The differential spatiotemporal expression pattern of shelterin genes

throughout lifespan

Kay-Dietrich Wagner[1,2#], Yilin Ying[1,3#], Waiian Leong[3#], Jie Jiang[3], Xuefei Hu[3], Yi Chen[3],

Jean-François Michiels[1,4], Yiming Lu[3,6], Eric Gilson[1,3,5*], Nicole Wagner[1,2*] and Jing Ye[3,6*]

[1]Institut for Research on Cancer and Aging, Nice (IRCAN), University of Nice Sophia-Antipolis, CNRS UMR7284/INSERM U1081, Faculty of Medicine, Nice, France,
[2]Institute of Biology Valrose, CNRS UMR7277/INSERM U1091, Faculty of Science, University of Nice Sophia-Antipolis, Nice, France,
[3]International Laboratory in Hematology and Cancer, Shanghai Jiao Tong University School of Medicine/Ruijin Hospital/CNRS/INSERM/Nice University, Pôle Sino-Français de Recherche en Sciences du Vivant et Génomique, Shanghai Ruijin Hospital, Shanghai 200025, P.R. China.
[4]Department of Pathology, CHU Nice, Nice, France,
[5]Department of Medical Genetics, CHU Nice, France,
[6]Shanghai Ruijin Hospital North, Shanghai, P.R. China.

# both authors contributed equally

*: co-senior authorship

Running title: Expression of Shelterin Subunits

Correspondence:

Jing Ye
Shanghai Ruijin Hospital, affiliated to Shanghai JiaoTong University, School of Medicine
No.197 Ruijin Er Road, Shanghai, People's Republic of China China, 200025
E-mail : yj11254@rjh.com.cn

Eric Gilson
Institut of Research on Cancer and Ageing, Nice (IRCAN)
UMR CNRS 7284 INSERM U1081 UNS
28, Avenue Valombrose
06107 Nice Cedex 2, France
Phone: +33 493 377088
E-mail: eric.gilson@unice.fr

Nicole Wagner
Institute of Biology Valrose (iBV)
UNS-CNRS UMR7277
28, Avenue Valombrose
06107 Nice Cedex 2, France
Phone: +33 (0) 493 37 76 65
E-mail: nwagner@unice.fr

1

Abstract

Shelterin forms the core complex of telomere proteins and plays critical roles in protecting telomeres against unwanted activation of the DNA damage response and in maintaining telomere length homeostasis. Although shelterin expression is believed to be ubiquitous for stabilization of chromosomal ends, some evidence suggests that some shelterin subunits have tissue-specific functions. However, very little is known regarding how shelterin subunit gene expression is regulated during development and aging. Using two different animal models, the mouse and zebrafish, we reveal herein that shelterin subunits exhibit distinct spatial and temporal expression patterns that do not correlate with the proliferative status of the organ systems examined. Together, this work shows that the shelterin subunits exhibit distinct spatiotemporal expression patterns, suggesting important tissue-specific functions during development throughout the lifespan.

Keywords shelterin, telomere, brain aging, development

Introduction

Telomeres are specialized chromatin structures, which cap chromosome ends and provide chromosome stability. The maintenance of telomeres requires accurate protections against DNA damage response (DDR) that would otherwise permanently stop cell division by checkpoint activation [ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR) signaling] and lead to end-to-end chromosomal fusions by non-homologous end joining (NHEJ). Another threat to genome integrity stems from the inability of the conventional replication machinery to fully replicate the extremities of parental DNA, erosion compensated for by telomerase or recombination mechanisms (1,2).

To achieve chromosome end protection, telomeres are composed of repetitive DNA sequences that can fold into a terminal loop (t-loop), nucleosomes, the non-coding

telomeric repeat-containing RNA (TERRA), the protein complex shelterin, and an ill-defined network of nuclear factors (3). Shelterin is essential for telomere protection and is composed of six subunits: three bind specifically to telomeric DNA (TRF1, TRF2, and POT1) and three establish protein–protein contacts: RAP1 with TRF2, TIN2 with TRF1 and TRF2, and TPP1 with TIN2 and POT1. Each shelterin subunit appears to have a specific role in telomere protection, i.e., TRF2 blocks ATM signaling and NHEJ, while POT1 blocks ATR signaling (4).

Importantly, telomeres are dynamic structures during development, cancer and aging (5-7). Indeed, the expression of telomerase is repressed in somatic tissues, leading to a progressive and cumulative telomere shortening with cell division ultimately leading to critically short telomeres triggering DDR and cellular senescence (8). Therefore, telomeres have emerged as a key driver of aging.

In addition to their role in chromosome end protection, shelterin subunits are able to localize outside telomeric regions, where they can regulate the transcription of genes involved in metabolism, immunity and neurogenesis (9). This delineates a signaling pathway by which telomeric changes (i.e. telomere shortening) control the ability of their associated factors to regulate transcription throughout the nucleus. This coupling between telomere protection, and tissue-specific transcriptional control might reflect the necessity of tissue homeostasis to rely on 'fine-tuned' coordination between telomeric dynamic (reflecting replicative history and the cumulative effects of various types of stress affecting telomere structure), cellular senescence and differentiation (9).

However, although many molecular and animal studies have manipulated the expression levels of shelterin subunits, only a few have examined the organ specificity of shelterin protein expression. We measured here shelterin gene expression levels in various tissues during development and early adulthood of mice and throughout the lifespan of zebrafish.

This revealed distinct spatiotemporal regulation patterns of shelterin subunits during development and aging.

Materials and Methods

All animals were used in accordance with the guidelines of the French Coordination Committee on Cancer Research and local Home Office regulations.

More specific details on the experimental procedures used for immunohistology and qRT-PCRs are given in the Supplemental Experimental Procedures. Data are expressed as means ± SEMs. ANOVA together with the Bonferroni post-hoc or Mann-Whitney test was performed as indicated. A p value < 0.05 was considered to reflect statistical significance.

Results

Shelterin genes are differentially expressed in mouse tissues

We measured the mRNA levels of mouse shelterin genes (*TERF1, TERF2, RAP1, TPP1, TINF2, POT1a,* and *POT1b*) in mouse brains, hearts, livers, and kidneys, commencing on embryonic day 10 (E10), at 2-day intervals up to postnatal day 1 (P1), and then on P7, P21, and P100 (Figure 1). The proliferating cell nuclear antigen (*PCNA*) gene served as a marker of proliferation in each organ system. In young mice, the relative expression levels of shelterin genes differed among the four tissues evaluated (Figure 1 and Table I). *TPP1* was most prominently expressed in all tissues; whereas *TERF2* and *RAP1* were more highly expressed than was *TINF2* and *POT1a/b* in the brain but not the heart, liver, or kidney. This differential expression pattern appeared to be established during development. Indeed, we observed downregulation of *POT1a/b*, unchanged expression of *TINF2* and *TERF1*, and a significant increase in *TERF2, RAP1,* and *TPP1* expression through to adulthood (Figure 1, Table II). All shelterin components except *POT1a* were expressed at low levels in the heart, and the *POT1a* level decreased significantly during

4

adulthood. The levels of *TPP1, RAP1*, and *TERF1* were higher in the liver than in the heart, and *POT1a/b* expression levels decreased during development. Kidney *TPP1* and *RAP1* levels were upregulated during adulthood; *POT1a/b* expression decreased, but no significant changes in the levels of *TERF1*, *TERF2*, or *TINF2* were observed (Figure 1). As expected, *PCNA* gene expression decreased significantly through to adulthood in all organs evaluated, indicating that the expression levels of the various shelterin genes were not associated with the proliferative status of the various tissues.

TRF2 is highly expressed in the mouse neuronal system during both development and adulthood

To evaluate in detail differential TRF2 protein expression during development, we stained mouse tissues for TRF2 during development until young adulthood (i.e., from E10 to P100). TRF2 was highly expressed in all tissues until E16, at which time expression began to decrease in the heart, liver, and kidney but remained high in the brain (Figure 2). This finding is in contrast to what was reported by Cheng et al. (10), who found that TRF2 was not detected in the brain to E18. This may be attributable to technical problems, as the authors used a mouse-derived antibody to examine mouse tissues. In the absence of extensive blocking procedures, this may create false-negative results caused by an enhanced background. During later development and young adulthood, we found that TRF2 expression decreased in the heart, liver, and kidney but remained stable in the brain; the protein was highly expressed in neurons (Figure 3, Supplementary Figure 1). In the heart, TRF2 continued to be expressed in some endothelial cells of the subepicardial vessels, but in the kidney, the expression thereof became restricted to glomerular podocytes and juxtaglomerular cells (Figure 3), in agreement with the recently described angiogenic properties of the protein and its expression regulation by the Wilms' tumor suppressor WT1 (11).

The persistent high level of TRF2 expression in the brain during both development and adulthood is in line with previous work showing that TRF2 expression specifically increases upon neural differentiation (12) (10,13). Such expression was accompanied by production of a brain-specific cytoplasmic form of TRF2, termed TRF2-S, which lacks both the DNA-binding domain and the nuclear localization signal (14) (15). However, we failed to detect marked cytoplasmic staining of TRF2 in neurons; the staining was predominantly nuclear (Figures 2-3). Overall, the results suggest that the nuclear form of TRF2 plays a key role in brain development and function.

Shelterin genes are differentially expressed in zebrafish tissues throughout lifespan

The subunit composition of zebrafish shelterin is similar to that of humans; the complex is composed of the six subunits TRF1, TRF2 (termed TRFA in zebrafish), RAP1, TIN2, TPP1, and POT1. We determined the relevant mRNA levels in various tissues of 6 female fishes, from the young adult stage (3 months) to aged fish (36 months) (Figure 4). We confirmed tissue identities using specific markers (Supplementary Figure 2). As in the mouse (Figure 1), the relative expression levels of shelterin genes varied among tissues. Thus, the relative levels of *TERFA* mRNA were highest in brain and muscle and lowest in liver; *TPP1* mRNA showed highest expression in the heart and lowest expression in the intestine and ovaries (Figure 4, Table I). During aging, we observed a trend toward general downregulation of shelterin gene expression (Figure 4, Table II); this was particularly marked in the brain and ovaries. The relative shelterin gene expression pattern was usually preserved, with the exception of the *RAP1*, which decreased in mRNA expression more rapidly than did the other shelterin genes in the intestine and the gill.

We performed whole-mount *in situ* hybridization of zebrafish embryos for *TERFA* mRNA using a cDNA probe (Figure 5). The signal corresponding to *TERFA* mRNA was present throughout the entire embryo from the blastula (4 hpf) to the gastrula (8 hpf) stage. In

6

contrast, the neuronal marker, Neurog1 (16), was detected only in neuronal tissues and only from 12 hpf to hatching at 72 hpf, whereas the hematopoiesis factor c-MYB was detected only in hematopoietic tissue and then only during the late stages of development (17)(Figure 5). Interestingly, high expression of *TERFA* mRNA in the nervous system was maintained from the beginning of the somite stage to the time of hindbrain formation (20 hpf) and thereafter (Figure 5). As was also true of Neurog1, at 20 hpf, *TERFA* mRNA appeared to be expressed prominently in the dorsal root ganglion and midbrain boundary,

in the regions of the neural tube that give rise to the neocortex, midbrain, and hindbrain, in the dorsal and ventral spinal cord, and in regions of the peripheral nervous system. These results are in agreement with the quantitative reverse-transcription PCR (qRT-PCR) data on zebrafish tissues (Figure 4) and the specific neuronal staining of TRF2 during mouse development (Figures 2–3 and supplementary Figure 2). In summary, TRF2 expression appears to be ubiquitous during early development but becomes progressively more restricted to neuronal tissues during later stages of development and into young adulthood.

Discussion

We unveil here a tissue-specific shelterin gene expression pattern that is largely conserved between mouse and zebrafish (Table I). In particular, the relative expression level of *TPP1* mRNA was high in most mouse and fish tissues, whereas the *TERF2/TERFA* mRNA levels were specifically elevated in the brain. With the exception of a study on tissue-specific expression of human shelterin genes in response to physiological stress (18), the present study is, to the best of our knowledge, the first to show that shelterin gene expression levels change in a tissue-specific manner during development and aging. We propose that this spatiotemporal expression pattern of shelterin gene expression plays important roles during development, tissue homeostasis, and aging. The fact that TRF2/TRFA is highly expressed in the brain, a tissue of low

7

proliferative activity, indicates that the tissue-specific roles played by TRF2 (and probably other shelterin components) may (at least in part) be independent of the functions in telomere protection.

Our study had certain limitations. First, the tissues evaluated are composed of many different cell types, and thus we cannot clearly conclude whether the changes noted are suggests that the more proliferative cell types do not significantly express shelterin genes. Second, most of our significant findings were detected at the mRNA level. However, we found that the TRF2 spatiotemporal expression pattern was identical, both, on the mRNA and the protein level, during mouse development, suggesting that any effects of post-transcriptional regulation may be limited. Overall, our results suggest that important tissue-specific shelterin subcomplexes exist. This is consistent with previous studies on the tissue-specific roles played by TRF2 (13,14) and RAP1 (19) (20). Consequently, the mechanisms by which shelterin and telomere structures affect cell fate may be more varied than previously thought; specific shelterin subcomplexes may be associated with different cell fates.

The existence of tissue-specific shelterin gene expression brings to light the view that tissue homeostasis relies on 'fine-tuning' of the coordination between the telomeric state and cell-type-specific functions (9). These mechanisms may explain the broad contributions made by telomeres and telomerase to normal development and aging, as well as the roles played by dysregulation of telomeres and telomerase in cancer and various other tissue-specific pathologies (6).

Our data form a solid foundation for future studies exploring the physiological role and regulation of the shelterin complex during development and aging. The work raises several questions. How is the spatiotemporal pattern of shelterin gene expression involved in tissue development, renewal, and function? How is shelterin gene expression regulated during development and over the subsequent lifespan? In this context, we have previously

shown that the TERF2 gene is a direct target of Wnt/beta-catenin and WT1 in both mouse and human cells (21) (11), suggesting that these signaling pathways play important roles in the spatiotemporal expression of shelterin during development and aging. If shelterin plays a critical role in telomere protection, how do the extratelomeric functions of shelterin subunits contribute to cell-type-specific functions? Interestingly, a global decrease in shelterin gene expression during aging was evident in most of the tissues evaluated. How is this decrease triggered during aging? Does the decrease actually cause aging, and, if so, can we stop aging by restoring normal levels of shelterin subunits? Future experiments addressing these questions will certainly shed new light on the increasingly complex and dynamic interaction between telomeres and lifespan.

Author Contributions

NW, KDW, EG, and JY designed the experiments. KDW, YLY, NW, WL, JJ, YC, and XFH performed the experiments. YE, EG, KDW, NW, YLY, and JFM analyzed the data. NW, KDW, EG, and YE wrote the paper. EG, NW and JY are co-senior authors because of their specific roles in coordinating the whole study (EG), the experiments with mice (NW) and the experiments with zebrafishes (JY). JY is the lead author.

References

1. Gilson, E. and Geli, V. (2007) How telomeres are replicated. *Nat Rev Mol Cell Biol*, **8**, 825-838.
2. Gomes, N.M., Shay, J.W. and Wright, W.E. (2010) Telomere biology in Metazoa. *FEBS Lett*, **584**, 3741-3751.
3. Giraud-Panis, M.J., Pisano, S., Benarroch-Popivker, D., Pei, B., Le Du, M.H. and Gilson, E. (2013) One identity or more for telomeres? *Front Oncol*, **3**, 48.
4. Palm, W. and de Lange, T. (2008) How shelterin protects mammalian telomeres. *Annu Rev Genet*, **42**, 301-334.
5. Campisi, J., Kim, S.H., Lim, C.S. and Rubio, M. (2001) Cellular senescence, cancer and aging: the telomere connection. *Exp Gerontol*, **36**, 1619-1637.
6. Armanios, M. and Blackburn, E.H. (2012) The telomere syndromes. *Nat Rev Genet*, **13**, 693-704.
7. Blackburn, E.H., Epel, E.S. and Lin, J. (2015) Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science*, **350**, 1193-1198.
8. Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. (1998) Extension of life-span by introduction of telomerase into normal human cells [see comments]. *Science*, **279**, 349-352.
9. Ye, J., Renault, V.M., Jamet, K. and Gilson, E. (2014) Transcriptional outcome of telomere signalling. *Nat Rev Genet*, **15**, 491-503.
10. Cheng, A., Shin-ya, K., Wan, R., Tang, S.C., Miura, T., Tang, H., Khatri, R., Gleichman, M., Ouyang, X., Liu, D. *et al.* (2007) Telomere protection mechanisms change during neurogenesis and neuronal maturation: newly generated neurons are hypersensitive to telomere and DNA damage. *J Neurosci*, **27**, 3722-3733.
11. El Mai, M., Wagner, K.D., Michiels, J.F., Ambrosetti, D., Borderie, A., Destree, S., Renault, V., Djerbi, N., Giraud-Panis, M.J., Gilson, E. *et al.* (2014) The Telomeric Protein TRF2 Regulates Angiogenesis by Binding and Activating the PDGFRbeta Promoter. *Cell Rep*, **9**, 1047-1060.
12. Jung, Y., Lee, S., Bang, S., Kim, S., Choi, K., Lee, C., Lee, S.G., Kim, C.J., Song, K. and Lee, I. (2004) TRF2 is in neuroglial cytoplasm and induces neurite-like processes. *FEBS Lett*, **557**, 129-132.
13. Ovando-Roche, P., Yu, J.S., Testori, S., Ho, C. and Cui, W. (2014) TRF2-mediated stabilization of hREST4 is critical for the differentiation and maintenance of neural progenitors. *Stem Cells*, **32**, 2111-2122.
14. Zhang, P., Casaday-Potts, R., Precht, P., Jiang, H., Liu, Y., Pazin, M.J. and Mattson, M.P. (2011) Nontelomeric splice variant of telomere repeat-binding factor 2 maintains neuronal traits by sequestering repressor element 1-silencing transcription factor. *Proc Natl Acad Sci U S A*, **108**, 16434-16439.

15. Zhang, P., Abdelmohsen, K., Liu, Y., Tominaga-Yamanaka, K., Yoon, J.H., Ioannis, G., Martindale, J.L., Zhang, Y., Becker, K.G., Yang, I.H. *et al.* (2015) Novel RNA- and FMRP-binding protein TRF2-S regulates axonal mRNA transport and presynaptic plasticity. *Nat Commun*, **6**, 8888.

16. Fode, C., Ma, Q., Casarosa, S., Ang, S.L., Anderson, D.J. and Guillemot, F. (2000) A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev*, **14**, 67-80.

17. Zhang, Y., Jin, H., Li, L., Qin, F.X. and Wen, Z. (2011) cMyb regulates hematopoietic stem/progenitor cell mobilization during zebrafish hematopoiesis. *Blood*, **118**, 4093-4101.

18. Laye, M.J., Solomon, T.P., Karstoft, K., Pedersen, K.K., Nielsen, S.D. and Pedersen, B.K. (2012) Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol (1985)*, **112**, 773-781.

19. Yeung, F., Ramirez, C.M., Mateos-Gomez, P.A., Pinzaru, A., Ceccarini, G., Kabir, S., Fernandez-Hernando, C. and Sfeir, A. (2013) Nontelomeric role for Rap1 in regulating metabolism and protecting against obesity. *Cell Rep*, **3**, 1847-1856.

20. Martinez, P., Gomez-Lopez, G., Garcia, F., Mercken, E., Mitchell, S., Flores, J.M., de Cabo, R. and Blasco, M.A. (2013) RAP1 protects from obesity through its extratelomeric role regulating gene expression. *Cell Rep*, **3**, 2059-2074.

21. Diala, I., Wagner, N., Magdinier, F., Shkreli, M., Sirakov, M., Bauwens, S., Schluth-Bolard, C., Simonet, T., Renault, V.M., Ye, J. *et al.* (2013) Telomere protection and TRF2 expression are enhanced by the canonical Wnt signalling pathway. *EMBO Rep*, **14**, 356-363.

Figure legends

Figure 1. Shelterin components are differentially expressed during development and adulthood

Quantitative RT-PCRs for Shelterin components Trf1, Trf2, Rap1, Tpp1, Tin2, Pot1a, and Pot1b and PCNA as a marker for proliferation in mouse brains, hearts, livers, and kidneys at different time-points of development and in adulthood (*n*=4 each, the four samples for E10 were each pooled from 7 organs, at E12 and 14 the four samples were pooled from four organs each). Significance was tested between E10 and P100 (adult). Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

Figure 2. TRF2 is Highly and Ubiquitously Expressed During Embryonic Development Up to E16 and Persists Afterwards Specifically in the Brain

Representative photomicrographs of TRF2 immunostaining on sections of mouse embryos (3,3' diaminobenzidine (DAB) substrate, brown, hematoxylin counterstaining) at different

11

stages before birth. B: brain, H: heart, L: liver, K: kidney. Unless otherwise indicated, scale bars represent 50μm.

Figure 3. TRF2 Expression Remains High in the Brain During Adulthood

Representative photomicrographs of TRF2 immunostaining for the brain, heart, liver, and kidney (3,3' diaminobenzidine (DAB) substrate, brown, hematoxylin counterstaining) at different stages after birth. Note the persistent high expression of TRF2 in neurons of the brain (see also Figure S3), the specific expression in subepicardial endothelial cells of the heart, and glomerular podocytes and juxta-glomerular cells of the kidney (arrows). Scale bars indicate 50μm.

Figure 4. Shelterin Genes are Differentially Expressed during Zebrafish Life Span

Quantitative RT-qPCRs for Shelterin components TRF1, TRF2 (TERFA), RAP1, TPP1, TIN2 and POT1 in zebrafish's brain, heart, liver, intestine, muscle, gill, tail and ovary at different time-points of life span from 3 month to 36 month (n=6 each). Significance was tested between 3 month and 36 month. Data are mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Figure 5. TRF2 (TERFA) Expression Increase since Neural Development at Embryonic Stage and Remains High in the Brain during Larval Stage Development

Representative photomicrographs of whole-mount in situ hybridization of TRF2 (TERFA), Neurog1 and c-MYB. The RNA probe labelled with DIG was stained in dark blue. The green arrow indicates the dorsal root ganglion neuron and the blue arrow indicates the lateral line neuromasts. The black arrow in 24hpf indicates the midbrain boundary. The red arrow indicates the c-MYB signal marked hematopoietic tissue.
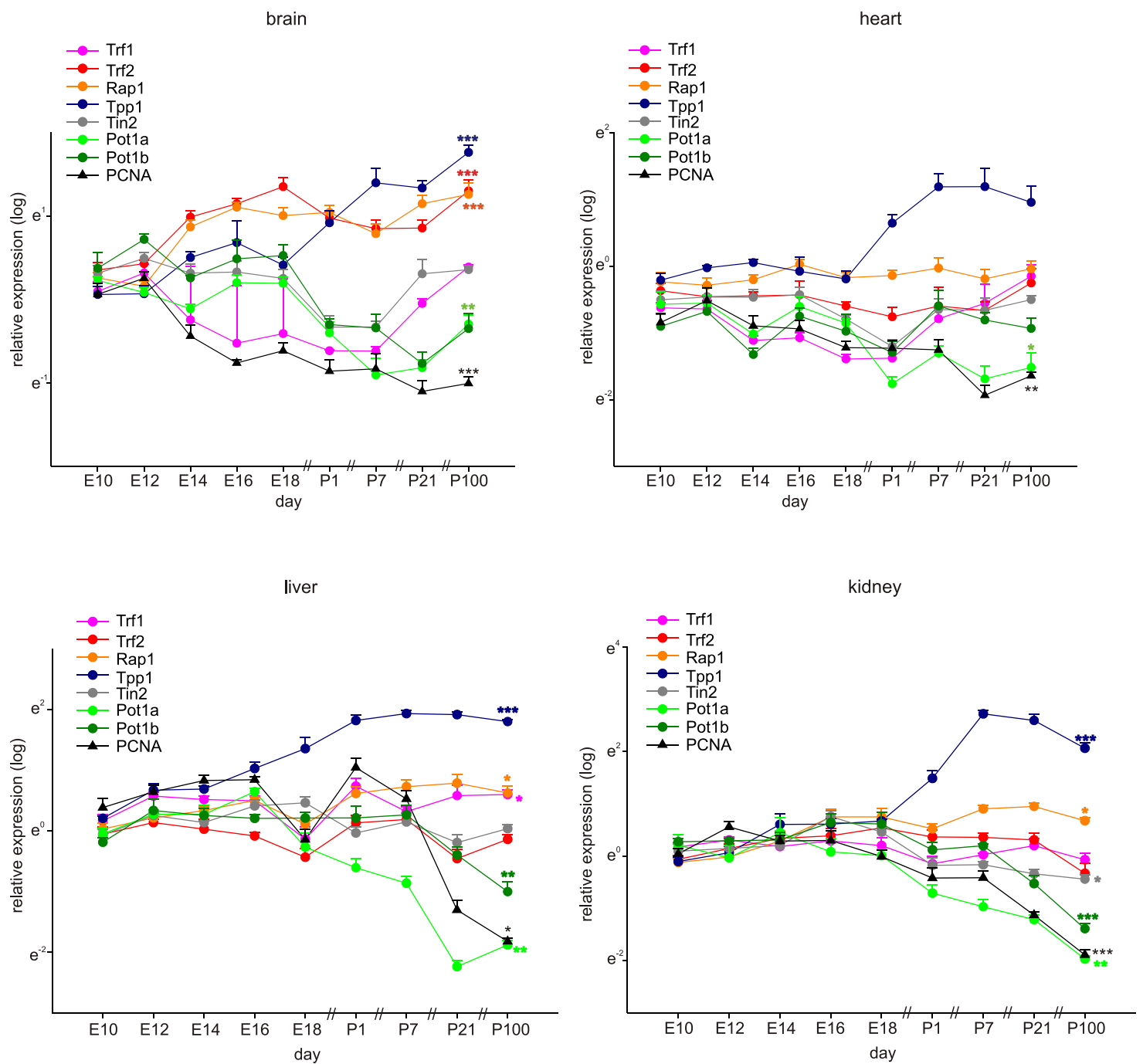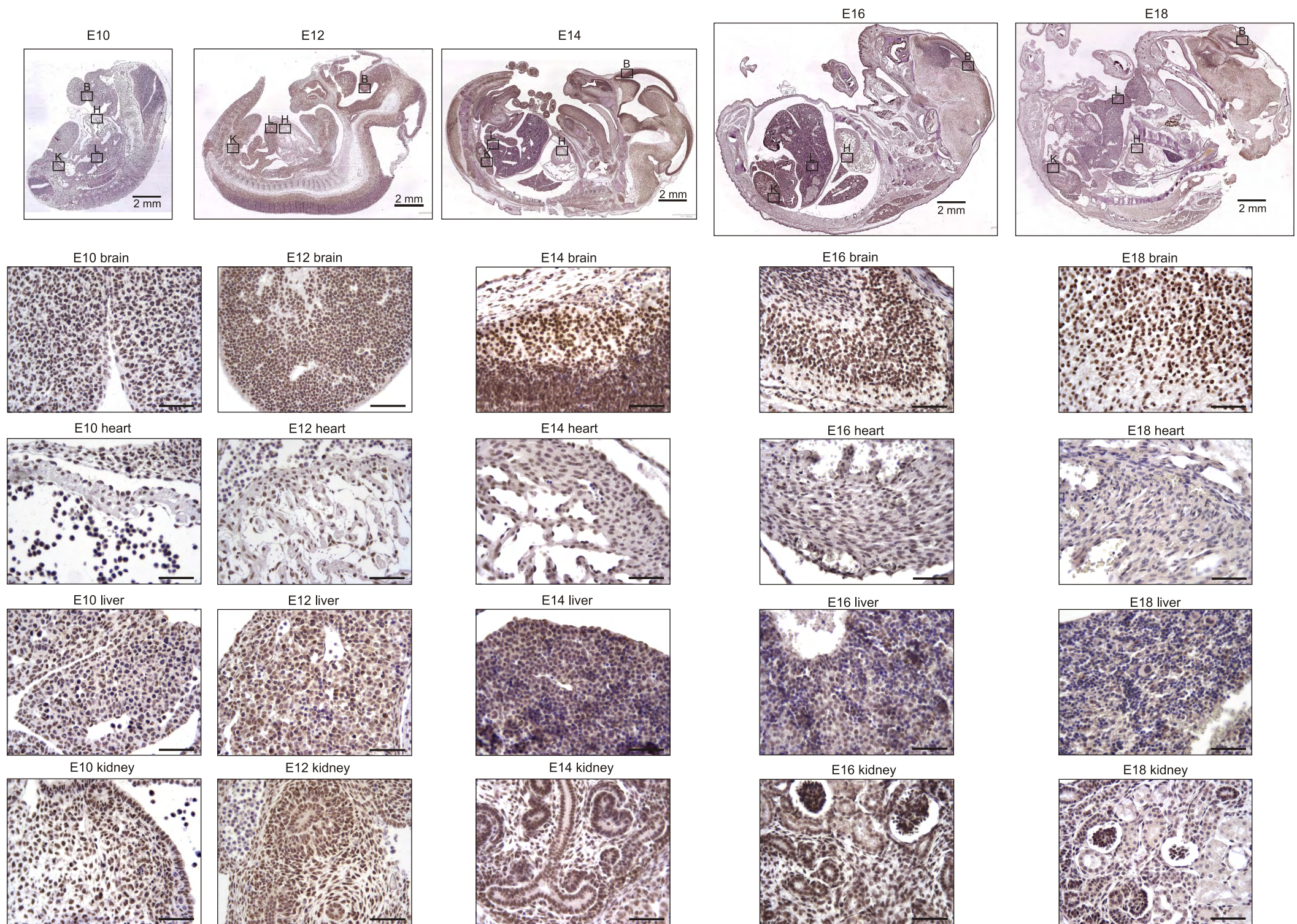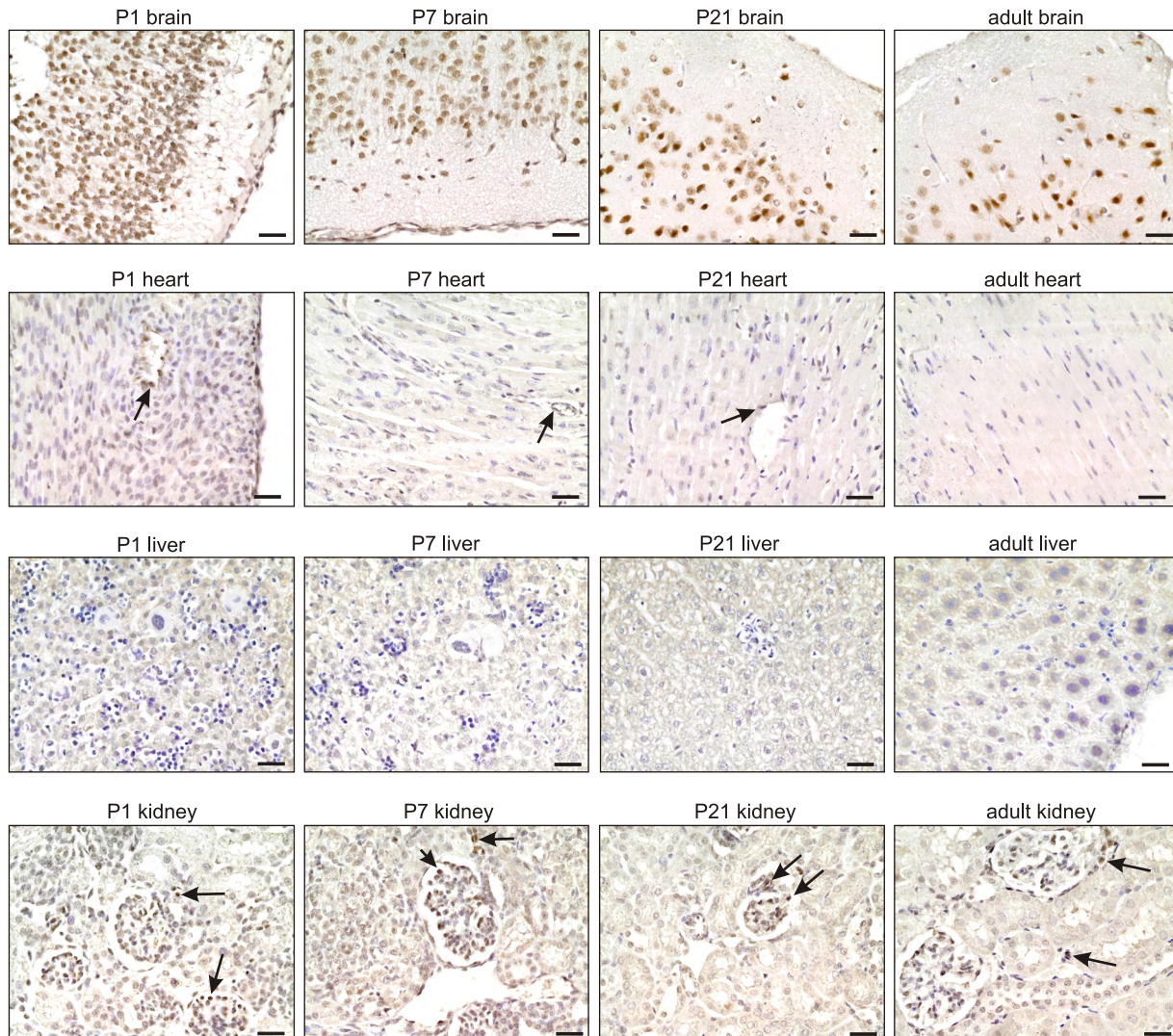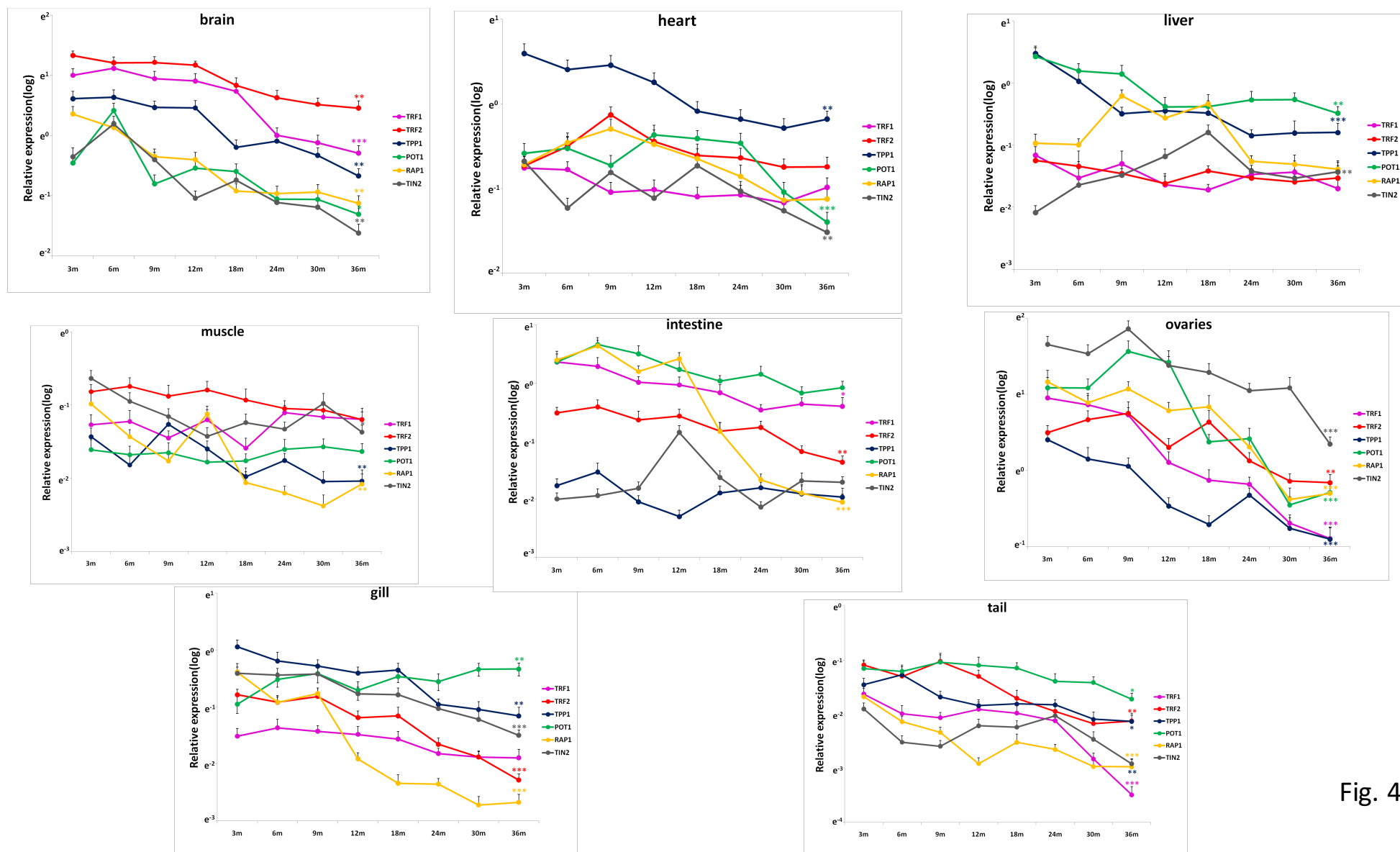
Table

Figure 1

Figure 2

Figure 3

Fig. 4

TRF2

4hpf 8hpf 12hpf 20hpf 24hpf 72hpf

neurog1

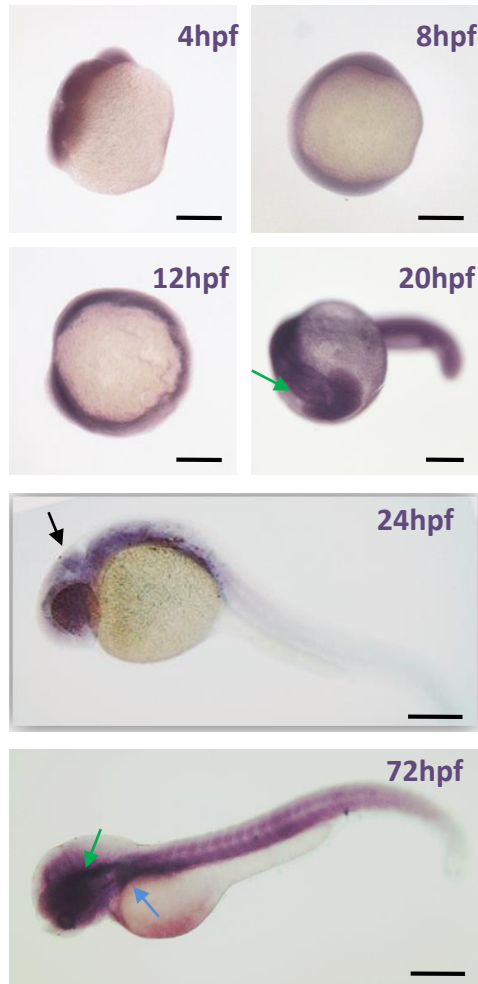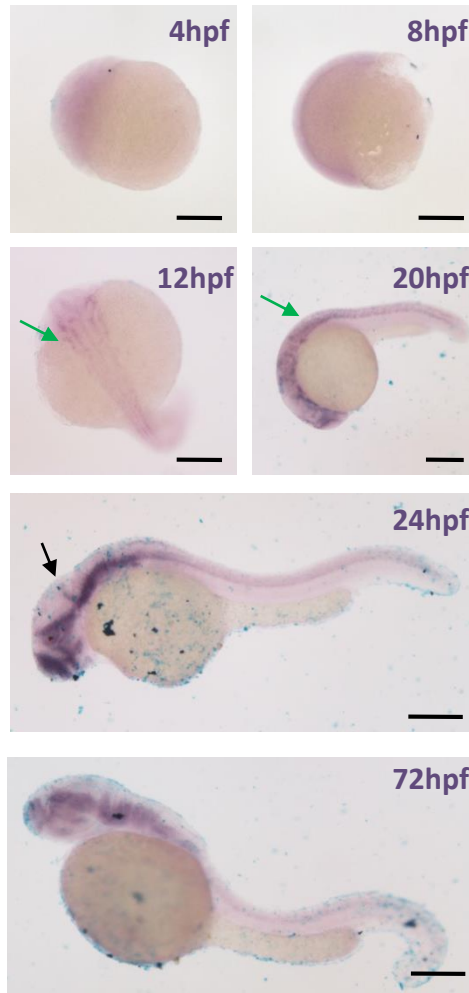4hpf 8hpf 12hpf 20hpf 24hpf 72hpf
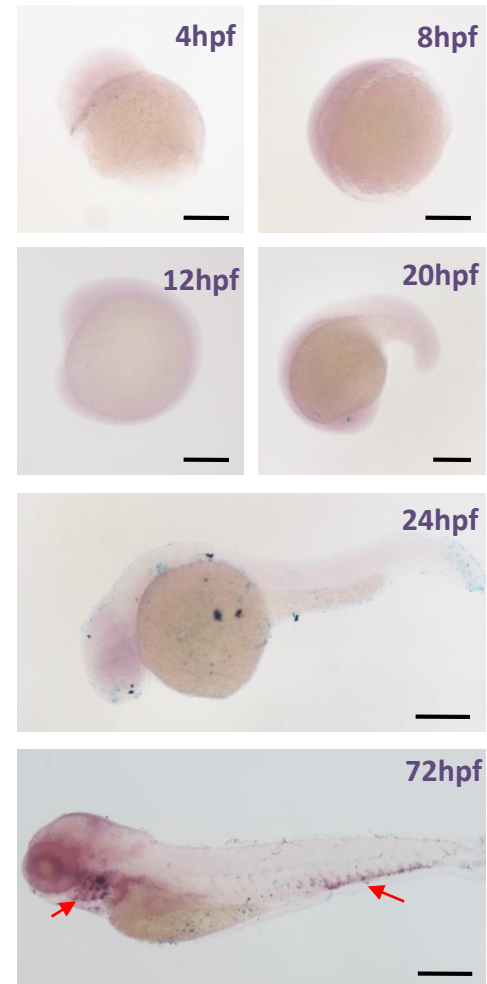
c-myb

4hpf 8hpf 12hpf 20hpf 24hpf 72hpf

Scale bar=500μm

Green:dorsal root ganglion
Blue: lateral line neuromasts

Table I   Relative expression of shelterin genes

Shelterin genes	Mouse development and young adulthood				Zebrafish adulthood and aging							
	Brain	Heart	Liver	Kidney	Brain	Gill	heart	Intestine	Liver	Muscle	Tail	Ovaries
TERF1	-	+/-	+/-	+/-	+/-	-	-	+	-	+	-	-
TERF2(m) *TERFA* (zf)	+	+/-	+/-	+/-	+	-	-	-	-	+	+/-	+/-
RAP1	+	+/-	+/-	+/-	-	-	-	+/-	-	-	-	+/-
TINF2	-	+/-	+/-	+/-	-	+	-	-	-	+	-	+
TPP1	+	+	+	+	+/-	+	+	-	+	-	+/-	+/-
POT1a (m)	-	-	-	-	na							
POT1b (m)	-	+/-	+/-	-	na							
POT1 (zf)	na				-	+	-	+	+	+/-	+	-

Table II : Trend in shelterin gene expression during development, adulthood and aging

Shelterin genes	Mouse development and young adulthood				Zebrafish adulthood and aging							
	Brain	Heart	Liver	Kidney	Brain	Gill	heart	Intestine	Liver	Muscle	Tail	Ovaries
TERF1	s	s	UP	s	DOWN	DOWN	s	DOWN	s	s	DOWN	DOWN
TERF2(m) *TERFA* (zf)	UP	s	s	s	DOWN	DOWN	s	DOWN	s	DOWN	DOWN	DOWN
RAP1	UP	s	UP	UP	DOWN	DOWN	s	DOWN	DOWN	DOWN	DOWN	DOWN
TINF2	s	s	s	s	DOWN	DOWN	DOWN	s	UP	DOWN	DOWN	DOWN
TPP1	UP	s	UP	UP	DOWN	DOWN	DOWN	s	DOWN	DOWN	DOWN	DOWN
POT1a (m)	DOWN	DOWN	DOWN	DOWN	na							
POT1b (m)	DOWN	s	DOWN	DOWN	na							
POT1 (zf)	na				DOWN	s	DOWN	DOWN	DOWN	s	DOWN	DOWN

13