Supplemental Figures

Fig. S1: Expression of S1PL in lung tissues obtained from irradiation-challenged mice. (A) Representative Western blot of S1PL expression in lung tissue from mice challenged with radiation. Lung tissues from irradiated mice (0-17 weeks post irradiation, 20Gy) were homogenized and subjected to Western blotting. **(B)** Quantification of S1PL expression in lung tissue post irradiation. The intensity of each band was quantified using an anti-S1PL antibody and normalized to GAPDH expression. *P < 0.05, n = 4 per group.

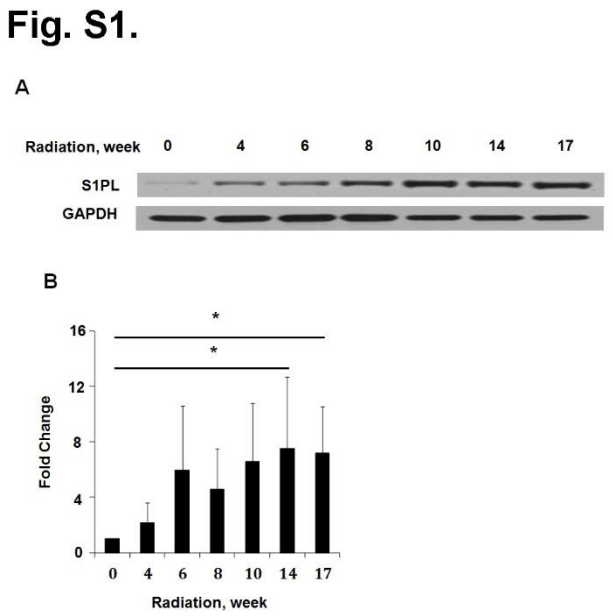

Fig. S2: Effect of S1PL overexpression on autophagosome formation in human lung fibroblasts. Human lung fibroblasts, infected with adenoviral vector control or *hSGPL1* (5 MOI, 24 h), were starved overnight in serum free DMEM medium followed by TGF-β (0 -5 ng/ml) treatment for another 48 h. The autophagosome formation was assessed by immunofluorescence staining of LC3 using confocal microscopy, and formation of LC3 (autophagosome) puncta was quantified. *P < 0.05, n = 10 per group.

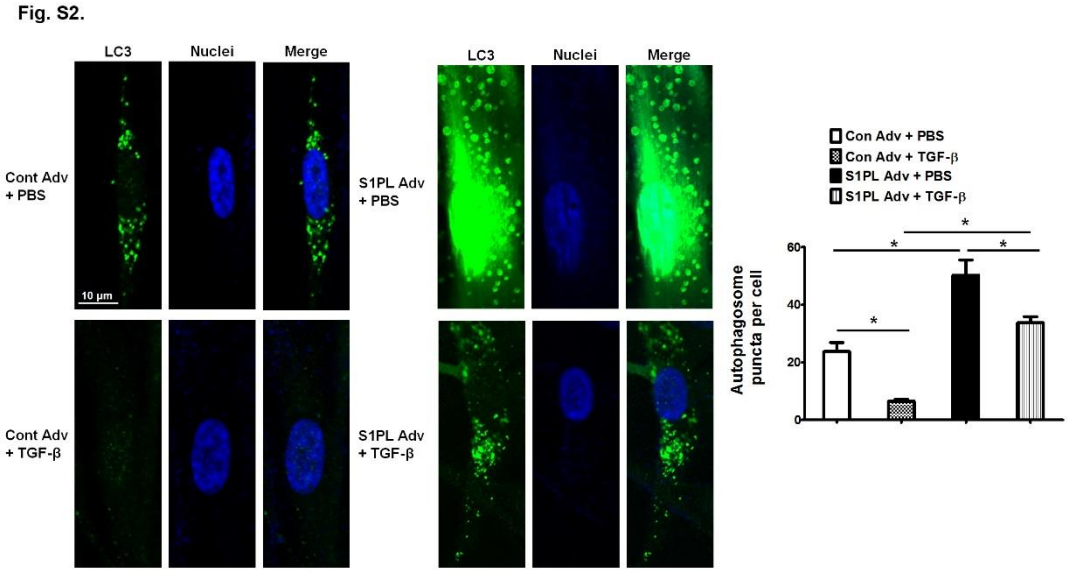

Fig. S3: Effect of S1PL overexpression on S1P-induced gene expression in human lung fibroblasts. Human lung fibroblasts post-*SGPL1* adenoviral infection (5 MOI, 24 h) were treated with S1P (0-1 μM) for 20 h, and the mRNA levels of beclin1, LC3, α-SMA and FN were quantified by real time RT-PCR as described in methods section and normalized to GAPDH. *P < 0.05, **P < 0.01; n.s, non-significant; n = 4 per group.

Fig. S3.

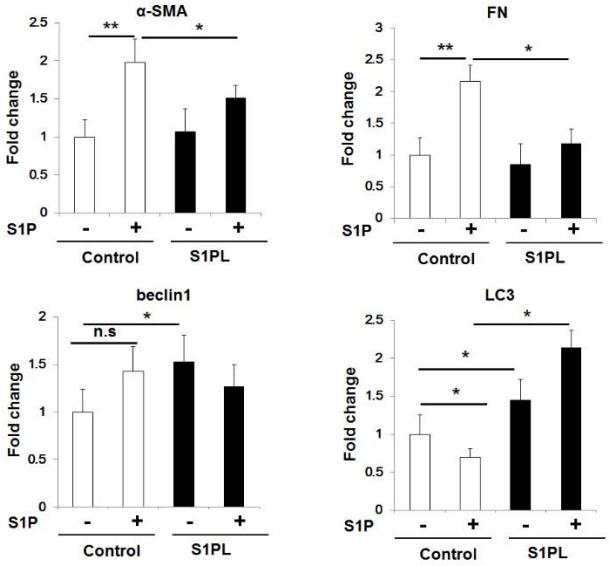

Fig. S4: Effect of S1P on LC3 accumulation in autophagosome in human lung fibroblasts. Human lung fibroblasts, transfected with LC3-GFP plasmid (3 μg/ml, 48 h), were starved overnight in serum free DMEM medium followed by treatment of S1P (0 or1 μM) for another 48 h, accumulation of LC3 in autophagosome was evaluated by confocal microscopy, and the number of LC3-GPF puncta in autophagosome were quantified. ***P < 0.001; n = 10 per group.

Fig. S4.

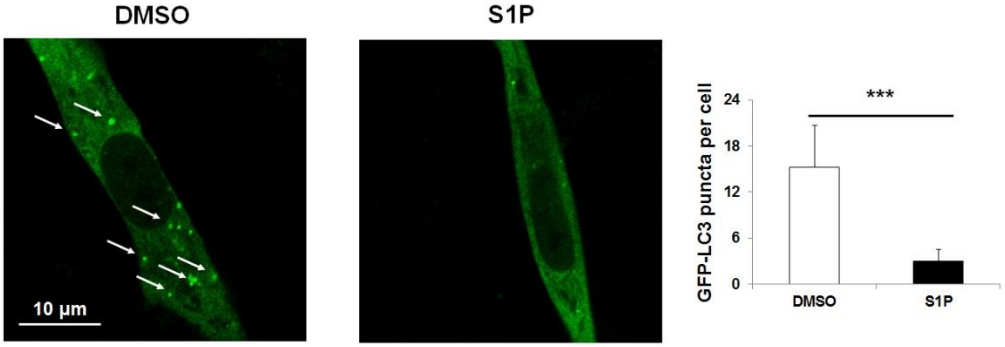

Fig. S5: Effects of Rapamycin on TGF-β-mediated differentiation of human lung fibroblasts. Human lung fibroblasts were starved overnight in serum free DMEM medium prior to challenge with DMSO or Rapamycin (Rap, 150 nM) for 1 h, followed by TGF-β (0 or 5 ng/ml) treatment for an additional 48 h, and the expression of FN, α-SMA and GAPDH was determined by Western blotting with specific antibodies. The intensity of each band was quantified and normalized to GAPDH. *P < 0.05; n = 4 per group.

Fig. S5.

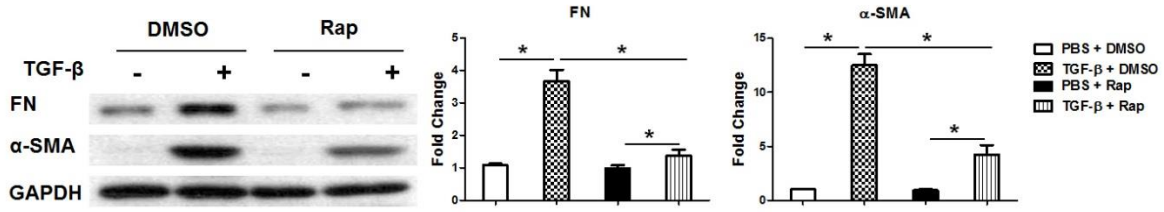

Fig. S6: Effects of chloroquine on TGF-β-mediated differentiation of human lung fibroblasts. Human lung fibroblasts, starved overnight in serum free DMEM medium, were pre-treated with DMSO or chloroquine (CQ,10 μM) for 1 h, followed by treatment of TGF-β (0 or 5 ng/ml) for another 48 h, and the expression of FN, α-SMA, LC3 II and GAPDH were analyzed by Western blotting. The intensity of each band was quantified and normalized to GAPDH. *P < 0.05; n = 4 per group.

Fig. S6.

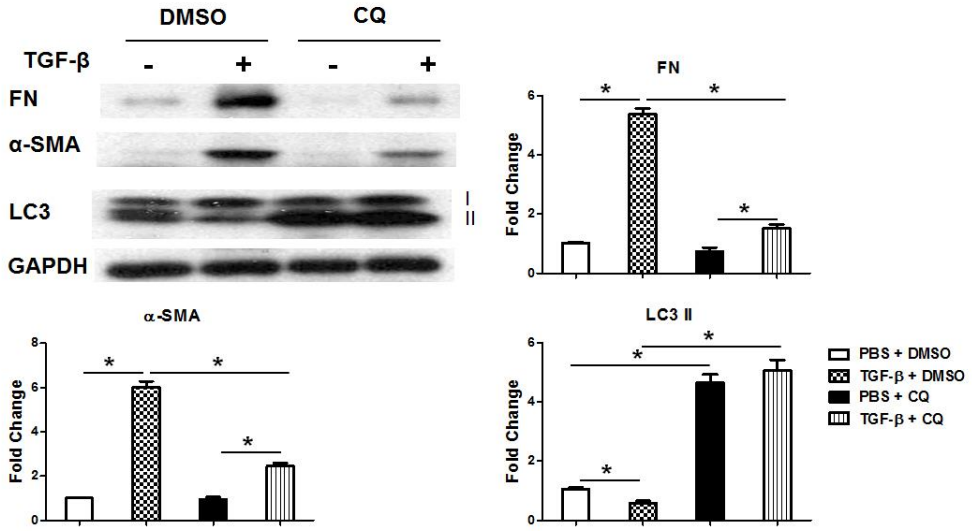

Fig. S7: Effect of chloroquine on S1PL induced inhibition of TGF-β-mediated differentiation of human lung fibroblasts. Human lung fibroblasts, infected with adenoviral vector control or *hSGPL1* (5 MOI, 24 h), were starved overnight in serum free DMEM medium followed by pre-treatment with DMSO or chloroquine (CQ, 10 μM) for 1 h, and then cells were treated with TGF-β (5 ng/ml) for another 48 h. Cell lysates were analyzed for the expression of FN, α-SMA, S1PL, LC3 and GAPDH by Western blotting. The intensity of each band was quantified and normalized to GAPDH. *P < 0.05; n = 4 per group.

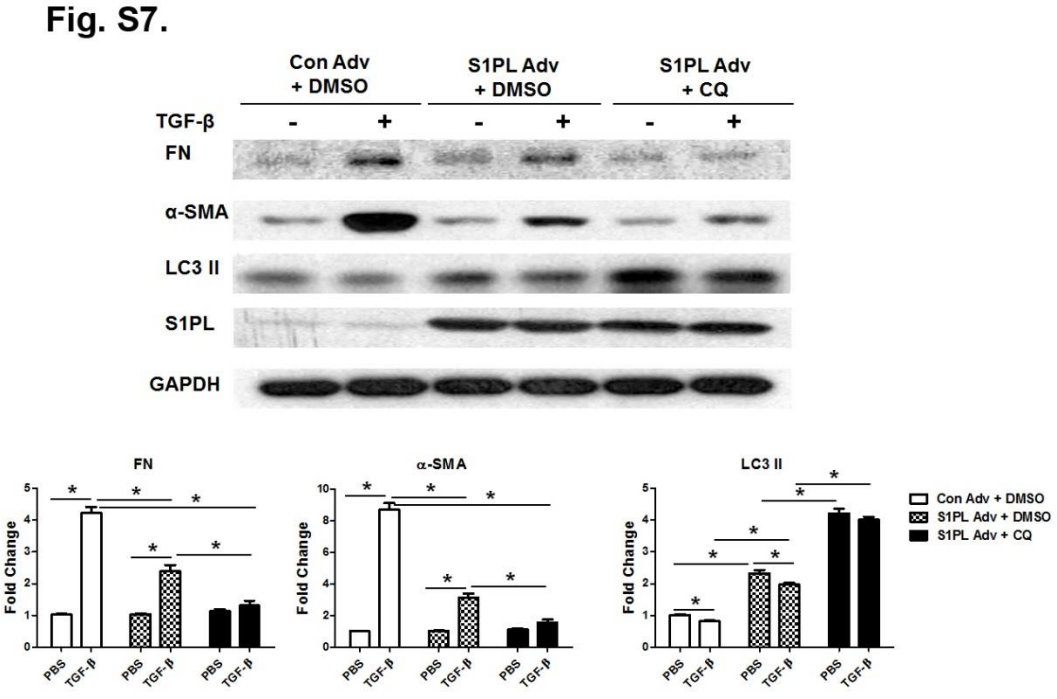

Fig. S8: Effects of knockdown of Beclin1 on S1PL mediated inhibition of TGF-β-mediated differentiation of human lung fibroblasts. Human lung fibroblasts were transfected with control or human Beclin1 shRNA (3 μg/ml, 24 h), after starvation overnight in serum free DMEM medium followed by treatment with TGF-β (5 ng/ml) for another 48 h. Cell lysates were analyzed for the expression of FN, α-SMA, Beclin1, S1PL, LC3 and GAPDH by Western blotting. The intensity of each band was quantified and normalized to GAPDH. *P < 0.05; n = 4 per group.

Fig. S8.

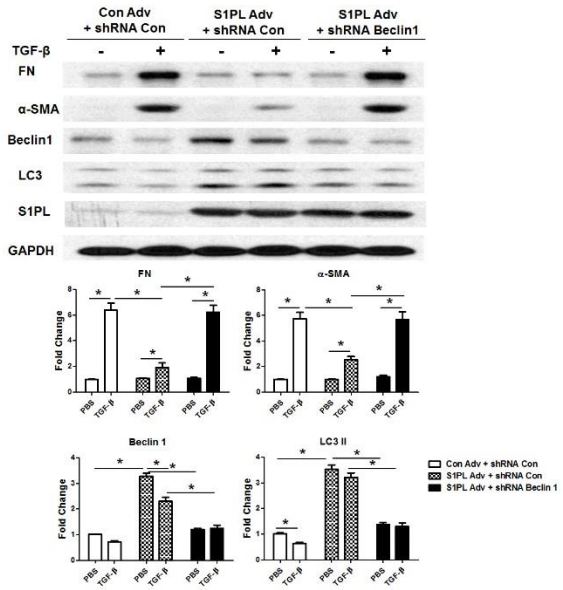

Fig. S9: Effect of S1PL overexpression on S1P induced activation of ERK, p38 and JNK in human lung fibroblasts. Human lung fibroblasts, infected with adenoviral vector control or *hSGPL1* constructs (5 MOI, 24 h), were challenged with S1P (1 μM) for 15 min, and the activation of MAPKs (ERK, p38 and JNK) were analyzed by Western blotting. **(A)** Representative Western blot depicting ERK, p38 and JNK activation in human lung fibroblasts with or without over-expression of S1PL. **(B-D)** Quantification of S1P induced activation of ERK **(B),** JNK **(C)** and p38 **(D)** in human lung fibroblasts. The intensity of each band was quantified and normalized to total ERK1/2, p38 or JNK protein, respectively. *P < 0.05, **P < 0.01; n = 4 per group.

Fig. S9.

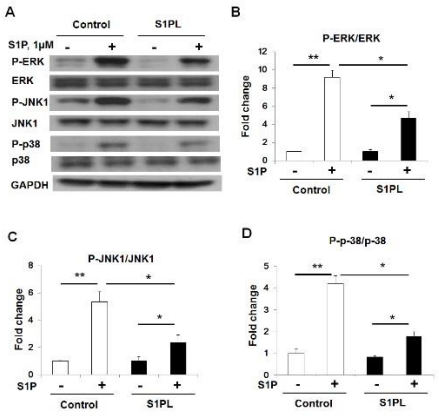

Fig. S10: Effect of genetic deletion of S1PL (*Sgpl1*$^{+/-}$) on LC3, beclin1 expression and the accumulation of autophagosomes in mouse lung fibroblasts. Primary mouse lung fibroblasts were isolated from mouse WT or *Sgpl1*$^{+/-}$ mice (8 weeks old male mice) as described in methods section, and expression of LC3, Beclin1 and S1PL were analyzed by western blot. **(A)** Representative Western blot and quantification of LC3 II, beclin1 and S1PL in primary lung fibroblasts from WT and *Sgpl1*$^{+/-}$ mice. The intensity of each band was quantified and normalized to total actin. *P < 0.05, n = 3 per group. **(B)** Accumulation of LC3 in autophagosomes of mouse lung fibroblasts. Lung fibroblasts from wild type and *Sgpl1*$^{+/-}$ mice were transfected with GFP-tagged LC3 plasmid (3 μg/ml) for 48 h, accumulation of GFP-tagged LC3 puncta in autophagosomes was checked by confocal microscopy, and the number of LC3-GPF puncta in cells were quantified. ***P < 0.001; n = 10 per group.

Fig S10.

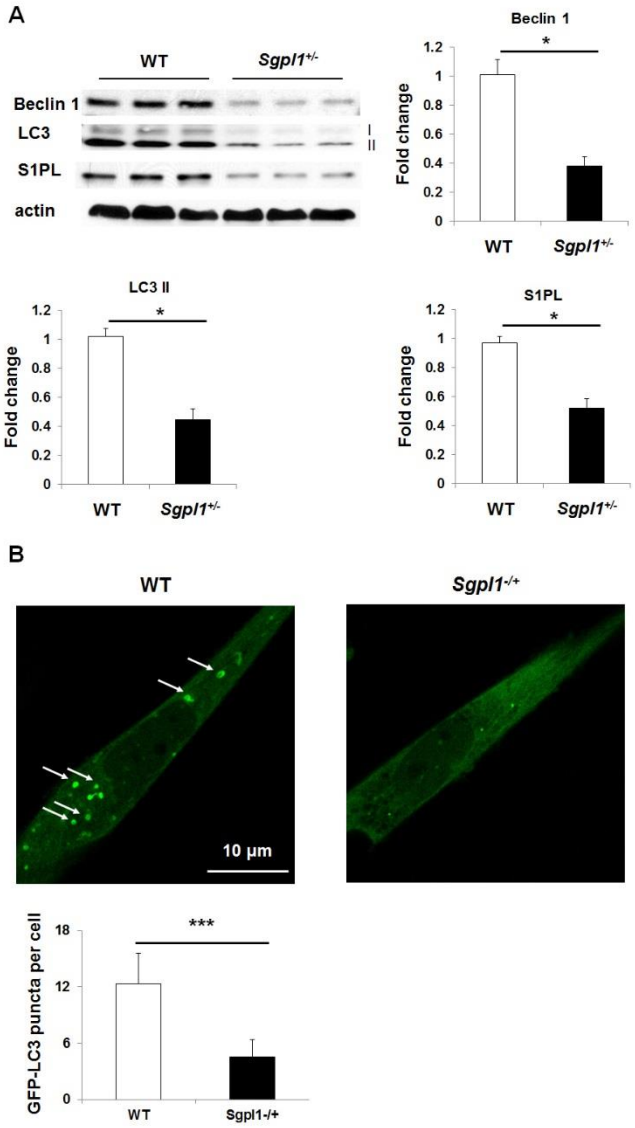

Fig. S11: Effect of genetic deletion of S1PL (*Sgpl1*$^{+/-}$) on chloroquine induced autophagosomes formation in mouse lung fibroblasts. Primary mouse lung fibroblasts were isolated from 8 week old WT or *Sgpl1*$^{+/-}$ mice as described in Methods section. Cells were starved overnight in serum free DMEM medium and treated with either DMSO or chloroquine (CQ, 10 μM) for 3 h, autophagosome formation was evaluated by immunofluorescence staining of LC3 using confocal microscopy, and the autophagosomes puncta were quantified and analyzed. *P < 0.05, n = 10 per group.

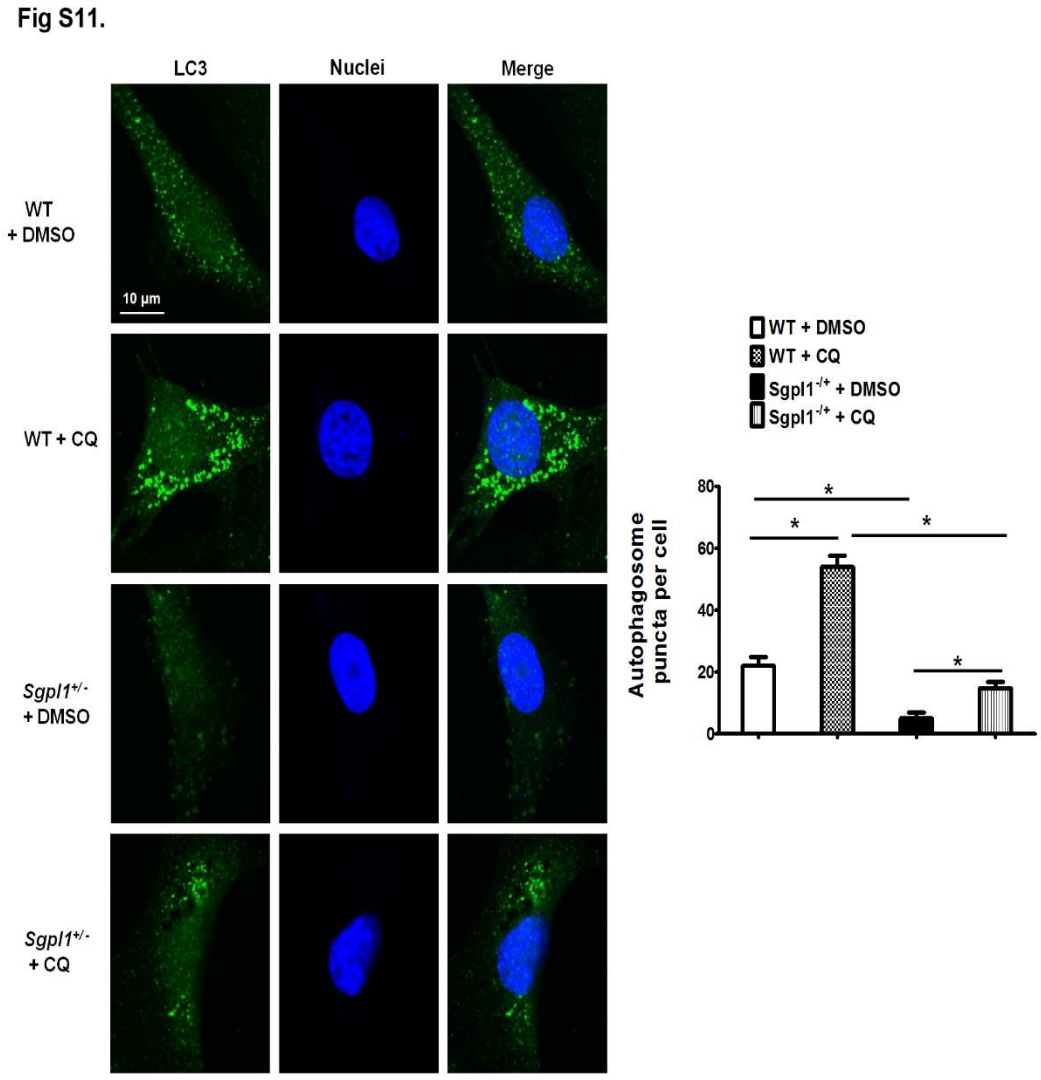

Fig. S12: Effect of S1PL deficiency (*Sgpl1*$^{+/-}$) on bleomycin-mediated inflammatory changes in mouse lung. *Sgpl1*$^{+/-}$ or WT (129SV) mice (male, 8 weeks) were intratracheally instilled with PBS or bleomycin (2 U/kg in 50 µl of PBS) and were sacrificed on day 14 post bleomycin administration. Lungs were lavaged using sterile PBS, and the BAL fluids were analyzed as described in Methods. **(A)** Total protein levels, **(B)** total cell number, **(C)** total neutrophils, **(D)** total macrophages, **(E)** total lymphocytes and **(F)** IL-6 levels in BAL fluids were expressed as mean ± SEM. *P < 0.05, **P < 0.01; n=4-6 per group.

Fig. S12.