N°Anonymat:

The candidate will provide (i) a title to the article, (ii; iii) a short description of the biological context and of the specific problematics of the article and (iv) an analysis of the data (for each panel, describe the aim of the experiment, the general methodology, the results and conclude). Finally the candidate will propose a model (v) and formulate 2 to 4 questions raised by the study (vi).

Cell Reports
Article

OPEN ACCESS
CellPress

Cell Reports *17*, 1383–1398, October 25, 2016

(i) TITLE?????

INTRODUCTION

(ii)- Biological context of the article

In zebrafish, widespread neurogenesis is maintained in adult stages in several brain regions, including the dorsal telencephalon (or pallium) (**Figure 1A**). In this region, radial glial cells (RG) have been identified as neural stem cells (NSCs). A distinguishing feature of adult NSCs when compared with embryonic neural progenitor cells (NPCs) is their relative quiescence. Recent works further demonstrated that quiescent NSCs are heterogeneous in their activation potential, their reactivity to various stimuli and, once activated, their division rate and ability to re-enter into quiescence.
MicroRNAs are small regulatory RNAs that are known to post- transcriptionally negatively regulate gene expression and are thus attractive candidates for integrating environmental and internal cues in the regulation of NSCs quiescence/activation balance. MicroRNA-9 (miR-9) is a determinant regulator of NPCs during vertebrate embryonic development. It was shown to promote the transition of NPCs from a proliferative to a neurogenic mode and to be crucial in the timing of their cell-cycle exit…

(iii)- Specific problematics of the article

RESULTS

(iv)- Data analysis (Fig. 1 to 3)

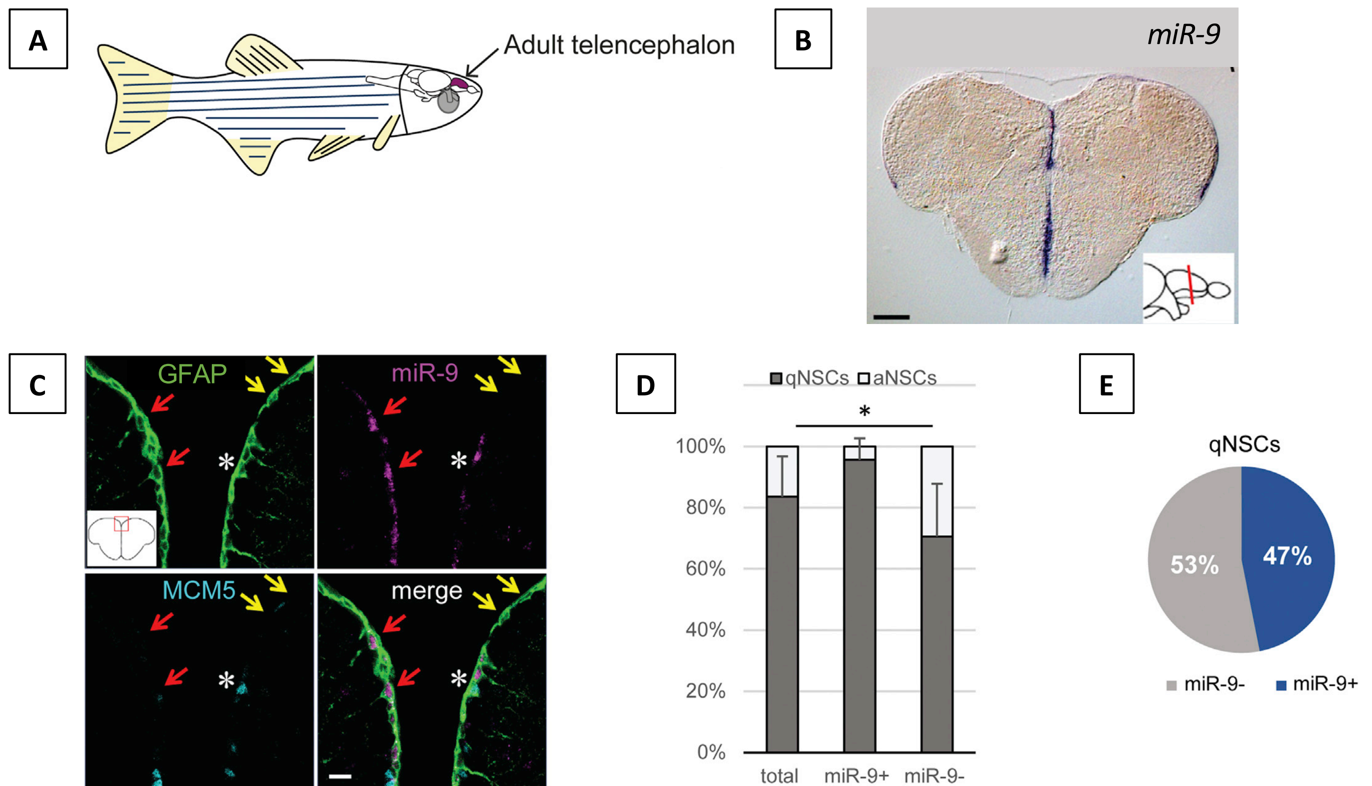

Figure 1. Analysis of miR-9 expression in NSCs
(A) Schematic showing the anatomy of the zebrafish brain.
(B) *In situ* hybridization (ISH) against miR-9 on a cross-section of the telencephalon. miR-9 expression (blue) is restricted to the first cellular row lining the brain ventricle, which is composed of the radial glia (RG). Scale bar: 100 mm.
(C-E) Analysis of mir-9 expression in quiescent and activated NSCs. (C) Sections through the medial region of the dorsal telencephalon showing ISH with a miR-9 probe (magenta) and double fluorescent immunostaining for GFAP (green, RG marker in the zebrafish telencephalon) and MCM5 (light blue, cycling cell marker). MiR-9^{+} cells are indicated with red arrows and miR-9^{-} cells are indicated with yellow arrows. An asterisk points a mir-9^{-}/GFAP^{+}MCM5^{+} cell. (D) Quantification of quiescent NSCs (qNSCs, GFAP^{+}MCM5^{-}; dark gray) and activated NSCs (aNSCs, GFAP^{+}MCM5^{+} ; white) among total cells or within the miR-9^{+} or miR-9^{-} NSC populations. (E) Quantification of qNSCs that are miR-9^{+} or miR-9^{-}.
*$p < 0.05$; one-way ANOVA with Bonferroni post hoc correction. Data are represented as mean ± 95% confidence interval (CI); n = 3 brains per condition.

k

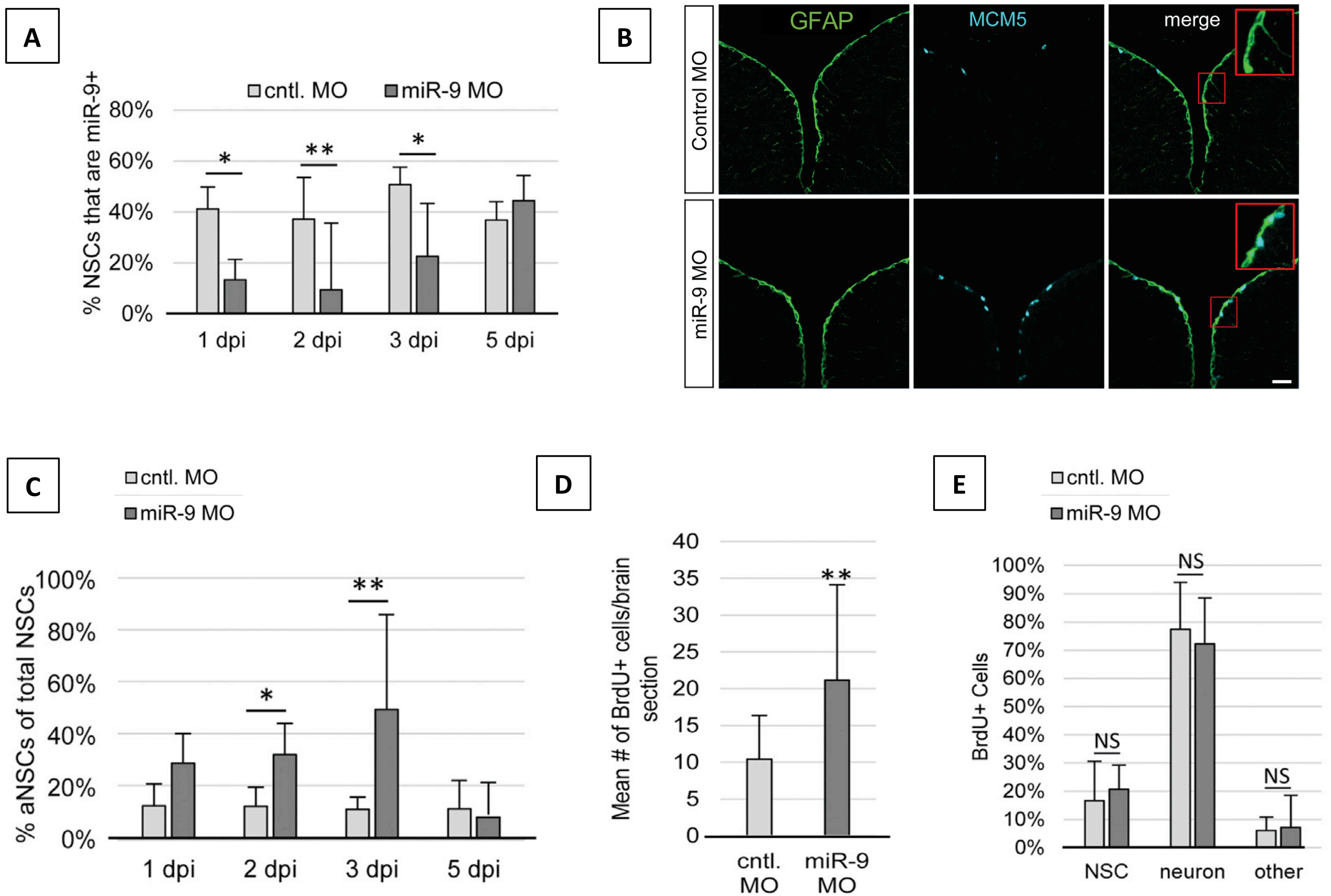

Figure 2: Analysis of miR-9 knockdown.

(A) Validation of miR-9 knockdown. NSCs expressing miR-9 were quantified 1, 2, 3, and 5 days post injection (dpi) of control morpholino (cntl.Mo, light gray bars) or miR-9 morpholino (miR-9 MO, dark gray bars) into the brain ventricle. Morpholinos are antisense oligonucleotides commonly used to block mRNA translation of targeted genes. Here, miR-9 MO was designed to bind to miR-9 and inhibit its maturation rendering it inactive and undetectable by ISH. It was also chemically-modified to enter into cells without the need for transfection.

(B, C) Analysis of Mir-9 knockdown effects on NSCs. (B) Double immunostaining for GFAP (green) and MCM5 (light blue) in control (top) and miR-9 (bottom) MO-injected brains. Scale bar: 20 mm. (C) Quantification of aNSCs ($GFAP^+MCM5^+$) over the total NSCs population ($GFAP^+$) 1, 2, 3, and 5 days post injection of the control MO (light gray bars) or miR-9 MO (dark gray bars).

(D, E) Fate analysis of activated cells upon miR-9 downregulation. Fish were administered BrdU for 48 hr following injection of MOs and analyzed after 30 days of chase. Such a pulse-chase experiment allows to follow BrdU-labelled cells (those that progressed through S-phase during the 48 hr-pulse) over time. (D) Mean number of $BrdU^+$ per brain section in control (light gray bar) and miR-9 (dark gray bar) MO-injected brains. (E) Percentage of $BrdU^+$ cells that remain NSCs ($GFAP^+$), or acquired a neuronal fate (HuC/D^+) or other fate in control (light gray bar) and miR-9 (dark gray bar) MO-injected brains after a 30 day chase.

*$p < 0.05$, **$p < 0.01$; one-way ANOVA with Bon- ferroni post hoc correction. Data are represented as mean ± 95% CI; n = 3 brains per condition.

NB: It is known that telencephalic NSC quiescence is maintained through active Notch signalling and that pharmacological inhibition of Notch signalling pushes qNSCs into cell cycle re-entry.

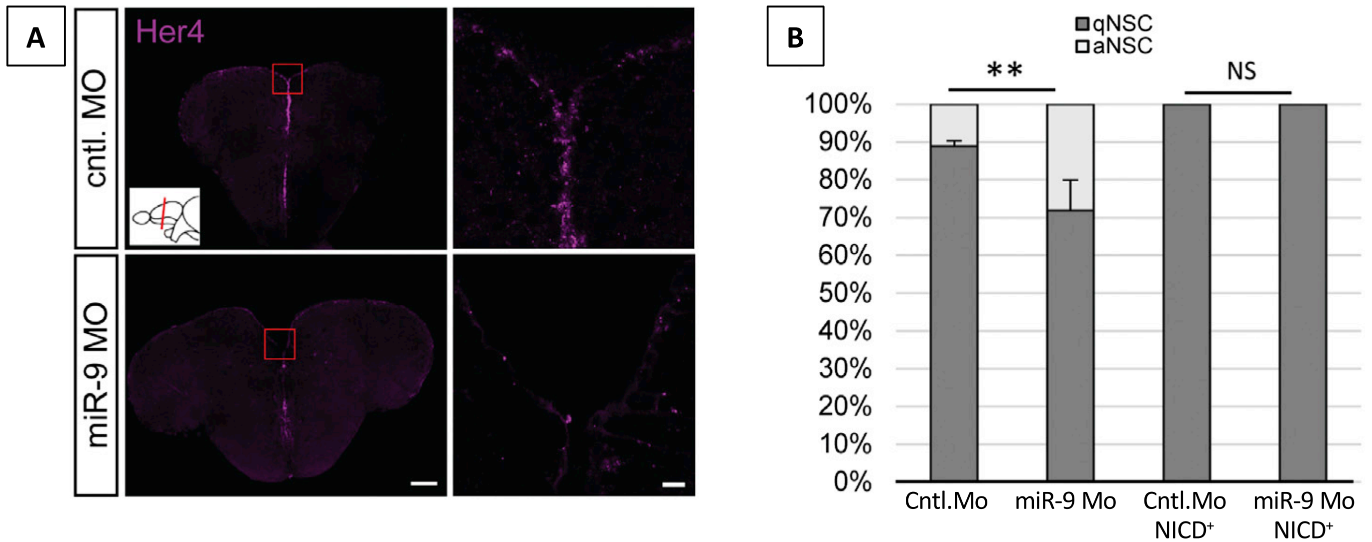

Figure 3: Analysis of miR-9 interaction with Notch signalling.
(A) ISH analysis of *her4* expression following control (top) or miR-9 (bottom) MO injection. *Her4* is a target gene of Notch signalling which is transcribed when the pathway is active. Right panels are close-ups of the region within the red box. Scale bars: 100 mm (left); 50 mm (right).
(B) Epistasis experiment combining miR-9 downregulation with overexpression of Notch intracellular domain (NICD). NICD overexpression leads to an activation of Notch signalling. The graph shows quantification of qNSCs (dark gray) and aNSCs (white) in control or miR-9 MO-injected cells that overexpress NICD (NICD+) or not.
*$p < 0.05$, **$p < 0.01$; one-way ANOVA with Bonferroni post hoc correction. Data are represented as mean ± 95% CI; n = 3 brains per condition.

NB: In general, mature microRNAs influence gene expression *via* post-transcriptional regulation of mRNA in the cytoplasm. There, they associate with Argonaute (Ago) proteins, the main effectors of the microRNA silencing pathway.
In contrast to this canonical situation, authors made the surprising observation that mature miR-9 molecules were strongly concentrated in the nuclei of qNSCs.

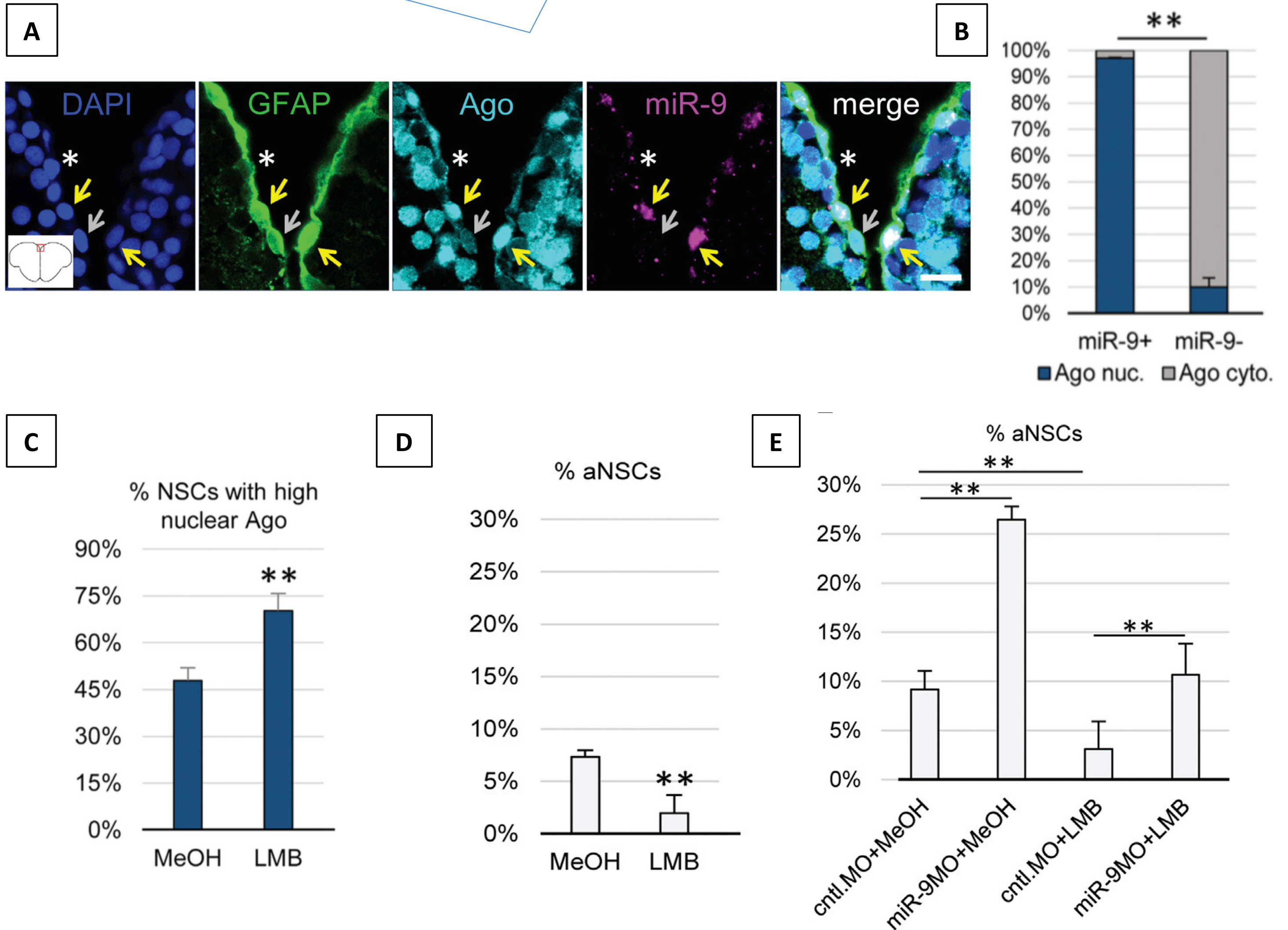

Figure 4: Analysis of miR-9 and Argonaute (Ago) subcellular localization.

(A, B) Analysis of miR-9 and Ago expression. (A) ISH detection of miR-9 (magenta) coupled with double fluorescent immunostaining for GFAP (green) and Ago (light blue). Nuclei are counterstained with DAPI (dark blue). Yellow arrows indicate miR-9$^+$ NSCs; asterisk and gray arrow indicate miR-9$^-$ NSCs. Scale bar, 20 mm. (B) Proportion of cells showing nuclear (blue) or cytoplasmic (light gray) localization of Ago in miR-9$^+$ *versus* miR-9$^-$ NSC populations.

(C-E) Effects of nuclear export inhibition through Leptomycin B (LMB) injection into the brain ventricle. Negative control brains are injected with methanol (MeOH), the diluent of LMB. (C) Quantification of NSCs with high nuclear Ago signal following MeOH or LMB injection. (D) Quantification of aNSCs among total NSCs following MeOH or LMB injection. (E) Percentage of aNSCs among total NSCs upon combination of LMB or MeOH injections together with control or miR-9 MO injections.

**p < 0.01; one-way ANOVA with Bonferroni post hoc correction. Data are represented as mean ± 95% CI; n = 3 brains per condition.

CONCLUSION AND DISCUSSION

(v) Using the informations of the introduction and all the results obtained, propose a simple model illustrating the regulation of NSCs quiescence/activation balance in the zebrafish telencephalon

(vi)- Ask questions