Supplementary Figure 1. Circadian Locomotor Behavior in \(Klb^{\Delta \text{CNS}}\) mice

Representative light-dark double plotted actograms of wheel-running activity for control (left) and \(Klb^{\Delta \text{CNS}}\) mice (right) (A). Average wave plots summarizing wheel-running light-dark activity (B) and % lights on activity (C) (n=5-8). Representative free-running double plotted actograms in constant dark (D). Representative \(\chi^2\) periodogram (E) and average alpha length (F; n=5-7) in control and \(Klb^{\Delta \text{CNS}}\). All data are represented as mean ± SEM. *p<0.05, Student’s t-test
Supplementary Figure 2. Diet-induced obesity and GCGR agonism in KlbΔCNS mice

*Klb* expression in lean, chow-fed mice (8wk-old n=5-7) and DIO, HFD-fed mice (16wk-old mice n=4-6) (A). Absolute body weight (B) in control and *KlbΔCNS* fed HFD for 8wk (n=10-14). Diurnal EE (C), respiratory quotient (D), food intake (E) and % light food intake (E inset), and locomotor activity (F) in control and *KlbΔCNS* for remaining 7d of HFD after 3d chow (n=5-7). Absolute body weight (G) in control and *KlbΔCNS* mice treated with IUB288 for 12d. Liver Fgf21 expression (H) after 12d IUB288 in control and *KlbΔCNS* mice. All data are represented as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 compared to lean vehicle controls, two-way ANOVA. Panel A: Main effect of genotype (p<0.01). Panel B: Main effect of genotype in hypothalamus (p<0.01). Panel G: Main effect of treatment (p<0.05). GCGR Agonist: IUB288
Supplementary Figure 3. GCGR-agonism in mice with KLB Antagonism

Glucose tolerance test (A, 5h fast) and iAUC (B) in 10wk-old lean mice (n=10). 1153 (0.3mg/kg or 3mg/kg) or vehicle administered at t=−70m and FGF21 or vehicle administered at t=−60m, before glucose (2g/kg) administered at t=0m. Absolute body weight in control and 1153 DIO mice from ICV surgery start (C; t=−2d) and from IUB288 start (D) (n=12-14). Dotted line indicates start of IUB288 (t=1d). Representative BAT UCP1 from 6 individual samples (E) and gene expression of Liver Fxr and downstream Fxr-targets (F; n=7-9). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to respective genotypic controls. #p<0.05, ##p<0.01, ###p<0.001 between IUB288 and 1153 + IUB288 groups, two-way ANOVA. Panel E: main effect of 1153 (p<0.01) on Fxr and main effect of treatment on Cyp7a1 (p<0.01). GCGR Agonist: IUB288. KLB Antagonist: 1153.
Supplementary Table 1. qPCR primers

| Gene   | Forward (5’-3’)                      | Reverse (5’-3’)                      |
|--------|--------------------------------------|--------------------------------------|
| Abca1  | CGT TTC CGG GAA GTG TCC TA           | GCT AGA GAT GAC AAG GAG GAT GGA     |
| Acaca  | CTT CCT GAC AAA CGA GTC TGG          | CTG CCG AAA CAT CTC TGG GA           |
| Hmgcr  | GTG TTC AAG GAG CAT GCA AAG          | AGC CAT CAC AGT GCC ACA TAC          |
| Srebp-1| GAG GAC CTT TGT CAT TGG CTG          | TAC AGA GCA AGA GGG TGC CAT          |
| Fxr    | CAC AGC GAT CGT CAT CCT CTC T        | TCT CAG GCT GTT ACA TCT TGC A        |
| Cyp7a1 | GGG ATT GCT GTG GTA GTG AGC          | GGT ATG GAA TCA ACC CGT TGT C        |
| Shp    | CAT GGC CTC TAC CCT CAA GAA C        | GTC ACC TCA GCA AAA GCA TGT C        |
| Rps18  | TTC TGG CCA ACG GTC TAG ACA AC       | CCA GTG GTC TTG GTG TGC TGA          |