Figure S1

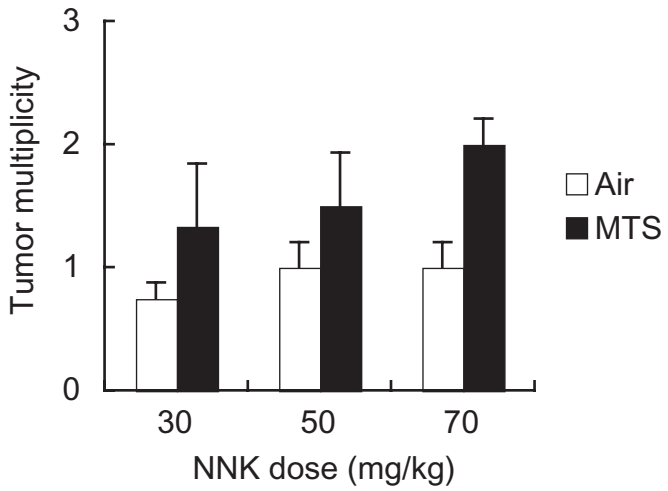

Figure S1. Lung tumor multiplicity in male A/J mice given the indicated doses of NNK and 2 cig./day MTS exposure for 2 months followed by 1 cig./day for 3 months

Tumor multiplicity was determined by serial sectioning at 350 mm intervals after a final 4 months recovery period. Results are means ± S.E. (n = 4 for each group).

Figure S2

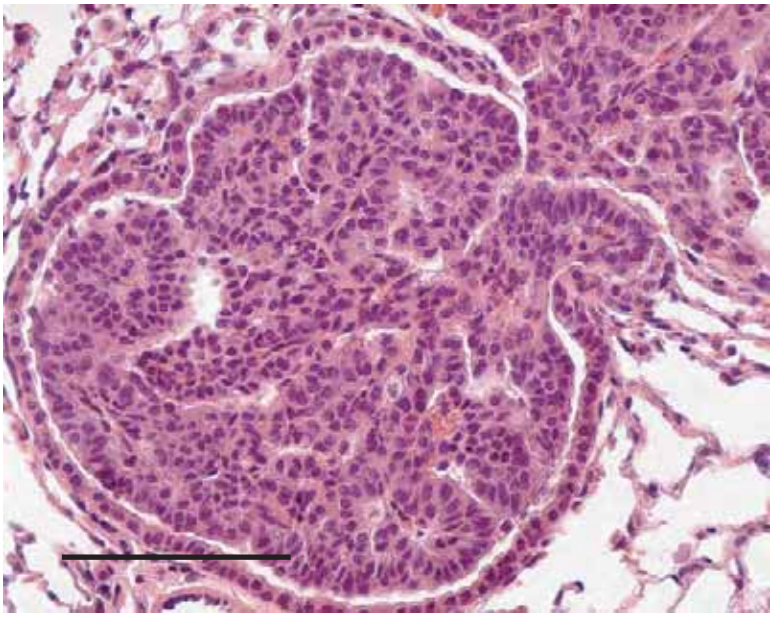

Figure S2. Histological appearance of adenocarcinoma in NNK+MTS-treated mice
An adenocarcinoma with bronchial invasion found in NNK+MTS-treated A/J mouse. Scale bar = 100 μm.

Figure S3

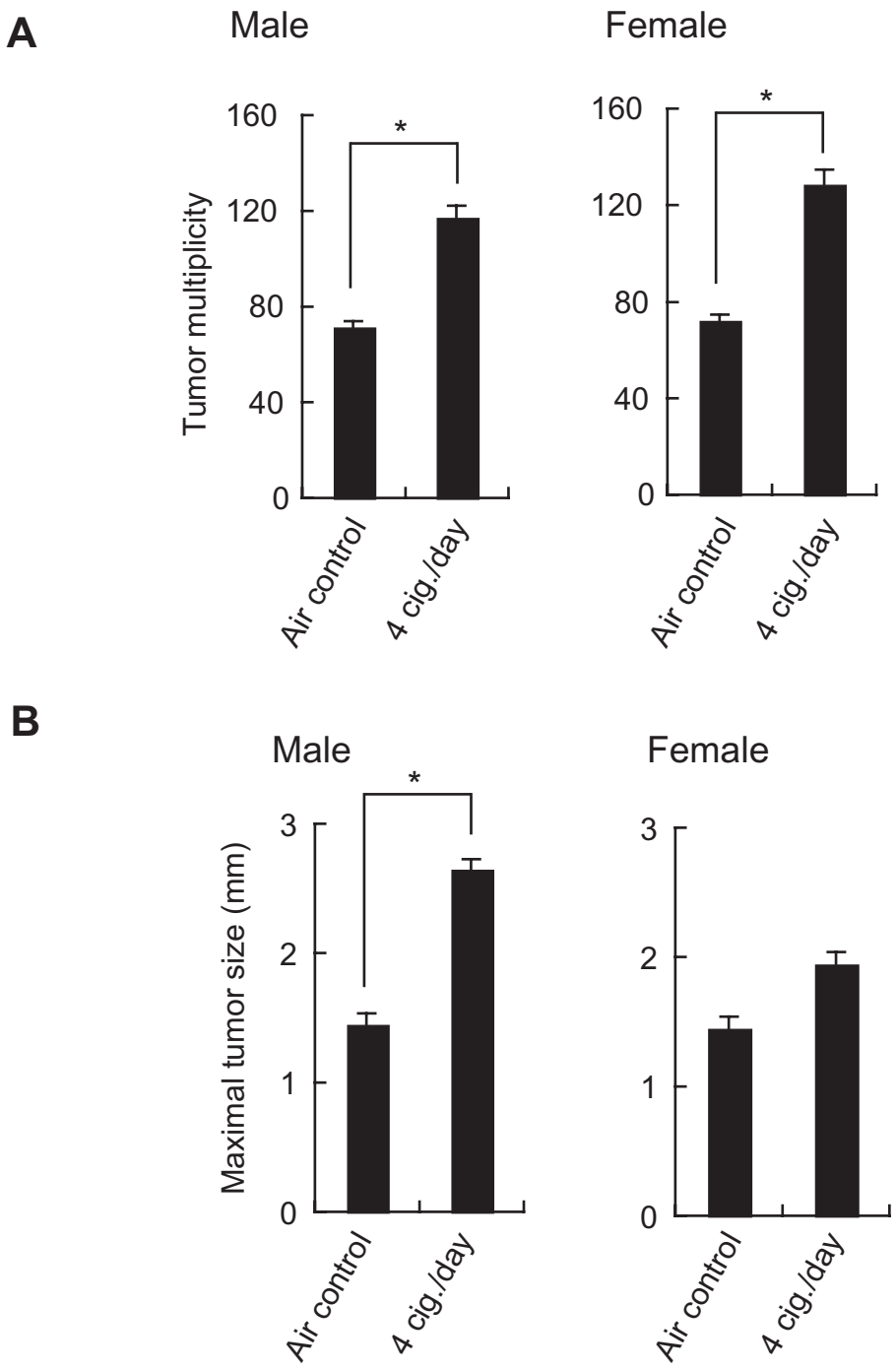

Figure S3. MTS exposure promotes lung tumor development in male and female K-ras^{LA2} mice

Lung tumor multiplicity (A) and maximal tumor sizes (B) were determined by serial sectioning. Results are means ± S.E. (male air control: n = 8; male 4 cig./day: n = 10; female air control: n = 8; female 4 cig./day: n = 8). Significant difference, *P < 0.02.

Figure S4

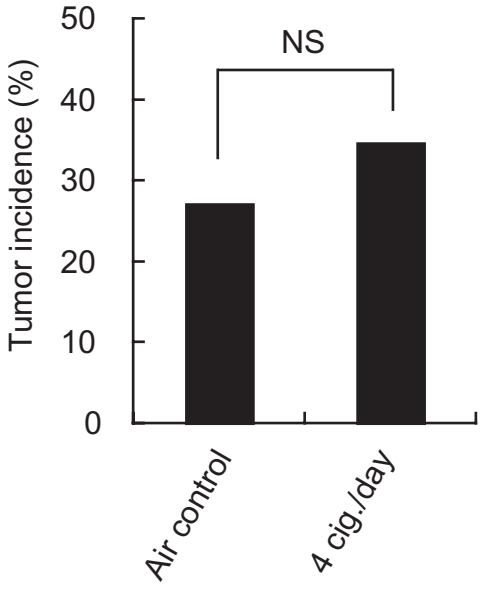

Figure S4. Thymic lymphoma incidence in air- or MTS-exposed K-ras^{LA2} mice
Sex-matched K-ras^{LA2} mice were exposed to MTS as in Fig. 2A and analyzed at 5 months of age for thymic lymphoma (air control: n = 22; 4 cig./day: n = 23). NS, insignificant difference.

Figure S5

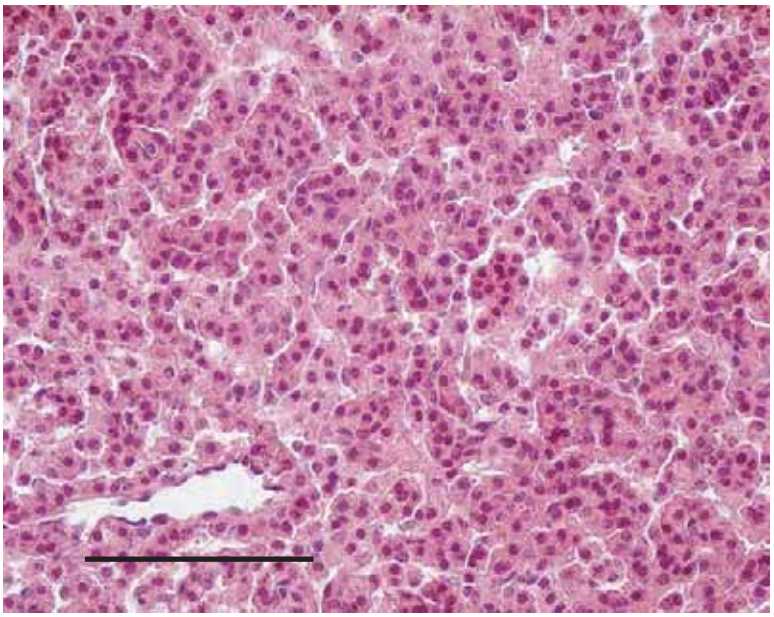

Figure S5. Histological appearance of alveolar adenoma in MTS-exposed K-ras^{LA2} mice
Alveolar adenoma found in MTS-exposed K-ras^{LA2} mouse. Scale bar = 100 μm.

Figure S6

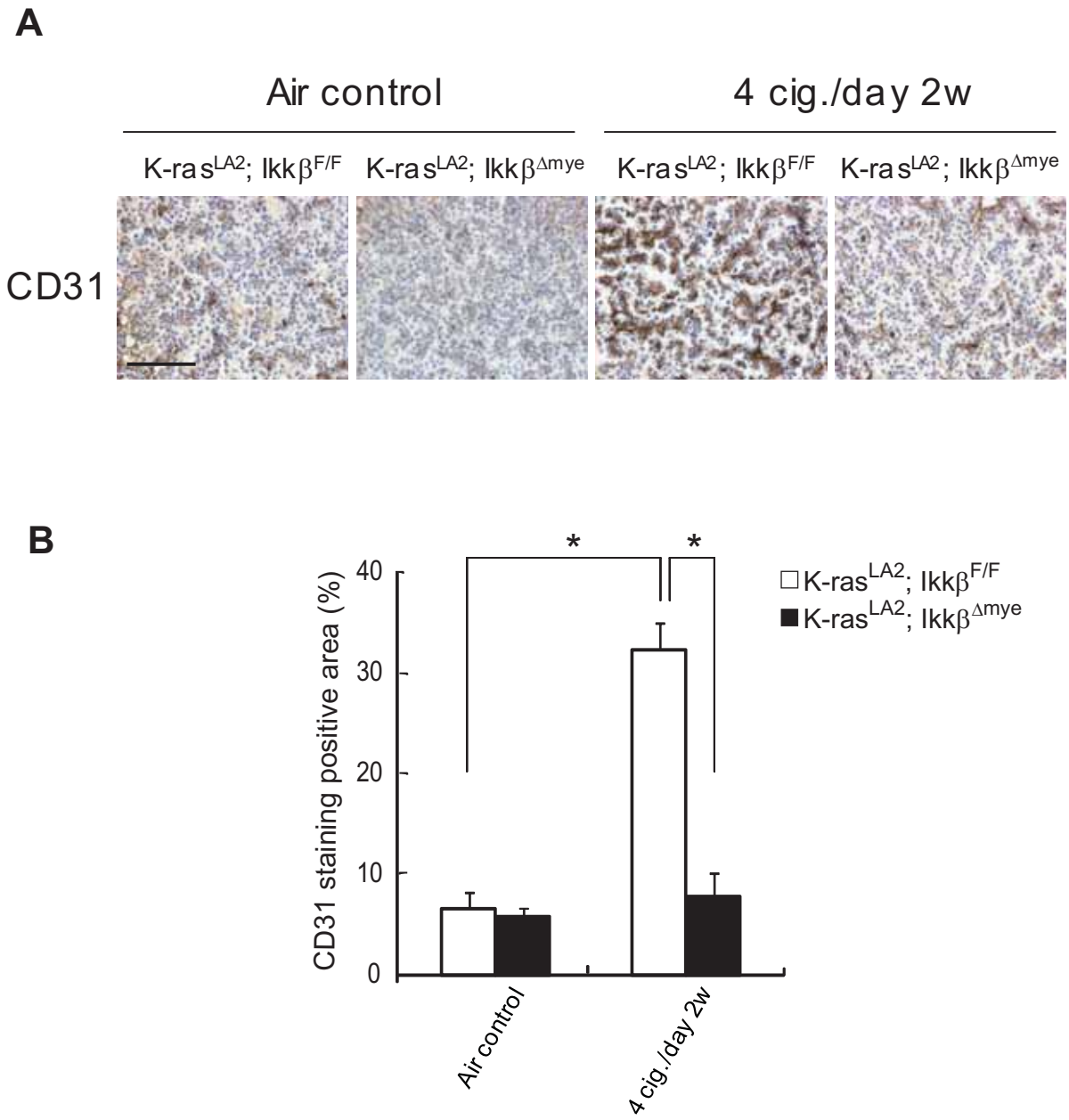

Figure S6. Myeloid cell IKKβ deletion decreases MTS-enhanced tumor angiogenesis.
Angiogenesis in lung adenomas in air- or MTS-exposed mice was determined by CD31 staining.
(A) Typical images of CD31-stained lung tumors in mice of the indicated genotypes.
Scale bar = 100 μm.
(B) CD31-positive areas were quantitated by an image analyzer. Results are means ± S.E.
K-ras^{LA2}; *Ikk*β$^{F/F}$ air control: $n = 5$; *K-ras*^{LA2}; *Ikk*β$^{F/F}$ 4 cig./day 2w: $n = 5$; *K-ras*^{LA2}; *Ikk*β^{Δmye} air control: $n = 6$; *K-ras*^{LA2}; *Ikk*β^{Δmye} 4 cig./day 2w: $n = 6$. Significant difference, $^{*}P < 0.01$.

Figure S7

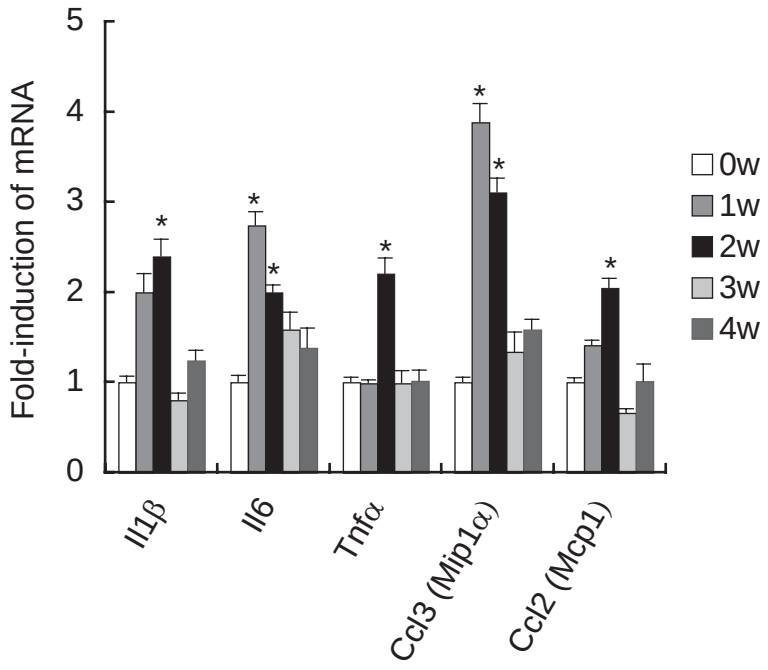

Figure S7. Induction of inflammatory cytokine and chemokine mRNAs in lungs of C57BL6 males exposed to 2 cig./day MTS

Lung RNA was isolated at indicated times after initiation of MTS exposure. Results are means ± S.E. (air control: n = 8; 2 cig./day 1w: n = 9; 2 cig./day 2w: n =7; 2 cig./day 3w: n = 4; 2 cig./day 4w: n = 4). Significant difference, *P < 0.04.

Figure S

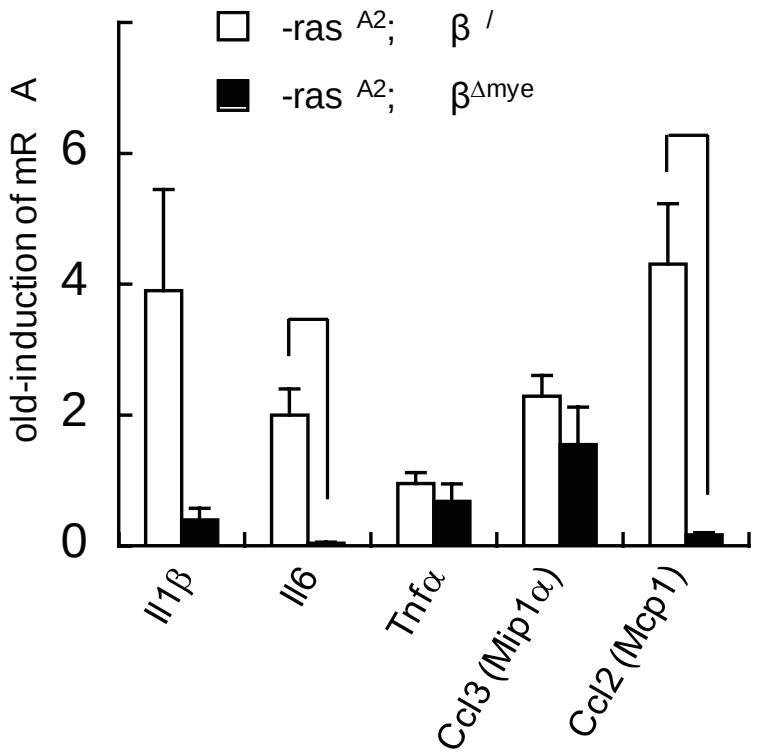

Figure S . Myeloid cell IKK β deletion decreases MTS-induced in lammationin *K-ras* tumor- earing mice

nduction of inflammatory cyto ine and chemo ine mR As in lungs of MTS-exposed K-ras A2; Ikkβ / (n = 6) and K-ras A2; Ikk$\beta^{\Delta mye}$ (n = 5) mice was examined by -RT- CR 24 hrs after the last 2 w round of MTS exposure. Results are means ± S.E. Significant difference, P < 0.00 .

Figure S9

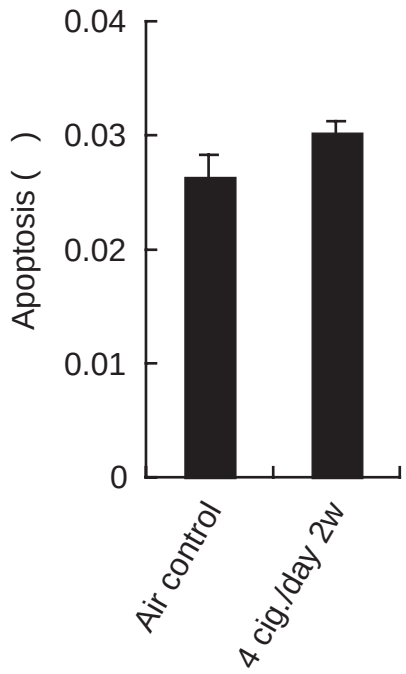

Figure S . o tosis in lungs o air- or MTS-e osed mice as determined y T staining

o induction of apoptosis above bac ground could be detected 2 wee s after initiation of MTS exposure. Results are means ± S.E. (air control: *n* = ; 4 cig./day: *n* =).

Figure S10

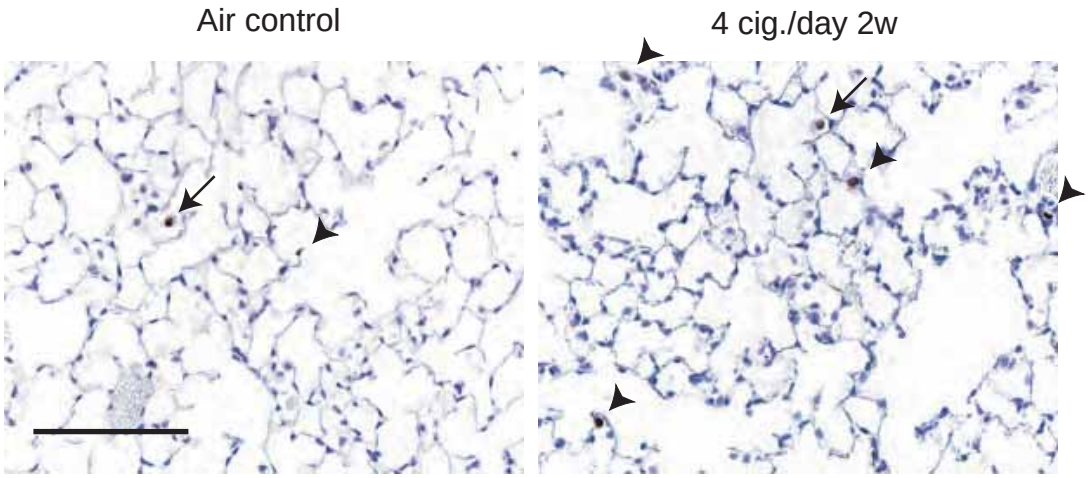

Figure S10. Proliferating cells in lung sections of air- or MTS-exposed mice were visualized by BrdU labeling
BrdU was incorporated into immune cells (arrows) and alveolar epithelial cells (arrow heads). Scale bar = 100 μm.

Figure S

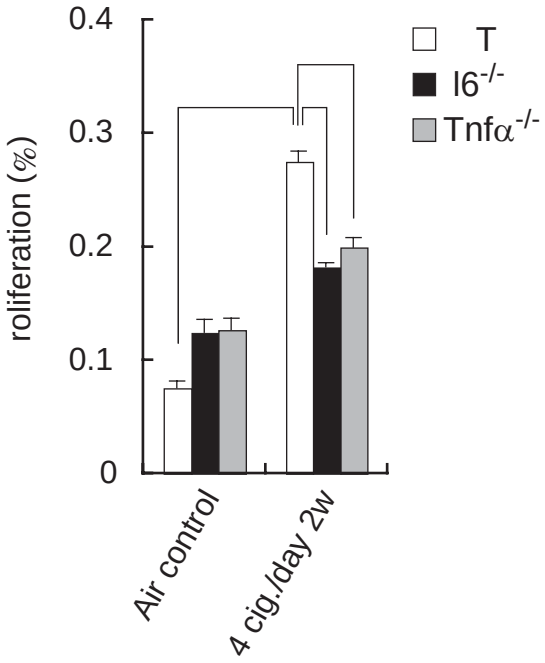

Figure S . lation o I -6 or T F-α reduces MTS-induced ulmonary cell roli eration requencies of proliferating cells in lungs of air- or MTS-exposed mice were determined by Brd labeling as above. Results are means ± S.E. (T air control: n = ; T 4 cig./day: n = ; Il6$^{-/-}$ air control: n = 5; Il6$^{-/-}$ 4 cig./day: n = ; Tnfα$^{-/-}$ air control: n = 6; Tnfα$^{-/-}$ 4 cig./day: n =). Significant difference, *P < 0.05.

Figure S12

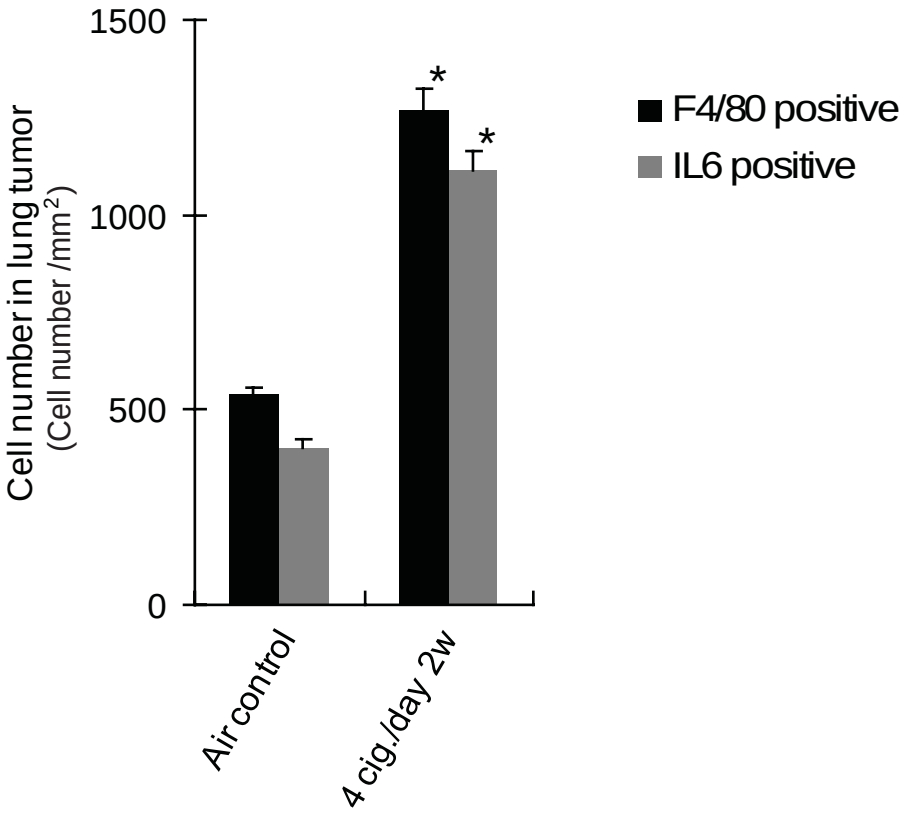

Figure S12. MTS exposure increases the number of IL-6-producing and F4/80- positive cells within tumors

Lung sections prepared at 24 hrs after last MTS exposure of K-ras^{LA2} mice were immunostained for F4/80 and IL-6. The numbers of F4/80- or IL-6-positive cells in randomly selected lung adenomas were counted. Results are means±S.E.(n = 7 for each group) Significant difference, * P <0.002 vs. air control.

Figure S

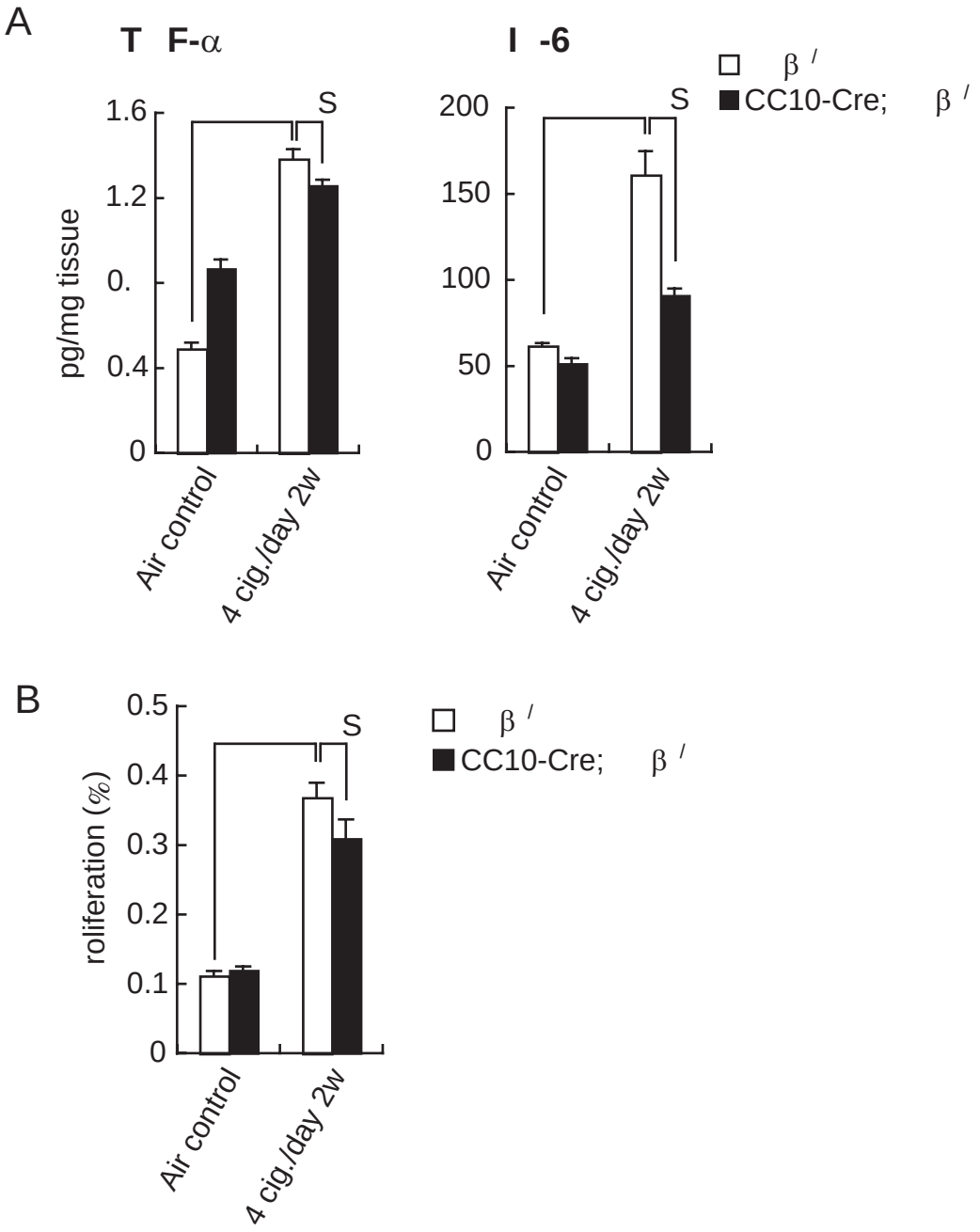

Figure S . lation o IKKβ in air ay e ithelium results in a modest and statistically insigni icant reduction in MTS-induced cyto ine secretion and cell roli eration

(A) resh lungs isolated from air- or MTS-exposed mice were cut into small pieces were incubated in culture medium at 3 C for 4 hrs and cyto ines secreted into culture supernatants were measured by E SA. Results are means ± S.E. (β / air control: n = 14; β / 4 cig./day: n = ; CC10-Cre; β / air control: n = 10; CC10-Cre; β / 4 cig./day: n =). Significant difference, < 0.05. S, insignificant difference.

(B) roliferating cells in lungs of air- or MTS-exposed mice were visualized by Brd labeling. Results showing frequencies of proliferating cells are means ± S.E. (n = per group). Significant difference, < 0.006. S, insignificant difference.

Figure S14

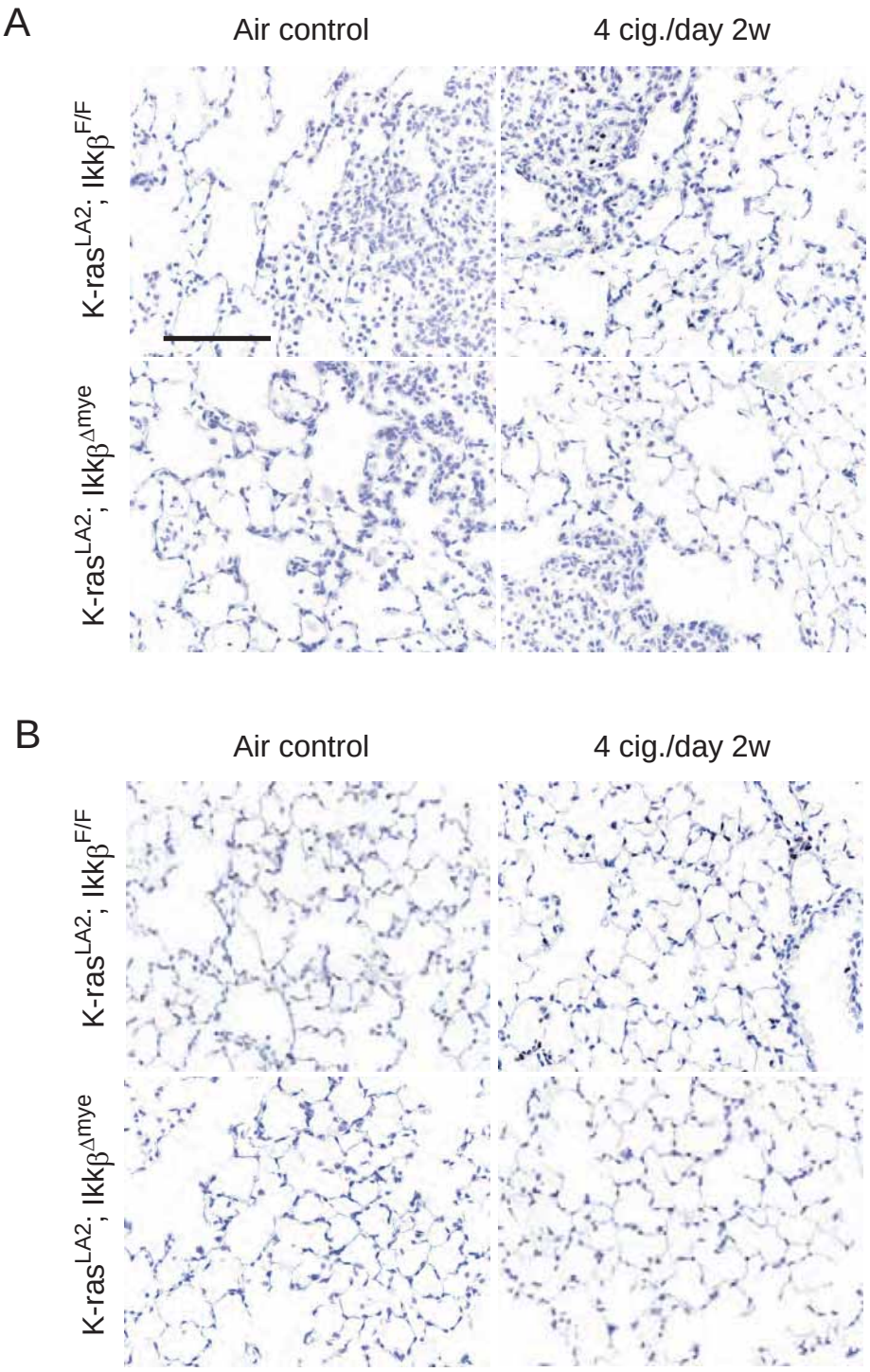

Figure S14. Myeloid cell IKKβ **deletion decreases MTS-induced ST T acti ation** ung sections prepared 24 hrs after last MTS exposure of the indicated mouse strains were analyzed by immunostaining for phospho-STAT3 (blac). (A) Tumor margin. (B) ormal lung. Scale bar = 100 μm.

Figure S15

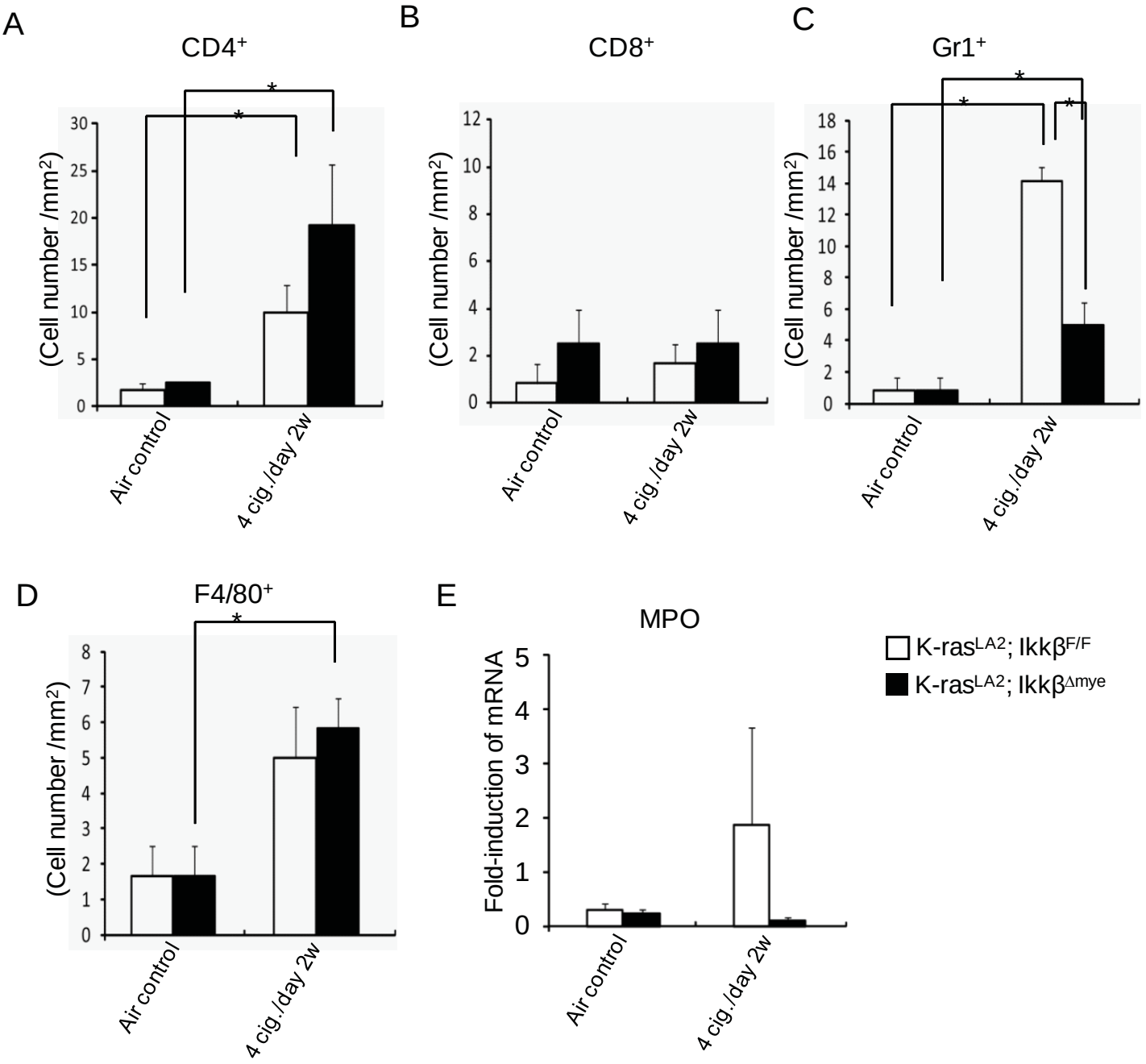

Fig. S . resence o leu ocytes in lungs o air- and MTS-e osed tumor- earing mice
(A-D) Presence of CD4+(A), CD8+(B), Gr1+(C) and F4/80+(D) cells in lungs was determined by immunohistochemical analysis of frozen sections. Results are means±S.E. K-ras^{LA2};Ikk$\beta^{F/F}$air control: n = 3; K-ras^{LA2};Ikk$\beta^{F/F}$4 cig./day 2w:n = 3; K-ras^{LA2};Ikk$\beta^{\Delta mye}$air control:n = 3; and K-ras^{LA2};Ikk$\beta^{\Delta mye}$ 4cig./day 2w:n= 3. Significant difference,*P < 0.05. (E) Expression of MPO mRNA in lungs was determined by QRT- PCR. Results are means ±S.E.K-ras^{LA2};Ikk$\beta^{F/F}$air control:n = 6; K-ras^{LA2};Ikk$\beta^{F/F}$ 4cig./day 2w:n= 6; K-ras^{LA2};Ikk$\beta^{\Delta mye}$ air control: n = 4 and K-ras^{LA2}; Ikk$\beta^{\Delta mye}$4 cig./day 2wn = 5.

Figure S16

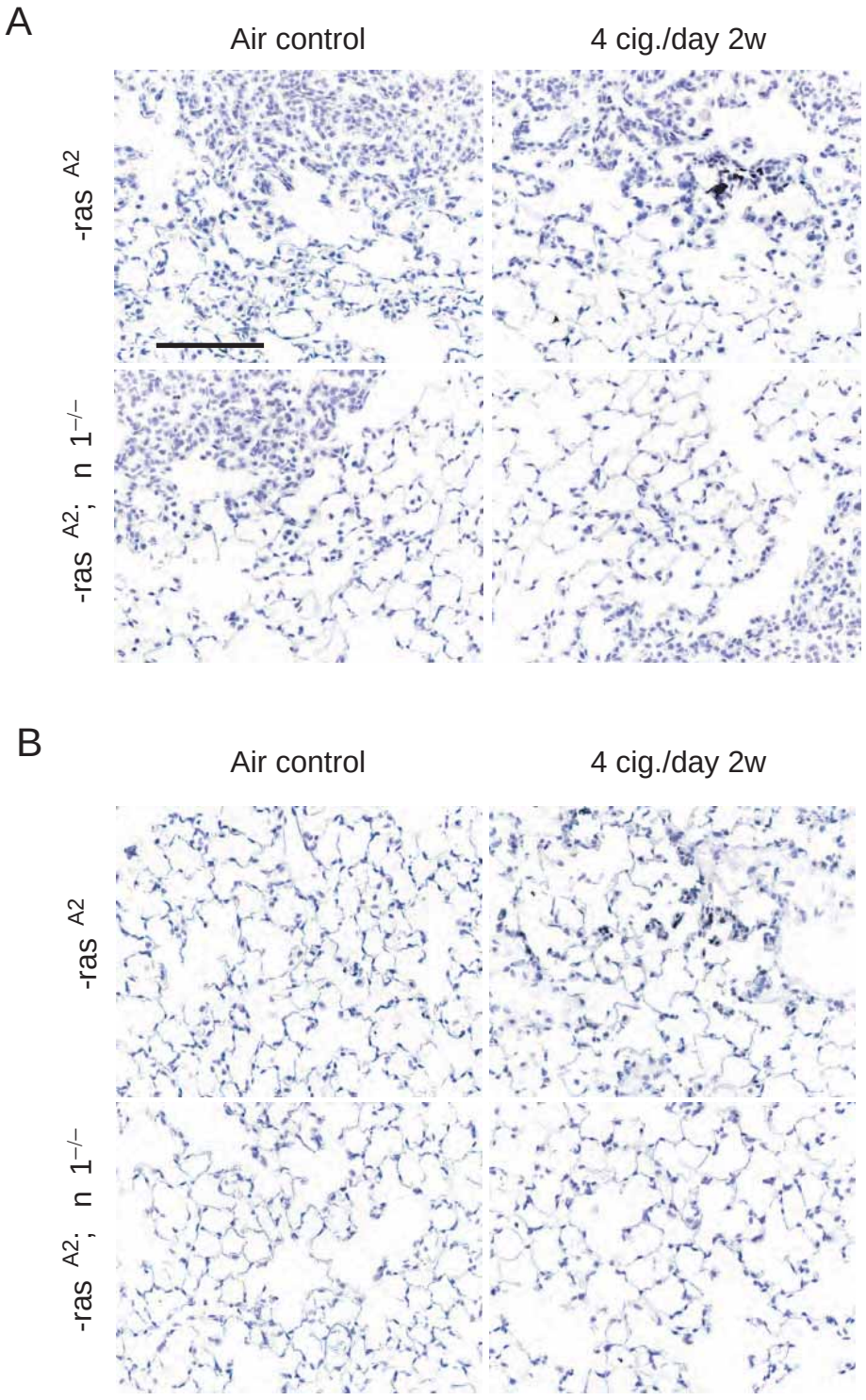

Figure S16. Deletion of JNK1 decreases MTS-induced STAT3 activation
ung sections prepared 24 hrs after last MTS exposure of the indicated mouse strains were analyzed by immunostaining for presence of phospho-STAT3 (blac). (a) Tumor margin. (b) ormal lung. Scale bar = 100 μm.