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# Supplemental Data

# Loss of GABAergic Signaling by AgRP Neurons to the Parabrachial Nucleus Leads to Starvation

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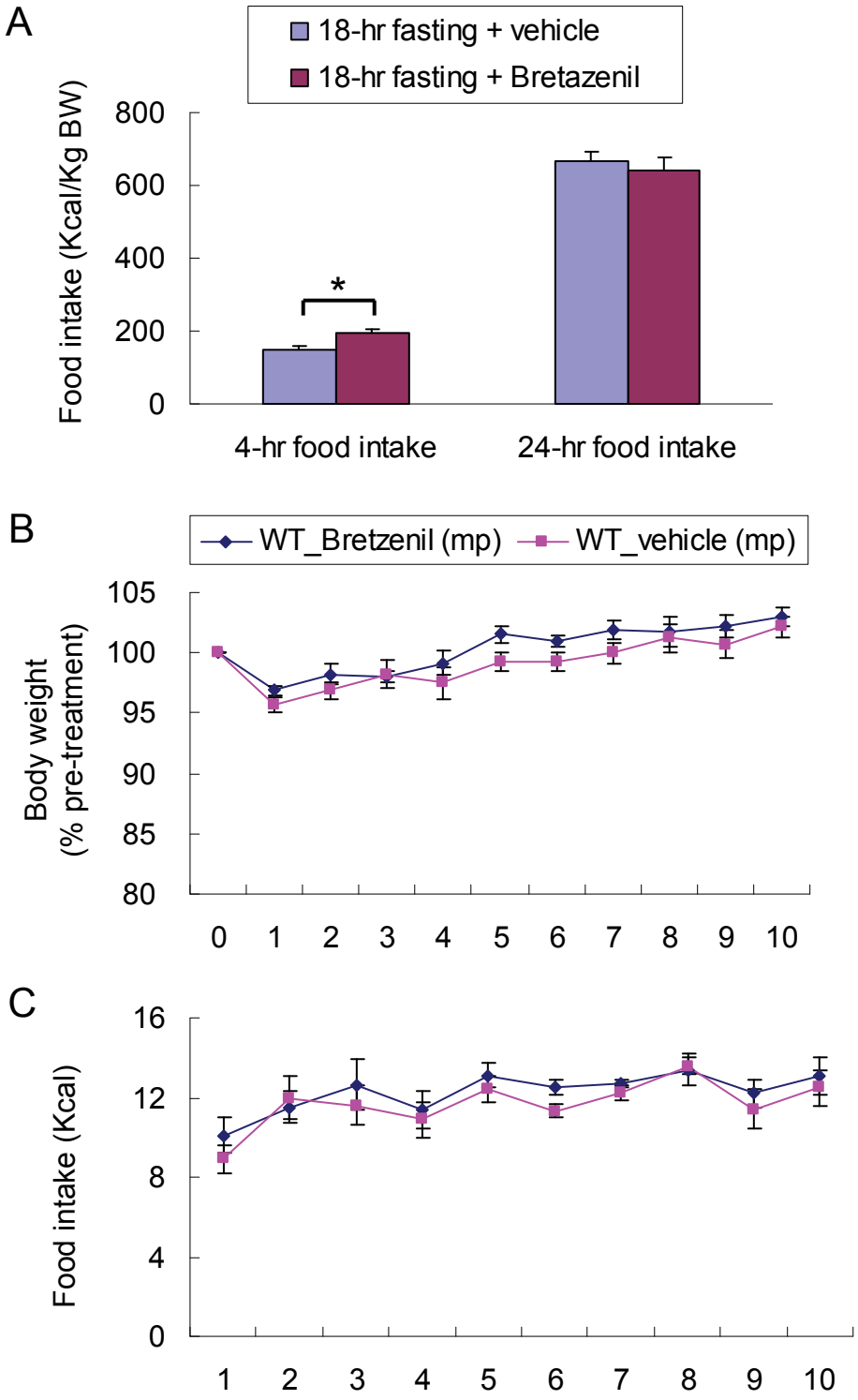


**Figure S1. Short- and long-term effects of bretazenil on the regulation of food intake and body weight.**

(***A***) Cumulative food intake of fasting wild-type mice after treatment of bretazenil (0.2 mg/kg, ip) or vehicle.

(***B***) Percentage of initial body weight of wild-type mice which are implanted mini pumps filled with either bretazenil (2.5 mg/ml) or vehicle.

(***C***) Intake of liquid diet by the mice described in ***B***.

N = 6 for each group. *, $p < 0.05$, ANOVA. Error bars represent the SEM.

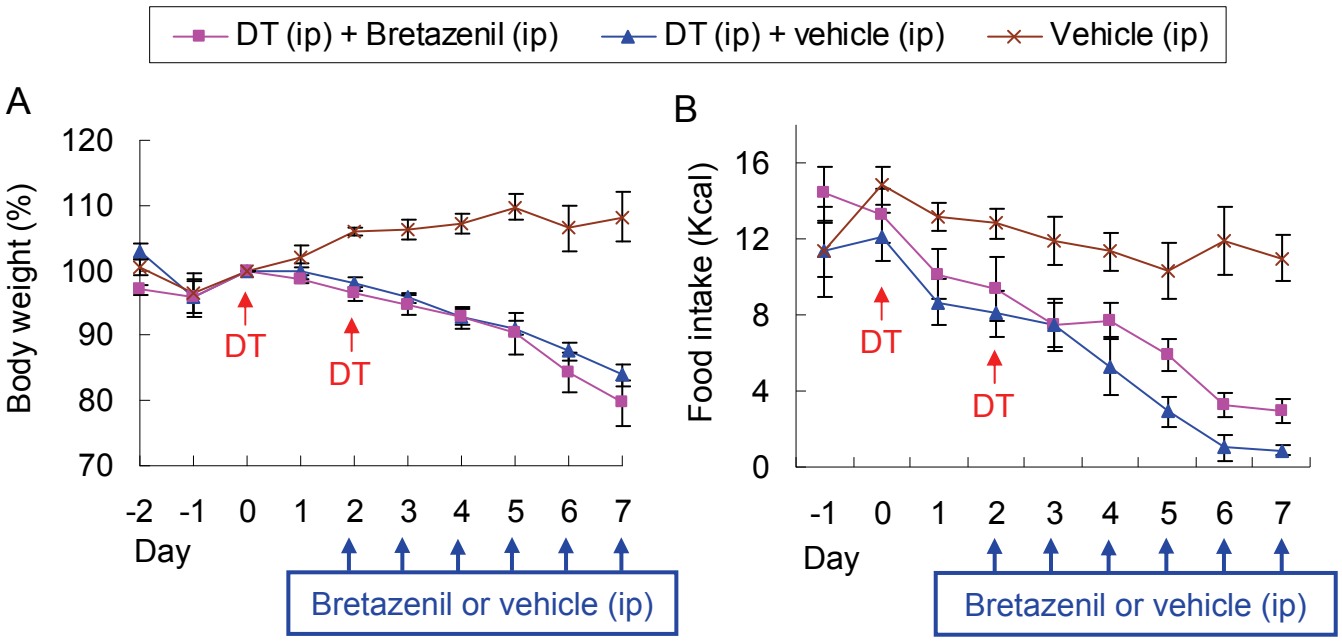


**Figure S2. Effects of daily intraperitoneal injection of bretazenil on body weight and food intake.**
(***A***) Percentage of initial body weight of *Agrp*$^{DTR/+}$ mice after two intramuscular injections of DT or vehicle, then followed by i.p. injection of either bretazenil (0.2 mg/kg) or vehicle for 6 consecutive days.
(***B***) Intake of liquid diet by the mice described in ***A***.
N = 6 - 8 for each group. Error bars represent the SEM.

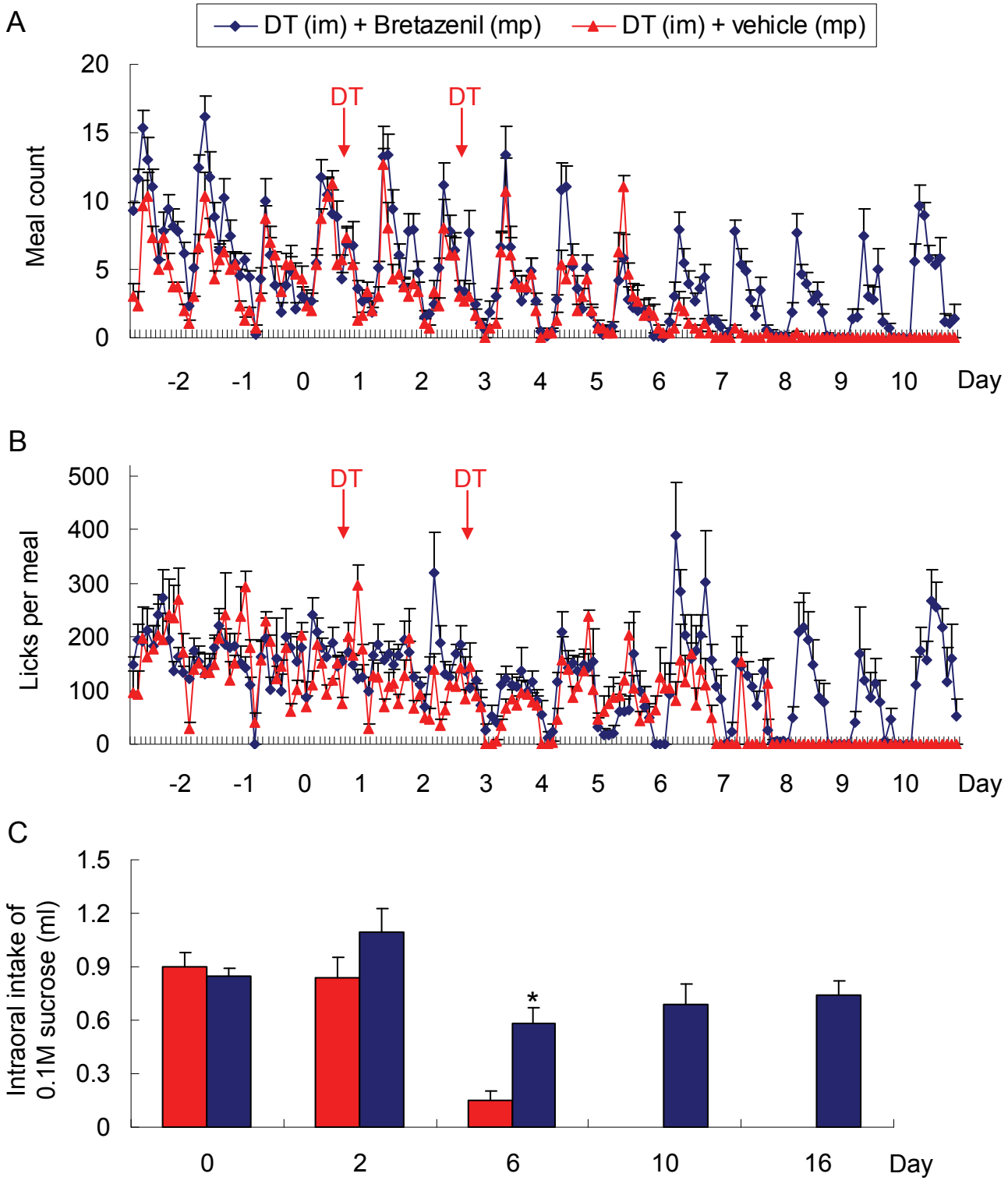


**Figure S3. Appetitive and consummatory responses in AgRP-ablated mice are rescued by chronic administration of bretazenil**

(***A, B***) Total number of meals (***A***) and average meal size (as shown by licks per meal, ***B***) were measured in DT-treated, *Agrp*$^{DTR/+}$ mice after subcutaneous implantation of mini-osmotic pumps loaded with either bretazenil (n = 12) or vehicle (n = 10). Meals are defined as the number of consecutive 10-s bins that contain five or more licks, and each meal must be separated by 12 or more 10-s bins in which no significant licking activity was recorded.

(***C***) Consumption of 0.1 M sucrose solution infused through cheek fistulas was measured in the mice described in ***A, B***. The control animals without bretazenil (gray bars) did not survive beyond day 6. *, $p < 0.01$, ANOVA. Error bars represent the SEM.

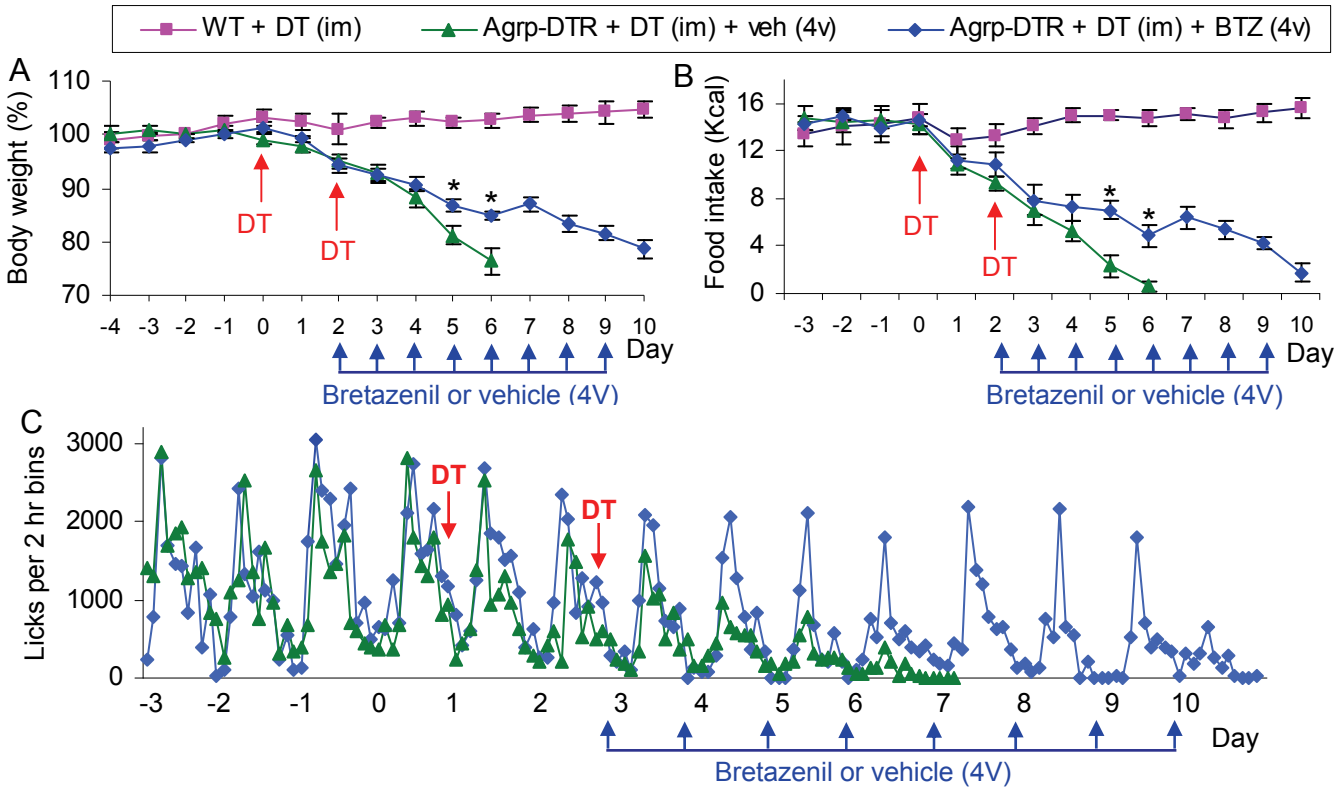


**Figure S4. 4th ventricle administration of bretazenil alleviates starvation in adult mice after ablation of AgRP neurons.**

(***A***) Percentage of initial body weight of wild-type mice and *Agrp*$^{DTR/+}$ mice after intramuscular injections of DT, then followed by daily 4v injection of either bretazenil (1 μg/mouse/day) or vehicle.

(***B***) Liquid diet intake of the mice described in ***A***.

(***C***) Licking activity of two groups of *Agrp*$^{DTR/+}$ mice described in ***A***. The average number of licks in 2-hr bins is plotted in both groups. *, $p < 0.01$, ANOVA.

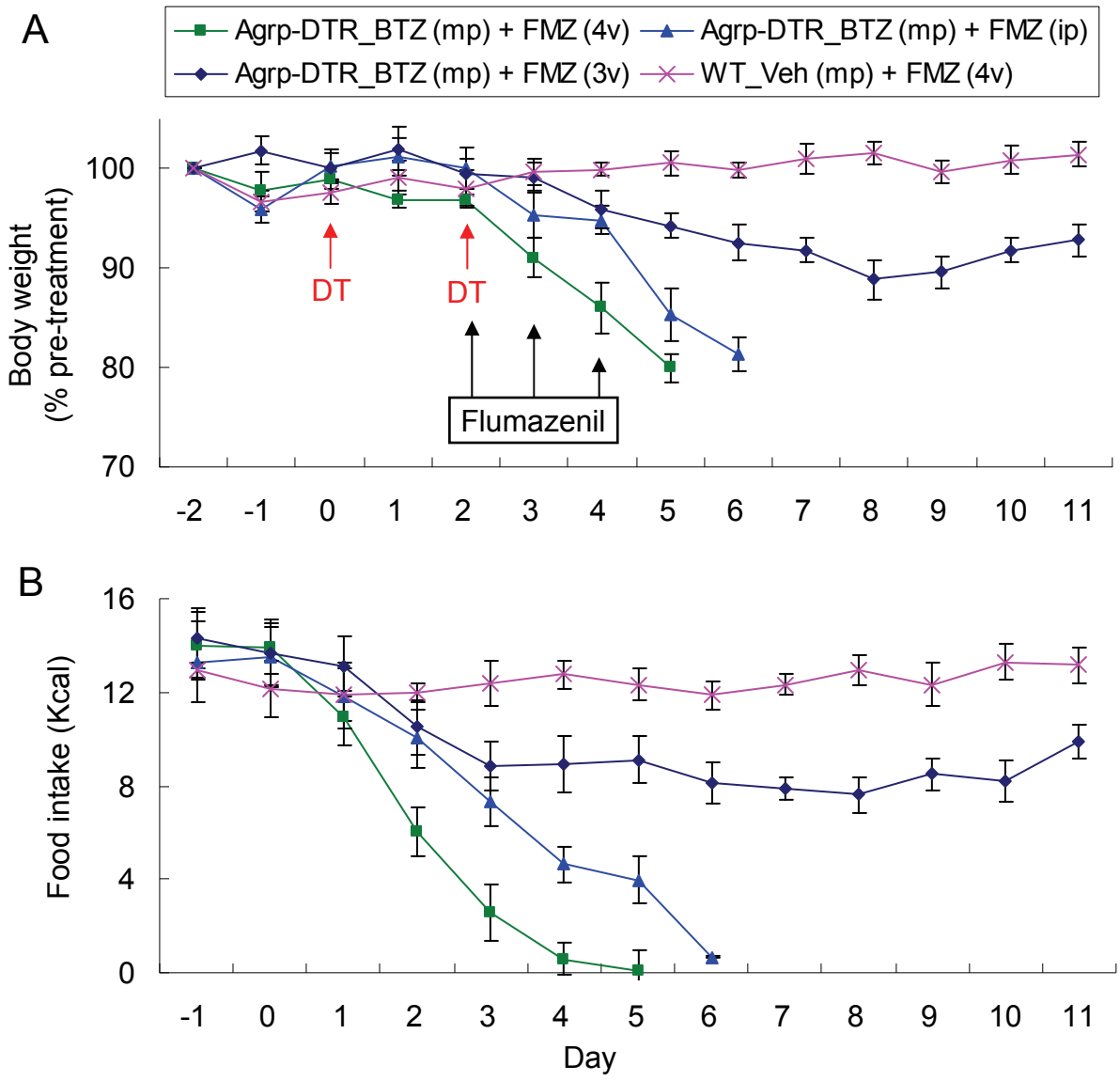


**Figure S5. Systemic or 4th ventricle administration of flumazenil prevents bretazenil-mediated restoration of body weight**

(***A***) Percentage of initial body weight of DT-treated, *Agrp*$^{DTR/+}$ and wide-type mice after implantation of minipumps loaded with bretazenil or vehicle, followed by administration of flumazenil either intraperitoneally or into the 3rd or 4th ventricle during Day 2 – Day 4.

(***B***) Intake of liquid diet by the mice described in ***A***.

N = 6 - 10 per group. Error bars represent the SEM.

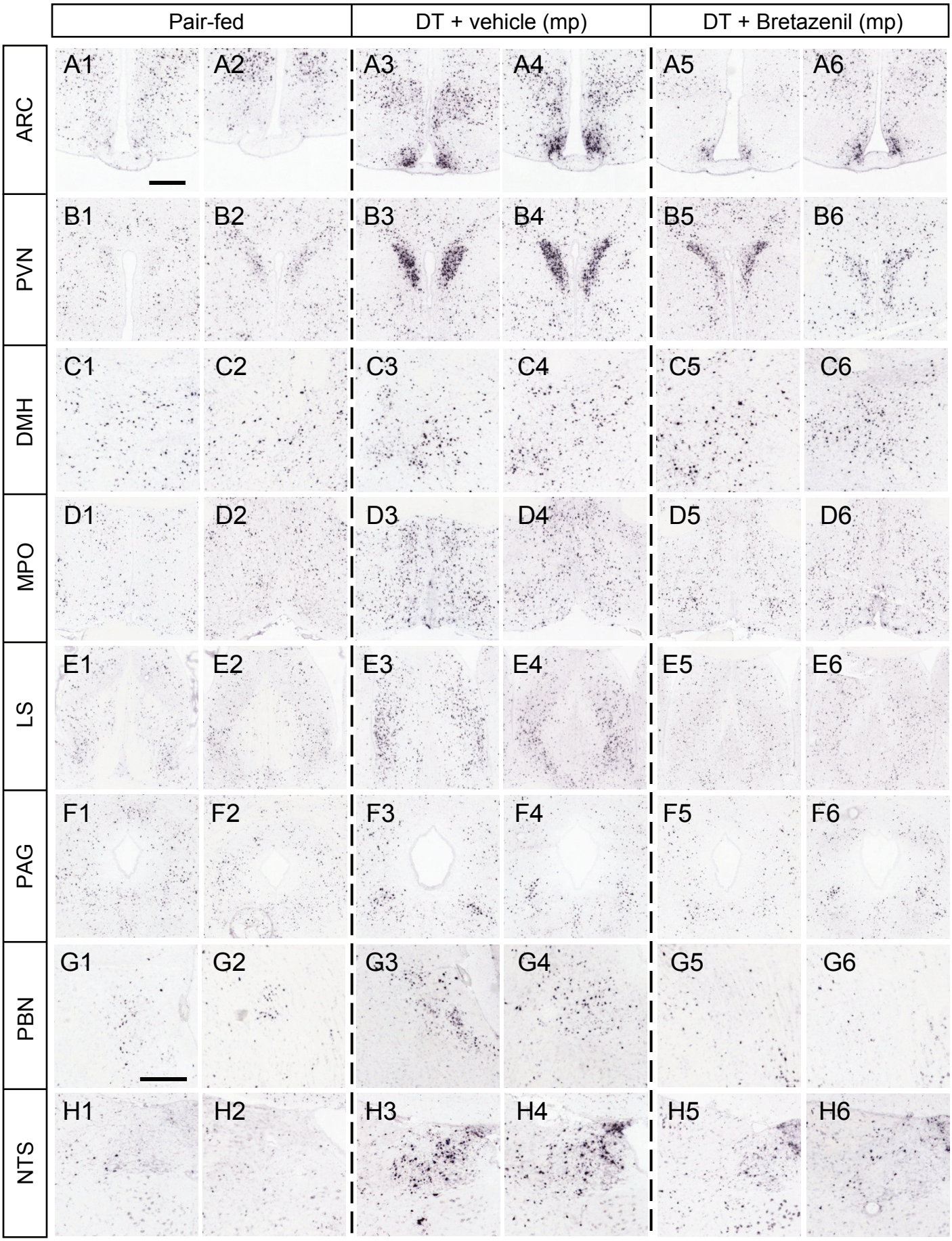
Pair-fed
DT + vehicle (mp)
DT + Bretazenil (mp)
ARC
PVN
DMH
MPO
LS
PAG
PBN
NTS
A1
A2
A3
A4
A5
A6
B1
B2
B3
B4
B5
B6
C1
C2
C3
C4
C5
C6
D1
D2
D3
D4
D5
D6
E1
E2
E3
E4
E5
E6
F1
F2
F3
F4
F5
F6
G1
G2
G3
G4
G5
G6
H1
H2
H3
H4
H5
H6

**Figure S6. Chronic administration of bretazenil downregulates *Fos* activation in selected brain regions of mice in which AgRP neurons have been ablated.**
(***A1-A6***) Representative pictures of *Fos* in situ hybridization in the ARC of pair-fed *Agrp*$^{DTR/+}$ mice (***A1, A2***) and DT-treated *Agrp*$^{DTR/+}$ mice that receive subcutaneous infusion of either vehicle (***A3, A4***) or bretazenil (***A5, A6***) via mini pumps.
(***B1-B6, C1-C6, D1-D6, E1-E6, F1-F6, G1-G6, H1-H6***) Representative pictures of *Fos* in situ hybridization in the PVN, DMH, MPO, LS, PAG, PBN, and NTS, of the mice described in ***A1-A6***, respectively.
Scale bar (in ***A1***) for ***A-F***, 400 μm; scale bar (in ***G1***) for ***G-H***, 400 μm; n = 4-6 for each group. See **Figure 3** of the main text for quantified results.

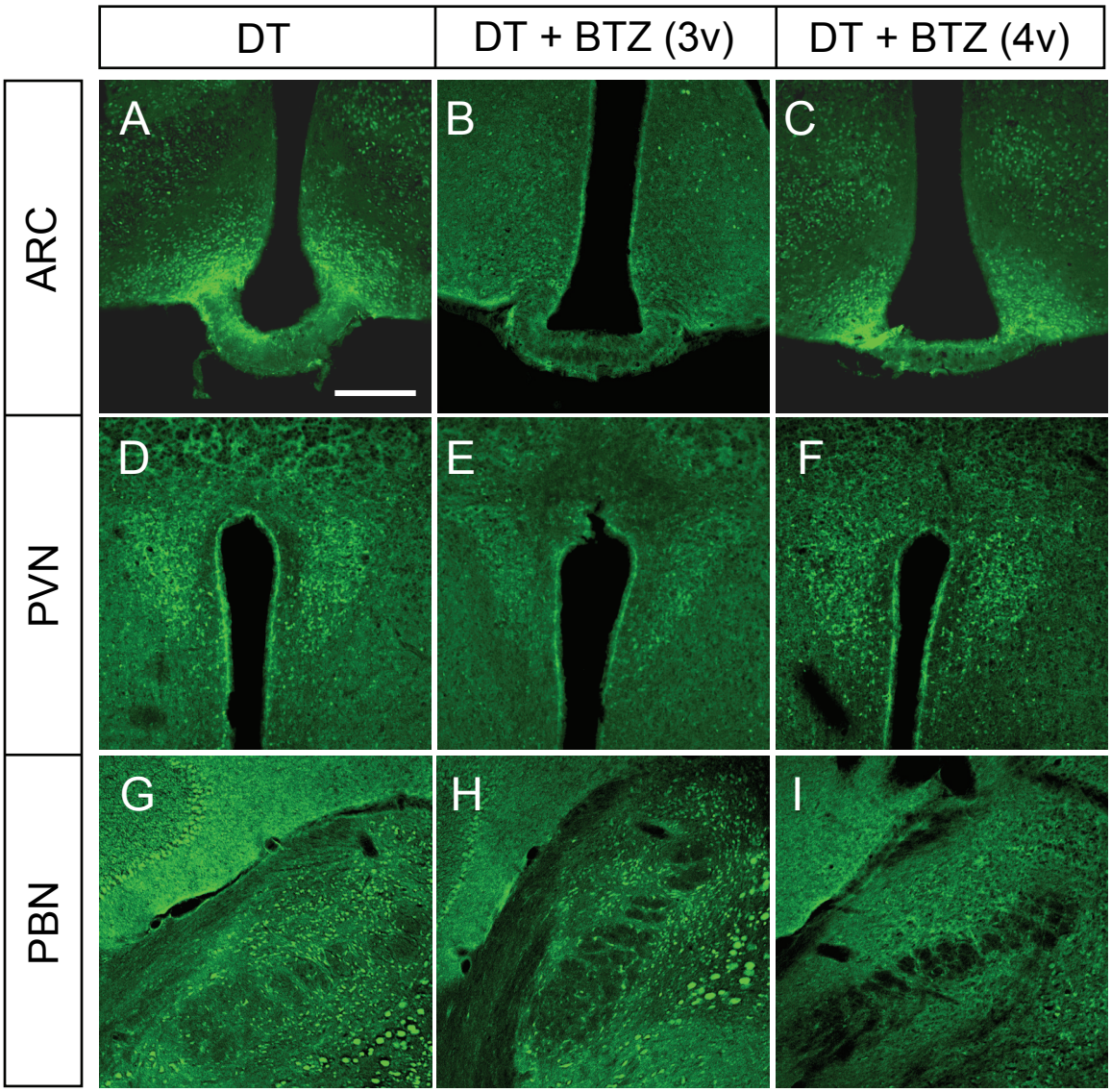


**Figure S7. Differentiated effects of chronic icv infusion of bretazenil on Fos activation in AgRP neuron-ablated mice.**
(***A-C***) Anti-Fos immunostaining in the ARC of DT-treated *Agrp*$^{DTR/+}$ mice, in some that also receive either 3$^{rd}$ or 4$^{th}$ ventricle administration of bretazenil via mini pumps.
(***D-I***) Fos immunostaining in the PVN, and PBN, of the mice described in ***A-C***, respectively. Scale bar (in ***A***) for ***A-I***, 400 μm; n = 6 for each group.

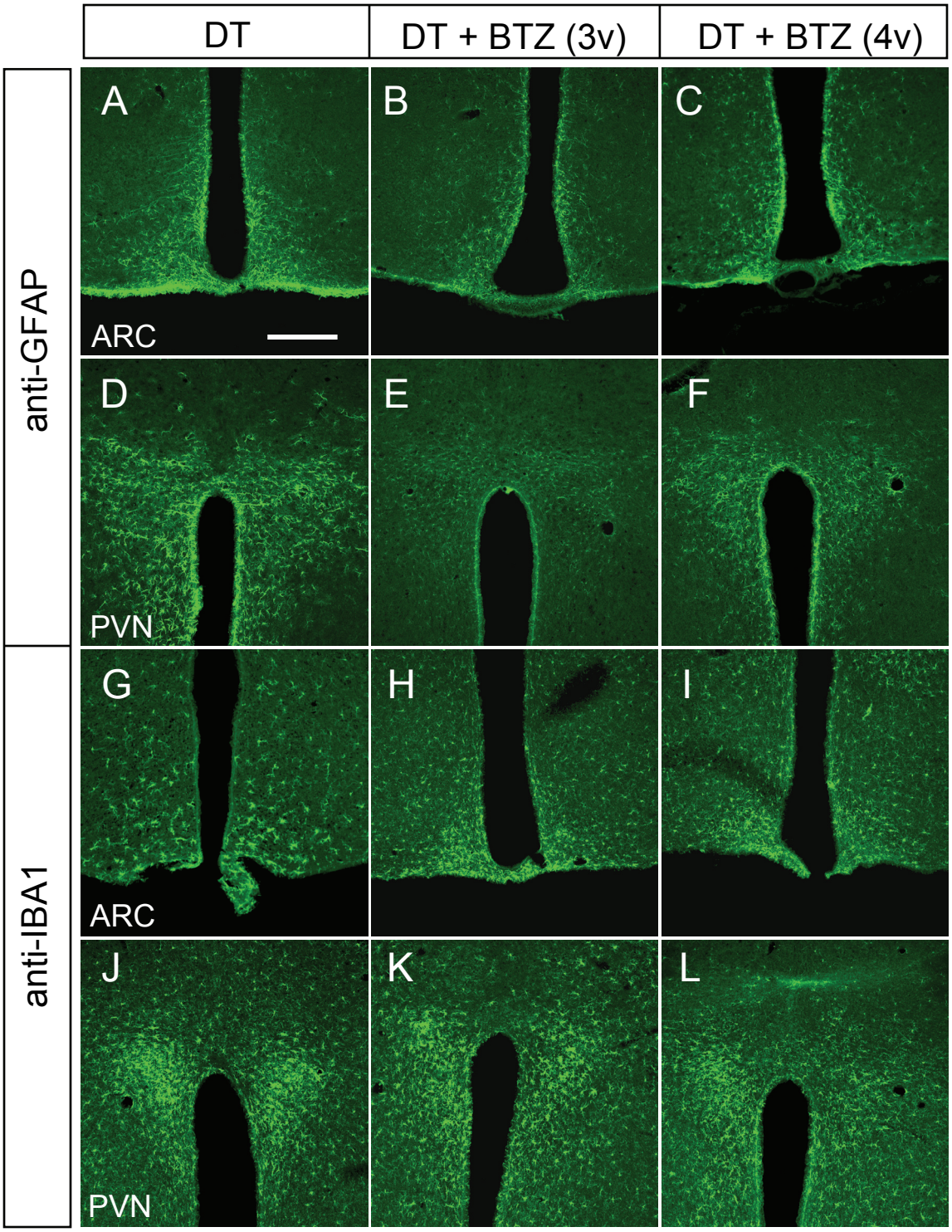


**Figure S8. Differentiated effects of chronic icv infusion of bretazenil on gliosis in AgRP neuron-ablated mice.**
(***A-C***) Anti-GFAP immunostaining in the ARC of DT-treated *Agrp*$^{DTR/+}$ mice, in some that also receive either 3$^{rd}$ or 4$^{th}$ ventricle administration of bretazenil via mini pumps.
(***D-F***) GFAP immunostaining in the PVN of the mice described in ***A-C***.
(***G-I***) Anti-IBA1 immunostaining in the ARC of the mice described in ***A-C***.
(***J-L***) Anti-IBA1 immunostaining in the PVN of the mice described in ***A-C***.
Scale bar (in ***A***) for ***A-L***, 400 μm; n = 6 for each group.

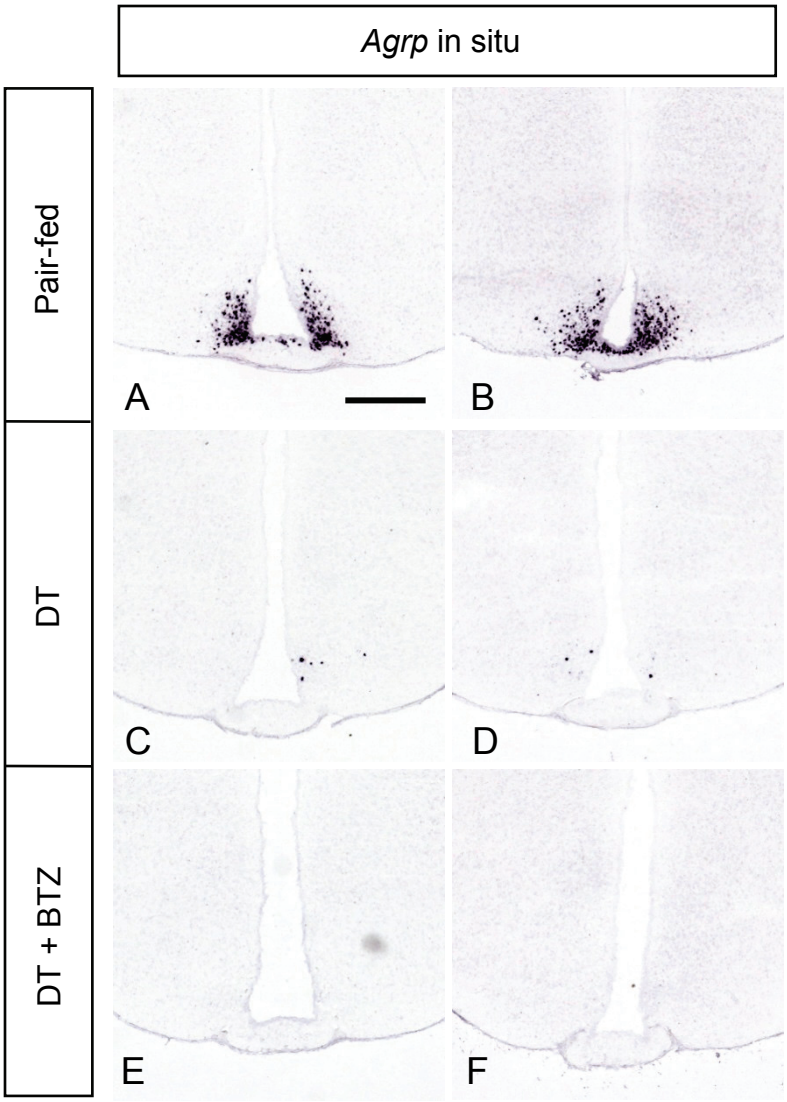


**Figure S9. Loss of *Agrp* mRNA following DT treatment**
(***A-F***) Representative pictures of *Agrp* in situ hybridization in the ARC of $Agrp^{DTR/+}$ mice after either pair feeding (***A, B***), DT treatment (***C, D***), or DT treatment plus chronic administration of bretazenil (***E, F***).
Scale bar (in ***A***) for ***A-F***, 400 µm; n = 4 - 6 for each group.

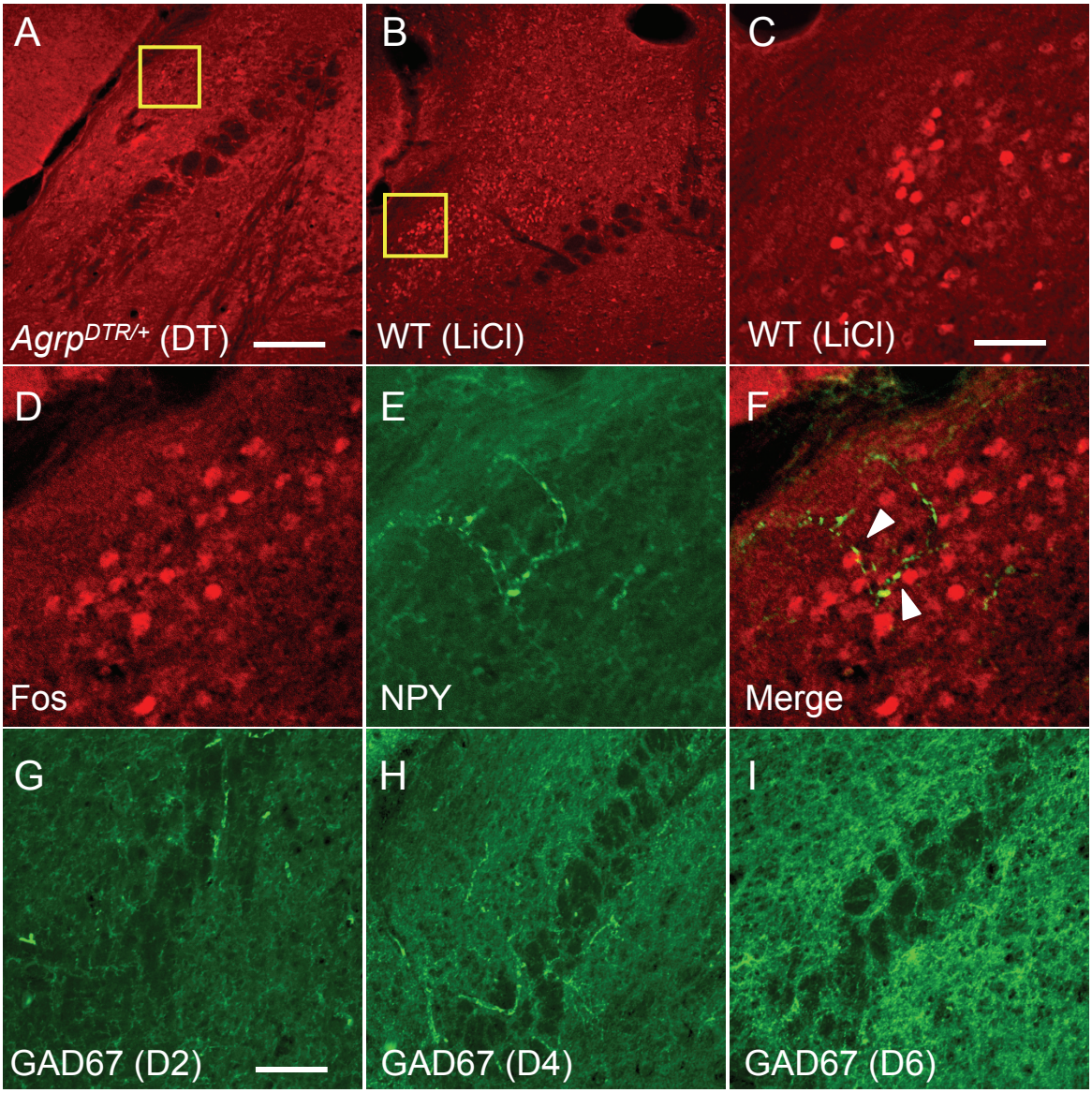


**Figure S10. AgRP neurons innervate Fos-positive cells in the PBN**
(***A***) Fos immunostaining in the PBN of *Agrp*$^{DTR/+}$ mice 4 days after injecting DT.
(***B***) Fos immunostaining in the PBN of wild-type mice 24 h after injecting LiCl (0.12 g/kg body weight, ip).
(***C***) Magnified view of the boxed area in ***B.***
(***D***) Magnified view of the boxed area in ***A.***
(***E***) NPY immunostaining in the same region as ***D.***
(***F***) Merged view of ***D*** and ***E***. Arrowheads indicate that varicosities stained with NPY antibody are in close proximity to the Fos-positive neurons in the dorsal lateral region of the PBN.
(***G-I***) GAD67 staining in the PBN at 2, 4 and 6 days after DT treatment, respectively.
Scale bar in (***A***) for ***A***, ***B***, 300 μm; in (***C***) for ***C-F***, 50 μm; in (***G***) for ***G-I*** , 150 μm**.**