

Immune-focused transcriptional profiling identifies a B-cell-associated programme in resectable lung adenocarcinoma

Marcella Barbarino

marcella.barbarino@unisi.it

University of Siena

Laura Bianchi

University of Siena

Alfonso Carleo

University of Salerno

Rosanna Salvo

University of Siena

Diletta Rosati

University of Siena

Mariachiara Siciliano

University of Siena

Matilde Pesetti

University of Siena

Andrea Quaranta

University of Bari

Gabriella Serio

University of Bari

Antonio Graziano

Sbarro Institute for Cancer Research and Molecular Medicine Italia Foundation ETS

Salvatore Cortellino

Scuola Superiore Meridionale

Luca Bini

University of Siena

Piero Paladini

Azienda Ospedaliera Universitaria Senese

Cristiana Bellan

University of Siena

Antonio Giordano

Article

Keywords: lung adenocarcinoma, tumour immune microenvironment, B cells, immune transcriptomics, resectable non-small-cell lung cancer, costimulation

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11203317/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

B cells and organised lymphoid immune niches contribute to antitumour immunity in lung cancer, but their transcriptional architecture in resectable lung adenocarcinoma (LUAD) remains incompletely defined. We profiled 730 immune-oncology genes in paired tumour and adjacent non-tumour tissues from 23 treatment-naïve patients with stage I–IIIA LUAD, integrating differential-expression, clinical–molecular, immune-cell, pathway and interaction-network analyses. Sixty-five immune-related genes differed between tumour and non-tumour tissues. The dominant functional convergence involved B-cell lineage, lymphocyte-recruitment and costimulatory components, including increased tumour expression of *CD19*, *CD79A*, *CD79B*, *MS4A1*, *CXCL13*, *CXCR5*, *CCR7* and *LTB*. B-cell signalling, tumour-infiltrating B-cell immunity, BAFF-induced signalling and T-cell costimulation were enriched, while relative immune-cell estimates were directionally concordant with higher plasma-cell and follicular-helper T-cell fractions in tumour tissue. Immune-related transcript abundance was associated with clinicopathological features, *KRAS* and *EGFR* status. Network analysis prioritized *TAL1*, *POU2F2*, *PAX5*, *CDK1* and *PPARG* as highly connected, network-derived hubs. In exploratory external anti-PD-1-treated LUAD and LUSC cohorts, these hubs and BAFF/APRIL-axis components showed opposite directional survival associations. These findings identify a B-cell-associated tumour immune programme in resectable LUAD and highlight B-cell-associated, costimulatory and BAFF-related components for spatially resolved and treatment-linked evaluation.

INTRODUCTION

Resectable non-small-cell lung cancer (NSCLC) comprises biologically heterogeneous tumours for which surgery remains the cornerstone of potentially curative treatment [1,2]. However, recurrence after complete resection remains a major clinical challenge, particularly in patients with node-positive or locally advanced disease. Although platinum-based perioperative chemotherapy provides a survival benefit in selected patients, its absolute benefit is modest, underscoring the need to better define the biological features that influence relapse risk and treatment sensitivity [3,4]. The introduction of immune checkpoint blockade into the perioperative setting has further changed the clinical context of resectable NSCLC. In selected patients, neoadjuvant chemoimmunotherapy improves pathological response and event-free survival compared with chemotherapy alone and has become a standard-of-care option [5–14]. These therapeutic advances have increased interest in the immune states present in resectable tumours and in their relationship with tumour biology.

Lung adenocarcinoma (LUAD), the most common histological subtype of NSCLC, comprises molecularly distinct tumours with different oncogenic drivers and tumour-immune features [15,16]. Understanding how the tumour immune microenvironment is organised in resectable LUAD may help distinguish immune programmes linked to effective adaptive immune activity from those associated with incomplete or context-dependent immune organisation.

In lung cancer, tumour-infiltrating lymphocytes, tertiary lymphoid structures (TLS), and the balance between costimulatory and inhibitory pathways have all been associated with clinical outcome [17,18]. B cells are now recognized as active components of this landscape rather than passive bystanders. Histological, transcriptomic and single-cell studies have shown that B-cell and plasma-cell states are remodelled during LUAD development [19,20].

These observations establish the relevance of B-cell-rich immune niches in lung cancer, but the tumour-associated transcriptional architecture connecting B-cell identity, lymphocyte-recruitment signals, costimulatory transcripts and clinicopathological or oncogenic context remains incompletely characterised in resectable LUAD.

In this study, we profiled 730 immune-oncology genes in tumour and patient-matched adjacent non-tumour tissues from 23 treatment-naïve patients with stage I-IIIa LUAD. Differential-expression analysis was used to define the tumour-associated immune signature, and GLM was used to examine its molecular associations. Immune-cell deconvolution, pathway enrichment and interaction-network analysis then provided complementary characterization of the resulting transcriptional profile. Network-prioritized genes and BAFF/APRIL-axis components were also queried in external anti-PD-1-treated LUAD and LUSC cohorts as an exploratory clinical-context analysis.

RESULTS

A tumour-associated immune transcriptional signature distinguishes LUAD from adjacent non-tumour lung tissue

Sixty-five immune-related genes met the predefined differential-expression criteria between tumour and adjacent non-tumour tissue conditions: 44 were higher and 21 were lower in tumour tissue (Fig. 1; Supplementary Table S2). This tumour-associated DEG set provided the basis for subsequent functional characterization and analysis of clinical and molecular associations.

Principal component analysis (PCA) of the 65-gene signature showed separation of tumour and adjacent non-tumour samples (K and N, respectively) along PC1, which accounted for 52.5% of total variance (Fig. 2); PC2 and PC3 accounted for 9.6% and 4.2%, respectively. Adjacent non-tumour samples formed a relatively compact distribution, whereas tumour samples showed broader dispersion along PC2 and PC3, indicating greater interpatient heterogeneity within the selected immune signature. When the PCA was annotated by smoking history, pathological T category and sex, no dominant visual grouping by these variables was apparent (Supplementary Figure S1).

Unsupervised hierarchical clustering of the 65 DEGs visualized both gene-level expression differences and sample-to-sample variability across tumour and patient-matched adjacent non-tumour tissues (Fig. 3). Consistent with the PCA, clustering primarily reflected tissue condition, with tumour and adjacent non-tumour samples forming distinct groups (Fig. 3, red and blue bars indicating tumour and

non-tumour samples, respectively). Adjacent non-tumour samples showed relatively homogeneous expression profiles, whereas tumour samples displayed greater within-group heterogeneity, particularly among the tumour-upregulated genes in the vertical dendrogram. No dominant visual clustering by pathological N category was apparent in the heatmap annotation (Fig. 3, horizontal gray-black bar).

GLM identifies clinicopathological and oncogenic associations of immune transcriptional variation

In addition to genes identified as differentially expressed between tumour and adjacent non-tumour tissue, the analysis identified transcripts that, although not differentially expressed in pairwise comparisons, met the predefined descriptive GLM thresholds in tumour tissue (an absolute regression coefficient ≥ 0.6 , nominal $p \leq 0.05$ and AUC ≥ 0.80 ; Supplementary Table S3A).

Their expression profiles showed consistent, gradual shifts and partially overlapping distributions across clinical and molecular categories rather than sharply discrete group separation (Supplementary Figure S2). These patterns describe transcript abundance variation associated with the clinicopathological and oncogenic features represented in this cohort.

Smoking history was associated with opposing expression trends of *BID* and *LTF* genes (Supplementary Figure S2 A). Former and current smokers presented similar distributions that differed from those of never smokers.

Lymph-node involvement, assessed both by nodal-dissemination annotation and by pathological N category (N0-N2), was associated with coherent abundance shifts across a shared set of immune-related transcripts (Supplementary Figure S2 B and C, respectively). These included genes related to innate and adaptive immune recognition, stimulation, immune-cell recruitment and signalling, and inflammatory responses (*MAVS*, *CYBB*, *IL13RA2*, *TLR8*, *C6*, *C3AR1*, *CCL16*, *BST2*, *CLEC5A*, *OAS3*, *NCAM1*, *ITGAM*, *PLA2G6*, *IGF1R*, *CKLF*, *ITGB2*). Notably, several of these genes have been reported to be involved in the TME's immune infiltration and inflammation, as well as in immune evasion, cancer progression, and poor prognosis in different cancers (e.g. *IL13RA2* [21,22]; *TLR8* [23,24], *ITGAM* [25], *NCAM1* [26], *MAVS* [27], *CCL16* [28,29], *CLEC5A* [30]). The distributions across nodal categories were partially overlapping and showed gradual shifts, consistent with modulation of immune and inflammatory transcriptional features across nodal extent of disease.

Tumour history was correlated with a significant trend for a single transcript (*CCL16*). Pathological T category (T1-T2 versus T3) was associated with coordinated variation in transcripts related to cytokine and interleukin signalling (*IL1R2*, *IL32* and *IL11RA*), immune regulation, cell-cell interaction (*SPN*, *CD74* and *SSX1*), and adhesion or stress-associated processes (*ITGA5*, *SYT17* and *MICA*) (Supplementary Figure S2 D-E; Supplementary Table S3A). Collectively, these T category-associated expression shifts place inflammatory signalling, immune-cell interaction and tumour-microenvironment-related transcriptional features within the variation observed across local tumour extent in this cohort. In

particular, the association of *SPN*, a gene linked to leukocyte adhesion and migration, provides a potential connection between local tumour extent and immune-cell trafficking.

Tumour grade (G2 versus G3) was associated with transcriptional variation in genes related to antigen presentation, immune cell recruitment and activation, interferon signalling, and inflammatory responses, including *FCER1A*, *CD1C*, *CD1E*, *CD207*, *LY86*, *CX3CR1*, *FYN*, *TBK1*, *IFIT1*, *C7* and *A2M*. Additional grade-associated transcripts included regulators of cell-cycle and survival signalling (*DUSP4*, *AKT3*, *PSMB7* and *TGFB2*) and genes related to adhesion, glycosylation and extracellular-matrix interactions (*AMICA1* and *FUT5*) (Supplementary Figure S2F).

KRAS and *EGFR* status were associated with distinct immune-related transcriptional profiles (Supplementary Figure S2 G-H; Supplementary Table S3A).

For *KRAS*, the associated genes included components of innate and inflammatory signalling, such as *TLR6*, *TLR10*, *IL6*, *IL17RA* and *TRAF6*, together with genes involved in immune-cell activation or antigen recognition (*SIGLEC1* and *CR2*) and antigen processing and presentation (*HLA-DOB*). (Supplementary Figure S2 G). The association of *CR2* with *KRAS* status links a B-cell-relevant transcript to tumour-intrinsic molecular context.

Similarly, *EGFR* associated genes encompassed cytokine and interferon signalling (*IL6ST*, *IL12A* and *IRF1*), innate immune and NF- κ B-related pathways (*CD59*, *TLR2* and *NFKBIA*), immune cell activation, polarization, and signalling (*TNFRSF9*, *IL5RA* and *LAG3*), chemokine-mediated trafficking (*CX3CL1* and *CCL17*), and antigen processing and presentation (*HLA-DMA*, *HLA-DPB1*, *HLA-DRB3* and *HLA-B2M*) (Supplementary Figure S2 H). In particular, *CEACAM6*, a cell-surface protein implicated in immune evasion, anoikis resistance and therapeutic resistance in NSCLC, and whose expression has been associated with clinical outcome in EGFR-mutated disease, was also associated with *EGFR* mutation status in our cohort (33–35). Together, these observations place *EGFR*-associated transcriptional variation within the broader immune and tumour-associated heterogeneity of resectable LUAD.

Finally, to assess whether the transcriptional features identified in tumour tissue were also associated with transcript abundance variation in the matched non-tumour lung tissue, we applied the same GLM framework to N samples using the identical criteria (absolute regression coefficient ≥ 0.6 , $p \leq 0.05$ and $AUC \geq 0.80$). The complete N-tissue results are reported in Supplementary Table S3 B. Fifty transcripts in adjacent non-tumour tissue and 81 transcripts in tumour tissue met these criteria, with only five transcripts shared between compartments including *ATM*, *BST2*, *CR2*, *IFIT1* and *TGFB2* (Supplementary Table S3C). Among these shared transcripts, the patterns of association differed between compartments; in non-tumour tissue these genes were associated mainly with tumour history and interferon/inflammatory signalling (*IFIT1*, *BST2*, *TGFB2*), whereas in tumour tissue, they were linked to progression-related features such as lymph node involvement (*BST2*), grading (*IFIT1*, *TGFB2*), and the *EGFR/KRAS* mutation status (*ATM*, *CR2*).

Immune-cell deconvolution provides relative compositional context for the tumour-associated signature

The expression heatmap of DEGs intersecting the LM22 immune-cell reference matrix provided gene-level context for the inferred immune populations (Fig. 4). CIBERSORT-based deconvolution then estimated relative immune-cell compositions in tumour and patient-matched adjacent non-tumour tissue.

Tumour tissue showed higher visualized mean estimated fractions of plasma cells, memory CD4 + T cells and follicular helper T cells than adjacent non-tumour tissue (Figs. 5–6). This pattern provides relative compositional context for the B-cell lineage, lymphocyte-recruitment and costimulatory transcripts identified in the DEG and pathway analyses. In particular, the tumour-associated expression of *CD19*, *MS4A1/CD20*, *CCR7* and *CD28* was directionally consistent with this adaptive immune profile (Fig. 4).

Conversely, neutrophils, resting NK cells, monocytes and M0 macrophages showed higher visualized mean estimated fractions in adjacent non-tumour tissue (Figs. 6 and 7). Notably, both M1 and M2 macrophages were enriched in tumour samples, with M1 macrophages showing a more pronounced increase than M2 macrophages in matched non-tumour tissues (Fig. 7).

Together, the deconvolution estimates provide relative compositional support for an adaptive immune transcriptional signature in resectable LUAD that incorporates B-cell lineage, lymphocyte-recruitment and costimulatory components.

Functional enrichment identifies broad immune, inflammatory, apoptotic and lung-cancer-related categories

The biological functions of the DEGs were explored by enrichment analysis using the MetaCore “map folders” ontology. Among the most significantly enriched terms ($FDR \leq 1.5 \times 10^{-3}$), four categories emerged, in agreement with the GLM framework, and are closely related to the main molecular and pathological features of the investigated samples: “immune system response” (58% of DEGs), “inflammatory response” (49%), “apoptosis” (38%), and “lung cancer” (42%) (Table S4 and Supplementary Figure S3). These categories provide a broad functional context for the tumour-associated transcriptional signature and indicate that its immune component coexists with inflammatory, survival-related and lung-cancer-associated processes.

To evaluate their direct functional correlations, we further processed the DEGs annotated with the above enriched terms by applying the build network tool and the direct interaction algorithm (DIA) from the MetaCore resource (Supplementary Figure S3, panels C, E, G, and I). PPAR γ , PAX5, and survivin emerged as central nodes across all generated DINs (except for PAX5 in “apoptosis” and survivin in “inflammatory

response”), underscoring their potential role in coordinating immune, inflammatory, and prosurvival responses within the lung cancer microenvironment.

B-cell-associated transcripts form the dominant functional programme in tumour tissue

DEGs were further subjected to enrichment analysis using the MetaCore “pathway maps” ontology to define the immune processes represented within the tumour-associated signature. The strongest functional convergence involved B-cell-associated pathways. Significantly enriched maps included “*B-cell signalling in hematological malignancies*”, “*role of tumor-infiltrating B-cells in anti-tumor immunity*”, “*B-regulatory cells and tumor cell intercellular interaction*”. A significant enrichment of DEGs was also observed in the “*immune response_BAFF (TNFSF13B)-induced signalling*” pathway (Table S5 in Supplementary Figure S4). Together, these maps linked the DEG set to B-cell receptor-associated signalling, tumour-infiltrating B-cell-related functions, B-cell-tumour cell interactions and BAFF-related signalling.

To assess functional relationships among the DEGs included in the enriched pathway maps, network analysis was performed by generating corresponding SPNs via the MetaCore resource (Supplementary Figure S4, panels F-I). While DEGs from the “*B-regulatory cell-tumor cell intercellular interaction*” pathway map were not interconnected according to SPA, those from the other three pathway maps formed significant SPNs. The factors added by the programme to cross-link these DEGs included PKC, Lyn, Fyn, and TRAF6. Of particular interest was the identification of PKC among the connector nodes generated by the SPN analysis. Accumulating evidence supports a central role for PKC signalling in lung cancer, where it contributes not only to tumour neoangiogenesis, including hypoxia-driven PKC α activation in endothelial cells but also to the modulation of the tumour microenvironment and immune-tumour cell crosstalk, ultimately influencing disease progression [34]. Overall, these pathways included 14 upregulated genes (i.e. *TACI (TNFSF13B)*, *CD79A*, *CD79B*, *CD20 (MS4A1)*, *BCMA (TNFSF17)*, *BAFF-R (TNFSF13C)*, *CD27 (TNFSF7)*, *CD70 (TNFSF7)*, *CD19*, *CXCR5*, *CXCL13*, *CD28*, *LTB*, *OX40 (TNFSF4)*, and two downregulated genes, namely, *IL1RL1 (ST2)* and *FPRL1 (FPR2)*, which are known to be involved in the survival, differentiation and activation of B cells [35–49].

T-cell-related pathway maps reveal costimulatory and differentiation-associated components

Furthermore, in the “pathway maps” ontology, we also detected a significant enrichment of DEGs in pathways related to T cells: “*immune response_T-cell cosignaling receptors, schema*”; “*immune response_Differentiation and clonal expansion of CD8 + T cells*”; “*immune response_T-cell subsets: cell surface markers*”; “*immune response_Differentiation of natural regulatory T cells*”; and “*immune response_Generation of memory CD4 + T cells*” (Table S6 in Supplementary Figure S5). Similar to what was performed for B-cell-related pathway maps (Supplementary Figure S4), DEGs included in the

enriched T-cell pathways were further analysed through network reconstruction to assess functional connectivity (Supplementary Figure S5, panels G-K). The relatively low number of DEGs within individual pathway maps limited, except for “*immune response_T-cell cosignaling receptors, schema*”, the generation of complex interaction networks, revealing a lack of functional connectivity among some of the experimental factors. Despite these limitations, the enriched pathways remain highly relevant to the biological context under investigation, highlighting the involvement of specific T-cell populations. Network connectivity was established through intermediary molecules, including SHP-1, Lck, TRAF2, and the IL2 receptor. Interestingly, all DEGs included in the T-cell pathway maps were upregulated in the K samples, except for ST2 (ST2(L) in MetaCore; L means the long transmembrane isoform), which was downregulated. The proteins corresponding to DEGs annotated in significantly enriched T-cell-related pathway maps included 11 molecules: BTLA, CD137 (TNFRSF9), CD27 (TNFRSF7), CD28, CD70 (TNFSF7), collagen III, GITR, OX40, CCR7, IL-2R alpha chain, and ST2(L). Five DEGs, i.e. CD27 (TNFRSF7), CD70 (TNFSF7), CD28, OX40, and ST2 (IL1RL1), were shared between the enriched B-cell- and T-cell-related pathway maps. This overlap likely reflects well-established roles for such receptors and ligands in both B and T lymphocyte biology. For example, the CD27-CD70 axis functions as a key costimulatory pathway that promotes activation, survival, and differentiation in both B cells and T cells and contributes to effector and memory immune responses across lymphocyte subsets [49,50]. Similarly, OX40 and its ligand participate in T-cell costimulation and in T-B interactions during adaptive immune responses [51]. Although IL1RL1 has been characterized more extensively in T-cell subsets and innate lymphoid cells, its expression in broader lymphoid contexts has been documented and suggests pleiotropic functions in type 2 and regulatory immune responses [48,52]. Despite the overlap in these key molecules, the distinct arrangement of other enriched pathway elements reflects lineage-specific molecular programmes that differentiate B cells from T cells.

Network analysis prioritizes B-cell-associated and broader regulatory nodes

Database-derived interaction-network analysis incorporated approximately 75% of the DEG-associated functional interactions and prioritized TAL1, POU2F2, PAX5, CDK1 and PPARG as highly interconnected nodes within the MetaCore network (Fig. 8). PAX5 and POU2F2 link the inferred network to B-cell-associated regulatory processes, whereas CDK1, TAL1 and PPARG extend it towards proliferative and broader regulatory functions. Together, these network-prioritized nodes place the B-cell-associated component within a wider tumour-immune transcriptional architecture.

In an exploratory external CIDE query, gene-level Cox proportional-hazards z-scores were examined in independent anti-PD-1-treated LUAD and LUSC cohorts. In the LUAD cohort, PAX5 ($z = -1.73$), CDK1 ($z = -0.90$), TAL1 ($z = -0.89$) and POU2F2 ($z = -0.27$) showed directionally favourable survival associations, whereas PPARG showed an unfavourable association ($z = +0.78$). In the LUSC cohort, the direction of association was reversed for each of the five network-prioritized genes.

Among BAFF/APRIL-axis components, BAFF/TNFSF13B ($z = -0.75$), BAFF-R/TNFRSF13C ($z = -1.26$), TACI/TNFRSF13B ($z = -0.70$) and BCMA/TNFRSF17 ($z = -0.63$) showed directionally favourable associations in the LUAD cohort, whereas APRIL/TNFSF13 showed an unfavourable association ($z = +0.65$). The direction of association was reversed for all five BAFF/APRIL-axis components in the LUSC cohort (Supplementary Table S4).

This histology-dependent reversal provides exploratory external clinical context for the network-prioritized genes and supports histology-specific evaluation in treatment-linked LUAD cohorts.

DISCUSSION

The present study identifies a tumour-associated immune transcriptional programme in resectable LUAD in which B-cell lineage, lymphocyte-recruitment and costimulatory components converge. The coordinated differential expression of *CD19*, *CD79A*, *CD79B* and *MS4A1/CD20*, together with *CXCL13*, *CXCR5*, *LTB* and *CCR7*, is consistent with an adaptive immune context characterized by B-cell-associated identity and lymphocyte organization. The enrichment of B-cell signalling and tumour-infiltrating B-cell immunity pathways, together with the directionally higher estimated relative fractions of plasma cells, memory CD4 + T cells and follicular helper T cells in tumour tissue, further supports this interpretation. In this context, *CXCL13* is of particular interest because it is linked both to B-cell recruitment within TLSs and to a functionally distinct PD-1-high CD8 + T-cell population associated with response and survival in NSCLC treated with PD-1 blockade [92]. However, the clinical significance of *CXCL13* appears to depend on its compartmental localization: *CXCL13* + cells within TLSs have been associated with favourable outcomes, whereas extra-TLS *CXCL13* expression in the tumour core or invasive margin has been linked to poor prognosis in advanced NSCLC [53]. *CXCL13* may also act directly on *CXCR5*-expressing tumour cells to promote migration and invasion through PLC β /PKC α /NF- κ B signalling [54]. These observations support a context-dependent interpretation of *CXCL13* in LUAD, in which immune-organizing and tumour-promoting activities may coexist in spatially distinct tumour-microenvironmental niches. BAFF-induced signalling should be interpreted in the same framework. The presence of *TACI*, *BAFF-R* and *BCMA* within the tumour-associated signature may be compatible with B-cell-associated immune organization, whereas previous evidence of BAFF-related receptor expression in NSCLC carcinoma cells and cancer-associated fibroblasts indicates that this axis may also operate in tumour- or stroma-associated compartments [55–57]. Defining its spatial distribution may be critical to clarify whether BAFF/APRIL-related signalling contributes predominantly to lymphoid immune organization, tumour-associated biology, or both. Consistent with this context-dependent interpretation, the exploratory CIDE analysis showed directionally favourable anti-PD-1 survival associations in LUAD for *BAFF*, *BAFF-R*, *TACI* and *BCMA*, whereas *APRIL* showed an unfavourable association; the direction of each association was reversed in the LUSC cohort (Supplementary Table S4). Although these external gene-level associations do not establish a predictive role, they support evaluation of BAFF/APRIL-related signalling in a histology- and compartment-specific manner.

The B-cell-associated component was accompanied by a broader programme of lymphocyte costimulation and differentiation. *CD27*, *CD70*, *CD28*, *OX40* and *IL1RL1/ST2* were shared between B-cell- and T-cell-related pathway maps, while the enriched T-cell pathways included co-signalling receptors, CD8 + T-cell differentiation and clonal expansion, T-cell subset markers, regulatory T-cell differentiation and memory CD4 + T-cell generation. The CD27-CD70 axis and OX40-related pathways regulate lymphocyte activation, survival and differentiation [42,46,49,51]. However, their presence in enriched pathways does not, by itself, establish the direction or functional consequences of signalling since persistent or dysregulated CD70 and OX40 signalling has also been linked to T-cell dysfunction and exhaustion [50]. The coexistence of costimulatory and regulatory components in the present cohort therefore suggests an immune landscape in which lymphocyte activation and organization coexist with the potential for functional drift towards dysfunction. This interpretation is also consistent with evidence that TLS-positive tumours can contain T cells in heterogeneous functional states and do not uniformly achieve optimal antitumour immunity [58,59]. The associations of immune-recognition, inflammatory, antigen-presentation and tumour/stroma-related transcripts with nodal involvement, pathological T category, tumour grade, *KRAS* and *EGFR* status further place this immune architecture within the biological heterogeneity of resectable LUAD. In particular, *CR2* links a B-cell-relevant transcript to *KRAS* status, whereas *EGFR*-associated variation includes cytokine-, interferon-, trafficking- and antigen-presentation related genes, together with *CEACAM6*. These findings indicate that clinical and molecular features are associated with particular immune-related transcriptional patterns without accounting for the overall architecture of tumour heterogeneity observed in the unsupervised analyses.

The network-prioritized hub proteins TAL1, POU2F2, PAX5, CDK1 and PPARG provide a further conceptual link between the B-cell-associated component and broader tumour-immune processes. PAX5 and POU2F2 are established regulators of B-cell biology [60,61], making their central network position coherent with the B-cell-associated programme described here, whereas CDK1, TAL1 and PPARG connect this framework with proliferative and broader regulatory processes [62–64]. The divergent survival associations observed for these hubs in anti-PD-1-treated LUAD and LUSC cohorts further suggest that their clinical relevance may be histology dependent.

Several limitations should be acknowledged. The targeted bulk-transcriptomic approach does not capture the full transcriptomic complexity of the tumour immune microenvironment, and the absence of spatial resolution does not permit assignment of the detected signals to specific cellular or tissue compartments. The modest cohort size may also limit statistical power for subgroup analyses. External validation in independent cohorts, together with orthogonal spatial and functional assays, will be essential to confirm the identified associations and to determine whether the TLS-like programmes observed here represent productive antitumour immunity or a partially organised yet functionally insufficient immune state. These studies may also clarify whether the identified immune pathways can be therapeutically reinforced during the neoadjuvant treatment window.

CONCLUSIONS

Resectable LUAD displays a tumour-associated transcriptional programme encompassing B-cell lineage markers, lymphocyte-recruitment signals, BAFF-related components and lymphocyte costimulatory pathways. Its clinicopathological and oncogenic associations place this programme within the broader immune heterogeneity of resectable LUAD. B-cell-associated, costimulatory and BAFF-related components merit evaluation in spatially resolved and treatment-linked cohorts.

MATERIAL AND METHODS

Cohort selection

LUAD samples were collected at AOUS Le Scotte, Siena, Italy. The cohort included 46 fresh tissue samples from 23 patients with resectable stage I-IIIa LUAD. For each patient, one tumour specimen and one patient-matched adjacent non-tumour lung specimen were collected. All patients underwent curative-intent lung resection at the Thoracic Surgery Unit of AOUS during 2022. None received neoadjuvant therapy.

Fresh tissue samples were collected immediately after resection under the supervision of an expert pulmonary pathologist. Histopathological diagnosis was established on corresponding formalin-fixed, paraffin-embedded sections according to the fifth edition of the WHO Classification of Thoracic Tumours. Areas of necrosis were excluded. Clinical information was collected from medical records and is summarized in Supplementary Table S1.

The study was approved by the AOUS Ethics Committee (19 April 2021; protocol 19317; code MI-PP), conducted in accordance with the Declaration of Helsinki and its later amendments. Written informed consent was obtained from all patients.

NanoString gene-expression profiling and statistical analysis

RNA was extracted from fresh tissues as previously described [65]. Digital multiplexed gene-expression profiling of 730 immune-related genes and 40 housekeeping genes was performed using the nCounter PanCancer Immune Profiling Panel (NanoString Technologies, Seattle, WA, USA) according to the manufacturer's instructions. No quality-control flags were detected, indicating that samples met the assay-performance and data-quality criteria specified in the NanoString guidelines. Statistical analysis was performed in RStudio Desktop 1.1463 (Integrated Development for RStudio, Inc., Boston, MA, USA; last accessed April 2026). Log₂-transformed counts per million were normalized and analysed using limma (v.4.4) [66]. Differential expression was assessed between tumour (K) and adjacent non-tumour (N) tissue conditions. Genes were considered differentially expressed at an absolute fold change of at least 1.3 ($|\log_2FC| \geq 0.3$) and a Benjamini-Hochberg false-discovery rate (FDR) ≤ 0.03 .

The 730 analysed genes were visualized in a volcano plot. Differentially expressed genes (DEGs) were clustered by Ward's method with Euclidean distance and displayed as a heatmap. Principal component

analysis (PCA) and hierarchical clustering were used as unsupervised visualizations of the selected DEG signature.

To explore associations between tumour-tissue gene expression and clinical variables, logistic regression models were fitted within a generalized linear model (GLM) framework. Model performance was assessed by receiver-operating-characteristic curve analysis. Transcripts meeting the predefined descriptive thresholds of an absolute regression coefficient ≥ 0.6 , nominal $p \leq 0.05$ and area under the curve ≥ 0.80 were reported. These analyses were used to describe clinical and molecular associations within the cohort, not to establish predictive models.

CIBERSORT immune-cell deconvolution

Relative immune-cell fractions were estimated from the normalized expression matrix with CIBERSORT [67] and the LM22 reference signature matrix representing 22 human immune-cell subtypes. Duplicate genes were removed, gene symbols were standardized, and genes shared by the experimental matrix and LM22 were retained. The analysis used 100 permutations, and samples with CIBERSORT $p < 0.05$ were retained for downstream visualization. For each sample, CIBERSORT provided estimated relative fractions, p values, Pearson correlation coefficients and root mean square errors.

Immune-cell fractions were merged with clinical metadata, organised by cell type and sample, row-standardized for heatmap visualization, and annotated by pathological condition. Group-level displays were based on mean estimated immune-cell fractions and normalized percentage variation, calculated as the difference between conditions divided by the larger group mean. Cell types were ordered by absolute variation and changes greater than 5% were annotated. A complementary heatmap was generated from DEGs intersecting the LM22 signature matrix. For this visualization, the LM22 matrix was binarized at z score > 1.5 to assign each gene to an associated immune-cell type, and normalized expression of the resulting gene set was displayed with sample annotations for tissue condition and gene annotations for the associated immune-cell type. Visualizations were generated using the pheatmap and ggplot2 packages in R.

Functional enrichment and network analysis

Functional enrichment of DEGs was performed with MetaCore v23.3 (Clarivate Analytics, Boston, MA, USA) in the “pathway maps” and “map folders” ontologies and terms with $FDR \leq 0.05$ were retained. Direct-interaction and shortest-path networks were generated with the MetaCore Network Building tool using the direct interaction algorithm (DIA) or shortest path algorithm (SPA), respectively, with two allowed steps. The resulting direct-interaction networks summarize curated functional connections among DEG-encoded proteins within the biological processes represented by the DEG set. Highly connected nodes were used to prioritize candidate transcriptional components within the database-derived DEG interaction network.

External CIDE query

The five network-prioritized genes (TAL1, POU2F2, PAX5, CDK1 and PPARG) and five BAFF/APRIL-axis components (TNFSF13B/BAFF, TNFRSF13C/BAFF-R, TNFRSF13B/TACI, TNFRSF17/BCMA and TNFSF13/APRIL) were queried in the Cancer Immunology Data Engine (CIDE; <https://cide.ccr.cancer.gov>; accessed 11 June 2026). The NSCLC.Adeno_RNA-seq_Cox-PH_anti-PD1_Pre-treat_bulk_tumor and NSCLC.Squamous_RNA-Seq_Cox-PH_anti-PD1_Pre-treat_bulk_tumor datasets from Ravi et al. were used [68].

Data availability

All data supporting the findings of this study are available within the article and its Supplementary Information.

Declarations

Additional Information

Competing interests

The authors declare no competing interests.

Funding

This research was funded by Human Brain Wave srl as industrial partner of the Digital Technology for Lung Cancer Treatment project, granted by Programma Operativo Regionale “Investimenti a favore della crescita e dell’occupazione” F.E.S.R. 2014/2020.

Authors’ contributions

Marcella Barbarino and Laura Bianchi contributed to conceptualisation, data curation, data analysis, interpretation of data and preparation of the original draft. Marcella Barbarino and Laura Bianchi supervised the study. Marcella Barbarino and Cristiana Bellan contributed to project administration. Laura Bianchi and Alfonso Carleo performed formal analysis. Laura Bianchi, Rosanna De Salvo, Alfonso Carleo and Marcella Barbarino contributed to data acquisition and investigation. Mariachiara Siciliano and Matilde Pesetti performed experimental procedures and contributed to data acquisition. Diletta Rosati performed immune-cell deconvolution. Antonio Graziano, Laura Bianchi and Cristiana Bellan contributed to funding acquisition. Cristiana Bellan and Piero Paladini provided biological samples. Cristiana Bellan conducted tissue sampling and pathological evaluation. Andrea Quaranta, Gabriella Serio, Salvatore Cortellino and Antonio Giordano contributed to data interpretation and critically revised the manuscript. All authors read and approved the final manuscript.

References

1. Postmus, P. E. *et al.* Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **28**, iv1–iv21 (2017).
2. Jamal-Hanjani, M. *et al.* Tracking the Evolution of Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **376**, 2109–2121 (2017).
3. Preoperative chemotherapy for non-small-cell lung cancer: a systematic review and meta-analysis of individual participant data. *The Lancet* **383**, 1561–1571 (2014).
4. Pignon, J.-P. *et al.* Lung Adjuvant Cisplatin Evaluation: A Pooled Analysis by the LACE Collaborative Group. *J. Clin. Oncol.* **26**, 3552–3559 (2008).
5. Forde, P. M. *et al.* Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer. *N. Engl. J. Med.* **386**, 1973–1985 (2022).
6. Provencio, M. *et al.* Neoadjuvant chemotherapy and nivolumab in resectable non-small-cell lung cancer (NADIM): an open-label, multicentre, single-arm, phase 2 trial. *Lancet Oncol.* **21**, 1413–1422 (2020).
7. Tong, B. C. *et al.* Perioperative outcomes of pulmonary resection after neoadjuvant pembrolizumab in patients with non–small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* **163**, 427–436 (2022).
8. Cascone, T. *et al.* Neoadjuvant nivolumab or nivolumab plus ipilimumab in operable non-small cell lung cancer: the phase 2 randomized NEOSTAR trial. *Nat. Med.* **27**, 504–514 (2021).
9. Provencio, M. *et al.* OA20.01 Long Term Survival in Operable Stage Iiia Nsclc Patients Treated With Neoadjuvant Nivolumab Plus Chemotherapy - Nadim Study. *J. Thorac. Oncol.* **16**, S883 (2021).
10. Felip, E. *et al.* Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB–IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. *The Lancet* **398**, 1344–1357 (2021).
11. Lei, J. *et al.* Neoadjuvant Camrelizumab Plus Platinum-Based Chemotherapy vs Chemotherapy Alone for Chinese Patients With Resectable Stage IIIA or IIIB (T3N2) Non–Small Cell Lung Cancer. *JAMA Oncol.* **9**, 1348–1355 (2023).
12. Cascone, T. *et al.* Neoadjuvant Durvalumab Alone or Combined with Novel Immuno-Oncology Agents in Resectable Lung Cancer: The Phase II NeoCOAST Platform Trial. *Cancer Discov.* **13**, 2394–2411 (2023).
13. Provencio, M. *et al.* Perioperative Nivolumab and Chemotherapy in Stage III Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* **389**, 504–513 (2023).
14. Ettinger, D. S. *et al.* NCCN Guidelines® Insights: Non–Small Cell Lung Cancer, Version 2.2023: Featured Updates to the NCCN Guidelines. *J. Natl. Compr. Canc. Netw.* **21**, 340–350 (2023).
15. Hartner, S. *et al.* Oncogenic driver mutations underlie the spatial tumour immune landscape of non-small cell lung cancer. *Nat. Commun.* **16**, 8402 (2025).
16. Frontiers | Targetable driver gene–tumor immune microenvironment axis in non-small cell lung cancer: from molecular pathological mechanisms to precision immunotherapy stratification

strategies.

<https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2026.1801943/full>.

17. Helmink, B. A. *et al.* B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* **577**, 549–555 (2020).
18. Sautès-Fridman, C., Petitprez, F., Calderaro, J. & Fridman, W. H. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat. Rev. Cancer* **19**, 307–325 (2019).
19. Hao, D. *et al.* The Single-Cell Immunogenomic Landscape of B and Plasma Cells in Early-Stage Lung Adenocarcinoma. *Cancer Discov.* **12**, 2626–2645 (2022).
20. Liu, W. *et al.* An immune cell map of human lung adenocarcinoma development reveals an anti-tumoral role of the Tfh-dependent tertiary lymphoid structure. *Cell Rep. Med.* **5**, 101448 (2024).
21. Zannikou, M. *et al.* Bi-specific T cell-engaging antibody triggers protective immune memory and glioma microenvironment remodeling in immune-competent preclinical models. *J. Immunother. Cancer* **13**, e011714 (2025).
22. Yan, X. *et al.* Killing effect of interleukin-13 receptor alpha 2 (IL-13Ralpha2) sensitized DC-CTL cells on human glioblastoma U251 cells. *Cell. Immunol.* **263**, 172–175 (2010).
23. Chen, H. *et al.* Hypoxia is correlated with the tumor immune microenvironment: Potential application of immunotherapy in bladder cancer. *Cancer Med.* **12**, 22333–22353 (2023).
24. Veneziani, I., Alicata, C., Moretta, L. & Maggi, E. Human toll-like receptor 8 (TLR8) in NK cells: Implication for cancer immunotherapy. *Immunol. Lett.* **261**, 13–16 (2023).
25. Ke, J. *et al.* CD11b/CD86 involved in the microenvironment of colorectal cancer by promoting Wnt signaling activation. *Cancer Med.* **13**, e70245 (2024).
26. Zhang, F. *et al.* Immunosenescence-Related Gene MAFF is Involved in Immune Regulation and Malignant Progression in Pancreatic Adenocarcinoma. *Biol. Proced. Online* **27**, 26 (2025).
27. Soliman, N. *et al.* Targeting Intracellular Innate RNA-Sensing Systems Overcomes Resistance to CAR T-cell Therapy in Solid Tumors. *Cancer Res.* **85**, 2679–2693 (2025).
28. Chen, K. *et al.* Tumor-Derived CCL16 Normalizes Tumor Vasculature through Macrophage ICAM-1 Receptor and Enhances Immunotherapy Efficacy in Hepatocellular Carcinoma. *Cancer Res.* **85**, 3633–3650 (2025).
29. Chi, H.-C. *et al.* CCL16 is a pro-tumor chemokine that recruits monocytes and macrophages to promote hepatocellular carcinoma progression. *Am. J. Cancer Res.* **14**, 3600–3613 (2024).
30. Hu, B. *et al.* CLEC5A suppresses cell growth and metastasis via interfering with the calcineurin/NFATc1 signaling pathway in osteosarcoma. *Exp. Cell Res.* **450**, 114650 (2025).
31. Wu, G. *et al.* The emerging roles of CEACAM6 in human cancer (Review). *Int. J. Oncol.* **64**, 27 (2024).
32. Ramlau, R. *et al.* Safety of unconventional antibody-drug conjugate L-DOS47 in a phase I/II monotherapy study targeting advanced NSCLC. *Front. Oncol.* **15**, 1544967 (2025).
33. Chen, Y. *et al.* Deciphering tumor-intrinsic and immune characteristics in resectable non-small cell lung cancer treated with neoadjuvant pembrolizumab and chemotherapy. *J. Transl. Med.* **23**, 1082

(2025).

34. Luo, H. *et al.* Cyclooxygenase-2 up-regulates vascular endothelial growth factor via a protein kinase C pathway in non-small cell lung cancer. *J. Exp. Clin. Cancer Res.* **30**, 6 (2011).
35. Liu, X., Asokan, S. B., Bear, J. E. & Haugh, J. M. Quantitative analysis of B-lymphocyte migration directed by CXCL13. *Integr. Biol.* **8**, 894–903 (2016).
36. López-Guerra, M., Xargay-Torrent, S. & Colomer, D. CXCR5-Mediated Shaping of the Lymphoid Follicle in Chronic Lymphocytic Leukemia.
<https://aacrjournals.org/cancerdiscovery/article/4/12/1374/4472/CXCR5-Mediated-Shaping-of-the-Lymphoid-Follicle-in>.
37. Terkelsen, T. *et al.* Secreted breast tumor interstitial fluid microRNAs and their target genes are associated with triple-negative breast cancer, tumor grade, and immune infiltration. *Breast Cancer Res.* **22**, 73 (2020).
38. Wei, F. *et al.* Intratumoural CD8+ CXCR5+ follicular cytotoxic T cells have prognostic value and are associated with CD19+ CD38+ B cells and tertiary lymphoid structures in colorectal cancer. *Cancer Immunol. Immunother.* **74**, 36 (2024).
39. Shang, T. *et al.* Tertiary lymphoid structures predict the prognosis and immunotherapy response of cholangiocarcinoma. *Front. Immunol.* **14**, (2023).
40. Gao, M. *et al.* Integrative Analysis of TLS-Associated Gene Signatures, Immune Infiltration and Drug Sensitivity in Pancreatic Cancer. *IET Syst. Biol.* **19**, e70038 (2025).
41. Gao, M. *et al.* Integrative Analysis of TLS-Associated Gene Signatures, Immune Infiltration and Drug Sensitivity in Pancreatic Cancer. *IET Syst. Biol.* **19**, e70038 (2025).
42. Thapa, B. *et al.* OX40/OX40 ligand and its role in precision immune oncology. *Cancer Metastasis Rev.* **43**, 1001–1013 (2024).
43. Damle, N. K., Linsley, P. S. & Ledbetter, J. A. Direct helper T cell-induced B cell differentiation involves interaction between T cell antigen CD28 and B cell activation antigen B7. *Eur. J. Immunol.* **21**, 1277–1282 (1991).
44. Cheng, Q. *et al.* Lipoxin A4 protects against lipopolysaccharide-induced sepsis by promoting innate response activator B cells generation. *Int. Immunopharmacol.* **39**, 229–235 (2016).
45. Chu, V. T., Enghard, P., Riemekasten, G. & Berek, C. In vitro and in vivo activation induces BAFF and APRIL expression in B cells. *J. Immunol.* **179**, 5947–5957 (2007).
46. Starzer, A. M. & Berghoff, A. S. New emerging targets in cancer immunotherapy: CD27 (TNFRSF7). *ESMO Open* **4**, e000629 (2019).
47. Ramon, S., Bancos, S., Serhan, C. N. & Phipps, R. P. Lipoxin A4 decreases human memory B cell antibody production via an ALX/FPR2-dependent mechanism: A link between resolution signals and adaptive immunity. *Eur. J. Immunol.* **44**, 357–369 (2014).
48. Larsen, K. M., Minaya, M. K., Vaish, V. & Peña, M. M. O. The Role of IL-33/ST2 Pathway in Tumorigenesis. *Int. J. Mol. Sci.* **19**, 2676 (2018).

49. Hintzen, R. Q. *et al.* Regulation of CD27 expression on subsets of mature T-lymphocytes. *J. Immunol.* **151**, 2426–2435 (1993).
50. Yang, Z.-Z., Novak, A. J., Ziesmer, S. C., Witzig, T. E. & Ansell, S. M. CD70+ non-Hodgkin lymphoma B cells induce Foxp3 expression and regulatory function in intratumoral CD4+CD25 T cells. *Blood* **110**, 2537–2544 (2007).
51. Watts, T. H., Yeung, K. K. M., Yu, T., Lee, S. & Eshraghisamani, R. TNF/TNFR Superfamily Members in Costimulation of T Cell Responses-Revisited. *Annu. Rev. Immunol.* **43**, 113–142 (2025).
52. Peine, M., Marek, R. M. & Löhning, M. IL-33 in T Cell Differentiation, Function, and Immune Homeostasis. *Trends Immunol.* **37**, 321–333 (2016).
53. Vahidian, F. *et al.* CXCL13 Positive Cells Localization Predict Response to Anti-PD-1/PD-L1 in Pulmonary Non-Small Cell Carcinoma. *Cancers* **16**, (2024).
54. Chao, C.-C. *et al.* CXC chemokine ligand-13 promotes metastasis via CXCR5-dependent signaling pathway in non-small cell lung cancer. *J. Cell. Mol. Med.* **25**, 9128–9140 (2021).
55. Dou, H., Yan, Z., Zhang, M. & Xu, X. APRIL, BCMA and TACI proteins are abnormally expressed in non-small cell lung cancer. *Oncol. Lett.* **12**, 3351–3355 (2016).
56. Dimitrakopoulos, F.-I. D. *et al.* Expression of Immune System-Related Membrane Receptors CD40, RANK, BAFFR and LT β R is Associated with Clinical Outcome of Operated Non-Small-Cell Lung Cancer Patients. *J. Clin. Med.* **8**, (2019).
57. Warakomska, M. *et al.* The effects of BAFF and APRIL signaling on non-small cell lung cancer cell proliferation and invasiveness. *Oncol. Lett.* **22**, 1–8 (2021).
58. Zhao, X. *et al.* 4P Spatially resolved transcriptome elucidates bidirectional tertiary lymphoid structure interacts with tumor microenvironment of non-small cell lung cancer. *Ann. Oncol.* **34**, S188 (2023).
59. Jiang, W. *et al.* Exhausted CD8+T Cells in the Tumor Immune Microenvironment: New Pathways to Therapy. *Front. Immunol.* **11**, (2021).
60. Nutt, S. L., Eberhard, D., Horcher, M., Rolink, A. G. & Busslinger, M. Pax5 determines the identity of B cells from the beginning to the end of B-lymphopoiesis. *Int. Rev. Immunol.* **20**, 65–82 (2001).
61. Hodson, D. J. *et al.* Regulation of normal B-cell differentiation and malignant B-cell survival by OCT2. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E2039–E2046 (2016).
62. Delogu, A. *et al.* Gene Repression by Pax5 in B Cells Is Essential for Blood Cell Homeostasis and Is Reversed in Plasma Cells. *Immunity* **24**, 269–281 (2006).
63. Luo, R., Zhuo, Y., Du, Q. & Xiao, R. POU2F2 promotes the proliferation and motility of lung cancer cells by activating AGO1. *BMC Pulm. Med.* **21**, 117 (2021).
64. Shi, H., Wang, W., Luo, J., Song, G. & Han, R. POU2F2+ B cells enhance antitumor immunity and predict better survival in non small cell lung cancer. *Sci. Rep.* **15**, 6549 (2025).
65. Barbarino, M. *et al.* Comparison of two mechanical disaggregation methods of fresh lung tissues for extraction of high-quality RNA. *Instrum. Sci. Technol.* **52**, 609–621 (2024).

66. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43**, e47 (2015).
67. Newman, A. M. *et al.* Robust enumeration of cell subsets from tissue expression profiles. *Nat. Methods* **12**, 453–457 (2015).
68. Ravi, A. *et al.* Genomic and transcriptomic analysis of checkpoint blockade response in advanced non-small cell lung cancer. *Nat. Genet.* **55**, 807–819 (2023).

Figures

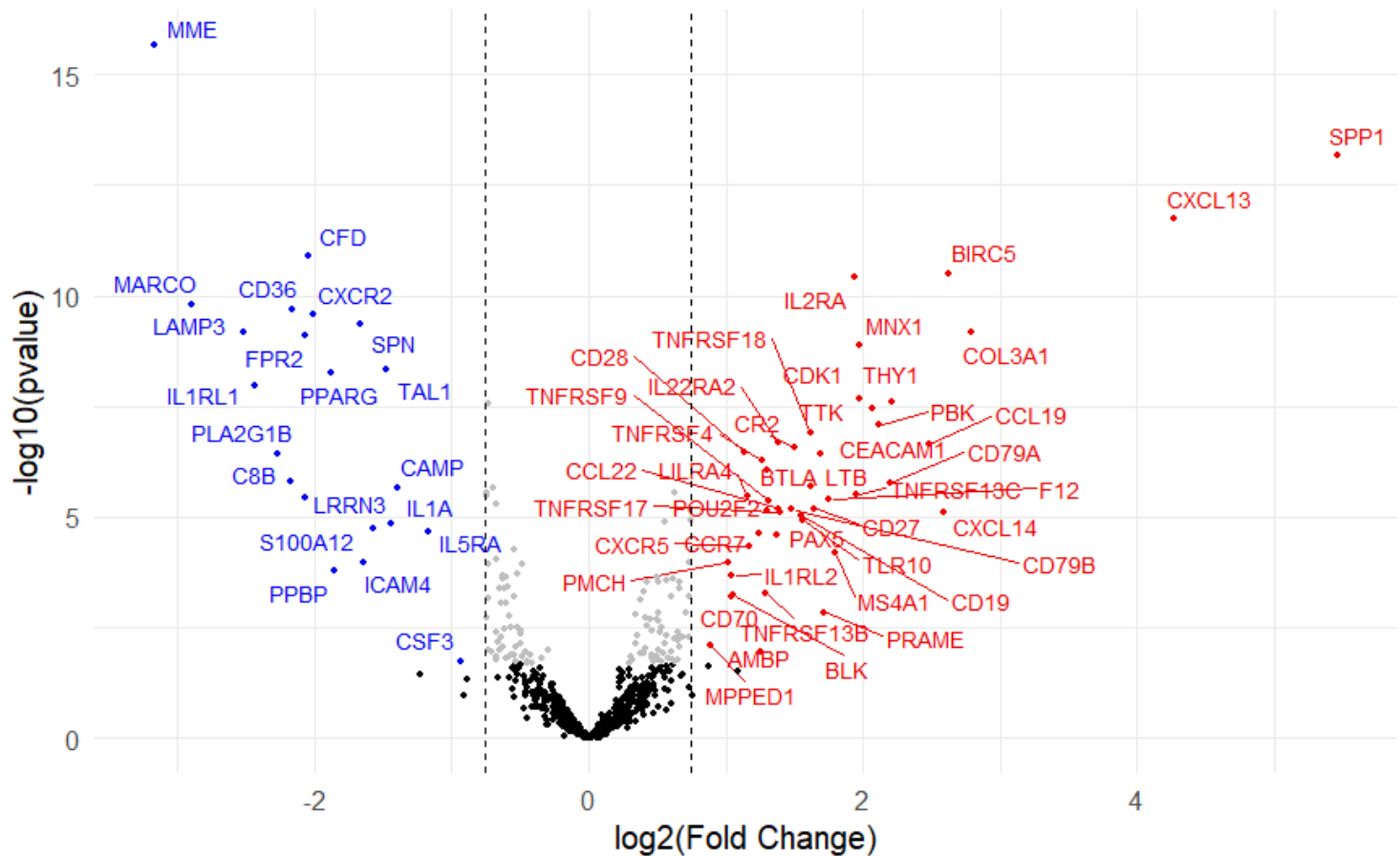


Figure 1

Volcano plot of expression changes between LUAD tumour and adjacent non-tumour tissue conditions. The 65 DEGs ($FDR \leq 0.03$ and $|\log_2 FC| \geq 0.3$) are shown in blue (lower in tumour) and red (higher in tumour).

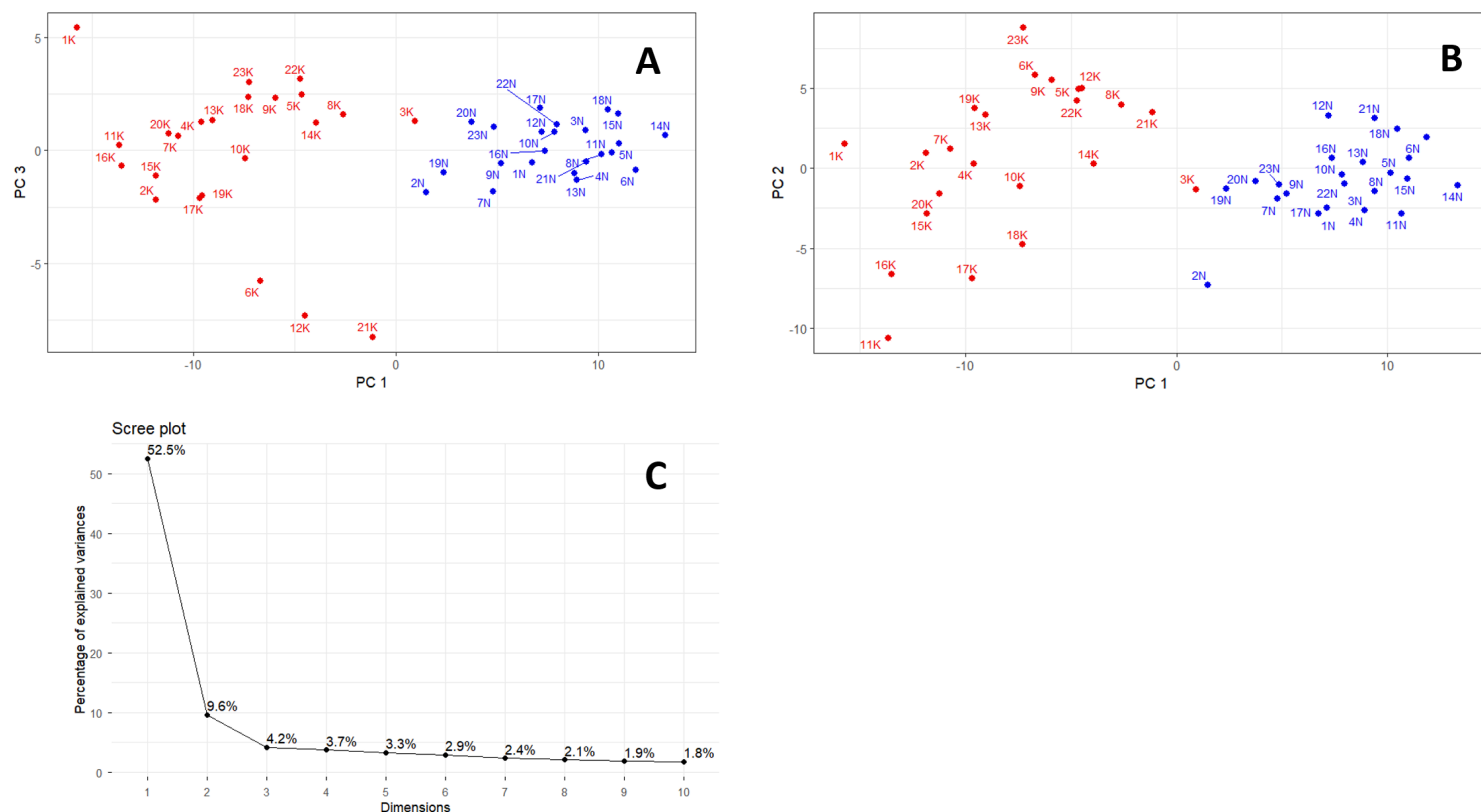


Figure 2

PCA of the 65 DEGs detected between 23 LUAD tumour (K) and 23 adjacent non-tumour (N) samples. Panels A and B display PC1 versus PC2 and PC1 versus PC3, respectively; panel C displays the scree plot. PC1, PC2 and PC3 account for 52.5%, 9.6% and 4.2% of the variance, respectively. Because PCA was performed on the DEG set selected from the tumour-versus-non-tumour comparison, it visualizes the structure of that signature. Tumour and adjacent non-tumour specimens from the same patient share the same identifier.

non-tumour (N, blue) tissue. The upper annotation bar indicates pathological N category (N0, N1 or N2). Tumour and adjacent non-tumour samples form distinct major clusters, while tumour samples show greater within-group expression heterogeneity. No dominant visual clustering by N category is apparent.

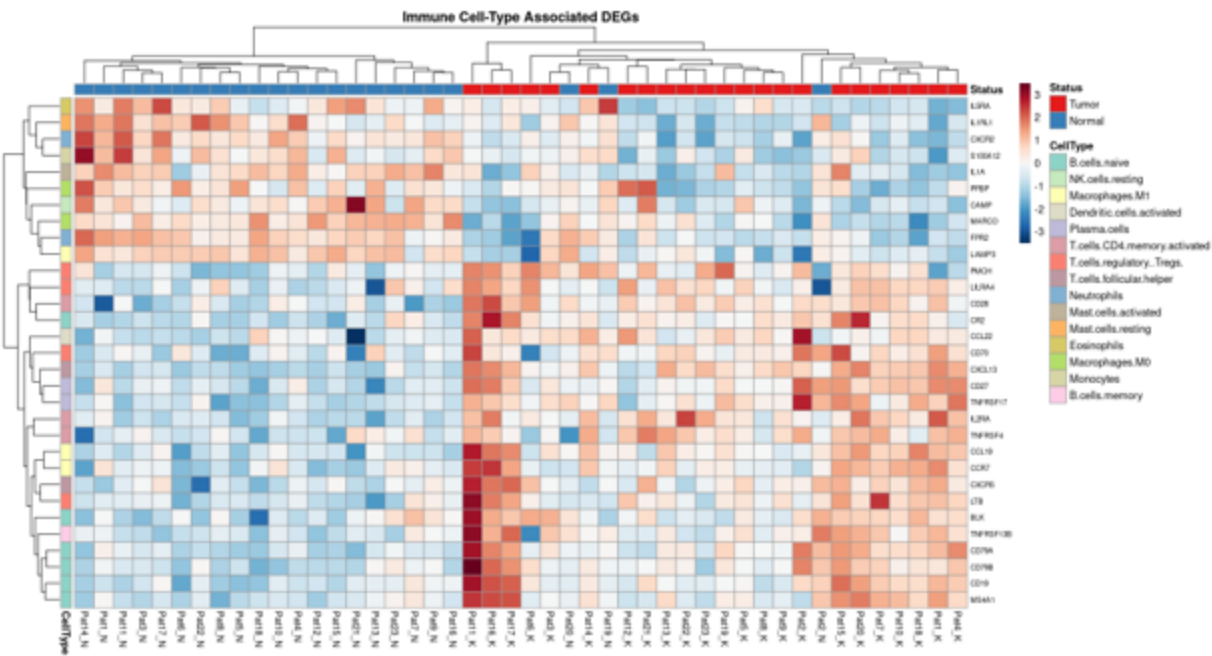


Figure 4

Heatmap of DEGs annotated to immune-cell types according to the LM22 signature matrix. Rows represent genes and columns represent samples. The figure visualizes normalized expression of LM22-overlapping DEGs and their LM22-associated annotation; it is not a direct measurement of immune-cell abundance.

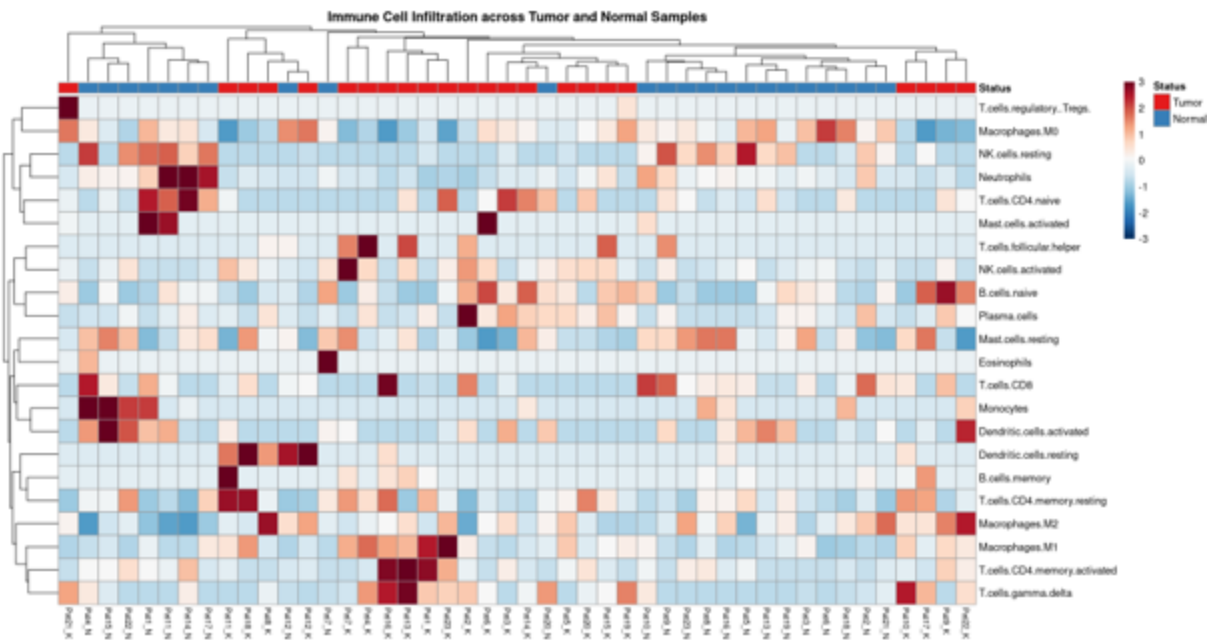


Figure 5

Normalized variation in CIBERSORT-estimated relative immune-cell fractions between tumour and adjacent non-tumour tissue conditions. Positive values indicate a higher mean estimated fraction in tumour tissue and negative values a higher mean estimated fraction in adjacent non-tumour tissue. Values are descriptive relative deconvolution estimates.

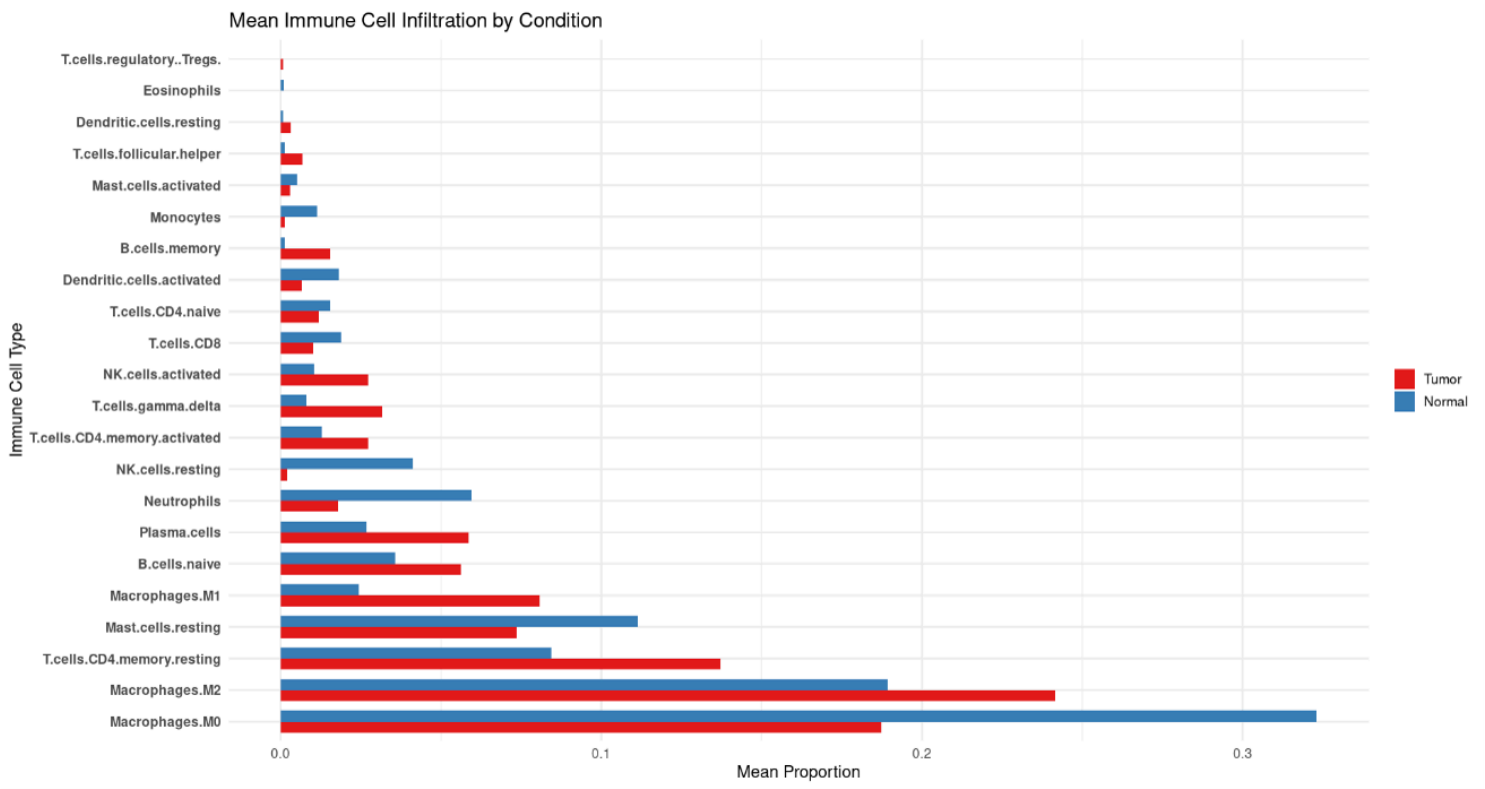


Figure 6

Mean CIBERSORT-estimated relative immune-cell fractions in tumour and adjacent non-tumour tissue conditions. Cell types are ordered by mean estimated fraction. The figure provides descriptive comparison of relative fractions and does not establish absolute immune-cell abundance.

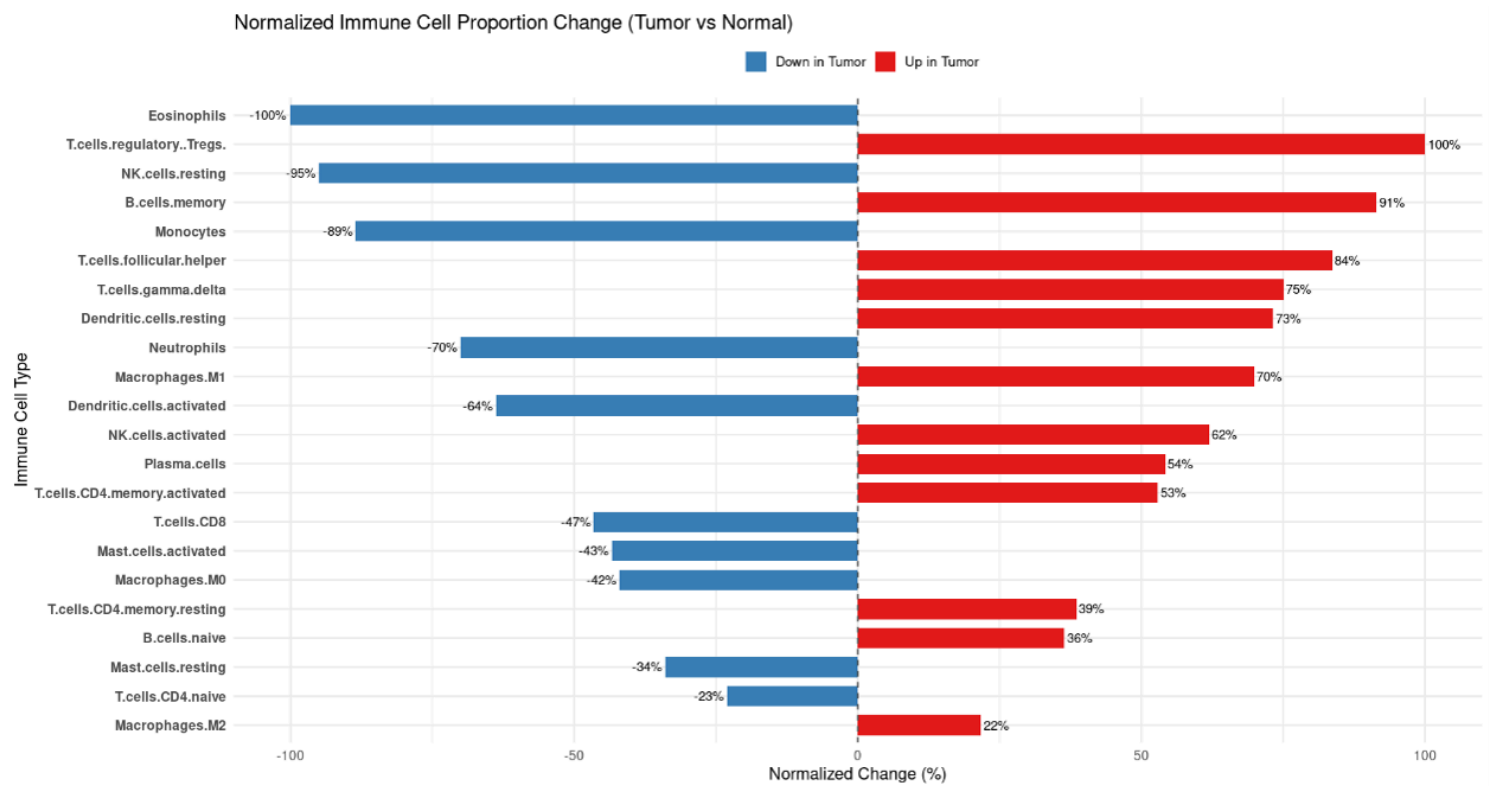


Figure 7

Heatmap of CIBERSORT-estimated relative fractions of 22 immune-cell types across tumour and adjacent non-tumour samples. Fractions were row-standardized and clustered for visualization; samples are annotated by tissue condition.

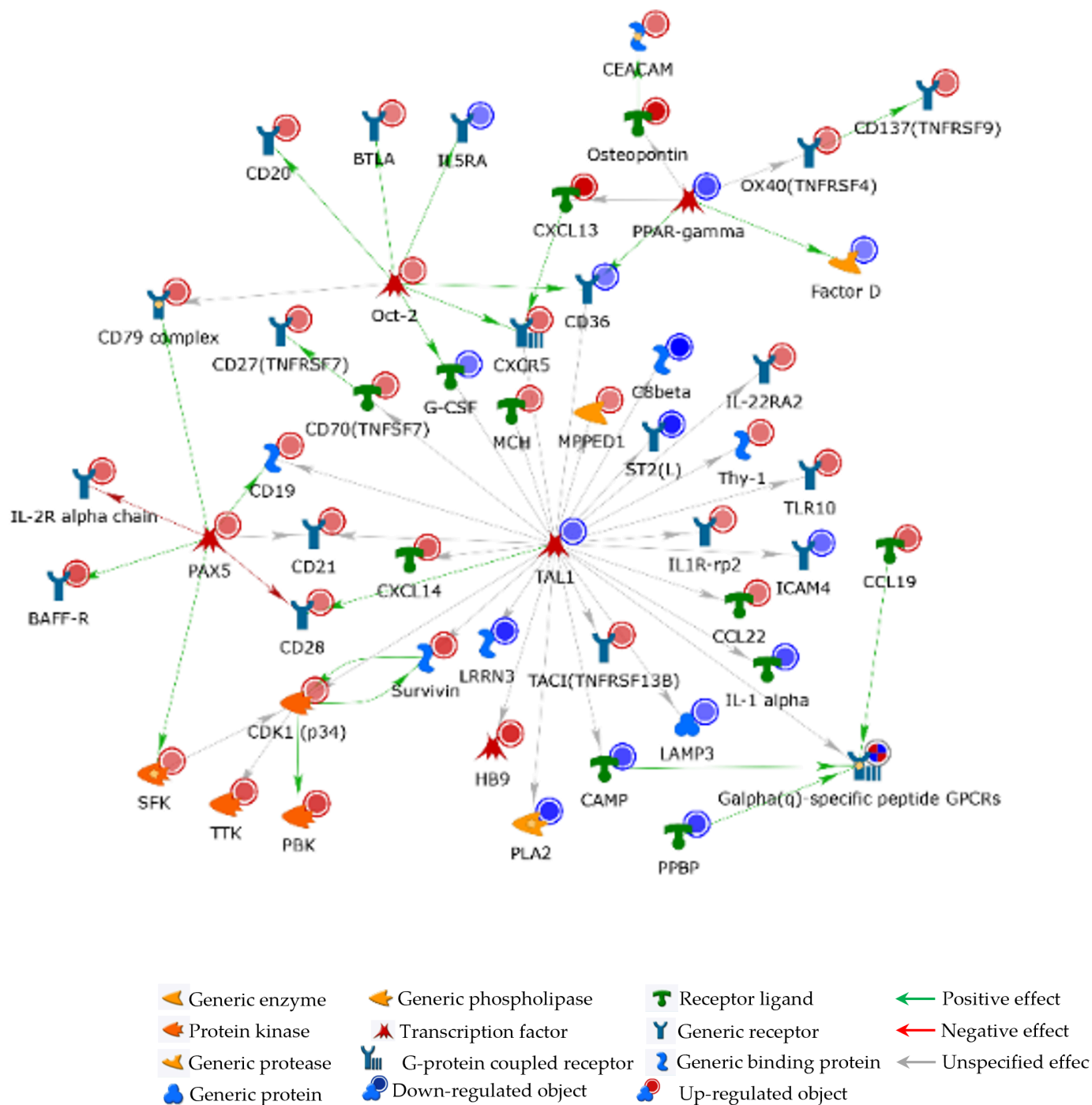


Figure 8

Database-derived direct-interaction network constructed from LUAD DEGs. Node colour indicates higher (red) or lower (blue) expression in tumour relative to adjacent non-tumour tissue. Edge colour denotes the curated interaction type and arrows indicate directionality. The network depicts curated connectivity among DEG-encoded proteins.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary.pdf](#)