



Exploring the Immunomodulatory Role of MUC5AC in KRAS Mutant Lung Adenocarcinoma as A Potentially Novel Immune Checkpoint

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Introduction

Among various oncogenes, KRAS contributes to the most cases of lung adenocarcinoma (LUAD), and despite growing advancements in treatment, KRAS-mutant LUAD (KM-LUAD) remains poorly treatable. Immune dysregulation in KM-LUAD contributes to a tumor-permissive microenvironment that promotes tumor growth, limited response to immune checkpoint blockade (ICB), and recurrence.

MUC5AC is the main gel-forming airway secretory mucin. MUC5AC has low expression at baseline but is overexpressed in lung inflammatory diseases such as chronic obstructive pulmonary disease. It is also overexpressed in KM-LUAD, and its overexpression is strongly associated with poor clinical prognosis.

Previous preclinical studies by our group established that genetic deletion of Muc5ac (knockout) leads to lower tumor burden in KM-LUAD murine models. Therefore, understanding the functional role of MUC5AC in KM-LUAD may allow for exploring it as a potential preventive and therapeutic target.

Objective

We aim to explore the functional effect of MUC5AC on tumors and the tumor microenvironment in KM-LUAD by inhibiting its expression using a specific antisense oligonucleotide (ASO), which could easily be translated to the clinical setting.

Methods

5'...ATG C C T G G A C T T C A...3 Sense strand of DNA
3'...T A C G G A C C T G A A G T...5 Antisense strand of DNA

Transcription (in the nucleus) of antisense strand

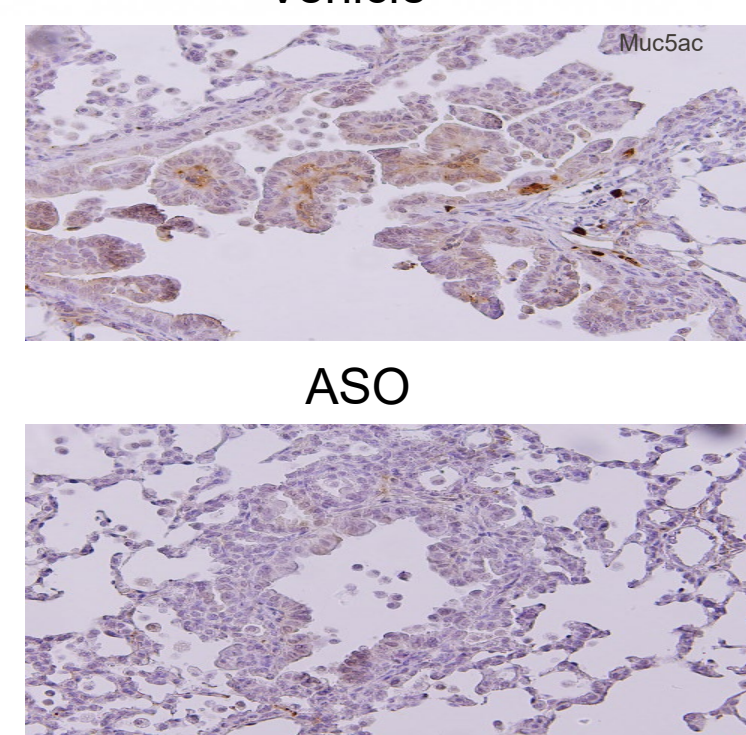
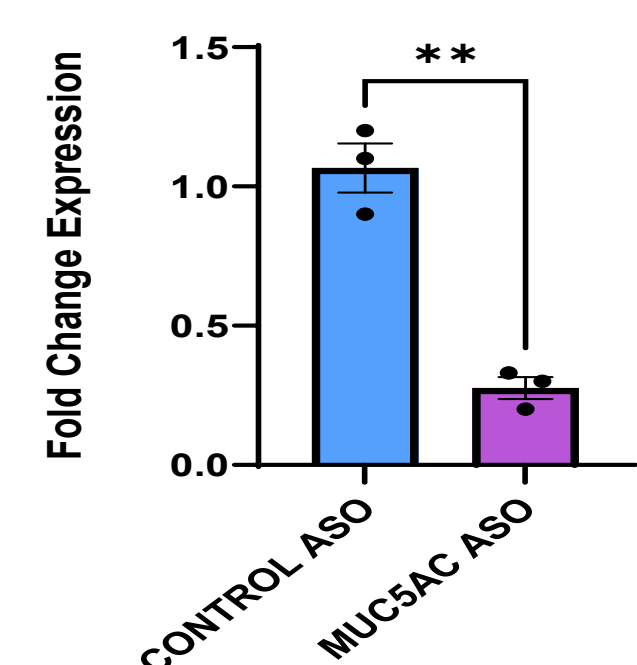
3'...A U G G C C U G A C U U C A...3 mRNA (= sense) in cytoplasm
3'...T A C C G A C T G T A G T...5 oligodeoxynucleotide

Translation of mRNA blocked

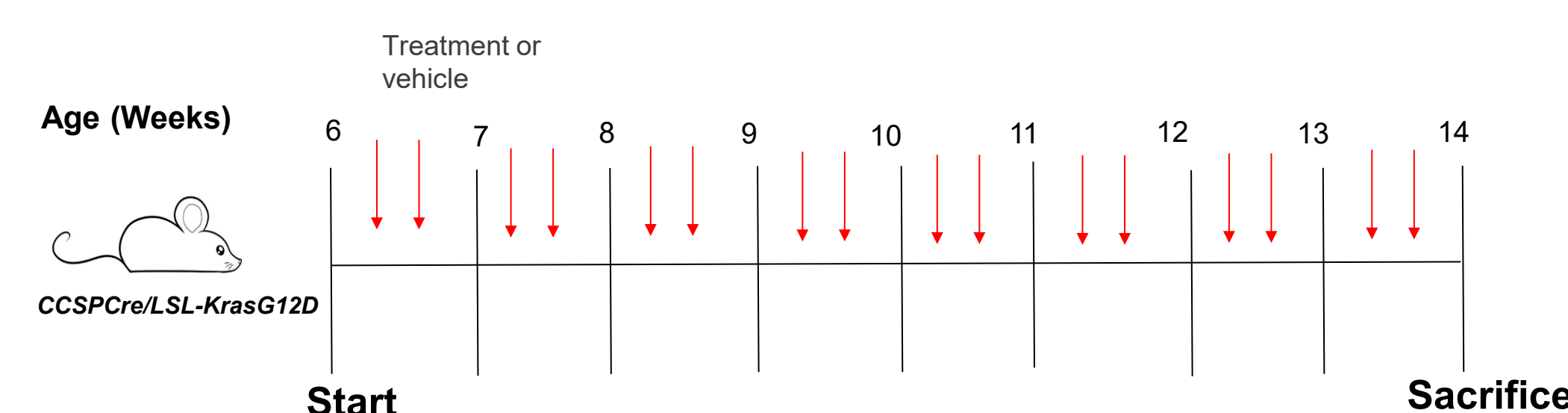
Met - Asp - Thr - Ser Peptide

Vehicle

Muc5ac Expression



Treatment Schematic



MUC5AC ASO or Vehicle	Delivery	Intratracheal
	Dosage	20 µg/kg twice per week

Figure 1: Cohorts of Kras mutant mice were given two weekly intratracheal doses of control or MUC5AC ASO from 6 weeks to 14 weeks of age. Tumors were assessed, and lungs and bronchoalveolar lavage fluid (BALF) were collected.

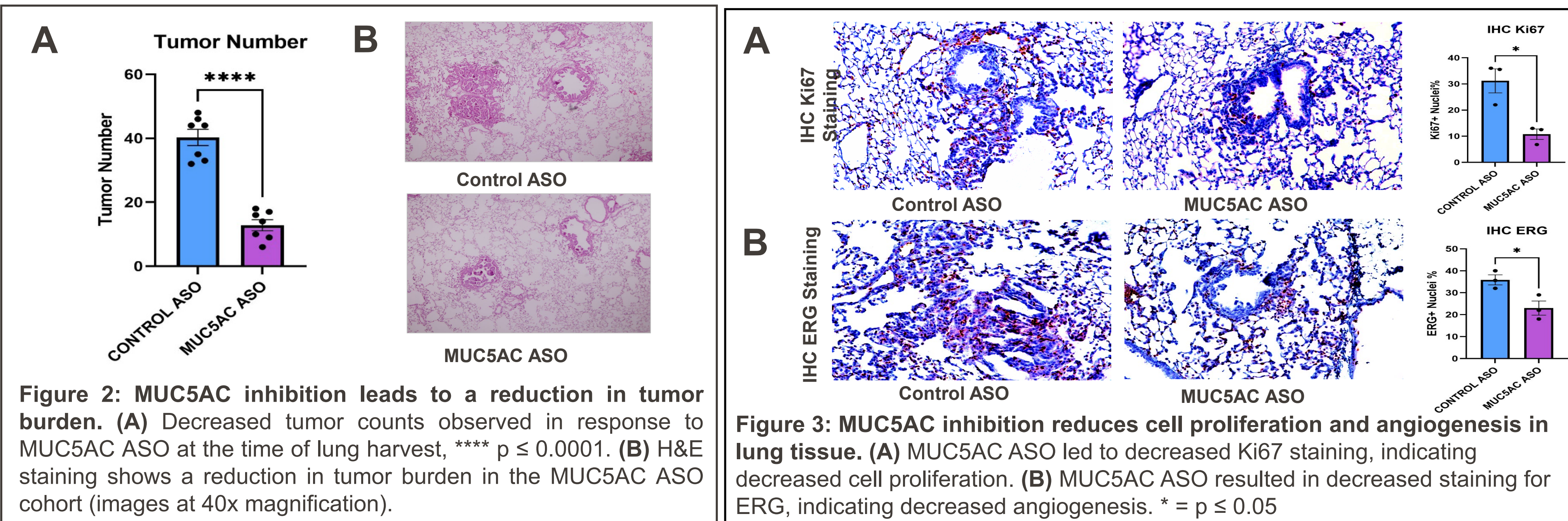


Figure 2: MUC5AC inhibition leads to a reduction in tumor burden. (A) Decreased tumor counts observed in response to MUC5AC ASO at the time of lung harvest, **** $p \leq 0.0001$. (B) H&E staining shows a reduction in tumor burden in the MUC5AC ASO cohort (images at 40x magnification).

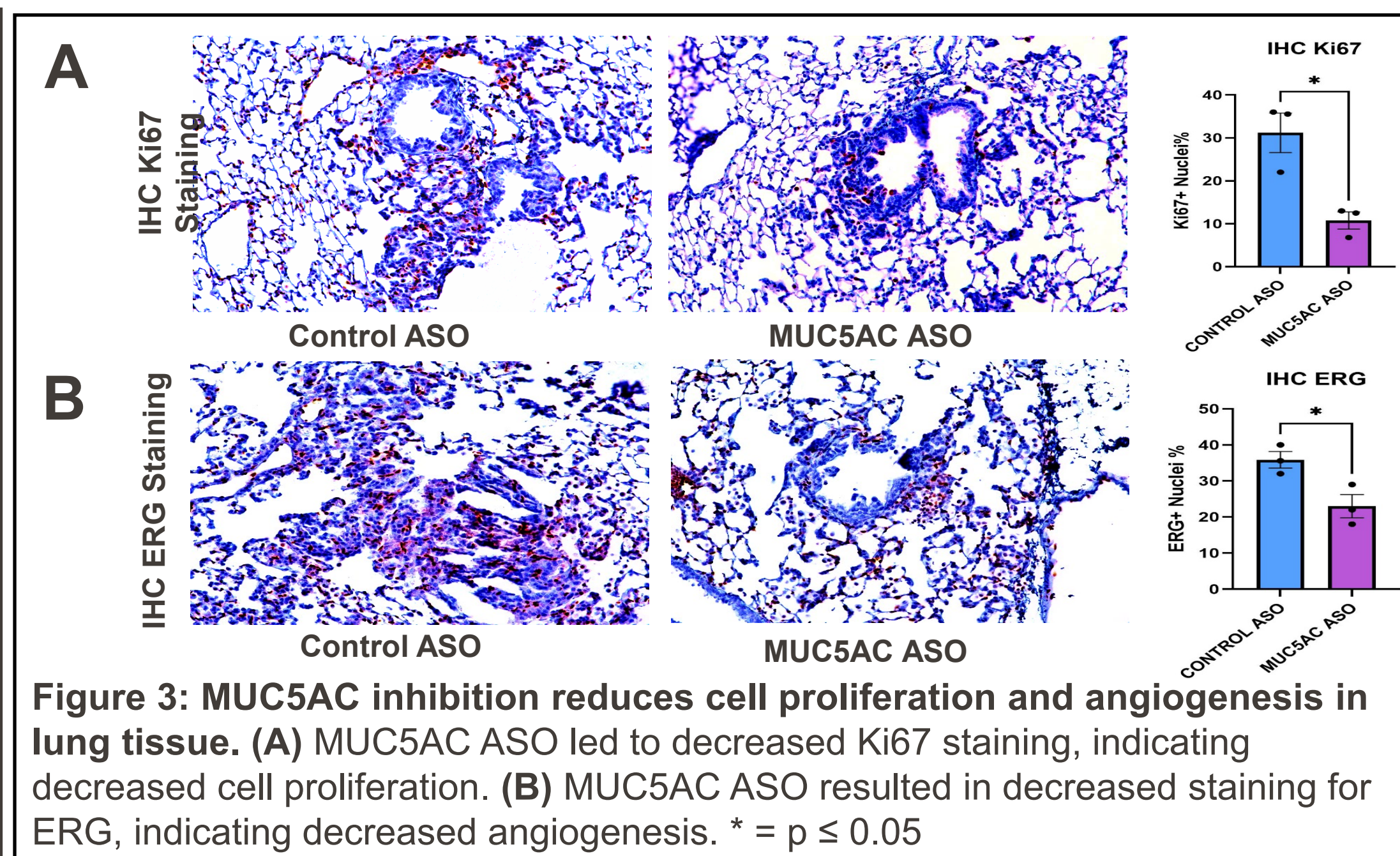


Figure 3: MUC5AC inhibition reduces cell proliferation and angiogenesis in lung tissue. (A) MUC5AC ASO led to decreased Ki67 staining, indicating decreased cell proliferation. (B) MUC5AC ASO resulted in decreased staining for ERG, indicating decreased angiogenesis. * $p \leq 0.05$

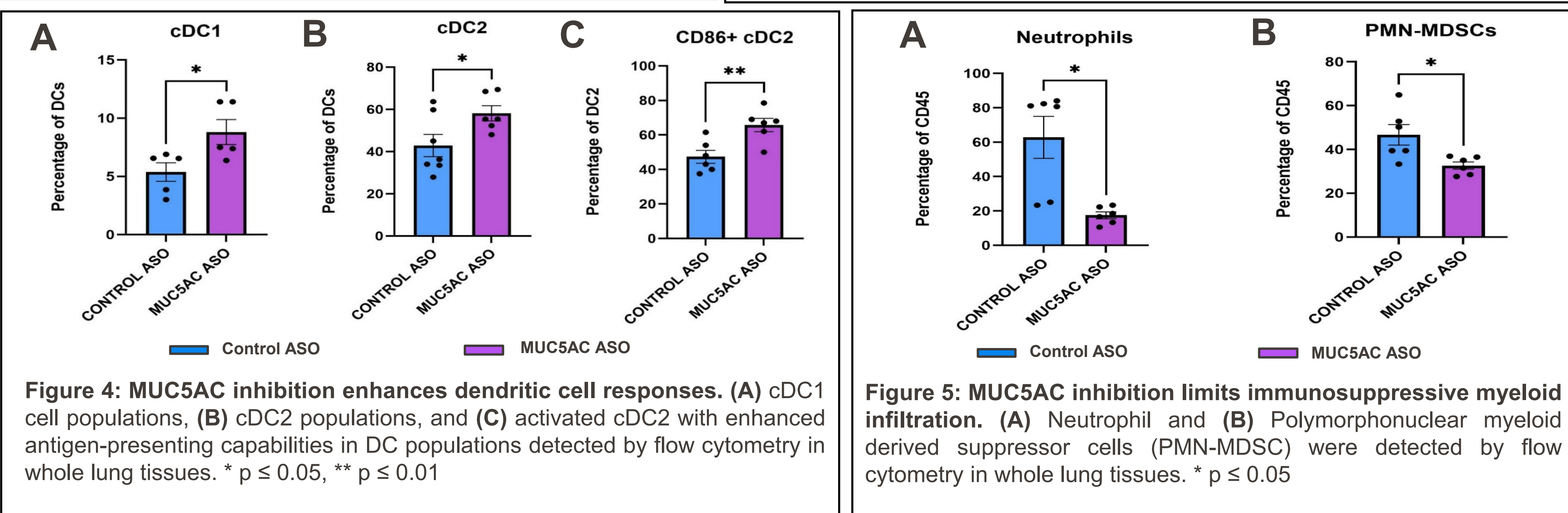


Figure 4: MUC5AC inhibition enhances dendritic cell responses. (A) cDC1 cell populations, (B) cDC2 populations, and (C) activated cDC2 with enhanced antigen-presenting capabilities in DC populations detected by flow cytometry in whole lung tissues. * $p \leq 0.05$, ** $p \leq 0.01$

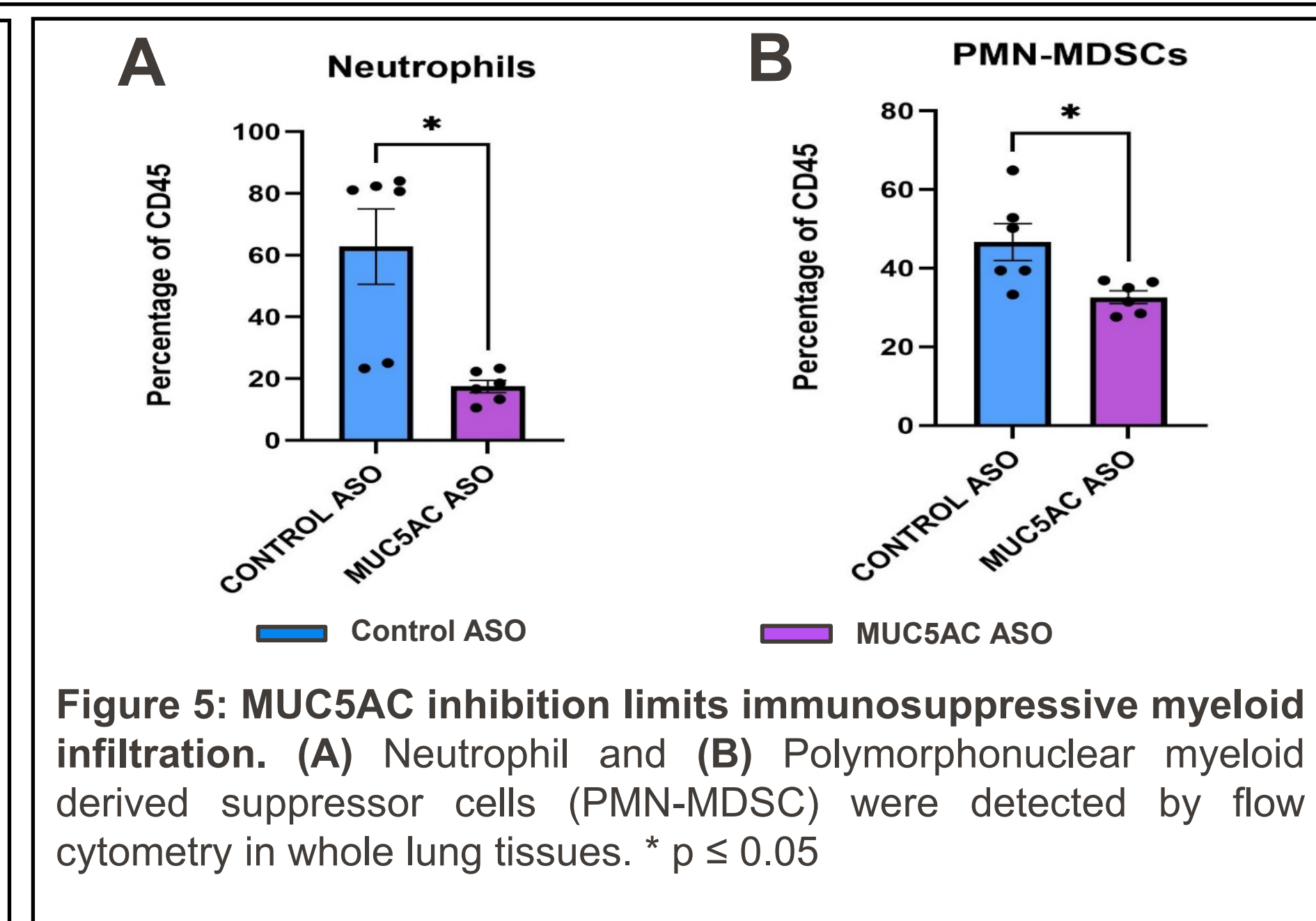


Figure 5: MUC5AC inhibition limits immunosuppressive myeloid infiltration. (A) Neutrophil and (B) Polymorphonuclear myeloid derived suppressor cells (PMN-MDSC) were detected by flow cytometry in whole lung tissues. * $p \leq 0.05$

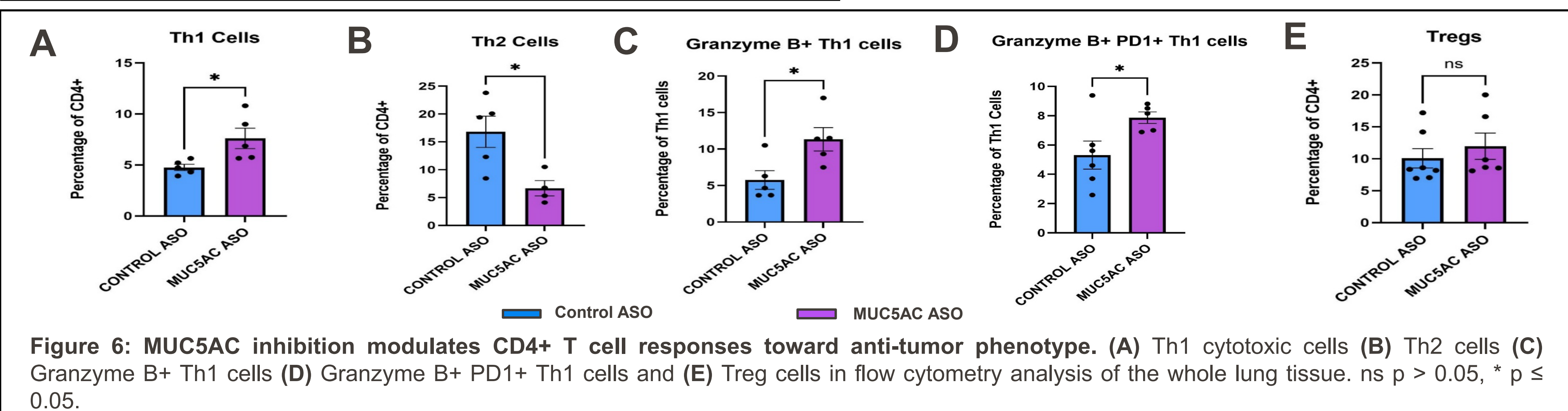


Figure 6: MUC5AC inhibition modulates CD4+ T cell responses toward anti-tumor phenotype. (A) Th1 cytotoxic cells (B) Th2 cells (C) Granzyme B+ Th1 cells (D) Granzyme B+ PD1+ Th1 cells and (E) Treg cells in flow cytometry analysis of the whole lung tissue. ns $p > 0.05$, * $p \leq 0.05$.

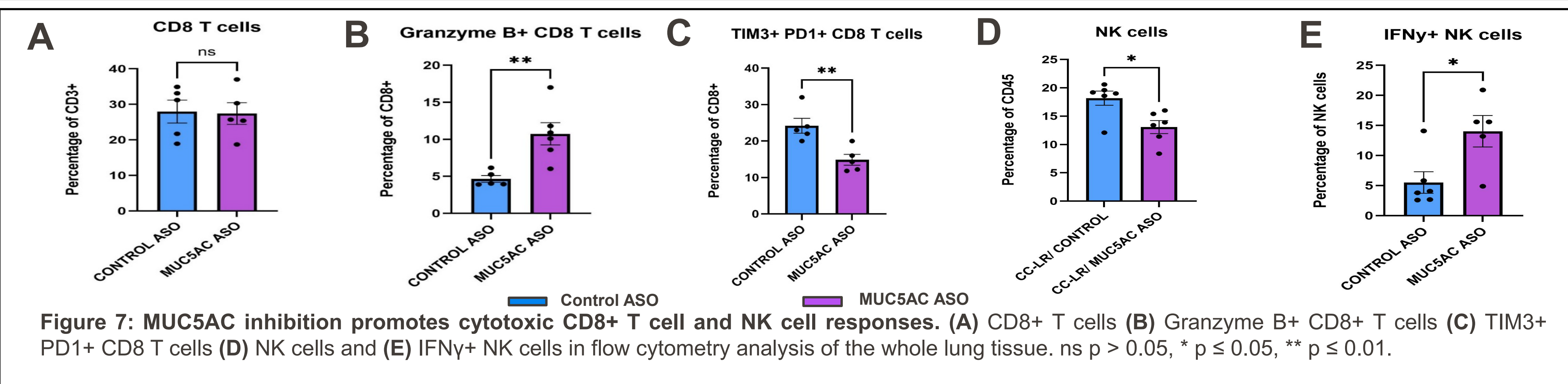


Figure 7: MUC5AC inhibition promotes cytotoxic CD8+ T cell and NK cell responses. (A) CD8+ T cells (B) Granzyme B+ CD8+ T cells (C) TIM3+ PD1+ CD8 T cells (D) NK cells and (E) IFNγ+ NK cells in flow cytometry analysis of the whole lung tissue. ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$.

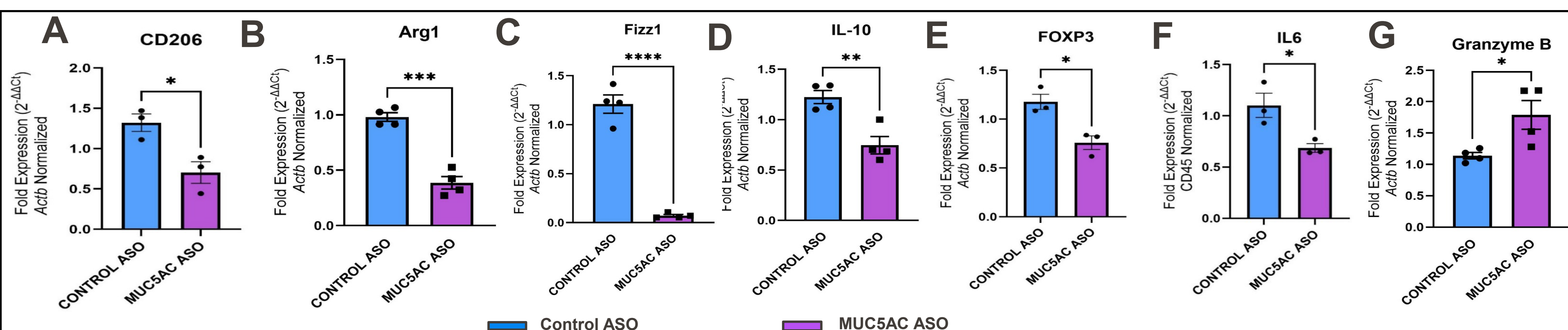


Figure 8: MUC5AC inhibition leads to reduced immunosuppressive marker expression and increased expression of the cytotoxic marker Granzyme B. (A) CD206, (B) Arg1, (C) Fizz1 (D) IL-10, (E) FOXP3, (F) IL6, and (G) anti-tumor Granzyme B in Q-PCR analysis of whole lung tissue. ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$.

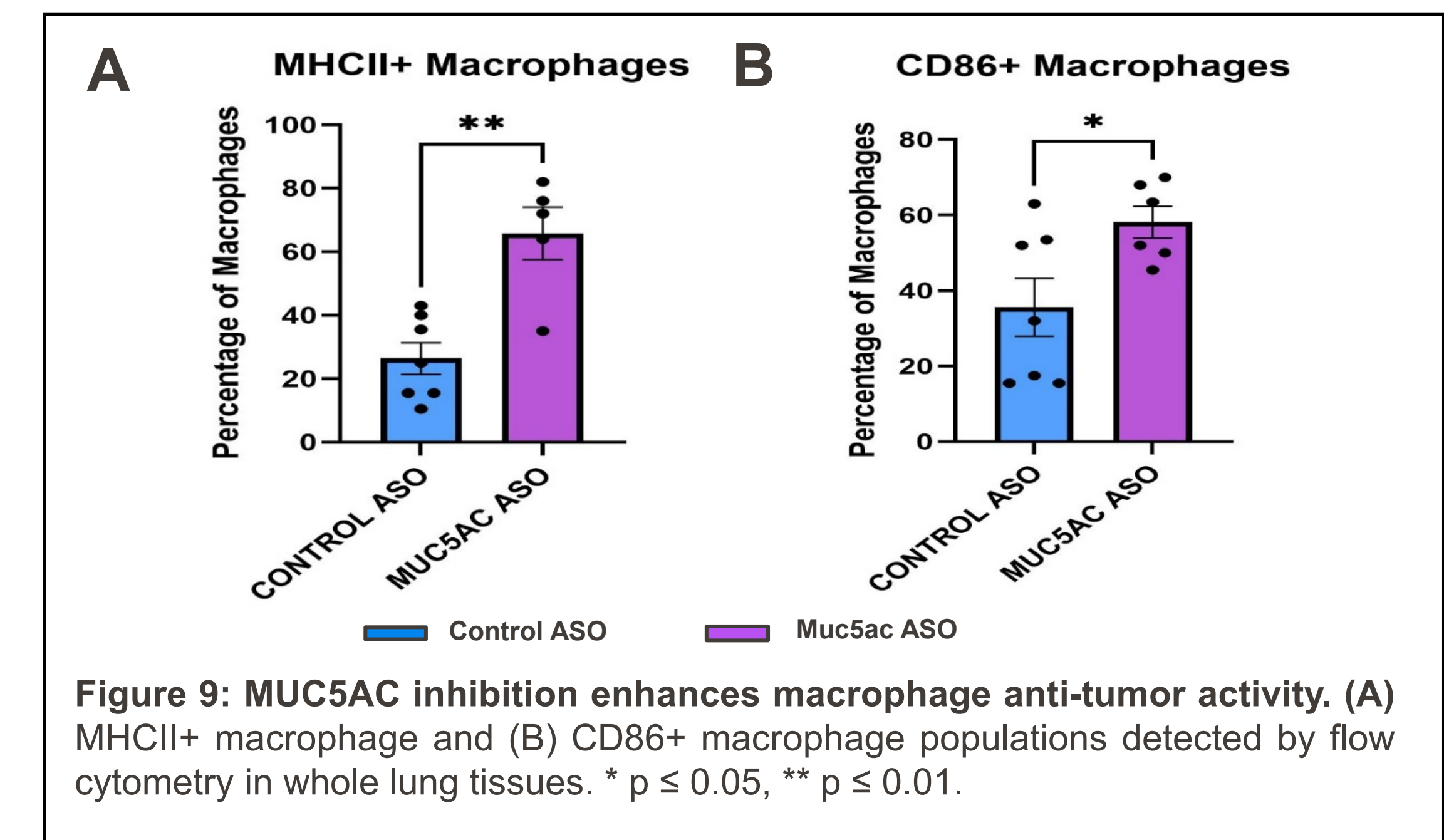


Figure 9: MUC5AC inhibition enhances macrophage anti-tumor activity. (A) MHCII+ macrophage and (B) CD86+ macrophage populations detected by flow cytometry in whole lung tissues. * $p \leq 0.05$, ** $p \leq 0.01$.

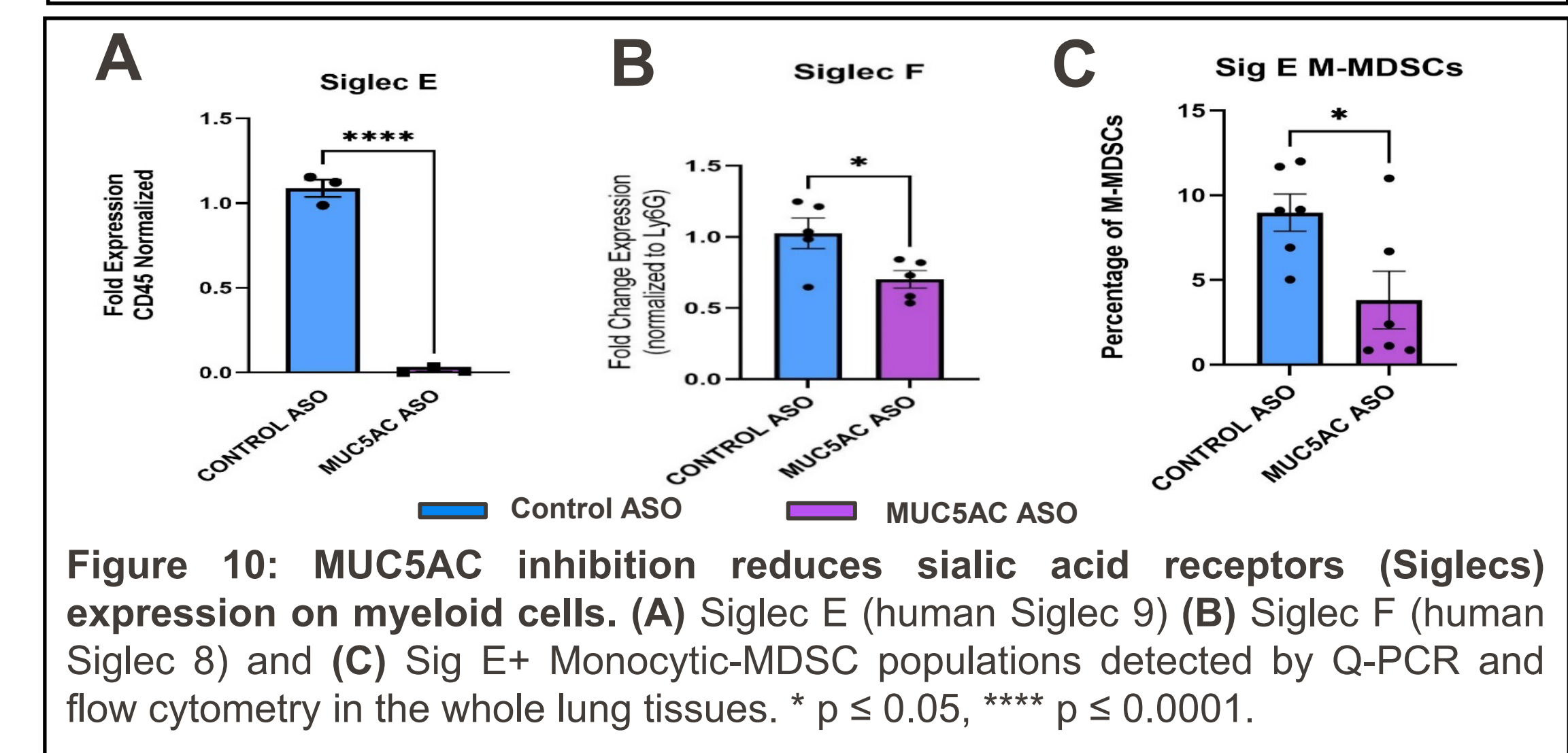


Figure 10: MUC5AC inhibition reduces sialic acid receptors (Siglecs) expression on myeloid cells. (A) Siglec E (human Siglec 9) (B) Siglec F (human Siglec 8) and (C) Sig E+ Monocytic-MDSC populations detected by Q-PCR and flow cytometry in the whole lung tissues. * $p \leq 0.05$, **** $p \leq 0.0001$.

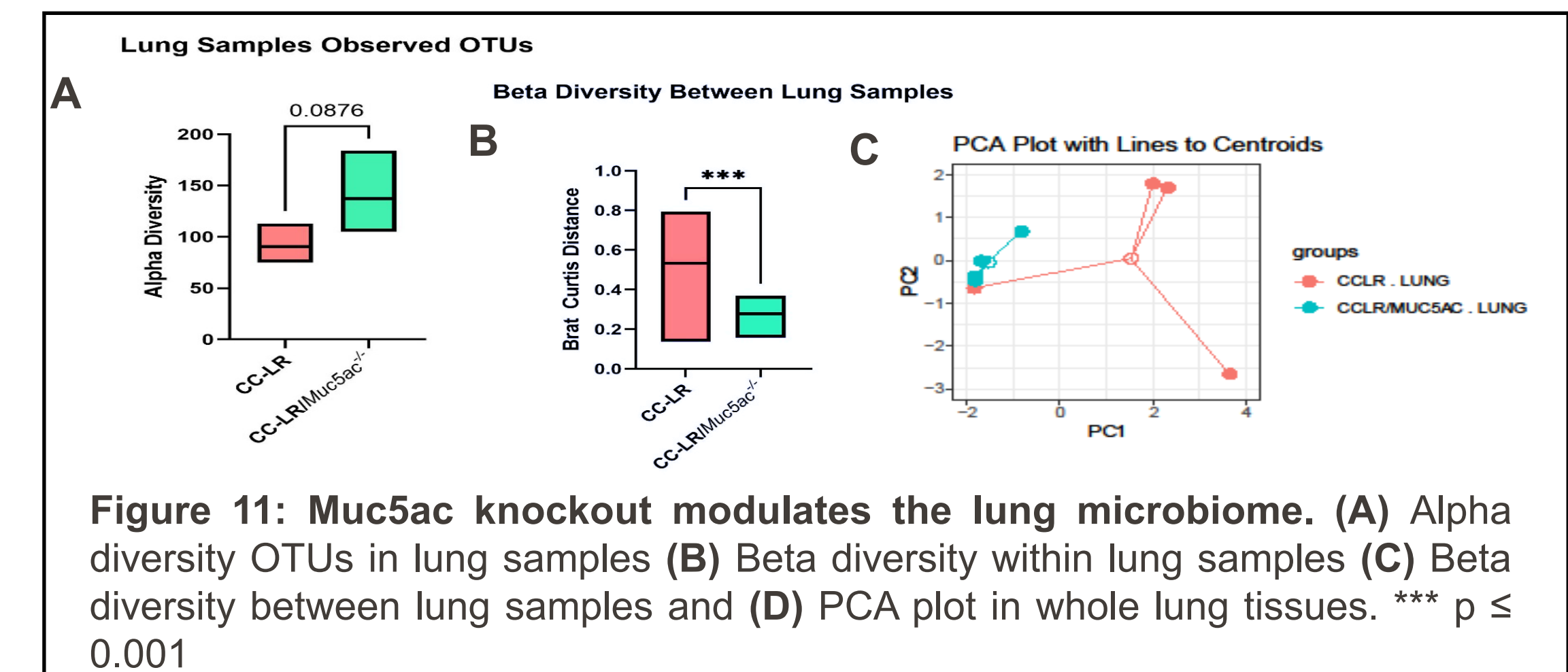


Figure 11: Muc5ac knockout modulates the lung microbiome. (A) Alpha diversity OTUs in lung samples (B) Beta diversity within lung samples (C) Beta diversity between lung samples and (D) PCA plot in whole lung tissues. **** $p \leq 0.0001$

Summary & Conclusion & Discussion

In summary, MUC5AC inhibition reduces Kras-mutant lung tumor burden, tumor cell proliferation, and angiogenesis by shifting the lung microenvironment from a pro-tumor to an anti-tumor immune phenotype.

We conclude that MUC5AC plays a critical role in KM-LUAD growth by impairing anti-tumor immune response and shifting the lung tumor microenvironment toward an immunosuppressive phenotype.

This might be mediated by upregulating Siglecs and modulating the tumor microenvironment by reprogramming myeloid cells. In addition, MUC5AC overexpression may cause microbial dysbiosis, disrupting normal lung immune homeostasis and promoting immune suppression.

Overall, these findings highlight MUC5AC as a potential new immune checkpoint and a promising preventive/therapeutic target in KM-LUAD, with high translational potential that could be combined with currently available interventions, including ICB or KRAS-specific targeted therapies.

Acknowledgment: IONIS Pharma synthesized and provided Muc5ac and control ASO under an approved MTA with SJM.

Funding: NCI (R01CA225977 to SJM), JK was supported by the UT MD Anderson SOAR Program (R25CA265800-01A1)

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