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***Ctip1* controls acquisition of sensory area identity and establishment of sensory input fields in the developing neocortex**

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SUMMARY

While transcriptional controls over the size and relative position of cortical areas have been identified, less is known about regulators that direct acquisition of area-specific characteristics. Here, we report that the transcription factor *Ctip1* functions in primary sensory areas to repress motor and activate sensory gene expression programs, enabling establishment of sharp molecular boundaries defining functional areas. In *Ctip1* mutants, abnormal gene expression leads to aberrantly motorized corticocortical and corticofugal output connectivity. *Ctip1* critically regulates differentiation of layer IV neurons, and selective loss of *Ctip1* in cortex deprives thalamocortical axons of their receptive “sensory field” in layer IV, which normally provides a tangentially and radially defined compartment of dedicated synaptic territory. Therefore, although thalamocortical axons invade appropriate cortical regions, they are unable to organize into properly configured sensory maps. Together, these data identify *Ctip1* as a critical control over sensory area development.

INTRODUCTION

The neocortex is responsible for processing and integrating all modalities of sensory information, generating precise motor output, and orchestrating higher-order cognitive tasks, a broad range of functions made possible by its tangential organization into specialized areas with distinct cytoarchitecture, connectivity, and patterns of gene expression (Rakic, 1988). In rodents, the neocortex is organized into four primary areas: motor (M1), somatosensory

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AUTHOR CONTRIBUTIONS

L.C.G. and J.D.M. conceived the project; L.C.G. and M.B.W. designed and performed all experiments; L.C.G., M.B.W., and J.D.M. analyzed and interpreted the data; and L.C.G. and M.B.W. made figures. C.G. performed embryonic *in situ* hybridization experiments. L.C.G., M.B.W., and J.D.M. synthesized and integrated the findings, and wrote and revised the paper. All authors read and approved the final manuscript.

(S1), visual (V1), and auditory (A1). Area identity begins to be specified early in development by morphogens and signaling molecules secreted from patterning centers (O'Leary et al., 2007), which induce graded ventricular zone expression of several key transcription factors, including *Emx1/Emx2*, *Couptf1*, *Pax6*, *Sp8*, and *Tbr2* (O'Leary et al., 2007; Woodworth et al., 2012; Elsen et al., 2013). These regulators appear to determine the final relative size and general position of cortical areas (Hamasaki et al., 2004; Armentano et al., 2007; Zembrzycki et al., 2013). Recent work has demonstrated that these early specification decisions are not made exclusively at the progenitor level, and that postmitotic neurons, while partially programmed, are sufficiently plastic to fully change their area identity (Alfano et al., 2014).

Substantially less is known about molecular controls that drive differentiation of cortical neurons toward particular area identities, including expression of area-specific genes, extension of axonal projections to area-specific targets, and interactions with thalamocortical input that enable its organization into modality-specific topographic maps. Recently, two transcription factors, *Bhlhb5* and *Lmo4*, have been shown to influence acquisition of molecular area identity (Joshi et al., 2008; Sun et al., 2005). However, neither control is critical for establishment of cardinal features of area-specific connectivity, or for organization of sensory maps (Ross et al., 2012; Huang et al., 2009; Cederquist et al., 2013). A third transcription factor, *Tbr1*, is expressed by postmitotic neurons and regulates early gene expression gradients, but *Tbr1* null mice die soon after birth, complicating investigation of later functions (Bedogni et al., 2010). Taken together, these studies begin to define the molecular mechanisms that direct area-specific differentiation in the cerebral cortex, but many important questions remain to be addressed.

Here, we identify that the transcription factor *Ctip1* acts postmitotically to regulate acquisition of sensory area identity. *Ctip1* was first described as an interacting partner of COUP transcription factors (COUP-TFs), which recruit *Ctip1* to potentiate transcriptional repression (Avram et al., 2000), but it can also regulate gene expression independently of COUP-TFs by binding specific DNA sequence motifs through its zinc finger domains (Avram et al., 2002). *Ctip1* has been most extensively studied in the hematopoietic system, where it controls specification of B-cells (Liu et al., 2003), and regulates the developmental switch from γ -globin to β -globin in red blood cells (Sankaran et al., 2008; Xu et al., 2011; Bauer et al., 2013). In the nervous system, *Ctip1* regulates dorsal interneuron morphogenesis and sensory circuit formation in the spinal cord (John et al., 2012), and *in vitro* studies also support a role in hippocampal neuron dendrite outgrowth and axon branching (Kuo et al., 2010). *Ctip1* also directs cortical projection neuron migration by regulating expression of *Sema3c* (Wiegrefe et al., 2015).

We report that *Ctip1* is a critical postmitotic control over sensory area identity acquisition in the neocortex, including the establishment of sensory-specific gene expression and output connectivity, as well as the organization of thalamocortical input into sensory maps. Expression of CTIP1 in the cortical plate initially follows a high-caudomedial to low-rostralateral gradient, and is further refined postnatally to be expressed most highly in primary sensory areas. In the absence of *Ctip1* function, there is a striking failure of molecular differentiation in primary sensory areas, as indicated by absent or reduced

expression of sensory genes, and by ectopic expression of motor genes. These molecular abnormalities result in abnormal area-specific connectivity of callosal, subcerebral, and corticothalamic projection neurons. In addition, *Ctip1* regulates differentiation of layer IV neurons, and is, therefore, critical for the establishment of a receptive “sensory field” for thalamocortical input. Cortex-specific deletion of *Ctip1* deprives thalamocortical afferents of this dedicated synaptic territory, resulting in apoptosis of thalamocortical neurons in sensory thalamic nuclei and severely disrupted organization of sensory maps in cortex. In an accompanying manuscript, we report that *Ctip1* functions as a critical control over projection neuron subtype specification in deep layers (Woodworth et al., submitted to Cell Reports), thereby linking sensory area identity acquisition to the generation of area-appropriate proportions of neuronal subtypes (Ramón y Cajal, 1899; Brodmann, 1909).

RESULTS

***Ctip1* is highly expressed in primary sensory areas during development**

CTIP1 can first be detected by immunocytochemistry in the cortical plate at approximately E12.5, and, by E15.5, it is also evident in the postmitotic migratory neurons of the intermediate zone (Figure 1A–1B and Woodworth et al., submitted to Cell Reports). Notably, expression of CTIP1 initially follows a high-caudomedial to low-rostralateral gradient in both the intermediate zone and the cortical plate, with an approximately 1.5-fold difference in mRNA expression along both axes ($p < 0.01$; Figure 1A–1B; Figure S1A–S1B). Over the course of development, CTIP1 expression undergoes progressive refinement to primary sensory areas. By P7, CTIP1 expression is quite low in motor cortex, and staining becomes sparser across all layers, although areal differences between motor and sensory areas are most striking in layers VI, IV, and deeper II/III (Figure 1C–1D). In tangential sections through layer IV, CTIP1 distinctly labels primary somatosensory, visual, and auditory cortex (Figure 1E–1F). The areal boundaries of CTIP1 expression are similar to those of BHLHB5, with strongest expression of both transcription factors in primary sensory areas (Figure S1E–S1H), and are complementary to those of LMO4, which is expressed in motor cortex and higher-order visual areas (Figure S1I–S1L).

Acquisition of sensory molecular area identity is disrupted in the absence of *Ctip1*

The initial gradient and progressive tangential refinement of CTIP1 expression motivated us to investigate potential functions of *Ctip1* in acquisition of area identity. Because *Ctip1*^{-/-} mice die at P0 (Liu et al., 2003), but cortical arealization continues to unfold over the first week of postnatal development, we pursued loss-of-function studies using *Ctip1*^{fl/fl}; *Emx1-Cre* cortex-specific conditional null mice, which survive to adulthood. As a first step toward interrogating area development in the absence of *Ctip1* function in these cortex-specific mutants, we performed *in situ* hybridization (ISH) for genes with areally-restricted expression patterns.

In *Ctip1*^{fl/fl}; *Emx1-Cre* brains, expression of motor-specific genes expands into sensory areas, but sensory-specific genes do not undergo complementary caudal shifts, though they are expressed at severely reduced levels. For example, expression of *Epha7*, which is normally present in motor cortex and higher order visual areas, but excluded from

somatosensory cortex (Figure 2A), expands to fill this “gap” (Figure 2B). Conversely, expression of *EfnA5*, normally quite high in somatosensory cortex, is strikingly and specifically reduced (Figure 2C–2D). Expression of the cell adhesion molecule *Cdh6* expands caudally into higher order visual areas in layer I/III, while expression of the transcription factor *Id2* is lost in layer II/III in the same territory (Figure 2E–2H). *Cyp26b1* and *Bhlhe40*, both of which are normally restricted to motor cortex, undergo striking caudal expansions in their expression domains (Figure 2I–2J, 2M–2N). In contrast, expression of *Mdga1* and *Cux1* in somatosensory cortex, is severely reduced, but does not appear to be shifted to more caudal positions (Figure 2K–2L, 2O–2P). Overall, primary sensory areas are partially motorized, with motor-specific genes expanding caudally and sensory areas failing to express sensory-specific genes at appropriately high levels.

To monitor acquisition of molecular area identity globally throughout cortex, we performed whole-mount β -galactosidase staining on brains carrying *LacZ* reporter alleles for either *Bhlhb5* or *Lmo4* (Feng et al., 2006; Deng et al., 2010). In wild-type mice at P7, *Bhlhb5* is expressed only in primary sensory areas (Figure 2S), while *Lmo4* is highly expressed rostrally in motor cortex, medially in cingulate cortex, and caudally in higher-order visual areas, but is excluded from primary sensory areas, with sharp boundaries between these regions (Figure 2Q). Strikingly, in *Ctip1* conditional null mice, expression of *Bhlhb5* becomes faint and diffuse, such that primary somatosensory, visual, and auditory cortex are no longer discernible (Figure 2T). The boundary between sensory and motor cortex is still present, and its position is not shifted either rostrally or caudally. In contrast, expression of *Lmo4* in motor and cingulate cortex expands into somatosensory territory, such that the boundary between them shifts caudally (Figure 2R). Importantly, establishment of molecular boundaries is comparatively preserved in the absence of either *Lmo4* or *Bhlhb5* function, using the same reporter alleles in *Lmo4^{fl/fl};Emx1-Cre* and *Bhlhb5^{fl/fl};Emx1-Cre* mice (Figure S2Q–S2V). Taken together, our data indicate that *Ctip1* acts after the initial positions of cortical areas have been at least partially established, and plays a critical role repressing motor identity and driving sensory differentiation.

To more rigorously examine gene expression changes, we performed RNA-seq on microdissected cortical areas. First, we compared gene expression between microdissected frontal (motor) and parietal (somatosensory) cortex in wild-type mice at P4, to prospectively identify genes more highly expressed in each area, and designated these differentially expressed transcripts M1 genes and S1 genes, respectively (Figure 2U; Table S1). We then compared gene expression between microdissected wild-type parietal cortex and *Ctip1^{fl/fl};Emx1-Cre* parietal cortex, and specifically examined how loss of *Ctip1* function affects expression of M1 genes and S1 genes (Figure 2V; Table S2). Of 329 M1 genes abnormally expressed in parietal cortex of *Ctip1* conditional nulls, 298 are upregulated and 31 are downregulated. Conversely, of 238 S1 genes abnormally expressed in parietal cortex of *Ctip1* conditional nulls, 210 are downregulated and 28 are upregulated. These data strongly reinforce the conclusion that *Ctip1* acts in somatosensory cortex to repress expression of motor genes and activate expression of sensory genes.

***Ctip1* functions cell-autonomously to establish sensory-area-specific callosal connectivity**

Callosal projection neurons (CPN) extend axons to mirror-image locations in corresponding cortical areas of the contralateral hemisphere, enabling highly organized inter-hemispheric transfer and integration of motor, somatosensory, visual, and auditory information (Richards et al., 2004). Because *Ctip1* is highly expressed by CPN, and because CPN molecular area identity is severely disrupted in *Ctip1* mutants, we hypothesized that *Ctip1* might be important for establishment of areally precise connectivity by sensory cortex CPN. We first investigated the effects of global loss of *Ctip1* function throughout cortex by focally injecting adeno-associated virus expressing EGFP (AAV-EGFP) into somatosensory cortex of *Ctip1^{fl/fl};Emx1-Cre* and control mice (Figure S3). In *Ctip1^{fl/fl};Emx1-Cre* brains, CPN axons from S1 continue to project to approximately correct coordinates. However, the distinct clusters of innervation to the forelimb (S1FL) and upper lip (S1ULp) regions seen in wild-type mice are no longer discernible and innervation becomes more uniform across the target field. This lack of precision in targeting might result from a corresponding lack of definition in the expression gradients of axon guidance molecules, such as *Epha7*, *Efna5*, and *Cdh6* (Figure 2A–2F).

Because any gradients controlling homotypic connectivity would change symmetrically in the absence of *Ctip1* function across the cortex, affecting both CPN and their contralateral targets, CPN targeting is less sharply defined, but relatively intact, as we observed in the experiments described above. Therefore, in a second set of experiments, we disrupted *Ctip1* expression unilaterally, to investigate whether this might create an areal mismatch between the two hemispheres, resulting in more aberrant re-routing of CPN axons. We electroporated *Cre* and floxed(STOP)-*EGFP* expression constructs into the ventricular zone of wild-type and *Ctip1^{fl/fl}* embryos at E14.5, and collected brains with matched S1 electroporations at P7 (Figure 3A). In control experiments, *Cre*-electroporated wild-type CPN project almost exclusively homotypically to S1, with distinct clusters of innervation in S1FL and S1ULp (Figure 3B). In striking contrast, *Cre*-electroporated *Ctip1^{fl/fl}* CPN project across the entire medio-lateral extent of the contralateral hemisphere, without distinct clusters at S1FL and S1ULp (Figure 3C). Innervation of S1FL and S1ULp by S1 CPN lacking *Ctip1* is significantly reduced, and their axons are misrouted to M1/2 and InsCx ($p < 0.05$ in each region; Figure 3D–3F). Taken together with our gene expression data, these results indicate that *Ctip1* is critical for proper areal specification of somatosensory cortex CPN, enabling precise targeting and refinement of connectivity between homotypic locations in the two cerebral hemispheres.

***Ctip1* represses motor identity in sensory area corticothalamic projection neurons**

The strong differential expression of CTIP1 between motor and sensory cortex in layer VI prompted us to investigate whether *Ctip1* might also direct acquisition of sensory area identity by corticothalamic projection neurons (CThPN), instructing them to establish connections with sensory thalamic nuclei. To investigate this connectivity, we anterogradely labeled CThPN axons from M1 and S1 of *Ctip1^{fl/fl};Emx1-Cre* and wild-type pups at P1 (Figure 4A) and examined thalamic sections at P7. In wild-type mice, motor CThPN project to the ventral lateral nucleus (VL), while somatosensory CThPN project to the ventral posterior nucleus (VP), with little overlap between the axons of these two populations

(Figure 4B–4C). In *Ctip1^{fl/fl};Emx1-Cre* mice, motor CThPN project normally to VA/VL (Figure 4D–4E), consistent with the lack of significant molecular abnormalities in motor cortex. In contrast, although a subset of somatosensory CThPN still project to VP in the absence of *Ctip1* function, many aberrantly innervate VL, leading to a striking increase in the overlap of somatosensory and motor cortical input in motor thalamus ($p < 0.01$; Figure 4F), consistent with *Ctip1* functioning to repress motor identity in sensory areas. To directly investigate acquisition of molecular area identity by CThPN, we examined genes specifically expressed in either S1 or M1 of layer VI. We find that, in the absence of *Ctip1* function, there is a striking expansion in the expression of motor-specific CThPN genes (Figure S4A–F), with a reciprocal reduction in expression of sensory CThPN genes (Figure S4G–S4H). Taken together, these data indicate that *Ctip1* is required to repress motor identity in sensory CThPN.

In contrast, anterograde labeling of S1 and V1 (Figure 4G) reveals proper segregation of somatosensory and visual axons in both wild-type and *Ctip1^{fl/fl};Emx1-Cre* brains, with somatosensory CThPN projecting to VP and visual CThPN projecting to the dorsolateral geniculate nucleus (Figure 4H–4K) and no significant difference in the overlap of somatosensory and visual input (Figure 4L). These results are consistent with the equivalently high levels of CTIP1 expression by CThPN in S1 and V1, which point toward a common function across all primary sensory areas. Expression of CTIP1 is necessary to prevent somatosensory CThPN from establishing connections with motor thalamic nuclei, but not for somatosensory and visual CThPN to establish connections with appropriate modality-specific sensory thalamic nuclei. Taken together, these data support the interpretation that *Ctip1* is specifically required for the broad distinction between CThPN specialized for motor output and those specialized for sensory output, but is not required for CThPN to become specialized for distinct sensory modalities.

***Ctip1* represses motor identity in sensory area subcerebral projection neurons**

Initially, subcerebral projection neurons (SCPN) in all areas of cortex send axons to the spinal cord, establishing collaterals to midbrain, pontine, and cerebellar nuclei along the way. This connectivity undergoes extensive pruning in the early postnatal period (P1–P21), such that SCPN in motor cortex maintain projections to the spinal cord and to the deep layers of the superior colliculus, while SCPN in visual cortex prune their projections to the spinal cord, and maintain projections to the superficial layers of the superior colliculus (Stanfield et al., 1982). Interestingly, while CTIP1 is progressively excluded from SCPN in motor cortex (Woodworth et al., submitted to Cell Reports), it continues to be expressed at low levels by SCPN in somatosensory and visual cortex, indicating that it might function in area-specific SCPN collateral pruning. At P4, only $6 \pm 2\%$ of SCPN in motor cortex express CTIP1, while $73 \pm 3\%$ of SCPN in sensory cortex express CTIP1 (Figure S4I–S4K).

To investigate the hypothesis that *Ctip1* might repress motor identity in sensory SCPN, we anterogradely labeled M1 and V1 SCPN in wild-type and *Ctip1^{fl/fl};Emx1-Cre* mice (Figure 4M). In wild-type mice, motor cortex SCPN project to the spinal cord and to the deep layers of the superior colliculus (Figure 4N–4R), and this precise area-specific connectivity is also evident in *Ctip1^{fl/fl};Emx1-Cre* mice (Figure 4S–4W). Visual cortex SCPN rarely extend

axons beyond the midbrain in wild-type mice (Figure 4P'-4Q'). In striking contrast, they aberrantly project into pons, through the medulla, and enter the dorsal corticospinal tract in *Ctip1^{fl/fl};Emx1-Cre* mice (Figure 4U'-4V'). We quantified this phenotype by retrogradely labeling from the dorsal corticospinal tract at P21: an equal number of neurons in motor cortex maintain spinal projections in the absence of *Ctip1* function (Figure S4L-S4N), but there is a five-fold increase in somatosensory cortex ($p < 0.05$), and a 20-fold increase in visual cortex ($p < 0.01$; Figure S4O-S4T). These results clearly indicate that output connectivity of somatosensory and visual cortex SCPN becomes partially motorized in the absence of *Ctip1* function. Further, expression of genes specific to SCPN in motor areas increases significantly in *Ctip1^{fl/fl};Emx1-Cre* sensory areas compared with wild-type ($p < 0.01$; Figure S4U-Z). These data indicate that SCPN in sensory cortex become motorized in the absence of *Ctip1* function, and that this change in their transcriptionally specified area identity causes corresponding changes in connectivity.

Postnatal overexpression of *Ctip1* is sufficient to repress motor identity in subcerebral projection neurons

We next investigated whether *Ctip1* is sufficient to repress motor identity in postmitotic SCPN, performing gain-of-function *in utero* electroporation experiments. Because CAG-promoter-driven overexpression of *Ctip1* starting in progenitors drastically reduces SCPN specification (Woodworth et al., submitted to Cell Reports), we used a tamoxifen-inducible form of Cre recombinase (*ERT2-Cre-ERT2*) to initiate expression of *EGFP* and/or *Ctip1* at P2, after subtype identity has already been established (Figure S5A-S5C). We find that postnatal misexpression of *Ctip1* in motor cortex dramatically changes the pruning decisions of SCPN in this area, which normally do not express *Ctip1* during early postnatal development. While most SCPN electroporated with *EGFP* maintain their spinal projections at P21 (Figure 5D-5G), remarkably few *EGFP/Ctip1*-electroporated SCPN maintain their spinal projections at P21 (Figure 5H-5K). This four-fold reduction in projections ($p < 0.01$; Figure 5L) indicates that downregulation of *Ctip1* by SCPN in motor cortex during normal development is necessary to maintain a spinal projection. Together, our loss- and gain-of-function data demonstrate that *Ctip1* represses motor identity in sensory area SCPN, instructing appropriate gene expression and final patterns of connectivity.

Thalamocortical input fails to organize into sensory maps in the absence of cortical *Ctip1* function

Because sensory areas undergo striking motorization of output connectivity in the absence of *Ctip1* function, we next examined whether organization of sensory thalamocortical input is also affected. Using serotonin (5HT) immunohistochemistry, we stained thalamocortical afferents, revealing sensory maps on tangential sections through layer IV of cortex. In wild-type brains, the triangular visual map is positioned in occipital cortex, and parietal cortex contains the somatotopic representations of the hindlimb, forelimb, and lower jaw, as well as the field of vibrissal barrels, arranged precisely to reflect the positions of whiskers on the snout (Figure 6A). In *Ctip1^{fl/fl};Emx1-Cre* mice, the entire sensory map is strikingly disorganized (Figure 6B). Blurry and imprecise representations of the hindlimb, forelimb, and lower jaw are still recognizable, but there are no similarly identifiable remnants of the visual map or the posteromedial barrel subfield. In spite of this severe disorganization, the

overall size of the sensory and motor domains does not change (Figure 6C). Importantly, areal refinement of *Rorb* and *Cdh8* expression, which depends on thalamocortical input (Chou et al., 2013; Vue et al., 2013), continues to reflect the overall configuration of sensory maps in *Ctip1^{fl/fl};Emx1-Cre* mice (Figure S6A–S6E). These data demonstrate that cortex-specific deletion of *Ctip1* is sufficient to dramatically disrupt thalamocortical organization into sensory maps, but does not affect the relative size of the specified sensory and motor domains, or the ability of cortical neurons to modify their gene expression in response to signals from thalamocortical axons.

We next investigated the possibility that abnormal subplate development in *Ctip1^{fl/fl};Emx1-Cre* mice might impair sensory map formation by disrupting navigation or topographic organization of thalamocortical axons *en route* to cortex, as successful invasion and preordering are both critical for subsequent map formation (Lokmane et al., 2013). First, we examined the earliest stages of thalamocortical development by genetically labeling sensory thalamic nuclei in E14.5 wild-type and *Ctip1* null embryos using *Gbx2-CreERT2*. These anterograde labeling experiments demonstrate that thalamocortical axons project to cortex along a normal trajectory, and that a comparable number have reached the cortical plate at E14.5 in both wild-type and *Ctip1* null mice (Figure S6F–S6J). We also directly visualized thalamocortical input at P1 on flattened cortices. In wild-type mice, diffuse 5HT staining roughly delineates presumptive V1, A1, and S1 (Figure S6K). Although organization of thalamocortical input is already less defined in *Ctip1^{fl/fl};Emx1-Cre* brains at P1, the boundary between the motor and sensory domains remains sharp (Figure S6L). These data indicate that thalamocortical axons successfully reach appropriate regions of cortex in the absence of cortical *Ctip1* function, but are subsequently unable to refine into topographically-organized sensory maps.

To address the possibility that *Ctip1* function in *Emx1*-expressing cells outside the cerebral cortex might cause or contribute to this phenotype, we investigated formation of sensory maps in the brainstem and thalamus. We find that brainstem and thalamic sensory maps are indistinguishable in wild-type and *Ctip1^{fl/fl};Emx1-Cre* brains (Figure 6D–6I), although the VP nucleus of thalamus is smaller in *Ctip1^{fl/fl};Emx1-Cre* brains, making fine details more difficult to discern. Thalamic nuclei are normally sized in *Ctip1^{fl/fl};Emx1-Cre* brains at P0, but shrink approximately 40% by P7 (Figure 6J–6N; $p < 0.01$), possibly due to atrophy via top-down remodeling (Zembrzycki et al., 2013). Indeed, there is increased cell death by apoptosis in sensory, but not motor, thalamic nuclei (Figure 6O–6S and data not shown). Taken together, these data indicate that, despite reaching appropriate cortical areas, sensory thalamocortical axons are unable to organize into topographic maps in the absence of cortical *Ctip1* function.

***Ctip1* regulates differentiation of layer IV neurons, and is necessary for their proper integration into barrels**

Thalamocortical axons interact extensively with layer IV neurons as they organize into precise topographic representations within a permissive sensory cortical field established under precise regulation by cortex-intrinsic transcriptional programs. We hypothesized that sensory maps might fail to form in *Ctip1^{fl/fl};Emx1-Cre* mice due to abnormal development

of this permissive sensory field more broadly, and of layer IV neurons in particular, since nearly all layer IV granule neurons in barrel cortex express *Ctip1* (83±3%; Figure S7A–S7C). In the absence of *Ctip1* function, cytoarchitectural organization of layer IV neurons in barrel cortex is entirely lost (Figure 7A–7D). Although layer IV remains readily identifiable by *Rorb* expression in coronal sections, we find strikingly reduced or entirely absent expression of other layer IV-specific genes (Figure 7E–7L). Concomitantly, expression of several genes that are normally excluded from layer IV markedly increases (Figure S7D–S7I). To investigate whether these molecular abnormalities affect compartmentalization of input within cortex, we combined 5HT immunostaining, to label thalamocortical input, with contralateral injection of AAV-EGFP, to label callosal input (Figure 7M). In wild-type mice, thalamocortical input clusters into distinct barrels, while callosal input from contralateral S1 is clearly excluded from the barrel compartment (Figure 7N–7R). In *Ctip1^{fl/fl};Emx1-Cre* mice, thalamocortical input is still restricted to layer IV, though barrels are absent and staining is markedly reduced; even more strikingly, there is robust callosal input to layer IV (Figure 7P–7R). These data indicate that *Ctip1* regulates expression of layer IV-specific genes, and is required for layer IV neurons to select specific sensory thalamocortical input over contralateral cortical input and to successfully establish the appropriate cytoarchitectural organization to receive this input.

To gain further insight into the abnormal development of layer IV neurons in *Ctip1^{fl/fl};Emx1-Cre* mice, we investigated the effects of cell-autonomous loss of *Ctip1* function. For these experiments, we electroporated *CAG-Cre* and *CAG-floxed(STOP)-EGFP* expression constructs into somatosensory cortex of *Ctip1^{fl/fl}* embryos or wild-type controls at E14.5 to target layer IV neurons (Figure 7S). *Cre*-electroporated neurons in wild-type brains adopt an unbiased distribution across barrel cortex, although more electroporated neurons are present in cell-dense barrel walls (Figure 7T). Remarkably, *Cre*-electroporated neurons in *Ctip1^{fl/fl}* brains completely avoid barrels, and instead position themselves exclusively between barrels, within the septae (Figure 7U). In addition, while most wild-type layer IV neurons orient their dendrites toward the center of each barrel to synapse with thalamocortical axons (Figure 7W, 7V), the dendritic trees of *Ctip1* null neurons accumulate in septae, completely avoiding barrels (Figure 7X, 7V). We also performed electroporations at E13.5 to evaluate radial positioning of electroporated neurons across layers. While *Cre*-electroporated neurons in wild-type brains found at high density in layer IV, *Cre*-electroporated neurons in *Ctip1^{fl/fl}* brains primarily found deep or superficial to layer IV, with the few neurons in layer IV residing primarily between barrels, within the septae (Figure S7O–S7Q). Importantly, mosaic loss of *Ctip1* function in a subset of layer IV neurons does not interfere with the ability of thalamocortical axons to organize into properly configured sensory maps (Figure S7J–S7N). These experiments indicate that *Ctip1* function is necessary for the integration of layer IV neurons into barrels, and for their assembly into the circuits that receive sensory information from thalamocortical afferents.

***Ctip1* functions in layer IV to establish a permissive cortical field for sensory map formation**

To determine whether *Ctip1* acts directly downstream of transcriptional programs that promote specification of sensory area identity, we investigated its genetic interactions with

Couptf1 (Armentano et al., 2007; Alfano et al. 2014). Most CTIP1-positive neurons in sensory areas also express COUPTF1 (Figure S8A–S8E; 71±4%), suggesting that *Couptf1*, which is expressed in progenitors, might activate expression of *Ctip1*. To test this hypothesis, we analyzed CTIP1 immunostaining in *Couptf1^{fl/fl};Emx1-Cre* mice, and find that it is reduced in areas that have been respecified to acquire motor identity, but preserved in the caudally displaced sensory domain (Figure S8F–S8G). These data indicate that *Ctip1* expression is responsive to changes in area identity specification, but is expressed within the sensory domain independently of *Couptf1* function. Conversely, in *Ctip1^{fl/fl};Emx1-Cre* mice, COUPTF1 expression is clearly preserved, though at somewhat reduced levels (Figure S8H–S8I). These data indicate that regulation of sensory area identity acquisition by *Ctip1* increases *Couptf1* expression levels.

Since neither transcription factor is required for expression of the other, we investigated their epistatic relationship by generating double conditional null mice (*Ctip1^{fl/fl};Couptf1^{fl/fl};Emx1-Cre*). We focused on sensory map formation, because the single conditional mutants have largely non-overlapping phenotypes, with caudal displacement of thalamocortical input in *Couptf1^{fl/fl};Emx1-Cre* mice (Figure 8B, 8F, 8G; Armentano et al., 2007; Alfano et al. 2014), but not in *Ctip1^{fl/fl};Emx1-Cre* mice (Figure 6B), and a dramatic failure in organization of topographic maps in *Ctip1^{fl/fl};Emx1-Cre* mice, but not in *Couptf1^{fl/fl};Emx1-Cre* mice. Interestingly, combined loss of cortical *Ctip1* and *Couptf1* function results in a largely additive phenotype, with thalamocortical input both caudally displaced and unable to organize into topographic maps (Figure 8C, 8H, 8I). Taken together, these data suggest that *Couptf1* and *Ctip1* have largely independent functions, with *Couptf1* determining the size and position of the sensory domain, and *Ctip1* regulating subsequent differentiation within that domain.

To investigate whether the requirement for *Ctip1* function in sensory map formation is primarily related to its function in layer IV neuron differentiation, we performed *in utero* electroporation surgeries to restore *Ctip1* expression exclusively in superficial layers of *Ctip1^{fl/fl};Emx1-Cre* cortex (Figure 8J). In control *Egfp*-only experiments, thalamocortical afferents established substantially disorganized maps, with rudimentary somatotopic representations, but no recognizable barrel field, as expected (Figure 8K–8M). Remarkably, however, *Ctip1* expression in superficial layers was sufficient to rescue organization of thalamocortical afferents into precisely arranged rows of distinctly formed barrels in the most densely electroporated portion of somatosensory cortex (Figure 8N–8P). In combination with our earlier data on layer IV neuron differentiation, these experiments demonstrate that *Ctip1* acts cell-autonomously to establish a receptive field of dedicated synaptic territory in layer IV that enables thalamocortical afferents to organize into properly configured sensory maps.

DISCUSSION

Elucidating transcriptional programs that direct specialization of cortical areas is critical for understanding the emergence of area-specific connectivity, cytoarchitecture, and function, as well as the diversification of neuronal subtypes and their precise differentiation during cortical development. We report here that *Ctip1* is highly expressed in primary sensory

areas, and that it is a critical control over acquisition of sensory molecular area identity (Figure 8Q). By repressing motor programs, *Ctip1* prevents projection neurons in sensory cortical areas from establishing aberrant connections to motor-specific targets. In addition, *Ctip1* regulates differentiation of layer IV neurons and their integration into thalamorecipient circuits, which is critical, in turn, for organization of thalamocortical afferents into appropriately-configured topographic maps. Taken together, our findings indicate that *Ctip1* acts across multiple neuronal subtypes to coordinately regulate sensory-specific gene expression, thus linking output connectivity, cytoarchitectural organization, and sensory map formation during development.

Postmitotic controls intersectionally regulate acquisition of molecular area identity

Genes belonging to diverse functional categories are expressed in an area-specific fashion, including transcriptional regulators (e.g., *Id2* and *Rorb*), cell adhesion molecules (e.g., *Cdh6* and *Cdh8*), intracellular signaling molecules (e.g., *Plcb1*), and axon guidance molecules (e.g., *Epha7* and *Efna5*) (Greig et al., 2013). Although they are useful readouts of area identity, the complex expression patterns of these genes represent the final outcome of intersectional regulation by multiple transcriptional programs acting in specific cortical layers and areas, rather than directly illuminating the basic organizational principles governing cortical arealization. The identification of transcriptional controls over postmitotic acquisition of area identity will enable deconstructing this complex regulatory network into individual “developmental vectors” acting within defined tangential and laminar domains to intersectionally establish the more complex expression patterns of downstream genes.

We show here that *Ctip1* is critical for the establishment of molecular boundaries between motor and sensory areas, which are comparatively preserved in the absence of either *Lmo4* or *Bhlhb5* function (Figure S2). Broadly speaking, our RNA-seq data demonstrate that *Ctip1* acts within the sensory domain to repress expression of motor genes and to activate expression of sensory genes (Figure 2U–2V). However, individual genes demonstrate complex patterns of misexpression in the absence of *Ctip1*. For example, *Lmo4* expands from motor and cingulate cortex into somatosensory territory, and recedes from higher-order visual areas into a narrow caudal band (Figure 2Q–2R). These symmetrically opposite changes suggest that *Lmo4* expression in motor and cingulate cortex might be established by mechanisms independent from those acting in higher-order visual areas, with *Ctip1* acting intersectionally with other regulators to activate or repress *Lmo4* expression, depending on context. Moreover, *Ctip1* functions in a number of distinct subtype-specific cellular contexts, potentially cooperating and competing with distinct sets of transcription factors in each neuronal subtype. Some of the changes in area-specific gene expression we have identified are consistent across subtypes (Figure 2A–2D), while others affect one specific neuronal subtype, while spare other neuronal subtypes, (Figure 2E–2H). These data suggest that *Ctip1* controls area identity both by regulating a common set of genes across multiple neuronal subtypes (acting autonomously or interacting with broadly expressed transcriptional regulators), and by regulating other sets of genes in a subtype-specific fashion (interacting with subtype-specific transcriptional regulators).

***Ctip1* and *Bhlhb5* act in parallel to regulate sensory area development**

The similar temporal and areal expression patterns of *Ctip1* and *Bhlhb5* suggest that they act downstream of a common set of regulators (Figure S1E–S1H). However, the two transcription factors have largely non-overlapping functions. First, molecular abnormalities in *Bhlhb5* null mice are much more limited (Joshi et al., 2008; Figure S2Q–S2V), and do not indicate overall motorization of the sensory domain that we identify here in *Ctip1^{fl/fl};Emx1-Cre* mice (Figure 2A–2T). Second, while most SCPN axons fail to reach the pyramidal decussation in *Bhlhb5* nulls (Joshi et al., 2008; Ross et al., 2012), loss of *Ctip1* function has the opposite effect, and more neurons project to the spinal cord (Figure S4L–S4T). These findings reinforce that *Bhlhb5* plays a broad role promoting SCPN axon outgrowth, especially in caudal motor cortex, while *Ctip1* instructs SCPN to acquire sensory area identity. One potentially shared function of *Bhlhb5* and *Ctip1* is barrel development, since cytoarchitectural organization in layer IV is disrupted in both *Bhlhb5* null and *Ctip1^{fl/fl};Emx1-Cre* mice (Joshi et al., 2008; Figure 7A–7D). However, these abnormalities are considerably more severe in *Ctip1^{fl/fl};Emx1-Cre* mice, with abnormal layer IV neuron differentiation also preventing thalamocortical axons from clustering into barrels (Figure 6A–6B). Overall, *Ctip1* regulates multiple critical aspects of postmitotic acquisition of sensory area identity independently of *Bhlhb5*, though both are involved in cytoarchitectural organization of layer IV.

Cortex-intrinsic regulation of layer IV neuron differentiation and sensory map formation

We report here that *Ctip1* links earlier cortex-intrinsic regulators that specify area identity with later cortex-extrinsic signals that are required for refinement of area identity, by directing differentiation of layer IV neurons within the sensory domain. Deletion of *Ctip1* from cortex results in a striking failure of sensory map formation, with no discernible organization of thalamocortical axons into barrels (Figure 6B). Layer IV neurons lack cytoarchitectural organization (Figure 7B) and fail to express layer IV-specific genes (Figure 7E–7L). In addition, axon terminals of contralateral callosal projection neurons invade layer IV of *Ctip1^{fl/fl};Emx1-Cre* cortex, infringing on synaptic territory normally reserved for thalamocortical input (Figure 7P–7R). Cell-autonomous loss of *Ctip1* function in layer IV neurons completely disrupts their integration into barrels and assembly into thalamorecipient circuits (Figure 7S–7X). Taken together, these data indicate that *Ctip1* is a cortex-intrinsic control acting within the sensory domain to regulate layer IV neuron differentiation, enabling appropriate spatial organization of their dendritic trees and interactions with thalamocortical input, which are in turn critical for subsequent stages of sensory area development.

Multiple lines of investigation have identified that thalamocortical axons provide information required both for sensory map formation, and for parallel changes in layer IV neuron cytoarchitectural organization and molecular area identity during the final stages of cortical arealization (Wu et al., 2011; Li and Crair, 2011). In *barrelless* (*Adcy1* null) mice, thalamocortical axons and layer IV neurons fail to segregate into individual barrels (Van der Loos et al., 1986; Abdel-Majid et al., 1998) due to the loss of adenylyl cyclase-dependent signals normally conveyed by thalamocortical axons (Iwasato et al., 2008). Genetically blocking neurotransmission at thalamocortical synapses disrupts barrel organization and

molecular differentiation of layer IV neurons (Li et al., 2013). Additionally, genetic ablation of sensory thalamic nuclei disrupts refinement of gene expression (Chou et al., 2013; Pouchelon et al., 2014). Together, these data highlight the importance of extrinsic signals for proper postnatal differentiation of cortical neurons.

Cortex-intrinsic programs acting at early stages of arealization have also been extensively investigated. Thalamocortical axons are attracted to specific regions of cortex by areally-specified cortical cues, which can be shifted rostrally or caudally by altering expression of transcription factors that specify area identity, like *Emx2* and *Couptf1* (O'Leary et al., 2007; Armentano et al., 2007). Once thalamocortical axons reach the cortical plate, however, their organization into sensory maps proceeds largely normally, regardless of the size or position of the respecified sensory domain (Hamasaki et al., 2004; Armentano et al., 2007; Zembrzycki et al., 2013). Loss of transcriptional controls over acquisition of sensory area identity has been associated with only subtle defects in cortical barrel cytoarchitecture and clustering of thalamocortical axons (Joshi et al., 2008). It has therefore remained unclear whether cortex-intrinsic programs direct differentiation of neurons within the sensory domain in order to enable the complex interactions with thalamocortical axons that are required for sensory map formation and further refinement of gene expression. We show here that *Ctip1* is a critical control over precisely this process in layer IV of sensory areas.

The complex interplay of cortex-intrinsic and cortex-extrinsic mechanisms in the specification of projection neuron area identity has important implications for a variety of neurodevelopmental disorders, but the precise molecular controls underlying these interactions remain poorly understood. Identification of *Ctip1* as a central regulator of layer IV neuron differentiation represents an important first step toward defining specific molecules that direct layer IV dendritic development, cytoarchitectural organization, and circuit integration. It will be of particular interest, in future studies, to investigate potential interactions between cortex-intrinsic transcription factors that control neuronal subtype and area identity, and transcription factors operating under the influence of thalamocortical activity. Further elucidation of the transcriptional mechanisms and molecular programs by which *Ctip1* regulates cortex-intrinsic aspects of arealization will offer important insights into the evolutionary and functional compartmentalization of cortex into distinct areas, and will also identify potential nodes of intersection with recently delineated maturational processes driven by thalamocortical input.

EXPERIMENTAL PROCEDURES

Animals

All mouse studies were approved by the Harvard University IACUC, and were performed in accordance with institutional and federal guidelines. The date of vaginal plug detection was designated E0.5, and the day of birth as P0. Further details are listed in Supplemental Experimental Procedures.

Immunocytochemistry, histology, and *in situ* hybridization

Mice were transcardially perfused with 4% paraformaldehyde, and brains were dissected and post-fixed at 4°C overnight. Tissue was sectioned at 50µm on a vibrating microtome (Leica). Embryonic brains were fixed at 4°C overnight, dissected, cryoprotected in 30% sucrose, and sectioned at 20µm on a cryostat (Leica). Riboprobe synthesis and nonradioactive *in situ* hybridization were performed using standard methods (Tiveron et al., 1996). Antibodies used and probe sources/primer sequences are listed in Supplemental Experimental Procedures.

In utero electroporation

Surgeries were performed as previously described (Molyneaux et al., 2005). Further details about procedure and plasmids are provided in the Supplemental Experimental Procedures.

Anterograde labeling with AAV

All virus work was approved by the Harvard Committee on Microbiological Safety, and conducted according to institutional guidelines.. Further details on procedure and viral constructs are listed in Supplemental Experimental Procedures.

RNA sequencing

Frontal (motor) and parietal (somatosensory) cortex was dissected from P4 wild-type and *Ctip1^{fl/fl};Emx1-Cre* mice, mRNA was isolated using mRNA Direct beads (Invitrogen), and libraries were prepared using NEBNext RNA Library Prep Kit (New England Biolabs). Sequencing was performed on an Illumina HiSeq 2500 at the Harvard Bauer core facility. Data was analyzed using Tuxedo tools (Trapnell et al., 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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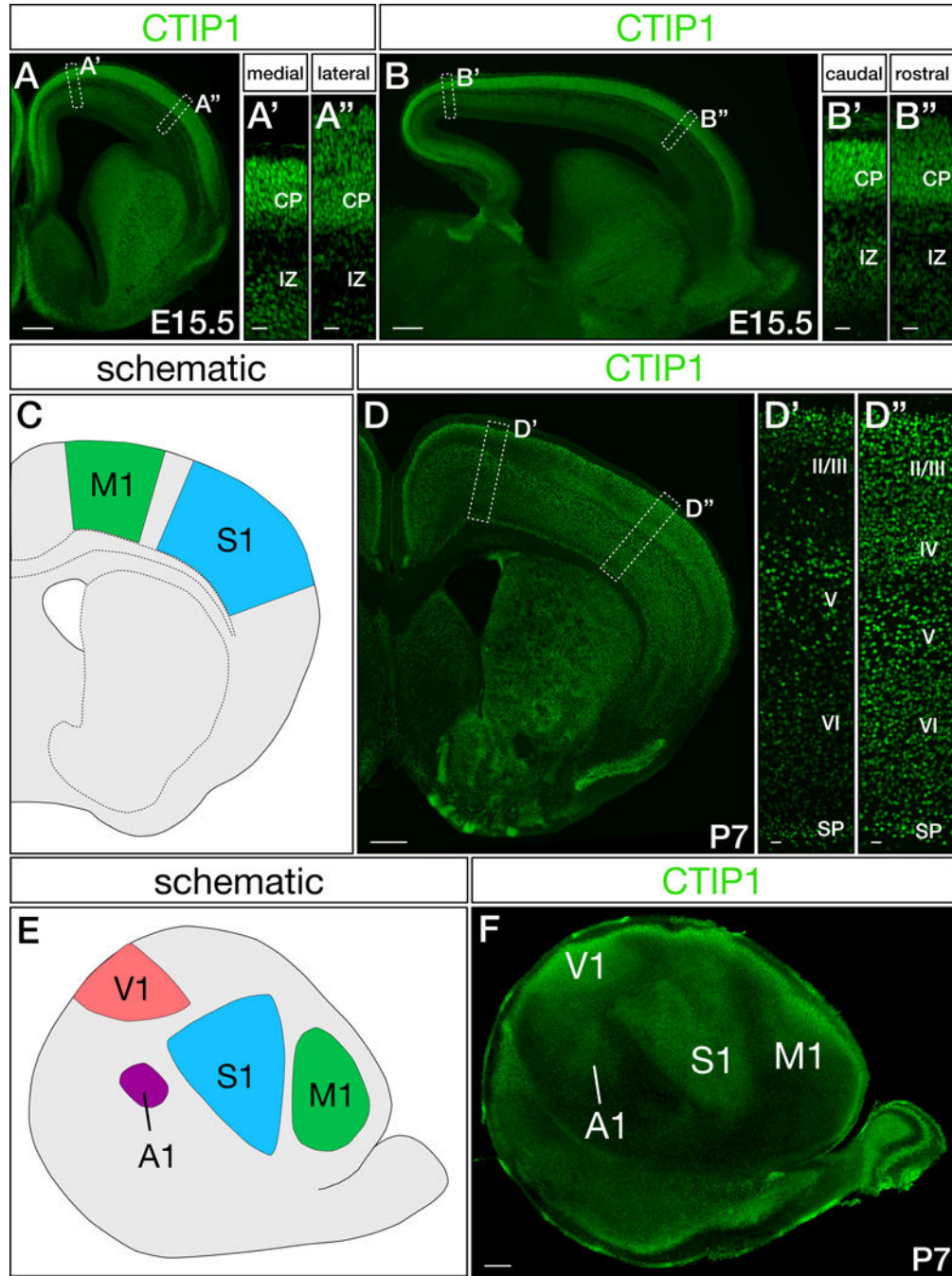


Figure 1. Neocortical expression of CTIP1 becomes areally patterned as the neocortex matures (A–B) At E15.5, CTIP1 is expressed by newly postmitotic projection neurons in a low-rostralateral to high-caudomedial gradient across the cortex. In coronal sections (A), CTIP1 is expressed by postmitotic projection neurons at higher levels medially (A') and lower levels laterally (A''), while in sagittal sections (B), CTIP1 is expressed at higher levels caudally (B') and lower levels rostrally (B''). (C–F) By P7, CTIP1 is highly expressed in primary sensory areas. In coronal sections, a distinct boundary of CTIP1 expression exists between primary somatosensory and primary

motor cortex (C), with sparser expression of CTIP1 in primary motor cortex (D') compared to primary sensory cortex (D''), especially in layer VI and deep layer II/III. In tangential sections of flattened cortex (E, F), CTIP1 expression clearly delineates primary sensory areas (V1, A1, and S1), and is absent from primary motor cortex (M1) and higher-order sensory areas.

A1, primary auditory cortex; M1, primary motor cortex; S1, primary somatosensory cortex; V1, primary visual cortex.

Scale bars: 500 μ m (D, F), 250 μ m (A, B), 25 μ m (A'-A'', B'-B'' and D'-D'')

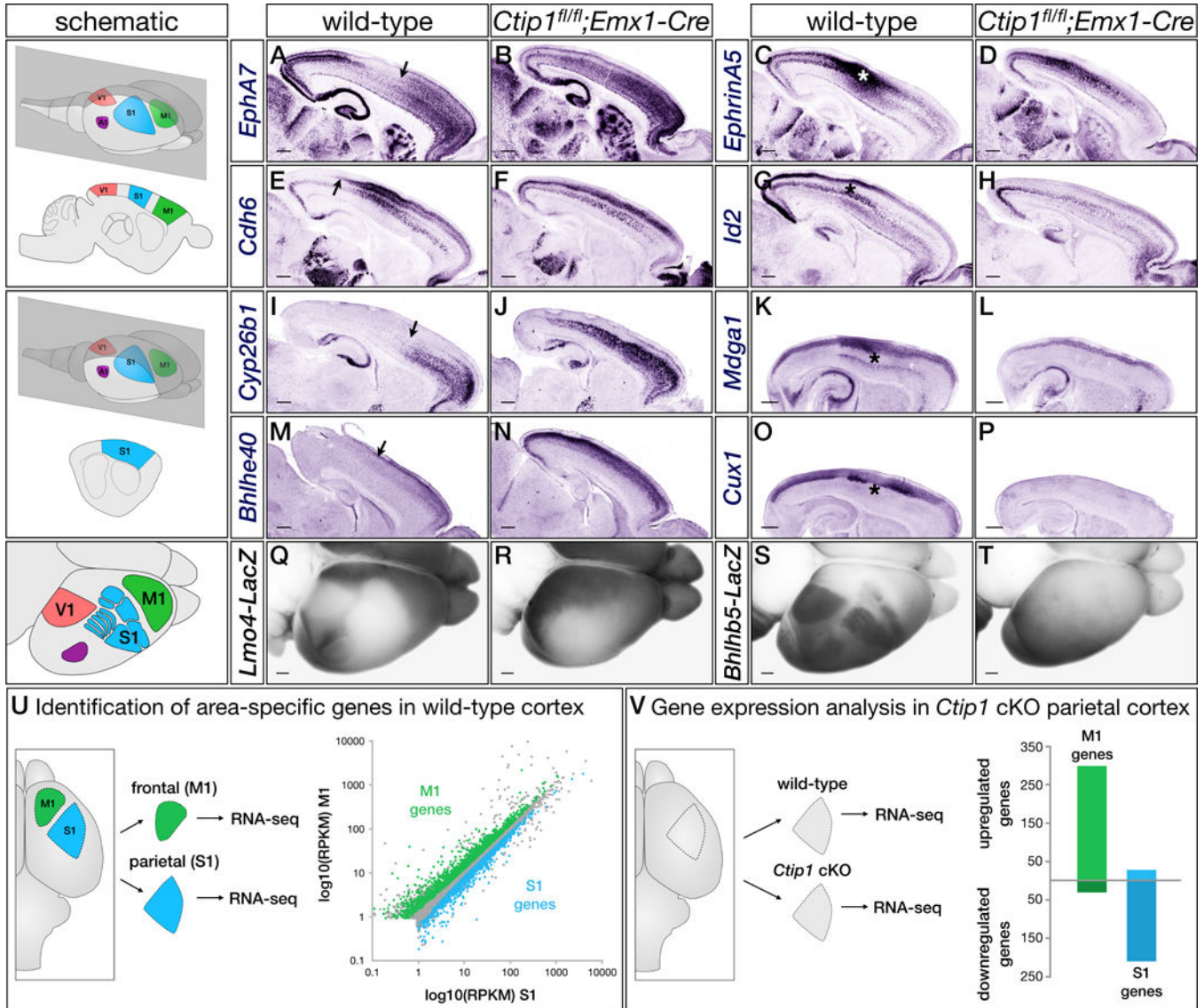


Figure 2. Establishment of precise gene expression programs delineating motor and sensory areas is severely disrupted in *Ctip1^{fl/fl};Emx1-Cre* cortex
 (A–P) In the absence of *Ctip1* function, molecular area identity in somatosensory cortex is severely disrupted. *EphA7*, largely excluded from wild-type somatosensory cortex (A), is extensively expressed in *Ctip1^{fl/fl};Emx1-Cre* somatosensory cortex (B). The sharp caudal boundaries of wild-type *EfnA5*, *Cdh6*, *Cyp26b1*, and *Bhlhe40* expression (C, E, I, M) are replaced by gradual gradients that aberrantly invade occipital cortex in *Ctip1^{fl/fl};Emx1-Cre* brains (D, F, J, N). Superficial-layer expression of *Id2* in wild-type somatosensory and visual cortex (G) is lost in *Ctip1^{fl/fl};Emx1-Cre* cortex (H), while strong expression of *Mdga1* and *Cux1* in wild-type somatosensory cortex (K, O) is lost or greatly reduced in *Ctip1^{fl/fl};Emx1-Cre* cortex (L, P). (Q–T) Expression domains of *Bhlhb5* and *Lmo4* are strikingly abnormal in the absence of *Ctip1* function. At P7, when area identity refinement is normally complete, distinct primary and higher-order visual areas in wild-type cortex (Q) are not discernible in *Ctip1^{fl/fl};Emx1-Cre* cortex (R) by *Lmo4*-driven *LacZ* expression. *Bhlhb5* expression is

almost absent from *Ctip1^{fl/fl};Emx1-Cre* cortex (T), compared with sharply-delineated sensory area expression in wild-type cortex (S). (U–V) *Ctip1^{fl/fl};Emx1-Cre* parietal cortex expresses genes characteristic of wild-type motor cortex, but not somatosensory cortex. We compared gene expression between wild-type frontal (M1) and parietal (S1) cortex at P4 by RNA-seq, designating genes enriched in one area as M1 or S1 genes, respectively (U). When wild-type and *Ctip1^{fl/fl};Emx1-Cre* parietal cortex are compared, 298 of 329 differentially expressed M1 genes are increased in expression in *Ctip1* conditional mutants, and 210 of 238 differentially expressed S1 genes are decreased in expression (V).

A1, primary auditory cortex; M1, primary motor cortex; S1, primary somatosensory cortex; V1, primary visual cortex.

Scale bars: 500 μ m, all panels.

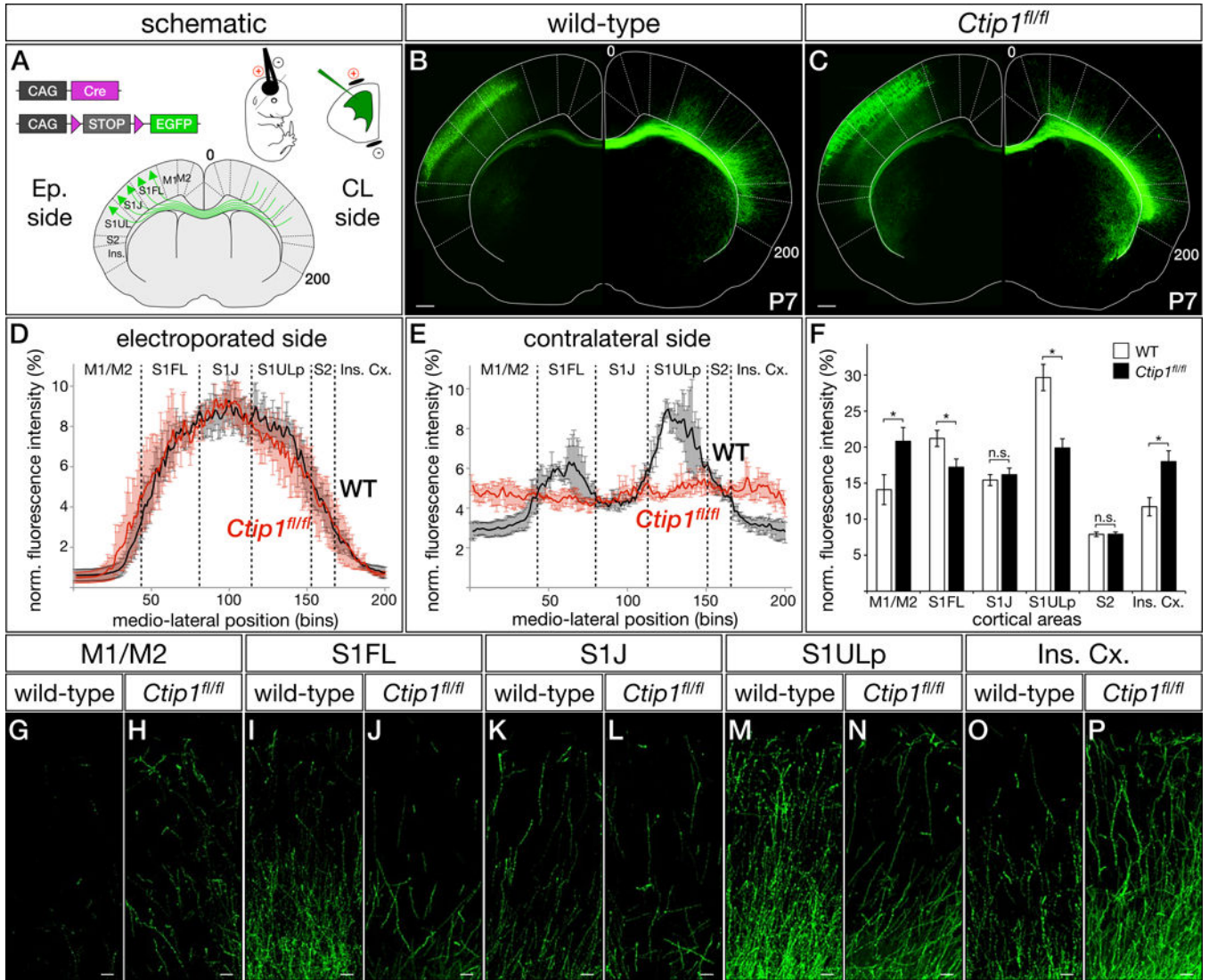


Figure 3. In the absence of *Ctip1* function, callosal projection neurons fail to project to homotypic locations in the contralateral hemisphere
 (A) Schematic of experimental approach. Embryos were electroporated at E14.5 with CAG-Cre and CAG-floxed(STOP)-EGFP plasmids, and brains were collected at P7. For quantification, images of electroporated and contralateral hemispheres of cortex were divided into 200 bins from the M1/S1FL boundary medially to the Ins. Ctx./Pir. Ctx. boundary laterally. Fluorescence intensity was quantified in each bin using ImageJ.
 (B–C) While axons of Cre-electroporated wild-type CPN cluster in S1FL and S1ULp (B), axons of Cre-electroporated *Ctip1^{fl/fl}* CPN exhibit little areal specificity (C).
 (D–F) Quantification of data in B–C. Although Cre electroporations into wild-type and *Ctip1^{fl/fl}* brains were similar in size and location (D), axons of electroporated wild-type neurons clearly target specific areas of contralateral cortex preferentially, while axons of electroporated *Ctip1^{fl/fl}* neurons distribute evenly across the medio-lateral extent of cortex (E). Significantly more axons from Cre-electroporated *Ctip1^{fl/fl}* neurons aberrantly target

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motor cortex and insular cortex, and significantly fewer target forelimb and upper lip regions of primary somatosensory cortex (F). *, $p < 0.05$.

(G–P) High magnification images of axons from B–C. Wild-type axons preferentially innervate S1FL and S1ULp (I, M), and avoid M1/M2, S1J, and Ins. Ctx. (G, K, O), while Cre-electroporated *Ctip1^{fl/fl}* axons innervate all areas approximately equally (H, J, L, N, P). M1, primary motor cortex; M2, secondary motor cortex; S1FL, primary somatosensory cortex, forelimb region; S1J, primary somatosensory cortex, jaw region; S1ULp, primary somatosensory cortex, upper lip region; S2, secondary somatosensory cortex; Ins. Ctx., insular cortex; Pir. Ctx., piriform cortex; CL, contralateral.

Scale bars: 100 μ m (B, C), 10 μ m (G–P). Data are represented as mean \pm SEM.

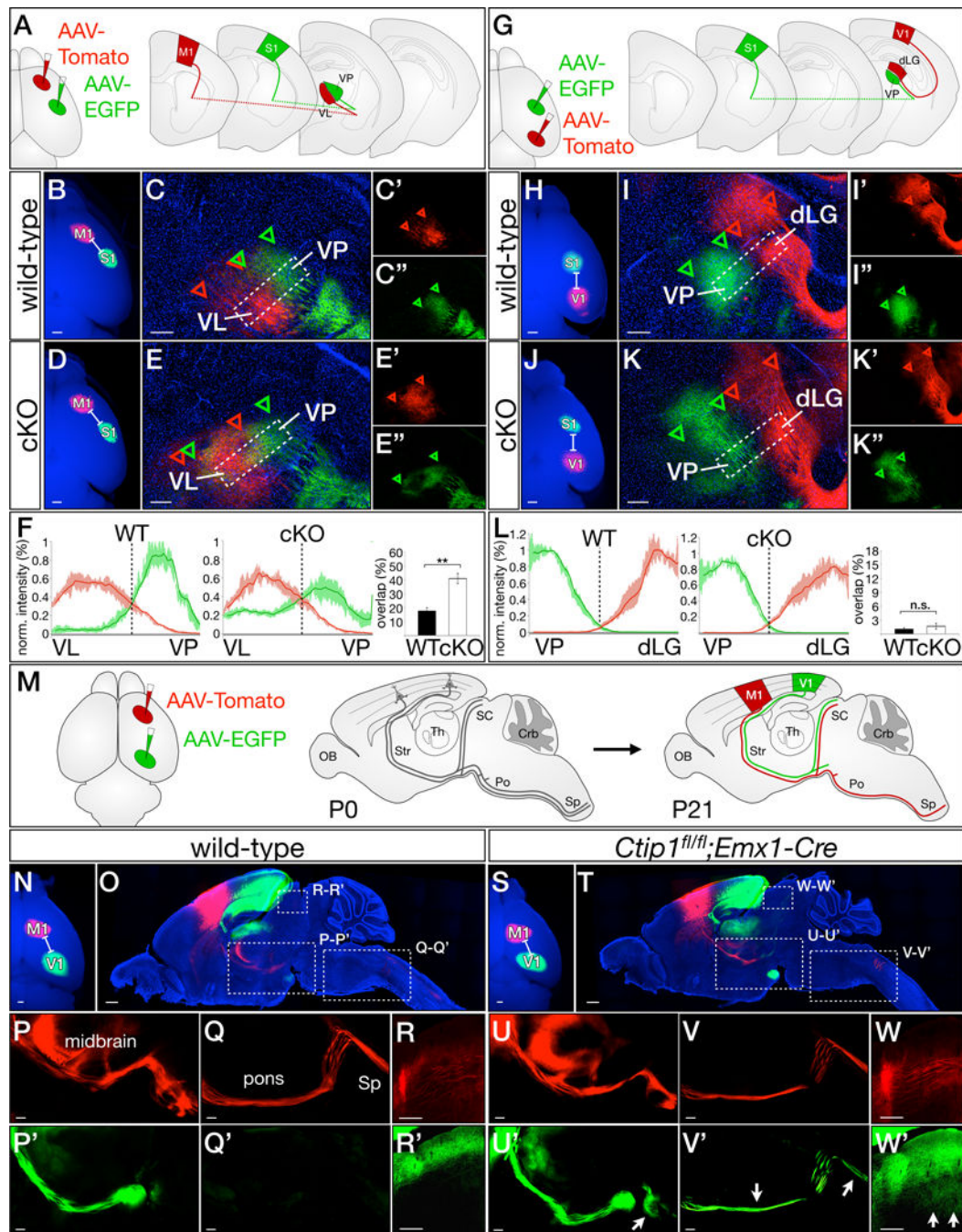


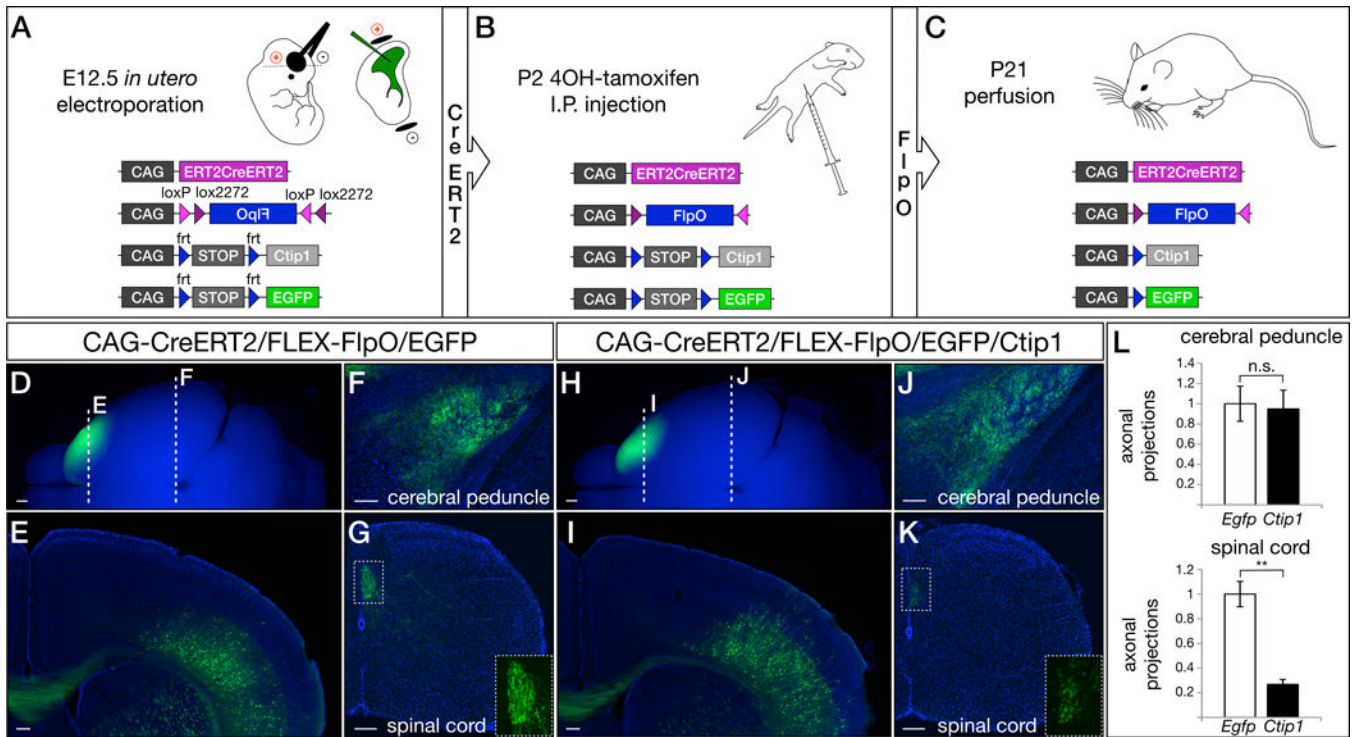
Figure 4. *Ctip1* represses motor identity in sensory cortex subcerebral and corticothalamic projection neurons

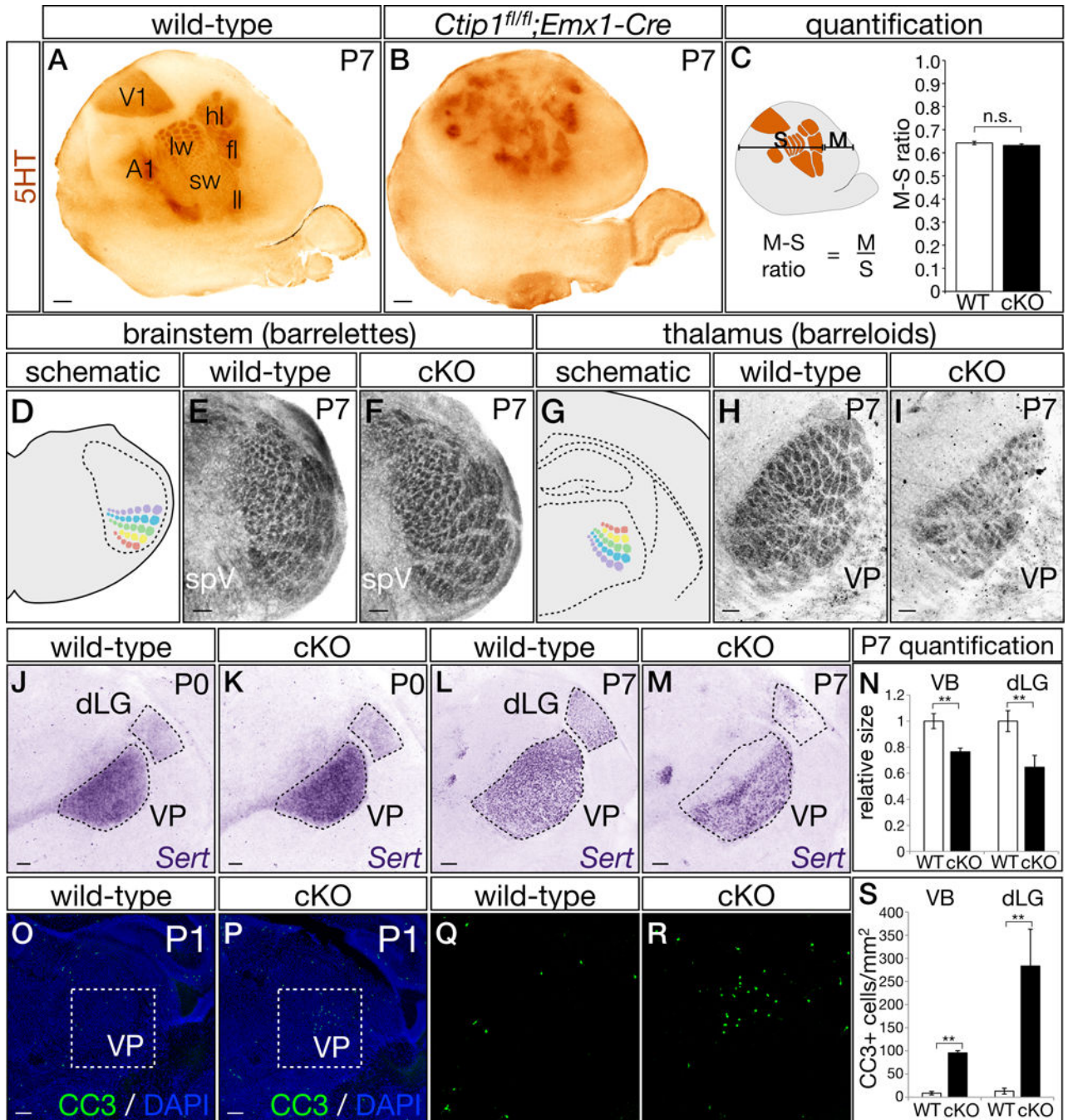
(A–F) *Ctip1* is necessary for corticothalamic axons from somatosensory cortex to repress motor identity and project specifically to sensory thalamic nuclei. Schematic of AAV labeling approach (A). In wild-type mice, axons anterogradely labeled by AAV injection into motor or somatosensory cortex sort into VL (motor; red) or VP (sensory, green) (B, C), but axons from *Ctip1^{fl/fl};Emx1-Cre* somatosensory cortex project to both VL and VP (D, E). Extent of AAV-tdTomato and AAV-EGFP labeling is indicated by red and green arrowheads, respectively.

Quantification of normalized fluorescence intensity, demonstrating significantly more overlap between motor and somatosensory injections in *Ctip1^{fl/fl};Emx1-Cre* mutants (F). **, $p < 0.01$ (G–L) *Ctip1* is not required for corticothalamic axons from distinct sensory areas to sort into appropriate sensory thalamic nuclei. Schematic of AAV labeling approach (G). In wild-type mice, axons anterogradely labeled by AAV injection into somatosensory and visual cortex project to distinct thalamic nuclei in both wild-type (H–I) and *Ctip1^{fl/fl};Emx1-Cre* (J–K) mice. Visual cortex CThPN (red) project to dLG, while somatosensory cortex CThPN (green) project to VP. Extent of AAV-tdTomato and AAV-EGFP labeling is indicated by red and green arrowheads, respectively. Quantification of normalized fluorescence intensity, demonstrating minimal overlap between somatosensory and visual injections in both wild-type mice and *Ctip1^{fl/fl};Emx1-Cre* mutants (L).

(M–W) *Ctip1* is required for repression of motor identity by visual subcerebral projection neurons. Schematic of labeling approach (M). P1 injection of AAV-tdTomato into motor cortex and AAV-EGFP into visual cortex labels descending projections in P21 wild-type (N–O) and *Ctip1^{fl/fl};Emx1-Cre* (S–T) cortex. Motor cortex SCPN from both wild-type and *Ctip1^{fl/fl};Emx1-Cre* brains project to the cerebral peduncle in the midbrain (P, U), and continue into the pons and spinal cord (Q, V). In wild-type brain, visual cortex SCPN do not project beyond the midbrain (arrow in P', Q'). However, in *Ctip1^{fl/fl};Emx1-Cre* brains, visual cortex SCPN aberrantly project past the midbrain to enter the pons (arrow in U'), then continue into the spinal cord (V'). Axons from motor cortex SCPN project to deep layers of the superior colliculus in wild-type and *Ctip1^{fl/fl};Emx1-Cre* brains (R and W). Axons from visual cortex SCPN project exclusively to superficial layers of the superior colliculus in wild-type brains (R'). However, while visual cortex SCPN still project to superficial layers of the superior colliculus in *Ctip1^{fl/fl};Emx1-Cre* brains, they also aberrantly innervate deep layers (arrows in W').

dLG, dorsal lateral geniculate nucleus of thalamus; M1, primary motor cortex; S1, primary somatosensory cortex; SC, superior colliculus; Sp, spinal cord; V1, primary visual cortex; VL, ventral lateral nucleus of thalamus; VP, ventral posterior nucleus of thalamus
Scale bars: 500 μ m (B, H), 200 μ m (D–F, I–K). Data are represented as mean \pm SEM.





(D–I) Sensory maps in thalamic and brainstem nuclei that convey information from whiskers develop in the absence of cortical *Ctip1* function, but later attenuate. Cytochrome oxidase-stained barrelettes in brainstem (schematic, D) are well-organized in both wild-type and *Ctip1^{fl/fl};Emx1-Cre* mice (E, F). Barreloids in the ventral posterior nucleus of thalamus (schematic, G) are smaller in *Ctip1^{fl/fl};Emx1-Cre* (I) compared with wild-type (H), potentially as a result of “top-down” plasticity, though clear organization is still evident. (J–S) Thalamic barreloids atrophy in the absence of cortical *Ctip1* function following initial appropriate development. At P0, VB and dLG are the same size in wild-type and *Ctip1^{fl/fl};Emx1-Cre* thalamus (outlines, J–K), but by P7, *Ctip1^{fl/fl};Emx1-Cre* VB and dLG have atrophied in comparison with wild-type VB and dLG (outlines, L–M). Quantification (N). This atrophy is due in part to apoptosis, which occurs at higher rates in *Ctip1^{fl/fl};Emx1-Cre* sensory thalamus than in wild-type (O–S). **, $p < 0.01$.

A1, auditory cortex; CC3, cleaved caspase 3; dLG, dorsal lateral geniculate nucleus of thalamus; fl, forelimb; hl, hindlimb; ll, lower lip; lw, large whiskers; M, motor cortex; S, sensory cortex; SpV, spinal trigeminal nucleus; sw, small whiskers; V1, primary visual cortex; VP, ventral posterior nucleus of thalamus

Scale bars: 500 μ m (A and B), 200 μ m (E–I), 100 μ m (J–P). Data are represented as mean \pm SEM.

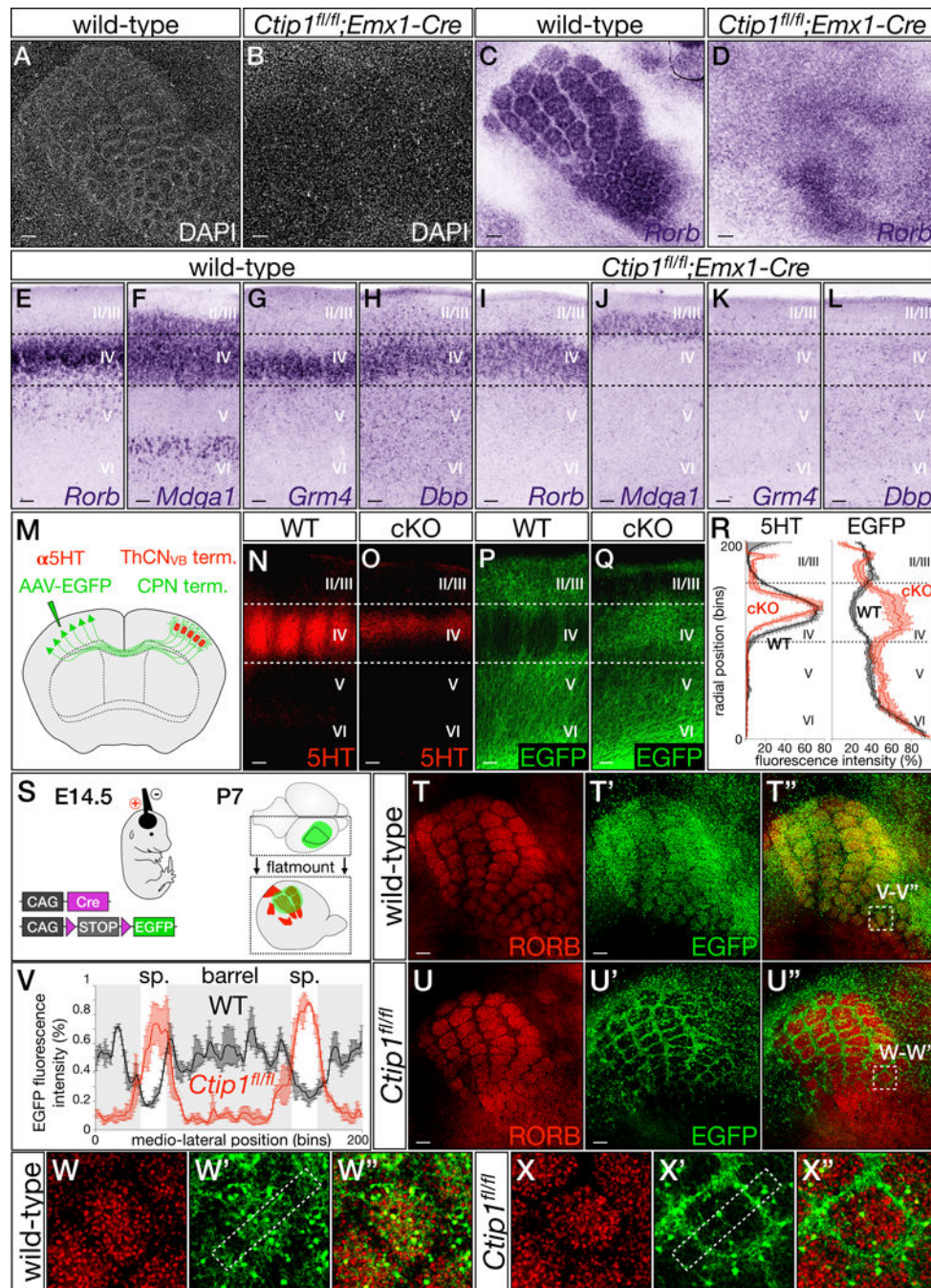


Figure 7. Layer IV granule neuron differentiation is impaired in the absence of *Ctip1* function, and cell-autonomous loss of *Ctip1* function leads to their exclusion from barrels
 (A–D) At P7, wild-type layer IV granular neurons are oriented into barrel structures visible by DAPI staining (A) or *Rorb* expression (C) in tangential sections. In layer IV of *Ctip1^{fl/fl};Emx1-Cre* cortex, neurons do not organize into barrels (B) or express *Rorb* in periodic clusters (D). (E–L) Genes expressed in barrel cortex by wild-type layer IV granule neurons, including *Rorb* (E), *Mdga1* (F), *Grm4* (G), and *Dbp* (H), are expressed in *Ctip1^{fl/fl};Emx1-Cre* barrel cortex at reduced levels (I) or absent entirely (J–L).

(M–R) In the absence of *Ctip1* function, axons of contralateral CPN compete with thalamocortical axons for synaptic partners in layer IV. Schematic of approach (M). CPN terminals are labeled with AAV-EGFP injected into the contralateral hemisphere, and thalamocortical axon terminals (ThCN_VB) are labeled with 5HT immunostaining. Wild-type layer IV granule neurons are receptive to synaptic contact with thalamocortical axons (N) and avoid contact with contralaterally-projecting CPN (P). In contrast, *Ctip1^{fl/fl};Emx1-Cre* layer IV neurons attract few ThCN_VB axon terminals (O), and layer IV is instead occupied by CPN axon terminals (Q). Quantification of 5HT and EGFP fluorescence as percentage of maximum fluorescence intensity (R).

(S–W) Mosaic loss of *Ctip1* function causes layer IV neurons and their dendrites to be excluded from barrels. Wild-type layer IV neurons electroporated with Cre primarily populate barrels (T-T', W-W'), but *Ctip1^{fl/fl}* neurons electroporated with Cre are excluded from barrels (U-U', X-X'). Schematic of experimental approach (S) and quantification (V, measuring across dashed boxes in W', X').

Scale bars: 200µm (A–D and T–U), 100µm (E–Q). Data are represented as mean ± SEM.

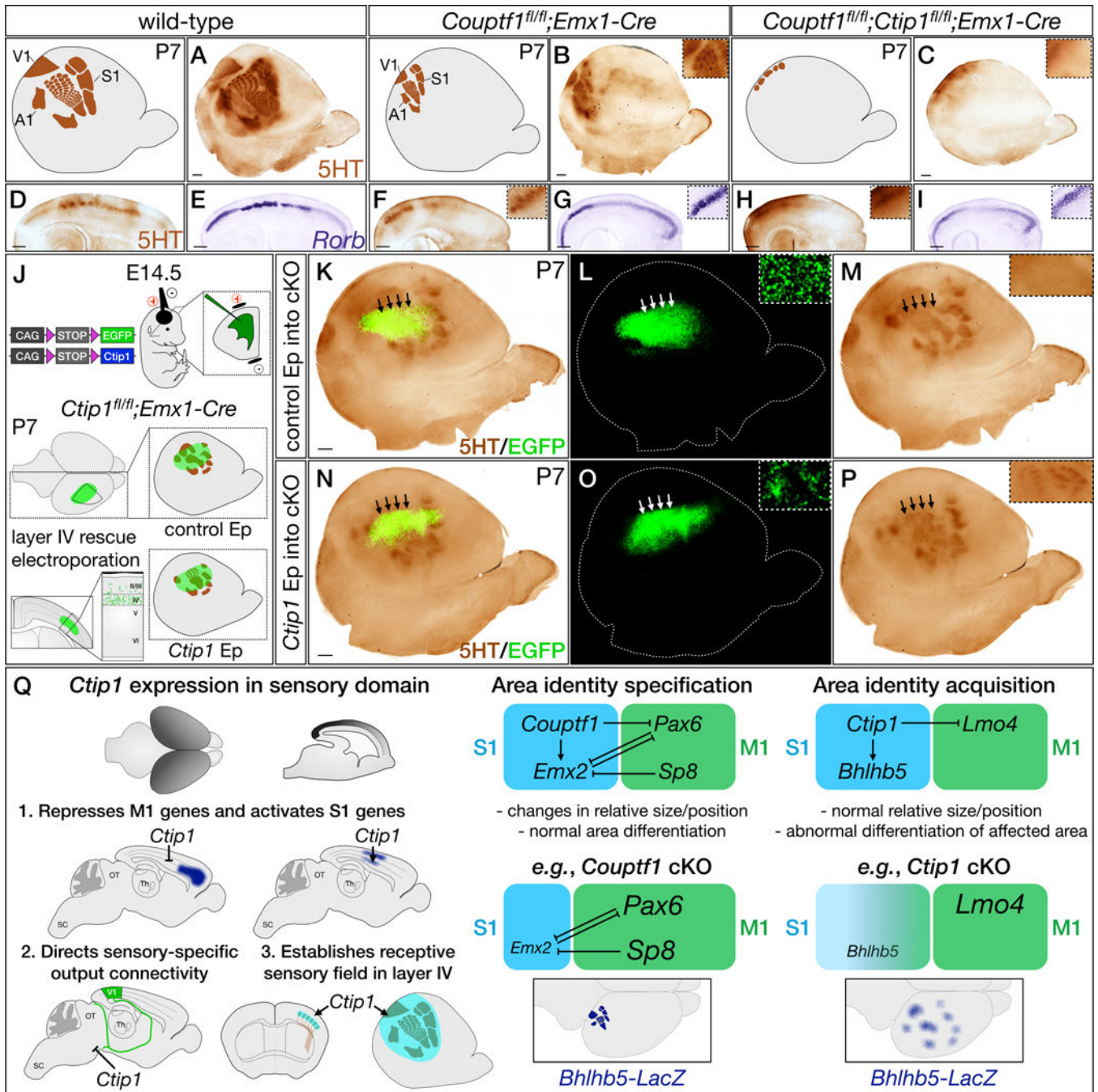


Figure 8. *Ctip1* functions independently of *Couptf1*, and restoring expression of *Ctip1* in layer IV of *Ctip1^{fl/fl};Emx1-Cre* cortex is sufficient to rescue sensory map formation

(A–I) CTIP1 and COUPTF1 act sequentially to regulate the position and topography of sensory maps. In wild-type somatosensory cortex, thalamocortical afferents and clusters of *Rorb*-expressing neurons form barrels (A, D–E). In *Couptf1^{fl/fl};Emx1-Cre* cortex, sensory representations are miniaturized, and shifted to the caudal extreme of cortex, but maintain their periodic organization (B, F–G). In double mutant *Ctip1^{fl/fl};Couptf1^{fl/fl};Emx1-Cre* cortex, sensory representations are shifted caudally, as in *Couptf1^{fl/fl};Emx1-Cre* cortex, but are also completely disorganized, as in *Ctip1^{fl/fl};Emx1-Cre* cortex (C, H–I).

(J–P) Mosaic *Ctip1* misexpression partially compensates for cortex-wide loss of *Ctip1* function. Schematic of approach (J). CAG-floxed(STOP)-*Egfp* and CAG-floxed(STOP)-*Ctip1* are co-electroporated into E14.5 *Ctip1^{fl/fl};Emx1-Cre* cortex, and electroporated brains are recovered and flatmounted at P7. *Ctip1^{fl/fl};Emx1-Cre* barrel cortex electroporated with EGFP alone contains neither clustering of electroporated neurons nor discernible periodic organization of thalamocortical afferents (K–M, insets in L and M), but *Ctip1^{fl/fl};Emx1-Cre* barrel cortex electroporated with both EGFP and *Ctip1* contains clustered electroporated neurons and rudimentary barrel-like structures in barrel cortex (N–P, insets in O and P). (Q) Model. *Ctip1* is expressed in sensory cortical areas, and controls sensory area gene expression, output connectivity, and establishment of receptive fields for sensory input connectivity.

Scale bars: 500 μ m (A–C) and 200 μ m (D–I).