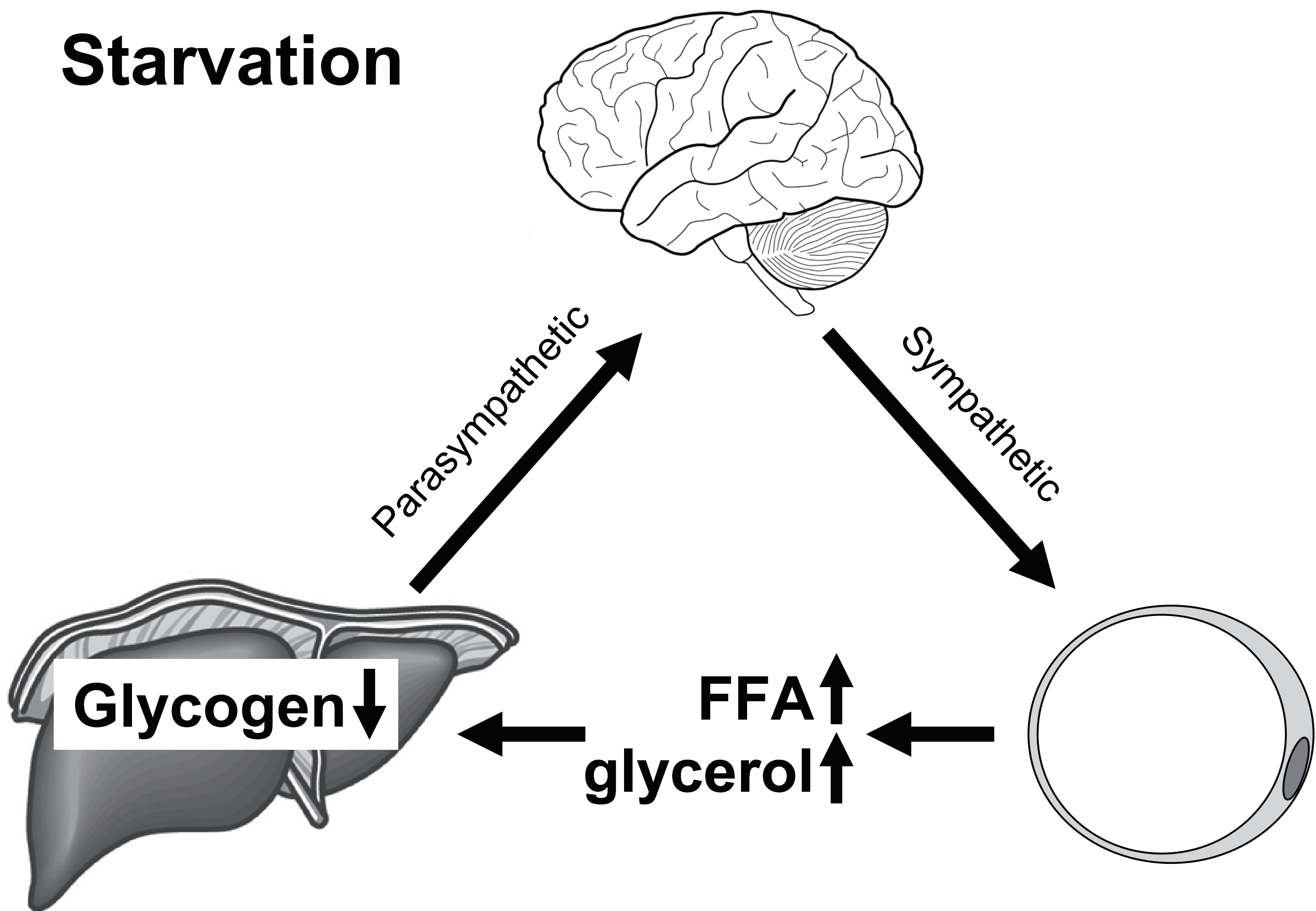
**Supplementary Information**

# Glycogen shortage during fasting triggers liver-brain-adipose neurocircuitry to facilitate fat utilization

Supplementary Figure S1.



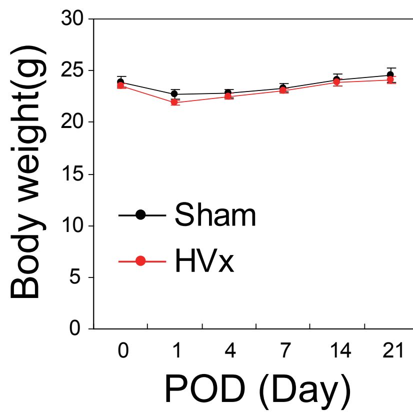

Supplementary Figure S1.

**Graphical abstract.**

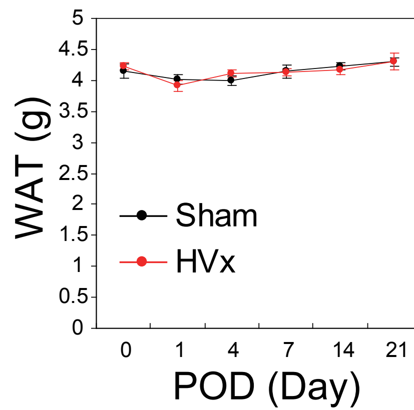
Liver glycogen shortage activates a liver-brain-adipose neural axis composed of afferent vagal and efferent sympathetic nerves, promoting fatty acid and glycerol release from white adipose tissue as a response to starvation.

# Supplementary Figure S2.

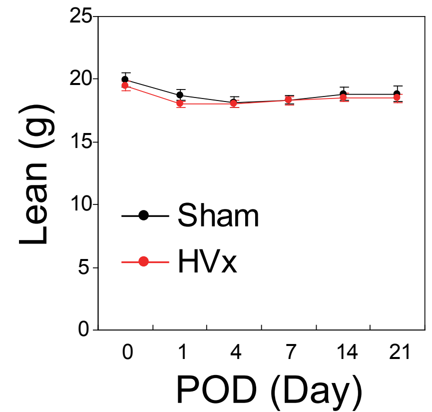
a




No significant difference vs Sham

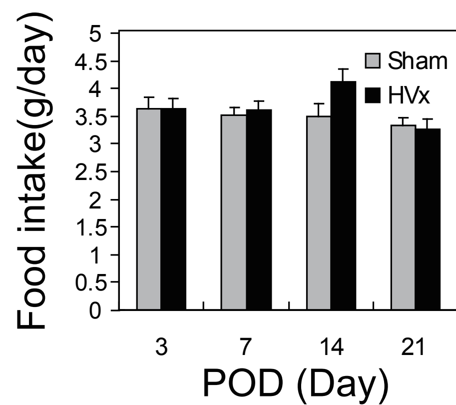



No significant difference vs Sham




No significant difference vs Sham

b



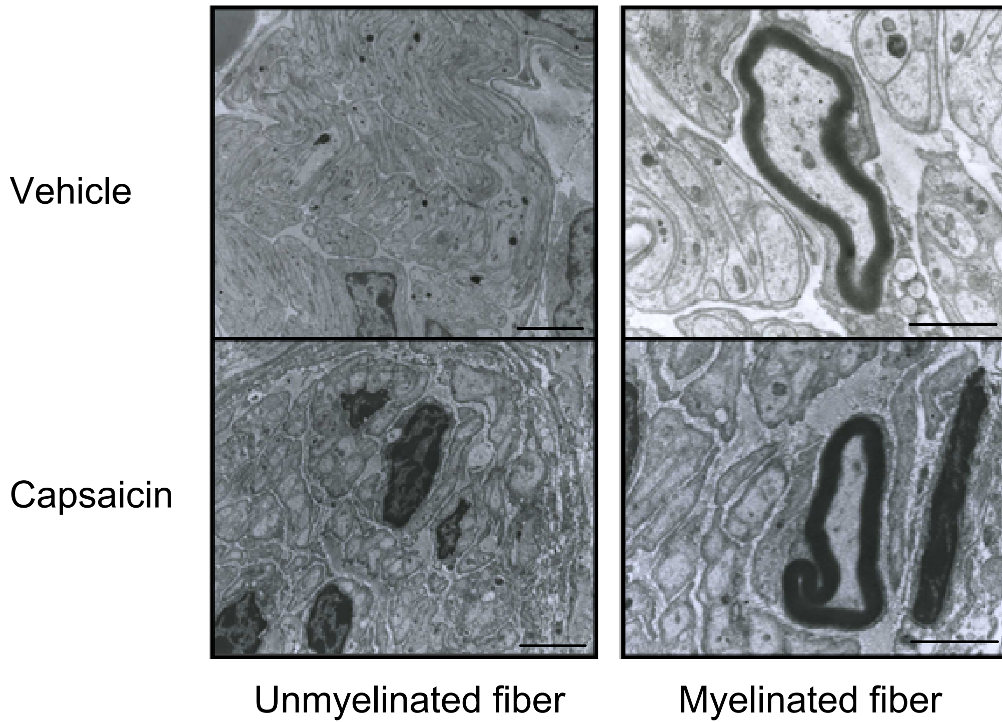

No significant difference vs Sham

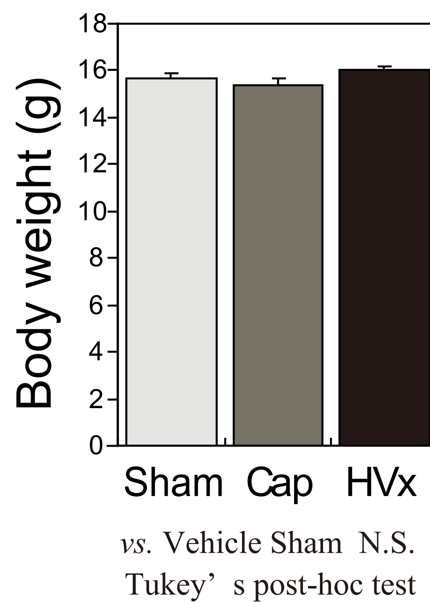
Supplementary Figure S2.

**Hepatic vagotomy does not affect post-operated body weight, epididymal fat weight and lean body weight and the amount of food intake.**

**a**, Sequential body weight, epididymal fat weight and lean body weight were measured by dual energy X-ray analysis (DEXA). No significant difference. Error bars, S.E.M. (n=7) **b**, The amount of food consumption was measured after hepatic vagus nerve resection (HVx). No significant difference. Error bars, S.E.M. (n=7)

Supplementary figure S3.



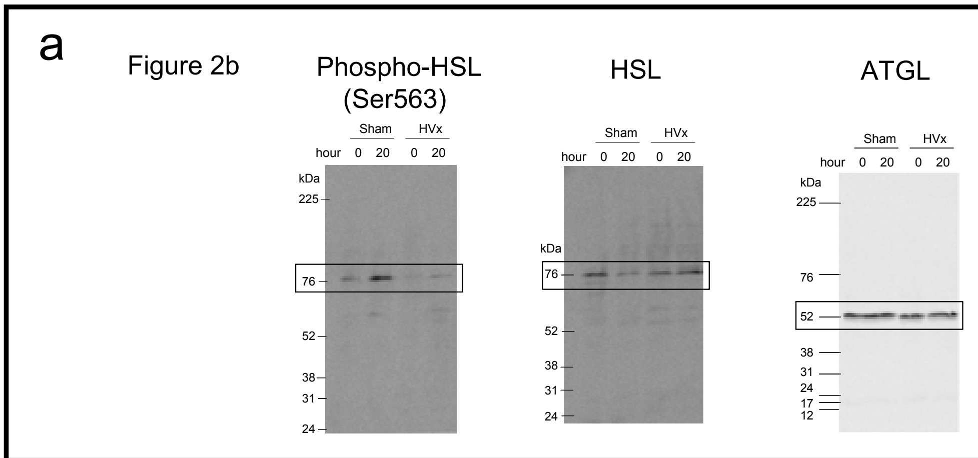

*vs.* Vehicle Sham N.S.
Tukey' s post-hoc test

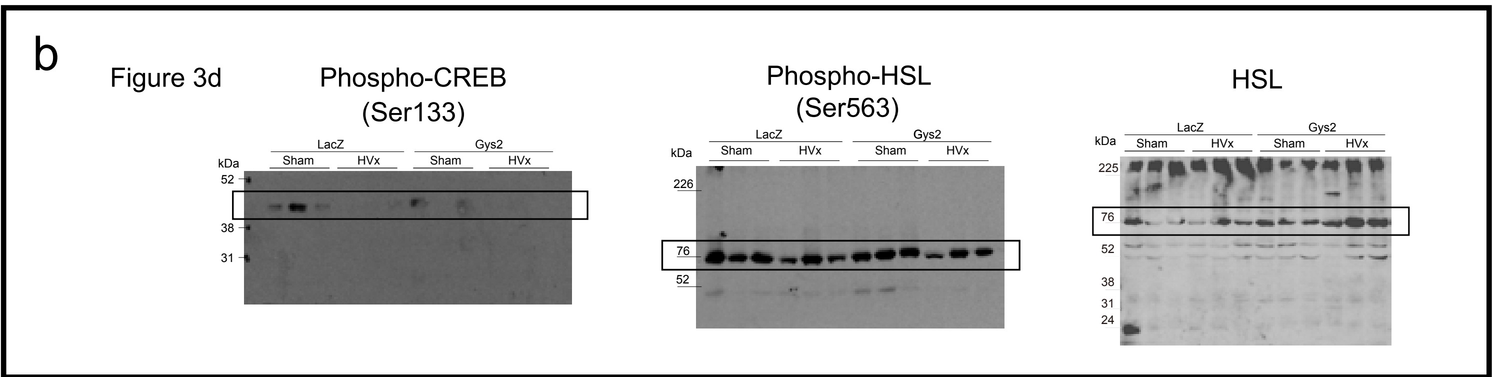
Supplementary Figure S3.

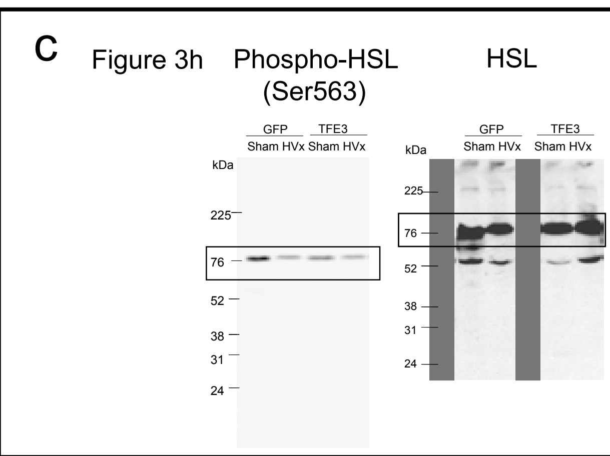
**Effect of neural circuit blockade with hepatic vagotomy or capsaicin.**

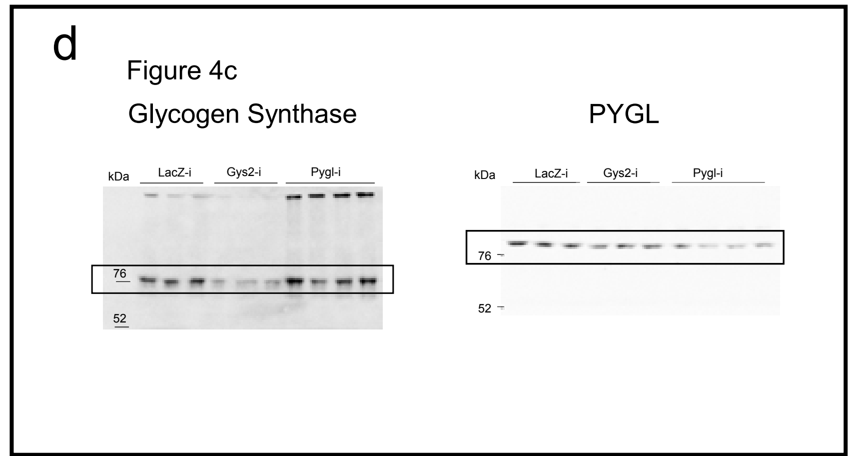
**a,** Trans Emission Microscopy (TEM) appearance of vehicle or capsaicin treated hepatic vagus nerve Upper, vehicle treated; lower, capsaicin treated group. Left, unmyelinated fiber x5000, Bar 10μm; right, myelinated fiber x10000, Bar 2μm **b,** Body weight of sham operated (Sham), capsaicin treated (CAP), or vagotomized (HVx) mice. Statistical comparisons were made by Tukey's post-hoc test. No significant difference. Error bars, S.E.M. (n=4-12)

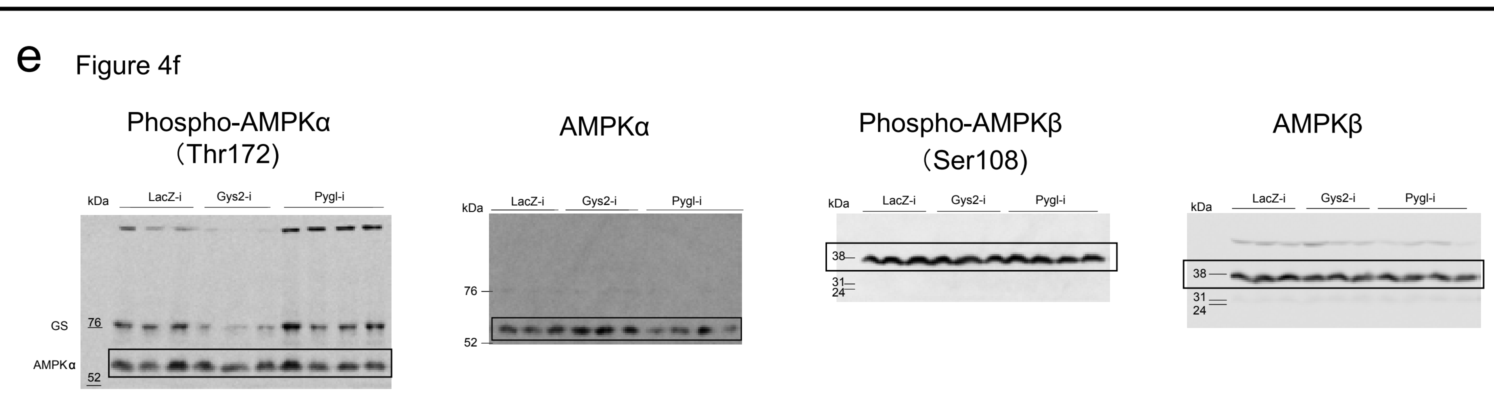
# Supplementary Figure S4.



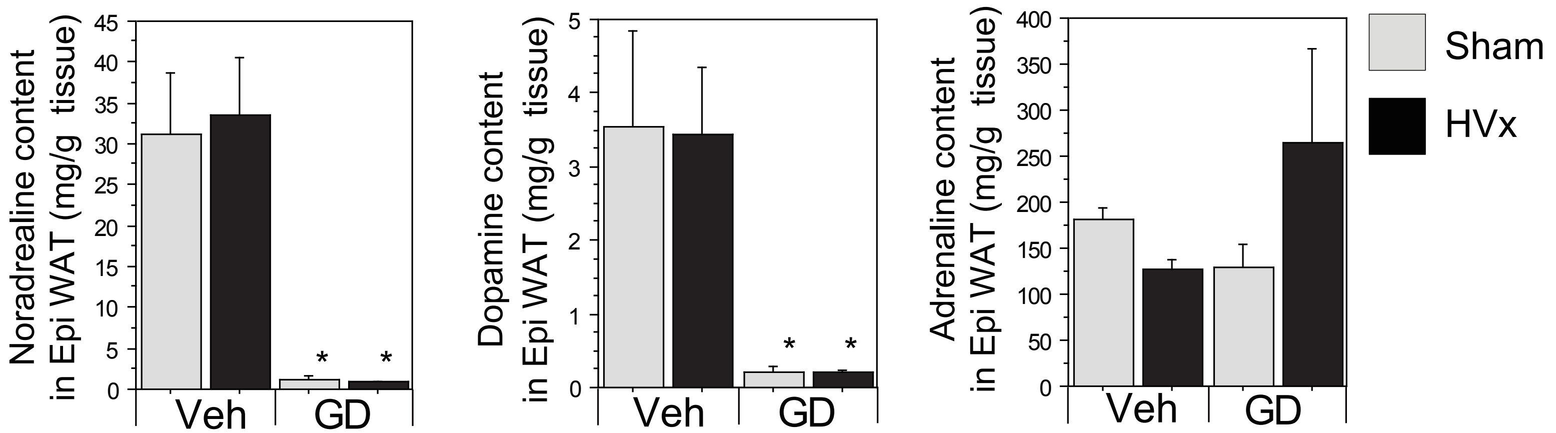




Supplementary Figure S4. Full length images of immunoblotting.

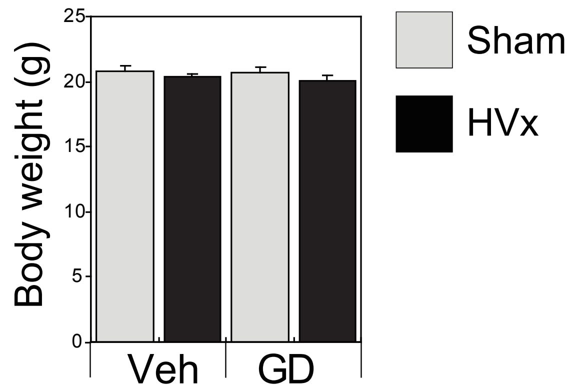
Supplementary Figure S5.

a




*vs*. Vehicle Sham * p<0.05
Tukey' s post-hoc test

b



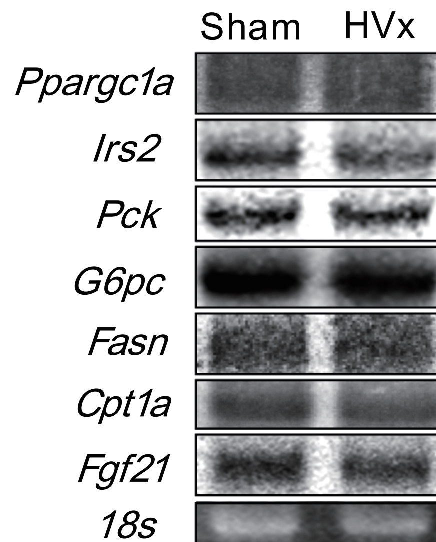

*vs*. Vehicle Sham N.S.
Tukey' s post-hoc test

Supplementary Figure S5.

**Effect of sympathetic neural circuit blockade on epididymal fat tissue.**

**a,** Catecholamine content of epididymal white adipose tissue in mice, either sham or hepatic vagotomized model ablated with sympathetic nerve neurotoxin guanethidine (GD) or vehicle administration (Veh). **b,** Body weight of sham and hepatic vagotomized mice administrated with vehicle or guanethidine. *p<0.05 by Tukey's post-hoc test. Error bars, S.E.M. (n=5)

Supplementary Figure S6.



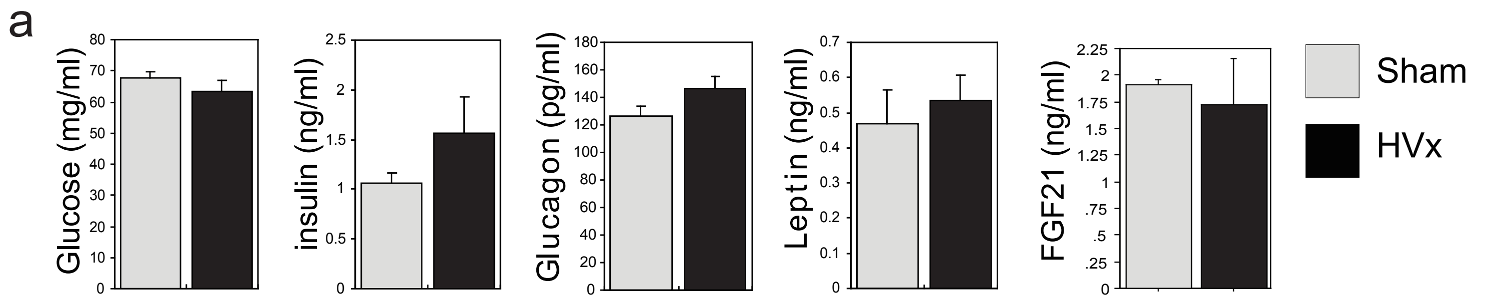

Supplementary Figure S6.

**Liver mRNA expression after selective hepatic vagotomy**

Effects of hepatic-vagotomy on hepatic gene expression was analyzed by northern blotting of livers in hepatic-vagotomized mice after 24h fasting (pooled individual three samples).

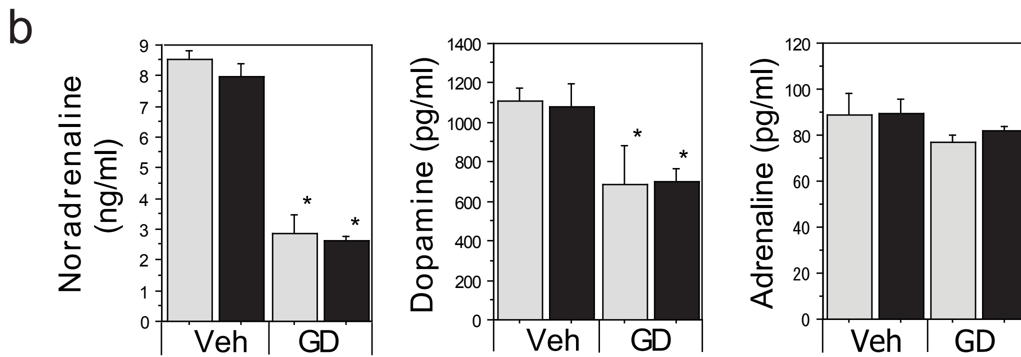
# Supplementary Figure S7.

a




*vs.* Sham N.S. Student's t-test

b



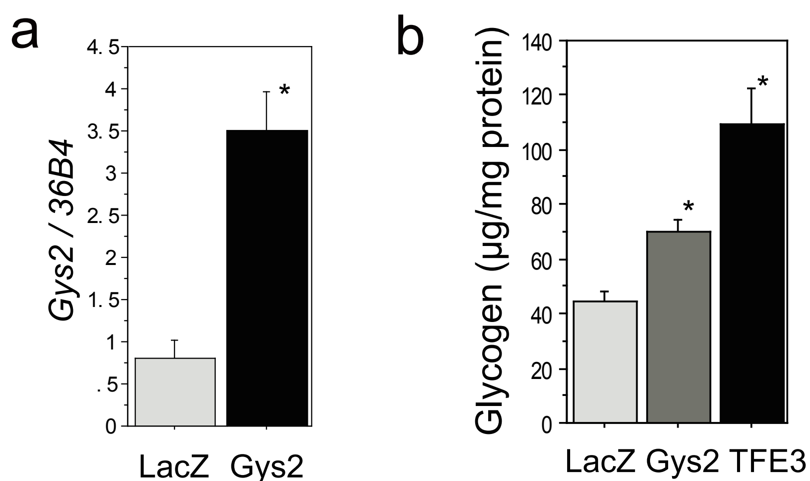

*vs.* Vehicle Sham * p<0.05 Tukey's post-hoc test

Supplementary Figure S7.

**Plasma hormone profiles in the experimental mice.**

**a,** Plasma glucagon, leptin, and FGF21 levels in hepatic vagus nerve transected mice fasted for 24 hours. **b,** Plasma catecholamine levels in vagotomized mice with or without guanethidine treatment on a 24-hour fasting condition. *$p<0.05$ by Tukey's post-hoc test. Error bars, S.E.M. (n=4-6)

# Supplementary Figure S 8.




*vs.* LacZ

* $p<0.05$ Tukey’ s post-hoc test

Supplementary Figure S8.

**Effect of glycogen accumulation in primary isolated hepatocytes infected with Ad-CMV-Gys2 or Ad-CMV-TFE3** ***in vitro*****.**

**a,** Primary hepatocytes, isolated from wild mouse were infected with adenovirus vectors, at titer of $5 \times 10^9$ pfu expressing *LacZ, Gys2* or *TFE3*. Gys2 mRNA steady state levels were assessed by qRT-PCR analysis and quantified by densitometric measurement using 36B4 mRNA as a reference. There was an Gys2 dose-dependent increase in Ad-CMV-Gys2 transduced livers. **b,** Glycogen content in primary hepatocytes infected with Ad-LacZ, Ad-Gys2, or Ad-TFE3. *$p<0.05$ by Tukey’s post-hoc test. Error bars, S.E.M. (n=4)

# Supplementary Table S1 .

| | Sham | | HVx | | |
|---|---|---|---|---|---|
| | LacZ | Gys2 | LacZ | Gys2 | |
| Body Weight (g) | 18.9±1.0 | 20.7±0.7 | 20.8±0.3 | 20.2±0.3 | n.s. *vs.* LacZ-Sham |

| | Sham | | HVx | | |
|---|---|---|---|---|---|
| | LacZ-i | Gys2-i | LacZ-i | Gys2-i | |
| Body Weight (g) | 19.4±0.3 | 18.3±0.5 | 20.2±0.4 | 19.9±0.4 | n.s. *vs.* LacZ-i -Sham |

| | Sham | | HVx | | |
|---|---|---|---|---|---|
| | LacZ-i | Pygl-i | LacZ-i | Pygl-i | |
| Body Weight (g) | 19.4±0.3 | 20.4±0.3 | 20.2±0.4 | 20.5±0.3 | n.s. *vs.* LacZ-i-Sham |

Supplementary Table S1.

**Body weight change after administration of adenovirus vectors overexpressing *Gys2* or those expressing short-hairpin RNA targeted for *Gys2* or *Pygl* .**

Body weight change after 24 hour fasting. v.s. each sham-control vectors. Tukey's post-hoc test. No significant difference. Mean± S.E.M. (n=4)

Supplementary Table S2.

## Northern blot reference set

| *Probe* | *Sequence* |
|---|---|
| *Gys2* | NM_145572.2:1669-2252 |
| *Tcfe3* | NM_172472: 911-3266 |
| *Ppargc1a* | NM_008904: 140-2533 |
| *Irs2* | NM_001081212: 1971-3442 |
| *Pck1* | NM_011044: 126-523 |
| *G6pc* | U00445: 25-1169 |
| *Fasn* | NM_007988: 6189-7453 |
| *Cpt1a* | NM_013495: 1859-2358 |
| *Fgf21* | NM_020013: 185-817 |

Supplementary Table S2.
List of cDNA probes used for Northern blotting.

Supplementary Table S3.

| | *Sequences of qPCR Primers* |
|---|---|
| *Srebf1c* | 5’-CGGCGCGGAAGCTGT-3’<br>5’-TGCAATCCATGGCTCCGT-3’ |
| *Lipe (HSL)* | 5’-TGGTTCAACTGGAGAGCGGAT-3’<br>5’-TGATGCAGAGATTCCCACCTG-3’ |
| *Atgl* | 5’-AACACCAGCATCCAGTTCAA-3’<br>5’-GGTTCAGTAGGCCATTCCTC-3’ |
| *Pdk4* | 5’-AGAAGACCAGAAAGCCCTGTCA-3’<br>5’-GCCATTGTAGGGACCACATTATG-3’ |
| *Gys2* | 5’-GGAAGAAACTCTATGACGGGTTATT-3’<br>5’-TCATCGATCATATTGTGAGTGGTC-3’ |
| *Pygl* | 5’-GCACTACTACGACAAGTGTCCC-3’<br>5’-TAAATGGCCTCATCGCAG-3’ |
| *Cpt1a* | 5’-CCTGGGCATGATTGCAAAG-3’<br>5’-GGACGCCACTCACGATGTT-3’ |
| *Rplp0(36B4)* | 5’-GAAGACAGGGCGACCTGGAA -3’<br>5’-TTGTCTGCTCCCACAATGAAGC-3’ |

Supplementary Table S3.
List of primer sets used for Q-RT PCR.

# Supplementary Table S4.

| *Antibody* | *Company, Cat. Number* | *Dilution* |
|---|---|---|
| Phospho-AMPKα (Thr172) Antibody | Cell Signaling technology #2535 | x1000 |
| AMPKα Antibody | Cell Signaling technology #2603 | x1000 |
| Phospho-AMPKβ1 (Ser108) Antibody | Cell Signaling technology #4181 | x1000 |
| AMPKβ1/2 Antibody | Cell Signaling technology #4150 | x1000 |
| HSL Antibody | Cell Signaling technology #4107 | x1000 |
| Phospho-HSL (Ser563) Antibody | Cell Signaling technology #4139 | x1000 |
| ATGL Antibody | Cell Signaling technology #2138 | x1000 |
| Phospho-CREB (Ser133) Antibody | Cell Signaling technology #9198 | x250 |
| Glycogen Synthase Antibody | Cell Signaling technology #3893 | x1000 |
| PYGB/L/M Antibody | Santa Cruz technology sc-46347 | x1000 |

Supplementary Table S4.
List of Antibodies used for immunoblotting

## Supplementary Methods

***Transmission electron microscopic analyses of vagal nerves*** — Seven days after perivagal treatment with capsaicin or vehicle, nerves were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4), and postfixed in 1% osmium tetroxide. They were dehydrated in graded alcohols and embedded in Epon812. Ultrathin sections were stained with uranyl acetate and lead, and then examined under a transmission electron microscope (H-7600, Hitachi, Tokyo, Japan).

***Metabolic measurements*** — Epinephrine, norepinephrine and dopamine were measured in plasma using a LaChrom Elite® HPLC system (Hitachi, Tokyo, Japan) according to the manufacturer's instructions.

***Isolation and culture of primary hepatocytes and glycogen synthesis*** — We prepared primary cultures of mouse hepatocytes and subjected them to adenovirus infection as previously described [5]. Briefly, primary hepatocytes were isolated from 7-week-old male ICR mouse (CLEA, Tokyo, Japan). Cells were resuspended in William's E medium containing 100 nM insulin and 10 nM dexamethasone on 100-mm collagen-coated dishes at a final density of 8 x $10^6$ cells. After incubation for 20 h to allow attachment, the medium was replaced with high glucose DMEM with 100 nM insulin containing each 5 MOI adenovirus. Using mouse hepatocytes, studies of glycogen synthesis, gene expression and immunoblotting were performed as described previously [5]. Briefly, Glycogen storage in hepatocytes were determined after washing the cells twice with ice-cold saline and by using amyloglucosidase digestion and subsequent glucose measurement. For RNA analysis, we incubated cells at 37 °C for 48 h after adenovirus infection at 5 MOI. For protein analysis, we stimulated adenovirus-infected cells with 100 nM insulin after a 48-h incubation.

***Assessment of sympathetic activity by measurement of catecholamine content after blockade of the sympathetic nervous system*** — Measurement of catecholamine content in tissues was according to previously published methods [17]. Briefly, epididymal white adipose tissue (WAT) was removed and frozen. Free catecholamines were extracted from WAT as follows: 100 mg WAT was homogenized on ice in 3 ml of 0.4 N perchloric acid with 0.2% EDTA-2Na and 0.002% L- ascorbic acid. Homogenized samples were centrifuged at 3500 r.p.m. for 20 min on ice, and the supernatant was used. The concentration of catecholamines was determined using a

LaChrom Elite® HPLC system (Hitachi, Tokyo, Japan) with electrochemical detection.

## Supplementary References

5. Nakagawa, Y. et al. TFE3 transcriptionally activates hepatic IRS-2, participates in insulin signaling and ameliorates diabetes. *Nat Med* **12**, 107-13 (2006).

17. King, V. L. et al. Cold exposure regulates the norepinephrine uptake transporter in rat brown adipose tissue. *Am J Physiol* **276**, R143-R151 (1999).