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Supplemental Information

MCH Neurons Regulate Permeability of the Median Eminence Barrier

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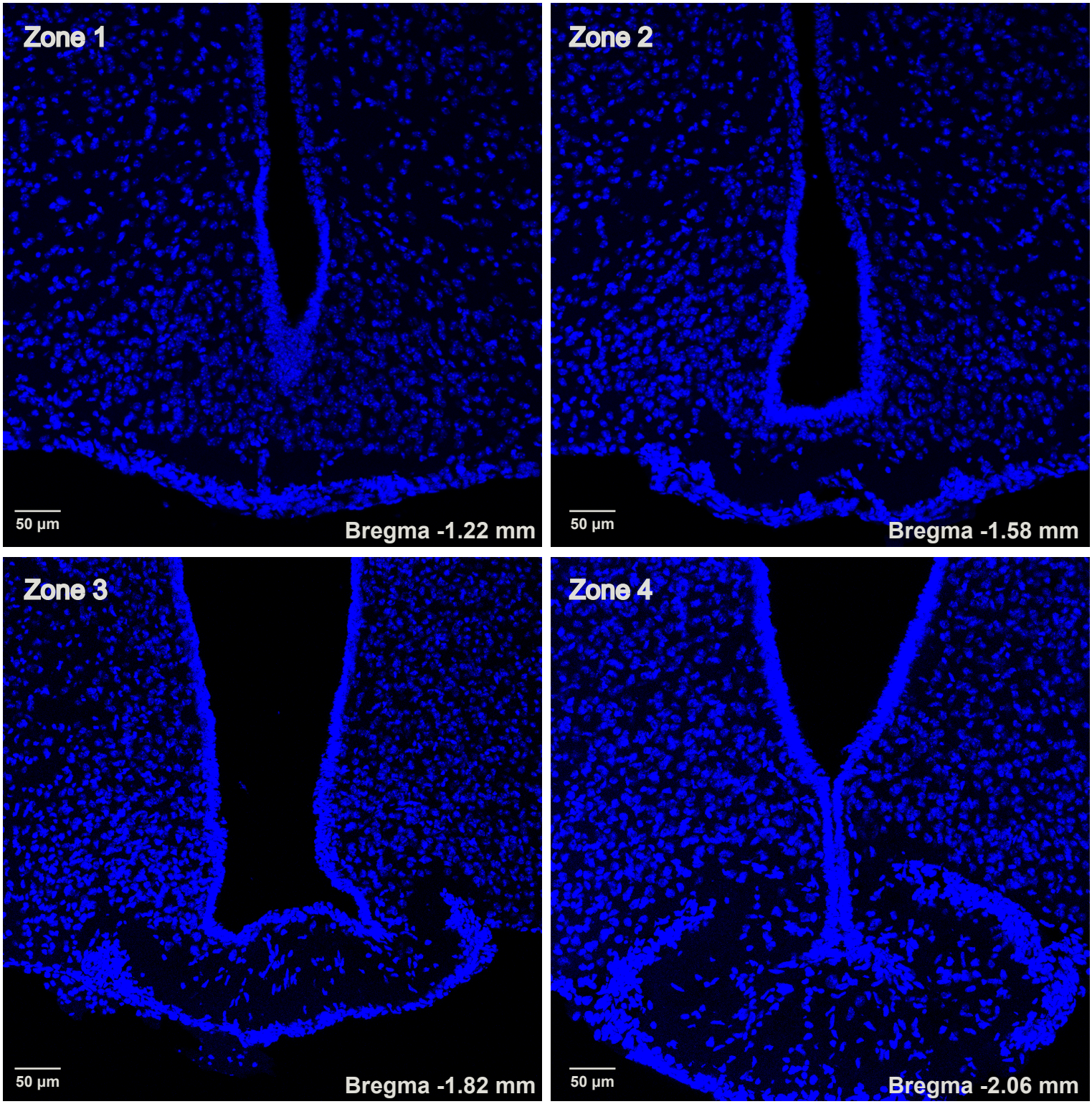


Figure S1. Related to Figure 1. Classification of four zones in the ARC. Representative anatomical location for each zone of the ARC from rostral to caudal. Scale bar: 50 µm.

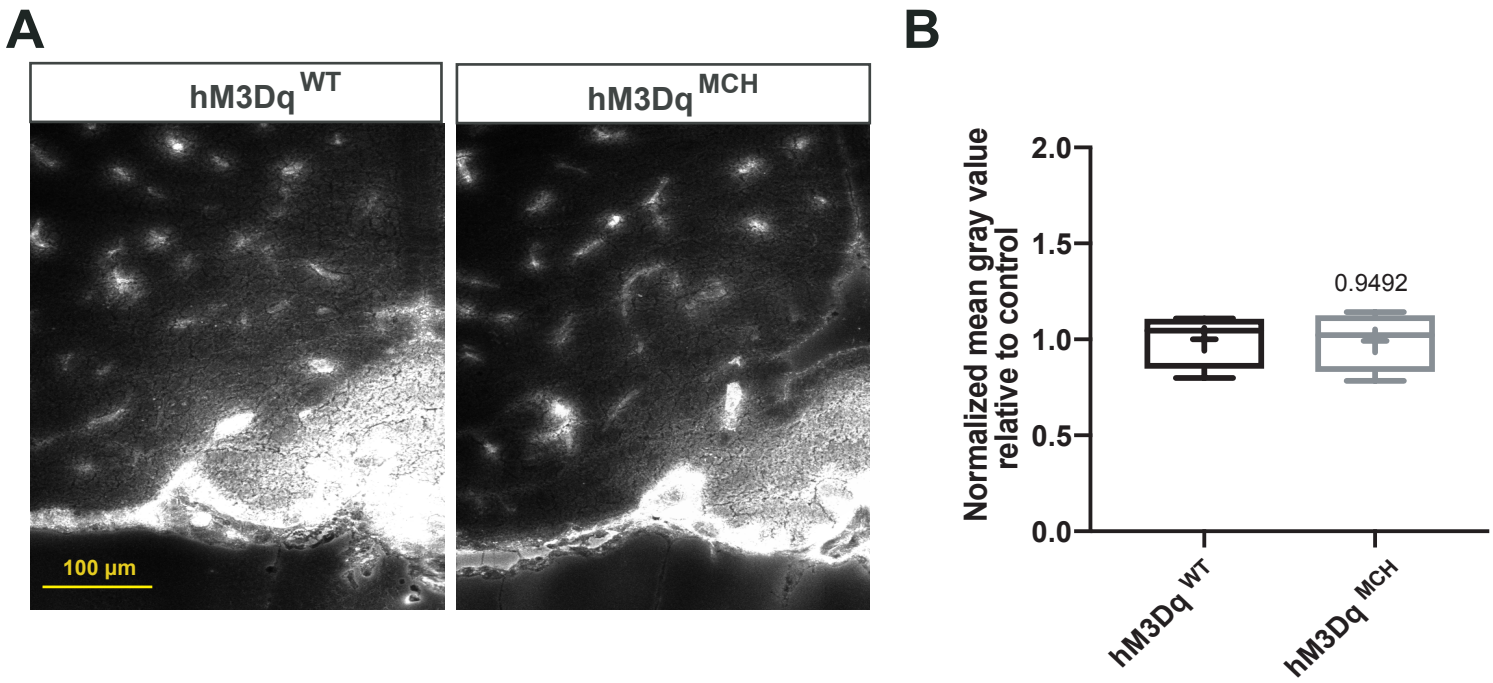


Figure S2. Related to Figure 2. Evans blue diffusion showed no detectable difference in vehicle-treated mice. (A) Representative images of Evans blue dye diffusion into the ARC region of fasted and 4 hrs vehicle-injected control and hM3Dq^{MCH} mice. (B) Quantification of Evans blue dye diffusion in the ARC region in vehicle-treated control (n=4) and hM3Dq^{MCH} (n=4) mice. Statistics: unpaired Student's T-test.

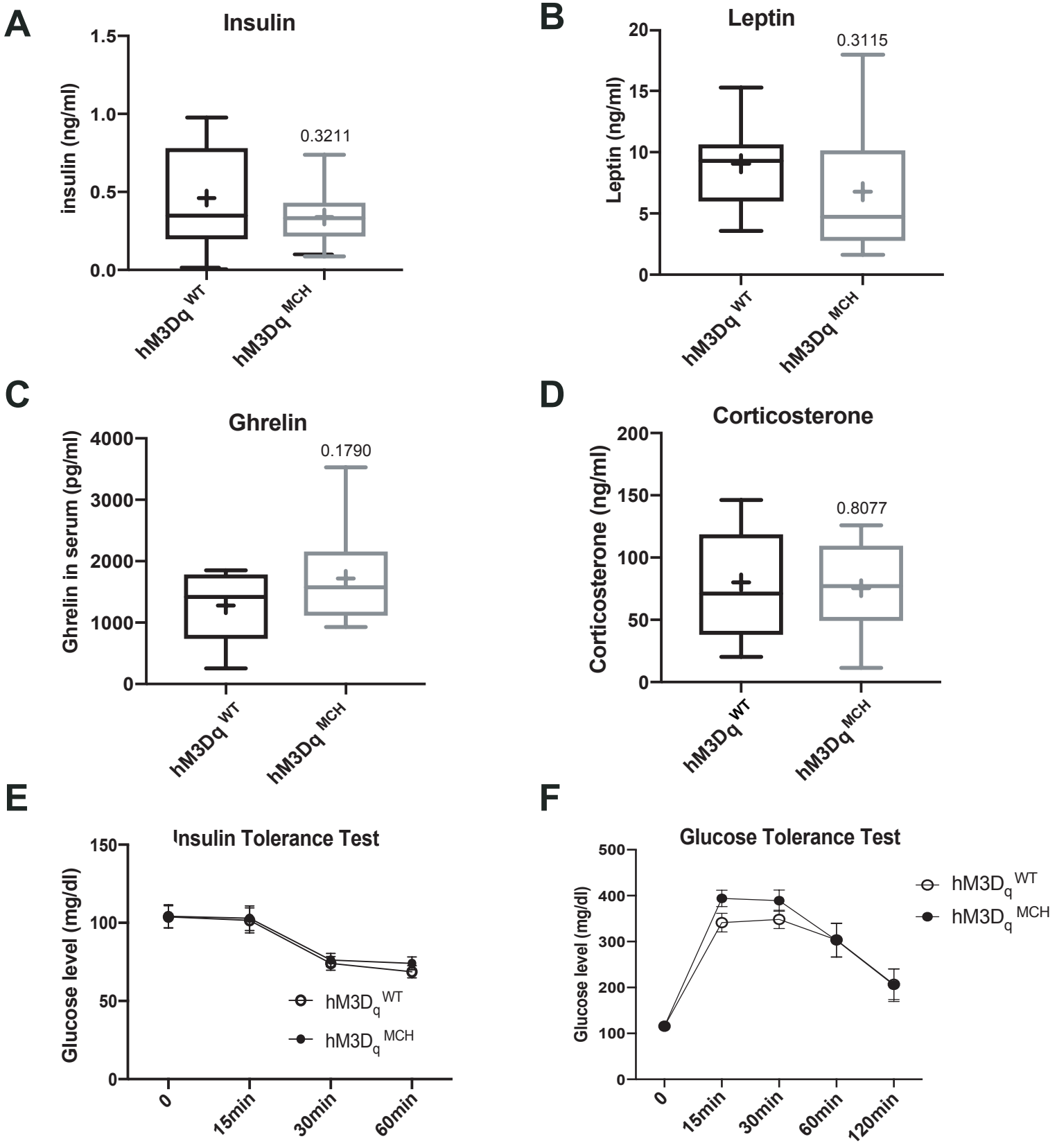


Figure S3. Related to Figure 2 and 3. Systemic metabolic parameters in CNO-treated control and hM3Dq^{MCH} mice. (A)-(D) Serum Insulin, Leptin, Ghrelin and Corticosterone levels of fasted and 4 hrs CNO-treated control (n=8-10) and hM3Dq^{MCH} mice (n=10-13). (E) and (F) Insulin tolerance test and Glucose tolerance test in fasted and 4 hrs CNO-treated control (n=10) and hM3Dq^{MCH} mice (n=13). Statistics: unpaired student T-test,for A-D; Two way ANOVA Sidack Post hoc test, for E and F.

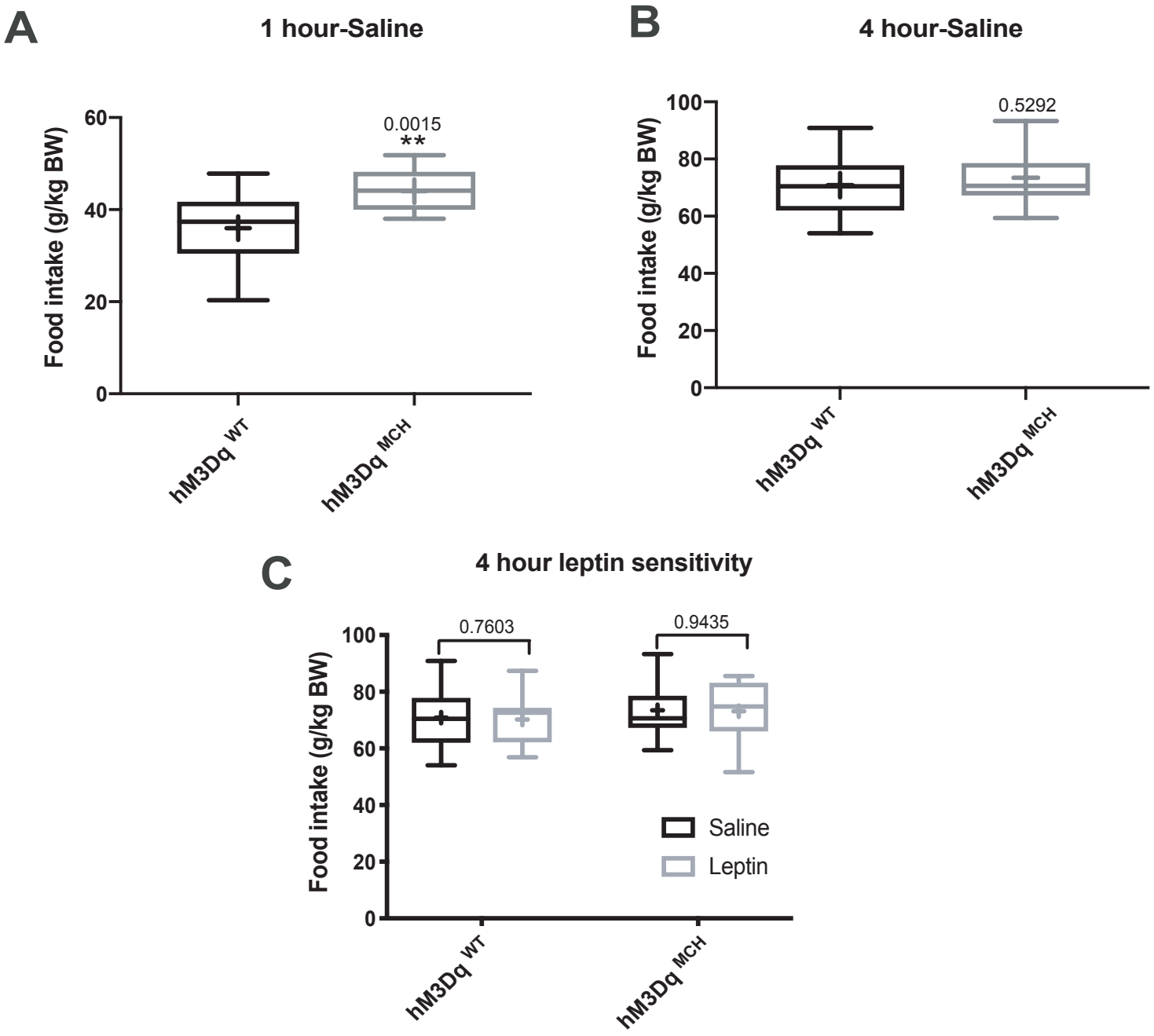
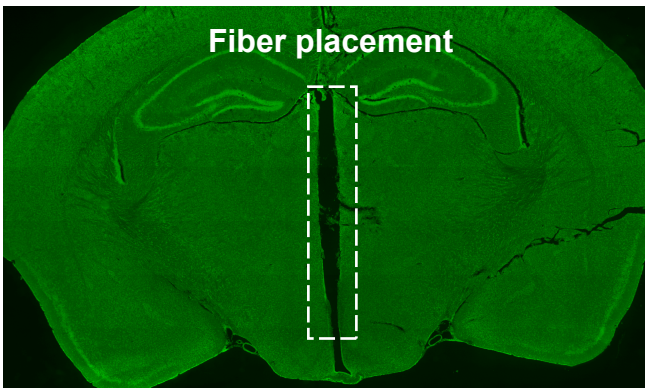
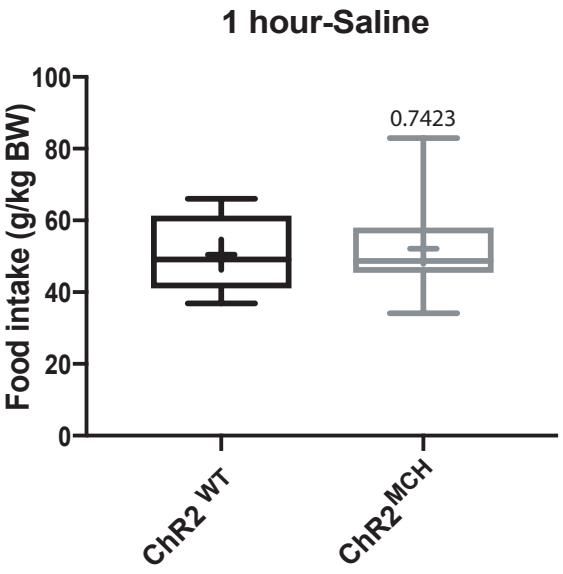


Figure S4. Related to Figure 4. Feeding regulation in hM3Dq^{MCH} mice .
(A) and (B), 1 hr and 4 hrs food intake in fasted and 4 hrs CNO-treated control (n=13) and hM3Dq^{MCH} mice (n=13) with saline (leptin vehicle) injected at half hour before refeeding. (C), Leptin sensitivity at 4 hrs refeeding in fasted and 4 hrs CNO-treated control and hM3Dq^{MCH} mice. Statistics: unpaired Student's T-test, **p < 0.01, except paired Student's T-test in leptin sensitivity.

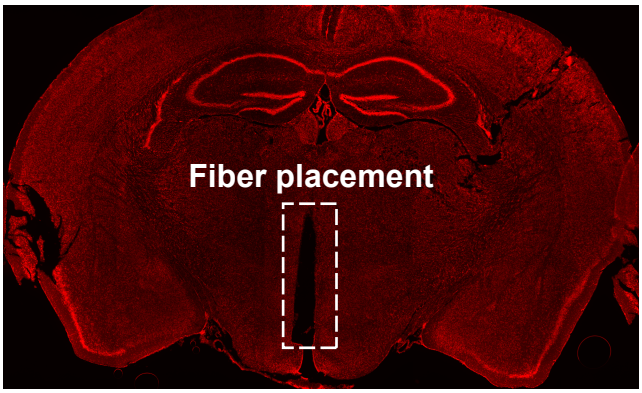
A



B

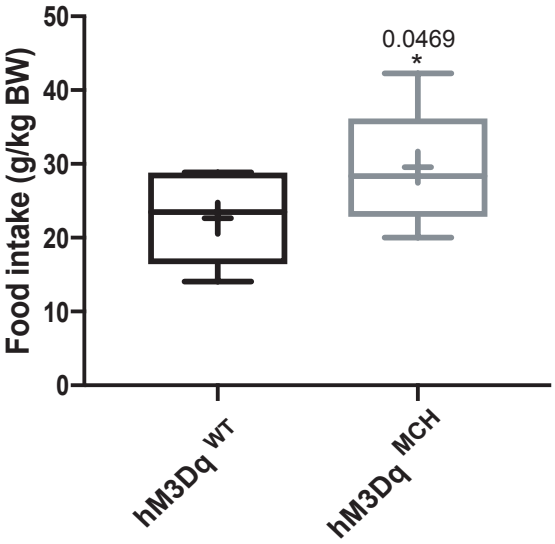


C

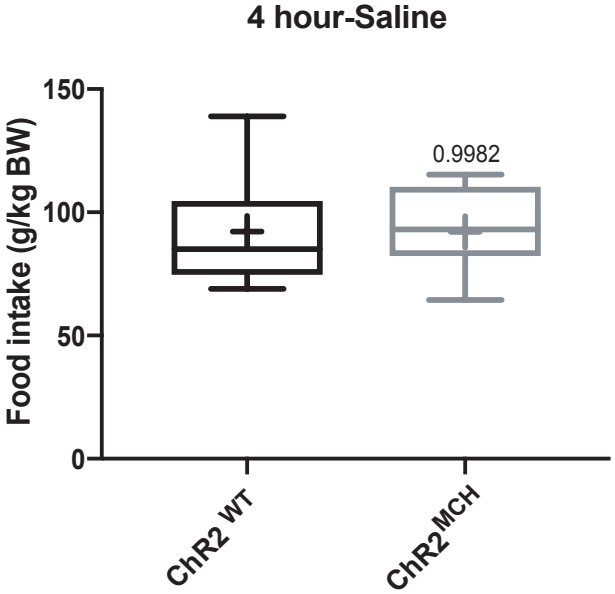


hM3Dq WT or MCH mice

D



E



F

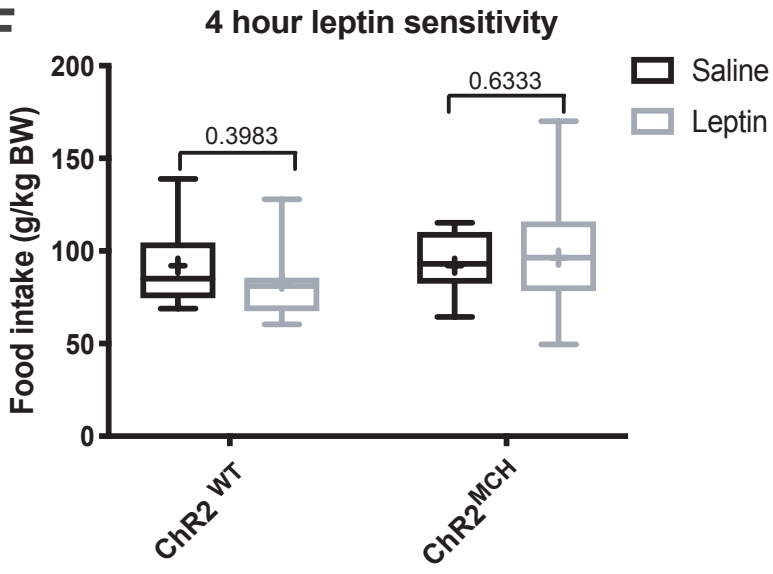


Figure S5. Related to Figure 5. Optogenetic MCH-ME projection stimulation does not change refeeding upon the saline injection. (A) Placement of optical fiber implantation into third ventricle in optogenetic experiments. Green: neuronal tracer stained section from perfused mice with optical fiber implanted. Dashed white square illustrates the fiber trace. (B) 1 hr refeeding in fasted and 3.5 hrs light illuminated control (n=12) and $ChR2^{MCH}$ mice (n=11) injected with saline (leptin vehicle) half hour before refeeding. (C) Placement of optical fiber into control and $hM3Dq^{MCH}$ mice. Red: neuronal tracer stained section from perfused mice with optical fiber implanted. (D) 1 hr food intake in fasted and 4 hrs CNO-treated and fiber implanted control (n=6) and $hM3Dq^{MCH}$ mice (n=8). (E) and (F) 4 hr refeeding in fasted and 3.5 hrs light illuminated control (n=12) and $ChR2^{MCH}$ mice (n=11) injected with saline (leptin vehicle) half hour before refeeding and 4 hrs leptin sensitivity in those mice. Statistics: unpaired Student's T-test, *$p < 0.05$, except paired Student's T-test in leptin sensitivity.

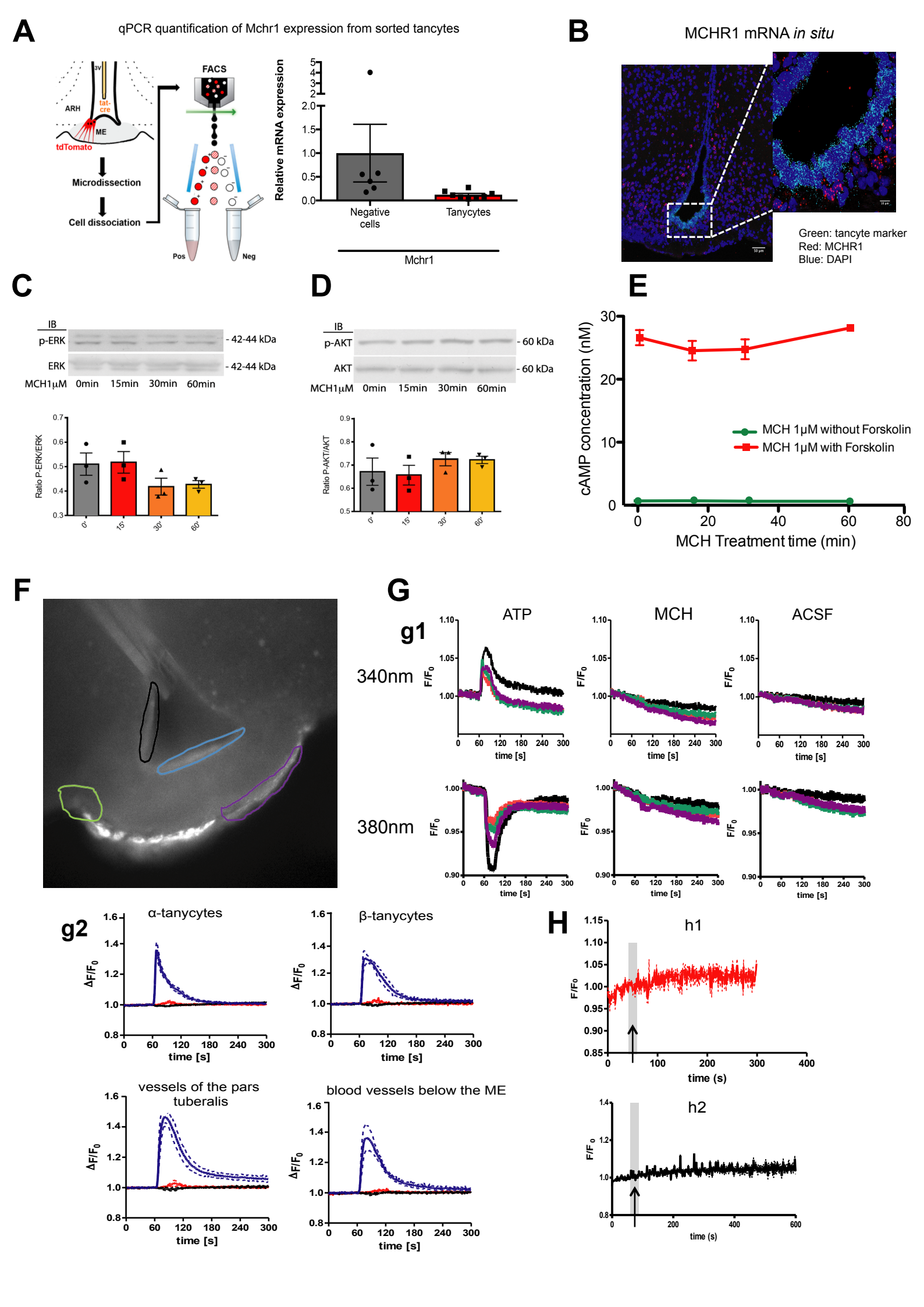
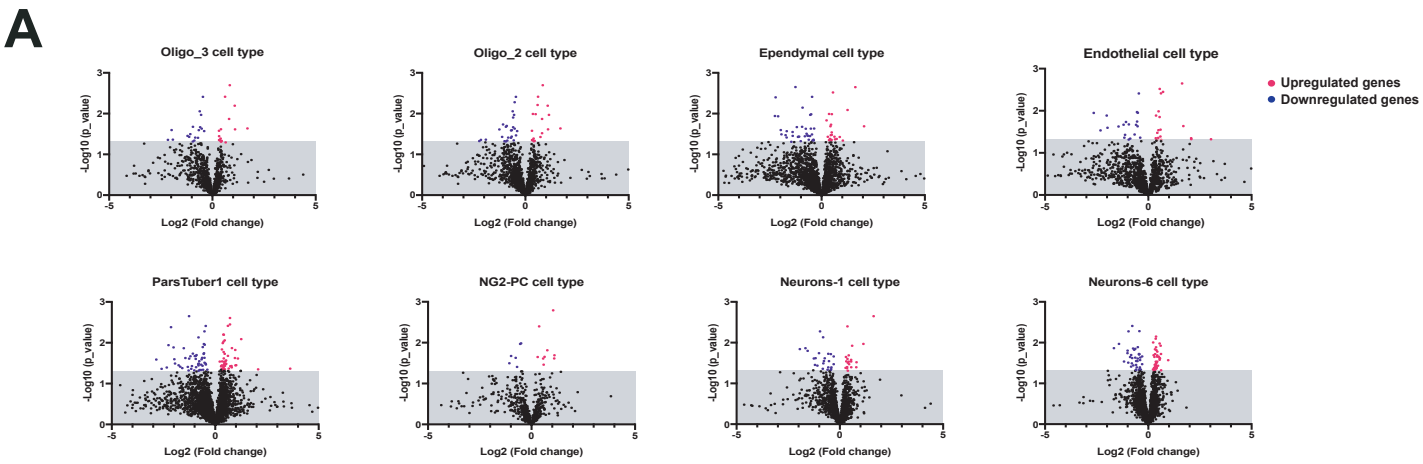
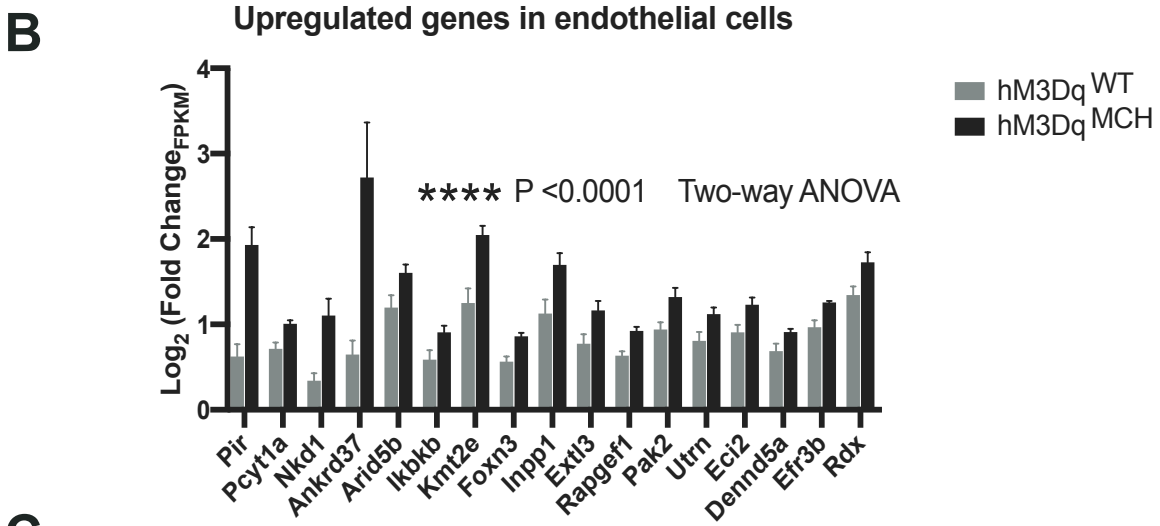

A
qPCR quantification of Mchr1 expression from sorted tancytes
3V
tat-cre
ARH
ME
tdTomato
Microdissection
Cell dissociation
FACS
Pos
Neg
Relative mRNA expression
Negative cells
Tanycytes
Mchr1
B
MCHR1 mRNA in situ
Green: tancyte marker
Red: MCHR1
Blue: DAPI
C
IB
p-ERK
ERK
42-44 kDa
MCH1μM 0min 15min 30min 60min
Ratio P-ERK/ERK
D
IB
p-AKT
AKT
60 kDa
MCH1μM 0min 15min 30min 60min
Ratio P-AKT/AKT
E
cAMP concentration (nM)
MCH 1μM without Forskolin
MCH 1μM with Forskolin
MCH Treatment time (min)
F
G
g1
ATP
MCH
ACSF
340nm
380nm
F/F0
time [s]
g2
α-tanycytes
β-tanycytes
vessels of the pars tuberalis
blood vessels below the ME
ΔF/F0
H
h1
h2
F/F0
time (s)

Figure S6. Related to Figure 6 and 7. MCH does not alter tanycyte signaling.
(A) Illustration of FACS sorted purified tanycytes from the ME (left) and qPCR quantification of Mchr1 expression in purified tdTomato marked tanycytes and non-tdTomato cells of the ME (right). (B) mRNA expression of Mchr1 mRNA by *in situ* hybridization on ME sections. Red, Mchr1; Green, tanycyte marker, Ppp1r1b; Blue, DAPI. (C) Western blotting of ERK and pERK expression after 0, 15 min, 30 min and 60 min of 1 µM MCH incubation (upper panel) and ratio of pERK/ERK expression (lower panel). (D) Western blotting of AKT and pAKT protein expression after 0, 15 min, 30 min and 60 min of 1 µM MCH incubation (upper panel) and ratio of pAKT/AKT comparison (lower panel). (E) cAMP concentration changes after 1 µM MCH 0, 15 min, 30 min and 60 min of incubation with and without forskolin.
(F) Illustration of the regions of interest for calcium imaging in ME slices. (G) Representative time course of fluorescence changes in a living brain slice loaded with Fura2 emitted with 340 nm (g1 upper panel) and 380 nm (g1 lower panel) after direct stimulation with ATP (1 mM, 2 µl over 30 s; a and b), MCH (1 mM, 2 µl over 30 s; c and d) and ACSF (2 µl over 30 s; e and f). Black, alpha tanycytes; red, beta tanycytes; green, blood vessels in the pars tuberalis; purple, blood vessels beneath the median eminence. g2, Average time curves of ΔF/F0 in α-tanycytes, β-tanycytes, vessels of the pars tuberalis and blood vessels beneath the median eminence of 3 brain slices loaded with Fura2 after direct stimulation with ATP (1 mM, 2 µl over 30 s; blue), MCH (1 mM, 2 µl over 30 s; red) and ACSF (2 µl over 30 s; black).
(H) Average of ΔF/F0 in ME-tanycytes of 3 brain slices loaded with Fura2 after direct stimulation with Glutamate (100 µM, 100 µl over 30 s; red) (h1) and baclofen (1 mM, 100 µl over 30 s; black) (h2).


A
Oligo_3 cell type
Oligo_2 cell type
Ependymal cell type
Endothelial cell type
ParsTuber1 cell type
NG2-PC cell type
Neurons-1 cell type
Neurons-6 cell type
-Log10 (p_value)
Log2 (Fold change)
Upregulated genes
Downregulated genes


B
Upregulated genes in endothelial cells
Log2 (Fold ChangeFPKM)
hM3Dq WT
hM3Dq MCH
**** P <0.0001 Two-way ANOVA
Pir
Pcyt1a
Nkd1
Ankrd37
Arid5b
Ikbkb
Kmt2e
Foxn3
Inpp1
Extl3
Rapgef1
Pak2
Utrn
Eci2
Dennd5a
Efr3b
Rdx

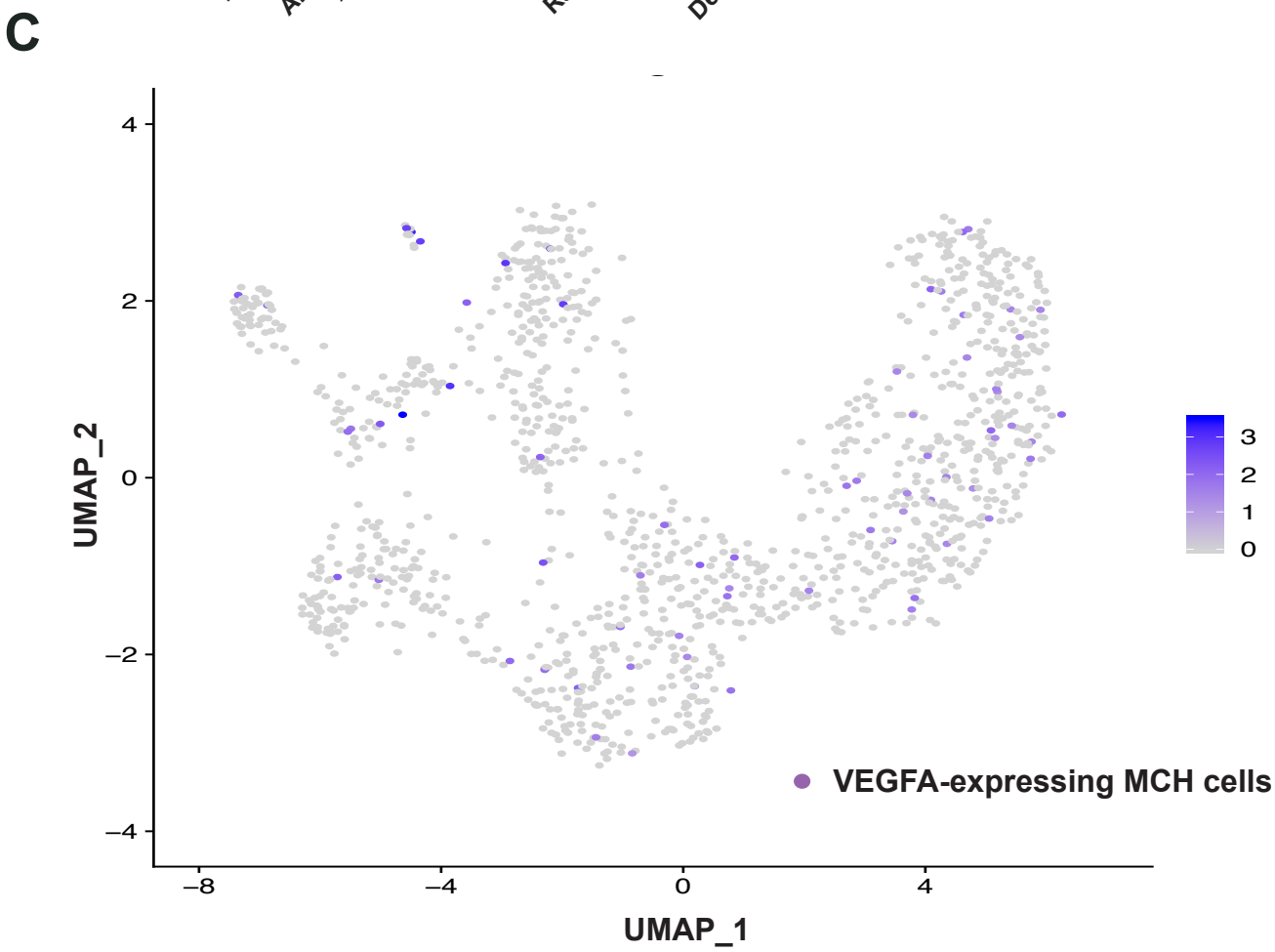

C
UMAP_2
UMAP_1
VEGFA-expressing MCH cells

Figure S7. Related to Figure 6. PhosphoRibotrap-based analysis of all cell types regulated by MCH neuron activation. (A) Neuronal, glia and endothelial cell types in the ARC were regulated in 1 hr CNO treated hM3Dq^{MCH} mice. (B) Enriched gene expression of endothelial cell cluster in control and hM3Dq^{MCH} mice. (C) VEGFA expressing cells are distributed cross all the clusters of MCH neurons. Purple dots represent VEGFA positive cells.