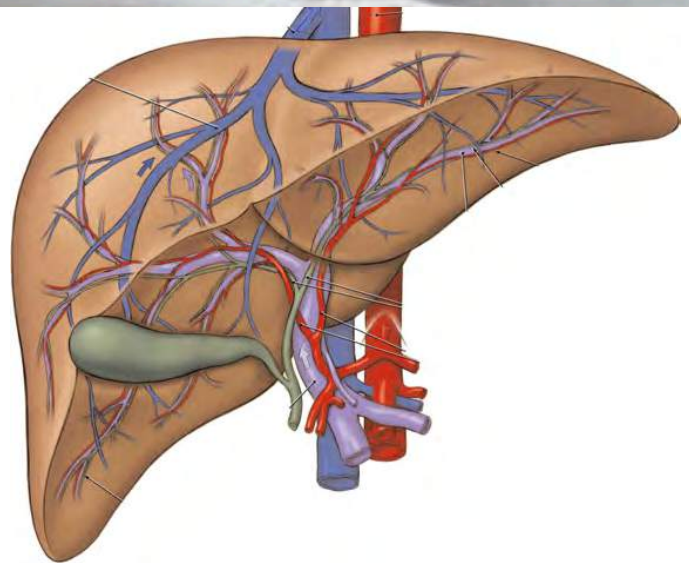
Mécanismes moléculaires de la zonation métabolique du foie

Sabine COLNOT
sabine.colnot@inserm.fr
Paris, France

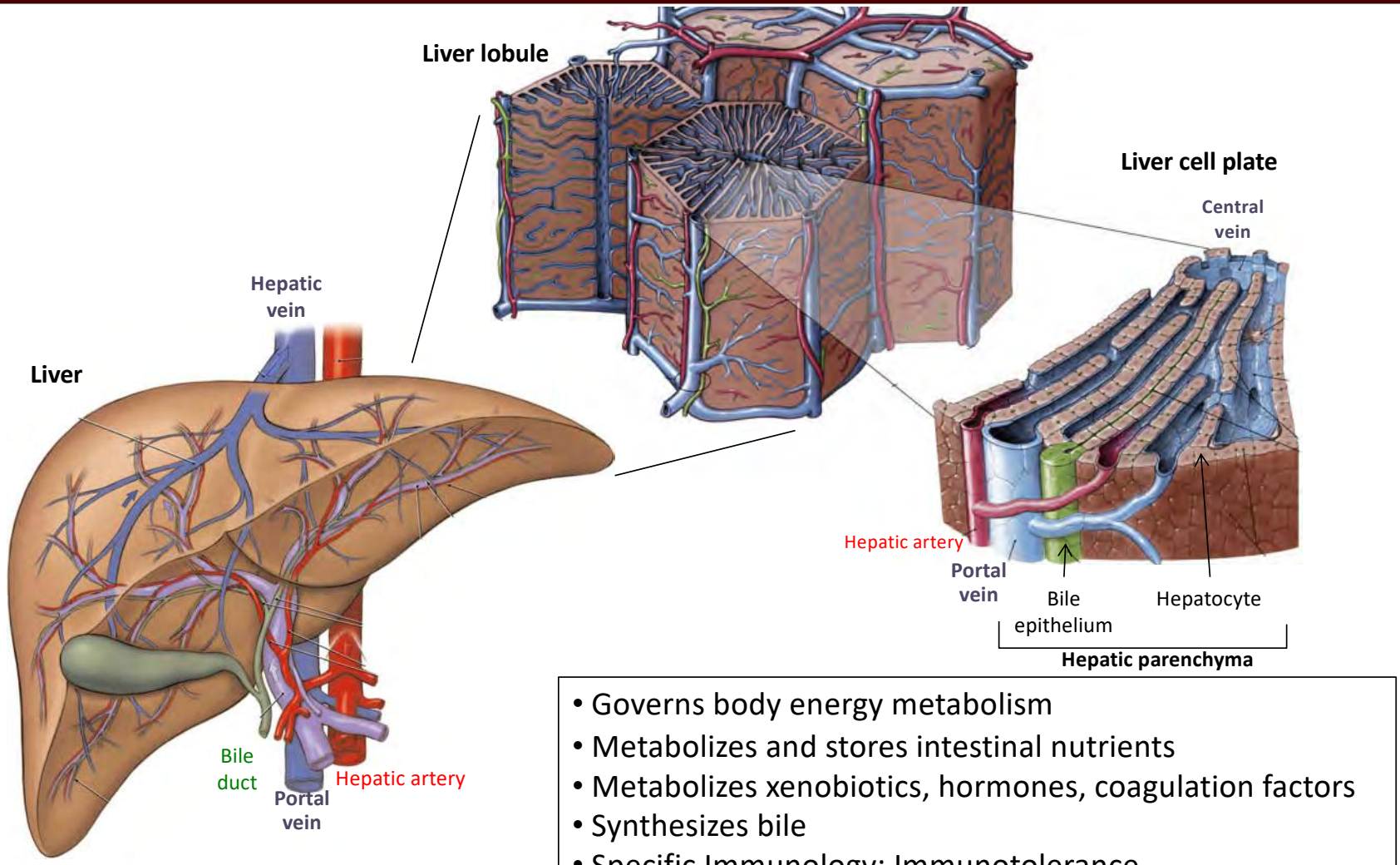
Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

Ode au Foie
de Pablo Neruda



Ami modeste et organisé.
Travailleur profond, permets que je te donne l'aile de mon chant.
Le coup d'air, le bondissement de mon Ode.
Elle naît de ton invisible machine et prend son vol dans ton infatigable et secret moulin, entraille délicate et puissante toujours vive et obscure.
Tandis que le cœur sonne et s'arroge la partition de la mandoline, à l'intérieur toi tu filtres, distribues, sépares, divises, multiplies, graisses, fais monter et recueilles les filets et les grâces de la vie.

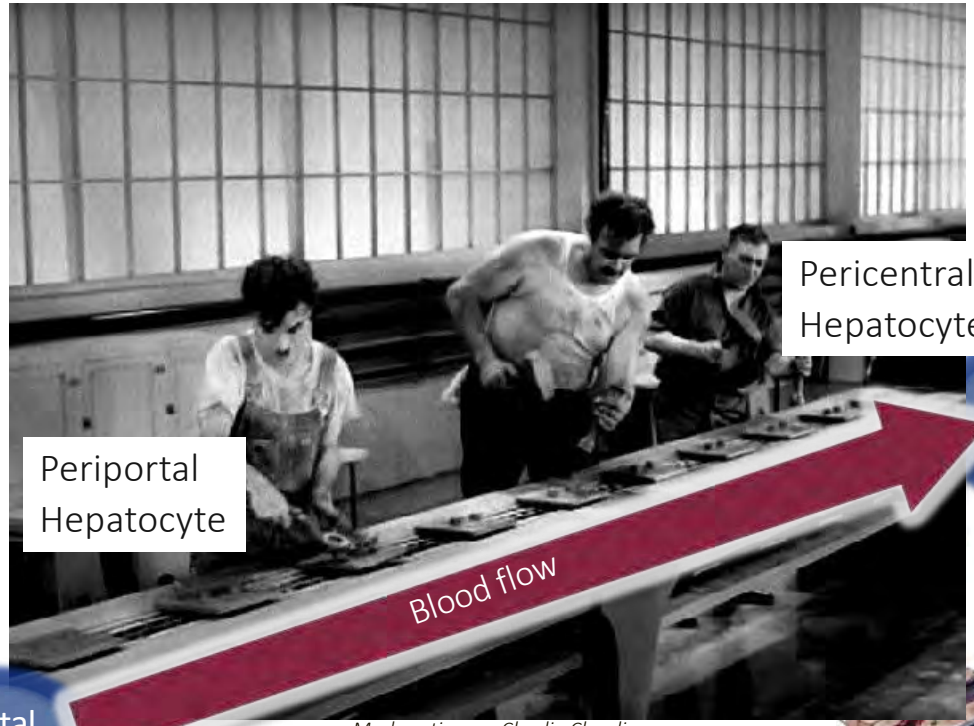
The adult liver: an architecture suited to its metabolic role



- Governs body energy metabolism
- Metabolizes and stores intestinal nutrients
- Metabolizes xenobiotics, hormones, coagulation factors
- Synthesizes bile
- Specific Immunology: Immunotolerance

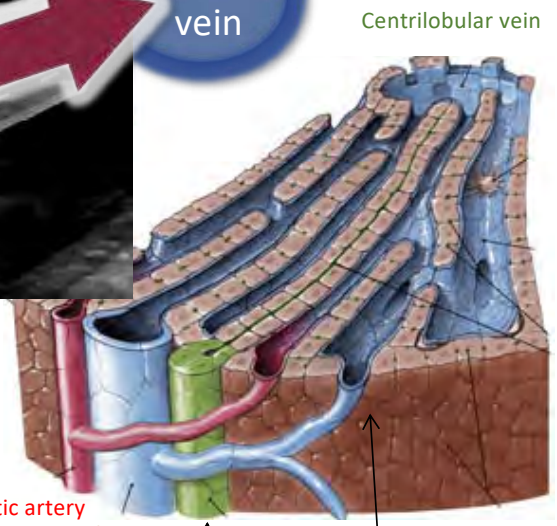
Liver zonation : a division of labour to achieve efficient metabolic functions

Halpern, Nature 2017

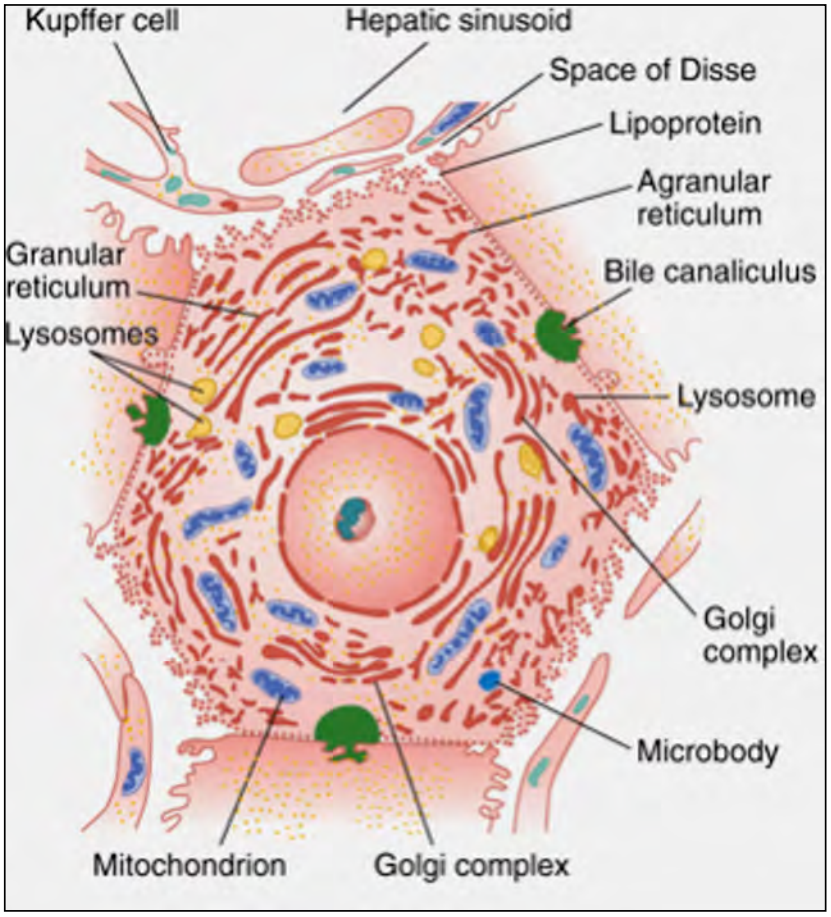


« Modern times » Charlie Chaplin

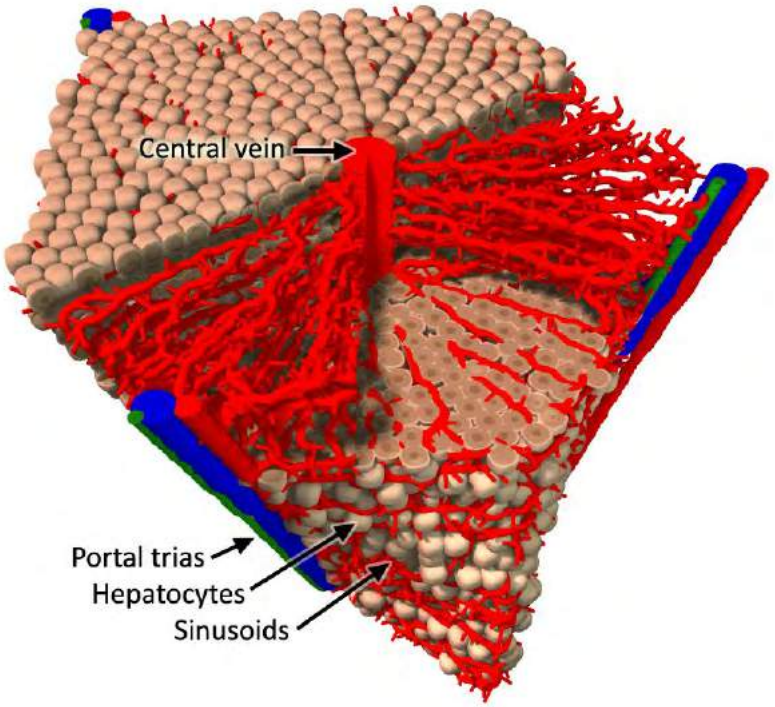
Oxygen
Insulin
Nutrients

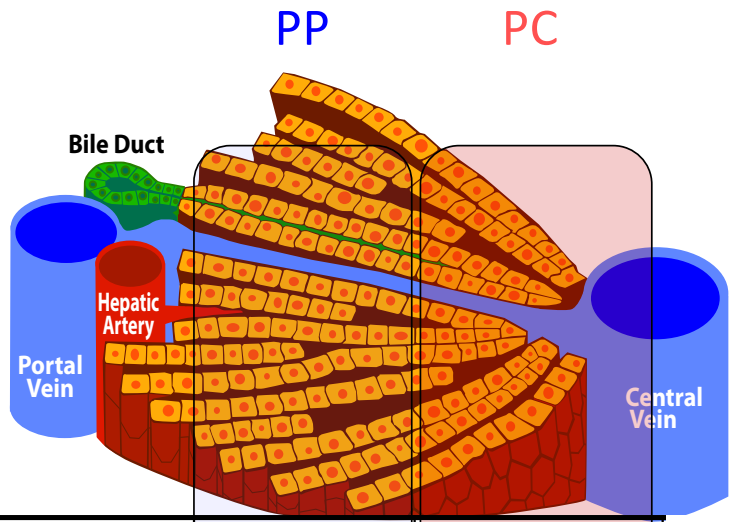


The hepatocytes (60% of liver cells) are quiescent and fulfill hepatic metabolic functions



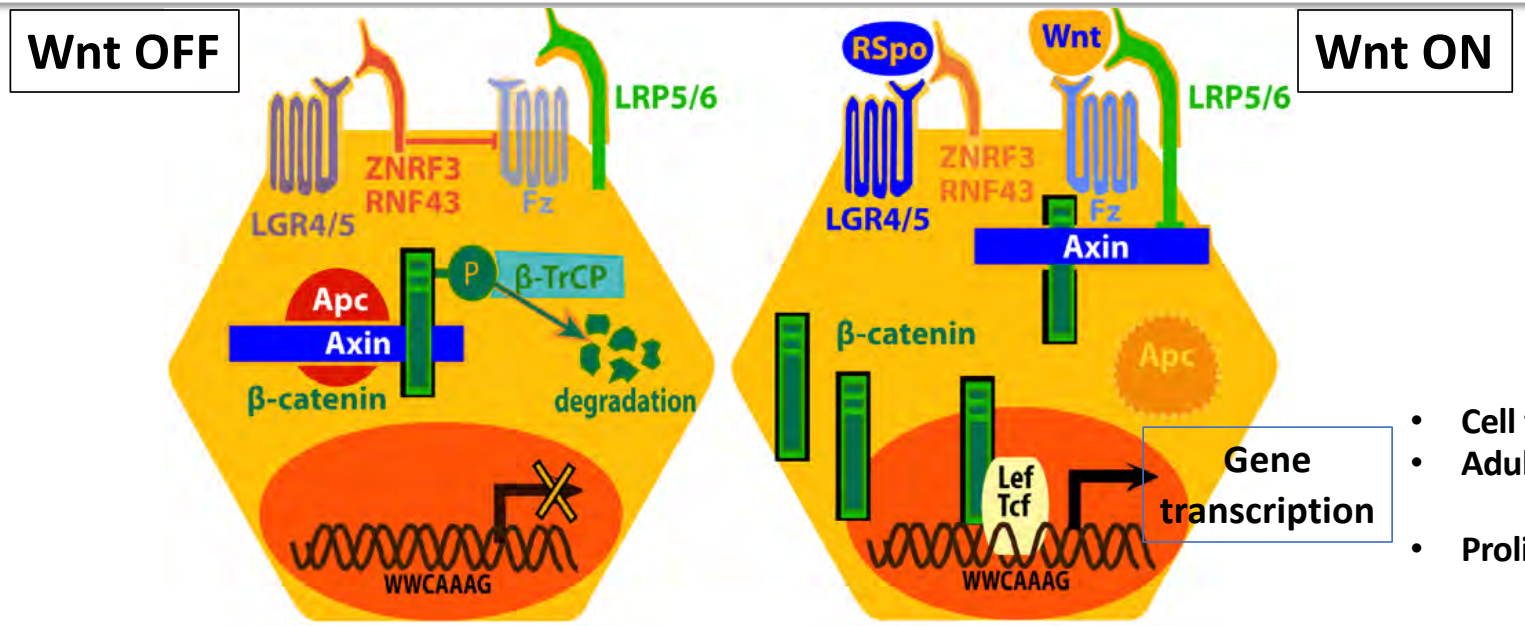
Transcriptional control of adult liver metabolic zonation





Metabolism	Periportal Hepatocytes PP	Pericentral Hepatocytes PC
ammonia, aminoacid metabolism	urea Cps1, Arg1 AA Hal, Gls2	glutamine GS, Oat, Glt1
glucidic	gluconeogenesis Pepck1, G6Pase	glycolysis GK, PK, Ldh
xenobiotics		CypP450 Cyp2e1, Cyp1a1/2
lipids		Bile acid Cyp7a1

Wnt/β-catenin signalling is the “zonation-keeper” of the liver



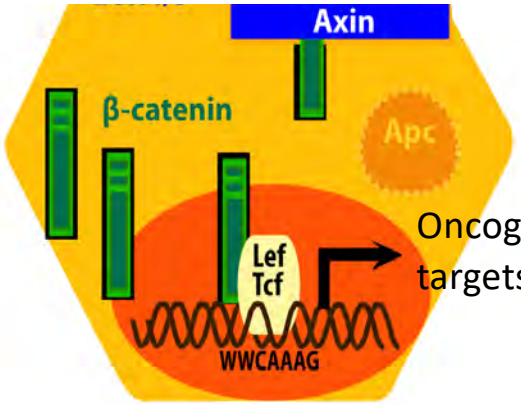
- Cell fate (embryo)
- Adult tissue/stem cell homeostasis
- Proliferation

β-catenin, a master oncogene in the adult liver

LIVER CANCER

> 30% of HCC

Stable β-catenin
β-catenin dependent transcription



Oncogenic targets

CTNNB1 32.8%

Gain-of-function mutations
Oncogene

AXIN1 15.2%

APC 1.6%

Loss-of-function mutations
Tumor Suppressors

β-catenin, a master oncogene in the adult liver

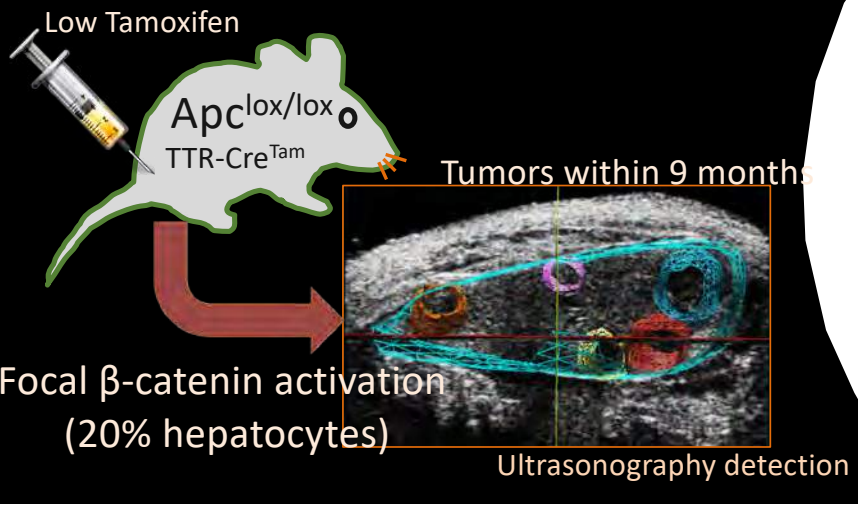
LIVER CANCER

> 30% of HCC

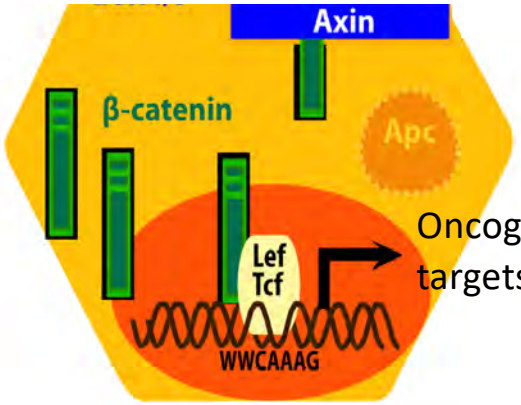
Hepatospecific Tamoxifen-inducible mice models

Colnot et al., PNAS 2004

Apc^{ko}



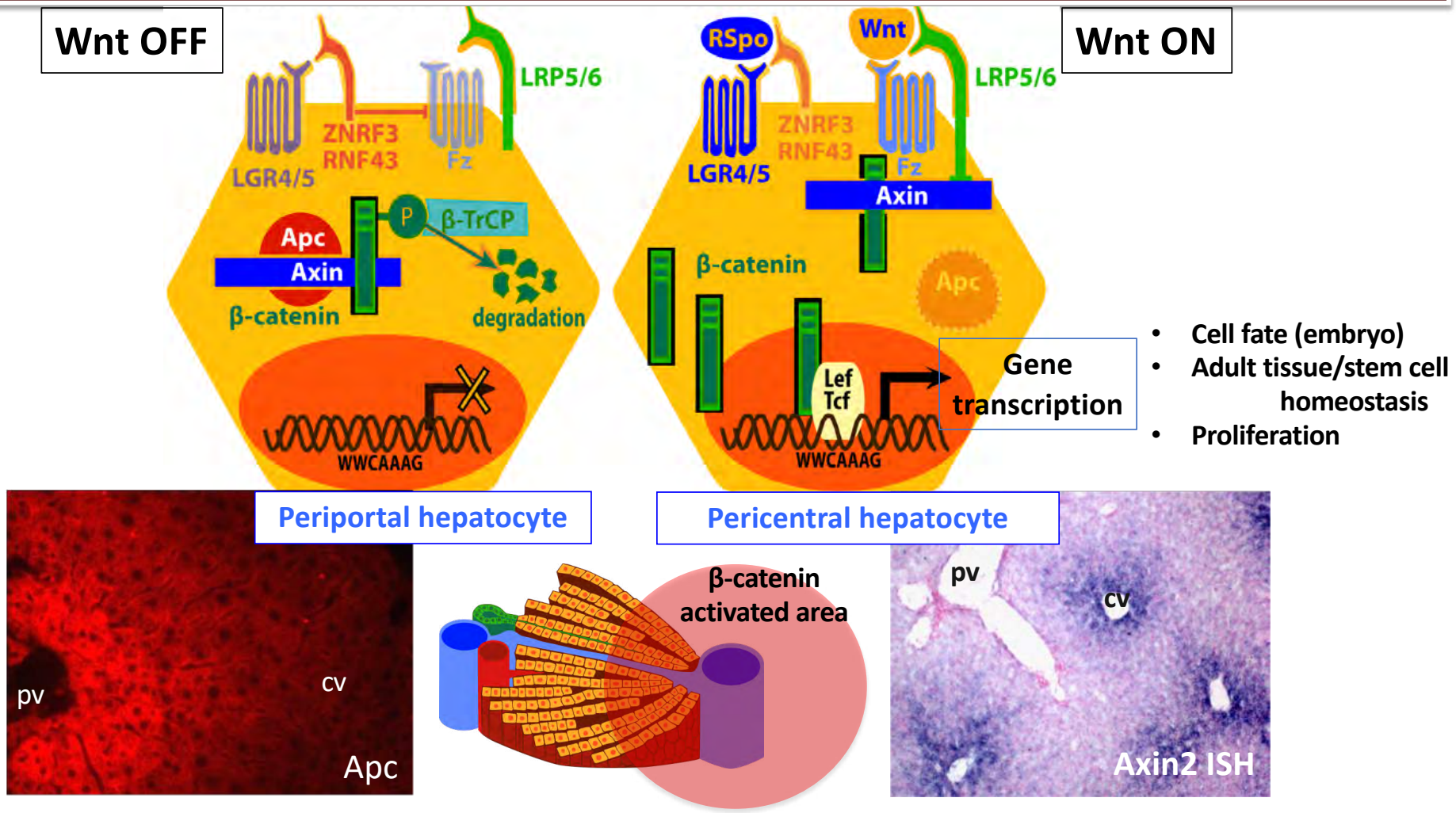
Stable β-catenin
β-catenin dependent transcription



CTNNB1 32.8%
Gain-of-function mutations
Oncogene

AXIN1 15.2%
APC 1.6%
Loss-of-function mutations
Tumor Suppressors

Wnt/β-catenin signalling is the "zonation-keeper" of the liver

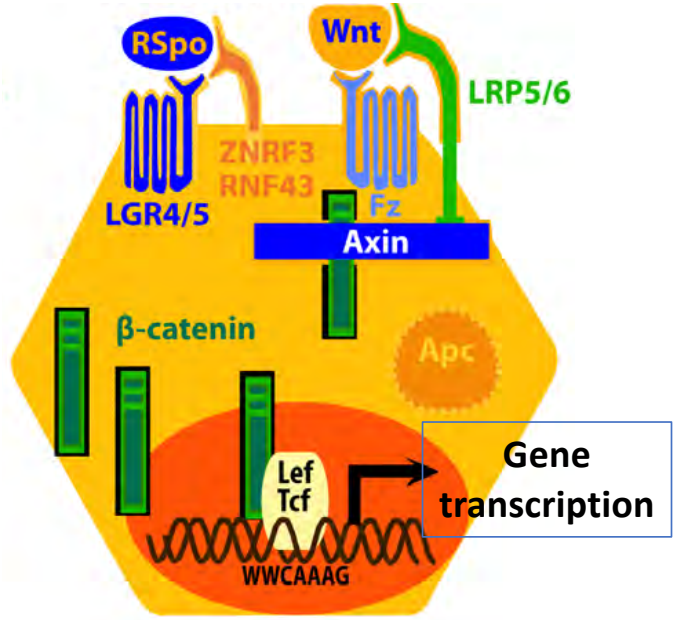


- Cell fate (embryo)
- Adult tissue/stem cell homeostasis
- Proliferation

Benhamouche et al., Dev Cell 2006

How does β-catenin zonal activation occur? Modulation of Wnt/Spondin ligands

Liver zonation is Wnt-dependent	*Benhamouche, Dev Cell 2006*	Forcing DKK-mediated loss of Wnts
Liver zonation is Wnt AND Spondin-dependent	*Rocha, Cell Rep 2015*	Endothelial-specific loss of R-spondin3
Orchestration of liver zonation	*Planas-Paz, Nat Cell Biol 2016*	Hepatospecific loss of Lgr4/5, Rnf43/ZNRF3

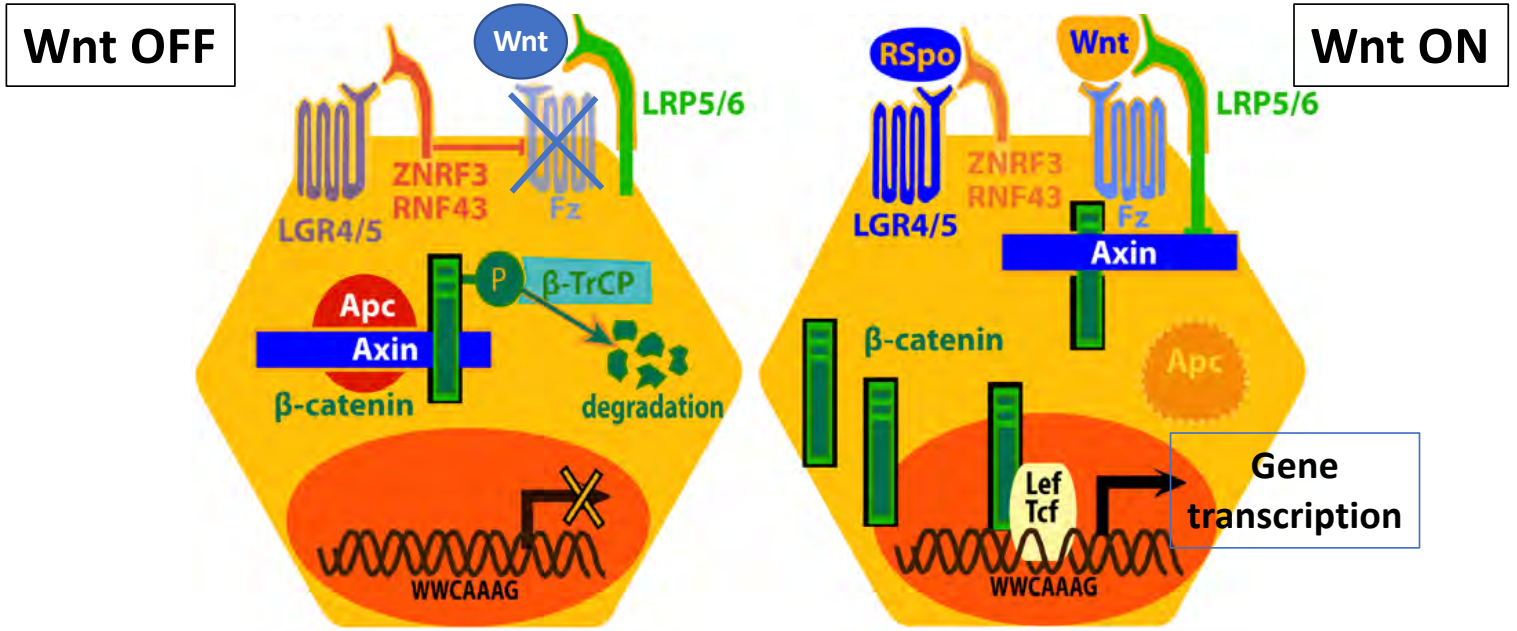


Pericentral hepatocyte

Wnt^{+}, **Spondin^{+}**, **Lgr5^{+}**, Lgr4^{+}

How does β-catenin zonal activation occur? Modulation of Wnt/Spondin ligands

Liver zonation is Wnt-dependent	*Benhamouche, Dev Cell 2006*	Forcing DKK-mediated loss of Wnts
Liver zonation is Wnt AND Spondin-dependent	*Rocha, Cell Rep 2015*	Endothelial-specific loss of R-spondin3
Orchestration of liver zonation	*Planas-Paz, Nat Cell Biol 2016*	Hepatospecific loss of Lgr4/5, Rnf43/ZNRF3

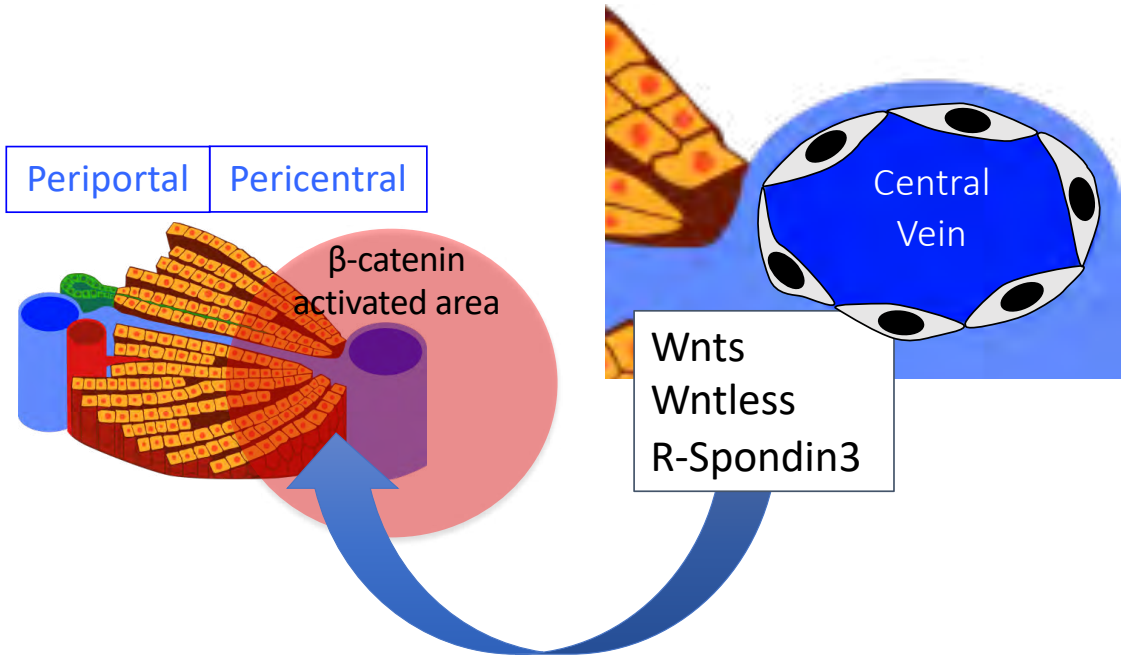


Periportal hepatocyte
Wnt^{+}, Lgr4^{+}, Spondin^{-}

Pericentral hepatocyte
Wnt2/Wnt9b^{+}, Spondin^{+}, Lgr5^{+}, Lgr4^{+}

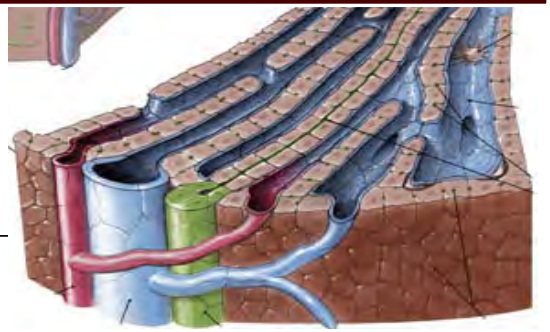
β-catenin zonal activation is due to the synthesis of Wnt, Wntless and R-Spondin 3 by the pericentral endothelial cells allowing Wnt paracrine signaling in the pericentral hepatocytes

Wnts are secreted by endothelial cells *Wang, Nature 2015* Endothelial-specific loss of Wntless
Liver zonation is Wnt AND Spondin-dependent *Rocha, Cell Rep 2015* Endothelial-specific loss of R-spondin3

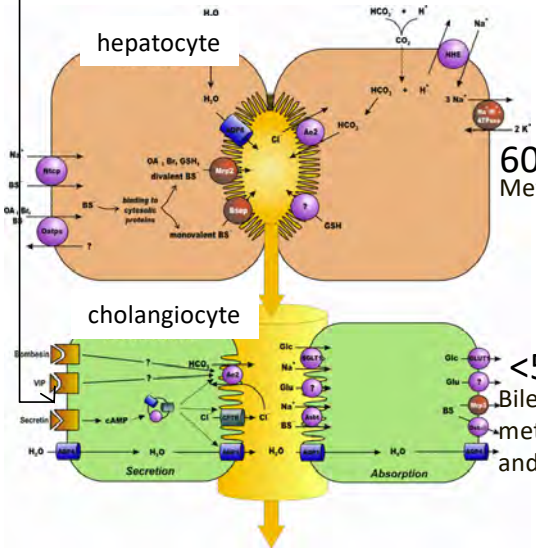


Zonation of the other Hepatic lineages

Parenchymal cells



Bile epithelium



60% Metabolism

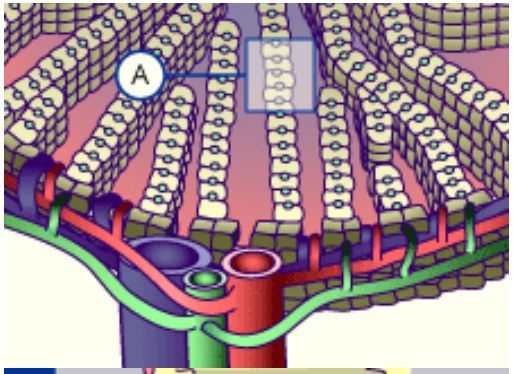
PC, midlobular, PP Hepatocytes

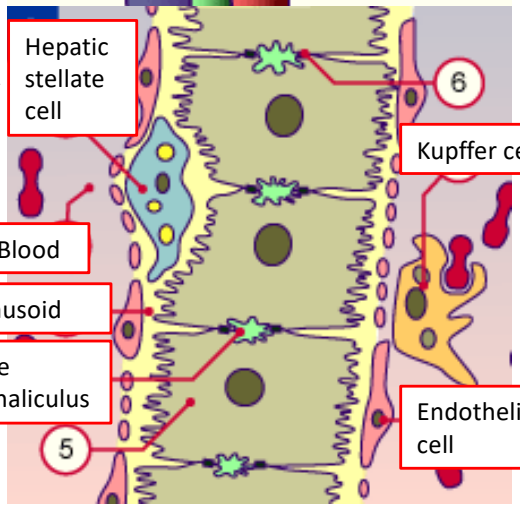
Halpern, Nature 2017

<5% Bile metabolism and transport

PP Cholangiocytes

Non-parenchymal cells





5% Vitamin A storage Pro-Fibrotic

Hepatic stellate cell

Kupffer cell

15% Macrophage

Blood

Sinusoid

Bile canaliculus

Endothelial cell

20% Fenestrated

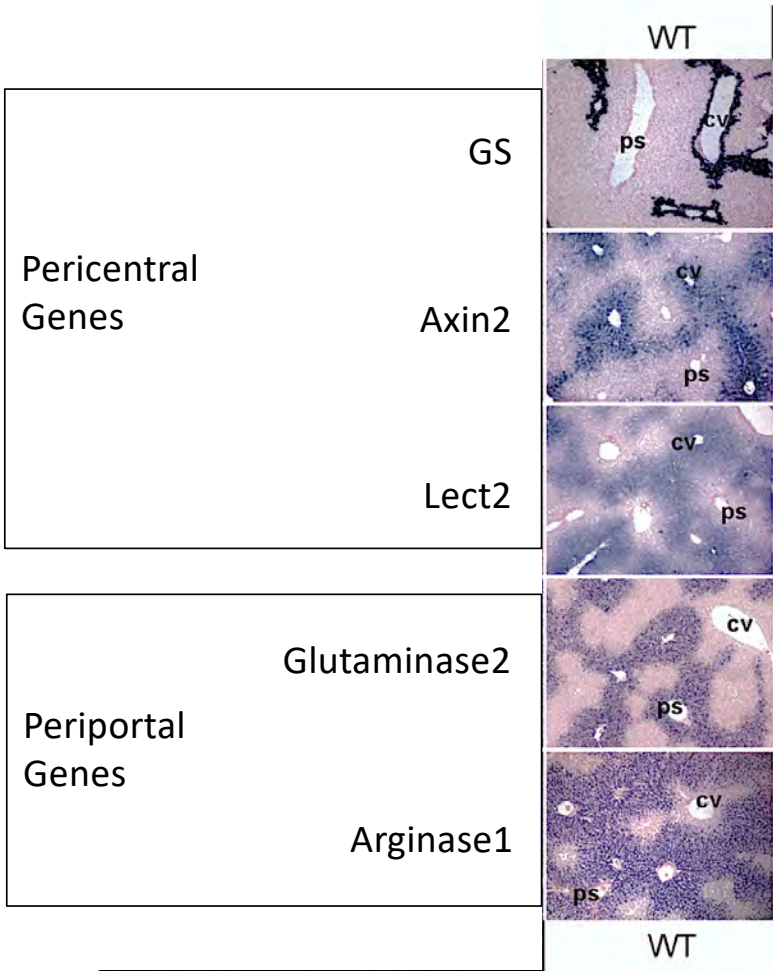
Microbiota-dependent PP Immune zonation of myeloid/lymphoid cells

Gola, Nature 2020

PC, midlobular, PP Endothelial Cells

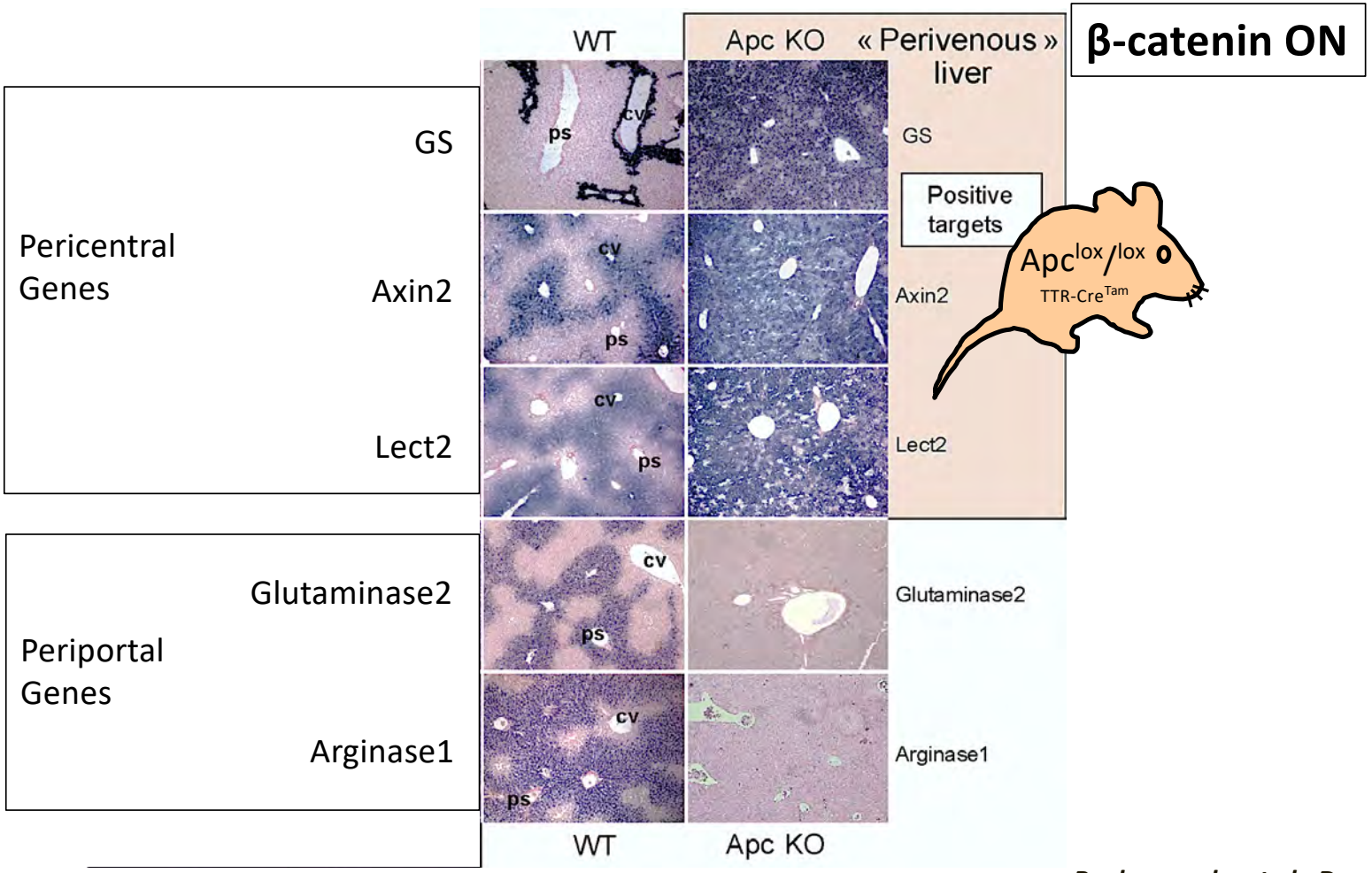
Halpern, Nat. Biotechnol 2018

Zonal gene expression in the liver lobule: *in situ* hybridizations



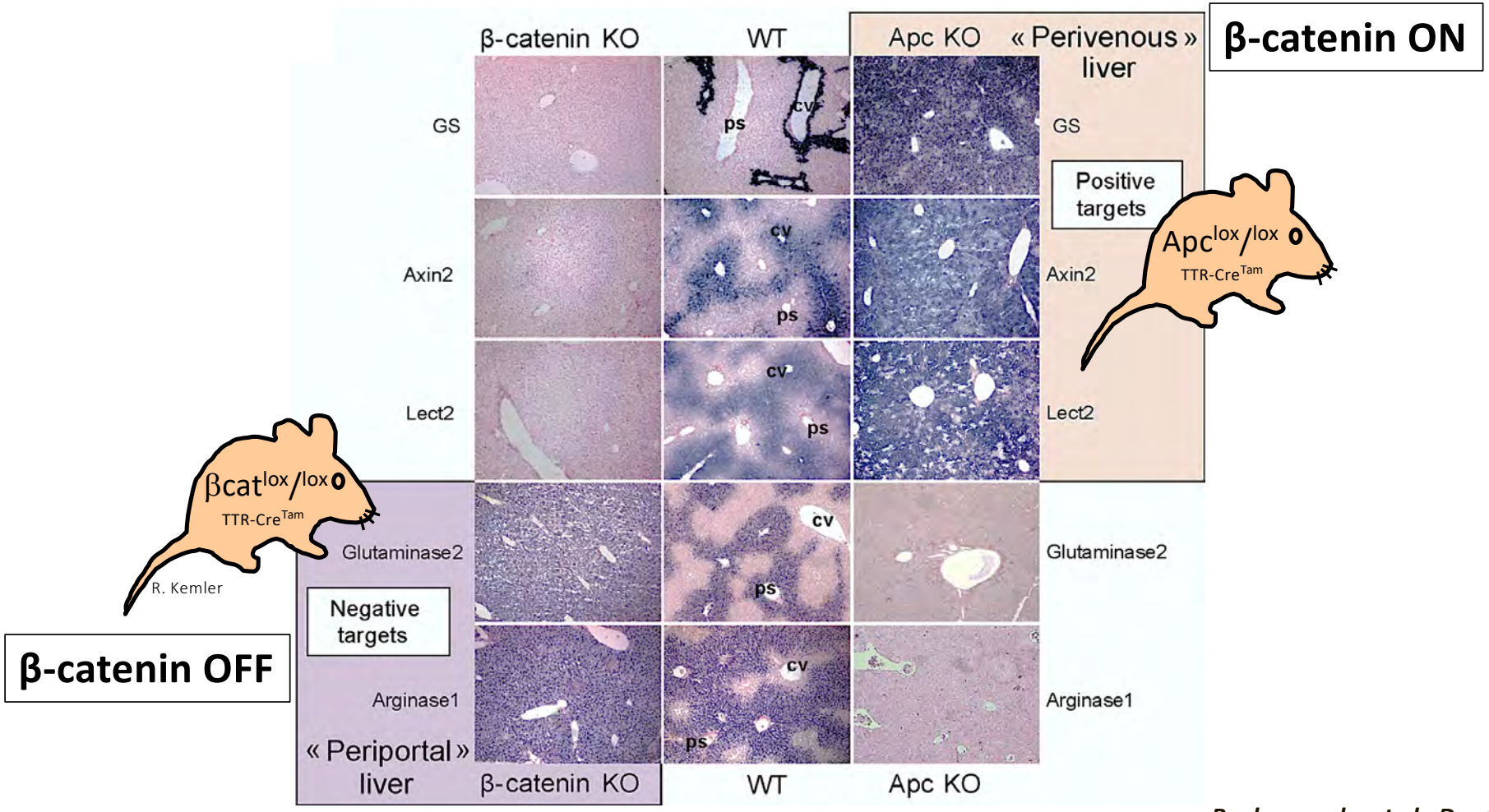
Benhamouche et al., Dev Cell 2006

Modulation of β-catenin signaling modifies zonal gene expression in the liver lobule



Benhamouche et al., Dev Cell 2006

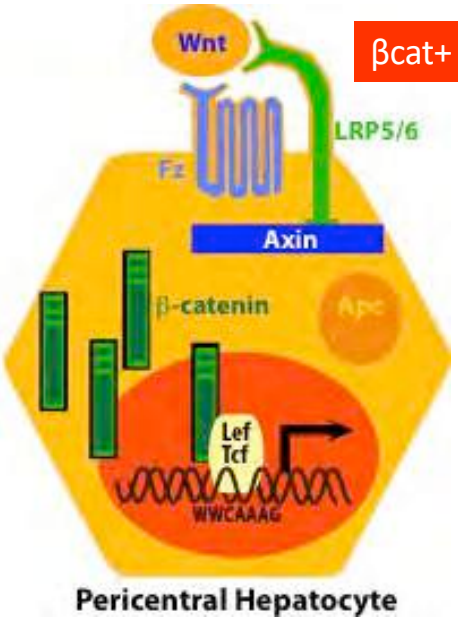
Modulation of β-catenin signaling modifies zonal gene expression in the liver lobule

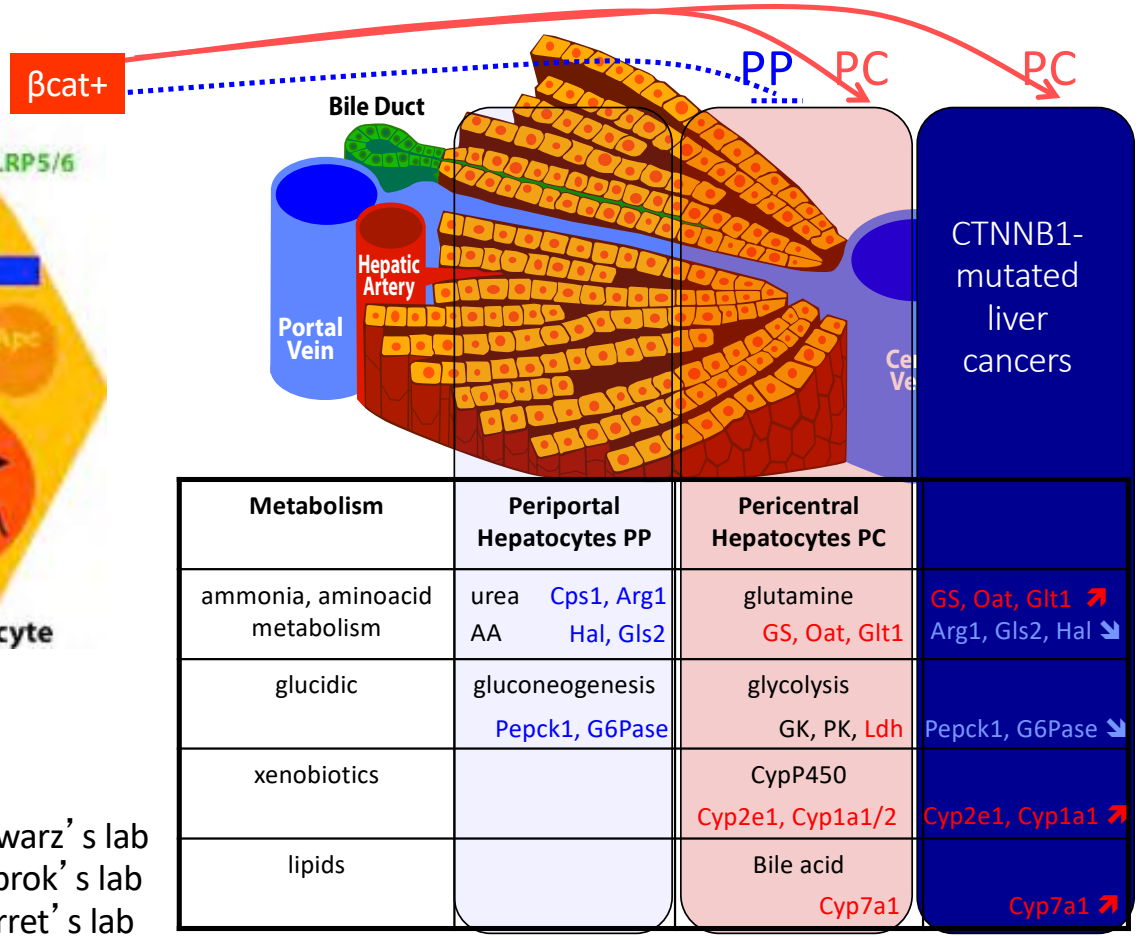


Benhamouche et al., Dev Cell 2006

Wnt/β-catenin in liver zonation: a prominent role in metabolism… and cancer

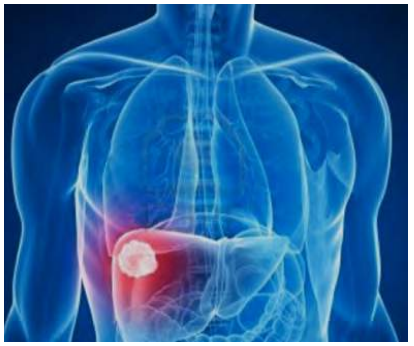
Glutamine Synthetase Immunostaining in HCC is a signature of β-catenin-activated HCC



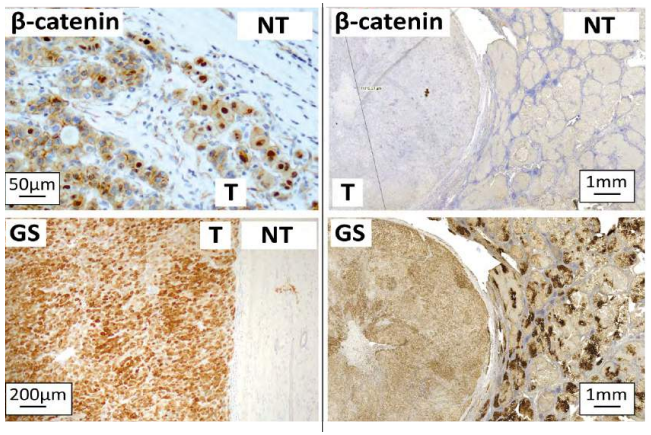


Metabolism	Periportal Hepatocytes PP	Pericentral Hepatocytes PC	
ammonia, aminoacid metabolism	urea Cps1, Arg1 AA Hal, Gls2	glutamine GS, Oat, Glt1	GS, Oat, Glt1 ↗ Arg1, Gls2, Hal ↘
glucidic	gluconeogenesis Pepck1, G6Pase	glycolysis GK, PK, Ldh	Pepck1, G6Pase ↘
xenobiotics		CypP450 Cyp2e1, Cyp1a1/2	Cyp2e1, Cyp1a1 ↗
lipids		Bile acid Cyp7a1	Cyp7a1 ↗

Schwarz's lab
Hebrok's lab
Perret's lab

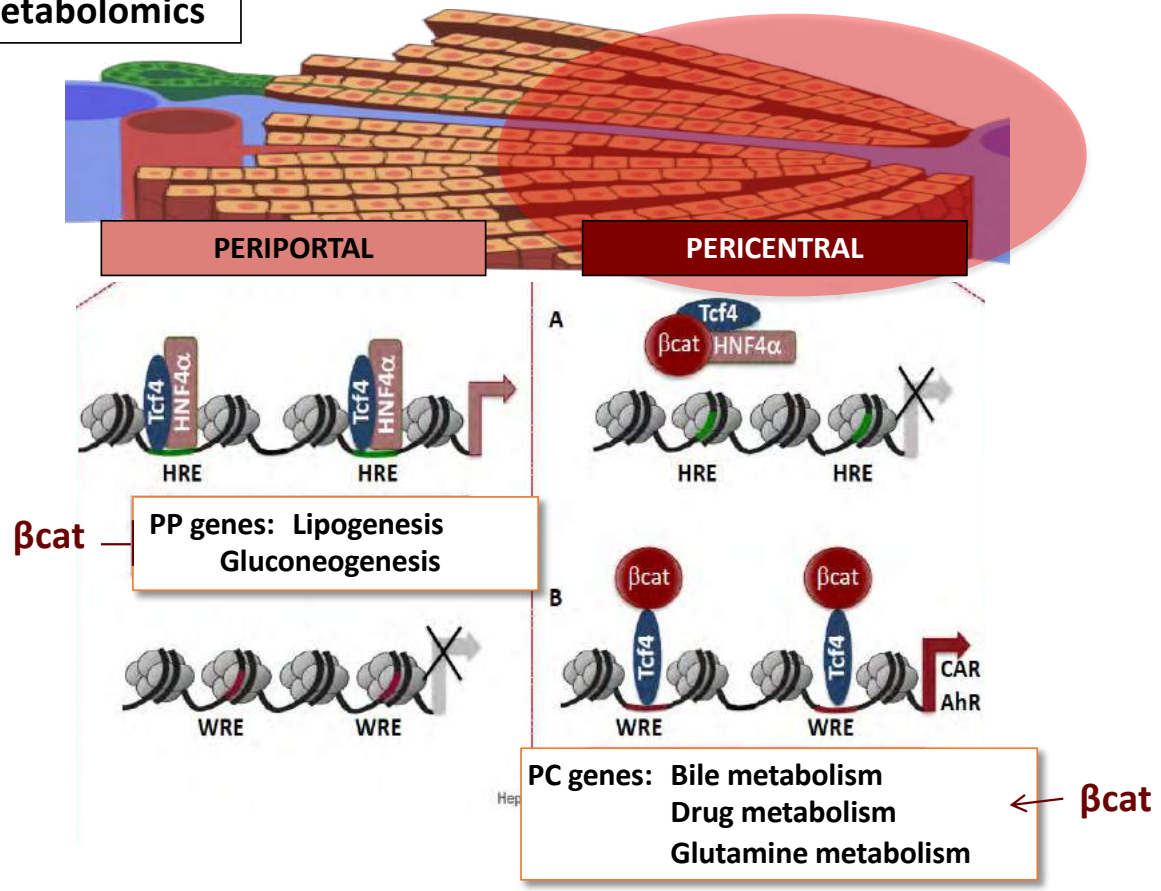


Human CTNNB1-mut HCC | **Human CTNNB1-wt HCC**



β-catenin interacts with Hnf4α to control a transcriptional program highly specialized in metabolism

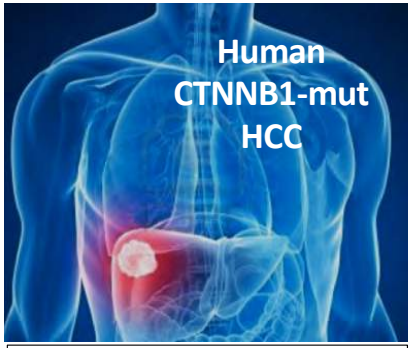
In vivo **CHIPseq RNAseq Metabolomics**



Gougelet, Torre et al., Hepatology 2014

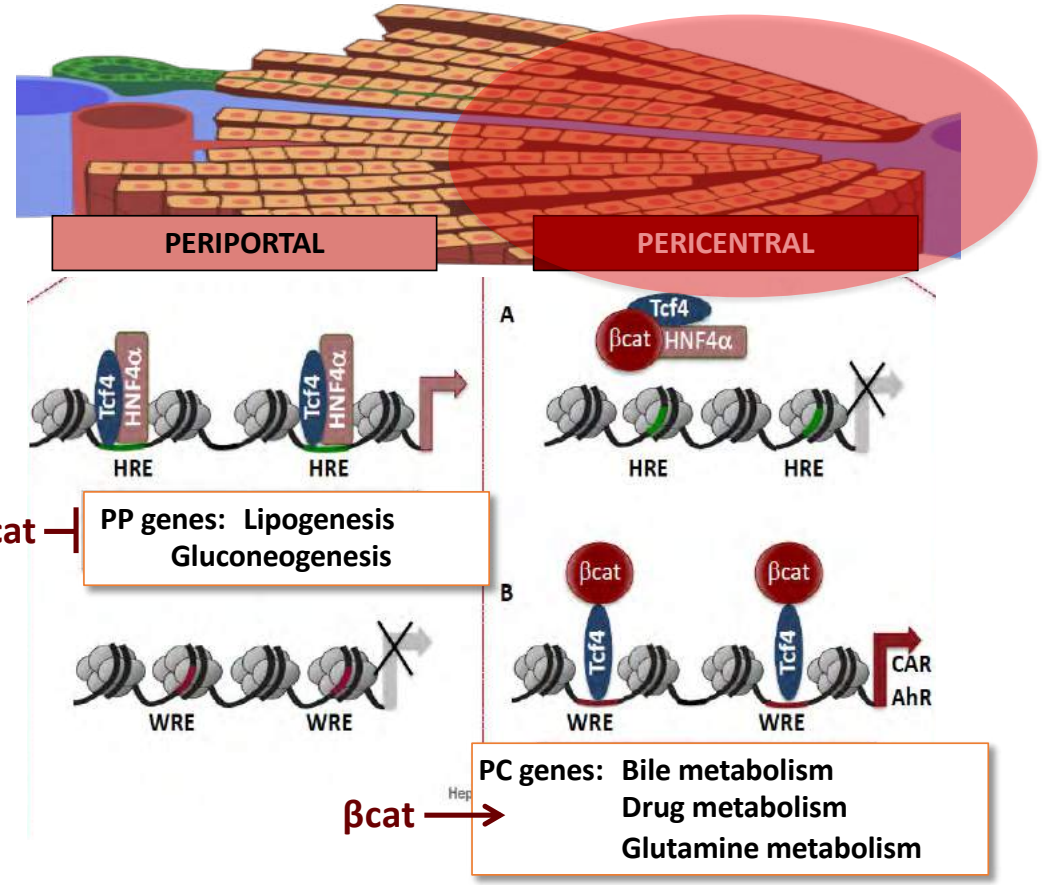
β-catenin signalling has a profound impact on metabolism in a normal or a cancerous liver

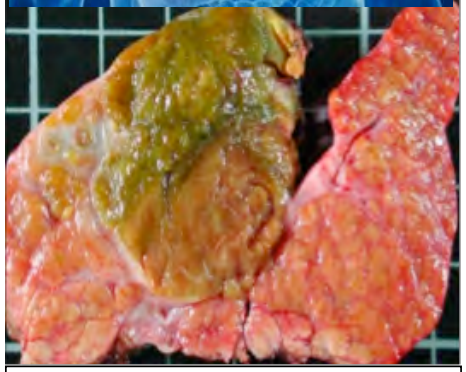
In vivo **CHIPseq RNAseq Metabolomics**



No intratumoral steatosis

Audard et al., J. Pathol. 2007
Calderaro et al., J Hepatol 2017

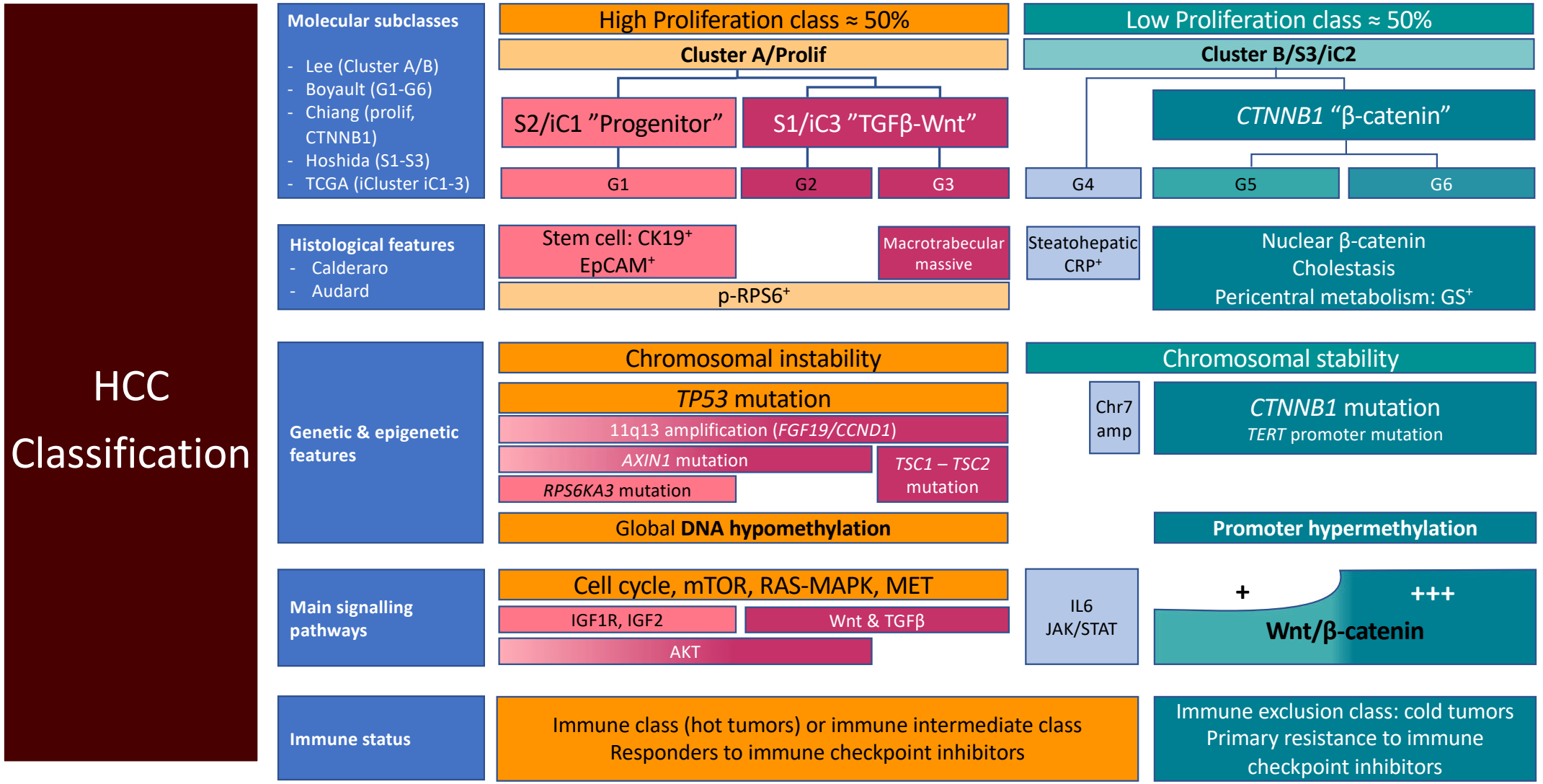




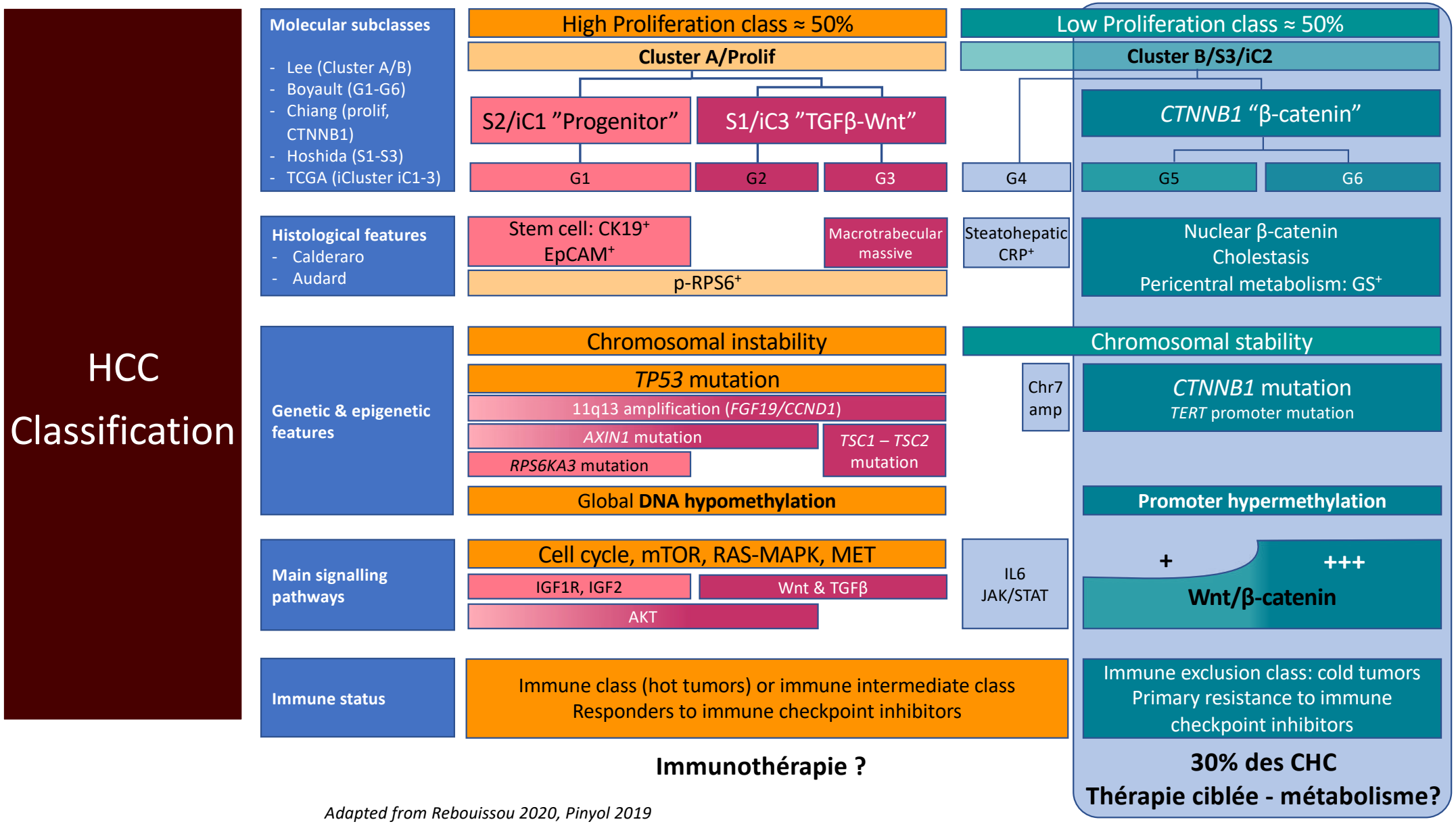
Intratumoral cholestasis

Audard et al., J. Pathol. 2007
Calderaro et al., J Hepatol 2017

Gougelet, Torre et al., Hepatology 2014



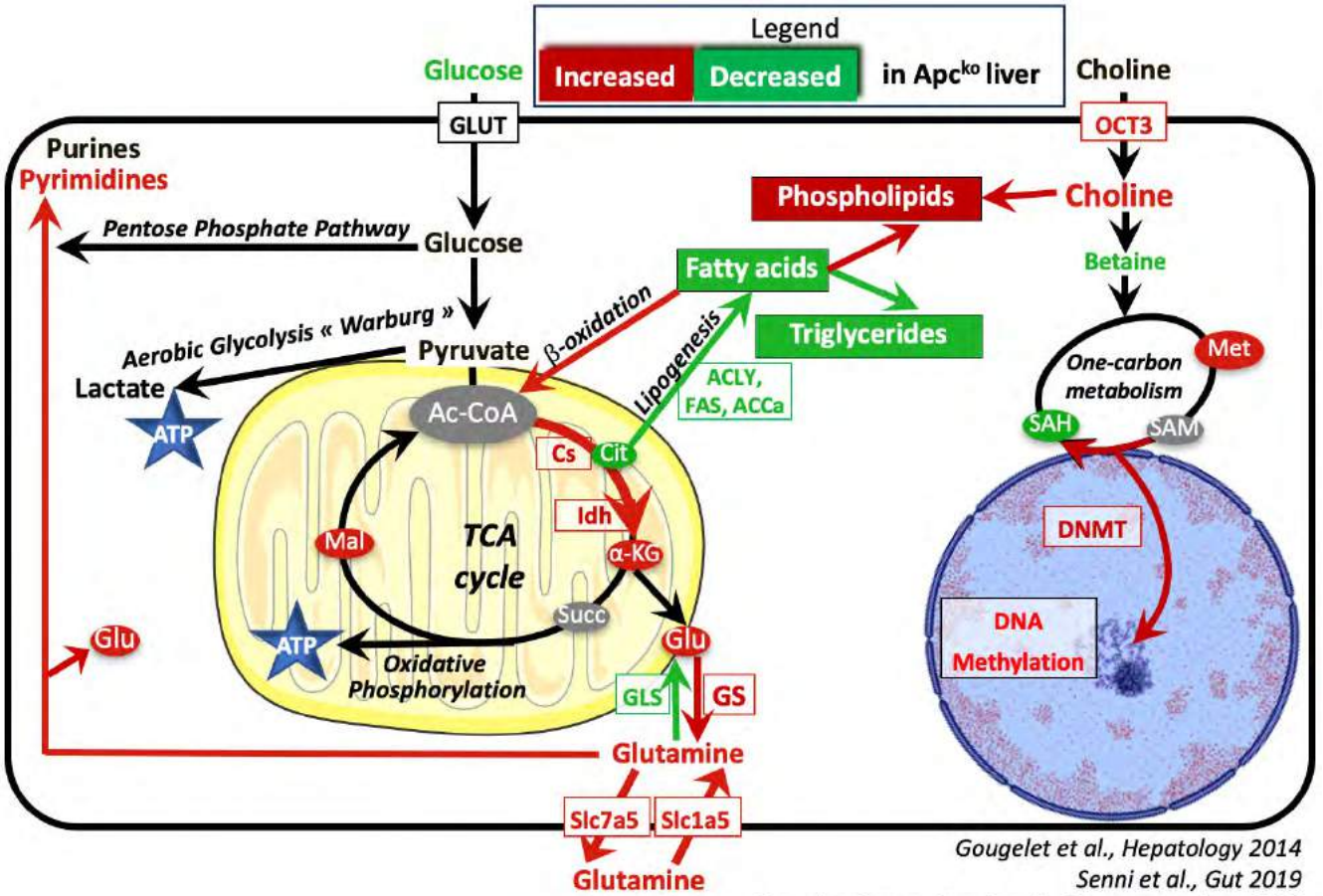
Adapted from Rebouissou 2020, Pinyol 2019



Adapted from Rebouissou 2020, Pinyol 2019

β-catenin impacts on normal and tumoral liver metabolism

In vivo **CHIPseq RNAseq Metabolomics**



Gougelet et al., Hepatology 2014
Senni et al., Gut 2019

Glutamine synthesis ↑

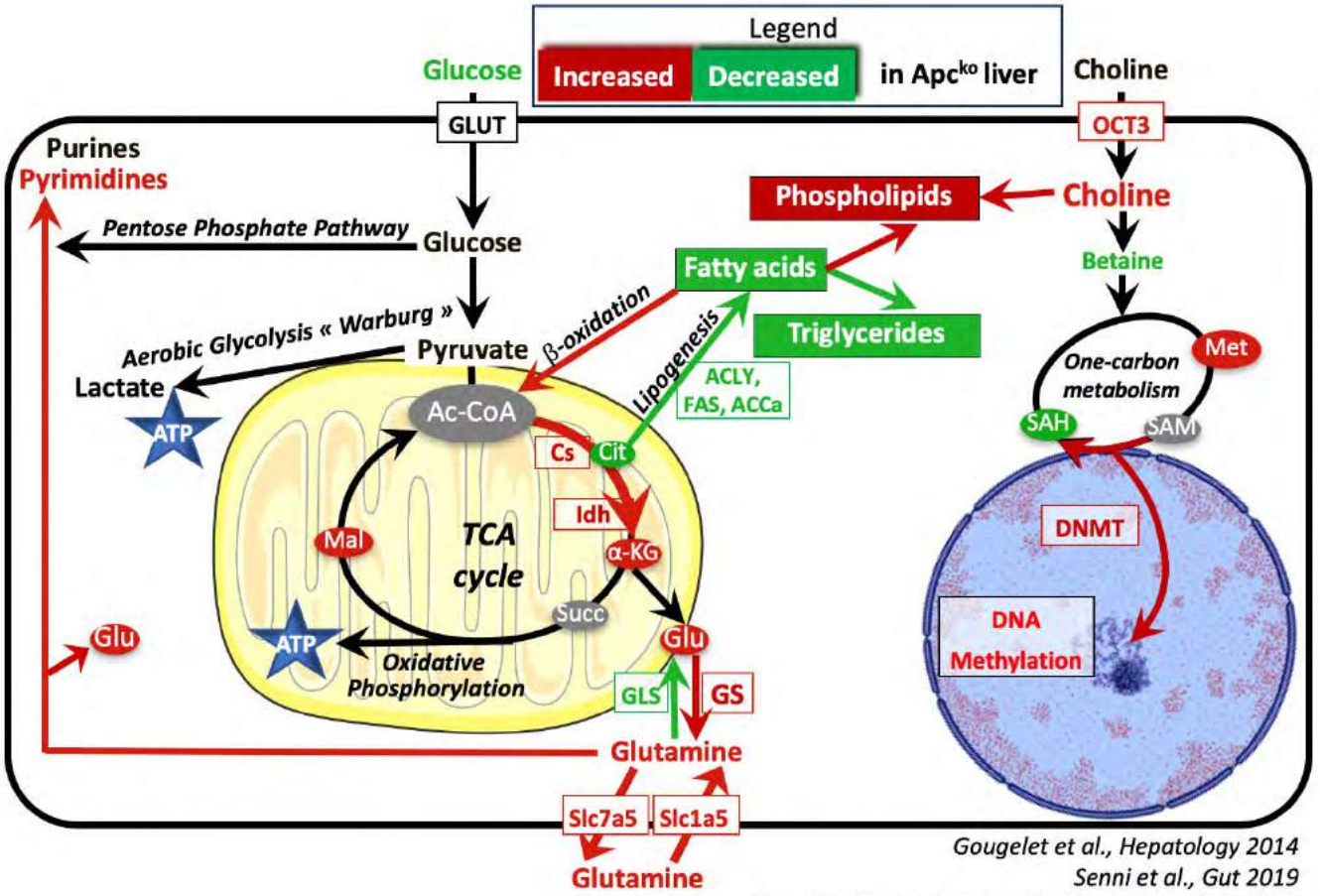
Lipogenesis ↓

Use of lipids as a source of energy ↑

The uptake and use of dietary Choline is enhanced in β-catenin-activated hepatocytes

In vivo **CHIPseq RNAseq Metabolomics**

Gougelet, Sartor, Senni et al., Gastroenterology 2019
Coll. St-Antoine, Créteil, Tenon Hospitals

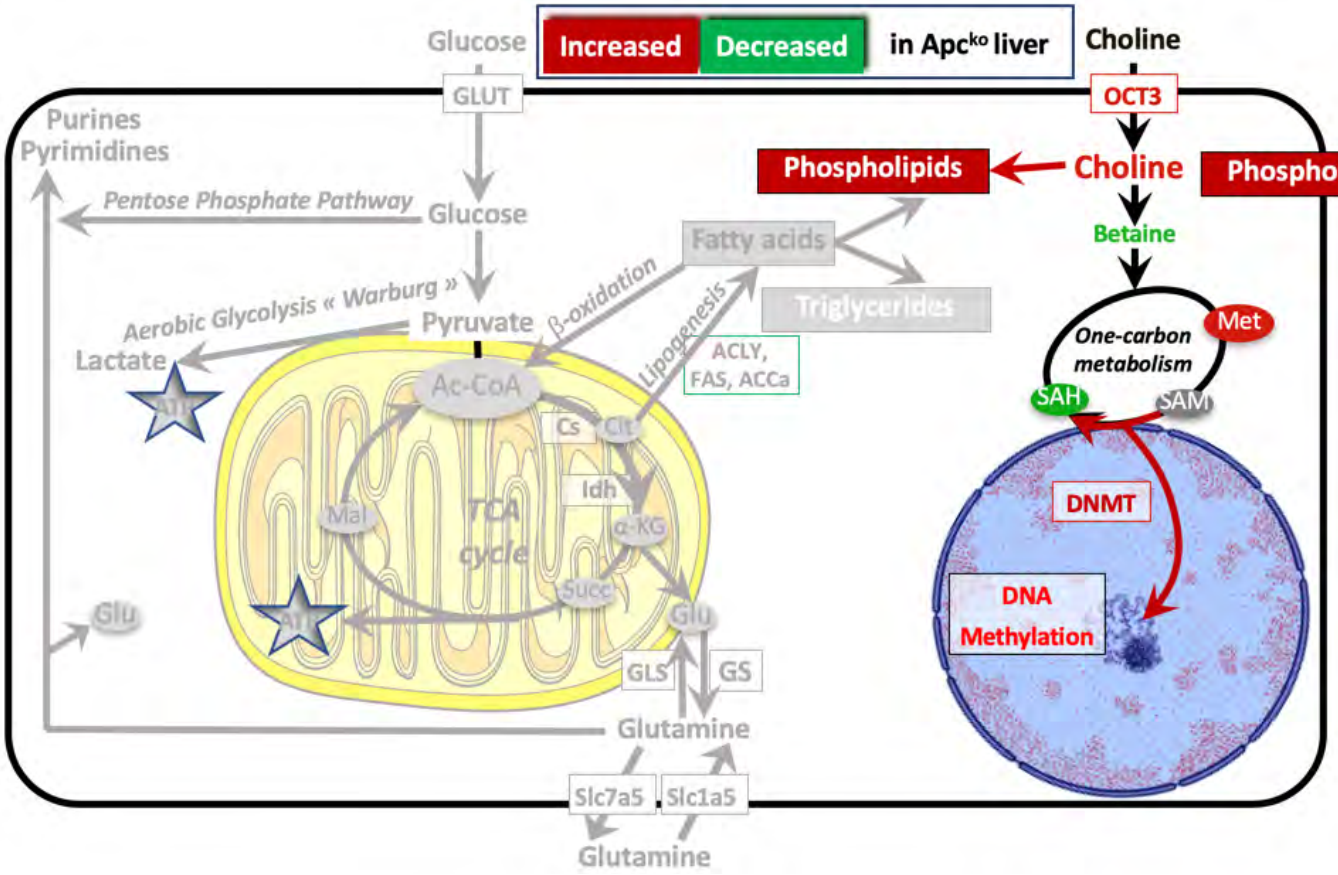


Gougelet et al., Hepatology 2014
Senni et al., Gut 2019

The uptake and use of dietary Choline is enhanced in β-catenin-activated hepatocytes

In vivo **CHIPseq RNAseq Metabolomics**

Gougelet, Sartor, Senni et al., Gastroenterology 2019
Coll. St-Antoine, Créteil, Tenon Hospitals

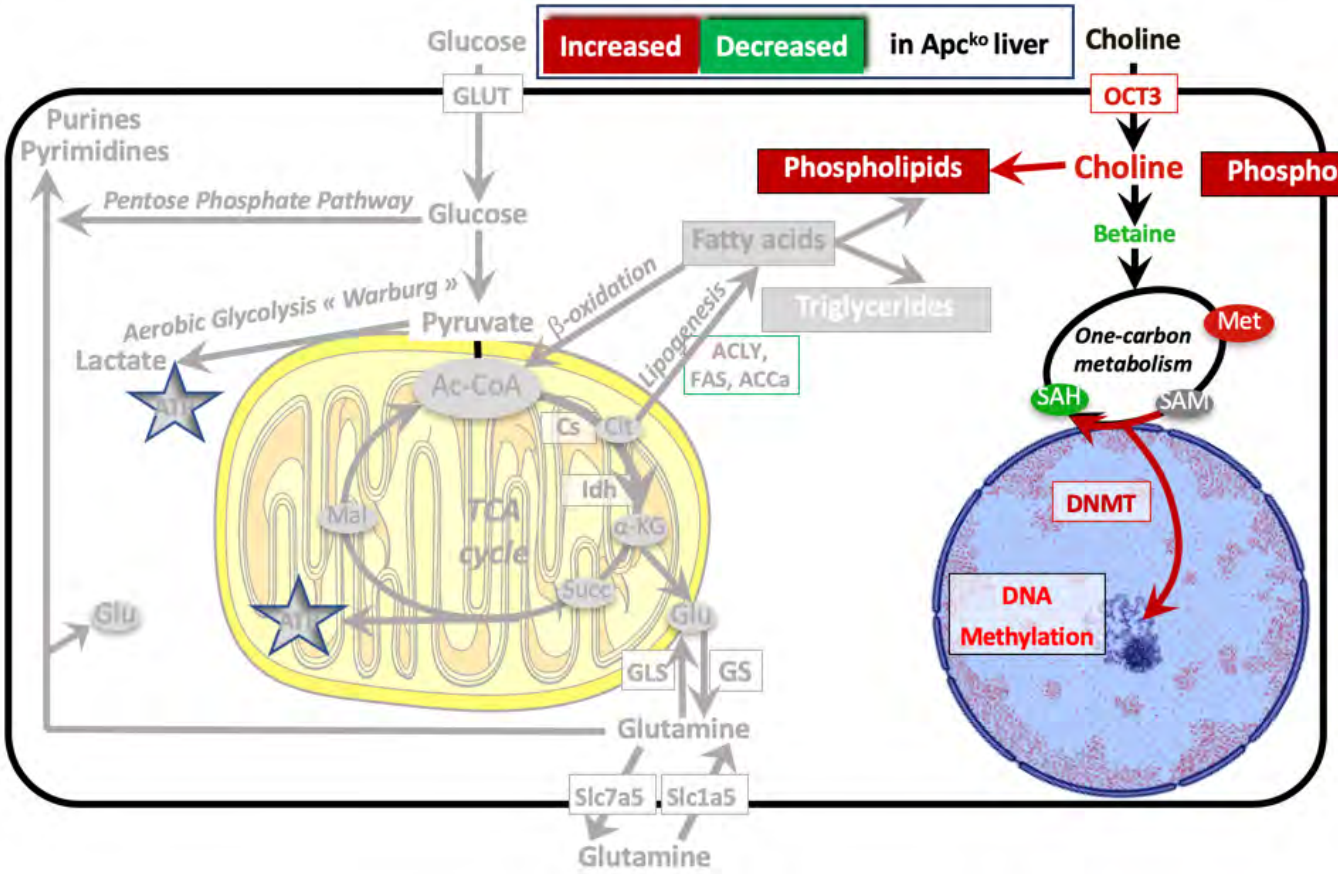


- **Membrane component (cell proliferation)**
- **Intracellular signaling**
- **Bile and VLDL component**

The uptake and use of dietary Choline is enhanced in β-catenin-activated hepatocytes

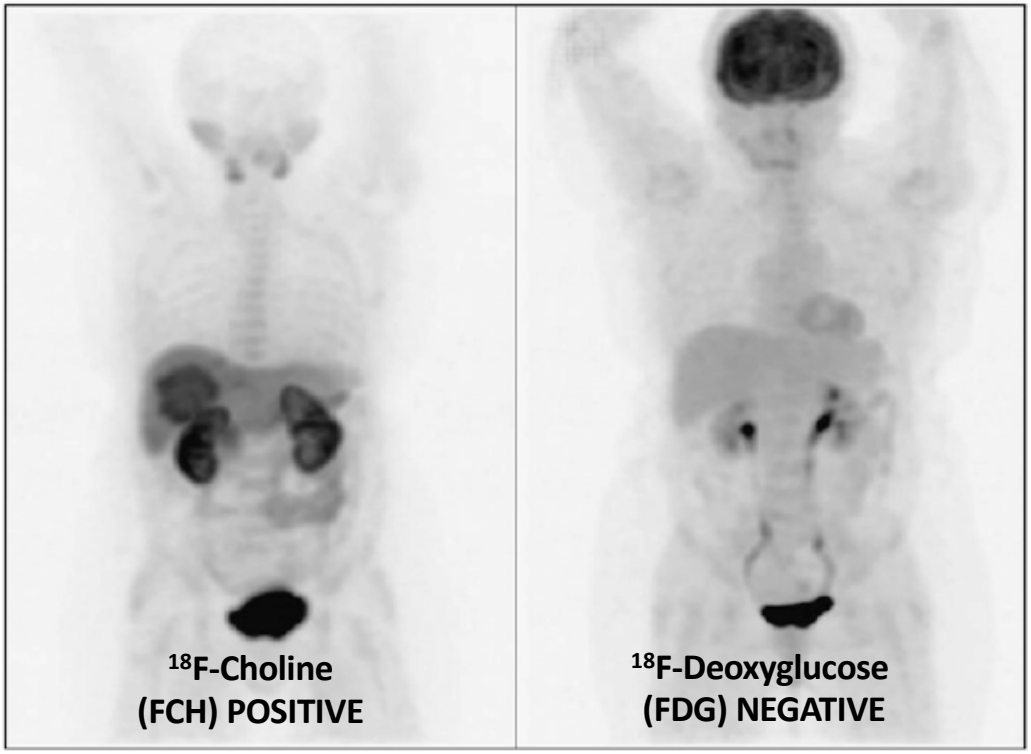
In vivo **CHIPseq RNAseq Metabolomics**

Gougelet, Sartor, Senni et al., Gastroenterology 2019
Coll. St-Antoine, Créteil, Tenon Hospitals



- **Membrane component (cell proliferation)**
- **Intracellular signaling**
- **Bile and VLDL component**

Increased uptake of choline is detected by Positron Emission Tomography in a subset of human liver cancers

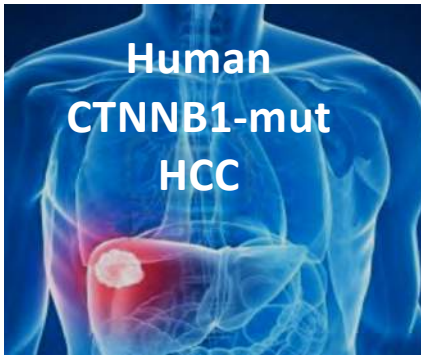


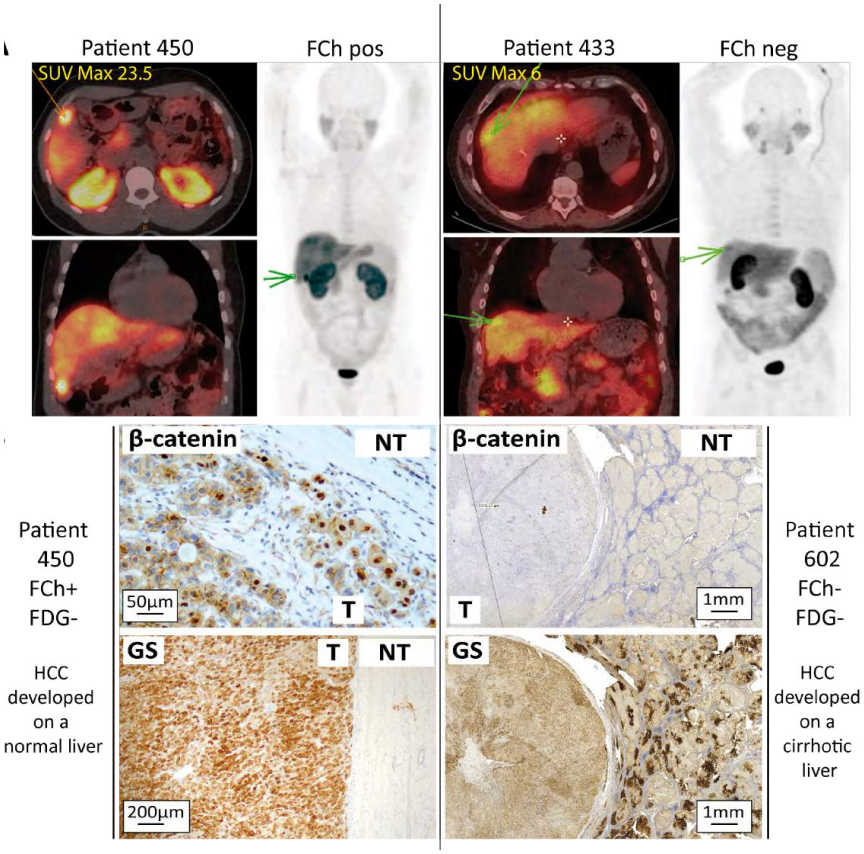
30% of HCCs can be detected by their increased uptake of ^{18}Fluoro-Choline (FCH) and are FDG-negative. They have no addiction to glucose, and thus no Warburg effect.

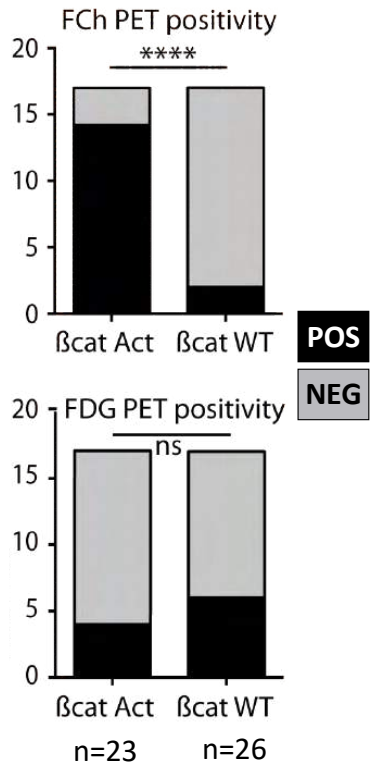
Talbot, J Nucl Med 2010
Fartoux, Nucl Med Com 2012

Do they correspond to CTNNB1-mutated HCCs?

Are β-catenin mutated HCCs choline-addicted? A Positron Emission Tomography approach (St-Antoine, Créteil & Tenon Hospitals)





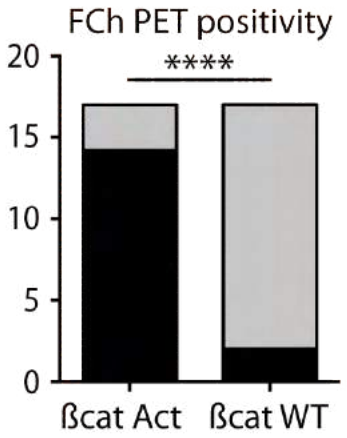


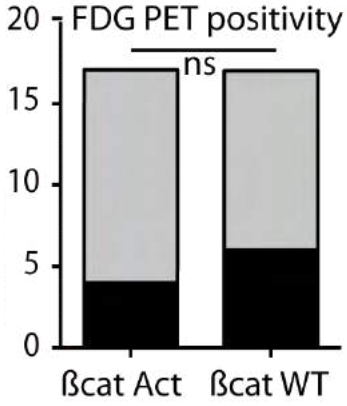
49 HCC analyzed
23 β-catenin-activated
26 β-catenin-WT

82% β-catenin-activated
12% β-catenin-WT are FCh+

Imaging of tumors by Positron Emission Tomography (PET) reveals an increased uptake of choline in β-catenin mutated HCCs both in human and mice

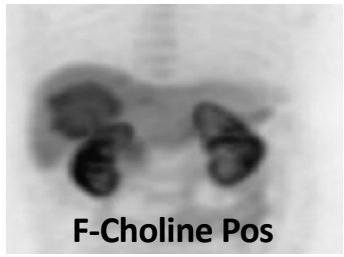
HUMANS

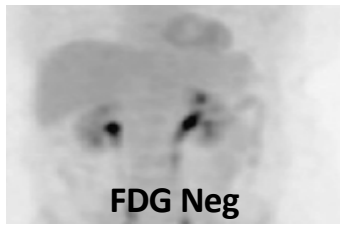




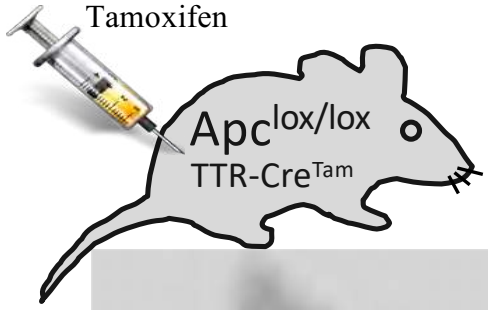
POS

NEG

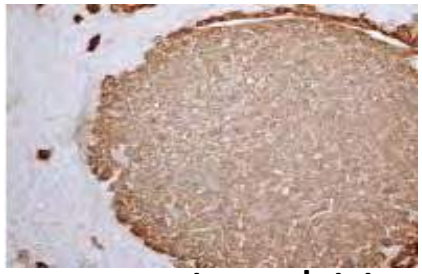




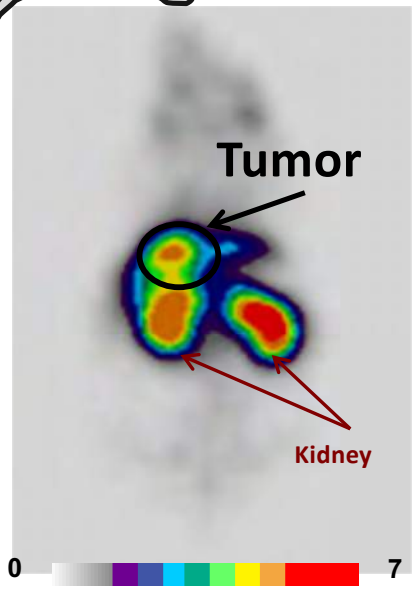
MICES



MONTH 6



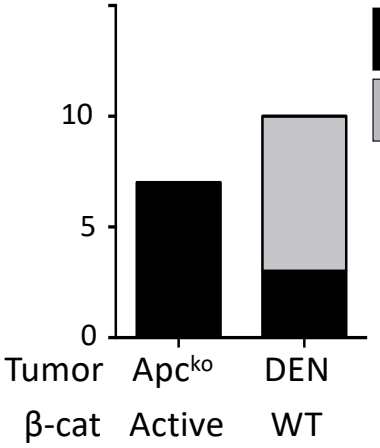
tumoral state



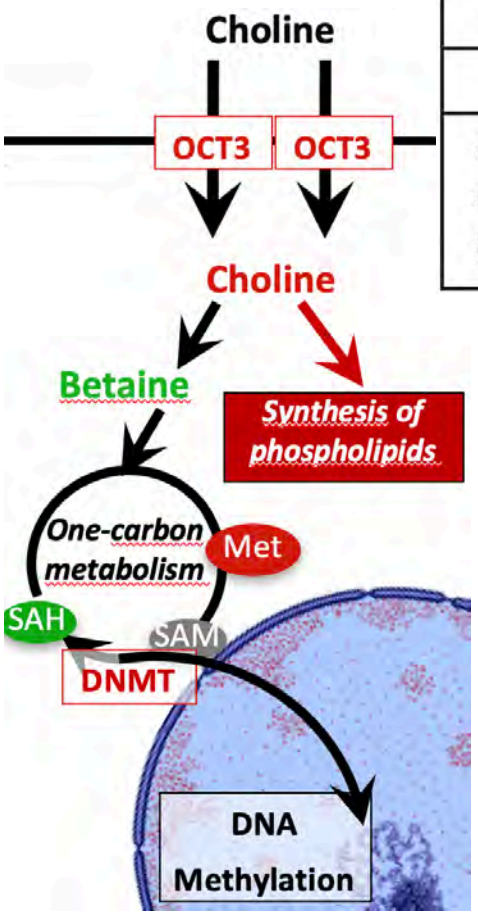
F-CHOLINE PET

POS

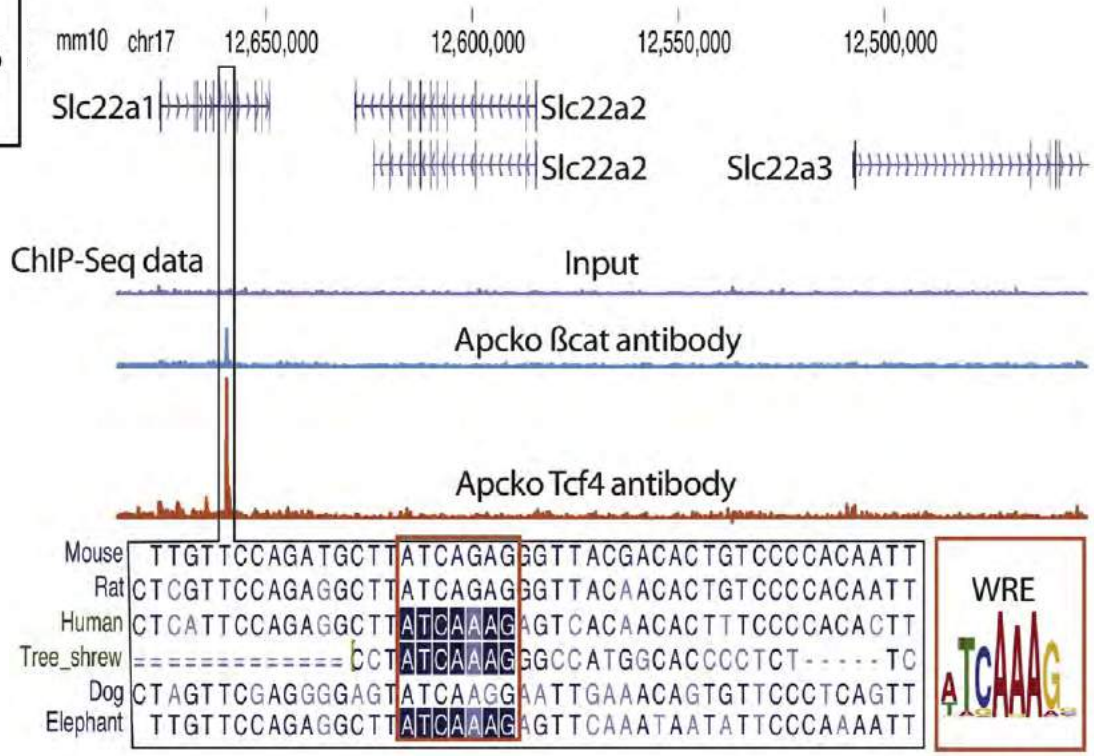
NEG



OCT3/SLC22A3 is a transporter with pericentral expression, is directly controlled by β-catenin and could be responsible for the increased uptake of Choline



mRNA-Seq Apcko T/NT		
	FC	*P*-value
Oct1	2.08	.005
Oct3	26.3	1.06E-06
Ctl3	2.13	.013

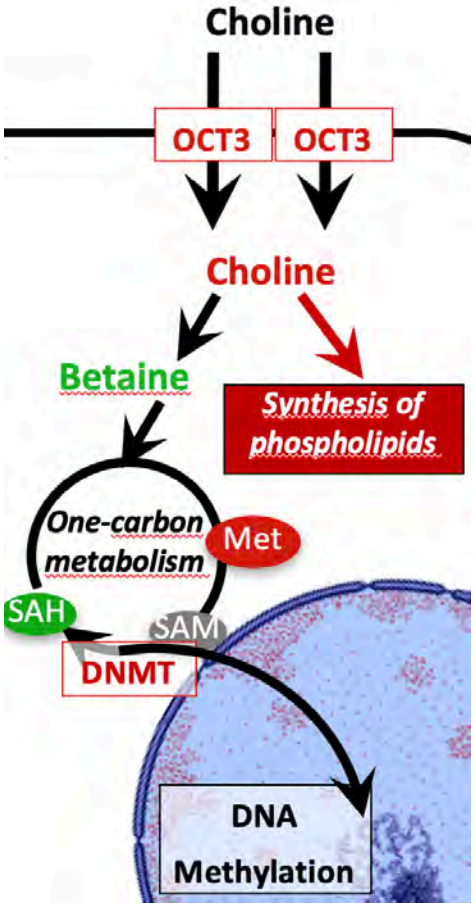


[Methyl]^{14}C-Choline

Explants from Apc^{ko} & DEN tumors

βcat active **βcat inactive**

What is the fate of β-catenin-dependent increased uptake of choline?

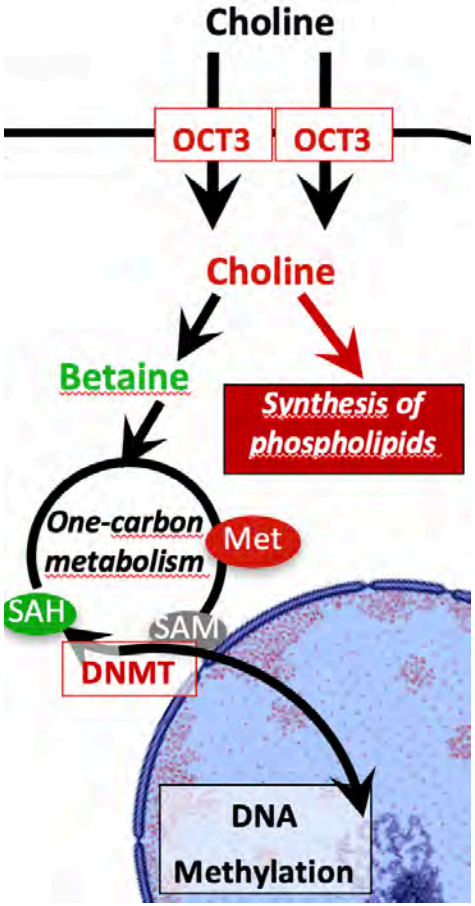


[Methyl]^{14}C-Choline

Explants from Apc^{ko} & DEN tumors

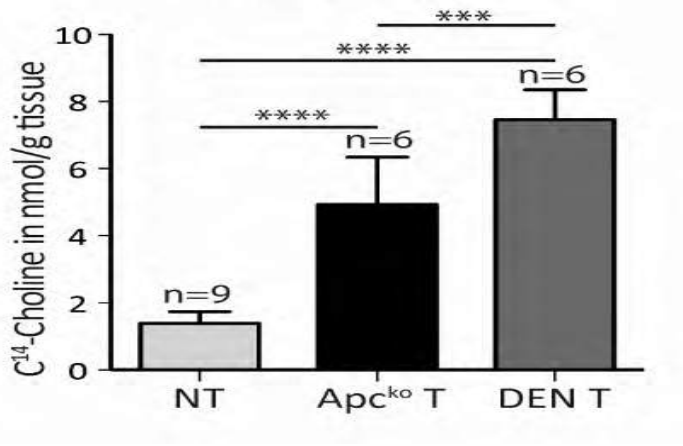
βcat active **βcat inactive**

What is the fate of β-catenin-dependent increased uptake of choline?



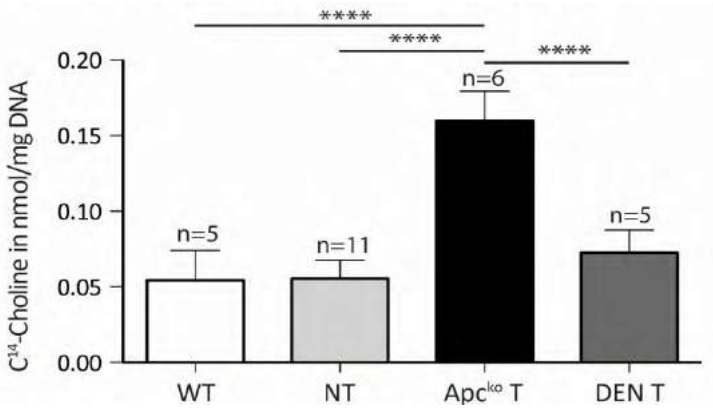
Increased incorporation of ^{14}C-Choline in phospholipids in tumors

- **Impact on tumor cell proliferation?**

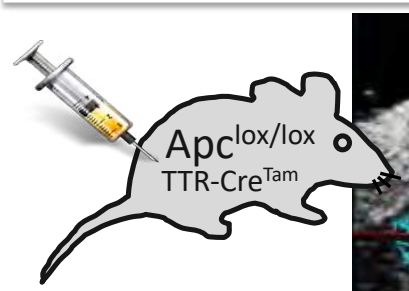


Increased incorporation of ^{14}C from Choline in DNA in β-catenin-activated tumors

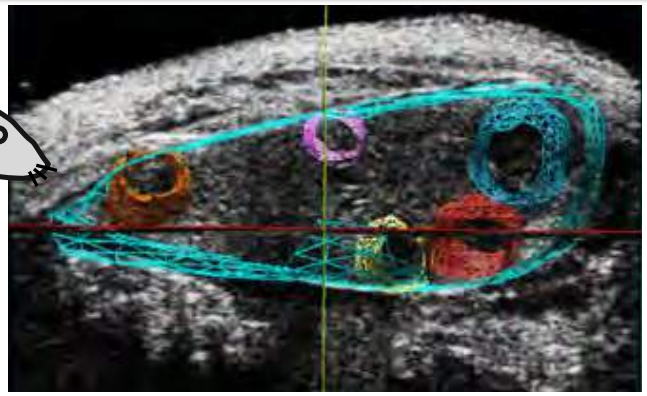
- **Impact on DNA methylation in tumors?**



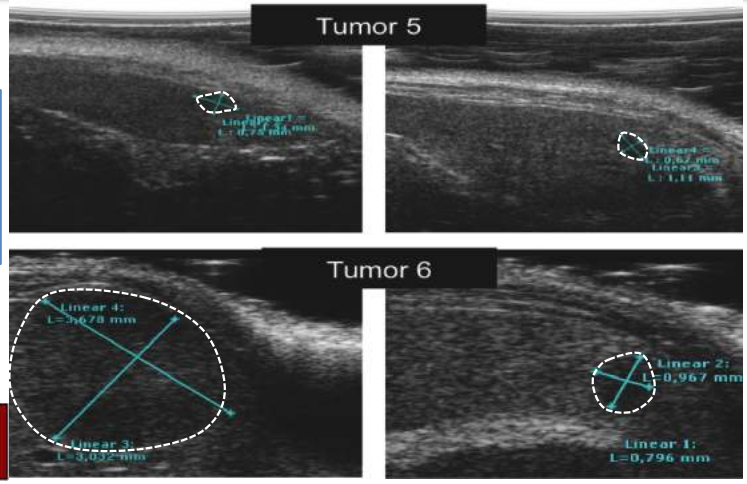
Blocking dietary intake of Choline in tumour-bearing mice makes Apc^{ko} tumors regress and relieves DNA methylation in tumors.



Tamoxifen

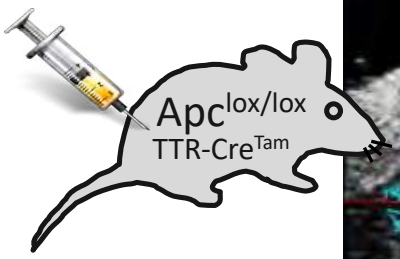


Choline-Deficient Diet

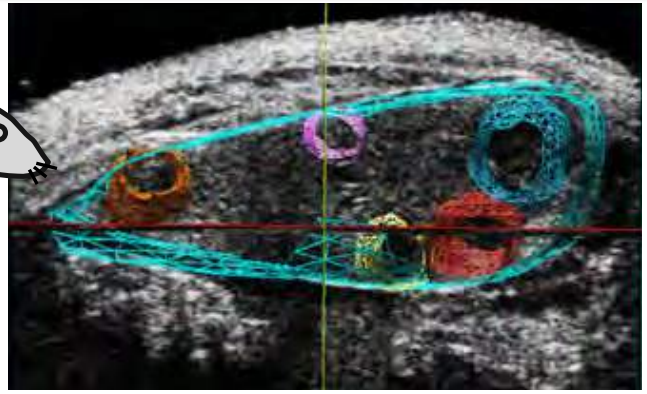


D0 M4 **ULTRASOUND FOLLOW-UP**

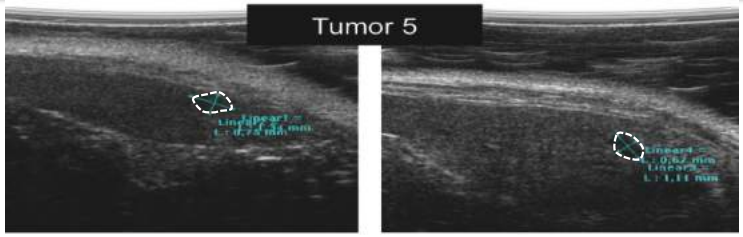
Blocking dietary intake of Choline in tumour-bearing mice makes Apc^{ko} tumors regress and relieves DNA methylation in tumors.

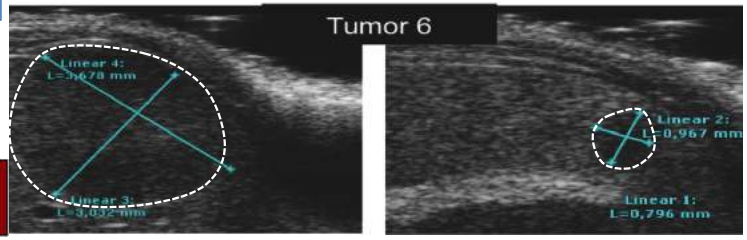


Tamoxifen



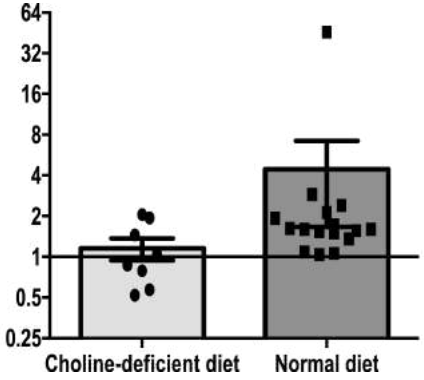
Choline-Deficient Diet

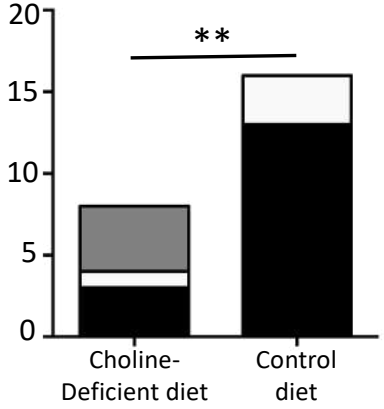


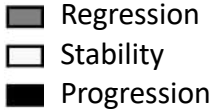


D0 M4 **ULTRASOUND FOLLOW-UP**

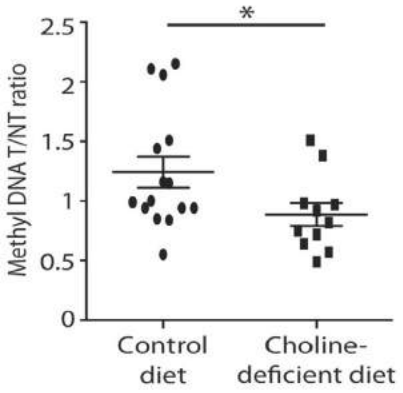
Growth of tumors in 15 days







DNA methylation in tumors



β-catenin signalling in the adult liver

CANCER

30% of HCC

Perret's lab: de la Coste, Romagnolo et al. PNAS 1998

Activating mutations in *CTNNB1* encoding β-catenin

LIVER PATTERNING

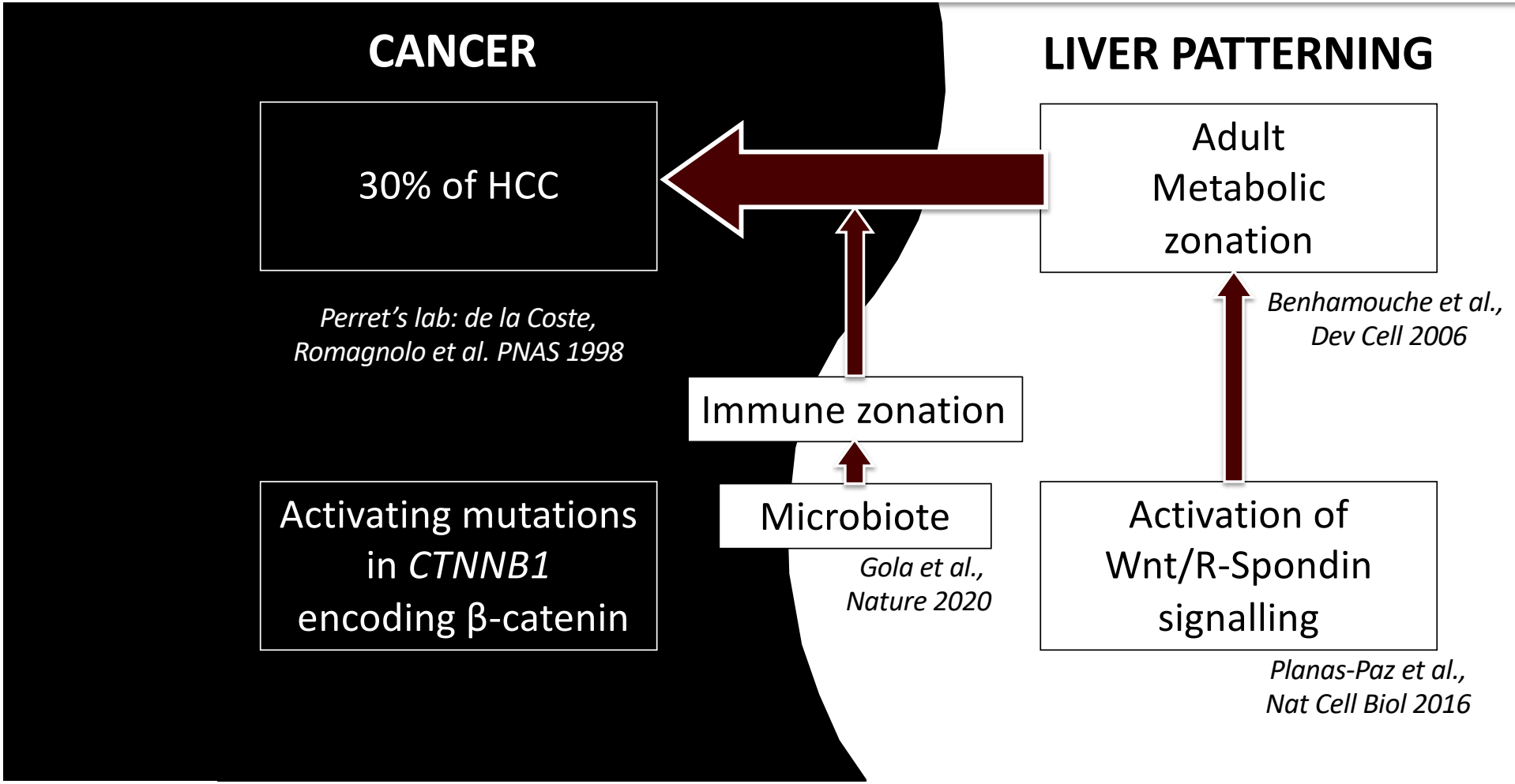
Adult Metabolic zonation

Benhamouche et al., Dev Cell 2006

Activation of Wnt/R-Spondin signalling

Planas-Paz et al., Nat Cell Biol 2016

Disturbing zonal metabolism impacts HCC pathogenesis with Wnt/β-catenin activation, and is a therapeutic option for these HCCs



Collaborators

Christine Perret, Paris
J. Zucman-Rossi, Paris
T. Tordjmann, Orsay

Metabolism: P. Bossard, Paris

Clinicians:

JN Talbot, *Tenon Hospital*
O. Rosmorduc, L. Fartoux,
D. Wendum, M. Lequoy, *St-Antoine Hospital*
J. Calderaro, G. Amaddeo, J. Chalaye, *Créteil Hospital*

Alumni:
Samira Benhamouche
Thomas Decaens
Cyril Torre

Angélique Gougelet
Christèle Desbois-Mouthon
Cécile Godard
Chiara Sartor
Nadia Senni
Robin Loesch
Rozenn Riou
Julie Sanceau
Lucie Poupel