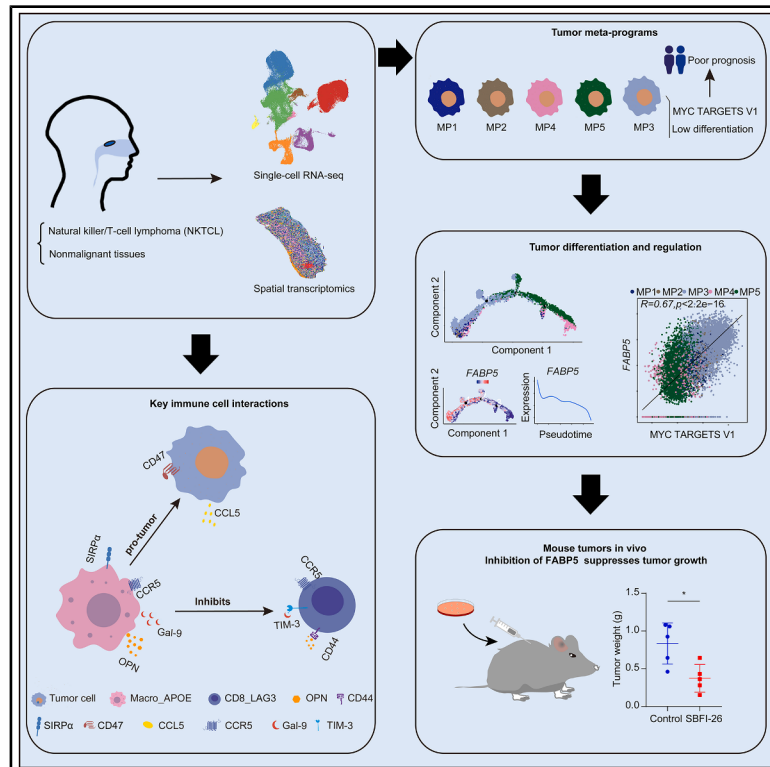


Single-cell and spatial transcriptomics reveal intratumor heterogeneity and immune evasion in natural killer/T cell lymphoma

Graphical abstract



Authors

Shuyun Ma, Biaojie Huang,
Jiahui Wang, ..., Wenlong Zhong,
Lei Wang, Qingqing Cai

Correspondence

liguibo@genomics.cn (G.L.),
zhongwlong3@mail.sysu.edu.cn (W.Z.),
wanglei3@genomics.cn (L.W.),
caiqq@sysucc.org.cn (Q.C.)

In brief

Cancer; Microenvironment;
Transcriptomics

Highlights

- Integrative multi-omics reveal five distinct malignant subtypes in NKTCL
- The MYC-hyperactivated malignant subtype correlates with poor prognosis in NKTCL
- FABP5 inhibition dampens MYC signaling and curbs tumor growth *in vitro* and *in vivo*
- APOE⁺ macrophages contribute to immune evasion and T cell activity inhibition in TME



Article

Single-cell and spatial transcriptomics reveal intratumor heterogeneity and immune evasion in natural killer/T cell lymphoma

Shuyun Ma,^{1,2,12} Biaojie Huang,^{3,4,12} Jiahui Wang,^{1,12} Ru Lv,^{3,4,12} Dan-Ling Dai,¹ Qian Zhong,¹ Yi Xia,¹ Panpan Liu,¹ Youqiong Ye,¹⁰ Kui Wu,⁶ Shichen Dong,⁵ Jun Cai,¹ Qihua Zou,¹ Yuchen Zhang,¹ Lirong Li,¹ Guibo Li,^{3,9,*} Wenlong Zhong,^{11,*} Lei Wang,^{3,5,7,8,*} and Qingqing Cai^{1,13,*}

¹Department of Oncology, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Collaborative Innovation Center of Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

²Department of Oncology, The Third Xiangya Hospital of Central South University, Changsha 410013, China

³BGI Research, Chongqing 401329, China

⁴College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

⁵BGI Research, Shenzhen 518083, China

⁶Institute of Intelligent Medical Research (IIMR), BGI Genomics, Shenzhen 518083, China

⁷Shenzhen Key Laboratory of Single-Cell Omics, BGI Research, Shenzhen 518083, China

⁸Guangdong Provincial Key Laboratory of Human Disease Genomics, Shenzhen Key Laboratory of Genomics, BGI Research, Shenzhen 518083, China

⁹State Key Laboratory of Genome and Multi-omics Technologies, BGI Research, Shenzhen 518083, China

¹⁰Shanghai Institute of Immunology, Faculty of Basic Medicine, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

¹¹Department of Urology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510275, China

¹²These authors contributed equally

¹³Lead contact

*Correspondence: liguibo@genomics.cn (G.L.), zhongwlong3@mail.sysu.edu.cn (W.Z.), wanglei3@genomics.cn (L.W.), caiqq@sysucc.org.cn (Q.C.)

<https://doi.org/10.1016/j.isci.2025.113626>

SUMMARY

Natural killer/T cell lymphoma (NKTCL), an Epstein-Barr virus-associated malignancy, is a highly aggressive subtype of non-Hodgkin lymphoma. However, the intratumoral heterogeneity and the interaction within the tumor microenvironment (TME) remain insufficiently understood. Here, we utilized single-cell and spatial transcriptomics to analyze tissues from NKTCL patients, identifying five malignant meta-programs (MPs) with distinct functional pathways, differentiation trajectories, and spatial distributions. Notably, the MP3 subgroup emerges at the early stages of tumor differentiation, characterized by the hyperactivation of MYC signaling and an association with poor prognosis. Intriguingly, pharmacologic inhibition of fatty acid-binding protein 5 (FABP5) leads to the downregulation of c-Myc and significantly impairs tumor growth both *in vitro* and *in vivo*. Furthermore, ligand-receptor interaction analysis reveals that tumor-associated macrophages (TAMs) may facilitate immune evasion and suppress T cell activity within the TME. Collectively, our findings elucidate the cellular heterogeneity and immune landscape of NKTCL, offering potential targets for therapeutic intervention.

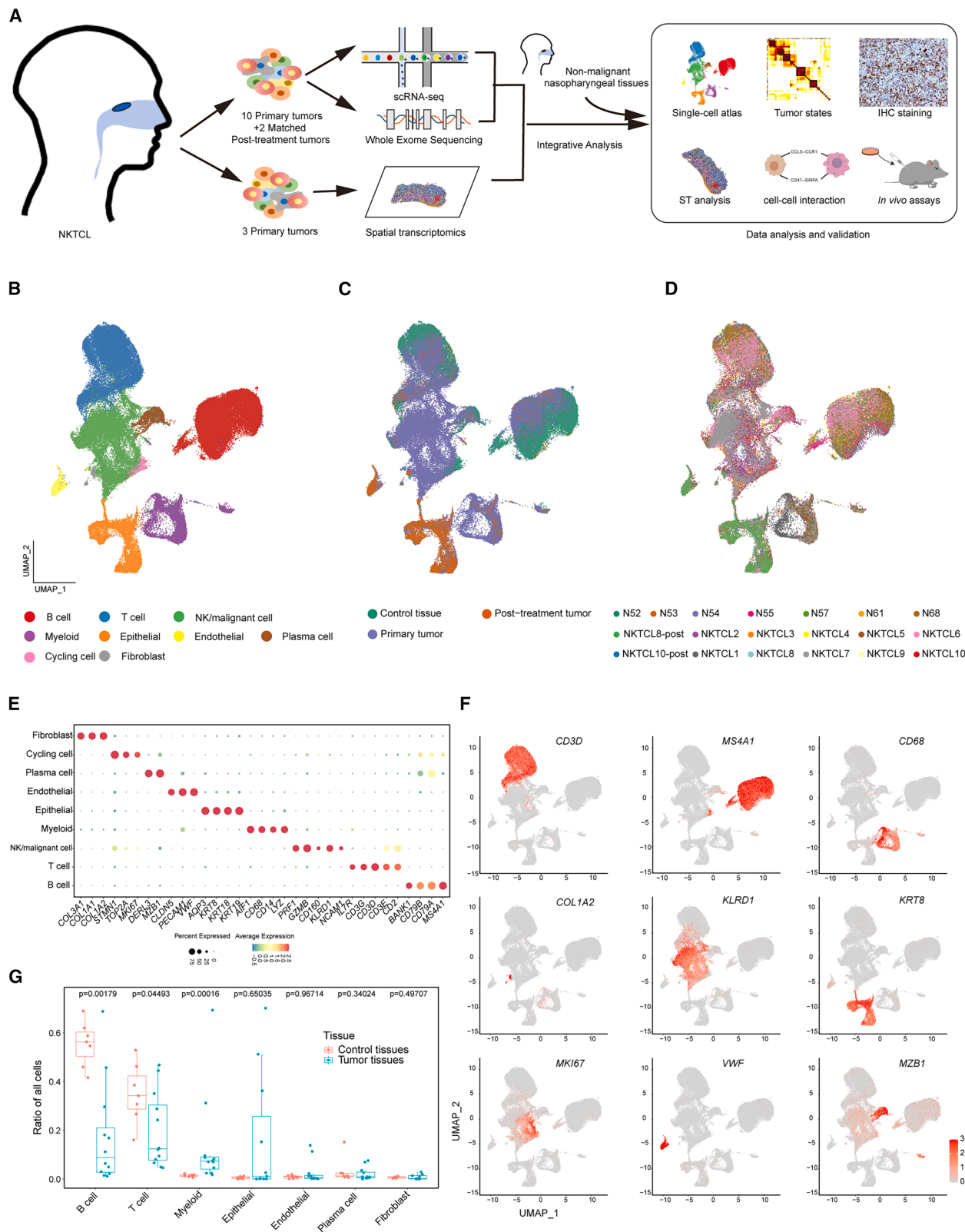
INTRODUCTION

Natural killer/T cell lymphoma (NKTCL) is a highly aggressive and clinically heterogeneous non-Hodgkin's lymphoma that is rare in North America and Europe but prevalent in South-east Asia.¹ Combination chemotherapy based on L-asparaginase remains the standard treatment^{2,3}; however, approximately 50% of patients exhibit resistance to this regimen.^{4,5} Recent advances have demonstrated the potential of immunotherapy to improve outcomes in patients with relapsed or refractory disease.^{6–9} Nevertheless, limited understanding of the tumor microenvironment (TME) continues to

hinder the development of more effective therapeutic strategies.¹⁰

The majority of malignant cells in NKTCL originate from natural killer (NK) cells, with a minority deriving from T cells.¹ These tumors often display the elevated expression of NK-related and cytotoxic genes, reflecting an activated cellular state.^{11,12} However, the absence of specific markers to define malignant cells poses a significant challenge in distinguishing these cells from normal NK cells, thereby impeding a deeper understanding of the intratumoral heterogeneity and the immune landscape of the TME in NKTCL. Previous studies have revealed the genetic heterogeneity and multiple molecular subtypes in NKTCL.^{13–15}





(legend on next page)

Furthermore, single-cell RNA sequencing (scRNA-seq) has revealed Epstein-Barr virus (EBV)-driven lymphomagenesis and immune remodeling.^{16,17} However, these techniques are inherently limited by the irreversible loss of spatial information within tumor tissues, which hinders a thorough characterization of the cellular composition and intercellular interaction networks within the tumor.

Here, we enrolled 13 patients with newly diagnosed NKTCL and subjected their tumor tissues to single-cell RNA sequencing and spatial transcriptomic profiling to delineate intratumoral heterogeneity and spatial architecture. To facilitate a comprehensive comparative analysis, we integrated these data with publicly available single-cell RNA-seq datasets from seven non-malignant nasopharyngeal tissues.¹⁸ This integrated approach allowed us to precisely map malignant and immune cells, analyzing their expression pattern, spatial distribution, and functional states. Our findings provide a deeper understanding of the intratumoral heterogeneity in NKTCL, decipher the intricate TME involved in immune evasion and provide new insights into the development of potential targets for immunotherapeutic intervention.

RESULTS

Cell populations and transcriptomic profiles in natural killer/T cell lymphoma tumors versus control tissues

To comprehensively characterize the cellular heterogeneity of NKTCL tumors, we performed single-cell RNA sequencing on tumor specimens from 10 patients with primary NKTCL and from two of them with post-treatment paired specimens (Figure 1A). The clinical and histopathological characteristics of these patients are shown in Table S1. In addition, 7 non-malignant nasopharyngeal tissue samples were also analyzed as controls.¹⁸ After stringent quality control (see STAR Methods), a total of 96369 cells were retained for subsequent analysis, including 66,873 cells from NKTCL tissues and 29,496 cells from control tissues. Based on the expression of canonical marker genes, we identified eight major cell types (e.g., epithelial cells, fibroblasts, endothelial cells, myeloid cells, T and B lymphocytes, NK/malignant cells, and plasma cells) and a group of cells with proliferative characteristics (cycling cells) (Figures 1B, 1E, and 1F). These cell types were consistently observed across different tissue origins and individual patients (Figures 1C and 1D; Figure S1A). The proportions of myeloid, B cell, and T cell differed significantly between NKTCL tissues and control tissues, with NKTCL tissues

exhibited a higher proportion of myeloid cells and a lower proportion of B cells and T cells (Figure 1G). In addition, diagnostic markers (CD56, CD3e, CD2, and granzyme B)¹⁹ and EBV-encoded genes were highly expressed in NK/malignant cells (Figures S1B and S1C), consistent with the clinicopathological features and EBV-associated characteristics of NKTCL.¹⁷

Intra-tumoral heterogeneity reveals the activation of MYC signaling associated with poor prognosis in meta-program3 subpopulations

To distinguish malignant from non-malignant cells, large-scale chromosomal copy number variations (CNVs) were inferred from transcriptome sequencing data. Malignant cells were defined as exhibiting evident CNVs, and the CNV patterns of malignant cells showed a high degree of concordance with those generated from paired whole-exome sequencing data (Figure 2A). Notably, deletions at chromosome 6q21, a region previously implicated in NKTCL,^{11,20} were observed in several samples (Figure 2A). In total, we identified 14,658 malignant cells from seven patients, each containing at least 200 malignant cells, which were then used for further analysis.

To characterize intratumoral heterogeneity in NKTCL, consensus non-negative matrix factorization (cNMF) was applied to 7 tumors with abundant malignant cells to elucidate potential programs. 37 intra-tumoral programs were generated, and the Pearson correlation coefficient was used to classify these programs into five “meta-programs” (MPs), which reflected common features of malignant cells (Figure 2B). These MPs existed in multiple samples and showed distinct functional pathway annotated by the 50 top-ranking genes (Table S2), including G2M checkpoints (MP1: *CCNB1*, *CDC20*, and *TPX2*), E2F targets (MP2: *HIST1H4C*, *HIST1H1D* and *NUSAP1*), MYC targeted V1 (MP3: *NPM1*, *FABP5* and *PTMA*) (Figures 2B and 2C). Notably, the hyperactivation of MYC (MP3) was associated with worse prognosis (MP3: HR 3.71, $p = 0.022$) (Figure 2D; Figure S2A), as determined by evaluating the top 50 signature genes of each MP in an independent cohort of 97 bulk RNA-seq datasets.¹³ Interestingly, MP5 highly expresses *NKG7*, *CCL5*, and *GZMK*, and was enriched in the TNF- α via NF- κ B pathway (Figures 2B and 2C). MP4, which expresses *MACF1*, and *NKTR*, *GOLGA4*, showed no significant enrichment in hallmark pathways (Figures 2B and 2C). These cells are likely damaged and will not perform extensive analysis. These results indicate that malignant cells exhibit a high degree of intra-tumor heterogeneity.

Figure 1. Single-Cell profiling from NKTCL and control tissues

- Workflow diagram shows the collection and processing of NKTCL tumors and control tissues.
- Uniform manifold approximation and projection (UMAP) plot of malignant and control tissue cells from 19 samples profiled in this study, colored by broad cell type.
- The UMAP plot of malignant and control tissue cells from 19 samples profiled in this study, colored by broad control tissue, primary tumor, and post-treatment tumor.
- The UMAP plot of malignant and control tissue cells from 19 samples profiled in this study, colored by broad patient.
- Dot plot of mean expression of canonical marker genes for nine major cell lineages from all samples. The dot size represents the percentage of cells expressing the genes, and the color represents the mean expression of marker genes in each cell type.
- UMAP plot shows the expression of selected marker genes for defined cell types. Red indicates high gene expression, while gray represents low gene expression.
- Boxplot shows the fraction of cell types in control (red) and tumor (blue) tissues. Box middle lines, median; box limits, 25th to 75th percentiles; box whiskers, 1.5 $\times$ the interquartile range. Comparison was made using the Wilcoxon rank-sum test.

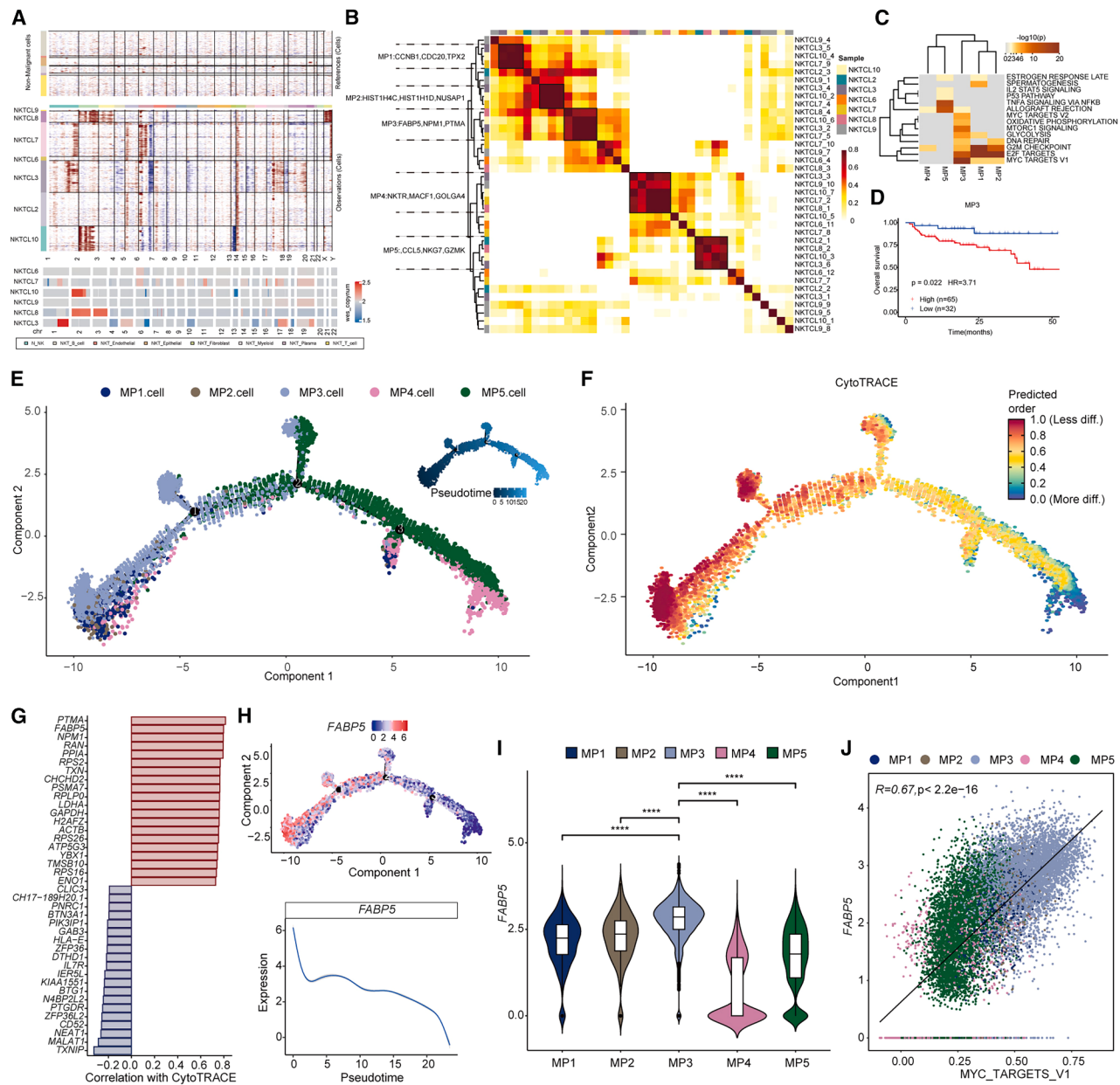


Figure 2. Intra-tumoral heterogeneity indicates MYC signaling activation linked to poor prognosis in MP3

(A) The chromosomal landscape of inferred large-scale CNVs. The inferred single-cell CNVs (top) for 7 samples are shown, with single cells (rows) and chromosomal regions (columns); red indicates CNV gains, and blue indicates CNV losses. The WGS CNVs (bottom) for 6 samples were consistent with single-cell inference results.

(B) A heatmap depicts the pairwise correlations of 37 intra-tumoral programs derived from 7 representative tumors using cNMF. 5 coherent expression programs across patients were identified.

(C) Heatmap shows pathway enrichment of 5 meta-programs analyzed using metascape.

(D) Overall survival of patients with the high expression of the gene signatures (top 50 genes) of MP3. P-value was calculated by the log rank test.

(E) Monocle2 pseudotime trajectory analysis of malignant cells with differential genes. Each dot on the pseudotime curve represents one single cell and is colored according to its meta-programs label.

(F) Pseudotime describes the distribution of CytoTRACE scores in tumor cells. Dark green indicates a low score (high differentiation) and dark red indicates a high score (low differentiation).

(G) Bar plot shows the top 20 less differentiated (red) and more differentiated (blue) genes correlated with CytoTRACE.

(H) Two-dimensional plots show the dynamic expression of *FABP5* (top panel), along with the pseudotime (below panel).

(legend continued on next page)

To further investigate the developmental trajectory and differentiation status of malignant cells, each malignant cell was classified into different MP subgroups based on the expression score of its corresponding top 50 signature genes (See [STAR Methods](#)). Pseudotime analysis revealed a continuous differentiation trajectory, beginning with a high-proliferative state and transitioning toward phenotypes associated with cytotoxicity ([Figures 2C and 2E](#)). CytoTRACE scores indicated that cells at the origin of the trajectory were less differentiated, while those at the terminus were more differentiated ([Figure 2F](#)), suggesting that MP3 cells are poorly differentiated, whereas MP5 cells are more differentiated.

In the progression of lymphoma, the reprogramming of metabolic pathways plays an essential role.²¹ Metabolic pathway enrichment showed that MP3 cells exhibit higher levels of fatty acid metabolism, which is critical for driving cancer progression and contributes to an immunosuppressive tumor microenvironment²² ([Figure S2B](#)). Fatty acid-binding protein 5 (*FABP5*), a lipid metabolism-related gene, demonstrated a strong correlation with a lower degree of differentiation ([Figure 2G](#)). Its expression decreased along the differentiation trajectory ([Figure 2H](#)) and was significantly elevated in MP3 cells compared to other cells ([Figure 2I](#)). Furthermore, a positive correlation between *FABP5* expression and the MYC-targeted V1 signaling in malignant NKTCL cells was observed, especially in MP3 cells ($R = 0.67$, $p < 0.001$; [Figure 2J](#)), suggesting a potential regulatory relationship between *FABP5* and MYC signaling in NKTCL tumor cells.

Previous studies have indicated that dysregulated lipid metabolism can impair NK cell function.²³ Consistently, gene expression analysis demonstrated elevated *FABP5* levels in malignant cells ([Figure S2C](#)), and immunohistochemical staining confirmed *FABP5* overexpression in NKTCL patient tissues compared to healthy controls ([Figure S2D](#)). These findings suggest that aberrant fatty acid metabolism is a hallmark of highly proliferative, poorly differentiated malignant cells in NKTCL.

Fatty acid-binding protein 5 inhibition downregulates MYC signaling and suppresses tumor cell proliferation

To further validate the functional role of *FABP5*, we overexpressed *FABP5* in YT cell lines, resulting in a marked increase in cell proliferation relative to vector controls (VC) ([Figure 3A](#)). Conversely, the pharmacologic inhibition of *FABP5* with the selective *FABP5* inhibitor SBFI-26²⁴ suppressed cell growth in a dose-dependent manner ([Figure 3B](#); [Figure S3A](#)), suggesting that *FABP5* promotes the proliferative capacity of NKTCL cells. Consistently, the *in vivo* administration of SBFI-26 significantly attenuated tumor progression in a YT xenograft model, as evidenced by reduced tumor volume and weight ([Figure 3C](#)). Histological analysis further confirmed that SBFI-26 treatment reduced Ki-67 positivity and enhanced apoptosis, as shown by increased cleaved caspase-3 staining ([Figure 3D](#)). Importantly, c-Myc protein levels were also decreased following either phar-

macological inhibition or genetic knockdown of *FABP5* ([Figures 3E and 3F](#); [Figure S3B](#)). Importantly, co-immunoprecipitation assays revealed a physical association between *FABP5* and c-Myc proteins ([Figure 3G](#)), suggesting that *FABP5* may modulate MYC signaling through a post-translational mechanism. These findings support the notion that *FABP5* acts upstream of c-Myc, stabilizing its expression in NKTCL cells.

Together, these results establish *FABP5* as a critical regulator of NKTCL proliferation and tumor growth, at least in part through maintaining the stability of c-Myc, consistent with the previous report.²⁴ Thus, targeting *FABP5* may represent a promising therapeutic strategy in MYC-driven NKTCL.

Tumor-associated macrophages (TAMs) accumulate within TME and promote tumor evasion

Compared with control tissues, NKTCL samples were enriched with a large proportion of myeloid cells ([Figure 1G](#)). A total of 8030 myeloid cells were clustered into ten subclusters according to specific signature markers, including mast cells (*TPSB2*, *TPSAB1*), two clusters for monocytes (*S100A9* and *RETN*), three for macrophages (*APOE*, *C1QA* and *CXCL10*), two for DCs (*XCR1* and *CD1C*), plasmacytoid dendritic cells (pDC) (*LILRA4*, *GZMB*) and neutrophils (*CXCL8*, *CXCL2*), which derived from multiple samples ([Figures 4A and 4B](#); [Figure S4A](#)). Among them, the two macrophage subpopulations expressing high levels of *APOE* (Macro_ *APOE*) and *CXCL10* (Macro_ *CXCL10*), respectively, were more abundant in tumor tissue ([Figure 4C](#)) and accounted for a larger proportion within the myeloid ([Figure S4B](#)). These findings suggest a significant myeloid cell expansion within the tumor microenvironment, similar to observations in murine models where EBV-encoded latent membrane protein 1 (*LMP1*) expression induces myeloid propagation via interferon- γ signaling.²⁵ With the treatment advancing, a significant reduction in the ratio of Macro_ *APOE* and Macro_ *CXCL10* was observed ([Figure S4C](#)). Differential gene expression analysis revealed significant differences between these macrophage subsets. Macro_ *APOE* cluster expressed high levels of *CD163*, *MSR1* and matrix metalloproteinase-9 (*MMP9*), involved in anti-inflammatory response and angiogenesis ([Figures S4D and S4E](#); [Table S3](#)), and was more prone to the M2 phenotype ([Figure 4D](#)). In contrast, Macro_ *CXCL10* expressed interferon-stimulated genes, such as guanylate-binding proteins (*GBPs*), interferon induced transmembrane protein 3 (*IFITM3*), and indoleamine 2,3-dioxygenase 1 (*IDO1*), involved in interferon-related signaling ([Figures S4D and S4E](#); [Table S3](#)), and was toward the M1 phenotype ([Figure 4D](#)). Partition-based graph abstraction (PAGA) analysis suggested that monocytes differentiate into Macro_ *CXCL10* and Macro_ *C1QA*, which can further transition into the pro-tumorigenic Macro_ *APOE* subtype ([Figure 4E](#)), indicating a phenotypic shift from an anti-tumorigenic to a protumorigenic state. These findings align with recent observations that high infiltration of CD68⁺ TAMs is correlated with poor

(I) Violin plot depicts the expression levels of *FABP5* across five subtypes of MP tumor cells. Statistical significance was determined using the Wilcoxon rank-sum test. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

(J) Scatterplot shows the correlation between *FABP5* expression and MYC_TARGETS_V1 gene set score in MP tumor cells. Each dot represents an individual tumor cell, colored according to its assigned MP subtype (MP1–MP5). A significant positive correlation was observed (Pearson correlation coefficient $R = 0.67$, $p < 2.2 \times 10^{-16}$). The dashed line represents the linear regression fit.

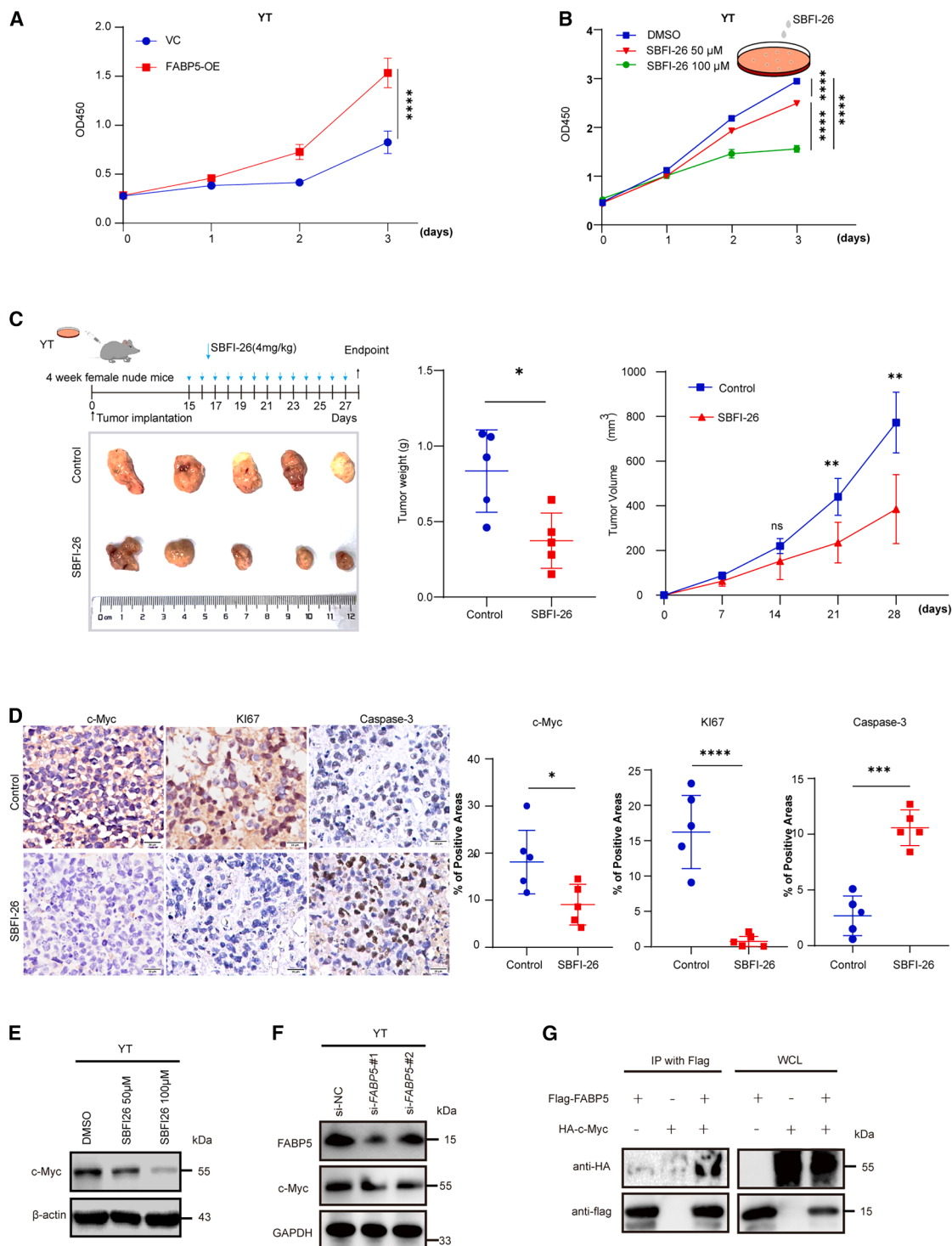


Figure 3. FABP5 affects NK/T cell lymphoma cell proliferation and tumor growth through c-Myc signaling

(A) Cell proliferation of YT cells overexpressing FABP5 or vector control (VC) was assessed by CCK-8 assay. Data are presented as mean $\pm$ SD. Statistical significance was determined by two-way ANOVA. **** p < 0.0001.

(B) YT cells were treated with SBFI-26 (50 μ M or 100 μ M) or DMSO control. Proliferation was measured by CCK-8 assay. Data represent mean $\pm$ SD, **** p < 0.0001 by two-way ANOVA.

(C) Nude mice were subcutaneously injected with YT cells to establish xenografts, followed by daily intraperitoneal administration of SBFI-26 (4 mg/kg) or corn oil vehicle (n = 5/group). Representative images of harvested tumors, tumor weights at the study endpoint, and tumor growth curves are presented. Tumor volumes

(legend continued on next page)

prognosis in extranodal NK/T cell lymphoma, nasal type.²⁶ This correlation suggests that macrophage polarization may play a critical role in tumor progression. In addition, high levels of immune checkpoint molecules were observed in myeloid cell subsets (Figure 4F). Macro_APOE expressed high levels of *SIRPA* and *VSIG4*. *VSIG4* was previously reported to inhibit the activation of pro-inflammatory macrophages and T cells.^{27,28} Further, higher levels of Macro_APOE were associated with poorer survival in patients with NKTCL (HR 2.63, $p = 0.044$) (Figure 4G). Next, the interactions were assessed between tumor and myeloid subsets (Figure 4H). Macro_APOE show a strong ligand receptor response with tumor cells, for example, SPP1-CD44, SIRP α -CD47. SPP1-CD44 interactions were reported to drive chemoresistance in triple-negative breast cancer.²⁹ The SIRP α -CD47 serves as a pivotal checkpoint in the evasion of tumor immunity, effectively dampening the macrophages' phagocytic capabilities and enabling tumor cells to circumvent immunological detection.^{30,31}

In addition, B cells and T cells were sub-clustered (Figures S5A–S5H; Tables S4, S5, and S6) and there was a significant increase in the proportions of CD8_LAG3 compared to the control tissue (Figure S5E). Furthermore, a significant positive correlation was observed between Macro_APOE score and CD8_LAG3 exhausted score in bulk RNA data,¹³ which suggested a potential immunosuppressive regulation (Figure 4I). Ligand-receptor analysis showed the SPP1-CD44 axis, which suppresses the activation of memory T cells,³² and Galectin-9/TIM-3 (LGALS9-HAVCR2) signaling, which regulates T cell apoptosis³³ (Figure S4F).

In summary, these analyses underscored the potential of the key ligand-receptor pair to impede the phagocytic capability of TAMs against tumor cells, and indicated that TAMs may regulate T cell activity, thereby promoting the immune evasion of tumor cells.

The spatial composition and the interrelationship between tumors and immunity in natural killer/T cell lymphoma

To further elucidate the spatial interplay between malignant and immune cells, spatially resolved transcriptomic analysis was performed on three primary NKTCL samples utilizing Stereo-seq technology.³⁴ After quality control, the SPOTlight³⁵ was used to deconvolute the cell-type composition for the remaining 27,129 bin50 spots (50 × 50 DNB bins, 25 μ m diameter). The spots were annotated as MP1-5 malignant cells, T cells, B cells, plasma cells, myeloid cells, fibroblasts, endothelial and epithelial cells, which localized to specific regional compartments within the tissue (Figure 5A; Figures S6A and S6C). Dotplots incorporating specific genes from scRNA-seq for each section, showed high consistency with the spotlight mapping results (Figure 5B;

Figures S6B and S6D). The MP3 tumors exhibited the high proportion among the three sections and were notably characterized by higher MYC target V1 and fatty acid metabolism pathways (Figure 5C; Figures S6E and S6F). *FABP5* showed a similar distribution pattern with MP3 tumor cells (Figure 5D; Figures S6G and S6H).

The spatial distribution of tumor cells and the immune microenvironment exhibited distinct patterns, which may facilitate tumor immune evasion. By conducting cell neighborhood (CN) analysis based on the deconvolution-derived cell type proportions, six neighborhoods (CN0–CN5) were identified (Figure 5E; Figures S6I and S6J), highlighting the spatial architecture of the NKTCL tumor microenvironment. The results showed that CN1 and CN2 were enriched with tumor cells, myeloid cells, and stromal cells, and CN5 was characterized by MP3 tumor cells and B cells. In addition, CN4 was dominated by epithelial cells, reflecting stromal-dominated regions. Notably, CN0 and CN3 exhibited high proportions of MP3 tumor cells and myeloid cells, implying potential interactions between tumor cells and myeloid cells (Figure 5F). The spatial distribution of Macro_APOE was found to correspond to myeloid cells, consistent with single-cell analysis showing Macro_APOE as the predominant myeloid subsets (Figures S4B and S6K). This indicates that tumor cells predominantly interact spatially with Macro_APOE. Compared to MP3 tumor cells in CN0, those in CN3 exhibited higher cytotoxicity features, indicating a potential inclination for the MP5-like differentiation of MP3 tumor cells in CN3 (Figure 5G). Interestingly, both Macro_APOE and CD8_LAG3 scores were significantly higher in CN0 and CN3 compared to other neighborhoods (Figure 5H). Dot plots analysis further highlighted the upregulated expression of *CD47*, *SIRPA*, *CCL5*, and *CCR1* in CN0 and CN3 (Figure 5I). The widespread expression of the SIRP α -CD47 and CCL5-CCR1 ligand-receptor pairs in three samples suggests that tumor cells recruit Macro_APOE through CCL5 secretion and interact with Macro_APOE via SIRP α -CD47, establishing an immunosuppressive microenvironment³⁶ (Figure S7A). This underscores the critical role of SIRP α -CD47 interaction in tumor immune evasion.

In conclusion, spatial transcriptome data analysis showed that highly activated tumor areas and tumor metastasis areas had unique spatial distribution patterns and immune microenvironments. These features and the potential interactions between malignant and immune cells are crucial for the development and immune escape of malignant cells.

DISCUSSION

Natural killer/T cell lymphoma (NKTCL) is a highly aggressive malignancy characterized by marked heterogeneity and limited therapeutic options. Leveraging single-cell and spatial

and Tumor weights were compared by two-tailed Student's t test (** $p < 0.01$ on day 28). Error bars show the mean $\pm$ SD, ns, not significant; * $p < 0.05$; ** $p < 0.01$ by two-tailed Student's t test.

(D) Immunohistochemical staining of Ki67, Caspase-3, and c-Myc expression in tumor tissues from mice treated with SBFI-26 or PBS control. Bar graphs indicate the quantification of positive staining areas. Scale bars, 20 μ m. Error bars show the mean $\pm$ SD, * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$ by two-tailed Student's t test.

(E) Western blot analysis of c-Myc protein levels in YT cells treated with SBFI-26.

(F) Western blot analysis of c-Myc expression in YT cells following FABP5 knockdown by two independent siRNAs (si-FABP5#1 and si-FABP5#2).

(G) Co-immunoprecipitation analysis of HEK293T cells co-transfected with Flag-FABP5 and HA-c-Myc plasmids. All cell experiments were repeated three times.

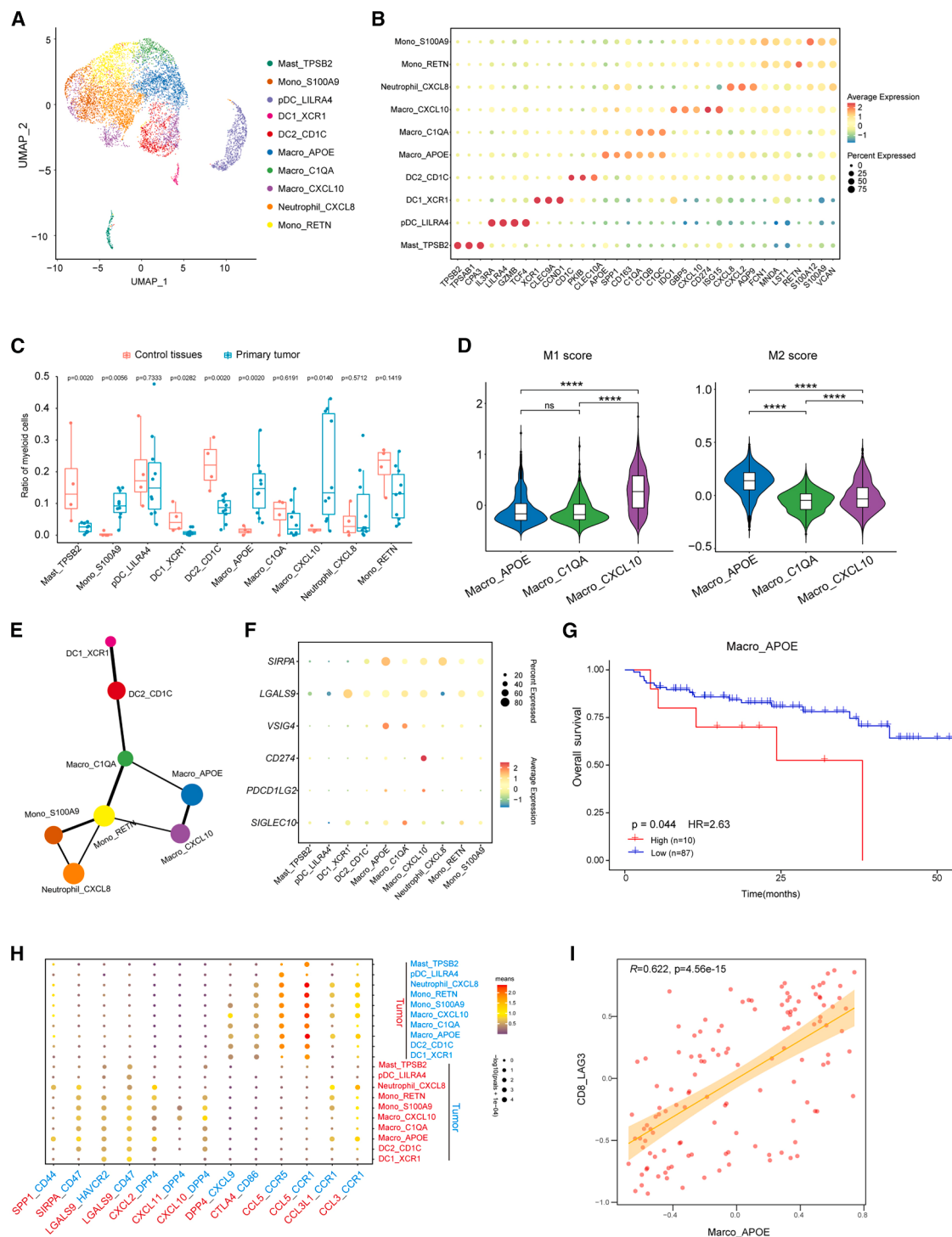


Figure 4. Tumor-associated macrophages (TAMs) aggregate within the TME and facilitate tumor evasion

(A) UMAP plot of the 8,030 myeloid cells landscape colored by subcluster. Cell type annotations are provided in the figure.

(B) Dot plot shows the expression level of marker genes in each myeloid subset. The dot size represents the percentage of cells expressing the genes, and the color represents the mean expression of marker genes in each cell type.

(C) Boxplot shows the fraction of cell subtypes of myeloid cells in control tissue (red) and primary tumor (blue). Box middle lines, median; box limits, 25th to 75th percentiles; box whiskers, 1.5× the interquartile range. Comparison was made using the Wilcoxon rank-sum test.

(legend continued on next page)

transcriptomics, our study provides a comprehensive analysis of the intratumoral cellular architecture, spatial distribution, and microenvironmental interactions in NKTCL. We identified five distinct malignant subpopulations with significant heterogeneity, elucidating the differentiation trajectory and progressive features within the tumor. Additionally, we observed the enrichment of various myeloid subpopulations with different functions and T cell subpopulations exhibiting immuno-suppressive characteristics within the tumor tissue. The complex and dynamic interplay between these cells at different developmental stages jointly contributed to the formation of an immunosuppressive microenvironment, suggesting a potential mechanism of tumor immune evasion.

Among these five malignant subpopulations, the MP3 subpopulation, which is characterized by high proliferation, low differentiation, and abnormal activation of fatty acid metabolism, emerged as the predominant malignant state. This subpopulation showed strong MYC signaling activation and was associated with an unfavorable prognosis. Importantly, *FABP5* was significantly upregulated in this key malignant subpopulation and was shown to be correlated with MYC signaling pathway activation. *FABP5* inhibition led to a decrease in c-Myc protein abundance, suggesting that *FABP5* may contribute to the stabilization or functional dependency of c-Myc rather than its transcriptional regulation. These findings align with previous reports in prostate, breast, and lung cancers,^{37–39} where *FABP5* was shown to support oncogenic signaling and metabolic reprogramming. Given that MYC lacks enzymatically active domains, which limits direct therapeutic targeting,⁴⁰ *FABP5* may represent a viable and druggable surrogate node in MYC-driven NKTCL.

Our pharmacological data demonstrated that the inhibition of *FABP5* by SBFI-26 not only impaired proliferation and induced apoptosis *in vitro* but also significantly suppressed tumor growth *in vivo*. These findings support the use of *FABP5* as a potential therapeutic target in NKTCL. However, further studies with larger cohorts are warranted to validate the efficacy and safety of *FABP5* inhibition in clinical settings.

Our single-cell and spatial analyses also unveiled an immunologically complex TME, enriched with diverse myeloid subsets and CD8⁺ T cells. Cell interaction analysis identified multiple chemokine-associated ligand-receptor interactions (CCL5-CCR5, CCL5-CCR1) between malignant cells and immune cells, indicating that these interactions are crucial for shaping the tumor microenvironment, recruiting immune cells,

and modulating tumor immune responses.⁴¹ Additionally, M2-like macrophages (Macro_APOE) may interact with malignant cells through SIRP α -CD47 signals. Malignant cells may transmit a “don’t eat me” signal to macrophages via this signaling pathway, reducing phagocytosis and other immune responses, thus leading to immune evasion.^{42,43} Furthermore, the interaction between malignant cells and Macro_APOE was validated within the spatial neighborhood. Blocking macrophage-associated checkpoints such as CD47-SIRP α or interfering with chemokine signaling may offer novel immunotherapeutic strategies. Besides, the infiltration levels of Macro_APOE cells were positively correlated with immunosuppressive T cells, suggesting that macrophages mediate malignant cell escape through complex interactions with malignant cells and lymphocytes.

In conclusion, our findings delineate the cellular heterogeneity and spatial organization of NKTCL tumors, identify *FABP5* as a critical regulator of MYC-driven proliferation, and highlight macrophage-mediated immune evasion as a key feature of the TME. These insights provide a foundation for the development of more effective molecular and immunotherapeutic interventions for NKTCL.

Limitations of the study

This study has several limitations. First, the relatively small sample size restricts generalizability, so expanded patient cohorts are needed to confirm the consistency of the identified malignant subpopulations and their interactions. Second, owing to the limited biopsy material from nasopharyngeal lesions, the functional validation of specific cell-cell interactions within the TME is challenging. In the future, integrating EBV-specific molecular profiling with *in vivo* models that have sufficient immune components, including *FABP5* and CD47-SIRP α inhibitors, will be crucial. This integration can help explore how EBV shapes immunosuppressive interactions, understand viral oncogenesis, and test TME-targeted therapies.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Qingqing Cai (caiqq@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

(D) Violin plots illustrate the distribution of M1 and M2 scores across different gene conditions in macrophage cells. Each plot represents the expression levels of M1_score and M2_score under the conditions of Macro_APOE, Macro_C1QA, and Macro_CXCL10. Statistical significance was determined using the Wilcoxon rank-sum test. ns, not significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001.

(E) PAGA analysis showing the potential developmental connectivity between different myeloid subsets.

(F) Dot plot shows the expression levels of selected genes across each myeloid subset. The dot size represents the percentage of cells expressing the genes, and the color represents the mean expression of marker genes in each cell type.

(G) Kaplan-Meier survival analysis based on overall survival rates at Macro_APOE characteristic gene expression levels, respectively. The P-value was calculated using the log rank test.

(H) Dot plots shows selected ligand-receptor interactions between malignant and myeloid subclusters. The ligand-receptor interactions and cell-cell interactions are indicated in columns and rows, respectively. The means of the average expression levels of two interacting molecules are indicated by a color heatmap, with purple to red representing low to high expression. The $-\log_{10}(p\text{vals}+1e-04)$ were indicated by circle size.

(I) Correlation of CD8_LAG3 exhausted score with Macro_APOE score in NKTCL from bulk RNA data. Each point represents a sample. Using Pearson correlation analysis.

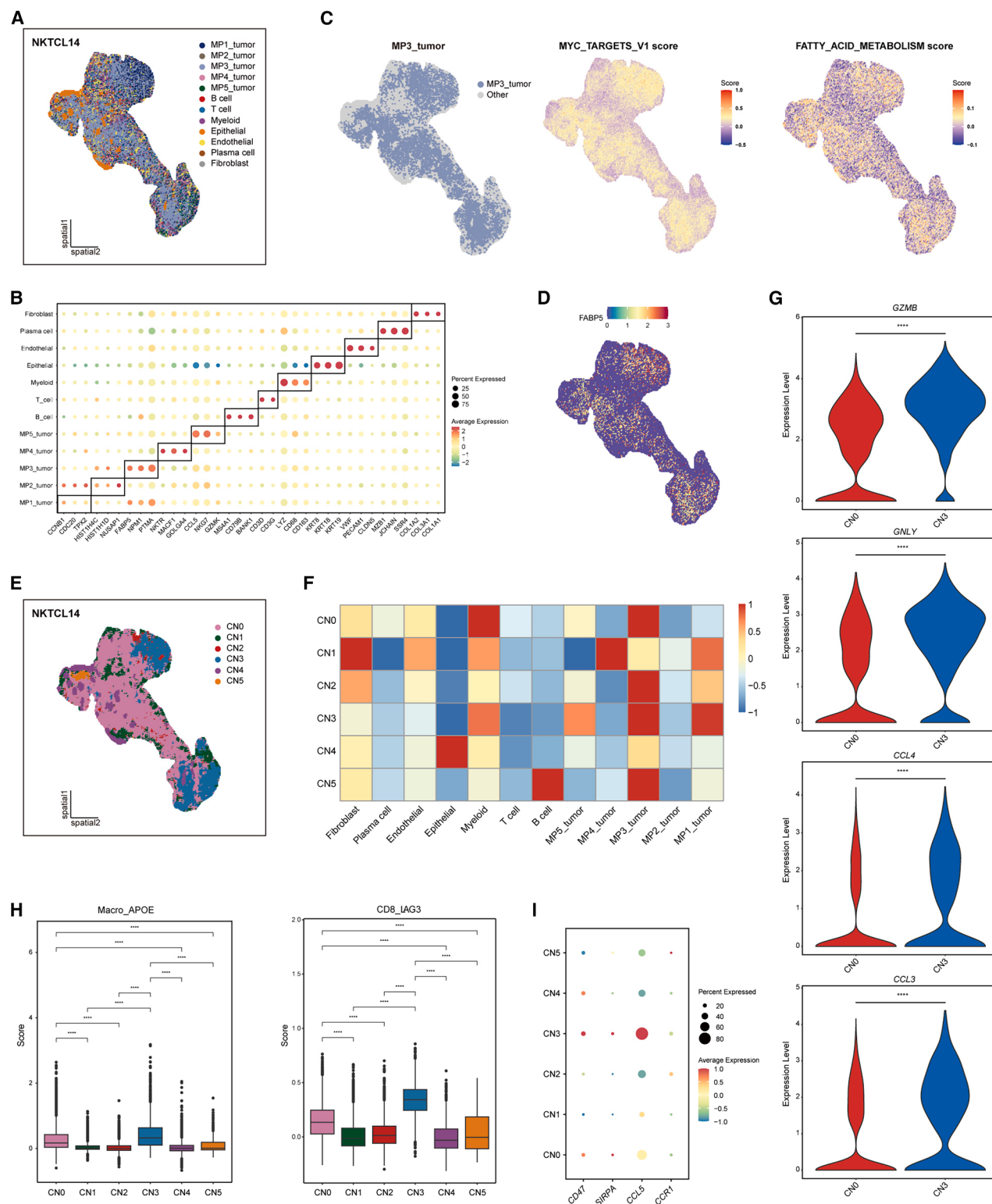


Figure 5. Cellular identification in spatial transcriptomics

(A) The SpatialDimPlot function shows the distribution of major cell types annotated by SPOTlight for NKTL14. Each spot is assigned to a specific cell type with the highest proportion.

(legend continued on next page)

Data and code availability

- The raw data from the scRNA-seq, whole exome sequencing, and spatial transcriptomics are under controlled access in designated repositories due to privacy and ethical considerations associated with human genetic information. The scRNA-seq and whole exome sequencing data are deposited in the Genome Sequence Archive for Human (GSA-Human) under accession number HRA007314 and in the CNGB Sequence Archive (CNSA)⁴⁴ of the China National GeneBank DataBase (CNGBdb)⁴⁵ under accession number CNP0005654. The spatial transcriptomics data reported in this article have been deposited in the OMIX, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (<https://ngdc.cncb.ac.cn/omix>; accession no. OMIX006113) and in STOmics DB⁴⁶ of CNGBdb⁴⁵ with accession number STT0000114. Researchers may request access through the respective data access committees, which evaluate applications based on intended use and compliance with ethical guidelines. Data are permitted for non-commercial academic research only. According to the guidelines of GSA-human, all non-profit researchers are allowed access to the data, and the Principal Investigator of any research group can apply for the data following the guidelines at the GSA database portal (<https://ngdc.cncb.ac.cn/gsa-human/>). The response time for access requests is approximately 10 working days. Once access has been granted, the data will be available for download within one month. The user can also contact the corresponding author directly for enquiries.
- This article does not report original code.
- Any additional information required to reanalyze the data reported in this article is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program (2022YFC2502602), the National Natural Science Foundation of China (82230001, 82270199 and 82400236), the Guangzhou Science and Technology Program (2024B03J1291), the Sun Yat-Sen University Clinical Research 5010 Program (2020009), the Beijing Xisike Clinical Oncology Research Foundation (Y-SY2021ZD-0110), the Hunan Provincial Natural Science Foundation of China (2023JJ40897), the Science and Technology Innovation Key R&D Program of Chongqing (CSTB2024TIAD-STX0006), the Guangdong Provincial Key Laboratory of Human Disease Genomics (2020B1212070028), the Shenzhen Key Laboratory of Single-Cell Omics (ZDSYS20190902093613831), and the China National GeneBank (CNGB). We would like to thank DCS Cloud (<https://cloud.stomics.tech/>) for providing the computational resources and software support necessary for this study.

AUTHOR CONTRIBUTIONS

Conceptualization: Q.Q.C., L.W., W.L.Z., and G.B.L.; supervision: Q.Q.C., L.W., W.L.Z., and G.B.L.; resources: S.Y.M. and J.H.W. (Human tissue acquisition, Pathology review); D.L.D., Q.Z., Y.X., P.P.L., Y.Q.Y., H.F.W., S.C.D., J.C., Q.H.Z., Y.C.Z., and L.R.L. (sample collection); methodology: S.Y.M.

and J.H.W. (sample preparation, experimental procedures); R.L. and B.J.H. (bioinformatics analysis); investigation: S.Y.M. and J.H.W. (experimental execution); R.L., B.J.H., and L.W. (data analysis); writing – original draft: All authors; writing – review and editing: all authors; project administration: Q.Q.C.; publication responsibility: Q.Q.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS**
 - Patients and tissue samples
 - Cell culture
 - Experimental animals
- **METHOD DETAILS**
 - Preparation of single-cell suspensions
 - Single-cell RNA sequencing
 - Whole-exome sequencing
 - Spatial transcriptomics
 - Identification of malignant cells
 - Transcriptomic “meta-programs” of malignant cells in NKTL tumors
 - Identification of cells infected by EBV
 - Identification of T, B and myeloid cell populations
 - Classification of CD8⁺ cells by functional state
 - Pathway enrichment analysis
 - Developmental trajectories of malignant cells, CD8⁺ T and myeloid cells in NKTL
 - Correlation of gene expression signatures with patient survival
 - Analysis of cell–cell interactions in the microenvironment of NKTL tumors
 - Xenograft tumor model
 - Cell proliferation assay
 - Immunohistochemistry (IHC)
 - Western blot analysis
 - FABP5 knockdown and overexpression
 - Co-immunoprecipitation (Co-IP)
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2025.113626>.

- (B) Dot plot shows the expression level of marker genes in major cell types. The dot size represents the percentage of cells expressing the genes, and the color represents the mean expression of marker genes in each cell type.
- (C) Spatial distribution of MP3 tumor cells (left), MYC_TARGETS_V1 gene set scores (middle), and FATTY_ACID_METABOLISM gene set scores (right) in NKTL14. The panel represents the spatial distribution of a specific gene set score, with colors indicating the score intensity (red for high, blue for low).
- (D) Spatial distribution of gene expression patterns for FABP5 in NKTL14, with colors indicating expression levels (red for high, blue for low).
- (E) Spatial distribution of cellular neighborhoods (CN0–CN5) in NKTL14. Each color represents a distinct cellular neighborhood.
- (F) Heatmap shows the major cell composition characteristics in 6 CNs.
- (G) Violin plots show the expression levels of GZMB, GNLY, CCL3, and CCL4 in MP3 tumor cells of CN0 and CN3. Each plot compares the expression distribution of the respective gene between CN0 (red) and CN3 (blue). Statistical significance was determined using the Wilcoxon rank-sum test. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
- (H) Boxplots show the score of Macro_APOE gene set (left) and CD8_LAG3 gene set (right) across different cellular neighborhoods (CN0–CN5), with statistical significance determined using the Wilcoxon rank-sum test. Box middle lines, median; box limits, 25th to 75th percentiles; box whiskers, 1.5× the interquartile range. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
- (I) Dot plot shows the expression levels of CD47, SIRPA, CCL5, and CCR1 genes in each CN. The dot size represents the percentage of cells expressing the genes, and the color represents the mean expression of marker genes in each cell type.

Received: December 19, 2024
Revised: June 2, 2025
Accepted: September 19, 2025
Published: September 22, 2025

REFERENCES

- Wang, H., Fu, B.B., Gale, R.P., and Liang, Y. (2021). NK-/T-cell lymphomas. *Leukemia* 35, 2460–2468. <https://doi.org/10.1038/s41375-021-01313-2>.
- Wang, X., Zhang, L., Liu, X., Li, X., Li, L., Fu, X., Sun, Z., Wu, J., Zhang, X., Yan, J., et al. (2022). Efficacy and Safety of a Pegasparginase-Based Chemotherapy Regimen vs an L-asparaginase-Based Chemotherapy Regimen for Newly Diagnosed Advanced Extranodal Natural Killer/T-Cell Lymphoma: A Randomized Clinical Trial. *JAMA Oncol.* 8, 1035–1041. <https://doi.org/10.1001/jamaoncol.2022.1968>.
- Zhang, Y., Ma, S., Cai, J., Yang, Y., Jing, H., Shuang, Y., Peng, Z., Li, B., Liu, P., Xia, Z., et al. (2021). Sequential P-GEMOX and radiotherapy for early-stage extranodal natural killer/T-cell lymphoma: A multicenter study. *Am. J. Hematol.* 96, 1481–1490. <https://doi.org/10.1002/ajh.26335>.
- Cai, J., Liu, P., Huang, H., Li, Y., Ma, S., Zhou, H., Tian, X., Zhang, Y., Gao, Y., Xia, Y., et al. (2020). Combination of anti-PD-1 antibody with P-GEMOX as a potentially effective immunochemotherapy for advanced natural killer/T cell lymphoma. *Signal Transduct. Target. Ther.* 5, 289. <https://doi.org/10.1038/s41392-020-00331-3>.
- Yang, Y., Zhang, Y.J., Zhu, Y., Cao, J.Z., Yuan, Z.Y., Xu, L.M., Wu, J.X., Wang, W., Wu, T., Lu, B., et al. (2015). Prognostic nomogram for overall survival in previously untreated patients with extranodal NK/T-cell lymphoma, nasal-type: a multicenter study. *Leukemia* 29, 1571–1577. <https://doi.org/10.1038/leu.2015.44>.
- Kim, S.J., Lim, J.Q., Laurensia, Y., Cho, J., Yoon, S.E., Lee, J.Y., Ryu, K.J., Ko, Y.H., Koh, Y., Cho, D., et al. (2020). Avelumab for the treatment of relapsed or refractory extranodal NK/T-cell lymphoma: an open-label phase 2 study. *Blood* 136, 2754–2763. <https://doi.org/10.1182/blood.2020007247>.
- Li, X., Cheng, Y., Zhang, M., Yan, J., Li, L., Fu, X., Zhang, X., Chang, Y., Sun, Z., Yu, H., et al. (2018). Activity of pembrolizumab in relapsed/refractory NK/T-cell lymphoma. *J. Hematol. Oncol.* 11, 15. <https://doi.org/10.1186/s13045-018-0559-7>.
- Xiong, J., Cheng, S., Gao, X., Yu, S.H., Dai, Y.T., Huang, X.Y., Zhong, H.J., Wang, C.F., Yi, H.M., Zhang, H., et al. (2024). Anti-metabolic agent pegaspargase plus PD-1 antibody sintilimab for first-line treatment in advanced natural killer T cell lymphoma. *Signal Transduct. Target. Ther.* 9, 62. <https://doi.org/10.1038/s41392-024-01782-8>.
- Tian, X.P., Cai, J., Xia, Y., Zhang, Y.C., Wang, L., Liu, P.P., Huang, H.Q., Li, Y.J., Zhou, H., Li, Z.M., et al. (2024). First-line sintilimab with pegaspargase, gemcitabine, and oxaliplatin in advanced extranodal natural killer/T cell lymphoma (SPIRIT): a multicentre, single-arm, phase 2 trial. *Lancet. Haematol.* 11, e336–e344. [https://doi.org/10.1016/s2352-3026\(24\)00066-8](https://doi.org/10.1016/s2352-3026(24)00066-8).
- Liao, Z., Lv, X., Liu, S., He, Z., Chen, S., Wang, L., Li, W., and Li, Y. (2018). Different aberrant expression pattern of immune checkpoint receptors in patients with PTCL and NK/T-CL. *Asia Pac. J. Clin. Oncol.* 14, e252–e258. <https://doi.org/10.1111/ajco.12850>.
- Huang, Y., de Reyniès, A., de Leval, L., Ghazi, B., Martin-Garcia, N., Traver, M., Bosq, J., Brière, J., Petit, B., Thomas, E., et al. (2010). Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. *Blood* 115, 1226–1237. <https://doi.org/10.1182/blood-2009-05-221275>.
- Mundy-Bosse, B.L., Weigel, C., Wu, Y.Z., Abdelbaky, S., Youssef, Y., Casas, S.B., Polley, N., Ernst, G., Young, K.A., McConnell, K.K., et al. (2022). Identification and Targeting of the Developmental Blockade in Extranodal Natural Killer/T-cell Lymphoma. *Blood Cancer Discov.* 3, 154–169. <https://doi.org/10.1158/2643-3230.Bcd-21-0098>.
- Xiong, J., Cui, B.W., Wang, N., Dai, Y.T., Zhang, H., Wang, C.F., Zhong, H.J., Cheng, S., Ou-Yang, B.S., Hu, Y., et al. (2020). Genomic and Transcriptomic Characterization of Natural Killer T Cell Lymphoma. *Cancer Cell* 37, 403–419.e6. <https://doi.org/10.1016/j.ccell.2020.02.005>.
- Chen, Z., Huang, H., Hong, H., Huang, H., Weng, H., Yu, L., Xiao, J., Wang, Z., Fang, X., Yao, Y., et al. (2024). Full-spectral genome analysis of natural killer/T cell lymphoma highlights impacts of genome instability in driving its progression. *Genome Med.* 16, 48. <https://doi.org/10.1186/s13073-024-01324-5>.
- Lin, G.W., Xu, C., Chen, K., Huang, H.Q., Chen, J., Song, B., Chan, J.K.C., Li, W., Liu, W., Shih, L.Y., et al. (2020). Genetic risk of extranodal natural killer T-cell lymphoma: a genome-wide association study in multiple populations. *Lancet Oncol.* 21, 306–316. [https://doi.org/10.1016/s1470-2045\(19\)30799-5](https://doi.org/10.1016/s1470-2045(19)30799-5).
- Xiong, J., Dai, Y.T., Wang, W.F., Zhang, H., Wang, C.F., Yin, T., Cheng, S., Zhong, H.J., Yu, S.H., Jiang, L., et al. (2023). GPCR signaling contributes to immune characteristics of microenvironment and process of EBV-induced lymphomagenesis. *Sci. Bull.* 68, 2607–2619. <https://doi.org/10.1016/j.scib.2023.09.029>.
- Li, Y.Q., Luo, C.L., Jiang, J.X., He, S., Liu, Y., Yan, W.X., Xia, Y., Cui, Q., Huang, Y., Lim, J.Q., et al. (2023). Single-Cell Analysis Reveals Malignant Cells Reshape the Cellular Landscape and Foster an Immunosuppressive Microenvironment of Extranodal NK/T-Cell Lymphoma. *Adv. Sci.* 10, e2303913. <https://doi.org/10.1002/adv.202303913>.
- Jin, S., Li, R., Chen, M.Y., Yu, C., Tang, L.Q., Liu, Y.M., Li, J.P., Liu, Y.N., Luo, Y.L., Zhao, Y., et al. (2020). Single-cell transcriptomic analysis defines the interplay between tumor cells, viral infection, and the microenvironment in nasopharyngeal carcinoma. *Cell Res.* 30, 950–965. <https://doi.org/10.1038/s41422-020-00402-8>.
- Tse, E., and Kwong, Y.L. (2016). Diagnosis and management of extranodal NK/T cell lymphoma nasal type. *Expert Rev. Hematol.* 9, 861–871. <https://doi.org/10.1080/17474086.2016.1206465>.
- Tse, E., and Kwong, Y.L. (2017). The diagnosis and management of NK/T-cell lymphomas. *J. Hematol. Oncol.* 10, 85. <https://doi.org/10.1186/s13045-017-0452-9>.
- Song, W., Gao, Y., Wu, J., Li, H., Shi, Z., Gong, C., Zhang, Z., Li, Z., and Zhang, M. (2024). LMP1 enhances aerobic glycolysis in natural killer/T cell lymphoma. *Cell Death Dis.* 15, 604. <https://doi.org/10.1038/s41419-024-06999-7>.
- Koundouros, N., and Poulogiannis, G. (2020). Reprogramming of fatty acid metabolism in cancer. *Br. J. Cancer* 122, 4–22. <https://doi.org/10.1038/s41416-019-0650-z>.
- Kobayashi, T., Lam, P.Y., Jiang, H., Bednarska, K., Gloury, R., Murigneux, V., Tay, J., Jacquelinot, N., Li, R., Tuong, Z.K., et al. (2020). Increased lipid metabolism impairs NK cell function and mediates adaptation to the lymphoma environment. *Blood* 136, 3004–3017. <https://doi.org/10.1182/blood.2020005602>.
- Farrell, M., Fairfield, H., Karam, M., D'Amico, A., Murphy, C.S., Falank, C., Pistofidi, R.S., Cao, A., Marinac, C.R., Dragon, J.A., et al. (2023). Targeting the fatty acid binding proteins disrupts multiple myeloma cell cycle progression and MYC signaling. *eLife* 12, e81184. <https://doi.org/10.7554/eLife.81184>.
- Koya, J., Tanigawa, T., Mizuno, K., Kim, H., Ito, Y., Yuasa, M., Yamaguchi, K., Kogure, Y., Saito, Y., Shingaki, S., et al. (2024). Modeling NK-cell lymphoma in mice reveals its cell-of-origin and microenvironmental changes and identifies therapeutic targets. *Nat. Commun.* 15, 9106. <https://doi.org/10.1038/s41467-024-53376-1>.
- Wang, H., Li, P., Wang, L., Xia, Z., Huang, H., Lu, Y., and Li, Z. (2015). High numbers of CD68+ tumor-associated macrophages correlate with poor prognosis in extranodal NK/T-cell lymphoma, nasal type. *Ann. Hematol.* 94, 1535–1544. <https://doi.org/10.1007/s00277-015-2401-4>.
- Vogt, L., Schmitz, N., Kurrer, M.O., Bauer, M., Hinton, H.I., Behnke, S., Gatto, D., Sebbel, P., Beerli, R.R., Sonderegger, I., et al. (2006). VSIG4,

- a B7 family-related protein, is a negative regulator of T cell activation. *J. Clin. Investig.* 116, 2817–2826. <https://doi.org/10.1172/jci25673>.
28. Li, J., Diao, B., Guo, S., Huang, X., Yang, C., Feng, Z., Yan, W., Ning, Q., Zheng, L., Chen, Y., and Wu, Y. (2017). VSIG4 inhibits proinflammatory macrophage activation by reprogramming mitochondrial pyruvate metabolism. *Nat. Commun.* 8, 1322. <https://doi.org/10.1038/s41467-017-01327-4>.
 29. Liu, F., Zhang, J., Gu, X., Guo, Q., and Guo, W. (2024). Single-cell transcriptome sequencing reveals SPP1-CD44-mediated macrophage-tumor cell interactions drive chemoresistance in TNBC. *J. Cell Mol. Med.* 28, e18525. <https://doi.org/10.1111/jcmm.18525>.
 30. van den Berg, T.K., and van der Schoot, C.E. (2008). Innate immune ‘self’ recognition: a role for CD47-SIRPα interactions in hematopoietic stem cell transplantation. *Trends Immunol.* 29, 203–206. <https://doi.org/10.1016/j.it.2008.02.004>.
 31. Barclay, A.N., and Brown, M.H. (2006). The SIRP family of receptors and immune regulation. *Nat. Rev. Immunol.* 6, 457–464. <https://doi.org/10.1038/nri1859>.
 32. Klement, J.D., Paschall, A.V., Redd, P.S., Ibrahim, M.L., Lu, C., Yang, D., Celis, E., Abrams, S.I., Ozato, K., and Liu, K. (2018). An osteopontin/CD44 immune checkpoint controls CD8+ T cell activation and tumor immune evasion. *J. Clin. Investig.* 128, 5549–5560. <https://doi.org/10.1172/jci123360>.
 33. Yang, R., Sun, L., Li, C.F., Wang, Y.H., Yao, J., Li, H., Yan, M., Chang, W.C., Hsu, J.M., Cha, J.H., et al. (2021). Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. *Nat. Commun.* 12, 832. <https://doi.org/10.1038/s41467-021-21099-2>.
 34. Chen, A., Liao, S., Cheng, M., Ma, K., Wu, L., Lai, Y., Qiu, X., Yang, J., Xu, J., Hao, S., et al. (2022). Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* 185, 1777–1792.e21. <https://doi.org/10.1016/j.cell.2022.04.003>.
 35. Elosua-Bayes, M., Nieto, P., Mereu, E., Gut, I., and Heyn, H. (2021). SPOT-light: seeded NMF regression to deconvolute spatial transcriptomics spots with single-cell transcriptomes. *Nucleic Acids Res.* 49, e50. <https://doi.org/10.1093/nar/gkab043>.
 36. Zimarino, C., Moody, W., Davidson, S.E., Munir, H., and Shields, J.D. (2024). Disruption of CD47-SIRPα signaling restores inflammatory function in tumor-associated myeloid-derived suppressor cells. *iScience* 27, 109546. <https://doi.org/10.1016/j.isci.2024.109546>.
 37. O’Sullivan, S.E., and Kaczocha, M. (2020). FABP5 as a novel molecular target in prostate cancer. *Drug Discov. Today*, S1359-6446(20)30375-5. <https://doi.org/10.1016/j.drudis.2020.09.018>.
 38. Chen, J., Alduais, Y., Zhang, K., Zhu, X., and Chen, B. (2021). CCAT1/FABP5 promotes tumour progression through mediating fatty acid metabolism and stabilizing PI3K/AKT/mTOR signalling in lung adenocarcinoma. *J. Cell Mol. Med.* 25, 9199–9213. <https://doi.org/10.1111/jcmm.16815>.
 39. George Warren, W., Osborn, M., Yates, A., Wright, K., and O’Sullivan, S.E. (2023). The emerging role of fatty acid binding protein 5 (FABP5) in cancers. *Drug Discov. Today* 28, 103628. <https://doi.org/10.1016/j.drudis.2023.103628>.
 40. Llombart, V., and Mansour, M.R. (2022). Therapeutic targeting of “undruggable” MYC. *EBioMedicine* 75, 103756. <https://doi.org/10.1016/j.ebiom.2021.103756>.
 41. Aldinucci, D., Borghese, C., and Casagrande, N. (2020). The CCL5/CCR5 Axis in Cancer Progression. *Cancers* 12, 1765. <https://doi.org/10.3390/cancers12071765>.
 42. Yang, H., Shao, R., Huang, H., Wang, X., Rong, Z., and Lin, Y. (2019). Engineering macrophages to phagocytose cancer cells by blocking the CD47/SIRPα axis. *Cancer Med.* 8, 4245–4253. <https://doi.org/10.1002/cam4.2332>.
 43. Li, Z., Li, Y., Gao, J., Fu, Y., Hua, P., Jing, Y., Cai, M., Wang, H., and Tong, T. (2021). The role of CD47-SIRPα immune checkpoint in tumor immune evasion and innate immunotherapy. *Life Sci.* 273, 119150. <https://doi.org/10.1016/j.lfs.2021.119150>.
 44. Guo, X., Chen, F., Gao, F., Li, L., Liu, K., You, L., Hua, C., Yang, F., Liu, W., Peng, C., et al. (2020). CNSA: a data repository for archiving omics data. Database 2020, baaa055. <https://doi.org/10.1093/database/baaa055>.
 45. Chen, F.Z., You, L.J., Yang, F., Wang, L.N., Guo, X.Q., Gao, F., Hua, C., Tan, C., Fang, L., Shan, R.Q., et al. (2020). CNGBdb: China National GeneBank DataBase. *Yi Chuan* 42, 799–809. <https://doi.org/10.16288/j.ycz.20-080>.
 46. Xu, Z., Wang, W., Yang, T., Li, L., Ma, X., Chen, J., Wang, J., Huang, Y., Gould, J., Lu, H., et al. (2024). STOmicsDB: a comprehensive database for spatial transcriptomics data sharing, analysis and visualization. *Nucleic Acids Res.* 52, D1053–D1061. <https://doi.org/10.1093/nar/gkad933>.
 47. Xiong, K.X., Zhou, H.L., Lin, C., Yin, J.H., Kristiansen, K., Yang, H.M., and Li, G.B. (2022). Chord: an ensemble machine learning algorithm to identify doublets in single-cell RNA sequencing data. *Commun. Biol.* 5, 510. <https://doi.org/10.1038/s42003-022-03476-9>.
 48. Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W.M., 3rd, Zheng, S., Butler, A., Lee, M.J., Wilk, A.J., Darby, C., Zager, M., et al. (2021). Integrated analysis of multimodal single-cell data. *Cell* 184, 3573–3587.e29. <https://doi.org/10.1016/j.cell.2021.04.048>.
 49. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
 50. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernyt, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297–1303. <https://doi.org/10.1101/gr.107524.110>.
 51. Tirosh, I., Izar, B., Prakadan, S.M., Wadsworth, M.H., 2nd, Treacy, D., Trombetta, J.J., Rotem, A., Rodman, C., Lian, C., Murphy, G., et al. (2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196. <https://doi.org/10.1126/science.aad0501>.
 52. Kotliar, D., Veres, A., Nagy, M.A., Tabrizi, S., Hodis, E., Melton, D.A., and Sabeti, P.C. (2019). Identifying gene expression programs of cell-type identity and cellular activity with single-cell RNA-Seq. *eLife* 8, e43803. <https://doi.org/10.7554/eLife.43803>.
 53. Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinform.* 14, 7. <https://doi.org/10.1186/1471-2105-14-7>.
 54. Qiu, X., Mao, Q., Tang, Y., Wang, L., Chawla, R., Pliner, H.A., and Trapnell, C. (2017). Reversed graph embedding resolves complex single-cell trajectories. *Nat. Methods* 14, 979–982. <https://doi.org/10.1038/nmeth.4402>.
 55. Gulati, G.S., Sikandar, S.S., Wesche, D.J., Manjunath, A., Bharadwaj, A., Berger, M.J., Ilagan, F., Kuo, A.H., Hsieh, R.W., Cai, S., et al. (2020). Single-cell transcriptional diversity is a hallmark of developmental potential. *Science* 367, 405–411. <https://doi.org/10.1126/science.aax0249>.
 56. Garcia-Alonso, L., Handfield, L.F., Roberts, K., Nikolakopoulou, K., Fernando, R.C., Gardner, L., Woodhams, B., Arutyunyan, A., Polanski, K., Hoo, R., et al. (2021). Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat. Genet.* 53, 1698–1711. <https://doi.org/10.1038/s41588-021-00972-2>.
 57. Su, M., Pan, T., Chen, Q.Z., Zhou, W.W., Gong, Y., Xu, G., Yan, H.Y., Li, S., Shi, Q.Z., Zhang, Y., et al. (2022). Data analysis guidelines for single-cell RNA-seq in biomedical studies and clinical applications. *Mil. Med. Res.* 9, 68. <https://doi.org/10.1186/s40779-022-00434-8>.
 58. Becht, E., McInnes, L., Healy, J., Dutertre, C.A., Kwok, I.W.H., Ng, L.G., Ginhoux, F., and Newell, E.W. (2018). Dimensionality reduction for visualizing single-cell data using UMAP. *Nat. Biotechnol.* 37, 38–44. <https://doi.org/10.1038/nbt.4314>.
 59. Sun, Y., Wu, L., Zhong, Y., Zhou, K., Hou, Y., Wang, Z., Zhang, Z., Xie, J., Wang, C., Chen, D., et al. (2021). Single-cell landscape of the ecosystem in

- p>early-relapse hepatocellular carcinoma.
- Cell*
- 184, 404–421.e16.
- <https://doi.org/10.1016/j.cell.2020.11.041>
- .
60. Chen, Y.P., Yin, J.H., Li, W.F., Li, H.J., Chen, D.P., Zhang, C.J., Lv, J.W., Wang, Y.Q., Li, X.M., Li, J.Y., et al. (2020). Single-cell transcriptomics reveals regulators underlying immune cell diversity and immune subtypes associated with prognosis in nasopharyngeal carcinoma. *Cell Res.* 30, 1024–1042. <https://doi.org/10.1038/s41422-020-0374-x>.
 61. Rao, A., Barkley, D., França, G.S., and Yanai, I. (2021). Exploring tissue architecture using spatial transcriptomics. *Nature* 596, 211–220. <https://doi.org/10.1038/s41586-021-03634-9>.
 62. Chang, J., Lu, J., Liu, Q., Xiang, T., Zhang, S., Yi, Y., Li, D., Liu, T., Liu, Z., Chen, X., et al. (2025). Single-cell multi-stage spatial evolutionary map of esophageal carcinogenesis. *Cancer Cell* 43, 380–397.e7. <https://doi.org/10.1016/j.ccell.2025.02.009>.
 63. Puram, S.V., Tirosh, I., Parkh, A.S., Patel, A.P., Yizhak, K., Gillespie, S., Rodman, C., Luo, C.L., Mroz, E.A., Emerick, K.S., et al. (2017). Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. *Cell* 171, 1611–1624.e24. <https://doi.org/10.1016/j.cell.2017.10.044>.
 64. Tripathi, S., Pohl, M.O., Zhou, Y., Rodriguez-Frandsen, A., Wang, G., Stein, D.A., Moulton, H.M., DeJesus, P., Che, J., Mulder, L.C.F., et al. (2015). Meta- and Orthogonal Integration of Influenza “OMICs” Data Defines a Role for UBR4 in Virus Budding. *Cell Host Microbe* 18, 723–735. <https://doi.org/10.1016/j.chom.2015.11.002>.
 65. Fan, J., Lu, F., Qin, T., Peng, W., Zhuang, X., Li, Y., Hou, X., Fang, Z., Yang, Y., Guo, E., et al. (2023). Multiomic analysis of cervical squamous cell carcinoma identifies cellular ecosystems with biological and clinical relevance. *Nat. Genet.* 55, 2175–2188. <https://doi.org/10.1038/s41588-023-01570-0>.
 66. Bost, P., Giladi, A., Liu, Y., Bendjelal, Y., Xu, G., David, E., Blecher-Gonen, R., Cohen, M., Medaglia, C., Li, H., et al. (2020). Host-Viral Infection Maps Reveal Signatures of Severe COVID-19 Patients. *Cell* 181, 1475–1488.e12. <https://doi.org/10.1016/j.cell.2020.05.006>.
 67. Mabrouk, N., Tran, T., Sam, I., Pourmir, I., Gruel, N., Granier, C., Pineau, J., Gey, A., Kobold, S., Fabre, E., and Tartour, E. (2022). CXCR6 expressing T cells: Functions and role in the control of tumors. *Front. Immunol.* 13, 1022136. <https://doi.org/10.3389/fimmu.2022.1022136>.
 68. Zhang, L., Li, Z., Skrzypczynska, K.M., Fang, Q., Zhang, W., O’Brien, S.A., He, Y., Wang, L., Zhang, Q., Kim, A., et al. (2020). Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. *Cell* 181, 442–459.e29. <https://doi.org/10.1016/j.cell.2020.03.048>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit monoclonal anti-FABP5	Abcam	Cat# ab255276
Mouse monoclonal anti-c-Myc	Thermo Fisher Scientific	Cat#MA1-16637; RRID:AB_568496
Rabbit monoclonal anti- β -actin	Cell Signaling Technology	Cat#4970; RRID:AB_2223172
Anti-Ki67 antibody	ZSGB Bio	Cat#ZM-0167; RRID:AB_2920617
Rabbit polyclonal anti-cleaved Caspase-3	Cell Signaling Technology	Cat#9661; RRID:AB_2341188
Rabbit monoclonal anti-HA-Tag	Cell Signaling Technology	Cat#3724; RRID:AB_1549585
Rabbit monoclonal anti-GAPDH	Cell Signaling Technology	Cat#2118; RRID:AB_561053
Bacterial and virus strains		
pHAGE-CMV lentiviral vector	Sun Yat-sen University Cancer Center	N/A
pCAGGS-CMV lentiviral vector	Sun Yat-sen University Cancer Center	N/A
Biological samples		
NKTCL samples	Sun Yat-sen University Cancer Center	See Table S1 for details
Peripheral blood from NKTCL patients	Sun Yat-sen University Cancer Center	N/A
Chemicals, peptides, and recombinant proteins		
SBFI-26	Selleck	S9957; CAS:1541209-75-9
Dimethyl sulfoxide	Sigma-Aldrich	Cat# D2650
RPMI 1640	Thermo Fisher Scientific	Cat# 31870082
Anti-FLAG® M2 Magnetic Beads	Sigma-Aldrich	Cat# M8823
PBS	Thermo Fisher Scientific	Cat# 70011069
Lipofectamine RNAiMAX	Thermo Fisher Scientific	Cat# 13778150
IP lysis buffer	Beyotime	Cat# P0013
RIPA lysis buffer, 10×	Millipore	Cat# 20-188
Protease and phosphatase inhibitors	Thermo Fisher Scientific	Cat# 78441
Critical commercial assays		
Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1	10X Genomics	Cat #1000121
DNeasy Blood & Tissue Kit	Qiagen	Cat #69504
Human All Exon 1.0 kit	Shbio	Cat #ShY1001B
Stereo-seq chips v1.2	BGI Shenzhen	Cat#201ST114 (4RXNs)
Pierce BCA protein assay kit	Thermo Fisher	Cat# 23227
Cell Counting Kit-8	APEXIO	Cat# K1018
Deposited data		
scRNA-seq and WES data (raw data)	This paper	GSA-Human:HRA007314;CNSA:CNP0005654
spatial RNA-seq data (processed data)	This paper	OMIX:OMIX006113;STOmics DB:STT0000114
scRNA-seq of 7 non-cancerous nasopharyngeal samples from normal subjects	Jin et al., ¹⁸ 2020	GSA: HRA000087(https://ngdc.cncb.ac.cn/gsa-human/)
Bulk RNA-seq datasets of 102 NKTCL patients	Xiong et al., ¹³ 2020	Mendeley Data: https://data.mendeley.com/datasets/7gwtb7mgr/draft?a=85eac518-0f19-41f8-aa58-69ed36b66e41
Experimental models: Cell lines		
YT	Sun Yat-sen University Cancer Center	N/A
NKYS	Sun Yat-sen University Cancer Center	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
BALB/c Nude mice	Gempharmatech Co., Ltd	N/A
Oligonucleotides		
Human-FABP5-siRNA1 sense- CUUUGGACAGGAGUAAUUA	This manuscript	N/A
Human-FABP5-siRNA1 antisense- UAAUUAACUCCUGUCCAAAG	This manuscript	N/A
Human -FABP5-siRNA2 sense- GUGGAGUGUGUCAUGAACAAU	This manuscript	N/A
Human -FABP5-siRNA2 antisense- AUUGUUAUGACACACUCCAC	This manuscript	N/A
Human -siNC sense- UUCUCCGAACGUGUCACGUTT	This manuscript	N/A
Human -siNC antisense- ACGUGACACGUUCGGAGAATT	This manuscript	N/A
Recombinant DNA		
pCAGGS-CMV-c-MYC-HA	This manuscript	N/A
pCAGGS-CMV-FABP5-flag	This manuscript	N/A
pHAGE-CMV-FABP5	This manuscript	N/A
Software and algorithms		
Cell Ranger v3.0.1	10x genomics	https://support.10xgenomics.com/single-cell-gene-expression/software/
Chord	Xiong et al. ⁴⁷	https://github.com/13308204545/Chord/
Seurat v4.1.0	Hao et al. ⁴⁸	https://satijalab.org/seurat/
BWA v0.7.13	Li et al. ⁴⁹	https://github.com/lh3/bwa
Genome Analysis Toolkit v3.5	McKenna et al. ⁵⁰	https://gatk.broadinstitute.org
SAW	BGI	https://github.com/BGIResearch/SAW
SPOTlight v1.5.1	Elosua-Bayes et al. ³⁵	https://marcelosua.github.io/SPOTlight/
infercnv v1.6.0	Tirosh et al. ⁵¹	https://github.com/broadinstitute/inferCNV
cNMF	Kotliar et al. ⁵²	https://github.com/dylkot/cNMF
GSVA v1.38.2	Hänzelmann et al. ⁵³	https://www.bioconductor.org/packages/release/bioc/html/GSVA.html
monocle v2.18.0	Qiu et al. ⁵⁴	https://github.com/cole-trapnell-lab/monocle-release
CytoTRACE v0.3.3	Gulati et al. ⁵⁵	https://cytotrace.stanford.edu/
survival v3.3.1	Therneau et al., 2024	https://cran.r-project.org/web/packages/survival/index.html
CellPhoneDB v3.1.0	Garcia-Alonso et al. ⁵⁶	https://github.com/Teichlab/cellphonedb
Prism 9.0	GraphPad Software	https://www.graphpad.com/
ImageJ	NIH	https://imagej.nih.gov/ij/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Patients and tissue samples

This study included biopsy samples from 13 patients diagnosed with NKTCL based on histopathological evaluation. Two of these patients underwent tumor biopsies both prior to and following treatment. Of the cohort, 6 patients were treated with the P-GemOx chemotherapy regimen, while 4 patients received immuno-chemotherapy. Paired samples, NKTCL8 and NKTCL8-post, were collected before and after immuno-chemotherapy, and matched samples, NKTCL10 and NKTCL10-post, were obtained from patients treated with chemotherapy (Table S1). Additionally, spatial transcriptomics was performed on samples from three patients, and whole-exome sequencing (WES) was conducted on 6 patients. Non-malignant nasopharyngeal tissues from seven healthy individuals were analyzed as controls,¹⁸ with complete clinicopathological profiles provided in Table S1. The study was

approved by the Institutional Review Board of Sun Yat-sen University Cancer Center (SYSUCC) and received ethical approval from the Ethics Review Committee of SYSUCC (Approval No. B2021-360-Y02). The raw data have been deposited in the Research Data Deposit (RDD). All participants provided written informed consent.

Cell culture

The NKTCL cell lines (NKYS and YT) were maintained at the SYSUCC and incubated in a humidified environment with 5% CO₂ at 37°C. NKYS cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS)(Gibco), IL-2 (100 IU/ml) and 1% penicillin/streptomycin. YT cells were maintained in IMDM (Gibco, USA) containing 20% FBS and antibiotics.

Experimental animals

Female BALB/c nude mice (4–5 weeks old, 18–20 g) were obtained from GemPharmatech Co., Ltd. (Guangdong, China) and housed at SYSUCC. Animal experiments were approved by the Animal Ethical and Welfare Committee at the same institution (Approval No. L025501202401010) and conducted according to institutional guidelines.

METHOD DETAILS

Preparation of single-cell suspensions

Tissue samples were washed twice with phosphate-buffered saline (PBS) containing 0.04% bovine serum albumin, minced into pieces <1 mm³, transferred into clean tubes containing enzymes H, R and A in RPM 1640 medium (Invitrogen, Waltham, MA, USA), gently agitated, and incubated. The digested tissue was filtered through screens with pore diameters of 70 μm and then 40 μm, and centrifuged at 400 g for 5 min. The supernatant was discarded and the cell pellet was resuspended in red blood cell lysis buffer at room temperature with gentle mixing. The lysate was mixed with PBS and inverted, the mixture was centrifuged and the supernatant was discarded. The cells were resuspended in PBS and centrifuged again. Following the removal of the supernatant, the cells were resuspended in an appropriate volume of PBS, from which an aliquot was taken and mixed with 0.4% trypan blue, and the number and viability of cells were evaluated using a blood cell counter under a microscope. The sample was then centrifuged and resuspended in serum-free PBS for cell staining and flow cytometry.

Single-cell RNA sequencing

A single-cell 3'-library was constructed using the Chromium Single-Cell Assay and Chip Assay (10X Genomics, Pleasanton, CA, USA). Cell suspensions were loaded onto a single-cell chip and mixed with reverse transcription master mix and single-cell 3'-gel beads at $2-8 \times 10^3$ cells per reaction. Samples were processed using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 (10X Genomics). After cell lysis, first-strand cDNA was synthesized and amplified for 12 cycles. Libraries were sequenced using a HiSeq X 10 system (Illumina, Inc., San Diego, CA, USA). Single-cell RNA sequences were processed using Cell Ranger 3.0.1 (<https://support.10xgenomics.com/single-cell-gene-expression/software/>) and a raw gene expression matrix was generated based on the human reference genome GRCh38. The average sequencing depth of our data exceeded 33000 reads per cell. To exclude data from droplets containing multiple cells, Chord (<https://github.com/13308204545/Chord/>)⁴⁷ was used to identify and remove potential doublets in each sample.⁴⁷ Gene expression matrices for all tumor samples or for all control samples were combined and converted into "objects" using the Seurat routine in R 4.1.0. Samples were excluded from analysis if they contained fewer than 700 genes, if they contained fewer than 1000 UMI, if sequences for each gene came from an average of fewer than three cells, or if more than 20% of the sequenced genes came from mitochondria. For the samples retained in the final analysis, gene expression matrices were log-normalized and corrected for different numbers of total transcripts, different fractions of mitochondrial transcripts, different fractions of ribosomal transcripts, and different phases in the cell cycle. These preprocessing steps were applied in both the overall clustering and subclustering analyses of cells. Tumor samples and control samples were processed, respectively, using V3 and V2 Chemistry reagents (10X Genomics, Pleasanton, CA, USA), and in both cases potential batch effects were removed using canonical correlation analysis with the FindIntegrationAnchors and IntegrateData function in the Seurat (V4) routine⁵⁷ based on 2000 variable features in the overall clustering as well as in the myeloid and T cell subclustering.

Principal component analysis (PCA) was conducted on the expression matrix after integration transformation, and the top 30 principal components and a resolution of 2.5 were used to perform unsupervised clustering in two dimensions using uniform manifold approximation and projection (UMAP).⁵⁸ Probable cell doublets and multiplets were removed based on expression of the following canonical cell type markers^{59,60}: B cells, *MS4A1*, *BANK1*, *CD79A*, and *CD79B*; plasma cells, *MZB1* and *DERL3*; T cells, *CD2*, *CD3D*, *CD3E*, *IL7R* and *CD3G*; NK cells, *NCAM1*, *CD160*, *GZMB*, *KLRD1* and *PRF1*; myeloid cells, *LYZ*, *CD68*, *CD14* and *AIF1*; cancer-associated fibroblasts, *COL1A1*, *COL1A2* and *COL3A1*; endothelial cells, *CLDN5*, *PECAM1* and *VWF*; epithelial cells, *KRT19*, *KRT18*, *KRT8* and *AQP3*; and proliferating cells, *MKI67*, *TOP2A* and *STMN1*.

Whole-exome sequencing

Using the DNeasy Blood and Tissue Kit (Qiagen, Venlo, The Netherlands), genomic DNA was extracted from blood and tumor tissue from the same NKTCL patient. The exome was captured using the Human All Exon 1.0 kit (Shbio, Shanghai, China), and libraries were quantified using a fluorimeter (Qubit 2.0). Product size and quality were assessed using a 2100 Bioanalyzer (Agilent Technologies)

and High Sensitivity DNA Assay (For Illumina). Suitable libraries underwent paired-end sequencing (2×150 bp) on a NovaSeq 6000 platform (Illumina). The resulting FASTQ files were aligned to the hg19/GRCh37 (human reference genome) using BWA 0.7.13,⁴⁹ then analyzed using the Genome Analysis Toolkit 3.5 (<https://gatk.broadinstitute.org>).⁵⁰

Spatial transcriptomics

To analyze the spatial distribution of cell types within NKTCL tumors, we processed freshly frozen biopsies from primary tumors from three patients using Stereo-seq,^{34,61} and adjacent tissue sections were stained using hematoxylin and eosin.

Within 30 min after sampling fresh tumor tissue, excess fluid was removed and the tissue was embedded in OCT, and stored at -80°C before the preparation of tissue sections ($10\ \mu\text{m}$) for overlaying onto Stereo-seq chips v1.2 (BGI Shenzhen, <https://www.stomics.tech/products/StereoseqChipsandReagentKits>). cDNA libraries were constructed following the Stereo-seq protocol and sequenced on a DNBSEQ-Tx sequencer (MGI, Research, Shenzhen, China). Using the SAW pipeline (<https://github.com/BGIResearch/SAW>), the resulting FASTQ files, which contained the coordinate identity (CID), molecular identity (MID), and cDNA sequences, were processed to generate a CID-containing expression profile matrix, which was converted to pseudo-spots (bin 50, 50×50 DNB bins, $25\ \mu\text{m}$ diameter) and imported into Seurat for analysis after excluding bins containing fewer than 200 genes. The SPOTlight³⁵(version 1.5.1) software was used to deduce the celltype composition of each bin50 spot, factorizing cell type-specific topic profiles from corresponding scRNA-seq data. The potential composition of each spot was refined and renormalized, with the top celltype being prioritized based on descending probabilities, and the predominant celltype was assigned for visualization. The spatial distribution of annotated bins and gene expression was visualized using the SpatialFeaturePlot function. The expression scores of MYC_target_V1 and fatty acid metabolism geneset were calculated utilizing the AddModuleScore function with default parameters and were visualized using SpatialFeaturePlot.

To delineate spatial organizational patterns of cellular communities, cell neighborhood analysis was performed. For each cell across three samples, the cell neighborhood region was defined as the collection of cells within a predefined radial distance⁶² (extending outward by one cell diameter). Leveraging two-dimensional spatial coordinates, a neighborhood feature matrix was constructed by quantifying the aggregate features (cellular type composition) of neighboring cells within a defined spatial range. After acquiring the adjacency matrix, the feature matrix was converted into Seurat-compatible objects, followed by dimensionality reduction and unsupervised clustering using the Seurat package, which eventually divided cellular neighborhoods into six distinct clusters (see Figures 5E, S6I, and S6J). Cluster-specific cellular composition was visualized via heatmaps to highlight cell-type enrichment patterns across neighborhoods. Additionally, dot plots, boxplots, and violin plots were employed to statistically compare quantitative features among cell neighborhoods.

Identification of malignant cells

Non-malignant cells (NK cells derived from control tissues, B cells, epithelial cells, endothelial cells, fibroblasts, myeloid cells, plasma cells and T cells) were used as a reference to identify malignant cells based on inferred CNVs, as determined using the Infercnv routine in R (infercnv package, version 1.6.0)⁵¹ with default parameters. CNVs were analyzed in seven biopsies, each of which contained more than 200 malignant cells, and compared to those determined by WES in six biopsies. The CNVs levels of all T cell from each patient were also estimated in the same manner using NK cells derived from control tissues as a reference but no significant CNVs were observed (results not shown).

Transcriptomic “meta-programs” of malignant cells in NKTCL tumors

Consensus Non-negative Matrix factorization (cNMF) was conducted to analyze intratumor heterogeneity of NKTCL (<https://github.com/dylkot/cNMF>).⁵² For each of 7 tumors, cNMF was applied to relative expression matrix and all negative values were replaced with zero.¹⁸ Briefly, cNMF was run 200 times and k which indicates the number of expression programs that each expression matrix was decomposed into after cNMF was set from 3 to 20, and computed a set of consensus programs by aggregating results and computed a stability and reconstruction error. An optimal k was selected by considering the trade-off between stability and error for each sample. And eventually a total of 37 programs were obtained from 7 patients. To identify common programs among the 37 programs, hierarchical clustering was performed, utilizing one minus the Pearson correlation coefficient over all gene scores as a distance metric.⁶³ Five “meta-programs” were manually identified based on Pearson correlation coefficient (cut off >0.6). For each meta-program, the average loadings of each gene across the programs were calculated and genes were ranked based on average loadings.⁶⁰ The top 50 genes with the highest loading were defined as the signature for the metaprograms (Table S2). Enrichment of biological processes in the meta-programs was assessed using Metascape (<http://metascape.org>).⁶⁴ In order to classify each malignant cell into a certain MP subgroup, each malignant cell was scored for the top 50 gene set of each MP. If 85% of the highest score exceeds the second-highest score, each malignant cell is assigned to the MP with the highest score, otherwise the malignant cell is assigned to an undefined one.⁶⁵

Identification of cells infected by EBV

Cells in NKTCL patients that were infected with EBV were detected using Viral-Track, which scans unmapped single-cell RNA sequencing data for EBV viral sequences (NC_007605.1).⁶⁶

Identification of T, B and myeloid cell populations

Cells from the 12 tumor biopsies and seven non-malignant nasopharyngeal tissue showing expression levels greater than 0.5 for marker genes of cell types other than T, B or myeloid lineages were removed. CD8⁺ and CD4⁺ T cells were identified based on the T cell subcluster and expression of *CD8A*, *CD8B* and *CD4*. Cell subclusters were identified using the top 10 principal components at a resolution of 0.4 for CD8⁺ and CD4⁺ T cells or 0.5 for B cells and myeloid cells. Clusters were annotated based on expression of marker genes with the FindAllMarkers function in Seurat. Signature genes of subcluster were defined: (1) fold difference > 1.5 (with adjusted *P* < 0.01) between clusters; (2) gene expressed in at least 25% of cells (Tables S3 and S4).

Classification of CD8⁺ cells by functional state

CD8⁺ T cells in all tissues were classified by functional state according to levels of expression of genes associated with a resident phenotype (marker genes *ITGAE*, *CD69*, *CXCR6*, *RUNX3*),^{59,67} cytotoxic phenotype (*PRF1*, *IFNG*, *NKG7*, *GZMB*, *GZMA*, *CST7*, *GZMK*),^{59,60} exhausted phenotype (*CTLA4*, *HAVCR2*, *LAG3*, *PDCD1*, *TIGIT*)⁶⁰ and costimulatory phenotype (*ICOS*, *CD27*, *TNFRSF14* *TNFRSF9*).⁶⁰ Macrophages were classified to M1 versus M2 phenotype as previously described.⁶⁸

Pathway enrichment analysis

To evaluate the scoring of specific gene sets within cells, we utilized the Gene Set Variation Analysis (GSVA) package (version 1.38.2) with its default parameters. The analysis was based on the Hallmark pathways delineated in the Molecular Signatures Database (MSigDB; <https://www.gsea-msigdb.org/gsea/msigdb>), facilitating the comparative assessment of differences in signaling pathway enrichment across individual cells.

Developmental trajectories of malignant cells, CD8⁺ T and myeloid cells in NKTCL

Developmental trajectories of malignant cells in NKTCL were simulated in pseudotime using the monocle routine (version 2.18.0)⁵⁴ in R. Genes differentially expressed between cell types were identified using the differentialGeneTest function (Benjamini-Hochberg-corrected *P* < 0.0001), and the malignant cells were ranked based on 1500 different genes between MP cells. The R package CytoTRACE (v0.3.3)⁵⁵ (<https://cytotrace.stanford.edu/>) was used to determine the extent of differentiation of MP cells along the trajectory with default parameter. Genes related to stemness and differentiation can be predicted based on their correlation with CytoTRACE Score.

Correlation of gene expression signatures with patient survival

97 patients in an independent NKTCL test cohort (total 102 patients, 5 lacked survival information) were used to evaluate the patient prognosis. The signature genes (defined as fold difference > 2 with adjusted *P* < 0.01) of cell subsets and classical cell type marker genes (B cells, *MS4A1*, *CD79A* and *CD79B*; myeloid cells, *CD68*, *CD14*, *LYZ* and *AIF1*; CD4⁺ T cells, *CD4*; and CD8⁺ T cells, *CD8A* and *CD8B*) were used together to assess patient prognosis. Patients were stratified based on gene set expression (low versus high) using the optimal cutpoint determined by the *surv_cutpoint* function in the survival routine (version 3.3.1) in R. Kaplan-Meier survival curves were plotted using the *ggsurvplot* function in R.

Analysis of cell-cell interactions in the microenvironment of NKTCL tumors

Potential interactions among cell types within the microenvironment of NKTCL tumors were investigated using CellPhoneDB 3.1.0⁵⁶ (<https://github.com/Teichlab/cellphonedb>). The gene expression matrixes of malignant cells, myeloid cells and T cells were input into CellPhoneDB for cell-cell interaction analysis. We identified the most relevant cell-type specific ligand receptor interactions, taking into account only those ligands and receptors that were expressed in more than 10% of the cells within their respective subtypes. Biologically relevant interactions were selected based on interaction scores and p-values. Significant ligand-receptor interactions between cell types were visualized using bubble plots generated with the *ggplot* function.

Xenograft tumor model

Female BALB/c nude mice (4–5 weeks old) were injected with 1×10^7 YT cells in the flank. Tumor growth became palpable around day 14, at which point the mice were randomly assigned to receive either daily intraperitoneal injections of SBFI-26 (4 mg/kg) or vehicle control (corn oil) for 14 consecutive days. Tumor volume was assessed every 1–2 days using digital calipers, applying the formula: (length $\times$ width²)/2. At the experiment's conclusion, mice were euthanized, and tumors were harvested for imaging and weight measurement.

Cell proliferation assay

Cell viability and proliferation were quantified using a commercial CCK-8 kit (APExBio, K1018). YT/NKYS cells were plated in 96-well plates at 5×10^3 cells/well and exposed to interventions: transfection with FABP5-OE plasmid or empty vector (VE); treatment with the FABP5 inhibitor SBFI-26 (50 or 100 μ M); or vehicle control (0.1% DMSO). Post-intervention, 10 μ L CCK-8 reagent was slowly added along the well wall to avoid bubbles, followed by 2-hour incubation at 37°C protected from light. Absorbance at 450 nm (OD₄₅₀) was measured using a Tecan Infinite M200 Pro microplate reader, with 650 nm as reference wavelength to correct for background interference.

Immunohistochemistry (IHC)

Immunohistochemistry was conducted to assess protein expression in tissue sections. Formalin-fixed, paraffin-embedded samples were dewaxed, rehydrated, and processed for peroxide blocking, antigen retrieval, and blocking. The sections were incubated overnight at 4°C with primary antibodies: anti-FABP5 (1:4000; abcam, ab255276) and anti-c-Myc (1:100; Thermo Fisher Scientific, MA1-16637), anti-Ki67 (1:300, ZSGB Bio, ZM-0167), anti-cleaved Caspase-3 (1:400; CST, 9661), followed by incubation with secondary antibodies the next day. Chromogenic detection was performed using DAB staining. Positively stained areas were quantified using ImageJ software, based on analysis of multiple randomly selected fields.

Western blot analysis

Proteins were extracted from cultured cells with RIPA buffer and quantified by BCA assay. Equal protein amounts (20 µg per lane) were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% non-fat milk, membranes were incubated overnight at 4°C with primary antibodies against FABP5 (1:1000; Abcam, ab255276), c-Myc (1:1000; Thermo Fisher Scientific, MA1-16637), GAPDH (1:1000; CST, 2118), and β-actin (1:1000; CST, 4970). Membranes were incubated with HRP-conjugated secondary antibodies, developed with ECL, and analyzed using ImageJ for band intensity quantification.

FABP5 knockdown and overexpression

For FABP5 knockdown, YT cells were transfected with two siRNAs targeting FABP5 or a control using Lipofectamine RNAiMAX (Thermo Fisher). After 48 h, Western blot was used to assess knockdown efficiency and c-MYC expression. For overexpression, FABP5 cDNA was transfected into cells with Lipofectamine RNAiMAX, and Western blot was performed after 48 h.

Co-immunoprecipitation (Co-IP)

To examine the interaction between FABP5 and c-Myc, pCAGGS-CMV-FABP5-FLAG and pCAGGS-CMV-c-Myc-HA plasmids were co-transfected into HEK293T cells with Lipofectamine RNAiMAX. After 48 h, cells were lysed (Beyotime, P0013), and lysates were incubated overnight with anti-Flag magnetic beads (Sigma-Aldrich, M8823). Immunoprecipitated proteins were then analyzed by Western blot with anti-Flag antibodies.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed as described in Figure legends. Differences in cell proportions in tumor and control tissue were compared using the Wilcoxon rank sum test. Other statistical methods are described in the corresponding figure legends. $p < 0.05$ was considered statistically significant. All analyses were conducted using R 4.1. Experimental data are presented as the mean ± SD. Data analysis and visualization were performed using GraphPad Prism 8. The statistical significance was analyzed by 2-tailed Student's t test, two-way ANOVA.