Supplementary Information

F-box protein FBXL19-mediated ubiquitination and degradation of the interleukin-33 receptor limits pulmonary inflammation

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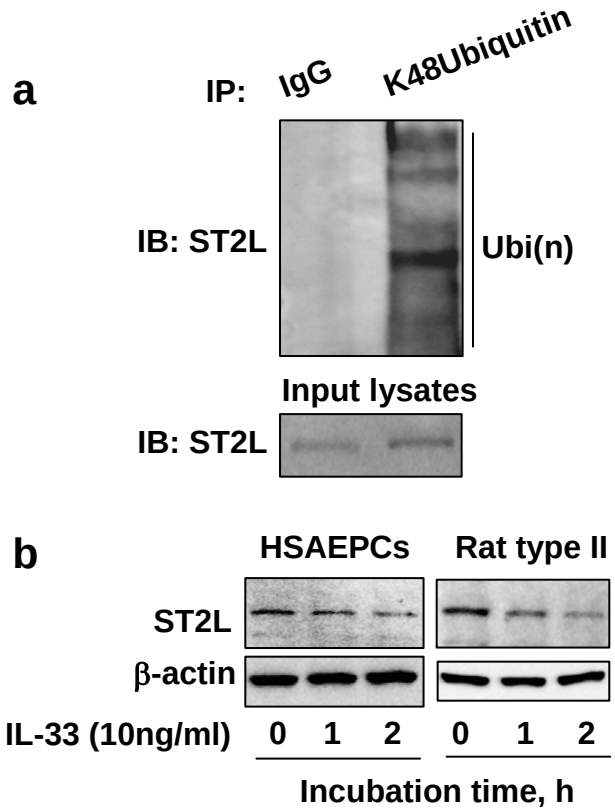

Supplementary Figure 1. mST2L degradation is mediated by a classic K48-linked polyubiquitination. (a) MLE12 cells were incubated with MG-132 for 8 h and cells were lysed in the presence of 1 μg/ml ubiquitin aldehyde and subjected to immunoprecipitation with IgG or K48 ubiquitin antibody. The precipitates were subjected to ST2L immunoblotting. **(b)** HSAEPCs and rat type II epithelial cells were treated with IL-33 (10 ng/ml) for 1 and 2 h. Cell lysates were subjected to ST2L and β-actin immunoblotting. Data are from one experiment representative of three.

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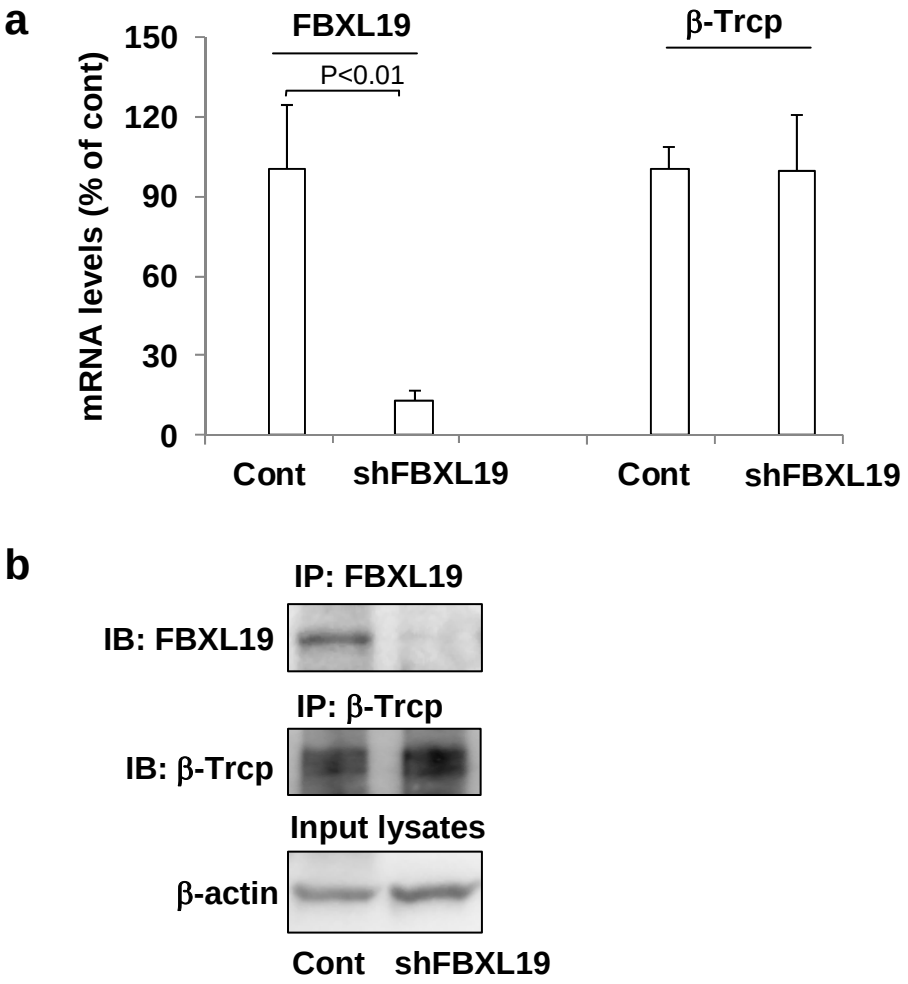

Supplementary Figure 2. shFBXL19 reduces FBXL19 expression. MLE12 cells were transfected with shFBXL19 for 72 h. **(a)** Total cellular RNA was extracted and mRNA levels of FBXL19 and β-Trcp were determined by real-time PCR. **(b)** Cell lysates were subjected to immunoprecipitation with a FBXL19 antibody or β-Trcp antibody. The precipitates were subjected to FBXL19 or β-Trcp immunoblotting. Data are from one experiment representative of three.

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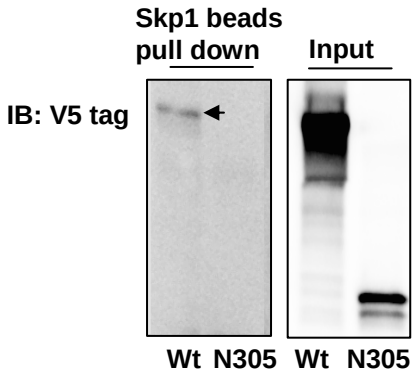
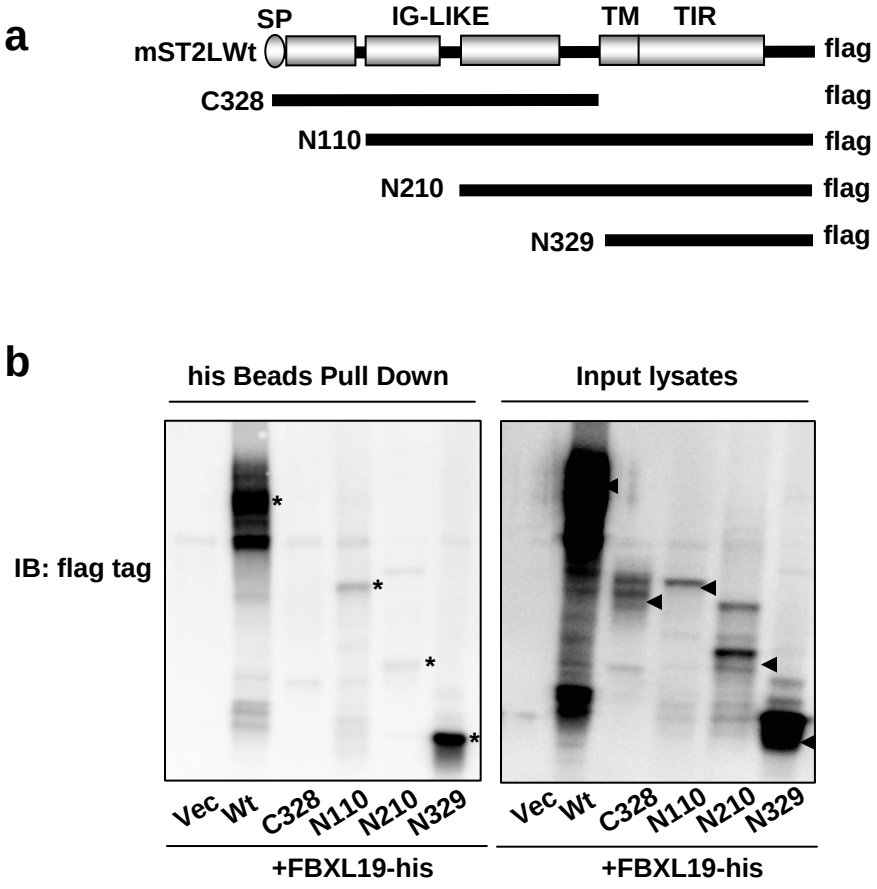


Supplementary Figure 3. FBXL19 binds to Skp1 through the F-box protein motif. MLE12 cells were transfected with plasmids encoding a V5 tagged FBXL19 wild type or a N-terminus plus F-box motif deleted mutant (N305) plasmid for 24 h. Cell lysates were subjected to Skp1 agarose bead pulldown and precipitates were subjected to V5 tag immunoblotting. Data are from one experiment representative of three.

Supplementary. Fig 3



Supplementary Figure 4. FBXL19 binds to ST2L within the C-terminus of ST2L. (a) Map of the flag-tagged mST2L and the deletion mutants. **(b)** Cell lysates from flag tagged mST2L wild type or mutants over-expressed HEK293 cells were incubated with the lysates from HEK293 cells expressing his tagged FBXL19 for 1 h; his tagged FBXL19 was pulled down using his beads. The immunoprecipitates (left panel) and input cell lysates (right panel) were subjected to flag immunoblotting. The positive immunoreactive bands are shown by asterisks or arrows. Data are from one experiment representative of three.

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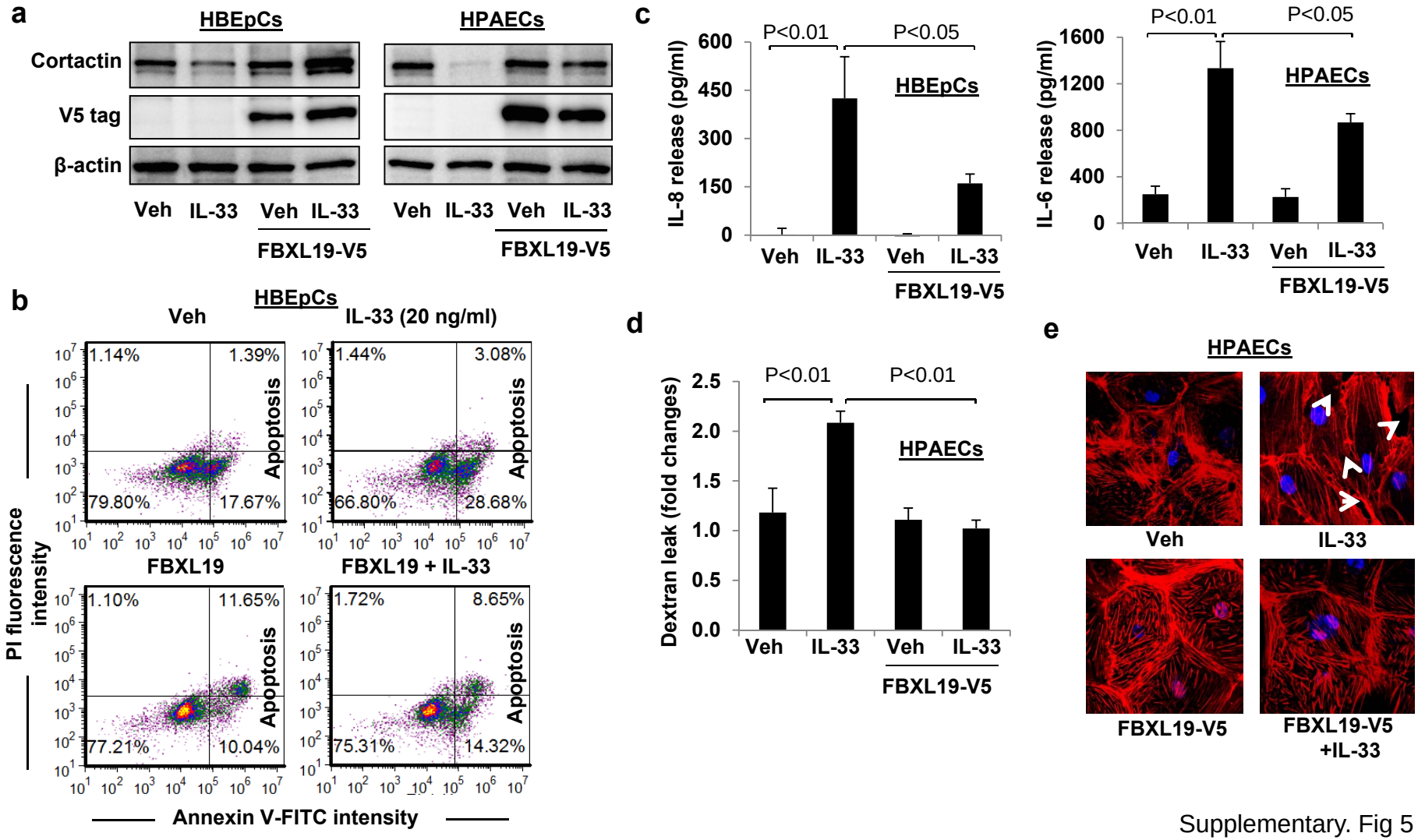

Supplementary. Fig 5

Supplementary Figure 5. FBXL19 attenuates IL-33-induced biological effects. (a) HBEpCs and HPAECs were transfected with V5 tagged FBXL19 prior to IL-33 treatment (20 ng/ml, 2 h) and cell lysates were subjected to cortactin, β-actin, and V5 tag immunoblotting. Data are from one experiment representative of three. **(b)** HBEpCs were transfected with V5 tagged FBXL19 prior to IL-33 treatment (20 ng/ml, 2 h) and apoptosis were analyzed by flow cytometry analysis. Data are from one experiment representative of three. **(c)** HBEpCs and HPAECs were transfected with V5 tagged FBXL19 prior to IL-33 treatment (20 ng/ml, 6 h), IL-8 or IL-6 levels in medium were measured by ELISA kits. **(d)** HPACEs were transfected with V5 tagged FBXL19 permeable inserts containing 0.4-μm pores. IL-33 were added into upper chamber, followed by addition of FITC-dextran (4 kDa). The leak of dextran into lower chamber in 6h were measured by fluorescence microplate reader. **(e)** HPAECs growth on glass bottom chamber were transfected with V5 tagged FBXL19 prior to IL-33 treatment (20 ng/ml, 2 h). Actin filaments were stained with FITC-phalloidin. Arrows indicate gaps. Shown are representative images (n=4).

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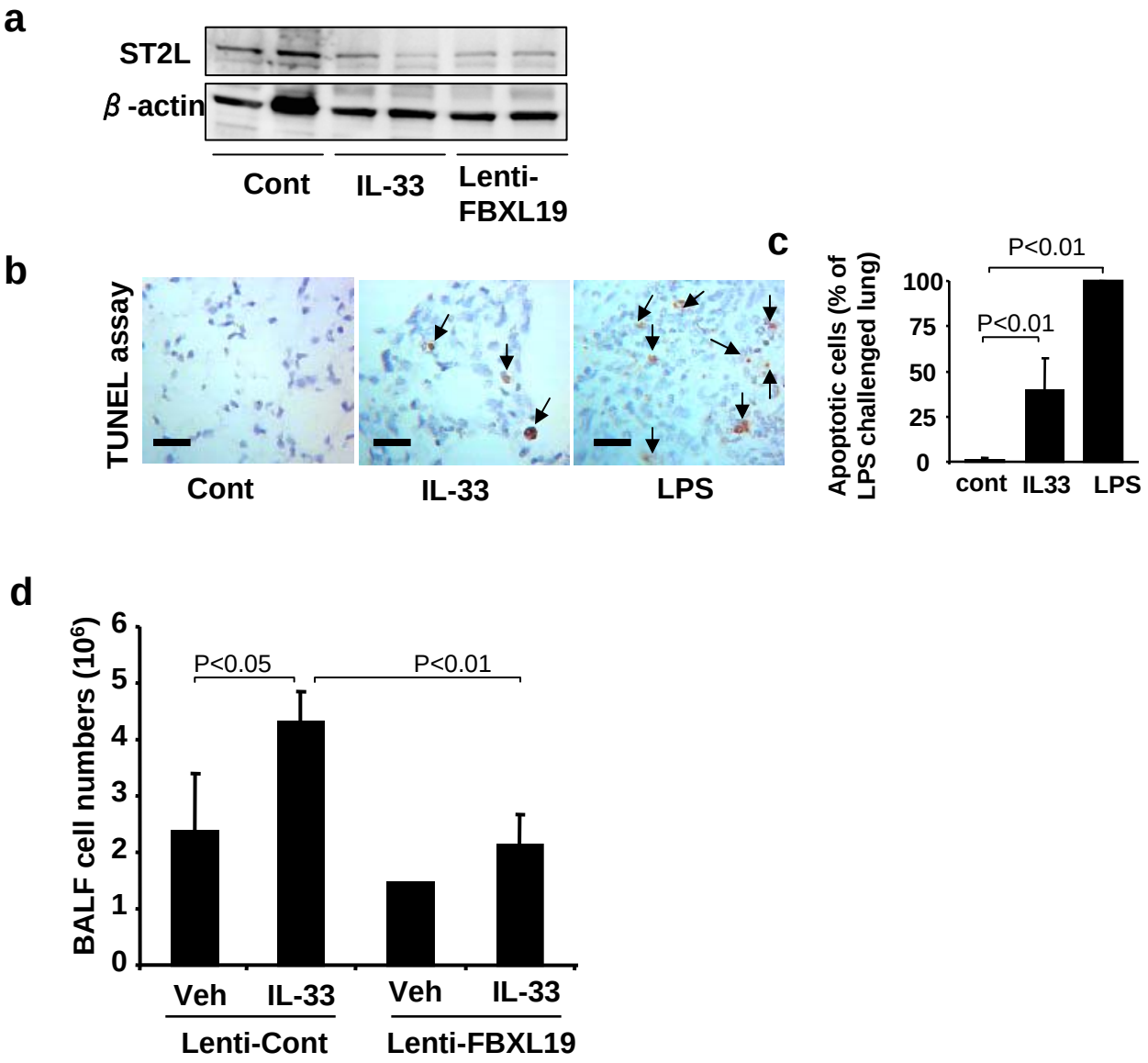

Supplementary Figure 6. IL-33 induces ST2L degradation and apoptosis in lung tissue. **(a)** C57/BL6 mice (4-6/ group) were given i.t. IL-33 (4 µg/mouse, 24 h) or Lenti-FBXL19 vector (10^9 pfu/mouse, 5 days). Lung tissue lysates were subjected to ST2L and β-actin immunoblotting**.** Data are from one experiment representative of three. **(b, c)** C57/BL6 mice were given i.t. IL-33 (4 µg/mouse, 24 h) or LPS (1 mg/kg weight, 24 h). Lung tissues were fixed and TUNEL staining was performed. Arrows show TUNEL-positive cells. Scale bar, 10 µm. Shown are representative images (*n=4*). Apoptotic cells in lung tissue was quantified by ImageJ software. **(d)** C57/BL6 mice were given i.t. Lenti-control or Lenti-FBXL19 (10^9 pfu/mouse, 5 days) prior to i.t. administration of IL-33 (4 µg/mouse, 24 h). BAL fluid was collected and cell numbers were counted and quantified.

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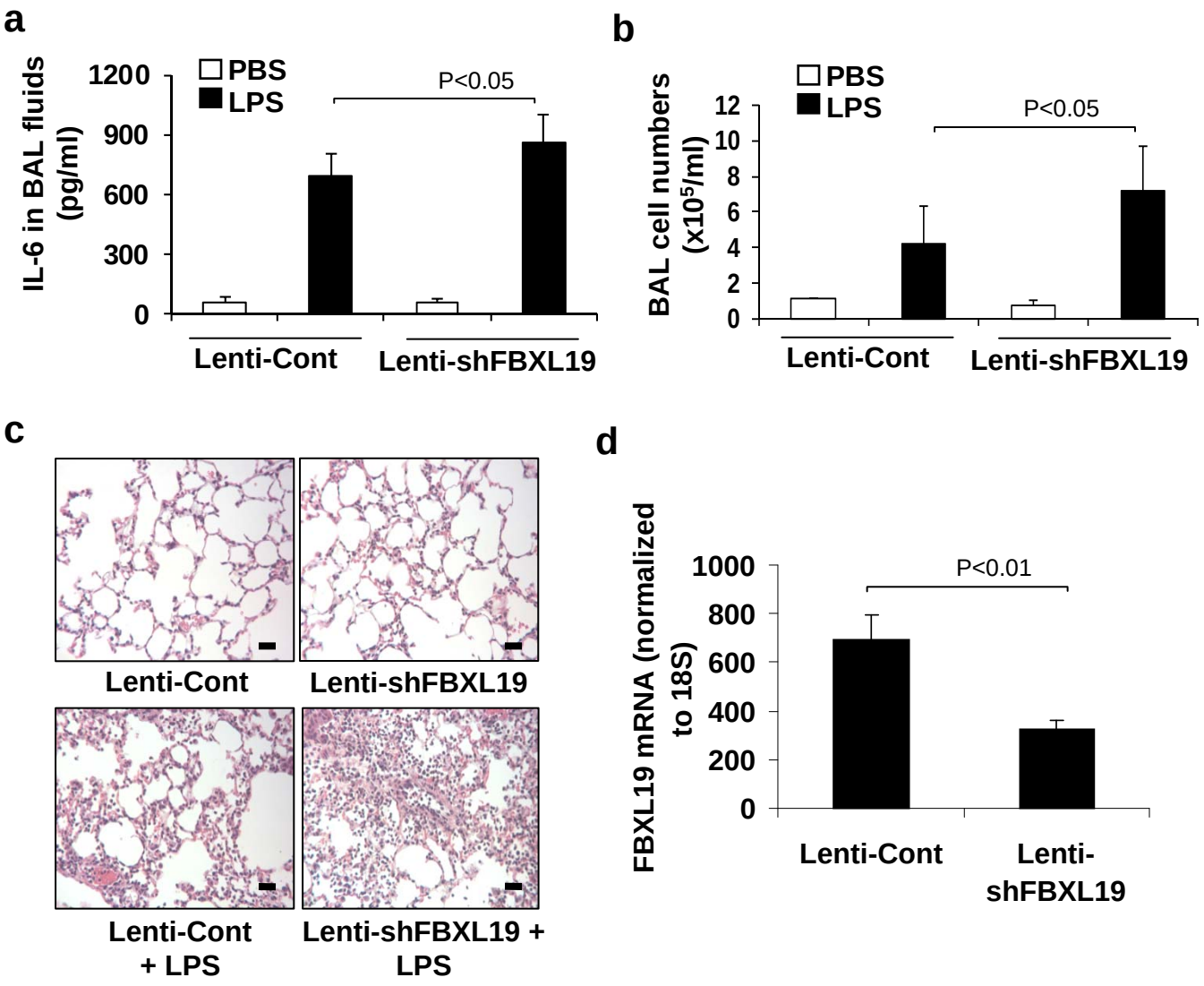

Supplementary Figure 7. shFBXL19 enhances LPS-induced lung inflammation. Control Lentivirus and Lenti-shFBXL19 were instilled by i.t. into C57/BL6 mice (4-6/ group) for 7 days, and then LPS (1 mg/kg weight) was given i.t. to Lenti-control or Lenti-FBXL19 infected mice. IL-6 **(a)** and cell numbers (**b)** in BAL fluid was analyzed by ELISA kit or cell counter. **(c)** Lung tissues were stained by H&E. Scale bar, 12.5 μm. Shown are representative images (*n=4*). **(d)** RNA was extracted from lung tissue and mRNA levels of FBXL19 was determined by real-time PCR.

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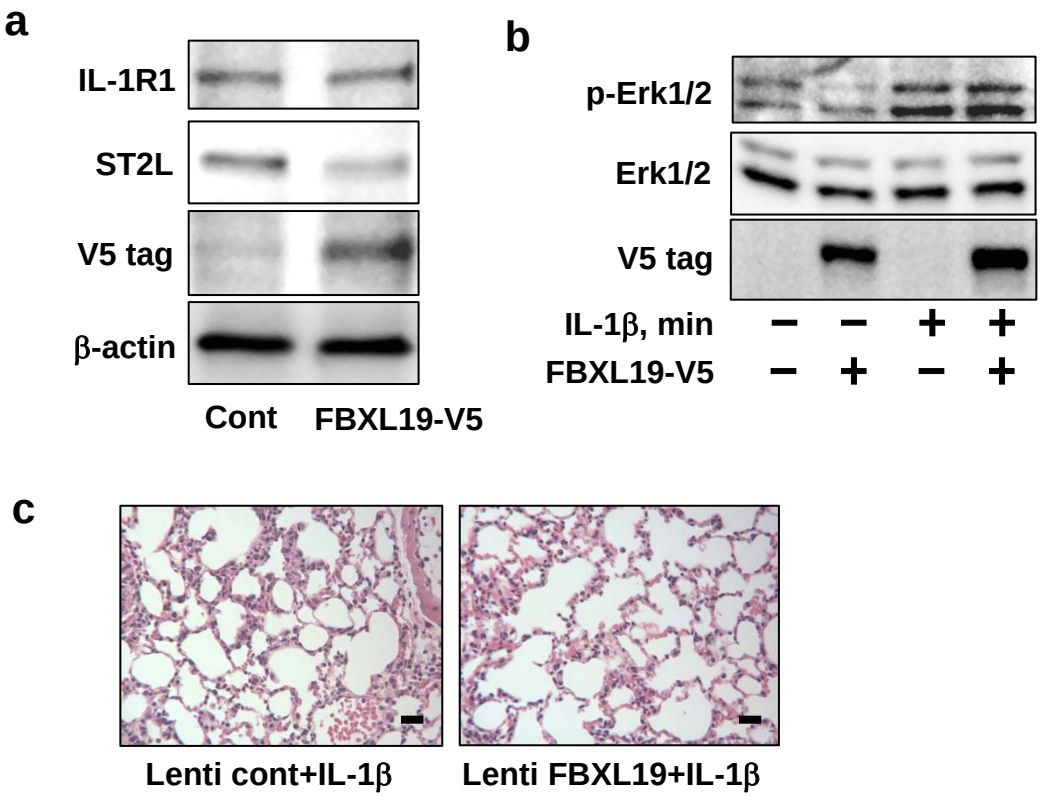

Supplementary Figure 8. FBXL19 does not alter IL-1β signaling. (a) MLE12 cells were transfected with FBXL19-V5 plasmid for 24 h. Cell lysates were subjected to IL-1R1, ST2L, V5 tag, and β-actin immunoblotting. **(b)** MLE12 cells were transfected with FBXL19-V5 plasmid for 24 h prior to IL-1β treatment (20 ng/ml, 5 min). Cell lysates were subjected to phospho-Erk1/2, Erk1/2, or V5 tag immunoblotting. Data are from one experiment representative of three. **(c)** IL-1β (4 μg/mouse) was given i.t. to the Lenti-control or Lenti-FBXL19 infected mice. Lung tissues were stained by H&E. Scale bar, 12.5 μm. Shown are representative images (*n=4*).

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