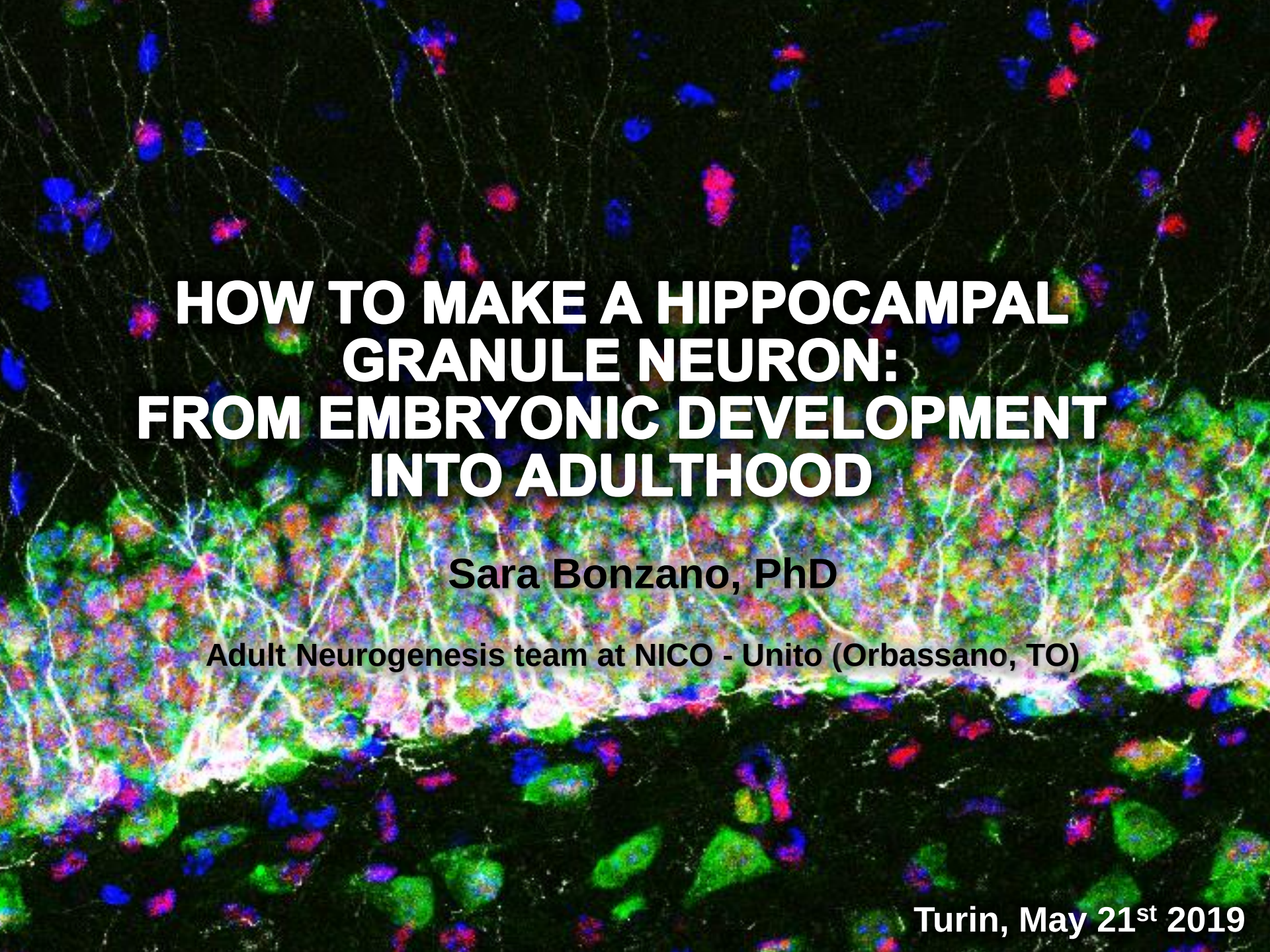


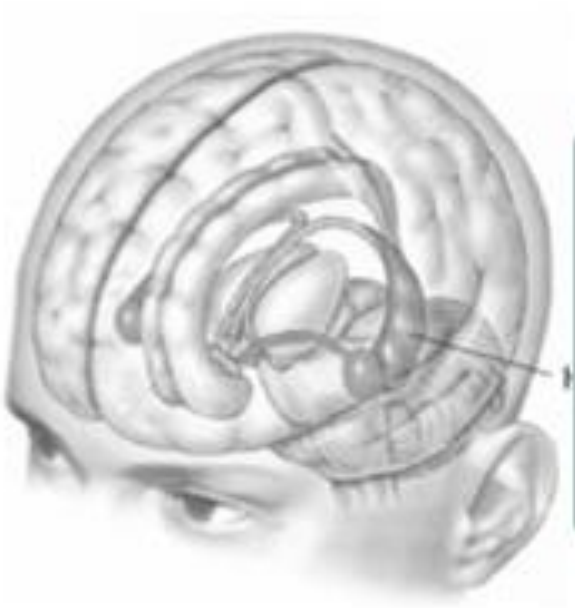
HOW TO MAKE A HIPPOCAMPAL GRANULE NEURON: FROM EMBRYONIC DEVELOPMENT INTO ADULTHOOD

Sara Bonzano, PhD

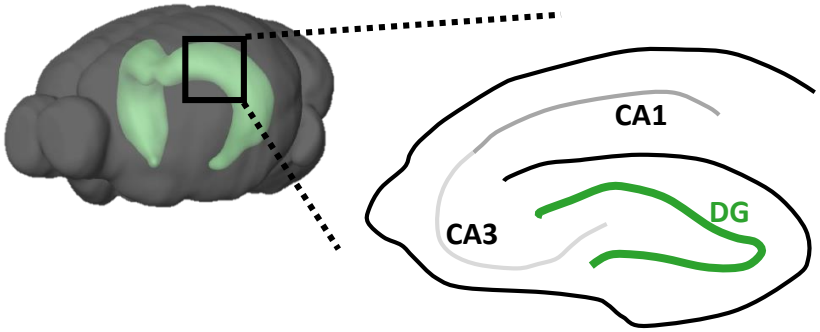
Adult Neurogenesis team at NICO - Unito (Orbassano, TO)

Turin, May 21st 2019

The hippocampus

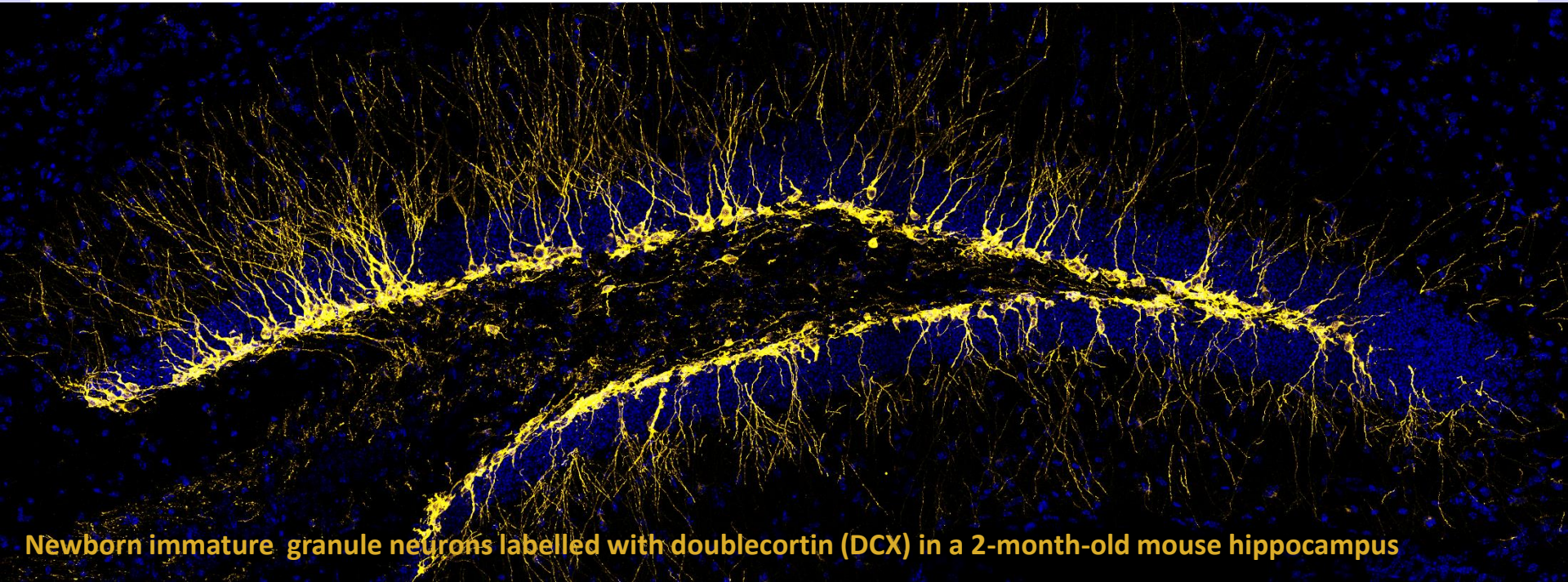


Adult hippocampal neurogenesis



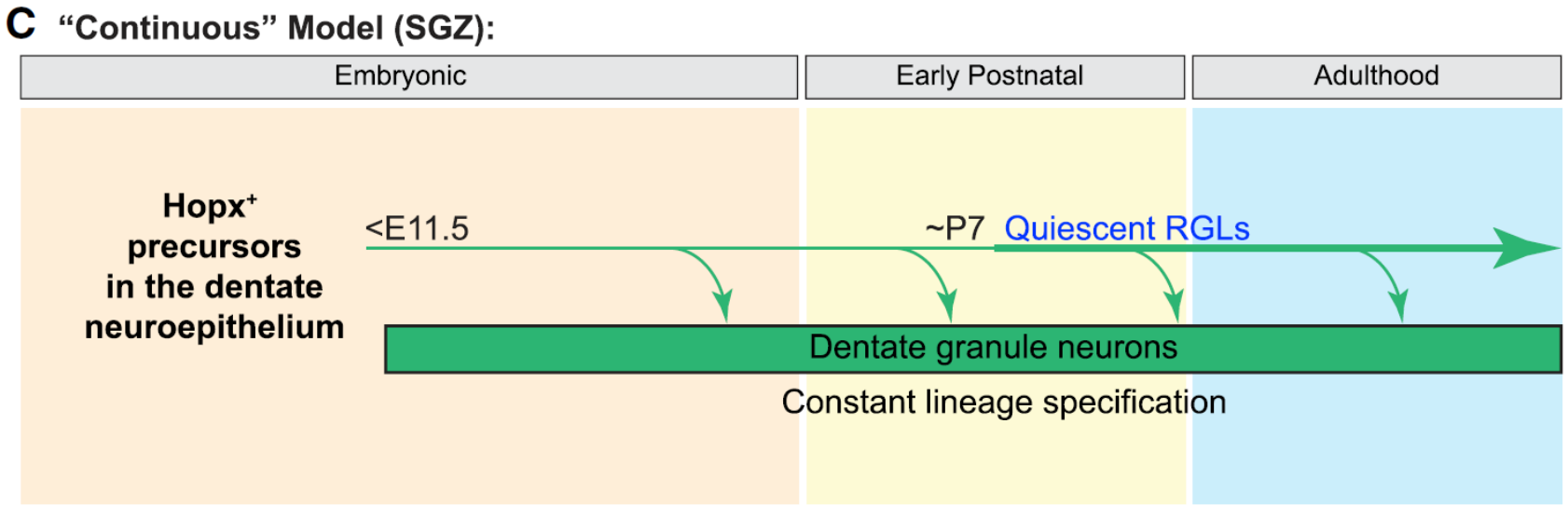
- ✓ Memory (spatial, short term)
- ✓ “Pattern separation” (discrimination)
- ✓ Emotions (anxiety, depression)

Bond et al., Cell Stem Cell 2015; Gonçalvez et al., Cell 2016



Newborn immature granule neurons labelled with doublecortin (DCX) in a 2-month-old mouse hippocampus

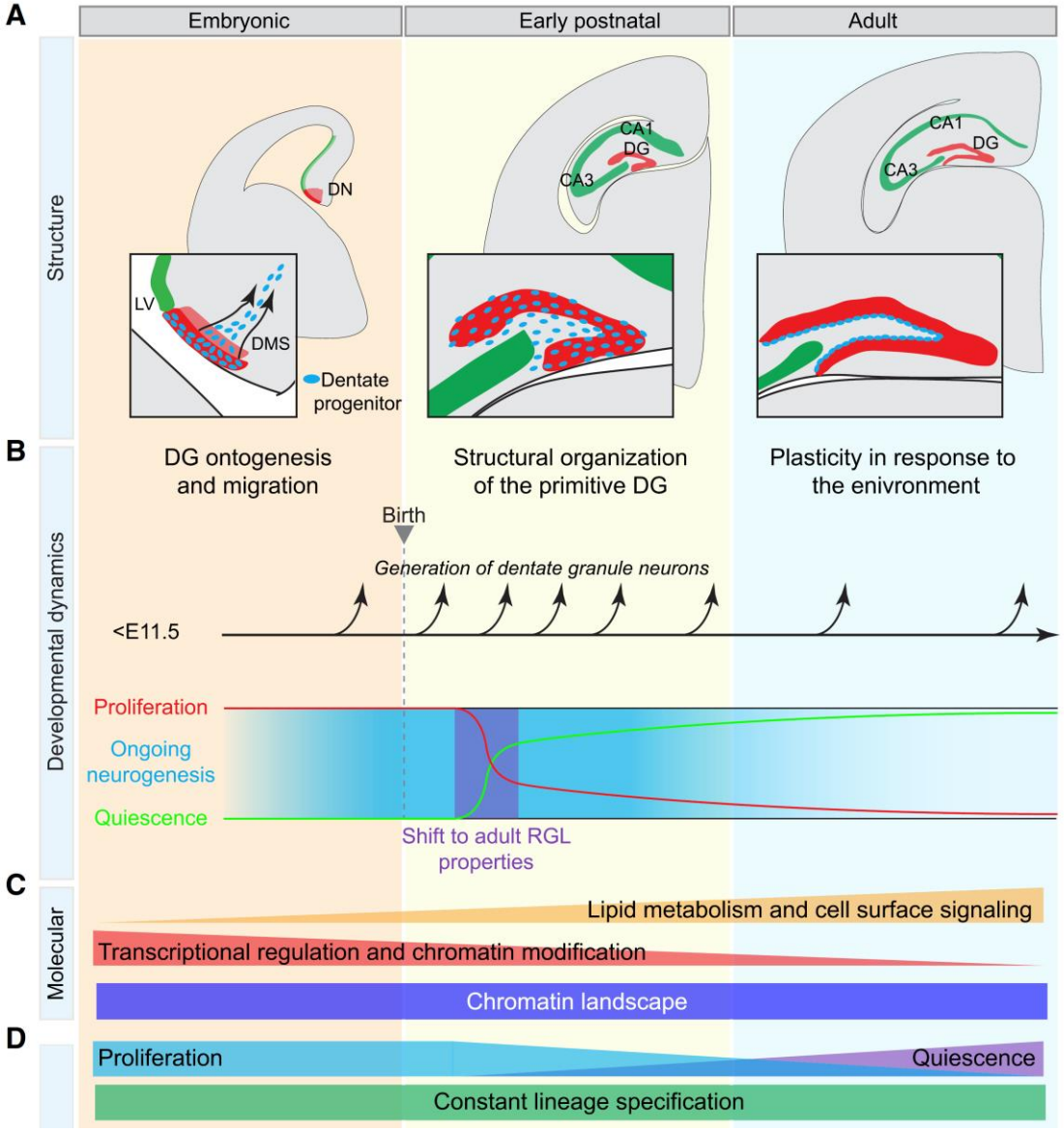
What's the origin of adult hippocampal NSCs?



- Dentate progenitors exhibit constant lineage specification across development
- Precursors to adult DG NSCs are not ''set aside'' in quescence during embryonic development (as SVZ), but they transit to a quiescent state during early PN period (P3-P7)
- Developmental and adult dentate neurogenesis are likely one continuous process

Berg et al., Cell 2019

What's the origin of adult hippocampal NSCs?

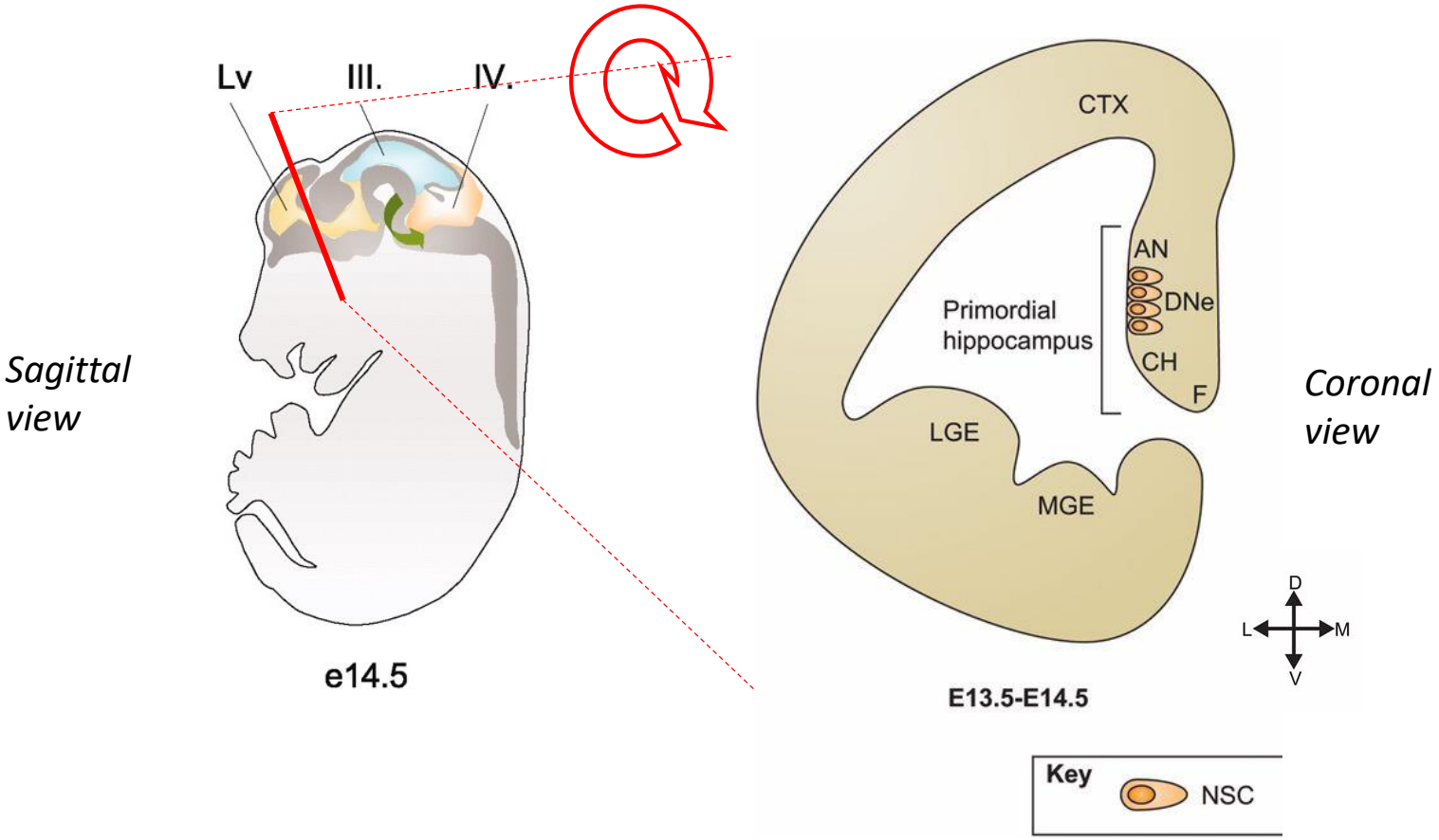


- The Hopx-CreERT2 line can label an embryonic origin (from E10.5-11) of adult dentate neural progenitors

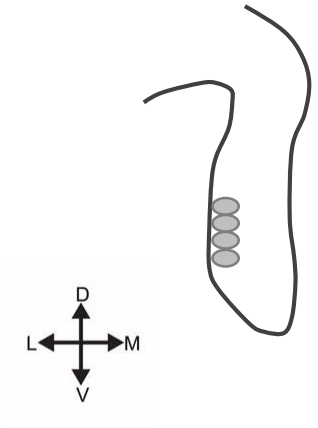
Berg et al., Cell 2019

Embryonic origin of the hippocampus

The hippocampus arises from the **caudomedial edge** of the **dorsal telencephalic neuroepithelium** adjacent to the cortical hem (CH, a transient structure acting as the “embryonic organizer” for the hippocampus)



Embryonic origin of the hippocampus

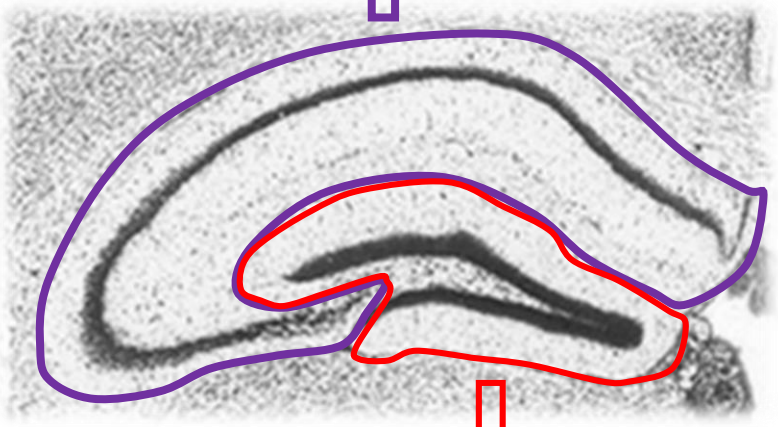


1 **Cornus ammonis and subiculum**

⇧

Ventricular germinative niche (VZ)

⇧



⇩

Dentate germinative niche (DNe)

⇩

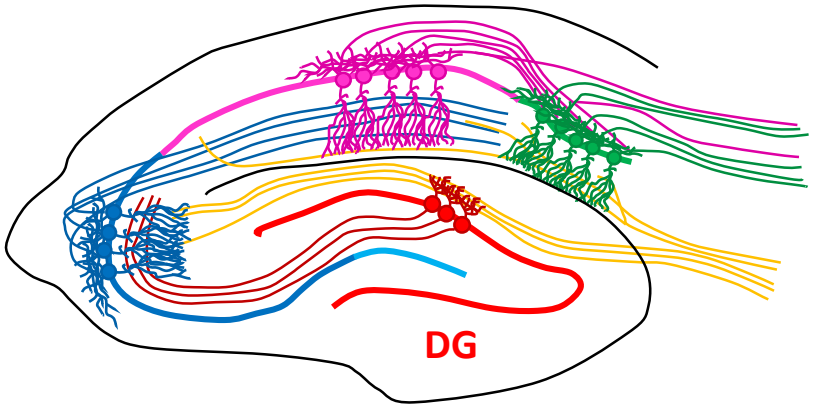
2 **Dentate gyrus**

Dentate gyrus ontogenesis during development

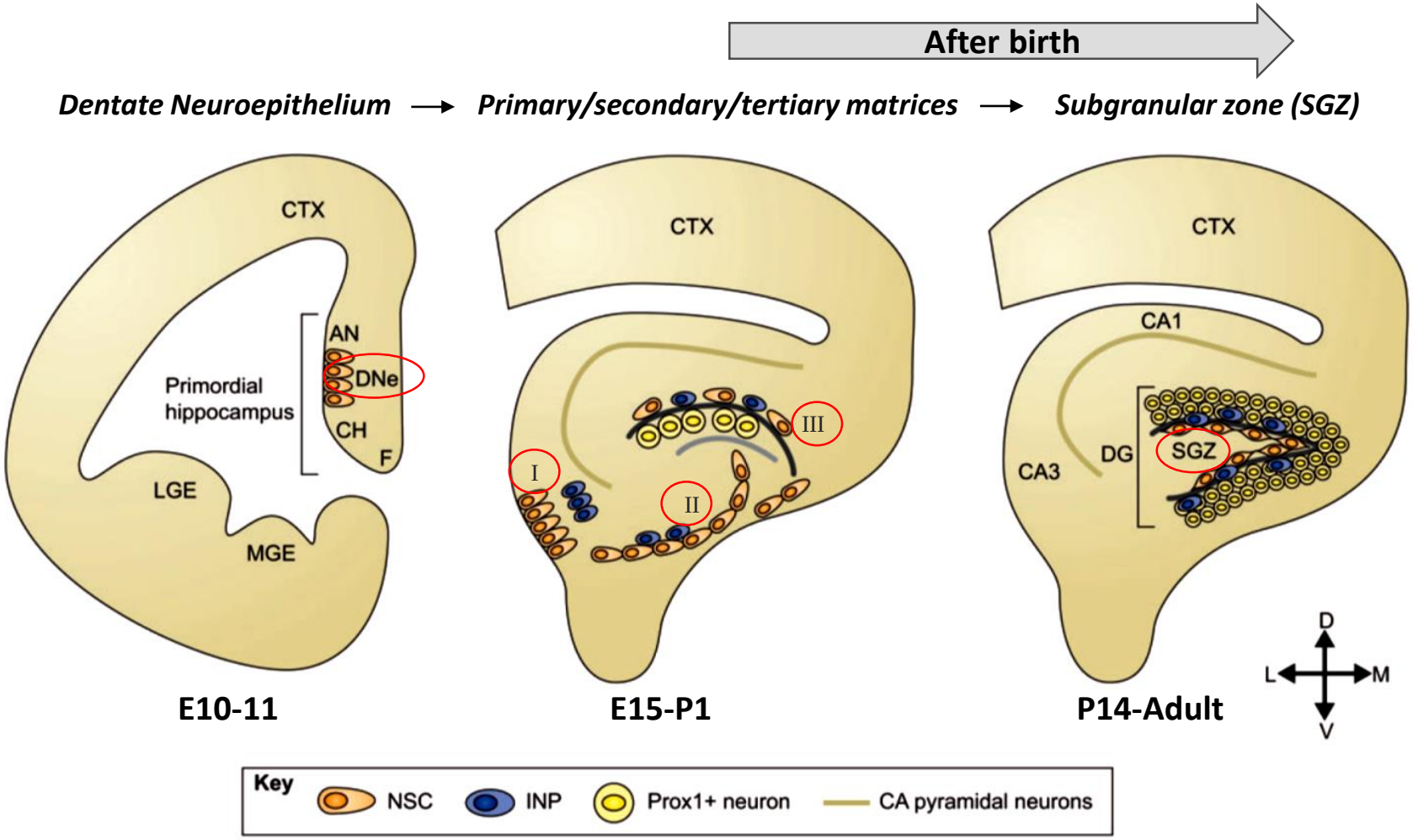
From a developmental point of view, the generation of the DG is **unique**

The formation of the DG involves **the generation of a dedicated progenitor cell source** away from the ventricular zone (VZ)

This **additional proliferative zone** remains **active during postnatal stages** and eventually becomes **the subgranular zone (SGZ)**, where adult hippocampal neural stem cells (NSCs) are located

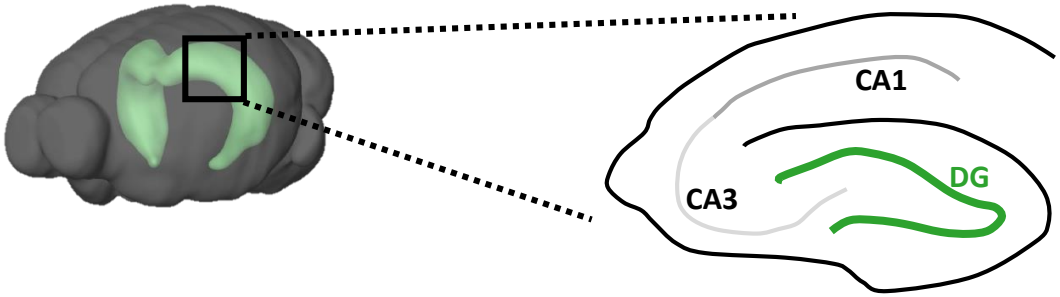


Dentate gyrus ontogenesis during development

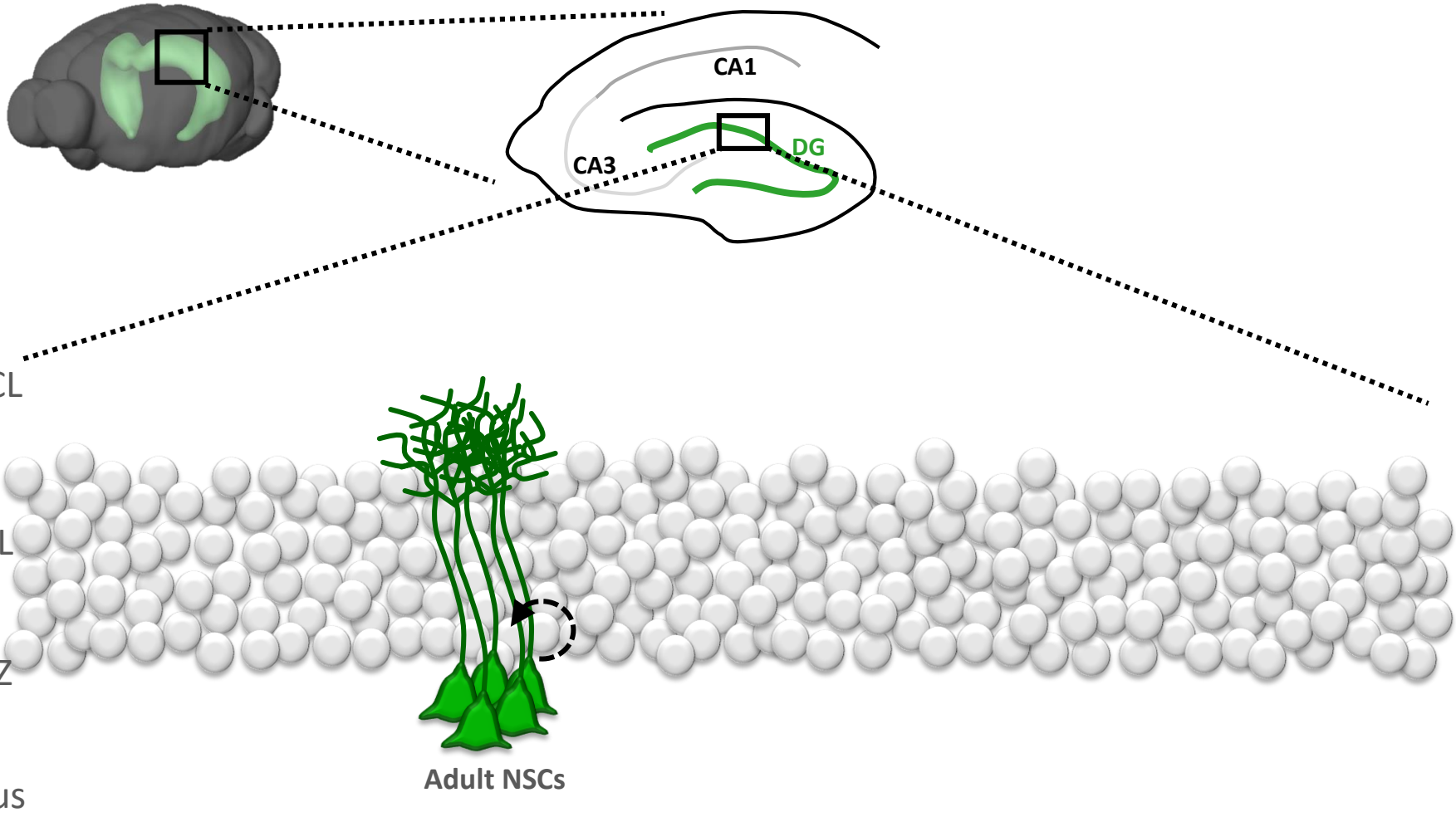


Modified from: Yu, Marchetto and Gage, Development 2014

The neurogenic niche in adult mouse dentate gyrus (DG)

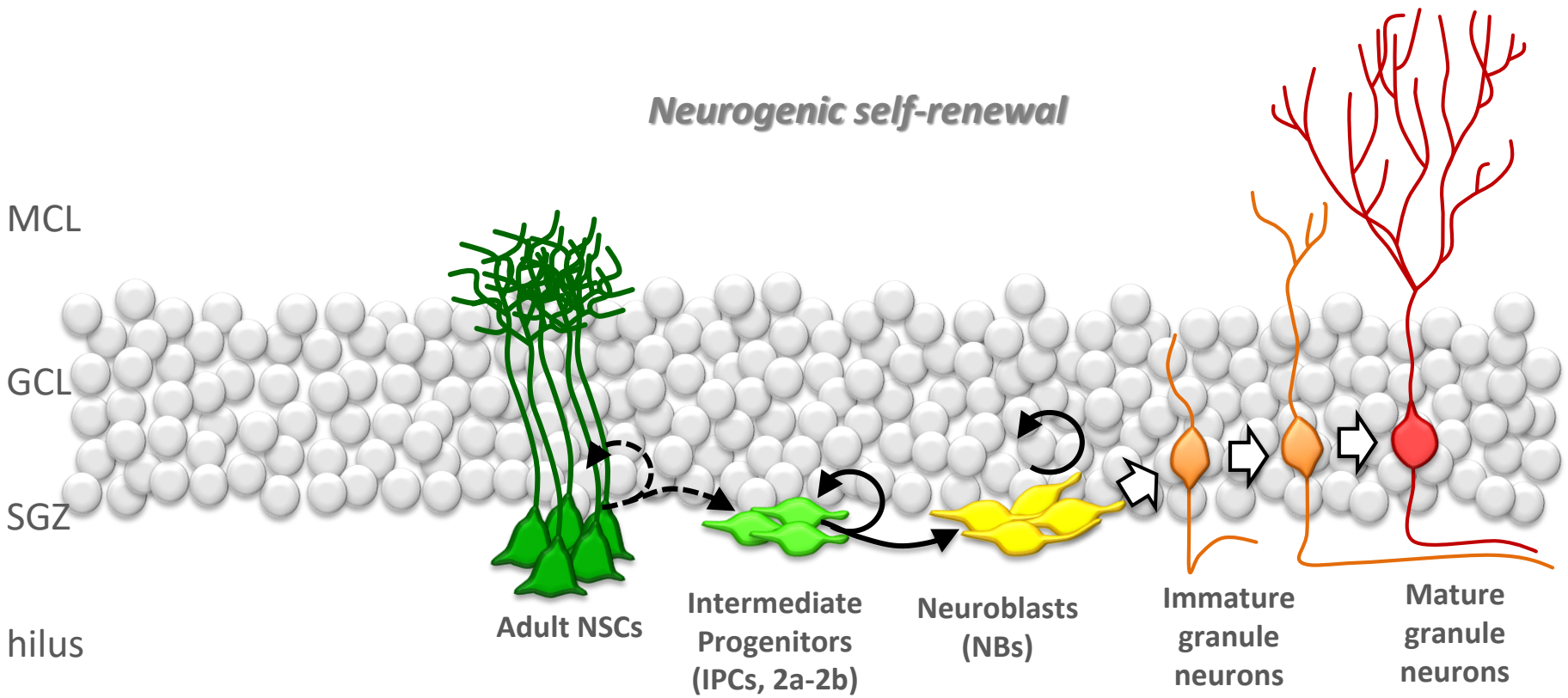


The neurogenic niche in adult mouse dentate gyrus (DG)



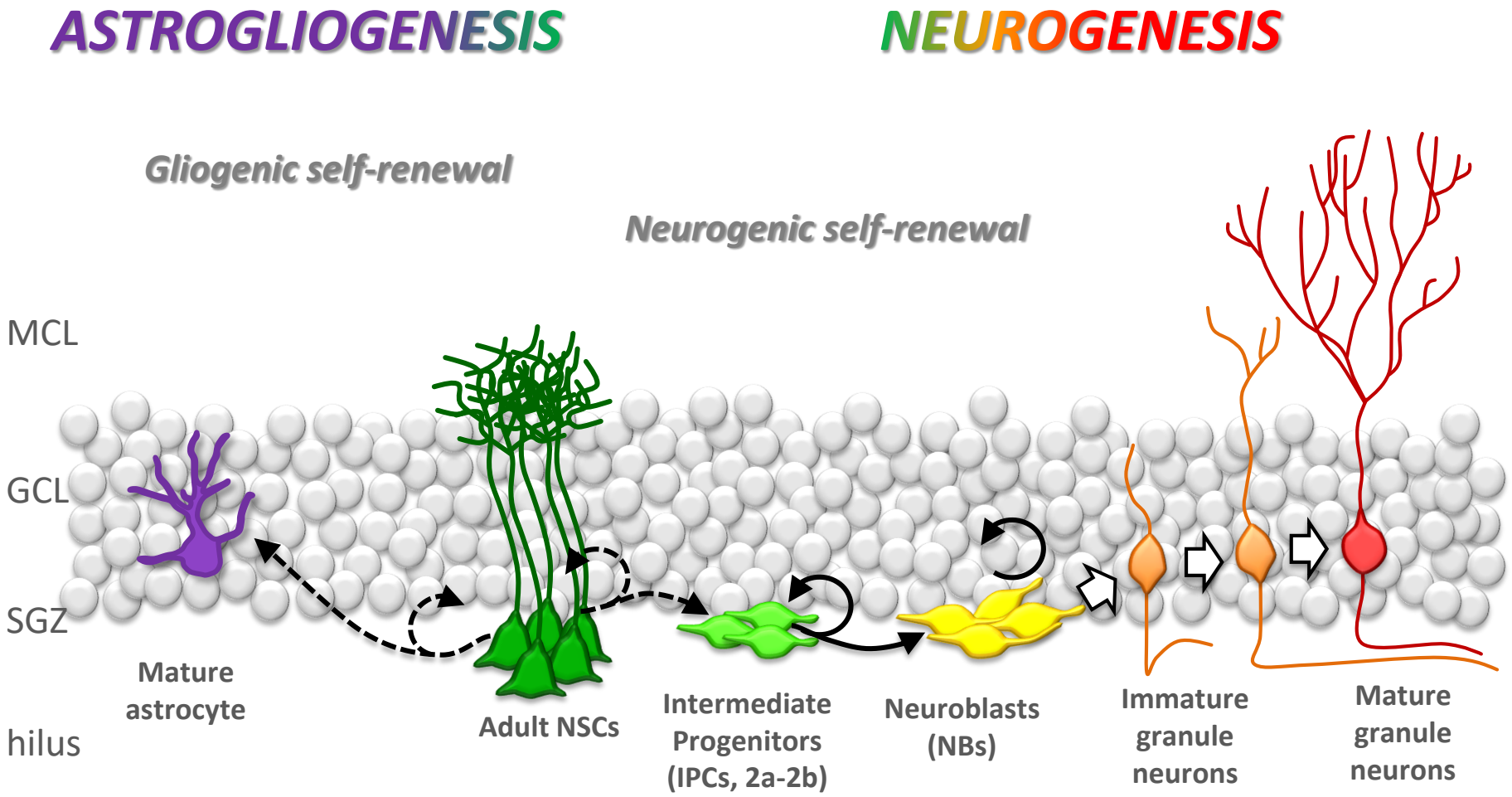
The neurogenic niche in adult mouse dentate gyrus (DG)

NEUROGENESIS

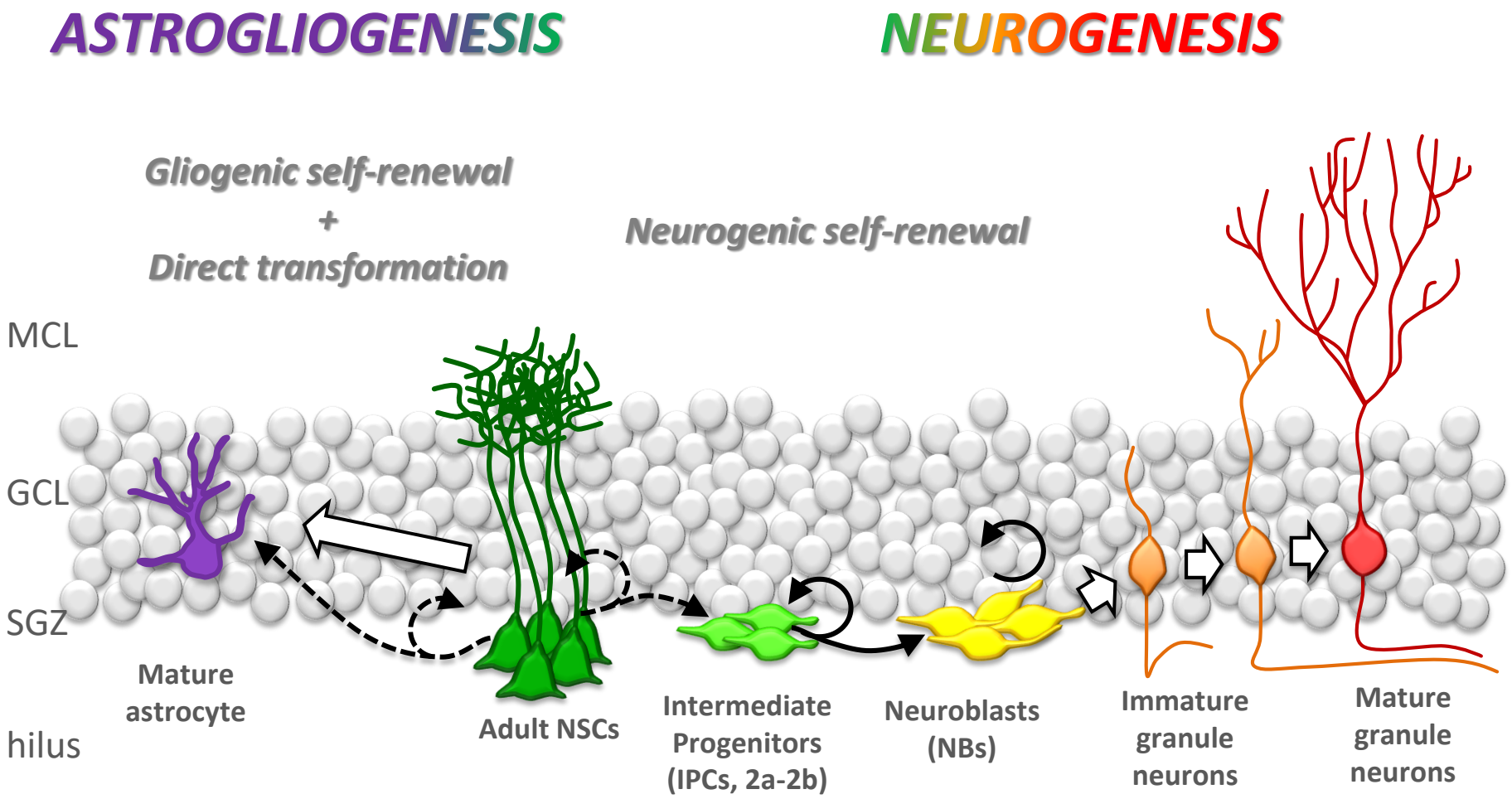


✓ ***Leaning and memory***

The neurogenic niche in adult mouse dentate gyrus (DG)

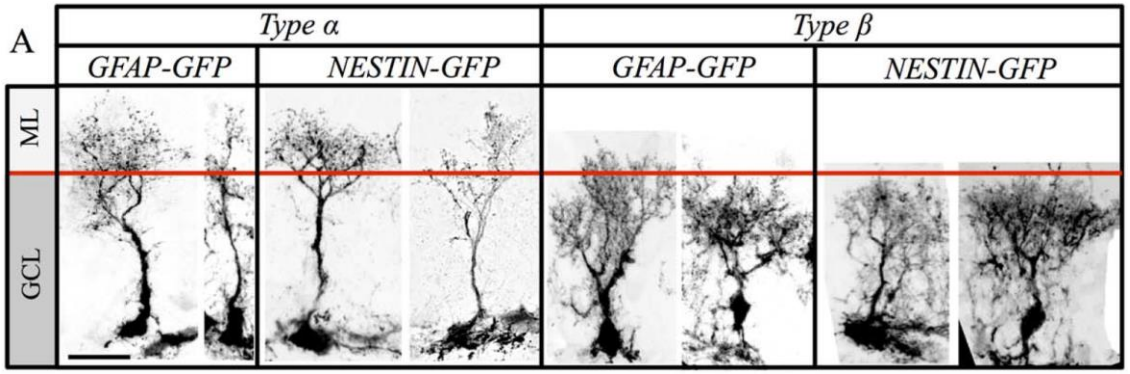


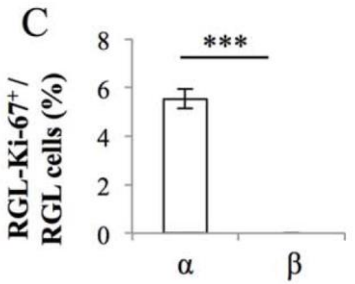
The neurogenic niche in adult mouse dentate gyrus (DG)



Heterogeneity of adult hippocampal NSCs

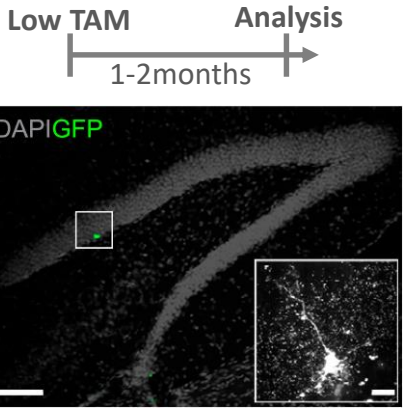
Morphological features and proliferative capabilities

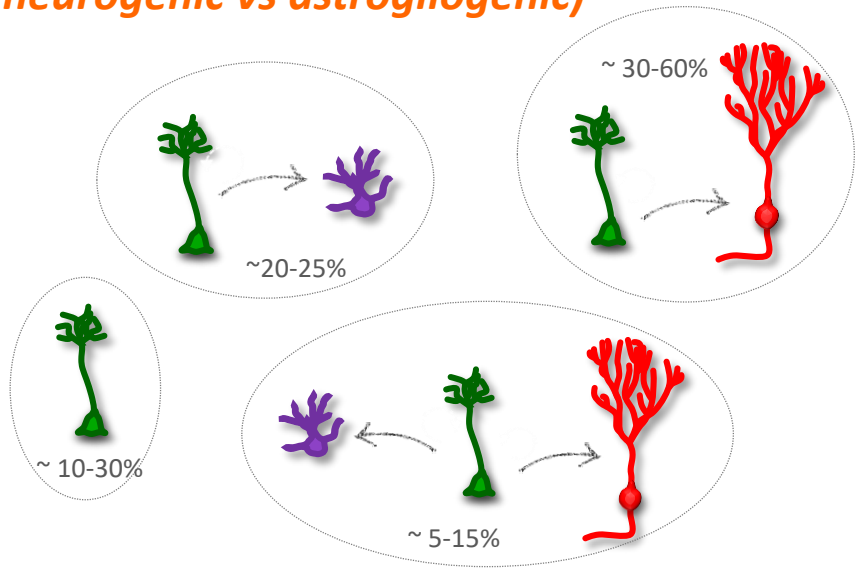




Gebara et al., Stem Cells 2016

Lineage potential (neurogenic vs astrogliogenic)

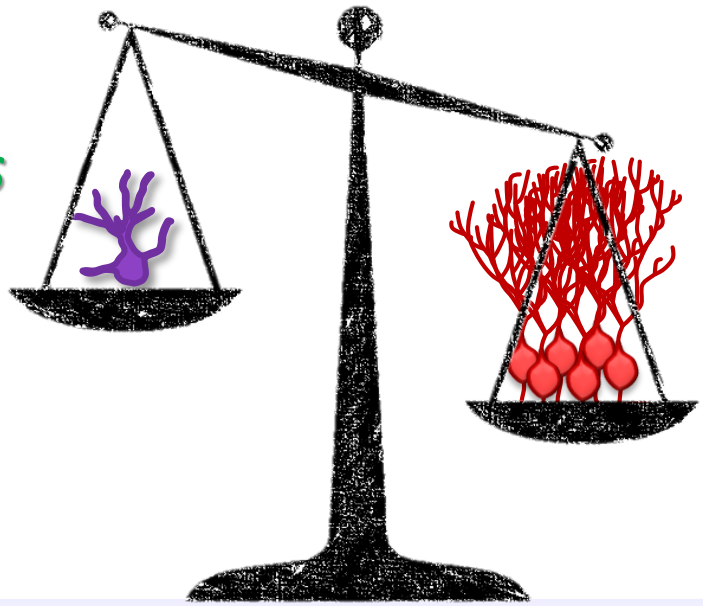




Bonaguidi et al., Cell 2011; Jeng et al., Cell Stem Cell 2013; Gebara e t al., Stem Cells 2016; Rolando et al., Cell Stem Cell 2016; Berg et al., Cell 2019

Physiological conditions

ASTROGLIOGENESIS



NEUROGENESIS

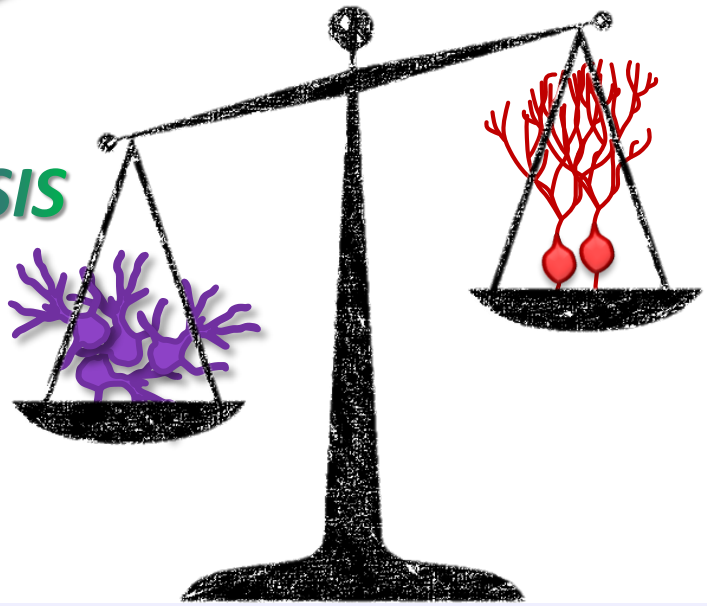
Pathological conditions disrupt the proper balance between DG neurogenesis and astrogliogenesis

AGING

INFLAMMATION

EPILEPSY

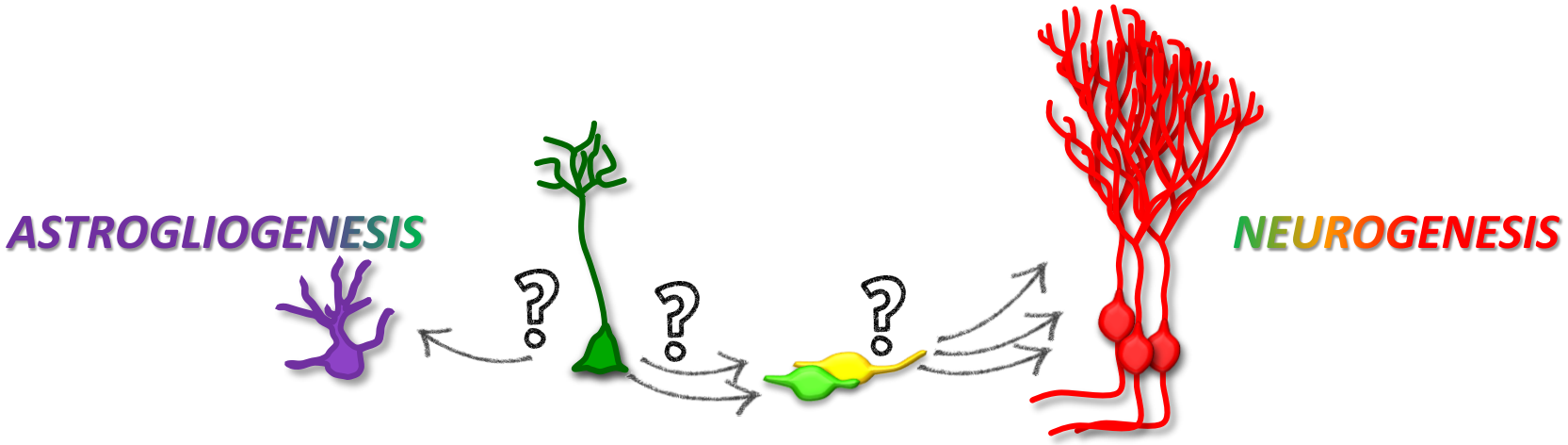
NEURODEGENERATION



ASTROGLIOGENESIS

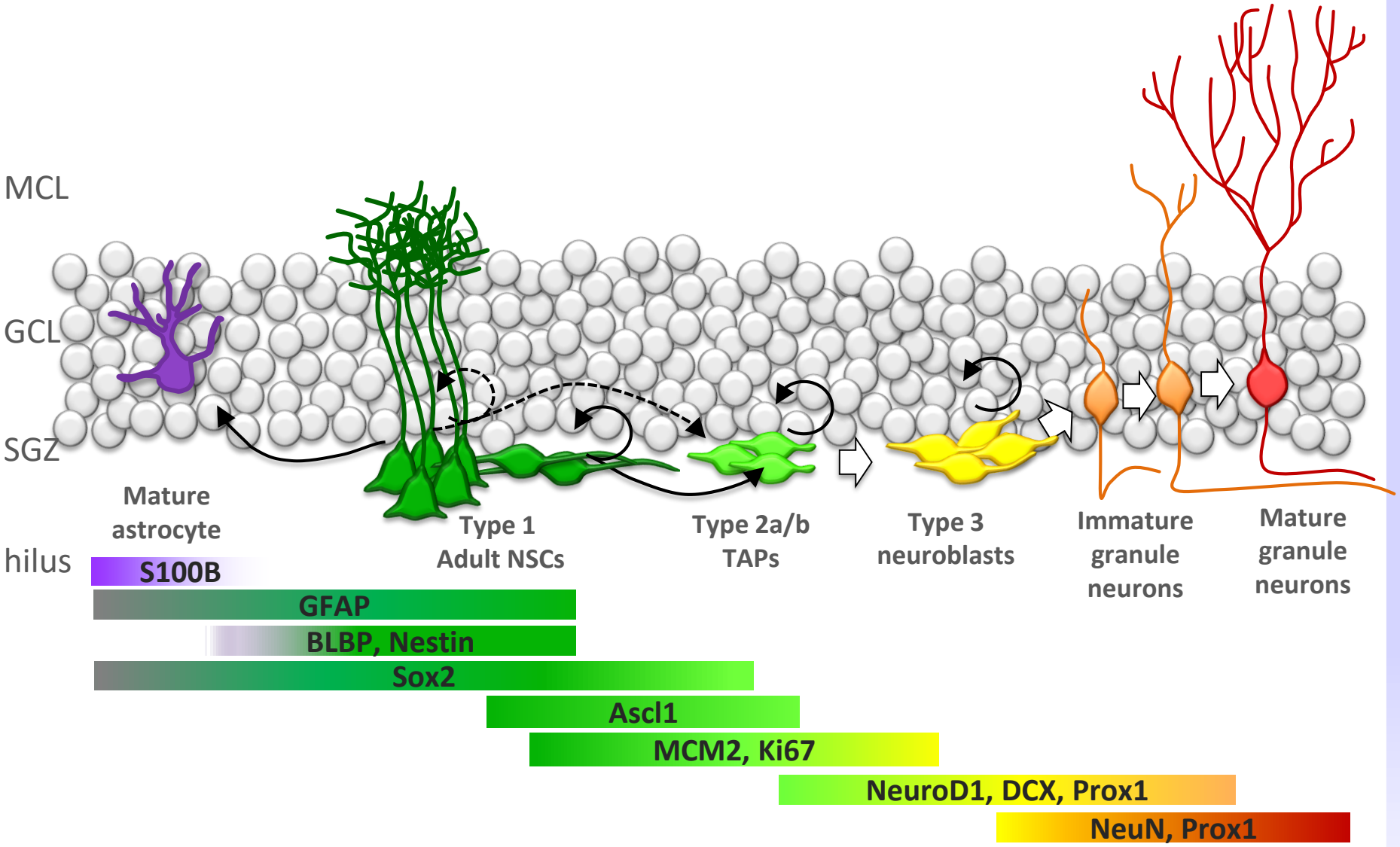
NEUROGENESIS

Which is the mechanism underlying adult DG neural stem cell/progenitor fate choice?

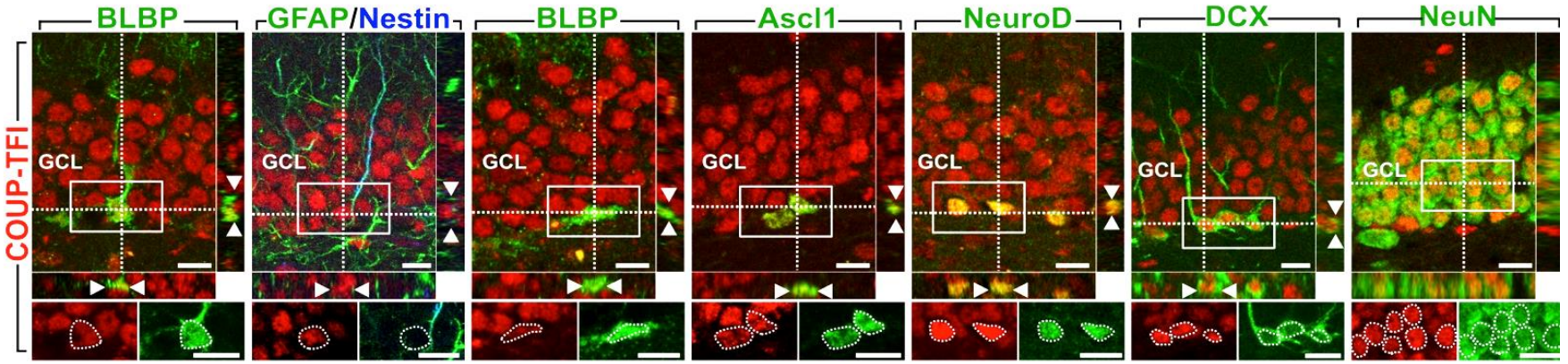


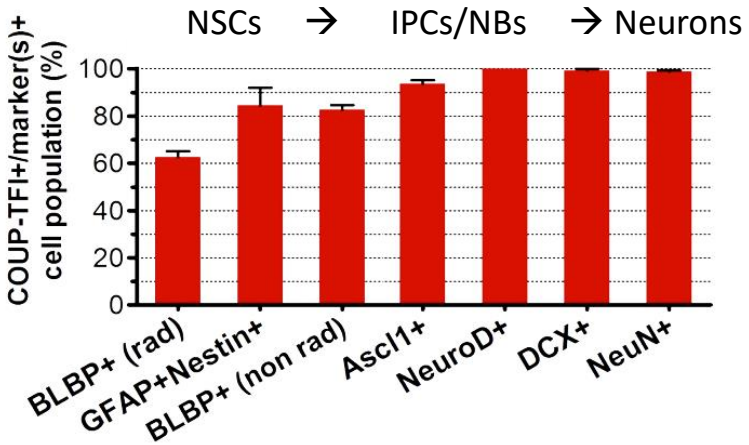
The transcription factor ***COUP-TFI/Nr2f1***

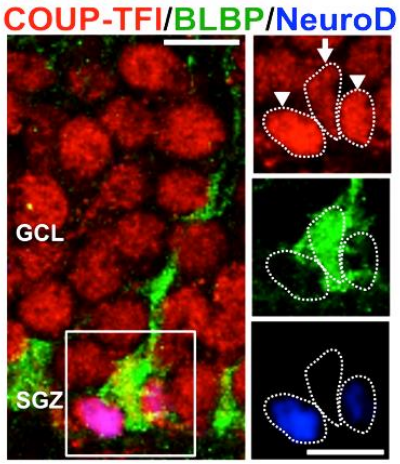
- ✓ **orfan nuclear receptor** of the steroid/thyroid hormone receptor family
- ✓ acts as an **activator and/or repressor** for target genes transcription
- ✓ **plays pleiotropic functions** during brain development
- ✓ emerging player in **adult brain plasticity**
- ✓ **its haploinsufficiency causes the BBSOAS** (OMIN#615722)

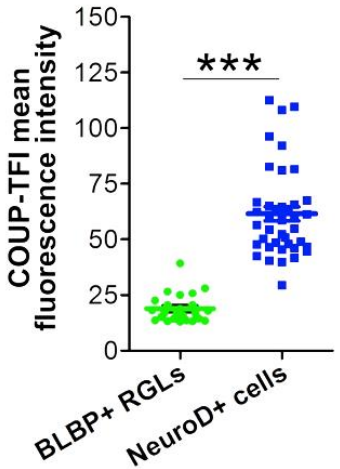

MCL
GCL
SGZ
hilus
Mature astrocyte
Type 1 Adult NSCs
Type 2a/b TAPs
Type 3 neuroblasts
Immature granule neurons
Mature granule neurons
S100B
GFAP
BLBP, Nestin
Sox2
Ascl1
MCM2, Ki67
NeuroD1, DCX, Prox1
NeuN, Prox1

COUP-TFI is expressed in a subset of hippocampal NSCs and upregulated during neuronal lineage progression



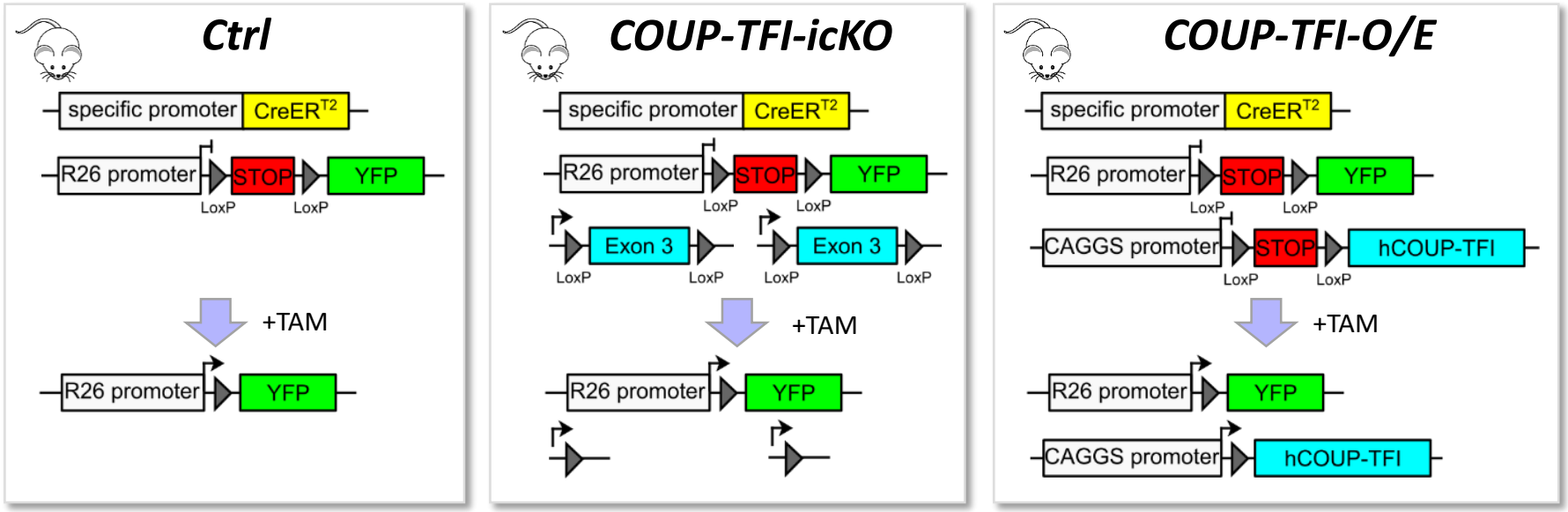




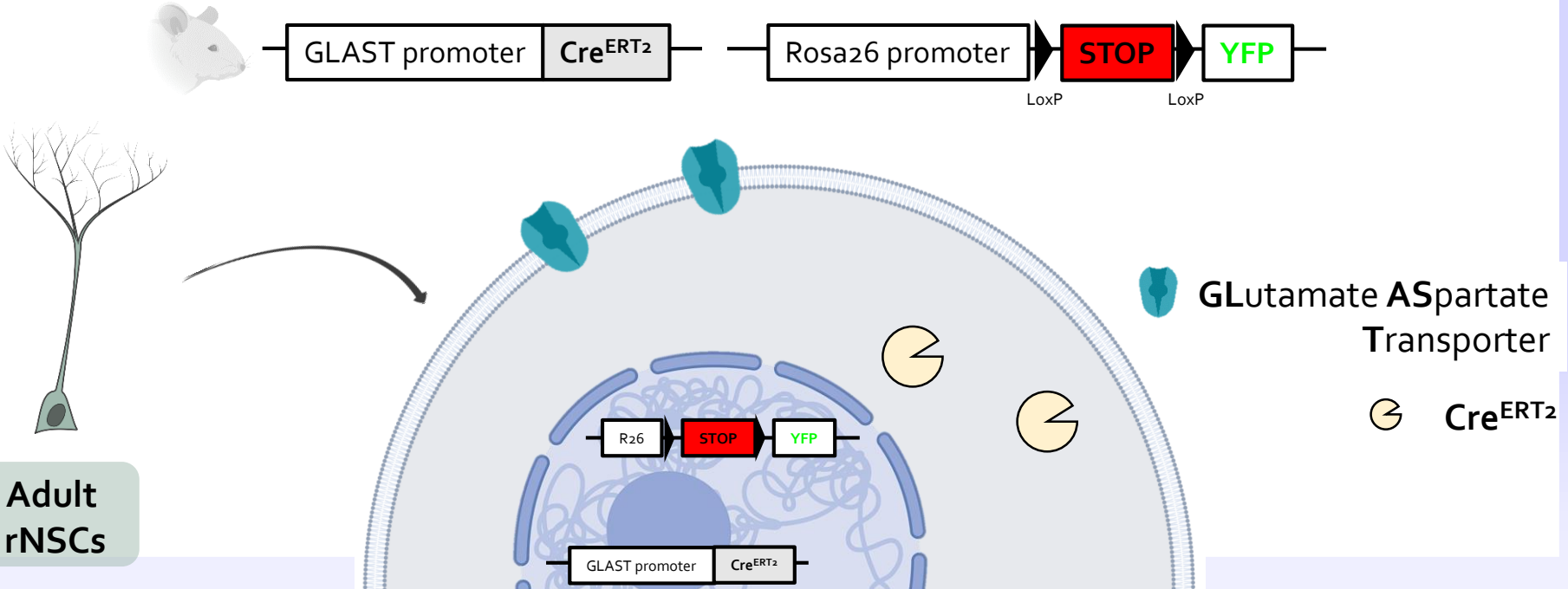


Bonzano et al., Cell Reports 2018

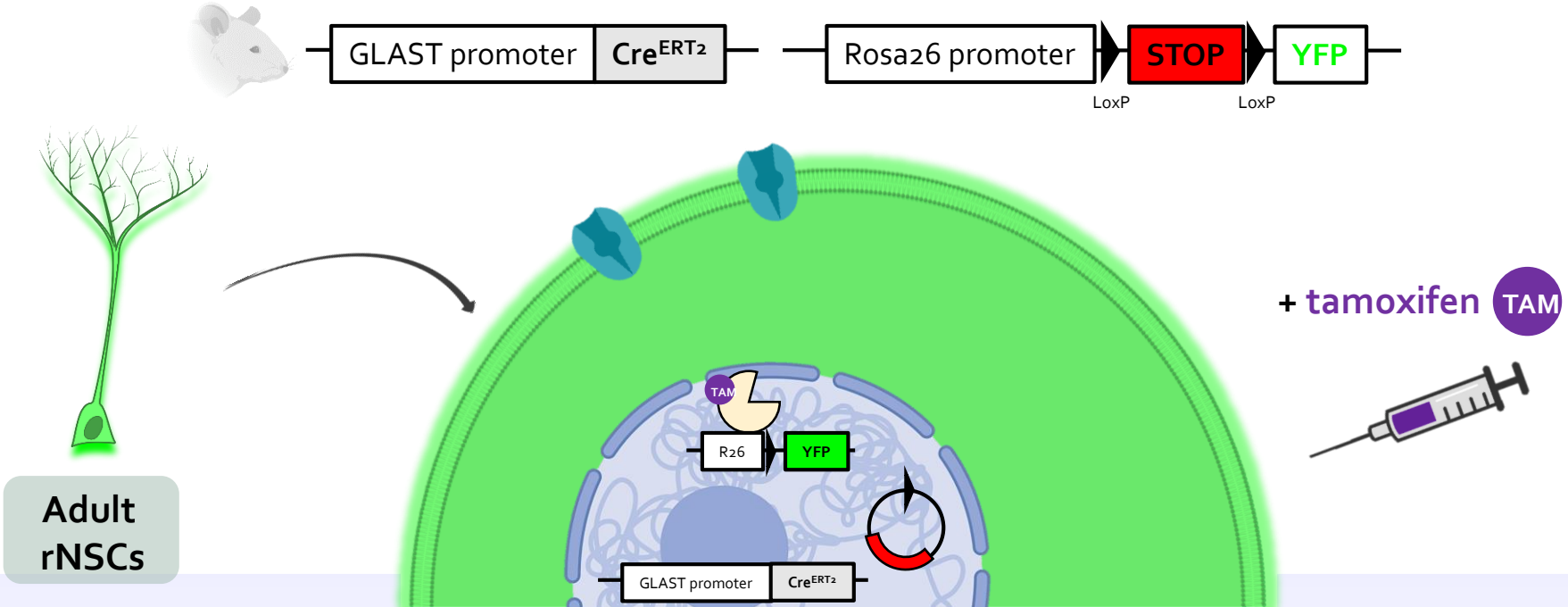
COUP-TFI loss- and gain-of-function in the DG niche through Cre/loxP technology coupled to genetic fate mapping



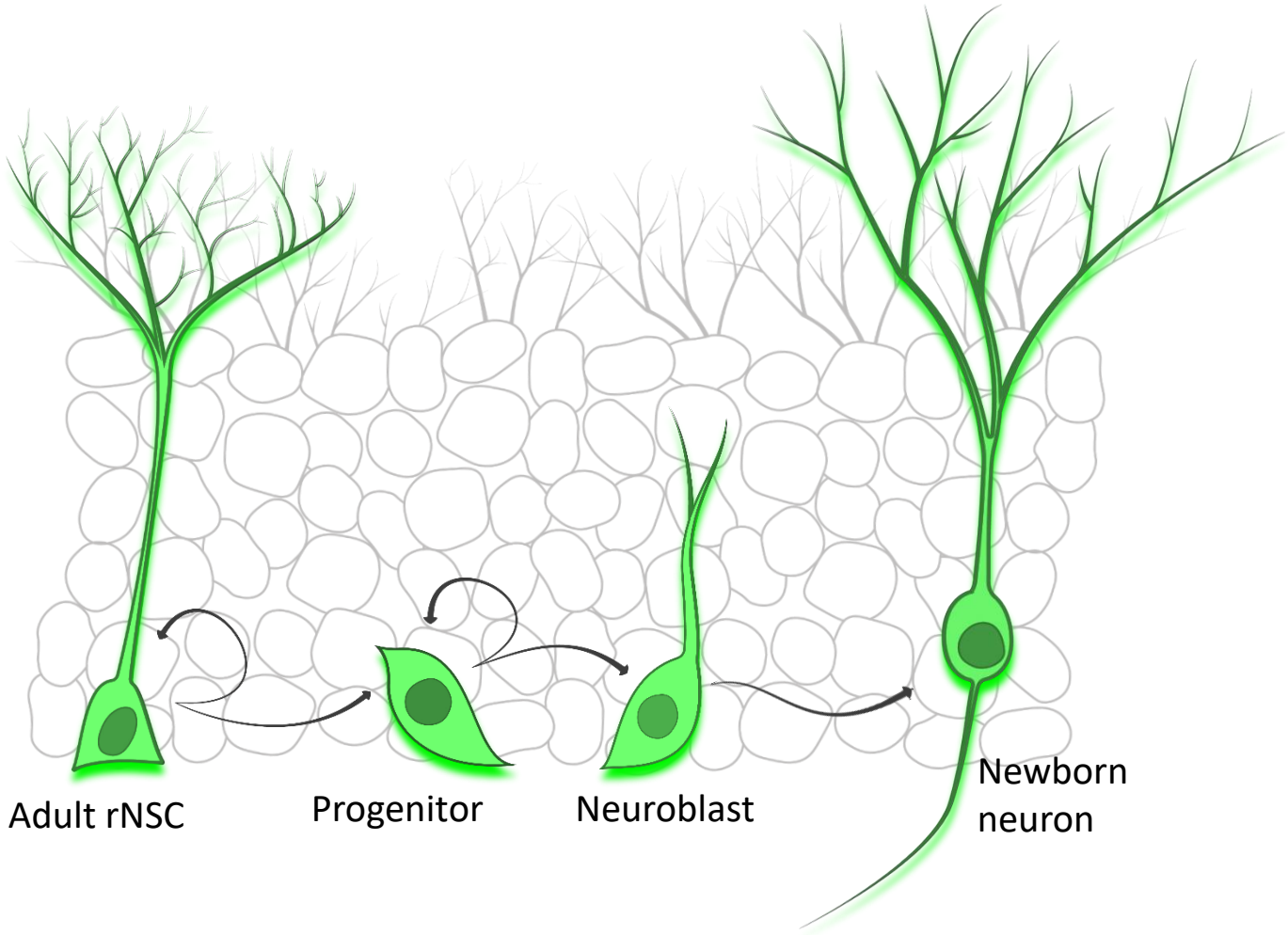
Cre/loxP dependent genetic fate mapping



Cre/loxP dependent genetic fate mapping



Cre/loxP dependent genetic fate mapping



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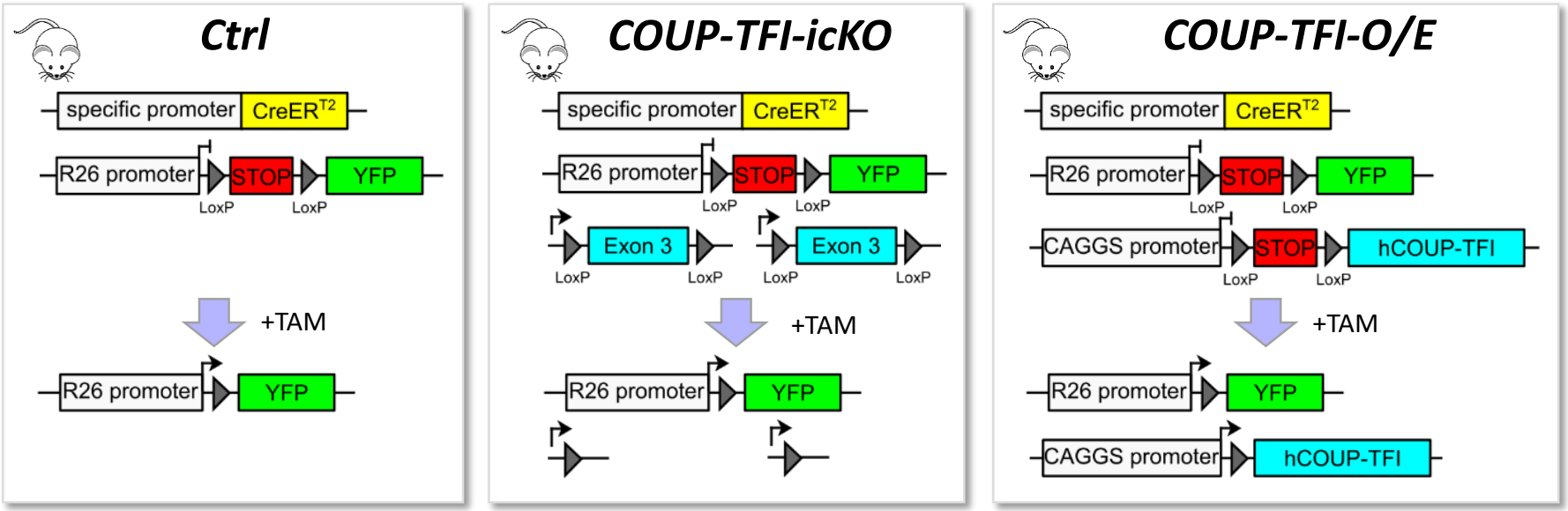
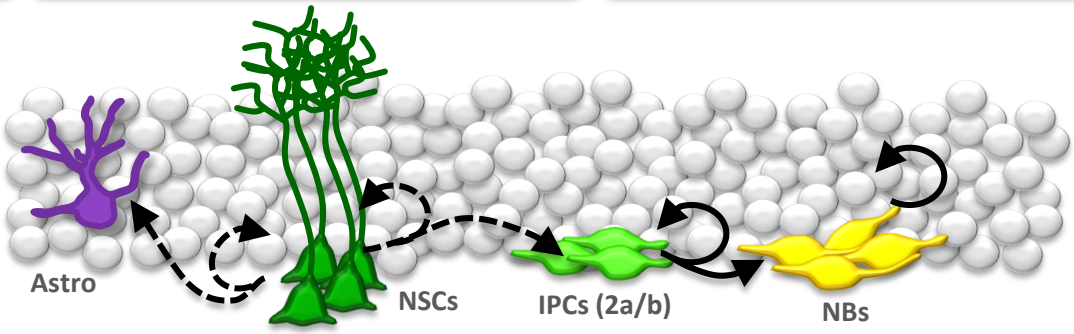


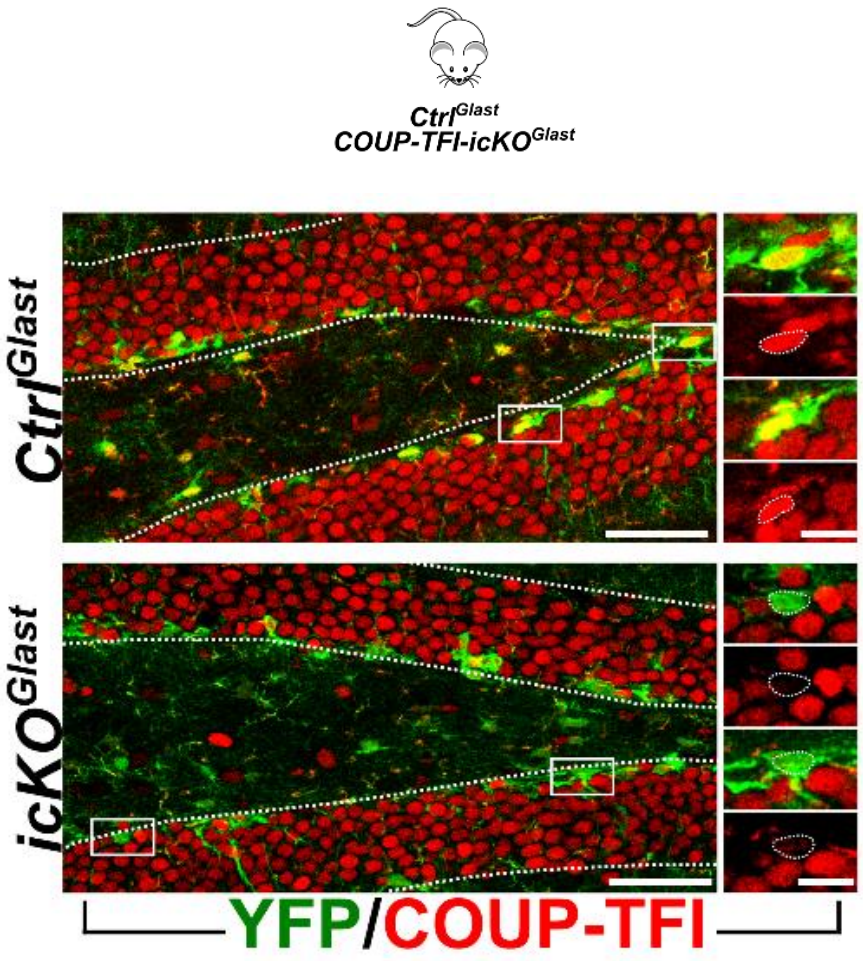


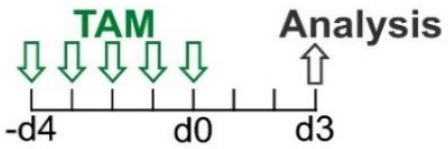
Glast-CreER^{T2} (+TAM) — *NSCs (both quiescent and active) + astrocytes*

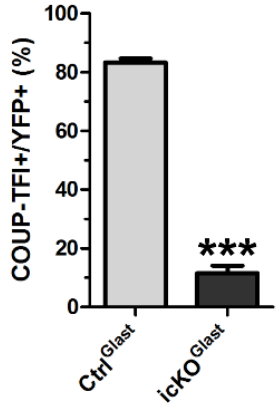
Ascl1-CreER^{T2} (+TAM) — *IPCs (2a) + NSCs (active; few)*

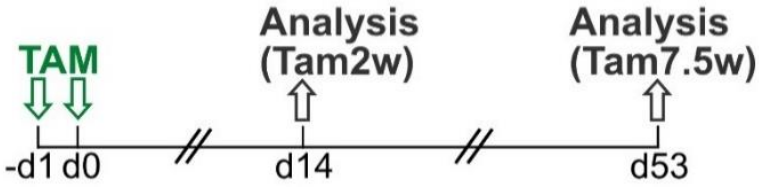
RetroVirus-Cre (Cre) — *IPCs/NBs*

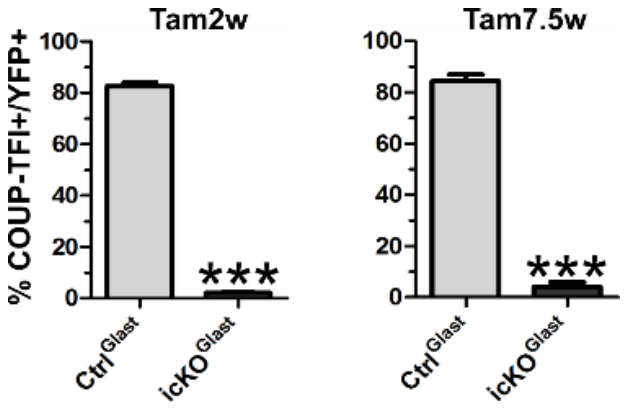
Efficient *COUP-TFI* deletion in the Glast lineage





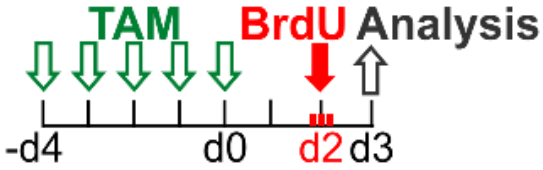


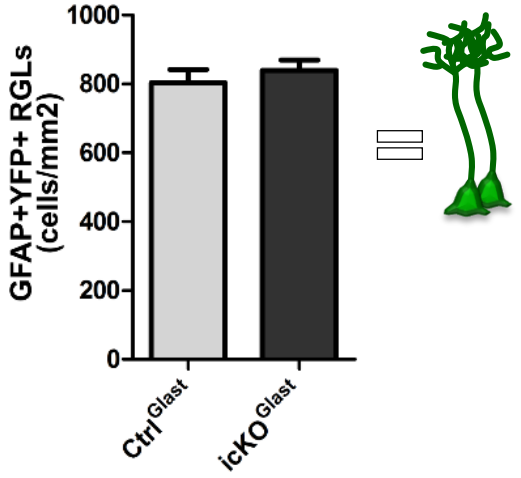


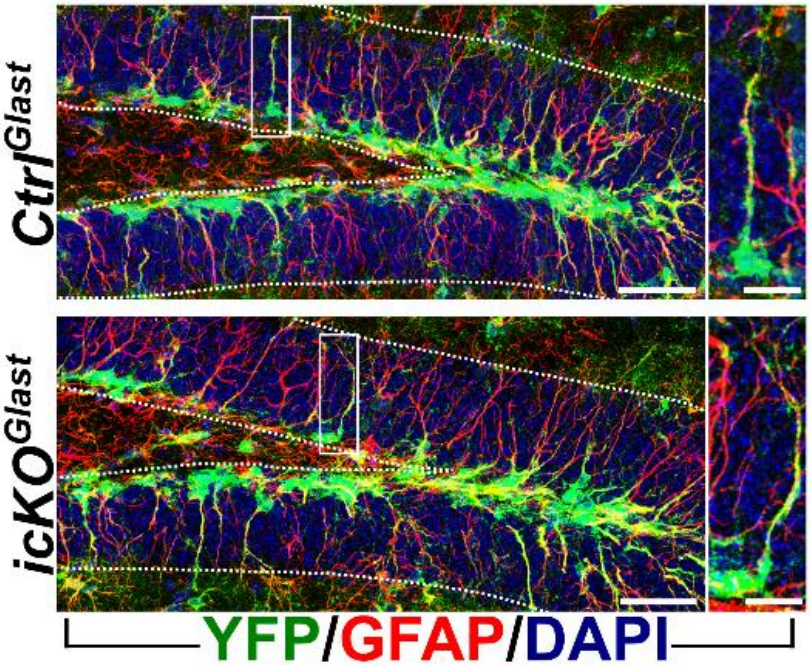


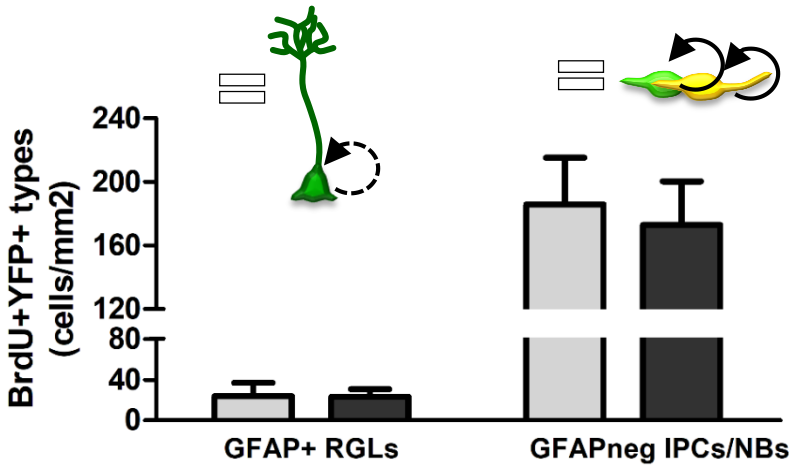
Bonzano et al., Cell Reports 2018

Short-term COUP-TFI loss of function does not alter radial NSC pool and progenitor cell proliferation



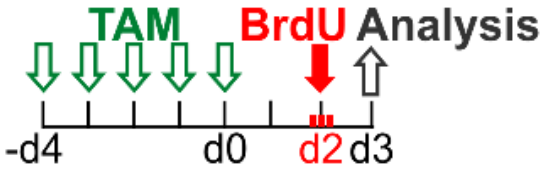


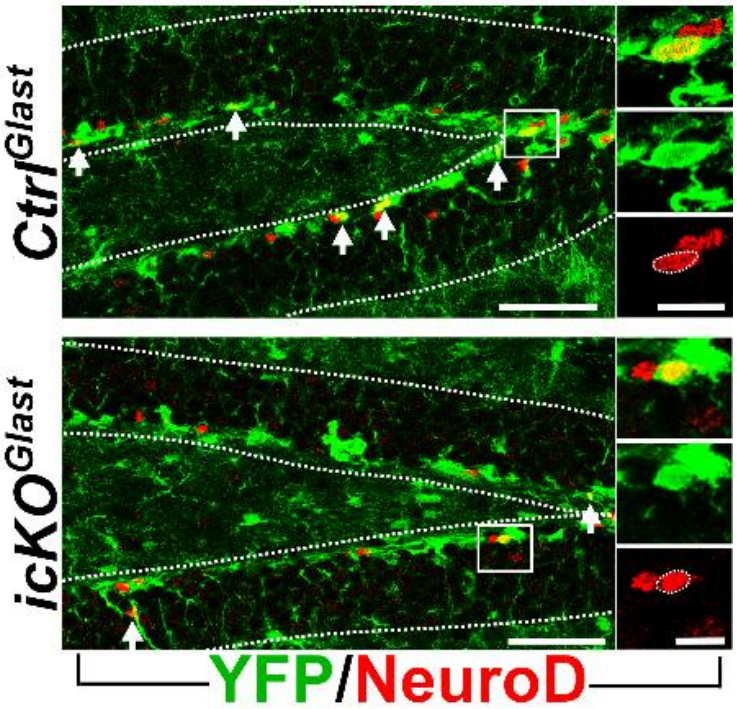


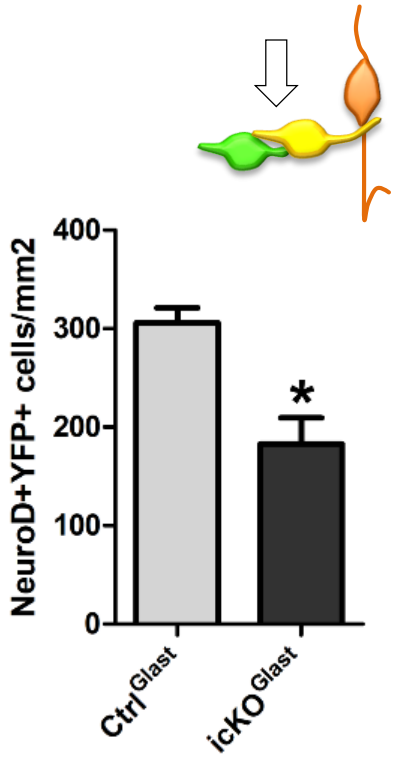


Bonzano et al., Cell Reports 2018 and unpublished

Short-term COUP-TFI loss of function reduces neuronal-committed progenitors and neuroblasts



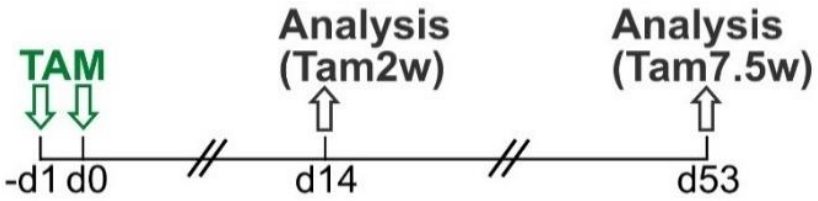


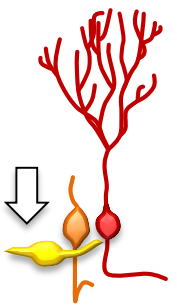


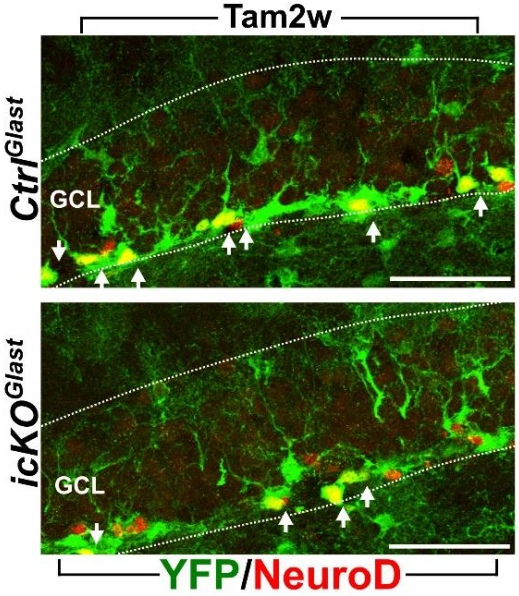
Bonzano et al., Cell Reports 2018

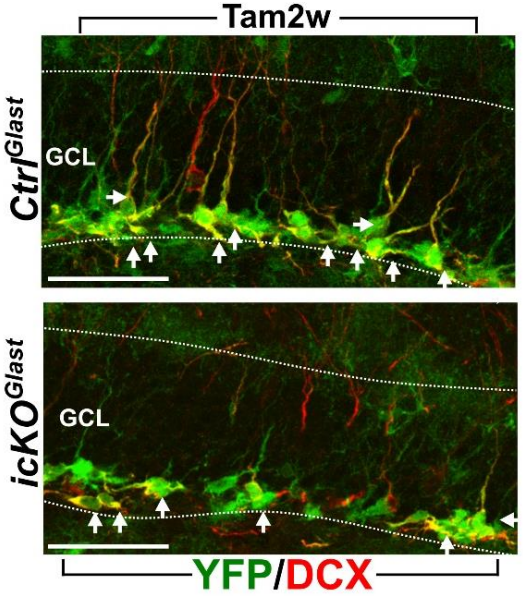
Long-term COUP-TFI deletion impairs DG neurogenesis

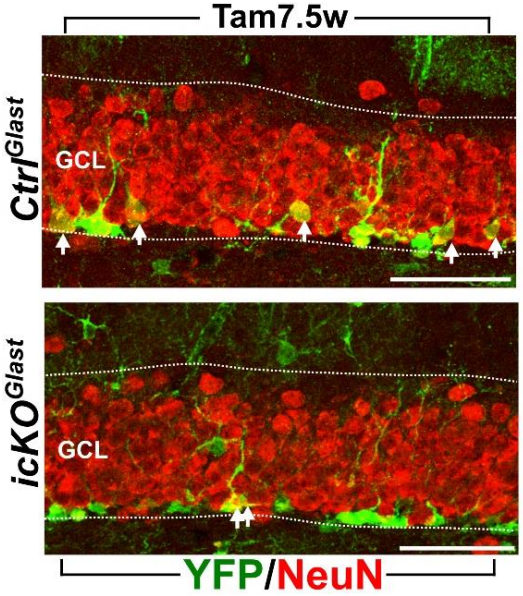
Ctrl^{Glast}
COUP-TFI-icKO^{Glast}

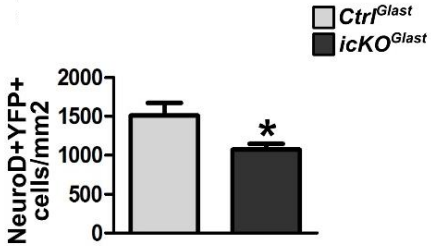


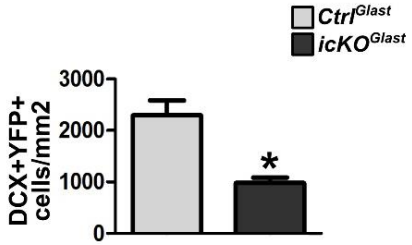


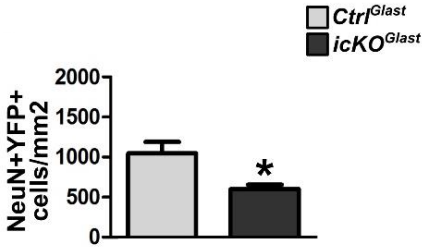




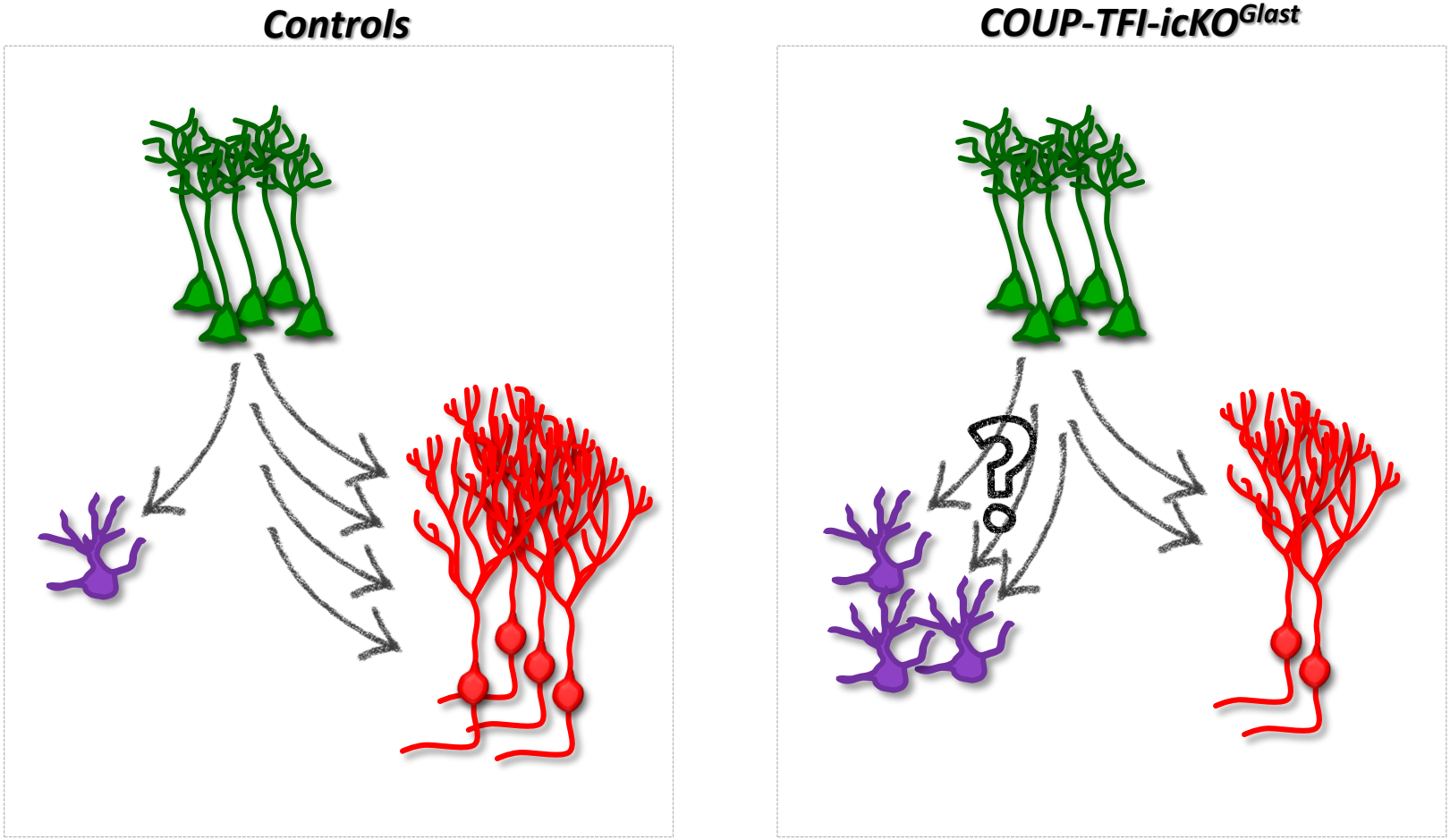




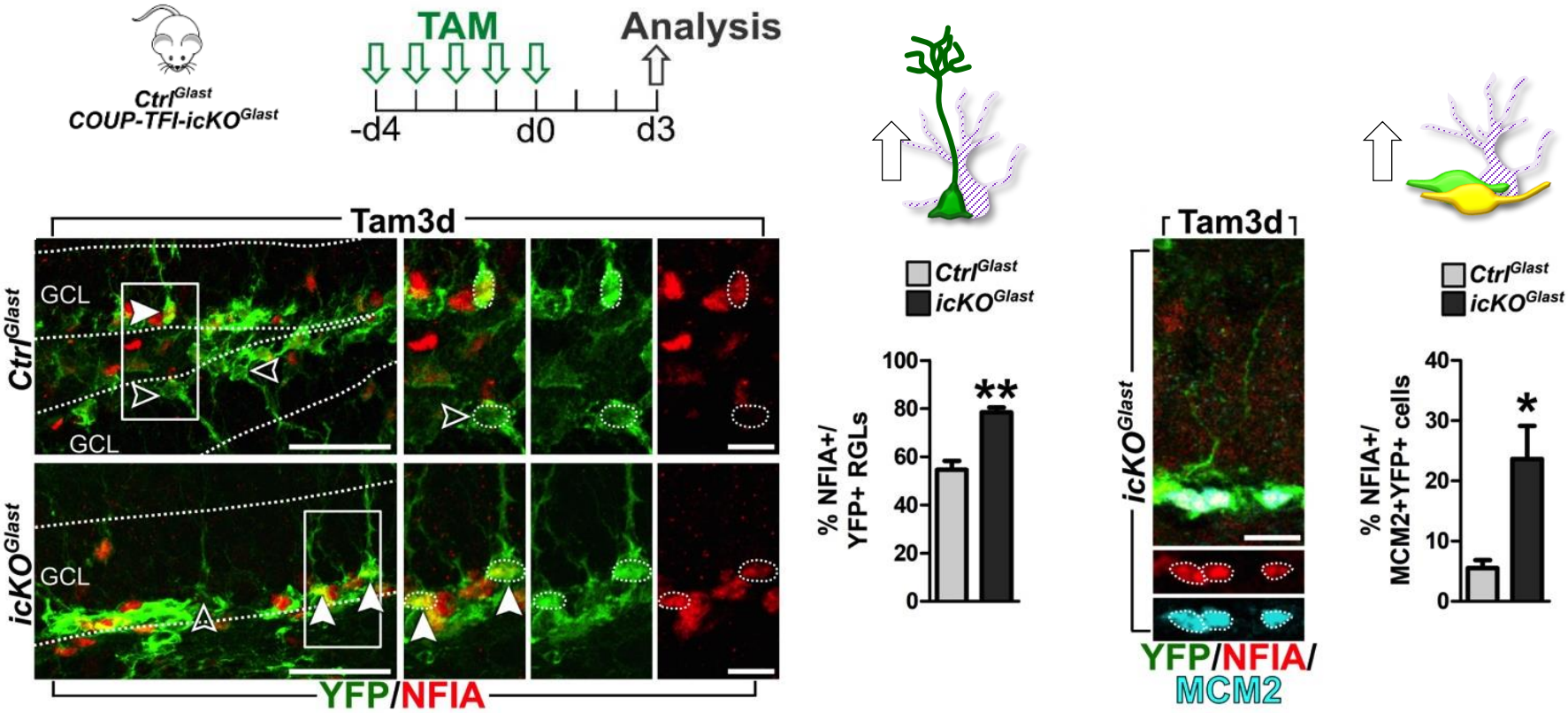




Bonzano et al., Cell Reports 2018


Controls
COUP-TFI-icKO Glast
?

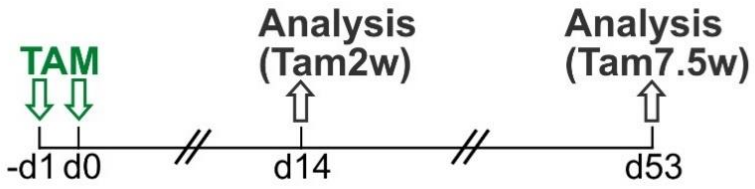
Adult COUP-TFI-depleted NSCs/progenitors increase the expression of the pro-astrogliogenic factor NFIA

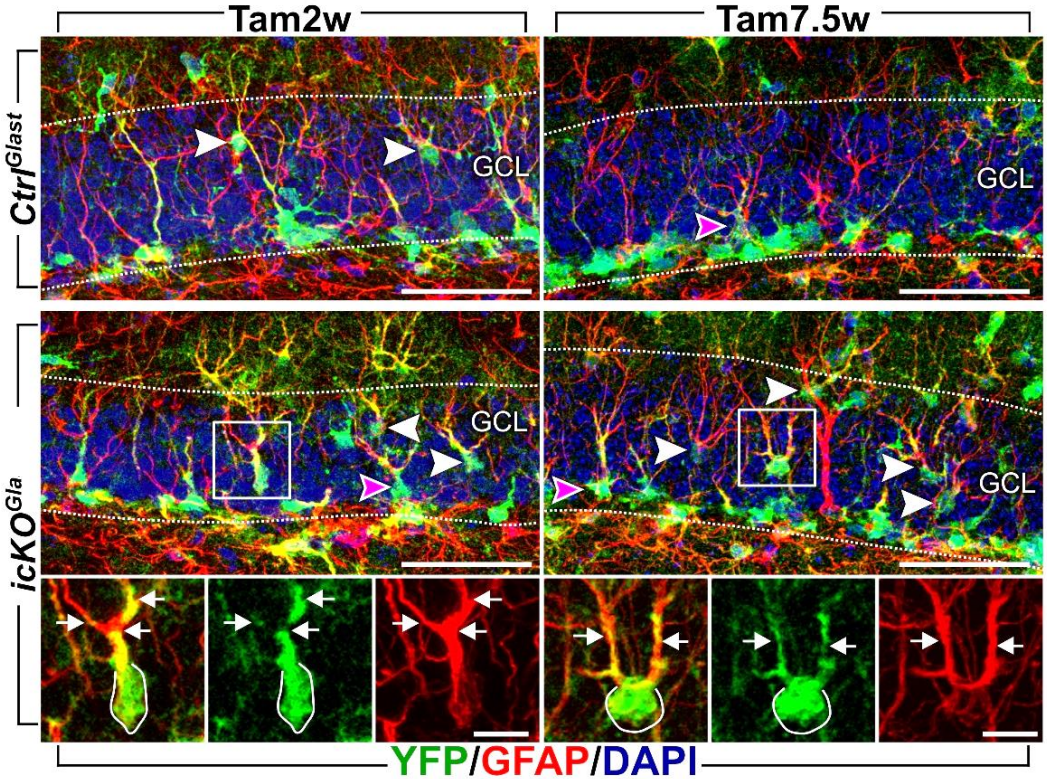


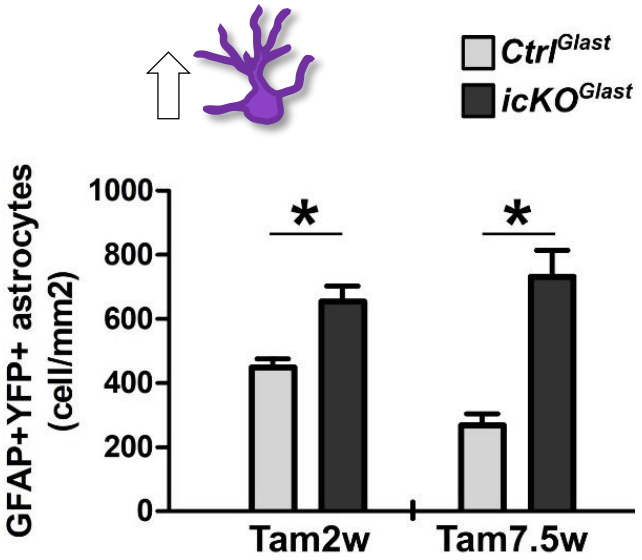
→ Switch of COUP-TFI deficient NSC/progenitor commitment towards an astroglial fate

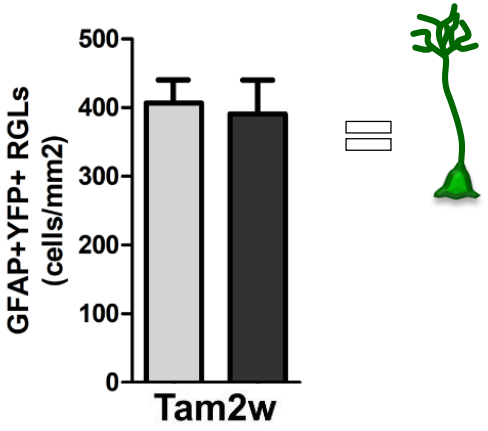
Bonzano et al., Cell Reports 2018

Enhanced astrogliogenesis in *COUP-TFI-icKO^{Glast}* DG







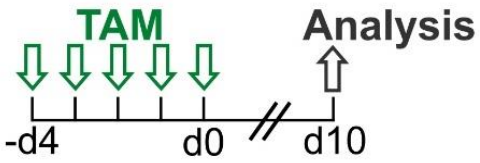


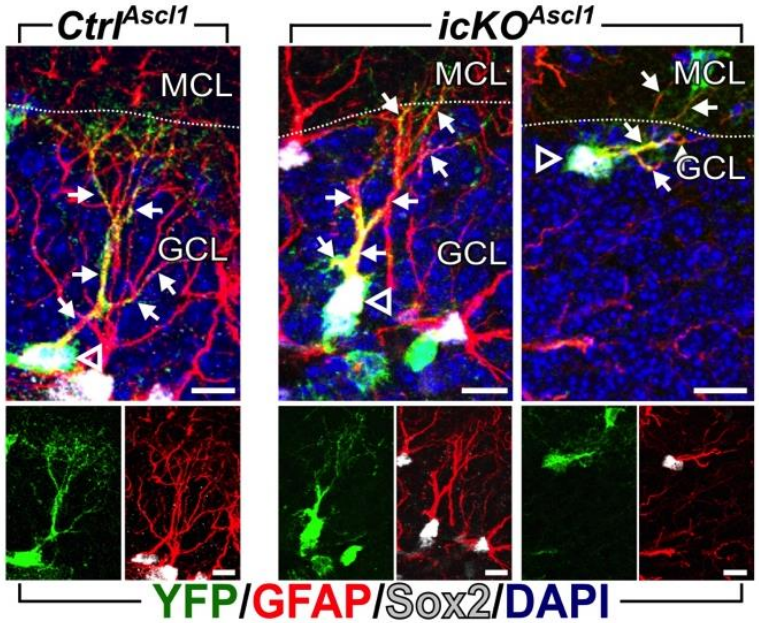
→ No direct transformation

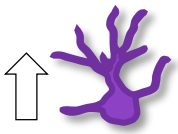
Bonzano et al., Cell Reports 2018

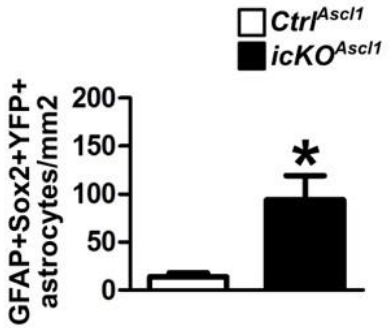
COUP-TFI-KO Ascl1+ active NSCs/neurogenic progenitors increase astrogliogenesis and decrease neurogenesis

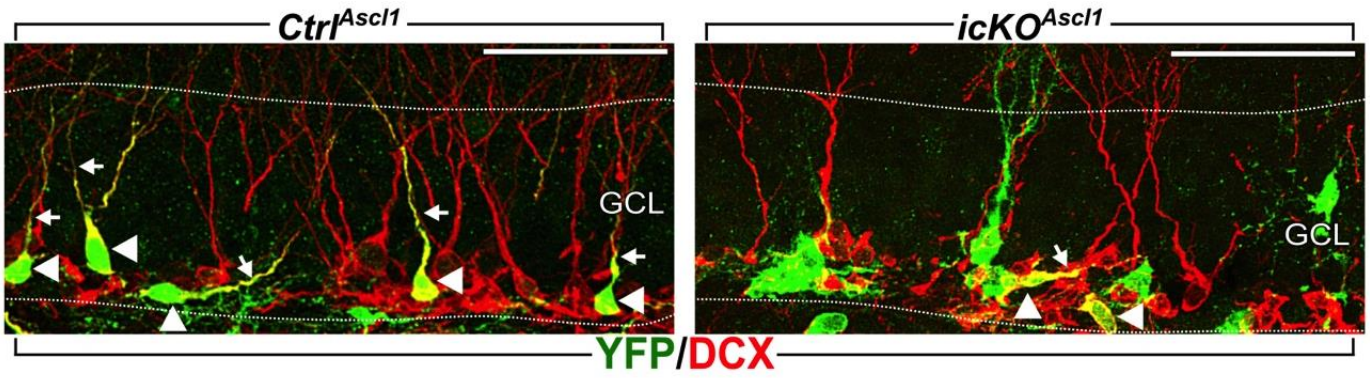
$Ctrl^{Ascl1}$
$COUP\text{-}TFI\text{-}icKO^{Ascl1}$

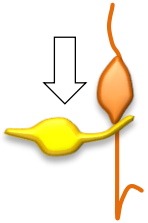


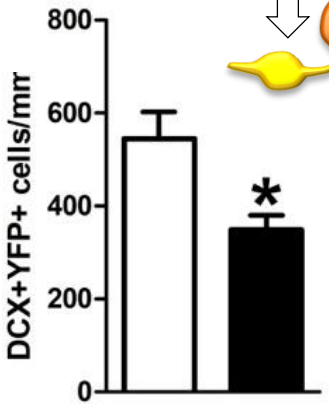






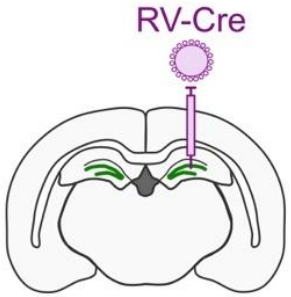




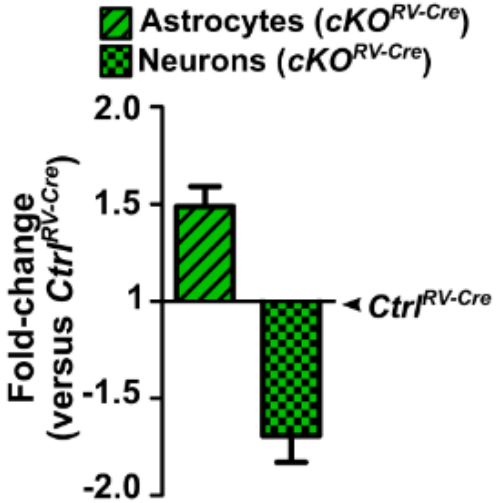


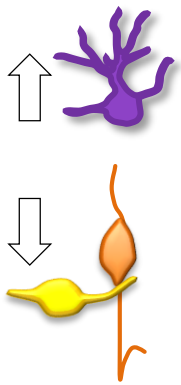
Bonzano et al., Cell Reports 2018

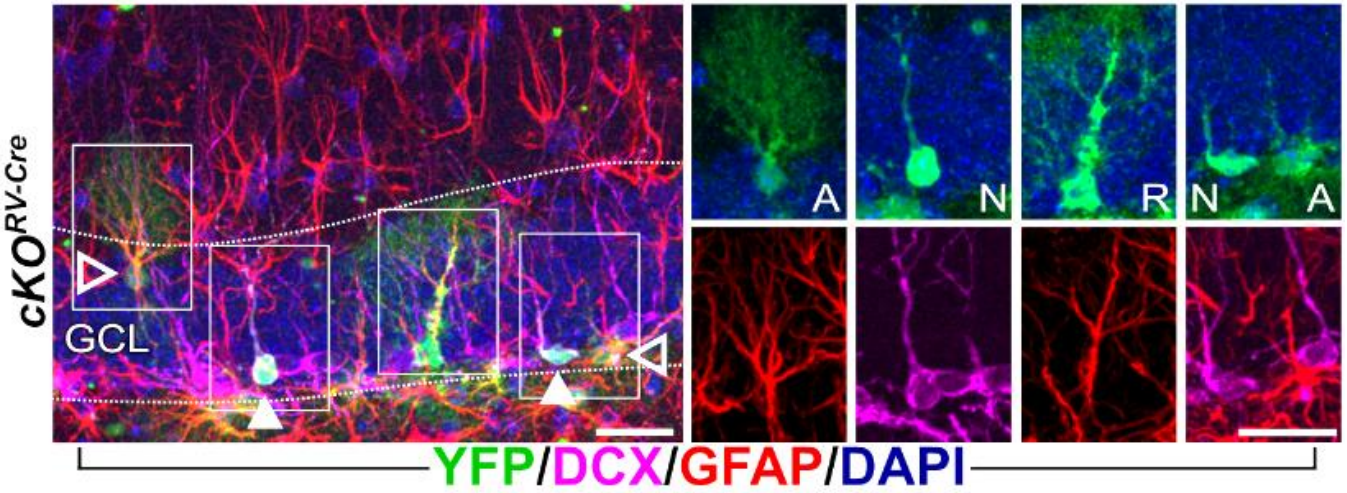
COUP-TFI restricts adult DG proliferating progenitor potential towards neurogenesis



1. *Ctrl$^{RV\text{-}Cre}$ (R26-floxed stop-YFP)*
2. *COUP-TFI-icKO$^{RV\text{-}Cre}$ (R26-floxed-stop-YFP;COUP-TFIfl/fl)*

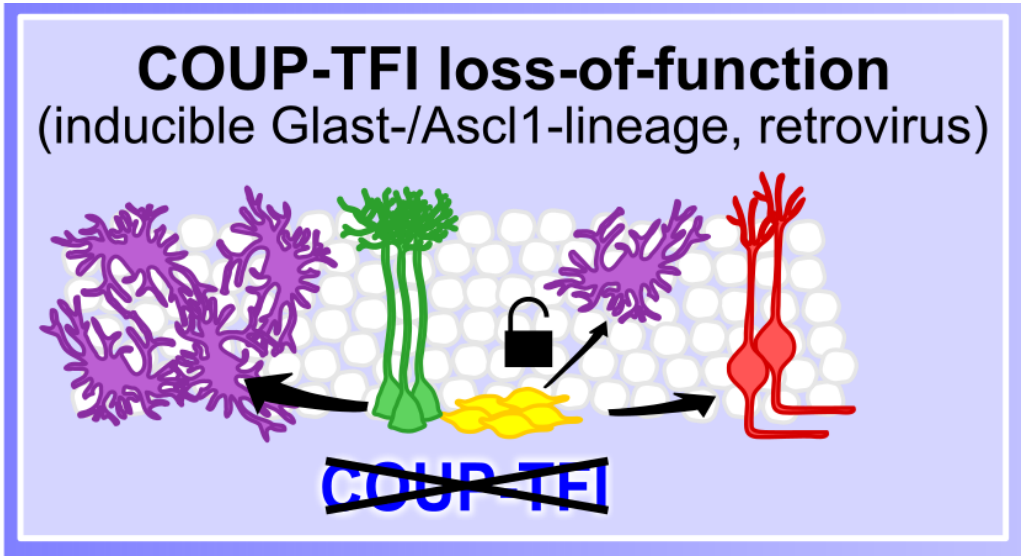






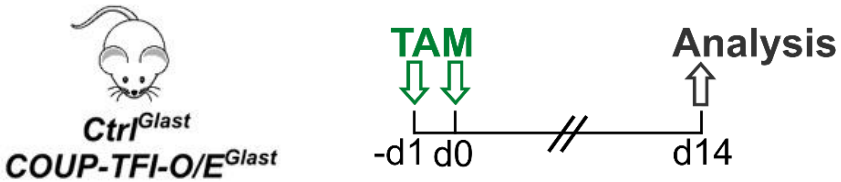
Bonzano et al., Cell Reports 2018

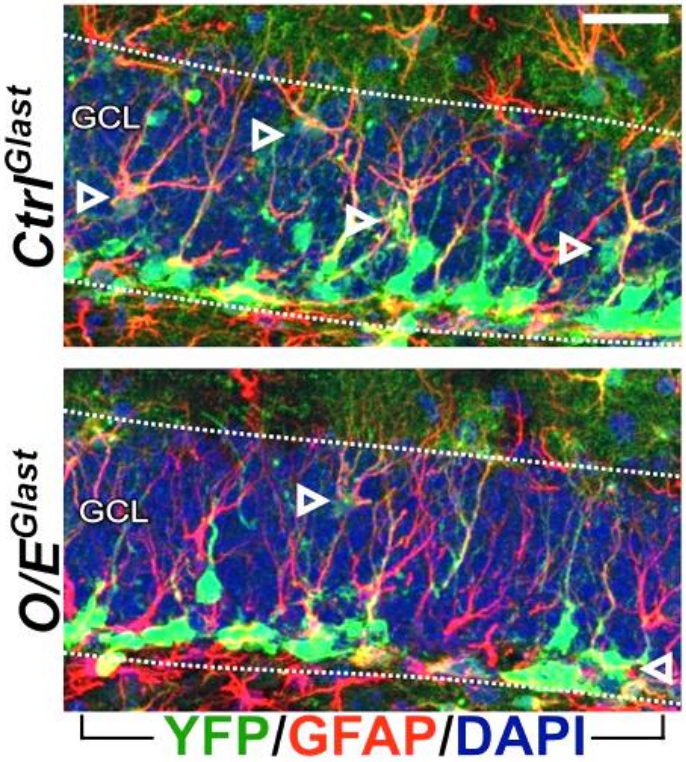
COUP-TFI deletion in RGL and neurogenic progenitors promotes astrogliogenesis at the expense of neurogenesis

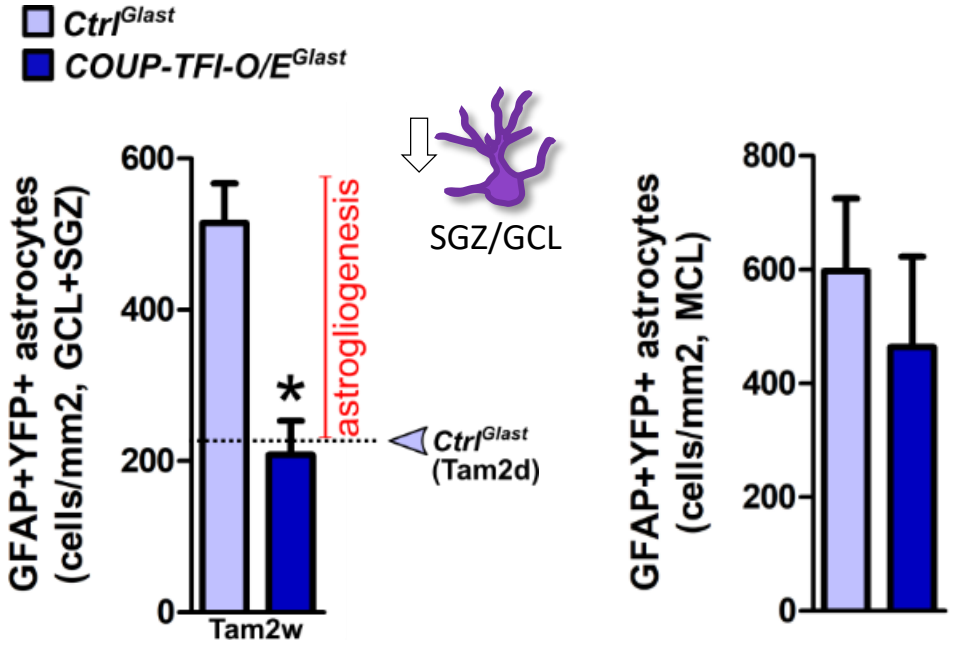


→ COUP-TFI sustains neurogenesis all along the neurogenic lineage by exerting an anti-astrogliogenic action on adult mouse hippocampal NSCs/progenitors

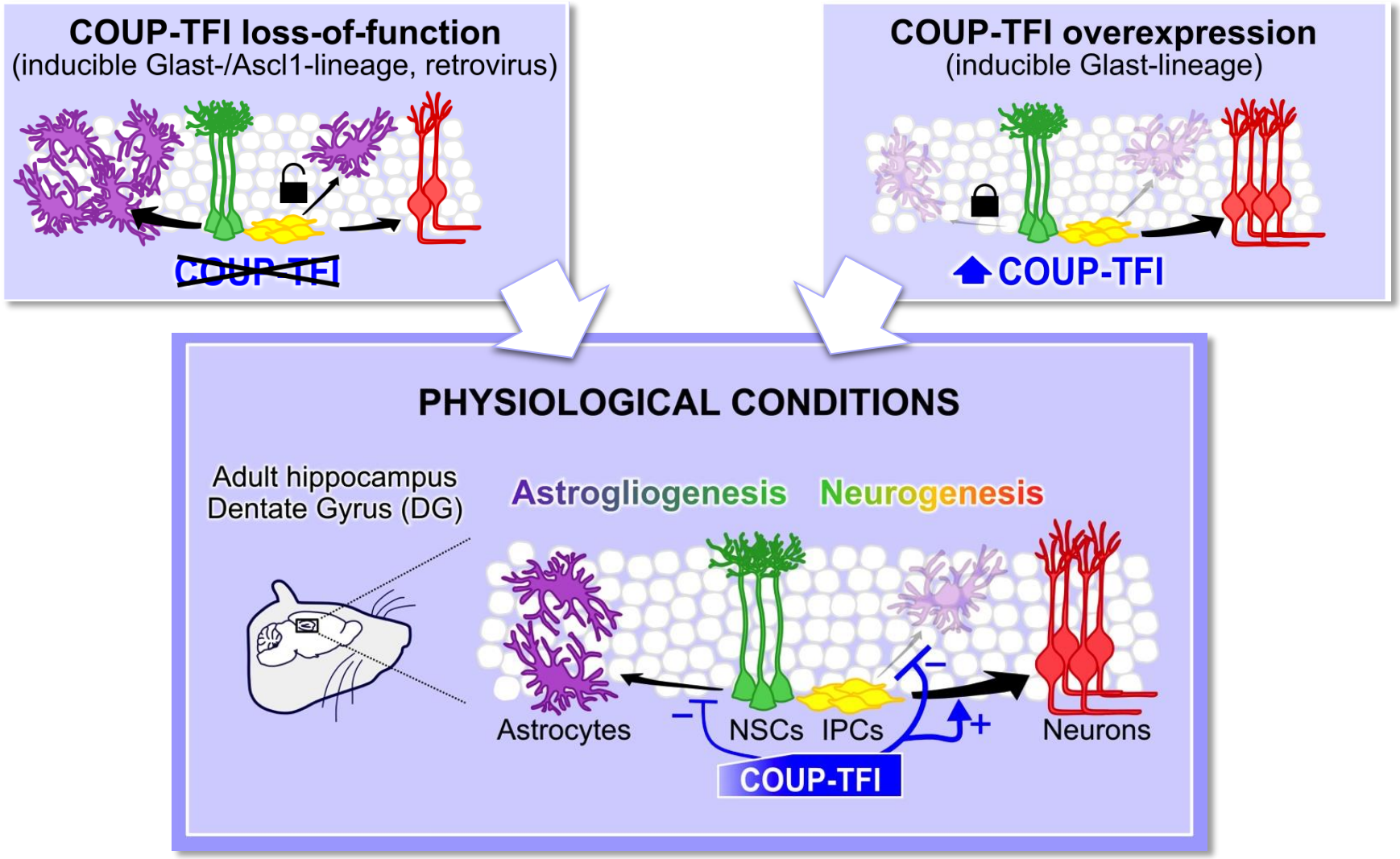
COUP-TFI overexpression (O/E) in the adult NSC lineage blocks hippocampal astrogliogenesis







Bonzano et al., Cell Reports 2018

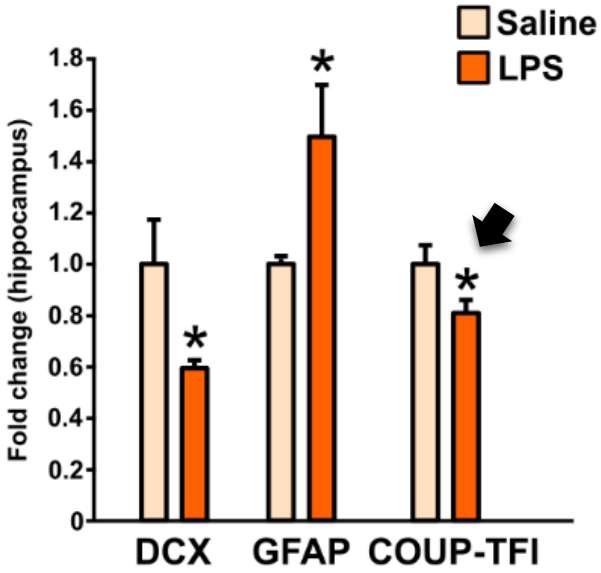


COUP-TFI is necessary to promote neurogenesis from **adult NSCs** and **neuronal committed progenitors** by repressing their commitment towards an astroglial fate

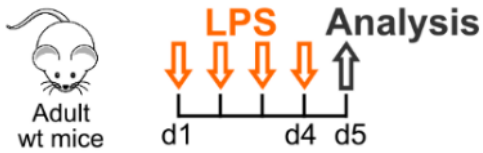
Neuroinflammation model: *E.coli* lipopolysaccharide (LPS)

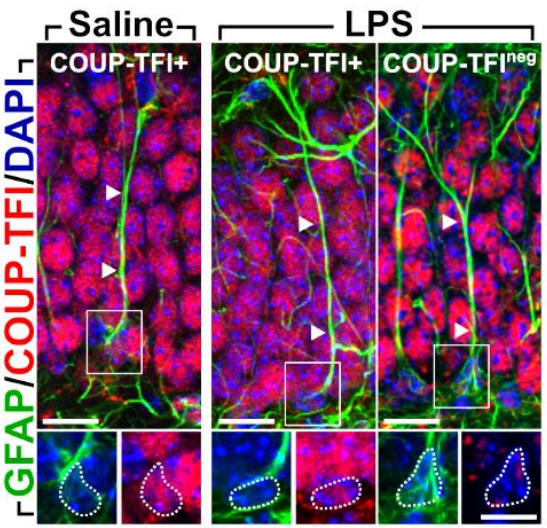
I. Crisci

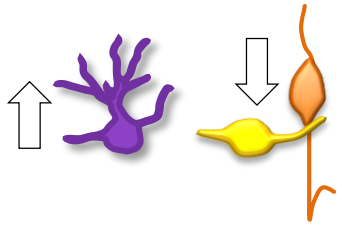
1. RT-qPCR:

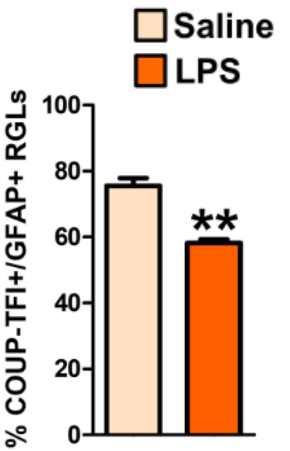


2. IFL:



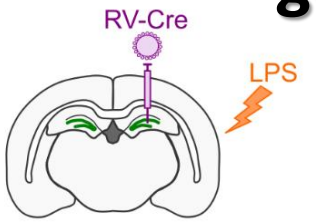


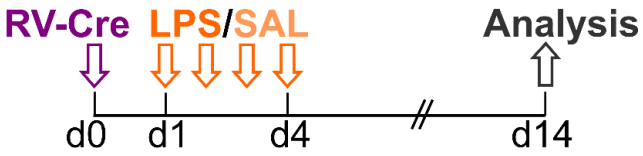




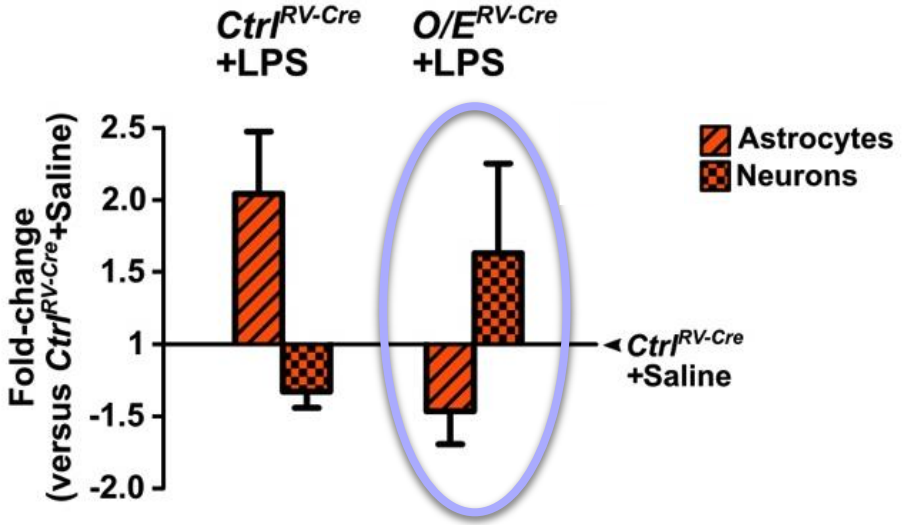
Bonzano et al., Cell Reports 2018

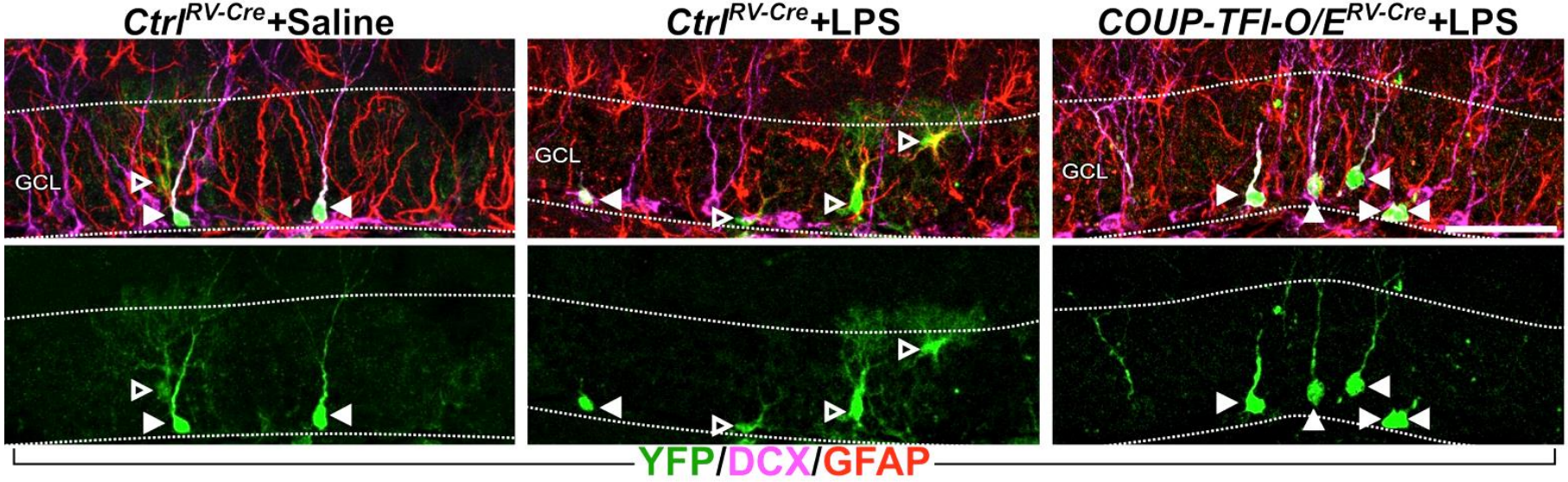
Forcing COUP-TFI expression rescues neuron-to-astrocyte generation shift upon neuroinflammation





1. $Ctrl^{RV\text{-}Cre}$ + Saline
2. $Ctrl^{RV\text{-}Cre}$ + LPS
3. $COUP\text{-}TFI\text{-}O/E^{RV\text{-}Cre}$ + LPS

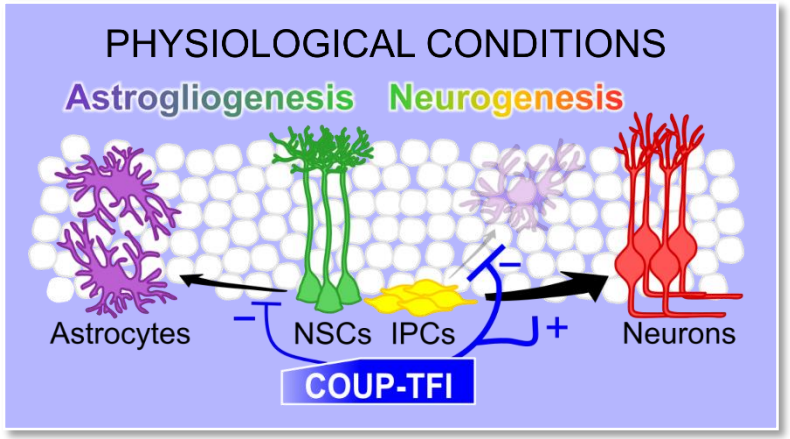


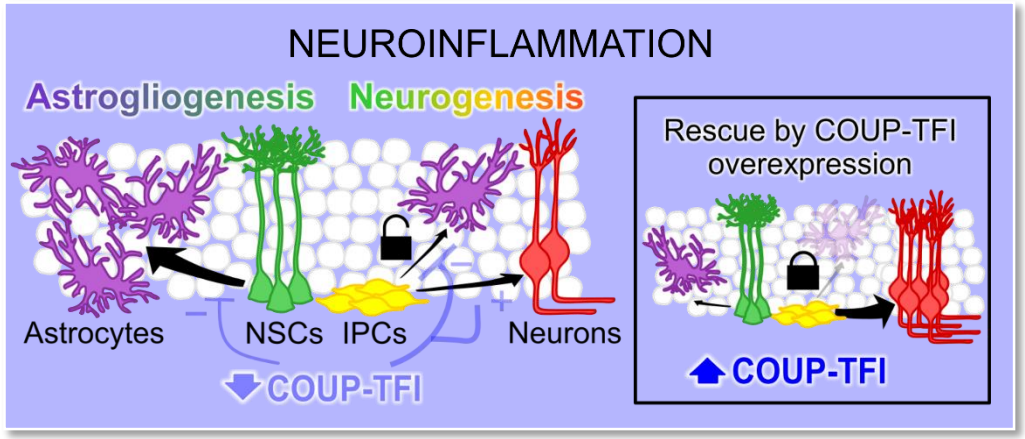


Bonzano et al., Cell Reports 2018

To sum up…

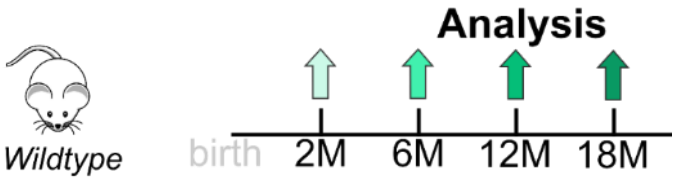
- ✓ COUP-TFI loss increases DG astrocytes by fostering astrogliogenesis from adult NSCs and unlocking a gliogenic potential in neuronal progenitors
- ✓ Forced COUP-TFI expression inhibit astrogliogenesis from adult hippocampal NSCs
- ✓ COUP-TFI is necessary and sufficient to restrict the entire adult DG neurogenic lineage towards neurogenesis
- ✓ Neurogenesis-to-astrogliogenesis switch is reverted by COUP-TFI gain-of-function upon neuroinflammation, suggesting that it may protect the DG niche from inflammatory insults

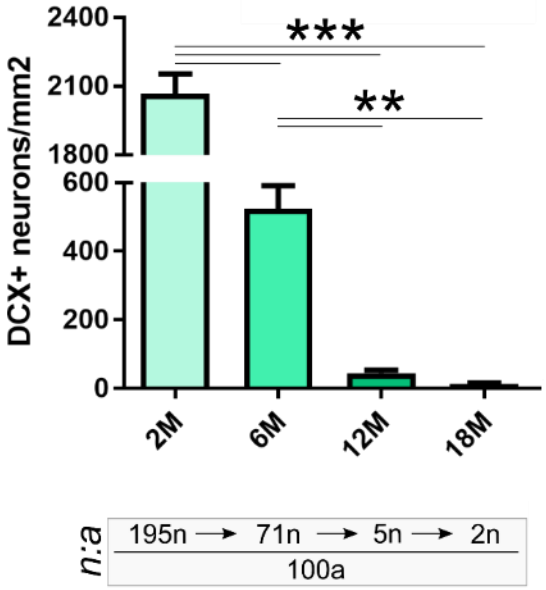


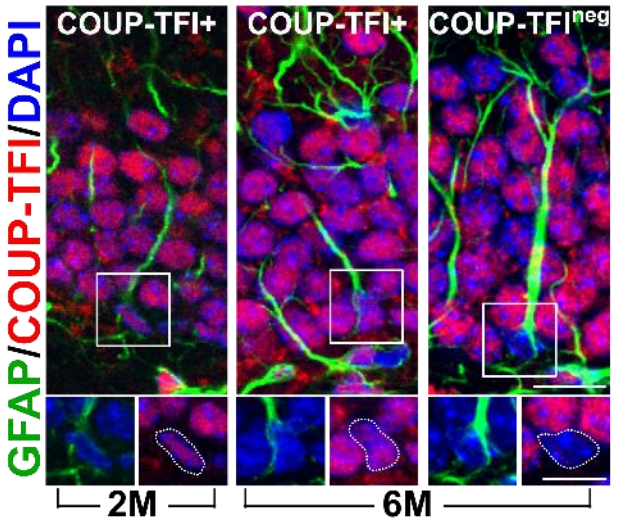


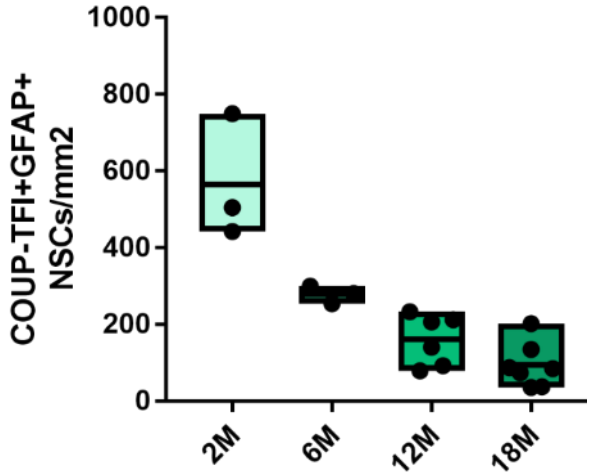
Ongoing analysis and future directions

Aging of the DG neurogenic niche: a role for COUP-TFI ?









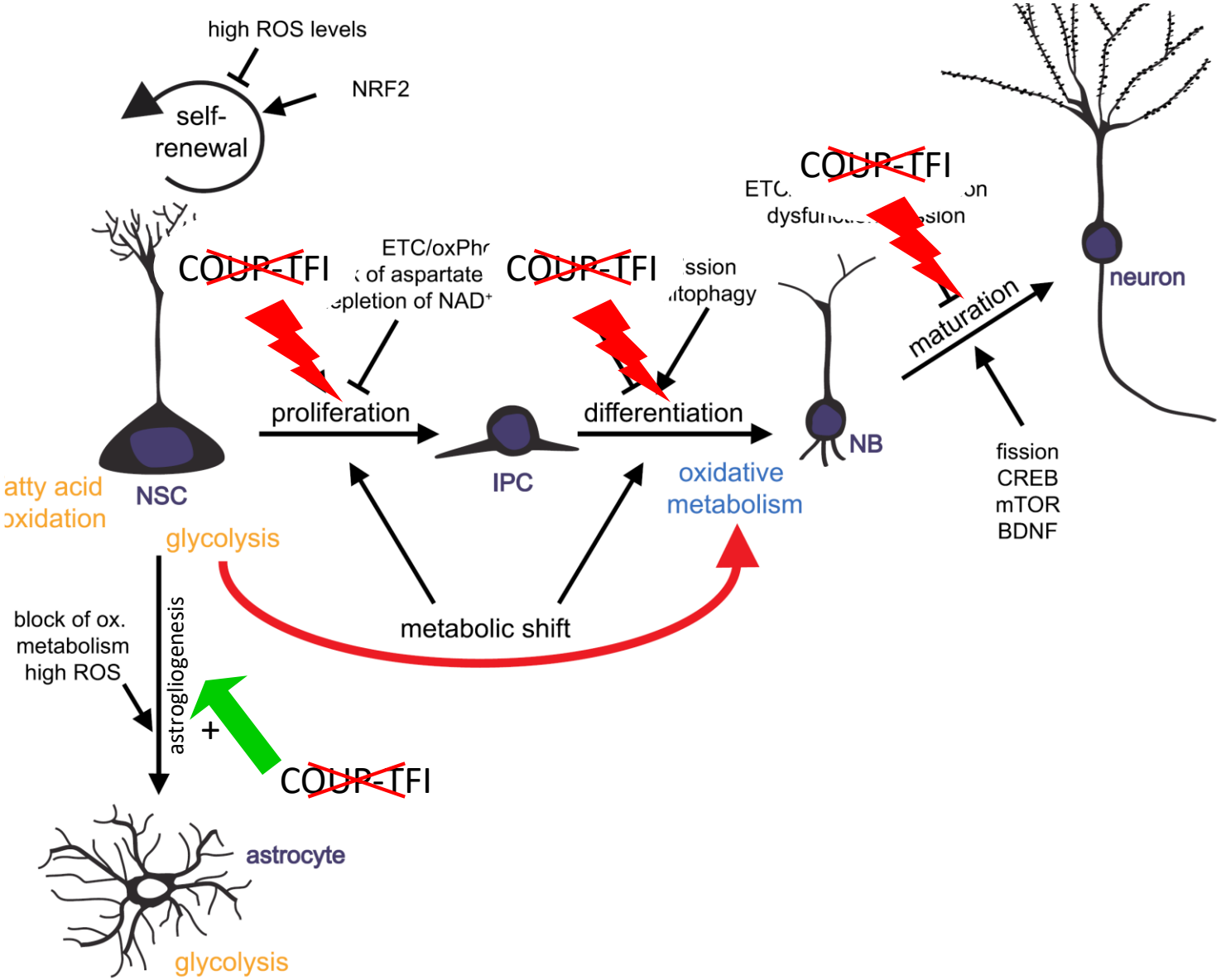
S. Bonzano/I. Crisci/D. Stajano - unpublished

Ongoing analysis and future directions

MitoCOUP Project

"COUP-TFI, mitochondria and adult NSCs: allies for brain plasticity"

Aging and mitochondria in adult hippocampal NSCs



Modified from Beckervordersandforth, Brain Plasticity 2017

COUP-TFI/Nr2f1 and mitochondria

COUP-TFI/Nr2f1 haploinsufficiency leads to impaired mitochondrial ETC functioning in a patient with BBSOAS

Journal of Human Genetics
https://doi.org/10.1038/s10038-017-0398-3 2018

BRIEF COMMUNICATION

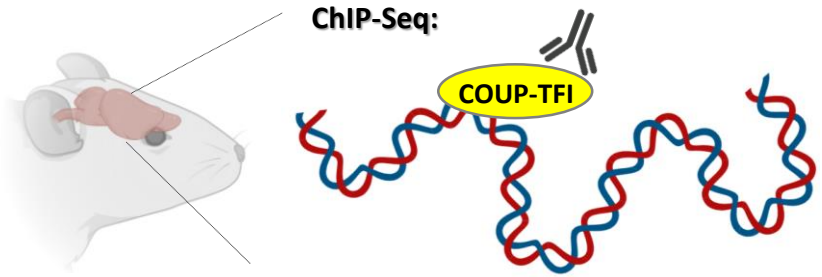
Mitochondrial involvement in a Bosch-Boonstra-Schaaf optic atrophy syndrome patient with a novel de novo *NR2F1* gene mutation

Elena Martín-Hernández[1,2] · María Elena Rodríguez-García[3] · Chun-An Chen[4,5] · Francisco Javier Cotrina-Vinagre[3] · Patricia Carnicero-Rodríguez[3] · Marcello Bellusci[1] · Christian P. Schaaf[4,5] · Francisco Martínez-Azorín[3,6]

COUP-TFI/Nr2f1 Loss of function

Mitochondrial defects

Several mitochondria-related genes are likely direct target genes for COUP-TFI



Enrichment Score: 15.17	G		Count	P_Value	Benjamini
mitochondrion ☆	RT		188	1.5E-23	7.1E-21
mitochondrion ☆	RT		133	9.8E-20	2.1E-17
transit peptide	RT		81	2.5E-13	2.7E-11
mitochondrial part ☆	RT		82	2.9E-12	6.6E-10
transit peptide:Mitochondrion	RT		79	1.4E-10	3.9E-7
Enrichment Score: 10.1	**G**		**Count**	**P_Value**	**Benjamini**
mitochondrion ☆	RT		133	9.8E-20	2.1E-17
mitochondrial part ☆	RT		82	2.9E-12	6.6E-10
organelle membrane	RT		109	8.6E-12	1.3E-9
mitochondrial envelope ☆	RT		63	4.1E-10	4.8E-8
organelle envelope	RT		78	5.4E-10	4.9E-8
envelope	RT		78	6.4E-10	4.9E-8
mitochondrial membrane ☆	RT		59	2.0E-9	1.1E-7
organelle inner membrane	RT		52	5.5E-9	2.8E-7
mitochondrion inner membrane ☆	RT		37	4.8E-8	2.9E-6
mitochondrial inner membrane ☆	RT		48	5.5E-8	2.1E-6

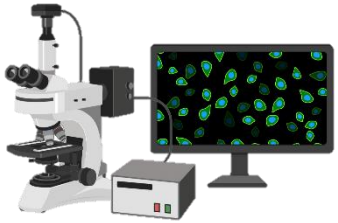
Preliminary data

Project workflow

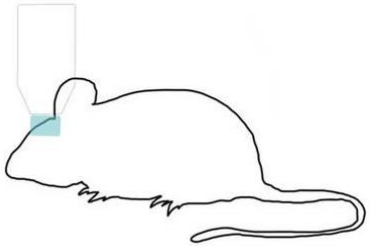
TASK1)
EVALUATE MITOCHONDRIA MARKER EXPRESSION IN COUP-TFI-icKO HIPPOCAMPAL TISSUES vs CTRLs

TASK2)
FUNCTIONAL IMAGING OF MITOCHONDRIA IN COUP-TFI-icKO DG NEUROGENIC NICHE

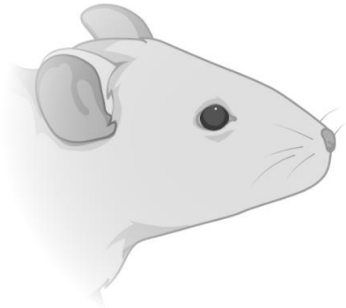
TASK3)
IDENTIFY COUP-TFI TARGETS IN ADULT NSCs AND THEIR POSSIBLE IMPLICATIONS DURING AGING



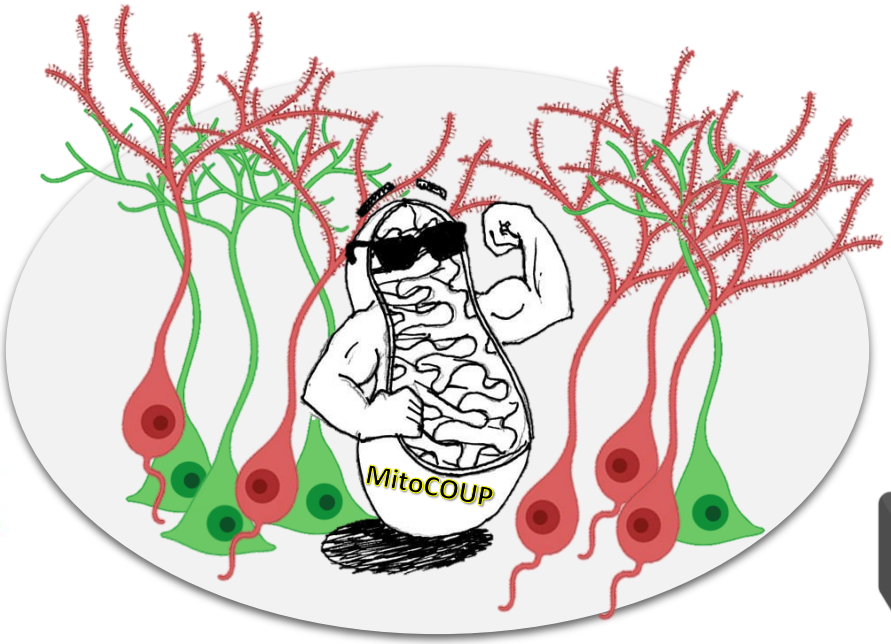
Confocal microscopy

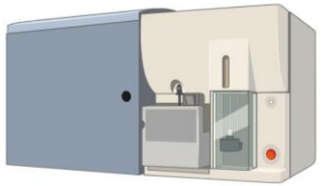


Two-photon functional imaging



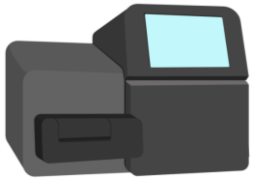
Trangenic mouse models





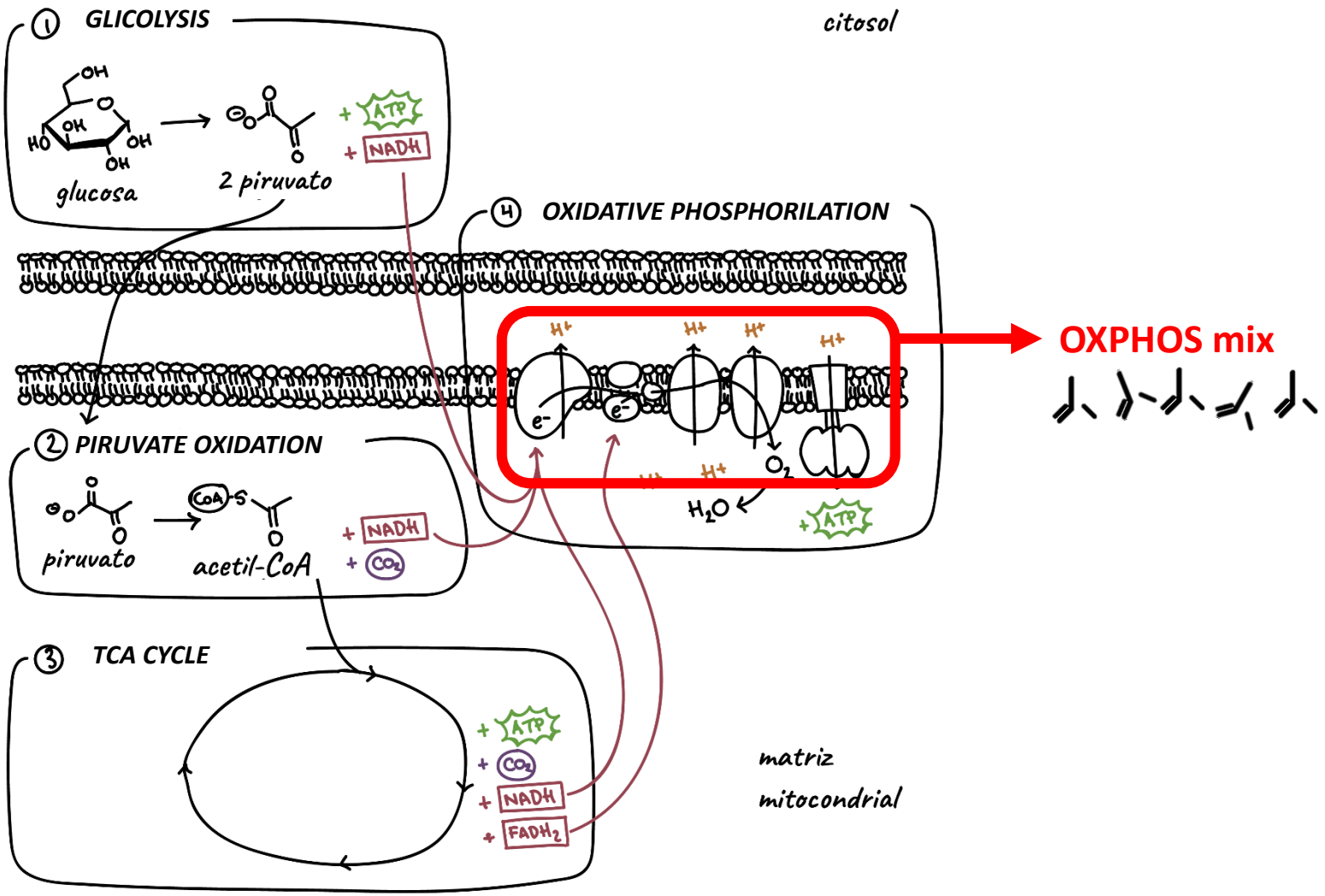
FACSorting

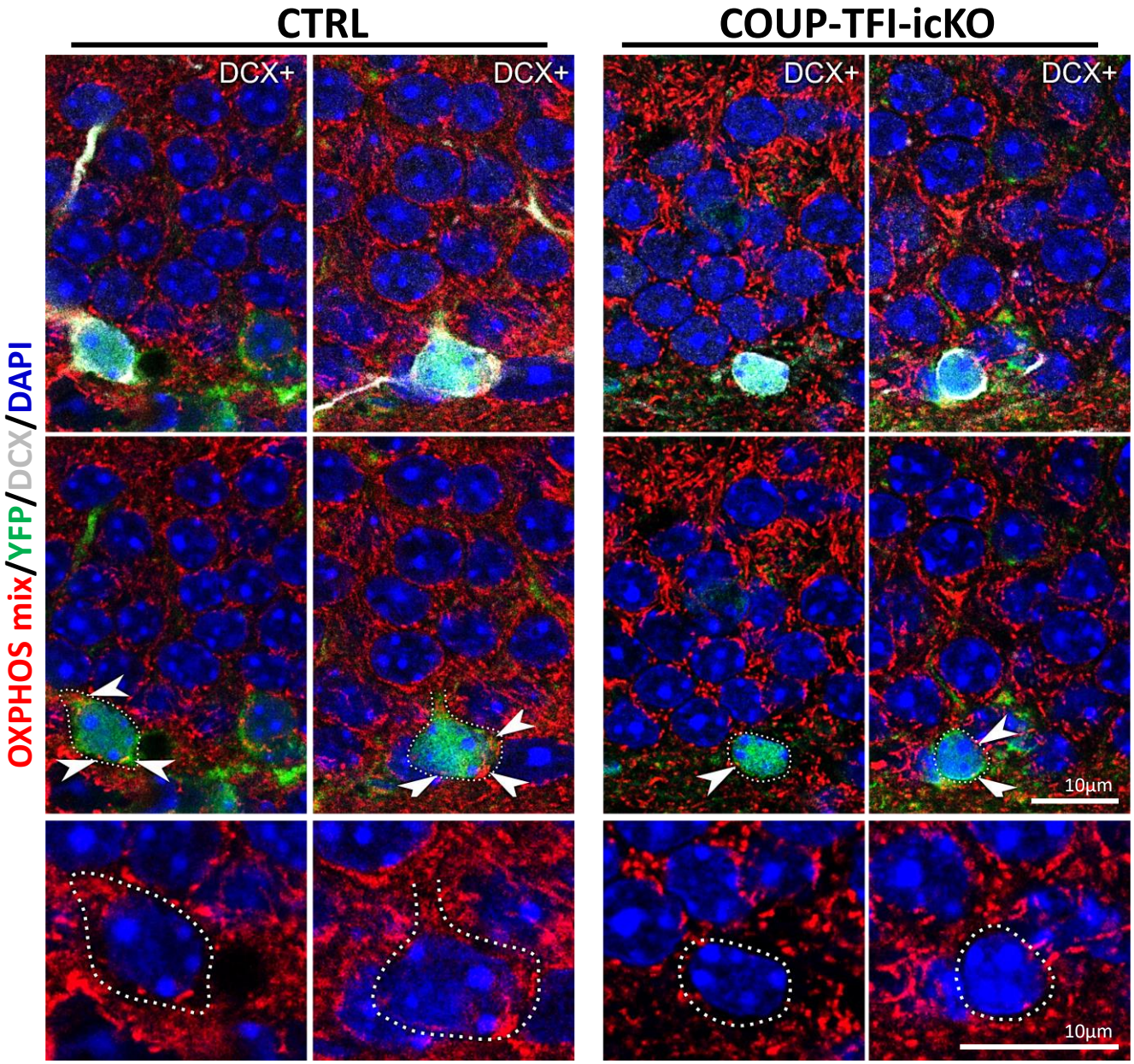
Retroviral vectors



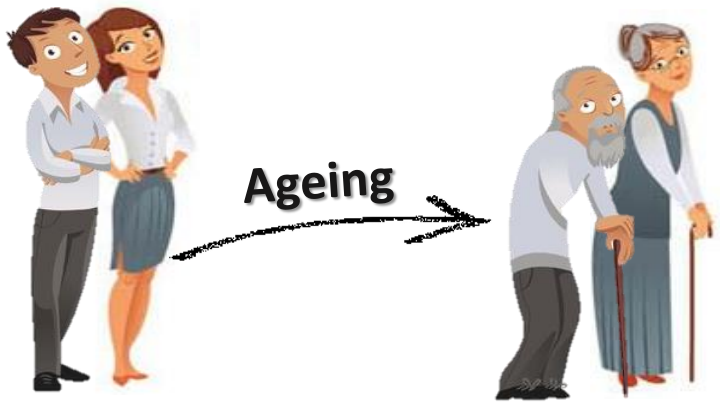
RNA-Sequencing

TASK1) EVALUATE MITOCHONDRIA MARKER EXPRESSION IN COUP-TFI-icKO HIPPOCAMPAL TISSUES vs CTRLs





YFP+DCX+=newborn neurons - OXPHOS mix=mitocondrial ETC - DAPI= nucleus



LEARNING / MEMORY

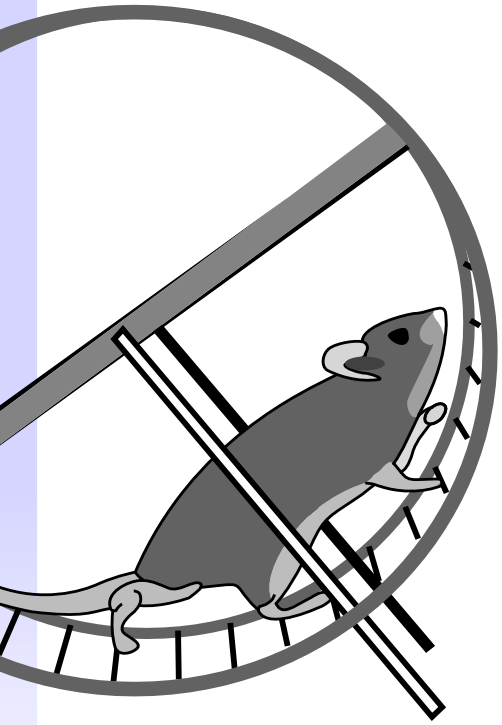
ENVIRONMENT ?

COUP-TFI

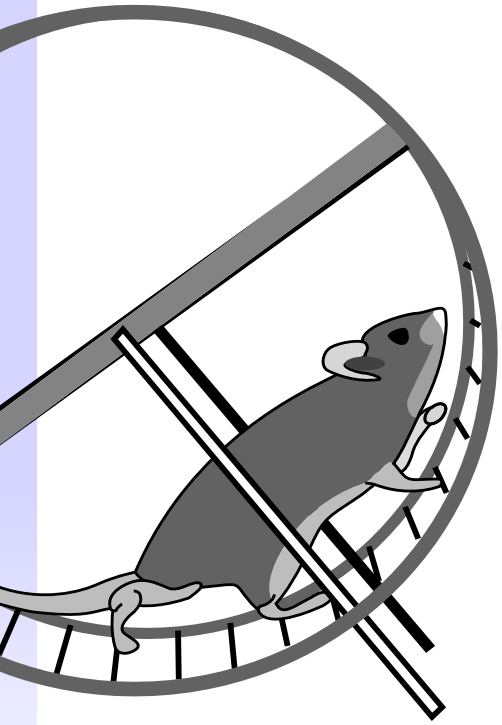
MITOCHONDRIAL METABOLISM

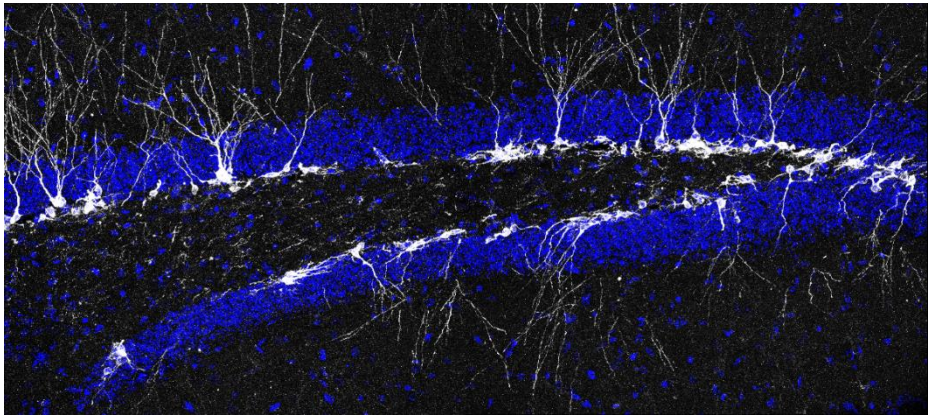
DG NEUROGENESIS

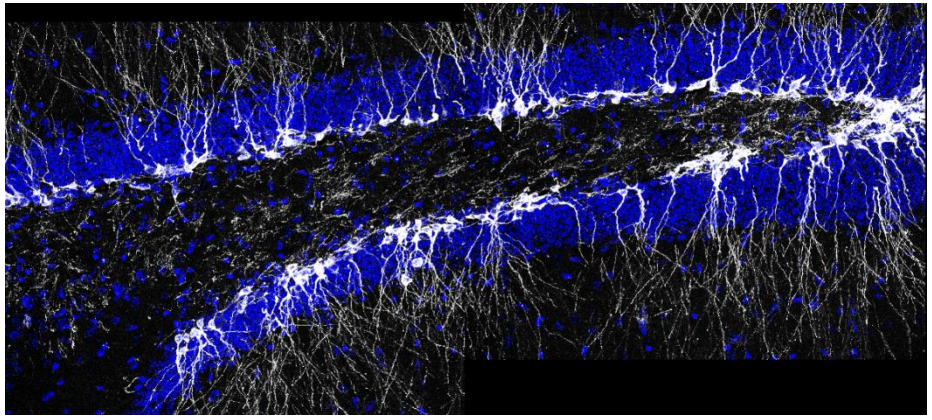
Enriched environment and running represent important **non-invasive strategies to increase brain plasticity** and to favor key cognitive functions (such as memory, learning, pattern separation)



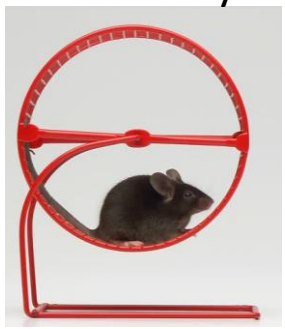
Enriched environment and running represent important **non-invasive strategies to increase brain plasticity** and to favor key cognitive functions (such as memory, learning, pattern separation)







Sedentary



Runner

Social interactions

Isolation

Complex enrivonment
(+/- running disc)

- ✓ Test whether COUP-TFI activity is modulated by experience
- ✓ Evaluate whether COUP-TFI is directly involved in activity-dependent regulation of adult DG neurogenesis

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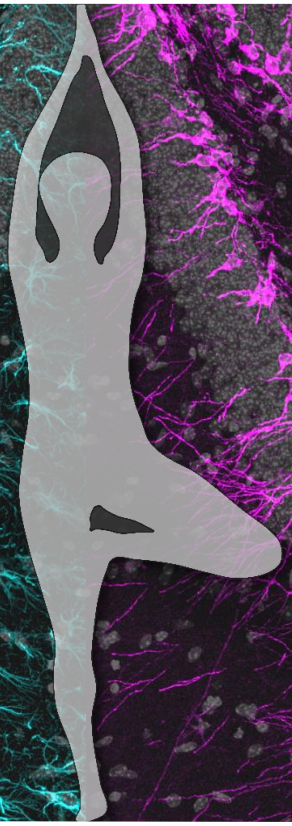

Collaborations

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