Usp11 controls cortical neurogenesis and neuronal migration through Sox11 stabilization.

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Advisor: Shen-Ju Chou and Ruey-Hwa Chen

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2 Institute of Biochemical Sciences, National Taiwan University, Taipei 100, Taiwan;
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中華民國兒童神經精神科學 勵翔獎
Young Investigator Award for Pediatric Neuropsychiatric Science

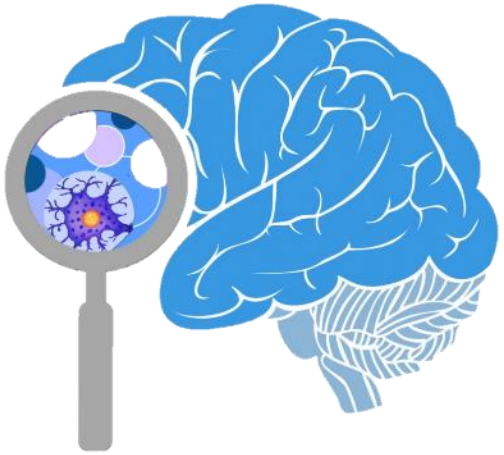
The role of ubiquitination in neuronal development.

The molecular mechanism of cortical development has been mostly focused on transcriptional regulation

↕

Post-translational regulation ?

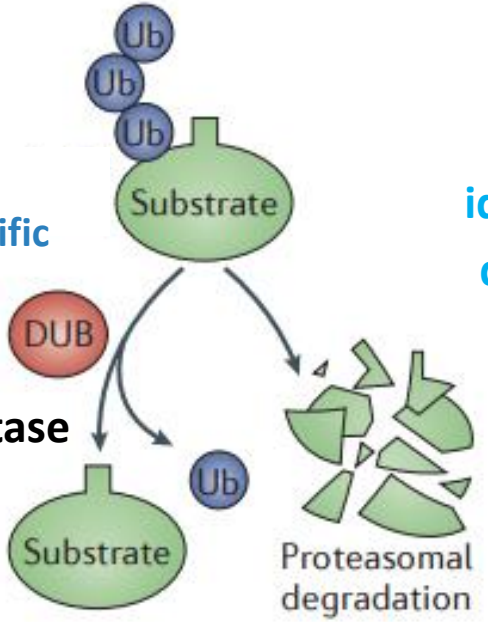
Ex. deubiquitination



Usp11

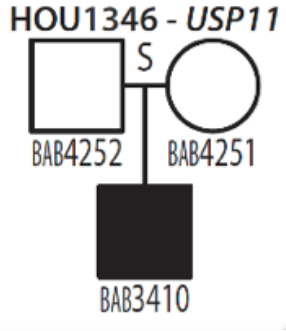
(Ubiquitin-specific protease 11)

deubiquinitase



Usp11 homozygous mutation was identified in patient with neurologic disorder and intellectual disability.

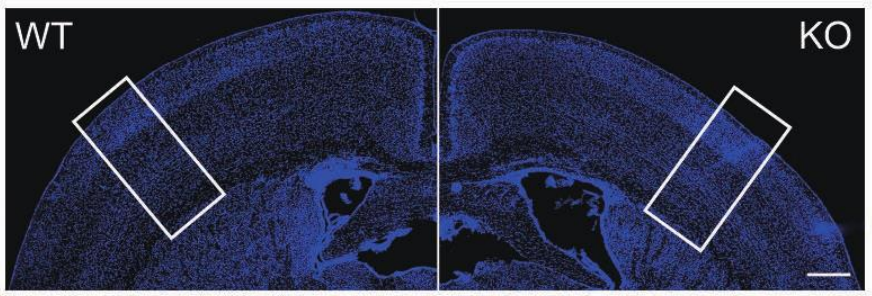
Neuron 2015 Nov 4;88(3):499-513.

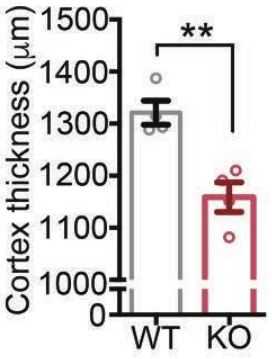


How does Usp11 affect cortical development ?

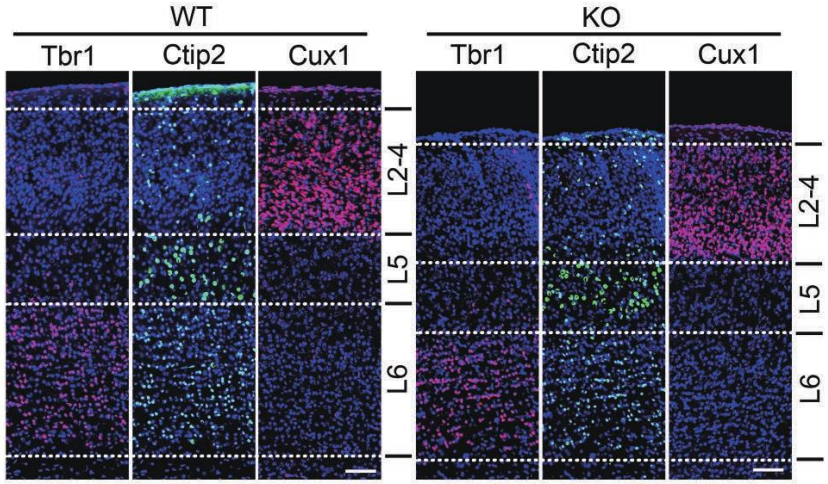
Usp11 KO reduces the thickness of embryonic cortex

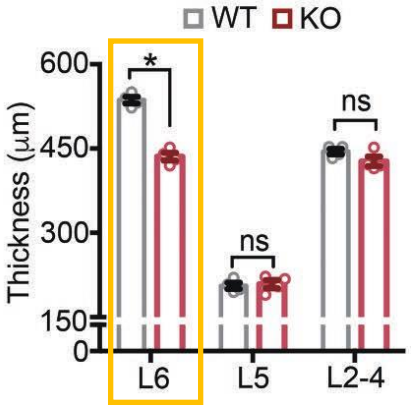
Usp11 conventional knockout mice at P7

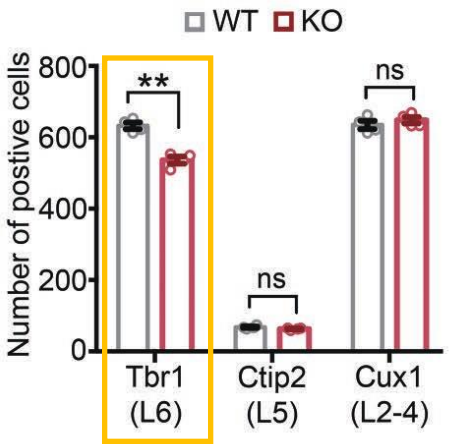




cortex	marker
L2-4	Cux1
L5	Ctip2
L6	Tbr1







These data suggest that Usp11 regulates layer 6 neurogenesis.

Disease-associated Usp11 mutant is defective in promoting early neurogenesis and late born neural migration.

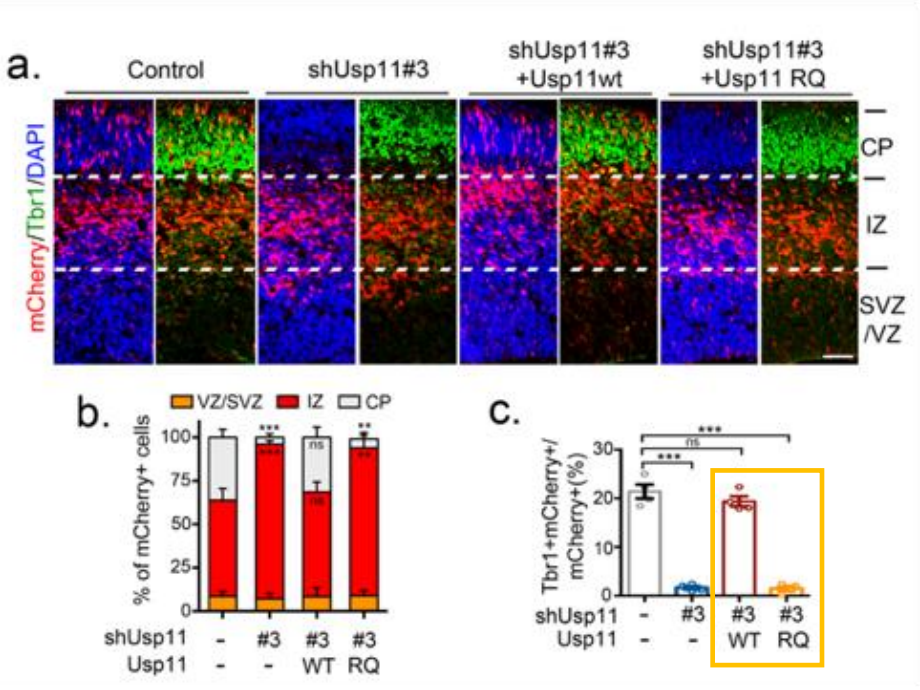
Whether Usp11 RQ mutant is functionally impaired in cortical development ?

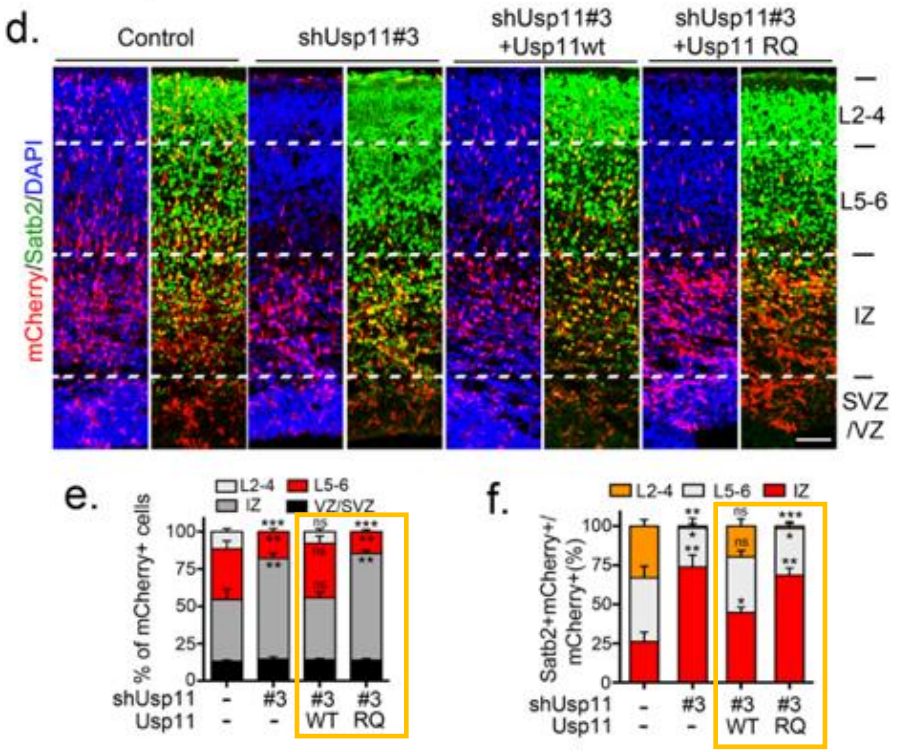
Neurogenesis

IUE at E12.5 → analyze at E15.5

Neuron migration

IUE at E15.5 → analyze at E18.5

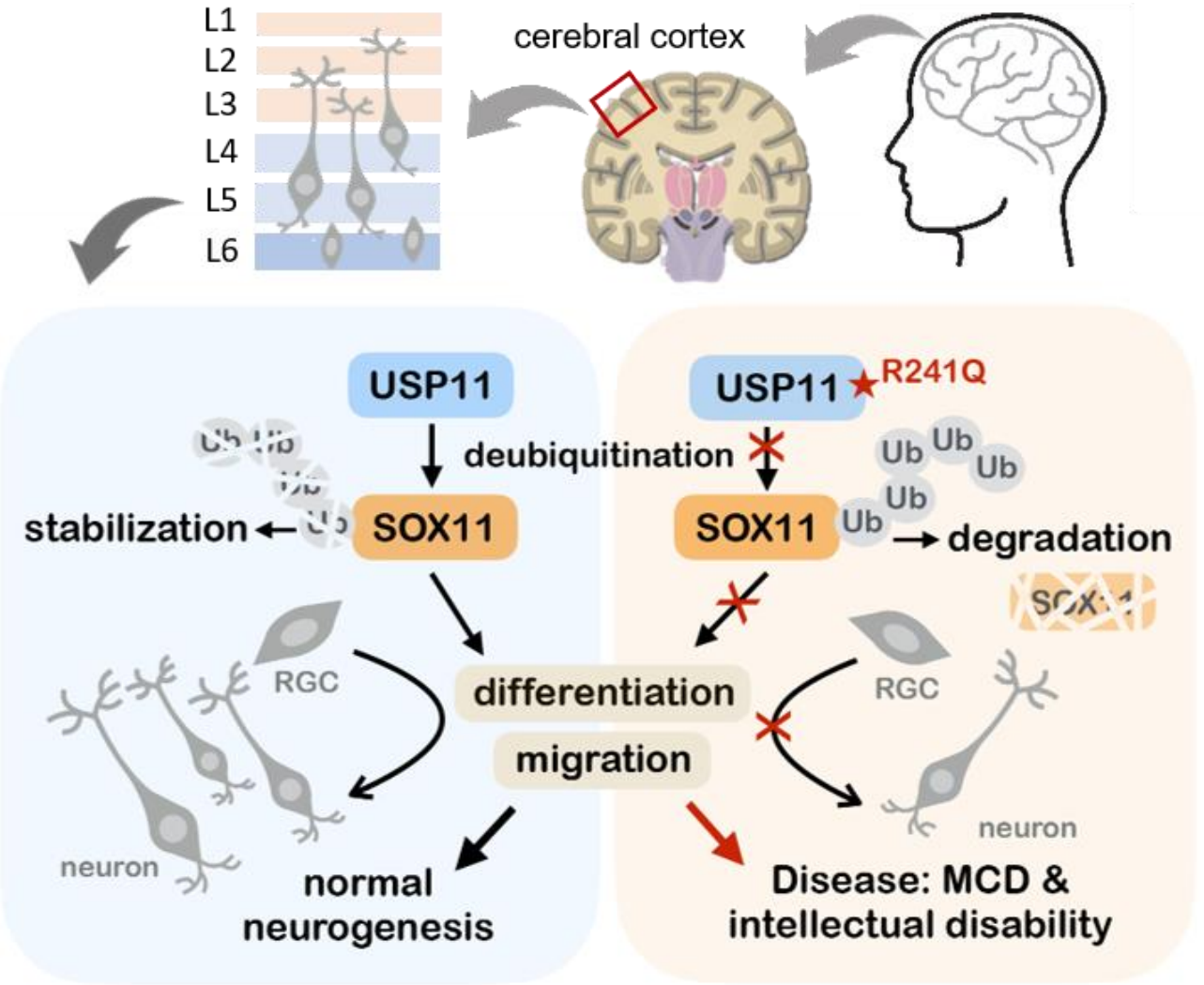




DEVELOPMENTAL NEUROSCIENCE

Usp11 controls cortical neurogenesis and neuronal migration through Sox11 stabilization

Conclusion



DEVELOPMENTAL NEUROSCIENCE

Usp11 controls cortical neurogenesis and neuronal migration through Sox11 stabilization

Shang-Yin Chiang[1,2], Hsin-Chieh Wu[1], Shu-Yu Lin[1], Hsin-Yi Chen[3], Chia-Fang Wang[4], Nai-Hsing Yeh[5], Jou-Ho Shih[5], Yi-Shuian Huang[5], Hung-Chih Kuo[4], Shen-Ju Chou[4]*, Ruey-Hwa Chen[1,2]*

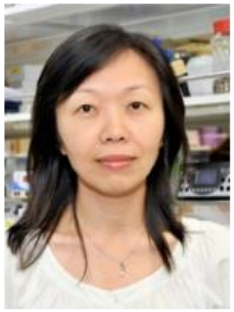
Acknowledgments

Dr. Ruey-Hwa Chen
Institute of Biological Chemistry

Dr. Shen-Ju Chou
Institute of Cellular and Organismic Biology

Dr. Hung-Chih Kuo
Institute of Cellular and Organismic Biology
neuronal induction from mES cells

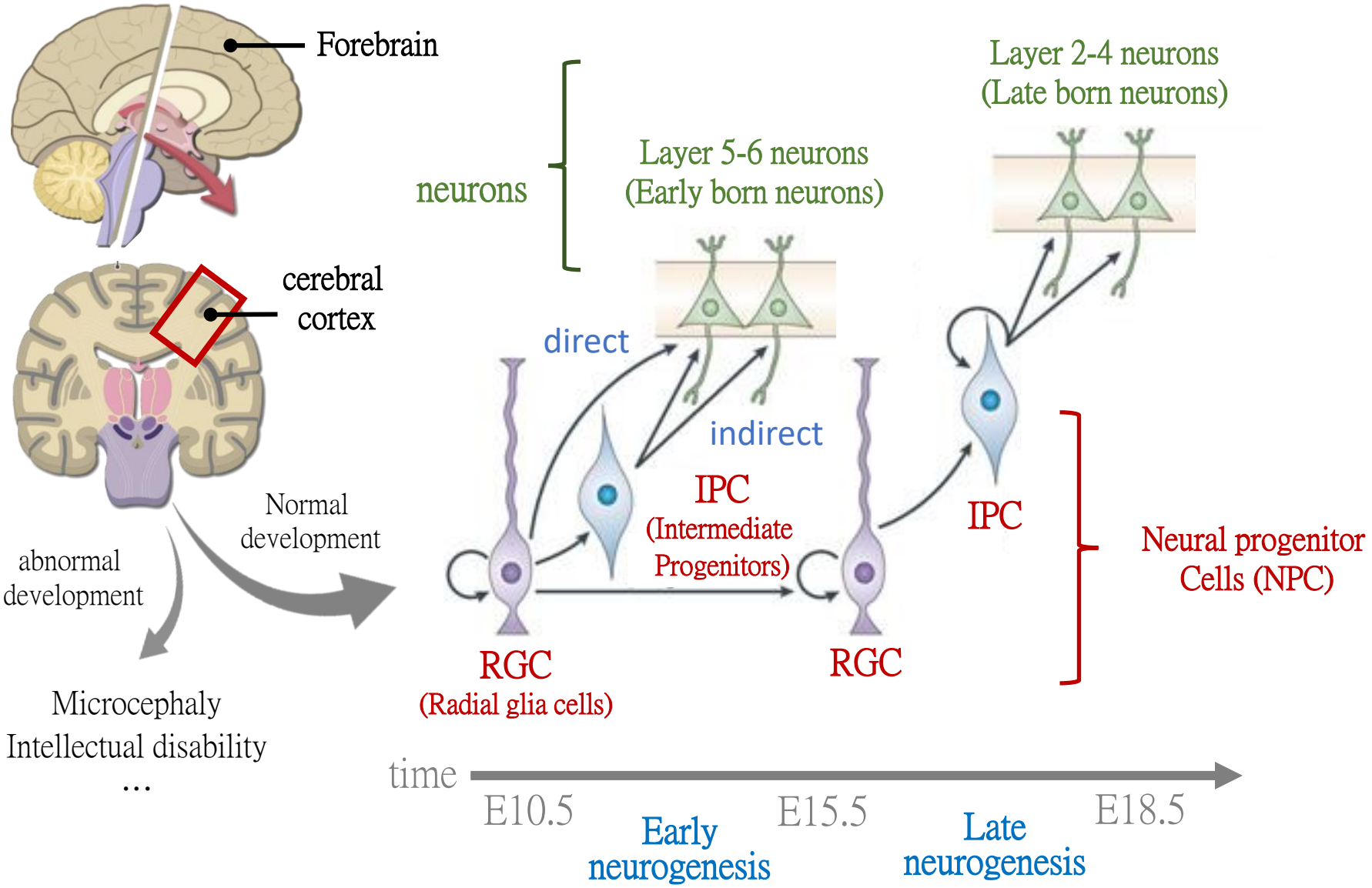
Dr. Hsin-Chieh Wu



Dr. Huang, Yi-Shuian
Institute of Biomedical sciences
mouse behavior

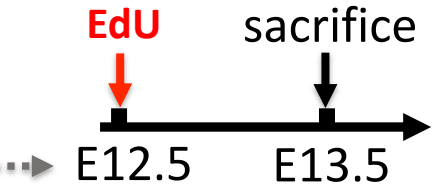
Hsin-Chieh Wu and Shu-Yu Lin: K-ε-GG profiling and proteome analysis by LC-MS/MS
Chia-Fang Wang, Nai-Hsing Yeh, Hsiang-Wei Hsing and Pei-Rung Wu: Technical instructions
Hsin-Yi Chen and Jou-Ho Shih : Statistical Analysis

Introduction: cortical neurogenesis

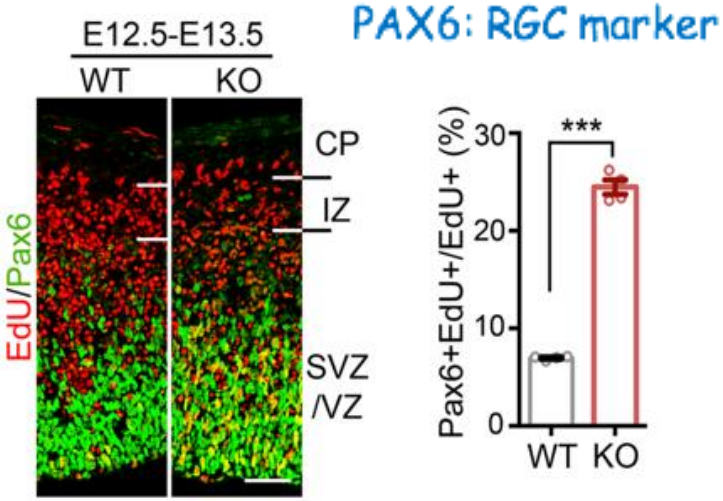


Usp11 affects RGC differentiation.

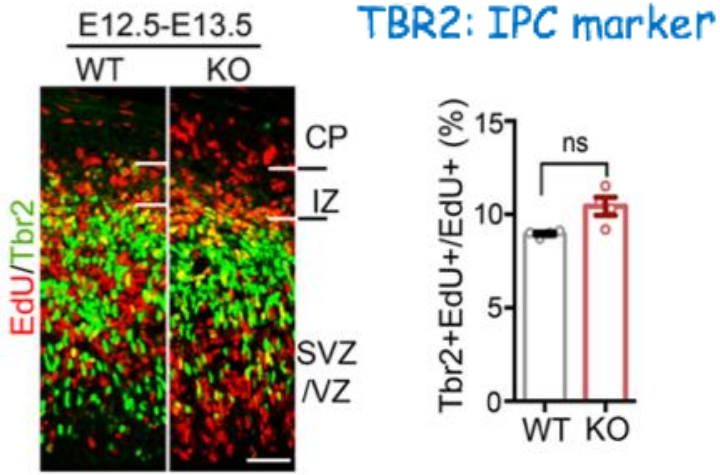
L6 neurogenesis
happens around E12.5



PAX6: RGC marker

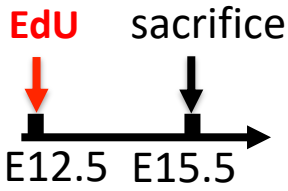


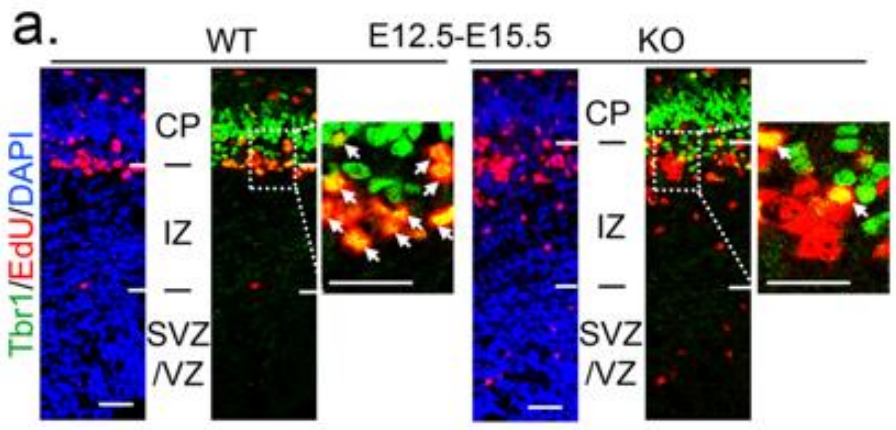
TBR2: IPC marker

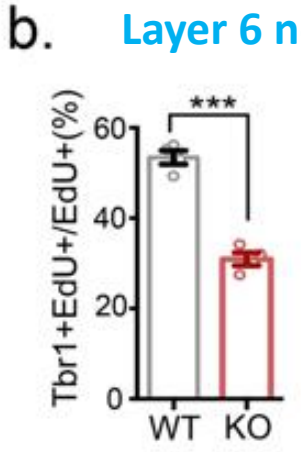


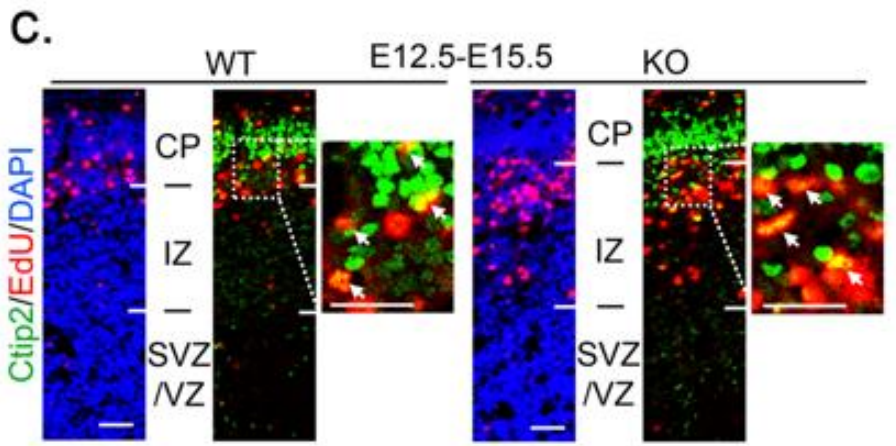
Usp11 inhibits the differentiation of E12.5 RGC, but does not affect IPC.

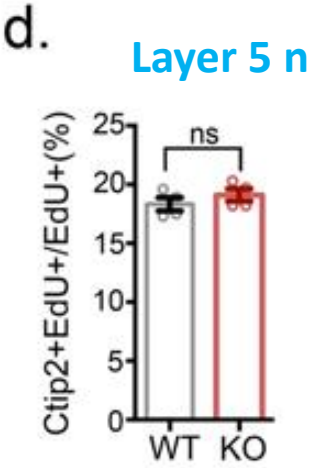
Usp11 functions in the layer 6 neurogenesis.







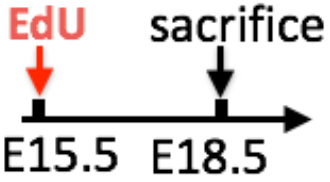


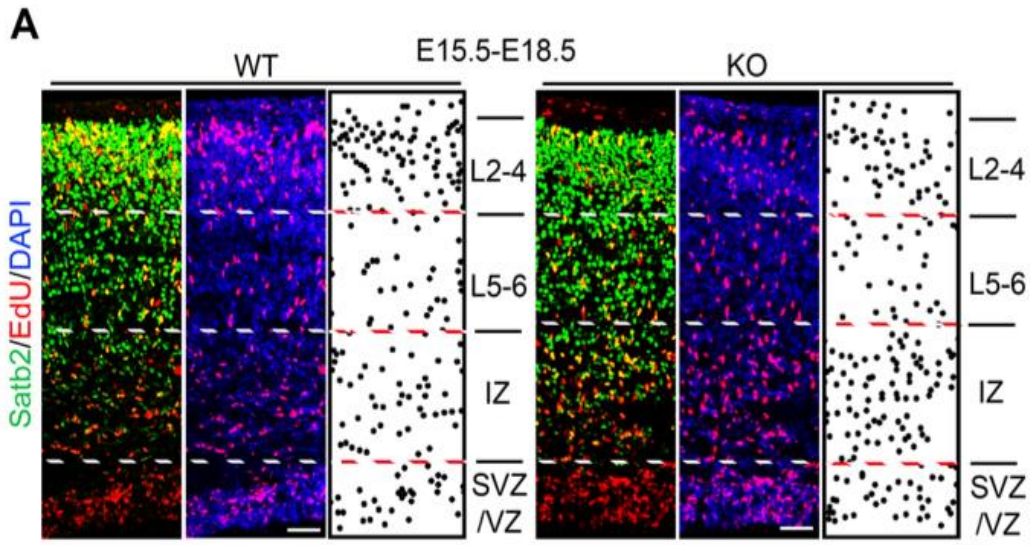


These data are also consistent with the reduced layer 6 thickness observed in Usp11 knockout mice.

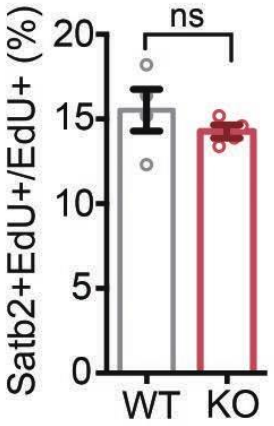
Usp11 KO impairs late-born neuron migration

We determined the effect of Usp11 on late neurogenesis.

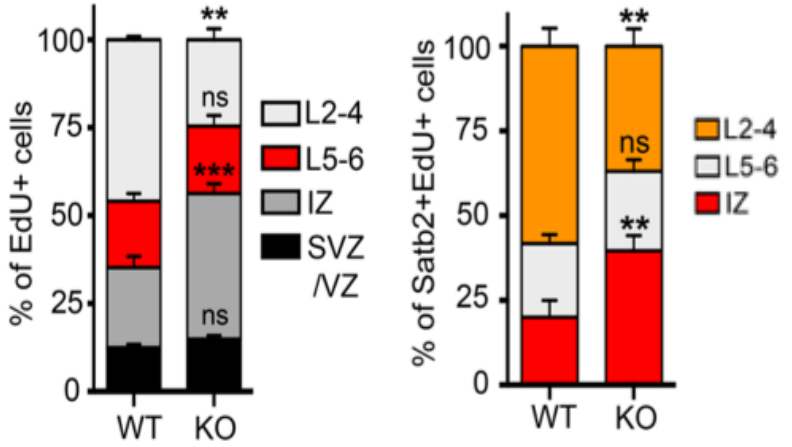




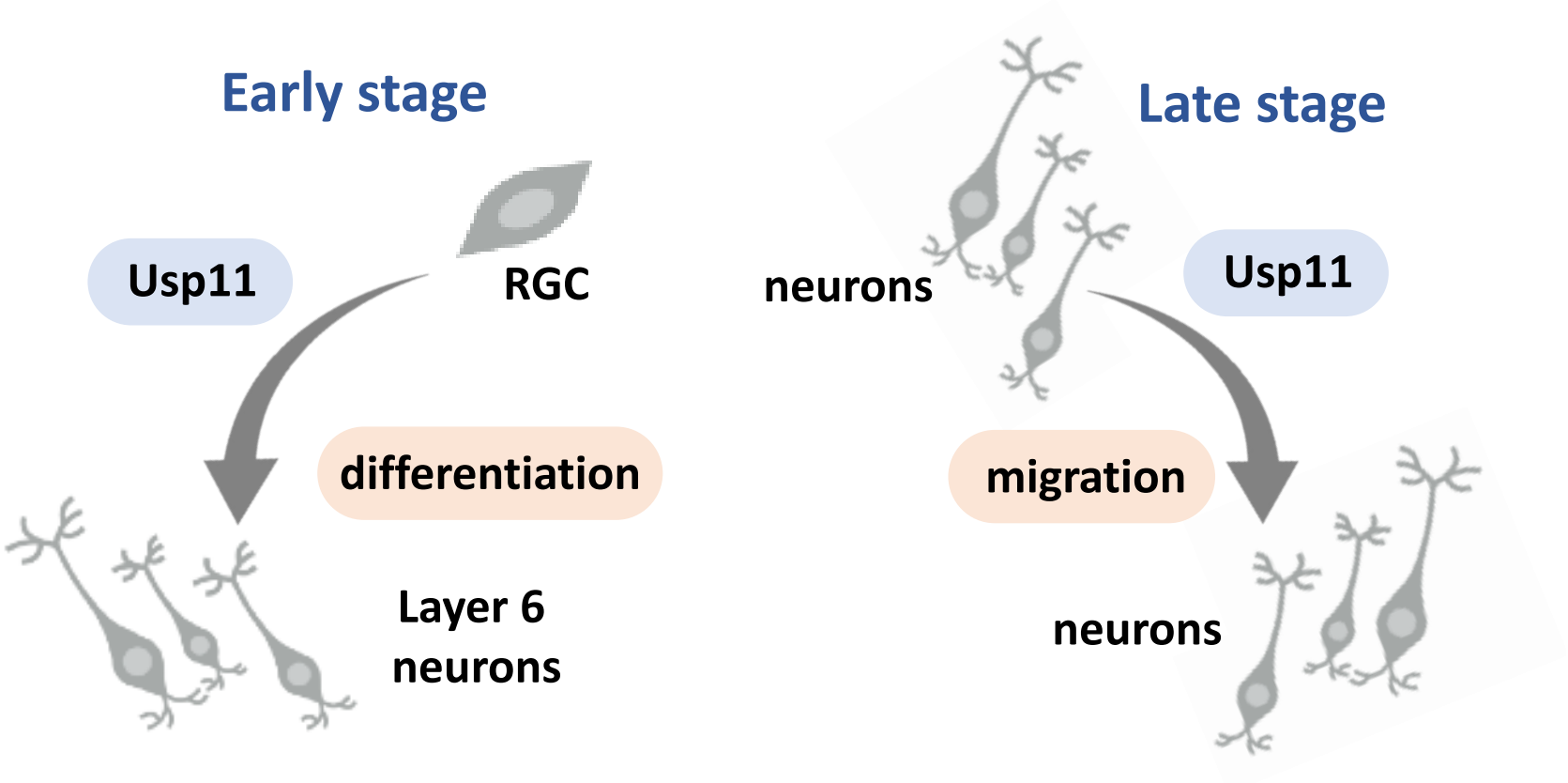
Upper layer neurons



Distribution



The dual roles of Usp11 in cortical development



Emx1 :*Usp11*$^{f/f}$ **Usp11 conditional knockout mice** *Nex* :*Usp11*$^{f/f}$

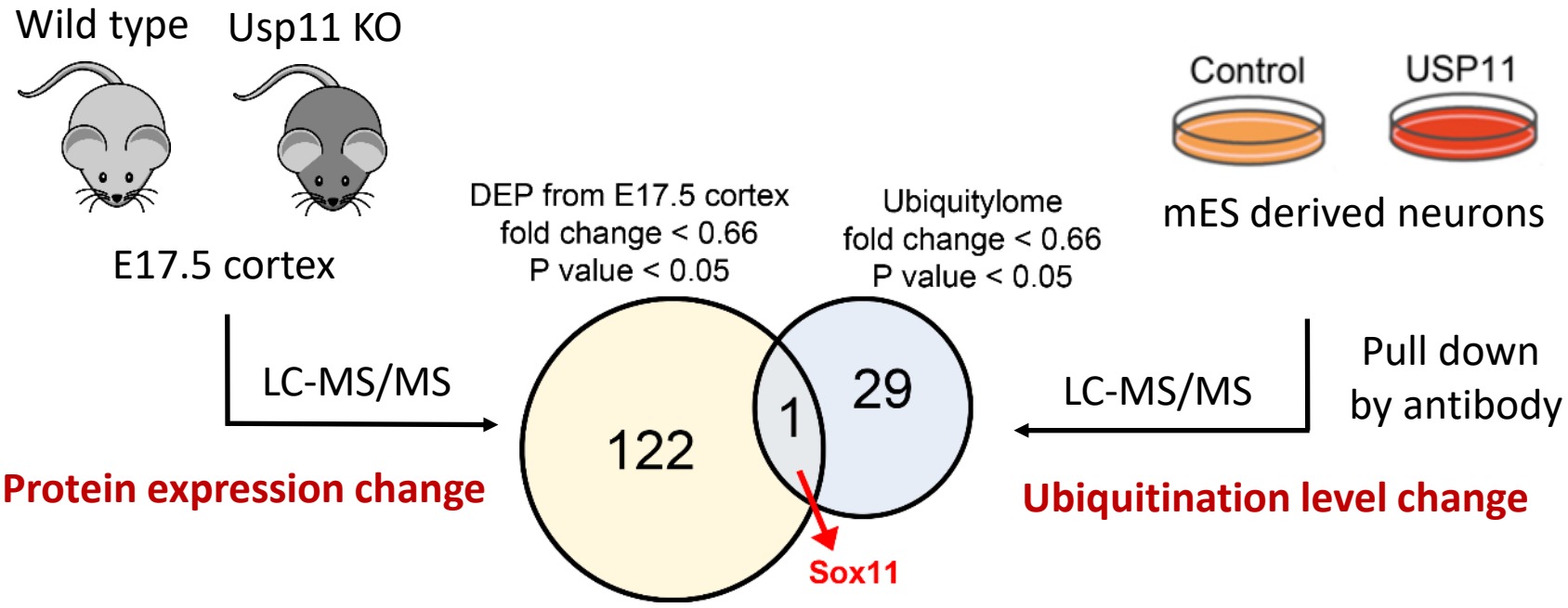
(cell autonomous effects)

Loss of Usp11 in NPC
→ defect in Layer 6 neurogenesis

Loss of Usp11 in post-mitotic neuron
→ defect in late born neuron migration

Identification of Usp11 substrates using omics analyses

To determine the molecular mechanism of Usp11 in cortical development.

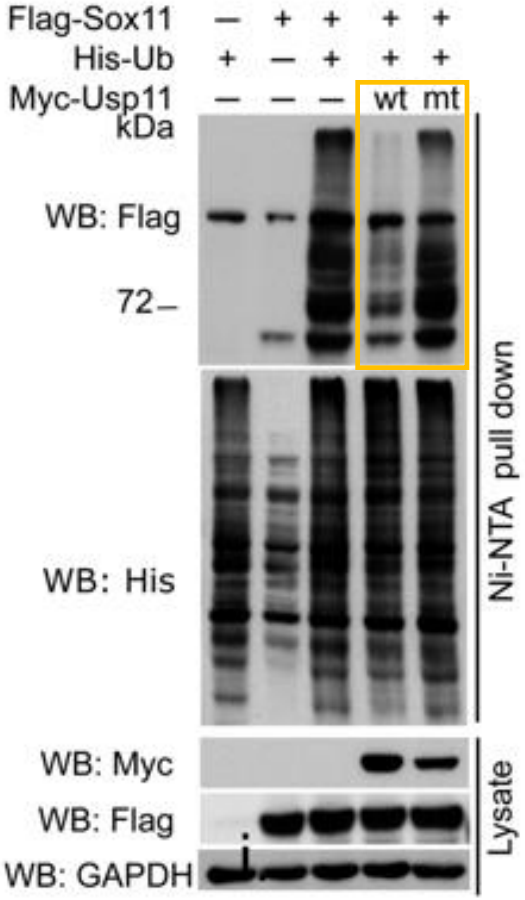


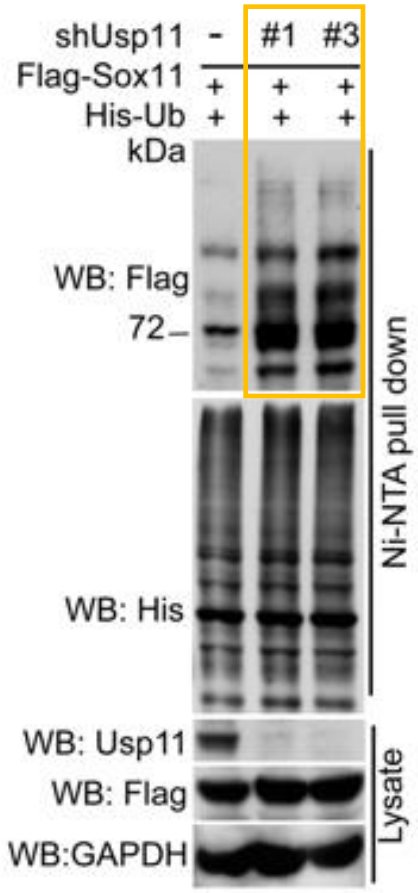
Sox11 is a transcriptional factor
and critical for neurogenesis and neuron migration.

J Neurosci. 2015 Jul 22; 35(29): 10629–10642.
J Neurosci. 2016 May 25;36(21):5775-84.

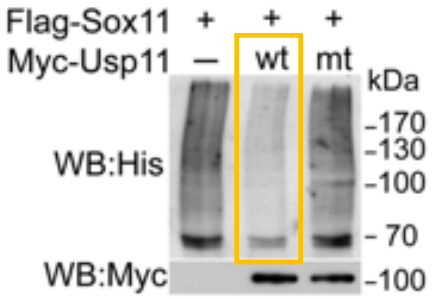
Usp11 binds and deubiquitinates Sox11

In vivo deUb assay

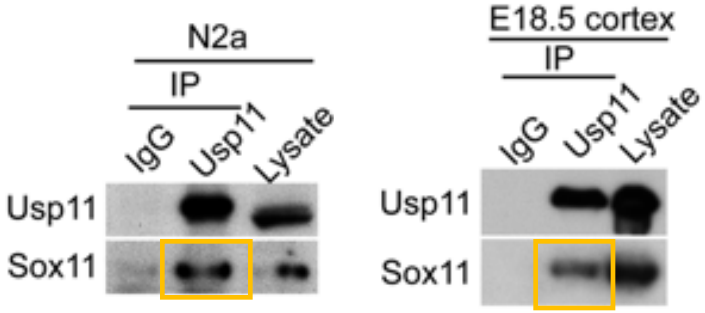




In vitro deUb assay



Endogenous co-IP

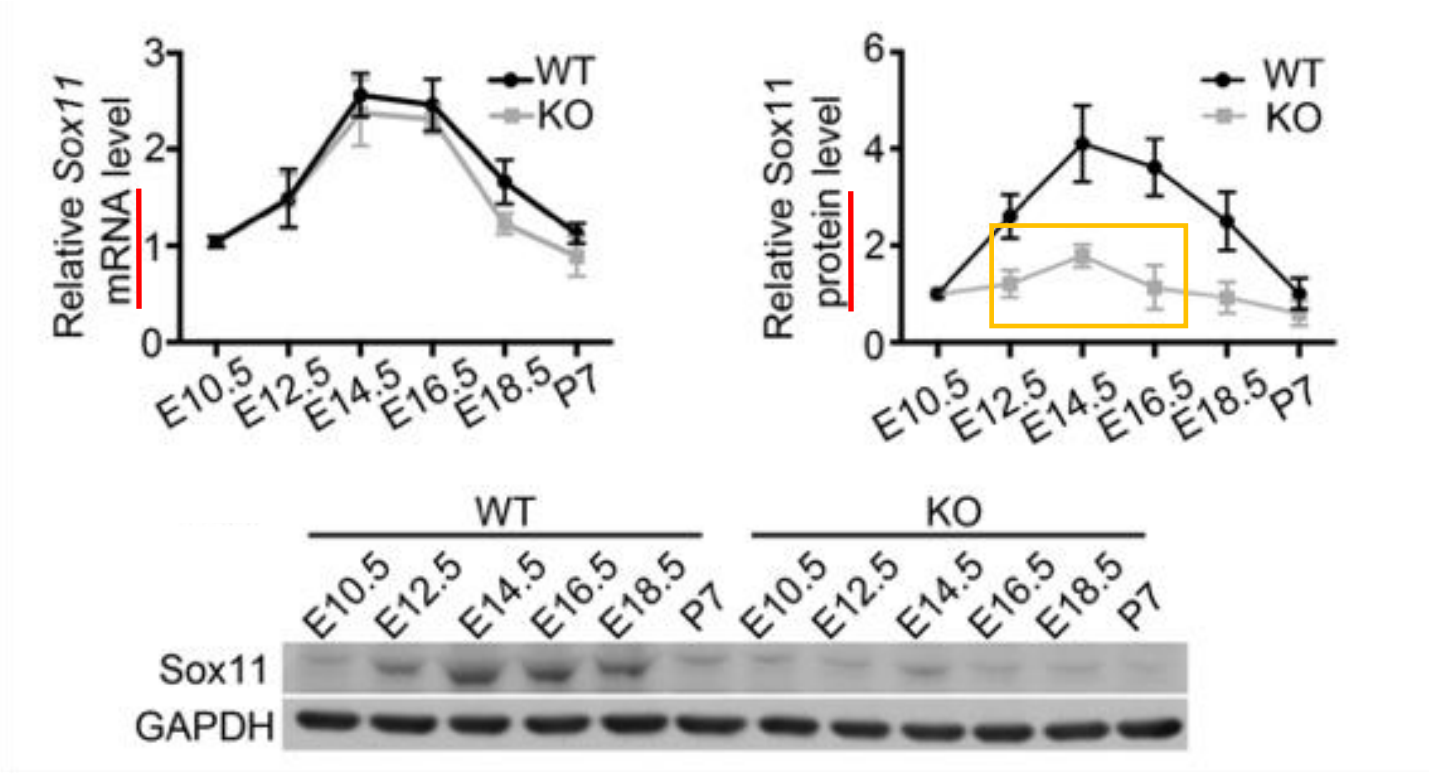


Sox11 is a substrate of Usp11.

Usp11-mediated Sox11 stabilization plays a major role in Sox11 induction during cortical development

Transcriptional regulation —previous→ Sox11 expression ←We found— post-translational regulation

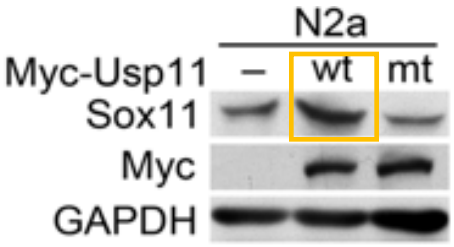
To determine the importance of this post-translational mechanism.

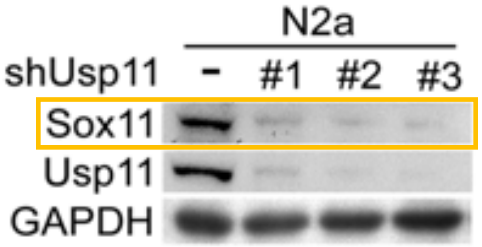


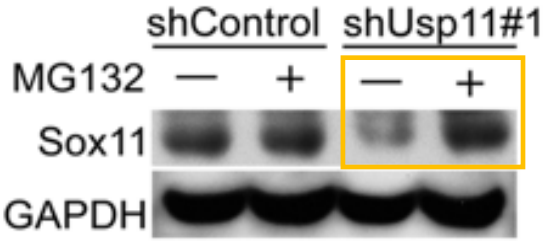
Usp11 prevents Sox11 from proteasomal degradation.

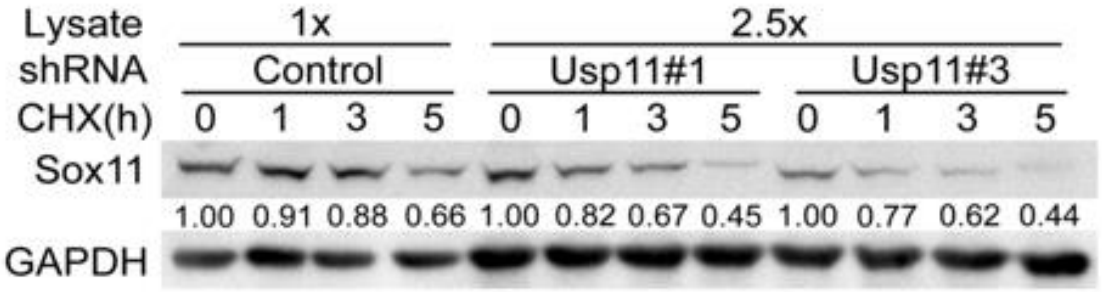
What is the outcome of Sox11 deubiquitination ?

MG132: proteasome inhibitor





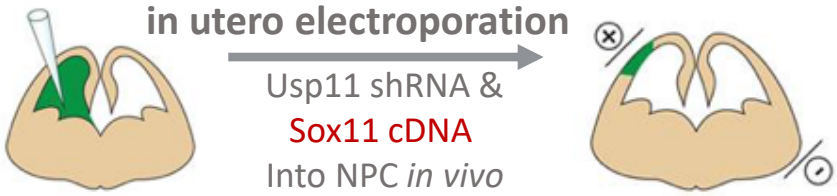




CHX: Cycloheximide, protein synthesis inhibitor

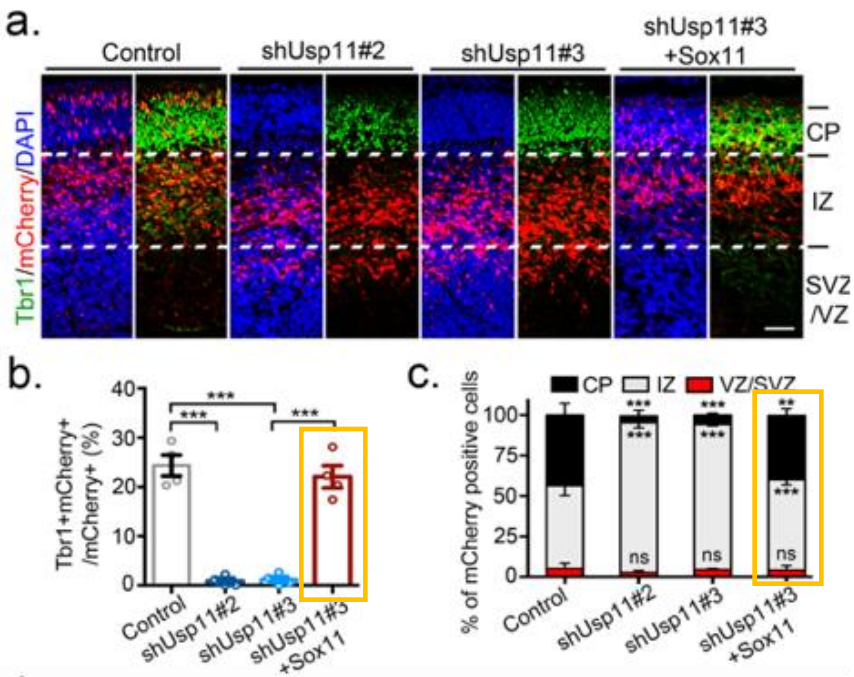
Sox11 overexpression rescues the early and late cortical developmental defects of Usp11 knockdown

Whether Sox11 contributes to the developmental defects of Usp11 KO?



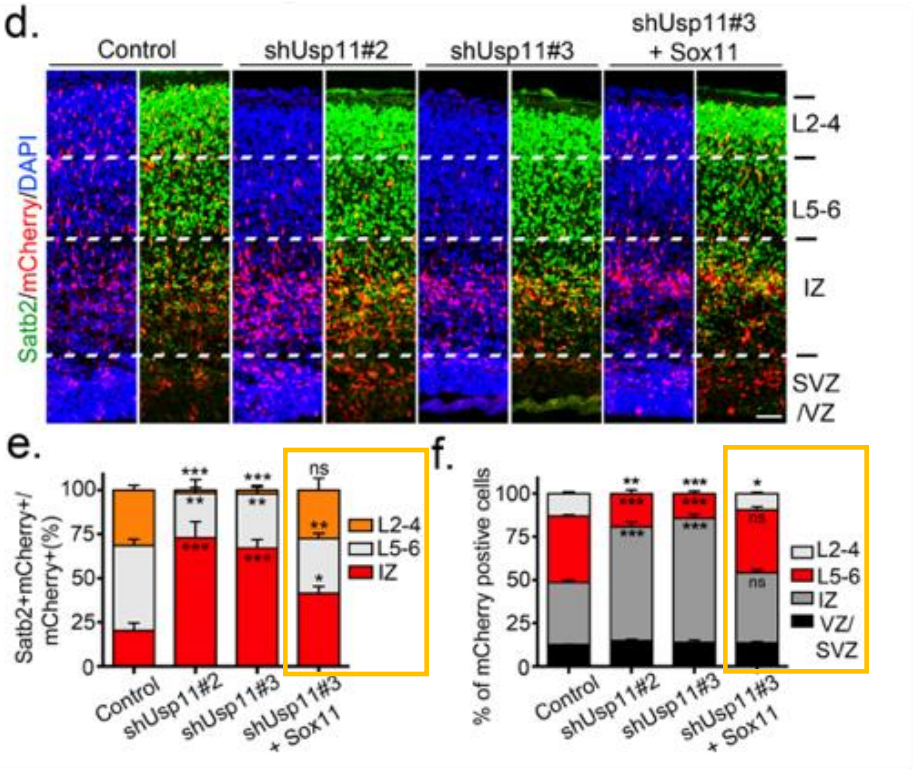
Neurogenesis

IUE at E12.5 → analyze at E15.5

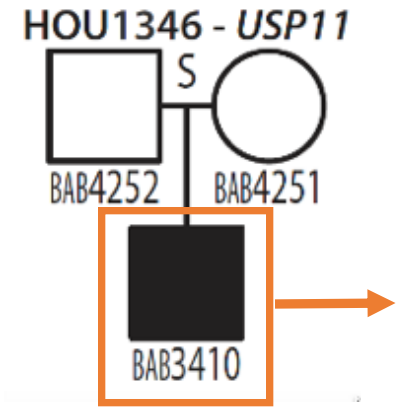


Neuron migration

IUE at E15.5 → analyze at E18.5



The disease relevance of Usp11/Sox11 axis.



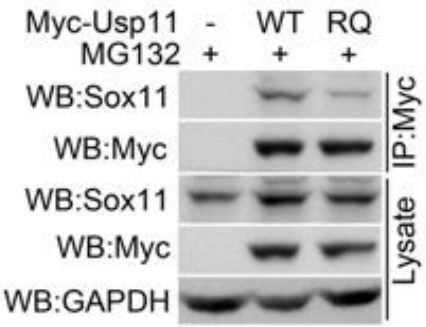
Neuron

Genes that Affect Brain Structure and Function Identified by Rare Variant Analyses of Mendelian Neurologic Disease

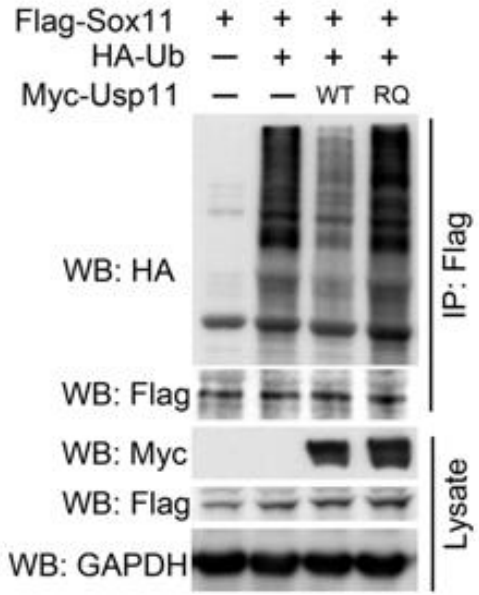
Karaca et al., 2015, Neuron *88*, 499–513

Previous study found Usp11 R241Q mutation in patient with corpus callosum agenesis, syntelencephaly and intellectual disability

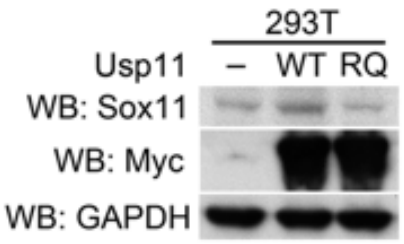
Binding by co-IP



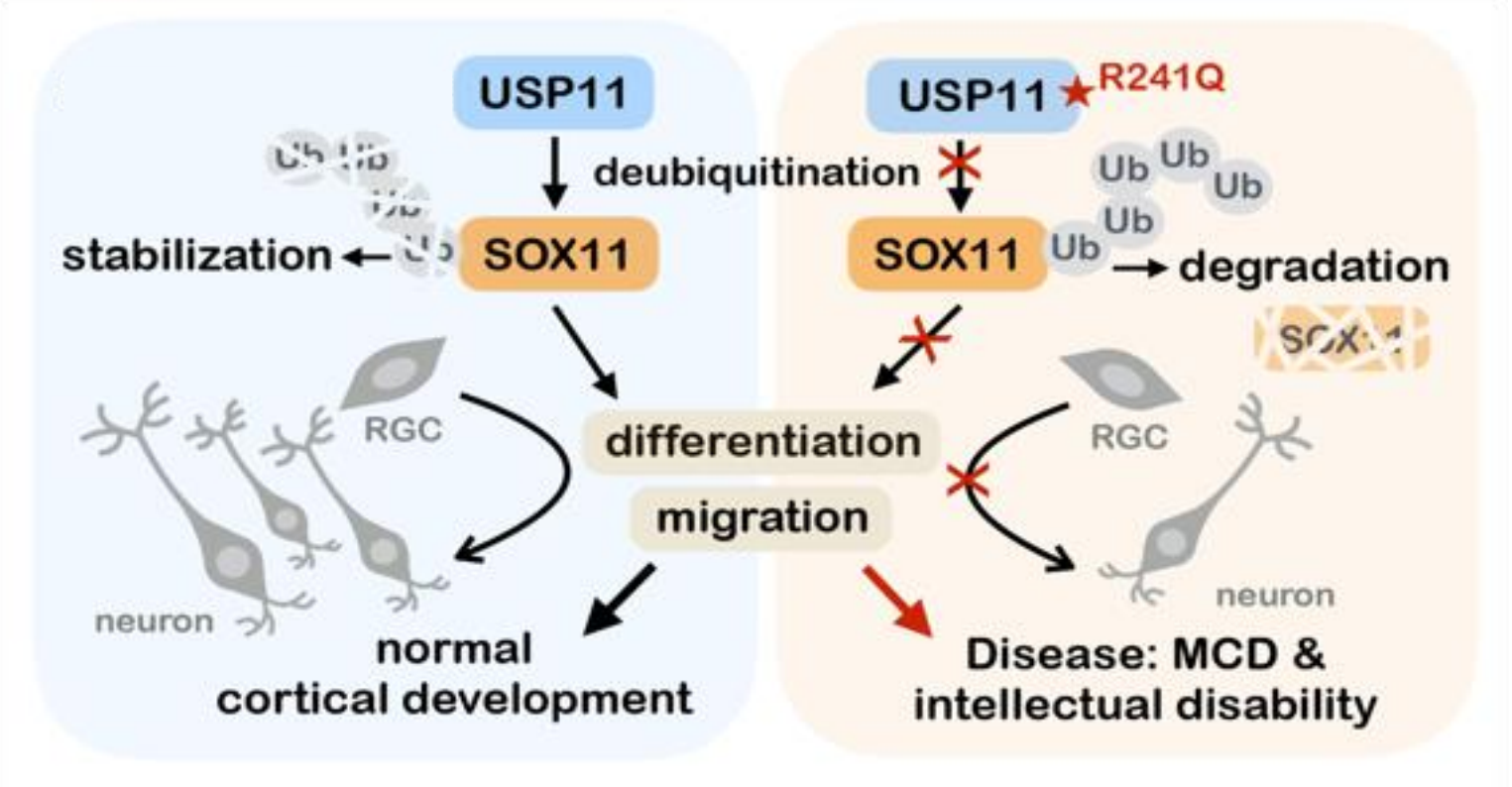
In vivo deUb assay



stablization



Conclusion



Highlight:

the importance of integration of protein stabilization mechanism into transcriptional program for a developmental process.

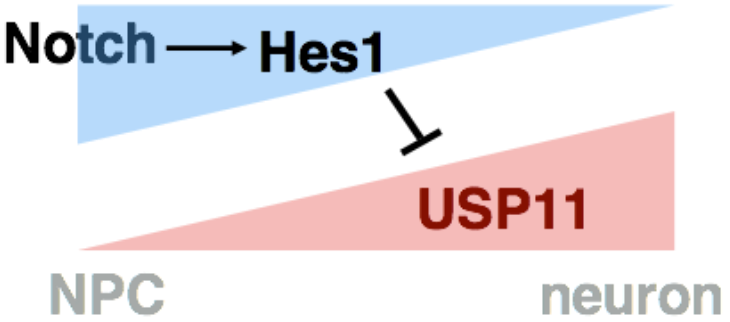
Science Advances (revision submitted)

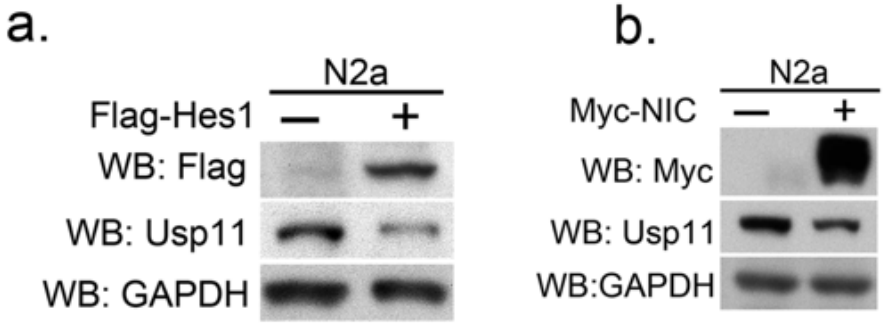
ScienceAdvances

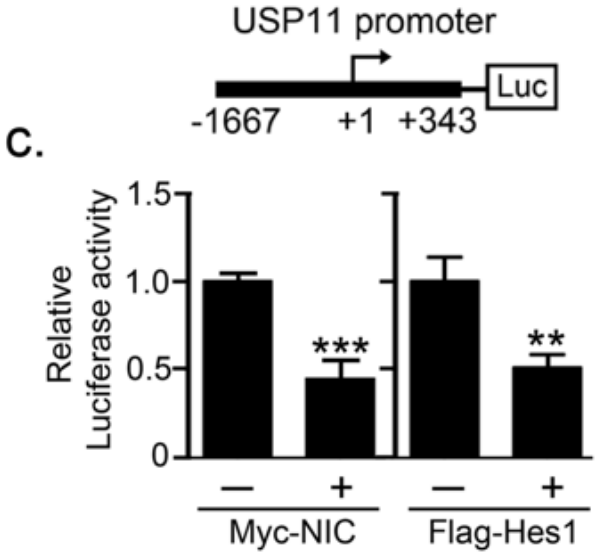
How the expression of Usp11 is regulated during cortical development?

Notch signaling plays an important role in the maintenance of neural progenitors.
(Artavanis-Tsakonas et al., 1999; Gaiano and Fishell, 2002; Honjo, 1996; Selkoe and Kopan, 2003)

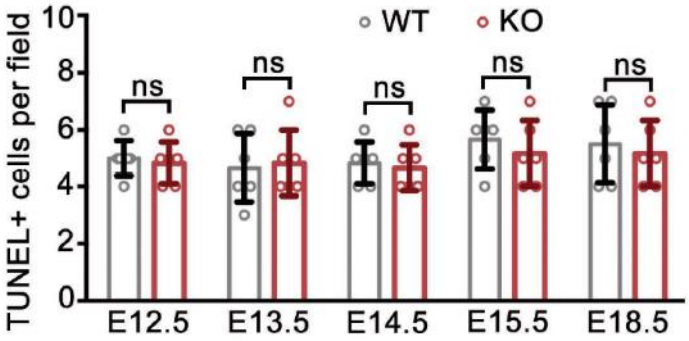
NICD activates expression of the basic helix-loop-helix transcriptional repressors Hes1 and Hes5, down-regulates proneural gene expression, and inhibits neuronal differentiation.
(Bertrand et al., 2002; Ross et al., 2003; Kageyama et al., 2007).





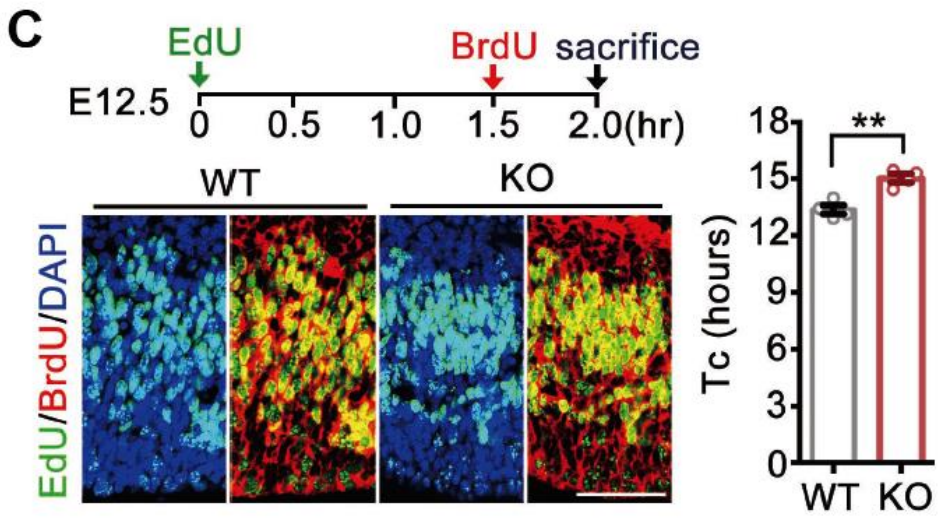


Besides proliferation and differentiation, whether does Usp11 KO affect the cell death?



We have analyzed the cell death by TUNEL assay during cortical neurogenesis, and then, the data suggest that Usp11 knockout doesn't affect the cell death.

What the details mechanism of usp11 mediated proliferation?

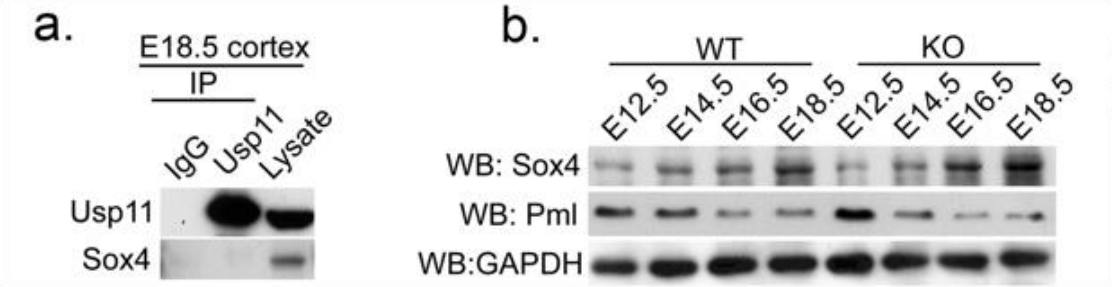


In the detail, we found usp11 KO increase the length of cell cycle in E12.5 NPC by double labeling the BrdU and EdU.

Therefore, increasing the cell cycle length results in increased cells staying in NPC. Conversely, fewer cells leave the cell cycle, so fewer cells differentiate into neurons.

Discussion: other possible substrates of Usp11 in brain development

Whether Usp11 regulates other substrates in the cortical neurogenesis ?



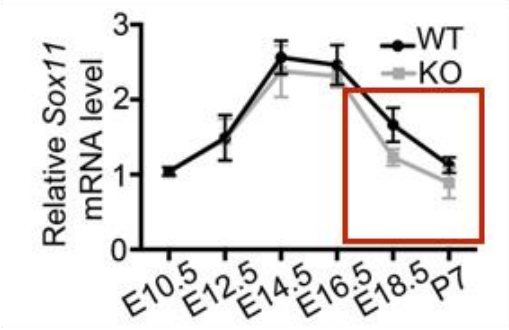
nature neuroscience

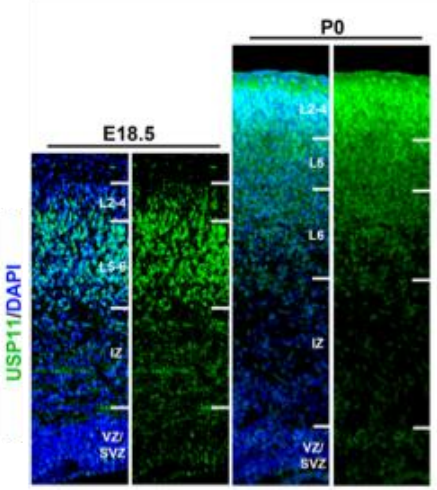
The tumor suppressor Pml regulates cell fate in the developing neocortex

Tarik Regad[1], Cristian Bellodi[1,2], Pierluigi Nicotera[1] & Paolo Salomoni[1]

Pml deficiency leads to an increase in NPC cycling and an impairment of the transition from RGCs to IPCs, thereby resulting in reduced neurogenesis and cortex thickness

Sox4 and Pml are not the substrates of Usp11 in the cortex.





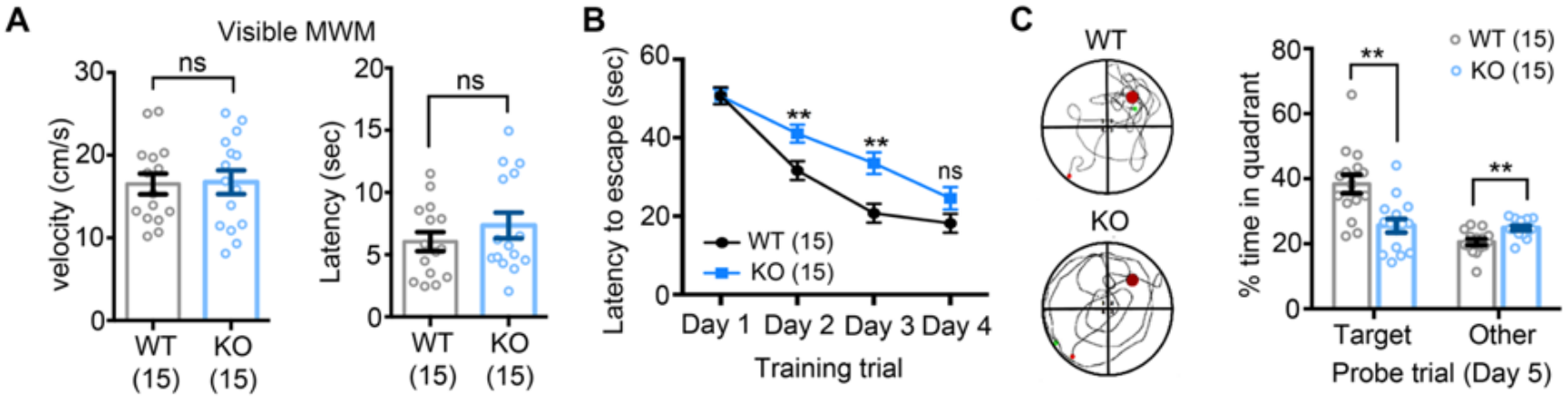
Usp11 still highly expressed at late stage

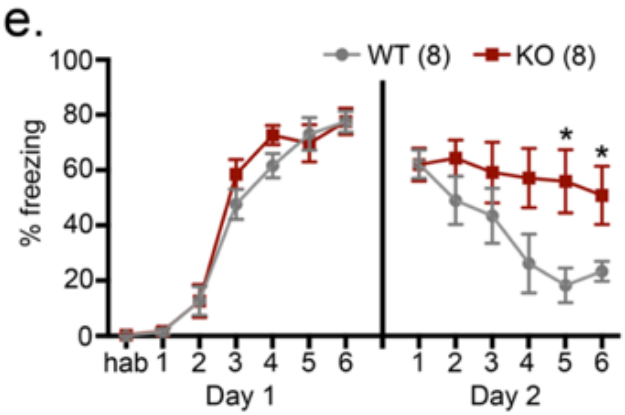
—> find other substrates by Mass spect from cortex sample

Usp11 KO mice exhibits behavior abnormalities about learning/memory.

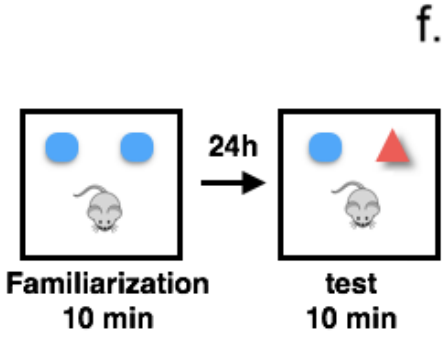
To investigate the cognitive function

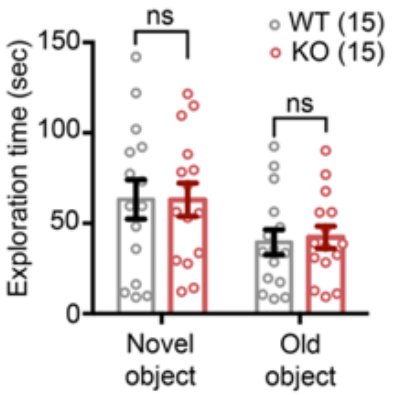
Morris water maze (MWM)





contextual fear conditioning (CFC)



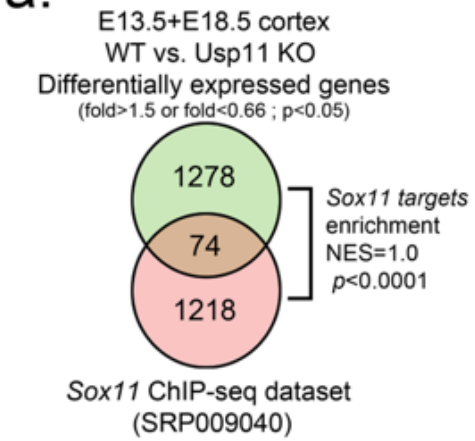


novel object recognition (NOR)

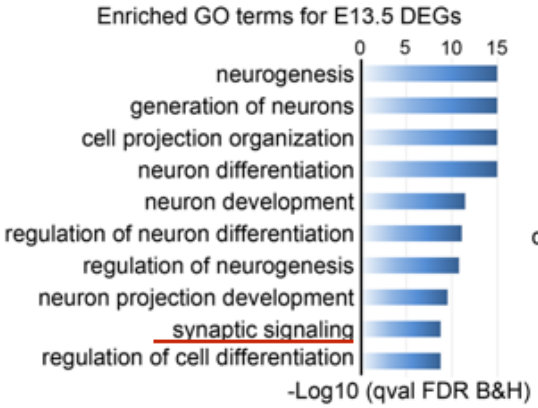
Discussion : other functions of Usp11/Sox11 in brain development

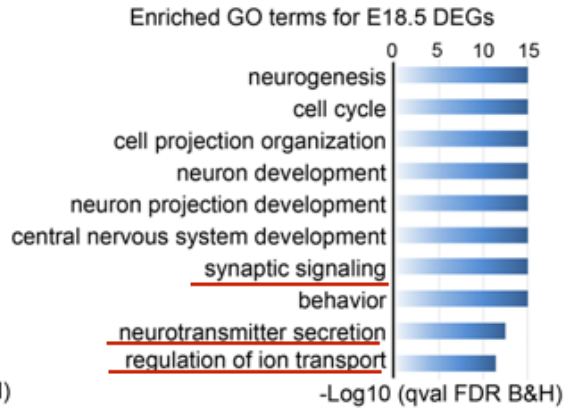
To investigate whether Usp11 regulate the synaptic transmission/signaling

a.

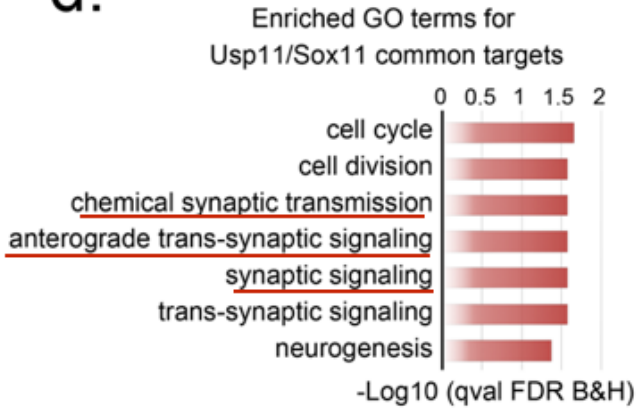


b.





d.



To analyze whether Usp11 KO affects the synaptic functions or structure?

Discussion: Whether Usp11 is involved in other developmental diseases?

Neuron

Genes that Affect Brain Structure and Function Identified by Rare Variant Analyses of Mendelian Neurologic Disease

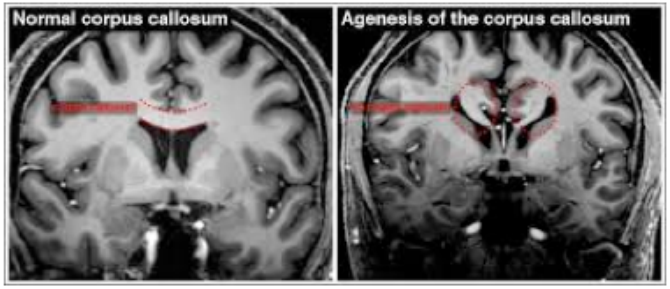
Karaca et al., 2015, Neuron *88*, 499–513

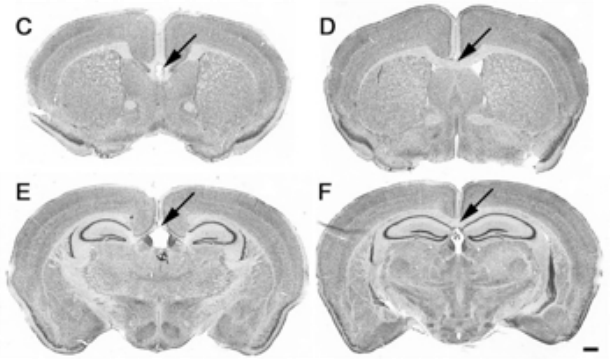
USP11 R241Q mutation was identified in rare familiar neurologic disorder

Phenotypes: **(1) intellectual disability**

(2) corpus callosum genesis 胼胝體發育不全症

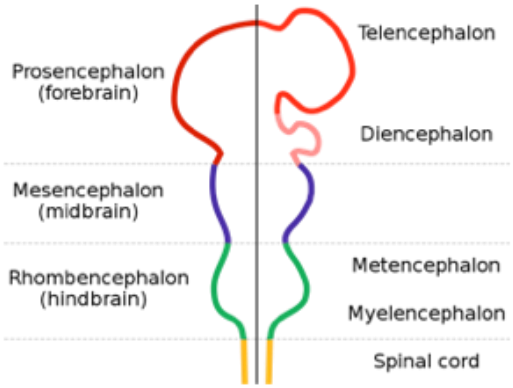
- metabolic factors
- Chromosome abnormality
- Genetic abnormalities
- ex: NF1 (Neurofibromatosis1)





(3)Syntelencephaly 端腦融合畸形
also known as a mild subtype of holoprosencephaly

-Environmental factors
-chromosomal abnormalities
-genetic abnormalities
(ex: TGIF, SHH, SIX3, ZIC2)



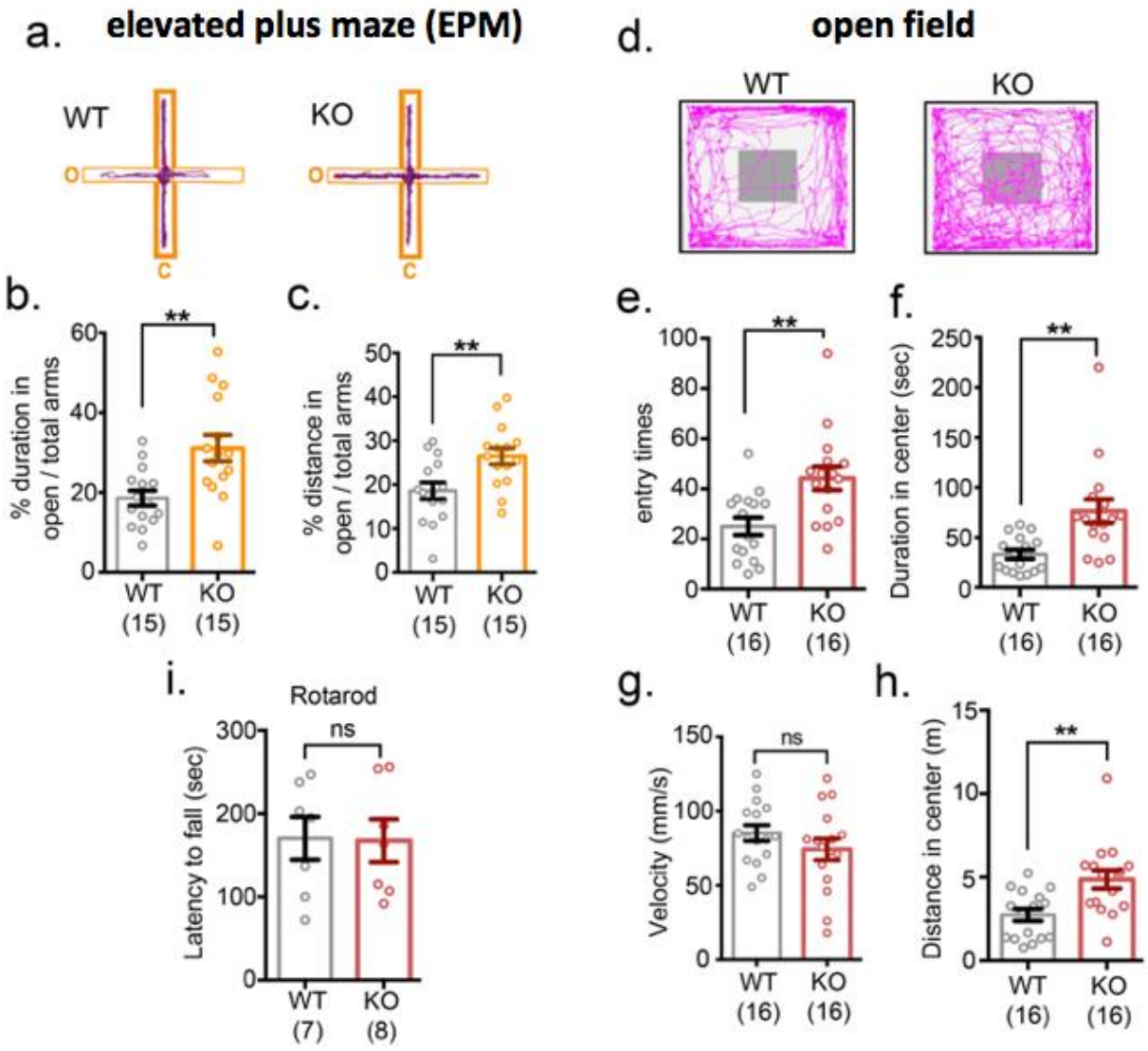
We doesn't observe corpus callosum genesis and syntelencephaly in the Usp11 KO mice

(1) Whether the expression pattern of Usp11 is different between mouse and human?

(2) Whether there are additional substrates of Usp11 in human?

Usp11 KO mice exhibits behavior abnormalities about anxiety and motor ability.

to assess anxiety-related behaviors



Introduction: the role of Sox11 in the cortical neurogenesis.

Sox11 is a Sox-family transcription factor

Transcription Factor Sox11 is Essential for both Embryonic and Adult Neurogenesis

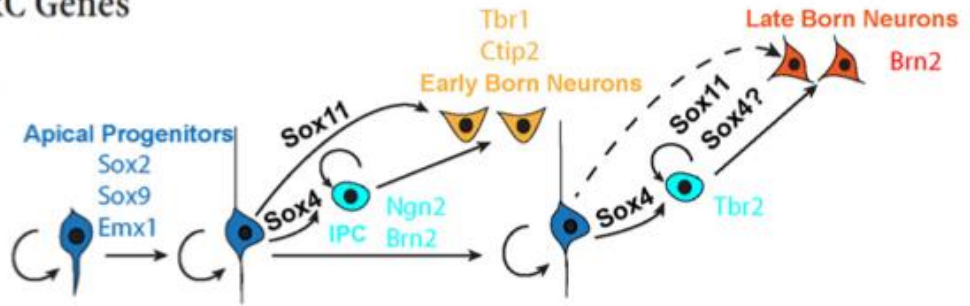
Developmental Dynamics
2 March 2013

- Analysis of Sox11 conventional KO mice found a reduction in cortex thickness at P0.
- This finding suggests the function of Sox11 in cortical neurogenesis.

Orchestration of Neuronal Differentiation and Progenitor Pool Expansion in the Developing Cortex by SoxC Genes

The Journal of Neuroscience, July 22, 2015

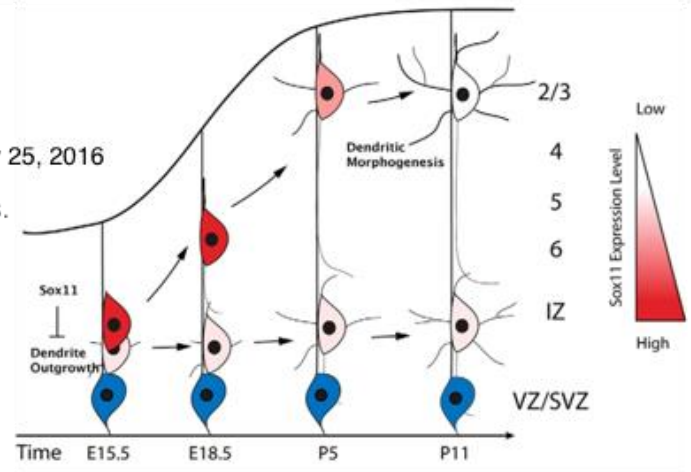
- Sox11 and its most closest family member Sox4 have nonoverlapping functions in early neurogenesis.
- Sox11 specifically promotes RGC differentiation into early born neuron without affecting IPC.
- However, Sox4 is important for IPC specification and maintenance.

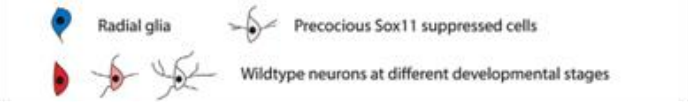


Sox11 Balances Dendritic Morphogenesis with Neuronal Migration in the Developing Cerebral Cortex

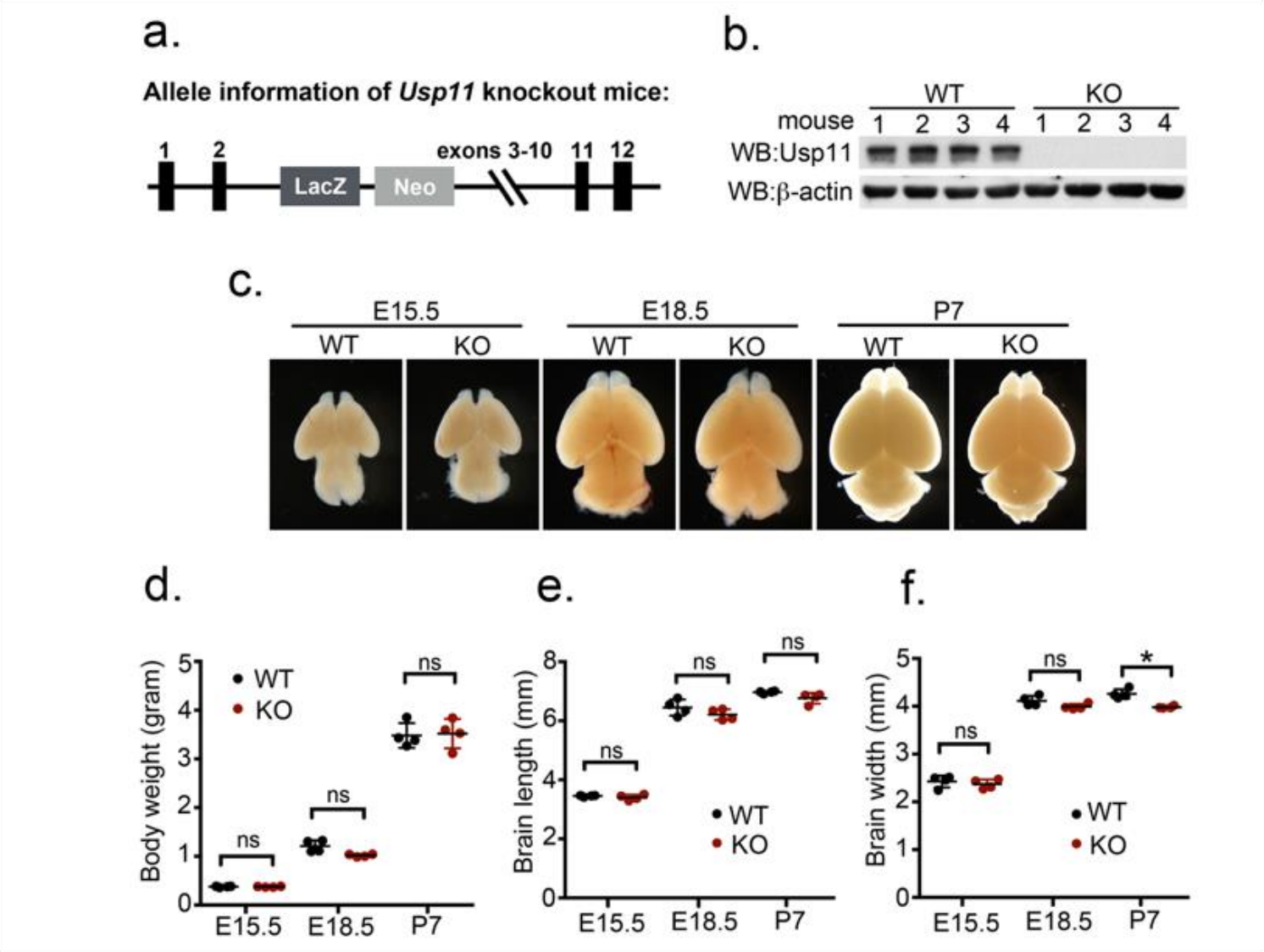
The Journal of Neuroscience, May 25, 2016

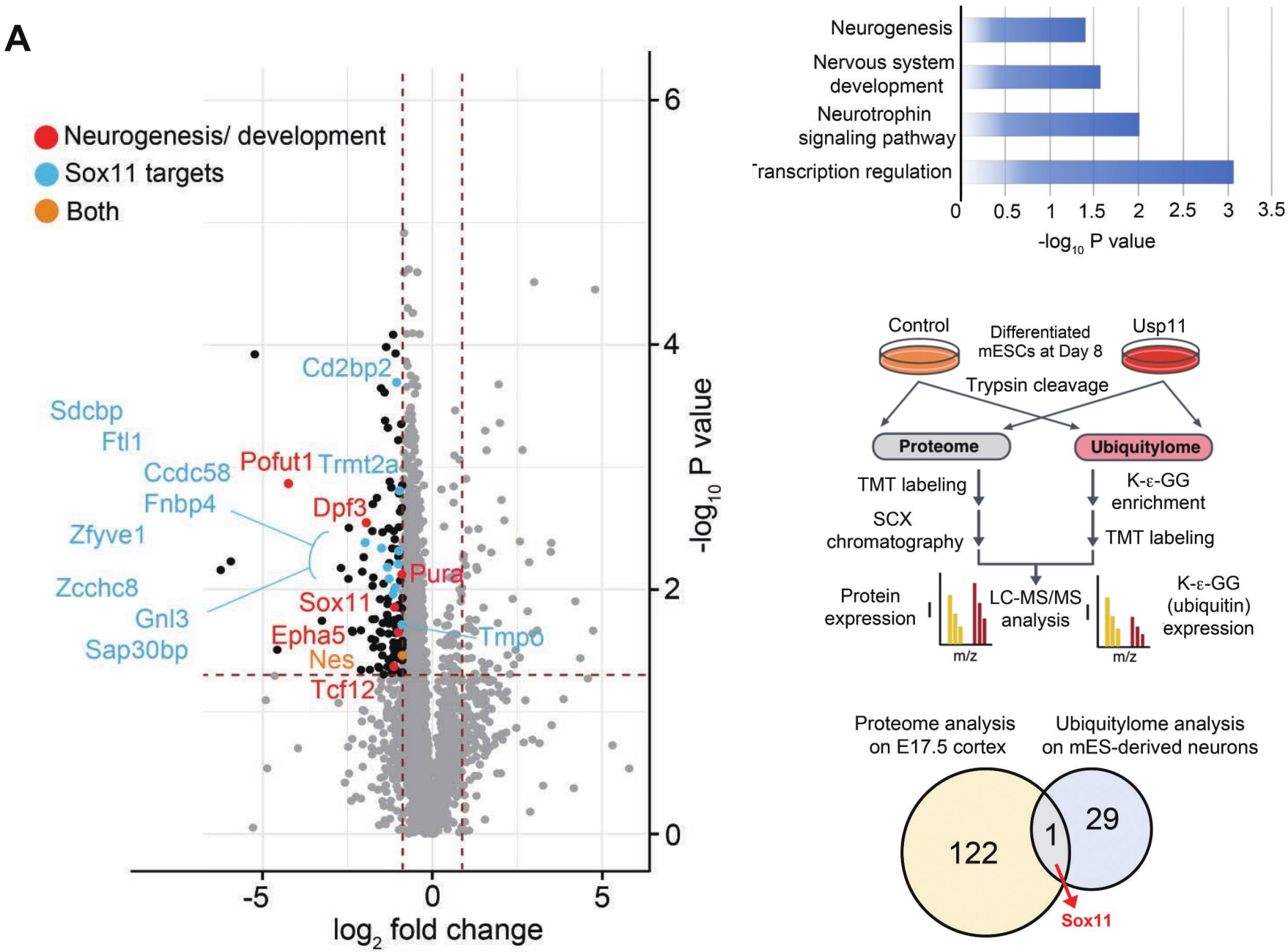
- They found that Sox11 does not affect the generate of late born neurons.
- Knockdown of Sox11 caused precocious branching of neurites and a neuronal migration defect on the late neurogenesis.





Characterization of the brain phenotype in Usp11 knockout mice.




A
Neurogenesis/ development
Sox11 targets
Both
Cd2bp2
Sdcbp
Ftl1
Ccdc58
Pofut1
Trmt2a
Fnbp4
Dpf3
Zfyve1
Pura
Zcchc8
Sox11
Gnl3
Epha5
Tmpo
Sap30bp
Nes
Tcf12
-log10 P value
log2 fold change
Neurogenesis
Nervous system development
Neurotrophin signaling pathway
Transcription regulation
-log10 P value
Control
Differentiated mESCs at Day 8
Usp11
Trypsin cleavage
Proteome
Ubiquitylome
TMT labeling
SCX chromatography
K-ε-GG enrichment
TMT labeling
Protein expression
LC-MS/MS analysis
K-ε-GG (ubiquitin) expression
m/z
m/z
Proteome analysis on E17.5 cortex
Ubiquitylome analysis on mES-derived neurons
122
1
29
Sox11