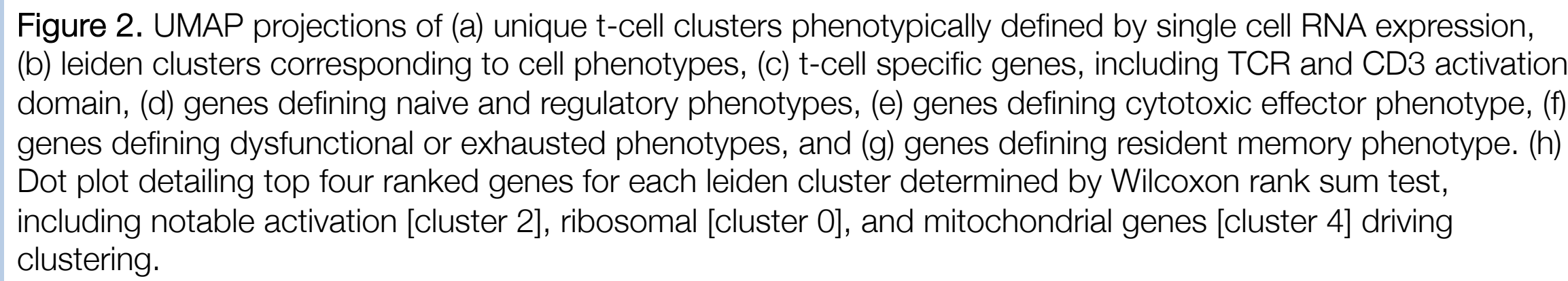


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While checkpoint inhibition (CI) has transformed the metastatic NSCLC treatment landscape, understanding of its role in the neoadjuvant space and how it augments tumor immune responses with the addition of stereotactic radiotherapy (SRT) is yet to be defined. Techniques in single cell RNA sequencing offer new improved resolution to define these cellular changes.

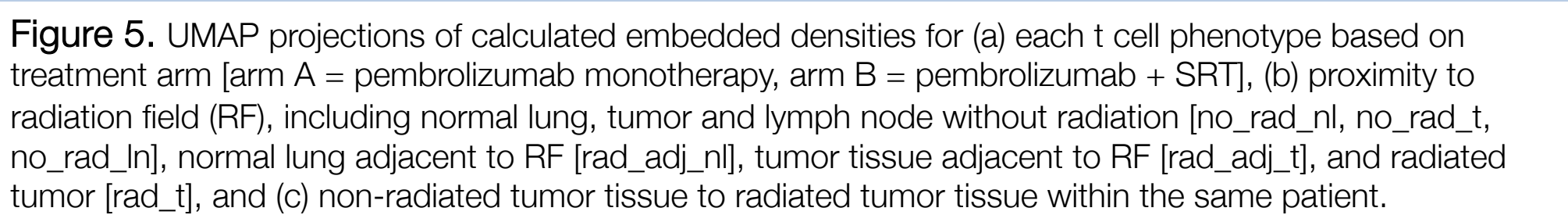
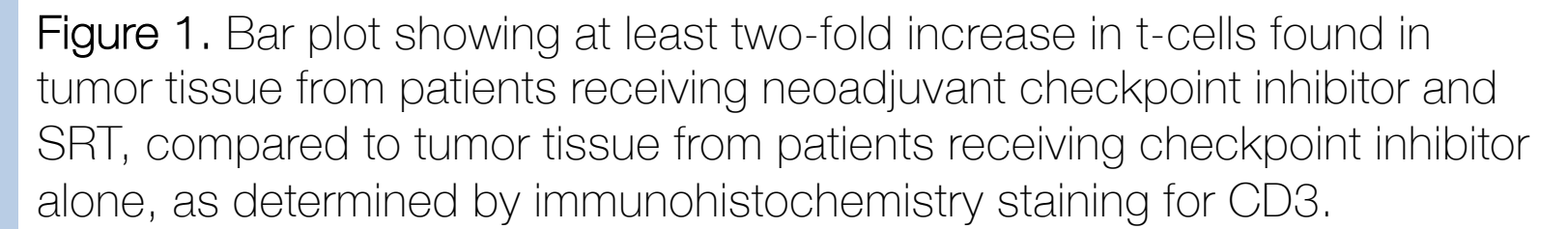
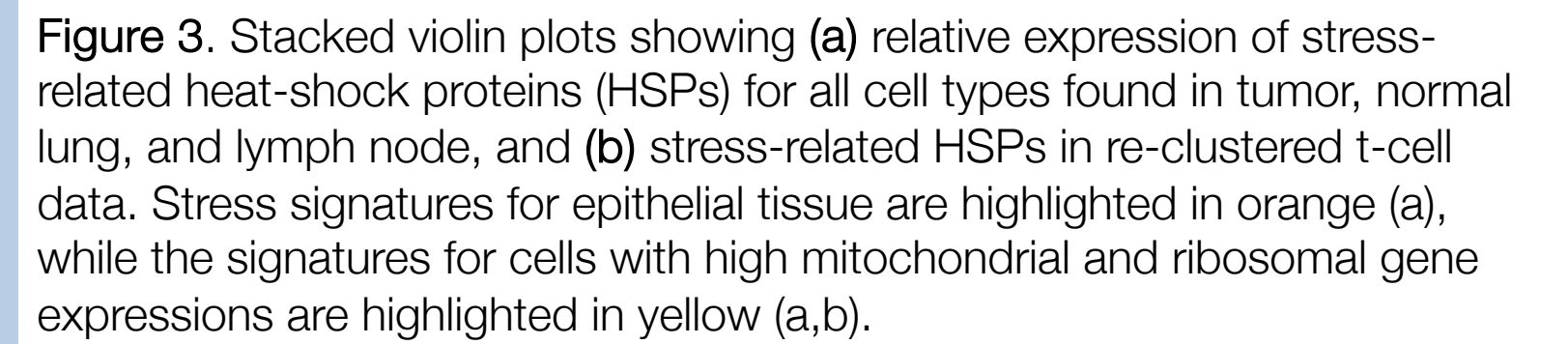
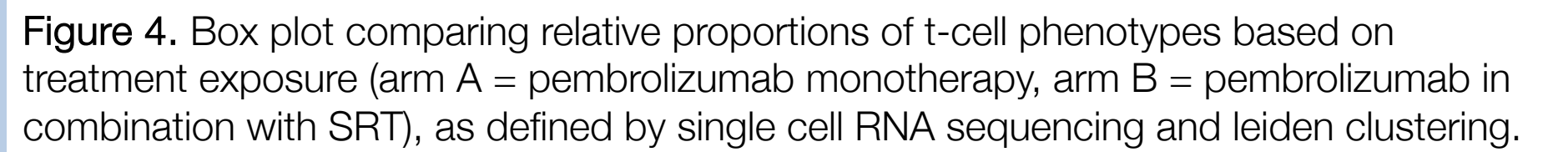
- NSCLC patients were enrolled in a randomized study to receive neoadjuvant CI monotherapy with pembrolizumab (Arm A) or CI and SRT (Arm B), prior to curative resection.
- 6 NSCLC resection samples (tumor, normal lung, and lymph tissue) were sequenced using Chromium single cell platform (10x Genomics) and Illumina (NovaSeq).
- Data preprocessing included scrublet doublet detection, cellbender ambient RNA removal, filtering for contamination cells and batch correction with harmony.
- Clustering was performed using scanpy python software and phenotypes defined by ranking genes and comparison of gene signatures to pre-defined single cell atlases.

- CI and SRT combination therapy increased CD3 T cell tumor infiltration by 2-fold (fig 1).
- 10 distinct t-cell phenotypes were found in tumor samples, including t-cells with ribosomal and mitochondrial gene signatures (fig 2).
- Ribosomal and mitochondrial gene signature did not correspond with markers of tissue stress response seen in lung epithelial cells (fig 3).
- A CD4 naïve population with ribosomal gene signature was the most prevalent cell type across both treatment arms (fig 4), specifically Arm A lymph node tissue and Arm B radiation-adjacent tumor tissue. These were absent in Arm A tumor tissue (fig 5b).
- SRT decreased exhausted CD8 t-cell phenotypes and increased CD8 effector and naïve types (fig 4).
- Directly radiated tissue revealed predominantly an activated CD8 effector phenotype, while adjacent tumor tissue displayed variety of t-cell phenotypes (fig 5c).



Overall, neoadjuvant checkpoint inhibition in combination with SRT appeared to decrease the proportion of relative exhausted t-cell phenotypes, while increasing naïve and effector phenotypes in tumor tissue. Direct effect of SRT on tumor tissue decreased t cell heterogeneity as compared to adjacent tumor tissue.

Mitochondrial and ribosomal gene signatures significantly influenced clustering and immune cell phenotyping. Though often associated with cell stress, these signatures did not correspond with the stress gene signatures found in the native lung tissue. The absence of stress response markers in certain t-cell populations may highlight new t-cell recruitment to the tumor microenvironment in the setting of immunomodulation.



Supplemental Table 1. Descriptive table of pertinent patient-related data, including treatment arm, tumor histology, PDL1 expression, pathologic staging at resection, and history of recurrence since undergoing therapy.

