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Supplemental information

Melanocortin-3 receptor expression in AgRP neurons is required for normal activation of the neurons in response to energy deficiency

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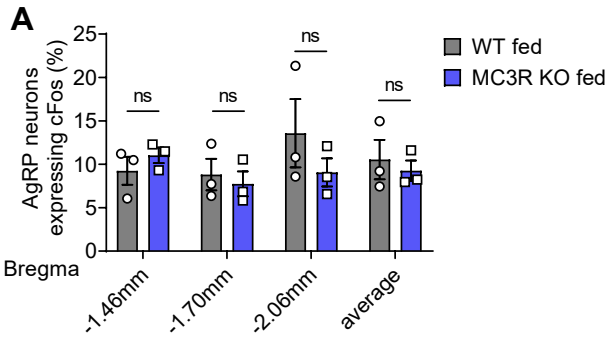
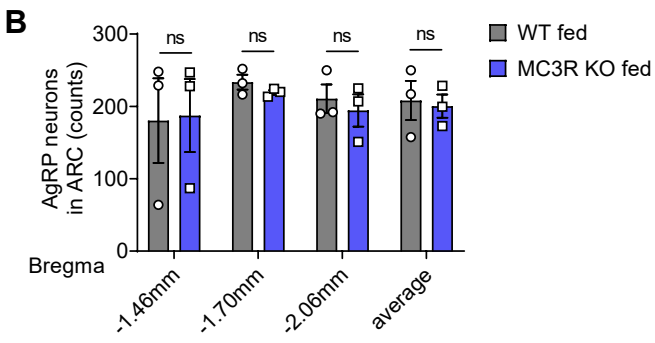
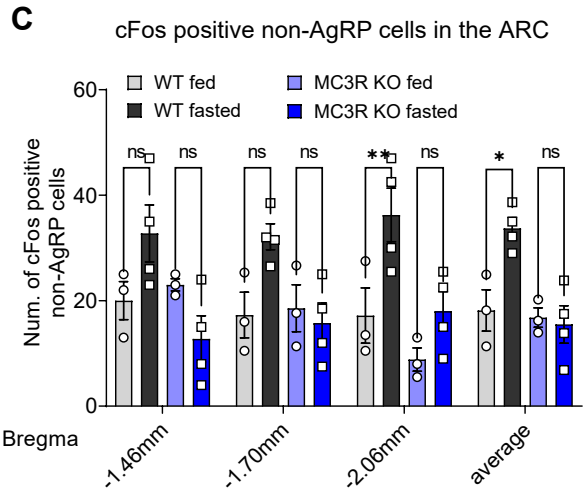
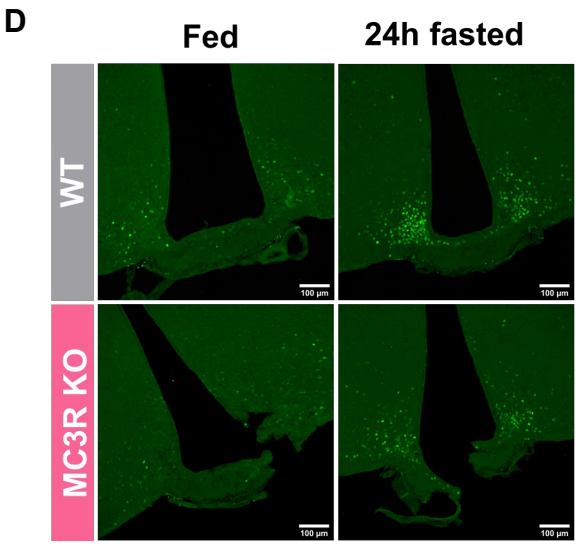
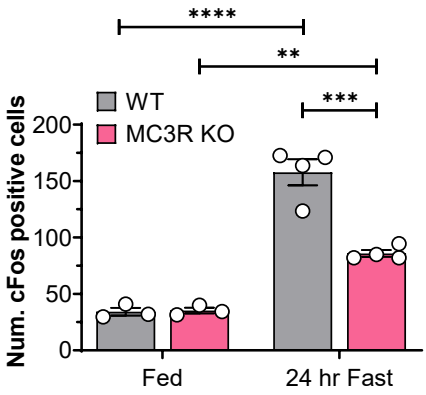

A
AgRP neurons expressing cFos (%)
25
20
15
10
5
0
ns
ns
ns
ns
WT fed
MC3R KO fed
Bregma
-1.46mm
-1.70mm
-2.06mm
average


B
AgRP neurons in ARC (counts)
300
200
100
0
ns
ns
ns
ns
WT fed
MC3R KO fed
Bregma
-1.46mm
-1.70mm
-2.06mm
average


C
cFos positive non-AgRP cells in the ARC
WT fed
WT fasted
MC3R KO fed
MC3R KO fasted
Num. of cFos positive non-AgRP cells
60
40
20
0
ns
ns
ns
ns
**
ns
*
ns
Bregma
-1.46mm
-1.70mm
-2.06mm
average


D
Fed
24h fasted
WT
MC3R KO
100 µm


E
****
**
***
WT
MC3R KO
Num. cFos positive cells
200
150
100
50
0
Fed
24 hr Fast

♀

Figure S1. MC3R is required for the activation of AgRP neurons by fasting. Related to Figure 1.

(A, B) Quantifications of the percentage of cFos-positive and the number of GFP cells in the ARC of *ad lib* fed WT and MC3R KO NPY-GFP male mice (n = 3 mice for all groups).

(C) Quantifications of the number of cFos positive non-GFP cells in the ARC of WT and MC3R KO NPY-GFP, fed or 24h-fasted male mice (n=3~4 mice for all groups).

(D) Representative images of cFos immunostaining in WT and MC3R KO fed or 24h-fasted female mice. Scale bar, 100 um.

(E) Quantifications of the number of cFos-positive cells in the ARC of WT and MC3R KO fed or 24h-fasted female mice (n = 3~4 mice for all groups).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 in two-way ANOVA with Sidak's posthoc test.

Figure S2. Defective AgRP neuron activation in fasted MC3R KO mice is not a result of developmental adaptation or differential reduction in leptin. Related to Figure 1.

(A) Schematic showing the unilateral injection of scramble or MC3R shRNA virus into the ARC of adult WT male mice.

(B) Representative images showing the expression of scramble or MC3R shRNA and cFos signals in the ARC of adult WT male mice following a 24h fast. Scale bar, 100um.

(C, D) Quantifications of the number of cFos-positive cells and fasting-induced fold change of cFos number in the ARC (n=4~9 mice for all groups).

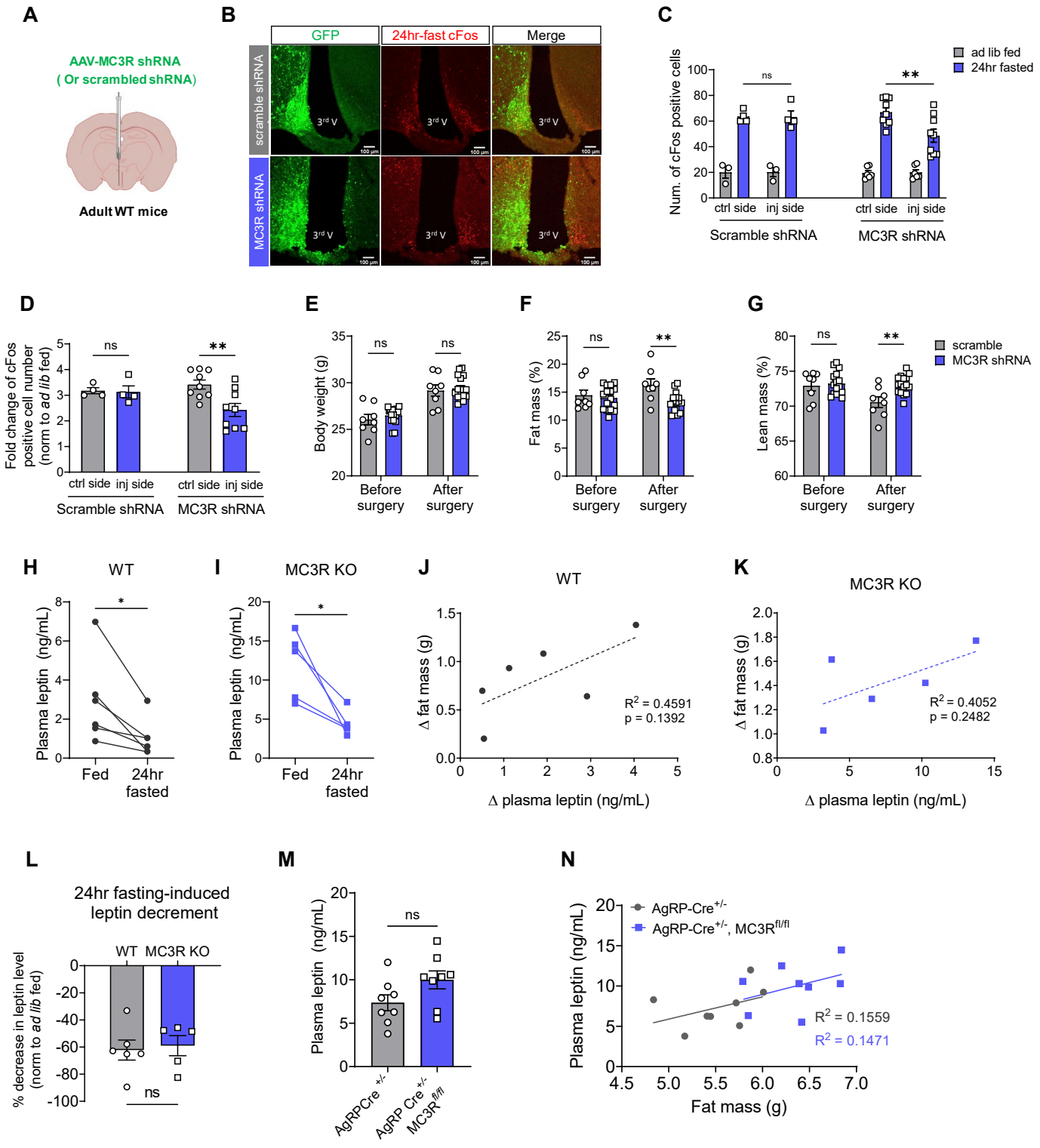
(E-G) Body weight and body composition of scramble or MC3R shRNA injected WT male mice (n = 8~16 mice for each group).

(H, I and L) Fasting-induced leptin level change in WT and MC3R KO male mice (n = 5~6 mice for each group).

(J, K) Relationship between decreased plasma leptin level and decreased fat mass in response to 24h fasting in WT and MC3R KO male mice.

(M, N) Plasma leptin levels and the relationship between leptin level and fat mass in AgRP-Cre and AgRP-specific MC3R KO male mice (n = 8 mice for each group).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; *p < 0.05; **p < 0.01 in unpaired Student's t-test and two-way ANOVA with Sidak's posthoc test.


A
AAV-MC3R shRNA
(Or scrambled shRNA)
Adult WT mice
B
GFP
24hr-fast cFos
Merge
scramble shRNA
MC3R shRNA
3rd V
C
ad lib fed
24hr fasted
Num. of cFos positive cells
ns
**
ctrl side
inj side
Scramble shRNA
MC3R shRNA
D
Fold change of cFos positive cell number (norm to ad lib fed)
E
Body weight (g)
Before surgery
After surgery
F
Fat mass (%)
G
Lean mass (%)
scramble
MC3R shRNA
H
WT
Plasma leptin (ng/mL)
Fed
24hr fasted
I
MC3R KO
J
WT
Δ fat mass (g)
Δ plasma leptin (ng/mL)
R2 = 0.4591
p = 0.1392
K
MC3R KO
R2 = 0.4052
p = 0.2482
L
24hr fasting-induced leptin decrement
WT MC3R KO
% decrease in leptin level (norm to ad lib fed)
M
AgRPCre +/-
AgRP Cre +/- MC3R fl/fl
N
AgRP-Cre+/-
AgRP-Cre+/-, MC3Rfl/fl
R2 = 0.1559
R2 = 0.1471
Fat mass (g)

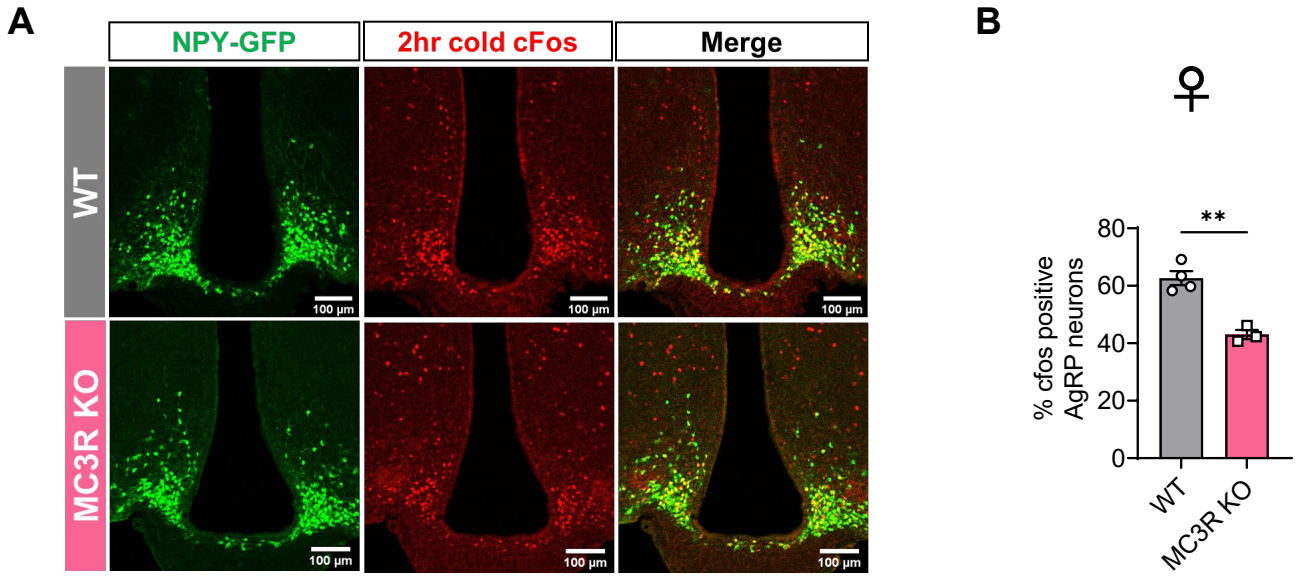


Figure S3. MC3R is required in female mice for the activation of AgRP neurons in response to cold. Related to Figure 2.

(A) Representative images of cFos immunostaining in WT and MC3R KO NPY-GFP female mice following 2h cold exposure. Scale bar, 100 um.

(B) Quantifications of the percentage of cFos-positive GFP cells in the ARC of WT and MC3R KO NPY-GFP female mice (n = 3~4 mice for each group).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; **p < 0.01 in unpaired Student's t-test.

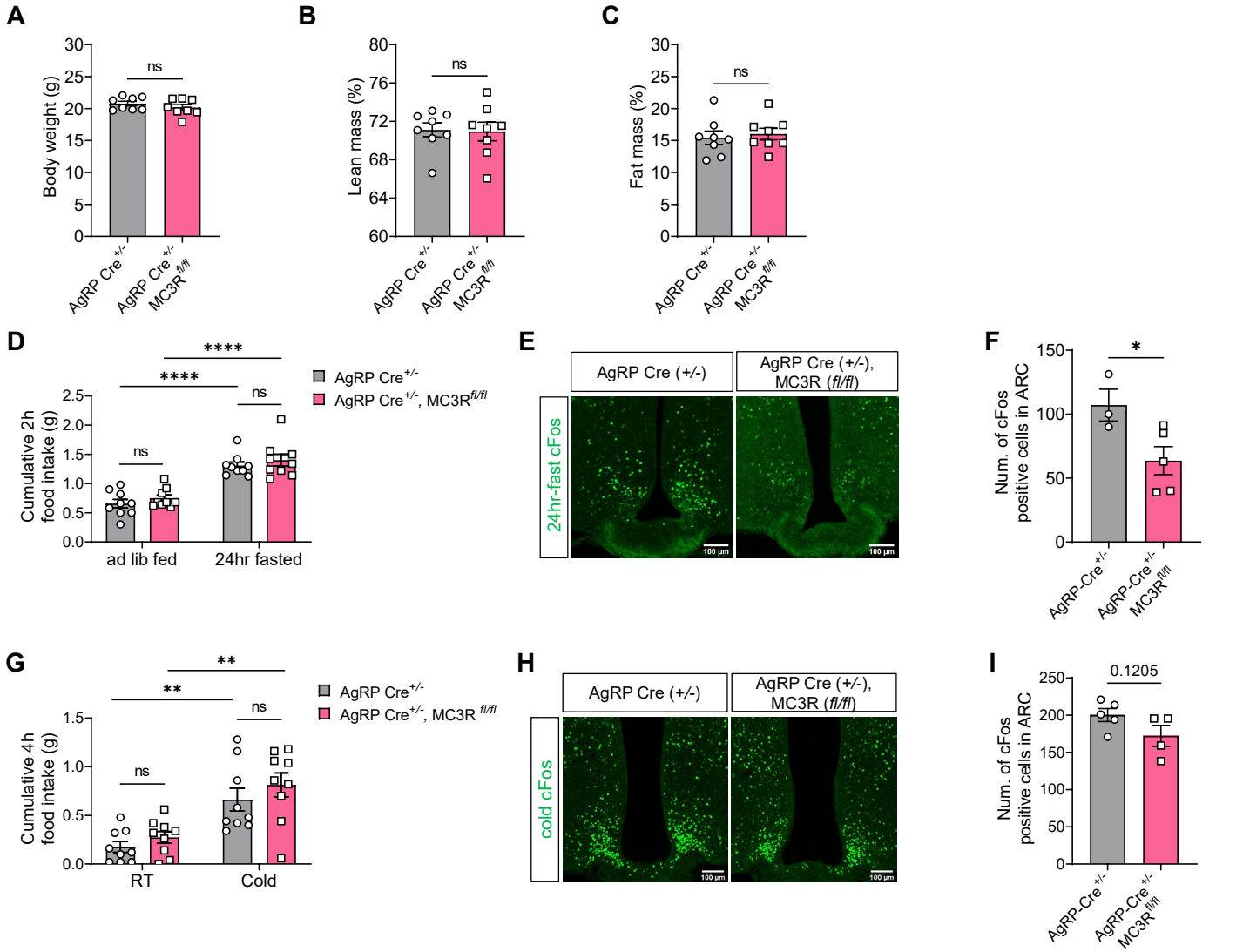


Figure S4. MC3R deletion in AgRP neurons impairs energy deficiency sensing in females in response to fasting, but not cold. Related to Figure 4.

(A-C) Body weight and body composition of AgRP-Cre and AgRP-specific MC3R KO female mice (n = 8 mice for each group).

(D) Two-hour food intake of AgRP-Cre and AgRP-specific MC3R KO female mice following a 24hr fast (n = 9 mice for each group).

(E, F) Representative images of cFos immunostaining, and quantifications of cFos-positive cell number in the ARC of 24h-fasted AgRP-Cre and AgRP-specific MC3R KO female mice. Scale bar, 100um (n = 3 or 5 mice for each group).

(G) Four-hour food intake of AgRP-Cre and AgRP-specific MC3R KO female mice under room temperature (RT, 22°C) or cold exposure (4°C) (n = 9 mice for each group).

(H, I) Representative images of cFos immunostaining, and quantifications of cFos-positive cell number in the ARC of cold-treated AgRP-Cre and AgRP-specific MC3R KO female mice. Scale bar, 100um (n = 4 or 5 mice for each group).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001 in unpaired Student's t-test and two-way ANOVA with Sidak's posthoc test.

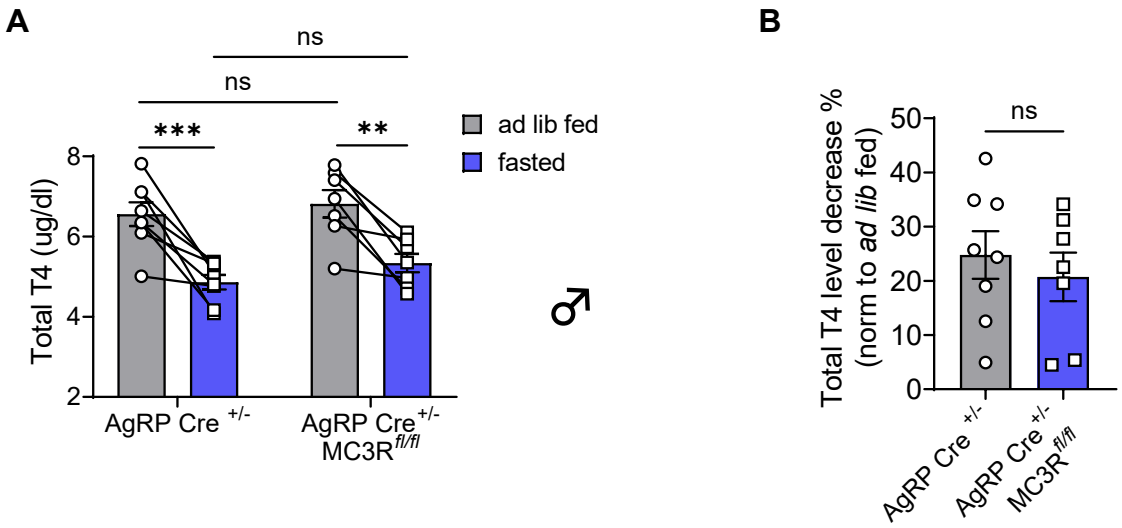


Figure S5. MC3R deletion in AgRP neurons does not impair fasting suppression of the HPT axis. Related to Figure 4.

(A) Serum total T4 levels measured by ELISA in fed versus 48h-fasted, AgRP Cre and AgRP-specific MC3R KO male mice (n =7 or 8 mice for each group).

(B) Decrement of serum total T4 levels upon 48hr fasting in AgRP Cre and AgRP-specific MC3R KO male mice (n =7 or 8 mice for each group).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; **p < 0.01; ***p < 0.001 in unpaired Student's t-test and two-way ANOVA with Sidak's posthoc test.

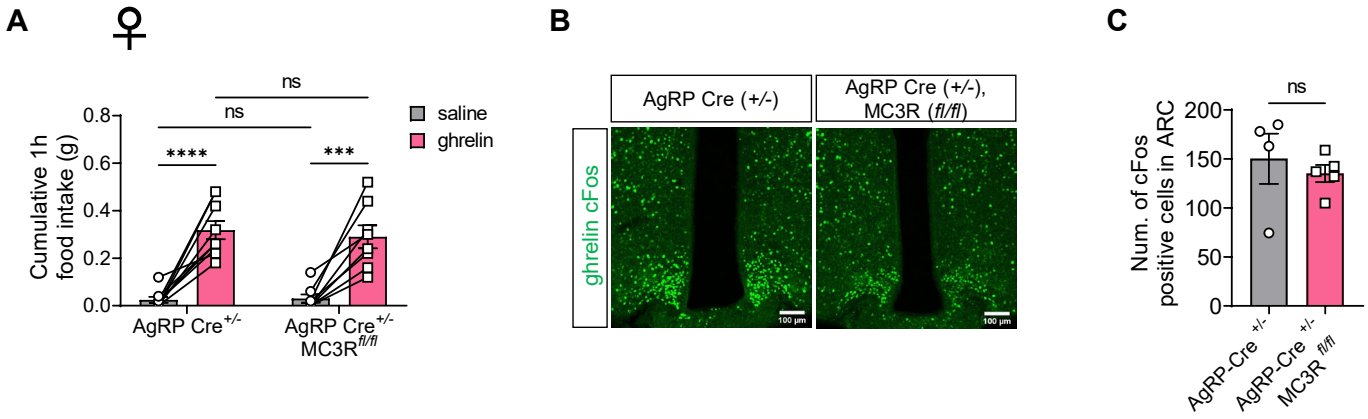


Figure S6. MC3R is not required for the orexigenic action of ghrelin on AgRP neurons in females. Related to Figure 6.

(A) One-hour food intake of AgRP-Cre and AgRP-specific MC3R KO female mice given saline or ghrelin injection (1.6 mg/kg, i.p.) (n = 8~9 mice for each group).

(B, C) Representative images of cFos immunostaining, and quantifications of cFos-positive cell number in the ARC of AgRP-Cre and AgRP-specific MC3R KO female mice in response to saline or ghrelin injection. Scale bar, 100um (n = 4~5 mice for each group).

Data are plotted as mean and all error bars represent the SEM. ns, non-significant; ***$p < 0.001$; ****$p < 0.0001$ in unpaired Student's t-test and two-way ANOVA with Sidak's posthoc test.