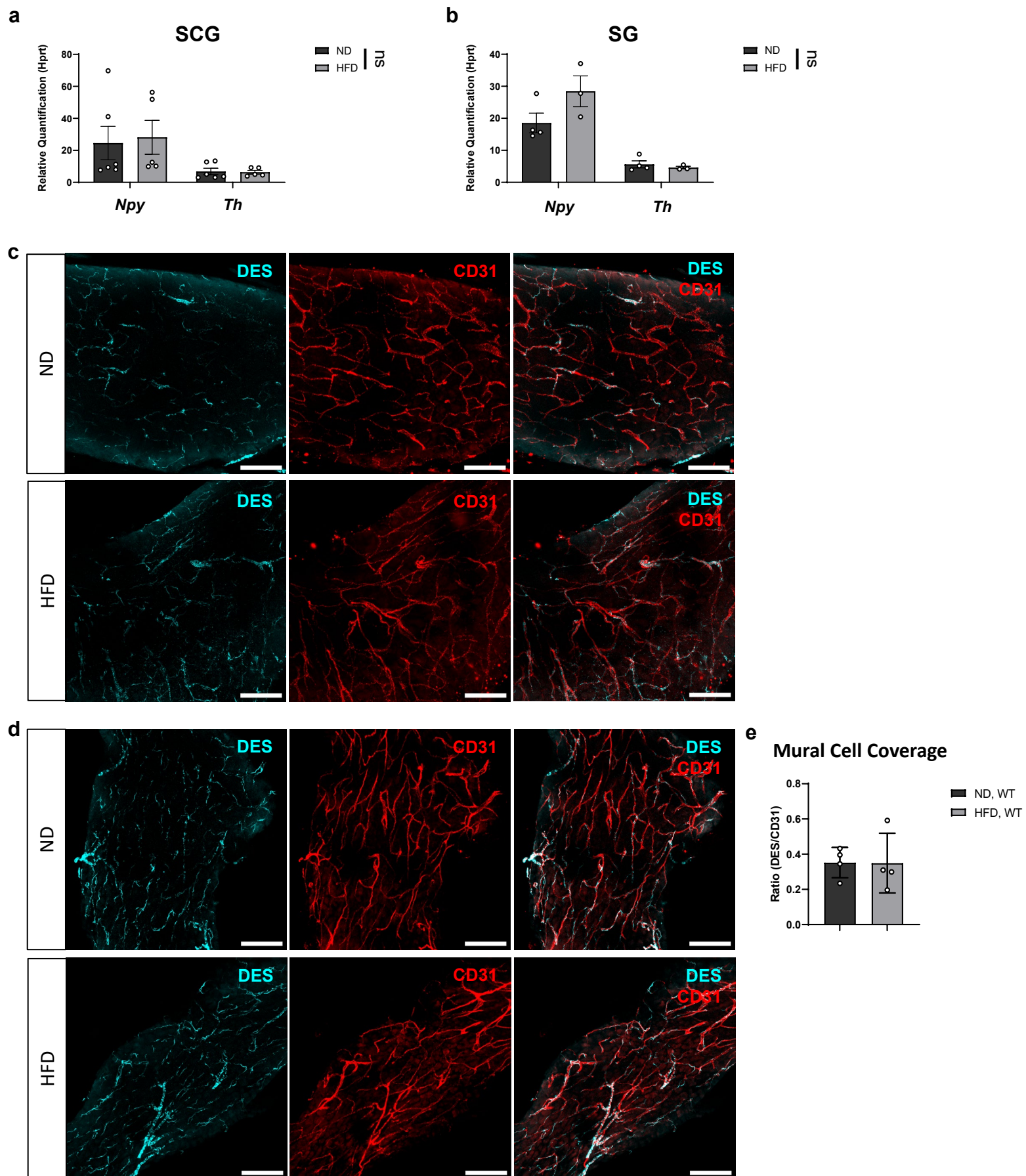
Supplementary Figure 3. Only UCP1⁺ multi-locular adipocytes are lineage-traced to NPY1R.

(a-c) Confocal images of (a) paraffin-embedded slides (scale bar=50 μ m) and (c) whole-mount vessels (scale bar=100 μ m) of iBAT and iWAT of 12-week-old male *Npy1r^{Cre}; Rosa26^{tdTomato}* mice stained with UCP1 (Cyan). (b) is zoomed-in (a) (scale bar=10 μ m). 488nm laser-excited autofluorescence (green), which is deviated from red and far-red (cyan) channels, indicates the presence of adipocytes. White arrows indicate multi-locular UCP1⁺ adipocytes in iWAT. Representative images are shown from 2 experiments.



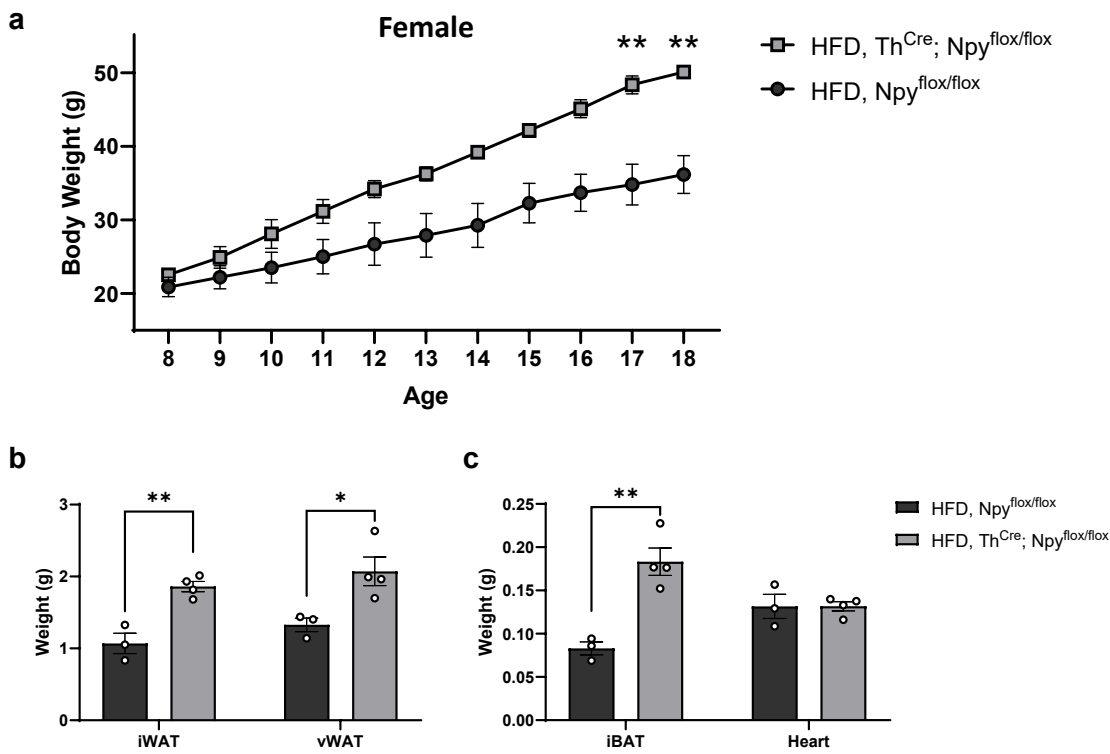
Supplementary Supplementary Figure 4. NPY does not affect proliferation or adipogenesis of 3T3-L1.

(a) The expression of *Npy1r*, *Npy2r*, and *Npy5r* in 3T3-L1 preadipocyte cell line (n=3 biologically independent samples). *Npy1r* vs. *Npy2r*, $P=0.0004$; *Npy1r* vs. *Npy5r*, $P=0.0008$. (b) The impact of NPY on the proliferation of 3T3-L1 preadipocytes revealed by the cell number after incubating with or without 1 μ M NPY for 24, 48, and 72 hours (n=3 biologically independent samples). (c-d) The expression of adipogenesis markers *Pparg* and *Plin1* in differentiated 3T3-L1 preadipocytes induced by induction medium (c) with or (d) without 1 μ g/mL insulin (n=9, 8, 8, 9 & 7 biologically independent samples per for *Pparg*, n=9, 9, 9, 9 & 7 biologically independent samples per for *Plin1*). The concentration of NPY is indicated in the plot. For all comparisons labelled, $P<0.0001$. All values are expressed as mean \pm SEM. Statistical comparisons were made using one-way ANOVA, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplementary Figure 5. Obesity does not affect *Npy* expression or mural cell coverage in sympathetic ganglia.

(a-b) The expression of *Npy* and *Th* in the (a) superior cervical ganglia (SCG, n=6&5 mice) and (b) stellate ganglia (SG, n=4&3 mice) of ND- and HFD-treated 17-week-old WT male mice. (c-d) The images of (c) cleared SCG and (d) cleared SG of ND- and HFD-treated 17-week-old WT male mice stained with anti-DES (cyan) and anti-CD31 (red) antibodies. Scale bar=100 μ m. (e) The quantification of mural cell coverage in SG (n=4 mice). All values are expressed as mean \pm SEM.



Supplementary Figure 6. Metabolic phenotype of female Th^{Cre}; Npy^{flox/flox} mice is similar to that of males.

(a) The weekly body weights of female Th^{Cre}; Npy^{flox/flox} mice and WT mice treated with high-fat diet (HFD) (n=4). At Week 17, P=0.0041; at Week 18, P=0.0019. (b-c) The weights of (b) iWAT (P=0.0029) and vWATs (P=0.0299), and (c) iBAT (P=0.0039) and hearts of HFD-treated 18-week-old female Th^{Cre}; Npy^{flox/flox} mice and Npy^{flox/flox} mice (n=3&4). All values are expressed as mean \pm SEM. Statistical comparisons were made using 2-tailed Student T-tests, *p<0.05, **p<0.01.

Supplementary Table 1. The Sequence of Primers for RT-qPCR

<i>Gapdh</i>	Forward	5'-AACTTTGGCATTGTGGAAGG-3'
	Reverse	5'-ACACATTGGGGGTAGGAACA-3'
<i>Hprt</i>	Forward	5'-CAGTCCCAGCGTCGTGATTA-3'
	Reverse	5'-TGGCCTCCCATCTCCTTCAT-3'
<i>Plin1</i>	Forward	5'-TAGAGTTCCTCCTGCCACCA-3'
	Reverse	5'-GTGCTGACCCTCCTCACAAG-3'
<i>Pparg</i>	Forward	5'-TCAAGGGTGCCAGTTTCG-3'
	Reverse	5'-GGAGGCCAGCATCGTGT-3'
<i>Des</i>	Forward	5'-TCCAGTCCTACACCTGCGAGAT-3'
	Reverse	5'-CGCAATGTTGTCCTGATAGCCA-3'
<i>Npy</i>	Forward	5'-CCCCAGAACAAGGCTTGAAG-3'
	Reverse	5'-TTGGAAAAGTCGGGAGAACAA-3'
<i>Npy1r</i>	Forward	5'-CTGATGGACCACTGGGTCTT-3'
	Reverse	5'-GAAGAAGCCACTGCAAGGAC-3'
<i>Npy2r</i>	Forward	5'-ATCATCTTGCTAGGGGTAGTTGG-3'
	Reverse	5'-CGGACCCATTTTCCACTCTCC-3'
<i>Npy5r</i>	Forward	5'-GTGAACTTTCTCATAGGCAACCT-3'
	Reverse	5'-GCATGATATGGCACATGGCTTT-3'
<i>Pdgrfb</i>	Forward	5'-AGGACAACCGTACCTTGGGTGACT-3'
	Reverse	5'-CAGTTCTGACACGTACCGGGTCTC-3'
<i>Rgs5</i>	Forward	5'-CAAATGGCGGAGAAGGCAA-3'
	Reverse	5'-CCAAGTCAAAGCTGCGAGGA-3'
<i>Tgfb1</i>	Forward	5'-TGATACGCCTGAGTGGCTGTCT-3'
	Reverse	5'-CACAAGAGCAGTGAGCGCTGAA-3'
<i>Cidea</i>	Forward	5'-TGCTCTTCTGTATCGCCCAGT-3'
	Reverse	5'-GCCGTGTTAAGGAATCTGCTG-3'
<i>Pgc1a</i>	Forward	5'-CCCTGCCATTGTTAAGAC-3'
	Reverse	5'-TGCTGCTGTTCTGTTTTTC-3'
<i>Ucp1</i>	Forward	5'-ACTGCCACACCTCCAGTCATT-3'
	Reverse	5'-CTTTGCCTCACTCAGGATTGG-3'
<i>Prdm16</i>	Forward	5'-CAGCACGGTGAAGCCATT-3'
	Reverse	5'-GCGTGCATCCGCTTGTG-3'
<i>Serca2b</i>	Forward	5'-ACCTTTGCCGCTCATTTTCCAG-3'
	Reverse	5'-AGGCTGCACACACTCTTTACC-3'
<i>Angpt1</i>	Forward	5'-TGCAGCAACCAGCGCCGAAA-3'
	Reverse	5'-CACGGCAGTTCCCGTCGTGT-3'
<i>Tnf</i>	Forward	5'-ATGAGCACAGAAAGCATGA-3'
	Reverse	5'-TACAGGCTTGTCACTCGAA-3'
<i>Il6</i>	Forward	5'-ACGGCCTTCCCTACTTCACA-3'
	Reverse	5'-CATTCCACGATTTCCAGA-3'

References

- 1 Henriques, F. *et al.* Single-cell RNA profiling reveals adipocyte to macrophage signaling sufficient to enhance thermogenesis. *Cell reports* **32**, 107998 (2020).
- 2 Shamsi, F. *et al.* Vascular smooth muscle-derived Trpv1+ progenitors are a source of cold-induced thermogenic adipocytes. *Nature metabolism* **3**, 485-495 (2021).