Table S1. Primer sequences for RT-qPCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	GGCACCACACCTTCTACAATGAGCT	GACAGCACAGCCTGGATGGCTAC
Acc1	GCAGTGTGGGCTGGCTGGG	ACATGGCCTGGCTTGGAGGG
Adipoq	CGTGATGGCAGAGATGGCACTC	AGCGATACACATAAGCGGCTTCTCC
C/ebpβ	GGACAAGCTGAGCGACGAGTACAAG	TGCTTGAACAAGTTCCGCAGGG
Dio2	TGTGTCTGGAACAGCTTCCTCCTAGA	AAGTCAAGAAGGTGGCATTCGGC
Ebf2	GCATTTCAGAGTCCACACAAGGAAATAA	ATGCTGTTGCTGGAGGTGCTGTAAT
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Fas	CGCTGCGGAAACTTCAGGAAAT	CCAGCAGTGTGCTCAGGTTCAGTT
Glut1	GCAGATGATGCGGGAGAAGAAGG	GCCTTCTCGAAGATGCTCGTTGA
Glut4	CTGATTCTGCTGCCCTTCTGTCCT	GACATTGGACGCTCTCTCTCCAACTT
Hsl	CATGGCTCAACTCCTTCCTGGAAC	TTCAAGGTATCTGTGCCCAGTAAGCC
Lpl	CCAGGATGCAACATTGGAGAAGC	GCAGGGAGTCAATGAAGAGATGAATG
Pgc-1a	ACGTCCCTGCTCAGAGCTTCTCA	ATGTTGCGACTGCGGTTGTGTATG
Ppara	ACAAGTGCCTGTCTGTCGGGATG	TCCGAATCTTTCAGGTCGTGTTCA
Pparg	AGGGCGATCTTGACAGGAAAGACA	AAATTCGGATGGCCACCTCTTTGC
Prdm16	CACAGCACGGTGAAGCCATTCA	TAGTGCTGAACATCTGCCCACAGT
Ucp1	AGCCACCACAGAAAGCTTGTCAAC	ACAGCTTGGTACGCTTGGGTACTG

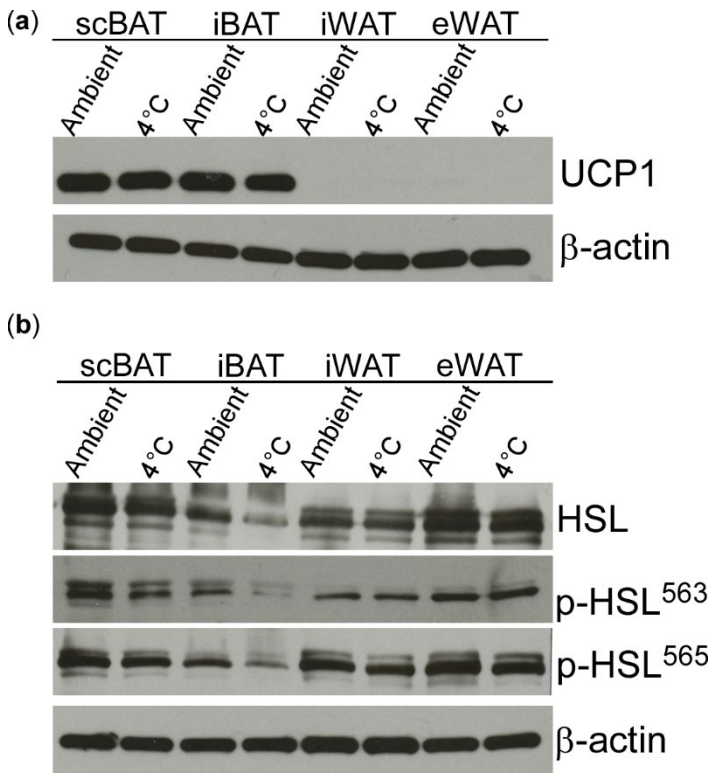

Figure S1. Protein levels of UCP1, HSL, and p-HSL in adipose tissues from mice housed at ambient temperature or after 6 hours of cold exposure. Representative images of western blot analysis of UCP1, HSL, and p-HSL (p-Ser^{563} and p-Ser^{565}) in scBAT, iBAT, iWAT, and eWAT dissected from an 8-week-old mouse housed at ambient temperature or after 6 hours of cold exposure. (**a**) UCP1, (**b**) HSL, p-HSL^{563}, and p-HSL^{565}. β-actin was used as a loading control. Three independent western blot analyses were performed for UCP1, HSL, and p-HSL^{565}. Two independent western blot analyses were performed for p-HSL^{563}. p-HSL^{565} has been shown to prevent phosphorylation of HSL at Ser^{563} [60].

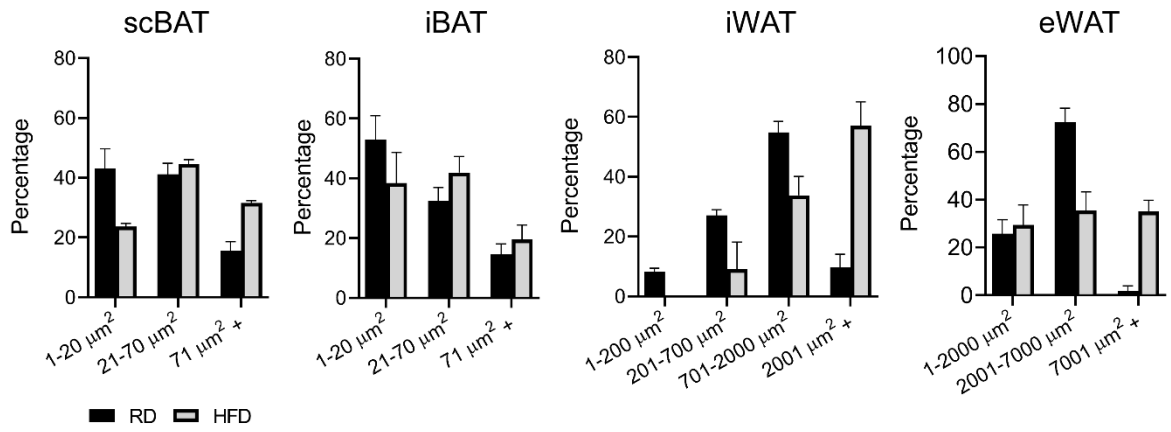

Figure S2. Size distributions for lipid droplets in scBAT, iBAT, iWAT, and eWAT from mice fed RD or 16 weeks of HFD. Lipid droplets were grouped based on their sizes (μm), small (BAT: 20 μm² or less, iWAT: 200 μm² or less, eWAT: 2000 μm² or less), medium (BAT: 21-70 μm², iWAT: 201-2000 μm², eWAT: 2001-7000 μm²), or large (BAT: 71 μm² +, iWAT: 2001 μm² +, eWAT: 7001 μm² +). The distributions are presented as percentage of lipid droplets that are small, medium, or large within the total amount of lipid droplets counted. The percentage of lipid droplets in the large size group is higher in mice fed with HFD than with RD. Adipose tissue sections from 3 mice were counted.

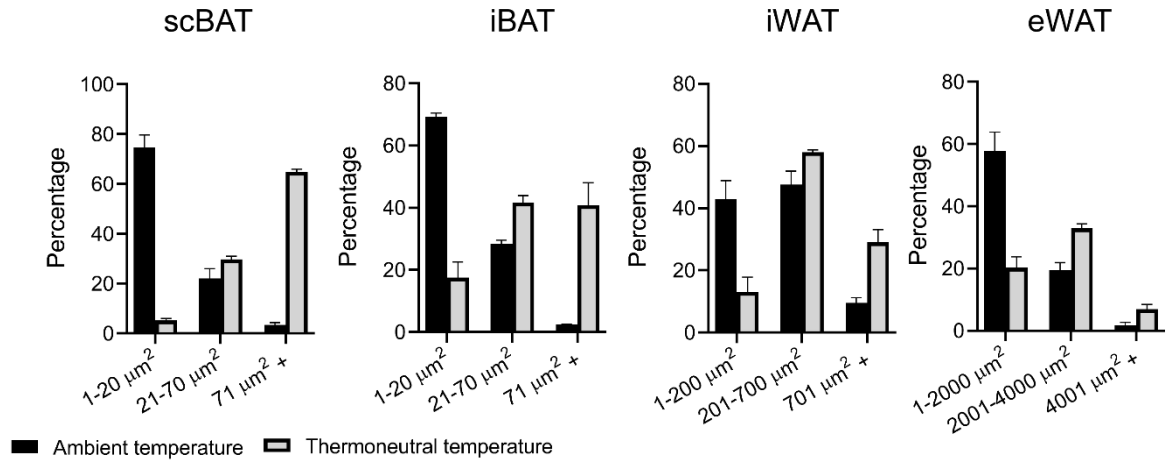

Figure S3. Size distributions for lipid droplets in scBAT, iBAT, iWAT, and eWAT from mice housed at ambient or thermoneutral temperature for 8 weeks. Lipid droplets were grouped based on their sizes (μm), small (BAT: 20 μm² or less, iWAT: 200 μm² or less, eWAT: 2000 μm² or less), medium (BAT: 21-70 μm², iWAT: 201-700 μm², eWAT: 2001-4000 μm²), or large (BAT: 71 μm² +, iWAT: 701 μm² +, eWAT: 4001 μm² +). The distributions are presented as percentage of lipid droplets that are small, medium, or large among the total amount of lipid droplets counted. The percentage of lipid droplets in large size group is higher in mice housed in thermoneutral than in ambient temperatures. Adipose tissue sections from 3 mice were counted.

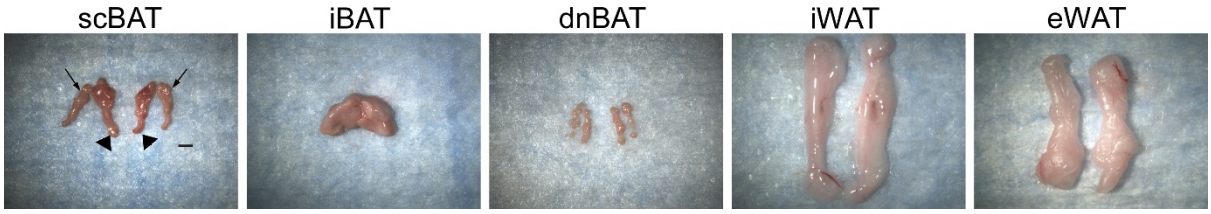

Figure S4. Adipose tissue images. Representative images of scBAT, iBAT, dnBAT, iWAT, and eWAT isolated from an 8-week-old male mouse. Scale bar: 1000 μm. Compared to scBAT and iBAT, dnBAT is much smaller. Arrowhead points to the part of scBAT that is positioned above the external jugular vein on the ventral side of the neck. Arrow points to the part of the scBAT that is positioned behind the external jugular vein.