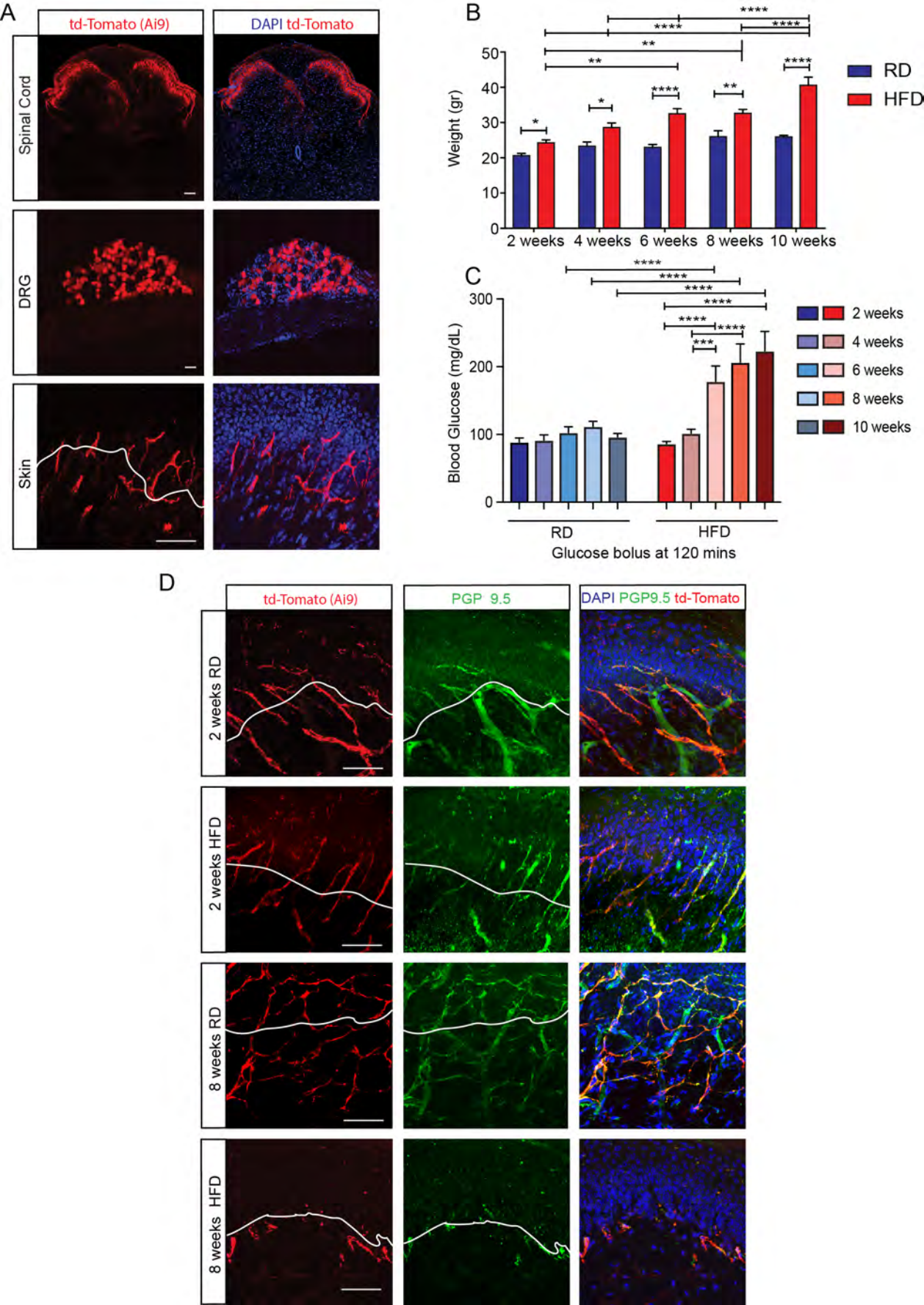
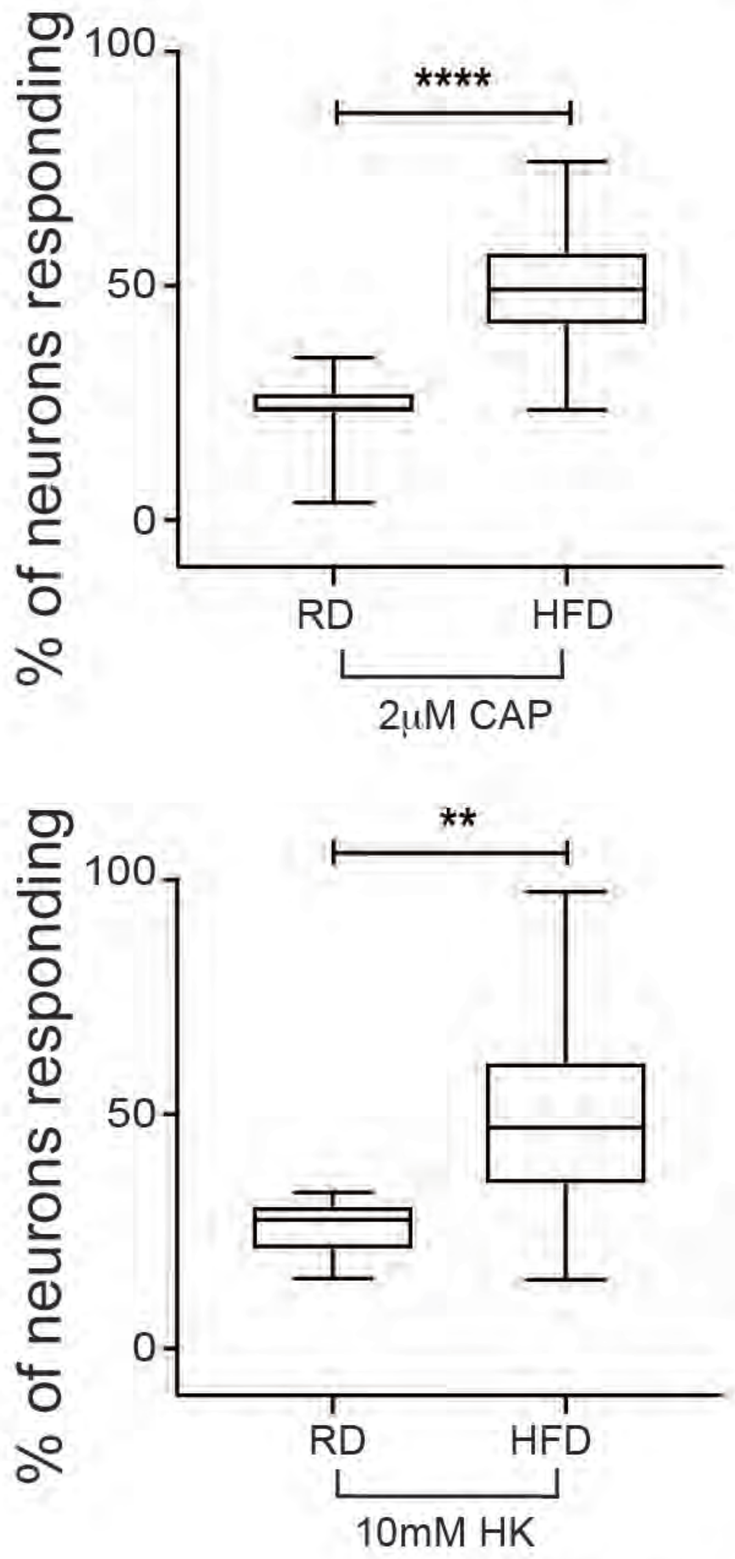
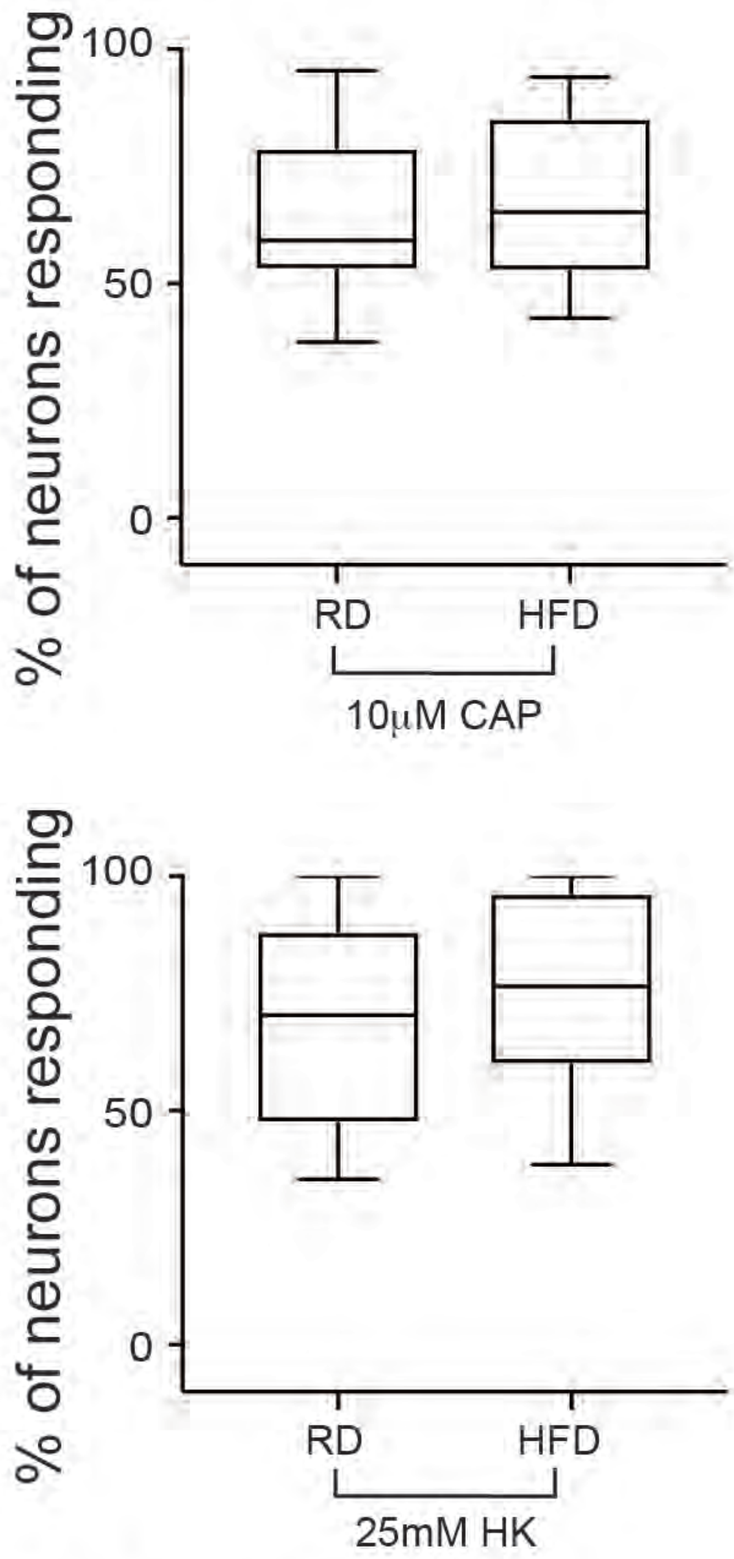

A
td-Tomato (Ai9)
DAPI td-Tomato
Spinal Cord
DRG
Skin
B
Weight (gr)
RD
HFD
2 weeks
4 weeks
6 weeks
8 weeks
10 weeks
C
Blood Glucose (mg/dL)
2 weeks
4 weeks
6 weeks
8 weeks
10 weeks
RD
HFD
Glucose bolus at 120 mins
D
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato
2 weeks RD
2 weeks HFD
8 weeks RD
8 weeks HFD

Supplemental Figure 1. Validation of the Na_v1.8-Cre system. (**A**) Confocal micrographs of spinal cord, DRG and skin taken from Na_v1.8-Cre;Ai9 mice showing Na_v1.8-positive neurons in the DRG and Na_v1.8-afferents in spinal cord and skin labeled with td-Tomato (**red**). Sections were co-labeled with a nuclear marker DAPI (**blue**). Magnification 10x (**top**), 20x (**middle**), 60x (**bottom**) (scale bar=50μm). (**B**) Weights of Na_v1.8-Cre;Ai9 mice in grams (gr) fed either RD (**blue**) or HFD (**red**) over a 10 week period (*, $p<0.05$, **, $p<0.01$, ****, $p<0.0001$.) (n= 8/group). (**C**) Blood glucose levels for both RD and HFD at various lengths of time on each diet, blood glucose levels were taken 120 minutes after injection of glucose (45% D-glucose solution (2mg glucose/1g animal body weight)) (***, $p<0.001$, ****, $P<0.0001$) (n=8/group). p-values were calculated using two-way ANOVA , Bonferroni multiple comparison test. (**D**) Confocal micrographs of skin taken from Na_v1.8-Cre;Ai9 mice that had been on either RD or HFD for 2 or 8 weeks showing td-Tomato (**red**), PGP 9.5 (**green**), and DAPI a nuclear marker (**blue**). At 8 weeks HFD mice showed a reduced number of nerve fibers crossing the epidermal-dermal junction (outlined in white). Magnification 60x (scale bar=50μm). Values are expressed as mean ± S.E.M.

A

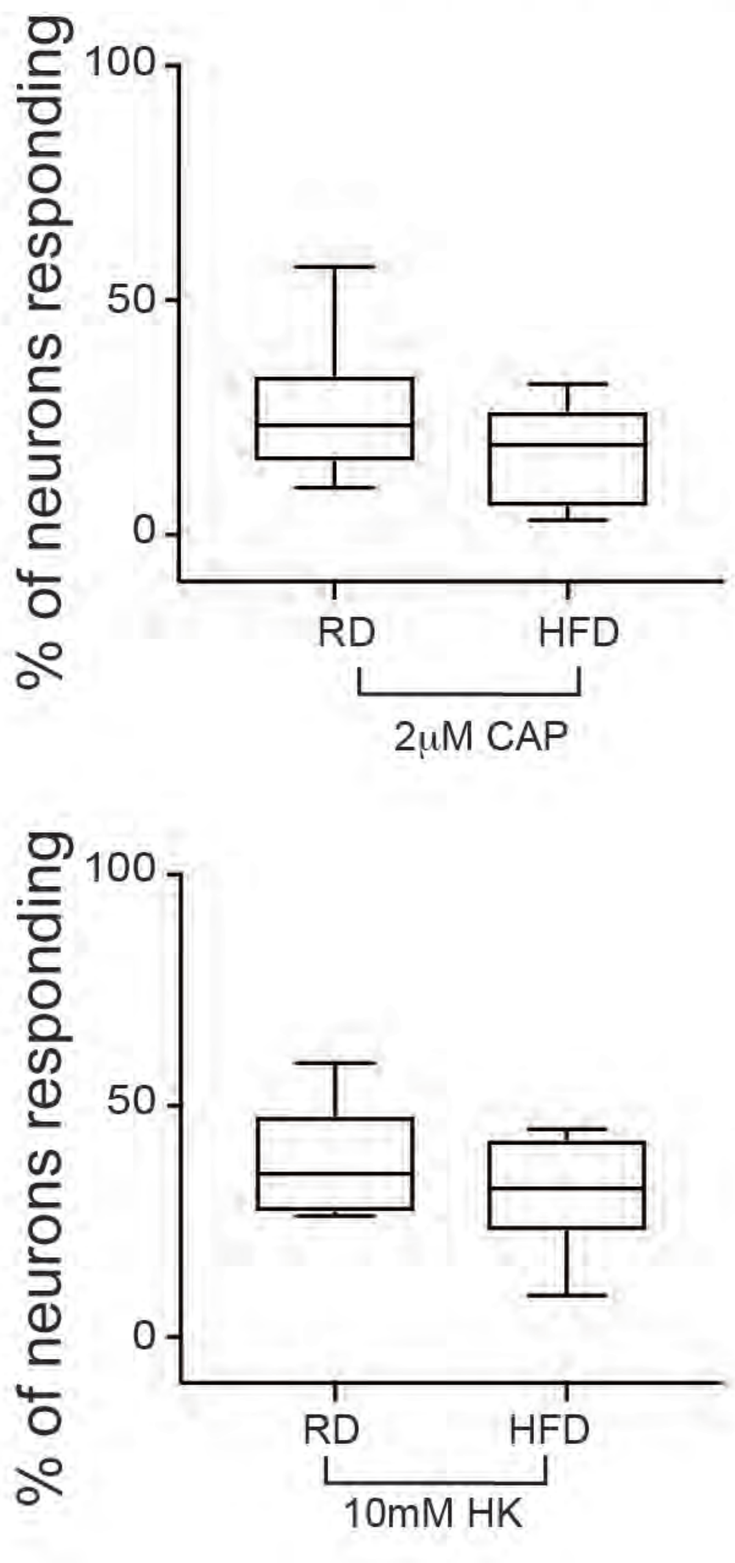
Pirt-GCaMP3 mice 6-12 weeks

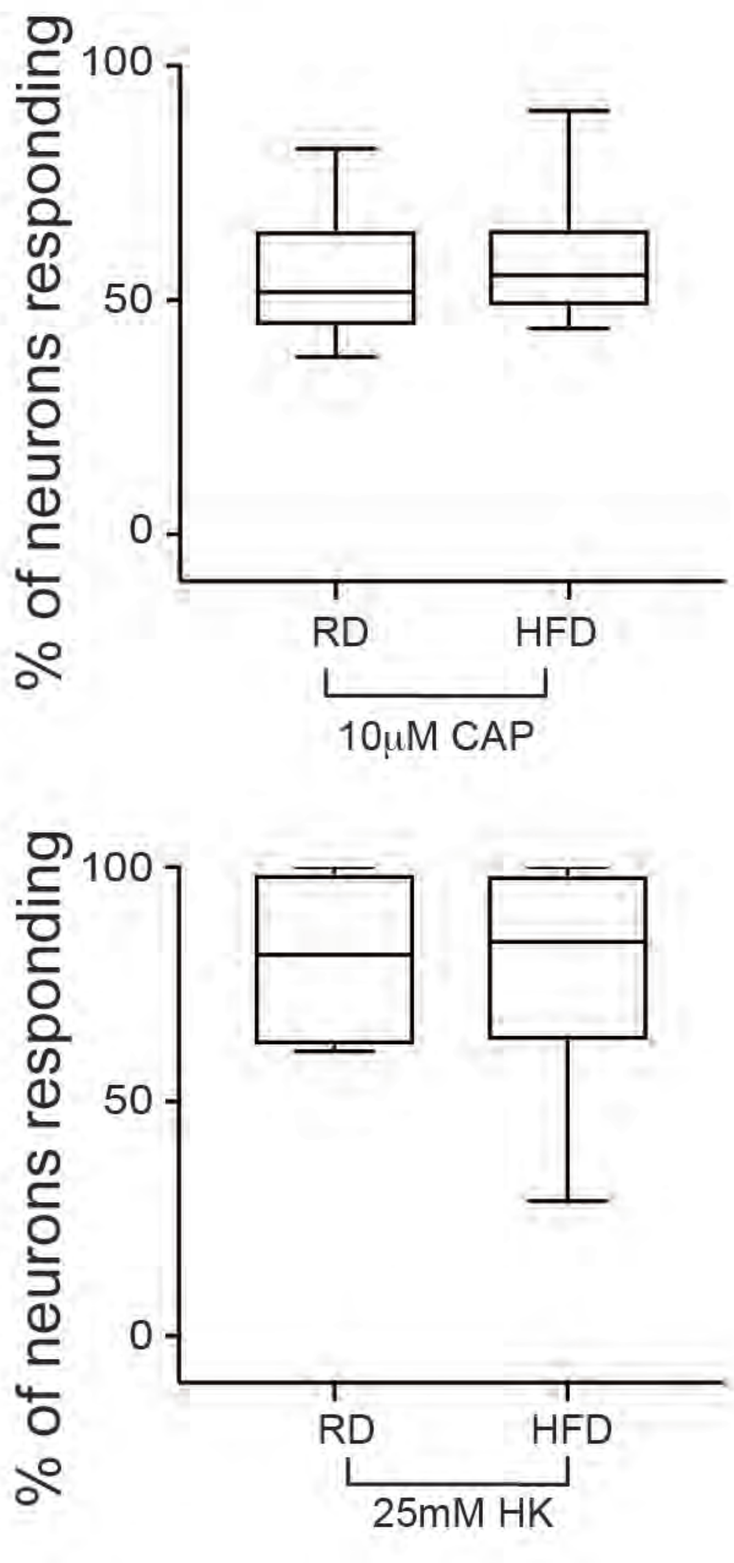




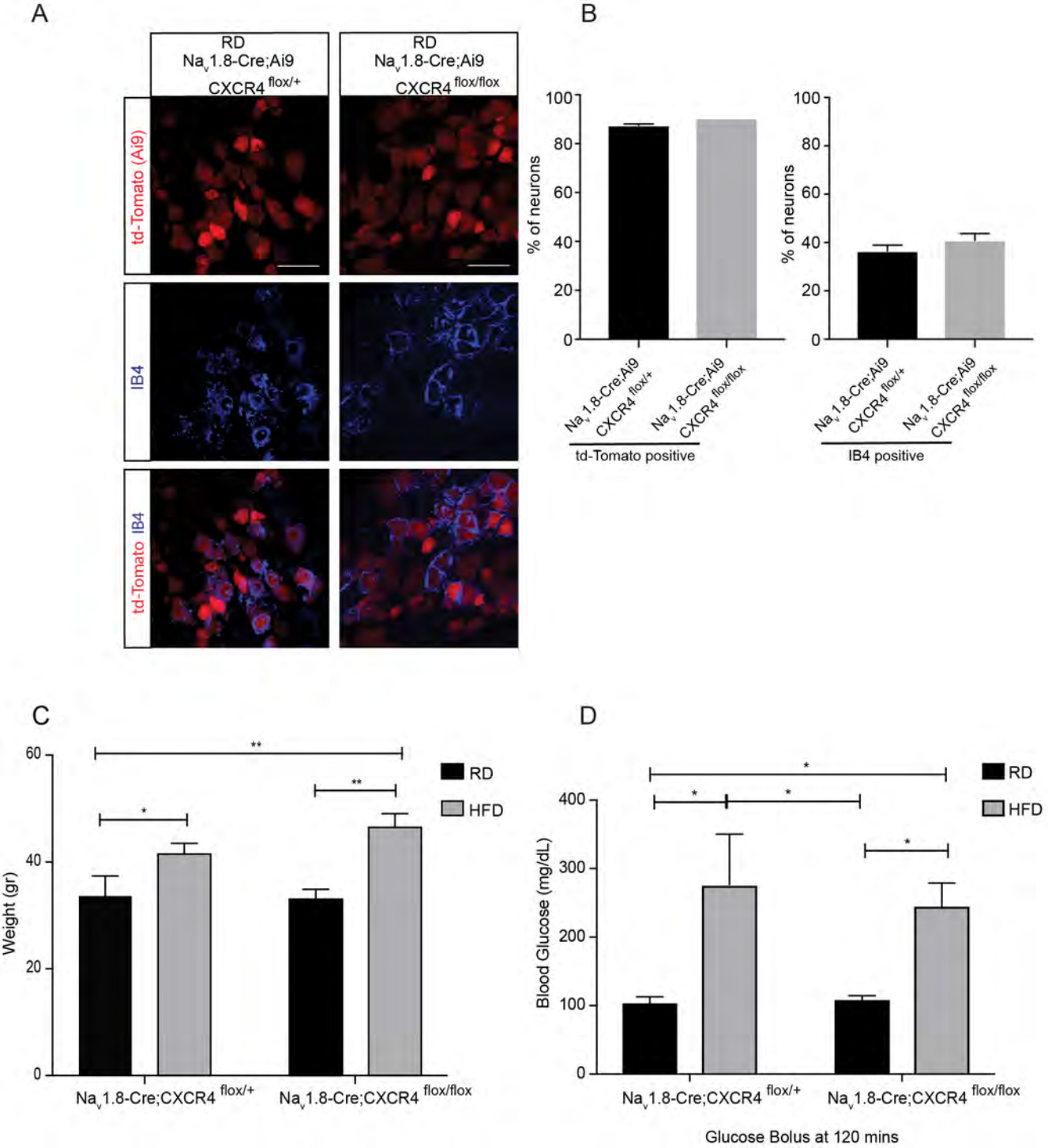
B

Pirt-GCaMP3 mice 2-4 weeks

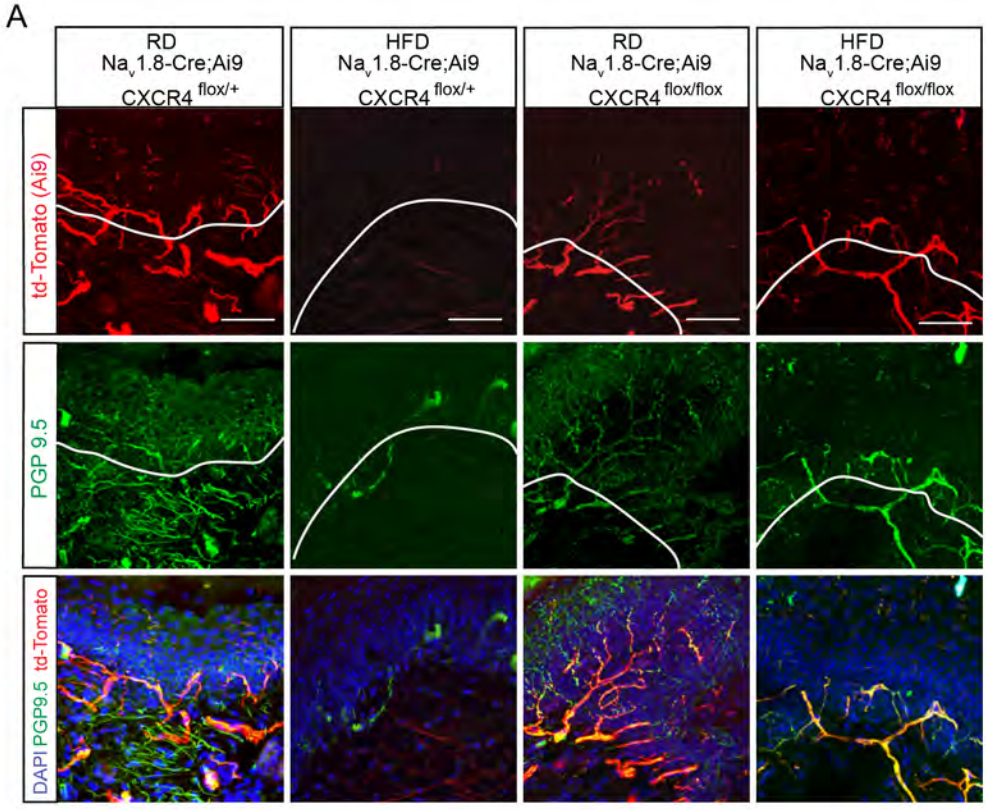


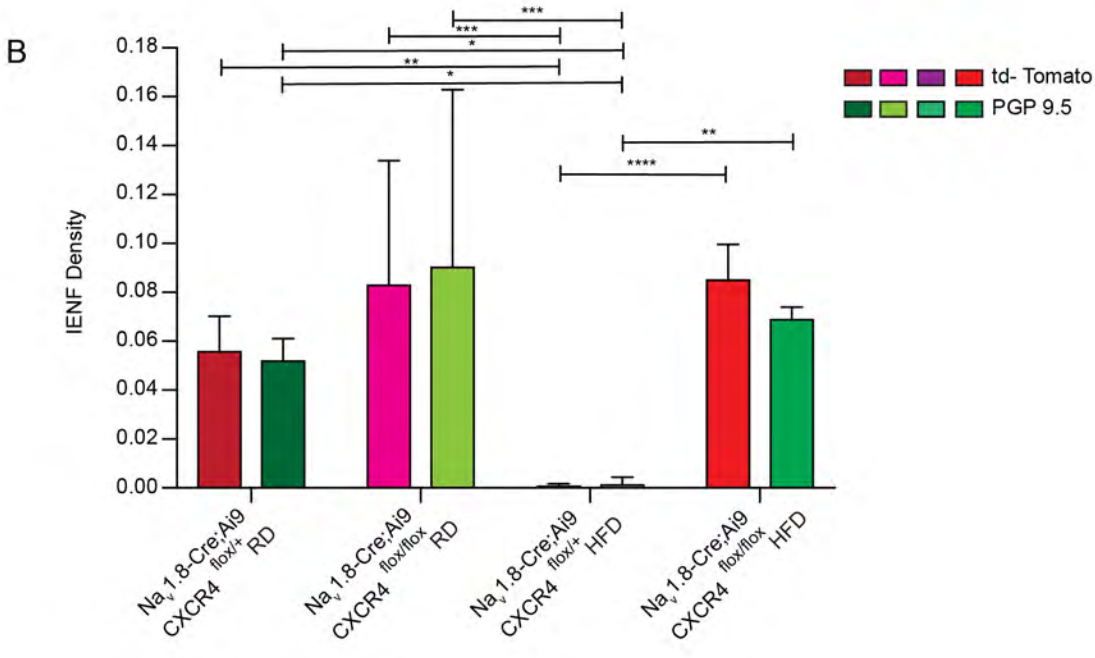


Supplemental Figure 2. Onset of increased $[Ca^{2+}]i$ responses in diabetic DRG explants after 6 weeks on HFD. (**A, B**) $[Ca^{2+}]i$ responses of acutely excised DRGs from Pirt-GCaMP3 mice to 2μM or 10μM capsaicin and 10mM, or 25mM high potassium buffer (HK). Data is shown as capsaicin or HK responsive DRG neurons as a percentage of total neurons that responded to 50mM HK. (**A**) Explants from HFD mice that had been on diet for 6-12 weeks showed increased responses to 2μM capsaicin compared to RD mice. There were also increased responses of HFD explants to 10mM HK compared to RD (**, $p<0.01$, ****, $p<0.0001$). At higher concentrations of capsaicin and HK there was no significant difference between RD and HFD mice (RD n=594 neurons n=18 explants; HFD n=844 neurons n=30 explants). (**B**) When these same experiments were done on explants from mice that had only been on RD or HFD for 2-4 weeks there was no significant difference in $[Ca^{2+}]_i$ responses to capsaicin or HK (RD n=347 neurons n=16 explants; HFD n=504 neurons n=20 explants). This showed that the increased $[Ca^{2+}]i$ responses of Pirt-GCaMP3 explants are evident after 6 weeks on HFD. Values are expressed as mean ± S.E.M. p-values were calculated by Mann-Whitney test.


A
RD
Na$_v$1.8-Cre;Ai9
CXCR4 flox/+
RD
Na$_v$1.8-Cre;Ai9
CXCR4 flox/flox
td-Tomato (Ai9)
IB4
td-Tomato IB4
B
% of neurons
100
80
60
40
20
0
Na$_v$1.8-Cre;Ai9 CXCR4 flox/+
Na$_v$1.8-Cre;Ai9 CXCR4 flox/flox
td-Tomato positive
IB4 positive
C
Weight (gr)
60
40
20
0
RD
HFD
*
**
**
Na$_v$1.8-Cre;CXCR4 flox/+
Na$_v$1.8-Cre;CXCR4 flox/flox
D
Blood Glucose (mg/dL)
400
300
200
100
0
RD
HFD
*
*
*
*
Na$_v$1.8-Cre;CXCR4 flox/+
Na$_v$1.8-Cre;CXCR4 flox/flox
Glucose Bolus at 120 mins

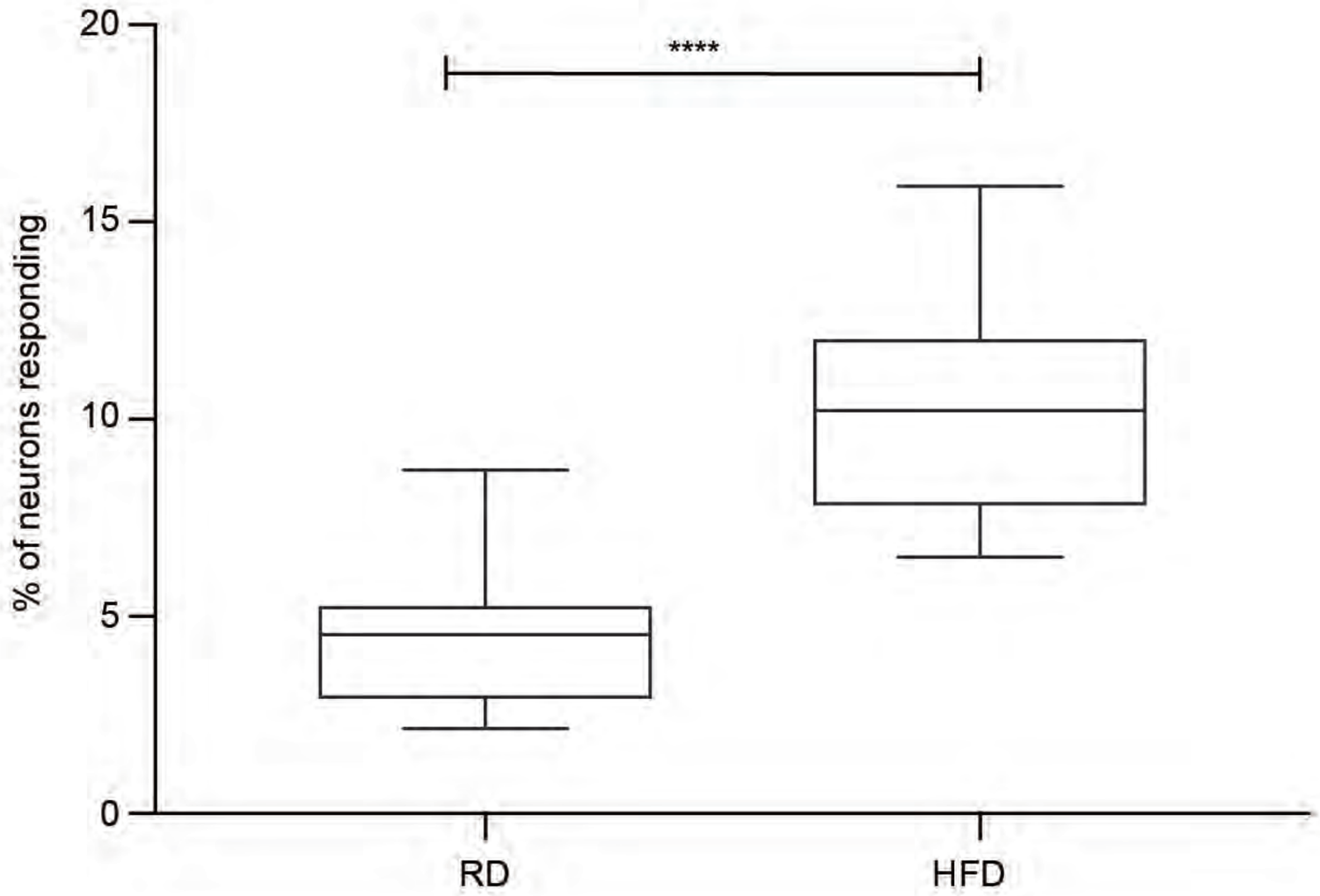
Supplemental Figure 3. Selective deletion of CXCR4 receptors from $Na_v1.8$-positve DRG neurons did not alter the DRG neuronal phenotype or metabolic profile of mice fed HFD. (**A**) Confocal micrographs from DRGs from mice with either heterozygous deletion of CXCR4 ($Na_v1.8$-Cre;Ai9;$CXCR4^{flox/+}$) or homozygous deletion ($Na_v1.8$-Cre;Ai9;$CXCR4^{flox/flox}$), showing td-Tomato (**red**) labeling $Na_v1.8$-positive DRG neurons some of which are also labeled with a marker for non-peptidergic DRG neurons, IB4 (**blue**). Magnification 60x (scale bar=50μm). (**B**) The numbers of td-Tomato positive and IB4 positive neurons were quantified and there were no significant differences between mice with heterozygous (td-Tomato 85.5$\pm$0.5, IB4 36.4$\pm$2.5) and homozygous (td-Tomato 87.3$\pm$2.8, IB4 35.8$\pm$2.9) selective CXCR4 deletions (n=177, 154 neurons respectively). p-values were calculated by Mann-Whitney test. (**C**) Weights of mice in grams (gr) with either heterozygous deletion of CXCR4 ($Na_v1.8$-Cre;Ai9;$CXCR4^{flox/+}$) or homozygous deletion ($Na_v1.8$-Cre;Ai9;$CXCR4^{flox/flox}$) of CXCR4 from $Na_v1.8$-positive neurons(*, $p<0.05$, **, $p<0.01$) (n=6/group). (**D**) Blood glucose levels of the same mice 120 minutes after injection of glucose (45% D-glucose solution (2mg glucose/1gr animal body weight)) (*, $p<0.05$) (n=6/group). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using one-way ANOVA, Bonferroni multiple comparison test.


A
RD
Nav1.8-Cre;Ai9
CXCR4 flox/+
HFD
Nav1.8-Cre;Ai9
CXCR4 flox/+
RD
Nav1.8-Cre;Ai9
CXCR4 flox/flox
HFD
Nav1.8-Cre;Ai9
CXCR4 flox/flox
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato

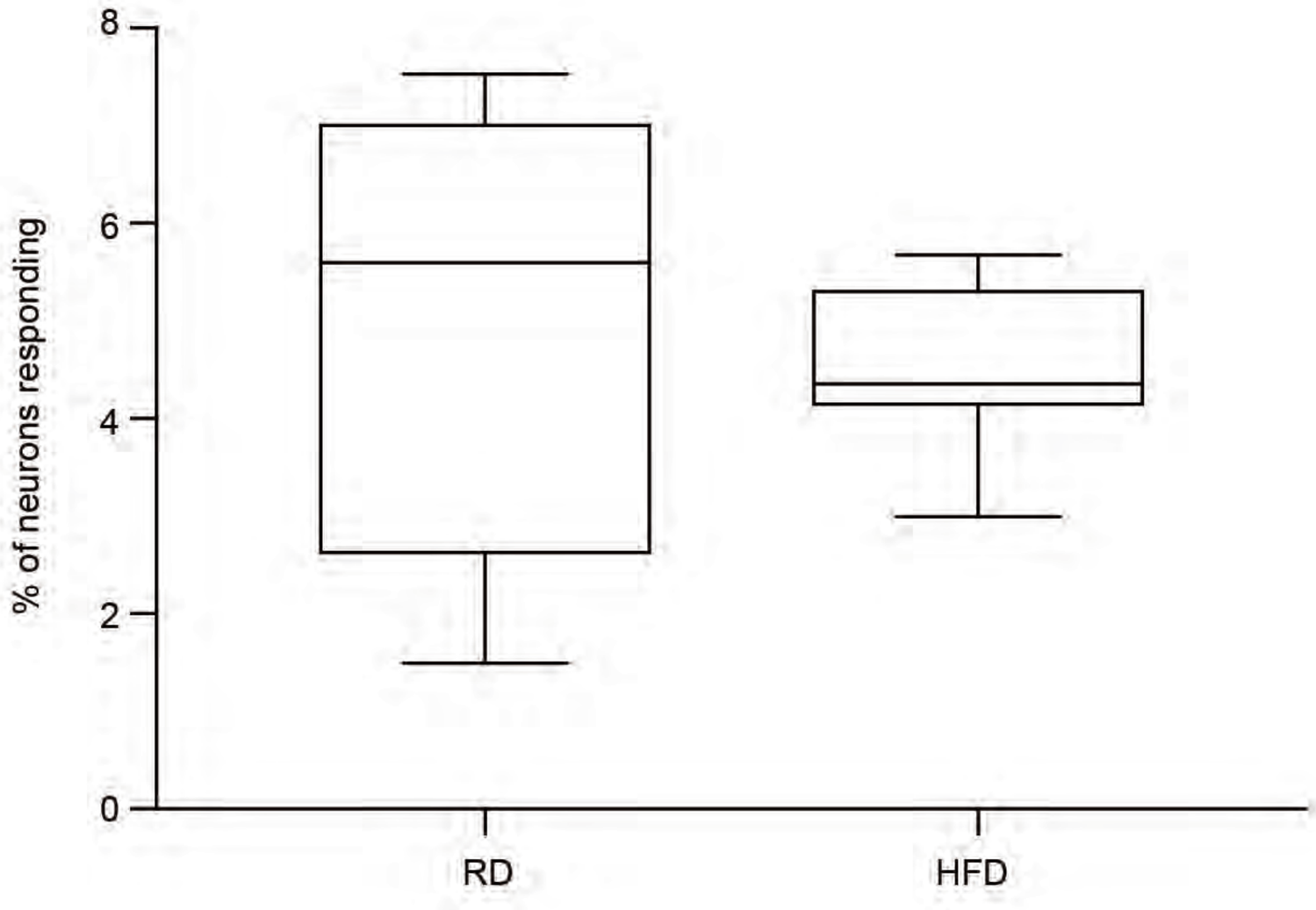

B
IENF Density
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
***
***
*
**
*
**
****
td- Tomato
PGP 9.5
Nav1.8-Cre;Ai9 CXCR4 flox/+ RD
Nav1.8-Cre;Ai9 CXCR4 flox/flox RD
Nav1.8-Cre;Ai9 CXCR4 flox/+ HFD
Nav1.8-Cre;Ai9 CXCR4 flox/flox HFD

Supplemental Figure 4. Selective chemokine receptor CXCR4 deletion from $Na_v1.8$-positive DRG neurons prevented the development of small-fiber degeneration in HFD-induced PDN. (**A**) Confocal analysis of skin from mice with either heterozygous ($Na_v1.8$-Cre;Ai9;CXCR4$^{flox/+}$) or homozygous deletion ($Na_v1.8$-Cre;Ai9;CXCR4$^{flox/flox}$) of CXCR4 on either RD or HFD showing td-Tomato (**red**), immunolabeling with antibody against the protein gene product 9.5 (PGP 9.5) (**green**), and merged images with the nuclear marker DAPI (**blue**). $Na_v1.8$-Cre;Ai9;CXCR4$^{flox/+}$ RD mice had normal skin innervation whereas the same mice on HFD had reduced innervation. However, selective homozygous deletion of CXCR4 for mice on HFD prevented small-fiber degeneration. Magnification 60x (scale bar=50μm). (**B**) This effect was quantified using intra-epidermal nerve density (IENF density) which is expressed as the number of nerves crossing the epidermal-dermal junction (outlined in white) as a function of epidermal-dermal junction length. IENF densities calculated using both td-Tomato labeled fibers (red or pink) and PGP 9.5 fibers (shades of green) (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$) (n=7 for all groups with 3 non-contiguous sections analyzed per sample). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using a two-way ANOVA with Dunnett's Multiple Comparison test.

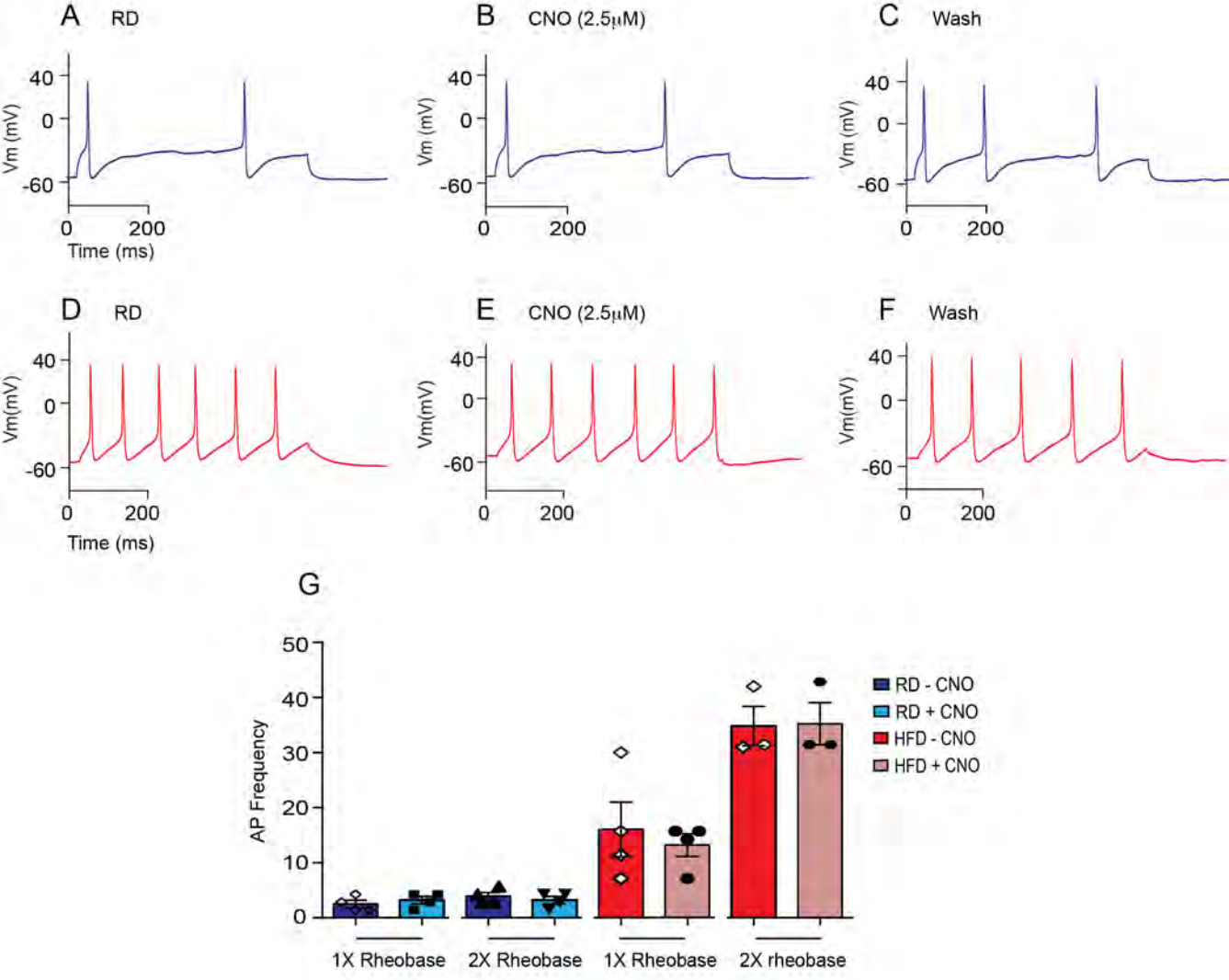
A Pirt-GCaMP3 mice 6-12 weeks CXCL12



B Pirt-GCaMP3 mice 2-4 weeks CXCL12

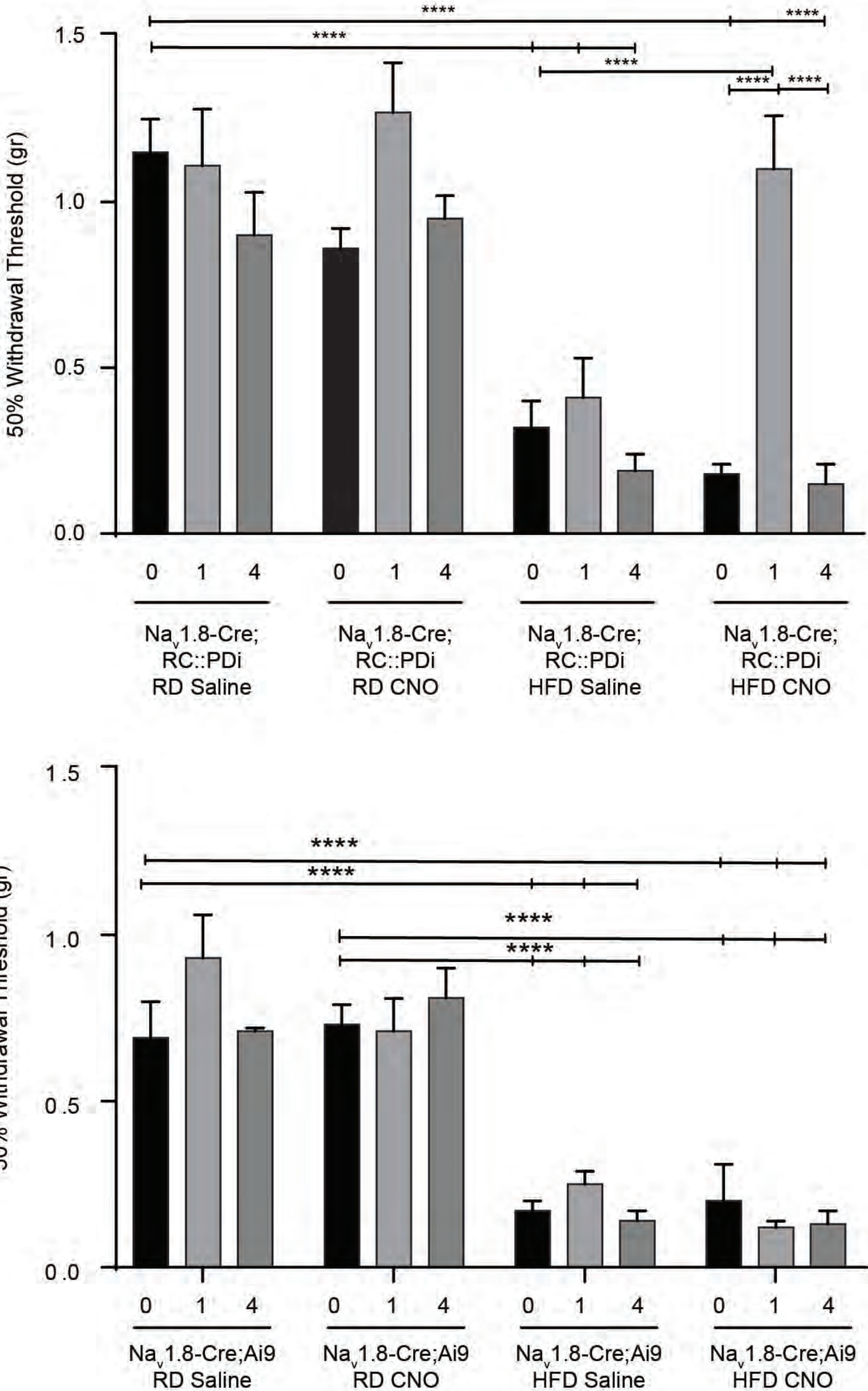


Supplemental Figure 5. Onset of increased $[Ca^{2+}]i$ responses following CXCL12 application after 6 weeks on HFD. (**A, B**) $[Ca^{2+}]i$ responses of acutely excised DRGs from Pirt-GCaMP3 mice to CXCL12. Data is shown as CXCL12 responsive DRG neurons as a percentage of total neurons that responded to a high potassium buffer (HK). (**A**) There were significantly more $[Ca^{2+}]i$ responses to CXCL12 (100 nM) in explants from HFD mice compared to RD fed non-diabetic controls after 6-12 weeks on diet (****, $p<0.0001$) (HFD n=844 neurons n=30 explants; RD n=594 neurons n=18 explants). (**B**) In contrast, $[Ca^{2+}]i$ responses to CXCL12 were not different after 2-4 weeks on HFD or on RD (HFD n=504 neurons n=20 explants; RD n=347 neurons n=16 explants). Values are expressed as mean ± S.E.M. p-values were calculated using Mann-Whitney test.


A
RD
B
CNO (2.5μM)
C
Wash
Vm (mV)
40
0
-60
0
200
Time (ms)
D
RD
E
CNO (2.5μM)
F
Wash
Vm(mV)
G
AP Frequency
50
40
30
20
10
0
RD - CNO
RD + CNO
HFD - CNO
HFD + CNO
1X Rheobase
2X Rheobase
1X Rheobase
2X rheobase

Supplemental Figure 6. $Na_v1.8$-positive DRG neurons that did not express DREADD receptors had no change in action potential frequency after CNO application. (**A-F**) Representative traces from a $Na_v1.8$-Cre;Ai9 primary cultured DRGs, which do not express DREADD receptors, from mice fed either RD (**A-C, blue**) or HFD (**D-F, red**). (**G**) In both RD and HFD application of 2.5μM CNO did not change the action potential frequency (AP frequency) at either 1X or 2X rheobase current injection (RD n=4 for 1X and 2X; HFD n=4 for 1X and n=3 for 2X). Values are expressed as mean ± S.E.M. p-values were calculated using Mann-Whitney test.

A


****
****
****
****
****
****
1.5
1.0
0.5
0.0
50% Withdrawal Threshold (gr)
0
1
4
0
1
4
0
1
4
0
1
4
Na$_v$1.8-Cre;
RC::PDi
RD Saline
Na$_v$1.8-Cre;
RC::PDi
RD CNO
Na$_v$1.8-Cre;
RC::PDi
HFD Saline
Na$_v$1.8-Cre;
RC::PDi
HFD CNO

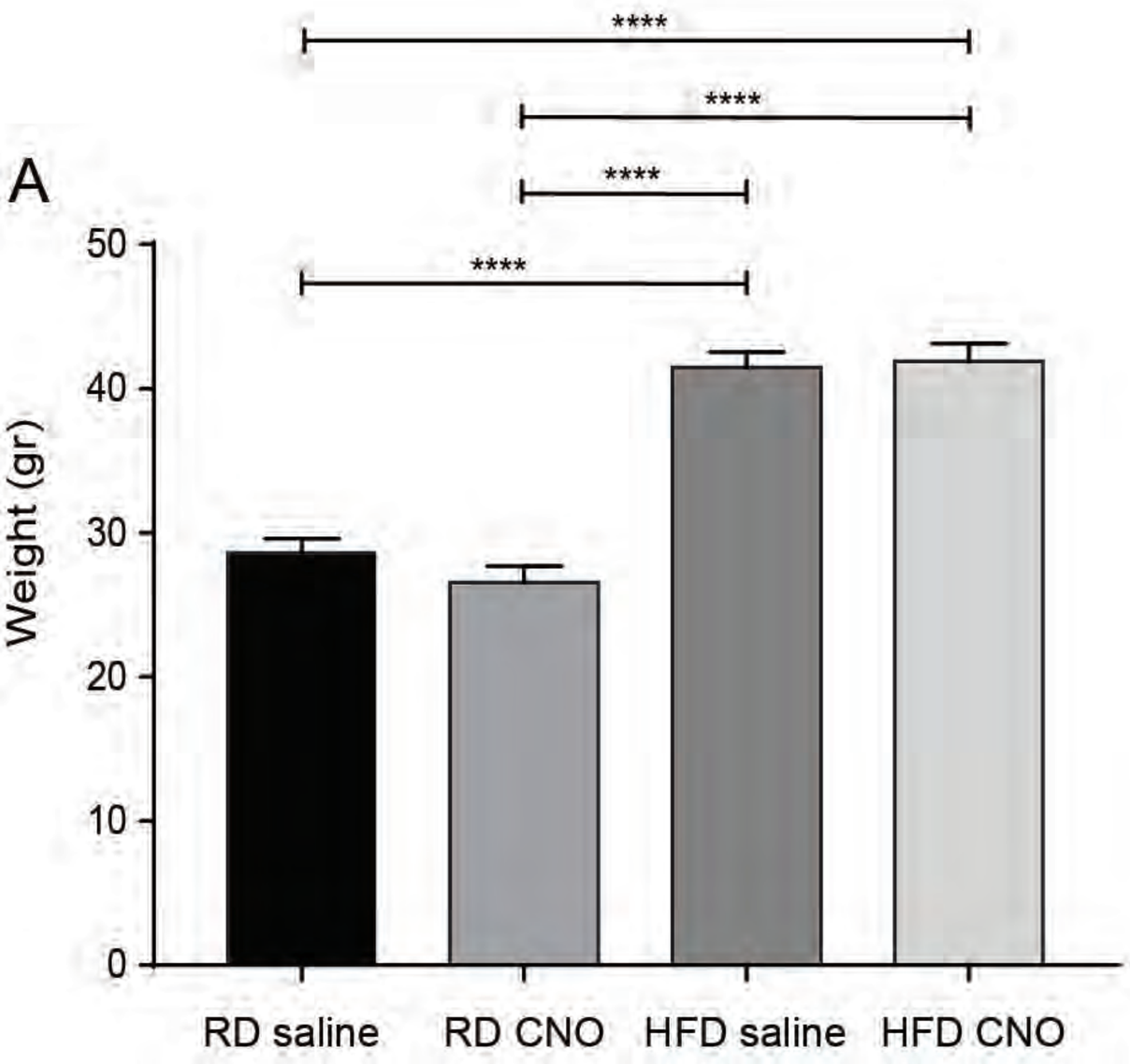
B

****
****
****
****
****
****
1.5
1.0
0.5
0.0
50% Withdrawal Threshold (gr)
0
1
4
0
1
4
0
1
4
0
1
4
Na$_v$1.8-Cre;Ai9
RD Saline
Na$_v$1.8-Cre;Ai9
RD CNO
Na$_v$1.8-Cre;Ai9
HFD Saline
Na$_v$1.8-Cre;Ai9
HFD CNO

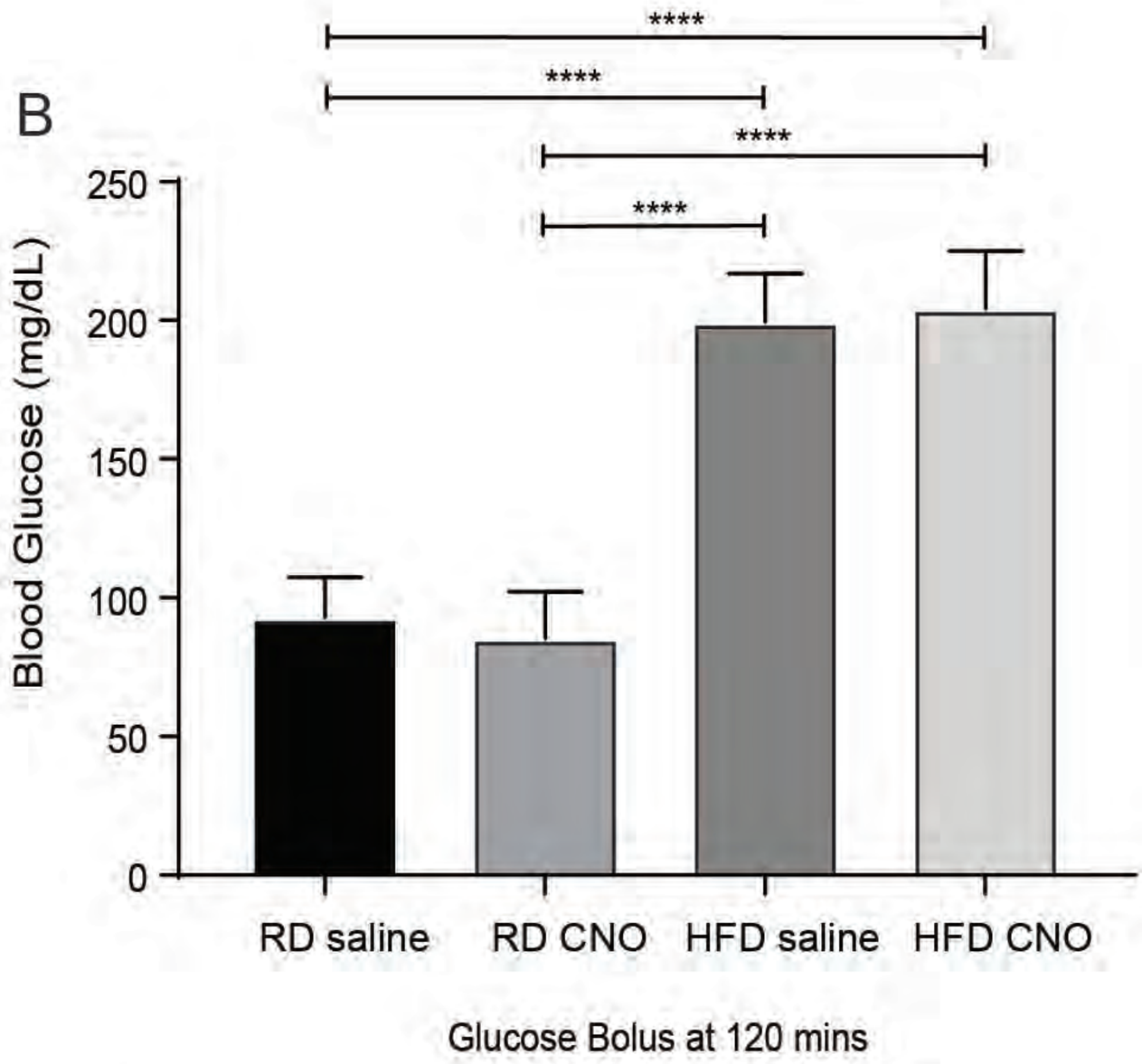
Supplemental Figure 7. Injection of CNO decreases mechanical allodynia in inhibitory PDi DREADD expressing mice on HFD and had no effect on mice not expressing DREADD receptors. (**A**) von Frey behavioral testing for $Na_V1.8$-Cre:RC::PDi mice on either RD or HFD injected with a CNO (10 mg/kg) or saline intraperitoneally (i.p.). These mice expressed inhibitory DREADD receptors, PDi, in their $Na_V1.8$-positive DRG neurons and fed a HFD showed an increase in pain withdrawal threshold one hour after CNO injection, this effect was absent four hours after injection (****, $p<0.0001$) (n=16/group). (**B**) von Frey behavioral testing was also performed on $Na_V1.8$-Cre;Ai9 mice that do not express inhibitory DREADD receptors. Mice were fed either RD or HFD and given an i.p. injection of either CNO (10 mg/kg) or saline. Mice on HFD had decreased withdrawal thresholds as expected and injection of CNO had no effect (****, $p<0.0001$) (n=8/group). For both genotypes behavioral testing was done before the injection (time=0), one hour after the injection (time=1hr) and four hours after (time=4hr). Values are expressed as mean ± S.E.M. p-values were calculated using a two-way ANOVA, Bonferroni multiple comparison test.

Na$_v$1.8-Cre;RC::PDi mice
(Inhibitory PDi)

A

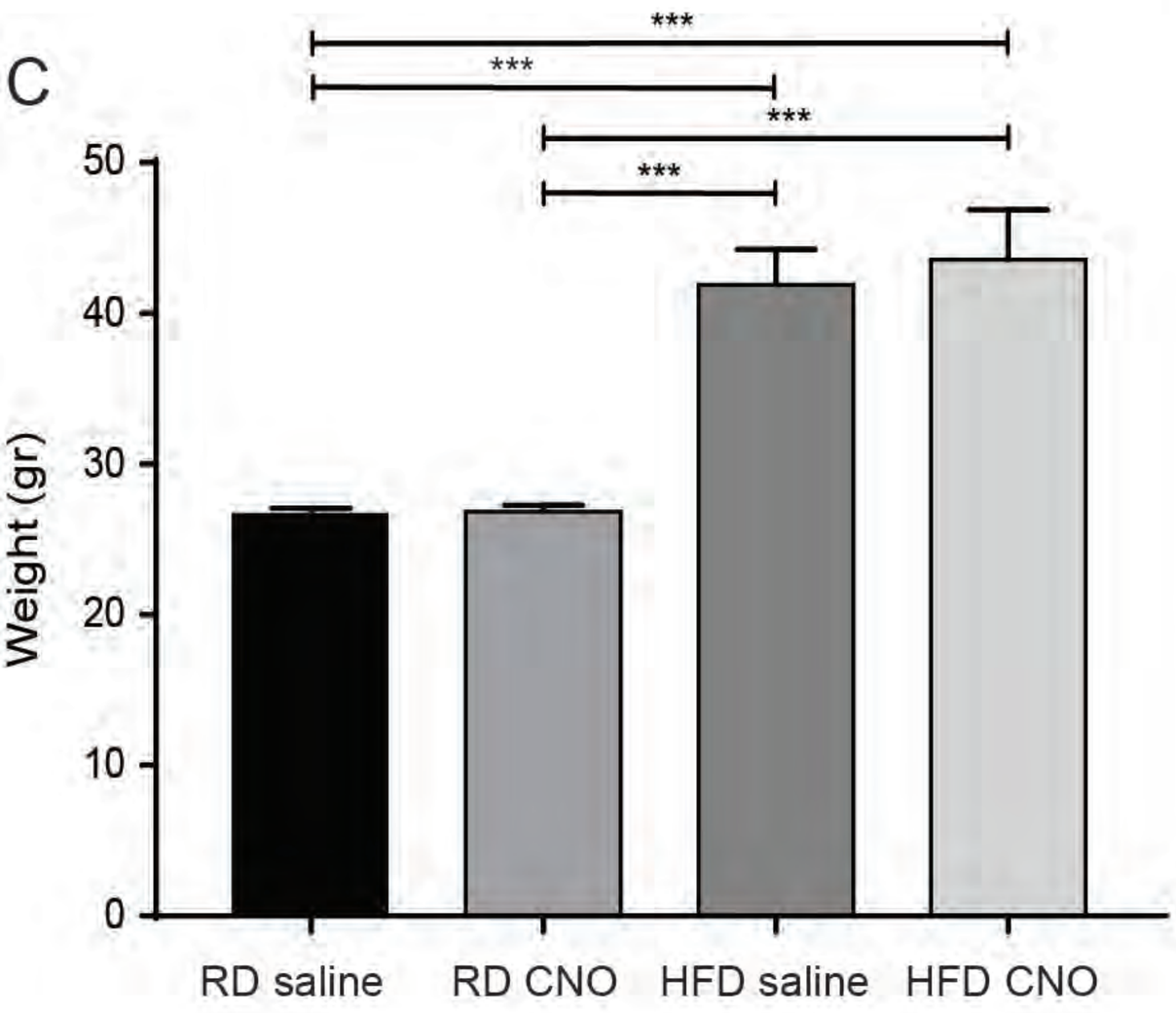


B

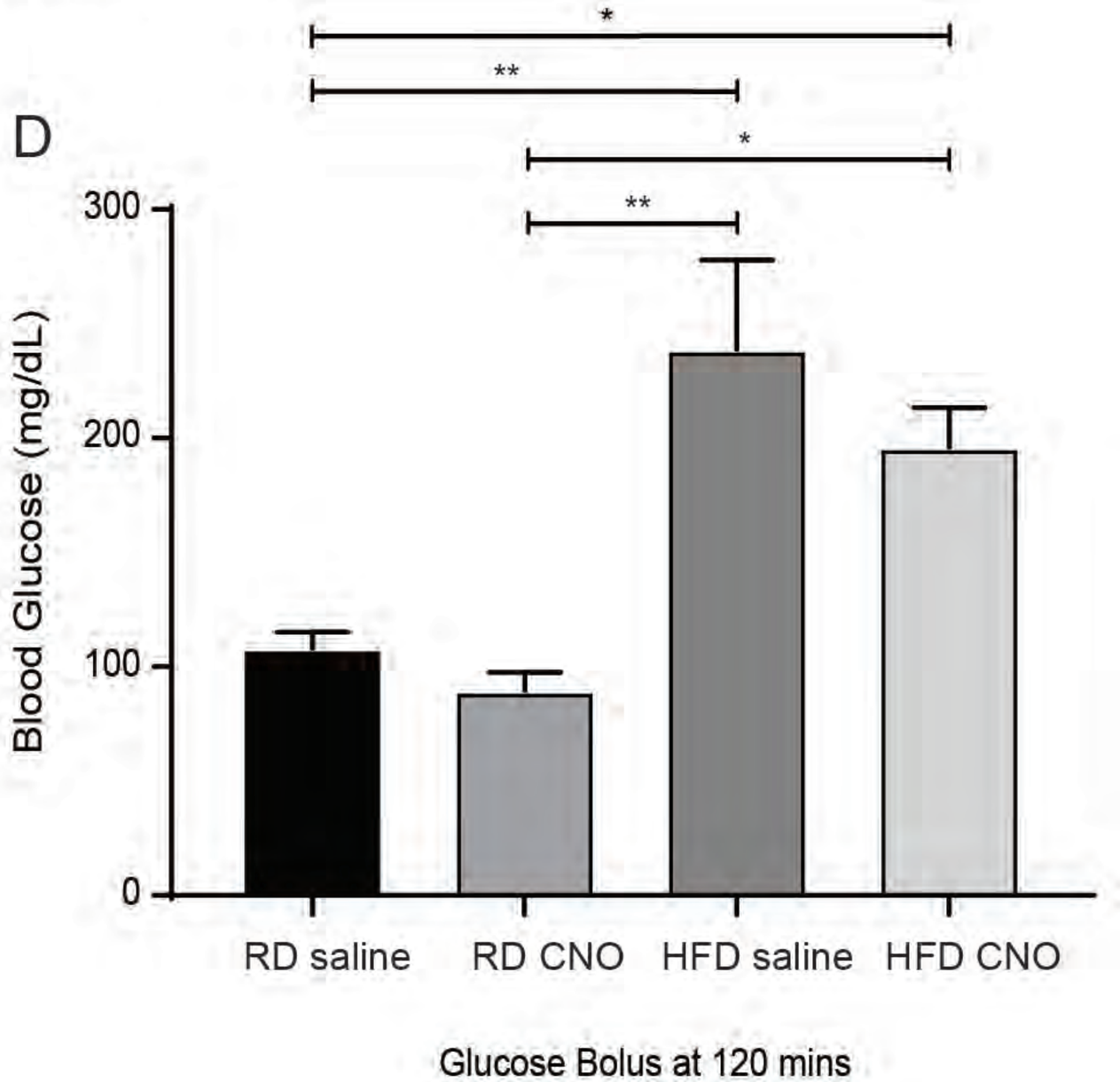


Na$_v$1.8-Cre;Ai9 mice

C

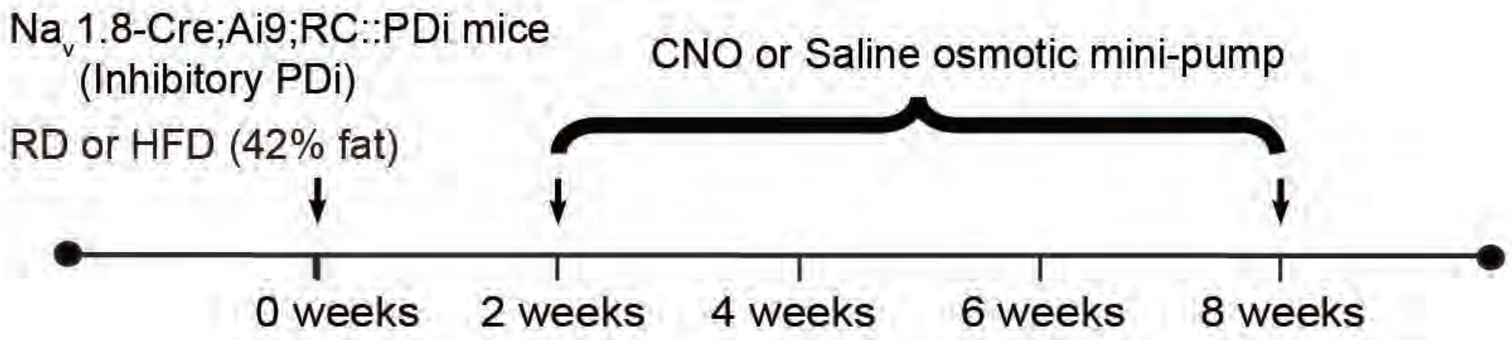


D

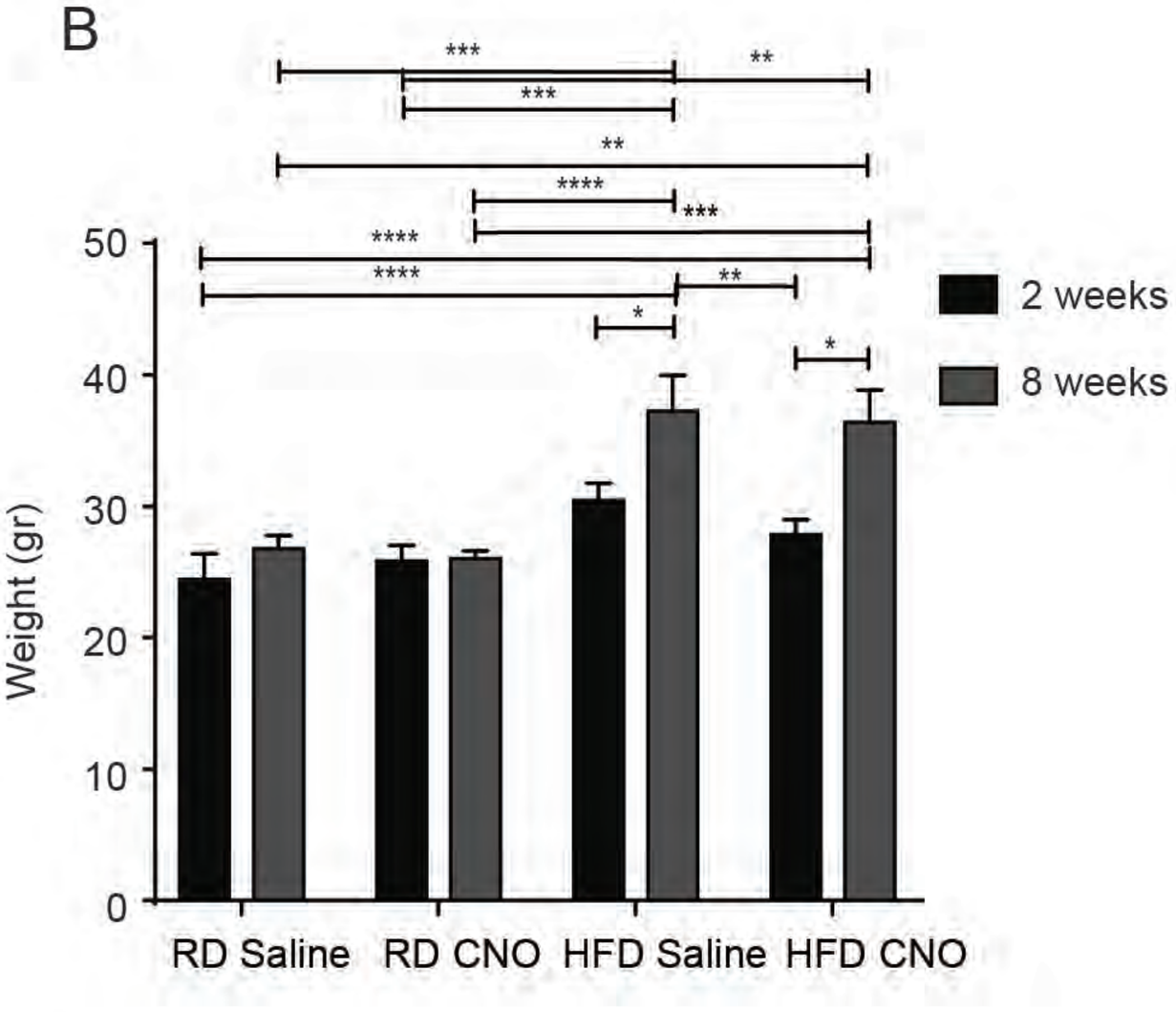


Supplemental Figure 8. Expression of inhibitory DREADD receptors, PDi in Na_V1.8-positive DRG neurons does not alter the metabolic profile in the HFD model. (**A**) Weight of Na_V1.8-Cre;RC::PDi mice in grams (gr) fed either RD or HFD for 10 weeks and injected with either CNO (10 mg/kg) or saline (****, $p<0.0001$) (n=6/group). (**B**) Blood glucose levels of the same mice 120 minutes after injection of glucose (45% D-glucose solution (2 mg glucose/1 g animal body weight)) (****, $p<0.0001$) (n=18/group). (**C**) Weight of Na_V1.8-Cre;Ai9 mice fed either RD or HFD and injected with either CNO (10 mg/kg) or saline (***, $p<0.001$) (n=6/group). (**D**) Blood glucose levels of the same mice 120 minutes after injection of glucose (*, $p<0.05$, **, $p<0.01$) (n=18/group). Values are expressed as mean ± S.E.M. p-values were calculated using one-way ANOVA, Bonferroni multiple comparison test.

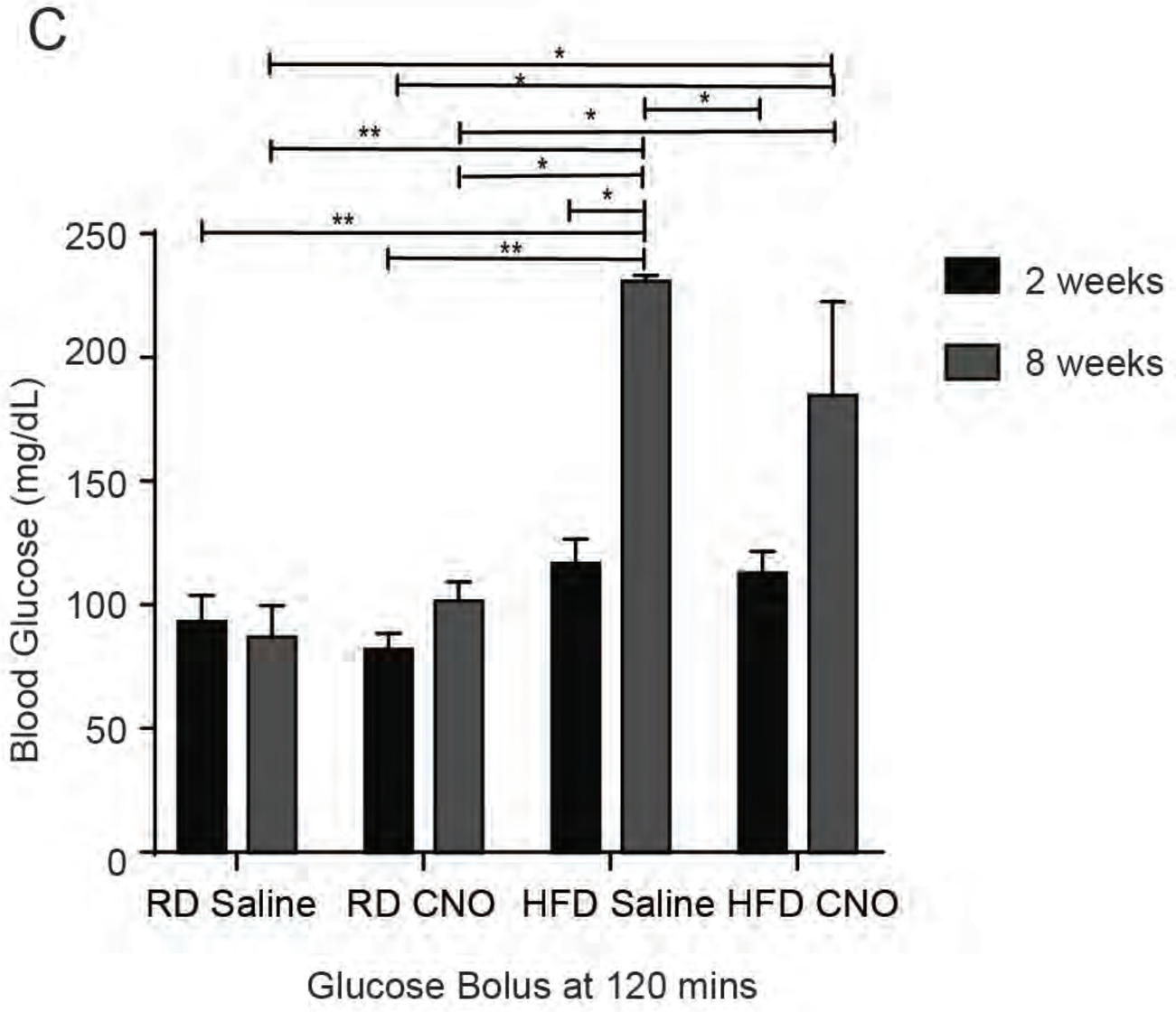
A


Na$_v$1.8-Cre;Ai9;RC::PDi mice
(Inhibitory PDi)
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
2 weeks
4 weeks
6 weeks
8 weeks

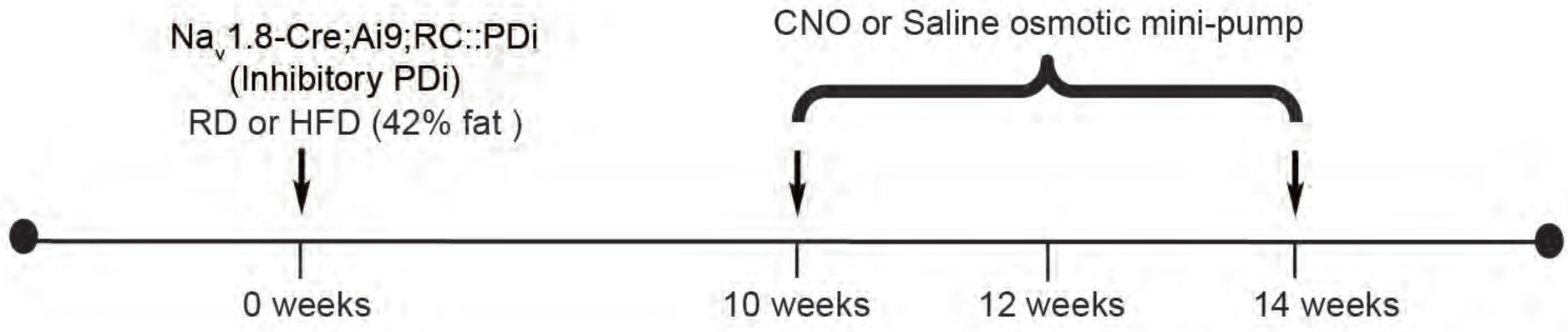
B


Weight (gr)
0
10
20
30
40
50
2 weeks
8 weeks
RD Saline
RD CNO
HFD Saline
HFD CNO

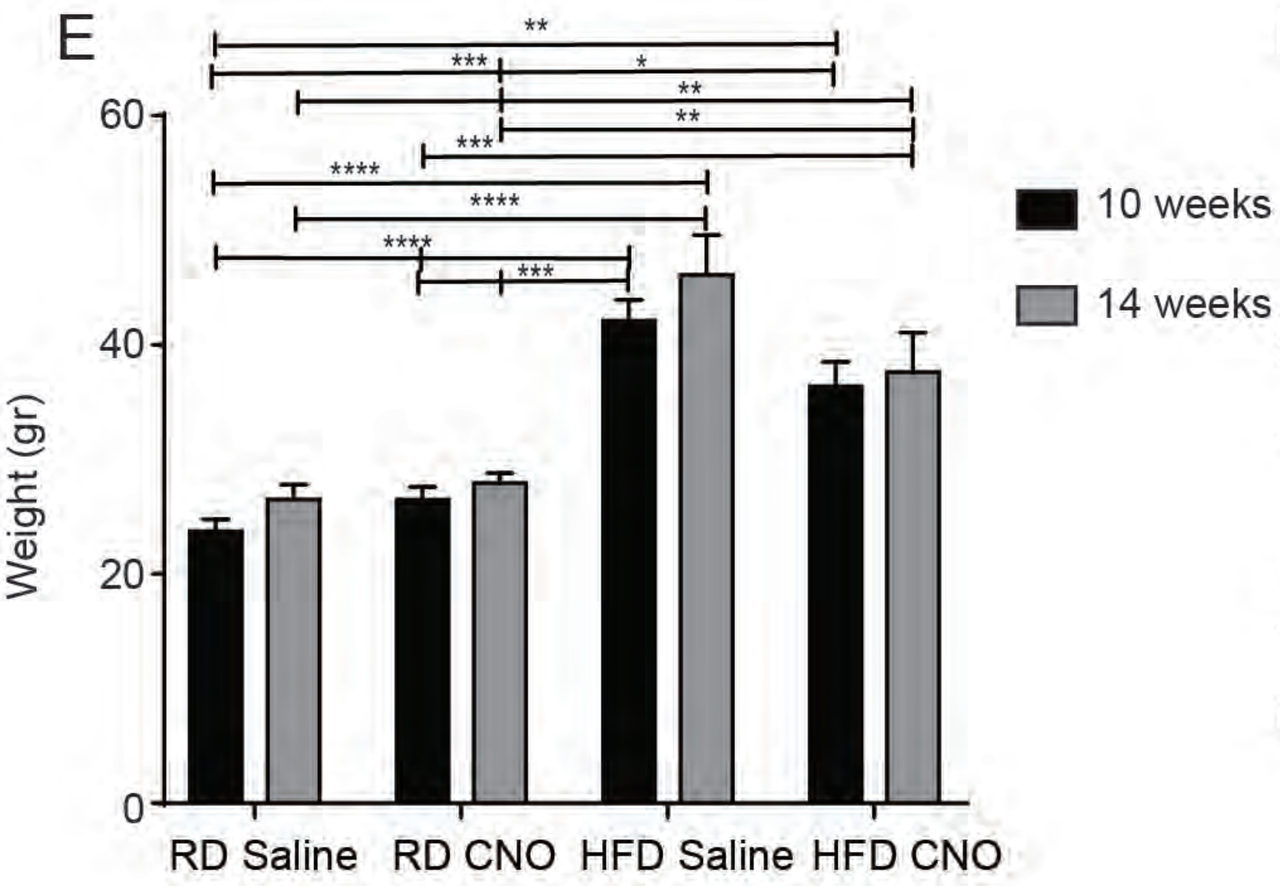
C


Blood Glucose (mg/dL)
0
50
100
150
200
250
2 weeks
8 weeks
RD Saline
RD CNO
HFD Saline
HFD CNO
Glucose Bolus at 120 mins

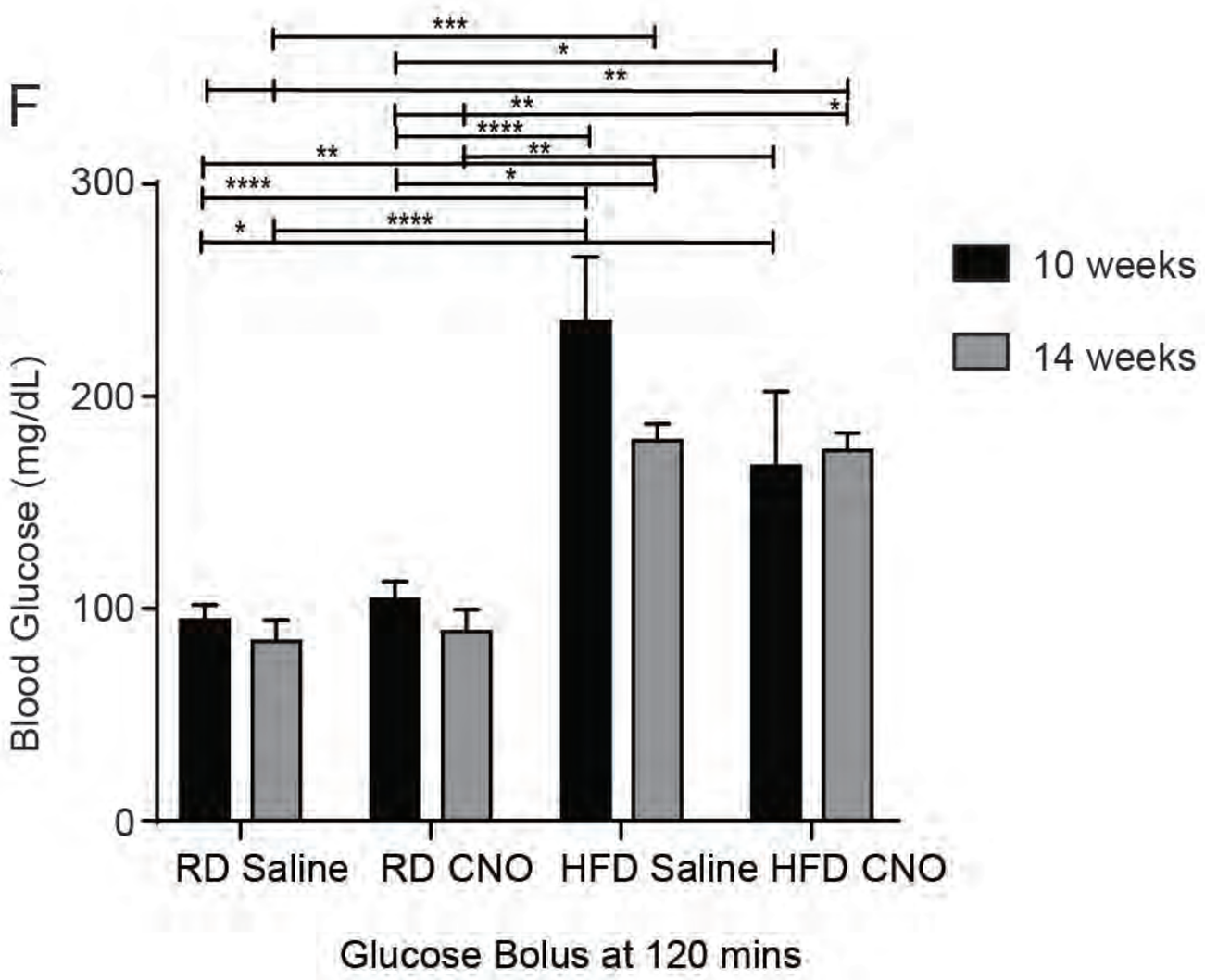
D


Na$_v$1.8-Cre;Ai9;RC::PDi
(Inhibitory PDi)
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
10 weeks
12 weeks
14 weeks

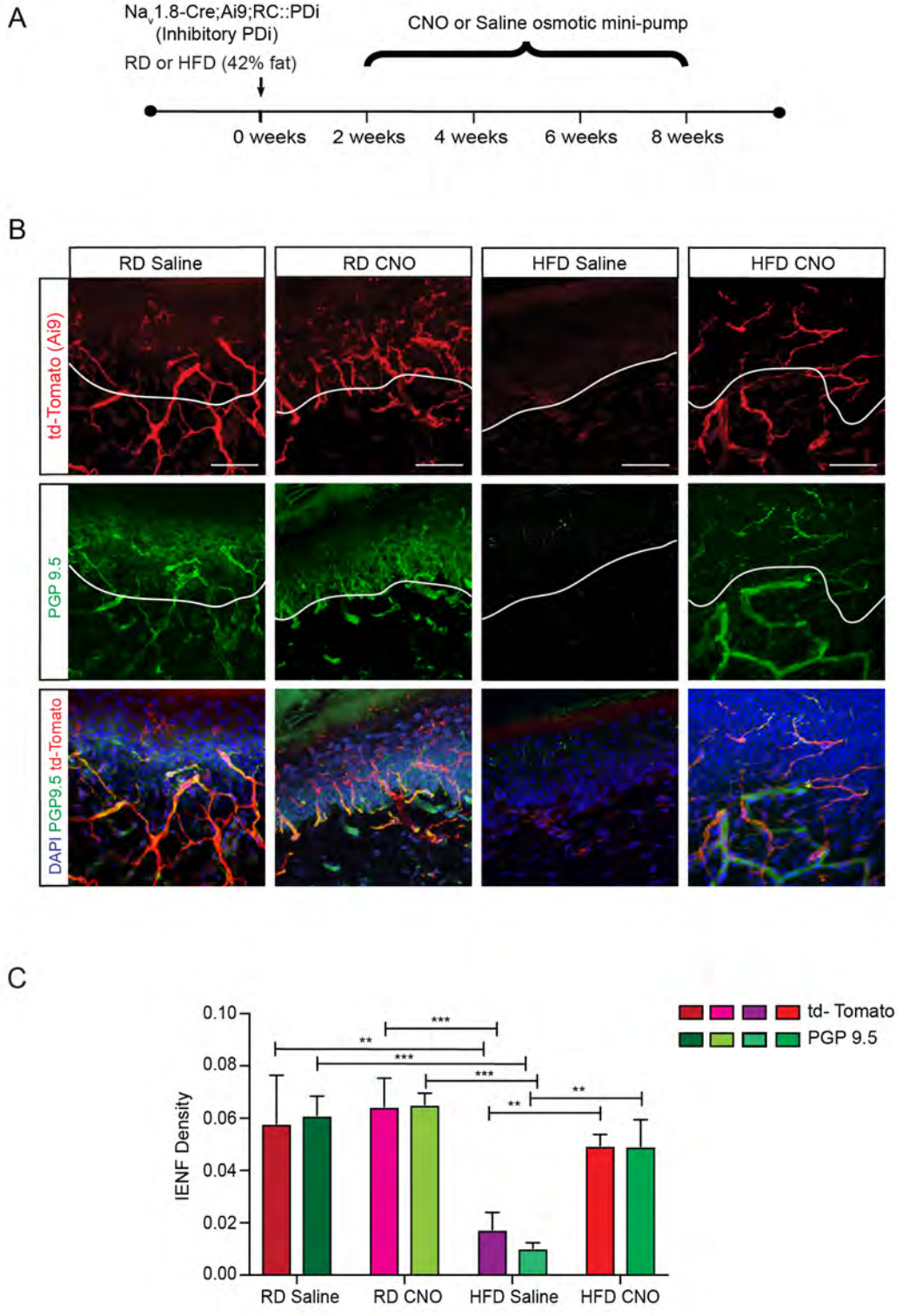
E


Weight (gr)
0
20
40
60
10 weeks
14 weeks
RD Saline
RD CNO
HFD Saline
HFD CNO

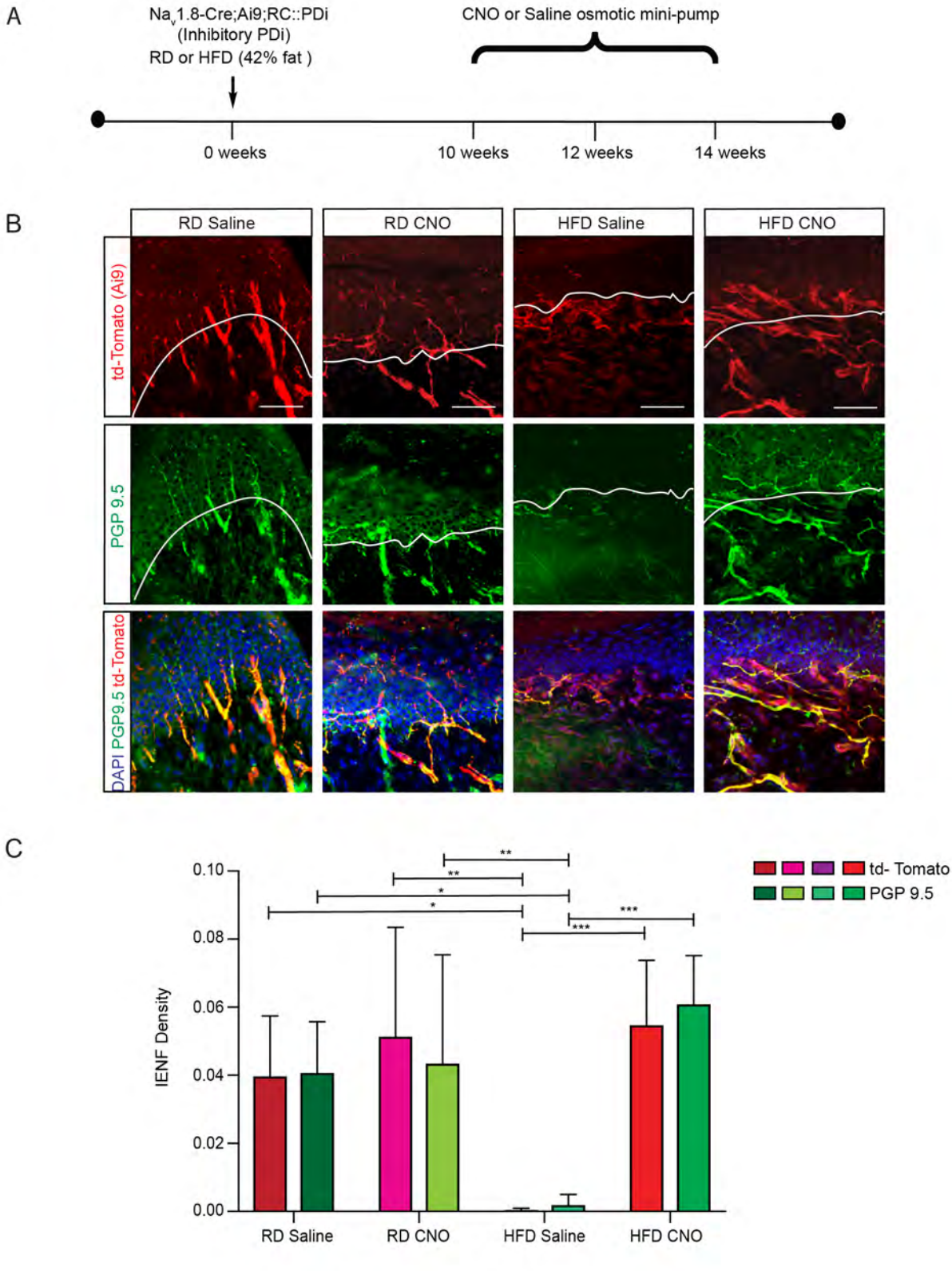
F


Blood Glucose (mg/dL)
0
100
200
300
10 weeks
14 weeks
RD Saline
RD CNO
HFD Saline
HFD CNO
Glucose Bolus at 120 mins

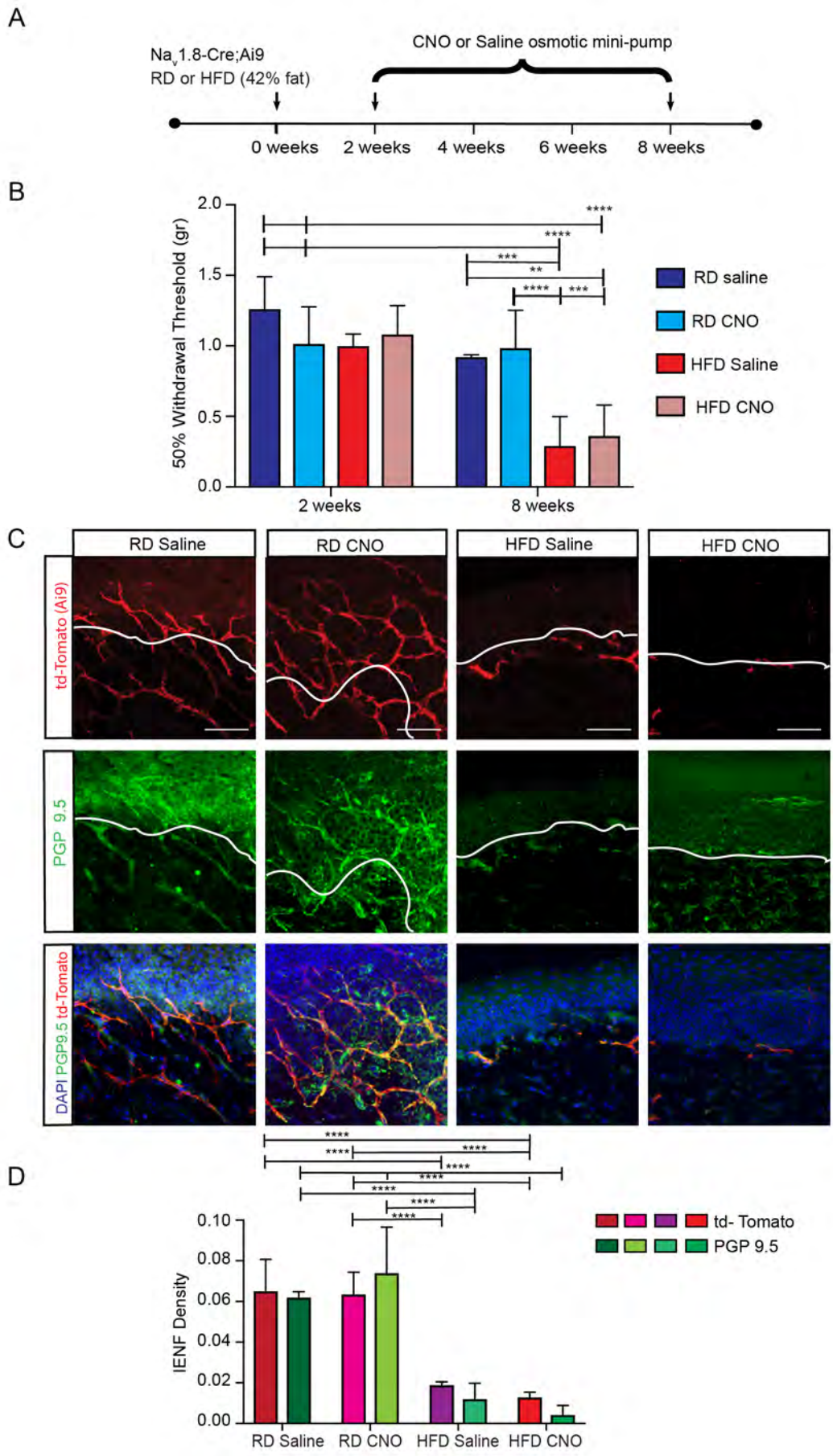
Supplemental Figure 9. Experimental plan, weights and blood glucose levels for prevention and reversal of PDN in mice that expressed inhibitory DREADD receptors, PDi. (**A**) Experimental timeline for the prevention set of experiments where Na_v1.8-Cre;Ai9;RC::PDi mice were administered CNO (10 mg/kg/day) or saline through an osmotic mini-pump concurrently with mice being fed either RD or HFD. Each arrow represents a time point when weight and blood glucose levels were measured. (**B**) Weights of these mice in grams (gr) after 2 or 8 weeks on the diet (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$) (n=6/group). (**C**) Blood glucose levels of these mice at 2 and 8 weeks on diet 120 minutes after injection of glucose (45% D-glucose solution (2mg glucose/1g animal body weight)) (*, $p<0.05$, **, $p<0.01$) (n=6/group). (**D**) Experimental timeline for the reversal set of experiments where Na_v1.8-Cre;Ai9;RC::PDi mice were administered CNO (10 mg/kg/day) or saline through an osmotic mini-pump after being fed on diet. Each arrow represents a time point when weight and blood glucose levels were measured. (**E**) Weights of these mice in grams (gr) after 10 or 14 weeks on RD or HFD (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$) (n=6/group). (**F**) Blood glucose levels of these mice at 10 and 14 weeks on diet (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$) (n=6/group). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using two-way ANOVA, Bonferroni multiple comparison test.


A
Na$_v$1.8-Cre;Ai9;RC::PDi
(Inhibitory PDi)
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
2 weeks
4 weeks
6 weeks
8 weeks
B
RD Saline
RD CNO
HFD Saline
HFD CNO
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato
C
IENF Density
0.10
0.08
0.06
0.04
0.02
0.00
***
**
***
***
**
**
td- Tomato
PGP 9.5
RD Saline
RD CNO
HFD Saline
HFD CNO

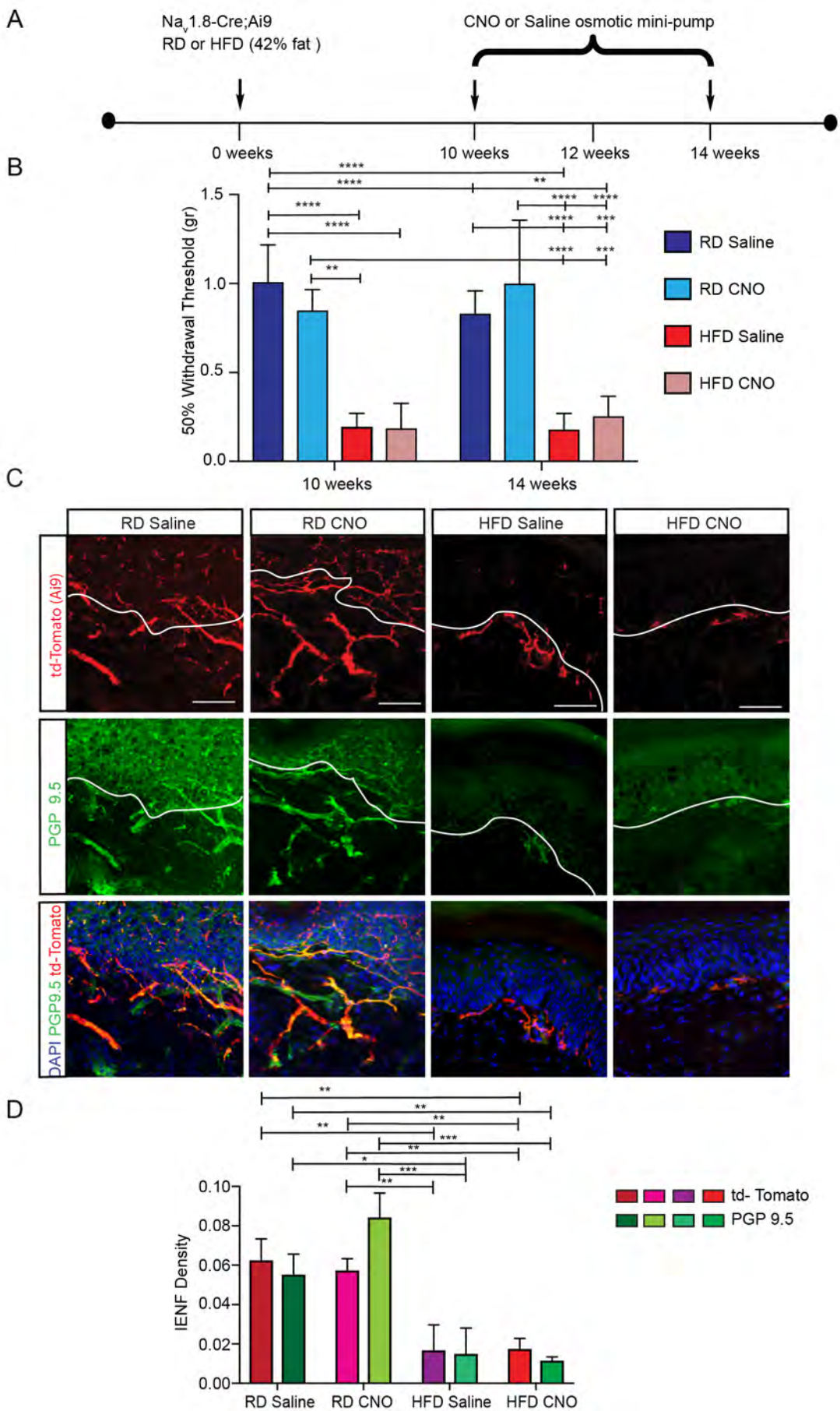
Supplemental Figure 10. Chemogenetic inhibition of Na_v1.8-positive DRG neurons can prevent small-fiber degeneration visualized by either td-Tomato positive fibers or fibers stained with PGP 9.5. (**A**) Experimental setup: osmotic mini-pumps infusing either CNO (10mg/kg/day) or saline were implanted i.p. in Na_v1.8-Cre;Ai9;RC::PDi between 2 and 8 weeks of RD or HFD. (**B**) Confocal analysis of skin from these mice that express the inhibitory DREADD receptor, PDi, fed either RD or HFD showing td-Tomato (**red**) in Na_v1.8-positive fibers, immunolabeling with antibody against PGP 9.5 (**green**), and merged images with the nuclear marker DAPI (**blue**). Mice on RD given either saline or CNO showed normal skin innervation. In diabetic mice given saline there was a reduction in skin innervation, but it was reversed for mice on HFD given CNO. CNO infusion prevented small-fiber degeneration of mice on HFD. Magnification 60x (scale bar=50μm). (**C**) This effect was quantified using intra-epidermal nerve density (IENF density) which is expressed as the number of nerves crossing the epidermal-dermal junction (outlined in white) as a function of epidermal-dermal junction length. IENF densities were calculated using both td-Tomato labeled fibers (shades of red) and PGP 9.5 labeled fibers (shades of green) (**, $p<0.01$, ***, $p<0.01$) (n=7 for all groups with 3 non-contiguous sections analyzed per sample). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using a two-way ANOVA with Dunnet Multiple comparison test.


A
Na$_v$1.8-Cre;Ai9;RC::PDi
(Inhibitory PDi)
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
10 weeks
12 weeks
14 weeks
B
RD Saline
RD CNO
HFD Saline
HFD CNO
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato
C
td- Tomato
PGP 9.5
IENF Density
0.10
0.08
0.06
0.04
0.02
0.00
**
**
*
*
***
***
RD Saline
RD CNO
HFD Saline
HFD CNO

Supplemental Figure 11. Chemogenetic inhibition of Na_v1.8-positive DRG neurons can reverse small-fiber degeneration visualized by either td-Tomato fibers or fibers stained with PGP 9.5. (**A**) Experimental setup for the reversal set of experiments. Na_v1.8-Cre;Ai9;RC::PDi were fitted with osmotic mini-pumps i.p. infusing either CNO (10mg/kg/day) or saline between 10-14 weeks of either RD or HFD. (**B**) Confocal analysis of skin from these mice that express the inhibitory DREADD receptor PDi fed either RD or HFD showing td-Tomato (**red**) in Na_v1.8-positive fibers, immunolabeling with antibody against the protein gene product 9.5 (PGP 9.5) (**green**), and merged images with the nuclear marker DAPI (**blue**). Control mice on a RD with saline or CNO pumps showed normal skin innervation. Diabetic mice on HFD implanted with a saline pump showed reduced skin innervation. However, diabetic mice on a HFD fitted with CNO pumps showed a significant improvement in skin innervation. (**C**) This effect was quantified using intra-epidermal nerve density (IENF density) which is expressed as the number of nerves crossing the epidermal-dermal junction (outlined in white) as a function of epidermal-dermal junction length. IENF densities were calculated using both td-Tomato fibers (shades of red) and with PGP 9.5 fibers (shades of green) (n=6 from each group with 3 non-contiguous sections analyzed per sample). (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$). Values are expressed as mean ± S.E.M. p-values were calculated using a two-way ANOVA with Dunnet Multiple comparison test.

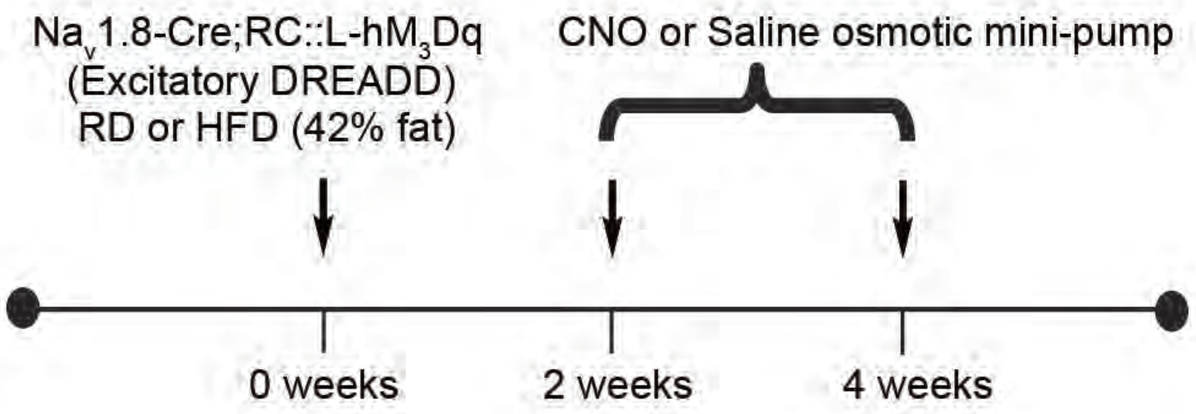

A
Na$_v$1.8-Cre;Ai9
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
2 weeks
4 weeks
6 weeks
8 weeks
B
50% Withdrawal Threshold (gr)
2.0
1.5
1.0
0.5
0.0
2 weeks
8 weeks
RD saline
RD CNO
HFD Saline
HFD CNO
C
RD Saline
RD CNO
HFD Saline
HFD CNO
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato
D
IENF Density
0.10
0.08
0.06
0.04
0.02
0.00
td- Tomato
PGP 9.5
RD Saline
RD CNO
HFD Saline
HFD CNO

Supplemental Figure 12. Long-term treatment with CNO to prevent PDN onset has no effect on mice that do not express DREADD receptors. (**A**) Experimental timeline for the prevention set of experiments where Na_v1.8-Cre;Ai9 mice, that do not express DREADD receptors, are administered CNO (10 mg/kg/day) or saline through an osmotic mini-pump implanted i.p. concurrently with being fed either RD or HFD. Each arrow represents a time point where pain behavior is assessed. (**B**) von Frey behavioral testing was done at 2 and 8 weeks showing that HFD mice show a decreased withdrawal threshold only after being on the diet for 8 weeks. Treatment with CNO pump did not change the decreased withdrawal threshold observed in HFD mice and also had no effect on RD mice (**, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$) (n=6/group). p-values were calculated using a two-way ANOVA with Bonferroni Multiple comparison test (**C**) Representative confocal micrographs taken from the skin of these animals showing the Na_v1.8-positive fibers labeled with td-Tomato (**red**), immunolabeling for PGP 9.5 (**green**) and merged images with the nuclear marker DAPI (**blue**). Mice on HFD given CNO showed no change in skin innervation. Magnification 60x (scale bar=50μm). (**D**) This effect was quantified using intra-epidermal nerve density (IENF density) which is expressed as the number of nerves crossing the epidermal-dermal junction (outlined in white) as a function of epidermal-dermal junction length. IENF densities were calculated using both td-Tomato fibers (shades of red) and with PGP 9.5 fibers (shades of green) (****, $p<0.0001$) (n=6/group with 3 non-contiguous sections analyzed per sample). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using a two-way ANOVA with Dunnet Multiple comparison test.

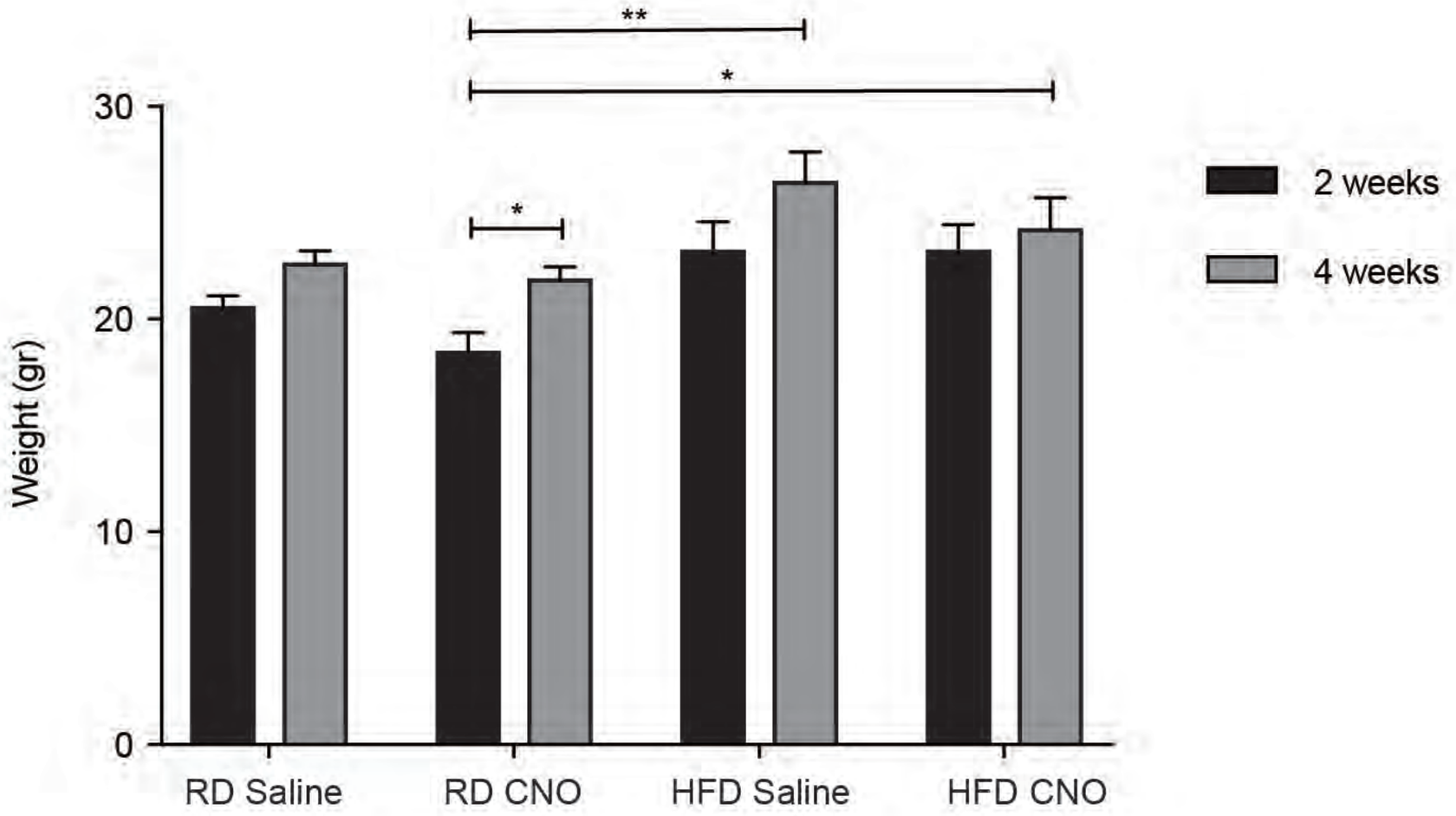

A
Na$_v$1.8-Cre;Ai9
RD or HFD (42% fat)
CNO or Saline osmotic mini-pump
0 weeks
10 weeks
12 weeks
14 weeks
B
50% Withdrawal Threshold (gr)
1.5
1.0
0.5
0.0
10 weeks
14 weeks
RD Saline
RD CNO
HFD Saline
HFD CNO
C
RD Saline
RD CNO
HFD Saline
HFD CNO
td-Tomato (Ai9)
PGP 9.5
DAPI PGP9.5 td-Tomato
D
IENF Density
0.10
0.08
0.06
0.04
0.02
0.00
RD Saline
RD CNO
HFD Saline
HFD CNO
td- Tomato
PGP 9.5

Supplemental Figure 13. Long-term treatment to reverse PDN with CNO has no effect on mice that do not express DREADD receptors. (**A**) Experimental timeline for the reversal set of experiments where $Na_V1.8$-Cre;Ai9 mice are administered CNO (10 mg/kg/day) or saline through an osmotic mini-pump implanted i.p. following being fed either RD or HFD for 10 weeks. Each arrow represents a time point where pain behavior was assessed. (**B**) von Frey behavioral testing was done at 10 and 14 weeks showing that, as expected, mice on HFD given saline have a much lower withdrawal threshold compared to RD mice. When HFD mice were given CNO there is no change in the withdrawal threshold (**, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$) (n=6/group). p-values were calculated using a two-way ANOVA, Bonferroni Multiple comparison test (**C**) Representative confocal micrographs taken from the skin of these animals showing the $Na_V1.8$-positive fibers labeled with td-Tomato (**red**), immunolabeling for PGP 9.5 (**green**) and merged images with the nuclear marker DAPI (**blue**). Mice on HFD given CNO showed no improvement in skin innervation. Magnification 60x (scale bar=50μm). (**D**) This effect was quantified using intra-epidermal nerve density (IENF density) which is expressed as the number of nerves crossing the epidermal-dermal junction (outlined in white) as a function of epidermal-dermal junction length. IENF densities were calculated using both td-Tomato fibers (shades of red) and with PGP 9.5 fibers (shades of green) (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$) (n=6/group with 3 non-contiguous sections analyzed per sample). Values are expressed as mean $\pm$ S.E.M. p-values were calculated using a two-way ANOVA with Dunnet Multiple comparison test.

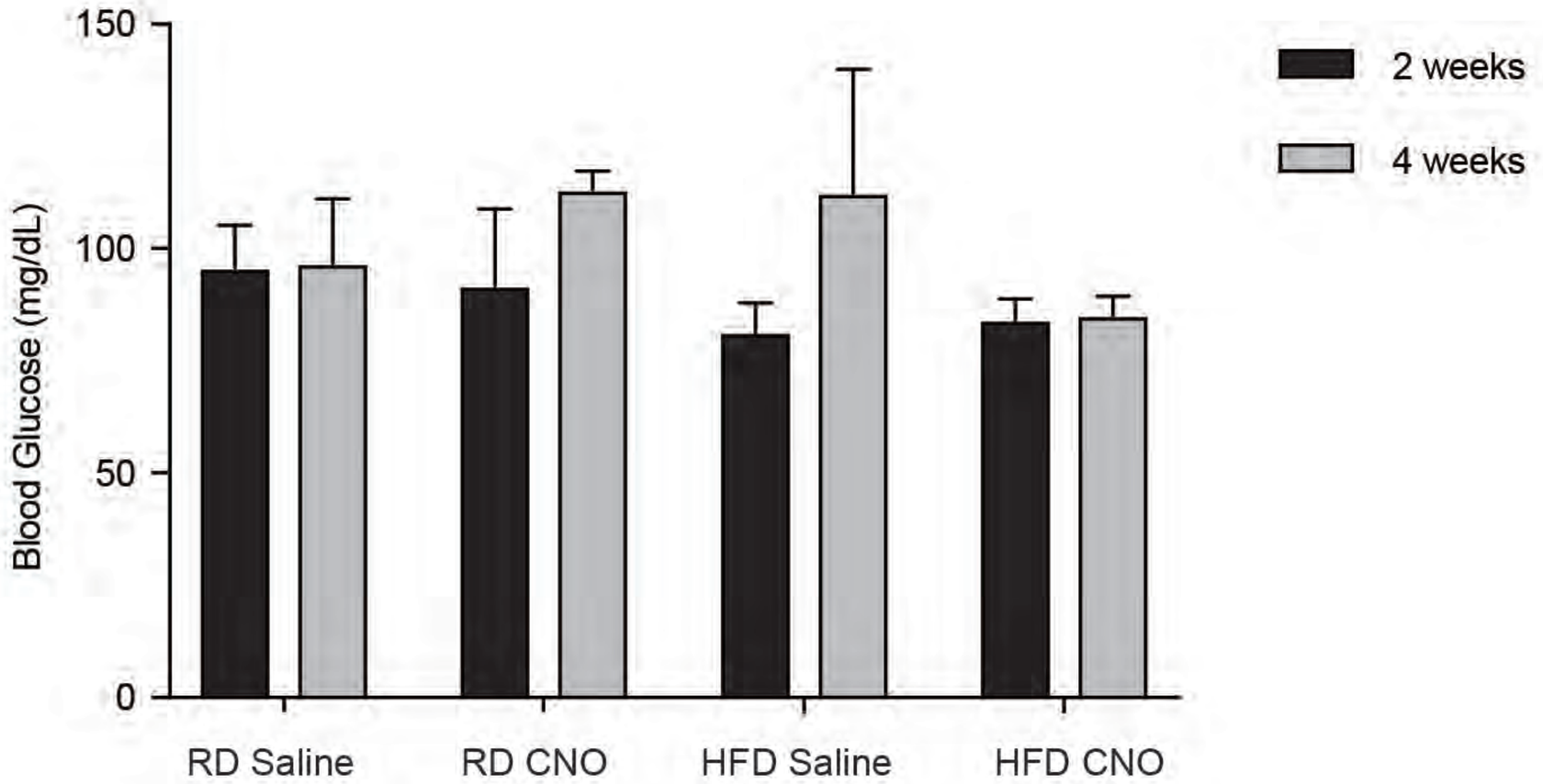
A



B



C



Glucose Bolus at 120 mins

Supplemental Figure 14. Continuous CNO infusion did not alter the metabolic profile of mice expressing hM$_3$Dq excitatory DREADD receptors. (**A**) Experimental setup of osmotic mini-pump implantation in Na$_v$1.8-Cre;RC::L-hM$_3$Dq mice. Na$_v$1.8-Cre;RC::L-hM$_3$Dq mice that expressed excitatory hM$_3$Dq DREADD receptors were fed either RD or HFD and had a osmotic mini-pump implanted intraperitoneally, which administered either saline or CNO (10mg/kg/day) for the period from 2 to 4 weeks following the commencement of HFD or RD. (**B**) Weights of these mice in grams (gr) after 2 or 4 weeks on the diet (*, $p<0.05$, **, <0.01) (n=6/group). (**C**) Blood glucose levels of these mice at two and four weeks on RD or HFD 120 minutes after injection of glucose (45% D-glucose solution (2mg glucose/1g animal body weight)) (n=6/group). Values are expressed as mean ± S.E.M. p-values were calculated using two-way ANOVA with Bonferroni Multiple comparison test.

Supplemental Table 1: Electrophysiological parameters of neurons used for recordings of Figure 11. Cells were recorded at culture days 2 – 4. For this dataset only a few medium and large neurons were included in the data. Values are expressed as mean ± S.E.M.

Genotype (n)	Vm (mV)	capacitance (pF)	smallest (pF)	biggest (pF)	Rin (MΩ)	rheobase (pA)
$Na_v1.8$-Cre;Ai9 (16)	-61.3 ± 0.6	51.3 ± 11.7	20	196	501 ± 59	318.5 ± 108.25
$Na_v1.8$-Cre;RC::L-hM_3Dq (28)	-62.8 ± 0.8	29.9 ± 1.7	19	53	641 ± 47	136 ± 22.7

Supplemental Table 2: [Ca^{2+}]i responses of parvalbumin-Cre::GCaMP6 explants to CXCL12 or to different concentration of potassium buffer after 8 weeks on either diet (RD, n=88 neurons , 6 explants; HFD n=118, 9 explants). Values are expressed as mean ± SEM. p-values were calculated using p- values were calculated using a Mann-Whitney test.

Parvalbumin-Cre::GCaMP6	CXCL12	HK10	HK25	HK50
RD	0	10.594±3.41	22.818±5.82	47.225±2.34
HFD	3.33±3.33	19.878±9.23	37.288±11.69	54.752±9.57