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CHL1 Regulates Cortical Neuron Identity and Laminar Formation during Stem Cell-derived Neurogenesis

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ABSTRACT

Background: Close Homolog of L1 (*CHL1*), a neural cell adhesion molecule, plays a critical role in cortical development, but its isoform-specific and stage-dependent functions remain poorly defined. This study examines the differential effects of *CHL1* presented either apically (*CHL(S)*) or basally (*CHL(B)*) on cortical neuron differentiation and maturation at key developmental stages.

Methods: Using human embryonic stem cell-derived cortical neurons, we performed quantitative gene expression analyses and morphometric assessments at Days 28, 35 and 42 to evaluate how *CHL1* presentation affects neuronal differentiation.

Result: At early differentiation (Day 28), *CHL1* showed minimal effects on gene expression. By Day 35, *CHL(S)* suppressed deep-layer markers (*Tbr1, Ctip2*) and *Tbr2*, suggesting inhibition of intermediate progenitor expansion. In contrast, *CHL(B)* maintained or elevated *Ctip2*, *Satb2* and *Brn2*, indicating support for laminar identity stabilization. By Day 42, orientation-specific effects persisted with *CHL(S)* suppressing *Cux1* and *CHL(B)* promoting *Ctip2*. Notably, *CHL1* did not affect neurite morphology at any stage examined. These findings position *CHL1* as a transcriptional regulator of cortical neuron identity, with orientation- and stage-specific effects on gene expression but not morphogenesis. The results highlight its role in cortical layering and support further investigation into *CHL1*-mediated signaling pathways in neuro developmental disorders.

Key words: Cell adhesion molecules, *CHL1* isoforms, Cortical development, Laminar identity, Neurogenesis regulation, Neuronal differentiation.

INTRODUCTION

Cortical development disorders affect approximately 1-3% of the population and represent a major cause of intellectual disability and epilepsy (Guerrini and Dobyns, 2014). The human cerebral cortex, containing ~16 billion neurons organized into six distinct layers, requires precise molecular orchestration during development to establish proper connectivity and function (Libé-Philippot and Vanderhaeghen, 2021). This complex process involves coordinated neural progenitor proliferation, neuronal differentiation, radial migration and layer-specific fate specification (Cossart and Garel, 2022). Disruptions at any stage can result in severe neurodevelopmental disorders including autism spectrum disorder, schizophrenia and cortical malformations (Subramanian *et al.,* 2020; Rajput *et al.,* 2021; Wu *et al.,* 2022; Salem *et al.,* 2023). Understanding the molecular mechanisms governing cortical lamination is therefore critical for developing therapeutic interventions.

Neural cell adhesion molecules (CAMs) play fundamental roles in cortical development by mediating cell-cell interactions, guiding migration and modulating differentiation signals. Among these, Close Homolog of L1 (*CHL1*), a member of the L1 family of CAMs, has emerged as a critical regulator of neural development (Holm *et al.,* 1996). *CHL1* influences multiple developmental processes including axon guidance (Guseva *et al.,* 2018), synapse formation (Andreyeva *et al.,* 2010) and neuronal migration

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(Katic *et al.,* 2014). Importantly, *CHL1* exists in two functionally distinct forms: a soluble form [*CHL(S)*] that acts as a diffusible signal and an adherent form [*CHL(B)*] that mediates direct cell-cell adhesion (Hillenbrand *et al.,* 1999). Previous studies suggest these isoforms exert differential effects on neuronal development (Zhou *et al.,* 2012), but their specific roles in human cortical layer formation remain unexplored.

Despite extensive research on *CHL1* in rodent models, critical knowledge gaps limit our understanding of its role in human cortical development. First, while both *CHL1* isoforms have been characterized biochemically, their differential functions in regulating cortical neuron fate specification have not been investigated. Second, the temporal dynamics of *CHL1* expression and function during human corticogenesis remain undefined. Third and most critically, how the spatial presentation of *CHL1* (apical versus basal) influences cortical layer identity has never been examined, despite growing evidence that the subcellular localization of adhesion molecules determines their signaling outcomes and biological effects. These gaps are particularly significant given that human cortical development differs substantially from rodent models in timing, complexity and molecular signatures.

The clinical relevance of understanding *CHL1* function is underscored by its association with multiple neuro developmental disorders. *CHL1* mutations have been linked to intellectual disability (IQ range 50-70) and increased schizophrenia susceptibility (Angeloni *et al.,* 1999). The *CHL1* gene, located on chromosome 3p26.1, falls within a region frequently deleted in 3p deletion syndrome, which presents with severe cortical malformations and intellectual disability. Recent genome-wide association studies have identified *CHL1* polymorphisms as risk factors for autism spectrum disorders, further highlighting its importance in human neurodevelopment (Paramasivam, 2021). However, the cellular and molecular mechanisms linking *CHL1* dysfunction to these clinical phenotypes remain poorly understood, hampering development of targeted therapeutic approaches.

To address these knowledge gaps, we employed human embryonic stem cell (hESC)-derived cortical neurons, which faithfully recapitulate key aspects of human corticogenesis including temporal gene expression patterns, layer-specific marker expression and extended developmental timelines (Espuny *et al.,* 2013). This model system offers unique advantages: (1) species-specific relevance for understanding human development, (2) precise control over *CHL1* spatial presentation and (3) ability to track the same cell populations longitudinally across developmental stages. By combining this model with comprehensive transcriptional profiling and morphometric analyses, we can dissect how *CHL1* isoforms differentially regulate cortical development.

Therefore, this study aimed to investigate how *CHL1* spatial presentation regulates human cortical neuron development. Specifically, we: (1) characterized temporal *CHL1* expression during cortical differentiation (Days 28, 35, 42); (2) examined how apical (*CHL(S)*) versus basal (*CHL(B)*) presentation affects deep-layer (*Tbr1*, *Ctip2*) and upper-layer (*Cux1, Satb2*) markers; (3) determined whether *CHL1* acts on progenitors or post-mitotic neurons and (4) assessed the relationship between transcriptional and morphological effects. This approach provides mechanistic insights into *CHL1*-mediated cortical patterning and its role in neurodevelopmental disorders.

MATERIALS AND METHODS

HESC cell lines and culture conditions

Human embryonic stem cells (H9 line) were used to generate cortical neurons through *in vitro* differentiation. Cells were cultured in mTeSR1 medium at 37°C with 5% CO_2. Differentiation was initiated once cultures reached approximately 95% confluence.

Differentiation of hESCs into cortical progenitors and neurons

Differentiation was initiated when cells reached approximately 95% confluence by replacing mTeSR1 with Cortical Mixture Media (CMM), comprising a 1:1 ratio of DMEM/F-12 and Neurobasal Medium, supplemented with L-Glutamine, Penicillin-Streptomycin, Non-Essential Amino Acids (NEAA), Insulin-Transferrin-Selenium (ITS-A), N-2 Supplement, B-27 Supplement and β-Mercaptoethanol. Unless otherwise specified, reagents were obtained from GIBCO. CMM was supplemented with SB431542 (1:1000, Cell Signaling, Cat. No. 14775) and LDN193189 (1:1000, Sigma, Cat. No. SML0559) during the neural induction phase (Days 1-11).

On Day 11, cells were passaged at a 1:2 ratio onto LN-521-coated wells using ReLeSR. After centrifugation (260 × g, 3 minutes), cells were resuspended in CMM containing 10 µM Y27632 and plated in 800-2000 µL volumes per well. From Days 12-17, CMM was supplemented with FGF2 (1:5000) to promote progenitor proliferation, with medium changes every other day. A second passage was performed on Day 17, after which cells entered the maturation phase (Days 18-34) and were maintained in CMM with medium refreshed every other day.

CHL1 presentation and treatment regimen

To assess the effects of soluble and adherent *CHL1* on cortical differentiation, two treatment conditions were employed. For soluble *CHL1*, recombinant human *CHL1* protein (R and D Systems, Cat. No. 2126-CH) was added to the culture medium (10 µg/mL) during the seeding step and maintained throughout the culture period with each medium change. For membrane-bound *CHL1*, LN-521-coated wells were first incubated overnight at 4°C with anti-His-tag antibody (Fc control), followed by PBS washes, blocking with 1% BSA (1 hour at 37°C) and incubation with recombinant *CHL1* protein (overnight, 4°C). After washing,

cells were seeded and cultured under standard differentiation conditions. Assessments were conducted at Day 28, Day 35 and Day 42. All treatments were performed with n= 3 independent biological replicates per condition.

Quantitative real-time PCR

Total RNA was extracted from differentiated cortical neurons using Trizol reagent (Sigma-Aldrich) following the manufacturer's instructions. RNA quality and quantity were assessed using spectrophotometry and 1 µg of RNA was reverse transcribed into cDNA using SuperScript IV First-Strand Synthesis System (Invitrogen, Cat. No. 18091050). qRT-PCR was conducted using PowerUp SYBR Green Master Mix (Applied Biosystems, Cat. No. A25742) on a QuantStudio 3 Real-time PCR System (Thermo Fisher Scientific). Primers targeting cortical development markers were used, including *Tbr1, Ctip2, Satb2, CHL1, Tbr2, Brn2, Cux1* and *Cux2. GAPDH* was used as a housekeeping gene for normalization (Table 1). Reactions were run in triplicate and gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method (Alsanie *et al.,* 2017).

Ethical approval

This study, along with all experimental procedures, was conducted according to the principles outlined by the ethics committee of the University of Taif (Approval No. TUZ-18-T-277).

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) from three independent biological replicates (n=3). Statistical analysis was conducted using Graph Pad Prism (v9, Graph Pad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro-Wilk test. For comparisons between two groups, unpaired two-tailed Student's t-test was used. For multiple group comparisons, one-way ANOVA followed by Tukey's post hoc test was performed. All qRT-PCR reactions were run in technical triplicates. Statistical significance was set at $p<0.05$. For multiple comparisons within the same dataset, p-values were adjusted using the Benjamini-Hochberg method to control false discovery rate. Effect sizes were calculated using Cohen's d for t-tests for ANOVA.

RESULTS AND DISCUSSION

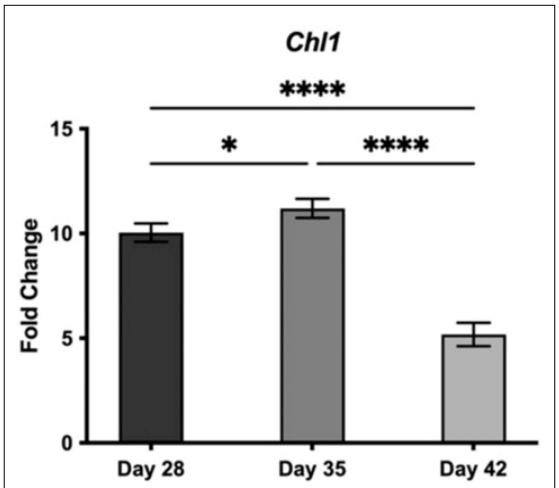
This study examined the effects of soluble (*CHL(S)*) and adherent (*CHL(B)*) forms of *CHL1* on cortical neuron development at Days 28, 35 and 42.

Temporal regulation of *CHL1* expression reveals a mid-developmental peak followed by rapid down regulation

To establish the temporal dynamics of *CHL1* during cortical development, we examined endogenous *CHL1* expression at three developmental stages (Fig 1). Expression was elevated at Day 28, peaked significantly at Day 35 ($p<0.05$), then declined dramatically by Day 42 ($p<0.0001$ versus both earlier timepoints). This temporal profile indicates that *CHL1* functions primarily during mid-stage cortical differentiation, with down regulation occurring as neurons mature.

CHL1 suppresses *Tbr1* expression in both apical and basal configurations at day 28

We first examined whether *CHL1* affects deep-layer neuron specification at the early differentiation stage. At Day 28, both *CHL1(S)* and *CHL1(B)* significantly decreased *Tbr1*



qRT-PCR analysis at Days 28, 35 and 42. Peak expression at Day 35 ($p<0.05$) followed by decline at Day 42 (**$p<0.0001$). Mean ±SEM, n=3. One-way ANOVA with Tukey's post hoc.

Fig 1: Temporal expression of *CHL1* during cortical differentiation.

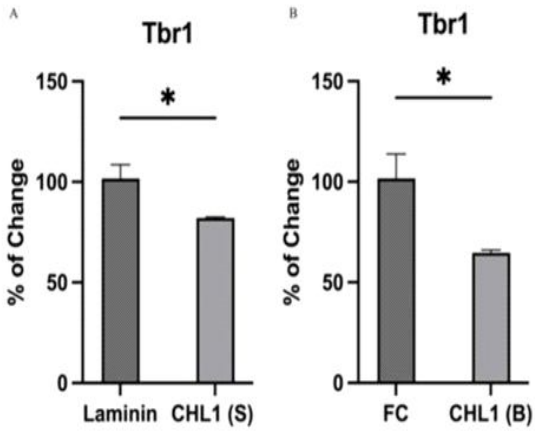
Table 1: List of primer sequences of the genes analyzed by qRT-PCR.

Gene	Accession number	Sense primer (5′→3′)	Antisense primer (5′ →3′)
Tbr1	NM_001013696.1	AGATCCAGGTGACAGGAGGA	TCCTCCATCAGGTCTCTTCC
Ctip2	NM_007555.3	CGGACATGGAGATGAGGAAG	TCTTGCTGTGGTGTGGAAGT
Satb2	NM_001163622.1	CGCTGGAGAAGAAAGAGAGC	CTGGTAGAGAGGGCTGGAAG
Tbr2	NM_001163630.1	GAGGAGGAGCTGAGGAGAAG	CTTCCTCCTCCTTGGTTTCC
Cux1	NM_009953.2	AGCAGAGAGGAACAGGAAGC	TGGGAGGTGAGGTTGATGAT
Cux2	NM_001168421.1	TGAGCCAGGAGGAGGAGATA	CTGGTGTAGGTGGTGATGTG
Brn2	NM_008898.2	CCCTTCATCTACCAGGATGG	AGTGGCTGTTGGTGAGGTAG
CHL1	NM_006614	AGAACAGGAGTGTTCGGCTGAC	TCCTTGGACTCTGGTCAGTTCC
GAPDH	NM_002046	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC

expression compared to their respective controls (Laminin and FC; $p<0.05$ for both) (Fig 2A and B). This indicates that *CHL1*, regardless of spatial orientation, initially suppresses this key layer VI marker during early cortical.

CHL1 does not alter the expression of early neurogenic transcription factors across surface conditions

To determine whether *CHL1's* effects extend to progenitor specification, we examined key neurogenic regulators at Day 28. Expression of *LHX2*, *FOXG1*, *TBR2, PAX6* and *TBR1* remained unchanged across all *CHL1* conditions (Fig 3). This indicates that *CHL1* does not interfere with core transcriptional machinery for progenitor identity, suggesting its regulatory effects are restricted to post-mitotic differentiation processes.



qRT-PCR at Day 28. (A) *CHL(S)* vs Laminin, (B) *CHL(B)* vs *FC*, both $p<0.05$. Mean ±SEM, n=3. Student's t-test.

Fig 2: *CHL1* suppresses *Tbr1* expression in both orientations.

Orientation-sensitive regulation of *Tbr1* and *Ctip2* reveals selective bias toward deep-layer lineage at day 35

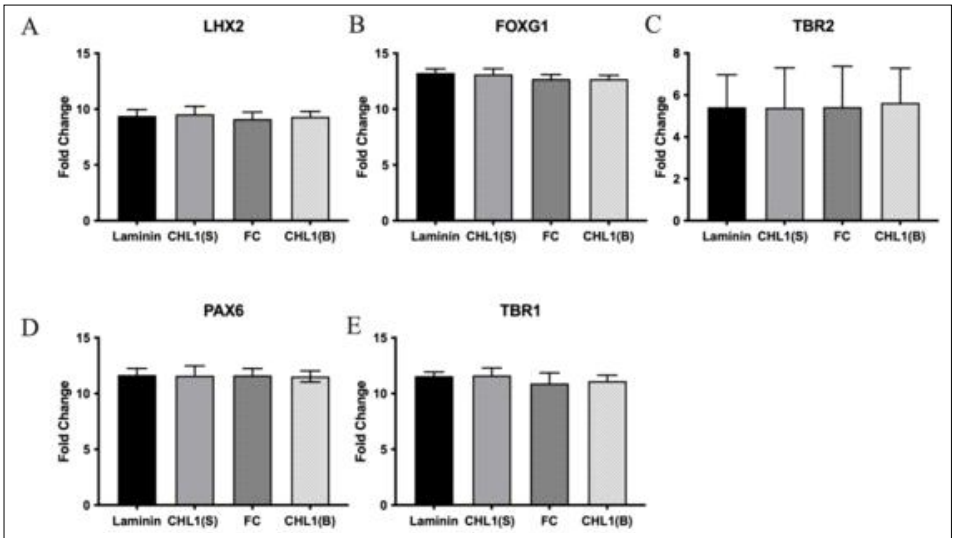
By Day 35, coinciding with peak *CHL1* expression, *CHL1* effects became orientation-specific (Fig 4). For *Tbr1*, *CHL1(S)* showed non-significant reduction versus Laminin (Panel A), while *CHL1(B)* significantly suppressed expression versus FC ($p<0.05$, Panel B). Conversely, *Ctip2* was significantly upregulated by *CHL1(S)* versus Laminin ($p<0.05$, Panel C), with no significant change in *CHL1(B)* versus FC (Panel D). These divergent effects demonstrate that *CHL1* spatial presentation differentially regulates deep-layer markers during peak differentiation.

CHL1 modulates a broad set of cortical identity markers with spatial precision at day 35

Comprehensive profiling at Day 35 revealed complex orientation-dependent effects (Fig 5). Remarkably, *Tbr1* expression was significantly higher in *CHL1(B)* compared to *CHL1(S)* ($p<0.01$, Panel A), representing a reversal from the uniform suppression observed at Day 28. This stage-specific switch suggests dynamic regulation of *CHL1* effects during development. *CHL1(B)* also elevated *Satb2* versus FC ($p<0.05$, Panel B), while *CHL1(S)* significantly decreased *Tbr2* versus controls ($p<0.01$, Panel C), indicating impaired intermediate progenitor expansion. *Ctip2* remained higher in *CHL1(B)* than *CHL1(S)* (Panel G), reinforcing orientation-specific effects on cortical fate determination.

CHL1 suppresses *Cux1* while promoting *ctip2*, indicating layer-specific fate programming

Fig 6 further clarifies *CHL1's* dual role in fate specification. Panel A shows *Tbr1* expression remained stable across all substrate conditions. Panel B displays slight increases



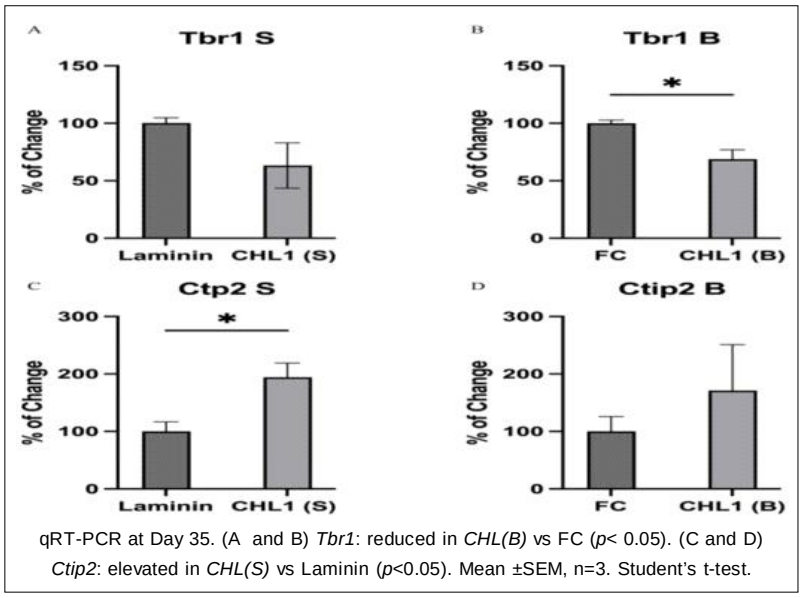
qRT-PCR at Day 28. (A-E) *LHX2*, *FOXG1*, *TBR2*, *PAX6, Tbr1* showed no significant changes across conditions. Mean ±SEM, n=3. One-way ANOVA.

Fig 3: *CHL1* does not affect early neurogenic markers.

in *Tbr2*, although no significance was achieved. Panel C reports no significant change in *Satb2*, while Panel D (*Cux2*) showed a mild but non-significant downward trend. Panel E (*Brn2*) remained unchanged. Panel F, however, revealed a significant reduction in *Cux1* expression in *CHL1(S)* compared to Laminin ($p<0.05$), suggesting a suppression of upper-layer identity. In contrast, Panel G showed that *Ctip2* was significantly elevated in *CHL1(B)* relative to *CHL1(S)* ($p<0.05$), highlighting an orientation-specific promotion of deep-layer fate. Together, these results underscore *CHL1's* ability to selectively repress superficial markers while enhancing deep-layer identities in a configuration-sensitive manner.

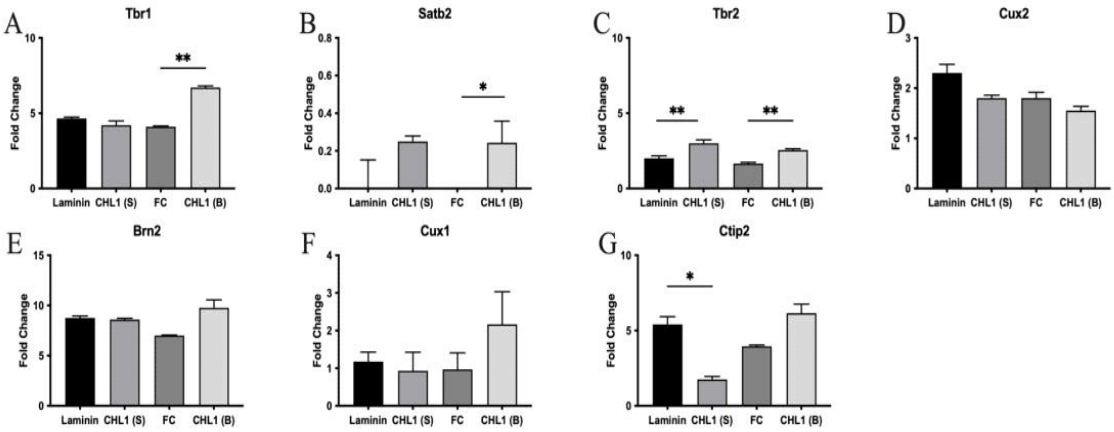
CHL1 does not significantly impact neurite morphology during cortical neuron development

Morphometric analysis was performed at Days 28, 35 and 42 across different substrate conditions (PDL, *CHL1(S)*, FC and *CHL1(B)*) (Fig 7). At all timepoints, no significant differences were observed in total neurite length, dominant neurite length, number of primary branches, or percentage of cells lacking neurites between *CHL1*-treated and control groups. While Day 35 showed minor trends in the *CHL1(B)* group, these remained statistically insignificant. These findings demonstrate that *CHL1* does not affect neurite morphology, branching architecture, or neuritogenesis timing throughout cortical



qRT-PCR at Day 35. (A and B) *Tbr1*: reduced in *CHL(B)* vs FC ($p< 0.05$). (C and D) *Ctip2*: elevated in *CHL(S)* vs Laminin ($p<0.05$). Mean ±SEM, n=3. Student's t-test.

Fig 4: Orientation-specific regulation of *Tbr1* and *Ctip2*.



qRT-PCR at Day 35. (A) *Tbr1*: *CHL(B)*>*CHL(S)*, $p<0.01$. (B) *Satb2*: *CHL(B)*>FC, $p<0.05$. (C) *Tbr2*: *CHL(S)*<controls, $p<0.01$. (D-F) No changes. (G) *Ctip2*: *CHL(B)*>*CHL(S)*. Mean ±SEM, n=3. One-way ANOVA with Tukey's post hoc.

Fig 5: *CHL1* modulates cortical markers in orientation-dependent manner.

neuron development, contrasting with its pronounced effects on transcriptional programs.

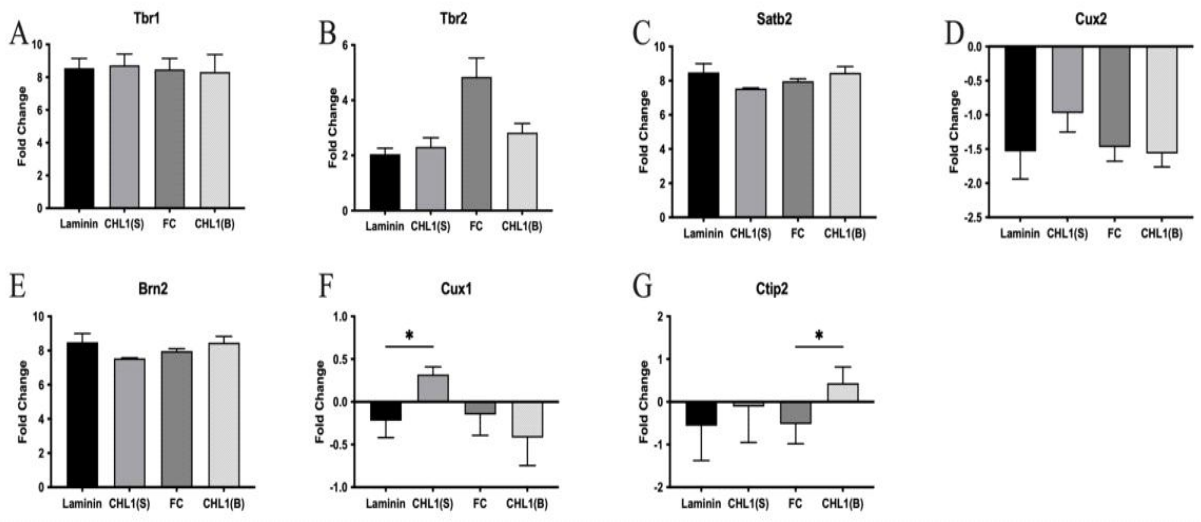
This study comprehensively delineates the spatially segregated and temporally dynamic regulatory functions of *CHL1* on cortical neuronal differentiation. Using a panel of transcriptional markers and morphometric analysis across key developmental timepoints, we demonstrate that *CHL1* operates not as a broad modulator of early neurogenesis, but as a precise, isoform-specific effector of cortical fate identity and maturation. The spatial orientation of *CHL1* presentation on the surface (*CHL(S)*) versus basally (*CHL(B)*) emerges as a critical determinant of its regulatory role, differentially affecting the expression of laminar markers, the timing of neurogenic transitions and the balance of differentiation.

At Day 28, *CHL1* orientation had no significant effect on cortical layer-specific gene expression or neurite morphology. This apparent lack of influence is consistent with previous findings that early stages of cortical development are primarily governed by intrinsic transcriptional programs and lineage-instructive signals, rather than substrate-mediated adhesion cues (Chen *et al.,* 1999; Maness and Schachner, 2007; Katic *et al.,* 2017). *CHL1's* functional role appears to be temporally downstream of progenitor identity specification, becoming relevant during the subsequent stages of fate consolidation and cortical layer patterning (Yang *et al.,* 2017).

By day 35, the effects of *CHL1* presentation became significantly more pronounced and distinct between its apical and basal configurations. *CHL(S)* consistently suppressed the expression of differentiation markers, including *Tbr1* and *Ctip2*. These findings align with previous literature identifying *CHL1* as a key regulator of neuronal positioning and identity, though its presentation-specific inhibitory effect in this context reveals a novel functional layer (Huang *et al.,* 2011).

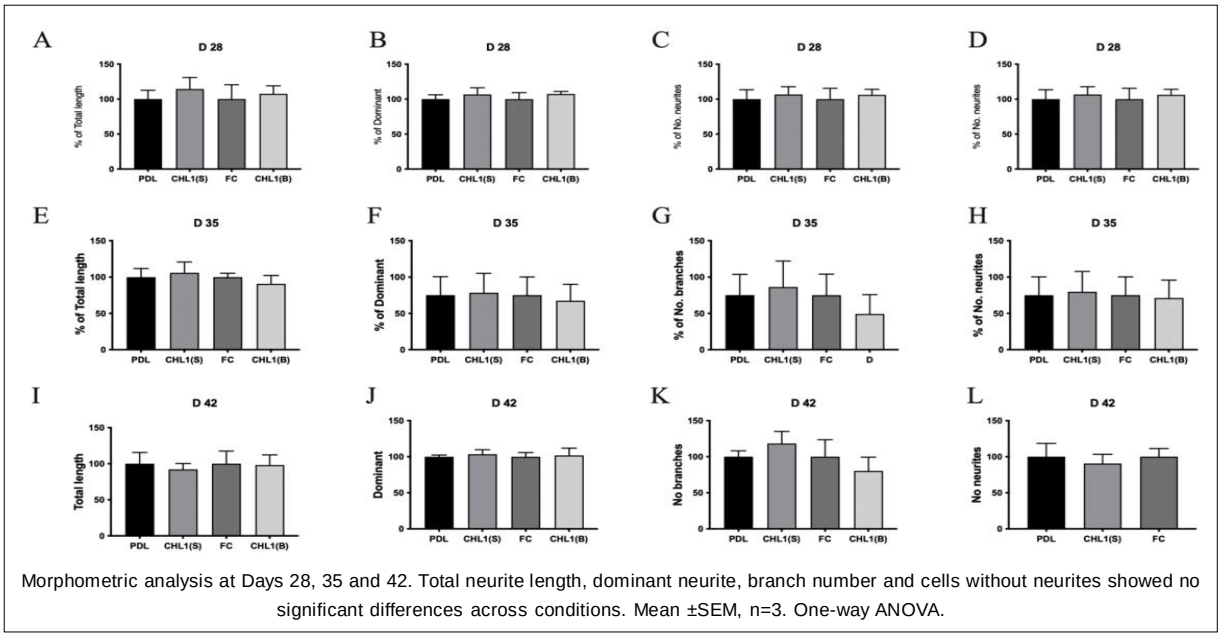
The reduction in Tbr2 under *CHL(S)* further supports the notion that this isoform impedes the neurogenic-to-neuronal transition by constraining the amplification of intermediate progenitors, thereby limiting output to both deep and upper cortical layers. In contrast, *CHL(B)* preserved or even enhanced the expression of deep-layer markers, particularly Ctip2, suggesting a supportive role in neuronal fate commitment. This effect likely reflects interactions with specific adhesion molecules such as NCAM or N-cadherin, both of which have been implicated in promoting deep-layer neuron differentiation and stabilizing their identity during cortical layer formation. *CHL(B)*'s ability to preserve Ctip2 expression, a key transcription factor for deep-layer cortical neurons, suggests its role in stabilizing cortical populations during layer formation. These findings align with previous studies showing that adhesion molecules play critical roles in deep-layer cortical neuron differentiation and organization (Demyanenko *et al.,* 2004; Togashi *et al.,* 2009; Klingler *et al.,* 2023).

CHL(B) also elevated the expression of upper-layer markers such as *Satb2* and *Brn2*, a finding not observed with *CHL(S)*. This dual promotion of both deep- and upper-layer markers under *CHL(B)* points to its role in supporting a broad range of neuronal identities during the peak period of cortical differentiation. This could reflect its ability to act as a permissive signal rather than a directive one, allowing for regionally specified transcriptional programs to unfold in response to positional cues. The suppression of *Cux1* by *CHL(S)*, in contrast, highlights the isoform's selective downregulation of superficial fate programs, reinforcing its role as a spatially localized suppressor of cortical identity.



qRT-PCR at Day 42. (A-E) No significant changes. (F) *Cux1*: *CHL(S)*<Laminin, $p<0.05$. (G) *Ctip2*: *CHL(B)*>*CHL(S)*, $p< 0.05$. Mean ±SEM, n=3. One-way ANOVA with Tukey's post hoc.

Fig 6: Selective modulation of cortical markers by *CHL1*.



Morphometric analysis at Days 28, 35 and 42. Total neurite length, dominant neurite, branch number and cells without neurites showed no significant differences across conditions. Mean ±SEM, n=3. One-way ANOVA.

Fig 7: *CHL1* does not affect neurite morphology.

By Day 42, both *CHL(S)* and *CHL(B)* began to influence the transition from proliferation to differentiation. Expression levels of Cux1 and Ctip2 were reduced across both configurations, consistent with previous reports that *CHL1* contributes to neuronal maturation and density regulation by facilitating cell cycle exit (Hillenbrand *et al.,* 1999; Fernandes *et al.,* 2023). However, the divergent effects on differentiation markers persisted. *CHL(S)* suppressed *Tbr1* and *Ctip2* expression, especially at intermediate stages (Day 35), reinforcing its inhibitory influence on early deep-layer fate. By contrast, *CHL(B)* exerted a milder effect on *Tbr1* and preserved Ctip2, suggesting support for cortical identity maintenance. This dual function-suppressing proliferation while sustaining fate markers positions *CHL(B)* as a potential stabilizer of cortical architecture during late-stage neurodevelopment.

Despite *CHL1's* pronounced effects on transcriptional programs, morphometric analyses revealed no impact on neurite morphology across all developmental stages. Total neurite length, branching patterns and dominant neurite extension remained unchanged between *CHL1*-treated and control conditions at Days 28, 35 and 42. This dissociation between molecular identity and structural features indicates that *CHL1* functions primarily as a transcriptional regulator rather than a morphogenic factor. Unlike other adhesion molecules that directly guide neurite extension (Jiang *et al.,* 2021; Scapin *et al.,* 2021), *CHL1* appears to modulate cortical fate specification without affecting neuronal architecture.

This isoform-specific and temporally regulated pattern was consistent throughout our analyses. The peak of *CHL1* expression at Day 35 (1) coincided with maximal transcriptional changes, where *CHL(S)* suppressed deep-layer markers (*Tbr1*, *Ctip2*) while *CHL(B)* maintained or enhanced their expression (5). Broader transcriptional profiling (5 and 6) confirmed that *CHL(B*) supported both deep-and upper-layer markers (*Ctip2, Satb2, Brn2*), whereas *CHL(S)* selectively suppressed Cux1. The absence of effects at Day 28 (3) distinguished *CHL1* as a downstream regulator of neuronal identity rather than an initiator of neurogenesis. Critically, these molecular changes occurred independently of morphological alterations (7), reinforcing *CHL1's* selective role in transcriptional regulation.

Our findings provide mechanistic insights into *CHL1*-associated neurodevelopmental disorders. The stage-specific and orientation-dependent effects explain how mutations affecting *CHL1* cleavage or membrane anchoring could disrupt cortical lamination, causing intellectual disability observed in patients. The temporal dynamics-peak expression at mid-differentiation-identify a therapeutic window for intervention.

The persistent effects on layer-specific markers after *CHL1* downregulation support the neurodevelopmental hypothesis of schizophrenia, explaining how early disruptions create lasting vulnerability. *CHL1* polymorphisms affecting spatial presentation could serve as disease biomarkers.

Therapeutically, these findings suggest biomaterial-based interventions controlling *CHL1* presentation. Promoting *CHL1(B)* could enhance differentiation in developmental delays, while *CHL1(S)* might benefit premature differentiation conditions. This precision approach, tailored to patient-specific genotypes, offers new strategies for treating neurodevelopmental disorders through targeted *CHL1* isoform manipulation.

CONCLUSION

In conclusion, *CHL1* acts as an orientation-dependent molecular switch that precisely regulates cortical neuron fate without affecting morphology. The opposing effects of

CHL(S) and *CHL(B)* suppressing versus promoting differentiation markers-combined with stage-specific regulation, reveal *CHL1* as a critical determinant of cortical layer identity. These findings establish a new paradigm for understanding how spatial presentation of adhesion molecules controls neurodevelopment and offer therapeutic targets for *CHL1*-related.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ashraf Albrakati, Majid Alhomrani and Walaa F. Alsanie. The first draft of the manuscript was written by Majid Alhomrani and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Data availability statement

All the data supportive stated results exist in the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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