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Supplemental information

Central FGF21 production regulates memory but not peripheral metabolism

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Supplemental Figures

Figure S1, related to Figure 1, FGF21 is expressed in the retrosplenial cortex but not the hypothalamus.

(A-B) Relative *Fgf21*(A) and *Cre* (B) mRNA levels from the liver of 10-12 week old male *Fgf21*-P2A-CRE mice and CRE negative littermate *ad libitum* fed or following a 24-hour fast (n=5-6/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(C) Representative fluorescence imaging for tdTomato-positive cells in the pancreas of 12-week-old male *Fgf21*-P2A-CRE;Ai-tdTomato mice. Scale bar = 500 µm.

(D) Representative fluorescence imaging for tdTomato-positive cells in the medial hypothalamus of female *Fgf21*-P2A-CRE;Ai14-tdTomato and CRE negative control mice following a 24-hour fast. Scale bars = 250 µm.

(E) RNAscope *in situ* hybridization for *Fgf21* and *tdTomato* mRNA in retrosplenial cortex (RSC) and hypothalamus from *Fgf21*-P2A-CRE;Ai-tdTomato mice. 3V = third ventricle. Scale bars = 75 µm.

(F) Representative immunofluorescence imaging for FGF21 and vimentin in the median eminence of male wild-type mice using primary (Rabbit anti-FGF21; Chicken anti-vimentin) and secondary (FGF21, Goat anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody Texas Red; Vimentin, Goat anti-Chicken IgY (H+L) Secondary Antibody Alexa Fluor 488) antibodies or FGF21 secondary antibody only.

(G) In silico single cell analysis of *Fgf21* expression in the hypothalamus in the indicated cell types.

(H) In silico single cell analysis of *Fgf21*-expressing cells in the thalamus based on the indicated marker genes.

Values are mean ± SEM.

Figure S2, related to Figure 2, Neuron-derived FGF21 does not regulate energy homeostasis.

(A) Representative fluorescence imaging for GFP-positive cells in 12-week-old $FGF21^{fl/fl}$;PHP.eB-eGFP-CRE mice. Scale bar = 1 mm.

(B) Daily measurement of sucrose intake during two-bottle choice of 10% sucrose versus water in male $FGF21^{fl/fl}$ mice injected with AAV-TBG-Con (null) or AAV-TBG-CRE virus (n=10/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(C-D) Plasma glucose (C) and plasma non-esterified fatty acid (NEFA) (D) levels in *ad libitum* fed and 24 hour fasted male $FGF21^{fl/fl}$ mice injected with AAV-TBG-Con (null) or AAV-TBG-CRE virus (n=7-10/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(E-F) Linear modeling of energy expenditure of 12-14 week old male $FGF21^{fl/fl}$;PHP.eB-GFP and $FGF21^{fl/fl}$;PHP.eB-CRE mice (n=7-9/group) during the light (E) or dark (F) cycles.

Values are mean ± SEM.

Figure S3, related to Figure 3, Role of FGF21 in learning and memory.

(A-B) Average of the four trials for latency to the escape chamber during training (A) and primary error to the escape chamber during training (B) in male $FGF21^{fl/fl}$ mice injected with PHP.eB-GFP

or PHP.eB-CRE virus (n=7-9/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(C-D) Average of the four trials for latency to the escape chamber during training (C) and primary error to the escape chamber during training (D) in male $FGF21^{fl/fl}$ mice injected with AAV-TBG-Con (null) or AAV-TBG-CRE virus (n=15/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(E) Representative fluorescent image of viral targeting of AAV-CMV-CRE to the retrosplenial cortex (RSC) of $FGF21^{fl/fl}$ mice. Scale bar = 1 mm.

(F-G) Average of the four trials for latency to the escape chamber during training (F) and primary error to the escape chamber during training (G) in male $FGF21^{fl/fl}$ mice injected with AAV-CMV-CRE and AAV-CMV-GFP virus in the RSC (n=15/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(H-J) Escape latency on test trials 24 hours (H), 7 days (I), and 14 days (J) after the training of male $FGF21^{fl/fl}$ mice which received stereotaxic injection of AAV-CMV-CRE and AAV-CMV-GFP virus compared to wild-type mice which received AAV-CMV-CRE virus into the retrosplenial cortex (RSC) (n=9-15group). Statistical analyses were conducted using a two-tailed t test (* = $p < 0.05$).

(K) Representative fluorescent image of viral targeting of AAV-hSyn-CRE-EGFP to the retrosplenial cortex (RSC) of $FGF21^{fl/fl}$ mice. Scale bar = 1 mm.

(L-M) Average of the four trials for latency to the escape chamber during training (L) and primary error to the escape chamber during training (M) in male $FGF21^{fl/fl}$ mice injected with AAV-hSyn-CRE and control virus in the RSC (n=15-16/group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(N-O) Average of the four trials for latency to the escape chamber during training (N) and primary error to the escape chamber during training (O) in wild-type male mice administered vehicle or FGF21 (1 mg/kg) each day of training (n=14-15group). Statistical analyses were conducted using a two-way ANOVA with multiple comparisons (* = $p < 0.05$).

(P-R) Escape latency on test trials 24 hours (P), 7 days (Q), and 14 days (R) after the training of 11-13 week old female C57BL/6J mice treated with FGF21 (1 mg/kg) or saline for 4 days via i.p. injection (n=13-15/group). Statistical analyses were conducted using a two-tailed t test (* = $p < 0.05$).

Values are mean ± SEM.

Figure S4, related to Figure 3, FGF21 administration consolidates spatial and fear memory.

Total freezing time of cued-dependent fear (A) and contextual (B) memory of 10-12 week old male C57BL/6 mice administered FGF21 (1mg/kg) or saline treatment via i.p. injection (n=10/group). Values are mean ± SEM.. Statistical analyses were conducted using a two-tailed t test (* = $p < 0.05$).

Video S1, related to Figure 1, Comprehensive evaluation of FGF21-expressing cells in the CNS.

Light sheet imaging of a brain from *Fgf21*-CRE;Ai14-tdTomato mice processed by DISCO clearing.

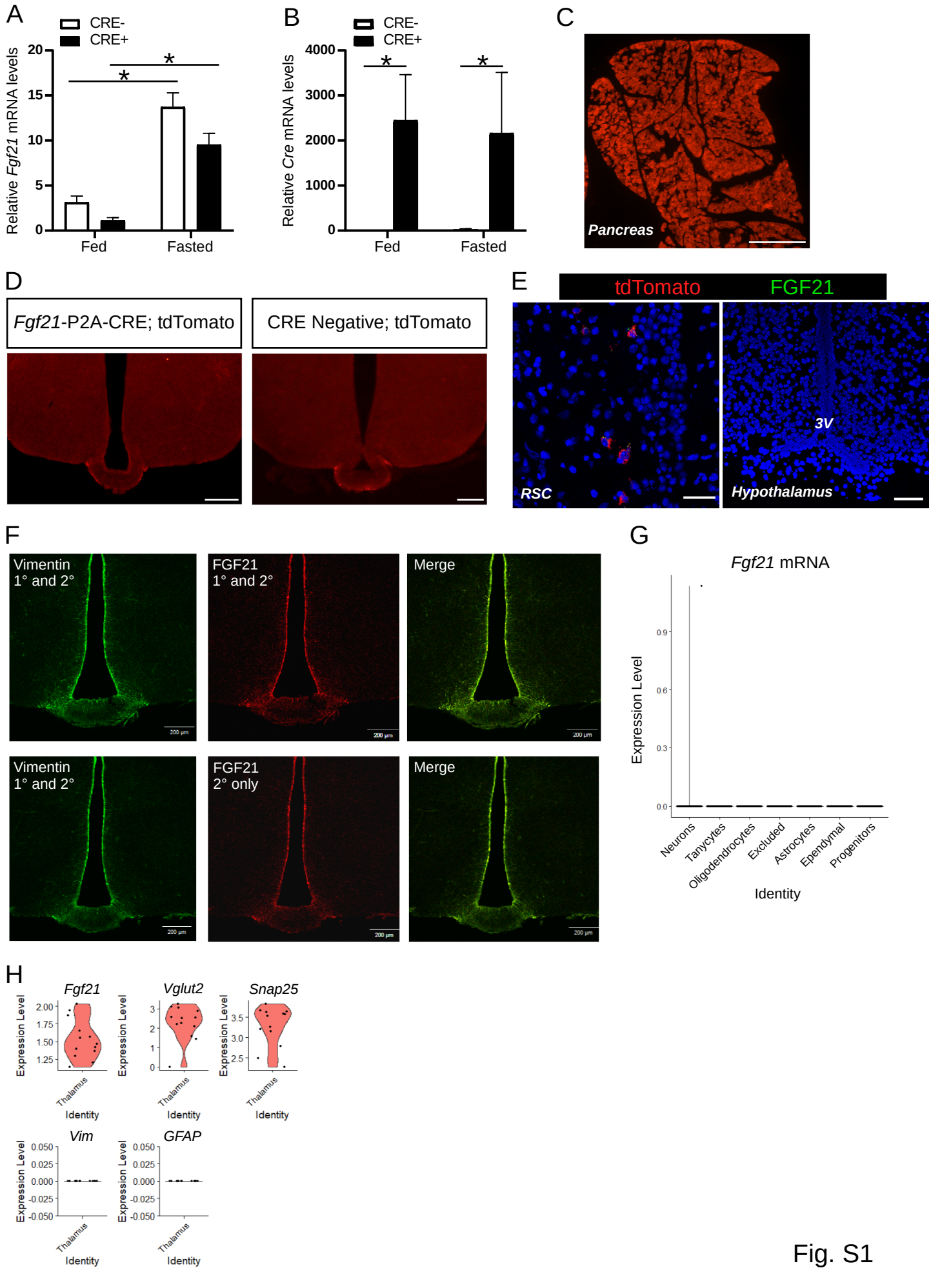
A
CRE-
CRE+
Relative Fgf21 mRNA levels
20
15
10
5
0
*
*
Fed
Fasted
B
CRE-
CRE+
Relative Cre mRNA levels
4000
3000
2000
1000
0
*
*
Fed
Fasted
C
Pancreas
D
Fgf21-P2A-CRE; tdTomato
CRE Negative; tdTomato
E
tdTomato
FGF21
3V
RSC
Hypothalamus
F
Vimentin
1° and 2°
FGF21
1° and 2°
Merge
Vimentin
1° and 2°
FGF21
2° only
Merge
200 µm
G
Fgf21 mRNA
Expression Level
0.9
0.6
0.3
0.0
Neurons
Tanycytes
Oligodendrocytes
Excluded
Astrocytes
Ependymal
Progenitors
Identity
H
Fgf21
Vglut2
Snap25
Vim
GFAP
Expression Level
Thalamus
Identity

Fig. S1

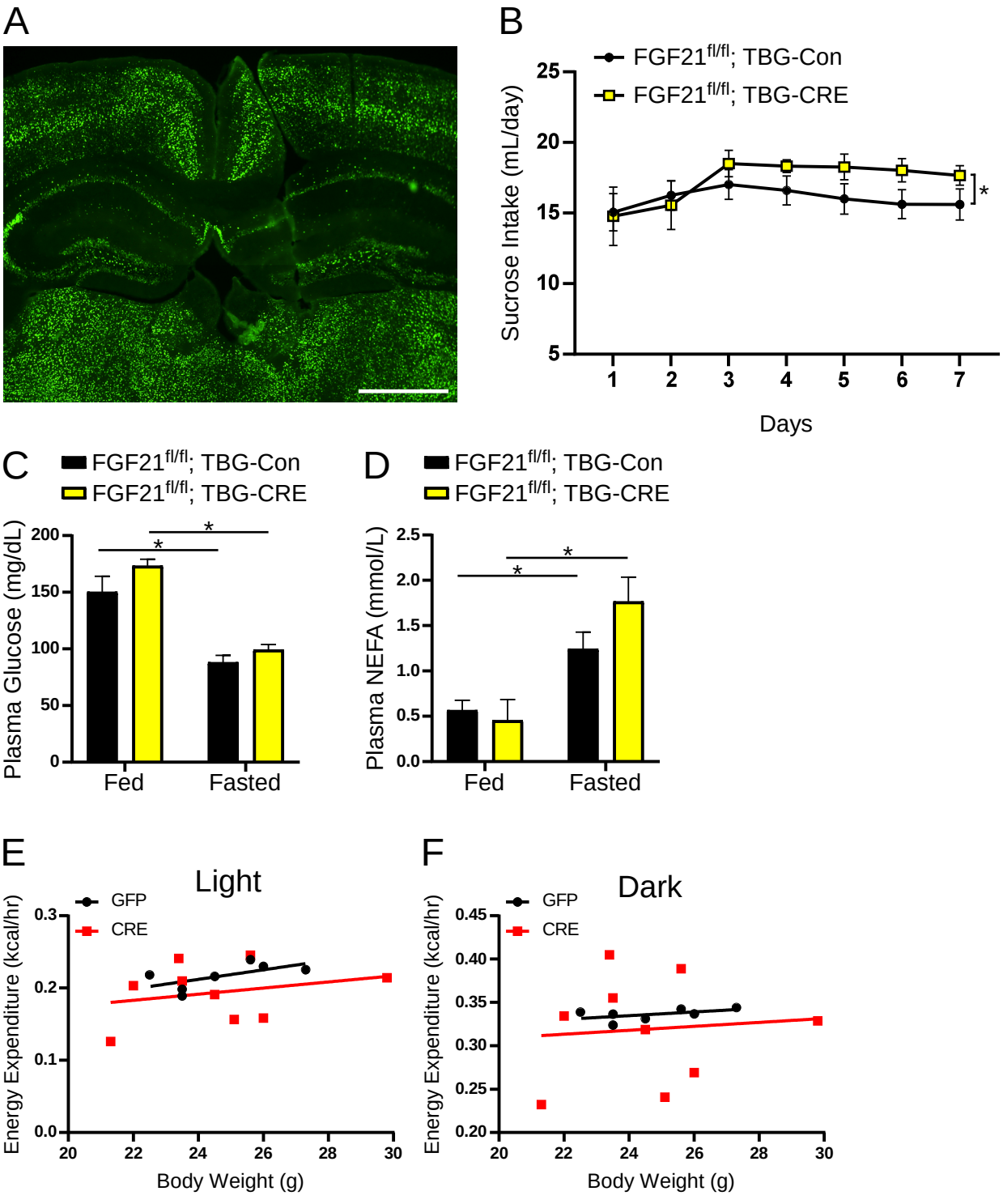
A
B
FGF21fl/fl; TBG-Con
FGF21fl/fl; TBG-CRE
Sucrose Intake (mL/day)
Days
C
D
Plasma Glucose (mg/dL)
Plasma NEFA (mmol/L)
Fed
Fasted
E
F
Light
Dark
GFP
CRE
Energy Expenditure (kcal/hr)
Body Weight (g)

Fig. S2

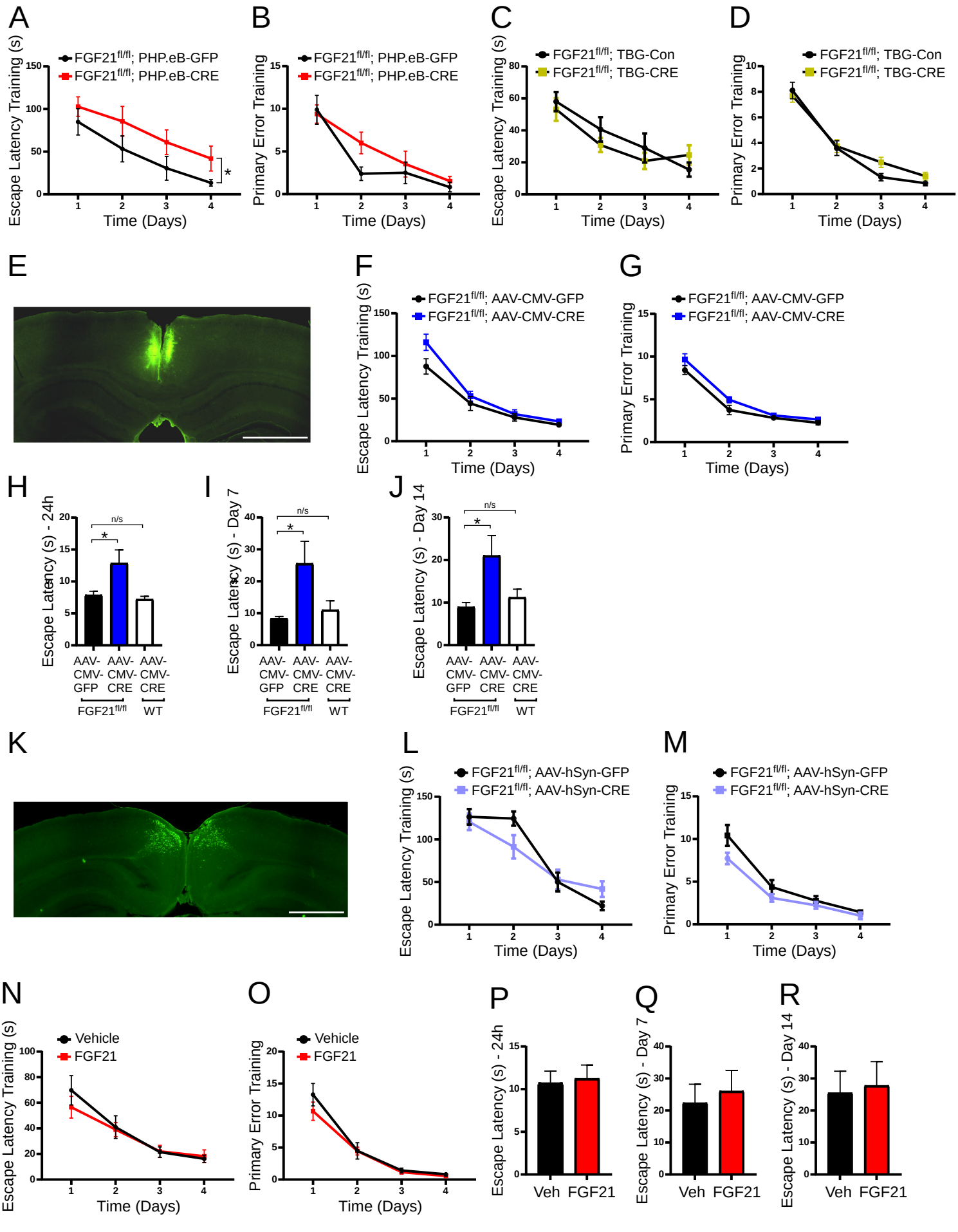
A
Escape Latency Training (s)
FGF21fl/fl; PHP.eB-GFP
FGF21fl/fl; PHP.eB-CRE
Time (Days)
B
Primary Error Training
FGF21fl/fl; PHP.eB-GFP
FGF21fl/fl; PHP.eB-CRE
Time (Days)
C
Escape Latency Training (s)
FGF21fl/fl; TBG-Con
FGF21fl/fl; TBG-CRE
Time (Days)
D
Primary Error Training
FGF21fl/fl; TBG-Con
FGF21fl/fl; TBG-CRE
Time (Days)
E
F
Escape Latency Training (s)
FGF21fl/fl; AAV-CMV-GFP
FGF21fl/fl; AAV-CMV-CRE
Time (Days)
G
Primary Error Training
FGF21fl/fl; AAV-CMV-GFP
FGF21fl/fl; AAV-CMV-CRE
Time (Days)
H
Escape Latency (s) - 24h
n/s
*
AAV-CMV-GFP
AAV-CMV-CRE
AAV-CMV-CRE
FGF21fl/fl
WT
I
Escape Latency (s) - Day 7
n/s
*
AAV-CMV-GFP
AAV-CMV-CRE
AAV-CMV-CRE
FGF21fl/fl
WT
J
Escape Latency (s) - Day 14
n/s
*
AAV-CMV-GFP
AAV-CMV-CRE
AAV-CMV-CRE
FGF21fl/fl
WT
K
L
Escape Latency Training (s)
FGF21fl/fl; AAV-hSyn-GFP
FGF21fl/fl; AAV-hSyn-CRE
Time (Days)
M
Primary Error Training
FGF21fl/fl; AAV-hSyn-GFP
FGF21fl/fl; AAV-hSyn-CRE
Time (Days)
N
Escape Latency Training (s)
Vehicle
FGF21
Time (Days)
O
Primary Error Training
Vehicle
FGF21
Time (Days)
P
Escape Latency (s) - 24h
Veh
FGF21
Q
Escape Latency (s) - Day 7
Veh
FGF21
R
Escape Latency (s) - Day 14
Veh
FGF21

Figure S3

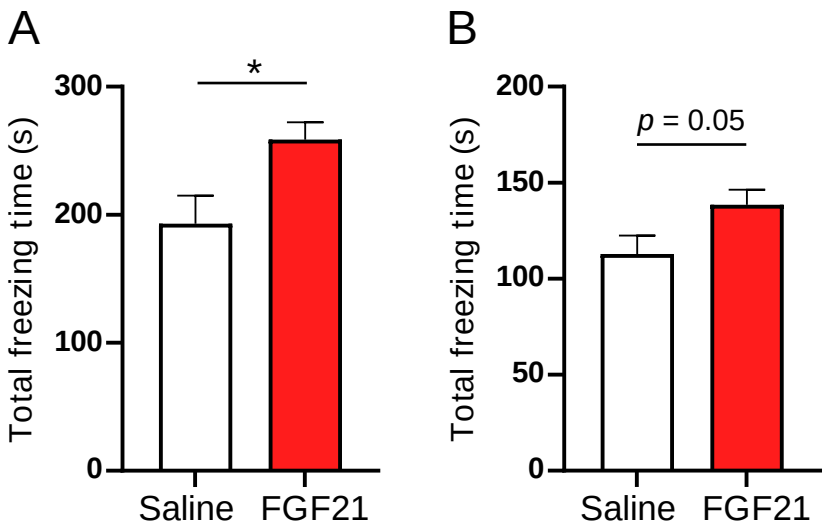
A
B
*
p = 0.05
Total freezing time (s)
300
200
100
0
150
50
Saline
FGF21

Figure S4