



## INTRODUCTION

Immune checkpoint inhibitors (ICIs) have become a standard treatment for patients with advanced non-small cell lung cancer (NSCLC). However, substantial interpatient variability in treatment efficacy remains, and reliable biomarkers predicting therapeutic response are still lacking. Recent studies have identified sarcopenia as an unfavorable prognostic factor in patients receiving ICIs.<sup>1</sup> In our previous prospective study, we demonstrated that plasma pentraxin 3 (PTX3) levels were significantly elevated in patients with muscle mass loss and that high PTX3 levels were independently associated with poor prognosis in patients with NSCLC treated with ICIs.<sup>2</sup> PTX3 is a soluble pattern-recognition molecule involved in innate immunity, inflammation, tissue repair, and cancer biology.<sup>3</sup> Nevertheless, its role in antitumor T-cell immunity and its influence on the efficacy of immune checkpoint blockade remain unclear. In the present study, we investigated the effects of PTX3 on antitumor T-cell responses and the therapeutic efficacy of PD-1 blockade using mouse tumor models.

## METHODS

### Survival analysis

Overall survival was analyzed using the GEPIA3 web server, which integrates RNA sequencing data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project.<sup>4</sup> Patients with NSCLC were stratified into high- and low-PTX3 expression groups according to the median PTX3 expression level, and Kaplan–Meier survival curves were generated.

### In vitro experiments

Tumor-draining lymph nodes (TDLNs) were harvested from C57BL/6 mice bearing MCA205 tumors. TDLN cells were cultured in complete medium in the presence or absence of recombinant PTX3. Following tumor-specific stimulation, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell proliferation, interferon- $\gamma$  (IFN- $\gamma$ ) production, expression of immune-related molecules, and apoptosis were evaluated by flow cytometry.

### In vivo experiments

To evaluate the effect of PTX3 on the therapeutic efficacy of PD-1 blockade, C57BL/6 mice bearing subcutaneous MC38 tumors were treated with anti-PD-1 antibody with or without PTX3 administration. Anti-PD-1 antibody was administered intraperitoneally at 10 mg/kg on day 5 after tumor inoculation, whereas PTX3 (25  $\mu$ g/mouse) was administered intraperitoneally once daily from days 2 to 9. Tumor growth was assessed by serial measurements of tumor surface area, calculated as the product of the longest and shortest tumor diameters.

## DISCUSSION AND CONCLUSION

- **PTX3 suppressed CD8<sup>+</sup> T cell proliferation and reduced IFN- $\gamma$  production in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, indicating an inhibitory role in antitumor T-cell immune responses. PTX3 administration was also associated with reduced efficacy of anti-PD-1 therapy in a mouse tumor model.**
- **These findings suggest that PTX3, which is elevated in muscle mass loss, contributes to poor responses to immunotherapy. PTX3 may therefore serve as a predictive biomarker and potential therapeutic target in cancer immunotherapy.**

## REFERENCES & ACKNOWLEDGEMENTS

### References

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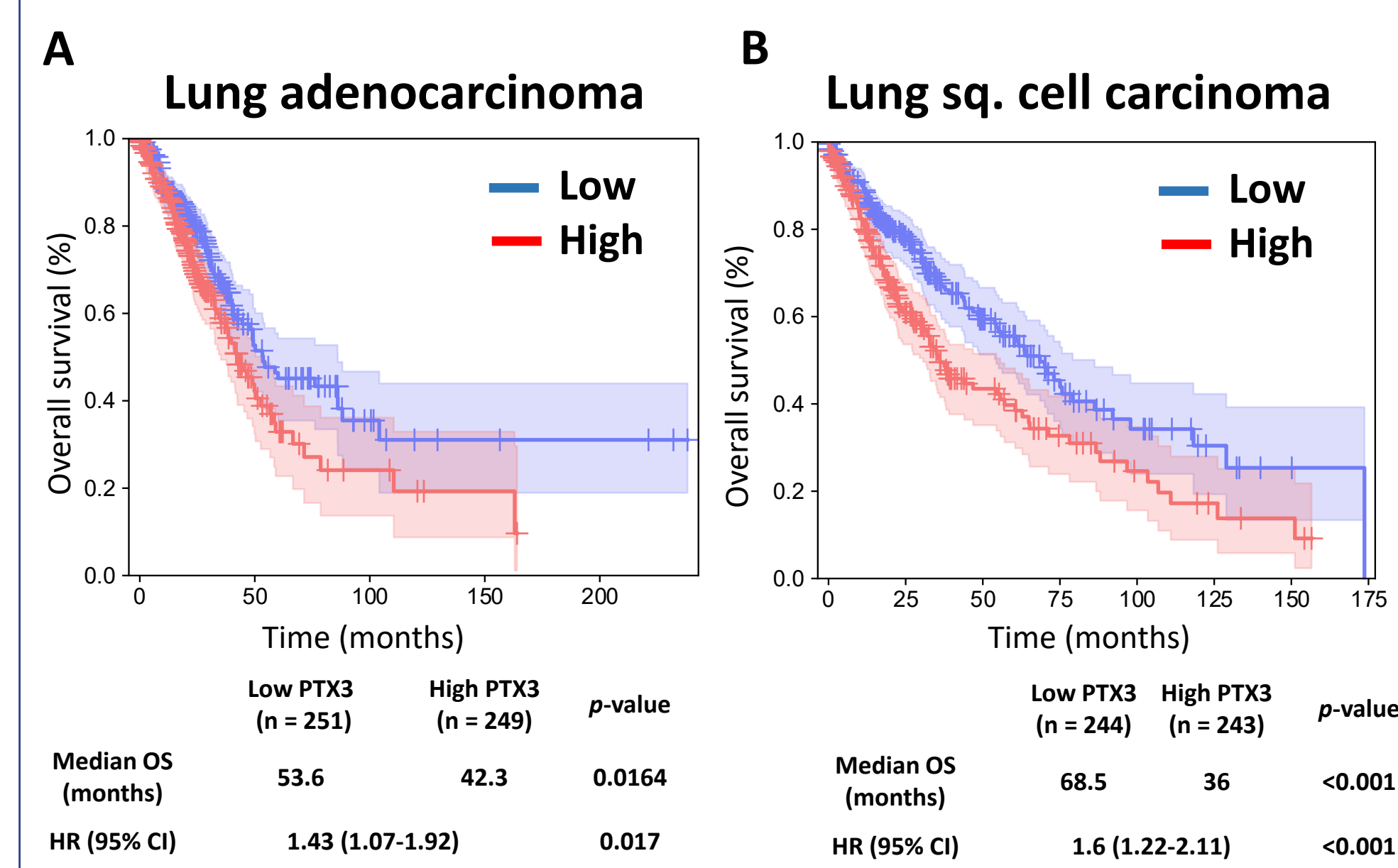
### AI Use Disclaimer

AI (ChatGPT, OpenAI) was used solely to assist with English language editing. All scientific content, data analysis, interpretation, and final approval were performed by the authors.

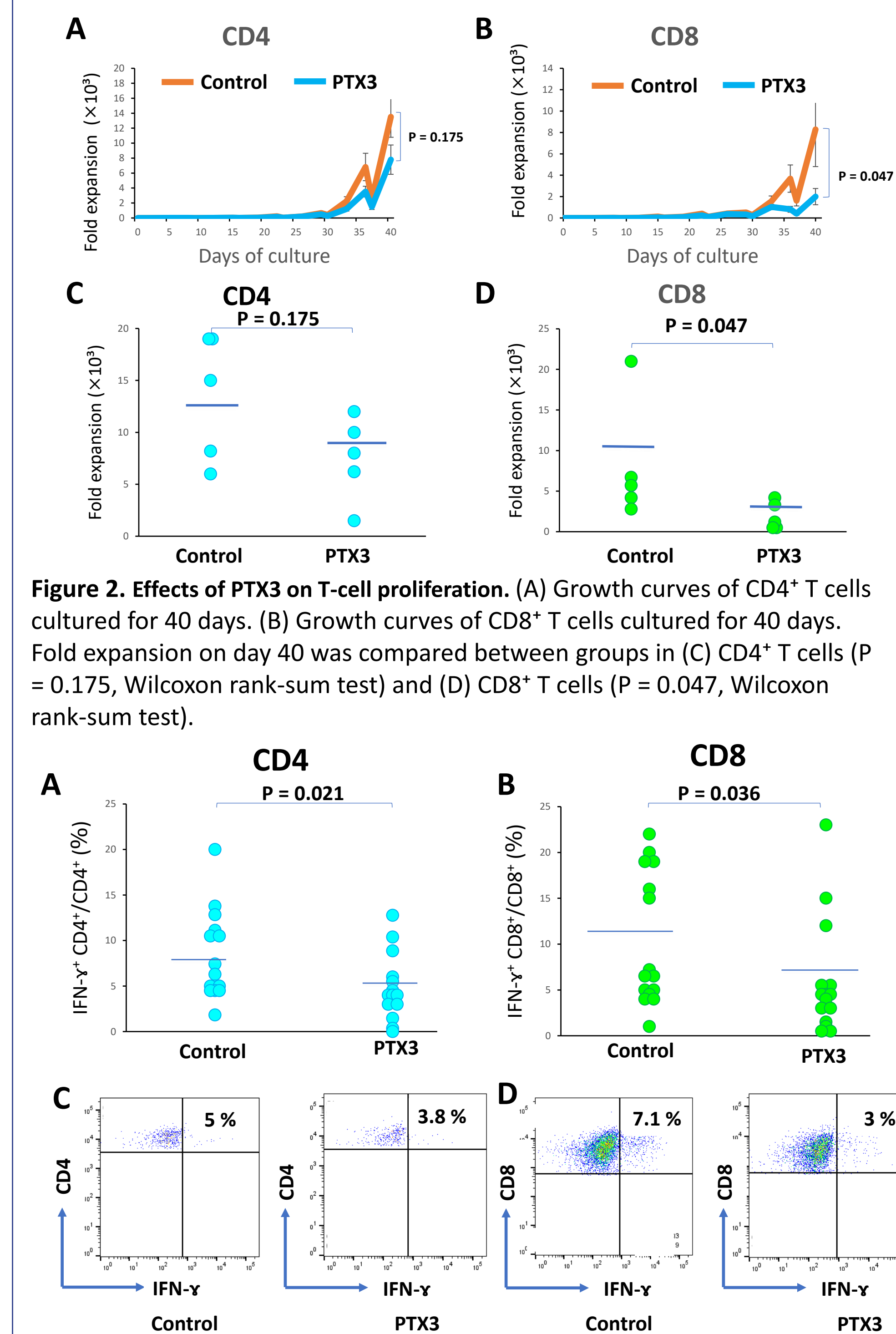
### Disclosure

The authors declare no conflicts of interest.

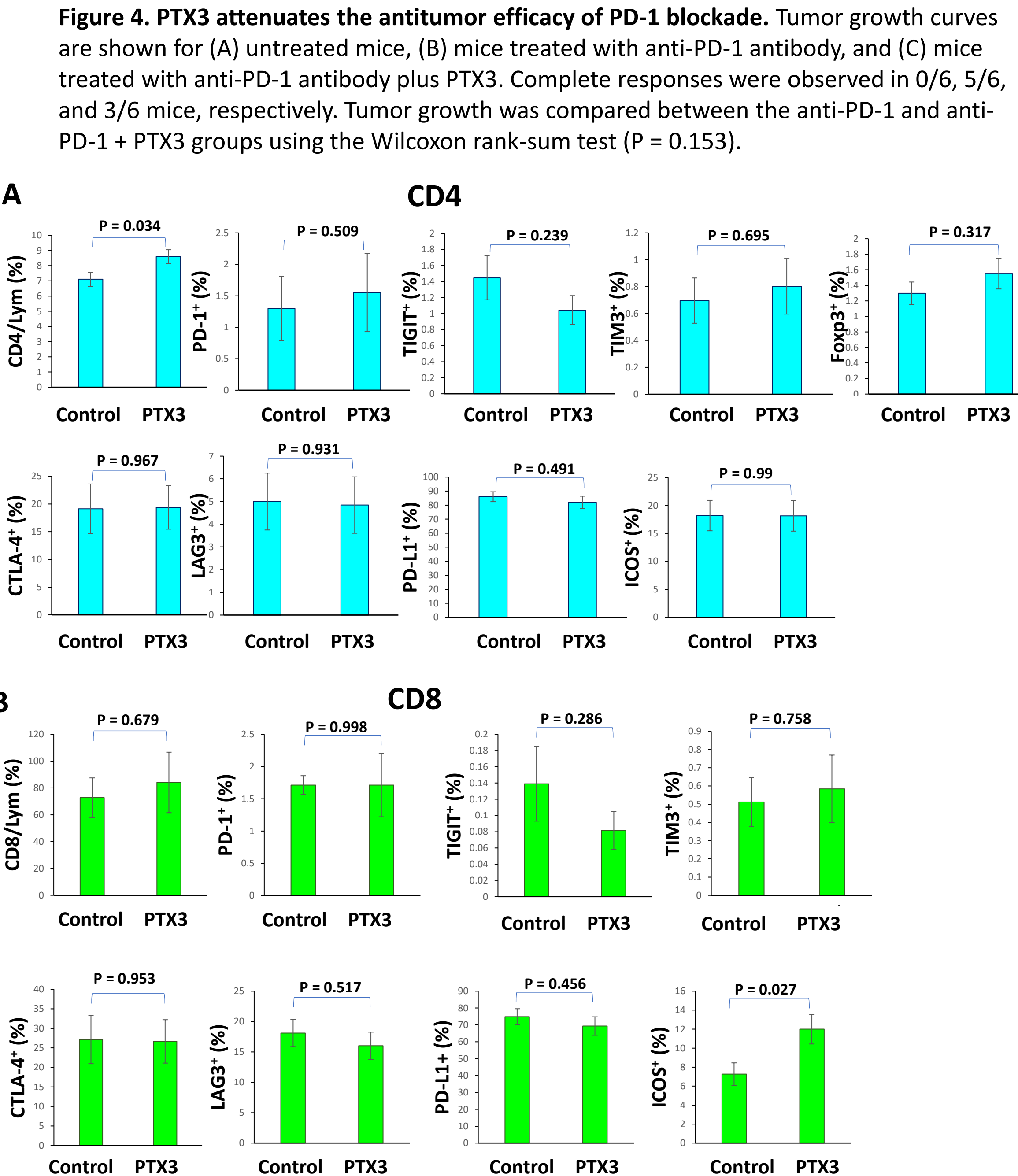
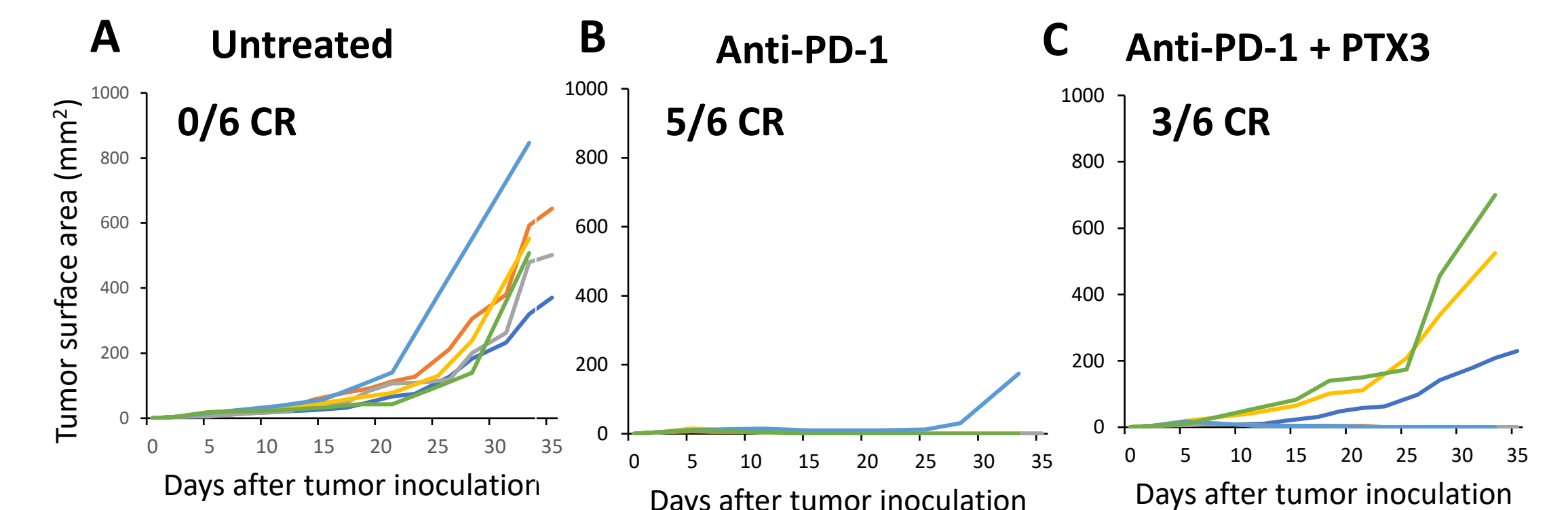
## RESULTS



**Figure 1. Overall survival was analyzed using GEPIA3.** Patients were stratified into high- and low-PTX3 expression groups according to the median PTX3 expression level. Kaplan–Meier survival curves are shown for (A) lung adenocarcinoma and (B) lung squamous cell carcinoma.



**Figure 3. Effects of PTX3 on IFN- $\gamma$  production in tumor-specific T cells.** (A) Percentage of IFN- $\gamma$  CD4<sup>+</sup> T cells (P = 0.021, Wilcoxon rank-sum test). (B) Percentage of IFN- $\gamma$  CD8<sup>+</sup> T cells (P = 0.036, Wilcoxon rank-sum test). (C and D) Representative flow cytometry plot of IFN- $\gamma$  expression in CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells.



**Figure 6. Effects of PTX3 on T-cell apoptosis.** Apoptosis was analyzed after 8 days of culture using Annexin V and 7-AAD staining. (A) CD4<sup>+</sup> T cells and (B) CD8<sup>+</sup> T cells were evaluated for early apoptosis (Annexin V<sup>+</sup>/7-AAD<sup>+</sup>) and late apoptosis or necrosis (Annexin V<sup>+</sup>/7-AAD<sup>+</sup>). Statistical analysis was performed using Welch's t-test.