Supplementary information for

Consequences of telomere dysfunction in fibroblasts, club and basal cells for lung fibrosis development

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Supplementary Fig. 1 Female mice with deletion of Trf1 in club cells exhibited lower lung inflammation and absence of airway remodeling. 

a Kaplan-Meier survival curves of Scgb1a1-Cre; Trf1+/+ke (Trf1+/+) and Scgb1a1-Cre; Trf1Δ/Δ (Trf1Δ/Δ) female mice. 

b Representative immunostainings for SCGB1A1 (blue) and TRF1 (brown) (red arrowheads indicate SCGB1A1+ club cells).
cells with deletion of TRF1) in lung sections from $\text{Ttf1}^{+/+}$ ($\text{Scgb1a1-Cre}; \text{Ttf1}^{+/+}$) and $\text{Ttf1}^{\Delta/\Delta}$ ($\text{Scgb1a1-Cre}; \text{Ttf1}^{+/+}$) female mice. c-g Quantification of total white blood cells (c), neutrophils (d), eosinophils (e), lymphocytes (f) and macrophages (g) in peripheral blood from $\text{Ttf1}^{+/+}$ and $\text{Ttf1}^{\Delta/\Delta}$ mice. h-i Lung resistance (LR) (h) and dynamic compliance (Cdyn) (i) evaluated by plethysmography in $\text{Ttf1}^{+/+}$ and $\text{Ttf1}^{\Delta/\Delta}$ mice. j Representative BALF cytospin preparations (May-Grünwald Giemsa (MGG)), Sirius Red staining and Vimentin and SMA immunostainings (airways) in lung sections from $\text{Ttf1}^{+/+}$ and $\text{Ttf1}^{\Delta/\Delta}$ mice. k-q Quantification of total (k) and differential BALF cell counts for neutrophils (l), lymphocytes (m) and macrophages (n), and quantification of airway collagen (Sirius Red) (o) and Vimentin (p) positive areas (%), and airway smooth muscle (SM) thickness (SMA) (µm) (q) in $\text{Ttf1}^{+/+}$ and $\text{Ttf1}^{\Delta/\Delta}$ mice. Data are expressed as mean ± SEM (the number of mice is indicated in each case). *p<0.05 (Mann-Whitney or unpaired t tests). Animal survival was assessed by the Kaplan-Meier analysis, using the log Rank (Mantel-Cox) test. Source data are provided as a Source Data file.
Supplementary Fig. 2 Female mice with deletion of *Trf1* in lung basal cells exhibited lower lung inflammation and absence of airway remodeling. **a** Kaplan-Meier survival curves of *p63-Cre; Trf1**+/+** (Trf1**+/+) and *p63-Cre; Trf1**Δ/Δ** (Trf1**Δ/Δ**) female mice. **b** Representative immunostainings for p63 (blue) and
TRF1 (brown) (red arrowheads indicate p63+ basal cells with deletion of TRF1) in lung sections from *Trf1*+/+ (p63-Cre; Trf1+/+) and *Trf1*ΔΔ (p63-Cre; Trf1+/+) female mice. c-g Quantification of total white blood cells (c), neutrophils (d), eosinophils (e), lymphocytes (f) and macrophages (g) in peripheral blood from *Trf1*+/+ and *Trf1*ΔΔ mice. h-i Lung resistance (LR) (h) and dynamic compliance (Cdyn) (i) evaluated by plethysmography in *Trf1*+/+ and *Trf1*ΔΔ mice. (j) Representative BALF cytospin preparations (May-Grünwald Giemsa (MGG)), Sirius Red staining and Vimentin and SMA immunostainings (airways) in lung sections from *Trf1*+/+ and *Trf1*ΔΔ mice. k-q Quantification of total (k) and differential BALF cell counts for neutrophils (l), lymphocytes (m) and macrophages (n), and quantification of airway collagen (Sirius Red) (o) and Vimentin (p) positive areas (%), and airway smooth muscle (SM) thickness (SMA) (µm) (q) in *Trf1*+/+ and *Trf1*ΔΔ mice. Data are expressed as mean ± SEM (the number of mice is indicated in each case). *p<0.05*, p<0.05 (Mann-Whitney or unpaired t tests). Animal survival was assessed by the Kaplan-Meier analysis, using the log Rank (Mantel-Cox) test. Source data are provided as a Source Data file.
Supplementary Fig. 3 Deletion of Trf1 in embryonic basal cells decreases survival and increases airway remodeling postnatally. a Generation of K5-Cre; Trf1+/+ (Trf1+/+, controls) and K5-Cre; Trf1ΔΔ (Trf1ΔΔ) mice in which Trf1 was deleted in basal cells from day E 11.5 of embryonic development onwards, using the Cre recombinase driven by the K5 promoter. b Kaplan-Meier survival curves of K5-Cre; Trf1+/+ (Trf1+/+) and K5-Cre; Trf1ΔΔ (Trf1ΔΔ) mice. c Representative immunostainings for K5 (blue) and TRF1 (brown) (red arrowheads indicate K5+ basal cells with deletion of TRF1), Sirius Red stainings and immunostainings for Vimentin and SMA (airways) in lung sections from Trf1+/+ and Trf1ΔΔ mice. d-g Quantification of p63+ -TRF1+ cells (%) (d), airway collagen (Sirius Red) (e) and Vimentin (f) positive areas (%),
and airway smooth muscle (SM) thickness (SMA) (µm) in \textit{Trf1}^{+/+} and \textit{Trf1}^{Δ/Δ} mice. Data are expressed as mean ± SEM (the number of mice is indicated in each case). *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney or unpaired t tests). Animal survival was assessed by the Kaplan-Meier analysis, using the log Rank (Mantel-Cox) test. Source data are provided as a Source Data file.

**Supplementary Table 1.** Primer sets used for qPCR.

| Gene | Accession No. | Forward primer (5´-3´) | Reverse primer (5´-3´) |
|------|---------------|-------------------------|------------------------|
| Ccl12| NM_011331.3   | TCCTCAAGGTATTGGCTGGAC   | GGCTGCTTTGATTCCTTGTA   |
| Col1a1| NM_007742.4  | CCGAGAAGAAGGAAACGAG   | CAGGGAAACACAGGCTAGC   |
| Col1a2| NM_007743.3  | GGAAACAAGGCTCACTGG   | CAAGGTCTCTGAGACCTGTA   |
| Col3a1| NM_009930.2  | CCAACCCAGAGATCCCATT | GGTCACCATTTCCTCCAGGA |
| Col4a1| NM_009931.2  | AAGTGGAGAGATGGAGGCC  | CTTCACCCTGCAACCTCGC |
| Col5a1| NM_015734.2  | TGAAACCCAGTAAAGCGAGAAG | CTTCCTCGTGTCCTCTCAT |
| Col6a1| NM_009933.4  | GCTCAGTGGATGGCTGTG   | GTTCTG TGGGTTGGGAGTA |
| Ilng | NM_008337.4   | TTCTCAGCAACAGCAAGGC | ACTCCTTTCCGCTCTCGA |
| Il1b | NM_008361.3   | GCAACTGTTCCTGAACCTCACT | ATCTTTGGGTTCCGTCAACT |
| Il4  | NM_021283.2   | CCTCAGACAGCAACAGAAC   | CGAAAGCCGAGAAAGTC |
| Il6  | NM_031168.1   | AGCCGTCTCCCTCTACTTCACA | CATTTCACGATTTCCTCAGA |
| Il10 | NM_010548.2   | GCTTTAGCAGAAATCTTCA  | TTTTCAGGGAGGAGATCG |
| Il13 | NM_008355.3   | GCCCTCAGGATAACAAAT | CTTCCTCCTCACCTCTCCT |
| Rn18s | NR_003278.3 | ATGCTCTTTGCTGAGGTCCCG | ATTTCTAGGAGGGATCCAG |
| Tnf  | NM_013693.3   | GCCCTTTTCTCATTCTGGCTTG | CTGATGAGGAGGAGGAGG |

Supplementary Table 1. Primer sets used for qPCR.
Supplementary methods

Generation of mutant mouse lines

To conditionally delete Trf1 in fibroblasts, club and basal cells, heterozygous \( \text{Trf}^1\text{lox}^+ / \text{Col}1\text{a2/Scgb1a1/p63-Cre}^\text{ERT2} \text{KFP}^\text{CAG-lox-STOP-lox} \) mice were crossed with \( \text{Trf}^1\text{lox/lo}x \) mice to obtain \( \text{Trf}^1\text{lox/lo}x / \text{Col}1\text{a2/Scgb1a1/p63-Cre}^\text{ERT2} \text{KFP}^\text{CAG-lox-STOP-lox} \) and \( \text{Trf}^1\text{lox/lo}x \text{KFP}^\text{CAG-lox-STOP-lox} \) mice. Tamoxifen (TMX) was dissolved in corn oil (Sigma Aldrich) to a concentration of 20 mg/ml and intraperitoneally (i.p.) injected to eight-to 10-week-old male \( \text{Trf}^1\text{lox/lo}x \text{KFP}^\text{ert2} \text{Cre}^\text{ert2} \) and \( \text{Trf}^1\text{lox/lo}x \text{KFP}^\text{ert2} \text{Cre}^\text{ert2} \) mice (75 mg/kg) to induce the deletion of Trf1 in fibroblasts, club and basal cells.

Additionally, we have generated the \( \text{Trf}^{1}\Delta/\Delta \text{K5-Cre p53}^{-/-} \) mouse model that constitutively express the Cre recombinase specifically in basal epithelial cells from day E 11.5 of embryonic development onwards (Martínez et al., 2009, Genes Dev. 23, 2060–2075). A p53-deficient background was used to bypass the perinatal lethality associated to constitutive Trf1 deletion from embryonic development.

In vivo measurement of lung function

The mice were anesthetized by intraperitoneal injection of 10 μl/g of a ketamine-medetomidine anesthetic combination in saline (75:1 mg/kg respectively). A MiniVent (Harvard Apparatus, Holliston, MA, USA) was connected to the plethysmograph and the tracheal cannula for animal ventilation at 10 ml/kg of tidal volume and 150 breaths per minute. Data were measured by 2 pressure transducers that detect pressure variations in the chamber (flow) and in the tracheal cannula (pressure).
Sample collection and processing

Animals were euthanized by intraperitoneal injection of 10 μl/g of a ketamine-xylazine anesthetic combination in saline (100:10 mg/kg respectively) after lung function assessment.

Serum was obtained by centrifugation at 3000 xg for 10 min at 4 °C and stored at -80°C. On the other hand, bronchoalveolar lavage fluid (BALF) was centrifuged at 10000 rpm for 5 min at 4 °C and the supernatants were stored at -80 °C to subsequently assess total protein concentration in BALF using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Hereafter, the BALF pellets were resuspended in 500 μl of ACK Lysing Buffer (Thermo Fisher Scientific) and centrifuged at 10000 rpm for 5 min at 4 °C after 10 min of incubation. The supernatants were discarded and 500 μl of PBS 1X were added to the pellet to prepare the BALF cytospin preparations by centrifugation of the slides at 1500 rpm for 5 min.

Quantification of BALF

Total cell number was counted and expressed as cells/ml of BALF, and differential cell counts were performed on May-Grünwald Giemsa (Sigma-Aldrich)-stained cytospins, counting a minimum of 300 cells per slide. Determination of differential cell counts was performed using standard morphology criteria.

Histopathological analyses and immunostaining

Fiji open source image processing software package v1.48r (http://fiji.sc) was used for the quantification of collagen (Sirius Red) and Vimentin, SMA, E-cadherin and Collagen I positive areas (percentage of
DAB), as well as to assess smooth muscle thickness (SMA) and epithelium length measurements. Quantifications in lung sections were performed in 4 different fields or bronchi, respectively in a random way.

Statistics

Statistical analyses were accomplished using SPSS Statistics Software v21 for Windows (IBM, Armonk, NY, USA). For all analyses, a p value < 0.05 was considered statistically significant. Results are shown as mean values ± standard error of the mean (SEM). For all analyses, a P value < 0.05 was considered statistically significant.