

Introduction of Single-Cell Analysis: A Different Perspective in Glioblastoma

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Adult diffuse gliomas (膠質瘤)

WHO CNS classification (2021):

- **IDH-mutant astrocytoma:**
IDH1, IDH2, ATRX, TP53, CDKN2A/B
- **IDH-mutant oligodendroglioma:**
IDH1, IDH2, 1p/19q, TERT promoter, CIC, FUBP1, NOTCH1
- **IDH-wt glioblastoma (GBM):**
TERT promoter, chromosomes 7/10, EGFR

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Identification of hypoxic macrophages in glioblastoma with therapeutic potential for vasculature normalization

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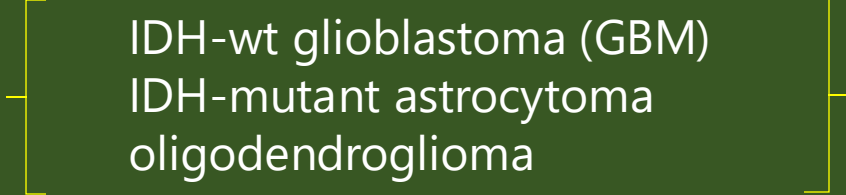
Neuro-Oncology

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The 2021 WHO Classification of Tumors of the Central Nervous System: a summary

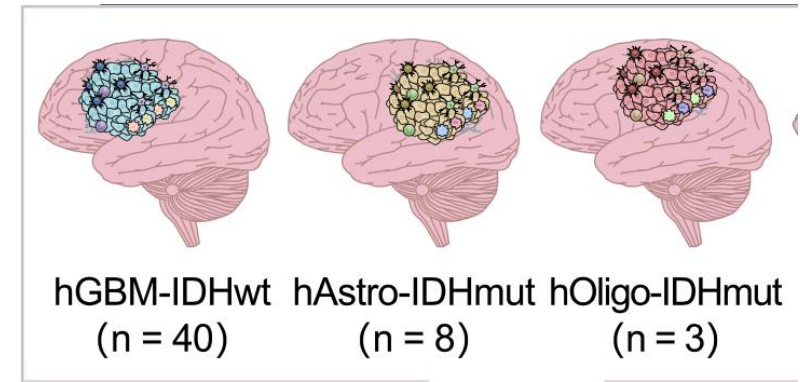
David N. Louis, Arie Perry, Pieter Wesseling[✉], Daniel J. Brat[✉], Ian A. Cree, Dominique Figarella-Branger, Cynthia Hawkins, H. K. Ng, Stefan M. Pfister, Guido Reifenberger, Riccardo Soffietti, Andreas von Deimling, and David W. Ellison

Introduction

1	Diffuse gliomas	 IDH-wt glioblastoma (GBM) IDH-mutant astrocytoma oligodendroglioma	malignant brain tumors
2	Tumor-associated macrophages (TAMs)	 - prevailing in diffuse gliomas - fuel tumor progression - architect of the immune-suppressive microenvironment	
3	TAM-targeting approaches		No compelling efficacy

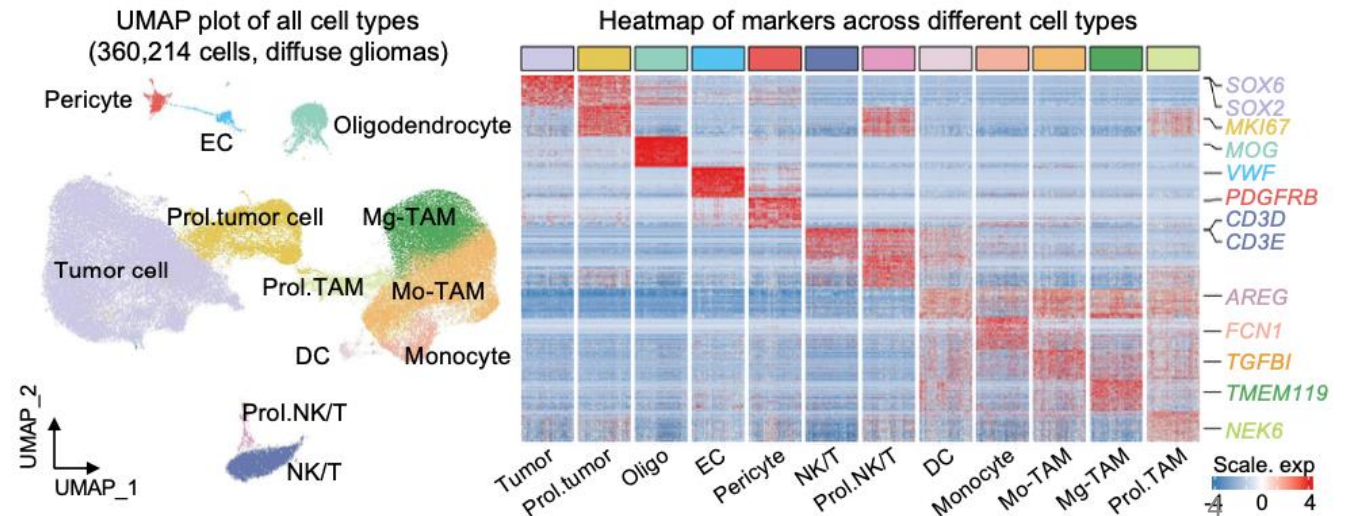
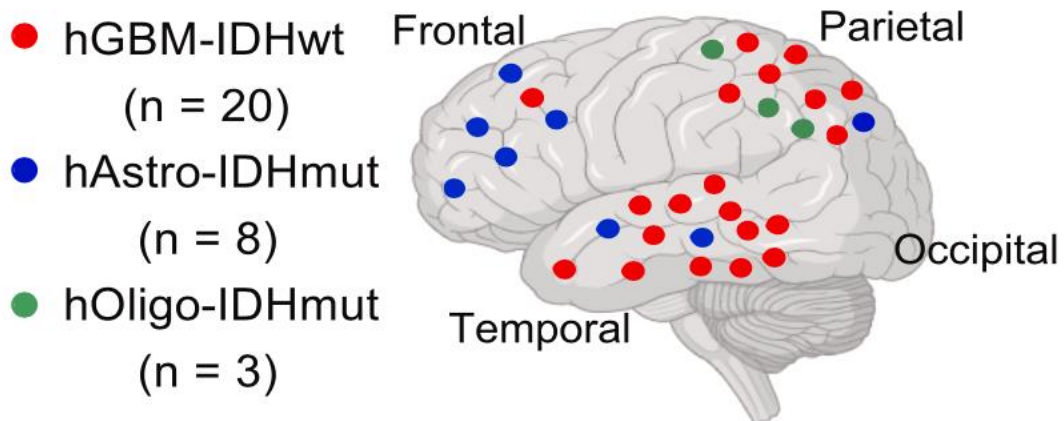
Introduction - Study Design

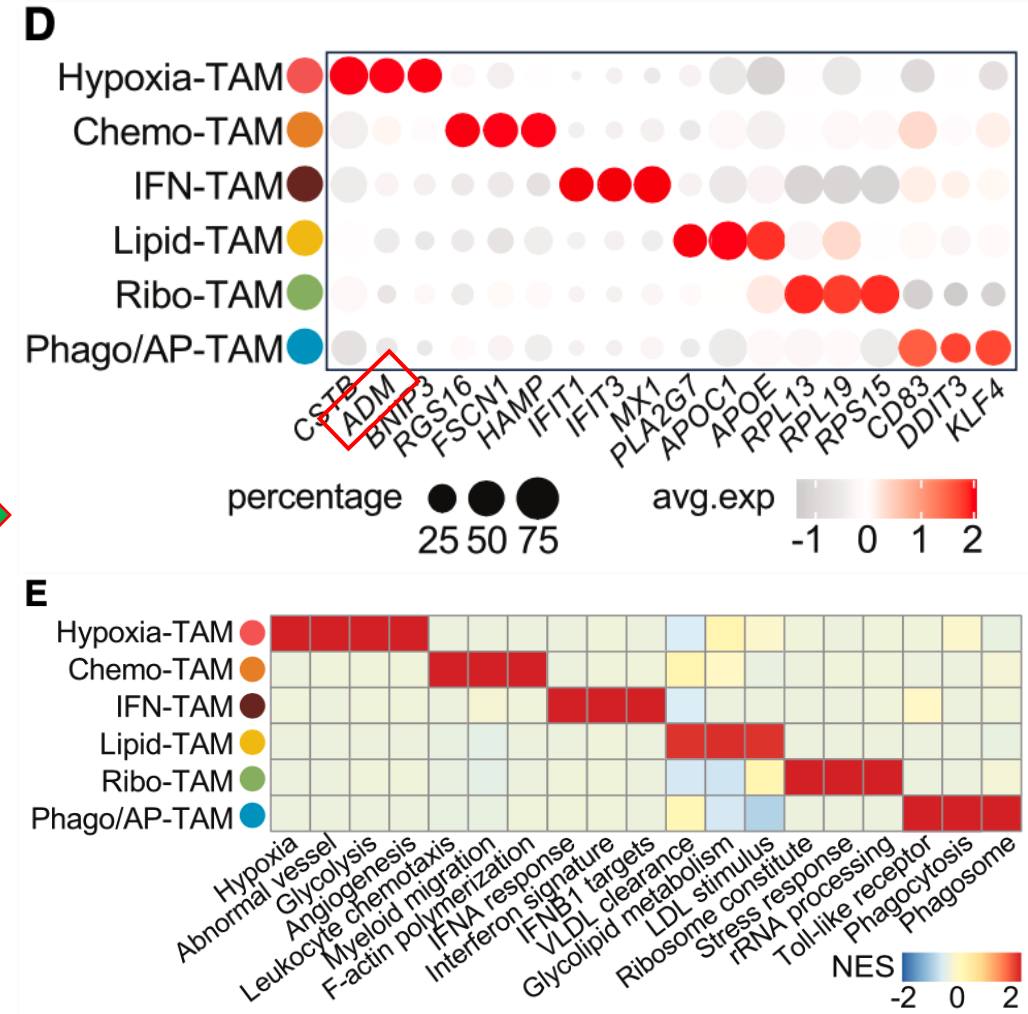
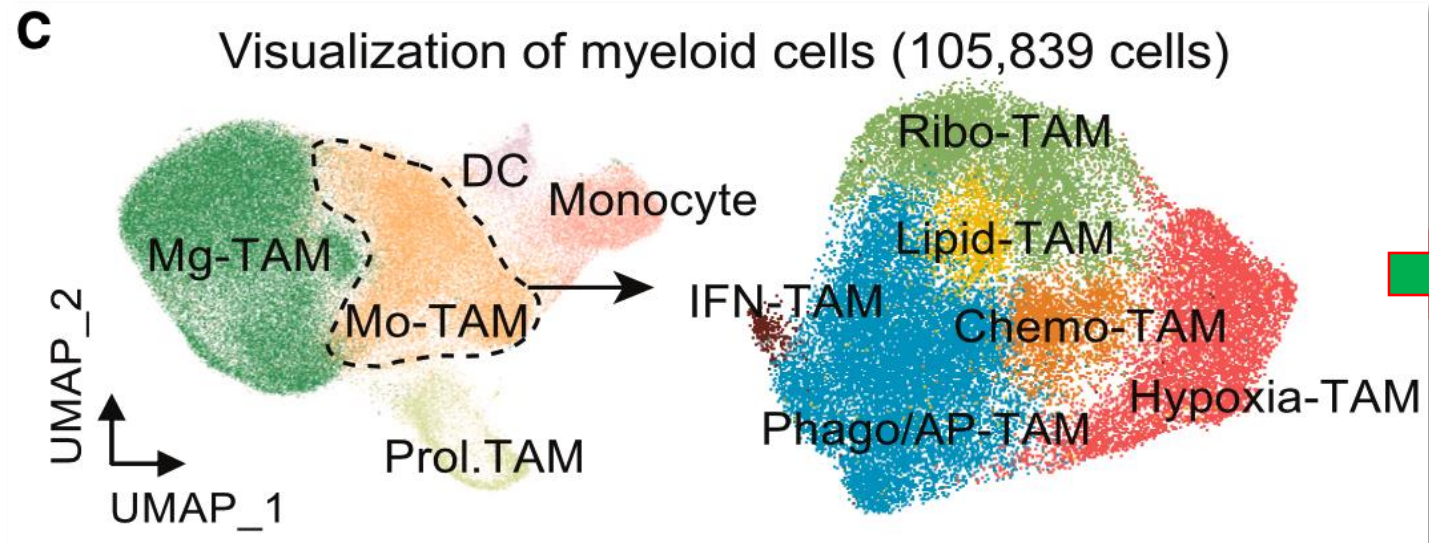
- 51 cases of diffuse gliomas in human (hGMB):



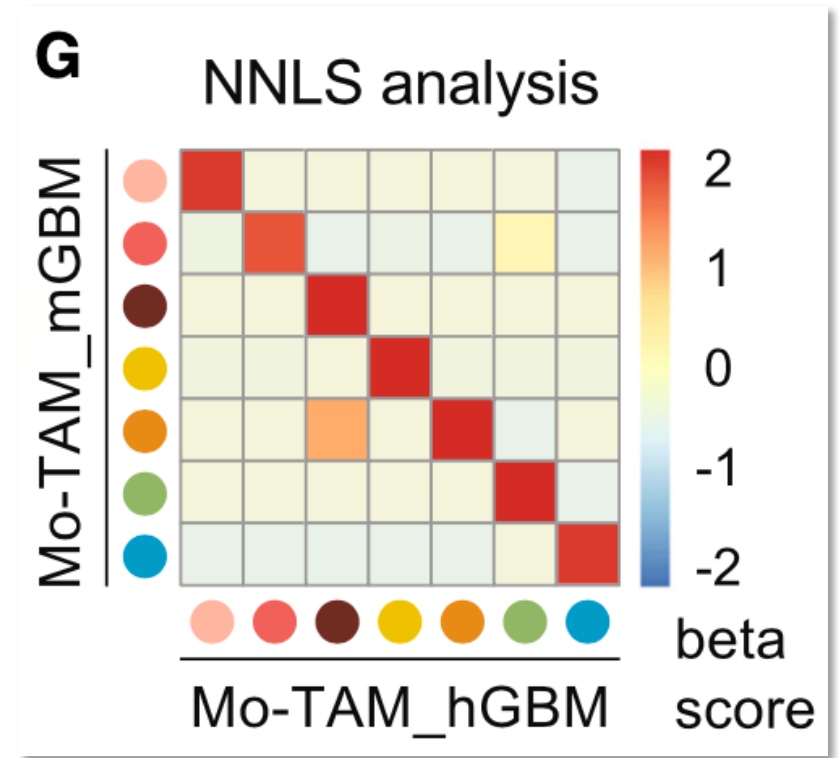
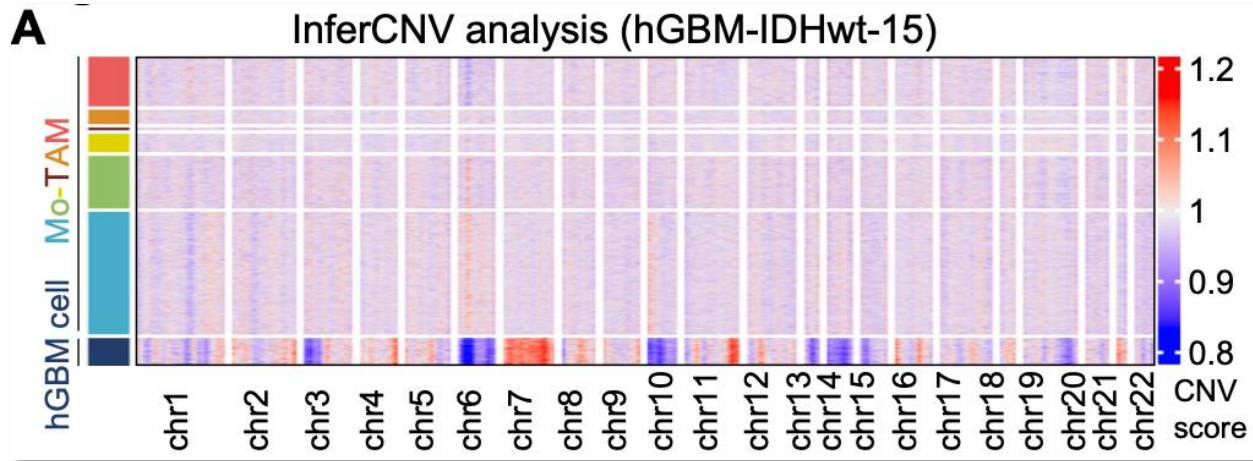
- 31 glioma specimens of our dataset were located in different lobes of the cerebrum
- identified 360,214 single cells organized into 12 main lineages

Sample location (our dataset)

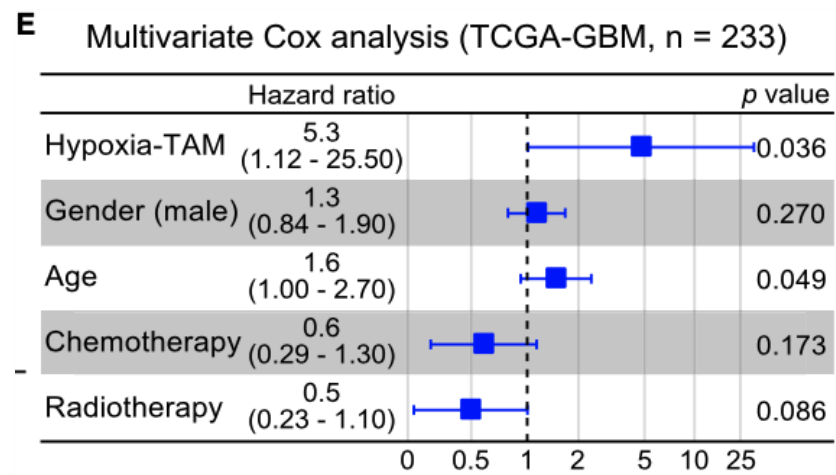
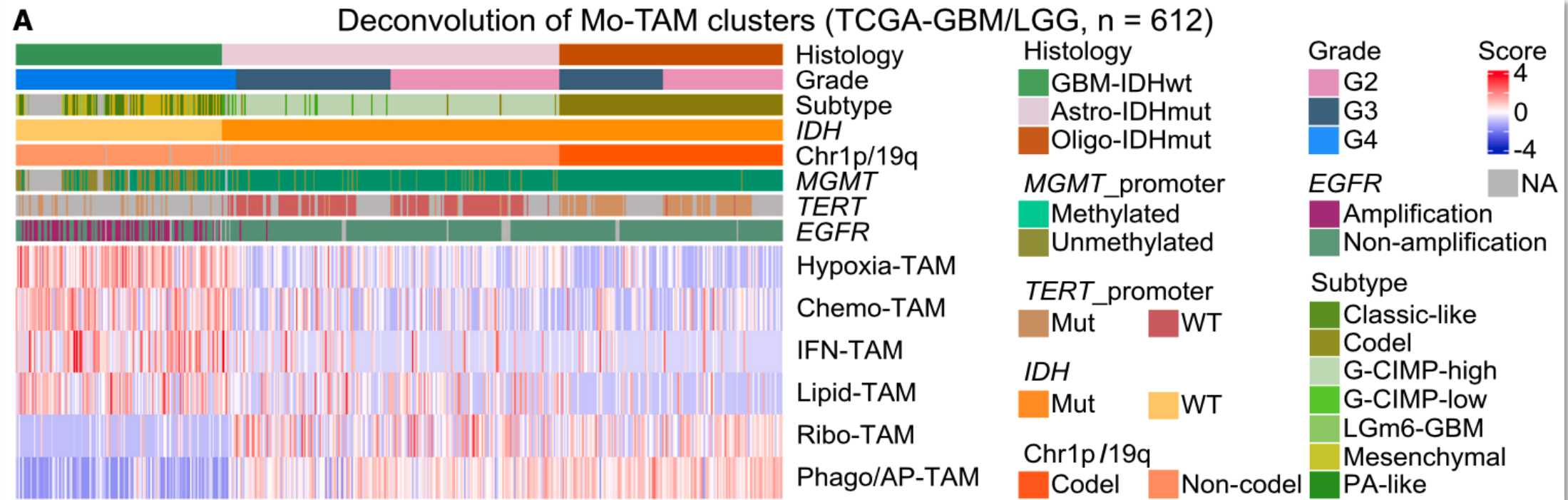




Mo-TAM clusters across diffuse gliomas are largely conserved but with remarkable differences in cellular composition

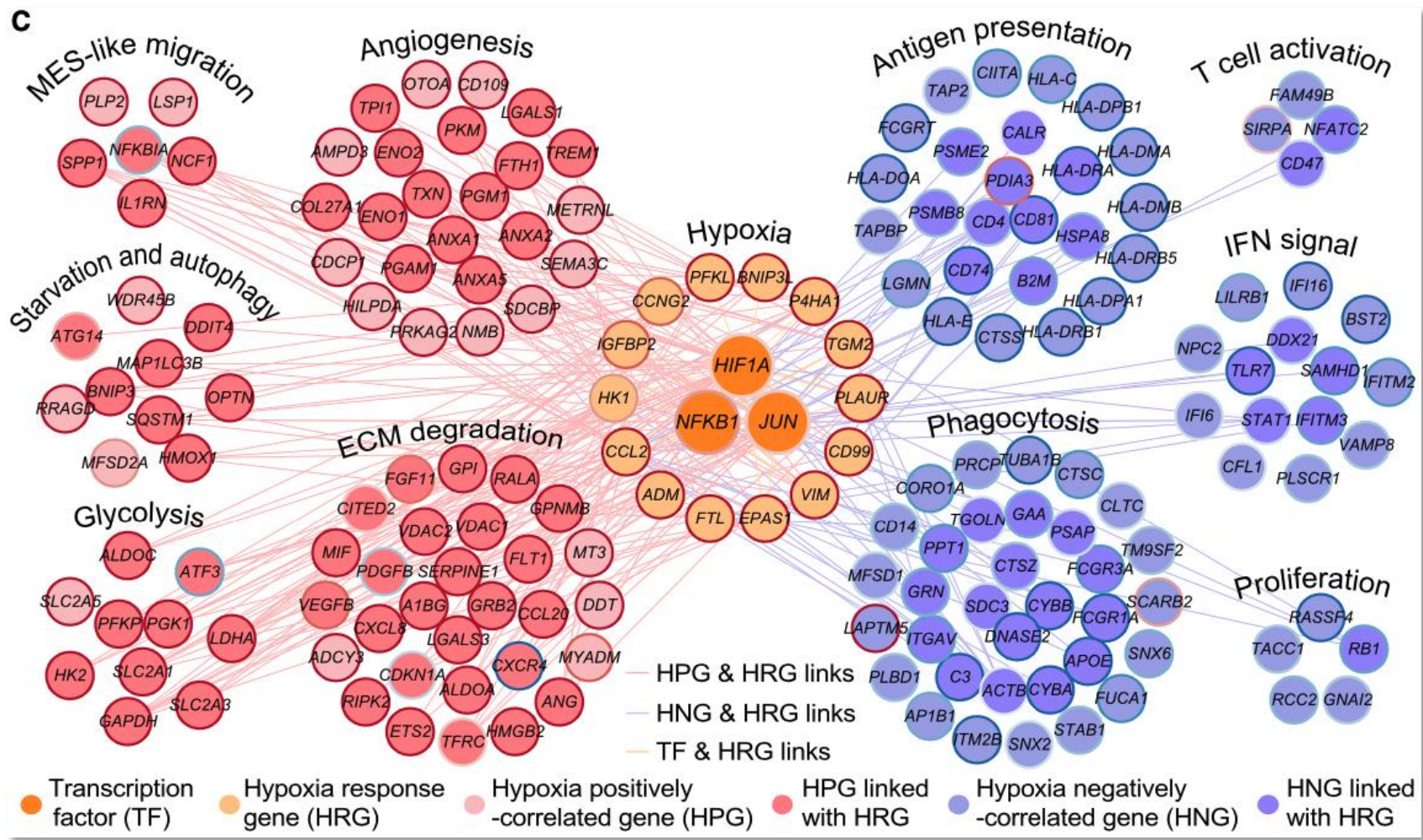


Clinical implications of Mo-TAM clustering

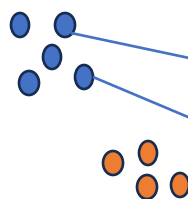


Hypoxia positively correlated genes

Hypoxia negatively correlated genes



Hypoxia-TAM



	Hypoxia Score	gene 1	gene 2
Hypoxia-TAM 1			
Hypoxia-TAM 2			

Correlation

Top100 genes

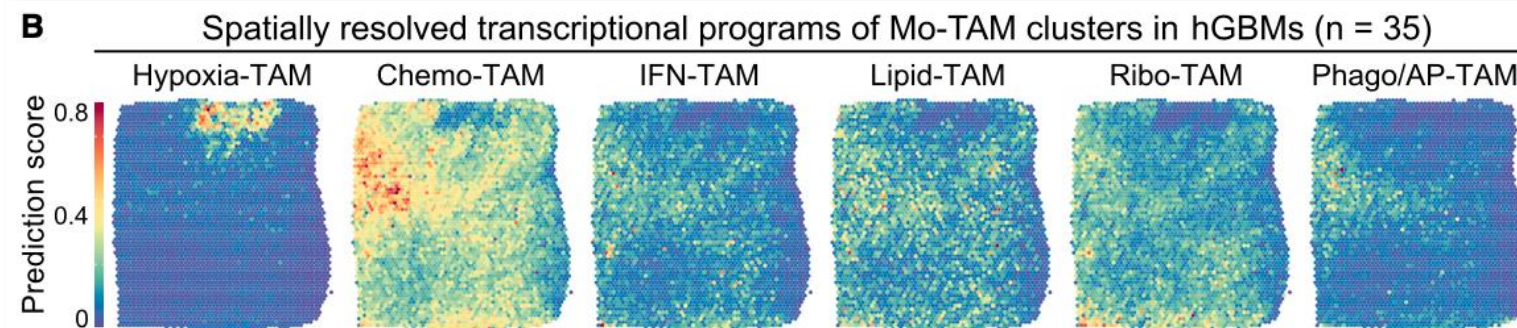
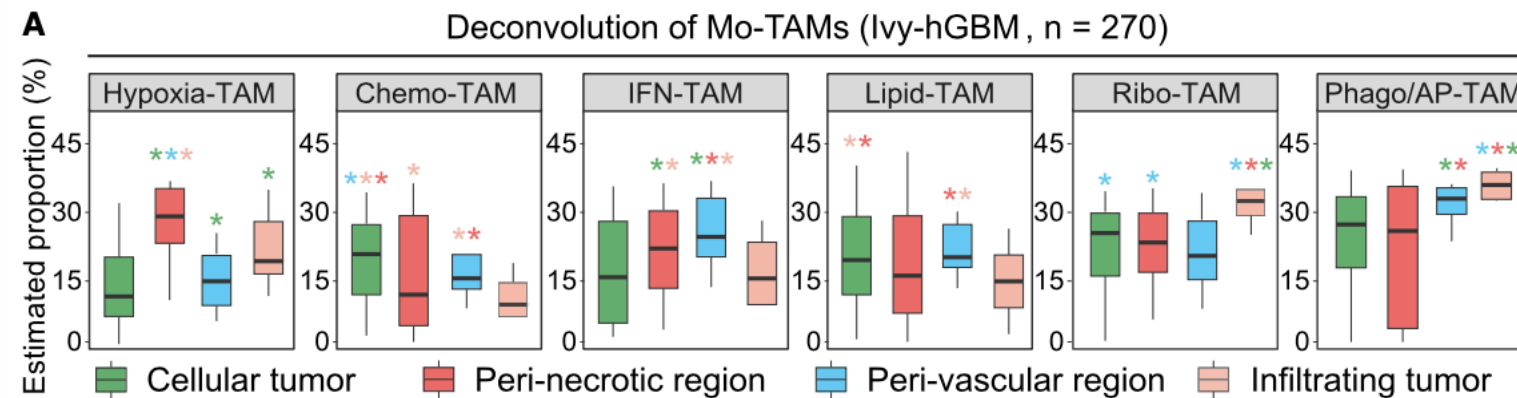
Hypoxia-TAM DEG

Where is Hypoxia-TAM?

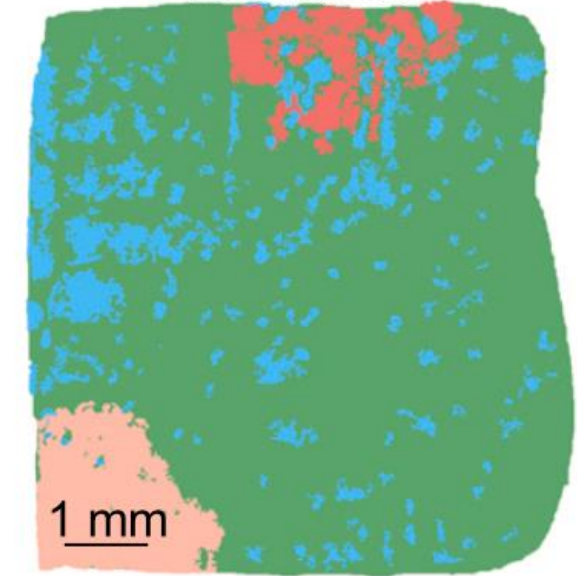
- **Ivy-hGBM (bulk RNA-seq)**
 - Cellular tumor
 - Peri-necrotic region
 - Peri-vascular region
 - Infiltrating tumor

IVY GAP

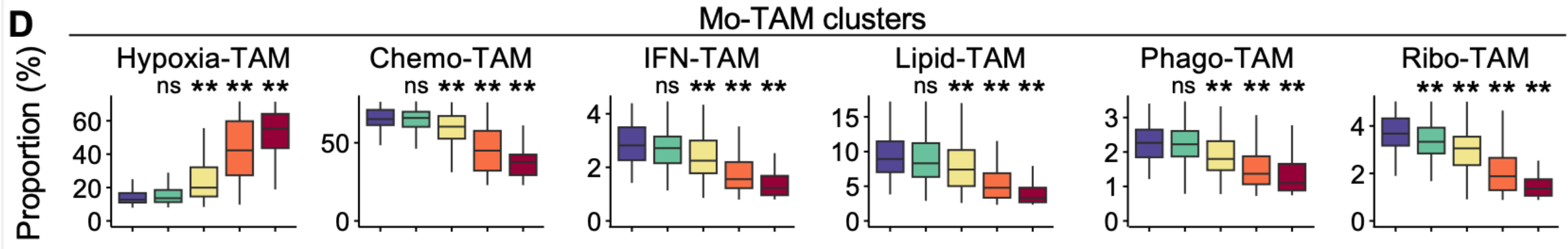
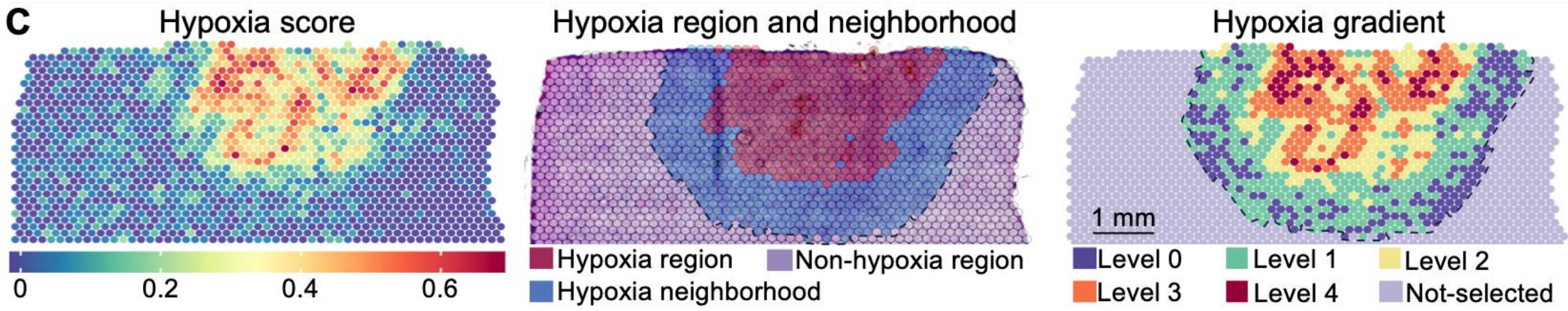
IVY GLIOBLASTOMA ATLAS PROJECT



C Histological features



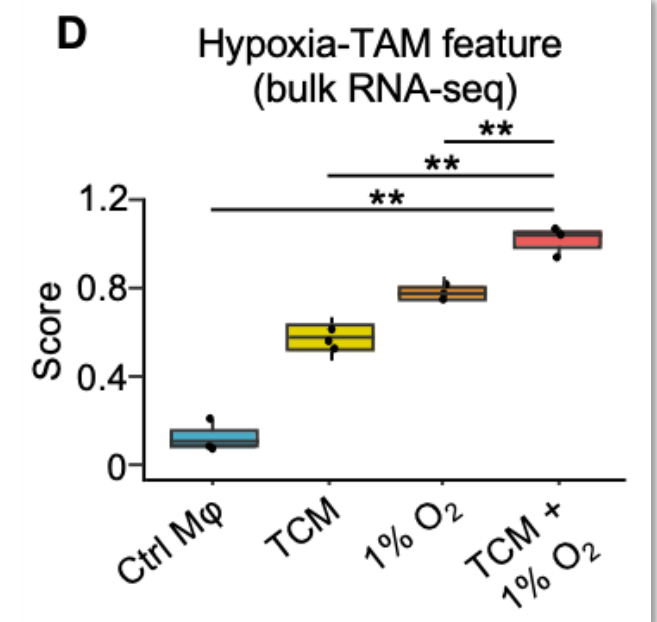
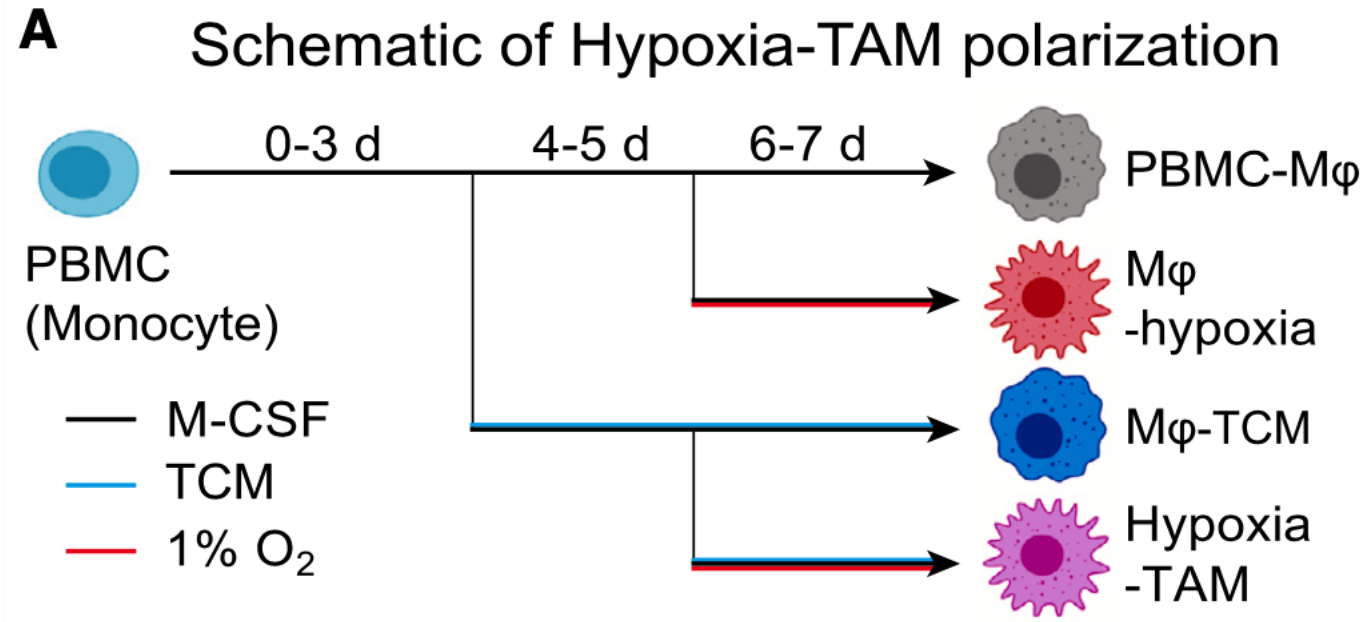
Hypoxia-TAM vs Hypoxia Gradient



ADM ↑

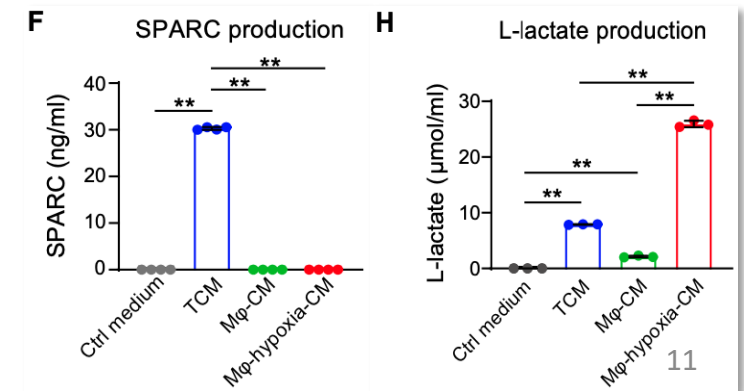
The polarization of Hypoxia-TAM could be driven by hypoxic tumor cues?

in vitro Mo-TAM polarization model

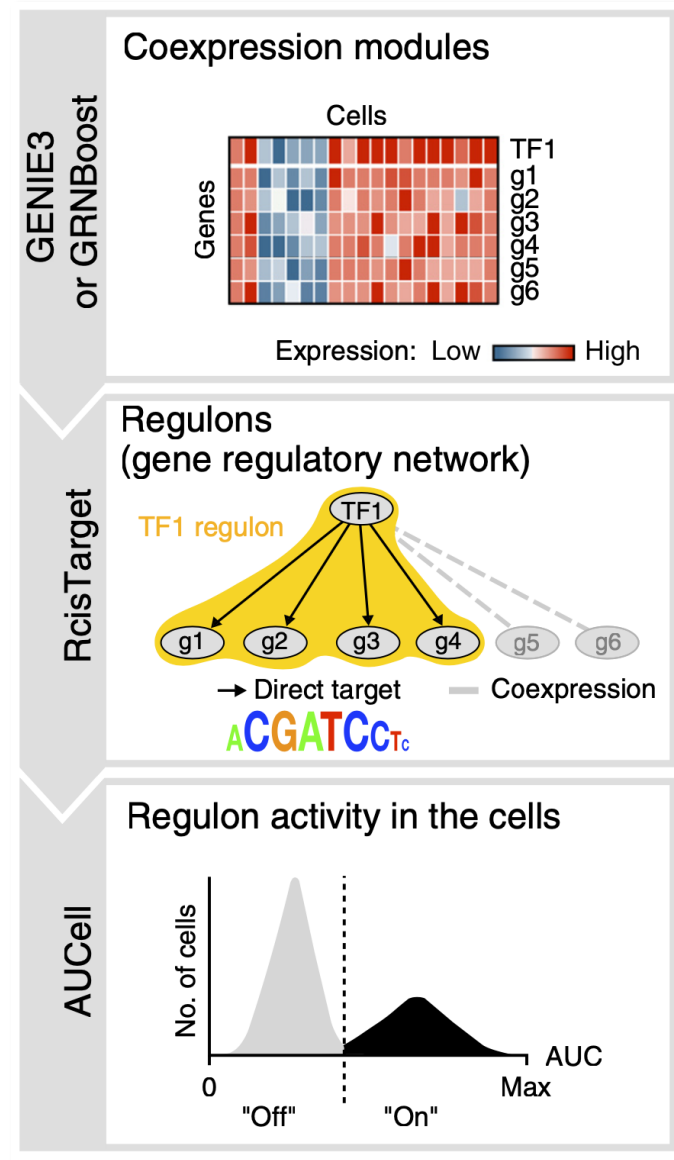


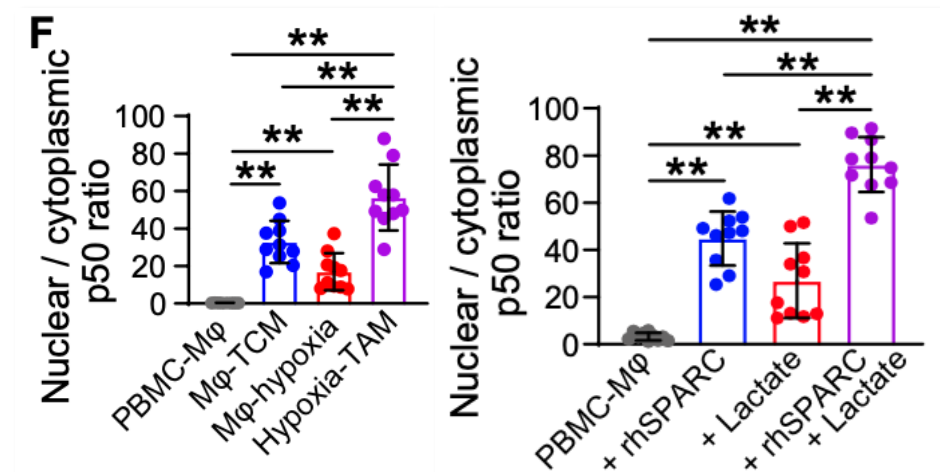
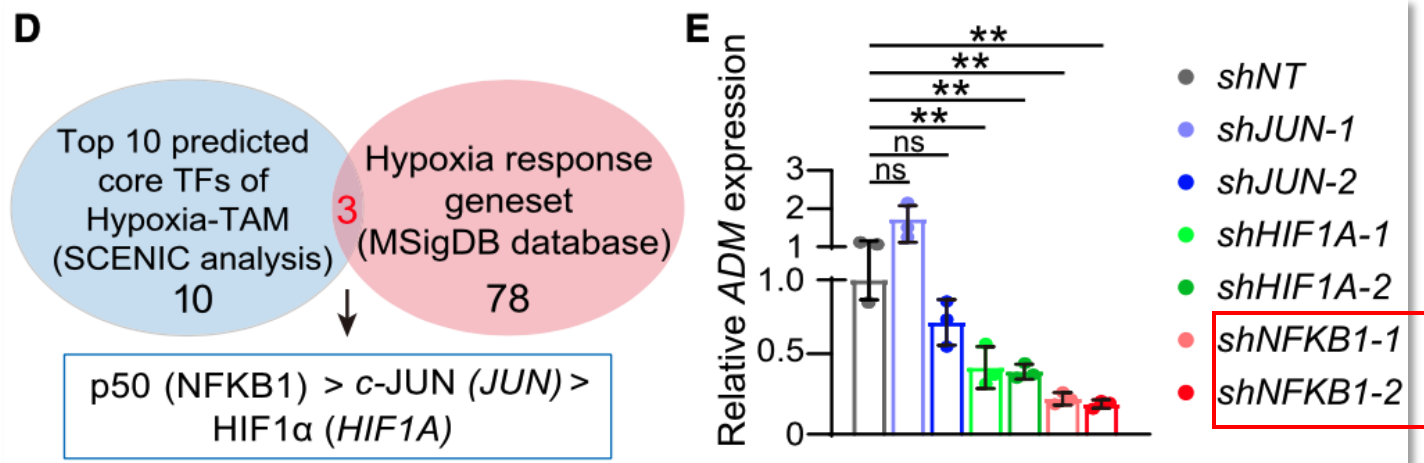
What are the critical niche factors?

- SPARC (Secreted Protein Acidic and Rich in Cysteine)
- Lactate

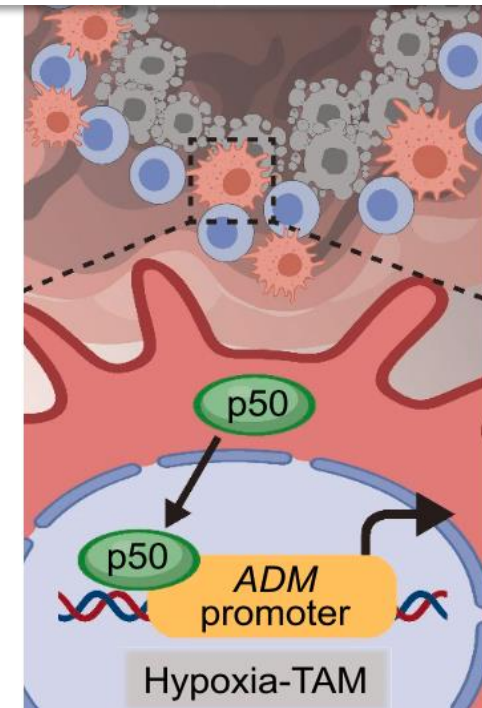


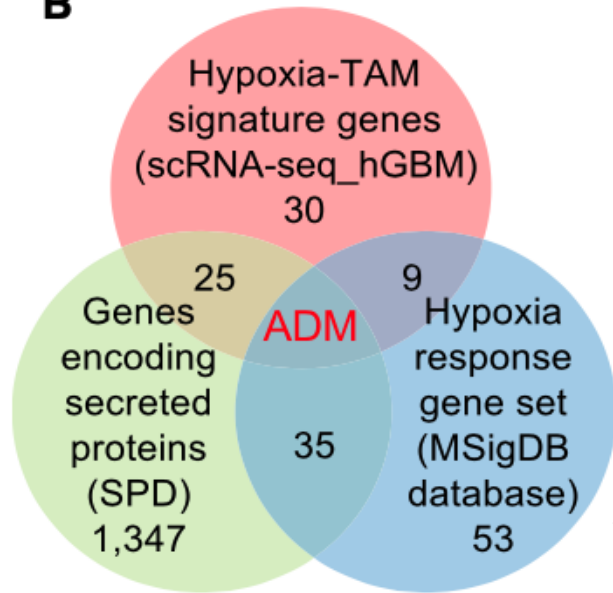
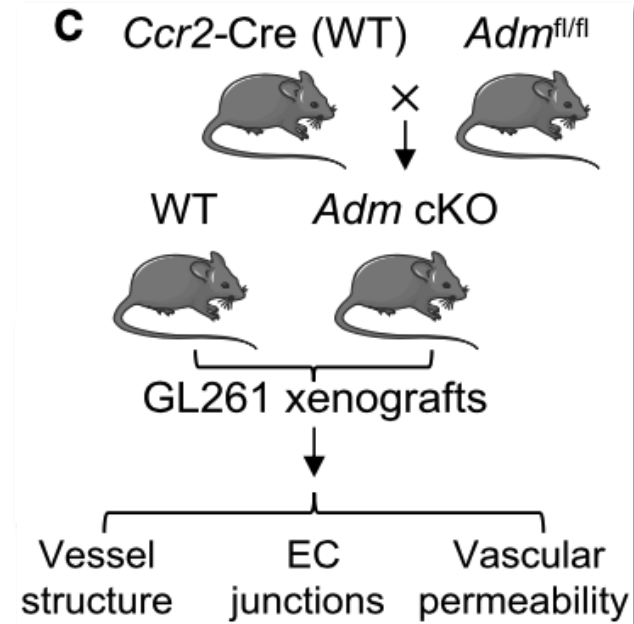
SCENIC (Single-Cell Regulatory Network Inference and Clustering)



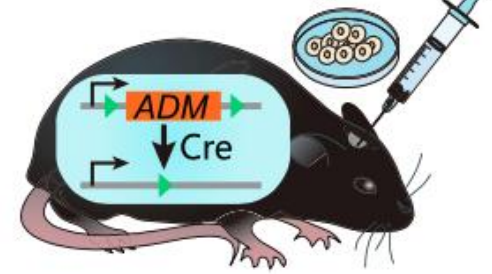


p50 nuclear translocation induced by SPARC and lactate upregulates the transcription of *ADM*

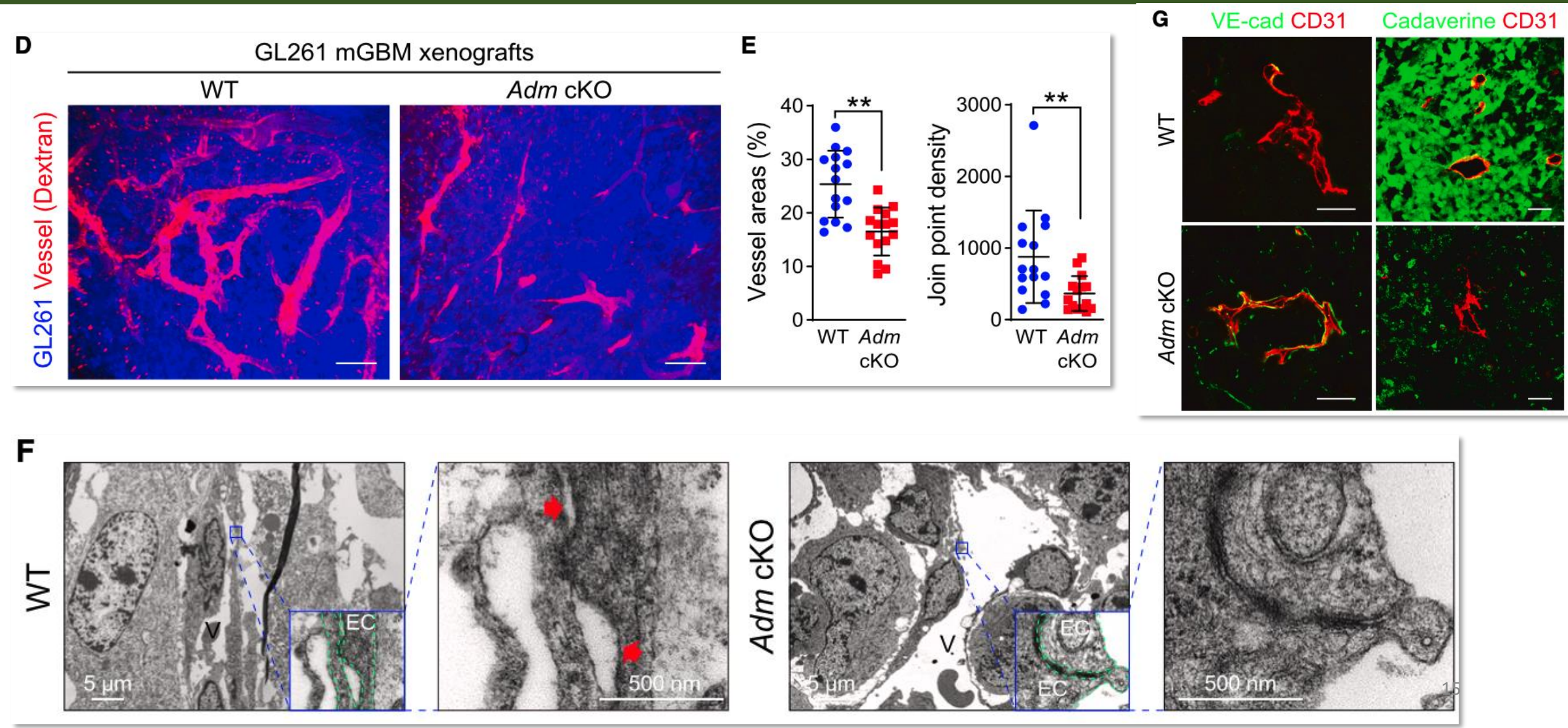


B**C**

mGBM xenograft

*Adm cKO* mice

Adm cKO restores tumoral vessel integrity and decreases vascular leakage in mGBM xenografts



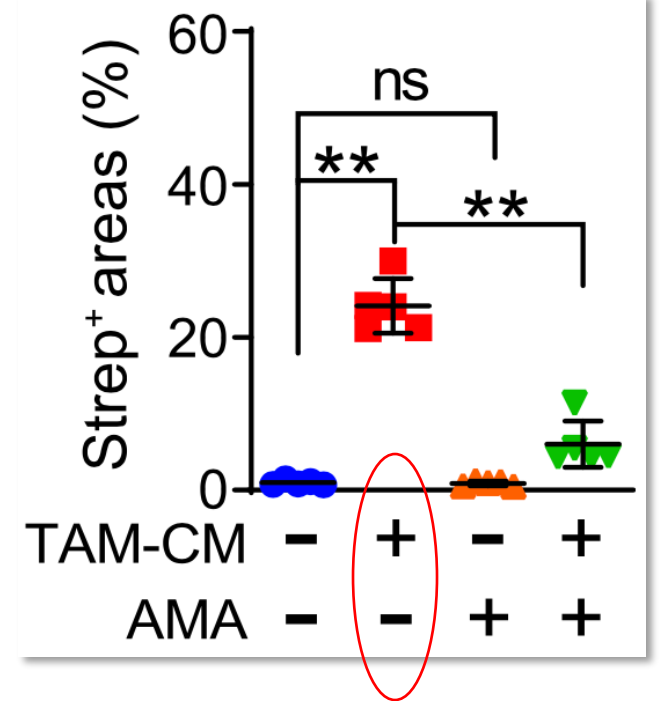
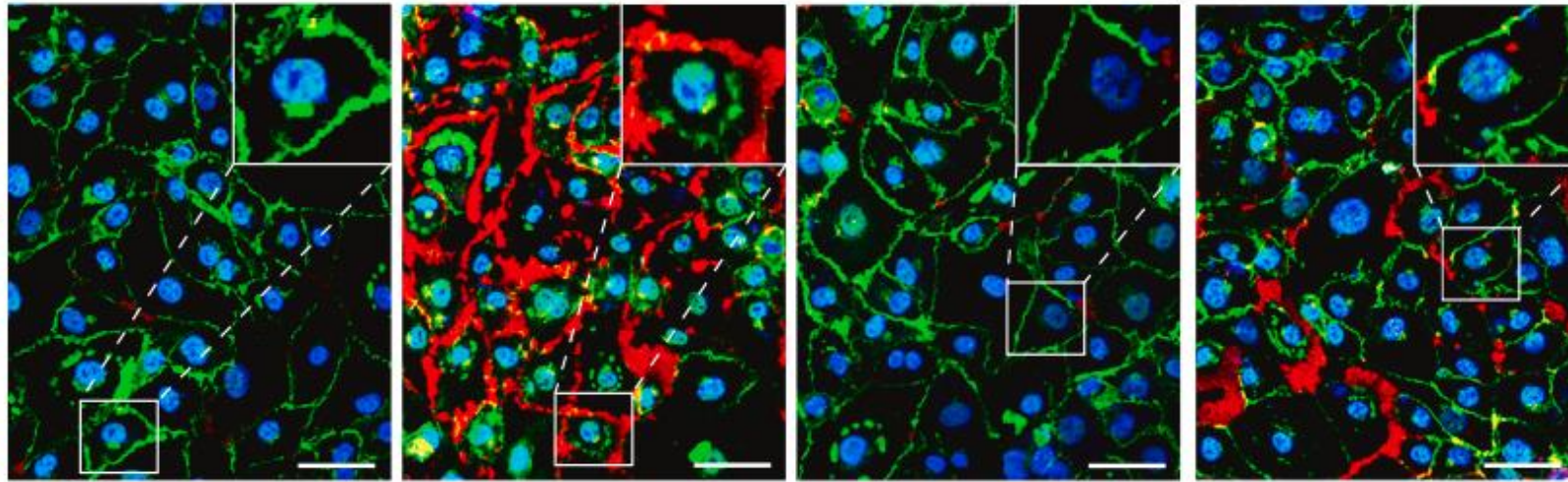
c

DAPI Strep VE-cad

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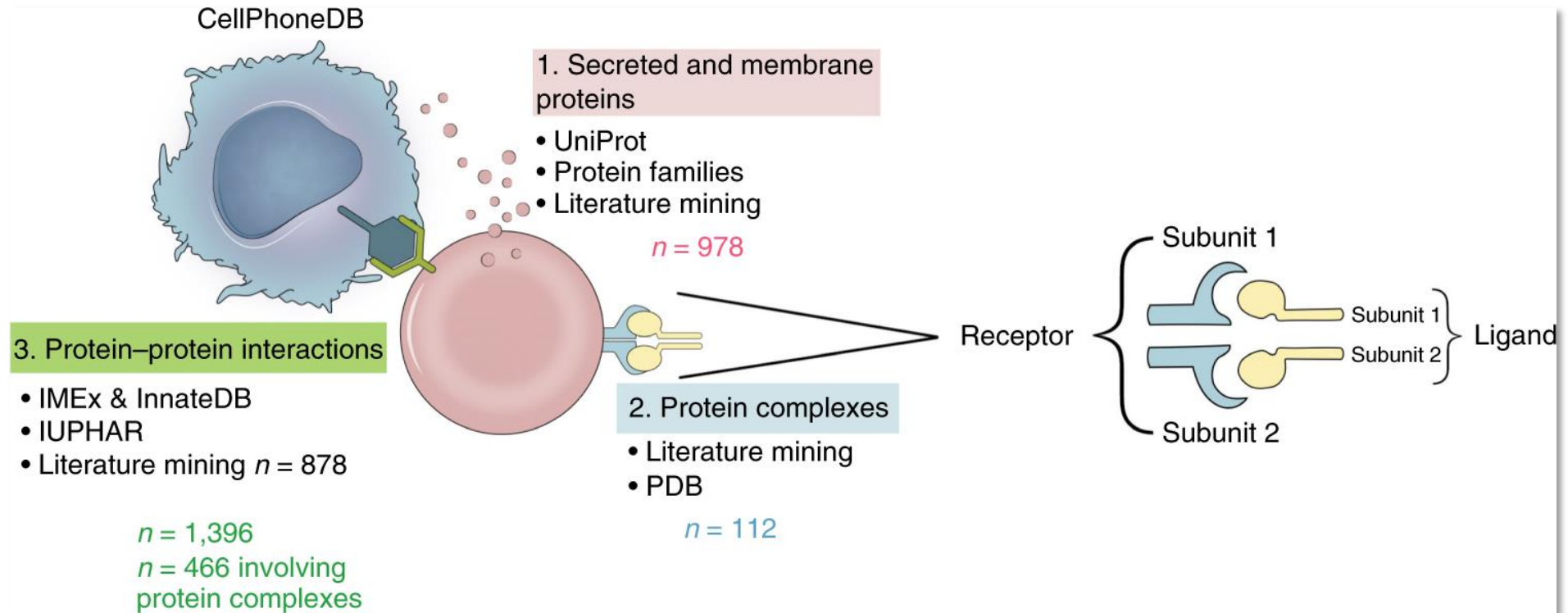
TAM-CM

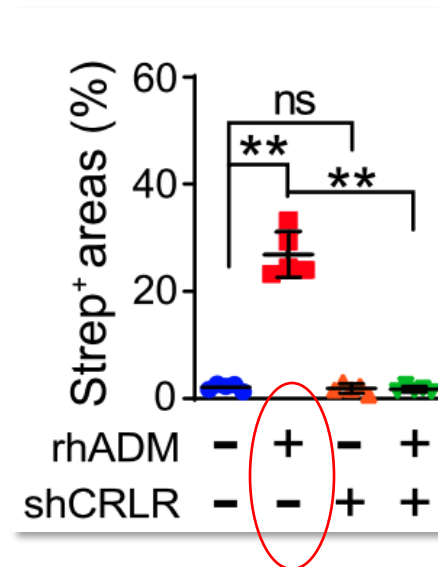
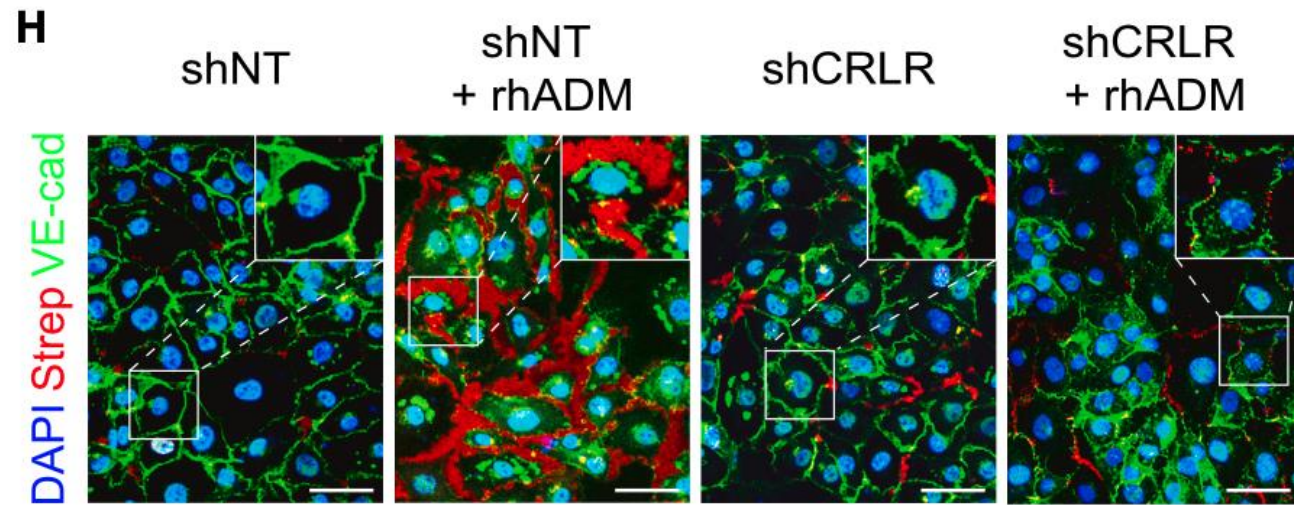
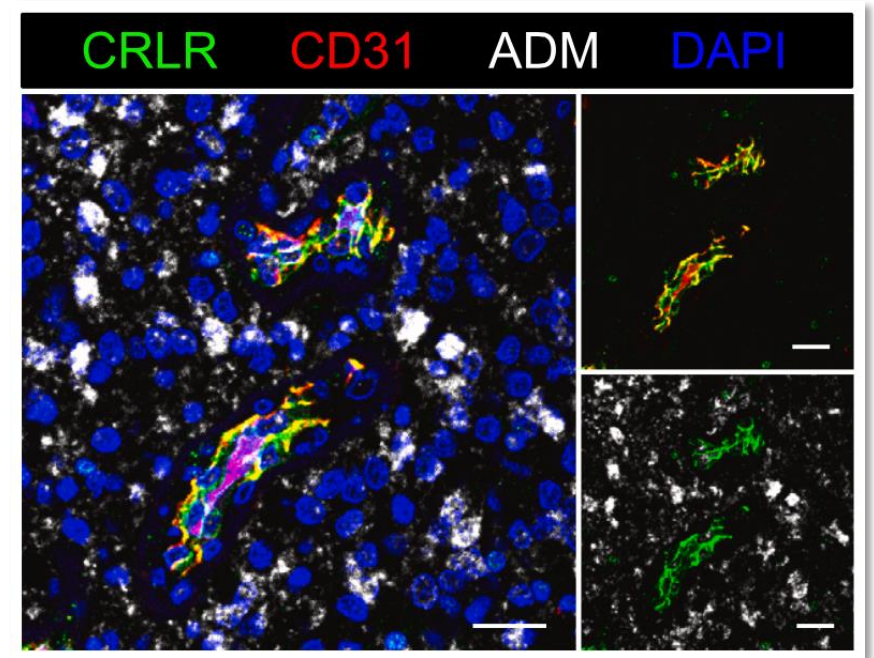
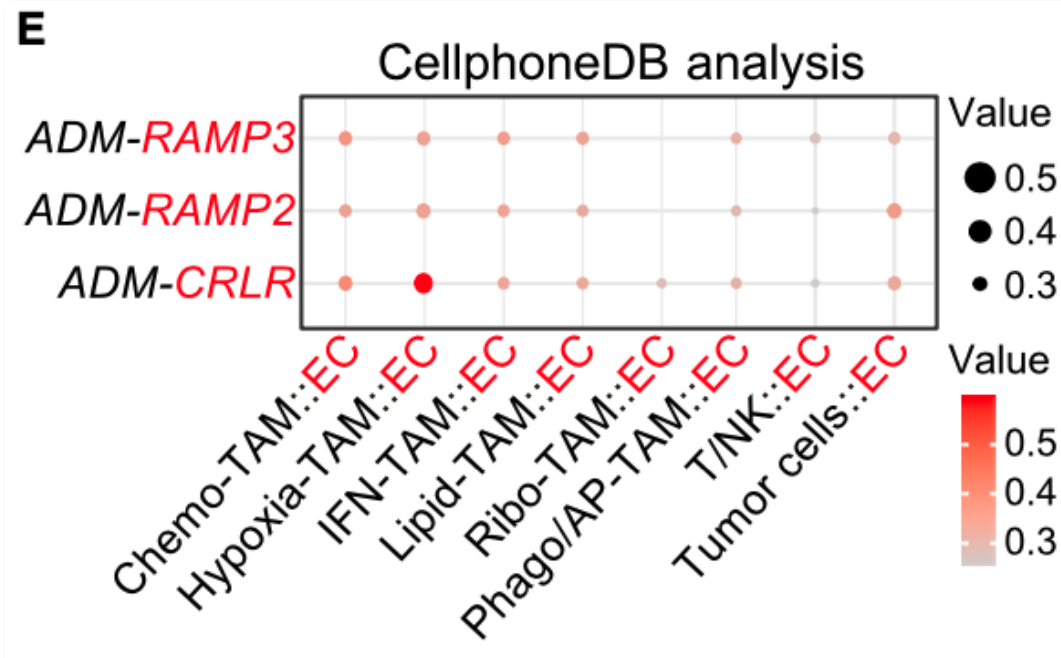
AMA

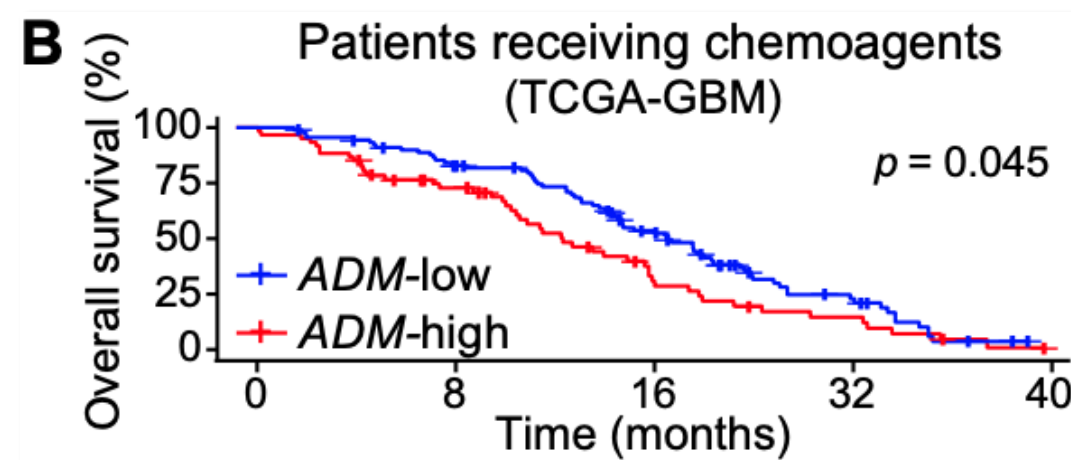
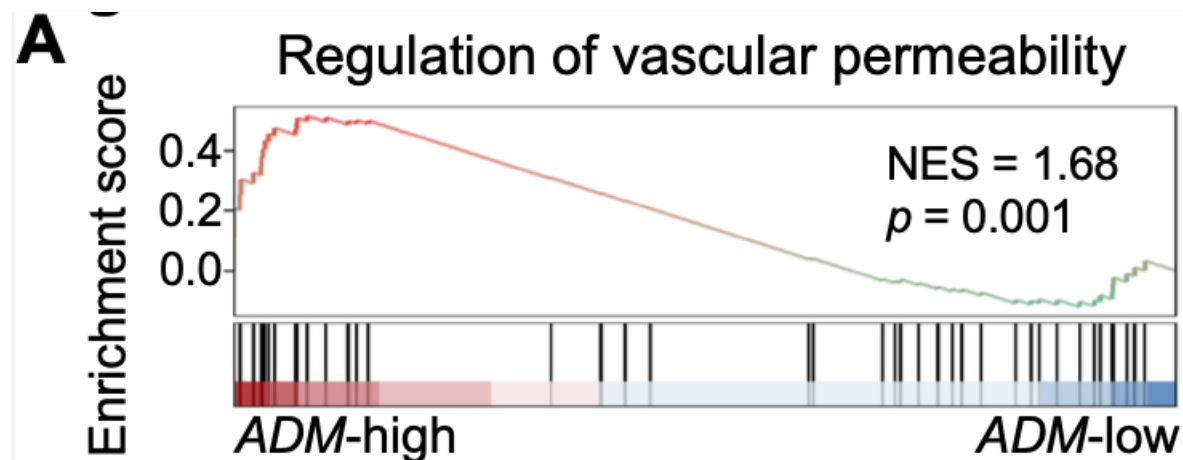
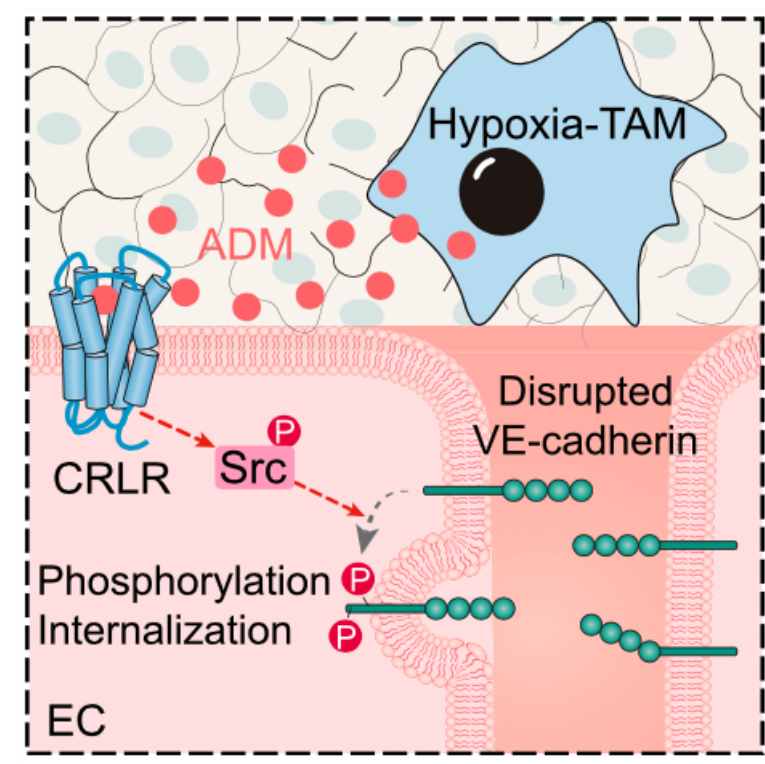
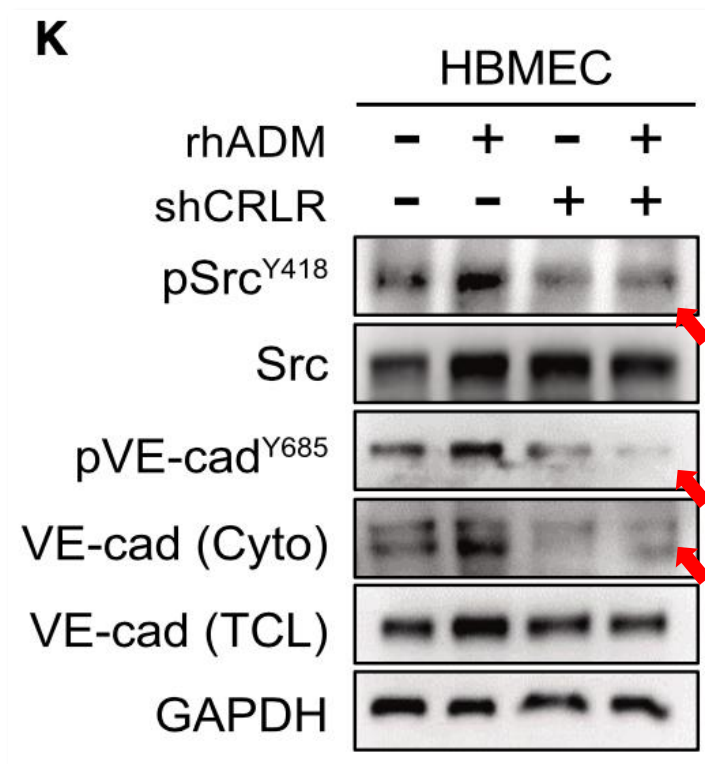
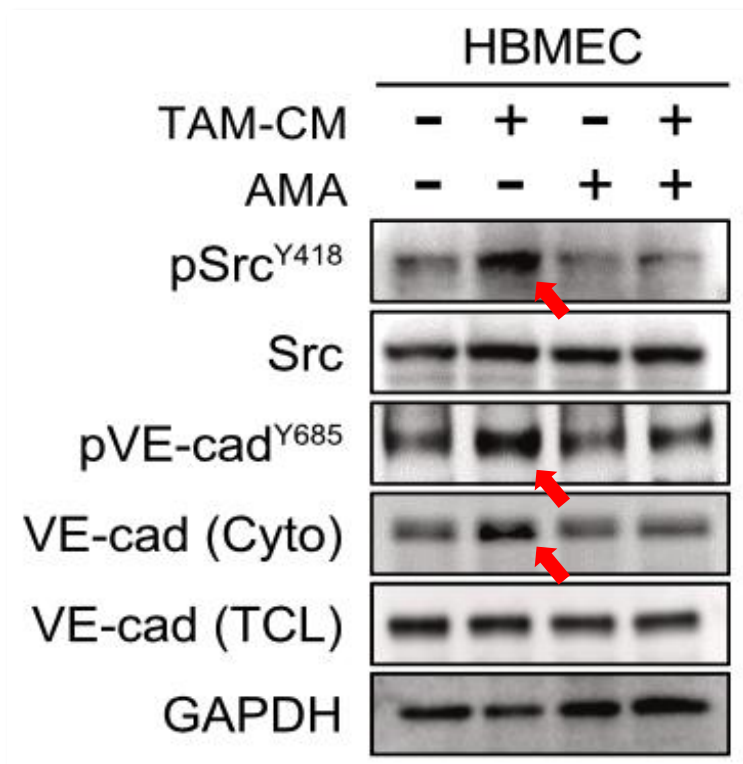
TAM-CM
+ AMA

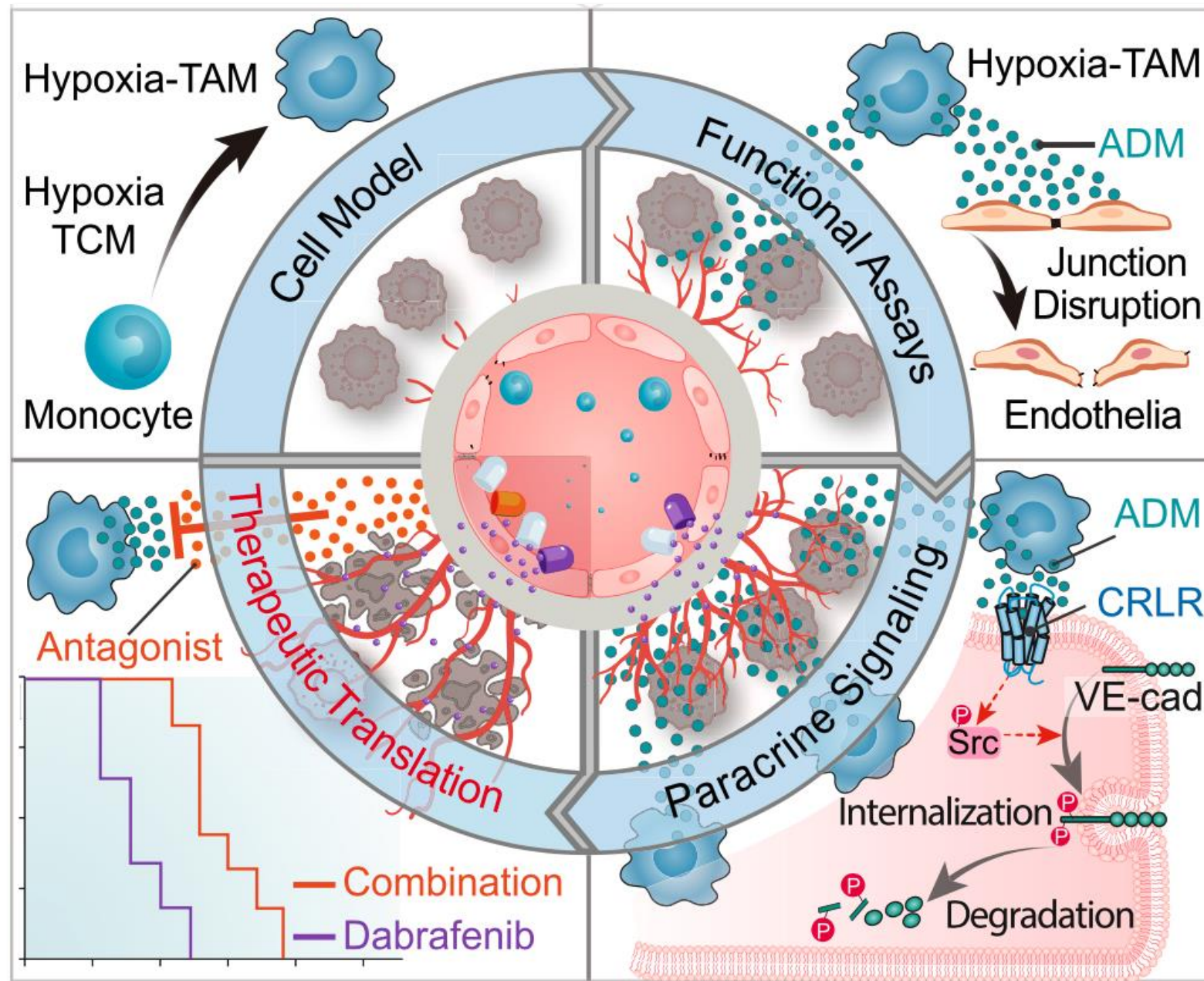


CellphoneDB







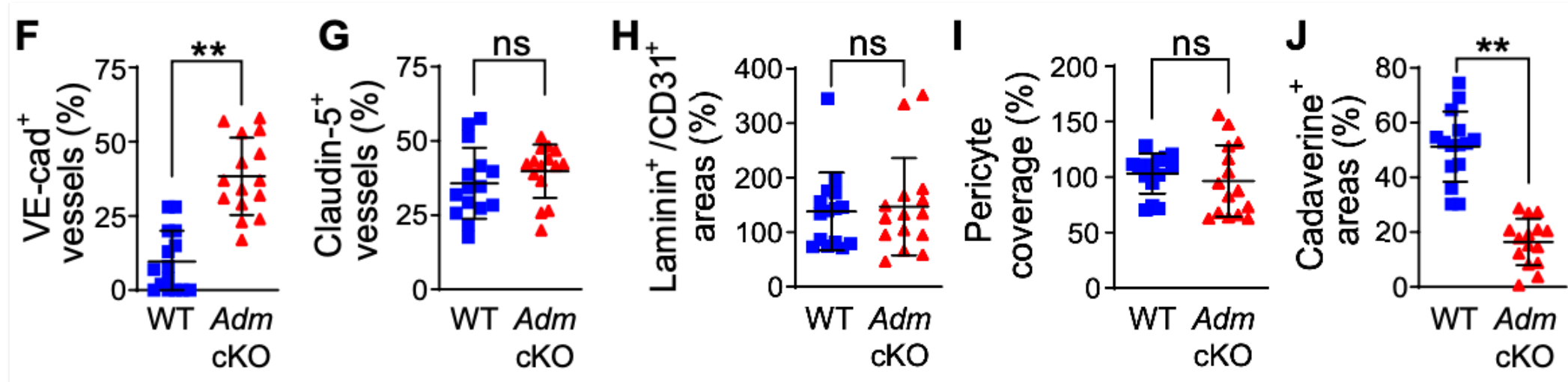
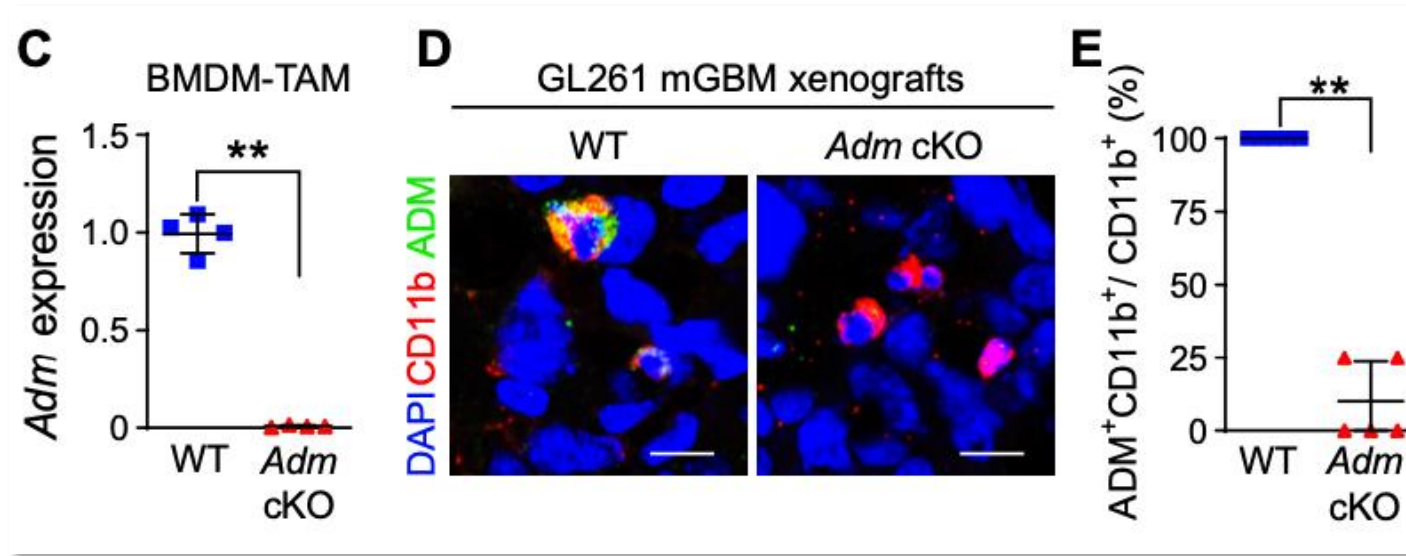


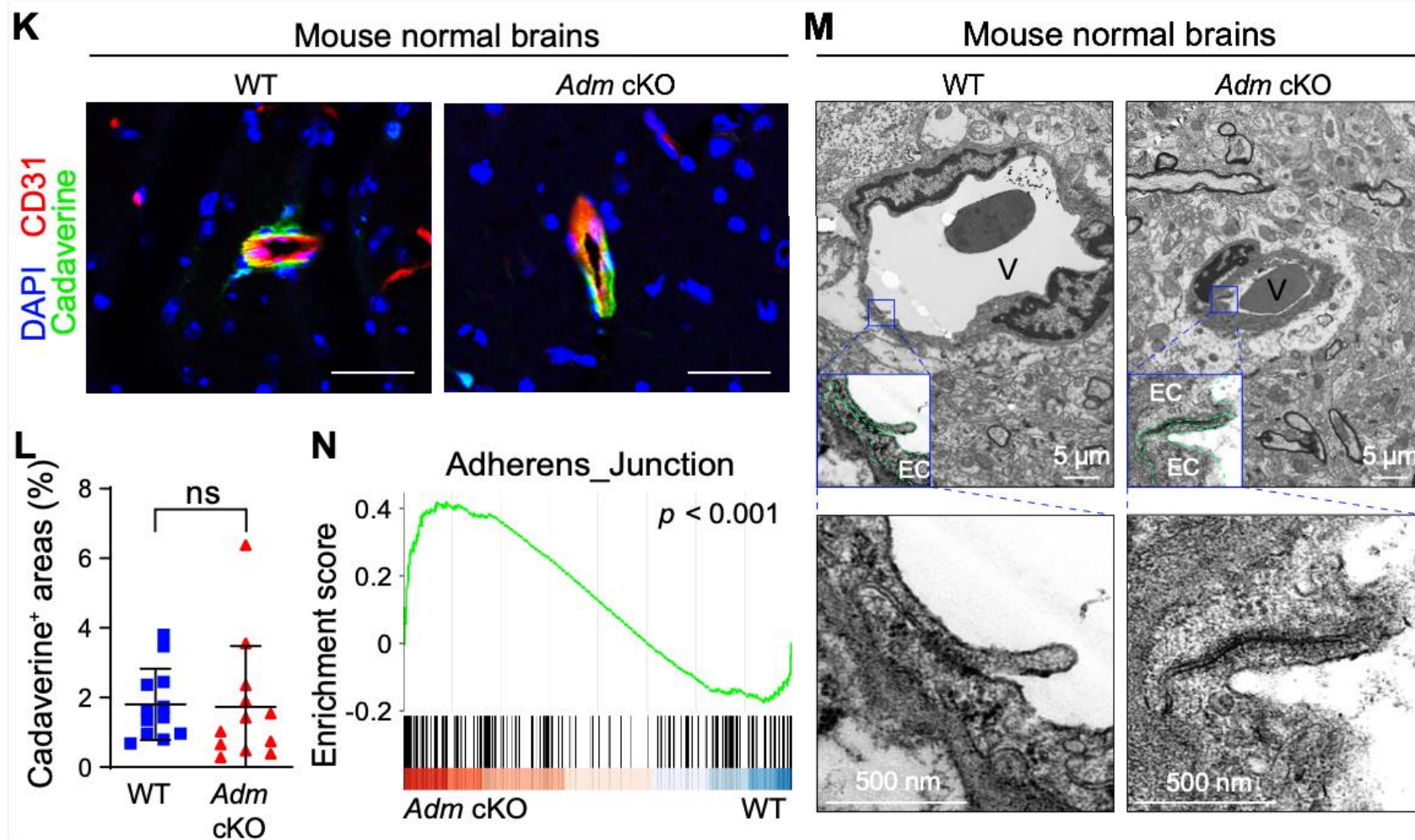
Take-Home messages

1. Hypoxia-TAM is a TAM subset in GBM with unique transcriptional profile.*
2. Hypoxia-TAMs are primarily located in peri-necrotic regions.*
3. The polarization of Hypoxia-TAMs is driven by SPARC and lactate, which activate the TF p50 (encoded by *NFKB1*), leading to the expression of *ADM*.*
4. *ADM*, which then interacts with the CRLR receptor on ECs, disrupting VE-cadherin junctions, disrupts tumor blood vessels and hindering drug delivery.*
5. Blocking *ADM* with AMA restores vascular integrity and improves drug delivery in GBM.

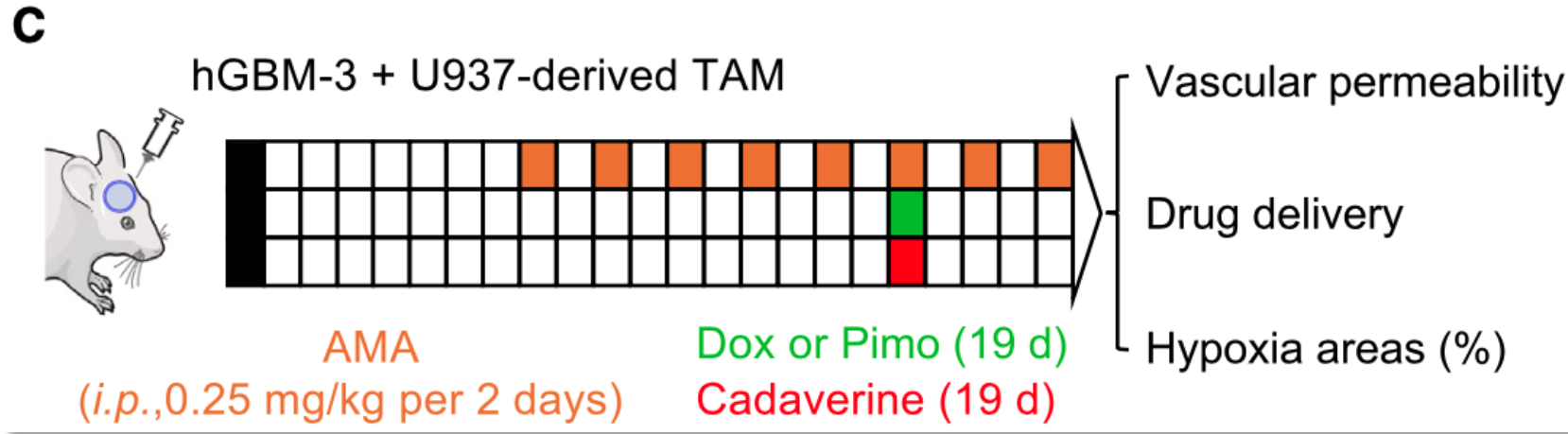
Thank you

ADM cKO efficacy



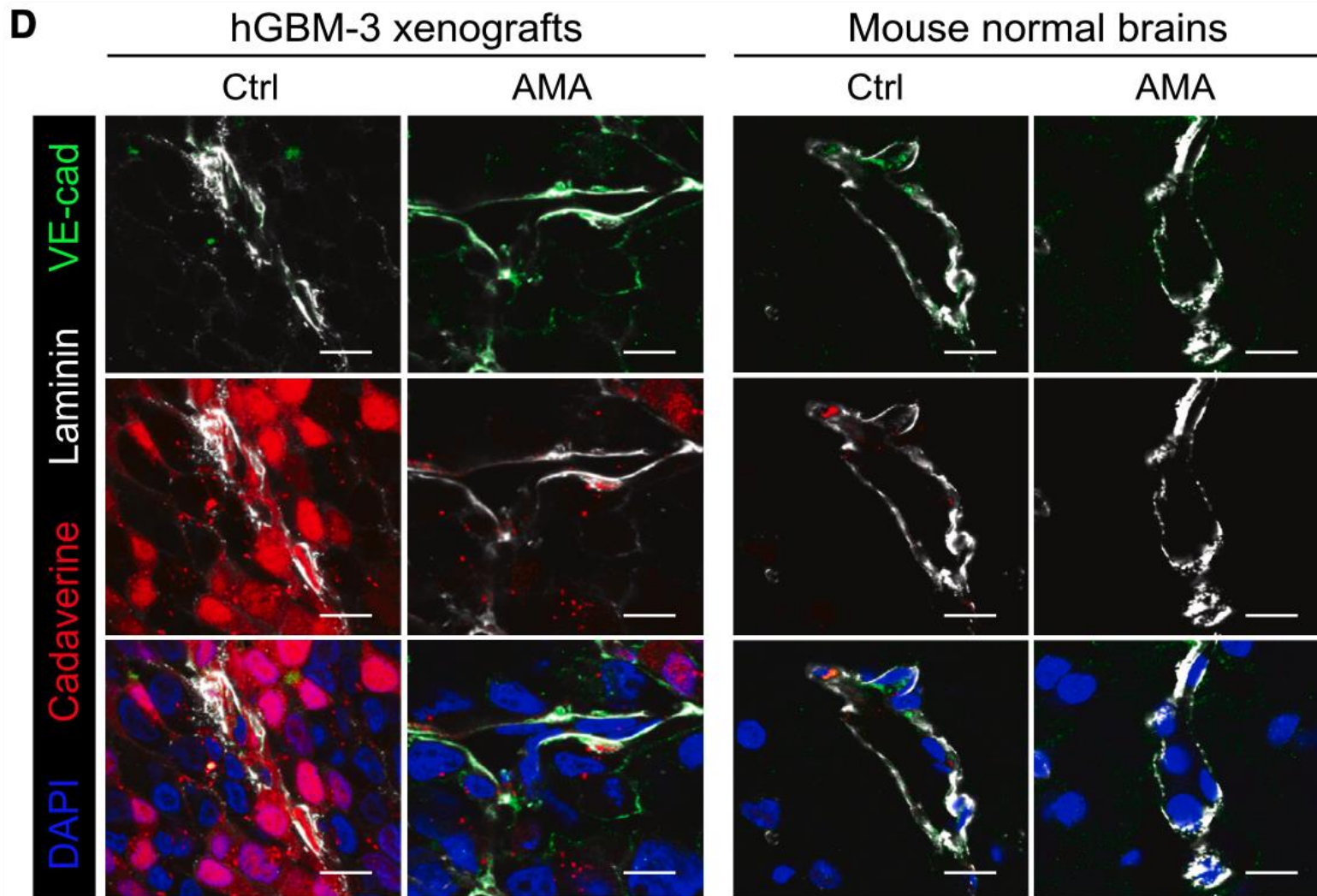


Blocking ADM through AMA could restore endothelial adherens junctions *in vivo*?

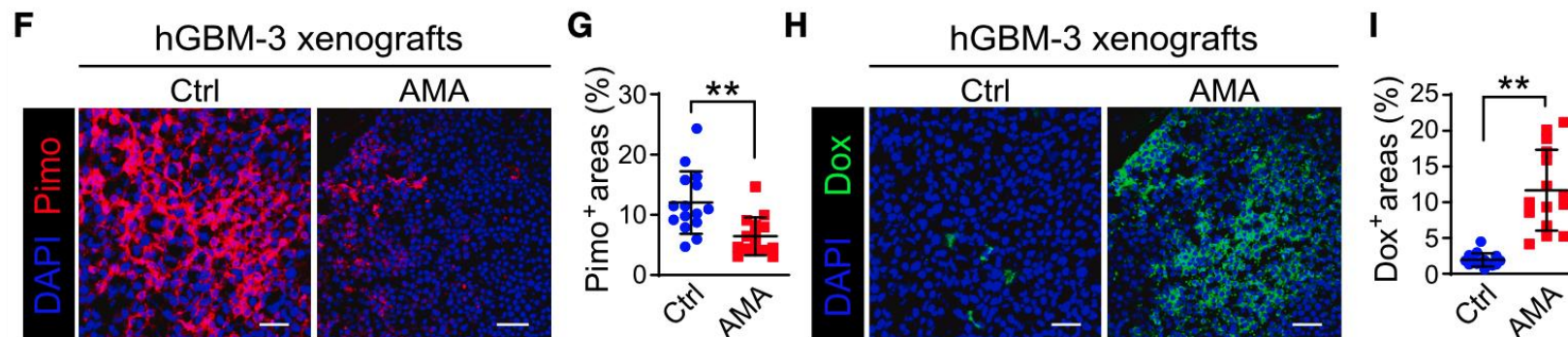


successfully mimicked the histology of the hypoxic niche in hGBMs:

- tumor necrosis
- neovasculature with impaired adherens junctions
- infiltration of ADM-producing TAMs



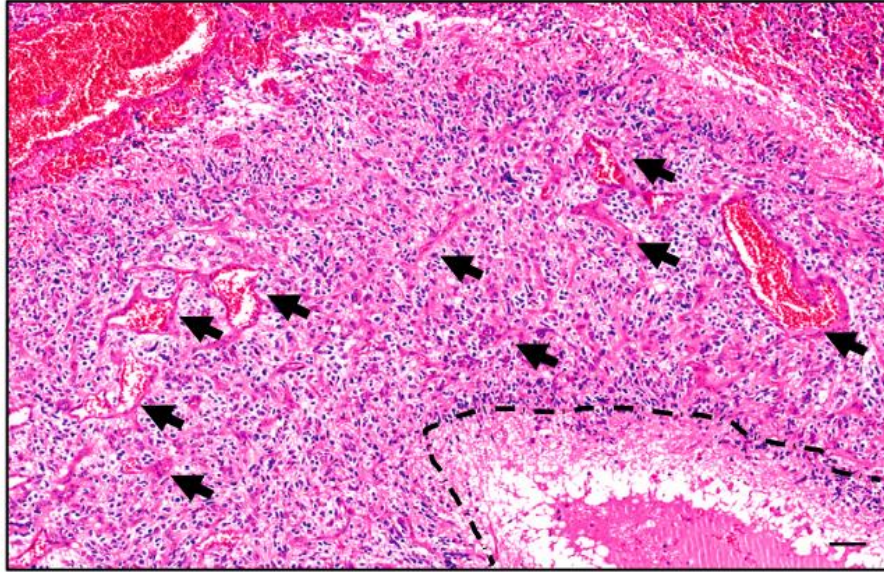
- restored VE-cadherin expression
- preserved endothelial junctions
- reduced cadaverine effusion
- negligible influence on mouse normal brain vasculature



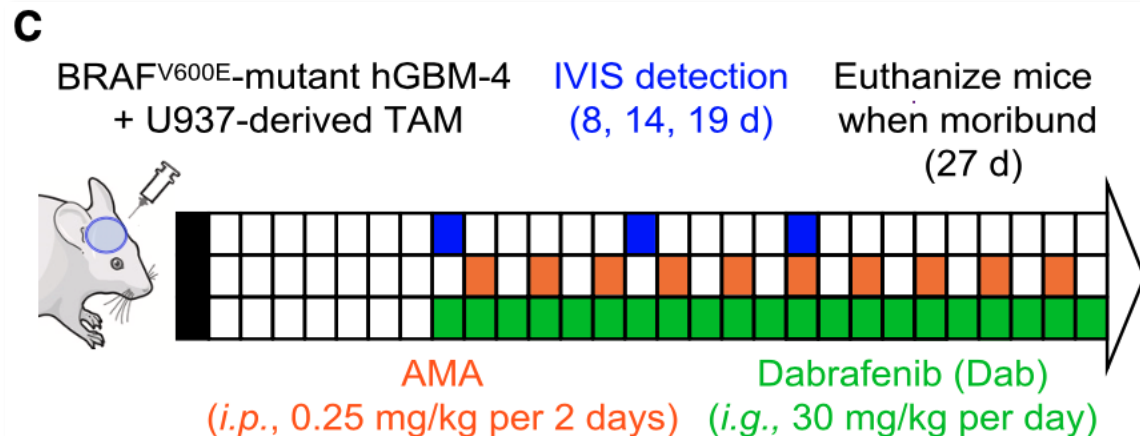
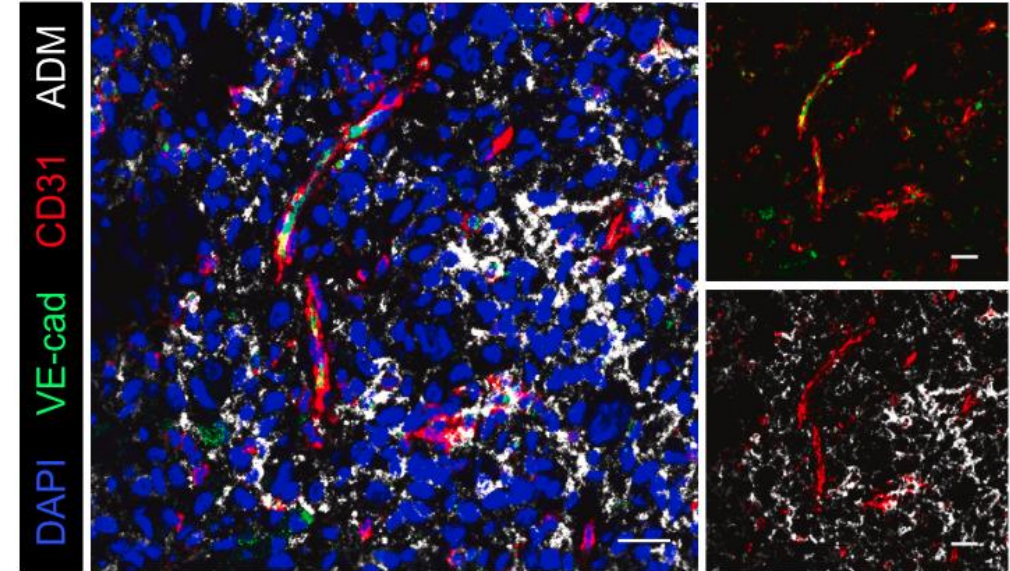
- reduced tumor hypoxia
- enhanced drug delivery

BRAF^{V600E}-mutant hGBMs

A H&E (hGBM-BRAF^{V600E} mutation)

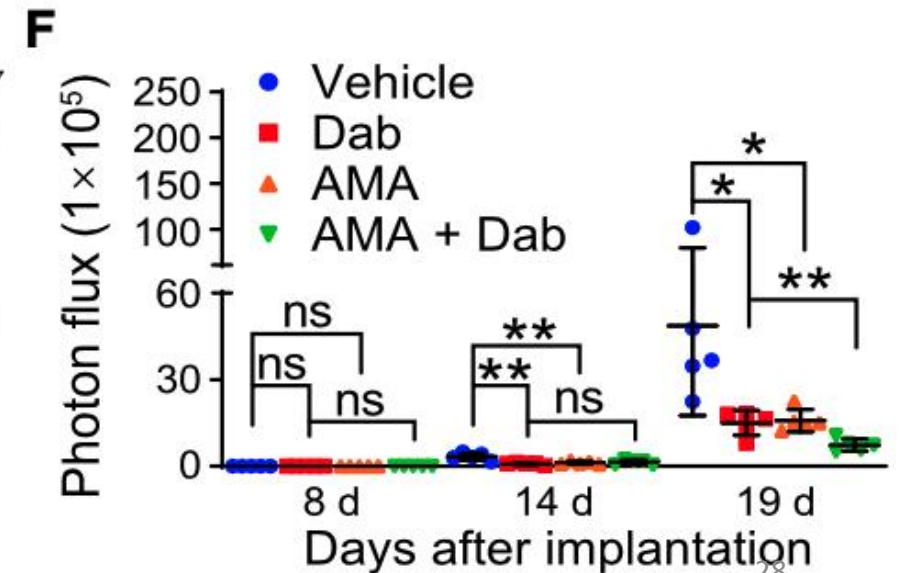
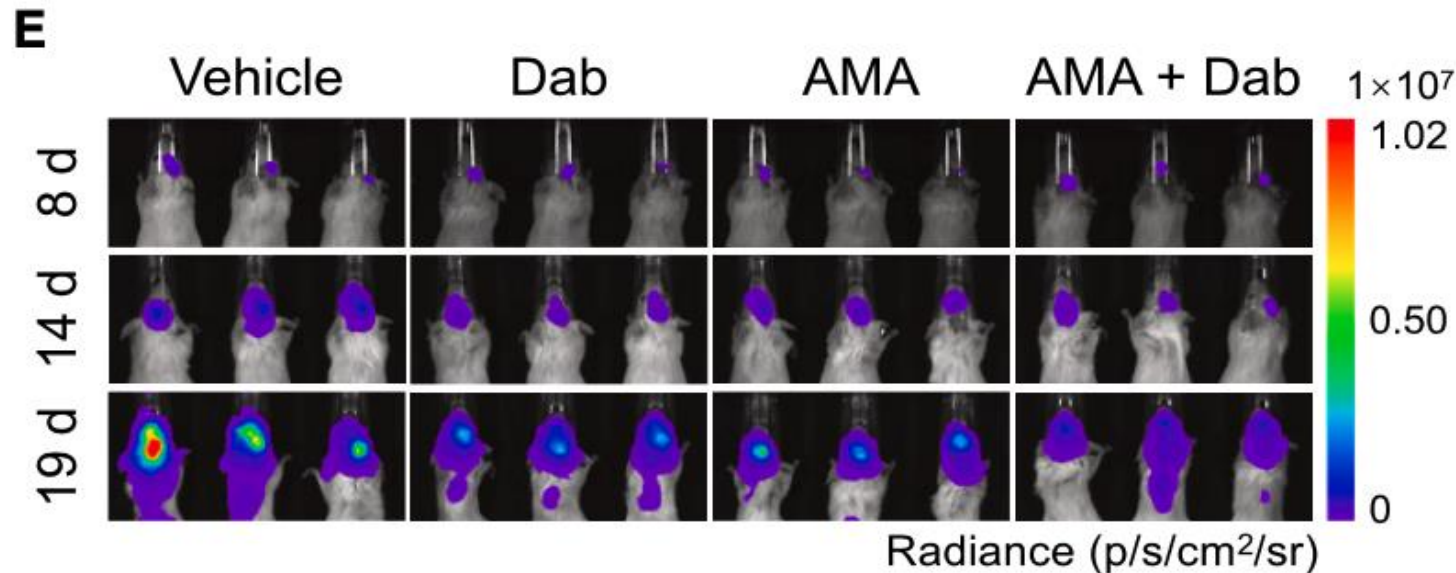
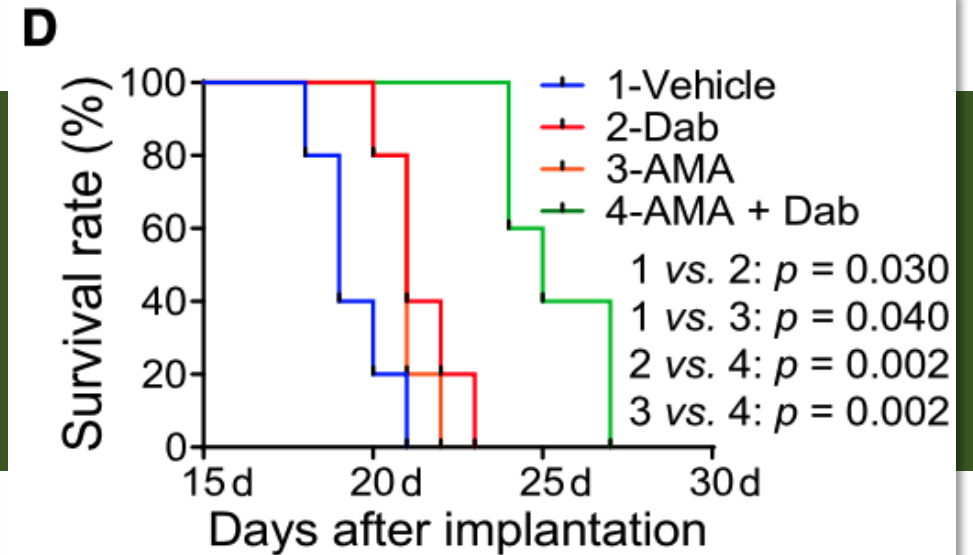


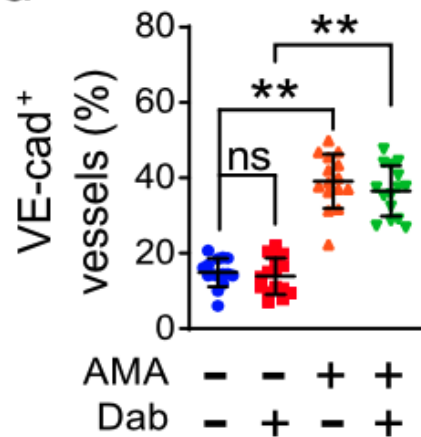
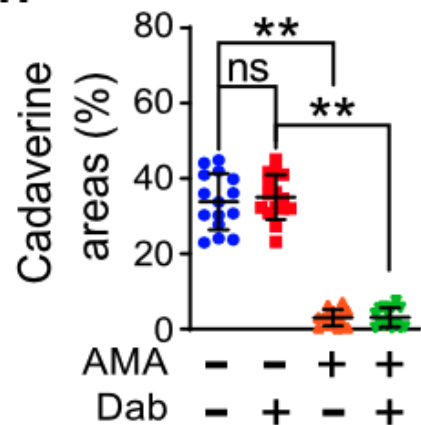
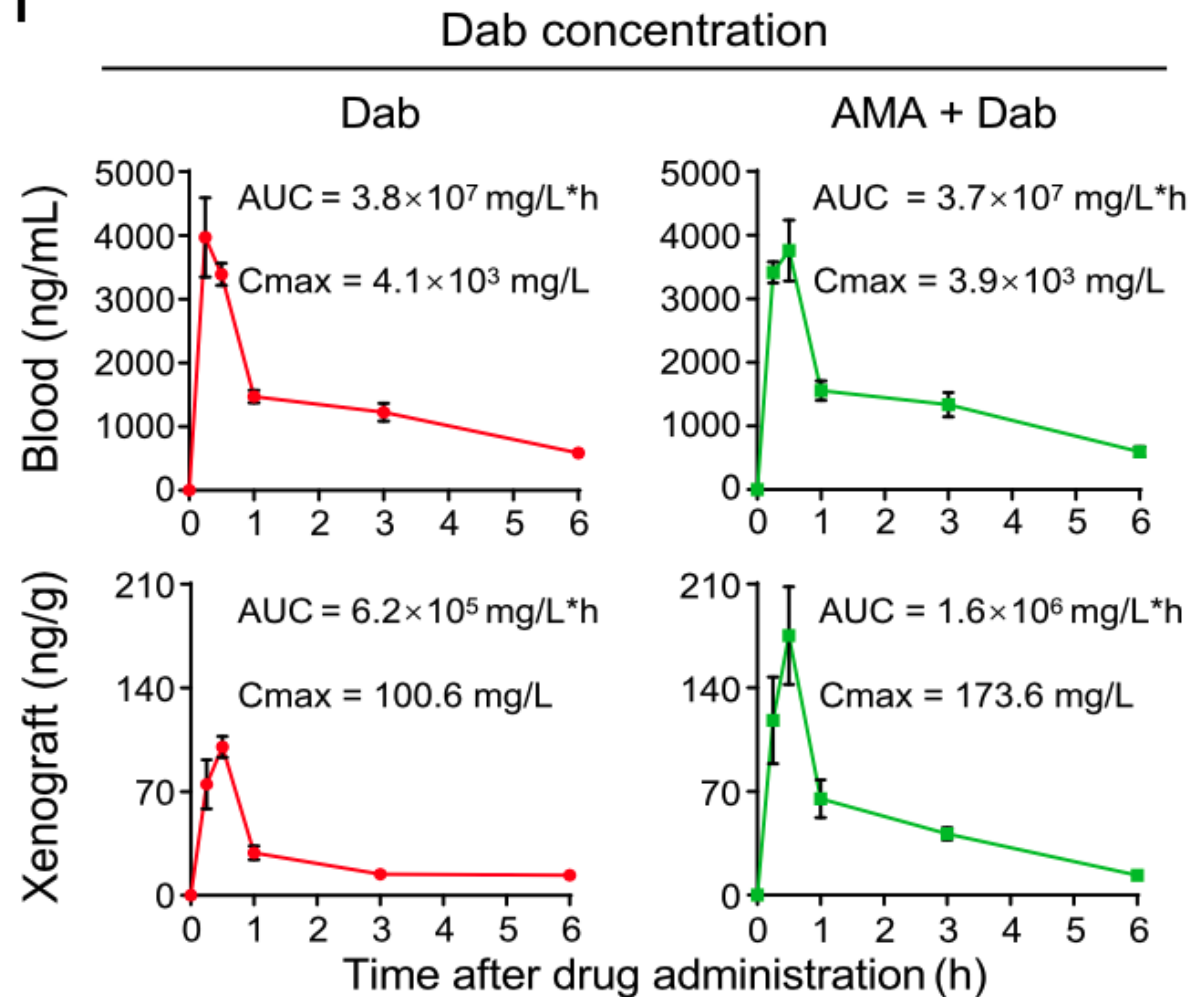
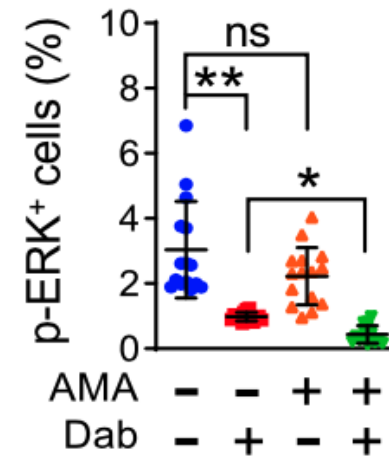
B hGBM2029 (BRAF^{V600E} mutation)



- Vehicle control
- AMA
- Dabrafenib
- AMA + Dabrafenib

“AMA administration with dabrafenib achieved combinatorial therapeutic benefits in extending animal survival and reducing tumor burden.”



G**H****I****J****K**