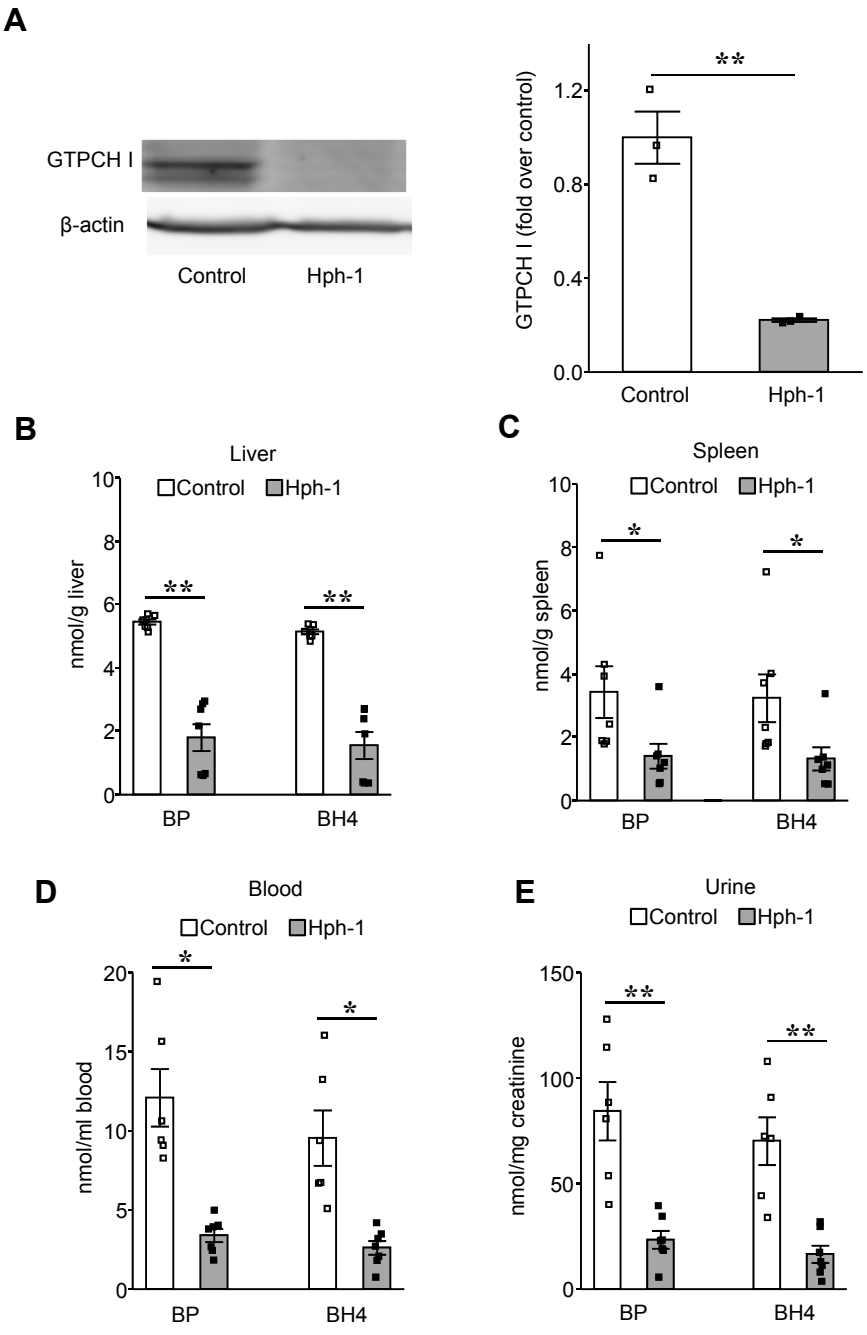
Supplemental Material

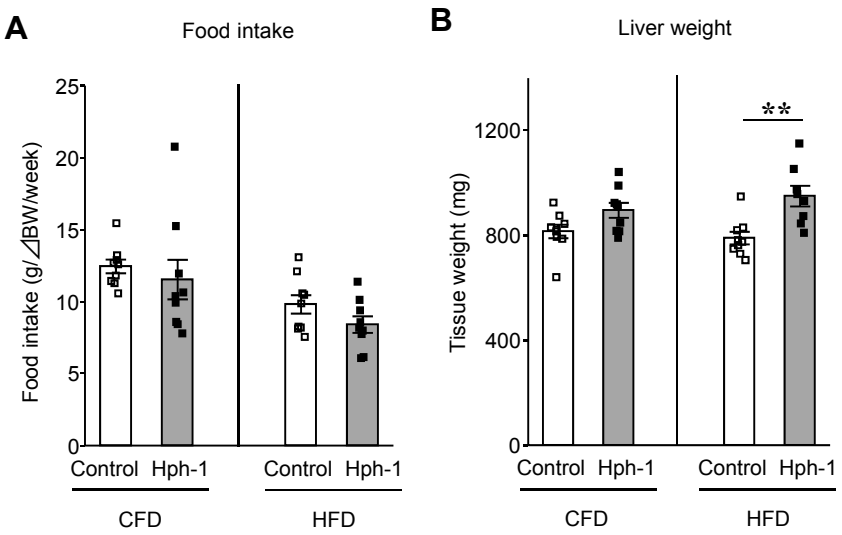
Supplemental Figure 1



Supplemental Figure 1. Phenotype of hph-1 mice.

(**A**) Protein expression levels of GTPCH I in liver tissue of hph-1 mice and control mice of the same background at 6 weeks of age (n = 3). (**B-E**) Total biopterin (BP) and tetrahydrobiopterin (BH4) levels in liver (**B**; n = 7), spleen (**C**; n = 7), blood (**D**; n = 6-7), and urine (**E**; n = 6-7) of hph-1 mice and control mice of the same background at 6 weeks of age. Values are means ± SEM. All statistical analysis was performed by Student t-test, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group.

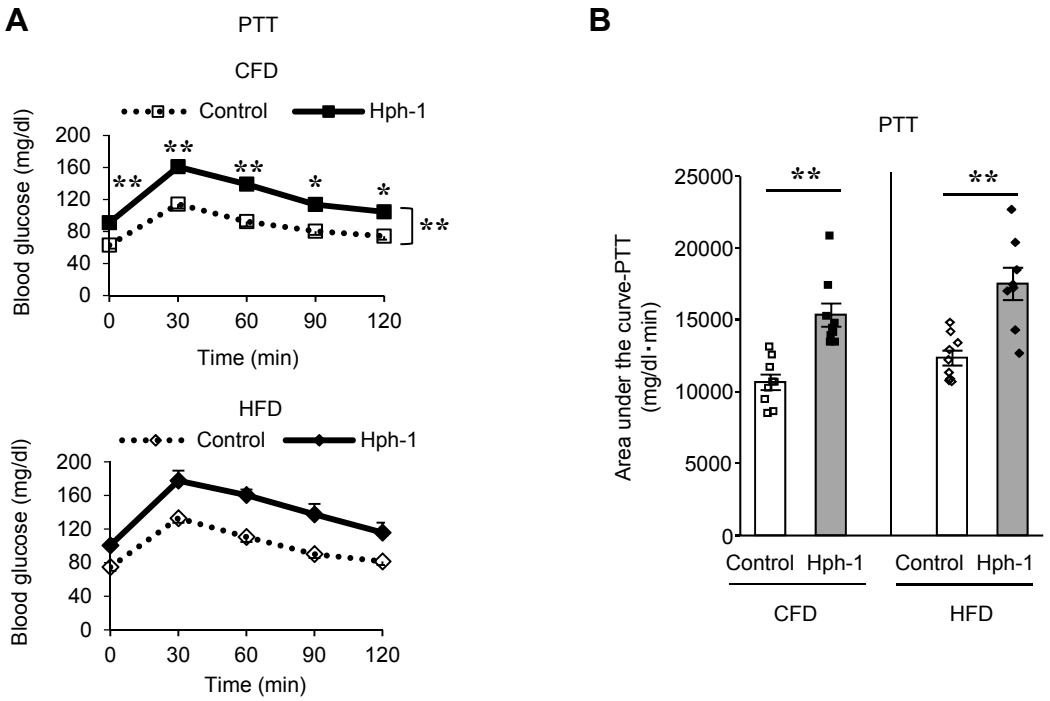
Supplemental Figure 2



Supplemental Figure 2. Phenotype of BH4-deficient mice under CFD or HFD.

(**A-B**) Food intake (**A**; $n = 9$) and liver weight (**B**; $n = 8$-9) in hph-1 mice and control mice of the same background under the control fat diet (CFD) or high fat diet (HFD) for 4 weeks from the age of 4 weeks. Values are means ± SEM. All statistical analysis was performed by Student t-test, $^{**}P < 0.01$ vs. control group.

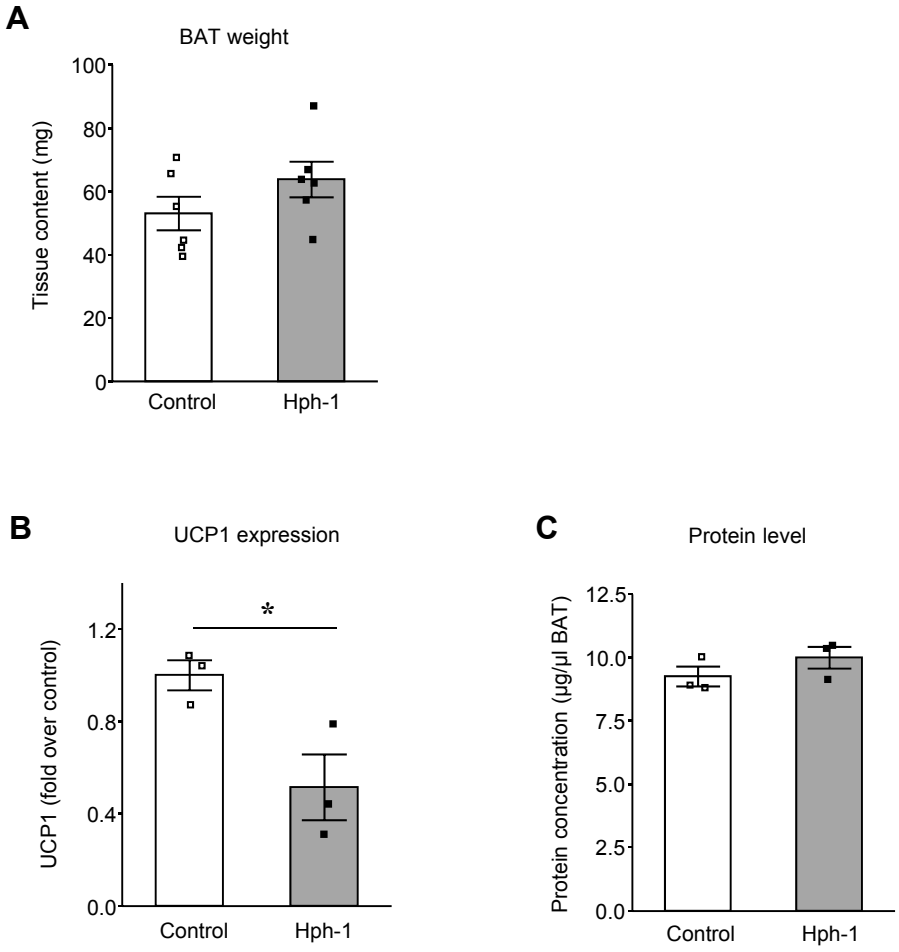
Supplemental Figure 3



Supplemental Figure 3. Phenotype of gluconeogenesis in BH4-deficient mice under CFD or HFD.

(**A-B**) Pyruvate tolerance test (PTT; 1 g/kg pyruvate) after 16 h fasting (**A-B**; n = 8-9) under control fat diet (CFD) or high fat diet (HFD) for 4 weeks from the age of 4 weeks. Values are means ± SEM. Statistical analysis was performed by two-way ANOVA with Bonferroni post-hoc test (**A**) or Student t-test (**B**), $*P < 0.05$, $**P < 0.01$ vs. control group.

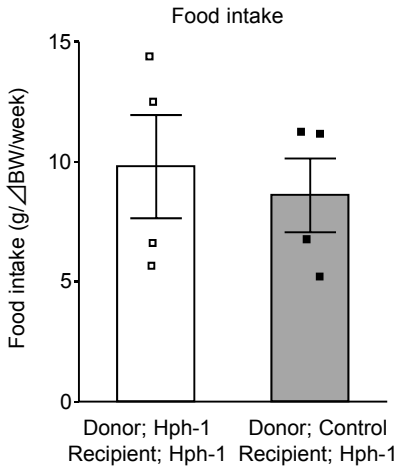
Supplemental Figure 4



Supplemental Figure 4. Phenotype of BAT in BH4-deficient mice.

(**A-C**) Brown adipose tissue (BAT) weight (**A**; n = 6), the total amount of UCP1 protein expression levels per mouse (**B**; n = 3) and protein concentrations (**C**; n = 3) in half of the interscapular BAT of hph-1 mice and control mice of the same background at 6 weeks of age under control fat diet. The total amount of UCP1 protein expression level per mouse was calculated as UCP1 protein expression per 10 μg × protein concentration per mouse. Values are means ± SEM. All statistical analysis was performed by Student t-test, *P < 0.05 vs. control group.

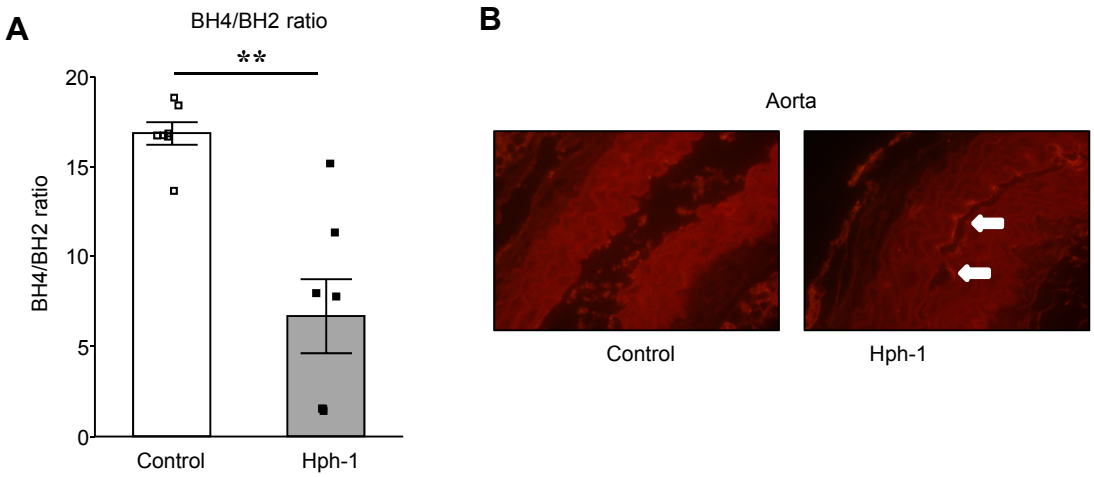
Supplemental Figure 5



Supplemental Figure 5. Food intake of BH4-deficient mice after BAT transplantation.

Food intake in hph-1 mice transplanted with 0.1 g brown adipose tissue (BAT) derived from hph-1 mice or control mice of the same background under high fat diet from the age of 8 weeks (n = 4). Values are means ± SEM. Statistical analysis was performed by Student t-test, vs. control group.

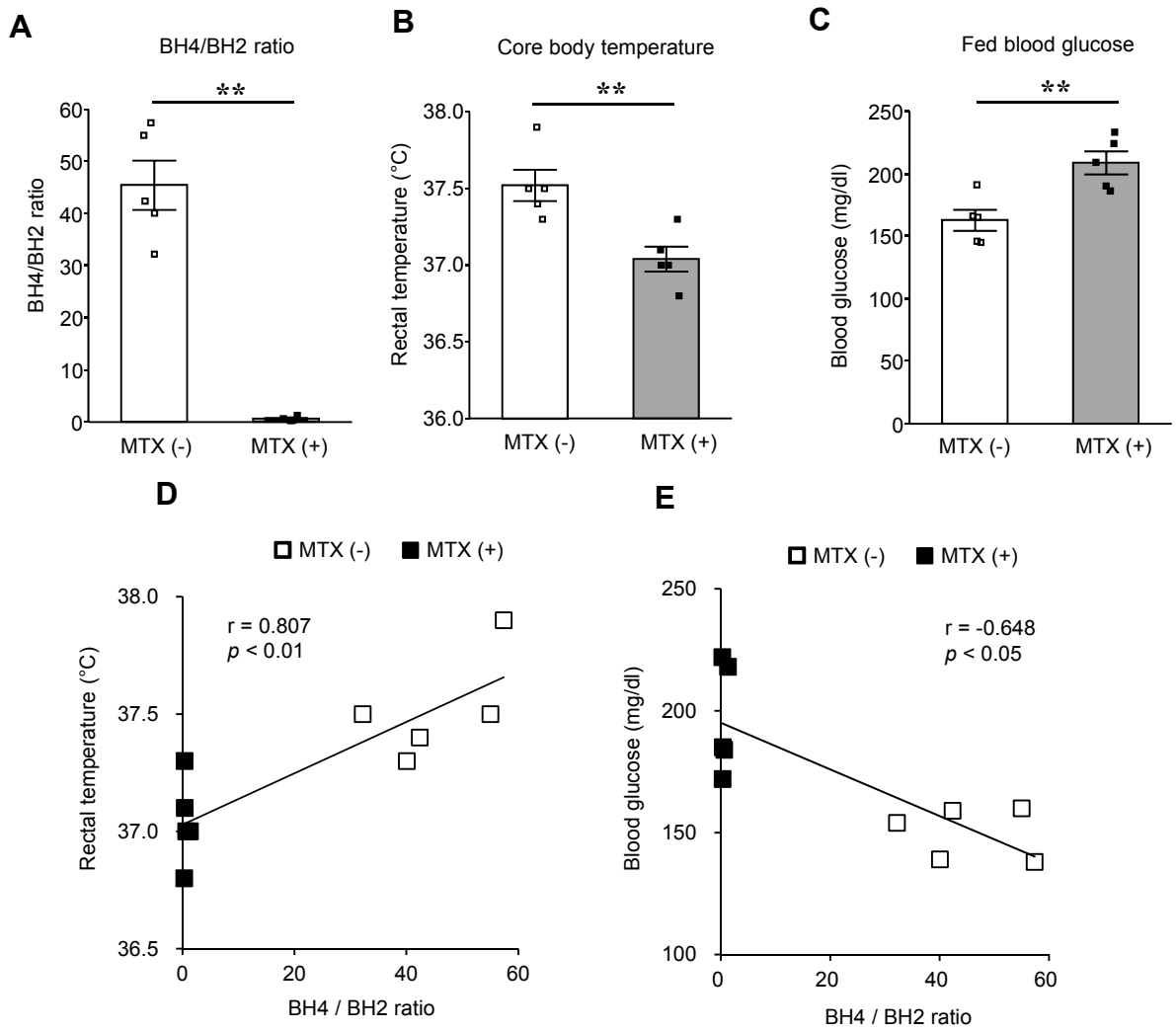
Supplemental Figure 6



Supplemental Figure 6. Indication of eNOS uncoupling in BH4-deficient mice.

(**A**) Tetrahydrobiopterin (BH4)/ 7,8-dihydrobiopterin (BH2) ratio in liver tissue of hph-1 mice and control mice of the same background at 6 weeks of age (n = 7). (**B**) Anti-nitrotyrosine staining in aorta of streptozotocin (STZ)-induced diabetic hph-1 mice and control mice of the same background, 4 weeks after the single administration of 120 mg STZ /kg body weight from the age of 6 weeks. Arrows indicate the stained areas with anti-nitrotyrosine antibody (original magnification, ×40). Values are means ± SEM. Statistical analysis was performed by Student t-test, $^{**}P < 0.01$ vs. control group.

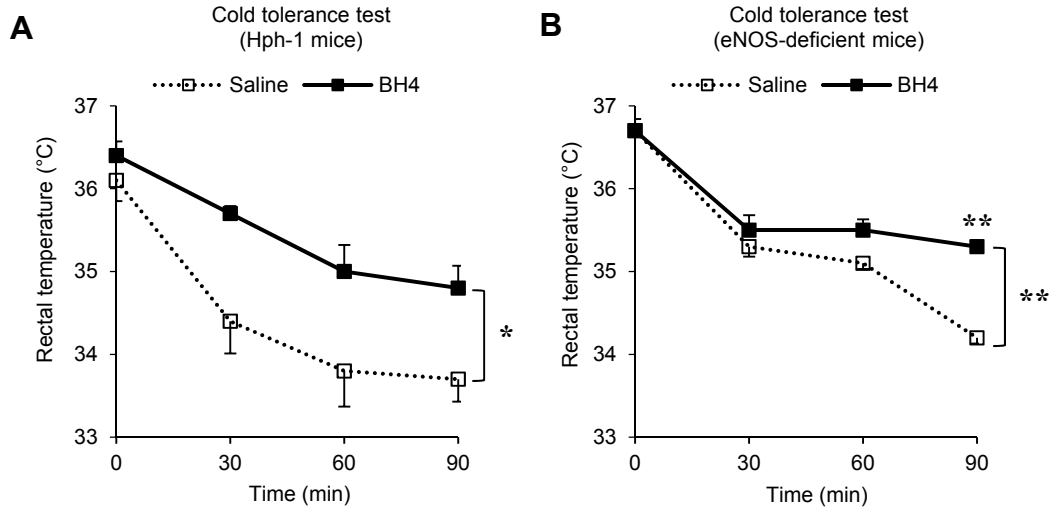
Supplemental Figure 7



Supplemental Figure 7. Relationship between BH4/BH2 ratio as a marker of eNOS uncoupling and energy metabolism.

(**A-E**) Tetrahydrobiopterin (BH4)/ 7,8-dihydrobiopterin (BH2) ratio in liver tissue (**A** ; $n = 5$), rectal temperature (**B** ; $n = 5$), fed blood glucose levels (**C** ; $n = 5$), and correlation between BH4/BH2 ratio and rectal temperature (**D** ; $n = 5$) and fed blood glucose levels (**E** ; $n = 5$) in hph-1 mice 2 weeks after the injection of 2 mg methotrexate (MTX) /kg body weight every 2 days from the age of 5 weeks. Values are means ± SEM. Statistical analysis was performed by Student t-test (**A-C**), $^{**}P < 0.01$ vs. control group, or Correlation coefficient, Spearman's rank correlation coefficient (**D-E**).

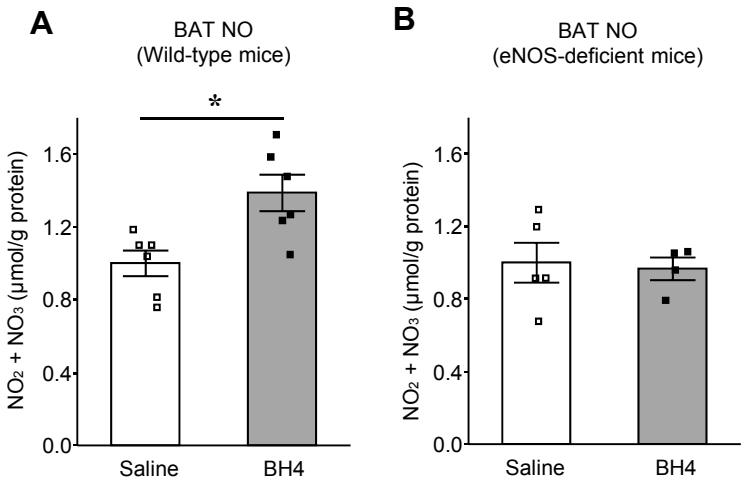
Supplemental Figure 8



Supplemental Figure 8. Effects of single administration of BH4 on thermogenic response in BH4-deficient mice and eNOS-deficient mice.

(**A-B**) Changes of rectal temperature in 4°C cold tolerance test in hph-1 mice (**A**; n = 5) and eNOS-deficient mice (**B**; n = 5-7) at 6 weeks of age after the single administration of 20 mg tetrahydrobiopterin (BH4)/kg body weight. Values are means ± SEM. All statistical analysis was performed by two-way ANOVA with Bonferroni post-hoc test, $*P < 0.05$, $**P < 0.01$ vs. saline group.

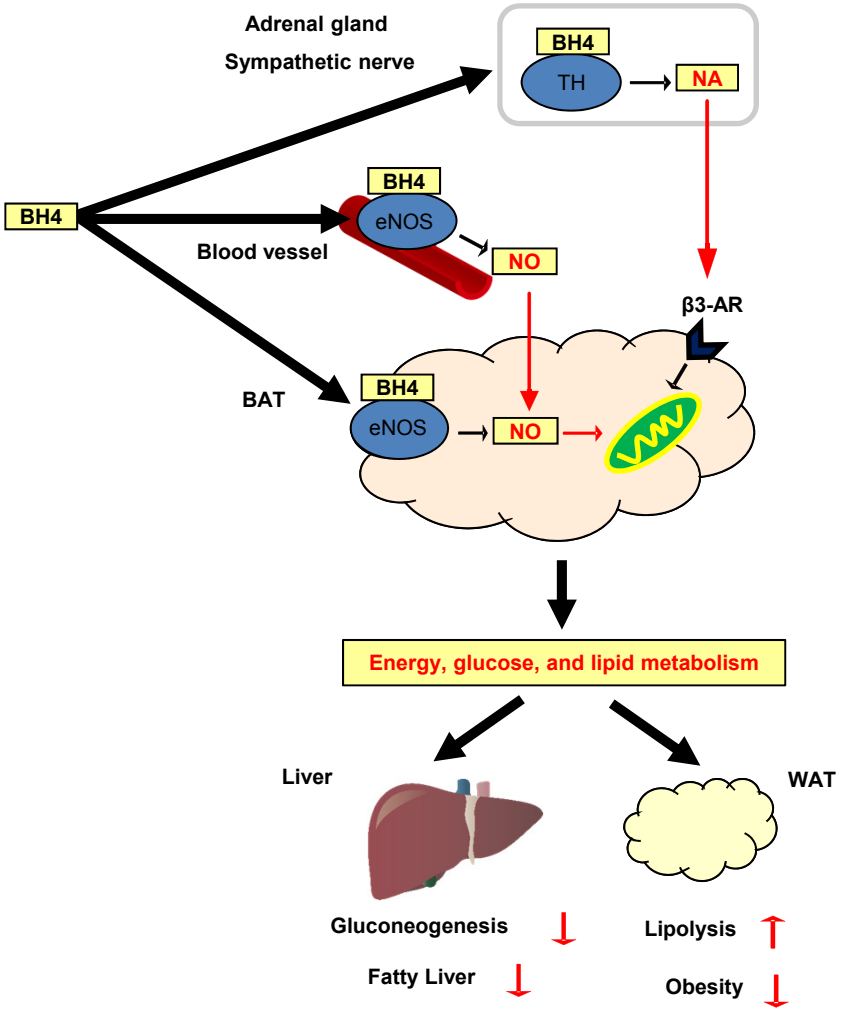
Supplemental Figure 9



Supplemental Figure 9. Effects of single administration of BH4 on NO production in BAT of eNOS-deficient mice.

(**A-B**) Nitric oxide (NO) contents of brown adipose tissue (BAT) in wild-type mice (**A**; $n = 6$) and eNOS-deficient mice at 6 weeks of age (**B**; $n = 4$-5) after treatment with or without 20 mg tetrahydrobiopterin (BH4)/kg body weight. Values are means ± SEM. All statistical analysis was performed by Student t-test, $^{*}P < 0.05$ vs. saline group.

Supplemental Figure 10



Supplemental Figure 10. Model of BAT-mediated metabolic regulation by BH4.

Supplemental tables

Supplemental Table 1. Primer sequences used in quantitative RT-PCR.

Name	Forward	Reverse
Gch1	CAAGCAAGTCCTTGGTCTCAG	ACCGCAATCTGTTTGGTGAG
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Dio2	AGCCCATGTAACCAGCACCGGA	CAGTCGCACTGGCTCAGGAC
Cpt1b	CTGTTAGGCCTCAACACCGAAC	CTGTCATGGCTAGGCGGTACAT
36B4	TGTGTGTCTGCAGATCGGGTAC	CTTTGGCGGGATTAGTCGAAG

Supplemental Table 2. Characteristics of hph-1 mice.

	Body weight (g)	Fed blood glucose (mg/dl)
Saline	16.00 ± 0.59	171.63 ± 4.46
BH4	16.08 ± 0.51	174.13 ± 4.47

Values are means ± SEM (n = 8).

No significant difference between saline and tetrahydrobiopterin (BH4) groups, Student t-test.