Cerebellin-2 neurons in the parafascicular thalamic nucleus regulate self-grooming behavior

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ABSTRACT

Self-grooming represents a stereotyped repetitive behavior which is commonly observed in various neuropsychiatric disorders. Deep brain stimulation (DBS) targeting the centromedian / parafascicular complex (CM/PF) has been shown to alleviate obsessive-compulsive disorder/behavior (OCD/OCB) symptoms in human. However, little is known about the neural circuits of the PF that are involved in the regulation of self-grooming behavior in rodents. Here, we report that *cerebellin-2* positive (*Cbln2*$^+$) neurons in PF bidirectionally encode self-grooming. Chemogenetic activation of *Cbln2*$^+$ PF neurons significantly reduces both physiological and pathological self-grooming, and chemogenetic inhibition of *Cbln2*$^+$ PF neurons increases physiological self-grooming. Moreover, activating synaptic excitatory inputs to *Cbln2*$^+$ PF neurons inhibits self-grooming, while synaptic inhibitory inputs enhance it. *Cbln2*$^+$ PF neurons independently transmit neural signals to the dorsal and ventral striatum. Activation of PF-dorsal striatum and PF-ventral striatum pathways both inhibit self-grooming behavior. Most excitingly, we alleviate excessive grooming in OCD mice by the activation of PF neurons using non-invasive ultrasound stimulation. Together, these data reveal that *Cbln2*$^+$ PF neurons are integral components of a brain network involved in self-grooming behavior and highly promising targets in the non-invasive therapy of OCD.

INTRODUCTION

Self-grooming is an evolutionarily conserved behavior observed in arthropods (Lefebvre, 1981; Richard and Dawkins, 1976), birds (Lefebvre, 1982; Lefebvre and Joly, 1982; van RHIJN, 1977), and mammals (Berridge, 1990; Colonnese et al., 1996). In rodents, self-grooming has a complex sequenced structure that consists of repeated stereotyped movements, which follows an innate cephalo-caudal progression, preceding by a postural cephalo-caudal transition from paw - head grooming to body grooming (Kalueff et al., 2007; Kalueff et al., 2016). Humans also exhibit repetitive patterned behaviors including excoriation (skin-picking) (Ekore and Ekore, 2021; Lochner et al., 2017) and trichotillomania (hair-pulling) (Grant and Chamberlain, 2016; Hoffman et al., 2021; Stein et al., 2006), which are in analogy with the stereotyped repetitive self-grooming behavior in rodents and share several common traits and brain circuits with rodents (Grant and Chamberlain, 2016; Graybiel and Rauch, 2000; Robbins et al., 2019; Robertson, 2000; Stein et al., 2008). Repetitive patterned behaviors in human frequently occur in several neural neuropsychiatric diseases including autism spectrum disorder (ASD) (Sharma et al., 2018), Tourette syndrome (Robertson, 2000), and obsessive-compulsive disorder (OCD) (Goodman et al., 2021), making the grooming behavior in rodents ideal to study the neuropsychiatric disorders (Liu et al., 2020; Peça et al., 2011; Welch et al., 2007).

Excessive self-grooming is frequently observed in genetic animal models of neuropsychiatric disorders, including obsessive compulsive disorder/behavior (OCD/OCB) (Piantadosi et al., 2024; Xue et al., 2022), Tourette' syndrome (TS) (Liu et al., 2020), autism spectrum disorder (Peñagarikano et al., 2011; Silverman et al., 2010), and Rett syndrome (Chao et al., 2010; Gold et al., 2024). Thus, understanding the neural circuitry that regulates self-grooming could provide a new framework for the research of the development neuropsychiatric disorders.

The parafascicular thalamic (PF) nucleus in rodents, known as the counterpart of centromedian/ parafascicular complex (CM/PF) in primates, belongs to the intralaminar system of the thalamus (Langlois et al., 2010; Smith et al., 2004). The PF nucleus play a role in limbic and associative network functions (Fallon et al., 2023; Lacey et al., 2007), and send glutamatergic projections to the striatum, which is involved in the completion of sequential

syntactic self-grooming chains (Cromwell and Berridge, 1996; Zhang et al., 2022). Deep brain stimulation (DBS) targeting the CM/PF has been shown to alleviate obsessive-compulsive disorder/behavior (OCD/OCB) symptoms in human (Servello et al., 2020). However, the neural circuit mechanism by which PF regulates repeated stereotyped behaviors, such as self-grooming, remains unclear. More importantly, whether non-invasive treatment, such as ultrasound stimulation of PF can modulate obsessive-compulsive disorder/behavior (OCD/OCB) symptoms is worth exploring (Darmani et al., 2022).

In the present study, we combined genetically encoded circuit analysis tools to identify a subset of PF neurons critical for both biological and pathological self-grooming in mice. *Cbln2*$^{+}$ PF neurons bidirectionally encode self-grooming. Activating synaptic inhibitory inputs to *Cbln2*$^{+}$ PF neurons enhances self-grooming, while synaptic excitatory inputs inhibit it. Moreover, *Cbln2*$^{+}$ PF neurons regulate self-grooming behavior through both dorsal and ventral striatum. Non-invasive ultrasound stimulation efficiently activates PF neurons while reducing time spent for spontaneous grooming in both WT mice and OCD mice. Our data reveal that *Cbln2*$^{+}$ PF neurons are integral components of a brain network involved in self-grooming behavior and ultrasound stimulation targeting the PF holds clinical therapeutic significance in OCD.

RESULTS

Cbln2^{+} PF neurons are required for the regulation of self-grooming

At the beginning of this study, we measured self-grooming by using a magnetic induction method (Inagaki et al., 2002; Xie et al., 2022) in parallel with video recording, this was modified from the methods described in previous work (Xie et al., 2022) (Fig. 1A). In previous study, the magnet was implanted in the forelimb to track the movements of forelimb during orofacial grooming stage, but it did not effectively indicate other types of grooming. Here, we implanted the magnet on the surface of the skull to track the movements of head during all types of self-grooming (Fig. 1A). The voltage threshold was calculated as before (mean ± 5 × SD) and used to identify the voltage pulses corresponding to the grooming behaviors. The magnetic inducing voltage signals efficiently indicated the grooming behaviors in each stage, including paw-licking, orofacial grooming and body grooming stage (Fig. 1B, and Movie S1). Then, we explored the role of PF nucleus in mediating self-grooming. By using RNAscope analysis, we found that the PF neurons are predominantly *Cbln2*$^{+}$ (more than 98% across 3 different sections, sFig.1A-B). To measure the contribution of *Cbln2*$^{+}$ PF neurons to self-grooming, we injected AAV-DIO-ChR2-mCherry into the PF of Cbln2-IRES-Cre mice, followed by optical fiber implantation above the PF bilaterally (Fig. 1C). The effect of photostimulation to trigger action potential firing in PF neurons expressing ChR2 was validated in acute brain slices (Fig. 1D). Optogenetic activation (473 nm, 10 s, 2 mW, 20 Hz) of *Cbln2*$^{+}$ PF neurons significantly suppressed spontaneous self-grooming (sFig.1C-J), including paw-licking (Fig. 1E), orofacial grooming (Fig. 1F), and body grooming (Fig. 1G) in mice (Movie S2). Furthermore, optogenetic activation of *Cbln2*$^{+}$ PF neurons also significantly suppressed oil-induced self-grooming (Xie et al., 2022) (Fig. 1H). These data suggested a preliminary conclusion that optogenetic activation of *Cbln2*$^{+}$ PF neurons inhibit both spontaneous and oil-induced self-grooming.

Next, we injected AAV-DIO-hM3Dq-mCherry or AAV-DIO-hM4Di-mCherry into the PF of Cbln2-IRES-Cre mice to verify the function of *Cbln2*$^{+}$ PF neurons in regulation of grooming behavior within a long period (Fig. 1I, K). The effect of CNO on chemogenetical modulation of action potential firing was validated (Fig. 1J, L). We found that chemogenetic activation of

122 *Cbln2*$^{+}$ PF neurons significantly inhibited spontaneous self-grooming, including paw-licking
123 (Fig. 1E), orofacial grooming (Fig. 1F), and body grooming (Fig. 1M and sFig.2B, D), as well
124 as oil-induced self-grooming (Fig. 1N), whereas chemogenetic inhibition of these neurons
125 enhanced both spontaneous and oil-induced self-grooming (Fig. 1M-N and sFig.2C, D),
126 suggesting that *Cbln2*$^{+}$ PF neurons regulate grooming behavior in a bidirectional manner
127 and potentially play key role in the maintenance of balance in the grooming behavior. At the
128 other hand, chemogenetic activation of *Cbln2*$^{+}$ PF neurons caused slight reduction of total
129 distance, while chemogenetic inhibition of *Cbln2*$^{+}$ PF neurons showed no effect on total
130 locomotion distance (sFig.2A). Besides, optogenetic activation of *Cbln2*$^{+}$ PF neurons also
131 did not significantly change the total distance or immobile time (sFig.1K, L), revealing that
132 the regulation of *Cbln2*$^{+}$ PF neurons on grooming behavior was not caused by the obvious
133 motor system dysfunction.

134 The *Sapap3*-knockout (*Sapap3*$^{-/-}$) mouse is a model used to study OCD, as it
135 recapitulates OCD-like compulsive behavior through excessive grooming (sFig.2E) (Hadjas
136 et al., 2020; Welch et al., 2007). We surprisingly found that chemogenetic activation of
137 *Cbln2*$^{+}$ PF neurons also strongly inhibit pathological self-grooming in *Sapap3*$^{-/-}$ mice (Fig.
138 1O). These data suggested that *Cbln2*$^{+}$ PF neurons are important components in the central
139 circuit module that broadly controls physiology and pathological self-grooming in mice.

140

141 **_Cbln2_$^{+}$ PF neurons encode self-grooming**

142 Next, we systematically characterized the physiological properties of *Cbln2*$^{+}$ PF
143 neurons. To assess the response properties of these neurons during self-grooming, we
144 recorded GCaMP signals in *Cbln2*$^{+}$ PF neurons using fiber photometric recording.
145 AAV-DIO-GCaMP7s was injected into the PF of Cbln2-IRES-Cre mice, followed by
146 implantation of an optical fiber above the *Cbln2*$^{+}$ PF neurons (Fig. 2A). Because the
147 spontaneous grooming behavior does not frequently occur, we sprayed the corn oil over the
148 mice from head to toe to induce more grooming behavior. In most trials, we found the mice
149 exhibited three major types of behavior, including stationary state, locomotion and grooming.
150 GCaMP fluorescence of *Cbln2*$^{+}$ PF neurons during grooming was higher than that of during
151 stationary state, and lower than that of during locomotion (sFig. 3A, B). Considering that the

grooming behavior is a kind of motor-related behavior, we chose to define the GCaMP fluorescence during the period of locomotion as the baseline. These results lead to a conclusion that *Cbln2*$^{+}$ PF neurons negatively regulate the grooming behavior, which is consistent with the results that opotogenetic or chemogenetic activation of *Cbln2*$^{+}$ PF inhibited grooming behavior.

In parallel with the oscillation of magnetic inducing voltage signal which indicated the period of grooming, the GCaMP fluorescence of *Cbln2*$^{+}$ PF neurons decreased immediately after the grooming behavior occurred, and stayed fairly low until grooming stopped. (Fig. 2B) We also noticed that the relative GCaMP fluorescence of *Cbln2*$^{+}$ PF showed negative correlation with the grooming duration (grooming duration is defined as the duration of a bout, pauses longer than 3 s constitute a new bout), suggesting that the *Cbln2*$^{+}$ PF neurons might also be involved in the maintenance of grooming. (Fig. 2C).

To further analyze the temporal relationship between GCaMP signal and self-grooming, we averaged the trials by aligning the GCaMP signal with the exact time points of initiation or termination of self-grooming. The GCaMP signal decreased immediately after the initiation of self-grooming (Fig. 2D-F). We defined the offset time of GCaMP signal as the time when the signal reaches 5% valley amplitude relative to the baseline. The average time when GCaMP signal began to decrease was 329 ± 71 ms after the initiation of self-grooming (Fig. 2G). The GCaMP signal began to recover to the baseline in prior to the termination of self-grooming (Fig. 2H-J). We defined the onset time of GCaMP signal as the time when the signal recovers to 95% valley amplitude relative to the baseline. The average time when GCaMP signal began to rise was 2531 ± 336 ms before the termination of self-grooming (Fig. 2K). These results suggest that *Cbln2*$^{+}$ PF neurons are more likely involved in the initiation, maintenance and termination of self-grooming.

To explore the neuronal activity of *Cbln2*$^{+}$ PF neurons at the single-cell level, we performed in vivo calcium imaging by using the miniscope system on free-moving mice (see methods for details, Fig. 2L). AAV-DIO-GCaMP7s was injected into PF, followed by the implantation of a gradient refractive index (GRIN) lens above the PF two weeks after viral injection (Fig. 2M). The example coronal brain section revealed the site of GCaMP expression was restricted to the PF, and the track of lens was exactly above the PF (Fig. 2N).

182 In the trials that recorded the initial stage of grooming (Movie S2), we detected three types of
183 GCaMP fluorescence response in *Cbln2*$^+$ PF neurons corresponding to the grooming
184 behavior (Fig. 2O, sFig. 3C). 75.00% of *Cbln2*$^+$ PF neurons (Type 1) showed decreased
185 response, 17.31% of *Cbln2*$^+$ PF neurons (Type 3) showed increased response and 7.61% of
186 *Cbln2*$^+$ PF neurons (Type 2) remained unchanged (Fig. 2P), consistent with the results of
187 fiber photometric recording that the total response of GCaMP fluorescence was decreased
188 during grooming compared with that during baseline period (Fig. 2D-F). In the trials that
189 recorded the terminate stage of grooming (Movie S2), the GCaMP fluorescence response in
190 *Cbln2*$^+$ PF neurons could also be classified into three types (Fig. 2Q, sFig. 3D). Most of the
191 *Cbln2*$^+$ PF neurons (70.51%, Type 1) showed increased response, 16.03% of *Cbln2*$^+$ PF
192 neurons (Type 3) showed deceased response and 13.46% of *Cbln2*$^+$ PF neurons (Type 2)
193 remained unchanged when the grooming behavior terminated (Fig. 2R), which was also
194 consistent with the results of fiber photometric recording which recorded the terminate stage
195 of grooming (Fig. 2H-J). Together, these data further confirmed the conclusion that *Cbln2*$^+$
196 PF neurons play a negative role in the regulation of self-grooming.
197

198 **Mapping the monosynaptic inputs of *Cbln2*$^+$ PF neurons**

199 Using recombinant rabies virus, we next performed monosynaptic retrograde tracing
200 (Wickersham et al., 2007) to examine how *Cbln2*$^+$ PF neurons are connected to brain areas
201 associated with self-grooming (Fig. 3A–C). A brain-wide survey revealed a series of
202 monosynaptic projections to the *Cbln2*$^+$ PF neurons (Fig. 3D, E). Among these brain regions,
203 we focused on the brain regions such as SC, SNr, ZI and LPB. First, robust monosynaptic
204 inputs arose from the superior colliculus (SC) (Fig. 3D2). Prenatal or early postnatal factors
205 could disrupt SC functions, thus resulting in autism (Jure, 2019; Liu et al., 2022), which was
206 reported to show excessive grooming behavior in rodents (Peça et al., 2011). Second,
207 *Cbln2*$^+$ PF neurons were monosynaptically innervated by neurons in the zona incerta (ZI)
208 and substantia nigra pars reticulata (SNr) (Fig. 3D3, D4), two brain areas that are involved in
209 the self-grooming behavior in mice (Jiang et al., 2024; Meyer-Luehmann et al., 2002; Zhu et
210 al., 2025). Third, *Cbln2*$^+$ PF neurons were also innervated by neurons in the lateral
211 parabrachial nucleus (LPB) (Fig. 3D1). Previous study showed that LPB glutamatergic (Glu)

212 neurons were involved in stress-induced self-grooming (Jia et al., 2023). These data
213 supported the hypothesis that *Cbln2*$^+$ PF neurons are within a brain network for
214 self-grooming behavior.
215

216 **Multiple *Cbln2*$^+$ PF neurons input pathways involved in self-grooming**

217 Next, we tested whether projections from multiple brain regions to *Cbln2*$^+$ PF neurons
218 were involved in the regulation of self-grooming. We injected AAV-DIO-hM3Dq- mCherry into
219 the SC (Fig. 4A, B) or LPB (Fig. 4E, F) of vGluT2-IRES-Cre mice, which followed by
220 cannulae implantation above the PF (Fig. 4C, G). By the perfusion of CNO into the PF
221 through the cannulae, the vGluT2$^+$ SC or LPB-PF pathway was activated. We found that
222 chemogenetic activation of either vGlut2$^+$ SC-PF pathway (Fig. 4D) or vGlut2$^+$ LPB-PF
223 pathway (Fig. 4H) significantly inhibited spontaneous self-grooming, including paw-licking,
224 orofacial grooming, and body grooming in mice (sFig. 4B, C). Then, we injected
225 AAV-DIO-hM3Dq-mCherry into the SNr (Fig. 4I, J) or ZI (Fig. 4M, N) of vGAT-IRES-Cre mice,
226 which followed by cannulae implantation above the PF (Fig. 4K, O). We found that
227 chemogenetic activation of either vGAT$^+$ SC-PF pathway (Fig. 4L) or vGAT$^+$ ZI-PF pathway
228 (Fig. 4P) significantly facilitated spontanous self-grooming, including paw-licking, orofacial
229 grooming, and body grooming in mice (sFig. 4D, E). These results verified the inhibitory role
230 of the glutamatergic inputs from SC or LPB to *Cbln2*$^+$ PF neurons and the excitatory role of
231 the GABAergic inputs from SNr or ZI to *Cbln2*$^+$ PF neurons on grooming behavior, further
232 elucidating that *Cbln2*$^+$ PF neurons are within a complex brain network and bidirectionally
233 regulate grooming behavior.
234

235 **The *Cbln2*$^+$ PF–striatum pathway is required for self-grooming**

236 Next, we injected AAV-DIO-EGFP into the PF of Cbln2-IRES-Cre mice to map the
237 efferent pathways of *Cbln2*$^+$ PF neurons involved in the grooming behavior. (Fig. 5A-B). We
238 found that the *Cbln2*$^+$ PF neurons divergently projected to various brain regions, including
239 the dorsal striatum (CPu), and the ventral striatum (aca, AcbC and Tu). (Fig. 5C), and other
240 brain regions (sFig. 5A-H). We tested the striatum as the possible relay station mediating
241 PF-related grooming behavior as following reasons: first, the activity of striatal cells is

242 reported to be orchestrated to represent the important timepoints in a grooming sequence
243 (Minkowicz et al., 2023); second, striatal dysfunction and defects in cortico-striatal function
244 have been observed in repetitive and compulsive grooming behaviors in autism model mice
245 (Peça et al., 2011) and striatal-specific expression of *Sapap3* rescues the over-grooming
246 behavior in OCD mice (Welch et al., 2007); third, ventral capsule/ventral striatum DBS
247 significantly reduced OCD symptoms in human (Graat et al., 2017; Welter et al., 2021).

248 The striatum, consisting of dorsal and ventral part, receives inputs from distinct
249 subpopulations of neurons in PF (Zhang et al., 2022). The ventral part of the striatum and
250 the ventral striatal islands of Calleja were particularly reported to be involved in the
251 regulation of grooming behavior in rodents (Xue et al., 2022; Zhang et al., 2021), however,
252 function of the dorsal part of striatum in grooming behavior was poorly elucidated. Hence it is
253 essential to verify if PF regulates grooming behavior through two distinct PF-striatum
254 pathways.

255 By using the strategy of retrograde labeling of cholera toxin subunit B (CTB) and wheat
256 germ agglutinin (WGA) (Fig. 5D), we labelled PF neurons which sent projections to the
257 dorsal striatum and the ventral striatum (Fig. 5E). The dorsal striatum-projecting PF neurons
258 and ventral striatum-projecting PF neurons were scarcely overlapped (double labelled
259 neurons were no more than 1.5%) in spatial arrangement (Fig. 5F-I). It would be essential to
260 clarify the role of these two distinct pathways in PF-mediating grooming behavior regulation.

261 Therefore, we injected AAV-DIO-hM3Dq- mCherry into the PF of Cbln2-IRES-Cre mice
262 (Fig. 5J, K, N, O), which followed by cannulae implantation above the dorsal striatum (Fig.
263 5L) or ventral striatum (Fig. 5P). We found that chemogenetic activation of either Cbln2^{+}
264 PF-dorsal striatum pathway (Fig. 5M) or Cbln2^{+} PF-ventral striatum pathway (Fig. 4H)
265 significantly inhibited spontaneous self-grooming, including paw-licking, orofacial grooming,
266 and body grooming in mice (sFig. 4F, G). Together, these data elucidated the pivotal role of
267 both the dorsal striatum and ventral striatum in the regulation of PF-mediating grooming
268 regulation.

269 In addition to the majority of GABAergic spiny projection neurons, there are many kinds
270 of interneurons in the striatum, which act on nearby circuits and shape functional output to

271 the basal ganglia (Muñoz-Manchado et al., 2018). Optogenetic activation of
272 parvalbumin-positive striatal interneurons reduced self-grooming events in *Sapap3*$^{-/-}$ mice
273 (Mondragón-González et al., 2024). By the injection of AAV-DIO-EGFP-Syb2 into the PF of
274 Cbln2-IRES-Cre mice (sFig.5I), we found that the striatal cells positive for Parvalbumin (PV),
275 Somatostatin (SST), Choline Acetyltransferase (ChAT) and Neuropeptide Y (NPY) in both
276 the dorsal striatum and ventral striatum were surrounded by the synaptic terminals from
277 *Cbln2*$^{+}$ PF neurons (sFig. 5J, K), which offered putative targets to divergently regulate the
278 grooming behavior. However, further work was essential to elucidate the functional
279 connections between *Cbln2*$^{+}$ PF neurons and these striatal cells and the role of these striatal
280 cells in grooming regulation under the innervation of *Cbln2*$^{+}$ PF neurons.
281

282 **Ultrasound stimulation targeting the PF results in activation of PF neurons and**
283 **alleviates over-grooming in OCD mice**

284 Activation of *Cbln2*$^{+}$ PF neurons significantly reduces time spent for grooming in both
285 WT mice and OCD mice (Fig. 1E-H, M-O), and nearly all the neurons are Cbln2 positive in
286 PF (sFig. 1A, B), making PF an ideal target for therapeutic intervention. Therefore, we
287 explored whether non-invasive approach, such as ultrasound stimulation, would efficiently
288 activate PF neurons and have impact on time spent for spontaneous grooming in WT or
289 OCD mice.

290 We conducted a preliminary experiment to determine whether ultrasound stimulation
291 targeting the PF could affect spontaneous grooming time in WT mice (Fig. 6A, B). Under the
292 schedule of 3 days of habituation followed by 7 days of daily 30-minute ultrasound
293 stimulation, we monitored and compared the grooming duration of mice after habituation
294 (Day 3) or each daily stimulation session (Day 4 – Day 10, sFig. 6A). We found a slight
295 decrease in spontaneous grooming time on Day 6, with significant reductions observed on
296 Day 8, 9, and 10. Therefore, in the formal experiment, we chose to apply daily 30-minutes
297 stimulation for 7 days and assessed grooming behavior only on the Day 3 (for baseline) and
298 Day 10 (Fig. 6C). We found that ultrasound stimulation targeting the PF not only increased
299 c-Fos expression in the PF region (Fig 6D, E), but also enhanced neuronal excitability of PF
300 neurons (Fig 6F, G). As expected, it also reduced spontaneous grooming duration in WT

301 mice (Fig 6H, I). Using the same stimulation schedule, we applied ultrasound stimulation to
302 OCD mice and observed a significant reduction in their excessive grooming behavior (Fig 6J,
303 K). Although the grooming time was not reduced to WT levels, this non-invasive approach
304 still offers promising potential as a therapeutic strategy for OCD.
305

306 **DISCUSSION**

307 Self-grooming is an innate behavior with important physiological functions including
308 hygiene maintenance, thermoregulation, social communication (Kalueff et al., 2016; Leonard
309 et al., 2005; Spruijt et al., 1992). Recently, the repetitive stereotyped behavior of mice was
310 reported to cope with stress and post-stress anxiety alleviation (Sun et al., 2022). In this
311 study, we identified that *Cbln2*$^+$ PF neurons encode self-grooming in a bidirectional manner.
312 Synaptic excitatory inputs that activate *Cbln2*$^+$ PF neurons inhibit spontaneous
313 self-grooming, and synaptic inhibitory inputs enhance it. Moreover, *Cbln2*$^+$ PF neurons
314 regulate self-grooming behavior through both dorsal and ventral striatum. Together, these
315 data elucidate that *Cbln2*$^+$ PF neurons are a central component of a brain network involved
316 in self-grooming behavior.

317 Our data also showed that chemogenetic activation of *Cbln2*$^+$ PF neurons substantially
318 decreased the excessive pathological self-grooming in OCD mice (Welch et al., 2007),
319 providing a solid neurobiological foundation for the improvement of OCD/OCB symptoms
320 under the treatment of DBS in PF in human (Servello et al., 2020). Meanwhile, our
321 preliminary findings indicated that activating PF neurons through non-invasive ultrasound
322 stimulation could also reduce excessive grooming behavior in OCD mice, which offers
323 additional options for clinical treatment of OCD.

324 The PF nucleus participates in sensorimotor coordination (Liu et al., 2008), pain and
325 nociception (Sims-Williams et al., 2016; Weigel and Krauss, 2004). Sensory perception is
326 one of the cases inducing grooming behavior (Xie et al., 2022). Among the brain regions we
327 mapped which send inputs to PF, SC and LPB are reported to mediate the circuits of
328 sensory perception (Choi et al., 2023; Nagashima et al., 2023), and ZI is believed to have
329 many integrative functions that link sensory stimuli with motor responses to guide behavior
330 (Hormigo et al., 2023). Optogenetic and chemogenetic activation of *Cbln2*$^+$ PF neurons
331 inhibit the oil-induced self-grooming, reveal the importance of the PF nucleus in
332 sensory-induced grooming behavior, while may also suggest that the PF nucleus is broadly
333 involved in the regulation of grooming behavior within a complex neural network.

334 *Cbln2*$^+$ PF neurons send projections to various brain regions, including the dorsal
335 striatum, the ventral striatum and others. Here we have proven that PF-dorsal striatum

pathway and PF-ventral striatum pathway are spatially independent but both involved in self-grooming regulation, specifying the effect of dorsal striatum on grooming behavior which was rarely reported in rodents. Besides the striatum receiving outputs from *Cbln2*$^{+}$ PF neurons, the periaqueductal gray (PAG) and the ventral tegmental area (VTA) are also potentially innervated by *Cbln2*$^{+}$ PF neurons and reported to implicate in grooming behavior (Gao et al., 2019; Sun et al., 2022). In future studies, it would be important to examine whether these two brain regions are involved in PF-mediating grooming regulation, thereby enriches the landscape of research on the regulation of grooming behavior.

There are certain limitations in our present study. First, the magnetic inducing voltage signals induced by embedding a magnet in the head only provide a relatively more comprehensive reflection of grooming behavior. However, further analysis on the characteristics of the voltage signals corresponding to different types of grooming will be helpful to automatically distinguish among them. Second, *Cbln2* is expressed in the vast majority of PF neurons, with the proportion exceeding 98%. Our miniscope data revealed three types of neurons with different responses to grooming behavior, addressing the question that classification of detailed subtypes of neurons in PF nucleus combining with single-cell sequence would be necessary to better elucidate how PF neurons encode self-grooming. Third, chemogenetic activation of *Cbln2*$^{+}$ PF neurons significantly alleviated the excessive grooming in OCD mice, while further electrophysiological, structural and biochemical studies are essential to refine the neural mechanism of *Cbln2*$^{+}$ PF neurons on pathological grooming regulation, which would prospectively bring new therapeutic target for OCD. Finally, we have only preliminary confirmed that ultrasound stimulation targeting PF reduces grooming time, future studies are required to determine how long the activation of PF neurons induced by ultrasound stimulation persist and the optimal stimulation parameters to prolong or enhance the activation effects.

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AUTHOR CONTRIBUTIONS

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558 **METHODS**

559 **Animals.** All the mice-using experimental procedures were conducted following the
560 protocols approved by the Administrative Panel on Laboratory Animal Care in the National
561 Institute of Biological Sciences (NIBS) in Beijing, China. Mouse lines used in this study,
562 including vGluT2-Cre and VGAT-Cre were imported from the Jackson Laboratory (JAX Mice
563 and Services). Cbln2-IRES-Cre mice were generated in NIBS and *Sapap3*$^{-/-}$ mice were
564 purchased from the GemPharmatech Co., Ltd. Mice were housed in groups (3–5 animals
565 per cage) and maintained under a 12-h light/12-h dark cycle with food and water available *ad*
566 *libitum*. Mice were separated 3 days before virus injection and housed individually after virus
567 injection until subsequent experiments were conducted. Primers used for genotyping are
568 listed in Supplementary Table 2.

569

570 **AAV vectors.** Viral particles used in this study were purchased from Shanghai Taitool
571 Bioscience Inc., BrainVTA Inc. and OBiO Technology Inc. Detailed information about the
572 AAVs were listed in the Supplementary Table 2. The final titer used for AAV injection was
573 5×10^{12} viral particles/ml.

574

575 **Stereotaxic injection.** Mice were anesthetized with an intraperitoneal injection of
576 tribromoethanol (125–250 mg per kg). Standard surgery was performed to expose the brain
577 surface above the target brain regions, including PF, SC, LPB, ZI, SNr, dorsal striatum and
578 ventral straitum. Coordinates used to inject PF were as follows: bregma -2.30 mm, lateral
579 ±0.65 mm and dura -3.20 mm. Coordinates used to inject SC were as follows: bregma -3.80
580 mm, lateral ±1.20 mm and dura -1.55 mm. Coordinates used for LPB injection were as
581 follows: bregma -5.43 mm, lateral ±1.35 mm and bregma -3.50 mm. Coordinates used for ZI
582 injection were as follows: bregma -1.79 mm, lateral ±1.28 mm and dura -4.09 mm.
583 Coordinates used for SNr injection were as follows: bregma -3.13 mm, lateral ±1.37 mm and
584 dura -4.36 mm. Coordinates used for dorsal striatum injection were as follows: bregma
585 +0.86 mm, lateral ±1.70 mm and dura -2.60 mm. Coordinates used for ventral striatum
586 injection were as follows: bregma +1.20 mm, lateral ±1.50 mm and dura -4.45 mm. The
587 AAVs, CTB-488 and WGA-555 were stereotaxically injected using a glass pipette connected

588 to a Nanoliter-Injector 201 (World Precision Instruments) at a slow flow rate of 0.15 μl min^{-1}
589 to avoid potential damage to local brain tissue. The pipette was withdrawn at least 20 min
590 after viral injection.
591 For optogenetic activation, AAV injections were bilateral. For chemogenetic
592 activation/inactivation, injections were bilateral. For chemogenetically synaptic activation,
593 injections were bilateral. Behavioral tests were conducted 3 weeks after viral injection. Slice
594 physiology and histology were conducted at least 3 weeks after AAV injection.
595
596 **Optical fiber implantation.** Thirty minutes after AAV injection, a ceramic ferrule with an
597 optical fiber (for optogenetics: 200 μm in diameter, numerical aperture (NA) of 0.37; for fiber
598 photometric recording: 200 μm in diameter, NA of 0.37) was implanted with the fiber tip on
599 top of the PF (bregma -2.30 mm, lateral ± 1.71 mm and bregma -2.55 mm with a 20° angle
600 towards the midline). The ferrule was then secured onto the skull with dental cement. After
601 implantation, the antibiotics were applied to the surgical wound. The optogenetic and fiber
602 photometric recording experiments were conducted 3 weeks after the optical fiber
603 implantation. For optogenetic stimulation, the output of the laser was measured and
604 adjusted to various intensities of 2 mW or 0.2 mW before each experiment. The pulse onset,
605 duration, and frequency of light stimulation were controlled by a programmable pulse
606 generator attached to the laser system.
607
608 **Cannula implantation and drug infusion.** Two cannulae were bilaterally implanted above
609 the PF, dorsal striatum or ventral striatum regions. The inner and outer diameters of the
610 cannula were 150 μm and 300 μm, respectively. Each cannula was fixed to the skull with
611 dental cement. During drug infusion, each cannula was connected with a catheter filled with
612 CNO (3 μM) or saline for injection. The other side of the catheter was connected to a
613 Hamilton syringe controlled by an infusion pump to drive the delivery of CNO or saline (50 nl
614 min−1). The catheter was removed 5 minutes after the delivery of CNO or saline was
615 completed. Then the mice were back to homecage for a 30-minute rest and used for the
616 subsequent behavior tests. At the end of the experimental session, the test mice were
617 perfused, and the coronal brain sections containing PF, dorsal striatum or ventral striatum

618 regions were inspected for the presence of cannula track. The mice with no cannula track
619 above the PF, dorsal striatum or ventral striatum were rejected from further analysis.
620
621 **Preparation of behavioral tests.** After AAV injection and fiber implantation, the mice were
622 housed individually for 3 weeks, and handled daily by the experimenters for at least 3 days
623 before the behavioral tests. On the day of the behavioral test, the mice were transferred to
624 the testing room and were habituated to the room conditions for 3 h before the experiments
625 started. The apparatus was cleaned with 20% ethanol, to eliminate odor cues from other
626 mice. All behavioral tests were conducted during the same circadian period (13:00–19:00).
627 All behaviors were scored by the experimenters, who were blinded to the animal treatments.
628
629 **Magnetic induction to record head movements during self-grooming.** The magnetic
630 induction system to measure self-grooming was constructed in the light of a magnetic
631 induction system to measure scratching behavior (Inagaki et al., 2002). For the mice without
632 fibers or cannulae, a small skin cut (1 mm) was made in the scalp under anesthesia. A metal
633 guide tube with sharp tip that carried a magnet cylinder (1 mm in diameter, 3 mm in length)
634 was inserted subcutaneously. Then the cylinder was pulled out, while leaving the magnet
635 inside the scalp. A drop of vet glue was applied to help the skin cut sealed well and healed.
636 The mice could be tested 3 days after the surgery. For the mice with fibers or cannulae, the
637 magnet cylinder was placed on the skull and wrapped in dental cement during the surgery of
638 fiber/cannula implantation. Then the behavior tests were conducted 3 weeks after the
639 surgery.
640 The tested mouse was placed in the chamber in a Helmholtz coil (230 mm in diameter,
641 CTXQ-230, Li Tian magnetoelectric Technology Inc.), or in the arena above a Helmholtz coil.
642 The electric potential induced in the coil by the movement of magnets was amplified (100x)
643 and recorded with Spike2 software (CED Inc., UK). In Spike2 software, the raw electrical
644 potential data was removed DC offset, passed through a digital low pass filter (Butterworth,
645 fourth order, corner: 40 Hz) and smoothed (time constant: 0.04 s). The mean voltage of
646 when the mice were stationary was calculated as the baseline. The threshold to identify the
647 head movement during self-grooming was defined as the mean voltage of baseline plus 5 x

648 SD (standard deviation).

649

650 **Recording the time spent for spontaneous self-grooming and oil-induced**
651 **self-grooming.** On the day of experiment, the test mouse was allowed to explore a cylinder
652 (15 cm in diameter) for 15 minutes. For oil-induced grooming, the experimenter sprayed corn
653 oil on the test mice from the top of the cylinder to make the mice covered with a thin layer of
654 oil after the freely exploration in the cylinder. The behavior of self-grooming was identified by
655 both visual inspection and the magnetic induction trace reflecting the rhythmic head
656 movements. The oil-induced self-grooming was quantified by measuring the time spent for
657 self-grooming during the 30 minutes after the spray of oil onto the whole body of the test
658 mice.

659

660 **Fiber photometric recording.** A fiber photometric system (ThinkerTech) was used to record
661 GCaMP signals from *Cbln2*$^{+}$ PF neurons. To induce fluorescence signals, a laser beams
662 from a laser tube (488 nm) was reflected by a dichroic mirror, focused by a 10 × (NA of 0.37)
663 lens and coupled to an optical commutator. A 2-m optical fiber (200 μm in diameter, NA of
664 0.37) guided the light between the commutator and the implanted optical fiber. To minimize
665 photobleaching, the power intensity at the fiber tip was adjusted to 0.02 mW. The GCaMP7s
666 fluorescence was band-pass filtered (MF525-39, Thorlabs) and collected by a
667 photomultiplier tube (R3896, Hamamatsu). An amplifier (C7319, Hamamatsu) was used to
668 convert the photomultiplier tube current output to voltage signals, which were further filtered
669 through a low-pass filter (40 Hz cut-off; Brownlee 440). The analog voltage signals were
670 digitized at 100 Hz and recorded by a Power 1401 digitizer and Spike2 (CED).

671 AAV-hSyn-DIO-GCaMP7s was stereotacically injected into the PF of Cbln2-IRES-Cre
672 mice followed by the implantation of an optical fiber above the *Cbln2*$^{+}$ PF neurons and the
673 implantation of magnet cylinder inside the dental cement. Three weeks after AAV injection,
674 fiber photometric recording was used to record GCaMP signals from *Cbln2*$^{+}$ PF neurons of
675 free moving mice in the magnetic induction system. A flasing LED triggered by 1s
676 square-wave pluse was simultaneously recorded to synchronize the video, GCaMP signals
677 and the magnetic inducing voltage signals. After the experiments, the optical fiber tip sites in

678 the PF were histologically examined in each mouse.

679

680 **Cell-type-specific RV tracing.** A modified RV-based three-virus system was used for
681 mapping whole-brain inputs to *Cbln2*$^{+}$ PF neurons (Wickersham et al., 2007). All viruses,
682 including AAV2/9-CAG-DIO-EGFP-2A-TVA (5 × 10^{12} viral particles per ml),
683 AAV2/9-CAG-DIO-RVG (5 × 10^{12} viral particles/ml), and EnvA-pseudotyped, glycoprotein
684 (RG)-deleted, and EGFP-expressing RVs (RV-EvnA-ΔG-DsRed) (5.0 × 10^{8} viral
685 particles/ml), were packaged and provided by BrainVTA Inc. (Wuhan, China). A mixture of
686 AAV2/9-CAG-DIO-EGFP-2A-TVA and AAV2/9-CAG-DIO-RVG (1:1, 300 nl) was injected into
687 the PF of Cbln2-IRES-Cre mice unilaterally. Two weeks after AAV helper injection,
688 RV-EvnA-ΔG-DsRed (300 nl) was injected into the same location in the PF of
689 Cbln2-IRES-Cre mice in a biosafety level-2 lab facility. Starter neurons were characterized
690 by the co-expression of DsRed and EGFP, which were restricted in the PF. One week after
691 injection of RV, the mice were perfused with saline, followed by 4% paraformaldehyde (PFA)
692 in phosphate-buffered saline (PBS). The brain was post-fixed in 4% PFA for 8 h and then
693 incubated in PBS containing 30% sucrose until they sank to the bottom. Coronal brain
694 sections (40-μm thickness) were prepared using a cryostat (Leica CM1900). All sections
695 were collected and stained with 4',6-diamidino-2-phenylindole (DAPI). The sections were
696 imaged with an Olympus VS120 epifluorescence microscope (10× objective lens) and
697 analyzed with ImageJ software. For quantification of subregions, boundaries were based on
698 the Allen Institute's reference atlas. We selectively analyzed retrogradely labeled dense
699 areas. The fractional distribution of total cells labeled with RV was measured.

700

701 **Cell-type-specific anterograde tracing.** For cell-type-specific anterograde tracing of
702 *Cbln2*$^{+}$ PF neurons, AAV-DIO-EGFP-syb2 (300 nl) was stereotaxically injected into the PF of
703 Cbln2-IRES-Cre mice. The mice were then maintained in a cage individually. Three weeks
704 after viral injection, mice were perfused with saline followed by 4% PFA in PBS. The brains
705 were post-fixed in 4% PFA for 8 hours and then incubated in PBS containing 30% sucrose
706 until they sank to the bottom. Coronal brain sections at 40 μm in thickness were prepared
707 using a cryostat (Leica CM1900). All coronal sections were collected and stained with

primary antibody against GFP and DAPI. The coronal brain sections were imaged with an Olympus VS120 epifluorescence microscope (10 × objective lens).

Slice physiological recording. Slice physiological recording was performed according to a previously published protocol. Brain slices containing the PF were prepared from adult mice anesthetized with isoflurane before decapitation. Brains were rapidly removed and placed in ice-cold oxygenated (95% O_2 and 5% CO_2) cutting solution (in mM: 228 sucrose, 11 glucose, 26 $NaHCO_3$, 1 NaH_2PO_4, 2.5 KCl, 7 $MgSO_4$ and 0.5 $CaCl_2$). Coronal brain slices (400 μm) were cut using a vibratome (VT 1200S, Leica Microsystems). The slices were incubated at 28°C in oxygenated artificial cerebrospinal fluid (ACSF containing in mM: 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 10 glucose and 2.5 $CaCl_2$) for 30 min, and then kept at room temperature under the same conditions for 1 h before transfer to the recording chamber. The ACSF was perfused at 1ml min^{-1}. The acute brain slices were visualized using a ×40 Olympus water immersion lens, differential interference contrast (DIC) optics (Olympus) and a CCD camera. Patch pipettes were pulled from borosilicate glass capillary tubes (Warner Instruments, no. 64-0793) using a PC-10 pipette puller (Narishige). For recording of action potentials (current clamp), pipettes were filled with a solution (in mM: 135 K-methanesulfonate, 10 HEPES buffer, 1 EGTA (ethylene glycol tetraacetic acid), 1 Na-GTP, 4 Mg-ATP and 2% neurobiotin (pH7.4)). The resistance of pipettes varied between 3.0 and 3.5 MΩ. The voltage signals were recorded using MultiClamp 700B and Clampex 10 data acquisition software (Molecular Devices). After establishment of the whole-cell configuration and equilibration of the intracellular pipette solution with the cytoplasm, series resistance was compensated to 10–15 MΩ. An optical fiber (200μm in diameter) was used to deliver light pulses, with the fiber tip positioned 500 μm above the brain slices. Light-evoked action potentials from ChR2-mCherry$^+$ neurons in the PF were triggered by a light-pulse train (473 nm, 2 ms, 10 Hz or 20 Hz, 5 mW) synchronized with Clampex 10 data acquisition software (Molecular Devices).

Miniscope calcium imaging and data analysis.

1) AAV injection and gradient index (GRIN) lens implantation

738 For miniscope calcium imaging studies, AAV-hsyn-DIO-jGCaMP7s was microinjected into
739 PF of Cbln2-IRES-Cre mice with following coordinates: anterior-posterior, -2.30 mm;
740 medial-lateral, -0.65 mm; dorsal-ventral, -3.2 mm from dura, then the GRIN lens (0.5 mm in
741 diameter × 6.1 mm in length, Inscopix) was implanted 2 weeks after viral injection. For the
742 implantation, the GRIN lens was connected to the imaging system of miniscope to monitor
743 the GCaMP fluorescence during slowly lowering (~100 μm/min) to the target brain area.
744 After the lens was placed at the appropriate coordinates to gain the optimal intensity of
745 GCaMP fluorescence and anchored to the skull with dental cement, the silicon adhesive
746 (Kwik-Sil, WPI) was used to cap the lens to avoid damage.
747 2) Measuring GCaMP fluorescence
748 Miniscope calcium imaging was conducted by using a miniaturized fluorescence microscopy
749 unit (miniscope) linked to a data acquisition device (Inscopix, nVista). At least one day
750 before behavior test and calcium imaging, awake mice with implanted lens were head-fixed
751 on a circular treadmill and a baseplate was implanted above the GRIN lens to get a suitable
752 field for imaging after mounted by the miniscope. Then the miniscope was replaced by a
753 fake one and the mice were returned to their homecages, which enabled the mice to be
754 habituated to the weight of miniscope.
755 On the day of behavior test and calcium imaging, two side-view cameras were used to
756 record the grooming behavior. Synchronization between imaging data and grooming
757 behavior was implemented by the video recording the flash of LED light and a Power 1401
758 digitizer which was used to mark the signal generated by the data acquisition device when
759 each session of imaging data was documented. Imaging data were acquired at a 20 Hz
760 frame rate.
761 3) Data acquisition and analysis
762 Imaging data were preliminarily processed by the Inscopix data processing software (version
763 1.6.0). Then the image data were exported as Tiff files. These Tiff files were further analyzed
764 by CNMF-E code in Matlab for the generation of example videos, or manually analyzed by
765 ImageJ software to read the dynamic changes of GCaMP fluorescence frame by frame for
766 each neuron.
767 For the trials which recorded the initial stage of grooming, the frame at which the mice

768 started to groom was denoted as 0 s, and normalized GCaMP fluorescence (ΔF/F) was
769 calculated over a baseline period of -3 s to 0 s.
770 For the trials which recorded the terminate stage of grooming, the frame at which the mice
771 stop grooming was denoted as 0 s, and normalized GCaMP fluorescence (ΔF/F) was
772 calculated over a baseline period of 0 s to 3 s.
773
774 **Ultrasound stimulation**
775 On the day prior to experimental procedures, the hair on the top of the mouse's head
776 was carefully removed using hair removal cream. The mice were anesthetized with 3%
777 isofurane in an induction chamber. After sedation, the mice were placed in a supine position
778 on a warming pad (38 °C) to maintain normothermia, and fixed to the three-dimensional
779 brain stereotaxic instrument by a head bar for ultrasound stimulation keeping in light
780 narcosis with 1% isofurane delivered via 100% O_2 mask inhalation (Li et al., 2019). The
781 schematic of ultrasound stimulation is shown in Fig. 6A. The ultrasound system used was
782 similar to those used in previous studies (Wang et al., 2024; Zhao et al., 2023). In the
783 experiment, a pulse signal generator (AFG3022C, Tektronix, USA) was used to continuously
784 transmit modulated sine wave electrical signals, and the signals were amplified by a power
785 amplifier (E&I 240 L, ENI Inc., USA) and then further amplified by a focused ultrasound
786 probe (V301-SU, focus [diameter, 25.4 mm; radius of curvature, 40 mm], Olympus, USA)
787 during ultrasound stimulation. A conical collimator (3D-printing, resin material, bottom
788 aperture: 4 mm) filled with ultrasonic coupling gel was fixed between the ultrasonic probe
789 and the mouse head, therefore the ultrasonic waves could penetrate the mouse brain, and
790 the ultrasound pressure was 0.36 MPa. The corresponding spatial-peak and pulse-average
791 intensity values were 4.3 W/cm^{-2} and the spatial-peak and time-average intensity value was
792 215.8 mW/cm^{-2} (Wang et al., 2024; Zhao et al., 2023). As shown in Fig. 6B, the
793 fundamental frequency (FF), pulse repetition frequency (PRF), number of cycle(NC), duty
794 cycle (DC), stimulation duration (SD), pulse duration (PD) and inter-stimulus interval (ISI) of
795 the ultrasound waves were 500 kHz, 1 Hz, 100000, 20%, 300 s, 200 ms and 300 s,
796 respectively. The stimulation was performed at the same time period every day, and each
797 stimulation was followed by a 5-min pause, for a total of three trials of 30 min (Fig. 6B).

798 Ultrasound stimulation for test mice (US) was continuously performed for 7 days (Fig. 6C).
799 All procedures for the control mice (NUS) were the same as that of the test mice (US) except
800 that the pulse signal generator of the control mice was turned off. Behavioral testing was
801 performed as described in Fig. 6C and sFig.6A. Briefly, for the preliminary experiment, mice
802 were acclimated to the ultrasound system for 30 minutes daily over three days. Grooming
803 time was tested on the third day (Day 3), then the mice received daily stimulation for 7
804 consecutive days, with each session lasting 30 min. Grooming time was assessed after each
805 simulation session (Day 4 - Day 10). For the formal experiment, mice were acclimated to the
806 ultrasound system for 30 minutes daily over three days and were recorded for grooming time
807 on Day 3 as baseline. Then the mice received 7 days of daily 30-minute ultrasound
808 stimulation, and were recorded for grooming time on Day 10. Mice were sacrificed on Day 10
809 immediately after recording. Only mice exhibiting significantly increased c-fos staining in the
810 PF region were included in the group of US for further analysis.
811
812 **Immunohistochemistry.** Mice were anesthetized with isoflurane and sequentially perfused
813 with saline and PBS containing 4% PFA. Post-fixation of the brain was avoided to optimize
814 immunohistochemistry. Brains were incubated in PBS containing 30% sucrose until they
815 sank to the bottom. Cryostat sections (40 μm) were collected, incubated overnight with
816 blocking solution (PBS containing 10% goat or donkey serum and 0.7% Triton X-100), and
817 then treated with primary antibodies diluted with blocking solution for 3 - 4 h at room
818 temperature. Primary antibodies used for immunohistochemistry are listed in the
819 Supplementary Table 2. Primary antibodies were washed three times with washing buffer
820 (PBS containing 0.7% Triton X-100) before incubation with secondary antibodies (tagged
821 with Alexa Fluor® 488 and Alexa Fluor® 546 dilution 1:500; Invitrogen) for 1 h at room
822 temperature. Sections were then washed three times with washing buffer, stained with DAPI,
823 and washed with PBS, transferred onto Super Frost slides, and mounted under glass
824 coverslips with mounting media.
825 Sections were imaged with an Olympus VS120 epifluorescence microscope (10 ×
826 objective lens) or a Zeiss LSM 800 confocal microscope (20 × and 63 × oil-immersion
827 objective lens). Samples were excited by 405, 488 and 561 nm lasers in sequential

828 acquisition mode to avoid signal leakage. Saturation was avoided by monitoring pixel
829 intensity with Hi-Lo mode. Confocal images were analyzed with ImageJ software.
830
831 **RNAscope.** Mice were perfused with PBS treated with 0.1% DEPC (Sigma, D5758),
832 followed by DEPC-treated PBS containing 4% PFA (PBS-PFA). Brains were post-fixed in
833 DEPC-treated PBS-PFA solution overnight and then placed in DEPC-treated 30% sucrose
834 solution at 4°C for 30h. Brain sections to a thickness of 20 μm were prepared using a
835 cryostat (Leica, CM3050S) and subsequently hybridized with the Cbln2 probe (Advanced
836 Cell Diagnostics, 428551) following the user manual of the RNAscope kit (Advanced Cell
837 Diagnostics, Doc No. 323100-USM). To visualize the NeuN signals, brain sections were
838 incubated with a primary antibody against NeuN at 4°C for 24 h and then with an Alexa
839 Fluor® 594-conjugated goat anti-rabbit secondary antibody (1:500, A11034, Invitrogen) at
840 room temperature for 2 h. Brain sections were imaged using a Zeiss LSM780 confocal
841 microscope or the Olympus VS120 Slide Scanning System. Detailed information about
842 probe and reagents are listed in the Supplementary Table 2.
843
844 **Data quantification and statistical analyses.** Data collection and analyses were
845 performed blinded to the conditions of the experiments. For statistical analyses of the
846 experimental data, two-sided Student's t-test was used. The "n" used for these analyses
847 represents the number of mice or cells.
848

849 **FIG.1 *Cbln2*-expressing PF neurons are essential for self-grooming**

850 (**A**, **B**) Schematic diagram (**A**) and example voltage pulses traces (**B**) showing the magnetic
851 induction system in parallel with a high-speed camera to record the stationary state,
852 locomotion, paw-licking, orofacial grooming and body grooming in mice. (**C**) Example
853 coronal section showing ChR2-mCherry expression in PF of Cbln2-IRES-Cre mice and
854 optical fiber tracks above the PF. (**D**) Light-pulse trains (473 nm, 10 s, 2 mW, 20 Hz / 10 Hz)
855 reliably evoked phase-locked spiking activity in ChR2-mCherry^{+} PF cells. (**E**-**G**) Quantitative
856 analyses of time spent for self-grooming within 10 second before (OFF) and during (ON)
857 photostimulation of *Cbln2*$^{+}$ PF neurons. Photostimulation was conducted when the mice
858 were in the sequential phases of paw-licking (**E**), orofacial grooming (**F**), and body grooming
859 (**G**). (**H**) Quantitative analyses of time spent for oil-induced self-grooming of mice within 10
860 second before (OFF) and during (ON) photostimulation of *Cbln2*$^{+}$ PF neurons. (**I**) Example
861 coronal section showing bilateral injection of AAV-DIO-hM3Dq-mCherry into the PF of
862 Cbln2-IRES-Cre mice. (**J**) Example trace of action potential firing (left) and quantitative
863 analysis of firing rate (right) showing the effectiveness of CNO to chemogenetically activate
864 hM3Dq-expressing PF neurons in acute brain slices. CNO was dissolved in artificial
865 cerebrospinal fluid (ACSF) (10 μM) and perfused to the brain slices. (**K**) Example coronal
866 section showing bilateral injection of AAV-DIO-hM4Di-mCherry into the PF of
867 Cbln2-IRES-Cre mice. (**L**) Example trace of action potential firing (left) and quantitative
868 analysis of firing rate (right) showing the effectiveness of CNO to chemogenetically silence
869 hM4Di-expressing PF neurons in acute brain slices. CNO was dissolved in artificial
870 cerebrospinal fluid (ACSF) (10 μM) and perfused to the brain slices. (**M**-**N**) Quantitative
871 analyses of time spent for spontaneous self-grooming (**M**) and oil-induced self-grooming (**N**)
872 within 30 min in mice treated with saline or CNO (1 mg/kg) to chemogenetically activate or
873 inactivate *Cbln2*$^{+}$ PF neurons. (**O**) Quantitative analyses of time spent for pathological
874 self-grooming within 30 min in mice treated with saline or CNO (1 mg/kg) to
875 chemogenetically activate *Cbln2*$^{+}$ PF neurons in Cbln2-IRES-Cre / *Sapap3*$^{-/-}$ mice. Scale
876 bars and numbers of mice are indicated in the graphs. Data in (**E** - **H**), (**J**), (**L**), (**M**) and (**N**)
877 are means ± SEM (error bars). Statistical analyses in (**E** - **H**), (**J**), (**L**), (**M**) and (**N**) were
878 performed using Student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$). For

879 *p* values, see Supplementary Table 1.

880 **FIG.2 *Cbln2*-expressing PF neurons respond to self-grooming**

881 (**A**) Representative micrographs showing the expression of GCaMP7s in PF, and the optical
882 fiber track above the GCaMP$^+$ PF neurons for fiber photometric recording in adult
883 Cbln2-IRES-Cre mice. (**B**) Example GCaMP signals and voltage pulses traces from
884 magnetic induction system in parallel with self-grooming. (**C**) Correlation analysis of average
885 GCaMP7s fluorescence change ($\Delta F_{(peak\text{-}valley)}/F_{peak}$) with grooming duration. (**D**, **H**) Heat-map
886 graphs of GCaMP7s fluorescence of the *Cbln2*$^+$ PF neurons aligned to the onset (**D**) and the
887 offset (**H**) of self-grooming. EGFP were used as control. (**E**, **I**) GCaMP response curve
888 aligned with the initiation (**E**) and termination (**I**) of self-grooming of an example mouse,
889 showing kinetics of GCaMP signal of *Cbln2*$^+$ PF neurons. EGFP were used as control. (**F**, **J**)
890 Quantitative analyses of average GCaMP7s fluorescence change (ΔF/F) with the initiation
891 (**F**) and termination (**J**) of self-grooming. (**G**, **K**) Quantitative analyses of distribution of the
892 time point at which the GCaMP signal begin to decrease during the initial stage of grooming
893 (**G**) and increase during the terminate stage of grooming (**H**). (**L**) Schematic diagram
894 showing the schedule for recording GCaMP7s fluorescence from *Cbln2*$^+$ PF single neurons
895 by miniscope. (**M**) Schematic diagram showing viral injection and GRIN lens implantation
896 strategy for calcium imaging of *Cbln2*$^+$ PF neurons. (**N**) Example coronal brain section
897 showing the track of GRIN lens above the GCaMP$^+$ PF neurons in Cbln2-IRES-Cre mice
898 (left). The field of view of raw GCaMP signals (right). (**O**) Heatmaps of the activities (ΔF/F0)
899 in individual PF neurons (n = 6 mice). The vertical white dashed line denotes the grooming
900 behavior onset (0 s). The aligned traces were sorted by the responsive time, and classified
901 to 3 different types depending on the average GCaMP fluorescence intensity difference
902 between the grooming period and the baseline period. Type1: decrease; Type2: no change;
903 Type3: increase. (**P**) Average GCaMP fluorescence response (ΔF/F0) curves of 3 different
904 types of neurons aligned with the initiation of grooming behavior (left). Proportion of PF
905 neurons from each category (right, n = 156 neurons from 6 mice). (**Q**) Heatmaps of the
906 activities (ΔF/F0) in individual PF neurons (n =6 mice). The vertical white dashed line
907 denotes the end of grooming behavior (0 s). The aligned traces were sorted by the
908 responsive time, and classified to 3 different types depending on the average GCaMP
909 fluorescence intensity difference between the grooming period and the baseline period.

910 Type1: increase; Type2: no change; Type3: decrease. (**R**) Average fluorescence response
911 (ΔF/F0) curves of 3 different types of neurons aligned with the termination of grooming
912 behavior (left). Proportion of PF neurons from each category (right, n = 156 neurons from 6
913 mice). Scale bars and numbers of mice are indicated in the graphs. Data in (**E**, **F**) and (**I**, **J**)
914 are means ± SEM (error bars). Statistical analyses in **(F)** and **(J)** were performed using
915 Student's t-test (****$p < 0.0001$). For p values, see Supplementary Table 1.
916

917 **FIG.3 Retrograde tracing of *Cbln2*$^{+}$ PF neurons with RV(A)** Schematic diagram showing
918 the viral injection strategy for the retrograde tracing of *Cbln2*$^{+}$ PF neurons using a
919 combination of AAV and rabies virus (RV). (**B-C**) Representative coronal section showing
920 that the viral injection center, as indicated by the co-expression of EGFP and DsRed, was
921 localized in the PF. Note that the double-labeled cells indicate starter cells **(C)**. **(D)** Example
922 micrographs showing DsRed^{+} cells in different brain regions, including the LPB (D1), the SC
923 (D2), the SNr (D3) and the ZI (D4). **(E)** Fraction of total RV-labeled cells in different brain
924 regions monosynaptically projecting to the *Cbln2*$^{+}$ PF neurons. Note that data in **(E)** were
925 normalized by dividing the total number of DsRed+ cells in these brain regions. Numbers of
926 mice **(E)** are indicated in the graphs. Scale bars are labeled in the graphs. Data in **(E)** are
927 means ± SEM (error bars). The experiment **(B-D)** was repeated four times independently
928 with similar results.
929
930

931 **FIG.4 *Cbln2*$^{+}$ PF input pathways involvement in self-grooming**

932 (**A, E**) Schematic diagram showing the injection of AAV-DIO-hM3Dq-mCherry into the SC or
933 LPB of vGluT2-IRES-Cre mice, followed by cannulae implantation above the PF to enable
934 cell-type-specific activation of the vGluT2^{+} SC–PF pathway (**A**) or vGluT2^{+} LPB–PF pathway
935 (**E**). (**B-C, F-G**) Example micrographs showing hM3Dq-mCherry expression in the SC (**B**) or
936 LPB (**F**) and the cannulae above hM3Dq-mCherry^{+} axon terminals in the PF (**C**, **G**). (**D, H**)
937 Quantitative analyses of time spent for spontaneous self-grooming within 30 min in mice
938 treated with saline or CNO (1 mg/kg) to chemogenetically activate vGluT2^{+} SC–PF pathway
939 (**D**) or vGluT2^{+} LPB–PF pathway (**H**). (**I, M**) Schematic diagram showing the injection of
940 AAV-DIO-hM3Dq-mCherry into the SNr or ZI of vGAT-IRES-Cre mice, followed by cannulae
941 implantation above the PF to enable cell-type-specific activation of the vGAT^{+} SNr–PF
942 pathway (**I**) or vGAT^{+} ZI–PF pathway (**M**). (**J-K**, **N-O**) Example micrographs showing
943 hM3Dq-mCherry expression in the SNr (**J**) or ZI (**N**) and the cannulae above
944 hM3Dq-mCherry^{+} axon terminals in the PF (**K**, **O**). (**L, P**) Quantitative analyses of time spent
945 for spontaneous self-grooming within 30 min in mice treated with saline or CNO (1 mg/kg) to
946 chemogenetically activate vGAT^{+} SNr–PF pathway (**L**) or vGAT^{+} ZI–PF pathway (**P**).
947 Scale bars and numbers of mice are indicated in the graphs. Data in (**D**), (**H**), (**L**), and (**P**)
948 are means ± SEM (error bars). Statistical analyses in (**D**), (**H**), (**L**), and (**P**) were performed
949 using Student's t-test (*p < 0.05, **p < 0.01, and ***p < 0.001). For p values, see
950 Supplementary Table 1.

951

952

953 **FIG.5 The role of *Cbln2*$^+$ PF–striatum pathway in self-grooming**

954 (**A**) Schematic diagram showing the viral injection strategy for labeling *Cbln2*$^+$ PF neurons
955 using AAV-DIO-ChR2-mCherry in adult Cbln2-IRES-Cre mice. (**B-C**) Representative
956 micrographs from the PF showing EGFP expression in the PF neurons (**B**) and
957 EGFP-positive axon terminals in the striatum (**C**). (**D**) Retrograde labelling of PF neurons
958 by injecting WGA555 into dorsal striatum and CTB488 into ventral striatum. (**E-H**) Example
959 coronal brain sections showing dorsal striatum-projecting WGA$^+$ PF neurons and ventral
960 striatum-projecting CTB+ neurons. (**I**) Quantitative analyses reflecting the overlap between
961 dorsal striatum- and ventral striatum-projecting PF neurons within different bregmas,
962 showing that dorsal striatum- projecting and ventral striatum-projecting *Cbln2*+ PF neurons
963 are largely segregated. (**J, N**) Schematic diagram showing the injection of
964 AAV-DIO-hM3Dq-mCherry into the PF of Cbln2-IRES-Cre mice, followed by cannulae
965 implantation above the dorsal striatum or ventral striatum to enable cell-type-specific
966 activation of the *Cbln2*$^+$ PF–dorsal striatum pathway (**J**) or *Cbln2*$^+$ PF–ventral striatum
967 pathway (**N**). (**K-L, O-P**) Example micrographs showing hM3Dq-mCherry expression in the
968 PF (**K**, **O**) and the cannulae above hM3Dq-mCherry$^+$ axon terminals in the dorsal striatum (**L**)
969 or ventral striatum (**P**). (**M**, **Q**) Quantitative analyses of time spent for spontaneous
970 self-grooming within 30 min in mice treated with saline or CNO (1 mg/kg) to
971 chemogenetically activate *Cbln2*$^+$ PF–dorsal striatum pathway (**M**) or *Cbln2*$^+$ PF–ventral
972 striatum (**Q**).

973 Scale bars and numbers of mice are indicated in the graphs. Data in (**M**) and (**Q**) are means
974 ± SEM (error bars). Statistical analyses (**M** and **Q**) were performed using Student's t-test
975 (*$p < 0.05$, and **$p < 0.01$). For p values, see Supplementary Table 1.

976

977

978 **FIG.6 Ultrasound stimulation targeting PF alleviates over-grooming in *Sapap3*$^{-/-}$ mice.**
979 (**A**) Schematic of ultrasound stimulation. (**B**) Parameters of ultrasonic stimulation,
980 inter-stimulus interval (ISI), fundamental frequency (FF), pulse repetition frequency (PRF),
981 number of cycle(NC), duty cycle (DC), pulse duration (PD) and stimulation duration (SD) of
982 the ultrasound signal. (**C**) Schematic diagram showing the schedule of ultrasound
983 stimulation and recording of grooming for test mice and control mice. (**D**, **E**) Example coronal
984 section showing c-fos staining of PF in the test mouse with ultrasound stimulation (US) (**D**)
985 and control mouse with no ultrasound stimulation (NUS) (**E**). (**F**) Example trace of
986 depolarization-induced spiking activity in PF neurons from test mice and control mice,
987 Injected current = 100 pA. (**G**) Quantitative analysis of the firing rates in PF neurons from
988 test mice and control mice in response to step depolarizing current injections. (**H**, **I**)
989 Quantitative analyses of time spent for spontaneous grooming of WT mice (**H**) and *Sapap3*$^{-/-}$
990 mice (**I**) on Day 3 (baseline) and Day 10 (with 30-min ultrasound stimulation from day 4 to
991 day 10 for each day). (**J**, **K**) Quantitative analyses of time spent for spontaneous grooming of
992 WT mice (**J**) and *Sapap3*$^{-/-}$ mice (**K**) on Day 3 (baseline) and Day 10 (only habituation for 30
993 mins from day 4 to day 10 for each day).
994 Scale bars and numbers of mice are indicated in the graphs. Data in (**G** - **K**) are means ±
995 SEM (error bars). Statistical analyses in (**H** - **K**) were performed using Student's t-test (*p <
996 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001). For p values, see Supplementary Table 1.
997
998

sFIG.1 Photostimulation of *Cbln2*$^{+}$ PF neurons inhibit spontaneous self-grooming but locomotion.

(**A**) Example micrographs showing the distributions of cbln2 mRNA in the PF of WT mice. (**B**) Quantitative analyses of number of PF cells expressing cbln2 mRNA (green) in coronal sections, as indicated by the distance to bregma. (**C, E, G, I**) Example voltage pulses traces showing the magnetic induction system in parallel with a high-speed camera to record spontaneous self-grooming, within 10 second before (OFF) and during (ON) photostimulation of *Cbln2*$^{+}$ PF neurons by various light-pulse trains (473 nm, 2 ms, 2 mW, 20 Hz) for (**C**), (473 nm, 2 ms, 2 mW, 2 Hz) for (**D**), (473 nm, 2 ms, 0.2 mW, 20 Hz) for (**G**), and (473 nm, 2 ms, 0.2 mW, 2 Hz) for (**I**). (**D, F, H, J**) Quantitative analyses of of time spent for spontaneous self-grooming within 10 second before (OFF) and during (ON) photostimulation of *Cbln2*$^{+}$ PF neurons by various light-pulse trains (473 nm, 2 ms, 2 mW, 20 Hz) for (D), (473 nm, 2 ms, 2 mW, 2 Hz) for (F), (473 nm, 2 ms, 0.2 mW, 20 Hz) for (H), and (473 nm, 2 ms, 0.2 mW, 2 Hz) for (**J**). (**K**, **L**) Quantitative analyses of time for total distance (L) and immobile time (L) of mice within 10 second before (OFF) and during (ON) photostimulation of *Cbln2*$^{+}$ PF neurons.

Scale bars are labeled in the graphs. Numbers of mice are indicated in the graphs (**B**, **D**, **F**, **H**, **J**, **K** and **L**). Data in (**B**), (**D**), (**F**), (**H**), (**J**), (**K**) and (**L**) are means ± SEM (error bars). Statistical analyses in (**B**), (**D**), (**F**), (**H**), (**J**), (**K**) and (**L**) were performed using Student's t-test (***$p < 0.001$, and ****$p < 0.0001$).

1022 **sFIG.2 Chemogenetically activation of *Cbln2*$^{+}$ PF neurons inhibit paw-licking,**
1023 **orofacial grooming and body grooming. (A)** Quantitative analyses of total distance within
1024 5 min in mice treated with saline or CNO (1 mg/kg) to chemogenetically activate or inactivate
1025 *Cbln2*$^{+}$ PF neurons. (**B**) Injection of AAV-DIO-hM3Dq-mCherry into PF of Cbln2-IRES-Cre
1026 mice, quantitative analyses of time spent for paw-licking, orofacial grooming, and body
1027 grooming of mice within 30 min treated with saline or CNO (1 mg/kg) to chemogenetically
1028 activate *Cbln2*$^{+}$ PF neurons. (**C**) Injection of AAV-DIO-hM4Di-mCherry into PF of
1029 Cbln2-IRES-Cre mice, quantitative analyses of time spent for paw-licking, orofacial
1030 grooming, and body grooming of mice within 30 min treated with saline or CNO (1 mg/kg) to
1031 chemogenetically inactivate *Cbln2*$^{+}$ PF neurons. (**D**) Injection of AAV-DIO-EYFP into PF of
1032 Cbln2-IRES-Cre mice, quantitative analyses of time spent for paw-licking, orofacial
1033 grooming, and body grooming of mice within 30 min treated with saline or CNO (1 mg/kg). (**E**)
1034 Quantitative analyses of time spent for excessive self-grooming within 30 min in *Sapap3*$^{+/-}$
1035 and *Sapap3*$^{-/-}$ mice.
1036 Numbers of mice are indicated in the graphs (**A**-**E**). Data in (**A**-**E**) are means ± SEM (error
1037 bars). Statistical analyses in (**A**-**E**) were performed using Student's t-test (*$p < 0.05$, **$p <$
1038 0.01, ***$p < 0.001$, and ****$p < 0.0001$).
1039
1040

1041 **sFIG.3 *Cbln2*-expressing PF neurons show decreased Ca^+ activity during**
1042 **self-grooming**

1043 **(A)** Example GCaMP signals from *Cbln2*$^+$ PF neurons during the stationary, locomotion, and
1044 self-grooming stages by fiber photometric recording. (**B**) Quantitative analyses of average
1045 GCaMP7s fluorescence change (ΔF/F) with during the stationary, locomotion, and
1046 self-grooming stages. (**C**) Representative GCaMP response curve from single *Cbln2*$^+$ PF
1047 neurons aligned with the initiation of self-grooming. (**D**) Representative GCaMP response
1048 curve from single *Cbln2*$^+$ PF neurons aligned with the termination of self-grooming.
1049 Numbers of mice are indicated in the graphs (**B**). Data in (**B**) are means ± SEM (error bars).
1050 Statistical analyses in (**B**) were performed using Student's t-test (**$p < 0.01$, and ***$p <$
1051 0.001).

1052

1053

1054 **sFIG.4 Chemogenetically activation of input pathway of *Cbln2*$^+$ PF neurons inhibit**
1055 **paw-licking, orofacial grooming and body grooming**
1056 **(A)** Example micrographs showing DsRed$^+$ cells in different brain regions, including the Sp5I
1057 (A1), the Pnc (A2), the DRD (A3), the DRV (A3), the PnO (A4), the VTA (A5), the MHb (A6),
1058 the LH (A7), the HDB (A8), the M1 (A9) and the M2 (A9). Scale bars are labeled in the
1059 graphs. Number of mice: four. (**B**-**G**) Quantitative analyses of time spent for paw-licking,
1060 orofacial grooming, and body grooming of mice within 30 min treated with saline or CNO (1
1061 mg/kg) to chemogenetically activate vGluT2$^+$ SC–PF pathway (**B**), vGluT2$^+$ LPB–PF
1062 pathway (**C**), vGAT$^+$ SNr–PF pathway (**D**), vGAT$^+$ ZI–PF pathway (**E**), Cbln2$^+$ PF–dorsal
1063 striatum pathway (**F**) or Cbln2$^+$ PF–ventral striatum (**G**). Numbers of mice are indicated in
1064 the graphs (**B**-**G**). Data in (**B**-**G**) are means ± SEM (error bars). Statistical analyses in (**B**-**G**)
1065 were performed using Student's t-test (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).
1066
1067

1068 **sFIG.5 Mapping the monosynaptic outputs of *Cbln2*$^{+}$ PF neurons**

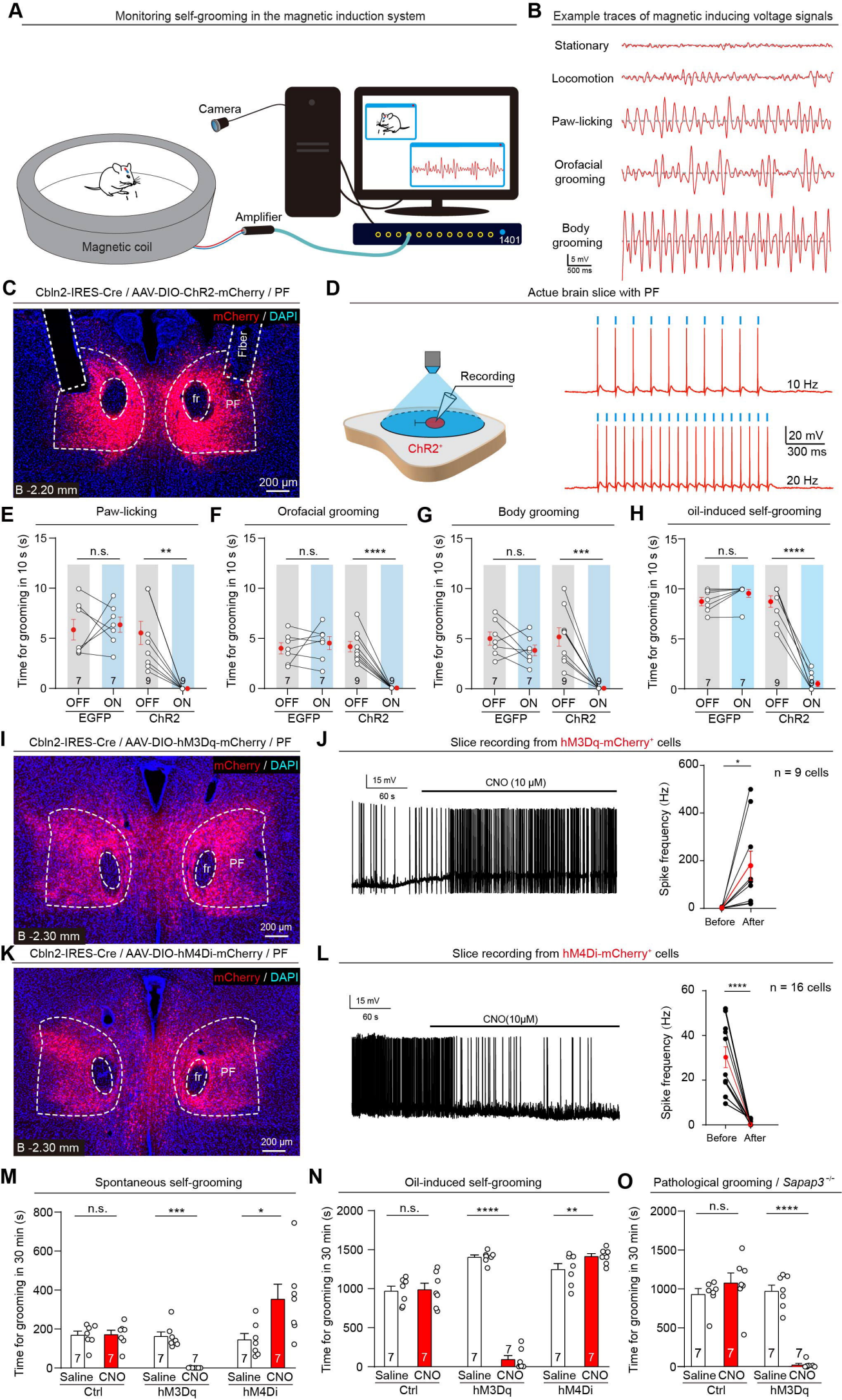
1069 (**A**-**H**) Example coronal brain sections showing EGFP^{+} synaptic terminals of *Cbln2*$^{+}$ PF
1070 neurons in the target brain regions, including the py (**A**), CGPn (**B**), PnV (**C**), LPAG (**D**),
1071 VLPAG (**D**), DRI (**D**), MnR (**D**), ZI (**E**), LC (**F**), CGA (**F**), LDTg (**F**), VTA (**G**), Re (**H**), AHC (**H**),
1072 and LH (**H**). (**I**) Antrograde labeling of synaptic terminals of *Cbln2*$^{+}$ PF neurons in the dorsal
1073 and ventral striatum. (**J** and **K**) Various subtypes of interneurons, including ChAT^{+}, NPY^{+},
1074 PV^{+} and SST^{+} in the dorsal stratum (**J**) and ventral stratum (**K**) were surrounded by the
1075 EGFP^{+} synaptic terminals from *Cbln2*$^{+}$ PF neurons.

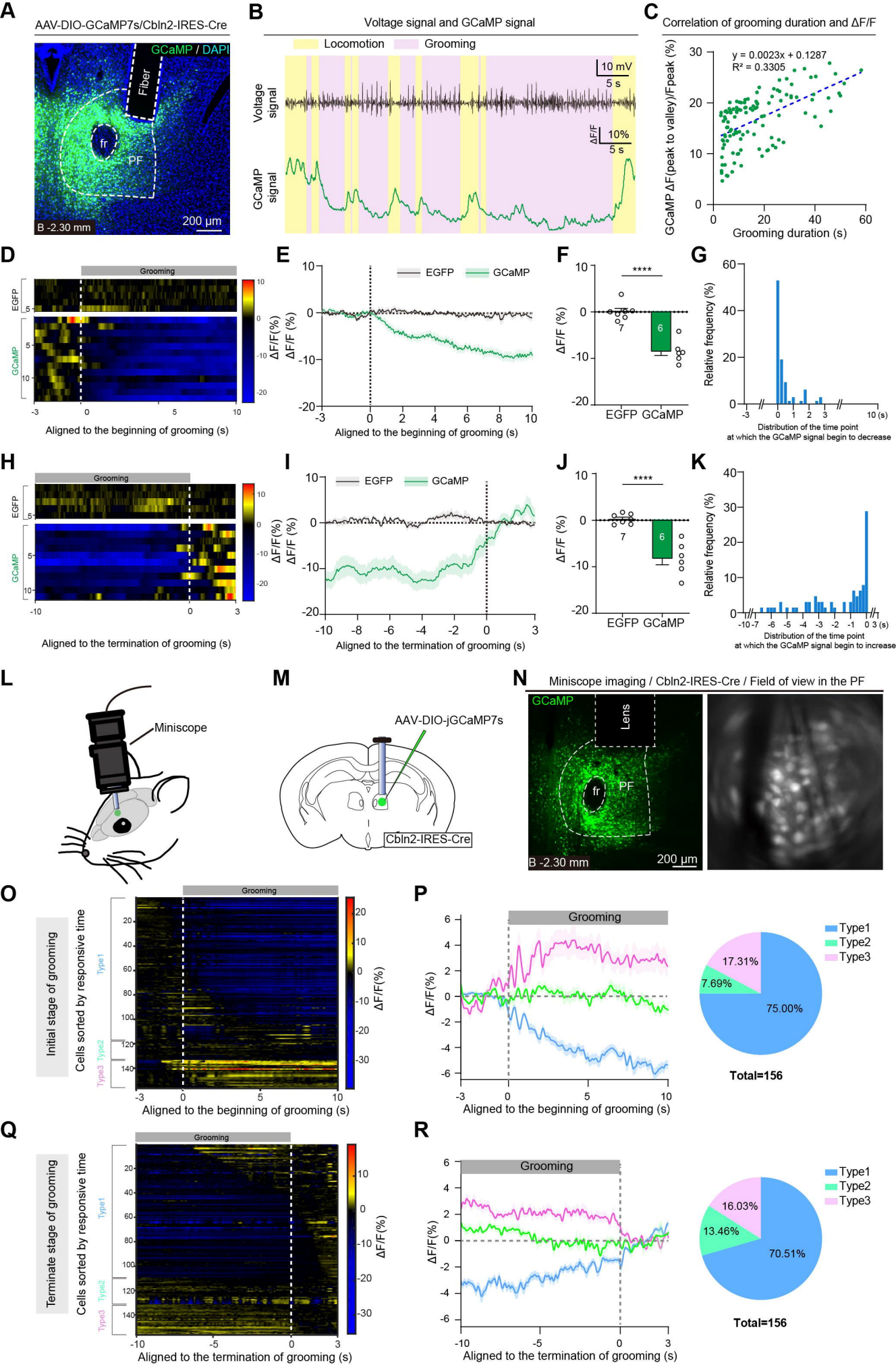
1076 Scale bars are labeled in the graphs.

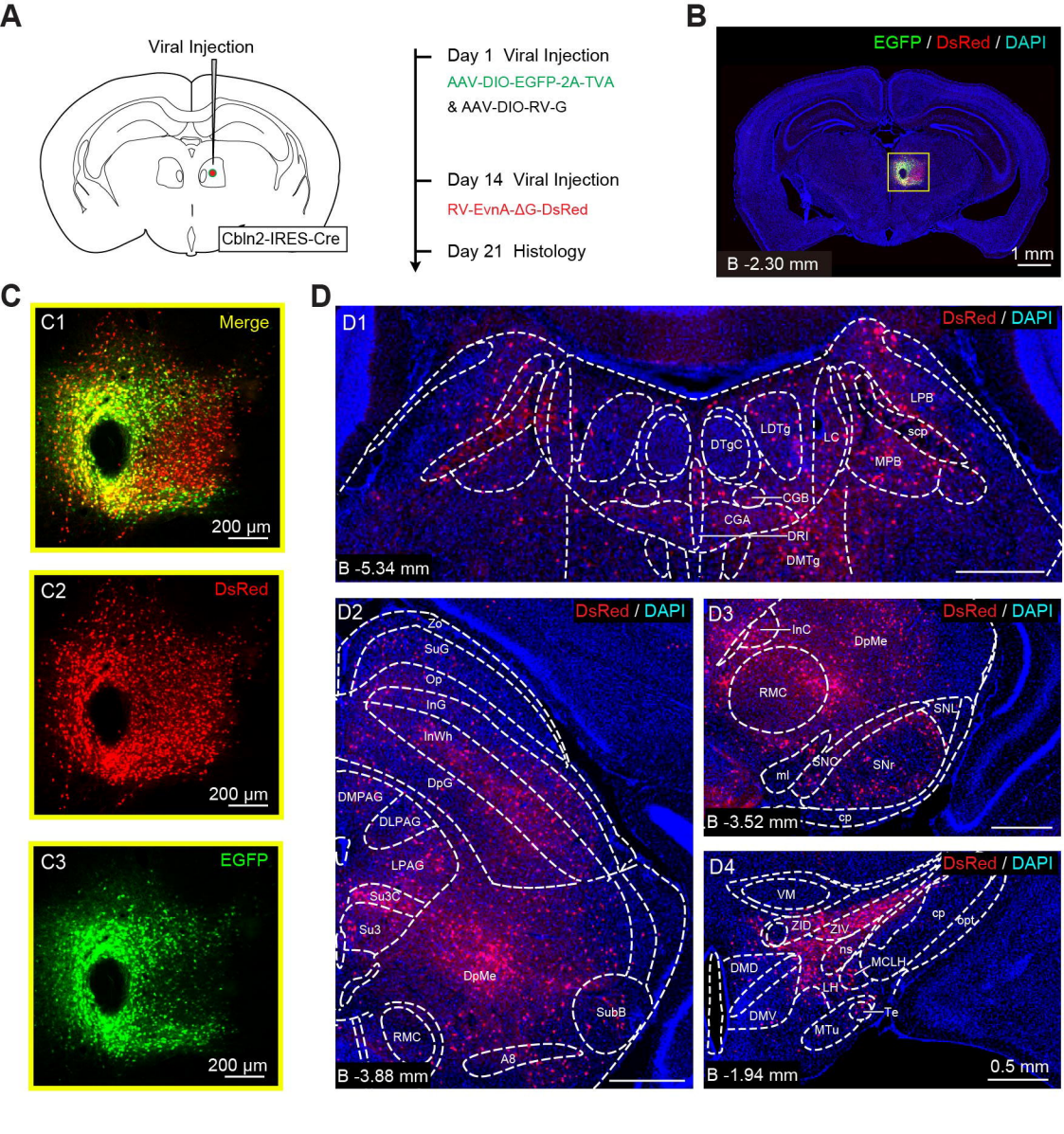
1077

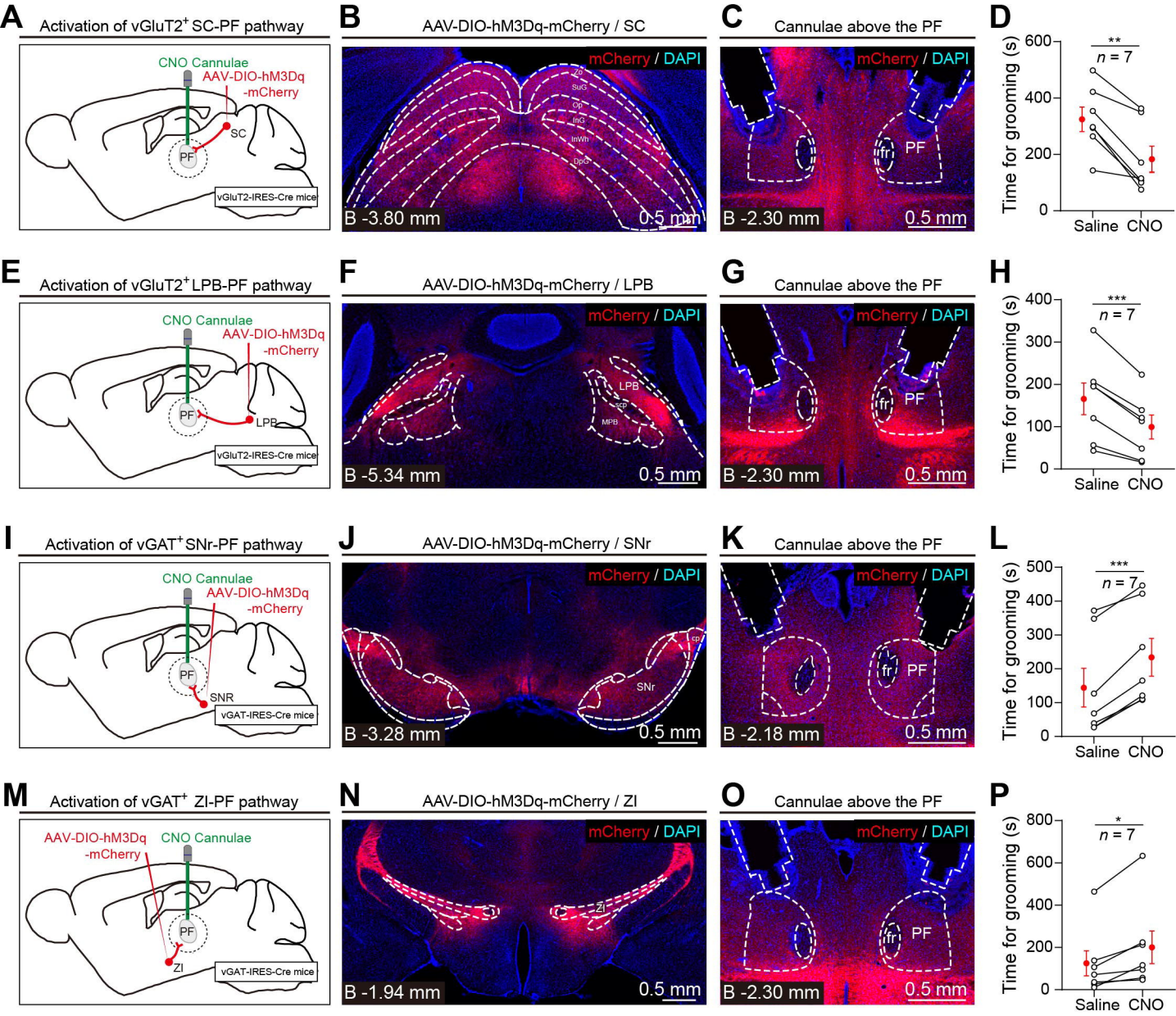
1078

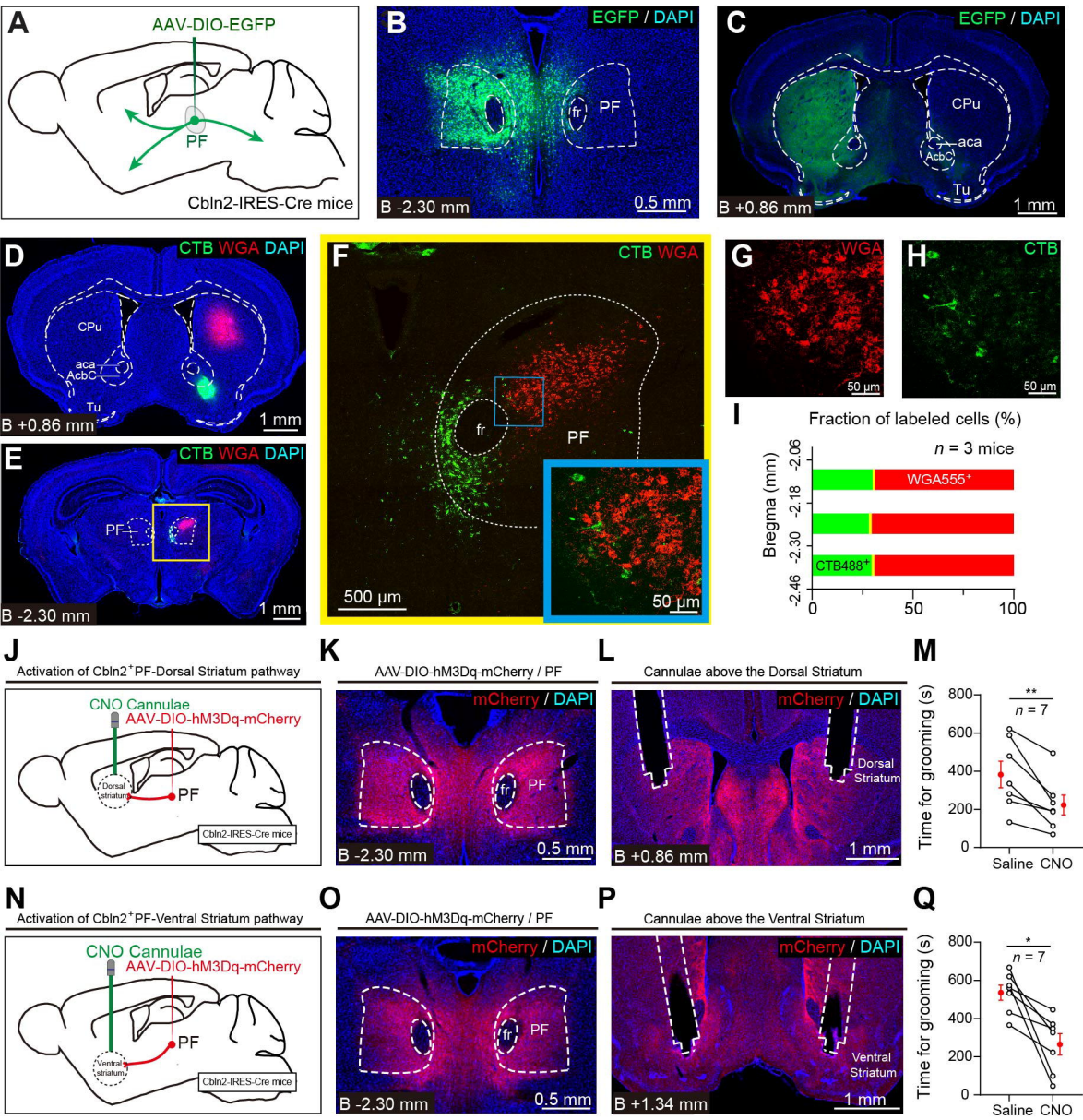
1079 **sFIG.6 Ultrasound stimulation targeting PF reduces time spent for total grooming in**
1080 **WT mice**
1081 (**A**) Schematic diagram showing the schedule for investigation of the effect of ultrasound
1082 stimulation on time spent for spontaneous grooming of WT mice. (**B**) Quantitative analyses
1083 of time spent for spontaneous grooming of WT mice on each day.
1084 Data in (**B**) are means ± SEM (error bars). Statistical analyses in (**B**) were performed using
1085 Student's t-test (*$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$).
1086

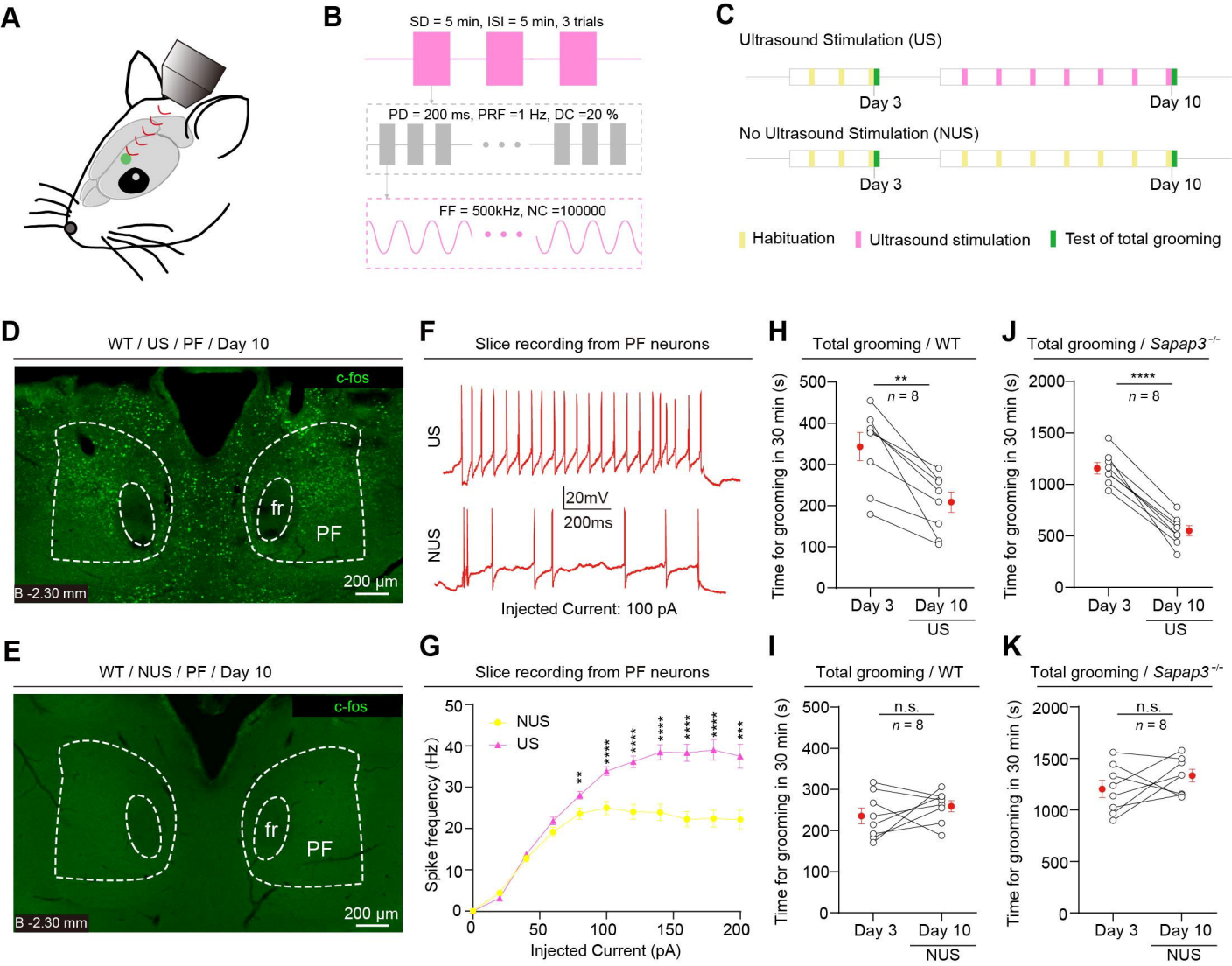

A
Monitoring self-grooming in the magnetic induction system
Camera
Amplifier
Magnetic coil
1401
B
Example traces of magnetic inducing voltage signals
Stationary
Locomotion
Paw-licking
Orofacial grooming
Body grooming
5 mV
500 ms
C
Cbln2-IRES-Cre / AAV-DIO-ChR2-mCherry / PF
mCherry / DAPI
Fiber
fr
PF
B -2.20 mm
200 μm
D
Actue brain slice with PF
Recording
ChR2+
10 Hz
20 mV
300 ms
20 Hz
E
Paw-licking
F
Orofacial grooming
G
Body grooming
H
oil-induced self-grooming
Time for grooming in 10 s (s)
n.s.
**
****
***
OFF
ON
EGFP
ChR2
I
Cbln2-IRES-Cre / AAV-DIO-hM3Dq-mCherry / PF
B -2.30 mm
J
Slice recording from hM3Dq-mCherry+ cells
15 mV
60 s
CNO (10 μM)
n = 9 cells
Spike frequency (Hz)
Before
After
K
Cbln2-IRES-Cre / AAV-DIO-hM4Di-mCherry / PF
L
Slice recording from hM4Di-mCherry+ cells
CNO(10μM)
n = 16 cells
M
Spontaneous self-grooming
Time for grooming in 30 min (s)
Saline
CNO
Ctrl
hM3Dq
hM4Di
N
Oil-induced self-grooming
O
Pathological grooming / Sapap3-/-

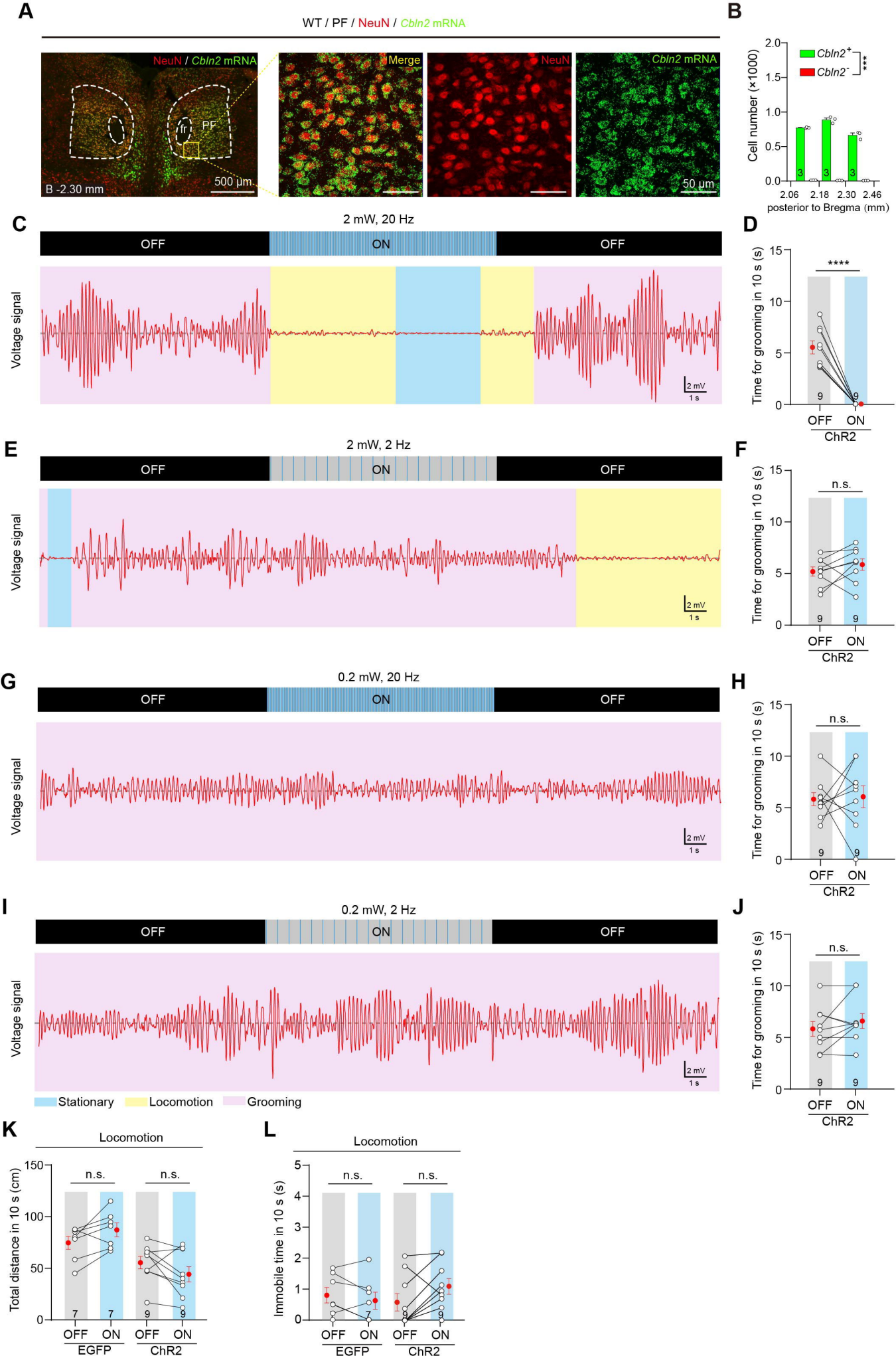

A
AAV-DIO-GCaMP7s/Cbln2-IRES-Cre
GCaMP / DAPI
Fiber
fr
PF
B -2.30 mm
200 μm
B
Voltage signal and GCaMP signal
Locomotion
Grooming
Voltage signal
GCaMP signal
10 mV
5 s
ΔF/F
10%
5 s
C
Correlation of grooming duration and ΔF/F
y = 0.0023x + 0.1287
R² = 0.3305
GCaMP ΔF(peak to valley)/Fpeak (%)
Grooming duration (s)
D
Grooming
EGFP
GCaMP
Aligned to the beginning of grooming (s)
E
EGFP
GCaMP
ΔF/F (%)
Aligned to the beginning of grooming (s)
F
****
ΔF/F (%)
EGFP GCaMP
G
Relative frequency (%)
Distribution of the time point at which the GCaMP signal begin to decrease
H
Grooming
EGFP
GCaMP
Aligned to the termination of grooming (s)
I
EGFP
GCaMP
ΔF/F (%)
Aligned to the termination of grooming (s)
J
****
ΔF/F (%)
EGFP GCaMP
K
Relative frequency (%)
Distribution of the time point at which the GCaMP signal begin to increase
L
Miniscope
M
AAV-DIO-jGCaMP7s
Cbln2-IRES-Cre
N
Miniscope imaging / Cbln2-IRES-Cre / Field of view in the PF
GCaMP
Lens
fr
PF
B -2.30 mm
200 μm
O
Initial stage of grooming
Cells sorted by responsive time
Grooming
Type1
Type2
Type3
ΔF/F(%)
Aligned to the beginning of grooming (s)
P
Grooming
ΔF/F(%)
Aligned to the beginning of grooming (s)
Type1
Type2
Type3
17.31%
7.69%
75.00%
Total=156
Q
Terminate stage of grooming
Cells sorted by responsive time
Grooming
Type1
Type2
Type3
ΔF/F(%)
Aligned to the termination of grooming (s)
R
Grooming
ΔF/F(%)
Aligned to the termination of grooming (s)
Type1
Type2
Type3
16.03%
13.46%
70.51%
Total=156

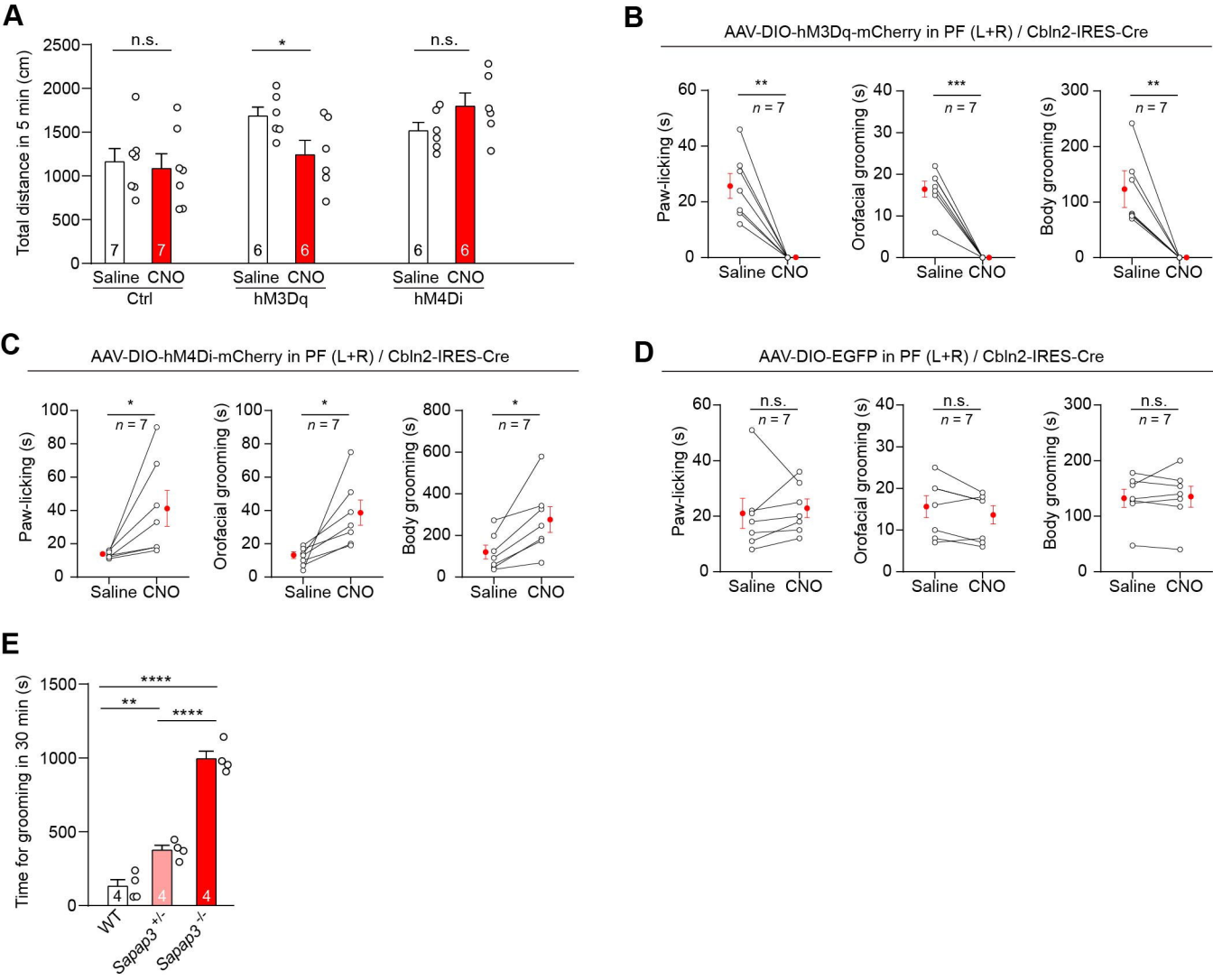

A
Viral Injection
Cbln2-IRES-Cre
Day 1 Viral Injection
AAV-DIO-EGFP-2A-TVA
& AAV-DIO-RV-G
Day 14 Viral Injection
RV-EvnA-ΔG-DsRed
Day 21 Histology
B
EGFP / DsRed / DAPI
B -2.30 mm
1 mm
C
C1
Merge
200 μm
C2
DsRed
200 μm
C3
EGFP
200 μm
D
D1
DsRed / DAPI
LPB
scp
LDTg
DTgC
LC
MPB
CGB
CGA
DRI
DMTg
B -5.34 mm
D2
DsRed / DAPI
Zo
SuG
Op
InG
InWh
DpG
DMPAG
DLPAG
LPAG
Su3C
Su3
DpMe
SubB
RMC
A8
B -3.88 mm
D3
DsRed / DAPI
InC
DpMe
RMC
SNL
SNC
SNr
ml
cp
B -3.52 mm
D4
DsRed / DAPI
VM
ZID
ZIV
cp
opt
ns
MCLH
DMD
LH
DMV
Te
MTu
B -1.94 mm
0.5 mm
E
Posterior
Anterior
n = 4 mice
Fraction of total cells (%)
25
20
15
10
5
0
Sp5I
Pnc
LPB
DRD
DRV
PnO
SC
DpMe
SNr
VTA
ZI
MHB
LHB
LH
HDB
M2
M1
S1FL

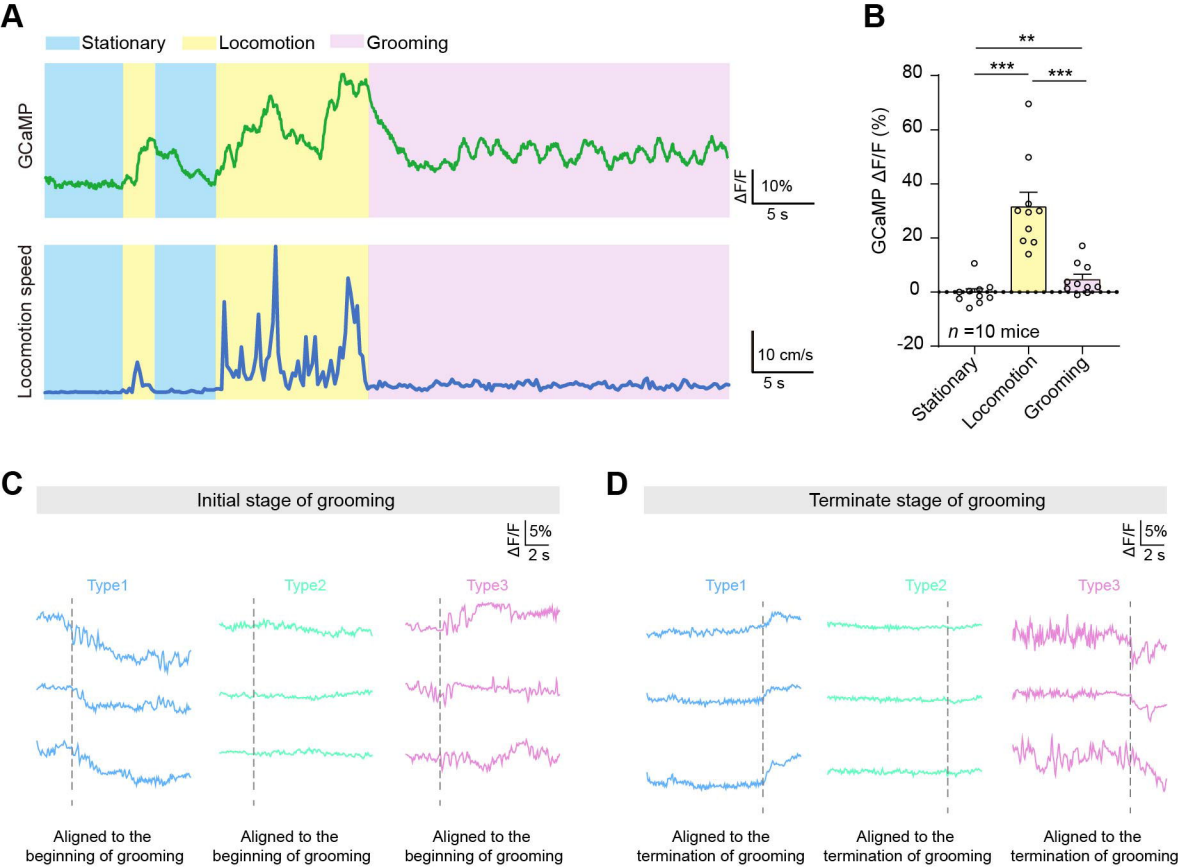

A
Activation of vGluT2+ SC-PF pathway
CNO Cannulae
AAV-DIO-hM3Dq -mCherry
SC
PF
vGluT2-IRES-Cre mice
B
AAV-DIO-hM3Dq-mCherry / SC
mCherry / DAPI
Zo
SuG
Op
InG
InWh
OpC
B -3.80 mm
0.5 mm
C
Cannulae above the PF
mCherry / DAPI
fr
PF
B -2.30 mm
0.5 mm
D
Time for grooming (s)
**
n = 7
Saline
CNO
E
Activation of vGluT2+ LPB-PF pathway
CNO Cannulae
AAV-DIO-hM3Dq -mCherry
PF
LPB
vGluT2-IRES-Cre mice
F
AAV-DIO-hM3Dq-mCherry / LPB
mCherry / DAPI
LPB
scp
MPB
B -5.34 mm
0.5 mm
G
Cannulae above the PF
mCherry / DAPI
fr
PF
B -2.30 mm
0.5 mm
H
Time for grooming (s)
***
n = 7
Saline
CNO
I
Activation of vGAT+ SNr-PF pathway
CNO Cannulae
AAV-DIO-hM3Dq -mCherry
PF
SNR
vGAT-IRES-Cre mice
J
AAV-DIO-hM3Dq-mCherry / SNr
mCherry / DAPI
cp
SNr
B -3.28 mm
0.5 mm
K
Cannulae above the PF
mCherry / DAPI
fr
PF
B -2.18 mm
0.5 mm
L
Time for grooming (s)
***
n = 7
Saline
CNO
M
Activation of vGAT+ ZI-PF pathway
AAV-DIO-hM3Dq -mCherry
CNO Cannulae
PF
ZI
vGAT-IRES-Cre mice
N
AAV-DIO-hM3Dq-mCherry / ZI
mCherry / DAPI
ZI
B -1.94 mm
0.5 mm
O
Cannulae above the PF
mCherry / DAPI
fr
PF
B -2.30 mm
0.5 mm
P
Time for grooming (s)
*
n = 7
Saline
CNO

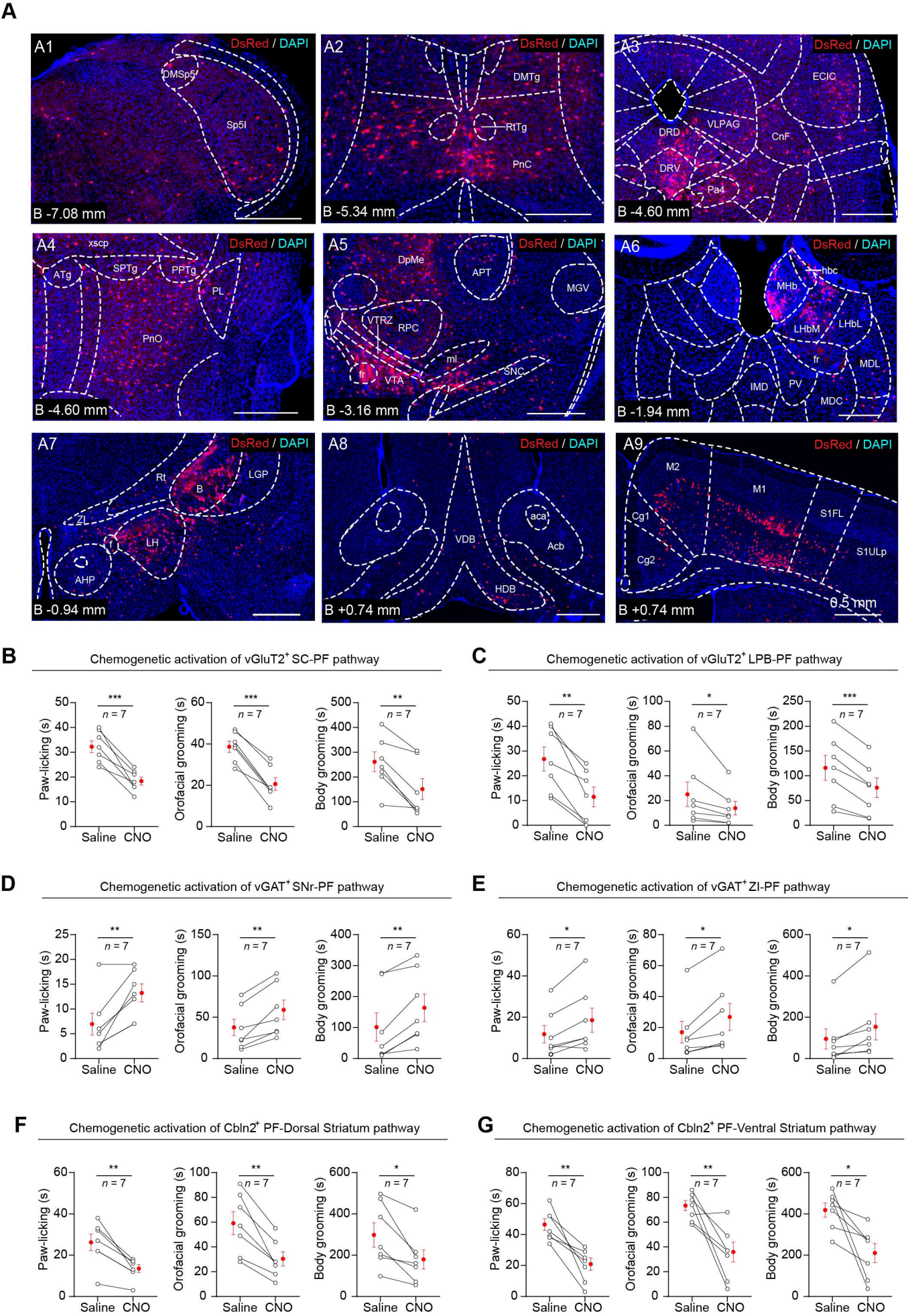

A
AAV-DIO-EGFP
PF
Cbln2-IRES-Cre mice
B
EGFP / DAPI
fr
PF
B -2.30 mm
0.5 mm
C
EGFP / DAPI
CPu
aca
AcbC
Tu
B +0.86 mm
1 mm
D
CTB WGA DAPI
CPu
aca
AcbC
Tu
B +0.86 mm
1 mm
E
CTB WGA DAPI
PF
B -2.30 mm
1 mm
F
CTB WGA
fr
PF
500 µm
50 µm
G
WGA
50 µm
H
CTB
50 µm
I
Fraction of labeled cells (%)
n = 3 mice
Bregma (mm)
-2.06
-2.18
-2.30
-2.46
WGA555+
CTB488+
0
50
100
J
Activation of Cbln2+PF-Dorsal Striatum pathway
CNO Cannulae
AAV-DIO-hM3Dq-mCherry
Dorsal striatum
PF
Cbln2-IRES-Cre mice
K
AAV-DIO-hM3Dq-mCherry / PF
mCherry / DAPI
fr
PF
B -2.30 mm
0.5 mm
L
Cannulae above the Dorsal Striatum
mCherry / DAPI
Dorsal Striatum
B +0.86 mm
1 mm
M
Time for grooming (s)
800
600
400
200
0
**
n = 7
Saline
CNO
N
Activation of Cbln2+PF-Ventral Striatum pathway
CNO Cannulae
AAV-DIO-hM3Dq-mCherry
PF
Ventral striatum
Cbln2-IRES-Cre mice
O
AAV-DIO-hM3Dq-mCherry / PF
mCherry / DAPI
fr
PF
B -2.30 mm
0.5 mm
P
Cannulae above the Ventral Striatum
mCherry / DAPI
Ventral Striatum
B +1.34 mm
1 mm
Q
Time for grooming (s)
800
600
400
200
0
*
n = 7
Saline
CNO

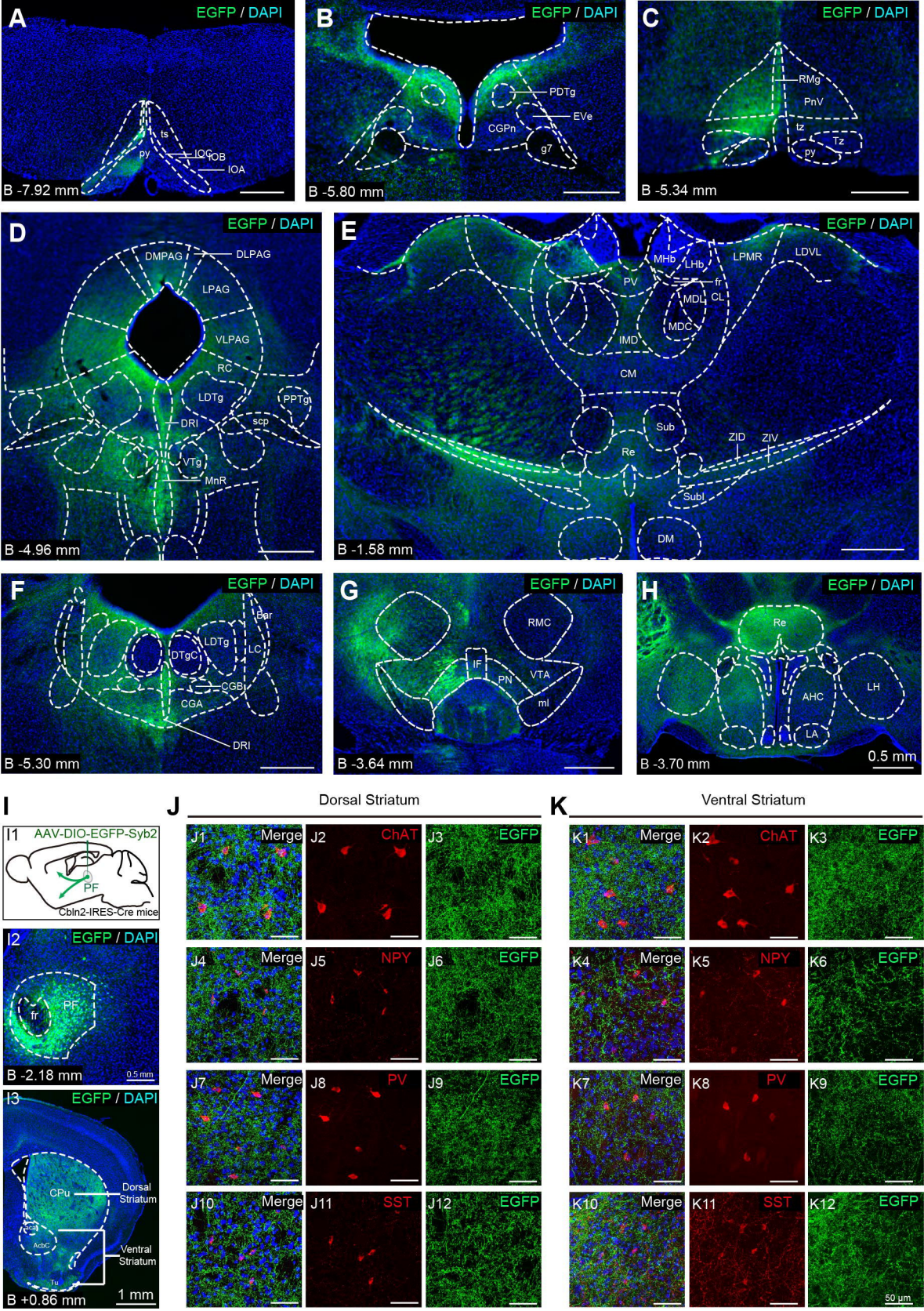

A
B
SD = 5 min, ISI = 5 min, 3 trials
PD = 200 ms, PRF =1 Hz, DC =20 %
FF = 500kHz, NC =100000
C
Ultrasound Stimulation (US)
Day 3
Day 10
No Ultrasound Stimulation (NUS)
Day 3
Day 10
Habituation
Ultrasound stimulation
Test of total grooming
D
WT / US / PF / Day 10
c-fos
fr
PF
B -2.30 mm
200 µm
E
WT / NUS / PF / Day 10
c-fos
fr
PF
B -2.30 mm
200 µm
F
Slice recording from PF neurons
US
NUS
20mV
200ms
Injected Current: 100 pA
G
Slice recording from PF neurons
NUS
US
Spike frequency (Hz)
Injected Current (pA)
H
Total grooming / WT
Time for grooming in 30 min (s)
**
n = 8
Day 3
Day 10
US
I
Total grooming / WT
Time for grooming in 30 min (s)
n.s.
n = 8
Day 3
Day 10
NUS
J
Total grooming / Sapap3-/-
Time for grooming in 30 min (s)
****
n = 8
Day 3
Day 10
US
K
Total grooming / Sapap3-/-
Time for grooming in 30 min (s)
n.s.
n = 8
Day 3
Day 10
NUS

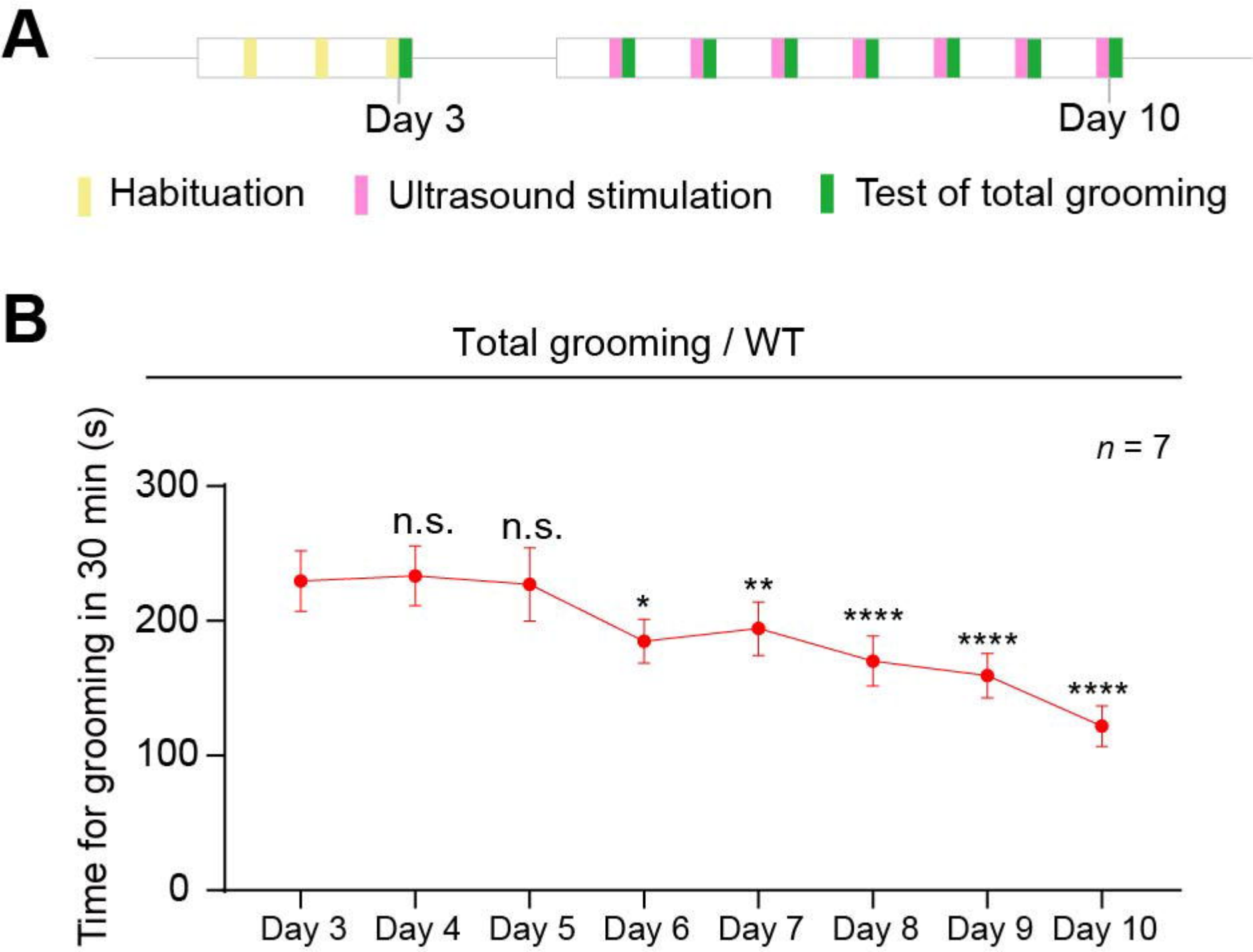

A
WT / PF / NeuN / Cbln2 mRNA
NeuN / Cbln2 mRNA
PF
fr
B -2.30 mm
500 μm
Merge
NeuN
Cbln2 mRNA
50 μm
B
Cbln2+
Cbln2-
***
Cell number (×1000)
posterior to Bregma (mm)
C
2 mW, 20 Hz
OFF
ON
OFF
Voltage signal
2 mV
1 s
D
****
Time for grooming in 10 s (s)
OFF
ON
ChR2
E
2 mW, 2 Hz
F
n.s.
G
0.2 mW, 20 Hz
H
I
0.2 mW, 2 Hz
J
Stationary
Locomotion
Grooming
K
Locomotion
Total distance in 10 s (cm)
EGFP
L
Immobile time in 10 s (s)


A
Total distance in 5 min (cm)
n.s.
*
n.s.
Saline CNO
Ctrl
hM3Dq
hM4Di
B
AAV-DIO-hM3Dq-mCherry in PF (L+R) / Cbln2-IRES-Cre
Paw-licking (s)
Orofacial grooming (s)
Body grooming (s)
n = 7
C
AAV-DIO-hM4Di-mCherry in PF (L+R) / Cbln2-IRES-Cre
D
AAV-DIO-EGFP in PF (L+R) / Cbln2-IRES-Cre
E
Time for grooming in 30 min (s)
WT
Sapap3 +/-
Sapap3 -/-


A
Stationary
Locomotion
Grooming
GCaMP
ΔF/F
10%
5 s
Locomotion speed
10 cm/s
5 s
B
**
***
***
GCaMP ΔF/F (%)
80
60
40
20
0
-20
n =10 mice
Stationary
Locomotion
Grooming
C
Initial stage of grooming
ΔF/F
5%
2 s
Type1
Type2
Type3
Aligned to the beginning of grooming
Aligned to the beginning of grooming
Aligned to the beginning of grooming
D
Terminate stage of grooming
ΔF/F
5%
2 s
Type1
Type2
Type3
Aligned to the termination of grooming
Aligned to the termination of grooming
Aligned to the termination of grooming


A
A1
DsRed / DAPI
DMSp5
Sp5I
B -7.08 mm
A2
DMTg
RtTg
PnC
B -5.34 mm
A3
ECIC
DRD
VLPAG
CnF
DRV
Pa4
B -4.60 mm
A4
xscp
ATg
SPTg
PPTg
PL
PnO
B -4.60 mm
A5
DpMe
APT
MGV
VTRZ
RPC
ml
fr
VTA
SNC
B -3.16 mm
A6
hbc
MHb
LHbM
LHbL
fr
MDL
IMD
PV
MDC
B -1.94 mm
A7
Rt
B
LGP
ZL
LH
AHP
B -0.94 mm
A8
aca
VDB
Acb
HDB
B +0.74 mm
A9
M2
M1
Cg1
S1FL
S1ULp
Cg2
0.5 mm
B +0.74 mm
B
Chemogenetic activation of vGluT2+ SC-PF pathway
Paw-licking (s)
Orofacial grooming (s)
Body grooming (s)
n = 7
Saline
CNO
C
Chemogenetic activation of vGluT2+ LPB-PF pathway
D
Chemogenetic activation of vGAT+ SNr-PF pathway
E
Chemogenetic activation of vGAT+ ZI-PF pathway
F
Chemogenetic activation of Cbln2+ PF-Dorsal Striatum pathway
G
Chemogenetic activation of Cbln2+ PF-Ventral Striatum pathway


A
EGFP / DAPI
ts
py
IOC
IOB
IOA
B -7.92 mm
B
EGFP / DAPI
PDTg
EVe
CGPn
g7
B -5.80 mm
C
EGFP / DAPI
RMg
PnV
tz
Tz
py
B -5.34 mm
D
EGFP / DAPI
DMPAG
DLPAG
LPAG
VLPAG
RC
LDTg
PPTg
DRI
scp
VTg
MnR
B -4.96 mm
E
EGFP / DAPI
MHb
LHb
LPMR
LDVL
PV
fr
MDL
CL
MDC
IMD
CM
Sub
Re
ZID
ZIV
SubI
DM
B -1.58 mm
F
EGFP / DAPI
Bar
LDTg
DTgC
LC
CGB
CGA
DRI
B -5.30 mm
G
EGFP / DAPI
RMC
IF
PN
VTA
ml
B -3.64 mm
H
EGFP / DAPI
Re
AHC
LH
LA
B -3.70 mm
0.5 mm
I
I1
AAV-DIO-EGFP-Syb2
PF
Cbln2-IRES-Cre mice
I2
EGFP / DAPI
fr
PF
B -2.18 mm
0.5 mm
I3
EGFP / DAPI
CPu
Dorsal Striatum
AcbC
Ventral Striatum
Tu
B +0.86 mm
1 mm
J
Dorsal Striatum
J1 Merge
J2 ChAT
J3 EGFP
J4 Merge
J5 NPY
J6 EGFP
J7 Merge
J8 PV
J9 EGFP
J10 Merge
J11 SST
J12 EGFP
K
Ventral Striatum
K1 Merge
K2 ChAT
K3 EGFP
K4 Merge
K5 NPY
K6 EGFP
K7 Merge
K8 PV
K9 EGFP
K10 Merge
K11 SST
K12 EGFP
50 μm


A
Day 3
Day 10
Habituation
Ultrasound stimulation
Test of total grooming
B
Total grooming / WT
n = 7
Time for grooming in 30 min (s)
300
200
100
0
n.s.
n.s.
*
**
****
****
****
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10