

Unravelling the role of NIPBL in the development and function of cortical interneurons

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1. NIPBL is mutated in more than 50% of Cornelia de Lange Syndrome patients

Cornelia de Lange Syndrome (CdLS) is a rare congenital disease characterized by growth retardation and multiple developmental defects such as facial dysmorphism, congenital heart disease, and neurological disorders among which, mental delay, anxiety, hyperactivity and repetitive self-injurious behaviours are the most commonly observed impairments. The pathological mechanisms underlying these neurological disorders remain understudied.

CdLS patients carry mutations in the genes encoding the cohesin complex or its regulators. Among the causal genes, *NIPBL* gene is mutated in more than 50% of patients, and is associated with the most severe form of the disease.

NIPBL is an evolutionary conserved protein being part of the cohesin loading complex and promoting cohesin DNA extrusion activity.

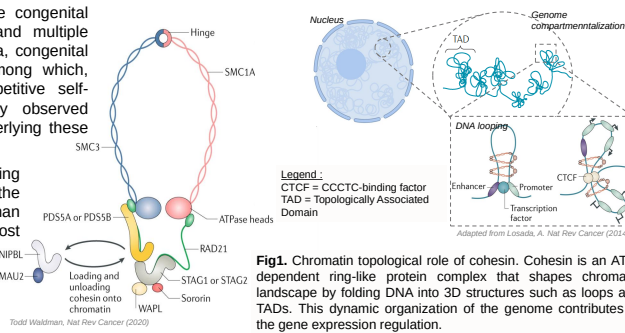


Fig1. Chromatin topological role of cohesin. Cohesin is an ATP-dependent ring-like protein complex that shapes chromatin landscape by folding DNA into 3D structures such as loops and TADs. This dynamic organization of the genome contributes to the gene expression regulation.

2. Cortical interneurons (cINs)

Cortical GABAergic interneurons (cINs) represent 20-30% of cortical neurons and are involved in the regulation of neuronal circuits activity. Recent evidence suggests a contribution of these neurons to neurodevelopmental disorders. During development, PV⁺ and SST⁺ cINs, the major cINs subtypes, originate from an early ventral telencephalic structure : the Medial Ganglionic

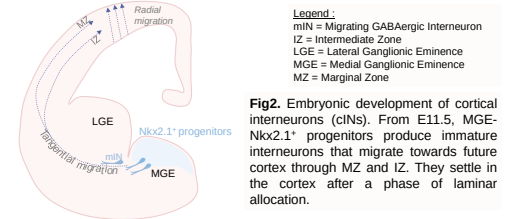
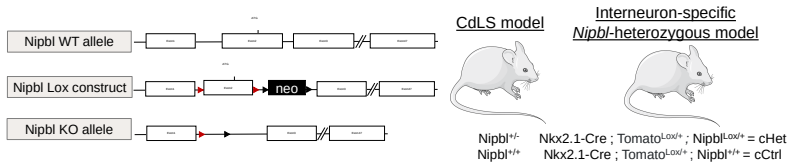


Fig2. Embryonic development of cortical interneurons (cINs). From E11.5, MGE- Nkx2.1⁺ progenitors produce immature interneurons that migrate towards future cortex through MZ and IZ. They settle in the cortex after a phase of laminar allocation.

3. Aims and mouse models for *Nipbl* invalidation

The study aims to decipher the role of *NIPBL* haploinsufficiency in cortical interneurons and assess the contribution of these cells to the onset of behavioural impairments associated with CdLS. Towards that purpose we are using two complementary mouse models derived from the same allele construct :



4. *Nipbl* is ubiquitously expressed in the developing brain

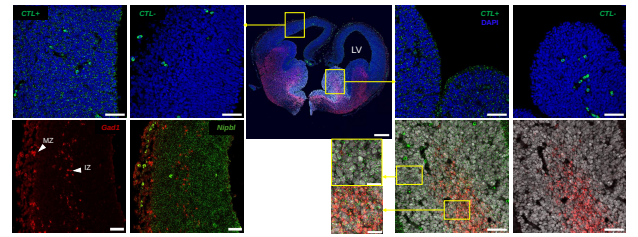


Fig3. *Nipbl* mRNA is expressed in the whole brain of E13.5 C57BL/6J embryo. RNAscopeTM ISH revealing in red Gad1⁺ cINs precursors in the MGE (right) and the MGNs in the pallium (left). Nkx2.1 immunofluorescence in white reveals the MGE-progenitors. All these cells express *Nipbl* (green).

5. Validation of NIPBL depletion

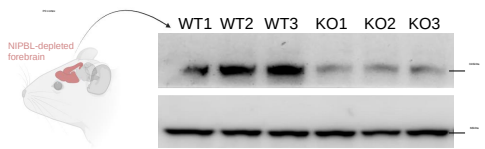


Fig4. Loss of NIPBL expression in the cortex of telencephalon-specific NIPBL-depleted mice. Western-blot of P0 cortex extracted proteins from 3 Foxg1-IRES-Cre; *Nipbl*^{lox/lox} mice (= KO) and 3 Foxg1-IRES-Cre; *Nipbl*^{+/+} mice (= WT).

6. *Nipbl*^{+/Lox} mice tend to show microcephaly

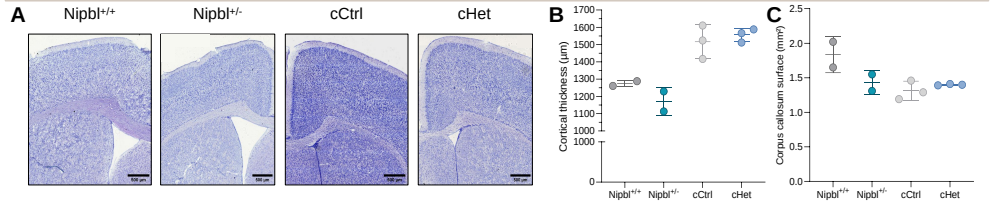


Fig5. *Nipbl*^{+/Lox} mice tend to exhibit microcephaly. (A) Histological staining of brain coronal sections with toluidine blue. 3-4-month *Nipbl*^{+/Lox} versus *Nipbl*^{+/+} old mice and P21 cCtrl versus cHet old mice were analysed (scale bar = 500μm). (B) Cortical thickness was measured and averaged from 3 medial sections per animal (n=2-3). (C) Corpus callosum surface was measured and averaged from 3 medial sections per animal (n=2-3).

7. No apparent defect in the number, layering and fate specification of NIPBL haploinsufficient cortical interneurons

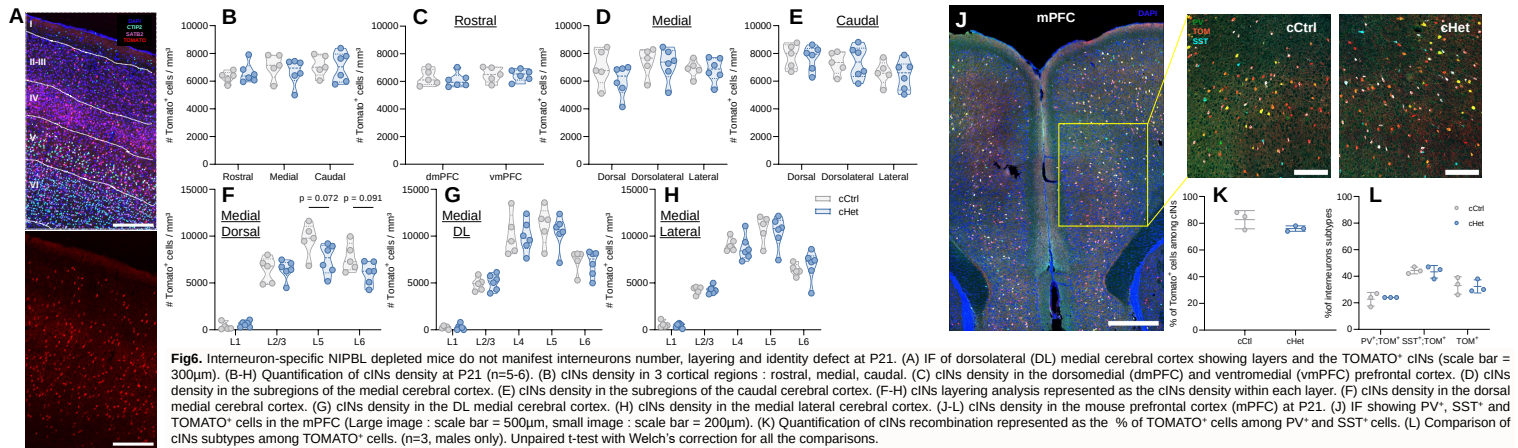


Fig6. Interneuron-specific NIPBL depleted mice do not manifest interneurons number, layering and identity defect at P21. (A) IF of dorsolateral (DL) medial cerebral cortex showing layers and the TOMATO⁺ cINs (scale bar = 300μm). (B-H) Quantification of cINs density at P21 (n=5-6). (B) cINs density in the dorsomedial (dmPFC) and ventromedial (vmPFC) prefrontal cortex. (C) cINs density in the subregions of the medial cerebral cortex. (D) cINs density in the subregions of the medial cerebral cortex. (E) cINs density in the subregions of the caudal cerebral cortex. (F-H) cINs layering analysis represented as the cINs density within each layer. (I) cINs density in the dorsal medial cerebral cortex. (J) cINs density in the DL medial cerebral cortex. (K) cINs density in the medial lateral cerebral cortex. (L) cINs density in the medial lateral cerebral cortex. (M) cINs density in the medial lateral cerebral cortex. (N) cINs density in the medial lateral cerebral cortex. (O) cINs density in the medial lateral cerebral cortex. (P) cINs density in the medial lateral cerebral cortex. (Q) cINs density in the medial lateral cerebral cortex. (R) cINs density in the medial lateral cerebral cortex. (S) cINs density in the medial lateral cerebral cortex. (T) cINs density in the medial lateral cerebral cortex. (U) cINs density in the medial lateral cerebral cortex. (V) cINs density in the medial lateral cerebral cortex. (W) cINs density in the medial lateral cerebral cortex. (X) cINs density in the medial lateral cerebral cortex. (Y) cINs density in the medial lateral cerebral cortex. (Z) cINs density in the medial lateral cerebral cortex.

8. *Nipbl*^{+/Lox} and cHet mice tend to behave differently from their WT counterparts

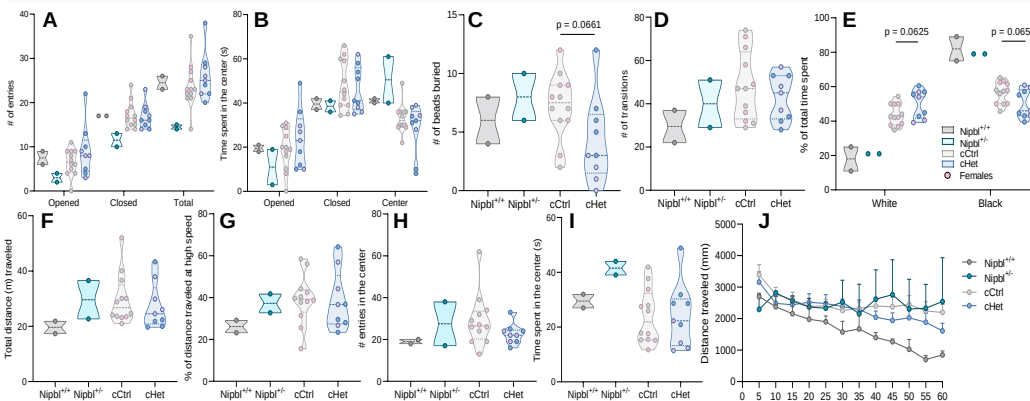


Fig7. Behavioural testing in the two models. (A-B) Elevated-Plus-Maze results. (A) Number of entries in the different zones. (B) Time spent in the different zones as the percentage of total time. (C) Number of beads buried in the Marble Burying test after 30min. (D-E) Light and dark box test results. (F) Number of transitions between the different zones. (G) Time spent in the different zones as the percentage of total time. (H-I) Open-Field test results (F) Total distance (m) traveled after 1 hour. (G) Percentage of total distance traveled at high speed. (H) Number of entries in the center during the first 10 minutes. (I) Time (s) spent in the center during the first 10 minutes. (J) Habituation measurement expressed as the distance traveled over time. An Unpaired t-test with Welch's correction was conducted for all the comparisons except for the habituation measurement (J) which required a 2-way ANOVA. Results expressed as mean ± SEM (J). (n=2-12 for all the tests).

Conclusion: Our preliminary results suggest that germline *NIPBL* depletion in mice interferes with normal behaviour. We plan to increment the number of animals to confirm these results. On the other hand, interneuron-specific *NIPBL* depleted mice tend to exhibit different behavioural issues without showing apparent defects in cINs development so far. Further *in vivo* studies in these two models might reveal whether cINs development is altered and whether the differences we observed between models rely on different mechanisms.

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