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Rbx2 regulates neuronal migration through different Cullin5-RING ligase adaptors

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Abstract

Morphogenesis requires the proper migration and positioning of different cell types in the embryo. Much more is known about how cells start and guide their migrations than how they stop when they reach their destinations. Here we provide evidence that Rbx2, a subunit of the Cullin5-RING E3 ubiquitin ligase (CRL5) complex, stops neocortical projection neurons at their target layers. Rbx2 mutation causes neocortical and cerebellar ectopias dependent on Dab1, a key signaling protein in the Reelin pathway. SOCS7, a CRL5 substrate adaptor protein, is also required for neocortical layering. SOCS7-CRL5 complexes stimulate the ubiquitylation and turnover of Dab1. SOCS7 is up-regulated during projection neuron migration and unscheduled SOCS7 expression stops migration prematurely. Cerebellar development requires Rbx2 but not SOCS7, pointing to the importance of other CRL5 adaptors. Our results suggest that CRL5 adaptor expression is spatio-temporally regulated to modulate Reelin signaling and ensure normal neuron positioning in the developing brain.

Introduction

Embryonic morphogenesis requires coordinated proliferation, migration and differentiation of cells to form tissues and organs. Importantly, migrating cells must stop in the correct positions before they differentiate. While contact repulsion or down-regulation of attractive signals may determine migration end-points, in many cases the mechanisms that stop migration are unclear (Kurosaka and Kashina, 2008). Precise cell positioning is particularly important for assembling complex structures in the mammalian brain, such as the six-layered neocortex (Medina and Abellan, 2009). Neocortical layers are formed when sequential cohorts of projection neurons migrate from the ventricular zone (VZ) and intermediate zone (IZ), between their older siblings in the cortical plate (CP), and stop moving when they reach the marginal zone (MZ), to form an inside-first, outside-last CP (Molyneaux et al., 2007). Cerebellar cortex layering also arises by coordinated movements of different cell types. First, the rhombic lip migratory stream (RLS) moves anteriorly below the pia while Purkinje cells (PCs) migrate from the cerebellar VZ to aggregate in a primordial cortical layer, the Purkinje cell plate (PCP) (Hevner, 2008; Sotelo and Rossi, 2013). Later, granule cells (GCs) replace the RLS to form an external granule layer (EGL)

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Supplemental Information
Supplemental information includes 4 figures and Supplemental Experimental Procedures and can be found online at XXX.

which expands enormously in the perinatal period. At this time, the PCP rearranges into a unicellular PC layer (PCL), and the GCs migrate inwards to form an inner granule layer (IGL). The assembly of such precisely organized anatomy requires high-level coordination of the birth, specification, migration, termination, differentiation and functional connection of different types of neurons.

Cullin 5 (Cul5) is one of seven Cullins that form Cullin-RING E3 ubiquitin ligase complexes (CRLs) (Figure 1A) (Petroski and Deshaies, 2005). CRLs are multi-subunit complexes nucleating around a single Cullin. Cullins bind a RING protein, either Rbx1/Roc1 or Rbx2/Rnf7/Sag, which recruits an E2 ligase. Recent evidence suggests that Rbx2 is the RING protein for Cul5 while Rbx1 is shared by the other members of the family (Huang et al., 2009). Cullins also bind substrate adaptor proteins that confer substrate specificity. CRL5 utilizes ElonginB/C (also known as Tceb2/Tceb1) subunits to associate with up to 38 different adaptor proteins (Okumura et al., 2012). CRL5 thus potentially targets many proteins, depending on which adaptor is present.

The Reelin signaling pathway regulates the positioning of projection neurons in the neocortex, PCs in the cerebellum and neurons in many other brain regions (Hevner, 2008; Tissir and Goffinet, 2003). Reelin is secreted during development in the MZ of the cortex and EGL of the cerebellum, binds to cell surface receptors and stimulates a core signaling complex composed of a tyrosine kinase, Fyn or Src, and a substrate protein, Dab1 (Tissir and Goffinet, 2003). Fyn/Src and tyrosine-phosphorylated Dab1 (pY.Dab1) are required for Reelin-dependent neuron migration and positioning.

Despite progress in understanding the molecular effects of Reelin, its cellular effects during brain development remain unclear. Reelin mutation causes inversion of neocortical layers and a reduced, disorganized cerebellum. An early idea to explain the neocortical phenotype was that Reelin in the MZ serves as a stop signal that locally inhibits neuron migration (Tissir and Goffinet, 2003). If neurons do not stop, others accumulate below, causing cortical inversion. Similarly, Reelin expressed in the outer part of the developing cerebellum may regulate PC migration (Hevner, 2008). However, exogenous or ectopic Reelin expression can partly rescue neocortical and cerebellar development, suggesting that the localization of Reelin is relatively unimportant for some stages of migration (Jossin et al., 2004; Magdaleno et al., 2002). In addition, recent evidence indicates that Reelin may have dual effects in the neocortex: first, promoting neuron migration from the IZ to the CP, and second, initiating the final step of somal translocation that ends at the top of the CP (Franco et al., 2011; Gil-Sanz et al., 2013; Jossin and Cooper, 2011; Sekine et al., 2011; Simo et al., 2010). This leaves open the question of what stops neurons at the top of the CP and raises new questions of how Reelin differentially regulates neurons in the IZ and CP.

Reelin is known to stimulate pY.Dab1 degradation, dependent on the proteasome (Arnaud et al., 2003a; Bock et al., 2004). Dab1 degradation requires Cul5 and inhibiting Cul5 increases migration cell-autonomously, suggesting that CRL5 stops migration by down-regulating Reelin signaling (Feng et al., 2007). Here we provide evidence that CRL5 regulates brain development in vivo. We report that pY.Dab1 binds to and is ubiquitylated by SOCS7-CRL5 complexes, and that mutation of either Rbx2 or SOCS7 induces a novel phenotype in the cortex attributable to excess pY.Dab1. In the cerebellum, CRL5 regulates PC positioning but SOCS7 is not required, suggesting a role for another SOCS protein in the cerebellum but not cortex. We further show that spatio-temporal control of SOCS7 expression by neocortical projection neurons permits Reelin-dependent passage through the IZ and restricts Reelin-induced somal translocation at the top of the CP.

Results

Rbx2 regulates cortical and cerebellar development

Previous experiments using shRNA provided evidence that Cul5 prevents "over-migration" of neurons at the top of the cortical plate (Feng et al., 2007; Simo et al., 2010). To confirm that CRL5 activity, and not just Cul5, was required to regulate neuron migration, we depleted Rbx2 by in utero electroporation of Rbx2 shRNA into progenitors at E14.5 and examined neuronal positioning at E19.5. While control neurons settled in layer III, neurons expressing Rbx2 shRNA remained at the top of the CP, creating an "over-migration" phenotype similar to that caused by Cul5 shRNA (Figure 1B). This suggests that Cul5 and Rbx2 are both important for terminating migration at the top of the CP and likely act together as members of a CRL5 complex.

To test the effect of globally inhibiting migration termination throughout development, we generated a floxed *rbx2* allele (Experimental Procedures, Figure 1C). In utero electroporation of Cre into the VZ of *rbx2* fl/fl embryos at E14.5 caused cell-autonomous over-migration similar to that caused by Rbx2 shRNA, implying that genetic deletion and shRNA knockdown have the same effects at the cellular level (data not shown). We then deleted *rbx2* from neuron progenitors from E10.5 onwards using a Nestin-Cre driver (Tronche et al., 1999). Resulting *rbx2* conditional knockout (*rbx2* cKO) mice were born at expected ratios and were viable, although they did not thrive (Figure S1A). Furthermore, around 90% of *rbx2* cKO mice analyzed (n=25) presented enlarged ventricles by P10–P15, with progressive hydrocephaly in 50–60% of mutant animals by 3–6 months of age. We analyzed the cerebral cortex at various stages of development. At E17.5 and birth, the cortical thickness, cell number and gross architecture were not detectably altered. The radial glia structure was indistinguishable from control by Nestin staining (Figure S1B). There were no indications of abnormal mitosis (phospho-histone H3, Figure S1C), or apoptosis (cleaved Caspase-3, Figure S1D). There was a clear MZ that contained Cajal-Retzius neurons, which express Reelin (Figure 1D). However, close examination with layer-specific markers (Molyneaux et al., 2007) showed ectopic lamination (Figure 1D and F). *rbx2* cKO neurons expressing Cux1 (layers II–IV), Ctip2 (layer V) and Tbr1 (layer VI and SP), were present in wildtype inside-out birth order, but the layers were significantly more diffuse than normal, with each layer overlapping its neighbors. At P5, Ctip2 and Cux1 neurons were still intermingled and layer II/III had split, perhaps due to continued migration of layer II cells (Figure 1E). We conclude that the absence of *rbx2* prevents the formation of tight laminae in the neocortex.

Rbx2 deletion also affected the cerebellum. Control cerebellum at P5 comprises an external granule layer (EGL), a unicellular PC layer (PCL), and an incipient internal granule layer (IGL) (Figure 1G, left images). In the mutant, the PCL was multi-layered and ectopic PCs resided in the white matter (Figure 1G, right images and left graph). The cells in the multi-layered PCL had normal dendritic arbors and were oriented with their Golgi apparatus facing the pia, as in controls (Figures S1F and S1Gc). The ectopic PCs in the white matter were also differentiated but were randomly oriented (Figure S1Gd). PC migration from the VZ to form the PCP occurs from approximately E13.5 to E17.5 (Hevner, 2008). Later, the PCs stimulate proliferation of GCs in the EGL, and expansion of the EGL is accompanied by reorganization of the PCP into the unicellular PCL. We monitored PC location between E13.5 and E17.5 using antibodies to PC markers Lhx1/5 (Morales and Hatten, 2006). Abnormal PC positioning in the *rbx2* mutant PCP was evident at E17.5 (Figure S1H). At P5, the area of the cerebellum and the number of mitotic GCs were both significantly reduced, consistent with impaired signaling from the misplaced PCs to the GCs (Figure S1E and Figure 1G, right graph).

Overall, the results show that *rbx2* deletion causes a unique phenotype with mingling of layers in the cortex and ectopic PCs in the cerebellum. Interestingly, the cells affected - projection neurons in the cortex and PCs in the cerebellum - are the same cells whose migrations are regulated by Reelin-Dab1 signaling (Tissir and Goffinet, 2003).

Rbx2 regulates Reelin-Dab1 signaling in vivo

Cul5 shRNA inhibits Reelin-induced turnover of pY.Dab1 in cultured neurons and after in utero electroporation (Feng et al., 2007). To test whether Rbx2 regulates Dab1 levels, cortical samples from E17.5 *rbx2* cKO embryos and control *rbx2* fl/fl littermates were analyzed by Western blotting. As expected, *rbx2* deletion inhibited Rbx2 expression and significantly increased the levels of Dab1 and pY.Dab1 (Figure 2A). Interestingly, Fyn, which phosphorylates Dab1 (Arnaud et al., 2003b; Bock and Herz, 2003), also accumulated (Figure 2A). Dab1 and Fyn RNA levels were unchanged (Figure S2A). Expression levels of Reelin, other members of the Reelin-Dab1 pathway (VLDLR and N-Cadherin), and another Cul5 substrate (p130Cas) were unaffected (Figure S2B) (Jossin and Cooper, 2011; Tissir and Goffinet, 2003)(Laszlo et al, submitted). The results suggest that Rbx2 controls turnover of active phosphorylated Dab1 and its tyrosine kinase, Fyn, in vivo.

Unexpectedly, Cul5 protein, but not RNA, decreased approximately 10-fold in *rbx2* cKO embryos (Figure 2A, Figure S2A). Levels of the closely related Cullin, Cul2, were unchanged (Figure S2B). These results suggest that Rbx2 regulates Cul5 but not Cul2 stability and that Rbx2 is a core component of CRL5.

To identify brain regions where Rbx2 regulates Dab1 turnover, we performed immunofluorescence on *rbx2* cKO and control littermates. Dab1 protein was increased in the neocortex, cerebellum, superior colliculus and thalamus but not the striatum or medulla (Figure 2B). In the neocortex, Dab1 was increased in the developing CP, IZ and SVZ but not VZ (Figure 2C). We also examined Dab1 in the cerebellum at P5. Dab1 was increased in the PCs but not GCs (Figure 2D). Increased Dab1 was detected in the PCP at E17.5 but not earlier (Figure S1H), coinciding with the time when PCP abnormalities were first detected. These results are consistent with Rbx2 negatively regulating Dab1 in the same brain regions and cell types where Rbx2, Reelin and Dab1 regulate neuron migration.

To determine whether Rbx2 regulates lamination by destabilizing Dab1, we tested for epistasis. When *rbx2* and *dab1* were both absent (*rbx2* cKO; *dab1* −/−), the phenotype resembled *dab1* −/−, with an absent MZ, inverted CP and underdeveloped cerebellum (Tissir and Goffinet, 2003), showing that *rbx2* requires *dab1* to regulate lamination (Figure 3A). Since the *rbx2* cKO phenotype may be due to excessive Dab1, we tested whether reducing the *dab1* gene dosage would suppress the phenotype. *dab1* heterozygosity in *rbx2* cKO (*rbx2* cKO; *dab1* +/−) decreased Dab1 level to approximately twice that in *dab1*+/+ samples (Figure S3B) and significantly rescued the distribution of Cux1 and Ctip2 positive neurons in the neocortex (Figure 3A). Both Purkinje cell positioning and cerebellum size were also significantly rescued by *dab1* heterozygosity (Figure 3B and Figure S3A).

Overall, our results suggest that *rbx2* requires *dab1* to suppress dispersion of neocortical projection neurons and PCs, and that *rbx2* mutant phenotypes are caused by Dab1 accumulation. While we cannot exclude the possibility that other Rbx2 interactions may also be required, it is likely that the main role for Rbx2 during cortical development is to regulate Dab1 turnover.

CRL5 adaptor protein SOCS7 regulates neuron migration and binds Dab1

CRL5 stimulates degradation of a wide range of proteins through its many substrate adaptor proteins. If the *rbx2* cKO phenotype is due to reduced Dab1 turnover, we reasoned that mutation of a Dab1-specific CRL5 substrate adaptor should cause a similar phenotype, while reducing potential *rbx2* cKO pleiotropic effects.

We focused on the SOCS sub-family of CRL5 substrate adaptors because they contain SH2 domains and can bind to sequences that contain phosphotyrosine (Okumura et al., 2012). We first used RT-qPCR to detect SOCS gene expression in the cortex at different times during development. SOCS4, 5, 6 and 7 mRNAs were readily detected throughout development, together with Cul5, Cul2, Rbx1 and Rbx2 (Figure S4A). CisH expression was low and SOCS1, 2 and 3 were barely detected above background. Interestingly, SOCS5 and 7 levels increased and SOCS4 and 6 decreased during development, suggesting that SOCS genes are differentially controlled at the level of transcription or mRNA stability.

We compared the SOCS proteins that are expressed in developing cortex for binding to pY.Dab1 in vitro. SOCS6 and 7 bound approximately ten-times more pY.Dab1 than SOCS4, 5 or CISH (Figure 4A). In utero electroporation of shRNA against SOCS7 but not SOCS6 caused “over-migration” (Figure 4B), similar to that caused by Rbx2 or Cul5 shRNA (Figure 1A). This result suggests that migration of projection neurons is controlled by a CRL5 complex containing SOCS7, Cul5 and Rbx2.

To test whether SOCS7 can regulate Dab1 levels we used HEK293T cells which express other CRL5 components (Bennett et al., 2010). Dab1-EGFP was transfected together with Fyn to drive Dab1 phosphorylation. When T7-SOCS7 WT was co-expressed, the level of Dab1-EGFP decreased, consistent with increased degradation (Figure 5A, compare lanes 4 and 5). As a control, we made T7-SOCS7-LCQQ, containing mutations predicted to inhibit binding to CRL5 (Piessevaux et al., 2006). T7-SOCS7-LCQQ did not inhibit Dab1-EGFP expression (Figure 5A, lane 6). Moreover, when Dab1 was not phosphorylated because Fyn kinase was absent or because the Dab1 phosphorylation sites were mutated, SOCS7 did not inhibit Dab1 expression (Figure 5A, lanes 1–3 & 7–9).

To test whether SOCS7-CRL5 complexes could directly ubiquitylate pY.Dab1, we incubated pY.Dab1 with SOCS7-CRL5 complexes in vitro. T7-SOCS7 WT or T7-SOCS7-LCQQ was over-expressed in 293T cells along with ElonginB/C, Cul5 and Rbx2, followed by anti-T7 immunoprecipitation. Cul5 co-precipitated with T7-SOCS7 WT but not LCQQ, as expected (Figure 5B). A HA-tagged Dab1 fusion protein containing the known tyrosine phosphorylation and ubiquitylation sites (Simo et al., 2010) was purified from bacteria and phosphorylated with purified Src kinase. The SOCS7 immunoprecipitates were then incubated with pY.Dab1, E1 and E2 enzymes and ubiquitin (Figure 5C). When SOCS7 WT immunoprecipitates, containing CRL5, were used in the ubiquitylation assay, pY.Dab1 was polyubiquitylated, shown by the ladder of HA-Dab1 (Figure 5C, lane 5). As predicted, SOCS7-LCQQ immunoprecipitates, lacking CRL5, were unable to catalyze Dab1 ubiquitylation (Figure 5C, lane 3). To test whether Dab1 phosphorylation was required for ubiquitylation, wild-type or mutant Dab1-4F, lacking pY sites, was incubated in the absence or presence of Src before the ubiquitylation assay. Either absence of Src (Figure 5D, lane 3), or mutation of the known pY sites (lane 5) inhibited ubiquitylation. Overall, these results showed that SOCS7 is a substrate adaptor for pY.Dab1, stimulating Cul5-dependent polyubiquitylation and degradation.

SOCS7 regulates cortical lamination and Dab1 accumulation

If SOCS7 is the CRL5 adaptor that recruits pY.Dab1 for ubiquitylation and proteolysis, then mutation of the *socs7* gene should cause a phenotype similar to that caused by *rbx2* mutation. Moreover, potential Dab1-independent effects of *rbx2* deletion would be minimized in a *socs7* mutant. A *socs7* knockout mouse was previously reported to have indistinct cortical layering, but was not analyzed further due to extreme hydrocephaly (Krebs et al., 2004). *socs7* deletion also causes severe cutaneous disease related to autoimmunity (Knisz et al., 2009).

To avoid hydrocephaly and autoimmunity, we deleted *socs7* from neuronal progenitors using a floxed *socs7* allele and Nestin-Cre (Figure 6A). As expected, Dab1 and pY.Dab1 increased in cortex samples from *socs7* cKO (Figure 6B, C), similar to the increase in *rbx2* cKO (Figure 2A). Similar to *rbx2* cKO, Dab1 was increased in the *socs7* cKO CP but not in the VZ, although unlike the *rbx2* cKO Dab1 only increased slightly in the SVZ and IZ (Figure 6D). Also unlike the *rbx2* cKO, neither Fyn nor Cul5 levels changed in the *socs7* cKO (Fig 6B, C). These results suggest that SOCS7 regulates Dab1 turnover in CP but not in the SVZ or IZ. Moreover, CRL5 substrate adaptors other than SOCS7 regulate Fyn turnover in the cortex, and Cul5 stability is independent of SOCS7.

In the neocortex, *socs7* deletion caused less disruption to the Tbr1-positive layer VI and SP neurons than *rbx2* deletion when analyzed at P0 (Figure 6F). However, *socs7* deletion caused conspicuous disruption to Ctip2-expressing layer V neurons and Cux1-expressing layer II–IV neurons. Moreover, at P5, Ctip2 and Cux1 positive neurons were invading contiguous territories, resembling the *rbx2* cKO cortex (Figure 6G). These results suggest that increased Dab1 and pY.Dab1 is sufficient to cause dispersion of layers II–V, even without the increase in Fyn that is caused by *rbx2* deletion.

Interestingly, unlike *rbx2*, *socs7* did not regulate Dab1 abundance in PCs in the cerebellum, suggesting that Dab1 is regulated by a SOCS7-independent Rbx2-dependent mechanism in these cells (Figure 6E). Moreover, cerebellar development was normal, with a unicellular PCL below the EGL (Figure 6H). Therefore, *socs7* cKO resembled *rbx2* cKO in accumulating pY.Dab1 and disrupting layers II–V of the neocortex, but had little effect on layer VI or the cerebellum. We suggest that additional phenotypes in *rbx2* cKO are due to SOCS7-independent, Rbx2-dependent, CRL5 substrates, presumably recognized by alternative CRL5 substrate adaptor proteins.

SOCS7 expression is tightly controlled during neuronal migration

Since layer VI cortical neurons are relatively unaffected in the *socs7* cKO mouse, we tested where SOCS7 is expressed in the CP. We used a gene trap *socs7*-LacZ line, which expresses LacZ under the *socs7* promoter (Figure 6A). LacZ expression was only detected in the meninges at E14.5, but increased in the cortex during development, first in the subplate and layer VI at E16.5 and later in all layers by E18.5 (Figure 7A). No expression was detected in the lower areas of the cortex, including the VZ, SVZ or IZ, confirming previous in situ hybridization data (Krebs et al., 2004). This suggests that *socs7* is expressed at much lower levels in progenitors, and perhaps early migrating neurons, relative to mature neurons that have ceased migration. To test this hypothesis, we used Ribotag mice to measure the amount of *socs7* mRNA on polysomes (Sanz et al., 2009). Upon Cre recombination, Ribotag mice express HA-tagged Rpl22, a core ribosomal protein, allowing polysome immunoprecipitation and purification of actively translating mRNA. We expressed Cre by in utero electroporation at E14.5 and mRNA samples from HA-Rpl22-positive neurons were collected at E16.5 and E18.5. At E16.5, when neurons born on E14.5 have reached the IZ, less *socs7* mRNA was being translated than at E18.5, when they have reached the top of the

CP and are stopping migration (Figure 7B). The results suggest that SOCS7 synthesis increases during developmental time, and increases within individual neurons when they near the top of the CP.

Temporal regulation of *socs7* gene expression might be important for Reelin responses during neuron migration. Recent data suggest that cortical neurons first require Reelin to trigger radial migration from the IZ into the CP (Jossin and Cooper, 2011), and again for terminal translocation through the primitive cortical zone to the top of the CP (Franco et al., 2011; Sekine et al., 2011). Low or absent expression of SOCS7 early in migration may prevent pY.Dab1 turnover, maximizing Reelin responses as cells pass through the IZ. To test this possibility, we forced SOCS7 expression by electroporation in utero at E14.5 and measured the positions of neurons at E19.5. While the control neurons positioned in layer III (Figure 7Ca), ectopic SOCS7 arrested migration in the IZ (Figure 7Cb, quantified in Figure 7Cg). Ectopic expression of CISH, which does not bind Dab1, did not arrest migration (Figure 7Cc). We hypothesized that SOCS7 might inhibit a pro-migratory substrate such as Dab1, either by binding and inhibiting activity or by stimulating CRL5-dependent degradation. To distinguish these possibilities, we used SOCS7-LCQQ, which binds Dab1 but not CRL5 (Figure 5B). SOCS7-LCQQ did not inhibit migration (Figure 7Ce). Furthermore, inhibition of migration by ectopic SOCS7 requires Cul5 (Figure 7Cd). These results imply that ectopic SOCS7 induces arrest in the IZ by targeting degradation of a pro-migratory protein. To test whether the hypothetical pro-migratory protein might be Dab1, we co-expressed Dab1-EGFP with SOCS7 (Figure 7Cf). Migration was rescued, suggesting that ectopic SOCS7 inhibits migration in the IZ by targeting pY.Dab1 to CRL5 for degradation, thereby inhibiting Reelin responses.

Taken together, our data suggest that SOCS7 regulates the migration of layer II–V neocortical neurons while other SOCS proteins regulate layer VI neurons and PCs. In addition, temporal regulation of *socs7* expression is important for normal development. Low expression of *socs7* early in migration may maximize Reelin responses required to leave the IZ and enter the CP. Increased *socs7* expression when cells reach the top of the CP ensures efficient down-regulation of Reelin signaling, permitting neurons to stop migrating and differentiate in the correct layer.

Discussion

Our results show that CRL5 is required for normal layering in the cortex and cerebellum. When CRL5 activity is inhibited by deleting *rbx2*, projection neurons in the neocortex and PCs in the cerebellum position ectopically. Remarkably, neocortical layer order is preserved but layer boundaries are blurred. Genetic and biochemical evidence shows that the phenotypes are likely due to excess Dab1 activity. SOCS7 is a CRL5 adaptor that binds pY.Dab1 and targets it for polyubiquitylation in vitro. In vivo, *socs7* deletion had no effect on Fyn levels but caused pY.Dab1 accumulation and disorganization of the cortex. However, SOCS7 has little or no role in the Rbx2-dependent pY.Dab1 accumulation or positioning of layer VI neurons or PCs. Therefore, different CRL5 adaptors may regulate where and when Dab1 and Fyn turnover occurs, orchestrating neuron positioning in different brain regions and pointing to the importance of spatially regulated *socs* expression for normal development.

Proper temporal control of *socs* expression is also important. Premature expression of *socs7* traps neurons in the intermediate zone by destabilizing Dab1. Recent evidence points to Reelin-Dab1 requirements at two stages during migration of cortical neurons: First, during the transition from multipolar to bipolar migration near the top of the intermediate zone (Jossin and Cooper, 2011; Simo et al., 2010; Uchida et al., 2009), and second, during somal

translocation at the top of the cortical plate (Franco et al., 2011; Gil-Sanz et al., 2013; Sekine et al., 2011). Our results suggest a model in which dynamic regulation of CRL5 activity is relevant for both stages of migration (Figure 7D). In the intermediate zone, *socs7* is poorly expressed, limiting CRL5 activity on pY.Dab1, maximizing Reelin sensitivity and allowing neurons to enter into the cortical plate. *socs7* is then induced, ensuring feedback inhibition of Reelin signaling and stopping migration at the top of the cortical plate (Figure 7D). According to this model, spatio-temporal regulation of different CRL5 adaptors that target pY.Dab1, Fyn and potentially other proteins modulates Reelin signaling in different cell types and different times during development.

In vivo substrates of CRL5

Our results show for the first time that CRL5 regulates both Dab1 and Fyn in vivo. Dab1 protein but not RNA accumulates in the cortex when *rbx2* or *socs7* is deleted, consistent with decreased proteasome-dependent proteolysis of Dab1 by a CRL5 complex containing Cul5, Rbx2 and SOCS7. However, SOCS7 does not regulate Dab1 in the cerebellum, suggesting that another SOCS protein may be required. We found that SOCS6 and SOCS7 both bind pY.Dab1 strongly. Interestingly, SOCS6 and 7 are closely related, and their SH2 domains bind to similar sequences (Krebs et al., 2002). Therefore, SOCS6 and 7 potentially bind to the pY sites in Dab1 that are required for turnover. Clearly, SOCS6 is an excellent candidate to regulate Reelin signaling in PCs. Future studies will address the potential role of SOCS6 in PC positioning and cerebellum development.

We also found that CRL5 down-regulates Fyn. CRL5 regulates Src turnover in cultured cells, but the substrate adaptors linking CRL5 to Src are unknown (Laszlo and Cooper, 2009; Pan et al., 2011). SOCS6 was reported to bind another Src relative, Lck, but not Fyn (Choi et al., 2010). Importantly, *socs7* does not regulate Fyn, suggesting that either SOCS7 does not target Fyn to Cul5 or there is functional redundancy with one or more other SOCS proteins. Since *socs7* regulates Dab1 but not Fyn, it is likely that Dab1-Fyn signaling complexes dissociate before ubiquitylation. It will be important to identify adaptors that target Fyn to CRL5 in vivo since CRL5-dependent Fyn down-regulation could provide negative feedback for other Fyn-dependent signaling events.

Our results also shed light on the relationship between Rbx2 and Cul5. Initial studies indicated that Rbx1 and Rbx2 had overlapping specificity for different Cullins (Ohta et al., 1999). More recently, Rbx2 was reported to associate with Cul1 and its adaptor Fbxw7, permitting ubiquitylation and degradation of c-Jun and neurofibromatosis-1 (Gu et al., 2007; Tan et al., 2012). However, co-immunoprecipitation and knockdown experiments in cell lines showed that Cul5 normally interacts with Rbx2 (Kamura et al., 2004). In addition, Nedd8 modification of Cul5 requires Rbx2 while Nedd8 modification of other Cullins requires Rbx1 (Huang et al., 2009). Our results argue that Rbx2 works in the same pathway as Cul5 and that Rbx1 and Rbx2 are not redundant. Moreover, Rbx2 knockout in neuronal progenitors did not detectably alter cell proliferation, apoptosis or differentiation, suggesting that Rbx2 is unlikely to regulate Cul1. Importantly, Rbx2 deletion reduced the stability of Cul5 but not Cul2. Our results are consistent with a strict interdependence between Rbx2 and Cul5.

Phenotypes caused by excess Reelin signaling

Why does cell-autonomous over-migration, caused by excessive or prolonged Reelin-Dab1-Fyn signaling, lead to the observed phenotypes in *rbx2* and *socs7* mutant mice? Why is there not, say, inversion of neocortical layers or migration of PCs into the external granule layer?

Previous studies, using transient Cul5 knockdown or a stablized Dab1 mutant that lacks ubiquitylation sites, showed that inhibiting pY.Dab1 degradation causes over-migration, although neurons do not enter the marginal zone (Feng et al., 2007; Simo et al., 2010). This led to a model in which Reelin first stimulates somal translocation to the top of the cortical plate, then stimulates Dab1 degradation, turning off the Reelin responses and stopping migration (Cooper, 2008). We expected that global inhibition of CRL5 would cause first-born cortical neurons to continue migrating after they reach the top of the cortical plate, and their presence would impede subsequent neurons reaching the marginal zone and therefore fail to undergo terminal translocation. With time, this would likely lead to an inverted cortex. However, the current data show that CRL5 inhibition causes layer dispersion rather than inversion. One potential explanation is based on the presence of a physical barrier. Rbx2 deletion from layer VI projection neurons causes continued somal translocation. Some of the next cohort of neurons may translocate successfully and also continue migrating. However, others may be unable to get close enough to receive the Reelin signal, so do not initiate somal translocation and are left behind. Over time, increasing numbers of migrating cells at the top of the cortex create an increasing barrier for later neurons, and more cells are left deeper in the cortical plate, resulting in a “blurring” of boundaries. Another possibility that cannot be excluded is that *rbx2* deletion with Nestin-Cre allows time for compensation that does not occur when Cre or shRNA is electroporated into radial glia progenitor cells. In the mutant animals, Reelin signaling is upregulated from early stages in all the cells, which might blur layers rather than induce overmigration. Formal testing of these hypotheses will be possible using appropriate Cre drivers to delete *rbx2* at different stages of migration.

In the cerebellum, *rbx2* deletion caused Dab1 accumulation in PCs and an abnormal PC plate by E17.5. Later, GC proliferation, cerebellar expansion and the formation of a unicellular PC layer were impaired. The phenotype was suppressed by lowering the gene dosage of *dab1*, suggesting that it results from excess Reelin-Dab1 signaling. Transgenic expression of activated mutant Src also causes multiple layers of PCs (Kotani et al., 2006). However, the phenotypes are difficult to explain by over-migration of PCs. One possible explanation involves PC adhesion. Reelin regulates neuron-neuron interactions ex vivo and in vivo (Kubo et al., 2010; Ogawa et al., 1995). The single layer alignment of PCs may involve down-regulation of Reelin-stimulated PC-PC adhesion. Excess PC-PC adhesion in *rbx2* mutants might interfere with formation of the PC plate and its rearrangement into a unicellular layer. Candidate adhesion molecules include N-cadherin and integrin α5, which are upregulated in response to Reelin in the cortical intermediate zone and marginal zone respectively (Jossin and Cooper, 2011; Sekine et al., 2012).

rbx2 and *socs7* conditional knockouts may be useful for future investigation of where and when Dab1 is activated during development and in adulthood. Available anti-pY.Dab1 antibodies have not been sensitive or specific enough for detecting Dab1 activity, but the brain regions that accumulate Dab1 when Rbx2 is deleted are presumably regions where Dab1 is activated and down-regulated. In addition, Rbx2 and SOCS7 deletion from different neuron types after birth should increase Dab1-Fyn signaling cell autonomously, and may lead to additional phenotypes.

Experimental Procedures

Animals

B6N-Rnf7$^{tm1a(Eucomm)Wtsi}$/H (*rbx2*-LacZ) mice were obtained from the International Knockout Mouse Consortium (IKMC, http://www.knockoutmouse.org) (Skarnes et al., 2011). *rbx2*-LacZ/+ females were crossed to FLPeR males (Farley et al., 2000) to remove the gene-trap cassette and generate Rbx2 floxed mice (*rbx2* fl/+). Homozygous *rbx2* fl/fl were viable and fertile and were crossed with Nestin-Cre/+; *rbx2* fl/+ mice to delete *rbx2*

during brain development (Tronche et al., 1999). *rbx2* cKO animals (*rbx2* fl/fl; Nestin-Cre/+) were born at Mendelian ratio.

B6Brd;B6N-Tyr$^{c\text{-}Brd}$ Socs7$^{tm1a(EUCOMM)Wtsi}$/WtsiCnbc (*socs7*-LacZ/+) two-cell stage embryos were obtained from the IKMC and recovered by transplantation into foster mothers (Soriano, 1995). *socs7* fl/fl and *socs7* cKO animals were obtained as described above.

In utero microinjection and electroporation

In utero microinjection and electroporation were performed as described (Simo et al. 2010), in accordance with procedures approved by the Fred Hutchinson Cancer Research Center Institutional Animal Care and Use Committee.

Antibodies

The following antibodies were used for biochemistry: rabbit anti-Rbx2 (G-8), rabbit anti-Fyn (Fyn3), rabbit anti-Cul5 (H-300) and goat anti-Actin (I-19) (Santa Cruz Biotechnology); mouse anti-phosphotyrosine (4G10); rabbit anti-Dab1 (Millipore); mouse anti-T7 (EMD Biosciences); mouse anti-HA (HA.11) and mouse anti-Tuj1 (Covance). The following antibodies were used for immunofluorescence: rabbit anti-CDP (Cux1, Santa Cruz Biotechnology); rat anti-Ctip2 and rabbit anti-Tbr1 (Abcam); rabbit anti-Calbindin and mouse anti-Reelin (Calbiochem); rabbit anti-pS.H3 (Cell Signaling); mouse anti-Nestin and anti-GM130 (BD Bioscience); mouse anti-Lhx1/5 (4F2) (Developmental Studies Hybridoma Bank); and rabbit anti-Dab1 (Millipore).

Biochemistry

Binding of pY.Dab1 to different SOCS proteins was assayed in vitro. Dab1-EGFP and Fyn were co-transfected into HEK293T cells and lysates were mixed with various T7-SOCSs immobilized on Protein A/G beads (Santa Cruz Biotechnology). The T7-SOCSs were prepared by transfection of 293T cells and immunoprecipitation with anti-T7 antibodies. After mixing Dab1-EGFP with T7-SOCS beads for 3 h at 4°C, the beads were washed 5-times with RIPA buffer and analyzed by Western blotting with anti-pY (4G10) and anti-T7 antibodies.

Dab1 expression levels in the presence of Fyn and presence or absence of SOCSs were measured as described elsewhere (Feng et al., 2007).

In vitro polyubiquitylation

Duplicate 10-cm dishes of HEK293T cells were transfected with 5 μg of each of the following: pCAG-Cul5, pCAG-Rbx2, pCAG-ElonginB-T2A-ElonginC, and either wild-type pCAG-T7-SOCS7 or pCAG-T7-SOCS7-LCQQ. After 2 days, cells were lysed in 1 ml/dish of 20 mM HEPES pH 7.4, 0.5 M NaCl, 0.25% Triton X-100, 1 mM PMSF, 1 μg/ml aprotinin and 10 μg/ml leupeptin. T7-SOCS7 and T7-SOCS7-LCQQ were immunoprecipitated using 10 μg mouse anti-T7 antibody and Protein A beads, washed with lysis buffer and then with 20 mM HEPES pH 7.4, 10% glycerol, 0.2 M NaCl, 0.01% Triton X-100, 1 mM DTT and protease inhibitors, and snap frozen. Phosphorylated HA-Dab1 was obtained by 30 min incubation of the following components at 37°C: 16 μg/ml recombinant Src (Seeliger et al., 2005), 130 μg/ml recombinant Dab1 fusion protein tagged with HA, 50 mM HEPES pH 7.4, 10 mM $MgCl_2$, 1 mM ATP, 0.05% 2-mercaptoethanol, 1 mM Na_3VO_4. Ubiquitylation reactions contained, in a total volume of 30 μl: 10 μl of anti-T7 immunoprecipitate from 100 μg cell protein, 10 μl of kinase reaction containing 1.2 μg pY.HA-Dab1, 1.6 μg UbcH5b (kind gift of Ning Zheng), 800 ng GST-E1, 6 μg ubiquitin, 2

mM ATP, 10 mM $MgCl_2$ and 50 nM Bortezomib. Reactions were stopped after 1 h at 25°C and analyzed by SDS PAGE and Western blotting.

Polysome Immunoprecipitation

Embryos of Ribotag mice, which express HA-tagged ribosomal protein Rpl22 from a floxed cassette (Sanz et al., 2009), were electroporated in utero at E14.5 with 1 μg of pBOB-CAG-iCre-SD (Addgene) and 0.5 μg of pCAG-GFP. At E16.5 and E18.5, embryo brains were harvested and GFP-expressing regions were coarsely dissected and pooled from 3 to 6 individual embryos. Polysomes were prepared and immunoprecipitated with anti-HA antibody. *socs7*, *actin* and *gapdh* transcripts were quantified by qRT-PCR. Immunoprecipitated *socs7* mRNA and total *socs7* mRNA were normalized to the mean of *actin* and *gapdh* using the ΔΔCt method. The immunoprecipitated mRNA samples were compared to the input sample in each case.

Statistics

Statistical analyses were performed with Prism 6 (GraphPad Software). Statistical analysis used for each experiment is described in the corresponding figure legend. Student's t-test was used for two-population comparison, one-way ANOVA with Tukey's post-hoc test for multiple comparisons, and two-way ANOVA with Tukey's post-hoc test for comparing neuron distributions. n.s.; not significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Cul5, Rbx2 and SOCS7 regulate neuron positioning through signaling protein Dab1.
- SOCS7 and Rbx2 are subunits of Cullin5-RING E3 ubiquitin ligases in vivo.
- Rbx2 and SOCS7 regulate cortical migrations, but only Rbx2 regulates the cerebellum
- Spatial and temporal control of *socs* gene expression ensures timely end of migration

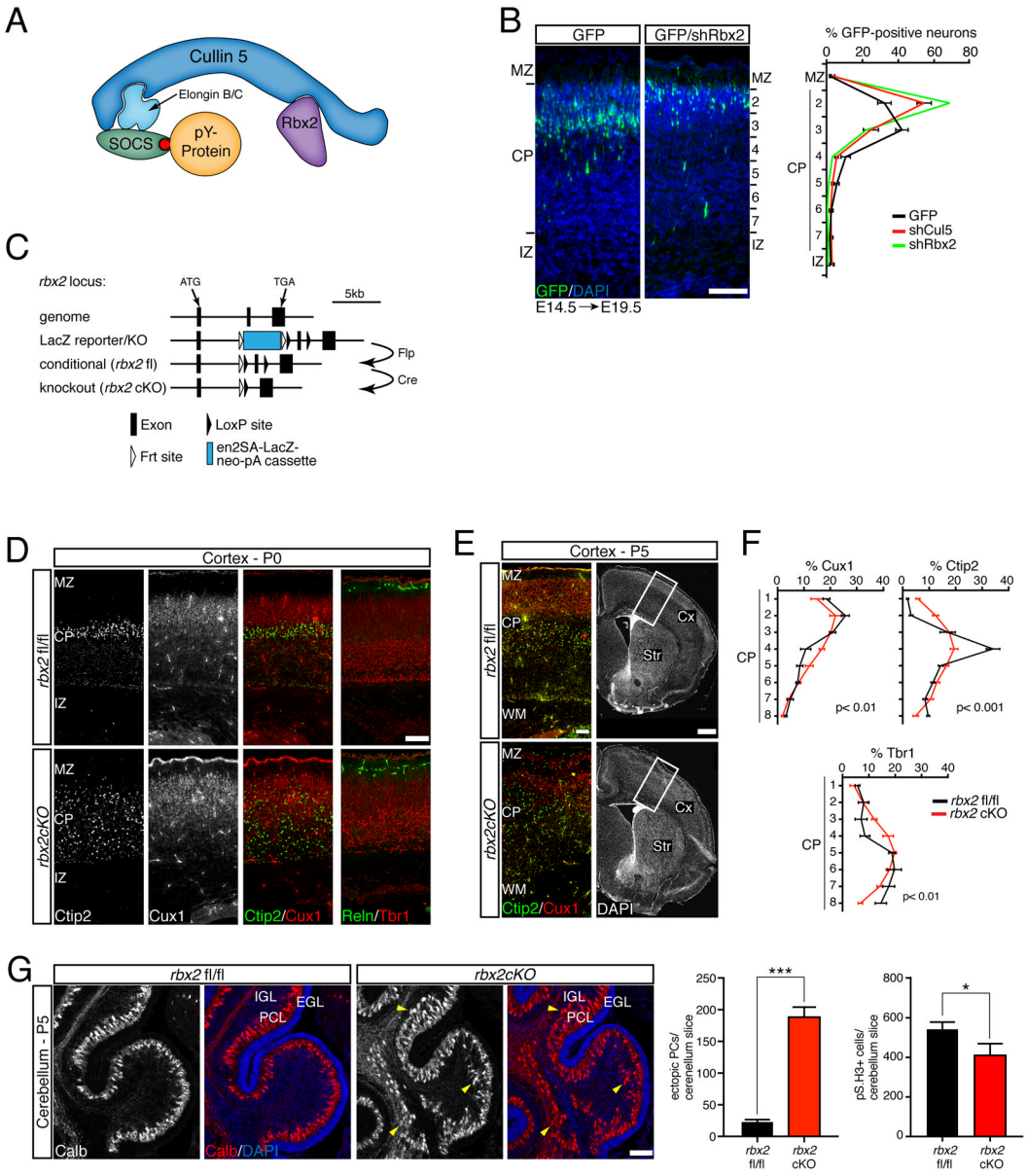

Figure 1. Rbx2 regulates neuron migration in the cortical plate and cerebellum

(A) CRL5 complex. Cul5 is the backbone protein that binds the adaptors subunits Elongin B and C, which in turn recruit substrate adaptor proteins. SOCS proteins are substrate adaptor proteins that bind to tyrosine phosphorylated substrate proteins. Rbx2 is a RING domain protein that binds Cul5.

(B) shRNA against Rbx2 induces over migration. pMX.puro vector or shRbx2 and pCAG-GFP were expressed by electroporation in utero at E14.5 and distribution of GFP-positive neurons analyzed on E19.5. Scale bar, 100 μm. Graph: Distribution of GFP-positive neurons. Mean±SEM for control (n=5) and shCul5 (n=7). Mean for shRbx2 (n=2).

(C) Generation of *rbx2* LacZ reporter, floxed (fl) and conditional knockout (cKO) mice.

(D) Dispersion of cortical layers at P0. Cortex of *rbx2* fl/fl or *rbx2* cKO at P0. Staining for Ctip2 (green), Cux1 (red), Tbr1 (red) and Reelin (green) as indicated. Scale bar, 100 μm.

(E) Developmental defects at P5. (Left) Increased dispersion of Ctip2- and Cux1-positive cells in the *rbx2* cKO cortex. Scale bar, 100 μm. (Right) Global cortical disorganization in the *rbx2* cKO visualized with DAPI. Scale bar, 500μm.
(F) Distribution of Cux1-, Ctip2- and Tbr1-positive neurons in the cortical plate at P0. Mean ±SEM (Cux1: *rbx2* fl/fl n=4 and *rbx2* cKO n=6; Ctip2: *rbx2* fl/fl n=5 and *rbx2* cKO n=6; and Tbr1: *rbx2* fl/fl n=3 and *rbx2* cKO n=4). Two-way ANOVA with Tukey's post-hoc test.
(G) Cerebellar defects. Sagittal sections of P5 cerebella from *rbx2* fl/fl and *rbx2* cKO stained with anti-Calbindin (red). Region of folium X is shown. Arrowheads: ectopic PCs. Scale bar, 100 μm. Graphs: Mean±SEM of number of ectopic PCs (*rbx2* fl/fl n=8 and *rbx2* cKO n=9). Mean±SEM of number of mitotic pS.H3-positive granule cells (*rbx2* fl/fl n=3 and *rbx2* cKO n=3). ***, $p<0.001$; *, $p<0.05$. Student's t-test.
See also Figure S1.

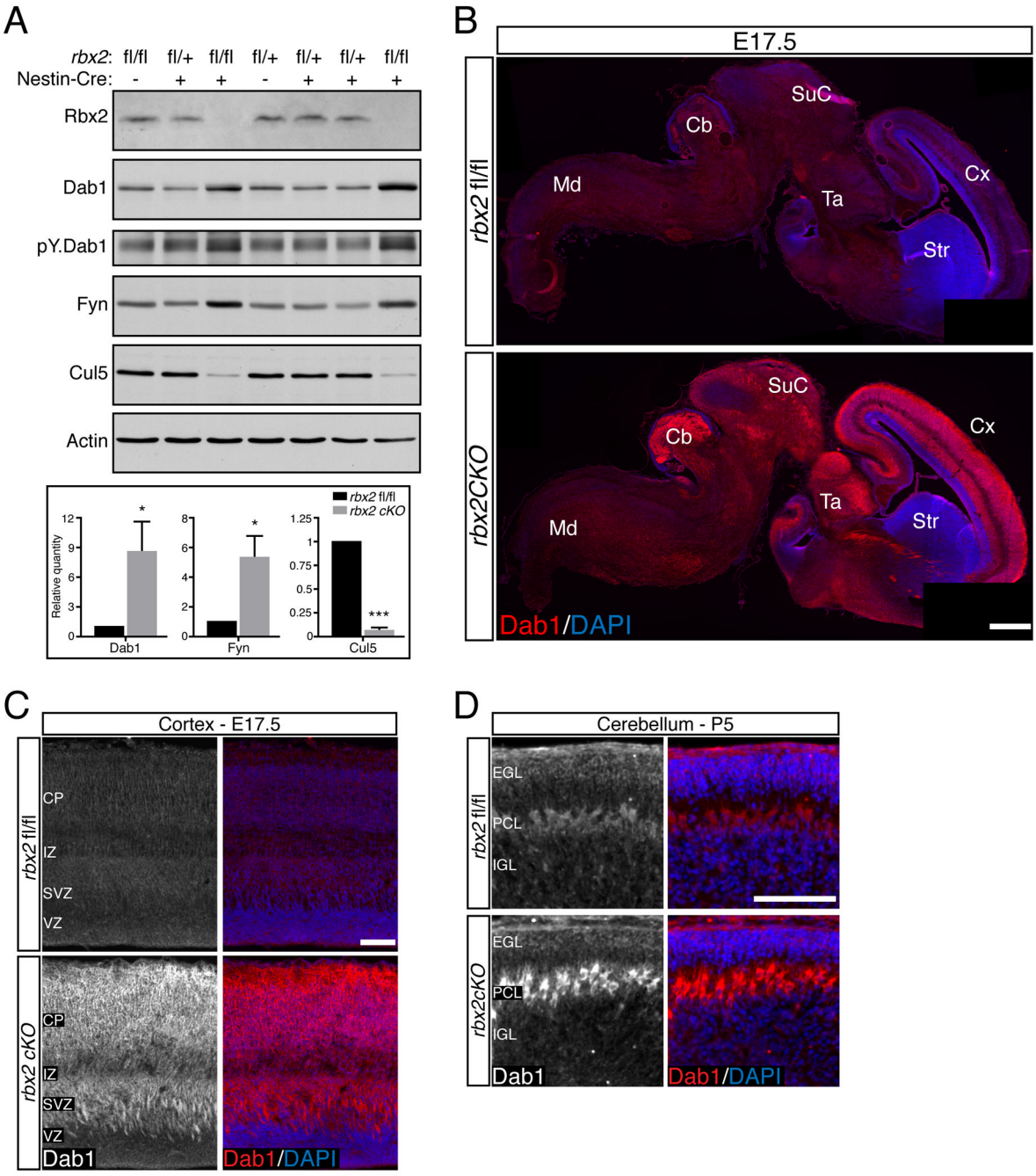

Figure 2. Rbx2 regulates Dab1 in cortical neurons and Purkinje cells

(A) E17.5 cortex lysates were analyzed by Western blotting with antibodies to Rbx2, Dab1, pY (4G10), Fyn, Cul5 and Actin. Samples represent a full litter. Graph: Mean±SEM of Dab1, Fyn and Cul5 levels in *rbx2* fl/fl versus *rbx2* cKO (*rbx2* fl/fl; Nestin-Cre/+) from three embryos born from different litters. ***, p<0.001; *, p<0.05. Student's t-test.

(B) Sagittal sections of E17.5 *rbx2* fl/fl or *rbx2* cKO brains stained with anti-Dab1 antibody (red). Scale bar, 500μm. Cb: cerebellum, Cx: cortex, Md, medulla, Str: striatum, SuC: superior colliculus,Ta: thalamus.

(C) Dab1 accumulated in all areas of the cortex except the VZ in E17.5 *rbx2* cKO relative to control. Sagittal sections. SVZ: subventricular zone and VZ: ventricular zone.

(D) Dab1 accumulated in PCs of P5 cerebellum of *rbx2* cKO. Sagittal sections showing folium X.
All scale bars, 100 μm.
See also Figure S2.

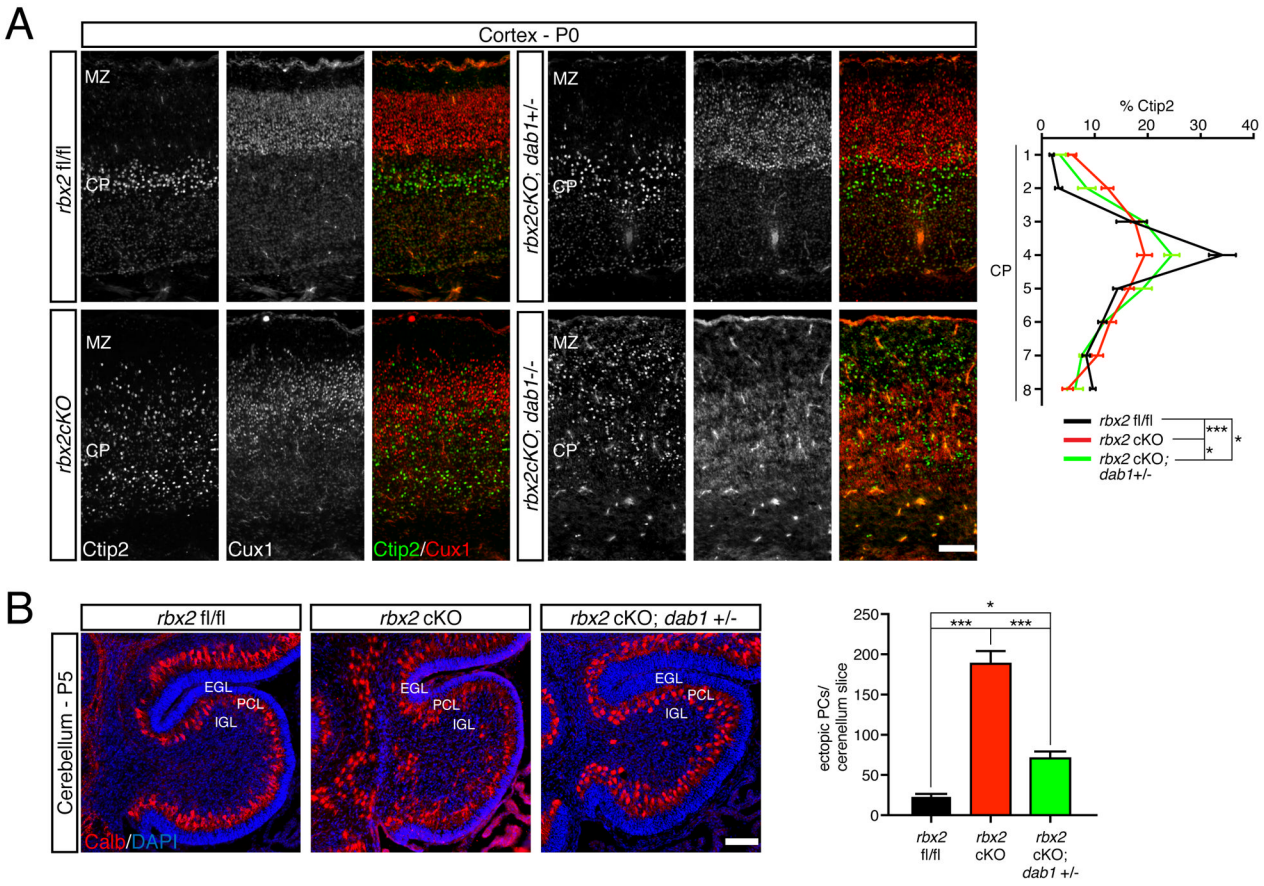

Figure 3. Rbx2 phenotype requires Dab1

(A) Suppression of *rbx2* cKO cortex phenotype by reduced *dab1* gene dosage. Coronal sections of *rbx2* fl/fl, *rbx2* cKO, *rbx2* cKO; *dab1* +/−, and *rbx2* cKO; *dab1*−/− cortex samples at P0 stained with antibodies to Ctip2 (green) and Cux1 (red). Note cortical inversion in *rbx2* cKO; *dab1*−/−. Scale bar, 100 μm. Graph: Ctip2-positive cells in the cortical plate of *rbx2* fl/fl (n=5), *rbx2* cKO (n=6), and *rbx2* cKO; *dab1*+/− (n=4). Mean ± SEM. *, p<0.05 and ***, p<0.001, two-way ANOVA with Tukey's post-hoc test.

(B) Suppression of *rbx2* cKO cerebellum phenotype by reduced *dab1* gene dosage. Sagittal sections of *rbx2* fl/fl, r*bx2* cKO and *rbx2* cKO; *dab1* +/− cerebellum samples at P5 were stained with anti-Calbindin (red). All panels show folium X. Scale bar, 100 μm. Graph: Mean±SEM of number of ectopic PCs per slice for *rbx2* fl/fl (n=8), *rbx2* cKO (n=9), and *rbx2* cKO; *dab1*+/− (n=6). *, p<0.05 and ***, p<0.001, one-way ANOVA with Tukey's post-hoc test.

See also Figure S3.

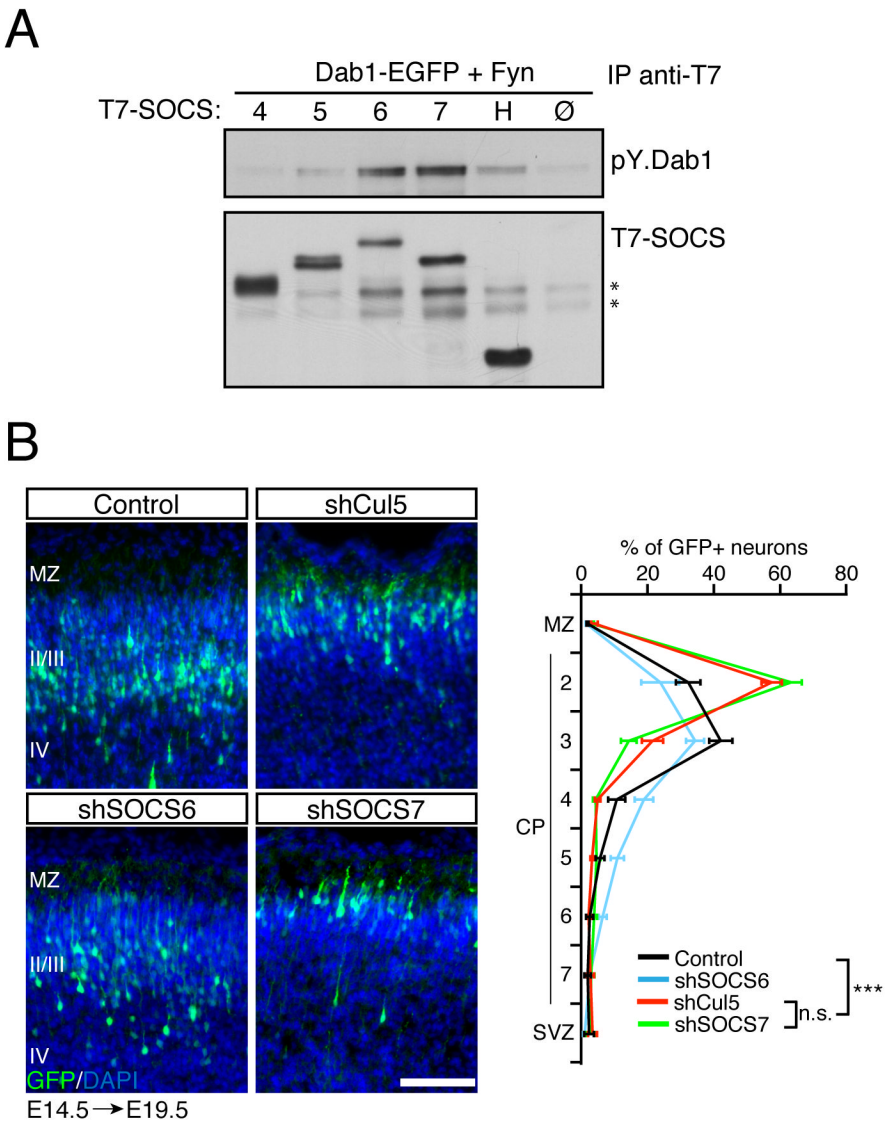

Figure 4. SOCS6 and 7 bind Dab1 and SOCS7 inhibits over-migration

(A) SOCS6 and SOCS7 bind to pY.Dab1 *in vitro*. Immobilized T7-SOCS4, SOCS5, SOCS6, SOCS7, CISH (H) or control (Ø) were mixed with equal quantities of phosphorylated Dab1 (pY.Dab1) and bound proteins were analyzed by Western blotting with anti-pY (4G10) and anti-T7 antibodies. Asterisks (*) indicate nonspecific bands. (B) shRNA against SOCS7 but not SOCS6 induces over-migration. pMX.puro-shCul5, shSOCS6, shSOCS7, or vector and pCAG-GFP were expressed by electroporation in utero at E14.5 and distribution of GFP-positive neurons analyzed at E19.5. Micrographs: marginal zone (MZ) and top part of cortical plate. Scale bar, 100 μm. Graph: distribution of GFP-expressing neurons for control (n=5), shCul5 (n=6), shSOCS6 (n=5) and shSOCS7 (n=6). Mean ± SEM. ***, $p<0.001$, two-way ANOVA with Tukey's post-hoc test.
See also Figure S4.

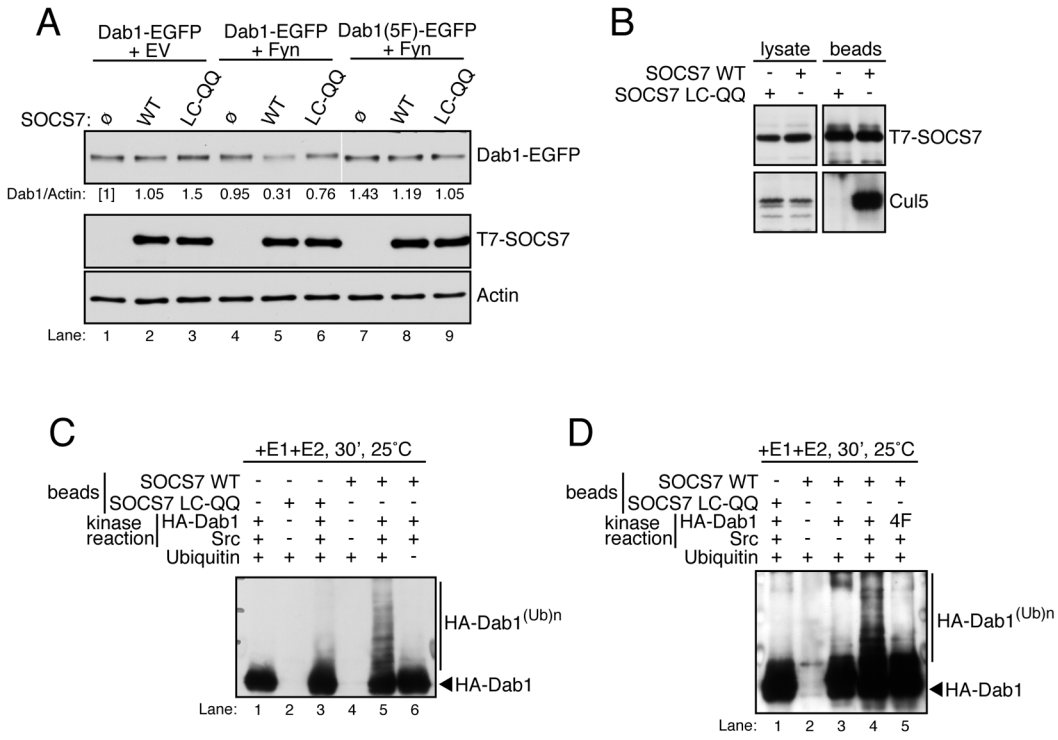

Figure 5. SOCS7 promotes Dab1 polyubiquitylation and degradation

(A) SOCS7 promotes pY.Dab1 degradation. HEK293T cells were transfected with wild-type Dab1-EGFP or Dab1(5F)-EGFP; Fyn or empty vector (EV); and wild-type SOCS7 (WT), mutant SOCS7 (LC-QQ) or empty vector (ø), as indicated. Cells were lysed 16 h after transfection, and lysates were analyzed by Western blotting with antibodies to GFP, T7 and actin. Dab1 to Actin ratio shows that Dab1 levels are reduced when expressed with SOCS7 WT and Fyn (lane 5).

(B) SOCS7 but not SOCS7-LCQQ binds CRL5. HEK293T cells were transfected with Cul5, Rbx2, ElonginB/C, and T7-SOCS7 WT or T7-SOCS7-LCQQ. After 2 d, T7-SOCS7 and T7-SOCS7-LCQQ were immunoprecipitated with anti-T7 antibodies and analyzed by Western blotting with antibodies to T7 and Cul5.

(C, D) Polyubiquitylation of Dab1 by SOCS7-Cul5 complexes in vitro requires tyrosine phosphorylation. Beads containing T7-SOCS7 WT or T7-SOCS7-LCQQ from (B) were incubated with purified E1, E2 and ubiquitin and various combinations of HA-Dab1 or HA-Dab1(4F) that had previously been phosphorylated by incubation with Src in kinase reactions. Reaction products were analyzed by Western blotting with anti-HA antibody. Polyubiquitylated HA-Dab1 (HA-Dab1$^{(Ub)n}$) forms a ladder above unmodified HA-Dab1.

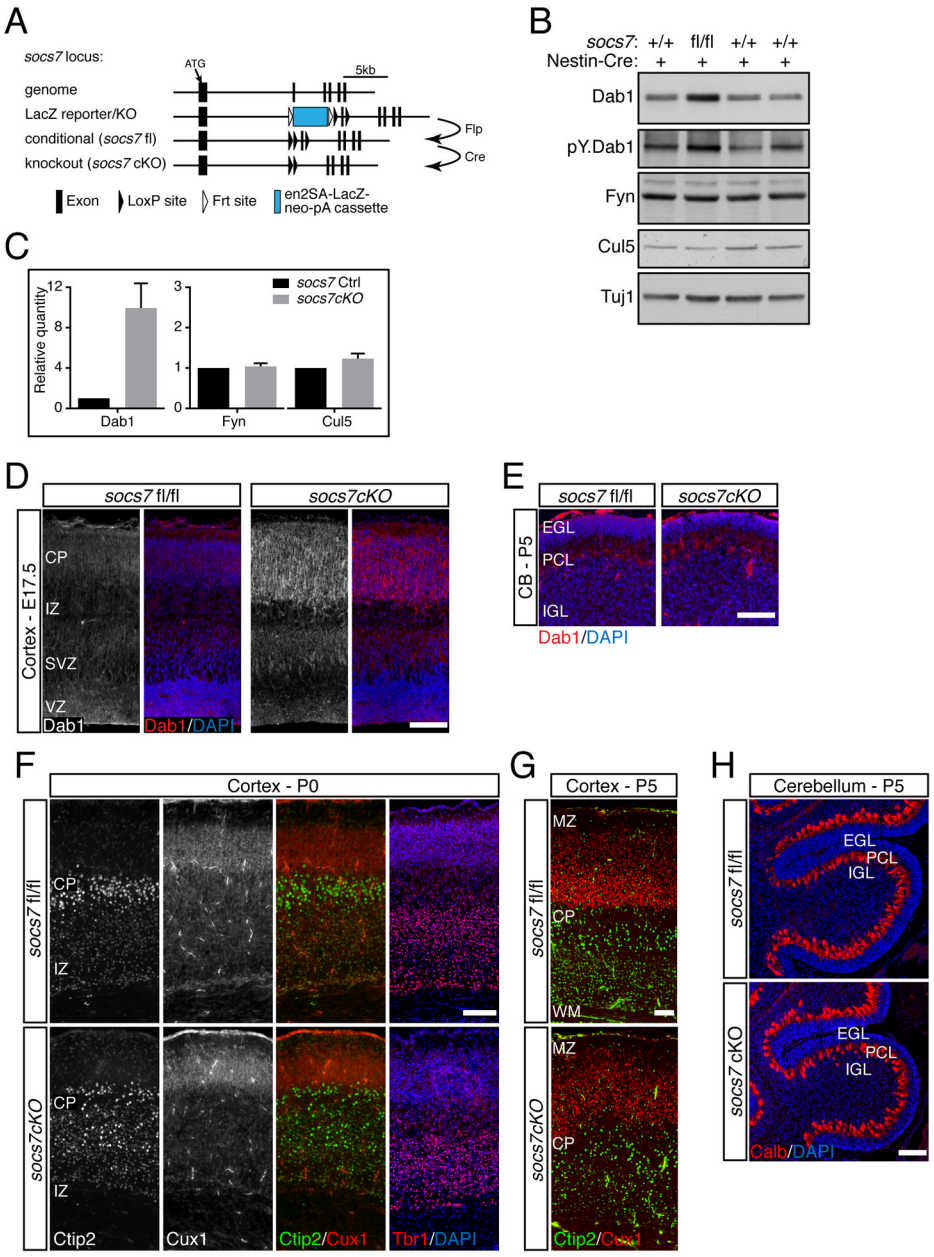

Figure 6. SOCS7 regulates Dab1 levels and cortical but not cerebellar development

(A) Generation of *socs7* LacZ reporter, floxed (fl) and conditional knockout (cKO) mice.

(B) E17.5 cortex lysates from control (+/+) or *socs7* cKO mutant (socs7 fl/fl; Nestin-Cre/+) embryos were analyzed by Western blotting with antibodies to Dab1, pY (4G10), Fyn, Cul5 and neuron-specific class III β-tubulin (Tuj1).

(C) Dab1, Fyn and Cul5 abundance, relative to β-tubulin, in *socs7* cKO (n=3) relative to control (n=5). Mean ± SEM.

(D, E) Dab1 increases in cortical plate but not cerebellum. E17.5 neocortex (D) and P5 cerebellum (E) from *socs7* fl/fl or *socs7* cKO mice were stained with Dab1 antibodies (red).

(F, G) Cortex from P0 (F) and P5 (G) *socs7* fl/fl or *socs7* cKO mice stained with anti-Ctip2 (green)(F,G), anti-Cux1 (red)(F,G), and anti-Tbr1 (red)(F).

(H) P5 cerebella from *socs7* fl/fl and *socs7* cKO mice stained with anti-Calbindin (red).

All scale bars, 100 μm.

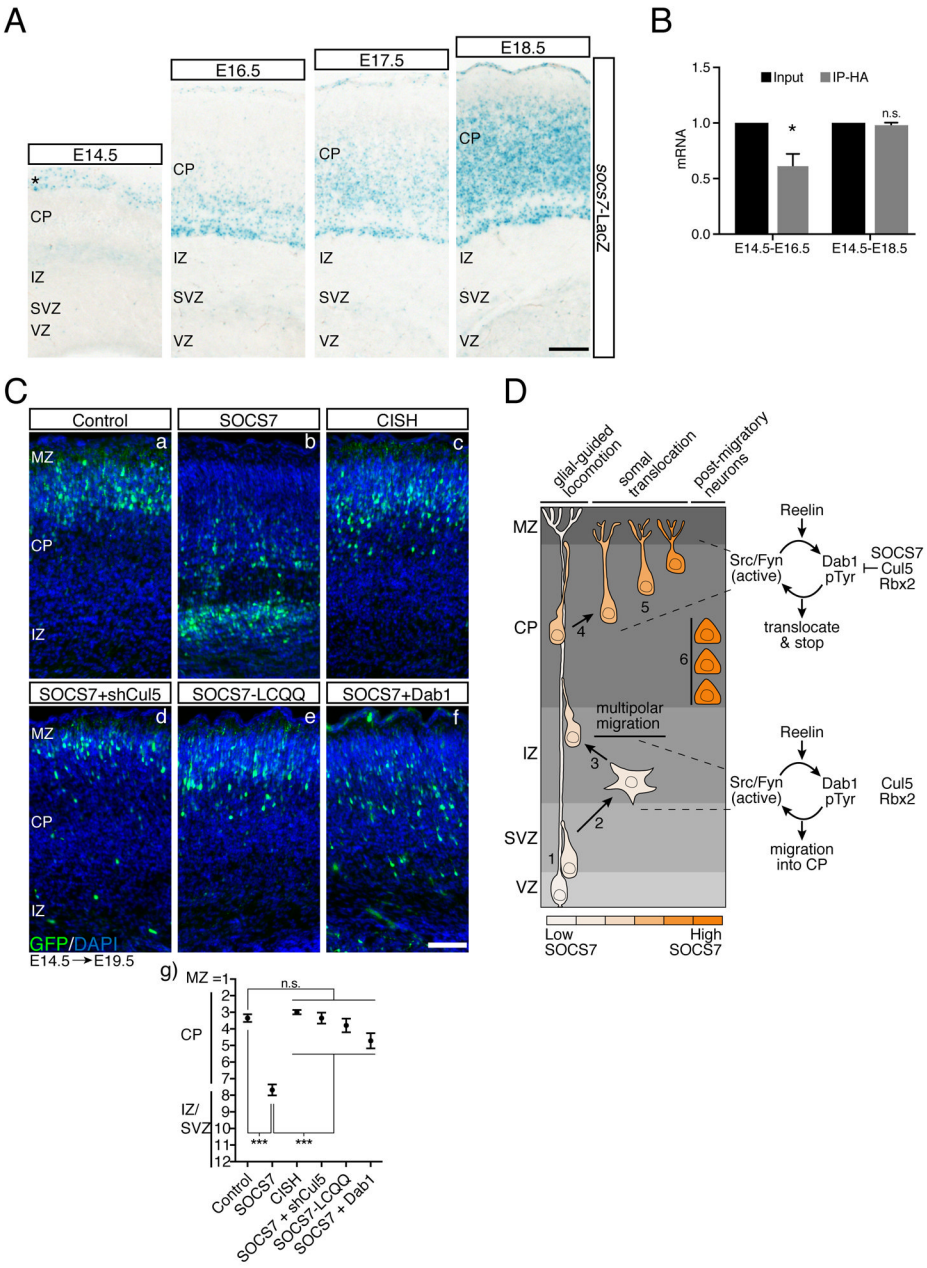

Figure 7. SOCS7 expression increases in the cortical plate during development and ectopic expression inhibits migration

(A) *socs7* expression in the neocortex. X-gal staining of coronal sections of *socs7*-LacZ/+ embryos at E14.5, E16.5, E17.5 and E18.5.

(B) *socs7* mRNA translation. Heterozygous Ribotag embryos were electroporated in utero at E14.5 with a Cre-expressing vector. Brains were harvested at E16.5 or E18.5. Polysomal *socs7* mRNA was quantified relative to the mean of *gapdh* and *actin* mRNAs. Results are mean±SEM of three independent experiments. *, p<0.05, Student's t-test.

(C) Ectopic expression of SOCS7. E14.5 wild-type embryos were electroporated in utero with GFP alone (a), or with T7-SOCS7(b), T7-CISH(c), T7-SOCS7 and shCul5(d), T7-SOCS7-LC-QQ(e), and T7-SOCS7 and Dab1-EGFP(f). Brains were analyzed at E19.5. (g) Average positions of GFP-positive neurons. Data from 3 to 10 independent embryos were

quantified per condition. Results are mean±SEM. ***, p<0.001. One-way ANOVA with Tukey's post-hoc test.

(D) Model. SOCS7 controls neuron migration through pY.Dab1. (1) Radial glia cells give rise to the majority of projection neurons in the cortex. (2) Neurons become multipolar when reach the IZ. (3) Low expression of SOCS7 (pale orange) inhibits pY.Dab1 turnover by CRL5, allowing low levels of Reelin in the IZ to induce re-polarization and radial migration. (4) When neurons near the top of the CP, Reelin in the MZ promotes detachment from the radial glia and (5) stimulates somal translocation. (6) Increased expression of SOCS7 (deeper orange) allows SOCS7-CRL5 to degrade pY.Dab1, stopping migration. Continued high expression of SOCS7 in post-migratory neurons limits the duration of further Reelin-induced responses.