Sustained remission of diabetic hyperglycemia induced by central injection of fibroblast growth factor 1

Jarrad M. Scarlett[1,2,10], Jennifer M. Rojas[1,10], Miles E. Matsen[1], Karl J. Kaiyala[3], Darko Stefanovski[4], Richard N. Bergman[5], Hong T. Nguyen[1], Mauricio D. Dorfman[1], Louise Lantier[6], David H. Wasserman[6], Zaman Mirzadeh[7], Terry G. Unterman[8,9], Gregory J. Morton[1], and Michael W. Schwartz[1].

SUPPLEMENTARY FIGURE LEGENDS

SUPPLEMENTARY FIGURE 1 Effect of i.c.v. FGF1 on glucoregulatory hormones in diabetic mice. (**a**) Plasma levels of insulin (left) and glucagon levels (right) in *ob/ob* (B6) mice 18 weeks after a single i.c.v. injection of either mFGF1 (3 μg; black bars, $n = 9$) or Veh (open bars, $n = 8$). Data are mean ± s.e.m. P=ns, i.c.v. mFGF1 *vs.* Veh as determined by two-tailed t-test. (**b**) Time course of changes of blood glucose levels (left), food intake (middle) and body weight (right) of *ob/ob* (B6) mice following a single injection of mFGF1 (3 μg; black symbols; $n = 10$) or Veh (open symbols; $n = 8$) into the 3rd ventricle. Data are the mean ± s.e.m. $^{**}P < 0.01$ for group (Veh *vs.* FGF1) by repeated measures designs by linear mixed model analyses. $^{*}P<0.05$, icv mFGF1 *vs.* Veh by mixed factorial analyses.

SUPPLEMENTARY FIGURE 2 Effect of i.c.v. FGF1 on plasma corticosterone levels in diabetic mice. Plasma levels of corticosterone obtained 6 h after injection of mFGF1 (3 μg; black bars) or Veh (open bars) into either (**a**) the 3rd ventricle (3V; Veh, $n = 8$; FGF1, $n = 10$) or (**b**) the lateral ventricle (LV; Veh, $n = 12$; FGF1, $n = 12$) of *ob/ob* (B6) mice. (**c**) Plasma levels of corticosterone levels in *ob/ob* (B6) mice 18 wk after a single i.c.v. injection of either mFGF1 (3 μg; black bars, $n = 9$) or Veh (open bars, $n = 8$). In all instances, plasma was obtained during mid-light cycle following a 6 h fast. Data are mean ± s.e.m. P=ns, i.c.v. mFGF1 *vs.* Veh as determined by two-tailed t-test.

SUPPLEMENTARY FIGURE 3 Effect of i.c.v. FGF1 on food intake and body weight across multiple rodent models of T2D. (**a**) Time course of changes of food intake (left) and body weight (right) in *db/db* mice following i.c.v. injection of either mFGF1 (3 µg; *n* = 6) or Veh (*n* = 9). (**b**) Time course of changes of food intake (left) and body weight (right) in DIO WT mice in which hyperglycemia was induced with low-dose STZ (DIO-LD STZ) following i.c.v. injection of either mFGF1 (3 µg; *n* = 8) or Veh (*n* = 8). (**c**) Time course of changes of food intake (left) and body weight (right) of *ob/ob* (B6) mice following a single i.c.v. injection of hFGF1 (3 µg; grey symbols; *n*=6), mFGF1 (3 µg; black symbols; *n* = 6) or Veh (open symbols; *n* = 4). Data are mean ± s.e.m. **P*<0.05, i.c.v. mFGF1 *vs.* Veh; $^{\#}$*P*<0.05, i.c.v. hFGF1 *vs.* Veh by mixed factorial analyses. (**d**) Time course of changes of food intake (left), body weight (middle), and fat mass (right) from *ad libitum*-fed ZDF rats following a single i.c.v. injection of either rFGF1 (3 µg; *n* = 10; black symbols) or Veh (*n* = 10; open symbols). Data are the mean ± s.e.m. Significant main effects in (left) (*P*<0.0001) and (middle) (*P* = 0.028) reflected group differences at earlier time points (treatment by day interaction is significant (*P*<0.0001). P-values for group (Veh *vs.* FGF1) by repeated measures designs by linear mixed model analyses.

SUPPLEMENTARY FIGURE 4 Effect of i.c.v. FGF1 on hepatic *Ucp1* gene expression and plasma lipid levels. (**a**) brown adipose tissue (BAT) *Ucp1* gene expression in *ob/ob* (B6) mice that underwent a basal glucose turnover study followed by a FSIGT 7 d after a single i.c.v. injection of mFGF1 (3 µg, black symbols; *n* = 13) or Veh (open symbols; *n* = 9). (**b**) Plasma levels of triglycerides (left), cholesterol (middle) and non-esterified free fatty acids (NEFA; right) in *ob/ob* (B6) mice in samples obtained 28 d following a single i.c.v. injection of hFGF1 (3 µg; grey symbols; *n* = 6), mFGF1 (3µg; black symbols; *n* = 6) or Veh (open symbols; *n* = 4). (**c**) Plasma levels of triglycerides (left), cholesterol (middle), and NEFA (right) from *db/db* mice on samples obtained 28 d following a single i.c.v. injection of either mFGF1 (3 µg; *n* = 6) or Veh (*n* = 9). Data are mean ± s.e.m. $^{\#}$*P*<0.05, i.c.v. hFGF1 *vs.* Veh as determined by one-way ANOVA.

SUPPLEMENTARY FIGURE 5 Effect of severe hyperglycemia on central FGF1-mediated glucose lowering. (**a**) Time course of blood glucose levels in more severely hyperglycemic, *ad libitum*-fed *ob/ob* (BTBR) mice following i.c.v. injection of mFGF1 (black symbols; $n = 8$) or Veh (open symbols; $n = 8$). (**b**) Time course of blood glucose levels in more severely hyperglycemic, *ad libitum*-fed *db/db* mice following i.c.v. injection of mFGF1 (black symbols; $n = 4$) or Veh (open symbols; $n = 9$). (**c**) Time course of blood glucose levels in more severely hyperglycemic, *ad libitum*-fed DIO WT mice treated with high dose-STZ (DIO-HD STZ) following i.c.v. injection of mFGF1 (black symbols; $n = 4$) or Veh (open symbols; $n = 3$). (**d**) Food intake (left), body weight (middle) and blood glucose (right) levels from *ad libitum*-fed DIO WT mice receiving continuous s.c. infusion of the insulin receptor antagonist S961 that received i.c.v. injection of either Veh (open symbols; $n = 10$) or mFGF1 (3 µg; black symbols; $n = 11$). Data are mean ± s.e.m. For Fig. 4d (left), the groups differed on days 1 and 2 ($P<0.0001$ and $P = 0.043$, respectively). *P-values for group (Veh *vs.* FGF1) by repeated measures designs by linear mixed model analyses.

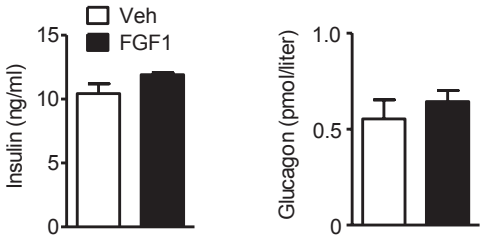
SUPPLEMENTARY FIGURE 6 Effect of i.c.v. FGF1 in LIRFKO and IRF fl/fl control mice made hyperglycemic by systemic administration of the insulin receptor antagonist S961. Food intake (left) body weight (middle) and blood glucose levels (right) from *ad libitum*-fed IRF fl/fl littermate controls (**a**) and LIRFKO mice (**b**) in which hyperglycemia was induced by continuous s.c. infusion of the insulin receptor antagonist S961, following i.c.v. injection of either Veh (open symbols; IRF fl/fl, $n = 12$; LIRFKO, $n = 10$) or mFGF1 (3 µg; black symbols; IRF fl/fl, $n = 13$; LIRFKO, $n = 11$). Data are mean ± s.e.m. *$P<0.05$, i.c.v. mFGF1 *vs.* Veh by mixed factorial analyses.

SUPPLEMENTARY FIGURE 7 (**a**) Representative immunofluorescence images of c-Fos (red) in the hypothalamus of mice euthanized 90 min after a single i.c.v. injection of either Veh saline (left, of $n = 20$ images), mFGF1 (3 µg; middle, of $n = 18$ images) or (**c**) hFGF19 (3 µg; right, of $n = 16$ images) (original

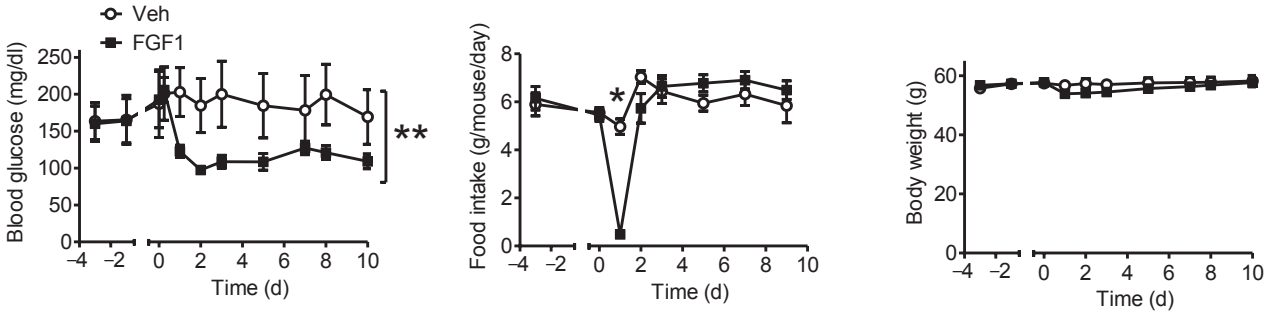
magnification, x20; Scale bar: 20 μM). (**b**) C-Fos immunoreactive nuclei quantified from cells (both ependymal and tanycytes) lining the 3rd ventricle (3V) from anatomically matched sections from the hypothalamus of mice treated with either i.c.v. Veh (open bars; $n = 4$), mFGF1 (grey bars; $n = 4$) or hFGF19 (black bars; $n = 4$). (**c**) Hypothalamic levels of mRNA encoding HSP25 obtained from chow-fed WT mice 24h after a single i.c.v. injection of either Veh ($n = 9$) or mFGF1 (3 μg; $n = 8$). (**d**) Western blot image (left) and densitometric quantification of synaptophysin protein levels (right) in hypothalamus (normalized to the loading control, β-tubulin III) of *ob/ob* mice obtained 1 week following a single i.c.v. injection of mFGF1 (3 μg; $n = 6$) or Veh ($n = 5$). Data are mean ± s.e.m. *$P<0.05$, i.c.v. mFGF1 (or hFGF19) *vs.* Veh as determined by one-way ANOVA or by two-tailed t-test.

Supplementary Figure 1

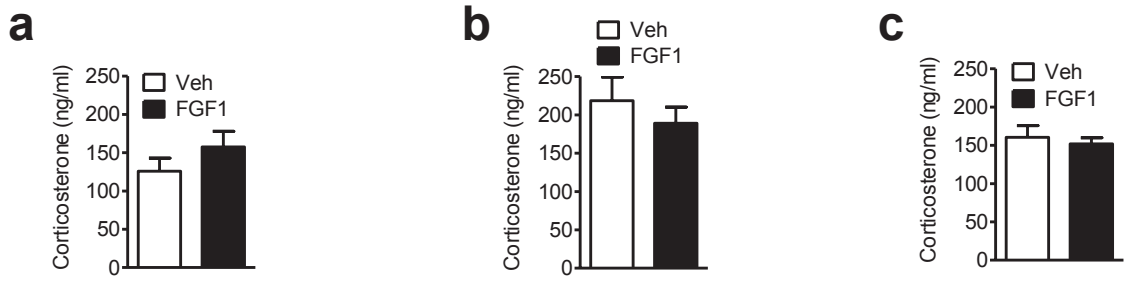
a



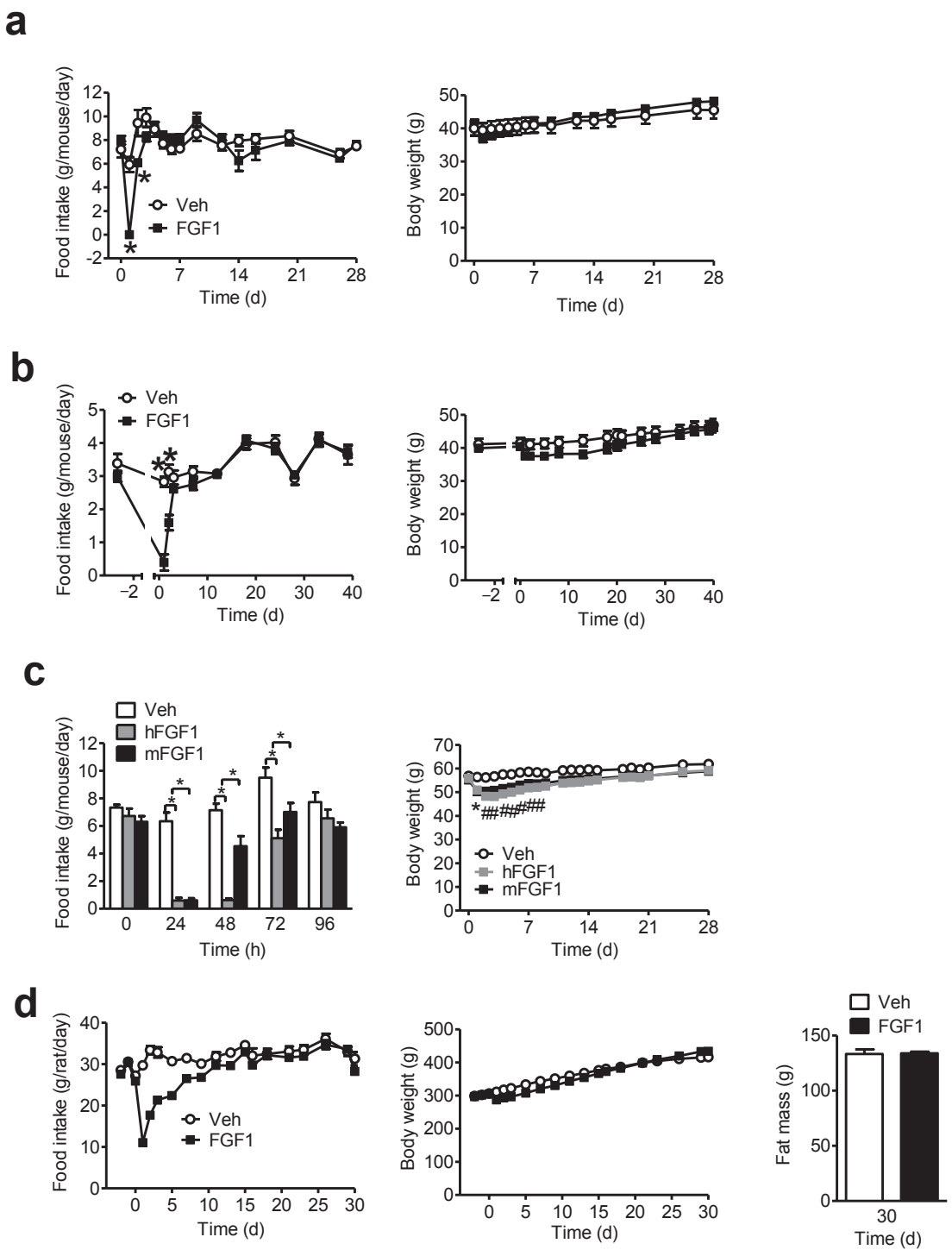
b



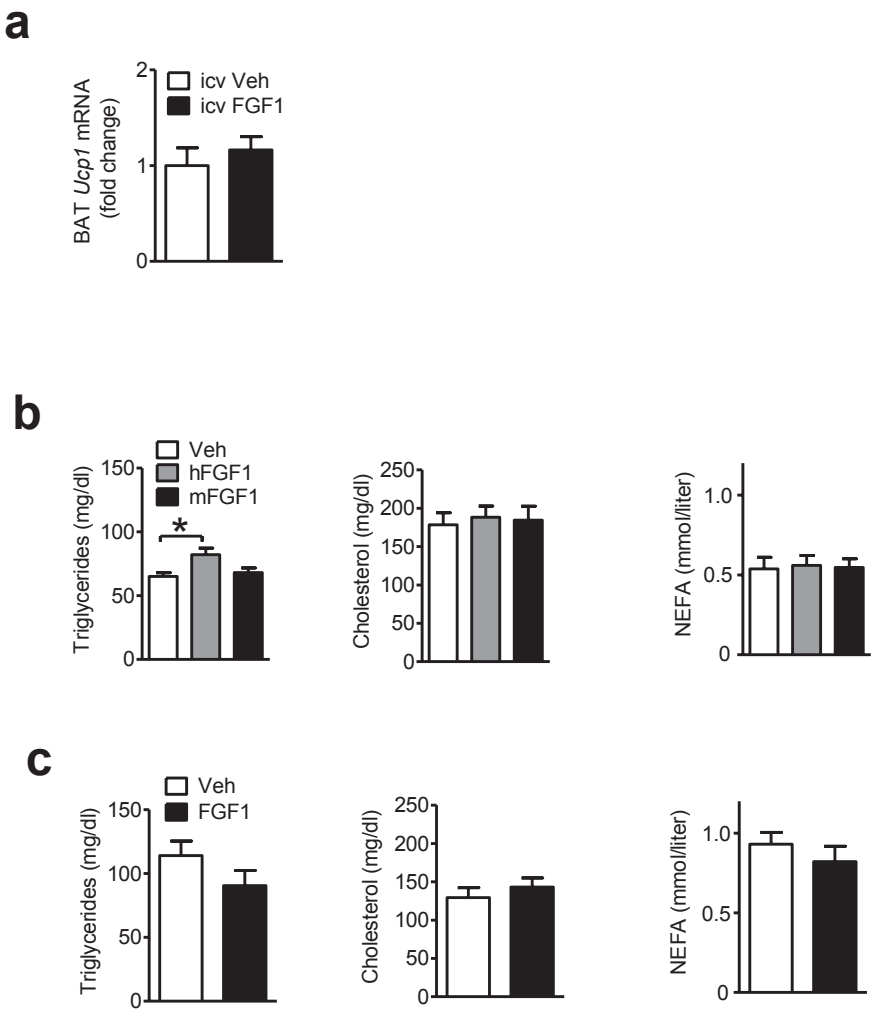
Supplementary Figure 2



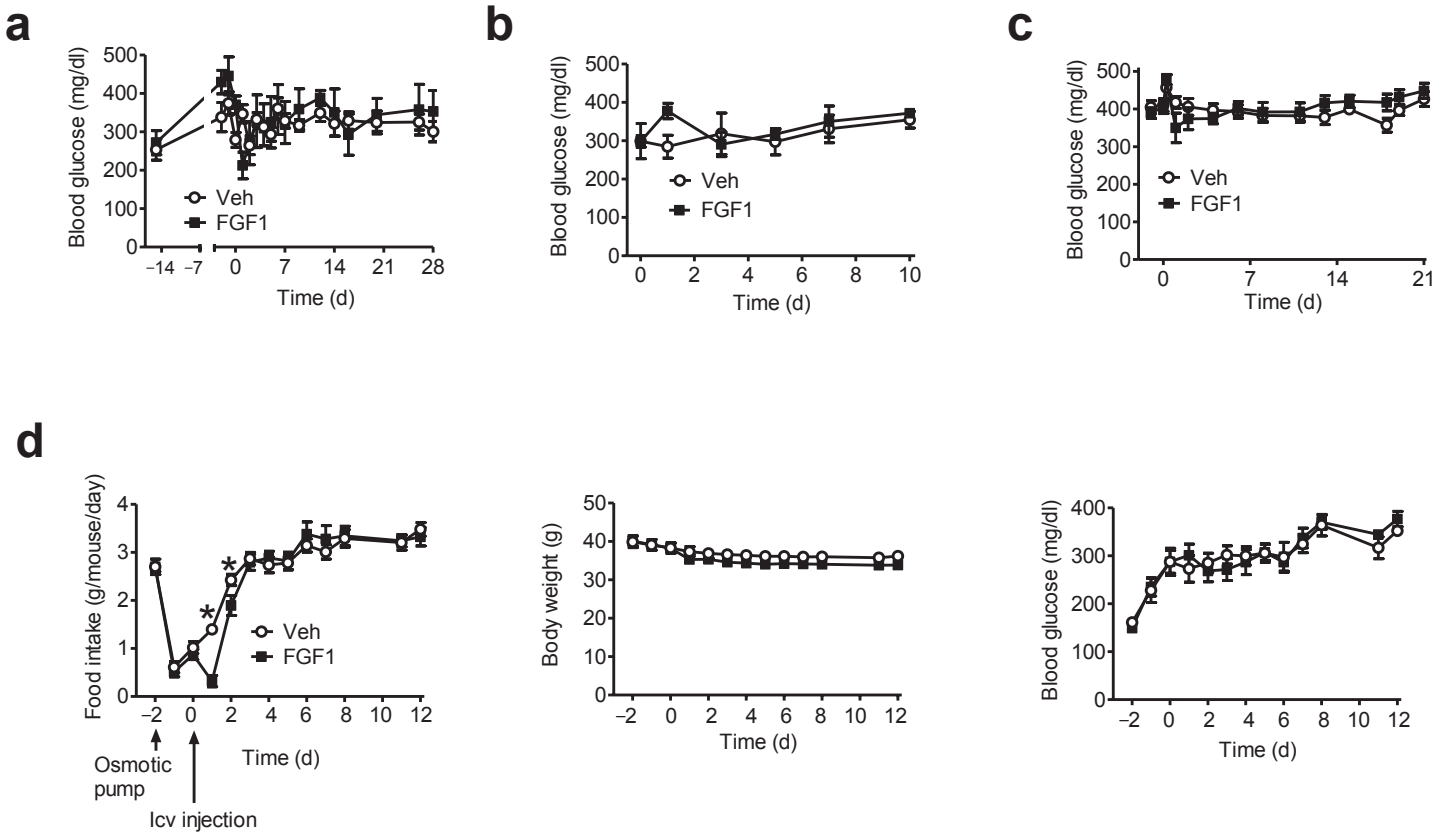
Supplementary Figure 3



Supplementary Figure 4

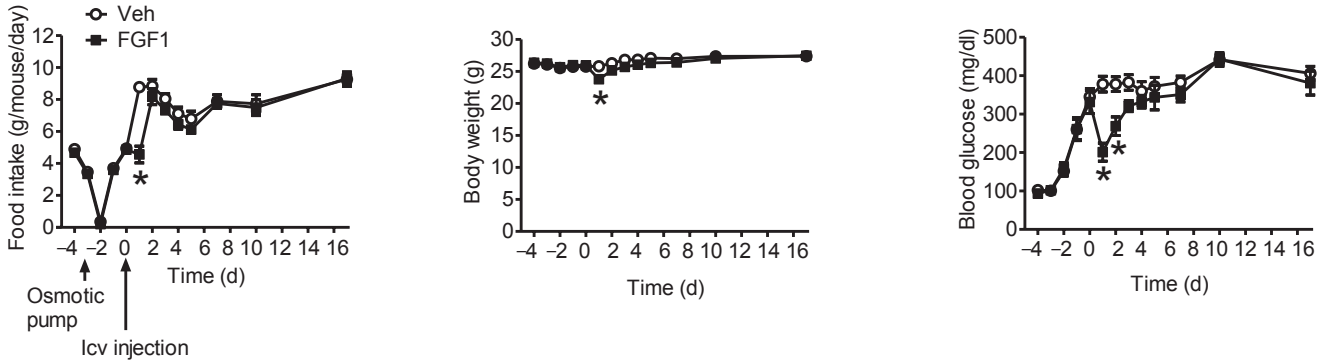


Supplementary Figure 5

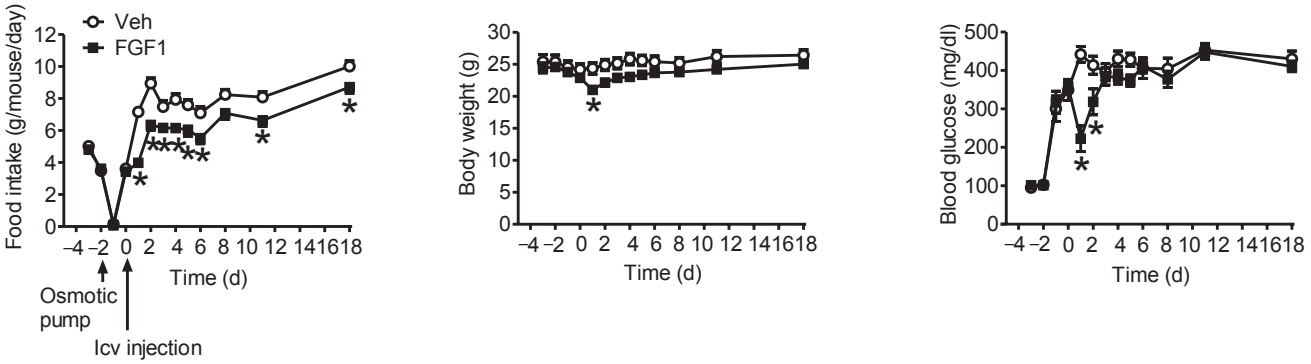


Supplementary Figure 6

a



b



Supplementary Figure 7