Study: Treatment of pulmonary fibrosis should focus on the telomeres of the cells that regenerate the lungs

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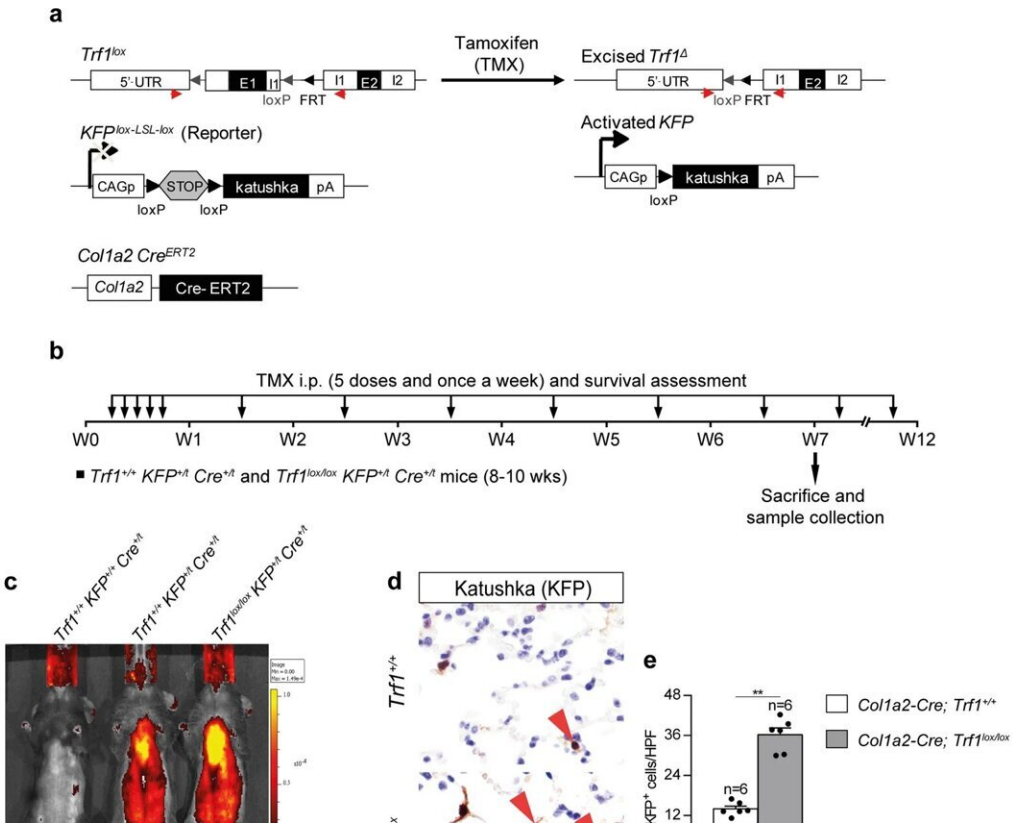

Efficient Trf1 deletion in lung fibroblasts upon tamoxifen administration. **a** Generation of the conditional knockout mouse model in which *Trf1* was deleted in fibroblasts using the Cre recombinase driven by the *Col1a2* promoter. $Trf1^{lox}$, $KFP^{Lox\text{-}LSL\text{-}Lox}$, and $Col1a2\text{-}Cre^{ERT2}$ alleles are depicted before and after Cre-mediated excision. **b** Tamoxifen (TMX) treatment, survival rate assessment and sample collection. Eight-to 10-week-old male $Trf1^{+/+}$ $KFP^{+/t}$ $Cre^{+/t}$ (*Col1a2-Cre;*

Trf1$^{+/+}$) and *Trf1*$^{lox/lox}$ *KFP*$^{+/t}$ *Cre*$^{+/t}$ (*Col1a2-Cre; Trf1*$^{lox/lox}$) mice were i.p. injected with TMX for five consecutive days during the first week and once a week until the sacrifice and sample collection on week (W) 7, and during the follow-up of survival until W12. **c** Representative images of fluorescence intensity of katushka fluorescent protein (KFP) in *Trf1*$^{+/+}$ *KFP*$^{+/+}$ *Cre*$^{+/t}$, *Trf1*$^{+/+}$ *KFP*$^{+/t}$ *Cre*$^{+/t}$ and *Trf1*$^{lox/lox}$ *KFP*$^{+/t}$ *Cre*$^{+/t}$ mice. Representative immunostainings for KFP (**d**), and quantification of KFP positive cells per 40X high-power field (HPF) (**e**) in lung sections from *Trf1*$^{+/+}$ *KFP*$^{+/t}$ *Cre*$^{+/t}$ and *Trf1*$^{lox/lox}$ *KFP*$^{+/t}$ *Cre*$^{+/t}$ mice. **f** Kaplan–Meier survival curves of *Col1a2-Cre; Trf1*$^{+/+}$ (*Trf1*$^{+/+}$, controls) and *Col1a2-Cre; Trf1*$^{\Delta/\Delta}$ (*Trf1*$^{\Delta/\Delta}$) mice upon TMX treatment. **g** Representative immunofluorescence stainings for COL1A2 (green) and TRF1 (red) (white arrowheads indicate COL1A2^{+} fibroblasts with deletion of TRF1), and immune-telomere-Q-FISH in COL1A2^{+} fibroblasts (Cy3Tel probe (red), COL1A2^{+} cells (green), and nuclei stained with DAPI (blue)) in lung sections from *Trf1*$^{+/+}$ and *Trf1*$^{\Delta/\Delta}$ mice. Quantification of the proportion of double COL1A2^{+}-TRF1^{+} fibroblasts (**h**) and mean telomere spot intensity (**i**) and average number of telomeres (**j**) in COL1A2^{+} cells from *Trf1*$^{+/+}$ and *Trf1*$^{\Delta/\Delta}$ mice. Data are expressed as mean ± SEM (the number of mice is indicated in each case). ***p*