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STRAUB-NAPOK

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MTA SZEGEDI BIOLÓGIAI KUTATÓKÖZPONT
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INSTITUTE OF ENZYMOLOGY

Reconstructing tumor evolution by multi-region exome sequencing

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Validation of SNP-s influencing response to Tamoxifen using next-generation sequencing

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INSTITUTE OF GENETICS

Development of an *in vitro* RNAi efficiency monitoring system

Dávid Pusztai, Katalin Hegedűs, Ildikó Fekete and Lajos Mátés

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A novel somatic transgenesis method in mice

Liza Hudoba¹, Andrea Nagy¹, Gergely Imre¹, Dávid Pusztai¹, Róbert Katona¹, Katalin Jemnitz², Thomas Rüllicke³ and Lajos Mátés¹

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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

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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

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Nuclear function for the actin binding cytoskeletal protein, Moesin

Ildikó Kristó¹, Csaba Bajusz¹, Henrik Gyurkovics¹, Maria K. Vartiainen² and Péter Vilmos¹

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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

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Development of a novel protein degradation technique in *Drosophila melanogaster*

Melinda Bence¹, Ferenc Jankovics¹, Tamás Lukácsovich² and Miklós Erdélyi¹

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Rybp-deficient cardiogenesis reveals disruption of sarcomere remodeling

Viktória Szabó, Gergő Kovács and Melinda K. Pirity

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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

Parveen Akhtar¹, Márta Dorogi¹, Rabail Razzaq¹, Krzysztof Pawlak¹, László Kovács¹, Attila Bóta², Teréz Kiss², Győző Garab¹ and Petar H. Lambrev¹
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How does chloramphenicol affect PSII photoinhibition in isolated spinach thylakoid membranes?

Sandeesh Kodru, Ateeq ur Rehman and Imre Vass
(*Institute of Plant Biology, BRC HAS, Szeged*)

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
(*Institute of Plant Biology, BRC HAS, Szeged*)

Thylakoid membrane ultrastructural changes during photosynthesis *in vivo* – revealed by small angle neutron scattering

Renáta Ünnepe^{1,2}, Ottó Zsiros³, László Kovács³, Noémi K. Székely⁴, Gergely Nagy^{1,2} and Győző Garab³
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Rybp-deficient cardiogenesis reveals disruption of sarcomere remodeling

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BACKGROUND

The congenital heart defects arise at an early stage of embryonic development caused by abnormal changes of molecular pathways that governing embryonic heart development. Sarcomeric disfunction plays a central role in reduced cardiac pump function in heart failure. Our laboratory focuses on the Ring1 and Yy1 binding protein (Rybp; also known as DEDAF (Death effector domain associated factor), UniGene Mm.321633; MGI:1929059), which is a transcriptional regulator with essential role in embryonic development [1]. The objective of this work was to determine the role of Rybp in murine cardiac differentiation and sarcomere remodeling in Embryonic Stem (ES) cells based model system [2].

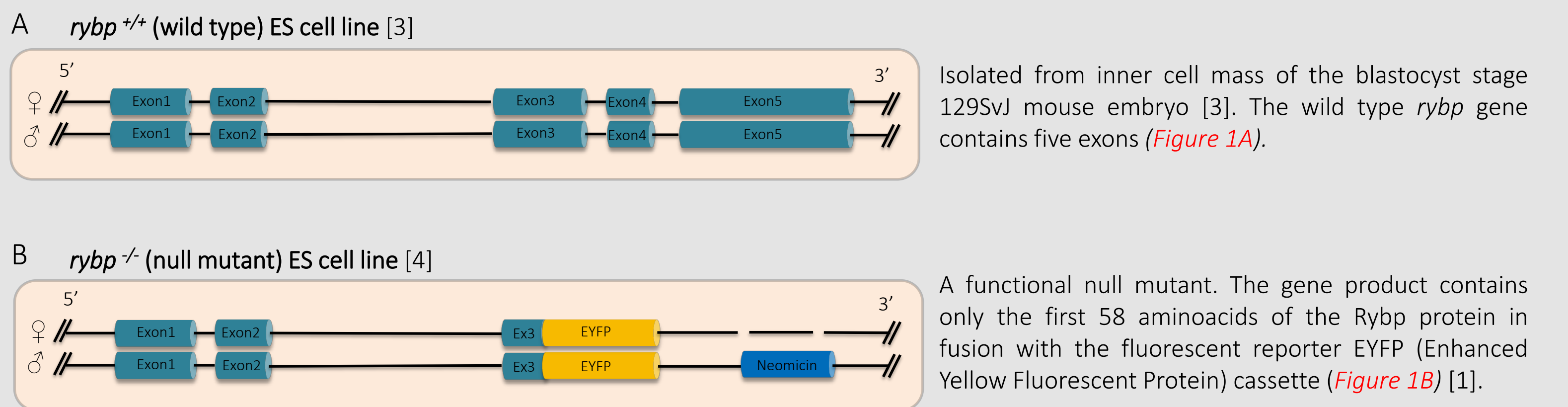


Figure 1: Stem Cell Lines

METHODS

In vitro cardiac differentiation via hanging drop method:

We utilized *rybp*^{+/+} (wild type) [4] and *rybp*^{-/-} (null mutant) [5] mouse ES cells (**Figure 1**) and differentiated them towards cardiac lineages *in vitro* (**Figure 2**) [5]. Embryoid Bodies (EBs) were formed using 1000 cells per drop (4x10⁴ cells/ml). Individual EBs were placed into wells of a 24-well plate containing 0.1 % gelatin coated cover-slips on the second day (d2). The differentiation medium was changed every second day. Cardiomyocytes were grown for 21 days and observed for beating daily, under a phase-contrast microscope. Sampling time points were at day 0, 7 and 14 for further gene expression studies (see below) and at day 14, 18, 21 for immunocytochemistry (see below) (**Figure 2**).

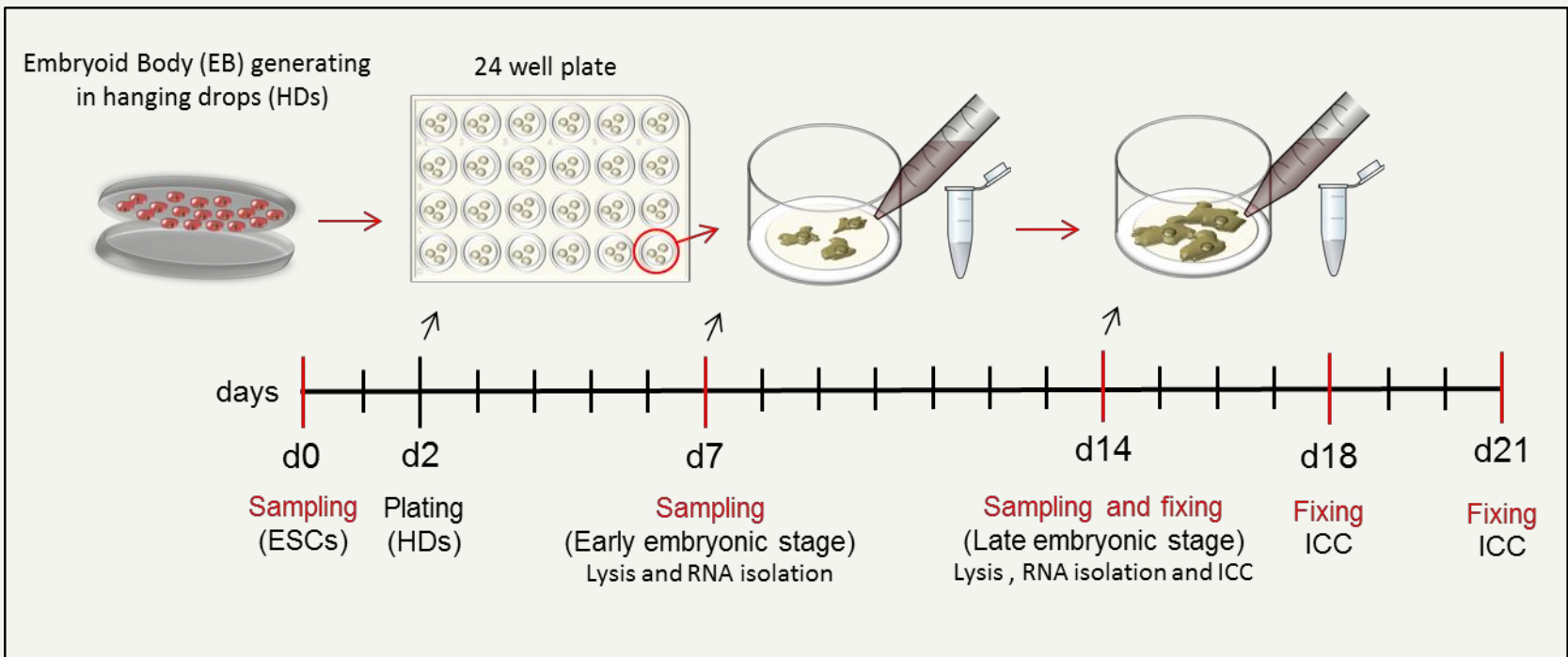


Figure 2: Time line of the cardiac differentiation

Quantitative Real-Time PCR (qRT-PCR):

RNA was isolated from the cardiomyocytes colonies using Gene Jet RNA Purification Kit (Thermo Scientific, Cat. K0739) according to the manufacturer's protocol. RNA was translated to cDNA with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Ca. 4368814). We used the cDNA in qRT-PCR reaction to determine gene expression changes (Rybp, Oct4, Zac1, Tnnt2). Reactions were performed in SYBR® Select Master Mix for CFX (Applied Biosystems, REF 4472942) using Bioer LineGeneK Real-time PCR System machine (Bioer, China). Relative gene expression changes were quantified using the $\Delta\Delta C_t$ method. The expression of the indicated markers were normalized to Hprt expression, which used as an internal control. To calculate fold change, the values compared to undifferentiated samples (d0, *rybp*^{+/+}) (**Figure 3**).

Immunocytochemistry (ICC) of cardiomyocytes:

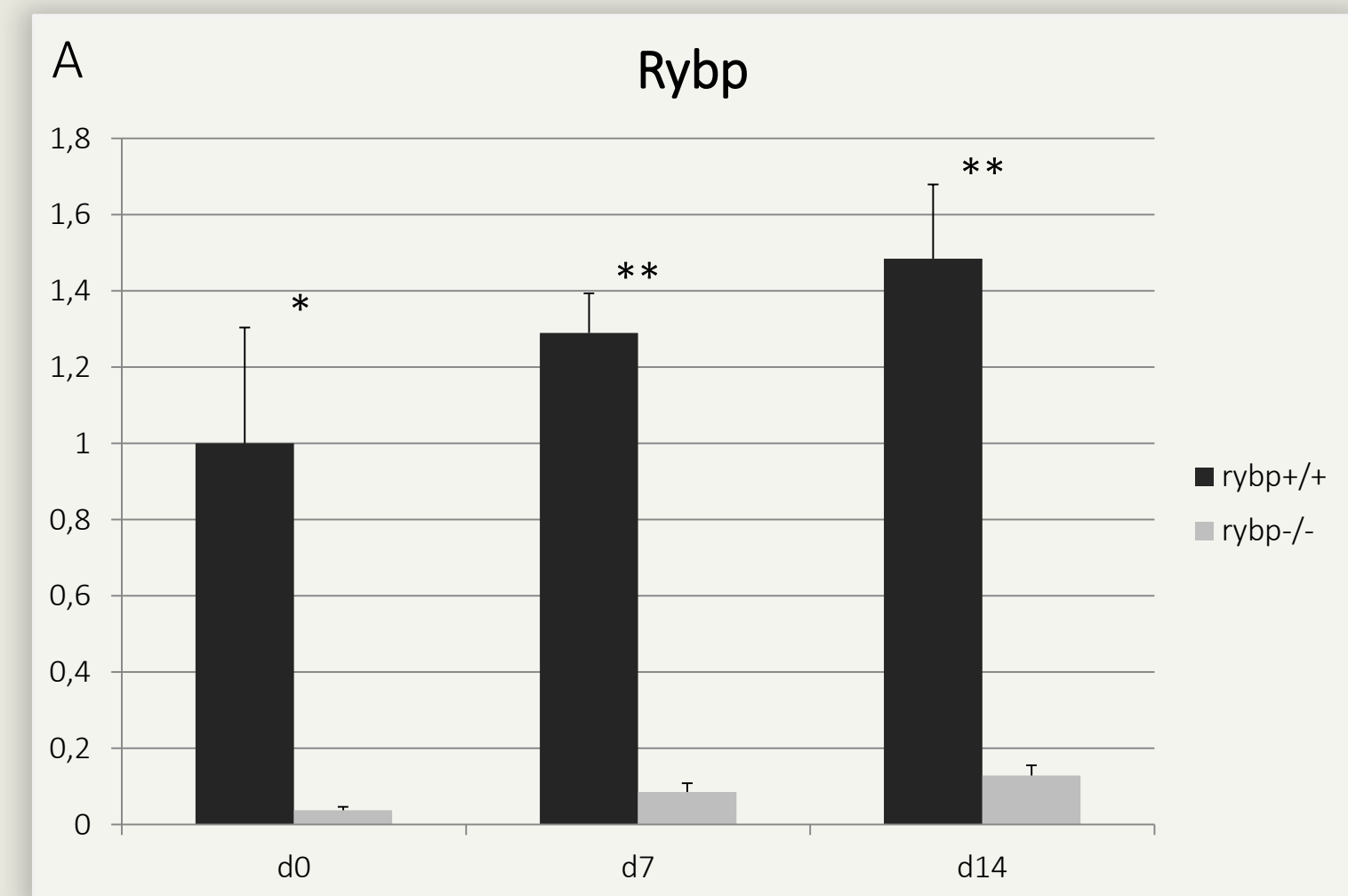
Cardiomyocytes were fixed with 4% (v/v) paraformaldehyde for 20 min at room temperature (RT), permeabilized with 0.2% Triton X-100 for 20 min at RT and blocked with blocking buffer (5% Bovine Serum Albumin) for 1h at RT, then sequentially incubated with the following primary antibodies at 4°C:

- Alpha-actinin (Anti-alpha Actinin antibody; [MAC276], BABRAM, Cat. BT-GB-276P, 1:400)
 - Zac1 (ZAC1 (M-300); Santa Cruz Biotechnology, Cat. sc-22812, 1:1000)
 - Rybp (Rbx DEDAF; Millipore, Cat. AB3637, 1:1000)
 - Oct4 (Octamer-binding transcription factor 4, Mouse Oct-3/4 (C-10); Santa Cruz, Cat. Sc-5279, 1:100)
 - Cardiac Troponin T (Anti-Cardiac Troponin T; ABCAM, Cat. ab8295, 1:2000)
- After that the cultures were incubated with fluorescent-labeled secondary antibodies for 1h:

- Alexa Fluor 546 G-A-M IgG, Cat. A-11030
- Alexa Fluor 546 G-A-R IgG, Cat. A-11081
- Alexa Fluor 647 G-A-R IgG, Cat. A-21244
- Alexa Fluor 647 D-A-M IgG, Cat. A-31571

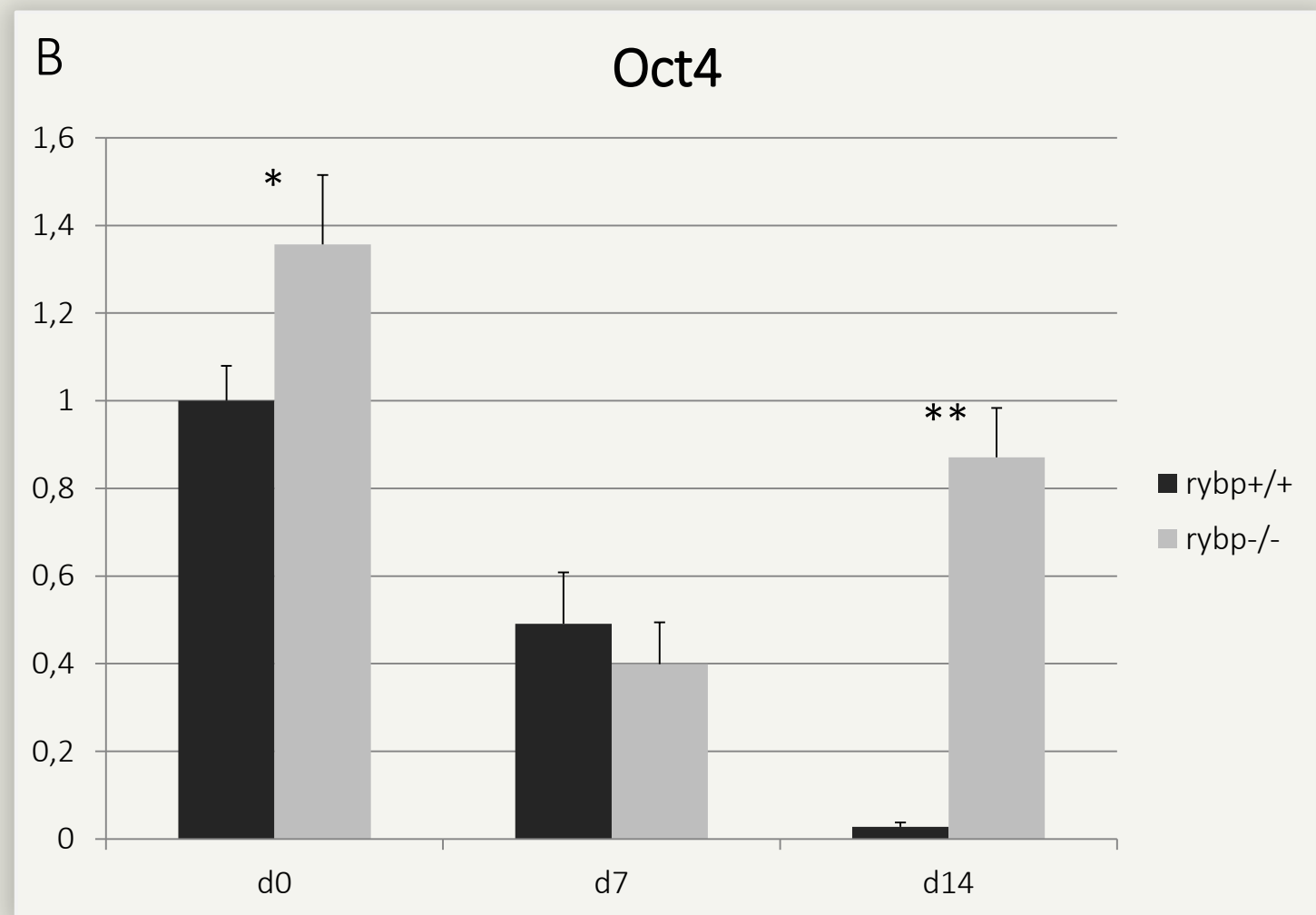
The cells were rinsed in 4',6'-diamidino-2-phenylindole (DAPI; Vector Laboratories, Cat. H-1200) for 20 min and mounted in 50% glycerol. The images were obtained using Olympus Confocal LSM (**Figure 6**), Leica Confocal LSM (**Figure 7**), and Olympus CellR Imaging microscope (**Figure 4,5,8**).

RESULTS



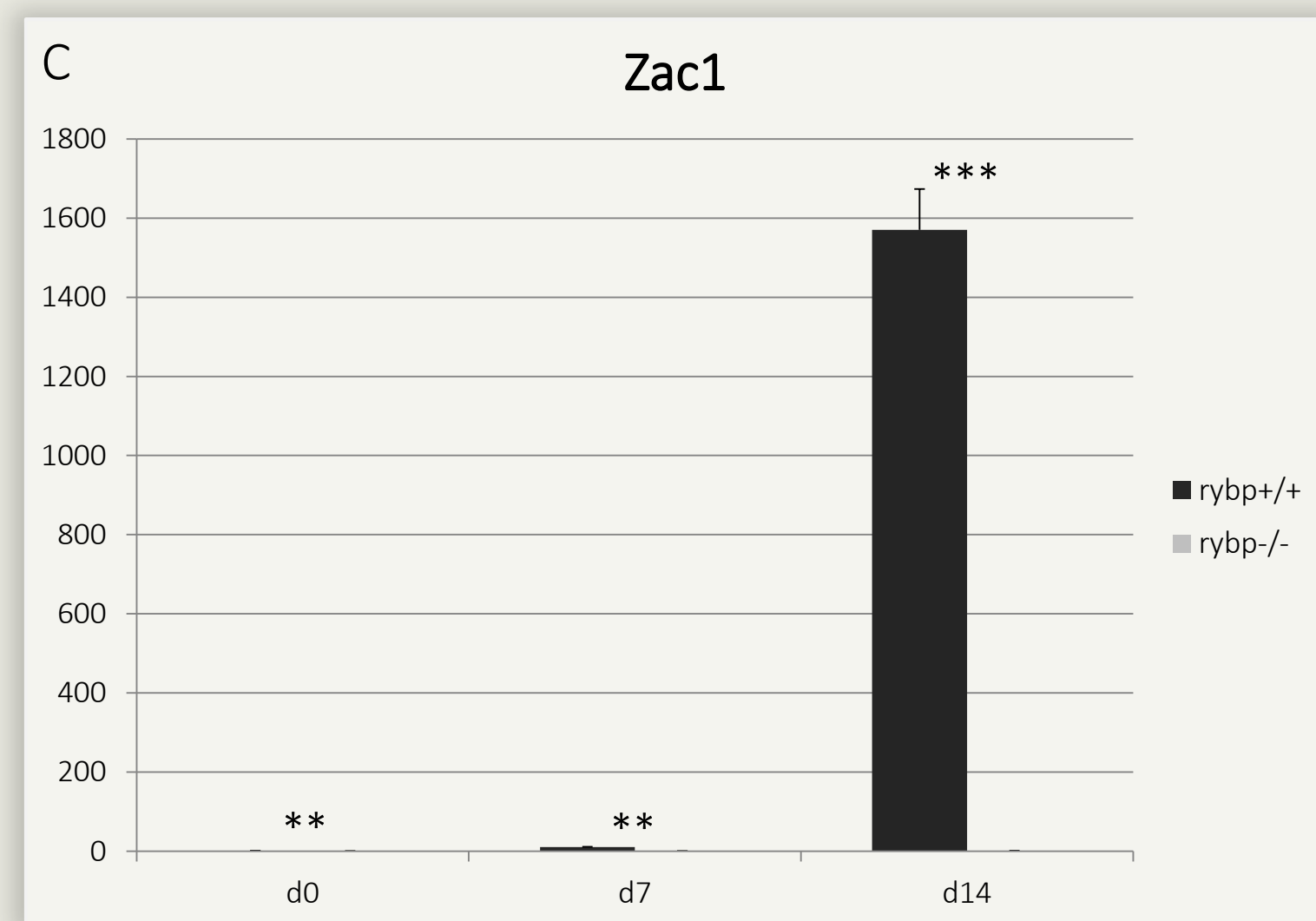
Expression of Rybp during *in vitro* cardiac differentiation.

Rybp (Ring1 and YY1 binding protein - UniGene Mm.321633; MGI:1929059) is not detectable in the *rybp* null mutants.



Expression of Oct4 during *in vitro* cardiac differentiation.

Pluripotency marker Oct4 (Octamer binding factor 4 - UniGene Mm.17031; MGI:101893) is downregulated in both cell lines during differentiation. [7]

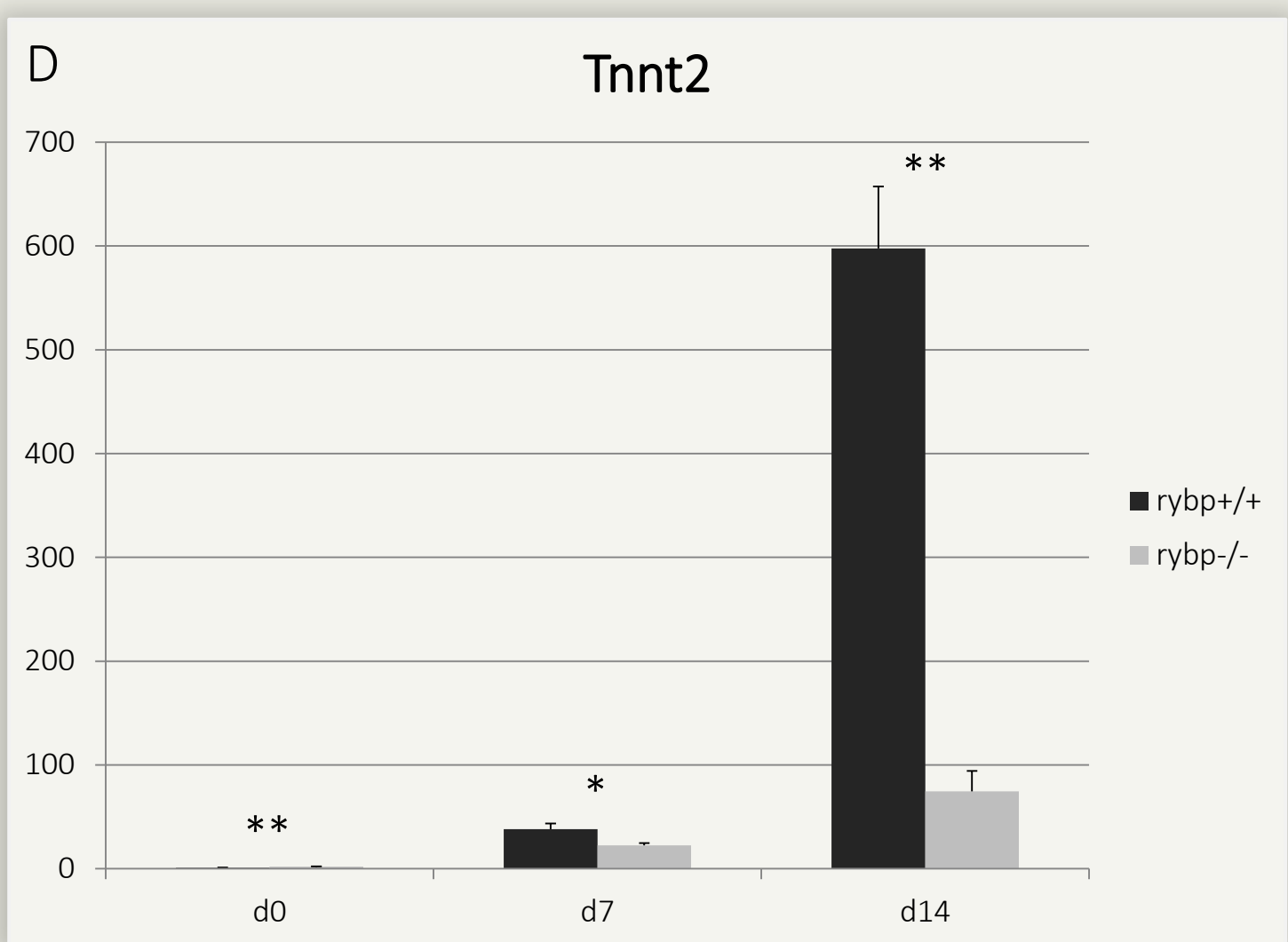


Expression of Zac1 during *in vitro* cardiac differentiation.

Zac1 (Zinc finger protein regulating apoptosis and cell-cycle arrest - UniGene Mm.287857; MGI:1100874) is elevated at later stage of cardiac differentiation in wild type, while its nearly absent in the mutant at all time points. [8]

Figure 3: Relative gene expression changes during *in vitro* cardiac differentiation

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



Expression of Tnnt2 during *in vitro* cardiac differentiation.

Expression of terminal cardiogenic marker, Tnnt2 (Cardiac Troponin T - UniGene Mm.247470; MGI:104597) is upregulated in wild type during cardiac differentiation, but remains low in the *rybp* deficient cells. [9]

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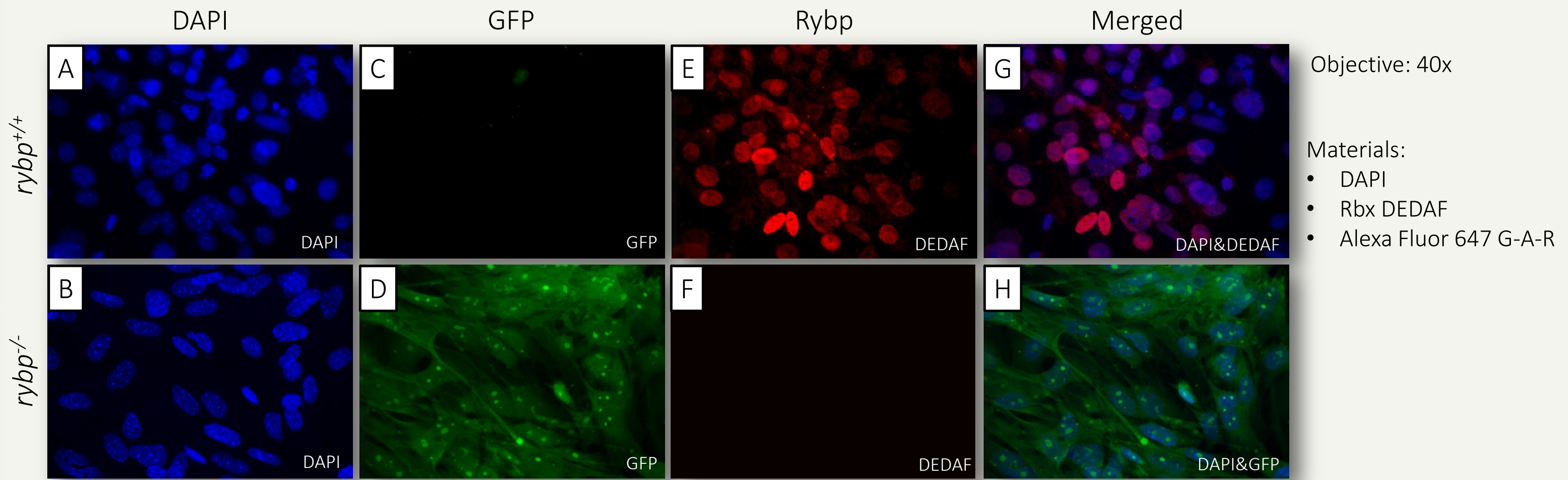


Figure 4: Rybp immunostaining analysis of cardiomyocytes derived from *rybp*^{+/+} and *rybp*^{-/-} ES cells

Cardiomyocytes were stained with anti-Rybp antibody (red; **4E,F**). Nuclei were stained with DAPI (blue; **4A,B**). The subcellular localisation of the knock-in EYFP construct in the *rybp*^{-/-} cells is cytoplasmic (green, **4D**). The wild type cells express endogenous *Rybp* which is also nuclear (**4E**). As expected, Rybp is not detectable in the *rybp* null mutants (**4F**).

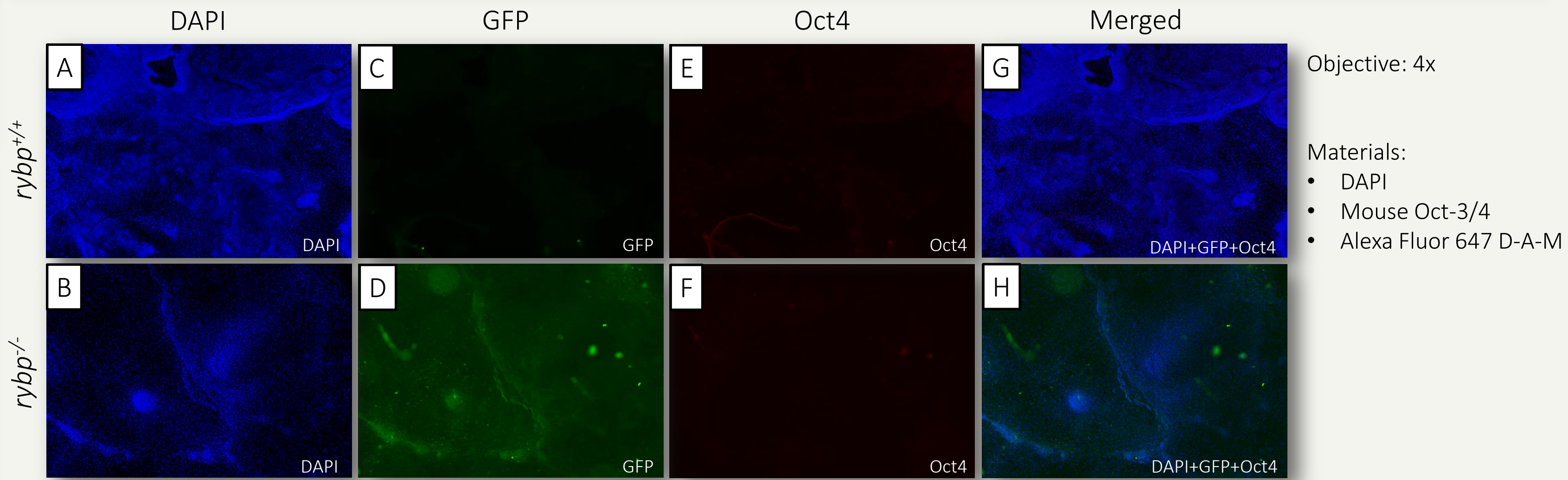


Figure 5: Immunostaining of cardiomyocytes derived from *rybp*^{+/+} and *rybp*^{-/-} ES cells for the pluripotency marker Oct4

Cardiomyocytes were stained with anti-Oct4 antibody (**5E,F**). Nuclei were stained with DAPI (blue; **5A,B**). During cardiac differentiation silencing of Oct4 is properly executed in both cell lines (**5E,F**).

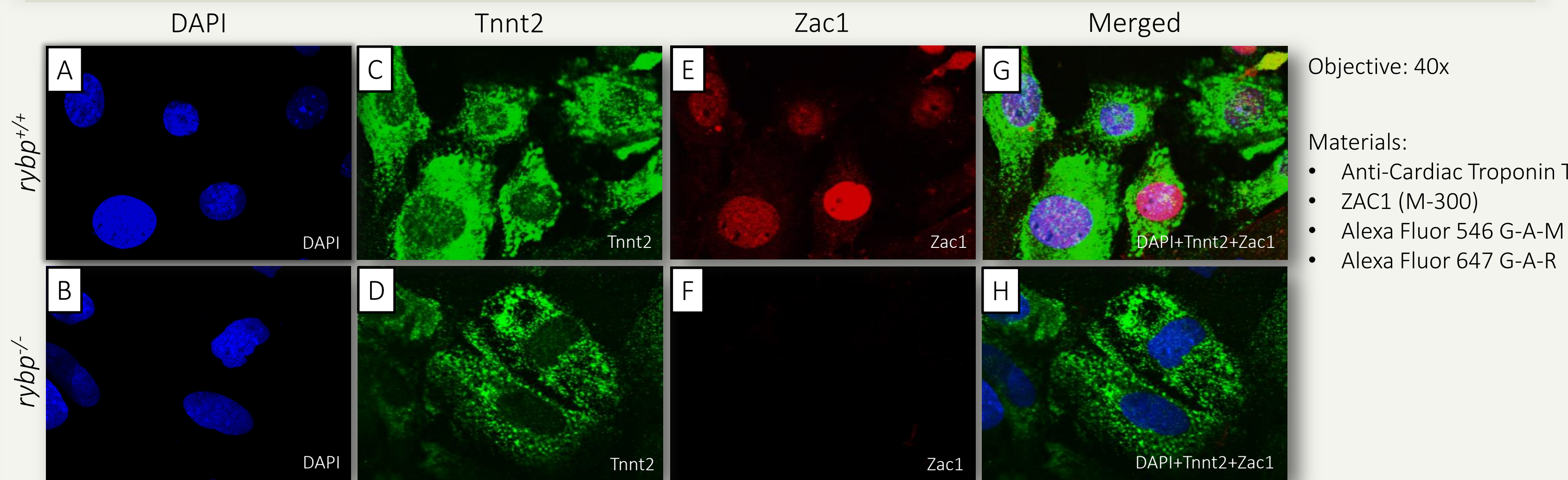


Figure 6: Immunostaining of cardiomyocytes derived from *rybp*^{+/+} and *rybp*^{-/-} ES cells for the terminal cardiac marker troponin T (Tnnt2) and for Zac1

Cardiomyocytes were stained with anti-Tnnt2 antibody (green; **6C,D**) and with anti-Zac1 antibody (red; **6E,F**). Nuclei were stained with DAPI (blue; **6A,B**). Cardiac Tnnt2 is down-regulated in the mutant and showed a complete absence of Zac1 protein (**6E,F**).

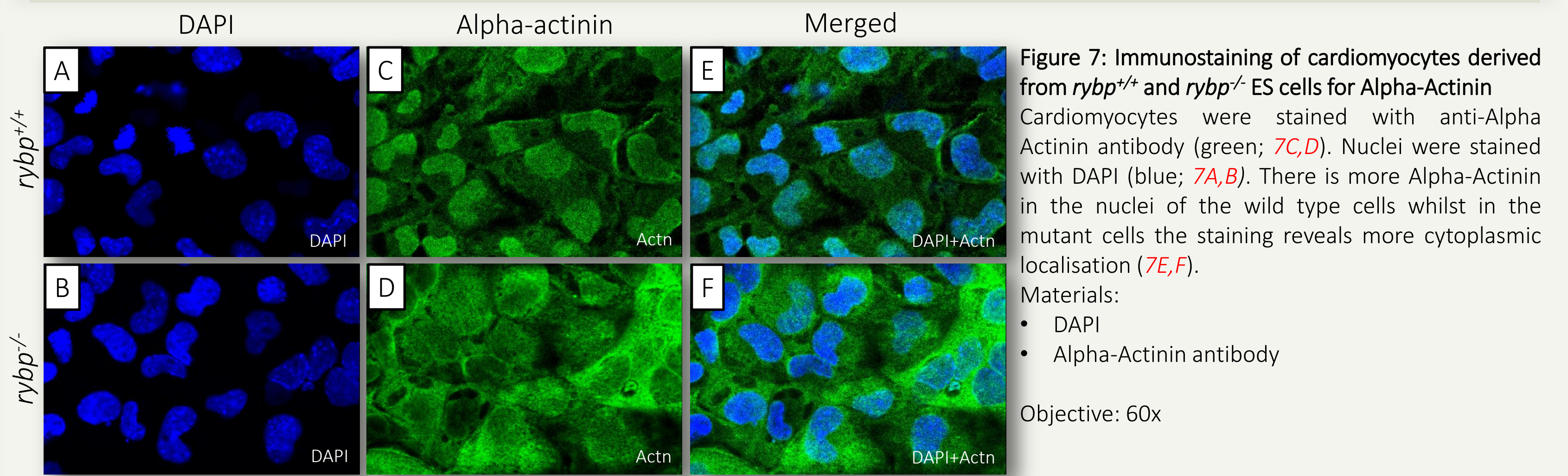


Figure 7: Immunostaining of cardiomyocytes derived from *rybp*^{+/+} and *rybp*^{-/-} ES cells for Alpha-Actinin

Cardiomyocytes were stained with anti-Alpha Actinin antibody (green; **7C,D**). Nuclei were stained with DAPI (blue; **7A,B**). There is more Alpha-Actinin in the nuclei of the wild type cells whilst in the mutant cells the staining reveals more cytoplasmic localisation (**7E,F**).

- DAPI
- Alpha-Actinin antibody

Objective: 60x

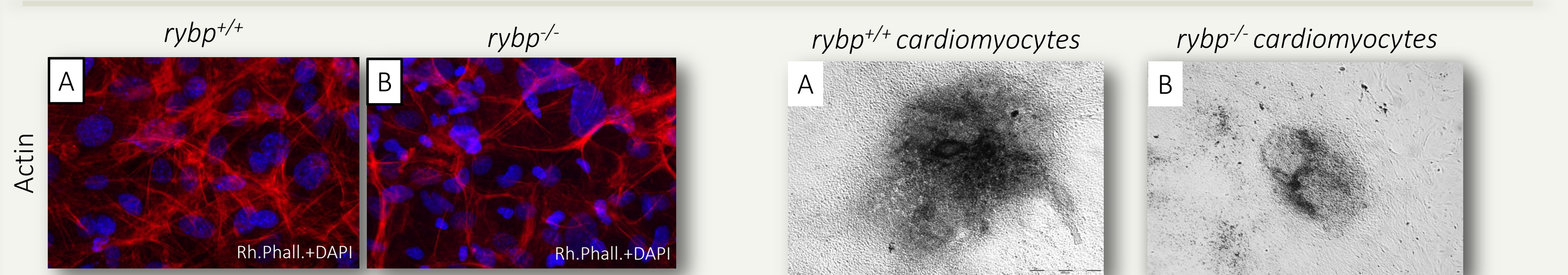


Figure 8: Cardiac differentiation potential of EBs derived from *rybp*^{+/+} and *rybp*^{-/-} cells

Cardiomyocytes were stained with rhodamine-phalloidin for actin (red; **8A,B**). Nuclei were stained with DAPI (blue; **8A,B**). Rhodamine-phalloidin staining was applied to visualize distribution of F-actin, microfilament of the sarcomere. There is underdeveloped actin-filament structure in the *rybp*^{-/-} cells (**8B**). Objective: 40x

- DAPI
- Rhodamine-phalloidin

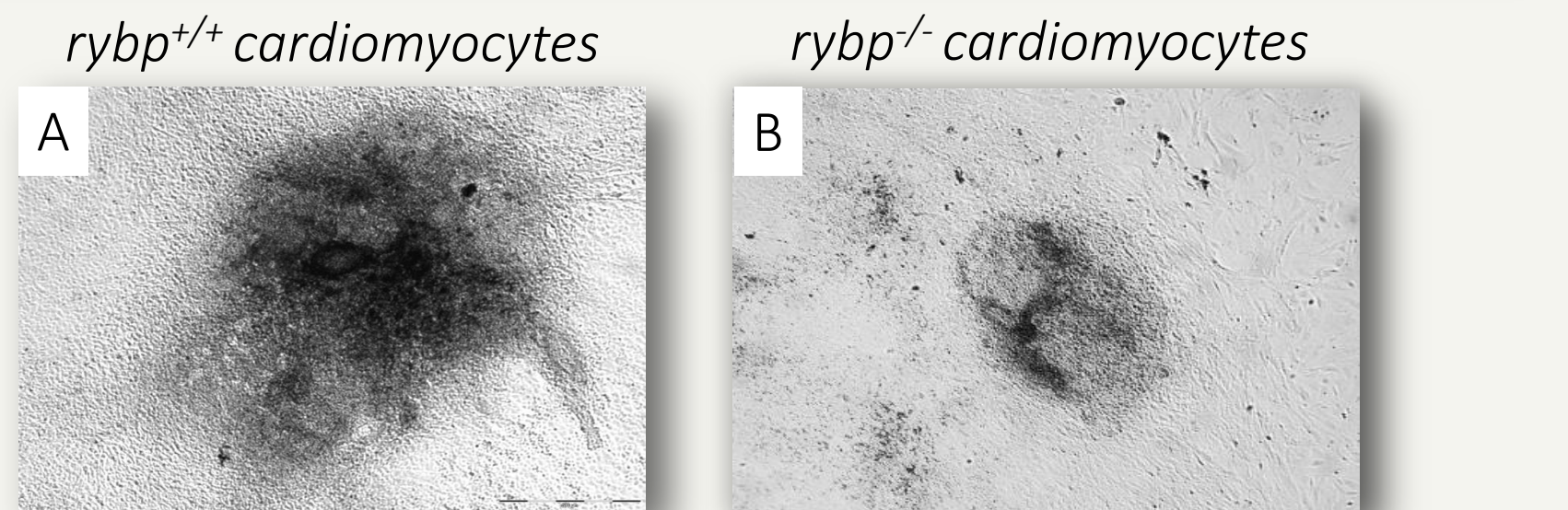


Figure 9: Microscopic analysis of 14 day old cardiomyocytes

- *rybp*^{+/+} cardiomyocytes grow in multiple layers and show contractile activity (**Figure 9A**).
- *rybp*^{-/-} cardiomyocytes are scattered, thin and do not show contractile activity (**Figure 9B**).

Objective: 4x

CONCLUSIONS

ES cells exhibit defects in cardiac differentiation in the absence of Rybp. The lack of Rybp is affecting the level of Tnnt2 during *in vitro* cardiac differentiation and may be important for the subcellular localisation/assembly of Tnnt2 and subsequent sarcomerogenesis. Alpha-actinin expressed in the mutant but it has different subcellular localisation than the wild type and the actin filament structure is underdeveloped, as well. Zac1 is an essential transcription factor for cardiac morphogenesis [8] and when *rybp* is not present (*rybp*^{-/-} cardiomyocytes) Zac1 is not expressed and sarcomerogenesis is impaired. We hypothesize that Rybp is an upstream regulator of Zac1 and regulates Zac1 during sarcomerogenesis. These findings are likely to place Rybp in processes of cardiac differentiation and have implications for the understanding of heart diseases. Together our data provide evidence for a previously unrecognized function of Rybp in heart development and sarcomerogenesis (**Figure 10**).

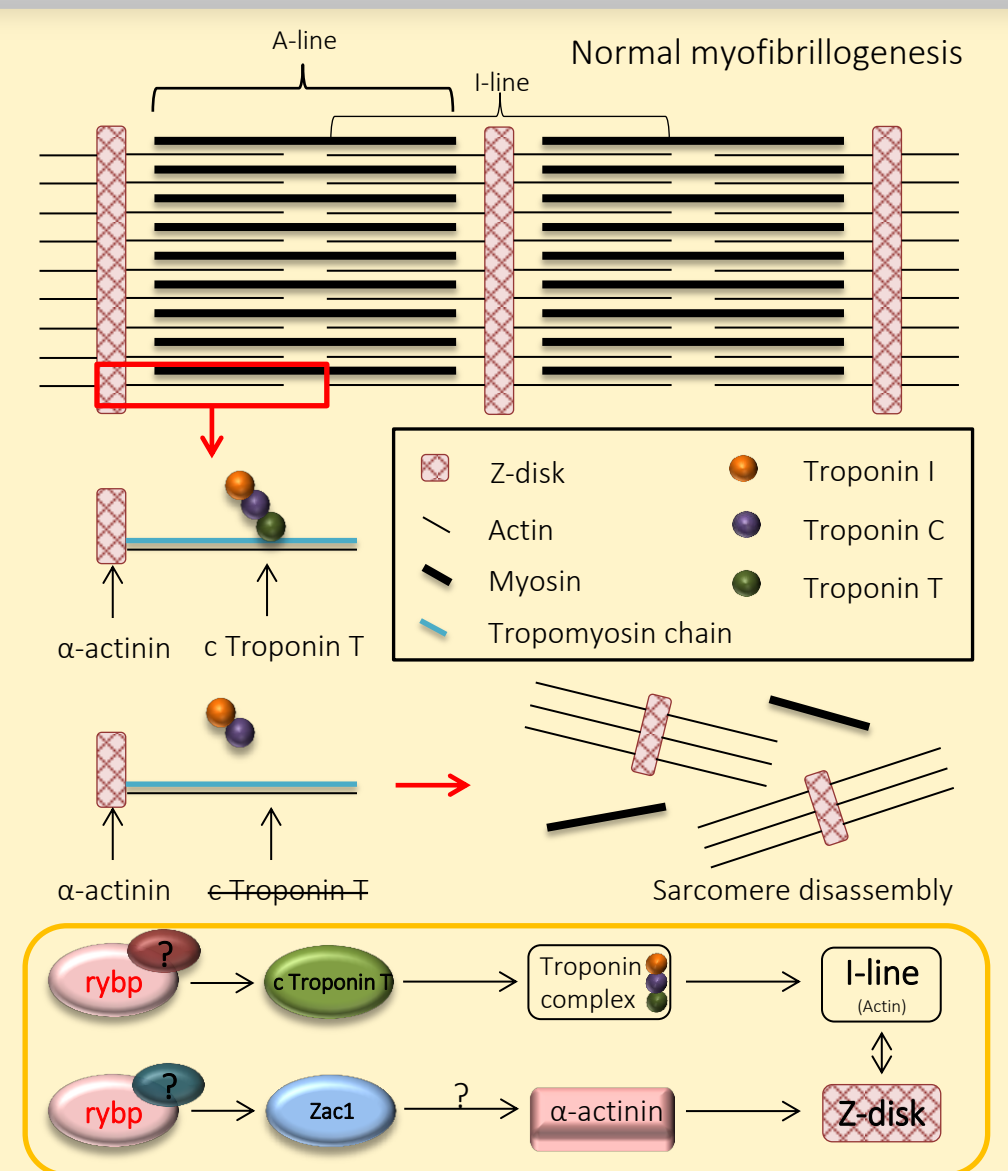


Figure 10: Schematic model for the disruption of sarcomerogenesis

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