

Allele-Specific Disruption of Spatial B Cell-Immune Networks in KRAS-mutant Non-Small Cell Lung Cancer

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INTRODUCTION

KRAS is the most commonly mutated oncogenic driver in lung adenocarcinoma, occurring in approximately 25% of cases. Relative to other driver-defined subtypes, KRAS-mutant tumours are characterized by higher tumour mutational burden, denser T cell infiltration, and more favourable responses to immune checkpoint blockade.

However, KRAS G12D is consistently different, arising predominantly in patients with limited smoking exposure and associated with low tumour PD-L1 expression, reduced lymphocyte infiltration, and inferior outcomes with checkpoint inhibition.

Whether KRAS alleles differ in the spatial organization of the tumour immune compartment, as distinct from its composition, has not been established. We applied single-cell resolved multiplex immunofluorescence to determine whether KRAS mutation status, and the G12D allele specifically, is associated with distinct patterns of direct cell-to-cell contact in resected NSCLC.

METHODS

Retrospective analysis of resected, treatment-naïve stage I to III NSCLC from 62 patients (all adenocarcinoma), profiled by 14-plex cyclic immunofluorescence (mIF) on the Lunaphore COMET platform across 124 TMA cores, two per patient.

Cells were segmented with an in-house machine-learning model using pathologist-guided thresholds; 13 phenotypes were assigned a priori. Direct contacts were quantified from Voronoi neighbourhoods as the proportion of cells of one phenotype touching at least one cell of a second.

Tumours were categorized into KRAS-mutant (n = 18; G12C 8, G12D 3, other 7) and KRAS wild-type (n = 44). Features were compared by rank-based linear regression with cluster-robust standard errors by patient, adjusted for age, sex, stage, and smoking, with Benjamini-Hochberg FDR correction within panel.

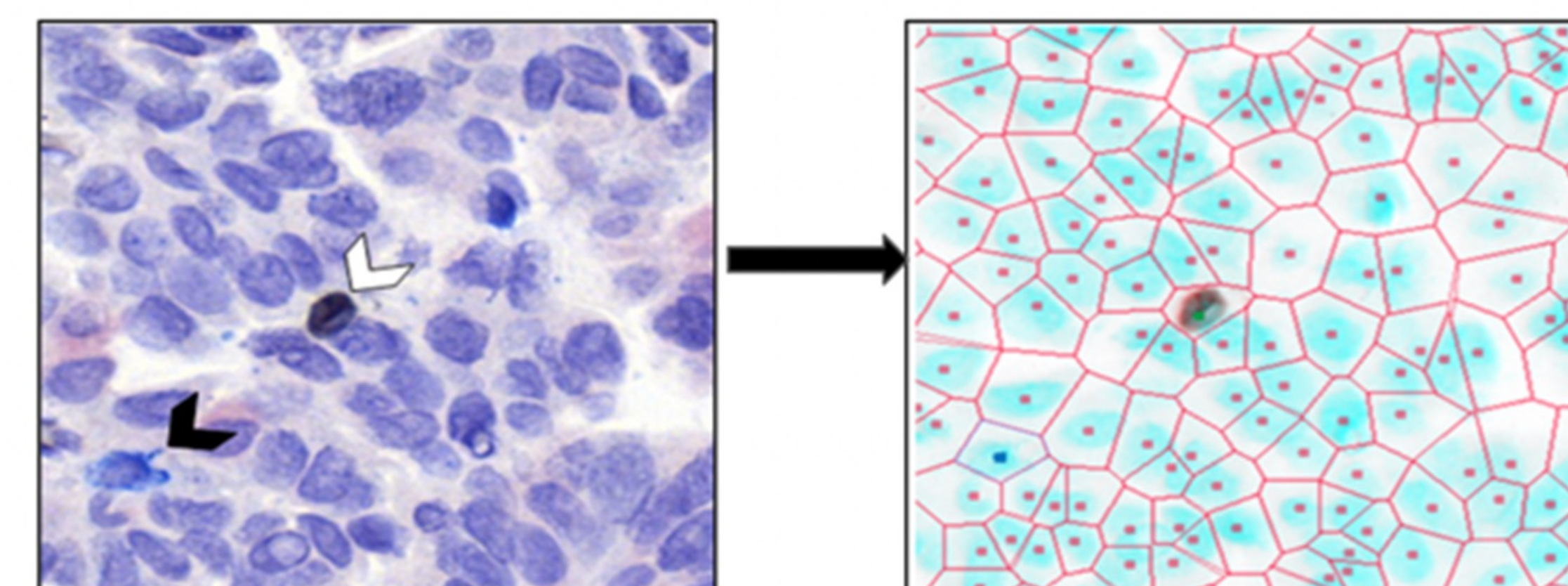


Figure 1. Cell neighbourhood analysis. Tissue (left) is converted to a Voronoi tessellation (right) from which spatial relationships and contact metrics are extracted.

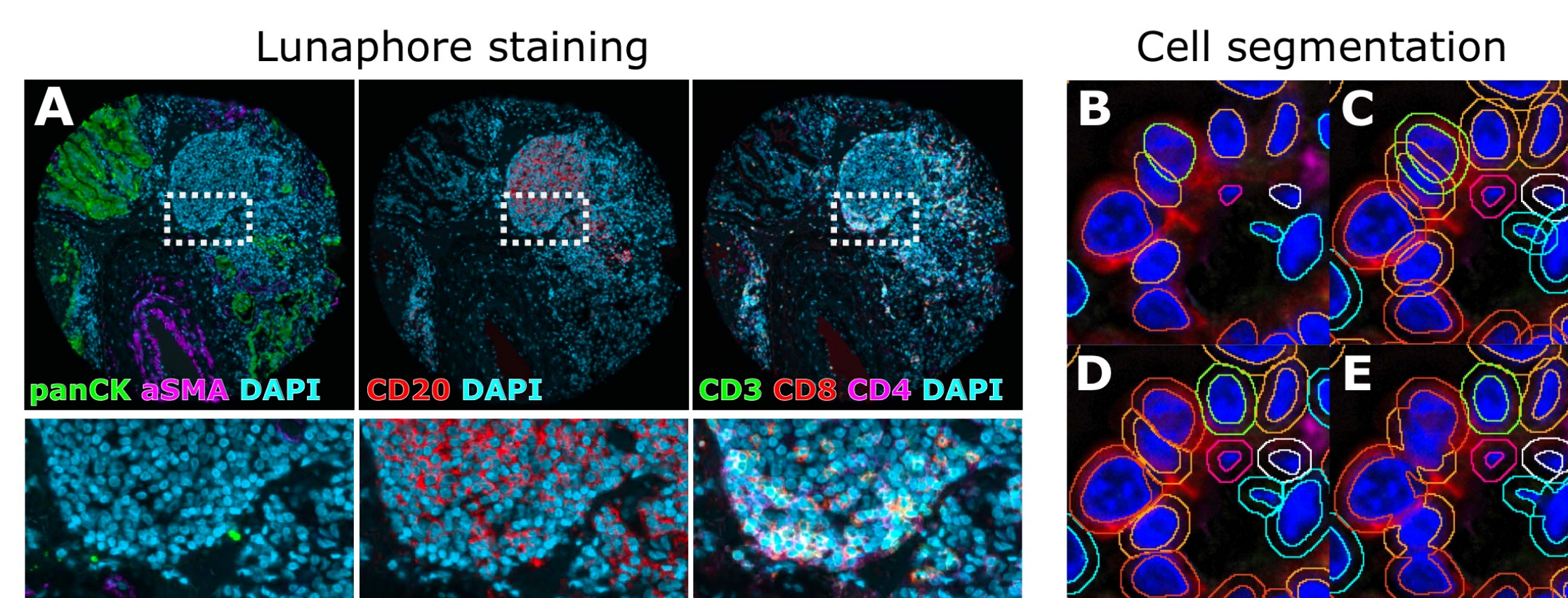


Figure 2. Multiplex immunofluorescence and cell segmentation workflow. (A) Representative 14-plex mIF staining on the Lunaphore COMET. (B) Cell segmentation masks generated by an in-house machine-learning model with pathologist-guided thresholds.

RESULTS

Depletion of B-Cell Centric Immune Colocalization in KRAS G12D Tumours

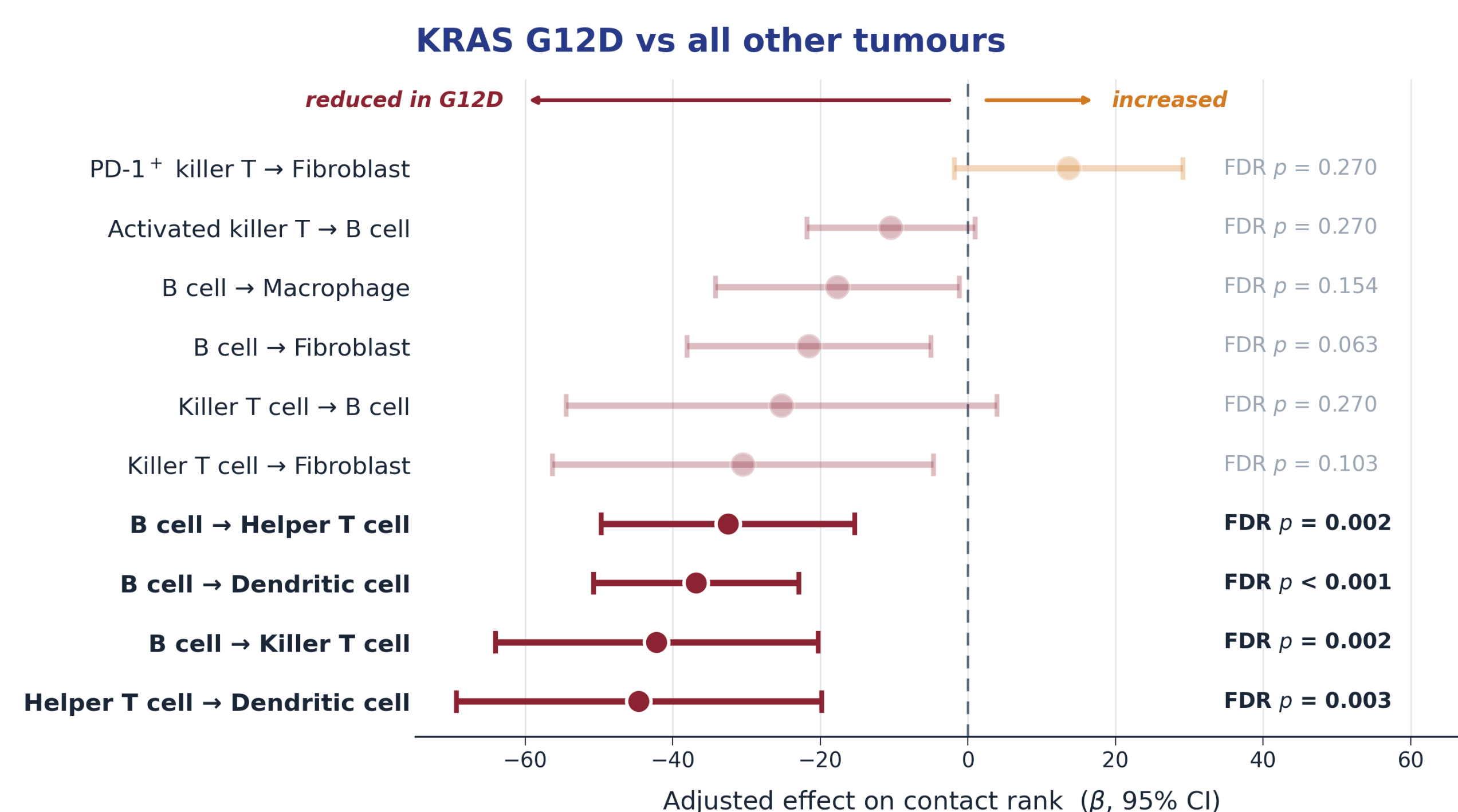


Figure 3. Spatial immune interactions in KRAS G12D tumours. Adjusted effect of G12D status on the rank of each contact feature, from rank-based linear regression with cluster-robust standard errors by patient, adjusted for age, sex, stage, and smoking status. Points are β coefficients with 95% confidence intervals; the ten features with the smallest p -values are shown. Solid markers indicate FDR $p < 0.05$; faded markers do not reach it. Negative values indicate reduced contact in KRAS G12D compared to all other tumours. Analysis restricted to cores in which both partner phenotypes were present.

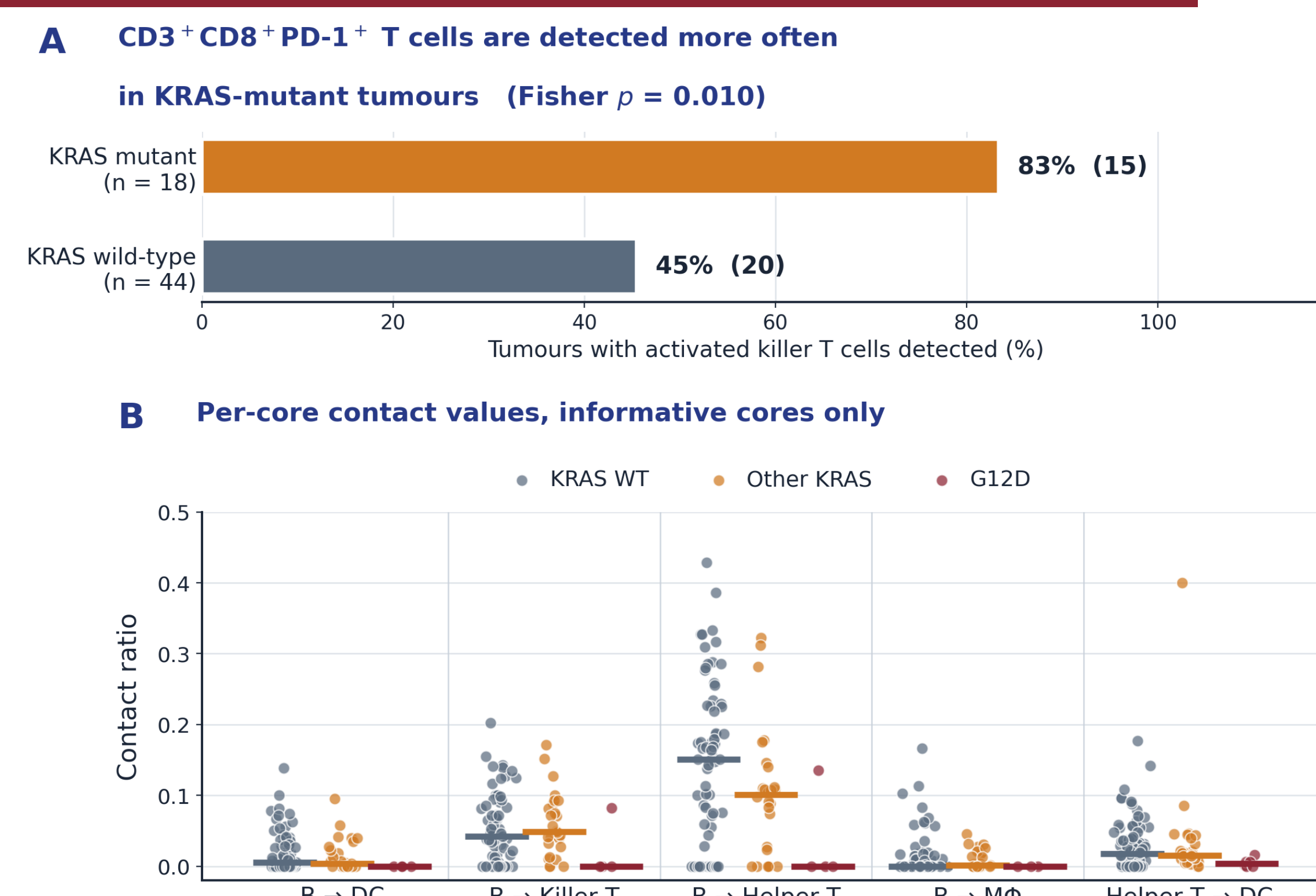


Figure 4. Cell detection and per-core contact values. (A) Proportion of tumours in which CD3⁺CD8⁺PD-1⁺ activated killer T cells were detected in at least one core, by KRAS status; Fisher exact test. (B) Per-core contact ratios for the five features most strongly associated with G12D, split into KRAS wild-type, other KRAS-mutant, and G12D tumours. Each point is one core; horizontal bars are group medians. Cores in which either partner phenotype was absent are excluded. DC, dendritic cell; M Φ , macrophage.

DISCUSSION AND CONCLUSION

KRAS-mutant tumours showed the immunologically active phenotype described in the literature. CD3⁺CD8⁺PD-1⁺ activated killer T cells were detected in 83% of KRAS-mutant tumours versus 45% of wild-type ($p = 0.010$), while no individual contact feature survived FDR correction after covariate adjustment.

The allele-level picture differed in kind. KRAS G12D tumours showed a **coordinated loss of B cell-centred contacts**: B cell to dendritic cell (FDR $p < 0.001$), to killer T cell and to helper T cell (both FDR $p = 0.002$), alongside reduced helper T cell to dendritic cell association (FDR $p = 0.003$). These are precisely the interactions that suggest local antigen presentation and tertiary lymphoid structure formation. Their simultaneous loss, could point to a failure to assemble organised immune aggregates rather than depletion of any one population. PD-L1 expression in the tested G12D cases was concordantly low (TPS <1%). The strategy of these TMAs was not designed to capture TLS and this will be further investigated.

Several caveats bound this reading. The G12D group comprised three patients contributing four informative cores, with B cell counts of 38, 3, 1, and 1, so absence of B cells and disengagement of B cells cannot be cleanly separated. Dendritic cell abundance was itself reduced in G12D (FDR $p = 0.035$), which may carry part of the B cell to dendritic cell effect. FDR correction was applied within panels rather than across all comparisons.

Against those limits, the effects were consistent in direction across five independent features, stable across three approaches to handling cores with absent phenotypes, and concordant with PD-L1 status. This suggests that **allele-specific immune architecture, and not only immune cell abundance, differs within KRAS-mutant NSCLC**. Validation in a larger G12D cohort with explicit tertiary lymphoid structure assessment is the necessary next step.