Boundary-Specific *Remodeling* Defines Divergent *TLS* Maturation in *Colorectal Cancer*

Afrooz Jahedi, PhD[1,6]; Mesut Unal, PhD[2]; Yu Ding, PhD[3]; Amina Nurmammadova, MS[5]; Yunhe Liu, PhD[1]; Linghua Wang, PhD[1,6]; Garry P. Nolan, PhD[4]

[1]Genomic Medicine Department, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
[2]Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
[3]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
[4]Department of Pathology, Stanford University, Palo Alto, California, USA.
[5]Institute for Cell Therapy Discovery and Innovation, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.
[6]Institute for Data Science In Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

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Introduction and Aim

- Immunotherapy has shown major success in cancers such as **melanoma** and **lung cancer**, but most **colorectal cancers (CRC)** do not respond effectively.
- **Tertiary lymphoid structures (TLSs)** are organized clusters of immune cells that form within tumors and can **enhance local antitumor immunity**.
- The **maturation process** and **microenvironmental organization** of TLSs in colorectal cancer remain **poorly understood**.
- **Hypothesis:** TLS maturation involves **coordinated structural and gene expression remodeling**, which differs between **immunotherapy-responsive,** colorectal-like responsive (CLR), and **non-responsive,** diffuse immune-inactive (DII), colorectal cancers.

Data & Methods

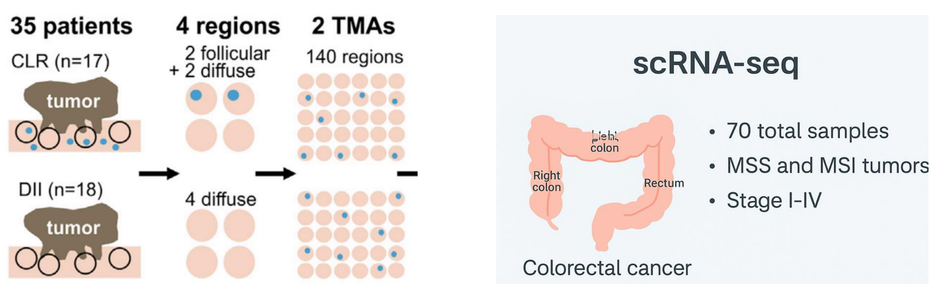

Figure 1. Multi-omics datasets for TLS analysis in colorectal cancer.
Spatial proteomics data and single-cell RNA sequencing data were integrated to map structural and transcriptional features of tertiary lymphoid structure maturation.

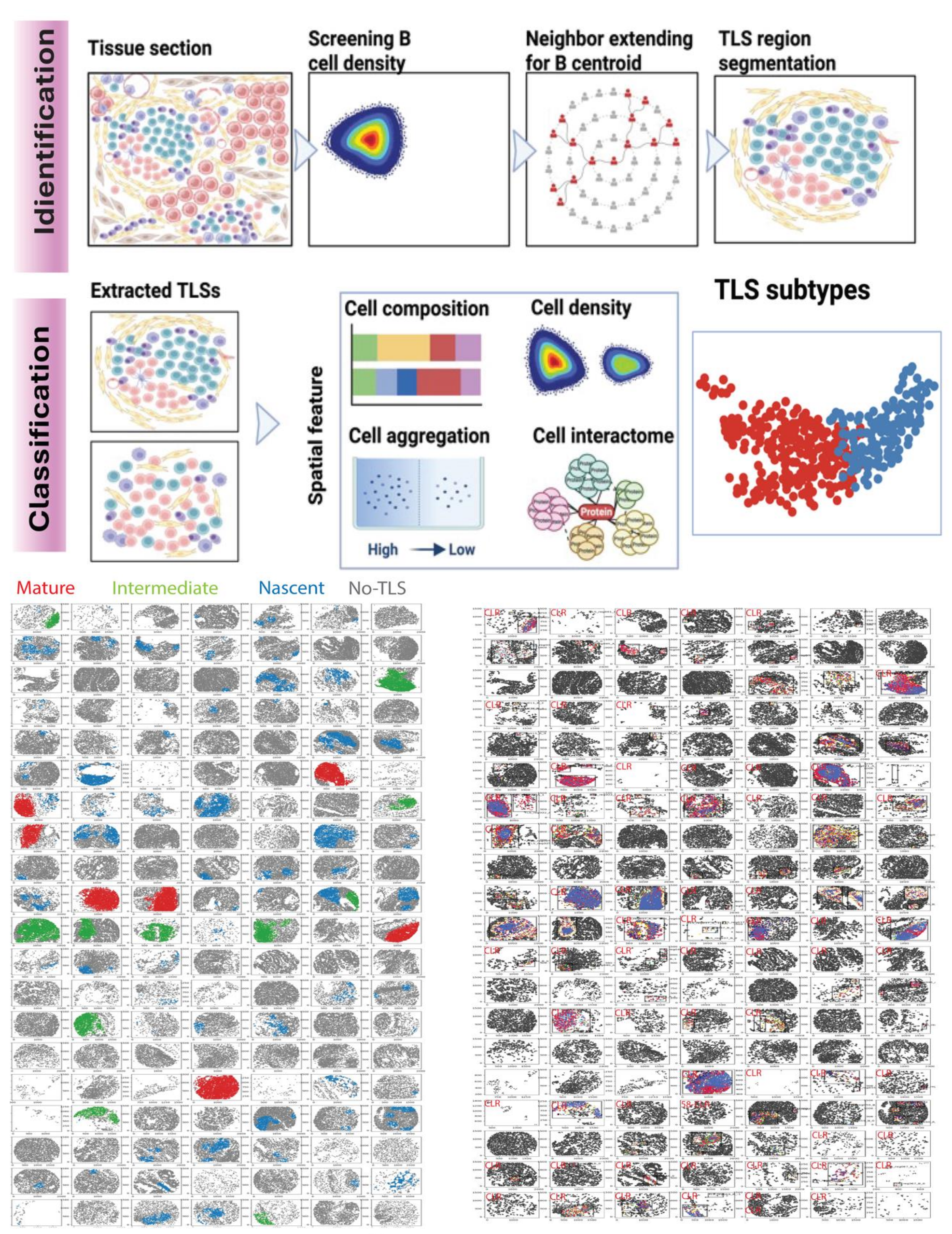

Figure 2. Identification and classification of TLS maturation states.
Workflow for TLS detection and classification. TLS regions were identified by screening B-cell density, expanding neighbors around B-cell centroids, and segmenting contiguous clusters into TLS regions. Extracted TLSs were further characterized by cell density, aggregation, composition, and interactome features. Representative TLSs across TMA cohorts A and B illustrate classification into nascent (blue), intermediate (green), and mature (red) maturation states. Cell composition derived by the classification algorithm provides reference distributions of immune subsets across TLS subtypes.

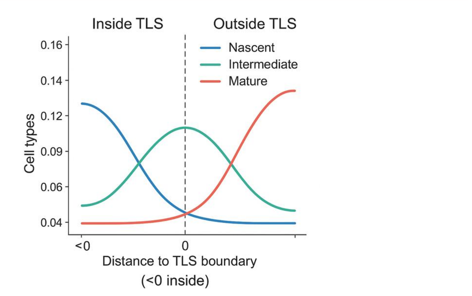

Figure 3. Multi-omics datasets for TLS analysis in colorectal cancer.
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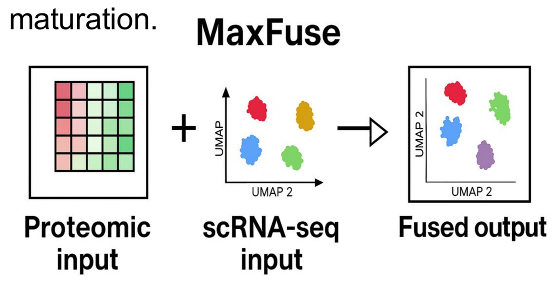

Figure 4. Multi-omics datasets for TLS analysis in colorectal cancer.
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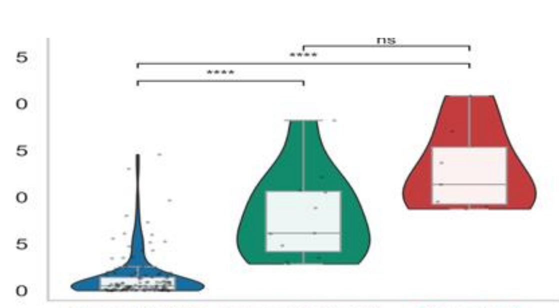

Figure 5: TLS number and area
Quantification of TLS number and area per maturation stage demonstrates progressive expansion and consolidation of TLS structures.

Results

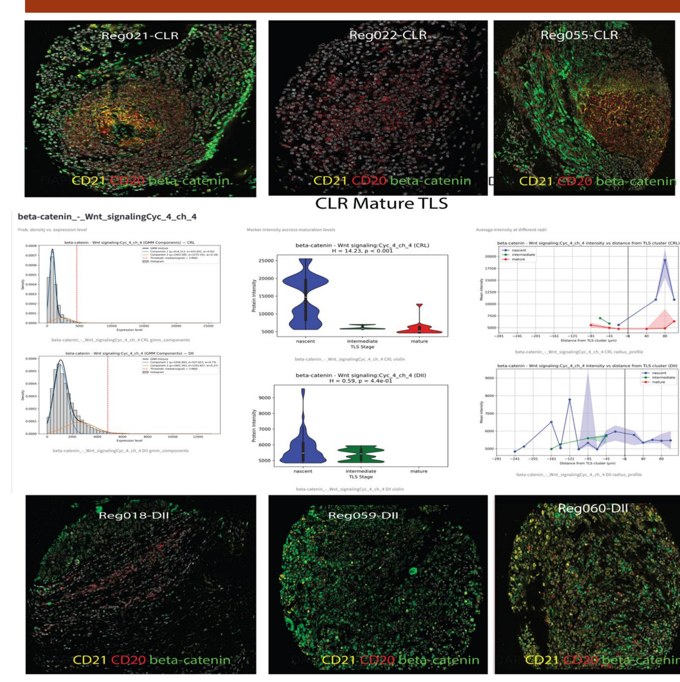

Figure 6. β-catenin expression.
CLR TLSs show **β-catenin suppression inside TLSs and enrichment outside boundaries**, indicating boundary-specific Wnt signaling, whereas **DII TLSs lack clear spatial gradients** and display diffuse β-catenin expression.

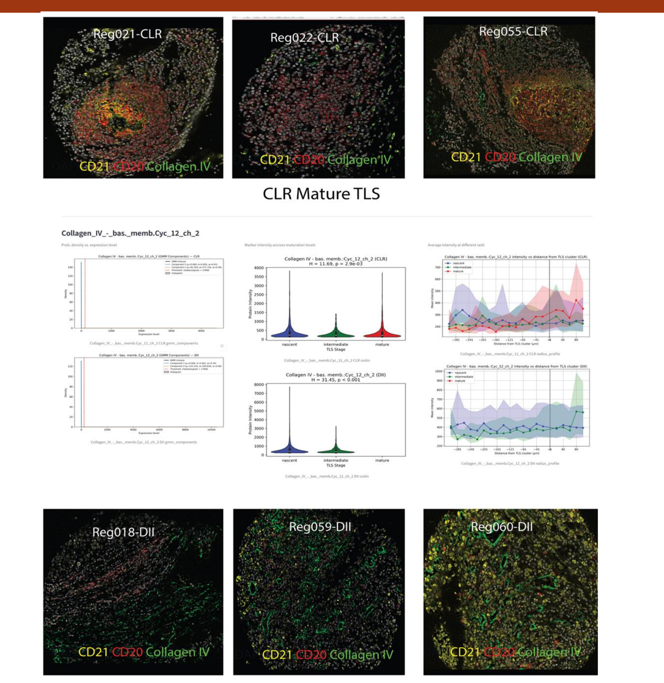

Figure 7. Collagen IV expression.
Mature CLR TLSs show **structured Collagen IV deposition at TLS boundaries**, indicating extracellular matrix remodeling, while **DII TLSs display diffuse, unorganized Collagen IV expression** lacking boundary formation.

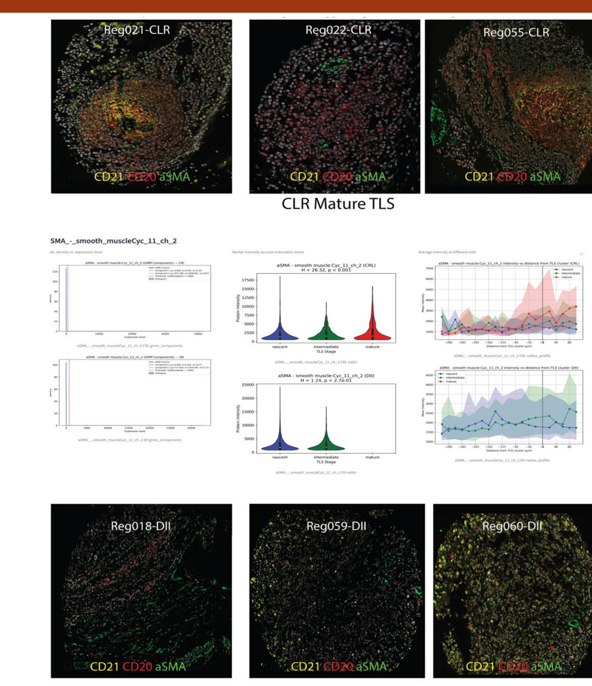

Figure 8. αSMA expression.
Mature CLR TLSs show strong **αSMA⁺ stromal encapsulation** around TLS boundaries, whereas **DII TLSs lack structured αSMA organization** and display diffuse, low expression.

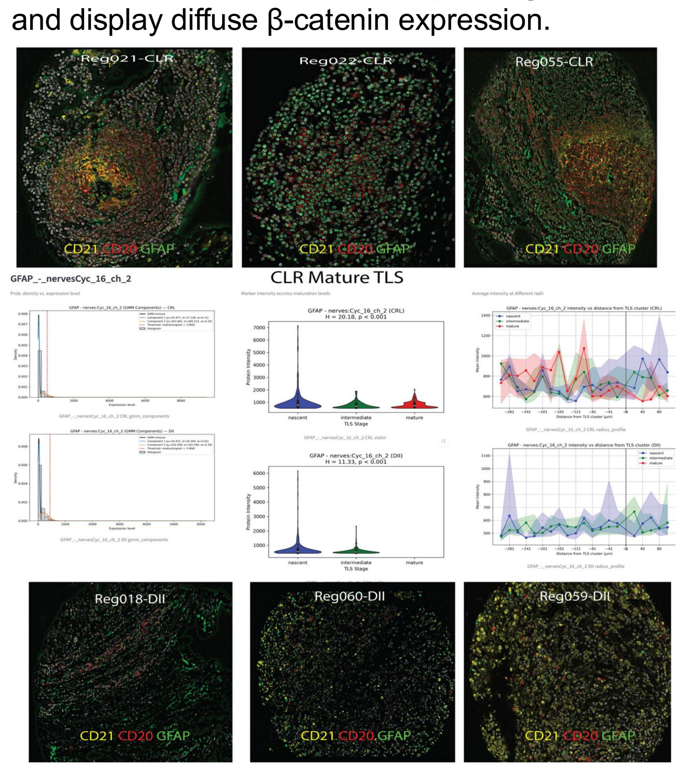

Figure 9. GFAP expression.
CLR TLSs show strong **GFAP enrichment inside mature TLSs,** indicating localized stromal activation, whereas DII TLSs display **diffuse, unpatterned GFAP expression** lacking spatial organization.

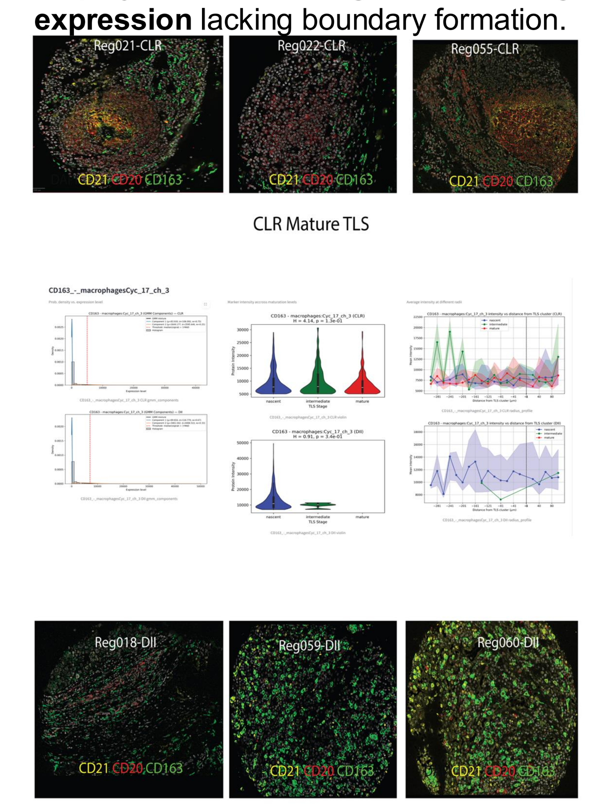

Figure 10. CD163 expression.
CD163⁺ macrophages are **enriched in nascent DII TLSs** and decline with maturation, while **CLR TLSs show consistently low, diffuse expression** across stages.

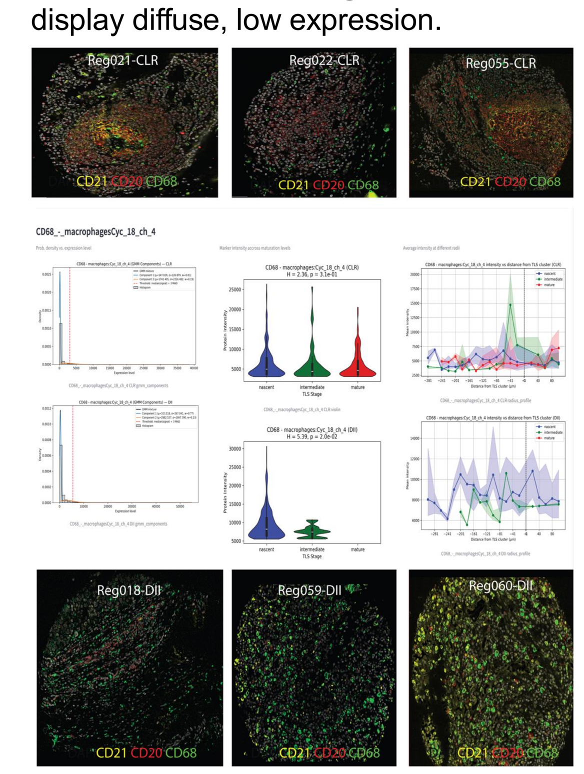

Figure 11. CD68 expression.
CD68⁺ macrophages are **enriched in nascent DII TLSs** and decline with maturation, while **CLR TLSs maintain uniformly low expression** without boundary-specific organization.

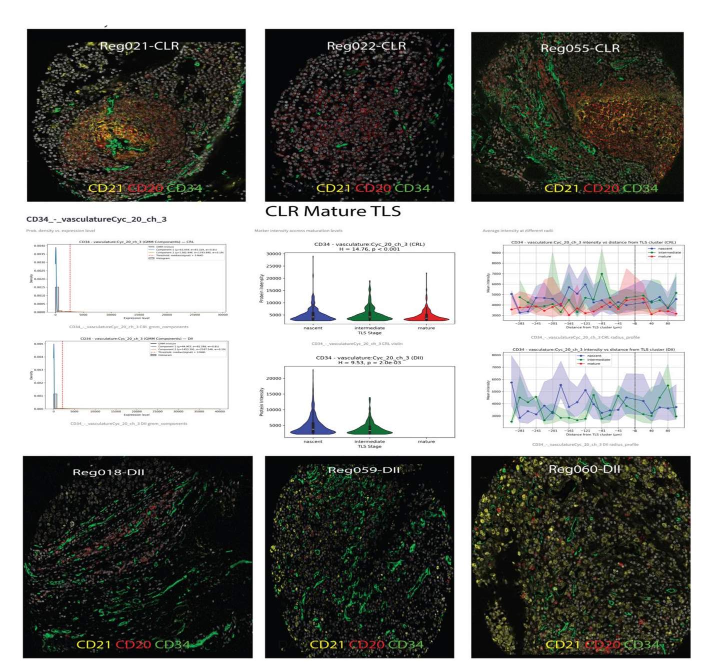

Figure 12. CD34 expression.
CLR TLSs show **reduced CD34⁺ vasculature inside mature TLSs,** indicating vascular exclusion, whereas **DII TLSs display higher, diffuse CD34 expression** consistent with persistent vascular activity.

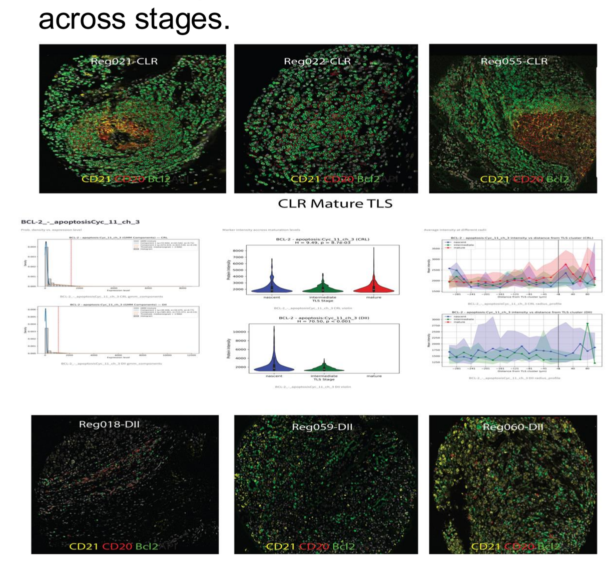

Figure 13. BCL-2 expression.
Mature CLR TLSs show a **BCL-2⁺ survival rim outside TLS boundaries,** indicating compartmentalized apoptotic regulation, while DII TLSs lack this spatial organization.

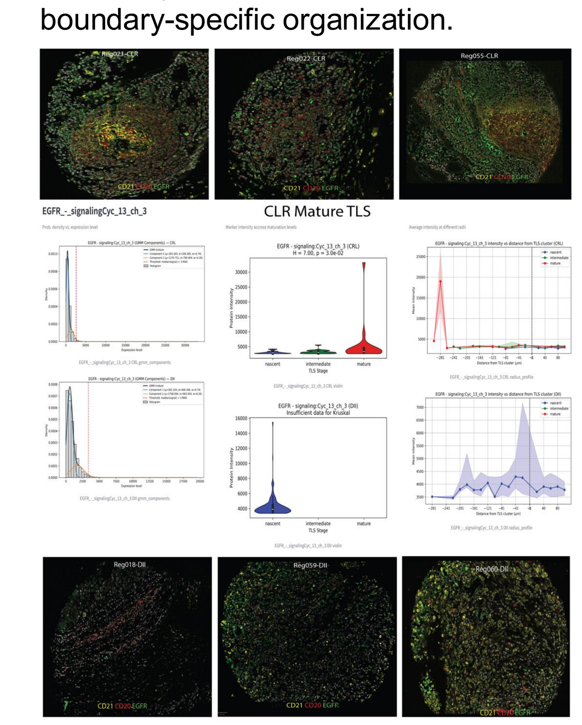

Figure 14. EGFR expression.
Mature CLR TLSs show a sharp **EGFR peak at the TLS boundary**, indicating boundary-restricted signaling, whereas DII TLSs display **diffuse, low EGFR expression** without spatial organization.

Results (Cont.)

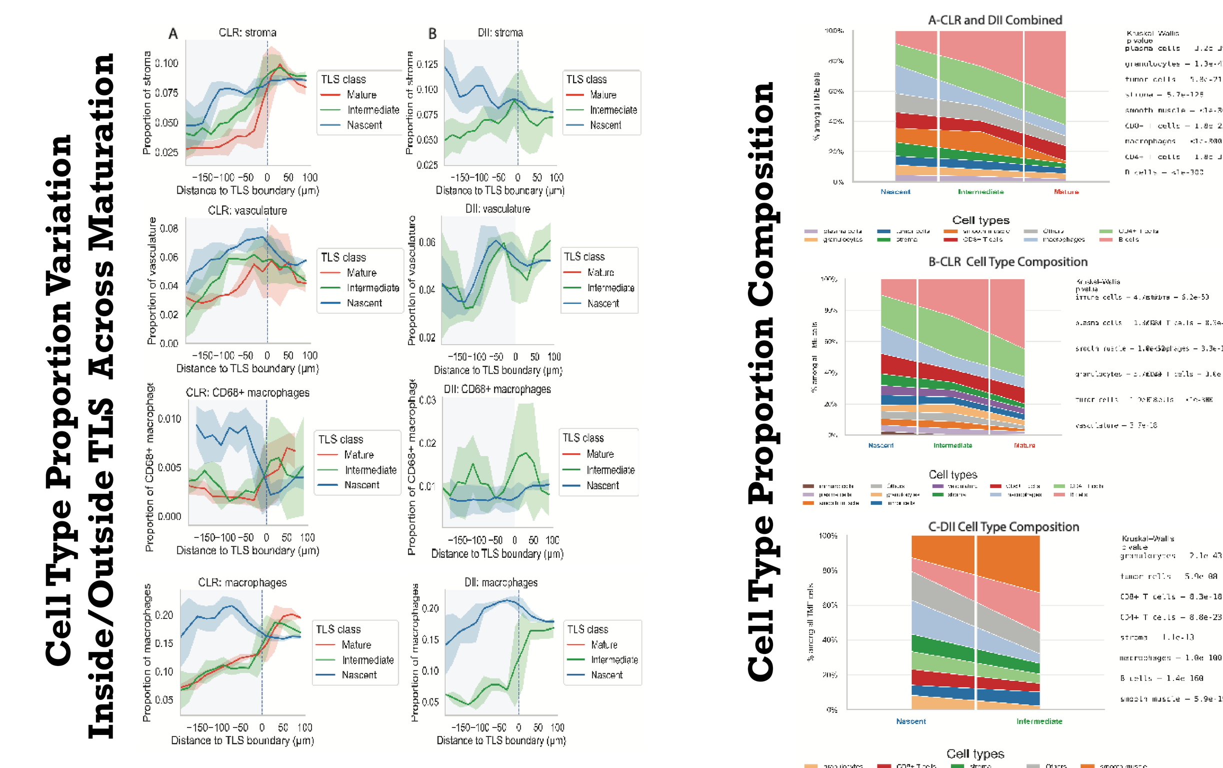

Figure 15. Cellular composition and spatial remodeling across TLS maturation.
Gradient plots (left) show spatial distributions of stroma, vasculature, and macrophages relative to the TLS boundary in **CLR** and **DII** patients, with lines indicating TLS stages—**nascent (blue)**, **intermediate (green)**, and **mature (red)**. CLR TLSs show organized boundary enrichment of stromal and macrophage populations, whereas DII TLSs remain diffuse. Stacked barplots (right) display overall cell-type composition changes across maturation, revealing decreased B and CD4⁺ T cells and increased plasma cells, macrophages, and stroma with TLS progression.

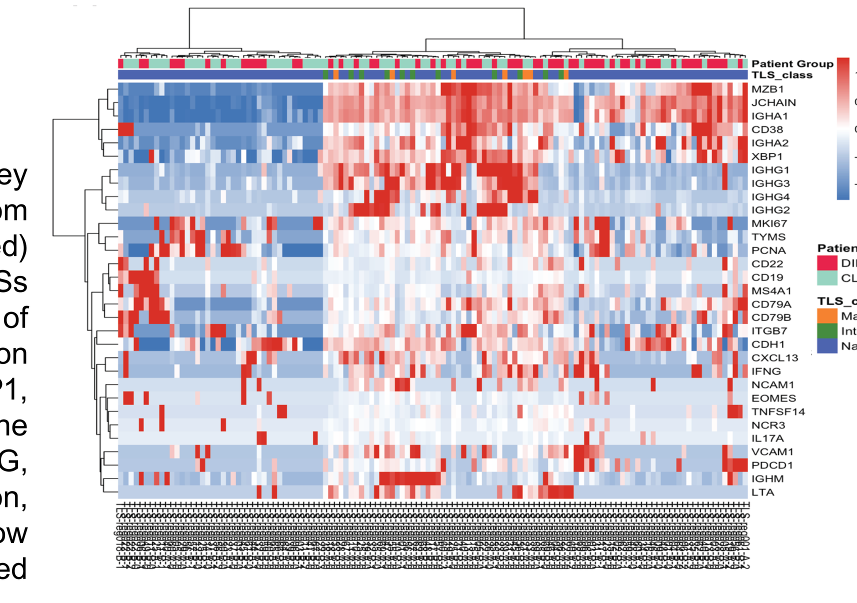

Figure 16. Gene expression patterns across TLS maturation in CRC
Heatmap showing key genes across TLSs from CLR (teal) and DII (red) patients. CLR TLSs display strong induction of B-cell differentiation (MZB1, XBP1, IGHA/IGHG) and immune activation (CXCL13, IFNG, PDCD1) with maturation, whereas DII TLSs show weaker, unstructured transcriptional programs.

Conclusions & Discussion

Conclusion

- TLS maturation in colorectal cancer involves **coordinated stromal**, **vascular**, and **immune remodeling**.
- Mature CLR TLSs form structured immune niches with αSMA⁺/Collagen IV⁺ capsules, boundary-restricted signaling (β-catenin, EGFR, BCL-2), and vascular exclusion.
- **DII TLSs** remain diffuse, lacking spatial and transcriptional compartmentalization.
- MaxFuse Integrated spatial proteomics and scRNA-seq revealed a shift from proliferative (MKI67⁺) nascent TLSs to plasma cell–rich (MZB1⁺, XBP1⁺) mature TLSs with enhanced immune effector activity.

Discussion

- Further work is needed to validate marker–cell type specificity and replicate findings in independent datasets.
- Future studies should integrate matched genomic and proteomic data from the same patients for deeper mechanistic insight.

Contact

[Afrooz Jahedi]
[University of Texas MD Anderson Cancer center]
[ajahedi@mdanderson.org]

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