Embryonic neural stem cells:

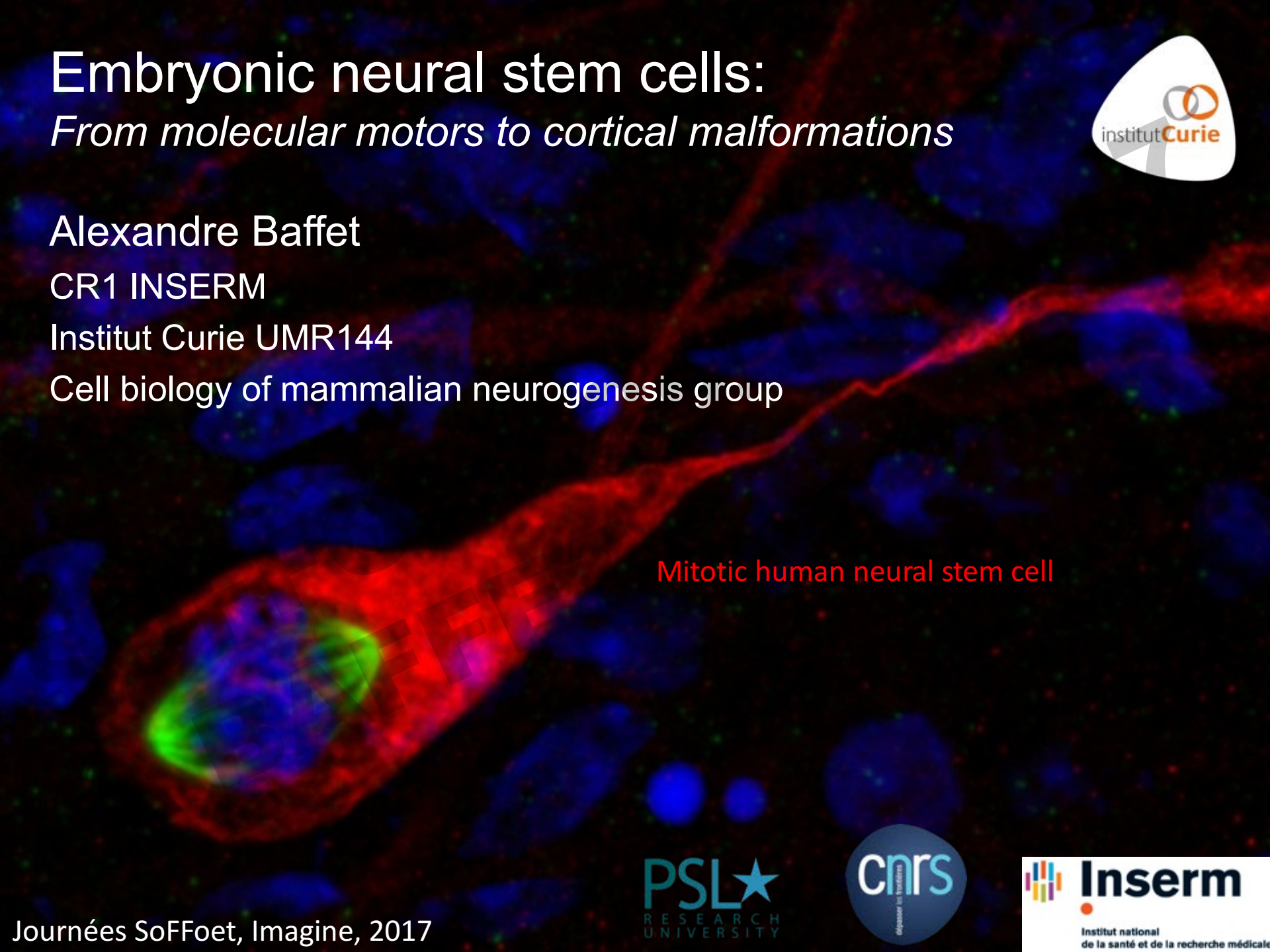
From molecular motors to cortical malformations

Alexandre Baffet

CR1 INSERM

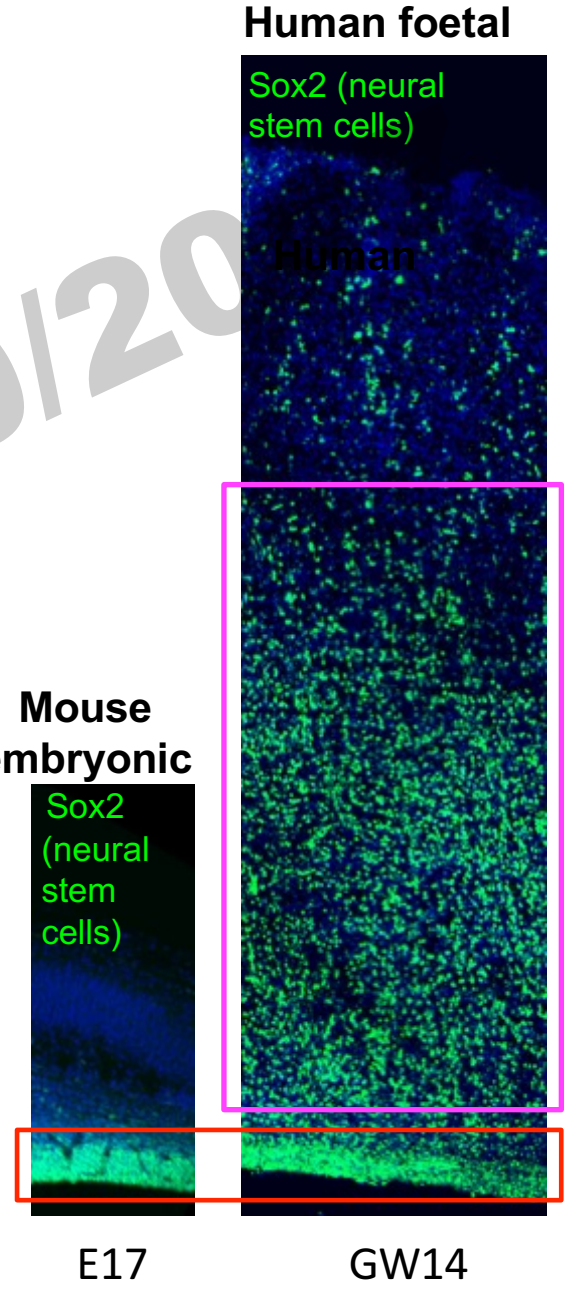
Institut Curie UMR144

Cell biology of mammalian neurogenesis group



Mitotic human neural stem cell

Human brain size expansion



Adult

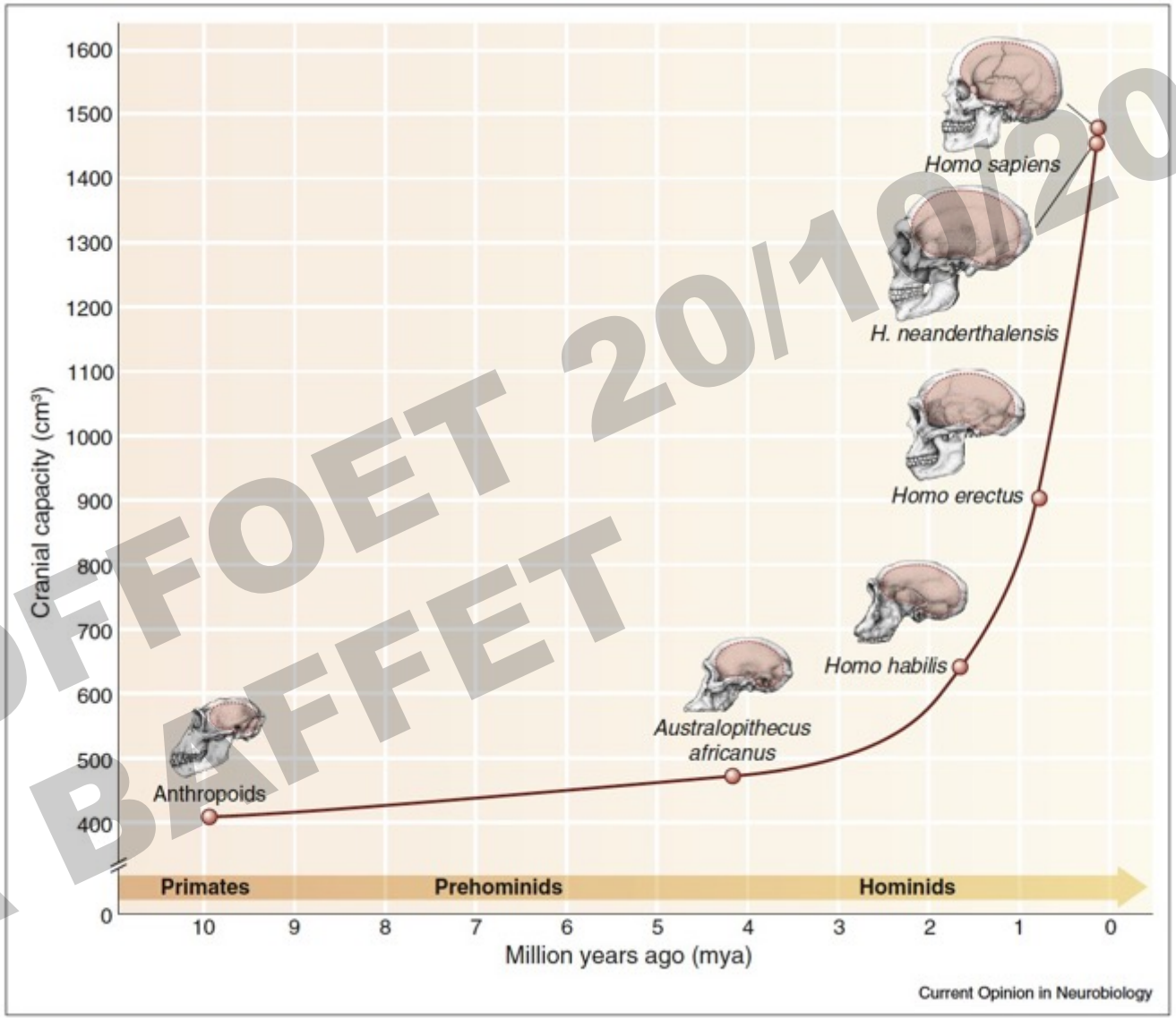
Increased amount of neurons

→

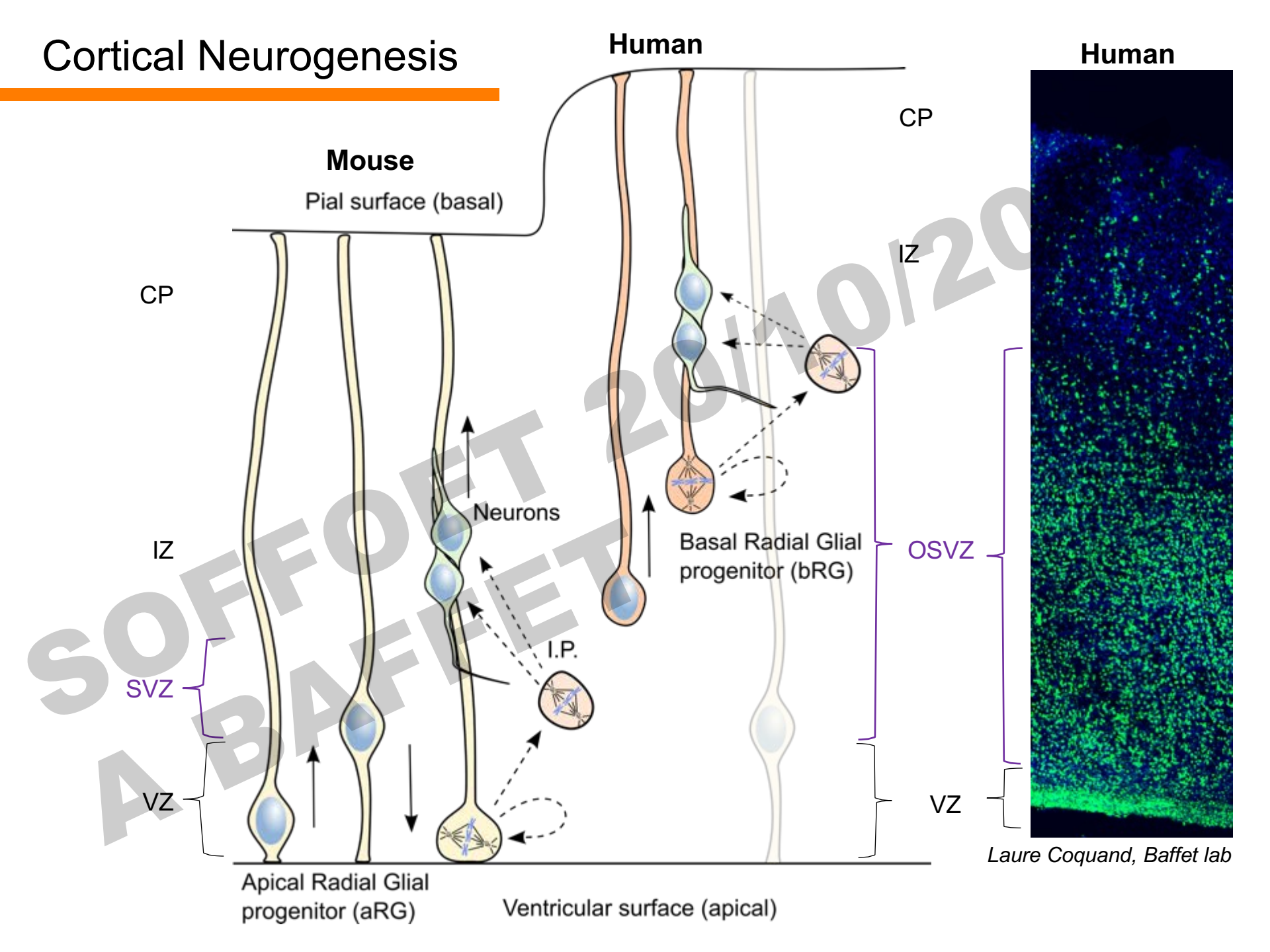
Embryo

Increased pool of neural stem cells

Brain expansion during human hominid evolution



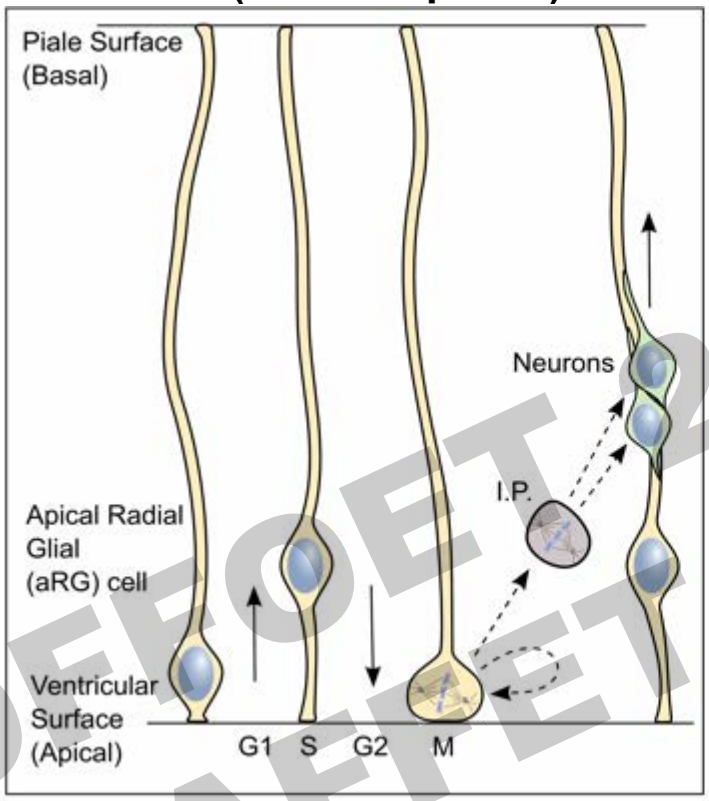
Cortical Neurogenesis

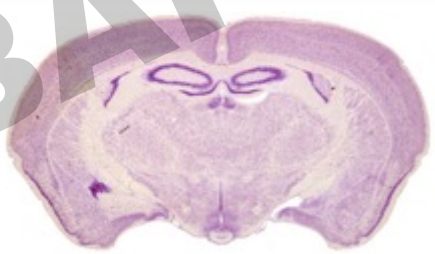


Laure Coquand, Baffet lab

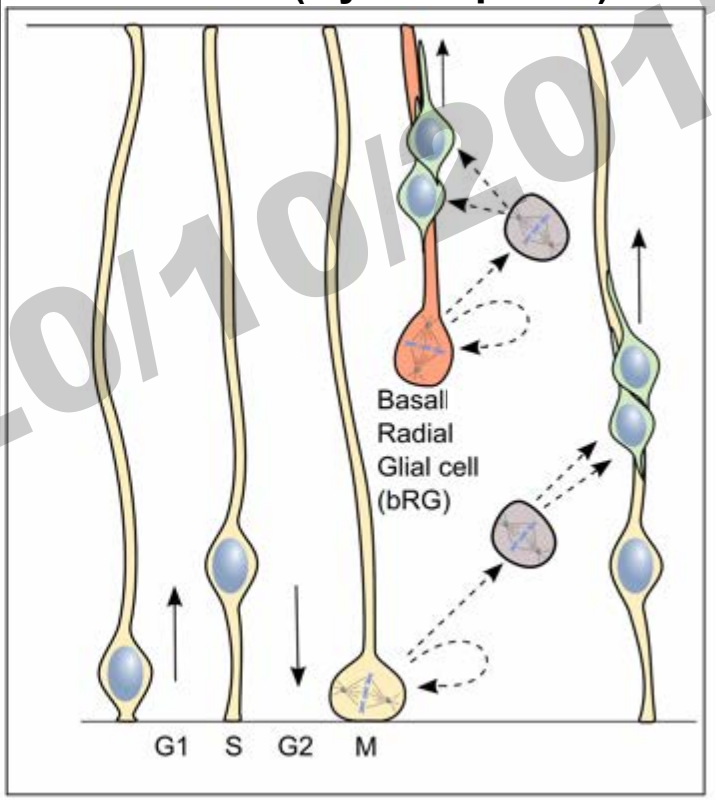
Brain folding

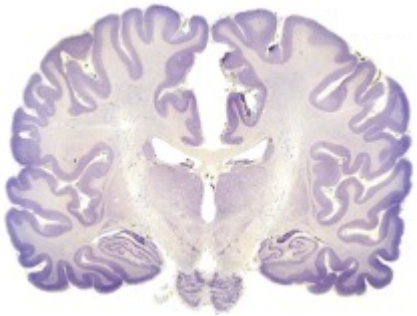
Mouse (Lissencephalic)



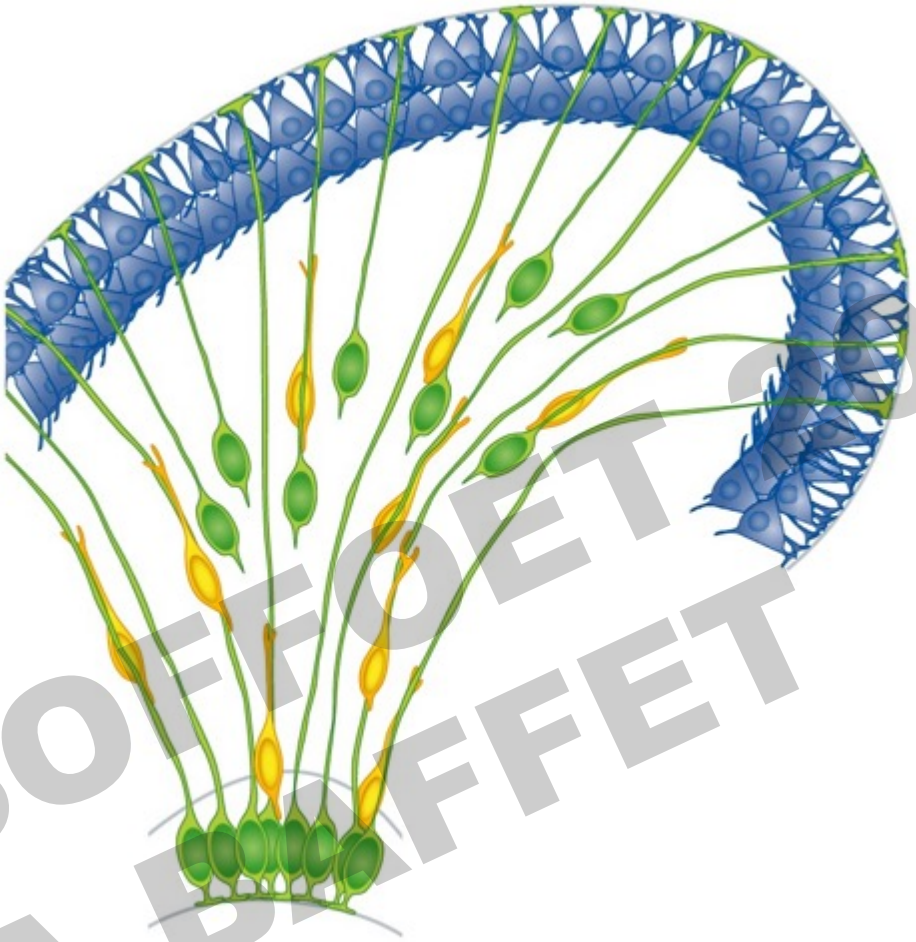


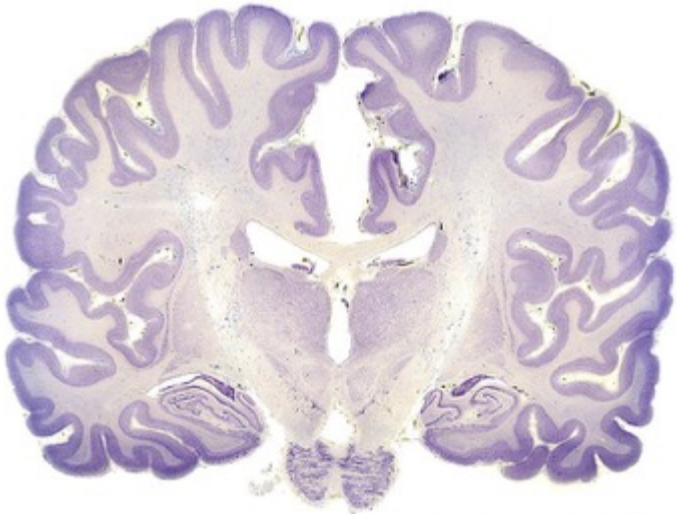
Human (Gyrencepahlic)





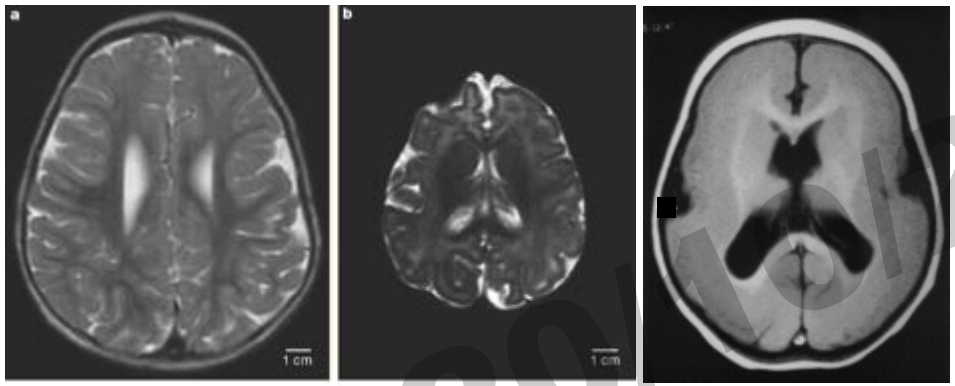
Brain folding





bRG cells and brain folding are not specific to humans

Neurogenesis defects can cause cortical malformations



Normal brain Microcephaly Lissencephaly

1/ Environmental causes

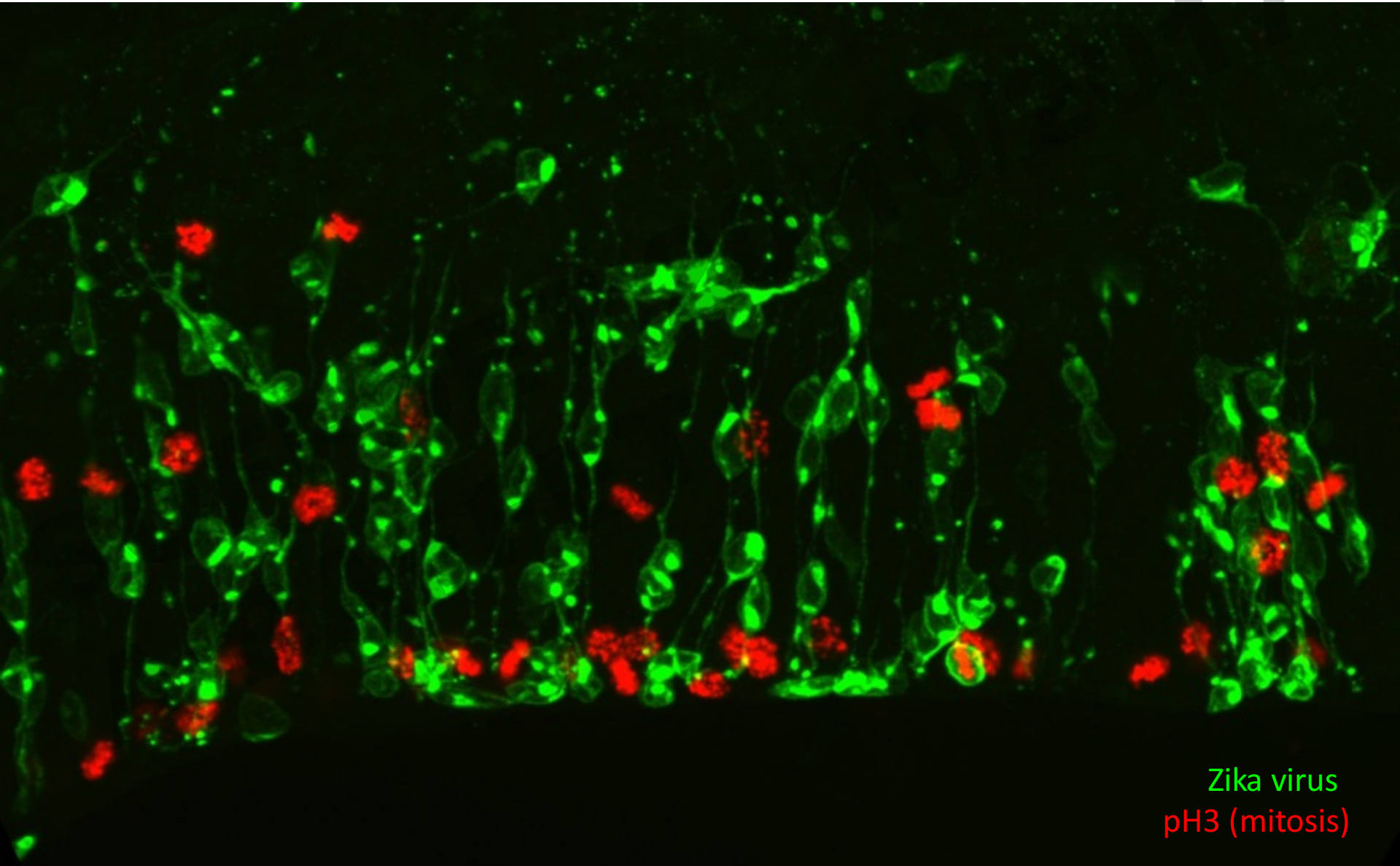
2/ Genetic causes

Microcephaly:
DYNC1H1 (dynein), ASPM, NDE1…

Lissencephaly:
LIS1, DCX, TUBB3…

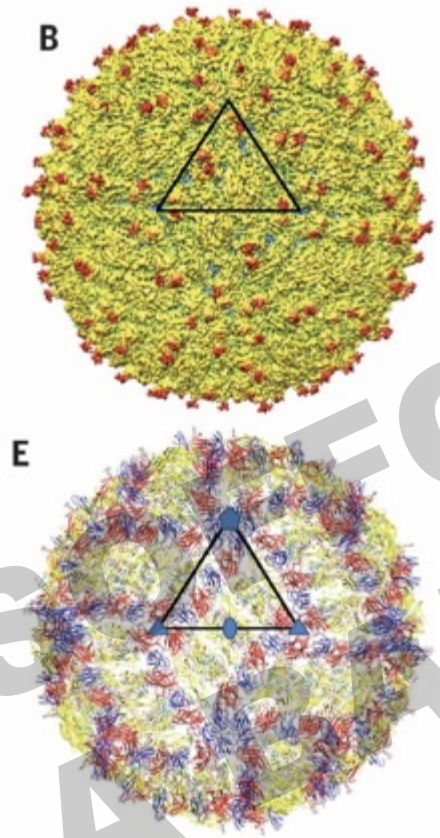
Microtubules and molecular motors

Neural stem cell infection by Zika virus in the mouse developing neocortex



Zika virus belongs to the flavivirus genus

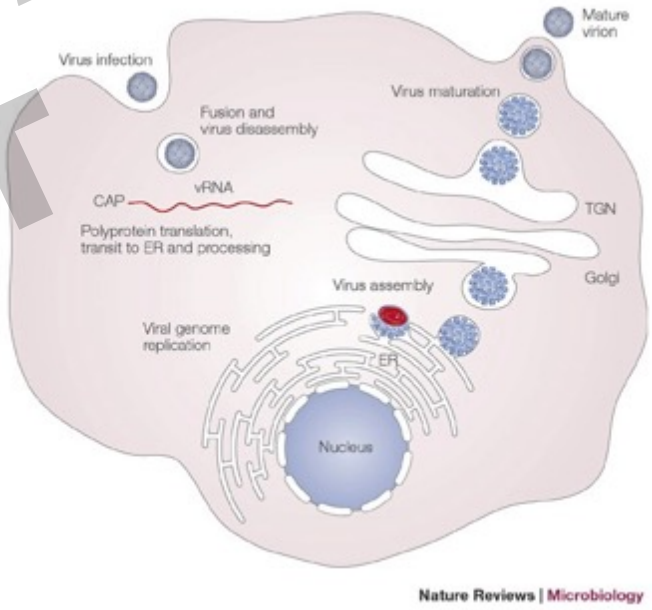
Zika virus



Flavivirus Genus (Yellow fever virus, West Nile virus, Dengue virus)

- Athropod-born (*Aedes* moquito)

- Single stranded positive-sens RNA viruses

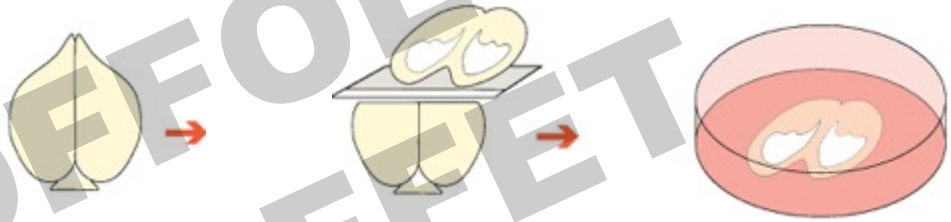


An assay to study Zika virus infection in the developing neocortex

E15 embryonic mouse brain

300 um-thick coronal brain slices

Live brain slices culture



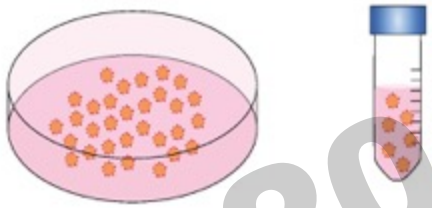
Collaboration with Nathalie Pardigon, Institut Pasteur

Brault, …, Baffet, *EBioMed,* 2016

An assay to study Zika virus infection in the developing neocortex

Virus amplification on *Aedes albopictus* C6/36 cells

Cécile Khou

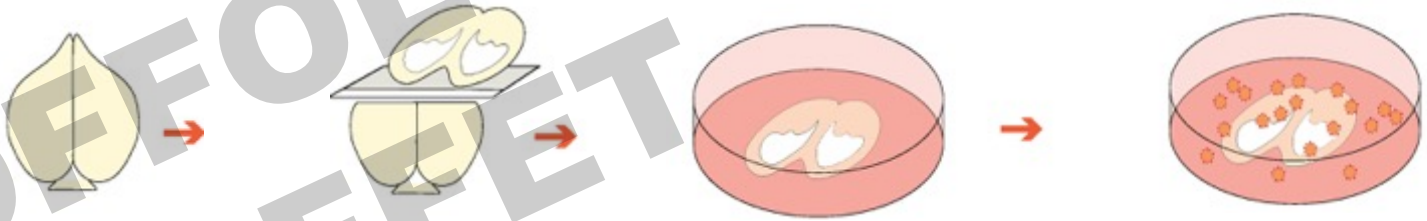


E15 embryonic mouse brain

300 um-thick coronal brain slices

Live brain slices culture

24-48 hours infection



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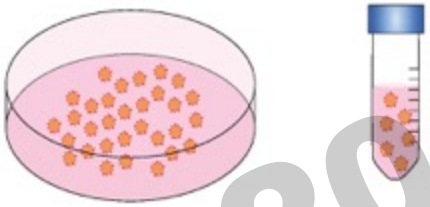


E15 embryonic mouse brain

300 um-thick coronal brain slices

Live brain slices culture

24-48 hours infection

Fixation

Immunostaining

Imaging with Spinning Wide (Yokogawa CSU-W1) microscope

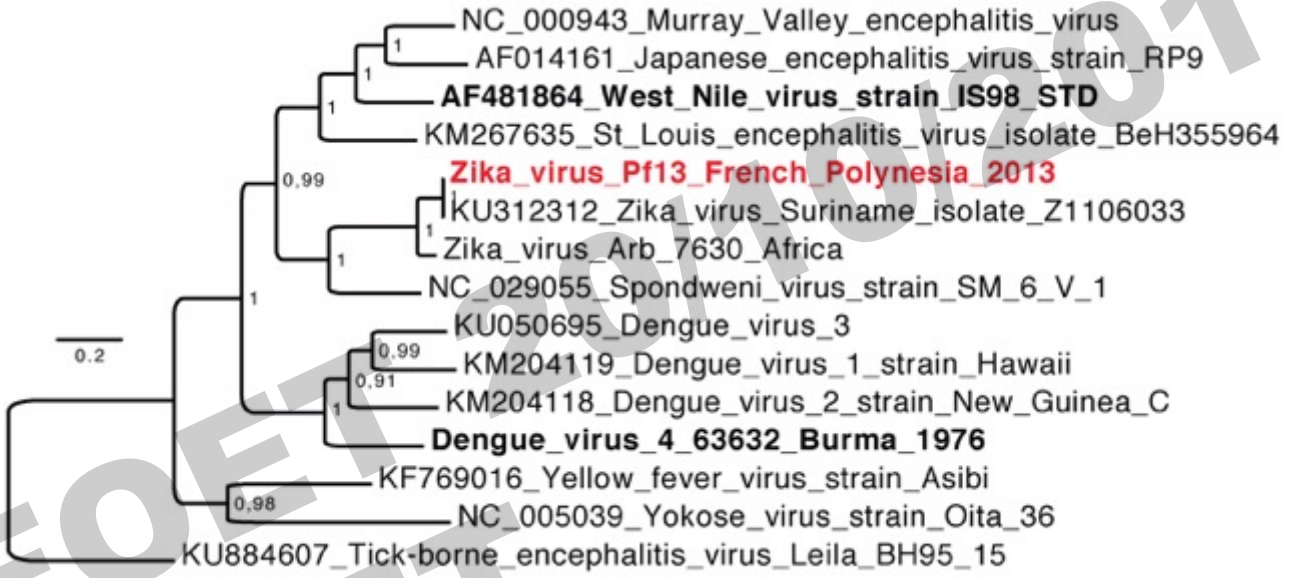
Data analysis

Collaboration with Nathalie Pardigon, Institut Pasteur

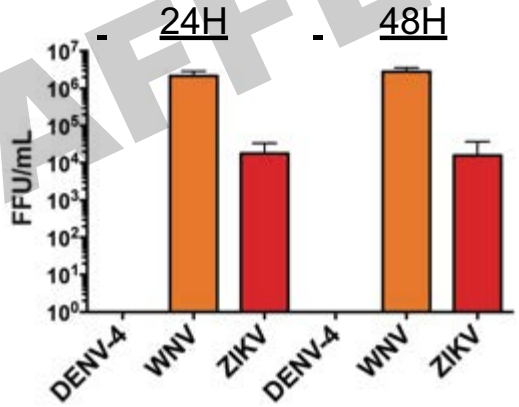
Brault, …, Baffet, *EBioMed,* 2016

An assay to investigate Zika virus effect during neocortex development

Flavivirus genus

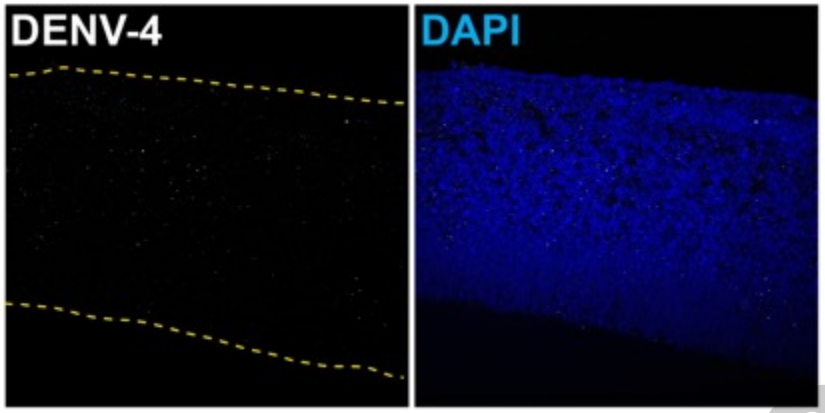


Virus titer



Brault, …, Baffet, *EBioMed,* 2016

An assay to investigate Zika virus effect during neocortex development



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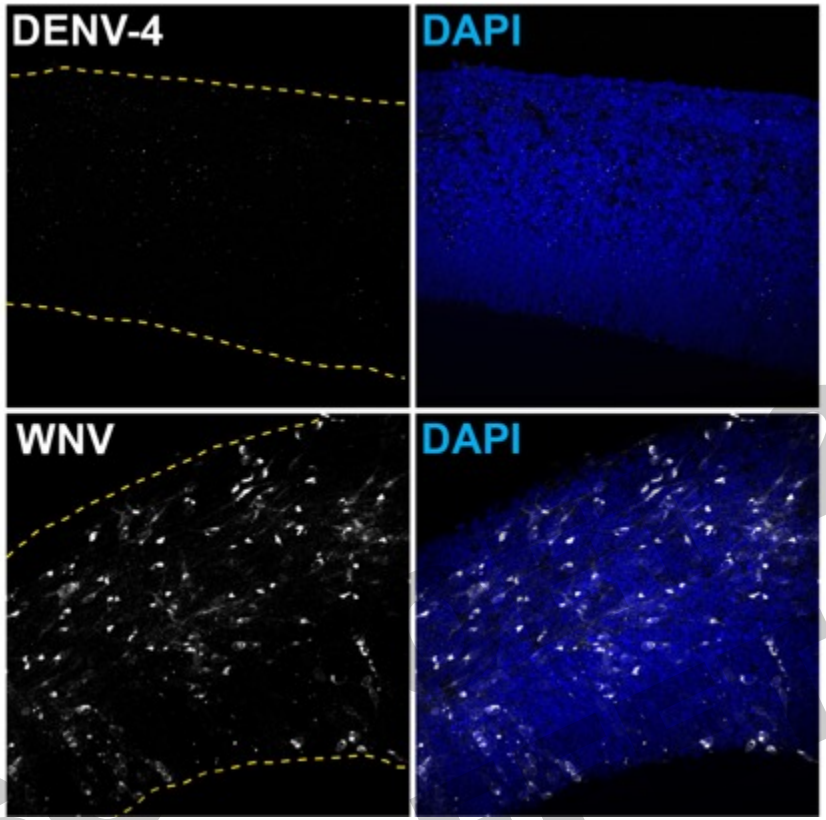
An assay to investigate Zika virus effect during neocortex development



Brault, …, Baffet, *EBioMed,* 2016

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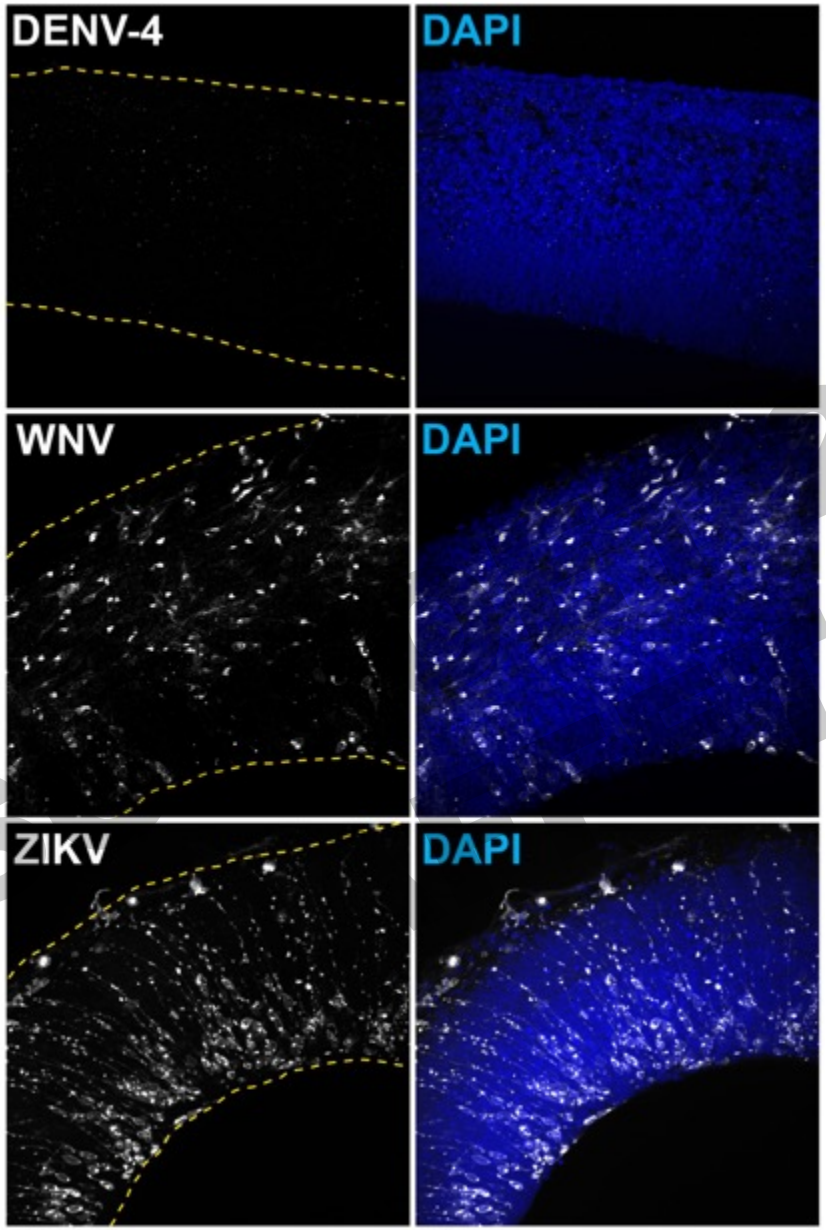
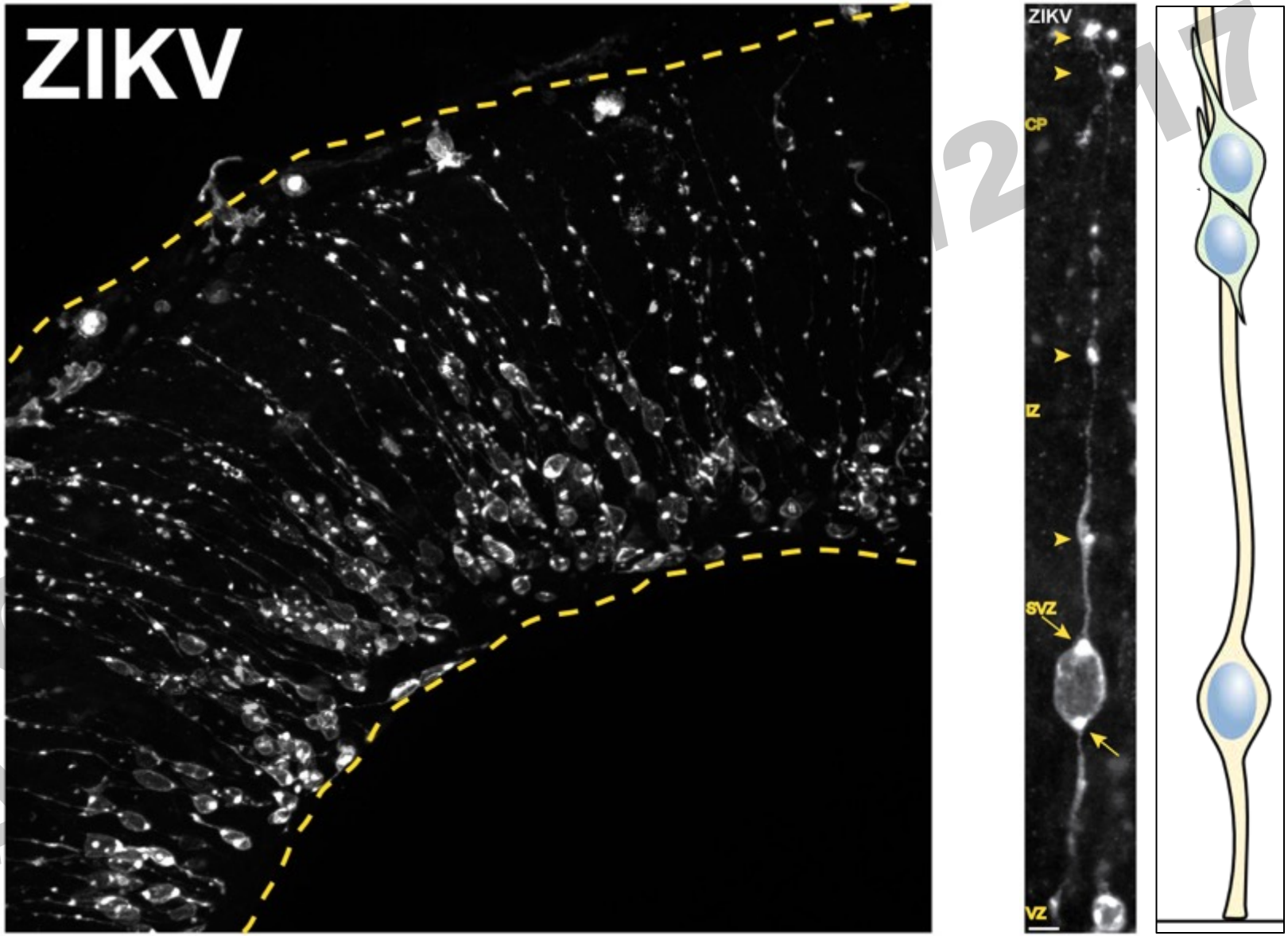


Brault, …, Baffet, *EBioMed,* 2016

Zika virus preferentially infect neural stem cells



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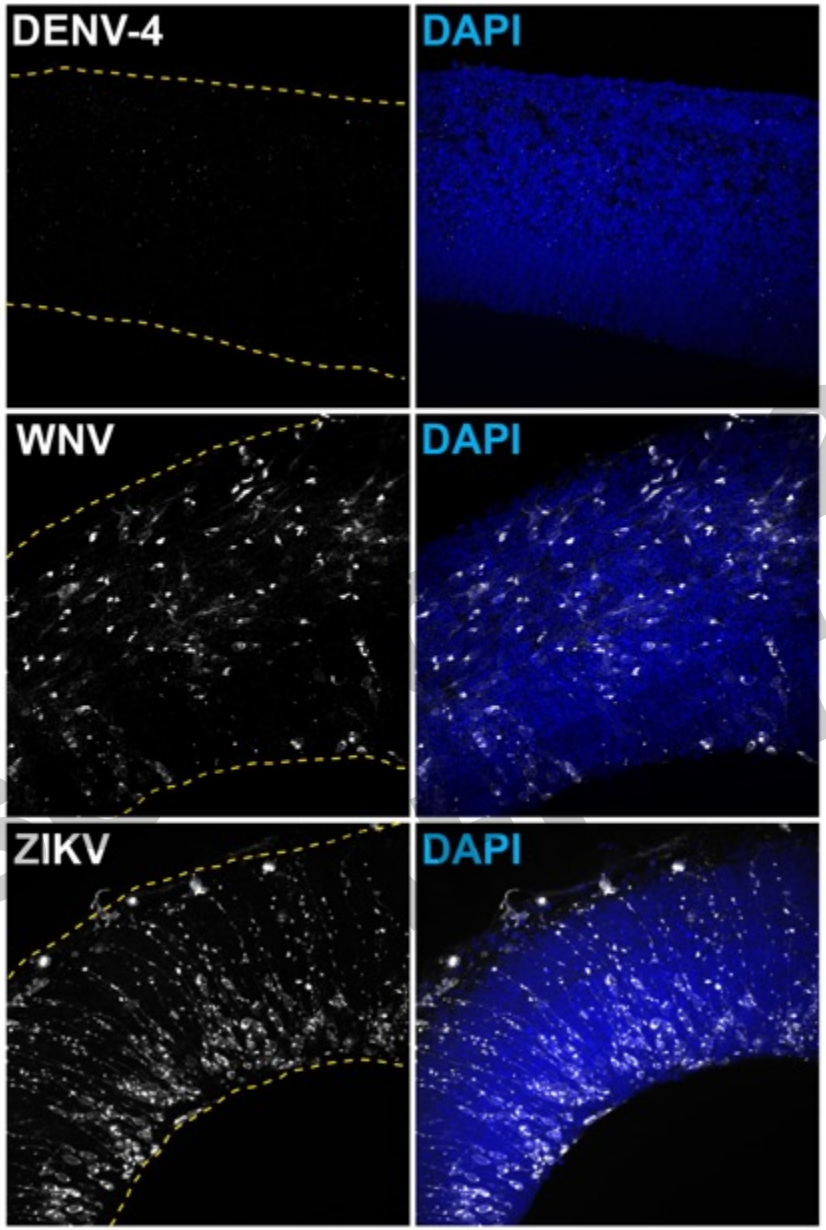

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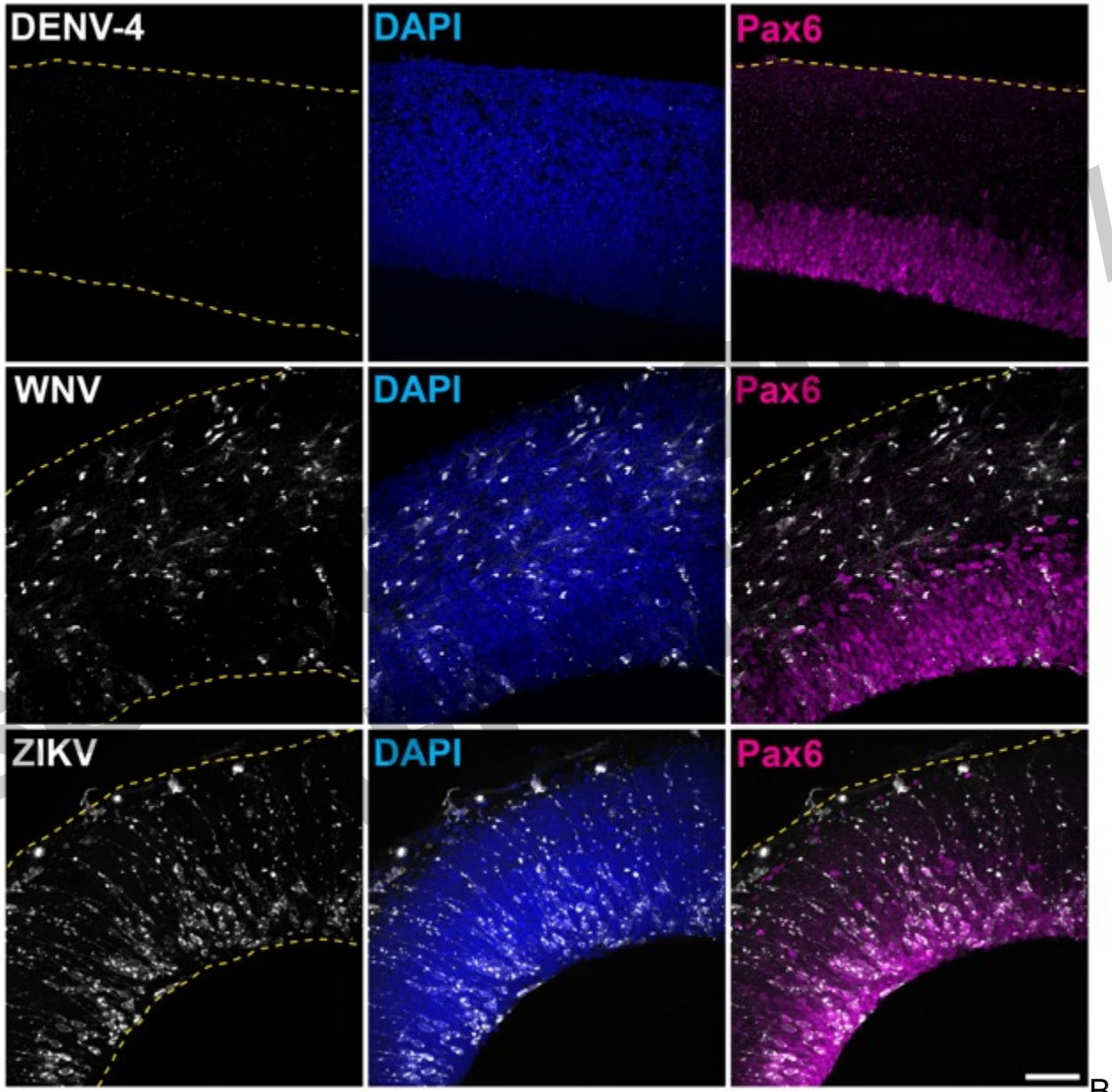
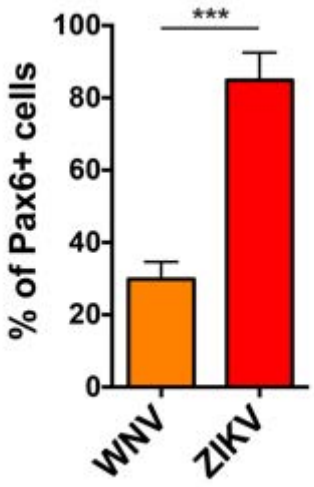
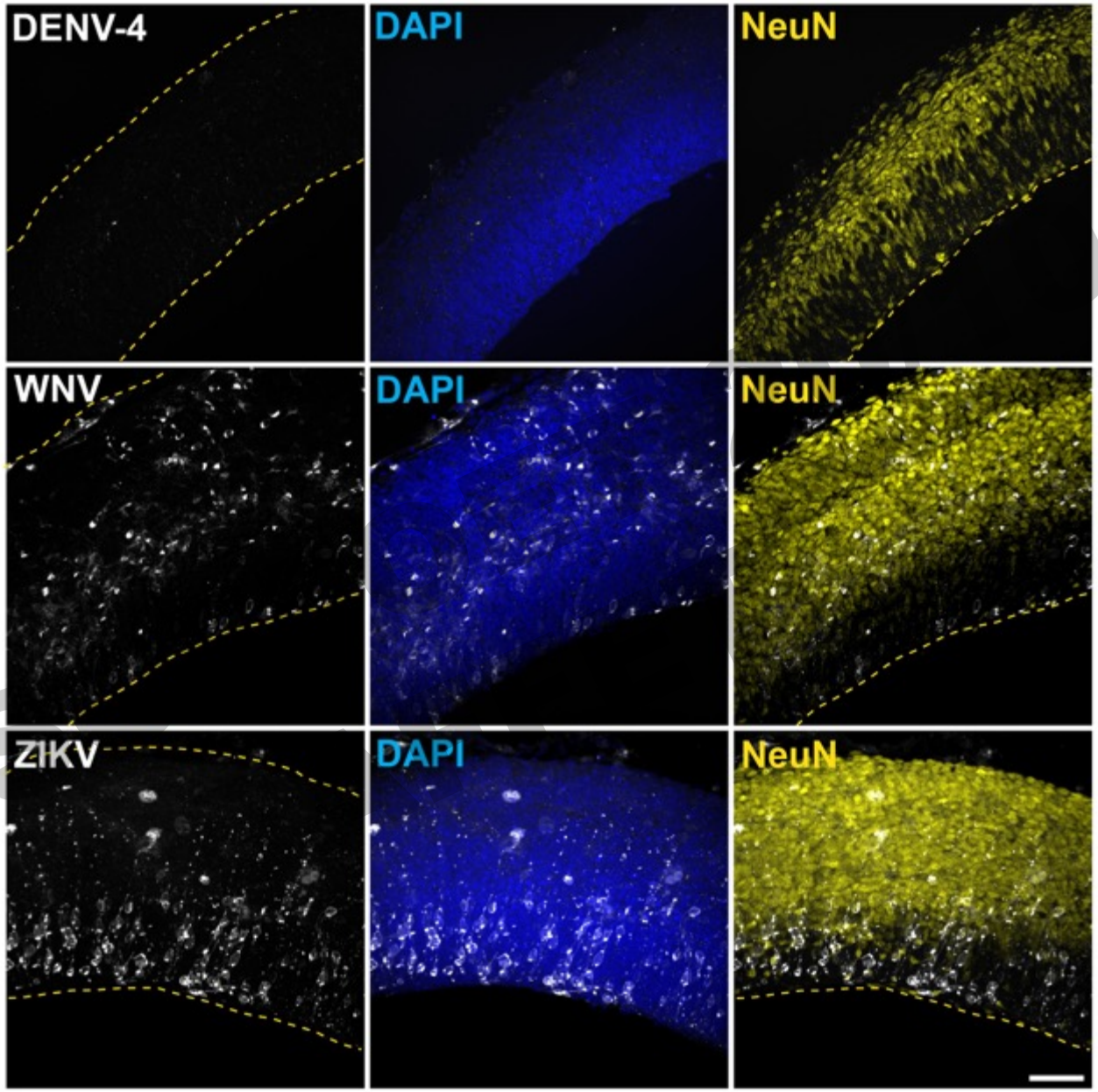
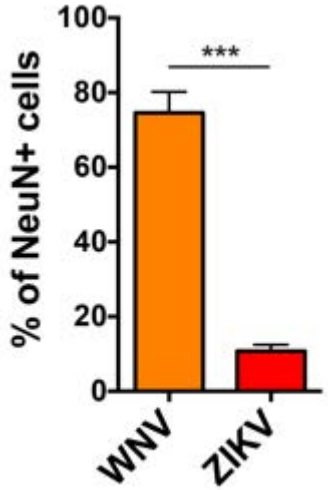




Brault, …, Baffet, *EBioMed,* 2016

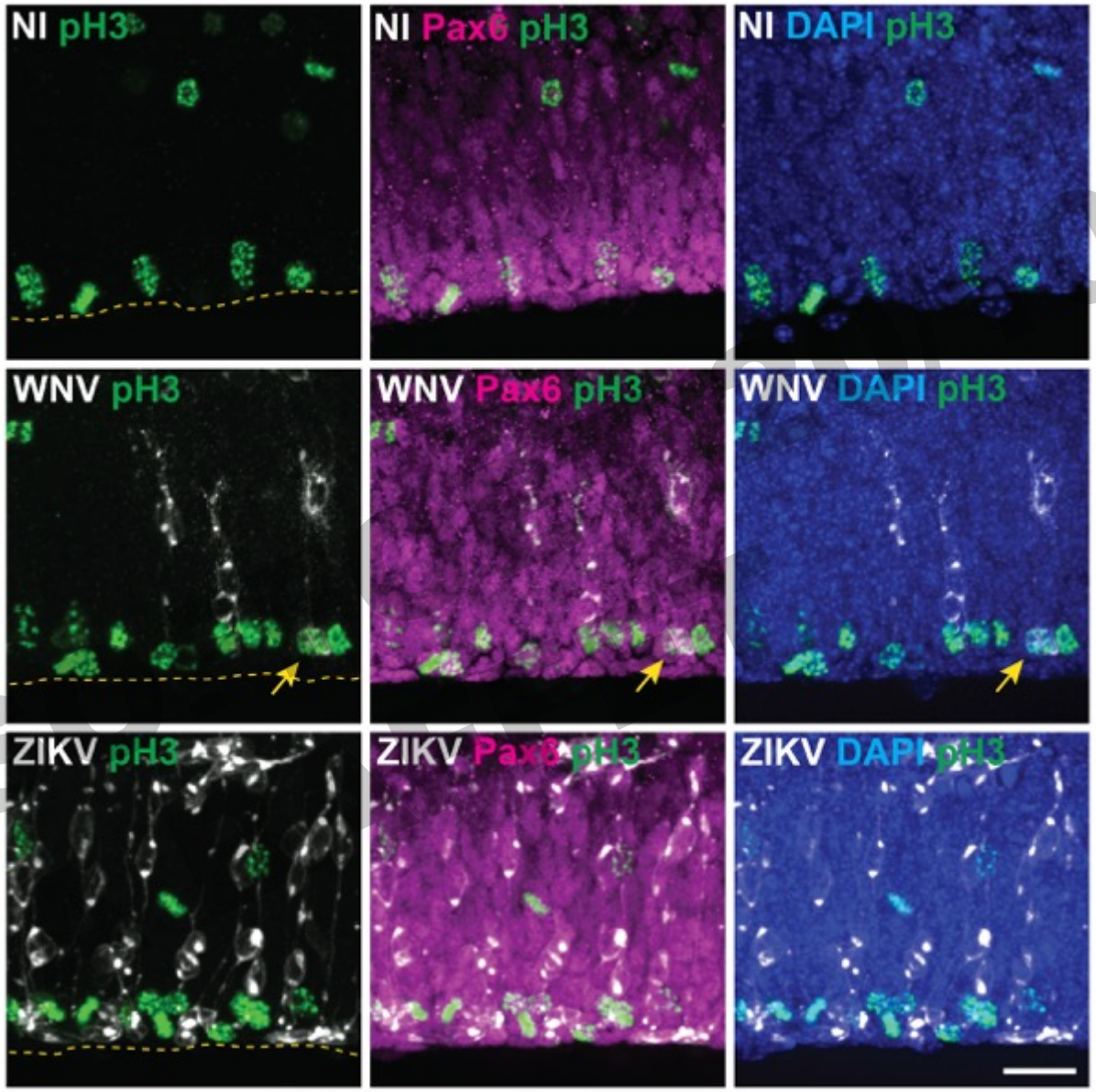
Zika virus poorly infects neurons

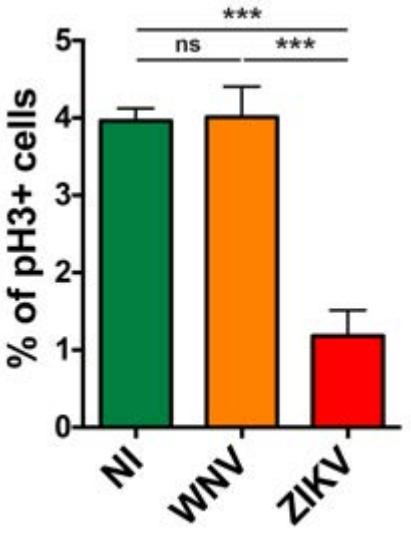




Brault, …, Baffet, *EBioMed,* 2016

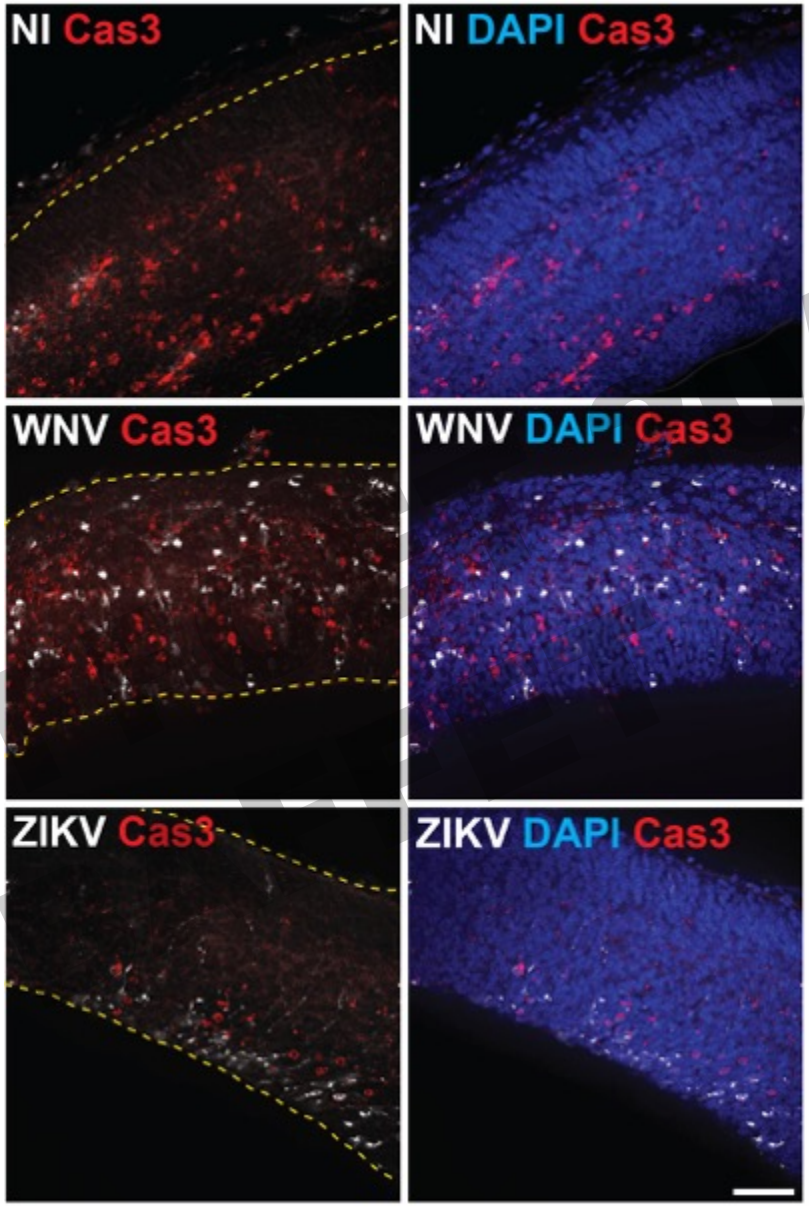
Zika virus impairs neural stem cell proliferation

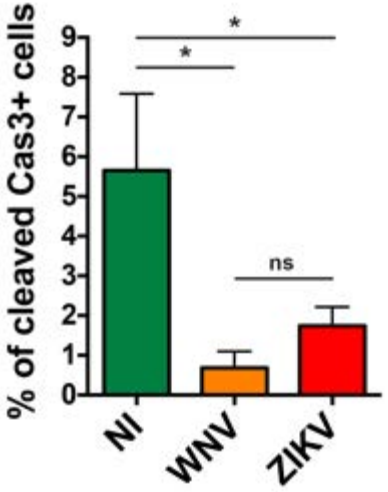




Brault, …, Baffet, *EBioMed,* 2016

Zika virus inhibits apoptosis at early stages of infection





Brault, …, Baffet, *EBioMed,* 2016

Conclusion

Zika virus, but not other closely related flaviviruses, has strong tropism for neural stem cells

Zika virus, but not West Nile virus, impairs neural stem cell proliferation Zika

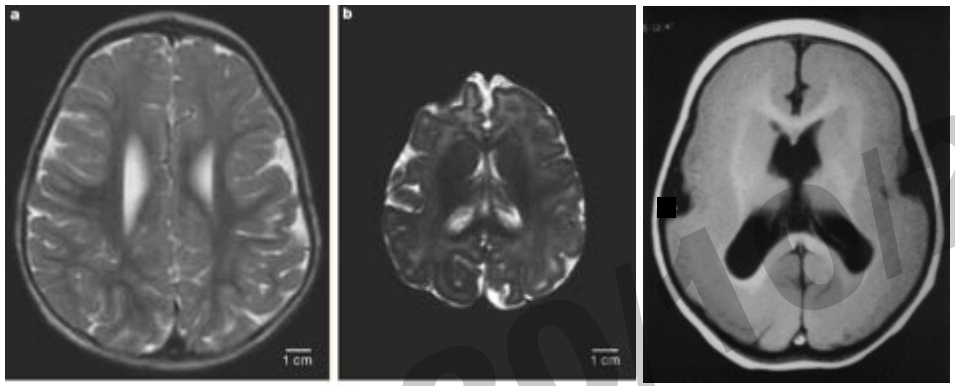
and West Nile viruses both inhibit apoptosis at early stages of infection

Mouse developing neocortex is a good model system

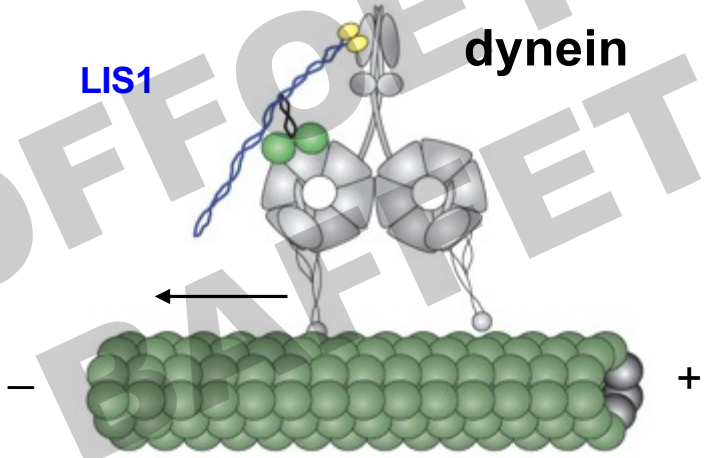
An assay to investigate Zika virus effect during neocortex development

Brault, …, Baffet, *EBioMed,* 2016

Neurogenesis defects lead to congenital brain malformations



Normal brain Microcephaly Lissencephaly



microtubule

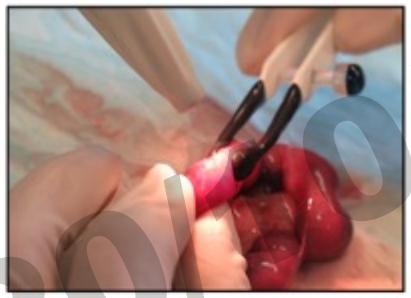
2/ Genetic causes

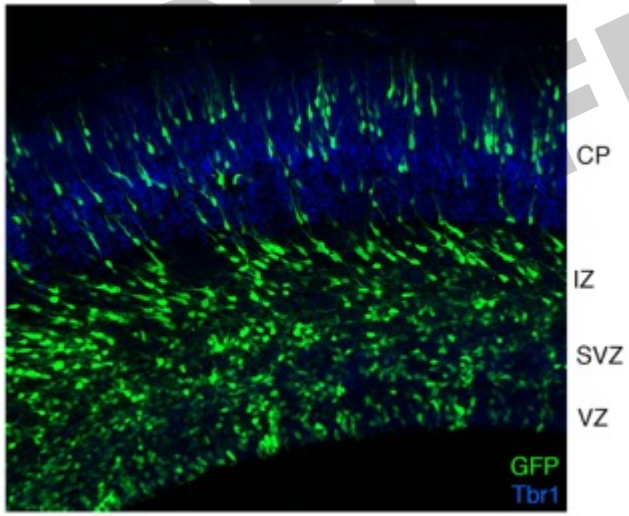
Microcephaly:
DYNC1H1 (dynein), ASPM, NDE1…

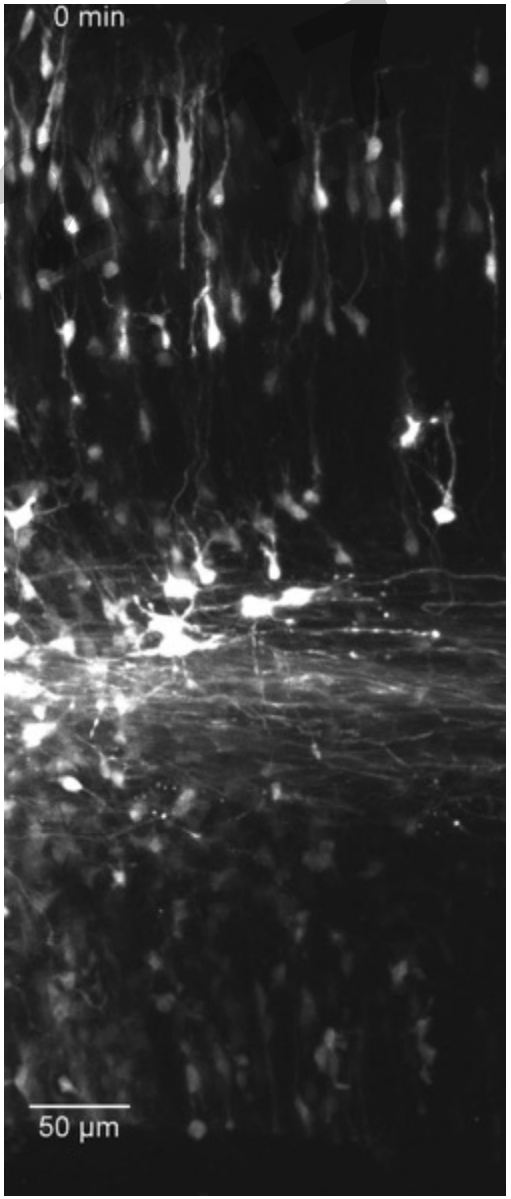
Lissencephaly:
LIS1, DCX, TUBB3…

Microtubules and molecular motors

In utero electroporation and live imaging of developing neocortex

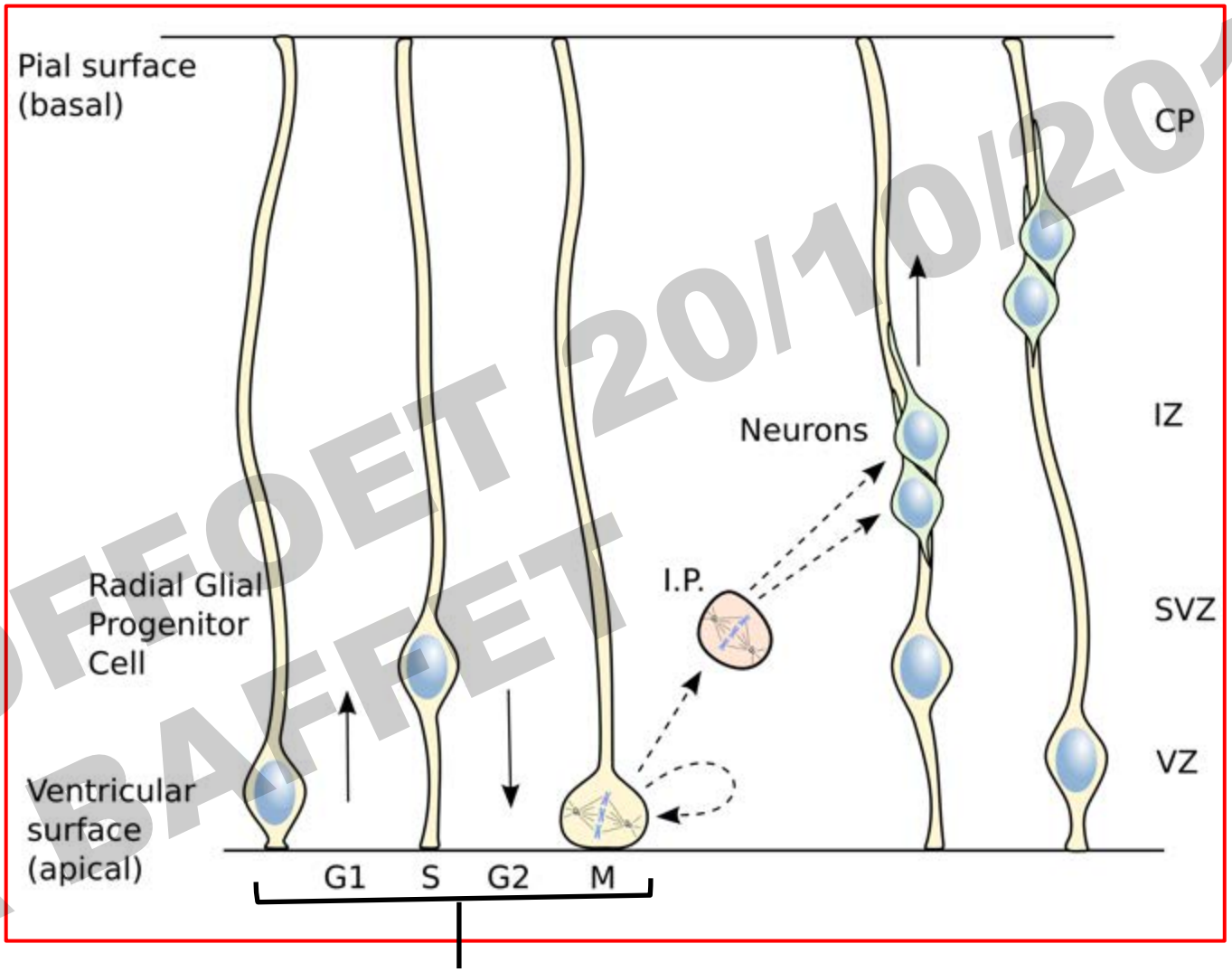






Baffet et al., **Methods in cell biology**, 2016

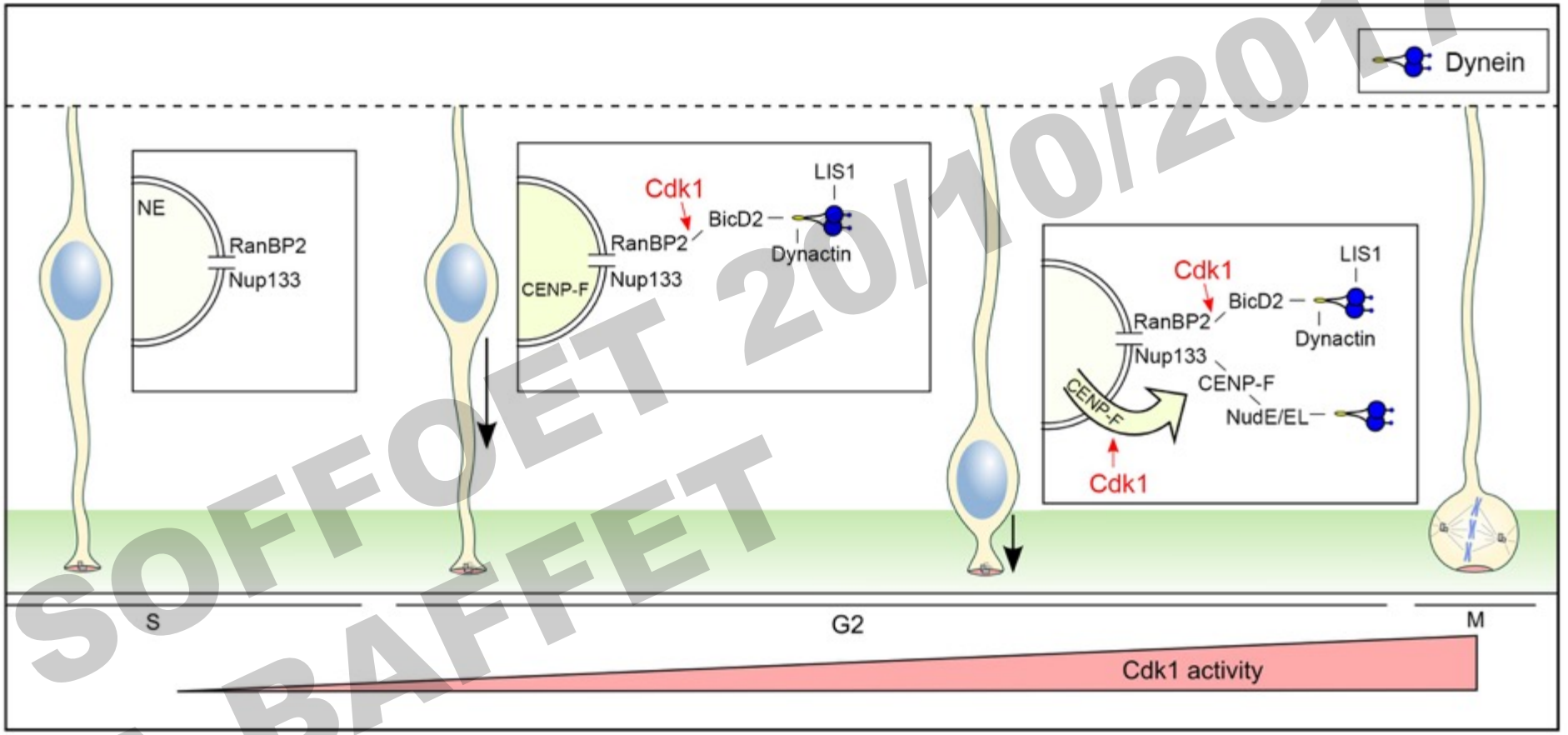
Interkinetic Nuclear Migration (INM) is microtubule-dependent



Interkinetic Nuclear Migration (INM)

Baffet A., Med. Sci., 2014

dynein recruitment to the nuclear pore complex triggers apical nuclear migration



Hu* D, Baffet* A et al, **Cell**, 2013

Baffet A., Hu D. and Vallee R., **Developmental Cell**, 2015

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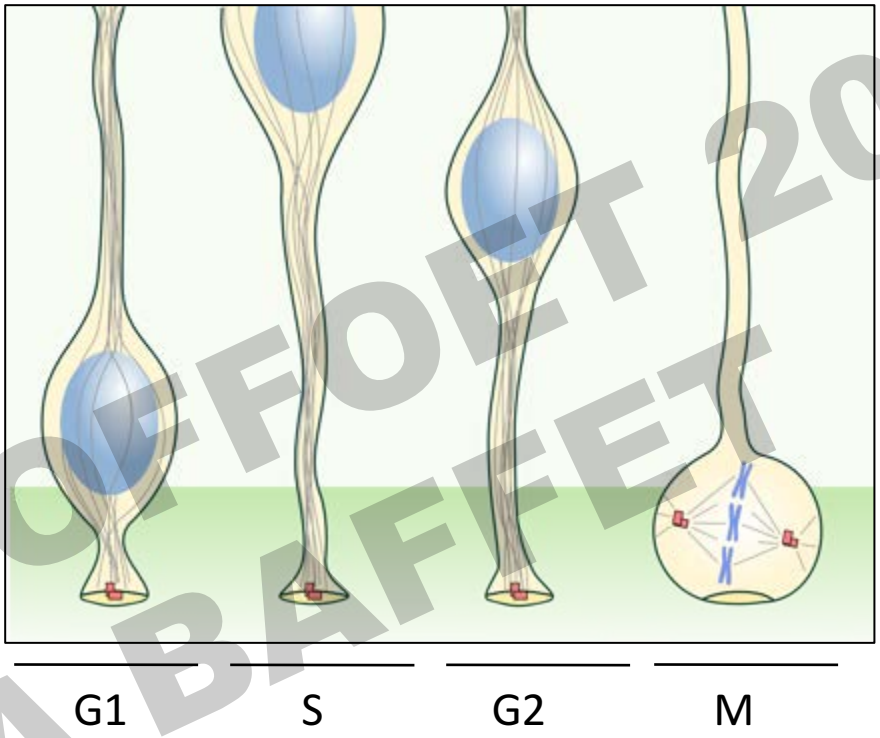
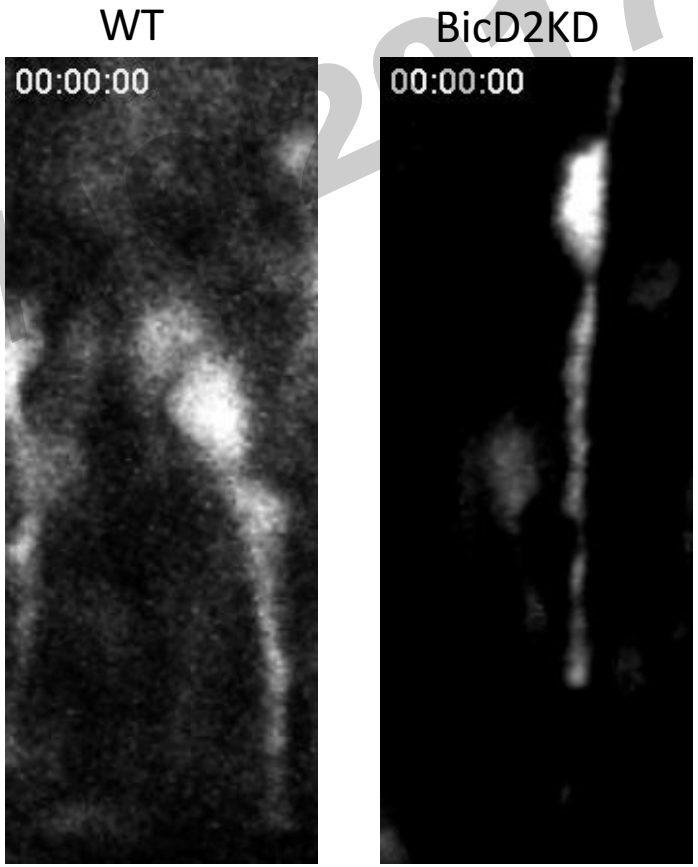
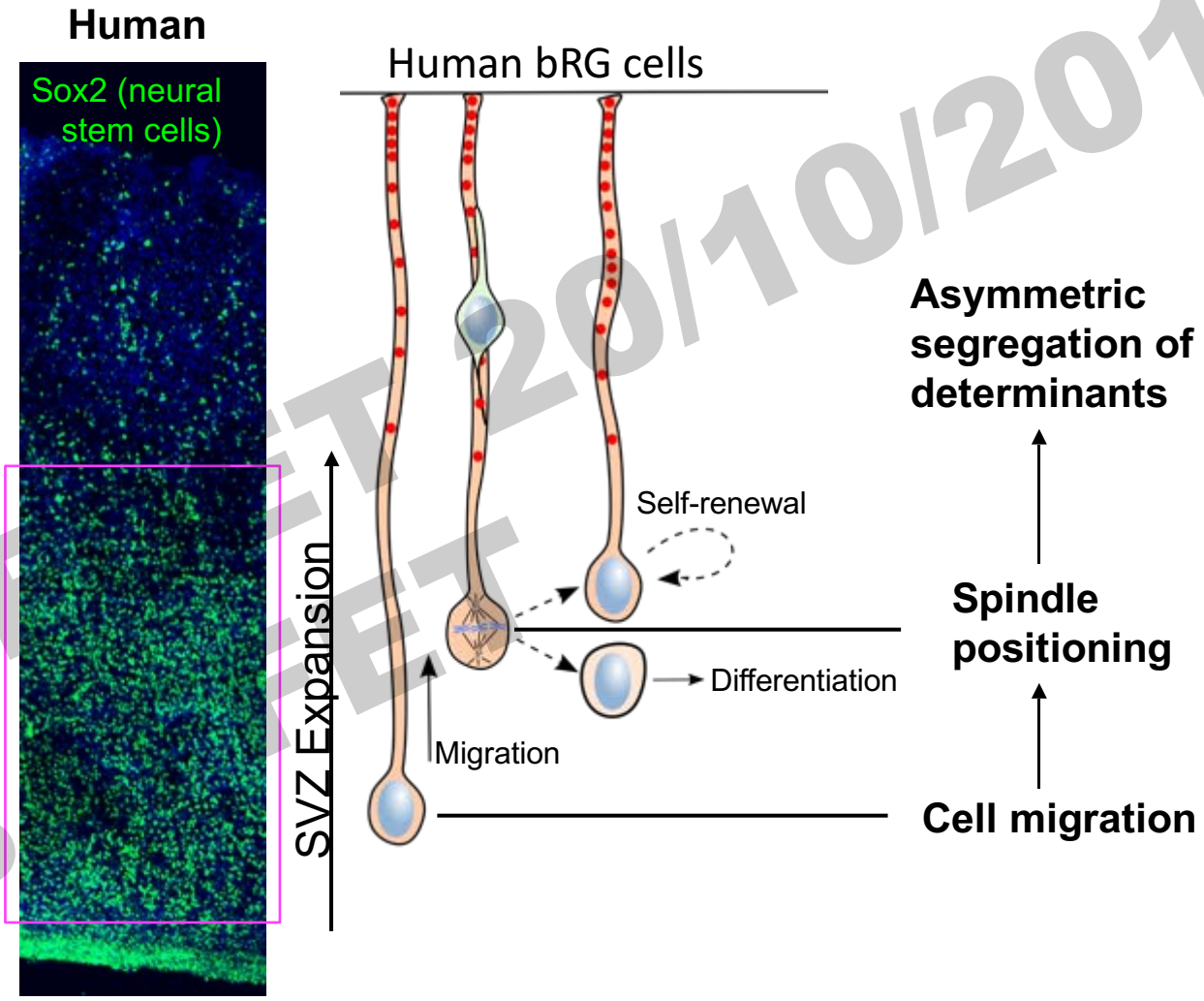
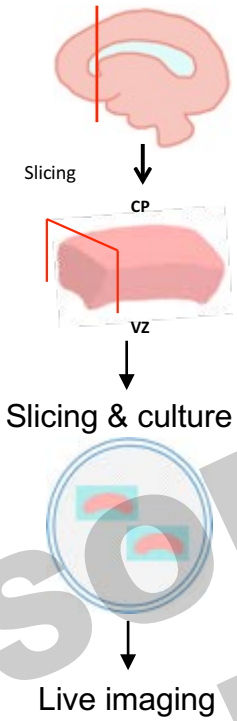




Basal radial glial cells (bRG): human

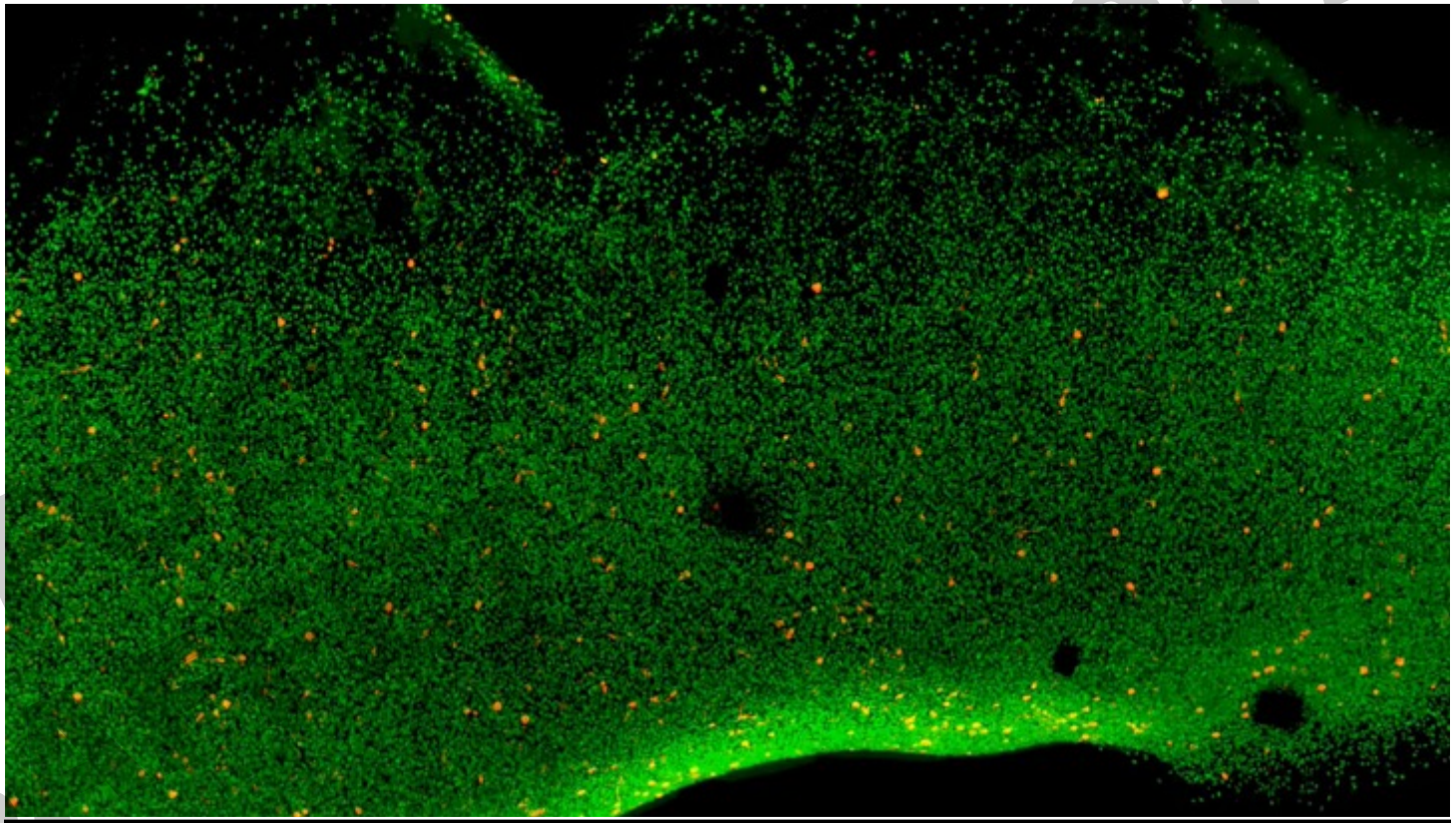


Investigating human bRG cells



Human fetal tissue

Gestation Week 14

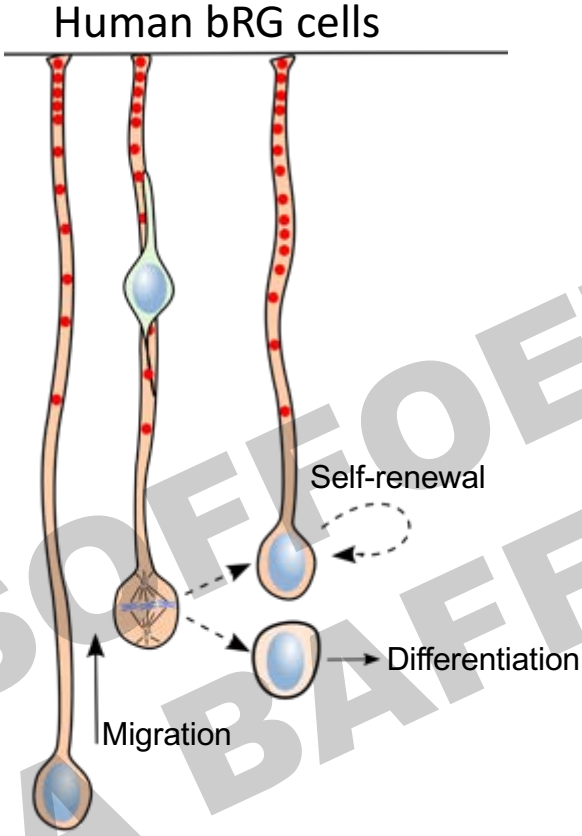


Sox2 (neural stem cells)
P-Vimentin (mitotic neural stem cells)
Microtubules (mitotic spindle)

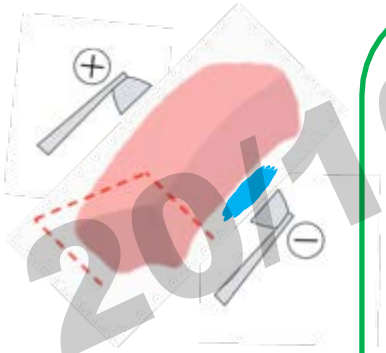
Laure Coquand

With Fabien Guimiot, Robert Debré
Nadia Bahi-Buisson, Imagine & Necker

Investigating human bRG cells in fetal brain samples



Cell migration



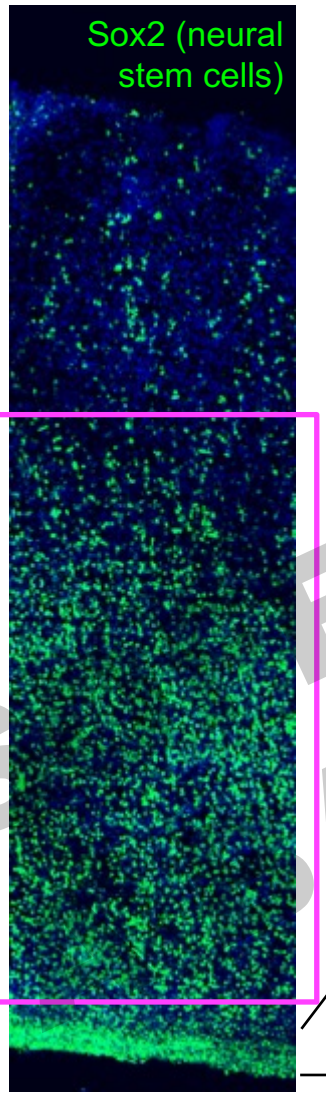
Live imaging of human bRG cells

Human fetal brain

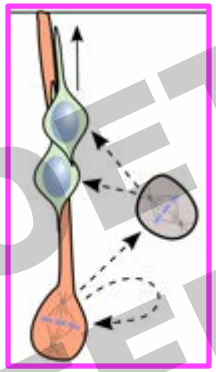
Laure Coquand

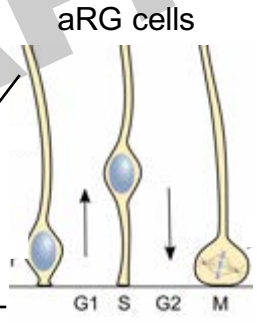
With Fabien Guimiot, Robert Debré

Investigating human bRG cells in fetal brain samples

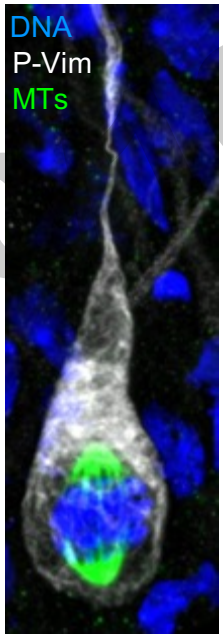


Human fetal brain (week 15)



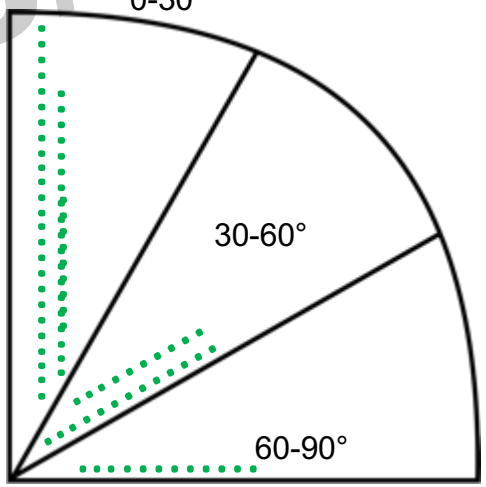


Human bRG cell



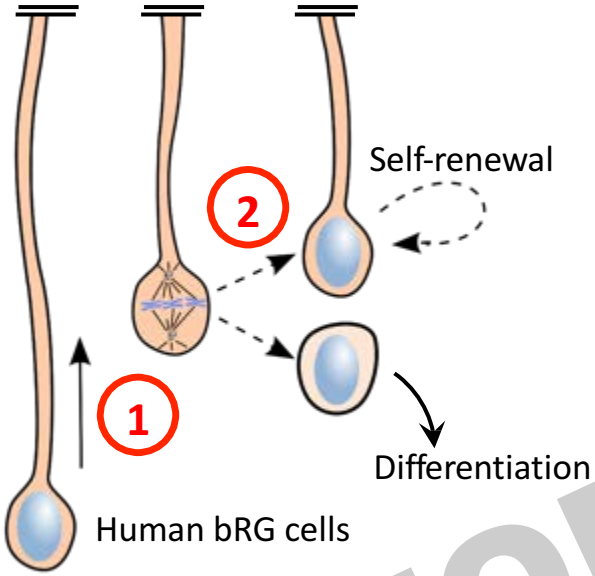
Laure Coquand

Spindle angles

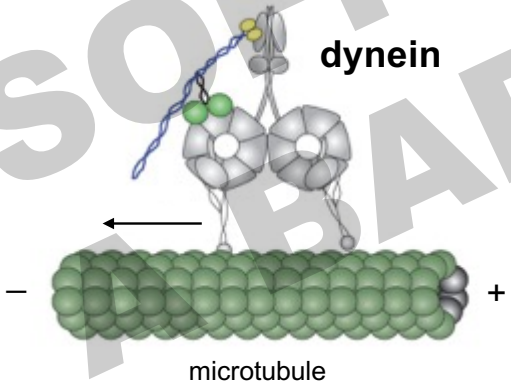


With Fabien Guimiot, Robert Debré

bRG cell migration and mitotic spindle orientation: Role of the dynein motor

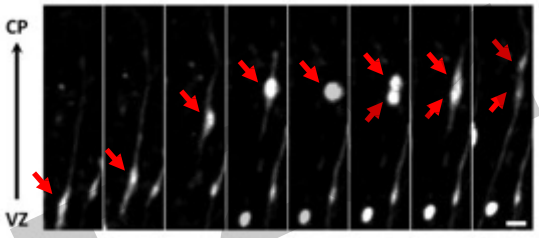


Role of Dynein-Lis1 complex

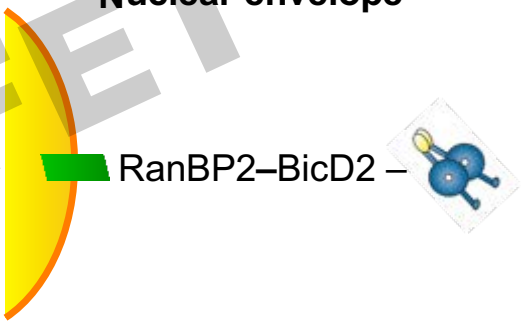


Aim1 Cell migration

Human fetal brain

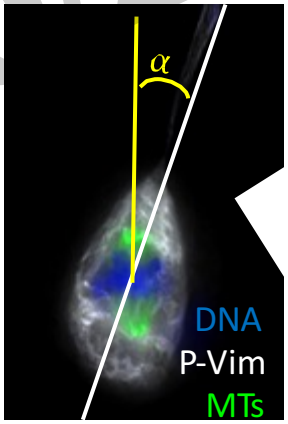


Nuclear envelope

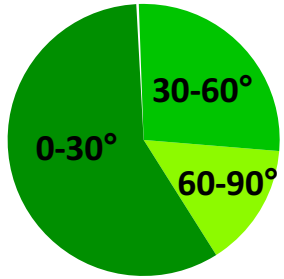


Aim2 Spindle orientation

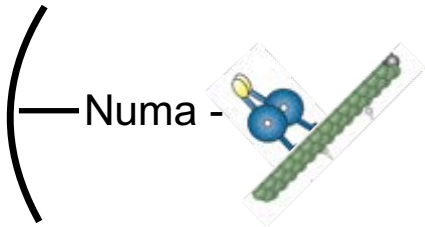
Human fetal brain



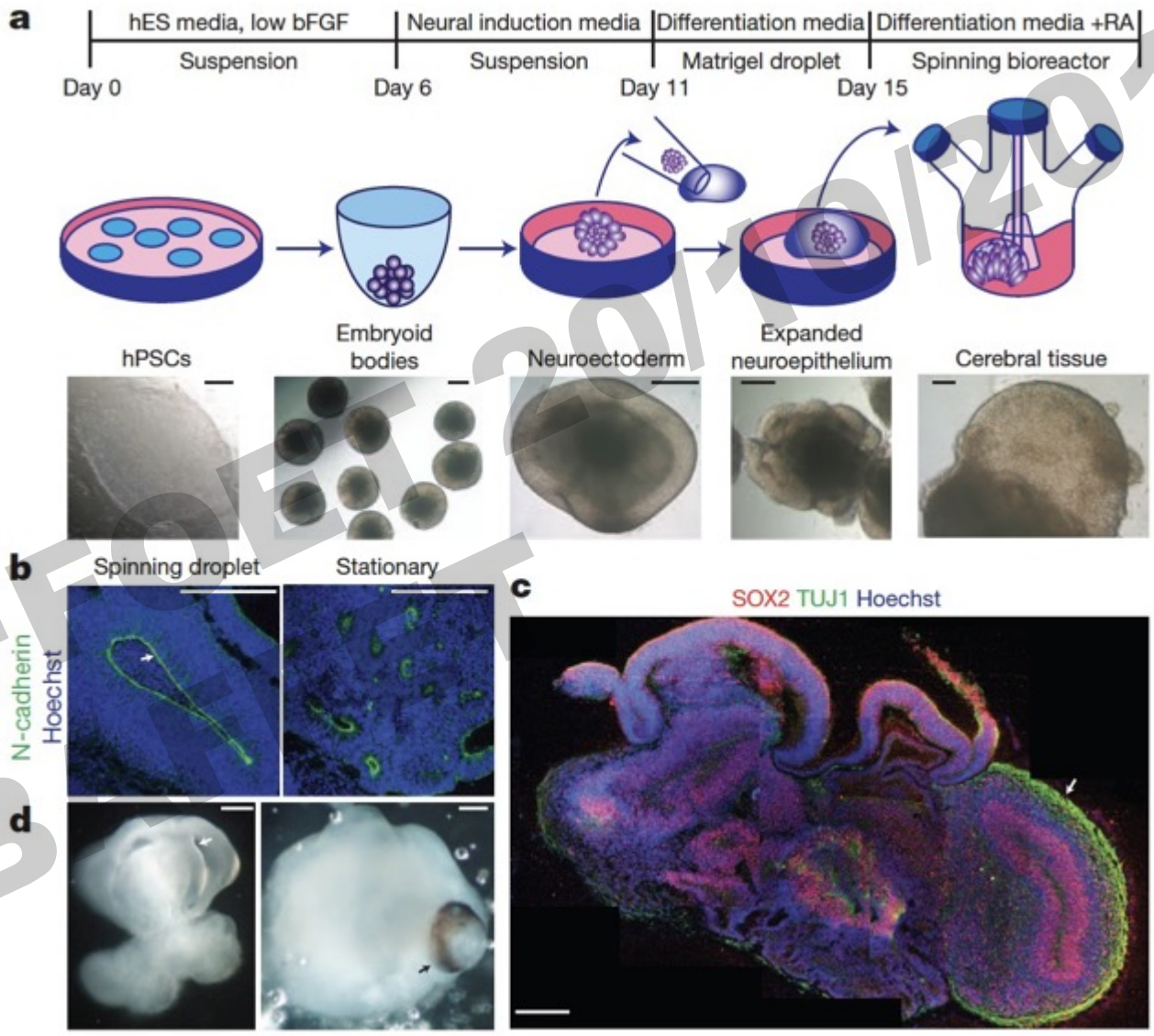
Spindle angles
N=1



Cell cortex

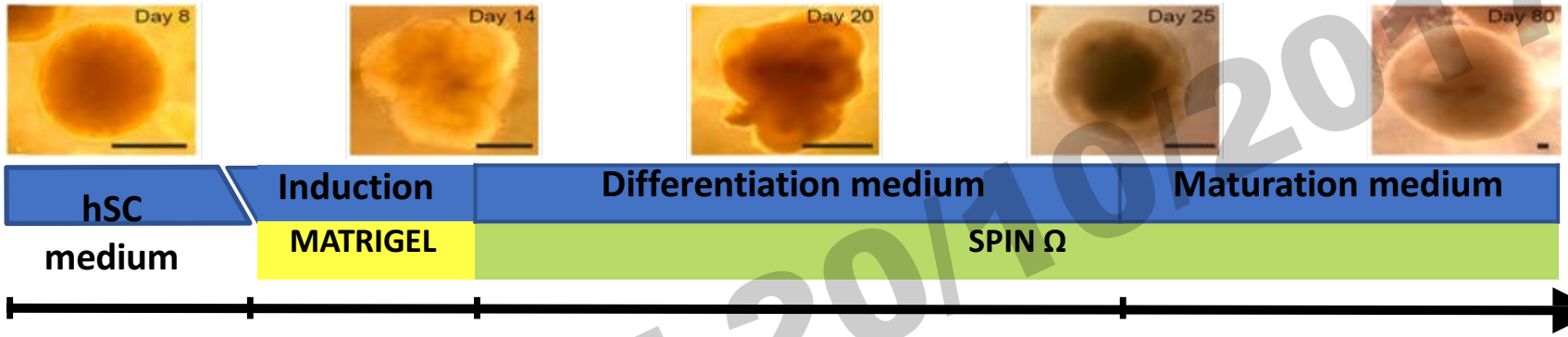


Cerebral organoids: Brains in a dish



Lancaster et al, Nature, 2013

Dorsal forebrain-specific organoids



day **1**

\+ Dorsomophin
+ A83
(**SMAD inhibitors**)

7

+ CHIR99021 (**GSK-3β inhibitor**)
+ WNT3A
+ Heparin (**Wnt pathway activator**)
+ SB431542 (**SMAD inhibitor**)

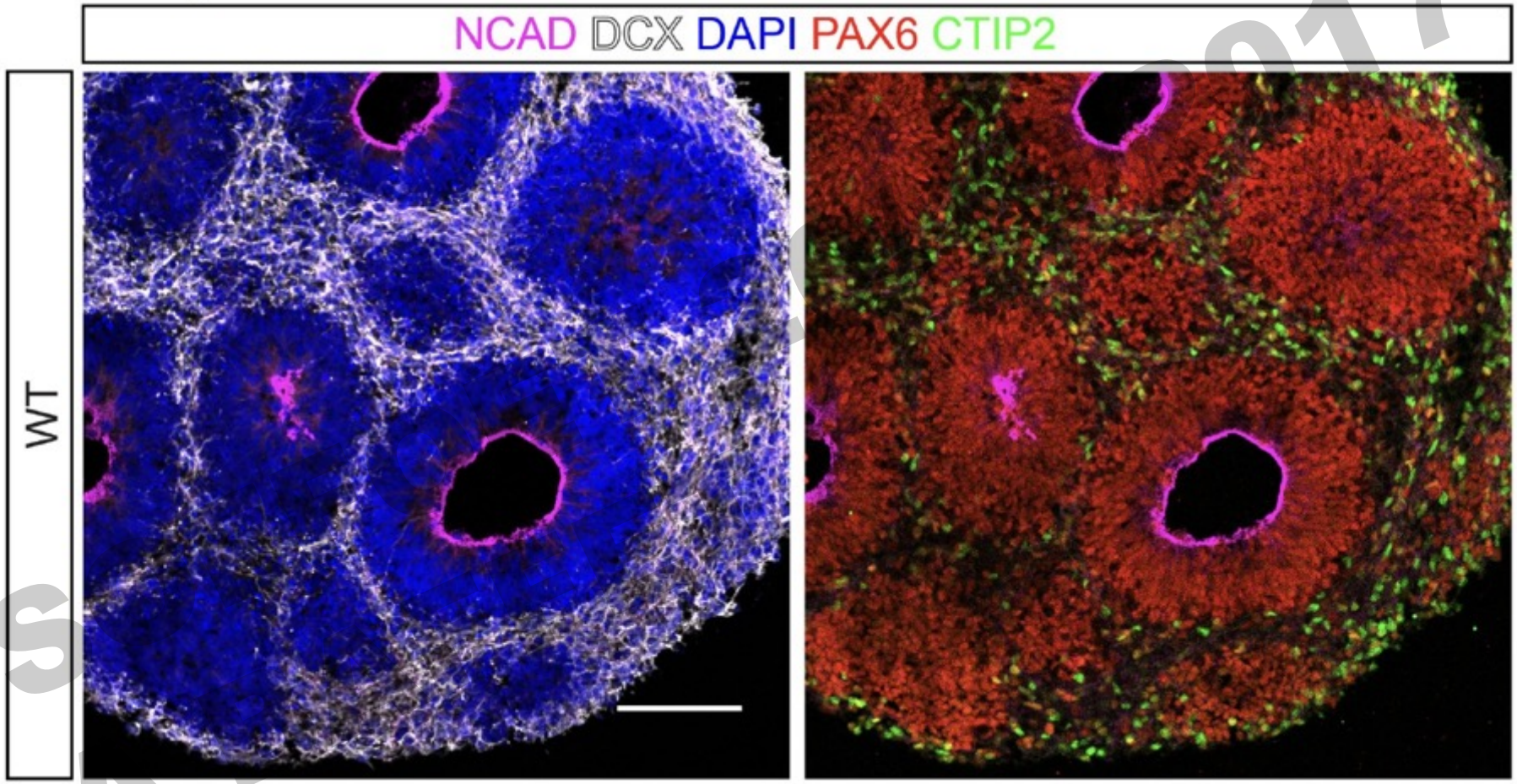
14

+ N2
+ B27 (plus VitA)
+ Insulin (**inhibits neuronal apoptosis**)

71

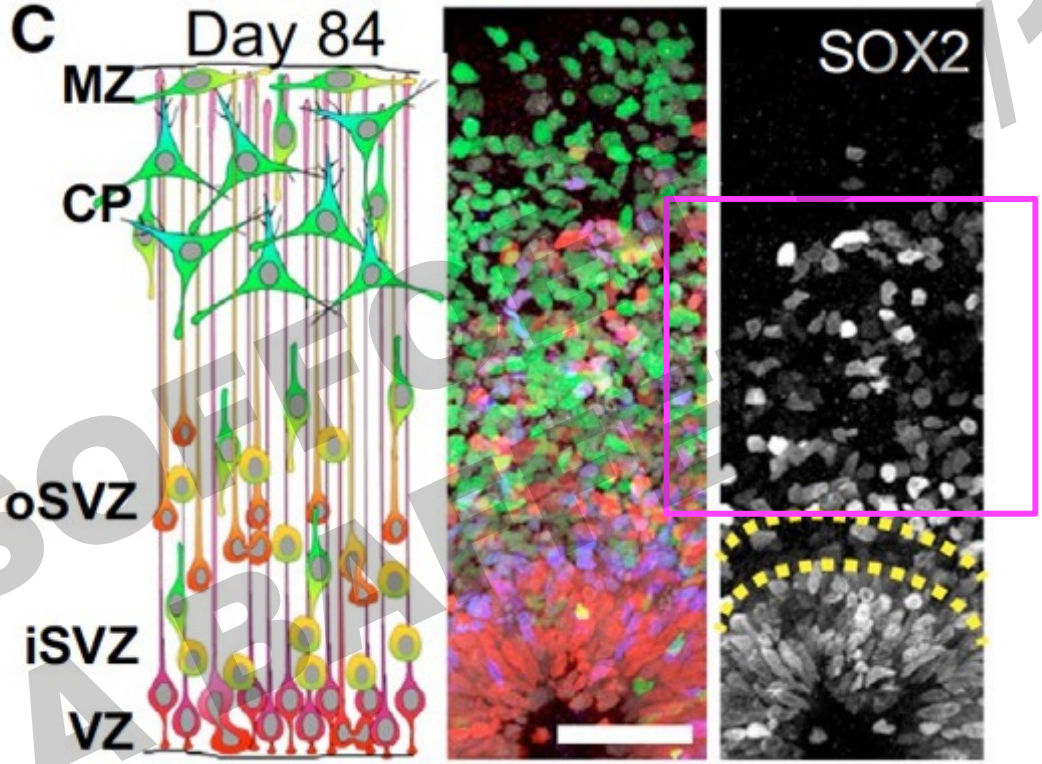
+ BDNF
Brain Derived Neurotrophic Factor
+ GDNF
Glial cell Derived Neurotrophic Factor
(**Promote survival and development of neurons**)
+ VitC
+ TGFβ
(**promotes neuronal differentiation**)
+ cAMP

Dorsal forebrain-specific organoids



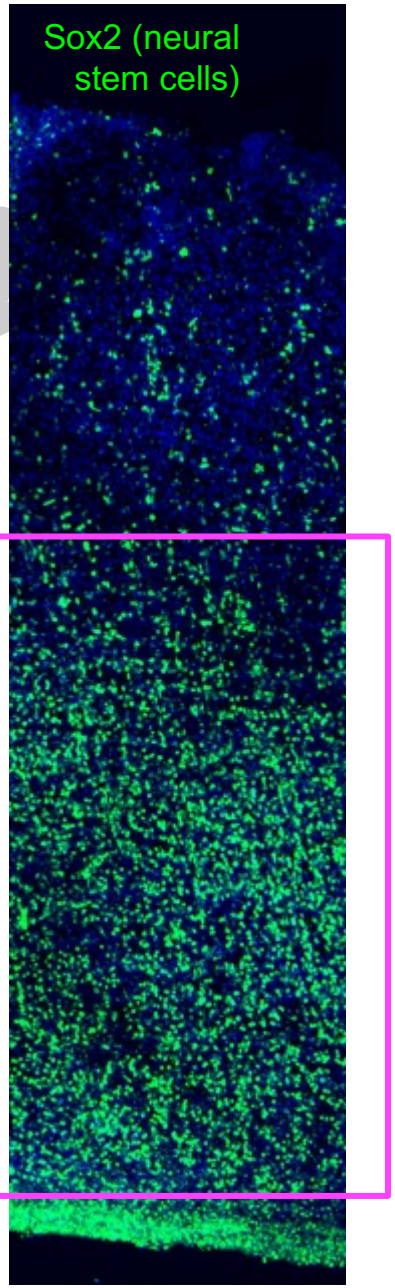
bRG-like cells in 3 months old organoids

Human cerebral organoid



Qian et al, 2016

Human foetal



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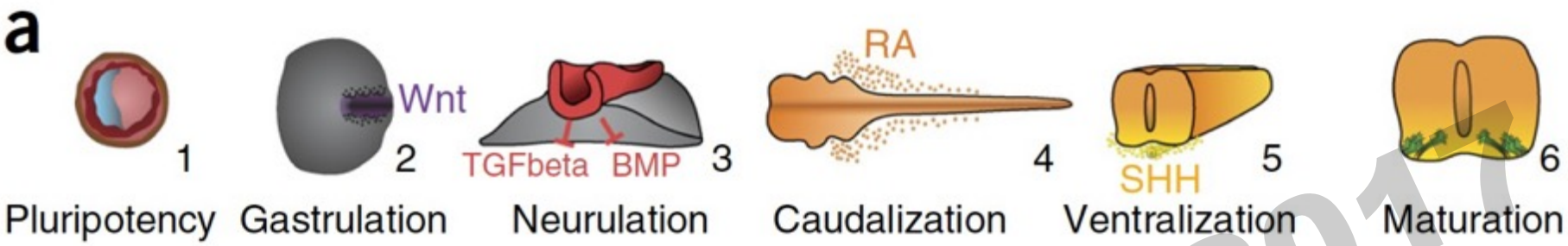
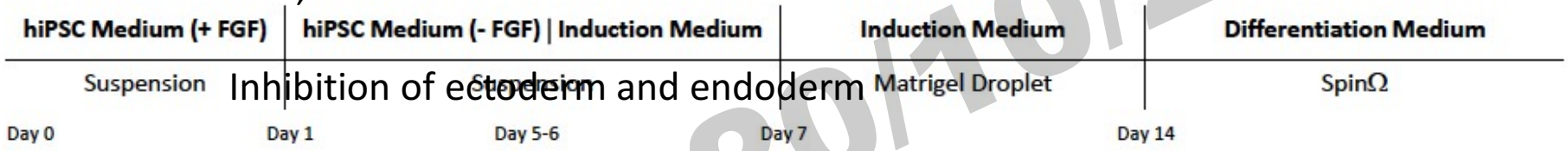
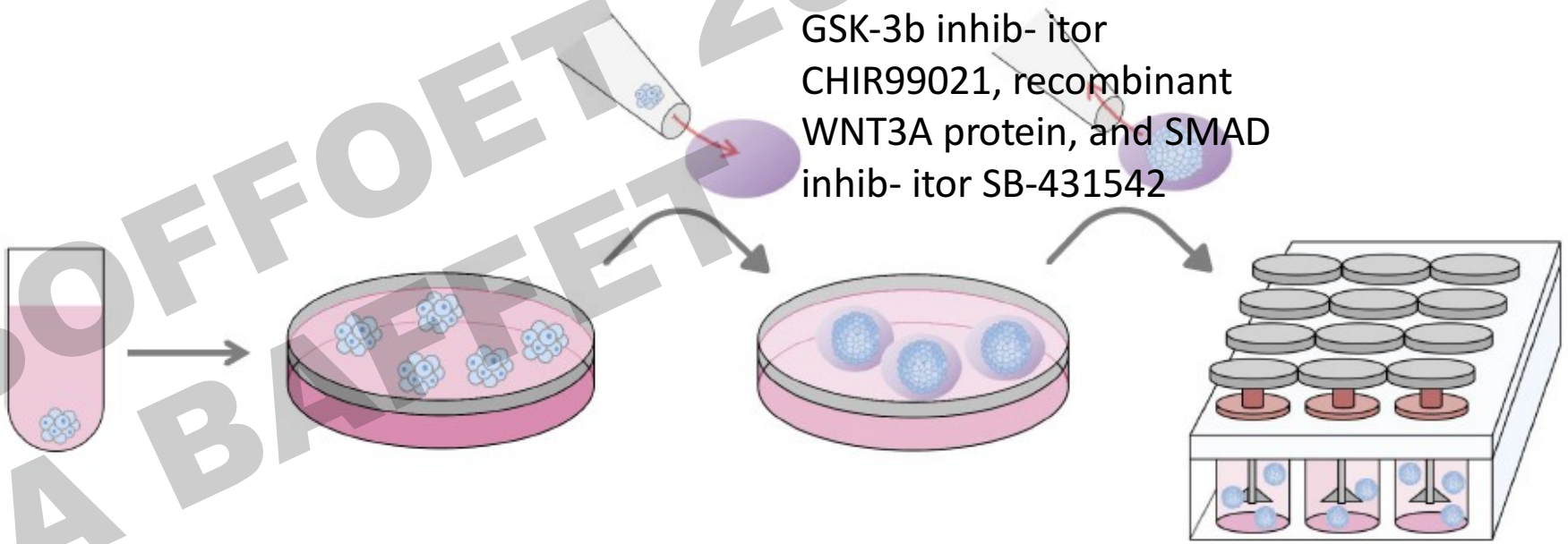


-FGF, SMAD inhibitors

Inhibition of ectoderm and endoderm



GSK-3b inhib- itor CHIR99021, recombinant WNT3A protein, and SMAD inhib- itor SB-431542



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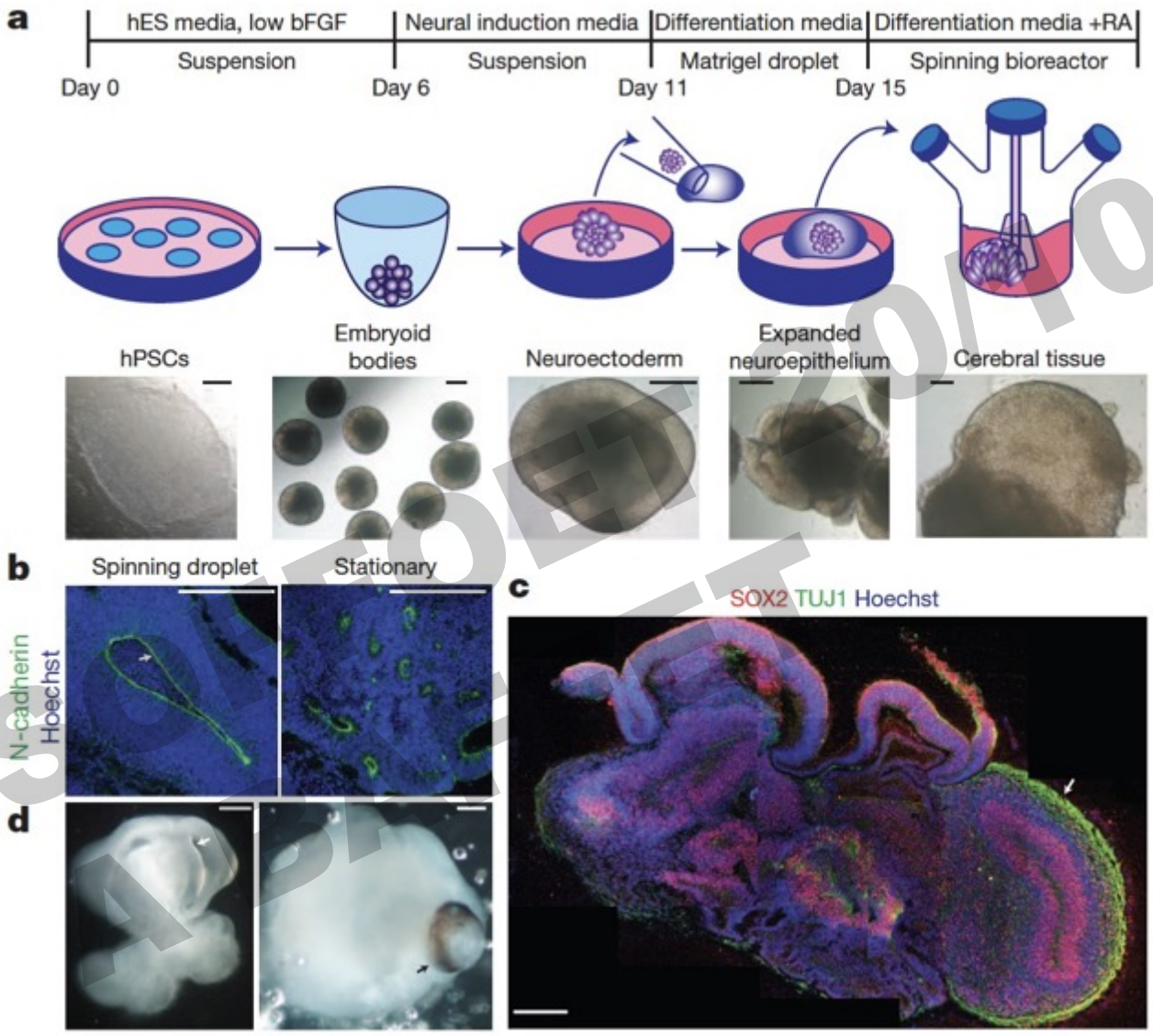

Lancaster et al, Nature, 2013

Qian et al, Cell, 2016