SUPPLEMENTAL FIGURE LEGENDS

Figure S1 is related to Figure 1. *Dbx1* expression in *Dbx1^{cKO}* embryonic telencephalon

(A-C) Schematic of coronal view of the embryonic forebrain at the level of the septum (A), ventral pallium (VP) (B) and preoptic area (POA) (C).

At E11.5, expression of *Dbx1* in developing septum and amygdala (VP and POA; Hirata et al., 2009) is unchanged between Ctrl (A.i-C.i) and *Dbx1^{cKO}* (A.ii-C.ii) embryos.

The scale bar represents 500 μm.

Figure S2 is related to Figure 2. Calbindin and Bat-Gal are co-expressed in embryonic *Dbx1^{cKO}* LH neurons

(A) Schematic of coronal views of the embryonic forebrain at the level of the LH with a red box indicating areas of corresponding immunohistochemical low magnification images in panels B and C.

(B-C.iii) Wnt-responsive Bat-Gal cells (green) and Calbindin+ (purple) cells in the LH of E15.5 *Dbx1$^{+/+}$;BAT-GAL$^{+/-}$* (B-B.iii) and *Dbx1$^{-/-}$;BAT-GAL$^{+/-}$* (C-C.iii) embryos. Arrowheads indicate co-labeled cells.

(D-F) Significant increases in the number of Wnt-responsive Bat-Gal+ cells co-labeling with Calbindin expression (D), the percent of co-labeled cells with respect to the total number of Calbindin+ cells (E) or the total number of Bat-Gal+ cells (F) are observed in the LH of E15.5 *Dbx1$^{-/-}$;BAT-GAL$^{+/-}$* embryos.

Mean ± SEM, n = 3, *p value < 0.05. The scale bar represents 50 μm in panels B and C and 15 μm in panels Bi-C.iii.

Figure S3 is related to Figure 2. Expression of embryonic PVN, VMH and PMN markers is unchanged in *Dbx1^{cKO}* and *Dbx1$^{-/-}$* embryos

(A, E, I) Schematic of a coronal view of the embryonic forebrain at the level of the PVN (A), VMH (E) and PMN (I) with a red box indicating the areas of corresponding ISH images.

No changes are observed in expression of *Avp*, *Sim1* and *Oxt* in the E17.5 PVN (B-D.ii); *Fezf1*, *Nr5a1* and *Lef1* in the E13.5 VMH (F-H.ii); or *Lef1* and *Sim1* in the E13.5 PMN (J-K.ii) in $Dbx1^{cKO}$ or $Dbx1^{-/-}$ brains.

Mean ± SEM; n = 3 - 6 per experimental group; all p values > 0.05 in comparisons between comparable groups for each probe. The scale bar represents 500 μm.

Figure S4 is related to Figure 3. Differential loss and sparing of Arc and LH protein expression in $Dbx1^{cKO}$ postnatal males and females

(A-F) Schematic of rostral to caudal coronal views of the postnatal brain with a red box indicating areas of corresponding immunohistochemical images taken under the same exposure conditions.

(A.i-F.i) Terminals of Agrp+ axons are present in multiple limbic nuclei in Ctrl mice at P21.

(A.ii-F.ii) $Dbx1^{cKO}$ brains show dramatically diminished Agrp+ terminals across all projection targets at P21.

(G) Summary diagram illustrating the requirement of *Dbx1* for Agrp+ projections.

Abbreviations: BNST (bed nucleus of stria terminalis); CeA (central amygdala); mPO (medial preoptic nucleus).

(H-J) In the Arc, no changes in expression of Pomc (H-H.ii), Cart (I-I.ii), or TH (J-J.ii) are observed at P21.

(K-M) In the LH, there is a significant decrease in the number of cells expressing Pmch (K-K.ii), Nesfatin (L-L.ii), or Cart (M-M.ii) in both male and female $Dbx1^{cKO}$ mice at P21.

Mean ± SEM; n = 3 per sex, per experimental group; *, p value < 0.05; **, p < 0.01.

The scale bar represents 250 μm in panels A.i-F.ii and 100 μm in panels H-M.i.

Figure S5 is related to Figure 3. Expression of postnatal PVN, VMH and PMN markers is unchanged in *Dbx1*^{cKO} postnatal male and female mice

(A, E, I) Schematic of a coronal view of the postnatal brain at the level of the PVN (A), VMH (E) and PMN (I) with a red box indicating corresponding areas of ISH images.

(B-D.iv) No changes are observed in expression of *Avp*, *Sim1* and *Oxt* in the PVN (B-D.ii); *Fezf1*, *Nr5a1* and *Lef1* in the VMH (F-H.ii); or *Lef1* and *Sim1* in the PMN (J-K.ii) in *Dbx1*^{cKO} males or females at P21.

Mean ± SEM; n = 3 - 6 per experimental group; all p values > 0.05 in comparisons between comparable groups for each probe. The scale bar represents 250 μm.

Figure S6 is related to Figure 5. Stress-feeding circuit connectivity and c-Fos expression in *Dbx1*^{cKO} mice after food stress

(A) Schematic of the dual AAV injection paradigm employed to label neurons in the LH projecting to the PVN.

(B) Expression of WGA-Cre helper virus (red) in the PVN.

(C, D, E) Low magnification (C) and high magnification (D, E) images of the LH with cells positive for the cre-dependent AAV virus carrying the floxed-stop YFP reporter (green).

(D.i-E.ii) High magnification of Pmch+ (D.i) and Hcrt+ (E.i) neurons co-labeling with the AAV-YFP reporter (D.ii and E.ii, respectively). Arrowheads indicate co-labeled neurons.

(F-H) Schematic of a coronal view of the postnatal brain at the level of the Arc (F), LH (G) and PVN (H) with a red box indicating corresponding areas of IHC images.

(F-H.iv) Representative images of c-Fos expression in the Arc, LH and PVN in a fed state (mice given regular chow diet *ad libitum*) (F.i-H.i and F.iii-H.iii) or 12 hr after fasting (F.ii-H.ii and F.iv-H.iv) in Ctrl (F.i-H.ii) and *Dbx1*^{cKO} (F.iii-H.iv) mice.

(I-N) Significant increases in the number of c-Fos+ cells in fasted Ctrl males and females compared to fed Ctrls are observed in the Arc (I, L), LH (J, M) and PVN (K, N) (white bars), with

no change in the number of c-Fos+ cells in female *Dbx1*^{cKO} Arc (L), male and female LH (J, M) and female PVN (N) (black and gray bars). The fold change in c-Fos+ cells after fasting is significantly lower in *Dbx1*^{cKO} male and female Arc (L.i, O.i), LH (M.i, P.i) and PVN (N.i, Q.i).
Mean ± SEM; n= 3-11, *p value < 0.05, **p<0.01, ***p<0.001

Figure S7 is related to Figure 6. Body length, metabolism and body fat composition is unchanged in *Dbx1*^{cKO} male and female mice on a regular chow diet

(A-D) Body length throughout post-weaning ages is unchanged in *Dbx1*^{cKO} males and females. No significant changes in oxygen consumption (E, H), carbon dioxide production (F, I), heat production (G, J), fat mass (K, N), lean mass (L, O), or percent body fat (M, P) are observed in P30 *Dbx1*^{cKO} males and females fed a regular chow diet.
Mean ± SEM; n = 9 - 15 per experimental group; all p values > 0.05 in comparisons between Ctrls and *Dbx1*^{cKO} in comparable measurements.

Figure S8 is related to Figure 7. Unchanged innate behaviors in *Dbx1*^{cKO} males and females

The following behaviors showed no significant differences between *Dbx1*^{cKO} and Ctrl mice:
male (A-C; n = 31 - 32) or female (I-K; n = 15 - 21) mating behaviors. Only one ejaculation event was observed in each group (A and I).
Male territorial aggression (D-F; n = 23 - 24) or female maternal aggression (L-N; n = 8 - 10).
Olfaction in males (G) or females (O) (n = 10 - 13).
Urine marking in males (H) or females (P) (n = 6 - 10).
(Q) Pup retrieval in females (n = 8 - 10).
Mean ± SEM. All p values > 0.05 in comparisons between Ctrls and *Dbx1*^{cKO} in comparable measurements.

Supplemental Movie.

Video clip showing behavior of female Ctrl and *Dbx1*^{cKO} mice in the presence of rat bedding. Video from each group was taken during the first three minutes of recording.

Table S1.

List of gene changes ≥ 2.0 fold in the embryonic and postnatal male and female *Dbx1*^{cKO} hypothalamus.

Table S2.

List of probes used to validate microarray screens.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

Mice were housed in the temperature- and light-controlled Children's National Medical Center animal care facility and given food and water *ad libitum*, unless otherwise stated. All animal procedures were approved by Children's National Medical Center's and the University of Pennsylvania's Institutional Animal Care and Utilization Committees (IACUC) and conformed to NIH Guidelines for animal use. Conditional-knockout mice (*Dbx1*^{cKO}: *Nkx2.1Cre*$^{+/-}$*;Dbx1*$^{c/-}$) and controls (Ctrl: *Nkx2.1Cre*$^{+/-}$*; Dbx1*$^{c/+}$) were obtained by crossing *Nkx2.1Cre*$^{+/-}$*; Dbx1*$^{+/-}$ males with *Dbx1*$^{flox/flox}$ females. *Dbx1* knockout mice (KO: *Dbx1*$^{-/-}$) and controls (WT: *Dbx1*$^{+/+}$ and Het: *Dbx1*$^{+/-}$) were obtained by crossing male and female *Dbx1*$^{+/-}$ mice. For analysis of Wnt-responsive cells, we carried out the above crosses with BAT-GAL transgenic mice (Jackson Labs strain *B6.Cg-Tg(BAT-lacZ)3Picc/J*) (Maretto et al., 2003). Mice were genotyped by Transnetyx Inc. Genotyping Services.

Gene Expression Profiling

RNA samples quantity and quality

E13.5 hypothalamic primordium or postnatal (3-4 month old) hypothalamic tissue was microdissected from 3 Ctrl and 3 *Dbx1*^{cKO} embryos from adult male and female mice (24 samples total) using Lumsden scissors in ice cold PBS (regions of dissections shown in **Figure 1**H-I). Embryos were sexed using PCR to amplify the Y-chromosome-specific *Sry* gene. RNA was isolated using a Qiagen kit (RNeasy kit 74104). RNA concentration of each sample was determined by NanoDrop® spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE). The quality of RNA samples was confirmed via RNA Integrity Number (RIN>6) with NanoChips on the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA).

Microarray

An aliquot of 200 ng of high-quality total RNA from each sample was used for expression profiling using Illumina® Gene Expression BeadChip Array technology (Illumina, Inc., San Diego, CA). Reverse Transcription of the first cDNA strand and synthesis of the second strand, followed by a single *in vitro* transcription (IVT) amplification, incorporating biotin-labeled nucleotides, were performed with Illumina® TotalPrep™ -96 RNA Amplification Kit (Ambion, Austin, TX), following the manufacturer's instructions. Quality of the amplified and labeled RNA (aRNA) was assessed with Bioanalyzer, and 1.5 µg of the IVT product was hybridized to Illumina MouseWG-6v2_BeadChip for 16 hr, followed by washing, blocking and streptavidin-Cy3 staining according to the Illumina Whole-Genome Gene Expression Direct Hybridization protocol. Arrays were scanned using Illumina HiScanSQ System, and the obtained decoded images were analyzed by GenomeStudio™ Gene Expression Module – Illumina integrated platform for the data visualization and analysis.

Expression values data generation

Generated in GenomeStudio, Illumina probe sets signal intensity values were uploaded (using Genome Studio *plug-in* option) into the Partek Genomics Suite, version 6.5 (Partek Incorporated, St. Louis, MO) to determine differently expressed genes, statistics analyses and data visualization. Partek automatically applies Robust Multi-array Average (RMA) and performs *log2* transformation for the generated expression values. One-way ANOVA statistical test was applied to the profiles to verify significance of the comparative results. Only expression values with a *p*-value cut off of $p < 0.05$ and fold change >1.5 were considered for further analyses.

***In situ* hybridization (*ISH*) and LacZ staining**

Postnatal animals (P21 and P90) and embryos ≥E15 were transcardially perfused with 4% paraformaldehyde (PFA) (wt/vol). E13.5 - 17.5 embryo and postnatal tissue was drop fixed with

4% PFA for 2 hr at 4°C, dehydrated in 30% sucrose (wt/vol), embedded in OCT embedding compound, and sectioned at 20 µm using a microtome cryostat (Leica HM525). Both control and mutant sections were mounted on the same slide for direct comparison. For *ISH*, sections were refixed with 4% PFA, treated with proteinase K, again refixed with 4% PFA, treated with triethanolamine containing acetic anhydride, and then hybridized with digoxigenin-UTP–labeled RNA probes overnight. The next day, the probes were washed with 2x SSC at 65°C. Signals were detected with an antibody to digoxigenin (Roche #11093274910) and BM purple (Roche #11442074001) (See **Table S2** for details of probes). For LacZ staining, E17.5 and E15.5 *Dbx1^{cKO};BAT-GAL* and *Dbx1$^{-/-}$;BAT-GAL* sections (with appropriate control sections on the same slide) were incubated in X-gal staining solution overnight at 37°C. For signal quantitation, positive puncta were counted in every 10th serial coronal section encompassing the entire anterior to posterior gene expression domain. Where positive puncta could not be counted, ImageJ was used to quantify positive signal by calculating total area of signal multiplied by the average intensity of signal (total area x mean pixel intensity) in gene expression domains of a section; every 10th section was quantified and added. Data from embryonic males and females were grouped, since no differences were detected between sexes. Data from *Dbx1$^{+/+}$* (WT) and *Dbx1$^{+/-}$* (Het) embryos were grouped, as no differences were detected between genotypes. Data was then presented as a percent (%) of control. Minimum n = 3 mice per sex, per genotype. Individual sample sizes (n) are noted in legends for **Figures 2**-**7** and **S4**-**S8**.

Immunohistochemistry (IHC)

Postnatal mice were perfused and postfixed with 4% PFA (wt/vol) for 16 hr at 4°C, then sectioned at 50 µm with a vibrating microtome (Leica VT1000S). For IHC, sections were incubated with the primary antibody for 16 hr at 4°C, washed and incubated with the corresponding fluorescent secondary antibodies, and mounted with DAPI Fluoromount (SouthernBiotech 0100-20). Primary antibodies used were rat anti-GFP (to detect YFP

expression, 1:1000, Nacalai 04404-84), sheep anti-Nesfatin (1:500, R&D Systems af6895), rabbit anti-Cart (1:20,000, Phoenix Pharmaceuticals), rabbit anti-TH (1:500, Santa Cruz sc14007), goat anti-Agrp (1:500, R&D Systems af634), goat anti-Pmch (1:500, Santa Cruz sc14509), rabbit anti-Hcrt (1:100, Millipore ab3096), goat anti-Pomc (1:100, Abcam ab322893), goat anti-Calbindin (1:500, Santa Cruz sc-7691) and rabbit anti-c-Fos (1:500, Santa Cruz sc-52). Quantification of total cells was obtained by counting positive cells (as determined by co-localization with DAPI) in every 6th serial coronal section of *Dbx1^{cKO}* and Ctrl, encompassing the entire domain of expression. Minimum n = 3 mice per sex, per genotype.

Stereotactic injection of viral tracers

The animals were anesthetized with intraperitoneal injections of a ketamine (80 mg/kg)/xylazine (15–20 mg/kg) cocktail (Sigma). As previously described (Gradinaru et al., 2010) the WGA-Cre AAV virus (AAV2-EF1α-mCherry-IRES-WGA-Cre; UNC vector core) was delivered into the PVN via a borosilicate glass pipette; the injection volume and flow rate (100 nl at 10 nl/min) were controlled with a pico injector (Harvard Apparatus). Five weeks later, the Cre-dependent AAV (pAAV-Ef1a-DIO EYFP; UNC vector core) was injected into the ipsilateral LH of the same animal. Coordinates used (relative to bregma) were -0.8/0.2/4.5 mm for the PVN and -1.5/1/4.8 mm or the LH. The glass pipette was left in place for 10 min after vector delivery and then was slowly withdrawn, to allow complete diffusion of the virus and avoid spillover or contamination of adjacent regions during pipette extraction.

Multiple Electrode Array Electrophysiology

Preparation of Acute Brain Slices

Ctrl and *Dbx1^{cKO}* male mice (P17-20) were anesthetized with isoflurane and transcardially perfused with 10 mL of ice-cold carbogenated (95% O_2, 5 % CO_2) HEPES-buffered artificial

cerebrospinal fluid (aCSF; composition, in mM: 92 NaCl, 2.5 KCl, 1.2 $NaH_2PO_4•H_20$, 30 $NaHCO_3$, 25 glucose, 20 HEPES, 5 Na-ascorbate, 3 Na-pyruvate, 2 thiourea, 10 $MgSO_4$, 0.5 $CaCl_2$; pH 7.4). The same aCSF solution was also utilized for perfusion, sectioning, and initial incubation of all brain slices. Mice were decapitated, and the brains were quickly removed and affixed to the stage of a vibrating microtome (Leica VT 1200S, Germany). Coronal brain sections containing the LH were obtained (300 μM thickness, from bregma -2.06 to -2.30 mm). The slices were incubated at 32°C for 15min. Slices were then transferred to a carbogenated, low-glucose aCSF (composition, in mM: 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 $NaH2PO_4•H_2O$, 2 $MgCl_2•6H_2O$, 2 $CaCl_2•2H_2O$, 0.2 glucose; pH 7.4) and maintained at ambient temperature (22-24^oC) until experimentation. Sucrose was added to the low-glucose aCSF to maintain osmolality (295-300 mOsm).

Electrophysiological recordings

Brain slices were incubated in oxygenated aCSF at ambient temperature for at least 1 hr before transfer to a 60-channel, 8 x 8 perforated MEA probe with 200 μM electrode spacing (Multi Channel Systems, Reutlingen, Germany). The slice was positioned such that the LH was covered by the array. A constant-vacuum pump was utilized to apply a gentle suction to stabilize the slice on the MEA probe. The chamber was maintained at 30°C under continuous perfusion (2 ml/min) of oxygenated, low-glucose (0.2 mM) aCSF for 1 hr prior to data acquisition. A 15-min recording of spontaneous spiking activity of each slice was acquired. The superfusion solution was then switched to a high-glucose (5.0 mM) aCSF solution, and a 1-hr recording was obtained. All recordings were obtained with a 60-channel amplifier (MEA-2100, Multi Channel Systems, Reutlingen, Germany) running MC Rack software (Multi Channel Systems, Reutlingen, Germany) at a sampling rate of 25 kHz.

Data processing

Event activity was filtered with a 125-Hz high-pass filter. Event detection was set to 5x the root mean square of the background noise for each channel. Events that surpassed this threshold were automatically detected utilizing the MC Rack software. Data were arranged into 60-sec bins, and the maximum spike frequency was calculated for each channel in each experimental condition.

Behavior Assays

All non-feeding behavior assays were video-recorded on digital video cassettes (Sony DVM60PRRJ) using a Sony MiniDV Digital Handycam (Sony DCRTRV33). Videos were uploaded as digital WMV files and scored independently by two investigators blind to genotype using the Scorevideo program for MatLab (Wu et al., 2009).

Mating

Sexually naïve experimental males were singly housed and left undisturbed for 1 week prior to the introduction of a hormonally primed, ovariectomized 129SvEv female. Sexually naïve hormonally primed ovariectomized experimental females were placed into the home-cage of a sexually experienced 129SvEv male. Each trial consisted of recordings from the first 30 min of behavior. Trials were repeated up to three times and separated by at least 1 week, as previously been described (Xu et al., 2012; Yang et al., 2013). (Male Mating: n ≥ 31 per group; total 63 trials: 32 trials from 11 Ctrl males, 31 trials from 11 *Dbx1*^{cKO} males; Female Mating: n ≥ 15 per group; total 36 trials: 15 trials from 5 Ctrl females, 21 trials from 7 *Dbx1*^{cKO} females).

Body Weight, Food Consumption and Body Length

Male and female mice were singly housed after weaning (P21). Weekly, body weight was taken at the same day and time. Whole chow pellets (regular chow diet; 7012 Teklad LM-485 Mouse/Rat Sterilizable Diet) were weighed to the nearest tenth of a gram, placed in the hopper

during weighing of the animal, and re-weighed at the next body-weight measurement. Daily food consumption was calculated by subtracting the weight of the remaining food from the amount given and then dividing that difference by seven (n ≥ 9 per group, total 48 mice: 9 male *Dbx1*^{cKO} mice, 9 female *Dbx1*^{cKO} mice, 15 Ctrl male mice, 15 Ctrl female mice). Fasting: food was removed from the hopper for 12 hr. Mice were weighed before and after the 12-hr fast, and food consumption was measured as the amount of food consumed within the 24 hr following re-feeding. A single cohort of 20 mice (5 Ctrl males, 5 *Dbx1*^{cKO} males, 5 Ctrl females, 5 *Dbx1*^{cKO} females) was used for the different feeding paradigms and was sequentially exposed to 4 weeks on the regular diet, 2 weeks on the restricted diet, and 7 weeks on the high fat diet. Regular diet: mice were fed regular chow diet *ad libitum*. Restricted diet: mice were fed regular chow diet from 17:00 – 12:00 the next day (nineteen hours), and then food was removed for five hours between 12:00 and 17:00. Body weights were measured daily, and food intake was measured 30 min and 24 hr after food was reintroduced. High-fat diet: mice were fed a special high-fat diet (Teklad TD.08811) for seven weeks. Body weights and food consumption was measured as described above. Body length was measured with a ruler from tip of the nose to the base of the tail in a naturally extended posture.

Body composition, Home-cage activity and Metabolic data

These experiments were performed at The Mouse Phenotyping, Physiology and Metabolism Core at the University of Pennsylvania Diabetes Endocrine Research Center. *Cold tolerance tests:* Implantable electronic transponders (Bio Medic Data Systems, Seaford, DE) were placed under the skin in the interscapular region of the animals. Temperatures measured by implanted transponders were recorded at room temperature. Mice were placed at 4º C for 6 hours and core temperature measured. Core temperature was also measured at room temperature and at 4º C using a rectal thermometer. *NMR*: Lean and fat mass were measured using Echo MRI 3-in-1 analyzer (Houston, TX). Body weight and fat composition was matched in each subject to give

294 a % fat for each subject. *DEXA*: Bone mineral density was measured using General Electric
295 Lunar PIXImus2. *CLAMS*: Animal activity monitoring was performed using Columbus Instrument
296 OXYMAX V4.47.
297
298 ***Open Field***
299 Singly housed P160 males and females were placed in the center of a clean open-field
300 apparatus (Coulbourn Instruments) and recorded for 30 min, as described previously (Bailey
301 and Crawley, 2009). Time spent in the center field was quantified at 5 min intervals over the 30
302 min of recording time. (n ≥ 9 per group, total 48 mice: 9 male *Dbx1*^{cKO} mice, 9 female *Dbx1*^{cKO}
303 mice, 15 Ctrl male mice, 15 Ctrl female mice).
304
305 ***Predator Avoidance***
306 P90 test subjects were video-recorded for 15 min after being given 12 g of clean 1/8" corncob
307 bedding (Harlan 7092) or corncob bedding from a soiled rat cage. Cardiac blood was taken from
308 anesthetized animals 45 min after the completion of the assay (1 hr after first exposure to
309 bedding) for ELISA of plasma Cort levels (n ≥ 11 per group, total 106 mice: 14 male Ctrl
310 mice+benign bedding, 14 male *Dbx1*^{cKO} mice+benign bedding, 13 male Ctrl mice+rat bedding,
311 14 male *Dbx1*^{cKO} mice+rat odor, 13 female Ctrl mice+benign bedding, 12 female *Dbx1*^{cKO}
312 mice+benign bedding, 11 female Ctrl mice+rat bedding, 15 female *Dbx1*^{cKO} mice+rat bedding).
313
314 ***Territorial Aggression***
315 Adult experimental males were singly housed and left undisturbed for 1 week prior to the
316 introduction of a smaller 129SvEv intruder male. Each trial consisted of 15 min of recorded
317 behavior; intruder was removed after each trial. Trials were repeated up to 2 times separated by
318 at least 1 week, following previous procedures (Yang et al., 2013). (n ≥ 23 per group, total 54
319 trials: 23 trials from 12 male Ctrl mice, 31 trials from 12 male *Dbx1*^{cKO} mice).

Maternal Aggression

Singly housed experimental females were mated and allowed to give birth to a litter of pups. On the day of experimentation, P3-10 pups were removed from the home-cage, and 129SvEv intruder male was placed into the home-cage. Each trial consisted of 15 min of recorded behavior, after which the intruder was removed and the pups were returned. Trials were repeated up to 2 times separated by at least 1 week as previously described (Xu et al., 2012). (n ≥ 8 per group, total 18 trials: 8 trials from 4 Ctrl dams, 10 trials from 5 *Dbx1*^{cKO} dams).

Pup Retrieval

Singly housed experimental females were mated and allowed to give birth to a litter of pups. In each trial, the dam was temporarily removed from the cage, and the pups (P3-10) were taken from the nest and placed in a far corner of the home-cage. The dam was returned to the cage, and the time required for her to retrieve two pups back to the nest was recorded. Trials were repeated up to 2 times separated by at least 1 week as previously described (Xu et al., 2012). (n ≥ 8 per group, total 18 trials: 8 trials from 4 Ctrl dams, 10 trials from 5 *Dbx1*^{cKO} dams).

Territorial Urine Marking

Bedding was removed from the home-cage and replaced with Whatman chromatography paper (GE Healthcare, UK 3030-6188), cut to fit the bottom of the home-cage. Test subjects were placed back in the cage for 1 hr. Urine marking was observed under UV light, and the number of urine spots was counted. (n ≥ 6 per group, total 30 mice: 10 Ctrl males, 8 *Dbx1*^{cKO} males, 6 Ctrl females and 6 *Dbx1*^{cKO} females).

Food Odor

All chow pellets were removed from the test subject's home-cage 12 hr prior to test. On the day of experimentation, the subject was placed in a clean cage containing 3 cm deep in which a cookie (Nabisco) was buried 1 cm below the surface in a random corner of the cage. A cookie was scored as uncovered when it was dug to the surface. (n ≥ 10 per group, total 48 mice: 15 Ctrl males, 13 *Dbx1*^{cKO} males, 10 Ctrl females and 10 *Dbx1*^{cKO} females).

ELISA

Serum from blood was collected 1 hr after first exposure to bedding (benign or rat). Samples were run in duplicate using corticosterone ELISA kits (Abcam ab108821) per manufacturer's recommendations. Values were read on a precision microplate reader (Emax, Molecular Devices) (n≥11 per group, total 98 mice: 12 male ctrl mice+benign bedding, 13 male *Dbx1*^{cKO} mice+benign bedding, 11 male ctrl mice+rat bedding, 14 male *Dbx1*^{cKO} mice+rat odor, 12 female ctrl mice+benign bedding, 13 female *Dbx1*^{cKO} mice+benign bedding, 11 female ctrl mice+rat bedding, 12 female *Dbx1*^{cKO} mice+rat bedding).

Figure S1

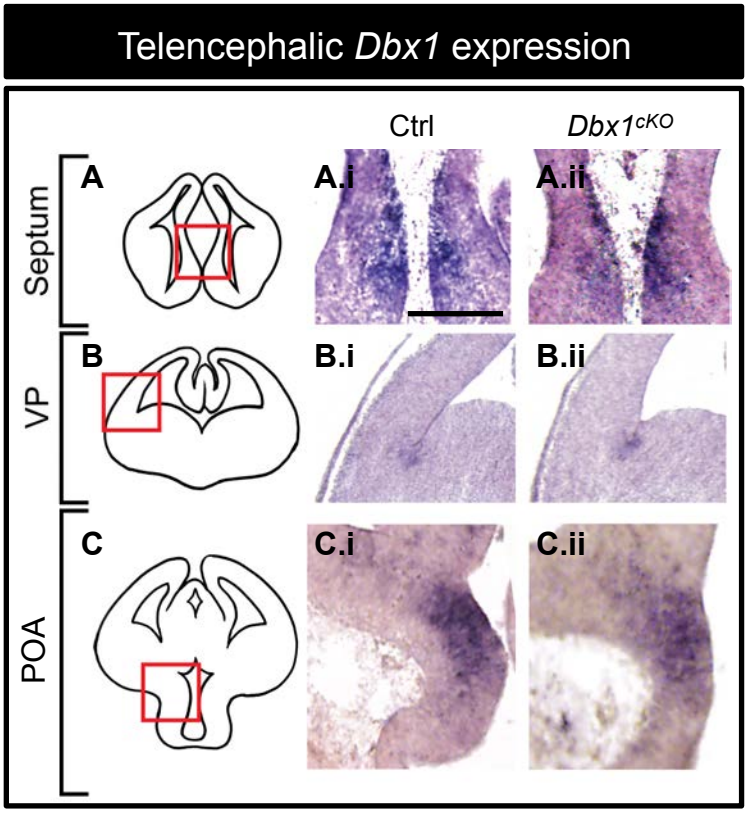

Telencephalic *Dbx1* expression
Ctrl
Dbx1^{cKO}
Septum
VP
POA
A
A.i
A.ii
B
B.i
B.ii
C
C.i
C.ii

Figure S2

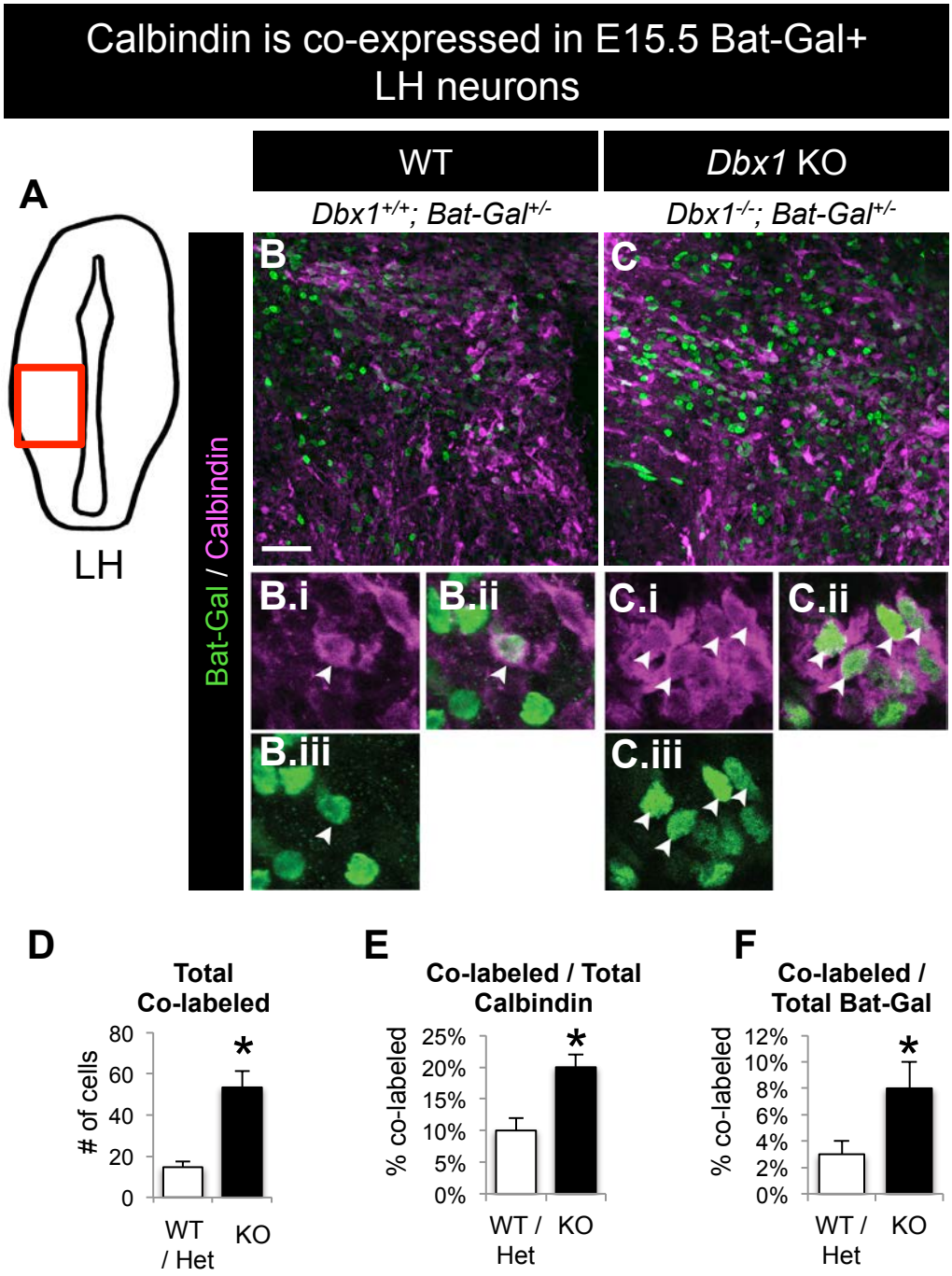

Calbindin is co-expressed in E15.5 Bat-Gal+ LH neurons
WT
Dbx1 KO
Dbx1+/+; Bat-Gal+/-
Dbx1-/-; Bat-Gal+/-
A
LH
Bat-Gal / Calbindin
B
C
B.i
B.ii
B.iii
C.i
C.ii
C.iii
D
Total Co-labeled
# of cells
80
60
40
20
0
WT / Het
KO
*
E
Co-labeled / Total Calbindin
% co-labeled
25%
20%
15%
10%
5%
0%
WT / Het
KO
*
F
Co-labeled / Total Bat-Gal
% co-labeled
12%
10%
8%
6%
4%
2%
0%
WT / Het
KO
*

Figure S3

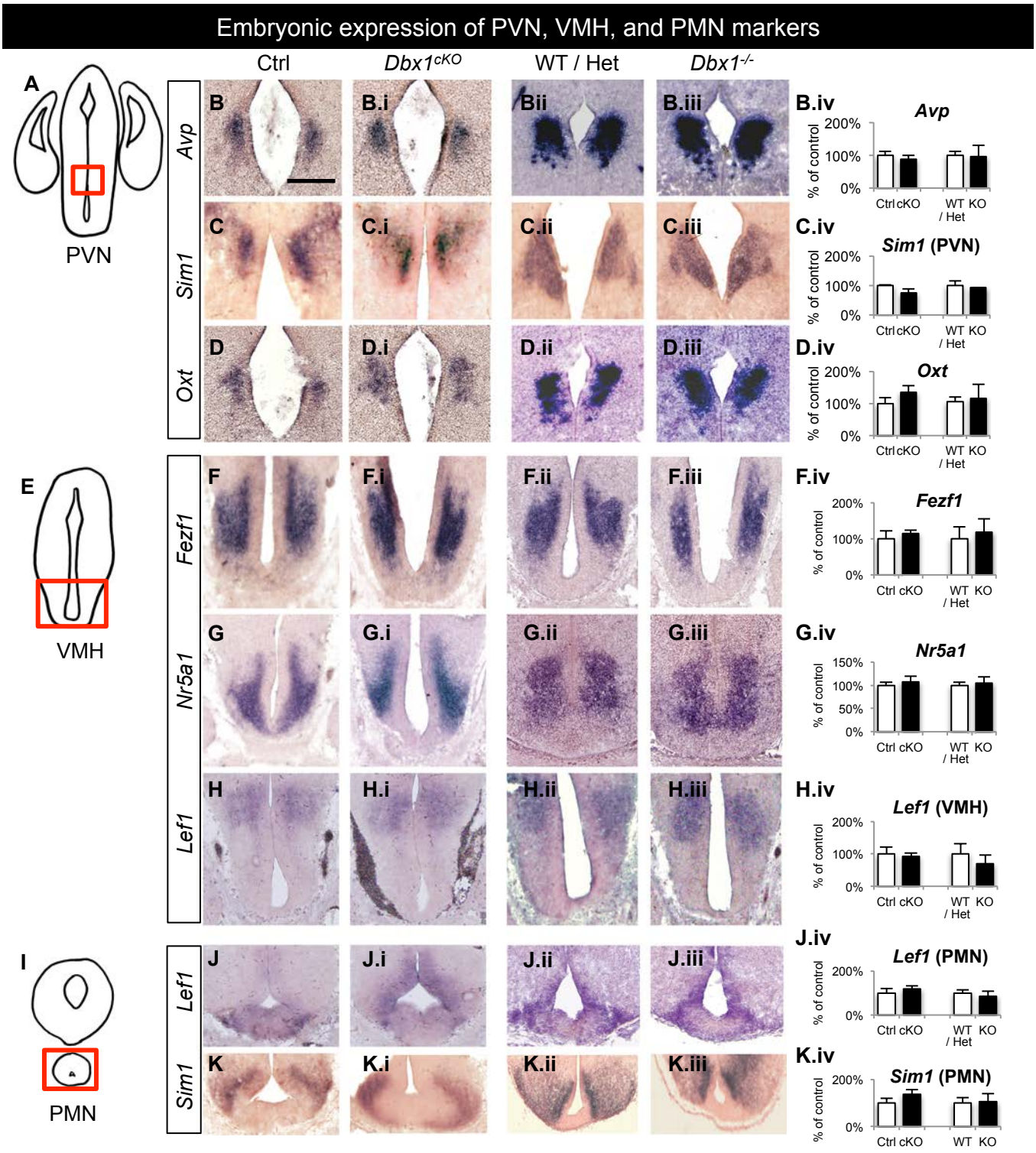

Embryonic expression of PVN, VMH, and PMN markers
Ctrl
Dbx1cKO
WT / Het
Dbx1-/-
A
PVN
Avp
Sim1
Oxt
B
B.i
Bii
B.iii
B.iv
Avp
C
C.i
C.ii
C.iii
C.iv
Sim1 (PVN)
D
D.i
D.ii
D.iii
D.iv
Oxt
E
VMH
Fezf1
Nr5a1
Lef1
F
F.i
F.ii
F.iii
F.iv
Fezf1
G
G.i
G.ii
G.iii
G.iv
Nr5a1
H
H.i
H.ii
H.iii
H.iv
Lef1 (VMH)
I
PMN
J
J.i
J.ii
J.iii
J.iv
Lef1 (PMN)
K
K.i
K.ii
K.iii
K.iv
Sim1 (PMN)
% of control
200%
150%
100%
50%
0%
Ctrl cKO
WT KO
/ Het

Figure S4

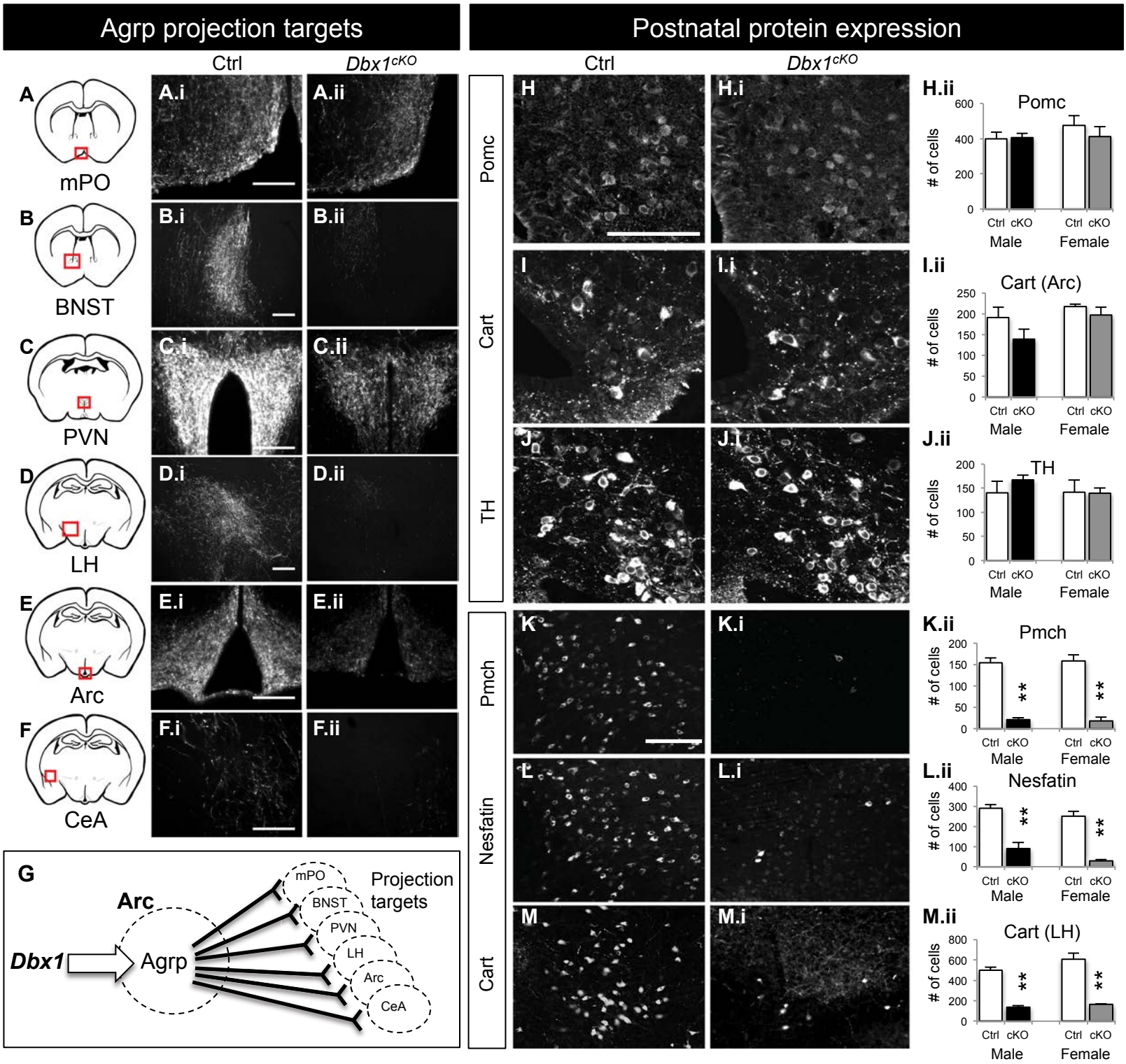

Agrp projection targets
Postnatal protein expression
Ctrl
Dbx1cKO
A
mPO
B
BNST
C
PVN
D
LH
E
Arc
F
CeA
A.i
A.ii
B.i
B.ii
C.i
C.ii
D.i
D.ii
E.i
E.ii
F.i
F.ii
G
Arc
Dbx1
Agrp
mPO
BNST
PVN
LH
Arc
CeA
Projection targets
Pomc
Cart
TH
Pmch
Nesfatin
Cart
H
H.i
H.ii
Pomc
I
I.i
I.ii
Cart (Arc)
J
J.i
J.ii
TH
K
K.i
K.ii
Pmch
L
L.i
L.ii
Nesfatin
M
M.i
M.ii
Cart (LH)
# of cells
Ctrl cKO Ctrl cKO
Male Female
**

Figure S5

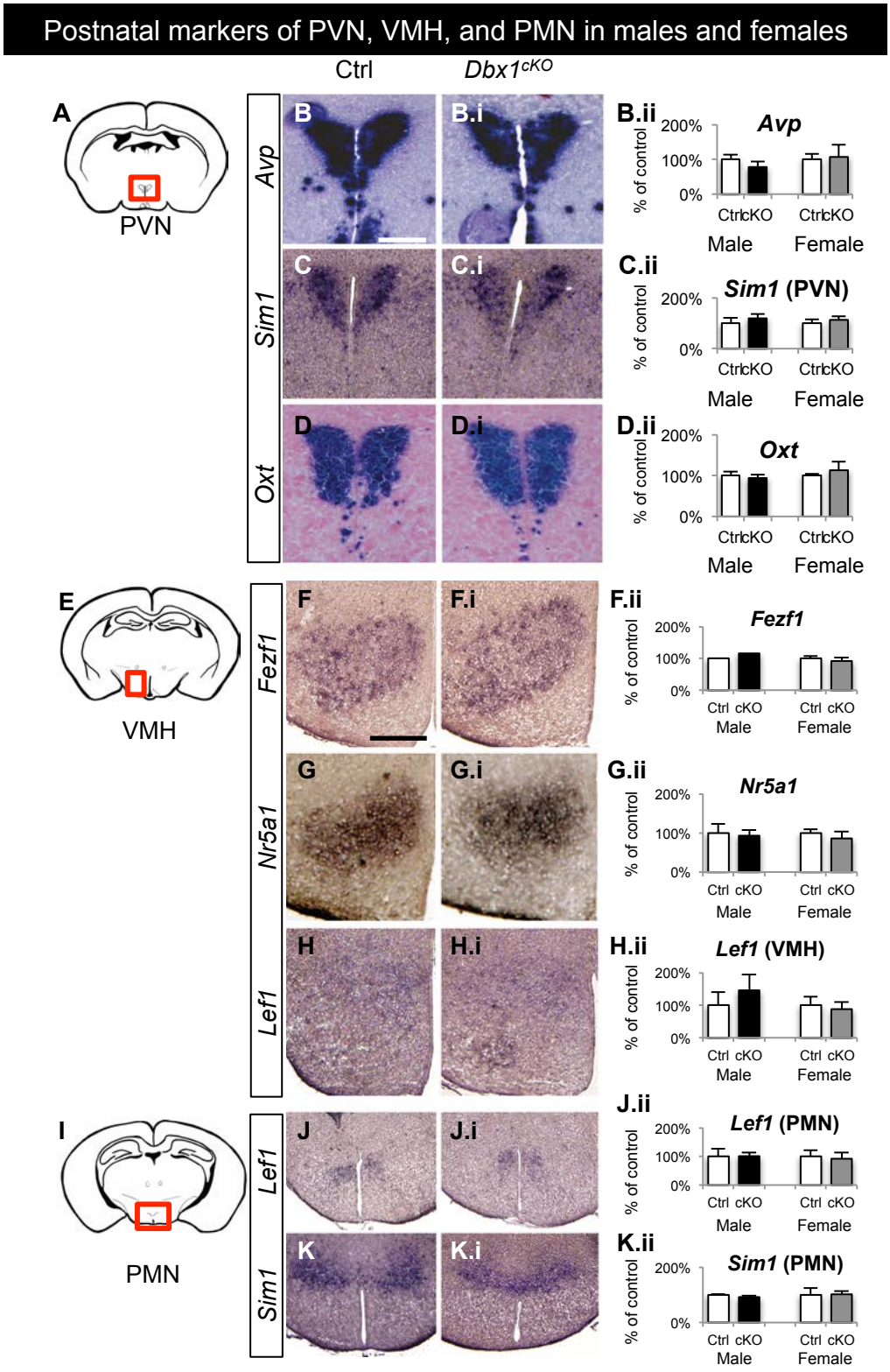

Postnatal markers of PVN, VMH, and PMN in males and females
Ctrl
Dbx1cKO
A
PVN
Avp
Sim1
Oxt
B
B.i
B.ii
Avp
C
C.i
C.ii
Sim1 (PVN)
D
D.i
D.ii
Oxt
% of control
200%
100%
0%
Ctrl cKO
Male
Female
E
VMH
Fezf1
Nr5a1
Lef1
F
F.i
F.ii
Fezf1
G
G.i
G.ii
Nr5a1
H
H.i
H.ii
Lef1 (VMH)
I
PMN
J
J.i
J.ii
Lef1 (PMN)
K
K.i
K.ii
Sim1 (PMN)

Figure S6

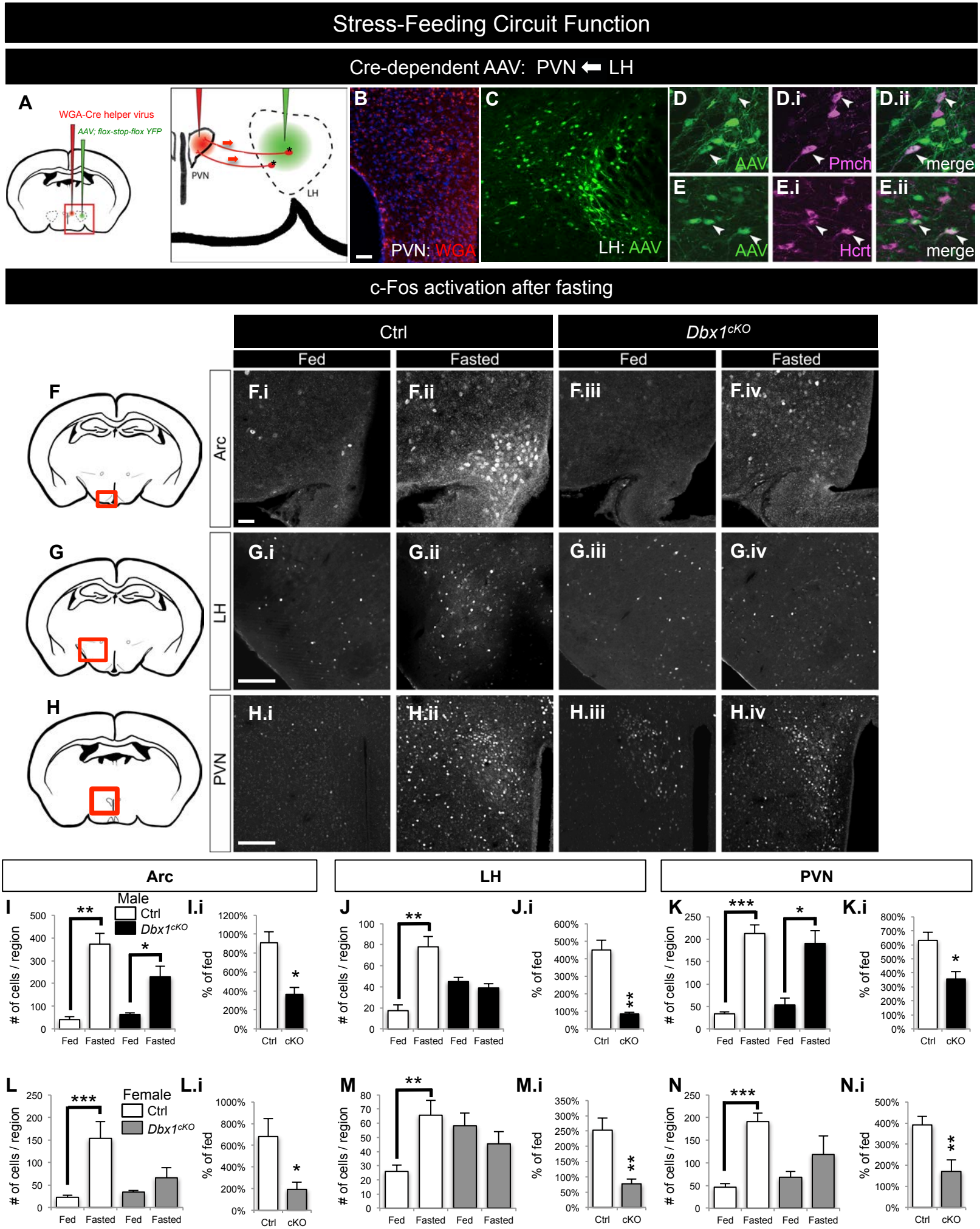

Stress-Feeding Circuit Function
Cre-dependent AAV: PVN ⬅ LH
A
WGA-Cre helper virus
AAV; flox-stop-flox YFP
PVN
LH
B
PVN: WGA
C
LH: AAV
D
AAV
D.i
Pmch
D.ii
merge
E
AAV
E.i
Hcrt
E.ii
merge
c-Fos activation after fasting
Ctrl
Dbx1cKO
Fed
Fasted
Fed
Fasted
F
Arc
F.i
F.ii
F.iii
F.iv
G
LH
G.i
G.ii
G.iii
G.iv
H
PVN
H.i
H.ii
H.iii
H.iv
Arc
LH
PVN
I
Male
Ctrl
Dbx1cKO
# of cells / region
Fed
Fasted
Fed
Fasted
I.i
% of fed
Ctrl
cKO
J
# of cells / region
J.i
% of fed
K
# of cells / region
K.i
% of fed
L
Female
Ctrl
Dbx1cKO
# of cells / region
L.i
% of fed
M
# of cells / region
M.i
% of fed
N
# of cells / region
N.i
% of fed

Figure S7

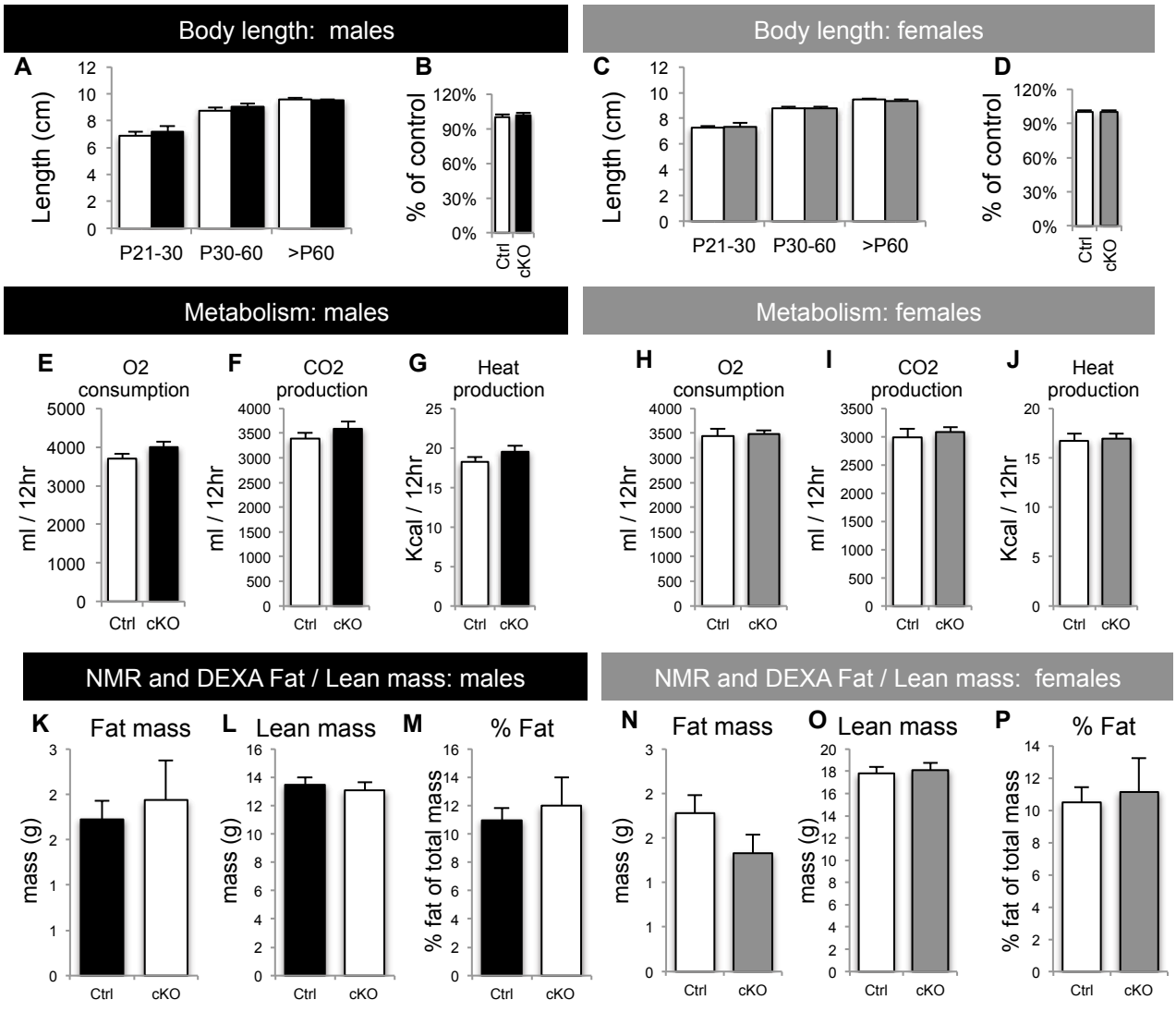
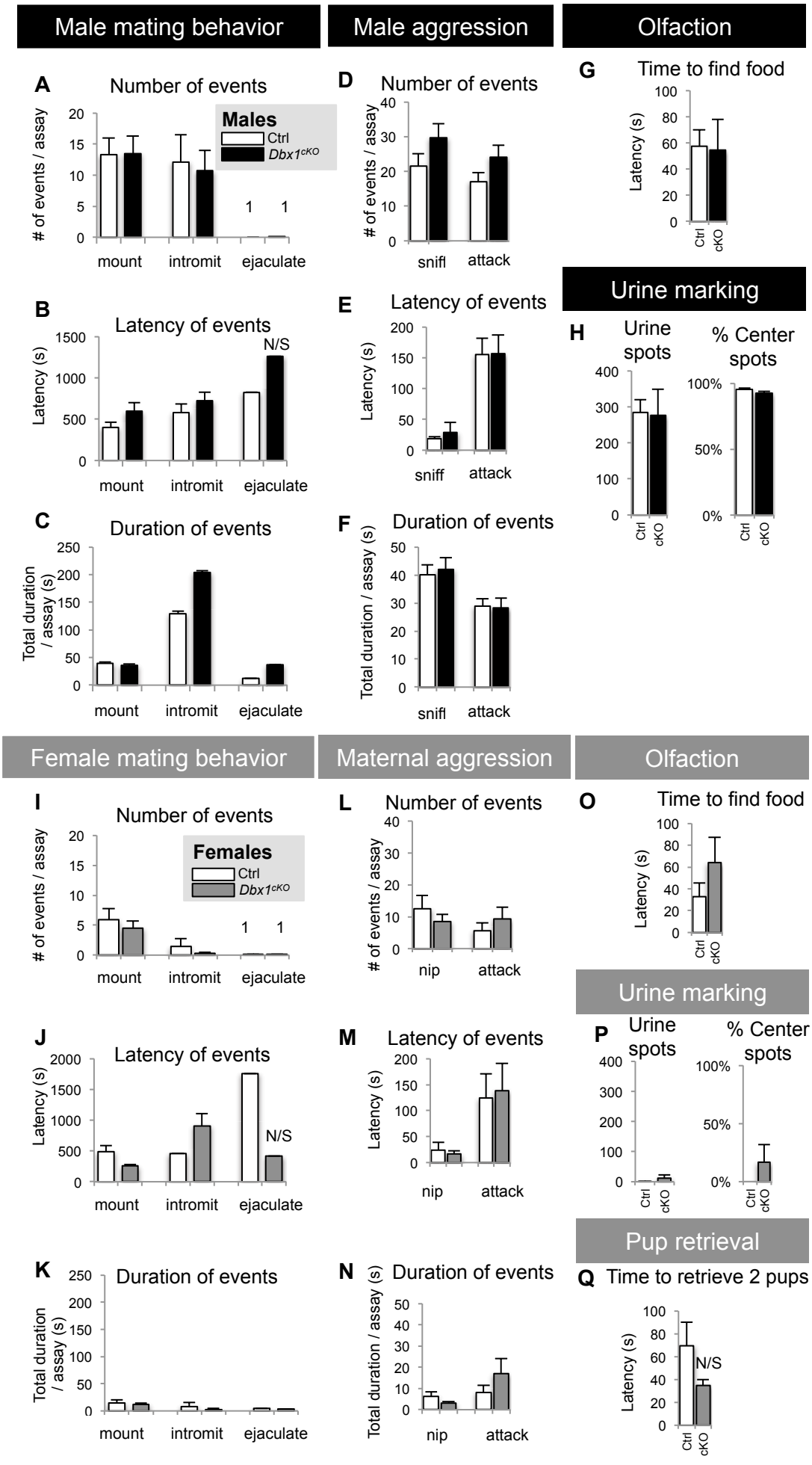

Body length: males
A
Length (cm)
P21-30
P30-60
>P60
B
% of control
Ctrl
cKO
Body length: females
C
Length (cm)
P21-30
P30-60
>P60
D
% of control
Ctrl
cKO
Metabolism: males
E
O2 consumption
ml / 12hr
F
CO2 production
ml / 12hr
G
Heat production
Kcal / 12hr
Metabolism: females
H
O2 consumption
ml / 12hr
I
CO2 production
ml / 12hr
J
Heat production
Kcal / 12hr
NMR and DEXA Fat / Lean mass: males
K
Fat mass
mass (g)
L
Lean mass
mass (g)
M
% Fat
% fat of total mass
NMR and DEXA Fat / Lean mass: females
N
Fat mass
mass (g)
O
Lean mass
mass (g)
P
% Fat
% fat of total mass

Figure S8


Male mating behavior
Male aggression
Olfaction
A
Number of events
# of events / assay
Males
Ctrl
Dbx1cKO
1
1
mount
intromit
ejaculate
D
Number of events
# of events / assay
sniff
attack
G
Time to find food
Latency (s)
Ctrl
cKO
B
Latency of events
Latency (s)
N/S
mount
intromit
ejaculate
E
Latency of events
Latency (s)
sniff
attack
Urine marking
H
Urine spots
% Center spots
C
Duration of events
Total duration / assay (s)
mount
intromit
ejaculate
F
Duration of events
Total duration / assay (s)
sniff
attack
Female mating behavior
Maternal aggression
Olfaction
I
Number of events
# of events / assay
Females
Ctrl
Dbx1cKO
1
1
mount
intromit
ejaculate
L
Number of events
# of events / assay
nip
attack
O
Time to find food
Latency (s)
Urine marking
J
Latency of events
Latency (s)
N/S
mount
intromit
ejaculate
M
Latency of events
Latency (s)
nip
attack
P
Urine spots
% Center spots
K
Duration of events
Total duration / assay (s)
mount
intromit
ejaculate
N
Duration of events
Total duration / assay (s)
nip
attack
Pup retrieval
Q
Time to retrieve 2 pups
Latency (s)
N/S