Single-cell multiomics guided mechanistic understanding of Fontan associated liver disease

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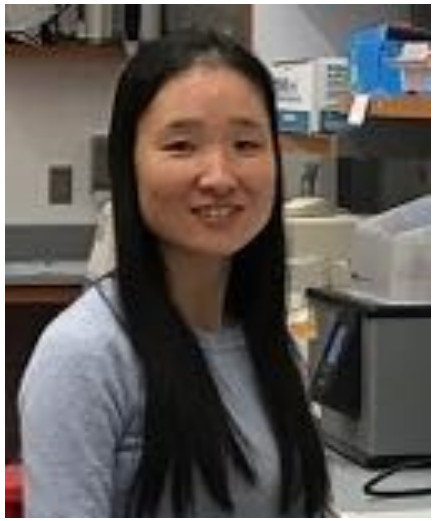
Disclosure

Nothing/Nada

Outline

1. Brief introduction of single-cell genomics

2. Fontan-associated liver disease (FALD)



Juanjuan Zhao

Po Hu

Jack Rychik

1. Single-cell genomics

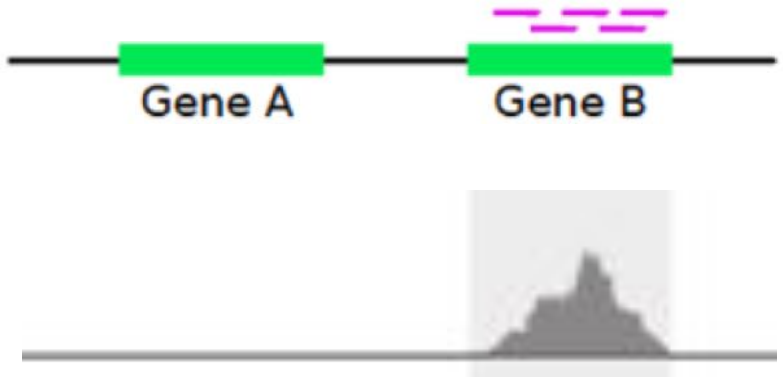
- Simultaneously analyze the genome-wide gene expression, epigenetic status and genome modification of thousands – millions of cells.

- Can include spatial and timing information.

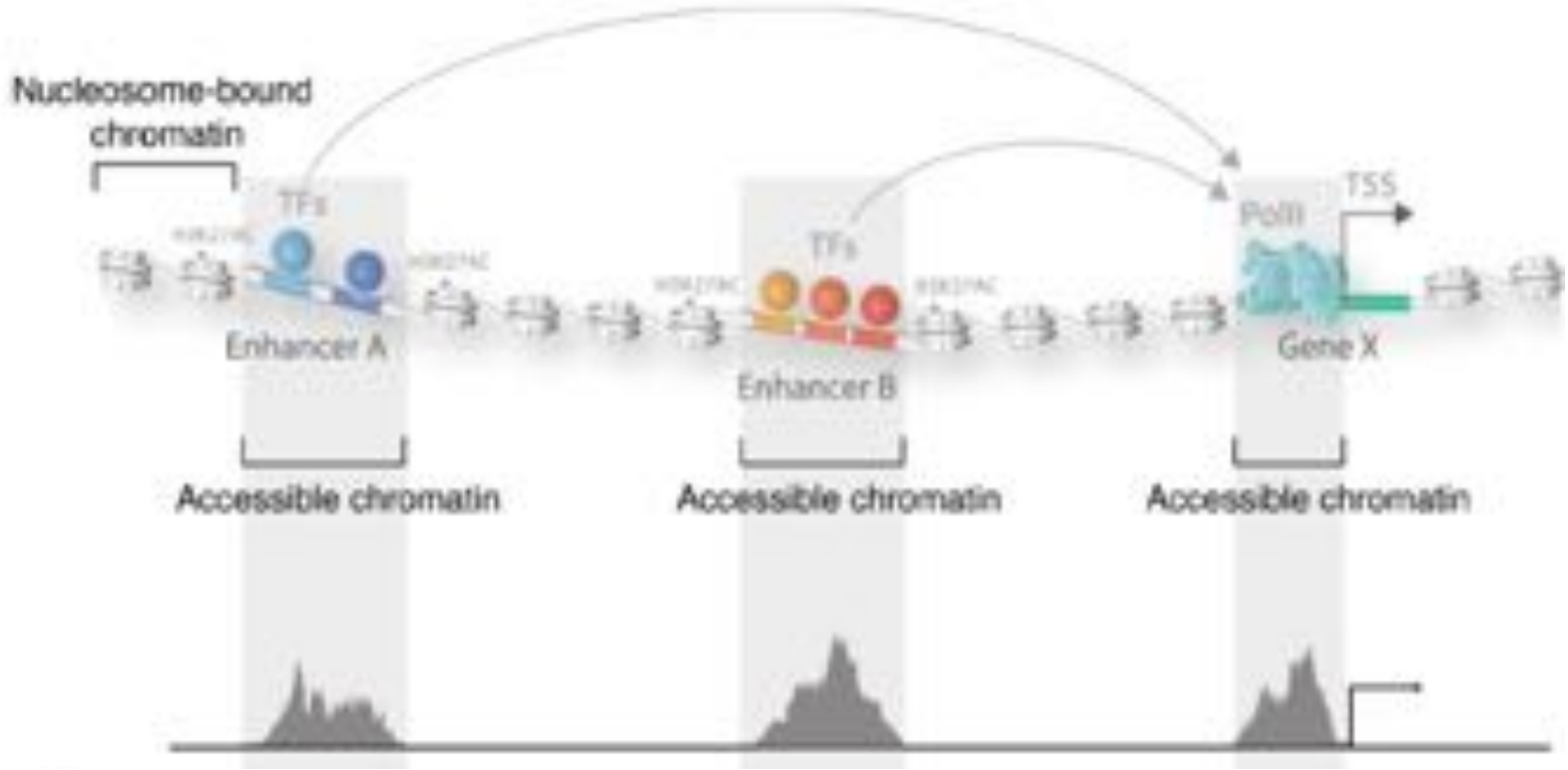
- New mechanistic insights into the underlying biology and disease, facilitating future mechanistic studies.

scRNA-ATAC-seq: gene expression and chromatin accessibility from the same cell, at single cell resolution

RNA-seq



ATAC-seq



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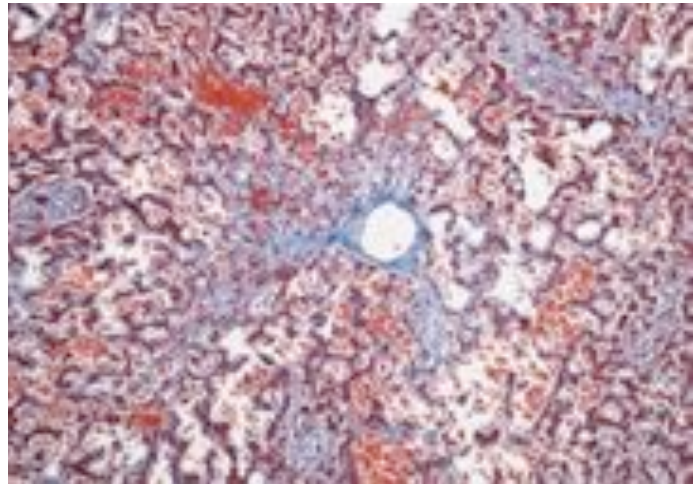
Fontan-associated liver disease (FALD)



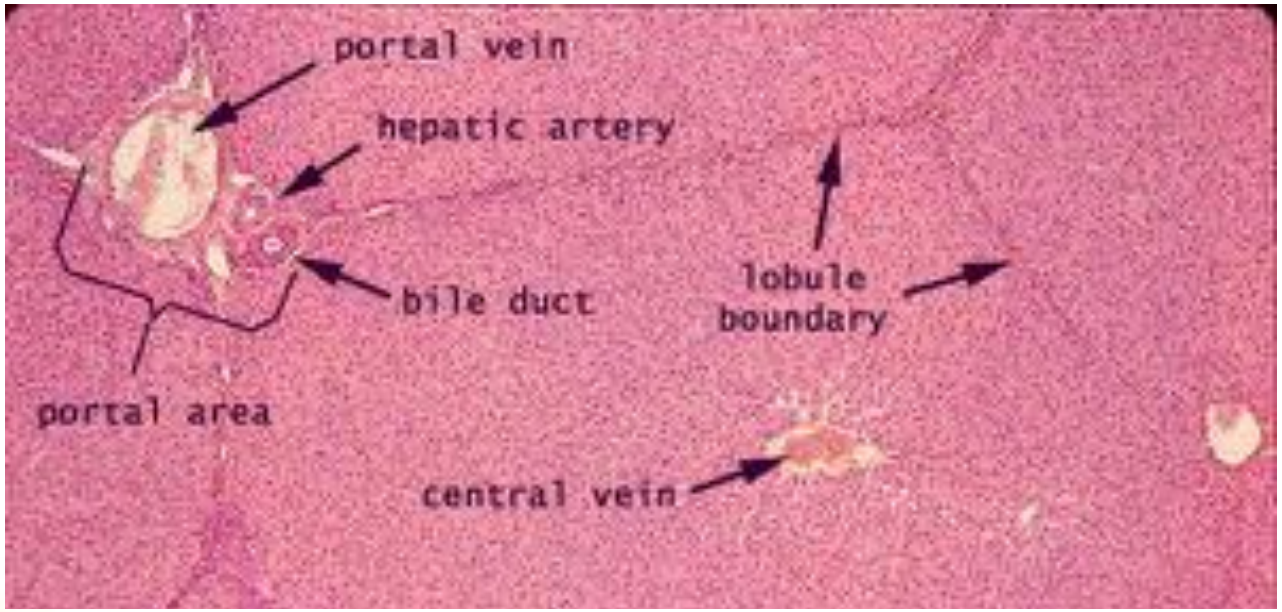
- **Severe and life-threatening hepatic fibrosis; no treatment:** often leads to liver cirrhosis and hepatocellular carcinoma and may require liver transplant
- **Almost 100% of Fontan patients develop FALD**
- **Universal, despite diverse genetic causes of original SVCHD**
- **Morphologically and mechanistically distinct from other liver fibrotic diseases (ALD, NASH, cirrhosis, etc.)**
- **Pathophysiology and mechanism not understood:** central venous hypertension? lymphatic congestion? low cardiac output?

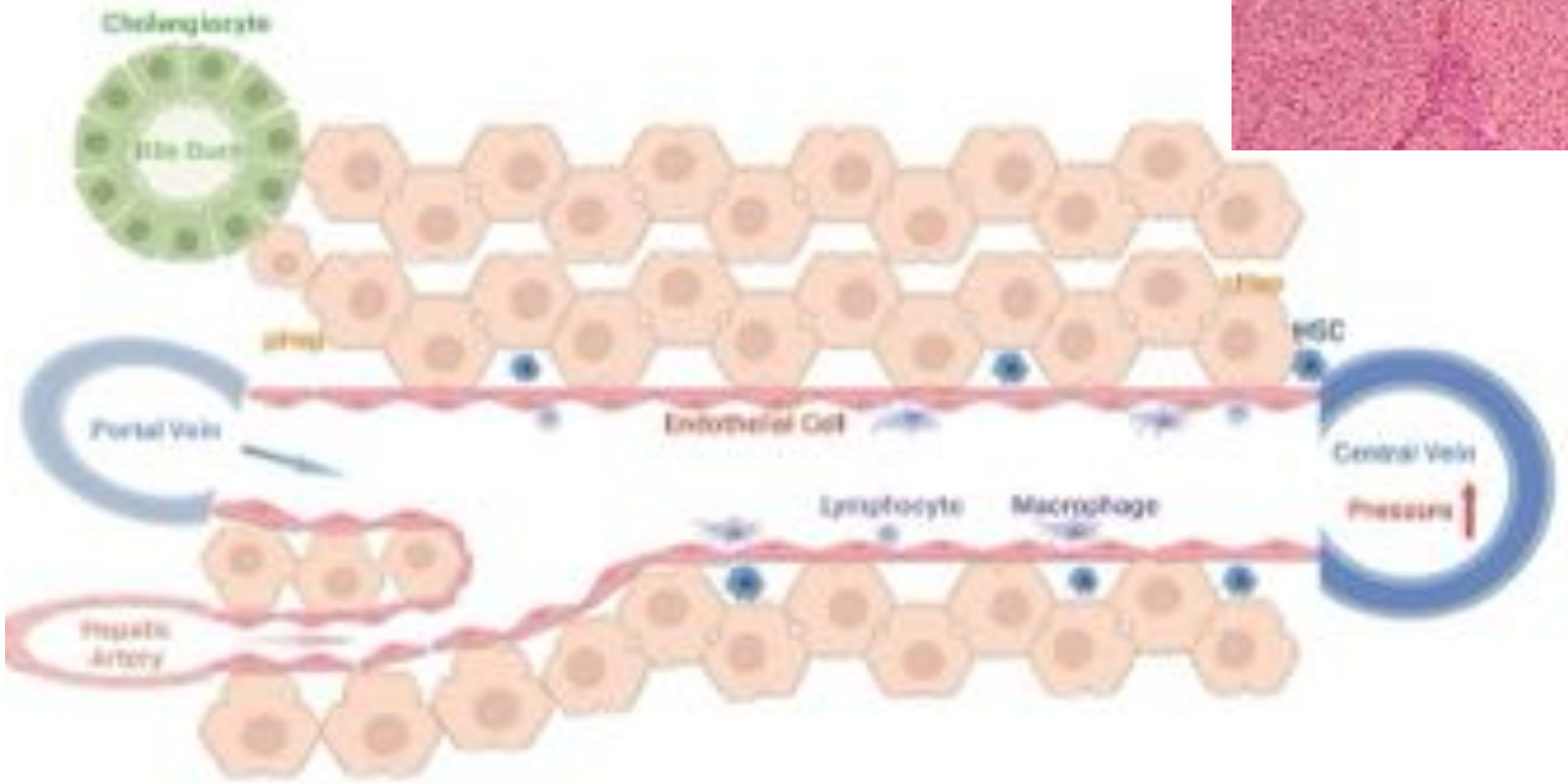
Liver biopsies from Fontan patients

	Fontan #1:	Fontan #2:	Fontan #3:	Fontan #4:
Single Ventricle Morphology	Left	Left	Right	Left
Cardiac Anatomy	Pulmonary atresia with intact ventricular septum, hypoplastic right ventricle	Double-inlet Left Ventricle, L-TGA	Hypoplastic left heart syndrome	Pulmonary atresia with intact ventricular septum, hypoplastic right ventricle
Age at Fontan	29 months	29 months	30 months	32 months
Fontan type	Extracardiac conduit with fenestration	Extracardiac conduit with fenestration	Lateral tunnel Fontan with fenestration	Extracardiac conduit with fenestration
Age at liver biopsy	16 yrs	18 yrs	13 yrs	15 yrs
General clinical status	Well, outpatient	Well, outpatient	Well, outpatient	Well, outpatient
ECHO Ventricular Function	Normal	Normal	Normal	Normal
ECHO AV Valve Regurgitation	Trivial	Trivial	Trivial	Trivial
Liver Ultrasound Echotexture/ additional findings	Normal liver echotexture/no additional findings	Multiple hyperechoic nodules consistent with regenerative liver nodules	Mildly increased echogenicity with nodular liver surface	Mild liver nodularity, gallstones present
IVC Pressure [mm Hg]	14	13	15	8
Hepatic Vein Wedge Pressure [mm Hg]	15	14	16	9

	Control #1:	Control #2:
Age at liver resection	14 yrs	13 yrs

Liver biology 101





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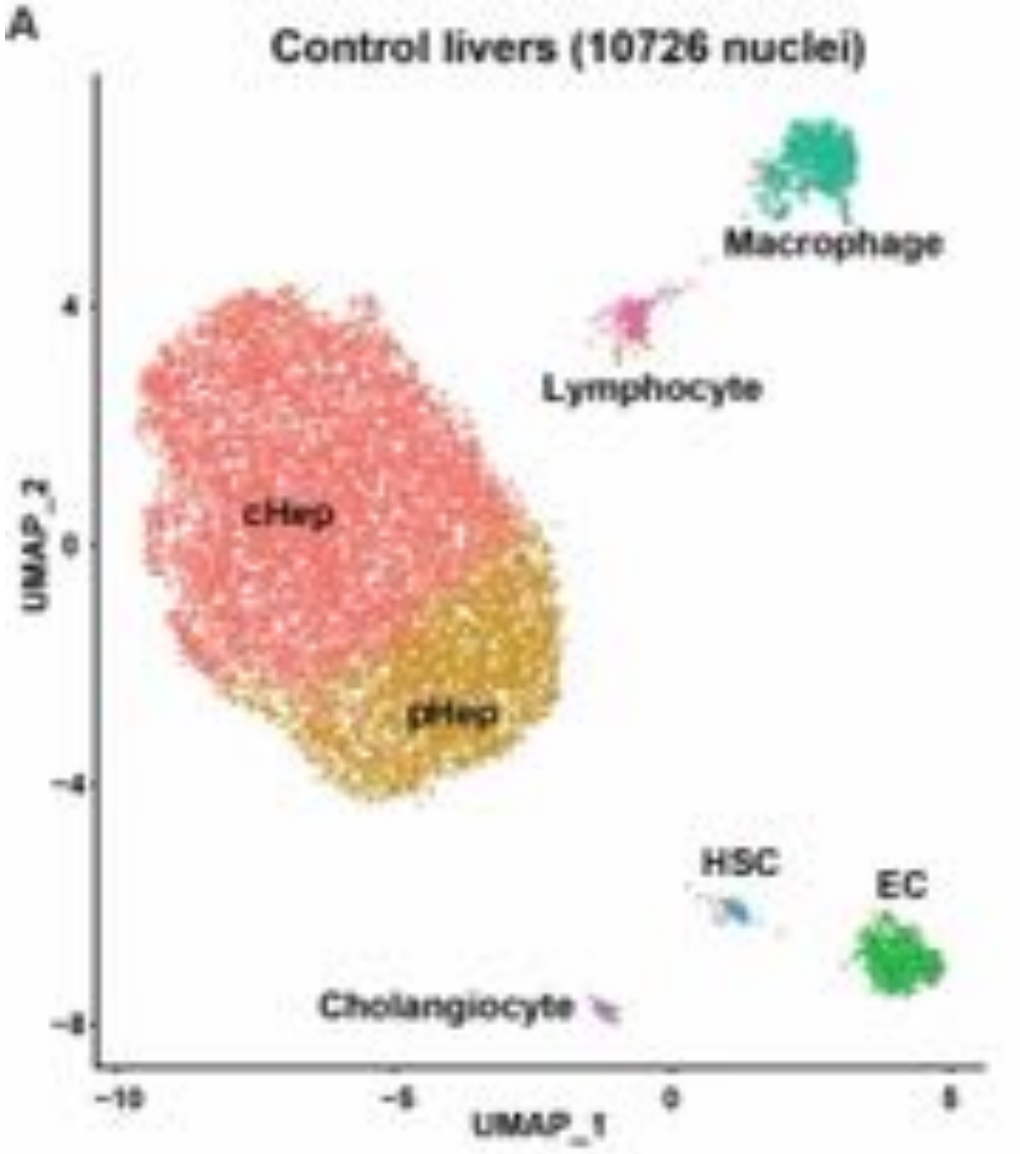
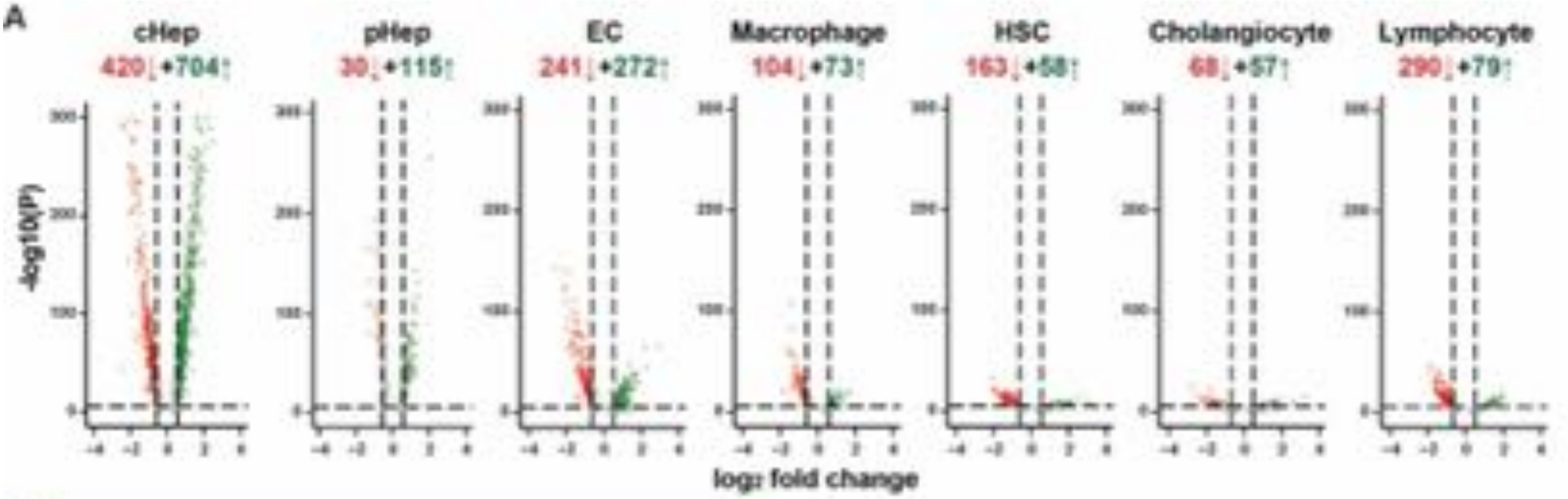
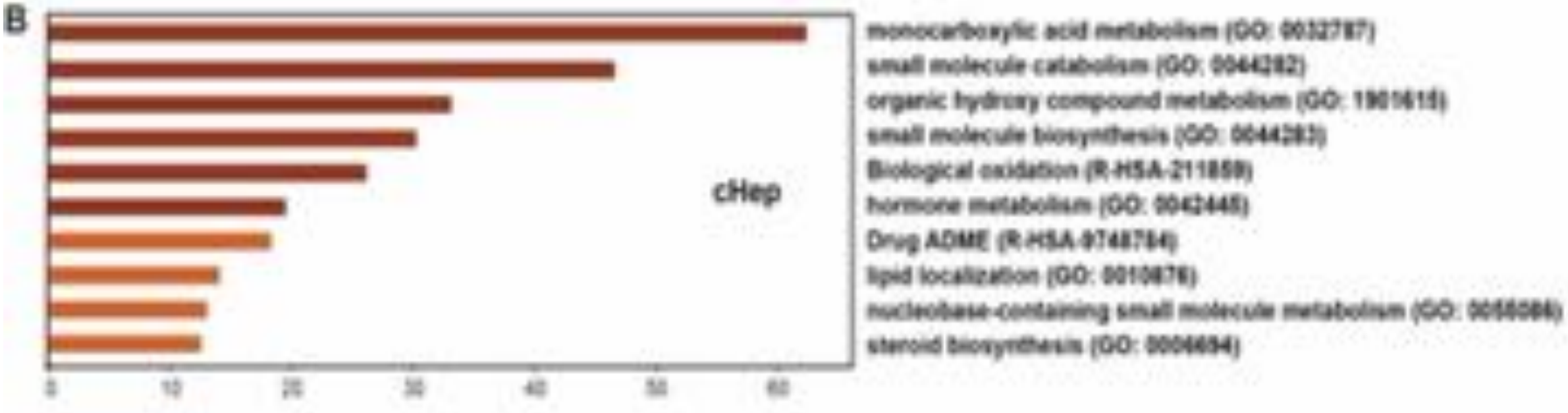
Single cell transcriptomic landscape of FALD



Cell type-specific transcriptional changes in FALD

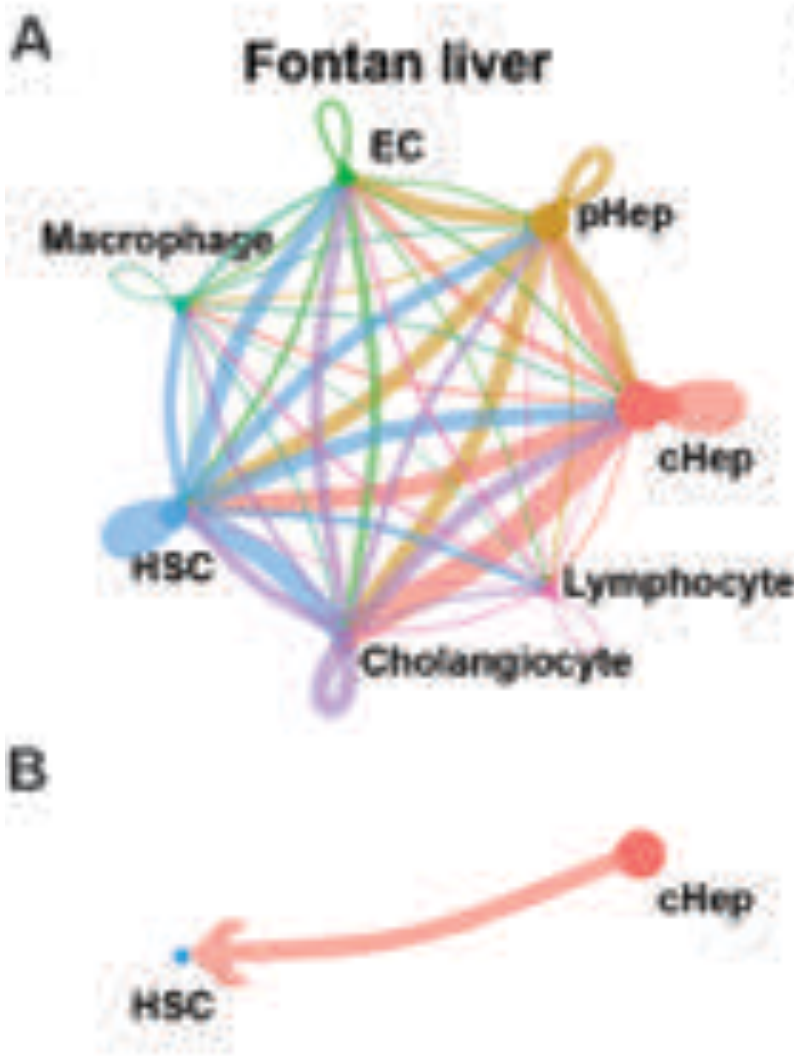


Cell type-specific functional changes in FALD



cHep likely the "first responder" in FALD pathogenesis

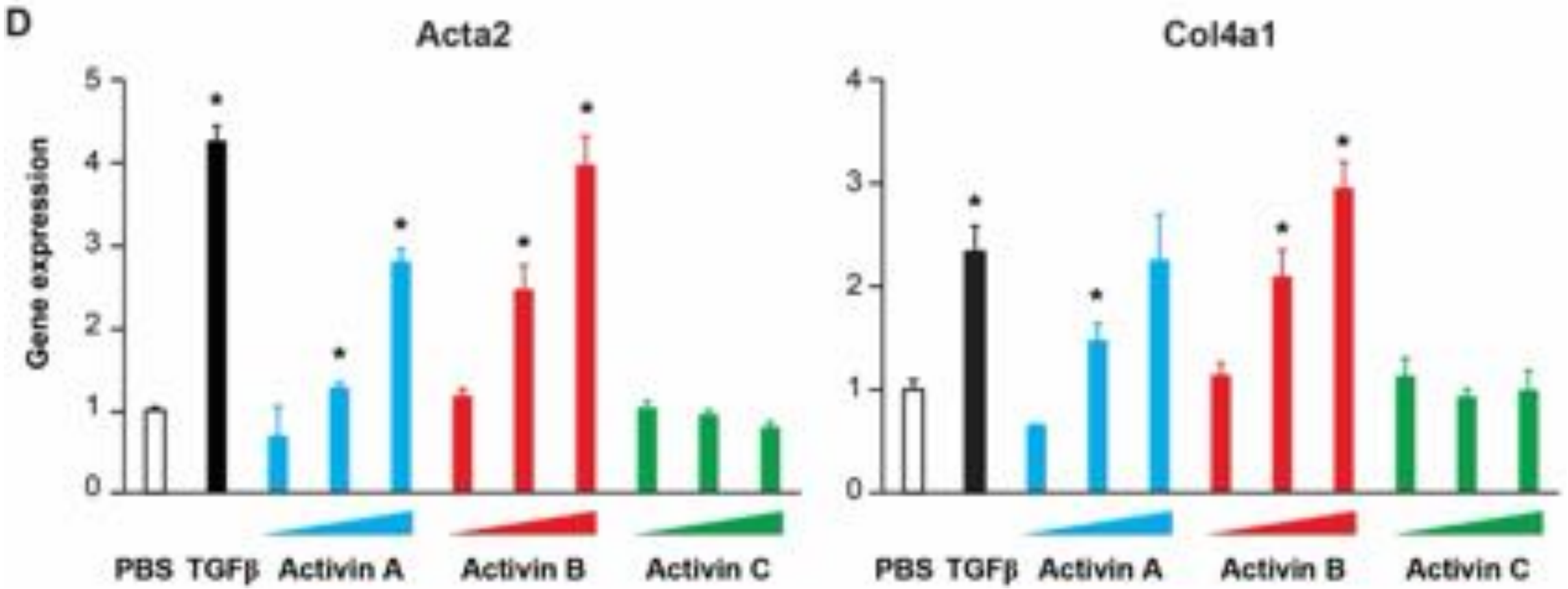
Identify intercellular signals that activate HSC



118 cHep ligand – HSC receptor pairs

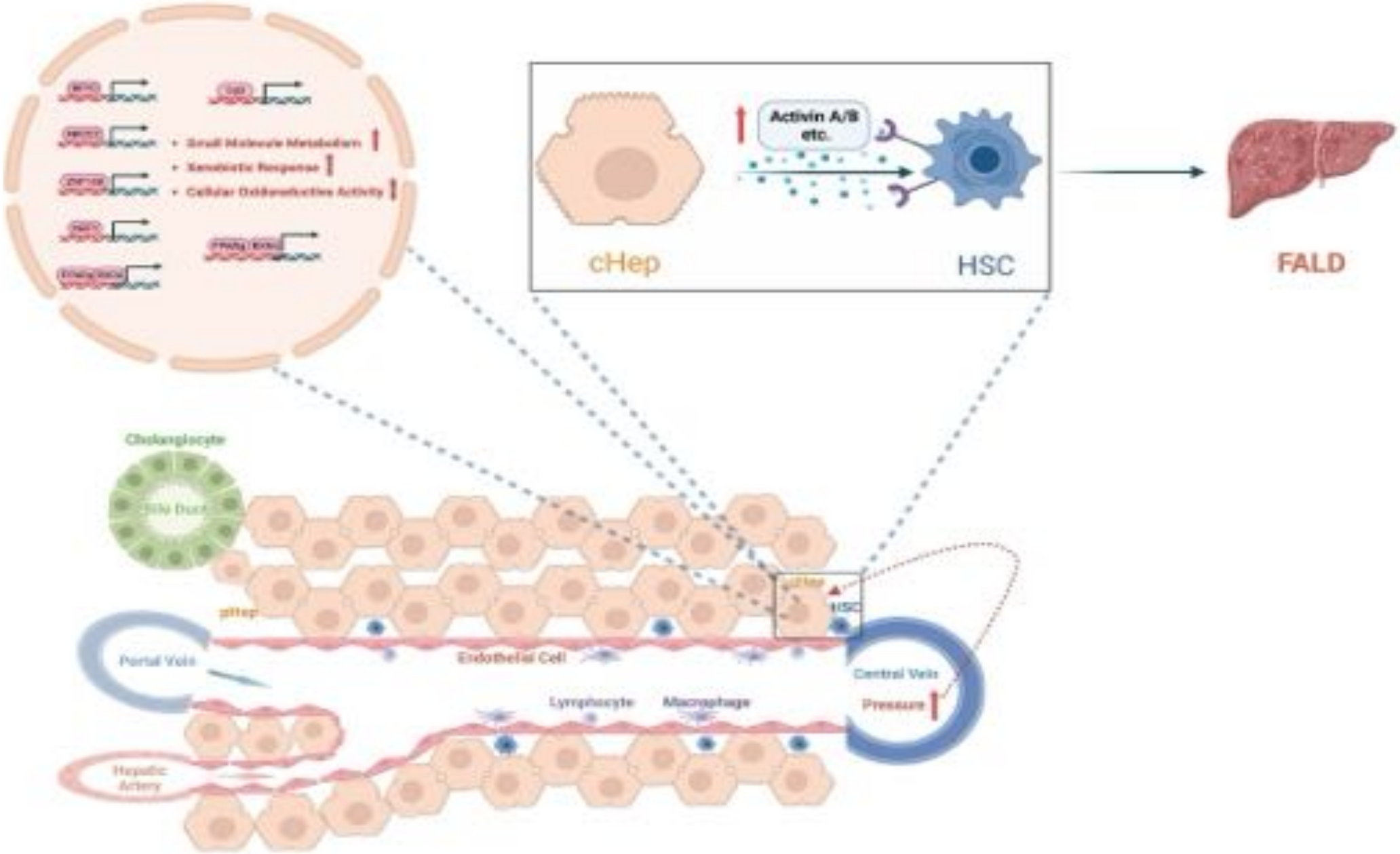
Rank	Source	Target	Ligand	Receptor	prob
1	cHep	HSC	GDF7	BMPR1A_ACVR2A	0.00116919
2	cHep	HSC	GDF7	BMPR1A_ACVR2B	0.00049838
3	cHep	HSC	GDF7	BMPR1A_BMPR2	0.00161189
4	cHep	HSC	GDF7	BMPR1B_ACVR2A	0.00068363
5	cHep	HSC	GDF7	BMPR1B_ACVR2B	0.00029127
6	cHep	HSC	GDF7	BMPR1B_BMPR2	0.00094278
7	cHep	HSC	INHBA	ACVR1B_ACVR2A	0.00479365
8	cHep	HSC	INHBB	ACVR1B_ACVR2A	0.00035679
9	cHep	HSC	INHBB	ACVR1B_ACVR2B	0.00015195
10	cHep	HSC	INHBB	ACVR1C_ACVR2A	5.36E-05
11	cHep	HSC	INHBB	ACVR1C_ACVR2B	2.28E-05
12	cHep	HSC	INHBC	ACVR1B_ACVR2A	0.00660538
13	cHep	HSC	INHBE	ACVR1B_ACVR2A	0.00119722
14	cHep	HSC	INHBE	ACVR1B_ACVR2B	0.00051015
15	cHep	HSC	INHBE	ACVR1C_ACVR2A	0.00018

Activins increase fibrotic gene expression *in vitro*



MRC-5 human fibroblasts, 3 biological replicates, 72h treatment

A model of FALD pathogenesis



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Mitch Lazar

Dan Kelly

Hao Wu

Katalin Susztak

Jon Epstein

Mark Kahn

Amita Sehgal

Boston Children's

Jeff Moffitt

Foerderer Award

ADDITIONAL VENTURES

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DEPARTMENT OF DEFENSE UNITED STATES OF AMERICA

HuBMAP The Human BioMolecular Atlas Program

American Heart Association.