

BACKGROUND

Gene expression based subtyping has consistently identified 3 distinct biologic subtypes in Lung Adenocarcinoma (AD), Terminal Respiratory Unit (TRU) formerly Bronchioid, Proximal Proliferative (PP) formerly Magnoid, and Proximal Inflammatory (PI) formerly Squamoid(1,2) and 4 subtypes within SQ, Primitive, Classical, Basal and Secretory(3,4) (See Figure 1). AD and SQ subtypes demonstrate key differences in genomic alterations, tumor drivers, prognosis, and likely response to various therapies(1-4).

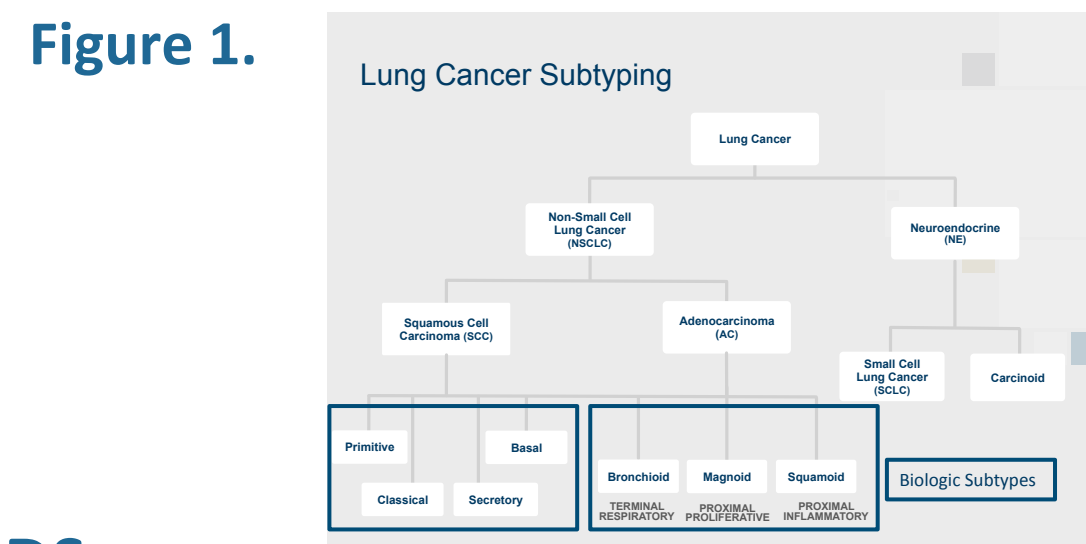


Figure 1.

METHODS

Using previously published Bindea et al. (5) immune cell gene signatures (24 in total) and AD and SQ subtyping gene expression signatures (1-4), several publically available lung AD and SQ datasets (2,4-9), were examined for immune cell features in relation to AD and SQ subtypes. Gene expression signatures of both Innate Immune Cells (IIC) and Adaptive Immune Cells (AIC), a 13 gene IFN signature (IFN), as well as single gene immune-biomarkers (*CTLA4*, *PDCD1*, and *CD274*(PD-L1), *PDCDLG2*(PD-L2)) were examined in the 3 AD subtypes (TRU, PP, and PI) and the 4 SQ subtypes (Primitive, Classical, Secretory, Basal). Immune cell signature associations with tumor subtype and with *CD274* expression were evaluated using linear regression. Hierarchical clustering of immune signatures and pairwise signature correlations were also analyzed. Survival-signature associations of Stages I-III samples were evaluated with stratified cox proportional hazard models allowing for different baseline hazards in each dataset.

Figure 2. Heatmaps of Bindea et al.(5) immune cell signatures and other immune markers in the TCGA Lung AD and SQ datasets.

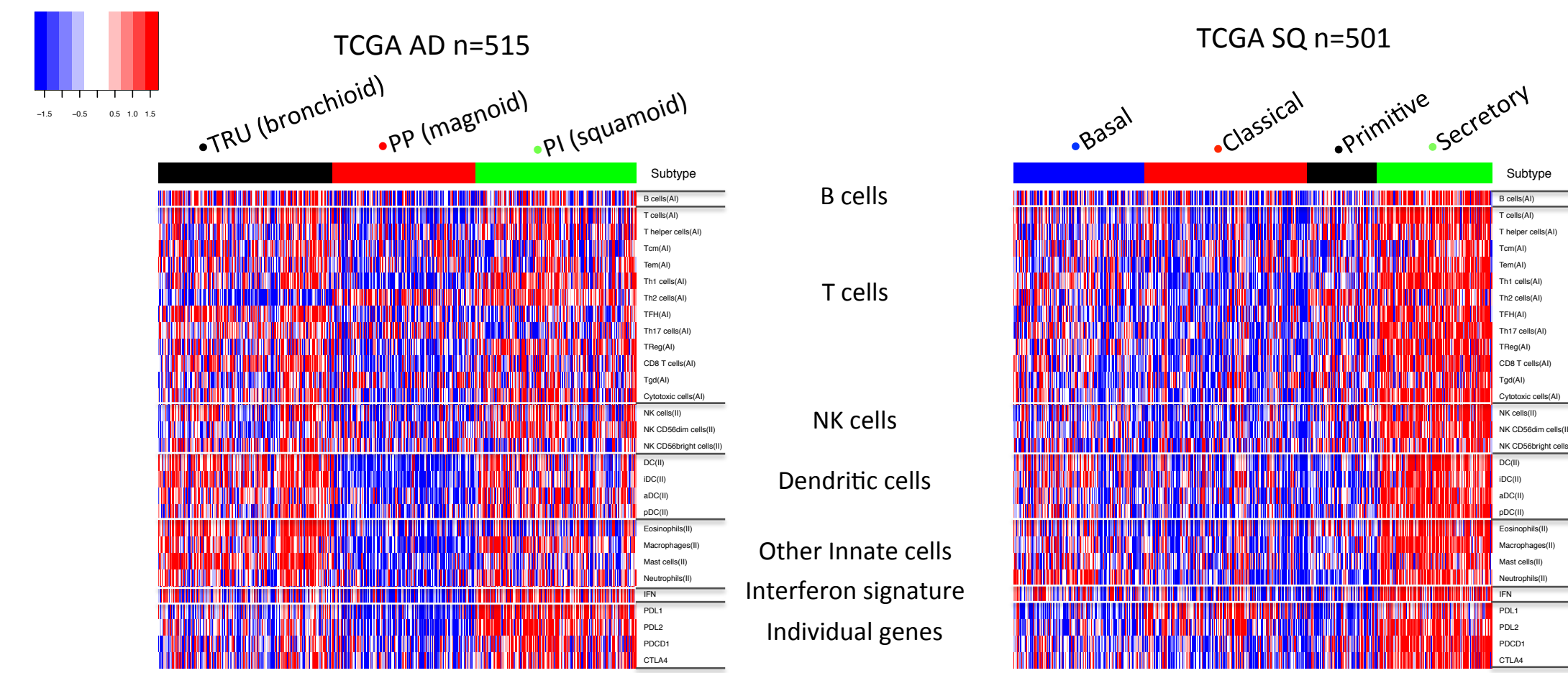


Figure 3. Correlation matrices of immune cell signatures in the TCGA AD and SQ dataset where signatures are arranged by hierarchical clustering.

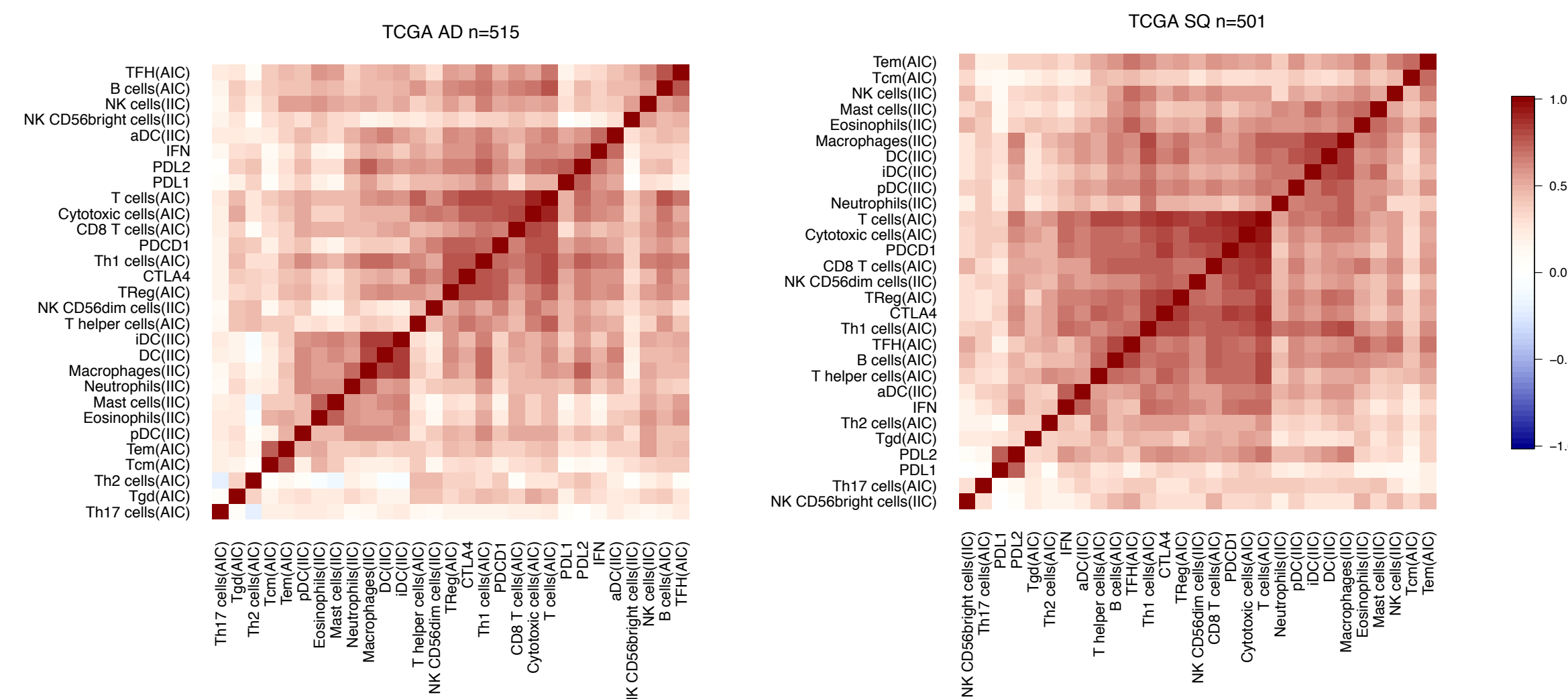


Figure 4. Reproducibility of T cell signature gene expression subtype patterns across multiple AD datasets (A) and SQ datasets (B).

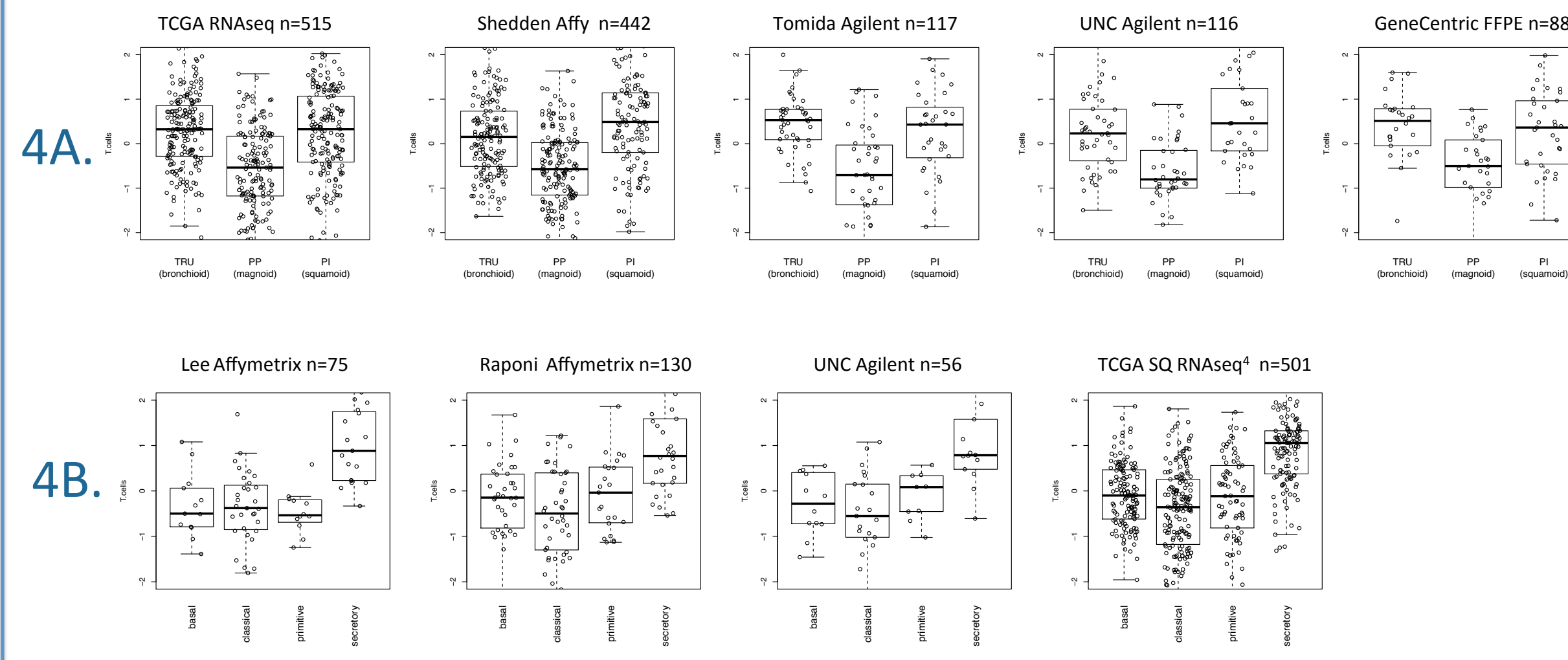


Figure 5. Association (adjusted R-squared) between CD274(PD-L1) expression and AIC signatures versus subtype and AIC signatures. In SQ, association is consistently greater for subtypes than for PD-L1.

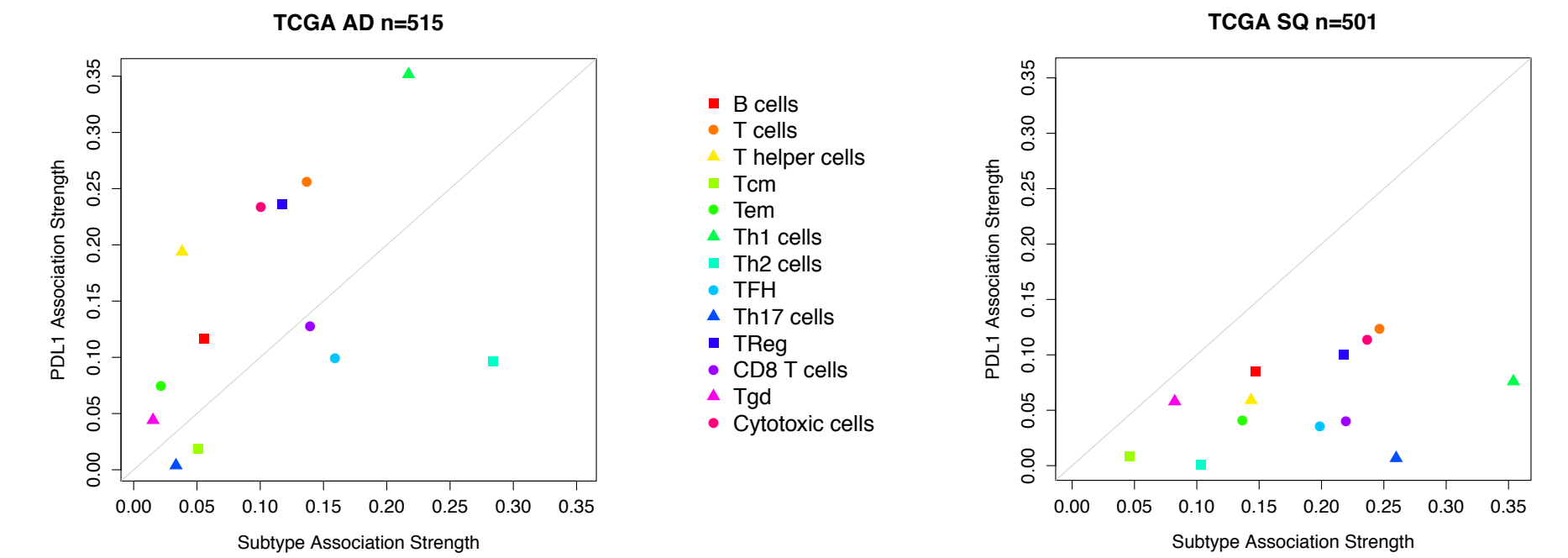
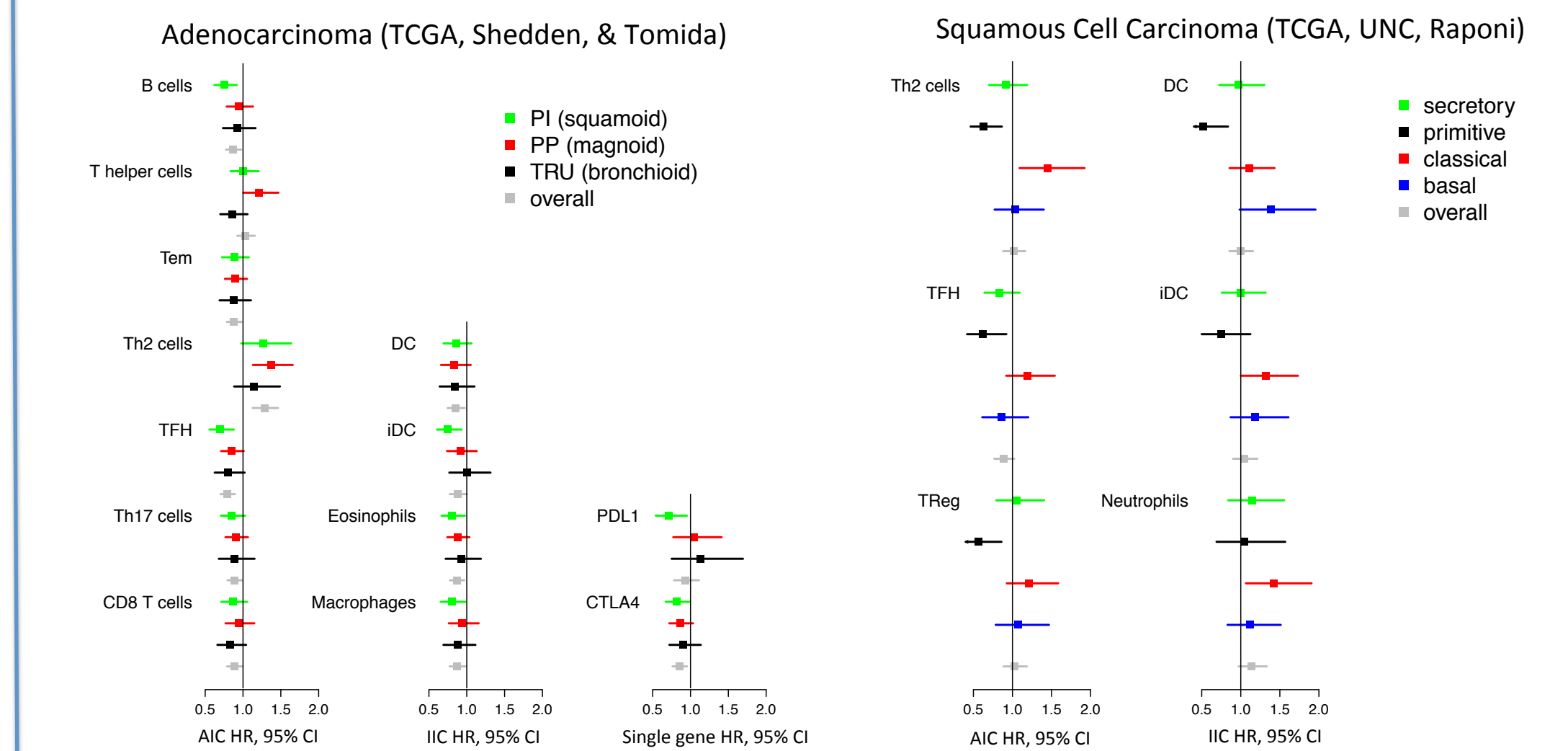


Figure 6. Signature-survival associations overall and by subtype. Hazard Ratios (HR) and confidence intervals calculated from stratified cox models. Subtype specific HR's are adjusted for stage (overall adjusted by stage and subtype) and only immune features with significant associations (p<0.05) are shown.



CONCLUSIONS

- Lung AD and SQ gene expression subtypes vary in their immune landscape.
- Adenocarcinoma PP (magnoid) subtype and SQ classical subtype show minimal immune infiltration suggesting reduced response to immunoRx.
- In SQ, subtype appears to be a better predictor of immune infiltration than *CD274*(PD-L1). *CD274* expression was not associated with AIC expression nor with improved survival in SQ.
- Adenocarcinoma PI subtype (squamoid) and the SQ primitive subtype showed immune feature expression associated with improved survival.
- Lung AD & SQ subtypes may be helpful as potential immunoRx biomarkers.

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ABBREVIATIONS

AD = Adenocarcinoma
 SQ = Squamous cell carcinoma
 TRU = Terminal Respiratory Unit
 PP = Proximal Proliferative
 PI = Proximal Inflammatory
 AIC = Adaptive Immune Cells
 IIC = Innate Immune Cells
 HR = Hazard Ratio