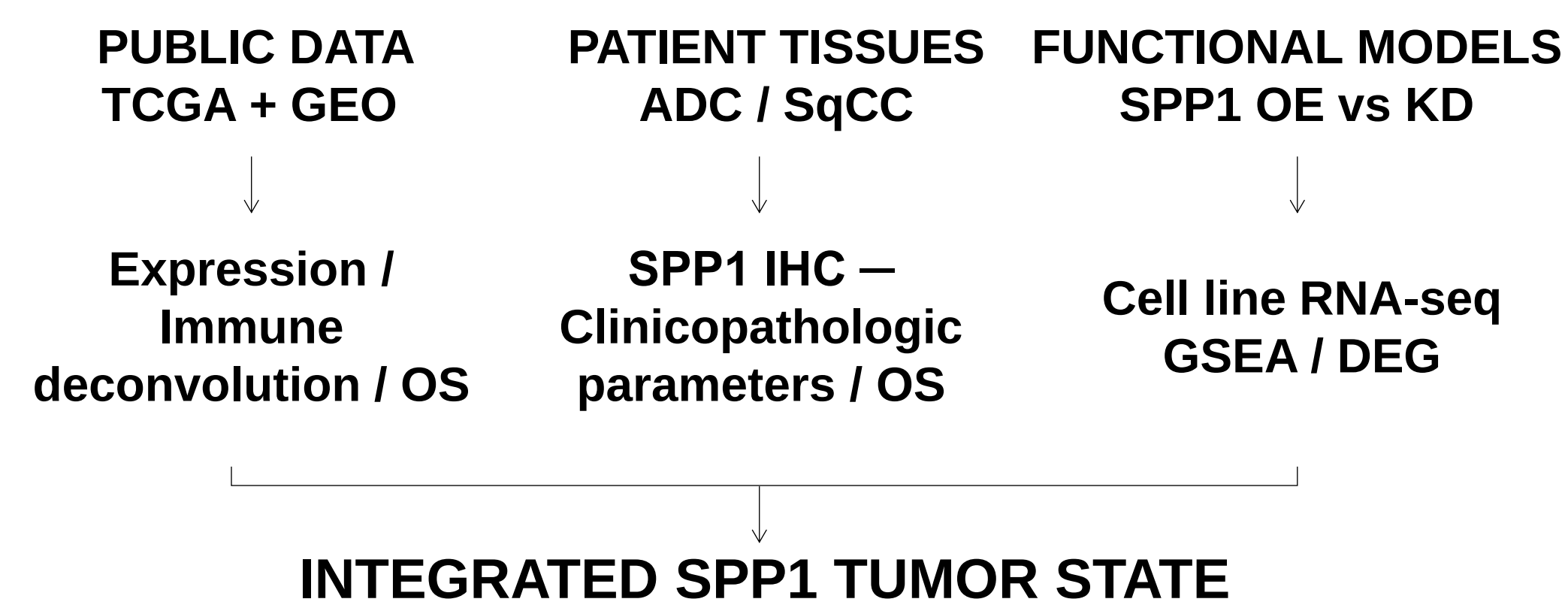


INTRODUCTION

- SPP1/osteopontin** has been associated with immunosuppressive tumor microenvironments, largely through studies of **SPP1-expressing tumor-associated macrophages**. However, the role of **tumor-cell-derived SPP1** in lung SqCC remains unclear.
- We investigated whether tumor-cell SPP1 defines a distinct biological state in lung cancer by integrating **public transcriptomic datasets**, **patient tissue cohorts**, **immune correlates**, and **SPP1 perturbation models**.

METHODS

Study-design



Public datasets: SPP1 expression, overall survival, and CIBERSORT-based immune-cell estimates were evaluated in TCGA-LUSC and independent GEO cohorts.

Patient tissues: Tumor-cell SPP1 was evaluated by IHC (clone EPR21139-316) and quantified using H-score and the percentages of tumor cells showing any, ≥2+, and 3+ staining. Cohorts included SqCC (n=143), ADC (n=167), ILD-associated SqCC (n=46), and ILD-associated ADC (n=23). Associations with clinicopathologic parameters, PD-L1, and survival were analyzed.

Functional studies: RNA sequencing was performed after SPP1 overexpression in Calu-1 cells and knockdown using two independent siRNAs in HCC95 cells. Differential expression and Hallmark GSEA were integrated to identify reproducible SPP1-associated programs.

DISCUSSION AND CONCLUSION

- Tumor-cell SPP1 defines a **squamous-enriched** state associated with **tumor-cell PD-L1 expression** and **immune remodeling**.
- SPP1 perturbation linked SPP1 to **EMT**, **KRAS-associated transcription**, **angiogenesis**, and reciprocal regulation of **NT5E/CD73** and **ELK3**.
- High SPP1 showed a tendency toward poorer overall survival across cohorts.
- Together, these findings support SPP1 as a marker of a PD-L1–associated, pro-tumorigenic state in lung SqCC and warrant further validation of the SPP1–CD73 axis.

Actionable Take Away Message

- Strong tumor-cell SPP1 identifies a biologically distinct, PD-L1–associated subset of lung SqCC.
- The SPP1–CD73 axis represents a testable candidate pathway linking tumor-cell state to immune remodeling.

Future Direction for Research

- Validate spatial relationships among SPP1, CD73, PD-L1, CD8 T cells, and macrophages using multiplex tissue imaging.
- Test the SPP1–CD73 axis using same-cell-line bidirectional perturbation and functional migration/invasion assays.
- Validate the SPP1 immune phenotype using patient-level FACS immune profiles and independent transcriptomic cohorts.

REFERENCES & ACKNOWLEDGEMENTS

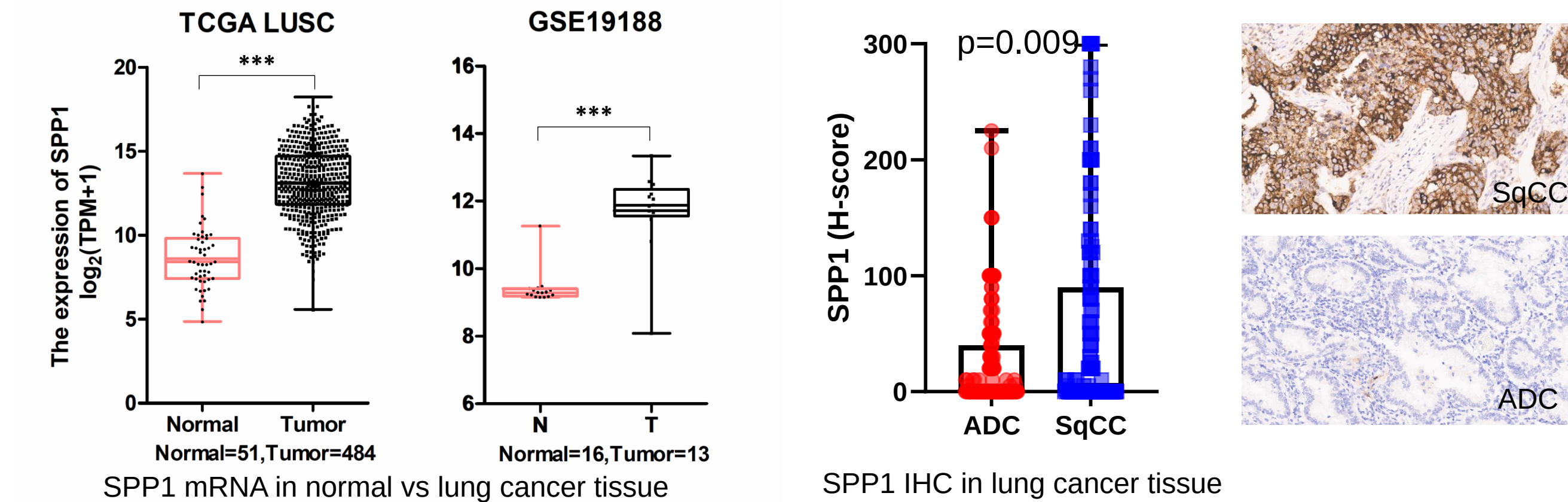
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Artificial Intelligence (AI) Use Disclaimer

Artificial Intelligence Use Disclosure: OpenAI ChatGPT (GPT-5.6 Sol) was used to assist with language editing and poster content/layout development.

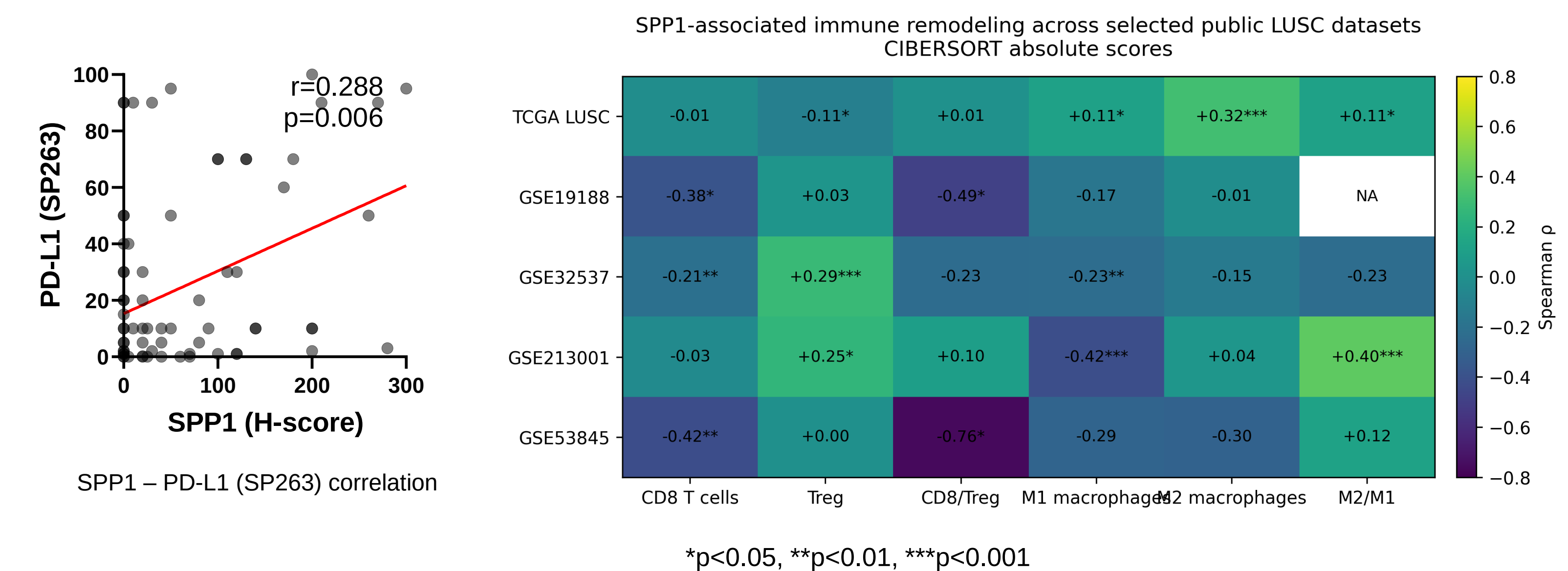
RESULTS

I. Tumor-cell SPP1 expression is enriched in SqCC



- SPP1 was upregulated in lung cancer in public datasets.
- In patient tissues, SPP1 expression was significantly higher in SqCC than in ADC.

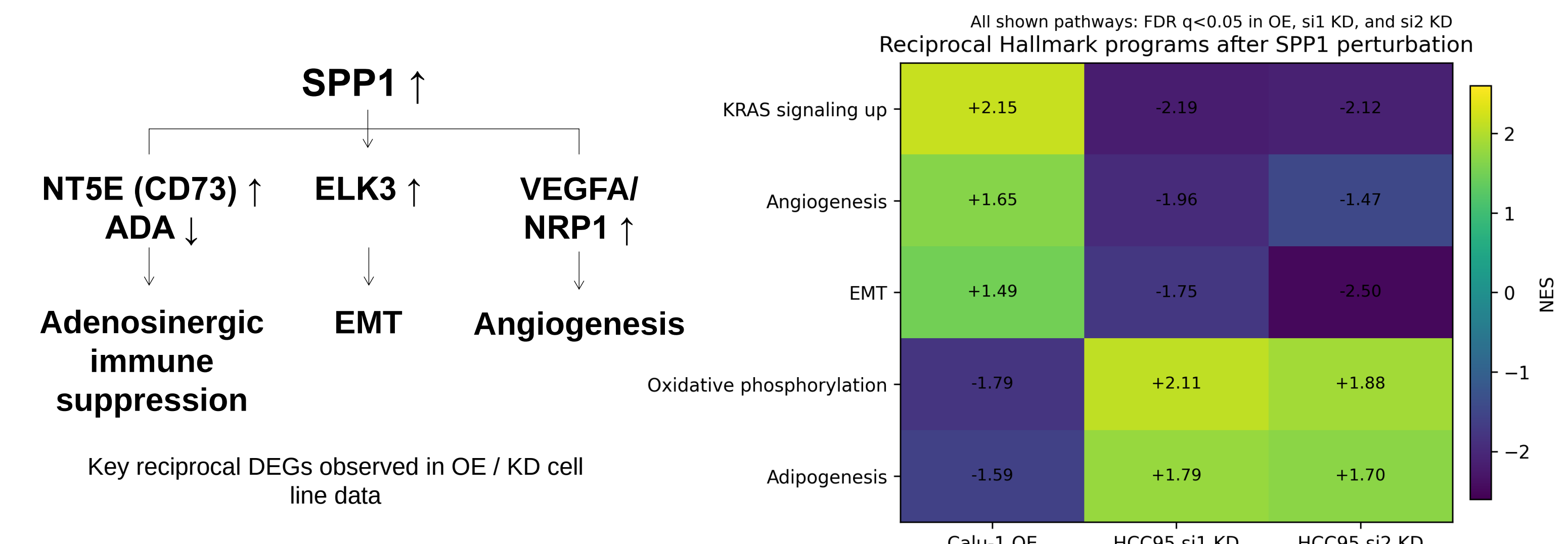
II. SPP1 is associated with tumor-cell PD-L1 and immune remodeling



- In SqCC, tumor-cell SPP1 correlated with PD-L1 expression, whereas no significant association was observed with immune-cell PD-L1.
- Public CIBERSORT analyses further showed recurrent CD8/M1-low and M2-associated immune remodeling.

III. SPP1 perturbation reveals aggressive transcriptional programs

- SPP1 overexpression enriched EMT, KRAS-associated, and angiogenic programs, with reciprocal changes after SPP1 knockdown.
- NT5E/CD73 and ELK3 emerged as robust reciprocal DEGs, with VEGFA/NRP1 supporting an angiogenic phenotype.



IV. High SPP1 shows a trend toward poorer survival across cohorts

