

*JCI insight – Research article*

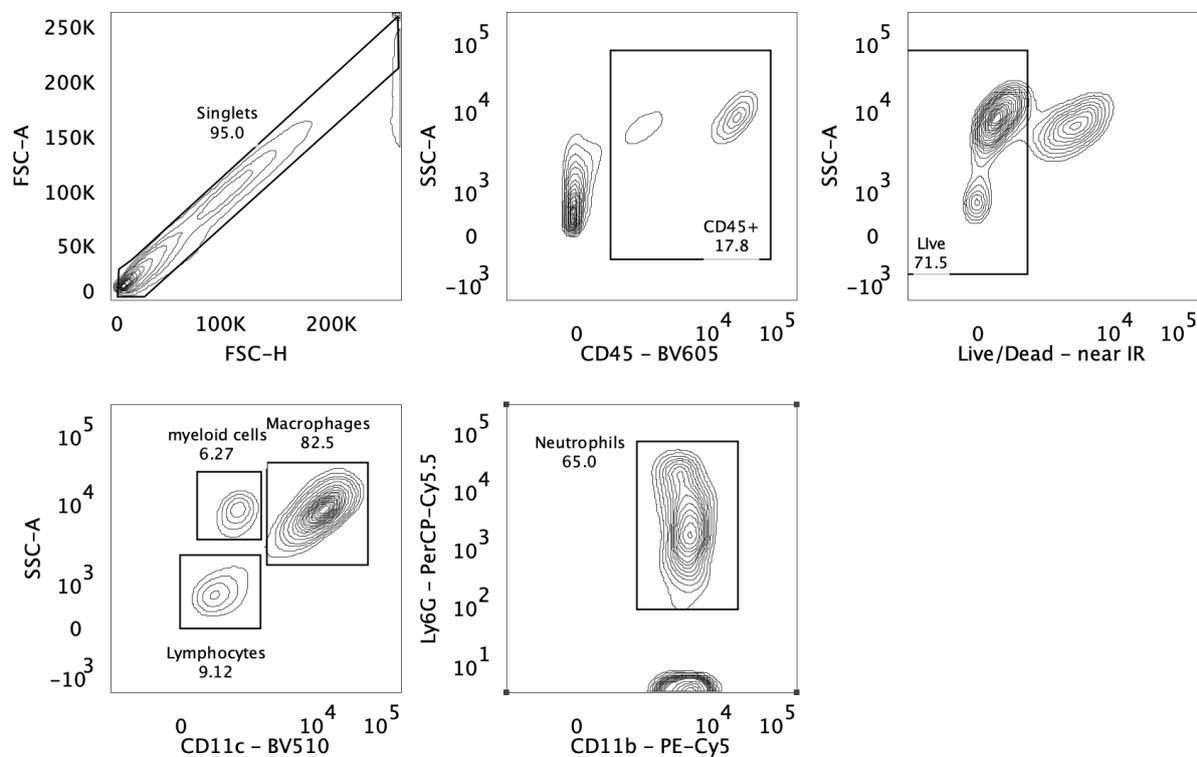
**An in vivo model for extracellular vesicle-induced emphysema**

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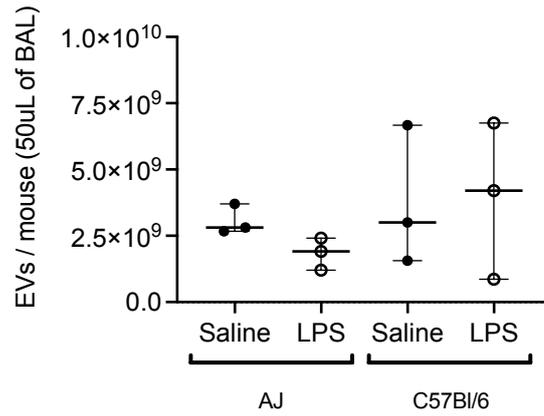
Gaggar A.<sup>1-4\*</sup>, Genschmer K.R.<sup>1,2#</sup>, Blalock J.E.<sup>1,3#</sup>

**Figures: 6**

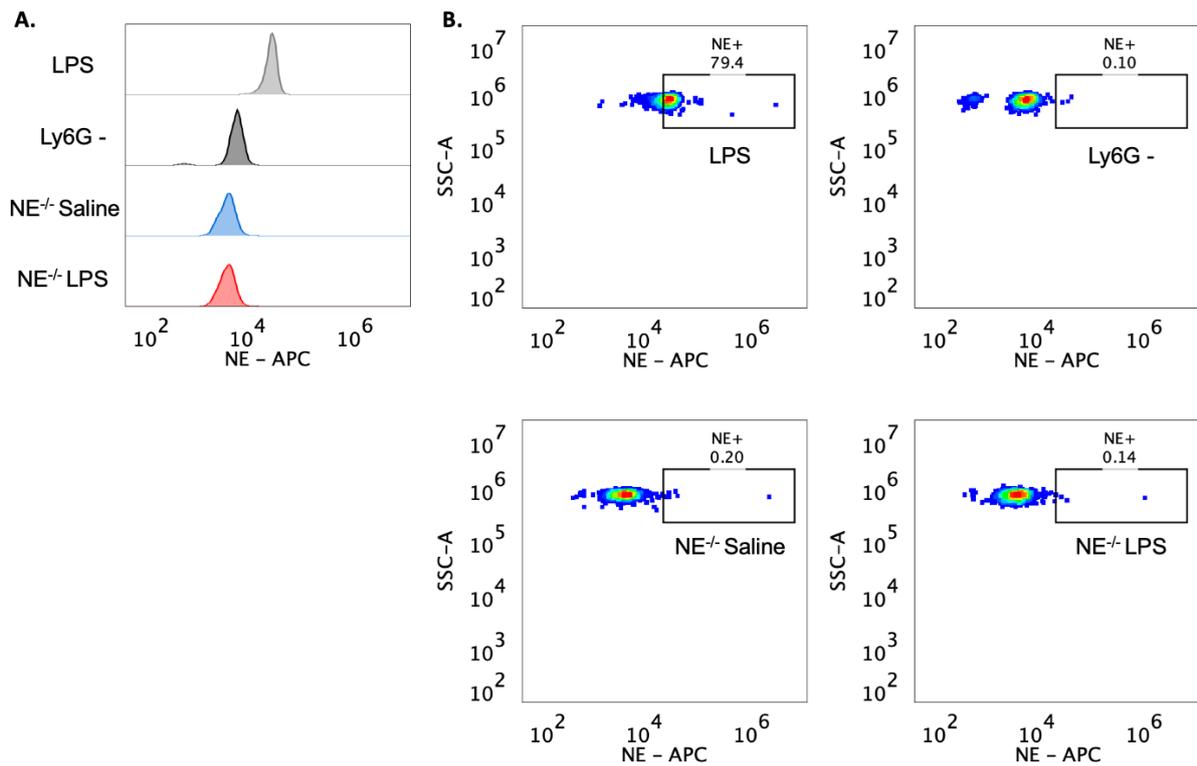
## Supplemental Figures



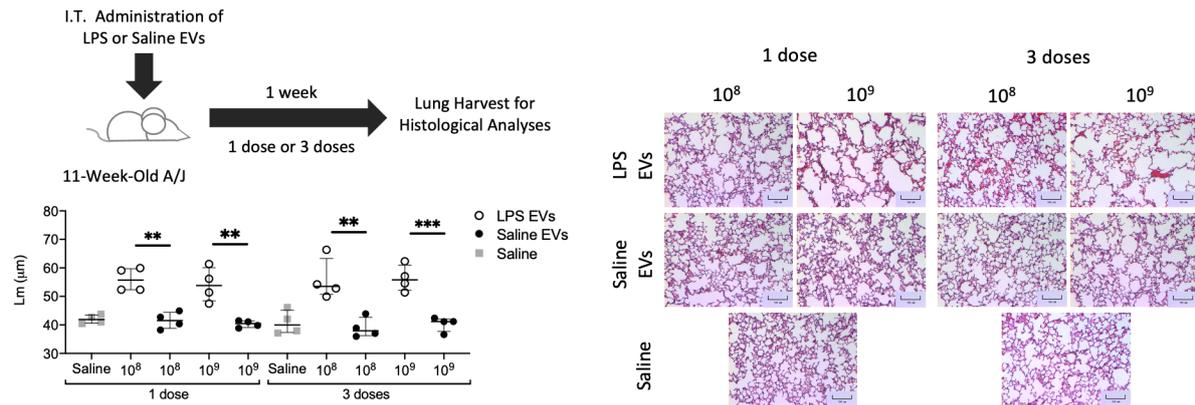
**Figure S1. Flow cytometry gating strategy.** Airway cells were isolated from BAL of saline or LPS-treated mice and analyzed by flow cytometry. Live neutrophils were identified as  $SSC-A^{hi}$   $CD11c^{-}$   $Ly6G^{+}$   $CD11b^{+}$ , macrophages as  $SSC-A^{hi}$   $CD11c^{+}$   $Ly6G^{-}$ , and lymphocytes as  $SSC-A^{lo}$   $CD11c^{-}$   $Ly6G^{-}$ .



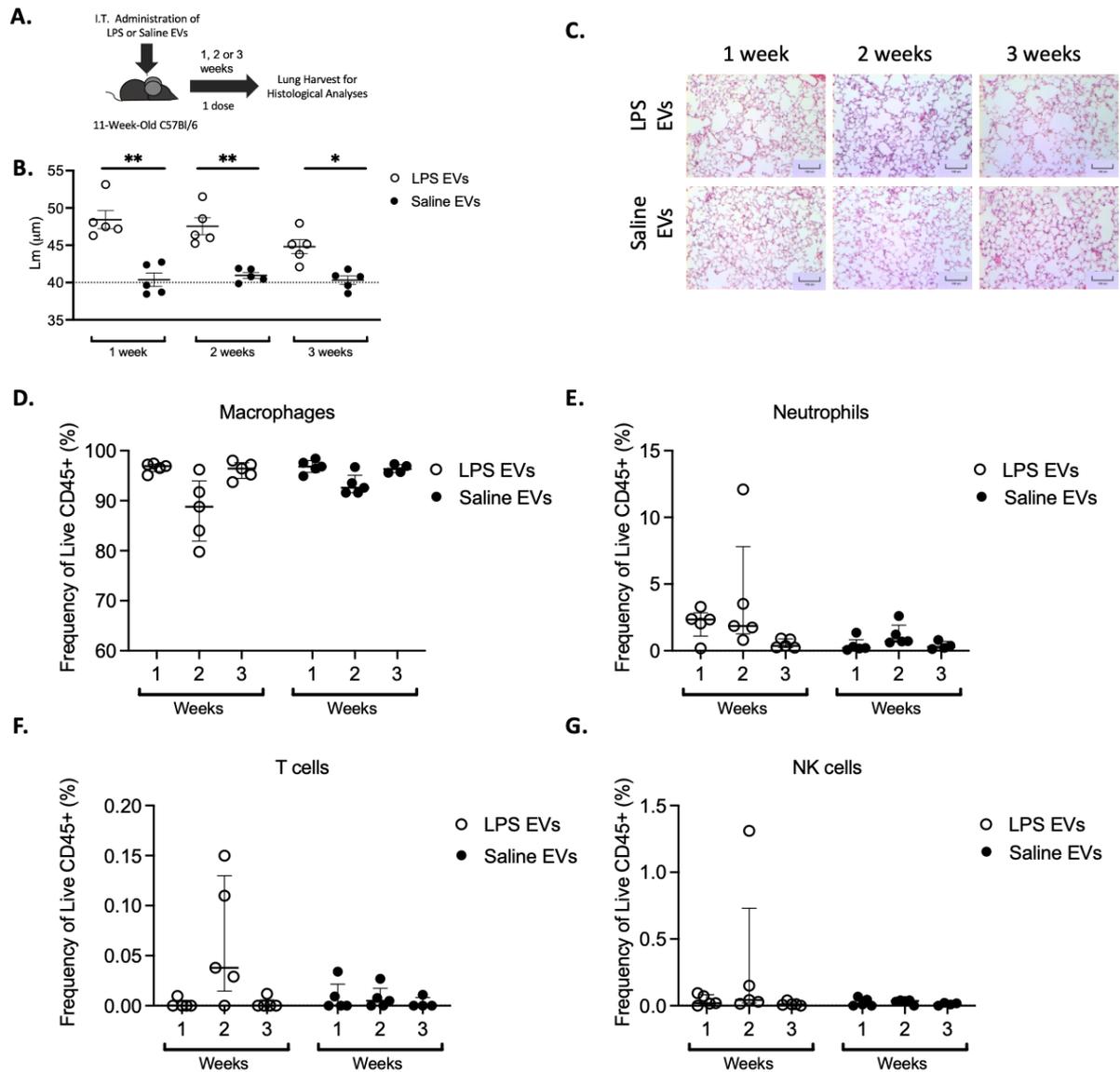
**Figure S2. Quantification of airway EVs.** EVs were harvested from BAL fluid 24 hours following saline or LPS (35ug) treatment of 11-week-old A/J mice or C57Bl/6 mice. Data are shown as median and interquartile range (n=3 independent experiments). Statistical analyses were performed using Wilcoxon signed-rank test, ns = not significant.



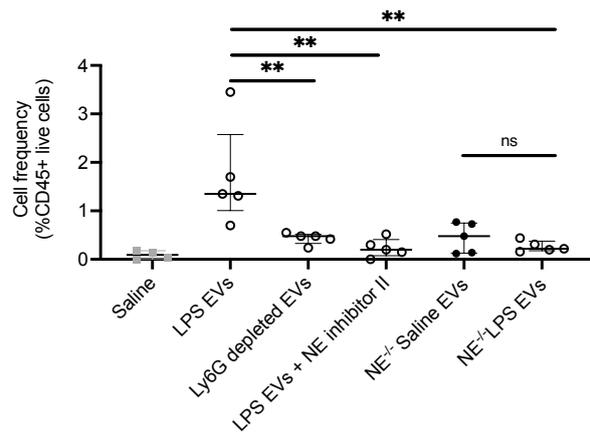
**Figure S3. Characterization of surface NE on mouse EVs.** (A) Surface NE quantification on mouse airway EVs by bead-based flow cytometric analysis. From top to bottom: LPS treated wild type mice - CD63 EV pull-down (grey); LPS treated wild type mice - CD63 EV pull-down after Ly6G<sup>+</sup> EV depletion (black); saline treated NE<sup>-/-</sup> mice - Ly6G pull-down (blue); LPS treated NE<sup>-/-</sup> mice - Ly6G pull-down (red). (B) Quantification of NE<sup>+</sup> EVs for the groups described in (A).



**Figure S4. Dosing strategy for saline or LPS intratracheal EVs delivery.** EVs were transferred intratracheally to 11-week old female A/J mice. Mice received  $10^9$  or  $10^8$  exosomes in either a single dose or over 3 doses for the duration of 1 week. Mean Linear Intercepts ( $L_m$ ) were quantified one week from the initial treatment as a measure of alveolar enlargement ( $n=4$  per group). Representative images (H&E) of EVs-treated mice (LPS and saline). Scale bars represent  $100\ \mu\text{m}$ . Data are shown as median and interquartile range ( $n=1$  experiment). Statistical analyses were performed using Wilcoxon signed-rank test, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns = not significant.



**Figure S5. Time course upon EVs delivery.** (A)  $10^7$  EVs were transferred intratracheally in a single dose to 11-week old female C57BL/6 mice. Lungs were harvested 1 week, 2 weeks and 3 weeks after the initial dose. (B)  $L_m$  were quantified one week from the initial treatment ( $n=5$  per group). (C) Representative images (H&E) of EV-treated mice (LPS and saline). Scale bars represent  $100 \mu\text{m}$ . (D-G) Immune cells in the BAL of mice treated with  $10^7$  Evs were characterized by flow cytometry as defined in Figure S1. T cells were identified as  $\text{CD3}^+$  and NK cells as  $\text{NK1.1}^+$ . Data are shown as median and interquartile range ( $n=1$  experiment). Statistical analyses were performed using Kruskal-Wallis test, \*\*  $p < 0.01$ , \*  $p < 0.05$ .



**Figure S6. Flow cytometry of BAL after EV delivery.** Neutrophils were quantified by flow cytometry in the BAL of mice treated with one  $10^7$  dose of saline or LPS EVs from WT or NE KO mice (n=5 per group). Data are shown as median and interquartile range (n=1 experiment). Statistical analysis was performed with Kruskal-Wallis test, \*\* p < 0.01, ns = not significant.