Figure S1

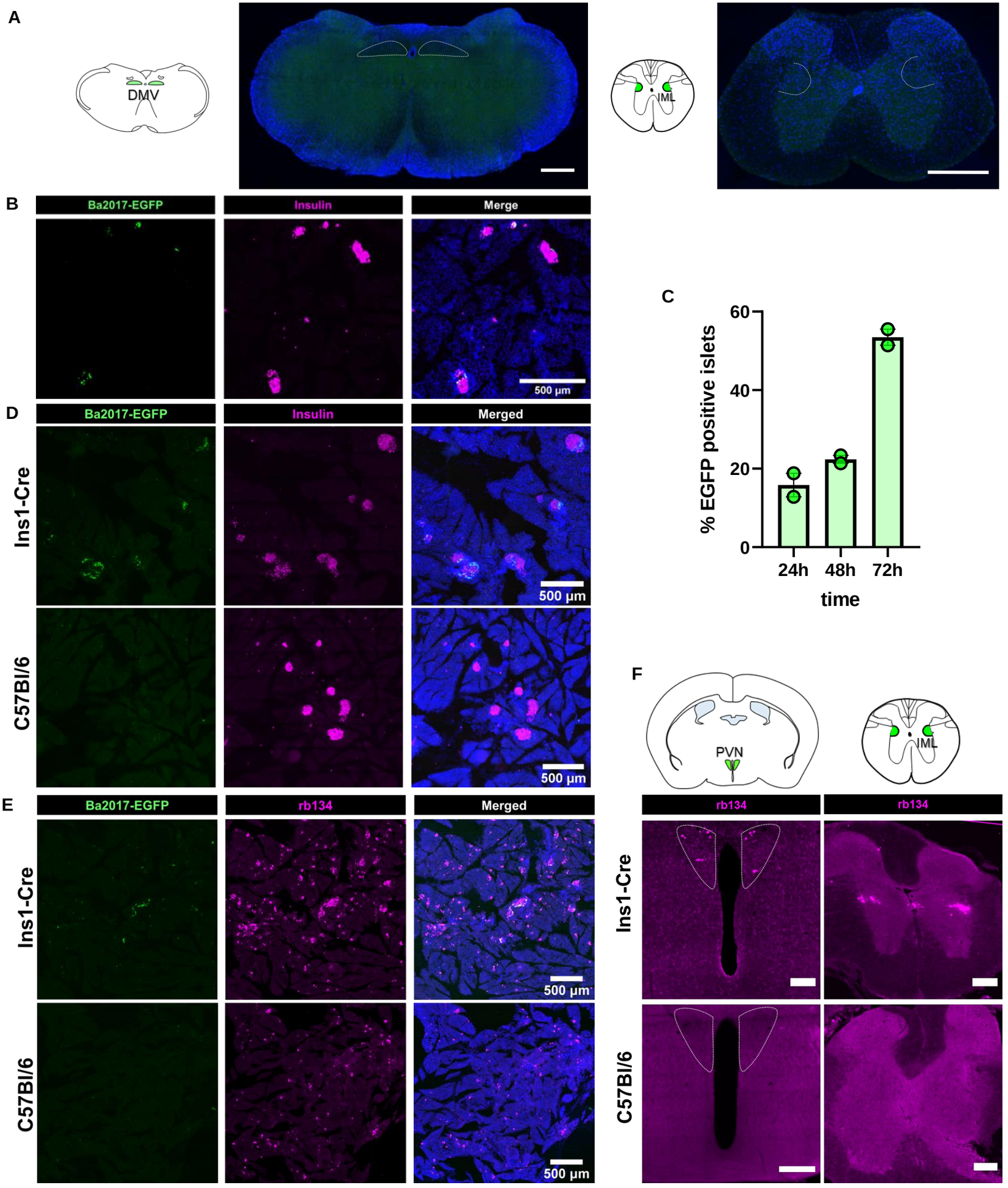
A
DMV
IML
B
Ba2017-EGFP
Insulin
Merge
500 µm
C
% EGFP positive islets
60
40
20
0
24h
48h
72h
time
D
Ba2017-EGFP
Insulin
Merged
Ins1-Cre
C57Bl/6
500 µm
E
Ba2017-EGFP
rb134
Merged
Ins1-Cre
C57Bl/6
500 µm
F
PVN
IML
rb134
rb134
Ins1-Cre
C57Bl/6

Figure S1. Related to Figures 1 and 2. Ba2017-EGFP localization 48-72 hours after injection.

(A) Representative images of brainstem (left; including the DMV-dorsal motor nucleus of the vagus) and spinal cord (right; including the IML-intermediolateral nucleus) from *Ins1-Cre* mice 48 hours after viral injection (*n* = 2, 5 images per mouse). Ba2017-EGFP fluorescence, green. (Scale bar = 500μm) Dashed areas represent the regions of interest in green identified in the brain section micrographs next to each image.

(B) Representative images of pancreas sections from *Ins1-Cre* mice 48 hours after viral injection (*n* = 2, 4 images per mouse). Insulin antibody staining, magenta; Ba2017-EGFP fluorescence, green. (Scale bar = 500μm)

(C) Quantification of % of EGFP positive islets 24, 48 and 72 hours after Ba2017 injection (*n* = 2 per time point).

(D) Representative images of pancreatic sections with $EGFP^+$ (green) and insulin (magenta) labeling from *Ins1*-Cre (top) and C57Bl6 (bottom) mice 72 hours after Ba2017 injection (*n* = 2 mice per condition, 4 images per mouse). (Scale bar = 500μm)

(E) Representative images of pancreatic sections with $EGFP^+$ (green) and rb134 (magenta) labeling from *Ins1*-Cre (top) and C57Bl6 (bottom) mice 72 hours after Ba2017 injection (*n* = 2 mice per condition, 4 images per mouse). (Scale bar = 500μm)

(F) Representative images of PVN and spinal cord sections with rb134 (magenta) labeling from *Ins1*-Cre (top) and C57Bl6 (bottom) mice 72 hours after Ba2017 injection (*n* = 2 mice per condition, 4 images per mouse). (Scale bar = 200μm) Dashed areas represent the regions of interest in green identified in the brain section micrographs above each image.

All sections were counterstained with nuclear marker DAPI (blue).

Figure S2

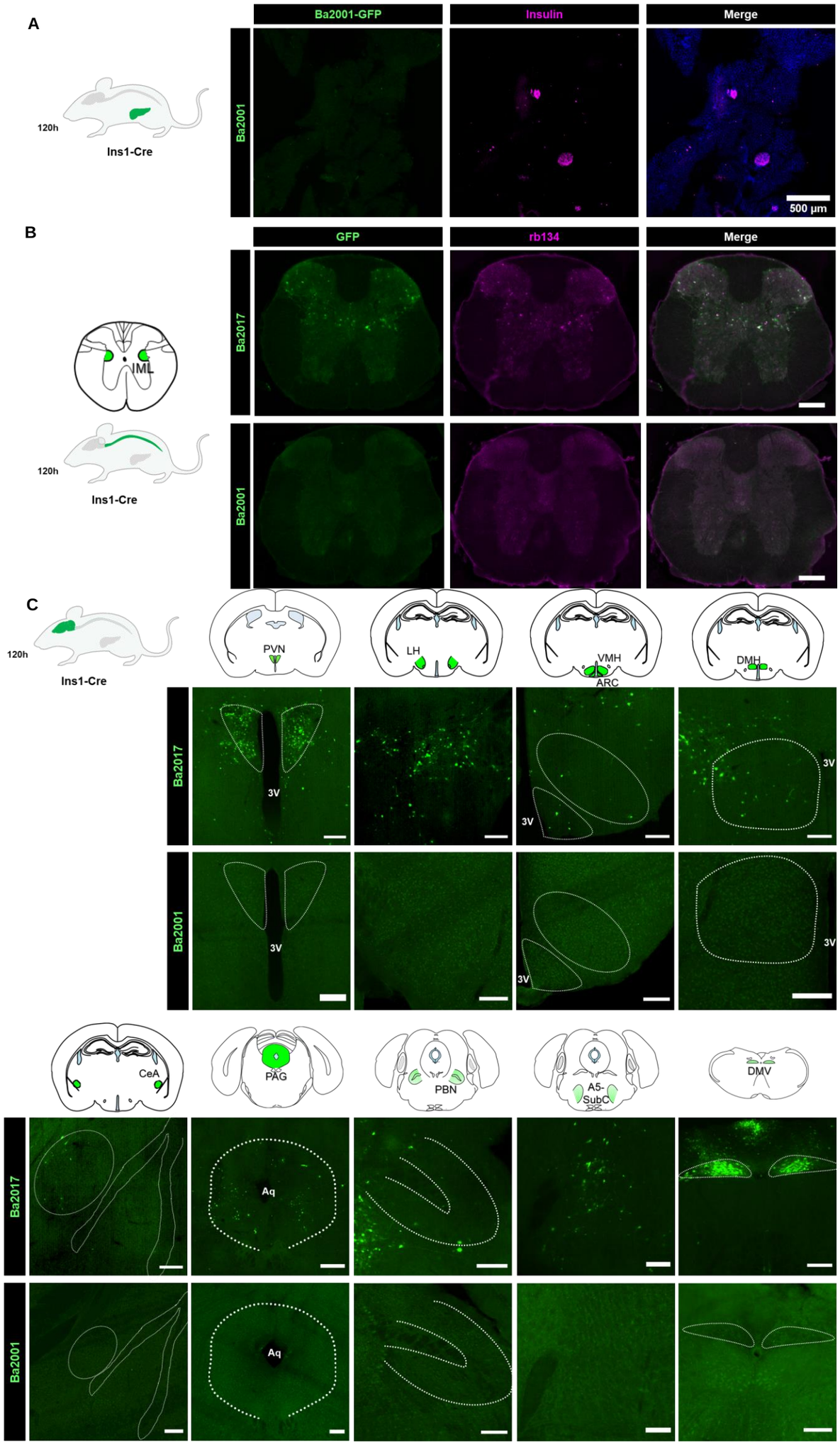
A
Ba2001-GFP
Insulin
Merge
120h
Ins1-Cre
Ba2001
500 μm
B
GFP
rb134
Merge
IML
120h
Ins1-Cre
Ba2017
Ba2001
C
120h
Ins1-Cre
PVN
LH
VMH
ARC
DMH
Ba2017
Ba2001
3V
CeA
PAG
PBN
A5-
SubC
DMV
Aq

Figure S2. Related to Figure 2. Comparison of Ba2017 and Ba2001 neuronal tracing.

(A) Representative images of pancreatic sections with GFP^+ (green) and insulin (magenta) immunostaining from *Ins1*-Cre mice 120h after injection with Ba2001 (*n* = 2 mice per condition, 4 images per mouse). (Scale bar = 500μm).

(B) Representative images of IML with GFP^+ (green) and rb134 (magenta) immunostaining from *Ins1*-Cre mice 120h after injection with Ba2017 (top; *n* = 4, 4 images per mouse) or Ba2001 (bottom; *n* = 3, 4 images per mouse). (Scale bar = 200μm).

(C) Representative images of $EGFP^+$ (Ba2017) fluorescent labeling or GFP^+ (Ba2001) immunostaining in various brain regions 120 hours after Ba2017 (top; *n* = 4, 4-8 images per region per mouse) or Ba2001 (bottom; *n* = 3, 4-8 images per region per mouse) administration. (Scale bar = 200μm). Dashed areas represent the regions of interest in green identified in the brain section micrographs above each image.

IML=intermediolateral nucleus of the spinal cord, PVN=Paraventricular nucleus of the hypothalamus, LH= Lateral hypothalamus, VMH=Ventromedial hypothalamic nucleus, DMH= Dorsomedial hypothalamic nucleus, CeA= Central nucleus of the amygdala, PAG= Periaqueductal grey, PBN= Parabrachial nucleus, SubC= Subcoereleus nucleus, DMV= Dorsal motor nucleus of the vagus, Aq= Aqueduct, 3V= 3rd Ventricle.

Figure S3

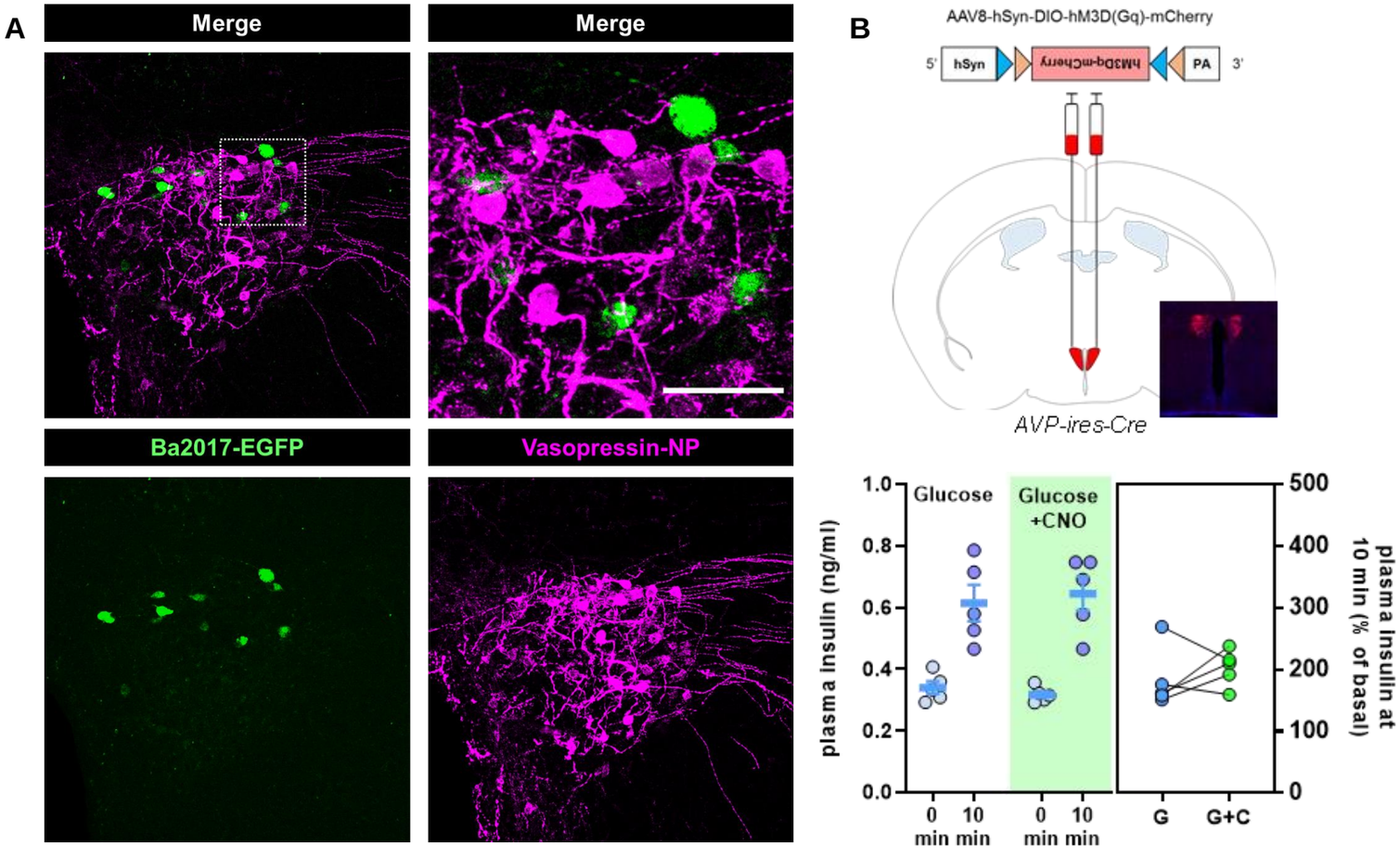

Figure S3. Related to Figure 3. PVN Vasopressin co-labeling with EGFP 72 hours after injection and chemogenetic stimulation.

(A) Representative image of β-cell-projecting PVN neurons expressing EGFP (green) and vasopressin (magenta) 72 hours after Ba2017 administration (*n* = 5, 4 images per mouse). Dashed square on upper left panel represents the area of the magnified image on the upper right panel. (Scale bar = 50μm).

(B) (Top) Schematic representation of DREADD virus injection site with representative image of viral mCherry expression in PVN^{AVP} neurons . (Bottom) Effect of PVN^{AVP} stimulation with CNO (0.5mg/kg) during GSIS on10-minute plasma insulin levels in $PVN^{AVP:hM3Dq}$ mice (*n* = 5).

Figure S4

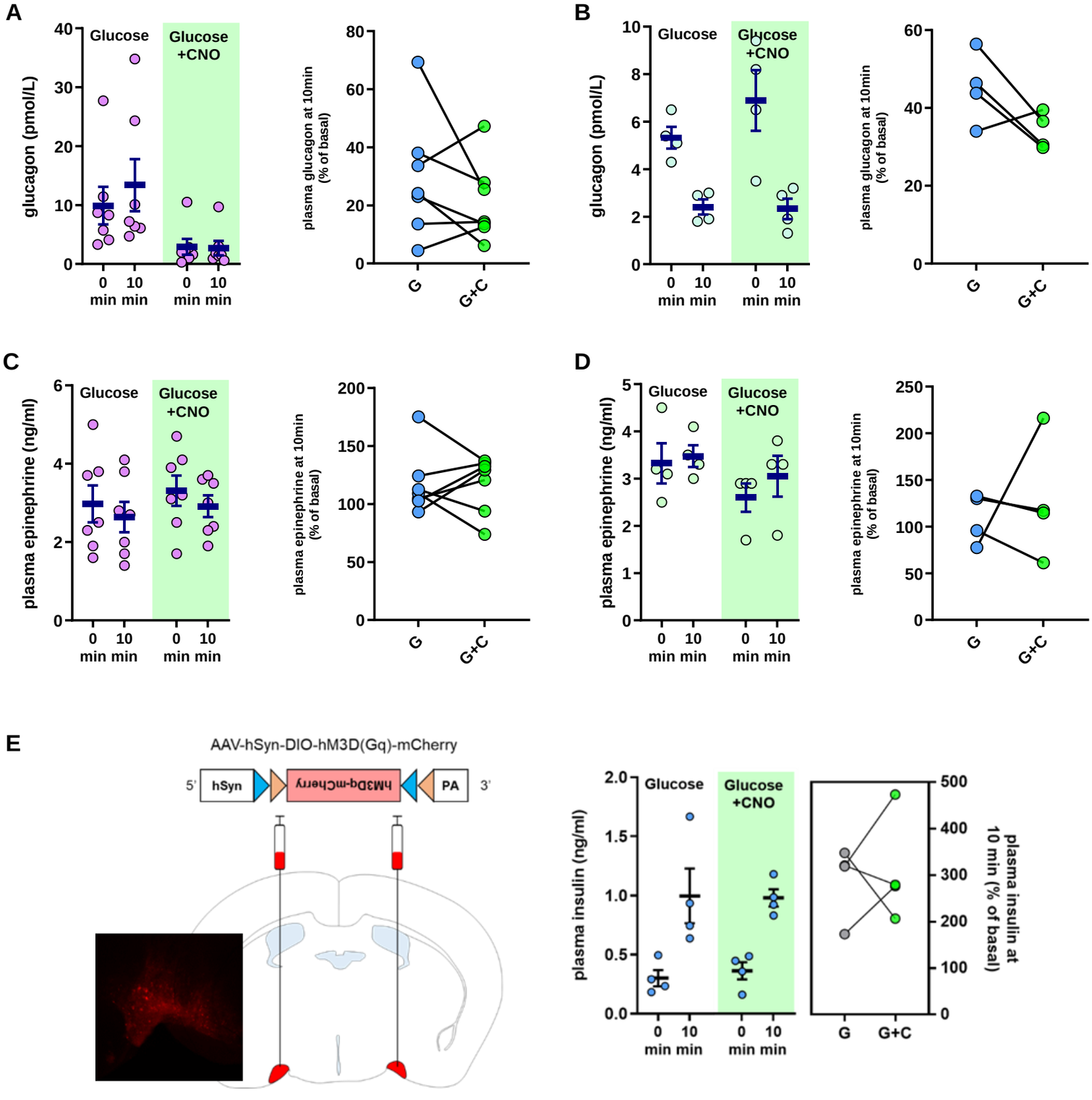
A
Glucose
Glucose +CNO
glucagon (pmol/L)
0 10 min min 0 10 min min
plasma glucagon at 10min (% of basal)
G
G+C
B
Glucose
Glucose +CNO
glucagon (pmol/L)
0 10 min min 0 10 min min
plasma glucagon at 10min (% of basal)
G
G+C
C
Glucose
Glucose +CNO
plasma epinephrine (ng/ml)
0 10 min min 0 10 min min
plasma epinephrine at 10min (% of basal)
G
G+C
D
Glucose
Glucose +CNO
plasma epinephrine (ng/ml)
0 10 min min 0 10 min min
plasma epinephrine at 10min (% of basal)
G
G+C
E
AAV-hSyn-DIO-hM3D(Gq)-mCherry
5'
hSyn
hM3Dq-mCherry
PA
3'
Glucose
Glucose +CNO
plasma insulin (ng/ml)
0 10 min min 0 10 min min
plasma insulin at 10 min (% of basal)
G
G+C

Figure S4. Related to Figure 3. Effect of PVN^{OXT} neuron stimulation on glucagon and epinephrine levels and effect of SON^{OXT} chemogenetic stimulation on insulin levels.

(A-B) Effect of chemogenetic stimulation of PVN^{OXT} neurons with CNO during GSIS on 10-minute plasma glucagon levels in mice injected with (A) hm3Dq (n = 7) or (B) control (n = 4) virus (mCherry). Graphs show mean ± sem.

(C-D) Effect of chemogenetic stimulation of PVN^{OXT} neurons with CNO during GSIS on 10-minute plasma epinephrine levels in mice injected with (C) hm3Dq (n = 7) or (D) control (n = 4) virus (mCherry). Graphs show mean ± sem.

(E) (Left) Schematic representation of DREADD virus injection site with representative image of viral mCherry expression and (Right) SON^{OXT} chemogenetic stimulation with CNO during GSIS on10-minute plasma insulin levels (n = 4, 4 images per mouse). Graphs show mean ± sem.

Figure S5

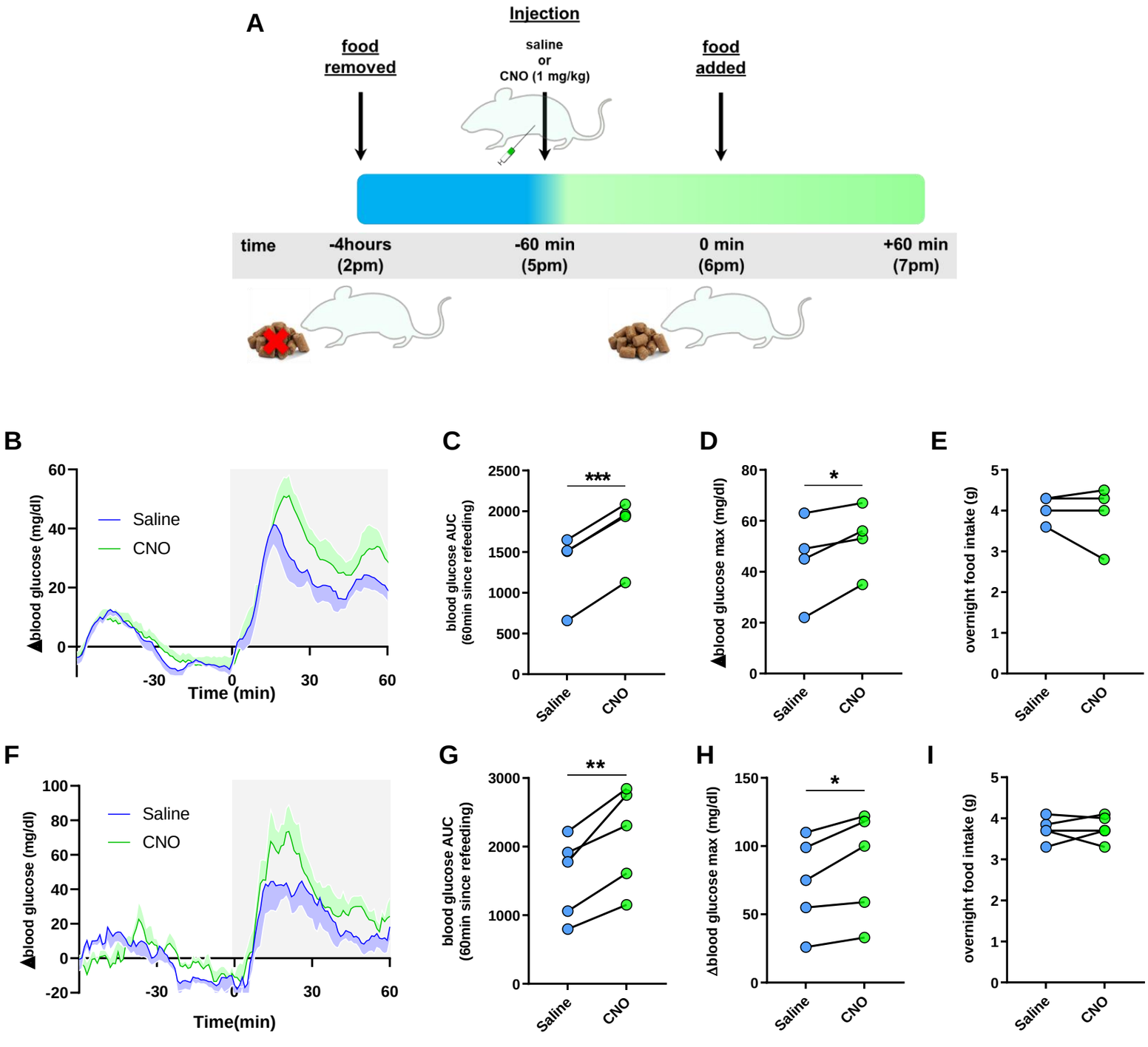
A
Injection
food removed
saline or CNO (1 mg/kg)
food added
time
-4hours (2pm)
-60 min (5pm)
0 min (6pm)
+60 min (7pm)
B
Δblood glucose (mg/dl)
Saline
CNO
Time (min)
C
blood glucose AUC (60min since refeeding)
***
D
Δblood glucose max (mg/dl)
*
E
overnight food intake (g)
F
Δblood glucose (mg/dl)
Saline
CNO
Time(min)
G
blood glucose AUC (60min since refeeding)
**
H
Δblood glucose max (mg/dl)
*
I
overnight food intake (g)

Figure S5. Related to Figures 3 and 4. Effect of PVN^{OXT} neuron stimulation on prandial glycemia.

(A) Schematic representation of the feeding protocol followed for testing prandial glucose levels. Food was removed from cages at 2pm. Mice were injected with either saline or CNO (1mg/kg) at 5pm and food was reintroduced at 6pm. Values were normalized with the average of the one-hour period before food reintroduction.

(B-E) Experiments with $PVN^{OXT:hM3Dq}$ mice.

(B) Normalized glycemic changes after injection of saline or CNO (1mg/kg) and during 1h post feeding. Glucose measurements inside the shaded area (1h post feeding, grey) were used for quantification of AUC and peak glycemia (blue and green shaded areas represent respective error bars). Baseline glucose (average glucose levels from injection to food placement; time -60 to 0): 85.77 ± 5.150 and 85.27 ± 9.189 respectively (n = 4). (C) Effect of CNO administration on prandial glycemic AUC, and (D) peak glycemia levels during the first 1h of feeding (n = 4) *P < 0.05, ***P < 0.001; paired t-test. (E) Effects of chemogenetic stimulation of PVN^{OXT} neurons on overnight food intake (n = 4).

(F-I) Experiments with $spPVN^{OXT:hM3Dq}$ mice.

(F) Normalized glycemic changes after injection of saline or CNO (1mg/kg) and during 1h post feeding. Glucose measurements inside the shaded area (1h post feeding, grey) were used for quantification of AUC and peak glycemia (blue and green shaded areas represent respective error bars) Baseline glucose (average glucose levels from injection to food placement; time -60 to 0): 121.3 ± 7.596 and 127.2 ± 6.563 respectively (n = 5). (G) Effect of CNO administration on prandial glycemic AUC, and (H) peak glycemia levels during the first 1h of feeding (n = 5) *P < 0.05, **P < 0.01; paired t-test. (I) Effects of chemogenetic stimulation of $spPVN^{OXT}$ neurons on overnight food intake (n = 5).