Expanded View Figures

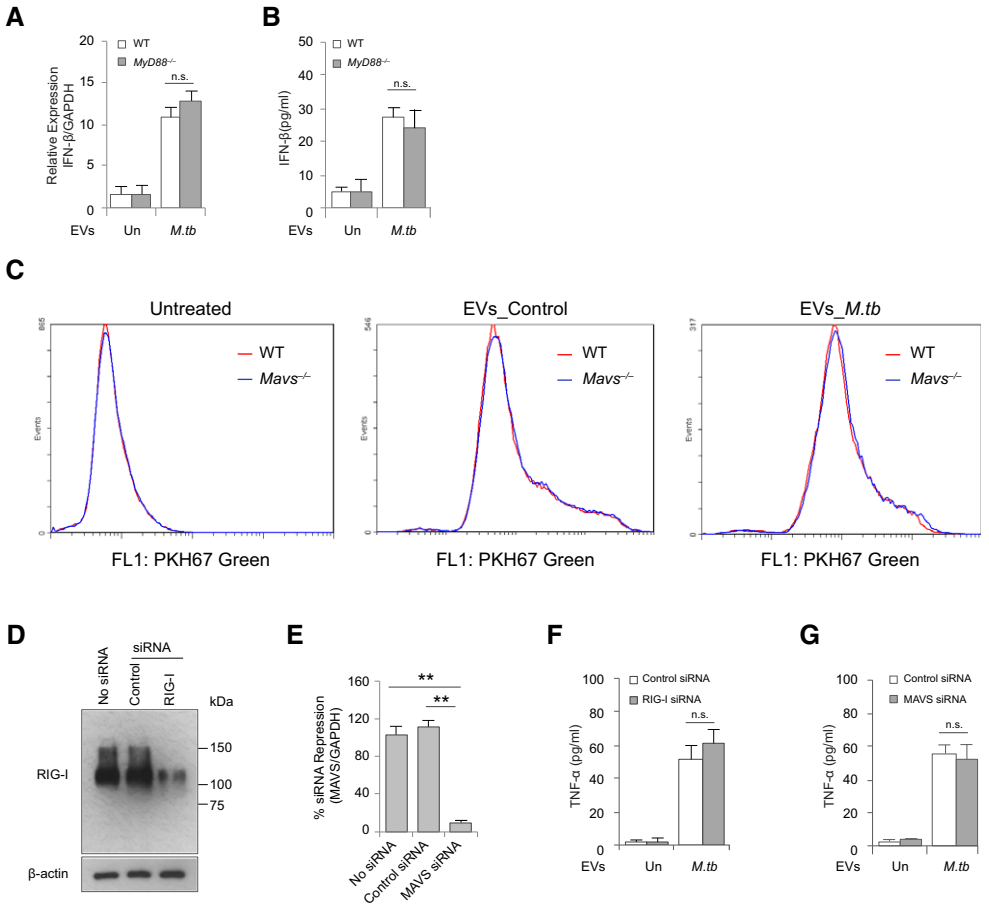

Figure EV1. Evaluating the role of MyD88, MAVS and Rig-1 in EV-induced cytokine production and EV uptake.

A Quantitative real-time PCR for IFN-β expression in WT and *MyD88*$^{-/-}$ BMMs at 5 h after treatment with EVs isolated from uninfected (Un) or *M.tb*-infected (*M.tb*) macrophages.

B Similar to (A), but IFN-β protein in BMM culture supernatant was measured using ELISA at 24 h.

C EV uptake assay. WT and *Mavs*$^{-/-}$ BMMs were treated with PKH67 green-labeled EVs for 4 h, and then, EV uptake rate was determined by flow cytometry.

D, E Western blot (D) and quantitative real-time PCR (E) to determine siRNA knockdown efficiency for RIG-I (D) and MAVS (E), respectively, in BMMs.

F, G ELISA analysis for TNF-α in the culture supernatant of BMMs pre-treated with control, RIG-I (F), or MAVS (G) siRNA, followed by EV treatment for 24 h.

Data information: Data shown in (A, B, and E–G) are the mean ± SD of three wells, and all results shown are representative of three independent experiments. n.s., not significant; $^{**}P < 0.01$ by Student's *t*-test (two-tailed).
Source data are available online for this figure.

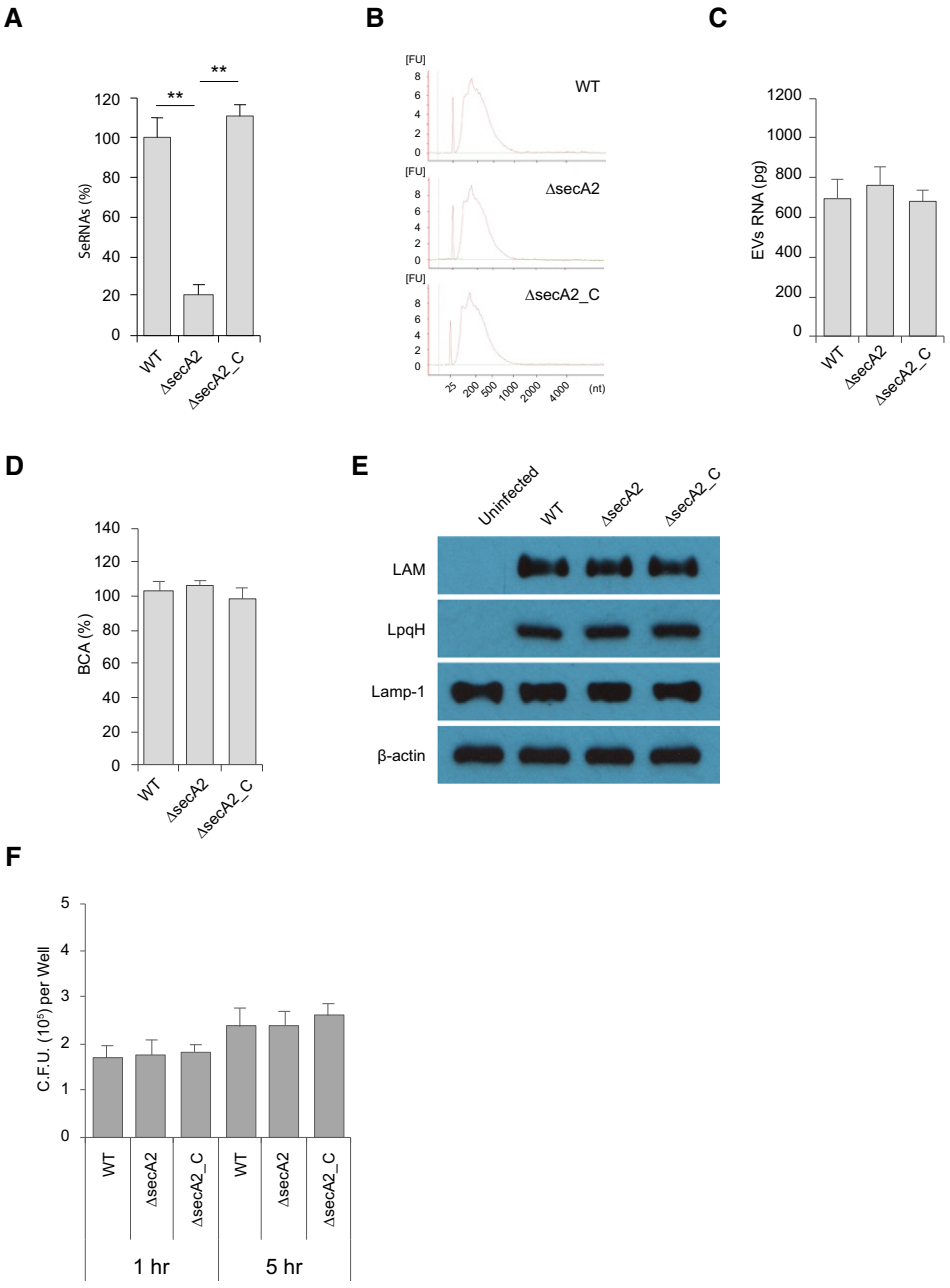

Figure EV2. Analyzing the affect of SecA2-deletion on mycobacterial and host RNA/protein/glycolipid transport to EVs.

A Released *M.tb* RNA in the culture supernatant from the same number of mycobacteria.
B RNA bioanalyzer analysis for total RNA isolated from EVs released by WT, ΔsecA2, or secA2-complemented (ΔsecA2_C) *M.tb* strains.
C Quantification of EV total RNA analyzed in (B).
D Quantification of total proteins of EVs from various *M.tb* strain-infected BMMs.
E Western blot analysis for host and *M.tb* proteins in EVs.
F *M.tb* survival assay in wild-type mouse BMMs.

Data information: Data shown in (A, C, D and F) are the mean ± SD of three independent cultures (A) or infections (C, D and F), and all results shown are representative of three independent experiments. $^{**}P < 0.01$ by Student's *t*-test (two-tailed).
Source data are available online for this figure.

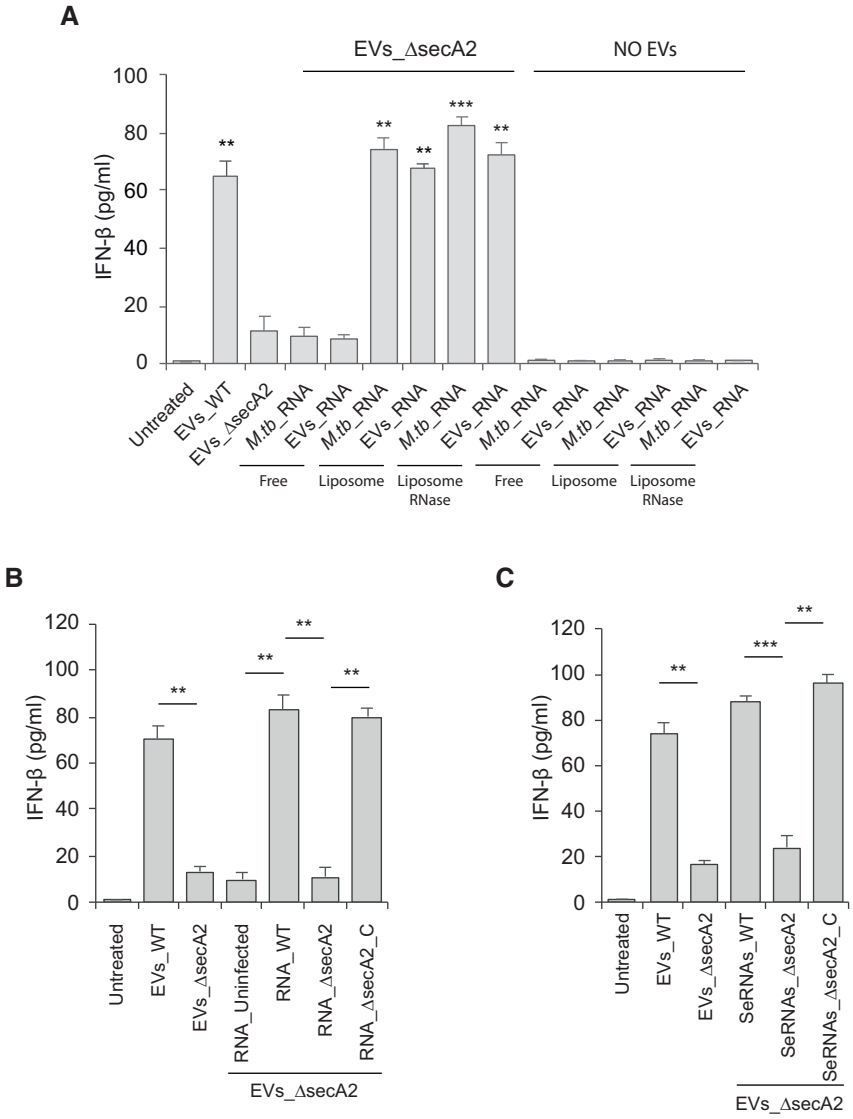

Figure EV3. EV-induced IFN-β production by BMMs requires the mycobacterial RNA to be lipid encapsulated.

A ELISA analysis for IFN-β secreted by BMMs treated with EVs from ΔsecA2-infected macrophages in combination with free or liposomal (with or without RNase treatment) *M.tb* RNA or RNA from EVs released by WT *M.tb*-infected macrophages. EVs_WT and EVs_ΔsecA2, EVs from WT or ΔsecA2 *M.tb*-infected macrophages, respectively; M.tb_RNA, RNA from *M.tb* cells; EVs_RNA, RNA from EVs released by WT *M.tb*-infected macrophages. $^{**}P < 0.01$ and $^{***}P < 0.001$, compared to EVs_ΔsecA2, by two-tailed Student's *t*-test.

B Similar to (A), but BMMs were co-treated with EVs_ΔsecA2 and liposomal RNA from EVs released by uninfected (RNA_Uninfected), WT (RNA_WT), ΔsecA2 (RNA_ΔsecA2), or secA2-complemented (RNA_ΔsecA2_C) *M.tb* strains.

C Similar to (B), but BMMs were co-treated with EVs_ΔsecA2 and liposomal RNA released (SeRNAs) by WT (SeRNAs_WT), ΔsecA2 (SeRNAs_ΔsecA2), or secA2-complemented (SeRNAs_ΔsecA2_C) *M.tb* strains.

Data information: In all tests, IFN-β production was measured 24 h post-treatment. Data shown are the mean ± SD of three independent treatments, and all results shown are representative of three independent experiments. n.s., not significant; $^{**}P < 0.01$ and $^{***}P < 0.001$ by Student's *t*-test (two-tailed).

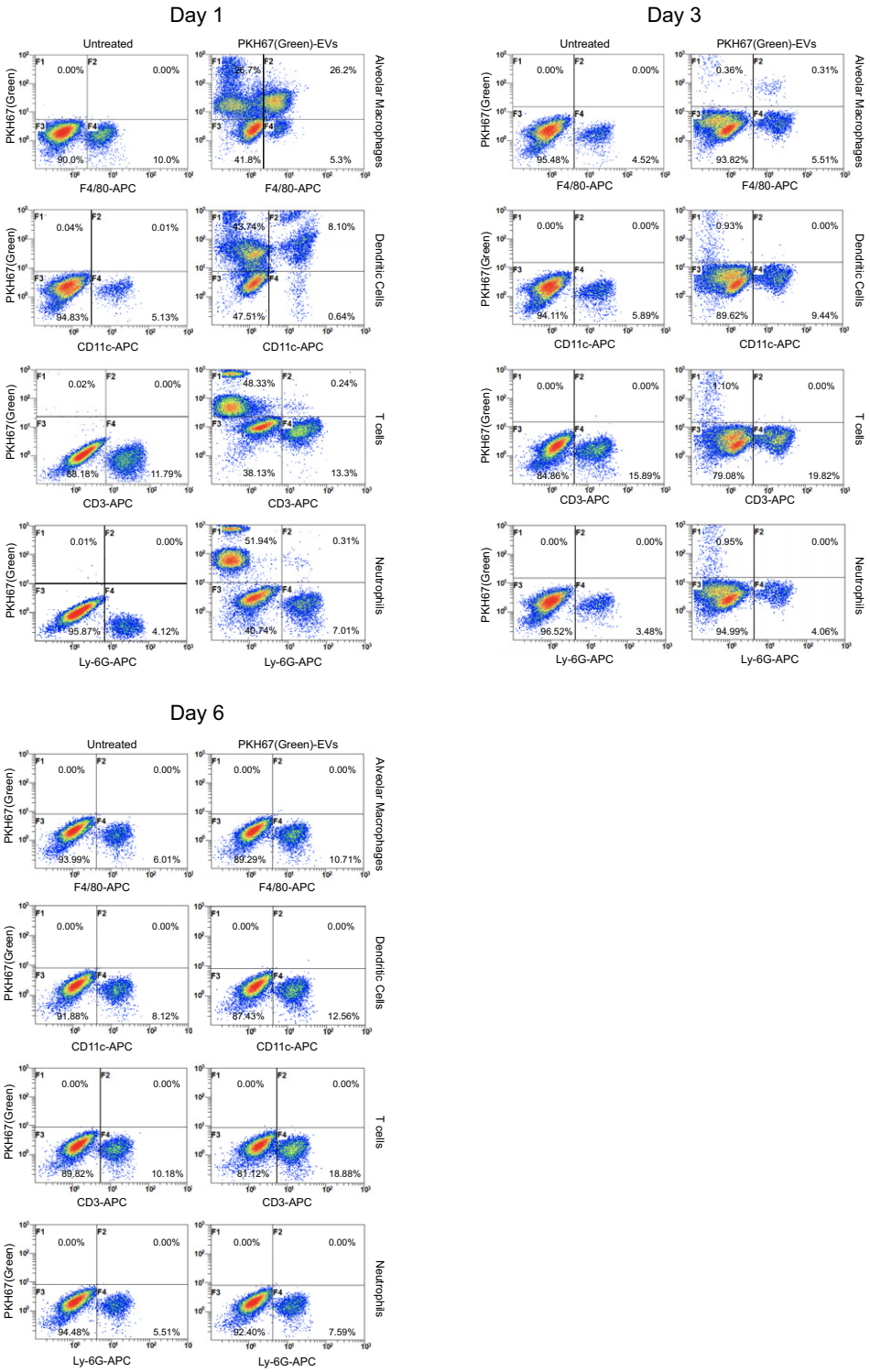

Figure EV4. EV uptake assay.

Wild-type C57BL/6 mice (n = 3 per group) were aerosol-infected with WT *M.tb* at a dose of 100–150 CFU/lung. Three weeks post-infection, PKH67 green-labeled EVs isolated from WT *M.tb*-infected macrophages were intratracheally injected into mice and the distribution of labeled EVs in different cell types of bronchoalveolar lavage (BAL) was determined by flow cytometry. Alveolar macrophages, APC-F4/80; DCs, APC-CD11c; T cells, APC-CD3; neutrophils, APC-Ly-6G. Data shown are representative of two independent experiments.