

Harnessing innate anti-tumour immunity using a *Klebsiella*-derived therapeutic to reduce tumour burden and improve outcomes in mouse models of lung cancer



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Abstract

Tumour regression and increased survival has been associated with certain acute microbial infections. Immune dysfunction contributes to the development and progression of lung cancer, and therapies that re-constitute anti-tumor immune responses provide an important means to effectively treat malignancies and improve health outcomes. We hypothesized that stimulating the innate immune system with bacterial-derived immunomodulators could induce protective anti-cancer immune responses. A *Klebsiella*-derived drug product, QBKPN (Qu Biologics), was used to specifically stimulate the innate immune niche in the lungs in established mouse models of lung cancer. Repeated subcutaneous administration with QBKPN significantly reduced lung tumor burden and increased survival. The protective action of QBKPN required prior exposure to *Klebsiella* through either environmental exposure or lung infection. However, this QBKPN-mediated anti-tumour response was independent of adaptive immunity, as the protective effect remained in RAG2-knockout mice. QBKPN intervention was characterized by a rapid, acute like systemic immune response, including increased circulatory inflammatory cytokines and innate immune cells, leading to recruitment of immune effector cells into the lung tissue, including macrophages and natural killer (NK) cells. In addition to recruitment of innate immune cells, QBKPN increased markers of classically activated macrophages and increased production of NK cell effector molecules. Together, these data suggest that QBKPN, a *Klebsiella*-derived immunomodulator, causes activation and recruitment of macrophages and NK cells into the lungs, reducing cancer tumour burden and improving survival outcomes.

Introduction

- Acute infection has been linked to spontaneous cancer remission throughout history
- Bacteria-induced immune mobilization in the context of malignancy is the first documented form of cancer immunotherapy
- The lack of sufficient understanding of the mechanisms driving this therapeutic effects, inability to elicit a consistent response, and safety issues, have prevented the full exploration of this therapeutic avenue
- QBKPN is a microbe-based investigational therapeutic (Qu Biologics) that is an immunomodulator derived from inactivated *Klebsiella*
- It was hypothesized that repeated subcutaneous QBKPN treatment would be able to induce an acute-like inflammatory response, resulting in anti-tumour efficacy

QBKPN reduced tumour burden in mouse model of lung cancer

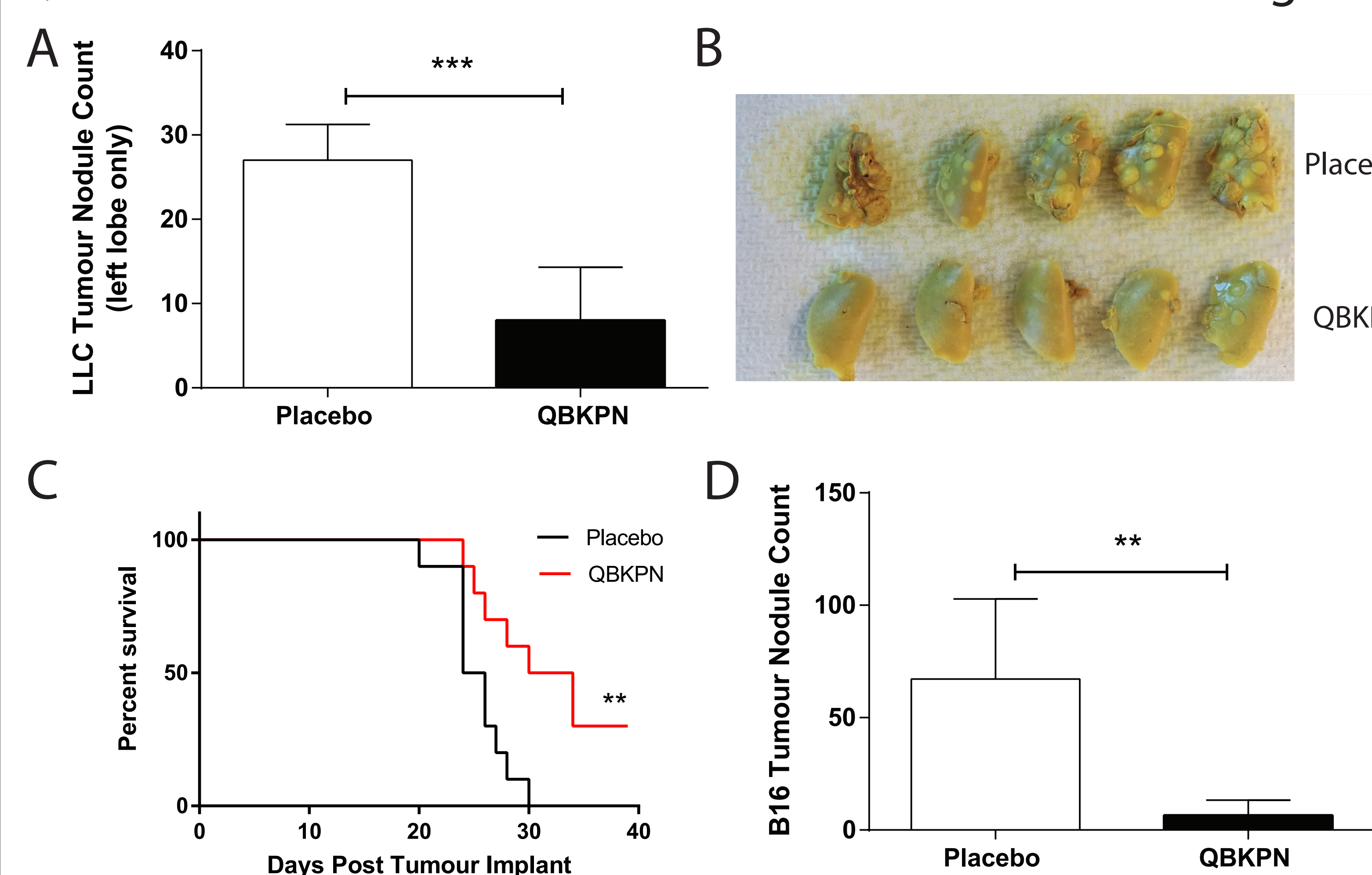


Figure 1: QBKPN efficacy in mouse models of lung cancer

Mice were treated by subcutaneous injection of Placebo or QBKPN every second day for 10 days before tumour inoculation, and continuing until mice were sacrificed. **A)** 14 days after Lewis Lung Carcinoma (LLC) cells were injected into the tail vein, QBKPN reduced tumour nodules counts in the lung. **B)** The decrease in tumour nodules was imaged by fixing the lungs in Bouin's solution. **C)** QBKPN also increased survival in the LLC challenged mice. **D)** In a B16F10 melanoma tail vein injected lung cancer model, QBKPN also decreased the tumour burden compared to Placebo. For tumor nodule counts and ID tumor size, mean \pm SD shown. ** $P < 0.01$; *** $P < 0.001$ by Student's *t*-test. For the survival curve, $n = 10$ mice per group. ** $P < 0.01$ by Log-rank test.

QBKPN efficacy required *Klebsiella* pre-exposure, but was independent of the adaptive immune response

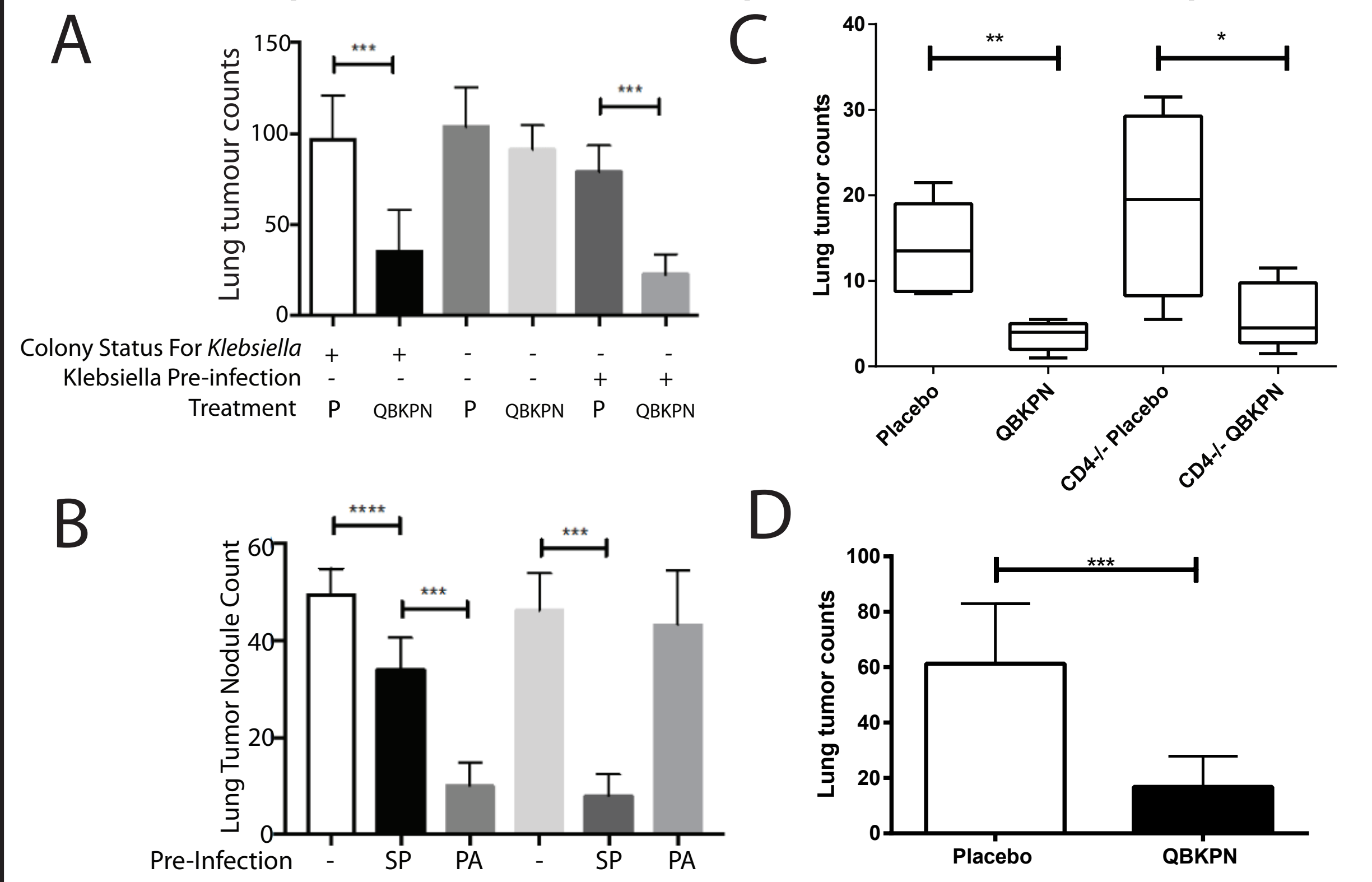


Figure 2: QBKPN anti-tumor efficacy requires host exposure to *Klebsiella*, but was independent of the adaptive immune response.

A) QBKPN, when compared to Placebo (P), was only efficacious in lung cancer models in mice sourced from facilities that were positive for *Klebsiella* environmental exposure, or when mice were given overt *Klebsiella* pre-infection. **B)** The link of efficacy to previous exposure by the same species was also seen for a *Streptococcus*-derived product (SP) and a *Pseudomonas*-derived product (PA). **C)** QBKPN efficacy was not dependent on present of CD25+ cells (which include adaptive memory cells). **D)** Rag2 knockout mice, which have no T or B cells, confirmed that QBKPN efficacy was not dependent on the adaptive immune response. $n = 5-10$ mice per group. ***, $P < 0.01$; ****, $P < 0.001$ by Student's *t*-test.

QBKPN altered the lung immune profile

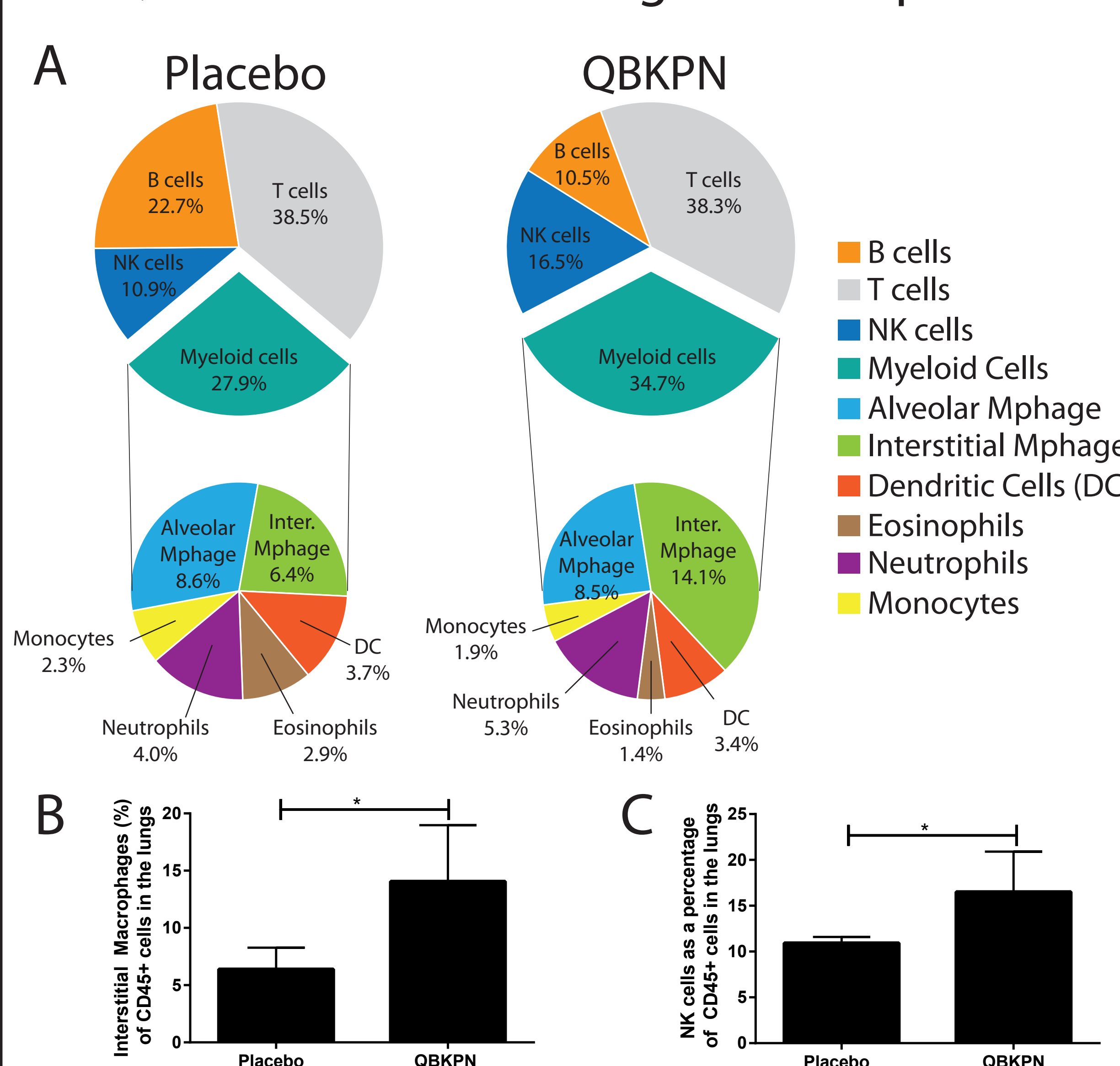


Figure 4: QBKPN recruitment of interstitial macrophages and NK cells into the lungs

A) QBKPN altered the lung immune profile in mice, treated with QBKPN or Placebo, in a day 17 B16F10 lung cancer model. Upper pie charts show the proportion of B cells, T cells, NK cells and myeloid cells. Lower pie charts show the proportion of different myeloid cells. Percentages reflect the percent of CD45+ defined cells. **B)** NK cells were increased in the lungs with QBKPN treatment, as were **(C)** Interstitial Macrophages. * $P < 0.05$ by Student's *t*-test

QBKPN induced a M2 to M1 shift in lung macrophages

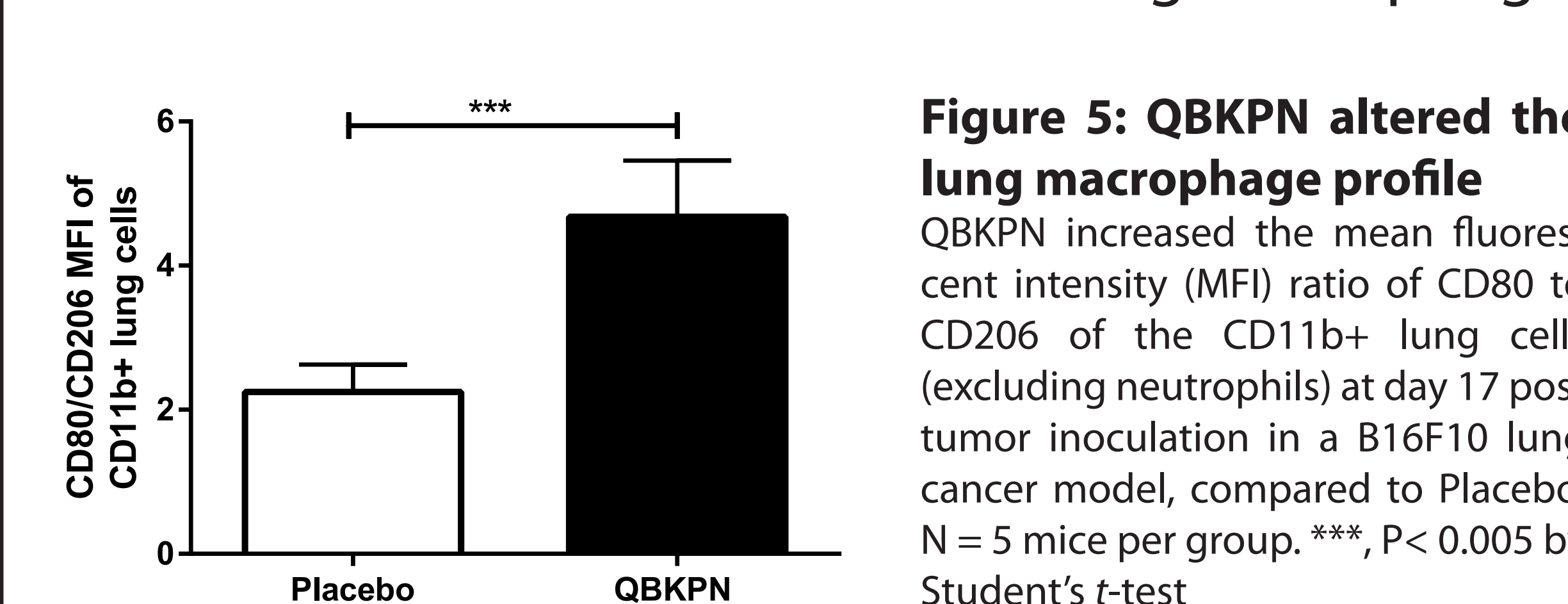


Figure 5: QBKPN altered the lung macrophage profile

QBKPN increased the mean fluorescent intensity (MFI) ratio of CD80 to CD206 of the CD11b+ lung cells (excluding neutrophils) at day 17 post tumor inoculation in a B16F10 lung cancer model, compared to Placebo. $N = 5$ mice per group. ***, $P < 0.005$ by Student's *t*-test

QBKPN stimulated an acute inflammatory response

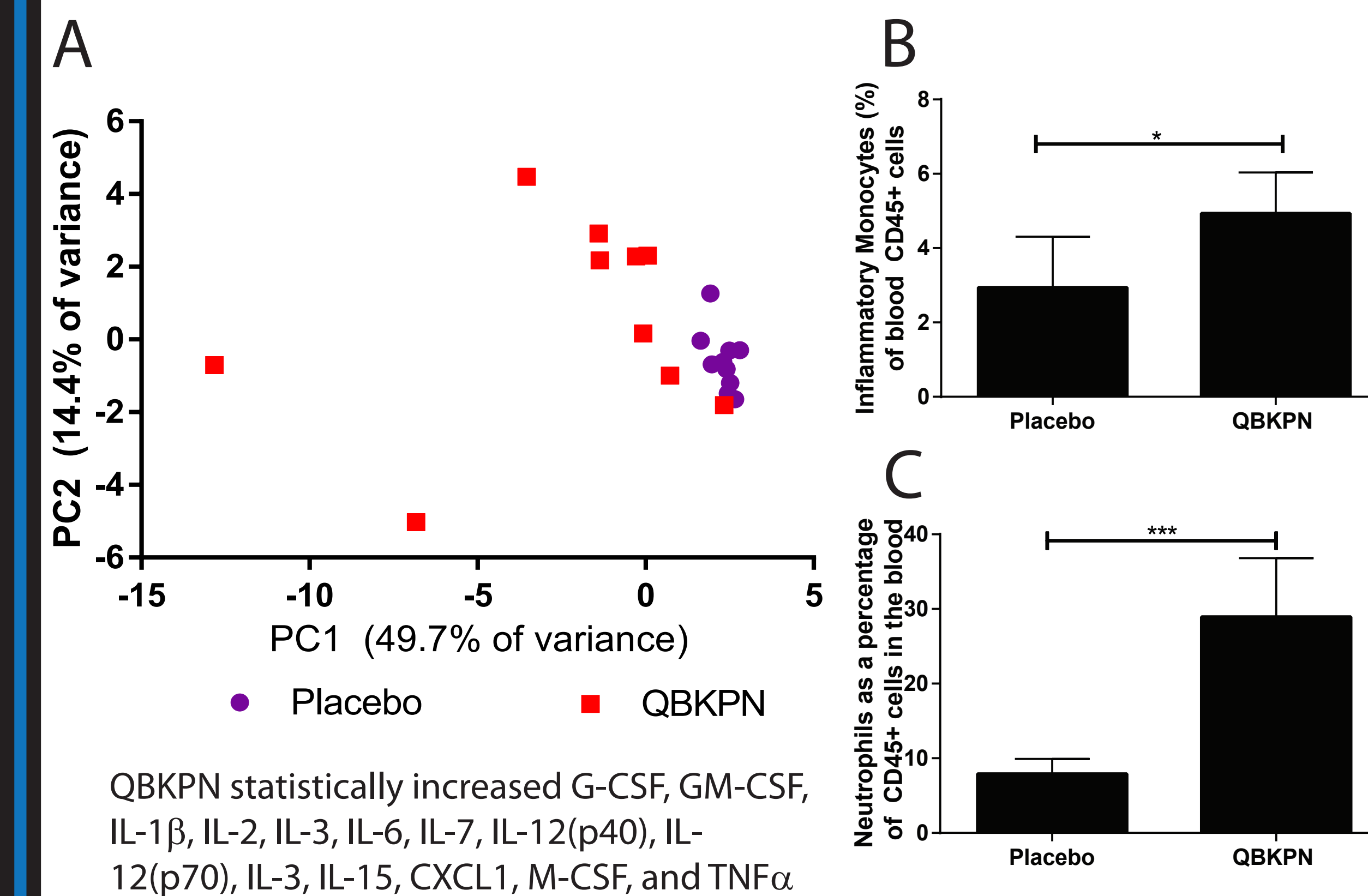


Figure 3: QBKPN treatment included an acute-like immune response 5 hours after a single dose of QBKPN

A) A principal component analysis of 31 cytokines/chemokines 5 hours after a single dose of QBKPN showed a difference in the cytokine profile between placebo (circles) or QBKPN (Squares) treated animals. **B)** The cytokine release led to an increase in inflammatory monocytes (CD11b+CCR2+CD115+Ly6C^{hi}) and **C)** Neutrophils (Ly6G+) as a percentage of CD45+ cells in the blood 5 hours after placebo or QBKPN treatment. $N = 5-10$ mice per group. *, $P < 0.05$; ***, $P < 0.005$ by Student's *t*-test

QBKPN efficacy was through an NKG2D and NK cell dependent mechanism

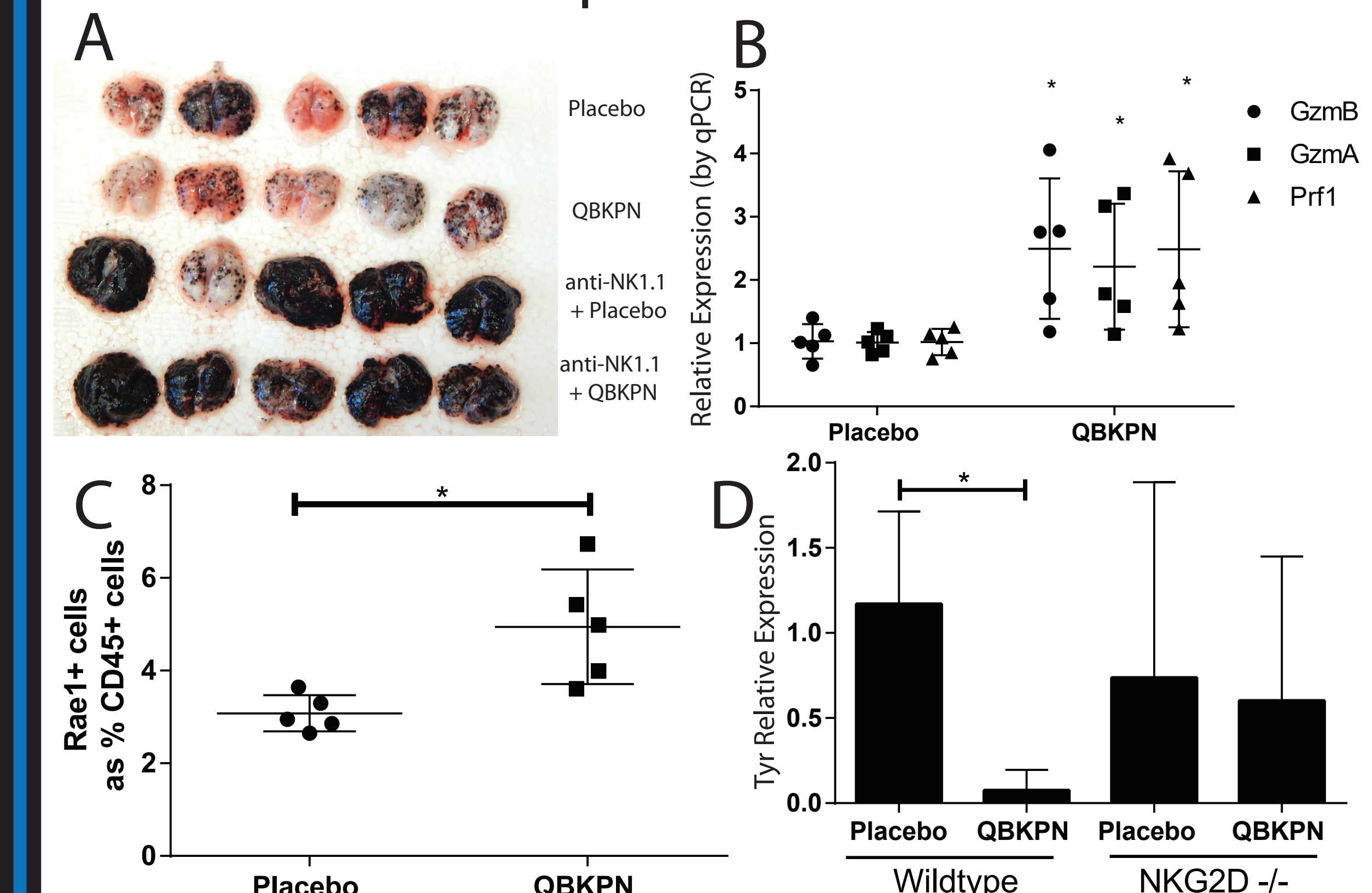


Figure 6: QBKPN anti-tumor efficacy requires NK cells and NKG2D

A) QBKPN efficacy in the B16F10 lung cancer model was lost when NK cells were blocked. **B)** QBKPN increased the expression of the NK cell cytotoxic pathways including Granzyme B (GzmB), Granzyme A (GzmA) and Perforin 1 (Prf1), in the lungs of mice treated with placebo or QBKPN at day 17 in a B16F10 model. **C)** QBKPN increased the expression of Rae1+ cells in the lungs, compared to placebo. Rae1 is a ligand for NKG2D, an activating receptor on NK cells. **D)** NKG2D was shown to be required for QBKPN efficacy in the B16F10 lung cancer model at day 17. $N = 5-10$ mice per group. *, $P < 0.05$; **, $P < 0.01$ by Student's *t*-test.

Conclusions

- QBKPN, derived for inactivated *Klebsiella*, reduced tumour burden and increased survival in mouse models of lung cancer
- This efficacy was dependent on *Klebsiella* pre-exposure, but independent on the adaptive immune response
- QBKPN induced an acute-like inflammatory response, which results in altering the lung immune environment
- QBKPN shifted the macrophage profile from an M2 to M1 profile
- This efficacy was dependent on NK cells, and the NKG2D pathway
- This study highlights the potential of using bacterial-derived products for innate immune stimulation as a therapeutic for lung cancer.

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