

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 delay and multiple developmental defects such as facial dysmorphia, congenital heart disease, and neurological disorders among which, intellectual disability, anxiety, hyperactivity and repetitive self-injurious behaviours are the most observed impairments. The mechanisms underlying these neurological disorders remain understudied.

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

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

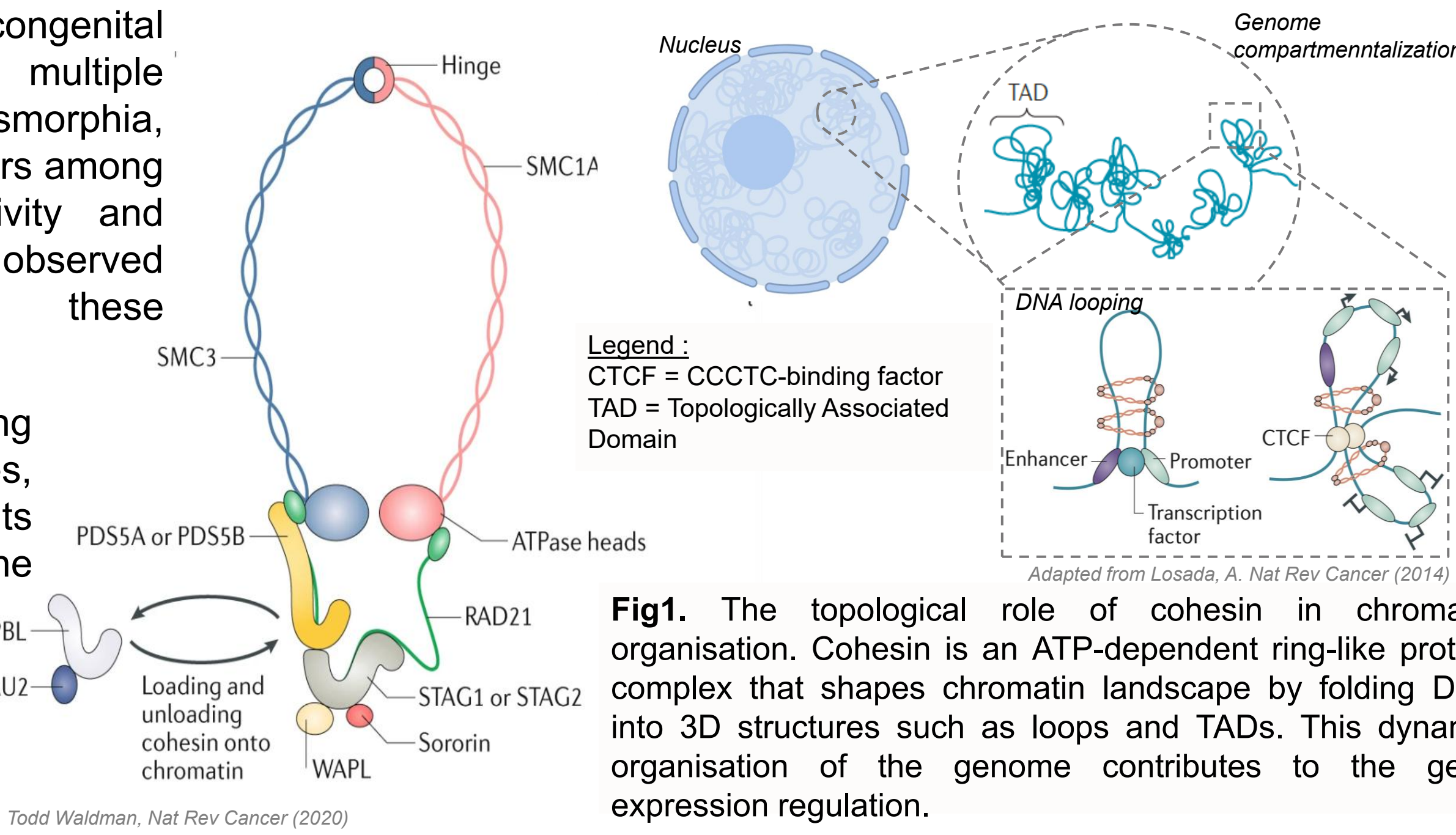


Fig1. The topological role of cohesin in chromatin organisation. Cohesin is an ATP-dependent ring-like protein complex that shapes chromatin landscape by folding DNA into 3D structures such as loops and TADs. This dynamic organisation of the genome contributes to the gene expression regulation.

2. Cortical interneurons (INs) development

GABAergic interneurons (INs) represent ~25% 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⁺ INs, the major subtypes, originate from an early ventral telencephalic structure : the Medial Ganglionic Eminence (MGE).

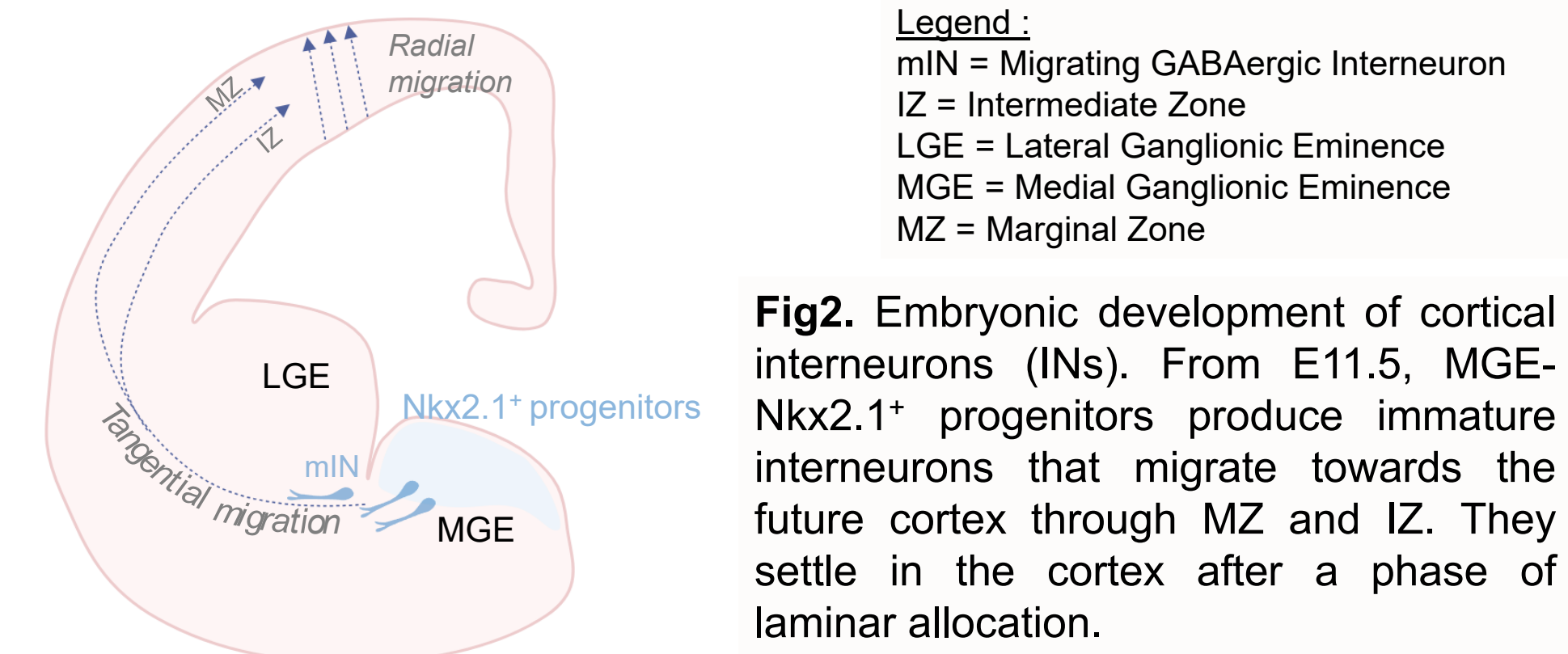
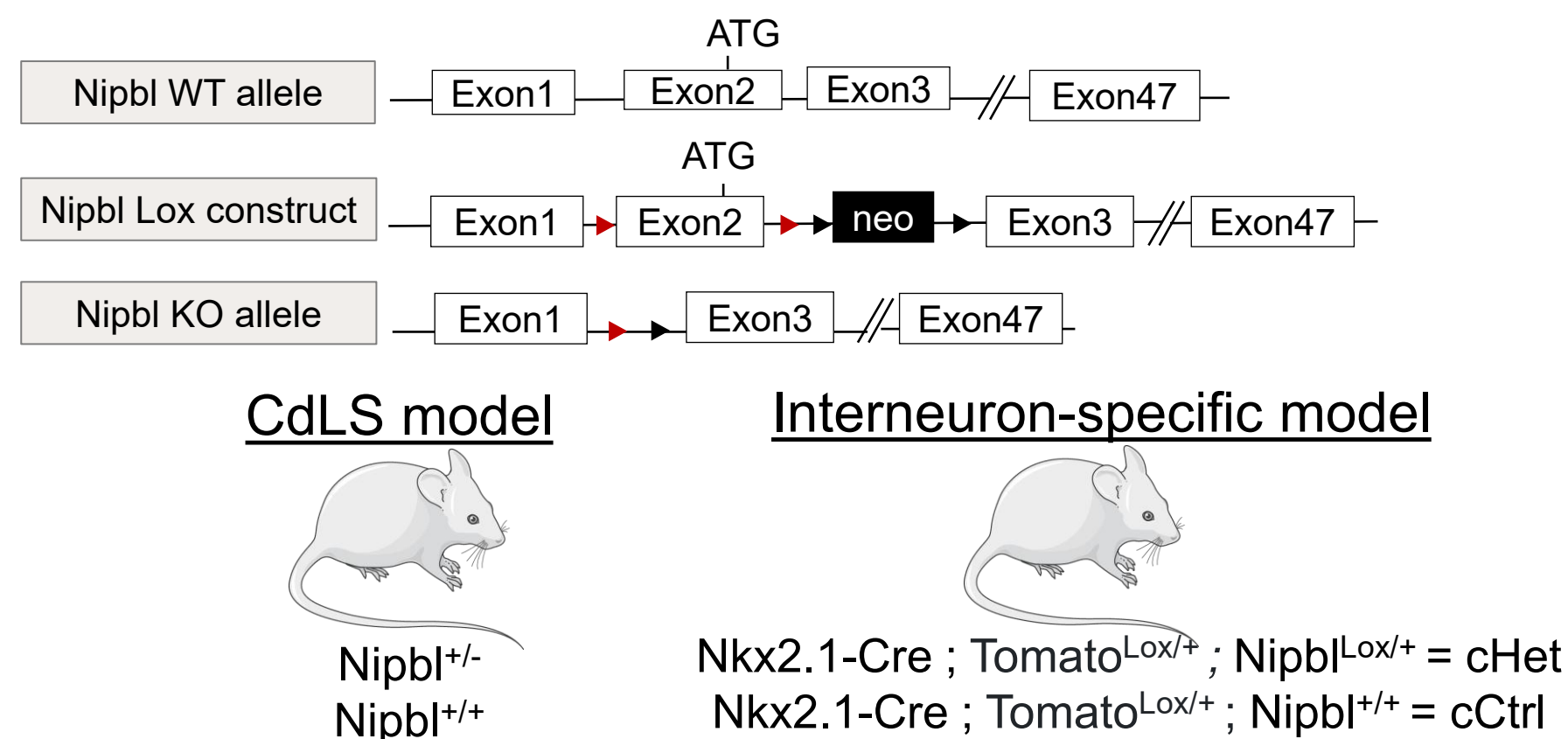


Fig2. Embryonic development of cortical interneurons (INs). From E11.5, MGE-Nkx2.1⁺ progenitors produce immature interneurons that migrate towards the future cortex through MZ and IZ. They settle in the cortex after a phase of laminar allocation.

3. Aims and mouse models

The study aims to decipher the role of NIPBL in INs and assess the contribution of these cells to the onset of behavioural impairments associated with CdLS. We are using two complementary mouse models derived from the same allele construct :



4. Nipbl is ubiquitously expressed

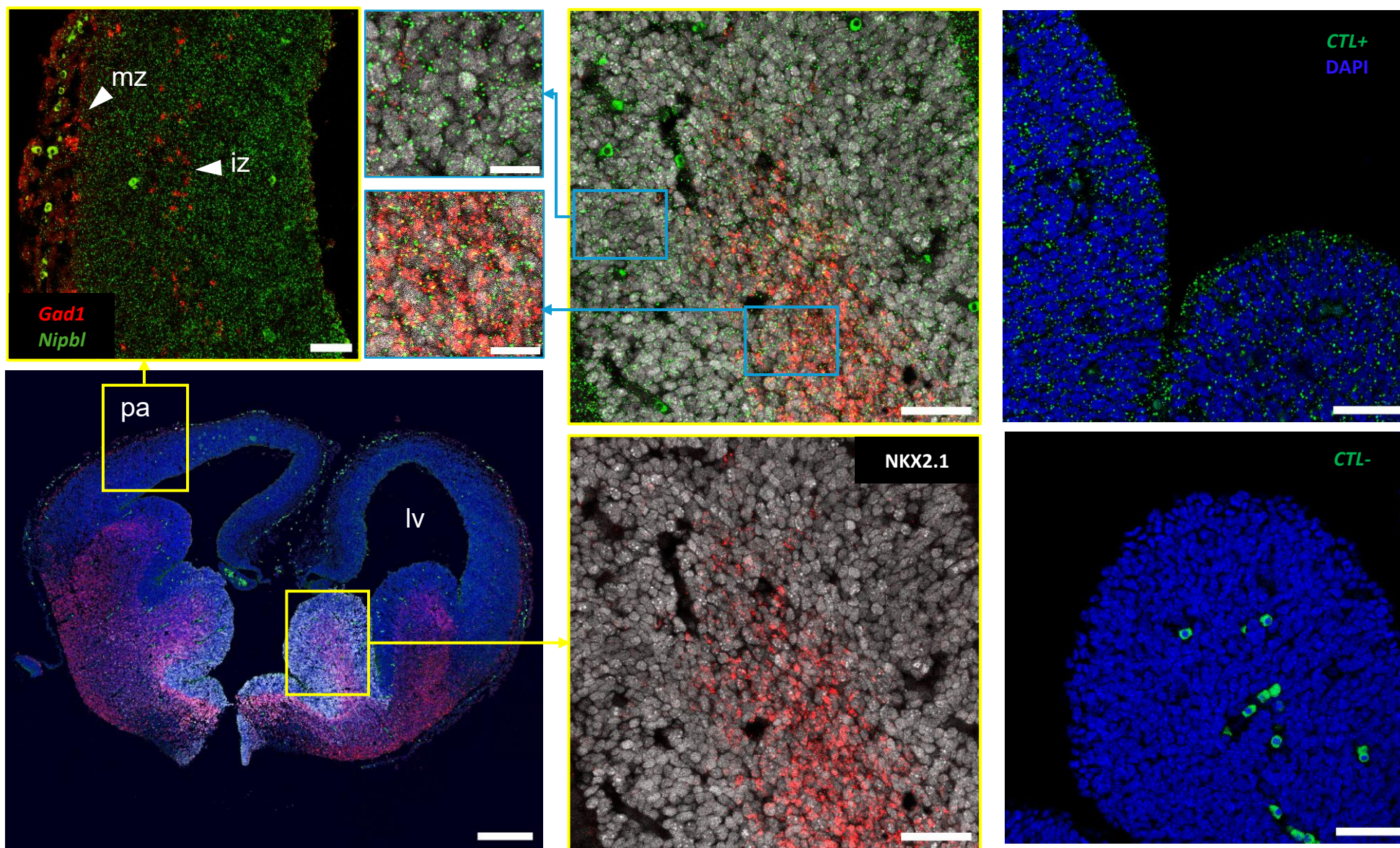


Fig3. *Nipbl* mRNA is expressed in the whole brain of E13.5 C57BL/6J embryo. RNAscope™ ISH revealing *Gad1*⁺ (red) INs precursors in the MGE and the mINs in the pallium. Nkx2.1 IF in white reveals the MGE-progenitors. All these cells express *Nipbl* (green). pa = pallium ; lv = lateral ventricle ; mz = marginal zone ; iz = intermediate zone

5. Validation of NIPBL depletion

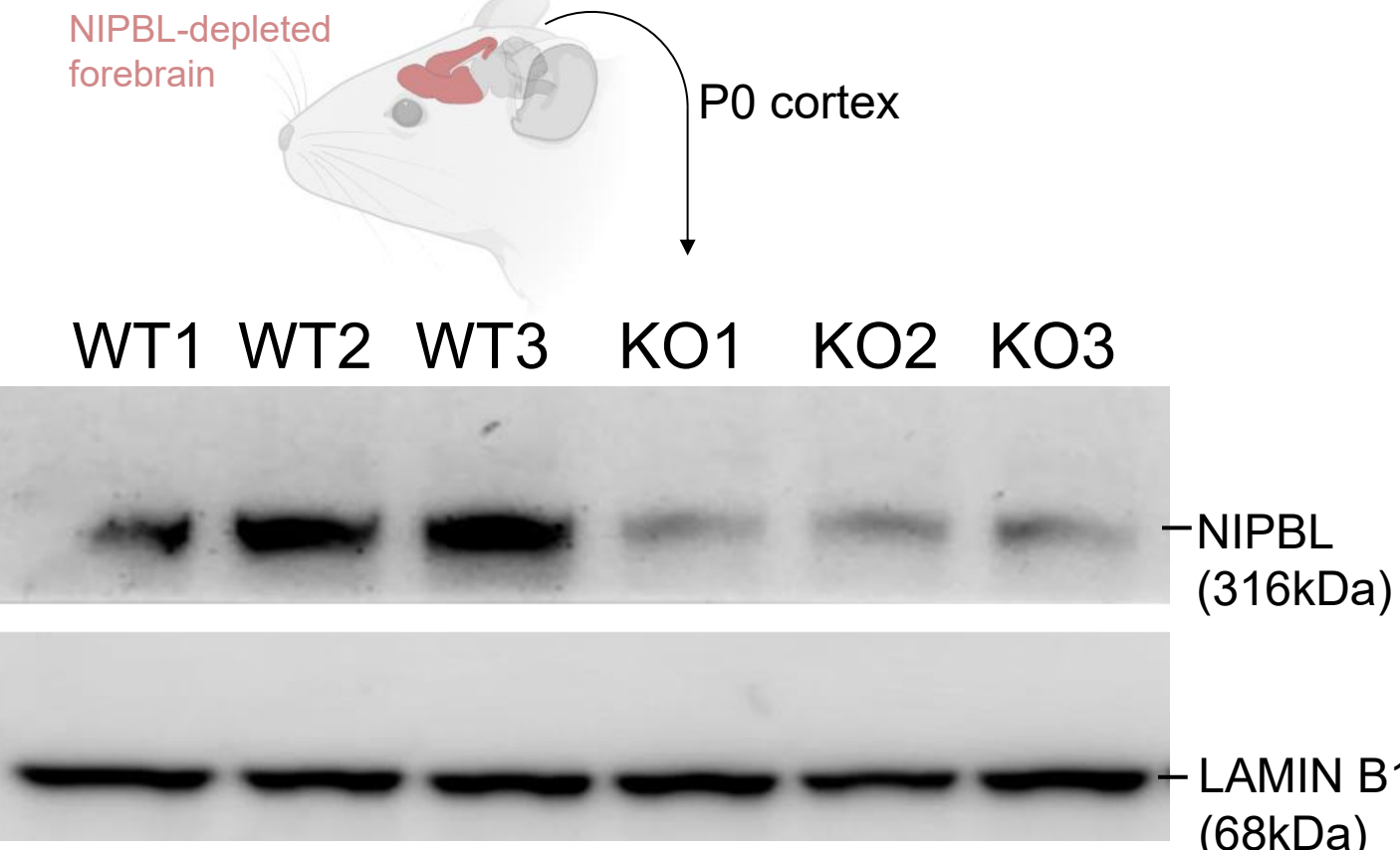


Fig4. Loss of NIPBL expression in the cortex of telencephalon-specific NIPBL-depleted mice. Western-blot of P0 cortex extracted proteins from 3 Foxg1-IRES-Cre ; Nipbl^{Lox/Lox} mice (= KO) and 3 Foxg1-IRES-Cre ; Nipbl^{+/+} mice (= WT).

6. Reduced weight in Nipbl^{Lox/+} mice

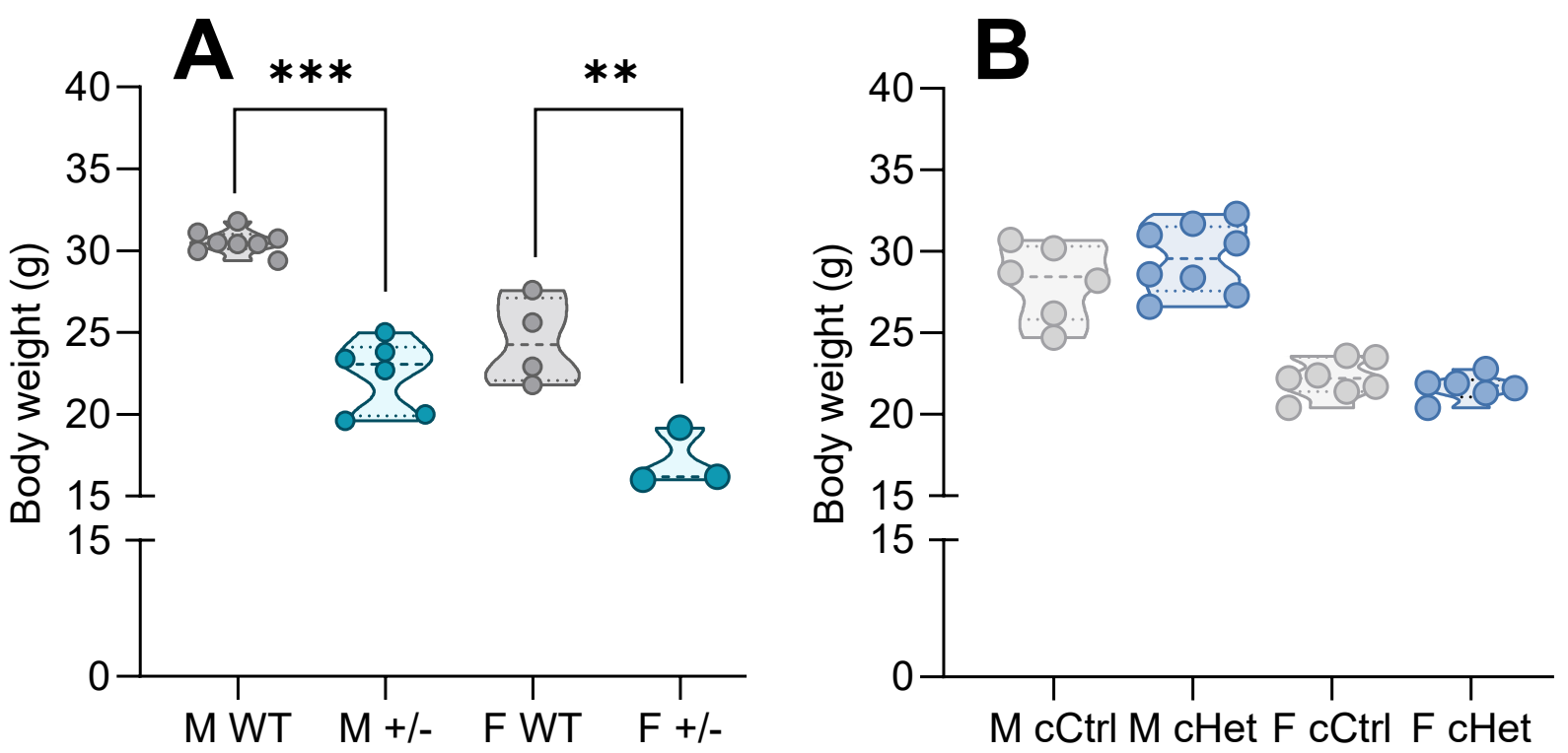


Fig5. Reduced body weight in *Nipbl*-deficient mice. (A-B) Body weight comparison at 3-4 months (A) *Nipbl*^{+/+} versus *Nipbl*^{Lox/+} mice. (B) cCtrl versus cHet mice.

7. Discordant anatomical measures of microcephaly in Nipbl-deficient mice at 3-4 months

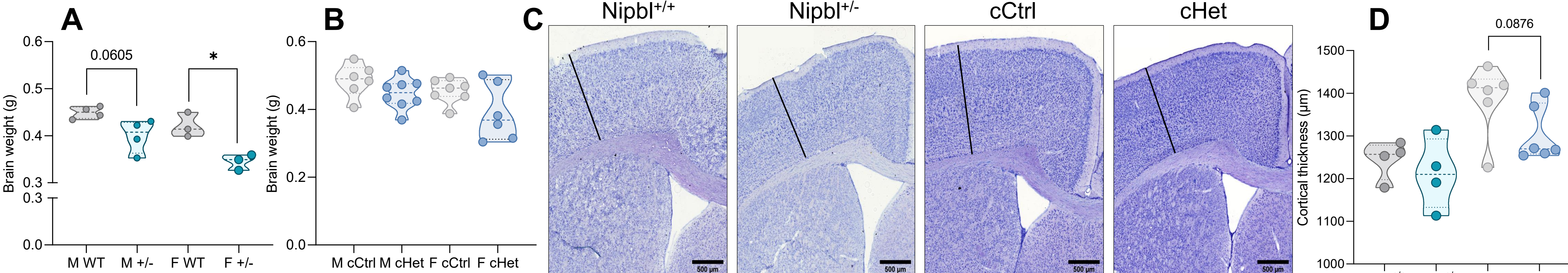


Fig6. Inconsistent evidence for microcephaly in *Nipbl*-deficient mice. (A-B) Brain weight comparison. (A) *Nipbl*^{+/+} versus *Nipbl*^{Lox/+} mice. (B) cCtrl versus cHet mice. (C) Coronal brain sections from 3-4-month-old mice (scale bar = 500µm). (D) Cortical thickness was measured and averaged from 3 medial sections per animal. Males only. M = male ; F = female.

8. Localized reduced SST⁺ cells in Nipbl^{Lox/+} mice

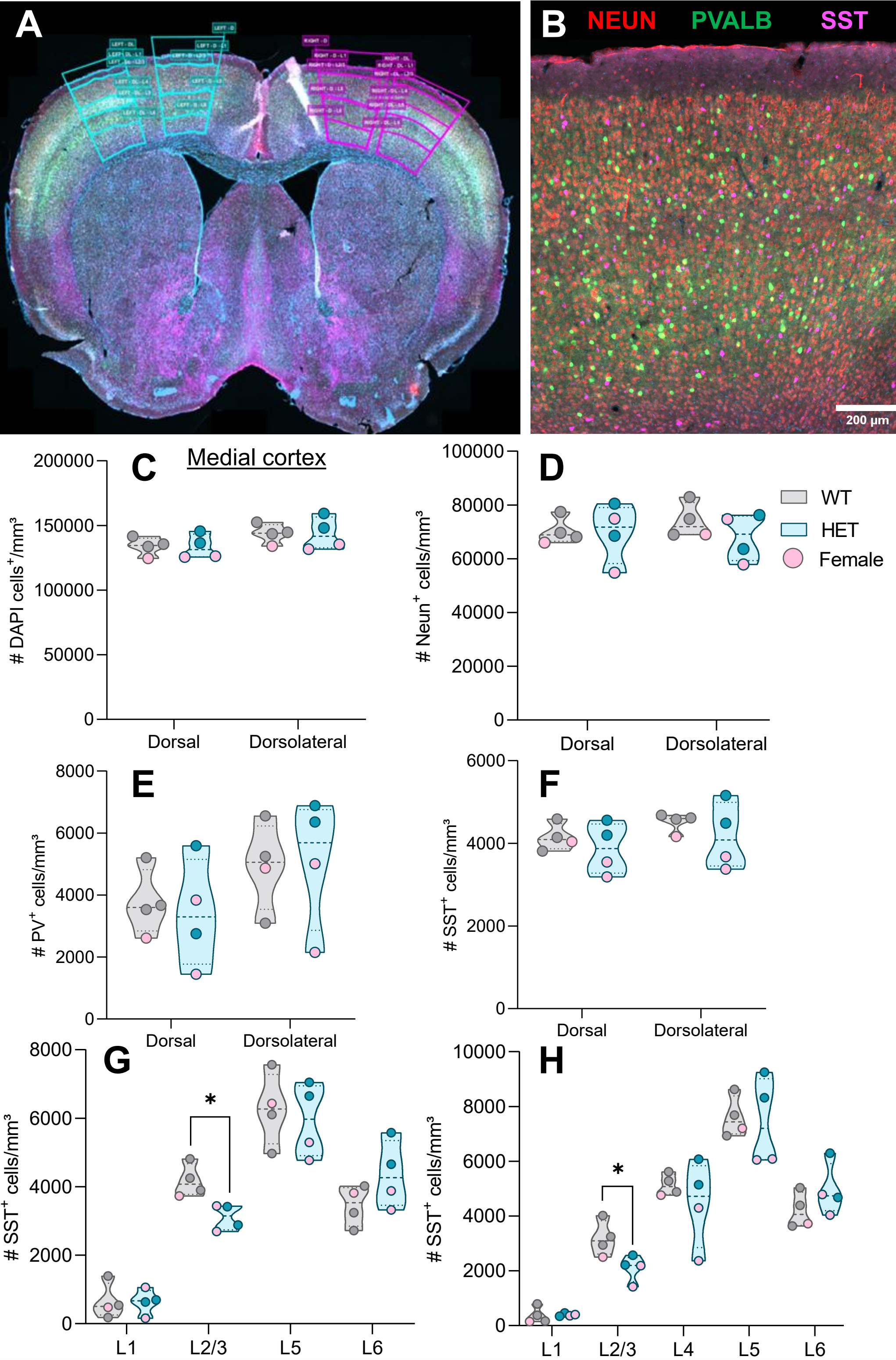
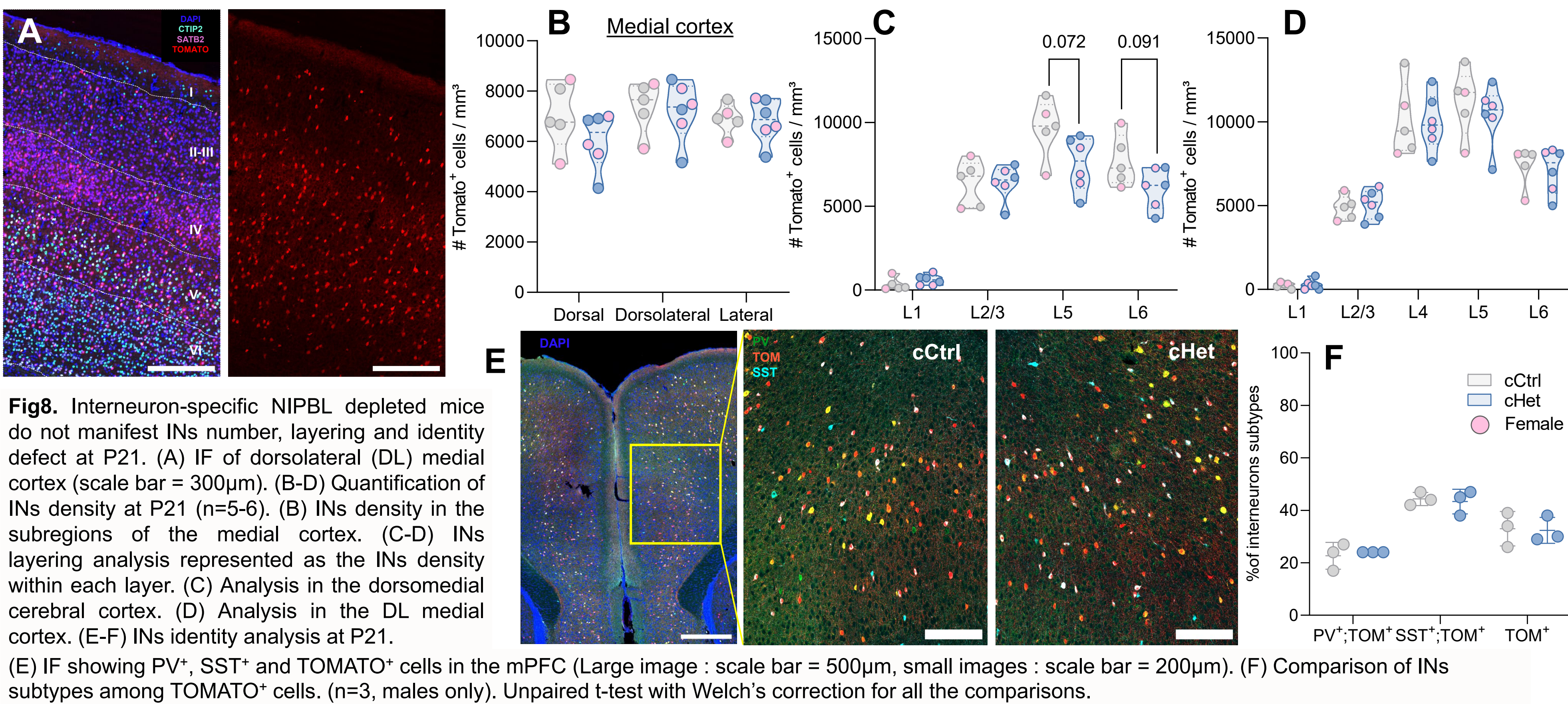


Fig7. Preliminary quantifications suggest a reduced SST⁺ cell density in cortical layer 2/3 at 3-4 months. (A) IF of the medial cortex. (B) Representative image of NEUN, PV and SST staining in the medial cortex. (C-F) Quantification of DAPI, NeuN⁺, PV⁺ and SST⁺ cell densities in the dorsal and dorsolateral medial cortex. (G-H) Quantification of SST⁺ cells in the layers of the medial dorsal and dorsolateral cortex. Unpaired t-test with Welch's correction for all the comparisons.

9. No defect in the number, layering and fate specification of NIPBL haploinsufficient INs



10. Nipbl^{Lox/+} mice show consistent hyperactivity in various tests but not cHet mice

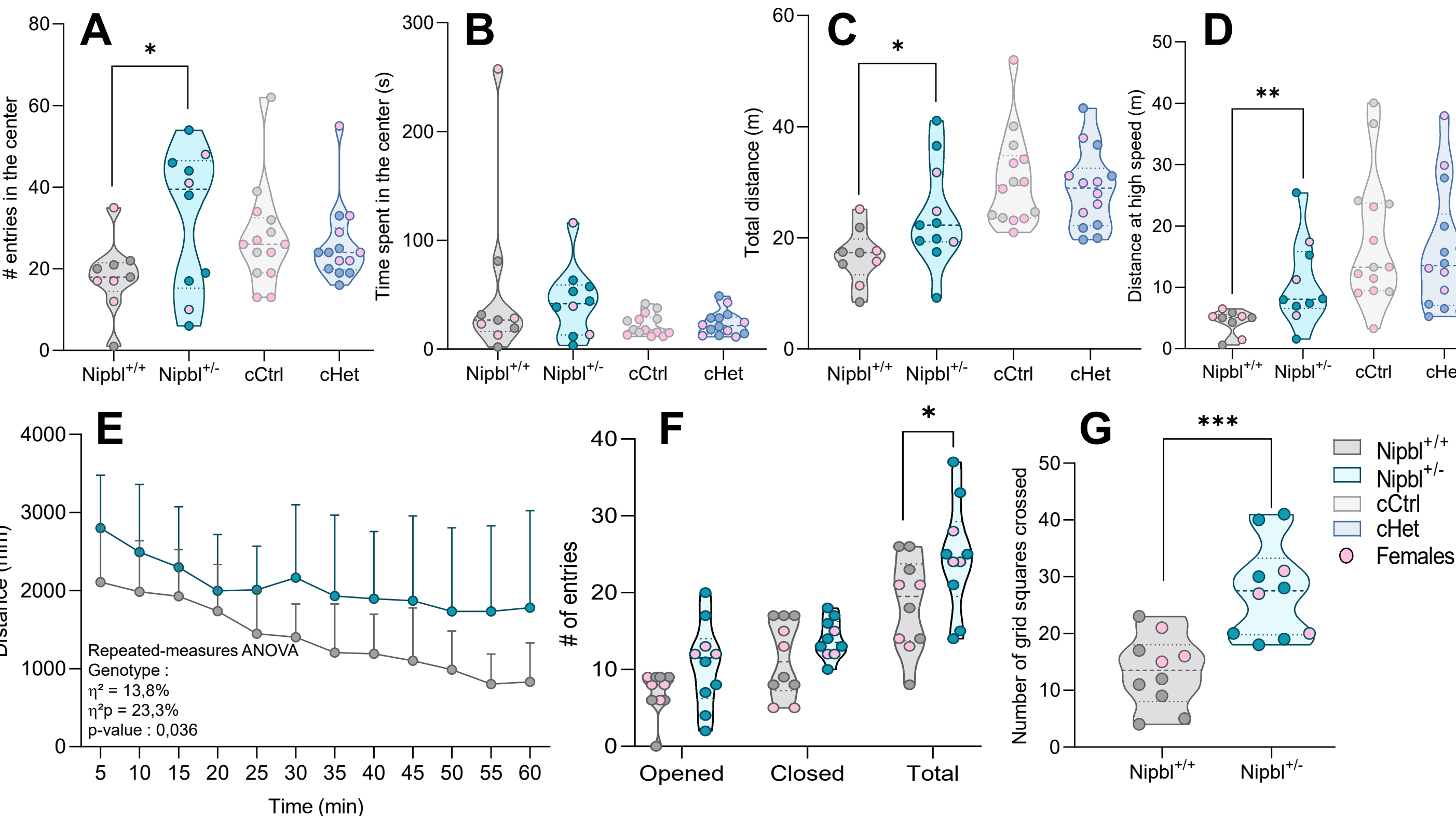


Fig9. Behavioural phenotyping of CdLS and interneurons-specific NIPBL-deficient models. (A-E) Open Field results. (A) Number of entries into the centre during the first 10min. (B) Time spent into the centre during the first 10min. (C) Total distance travelled (D) Distance travelled at high speed. (E) Distance travelled over time. Results expressed as mean ± SD. (F) Number of entries into the different arms of the Elevated Plus Maze. (G) Locomotion during SHIRPA assessment. (n=9-14). An Unpaired t-test with Welch's correction test was conducted for all the comparisons except for (E) which required a repeated-measures ANOVA.

Conclusion: Our results indicate that germline NIPBL depletion in mice interfere with normal behaviour and lead to hyperactivity. In addition, preliminary quantification of INs in the medial cortex suggest a localized depletion of SST⁺ interneurons. On the other hand, interneuron-specific NIPBL depleted mice do not show apparent defects in INs development and do not recapitulate behavioural issues observed in *Nipbl*^{Lox/+} mice. These findings suggest that any cell-autonomous defect in NIPBL-depleted interneurons is unlikely to underlie the behavioural impairments seen in the CdLS model.

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