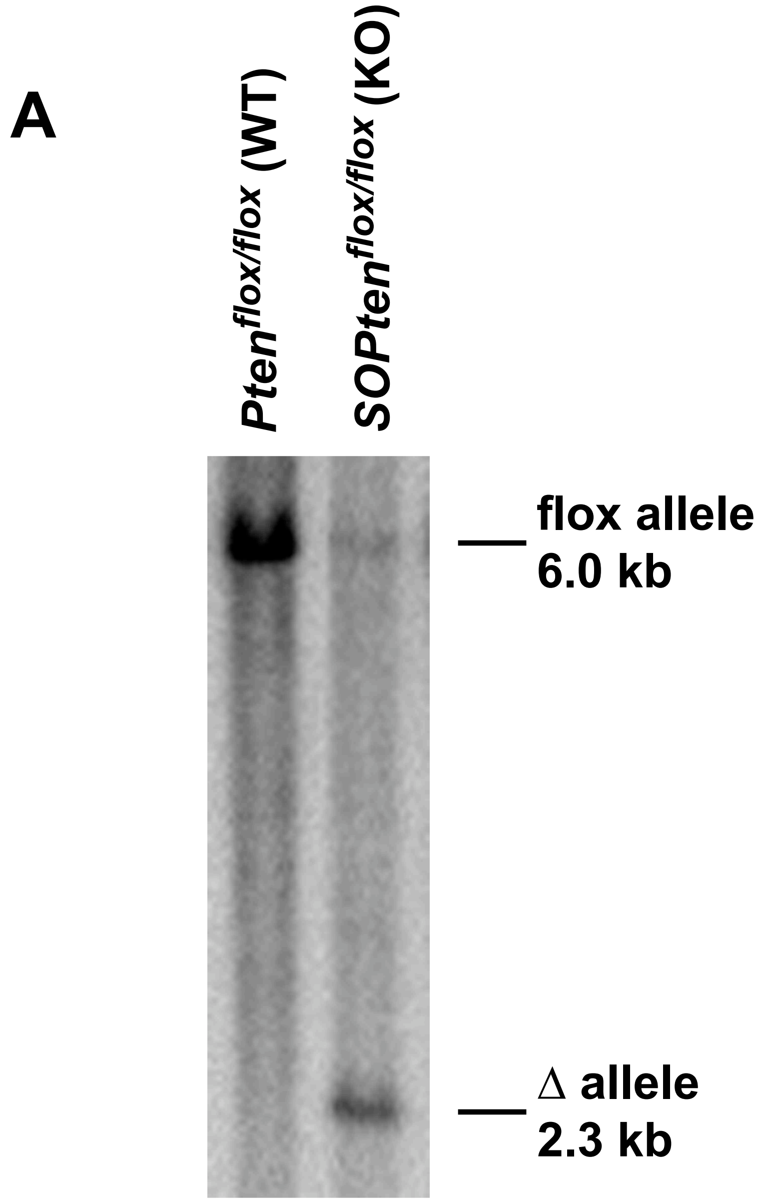
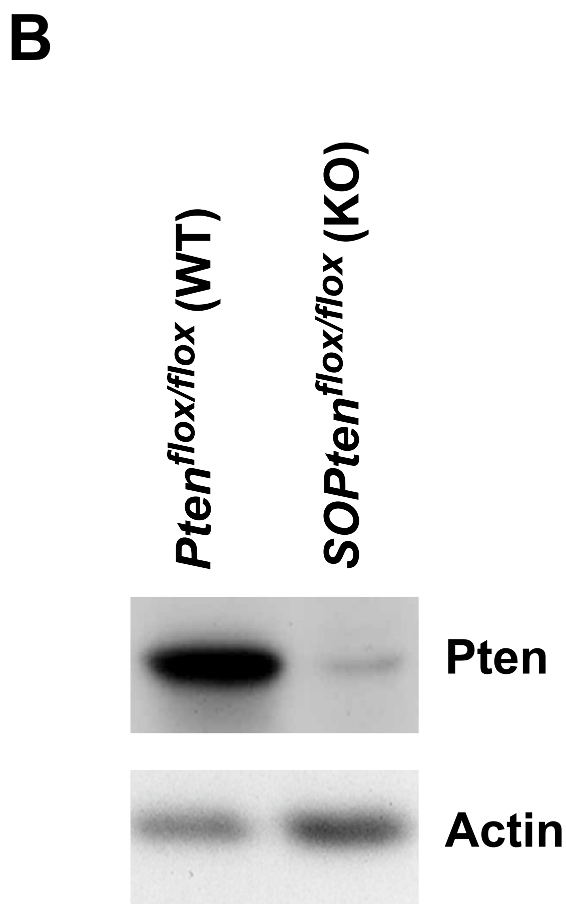
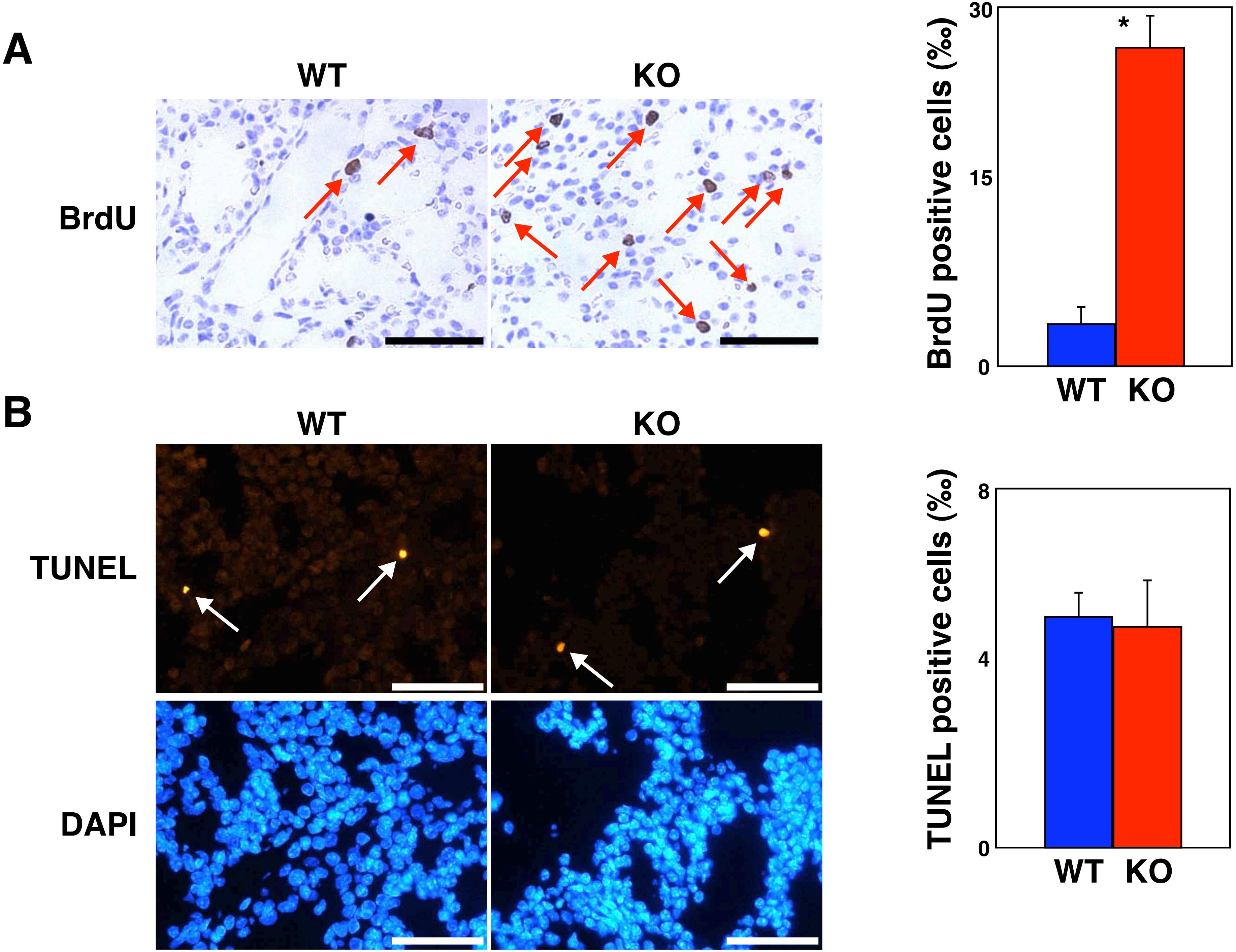
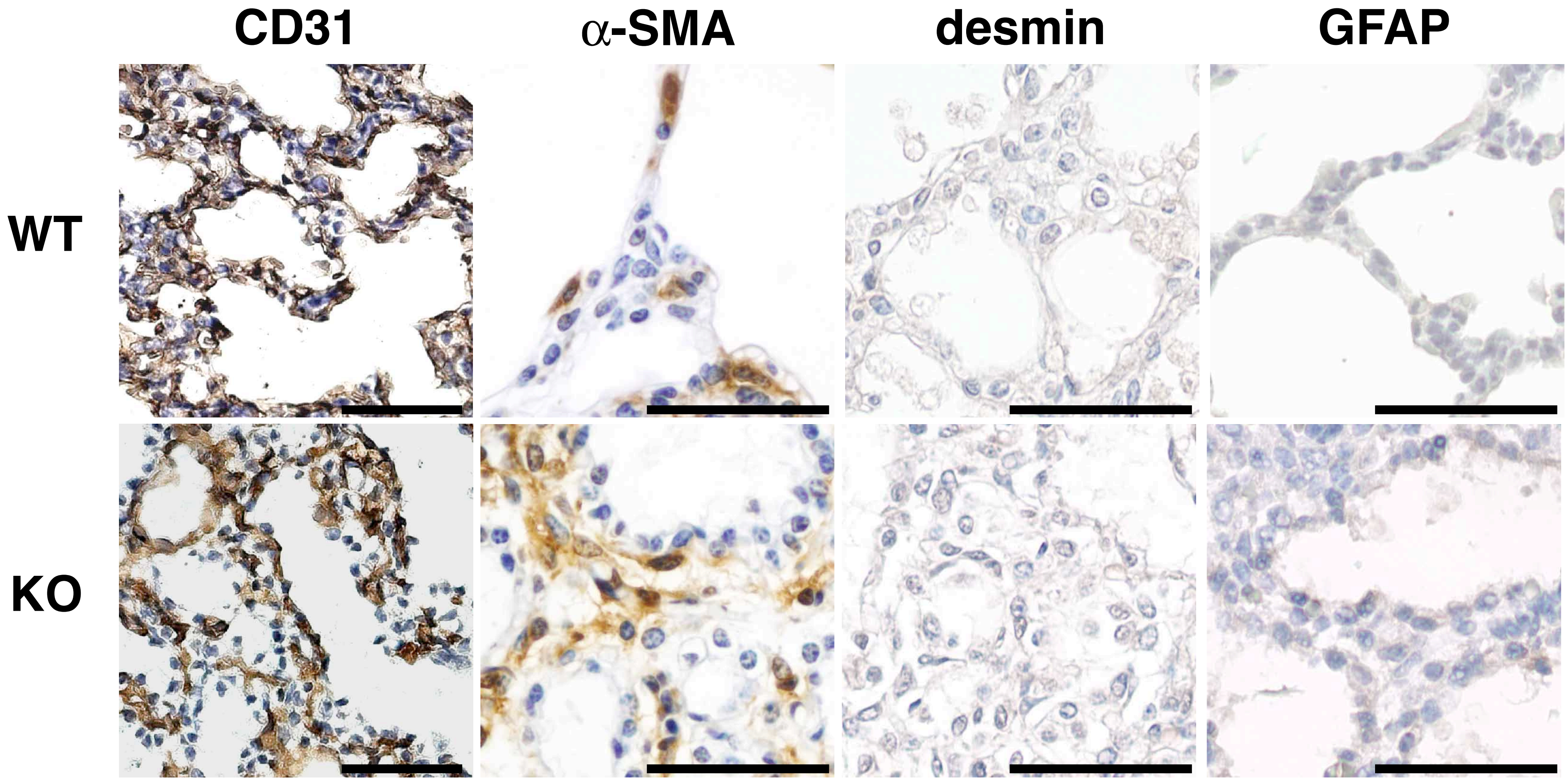




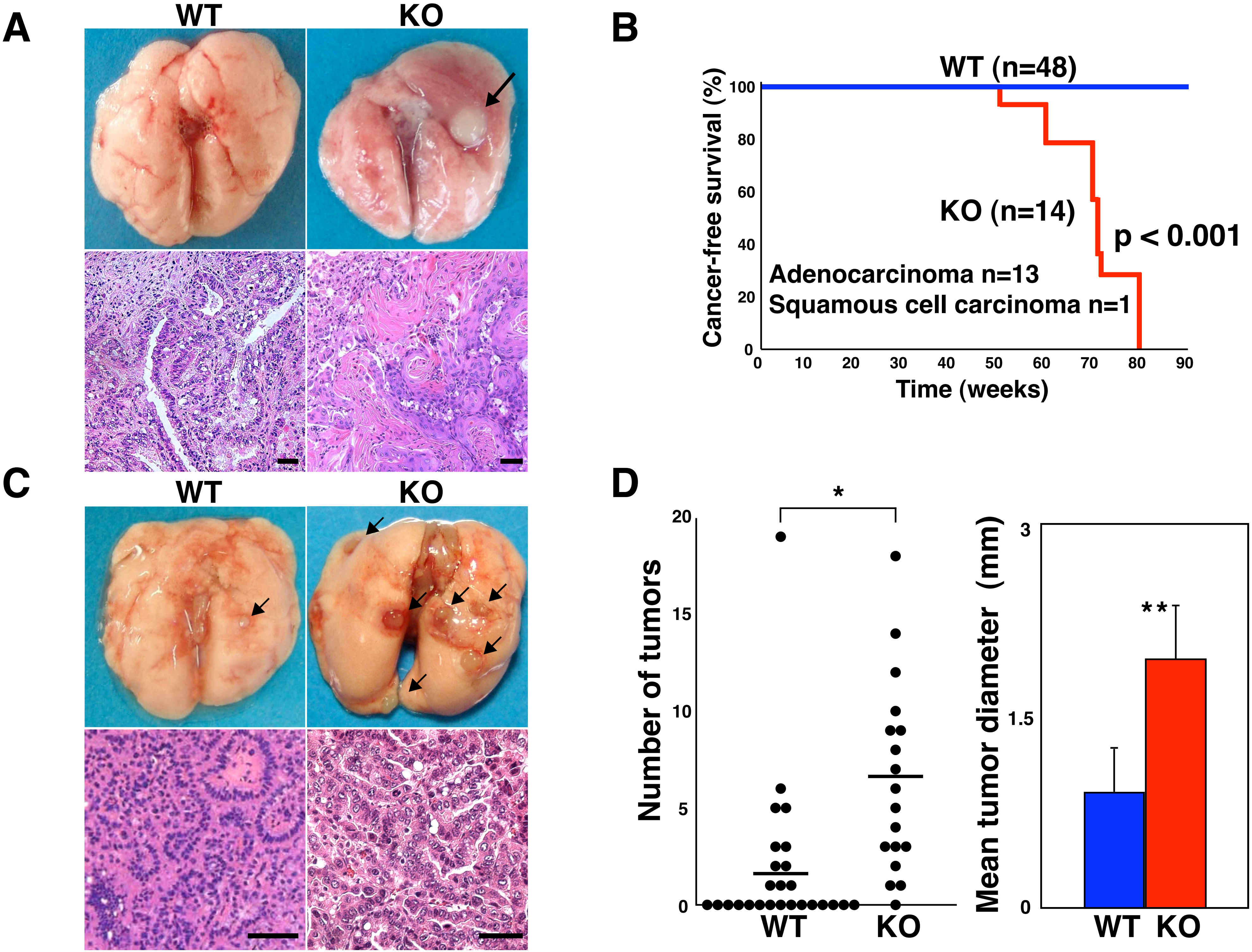
Supplementary Figure S1. Yanagi, et al.



Supplementary Figure S2. Yanagi, et al.



Supplementary Figure S3. Yanagi, et al.



Supplementary Figure S4. Yanagi, et al.

Supplementary Figure Legends

Supplementary Figure S1**. **Generation of SP-C-rtTA/(tetO)$_7$-Cre/Pten$^{flox/flox}$ (SOPten$^{flox/flox}$) mice. (A) Genomic Southern blot. DNA (20 μg) extracted from lungs of *Pten*$^{flox/flox}$(E10-16) (WT) and *SOPten*$^{flox/flox}$(E10-16) (KO) mice was digested with *Hind III* and hybridized to a previously described probe (24). Lungs from the majority of KO mice showed deletion of the *Pten* gene. The floxed (flox allele) and deleted (Δ allele) *Pten* alleles are shown. (B) Western blot analysis of Pten protein in lungs of 8-week-old WT(E10-16) and *SOPten*$^{flox/flox}$(E10-16) (KO) mice. Actin, loading control.

Supplementary Figure S2. Increased cell proliferation in SOPten$^{flox/flox}$(E10-16) lung

(A) Enhanced proliferation. Left panel: BrdU-positive lung cells (arrows) in lungs of E19.5 WT(E10-16) and *SOPten*$^{flox/flox}$(E10-16) (KO) mice were counted 2 hr after BrdU administration to their dams. Bars, 50 μm. Right panel: data were expressed as the mean number of BrdU-positive lung cells ± SD/1×10^3 cells per mouse in 4 mice/group. *, p<0.001, Student's t-test. (B) No effect on apoptosis. Upper left panels: TUNEL positive lung cells (arrows). Lower left panels: control DAPI staining. Bars, 50 μm. Right panel: data were expressed as the mean number of TUNEL-positive lung cells ± SD/1×10^3 cells/mouse in 4 mice/group.

Supplementary Figure S3. Increase in number of myofibroblast precursors in the alveolar septa of SOPten$^{flox/flox}$(E10-16) mice

Immunohistochemical analyses of α-SMA, a differentiation marker for smooth muscle cells and myofibroblasts, and desmin, a main component of the intermediate filaments of muscle cells, showed an increased number of α-SMA^{+} desmin^{-} cells in the septa of *SOPten*$^{flox/flox}$(E10-16) (KO) lungs. These cells are negative for GFAP, a marker protein for glial cells such as astrocytes. No obvious differences in the intensity or patterns of CD31 expression, a marker protein for vascular endothelial cells, were seen between WT(E10-16) and KO(E10-16) mice. Bars, 50 μm.

Supplementary Figure S4. Spontaneous lung adenocarcinomas and urethane-induced lung tumors in SOPten$^{flox/flox}$(E10-16) mice

(A) Gross and histological analyses of spontaneous tumors. Gross appearance of a lung in a 68-week-old WT(E10-16) mouse and representative lung carcinoma (arrow) in a lung of a 68-week-old *SOPten*$^{flox/flox}$(E10-16) (KO) mouse (upper panels). Lower left panel: spontaneous lung adenocarcinoma in a lung of a 72-week-old KO(E10-16) mouse, representative of tumors observed in 13/14 KO mice. Lower right panel: squamous cell carcinoma (1/14) in the lung of a 76-week-old KO(E10-16) mouse. Bars, 50 μm. (B) Kaplan-Meier curves showing cancer-free survival of untreated WT(E10-16) (n=48) and KO(E10-16) (n=14) mice. The statistical difference in survival was determined by the log-rank test. (C) Gross and histological analyses of urethane-induced tumors. WT(E10-16) (n=26) and KO(E10-16) mice (n=18) were injected i.p. with urethane. After 20 weeks, tumors were counted and their diameters measured from the lung surface. Upper panels: gross appearance of urethane-induced lung tumors (arrows) in WT(E10-16) and KO(E10-16) mice. Lower left panel: histology of a lung adenoma representative of those observed in both WT(E10-16) and KO(E10-16) mice. Lower right panel: histology of the one lung adenocarcinoma occurring in a KO(E10-16) mouse. Bars, 50 μm. (D) Numbers (left panel) and mean size (right panel) of urethane-induced tumors in WT(E10-16) and KO(E10-16) mice. The horizontal bars (left panel) represent the mean tumor numbers in the WT and KO groups. The right panel shows the mean diameter ± SD of tumors in the WT and KO groups. *, $p<0.005$, **, $p<0.001$, Welch's *t*-test.

Supplementary Table S1 Primers for PCR and RT-PCR experiments

PCR		
SP-C-rtTA	sense	5'-AAAATCTTGCCAGCTTTCCCC-3'
	antisense	5'-GACACATATAAGACCCTGGTCA-3'
$(tetO)_7$-Cre	sense	5'-TGCCACGACCAAGTGACAGCAATG-3'
	antisense	5'-AGAGACGGAAATCCATCGCTCG-3'
Pten wt and flox allele	sense	5'-CTCCCACCAATGAACAAACAGTC-3'
	antisense	5'-GTGAAAGTGCCCCAACATAAGG-3'
K-ras, exon 1	sense	5'-ATGACTGAGTATAAACTTGT-3'
	antisense	5'-CTCTATCGTAGGGTCGTACT-3'
K-ras, exon 2	sense	5'-ACTCCTACAGGAAACAAGT-3'
	antisense	5'-CTATAATGGTGAATATCTTC-3'
K-ras, exon 1, sequence		5'-CTCTATCGTAGGGTCGTACT-3'
K-ras, exon 2, sequence		5'-CTATAATGGTGAATATCTTC-3'
RT-PCR		
Spry2	sense	5'-TGTGAGGACTGTGGCAAGTGC-3'
	antisense	5'-TTTAAGGCAACCCTTGCTGG-3'
Shh	sense	5'-GAAGATCACAAGAAACTCCGAACG-3'
	antisense	5'-TGGATTCATAGTAGACCCAGTCGAA-3'
FGF7	sense	5'-CAAACGGCTACGAGTGTGAA-3'
	antisense	5'-TCCGCTGTGTGTCCATTTAG-3'
FGF10	sense	5'-GAGAAGAACGGCAAGGTCAG-3'
	antisense	5'-GGAGGAAGTGAGCAGAGGTG-3'
FGFR2	sense	5'-AAATACCAAATCTCCCAACC-3'
	antisense	5'-GCCGCTTCTCCATCTTCT-3'
BMP4	sense	5'-AGGAGGAGGAGGAAGAGCAG-3'
	antisense	5'-TGTGATGAGGTGTCCAGGAA-3'
Wnt7b	sense	5'-CCCACATGACAGATGGACAG-3'
	antisense	5'-GGACCCTGAAGCAGGTATCA-3'
Nkx2.1	sense	5'-AACCTGGGCAACATGAGCGAGCTG-3'
	antisense	5'-ATCTTGACCTGCGTGGGTGTCAGG-3'
GATA6	sense	5'-CTTCTAATTCAGATGACTGC-3'
	antisense	5'-GCACGGAGGATGTGACTTC-3'
Nfib	sense	5'-CGAACATGGCACGAAAGAGA-3'
	antisense	5'-TGGACTGGATGGGTCATAAG-3'
P300	sense	5'-GTGTCTCCACATGGTGCTTG-3'
	antisense	5'-GTGTCTCCACATGGTGCTTG-3'
CBP	sense	5'-CAGAGCGGATCATCCATGACTA-3'
	antisense	5'-GCTTCTCCATGGTGGCATAC-3'
Tcf21	sense	5'-AGCTACATCGCCCACTTGAG-3'
	antisense	5'-CGGTCACCACTTCTTTCAGG-3'
Cutl1	sense	5'-GGACGTAATGTCCGAAGATGC-3'
	antisense	5'-CTCAGAGGGCGAGGAAATTG-3'
Hoxa5	sense	5'-GTGCCAATGTTGTGTGTT-3'
	antisense	5'-CATACATCACTGAACTGCGC-3'

Foxa1	sense	5'-GAAAGGCTAGCCAGCTAGAGG-3'
	antisense	5'-AGATGCAGCTGAGATTCGTG-3'
Foxa2	sense	5'-CCCATTCCAGCGCTTCTC-3'
	antisense	5'-GTAATGGTGCTCGGGCTTC-3'
GR	sense	5'-CAAAGCCGTTTCACTGTCC-3'
	antisense	5'-ACAATTTCACACTGCCACC-3'
Adam17	sense	5'-GCGGCGTCTCCTCATCCT-3'
	antisense	5'-TTATATTCTGCCCCATCTGTGTTG-3'
HIF2α	sense	5'-GGCGACAATGACAGCTGACAAGGAG-3'
	antisense	5'-GAGTGAAGTCAAAGATGCTGTGTC-3'
eNOS	sense	5'-AAGACAAGGCAGCGGTGGAA-3'
	antisense	5'-GCAGGGGACAGGAAATAGTT-3'
GAPDH	sense	5'-TGATGGGTGTGAACCACGAG-3'
	antisense	5'-GTCATTGAGAGCAATGCCAG-3'