Supplemental Data: Tables, Figures, and Figure Legends

Telomere shortening in neural stem cells disrupts neuronal differentiation and neuritogenesis

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Table showing sequence of the primers, product size, and annealing temperature (T) for RT-PCR determination of different genes.

| Gene  | Forward (5´-3´)                  | Reverse (5´-3´)                  | Size (bp) | T  |
|-------|----------------------------------|----------------------------------|-----------|----|
| β-actin | CCGGGACCTGACAGACTACCT            | GCCATCTCCTGCTGGAAGTCTA           | 100       | 59 |
| Bax   | TGGAGCTGCAGAGGATGATT             | AAGTTGCATCGAAGCAGCAT            | 115       | 59 |
| Notch 1 | CCAGGAAACACTGCAAGAA              | AGTACTGACCCGTCCACTCC           | 90        | 59 |
| Notch 3 | CCGAATTGAGCCAGTTTACTT          | CTGCTCTGACATTCGTCAGAT         | 84        | 59 |
| Noxa  | CCACCTGAGTTCCAGCTCAA             | GTTGACACAGCTCGTCCTTCAA         | 130       | 60 |
| p21   | CAAAGTGTGCCGTCGTCCTCT             | CTGGACCTGATCCCGGAGA           | 160       | 60 |
| Perp  | TCATCCTGTGATCTGCTTTC             | GGGTTATCGTGAAAGCTGGA          | 180       | 60 |
| PUMA  | GACGACCTCAACGACAGTA              | CACCCTTTGGCTGCTCATCT          | 231       | 58 |
| Rock 1 | AGCTTTGTGTGGCAATGCAGC         | CCTGCAAGCTTTTATCCACA         | 122       | 60 |
| Rock 2 | AGCTGGAGATCAAAAGATGATGG         | AGTGGTTGTTGGCACAAGGCAAT      | 611       | 55 |
| Tert  | GAGAGATAGCCAGGGAAGGCAAG         | ATGCTCTGCTGATGACAAC         | 150       | 59 |
Table showing the relative numbers of calterinin (CR)+, calbindin (CB)+, and tyrosine hydroxylase (TH)+ neurons in layers of the olfactory bulb (OB) in the different genotypes. Every sixth section (180 μm) from vibratome coronal series through the OB was selected and stained for the different markers. Four predetermined areas (50 x 50 μm) in the granular or periglomerular cell layers were analyzed on each section and all immunopositive cells in these selected areas were counted. Data are expressed as mean number of cells ± sem. Three independent animals per genotype were analyzed. Notice significant reductions in the density of all types of interneurons in G3 Terca− mice that are restored in a p53 null background. Because of the changes in OB volume in the various genotypes (see Suppl. Fig. 1), differences in total numbers between WT and G3 Terca− are even larger. We observed similar differences when we scored BrdU-LRC neurons in the different interneuron classes (not shown).

| Genotype           | OB layer Marker | CR+   | CB+   | TH+   |
|--------------------|----------------|-------|-------|-------|
|                    | Granular       |       |       |       |
| WT                 | 73 ± 2         | 58 ± 9|       | 11 ± 1|
| G3 Terca−          | 55 ± 1**       | 41 ± 7**| 6 ± 0**|
| G3 Terca−;p53−     | 70 ± 4         | 65 ± 11| 9 ± 0 |
| p53−               | 117 ± 3        | 75 ± 9 | 9 ± 1 |
Supplementary Figure 1. Defects in proliferation and neurogenesis in the SEZ of telomerase-deficient mice are restored in a p53-deficient background. (A) Quantification of the total number of BrdU+ cells per SEZ in 2-m mice of different genotypes (n = 3 animals per genotype). (B) Quantification of the total number of βIII-tubulin+ cells per SEZ in 2-m mice of different genotypes (n = 3 animals per genotype). (C) Immunofluorescent detection of βIII-tubulin (red) and BrdU (green) in the SEZ of WT, G3 Terc−/−, G3 Terc−/−;p53−/− and p53−/− in adult mice. Empty arrows point at neuroblasts which have incorporated BrdU injected on the day of sacrifice. Inset shows a higher power micrograph of double-labelled cells. (D) Histogram showing the volume of the OB in wild-type (WT), G3 Terc−/−, G3 Terc−/−;p53−/− and p53−/− 2-m mice (n = 3 animals per genotype). (E) Quantification of the density of GFAP+ cells in the SEZ of 2-m mice of different genotypes (n = 3 animals per genotype). *p<0.05, **p<0.01, ***p<0.001. Scale bars: C, 20 µm.
Supplementary Figure 2. Defects in proliferation, self renewal, and differentiation in telomerase-deficient neurosphere cultures are restored in a p53-deficient background. (A) Histogram showing the numbers of neurospheres formed by equal numbers of dissociated neurosphere cells of different genotypes when seeded at low density (2.5 cell/µl). Secondary sphere formation in double p53 and telomerase-deficient animals (G3 Terc⁻/⁻; p53⁻/⁻) are normal relative to control animals (WT) (n = 3 independent cultures per genotype). Notice that, as previously reported, p53-deficient NSCs exhibit increased clonogenic capacity. (B) Growth curves corresponding to the expansion through consecutive passages of neurosphere cultures from wild-type (WT), G3 Terc⁻/⁻ and G3 Terc⁻/⁻; p53⁻/⁻ 2-m mice (n = 3 independent cultures per genotype). div, days in vitro. (C) Proportions of neurospheres of the different genotypes that produce three (tripotent), two (bipotent), or one (astrocytes-only; unipotent) cell derivatives (n = 3 cultures per genotype). *p<0.05, **p<0.01.
Supplementary Figure 3. Short telomeres result in reduced neurogenesis. (A) Table showing the percentages of neurons that co-express βIII-tubulin and MAP2 in cultures differentiated from embryonic and adult neurospheres (fetal and adult, respectively). After 7 div most βIII-tubulin+ neurons are also immunoreactive for the more mature marker MAP2. (B) Low power fluorescent micrographs of wild-type (WT) and G5 Terc^-/- differentiated cultures immunostained with anti-βIII-tubulin (red) and anti-MAP2 (green) antibodies. (C) Quantification of the relative number of βIII-tubulin+, MAP2+, and βIII-tubulin+/MAP2+ positive cells in wild-type (WT) and G5 Terc^-/- differentiated cultures (n = 3 independent cultures per genotype). Scale bars: B, 30 µm. *p<0.05, **p<0.01.
**Supplementary Figure 4. Negative effects of short telomeres on neuritogenesis are cell autonomous.** (A) Schematic drawing depicting the design of the co-culture experiments (left) and fluorescent micrographs of co-culture cells showing wild-type non-GFP+ βIII-tubulin+ neurons (red) together with GFP+ WT or G5 Terc^-/- βIII-tubulin+ neurons (green and red). (B) Quantification of the relative number of βIII-tubulin+ neurons (left) and of the number of end-points (EP) per neuron (right) in WT/G5 Terc^-/- co-cultures (n = 3 independent cultures per genotype). Quantifications in WT/WT co-cocultures indicated no significant differences; dotted lines indicate the mean value for neurons and EP/neurons in WT/WT cultures (GFP+ and GFP- data pooled together). Statistical significance according to a Student’s t-test (after arcsen transformation of relative values): *p<0.05, **p<0.01. Scale bars: A, 30 µm.
Supplementary Figure 5. Effects of Notch constitutive expression on astrogliogenesis and neurogenesis from adult neurosphere cells. (A) Fluorescent micrographs of 7 div differentiated cultures from adult neurosphere cells which had been retrovirally transduced with a constitutively active Notch intracellular fragment (pMXIE-NotchIC) or with the empty construct (pMXIE), after immunocytochemical staining for βIII-tubulin (red) and MAP2. (B) Quantification of the relative number of S100β+ astrocytes in pMXIE and pMXIE-NotchIC transduced cultures after a 7 div differentiation (n = 3 independent cultures). (C) Quantification of the relative number of βIII-tubulin+, MAP2+, and βIII-tubulin+/MAP2+ positive cells in pMXIE and pMXIE-NotchIC transduced cultures after a 7 div differentiation (n = 3 independent cultures). Statistical significance according to a Student’s t-test (after arcsen transformation of relative values): *p<0.05, **p<0.01. Scale bars: A, 30 µm.
Supplementary Figure 6. p53 and Notch regulate neuritogenesis. Representative drawings of βIII-tubulin+ neurons differentiated from WT and G5 Terc−/− (fetal cultures) and from WT and G3 Terc−/− (adult cultures) cells, untreated or treated with either the γ-secretase inhibitor (A) or the Rock1/2 inhibitor (B) at 1 µM.