Cell Reports, Volume *38*

Supplemental information

L-plastin enhances NLRP3 inflammasome assembly and bleomycin-induced lung fibrosis

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Figure S1

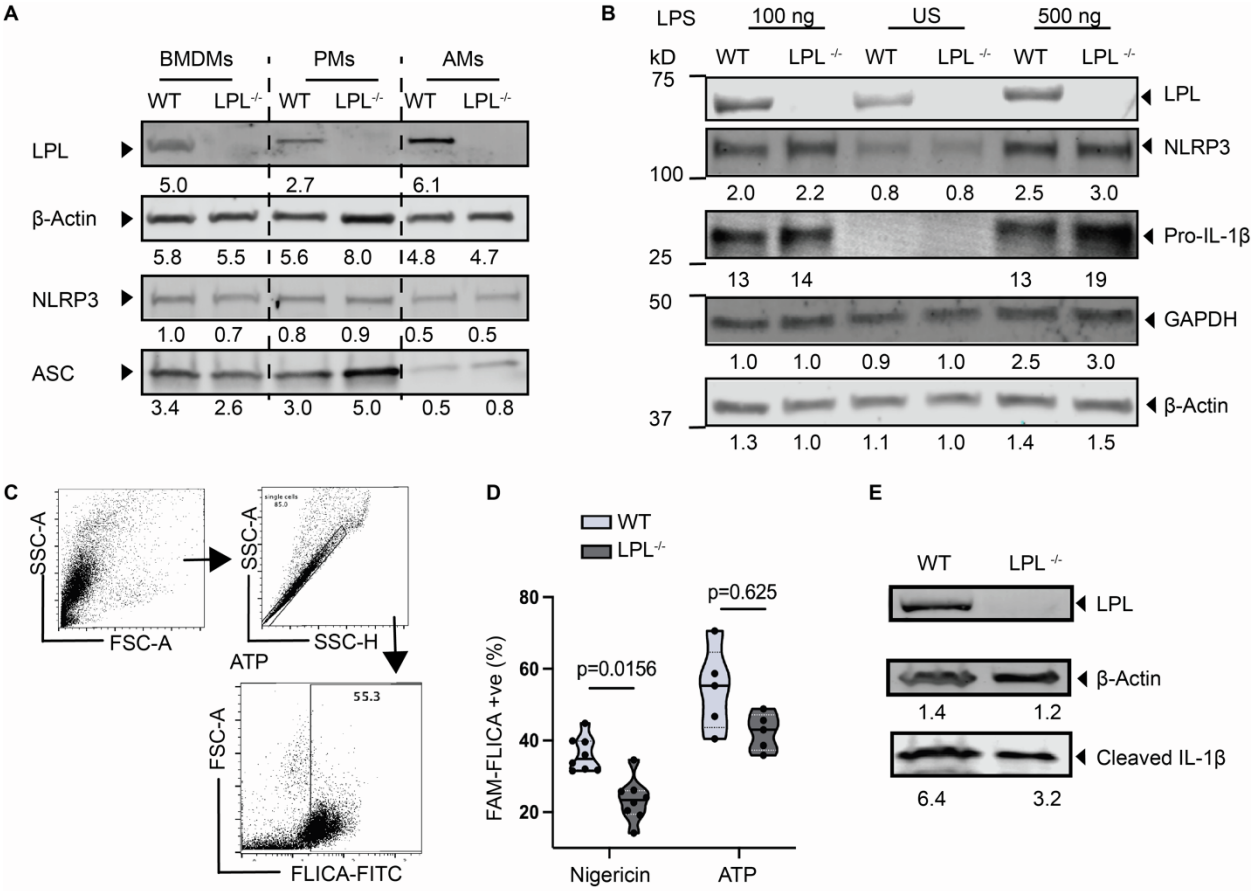

Figure S1. LPL$^{-/-}$ macrophages have normal expression of NLRP3 pathway proteins and priming signal while reduced caspase-1 activation and IL-1β cleavage upon NLRP3 activation, Related to Figure 1 and Figure 2.

(A) Cell lysates from freshly harvested BMDMs, peritoneal macrophages (PMs) and AMs were analyzed by western blotting. Expression of NLRP3 and ASC proteins were probed in WT and LPL$^{-/-}$ cells with quantification shown below. Here, β-actin serves as loading control and dotted lines were added to visually separate the different macrophage lineages. Results representing 2 independent replicate experiments are shown. **(B)** BMDMs were stimulated with LPS with indicated dose for 4 h and cell lysates probed for pro-IL-1β and NLRP3. Corresponding densities are shown in box below. Here, β-actin and GAPDH serve as cell lysate loading controls. Results representing 3 independent experiments are shown. **(C, D)** AMs exposed to nigericin and ATP to activate NLRP3 after LPS priming and stained with FAM-FLICA for detecting active caspase-1. Representative FACS analysis (C) and frequencies of FAM-FLICA containing cells (D). Results representing 3 biological replicates with data points, n=8 (nigericin), n=5 (ATP) shown. In graphs, solid line shown at median and dashed lines represents interquartile range. P-values calculated with Wilcoxon test **(E)** AMs primed with LPS and treated with nigericin (30 m) and cell lysates were analyzed for cleaved IL-1β. Densitometry values are shown below each band, β -actin serves as total cell control. Results representing 4 independent biological replicates.

Figure S2

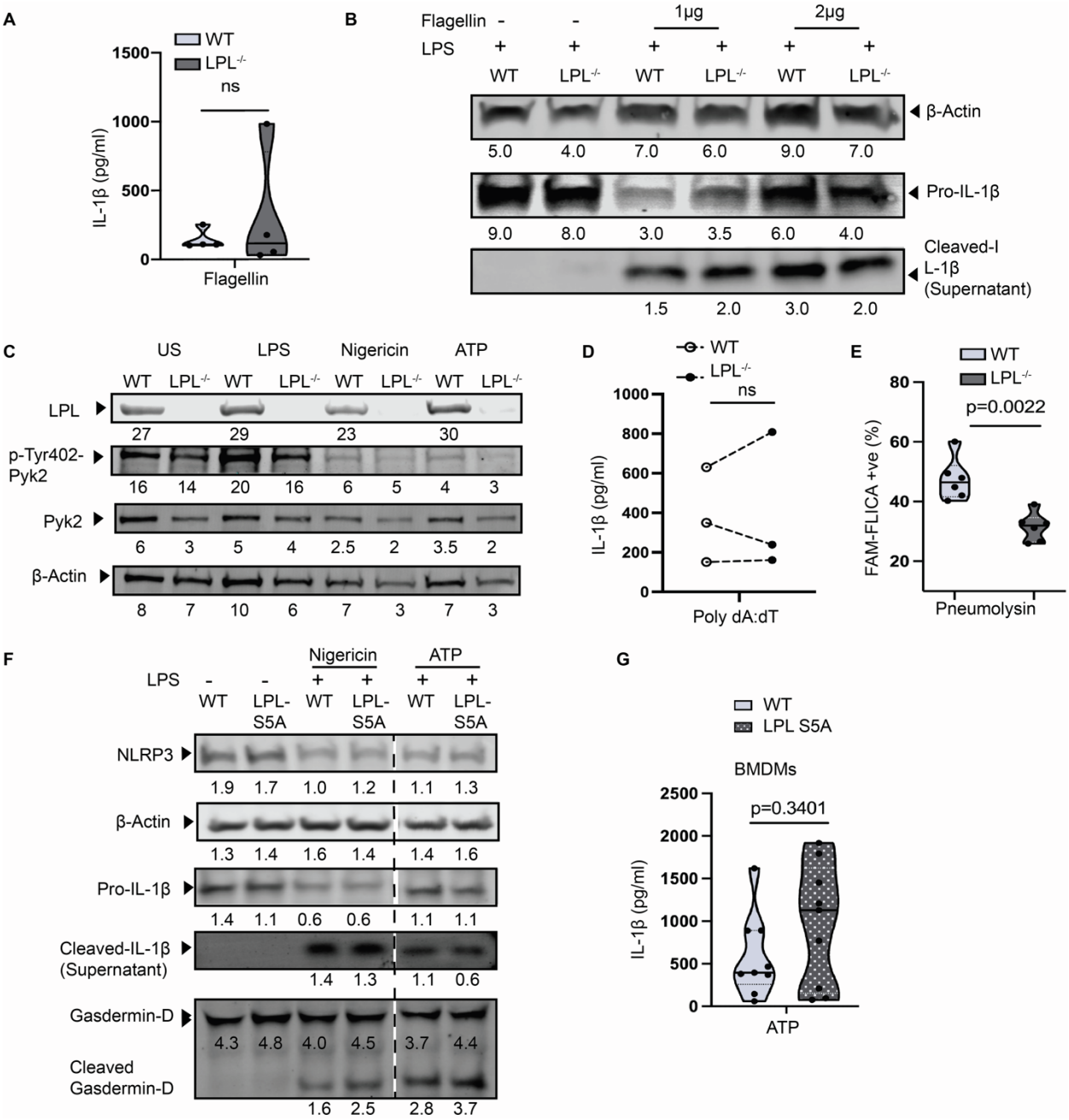

Figure S2. LPL is dispensable for NLCR4 and AIM2 inflammasome activation, and Ser-5 phosphorylation of LPL is not required for NLRP3 activation, Related to Figure 4 and Figure 5.

(A, B) BMDMs were primed with LPS (500ng/ml) for 4 hrs and then transfected with flagellin protein (1μg/ml, 1h) for NLRC4 inflammasome activation. Culture supernatants were analyzed for IL-1β using ELISA. Results representing n=4 from 3 independent experiments **(B)** BMDMs were LPS primed and transfected with indicated amount of flagellin protein. Culture supernatants and cell lysates subjected to immunoblot analysis to probe pro- IL-1β and cleaved IL-1β, respectively. Densities shown in box below each band. β-actin used as loading control. Representative immunoblot from 3 independent experiments

is shown. **(C)** BMDMs from WT and LPL-/- mice were stimulated with LPS for 4 h and then activated for NLRP3 via nigericin (20 μM) or ATP (5 mM) for 30 min. Cell lysates were resolved by SDS-PAGE and analyzed by immunoblotting for phosphorylated (Tyr- 402) Pyk2 and total Pyk2. Here, density of each band is shown below and β-actin used as loading control. Rrepresentative immunoblot from 4 independent experiments **(D)** BMDMs were primed with LPS (500ng/ml) and transfected with Poly(dA:dT) 5 ug/ml, 2 h) and culture supernatant were analyzed by ELISA for IL-1β detection. Results representing n=3 from 3 biological replicate experiments. (**E**) LPS primed AMs exposed to pneumolysin to activate NLRP3 and stained with FAM-FLICA for detecting active caspase-1 and frequencies of FAM-FLICA containing cells analyses by flowcytometry. Results showing n=6 data points from 3 independent experiments. **(F**) BMDMs from WT and LPL-S5A mice were primed with LPS and then activated with Nigericin or ATP. Cell lysates were analyzed by immunoblotting for gasdermin-D and culture supernatant by immunoblotting for cleaved IL-1β. Here, NLRP3 and pro-IL-1β indicate priming, and β-actin serves as loading control. Dotted line indicates cropping of duplicate samples from immunoblot. Reprentative immunoblot from 4 independent experiments. **(G)** Culture supernatant from NLRP3 activated (nigericin) WT and LPL S5A BMDMs analyzed for IL-1β using ELISA. Represented results from n=9 data points from 4 independents experiments are shown. In A, D, E and F, each dot represents individual data point with median (solid line) and interquartile range (dashed line). p-values calculated with Wilcoxon (A, D) and Mann Whitney (E, G).

Figure S3

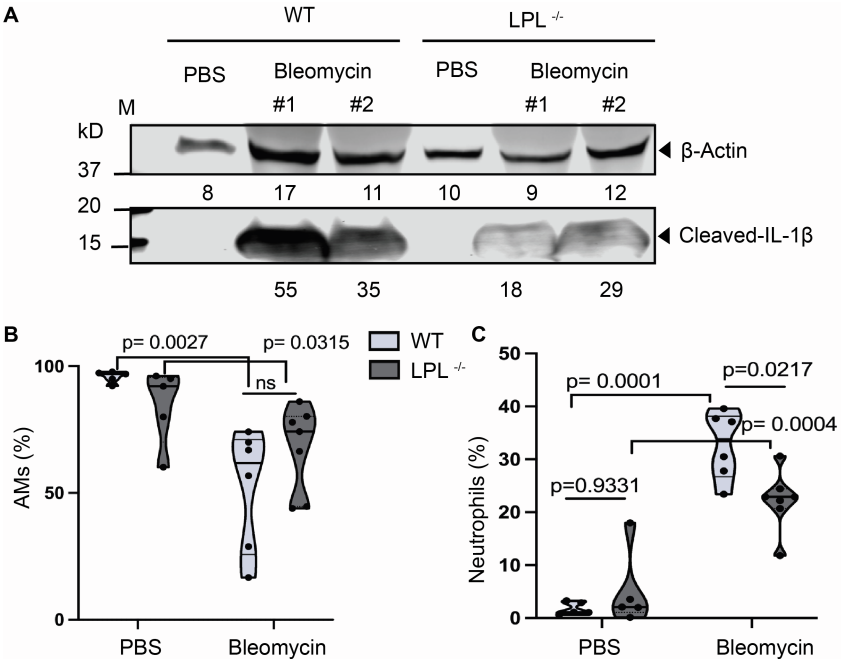

Figure S3. In LPL$^{-/-}$ mouse lungs, bleomycin-induced IL-1β production and recruitment of inflammatory cells are inhibited, Related to Figure 6.

(**A**) Mice were challenged (i. n.) with a single dose of bleomycin (2 mg/kg) and BAL obtained on day 21 after challenge. IL-1β was detected from BAL exudate by immunoblot analysis (density shown below bands) from PBS and 2 bleomycin-treated mice. β-actin used as loading control. **(B, C)** Post 24 h of bleomycin treatment, BALF from WT and LPL$^{-/-}$ mice was analyzed using flow cytometry. Frequencies of AMs (CD11c+ Siglec F+) (**B**) and neutrophils (CD11b+ Ly6G+) (**C**) in CD45+ population were quantified. Here, each symbol represents individual mice and data represent 3 independent experiments, showing median (solid line) and interquartile range (dashed line). p-values calculated from ANOVA test.

Figure S4

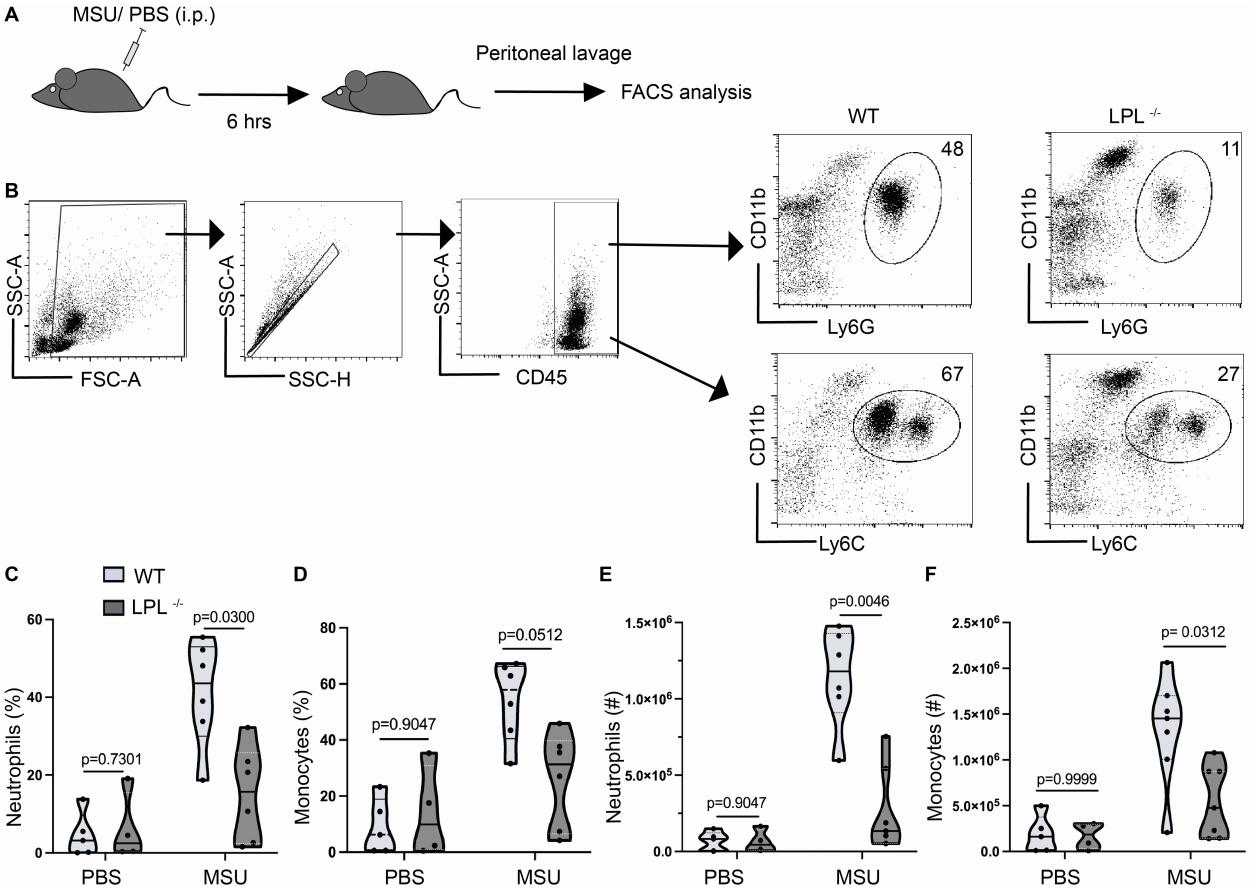

Figure S4. LPL$^{-/-}$ mice exhibit reduced peritoneal infiltration of inflammatory cells after MSU challenge, Related to Figure 6.

(A) Schematic representation of MSU induced peritonitis experiment wherein WT and LPL$^{-/-}$ mice are intraperitoneally injected with 250 μg MSU. **(B)** Representative flow cytometric analysis of peritoneal exudate from 3 independent experiments is shown Neutrophils identified as CD11b+ Ly6G+ and monocytes identified as CD11b+ Ly6C+. **(C, D)** Frequencies of neutrophils (C) and monocytes (D) in peritoneal exudate were obtained by flow cytometry analysis. **(E, F)** Absolute numbers of neutrophils (E) and monocytes (F) counted by flowcytometry. Values are graphed with median (at solid dashed line) and interquartile range (in light dashed line). Here, each symbol represents one mouse (n=6) and data combined from 3 independent experiments. p-values obtained from Mann Whitney test.