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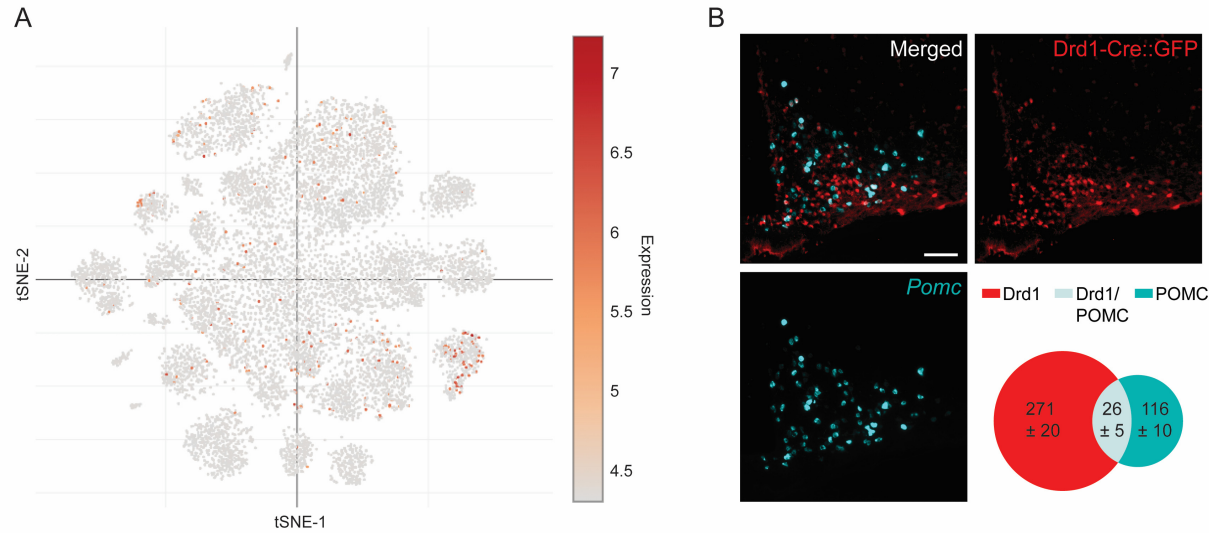
Supplemental information

Food-induced dopamine signaling in

AgRP neurons promotes feeding

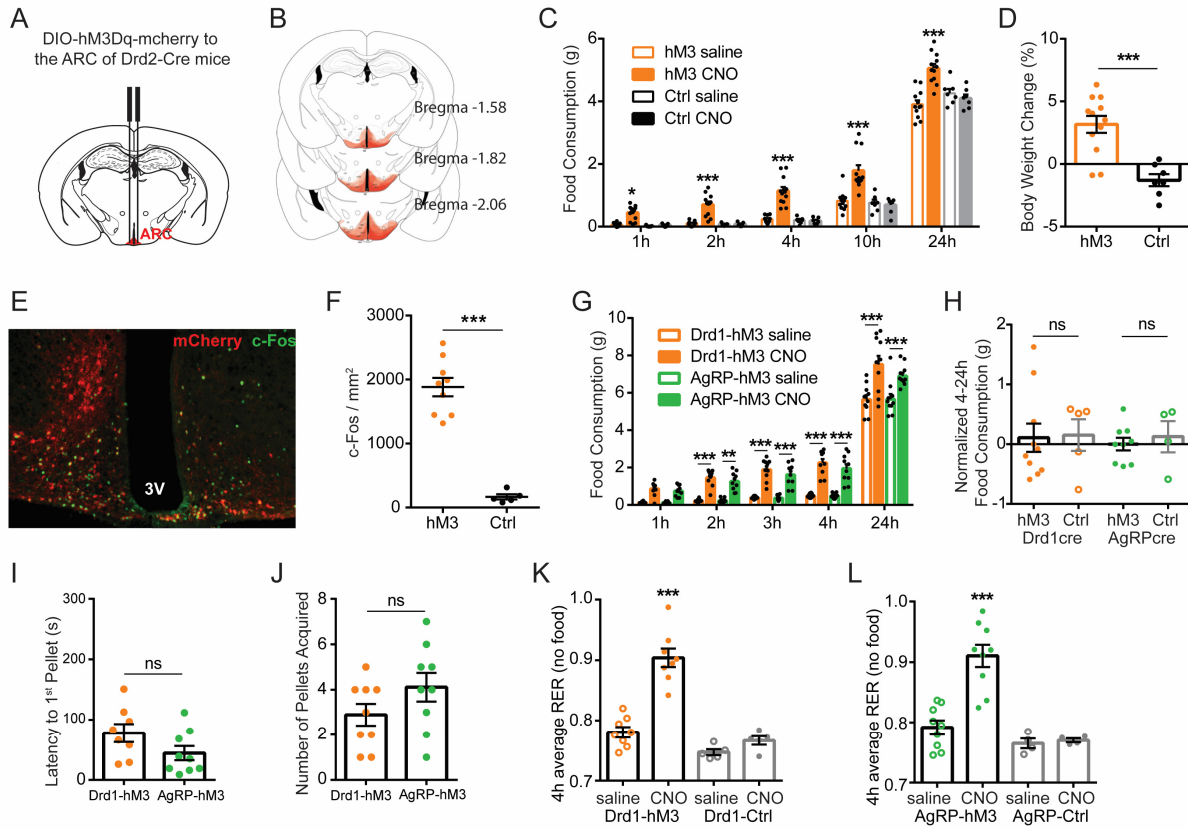
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Figure S1. The genetic profile of ARC neurons, Related to Figure 1



(A) A t-Distributed Stochastic Neighbor Embedding (t-SNE) plot showing the expression level of *Drd1* in the molecular census of the hypothalamic ARC-ME complex. (B) Representative images showing the ARC of *Drd1-Cre::GFP* mice. Scale bar represents 100 μ m. Venn diagram indicates the average number of cells expressing only the *Drd1-Cre::GFP* (red), only *Pomc* (cyan), and both *Drd1-Cre::GFP* and *Pomc* (light cyan). n = 3.

Figure S2. ARC^{Drd1} neurons regulate feeding and energy homeostasis, Related to Figures 2 - 4



(A) Schematic depicting Cre-dependent hM3Dq-mCherry expression in the ARC of Drd1-Cre mice.

(B) Expression pattern of hM3Dq-mCherry in the brain sections of Drd1-hM3Dq mice.

(C) Food consumption in Drd1-hM3Dq (orange; hM3) or control (black; Ctrl) mice 1, 2, 3, 4, and 24 hrs after saline (hollow) or CNO (solid) administration. Repeated-measures three-way ANOVA with Bonferroni post hoc comparison; $n = 7 - 12 / \text{group}$; $F_{\text{transgene}} (1, 17) = 17.84, P < 0.001$; $F_{\text{treatment}} (1, 17) = 37.31, P < 0.001$; $F_{\text{time}} (2.504, 42.58) = 1120, P < 0.001$.

(D) Percentage body weight change after 3 consecutive days of CNO injections in Drd1-hM3Dq mice Student's two-tailed t -test; $n = 7 - 12 / \text{group}$.

(E) Representative image of hM3Dq-mCherry and c-Fos expression after CNO administration in Drd1-hM3Dq mice. Scale bar represents 100 μm .

(F) Number of c-Fos-expressing cells in the ARC after CNO administration in Drd1-hM3Dq or control mice. Student's two-tailed t -test; $n = 5 - 9 / \text{group}$.

(G) Food consumption in the Drd1-hM3Dq (orange) or AgRP-hM3Dq mice (green) 1, 2, 3, 4, and 24 hrs after saline (hollow) or CNO (solid) administration. Repeated-measures three-way ANOVA with Bonferroni post hoc comparison; $n = 4 - 10 / \text{group}$; $F_{\text{genotype}} (1, 18) = 1.172, P = 0.2932$; $F_{\text{treatment}} (1, 18) = 120.8, P < 0.001$; $F_{\text{time}} (4, 72) = 810.6, P < 0.001$.

(H) Normalized food consumption from 4 hrs after injections until 24 hrs in Drd1-hM3Dq or control mice, or AgRP-hM3Dq or control mice. Responses were normalized by subtracting the average food consumption on day 1, 5, 6 after saline injections during the same period. Two-way ANOVA with Bonferroni post hoc comparison; $n = 4 - 10 / \text{group}$; $F_{\text{genotype}} (1, 24) = 0.08133, P = 0.7779$; $F_{\text{transgene}} (1, 24) = 0.1268, P = 0.7249$. One outlier in the AgRP-hM3Dq group was detected by ROUT outlier test ($Q=1\%$) and excluded for analysis and data presentation.

(I) Latency to the acquisition of the first pellet. Student's two-tailed t -test; $n = 8 - 9 / \text{group}$. One outlier in the Drd1-hM3Dq group was detected by ROUT outlier test ($Q=1\%$) and excluded for analysis and data presentation.

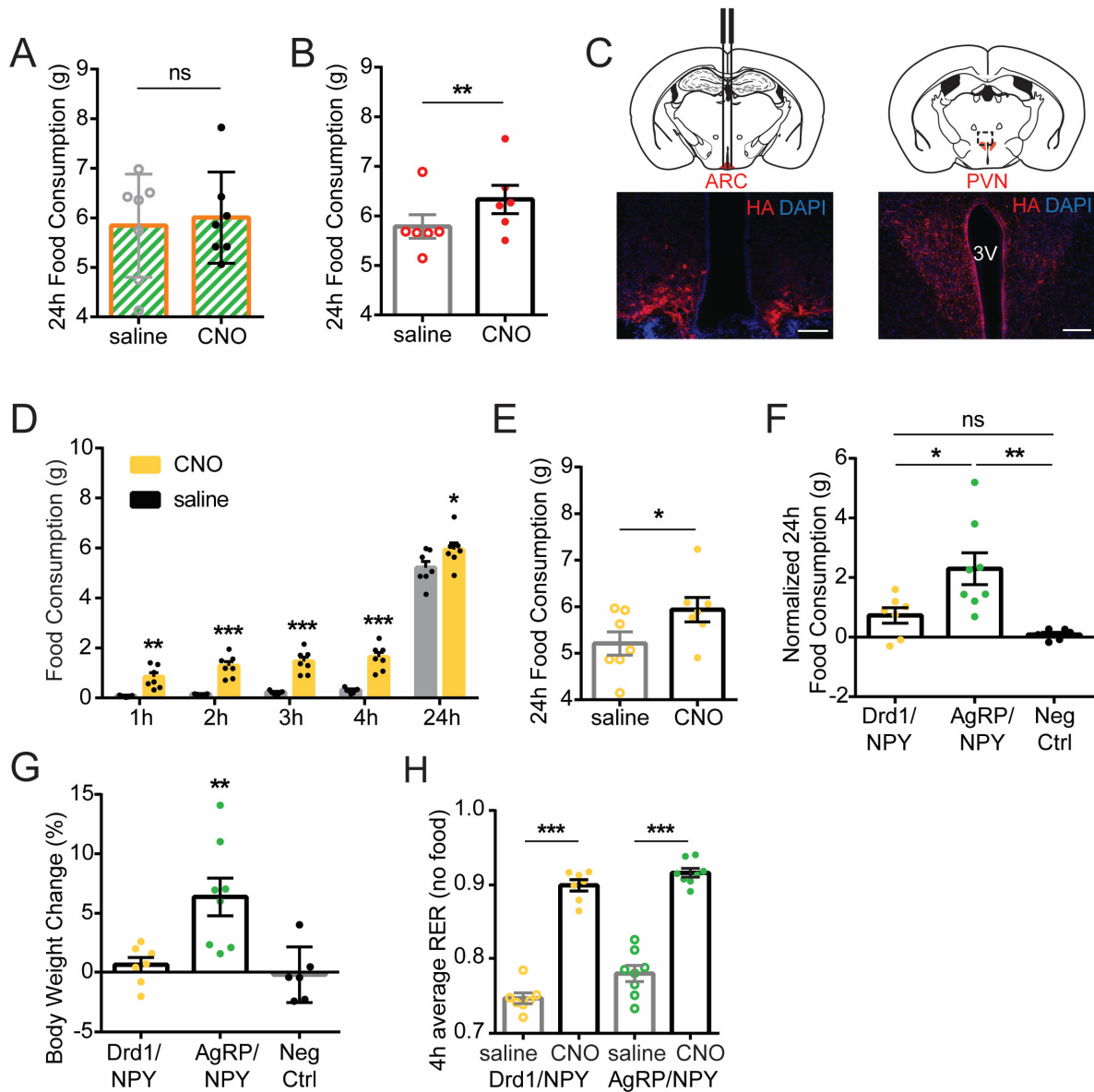
(J) Total numbers of food pellets acquired during the 20 min test. Student's two-tailed t -test; $n = 9 / \text{group}$.

(K) Average 4-hr RER in Drd1-hM3Dq and control mice following saline or CNO administration during food deprivation. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5 - 8 / \text{group}$; $F_{\text{transgene}} (1, 11) = 41.20, P < 0.001$; $F_{\text{treatment}} (1, 11) = 49.92, P < 0.001$.

(L) Average 4-hr RER in AgRP-hM3Dq and control mice following saline or CNO administration during food deprivation. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 4 - 9 / \text{group}$; $F_{\text{transgene}} (1, 11) = 26.79, P < 0.001$; $F_{\text{treatment}} (1, 11) = 12.20, P = 0.005$.

Data are represented as mean \pm SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

Figure S3. Stimulation of ARC^{Drd1} neurons expressing AgRP/NPY induces feeding acutely, Related to Figure 5



(A, B, E) 24-hr Food consumption following saline or CNO administration of Drd1-Cre;NPY-Flp mice with Cre-dependent hM3Dq-mCherry and Flp-dependent TeNT-GFP expression (A), Cre-on;Flp-off hM3Dq-HA expression (B), or Cre-on;Flp-on hM3Dq-HA expression (E) in the ARC. Student's two-tailed *t*-test; *n* = 6 - 7 / group.

(C) Schematic and representative image showing the Cre-on;Flp-on hM3Dq-HA expression in the ARC and PVN of Drd1-Cre;NPY-Flp mice. Scale bar represents 100 μ m.

(D) Food consumption after 1h, 2h, 3h, 4h, and 24 hrs of saline or CNO injections in Drd1/NPY-hM3Dq mice. Repeated-measures two-way ANOVA with Bonferroni post hoc

comparison; $n = 7$ / group; $F_{\text{treatment}} (1, 12) = 31.51, P < 0.001$; $F_{\text{time}} (4, 84) = 847.8, P < 0.001$.

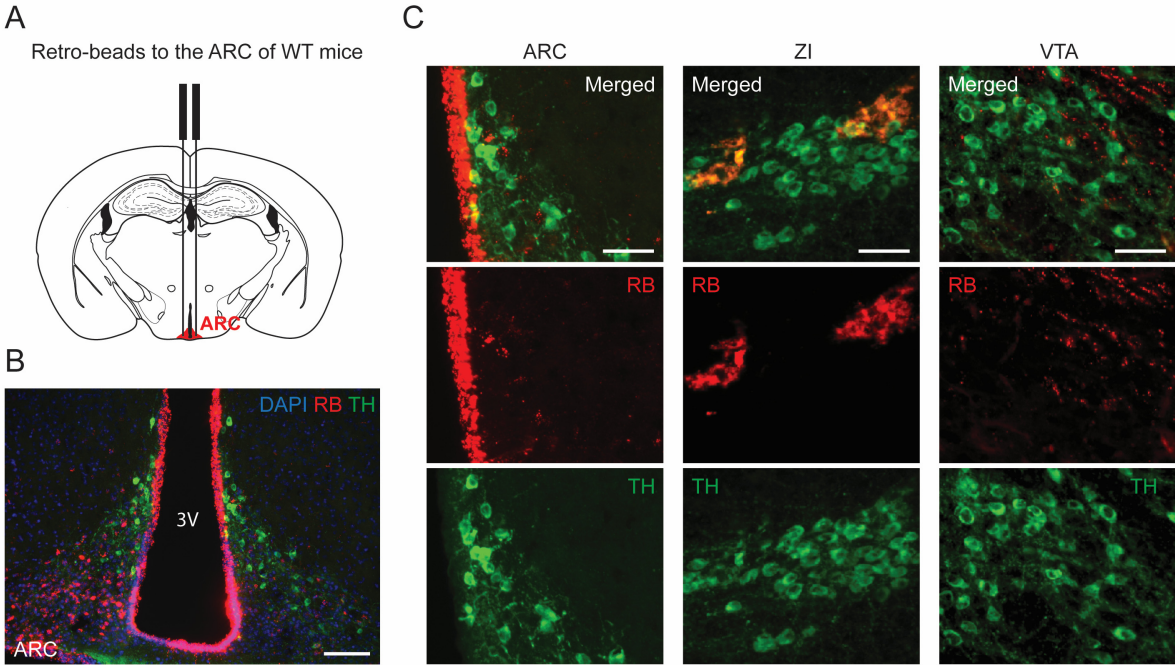
(F) Normalized 24 -hr food consumption in *Drd1*/NPY-hM3Dq, *AgRP*/NPY-hM3Dq, or negative control mice. One-way ANOVA with Bonferroni post hoc comparison; $n = 6 - 8$ / group; $F_{\text{genotype}} (2, 18) = 8.844, P = 0.0021$.

(G) Percentage body weight change in *Drd1*/NPY-hM3Dq, *AgRP*/NPY-hM3Dq, or negative control mice after 3 consecutive days of CNO injections. One-way ANOVA with Bonferroni post hoc comparison; $n = 6 - 8$ / group; $F_{\text{genotype}} (2, 18) = 9.278; P = 0.0017$.

(H) Average 4-hr RER in *Drd1*/NPY-hM3Dq and *AgRP*/NPY-hM3Dq mice following saline or CNO administration during food deprivation. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 7 - 8$ / group; $F_{\text{genotype}} (1, 13) = 8.120, P = 0.0137$; $F_{\text{treatment}} (1, 13) = 371.5, P < 0.001$.

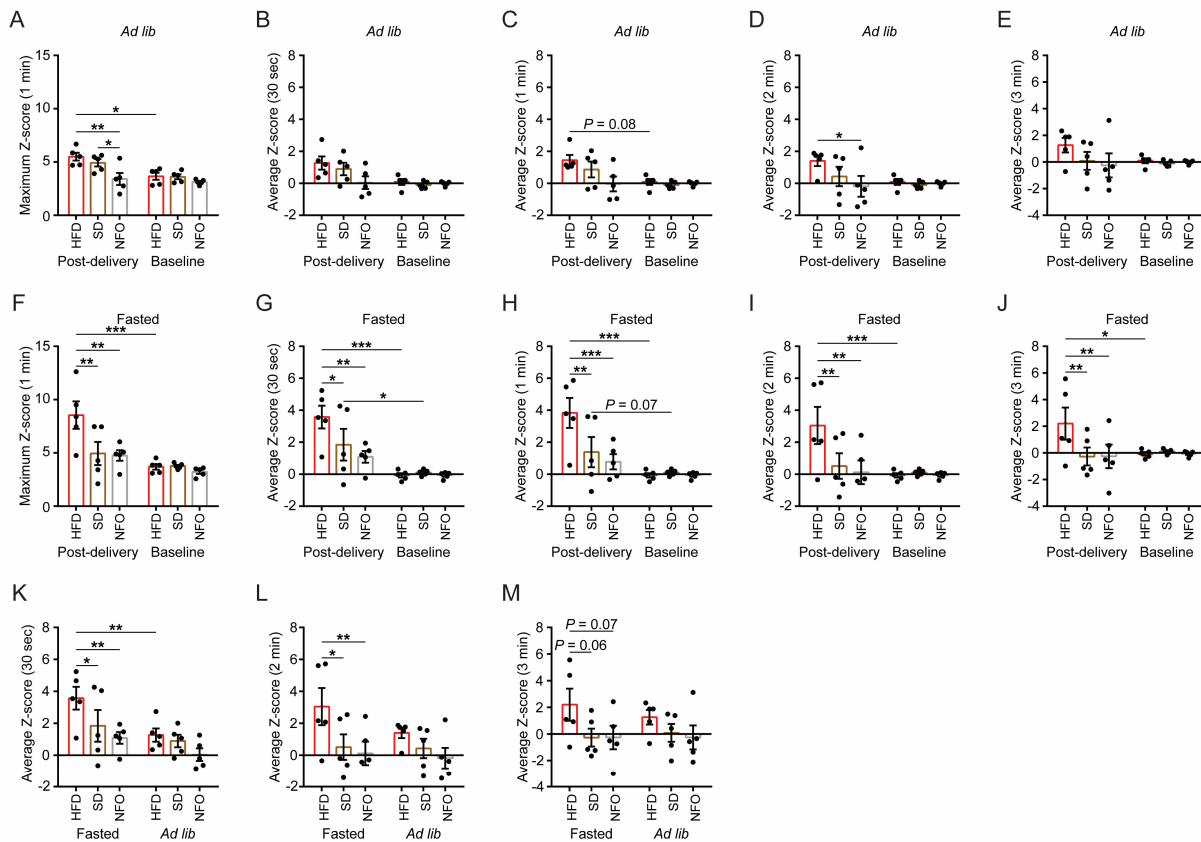
Data are represented as mean \pm SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

Figure S4. The ARC receives anatomical inputs from multiple dopaminergic neuronal populations, Related to Figure 6



(A) ARC retrograde tracing paradigms depicting retro-beads delivered to the ARC of WT mice.
(B) Representative images showing retro-beads in the ARC. Scale bar represents 100 μm .
(C) Representative images showing retro-beads and their co-localization with TH-expressing cells in the ARC (left), ZI (middle) and the VTA (right). Scale bar represents 50 μm .

Figure S5. DA levels in the ARC in response to food, Related to Figure 7



(A) Maximum Z-score during the baseline or the 1-min period following HFD (red), SD (brown), or NFO (gray) delivery in *ad lib* mice. Baseline is defined as the 90 - 30 sec window prior to food delivery (A-J). Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 11.11$, $P = 0.029$; $F_{\text{treatment}} (2, 8) = 14.98$, $P = 0.002$.

(B) Average Z-score during the baseline or the 30-sec period following HFD (red), SD (brown), or NFO (gray) delivery in *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 12.67$, $P = 0.0236$; $F_{\text{treatment}} (2, 8) = 3.2$, $P = 0.0953$.

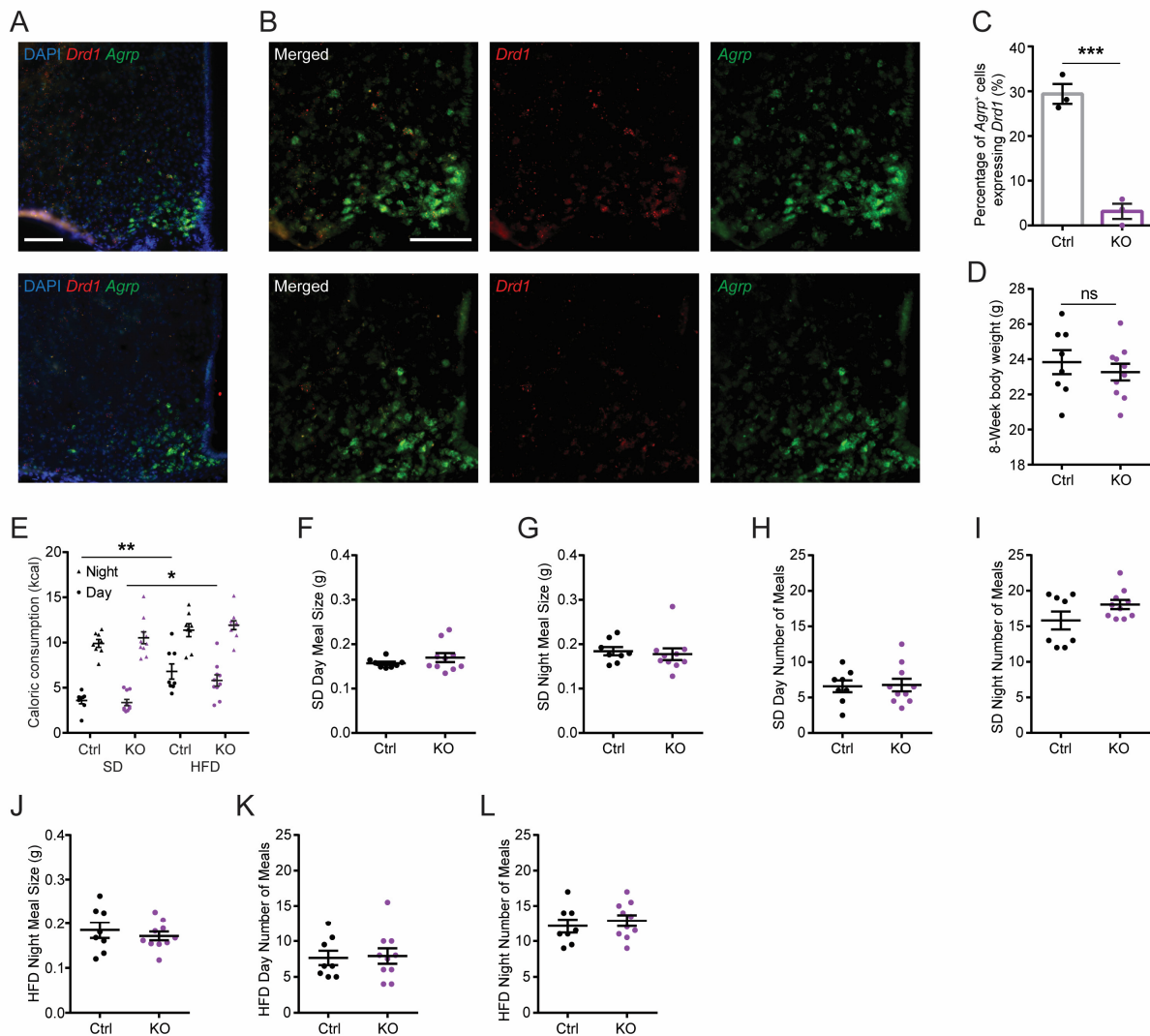
(C) Average Z-score during the baseline or the 1-min period following HFD (red), SD (brown), or NFO (gray) delivery in *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 9.282$, $P = 0.0382$; $F_{\text{treatment}} (2, 8) = 4.974$, $P = 0.0395$.

(D) Average Z-score during the baseline or the 2-min period following HFD (red), SD (brown), or NFO (gray) delivery in *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 2.383$, $P = 0.1975$; $F_{\text{treatment}} (2, 8) = 4$, $P = 0.0625$.

- (E) Average Z-score during the baseline or the 3-min period following HFD (red), SD (brown), or NFO (gray) delivery in *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 0.6314$, $P = 0.4713$; $F_{\text{treatment}} (2, 8) = 2.007$, $P = 0.1967$.
- (F) Maximum Z-score during the baseline or the 1-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 9.699$, $P = 0.0357$; $F_{\text{treatment}} (2, 8) = 9.381$, $P = 0.008$.
- (G) Average Z-score during the baseline or the 30-sec period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 12.58$, $P = 0.0239$; $F_{\text{treatment}} (2, 8) = 4.783$, $P = 0.043$.
- (H) Average Z-score during the baseline or the 1-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 7.806$, $P = 0.0491$; $F_{\text{treatment}} (2, 8) = 8.483$, $P = 0.0105$.
- (I) Average Z-score during the baseline or the 2-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 2.307$, $P = 0.2034$; $F_{\text{treatment}} (2, 8) = 7.759$, $P = 0.0134$.
- (J) Average Z-score during the baseline or the 3-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{time}} (1, 4) = 0.4723$, $P = 0.5297$; $F_{\text{treatment}} (2, 8) = 5.415$, $P = 0.0326$.
- (K) Average Z-score during the 30-sec period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted or *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{condition}} (1, 4) = 11.96$, $P = 0.0259$; $F_{\text{treatment}} (2, 8) = 4.931$, $P = 0.0402$.
- (L) Average Z-score during the 2-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted or *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{condition}} (1, 4) = 1.508$, $P = 0.2868$; $F_{\text{treatment}} (2, 8) = 10.91$, $P = 0.0052$.
- (M) Average Z-score during the 3-min period following HFD (red), SD (brown), or NFO (gray) delivery in overnight-fasted or *ad lib* mice. Repeated-measures two-way ANOVA with Bonferroni post hoc comparison; $n = 5$ / group; $F_{\text{condition}} (1, 4) = 0.1042$, $P = 0.763$; $F_{\text{treatment}} (2, 8) = 7.172$, $P = 0.0164$.

Data are represented as mean \pm SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

Figure S6. Feeding microstructures of mice with genetic ablation of *Drd1* in the ARC^{AgRP/NPY} neurons, Related to Figure 7



(A) Representative images of *Drd1* expression in control (Ctrl) (top) or *Drd1*^{AgRP-KO} (KO) (bottom) mice. Scale bar represents 100 μ m.

(B) Zoomed-in images of A. Scale bar represents 100 μ m.

(C) Percentage of *AgRP*-expressing cells in Ctrl or KO mice with *Drd1* expression. Student's two-tailed *t*-test; *n* = 3 / group.

(D) Body weights of the control (Ctrl; *AgRP-Cre*^{+/+}; *Drd1-Flox*^{fl/fl}; black) and *Drd1*^{AgRP-KO} (KO; *AgRP-Cre*^{cre/+}; *Drd1-Flox*^{fl/fl}; purple) littermates at 8 weeks of age. Student's two-tailed *t*-test; *n* = 8 - 10 / group.

(E) Day and night kilocalories (kcal) consumed by control or *Drd1*^{AgRP-KO} mice during two days when provided with *ad lib* access to SD and the first two days when switched to *ad lib* access HFD. Repeated-measures three-way ANOVA with Bonferroni post hoc comparison;

$n = 8 - 10 / \text{group}$; $F_{\text{time}} (1, 16) = 198.7, P < 0.001$; $F_{\text{genotype}} (1, 16) = 0.0007562, P = 0.9784$; $F_{\text{diet}} (1, 16) = 34.43, P < 0.001$.

(F, G) Average of day-time (F) or night-time (G) SD meal sizes of Ctrl or KO mice during two days when provided with *ad lib* access to SD. Student's two-tailed *t*-test; $n = 8 - 10 / \text{group}$.

(H, I) Average of day-time (H) or night-time (I) number of SD meals of control or *Drd1*^{AgRP}-KO mice during two days when provided with *ad lib* access to SD. Student's two-tailed *t*-test; $n = 8 - 10 / \text{group}$.

(J) Average of night-time HFD meal size of control or *Drd1*^{AgRP}-KO mice during the first two days when switched to *ad lib* access HFD. Student's two-tailed *t*-test; $n = 8 - 10 / \text{group}$.

(K, L) Average of day-time (I) or night-time (J) number of HFD meals of control or *Drd1*^{AgRP}-KO mice during the first two days when switched to *ad lib* access HFD. Student's two-tailed *t*-test; $n = 8 - 10 / \text{group}$.

Data are represented as mean \pm SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.