SUPPLEMENTAL INFORMATION

Figure S1. HFD pair-feeding does not impact glucose or food intake but deficiency of microglial inflammatory signaling tends to increase gluconeogenesis, related to Figure 2. (A) Blood glucose and (B) food intake in IKKβ-MGKO mice fed *ad libitum* (IKKβ-MGKO AL) vs. pair-fed control (Ctl PF). Measurements were obtained 4 hours into the dark cycle. N = 7/group. **(**C) Pyruvate tolerance test (2 g/kg pyruvate, i.p.) and (D) total glucose AUC at 11 weeks HFD in IKKβ-MGKO mice fed *ad libitum* vs. pair-fed control.

Figure S2. Validation of TNF antibody (abcam, ab1793), related to Figure 4. Representative images of hypothalamic sections showing Iba1 (green) and TNF (red) staining in wild-type (top) and TNF KO (bottom) mice treated with LPS (5 mg/kg) for 6 hours. Scale bars = 20 um.

Figure S3. Pharmacological inhibition of microglia worsens insulin resistance during DIO in rats, related to Figure 5. Male Wistar rats (N = 5/group) were fed HFD for 4 weeks and treated with minocycline (10 μg, i.c.v.) or saline vehicle administered 3 times over 2d. (A) Plasma insulin levels at t = 0 of ITT. (B) ITT (1.5 U/kg insulin, i.p.) performed 1h after the third dose of minocycline or vehicle. (C) Total glucose AOC for the ITT. Values are mean ± SEM. 2-way ANOVA with Bonferroni post-hoc test and Student's *t*-test, * $p < 0.05$, ** $p < 0.01$.

Figure S4. Chemogenetic microglial activation in hM3D mice is associated with increased pro-inflammatory signaling, related to Figure 5, Figure 6, and Figure 7. Cx3cr1$^{CreER/+}$::LSL-HA-hM3Dq-citrine (hM3D) mice were generated as an inducible model of *in vivo* microglial activation. (A) Representative hypothalamic sections immunostained for the microglial marker

(Iba1, green) and hM3D DREADD receptor (anti-HA tag, red) in tamoxifen-treated control and hM3D mice. Scale bars = 100 um. (B) Quantification of hM3D transgene expression by qRT-PCR in CNS and peripheral tissues in hM3D mice normalized to control ($Cx3cr1^{CreER/+}$) levels. N = 3-4/group. Student's *t*-test, ** $p < 0.01$. (C) Fura Red was used to detect changes in intracellular calcium. Isolated hM3D primary microglia were treated with CNO (0.01 μM or 0.1 μM) or saline control over 150 minutes. Fura Red fluorescence of the microglial cells was measured using a plate reader and plotted normalized to 0 minute baseline. Mean traces shown for clarity. (D) 30 minute AUC bins of data in C. N = 6-7 replicates/treatment. Two-way ANOVA with Bonferroni post-hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (E) Representative hypothalamic sections from hM3D mice immunostained for the microglial marker (Iba1, green), hM3D DREADD receptor (anti-HA tag, red), and DAPI nuclear stain in hM3D mice given CNO (1 mg/kg, i.p.) or saline and perfused 1 hour later. Scale bars = 100 um. (F-G) Quantification of $Iba1^{+}$ microglia by (F) total number and (G) cellular size after CNO treatment. N = 6-12/group. Student's *t*-test, **$p<0.01$. (H) Cytokine expression in FACS-isolated microglia from hM3D normalized to control levels in mice treated with CNO (1 mg/kg, i.p.). N = 6-7/genotype. Student's *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All values are mean ± SEM.

Figure S5. Acute CNO-mediated activation of microglial hM3D does not induce sickness behavior, related to Figure 5. (A) Dark cycle ambulatory activity was measured after saline or CNO injection (1 mg/kg, i.p.) in hM3D mice. Pretreatment line represents average of both groups of hM3D mice before injection (indicated by arrow). Dashed lines represent ± SEM. (B) 30 min AUC bins of activity for 240 min after injection. N = 13-19/group. (C) Food intake and

(D) body weight were measured 24 hours after saline or CNO injection (0.3mg/kg or 1mg/kg, i.p.) using a within subjects crossover design. N = 14/group.

Figure S6. Subthreshold peripheral CNO administration does not improve glucose tolerance in hM3D mice, related to Figure 5. (A) GTT (2 g/kg glucose, i.p.) and (B) AUC in CD-fed hM3D and Ctl mice administered systemic CNO at the same dose used for i.c.v. experiments (1 μg, i.p., 2 hours prior). N = 8/group. All values are mean ± SEM.

Figure S7. Chemogenetic activation of microglia in a Tmem119 Cre-based DREADD model improves glucose tolerance, related to Figure 5. (A-D) GTT and AUC of CD-fed Tmem-Gq mice and littermate controls treated with (A-B) saline or (C-D) systemic CNO (10 mg/kg, i.p. 2 hr prior; 2 g/kg glucose, i.p.). N = 8-9/group. Student's *t*-test, *** $p < 0.001$.

Figure S8. Chemogenetic activation of microglia improves glucose tolerance independently of central IL-6, IL-1, or CCR5 signaling and increases POMC neuron glucose sensing, related to Figure 6. (A-F) CD-fed control and hM3D mice were injected with CNO (1 mg/kg, i.p.) and i.c.v. treatment listed below (indicated in figures by arrow labeled i.c.v.) 2 hours prior to all GTTs (2 g/kg glucose, i.p.). (A) GTT and (B) AUCs of mice administered IL-6ab (150 ng, i.c.v.) or vehicle. (C) GTT and (D) AUC of mice administered IL-1RA (100 ng, i.c.v.) or vehicle. (E) GTT and (F) AUC of mice administered CCR5 antagonist maraviroc (CCR5ant) (500 ng, i.c.v.) or vehicle. Two-way ANOVA with Šidák correction for multiple comparisons. (G) Representative images from the ARC of control and hM3D mice 2 hours after CNO (5 mg/kg, i.p.) and 1 hour after saline or glucose (2 g/kg, i.p.) stained for β-endorphin (green) and c-Fos (red).

Scale bars = 50 um. (H) Quantification of c-Fos$^+$ cells as a percent of β-endorphin cells from G, N = 7-8 mice/group, 5 sections/mouse. Two-way ANOVA with post-hoc comparison. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All values are mean ± SEM.

Figure S9. Microglial chemogenetic activation increases first-phase insulin release, which is not prevented by sympathetic agonist administration, related to Figure 7. (A-D) Acute-phase glucose-stimulated insulin secretion in CD-fed control and hM3D mice treated with systemic CNO (5 mg/kg, i.p., 2 hours prior; 2 g/kg glucose, i.p.) at (A-B) 4 min and (C-D) 8 min post-glucose injection, N = 9-11/group. Two-way ANOVA with Šidák correction for multiple comparisons. (E-G) GTT (2 g/kg glucose, i.p.) performed in CD-fed control and hM3D mice 45 min after administration of (E) saline or (F) clonidine (0.01 mg/kg, i.p.) and 20 min after administration of low-dose CNO (0.5 mg/kg, i.p.). (G) AUC quantification of E and F, N = 7-8/group. Two-way ANOVA with Sidak correction for multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All values are mean ± SEM.

Table S1. Genotyping and real-time qPCR primers, related to STAR Methods.

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Real-time qPCR Primers		
Primers for 18S; *Forward:* CGGACAGGATTGACAGATTG *Reverse:* CAAATCGCTCCAACTAA		N/A
Primers for mouse TNFα; *Forward:* CATCTTCTCAAAACTCGAGTGACAA *Reverse:* TGGGAGTAGATAAGGTACAGCCC		N/A
Primers for mouse IL1β; *Forward:* TACAAGGAGAGACAAGCAACGACA *Reverse:* GATCCACACTCTCCAGCTGCA		N/A
Primers for mouse IL6; Forward: GTGGCTAAGGACCAAGACCA Reverse: GGTTTGCCGAGTAGACCTCA		N/A

Primers for mouse MIP1α; *Forward:* CTGCCCTTGCTGTTCTTCTC *Reverse:* GATGAATTGGCGTGGAATCT		N/A
Primers for mouse MIP1β; Forward: CTGCCCTCTCTCTCCTCTTG Reverse: AGGAAGTGGGAGGGTCAGAG		N/A
Primers for mouse MCP1; Forward: CTTCTGGGCCTGCTGTTCA Reverse: CAAGCCTACTCATGGGCTTC		N/A
Primers for mouse Slc2a1; Forward: CCCCGTCCTGCTGCTATTG Reverse: GCACCGTGAAGATGATGAAGAC		N/A
Primers for mouse UCP2; Forward: AAGCAGTTCTACACCAAGGGC Reverse: TCTCGTGCAATGGTCTTGTAG		N/A
Primers for mouse GCK; *Forward:* CAAGCTGCACCCGAGCTT *Reverse:* TGATTCGATGAAGGTGATTTCG		N/A
Primers for mouse G6pc; *Forward:* TCAACCTCGTCTTCAAGTGGATT *Reverse:* CTGCTTTATTATAGGCACGGAGCT		N/A
Primers for mouse Pck1; *Forward:* GGCGGAGCATATGCTGATCC *Reverse:* CCACAGGCACTAGGGAAGGC		N/A
Primers for mouse Gq DREADD; *Forward:* TCTGGCAAGTGGTCTTCATC *Reverse:* TTACCAGGATGTTGCCGATG		N/A
Genotyping Primers		
Primers for Ikbkb floxed allele; *Forward:* CCTTGTCCTATAGAAGCACAAC *Reverse:* GTCATTTCCACAGCCCTGTGA		N/A
Primers for CreER allele; *Common* *Forward:* AAGACTCACGTGGACCTGCT *Mutant* *Reverse:* CGGTTATTCAACTTGCACCA *Wild Type Reverse:* AGGATGTTGACTTCCGAGTTG		N/A
Primers for hM3D line; *Transgene* *Forward:* TCTGGCAAGTGGTCTTCATC *Transgene* *Reverse:* TTACCAGGATGTTGCCGATG *Int. Pos. Ctrl Forward:* CACGTGGGCTCCAGCATT *Int. Pos. Ctrl. Reverse:* TCACCAGTCATTTCTGCCTTTG		N/A

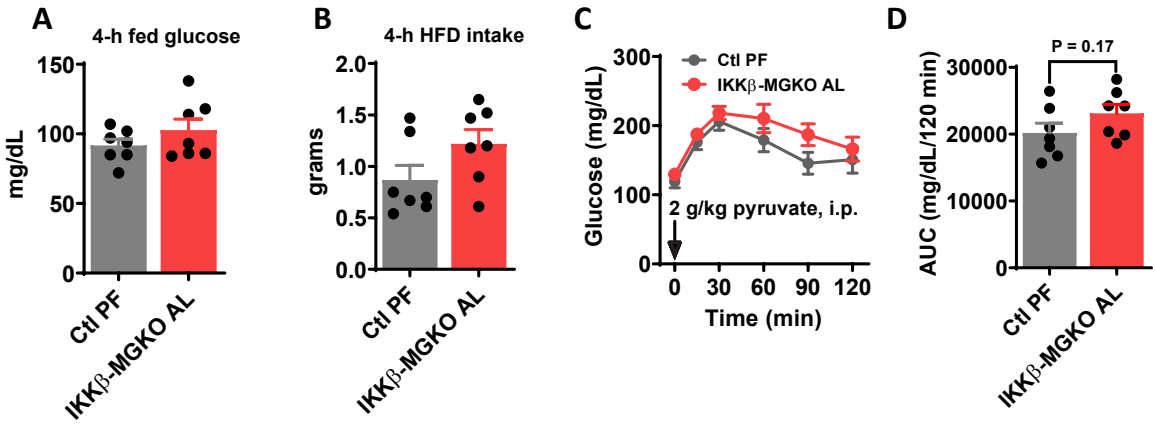
A
4-h fed glucose
mg/dL
150
100
50
0
Ctl PF
IKKβ-MGKO AL
B
4-h HFD intake
grams
2.0
1.5
1.0
0.5
0.0
Ctl PF
IKKβ-MGKO AL
C
Ctl PF
IKKβ-MGKO AL
Glucose (mg/dL)
300
200
100
0
2 g/kg pyruvate, i.p.
0
30
60
90
120
Time (min)
D
P = 0.17
AUC (mg/dL/120 min)
30000
20000
10000
0
Ctl PF
IKKβ-MGKO AL

Figure S1

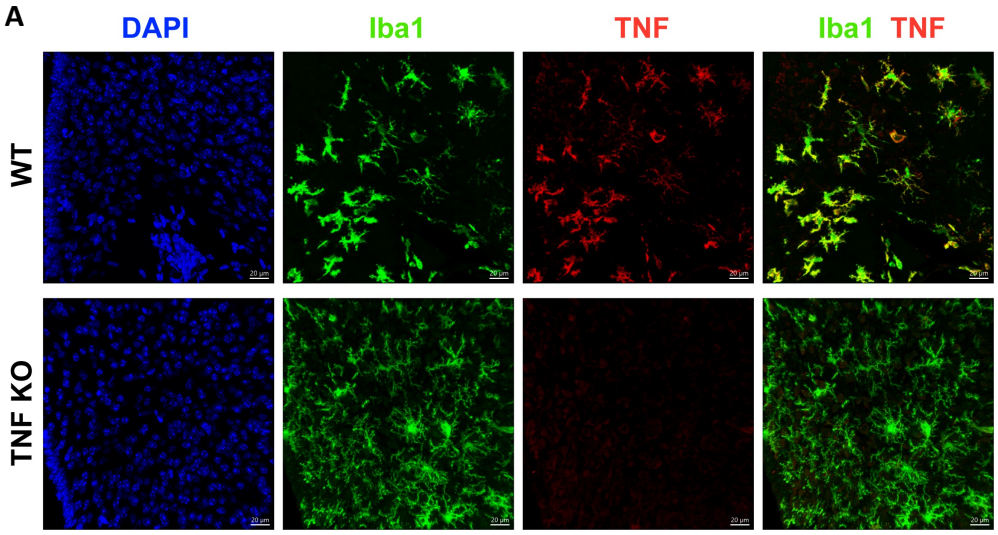

Figure S2

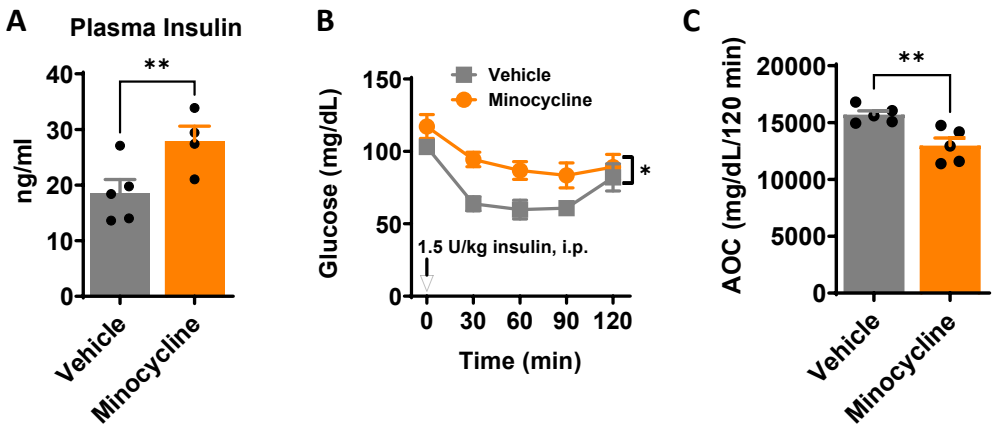
A
Plasma Insulin
ng/ml
40
30
20
10
0
**
Vehicle
Minocycline
B
Glucose (mg/dL)
150
100
50
0
Vehicle
Minocycline
1.5 U/kg insulin, i.p.
0
30
60
90
120
Time (min)
*
C
AOC (mg/dL/120 min)
20000
15000
10000
5000
0
**
Vehicle
Minocycline

Figure S3

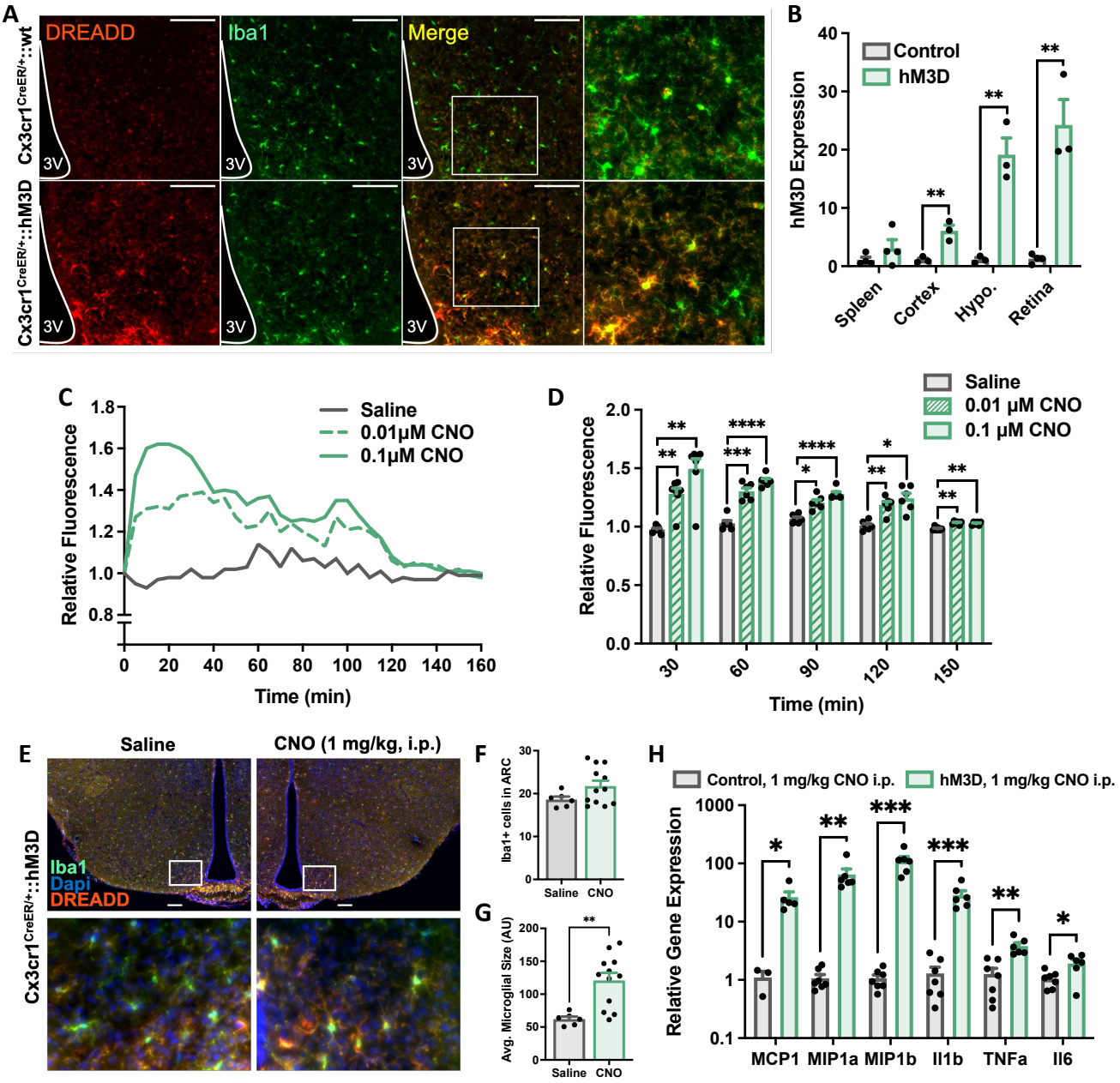
A
DREADD
Iba1
Merge
Cx3cr1CreER/+::wt
Cx3cr1CreER/+::hM3D
3V
B
Control
hM3D
hM3D Expression
Spleen
Cortex
Hypo.
Retina
C
Saline
0.01μM CNO
0.1μM CNO
Relative Fluorescence
Time (min)
D
Saline
0.01 μM CNO
0.1 μM CNO
Relative Fluorescence
Time (min)
E
Saline
CNO (1 mg/kg, i.p.)
Cx3cr1CreER/+::hM3D
Iba1
Dapi
DREADD
F
Iba1+ cells in ARC
Saline
CNO
G
Avg. Microglial Size (AU)
Saline
CNO
H
Control, 1 mg/kg CNO i.p.
hM3D, 1 mg/kg CNO i.p.
Relative Gene Expression
MCP1
MIP1a
MIP1b
Il1b
TNFa
Il6

Figure S4

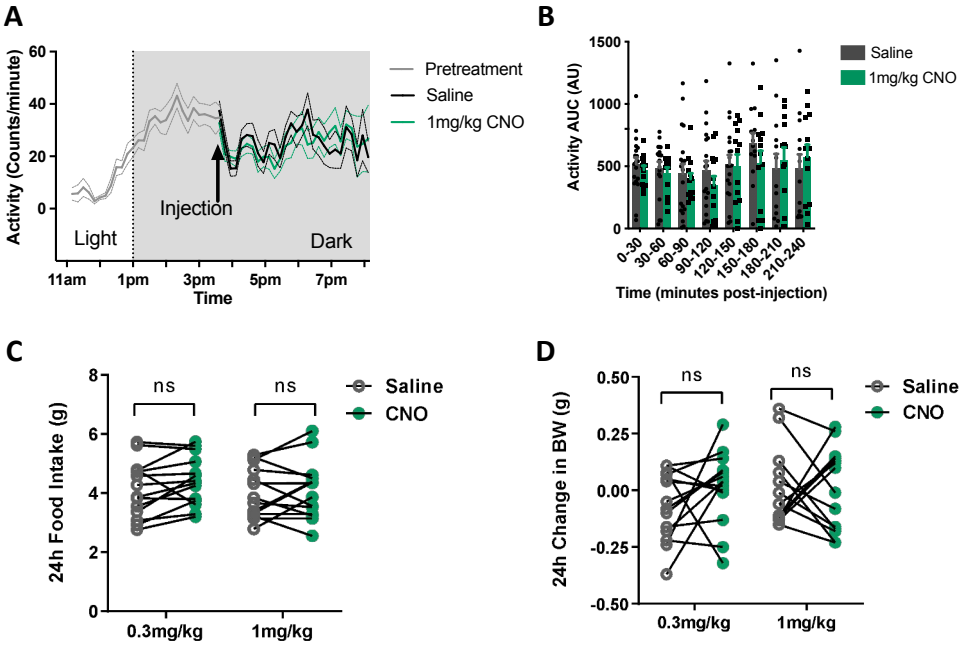
A
Activity (Counts/minute)
60
40
20
0
Light
Injection
Dark
11am
1pm
3pm
5pm
7pm
Time
Pretreatment
Saline
1mg/kg CNO
B
Activity AUC (AU)
1500
1000
500
0
0-30
30-60
60-90
90-120
120-150
150-180
180-210
210-240
Time (minutes post-injection)
Saline
1mg/kg CNO
C
24h Food Intake (g)
8
6
4
2
0
ns
ns
0.3mg/kg
1mg/kg
Saline
CNO
D
24h Change in BW (g)
0.50
0.25
0.00
-0.25
-0.50
ns
ns
0.3mg/kg
1mg/kg
Saline
CNO

Figure S5

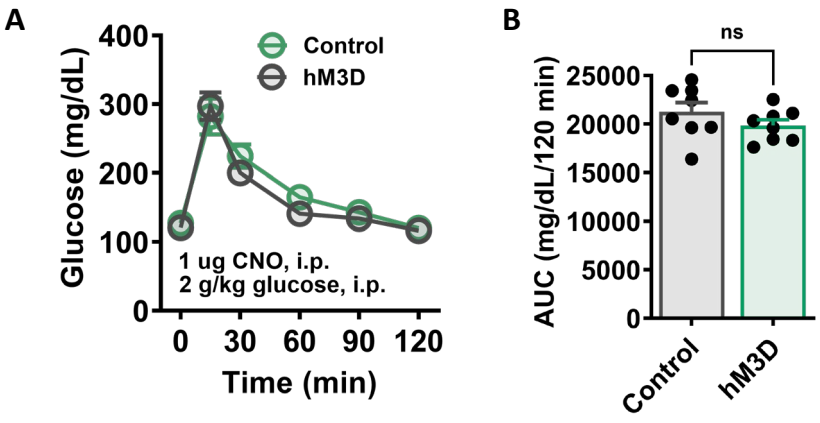
A
400
300
200
100
0
Glucose (mg/dL)
Control
hM3D
1 ug CNO, i.p.
2 g/kg glucose, i.p.
0
30
60
90
120
Time (min)
B
ns
25000
20000
15000
10000
5000
0
AUC (mg/dL/120 min)
Control
hM3D

Figure S6

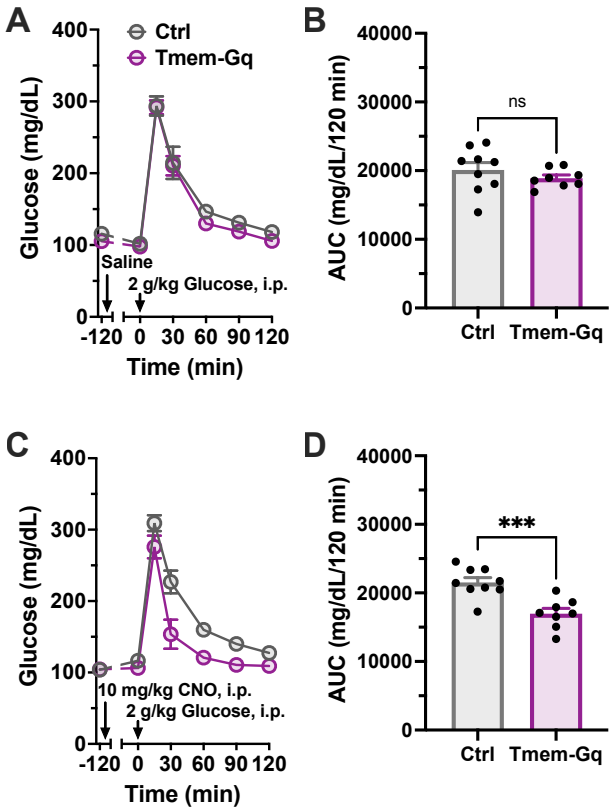
A
Ctrl
Tmem-Gq
Glucose (mg/dL)
400
300
200
100
0
Saline
2 g/kg Glucose, i.p.
-120 0 30 60 90 120
Time (min)
B
AUC (mg/dL/120 min)
40000
30000
20000
10000
0
ns
Ctrl Tmem-Gq
C
Glucose (mg/dL)
400
300
200
100
0
10 mg/kg CNO, i.p.
2 g/kg Glucose, i.p.
-120 0 30 60 90 120
Time (min)
D
AUC (mg/dL/120 min)
40000
30000
20000
10000
0
***
Ctrl Tmem-Gq

Figure S7

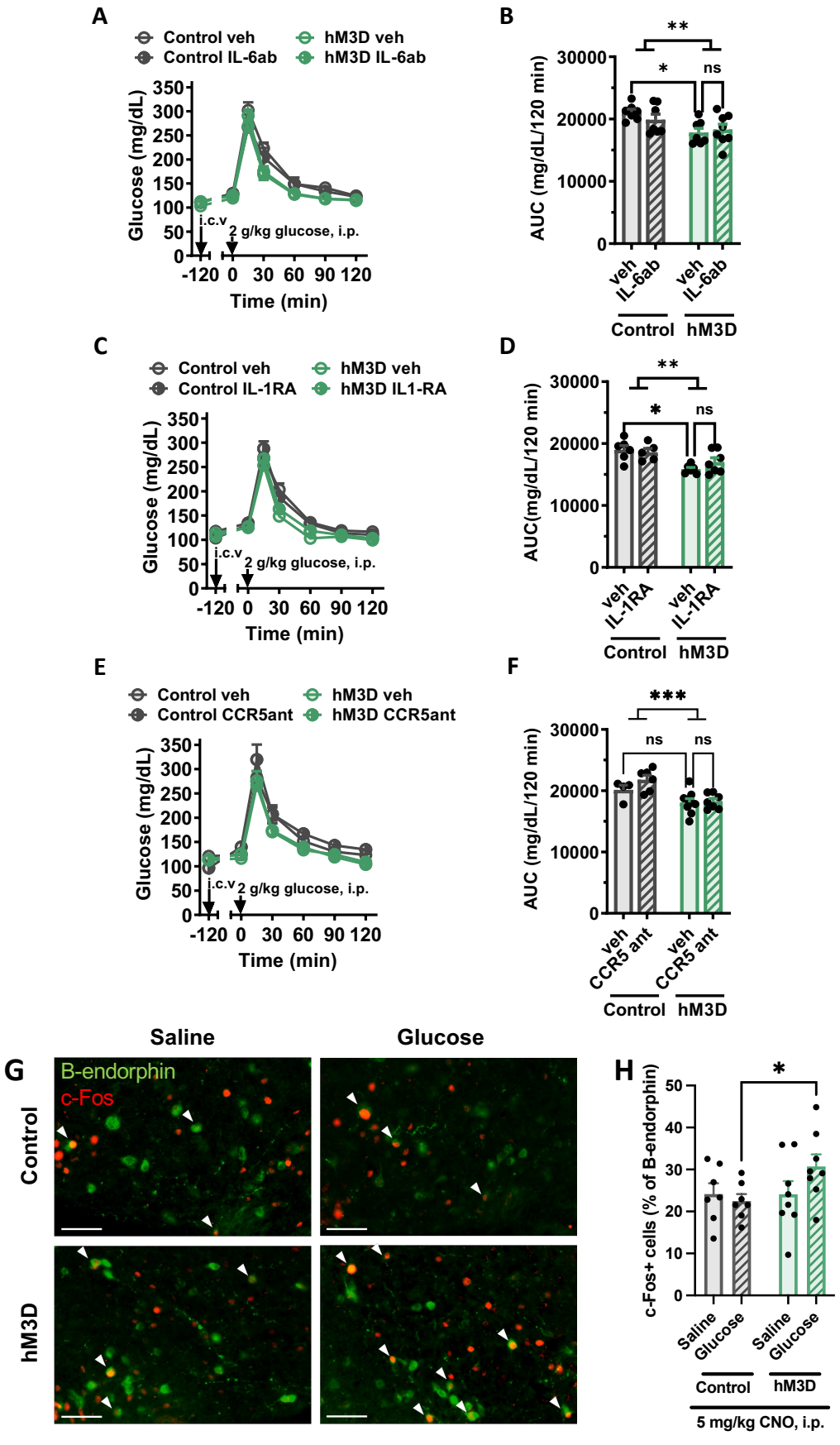
A
Control veh
Control IL-6ab
hM3D veh
hM3D IL-6ab
Glucose (mg/dL)
i.c.v
2 g/kg glucose, i.p.
Time (min)
B
AUC (mg/dL/120 min)
veh
IL-6ab
Control
hM3D
C
Control veh
Control IL-1RA
hM3D veh
hM3D IL1-RA
D
AUC(mg/dL/120 min)
IL-1RA
E
Control veh
Control CCR5ant
hM3D veh
hM3D CCR5ant
F
CCR5 ant
G
Saline
Glucose
B-endorphin
c-Fos
Control
hM3D
H
c-Fos+ cells (% of B-endorphin)
5 mg/kg CNO, i.p.

Figure S8

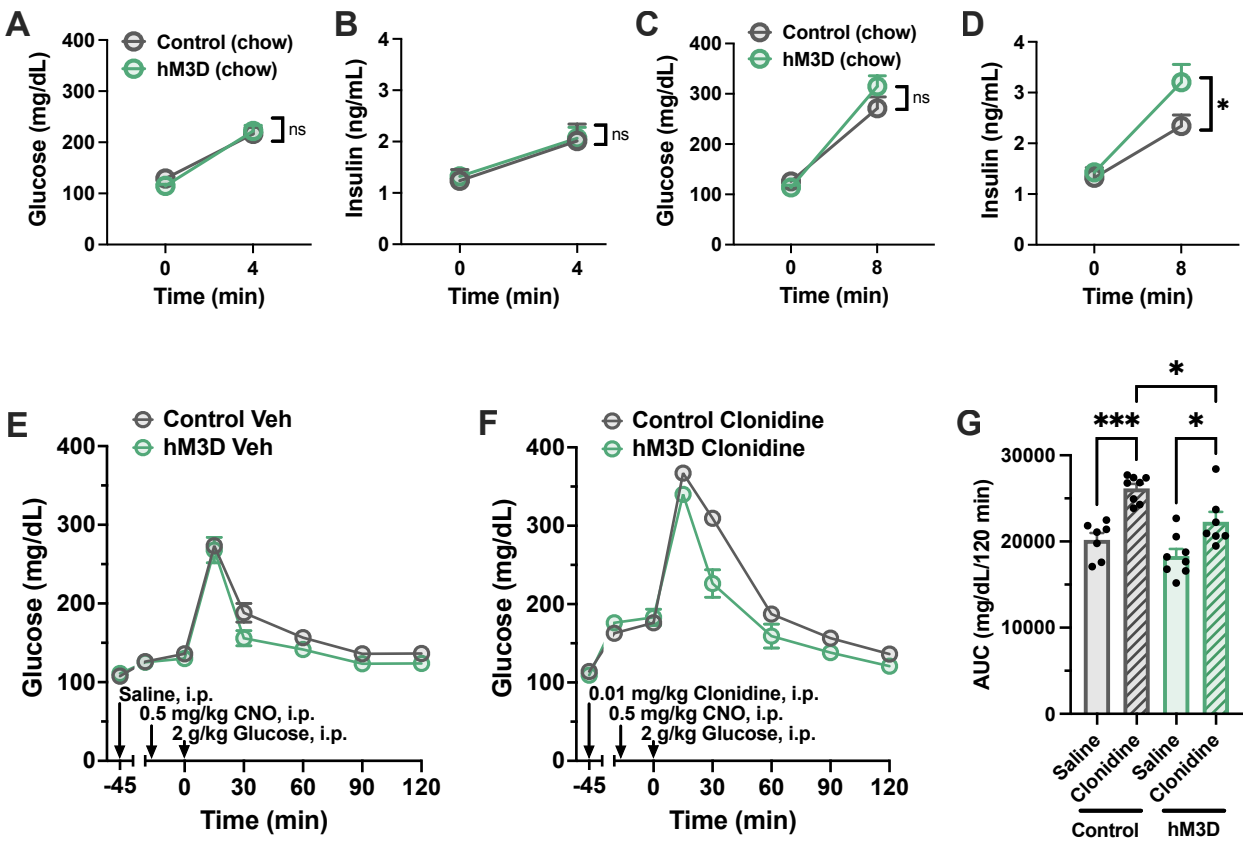
A
Control (chow)
hM3D (chow)
Glucose (mg/dL)
Time (min)
ns
B
Insulin (ng/mL)
Time (min)
ns
C
Control (chow)
hM3D (chow)
Glucose (mg/dL)
Time (min)
ns
D
Insulin (ng/mL)
Time (min)
*
E
Control Veh
hM3D Veh
Glucose (mg/dL)
Saline, i.p.
0.5 mg/kg CNO, i.p.
2 g/kg Glucose, i.p.
Time (min)
F
Control Clonidine
hM3D Clonidine
Glucose (mg/dL)
0.01 mg/kg Clonidine, i.p.
0.5 mg/kg CNO, i.p.
2 g/kg Glucose, i.p.
Time (min)
G
AUC (mg/dL/120 min)
***
*
*
Saline
Clonidine
Saline
Clonidine
Control
hM3D

Figure S9