Cell

Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging

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SUMMARY

A highly multiplexed cytometric imaging approach, termed co-detection by indexing (CODEX), is used here to create multiplexed datasets of normal and lupus (MRL/lpr) murine spleens. CODEX iteratively visualizes antibody binding events using DNA barcodes, fluorescent dNTP analogs, and an in situ polymerization-based indexing procedure. An algorithmic pipeline for single-cell antigen quantification in tightly packed tissues was developed and used to overlay well-known morphological features with de novo characterization of lymphoid tissue architecture at a single-cell and cellular neighborhood levels. We observed an unexpected, profound impact of the cellular neighborhood on the expression of protein receptors on immune cells. By comparing normal murine spleen to spleens from animals with systemic autoimmune disease (MRL/lpr), extensive and previously uncharacterized splenic cell-interaction dynamics in the healthy versus diseased state was observed. The fidelity of multiplexed spatial cytometry demonstrated here allows for quantitative systemic characterization of tissue architecture in normal and clinically aberrant samples.

Keywords

multiplexed imaging, multidimensional imaging, microenvironment, tissue architecture, autoimmunity, immune tissue

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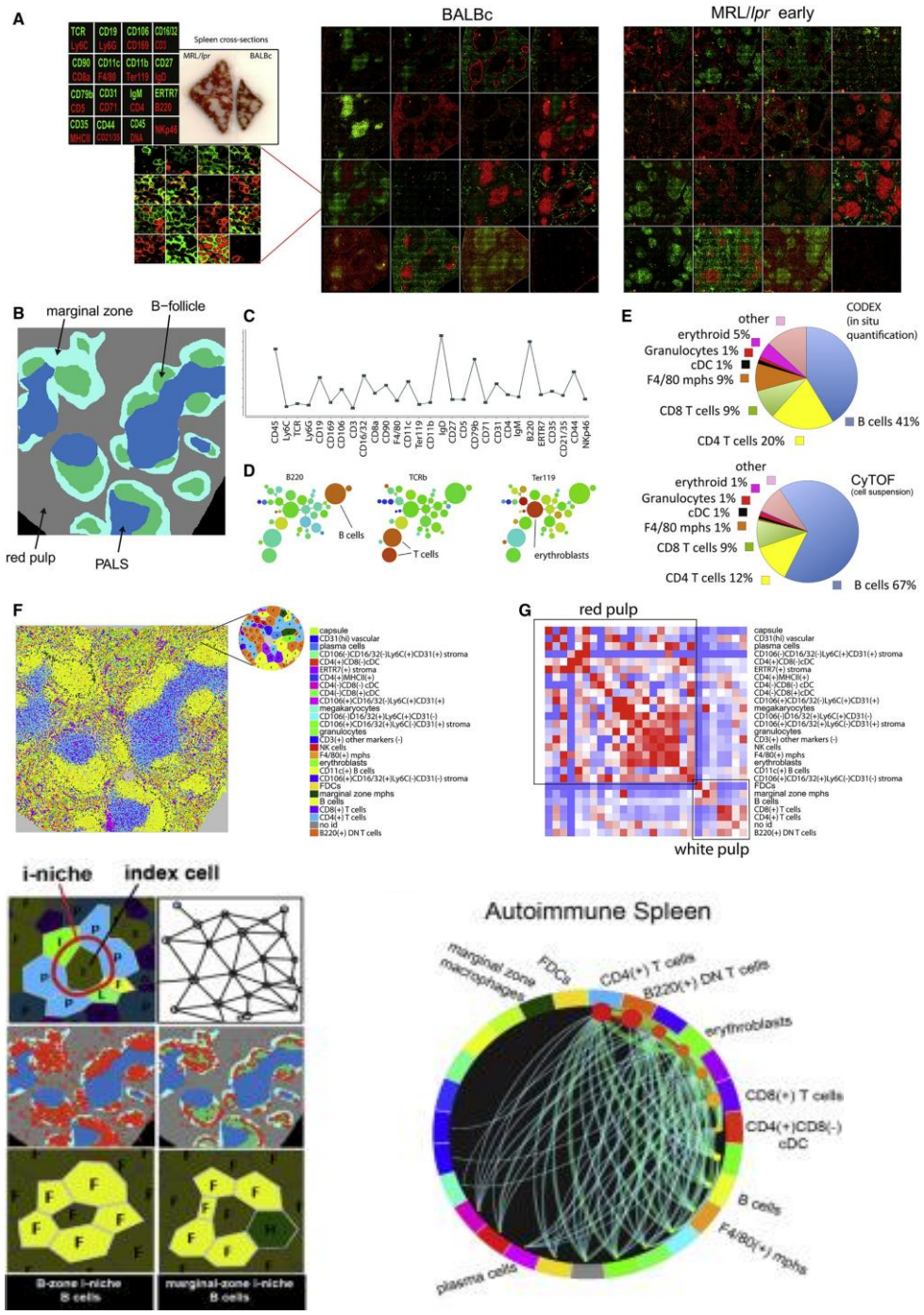


Cell

Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front

Christian M. Schürch [1, 2, 6], Salil S. Bhate [1, 2, 3, 6], Graham L. Barlow [1, 2, 6], Darci J. Phillips [1, 2, 4, 6], Luca Noti [5], Inti Zlobec [5], Pauline Chu [1, 2], Sarah Black [1, 2], Janos Demeter [1], David R. McIlwain [1, 2], Shigemi Kinoshita [1], Nikolay Samusik [1], Yury Goltsev [1, 2], Garry P. Nolan [1, 2, 7]

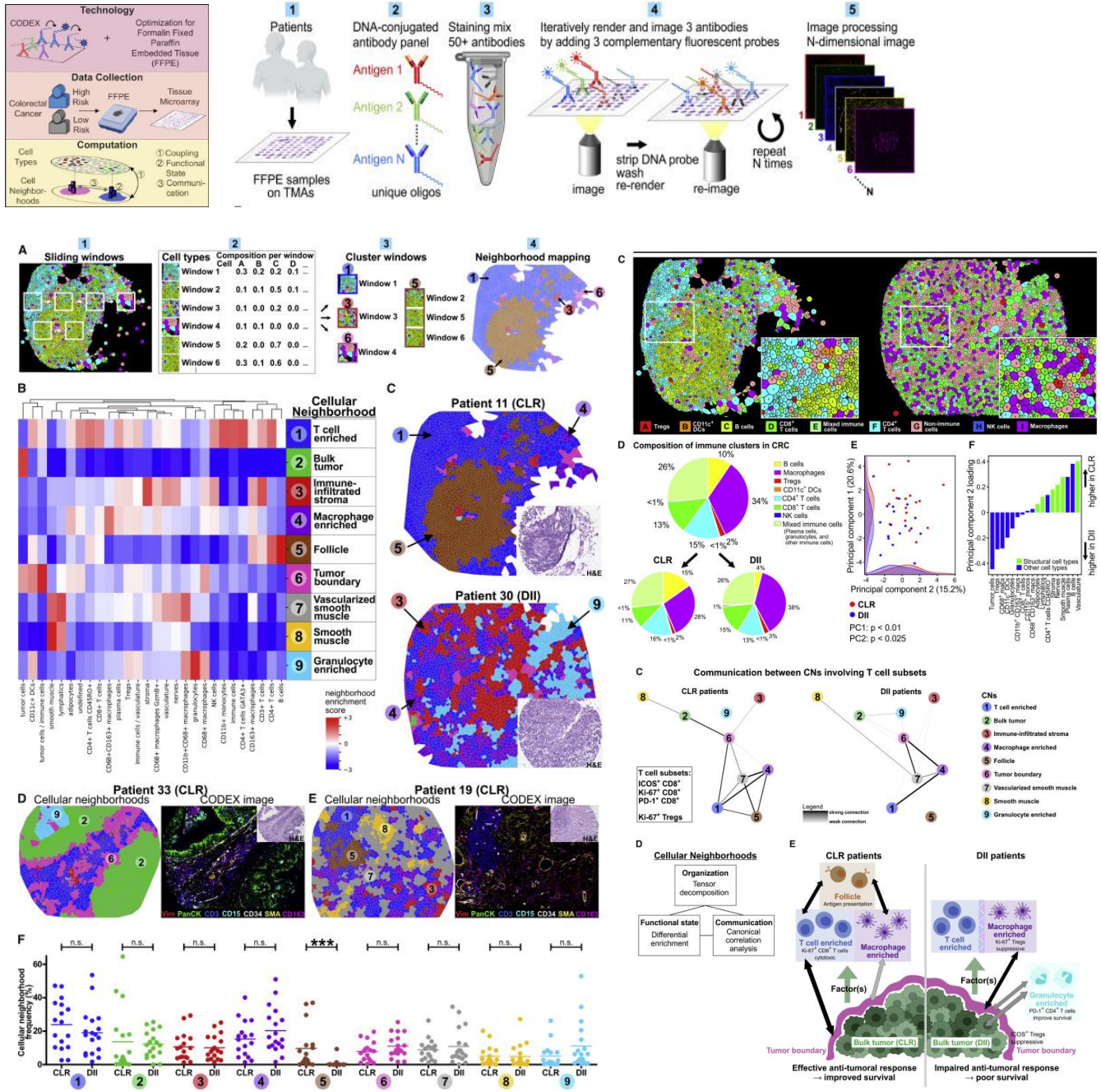
SUMMARY

Antitumoral immunity requires organized, spatially nuanced interactions between components of the immune tumor microenvironment (iTME). Understanding this coordinated behavior in effective versus ineffective tumor control will advance immunotherapies. We optimized CO-Detection by indEXing (CODEX) for para ffin-em bedded tissue microarrays, enabling profiling of 140 tissue regions from 35 advanced-stage colorectal cancer (CRC) patients with 56 protein markers simultaneously. We identified nine conserved, distinct cellular neighborhoods (CNs)–a collection of components characteristic of the CRC iTME. Enrichment of PD-1^{+}CD4^{+} T cells only within a granulocyte CN positively correlated with survival in a high-risk patient subset. Coupling of tumor and immune CNs, fragmentation of T cell and macrophage CNs, and disruption of inter-CN communication was associated with inferior outcomes. This study provides a framework for interrogating complex biological processes, such as antitumoral immunity, demonstrating an example of how tumors can disrupt imm une functionality through interference in the concerted action of cells and spatial domains.

Keywords

Antitumoral immunity, cellular neighborhoods, CODEX, colorectal cancer, FFPE, immune checkpoints, immune tumor microenvironment, multiplexed imaging, tertiary lymphoid structures, tissue architecture

DOI: 10.1016/j.cell.2020.07.005



bioRxiv
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Single-Cell Transcriptomic Analysis of mIHC Images via Antigen Mapping

Kiya W. Govek, Emma C. Troisi, Zhen Miao, Steven Woodhouse, Pablo G. Camara

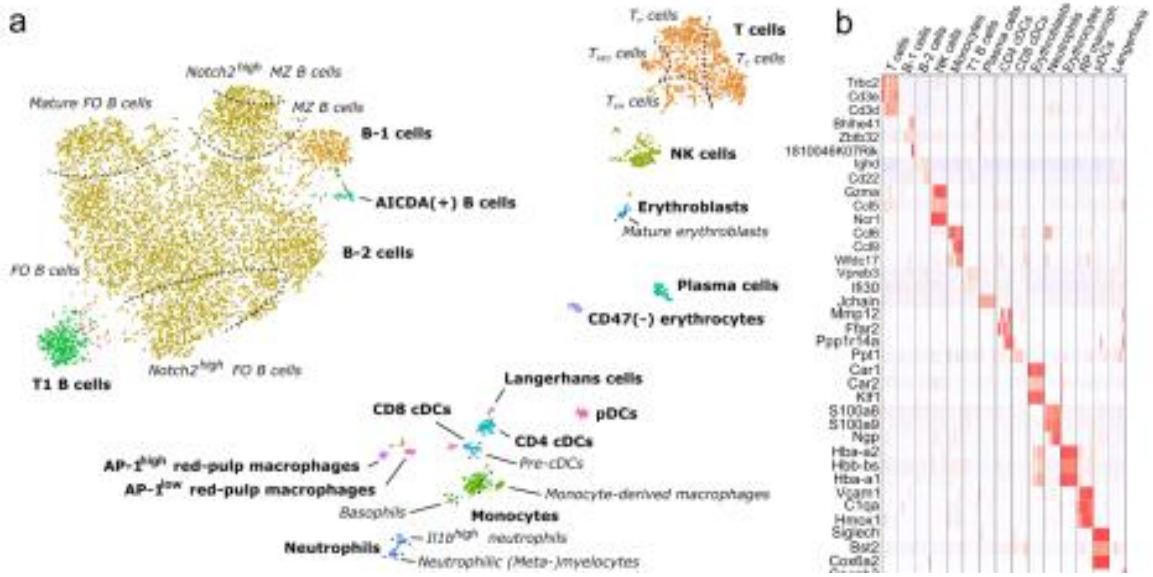
Abstract

Highly-multiplexed immunohistochemistry (mIHC) enables the staining and quantification of dozens of antigens in a tissue section with single-cell resolution. However, annotating cell populations that differ little in the profiled antigens or for which the antibody panel does not include specific markers is challenging. To overcome this obstacle, we have developed an approach for enriching mIHC images with single-cell RNA-seq data, building upon recent experimental procedures for augmenting single-cell transcriptomes with concurrent antigen measurements. Spatially-resolved Transcriptomics via Epitope Anchoring (STvEA) performs transcriptome-guided annotation of highly-multiplexed cytometry datasets. It increases the level of detail in histological analyses by enabling annotation of subtle cell populations, spatial patterns of transcription, and interactions between cell types. More generally, it enables the systematic annotation of cell populations in cytometry data. We demonstrate the utility of STvEA by uncovering the architecture of poorly characterized cell types in the murine spleen using published highly-multiplexed cytometry and mIHC data.

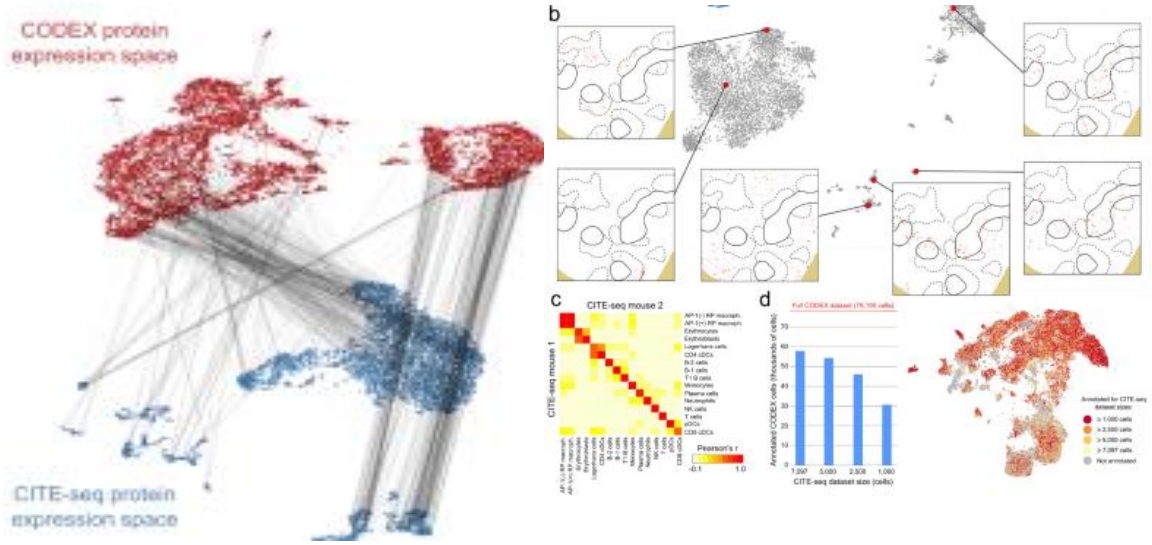
Keywords

CITE-seq, CODEX, RNAscope, immune cellular architecture, co-localization pattern, cell population interactions, mapping, mIHC

doi: https://doi.org/10.1101/672501



A high-resolution CITE-seq atlas of the murine spleen.



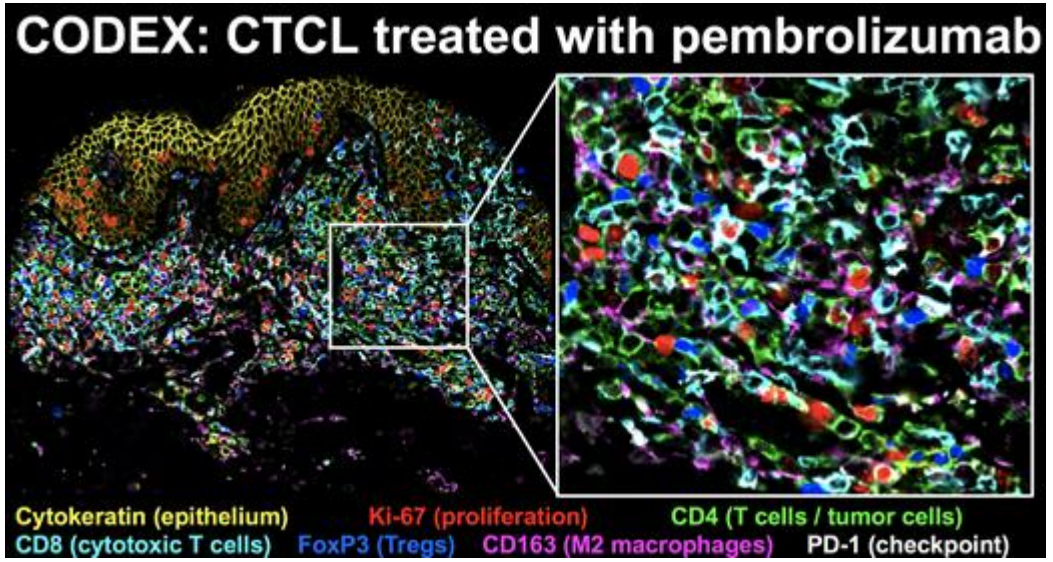
Mapping of the splenic CITE-seq atlas into histology sections profiled with CODEX.

blood

Dynamics of the Cutaneous T Cell Lymphoma Microenvironment in Patients Treated with Pembrolizumab Revealed By Highly Multiplexed Tissue Imaging

Christian M. Schuerch, MD PhD , Darci J. Phillips, MD PhD , Salil S. Bhate, MMath , Graham L. Barlow, BA , Steven P Fling, PhD , Nirasha Ramchurren , Robert Pierce, MD , Martin A Cheever, MD , Michael S. Khodadoust, MD PhD , Youn H. Kim, MD , Garry P. Nolan, PhD

Cutaneous T cell lymphoma (CTCL) is a CD4⁺ T cell malignancy of the skin with heterogeneous outcomes and limited treatment options. Monoclonal antibodies directed against PD-1, such as pembrolizumab, have shown impressive efficacy in multiple advanced malignancies, and are currently tested in clinical trials in patients with CTCL. Initial data indicate that about half of the patients experience treatment response, whereas the other half are non-responders. Non-responders can be further divided into patients with stable disease versus rapid progressors. It is currently unknown why some CTCL patients respond to pembrolizumab while others rapidly progress, and no predictive biomarkers are available. Single-cell analysis approaches to identify biomarkers of response, for example quantifying the expression of PD-1 on tumor cells vs. reactive immune cells, have not enabled stratification of patients. We therefore hypothesized that more complex spatial cellular interactions within the immune tumor microenvironment (iTME) of CTCL could provide insight into the mechanisms of pembrolizumab response and enable prediction. We applied CODEX (CO-Detection by indEXing) highly multiplexed tissue imaging to study the CTCL iTME in matched biopsies before and after pembrolizumab therapy in 7 responders and 7 non-responders (see the Figure). Using 54 markers simultaneously allowed discriminating malignant CD4⁺ tumor cells from reactive CD4⁺ T cells and identified 30 different cell clusters with spatial information, including an M2 macrophage cluster that was enriched in non-responders before therapy. Unexpectedly, in pembrolizumab responders compared to non-responders, PD-1 expression levels were higher in multiple clusters of tumor cells and reactive T cells. Computational spatial analysis revealed ten distinct, conserved cellular neighborhoods in the CTCL iTME that changed in composition and frequency during therapy. Interestingly, one cellular neighborhood to be presented dramatically increased after therapy only in responders. Therefore, highly multiplexed spatial analysis of the CTCL iTME allows discovering novel, predictive biomarkers of immunotherapy response and will pave the way for future studies that functionally address the identified cell types and cellular interactions.



Keywords
diagnostic imaging, lymphoma, t-cell, cutaneous, pembrolizumab, biological markers, tumor cells, biopsy, cancer, advanced, immunotherapy, monoclonal antibodies, skin cancer

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Landscape of infiltrating B cells and their clinical significance in human hepatocellular carcinoma

Zhao Zhang, Lijie Ma, Shyamal Goswami, Jiaqiang Ma, Bohao Zheng, Meng Duan, ...show all

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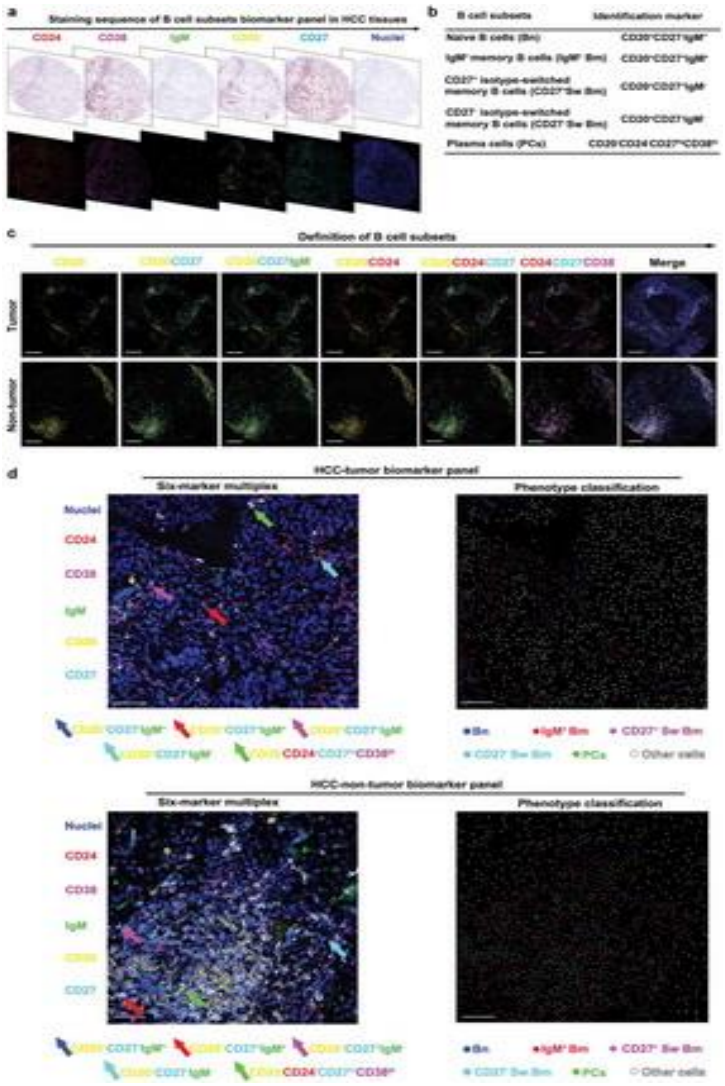
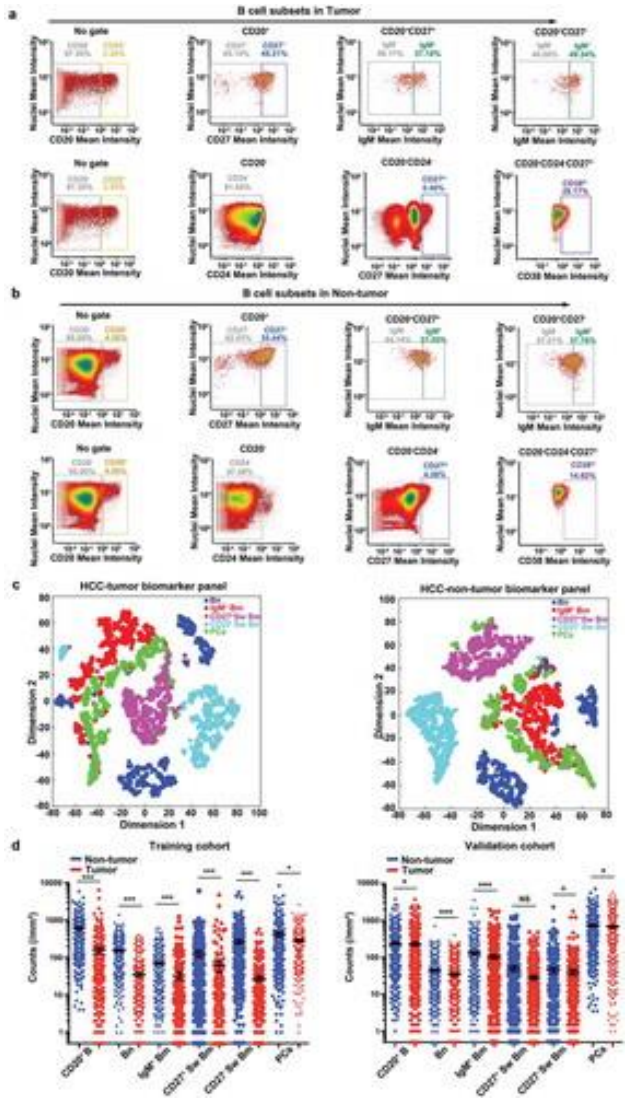
Published on Oncoimmunology.

Hepatocellular Carcinoma/Immuno-Oncology

Institute Pasteur of Shanghai, CAS

- System(s): CODEX, MAV
- Markers: CD20, CD24, CD27, CD38, IgM, and DAPI
- Keywords: Hepatocellular carcinoma (HCC),TMA
- purpose: To provide a comprehensive view of tumor-infiltrating B cells in HCC, fluorescent multiplexed immunohistochemistry was used to determine the subset, density, and distribution of B cells in tumor tissues and paired non-tumor liver tissues. Furthermore, flow cytometry analysis was used to validate the immunohistochemistry results.





nature

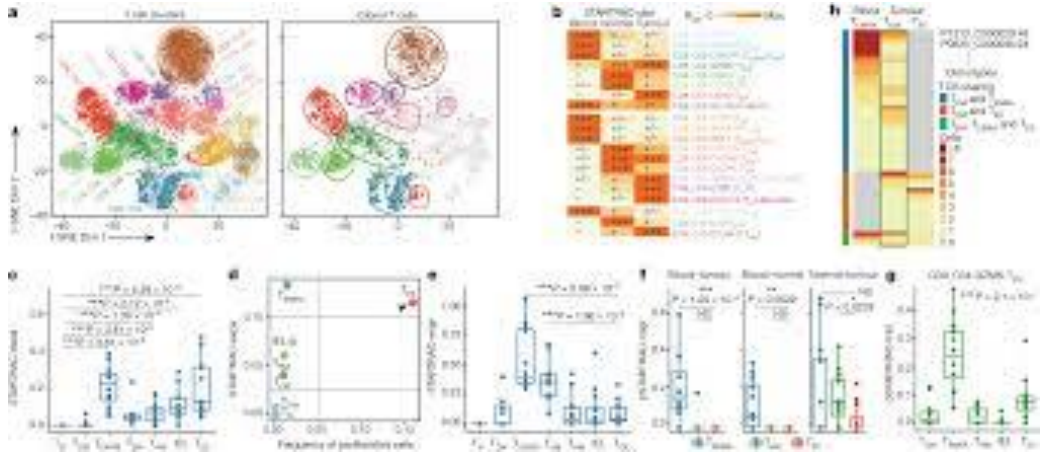
Published on Nature, 2018 October 29. IF= 43

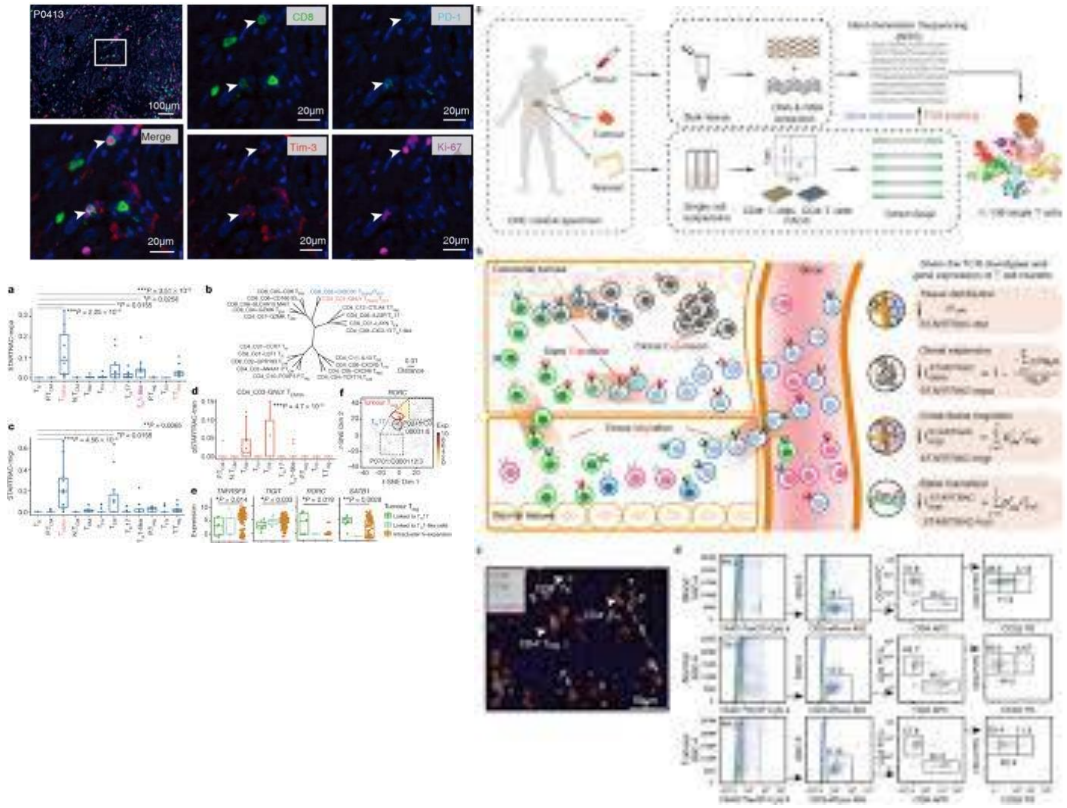
Colorectal Cancer/NGS+Phenoptics

Lineage tracking reveals dynamic relationships of T cells in colorectal cancer

Lei Zhang, Xin Yu, Liangtao Zheng, Yuanyuan Zhang, Yansen Li, Qiao Fang, Ranran Gao, Boxi Kang, Qiming Zhang, Julie Y. Huang, Hiroyasu Konno, Xinyi Guo, Yingjiang Ye, Songyuan Gao, Shan Wang, Xueda Hu, Xianwen Ren, Zhanlong Shen ✉, Wenjun Ouyang ✉ & Zemin Zhang ✉

Nature **564**, 268–272(2018) | Cite this article





- System(s): CODEX, MAV
- Markers: CD8,CD4, GZMK, EOMES, RUNX3, CXCL13, BHLHE40 etc…
- Keywords: colorectal cancer
- purpose: To investigate the dynamic relationships among 20 identified T cell subsets with distinct functions and clonalities.

Cell
Volume 179, Issue 4, 31 October 2019, Pages 829-845.e20

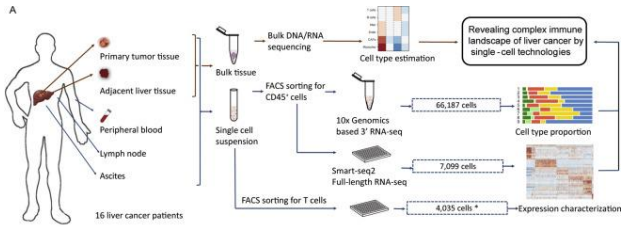
Article

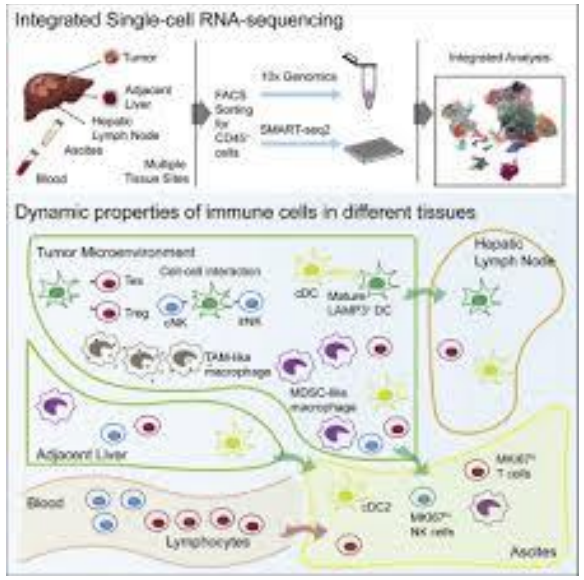
Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma

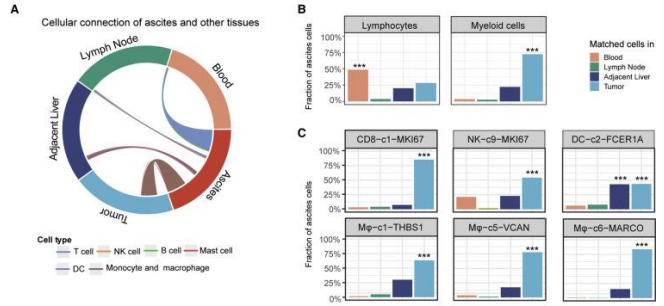
Qiming Zhang [1, 14], Yao He [2, 14], Nan Luo [3, 4, 14], Shashank J. Patel [5], Yanjie Han [1], Ranran Gao [1], Madhura Modak [6], Sebastian Carotta [7], Christian Haslinger [8], David Kind [8], Gregory W. Peet [5], Guojie Zhong [1], Shuangjia Lu [1], Weihua Zhu [9], Yilei Mao [10], Mengmeng Xiao [11], Michael Bergmann [12], Xueda Hu [1] ... Zemin Zhang [1, 2, 15]

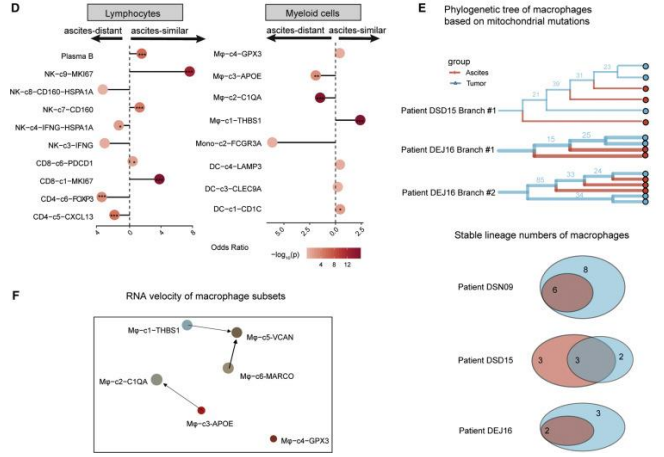
Hepatocellular Carcinoma NGS+Phenoptics (10x Genomics and SMART-seq2)

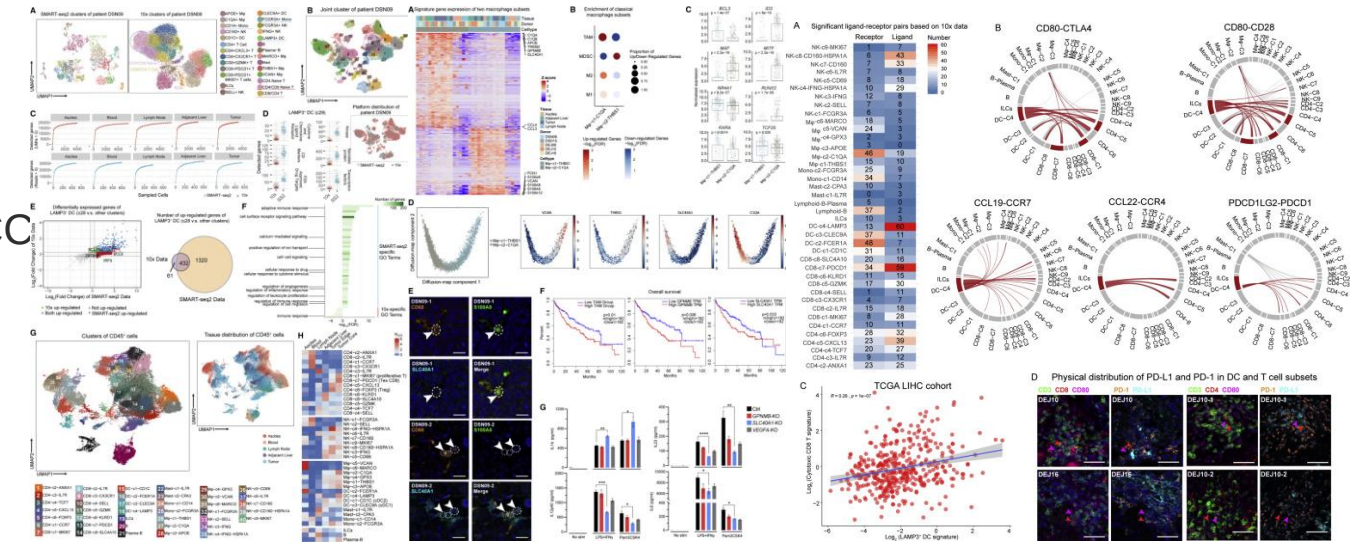
- System(s): CODEX, MAV
- Markers: CD4, CD8,CD45, CX3CR1, GZMK, LAMP3, CLEC9A, CD14, and FCGR3A
- Keywords: HepThe combination of the two scRNA-seq technologies and the inclusion of LNs and ascites allowed us to not only characterize the immune composition of HCC with high resolution but also to trace their dynamics.
- atocellular carcinoma (HCC),TMA
- purpose:











Cell

Article

Stress-Induced Metabolic Disorder in Peripheral CD4+ T Cells Leads to Anxiety-like Behavior

Ke-qi Fan,[1,9] Yi-yuan Li,[1,9] Hao-li Wang,[1] Xin-tao Mao,[1] Jin-xin Guo,[1] Fei Wang,[1] Ling-jie Huang,[2] Yi-ning Li,[1] Xiang-yu Ma,[3,4,5] Zheng-jun Gao,[1] Wei Chen,[6] Dan-dan Qian,[3] Wen-jin Xue,[3] Qian Cao,[2] Lei Zhang,[2] Li Shen,[1] Long Zhang,[1] Chao Tong,[1] Jiang-yan Zhong,[1] Wei Lu,[3] Ling Lu,[7] Ke-ming Ren,[2] Guisheng Zhong,[8] Yuan Wang,[6] Mingliang Tang,[3] Xin-Hua Feng,[1] Ren-jie Chai,[3,4,5,*] and Jin Jin[1,2,10,*]

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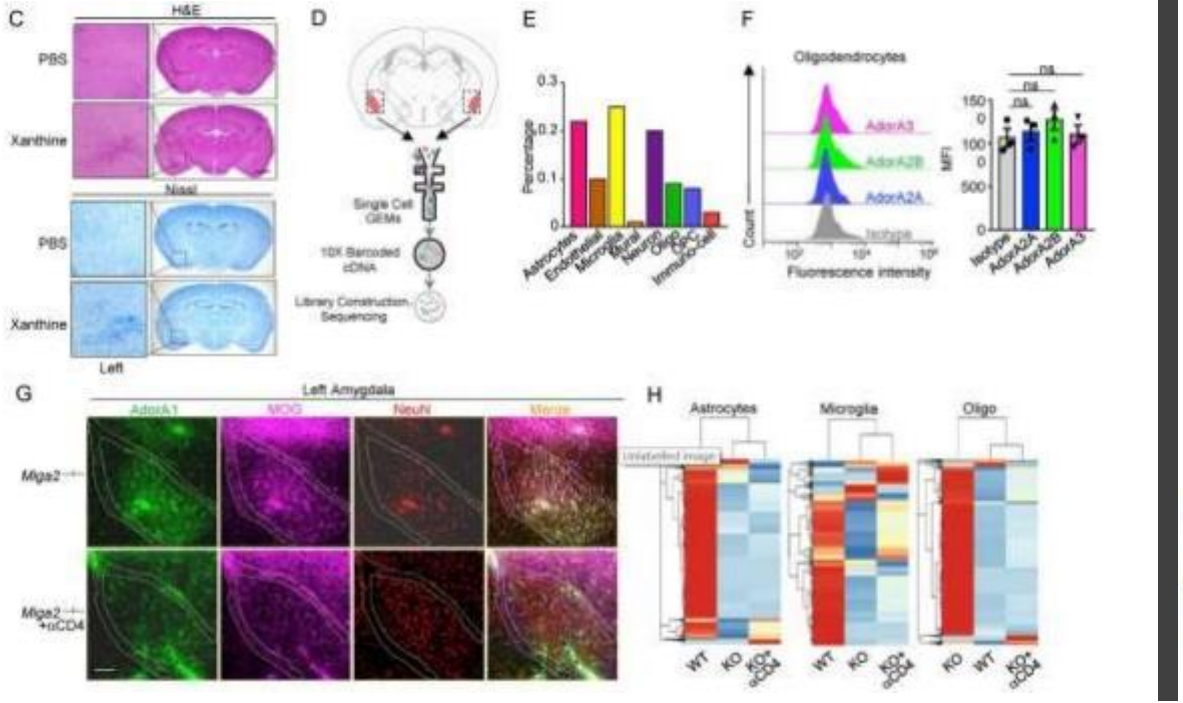
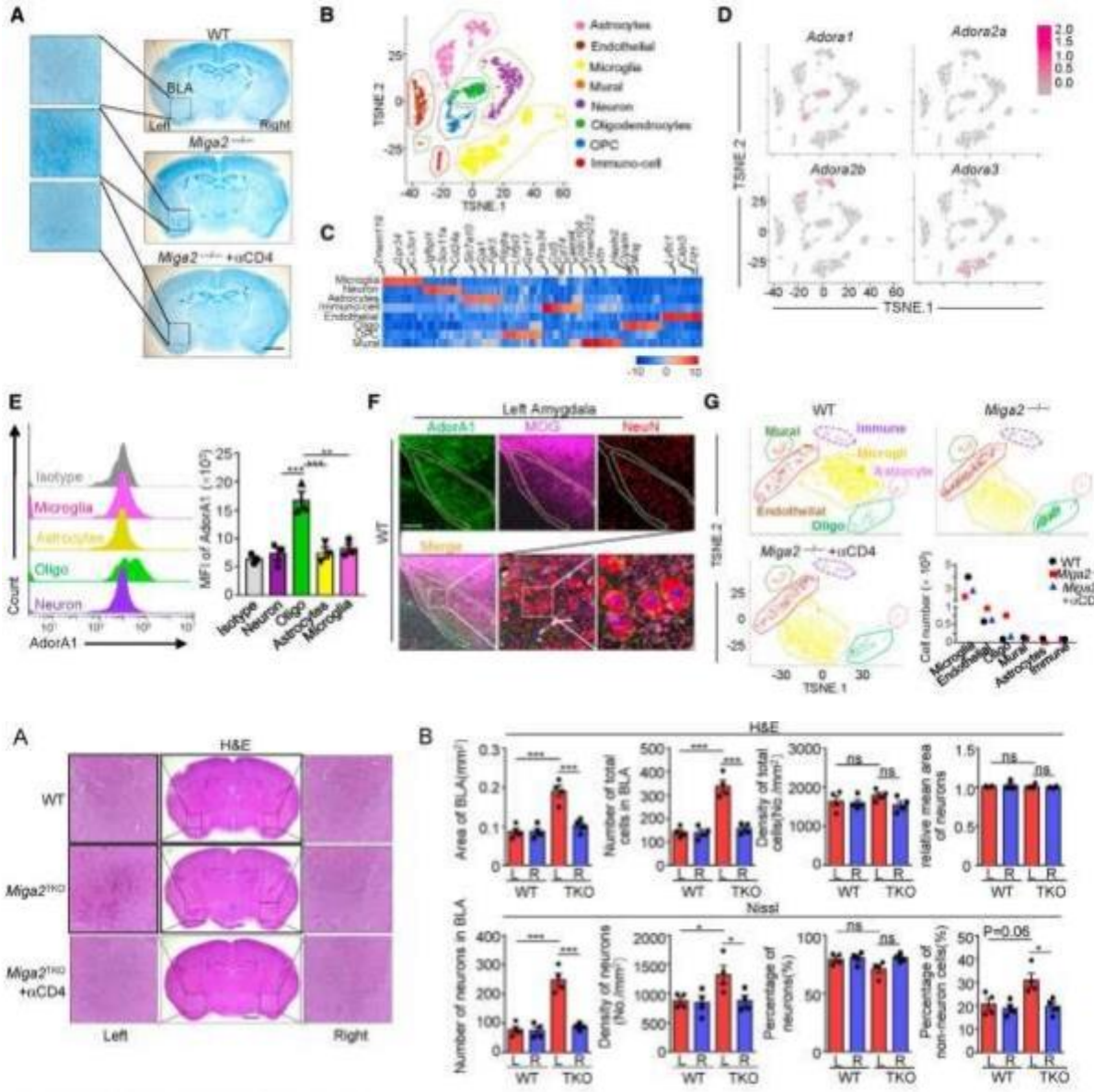
Neurosciences/Beyond I-O

Zhejiang University





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