

2015
JÚNIUS
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STRAUB-NAPOK

MEGHÍVÓ



MTA SZEGEDI BIOLÓGIAI KUTATÓKÖZPONT
AZ EURÓPAI UNIÓ KIVÁLÓSÁGI KÖZPONTJA
SZEGED, TEMESVÁRI KRT. 62., NAGYELŐADÓ



INSTITUTE OF ENZYMOLOGY

Reconstructing tumor evolution by multi-region exome sequencingLőrinc S. Pongor^{1,2}, Zsófia Sztupinszki³, Ádám Nagy¹ and Balázs Győrfy^{1,2}⁽¹⁾*Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest;*⁽²⁾*Semmelweis University 2nd Dept. of Pediatrics, Budapest;*⁽³⁾*Semmelweis University 1st Dept. of Pediatrics, Budapest)***Validation of SNP-s influencing response to Tamoxifen using next-generation sequencing**Ádám Nagy¹, Lőrinc S. Pongor^{1,2} and Balázs Győrfy^{1,2}⁽¹⁾*Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest;*⁽²⁾*Semmelweis University 2nd Dept. of Pediatrics, Budapest)*

INSTITUTE OF GENETICS

Development of an *in vitro* RNAi efficiency monitoring system

Dávid Pusztai, Katalin Hegedűs, Ildikó Fekete and Lajos Mátés

*(Institute of Genetics, BRC HAS, Szeged)***A novel somatic transgenesis method in mice**Liza Hudoba¹, Andrea Nagy¹, Gergely Imre¹, Dávid Pusztai¹, Róbert Katona¹, Katalin Jemnitz², Thomas Rüllicke³ and Lajos Mátés¹⁽¹⁾*Institute of Genetics, BRC HAS, Szeged;*⁽²⁾*Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Budapest;*⁽³⁾*Institute of Laboratory Animal Science, University of Veterinary Medicine, Vienna, Austria)***Non cell-autonomous differentiation in the hematopoietic compartments of *Drosophila melanogaster***Viktor Honti¹, Gergely I. B. Varga¹, Gábor Csordás¹, Ferenc Jankovics¹, Róbert Márkus¹, Tamás Lukácsovich², Éva Kurucz¹ and István Andó¹⁽¹⁾*Institute of Genetics, BRC HAS, Szeged;*⁽²⁾*Developmental Biology Center, University of California, Irvine, USA)***Identification of the genetic interaction partners of the formin dDAAM required for axonal growth in *Drosophila***

Gabriella Gerhát, Viktor Nagy, Rita Gombos, István Földi and József Mihály

*(Institute of Genetics, BRC HAS, Szeged)***Nuclear function for the actin binding cytoskeletal protein, Moesin**Ildikó Kristó¹, Csaba Bajusz¹, Henrik Gyurkovics¹, Maria K. Vartiainen² and Péter Vilmos¹⁽¹⁾*Institute of Genetics, BRC HAS, Szeged;*⁽²⁾*Institute of Biotechnology, University of Helsinki, Finland)***Application of the CRISPR-Cas9 system for the *in vivo* target specific modification of a *Drosophila* gene coding for an actin binding protein**

Csaba Bajusz, Ildikó Kristó and Péter Vilmos

*(Institute of Genetics, BRC HAS, Szeged)***Development of a novel protein degradation technique in *Drosophila melanogaster***Melinda Bence¹, Ferenc Jankovics¹, Tamás Lukácsovich² and Miklós Erdélyi¹⁽¹⁾*Institute of Genetics, BRC HAS, Szeged;*⁽²⁾*Department of Developmental and Cell Biology, University of California, CA, USA)***Rybp-deficient cardiogenesis reveals disruption of sarcomere remodeling**

Viktória Szabó, Gergő Kovács and Melinda K. Pirity

(Institute of Genetics, BRC HAS, Szeged)

Lack of rybp impairs neural differentiation in embryonic stem cells

Gergő Kovács, Viktória Szabó and Melinda K. Pirity
(*Institute of Genetics, BRC HAS, Szeged*)

Functional analysis of the proteins encoded by the Nimrod gene cluster in *Drosophila melanogaster*

Gyöngyi Cinege, János Zsámboki, Gábor Csordás, Viktor Honti, István Andó and Éva Kurucz
(*Institute of Genetics, BRC HAS, Szeged*)

Molecular definition of hemocyte subsets in the honey bee (*Apis mellifera*)

Erika Gábor¹, János Zsámboki¹, Gyöngyi Cinege¹, Tibor Török², Gábor Csordás¹, Viktor Honti¹, Éva Kurucz¹ and István Andó¹
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The short stop gene is required for efficient epithelial closure in *Drosophila melanogaster*

Zsanett Lakatos, Ferenc Jankovics and Miklós Erdélyi
(*Institute of Genetics, BRC HAS, Szeged*)

Induction of genes in astrocytes having roles in hypoxic adaptation by a novel clinical candidate against Alzheimer's disease

Orsolya Huzián¹, Liliána Z Fehér¹, Ágnes Zvara² and László G Puskás^{1,2}
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INSTITUTE OF PLANT BIOLOGY**Environment-induced changes in the energy transfer dynamics of Light-Harvesting Complex II**

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Pigment interactions in Light-Harvesting Complex II in different molecular environments

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How does chloramphenicol affect PSII photoinhibition in isolated spinach thylakoid membranes?

Sandeesh Kodru, Ateeq ur Rehman and Imre Vass
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Auxin-ethylene interactions in the *Arabidopsis thaliana crk5-1* mutant in dark

Abu Imran Baba, Norbert András, Teréz Górcsa, Gábor Rigó, László Szabados and Ágnes Cséplő
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Effect of silver nanoparticles on photosynthetic activity of the cyanobacterium *Synechocystis* PCC 6803

Sándor Kovács, Imre Vass and Zsuzsanna Deák
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Thylakoid membrane ultrastructural changes during photosynthesis *in vivo* – revealed by small angle neutron scattering

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LACK OF RYBP IMPAIRS NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS

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INTRODUCTION

Ring1 and Yy1 Binding Protein (Rybp, UniGene Mm.321633; MGI:1929059) is a member of the non-canonical Polycomb Repressive Complex 1 and the BCOR (BCL6 corepressor) complex. It has essential role in embryonic development. Alterations of Rybp dosage cause striking neural tube defects (NTDs) in mouse models (Pirity *et al*, 2005). Here, we characterized the possible molecular mechanisms underlying this phenotype.

MATERIALS AND METHODS

Neuronal differentiation of embryonic stem cells (Fig 1)

Rybp^{+/+} (wild type) (Nagy *et al*, 1993) and *rybp*^{-/-} (mutant) (Pirity *et al*, 2005) mouse embryonic stem (ES) cells were differentiated to neural lineages induced by retinoic acid (RA) according to Bibel *et al*, 2007.

Immunocytochemistry (ICC)

The cells fixed with 4% paraformaldehyde and permeabilized with 0.2% TritonX-100. Then sequentially incubated with the following primary antibodies: Nestin (Rat-401; Hybridoma Bank, Iowa, USA, 1:100), Tuj1 (Neuronal Class III β -Tubulin Polyclonal Antibody; Covance, Cat.No PRB-435P, 1:1000) and Zac1 (Zac1 (M-300); Santa Cruz Biotechnology, Cat.No sc-22812, 1:1000) and labeled with fluorescent-labeled secondary antibody (Alexa Fluor 647 G-A-R IgG, Invitrogen, Cat.No A-21244) and DAPI (4',6-diamidino-2-phenylindole, Vector Laboratories, Cat.No H-1200).

Real time PCR (qRT-PCR)

The isolated RNA was reverse-transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat.No 4368814). Primers were designed for the amplification of specific products (Cnd2, Crabp1, Crabp2, Gfap, Nanog, Nestin, NeuroD1, Oct4, Olig2, p21, Pax6, Rara, Stra6, Tubb3, Zac1). qRT-PCR reactions in SYBR Green for CFX (Applied Biosystems, Cat.No 4472942) were carried out in Bioer LineGeneK Real-time PCR System (Bioer, China). Relative gene expression changes were quantified using the $\Delta\Delta Ct$ method. The threshold cycle (Ct) values for each gene were normalized to expression level of Hprt. To calculate fold change, the values compared to undifferentiated samples (d0, *rybp*^{+/+}).

RESULTS AND CONCLUSIONS

Rybp^{+/+} and *rybp*^{-/-} ES cells were *in vitro* differentiated to neural lineages. Samples were taken for gross morphology (Fig 2A), ICC (Fig 2BCD) and qRT-PCR (Fig 3) analyses on day 0 (d0), day 3 (d3), day 7 (d7), day 10 (d10) and day 14 (d14).

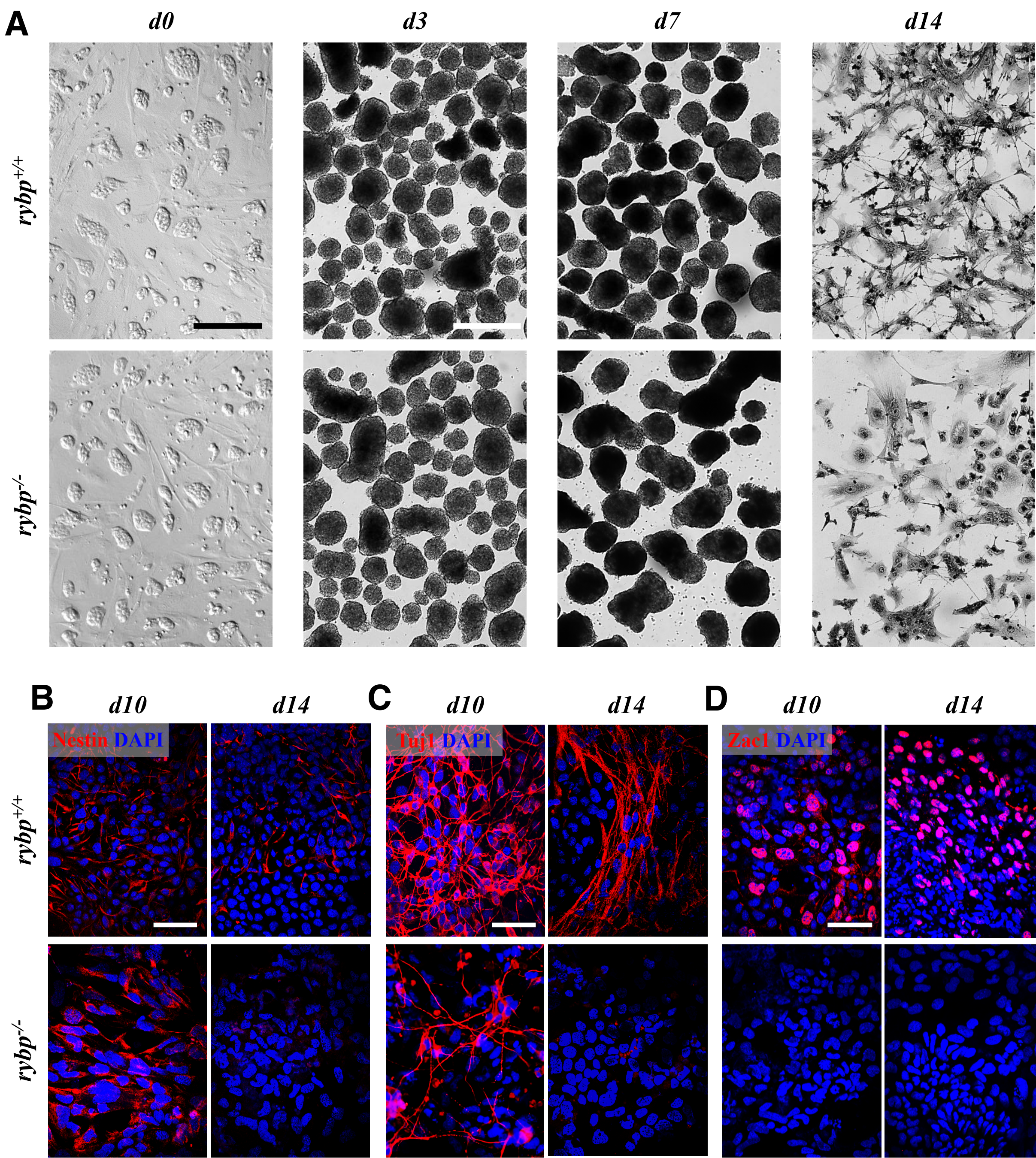


Figure 2 – EB formation and ICC analyses of the *rybp*^{+/+} and *rybp*^{-/-} ES cells during *in vitro* neural differentiation
(A) EB formation (d3, d7) is not compromised in the *rybp*^{-/-} cells but they grew less neurite processes (d10, d14) in comparison to the wild type. Images were obtained using Olympus cellR microscope (Olympus Corporation, Japan). Magnifications are 4x (d3, d7) and 10x (d0, d14). Scale bars represent: 200 μ m (d0) and 500 μ m (d3).
(B) Neural precursor (NPC) marker Nestin is abundantly present in the *rybp*^{-/-} neurons (d10) suggesting an accelerated precursor production in the mutant cells. By d14 Nestin expression declined in both cell lines. (C) Decreased amount of neurite processes leads to the formation of a less complex networks in the *rybp*^{-/-} neural cultures revealed by Tuj1 immunostaining. (D) Zac1 immunostaining reveals lack of cell-cycle and apoptosis regulator Zac1 (Abdollahi *et al*, 1997; Spengler *et al*, 1997) in the *rybp*^{-/-} neural cultures. Images were obtained using Olympus LSM confocal microscope (Olympus Corporation, Japan). Magnification is 60x. Scale bars represent 60 μ m.

SUMMARY

Our results demonstrate that normal level of Rybp is indispensable for the proper differentiation of neural cell types. In the absence of functional Rybp it is very likely that the balance of early and late neural processes are changed, which is probably linked to the upregulation of Pax6 and the complete loss of Zac1. The formation of neurite processes and their organization into complex network also influenced by the presence of Rybp. These results confirm our hypothesis that Rybp plays important role in the development of mature neurons. Together, these results support a continuing role for Rybp as regulator of neural circuit formation and maintenance, above and beyond its developmental function as regulator of neuronal and glial fate.

Acknowledgements

This work was supported by NKTH-OTKA FP7 “Mobility” HUMAN-MB08C-80205.

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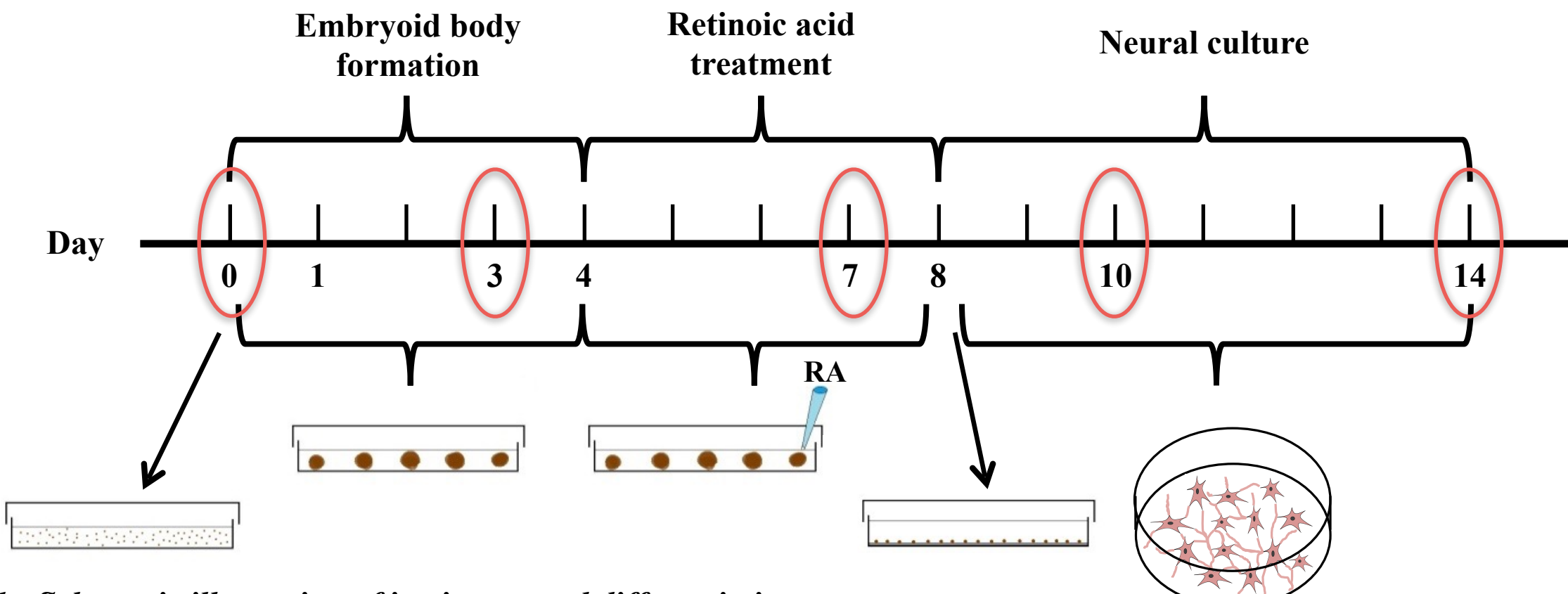


Figure 1 - Schematic illustration of *in vitro* neural differentiation
ES cells seeded as single cell suspension in ES medium without LIF then allowed to aggregate and form embryoid bodies (EBs) for 8 days. On the 4th day 5 μ M (RA) was added to the medium and EBs were cultured for a further 4 days in the presence of RA. Thereafter, 8-day-old EBs were dissociated and plated onto L-ornithine and laminin coated dishes in neural differentiation medium. The cells were harvested for further analyses on labeled time points (red circles).

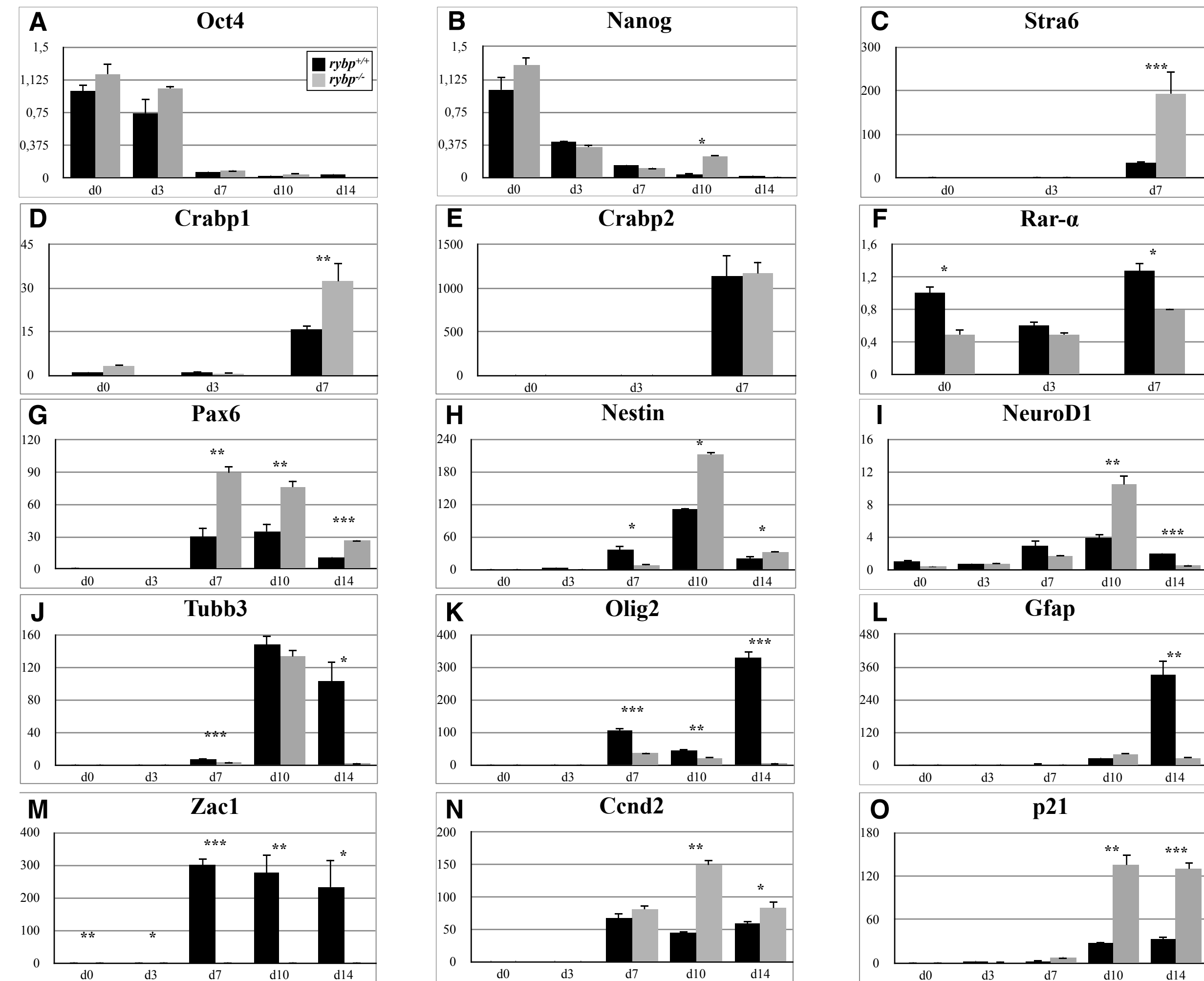


Figure 3 - Relative gene expression changes during *in vitro* neural differentiation
Oct4 (A) and Nanog (B) expression is downregulated in both cell lines suggesting proper silencing of pluripotency markers during differentiation. Crabp2 (E) and Rara (F) expression is unchanged but Stra6 (C) and Crabp1 (D) expression is higher in *rybp*^{-/-} cells compared to the wild type suggesting an increased RA sensitivity of the *rybp*^{-/-} cells. The expression of early neural markers (Pax6 (G), Nestin (H), NeuroD1 (I)) is elevated after neuroectodermal induction by RA (d7, d10) then decreased by the end of differentiation (d14). The expression of early neural markers was higher generally in the *rybp*^{-/-} cells. On the contrary, the expression of late neural markers (Tubb3 (J), Olig2 (K), Gfap (L)) are deficient (d14) in the mutant. These data suggest that the mutant cells form a larger NPC pool than wild-type cells (d10) but the formation of mature neural cell types (d14) from this pool is defective. Zac1 (M) is strongly induced after the RA treatment (d7, d10, d14) in the wild type but in the mutant its level remained low. The induction of cell-cycle markers (Cnd2 (N), p21 (O)) reaches higher peaks in the *rybp*^{-/-} cells (d7). These changes may cause altered cell-cycle processes in the mutant during *in vitro* neural differentiation (see Figure 4). Means are standard deviation $\pm$ SD. Values of $p < 0.05$ were accepted as significant (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Statistical method: T test type 3.

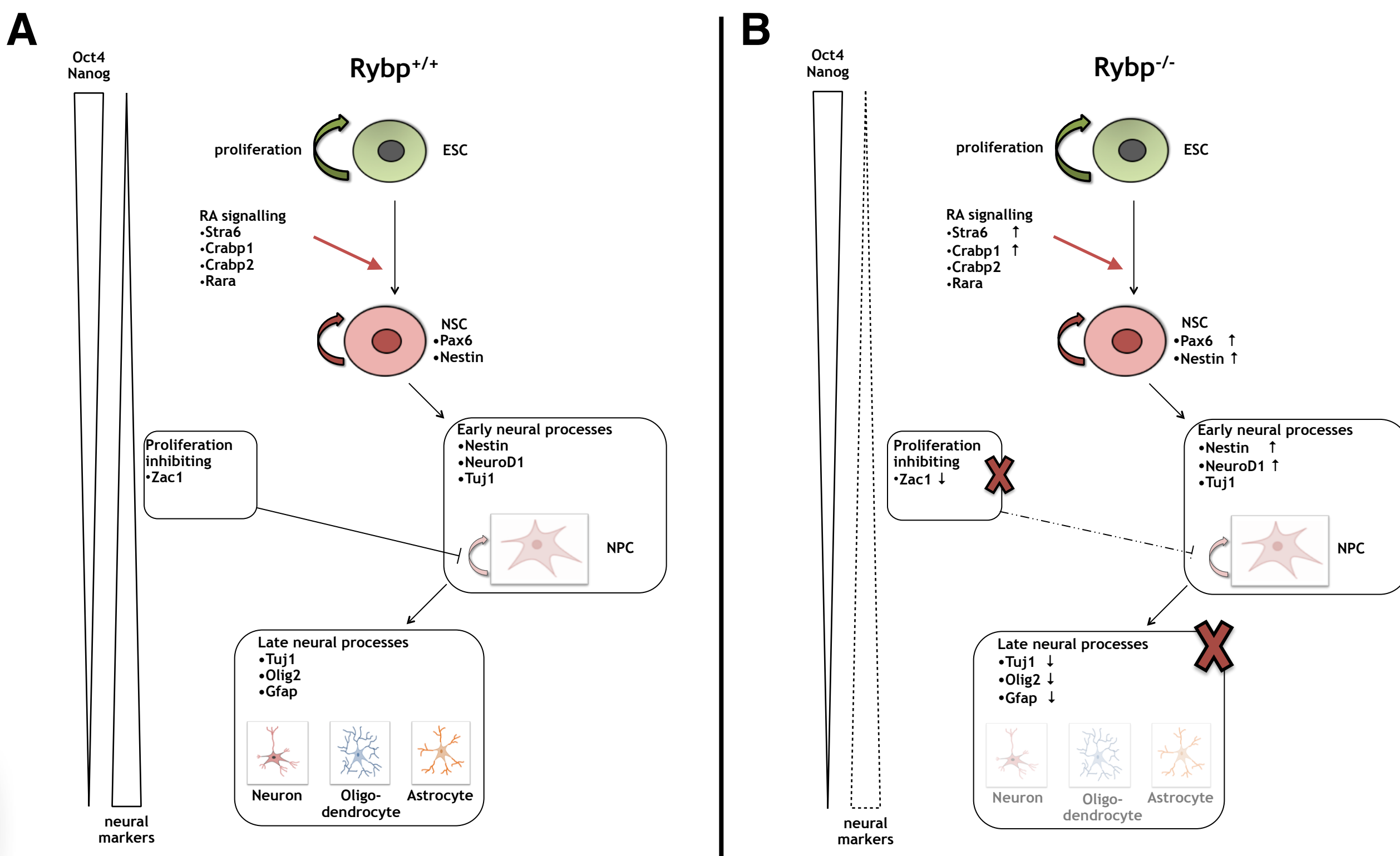


Figure 4 - Model of dosage dependent effect of Rybp during *in vitro* neural differentiation of ES cells
(A) Pluripotent ES cells express key pluripotency markers (e.g. Oct4, Nanog) in undifferentiated state, which are gradually decreasing during differentiation. Early neural markers (Pax6, Nestin) are upregulated in neural stem cells (NSCs), which will confer reduced proliferative and high differentiation capacity to the cells. In the presence of Rybp, upregulation of Zac1 helps to facilitate late neural differentiation and inhibits cell proliferation, which will facilitate the generation of terminally differentiated neural cell types.
(B) During neural differentiation of pluripotent ES cells in the absence of Rybp, RA pathway members and early neural markers exhibit elevated expression pattern. These will facilitate the generation of a bigger pool of NPCs with higher proliferative capacity. In the mutant, Zac1 expression is defective, which contributes to increased proliferative and decreased differentiation capacity of the NPCs. The formation of terminally differentiated neural cell types will be impaired in the lack of functional Rybp.