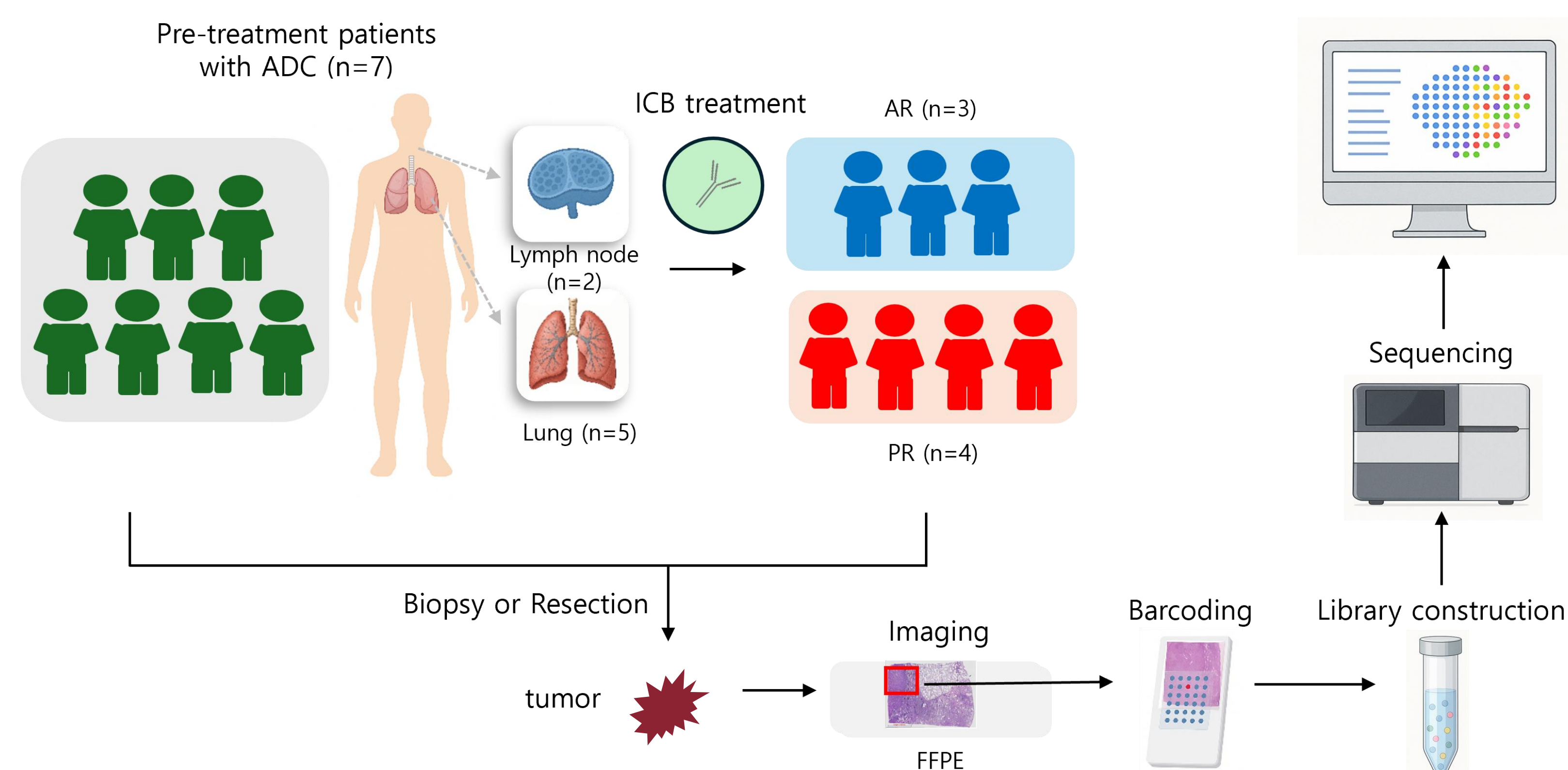


INTRODUCTION

- ❖ Although immune checkpoint blockade (ICB) has improved outcomes in lung cancer, many tumors remain resistant. It is still unclear how the tumor microenvironment (TME) is spatially remodeled during ICB and whether such remodeling differs according to treatment response and resistance.
- ❖ In this study, we investigated the spatial TME features associated with resistance in lung adenocarcinoma.

METHODS

- ❖ We performed 10x Genomics Visium spatial transcriptomic profiling of pre-treatment tumor samples from seven patients with lung adenocarcinoma treated with ICB, classified as acquired resistance (AR, n=3) or primary resistance (PR, n=4).



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Funding

This work is funded by National Research Foundation of Korea (NRF-2022-NR073743 and NRF-2026-25474955 to H.K.)

Artificial Intelligence (AI) Use Disclaimer

No AI or AI-assisted tools were used in any part of this work.

DISCUSSION AND CONCLUSION

Conclusion

- ❖ Spatial transcriptomic profiling revealed distinct TME programs associated with acquired versus primary resistance to ICB in lung adenocarcinoma.
- ❖ AR was characterized by TLS-enriched immune organization, whereas PR showed a neutrophil/MDSC-invasive epithelial program.

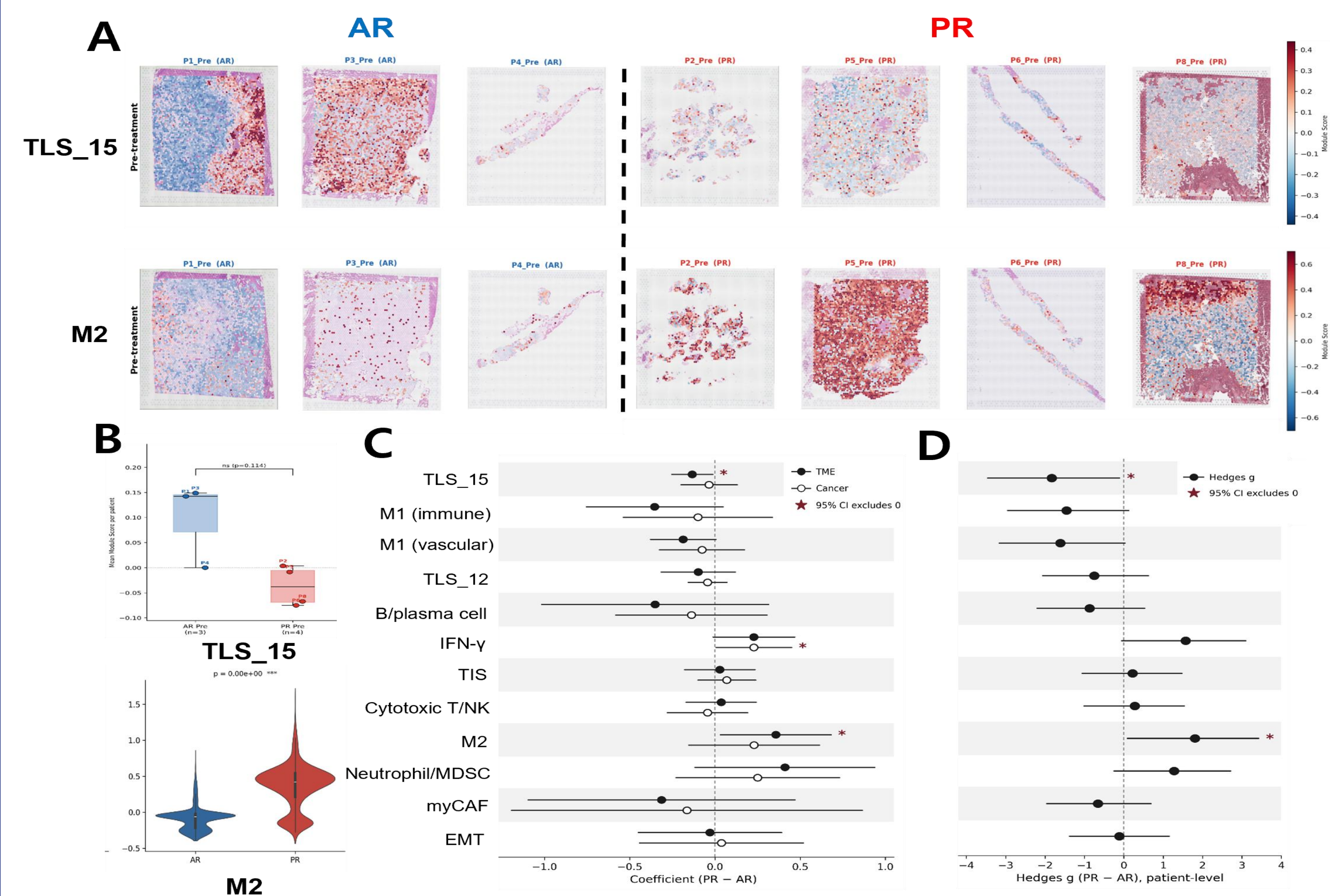
Future Direction for Research

- ❖ These findings highlight spatial microenvironmental organization as a potential source of predictive biomarkers for ICB benefit.

RESULTS

Figure 1. Distinct pretreatment TME programs characterize acquired versus primary resistance.

(A) Spatial maps of the TLS-15 signature and the M2 resistance program (B) Box-and-violin plots of TLS-15 and M2 scores in AR and PR. (C) Linear mixed-effects model estimates of module scores in TME and cancer compartments. (D) Patient-level Hedges' g effect sizes.

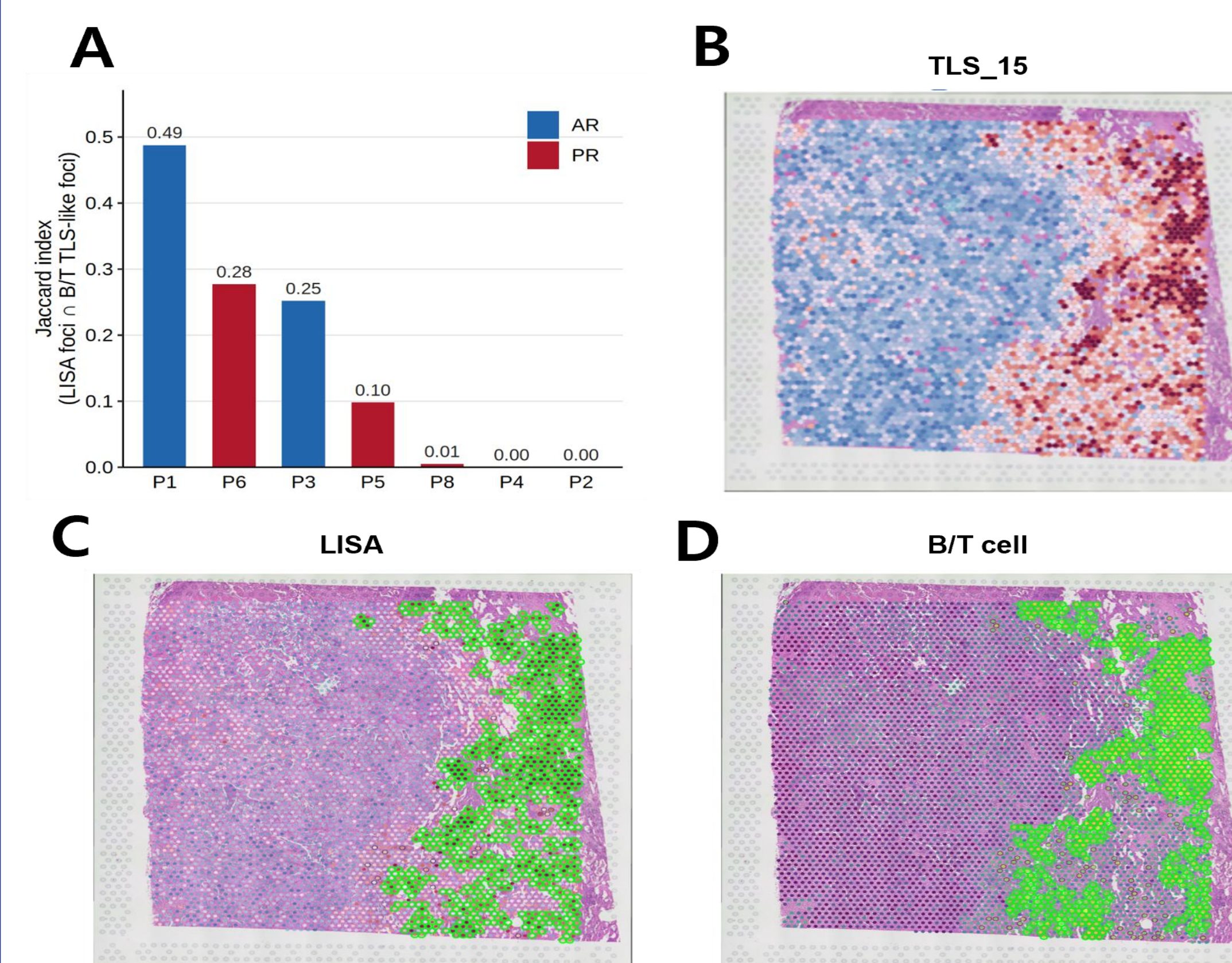


Abbreviations: AR, acquired resistance; PR, primary resistance; TME, tumor microenvironment; TLS, tertiary lymphoid structure; IFN-γ, interferon-gamma; TIS, tumor inflammation signature; T/NK, T cell/natural killer cell; MDSC, myeloid-derived suppressor cell; myCAF, myofibroblastic cancer-associated fibroblast; EMT, epithelial-mesenchymal transition; CI, confidence interval.

- ❖ In the TME compartment, TLS-15 scores were higher in AR and M2 scores were higher in PR by linear mixed-effects modeling, and patient-level Hedges' g effect sizes were consistent in direction and magnitude (TLS-15 $g = 1.83$, 95% CI 0.11–3.46; M2 $g = -1.81$, 95% CI -3.42 to -0.09).

- ❖ Neither difference, however, reached significance in the exact permutation test at the patient level after FDR correction - the pre-specified criterion for group-level significance in this $n = 3$ vs. 4 cohort (TLS-15: exact $p = 0.057$ [the theoretical floor for this design], $q = 0.40$; M2: exact $p = 0.143$, $q = 0.50$).

Figure 2. Spatial concordance between independently defined TLS foci. (A) Jaccard concordance index between LISA-derived hotspot foci and B/T-cell-derived TLS-like foci across all seven pre-treatment samples. (B) Representative section (P1, AR) with spatial map of the TLS-15 module score, (C) LISA-derived hotspot foci, and (D) B/T-cell-derived TLS-like foci for the same section.



- ❖ Concordance between two independently derived TLS foci definitions - one from spatial autocorrelation of the TLS-15 gene signature (LISA), one from co-enrichment of B and T cells - varied widely across the cohort, indicating that TLS spatial confirmation reflects case-level heterogeneity rather than a feature that distinguishes resistance phenotype.

- ❖ The section with the highest concordance, P1 (AR), is shown in detail, where the two independently derived foci sets overlap substantially along the TME compartment.

Abbreviations: AR, acquired resistance; PR, primary resistance; TLS, tertiary lymphoid structure; LISA, local indicators of spatial association; B/T, B-cell/T-cell.