

Figure S1, Related to Figure 1. (A, B) Immobility was measured in mice administered FGF21 or vehicle by osmotic minipump and then subjected to the forced-swim test (A) or tail-suspension test (B). Immobility times (seconds) are shown as the mean \pm S.E.M. (n = 6-9/group).

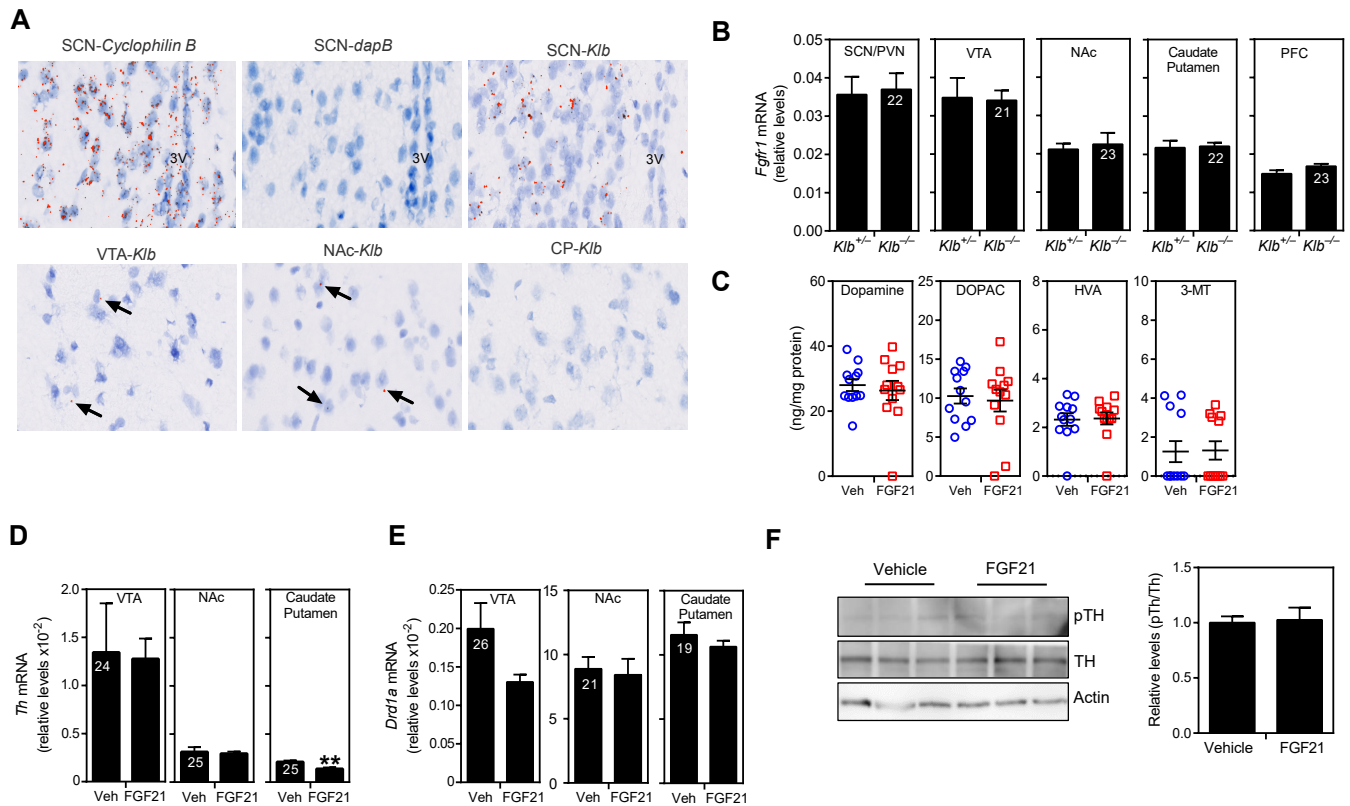


Figure S2, Related to Figure 3. (A) In situ hybridization of *Klb* in the suprachiasmatic nucleus (SCN; positive control), ventral tegmental area (VTA), nucleus accumbens (NAc) and caudate putamen (CP). The third ventricle (3V) in SCN, and *Klb* signal in NAc and VTA (arrows) are indicated. Positive control (*cyclophilin B*) and negative control (*dapB*) are shown for SCN. Brown chromogenic signal was highlighted in red using Image J and nuclei were counterstained with hematoxylin. Similar results were observed in three different mouse brains. (B) FGF receptor 1 (*Fgfr1*) mRNA levels in the suprachiasmatic nucleus/paraventricular nucleus (SCN/PVN) region of the hypothalamus, ventral tegmental area (VTA), nucleus accumbens (NAc), caudate putamen and medial prefrontal cortex (PFC) of *Klb*^{+/-} and *Klb*^{-/-} mice (n = 6/group). Values are means ±S.E.M. (C) Concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) in the CP of mice administered either FGF21 or vehicle by osmotic minipump for 2 weeks (n = 12/group). Means ±S.E.M are shown. (D, E) mRNA levels of tyrosine hydroxylase (*Th*) (D) and dopamine receptor 1 (*Drd1a*) (E) in VTA, NAc and caudate putamen of mice administered either vehicle or FGF21 by osmotic minipump for 2 weeks (n = 7-8/group). Ct values are shown. Values are means ±S.E.M. **, p<0.01. (F) Western blot analysis of total and phosphorylated (Ser40) tyrosine hydroxylase in VTA from mice treated with FGF21 or vehicle by osmotic minipump for 14 days. The ratio of phosphorylated to total tyrosine hydroxylase is shown in the panel on the right.

Table S1, Related to Figure 1A and B. Two-bottle sweet preference assays in wild-type (WT) and FGF21-transgenic (Tg) mice.

| 3% Sucrose bottle (ml/g) | | Water only bottle (ml/g) | | Total fluid (ml/g) | |
|--|----------------------------------|-----------------------------|----------------|--------------------|--------------|
| WT | Tg | WT | Tg | WT | Tg |
| 0.31 ± 0.040 | 0.20 ± 0.035 ^(p=0.07) | 0.01 ± 0.003 | 0.07 ± 0.010** | 0.32 ± 0.040 | 0.27 ± 0.035 |
| Mean body weight (g): WT (28.8 ± 1.17), Tg (14.7 ± 0.69) | | | | | |
| 0.2% Saccharin bottle (ml/g) | | Water only bottle (ml/g) | | Total fluid (ml/g) | |
| WT | Tg | WT | Tg | WT | Tg |
| 0.13 ± 0.011 | 0.10 ± 0.009* | 0.02 ± 0.005 | 0.05 ± 0.005** | 0.16 ± 0.011 | 0.15 ± 0.011 |

Mean body weight (g): WT (31.2 ± 1.35), Tg (15.5 ± 0.75).

Values are expressed as volume consumed in ml/g body weight (n = 10-11/group, mean ± S.E.M); *p<0.05, **p<0.01 versus WT controls. Representative 24 hour data from day 2 after initiating the assay are shown.

Table S2, Related to Figure 1C. Effect of FGF21 in the two-bottle saccharin preference assay in control (fl/fl) and CNS-specific β -Klotho knockout (Camk2a) mice.

| 0.2% Saccharin bottle (ml/g) | | | | Water only bottle (ml/g) | | | |
|------------------------------|-----------------|--------------|-----------------|--------------------------|-----------------|--------------|-----------------|
| fl/fl+Veh | fl/fl+F21 | Camk2a+Veh | Camk2a+F21 | fl/fl+Veh | fl/fl+F21 | Camk2a+Veh | Camk2a+F21 |
| 0.19 ± 0.023 | 0.07 ± 0.009*** | 0.28 ± 0.030 | 0.25 ± 0.037### | 0.03 ± 0.015 | 0.28 ± 0.029*** | 0.01 ± 0.005 | 0.00 ± 0.003### |

| Total fluid (ml/g) | | | |
|--------------------|----------------|--------------|--------------|
| fl/fl+Veh | fl/fl+F21 | Camk2a+Veh | Camk2a+F21 |
| 0.22 ± 0.018 | 0.35 ± 0.029** | 0.29 ± 0.033 | 0.25 ± 0.039 |

Mean body weight (g): fl/fl + Veh (24.0 ± 0.83), fl/fl + F21 (24.3 ± 0.65), Camk2a + Veh (22.8 ± 0.76), Camk2a + F21 (24.3 ± 1.19).

Values are expressed as volume consumed in ml/g body weight (n = 6-9/group, mean ±S.E.M), *p<0.05, **p<0.01, ***p<0.001 for vehicle versus FGF21-treated fl/fl mice; and #p<0.05, ##p<0.01, ###p<0.001 for fl/fl versus Camk2a mice treated with FGF21.

Representative 24 hour data from day 2 after initiating the assay are shown.

Table S3 Related to Figure 4. Two-bottle ethanol preference assay in wild-type (WT) and FGF21-transgenic (Tg) mice.

| Ethanol (%) | Ethanol bottle (ml/day/g) | | Water only bottle (ml/day/g) | | Total fluid (ml/day/g) | |
|-------------|---------------------------|-----------------|------------------------------|------------------|------------------------|-----------------------------------|
| | WT | Tg | WT | Tg | WT | Tg |
| 0 | | | | | 0.107 ± 0.011 | 0.129 ± 0.007 ^(p=0.12) |
| 2 | 0.040 ± 0.003 | 0.044 ± 0.005 | 0.070 ± 0.010 | 0.140 ± 0.017** | 0.111 ± 0.011 | 0.184 ± 0.014** |
| 4 | 0.036 ± 0.006 | 0.020 ± 0.002* | 0.075 ± 0.008 | 0.141 ± 0.022* | 0.111 ± 0.005 | 0.162 ± 0.021* |
| 8 | 0.051 ± 0.007 | 0.029 ± 0.001** | 0.085 ± 0.005 | 0.156 ± 0.019** | 0.136 ± 0.007 | 0.184 ± 0.016** |
| 12 | 0.036 ± 0.004 | 0.022 ± 0.002** | 0.064 ± 0.004 | 0.122 ± 0.010*** | 0.100 ± 0.006 | 0.142 ± 0.009** |
| 16 | 0.042 ± 0.006 | 0.024 ± 0.002* | 0.077 ± 0.008 | 0.092 ± 0.012 | 0.117 ± 0.011 | 0.121 ± 0.012 |

Mean body weight (g): WT (29.0 ± 0.48), Tg (14.3 ± 0.30).

Values represent the mean daily volume consumed during the 4 day assay and is expressed in ml/day/g body weight (n = 9/group, mean ± S.E.M.), *p<0.05, **p<0.01, ***p<0.001 versus WT control groups.