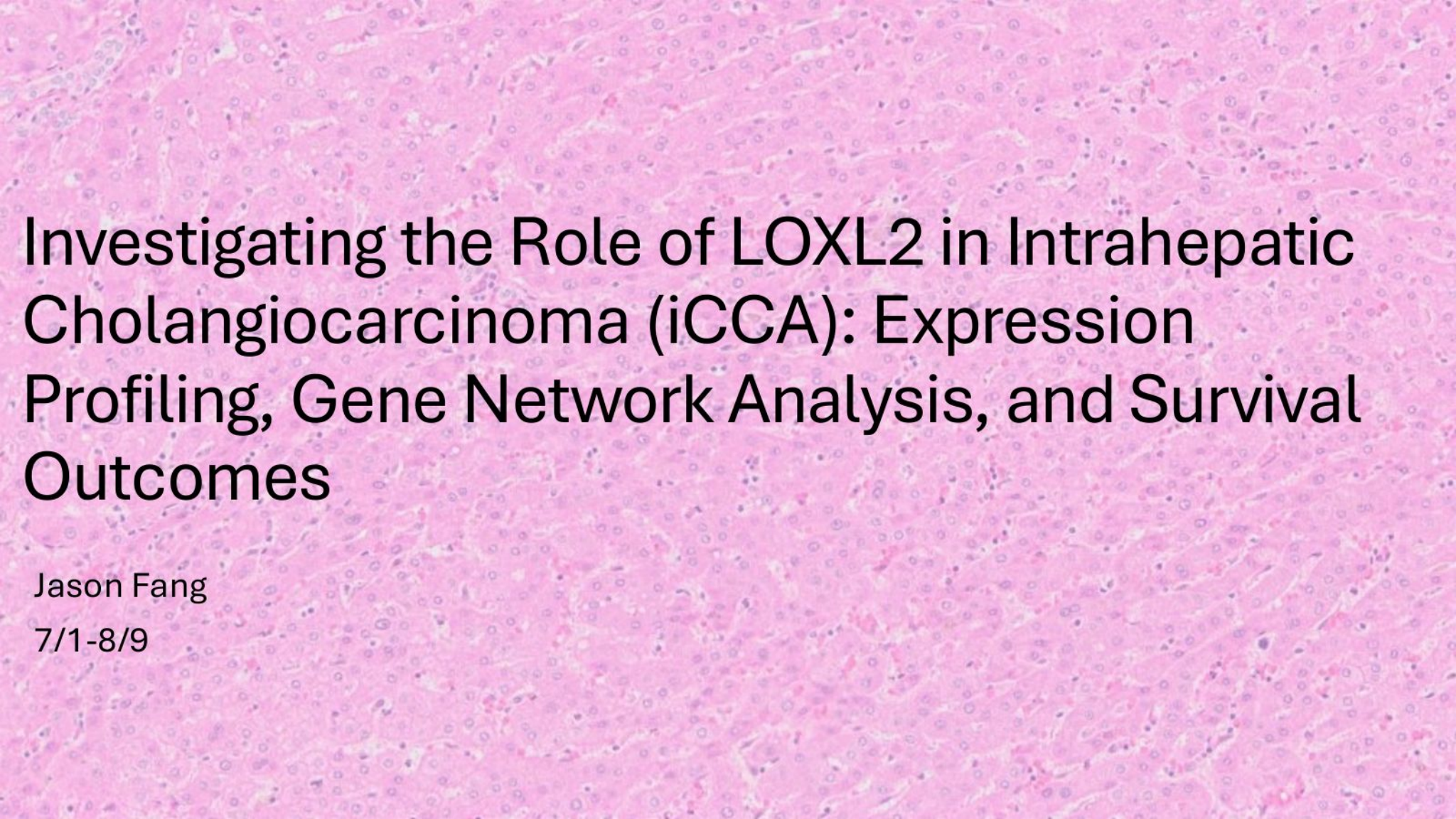




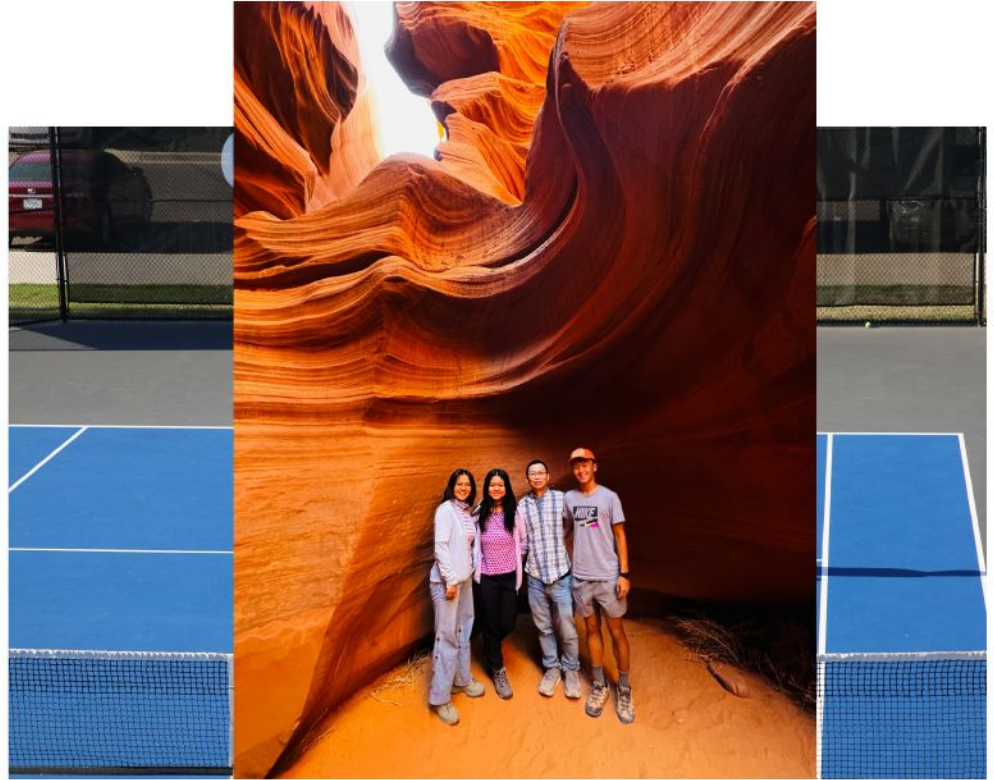
Investigating the Role of LOXL2 in Intrahepatic Cholangiocarcinoma (iCCA): Expression Profiling, Gene Network Analysis, and Survival Outcomes

Jason Fang

7/1-8/9

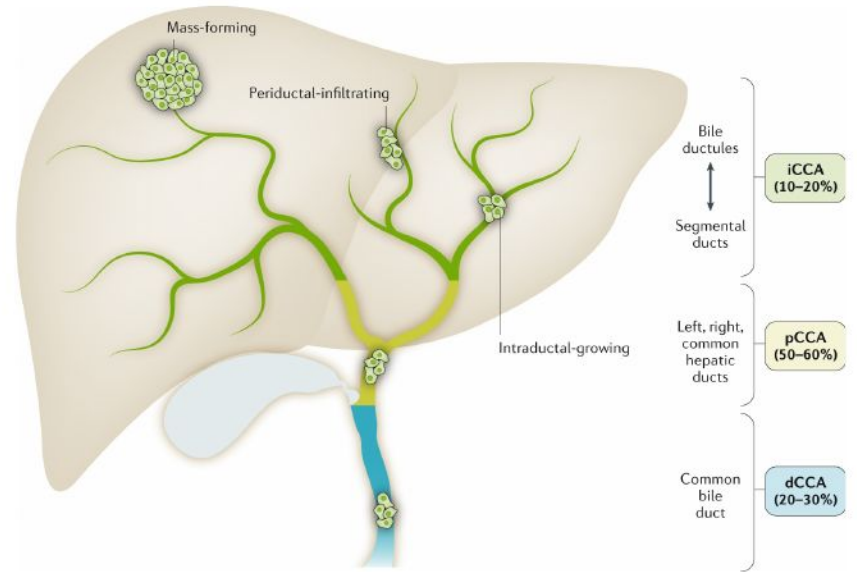
About Me

- Memphis, TN
- UPenn
- Tennis
- Hiking



iCCA: origin

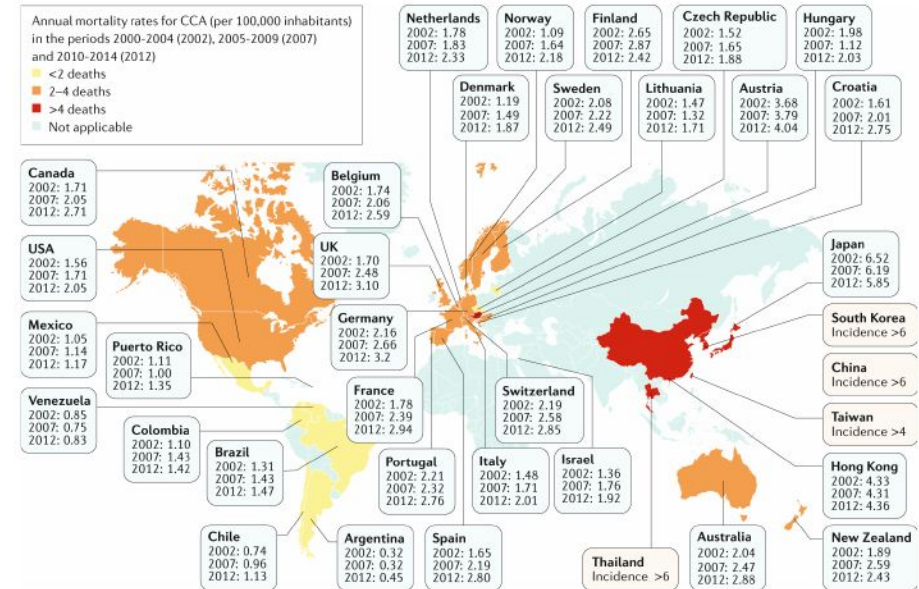
- **Cell of origin**: Cholangiocarcinoma (CCA) originates from **cholangiocytes**, the epithelial cells of bile duct.
- **Site of origin**: CCA can arise at any point of the **biliary tree**.
 - **Intrahepatic CCA**: the 2nd common primary **liver cancer** after HCC
 - Perihilar CCA
 - Distal CCA



Banales, Jesus M., et al. *Nature reviews Gastroenterology & hepatology* (2020)

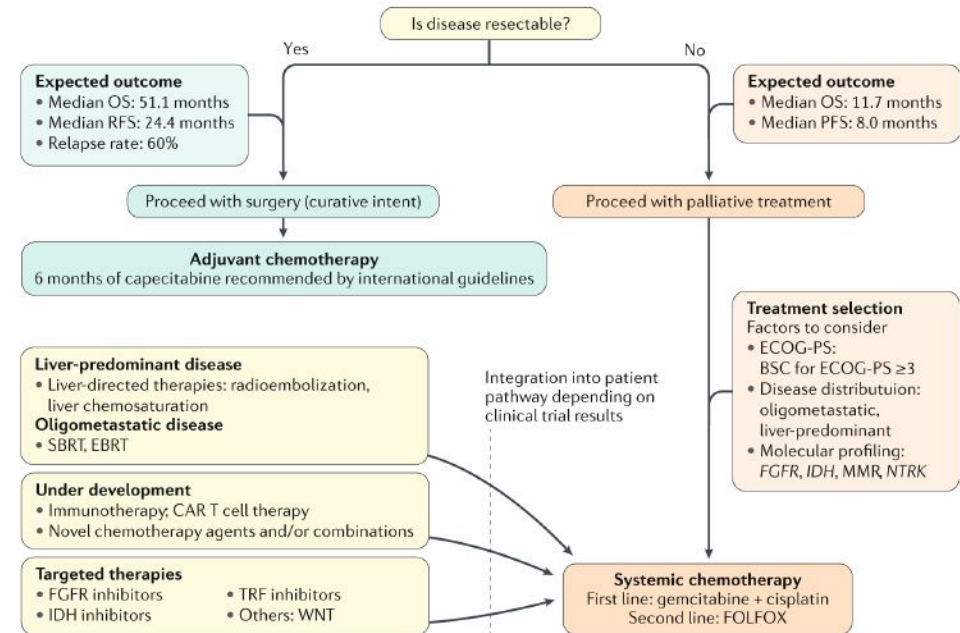
iCCA: epidemiology

- **Incidence:**
 - **0.3-6** per 100,000 inhabitants per year;
 - **>6** in some regions (e.g. East Asia)
- **Mortality:**
 - **1-6** per 100,000 inhabitants per year
 - **increasing** globally
- **5-year survival: <10%**
 - 24% if diagnosed at an early stage
 - 9% if spread to regional lymph nodes
 - **2% if metastasize to distant part**
- **Survival rate: 16.1 months**
 - 27.6 months for curative resection
 - 12.9 months for palliative chemoradiotherapy
 - 4.9 months for best supportive care



iCCA: treatment & relapse

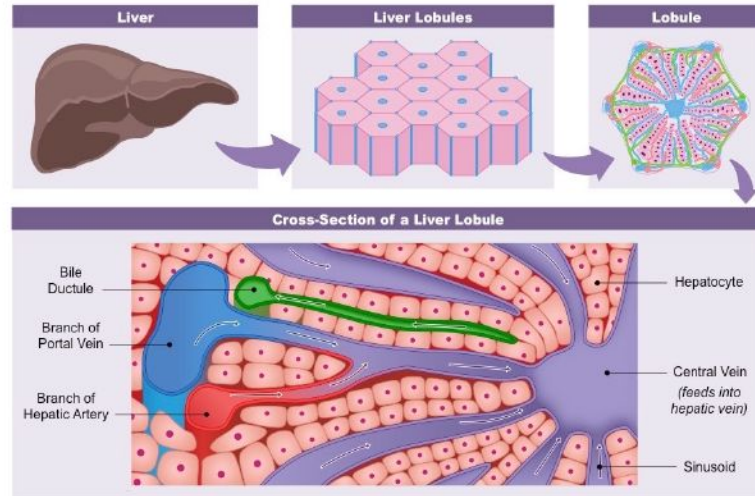
- **~40%** of iCCA patients carry targetable genetic alterations, but the improvement on survival is minimal.
- ***Surgical resection*** with negative margin is currently the only curative treatment for iCCA.
- ***Relapse:***
 - **60-70%** after curative resection
 - **3-6** months
 - **multiple liver lesions**



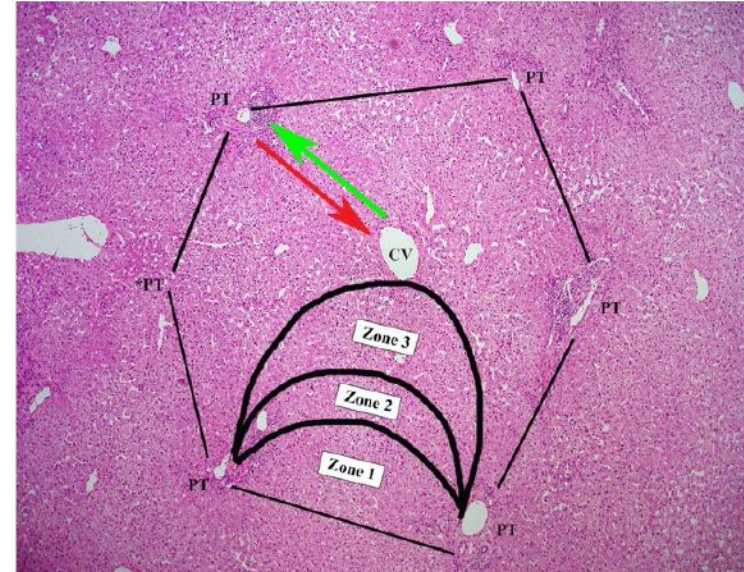
Banales, Jesus M., et al. *Nature reviews Gastroenterology & hepatology* (2020)

Early and extensive **tumor dissemination** of iCCA

Tumor microenvironment of iCCA: liver zonation

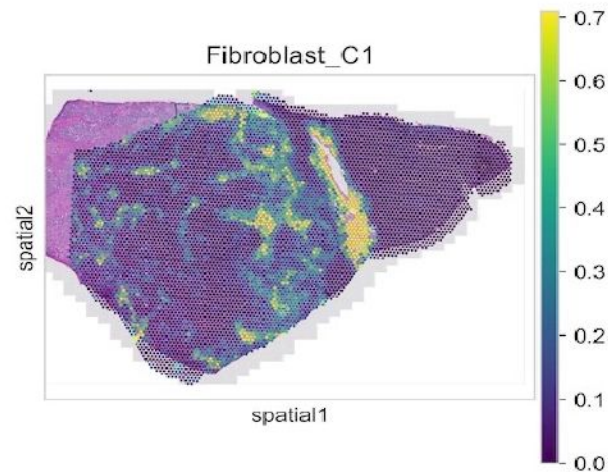
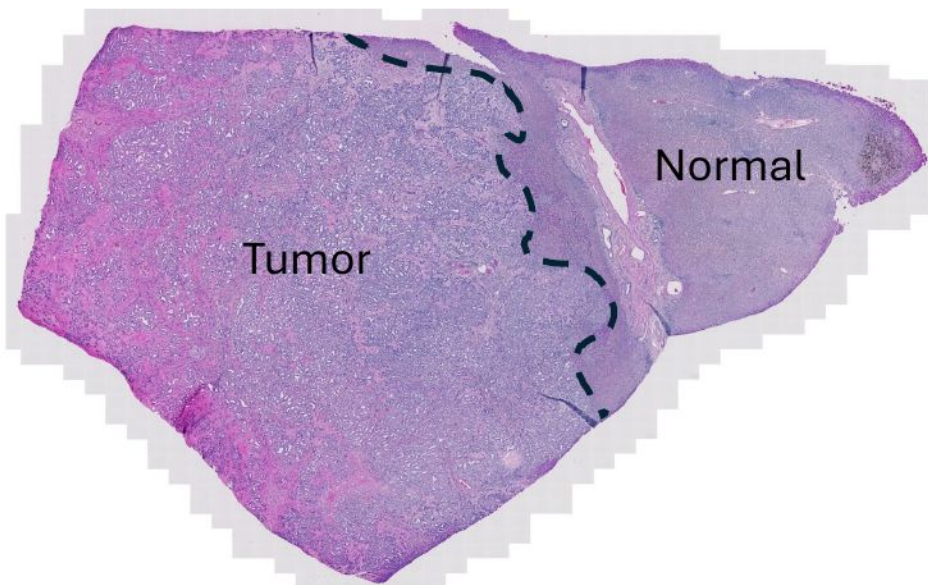


BioNinja: <https://ib.bioninja.com.au/options/option-d-human-physiology/d3-functions-of-the-liver/liver-structure.html>

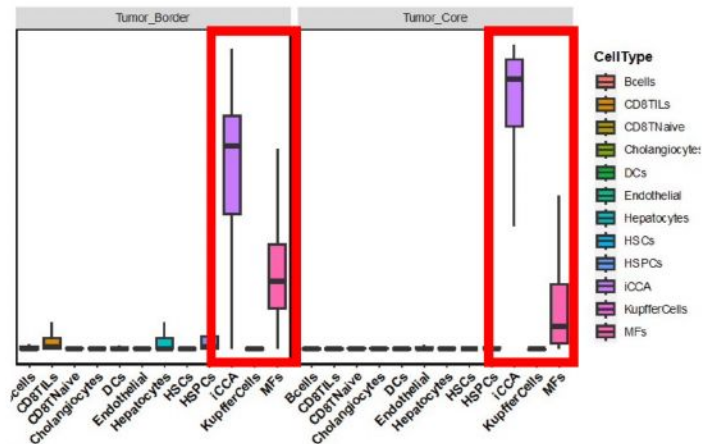


Portal Vein (PV) regions/ Periportal regions	Zone 1	cells in this zone first receive everything in blood, including oxygen, absorb nutrients, absorb xenobiotics
Central Vein (CV) regions/ Parenchymal regions	Zone 2	cells in this zone have characteristics intermediate between cells of Zone 1 and 3
	Zone 3	cells in this zone are close to central vein, and therefore are the last receiving and coming blood

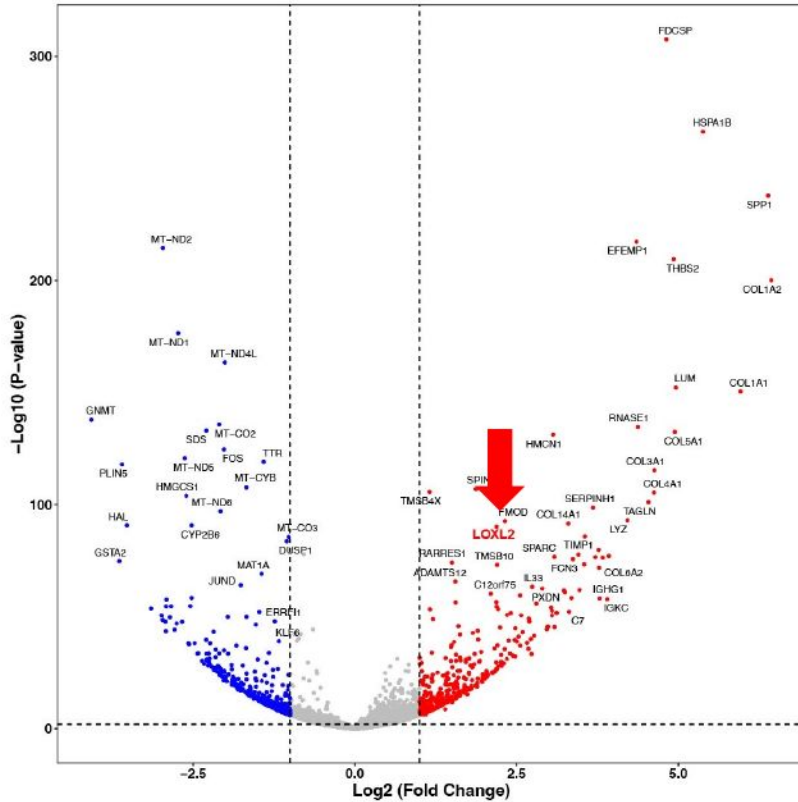
Myofibroblasts are colocalized with iCCA



Central hypothesis: the spatially defined damage responses of the host liver to iCCA development elicit early tumor cell dissemination along the **periportal myofibroblast network**



LOXL2 as a Potential Master Regulator



DEGs: Portal vs Central Vein

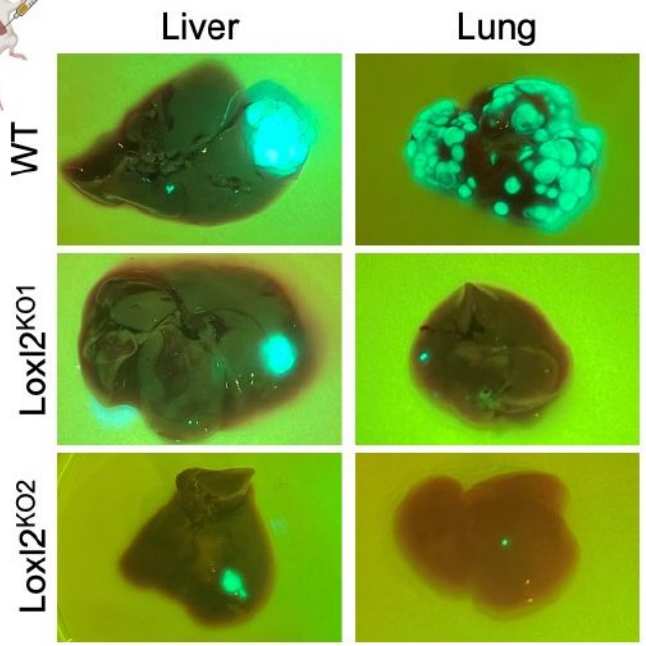
LOXL2

- Member of lysyl oxidase family
- Catalyzes collagen and elastin crosslinking in the ECM
- Overexpressed in various cancers (breast, lung, colon, liver)¹
- Associated with poor prognosis
- Drives epithelial-mesenchymal transition²
- Modifies the tumor microenvironment in a way that supports tumor growth and survival²

¹Cano et al., 2023, ²Wen et al., 2020

LOXL2 promotes iCCA tumor metastasis (Zhu Lab)

f

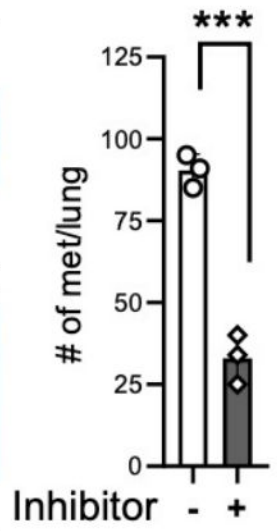
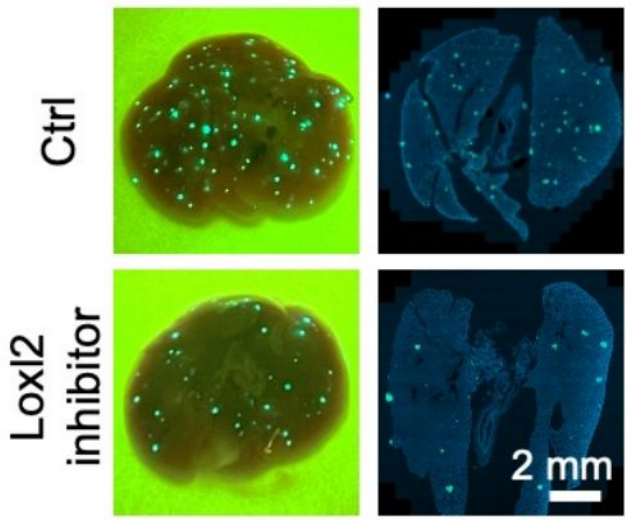


g



Gross
GFP

GFP/Dapi



Background

Gene expression profiling of iCCA and its microenvironment using scRNA-seq and ST identified LOXL2 as a potential master regulator of iCCA dissemination.



My Project

LOXL2 Expression
Profiling

related to intrahepatic cholangiocarcinoma

Survival Analysis



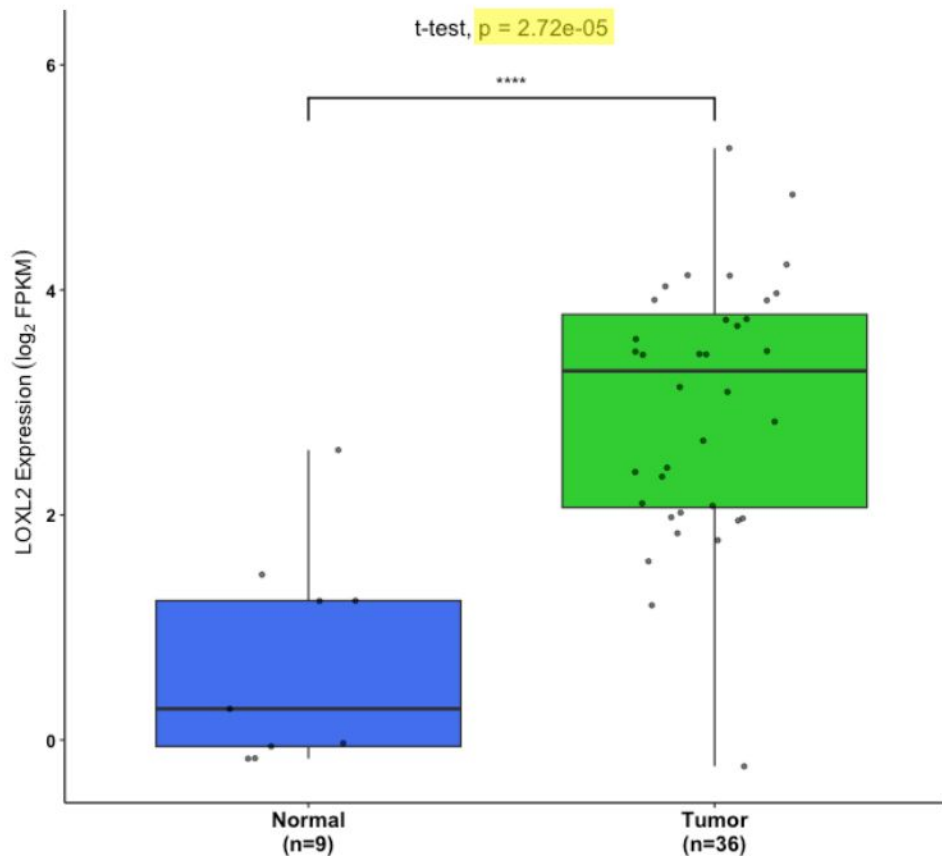
Ultimate Goal

Reveal the biology underlying the early and rapid **dissemination of iCCA**

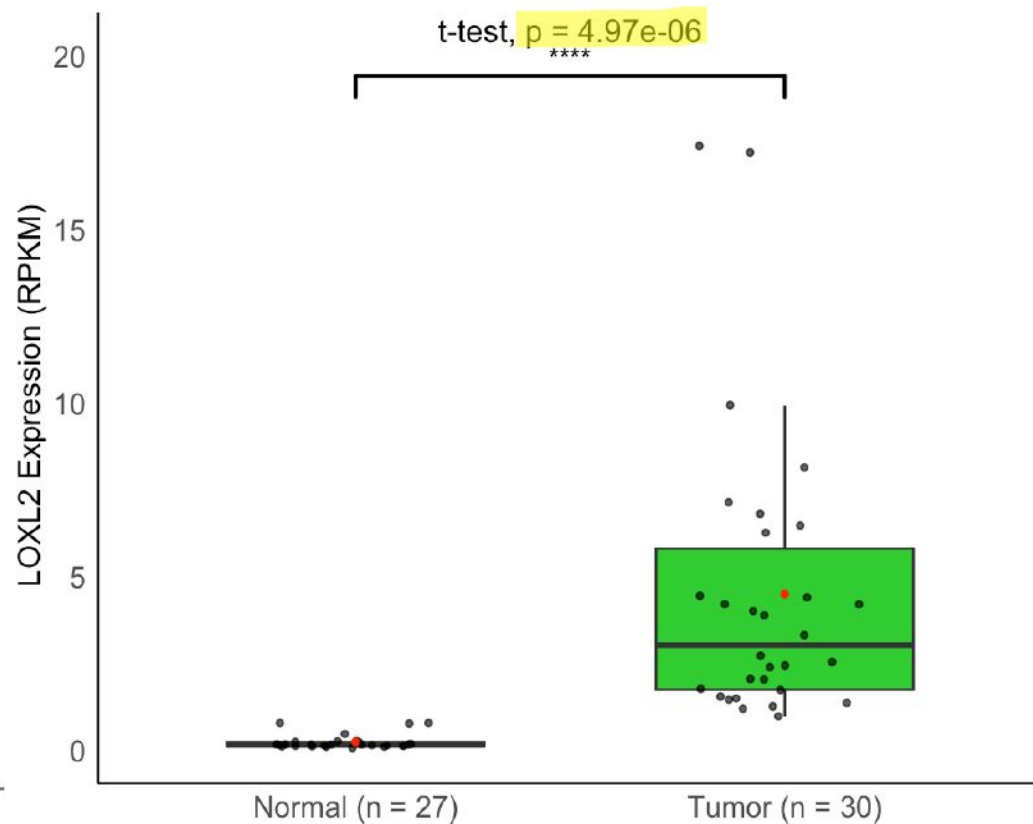
Datasets Utilized for Bulk RNA Expression Profiling and Survival Analysis

☐	Species	Data type	Category	Tissue/Tumor	Author	N	Normalization	Platform	Comp...	Material	Accession	Release da...	R2 date	Access
	Select	Select Filter	Select Filter	cholan			Select Filter		Selec	Selec				Select
☐	hs	Expression data	Mixed	Cholangiocarcinoma (2022-v...	tcga	44	tpm	gencode36	bulk	RNA		2000-01-01	2022-06-07	public
☐	hs	Expression data	Mixed	Intrahepatic cholangiocarcioma	Kang	57	DESeq2_rlog	igenomesegrch...	bulk	RNA	GSE107943	2018-12-01	2023-05-02	public
☐	hs	Expression data	Tumor	Cholangiocarcinoma (combat ...	Lim	120	custom	ilmnht12v4	bulk	DNA	GSE89749	2017-07-03	2022-06-20	public
☐	hs	Expression data	Tumor	Cholangiocarcinoma	TCGA	36	rsem	tcgars	bulk	RNA	CHOL	2000-01-01	2015-11-26	public
☐	hs	Expression data	Tumor	Cholangiocarcinoma - part A ...	Jasakul	48	custom	ilmnht12v4	bulk	DNA		2000-01-01	2018-05-04	public
☐	hs	Expression data	Tumor	Cholangiocarcinoma - part B ...	Jasakul	72	custom	ilmnht12v4	bulk	DNA	GSE89749	2017-07-03	2018-05-04	public
☐	hs	Expression data	Tumor	Cholangiocarcinoma / Hepato...	Nabila	109	rma_sketch	hta2t	bulk	RNA	E-MTAB-6...	2000-01-01	2023-01-19	public

LOXL2 Expression in Tumor vs Normal Samples

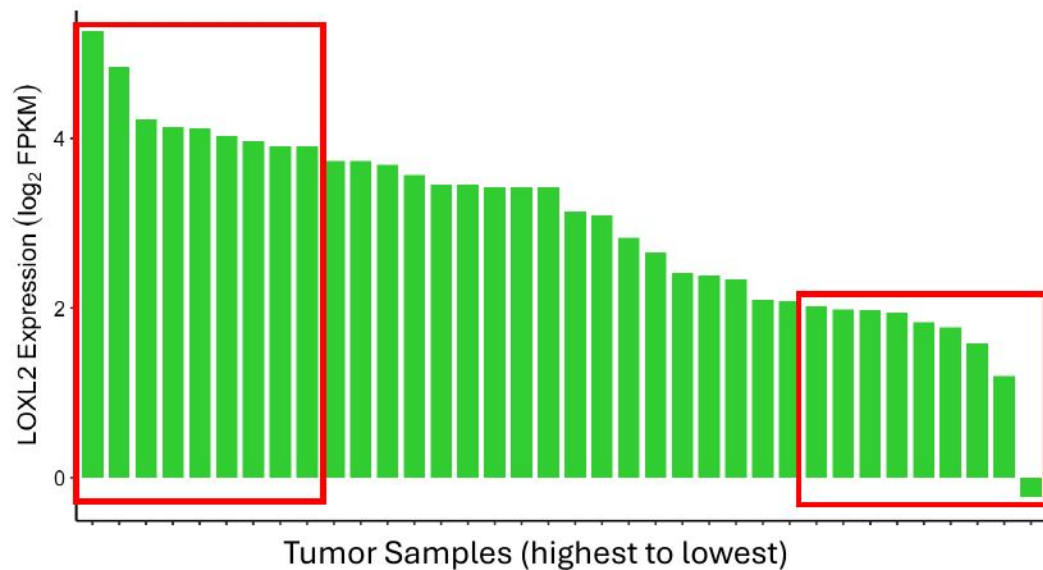


TCGA

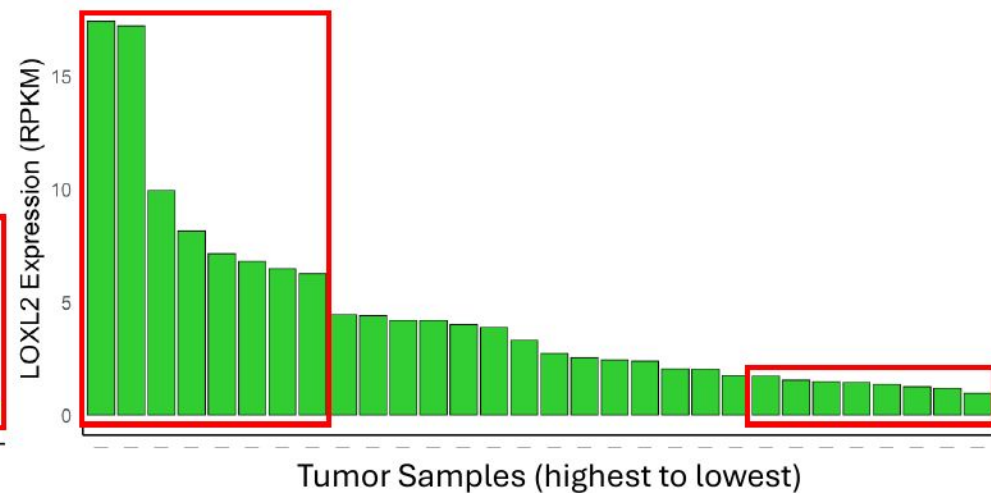


Kang

LOXL2 Expression in Tumor Samples

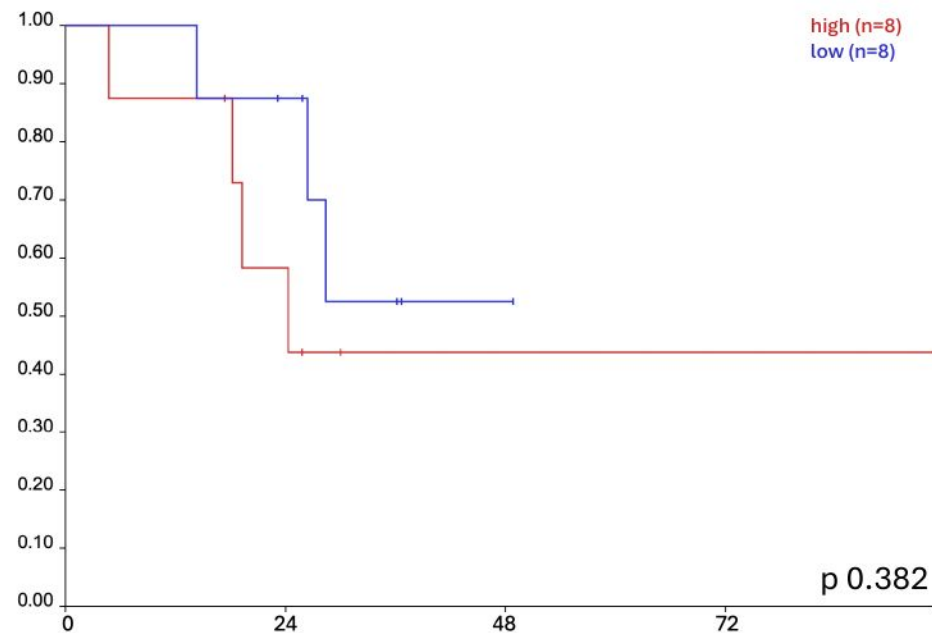
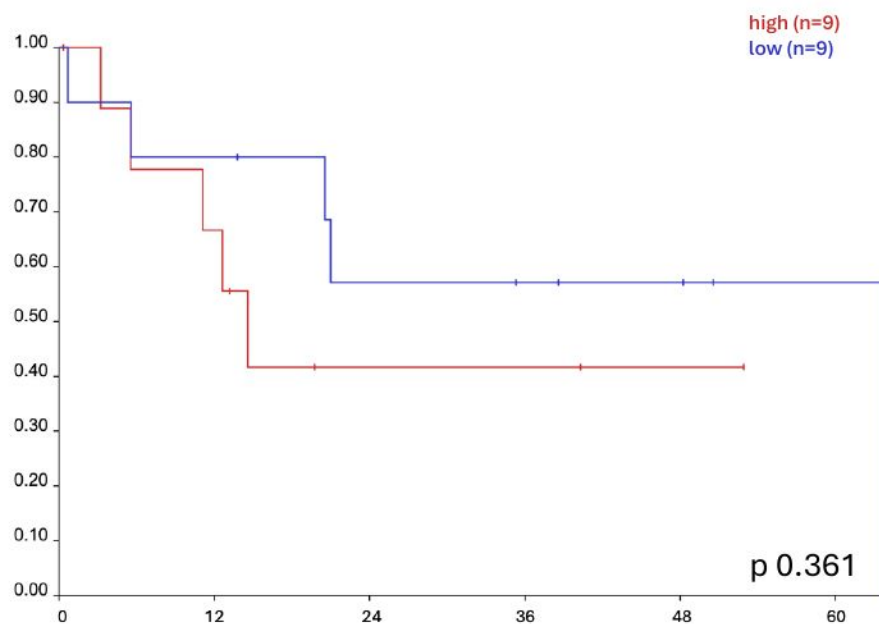


TCGA



Kang

Kaplan-Meier Analysis of High vs. Low LOXL2 Expression

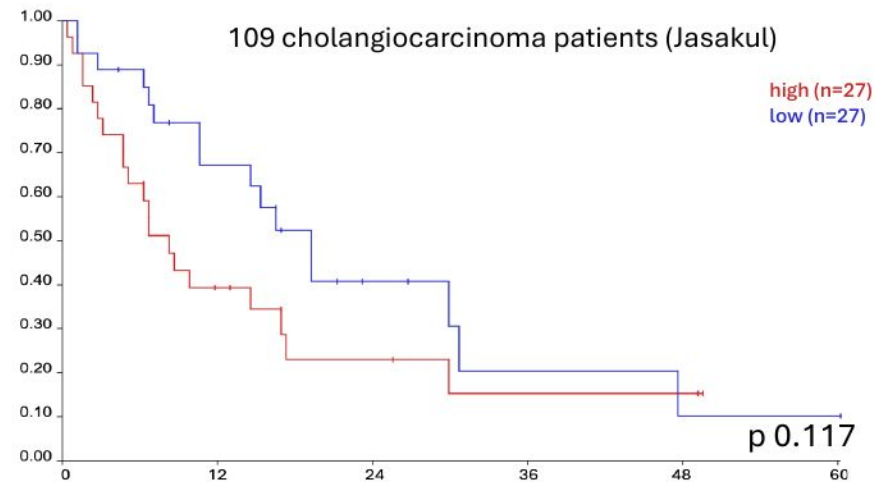
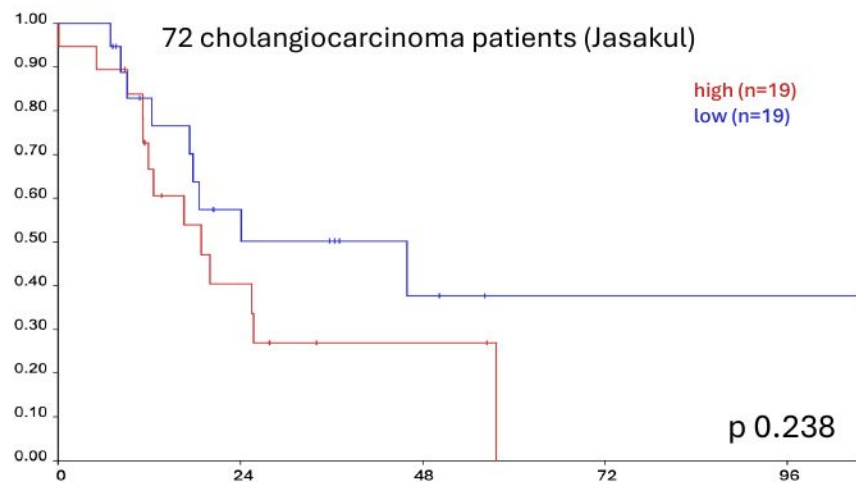
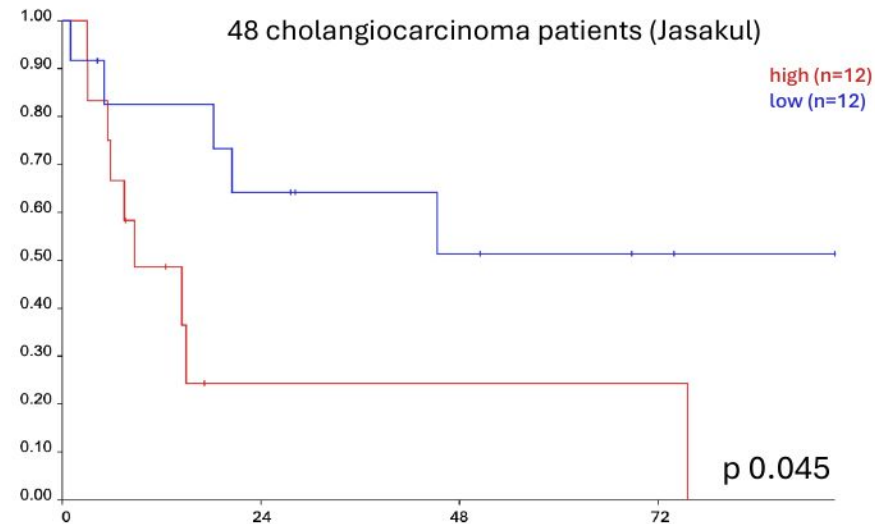
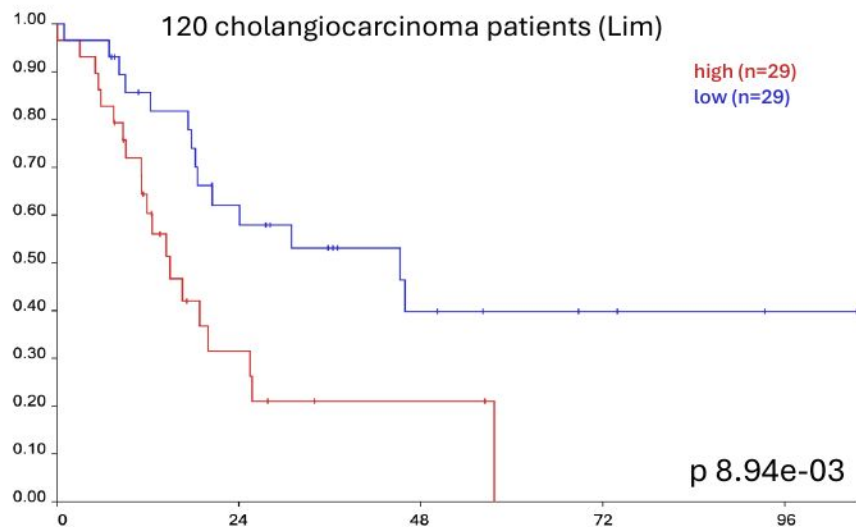


Survival probability
↑
Follow up (months)
→

TCGA

Kang

Kaplan-Meier Analysis of High vs. Low LOXL2 Expression



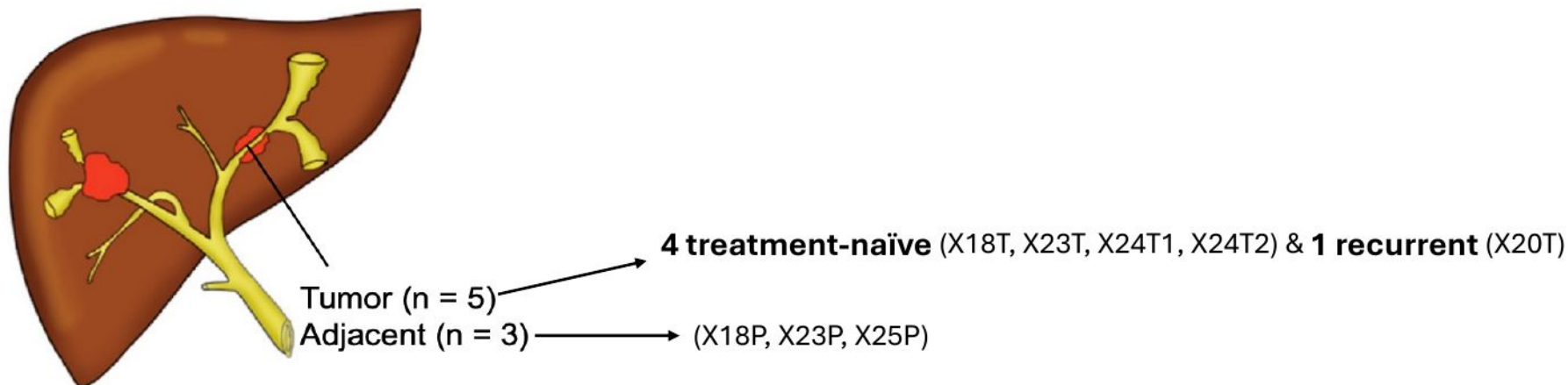
Clinical Relevance of LOXL2

- **Expression Differences: Significant** LOXL2 differences between normal and tumor cells in two datasets (Box Plots)
- **Survival Analysis:** High LOXL2 expression linked to **worse outcomes** in all 6 cohorts—2/6 significant (Kaplan-Meier)

Single-Cell RNA Sequencing Analysis of LOXL2 in iCCA Using



GSE138709¹



Intrahepatic cholangiocarcinoma
Paired tumor and adjacent tissues

Surgical resection samples

GSE138709

sourceSample	Total cells	Total genes	Genes per cell (mean)	UMIs per cell (mean)	Cells per gene (mean)
Combined	33991	19813	2199	9361	3773
X18P	10317	14647	1316	4032	927
X18T	4963	16423	3493	19281	1055
X20T	2714	14670	2304	9053	426
X23P	4425	15308	1621	5728	469
X23T	3504	16566	3005	14256	636
X24T	5909	17814	2889	12384	958
X25P	2159	13884	1303	3638	203

GSE138709

Filtration cutoffs estimated by scMINER

	# Cells per gene (nCell)	# UMIs per cell (nUMI)	# Features per cell (nFeature)	% Mitochondrial genes (pctMito)	% Spike-In RNAs (pctSpikeIn)
Minimum	169	342	259	0.0000	0
Maximum	Inf	115007	Inf	0.1052	0

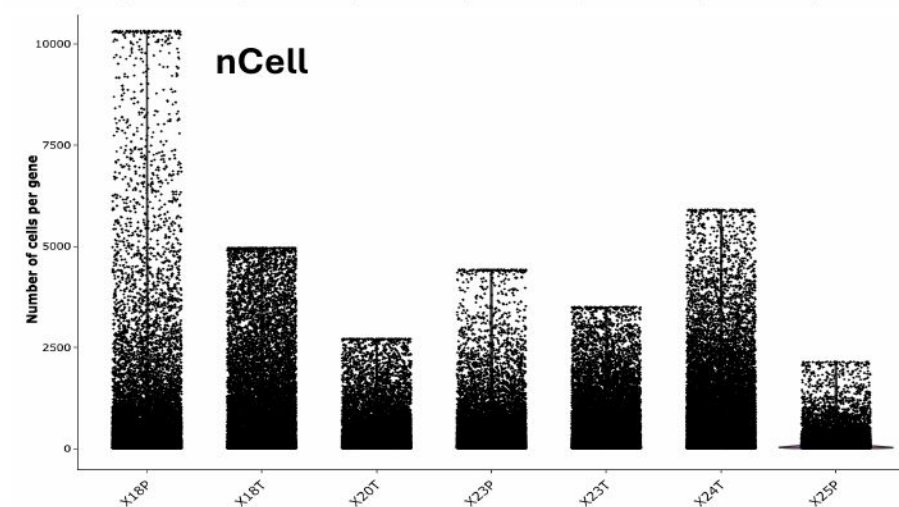
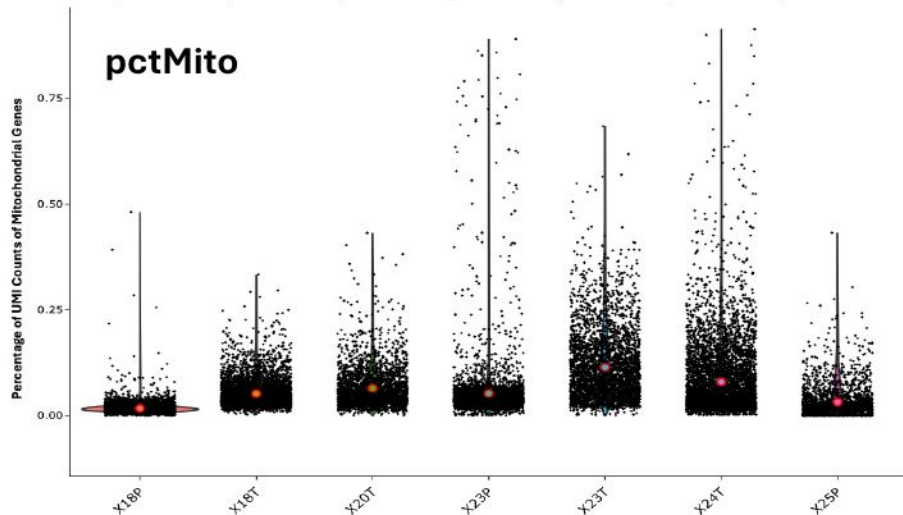
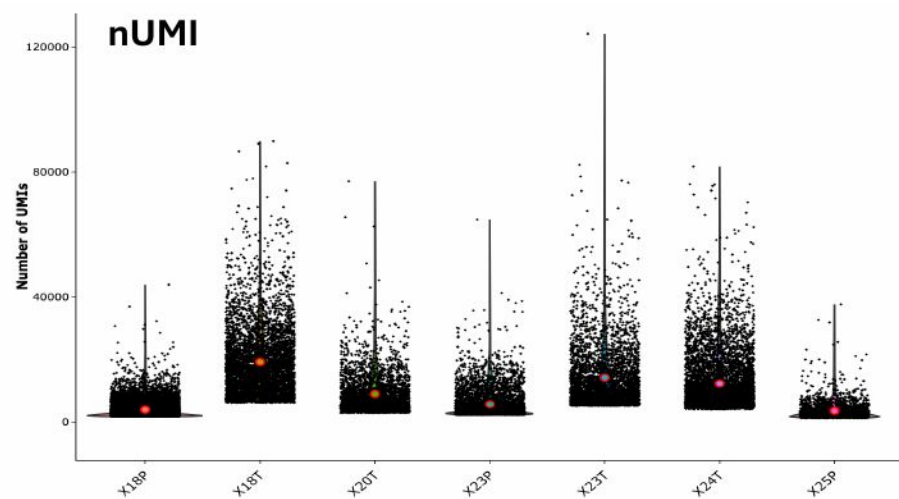
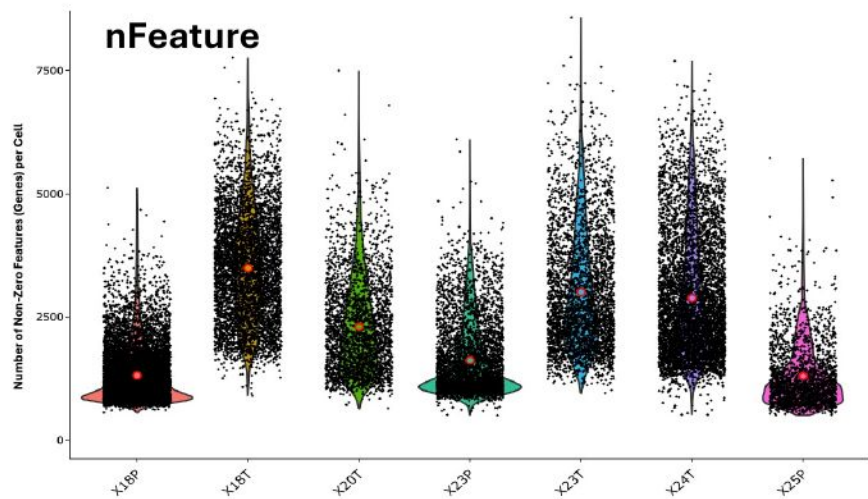
Gene filtration statistics

Metrics	nCell
Gene_total	19813
Gene_failed	4405(22.23%)
Gene_passed	15408(77.77%)

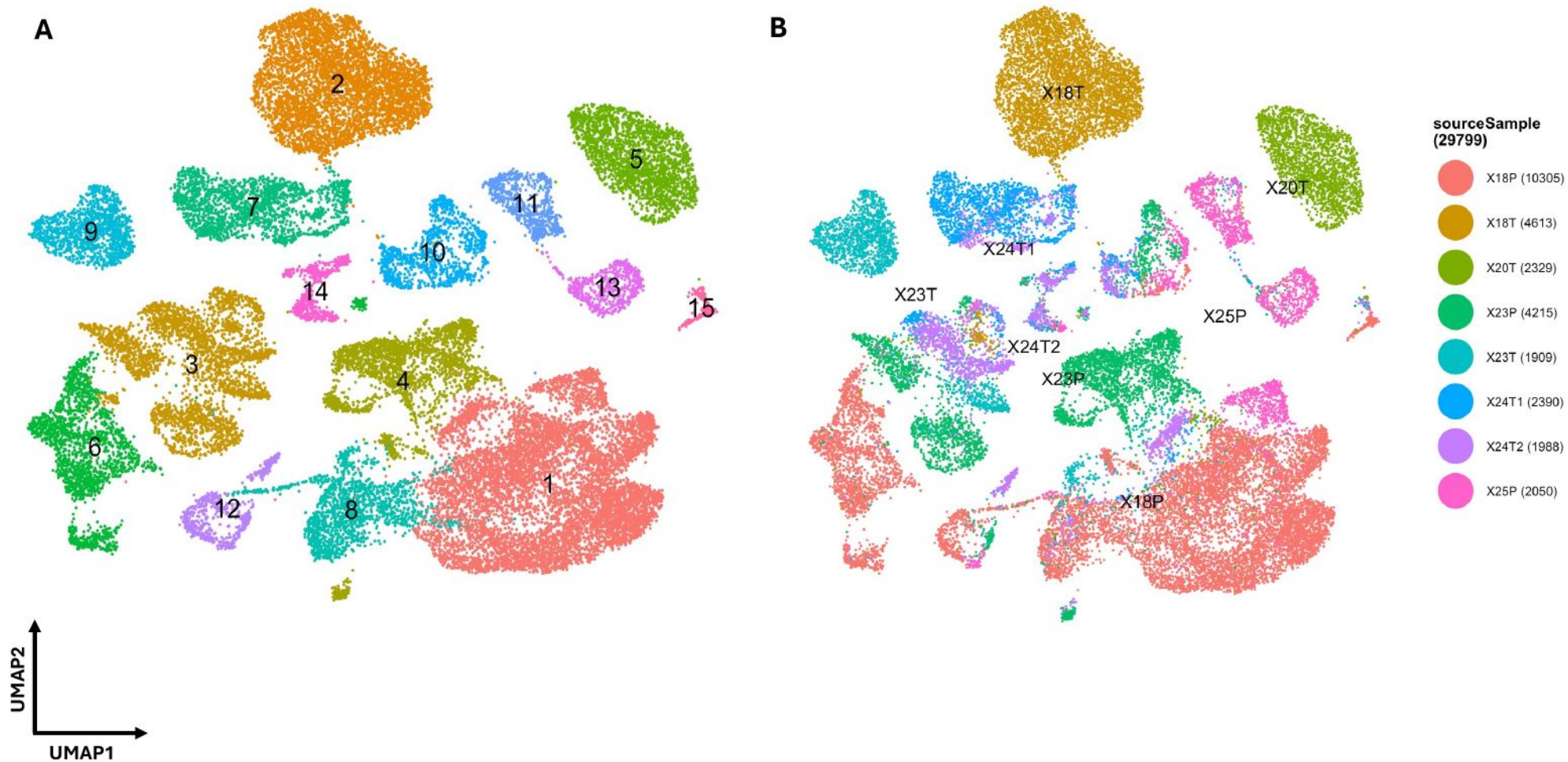
Cell filtration statistics

Metrics	Combined	nUMI	nFeature	pctMito	pctSpikeIn
Cell_total	33991	33991	33991	33991	33991
Cell_failed	4192(12.33%)	1(0%)	0(0%)	4191(12.33%)	0(0%)
Cell_passed	29799(87.67%)	33990(100%)	33991(100%)	29800(87.67%)	33991(100%)

Distributions from scMINER QC Report

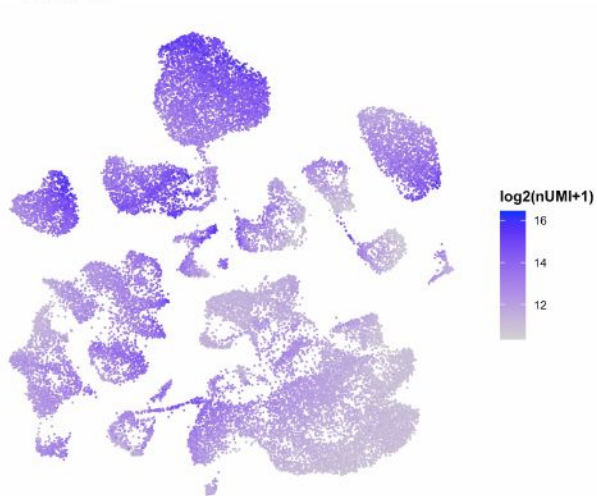


MICA output UMAP cluster (5.3)

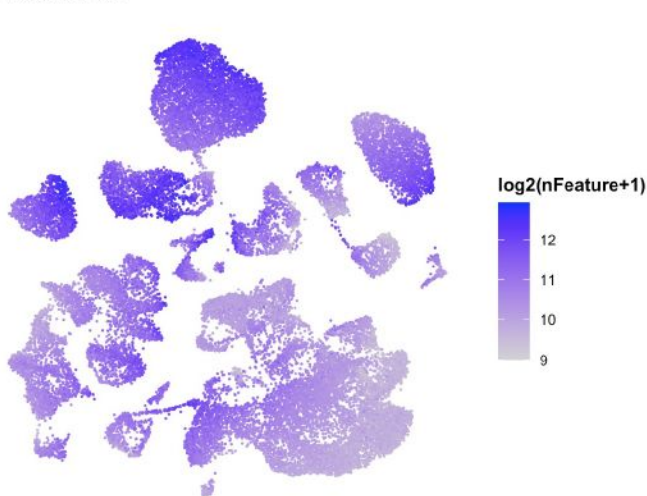


UMAP QC Metrics: nUMI, nFeature, pctMito

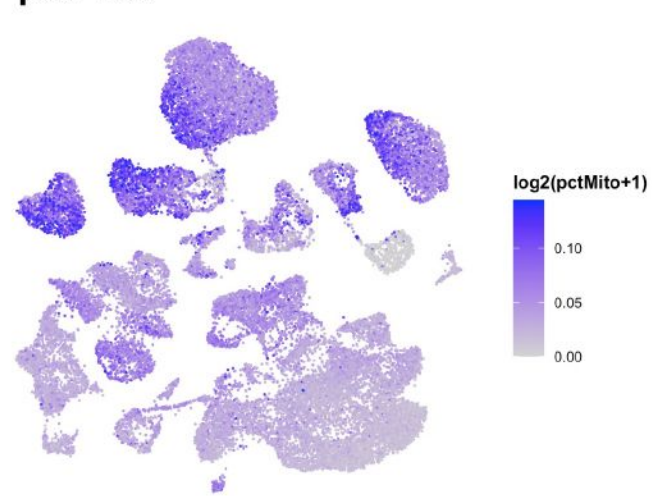
nUMI



nFeature



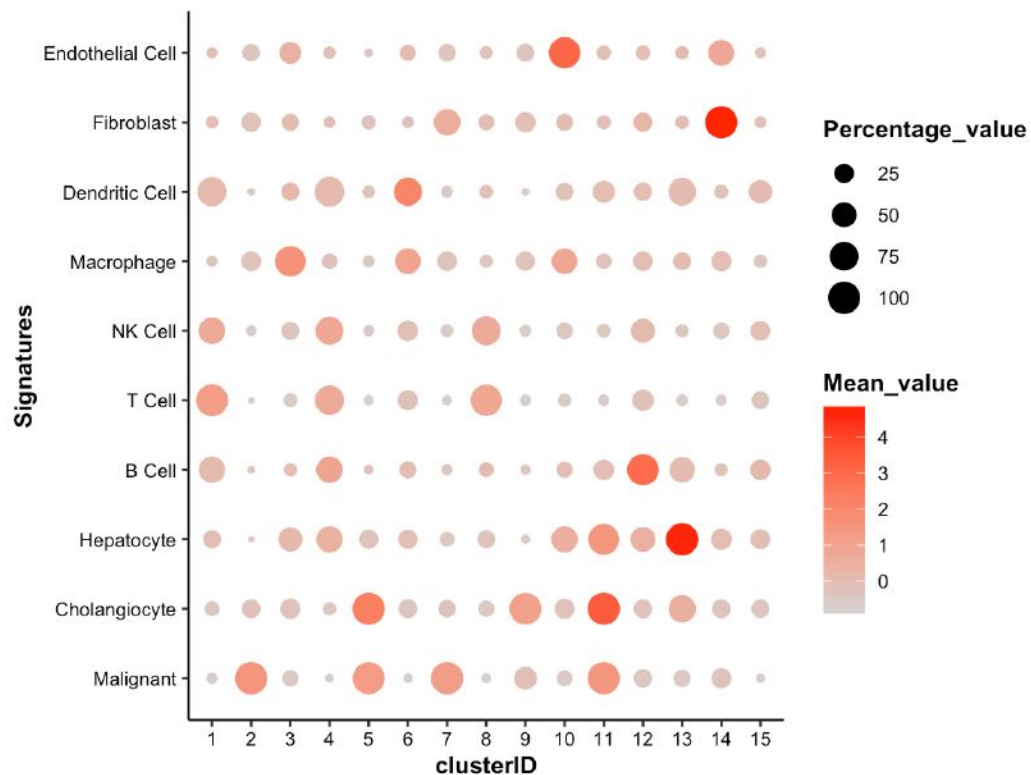
pctMito



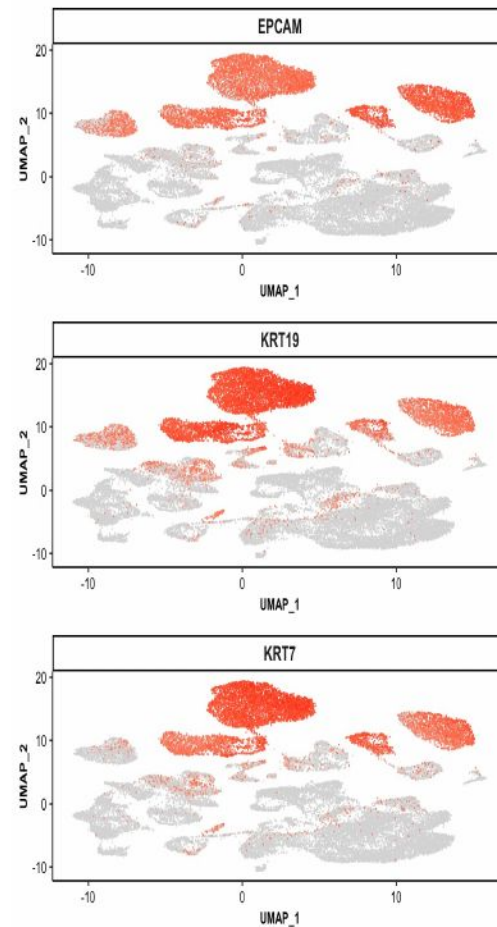
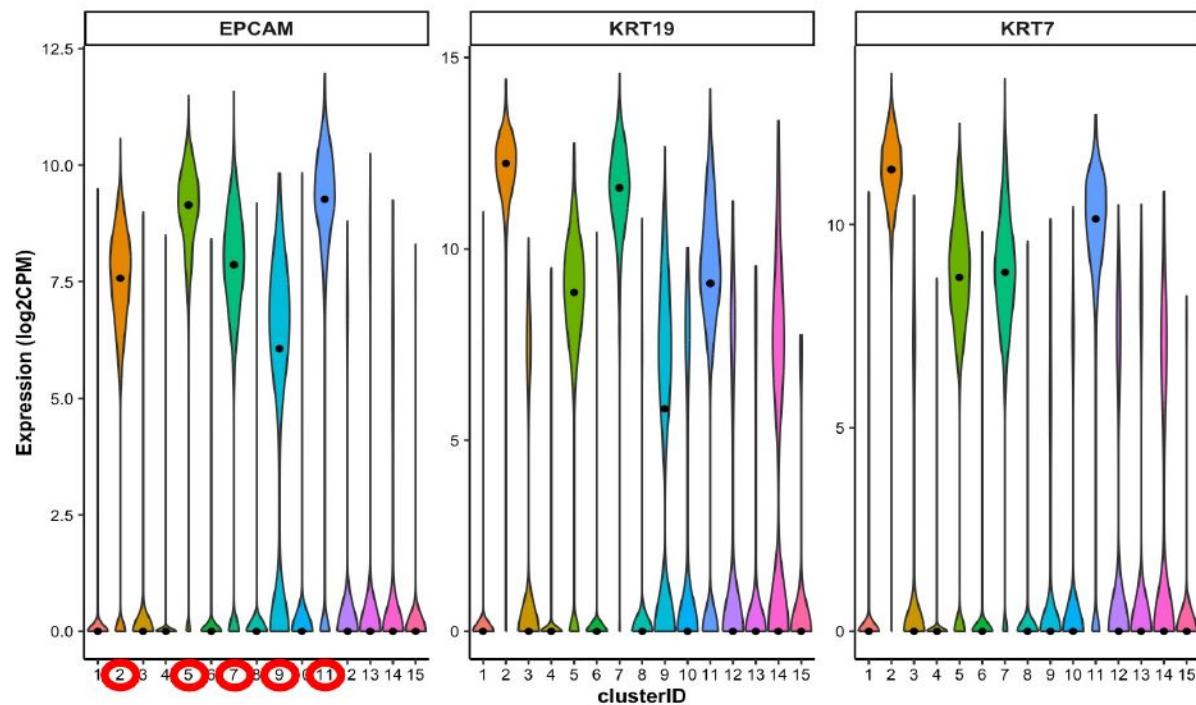
UMAP2
↑
UMAP1
→

Initial Curated Mark List¹

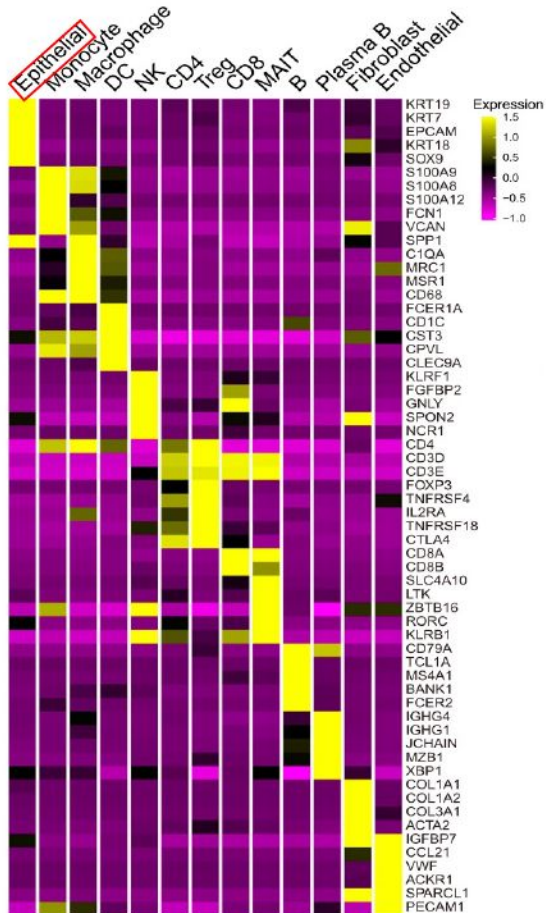
signature_name	signature_feature	weight
Malignant	EPCAM	1
Malignant	KRT19	1
Malignant	KRT7	1
Cholangiocyte	FYXD2	1
Cholangiocyte	TM4SF4	1
Cholangiocyte	ANXA4	1
Hepatocyte	APOC3	1
Hepatocyte	FABP1	1
Hepatocyte	APOA1	1
B Cell	MS4A1	1
B Cell	CD79A	1
T Cell	CD2	1
T Cell	CD3D	1
T Cell	CD3E	1
NK Cell	CD7	1
NK Cell	FGFBP2	1
NK Cell	KLRF1	1
Macrophage	CD14	1
Dendritic Cell	CLEC9A	1
Dendritic Cell	CD1C	1
Fibroblast	ACTA2	1
Fibroblast	COL1A2	1
Endothelial Cell	ENG	1
Endothelial Cell	vWF	1



Initial Markers for Malignant Cells



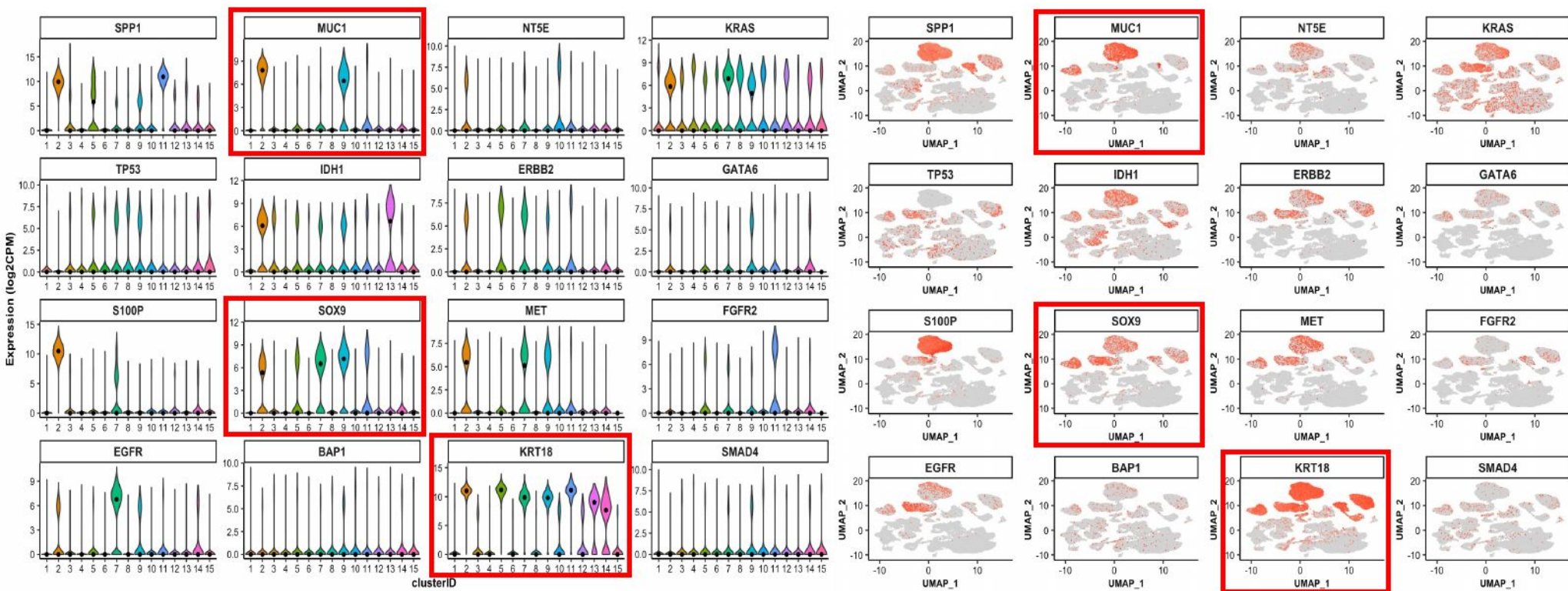
Supervised Cell Annotation



THE HUMAN PROTEIN ATLAS 

¹Song et al., 2022

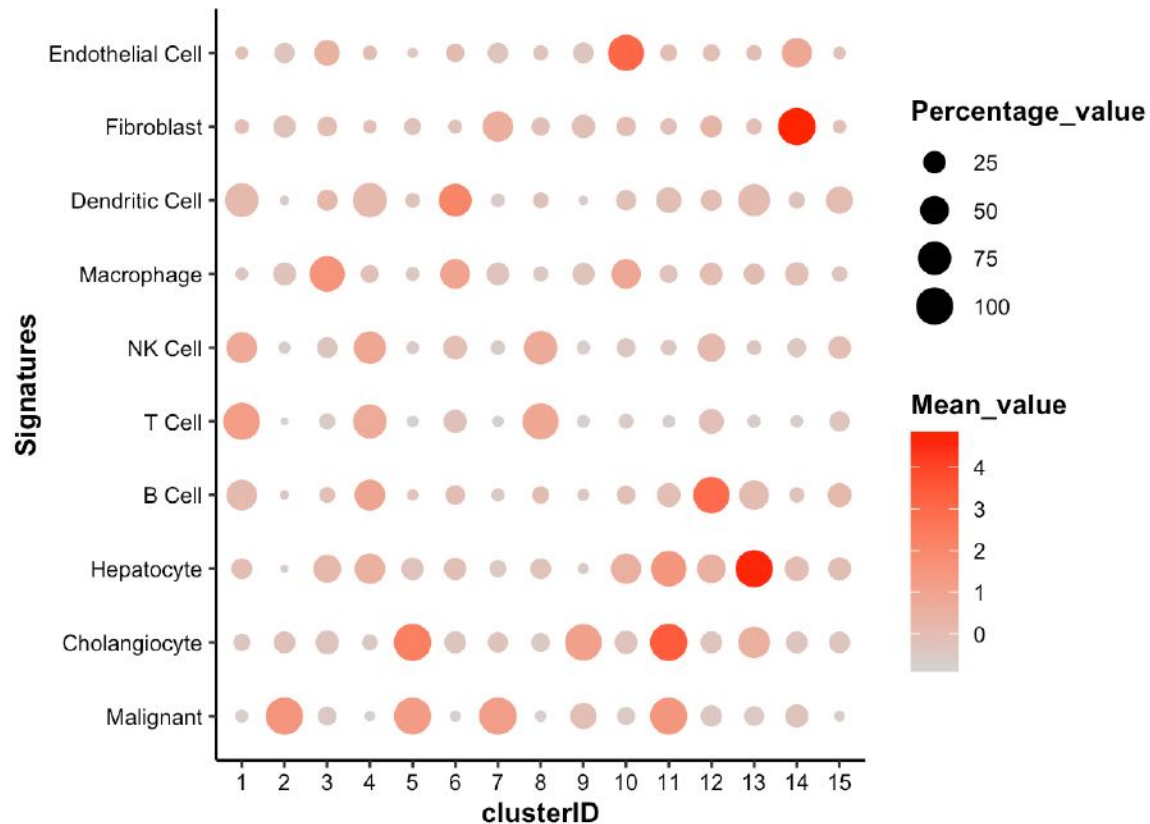
Analysis of Additional Markers for Malignant Cells



MUC1, SOX9, KRT18

Refined Mark List

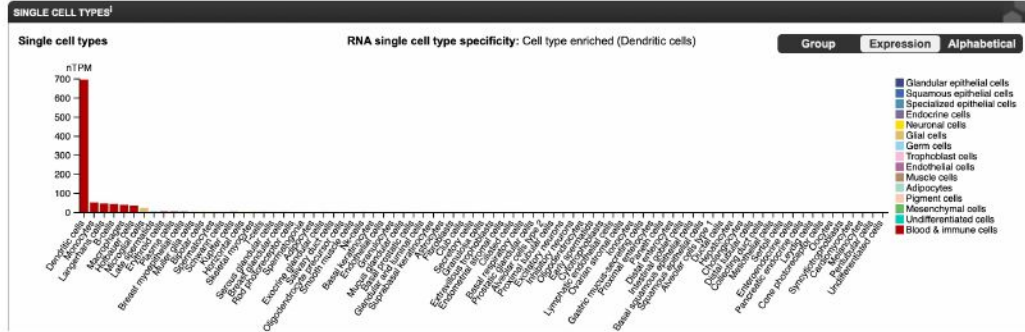
signature_name	signature_feature	weight
Malignant	EPCAM	1
Malignant	KRT19	1
Malignant	MUC1	1
Malignant	SOX9	1
Malignant	KRT18	1
Malignant	KRT7	1
Cholangiocyte	FXYD2	1
Cholangiocyte	TM4SF4	1
Cholangiocyte	AQP1	1
Cholangiocyte	HNF1B	1
Cholangiocyte	ANXA4	1
Hepatocyte	APOC3	1
Hepatocyte	FABP1	1
Hepatocyte	HAO1	1
Hepatocyte	APOA1	1
B Cell	FCER2	1
B Cell	MS4A1	1
B Cell	CD79A	1
T Cell	CD2	1
T Cell	CD3D	1
T Cell	CD8B	1
T Cell	CD3E	1
NK Cell	SH2D1A	1
NK Cell	CD7	1
NK Cell	FGFBP2	1
NK Cell	CD69	1
NK Cell	SPON2	1
Macrophage	CD4	1
Macrophage	C1QA	1
Macrophage	CD68	1
Macrophage	MSR1	1
Macrophage	CD14	1
Dendritic Cell	LILRB4	1
Dendritic Cell	CD300A	1
Dendritic Cell	IRF7	1
Dendritic Cell	PTCRA	1
Dendritic Cell	CD1C	1
Fibroblast	COL3A1	1
Fibroblast	COL1A1	1
Fibroblast	ACTA2	1
Fibroblast	COL1A2	1
Endothelial Cell	ENG	1
Endothelial Cell	SOX9	1
Endothelial Cell	IGFBP7	1
Endothelial Cell	SPARCL1	1



Unsupervised Cell Annotation

```
> de_t$feature[1:50]
```

```
[1] "GZMB" "IGJ" "PLD4" "LILRA4" "GPR183" "IRF8" "IRF7" "PLAC8" "ITM2C" "TCF4" "ALOX5AP" "RNASE6"  
[13] "C12orf75" "SELL" "IRF4" "TYROBP" "MZB1" "SERPINF1" "SPIB" "FCER1G" "LILRB4" "TPM2" "SOX4" "TSPAN13"  
[25] "PTGDS" "BCL11A" "IL3RA" "HLA-DQA1" "PTCRA" "RGS2" "CCDC50" "MPEG1" "HLA-DRA" "HLA-DQB1" "MYBL2" "HLA-DRB1"  
[37] "HLA-DPA1" "HLA-DPB1" "LDLRAD4" "MIR142" "RGS1" "RNASET2" "PTPRE" "HERPUD1" "CD74" "CLIC3" "PTPRS" "PRMT10"  
[49] "SLC15A4" "NR3C1"
```



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[Transcription](#) [Pathways](#) [Ontologies](#) [Diseases/Drugs](#) **[Cell Types](#)** [Misc](#) [Legacy](#) [Crowd](#)

Description No description available (44 genes)

CellMarker 2024

T Cell Lung Human
T Cell Stomach Human
Fibroblast Lung Mouse
Natural Killer Cell Human
T Cell B-cell Human

Descartes Cell Types and Tissue 2021

Lymphoid cells in Lung
Lymphoid cells in Stomach
Fibroblast Lung Mouse
Lymphoid cells in Bone
Lymphoid cells in Adrenal

CCLE Proteomics 2020

697 HAEMATOPOIETIC AND LYMPHOID TISS
697 HAEMATOPOIETIC AND LYMPHOID TISS
697 HAEMATOPOIETIC AND LYMPHOID TISS
697 HAEMATOPOIETIC AND LYMPHOID TISS
697 HAEMATOPOIETIC AND LYMPHOID TISS

Tabula Sapiens

Liver-ck Cell
Lung-ck Cell
Bone Marrow-ck Cell
Adipose-ck Cell
Pancreas-ck Cell

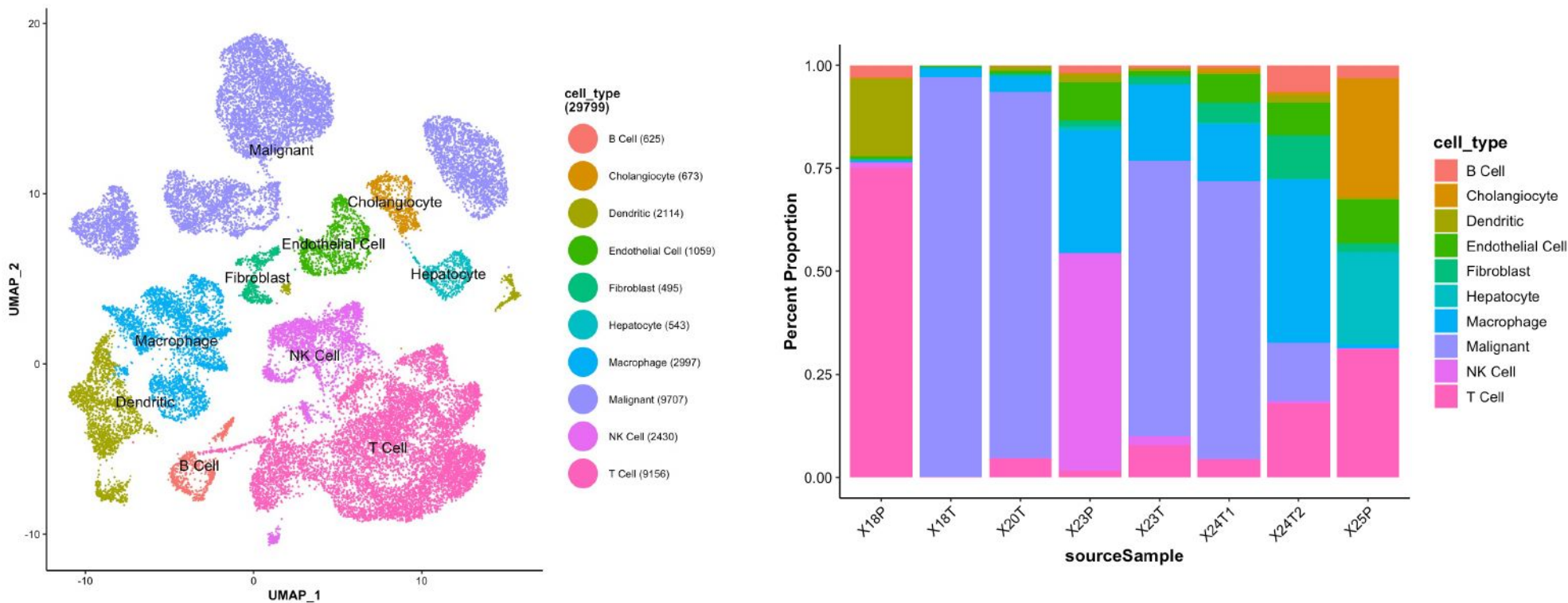
HuBMAP ASCTplusB augmented 2022

Natural Killer Cell - Respiratory System
CD8-positive, Alpha-Beta Memory T cell - Lyr
CD8-positive, Alpha-Beta Memory T cell - Lyr
CD8-negative
CD56-bright Natural Killer cell

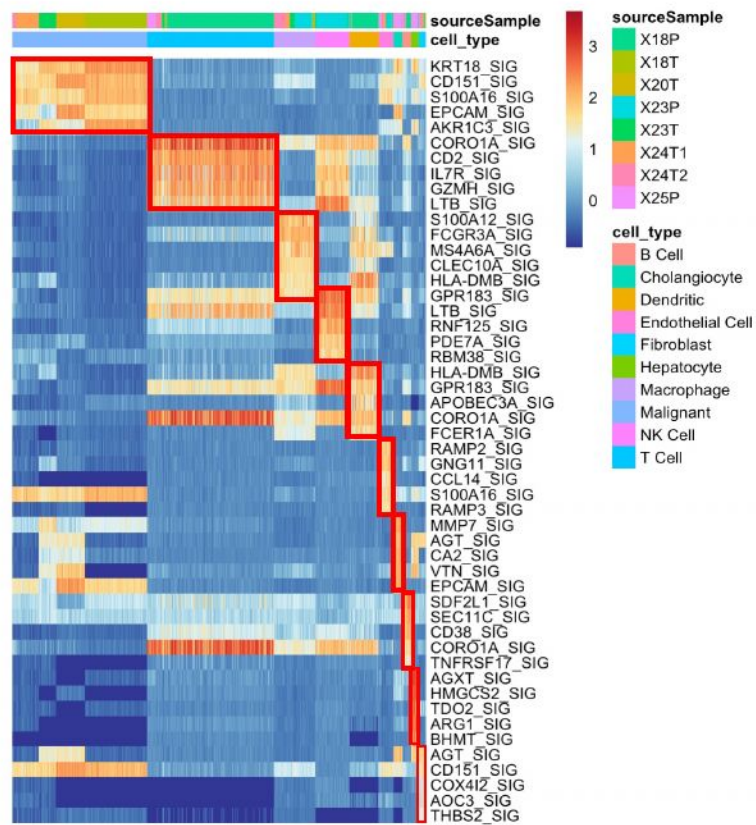
CellMarker Augmented 2021

CD8+ Regulatory T cell/Undefined
Memory T cell/Leukocyte
Memory T cell/Peripheral Blood
Naive T cell/Unfractionated Cord Blood
T cell/Blood

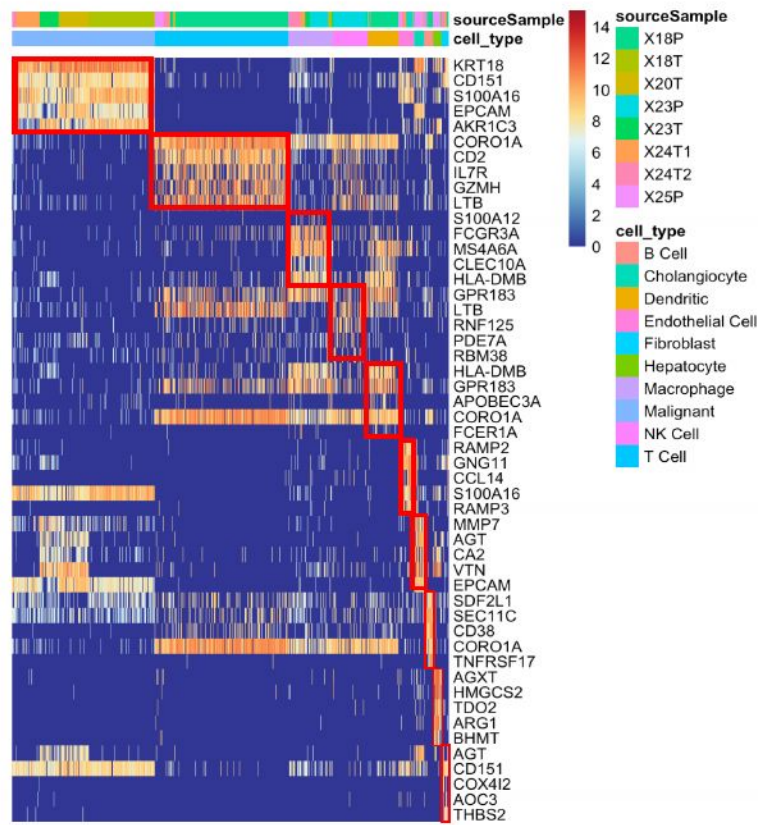
Cell Type Distribution and Composition: UMAP and Proportion Analysis



Top 5 Drivers per Cell Type: Expression & Activity Matrices

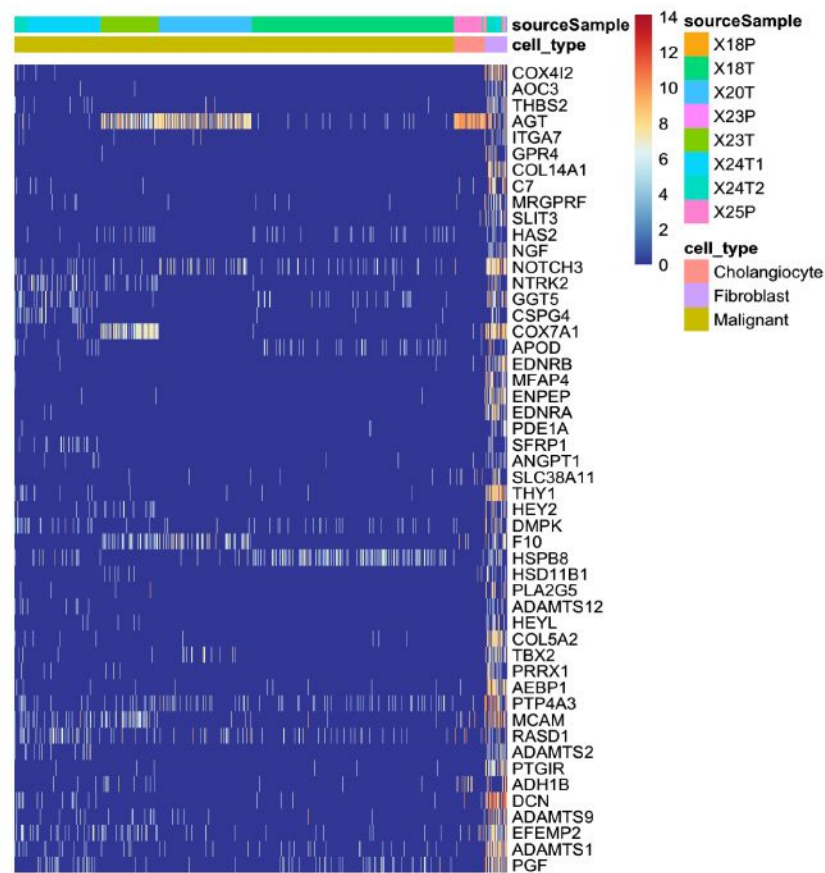
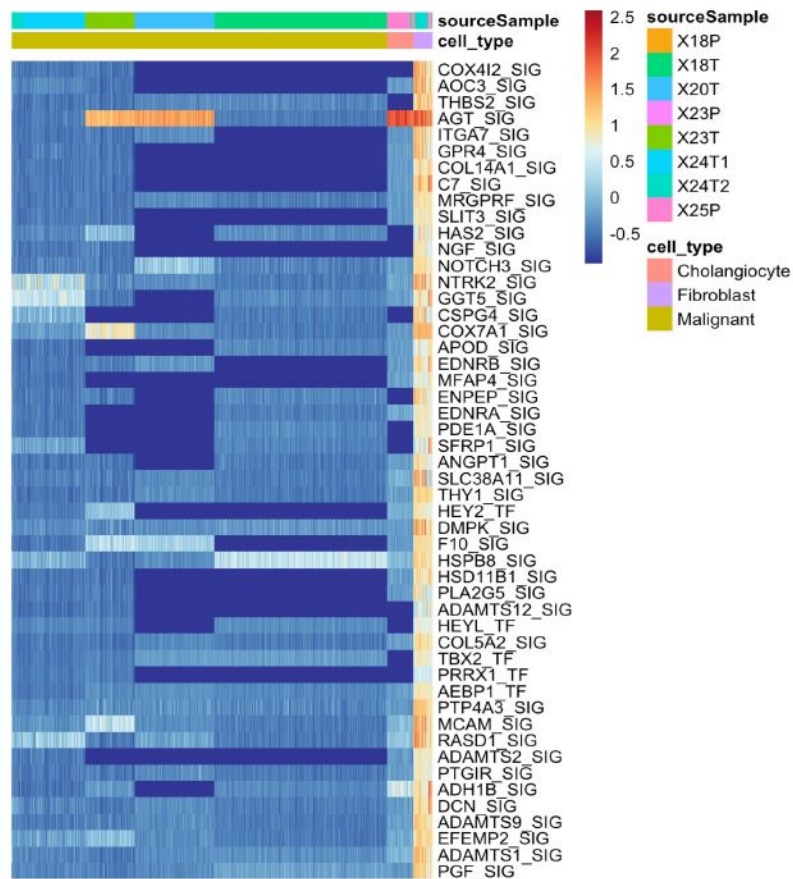


Activity



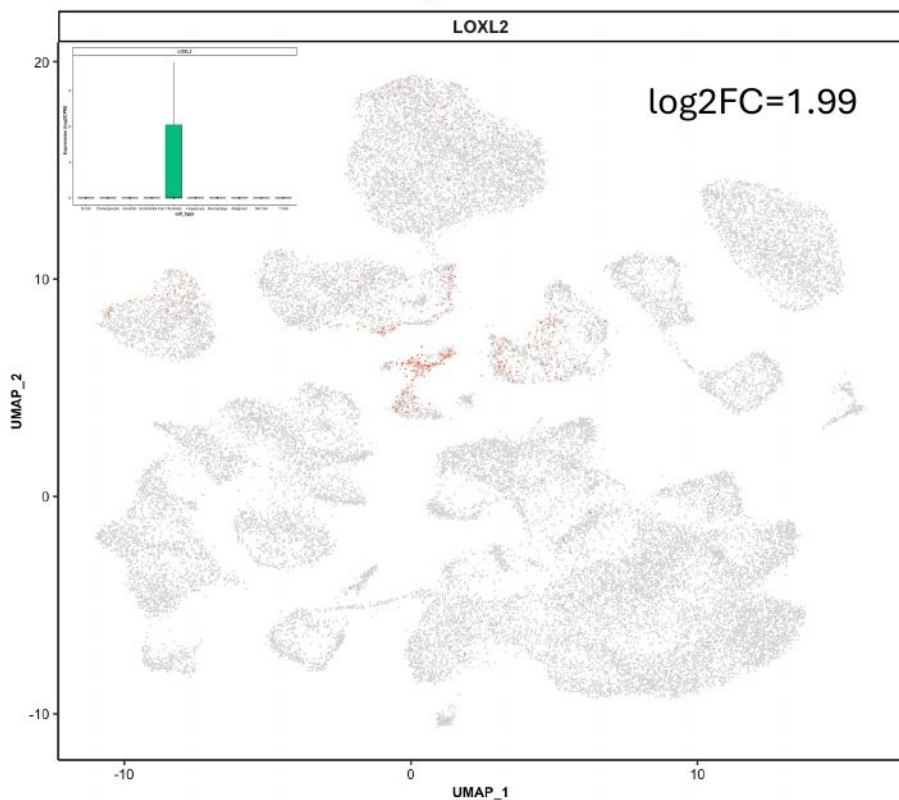
Expression

Top 50 Drivers in Fibroblasts: Expression & Activity Matrices

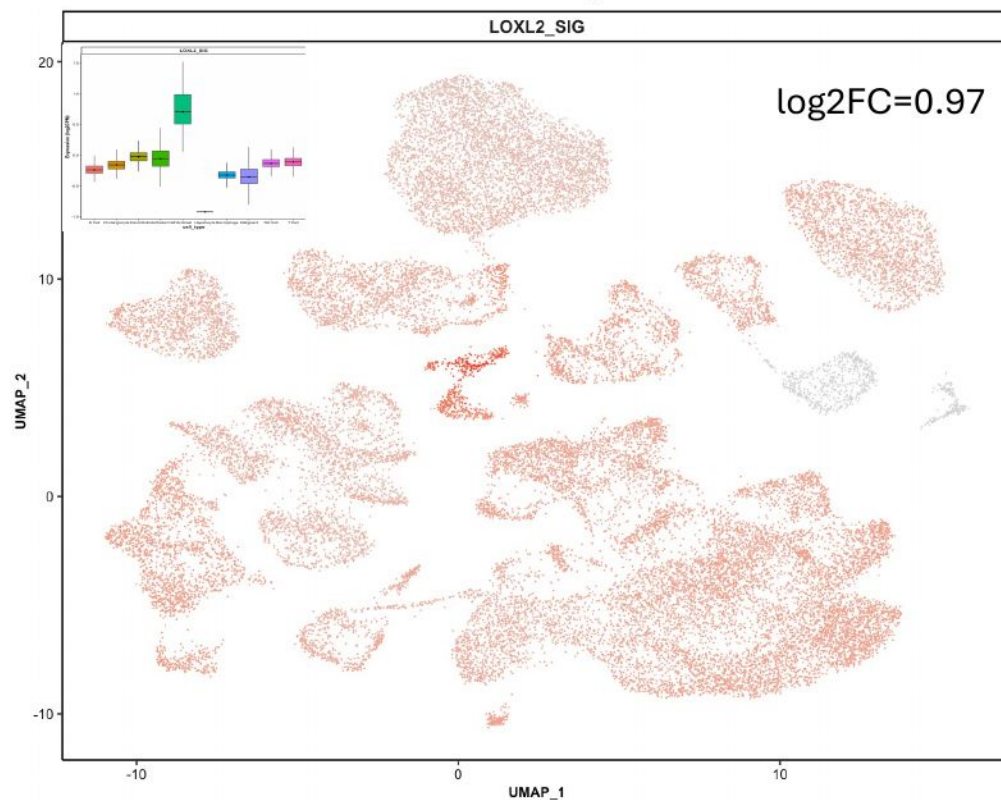


LOXL2 is a Potential Master Regulator

Expression

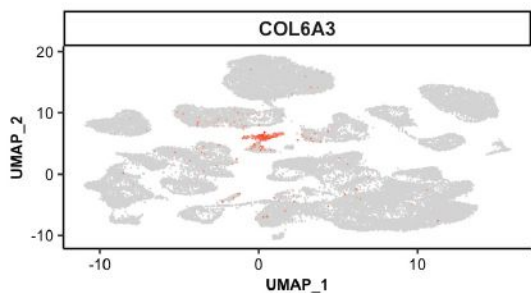
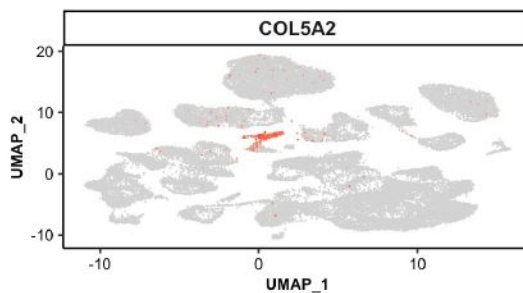
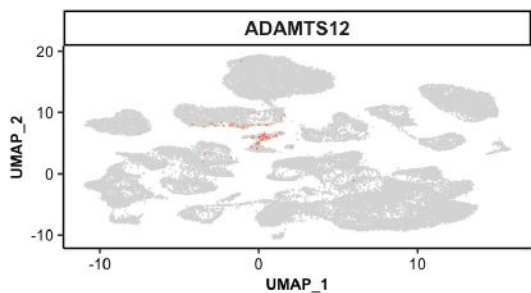
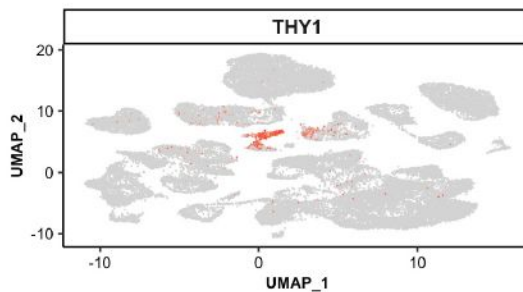
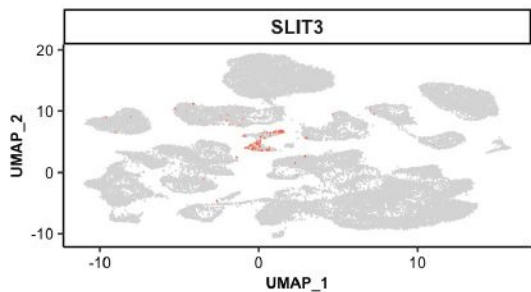


Activity

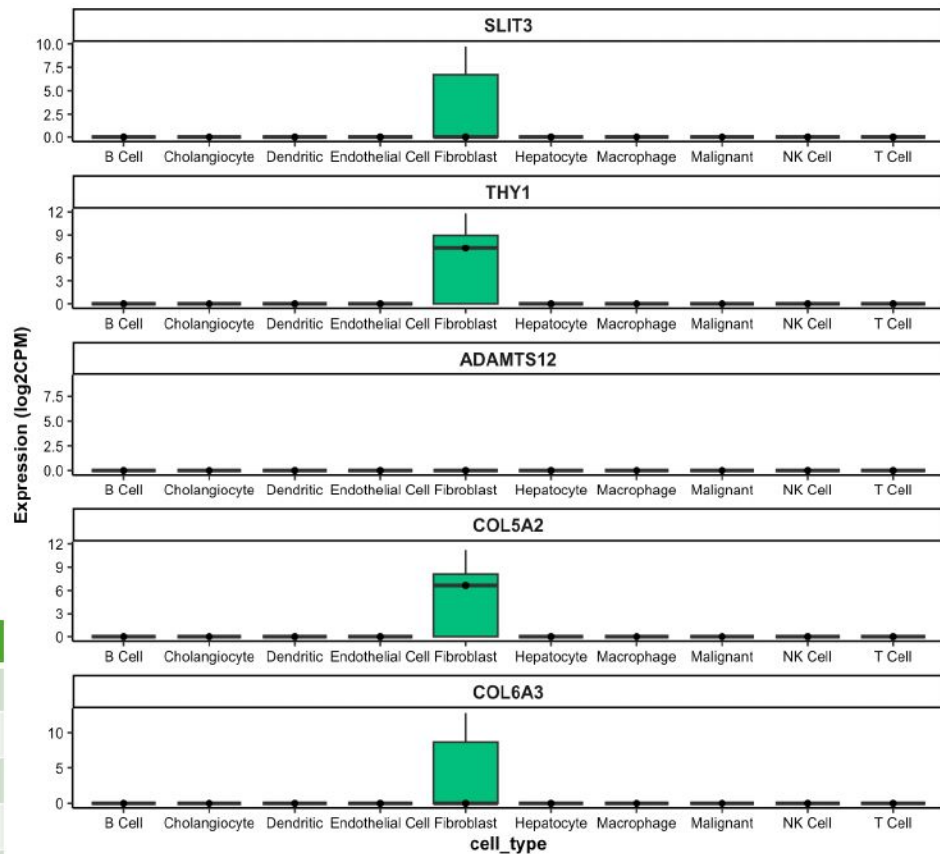


Central hypothesis: the spatially defined damage responses of the host liver to iCCA development elicit early tumor cell dissemination along the **periportal myofibroblast network**

Top 5 LOXL2-Regulated Genes Active in Fibroblasts



Feature	log2FC
SLIT3	1.532565543
THY1	1.397392715
ADAMTS12	1.332508929
COL5A2	1.323149776
COL6A3	1.252073950

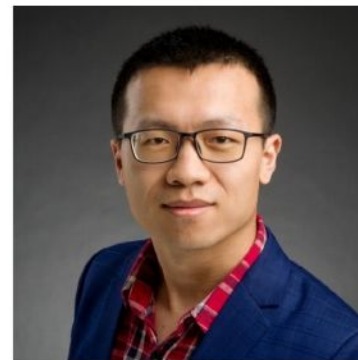
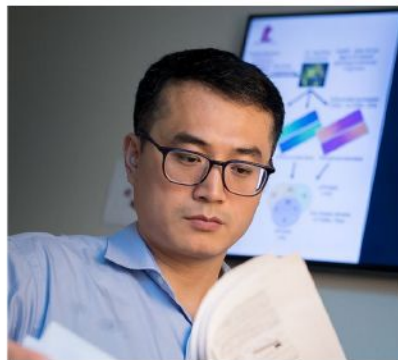


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