The differentiation and integration of the hippocampal dorsoventral axis are controlled by two nuclear receptor genes

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Abstract

The hippocampus executes crucial functions from declarative memory to adaptive behaviors associated with cognition and emotion. However, the mechanisms of how morphogenesis and functions along the hippocampal dorsoventral axis are differentiated and integrated are still largely unclear. Here, we show that *COUP-TFI* and *-TFII* genes are distinctively expressed in the dorsal and ventral hippocampus, respectively. The loss of *COUP-TFII* results in ectopic CA1/CA3 domains in the ventral hippocampus. The deficiency of *COUP-TFI* leads to the failed specification of dorsal CA1, among which there are place cells. The deletion of both *COUP-TF* genes causes almost agenesis of the hippocampus with abnormalities of trisynaptic circuit and adult neurogenesis. Moreover, *COUP-TFI*/*-TFII* may cooperate to guarantee appropriate morphogenesis and function of the hippocampus by regulating the *Lhx5-Lhx2* axis. Our findings revealed a novel mechanism that *COUP-TFI* and *COUP-TFII* converge to govern the differentiation and integration of distinct characteristics of the hippocampus in mice.

Keywords

Memory; Hippocampus; *COUP-TF* Gene; Place Cell; Trisynaptic Circuit

36 **Introduction**

37 Memory, including declarative and nondeclarative memory, unifies our mental world to
38 ensure the quality of life for people of all ages, from newborns to elderly individuals
39 (Eichenbaum & Cohen, 2014; Kandel, Dudai, & Mayford, 2014). The pioneering studies of
40 Milner and her colleagues revealed that the hippocampus is required for declarative memory
41 but not nondeclarative memory (Penfield & Milner, 1958; Scoville & Milner, 1957). The
42 discovery of activity-dependent long-term potentiation and place cells provides the
43 neurophysiological basis of hippocampal function (Bliss & Lomo, 1973; O'Keefe &
44 Dostrovsky, 1971). The rodent hippocampus can be divided into the dorsal and ventral
45 domains, corresponding to the posterior and anterior hippocampus in humans, respectively.
46 In recent decades, numerous studies have supported the Moser theory that the hippocampus is
47 a heterogeneous structure with distinct characteristics of gene expression, connectivity, and
48 functions along its dorsoventral axis (Bast, 2007; Fanselow & Dong, 2010; Moser & Moser,
49 1998; Strange, Witter, Lein, & Moser, 2014). The dorsal hippocampus, which connects and
50 shares similar gene expression with the neocortex (Fanselow & Dong, 2010), serves the "cold"
51 cognitive function associated with declarative memory and spatial navigation. The ventral
52 hippocampus, which connects and generates similar gene expression with the amygdala and
53 hypothalamus (Cenquizca & Swanson, 2007; Kishi et al., 2000; Pitkanen, Pikkarainen,
54 Nurminen, & Ylinen, 2000), corresponds to the "hot" affective states related to emotion and
55 anxiety (Fanselow & Dong, 2010; Tyng, Amin, Saad, & Malik, 2017). Nonetheless, to date,
56 the molecular and cellular mechanisms by which the morphogenesis, connectivity, and
57 functions along the dorsoventral axis of the hippocampus are differentiated and integrated are
58 largely unknown.

59 The hippocampus, a medial temporal lobe structure in the adult rodent forebrain, originates
60 from the medial pallium (MP) in the medial line of the early dorsal telencephalon. The

cortical hem (CH), which is located ventrally to the MP, functions as an organizer for hippocampal development (Hébert & Fishell, 2008; Schuurmans & Guillemot, 2002). It has been demonstrated that both extrinsic signals, such as *Wnts* and *Bmps*, and intrinsic factors, including *Emx1*, *Emx2*, *Lef1*, *Lhx2*, and *Lhx5*, are involved in the regulation of early morphogenesis of the hippocampus. As the earliest *Wnt* gene to be exclusively expressed in the cortical hem, *Wnt3a* is required for the genesis of the hippocampus (S. M. Lee, Tole, Grove, & McMahon, 2000); in addition, *Lef1* is downstream of Wnt signaling, and the hippocampus is completely absent in *Lef1-neo/neo* null mutant mice (Galceran, Miyashita-Lin, Devaney, Rubenstein, & Grosschedl, 2000). Wnt signaling is essential for early development of the hippocampus. *Emx1* and *Emx2* are mouse homologs of *Drosophila empty spiracles* (Simeone et al., 1992). Interestingly, the dorsal hippocampus is smaller in an *Emx1* null mutant (Yoshida et al., 1997), while *Emx2* is required for the growth of the hippocampus but not for the specification of hippocampal lineages (Tole, Goudreau, Assimacopoulos, & Grove, 2000). Moreover, *Lhx5*, which encodes a LIM homeobox transcription factor and is specifically expressed in the hippocampal primordium, is necessary for the formation of the hippocampus (Zhao et al., 1999). *Lhx2*, encoding another LIM homeobox transcription factor, is required for the development of both the hippocampus and neocortex (Mangale et al., 2008; Monuki, Porter, & Walsh, 2001; Porter et al., 1997). Intriguingly, deficiency of either *Lhx5* or *Lhx2* results in agenesis of the hippocampus, and more particularly, these genes inhibit each other (Hébert & Fishell, 2008; Mangale et al., 2008; Roy, Gonzalez-Gomez, Pierani, Meyer, & Tole, 2014; Zhao et al., 1999), indicating that the *Lhx5* and *Lhx2* genes may generate an essential regulatory axis to ensure the appropriate hippocampal development. Nevertheless, whether there are other intrinsic genes that participate in the regulation of morphogenesis and function of the hippocampus has not been fully elucidated.

COUP-TF genes, including *COUP-TFI* (also known as *NR2F1*) and *COUP-TFII* (also known as *NR2F2*), encode two transcription factor proteins belonging to the nuclear receptor superfamily (Yang, Feng, & Tang, 2017). Mutations of *COUP-TFI* are highly related to neurodevelopmental disorders (NDD), such as intellectual disability (ID) and autism spectrum disorders (ASD) (Bertacchi et al., 2020; Bosch et al., 2014; Contesse, Ayrault, Mantegazza, Studer, & Deschaux, 2019), and mutations of the *COUP-TFII* gene are associated with congenital heart defects (CHD) (High et al., 2016). By using animal models, our studies and others have demonstrated that *COUP-TF* genes participate in the regulation of the development of the central nervous system (Zhang et al., 2020). The *COUP-TFI* plays an essential role in the differentiation of cortical excitatory projection neurons and inhibitory interneurons, the development of the dorsal hippocampus, and cortical arealization (Armentano et al., 2007; Bertacchi et al., 2020; Del Pino et al., 2020; J. Feng et al., 2021; Flore et al., 2017; Lodato et al., 2011; C. Zhou et al., 1999; C. Zhou, Tsai, & Tsai, 2001). *COUP-TFII* plays a vital role in the development of the amygdala, hypothalamus, and cerebellum (S. Feng et al., 2017; Kim, Takamoto, Yan, Tsai, & Tsai, 2009; Tang, Rubenstein, Tsai, & Tsai, 2012). Nevertheless, whether and how *COUP-TFI* and/or *COUP-TFII* genes regulate the differentiation and integration of hippocampal morphogenesis, connectivity, and function is still largely unclear.

Here, our data show that *COUP-TFI* and *-TFII* genes are differentially expressed along the dorsoventral axis of the postnatal hippocampus. The loss of *COUP-TFII* results in ectopic CA1 and CA3 domains in the ventral hippocampus. In addition, the deficiency of *COUP-TFI* leads to not only dysplasia of the dorsal hippocampus but also failed specification and differentiation of the dorsal CA1 pyramidal neuron lineage. Furthermore, the deletion of both genes in the *RXCre*/+; *COUP-TFI*$^{F/F}$; *COUP-TFII*$^{F/F}$ double mutant mouse causes almost agenesis of the hippocampus, accompanied by compromised specification of the CA1, CA3,

and dentate gyrus (DG) domains. The components of the trisynaptic circuit are abnormal in the corresponding single-gene or double-gene mutant model. Moreover, *COUP-TF* genes may cooperate to guarantee the appropriate morphogenesis and function of the hippocampus by regulating the *Lhx5-Lhx2* axis.

Results

Differential expression profiles of *COUP-TFI* and -*TFII* genes along the dorsoventral axis in the developing and postnatal hippocampus

To investigate the functions of the *COUP-TFI* and *COUP-TFII* genes in the hippocampus, immunofluorescence staining was first performed to examine their expression in wild-type mice at postnatal month 1 (1M). In both the coronal and sagittal sections, COUP-TFI exhibited a septal/dorsal high-temporal/ventral low expression pattern along the hippocampus (Figure 1Aa, d, c, f, g, j, m, i, l, and o), and its expression is highest in the dorsal CA1 region (Figure 1Aa, d, c, f, j, and l), where place cells are mainly located (O'Keefe & Conway, 1978; O'Keefe & Dostrovsky, 1971), and the dorsal DG, where there are adult neural stem cells (NSCs) (Gould & Cameron, 1996). However, the expression of COUP-TFII was high in the temporal/ventral hippocampus but was barely detected in the septal/dorsal part of the hippocampus (Figure 1Ab, c, e, f, h, i, k, l, n, and o). The dorsal-high COUP-TFI and ventral-high COUP-TFII expression profiles were further verified in the postnatal hippocampi at 1M by western blotting assays (Figure 1Ap, q). At embryonic day 10.5 (E10.5), COUP-TFI was detected in the dorsal pallium (DP) laterally and COUP-TFII was expressed in the MP and CH medially (Figure 1—figure supplement 1Aa-b). At E11.5 and E12.5, the expression of COUP-TFII remained in the CH (Figure 1—figure supplement 1Ac-d, Bb-c). Interestingly, COUP-TFI and -TFII generated complementary expression patterns in the hippocampal primordium with COUP-TFI in the dorsal MP and COUP-TFII in the ventral CH at E14.5

135 (Figure 1—figure supplement 1Ba-f). Additionally, septal/dorsal-high COUP-TFI and
136 temporal/ventral-high COUP-TFII expression patterns were observed at postnatal day 0 (P0)
137 (Figure 1—figure supplement 1Bg-l). The data above revealed that the differential expression
138 patterns of *COUP-TFI* and *-TFII* genes were generated and maintained along the
139 dorsoventral axis in the early hippocampal primordium, the developing and postnatal
140 hippocampus, indicating that they could play distinct roles in the mediation of the
141 morphogenesis and functions of the hippocampus.

142 Next, to investigate the roles of *COUP-TF* genes in the hippocampus, an *RXCre* mouse was
143 used to excise the expression of the *COUP-TFI* and/or *COUP-TFII* genes (Swindell et al.,
144 2006; Tang et al., 2012). The deletion efficiency of RXCre recombinase was verified by
145 immunofluorescence assays. Compared with control mice, either *COUP-TFII* or *COUP-TFI*
146 could be excised in the postnatal hippocampus of corresponding single-gene mutants at 1M
147 (Figure 1—figure supplement 1Ca-i). In addition, compared with control mice, both the
148 *COUP-TFI* and *-TFII* genes were almost completely deleted in the hippocampal primordium
149 in mutant mice at E14.5 (Figure 1—figure supplement 1Cj-o). Since the LacZ expression
150 serves as an indicator for the deletion of *COUP-TFII* (Swindell et al., 2006; Tang et al.,
151 2012), we performed immunofluorescence staining with antibodies against COUP-TFII and
152 LacZ on the sagittal sections of *RXCre/+; COUP-TFII*$^{F/+}$ and *RXCre/+; COUP-TFII*$^{F/F}$ mice
153 at E11.5. COUP-TFII was readily detected at the hippocampal primordium of the
154 heterozygous mutant embryo at E11.5 (Figure 1—figure supplement 1Da, c, g); in contrast,
155 the expression of *COUP-TFII* was significantly reduced in the homozygous mutant (Figure
156 1—figure supplement 1Dd, f, j). In addition, compared with the heterozygous mutant embryo
157 (Figure 1—figure supplement 1Db-c, h), the LacZ signals clearly increased in the
158 hippocampal primordium of the homozygous mutant embryo at E11.5 (Figure 1—figure
159 supplement 1De-f, k), suggesting that RXCre recombinase can efficiently excise the *COUP-*

160 *TFII* gene in the hippocampal primordium as early as E11.5. Intriguingly, we observed that
161 the expression of *COUP-TFI* increased in the caudal hippocampal primordium of the *COUP-*
162 *TFII* homozygous mutant embryo at E11.5 (Figure 1—figure supplement 1Di, l), indicating
163 that similar to the observations in the early optic cup (Tang et al., 2010), *COUP-TFI* and -
164 *TFII* genes could be partially compensate with each other in the developing hippocampal
165 primordium. All the data above show that RXCre recombinase could efficiently excise
166 *COUP-TFI* and/or *COUP-TFII* in the early developing and postnatal hippocampus.
167

168 **The *COUP-TFII* gene is required for the appropriate morphogenesis of the ventral**
169 **hippocampus but not of the dorsal hippocampus**

170 Given that the *COUP-TFII* gene is highly and specifically expressed in the postnatal ventral
171 hippocampus and the CH of the hippocampal primordium (Figure 1 and Figure 1—figure
172 supplement 1), we asked whether the *COUP-TFII* gene is required for the appropriate
173 morphogenesis of the hippocampus, particularly the ventral hippocampus. To answer this
174 question, we conducted Nissl staining with samples from the *COUP-TFII* single-gene
175 (*RXCre/+*; *COUP-TFII*$^{F/F}$ or *RXCre/+*; *CII*$^{F/F}$) knockout mouse model. In coronal sections,
176 compared with the control at 1M, the septal hippocampus was normal; unexpectedly, an
177 ectopic CA-like region was observed medially in the temporal hippocampus in the *COUP-*
178 *TFII* mutant, where the prospective posterior part of the medial amygdaloid (MeP) nucleus
179 was situated, indicated by the star (Figure 1Ba-f). The presence of the ectopic CA-like region
180 in the ventral but not dorsal hippocampus of the mutant was further confirmed by the
181 presence of the prospective MeP and amygdalohippocampal area (AHi) in sagittal sections,
182 as indicated by the star (Figure 1Bg-l). Furthermore, immunofluorescence assays were
183 performed to verify whether specific lineages of the hippocampus were altered with sagittal
184 sections, in which the subregions of both the dorsal and ventral hippocampus were well

185 displayed and distinguished. Ctip2 is a marker for CA1 pyramidal neurons and DG granule
186 neurons, and HuB is a marker for CA3 pyramidal cells (Sugiyama, Osumi, & Katsuyama,
187 2014). The dorsal hippocampus appeared normal in both the control and mutant mice at 1M
188 (Figure 1Ca-l). Nonetheless, compared with the observations in control mice, an ectopic
189 HuB-positive CA3 pyramidal neuron lineage, indicated by the star, and a duplicated Ctip2-
190 positive CA1 pyramidal neuron lineage, indicated by the arrowhead, were observed in the
191 ventral hippocampal area in the mutant (Figure 1Ca-f, m-r), revealing that there were ectopic
192 CA1 and CA3 lineages in the *COUP-TFII* mutants.
193 Consistent with the previous report (Leid et al., 2004), the expression of Ctip2 was detected
194 in the amygdala including the AHi and posteromedial cortical amygdaloid nucleus (PMCo);
195 in addition, Ctip2 was also highly expressed in the dorsal part of the MeP (MePD) in the
196 control (Figure 1Cb, n).
197 Intriguingly, compared with the controls at 1M, there were ectopic CA domains in the mutant
198 ventral hippocampus with the expense of the Ctip2 positive AHi and MePD amygdaloid
199 nuclei (Figure 1Ce, q), indicated by the arrows. Clearly, all the data above suggested that the
200 *COUP-TFII* gene is necessary to ensure the appropriate morphogenesis of the ventral
201 hippocampus.
202 At early embryonic stages, *COUP-TFII* was preferentially expressed in the CH (Figure 1—
203 figure supplement 1Ab, d, 1Bb, e), the organizer of the hippocampus, and at postnatal 1-
204 month-old (1M) stage, *COUP-TFII* was also highly expressed in some amygdala nuclei such
205 as the AHi and medial amygdaloid nucleus, which are adjacent to the ventral/temporal
206 hippocampus (Figure 1Ae, h, n) (Tang et al., 2012). We would like to investigate the
207 correlation of the CH and/or amygdala anlage with the duplicated ventral hippocampal
208 domains in the *COUP-TFII* mutant in detail in our future study. The observations above
209 suggest that *COUP-TFII* is not only specifically expressed in the ventral hippocampus but is

210 also required for morphogenesis and probably the function of the ventral hippocampus. Since
211 the ventral hippocampus participates in the regulation of emotion and stress, mutations in the
212 *COUP-TFII* gene lead to CHDs, and the formation of the ventral hippocampus is disrupted in
213 *COUP-TFII* mutant mice at 1M, we wondered whether CHD patients with *COUP-TFII*
214 mutations also exhibit symptoms associated with psychiatric disorders such as depression,
215 anxiety, or schizophrenia.
216
217 **The *COUP-TFI* gene is required for the specification and differentiation of the dorsal**
218 **CA1 identity**
219 Next, we asked whether the deletion of the *COUP-TFI* gene by *RXCre* also affected the
220 development of the hippocampus. Consistent with the previous finding in *Emx1Cre/+*;
221 *COUP-TFI*$^{F/F}$ (*Emx1Cre/+*; *CI*$^{F/F}$) mutant mice (Flore et al., 2017), it was the septal/dorsal
222 hippocampus, not the temporal/ventral hippocampus, that was specifically shrunken in both
223 coronal (Figure 2Aa-f) and sagittal sections (Figure 2Ag-i) of *RXCre/+*; *COUP-TFI*$^{F/F}$
224 (*RXCre/+*; *CI*$^{F/F}$) mutant mice. Then, we asked whether the loss of the *COUP-TFI* gene
225 caused abnormal specification and differentiation of hippocampal lineages. Compared with
226 the control mice, *COUP-TFI* mutant mice had fewer HuB-positive CA3 pyramidal neurons,
227 as indicated by the star; intriguingly, Ctip2-positive CA1 pyramidal neurons failed to be
228 detected, as indicated by the arrowhead, with Ctip2-positive DG granule neurons unaltered in
229 the dorsal hippocampus (Figure 2Ba-l). The loss of the dorsal CA1 pyramidal neuron identity
230 in mutant mice was further confirmed by Wfs1, another dorsal CA1 pyramidal neuron-
231 specific marker (Takeda et al., 2001) (Figure 2Ca-r). Nonetheless, the HuB-positive and
232 Ctip2-positive lineages were comparable in the ventral hippocampus between the control and
233 mutant mice (Figure 2Ba-f, m-r), even though the low expression of COUP-TFI was detected
234 there. Indeed, *COUP-TFI* is not only expressed at the highest level in the dorsal CA1 but is

also required for the specification and differentiation of dorsal CA1 pyramidal neurons, among which place cells are essential for learning and memory (O'Keefe & Conway, 1978; O'Keefe & Dostrovsky, 1971).

Given that dysplasia of the dorsal hippocampus was generated in both the *Emx1Cre* and *RXCre* models (Flore et al., 2017) (Figure 2Aa-l), we asked whether the development of dorsal CA1 pyramidal neurons was also abolished in *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice. To answer this question, immunofluorescence staining was conducted first. Compared with that in the control mice, the proportions of either the Wfs1- or Ctip2-positive CA1 domain were reduced in the mutant mice at 3M (Figure 2—figure supplement 1Aa-h), indicating that the differentiation of the dorsal CA1 pyramidal neurons was also compromised in the *Emx1Cre* model, although it was less severe than that in the *RXCre* model. To make our findings more consistent with previous studies, we further conducted experiments with the *Emx1Cre* model. Afterward, to investigate the fine structure of the dorsal CA1 pyramidal neurons, Golgi staining was performed. Compared with those of control mice, the numbers of secondary dendrites and branch points of both the apical and basal dendrites were significantly reduced in the dorsal CA1 pyramidal neurons of mutant mice at 3M (Figure 2—figure supplement 1Ba-e). Then, the dorsal hippocampus-related spatial learning and memory behavior test, the Morris water maze, was performed (Vorhees & Williams, 2006). Consistent with a previous report (Flore et al., 2017), spatial learning and memory function was significantly impaired in adult *Emx1Cre*/+; *COUP-TFI*$^{F/F}$ mice, compared with the control mice (Figure 2—figure supplement 1C). The data above suggest that *COUP-TFI* is vital for the morphogenesis, lineage specification, and spatial learning and memory of the dorsal hippocampus, and particularly, the compromised dorsal CA1 lineage could contribute to the phenotypes associated with neurodevelopmental disorders, including ID or ASD.

260 **The *COUP-TFI* and -*TFII* genes coordinate to ensure the genesis of the hippocampus**

261 Given that the loss of either *COUP-TFI* or -*TFII* leads to dysplasia of the dorsal or ventral
262 hippocampus, respectively, we asked whether these genes compensate for each other to
263 regulate the morphogenesis of the hippocampus. To answer this question, the *RXCre/+*;
264 *COUP-TFI*$^{F/F}$; *COUP-TFII*$^{F/F}$ (*RXCre/+*; *CI*$^{F/F}$; *CII*$^{F/F}$) double-mutant mouse was generated,
265 and a few homozygous double-gene mutant mice survived for approximately 3 weeks (3W).
266 Nonetheless, the reason for the lethality of the double-mutant mice is still unknown. Nissl
267 staining data showed that compared with that of control mice, the septal hippocampus was
268 severely shrunken, as indicated by the star, and the temporal hippocampus was barely
269 observed in the double-mutant mouse brains (Figure 3Aa-h). Unexpectedly, an ectopic
270 nucleus was observed in the region of the prospective temporal hippocampus, indicated by
271 the arrowhead, in the double-mutant mice (Figure 3Ag-h). In addition, compared with those
272 of controls, the regions with HuB-positive CA3 pyramidal neurons and Ctip2-positive or
273 Prox1-positive DG granule neurons were diminished in the double mutants; in particular,
274 Ctip2-positive dorsal CA1 pyramidal neurons could not be detected in the double mutants
275 (Figure 3Ba-l). Furthermore, compared with the domains of controls, no HuB-positive, Ctip2-
276 positive, or Prox1-positive domains could be detected in the prospective temporal
277 hippocampus in the double mutants (Figure 3Ca-l). The results above suggest that the *COUP-*
278 *TFI* and *COUP-TFII* genes coordinate with each other to mediate the appropriate
279 morphogenesis of the entire hippocampus.

280

281 ***COUP-TF* genes and adult neurogenesis in the hippocampus**

282 Given that the *COUP-TFI* or -*TFII* gene was highly expressed in the dorsal or ventral DG,
283 respectively (Figure 1A), and that RXCre recombinase could efficiently delete either gene in
284 the DG (Figure 1—figure supplement 1Ca-i), we asked whether the loss of the *COUP-TFI*

285 or/and -*TFII* gene in the DG would affect hippocampal adult neurogenesis. To answer this
286 question, the ventral DG, dorsal DG, and septal DG were chosen to perform
287 immunofluorescence assays in the *COUP-TFII* mutant, *COUP-TFI* mutant, and double
288 mutant models, respectively. Adult NSCs in the subgranular zone (SGZ) of the DG express
289 both GFAP and Nestin, and newborn granule neurons express Dcx (Gao, Arlotta, Macklis, &
290 Chen, 2007). The numbers of NSCs and newborn neurons in the SGZ of the DG were
291 comparable between control mice and either the *COUP-TFII* or *COUP-TFI* mutant mice
292 (Figure 3—figure supplement 1Aa-p, Ba-h); nonetheless, compared with those of control
293 mice, the numbers of the NSCs and newborn granule neurons in the SGZ of the DG were
294 reduced in the double mutants (Figure 3—figure supplement 1Aq-x, Bi-l), and the reduction
295 in both lineages was significant (Figure 3—figure supplement 1Ca, b). The data above
296 suggest that *COUP-TF* genes may coordinate with each other to execute essential functions
297 for appropriate hippocampal adult neurogenesis in the DG.
298
299 **Hippocampal trisynaptic connectivity was impaired in postnatal *COUP-TFII* single-,**
300 ***COUP-TFI* single-, and double-mutant mice at about 1M**
301 Given that dysplasia of the hippocampus was observed in all three mouse models, we asked
302 whether the connectivity of the hippocampal trisynaptic circuit associated with the DG, CA3,
303 and CA1 regions (Amaral, 1993) was normal in these models. To answer this question, the
304 components of the trisynaptic circuit were characterized in the ventral hippocampus of
305 *COUP-TFII* mutants, the dorsal hippocampus of *COUP-TFI* mutants, and the septal
306 hippocampus of double mutants. Calretinin is a marker of mossy cells, Calbindin is a marker
307 of mossy fibers, and SMI312 is a marker of Schafer collaterals (Flore et al., 2017). Compared
308 with those of controls (Figure 4Aa, b, e, f, i, and j), the numbers of Calretinin-positive mossy
309 cells were reduced, Calbindin-positive mossy fibers were longer but thinner, and SMI312-

310 positive Schafer collaterals were thinner and discontinued in the ventral hippocampus of
311 *COUP-TFII* mutants at 1M (Figure 4Ac, d, g, h, k, and l). In addition, similar to the previous
312 report (Flore et al., 2017), the numbers of Calretinin-positive mossy cells were decreased,
313 Calbindin-positive mossy fibers were shorter and thinner, and SMI312-positive Schafer
314 collaterals were barely detected in the dorsal hippocampus of *COUP-TFI* mutants (Figure
315 4Ac, d, g, h, k, and l), compared with those of controls at 1M (Figure 4Ba, b, e, f, i, and j).
316 Moreover, compared with those of control mice (Figure 4Ca, b, e, f, i, and j), the numbers of
317 Calretinin-positive mossy cells were reduced; both Calbindin-positive mossy fibers and
318 SMI312-positive Schafer collaterals were barely detected in the prospective septal
319 hippocampus of the double mutants at 3W (Figure 4Cc, d, g, h, k, and l). Clearly, without
320 both *COUP-TF* genes, the connectivity of the hippocampal trisynaptic circuit was abolished
321 more severely. The observations above revealed that the formation of the trisynaptic circuit,
322 which is one of the fundamental characteristics of hippocampal neurophysiology (Basu &
323 Siegelbaum, 2015), was abnormal in all three mouse models, indicating that both the
324 morphology and functions of the hippocampus are most likely compromised in the loss of the
325 *COUP-TFI* and/or *COUP-TFII* gene.
326

327 **The expression of several essential regulatory genes associated with early hippocampal**
328 **development was abnormal in double mutants**

329 Given that the hippocampus was almost completely diminished in double mutants, we asked
330 how *COUP-TF* genes participated in the regulation of the early morphogenesis of the
331 hippocampus. To answer this question, total RNA isolated from the whole telencephalons of
332 control (n=5) and double-mutant-(n=3) embryos at E11.5 was used to generate cDNA, and
333 then real-time quantitative PCR (RT-qPCR) assays were performed. As expected, compared
334 with that of control mice, the expression of *COUP-TFI* and *COUP-TFII* was reduced

significantly in the double mutant mice (Figure 5A). Then, we mainly focused on the intrinsic regulatory networks by analyzing the expression profiles of two groups of transcription factor genes. The *Foxg1*, *Gli3*, *Lhx2*, *Otx1, Otx2*, and *Pax6* genes, which are highly related to the early patterning of the dorsal telencephalon (Hébert & Fishell, 2008), were in the first group; *Axin2*, *Emx1*, *Emx2*, *Lef1*, *Lhx5*, and *Tcf4* genes, which are associated with early hippocampal development (Galceran et al., 2000; Moore & Iulianella, 2021; Tole et al., 2000; Yoshida et al., 1997; Zhao et al., 1999), were in the other group. The expression of the *Foxg1*, *Gli3*, *Lhx2*, *Otx1, Otx2*, and *Pax6* genes was comparable between the controls and double mutants (Figure 5A), indicating that the early patterning of the dorsal telencephalon is largely unaltered. Compared with that of control mice, the expression of the *Axin2*, *Emx2*, *Lef1*, and *Tcf4* genes was normal in the double mutants; interestingly, the expression of the *Emx1* and *Lhx5* transcripts was decreased significantly in the double mutants at E11.5 compared to that in control mice (Figure 5A). Consistent with the downregulated expression of *Lhx5* transcripts in the double mutant, the expression of the Lhx5 protein was reduced in the CH in the double mutants at E11.5; moreover, the number of Lhx5-positive Cajal-Retzius cells decreased in the double mutant embryos at E11.5, E13.5 and E14.5 (Figure 5Ba-d, a'-d', a''-d'', i-l, i'-l', q-t, q'-t'). The expression of *Lhx2* was expanded ventrally into the choroid plexus in the *Lhx5* null mutant mice (Zhao et al., 1999), indicating that *Lhx5* could inhibit *Lhx2* expression locally. Consistent with RT-qPCR data, the expression of Lhx2 was comparable between the control and double-mutant mice at E11.5 (Figure 5Be-h, e'-h'). Interestingly, the expression of the Lhx2 protein was increased in the hippocampal primordium in the *COUP-TF* double-mutant mice at E13.5 and E14.5 (Figure 5Bm-p, m'-p', u-x, u'-x'). The upregulation of Lhx2 expression is most likely associated with the reduced expression of the *Lhx5* gene.

Next, we asked whether neural precursor cells (NPCs), intermediate progenitor cells (IPCs), or newborn neurons were affected in the early development of the hippocampus in double-mutant mice. Sox2 is a marker for NPCs, Tbr2 is a marker for IPCs, and NeuroD1 is a marker for newborn neurons (Yu, Marchetto, & Gage, 2014). The expression of Sox2 in the hippocampal regions was comparable between the control and double-mutant mice at E14.5 (Figure 5Ca-d, a'-d'), indicating that the generation of NPCs was normal. Nevertheless, compared with the control embryos, the numbers of Tbr2-positive IPCs and NeuroD1-positive newborn neurons were reduced in the double-mutant embryos (Figure 5Ce-l, e'-l'), and the reduction was significant (Figure 5D). Our observations were consistent with previous findings in *Lhx5* null mutant mice that the specification of the hippocampal NPCs was normal, but the later differentiation event was abolished (Zhao et al., 1999). All the data above suggest that *COUP-TF* genes may cooperate to ensure the early morphogenesis of the hippocampus by regulating the appropriate expression levels of *Lhx5* and *Lhx2* genes. Nevertheless, we could not exclude other possibilities that *COUP-TF* genes could also participate in the modulation of hippocampal development through *Emx1* or other genes.

Discussion

In our present study, we observed dorsal-high COUP-TFI and ventral-high COUP-TFII expression profiles in the postnatal hippocampus. The deletion of the *COUP-TFII* gene led to duplicated CA1 and CA3 domains of the ventral hippocampus. The loss of *COUP-TFI* resulted in the failed specification and differentiation of the dorsal CA1 pyramidal neuron lineage with a diminished dorsal hippocampus. Furthermore, the deficiency of both *COUP-TF* genes caused atrophy of almost the entire hippocampus, accompanied by compromised generation of the CA1, CA3, and DG identities. In addition, the dorsal trisynaptic components, ventral trisynaptic components, or entire trisynaptic components were abolished

384 in the corresponding *COUP-TFI* gene mutant, *COUP-TFII* gene mutant, or *COUP-TFI*/*-TFII*
385 double-gene mutant mice. Moreover, *COUP-TF* genes may cooperate to ensure the
386 appropriate morphogenesis and function of the hippocampus by regulating the *Lhx5-Lhx2*
387 axis.
388
389 **1. *COUP-TFII* governs the distinct characteristics of the ventral hippocampus**
390 Sixty years ago, the pioneering work of Milner and her colleagues discovered the essential
391 role of the hippocampus in declarative memory (Penfield & Milner, 1958; Scoville & Milner,
392 1957). Recently, accumulating evidence has supported the Moser theory that the
393 hippocampus is a heterogeneous structure with distinct characteristics of gene expression,
394 connectivity, and function along its dorsoventral axis (Bast, 2007; Fanselow & Dong, 2010;
395 Moser & Moser, 1998; Strange et al., 2014). The dorsal hippocampus marked in blue, in
396 which gene expression is similar to the neocortex, serves the "cold" cognitive function
397 associated with declarative memory and spatial navigation, and the ventral hippocampus
398 marked in red, in which gene expression is close to the hypothalamus and amygdala,
399 corresponds to the "hot" affective states related to emotion and anxiety (Figure 5—figure
400 supplement 1). The ventral hippocampus generates direct connectivity with the amygdala,
401 hypothalamus, medial prefrontal cortex (mPFC), and olfactory bulb (Cenquizca & Swanson,
402 2007; Hoover & Vertes, 2007; Kishi et al., 2000; Pitkanen et al., 2000; Roberts et al., 2007).
403 Nonetheless, thus far, the molecular and cellular mechanism of how the morphogenesis,
404 connectivity, and function of the ventral hippocampus is achieved has been largely unclear.
405 *COUP-TFII*, a nuclear receptor gene associated with heart disease (High et al., 2016), was
406 highly and exclusively expressed in the ventral hippocampus in 1-month-old mice and was
407 expressed ventrally in the CH of the hippocampal primordium in mouse embryos (Figure 1,
408 Figure 1—figure supplement 1, Figure 5Ea), indicating that the *COUP-TFII* gene may

409 participate in the regulation of the development and function of the ventral hippocampus.
410 First, deficiency of the *COUP-TFII* gene led to the duplication of the CA1 and CA3 domains
411 of the ventral hippocampus but not the dorsal hippocampus, which was confirmed both
412 morphologically and molecularly (Figure 1). Second, the formation of the trisynaptic circuit
413 was specifically abolished in the ventral hippocampus of *COUP-TFII* mutants (Figure 4),
414 indicating that the intrahippocampal circuit, information transfer, and function of the ventral
415 hippocampus could be compromised. Third, the ventral hippocampus generates neural
416 circuits with the mPFC, amygdala, nucleus accumbens, and hypothalamus, which are
417 associated with anxiety/behavioral inhibition, fear processing, pleasure/reward seeking, and
418 the neuroendocrine system, respectively (Anacker & Hen, 2017; Baik, 2020; Bryant &
419 Barker, 2020; Cenquizca & Swanson, 2007; Herman et al., 2016; Kishi et al., 2000; O'Leary
420 & Cryan, 2014; Pitkanen et al., 2000). These ventral hippocampal projections may be
421 important for processing information related to emotion and anxiety. Intriguingly, our
422 previous studies revealed that *COUP-TFII* is required for the development of the
423 hypothalamus, amygdala, and olfactory bulb (S. Feng et al., 2017; Tang et al., 2012; X. Zhou
424 et al., 2015), all of which generate functional neural circuits with the ventral hippocampus
425 (Fanselow & Dong, 2010). Particularly, both the hypothalamus and amygdala are also
426 diminished in *RXCre/+*; *COUP-TFII*$^{F/F}$ mutant mice (S. Feng et al., 2017; Tang et al., 2012),
427 indicating that their interconnectivities with the ventral hippocampus are abnormal. Thus, all
428 the findings above suggest that *COUP-TFII* is a novel and essential intrinsic regulator that
429 controls the morphogenesis, connectivity, and function of the ventral hippocampus.
430 Given that mutations of *COUP-TFII* are highly associated with CHDs and are required for
431 the distinct characteristics of the ventral hippocampus, we wondered whether CHD patients
432 carrying mutations of *COUP-TFII* also display symptoms of psychiatric disorders, such as
433 depression, anxiety, or schizophrenia, related to the ventral hippocampus. In our future study,

434 we would like to generate hippocampus-specific or hippocampal subdomain-specific
435 conditional knockout models to dissect distinct roles of the *COUP-TFII* gene in the
436 hippocampus, particularly in the ventral hippocampus, in detail.
437
438 **2. The *COUP-TFI* gene is required for the specification and differentiation of dorsal**
439 **CA1 pyramidal neurons**
440 The expression of *COUP-TFI*, another orphan nuclear receptor gene associated with
441 neurodevelopmental disorders (Bertacchi et al., 2020; Bosch et al., 2014; Contesse et al.,
442 2019), is high in the dorsal MP of the hippocampal primordium and is higher in the dorsal
443 hippocampus than in the ventral hippocampus (Figure 1, Figure 1—figure supplement 1,
444 Figure 5Ea) (Flore et al., 2017). Consistent with previous observations in *Emx1Cre/+*;
445 *COUP-TFI*$^{F/F}$ mutant mice (Flore et al., 2017), the dorsal hippocampus but not the ventral
446 hippocampus was specifically shrunken in *RXCre/+; COUP-TFI*$^{F/F}$ mice (Figure 2, Figure
447 5—figure supplement 1). *COUP-TFI* is expressed at the highest level in dorsal CA1
448 pyramidal neurons (Figure 1), indicating that the *COUP-TFI* gene may play a role in the
449 specification and differentiation of dorsal CA1 pyramidal neurons. As expected, the
450 expression of Ctip2 and Wfs1, two markers for dorsal CA1 pyramidal neurons, could not be
451 detected in the prospective dorsal CA1 domain in *RXCre/+*; *COUP-TFI*$^{F/F}$ mutant mice
452 (Figure 2); furthermore, *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice partially phenocopied the
453 compromised development of the dorsal CA1 lineage (Figure 2—figure supplement 1). It
454 seems that the spatiotemporal activity of RXCre recombinase is better or broader than that of
455 the Emx1Cre recombinase during the critical period of the specification of the dorsal CA1
456 pyramidal neuron identity. In addition, the Golgi staining assay revealed that the
457 development of the dendrites of the dorsal CA1 pyramidal neurons was abnormal (Figure 2—
458 figure supplement 1). All the observations above indicate that the *COUP-TFI* gene is not only

459 necessary for the morphogenesis of the dorsal hippocampus but is also required for the
460 specification and differentiation of the dorsal CA1 pyramidal neurons, among which there are
461 place cells. The identification of place cells fifty years ago was one of the most important
462 breakthroughs in understanding the role of the hippocampus in memory (O'Keefe &
463 Dostrovsky, 1971). Except for spatial information, place cells in the dorsal CA1 may also
464 encode nonspatial representations, such as time (Eichenbaum, 2017; Lisman et al., 2017).
465 Notably, 95% of patients carrying *COUP-TFI* mutations are associated with ID. Here, our
466 observations support the notion that the *COUP-TFI* gene is a novel intrinsic regulator that
467 specifies the dorsal CA1 pyramidal cell identity, which will benefit the understanding of both
468 neurophysiological functions of the hippocampus and the etiology of NDD including ID and
469 ASD.
470

471 **3. *COUP-TFI* and *-TFII* cooperate to ensure the appropriate morphogenesis of the**
472 **hippocampus by regulating the *Lhx5-Lhx2* axis in mice**

473 In wild-type mice, *COUP-TFI* and *-TFII* genes generated complementary expression profiles
474 in the embryonic hippocampal primordium with *COUP-TFI* in the dorsal MP, marked in
475 green; *COUP-TFII* in the ventral CH, marked in red (Figure 5Ea, Figure 1—figure
476 supplement 1); and in the postnatal hippocampus with high-COUP-TFI expression in the
477 dorsal, marked in green; and high-COUP-TFII expression in the ventral, marked in red
478 (Figure 5Ea, Figure 1). These findings indicated that *COUP-TF* genes may coordinate to
479 regulate hippocampal development. Indeed, as discussed above, the loss of either *COUP-TFI*
480 or *-TFII* only leads to dysplasia of the dorsal hippocampus (Flore et al., 2017) (Figure 2,
481 Figure 2—figure supplement 1) or ventral hippocampus (Figure 1), respectively; intriguingly,
482 while both genes are efficiently excised by *RXCre* in the hippocampal primordium (Figure
483 1—figure supplement 1), more severely shrunken hippocampi developed in the 3-week-old

484 double knockout mice (Figure 3). The dosage-dependent severity of hippocampal
485 abnormalities suggested that two nuclear receptor genes, *COUP-TFI* and *-TFII* could
486 cooperate with each other to execute an essential and intrinsic function in the development of
487 the hippocampus.

488 It is known that both extrinsic signals and intrinsic factors participate in the regulation of the
489 early development of the hippocampus. Notably, mutations of *Wnt3a* and *Lef1* eliminate the
490 entire hippocampus (Galceran et al., 2000; S. M. Lee et al., 2000). Given that the expression
491 of *Axin2*, *Lef1* and *Tcf4*, three Wnt-responsive transcription factor genes, was not altered in
492 the *COUP-TF* double mutant (Figure 5A), it is unlikely that abnormal Wnt signaling is the
493 cause of the compromised hippocampus. *Lhx5* is specifically expressed in the hippocampal
494 primordium and is required for the morphogenesis of the hippocampus (Zhao et al., 1999).
495 *Lhx2* is necessary for hippocampal development by repressing cortical hem fate (Mangale et
496 al., 2008; Monuki et al., 2001). Agenesis of the hippocampus is observed in either *Lhx5* or
497 *Lhx2* null mutant mice, and these genes particularly repress each other (Hébert & Fishell,
498 2008; Mangale et al., 2008; Roy et al., 2014; Zhao et al., 1999), indicating that the proper
499 expression levels of *Lhx5* and *Lhx2* genes are critical to maintain the appropriate
500 development of the hippocampus (Figure 5Eb). The transcriptional and protein expression
501 levels of *Lhx5* but not *Lhx2* were first reduced in the hippocampal primordium of *COUP-TF*
502 double-mutant mice at E11.5; later, enhanced expression of the Lhx2 protein was detected in
503 the hippocampal primordium of double-mutant mice at E13.5 and E14.5 (Figure 5A-B).
504 Moreover, the number of Lhx5-positive Cajal-Retzius cells was clearly reduced in the double
505 mutant embryos at E11.5, E13.5 and E14.5; consistent with the observations in the *Lhx5* null
506 mutant (Li et al., 2021; Miquelajáuregui et al., 2010), the generation of Sox2-positive
507 hippocampal NPCs was not affected, but the development of Tbr2-positive IPCs and
508 NeuroD1-positive newborn neurons was abnormal in *COUP-TF* double-mutant mice (Figure

5). Thus, our findings reveal a novel intrinsic regulatory mechanism that *COUP-TFI* and *COUP-TFII*, two disease-associated nuclear receptor genes, may cooperate with each other to ensure proper hippocampal morphogenesis by regulating the *Lhx5-Lhx2* axis. Intriguingly, compared with the adult *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice, the hippocampus was much smaller in the adult *Emx1Cre/+*; *COUP-TFI*$^{F/F}$; *COUP-TFII*$^{F/F}$ double-gene mutant mice; nevertheless, both the dorsal and ventral hippocampus were readily detected in double-gene mutants with *Emx1Cre* (our unpublished observations). In addition, the discrepancy between the shrunken dorsal hippocampus associated with the loss of *COUP-TFI* and the duplicated CA domains of the ventral hippocampus associated with the deficiency of *COUP-TFII* suggested that the regulatory network related to *COUP-TF* genes during the early morphogenesis of the hippocampus could be much more complicated than suspected and should be investigated in our future study.

4. *COUP-TF* genes are imperative for the formation of the trisynaptic circuit

The hippocampus and entorhinal cortex (EC) are interconnected through various neural circuits to mediate the flow of the information associated with declarative memory (Basu & Siegelbaum, 2015). Both direct and indirect glutamatergic circuits are involved in the relay of information from the EC to the hippocampal CA1, and the trisynaptic pathway is the most well-characterized indirect circuit. The EC sends sensory signals from association cortices via the perforant path to the DG, then the DG granule cells send excitatory mossy fiber projections to CA3 pyramidal neurons, and CA3 pyramidal neurons project to CA1 via the Schaffer collaterals (H. Lee, GoodSmith, & Knierim, 2020). Consistent with the high expression of COUP-TFI in the dorsal hippocampus and COUP-TFII in the ventral hippocampus (Figure 1), the dorsal trisynaptic circuit is specifically damaged in *COUP-TFI* mutants, as is the ventral trisynaptic circuit in *COUP-TFII* mutant mice. Moreover, the

534 hippocampal trisynaptic circuit was almost completely absent in the double mutants (Figure 4,
535 and our unpublished observations). The information transfer associated with the trisynaptic
536 circuits should be abolished particularly in the dorsal and/or the ventral hippocampus in the
537 above corresponding genetic mouse models. Interestingly, *COUP-TFI* is also required to
538 specify the medial EC cell fate (J. Feng et al., 2021). Therefore, the impaired formation and
539 function of trisynaptic circuits could be caused by the abnormal development of CA1, CA3,
540 DG or EC lineages. Nonetheless, given that newborn granule neurons are continuously
541 generated in the adult DG to integrate into the existing neural circuits essential for declarative
542 memory (Toda, Parylak, Linker, & Gage, 2019; Tuncdemir, Lacefield, & Hen, 2019) and that
543 hippocampal adult neurogenesis was severely compromised in the *COUP-TF* double mutant
544 (Figure 3—figure supplement 1), we could not exclude the possibility that impaired adult
545 neurogenesis may also contribute to the malformation and impaired function of the
546 trisynaptic pathway in double mutants.
547
548 The hippocampus is heterogeneous along its dorsoventral axis, and either the dorsal or
549 ventral hippocampus generates unique and distinguishable characteristics of gene expression
550 and connectivity, which enable the hippocampus to execute an integrative function from the
551 encoding and retrieval of certain declarative memory to adaptive behaviors. Lesions of the
552 dorsal hippocampus, which are essential for the cognitive process of learning and memory,
553 lead to amnesia and ID; while damage to the ventral hippocampus, which is central for
554 emotion and affection, is highly associated with psychiatric disorders including depression,
555 anxiety, and schizophrenia. Our findings in this study reveal novel intrinsic mechanisms by
556 which two nuclear receptor genes, *COUP-TFI* and *-TFII*, which are associated with NDD or
557 CHD, converge to govern the differentiation and integration of the hippocampus along the
558 dorsoventral axis morphologically and functionally. Furthermore, our present study provides

559 novel genetic model systems to investigate the crosstalk among the hippocampal complex in
560 gene expression, morphogenesis, cell fate specification and differentiation, connectivity,
561 functions of learning/memory and emotion/anxiety, adaptive behaviors, and the etiology of
562 neurological diseases. Nevertheless, many enigmas, such as whether and how the
563 abnormalities of either the dorsal or ventral hippocampus affect the characteristics of the
564 other, remain unsolved. In addition to the excitatory lineages and circuits, interneurons and
565 inhibitory circuits play vital roles in maintaining the plasticity and functions of the
566 hippocampus. We also wonder whether and how defects in interneurons and inhibitory
567 circuits could contribute to the compromised morphogenesis, connectivity, and functions of
568 the hippocampus and the etiology of psychiatric and neurological conditions including ID,
569 ASD, depression, anxiety, and schizophrenia.
570
571 **Materials and Methods**

572 **Animals**

573 *COUP-TFI-floxed* mice, *COUP-TFII-floxed* mice, *Emx1Cre* mice and *RXCre* mice (Swindell
574 et al., 2006) (PMID: 16850473) used in the study were of the C57B6/129 mixed background.
575 The noon of vaginal plug day was set as the embryonic day 0.5 (E0.5). All animal protocols
576 were approved by the Animal Ethics Committee of the Shanghai Institute of Biochemistry
577 and Cell Biology. All methods were performed in accordance with the relevant guidelines
578 and regulations. Only the littermates were used for the comparison.
579

580 **Nissl staining**

581 We used xylene to dewax paraffin sections, followed by rinsing with 100%, 95%, and 70%
582 ethanol. The slides were stained in 0.1% Cresyl Violet solution for 25 mins. Then the
583 sections were washed quickly in the water and differentiated in 95% ethanol. We used 100%

584 ethanol to dehydrate the slides, followed by rinsing with the xylene solution. Finally, the
585 neutral resin medium was used to mount the slides.
586
587 **Immunohistochemical (IHC) staining**
588 The paraffin sections were dewaxed and rehydrated as described above for Nissl staining.
589 The slides were boiled in 1×antigen retrieval solution (DAKO) under microwave conditions
590 for 15 min. After cooled to room temperature (RT), the slides were incubated with 3% H2O2
591 for 30 min. Then, the slides were treated with blocking buffer for 60 min at RT and then
592 incubated with the primary antibody in the hybridization buffer (10×diluted blocking buffer)
593 overnight (O/N) at 4 °C. The next day, the tyramide signal amplification kit (TSA)
594 (Invitrogen) was used according to the manufacturer's protocol. After being incubated with
595 1%TSA blocking buffer, the sections were treated with a biotinylated secondary antibody for
596 60 min at RT. After being washed with 1×PBS three times, the slides were incubated with
597 1×HRP-conjugated streptavidin for 1 h. Next, the tyramide working solution was prepared,
598 including the 0.15‰ H_2O_2 in distilled water, the 100×diluted tyramide substrate solution
599 (tyramide-488 or tyramide-594), and the amplification buffer. The sections were incubated
600 with the working solution for 10 min. Then the slides were counterstained with DAPI and
601 mounted with the antifade mounting medium (Southern Biotech) (S. Feng et al., 2017; Zhang
602 et al., 2020). Finally, the sections were observed and images were captured with a digital
603 fluorescence microscope (Zeiss).
604 The following primary antibodies were used in the study: mouse anti-COUP-TFI (1:1000,
605 R&D, Cat # PP-H8132-00), mouse anti-COUP-TFII (1:2000, R&D, Cat # PP-H7147-00),
606 rabbit anti-COUP-TFII (1:2000, a gift from Dr. Zhenzhong Xu, Zhejiang University, China),
607 rabbit anti-HuB (1:500, Abcam, Cat # ab204991), rat anti-Ctip2 (1:500, Abcam, Cat #
608 ab18465), rabbit anti-Wfs1 (1:500, ProteinTech, Cat # 11558-1-AP), goat anti-Prox1 (1:500,

609 R&D, Cat # AF2727), rabbit anti-Calretinin (1:500, Sigma, Cat # C7479), rabbit anti-
610 Calbindin (1:500, Swant, Cat # CB38), mouse anti-SMI312 (1:200, Covance, Cat # SMI-
611 312R), rabbit anti-Sox2 (1:500, Affinity BioReagents, Cat # PA1-16968), rat anti-Tbr2
612 (1:500, Thermo Fisher, Cat # 12-4875-82), goat anti-NeuroD1 (1:200, Santa Cruz, Cat # sc-
613 1084), goat anti-Lhx2 (1:200, Santa Cruz, Cat # sc-19344), goat anti-Lhx5 (1:200, R&D, Cat
614 # AF6290), goat anti-β-galactosidase (LacZ) (1:400, Biogenesis, Cat # 4600-1409), mouse
615 anti-GFAP (1:500, Sigma, Cat # G3893), rabbit anti-Nestin (1:200, Santa Cruz, Cat # 20978),
616 goat anti-Dcx (1:500, Santa Cruz, Cat # sc-8066). The following secondary antibodies were
617 used in the study: donkey anti-mouse IgG biotin-conjugated (1:400, JacksonImmuno, Cat #
618 715-065-150), donkey anti-rabbit IgG biotin-conjugated (1:400, JacksonImmuno, Cat # 711-
619 065-152), donkey anti-goat IgG biotin-conjugated (1:400, JacksonImmuno, Cat # 705-066-
620 147), donkey anti-rat IgG biotin-conjugated (1:400, JacksonImmuno, Cat # 712-065-150).
621

622 **Western blotting**

623 We homogenized the isolated dorsal and ventral hippocampus tissues from 1-month-old mice
624 respectively in the RIPA buffer (Applygen) with protease inhibitor cocktail (Sigma) and
625 phosphatase inhibitors (Invitrogen) and then centrifuged at the speed of 12,000 rpm for 30
626 mins. We collected the supernatants and analyzed the total concentrations by the BCA kit
627 (Applygen). Gradient SDS-PAGE gels were used to separate the same amounts of protein
628 sample (40 μg/lane), and then the proteins were transferred to the PVDF membranes
629 (Millipore). After being blocked by the 3% BSA (Sigma) for 2h, the membranes that
630 contained proteins were incubated by primary antibodies at 4 °C O/N. The membranes were
631 rinsed with 1×PBST three times for 10 mins and then treated with biotinylated secondary
632 antibodies for 2h at RT. After washing with 1×PBST, membranes were treated with the HRP-
633 conjugated streptavidin for 1h at RT. Finally, we used the chemiluminescence detection

634 system (Tanon) to detect the bands. The density of the protein band was analyzed by the
635 software Image J.
636 The primary antibodies were used in the experiment as below: mouse anti-COUP-TFI
637 (1:2000, R&D, Cat # PP-H8132-00), rabbit anti-COUP-TFII (1:3000, a gift from Dr.
638 Zhenzhong Xu, Zhejiang University, China). The following secondary antibodies were
639 applied in the study, including goat anti-mouse IgG biotin-conjugated (1:1000, KPL, Cat #
640 16-18-06), goat anti-rabbit IgG biotin-conjugated (1:1000, KPL, Cat # 16-15-06).
641
642 **Golgi staining**
643 Deep anesthesia was performed before sacrificing the control and mutant mice, and the brains
644 were immediately isolated. The FD rapid GolgiStain kit (FD NeuroTech) was used to process
645 the brain tissue samples, which were immersed in an equal volume of immersion solution
646 mixed with solutions A and B and stored in the dark for two weeks at RT. At least 5mL of
647 immersion solution was used for each cubic meter of the tissue. To achieve the best results,
648 the container of tissue was gently swirled from side to side twice a week during the
649 incubating period. Afterwards, the brain tissue was transferred to solution C in the dark at RT
650 for at least 72 hours (up to 1 week). Finally, the tissues were cut into 100 μm thick slices with
651 a cryostat at -20°C to -22°C and transferred to gelatin-coated microscope slides containing
652 solution C using a sample retriever. The slices were dried naturally at RT. The concrete
653 staining procedure of the kit was followed using the manufactory's protocol. Then, the
654 sections were rinsed twice with double-distilled water for 4 minutes each time. The slices
655 were placed in a mixture of one volume of solution D, one volume of solution E, and two
656 volumes of double distilled water for 10 minutes and were rinsed twice with distilled water
657 for 4 minutes each time. The sections were later dehydrated in 50%, 75%, and 95% ethanol
658 for 4 minutes respectively. Next, the slices were dehydrated in 100% ethanol 4 times for 4

659 minutes each time. Finally, the sections were cleared in xylene and mounted with a neutral
660 resin medium.
661
662 **Morris water maze**
663 By recording the time spent by the mice swimming in the water tank and finding the escape
664 platform hidden underwater, and the swimming trajectory, the Morris water maze test can
665 objectively reflect the spatial learning and memory ability of the mice. We poured tap water
666 into the water maze tank and added an appropriate amount of well-mixed, milky white food
667 dye. The height of the liquid level was about 1 cm higher than the escape platform, and the
668 water temperature was kept at about 25±1℃. At the same time, four markers of different
669 shapes were pasted on the four directions of the inner wall above the water tank to distinguish
670 different directions. The Morris water maze test was divided into the training phase and the
671 probe trial phase. The training phase lasted for 6 days, 4 times a day, and the interval between
672 each training was about 30 minutes. During training, the mice were placed into the tank from
673 the entry points of four different quadrants facing the inner wall, and their latency was
674 recorded from the time they entered the water to the time they found a hidden underwater
675 platform and stood on it. After the mouse found the platform, we let it stay on the platform
676 for 10 seconds before removing it. If the mouse failed to discover the platform 60 seconds
677 after entering the water, it was guided to find the platform and left to stay for 10 seconds.
678 Each mouse was placed into the water tank from four water entry points and recorded as one
679 training session. The probe trial was carried out on the seventh day, and the underwater
680 platform was removed. Each experimental mouse was put into the water tank at the same
681 water entry point and allowed to move for 60 seconds. The time that each mouse spent in the
682 quadrant, where the platform was originally placed, was recorded.
683

RNA isolation and quantitative real-time PCR

Total RNAs were prepared from the whole telencephalon of the control (n=5) and double mutant (n=3) mice at E11.5 respectively, with the TRIzol Reagent (Invitrogen) by following the manufactory's protocol. Reverse-transcription PCR and real-time quantitative PCR assays were performed as described previously (Tang et al., 2012). A student's t-test was used to compare the means of the relative mRNA levels between the control group and mutant group. Primer sequences are as follows:

Axin2-f, 5'-*ctgctggtcaggcaggag*-3', *Axin2-r*, 5'-*tgccagtttctttggctctt*-3'; *Coup-tfI-f*, 5'-*caaagccatcgtgctattca*-3', *Coup-tfI-r*, 5'-*cctgcaggctttcgatgt*-3'; *Coup-tfII-f*, 5'-*cctcaaagtgggcatgagac*-3', *Coup-tfII-r*, 5'-*tgggtaggctgggtaggag*-3'; *Emx1-f*, 5'-*ctctccgagacgcaggtg*-3', *Emx1-r,* 5'-*ctcagactccggcccttc*-3'; *Emx2-f*, 5'-*cacgcttttgagaagaacca*-3', *Emx2-r,* 5'-*gttctccggttctgaaacca*-3'; *Foxg1-f*, 5'-*gaaggcctccacagaacg*-3', *Foxg1-r*, 5'-*ggcaaggcatgtagcaaaag*-3'; *Gli3-f*, 5'-*tgatccatctcctattcctcca*-3', *Gli3-r*, 5'-*tctggatacgtcgggctact*-3'; *Lef1-f*, 5'-*tcctgaaatccccaccttct*-3', *Lef1-r*, 5'-*tgggataaacaggctgacct*-3'; *Lhx2-f*, 5'-*cagcttgcgcaaaagacc*-3', *Lhx2-r*, 5'-*taaaaggttgcgcctgaact*-3'; *Lhx5-f*, 5'-*tgtgcaataagcagctatcca*-3'; *Lhx5-r,* 5'-*caaactgcggtccgtaca*-3'; *Otx1-f*, 5'-*ccagagtccagagtccaggt*-3', *Otx1-r*, 5'-*ccgggttttcgttccatt*-3'; *Otx2-f*, 5'-*ggtatggacttgctgcatcc*-3', *Otx2-r*, 5'-*cgagctgtgccctagtaaatg*-3'; *Pax6-f*, 5'-*gttccctgtcctgtggactc*-3', *Pax6-r*, 5'-*accgcccttggttaaagtct*-3'; *Tcf4-f*, 5'-*aaatggccactgcttgatgt*-3', *Tcf4-r*, 5'-*gcaccaccggtactttgttc*-3'.

Quantification and statistical analysis

The number of specified immunofluorescent marker-positive cells was assessed by Image J Cell Counter in full image fields. Three brain sections per mouse were counted for each index. GraphPad Prism 7.0 (GraphPad) was used to perform statistical analysis. The data analysis used one-way analysis of variance (ANOVA), Dunnett's or Tukey's post hoc tests, and student's unpaired t-test. The data were expressed as the mean ± SEM. The data obtained

from at least three independent replicates were used for statistical analysis. $P < 0.05$ was considered the significant statistical difference.

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Author contributions

X.Y., W.R. designed and conducted the experiments. X.Y. and K.T. organized and wrote the manuscript. Z.W.L., S.F. and J.X.Y. assisted in conducting the experiments and analyzed the data. N.J. and K.T. conceived the project and approved the manuscript.

Competing interests

The authors declare no competing interests.

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950 **Figure legends**

951

952 **Figure 1. Duplicated CA1 and CA3 domains are generated in the ventral hippocampus**

953 **of *RXCre/+*; *CII$^{F/F}$* mutant mice.**

954 **A,** The expression of COUP-TFI (**a, d, g, j, m**) and COUP-TFII (**b, e, h, k, n**) in coronal

955 sections (**a-f**) and sagittal sections (**g-o**) of the hippocampus at postnatal month 1 (1M);

956 representative Western blots and quantitative densitometry data for the expression of COUP-

957 TFI and COUP-TFII in the dorsal and ventral hippocampus at 1M (**p-q**). **B,** In coronal

958 sections along the rostrocaudal axis (**a-f**) and sagittal sections along the lateral-medial axis

959 (**g-l**) of the hippocampus in mutant mice, compared with that in control mice (**a-c**, **g-i**), the

960 ectopic CA-like structure, indicated by the star, was observed in the ventral region in *COUP-*

961 *TFII* gene mutant (*RXCre/+*; *CII$^{F/F}$)* mice at 1M (**d-f**, **j-l**). **C,** The expression of HuB and

962 Ctip2 in the corresponding inserted area in **a-f** under a high magnification objective lens at

963 1M (**g-r**); compared with those of control mice (**a-c**, **g-i**, **m-o**), the duplicated HuB-positive

964 CA3 domain, indicated by the star, and Ctip2-positive domains, indicated by the arrowhead,

965 were specifically observed in the ventral hippocampus (**d-f, p-r**) but not in the dorsal

966 hippocampus (**d-f**, **j-l**) of *COUP-TFII* mutant mice at 1M; Ctip2 positive AHi and MePD

967 amygdaloid nuclei were barely observed in the *COUP-TFII* mutant mice, indicated by the

968 arrows, instead of the ectopic CA domains at the prospective amygdaloid regions (**e-f**, **q-r**).

969 Data are expressed as the mean ± SEM. *Student's t test*, $^{*}P<0.05$, $^{**}P<0.01$. AHi,

970 amygdalohippocampal area; AMY, amygdala nuclei; CTX, cortex; dCA1, dorsal CA1; dCA3,

971 dorsal CA3; dDG, dorsal dentate gyrus; dHPC, dorsal hippocampus; MePD, posterodorsal

972 part of the medial amygdaloid nucleus; PMCo, posteromedial cortical amygdaloid nucleus;

973 vCA1, ventral CA1; vCA3, ventral CA3; vDG, ventral dentate gyrus; vHPC, ventral

974 hippocampus. Scale bars, **Aa-c**, **Ad-f**, **Aj-o**, **Cg-r**, 100 μm; **Ag-i**, **Ba-l, Ca-f**, 200μm.

975

976 **Figure 1—figure supplement 1. The expression of *COUP-TF* genes in the early**
977 **developing hippocampus and different conditional knock mouse models.**
978 **A,** The expression of *COUP-TFI* and *COUP-TFII* genes in the forebrain at E10.5 (**a-b**) and
979 E11.5 (**c-d**). **B,** The expression of *COUP-TFI* and *COUP-TFII* genes in the developing
980 hippocampus at E12.5 (**a-c**), E14.5 (**d-f**), and P0 (**g-l**). **C,** Compared with that of control mice
981 (**a-c**), *COUP-TFII* is efficiently deleted by RXCre recombinase in the hippocampus of
982 *COUP-TFII* mutant mice at 1M (**d-f**); *COUP-TFI* is clearly deleted by RXCre recombinase
983 in the hippocampus of *COUP-TFI* mutant mice at 1M (**g-i**). Compared with that of control
984 mice (**j-l**), COUP-TFI and -TFII were efficiently deleted by RXCre recombinase at the
985 hippocampal primordium, including the MP and CH, in *COUP-TFI/-TFII* double-mutant
986 mice at E14.5 (**m-o**). **D,** Compared with that of the control mice (**a-c, g-h**), the expression of
987 *COUP-TFII* was significantly decreased in the hippocampal primordium of the homozygous
988 mutant mice at E11.5; meanwhile, the LacZ signals obviously increased in the *COUP-TFII*
989 homozygous mutant mice at E11.5 (**d-f, j-k**). Compared with that of the control mice (**i**), the
990 expression of COUP-TFI is activated in the caudal hippocampal primordium of the
991 homozygous mutant mice at E11.5 (**l**). CH, cortical hem; dHPC, dorsal hippocampus; HP,
992 hippocampal primordium; HPC, hippocampus; MP, medial pallium; vHPC, ventral
993 hippocampus. Scale bars, **Aa-d**, **Ba-f**, **Cj-o**, 200 μm; **Bg-l**, **Ca-i**, **Dg-l**, 100 μm; **Da-f**, 250 μm.
994

995 **Figure 2. The specification and differentiation of the dorsal CA1 lineage failed with the**
996 **dysplastic dorsal hippocampus in *RXCre/+*; *CI$^{F/F}$* mutant mice.**
997 **A,** In coronal sections along the rostrocaudal axis (**a-f**) and sagittal sections along the lateral-
998 medial axis (**g-l**) of the hippocampus, compared with that of control mice (**a-c**, **g-i**), the dorsal
999 hippocampus was shrunken, indicated by the star, in *COUP-TFI* gene mutant (*RXCre/+;CI$^{F/F}$*)
1000 mice at 1M (**d-f**, **j-l**). **B,** The expression of HuB and Ctip2 in the corresponding inserted area

1001 in **a-f** under a high magnification objective lens at 1M (**g-r**); compared with that of control
1002 mice (**a-c**, **g-i**, **m-o**), the HuB-positive CA3 domain was reduced in the dorsal hippocampus,
1003 especially the Ctip2-positive dorsal CA1, which was barely detected in *COUP-TFI* mutant
1004 mice at 1M (**d-f**, **j-l**), while their expression in the ventral hippocampus was comparable
1005 between the controls and mutants (**d-f**, **p-r**). **C,** The expression of HuB and Wfs1 in the
1006 corresponding inserted area in **a-f** under a high magnification objective lens at 1M (**g-r**); the
1007 expression of HuB and the dCA1 marker Wfs1 in the control (**a-c**, **g-i**, **m-o**) and *COUP-TFII*
1008 mutant mice (**d-f**, **j-l**, **p-r**) at 1M. Wfs1-positive dorsal CA1 could not be detected in *COUP-*
1009 *TFI* mutant mice at 1M, as indicated by the arrowhead. dHPC, dorsal hippocampus; HPC,
1010 hippocampus; vHPC, ventral hippocampus. Scale bars, **Aa-l**, **Ba-f**, **Ca-f,** 200 μm; **Bg-r**, **Cg-r,**
1011 100 μm.
1012
1013 **Figure 2—figure supplement 1. Defects in *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice.**
1014 **A,** Immunofluorescence staining data showed that compared with those of control mice (**a, c,**
1015 **e, g**), the proportions of either the Wfs1- or Ctip2-positive dorsal CA1 domain were reduced
1016 in *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice at 3M (**b, d, f, h**). **B,** Golgi staining showed that
1017 compared with those of controls, the numbers of branch points and secondary dendrites of
1018 both apical and basal dendrites were significantly reduced in the dorsal hippocampal CA1
1019 pyramidal neurons of *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice (**a-c**). **C,** The Morris water
1020 maze behavior test showed that compared with that of controls, spatial learning and memory
1021 were significantly damaged in *Emx1Cre/+*; *COUP-TFI*$^{F/F}$ mutant mice. Data are expressed as
1022 the mean ± SEM. *Student's t test*, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Scale bars, **Aa-h,** 100 μm;
1023 **Ba-b,** 50 μm.
1024

1025 **Figure 3. Defects in the hippocampus in *RXCre/+*; *CI$^{F/F}$*; *CII$^{F/F}$* double-gene mutant**
1026 **mice.**
1027 **A,** In coronal sections along the rostrocaudal axis, compared with control mice (**a-d**), the
1028 hippocampus was atrophic in *RXCre/+*; *CI$^{F/F}$; CII$^{F/F}$* double mutant mice, indicated by the
1029 star, and an ectopic unknown nucleus was observed in the caudal plates, indicated by the
1030 arrowhead (**e-h**). **B,** Compared with that of control mice (**a-c**, **g-i**), the expression of HuB,
1031 Ctip2, and Prox1 was decreased in the hippocampus of *COUP-TFI/-TFII* double-gene mutant
1032 mice at 3 weeks postnatal (3W) (**d-f**, **j-l**). **C,** Compared with that of control mice (**a-c**, **g-i**),
1033 the expression of HuB could not be detected in the presumptive CA3 domain, and the
1034 expression of Ctip2 or Prox1 could not be detected in the presumptive DG domain of the
1035 prospective ventral hippocampus of *RXCre/+*; *CI$^{F/F}$*; *CII$^{F/F}$* double mutant mice. Scale bars,
1036 **Aa-h**, 200 μm; **Ba-l**, **Ca-l,** 100 μm.
1037
1038 **Figure 3—figure supplement 1. Adult neurogenesis was abnormal in the hippocampi of**
1039 ***COUP-TFI/-TFII* double-gene mutant mice.**
1040 **A,** The expression of GFAP and Nestin, markers of NSCs, in the SGZ of the vDG in control
1041 and *COUP-TFII* mutant mice at 1M (**a-h**), in the SGZ of the dDG in control and *COUP-TFI*
1042 mutant mice at 1M (**i-p**) and in the SGZ of the DG in control and *COUP-TFI/-TFII* double-
1043 gene mutant mice at 3W (**q-x**). **B**, The expression of Dcx, a marker of newborn neurons, in
1044 the SGZ of the vDG in control and *COUP-TFII* mutant mice at 1M (**a-d**), in the SGZ of the
1045 dDG in control and *COUP-TFI* mutant mice at 1M (**e-h**) and in the SGZ of the DG in control
1046 and *COUP-TFI/-TFII* double-gene mutant mice at 3W (**i-l**). **C,** Quantitative analysis of
1047 GFAP/Nestin-positive cells (**a**) and Dcx-positive cells (**b**) in the SGZ of the DG in control
1048 and *COUP-TFI/-TFII* double-gene mutant mice at 3W. The numbers of GFAP and Nestin
1049 double-positive NSCs and Dcx-positive newborn neurons were significantly reduced in

1050 double-mutant mice. Data are expressed as the mean ± SEM. *Student's t test*, $^{**}P<0.01$,
1051 $^{***}P<0.001$. dDG, dorsal DG; NSC, neural stem cell; SGZ, subgranular zone; vDG, ventral
1052 DG. Scale bars, **Aa-x**, 50 μm; **Ba-l**, 100 μm.
1053
1054 **Figure 4. The impairment of hippocampal trisynaptic connectivity in *COUP-TFII***
1055 **single-gene, *COUP-TFI* single-gene, and *COUP-TFI/-TFII* double-gene mutant mice.**
1056 **A,** The expression of Calretinin, Calbindin, and SMI312 in the ventral hippocampus of the
1057 control (**a-b**, **e-f**, **i-j**) and *COUP-TFII* single-gene mutant mice (**c-d**, **g-h**, **k-l**). **B,** The
1058 expression of Calretinin, Calbindin, and SMI312 in the dorsal hippocampus of the control (**a-**
1059 **b**, **e-f**, **i-j**) and *COUP-TFI* single-gene mutant mice (**c-d**, **g-h**, **k-l**). **C,** The expression of
1060 Calretinin, Calbindin, and SMI312 in the hippocampus of the control (**a-b**, **e-f**, **i-j**) and
1061 *COUP-TFI*/*-TFII* double-gene mutant mice (**c-d**, **g-h**, **k-l**). dHPC, dorsal hippocampus; HPC,
1062 hippocampus; vHPC, ventral hippocampus. Scale bars, **Aa-l**, **Ba-l**, **Ca-l**, 100 μm.
1063
1064 **Figure 5. *COUP-TF* genes regulate the expression of key genes associated with early**
1065 **hippocampal development.**
1066 **A,** The expression profiles of genes involved in hippocampal development in control and the
1067 double mutant mice at E11.5. **B,** Compared with that of control mice (**a-b**, **a'-b', a''-b'', i-j,**
1068 **i'-j', q-r, q'-r'**), the expression of Lhx5 was reduced in double-mutant mice at E11.5 (**c-d**,
1069 **c'-d', c''-d''**), E13.5 (**k-l**, **k'-l'**) and E14.5 (**s-t**, **s'-t'**); the expression of Lhx2 was
1070 comparable between the control and double-mutant mice at E11.5 (**e-h**, **e'-h'**); and compared
1071 with that of control mice (**m-n**, **m'-n'**, **u-v**, **u'-v'**), the expression of Lhx2 was increased in
1072 double-mutant mice at E13.5 (**o-p**, **o'-p'**) and E14.5 (**w-x**, **w'-x'**). **C,** Compared with that of
1073 control mice (**a-b**, **a'-b'**), the expression of Sox2 was normal in double-mutant mice at E14.5
1074 (**c-d**, **c'-d'**); compared with that of control mice (**e-f**, **e'-f'**), the expression of Tbr2 was

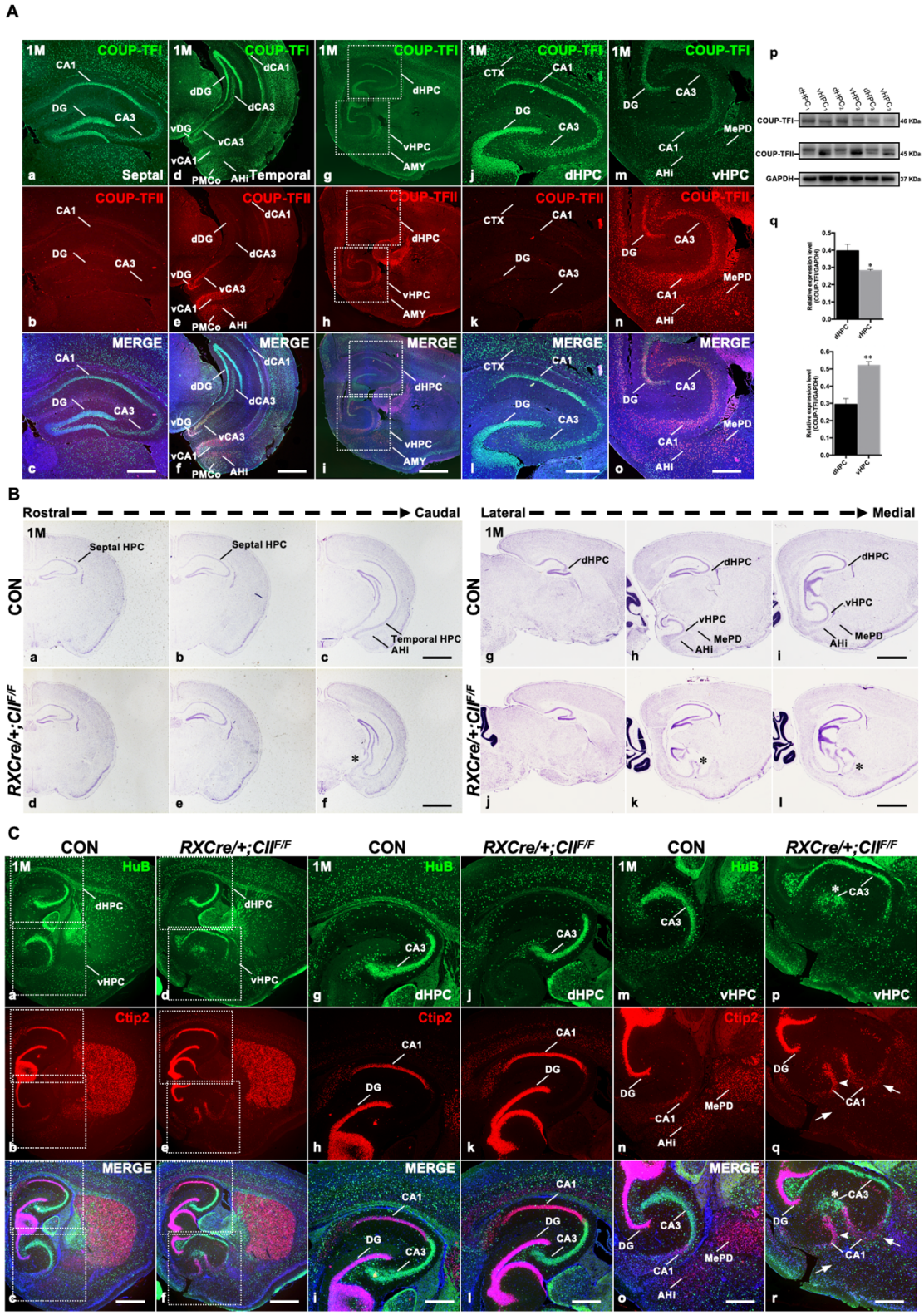
1075 decreased in *COUP-TF* mutant mice at E14.5 (**g-h**, **g'-h'**); compared with that of control
1076 mice (**i-j**, **i'-j'**), the expression of NeuroD1 was reduced in double-mutant mice at E14.5 (**k-l**,
1077 **k'-l'**). **D,** Quantitative analysis of Tbr2-positive cells and NeuroD1-positive cells in **Ce'-h'**
1078 and **Ci'-l'**. **E,** In the hippocampal primordium of the early embryo, *COUP-TFI* is expressed
1079 dorsally in the MP, and *COUP-TFII* is expressed ventrally in the CH. In the mature
1080 hippocampus, the expression of *COUP-TFI* is higher in the dorsal hippocampus, which is
1081 related to spatial learning and memory, and the expression of *COUP-TFII* is mainly in the
1082 ventral hippocampus, which is associated with emotion and anxiety (**a**). Our findings support
1083 a novel molecular mechanism by which *COUP-TFI* and *COUP-TFII* may cooperate to ensure
1084 the appropriate morphogenesis and functions of the hippocampus by modulating the *Lhx5-*
1085 *Lhx2* axis (**b**). Data are expressed as the mean ± SEM. *Student's t test*, $^{**}P<0.01$. CH, cortical
1086 hem; ChP, choroid plexus; DP, dorsal pallium; HP, hippocampal primordium; MP, medial
1087 pallium. Scale bars, **Ba-x, Ca-l**, 200 μm.

1088

1089 **Figure 5—figure supplement 1. *COUP-TFI* and *-TFII* genes coordinate to control**
1090 **distinct characteristics of the hippocampus.**

1091 Roles of *COUP-TFI* and *COUP-TFII* genes in the development and function of the
1092 hippocampus and the association with neurological diseases. *COUP-TFI* is required for the
1093 morphogenesis of the dorsal hippocampus and the specification of dorsal CA1 pyramidal
1094 neuron lineage, which are associated with neurodevelopmental disorders, including ID and
1095 ASD. *COUP-TFII* is required to prevent the duplication of the CA1 and CA3 lineages of the
1096 ventral hippocampus, which may be related to psychiatric diseases such as depression,
1097 anxiety, or schizophrenia. The *COUP-TFI* and *COUP-TFII* genes are novel intrinsic
1098 regulatory genes, which cooperate with each other to ensure the early morphogenesis of the
1099 hippocampus.

Figure 1, Yang et al.



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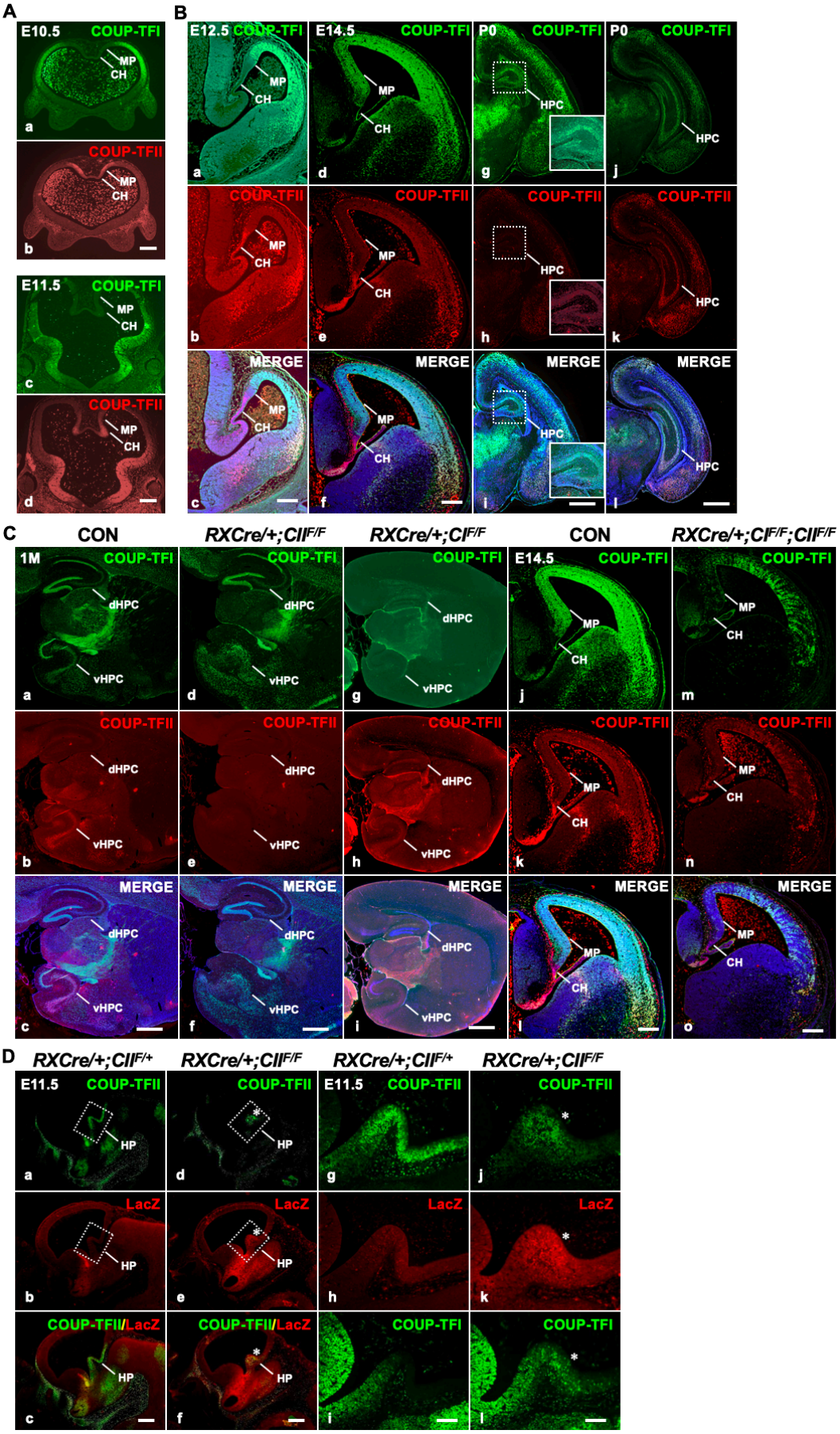
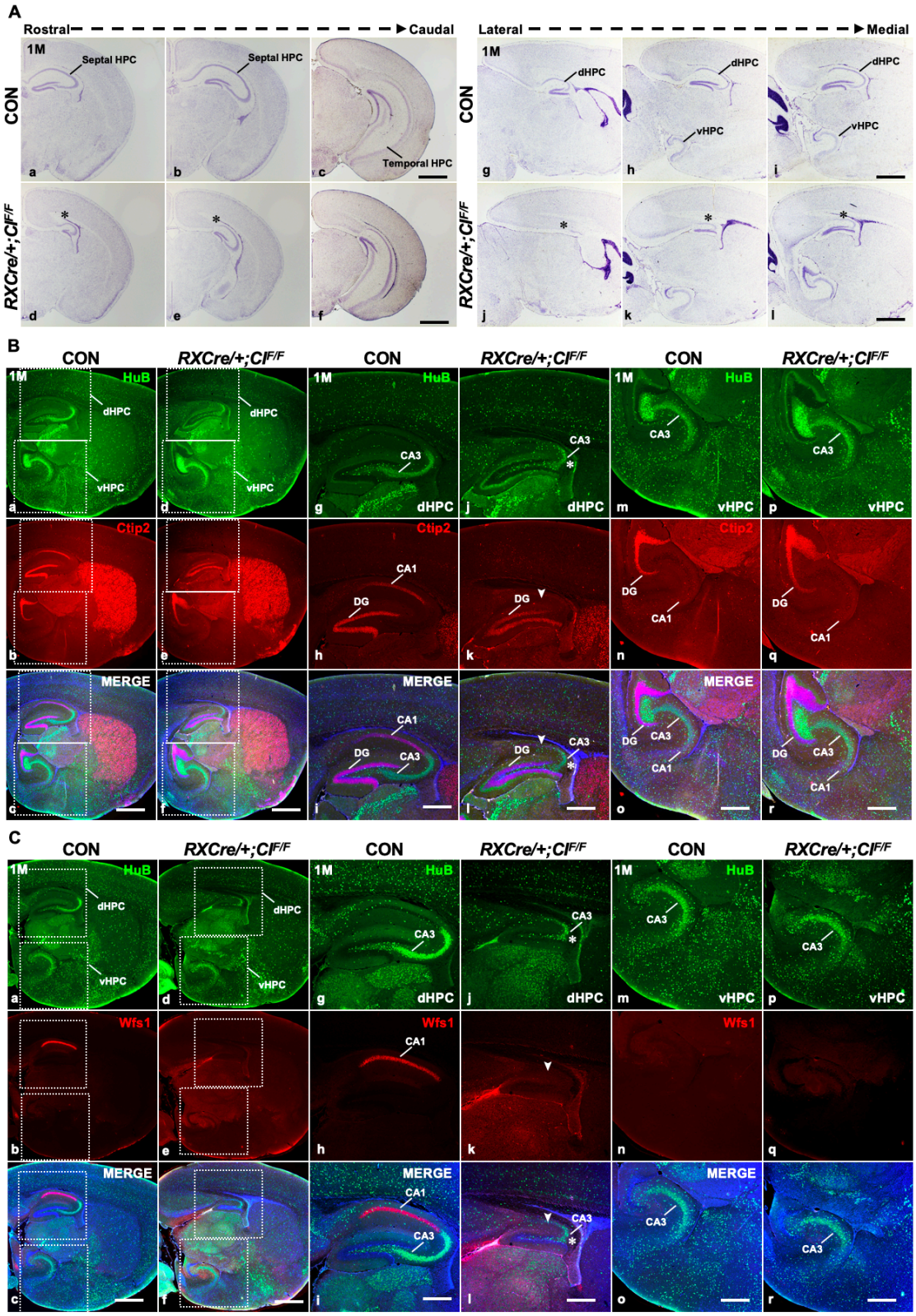


Figure 2, Yang et al.



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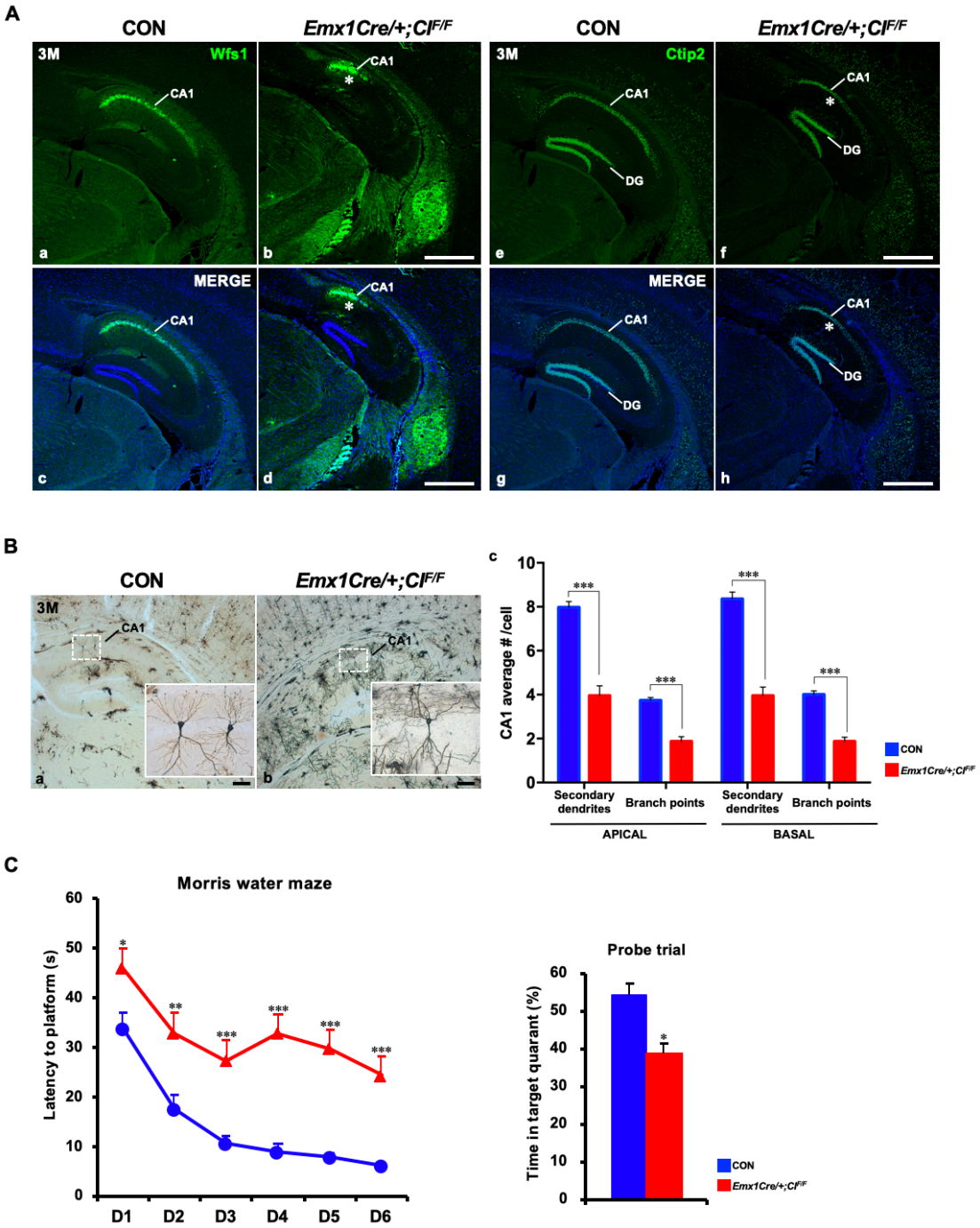

Figure 3, Yang et al.

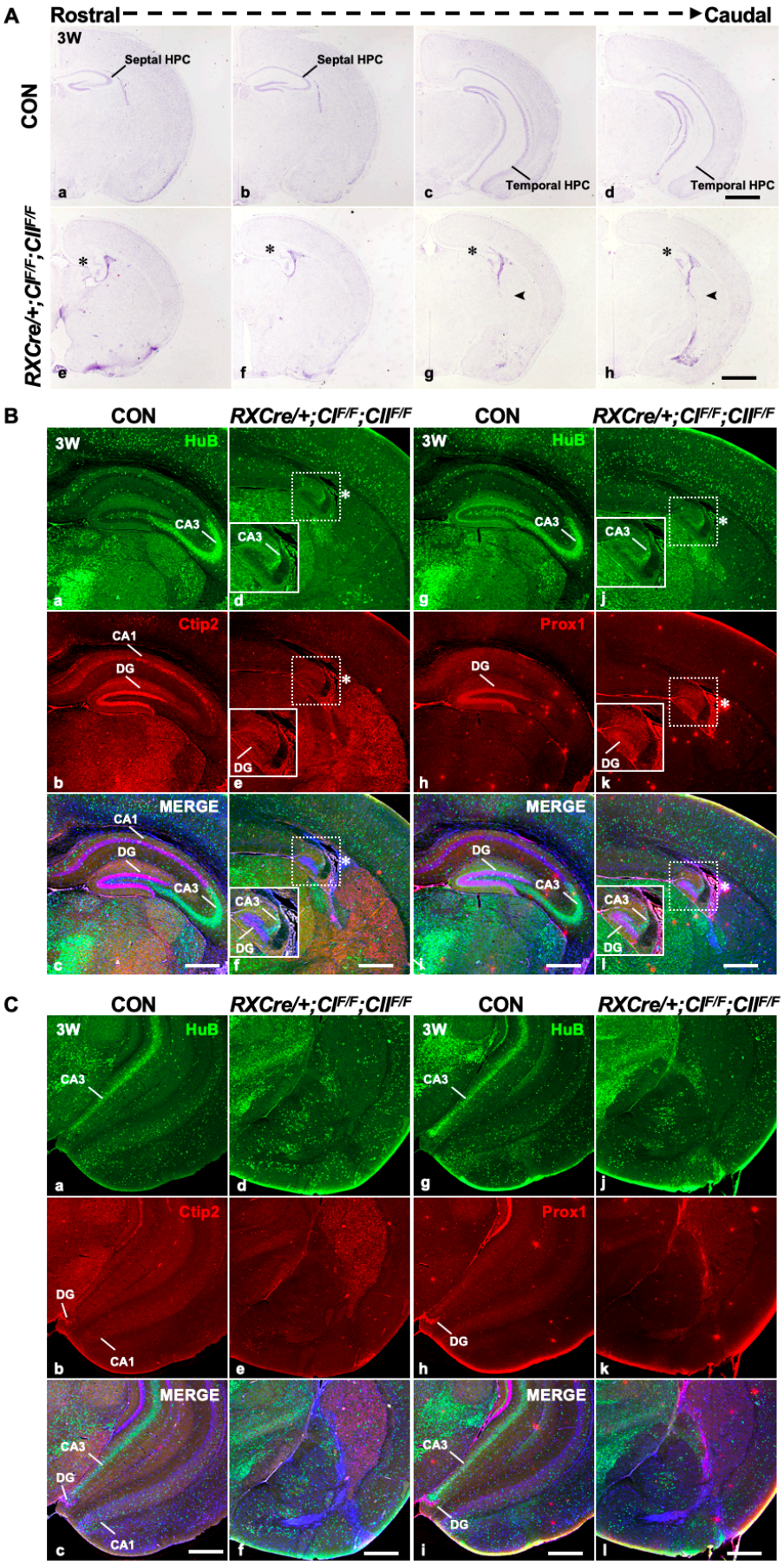

Figure 3—figure supplement 1, Yang et al.

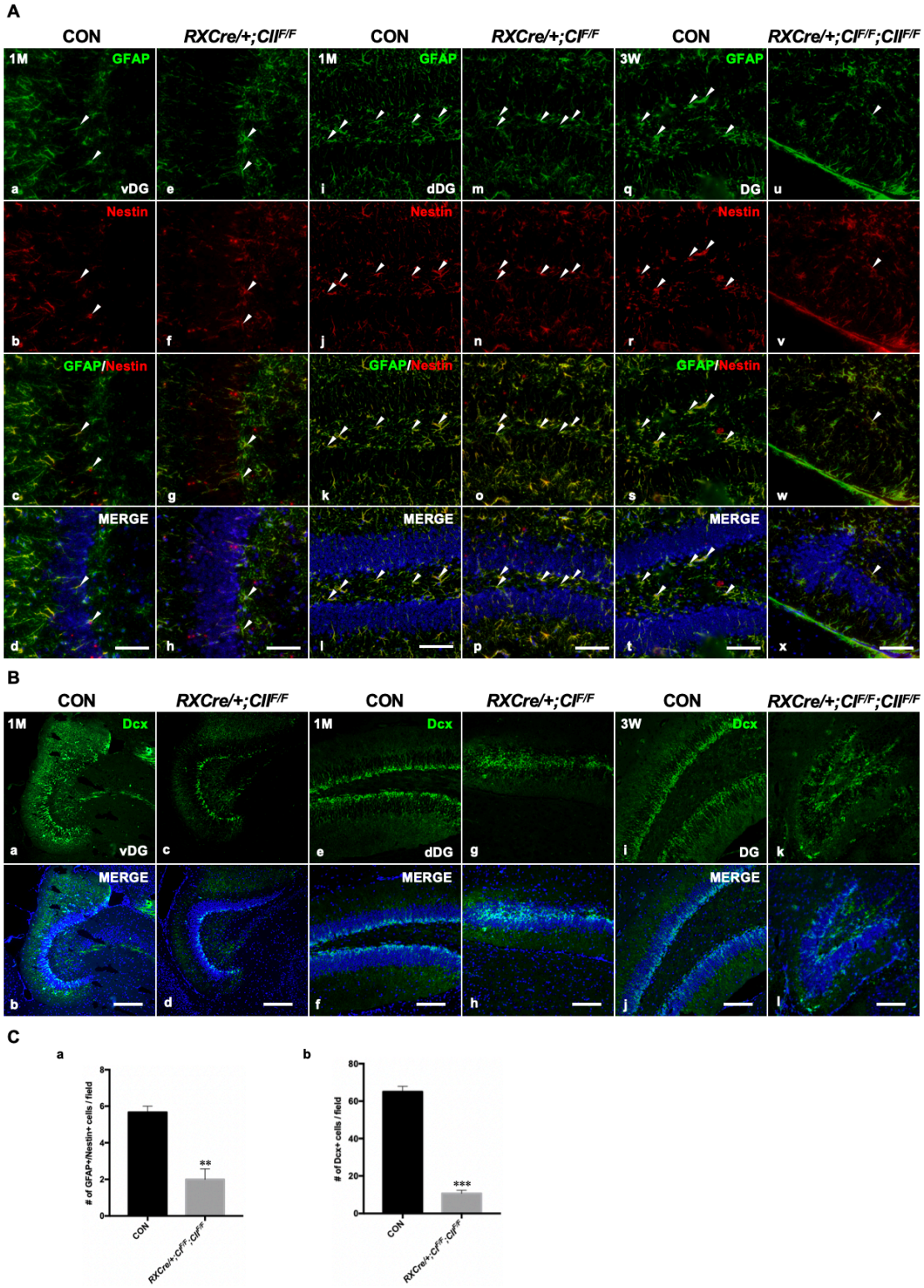

Figure 4, Yang et al.

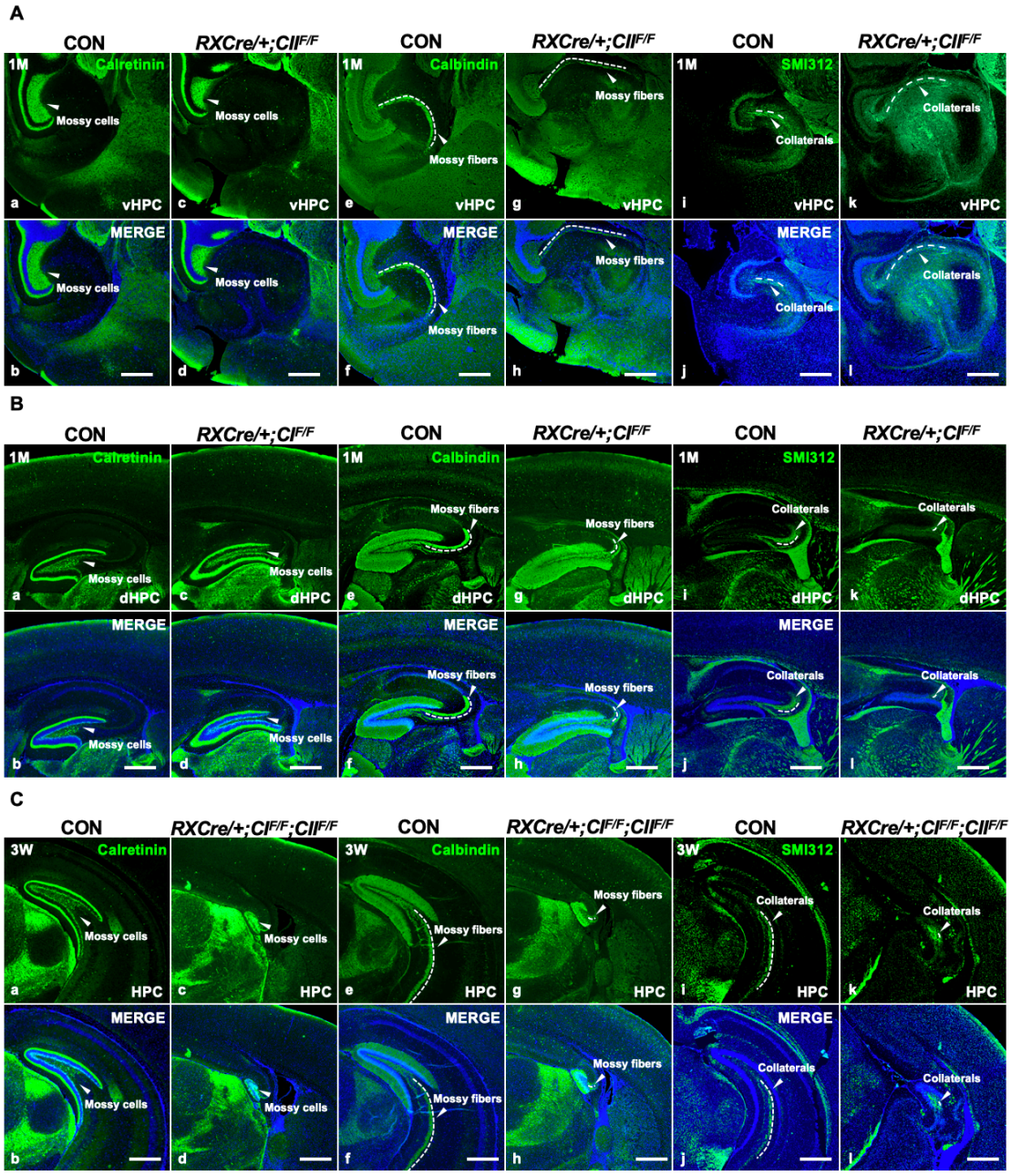

Figure 5, Yang et al.

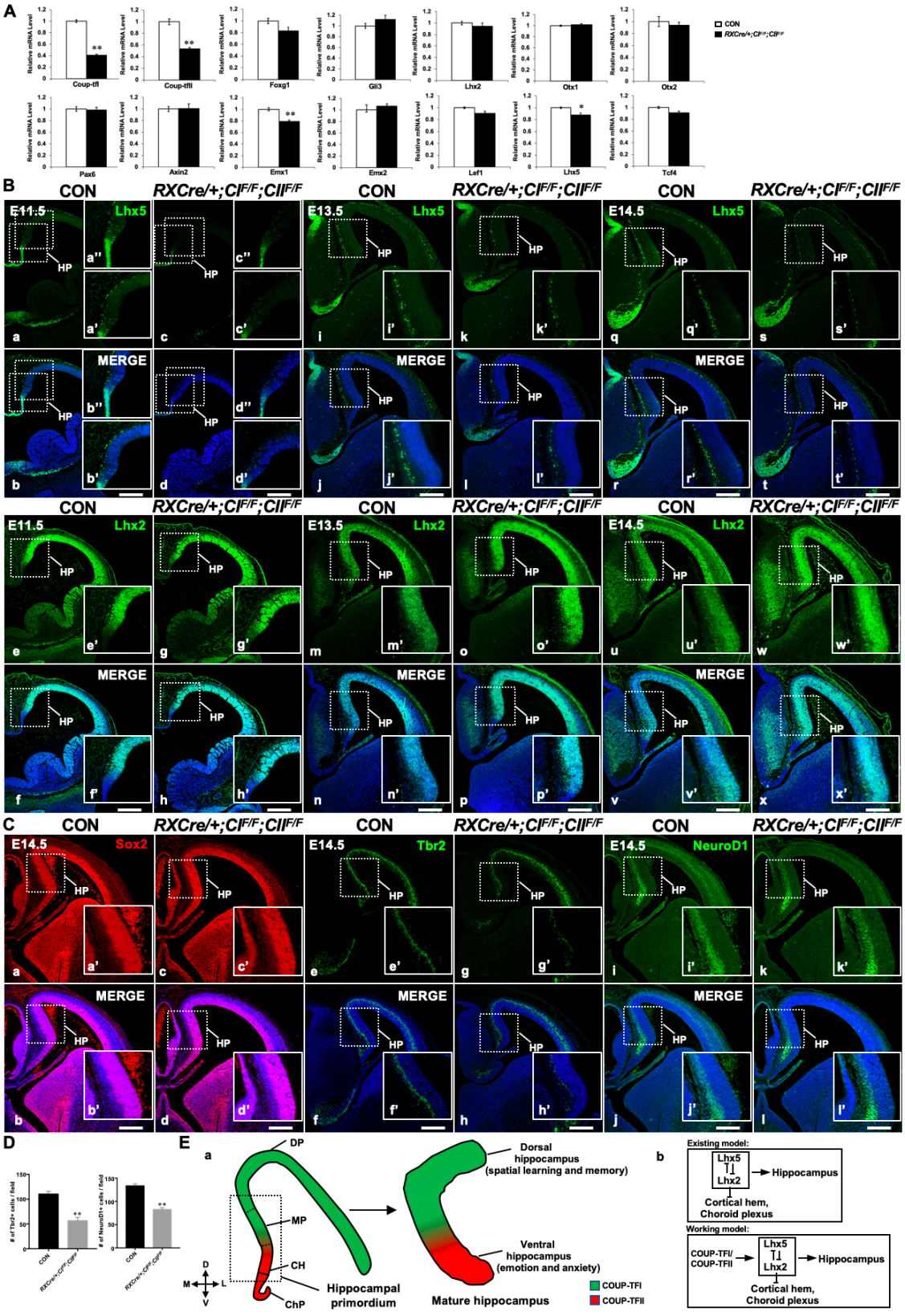
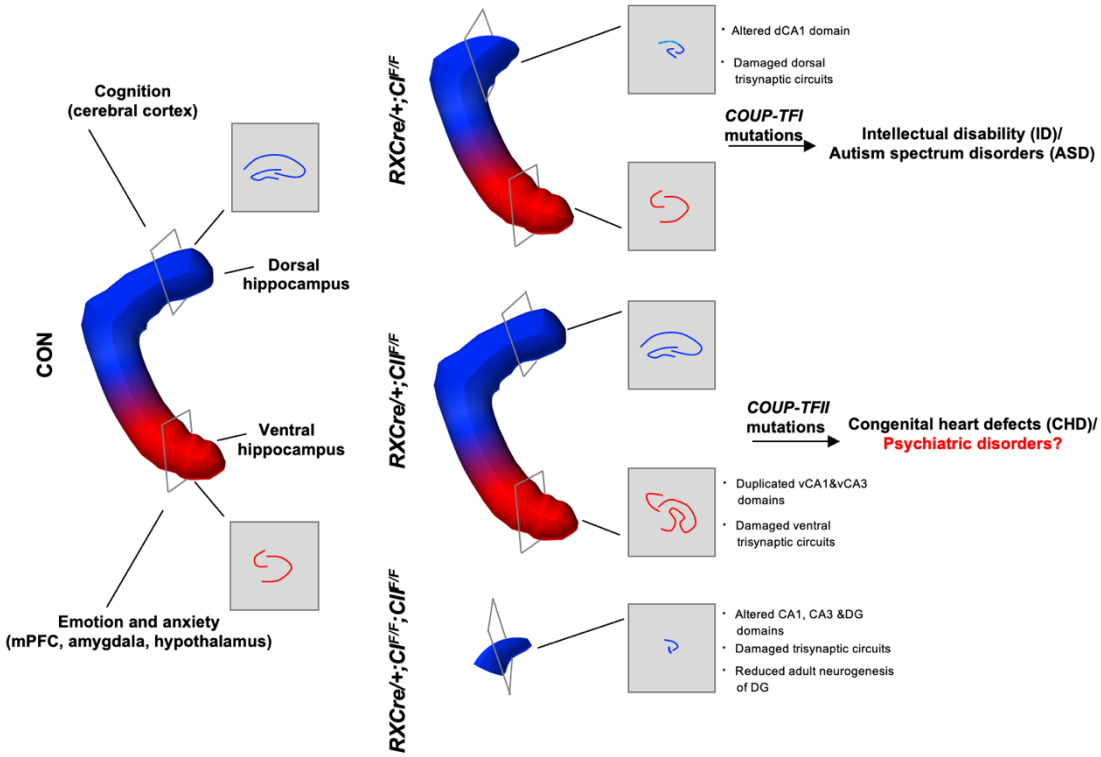
Figure 5—figure supplement 1, Yang et al.