Supplementary Information for
Prolactin-mediated restraint of maternal aggression in lactation

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Other supplementary materials for this manuscript include the following:

- Movie S1
Animals

VGlu2- and VGat-ires-Cre mice, originally developed by Brad Lowell\textsuperscript{1}, were purchased from Jackson Labs [and (Slc17a6\textsuperscript{tm2(cre)Jowl}/MwarJ, stock number 028863) and (Slc32a1\textsuperscript{tm2(cre)Jowl}/MwarJ, stock number 028862), respectively]. These mice were crossed with our Prlr\textsuperscript{lox/lox} mice to generate glutamatergic neuron-specific Prlr deleted mice (Prlr\textsuperscript{lox/lox}/VGlu2-Cre) and GABAergic neuron-specific Prlr deleted mice (Prlr\textsuperscript{lox/lox}/VGat-Cre). As Cre-mediated inversion deletes the Prlr gene and knocks in EGFP in its place in Prlr\textsuperscript{lox/lox} mice\textsuperscript{2}, EGFP expression in the brain was used as a marker for both successful recombination and for the normal pattern of receptor expression.

Prlr-iCre mice were crossed with Ai9 Cre-dependent tdTomato mice\textsuperscript{3}, generating mice that express tdTomato specifically in Prlr-expressing neurons (Prlr-iCre/tdTomato mice). To assess the effect of prolactin on changes in intra-cellular calcium, Prlr-iCre mice were crossed with Cre-dependent calcium indicator GCaMP6f (B6.Cg\textsuperscript{-Gt(ROSA)26Sor\textsuperscript{tm95.1(CAG-GCaMP6f)Hze}}, stock number 024105) mice from Jackson Labs, generating mice that express GCaMP6f specifically in Prlr-expressing neurons.

Immunohistochemistry

Immunohistochemistry for fluorescent pSTAT5 in brain tissue from Prlr\textsuperscript{lox/lox}/VGlu2-Cre mice and control Prlr\textsuperscript{lox/lox} mice was conducted as previously described\textsuperscript{4}. Briefly, an antigen retrieval procedure was performed on all tissue before immunohistochemistry for pSTAT5. Sections were incubated in anti-pSTAT5 primary antibody (1:1000; polyclonal rabbit anti-pSTAT5; Tyr-694; Cell Signalling Technology, Inc., Beverly, MA) for 48 h at 4 °C, followed by a 60-min incubation in biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Peterborough, United Kingdom). Sections were then incubated in Biotin-XX Tyramide (0.3%; Invitrogen, Carlsbad, CA), before being incubated in a Streptavidin 647 IgG (1:400, AlexaFluor; Invitrogen) for 2 h at 37 °C. For dual immunofluorescent labelling of GFP and ERα, sections from virgin and lactating Prlr\textsuperscript{lox/lox}/VGlu2-Cre mice (n = 4-6/group) were incubated in anti-GFP primary antibody (polyclonal chicken anti-GFP; 1:5000; Aves Labs) for 48 h 4 °C, followed by a 60-min incubation in biotinylated goat anti-chicken IgG (1:500; Vector Laboratories). Sections were then incubated in Biotin-XX Tyramide (0.3%; Invitrogen,), before being incubated in a Streptavidin 568 IgG (1:400, AlexaFluor; Invitrogen) for 2 h at 37 °C. Tissue was incubated in anti-ERα primary antibody (1: 5000; polyclonal rabbit anti-ERα, 06-935; Sigma-Aldrich) for 48 h at 4 °C, followed by a 4 h incubation in goat anti-rabbit 488 IgG (1:500, AlexaFluor; Invitrogen) for 4 h at 21 °C.

To evaluate transfection and loss of functional Prlr expression in the VMN of AAV-Cre injected Prlr\textsuperscript{lox/lox} mice, one series of brain tissue each was used to label GFP and pSTAT5 by chromogenic immunohistochemistry. To label GFP, sections were incubated in polyclonal rabbit anti-GFP (1:10 000; A-
645; Life Technologies) for 48 h at 4 °C. To label pSTAT5, sections were incubated in anti-pSTAT5 primary antibody as above. For GFP and pSTAT5 labelling, sections were incubated for 90 min in biotinylated goat anti-rabbit IgG (detailed above). Peroxidase labelling was visualized with nickel-diaminobenzidine tetrahydrochloride using glucose oxidase to produce a black nuclear precipitate. The GFP and prolactin-induced pSTAT5 labelling in the VMN were assessed in all AAV-Cre and AAV-control administered Prlrlox/lox and C57BL/6J mice. Only Prlrlox/lox/AAV-Cre mice showing widespread GFP labelling and the complete absence of pSTAT5 labelling throughout the VMN were included for analysis of maternal aggression.

To assess intruder-induced activation of Prlr-expressing neurons, groups of virgin and lactating (day 2-3 of lactation) Prlr-iCre/tdtomato female mice (n = 5-9/group) had a wildtype juvenile male mouse (19-25 days old) introduced to the home cage for 15 min at 1000 h. Separate control groups of virgin and lactating mice were generated where mice were not exposed to an intruder. Mice were anaesthetized 90 min following introduction of intruder and brains collected and processed as described above. One series of tissue was used to examine cFos immunoreactivity in tdtomato-expressing cells by immunofluorescence. Sections were incubated in anti-cFos primary antibody (1:5000; polyclonal rabbit anti-cFos; ab190289; Abcam, Cambridge, MA) for 48 h 4 °C, followed by a 60-min incubation in biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories). Sections were then incubated in Biotin-XX Tyramide (0.3%; Invitrogen), before being incubated in a Streptavidin 647 IgG (1:400, AlexaFluor; Invitrogen) for 2 h at 37 °C. Quantification of tdtomato and cFos labelling was undertaken by counting the total number of cFos-labelled cells with and without tdtomato, in two sections per animal. Sections were anatomically matched between each different animal.

To assess AAV-hM3Dq transfection, groups of Prlr-iCre/AAV-mCherry and Prlr-iCre/AAV-hM3Dq mice were administered with 1.5 mg/kg CNO (saline/0.5% DMSO; i.p.) 45 minutes prior to transcardial perfusion with 4% paraformaldehyde. Brains and sections were collected as above and one series of tissue used to label mCherry (as a marker of successful AAV transfection) and cFos (as a marker of CNO-induced cellular activation in AAV-hM3Dq administered mice) by dual label chromogenic immunohistochemistry. Briefly, sections were incubated in rabbit anti-cFos primary antibody (1:10 000; ab190289, Abcam) for 48 h at 4 °C, followed by a 60-min incubation in biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories). Peroxidase labeling was visualized with nickel-diaminobenzidine tetrahydrochloride using glucose oxidase to produce a black nuclear precipitate. Subsequently, sections were incubated in anti-mCherry primary antibody (1:10 000; rabbit anti-mCherry; ab167453, Abcam) for 48 h at 4 °C, followed by a 120-min incubation in peroxidase goat anti-rabbit IgG (1:200; Vector Laboratories). Peroxidase labelling was visualized with diaminobenzidine tetrahydrochloride using glucose oxidase to produce a brown cytoplasmic precipitate. Immunolabelling for mCherry and cFos were assessed in the VMN of all AAV-hM3Dq-injected and control AAV-mCherry-injected Prlr-iCre mice. Data was separately analyzed
for bilateral and unilateral AAV-hM3Dq transfection and no statistical difference was detected between these two groups. Mice showing both unilateral or bilateral AAV-hM3Dq transfection, as indicated by mCherry expression, were included for behavioral analysis (n=8-10/group).

To identify fiber projections from Prlr-expressing VMN cells, brains were collected from day 3 lactating Prlr-iCre/tdTomato mice injected with AAV5-EF1a-DIO-hChR2(H13R)-eYFP-WPRE. Two series of 30-μm-thick sections were cut throughout the forebrain and brainstem, and incubated with anti-GFP primary antibody (1:5000; polyclonal chicken anti-GFP; Aves Labs) for 48 h 4 °C, followed by a 3 h incubation in goat anti-chicken 488 (1:500, AlexaFluor; Invitrogen) at 37 °C. Only animals (n = 2-3) showing exclusive transfection of the VMNvl, with no cell body labelling present in the anatomically-close ARN, were included for analysis. Importantly, areas showing high levels of YFP-labelling of fibers did not show YFP-positive staining in cells bodies, which would have been indicative of retrograde transport of the marker, suggesting that the observed fiber staining was localized exclusively in projections from the VMN.

Fluorescent images for characterizing Prlr-expressing neurons were collected with a Nikon A1 Inverted Confocal microscope and x20 objective. Z stacks were taken 1.2 μm apart. Fluorescent images for assessing projections from Prlr-expressing VMN neurons were collected with a Ti2E Nikon Inverted microscope and x10 objective to acquire whole brain images. Briefly, the area containing a brain section was defined, the area scanned with two filters, individual images in the area were stitched together automatically and Z stacks were collected 4 μm apart. Chromogenic images were collected using an Olympus AX70 Light Microscope and x10 objective.

Resident-intruder test

All intruder mice were group housed. A yellow plastic block was used as a novel object. Animal behaviors were recorded from a profile view of the cage using a Canon Legria HF G50 camera. Manual behavioral annotation was performed on a frame-by-frame basis by a researcher blind to treatment and/or genotype. If a behavior was not observed during the test, then the test-duration of 15 min was assigned as latency time. In order to give an indicator of length of behavioral episodes, number of bouts and total time were reported separately. It should be noted that in these studies, changes in the number of attacking bouts consistently aligned with changes in total time attacking.

To investigate the effect of Prlr deletion from glutamatergic neurons in lactating mice, groups of Prlr^{lox/lox}/VGlut2-Cre and Cre-negative control Prlr^{lox/lox} mice (n = 8-10/group) underwent a resident-intruder test on day 2-3 of lactation. Separate groups of Prlr^{lox/lox}/VGlut2-Cre and Cre-negative control Prlr^{lox/lox} mice (n = 8-10/group) were generated and underwent the resident-intruder test to assess whether Prlr deletion from glutamatergic neurons altered intruder-directed aggression in virgin females (n = 5/group). Groups of GABAergic neuron-specific Prlr^{lox/lox}/VGat-Cre and Cre-negative control Prlr^{lox/lox}
mice were generated and underwent the resident-intruder test as virgins (n = 5-6/group) or during lactation day 2-3 (n = 4-5/group) to assess whether effects on intruder-directed aggression were specific to glutamatergic neurons.

**Elevated Plus Maze**

The elevated plus maze (EPM) was used to assess anxiety-related behavior in separate cohorts of lactating day 2-4 Prlr<sup>lox/lox</sup>/VGlut2-Cre and control Prlr<sup>lox/lox</sup> mice (n = 6-8/group). Animals were placed in the centre of the maze facing a closed arm. Behavior was recorded for 5 min, and recordings were analyzed using TopScan (CleverSys, Inc.). Analyzed parameters were the number of entries in each arm, the time spend in each arm and the total distance travelled in the maze. An arm entry was categorized when at least 75% of the animal’s body was in the arm.

**Ex vivo brain slice electrophysiology and calcium imaging**

Intracellular calcium imaging and electrophysiological recordings were made from adult (10-20 weeks) Prlr-iCre/GCaMP6f or Prlr-iCre/AAV-hM3Dq diestrus female and lactating mice. Mice were injected with a terminal dose of sodium pentobarbital (100 mg/kg, i.p.), decapitated, brains rapidly extracted and submerged in a choline chloride solution containing: 92mM choline chloride, 2.5mM KCl, 1.2mM Na<sub>H2</sub>PO<sub>4</sub>, 30mM NaHCO<sub>3</sub>, 20mM HEPES, 25mM Glucose, 2mM L-Ascorbic acid, 2mM Thiourea, 3mM sodium pyruvate, 10 mM MgCl<sub>2</sub>, 0.5mM CaCl<sub>2</sub>, and bubbled with 5% CO<sub>2</sub> and 95% O<sub>2</sub>. Coronal brain slices (200µm) were cut using a vibratome (VT1000S; Leica). The slices were incubated for 10 min at 30°C in the same choline chloride solution listed above. Slices recovered for a minimum of 1 h at room temperature in oxygenated HEPES holding solution containing: 92mM NaCl, 2.5mM KCl, 1.2mM Na<sub>H2</sub>PO<sub>4</sub>, 30mM NaHCO<sub>3</sub>, 20mM HEPES, 25mM Glucose, 2mM L-Ascorbic acid, 2mM thiourea, 3mM sodium pyruvate, 1.3mM MgCl<sub>2</sub>, 2.4mM CaCl<sub>2</sub>.

Coronal brain slices (200µm) were transferred to a chamber and visualised using an upright microscope (Olympus, Tokyo, Japan). Slices were perfused at a rate of approx. 1.5-2 ml/min with oxygenated warm (30°C) aCSF solution containing: 127mM NaCl, 1.9mM KCl, 1.2mM NaH<sub>2</sub>PO<sub>4</sub>, 26mM NaHCO<sub>3</sub>, 10mM Glucose, 1.3mM MgCl<sub>2</sub>, 2.4mM CaCl<sub>2</sub>. Slices were illuminated through a 40X immersion objective, using the xenon arc light source (300 W; filtered by a GFP filter cube, excitation 470–490 nm; Chroma) and shutter of a λDG-4 (Sutter Instruments, Novato, CA, USA). Epifluorescence images were (495 nm long pass and emission 500–520 nm) collected using a Hamamatsu ORCA-ER digital CCD camera (Hamamatsu Photonics, Shizuoka, Japan). The µ-manager 1.4 software controlled and synchronized the light source, shutter and camera.

For whole-cell recordings, glass pipettes were filled with an internal solution containing: 120mM K-gluconate, 15M KCl, 0.5mM Na<sub>2</sub>EGTA, 2mM Mg<sub>2</sub>ATP, 0.4mM Na<sub>2</sub>GTP, 10mM HEPES, and 5mM Na<sub>2</sub>-phosphocreatine (adjusted to pH 7.2 with KOH; adjusted to 290 mOsm with sucrose). Electrophysiological
signals were recorded using a Multiclamp 700B amplifier connected to a Digidata 1440A digitizer (Molecular Devices). Signals were low-pass filtered at 2 kHz and digitized at a rate of 10 kHz. Signal acquisition was carried out with pClamp 10 (Molecular Devices).

A focal plane including at least one fluorescent cell was chosen and acquisitions (100-ms light exposure at 2 Hz) were started. All pharmacological compounds were bath applied for 5 min following a 2-min baseline period. At the end of the recordings, the responsiveness of fluorescent cells was tested by bath application of 20mM KCl. The solutions were measured to reach the bath in 1 min. Changes in intracellular calcium concentration were estimated by measuring GCaMP6f fluorescence variations. Prolactin was purchased from Sigma (L6520, Sigma-Aldrich, St. Louis, MO, USA) and CNO was purchased from Advanced Molecular technologies (AMTA056, Rocco Drive, Scoresby, Victoria, 3179, Australia).

For calcium imaging analysis, time series of images were processed in ImageJ and regions of interest were selected. Average fluorescence intensity of in-focus individual neuronal cell bodies selections was measured in each individual frame. To correct for fluorescence bleaching, a linear regression was calculated using the slope of the signal. Fluorescence intensity data was analyzed and processed using Excel and Prism. Relative changes in fluorescence (ΔF/F) for each region of interest were calculated were F is the mean baseline fluorescence intensity. The fluorescence change was represented as a percentage. Prolactin was considered to have an effect on \([\text{Ca}^{2+}]_i\) if the GCaMP6f fluorescence increased by more than twice the standard deviation of the baseline period. If this criterion was not met, the effect of prolactin on the number of calcium spikes was analyzed instead. Cells were considered either excited or inhibited if the calcium spike frequency changed by at least 20% of the baseline event frequency. For calculating number of calcium events, total spike counts were collected using the pClamp 10 event threshold search.

**Statistical Analysis**

Data are reported as mean ± SEM (unless specified) and statistical analysis was undertaken using GraphPad Prism 9 (GraphPad Software, LLC.). Full statistical analysis for the data presented in figures has been reported in Source Data. Statistical significance was established as \(p < 0.05\). Differences in control and intruder-induced cFos immunolabelling in virgin and lactating animals were compared by two-way ANOVA and a Šidák multiple comparison test was used to identify statistically significant changes. One-way ANOVAs were used to compare the number of pSTAT5-labelled nuclei in groups of Prl\(^{lox/lox}\) and Prl\(^{lox/lox}\)/VGlut-Cre mice, and Fisher’s Uncorrected LSD test was used to identify statistically significant changes. Differences between vehicle and CNO injected animals in hM3Dq or mCherry-injected mice were compared by two-way ANOVA (Šidák post-hoc test).

All latency data were analyzed using survival analysis and curve comparison with the Mantel-Cox Log-rank test. When a significant main effect was found in the comparison of more than two curves, each two
individual curves were compared pairwise. Where correction for multiple comparisons was needed, \( p \)-values were adjusted with the Benjamini-Hochberg False Discovery Rate method, using an online calculator (Carbocation Corporation, 2016). Hazard ratios (HR) have been provided as an estimate of the effect size for the comparison of latency data (included in supplementary tables). Number of bouts and total time investigating or attacking were compared by \( t \)-test (when comparing 2 groups) or one-way ANOVA (Tukey post-hoc test). For calcium imaging, all group comparisons were performed using repeated measures multiple comparisons one-way ANOVA (Tukey post-hoc test) unless otherwise stated.
Lactating PrP<sup>PrP<sub>F</sub> / VGlut-Cre

Novel Object

a

Latency to attack (seconds)

Percent of animals

p = 0.7798

p = 0.6809

Attacking bouts (number)

p = 0.3911

Time attacking (seconds)

p = 0.1356

p = 0.0006

Percent of animals

Latency to investigate (seconds)

p = 0.8188

p = 0.8280

Investigating bouts (number)

p = 0.7664

p = 0.7101

Time investigating (seconds)

p = 0.2767

p = 0.9657

Virgin PrP<sup>PrP<sub>F</sub> / VGlut-Cre

♀ Juvenile intruder

Latency to attack (seconds)

Percent of animals

p = 0.6733

p = 0.297

Attacking bouts (number)

Time attacking (seconds)

p = 0.6672

p = 0.008

Percent of animals

Latency to investigate (seconds)

p = 0.8188

p = 0.8280

Investigating bouts (number)

p = 0.7664

p = 0.7101

Time investigating (seconds)

p = 0.2767

p = 0.9657

♂ Juvenile intruder
Fig. S1. Intruder or object-directed aggressive behavior (a-c) and investigative-behavior (d-f) in lactating Prlr<sup>lox/lox</sup>/VGlu-t-Cre mice and virgin Prlr<sup>lox/lox</sup>/VGat-Cre mice. Latencies to attack (a) or investigate (d), bouts of behavior (b, e) and total time undertaking attacking (c) or investigating (f) are reported.
Fig. S2. Fertility (a), and number of live pups per litter (b) in PrlR<sup>lox/lox</sup>/VGlut-Cre mice and control PrlR<sup>lox/lox</sup> mice. The time taken from introduction of a stud male to the presence of a copulatory vaginal plug (that led to a successful pregnancy) is used as an indicator of fertility (a). Number of live pups per litter (b) was recorded on day 3 of lactation where day of parturition was counted as day 1 of lactation. Litter size was adjusted to 6 pups per litter on day 3 of lactation and pup weight on day 4 and day 20 are reported (c). Pups from both PrlR<sup>lox/lox</sup>/VGlut-Cre and control PrlR<sup>lox/lox</sup> lactating mice significantly increased weight during lactation but there was no difference in pup weight gain between groups.
Fig. S3. Anxiety-behavior in the elevated plus maze was not different in lactating Prlr^{lox/lox}/VGlut-Cre mice compared to control Prlr^{lox/lox} mice.
Fig. S4. a-c) Intruder-directed investigative behavior in lactating Prl<sub>lox/lox</sub>/VGlut-Cre, virgin Prl<sub>lox/lox</sub>/VGlut-Cre mice and in lactating Prl<sub>lox/lox</sub>/VGat-Cre mice. Compared to lactating control Prl<sub>lox/lox</sub> mice, lactating Prl<sub>lox/lox</sub>/VGlut-Cre mice show reduced bouts of investigating (b) and spent less time investigating (c) a C57BL/6J juvenile male intruder.
Fig. S5. Fertility (a), and number of live pups per litter on day 3 of lactation (b) in Prlr<sup>lox/lox</sup> mice administered with an AAV-control, wildtype C57BL/6J mice administered with AAV-Cre and in mice with a VMN-specific deletion of the prolactin receptor (Prlr<sup>lox/lox</sup>/AAV-Cre). c-e) Intruder-directed investigative behavior in Prlr<sup>lox/lox</sup>/AAV-control, C57BL/6J/AAV-Cre (B6/AAV-Cre) and Prlr<sup>lox/lox</sup>/AAV-Cre mice. Compared to lactating control groups, lactating Prlr<sup>lox/lox</sup>/AAV-Cre mice show longer latencies to investigate (c) and spent significantly less time investigating (e) a C57BL/6J juvenile male intruder. Different letters represent statistically different groups ($P < 0.05$).
Fig. S6. Intruder-directed investigative behavior in virgin C57BL/6J female mice receiving either vehicle or prolactin (5 mg/kg, i.p., 45 minutes prior to testing) administration (a-c) and day 2-4 lactating C57BL/6J mice receiving either vehicle or a D2-receptor agonist, bromocriptine, to suppress endogenous prolactin release (5 mg/kg, i.p., 2 hours prior to testing; d-f).
Fig. S7. Intruder-directed investigative behavior in hM3Dq-injected and control mCherry-injected virgin (g-i) or lactating (j-l) Prlr-iCre mice following vehicle and CNO (1.5mg/kg; i.p.) administration. In hM3Dq-injected lactating females, CNO induced an increase in the total time spend investigating the intruder. Different letters represent statistically different groups ($P < 0.05$).
**Fig. S8.** Serial sections through the brain showing the distribution of GFP-labelled fibres (cyan) derived from prolactin-responsive neurons in the VMN, following unilateral administration of AAV5-EF1a-DIO-hChR2(H13R)-eYFP-WPRE into the VMN of Prlr-iCre mice. Note the predominately ipsilateral distribution extending from the AVPV (a-c), LPO (a), LS (a-c), VP (a,b), BNST (d,e), MPOA (d,e), PeVN (d,e), PVN (f), LH (d-g), SON (f), MeA (h,i), SNR (j), PAG (k,l), DpME (k), PBN (m,n), reticular nucleus (o). AVPV, anteroventral periventricular region; BNST, bed nucleus of the stria terminalis; DpME, deep mesencephalic nucleus; LH, lateral hypothalamic area; LPO, lateral preoptic area; LS, lateral septal nucleus; MPOA, medial preoptic area; MeA, medial amygdala; PAG, periaqueductal gray; PBN, parabrachial nucleus; PeVN, periventricular hypothalamic nucleus; PVN, paraventricular nucleus; SON, supraoptic nucleus; SNR, substantia nigra, reticular part; VMN, ventromedial nucleus; VP, ventral pallidum.
**Fig. S9.** Immunofluorescent labelling for Prlr-expressing glutamatergic neurons (indicated by GFP-labelling, cyan; a) and ERα (magenta; b) in the VMN and Arc of female Prlrl\textsuperscript{lox/lox}/VGlut-Cre mice (c shows composite image of GFP and ERα immunofluorescence). d) Approximately 80% of Prlr-expressing glutamatergic neurons express ERα in the VMN. Note the presence of many additional ERα-immunoreactive cells that do not co-express Prlr (a-c).
**Movie S1 (separate file).** Resident intruder test from a control C57Bl6/AAV-Cre dam (top panel) and a VMN specific Prlr deleted (Prlr^{lox/lox}/AAV-Cre) dam (bottom panel) following introduction of a juvenile male intruder.

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## Table S1. Figure 1 Statistical Analysis

| Figure | Panel | Test Description | N     | Statistics | Post-hoc Test | Statistics | p (post test) |
|--------|-------|------------------|-------|------------|---------------|------------|--------------|
|       | a     | Log-rank (Mantel-Cox) test | Virgin | c²         | p             | n/a        |              |
|       |       |                  | Lactating | 4.786     | 0.0287        | n/a        |              |
| b     |       | Paired t test    | Virgin | t, df      | p             | n/a        |              |
|       |       |                  | Lactating | t=3.055, df=19 | 0.0065       | n/a        |              |
| c     |       | Paired t test    | Virgin | t, df      | p             | n/a        |              |
|       |       |                  | Lactating | t=3.275, df=19 | 0.004        | n/a        |              |
| g     |       | Two-way ANOVA    | Prlr<sup>lox/lox</sup> virgin | 12 | F (DFn, DFd) | p             | Prlr<sup>lox/lox</sup>:Virgin vs. Prlr<sup>lox/lox</sup>:Lactating | 0.1731 |
|       |       |                  | Prlr<sup>lox/lox</sup> lactating | 8 | F (1, 30) = 3.579 | 0.0682     | Prlr<sup>lox/lox</sup>:Virgin vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Virgin | 0.2343 |
|       |       |                  | Prlr<sup>lox/lox</sup>/VGlut-Cre virgin | 9 | F (1, 30) = 18.17 | 0.0002     | Prlr<sup>lox/lox</sup>:Virgin vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Lactating | 0.1076 |
|       |       |                  | Prlr<sup>lox/lox</sup>/VGlut-Cre lactating | 5 | F (1, 30) = 0.6909 | 0.4413     | Prlr<sup>lox/lox</sup>:Lactating vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Virgin | 0.0041 |
|       |       |                  | Prlr<sup>lox/lox</sup>/VGlut-Cre lactating | 5 | F (1, 30) = 0.6909 | 0.4413     | Prlr<sup>lox/lox</sup>/VGlut-Cre:Virgin vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Lactating | 0.0028 |
| h     |       | Log-rank (Mantel-Cox) test | Prlr<sup>lox/lox</sup> | 10 | c²         | p             | n/a        |              |
|       |       | Lactating - male juvenile intruder | Prlr<sup>lox/lox</sup>/VGlut-Cre | 8 | 7.371     | 0.0066      | n/a        |              |
|       |       | Log-rank (Mantel-Cox) test | Prlr<sup>lox/lox</sup> | 9 | c²         | p             | n/a        |              |
|       |       | Lactating - male adult intruder | Prlr<sup>lox/lox</sup>/VGlut-Cre | 8 | 10.52     | 0.0012      | n/a        |              |
|       |       | Log-rank (Mantel-Cox) test | Prlr<sup>lox/lox</sup> | 11 | c²         | p             | n/a        |              |
|       |       | Lactating - female adult intruder | Prlr<sup>lox/lox</sup>/VGlut-Cre | 6 | 0         | >0.9999     | n/a        |              |
|       |       | Log-rank (Mantel-Cox) test | Prlr<sup>lox/lox</sup> | 5 | c²         | p             | n/a        |              |
|                  | **Prlr$^{lox/lox}$/VGlu-Cre** | 5 | 0 | >0.9999 |
|------------------|------------------------------|---|---|---------|
| **Virgin - male juvenile intruder** | **Prlr$^{lox/lox}$/VGlu-Cre** | 4 | c2 | p | n/a |
| **Log-rank (Mantel-Cox) test** | **Prlr$^{lox/lox}$/VGlu-Cre** | 5 | 0.2582 | 0.6114 |
| Lactating - male juvenile intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 10 | t, df | p | n/a |
| Lactating - male adult intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 8 | t=2.037, df=16 | 0.0585 |
| Unpaired t test | **Prlr$^{lox/lox}$/VGlu-Cre** | 9 | t, df | p | n/a |
| Lactating - female adult intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 11 | t, df | p | n/a |
| Unpaired t test | **Prlr$^{lox/lox}$/VGlu-Cre** | 5 | t, df | p | n/a |
| Virgin - male juvenile intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 4 | t=0.9155, df=7 | 0.3904 |
| **Unpaired t test** | **Prlr$^{lox/lox}$/VGlu-Cre** | 5 | t=2.96, df=15 | 0.0247 |
| Lactating - male juvenile intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 10 | t, df | p | n/a |
| Lactating - male adult intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 8 | t=2.756, df=16 | 0.014 |
| Unpaired t test | **Prlr$^{lox/lox}$/VGlu-Cre** | 9 | t, df | p | n/a |
| Lactating - female adult intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 11 | t=2.374, df=15 | 0.0314 |
| Unpaired t test | **Prlr$^{lox/lox}$/VGlu-Cre** | 6 | t, df | p | n/a |
| Lactating - female adult intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 5 | t=1,323, df=7 | 0.2276 |
| Virgin - male juvenile intruder | **Prlr$^{lox/lox}$/VGlu-Cre** | 4 | t, df | p | n/a |
Table S2. Figure 2 Statistical Analysis

| Figure | Panel | Test | N         | Statistics | Post-hoc test | Statistics | p (post test) |
|--------|-------|------|-----------|------------|---------------|------------|---------------|
| 2      | c     | Log-rank (Mantel-Cox) test | $Pr^{lox/lox}$/ AAV-control | 8 | $c^2$ | $p$ | 28.42 | $<0.0001$ | Benjamini-Hochberg False Discovery Rate | $Pr^{lox/lox}$/ AAV-control vs. $Pr^{lox/lox}$/ AAV-Cre | 0.0003 |
|        |       |      | $Pr^{lox/lox}$/ AAV-Cre | 8 | | | | | $Pr^{lox/lox}$/ AAV-control vs. B6/ AAV-Cre | 0.4978 |
|        |       |      | B6/ AAV-Cre | 6 | | | | | $Pr^{lox/lox}$/ AAV-Cre vs. B6/ AAV-Cre | 0.00045 |
| 2      | d     | One-way ANOVA | $Pr^{lox/lox}$/ AAV-control | 8 | F | $p$ | 11.72 | 0.0005 | Tukey's multiple comparisons test | $Pr^{lox/lox}$/ AAV-control vs. $Pr^{lox/lox}$/ AAV-Cre | 0.0014 |
|        |       |      | $Pr^{lox/lox}$/ AAV-Cre | 8 | | | | | $Pr^{lox/lox}$/ AAV-control vs. B6/ AAV-Cre | 0.9708 |
|        |       |      | B6/ AAV-Cre | 6 | | | | | $Pr^{lox/lox}$/ AAV-Cre vs. B6/ AAV-Cre | 0.0017 |
| 2      | e     | One-way ANOVA | $Pr^{lox/lox}$/ AAV-control | 8 | F | $p$ | 15.89 | $<0.0001$ | Tukey's multiple comparisons test | $Pr^{lox/lox}$/ AAV-control vs. $Pr^{lox/lox}$/ AAV-Cre | 0.0005 |
|        |       |      | $Pr^{lox/lox}$/ AAV-Cre | 8 | | | | | $Pr^{lox/lox}$/ AAV-control vs. B6/ AAV-Cre | 0.7519 |
|        |       |      | B6/ AAV-Cre | 6 | | | | | $Pr^{lox/lox}$/ AAV-Cre vs. B6/ AAV-Cre | 0.0002 |
Table S3. Figure 3 Statistical Analysis

| Figure | Panel | Test | N | Statistics | Post-hoc test | Statistics | p (post test) |
|--------|-------|------|---|------------|---------------|------------|--------------|
| b      | One-way RM ANOVA | 7 | F | 32.4 | <0.0003 | Tukey's multiple comparisons test | Baseline vs. Prolactin 250nM: 0.0035 Baseline vs. Wash: 0.7955 Prolactin 250nM vs. Wash: 0.0005 |
| c      | One-way RM ANOVA | 14 | F | 38.04 | <0.0001 | Tukey's multiple comparisons test | Baseline vs. Prolactin 250nM: <0.0001 Baseline vs. Wash: 0.5899 Prolactin 250nM vs. Wash: 0.0002 |
| d      | Gehan-Breslow-Wilcoxon test | 12 | c² | 0.6944 | 0.4047 | n/a | |
| e      | Paired t test | 12 | t, df | t=0.1421, df=11 | 0.8896 | n/a | |
| f      | Paired t test | 12 | t, df | t=0.3949, df=11 | 0.7004 | n/a | |
| g      | Log-rank (Mantel-Cox) test | Vehicle 10 | c² | 0.316 | 0.574 | n/a | |
| h      | Unpaired t test | Vehicle 10 | t, df | t=0.4289, df=19 | 0.6728 | n/a | |
| i      | Unpaired t test | Vehicle 10 | t, df | t=0.8655, df=19 | 0.3976 | n/a | |
| Figure | Panel | Test | N  | Statistics | Post-hoc test | Statistics | p (post test) |
|--------|-------|------|----|------------|--------------|------------|--------------|
| 4a     |       |      |    |            | Tukey's multiple comparisons test |            | 0.0001       |
| 4b     |       |      |    |            | Tukey's multiple comparisons test |            | 0.001        |
| 4g     |       |      |    |            | Benjamini-Hochberg False Discovery Rate |            |              |
| h      |       |      |    |            | Šídák’s multiple comparisons test |            | 0.3334       |
| i      |       |      |    |            | Šídák’s multiple comparisons test |            | 0.2654       |
| j      |       |      |    |            | Benjamini-Hochberg False Discovery Rate |            |              |
| k      |       |      |    |            | Šídák’s multiple comparisons test |            | 0.5785       |
| l      |       |      |    |            | Šídák’s multiple comparisons test |            | 0.7406       |

**Table S4. Figure 4 Statistical Analysis**

- Virgin no intruder vs virgin intruder
- Virgin no intruder vs lactating no intruder
- Lactating no intruder vs lactating intruder
- Virgin no intruder vs lactating intruder
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-Virgin no intruder vs virgin intruder
-Lactating no intruder vs lactating intruder
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Table S1. Supplementary Figure 1 Statistical Analysis

| Figure | Panel | Test | N | Statistics |
|--------|-------|------|---|------------|
| a      |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 9   | 0 >0.9999  |
|        |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Lactating - female juvenile intruder | Prllox/Lox/VGlut-Cre | 11  | 0.07815 0.7798 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Virgin - juvenile male intruder | Prllox/Lox/VGat-Cre | 5   | 1.2 0.2733 |
| b      |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 9   | n/a n/a   |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - female juvenile intruder | Prllox/Lox/VGlut-Cre | 11  | t=0.4188, df=16 0.6809 |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Virgin - juvenile male intruder | Prllox/Lox/VGat-Cre | 5   | t=1.108, df=9 0.2967 |
| c      |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 9   | n/a n/a   |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - female juvenile intruder | Prllox/Lox/VGlut-Cre | 11  | t=0.8815, df=16 0.3911 |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Virgin - juvenile male intruder | Prllox/Lox/VGat-Cre | 5   | t=1.108, df=9 0.2967 |
| d      |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 9   | 2.224 0.1358 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Lactating - female juvenile intruder | Prllox/Lox/VGlut-Cre | 11  | 11.73 0.0006 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/Lox | c2  | p          |
|        |       | Virgin - juvenile male intruder | Prllox/Lox/VGat-Cre | 5   | 0.1849 0.6672 |
| e      |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 11  | t=0.2324, df=18 0.8188 |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - female juvenile intruder | Prllox/Lox/VGlut-Cre | 11  | t=0.2234, df=16 0.826 |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Virgin - juvenile male intruder | Prllox/Lox/VGat-Cre | 5   | t=1.158, df=9 0.2767 |
| f      |       | Unpaired t test | Prllox/Lox | t, df | p |
|        |       | Lactating - novel object   | Prllox/Lox/VGlut-Cre | 10  | t=0.3022, df=16 0.7664 |
|        |       | Unpaired t test | Prllox/Lox | t, df | p |
| Intruder Type                  | Strain               | Sample Size | t-Value | df  | p-Value |
|--------------------------------|----------------------|-------------|---------|-----|---------|
| Lactating - female juvenile intruder | Prllox/lox/VGlut-Cre  | 7           | 0.3784  | 16  | 0.7101  |
| Unpaired t test                | Prllox/lox           | 5           |         |     |         |
| Virgin - juvenile male intruder | Prllox/lox/VGat-Cre  | 6           | 0.04423 | 9   | 0.9657  |
Table S6. Supplementary Figure 2 Statistical Analysis

| Figure | Panel | Test | N | Statistics | Post-hoc test | Statistics | p (post test) |
|--------|-------|------|---|------------|---------------|------------|--------------|
| S3     | a     | Unpaired t test | Prlr<sup>lox/lox</sup> | 12 | t, df | p | 0.1553 | n/a |
|        |       |                  | Prlr<sup>lox/lox</sup>/VGlut-Cre | 10 | t=1.477, df=20 | 0.1553 | n/a |
|        | b     | Unpaired t test | Prlr<sup>lox/lox</sup> | 12 | t, df | p | 0.1983 | n/a |
|        |       |                  | Prlr<sup>lox/lox</sup>/VGlut-Cre | 10 | t=1.331, df=20 | 0.1983 | n/a |
|        | c     | Two-way RM ANOVA | Day 4 - Prlr<sup>lox/lox</sup> | | F (DFn, DFd) | p | Šídák's multiple comparisons test | Prlr<sup>lox/lox</sup>:Day 4 vs. Prlr<sup>lox/lox</sup>:Day 20 | <0.0001 |
|        |       |                  | Day 20 - Prlr<sup>lox/lox</sup> | | F (1, 16) = 2.613 | 0.1255 | | Prlr<sup>lox/lox</sup>/VGlut-Cre:Day 4 vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Day 20 | <0.0001 |
|        |       |                  | Day 4 - Prlr<sup>lox/lox</sup>/VGlut-Cre | | F (1, 16) = 0.2114 | 0.6518 | Šídák's multiple comparisons test | Prlr<sup>lox/lox</sup>:Day 4 vs. Prlr<sup>lox/lox</sup>/VGlut-Cre:Day 4 | 0.3648 |
| Figure | Panel | Test               | N  | Statistics         |
|--------|-------|--------------------|----|--------------------|
| S3     | a     | Unpaired t test    | 8  | t, df              |
|        |       | Prl\(^{lox/lox}\)  |    | p                  |
|        |       | Prl\(^{lox/lox}\)/VGlut-Cre | 6 | t=1.646, df=12 | 0.1256 |
|        | b     | Unpaired t test    | 8  | t, df              |
|        |       | Prl\(^{lox/lox}\)  |    | p                  |
|        |       | Prl\(^{lox/lox}\)/VGlut-Cre | 6 | t=0.2556, df=12 | 0.8026 |
|        | c     | Unpaired t test    | 8  | t, df              |
|        |       | Prl\(^{lox/lox}\)  |    | p                  |
|        |       | Prl\(^{lox/lox}\)/VGlut-Cre | 6 | t=0.8801, df=12 | 0.3961 |
Table S8. Supplementary Figure 4 Statistical Analysis

| Figure | Panel | Test | N | Statistics |
|--------|-------|------|---|------------|
|        | a     | Log-rank (Mantel-Cox) test | Prllox/lox | c2 | 0.3577 0.5498 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/lox | c2 | 2.224 0.1358 |
|        |       | Lactating - male adult intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/lox | c2 | 1.024 0.3116 |
|        |       | Lactating - female adult intruder | Prllox/lox/VGlut-Cre | 6 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/lox | c2 | 0.0224 0.881 |
|        |       | Virgin - male juvenile intruder | Prllox/lox/VGat-Cre | 5 |
|        |       | Log-rank (Mantel-Cox) test | Prllox/lox | c2 | 3.186 0.0743 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Unpaired t test | Prllox/lox | t, df = 2.406, df = 16 | p 0.0286 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Unpaired t test | Prllox/lox | t, df = 2.091, df = 15 | p 0.054 |
|        |       | Lactating - male adult intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Unpaired t test | Prllox/lox | t, df = 0.5312, df = 15 | p 0.6031 |
|        |       | Lactating - female adult intruder | Prllox/lox/VGlut-Cre | 6 |
|        |       | Unpaired t test | Prllox/lox | t, df = 1.068, df = 8 | p 0.3168 |
|        |       | Virgin - male juvenile intruder | Prllox/lox/VGat-Cre | 5 |
|        |       | Unpaired t test | Prllox/lox | t, df = 2.382, df = 7 | p 0.0487 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 8 |
|        | b     | Unpaired t test | Prllox/lox | t, df = 3.699, df = 16 | p 0.0019 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Unpaired t test | Prllox/lox | t, df = 0.5183, df = 15 | p 0.6118 |
|        |       | Lactating - male adult intruder | Prllox/lox/VGlut-Cre | 8 |
|        |       | Unpaired t test | Prllox/lox | t, df = 1.490, df = 15 | p 0.1569 |
|        |       | Lactating - female adult intruder | Prllox/lox/VGlut-Cre | 6 |
|        |       | Unpaired t test | Prllox/lox | t, df = 1.805, df = 8 | p 0.1088 |
|        |       | Virgin - male juvenile intruder | Prllox/lox/VGat-Cre | 5 |
|        |       | Unpaired t test | Prllox/lox | t, df = 0.4232, df = 7 | p 0.6848 |
|        |       | Lactating - male juvenile intruder | Prllox/lox/VGlut-Cre | 5 |
Table S9. Supplementary Figure 5 Statistical Analysis

| Figure | Panel | Test | N   | Statistics | Post-hoc test | Statistics | p (post test) |
|--------|-------|------|-----|------------|---------------|------------|--------------|
|        | a     | One-way ANOVA | Prlrsamy/ AAV-control | 8 | F  p | Tukey's multiple comparisons test | Prlrsamy/ AAV-control vs. B6/ AAV-Cre | 0.7878 |
|        |       |                   | Prlrsamy/ AAV-Cre | 7 | 0.7262 0.4974 |   | B6/ AAV-Cre vs. Prlrsamy/ AAV-Cre | 0.4654 |
| S5     | c     | One-way ANOVA | Prlrsamy/ AAV-control | 8 | F  p | Tukey's multiple comparisons test | Prlrsamy/ AAV-control vs. B6/ AAV-Cre | 0.9733 |
|        |       |                   | Prlrsamy/ AAV-Cre | 8 | 0.9626 0.3997 |   | Prlrsamy/ AAV-control vs. Prlrsamy/ AAV-Cre | 0.4035 |
|        |       |                   | B6/ AAV-Cre | 6 |   |   | B6/ AAV-Cre vs. Prlrsamy/ AAV-Cre | 0.5874 |
|        | c     | Log-rank (Mantel-Cox) test | Prlrsamy/ AAV-control | 8 | c2  p | Benjamini-Hochberg False Discovery Rate | Prlrsamy/ AAV-control vs. Prlrsamy/ AAV-Cre | 0.5181 |
|        |       |                   | Prlrsamy/ AAV-Cre | 8 | 2.316 0.3141 |   | Prlrsamy/ AAV-control vs. B6/ AAV-Cre | 0.5956 |
|        |       |                   | B6/ AAV-Cre | 6 |   |   | Prlrsamy/ AAV-Cre vs. B6/ AAV-Cre | 0.49185 |
|        | d     | One-way ANOVA | Prlrsamy/ AAV-control | 8 | F  p | Tukey's multiple comparisons test | Prlrsamy/ AAV-control vs. Prlrsamy/ AAV-Cre | 0.0148 |
|        |       |                   | Prlrsamy/ AAV-Cre | 8 | 5.858 0.0104 |   | Prlrsamy/ AAV-control vs. B6/ AAV-Cre | 0.037 |
|        |       |                   | B6/ AAV-Cre | 6 |   |   | Prlrsamy/ AAV-Cre vs. B6/ AAV-Cre | 0.9779 |
|        | e     | One-way ANOVA | Prlrsamy/ AAV-control | 8 | F  p | Tukey's multiple comparisons test | Prlrsamy/ AAV-control vs. Prlrsamy/ AAV-Cre | 0.0034 |
|        |       |                   | Prlrsamy/ AAV-Cre | 8 | 12.04 0.0004 |   | Prlrsamy/ AAV-control vs. B6/ AAV-Cre | 0.5812 |
|        |       |                   | B6/ AAV-Cre | 6 |   |   | Prlrsamy/ AAV-Cre vs. B6/ AAV-Cre | 0.0007 |
### Table S10. Supplementary Figure 6 Statistical Analysis

| Figure | Panel | Test | N  | Statistics       |
|--------|-------|------|----|------------------|
| S6     | a     | Gehan-Breslow-Wilcoxon test | 12 | c², p             |
|        |       |      |    | 0.5198, 0.4709   |
|        | b     | Paired t test               | 11 | t, df, p         |
|        |       |      |    | t=1.221, df=10, 0.2503 |
|        | c     | Paired t test               | 12 | t, df, p         |
|        |       |      |    | t=2.669, df=11, 0.0218 |
|        | d     | Log-rank (Mantel-Cox) test  | Vehicle 10 | c², p          |
|        |       |      |    | 0.00914, 0.9238  |
|        |       |      |    | Bromocortine 11  |
|        | e     | Unpaired t test             | Vehicle 10 | t, df, p       |
|        |       |      |    | t=2.988, df=19, 0.0076 |
|        |       |      |    | Bromocortine 11  |
|        | f     | Unpaired t test             | Vehicle 10 | t, df, p       |
|        |       |      |    | t=0.1061, df=18, 0.9167 |
|        |       |      |    | Bromocortine 11  |
Table S11. Supplementary Figure 7 Statistical Analysis

| Figure | Panel | Test | N  | Statistics | Post-hoc test | p (post test) |
|--------|-------|------|----|------------|---------------|--------------|
|        |       |      |    |            | Benjamini-Hochberg False Discovery Rate |              |
| a      |       | Log-rank (Mantel-Cox) test | hM3Dq 13 | c² | df | p | hM3Dq-Veh vs hM3Dq-CNO | 0.6799 |
|        |       |      |    |            | mCherry-Veh vs mCherry-CNO | 0.9902 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.4552 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.9153 |
| b      |       | Two-way RM ANOVA | hM3Dq 13 | F (DFn, DFd) | p | Šídák's multiple comparisons test | hM3Dq-Veh vs hM3Dq-CNO | 0.9031 |
|        |       |      |    |            | Treatment x Virus | 0.9794 |
|        |       |      |    |            | Treatment | 0.9724 |
|        |       |      |    |            | Virus | 0.0649 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.136 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.143 |
| c      |       | Two-way RM ANOVA | hM3Dq 13 | F (DFn, DFd) | p | Šídák's multiple comparisons test | hM3Dq-Veh vs hM3Dq-CNO | 0.9757 |
|        |       |      |    |            | Treatment x Virus | 0.9289 |
|        |       |      |    |            | Treatment | 0.7798 |
|        |       |      |    |            | Virus | 0.2324 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.5044 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.5721 |
| d      |       | Log-rank (Mantel-Cox) test | hM3Dq 10 | c² | df | p | Benjamini-Hochberg False Discovery Rate | hM3Dq-Veh vs hM3Dq-CNO | 0.3149 |
|        |       |      |    |            | mCherry-Veh vs mCherry-CNO | 0.046 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.27 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.0548 |
| e      |       | Two-way RM ANOVA | hM3Dq 10 | F (DFn, DFd) | p | Šídák's multiple comparisons test | hM3Dq-Veh vs hM3Dq-CNO | 0.5295 |
|        |       |      |    |            | Treatment x Virus | 0.5711 |
|        |       |      |    |            | Treatment | 0.0571 |
|        |       |      |    |            | Virus | 0.0516 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.1669 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.8699 |
| f      |       | Two-way RM ANOVA | hM3Dq 10 | F (DFn, DFd) | p | Šídák's multiple comparisons test | hM3Dq-Veh vs hM3Dq-CNO | 0.0047 |
|        |       |      |    |            | Treatment x Virus | 0.0439 |
|        |       |      |    |            | Treatment | 0.0185 |
|        |       |      |    |            | Virus | 0.0654 |
|        |       |      |    |            | hM3Dq-Veh vs mCherry-Veh | 0.9488 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.9975 |
|        |       |      |    |            | hM3Dq-CNO vs mCherry-CNO | 0.0125 |
Table S12. Supplementary Figure 9 Statistical Analysis

| Figure | Panel | Test                  | N     | Statistics               |
|--------|-------|-----------------------|-------|--------------------------|
| S9     | d     | Unpaired t test - VMN | Virgin | 19 | t, df | p | t=0.2593, df=45 | 0.7966 |
|        |       | Lactating             | 28    |                           | t=0.5994, df=45 | 0.5519 |
|        |       | Unpaired t test - Arc | Virgin | 19 | t, df | p | t=0.2593, df=45 | 0.7966 |
|        |       | Lactating             | 28    |                           | t=0.5994, df=45 | 0.5519 |