Supplementary Tables S1 and S2: Clinical features and pathological variables of patients with chronic obstructive pulmonary disease and control smokers. Table 1 describes all patients and controls from whom lung samples were obtained. Cells were collected from a subset of 14 patients and 16 controls, whose characteristics are described in Table 2.

Tables S1

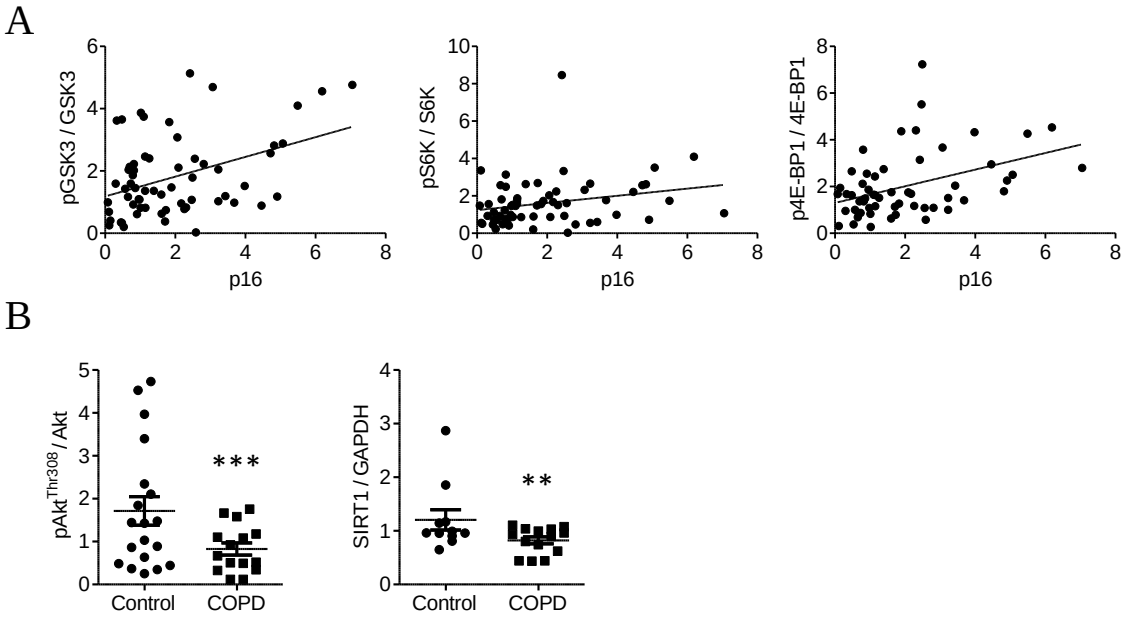
	Control subjects	Patients with COPD	
N	30	30	*P* value
Gold Stage 1	-	14	
Gold Stage 2	-	16	
females/males	14/16	7/23	
Age, yr	64.06 ± 2,20	60,59 ± 1.27	*0,152*
FEV %	94.06 ± 2.53	**78.22 ± 3,12 *** **	*0,000*
FVC%	96,12 ± 2,72	90,06 ± 4.95	*0,284*
FEV /FVC, %	81.69 ± 1,67	**66.19 ± 0,97 *** **	*0,000*
Pack-years	26,94 ± 3,90	**40,70 ± 4.91***	*0,031*
BMI	25,36 ± 0,76	24,60 ± 0,78	*0,643*

Tables S2

	Control subjects	Patients with COPD	
N	16	13	*P* value
females/males	10/6	6/7	
Age, yr	70,65 ±1,95	65,46 ±2,10	*0,079*
FEV %	92,79 ±4,00	**77,75 ± 4,79***	*0,010*
FVC%	100,57 ±6,74	101,00 ±13,57	*0,975*
FEV /FVC, %	78,50 ±2,93	**65,75 ±1,68****	*0,005*
BMI	25,47± 1,44	25,10±1,24	*0,842*

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FEV_1%, percentage of the predicted FEV_1 value; FVC, forced vital capacity; FVC%, percentage of the predicted FVC value.

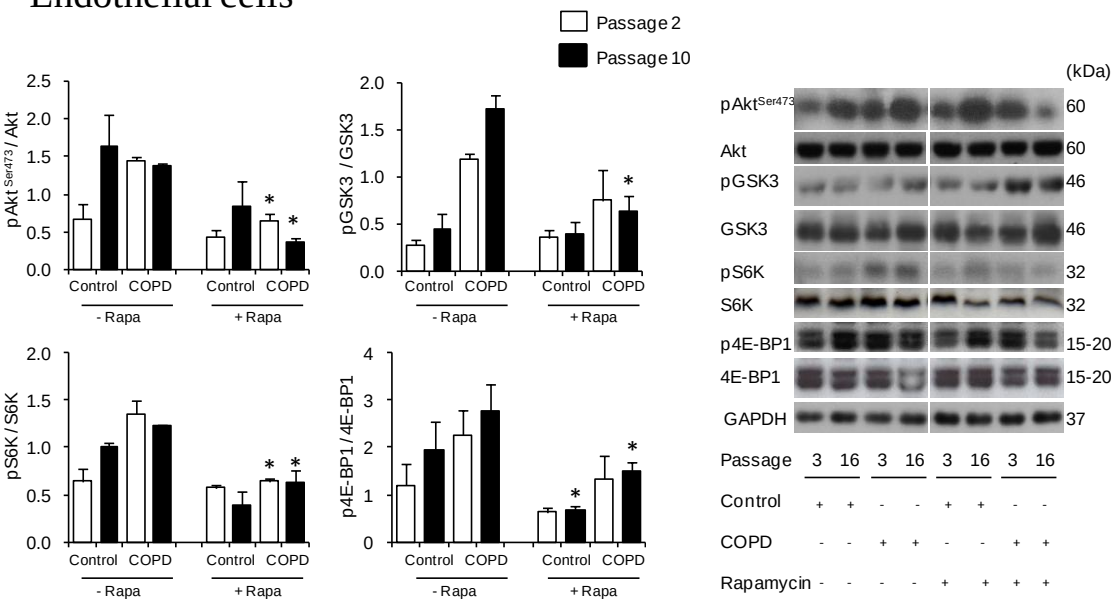
Supplementary Figure S1



Supplementary Figure S1:

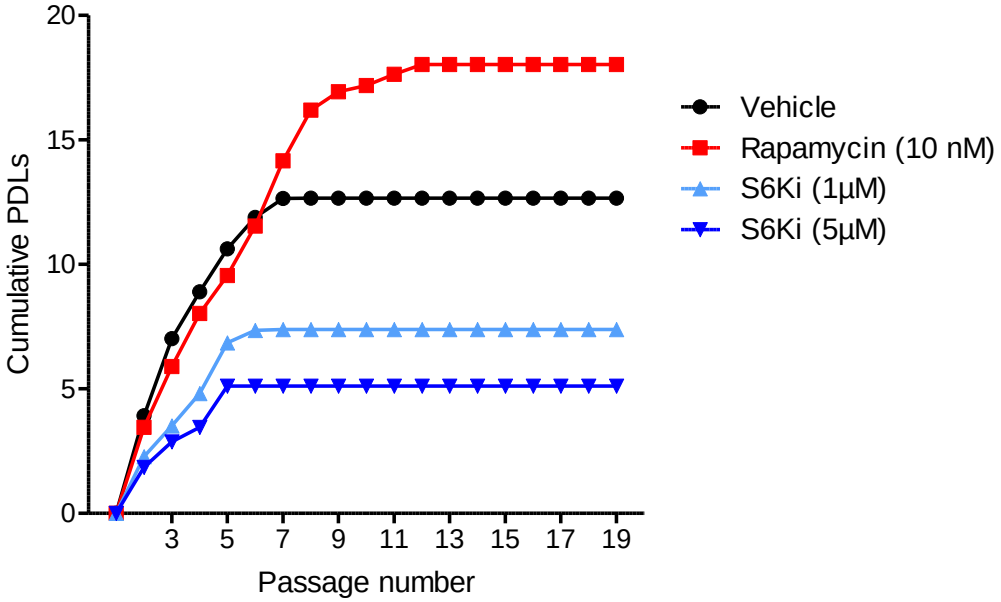
Analysis of lung samples from patients with COPD and controls. (A) Correlations between p16 protein levels and pGSK3 (spearman r=0.34, *P*<0.01), pS6K (r=0.26, *P*<0.05) and p4EBP1 (r=0.39, *P*<0.01) in patients with COPD and controls. (B) Graph of protein levels of phosphorylated Akt-Thr308 and SIRT1 in lungs from patients with COPD and controls. Values are means±SEM. ***P*<0.01 vs. controls.

Supplementary Figure S2



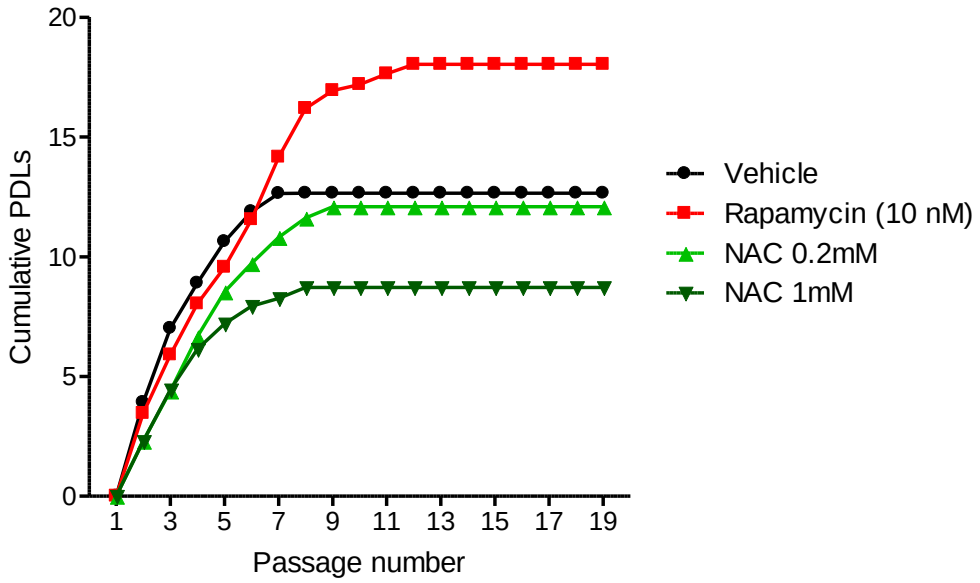
Supplementary Figure S2:
Graph of protein levels of phosphorylated Akt-Ser473, GSK3, S6K, and 4E-BP1 in cultured endothelial cells from 8 patients with COPD and 8 controls at passages 3 and 16, during vehicle or rapamycin treatment. Values are means±SEM. **P*<0.05 compared to vehicle-treated cells at the corresponding cell passage (analysis by 2-way ANOVA with Bonferroni posthoc test). These measurements were performed in cells deprived of serum for 24 hours.

Supplementary Figure S3



Supplementary Figure S3: Effect of treatment with the S6K1 inhibitor (PF478671) or with rapamycin on pulmonary vascular endothelial cells from patients with COPD and controls. Proliferating cultured cells were counted at each passage, and the population doubling level (PDL) was calculated for patients with COPD and controls. Mean values are represented at each passage. Values are from a representative experiment repeated 3 times. These measurements were performed in cells deprived of serum for 24 hours.

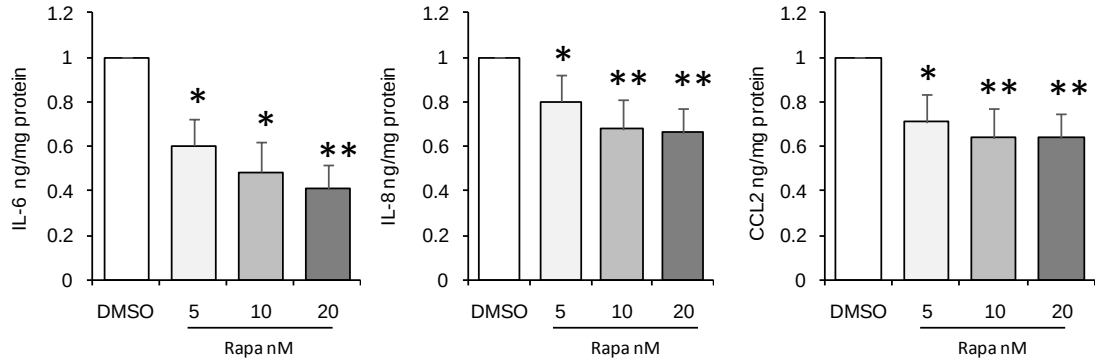
Supplementary Figure S4



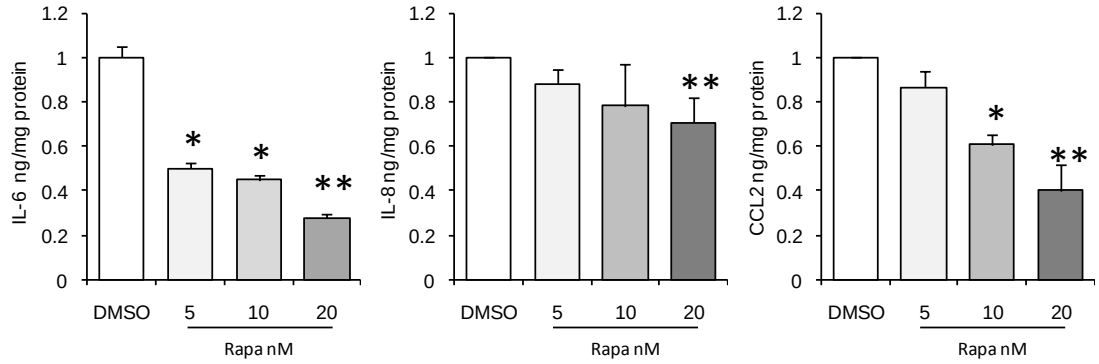
Supplementary Figure S4: Effect of treatment with 0.2mM and 1mM N-acetyl cysteine (NAC) on pulmonary vascular endothelial cells from patients with COPD and controls. Proliferating cultured cells were counted at each passage, and the population doubling level (PDL) was calculated for patients with COPD and controls. Mean values are represented for each passage. Values are from a representative experiment repeated 3 times. These measurements were performed in cells deprived of serum for 24 hours.

Supplementary Figure S5

A. Smooth Muscle Cells



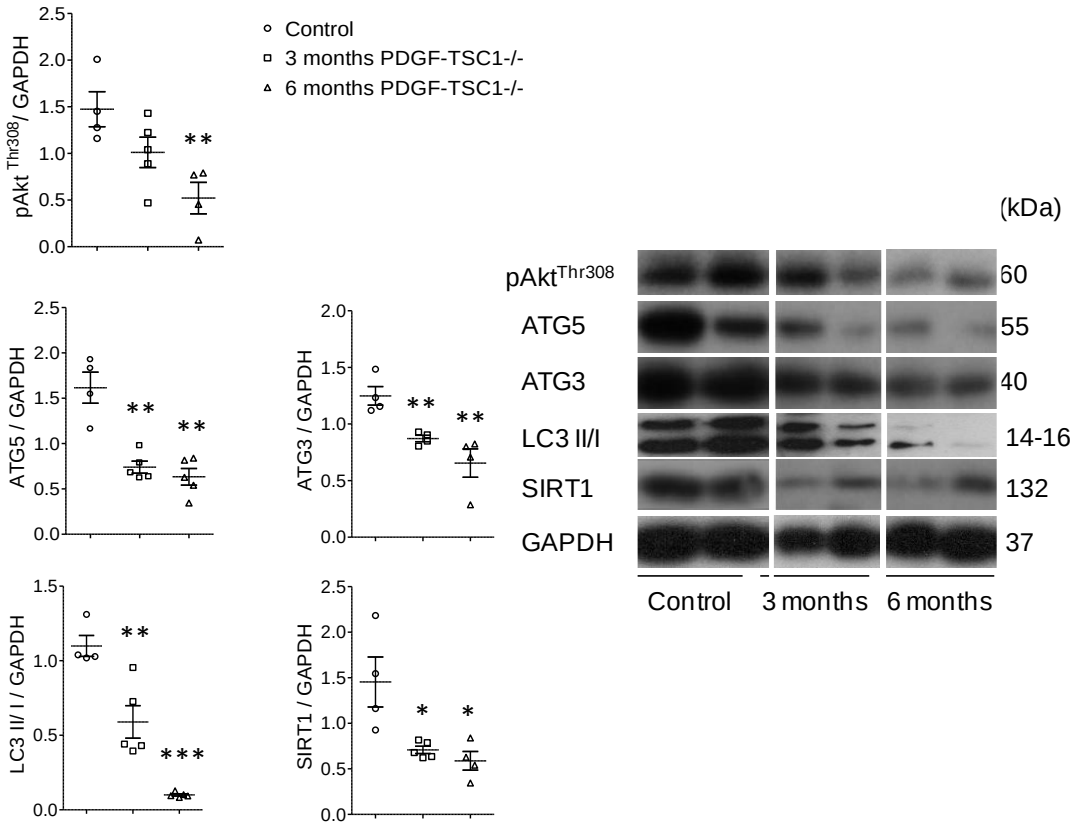
B. Endothelial Cells



Supplementary Figure S5: Effect of rapamycin treatment on the levels of proinflammatory cytokines (IL6, IL8, and CCL2) measured in conditioned media of PA-SMCs **(A)** from 7 patients with COPD and 8 controls and P-ECs **(B)** from 8 patients with COPD and 8 controls. Rapamycin was added to PA-SMC or P-EC cultures containing over 50% of β–gal-positive cells during 48 hours in doses ranging from 5 to 20 nM. **P*<0.05 and ***P*<0.01 versus vehicle- treated cells. These measurements were performed in cells deprived of serum for 24 hours (P-ECs) or for 48 hours (PA-SMCs).

Supplementary Figure S6

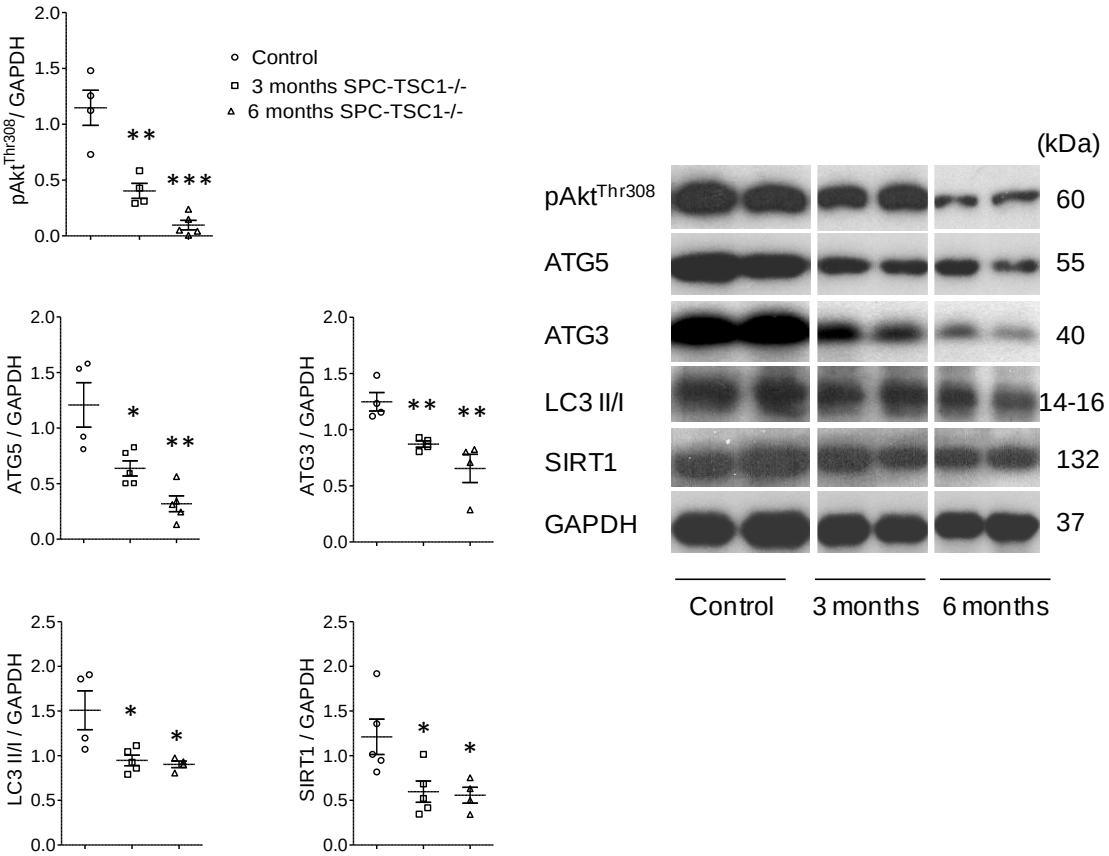
A



Supplementary Figure S6: Effects of mTOR overactivation in SPC-TSC1-/- and PDGF-TSC1-/- mice. TSC1 deletion was induced by intraperitoneal tamoxifen in PDGF-TSC1-/- mice and by treatment with doxycycline in drinking water in SPC-TSC1-/- mice. The mice were investigated 3 and 6 months later, and compared with vehicle-treated mice **(A)** Lung levels of pAkt-Thr308, ATG5, ATG3, LC3 II/I and SIRT1 proteins in PDGF-TSC1-/- mice 3 and 6 months after starting tamoxifen treatment, compared with vehicle-treated control mice. **(B)** Similar representations for SPC-TSC1-/- mice 3 and 6 months after starting doxycycline treatment.

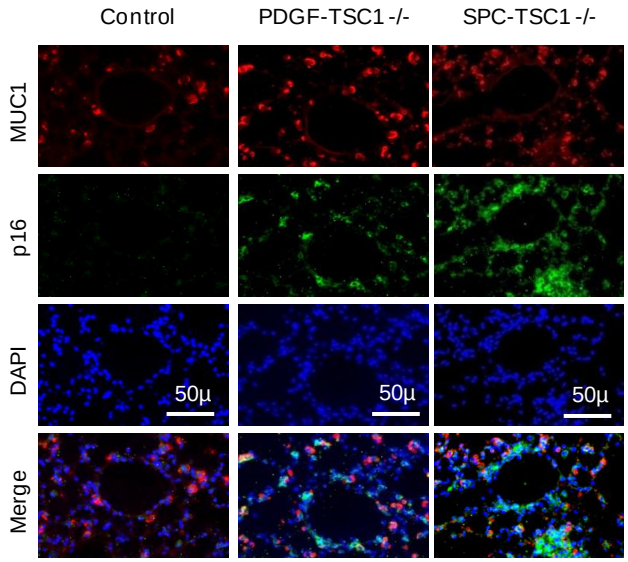
Supplementary Figure S6

B



Supplementary Figure S6: **(B)** Lung levels of pAkt-Thr308, ATG5, ATG3, LC3 II/I and SIRT1 proteins in SPC-TSC1-/- mice 3 and 6 months after starting doxycycline treatment, compared with vehicle-treated control mice.

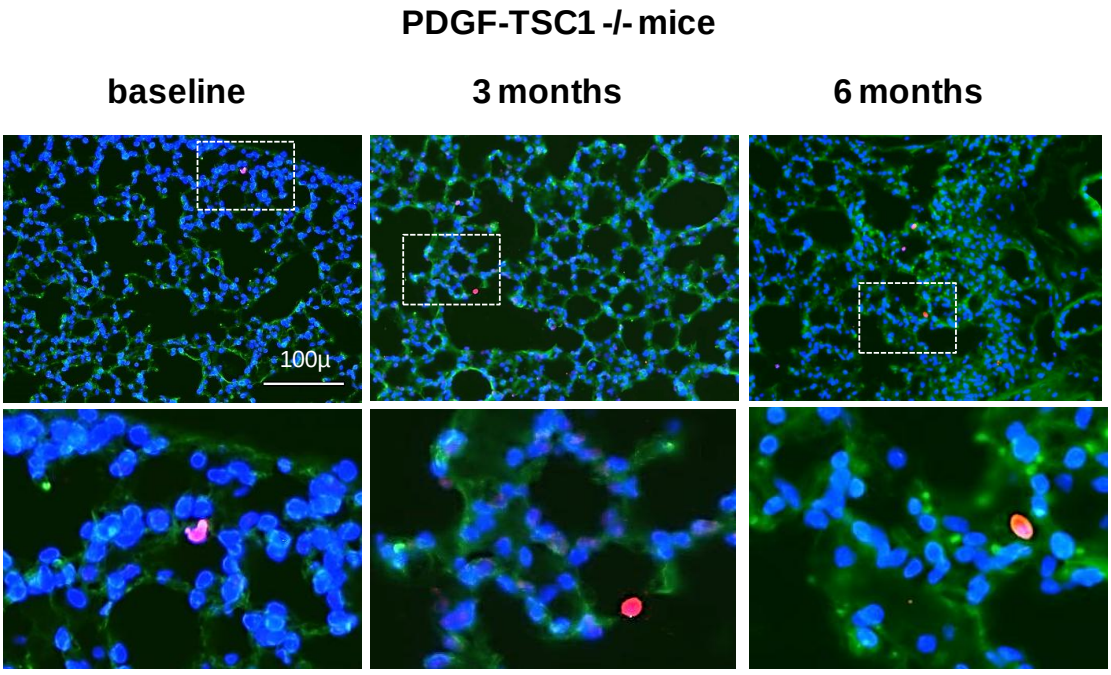
Supplementary Figure S7



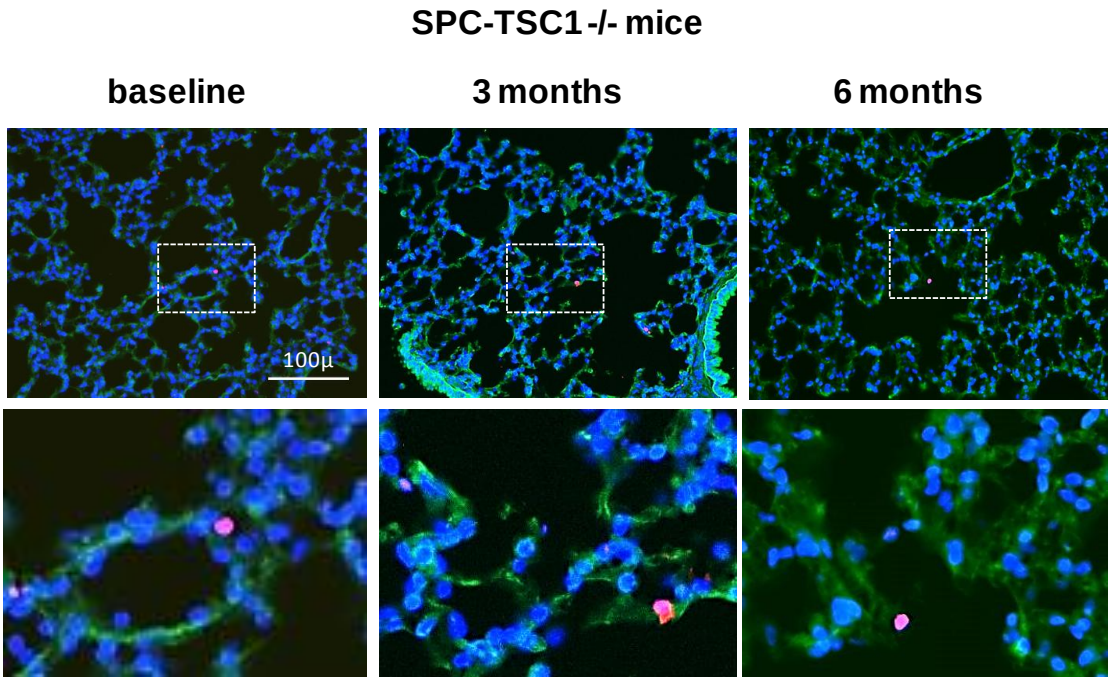
Supplementary Figure S7: Representative photographs of lung sections showing p16-positive cells and MUC1-stained cells in PDGF-TSC1-/- and SPC-TSC1-/- mice. Scale bars: 50µm.

Supplementary Figure S8

A



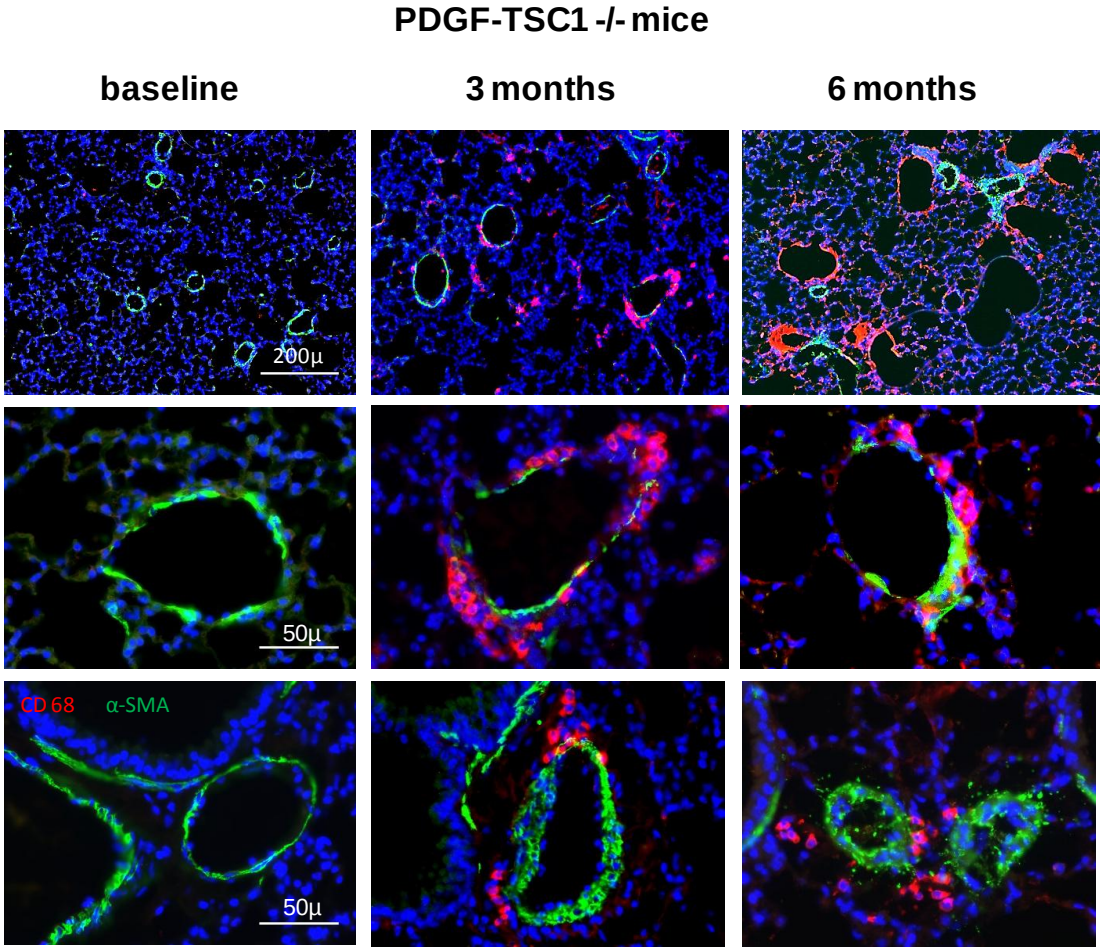
B

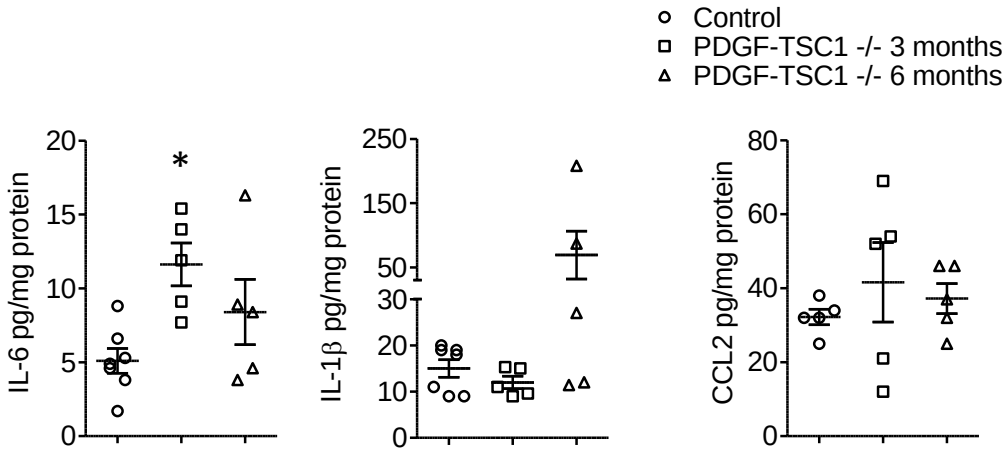


Supplementary Figure S8: Effects of mTOR overactivation in PDGF-TSC1-/- mice (A) and SPC-TSC1-/- mice (B) on apoptosis induction in lung tissue. Cell death (double DNA fragmentation) was detected *in situ* by terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL). Cells with positive TUNEL staining (red) were considered apoptotic. Nuclei were identified with DAPI (blue).

Supplementary Figure S9

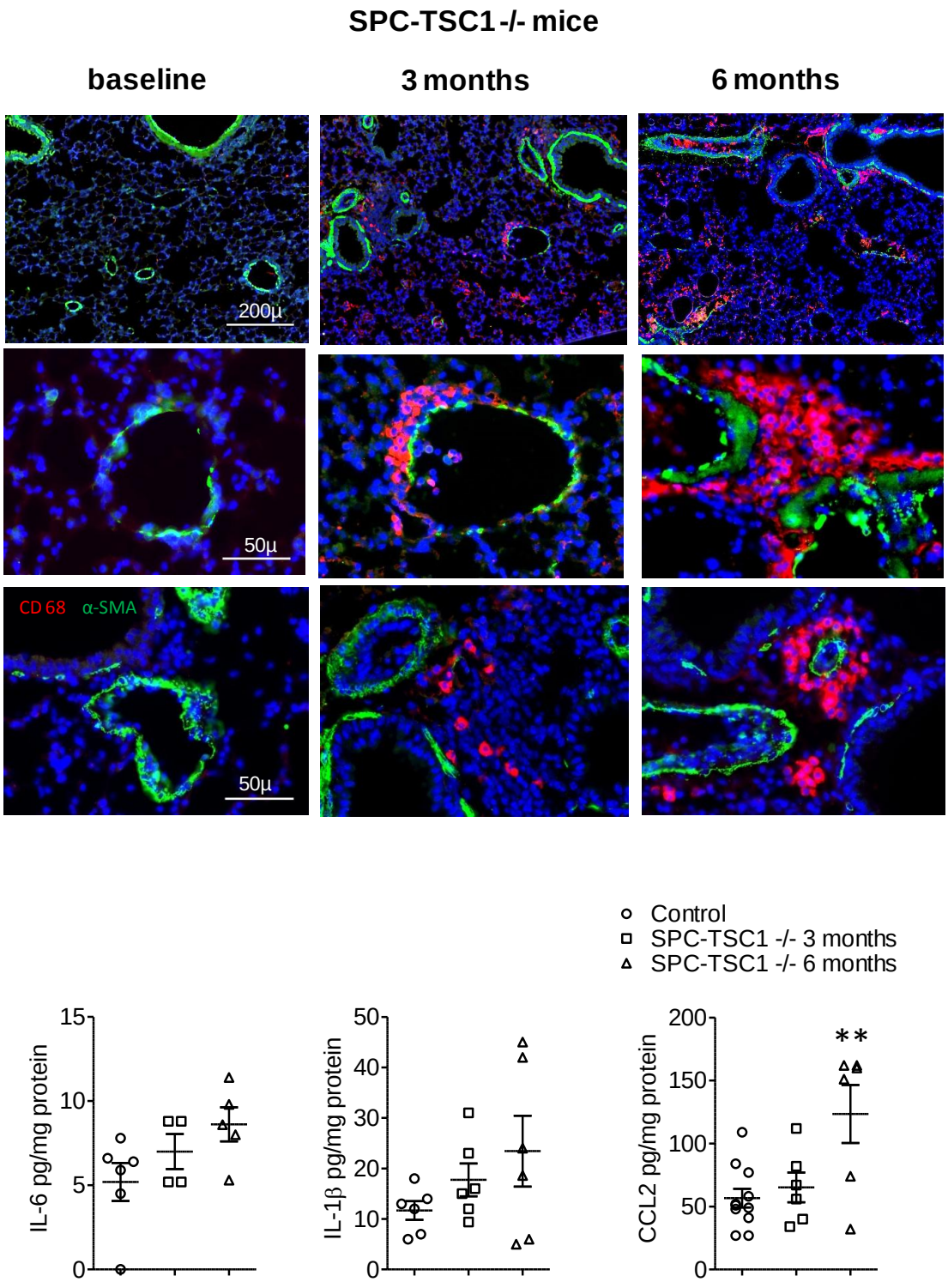
A





Supplementary Figure S9

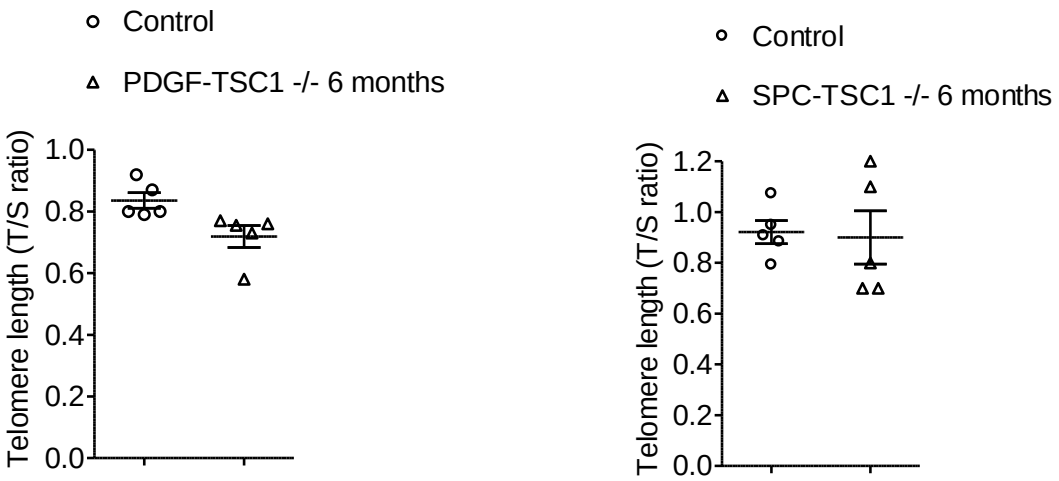
B



Supplementary Figure S9: Effects of mTOR overactivation in PDGF-TSC1-/- mice (A) and SPC-TSC1-/- mice (B) on inflammation. Upper panel: Representative micrographs showing macrophages infiltration in lung tissue at baseline, 3- and 6 months after TSC-1 deletion. Macrophages were identified by CD68 staining (red); smooth muscle cells were labelled with alpha-smooth muscle actin (α-SMA) (green); nuclei were labelled with DAPI (blue). Lower

panel: Lung levels of IL6, IL-1β and CCL2 proteins in PDGF-TSC1-/- and SPC-TSC1-/- mice studied 3 and 6 months after the induction of TSC1 deletion, compared with vehicle-treated control mice.

Supplementary Figure S10



Supplementary Figure S10: Telomere length measured in PDGF-TSC1-/- mice and SPC-TSC1-/- mice 6 months after TSC-1 deletion, compared with vehicle-treated control mice.