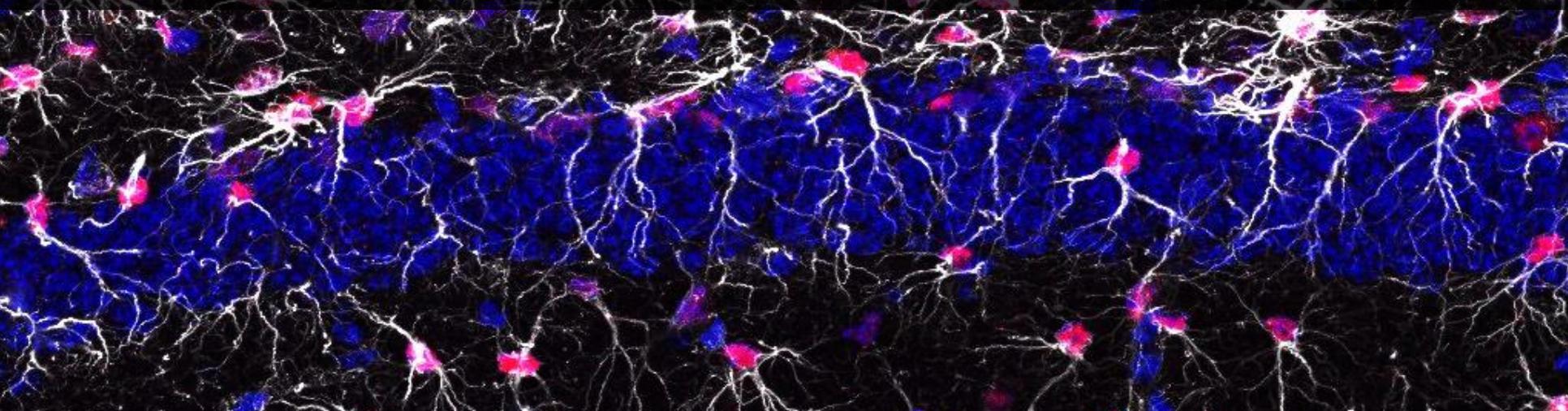
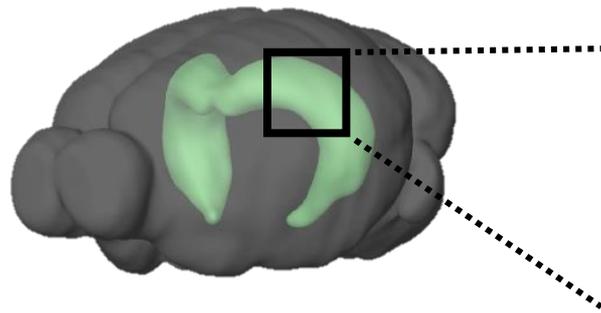


**“Neuron-astroglia cell fate decision in the adult mouse
hippocampal neurogenic niche is
cell-intrinsically controlled by COUP-TFI *in vivo*”**

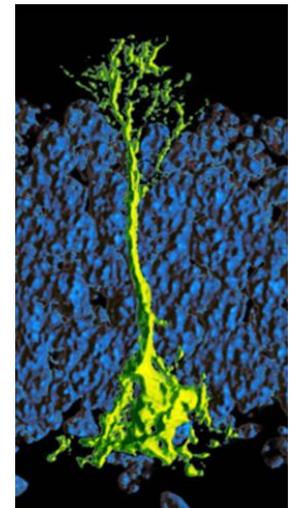
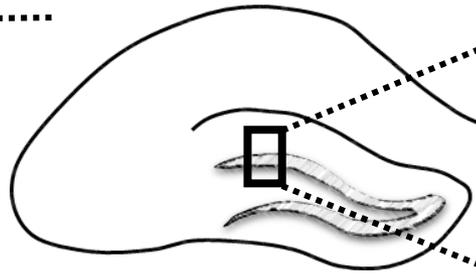
S. Bonzano, I. Crisci, A. Podlesny-Drabiniok, C. Rolando, W. Krezel, M. Studer, S. De Marchis



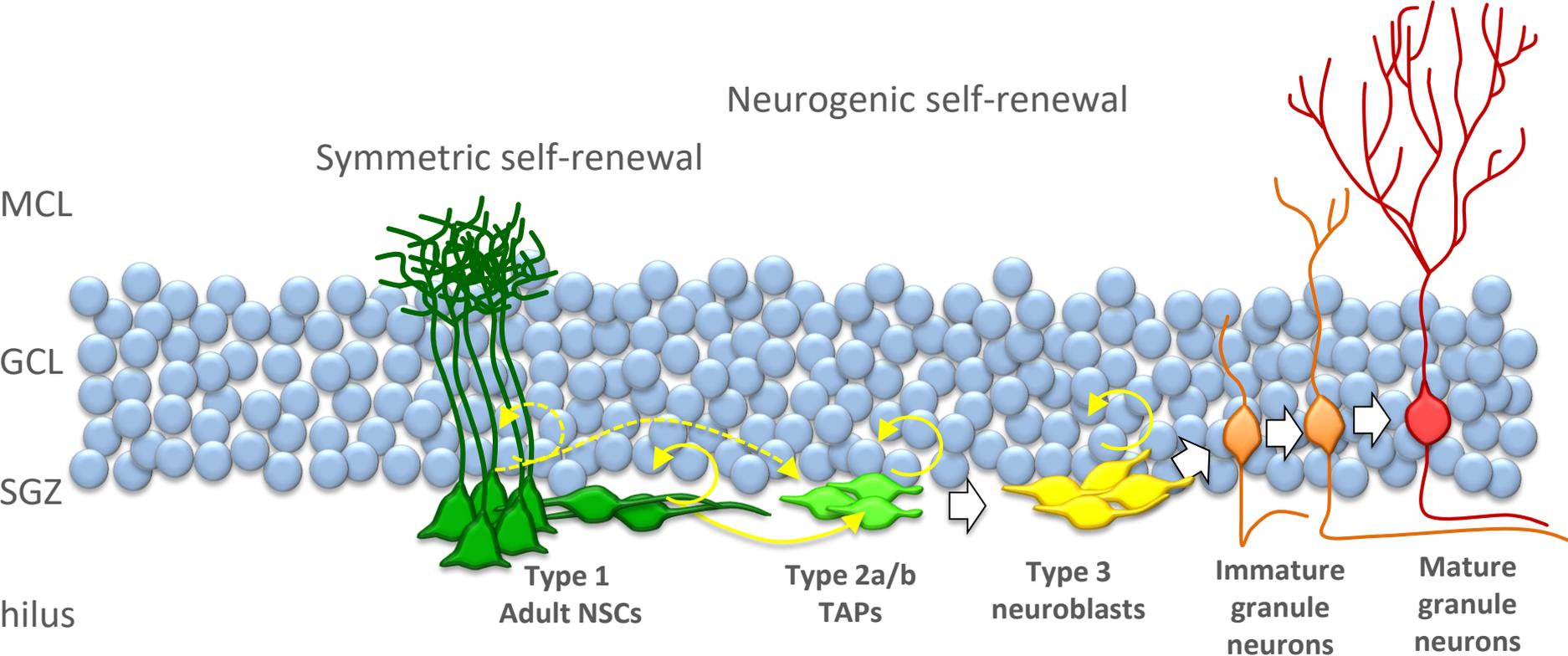
**Adult neural stem cell
(NSCs)**



Dentate gyrus

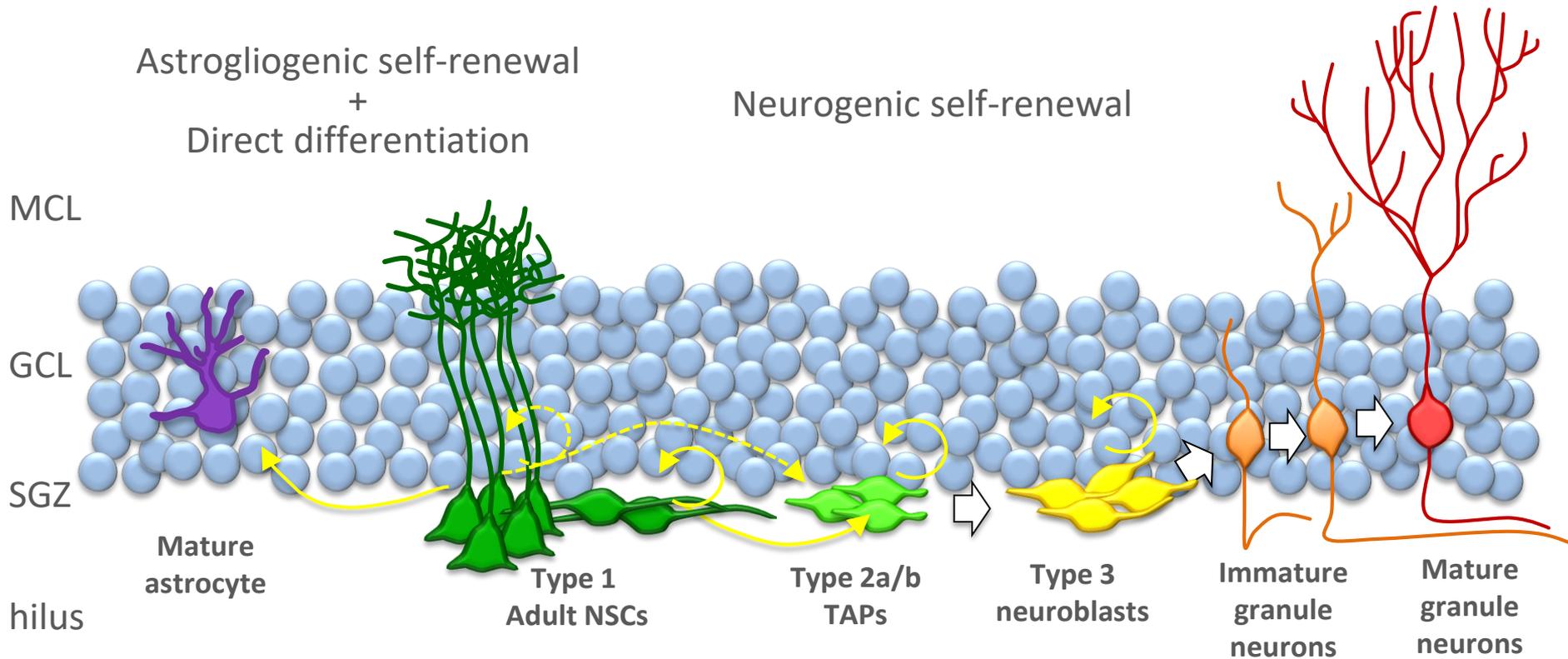


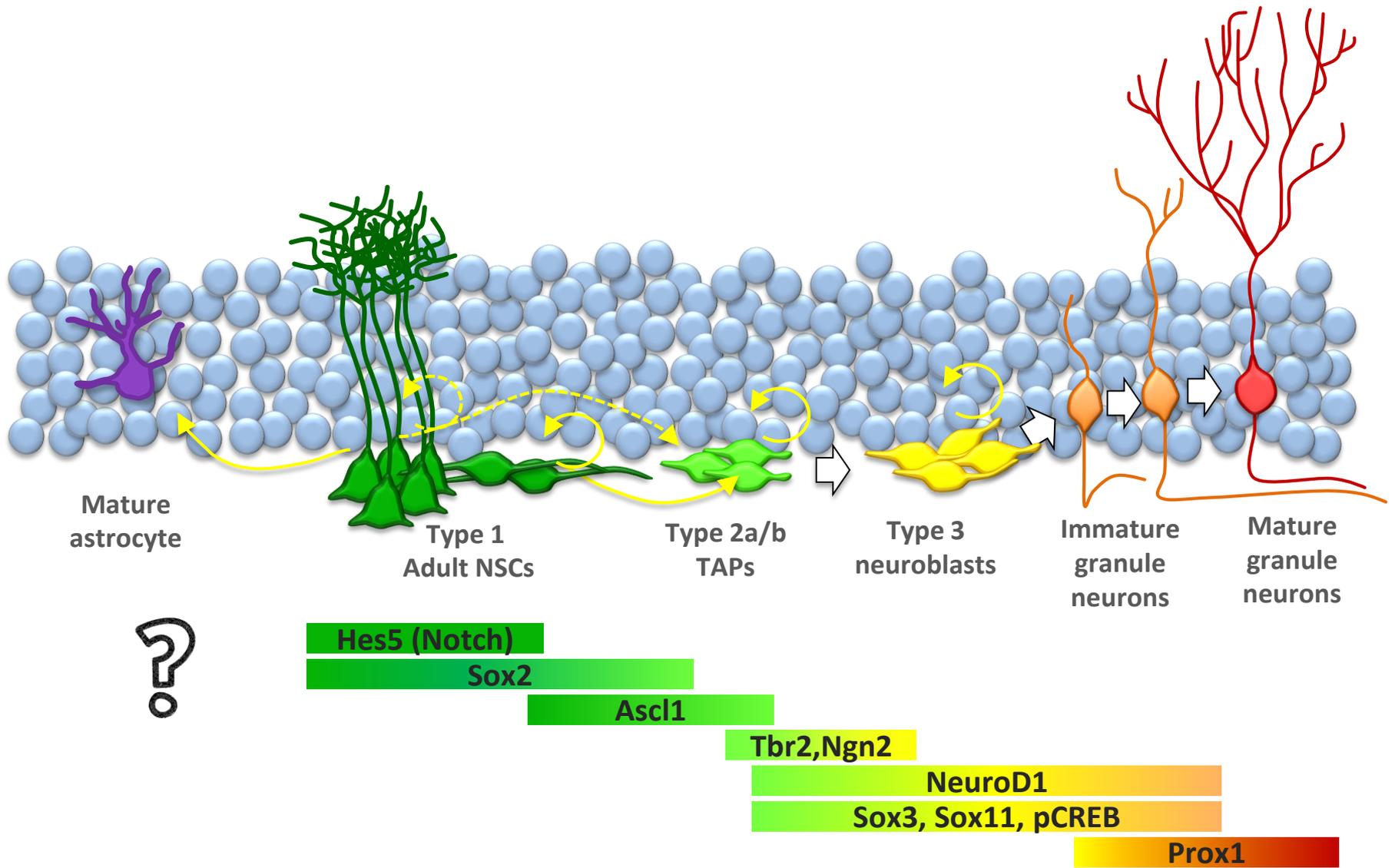
NEUROGENESIS



ASTROGLIOGENESIS

NEUROGENESIS





Astrocytes and adult-born neurons cover key roles in adult DG neuroplasticity

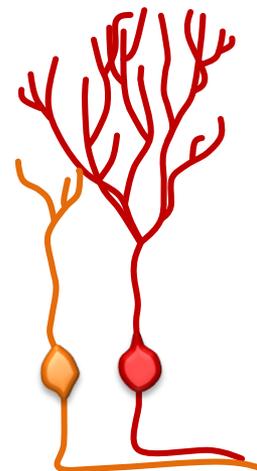
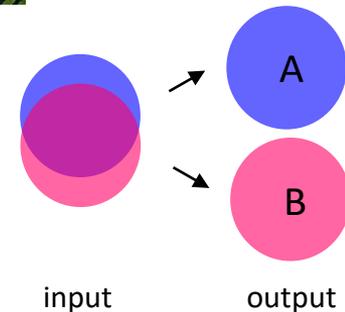
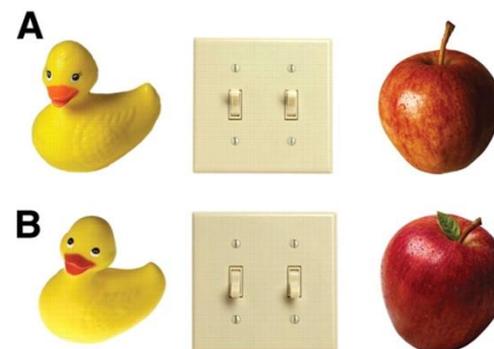
- ✓ Expansion of adult NSCs
- ✓ Dendritic maturation of adult-born neurons
- ✓ Synaptic integration of newly generated neurons



- ✓ Spatial navigation



- ✓ Pattern separation

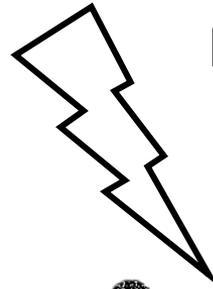


INFLAMMATION

AGING

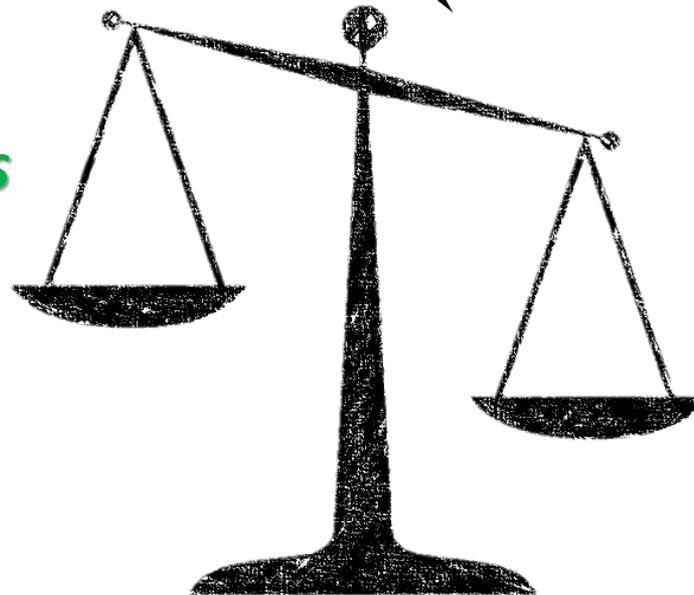
NEURODEGENERATION

EPILEPSY



ASTROGLIOGENESIS

NEUROGENESIS

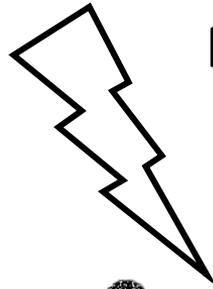


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AGING

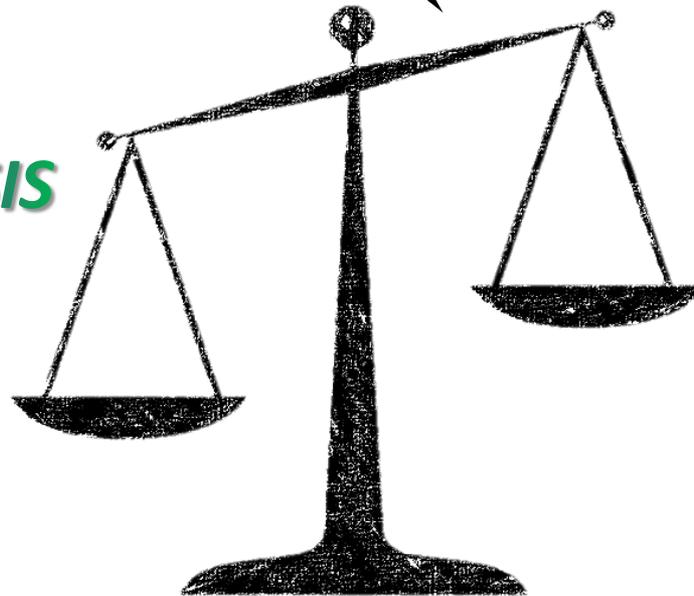
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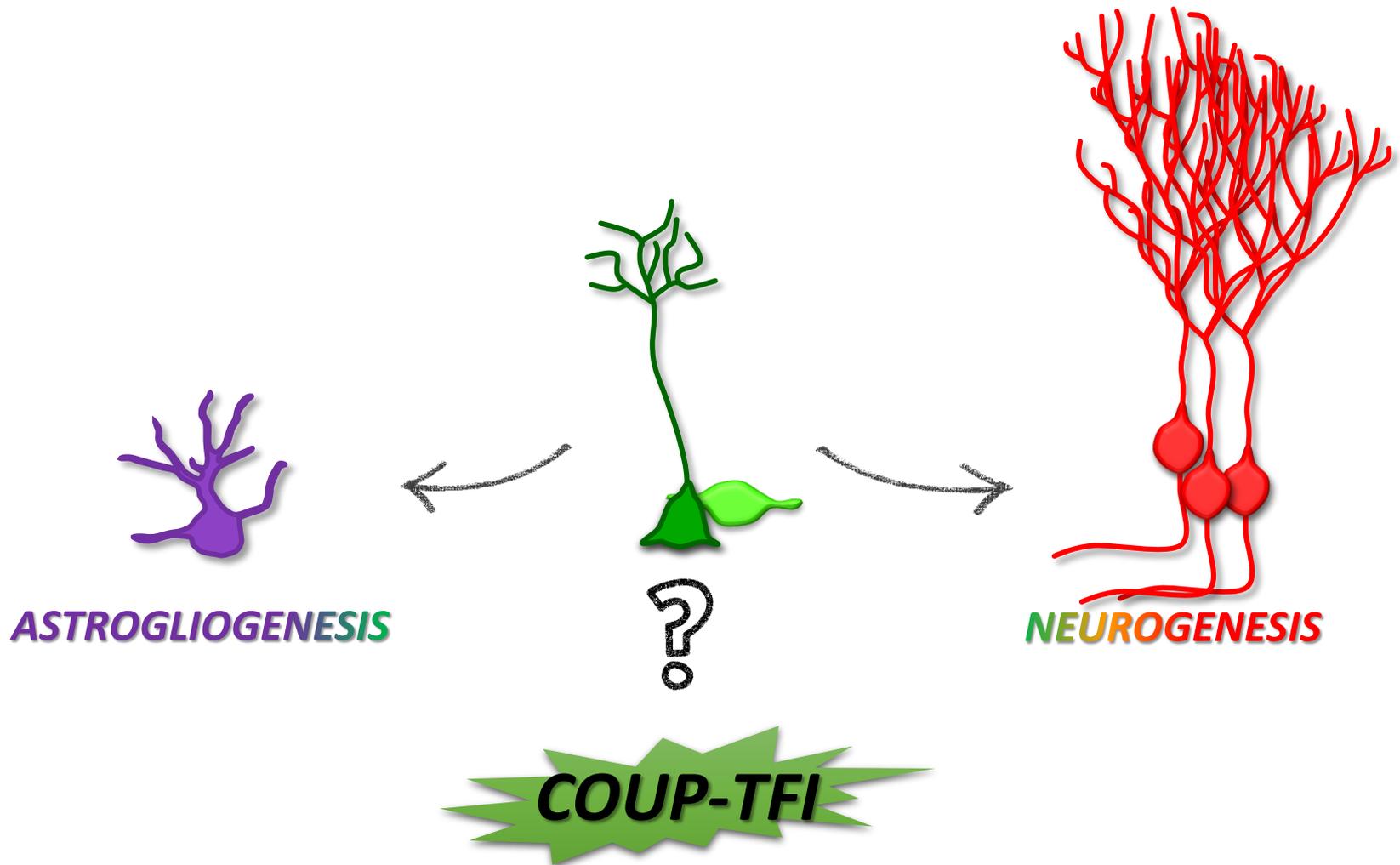


ASTROGLIOGENESIS

NEUROGENESIS



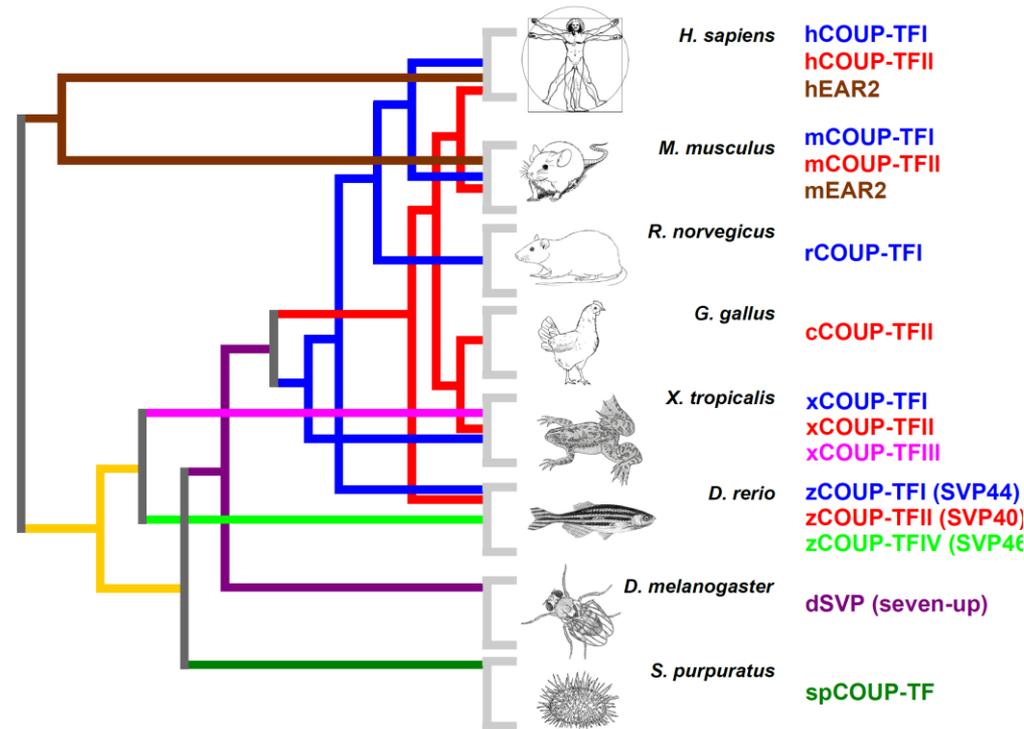
Which is the molecular mechanism underlying adult hippocampal NSC/progenitor fate choice?



COUP-TFs

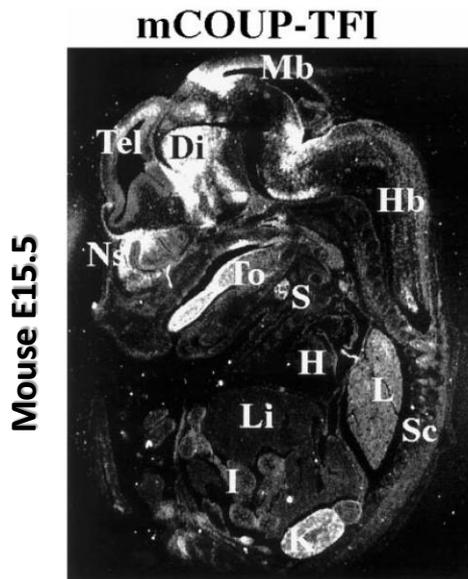
(chicken ovalbumin upstream promoter transcription factors)

- Major homologues in Vertebrates: **COUP-TFI (NR2F1)** and **COUP-TFII (NR2F2)**
- Highly **conserved during evolution**
- Orphan **nuclear receptors**
- Steroid/thyroid hormone receptor superfamily
- Function as **activators and/or repressors** for target gene transcription

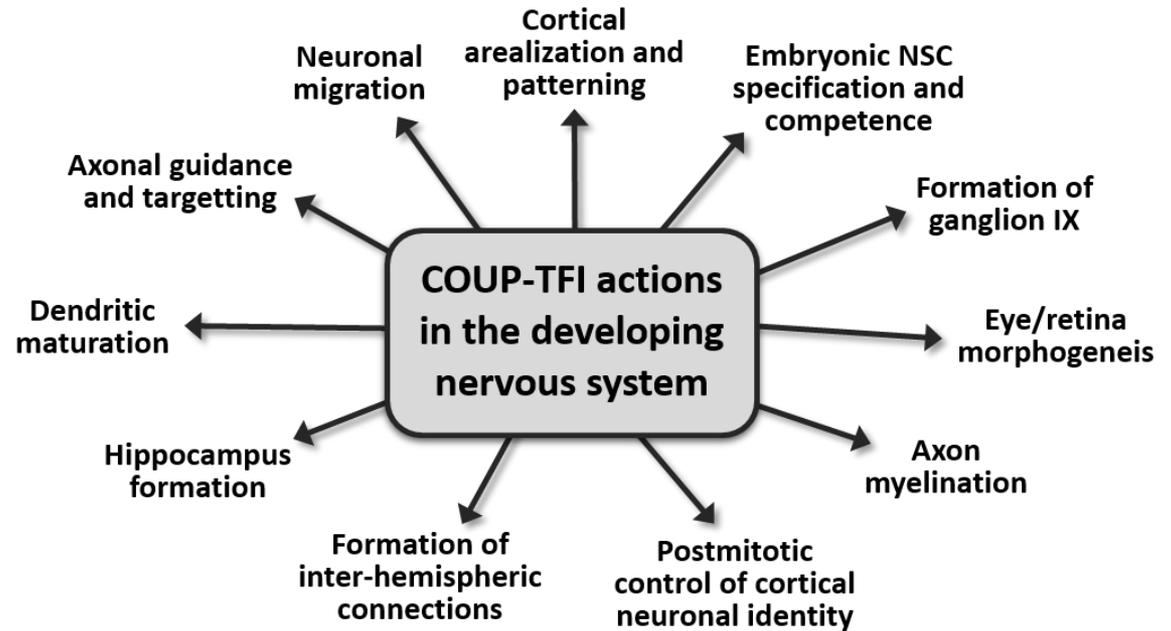


Modified from Alfano et al., Cell Mol Life Sci 2014

COUP-TFI plays pleiotropic functions in brain development



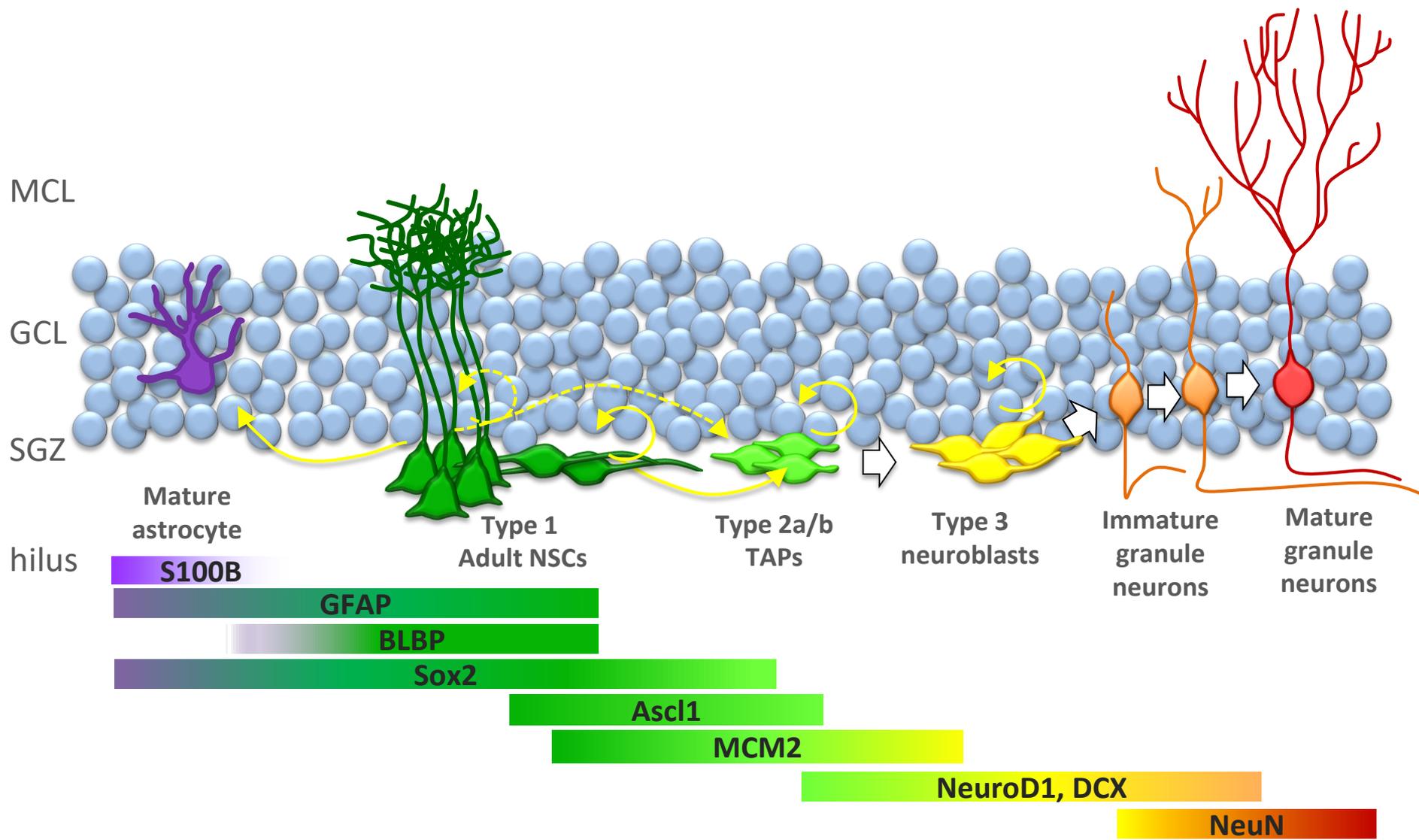
Tsai Y. T. and Tsai M., Endocr Rev 1997



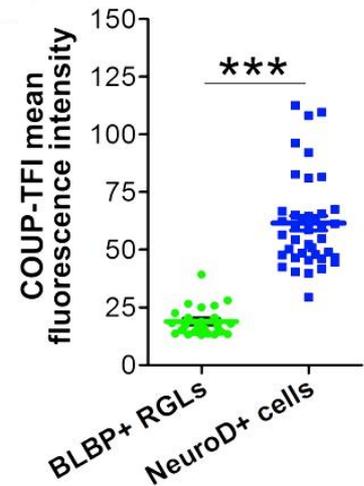
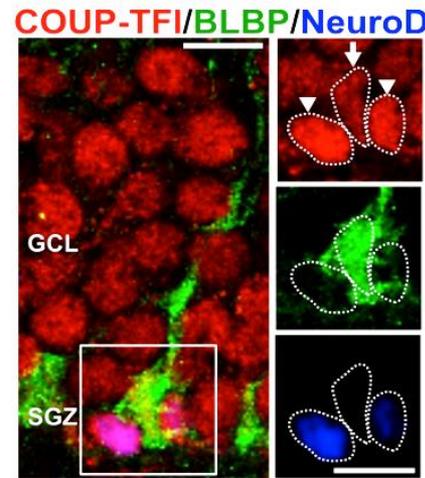
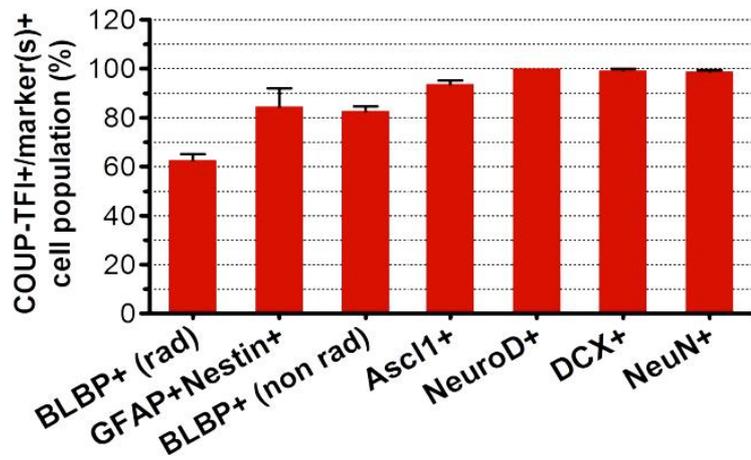
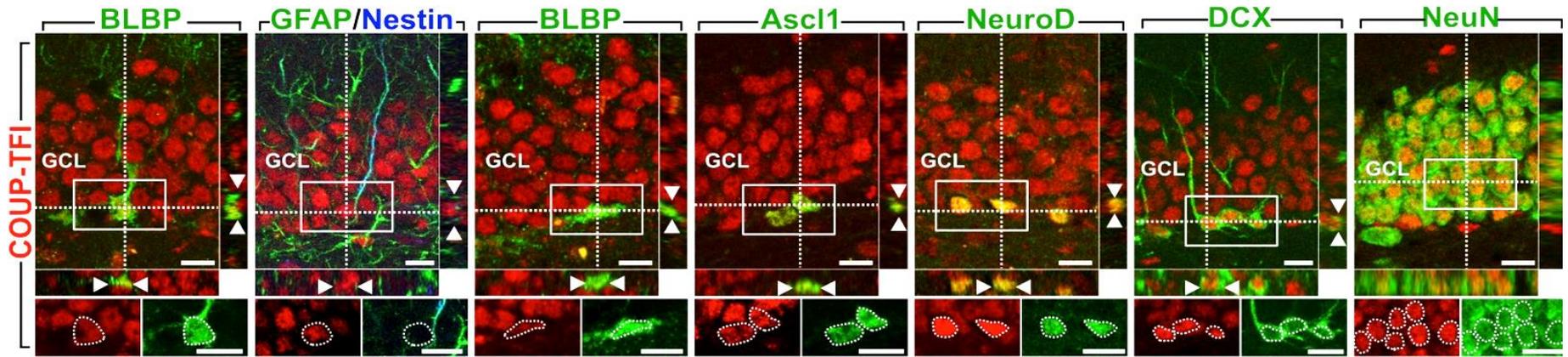
Modified from Park et al., Keio J Med 2003

Outline of the study

- 1. COUP-TFI expression pattern within the adult hippocampal DG*
- 2. COUP-TFI loss of function in the DG niche through Cre/loxP technology*
- 3. COUP-TFI overexpression and gain-of-function in NSCs/progenitors through Cre/loxP technology*



COUP-TFI is expressed in radial NSCs (RGLs) and upregulated in the neurogenic lineage of the adult DG



COUP-TFI is expressed in radial NSCs (RGLs) and upregulated in the neurogenic lineage of the adult DG

Cell Reports
Resource

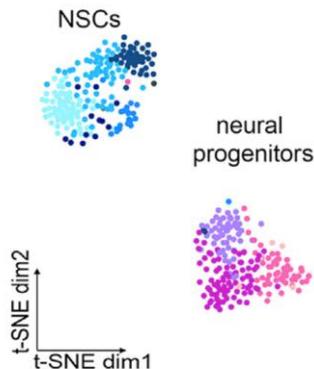
A Single-Cell RNA Sequencing Study Reveals Cellular and Molecular Dynamics of the Hippocampal Neurogenic Niche

Benedetta Artergiani,¹ Anna Lyubimova,¹ Mauro Muraro,¹ Johan H. van Es,¹ Alexander van Oudenaarden,¹ and Hans Clevers^{1,2,*}

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²Lead Contact

*Correspondence: h.clevers@hubrecht.eu
<https://doi.org/10.1016/j.celrep.2017.11.050>

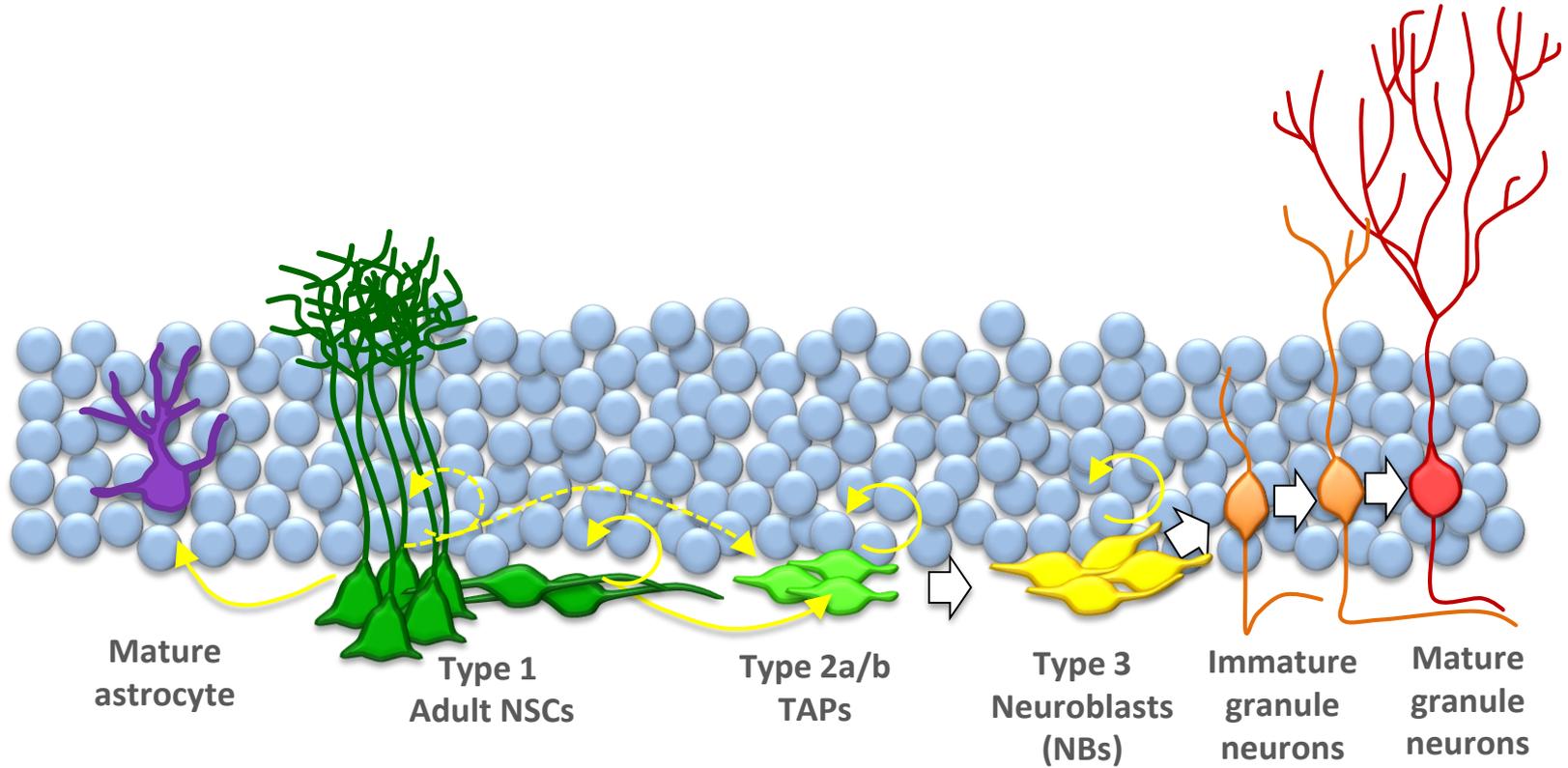


We found a high number of transcription factors among the genes specifically enriched in progenitors (Table S3). This probably underscores the importance of transcriptional cascades in the switch from NSCs to progenitors and neural progenitor development. We focused on transcription factors that were enriched in progenitors and on those that were differentially expressed between the two main progenitor populations (Table S3). Among these, there were known regulators of pattern specification (e.g., *Emx1*, *Emx2*, *Dlx2*, *Bmi1*, *Foxg1*, and *Eomes*) (Brill et al., 2008; Hodge et al., 2013; Yoshida et al., 1997) and neuronal differentiation (*Sox11*, *Neurog2*, *Neurod1*, and *Neurod2*) (Ninkovic et al., 2013) and chromatin remodelers (e.g., *Hdac2*, *Smarca4*, and *Smarca2*), but also many transcription factors whose function has not been described in the context of adult hippocampal neural development (e.g., *Hmgn2*, *Lhx2*, *COUP-TF1/Nr2f1*, *Pbx1*, *Cbx3*, *Maged1*, and *Ssbp3*). Thus, our data constitute a valuable resource for future studies on molecular mechanisms regulating neurogenesis.

Outline of the study

- 1. COUP-TFI expression pattern within the adult hippocampal DG*
- 2. COUP-TFI loss of function in the DG niche through Cre/loxP technology*
- 3. COUP-TFI overexpression and gain-of-function in NSCs/progenitors through Cre/loxP technology*

COUP-TFI loss of function in the DG niche through Cre/loxP

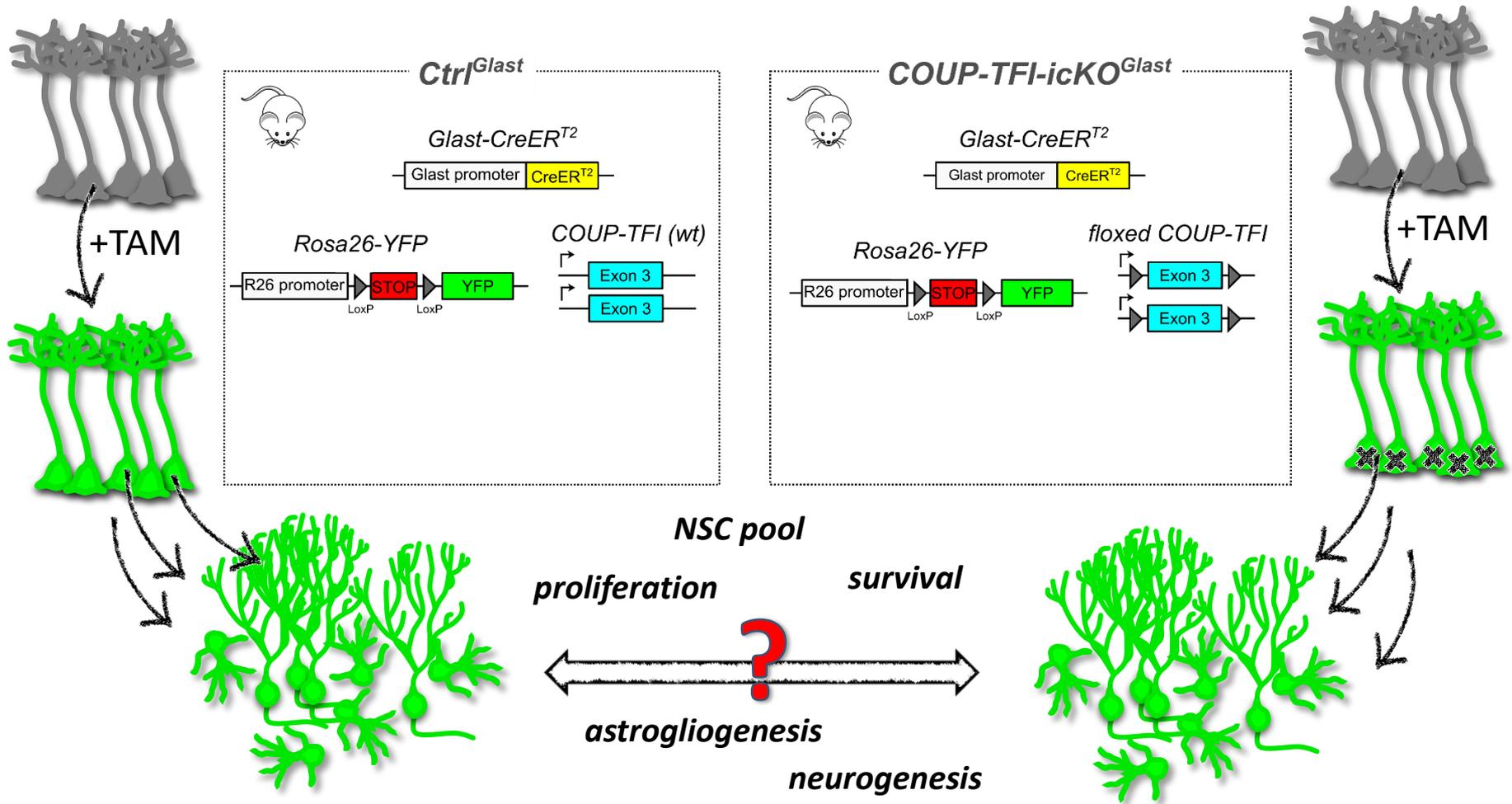


Glast-CreER^{T2}: NSCs (all) + astrocytes

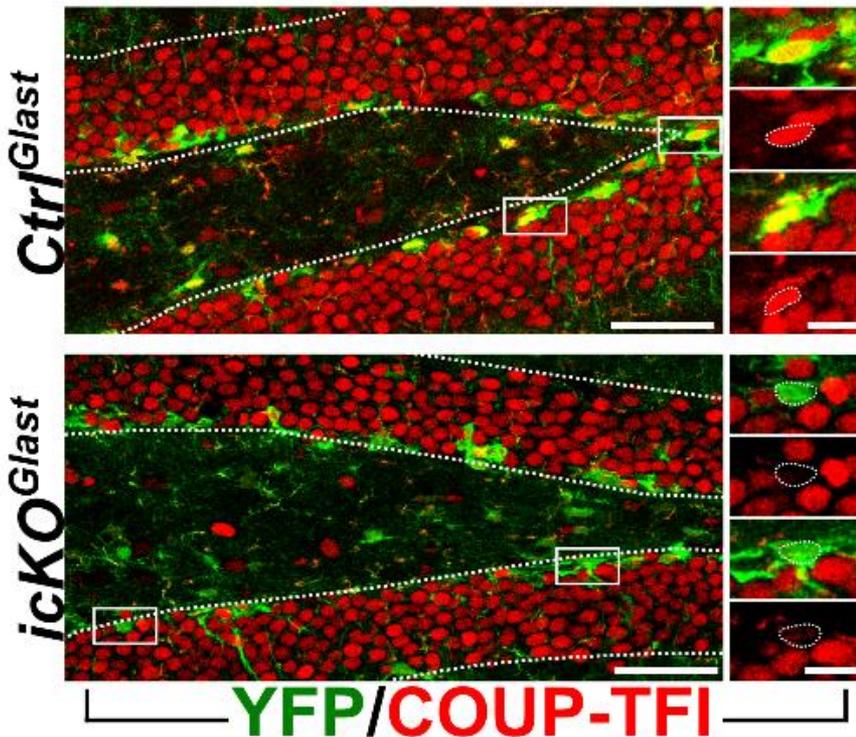
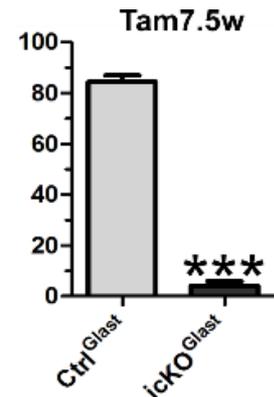
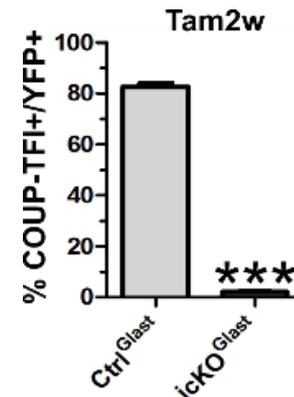
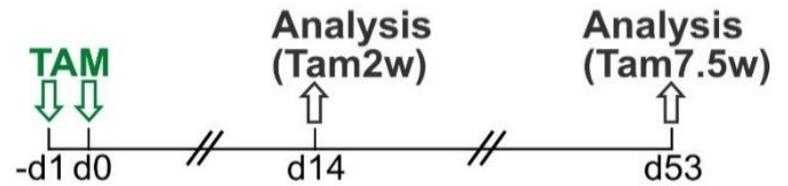
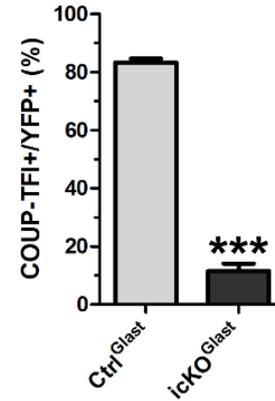
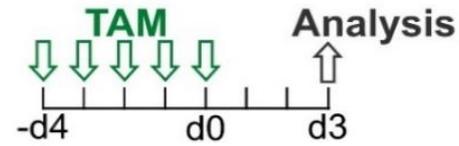
Ascl1-CreER^{T2}: NSCs (active) + TAPs (2a)

Retrovirus: NSCs (few) + TAPs (many) + NBs

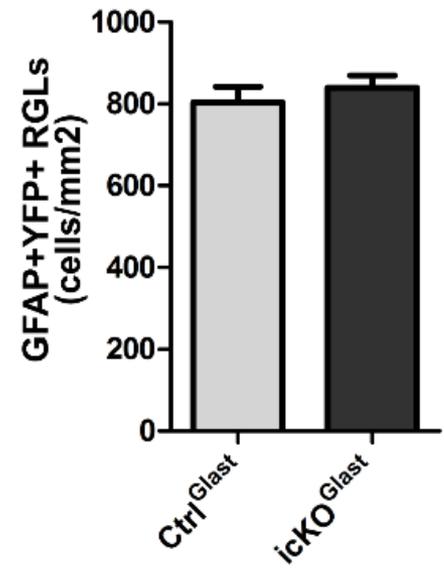
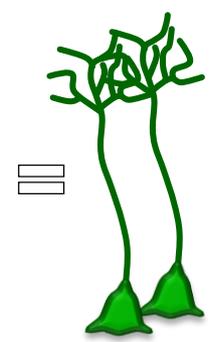
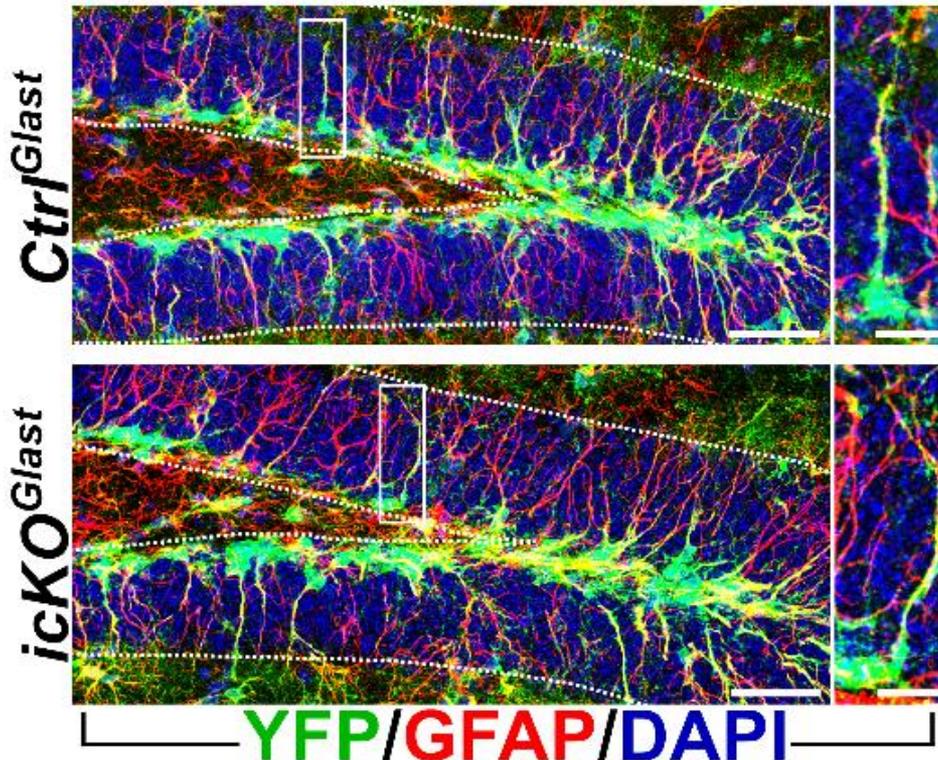
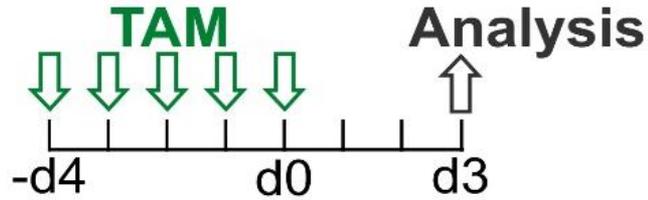
In vivo inducible COUP-TFI loss of function in adult *Glast*+ NSCs coupled to fate mapping



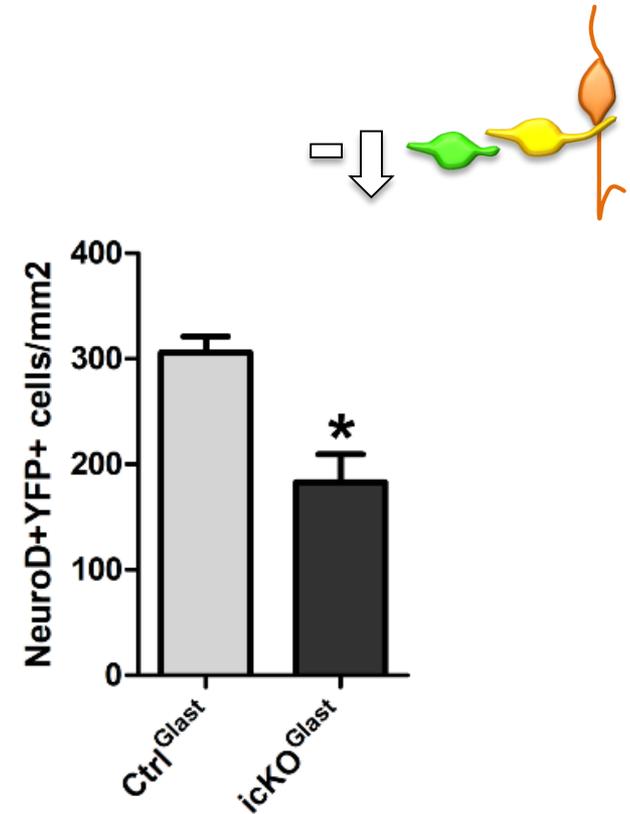
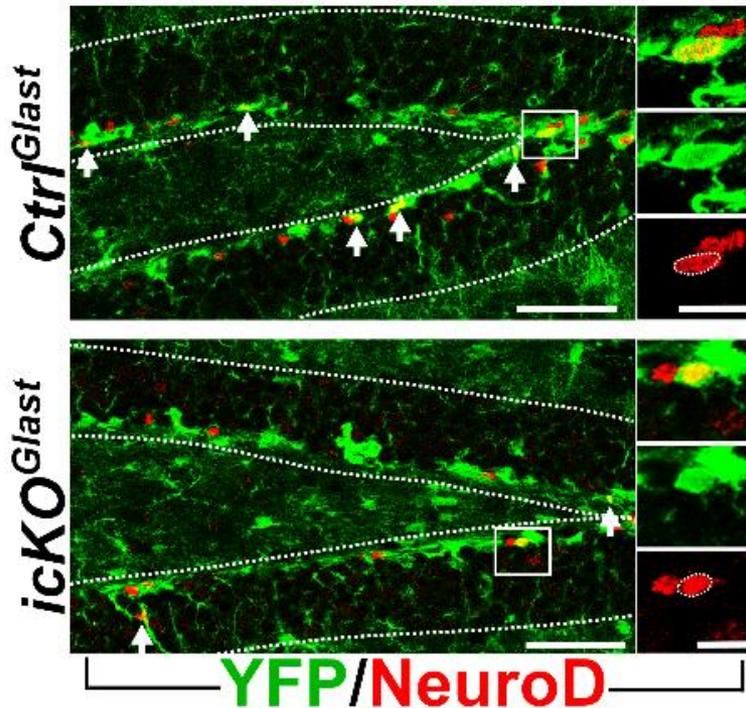
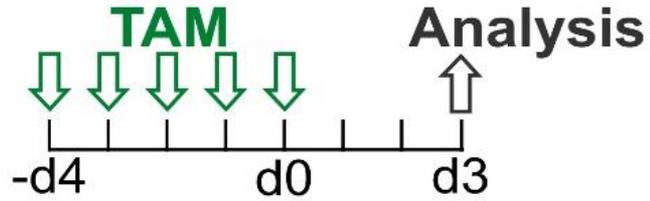
Efficient *COUP-TFI* deletion in the *Glast* lineage



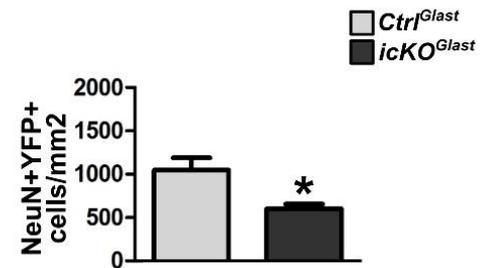
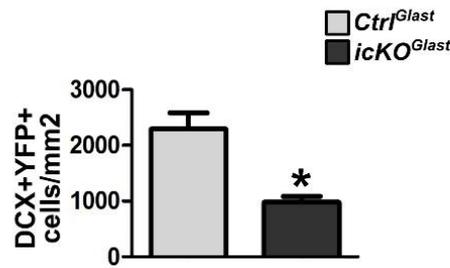
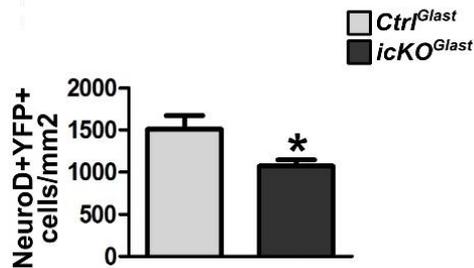
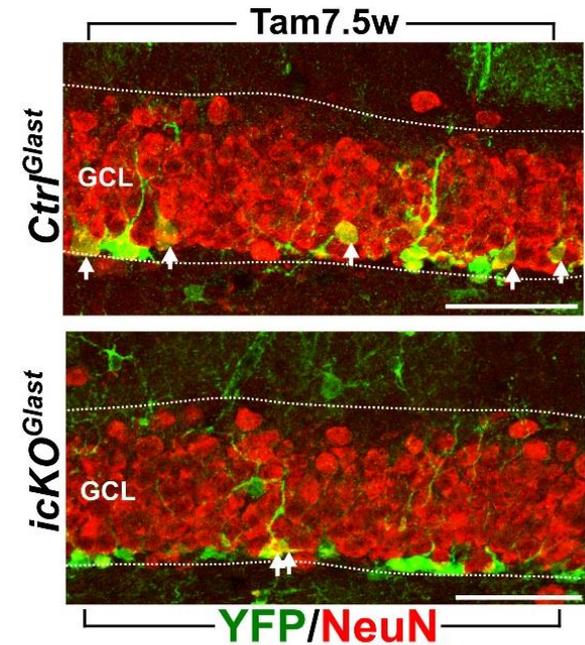
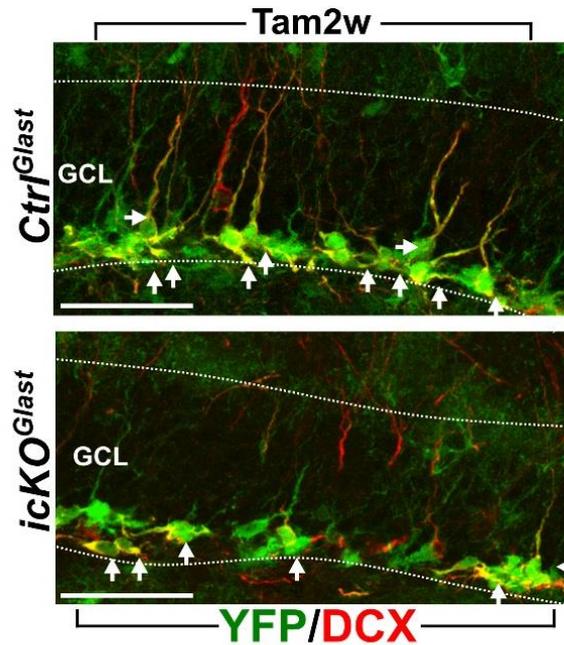
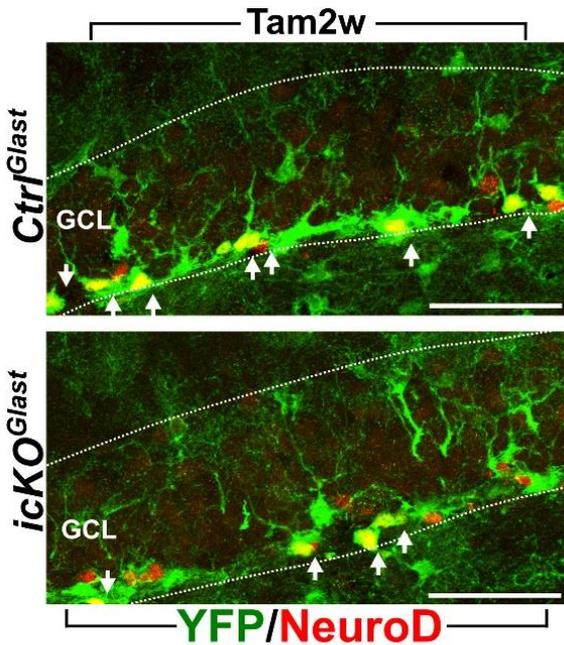
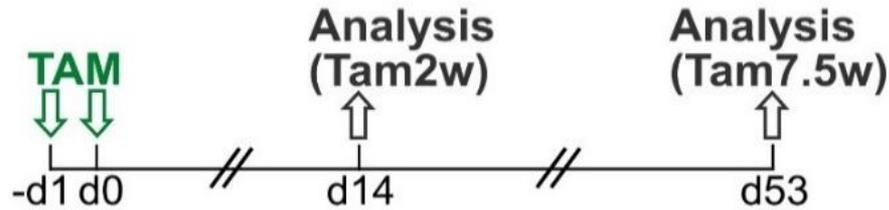
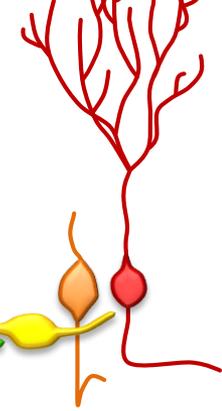
COUP-TFI loss does not alter the RGL cell pool



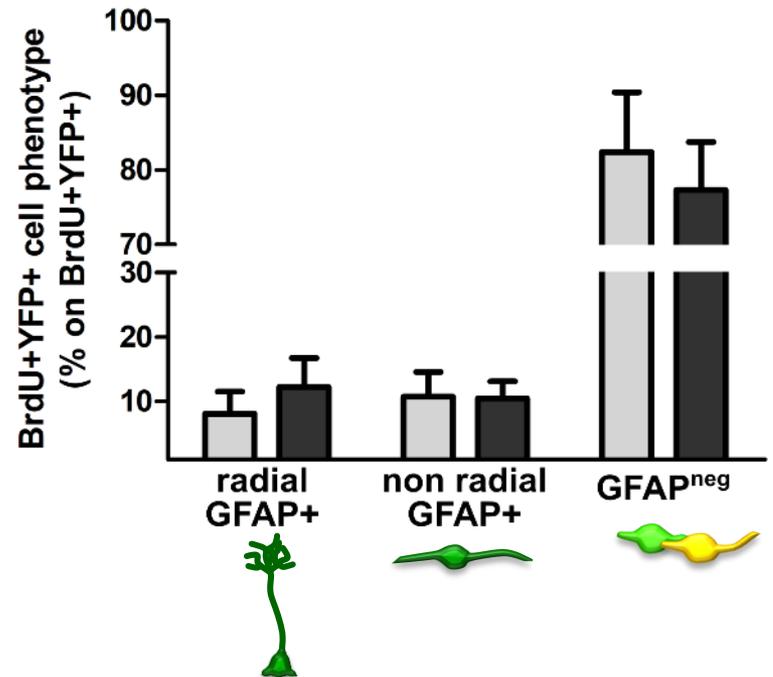
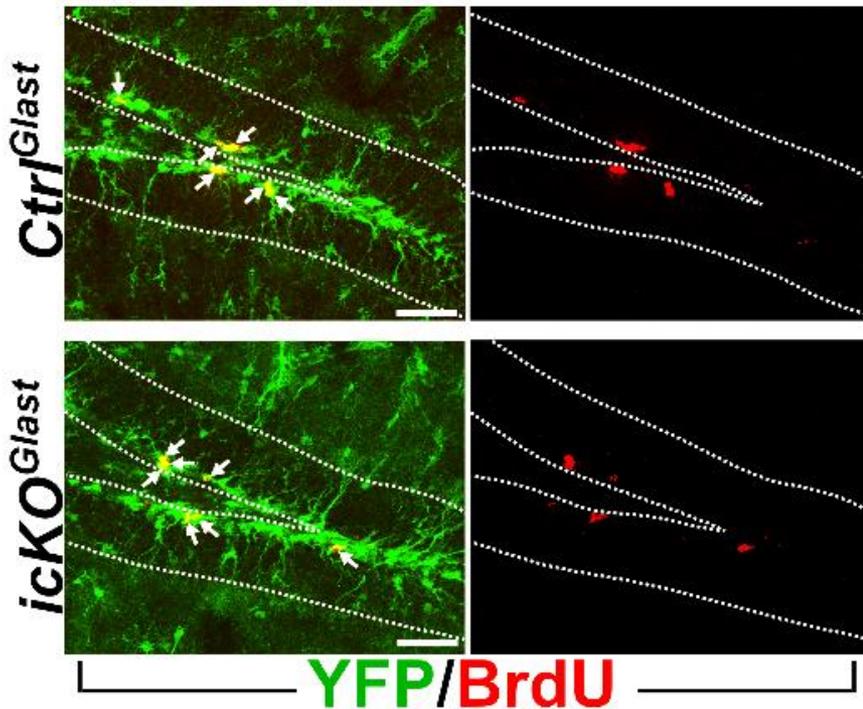
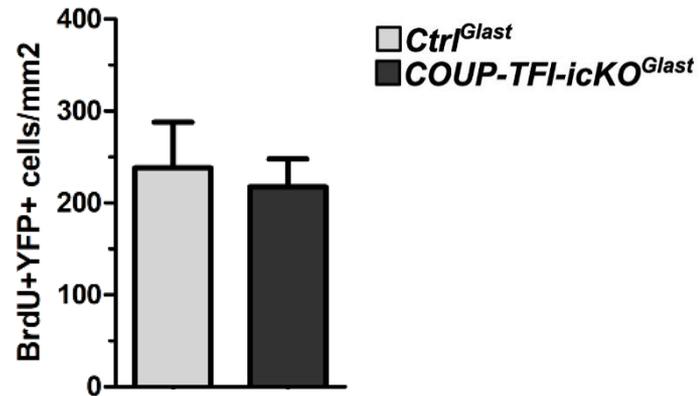
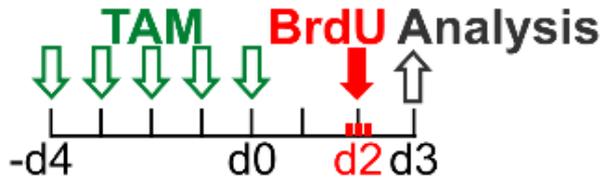
COUP-TFI loss reduces neuronal progenitors/neuroblasts



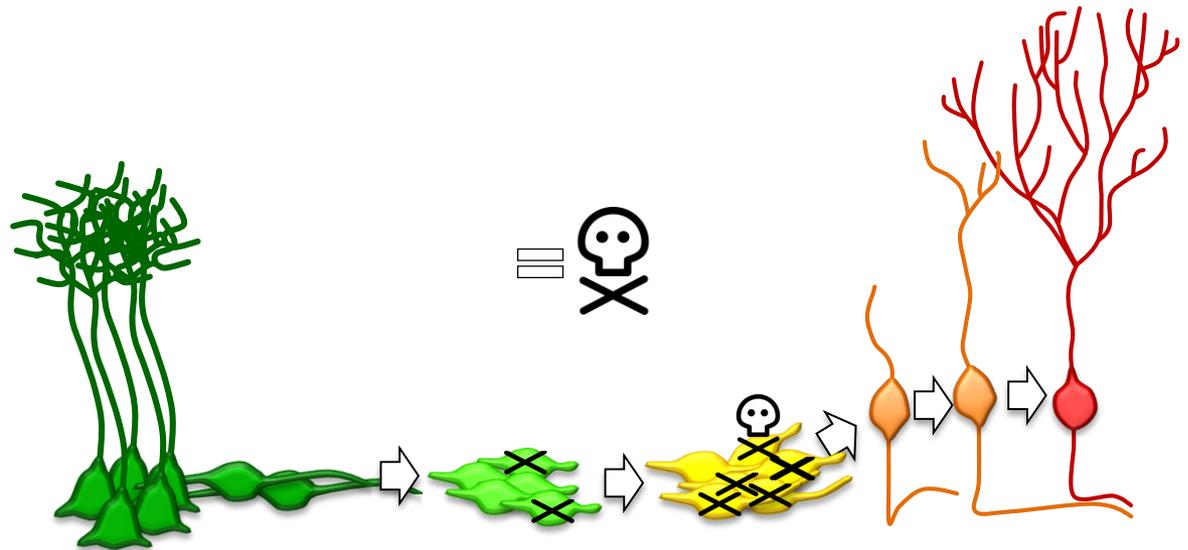
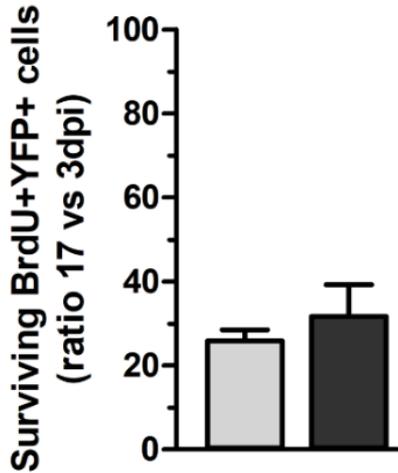
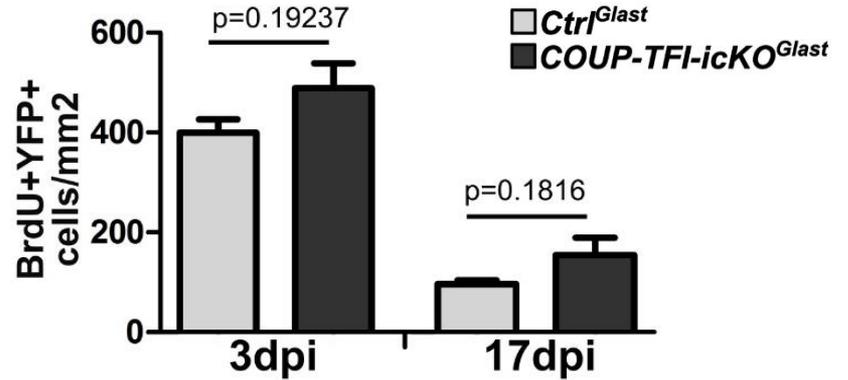
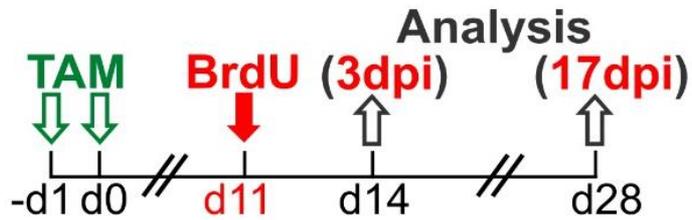
Mosaic COUP-TFI loss decreases DG neurogenesis



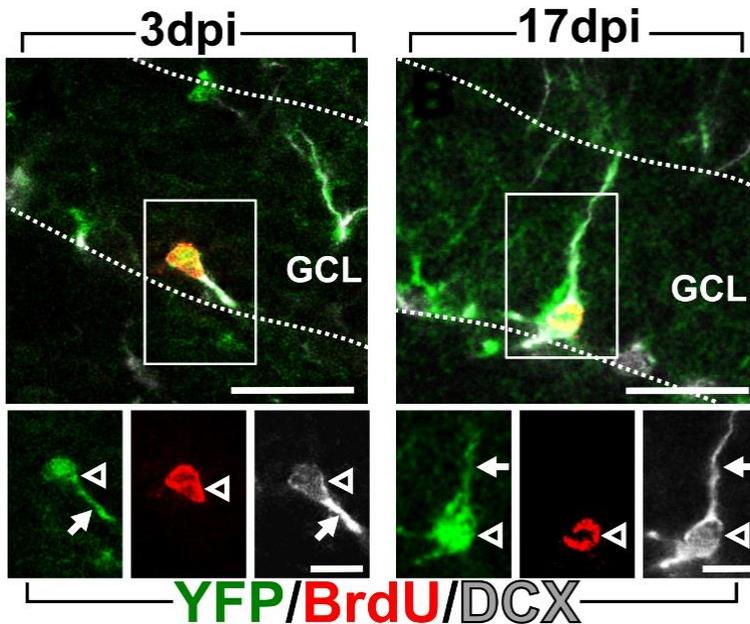
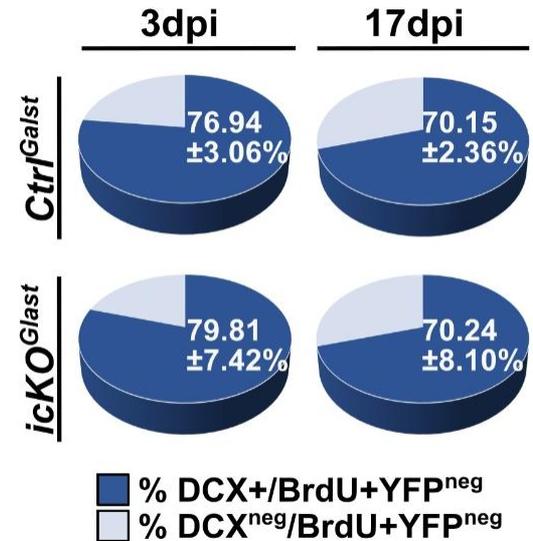
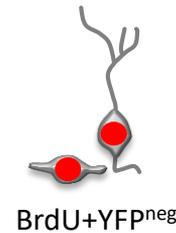
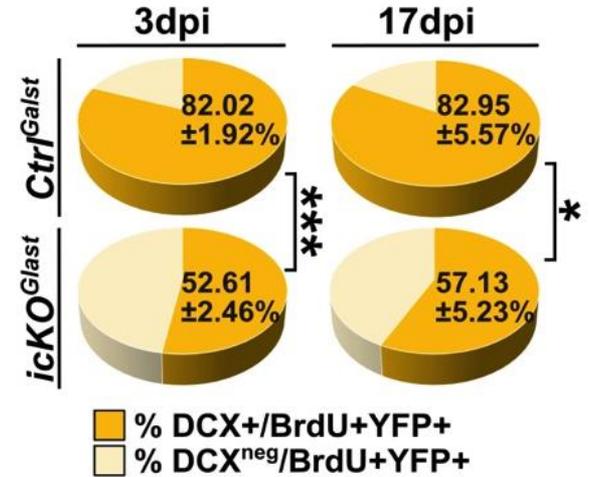
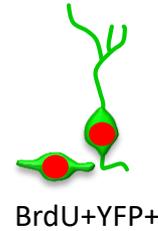
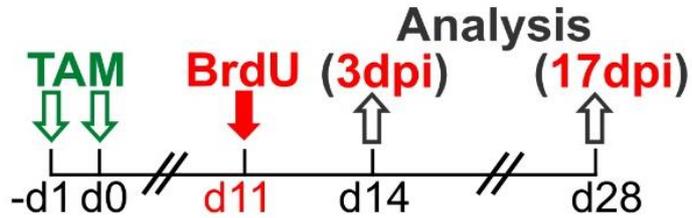
Reduced neurogenesis in COUP-TFI-icKO is not due to altered NSC/progenitor proliferation



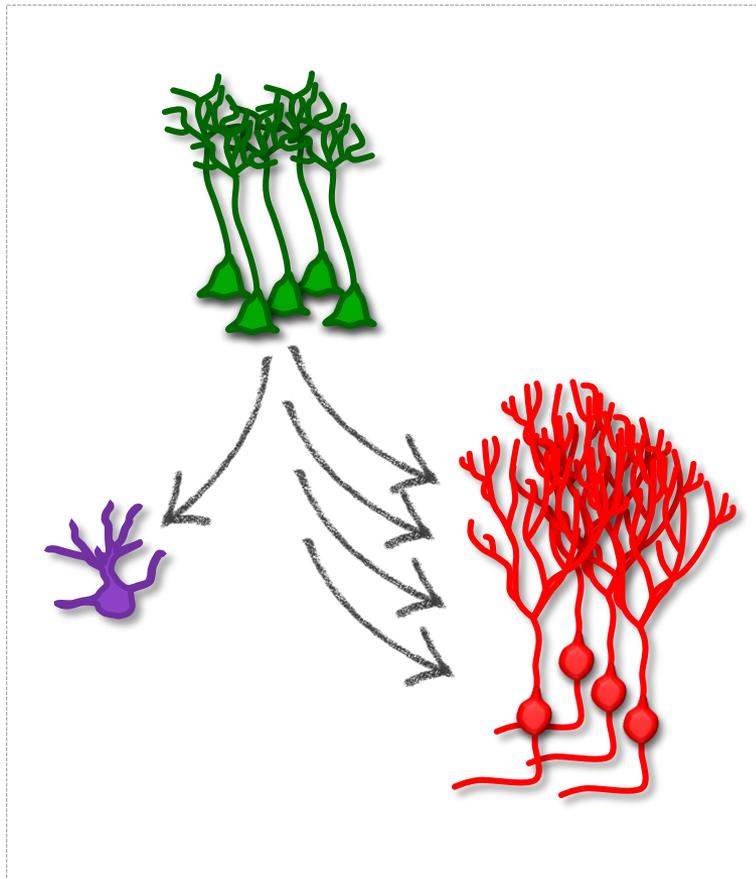
Diminished neurogenesis in COUP-TFI-icKO is not a consequence of newborn cell survival defects



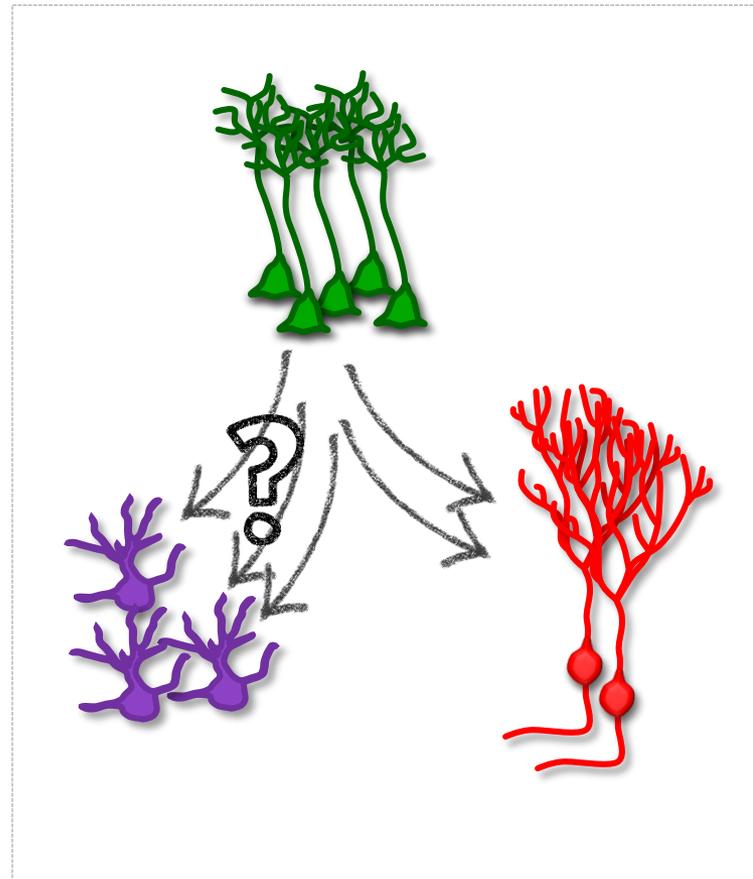
Adult neurogenesis is cell-autonomously impaired in COUP-TFI-icKO



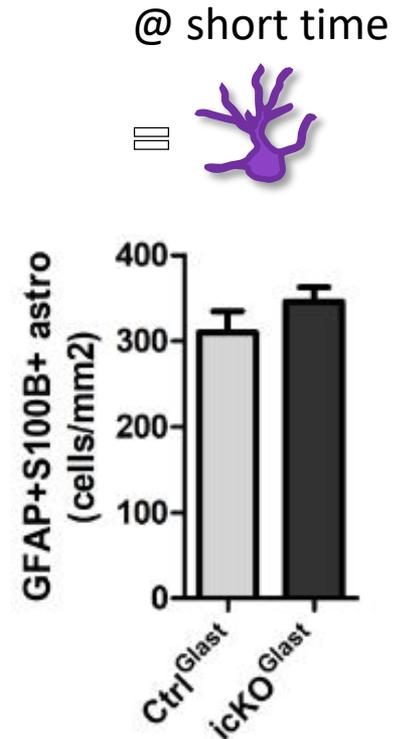
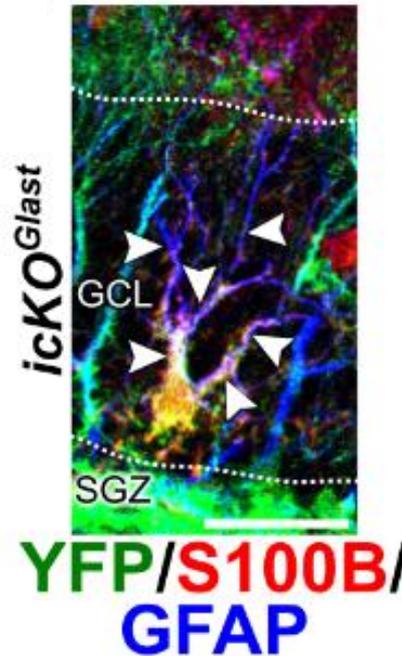
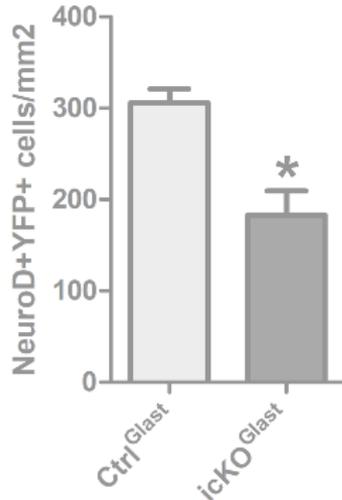
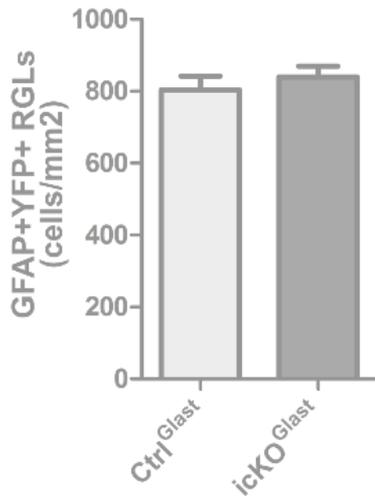
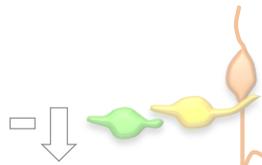
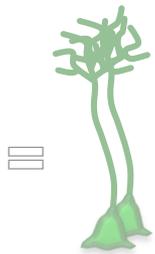
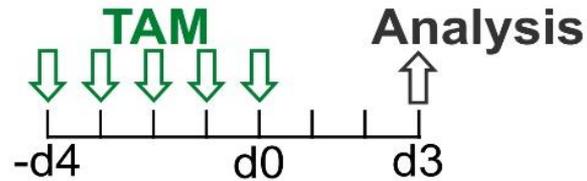
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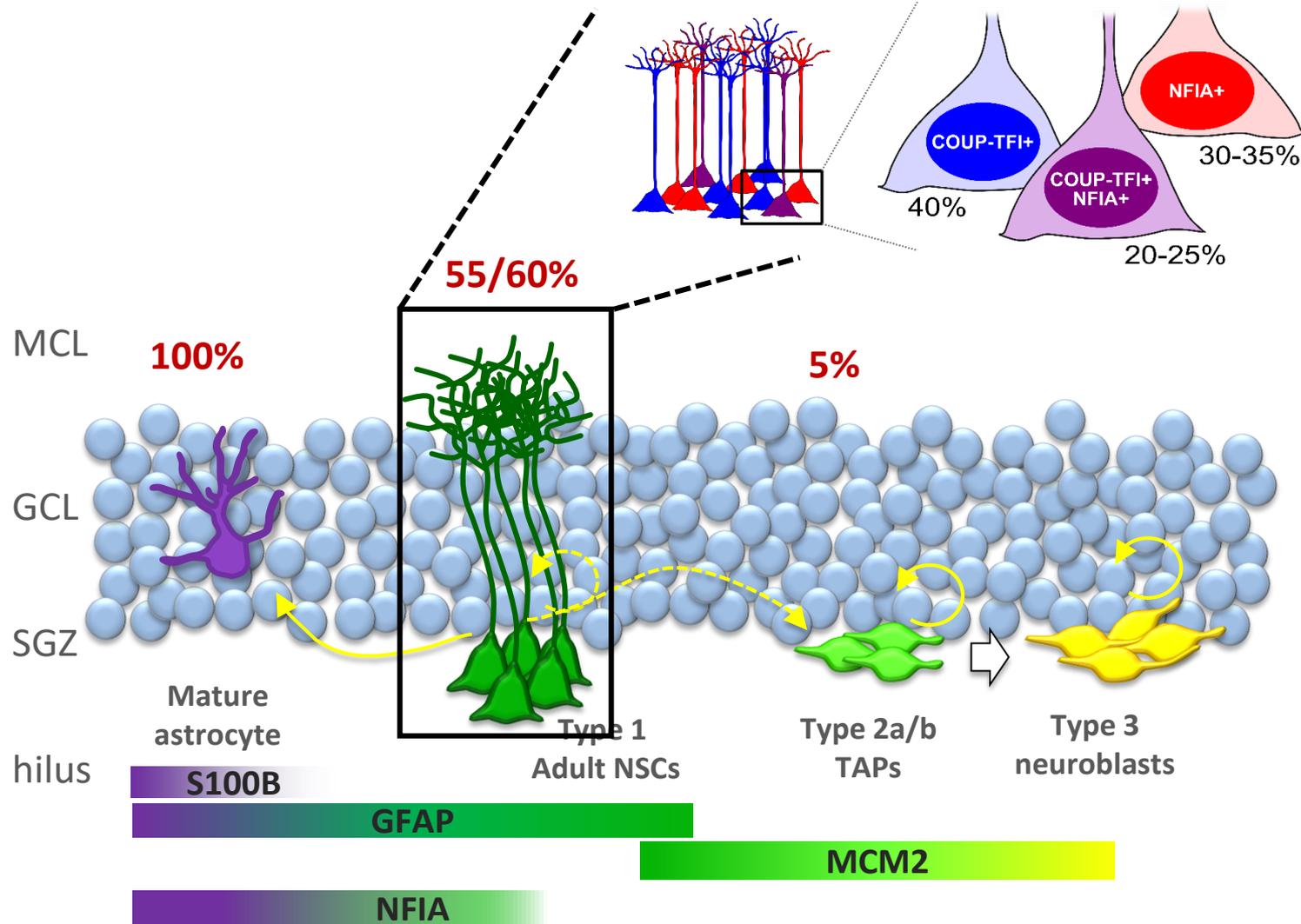
COUP-TFI-icKO^{Glast}



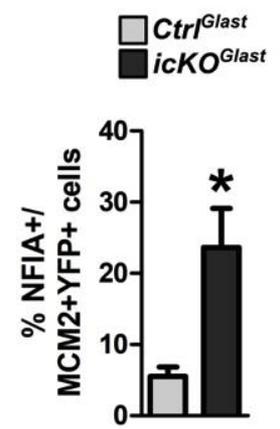
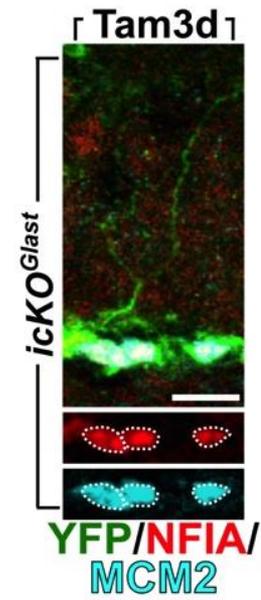
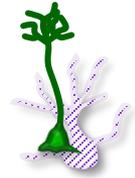
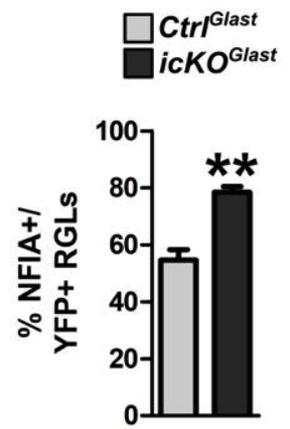
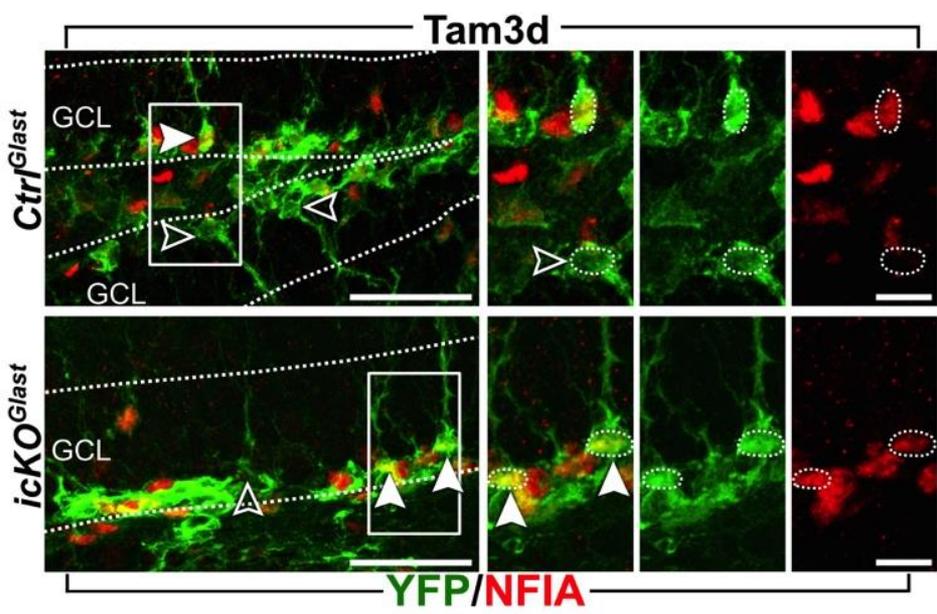
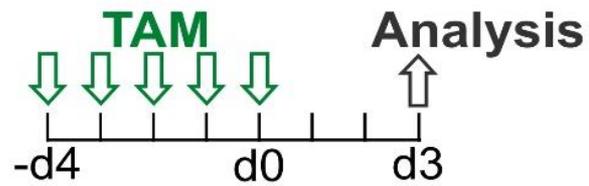
Direct differentiation of COUP-TFI-depleted radial NSCs is unlikely to occur in mutants



NFIA (Nuclear Factor I A), a pro-astroglial TF during development

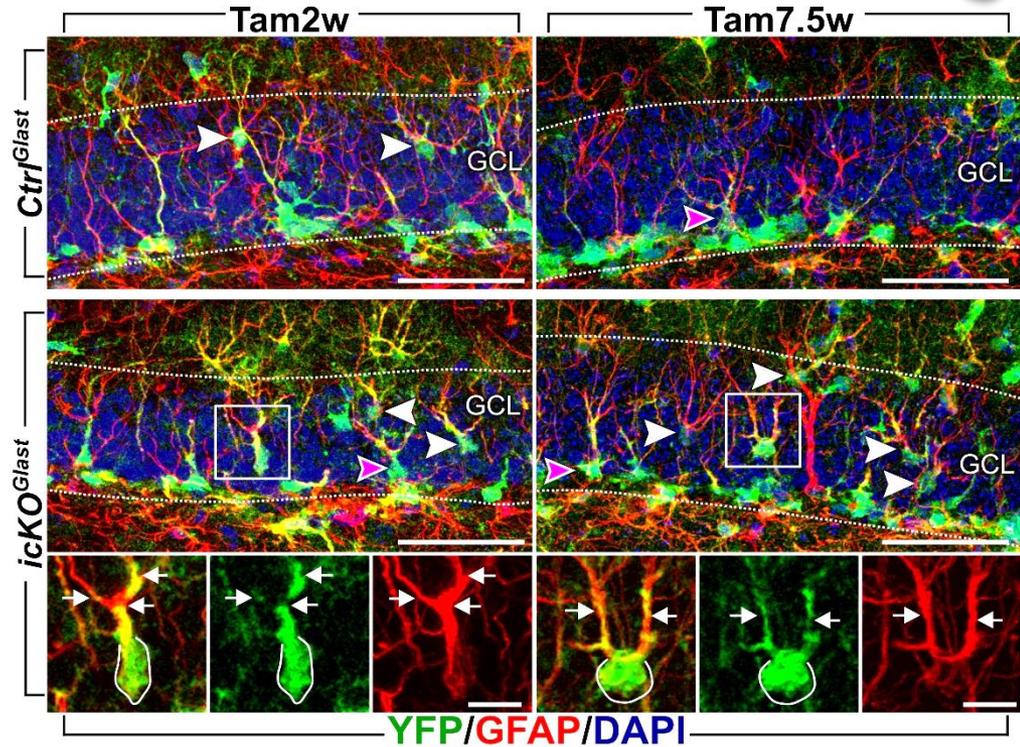
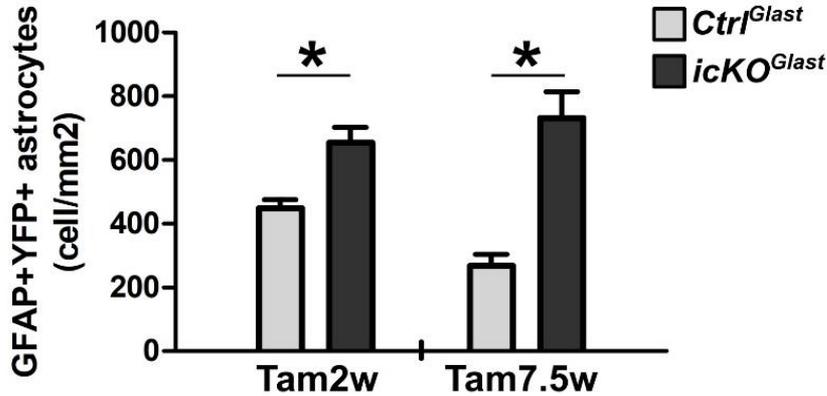
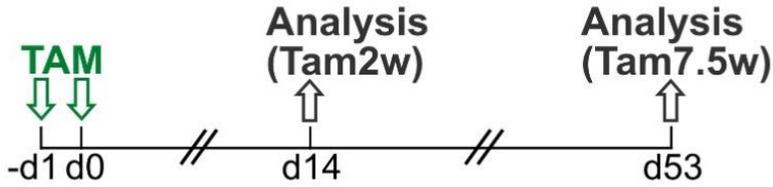
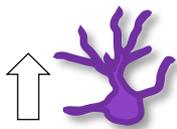


Upregulation of the pro-astroglial factor NFIA in COUP-TFI-deficient radial NSCs and progenitors



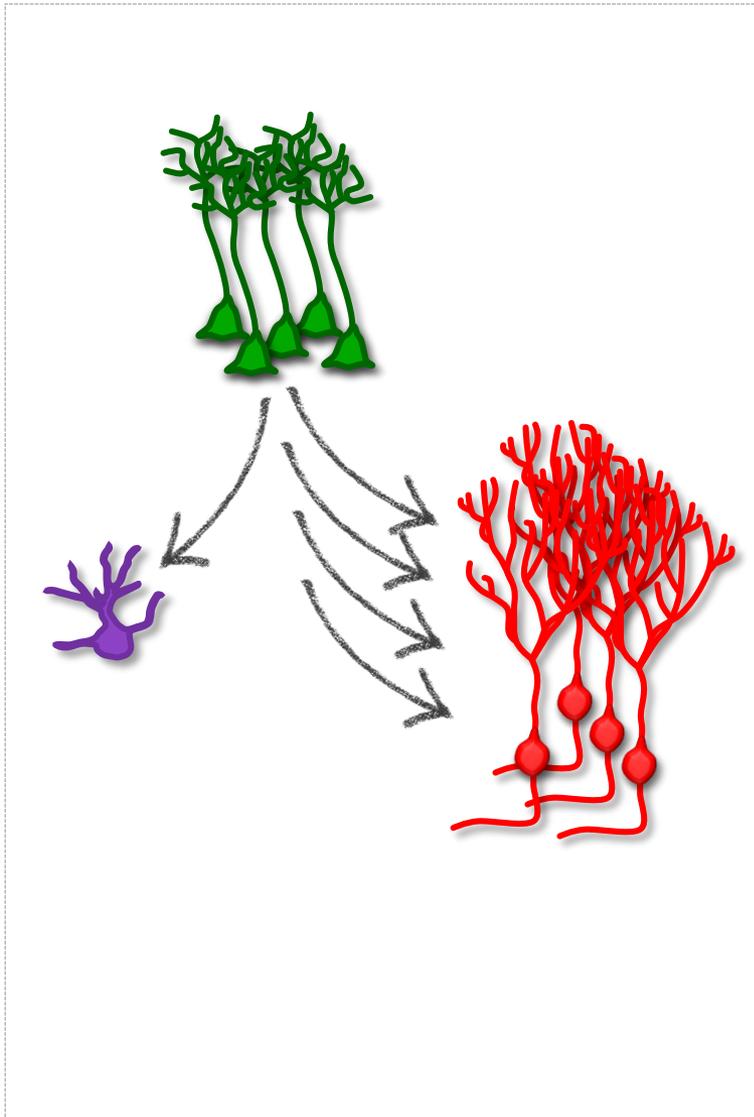
→ Switch of COUP-TFI deficient NSC/progenitor commitment towards an astroglial fate

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

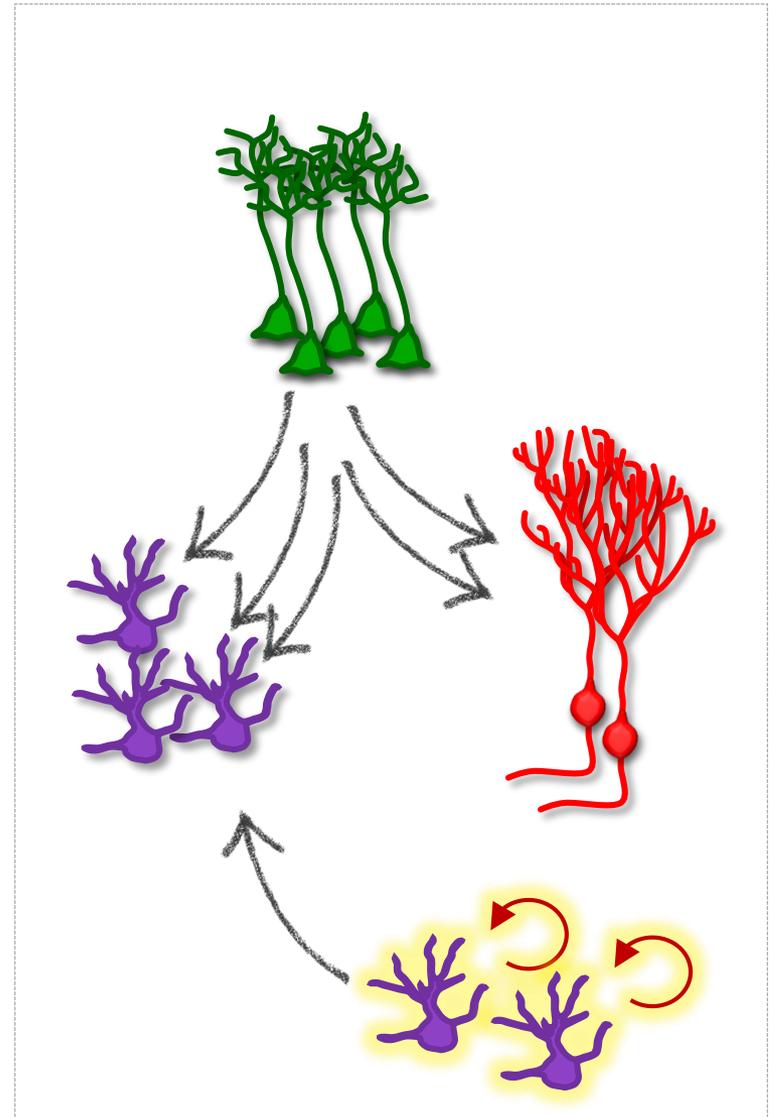


→ no depletion of the stem cell pool → no direct differentiation into astrocytes

Controls



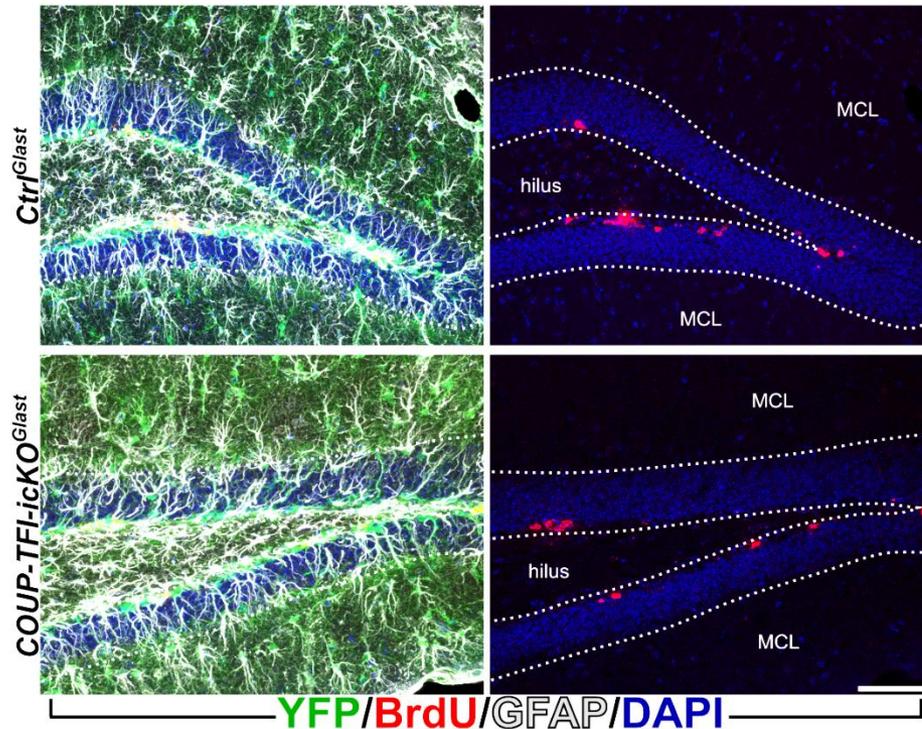
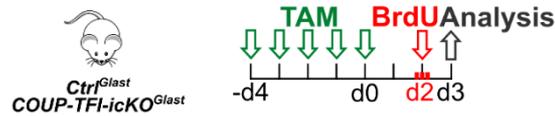
COUP-TFI-icKO^{Glast}



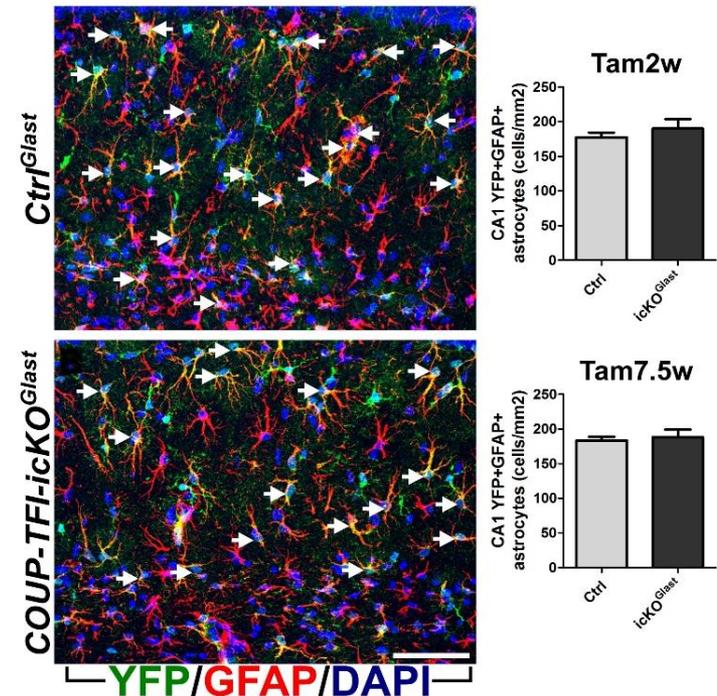
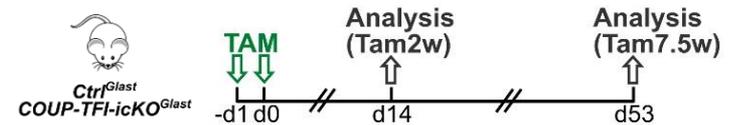


“Re-awakening” of mature parenchymal astrocytes does not occur in *COUP-TFI-icKO^{Glast}* hippocampi

BrdU incorporation in DG mature astrocytes:



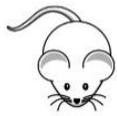
Astrocytes within the hippocampal CA1:



Selective COUP-TFI loss of function in neurogenic progenitors and mitotically active NSCs/progenitors

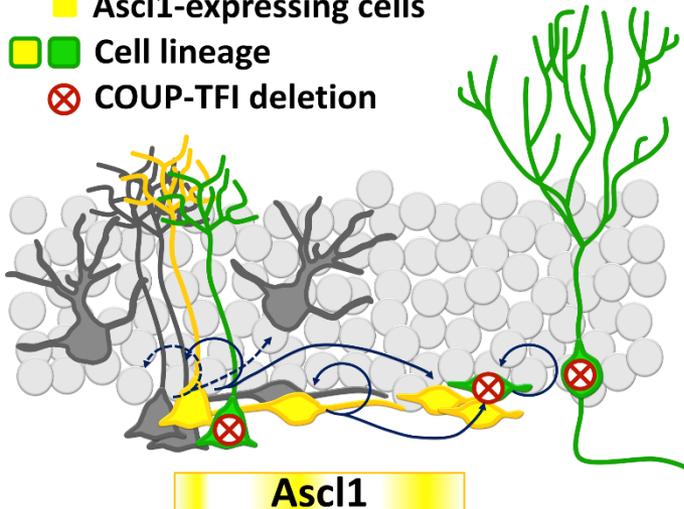
a

Ascl1 lineage-dependent COUP-TFI-icKO



Ctrl^{Ascl1}
COUP-TFI-icKO^{Ascl1}

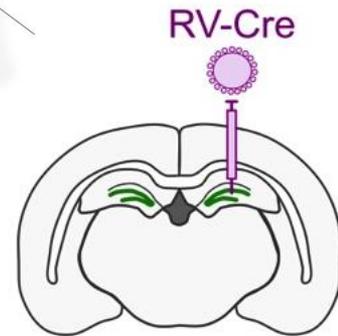
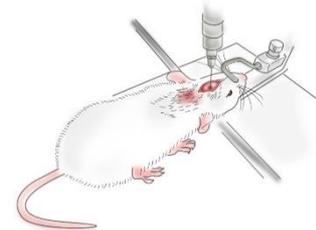
- Ascl1-expressing cells
- Cell lineage
- ⊗ COUP-TFI deletion



b

RV-Cre into the adult DG

DG stereotaxic injection of Cre-expressing Retrovirus (RV-Cre)

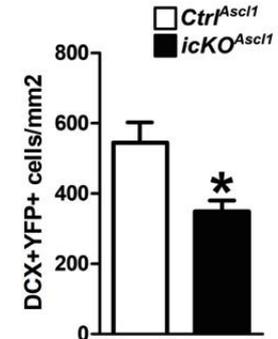
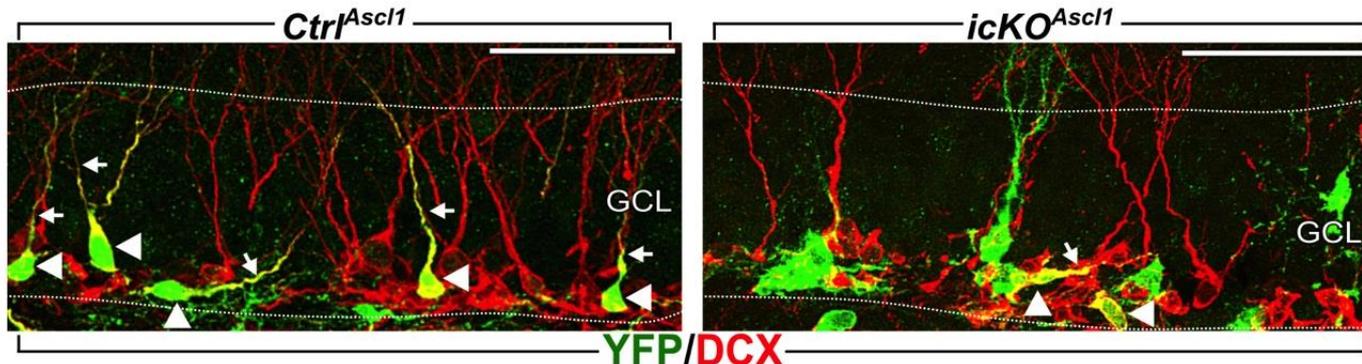
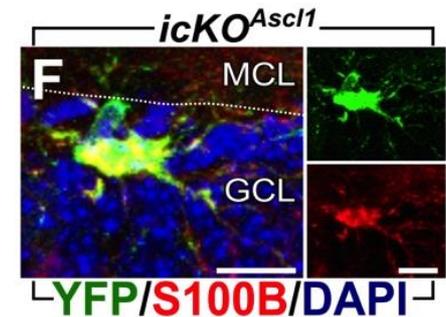
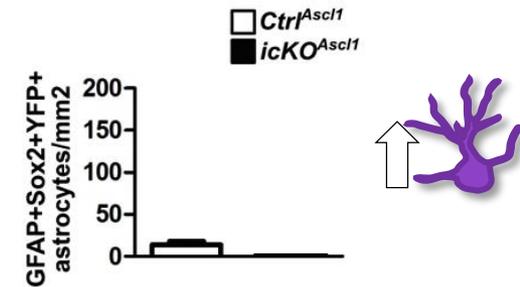
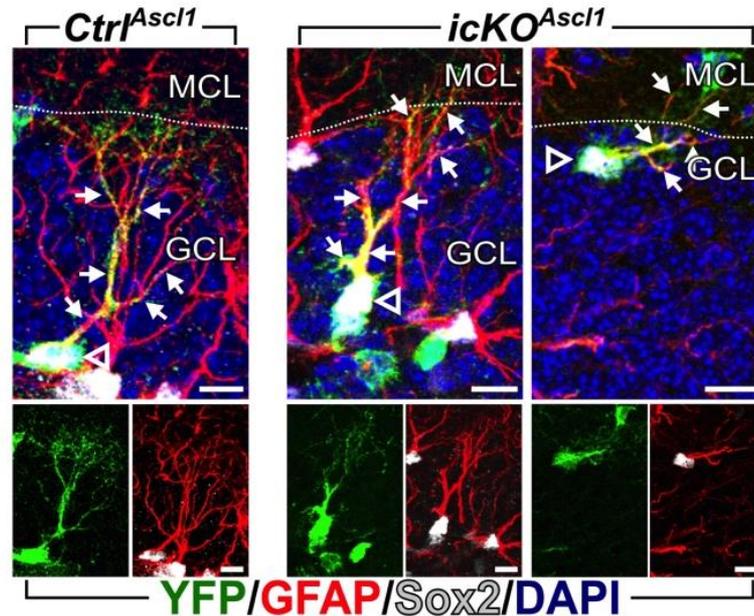
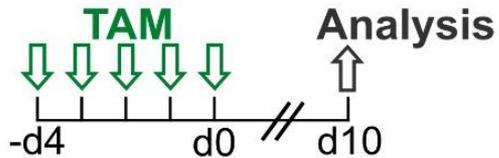


R26-YFP;COUP-TF1f1/f1 (cKO)
R26-YFP;wt (Ctrl)

a

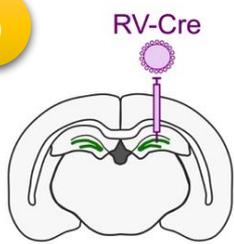
Ctrl^{Ascl1}COUP-TFI-icKO^{Ascl1}

Enhanced astrogliogenesis - decreased neurogenesis from Ascl1+ activated NSCs and neurogenic progenitors in COUP-TFI-icKO



→ COUP-TFI promotes neurogenesis by repressing astrogliogenesis in progenitors

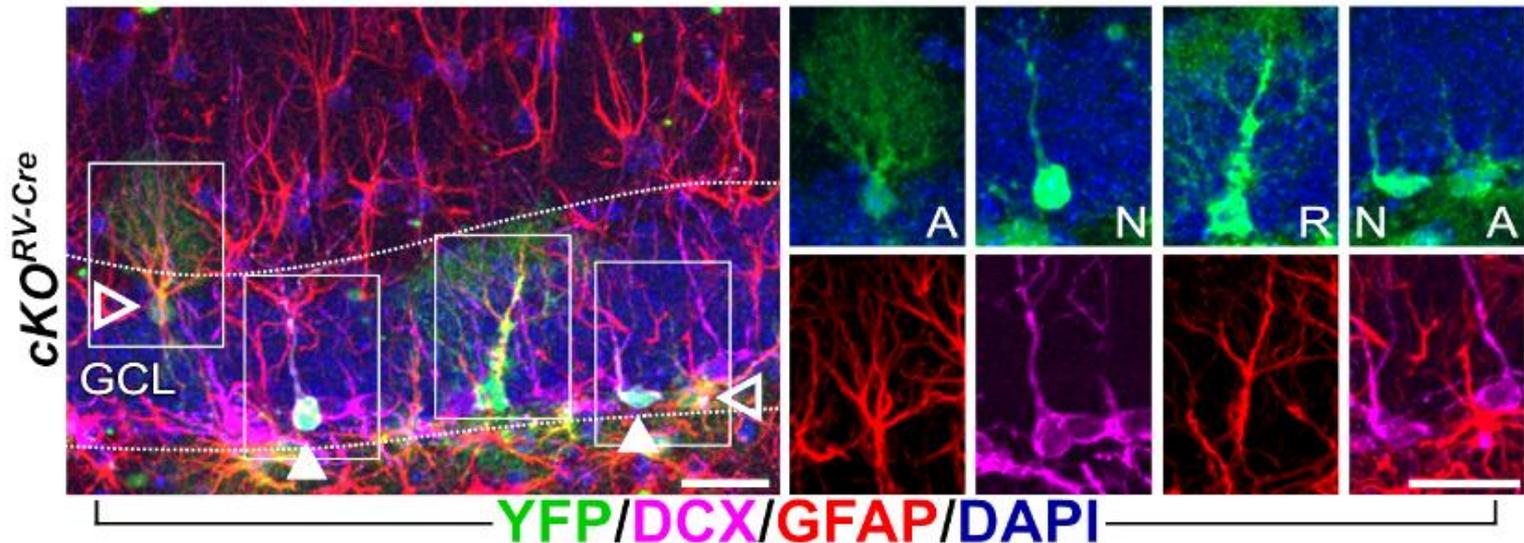
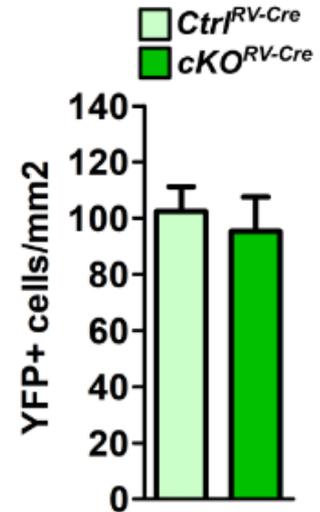
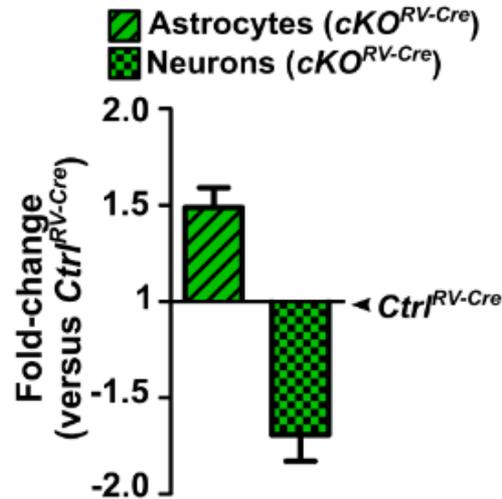
b



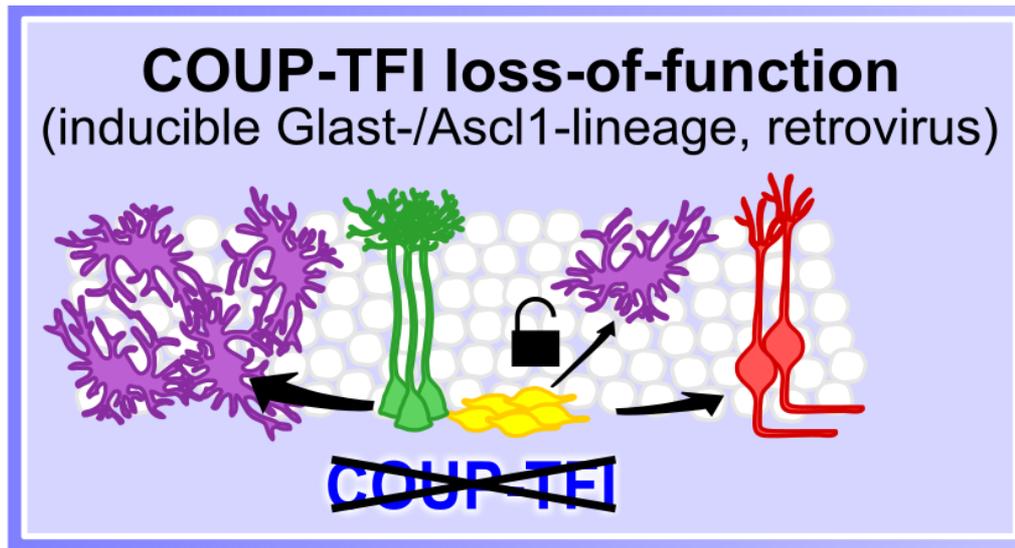
Enhanced astrogliogenesis and decreased neurogenesis following retrovirus-mediated COUP-TFI loss



1. *Ctrl^{RV-Cre} (R26-YFP;COUP-TFI^{wt/wt})*
2. *COUP-TFI-icKO^{RV-Cre} (R26-YFP;COUP-TFI^{fl/fl})*



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

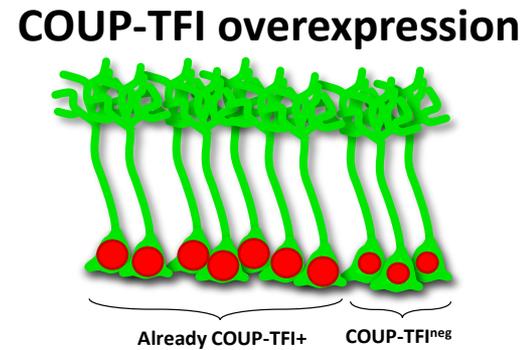
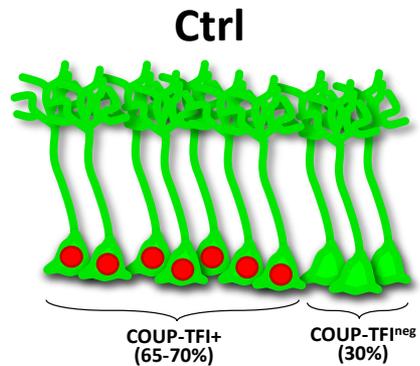
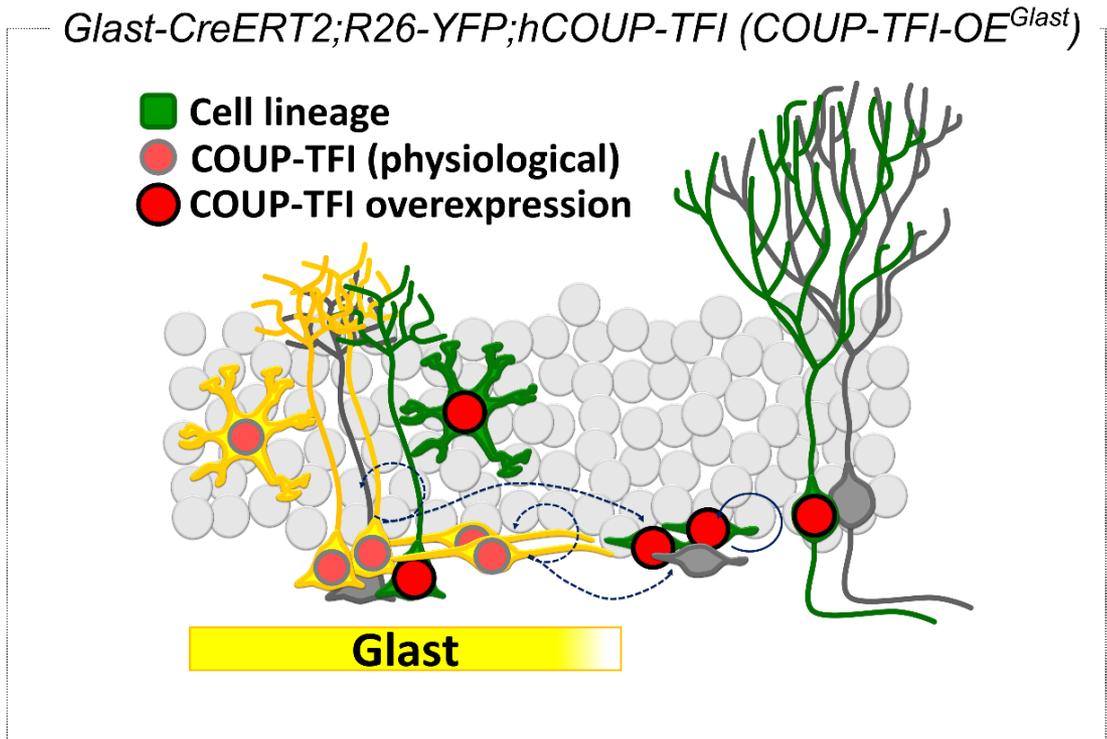
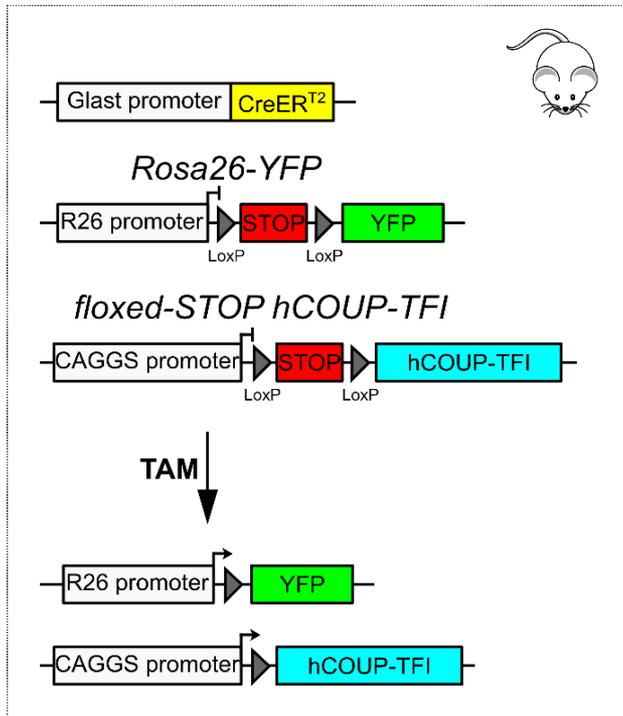


→ COUP-TFI is necessary to promote neurogenesis from RGL and progenitors by repressing their commitment towards an astroglial fate

Outline of the study

- 1. COUP-TFI expression pattern within the adult hippocampal DG*
- 2. COUP-TFI loss of function in the DG niche through Cre/loxP technology*
- 3. COUP-TFI overexpression and gain-of-function in NSCs/progenitors through Cre/loxP technology***

COUP-TFI overexpression

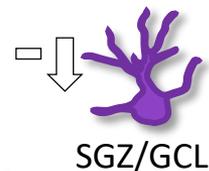
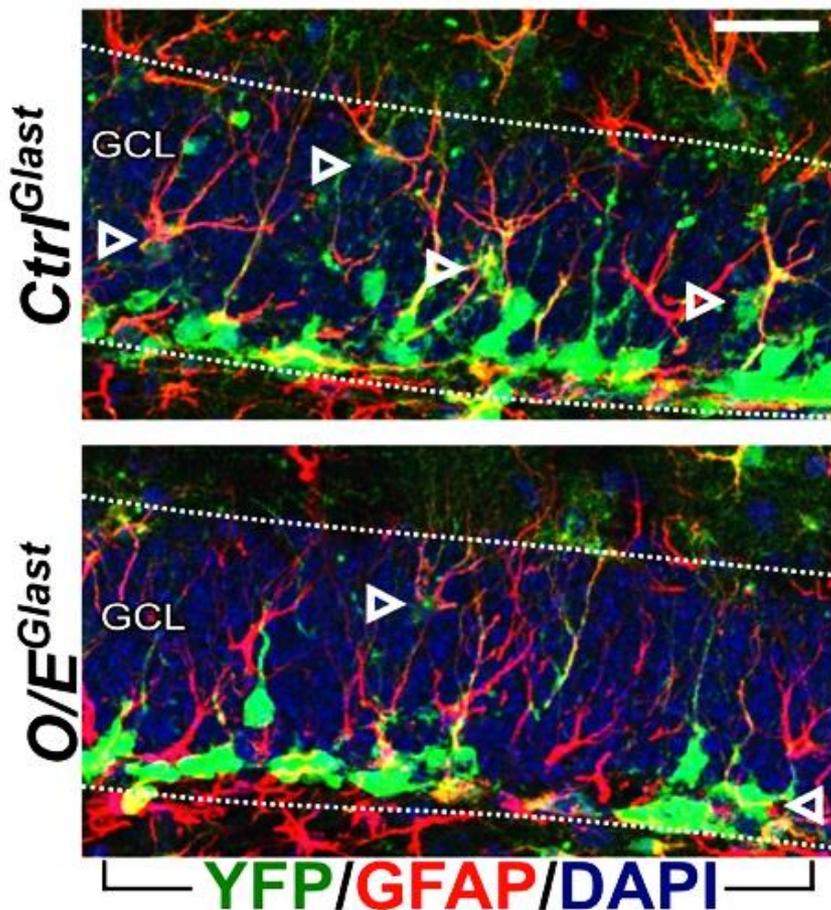
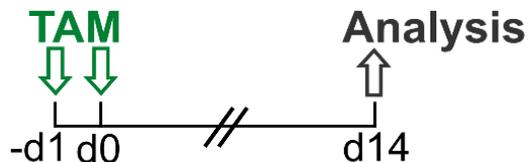




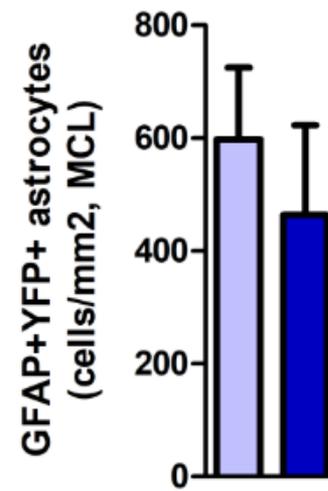
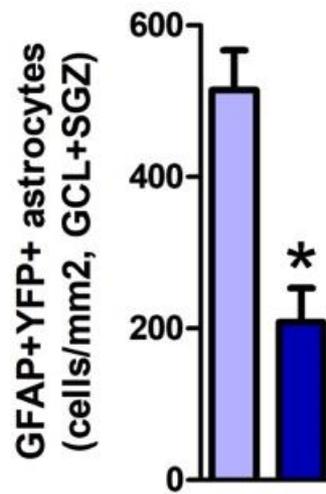
Ctrl^{Glast}

COUP-TFI-O/E^{Glast}

Forced COUP-TFI expression prevents astrogliogenesis



□ Ctrl^{Glast}
■ COUP-TFI-O/E^{Glast}

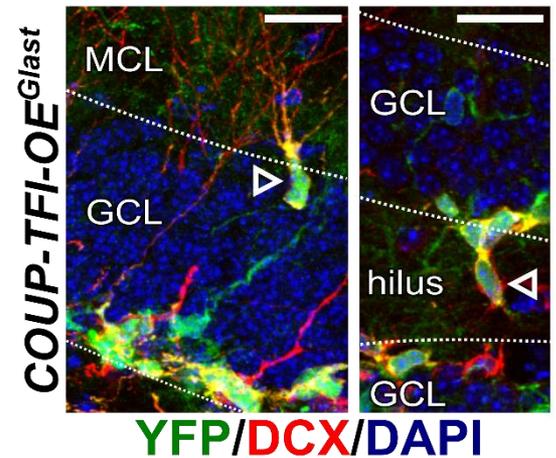
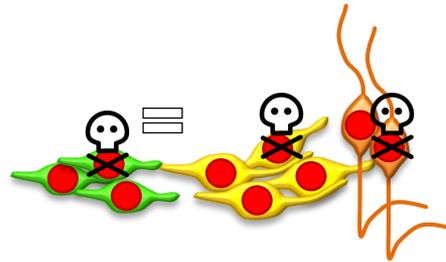
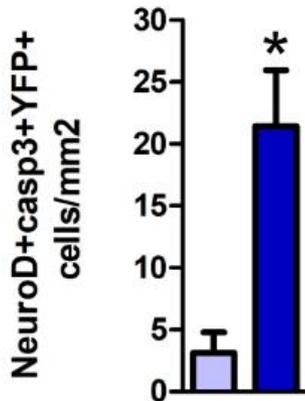
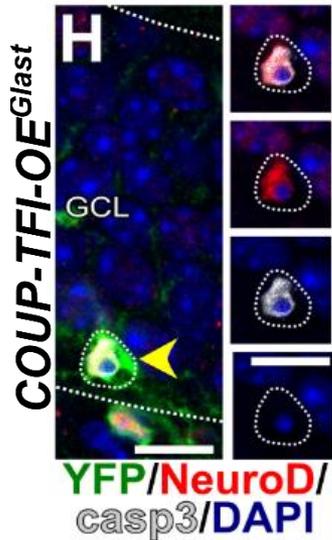
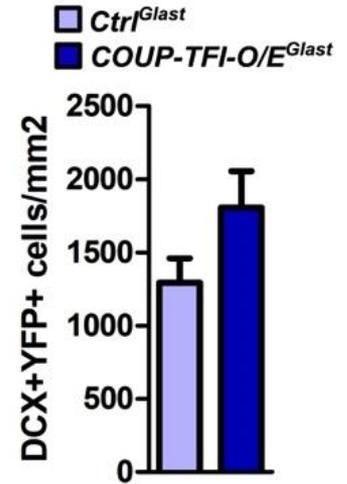
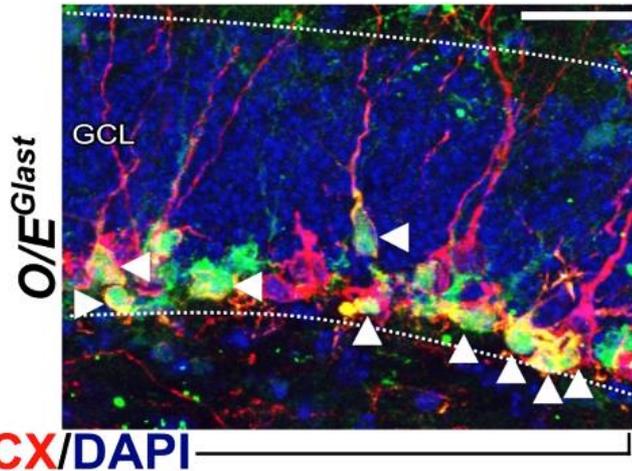
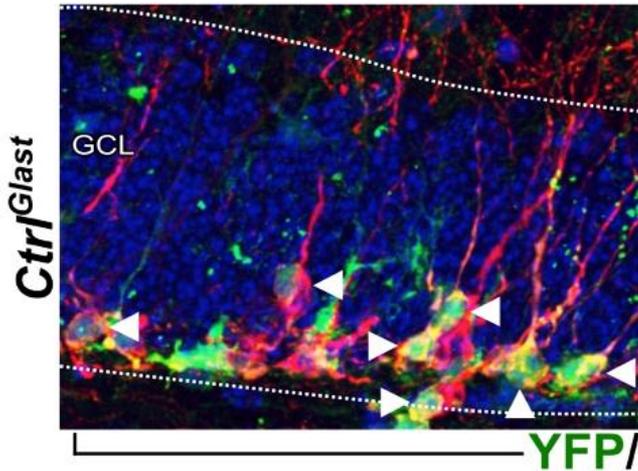




Ctrl^{Glast}

COUP-TFI-O/E^{Glast}

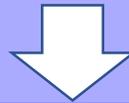
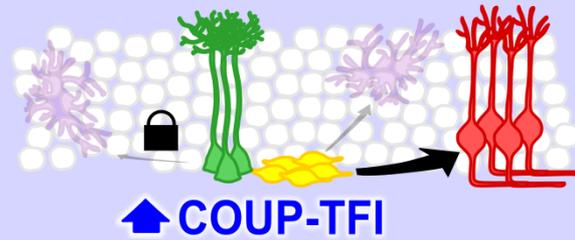
COUP-TFI overexpression dramatically increases apoptosis in newborn neurons



COUP-TFI loss-of-function
(inducible Glast-/Ascl1-lineage, retrovirus)

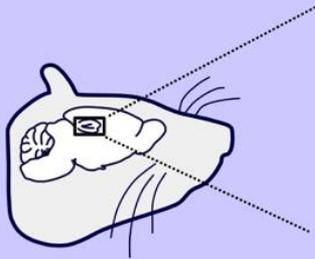


COUP-TFI overexpression
(inducible Glast-lineage)

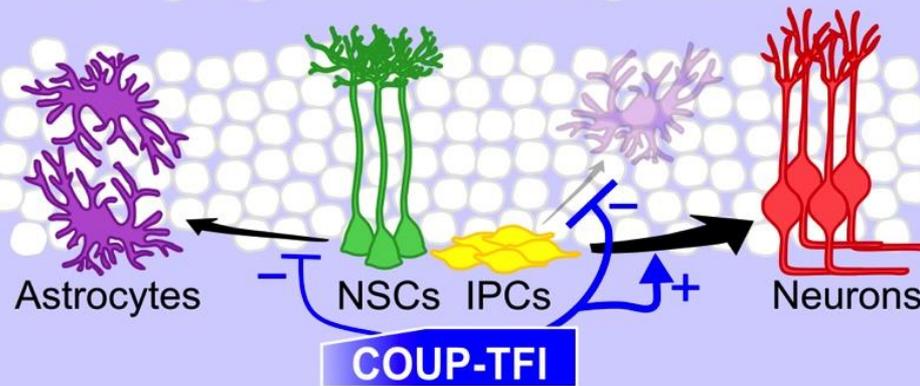


PHYSIOLOGICAL CONDITIONS

Adult hippocampus
Dentate Gyrus (DG)



Astroglialogenesis **Neurogenesis**

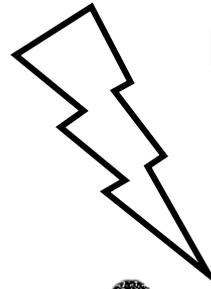


INFLAMMATION

AGING

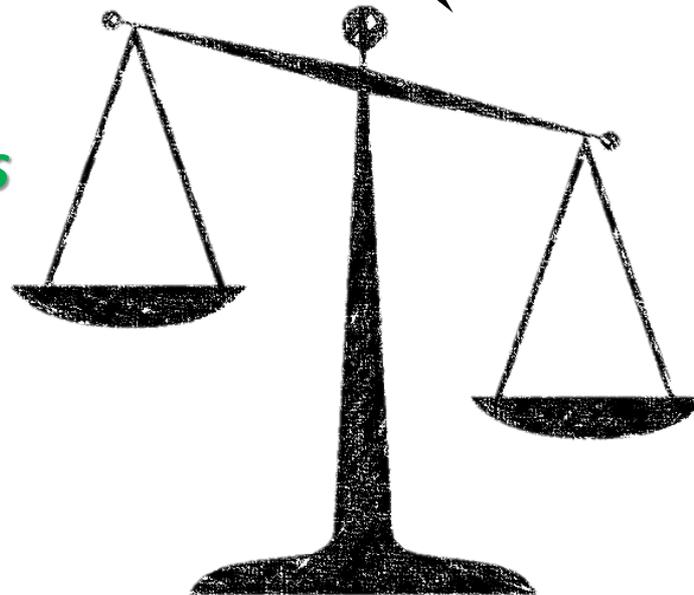
NEURODEGENERATION

EPILEPSY



ASTROGLIOGENESIS

NEUROGENESIS

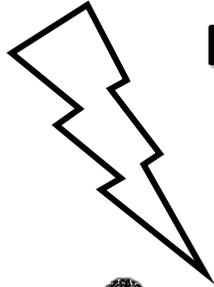


INFLAMMATION

AGING

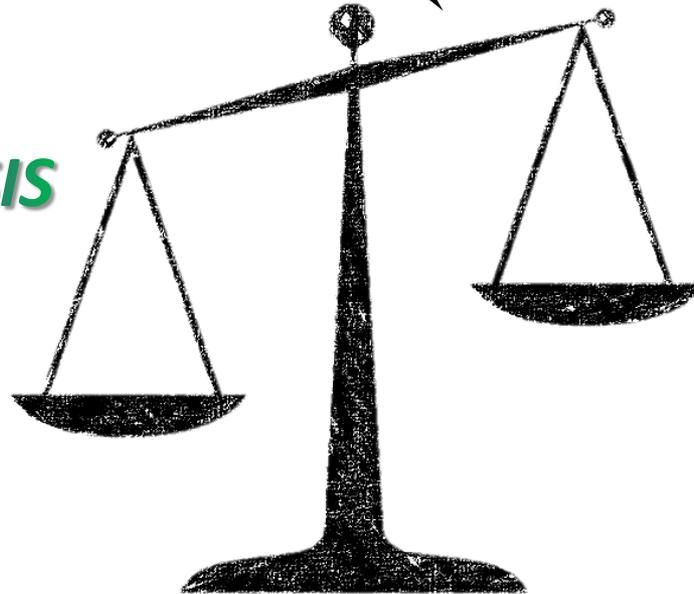
NEURODEGENERATION

EPILEPSY



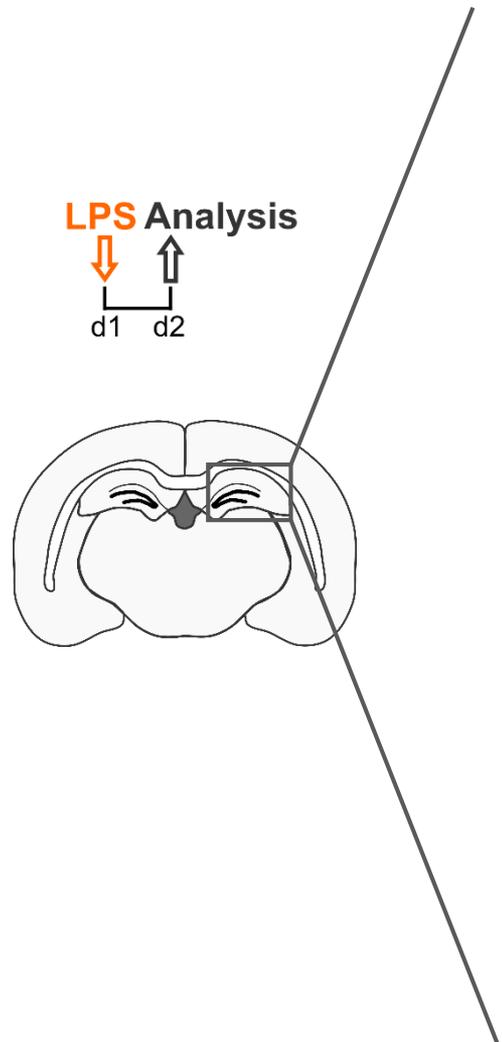
ASTROGLIOGENESIS

NEUROGENESIS

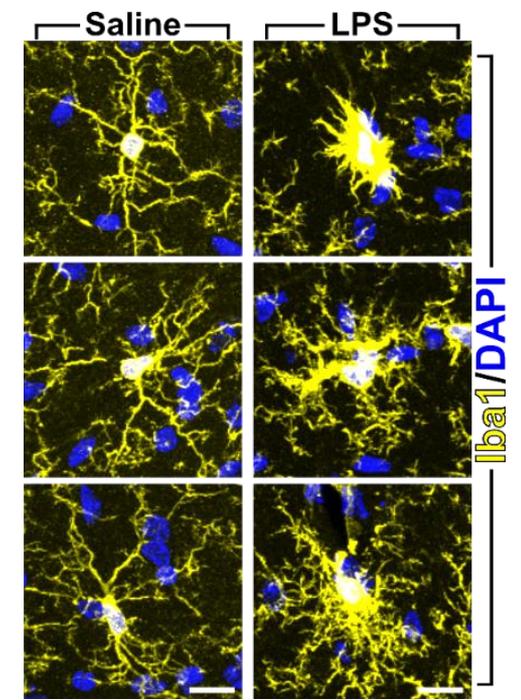
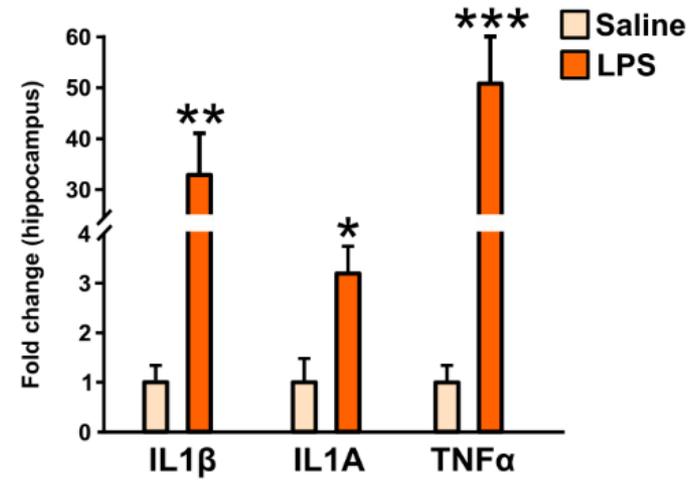




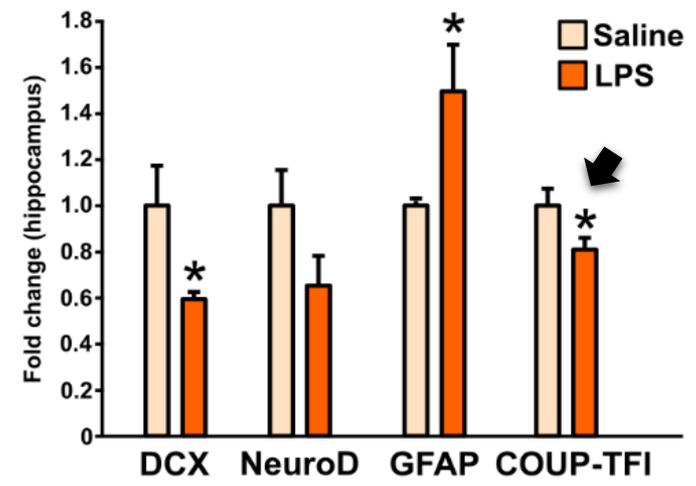
Neuroinflammation model: *E. coli*-derived lipopolysaccharide (LPS)



RT-qPCR:

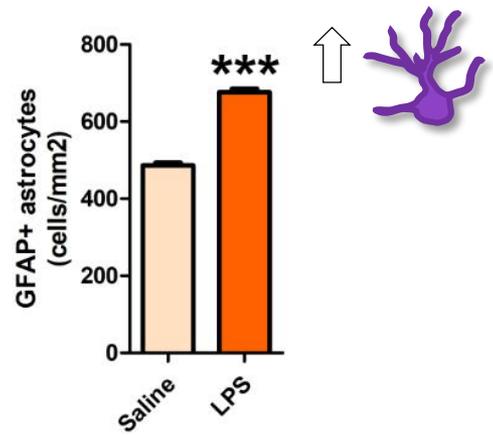
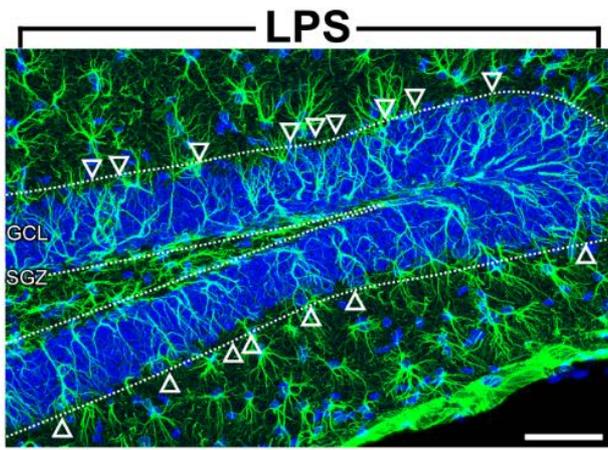
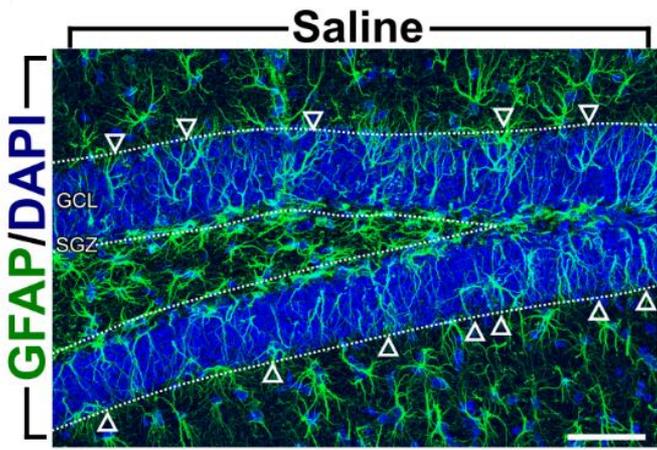
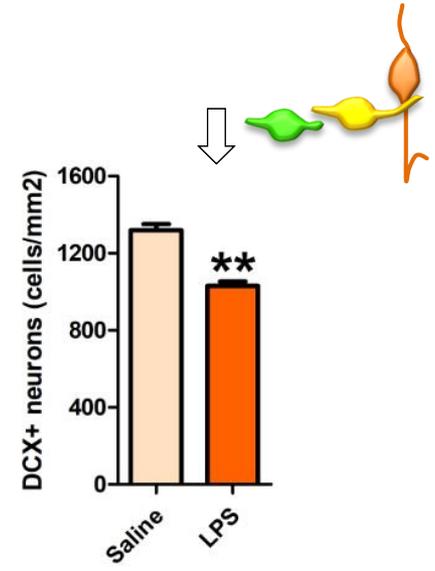
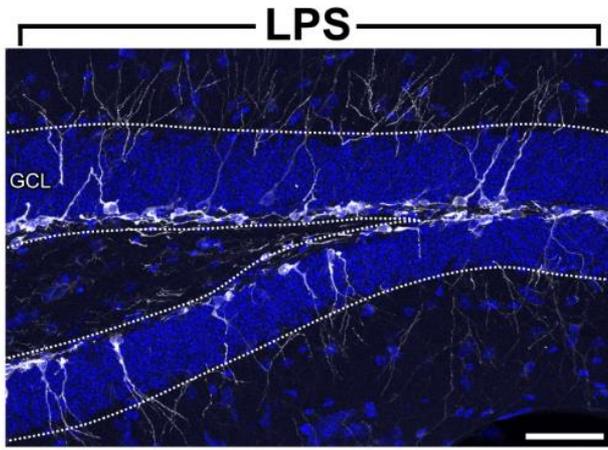
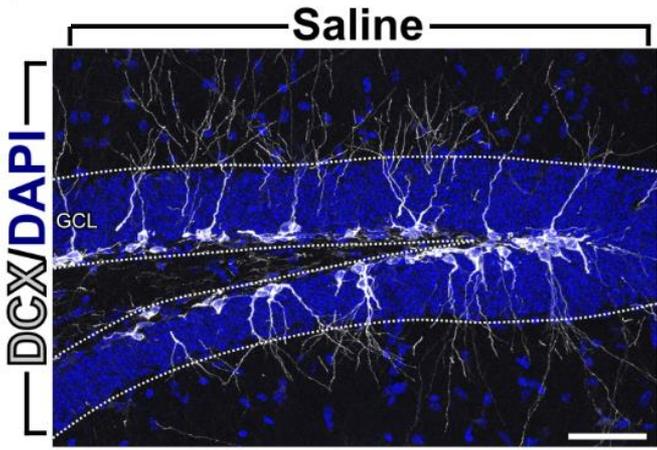


Activated microglia



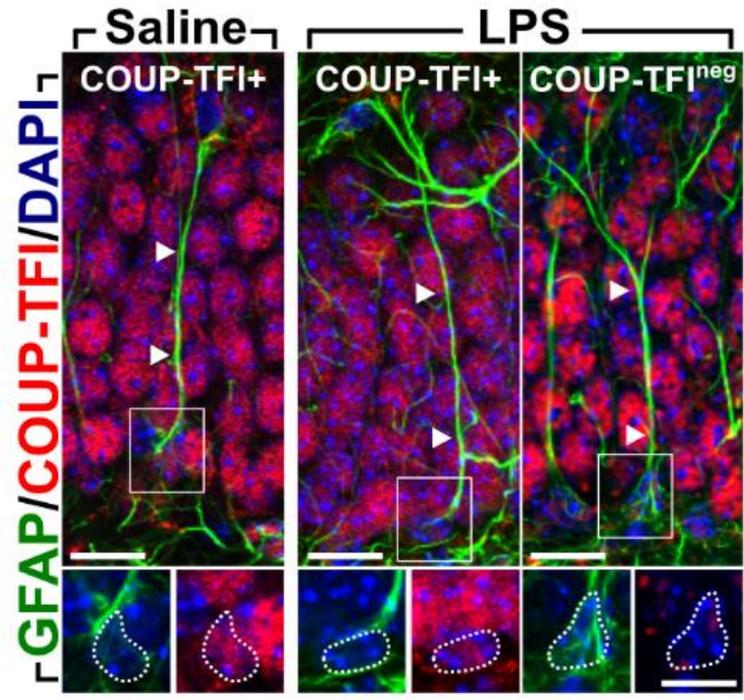


Neuroinflammation model: *E. coli*-derived lipopolysaccharide (LPS)

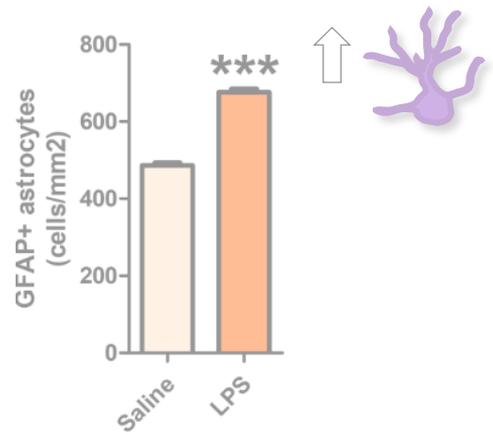
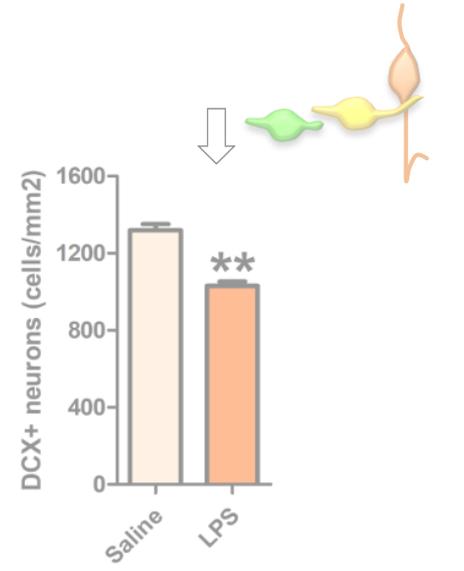
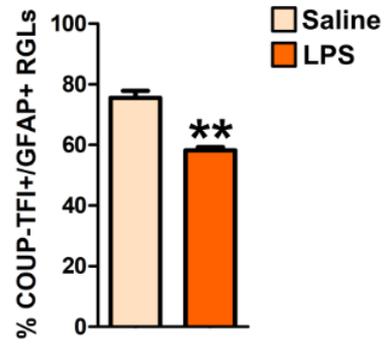


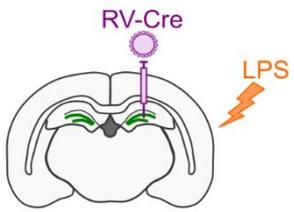


Neuroinflammation model: *E. coli*-derived lipopolysaccharide (LPS)



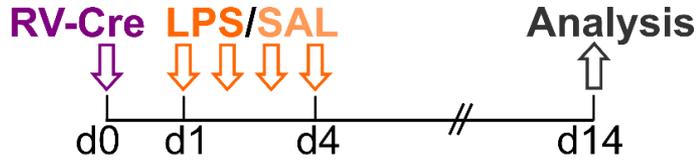
↓ Decreased COUP-TFI expression



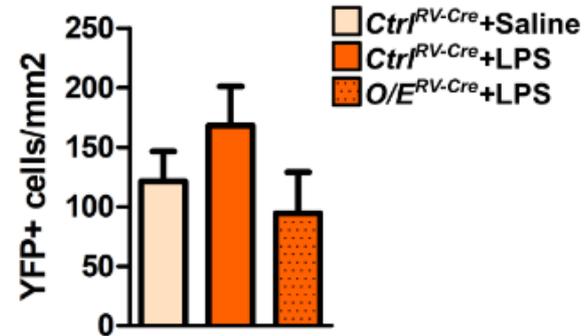


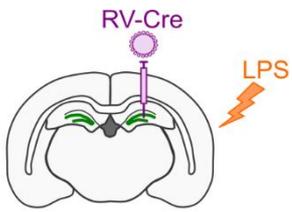
COUP-TFI gain of function rescues neuron-to-astrocyte generation upon neuroinflammation

R26-YFP;hCOUP-TFI (COUP-TFI-O/E)
R26-YFP;wt (Ctrl)



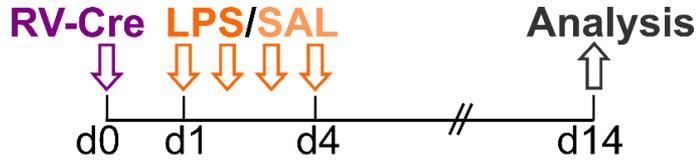
1. *Ctrl*^{RV-Cre} + Saline
2. *Ctrl*^{RV-Cre} + LPS
3. *COUP-TFI-O/E*^{RV-Cre} + LPS



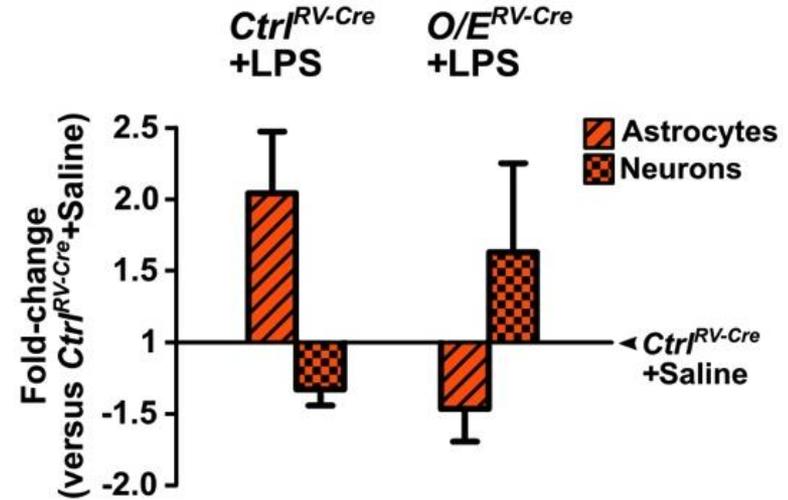


COUP-TFI gain of function rescues neuron-to-astrocyte generation upon neuroinflammation

R26-YFP;hCOUP-TFI (COUP-TFI-O/E)
R26-YFP;wt (Ctrl)



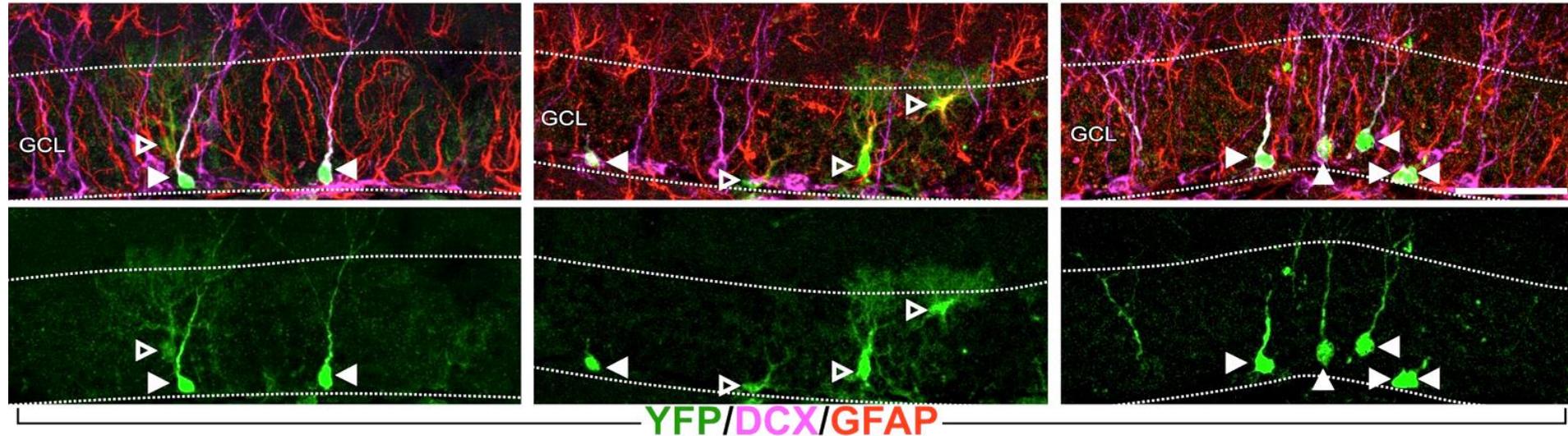
1. *Ctrl*^{RV-Cre} + Saline
2. *Ctrl*^{RV-Cre} + LPS
3. *COUP-TFI-O/E*^{RV-Cre} + LPS



Ctrl^{RV-Cre} + Saline

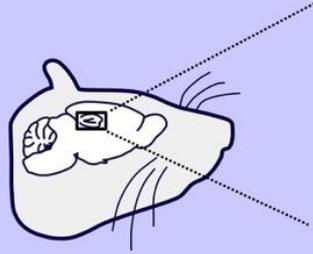
Ctrl^{RV-Cre} + LPS

COUP-TFI-O/E^{RV-Cre} + LPS

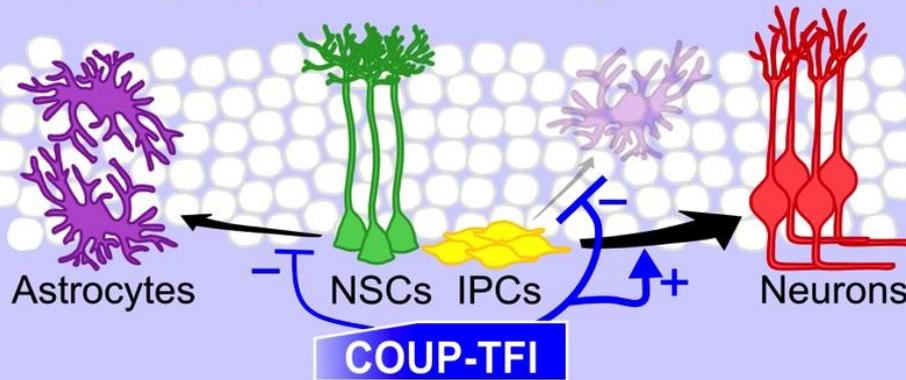


PHYSIOLOGICAL CONDITIONS

Adult hippocampus
Dentate Gyrus (DG)

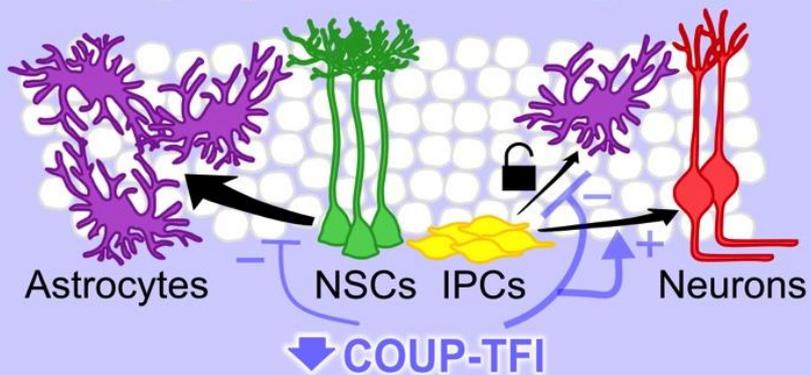


Astroglialogenesis Neurogenesis

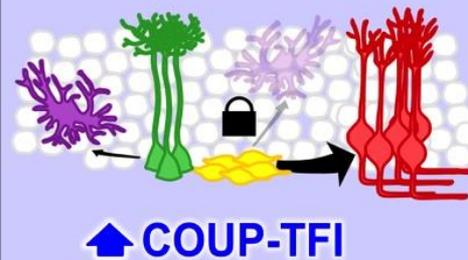


NEUROINFLAMMATION

Astroglialogenesis Neurogenesis



Rescue by COUP-TFI
overexpression



Adult neurogenesis lab at NICO, Orbassano (TO)

Silvia De Marchis
Isabella Crisci

Paolo Peretto
Luca Bonfanti
Federico Luzzati
Serena Bovetti
Giulia Nato
Chiara La Rosa
Marco Fogli

Filippo Michelon
Daniele Stajano
Eleonora Dallorto
Valentina La Monica
Mario Orts



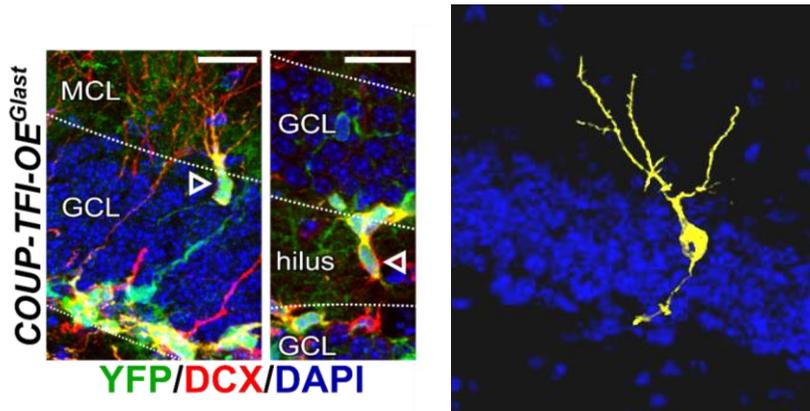
Collaborations

M. Studer, PhD (iBV, CNRS, Inserm, Univ. Nice)
W. Krezel, PhD (IGBMC- Illkirch, Univ. Strasbourg)
C. Rolando, PhD (Dept. Biomedicine, Univ. Basel)



Ongoing analysis and projects

Neuron morphology in COUP-TFI mutants

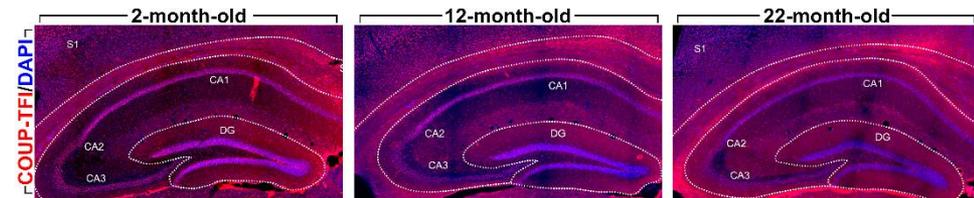


- ✓ migration/positioning (SGZ/GCL, ectopism)
- ✓ morphological features (total dendritic length, branching points, ...)

in *Ctrl^{Glast}*, *COUP-TFIO/E^{Glast}*, *COUP-TFI-het^{Glast}*,
COUP-TFI-icKO^{Glast}

Filippo Michelon

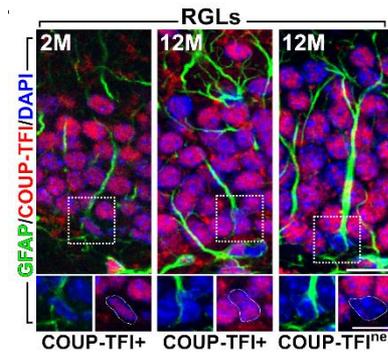
COUP-TFI in aging DG



COUP-TFI

DG neurogenesis

DG astroliogenesis??



Daniele Stajano

- ✓ characterization of COUP-TFI expression in the DG niche at different ages (2-24 months)
- ✓ lineage tracing in *Ctrl^{Glast}* at different ages
- ✓ long-term evaluation of TAM-treatments in young *Ctrl^{Glast}* vs *COUP-TFI-icKO^{Glast}*

Neuroinflammation, tamoxifen and COUP-TFI

Isabella Crisci, Valentina La Monica, Eleonora Dallorto

