Supplemental figures

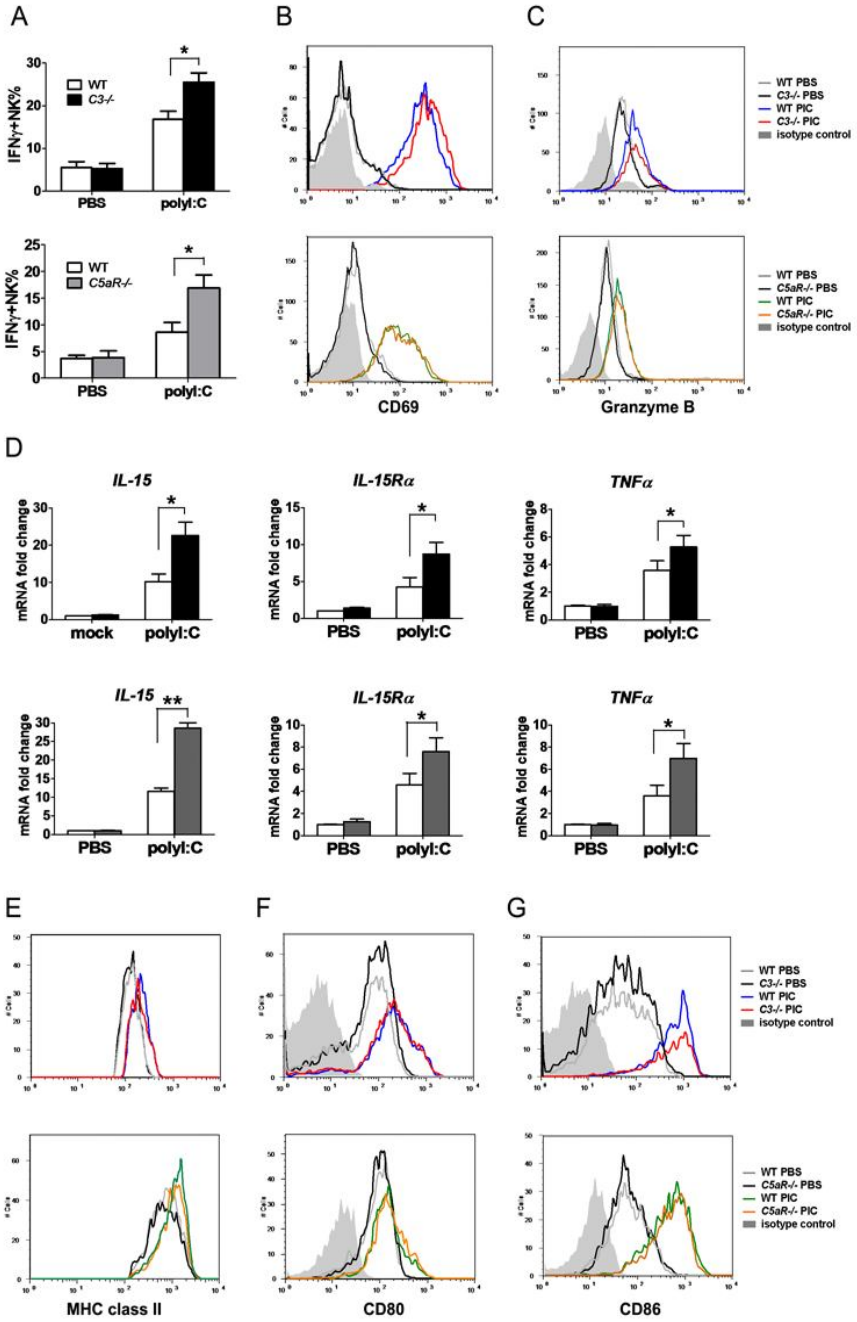

Fig. 1 In vivo activation of cDC and NK cells in complement deficient mice. C3-/- or C5aR-/- and corresponding WT mice were i.p. injected with 100 µg polyI:C. Mice were sacrificed 6 hr later. IFN-γ production (A), CD69 (B) and granzyme B (C) expression were analyzed in splenic NK cell population by FACS. Expression of MHC class II (E), CD80 (F) and CD86 (G) were examined in splenic cDCs by FACS. Flow-cytometry data represents 3 independent experiments with similar results. (D) Purified cDCs were analyzed for *IL-15*, *IL-15Rα* and *TNF-α* expression by qPCR. means ± s.d. n=3. Results are representative of 2 independent experiments with similar results. Student *t* test. * $P<0.05$

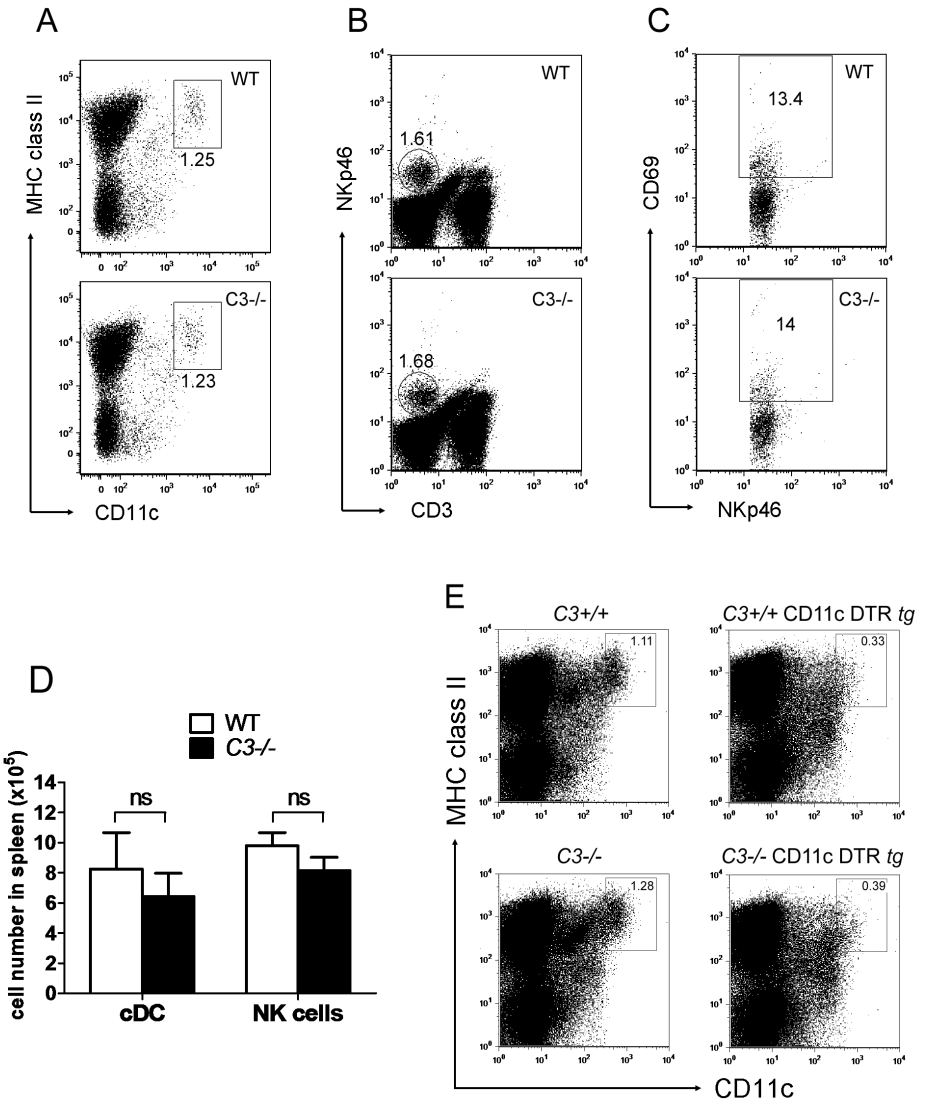

Fig. 2 Splenic distribution of cDCs and NK cells in WT and *C3-/-* mice. WT or *C3-/-* mice were analyzed for the percentage (A, B) and cell numbers (D) of $CD11c^{high}$MHC class II^{+} cDCs and $NKp46^{+}CD3^{-}$ NK cells in the spleen. Expression of early activation marker CD69 by NK cells were also compared (C). (E) Depletion of cDC in CD11c DTR tg mice was achieved by injection of DT. Splenocytes were analyzed for cDC 48 hr later. A, B, C, E data are representative of at least 6 mice of each strain. Numbers in the dot plots indicate the percentage of gated cells. D, means ± s.e.m. n=4-6 mice/group, ns, not significant, two-way unpaired Student *t*-test.

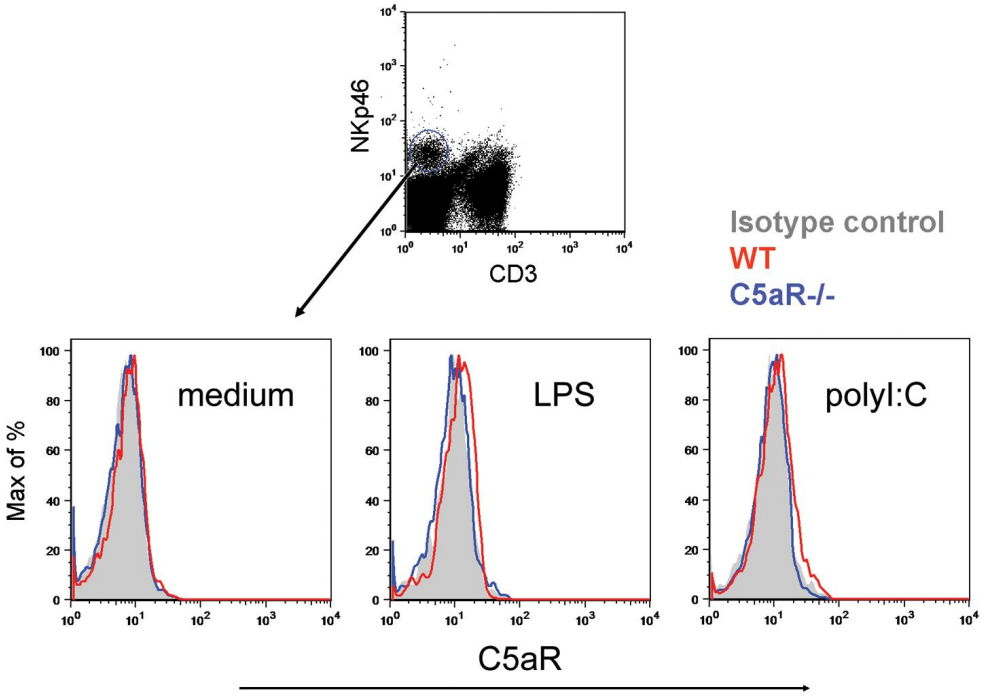

Fig. 3 Lack of C5aR expression on NK cells. WT or *C5aR-/-* mice were i.p. injected with PBS, 20 μg LPS or 100 μg polyI:C for 24 hr. Expression of C5aR on splenic NK cells (gated on $NKp46^{+}CD3^{-}$) were analyzed by FACS. Data are representative of 5 independent experiments with similar results.

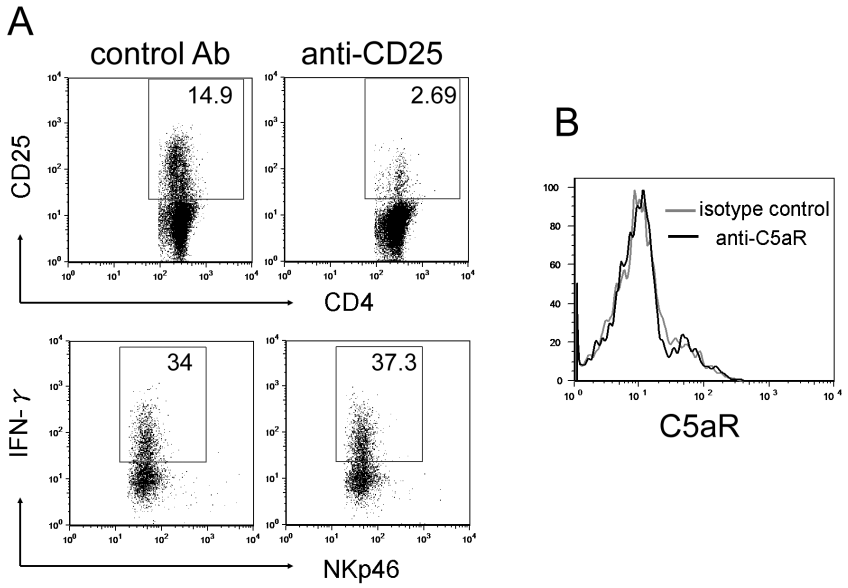

Fig. 4 The impact of Treg cell depletion on polyI:C induced cDC-NK activation. (A) C57BL/6 mice were i.p. injected with 100 µg anti-CD25 mAb (clone PC61) or an isotype-matched control mAb. Three days later, $CD4^+CD25^+$ cells were analyzed in the spleen, and total splenocytes were stimulated with polyI:C. IFN-γ positive NK cell percentage was determined. Numbers indicate the percentage of gated cells. (B) Expression of C5aR was examined on $CD4^+CD25^+$ cells in the spleen by FACS. Data are representative of 3 independent experiments with similar results

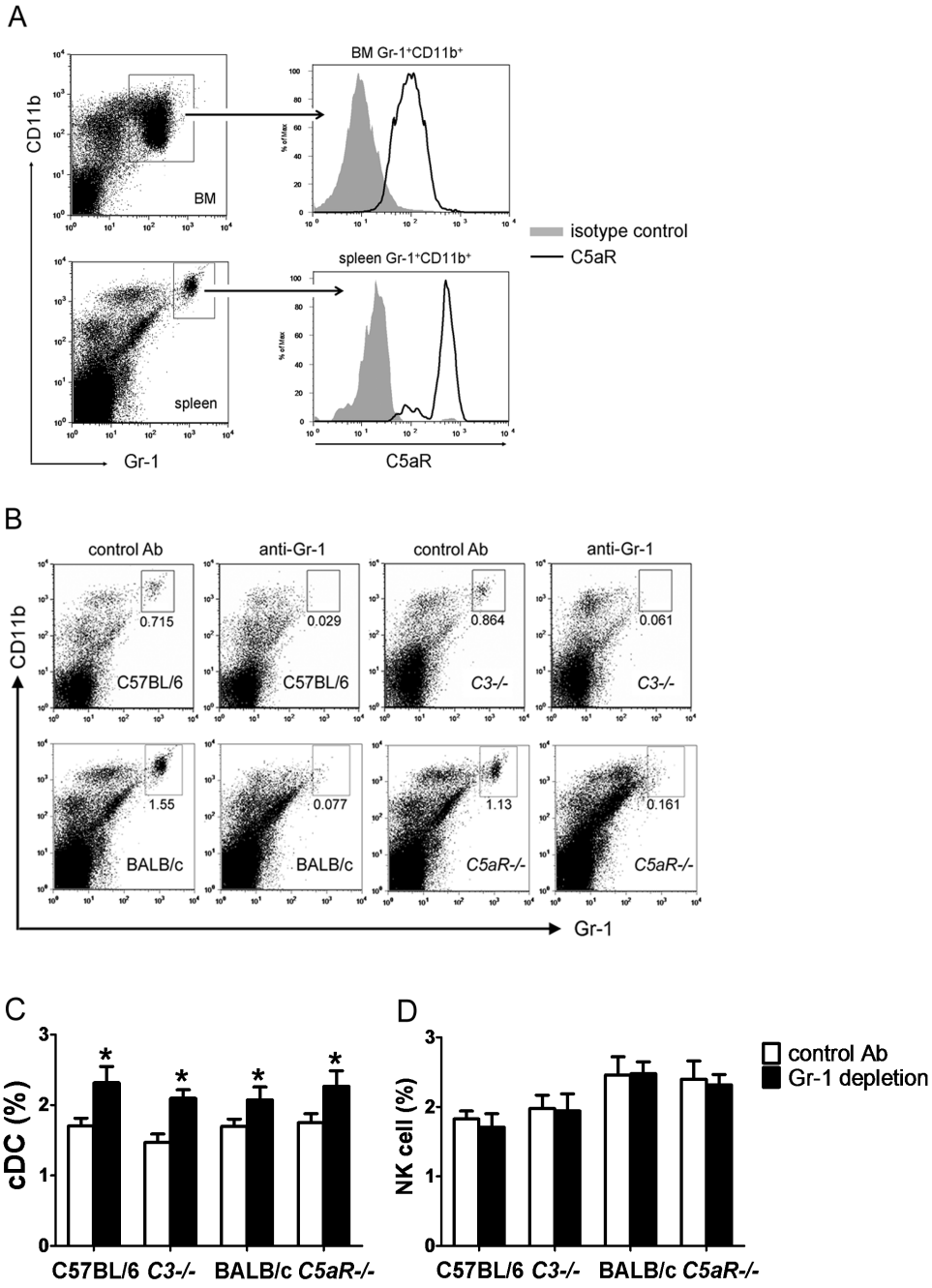

Fig. 5 C5aR expression on Gr-1$^+$CD11b$^+$ cells and Gr-1$^+$ cell depletion. (A) BM cells and splenocytes from WT mice were stained for Gr-1, CD11b and C5aR. C5aR expression was analyzed in the Gr-1$^+$CD11b$^+$ population by FACS. Data are representative of 4 independent experiments. (B-D) WT, *C3-/-* and *C5aR-/-* mice were injected with anti-Gr-1 mAb or control Ab. Three days later, Gr-1$^+$CD11b$^+$ cells (B), cDCs (C), and NK cells (D) were analyzed in the spleen. Numbers in the graph indicate the percentage of gated cells. mean ± s.e.m. n=4-5 mice/group. * P<0.05, one-way unpaired Student t-test.

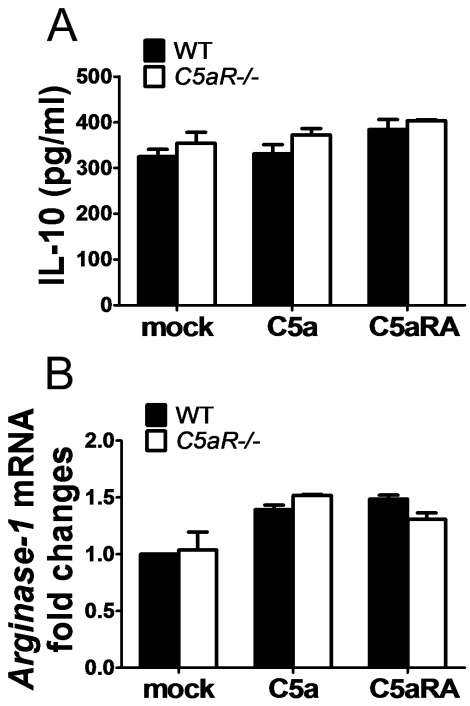

Fig. 6 IL-10 and arginase-1 production in BM Gr-1^{+}CD11b^{+} cells in the absence of C5aR. (A) IL-10 and (B) *arginase-1* were measured in BM Gr-1^{+}CD11b^{+} cells isolated from WT or *C5aR-/-* mice in the culture supernatant by ELISA and in the cell lysate by real-time PCR, respectively. mean ± s.d. n=3. Data are representative of 3 independent experiments with similar results.

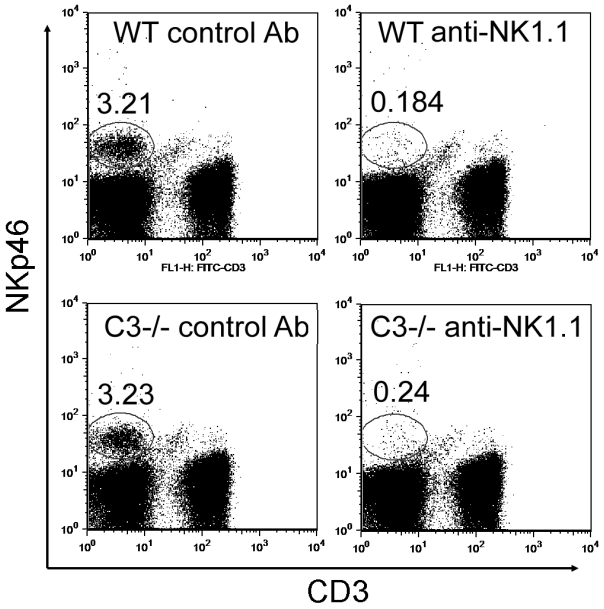

Fig. 7 Depletion of NK cells in WT and *C3-/-* mice. NK cells were depleted by i.p. injection of anti-NK1.1 mAb (clone PK136). Blood was collected for analysis of NK cell compartment 3 days after initial injection. In mice with B16 tumors, depletion of NK cells was performed on day -3, day 0, and day 7 of tumor cell inoculation. Numbers indicate the percentage of gated cells. Data shown represent 5 mice per group in 2 independent experiments with similar results.