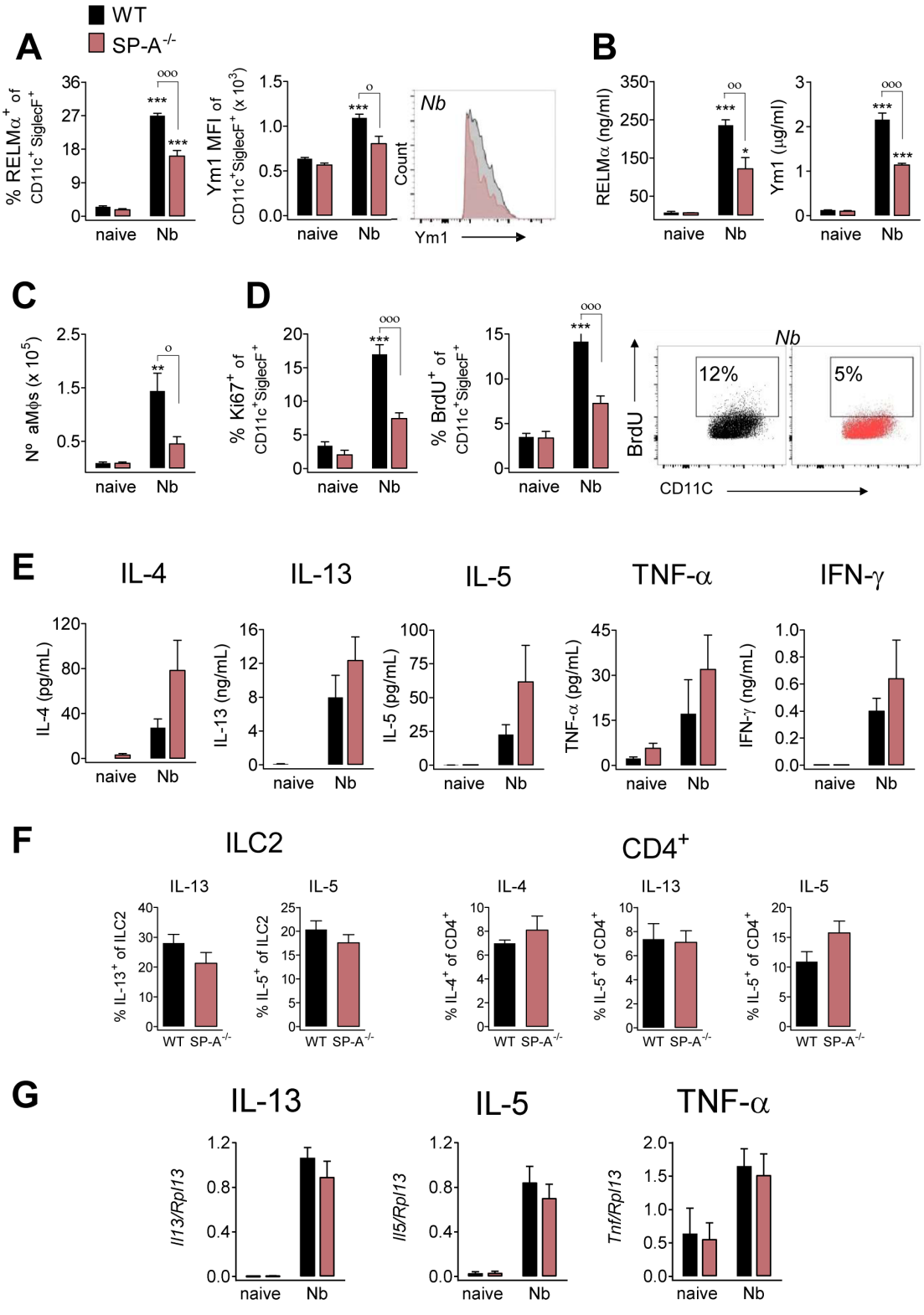


Fig. S1. Further characterization of WT vs. SP-A-deficient mice during *Nippostrongylus brasiliensis* infection. WT or SP-A$^{-/-}$ mice were left uninfected or infected with 250 *N. brasiliensis* L3's, and assessed at day 6 after

infection. **(A)** Expression of the M(IL-4) markers RELMα and Ym1, in alveolar macrophages from single-cell suspensions of lung tissue. **(B)** Analysis of secreted RELMα and Ym1 from BAL by ELISA. **(C)** Number of alveolar macrophages recovered in BAL. **(D)** Proliferation of alveolar macrophages from lung tissue, measured by Ki67 expression and BrdU incorporation. A representative plot showing BrdU staining in macrophages from WT and SP-A$^{-/-}$ infected animals is shown. **(E)** Levels of IL-4, IL-13, IL-5, TNFα, and IFN-γ in supernatants of thoracic lymph node cells from WT and SP-A$^{-/-}$ mice, cultured with *N. brasiliensis* antigen (1 μg/ml); results are normalized to those obtained for cells cultured with medium alone. **(F)** Expression of IL-13 and IL-5 by ILC2 and IL-4, IL-13 and IL-5 by CD4^{+} cells from single-cell suspensions of lung tissue stimulated *ex vivo* with PMA and ionomycin. **(G)** Amplification of *Il13, Il5 and Tnf*-encoding mRNA in lung homogenates is also shown. Results are representative from two independent experiments (means ± SEM) (naïve: 3 mice, *Nb*: 6 mice). ANOVA followed by the Bonferroni multiple-comparison test or Student's *t*-test (F) was used. $^{**}p < 0.01$ and $^{***}p < 0.001$, when compared with the uninfected group; $^{\circ}p < 0.05$ and $^{\circ\circ\circ}p < 0.001$ when WT vs. SP-A$^{-/-}$ infected groups are compared.

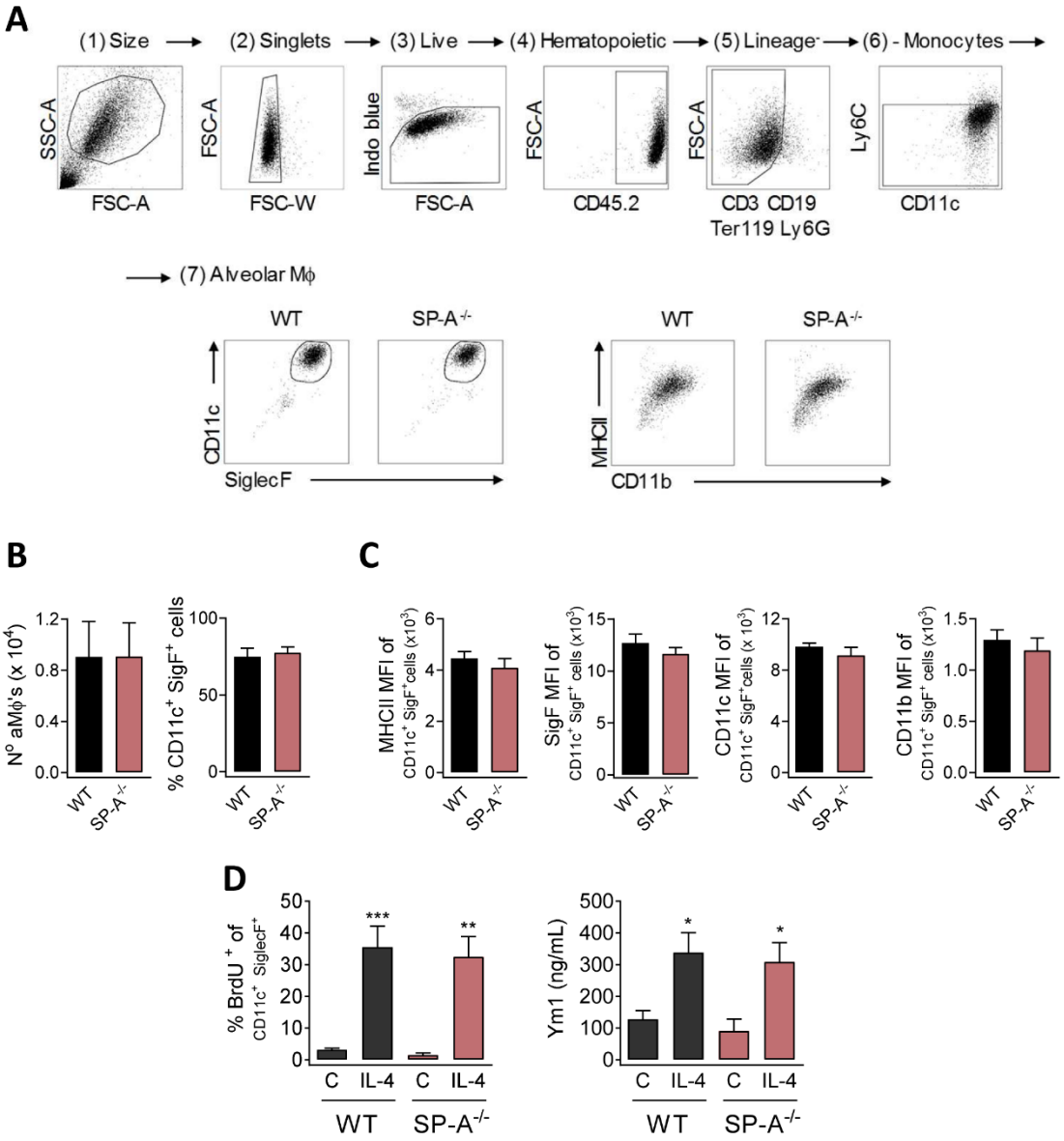


Fig. S2. Characterization of alveolar macrophages from SP-A-deficient mice. (A) Sequential identification of alveolar macrophages by flow cytometry. Cells first identified by size (1), and then as singlets (2) and live cells (3). Subsequently, hematopoietic cells selected by CD45.2 expression (4). Lin− cells (5) obtained by gating out CD3+, CD19+, Ly6G+, and Ter119+ cells (T cells, B cells, neutrophils and red blood cells, respectively). Monocytes successively excluded by expression of Ly6C (6). Lin− and Ly6C− populations were further subgated on the basis of the expression of CD11c versus SiglecF. Alveolar macrophages are identified as $CD11c^{hi}$ and $SiglecF^{hi}$ (7); a representative population of alveolar macrophages from WT and SP-A-deficient mice is shown. The expression of MHCII and CD11b by alveolar macrophages is also shown for both mice strains. All shown gates are children of the parent gates shown previously. **(B)** Number and percentage of alveolar macrophages recovered in BAL. **(C)** Expression of MHCII, SiglecF, CD11c and CD11b in alveolar macrophages from WT and SP-A-deficient mice. Data are representative from two independent experiments (mean ± SEM; WT: 4 mice, $SP\text{-}A^{-/-}$: 3 mice). Student's

t-test was used. **(D)** Purified alveolar macrophages from WT and SP-A-deficient mice were treated with or without IL-4 (1μg/ml) and exposed to BrdU for proliferation analysis. BrdU incorporation and secretion of Ym1 by resident alveolar macrophages are shown. The results are presented as means (± SEM) from two different cell cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, when compared with untreated macrophages.

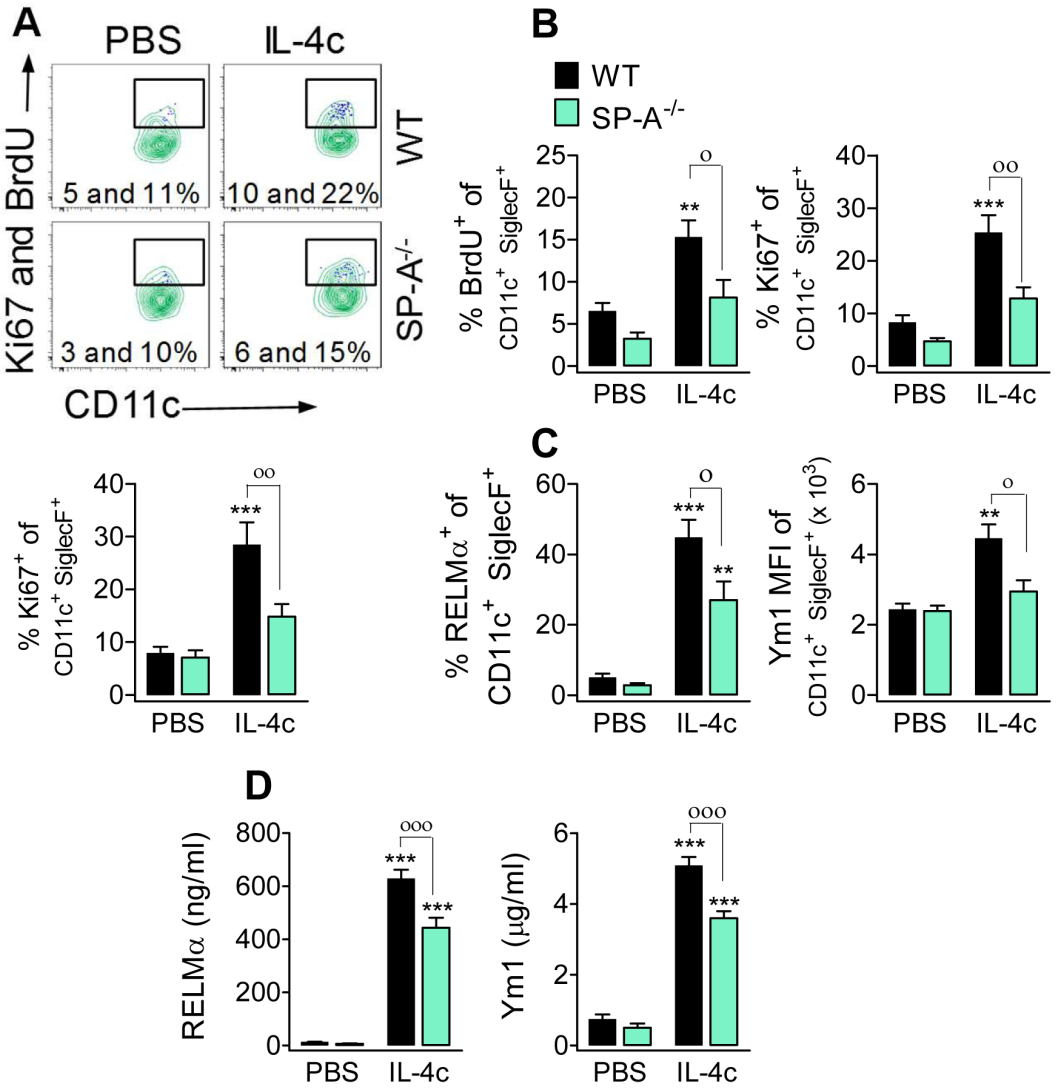


Fig. S3. Alveolar macrophages from SP-A-deficient mice show decreased IL-4-induced proliferation and M(IL-4) activation. WT and SP-A$^{-/-}$ mice were treated with 5μg of IL-4c (i.p.) at days 0 and 2; and samples were analyzed at day 4. **(A)** Expression of Ki67 by alveolar macrophages from BAL in response to IL-4c. A representative plot from WT and SP-A$^{-/-}$ mice treated with PBS or IL-4c is shown. Ki67 (green contour) and BrdU^{+} cells (Fig. 1Q) are overlaid as blue dots. Numbers below outlined areas indicate percent of BrdU^{+} and Ki67^{+} macrophages. **(B)** BrdU incorporation and expression of Ki67 by alveolar macrophages from lung tissue in response to IL-4c. (**C**) Expression of RELMα and Ym1 by alveolar macrophages from lung tissue. **(D)** Protein levels of RELMα and Ym1 in BAL measured by ELISA. Data were pooled from three independent experiments (means ± SEM) (PBS: 9 mice, IL-4c: 11 mice). ANOVA followed by the Bonferroni multiple-comparison test was used. *p < 0.05, **p < 0.01, and ***p < 0.001 when compared with the untreated group. °p < 0.05, °°p < 0.01 and °°°p < 0.001 when WT vs. SP-A$^{-/-}$ groups are compared.

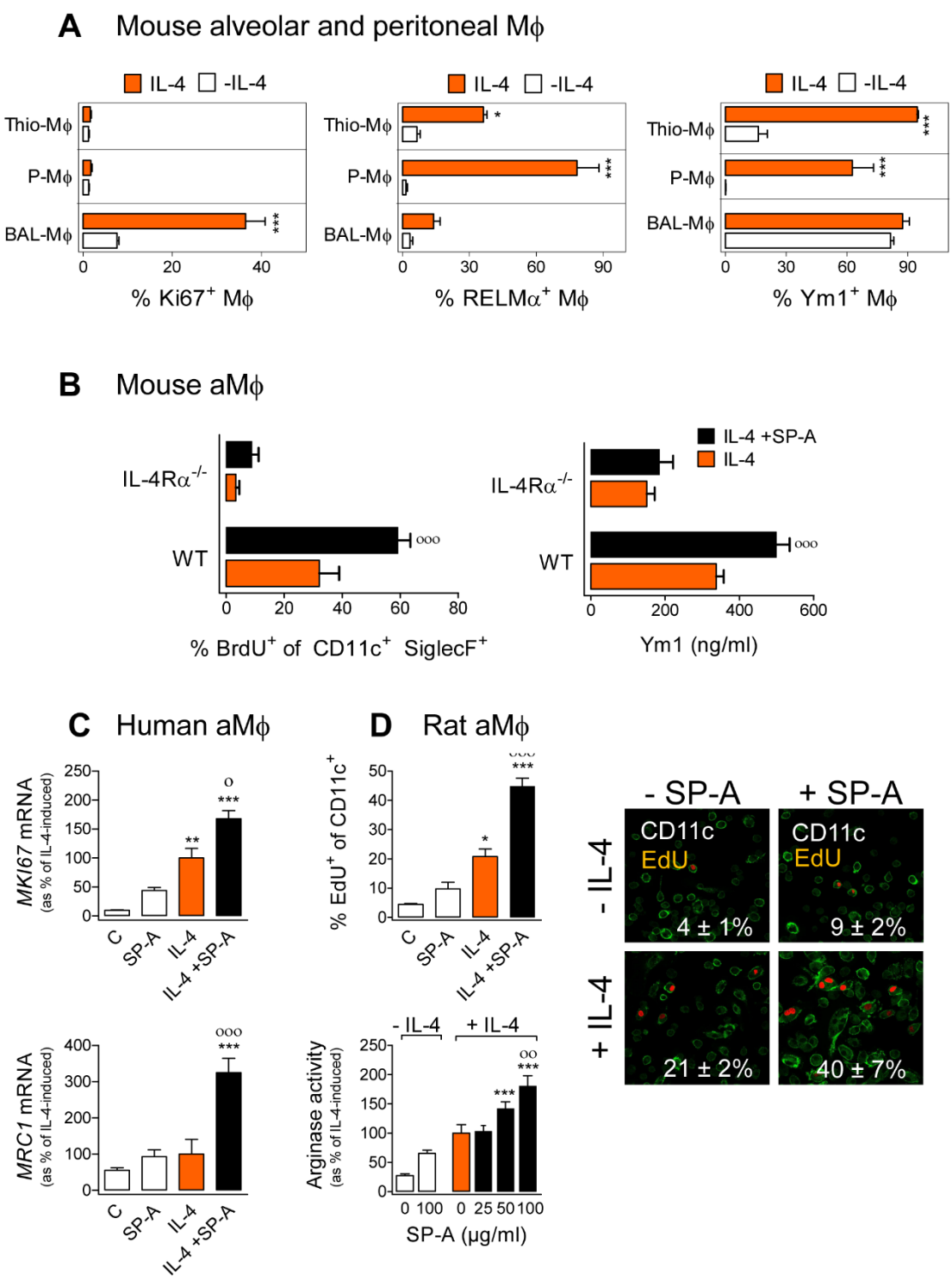


Fig. S4. Proliferation and activation of macrophages isolated from mice, humans, and rats. (A) Purified alveolar (BAL-Mϕ), peritoneal (resident (P-Mϕ) and thioglycollate-recruited (Thio-Mϕ) from mice were treated with or without IL-4 (0.5-1 μg/ml). Ki67, RELMα, and Ym1 expression was assessed by FACS. Note: cultured aMϕ are $Ym1^+$ regardless of IL-4 treatment. Purified aMϕ from mice **(B)**, humans **(C)**, or rats **(D)** were treated with or without IL-4 (0.5-1 μg/ml) in the presence or absence of SP-A. (**B**) BrdU incorporation and secretion of Ym1 by

cultured alveolar macrophages from WT and IL-4Rα$^{-/-}$ mice in the presence and absence of SP-A. **(C)** *MKI67* and *MRC1* mRNA expression by human alveolar macrophages as measured by qRT-PCR. (**D**) EdU incorporation analyzed by flow cytometry and arginase activity of rat alveolar macrophages. A representative confocal micrograph of rat alveolar macrophages immuno-stained with CD11c-FITC and EdU-Alexa Fluor 647 is shown. Numbers below indicate percentage ± SEM of EdU^{+} cells from three independent experiments. The results are presented as means (± SEM) from three different cell cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, when compared with untreated macrophages; $^{o}p < 0.05$, °°$p < 0.01$, and °°°$p < 0.001$, when SP-A+IL-4-treated macrophages were compared with IL-4-treated macrophages.

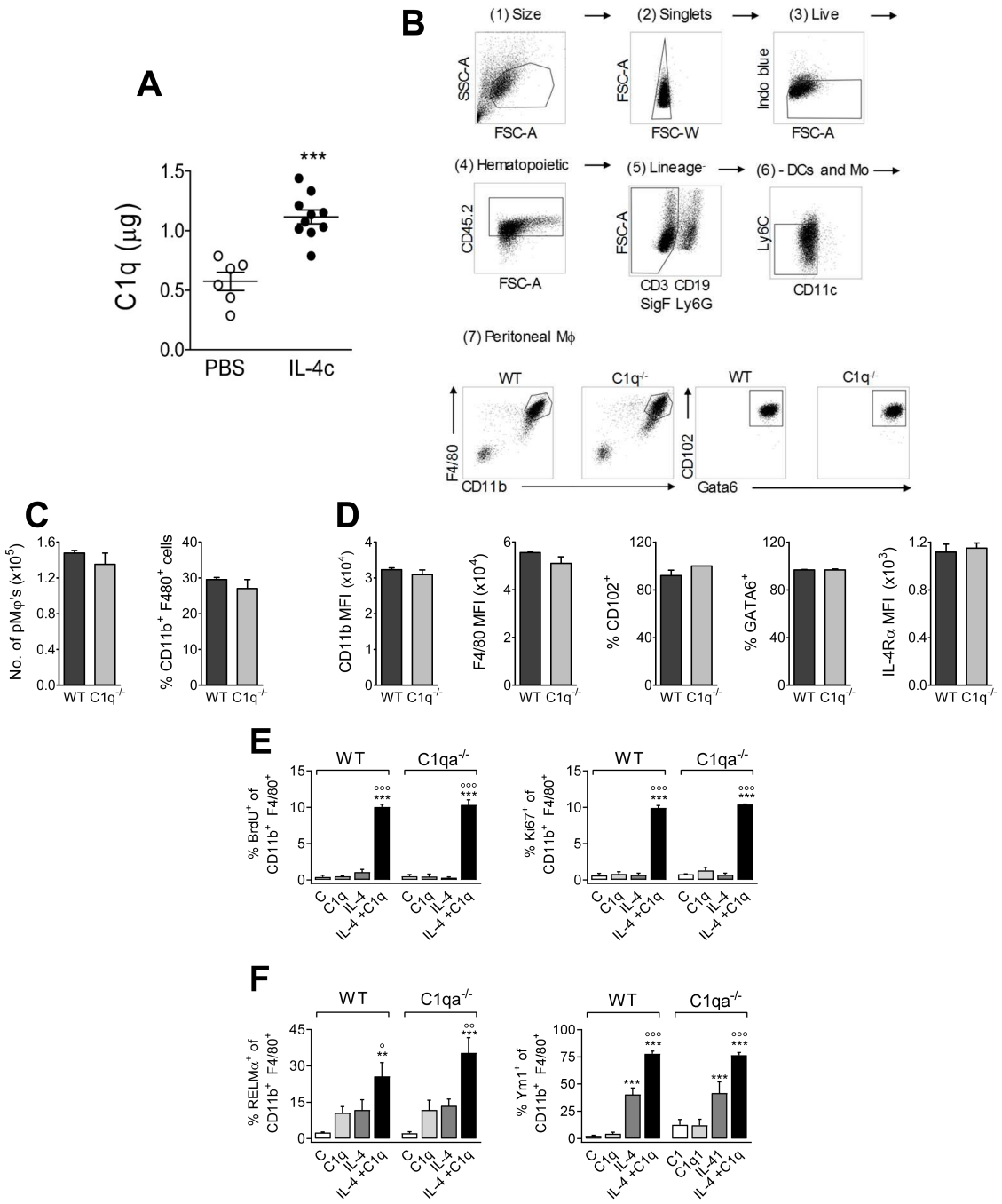


Fig. S5. C1q protein levels in the peritoneal cavity and characterization of peritoneal macrophages from C1q-deficient mice. (A) Following IL-4c delivery, C1q protein was measured by ELISA in WT mice. **(B)** Sequential identification of peritoneal macrophages by flow cytometry. Cells first identified by size (1), and then as singlets (2) and live cells (3). Subsequently, hematopoietic cells are selected by CD45.2 expression (4). Lin^- cells (5) are obtained by gating out $CD3^+$, $CD19^+$, $Ly6G^+$ and $SiglecF^+$ cells (T cells, B cells, neutrophils and eosinophils, respectively). Monocytes and dendritic cells are successively excluded by expression of Ly6C and CD11c (6). Lin^-, $CD11c^-$ and $Ly6C^-$ populations are further subgated on the basis of the expression of CD11b versus F4/80. Peritoneal macrophages are $CD11b^{hi}$ and $F4/80^{hi}$ (7); a representative population of peritoneal Mϕs from WT and

C1q-deficient mice is shown. The expression of CD102 and Gata6 by peritoneal Mϕs is also shown for both mice strains. All shown gates are children of the parent gates shown previously. **(C)** Number and percentage of peritoneal macrophages recovered in the peritoneal washes. **(D)** Expression of CD11b, F4/80, CD102, Gata6, and IL-4Rα in peritoneal macrophages from WT and C1q-deficient mice. Data are representative from two independent experiments (mean ± SEM; WT: 4 mice, $C1qa^{-/-}$: 4 mice). In C and D, Student's *t*-test was used. In E and F, purified peritoneal macrophages from WT and C1q-deficient mice were treated with or without IL-4 (1 μg/ml or 10 ng/ml) and exposed to BrdU. **(E)** Proliferation (BrdU incorporation and Ki67 expression) in response to 1 μg/ml of IL-4. **(F)** M(IL-4) activation (RELMα and Ym1 expression) in response to 10 ng/ml of IL-4 of pMϕs. The data shown are means ± SEM of two different peritoneal macrophage cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. $^{**}p < 0.01$, and $^{***}p < 0.001$, when compared with untreated cells (C); $^{\circ}\ p < 0.05$, $^{\circ\circ}p < 0.01$, and $^{\circ\circ\circ}p < 0.001$, when C1q+IL-4-treated macrophages were compared with IL-4-treated macrophages.

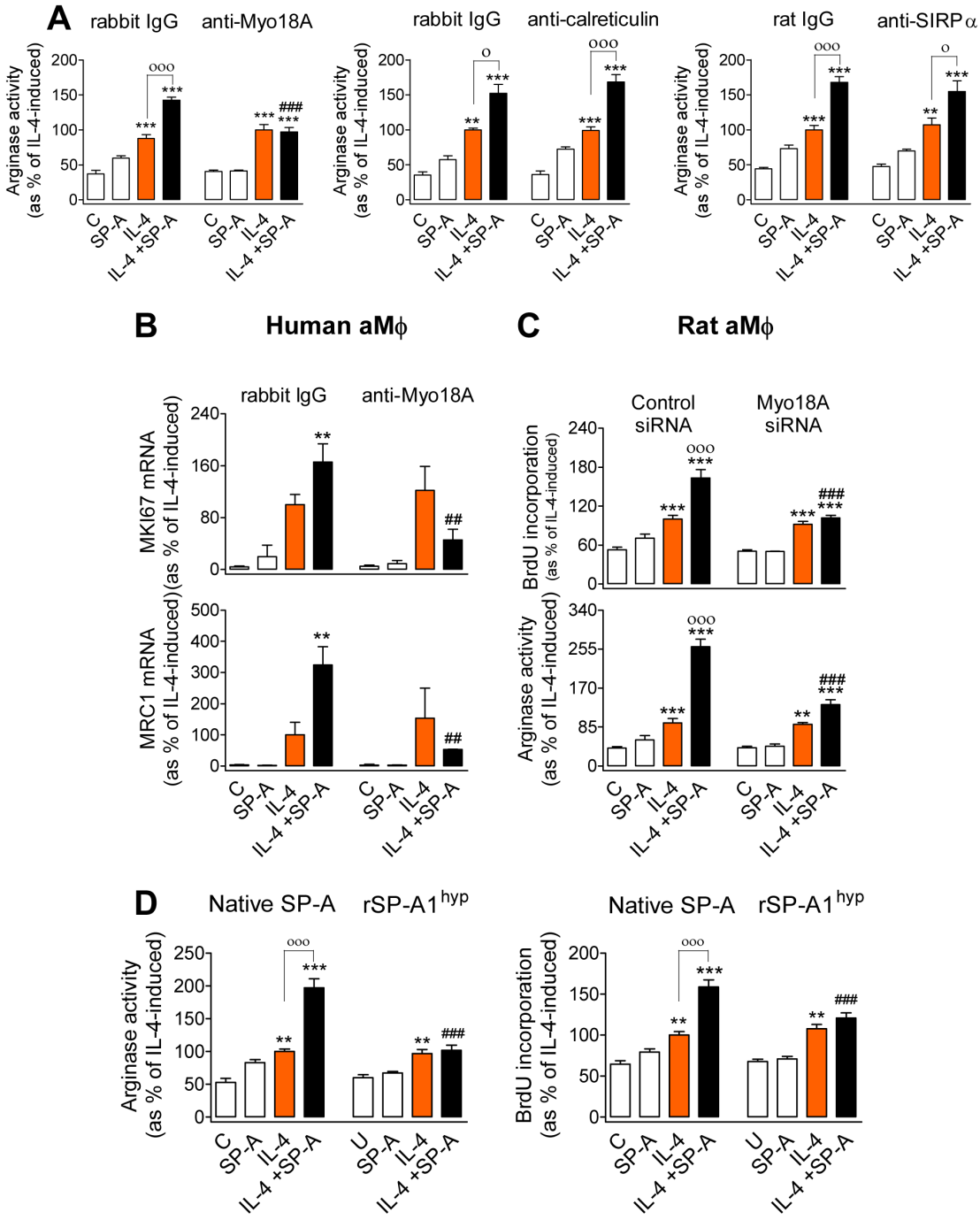


Fig. S6. (A-C) Myo18A mediates SP-A's effects on aMϕs isolated from humans and rats. (A) Purified rat alveolar macrophages were treated with either anti-Myo18A (10 μg/ml), anti-calreticulin (10-50 μg/ml), anti-SIRPα (10-50 μg/ml), or an isotype control for two hours. Next, cells were IL-4-stimulated with or without native SP-A. **(B)** mRNA expression of *MKI67* and *MRC1* by human alveolar macrophages treated with anti-Myo18A antibody. **(C)** BrdU incorporation and arginase activity in rat alveolar macrophages that were nucleofected with Myo18A

(RSS322720) or control siRNA. Similar results were found using RSS322721 Myo18A siRNA. **(D) The collagen-like domain of SP-A is required for functional interaction with Myo18A.** Purified rat alveolar macrophages were IL-4-stimulated with or without either native human SP-A or recombinant human SP-A1 expressed in insect cells ($SP\text{-}A1^{hyp}$). Recombinant human SP-A1 expressed in insect cells lacks the prolyl hydroxylation, resulting in improper folding of the collagen domain (31). The data shown are means ± SEM of three different alveolar or peritoneal macrophage cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. $^{**}p < 0.01$, and $^{***}p < 0.001$, when compared with untreated cells (C); $^{\circ}p < 0.05$, and $^{\circ\circ\circ}p < 0.001$, when SP-A+IL-4-treated macrophages were compared with IL-4-treated macrophages; $\#\#p < 0.01$, and $\#\#\#p < 0.001$, when either the effect of Myo18A siRNA vs. control siRNA or anti-Myo18A vs. rabbit IgG are compared or when the effects of native SP-A vs. $SP\text{-}A1^{hyp}$ on macrophage activation and proliferation are compared.

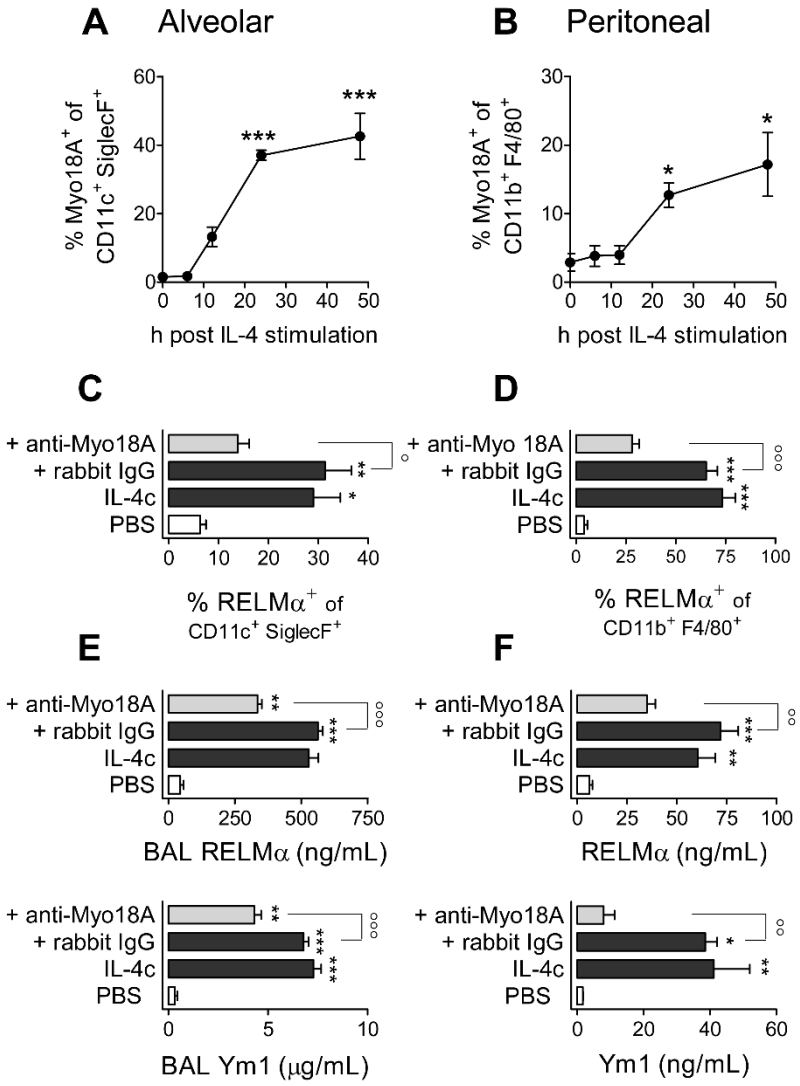


Fig. S7. Myo18A expression in response to IL-4 treatment and further characterization of blocking Myo18A on IL-4-mediated activation of macrophages *in vivo*. Time-dependent expression of Myo18A on the cell surface of alveolar **(A)** and peritoneal **(B)** macrophages in response to IL-4 treatment *in vitro*. The data shown are means ± SEM of three different alveolar or peritoneal macrophage cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. *$p < 0.05$, and ***$p < 0.001$, when compared with 0 hours post IL-4 stimulation. **(C, E)** For aMϕ analysis, WT or SP-A$^{-/-}$ mice received 5 μg of IL-4c (*i.p.*) at day 0 and 2. Simultaneously, some animals were intra-nasally treated with either anti-Myo18A or rabbit IgG antibody at day 2 and 3, and samples analyzed at day 4. IL-4-mediated activation (% RELMα^{+} aMϕ) **(C)** as well as concentration of RELMα and Ym1 in BAL **(E)** is shown. **(D, F)** For pMϕ analysis, WT or C1qa$^{-/-}$ mice received 1 μg of IL-4c (i.p.)

at day 0, and samples were analyzed at day 1. Some mice were also treated with either anti-Myo18A or rabbit IgG antibody (*i.p.*) 2 hours before IL-4c administration. IL-4-mediated activation (% RELMα^{+} aMϕ) **(D)**, as well as concentration of RELMα and Ym1 in the peritoneal cavity **(F)** is shown. Data were pooled from three independent experiments (means ± SEM) (PBS: 6 mice, other groups: 9 mice). ANOVA followed by the Bonferroni multiple-comparison test was used. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, when compared with PBS treated mice; °°$p < 0.01$, and °°°$p < 0.001$ when anti-Myo18A vs rabbit IgG treatment is compared in IL-4c-treated mice.

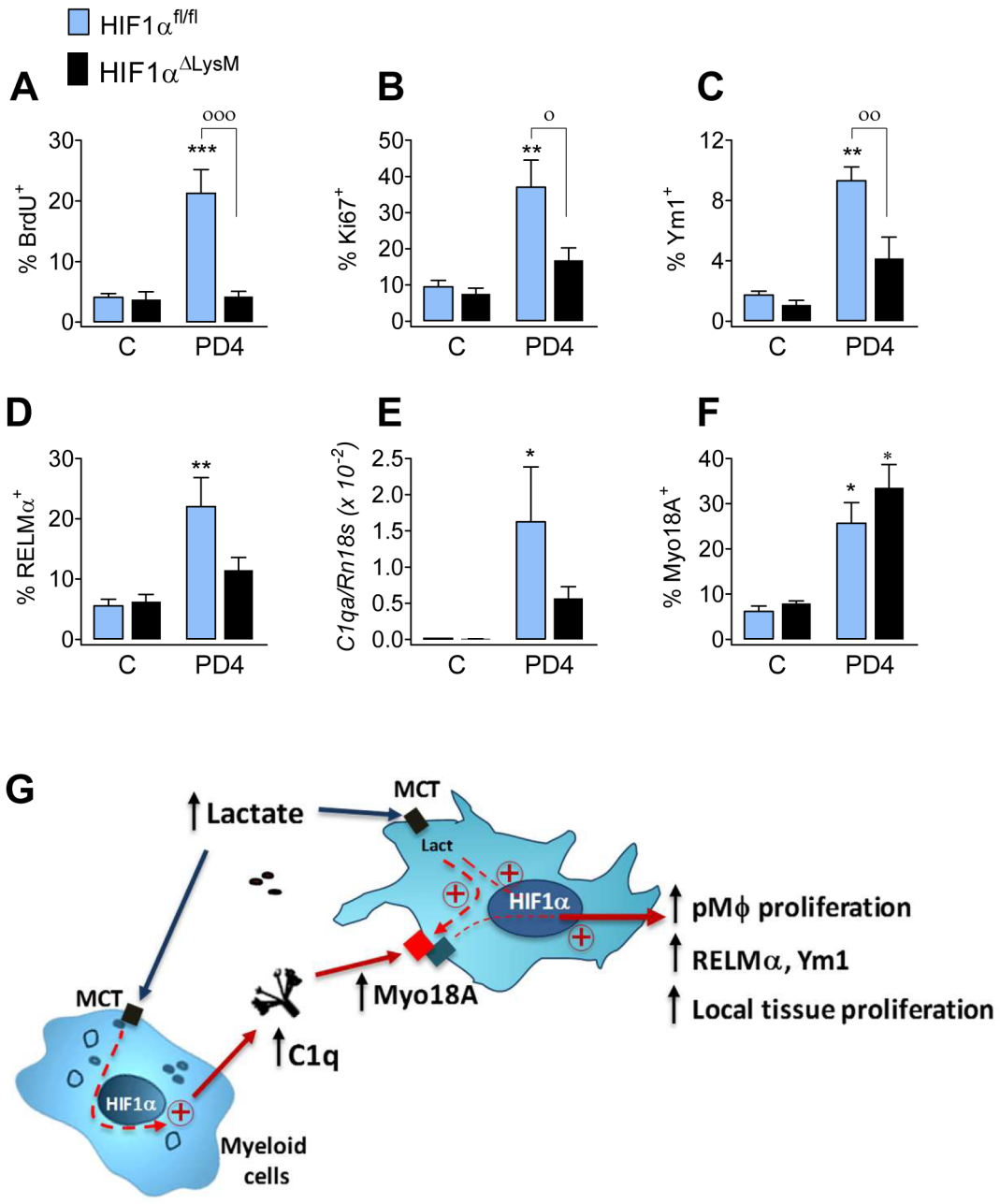


Fig. S8. Lactate-dependent proliferation and type 2 macrophage activation requires stabilization of HIF1α in a model of peritoneal dialysis. Hif1aflox/flox;LysMcre$^{+/+}$ mice and littermate WT controls (Hif1aflox/flox;LysMcre$^{-/-}$) were either untreated (control, C) or treated with 5 ip injections of Dianeal PD-4 every other day. Samples were analyzed a day after the last delivery. (**A**) BrdU incorporation in peritoneal macrophages. (**B**) Ki67expression. (**C**) Ym1 and (**D**) expression of RELMα. (**E**) Amplification of C1qa-encoding mRNA in peritoneal tissue. (**F**) Myo18A expression on the surface of peritoneal macrophages. Results are representative from two independent experiments (means ± SEM) (untreated: 3 mice, PD4: 4 mice). ANOVA followed by the

Bonferroni multiple-comparison test or Student's t-test (A) was used. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ when compared with control group; °$p < 0.05$, °°$p < 0.01$, and °°°$p < 0.001$ when WT vs. myeloid specific HIF-1α-deficient mice treated with Dianeal PD-4 are compared. (**G**) **Schematic of lactate-dependent promotion of a type 2 macrophage phenotype, which is dependent on HIF1α and C1q in the peritoneal cavity**. A lactate-based dialysate increases C1q levels in the peritoneal cavity and expression of myosin 18A on the surface of pMϕ. Lactate-induced proliferation of peritoneal macrophages and production of type 2 macrophage markers were blocked in C1q deficiency mice and animals lacking HIF1α in macrophages, but not in IL-4Rα deficient mice. C1q is required to amplify HIF1α-dependent lactate signaling, which promotes peritoneal fibrosis. (MCT: monocarboxylate transporter for lactate; HIF1α: hypoxia-inducible factor 1α)

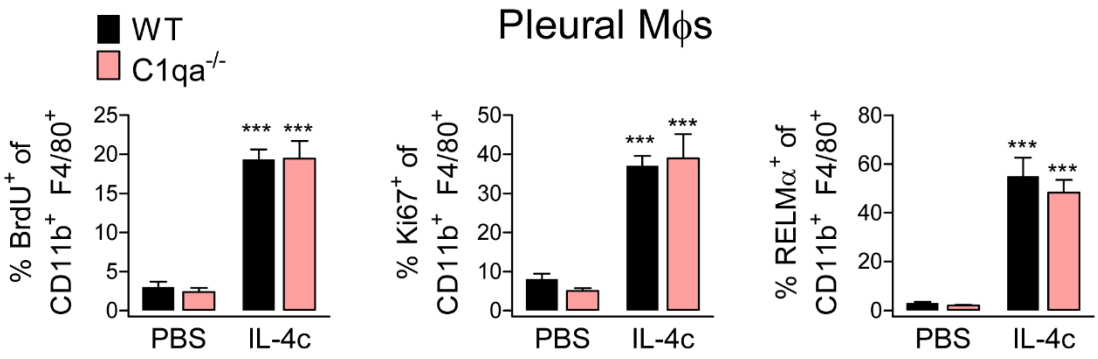


Fig. S9. IL-4 mediated proliferation and activation of pleural cavity macrophages is not enhanced by C1q. WT and C1qa$^{-/-}$ mice were treated with 1μg of IL-4c (i.p.) at days 0; and samples were analyzed at day 1. Proliferation (BrdU incorporation and Ki67 expression) and M(IL-4) activation (RELMα expression) of resident pleural macrophages are shown. Data were pooled from two independent experiments (means ± SEM) (PBS: 8 mice, IL-4c: 10 mice). ANOVA followed by the Bonferroni multiple-comparison test was used. ***$p < 0.001$ when compared with the untreated group.

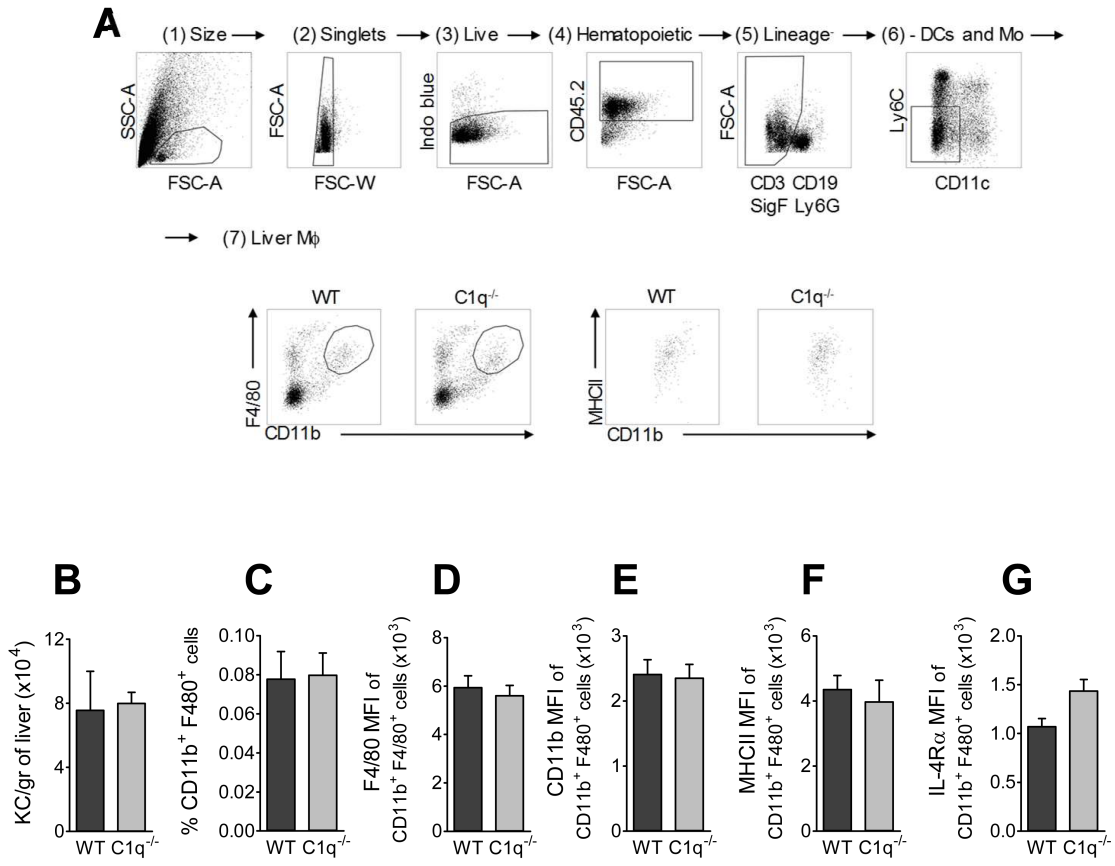


Fig. S10. Characterization of liver macrophages from C1q-deficient mice. (A) Sequential identification of liver macrophages by flow cytometry. Cells are first identified by size (1), and then as singlets (2) and live cells (3). Subsequently, hematopoietic cells are selected by CD45.2 expression (4). Lin^- cells (5) are obtained by gating out $CD3^+$, $CD19^+$, $Ly6G^+$ and $SiglecF^+$ cells (T cells, B cells, neutrophils and eosinophils, respectively). Monocytes and dendritic cells are successively excluded by expression of Ly6C and CD11c (6). Lin^-, $CD11c^-$ and $Ly6C^-$ populations are further subgated on the basis of the expression of CD11b versus F4/80. $CD11b^+$ and $F4/80^+$ cells are liver macrophages (7); a representative population of liver macrophages from WT and C1q-deficient mice is shown. The expression of MHCII and CD11b by liver macrophages is also shown for both mice strains. All shown gates are children of the parent gates shown previously. **(B)** Number and **(C)** percentage of liver macrophages from tissue homogenates. Expression of **(D)** F4/80, **(E)** CD11b, **(F)** MHCII, and **(G)** IL-4Rα in liver macrophages from WT and C1q-deficient mice. Data are representative of two independent experiments (mean ± SEM; WT: 4 mice, $C1qa^{-/-}$: 4 mice). Student's *t*-test was used.

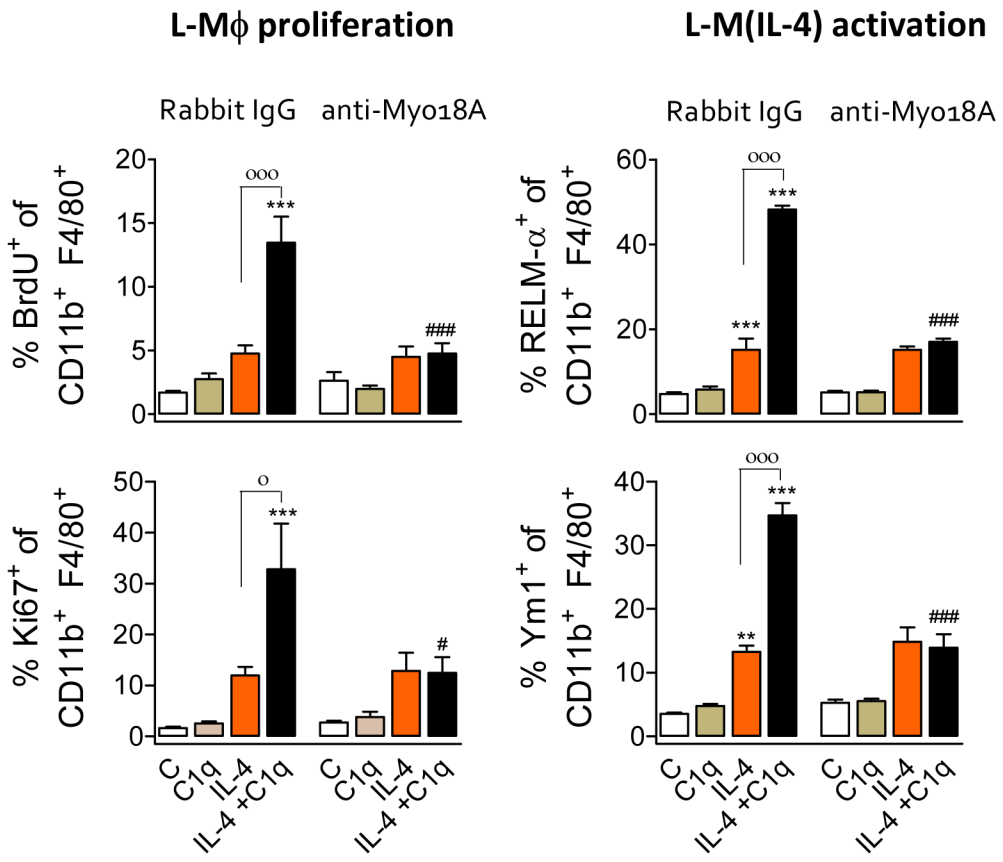


Fig. S11. Myo18A mediates C1q enhancement of IL-4-induced effects on liver macrophages. Purified mouse liver macrophages were treated with anti-Myo18A or rabbit IgG for one hour. Next, cells were IL-4-stimulated with or without C1q. Proliferation (BrdU incorporation and Ki67 expression) and M(IL-4) activation (RELMα and Ym1 expression) of liver macrophages are shown. The results are presented as means (± SEM) from three different cell cultures with at least three biological replicates. ANOVA followed by the Bonferroni multiple-comparison test was used. **$p < 0.01$, and ***$p < 0.001$, when compared with untreated cells; °$p < 0.05$ and °°°$p < 0.001$, when C1q+IL4-treated macrophages were compared with IL-4-treated macrophages; #$p < 0.05$, and ###$p < 0.001$, when the effect of anti-Myo18A antibody is compared in cells treated with C1q+IL4.

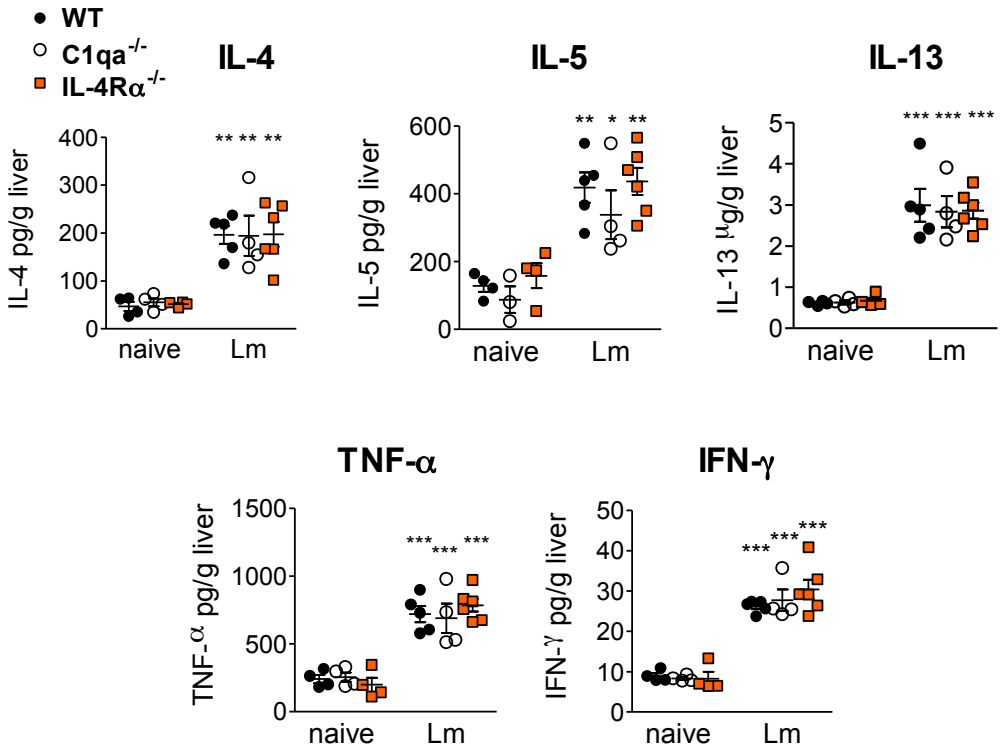
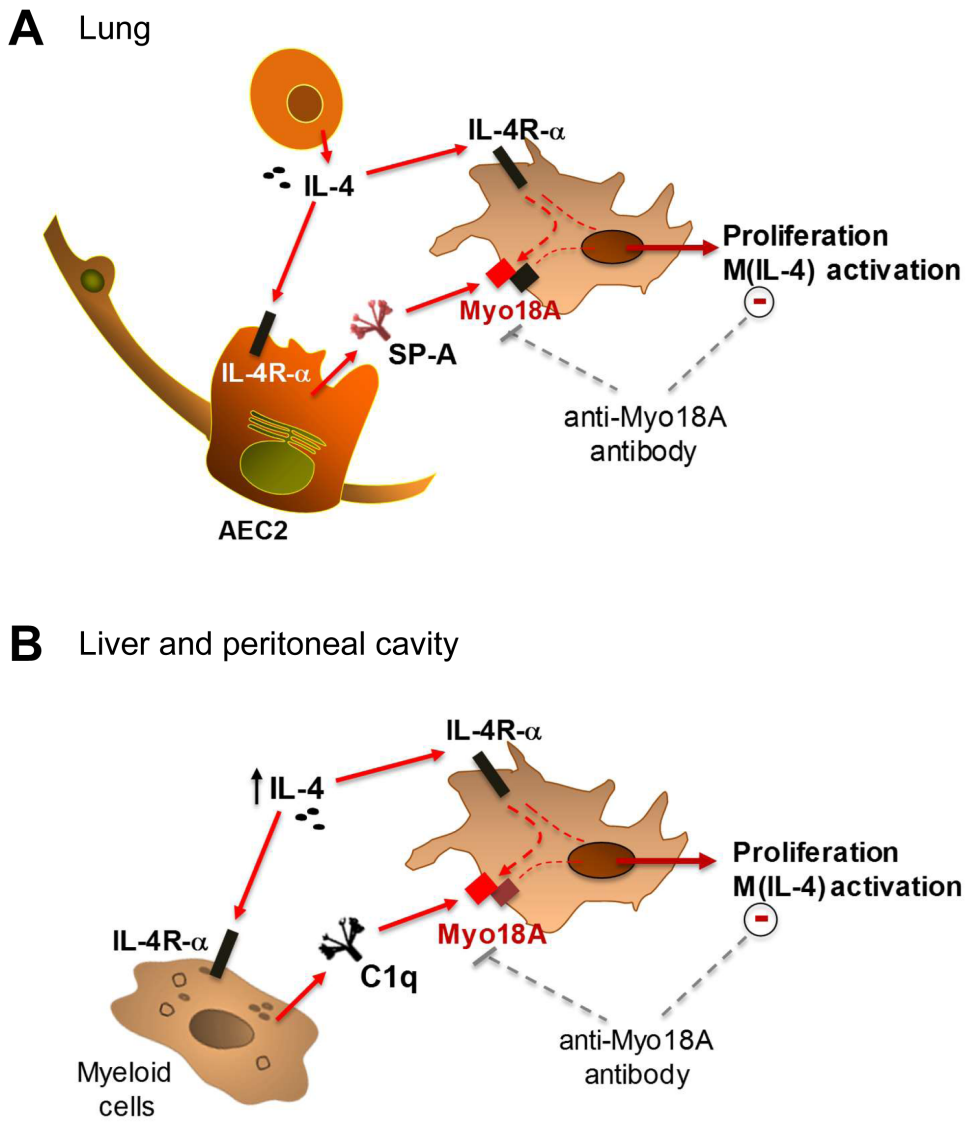


Fig. S12. Further characterization of WT vs. C1qa-deficient mice during *Listeria monocytogenes* infection. WT, C1qa$^{-/-}$, or IL-4Rα$^{-/-}$ mice were left uninfected or received intravenous infection with 10^4 *L. monocytogenes* c.f.u. and samples were assessed at day 3.5 after infection. Levels of IL-4, IL-13, IL-5, TNFα, and IFN-γ in the supernatants of liver homogenates from WT, C1qa$^{-/-}$, and IL-4Rα$^{-/-}$ mice. Data are representative from two independent experiments (mean ± SEM; naïve: 4 mice, *Lm*: 4-6 mice). ANOVA followed by the Bonferroni multiple-comparison test was used. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, when compared with the uninfected group.

Fig. S13.



Schematic of direct and indirect effects of IL-4. Rather than IL-4 signaling to macrophages alone, IL-4 drives the production of a second signal for full M(IL-4) activation and proliferation. (**A**) IL-4 increases the production of SP-A by alveolar epithelial type II cells (AEC2) and the expression of myosin 18A on the surface of aMϕ. SP-A deficiency or receptor blockade abrogated IL-4-induced proliferation of aMϕ and reduced M(IL-4) activation. **(B)** In the liver and the peritoneal cavity, IL-4 increases the production of C1q and the expression of myosin 18A receptor on the surface of liver and peritoneal macrophages. C1q deficiency or receptor blockade impedes full M(IL-4) activation and proliferation.

Supplementary Table 1. Genotyping of the *Sftpa1*$^{-/-}$ and *Sftpa1*$^{+/+}$ littermates

SP-A-deficient mice genotyping	
Primer	Sequence
Sftpa1 F neo	GTGGGGTGGGATTAGATAAATGC
Sftpa1 1743-1766	GCATTAGACGACAGAACTCCAGCC
Sftpa1 R 1981-1957	TACTGAGAGATGTGTGCTTGGTGAG

Supplementary Table 2. Primer sequences used for quantitative RT-PCR of genes of interest.

Gene	Primer	Sequence
Sftpa1	For	CTGGAGAACATGGAGACAAGG
	Rev	AAGCTCCTCATCCAGGTAAGC
Col3a1	For	AAGGGTGAAGTCGGTGCTC
	Rev	TCCAGCTCCACCTCTAGCA
Col1a1	For	TCTGGTCTCCAGGGTCCTC
	Rev	GTCTTTGCCAGGAGAACCAG
Acta2	For	CCAACCGGGAGAAAATGAC
	Rev	CAGTTGTACGTCCAGAGGCATA
Vegf	For	ACTCGGATGCCGACACGGGA
	Rev	CCTGGCCTTGCTTGCTCCCC
Mmp12	For	CAATTGGAATATGACCCCCTGT
	Rev	AGCAAGCACCCTTCACTACAT
Rpl13a	For	CATGAGGTCGGGTGGAAGTA
	Rev	GCCTGTTTCCGTAACCTCAA
Rn18s	For	GTAACCCGTTGAACCCCATT
	Rev	CCATCCAATCGGTAGTAGCG
MKI67	For	TCGACCCTACAGAGTGCTCA
	Rev	GTGGGGAGCAGAGGTTCTTC
MRC1	For	CAGATGCCCGGAGTCAGATC
	Rev	TTTATCCACAGCCACGTCCC
GAPDH	For	GATCATGAGCAATGCCTCCT
	Rev	TGTGGTCATGAGTCGTTCCA