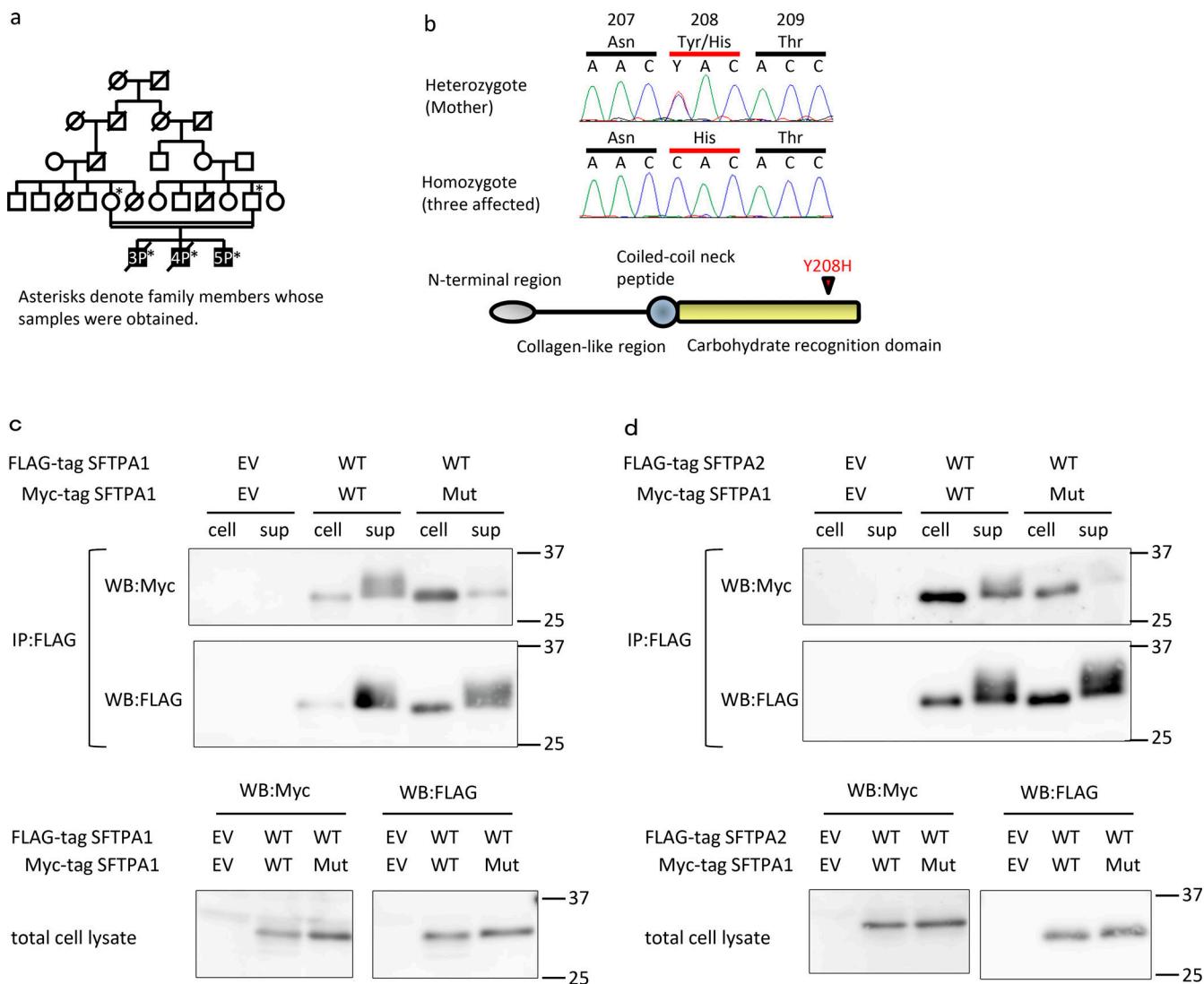
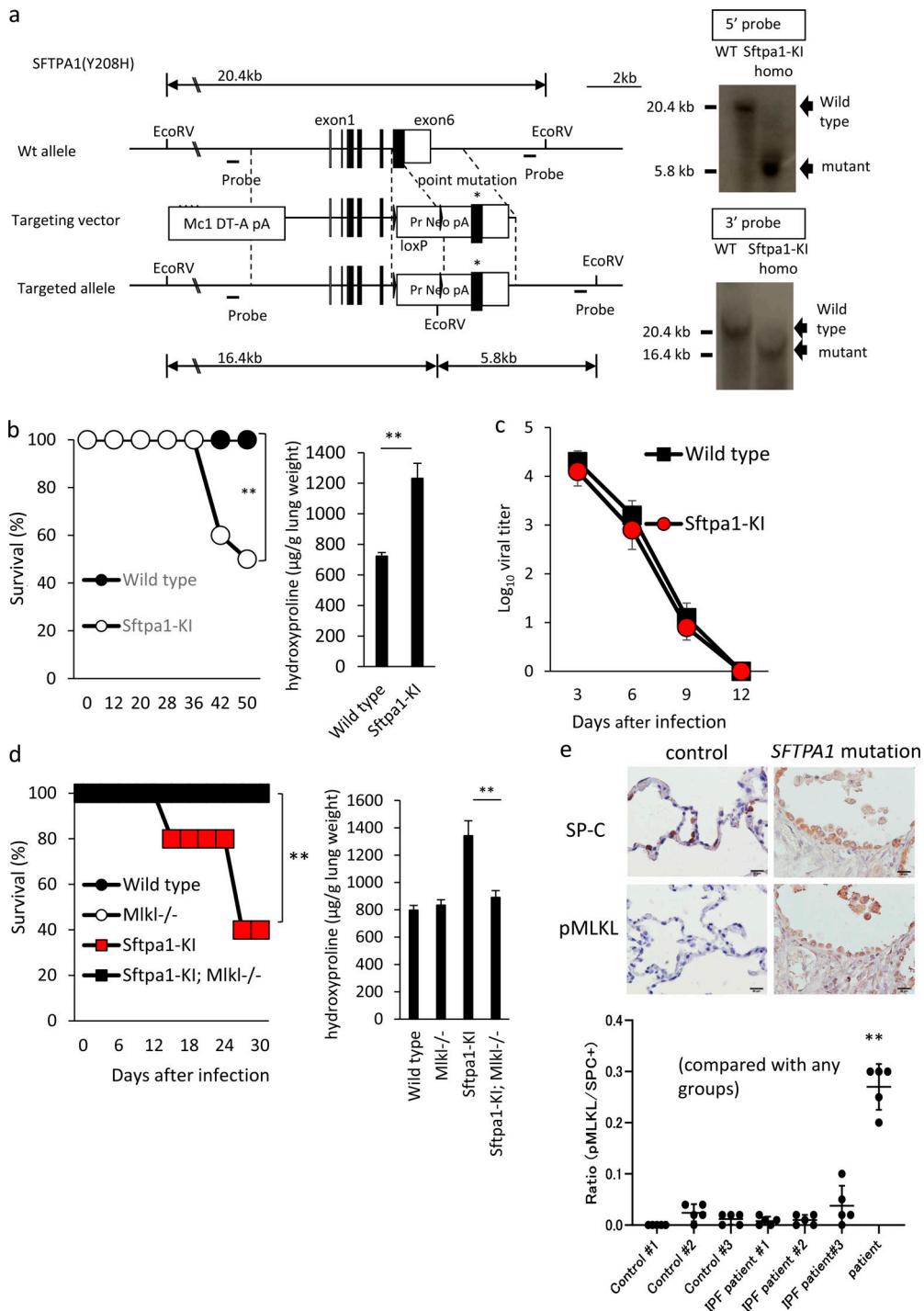


## Supplemental material

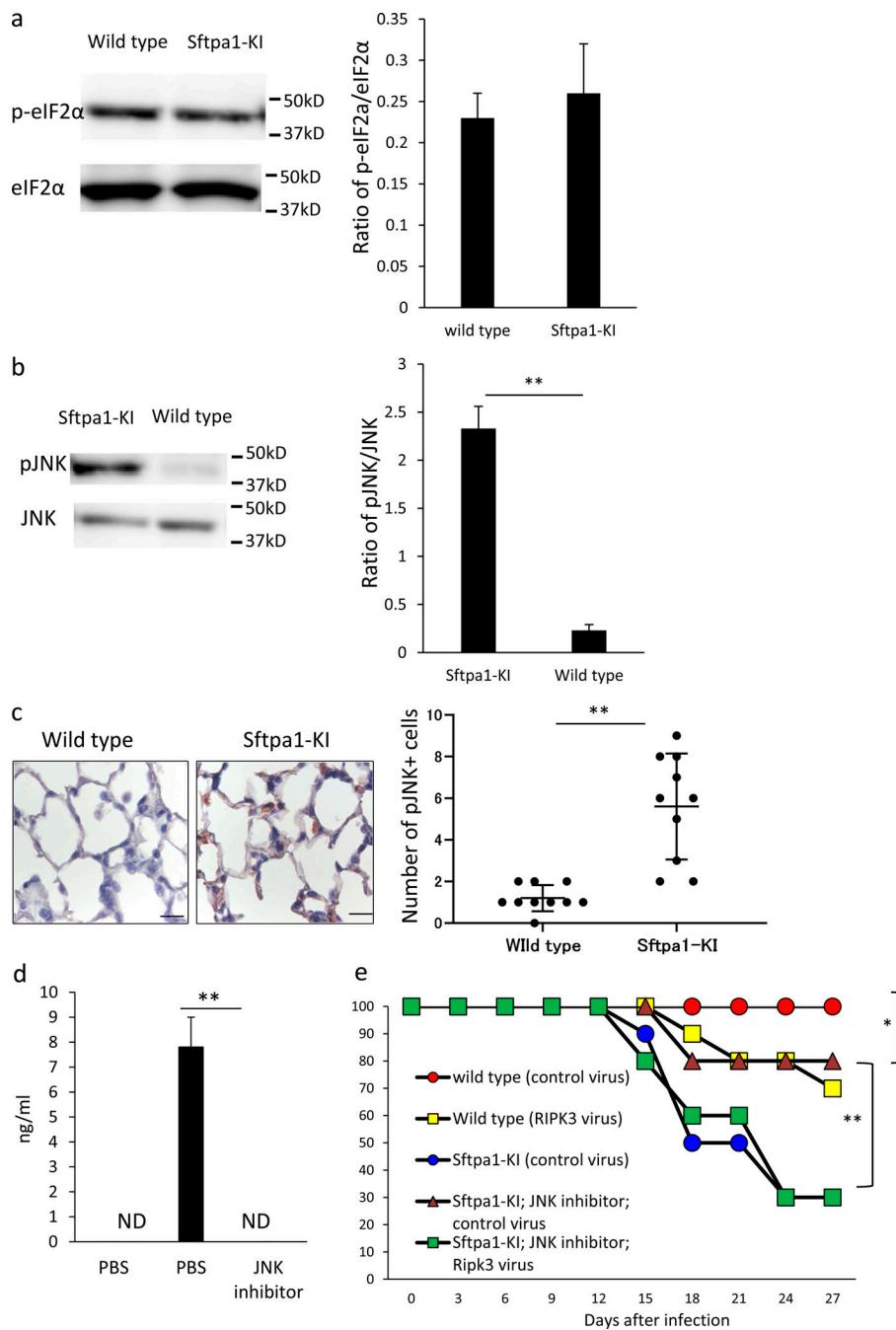
Takezaki et al., <https://doi.org/10.1084/jem.20182351>



**Figure S1. Identification of an SFTPA1 mutation in IPF patients and its effect on the secretion of SFTPA1.** **(a)** Pedigree of a Japanese IPF family: double lines indicate consanguinity, filled black indicates IPF patients, and diagonal lines indicate deceased siblings. P, patient. **(b)** The heterozygous (parents) or homozygous (patients) missense mutation in *SFTPA1* (Y208H) as shown by capillary sequencing. *SFTPA1* protein domains: the red arrowhead indicates position of the missense mutation. **(c and d)** The Flag-tagged SFTPA1 and Myc-tagged SFTPA1 (**c**; WT or mutant [Mut]) or Flag-tagged SFTPA2 and Myc-tagged SFTPA1 (**d**; WT or Mut) cDNA was transfected into A549 cells. As a negative control, empty vector (EV) was transfected. 1 d after transfection, the cell pellets (cell) or supernatant (sup) were immunoprecipitated with anti-Flag antibody, and the immunoprecipitates were blotted with an anti-Flag or Myc antibody. As a control, total cell lysates were blotted with the anti-Flag or Myc antibody. WB, Western blot. Data shown are representative of five (**c** and **d**) independent experiments.



**Figure S2. Increased necroptosis in Sftpa1-KI mice and IPF patients. (a)** The breeding scheme for the construction of Sftpa1-KI mice. The point mutation was inserted at position 208 (Y208H). Southern blot analysis of genomic DNA from WT and Sftpa1-KI mice. Genomic DNA from mice with homozygous (homo) mutant allele or WT mice was digested with EcoRI and blotted with 3' and 5' probes. DT-A, diphtheria toxin fragment A; pA, polyadenylation signal; Pr, promoter. **(b)** Survival analysis of WT (closed circles) or Sftpa1-KI mice (open circles; number of mice in each independent experiment is 20). \*\*, P < 0.01 by log rank test. Quantification of hydroxyproline contents in WT or Sftpa1-KI mice (number of mice in each independent experiment is seven) at the age of 35 wk. Data are means ± SD. \*\*, P < 0.01. **(c)** Virus titer in lung tissue was evaluated in WT (closed square) or Sftpa1-KI (red circle) mice infected with IAV. Results are shown as means ± SD. **(d)** Survival analysis of WT (closed circles), Mlkl<sup>-/-</sup> (open circles), Sftpa1-KI (red squares), and Sftpa1-KI mice deficient in Mlkl (Sftpa1-KI; Mlkl<sup>-/-</sup>; closed squares); number of mice in each independent experiment is 10). \*\*, P < 0.01 by log rank test. The data are representative of three independent experiments. Quantification of hydroxyproline contents in WT, Mlkl<sup>-/-</sup>, Sftpa1-KI, or Sftpa1-KI; Mlkl<sup>-/-</sup> mice (number of mice in each independent experiment is 10) 12 d after IAV infection. Data represent the means ± SD. \*\*, P < 0.01. **(e)** Histological section was stained with anti-SP-C or pMLKL antibody in controls (healthy part of lungs from three lung cancer patients) and three IPF patients without SFTPA1 mutation (#1, #2, and #3) and a patient with SFTPA1 mutation (3P in Fig. S1 a). The scale bar indicates 20 µm. The ratios of pMLKL-positive cells/SP-C-positive cells in five distinct parts of images are shown. Data are means ± SD. \*\*, P < 0.01. Data shown are representative of five (b–f) independent experiments.



**Figure S3. The inhibition of JNK blocks pulmonary fibrosis.** (a and b) The expression of phosphorylated eIF2 $\alpha$  (a) or phosphorylated JNK (b) in WT or Sftpa1-KI mice at the age of 35 wk was evaluated by Western blotting. Data are means  $\pm$  SD. \*\*, P < 0.01. (c) The expression of pJNK in WT or Sftpa1-KI mice at the age of 30 wk was evaluated by immunohistochemistry. The pJNK-positive cells (average of the number of pJNK-positive cells in 10 independent fields in right lung) was counted. Data are means  $\pm$  SD. \*\*, P < 0.01. The scale bar indicates 20  $\mu$ m. (d) WT mice were injected with LPS (50  $\mu$ g), and serum TNF $\alpha$  was measured 3 h after LPS injection. The JNK inhibitor (30 mg/kg, s.c.) was administered 3 h before LPS injection. Data are means  $\pm$  SD. \*\*, P < 0.01. The data are representative of two independent experiments. (e) This is an additional set of experiments for Fig. 3 b. Survival of 20-wk-old WT (red circles), WT infected with adenovirus carrying Ripk3 (yellow squares), Sftpa1-KI mice infected with control virus (blue circles), Sftpa1-KI mice infected with control virus and treated with a JNK inhibitor (brown triangles), or Sftpa1-KI mice infected with Ripk3 adenovirus and treated with a JNK inhibitor (green squares; number of mice in each independent experiment is 10). \*, P < 0.05; \*\*, P < 0.01 by log rank test. Data shown are representative of five (a-d) independent experiments.