Supplementary Material

A missense mutation in zinc finger homeobox-3 (ZFHX3) impedes growth and alters metabolism and hypothalamic gene expression in mice.

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Supplemental Figure 1: One year old \(Zfhx3^{Sc/+}\) mice fat pad mass do not differ to wildtype when corrected for body weight.

The Sci mutation in male and female mice does not alter fat pad mass / body weight (BWT). This corrected tissue mass is higher overall in female mice in all white adipose tissues measured (A-D), and sex did not affect brown adipose corrected mass (E). Plotted are mean ± SEM with individual values. Statistical comparison is by 2-way ANOVA, with overall comparisons indicated on the graphs. ****P<0.0001, NSD: no significant differences. iWAT: inguinal white adipose tissue, gWAT: gonadal white adipose tissue, prWAT: perirenal white adipose tissue, mWAT: mesenteric white adipose tissue, iBAT: interscapular brown adipose tissue.
Supplemental Table S1: Statistical Comparisons for Cumulative Food Intake Data (Figure 3).
Stated are the significantly different (or approaching significance) ANOVA comparison statistics at each timepoint for the data presented in figure 3.

| Figure | Comparison | F-Stat | P Value |
|--------|------------|--------|---------|
| 3 A    | Food Intake Week 9 Sex | F (1, 19) = 9.909 | P=0.0053 |
| 3 A    | Food Intake Week 9 Genotype | F (1, 19) = 12.18 | P=0.0024 |
| 3 A    | Food Intake Week 10 Sex | F (1, 19) = 17.50 | P=0.0005 |
| 3 A    | Food Intake Week 10 Genotype | F (1, 19) = 14.85 | P=0.0011 |
| 3 A    | Food Intake Week 11 Sex | F (1, 19) = 19.42 | P=0.0003 |
| 3 A    | Food Intake Week 11 Genotype | F (1, 19) = 14.19 | P=0.0013 |
| 3 A    | Food Intake Week 12 Sex | F (1, 19) = 21.47 | P=0.0002 |
| 3 A    | Food Intake Week 12 Genotype | F (1, 19) = 12.64 | P=0.0021 |
| 3 A    | Food Intake Week 13 Sex | F (1, 19) = 21.47 | P=0.0002 |
| 3 A    | Food Intake Week 13 Genotype | F (1, 19) = 12.64 | P=0.0021 |
| 3 A    | Food Intake Week 14 Sex | F (1, 19) = 20.86 | P=0.0002 |
| 3 A    | Food Intake Week 14 Genotype | F (1, 19) = 11.06 | P=0.0035 |
| 3 A    | Food Intake Week 15 Sex | F (1, 19) = 21.02 | P=0.0002 |
| 3 A    | Food Intake Week 15 Genotype | F (1, 19) = 10.14 | P=0.0049 |
| 3 A    | Food Intake Week 16 Sex | F (1, 19) = 20.29 | P=0.0002 |
| 3 A    | Food Intake Week 16 Genotype | F (1, 19) = 10.09 | P=0.0050 |
| 3 A    | Food Intake Week 17 Sex | F (1, 19) = 18.35 | P=0.0004 |
| 3 A    | Food Intake Week 17 Genotype | F (1, 19) = 8.977 | P=0.0074 |
| 3 A    | Food Intake Week 18 Sex | F (1, 19) = 19.54 | P=0.0003 |
| 3 A    | Food Intake Week 18 Genotype | F (1, 19) = 10.14 | P=0.0049 |
| 3 A    | Food Intake Week 19 Sex | F (1, 19) = 19.70 | P=0.0003 |
| 3 A    | Food Intake Week 19 Genotype | F (1, 19) = 9.839 | P=0.0054 |
| 3 A    | Food Intake Week 20 Sex | F (1, 21) = 14.15 | P=0.0011 |
| 3 A    | Food Intake Week 20 Genotype | F (1, 21) = 11.97 | P=0.0023 |
| 3 B    | Bodyweight Week 8 Sex | F (1, 42) = 4.721 | P=0.0355 |
| 3 B    | Bodyweight Week 8 Genotype | F (1, 42) = 2.144 | P=0.1506 |
| 3 B    | Bodyweight Week 9 Sex | F (1, 42) = 13.33 | P=0.0007 |
| 3 B    | Bodyweight Week 9 Genotype | F (1, 42) = 4.701 | P=0.0359 |
| 3 B    | Bodyweight Week 10 Sex | F (1, 42) = 21.39 | P=0.0001 |
| 3 B    | Bodyweight Week 10 Genotype | F (1, 42) = 4.813 | P=0.0338 |
| 3 B    | Bodyweight Week 11 Sex | F (1, 42) = 23.35 | P=0.0001 |
| 3 B    | Bodyweight Week 11 Genotype | F (1, 42) = 5.084 | P=0.0294 |
| 3 B    | Bodyweight Week 12 Sex | F (1, 42) = 27.12 | P=0.0001 |
| 3 B    | Bodyweight Week 12 Genotype | F (1, 42) = 4.652 | P=0.0368 |
| 3 B    | Bodyweight Week 13 Sex | F (1, 42) = 27.06 | P=0.0001 |
| 3 B    | Bodyweight Week 13 Genotype | F (1, 42) = 4.782 | P=0.0344 |
| 3 B    | Bodyweight Week 14 Sex | F (1, 42) = 26.07 | P=0.0001 |
| 3 B    | Bodyweight Week 14 Genotype | F (1, 42) = 3.509 | P=0.0680 |
| 3 B    | Bodyweight Week 15 Sex | F (1, 42) = 21.00 | P=0.0001 |
| 3 B    | Bodyweight Week 15 Genotype | F (1, 42) = 3.307 | P=0.0761 |
| 3 B    | Bodyweight Week 15 Interaction | F (1, 42) = 4.145 | P=0.0481 |
| 3 B    | Bodyweight Week 16 Sex | F (1, 42) = 19.12 | P=0.0001 |
| 3 B    | Bodyweight Week 16 Genotype | F (1, 42) = 3.660 | P=0.0626 |
| 3 B    | Bodyweight Week 17 Sex | F (1, 42) = 15.02 | P=0.0004 |
| 3 B    | Bodyweight Week 17 Genotype | F (1, 42) = 3.310 | P=0.0760 |
| 3 B    | Bodyweight Week 18 Sex | F (1, 42) = 13.87 | P=0.0006 |
| 3 B    | Bodyweight Week 18 Genotype | F (1, 42) = 3.903 | P=0.0548 |
| 3 B    | Bodyweight Week 18 Interaction | F (1, 42) = 4.342 | P=0.0433 |
Supplemental Figure 2: 8 week blood glucose, 10 week urinary corticosterone and 24 h food and water intake data from TSE Phenomaster Calorimetry in 12 week old female Zfhx3<sup>Sc/v</sup> mice.

Fasted blood glucose was not altered in Zfhx3<sup>Sc/v</sup> mice at 8 weeks old (E), and neither was urinary corticosterone at 10 weeks, with peak and trough values maintained (F). Food intake in 1 h bins (C) total intake over 24 h, and light and dark phase only (D) was not altered by genotype during the sampling period in TSE Phenomaster metabolic cages, carried out in the second 24 h single housed in metabolic cages, at 12 weeks of age. However, water intake was significantly reduced overall in Zfhx3<sup>Sc/v</sup> mice, with an interaction between time and genotype (E) since water intake was significantly reduced in the dark phase and not the light phase (F). In the 24 h period, activity approached significant reduction in the Zfhx3<sup>Sc/v</sup> mice and there was a significant interaction with time of sampling but this did not reach significance when considered in hourly bins (G), however when considering dark phase data only, this did the Zfhx3<sup>Sc/v</sup> mice did achieve significantly less activity (H). Plotted are mean ± SEM with (A, B, D, F, H) or without (C, E, G) individual values. Statistical comparison is by Mann-Whitney (A, B), Wilcoxon matched – pairs tests (between timepoints, B), or 2-way ANOVA, with overall comparisons indicated on the graphs (C, E, G), or unpaired t-test (D, F, H); ****P<0.0001, **P<0.01, *P<0.05.
Supplemental figure 3: Hypothalamic candidate gene expression not altered in female Zfhx3Sci/+ mice

Expression of Otp in the paraventricular nucleus (PVN) was reduced at ZT 15 (A), Sim1 (B) and Trh (B) expression in the PVN were not altered by genotype or time. Expression of Dio2 in the ventricular ependymal layer increased overall at ZT 15 (D) Trh-r1 expression in the ARC was unaltered by time or genotype (E), while Trh expression in the dorsomedial hypothalamus was increased at ZT 15 (F), Tbx3 (G) and Dlk1 (H) expression in the ARC were unaltered by time or genotype. Plotted are mean ± SEM with individual values overlaid. Example images are shown beneath each plot. Comparisons are by 2-way ANOVA with Šídák’s multiple comparison tests indicated where appropriate. Dio2 data were log transformed for statistical comparison. * P < 0.05, ** P < 0.01, NSD: no significant differences. N = 10 – 12.