

Fig. S1. (A and B) Balb/c mice-derived alveolar macrophages (3×10^5 /well) were infected with different MOIs of RSV (0.1 – 10) for 6 h at 37°C with 5% CO₂. Afterwards, cells were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry (A) or cell death was assessed by LDH release in macrophages supernatants and expressed as % LDH release (B). **(C)** Alveolar macrophages (3×10^5 /well) were infected with RSV (MOI 1) for 2, 4 or 6 h. Afterwards, cells were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry. **(D)** Balb/c mice-derived peritoneal macrophages (3×10^5 /well) were infected with different MOIs of RSV (0.1 – 10) for 6 h at 37°C with 5% CO₂. Afterwards, cells were harvested and labeled with

Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry. **(E)** Balb/c mice-derived peritoneal macrophages (3×10^5 /well) were infected with different MOIs of RSV (0.1 – 10) for 6 h. Then, cell death was assessed by LDH release in macrophages supernatants and expressed as % LDH release. **(F)** Peritoneal macrophages (3×10^5 /well) were infected with RSV (MOI 1) for 2, 4 or 6 h. After that, cell death was assessed by LDH release in macrophages supernatants and expressed as % LDH release. Data are representative of 3 independent experiments performed in triplicates and represent mean \pm SEM. Data were analyzed with one-way ANOVA with Tukey's post-hoc test. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

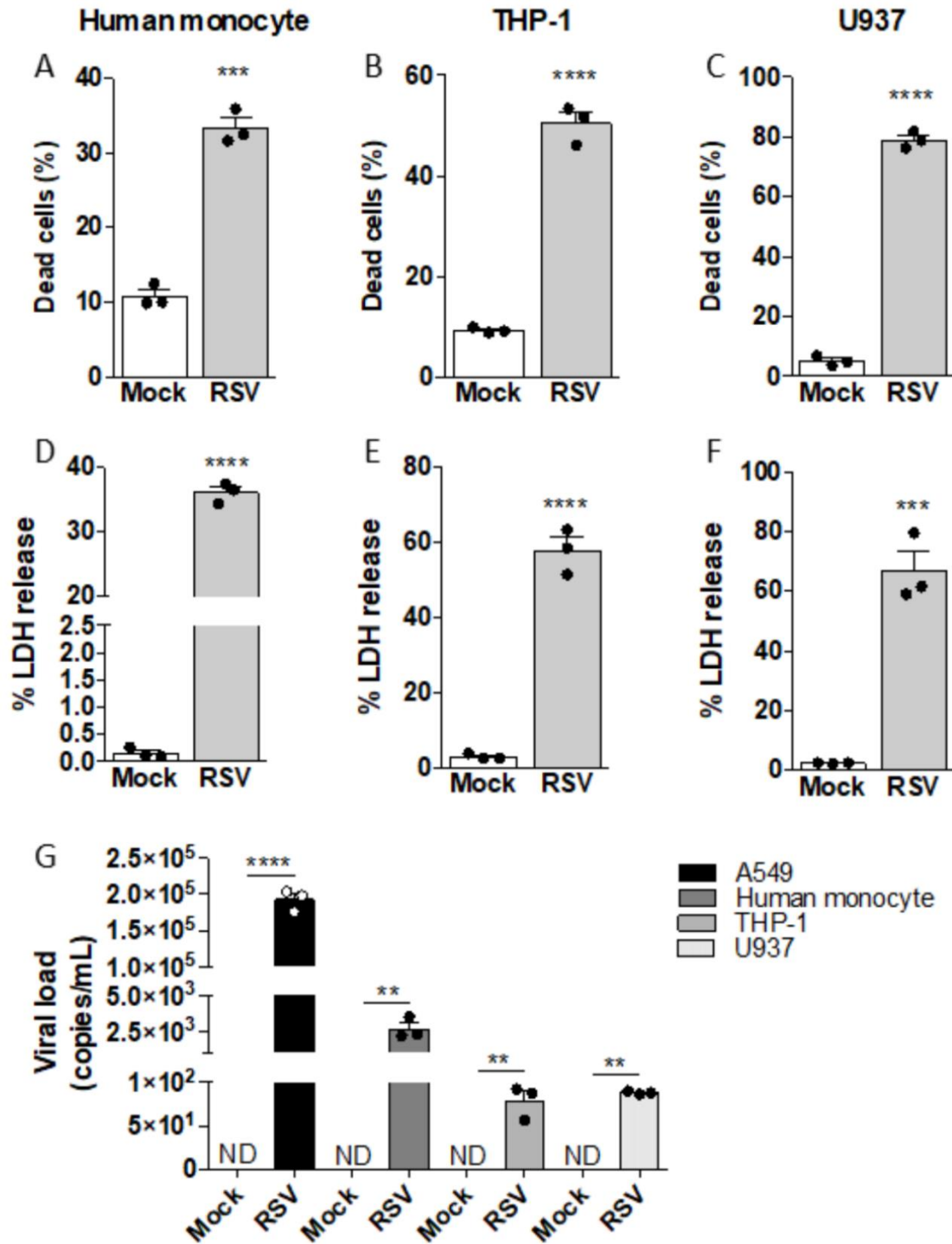


Fig. S2. (A, D) Human monocytes (3×10^5 /well) were infected with RSV (MOI 1) for 6 h. Afterwards, cells were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry (A) or cell death was assessed by LDH

release in cell supernatants and expressed as % LDH release (D). **(B, E)** THP-1 cells ($2 \times 10^5/\text{cm}^2$) were infected with RSV (MOI 1) for 6 h. After that, cells were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry (B) or cell death was assessed by LDH release in cell supernatants and expressed as % LDH release (E). **(C, F)** U937 cells ($2 \times 10^5/\text{cm}^2$) were differentiated to macrophage-like cells with PMA (50 ng/mL) for 24 h. After differentiation, cells were infected with RSV (MOI 1) for 6 h. Then, cells were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry (C) or cell death was assessed by LDH release in cell supernatants and expressed as % LDH release (F). **(G)** A549 cells, human monocytes, THP-1 cells and U937 cells ($3 \times 10^5/\text{well}$) were infected with RSV (MOI 1) for 6 h. Then, RNA was harvested and RSV viral loads were quantified the by real-time PCR and expressed as copies/mL. Data are representative of 3 independent experiments performed in triplicates and represent mean \pm SEM. Data were analyzed with unpaired Student's *t* test. ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001.

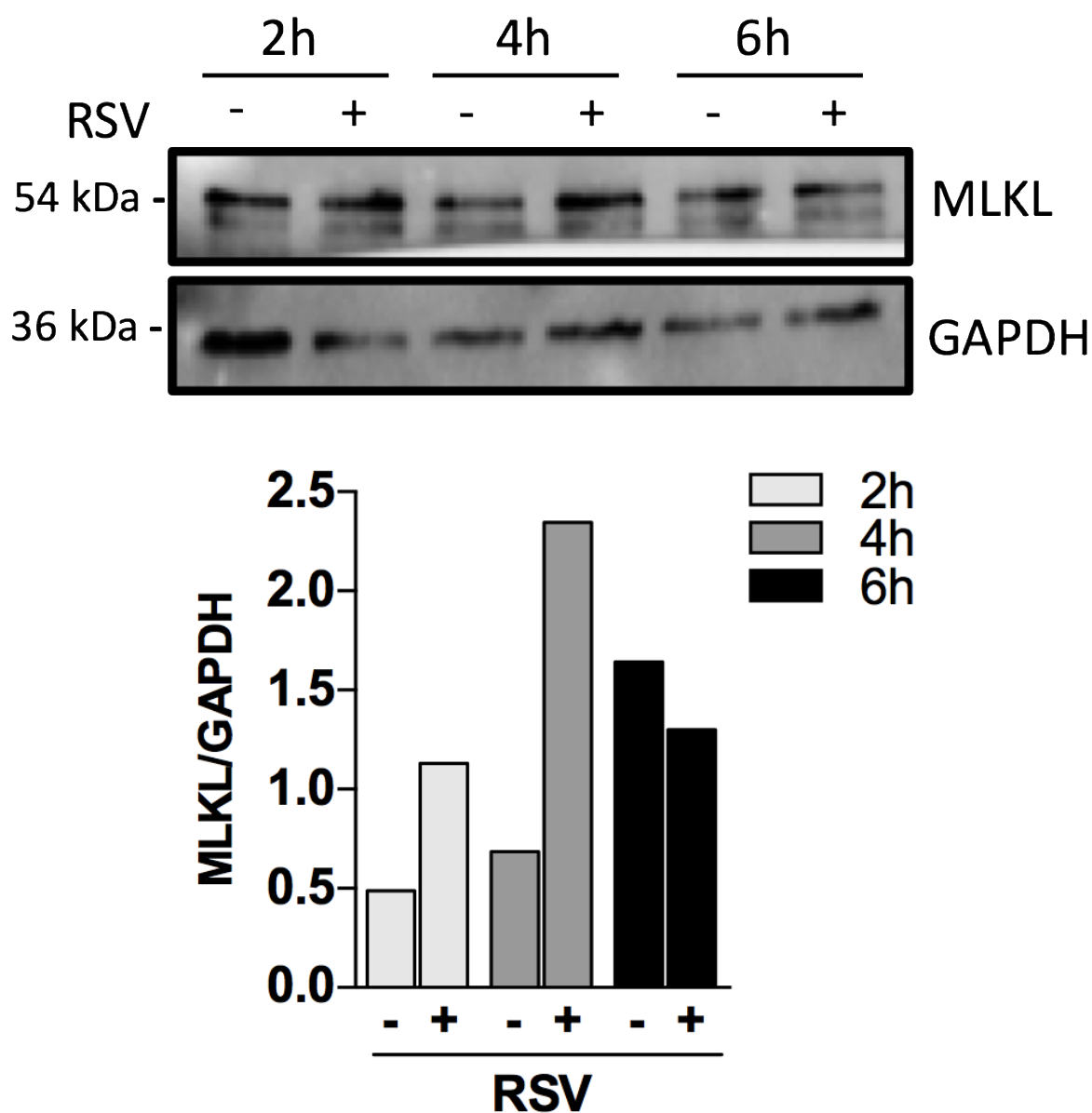


Fig. S3. Balb/c mice-derived alveolar macrophages were infected with RSV (MOI 1) for 2, 4 or 6 h. Cell lysates were examined for MLKL expression by western blot. The densitometry analysis was performed using ImageJ 1.43 software (NIH). MLKL bands were normalized to GAPDH bands. Data are representative of 2 independent experiments with similar results.

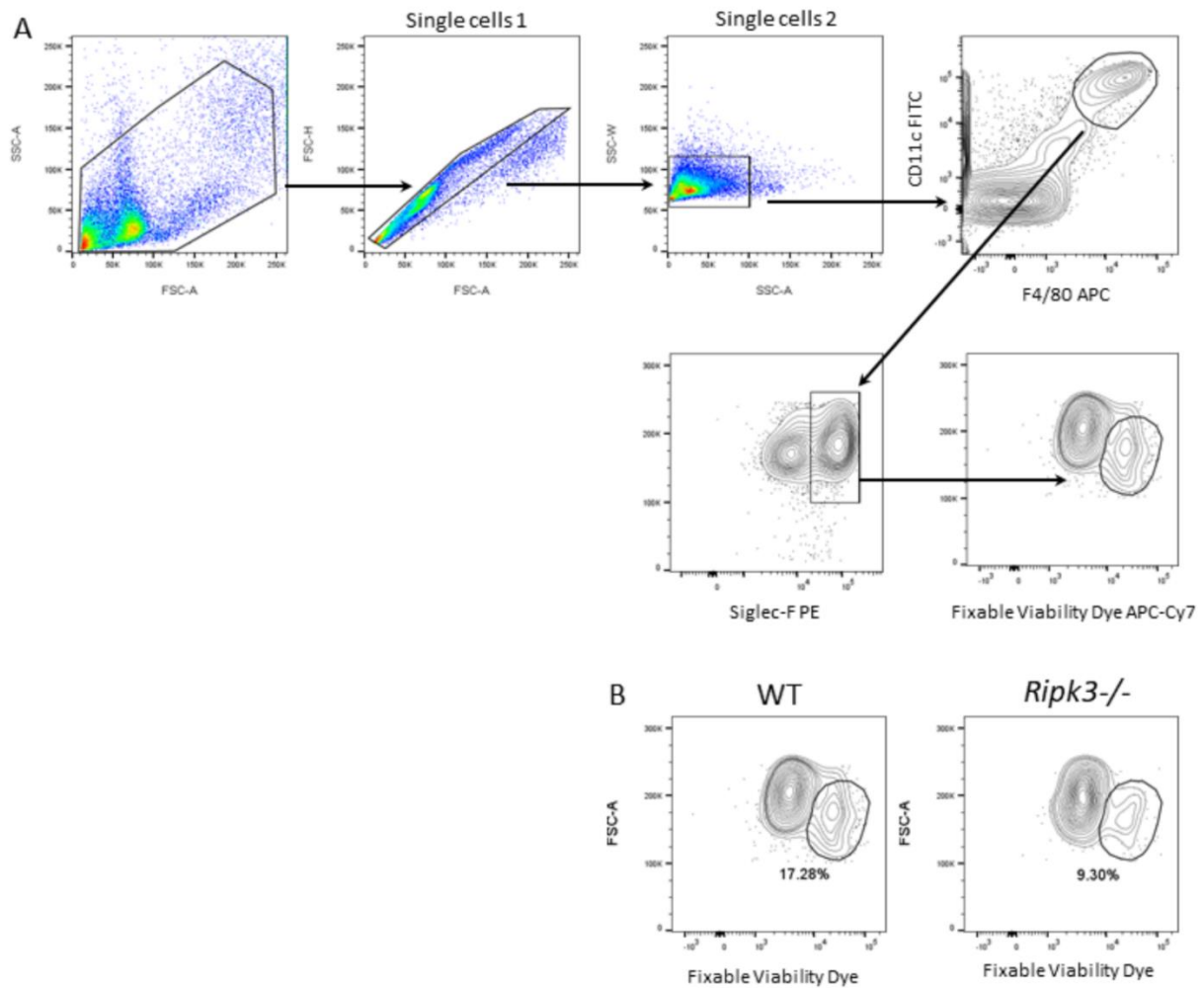


Fig. S4. Gating strategy used in the flow cytometry analysis of necrotic alveolar macrophages obtained from WT and *Ripk3*^{-/-} mice infected with RSV. **(A)** BAL cells were collected from infected WT and *Ripk3*^{-/-} mice and stained for CD11c, F4/80, Siglec-F and fixable viability dye. Single cells 1 were gated based on FSC-H x FSC-A plot. Single cells 2 were gated based on SSC-W x SSC-A plot inside single cells 1. CD11c⁺F4/80⁺ population was gated inside single cells 2. Siglec-F⁺ population was gated inside CD11c⁺F4/80⁺ gate. Fixable viability dye⁺ population (necrotic cells) was gated inside Siglec-F⁺ gate. **(B)** Representative FACS profile of necrotic WT and *Ripk3*^{-/-} alveolar macrophages (CD11c⁺F4/80⁺Siglec-F⁺fixable viability dye⁺) after RSV infection.

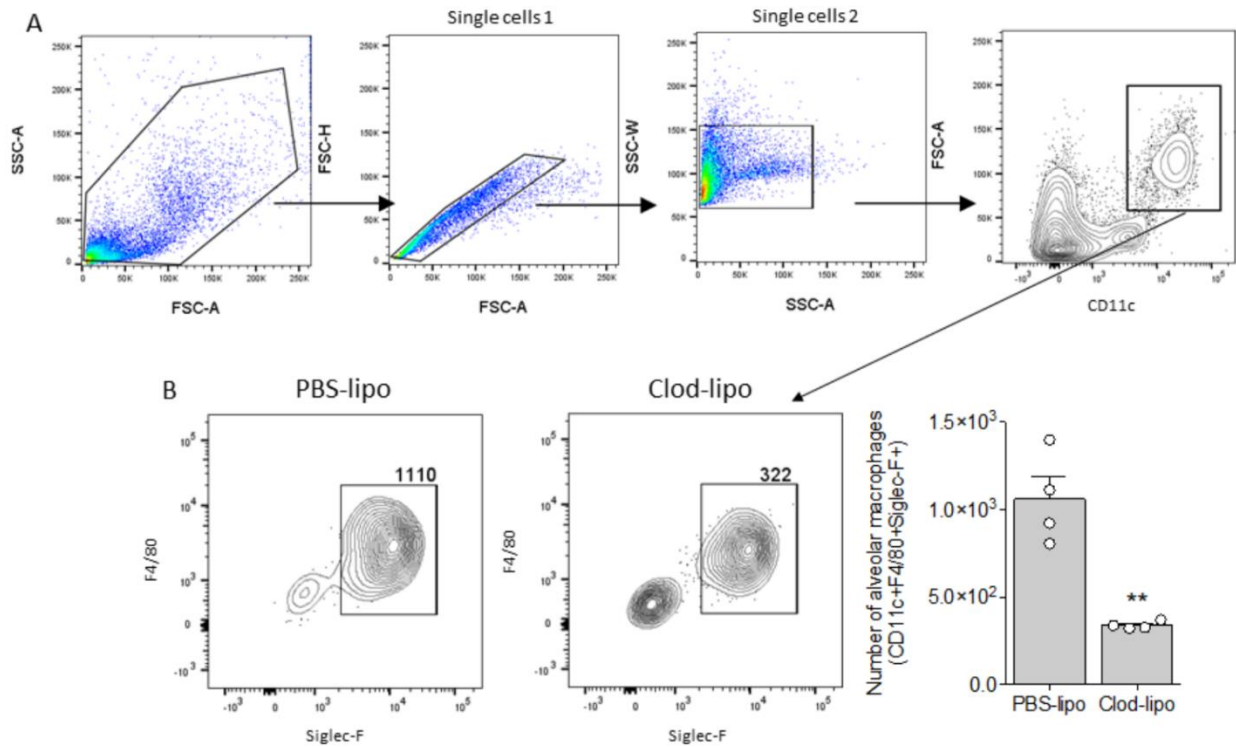


Fig. S5. Gating strategy used in the flow cytometry analysis of alveolar macrophage numbers in BAL fluid of mice treated with clodronate-liposomes or PBS-liposomes for 24 h (n = 4). **(A)** BAL cells were collected from mice treated with clodronate-liposomes or PBS-liposomes and stained for CD11c, F4/80 and Siglec-F. Single cells 1 were gated based on FSC-H x FSC-A plot. Single cells 2 were gated based on SSC-W x SSC-A plot inside single cells 1. CD11c⁺ population was gated inside single cells 2. F4/80⁺Siglec-F⁺ population was obtained inside CD11c⁺ gate. **(B)** Representative FACS profile and absolute numbers of alveolar macrophages (CD11c⁺F4/80⁺Siglec-F⁺) in BAL fluid of clodronate-liposomes or PBS-liposomes-treated mice. Data were analyzed with unpaired Student's *t* test. ***p* < 0.01.

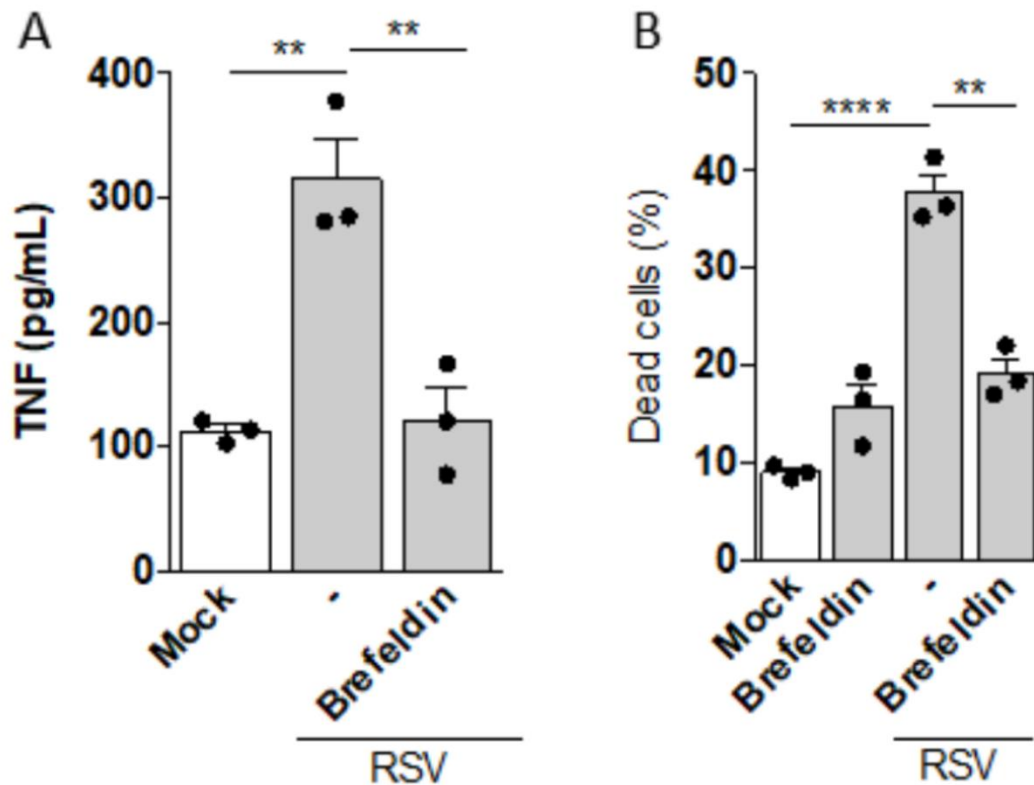


Fig. S6. (A) Balb/c mice-derived macrophages (3×10^5 /well) were infected with RSV (MOI 1) for 6 h alone or in the presence of brefeldin A (BD GolgiStop™, BD Bioscience, San Jose, CA, USA) (0.5 μ g/mL) added in the last 4 h. Afterwards, the supernatants were collected and TNF concentrations were measured by ELISA. **(B)** Macrophages (3×10^5 /well) were infected with RSV (MOI 1) for 6 h alone or in the presence of brefeldin A added in the last 4 h. Then, macrophages were harvested and labeled with Fixable Viability Dye eFluor® 780 and the percent of dead cells was analyzed by flow cytometry. Data are representative of 2 independent experiments performed in triplicates and represent mean \pm SEM. Data were analyzed with one-way ANOVA with Tukey's post-hoc test. **p < 0.01; ****p < 0.0001.

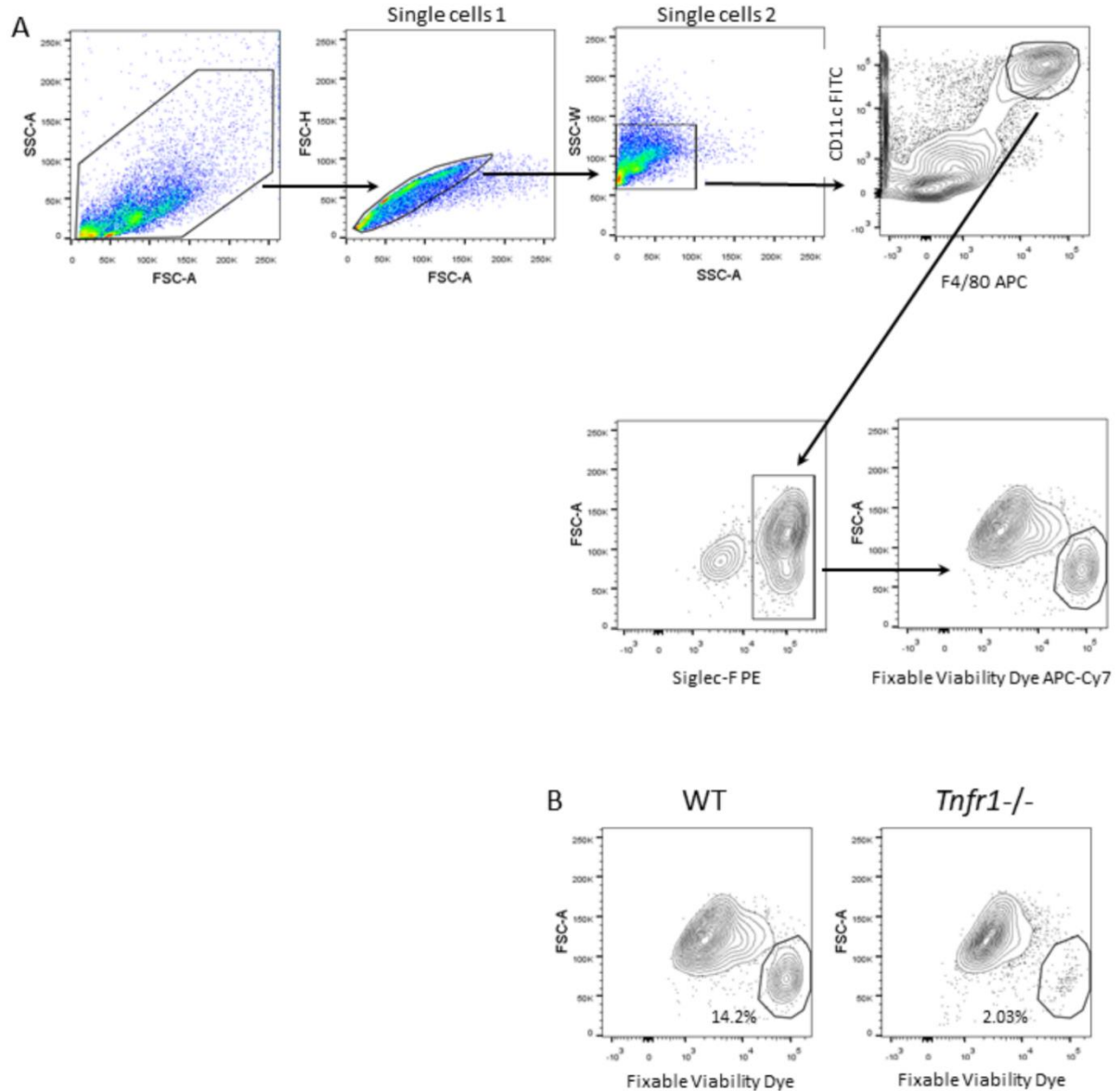


Fig. S7. Gating strategy used in the flow cytometry analysis of necrotic alveolar macrophages obtained from WT and *Tnfr1*^{-/-} mice infected with RSV. **(A)** BAL cells were collected from infected WT and *Tnfr1*^{-/-} mice and stained for CD11c, F4/80, Siglec-F and fixable viability dye. Single cells 1 were gated based on FSC-H x FSC-A plot. Single cells 2 were gated based on SSC-W x SSC-A plot inside single cells 1. CD11c⁺F4/80⁺ population was gated inside single cells 2. Siglec-F⁺ population was gated inside CD11c⁺F4/80⁺ gate. Fixable viability dye⁺ population (necrotic cells) was gated inside Siglec-F⁺ gate. **(B)** Representative FACS profile of

necrotic WT and *Tnfr1*^{-/-} alveolar macrophages (CD11c⁺F4/80⁺Siglec-F⁺fixable viability dye⁺)
after RSV infection.

Table S1. Characteristics of the study population

	RSV positive (n = 39)	RSV negative (n = 5)	95% CI	P value
Age (months)	3.434 ± 1.787	3.807 ± 2.665	-2.171 to 1.426	0.6784
Sex (M/F)	28/11	2/3		0.1507*
Birth weight (kg)	3.084 ± 848.5	3.336 ± 590.9	-420.9 to 925.8	0.4484

Values are presented as the mean ± standard deviation for age and birth weight (unpaired *t* test analysis). RSV, respiratory syncytial virus; CI, confidence interval; M, male; F, female. **P* value for chi-square test.