SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

For the C57BL/6NJcl mice, 58 total mice were used to characterize their torpor phenotype (12 females and 46 males). The age (mean ± SD) and body weight (mean ± SD) at the beginning of the experiment were as follows: 8.67 ± 0.39 weeks old and 19.6 ± 0.7 g for females; 8.34 ± 0.53 weeks old and 22.5 ± 1.2 g for males. For the C57BL/6J mice, 50 total mice were used to characterize the torpor phenotype (n = 12; 8 females and 4 males) and sampling tissues (n = 38, all males). The characteristics were: 8.43 ± 0.15 weeks old and 17.7 ± 0.6 g for females; 8.22 ± 0.39 weeks old and 23.0 ± 1.2 g for males. As described in the RESULTS section, for B6J mice, data recorded in a previous report (Sunagawa and Takahashi, 2016) (n = 43, all male mice, 8.07 ± 0.35 weeks old, 22.9 ± 1.2 g) were also included in the data analysis to characterize the thermoregulatory system. To test for torpor phenotype inheritance, B6J and B6N were crossed, and their offspring were evaluated. Male B6NJ mice (B6N females crossed with B6J males) and B6JN mice (B6J females crossed with B6N males) were used for this assessment. The characteristics of each strain were: n = 9, 8.90 ± 1.01 weeks old, 25.5 ± 2.9 g for B6NJ-F1 mice and n = 8, 8.73 ± 0.62 weeks old and 23.3 ± 0.9 g for B6JN-F1 mice.

During the experiments, each animal was housed in a temperature-controlled chamber (HC-100, Shin Factory). To record T_B continuously, a telemetry temperature sensor (TA11TA-F10, DSI) was implanted in the animal's abdominal cavity under general inhalation anesthesia at least 7 days before recording. The metabolism of the animal was continuously analyzed by respiratory gas analysis (ARCO-2000 mass spectrometer, ARCO system). During the experiment, the animal was monitored through a networked video camera (TS-WPTCAM, I-O DATA, Inc.). This video camera can detect infrared signals, which made it possible to monitor the animal's health during the dark phase without opening the chamber.

Daily Torpor Induction Experiment

Each daily torpor induction experiment was designed to record the animal's metabolism for

three days (Figure 1B) unless the tissues were sampled on day 2. The animals were introduced to the chamber the day before recording started (day 0). Food and water were freely accessible. The T_A was set as indicated on day 0 and kept constant throughout the experiment. A telemetry temperature sensor implanted in the mouse was turned on before placing the mouse in the chamber. The standard experimental design was as follows: on day 2, ZT-0, the food was removed to induce torpor. After 24 hours, on day 3, ZT-0, the food was returned to each animal. In the torpor-prevention experiment with food administration (Figure 3A), the food was not removed at day 2. In the torpor-deprivation experiment, one experimenter monitored the VO_2 and touched the mouse gently when the VO_2 started to drop. The metabolism monitoring for torpor deprivation was started at ZT-17 on day 2 and maintained until the mouse tissue was sampled at ZT-22.

Body Temperature and Oxygen Consumption Modelling for Daily Torpor Detection

To model the temporal variation of T_B and VO_2, we constructed the models in a Bayesian framework. From the first 24-hour recordings of T_B and VO_2 for each animal, we estimated the parameters using Markov Chain Monte Carlo (MCMC) sampling by Stan (Stan Development Team, 2016a) with the RStan library (Stan Development Team, 2016b) in R (R Core Team, 2017). The detailed methods were described previously (Sunagawa and Takahashi, 2016) and modified with software updates. In short, we used the 99.9% credible interval (CI) of the posterior distribution of the estimated metabolism, the T_B and VO_2, of the animal to detect outliers. That is, when the value was lower than the CI, that time point was defined as torpor due to an abnormally low metabolic status. In this study, when both T_B and VO_2 met the criteria in the second half of the day, the time point was labelled as torpor.

Parameter Estimation of the Thermoregulatory system

The thermoregulatory system was modelled as an integration of the heat loss and heat production of the animal. Three parameters G, T_R, and H were estimated from the metabolic stable state of the animal at various T_As. The details were described previously (Sunagawa and Takahashi, 2016).

Tissue Sampling and RNA Isolation

Dissected soleus muscles were rapidly frozen in liquid nitrogen. The RNA was isolated using an RNeasy Fibrous tissue kit (Qiagen) according to the manufacturer's instructions. The quality of the total RNA was evaluated using a Bioanalyzer 2100 (Agilent). The quantity and purity of the RNA were estimated using a NanoDrop Spectrophotometer. The lateral or both soleus muscles were used according to the total amount of RNA needed.

Data Processing

Data were processed in R (R Core Team, 2017) unless otherwise noted. The expression level of the 12,862 defined CAGE clusters was normalized by sample in TPM (tags per million) and then analyzed with the edgeR package (Robinson et al., 2010) with TMM (trimmed mean of M-value) normalization. For MDS (multidimensional scaling) plots, DE (differential expression), and GO and KEGG pathway enrichment analysis, several R packages were applied, including edgeR, clusterProfiler (Yu et al., 2012), and pathview (Luo and Brouwer, 2013). Muscle enhancers were predicted de novo by applying the FANTOM5 protocol (Andersson et al., 2014) to our mouse CAGE data and masked with ±500-bp regions from the 5' ends of annotated genes. The mouse CAGE data for muscles can be observed and are publicly available in the Zenbu browser (http://fantom.gsc.riken.jp/zenbu/gLyphs/#config=ylDd70XVLdPufetrnXzQkB). The DE results (reversible, hypometabolic, and torpor-deprivation-specific promoters) along with torpor-specific promoters are listed in Supplemental Table S1.

Basic Promoter Features Analysis

Promoter region features were analyzed in terms of GC content and SI (Hoskins et al., 2011). The SI and %GC were calculated for ±50 bp regions around the TSS position. CpG island muscle promoters were defined by searching for overlaps with the UCSC annotation using bedtools v2.25.

Motif Analysis

Transcription factor binding sites (TFBS) were predicted in -300/+100 bp regions around the TSS position using MEME Suite 4.11.2 and the JASPAR CORE motif library for vertebrates 2016. The position-dependent enrichment of these motifs was performed by the CentriMo tool.

SNP analysis

The single nucleotide polymorphisms data for the C57BL/6NJ strain were downloaded from the Mouse Genomes Project of the Sanger Institute (ftp://ftp-mouse.sanger.ac.uk/current_snps/strain_specific_vcfs/C57BL_6NJ.mgp.v5.snps.dbSNP142.vcf.gz). Originated from the C57BL/6N strain, the C57BL/6NJ mice were derived from embryos cryopreserved (F126) at the NIH in 1984, and C57BL/6NJcl mice were introduced to the Central Institute for Experimental Animals from the NIH at F121 in 1978, and then transferred to CLEA Japan at F146 in 1988. Of the C57BL/6NJ-specific SNPs, 89% are preserved in C57BL/6NJcl (Mekada et al., 2015). Overlaps of the SNPs with mouse transcripts, muscle promoters, and enhancers regions were performed using bedtools v2.25. All SNPs overlapping predicted promoter regions (-300/+100 bp from TSS), annotated RefSeq and Ensembl transcripts, including both coding and noncoding regions, were counted. The overrepresentation rate of SNPs in pathways was calculated by applying a hypergeometric test in R.

SUPPLEMANTAL DATA

Figure S1. Torpor Phenotype in Mice is Affected by Genetic Background, related to Figure 1.

(A) (B) Traces of T_B (red lines) and VO_2 (blues lines) of the B6N male mouse at various T_As.

(C) Posterior distribution of the estimated slope a_1 during the normal state and torpor.

(D) Posterior distribution of the estimated slope a_2 during the normal state and torpor.

(E) Relationship between minimum T_B and VO_2 seen during the normal and torpid states at various T_As in B6J mice. Darkness of the dots indicates the T_A. The horizontal intercept of the line indicates the theoretical set-point of T_B, which is T_R. Note that the slope of the T_B - VO_2 relationship during torpor was less steep for B6J than for B6N mice, indicating that B6J had less sensitivity to T_B during torpor, consistent with the observation that B6J had a lower minimal T_B during torpor than B6N.

(F) Distribution of the estimated ΔH, the difference in H during torpor for B6N versus B6J. Red line denotes 0, and the dashed lines denote the lower and upper range of the 89% HDPI of ΔH. Note that because the HDPI does not include 0, the ΔH is likely to be positive at the probability of more than 89%; this can be interpreted as it being highly probable that B6N has a larger H than B6J.

(G) Distribution of the estimated ΔT_R, the difference in of T_R during torpor of B6N versus B6J. Red line denotes 0, and dashed lines denote the lower and upper range of 89% HDPI of ΔT_R. Because the 89% HDPI do includes 0, the ΔT_R may be 0 at a probability of 89%; this can interpreted as it being highly probable that B6N and B6J do not have different T_Rs.

(H) (I) Traces of T_B (red lines) and VO_2 (blues lines) over time of B6J and B6N female mice at T_A = 20 °C.

(J) Posterior distribution of the difference in the estimated minimal T_B and VO_2 of B6N, B6J, B6NJ-F1, and B6JN-F1 mice during torpor. Red vertical line denotes 0, and the dashed vertical lines denote the lower and upper range of the 89% HDPI of ΔT_B or ΔVO_2. When 0 is not included in the HDPI, the index is highly probable to have a difference. B6N had a distinct phenotype for both the minimal T_B and VO_2 during torpor than from that of the other three strains.

Figure S2. Fasting-induced Torpor Shows a Reversible Transcriptome Signature, related to Figure 2.

(A) Boxplots for the VO_2 of animals at sampling in reversibility experiment #2. Each dot represents one sample from one animal. The results resembled the metabolic phenotypes as detected in experiment #1. See Figure 2B.

(B) MDS plot of the TSS-based distance in reversibility experiment #2. Each dot represents one sample from one animal. Note that the Mid group was clustered differently from the Pre and Post groups in the 1st dimension, as it were in Figure 2C.

(C) Hierarchical clustering heatmap based on the TPM of TSS detected in the reversibility experiment #2.

(D) The top thirty motifs enriched in the reversible promoters.

(E) Logos of the top ten motifs in the reversible promoters.

Figure S3. Torpor Prevention at High T_A Revealed Hypometabolism-associated Promoters, related to Figure 3.

(A) One B6J mouse in eight failed to enter torpor at T_A = 28 °C.

(B) At T_A = 32 °C, no mouse entered torpor (n = 4).

(C) Top thirty motifs enriched in the hypometabolic promoters.

(D) Logos of the top ten motifs enriched in the hypometabolic promoters.

Figure S4. Identification of Torpor-specific Promoters and their Dynamics, related to Figure 4.

(A) Top ten enriched GO terms in the torpor specific promoters.

(B) Of the 13 enriched KEGG pathways, the "mTOR signaling pathway" is shown as a representative example. Green and red denote up- and down-regulated genes, respectively.

(C) Top thirty motifs enriched in the torpor-specific promoters.

(D) Logos of the top ten enriched motifs in the torpor-specific promoters.

(E) Distribution of the %GC in the torpor-specific promoters compared to all muscle promoters. The three horizontal lines inside the violin denote the 1st, 2nd, and 3rd quartile of the distribution from the upmost line. No significant difference was detected in this dataset.

(F) Boxplots for the VO_2 of animals at sampling in the torpor deprivation experiment. Each dot represents one sample from one animal. Torpor-deprived animals (Dep group, n = 4) did not show an apparent change in VO_2 compared to the Mid group.

(G) MDS plot of the TSS-based distance in the torpor-deprivation experiment. Each dot represents one sample from one animal. A clear separation between the Mid and Dep groups was not found in this analysis.

(H) Distribution of motifs enriched in the torpor-specific promoters. The horizontal axis denotes the position of the motif density peak from the TSS. The vertical axis denotes the *p*-value of the enriched motif.

Figure S5. Genetic Link of Distinct Torpor Phenotypes in Inbred Mice, related to Figure 5.

(A) (B) Torpor-specific promoters that have B6N/B6J SNPs within the range of +400/-100 bp from the TSS. The vertical lines denote the SNPs, which are red when included in the promoters and black when not. Among the up-regulated torpor-specific promoters, *Plin5* and *Sik3* had one SNP each. Among the down-regulated promoters, *Creb3l1* and *Lrrn1* had one, and *Bhlhe40* and *Rrad* had two SNPs in the promoter region.

Table S1. Differentially Expressed Promoters During Torpor

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