Supplementary Information for

Cerebellin-2 Regulates a Serotonergic Dorsal Raphe Circuit that Controls Compulsive Behaviors

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EXTENDED MATERIALS AND METHODS

**Immunohistochemistry.** For all immunohistochemistry experiments, mice were anesthetized with isoflurane and their brains were fixed by transcranial perfusion of 4% paraformaldehyde (PFA). Following removal from the skull, brains were further incubated in 4% PFA for 2 h at room temperature (RT) with agitation. Brains were then cryoprotected with 30% sucrose (in 1X PBS) at 4°C for 48 h with rotation. The samples were frozen in OCT compound (TissueTek) on dry ice, and 25 μm coronal or sagittal sections were produced with a cryostat (CM 3050S; Leica Biosystems). Sections were carefully transferred to a 1.5 mL tube and blocked with 10% goat serum, 1% BSA, 0.3% Triton X-100 in 1X PBS for 1 h at RT with rocking. Sections stained for GFP only were incubated in primary antibody overnight at 4°C with rocking; all other antibodies were incubated for 48 h at 4°C. Sections were washed 3 times for 20 min with PBS containing 0.3% Triton X-100, and incubated for 2 h at RT in species-specific secondary antibodies coupled to Alexa Fluor 488, 546, 633, or 647 (Invitrogen; 1:400 dilution). Sections were washed and mounted on slides with Vectashield containing the nuclear stain DAPI (Vector Laboratories). Images were collected using a Nikon A1R confocal with a 10X, 20X or 60X objective and analyzed using NIS-Elements Advanced Research software (Nikon Instruments) and ImageJ/Fiji (NIH).

3-week-old Cbln2-mVenus mice were used for retrograde tracing and co-labeling experiments (Figure 3 and Supplementary Figure S3), and at least two mice and four sections per mouse were used for analysis.

~1-month-old Cbln2 KO and control littersmates were used for synapse quantification experiments in Figure 4. For each synaptic marker, data were collected from 3 mice/genotype, 3 sections/mouse. The density of vGluT1+ and vGluT2+ synapses was first normalized to the area of TPH2-labeled tissue and then to the density observed in the controls. The density of SERT+ and vGluT3+ synapses was first normalized to the area of SERT-labeled axons and then to the density observed in the controls.

~1-month-old Cbln2 KO mice and control littersmates were used for the NeuN and TPH2 neuron quantification experiments (Supplementary Figure S4) and for the SERT+ axon and puncta quantification experiments (Figure 4 and Supplementary Figure S5). Data were collected from 3 mice/genotype, 3 sections/mouse. SERT+ axon density was quantified using the DEFiNE plugin for ImageJ/Fiji (Powell et al., 2019) and was first normalized to the area of the region of interest (ROI) and then to the density observed in the controls. SERT+ puncta density was first normalized to the ROI area and then to the density observed in the controls.

~1-month-old Cbln2 KO mice were used for the Cbln2-His labeling experiments (Figure 5 and Supplementary Figure S7). Two mice and four sections per mouse were collected for each time point in the Cbln2 treated group and for the vehicle treated group.
**Quantification of 5-HT (serotonin), 5-HIAA, and dopamine by ELISA.** Concentrations of serotonin, 5-HIAA, and dopamine were measured in tissue samples using Serotonin High Sensitive ELISA kit (Eagle Biosciences), 5-HIAA ELISA kit (Novus Biologicals), and Dopamine ELISA kit (Eagle Biosciences), respectively, according to the manufacturer’s instructions. Following dissection, tissue samples were immediately flash frozen in liquid nitrogen and then stored at -80°C for later processing. Tissues were homogenized in 300 ul of Homogenization Buffer (25 mM Tris-Hcl, pH 7.4, 150 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100) and incubated on ice for 20 min. Homogenates were then centrifuged at 20,000 x g for 20 min at 4°C, and the supernatants were transferred to a clean, pre-chilled 1.5 mL tube. Samples were divided into small volume aliquots to avoid repeated freeze/thaw cycles and were stored at -80°C. The serotonin and 5-HIAA ELISA assays were carried out using samples diluted 1:2 in the provided Standard Buffer containing 0.2% ascorbic acid (for a final concentration of 0.1% ascorbic acid). Protein concentration was measured in samples diluted 1:2 in Homogenization Buffer without Triton X-100 using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific, USA). Monoamine concentrations were first normalized to the concentration of total protein in the sample and then to the values in the control samples. Samples were collected from 6 mice/genotype, and 3 samples/genotype were tested in two separate assays. In the first set of experiments, tissues were taken from ~1-month-old littermate control, Cbln1 KO, and Cbln2 KO mice (Figure 2a-b); in the second set of experiments, tissues were taken from ~1-month-old littermate control and Cbln2 KO mice (Figure 2c).

**Immunoblotting.** ~1-month-old Cbln2 KO mice and littermate controls were deeply anesthetized with isoflurane, and the dorsal raphe was dissected and immediately frozen in liquid nitrogen. Tissues were later homogenized in fresh, pre-chilled RIPA buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl) containing 1 mM PMSF protease inhibitor cocktail (Roche Applied Science) and incubated for 20 min on ice. Samples were then centrifuged at 20,000 x g for 30 min at 4°C, and the supernatants were transferred to pre-chilled 1.5 mL eppendorph tubes. Protein concentrations were determined using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific). 10 μg of protein were loaded onto a Criterion TGX 4-20% Tris-Glycine precast gel (Bio-Rad) and separated via SDS-PAGE at 120 V for 1.5h and then transferred onto a nitrocellulose membrane using the Trans-Blot Turbo transfer system (Bio-Rad) according to the manufacturer's instructions. Membranes were then blocked with 5% nonfat dry milk in TBS containing 0.1% Tween 20 (TBST) for 1h at RT with agitation, and then incubated in primary antibody overnight at 4°C with agitation. Membranes were washed 3X with TBST, 10 min/each at RT, and then incubated in fluorescently labeled secondary antibodies (donkey anti-rabbit IR dye 680CW, 1:5000; and donkey anti-mouse IR dye 800CW, 1:5000; LI-COR Bioscience). Membranes were scanned using an Odyssey Infrared Imager and analyzed with Odyssey software (LI-COR Biosciences). Intensity values for each protein of
interest were first normalized to β-actin and then to the values in the control samples. Samples were collected from 6 mice/group.

**Preparation of recombinant Cbln2.** Mouse Cbln2 (52-224aa) cDNA was inserted downstream of IgK signal peptide-6 × His- Avitag in pEG BacMam vector and expressed in Expi293F™ cells (Thermo Fisher Scientific) following manufacturer’s protocol. Briefly, 50 mL of Expi293F™ cells were grown to 2.5 × 10^6 cells/mL. These cells were transfected using 50 µg DNA using 135 µL GibcoTM ExpiFectamine™ 293 Reagent. Enhancers were added 18 hours post transfection and cells were harvested after another 72 hours. Cbln2 was purified by Ni^{2+}-NTA affinity column chromatography followed by size exclusion chromatography using 1 × HBS-C (10 mM HEPES, pH 7.2, 150 mM NaCl and 2 mM CaCl_2) buffer. Cbln2 was specifically biotinylated by BirA ligase at the N-terminal biotin-acceptor peptide before size exclusion chromatography. For the SDS-PAGE, Cbln2 was loaded with sample buffer without boiling.

**Mouse behavior.** *Open field activity:* Open field activity was measured using a 28x28 cm force-plate actometer. Changes in the center of force (movement) of the mouse is monitored and analyzed using an in-house program. The mice were allowed to freely explore the field for 15 min and the following behaviors were recorded and analyzed: Total distance: the line integral of movement of the center of force; a measure of straight running/walking activity. *Low-mobility bouts (LMB):* total number of bouts during which the center of force remained within a 30-mm diameter circle for 10+ seconds. *Stereotypy:* defined as the intensity of behavior occurring in “one place.” The movement of the center of force during LMBs divided by the total number of LMBs. *Rotations around the center:* Number of net rotations around the center of the force plate. *Time spent in center of the field:* Total time the mouse (center of force) remained inside the central square that occupies 25% of the actometer floor area. *Explosive jumping:* In addition to the automatic analysis, each 15 min session was video recorded and the number of explosive jumps was counted by hand.

*Elevated plus maze:* Mice were placed in the center of a maze standing 50 cm off the ground and consisting of four arms (two open, two enclosed with 25 cm high walls) measuring 30 cm long and 5 cm wide. Mice were allowed to freely explore the maze for 6 minutes, and the amount of time spent in each arm, number of entries into each arm, total distance traveled, and average velocity was measured using Viewer3 tracking software (Bioserve).

*Resident/Intruder test:* Food and nests were removed from the homecage of the test mouse (resident) 30 min before the start of the experiment. A juvenile (P21-25) CD1 male mouse (intruder) was placed in the resident’s homecage for 5 min. The interactions between the two mice were video recorded and later analyzed for resident-initiated aggressive behaviors. The latency to first attack and total number of attacks was scored.

*Marble burying:* 20 glass marbles (1.5 cm diameter) were arranged in a 5x4 (row x column) grid in the center of clean mouse cage filled with 5 mm sawdust shavings. Mice were placed
in the cage for 30 min and the number of marbles buried (at least 2/3 covered with sawdust) was counted.

**Nest building:** Normal nest building was assessed as described previously [41]. Briefly, single-housed mice were provided with a cotton nestlet and left undisturbed for 24 h. Nests were then given a score of 1-5 based on the amount of nestlet used in their nest and the overall quality of the nest, 1 being the worst and 5 being the best. Excessive nest building was similarly scored and was based on the amount of a paper tube the mouse had incorporated into their nest: 0=used <10% tube, 1=10-50%, 2=50-80%, and 3=80-100%.

**Effect of fluoxetine on nest building behavior:** Mice were single-housed and initially provided with 9 grams of cotton nestlets. Instead of normal drinking water, the mice were given *ad libitum* access to 2% sucrose in water. To minimize the influence of acute stress on nest building, the mice were given a 3-day acclimation period during which time their nests were not removed and they were not provided with any additional nesting material. Following acclimation, mice were provided 9 grams of cotton nestlets every day for 4 consecutive days and the amount of unused nestlets from the previous day was weighed, and the previous day’s nest was removed. Following this baseline period, mice were randomly assigned to continue receiving only 2% sucrose (control) or 50 mg/kg/day fluoxetine in 2% sucrose, and the protocol described above (including the 3 day acclimation period) was repeated.

**Effect of 5-HTP or fluoxetine on explosive jumping behavior:** Male and female mice aged P30-P35 were placed in a 28x28 cm open field arena (force-plate actometer) for 15 min to assess baseline explosive jumping behavior. 7 days later, mice were given an IP injection of either saline or 10 mg/kg 5-HTP (or in a separate experiment saline or 10 mg/kg fluoxetine). After 30 min, the mice were again placed in the 28x28 cm open field arena for 15 min. Each 15 min session was video recorded and the number of explosive jumps was counted by hand. Mice were randomly assigned to receive either drug or saline.

**Effect of infusing recombinant Cbln2 protein into the DR on explosive jumping behavior:** Male and female Cbln2 KO mice aged P28-P35 were placed in a 28x28 cm open field arena (force-plate actometer) for 15 min to assess baseline explosive jumping behavior. 7 days later, either vehicle or 4 ug/ul recombinant Cbln2 protein (0.5 ul at each depth; 8 ug total) was stereotaxically injected into the DR. 4 days after surgery, the mice were again placed in the 28x28 cm open field arena for 15 min. Each 15 min session was video recorded and the number of explosive jumps was counted by hand. In this experiment, only mice that engaged in explosive jumping during the baseline test were used in the subsequent test.

**Sucrose preference test.** Over 48h, mice were given free choice between two bottles, one containing tap water and the other containing a 2% sucrose solution. The location of the bottles was swapped after 24h, and the bottles were weighed daily. Preference was determined by calculating the relative amount of each solution consumed (as a percentage of total volume).
**Forced swim test.** Mice were placed in a transparent Plexiglas tank (30 cm height x 20 cm diameter) filled halfway with tap water (maintained at 25°C) for 3 min. The latency to immobility and total time spent immobile was recorded.

**Animal cohorts used for behavior and order of assays.**

Cohort 1: Male littermate WT, Cbln1 KO, Cbln2 KO mice. Tests started at 2 months of age. Assays (in order of testing) included: open field test (Fig. 1a-d), elevated plus maze (Fig. 1e-h and Supp. Fig. S1a), excessive nest building (Fig. 1n-o), normal nest building (Fig. 1l-m), sucrose preference (Supp. Fig. S1b), forced swim test (Supp. Fig. S1c), resident/intruder (Fig. 1i).

Cohort 2: Female and male littermate WT, Cbln1 KO, Cbln2 KO mice. Tests started at 1 month of age. Assays included: Explosive jumping (Fig. 1k), marble burying (Fig. 1j).

Cohort 3: Female and male littermate WT, Cbln2 KO mice. Mice aged 1 month. Assay: Explosive jumping rescue with 5-HTP (Fig. 2e).

Cohort 4: Female and male littermate WT, Cbln2 KO mice. Mice aged 1 month. Assay: Explosive jumping rescue with fluoxetine (Fig. 2e).

Cohort 5: Female and male littermate WT, Cbln2 KO mice. Mice aged 2 months. Assay: Excessive nest building rescue with fluoxetine (Fig. 2f).

**Statistics.** All statistical analysis was done using GraphPad Prism 6. Graphs depict mean ± S.E.M. Neuron, axon, and puncta quantifications were analyzed with Student’s t-test and significant differences are reported as p-values. ELISA measurements and behavior assays comparing control, Cbln1 KO, and Cbln2 KO mice were analyzed by one-way Kruskal-Wallis analysis of variance (ANOVA) for group differences and significant differences are reported as p-values. Differences between control and individual KO groups were then determined using Dunnett’s *post hoc* test, correcting for multiple comparisons, and are reported as adjusted p-values. ELISA measurements, immunoblots, and behavior comparing control and Cbln2 KO mice were analyzed by Student’s t-test and significant differences are reported as p-values. For the rescue experiments with 5-hydroxytryptophan or fluoxetine, data were analyzed by two-way ANOVA for group comparisons and significant differences are reported as p-values. Within group treatment comparisons were then determined using Tukey’s *post hoc* test, correcting for multiple comparisons, and significant differences are reported as adjusted p-values.
Supplementary Figure S1: Cbln1 but not Cbln2 KO mice exhibit decreased anxiety in the elevated plus maze, and neither Cbln1 KO nor Cbln2 KO mice showed evidence for depression-like behaviors (related to Fig. 1).

(a) Analysis of Cbln1 and Cbln2 KO mice in the elevated plus maze (left, Cbln1 KO mice \(n=8\)) spent significantly less time in the closed arms compared to littermate control mice (Ctrl., \(n=10\)) and to Cbln2 KO mice \(n=9\) \((^*P=0.030\), ANOVA\); right, Cbln1 and Cbln2 KO mice made more entries into the closed arms compared to control \((^*P=0.039\), ANOVA\).

(b) In the sucrose preference test, control mice \(n=10\); **** adj. \(P<0.0001\), Sidak’s post hoc), Cbln1 KO mice \(n=8\); ** adj. \(P=0.0017\), Sidak’s post hoc), and Cbln2 KO mice \(n=9\); ** adj. \(P=0.0008\), Sidak’s post hoc) all consumed significantly more 2% sucrose in water than water alone, and there was no significant difference between the groups.

(c) In the forced swim test, there was no difference between the groups in the time spent immobile (left) or the latency to immobility (right). Ctl mice \(n=10\), Cbln1 KO mice \(n=8\), and Cbln2 KO mice \(n=9\).

Data shown are mean ±SEM.
Supplementary Figure S2: Selective deletion of Cbln2 from only serotonergic neurons does not induce explosive jumping (related to Fig. 5).

(a) Schematic depicting the breeding strategy used to selectively delete Cbln2 from serotonergic neurons. Cbln2 cKO (Cbln2\textsuperscript{Flox/Flox}) mice were bred to mice that express Cre-recombinase under control of the serotonin transporter (SERT) promoter, restricting recombination and Cbln2 deletion to serotonergic neurons and cortical neurons that express SERT transiently during development (see Verney et al., 2002). Male and female littermates aged P30-P35 were tested for explosive jumping behavior.

(b) Summary graph showing that selectively deleting Cbln2 in serotonergic neurons does not result in the explosive jumping phenotype. Data shown are means ± SEM (Cbln2\textsuperscript{F/+} mice, n=8, Cbln2\textsuperscript{F/-} mice, n=8, Cbln2\textsuperscript{+/-} mice, n=5, and Cbln2\textsuperscript{-/-} mice, n=11).
Supplementary Figure S3: Injection sites of labeled cholera toxin B into the dorsal raphe (DR) for retrograde tracing experiments (a-c), and single-channel images from input mapping experiments (d) (related to Fig. 3)
(a) Coronal sections from Cbln2-mVenus reporter mice (green) showing expression of Cbln2 in the dorsal raphe (DR).

(b) Sagittal section from Cbln2-mVenus reporter mice following injection of the retrograde tracer cholera toxin B (CTB) into the DR, showing the location of CTB⁺ neurons that project to the DR.

(c) Coronal sections from the same mice as in (b) showing the injection site of CTB (magenta) into the DR.

(d) Single channel images showing neurons labeled for CTB (magenta; top), Cbln2 (green; middle), and/or both (bottom) in the agranular insular cortex (AI), orbital frontal cortex (ORB), prelimbic cortex (PrL), lateral habenula (LHb), and medial habenula (MHb). Cbln2 expression was visualized using an antibody against GFP to boost the intensity of the mVenus signal (sections are the same sections as those shown in Fig. 3).

Scale bars = 1 mm (b), 500 μm (a and c), and 100 μm (d). Abbreviations: Aq, cerebral aqueduct; Cl, claustrum; HY, hypothalamus; IC, inferior colliculus; MO, motor cortex; MR, median raphe; NAc, nucleus accumbens; PAG, periaqueductal gray; SC, superior colliculus; TH, thalamus; VTA, ventral tegmental area.
Supplementary Figure S4: No change in the number of TPH2+ neurons or TPH2 fluorescence signal intensity in the dorsal raphe of Cbln2 KO mice (related to Fig. 4).

(a) Representative images showing the dorsal raphe (DR) of ~1 month old control (top) and Cbln2 KO (bottom) mice. Sections were co-labeled with TPH2 (green) to identify serotonergic neurons and NeuN (magenta) to label all neurons. Scale bar = 50 μm.

(b) Summary graphs showing that Cbln2 deletion has no effect on total/NeuN+ neuron density (top left) or on serotonergic/TPH2+ neuron density measured as either a function of region of interest (ROI) area (top right) or the number of NeuN+ cells within the ROI (bottom left). TPH2 protein expression as measured by fluorescence signal intensity is also unchanged following Cbln2 deletion (bottom right). Data shown are means ± S.E.M. and were normalized to the values observed in the controls. Statistical analysis was performed by Student’s t-test (n = 3 sections/mouse, 3 mice/genotype).
Supplementary Figure S5: The serotonin system remains structurally intact in Cbln2 KO mice (related to Fig. 4).

(a-c) Loss of Cbln2 has no effect on the density of axons or puncta expressing serotonin reuptake transporter (SERT) in the striatum. (a) Schematic showing the location of the striatum sections shown in b and quantified in c. (b) Representative images showing SERT antibody labeling in the striatum of ~1 month old control (left) and Cbln2 KO (right) mice. Bottom images show a high magnification example of SERT⁺ puncta. Scale bars = 25 μm. (c) Summary graphs showing no change in the density of SERT⁺ axons (left) or in the density
(*middle*) or size (*right*) of SERT* puncta in the striatum of Cbln2 KO mice compared to control.

(*d*-f) Same as in a-c but for the CA1 hippocampus.

(*g*-i) Same as in a-c but for the basolateral amygdala (BLA).

For all, data shown are means ± S.E.M. Axon and puncta density were first normalized to the area of the region of interest and then to the levels observed in the controls. Statistical analysis was performed by Student’s *t*-test (*n* = 3 sections/mouse, 3 mice/genotype).
a  
Injection site of AAVs expressing cre-GFP into the dorsal raphe of Cbln2 cKO mice expressing IRES-mVenus

b  
Cre expression in the mPFC after injection of retro-AAVs expressing cre-GFP into the dorsal raphe of Cbln2 cKO mice expressing IRES-mVenus
Supplementary Figure S6: Injection site of AAVs expressing Cre-GFP into the dorsal raphe (DR) (a), and expression of Cre protein in prefrontal cortex (PFC) following injection of AAV-retro-Cre-GFP into the DR of Cbln2 conditional KO (cKO) mice (b-c) (related to Fig. 5).

(a) Coronal sections from a Cbln2 cKO mouse following injection of AAV-Cre-GFP into the dorsal raphe (DR) and co-labeled with the nuclear marker DAPI (blue). Images show the injection site and the extent of viral spread. GFP expression (green) is seen in both infected neurons and in neurons that express Cbln2 (Cbln2 cKO mice express mVenus, which is almost identical to GFP, from the endogenous Cbln2 gene when Cbln2 is not deleted; see ref. 38). (scale bar = 100 µm).

(b) Low-magnification overview of a coronal section from a Cbln2 cKO mouse that was injected with AAV-retro-Cre-GFP into the DR. The section was stained for Cre (black); box indicates the location from which the images in c were taken (scale bar = 500 µm).

(c) High-magnification images of coronal PFC sections stained for GFP (green) and Cre (magenta), taken from the boxed area in b. Images show co-labeling of GFP+ neurons (green) with Cre antibody (magenta). Because GFP expression is expected in both neurons that project to the DR (that were infected with AAV-retro-Cre-GFP) and in neurons that express Cbln2-mVenus, Cre labeling is only observed in a subset of GFP+ neurons but is similar to that found in retrograde tracing experiments (see Figure 3d). Arrowheads indicate double GFP+/Cre+ neurons, and stars indicate GFP+/Cre− neurons (scale bar = 100 µm).

Abbreviations: 3N, oculomotor nucleus; Aq, aqueduct; CB, cerebellum; CLi, caudal linear nucleus of the raphe; DR, dorsal raphe nucleus; DRC, caudal DR; DRD, dorsal DR; DRI, infrafascicular DR; DRV, ventral DR; MB, midbrain; mlf, medial longitudinal fasciculus; MnR, median raphe nucleus; PAG, periaqueductal gray; PMnR, paramedian raphe nucleus; scp, superior cerebellar peduncle.
Supplementary Figure S7: Distribution of Cbln2-His protein following injection of recombinant Cbln2-His protein into the dorsal raphe (DR) (related to Fig. 5).
Coronal sections showing anti-His tag (green) labeling in the DR of Cbln2 KO mice following stereotaxic injection of vehicle (a) or of recombinant Cbln2-His protein. Mice were analyzed 2 days (b), 4 days (c), 7 days (d'), or 14 days (e) after injections. Serotonergic neurons are labeled with antibodies to TPH2 (magenta). Scale bars = 100 μm.

(a”-e””) Higher magnification images from a’-e’ illustrating the punctate pattern of Cbln2-His labeling on the dendrites and cell bodies of TPH2+ neurons at 2 days post-injection (b”) and 4 days post-injection (c”). Labeling decreases 7 days post-injection (d”) and is absent 14 days post-injection (e”). Scale bars = 20 μm.
**Supplementary Table S1:** Quantification of TPH2\(^+\), Cbln2\(^+\), and double TPH2\(^+\)/Cbln2\(^+\) neurons in the dorsal and median raphe (related to Fig. 3).

| Region | %TPH2 only | %Cbln2 only | %Double labeled | %TPH2+ w/Cbln2 | %Cbln2+ w/TPH2 | Total neurons |
|--------|------------|-------------|-----------------|----------------|----------------|---------------|
| DRD    | 62.86      | 20.55       | 16.58           | 20.87          | 44.65          | 579           |
| DRV    | 45.32      | 3.42        | 51.26           | 53.07          | 93.75          | 556           |
| DRI    | 38.98      | 4.72        | 56.30           | 59.09          | 92.26          | 254           |
| PDR    | 37.24      | 19.31       | 43.45           | 53.85          | 69.23          | 154           |
| MR     | 46.19      | 37.80       | 16.01           | 25.74          | 29.76          | 381           |
| PMR    | 4.52       | 90.50       | 4.98            | 52.38          | 5.21           | 442           |

Table shows the percentage of total labeled neurons in each subnucleus expressing only TPH2, only Cbln2, or expressing both TPH2 and Cbln2, as well as the percentage of all TPH2\(^+\) neurons that co-express Cbln2 and the percentage of all Cbln2\(^+\) neurons that co-express TPH2.

Abbreviations: DR, dorsal raphe; DRD, dorsal DR; DRI, intrafascicular DR; DRV, ventral DR; MR, median raphe; PDR, posterodorsal raphe; PMR, paramedian raphe.
**Supplementary Table S2**: Quantification of CTB/Cbln2 double-labeled neurons projecting to the dorsal raphe (related to Fig. 3).

| Region         | CTB/Cbln2+ (% total CTB+) |
|----------------|---------------------------|
| **Cortical regions** |                            |
| Motor cortex    | 19.20 (n=495)             |
| ACC             | 20.31 (n=448)             |
| PrL             | 6.48 (n=571)              |
| IL              | 16.93 (n=567)             |
| ORBm            | 9.88 (n=435)              |
| ORBv            | 37.11 (n=353)             |
| ORBl            | 49.70 (n=503)             |
| Insula          | 44.55 (n=615)             |
| Claustrum       | 23.05 (n=269)             |
| Rsp             | 74.05 (n=131)             |
| **Hypothalamus** |                            |
| MEPO            | 18.60 (n=43)              |
| PVi             | 32.20 (n=118)             |
| MPO             | 20.57 (n=175)             |
| Combined        | 24.40 (n=336)             |
| **Habenula**    |                            |
| MHB             | 99.34 (n=304)             |
| LHb             | 80.71 (n=1127)            |

For each brain region, the percentage of total CTB+ neurons (n) co-expressing Cbln2 is shown.

Abbreviations: ACC, anterior cingulate cortex; IL, infralimbic cortex; LHb, lateral habenula; MEPO, median preoptic hypothalamic nucleus; MHB, medial habenula; MPO, medial preoptic hypothalamic nucleus; ORBI, lateral orbital frontal cortex; ORBm, medial orbital frontal cortex; ORBv, ventral orbital frontal cortex; PrL, prelimbic cortex; PVi, periventricular hypothalamic nucleus; Rsp, retrosplenial cortex.
Supplementary Video 1: Video depicts a Cbln2 KO mouse (aged ~P30) engaging in explosive jumping in an open field arena.

Supplementary Reference:
Verney C, Lebrand C, Gaspar P. Changing distribution of monoaminergic markers in the developing human cerebral cortex with special emphasis on the serotonin transporter. *Anat Rec.* 2002 Jun 1;**267**(2):87-93.