Supplemental Figure Legends

Supplemental Figure 1, related to Figure 1. FGF21 signaling to glutamatergic neurons is required to promote weight loss, but not improve insulin sensitivity, in DIO mice.

(a-c) 16–18 week-old diet-induced obese (DIO) WT and $KLB^{Vglut2\text{-}KO}$ mice were administered vehicle or FGF21 (1 mg/kg/day) by osmotic minipump for 2 weeks (n = 7-10/group). (a) Body weight on the day of minipump implants, (b) plasma glucose levels and (c) hepatic triglyceride levels.

(d) Plasma glucose levels in 12-14 week-old male WT and $KLB^{Vglut2\text{-}KO}$ mice co-injected with insulin and either vehicle or FGF21 (i.p, 1 mg/kg) (n = 6/group). (e) Quantification of the area under the curve for ITT in (d).

(f-n) 16-18 week-old DIO WT and $KLB^{Vglut2\text{-}KO}$ mice were administered vehicle for 5 days followed by 7 days of FGF21 treatment (i.p., 1 mg/kg/day) (n=7-8/group). (f) Correlation of heat production and body weight during the dark cycle, (g) average energy expenditure during the light cycle, (g) correlation of heat production and body weight during the light cycle, (i) average VO_2 during the dark cycle, (j) average VO_2 during the light cycle, (k) average water consumption during the dark cycle, (l) average water consumption during the light cycle, (m) average activity during the dark cycle, and (n) average activity during the light cycle.

Values are mean ± SEM. *$p < 0.05$. Statistical analyses were conducted using two-way ANOVA with Holms-Sidak's multiple comparisons test.

Supplemental Figure 2, related to Figure 1. FGF21 signaling to glutamatergic neurons is required for FGF21-mediated reductions in liver and BAT lipid accumulation. (a-b) 16–18 week-old diet-induced obese (DIO) WT and $KLB^{Vglut2\text{-}KO}$ mice were administered vehicle or

FGF21 (1 mg/kg/day) by osmotic minipump for 2 weeks (n = 3-4/group). (a) Histology of BAT and (b) liver.

Supplemental Figure 3, related to Figure 2. FGF21 signaling to GABAergic neurons is not required to promote weight loss in DIO mice.

(a-b) 16-18 week-old diet-induced obese (DIO) WT and $KLB^{Vgat\text{-}KO}$ mice were administered vehicle or FGF21 (1 mg/kg/day) by osmotic minipump for 2 weeks (n = 7-9/group). (a) Body weight on the day of minipump implants and (b) body weight on the last day of minipump treatment.

Values are mean ± SEM. *$p < 0.05$. Statistical analyses were conducted using two-way ANOVA with Holms-Sidak's multiple comparisons test.

Supplemental Figure 4, related to Figure 4. Leptin signals to KLB^{+} cells in the hypothalamus.

Percentage of leptin-induced pSTAT3 that colocalizes to tdTomato-expressing cells in the medial preoptic area (mPOA), lateral hypothalamus (LH), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC) and ventral premammillary nucleus (PMv) of KLB-Cre;tdTomato mice administered leptin (i.p., 100 μg) following an overnight fast.

Supplemental Figure 5, related to Figure 5. FGF21 signals to leptin-receptor expressing cells and enhance leptin signaling to promote weight loss

(a-h) 10-12 week-old chow-fed WT mice were administered vehicle, leptin (250 ng/hour), FGF21 (1 mg/kg/day) or leptin plus FGF21 by osmotic minipump for 2 weeks (n= 4-9/group). (a) Body weight on the day of minipump implants, (b) plasma FGF21 levels, (c) plasma leptin levels, (d)

brown adipose tissue *Ucp1* mRNA expression, (e) tail temperature and (f) representative infrared images as quantified in (e), (g) plasma glucose levels, and (h) plasma insulin levels.

(i-l) 10-12 week-old chow-fed WT mice were administered vehicle, FGF21 (1 mg/kg/day) or leptin plus FGF21 by osmotic minipumps for 2 weeks (n= 6-8/group). One group of FGF21 treated mice was pair fed to FGF21 + leptin group. (i) Body weight on the day of minipump implants, and (j-l) brown adipose tissue mRNA expression of (j) *Ucp1,* (k) *Bmp8b* and (l) *Elovl3.*

Values are mean ± SEM. *p < 0.05. Statistical analyses were conducted using either one-way ANOVA or two-way ANOVA with Holms-Sidak's multiple comparisons test.

Supplemental Figure 6, related to Figure 6. FGF21 requires central leptin signaling to fully promote body weight loss.

(a-b) 16-18 week-old diet-induced obese (DIO) WT mice were administered vehicle, leptin antagonist (LA; 8 μg/day), FGF21 (1 μg/day), or LA and FGF21 by ICV osmotic minipump for 7 days (n= 7-9/group). (a) Body weight on the day of ICV minipump implant, and (b) plasma leptin levels.

(c) Plasma leptin levels in 16-18 week-old DIO WT mice at the indicated times after administration of a single injection of vehicle or FGF21 (1 mg/kg) (n = 6-7/group).

(d-f) Plasma glucose levels (d), plasma insulin levels (e), and plasma triglyceride levels (f) from mice described in (a-b) (n = 6-9/group).

Values are mean ± SEM. *p < 0.05. Statistical analyses were conducted using one-way ANOVA.

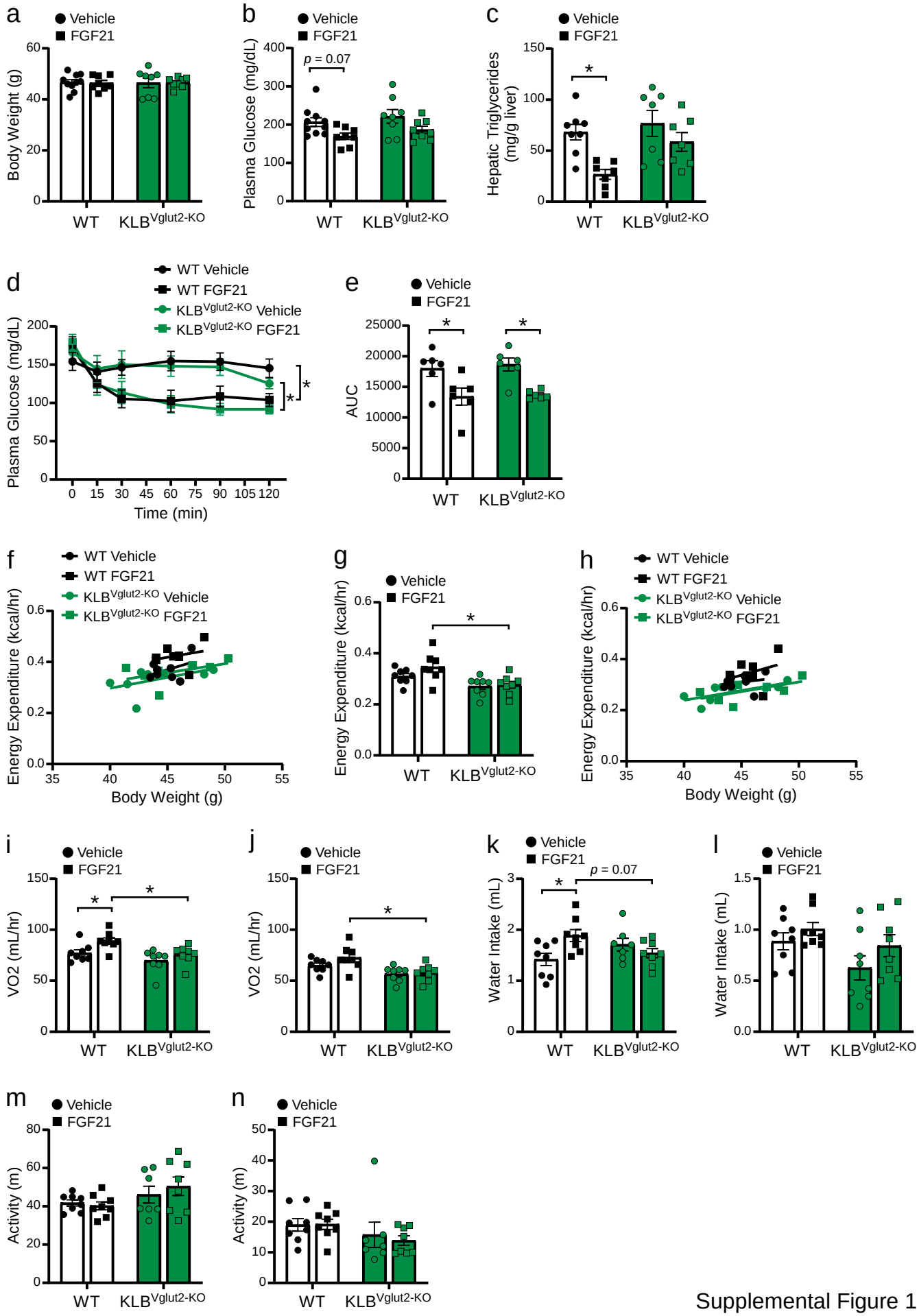
Supplemental Video 1, related to Video 1: Light sheet imaging of a brain from WT;Ai65-tdTomato (Cre negative) mice processed by DISCO clearing.

Supplemental Video 2, related to Video 1: Light sheet imaging of a brain from KLB-Cre;Vglut2-FLP;Ai65-tdTomato mice processed by DISCO clearing.

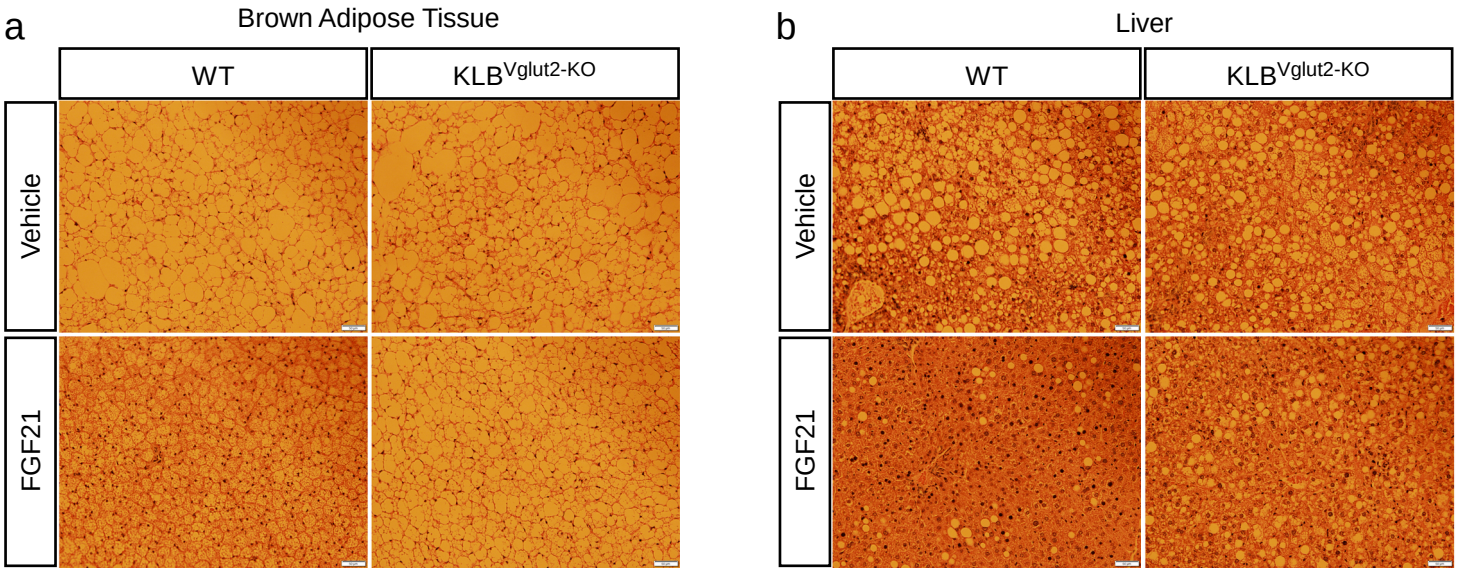
Supplemental Methods

An augmented DISCO protocol was utilized to clear whole brain tissue of KLB-Cre;Vglut2-Flp;Ai65 triple knock-in and CRE negative control mice [1]. Mice were anesthetized and transcardially perfused with pH 9.5 saline followed by pH 9.5 4% PFA. After post-fixing in PFA overnight, the brains were washed 3 times in saline and dehydrated with 50% tetrahydrofuran (THF) in dH_2O for 24 hours. Every 24 hours the brains were transferred to a higher concentration of THF (50%, 70%, 80%), all at pH = 9.5. Brains were incubated in 100% THF for 48 hours, and the solution was replaced every 12 hours to maximize tissue THF concentration. After the final incubation in 100% THF, brains were transferred to dichloromethane (DCM) for 3 hours followed by BABB-D15 for 4 hours, as previously [2]. All steps were performed at 4°C in the dark. Additionally, after transferring brains to a new solution, argon gas was flowed over the sample to minimize oxygen interaction with the sample. Cleared brains were imaged in BABB-D15 using a light sheet fluorescence microscope (LaVision BioTec Ultramicroscope II) interfaced with an Andor Neo sCMOS camera, an Olympus MVX10 zoom microscope body, and a MVPLAPO 2x dipping cap. Whole brain three-dimensional images were achieved using a 3x3 mosaic tile scan of a 2x optical zoom and a z-stack with step size of 2.5 μm. The raw lossless TIFF files of the collected tile scans were stitched using the ImspectorPro software and reconstructed into three dimensions with Imaris File Converter. Images and movies of brain samples were captured with Imaris.

[1] Claflin, K.E., Flippo, K.H., Sullivan, A.I., Naber, M.C., Zhou, B., Neff, T.J., et al., 2022. Conditional gene targeting using UCP1-Cre mice directly targets the central nervous system beyond thermogenic adipose tissues. Mol Metab 55:101405.
[2] Pan, C., Cai, R., Quacquarelli, F.P., Ghasemigharagoz, A., Lourbopoulos, A., Matryba, P., et al., 2016. Shrinkage-mediated imaging of entire organs and organisms using uDISCO. Nat Methods 13(10):859-867.



Supplemental Figure 1



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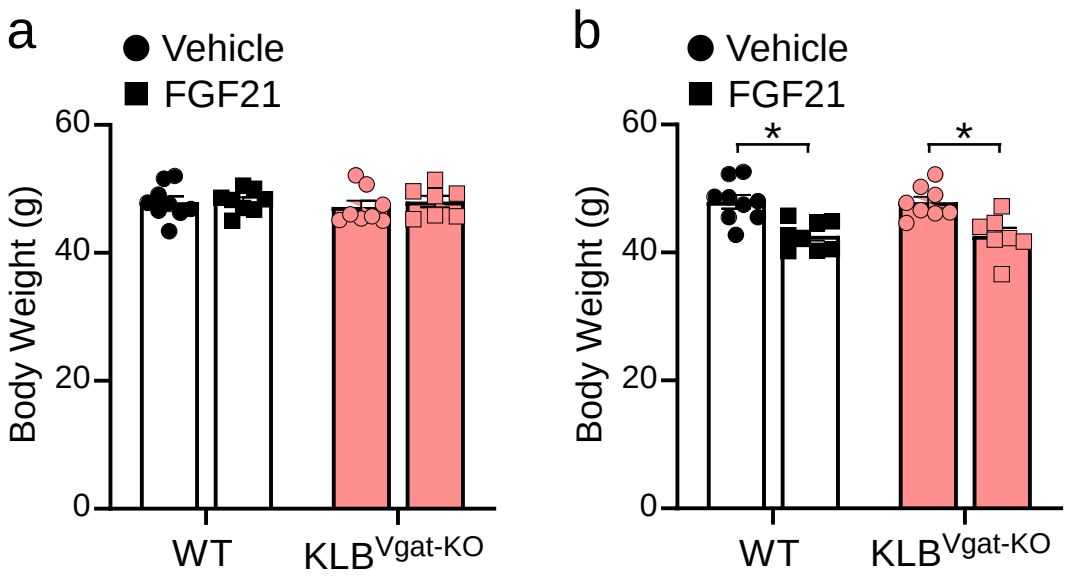

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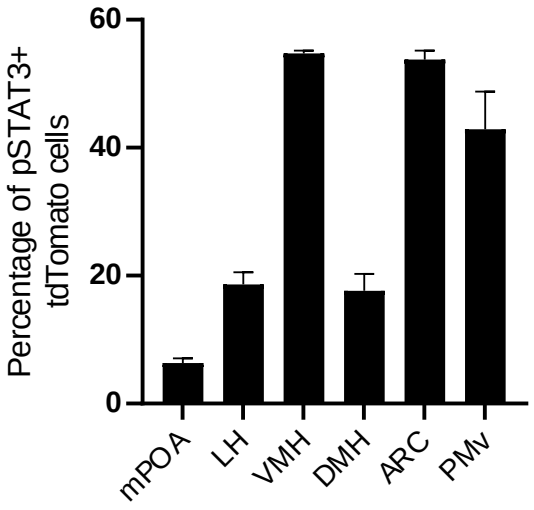
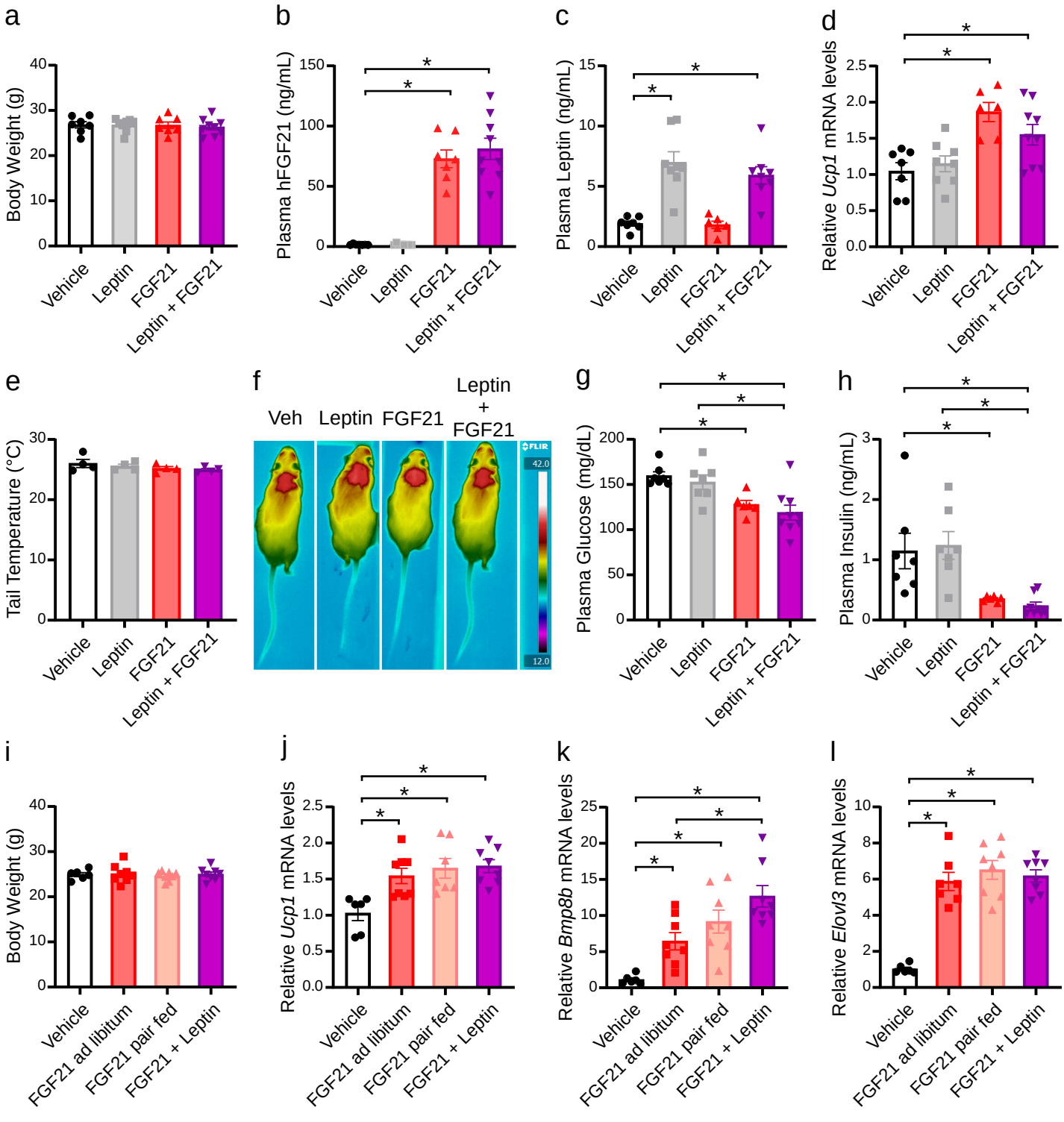
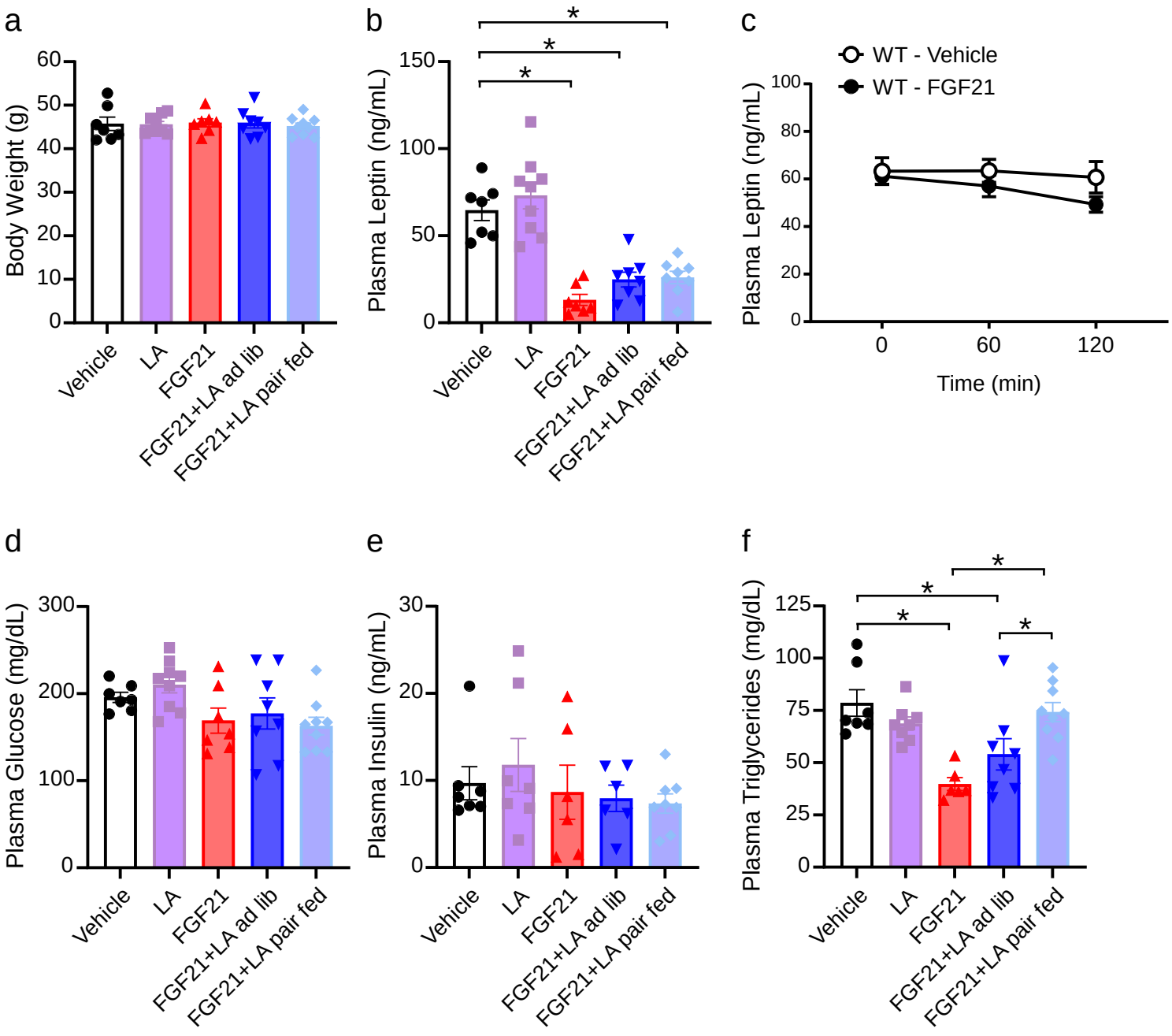


Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6