Liver Regeneration: Initiation and termination signals

George K. Michalopoulos
Dept. of Pathology
University of Pittsburgh


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Regeneration

Prometheus

Rat Liver Regeneration After 70% Partial Hepatectomy (PHx)

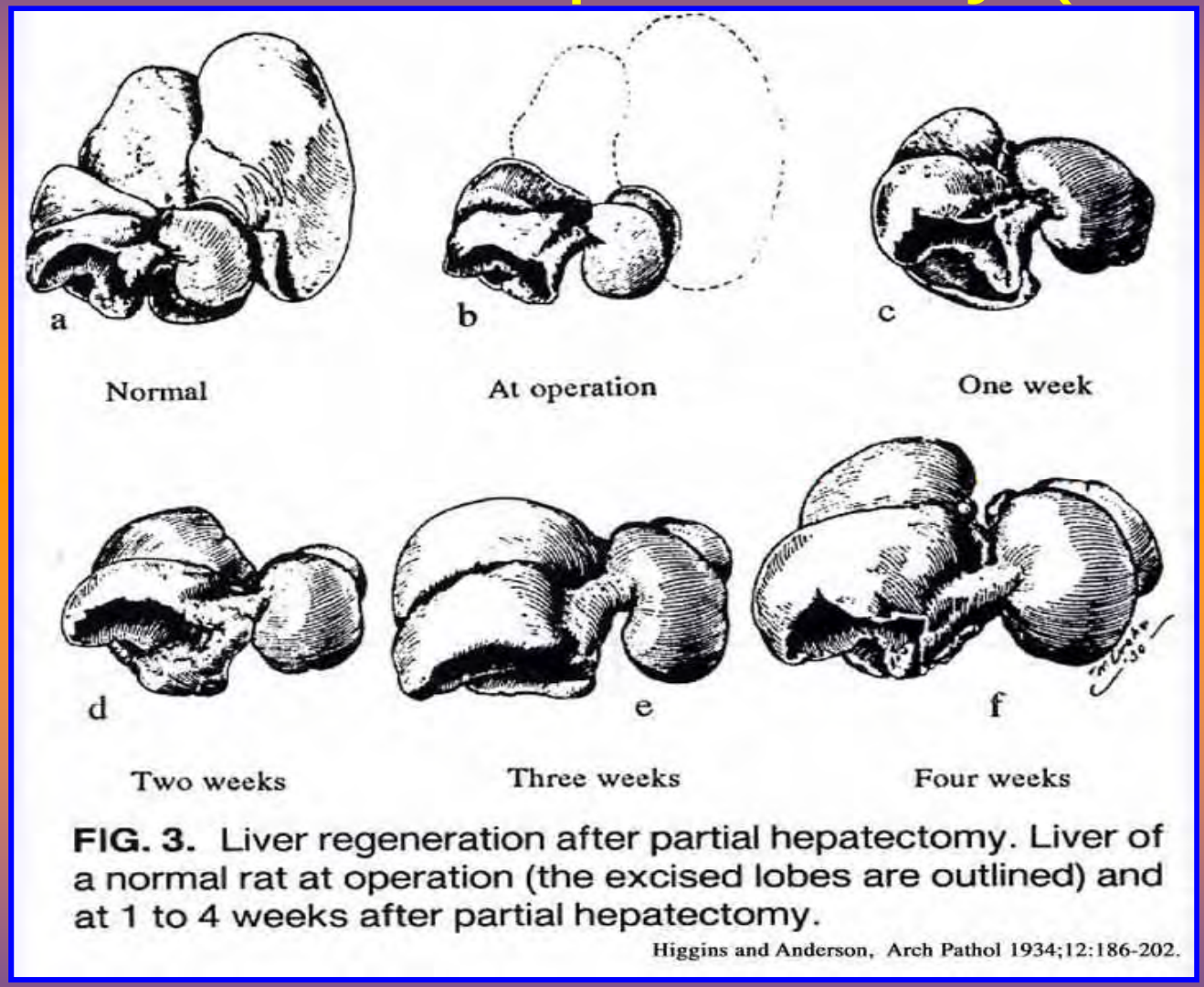
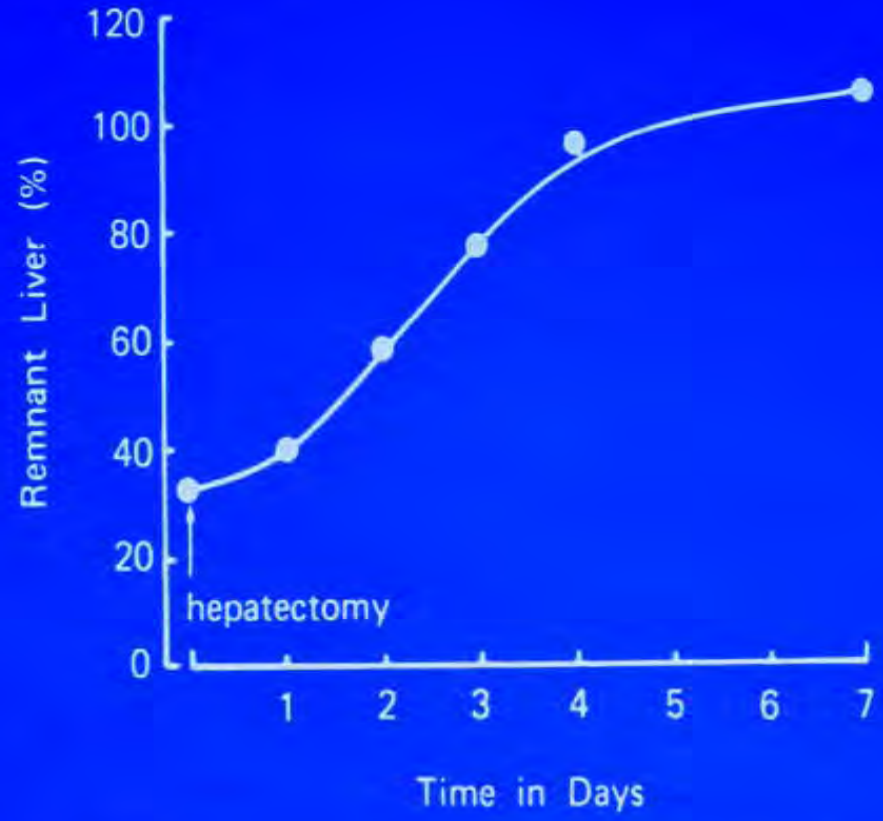


FIG. 3. Liver regeneration after partial hepatectomy. Liver of a normal rat at operation (the excised lobes are outlined) and at 1 to 4 weeks after partial hepatectomy.

Higgins and Anderson, Arch Pathol 1934;12:186-202.

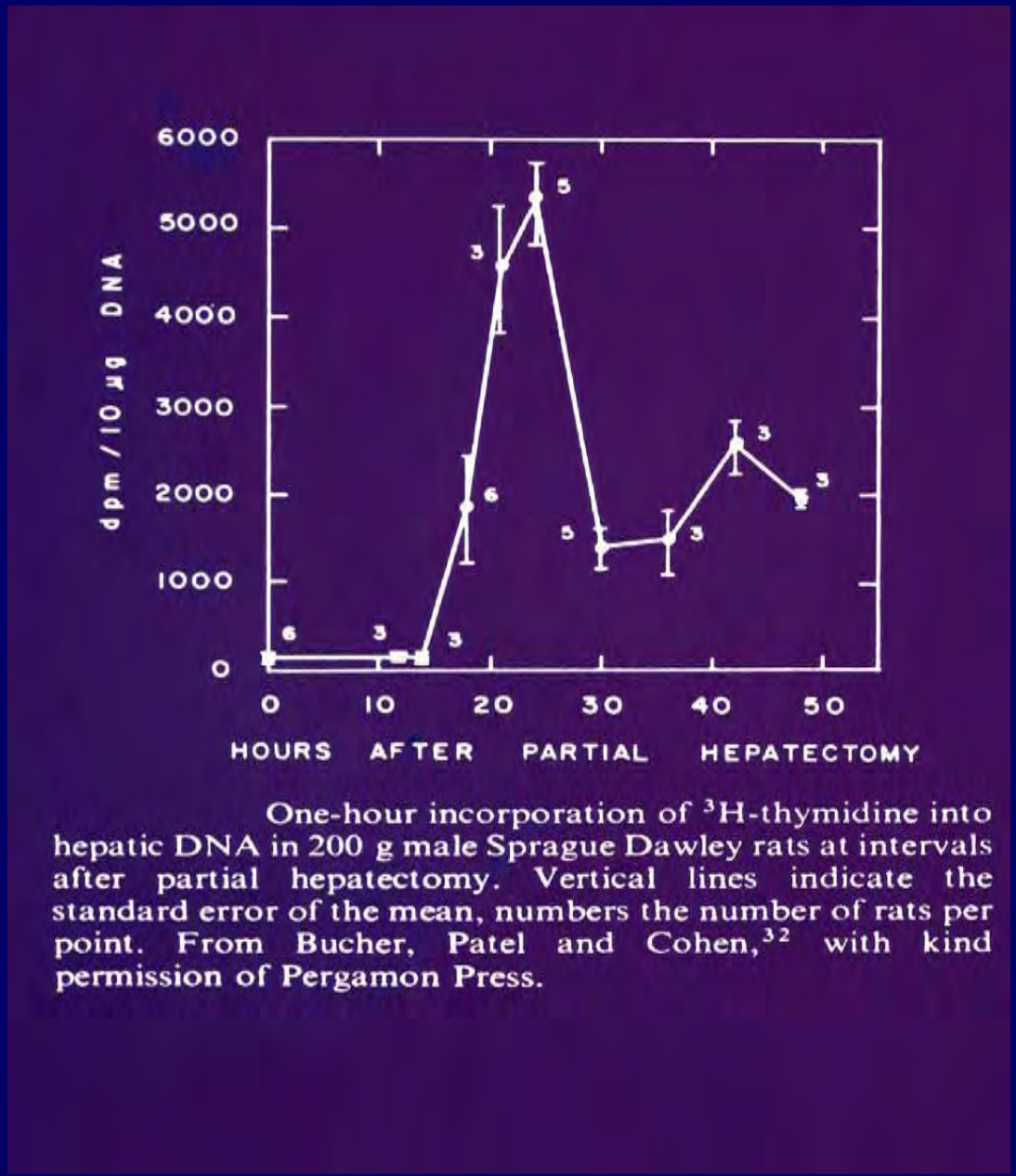
Compensatory hyperplasia of remnant lobes restores liver mass lost due to 70% PHx

Restoration of hepatic mass after partial hepatectomy in the rat.

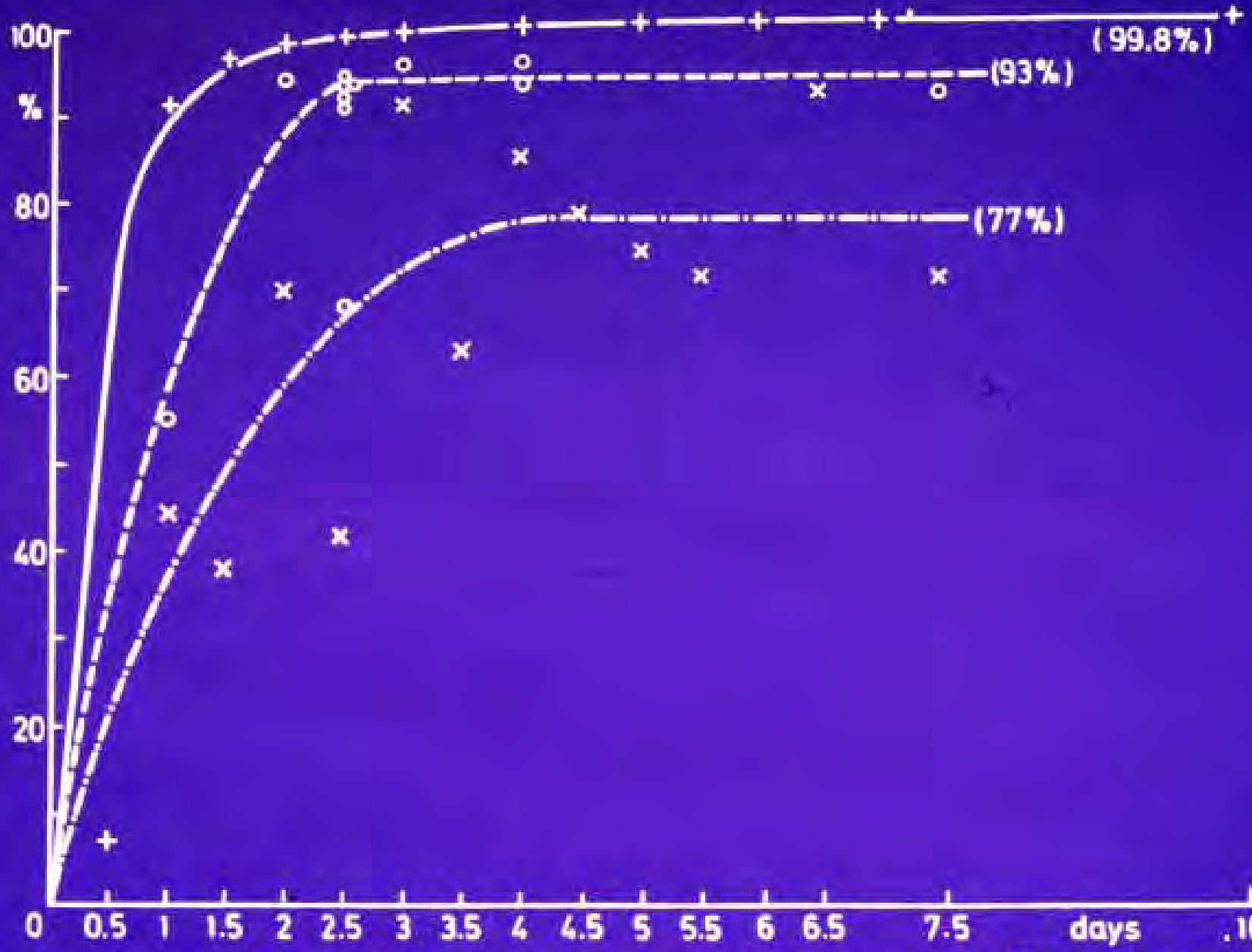


Wet weight of remnant liver. Regenerated rate of the hepatic remnant is shown as percent of the calculated preoperative whole liver weight.

DNA synthesis cycles after partial hepatectomy.

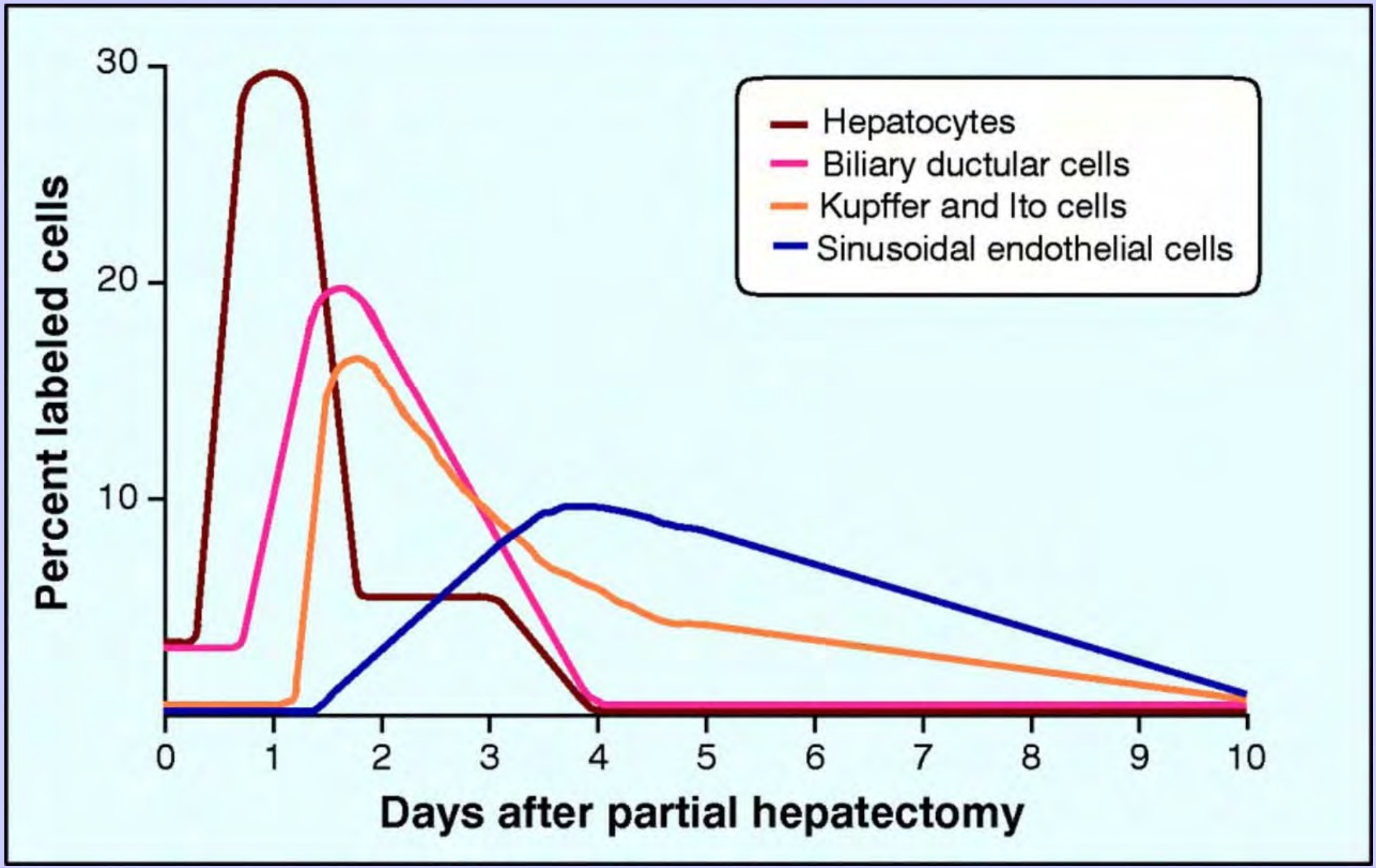


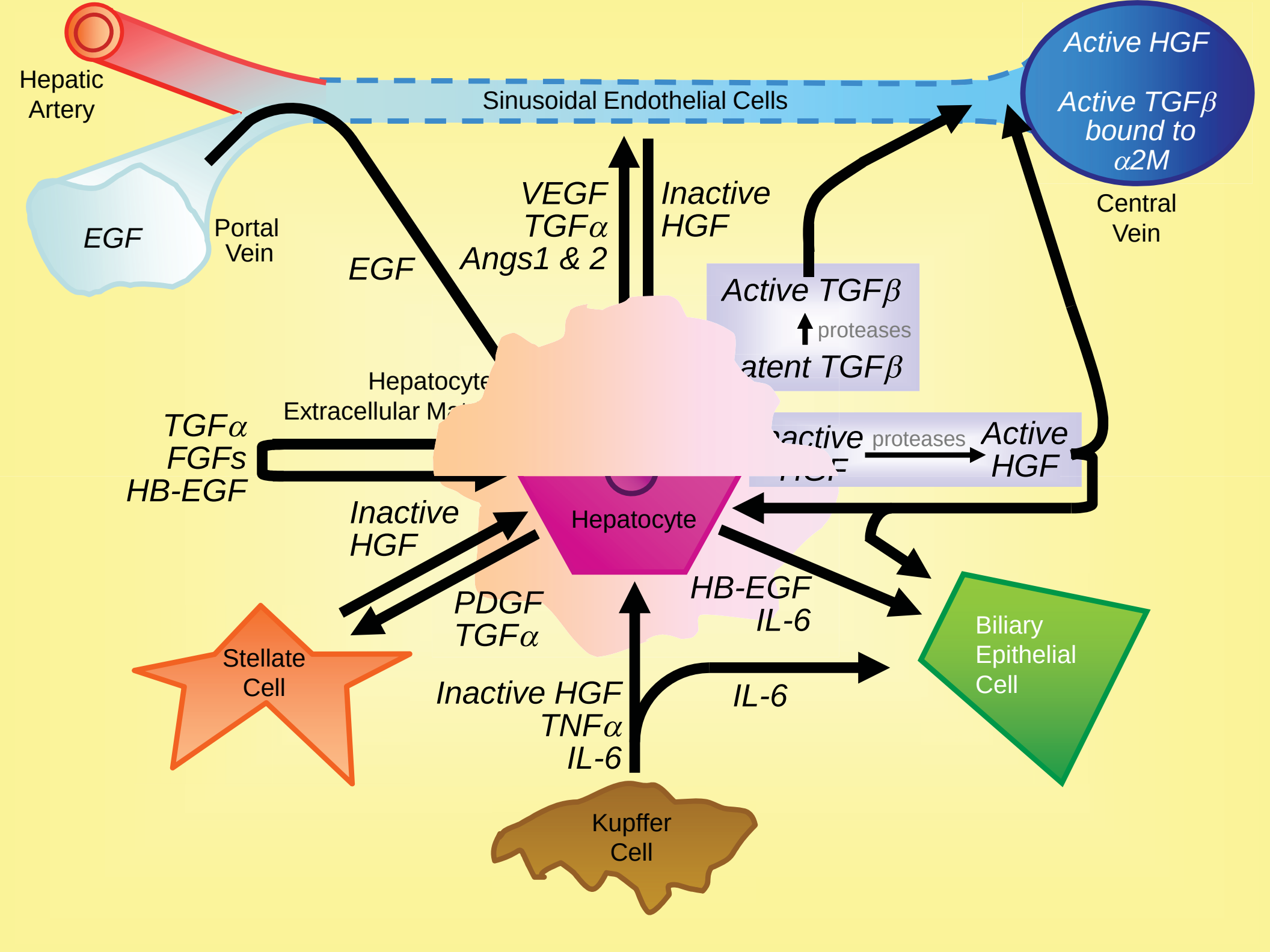
One-hour incorporation of ^{3}H-thymidine into hepatic DNA in 200 g male Sprague Dawley rats at intervals after partial hepatectomy. Vertical lines indicate the standard error of the mean, numbers the number of rats per point. From Bucher, Patel and Cohen,[32] with kind permission of Pergamon Press.



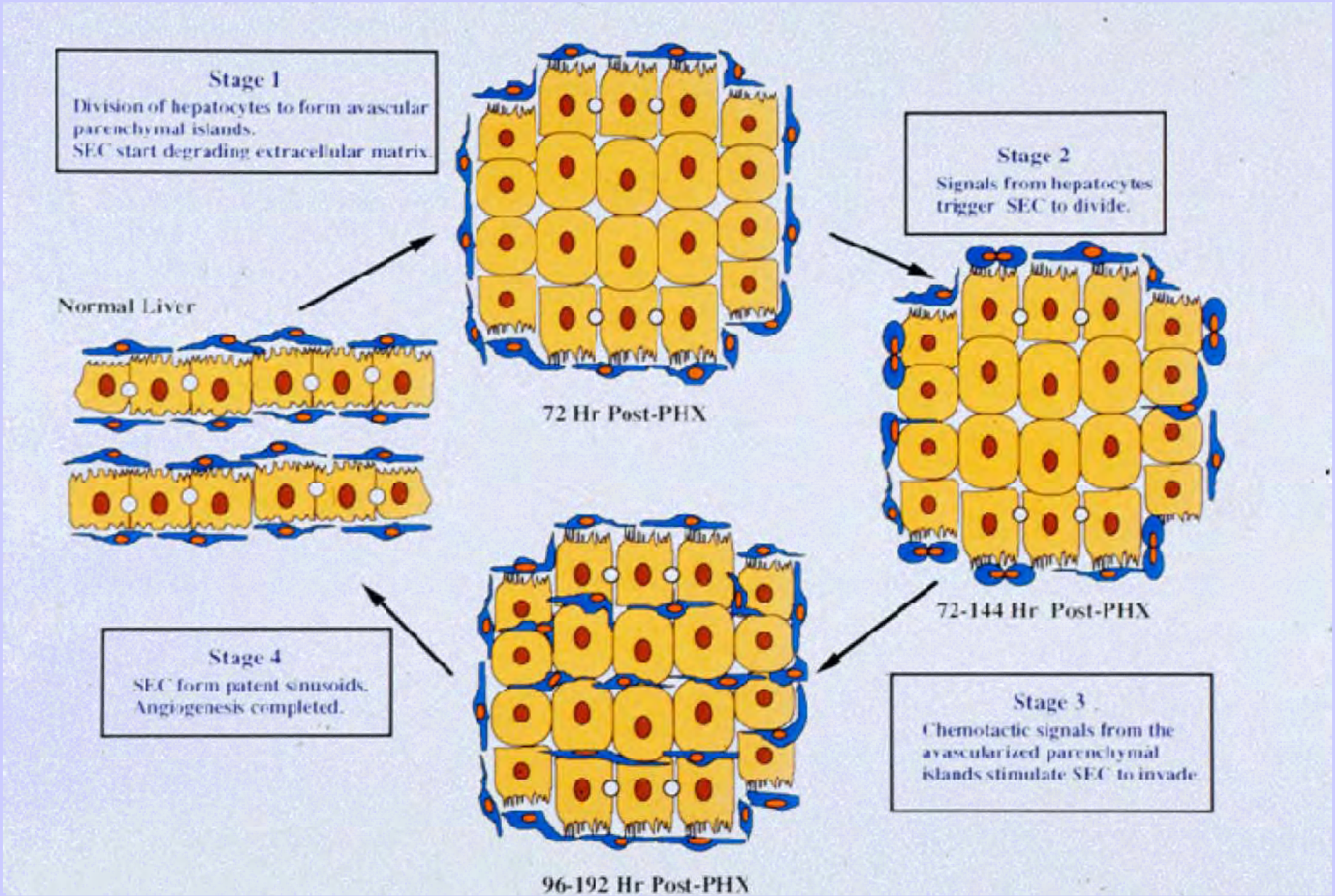
Percentage of labeled hepatocytes as a function of time after $^2/_3$ partial hepatectomy. –+–+– 3 to 4-week-old rats; –○–○– 2 to 4-month-old rats; –·–x–x– senescent rats (STÖCKER et al. 1972)

DNA synthesis of different hepatic cell types after partial hepatectomy.




Hepatic Artery
Sinusoidal Endothelial Cells
Active HGF
Active TGFβ bound to α2M
Central Vein
EGF
Portal Vein
EGF
VEGF
TGFα
Angs1 & 2
Inactive HGF
Active TGFβ
proteases
atent TGFβ
Hepatocyte
Extracellular Ma
TGFα
FGFs
HB-EGF
active
proteases
Active HGF
Inactive HGF
Hepatocyte
PDGF
TGFα
HB-EGF
IL-6
Stellate Cell
Biliary Epithelial Cell
Inactive HGF
TNFα
IL-6
IL-6
Kupffer Cell

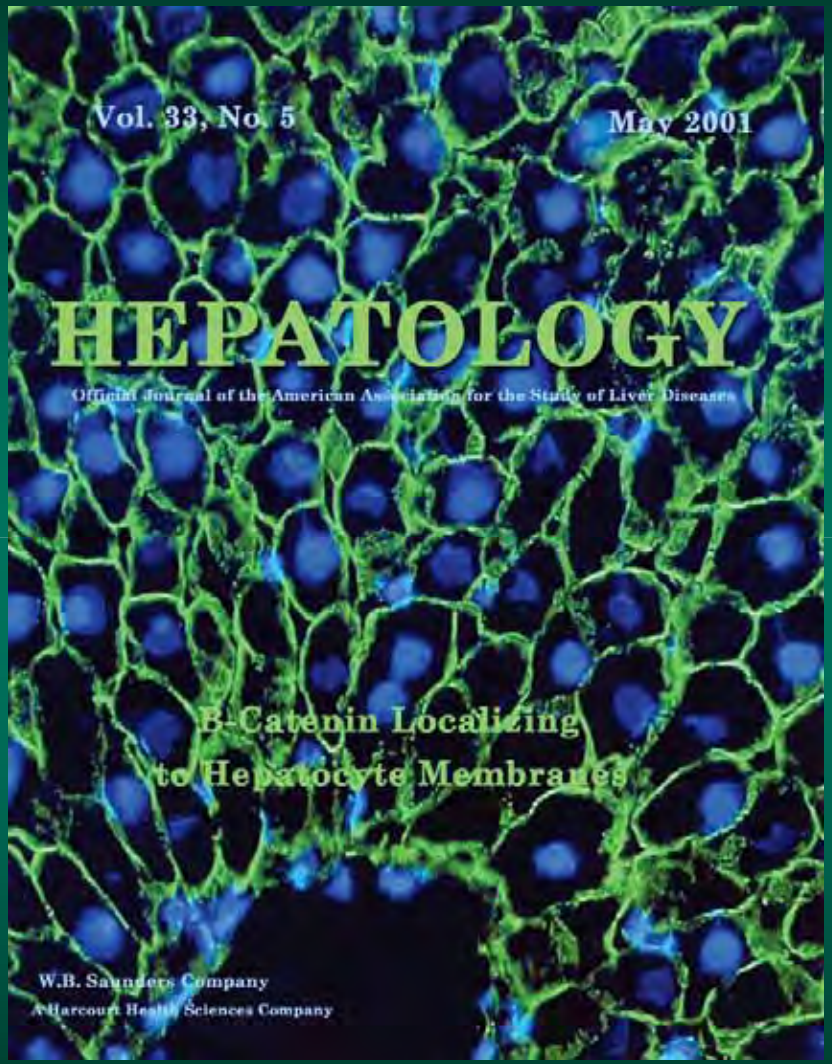
Angiogenesis cycle in Liver Regeneration



Chronology of **concurrent** early (first 1 hour) signaling events after PHx

- Multiple signaling pathways involving both growth factors, cytokines, paracrine signals (Notch/Jagged) and neuroendocrine factors (Norepinephrine) occur simultaneously within the first 60 minutes after PHx. Examples:

- Increase in urokinase activity (first 5 minutes)
- Translocation of N(otch)ICD to the nucleus (15 minutes)
- Translocation of beta-catenin to the nucleus (5-10 minutes to 6 hours)
- Decrease in HGF biomatrix stores (30 minutes to 3 hours)
- Activation of the HGF receptor (within 30-60 minutes)
- Activation of the EGF receptor (within 30-60 minutes)
- Increase of HGF, Norepinephrine, IL6, TNFa, TGFb1 and hyaluronic acid in the plasma.
- Activation of AP1, NFkB and STAT3
- Extensive gene expression reprogramming of hepatocytes within 30 minutes after PHx (Taub et al.).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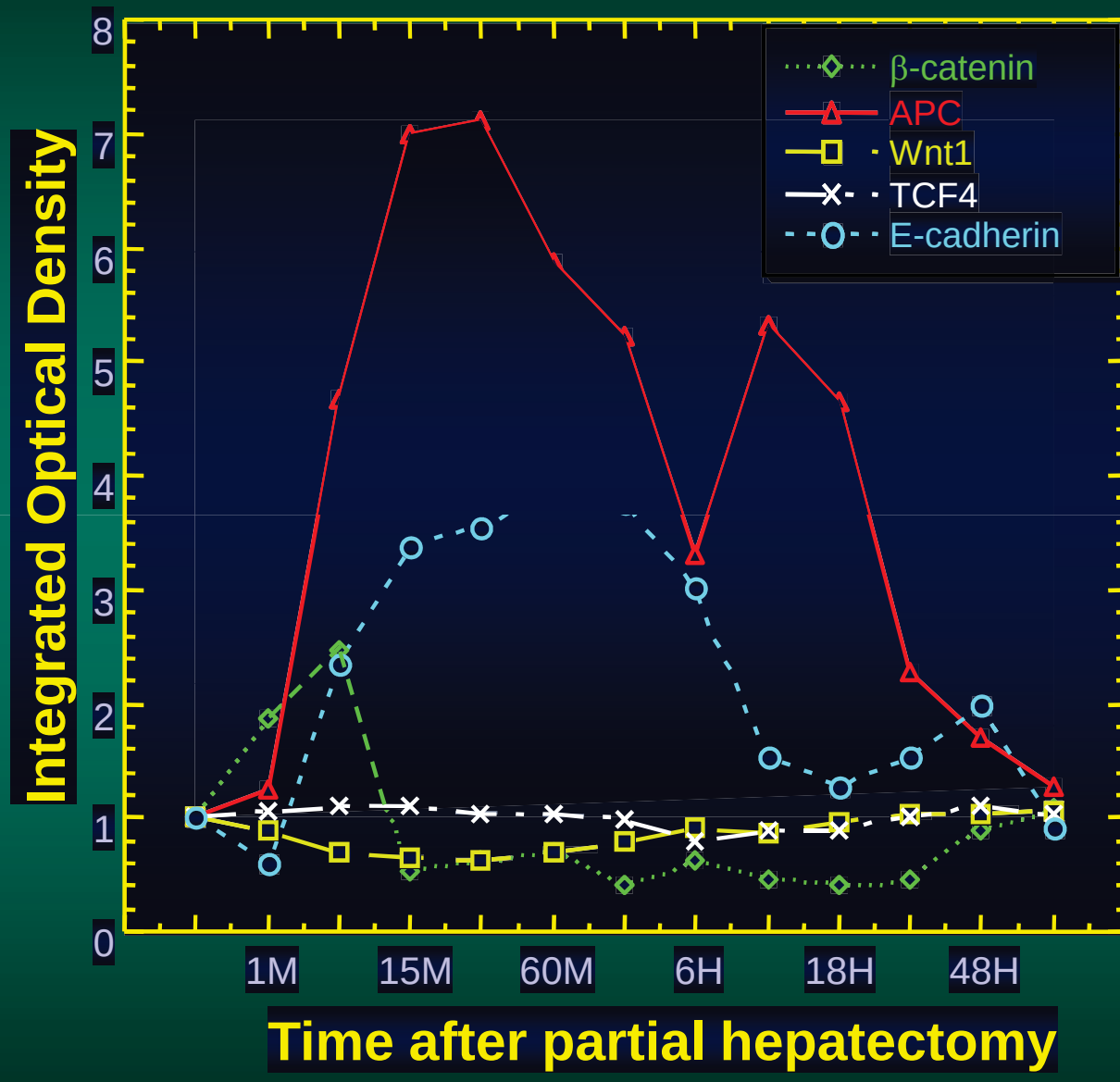
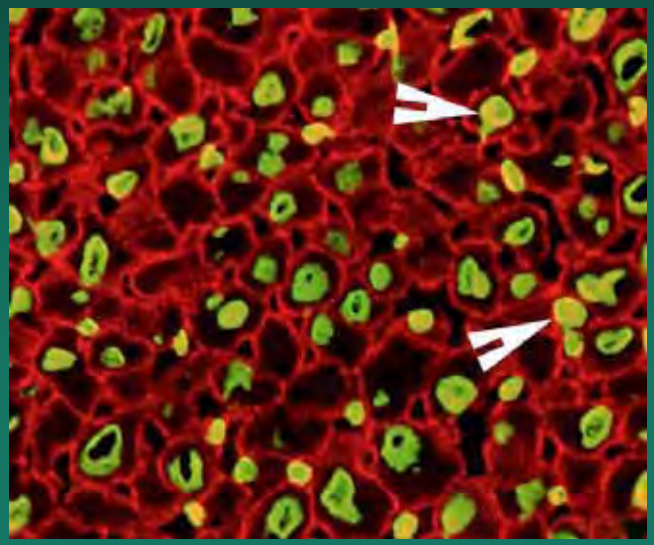
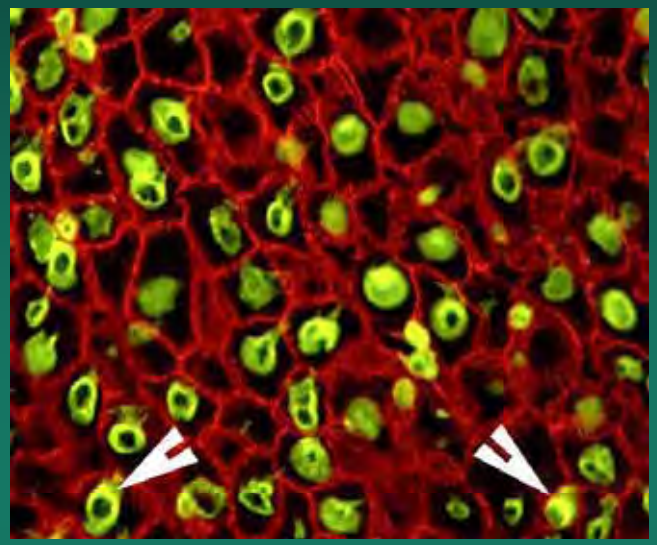
HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases
β-Catenin Localizing
to Hepatocyte Membranes
W.B. Saunders Company
A Harcourt Health Sciences Company


Integrated Optical Density
0
1
2
3
4
5
6
7
8
β-catenin
APC
Wnt1
TCF4
E-cadherin
1M
15M
60M
6H
18H
48H
Time after partial hepatectomy

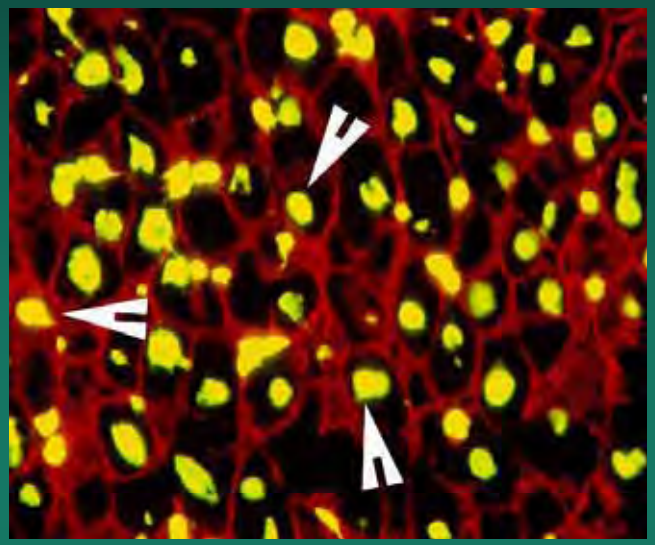
Increased translocation of residual β-catenin

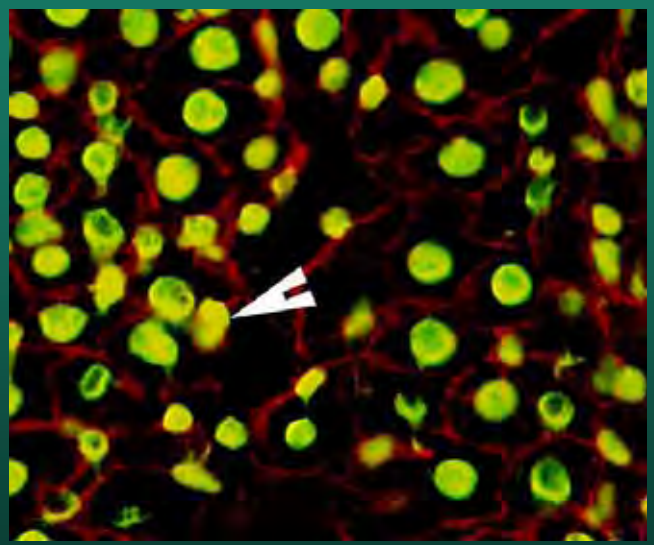


0 min.

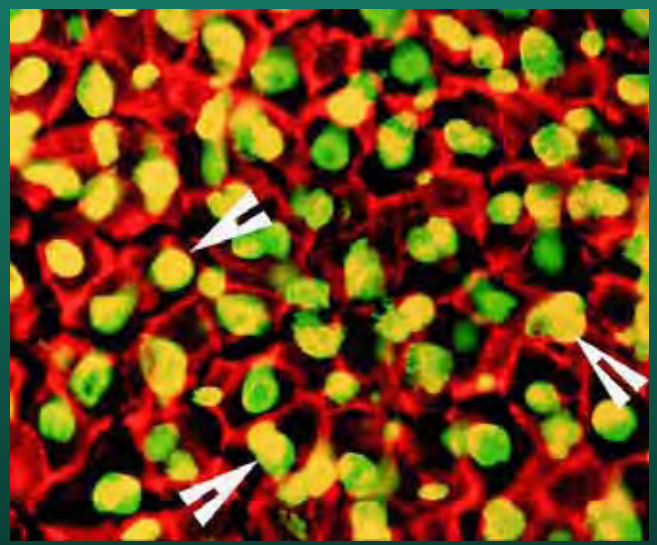


1 min.

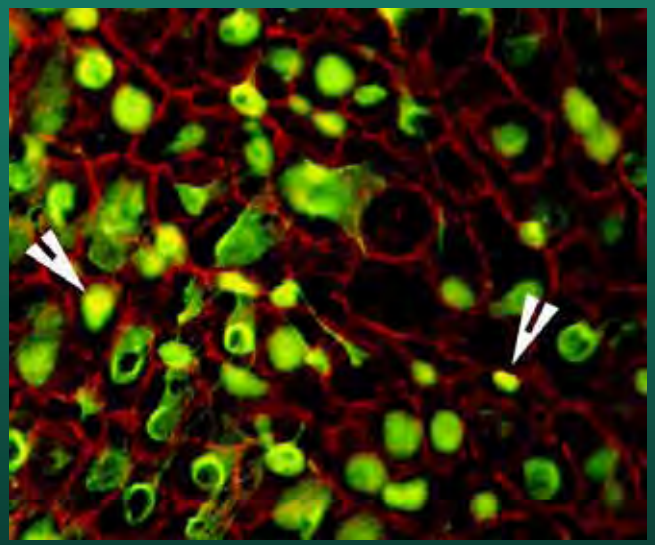




30 min.

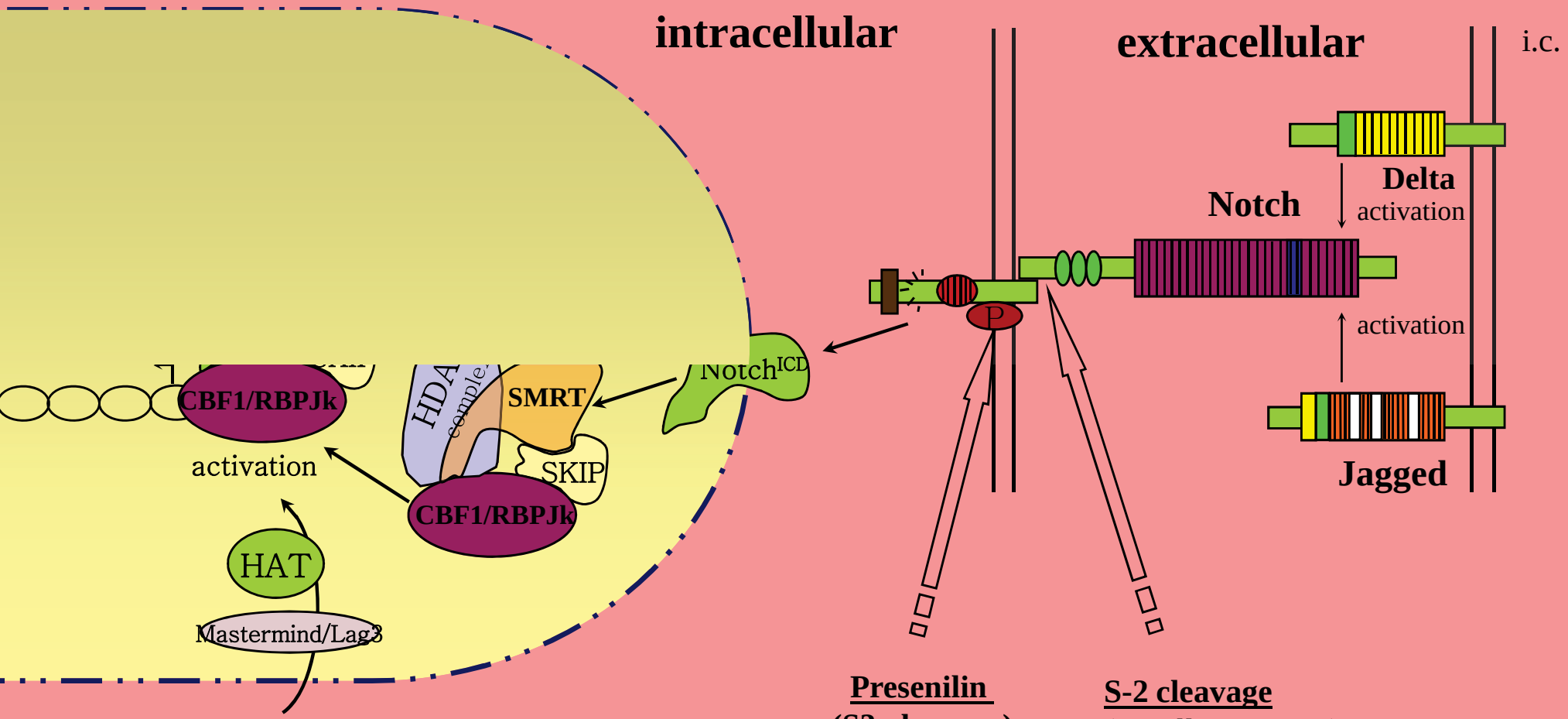


6 hrs.



48 hrs.

Notch/Jagged pathway



Presenilin
(S3-cleavage)

S-2 cleavage
(metalloproteases)

+ co-activators:
  - -Mastermind,
  - -GCN4 and PCAF
    histone acetylases (HATs)

EGF-repeats
= EGF 11-12 of Notch
= six ankyrin repeats
= Notch + Lin-12 repeats
= Pest sequence
= DSL domain
(Delta:Serrate:LAG-2 domain)

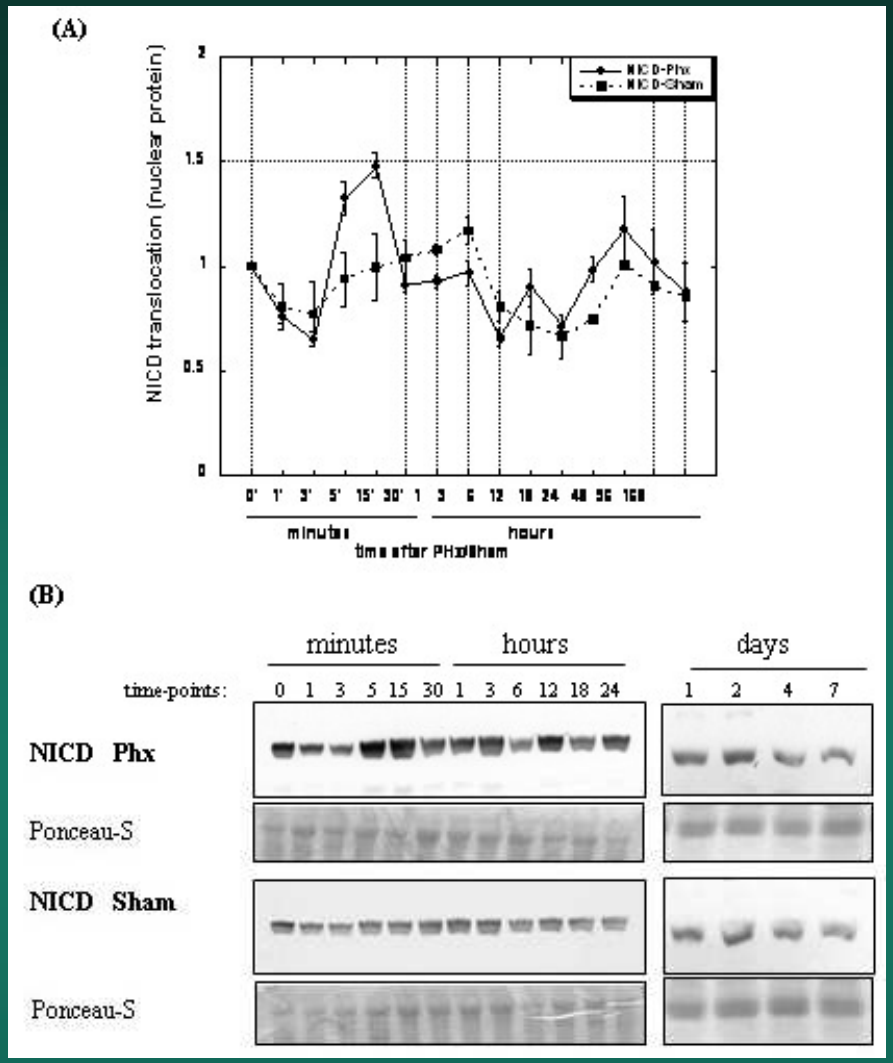


Fig. 5**. Detection of cytoplasmic domain of Notch (NICD) in nuclear protein (NP) extracts by Western blot analysis** (rabbit polyclonal antibody, Upstate). (A) Densitometric analysis of Western blots for NICD in nuclear protein (NP). Data are shown as mean _ SEM (n _ 3). (B) Representative Western blot of NICD detection in NP of rat liver. Ponceau-S stain of a band at 176 kDa are used as loading control. Numbers indicate time elapsed after operation in minutes, hours, and days

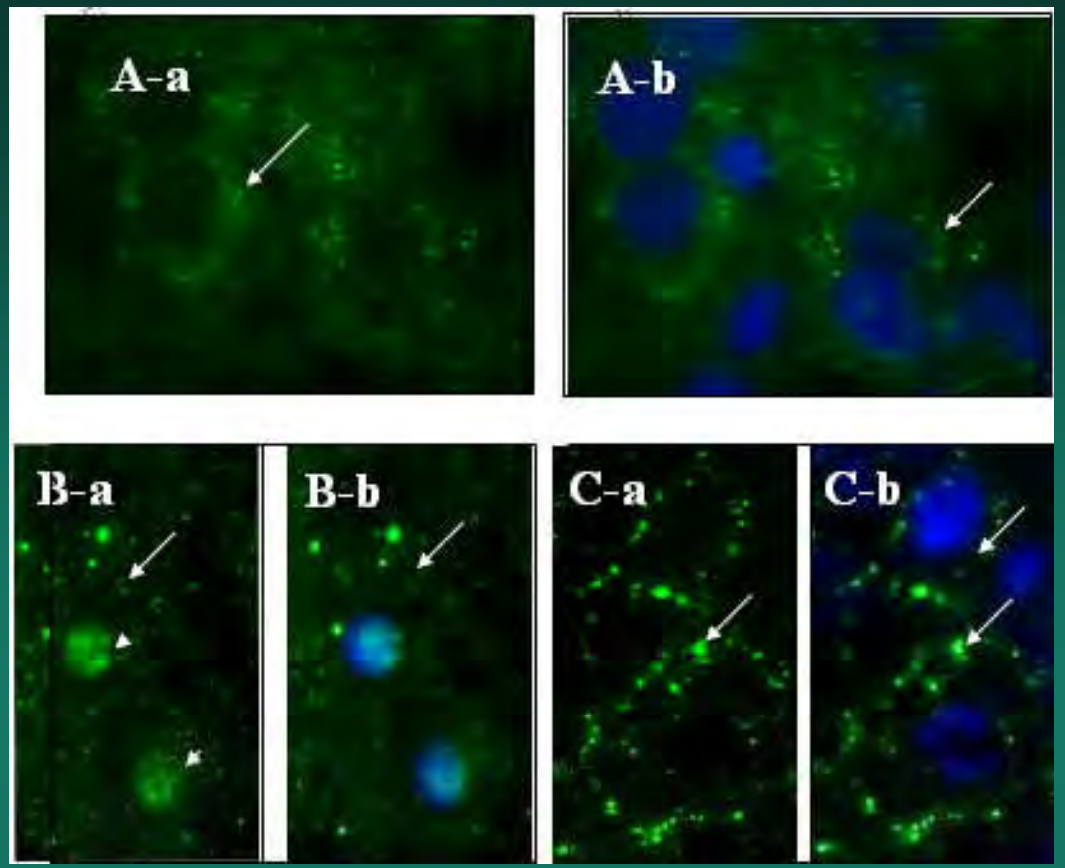
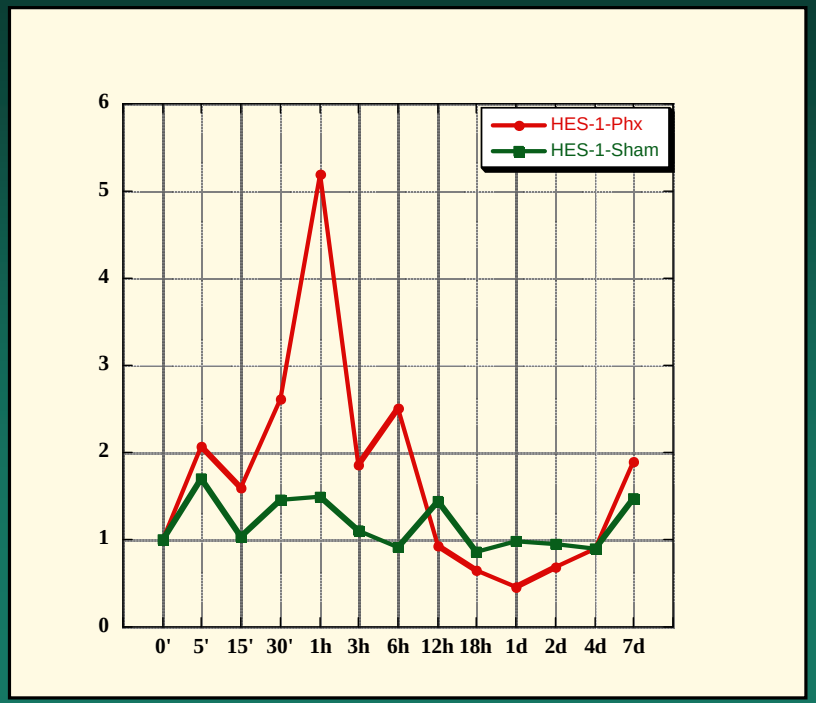
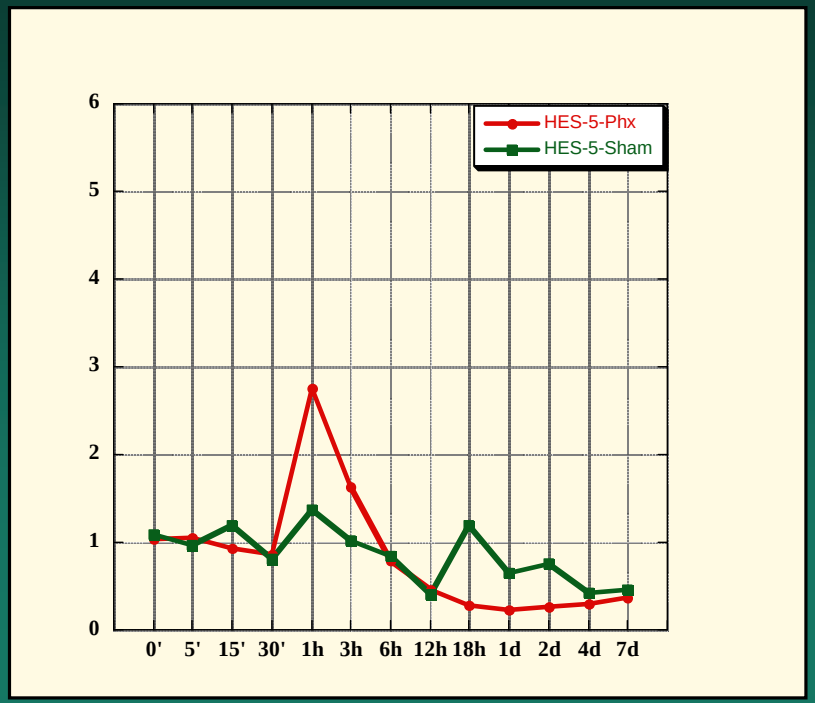


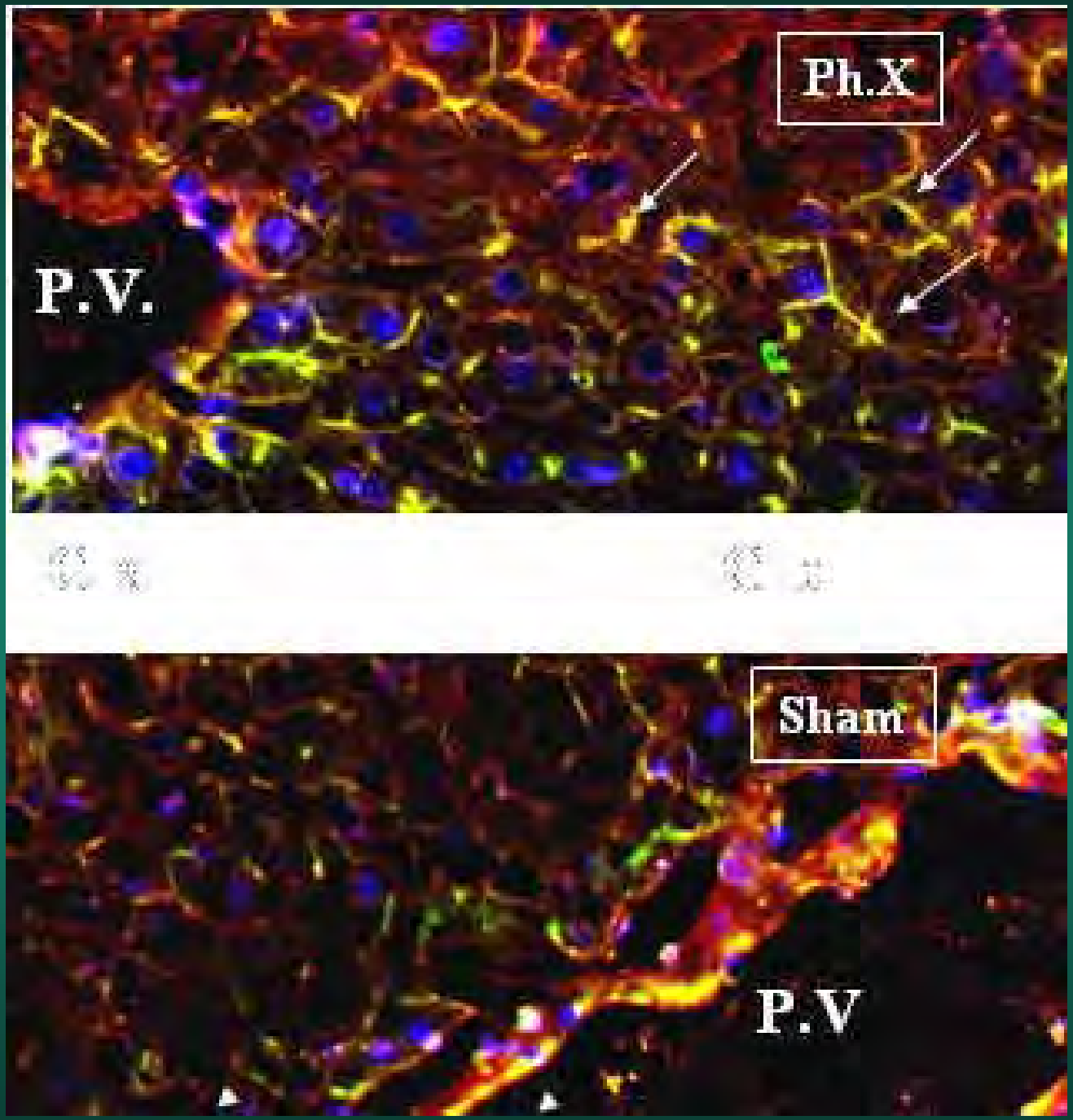
Fig. 6. **Detection of localization of the intra-cytoplasmic domain of Notch (NICD) in normal liver (A-a and A-b), liver at 15 minutes after partial hepatectomy (B-a and B-b), or sham operation (C-a and C-b). In normal liver and in sham-operated animals**, NICD is localized only on the cytoplasm or the plasma membrane. There is no green fluorescence in the nuclei. Green fluorescence is seen in the nuclei at 15 minutes after partial hepatectomy. The nuclei were counter-stained with Hoechst dye shown in Fig. A-b, B-b, and C-b, to serve as comparison with the corresponding (a) figures in order to facilitate visual localization of the nuclei. Cytoplasmic and membrane localization of NICD is shown by long arrows. Nuclear localization (seen only in B-a) is shown by short arrows





HES-1 = well expressed (Ct=23)
HES-5 = 1/30 of HES-1 expression

Figure 7. **Real-time PCR analysis of HES-1 and HES-5 gene expression in normal and regenerating liver.** (A) Expression of the Notch-1 target gene HES-1 was induced after PHx with a peak expression at 1 hour. Minor fluctuation (not statistically significant) are seen in sham operated animals. (B) Expression of HES-5. The only statistically significant increase over sham operated animals is a small increase at 6 hours after PHx. Data were normalized to expression in normal liver (CtHES-1=24.5; CtHES-5=35). Data represent the mean value ± SEM (n≥3).



Colocalization of Notch and Jagged in regenerating liver at Day 4 after partial hepatectomy.

The secondary antibodies used in the immunohistochemical stains for Notch and Jagged was conjugated with Alexa-488 (Notch) and CY-3 (Jagged). Notch (green) and Jagged (red) are colocalized (yellow) at the membrane of hepatocytes and endothelial cells in regenerating liver (A). Some of the points of colocalization on the plasma membrane are indicated by arrows. No significant degree of colocalization was noted in sham operated animals (B).

Table 1. Effect of Notch-1 and Jagged-1 silencing on proliferation of hepatocytes *in vivo*

Treatment	Days post-Phx	Mean of BrdU positive nuclei	Standard Error
normal liver	2	35.40	5.4
Scra-siRNA	2	40.70	3.6
J1-siRNA	2	7.30 ***	3.8
N1-siRNA	2	24.70 **	3.6
normal liver	3	59.60	7.6
Scra-siRNA	3	43.00	4.3
J1-siRNA	3	25.90 **	3.9
N1-siRNA	3	29.95 *	2.2
normal liver	4	68.50	3.2
Scra-siRNA	4	63.15	4.2
J1-siRNA	4	29.9 ***	2.4
N1-siRNA	4	35.4 ***	4.2

P-value of significance against Scra-siRNA treatment

*** = P < 0.0005
** = P < 0.005
* = P < 0.05

Extracellular Signals implicated in liver regeneration

- **Hepatocyte Growth Factor (HGF) and receptor c-Met)**
- **Ligands of the EGF R (EGF, TGFα, HB-EGF, Amphiregulin)**
- **Norepinephrine and the α1 adrenergic receptor.**
- **TNF and TNFR1.**
- **IL6**
- ***Notch and Jagged***
- **VEGF and receptors I and II.**
- **TGFβ1**
- **PDGF**
- **Angiopoietins 1 and 2**
- **Matrix remodeling and associated signals and mediators (urokinase uPA), uPAR, MMP9, MMP2 and MT MMP).**
- **Bile acids**
- **Serotonin**

Exchange of hepatotrophic factors through parabiotic circulation.

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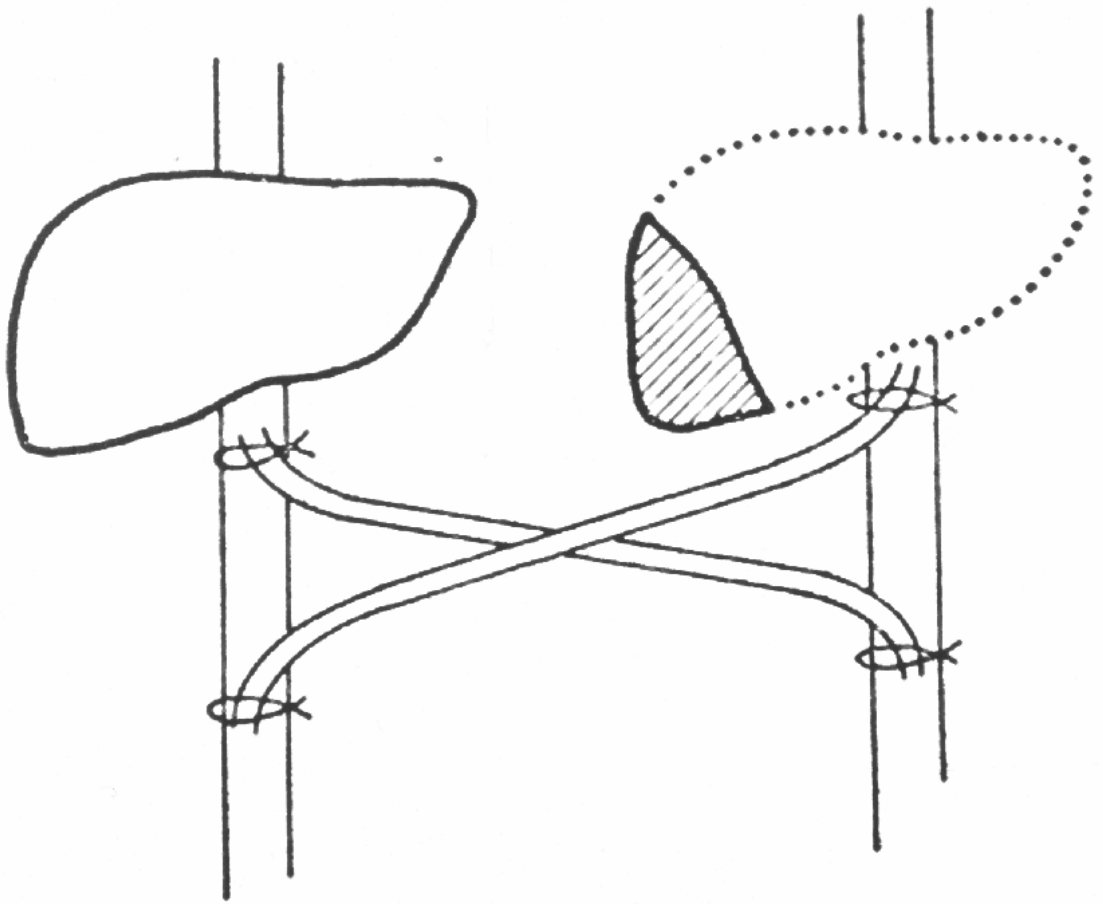
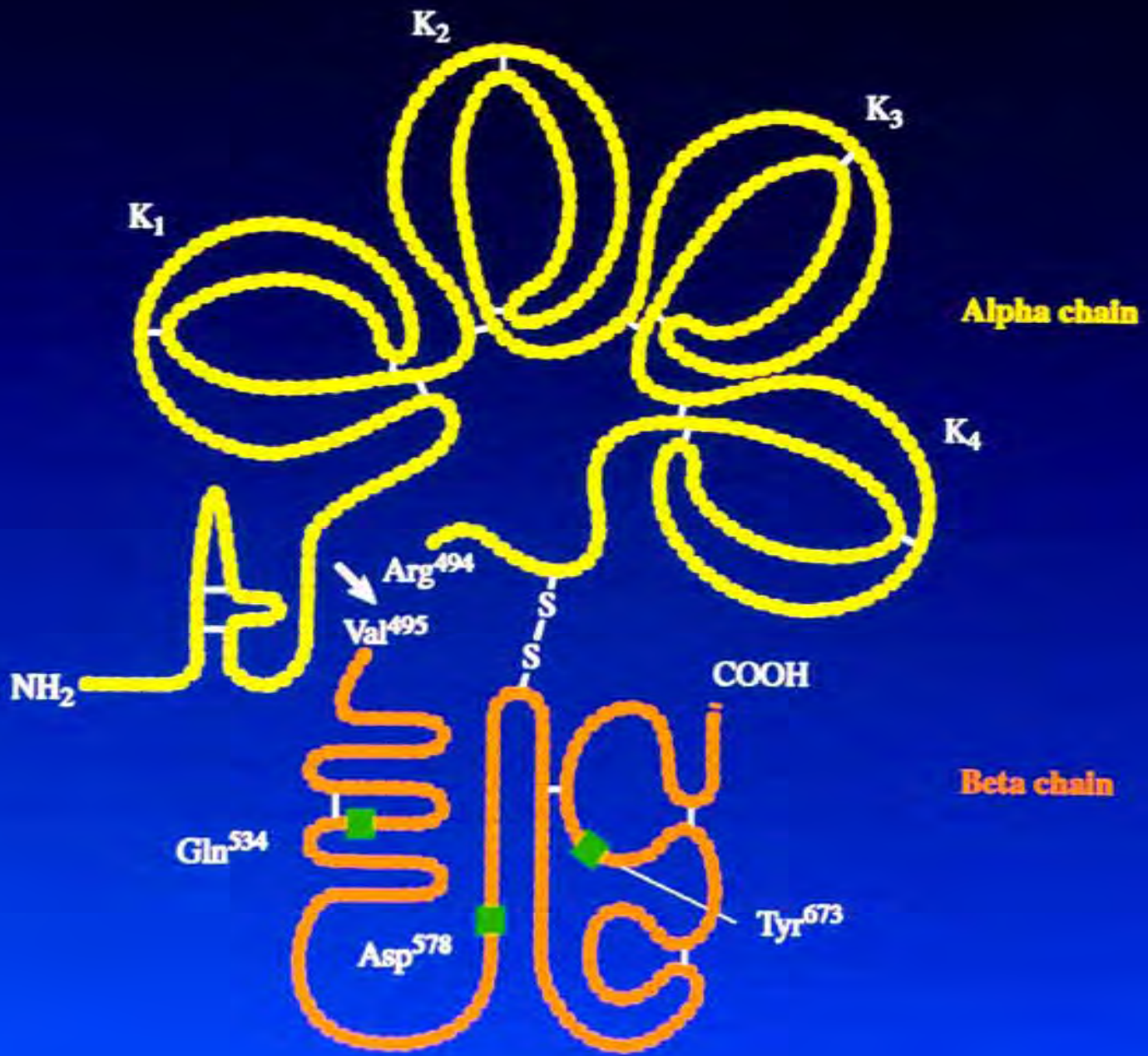


Fig. 1. Diagram of the basic experimental procedure: partial hepatectomy in a rat connected to a partner by chronic direct cross-circulation.

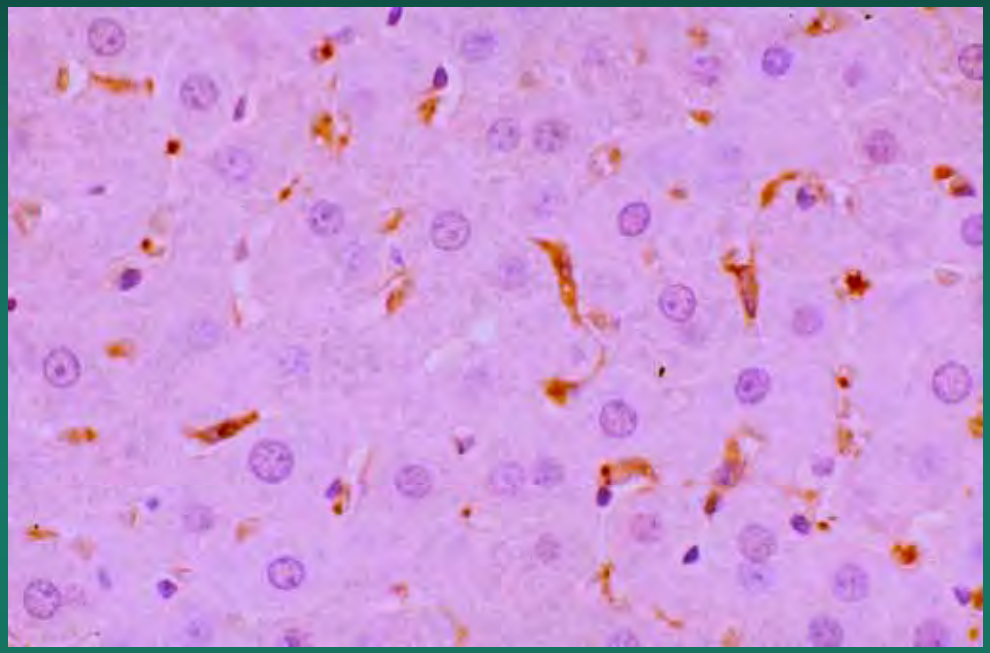


HGF structure cartoon

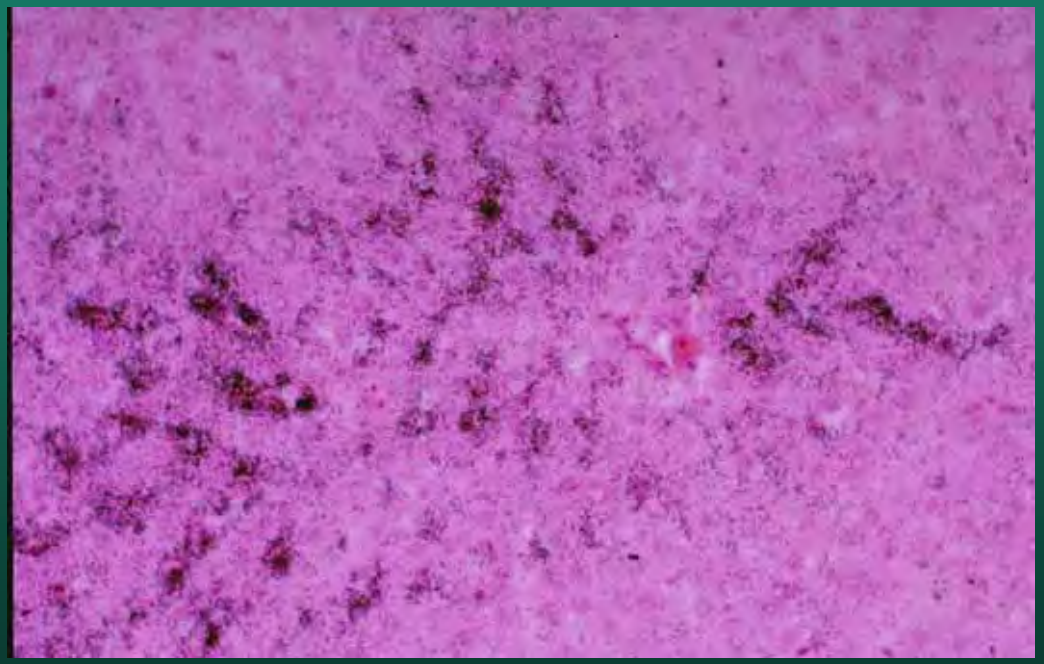
HGF as:

Mitogen
Motogen
Morphogen

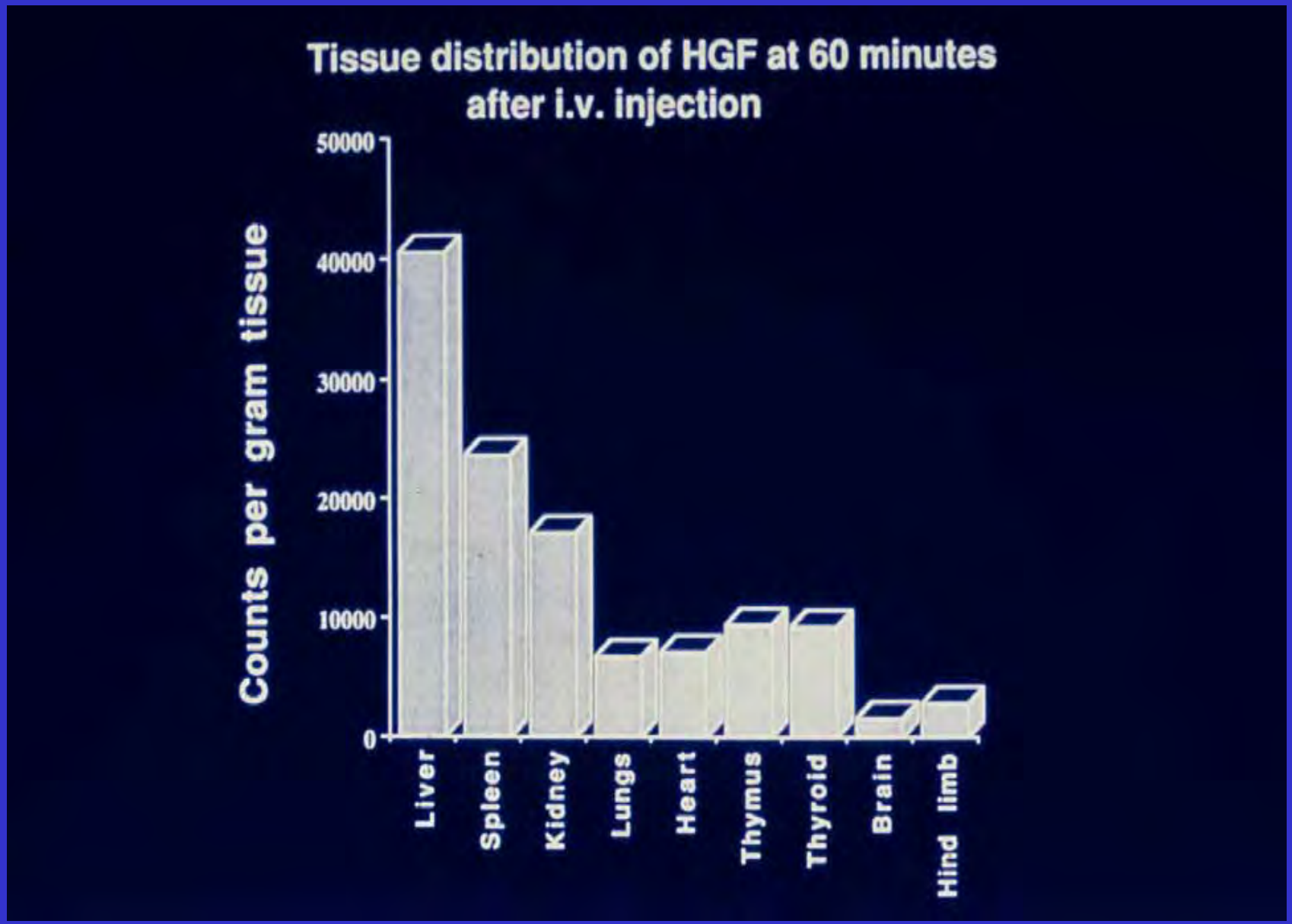
HGF in hepatic stellate cells



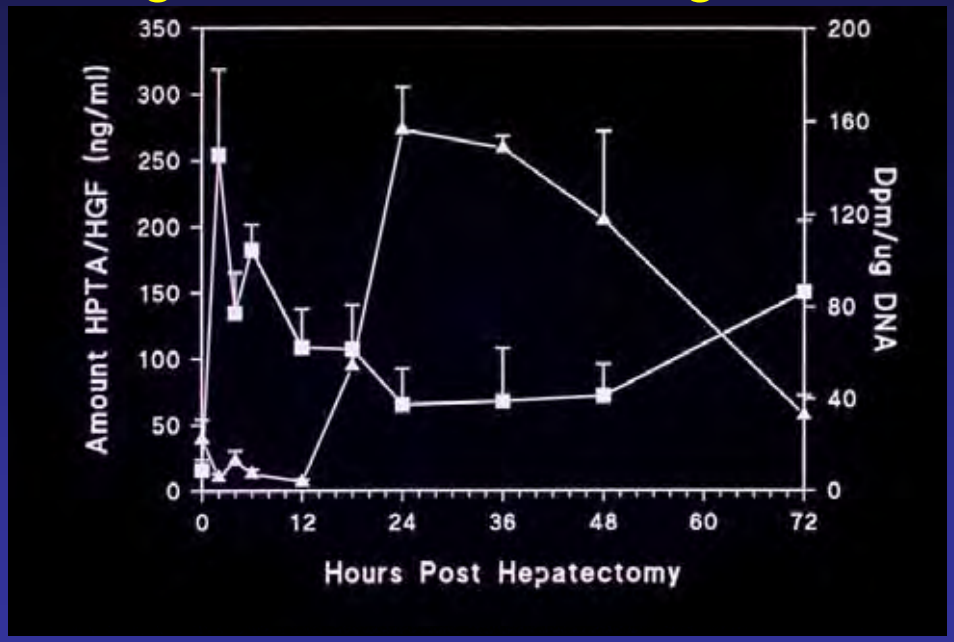
HGF is sequestered in periportal matrix after systemic injection.

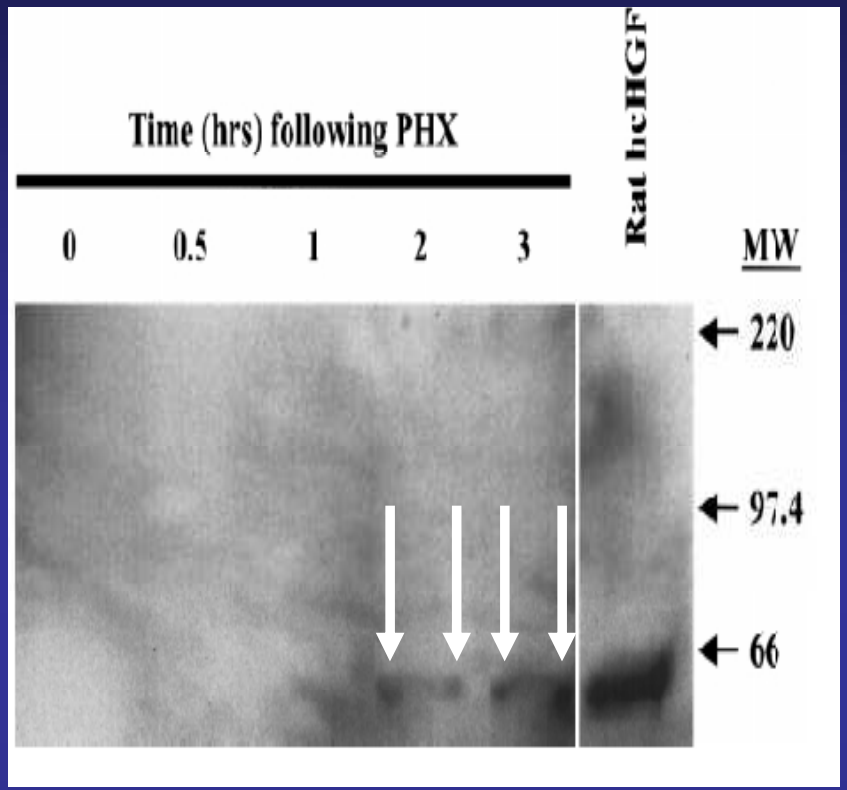


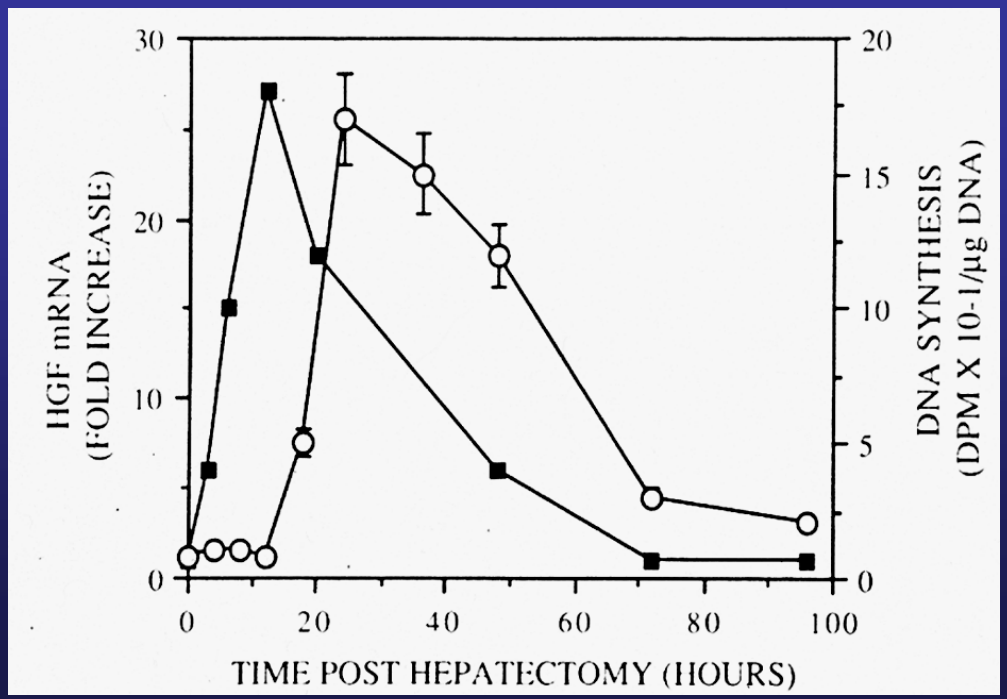
Sequestration of systemically injected 135I-HGF in tissues.



HGF was isolated from plasma of hepatectomized rats as the mitogenic substance rising in the blood after partial hepatectomy.

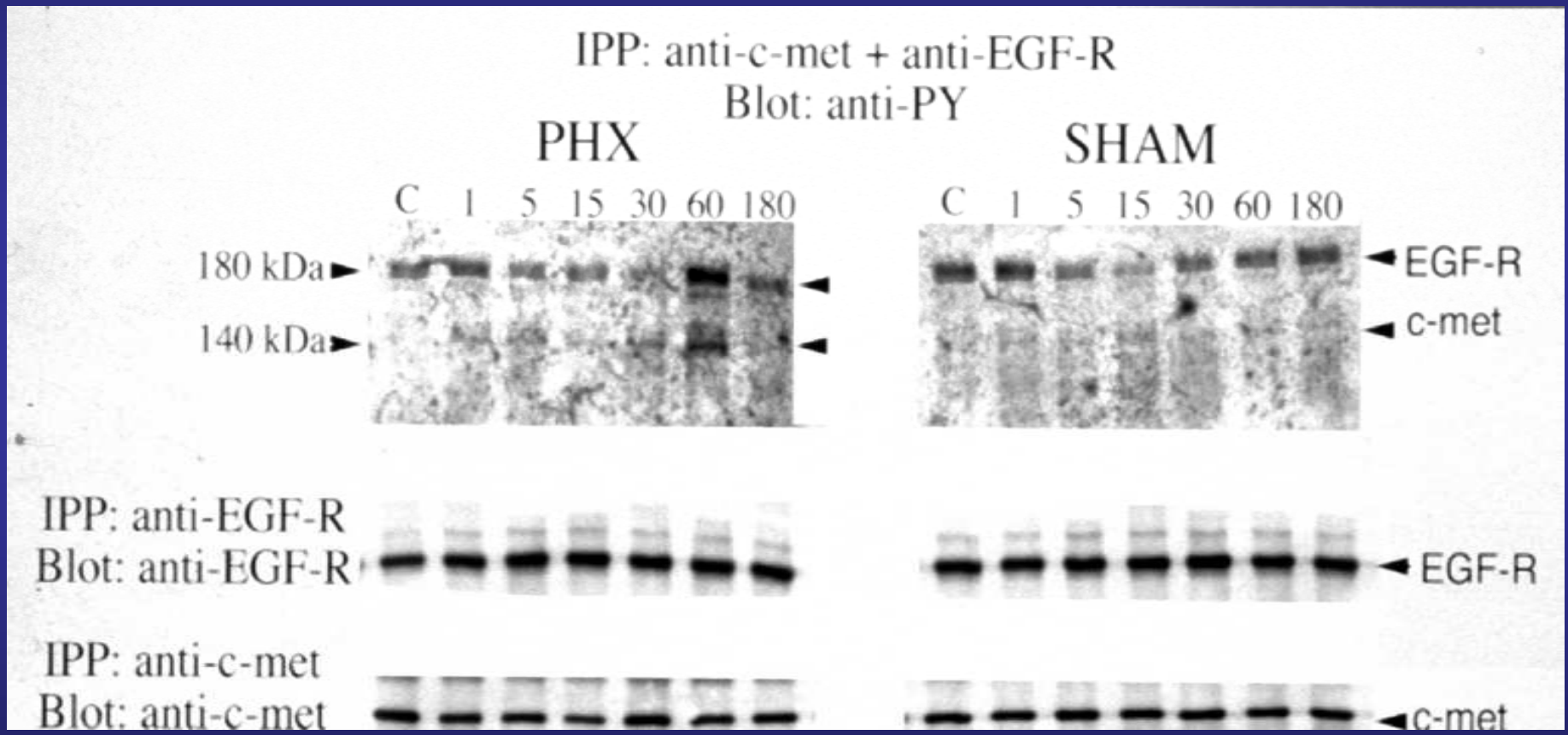




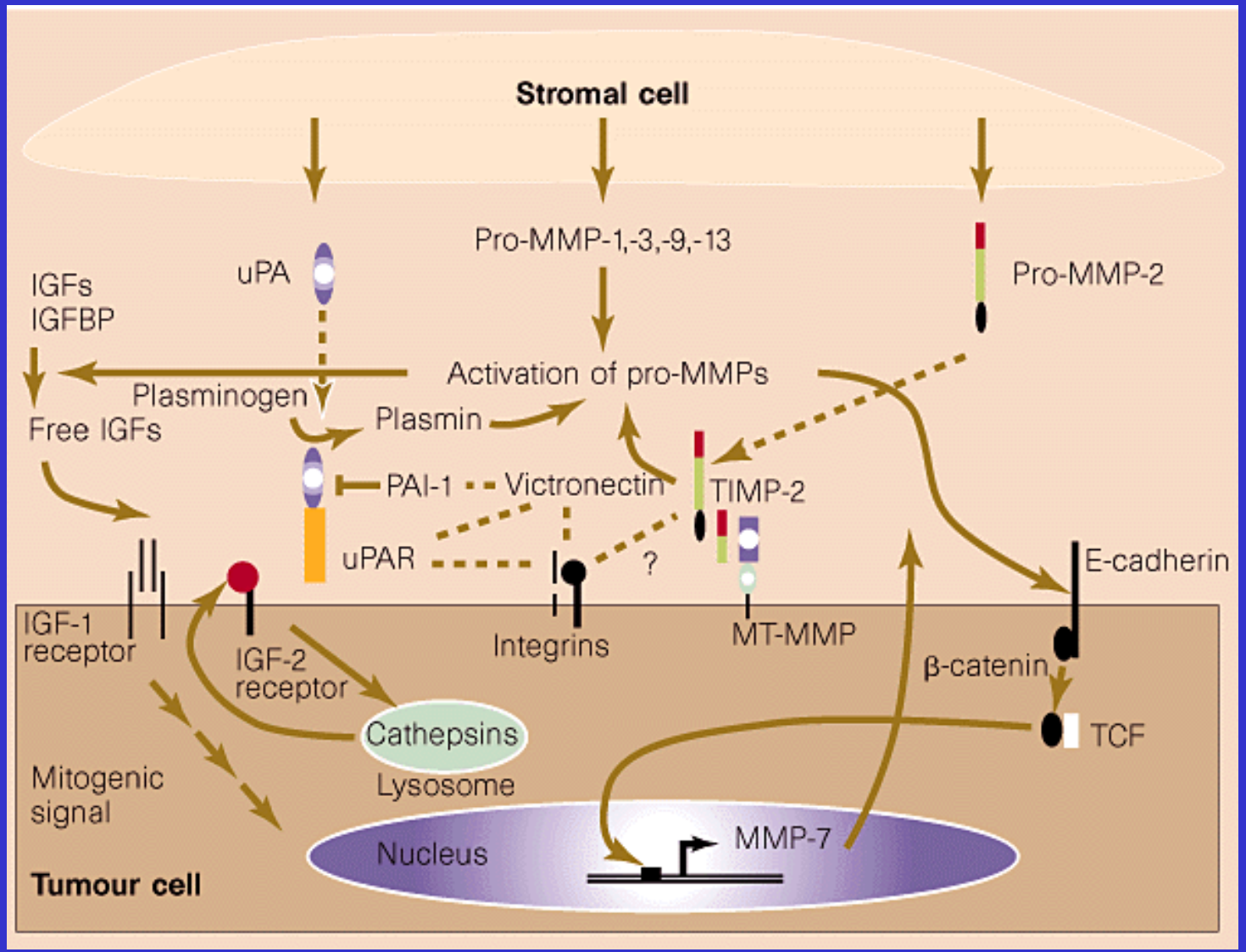


HGF in plasma increases 20-fold within 1 hour.
HGF mRNA expression in liver and lung starts at 3 hours peaking at 20-30 hours.
Where does the plasma HGF come from?

Tyrosine phosphorylation of Met and EGFR after PHx.

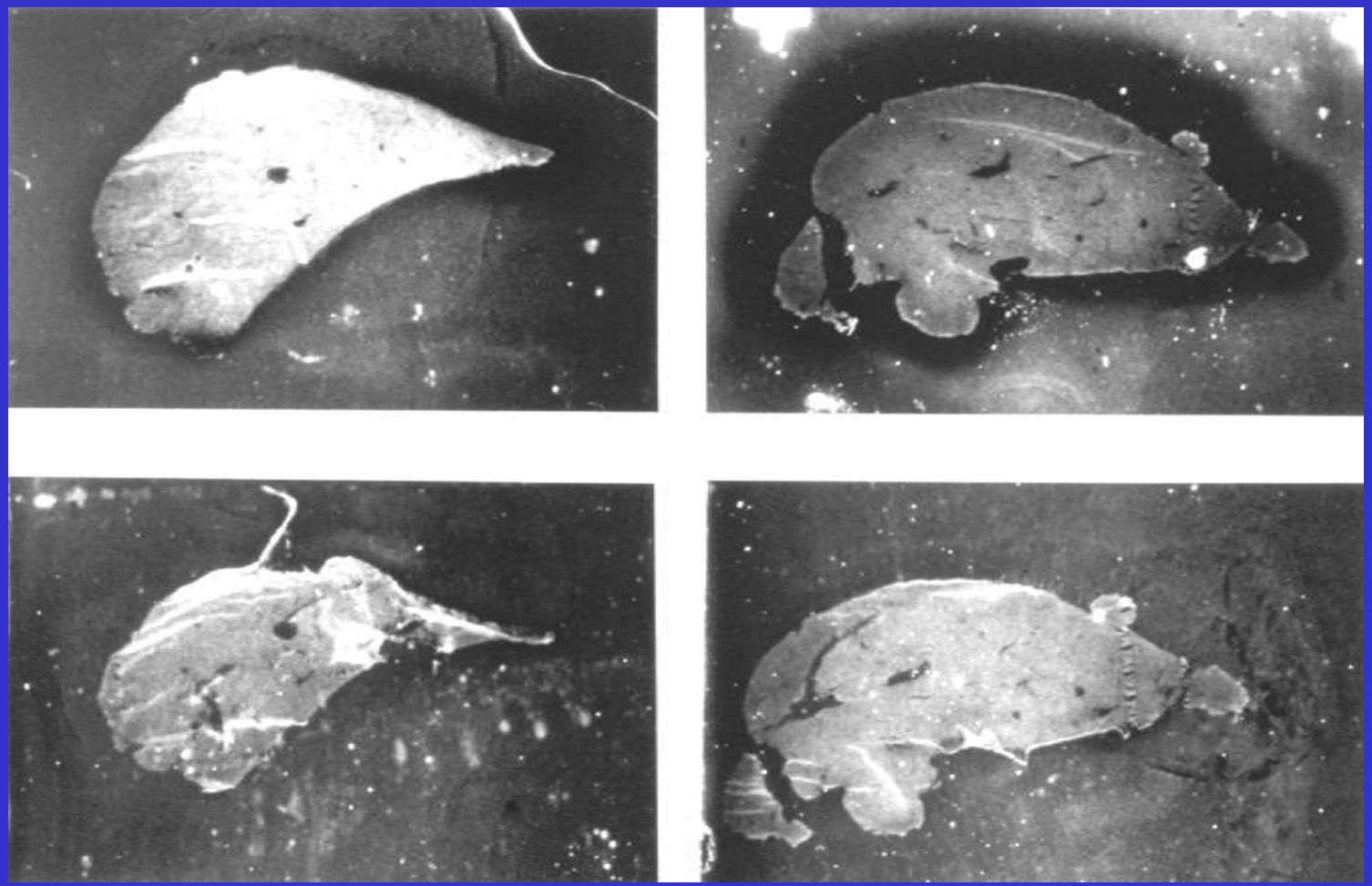


Proteolytic cascades involved in matrix remodeling.



uPA activity increases rapidly following partial hepatectomy.

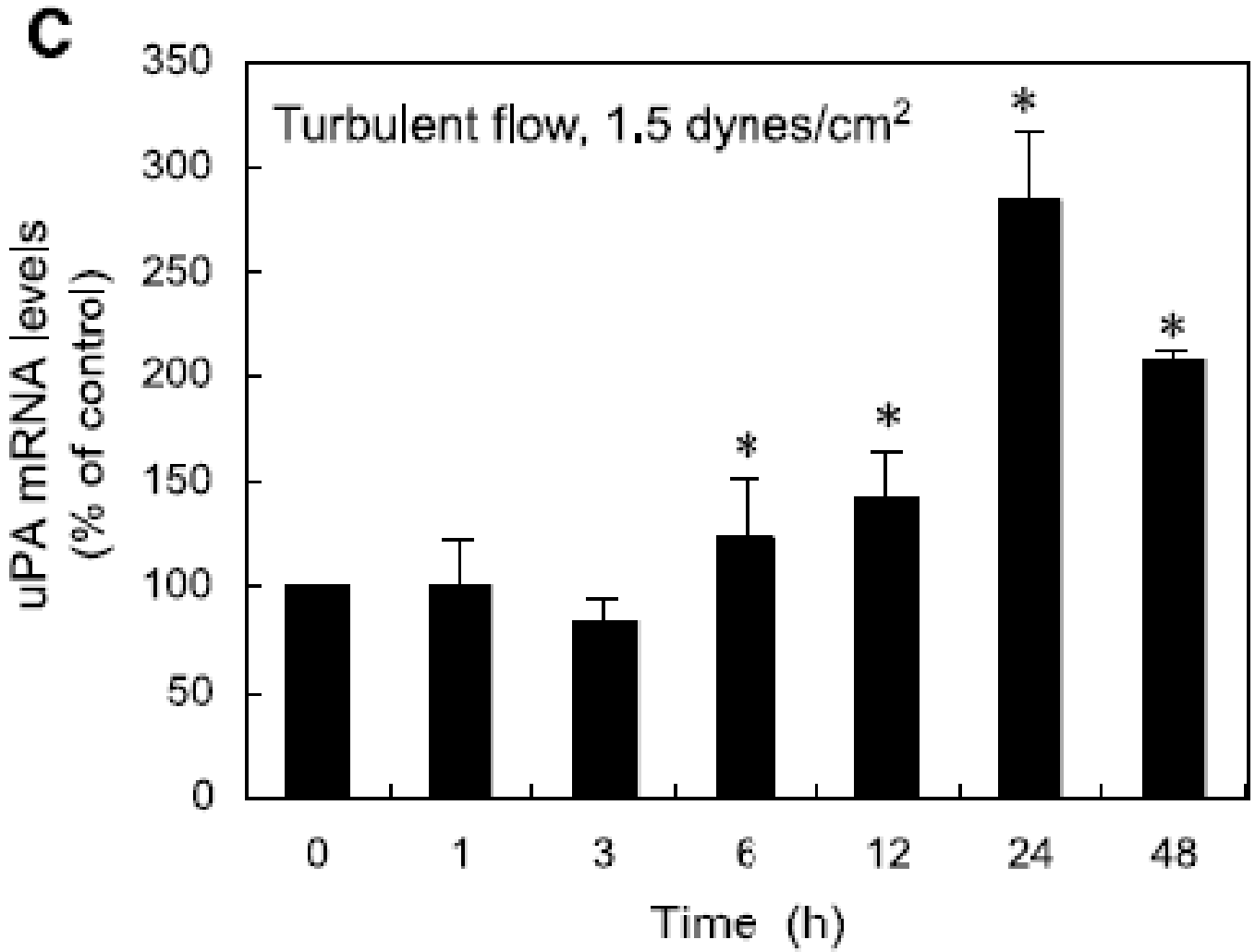
Normal Liver **One minute after PHx**



-anti uPA

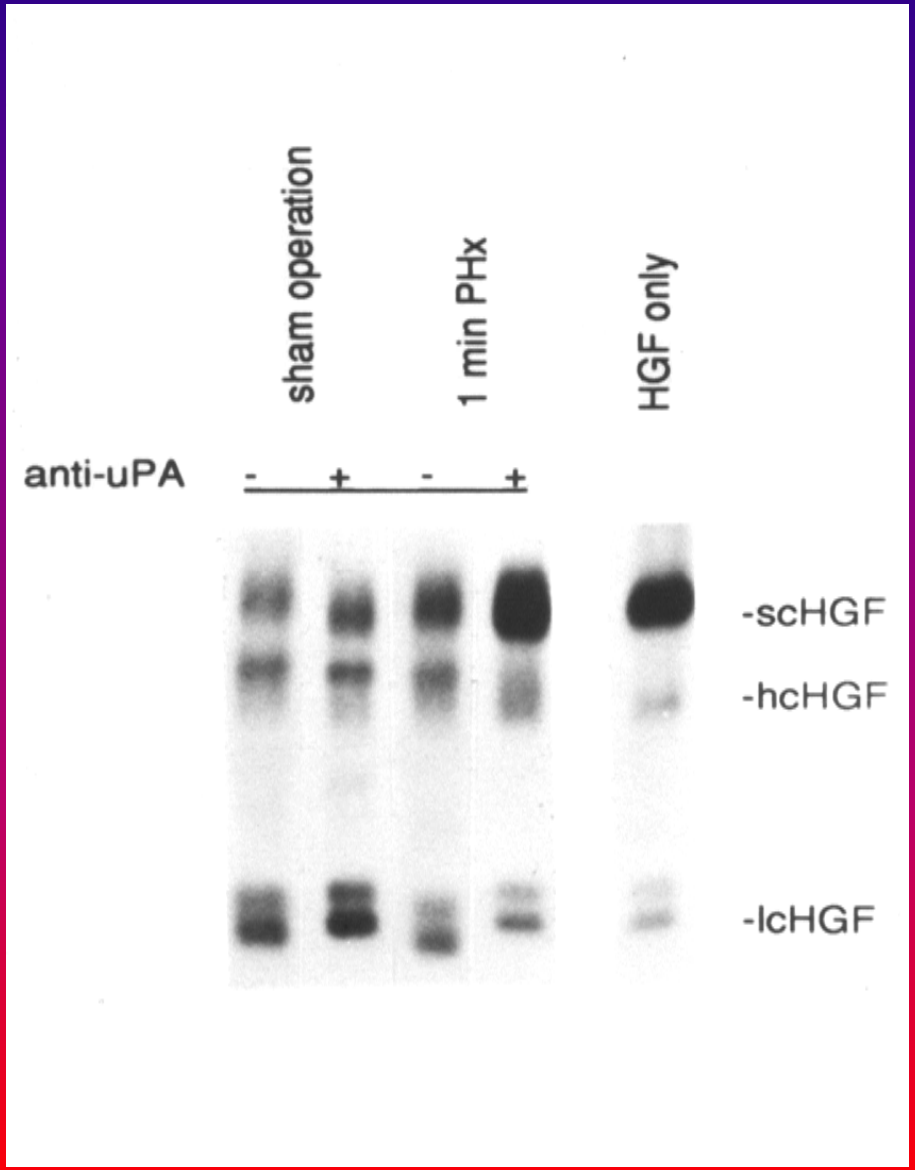
+ anti uPA

Effects of shear stress on urokinase-type plasminogen activator (uPA) mRNA levels in human coronary artery endothelial cells (HCAECs).



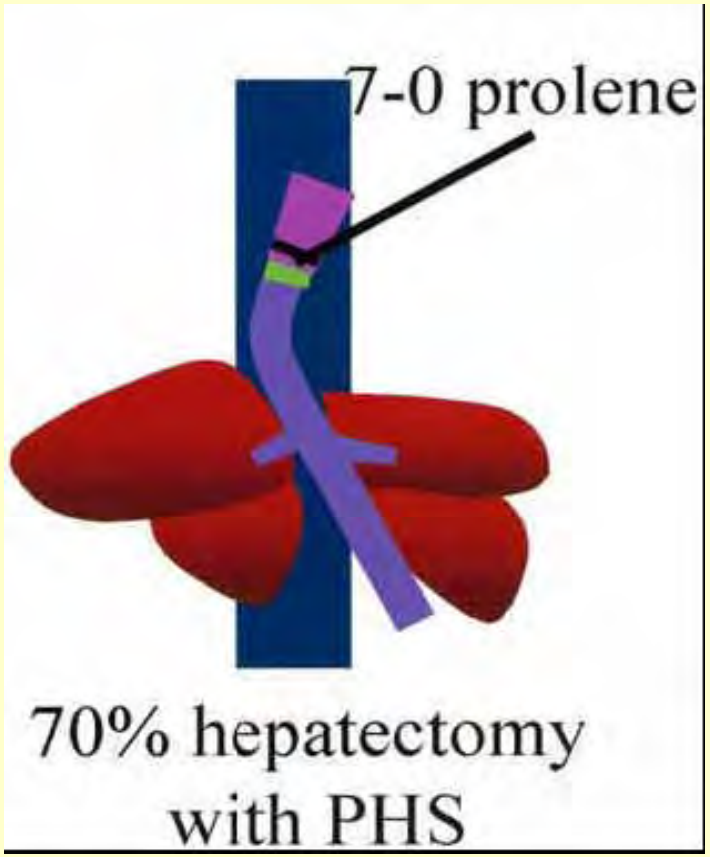
Takaaki Sokabe, Kimiko Yamamoto, Norihiko Ohura, Hideki Nakatsuka, Kairong Qin, Syotaro Obi, Akira Kamiya, and Joji Ando. Differential regulation of urokinase-type plasminogen activator expression by fluid shear stress in human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol 287: H2027–H2034, 2004.

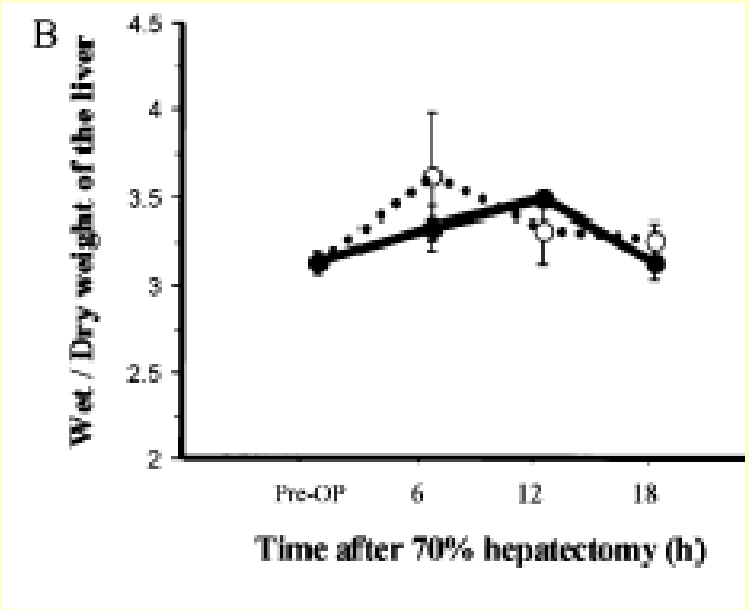
Urokinase activates scHGF to tcHGF after hepatectomy.

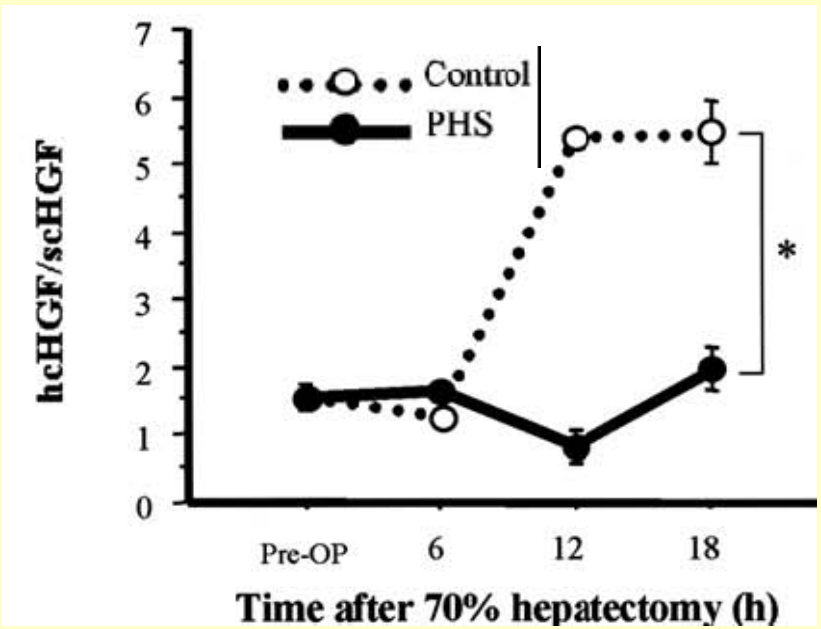


Effect of portal hemodynamics on liver regeneration studied in a novel portohepatic shunt rat model.

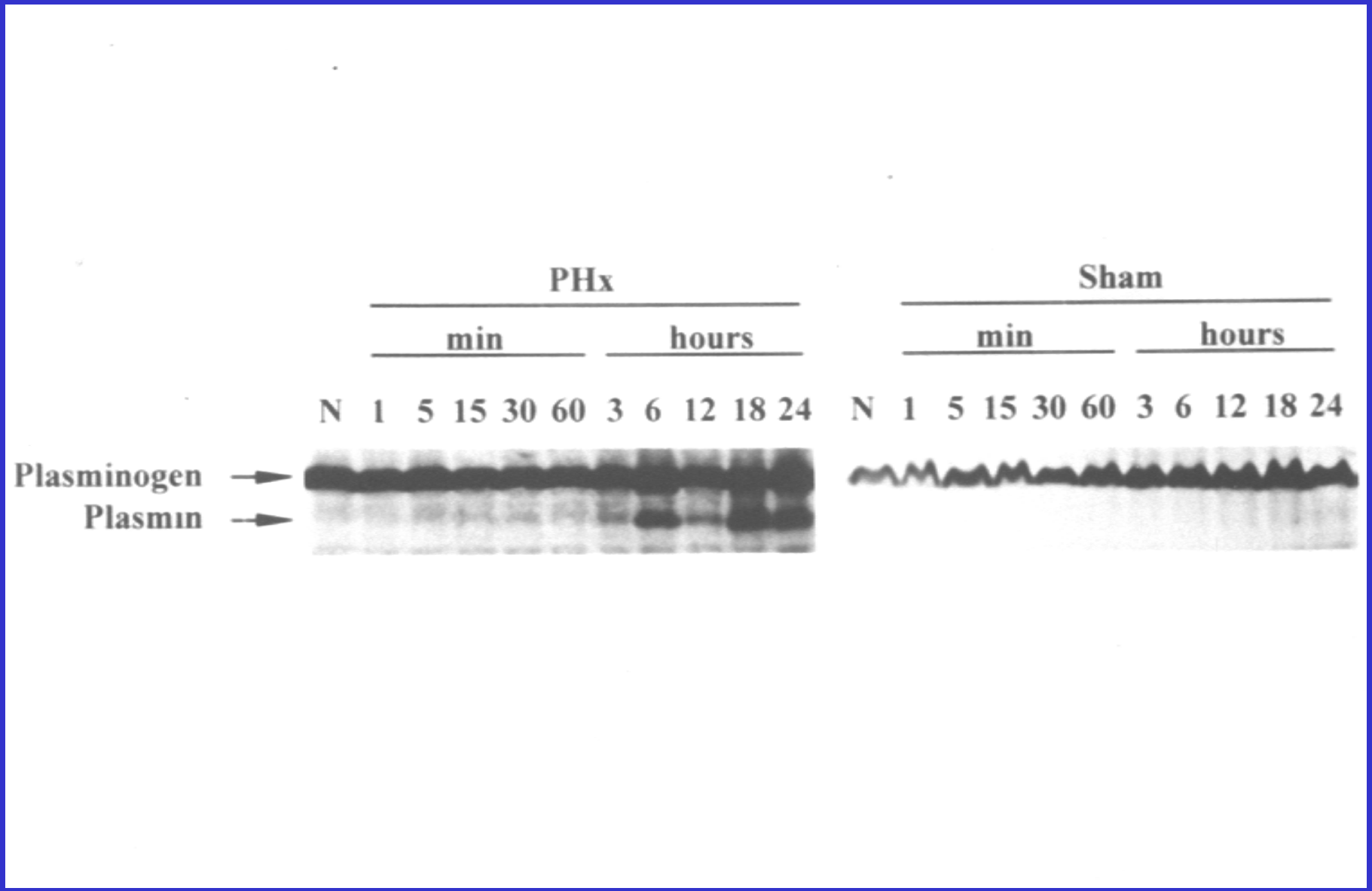
Marubashi et al., *Surgery*, 136:1028-1037, 2004





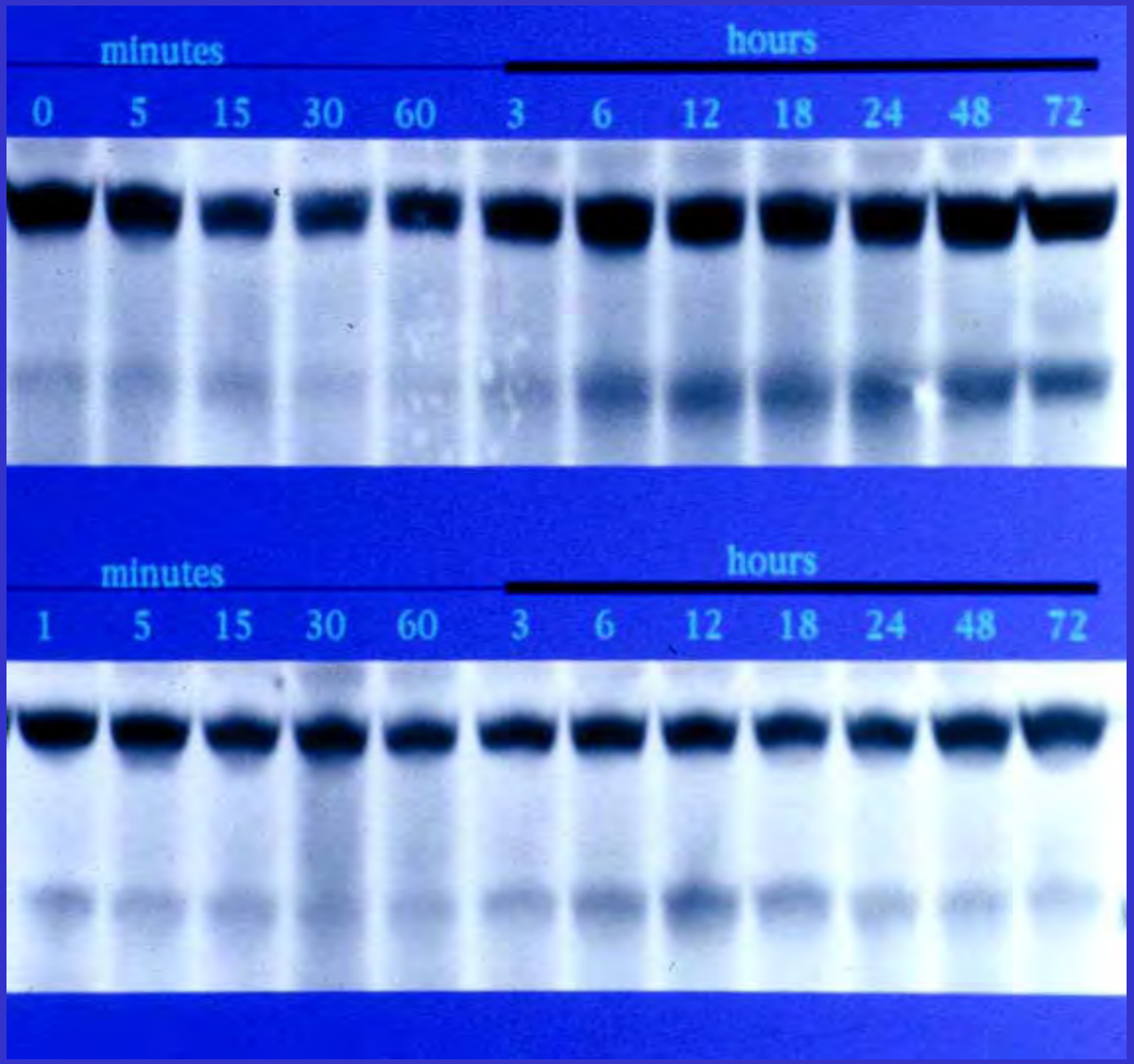


Activation of plasminogen to plasmin after PHx



scHGF and tcHGF during liver regeneration after partial hepatectomy.

PHx



Sham

Our data suggest that there two stages of HGF involvement during liver regeneration:

First phase: Consumption from existing stores in the biomatrix (cMet phosphorylation within 30-60 minutes after PHx).

Second phase: Newly synthesized HGF from stellate cells (and endothelial cells?).

If the first proliferation wave of hepatocytes is driven by preexisting stores of HGF in hepatic biomatrix, what is the role of the newly synthesized HGF?

Loss of HGF receptor cMet targeted to the liver eliminates hepatic regeneration after 2/3 partial hepatectomy

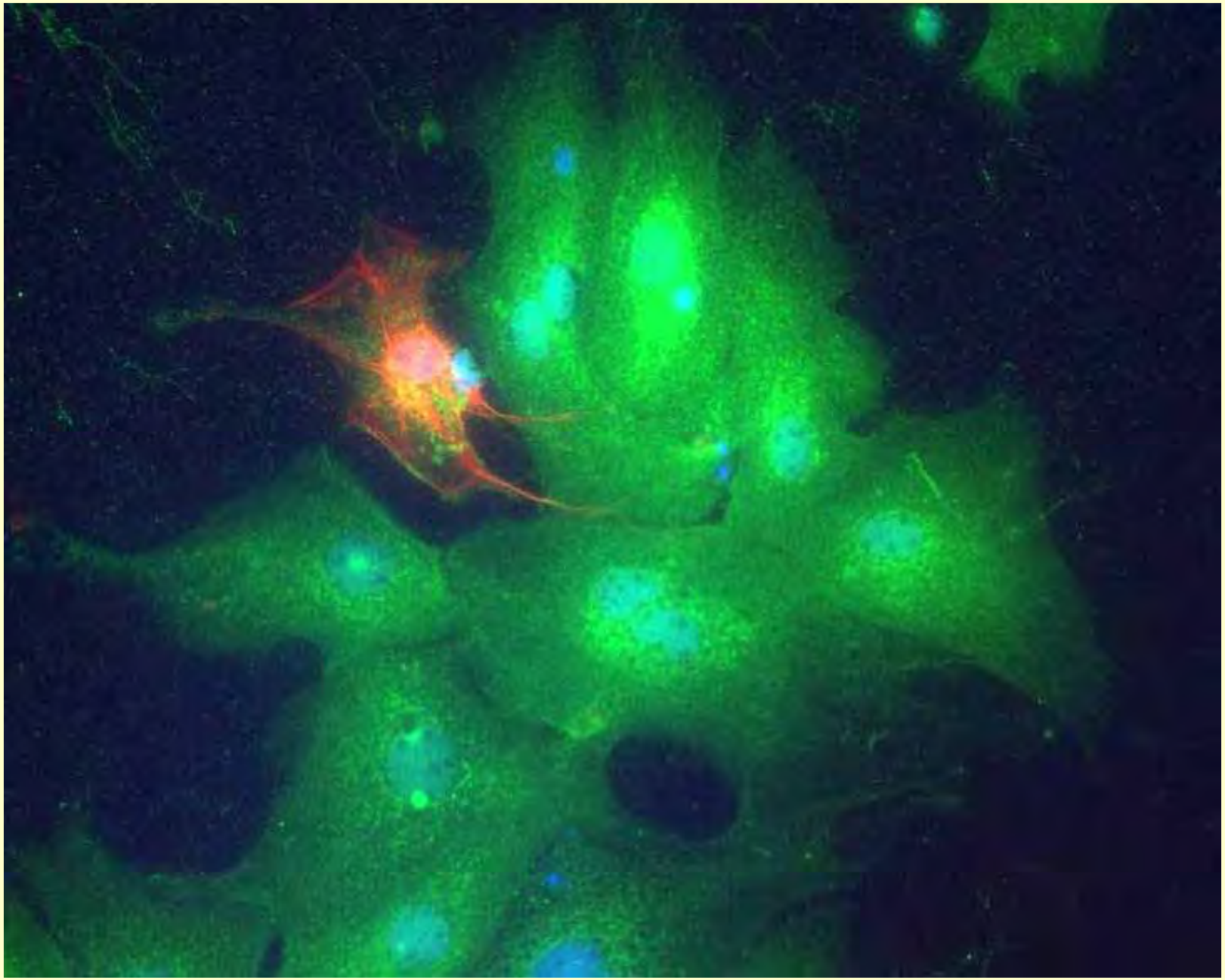
Oe S, Lemmer ER, Conner EA, Factor VM, Leveen P, Larsson J, Karlsson S,Thorgeirsson SS. **Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair.** Proc Natl Acad Sci U S A. 2004 Mar 30;101(13):4477-82.

Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. **Met provides essential signals for liver regeneration.** Proc Natl Acad Sci U S A. 2004 Jul 20;101(29):10608-13. Epub 2004 Jul.

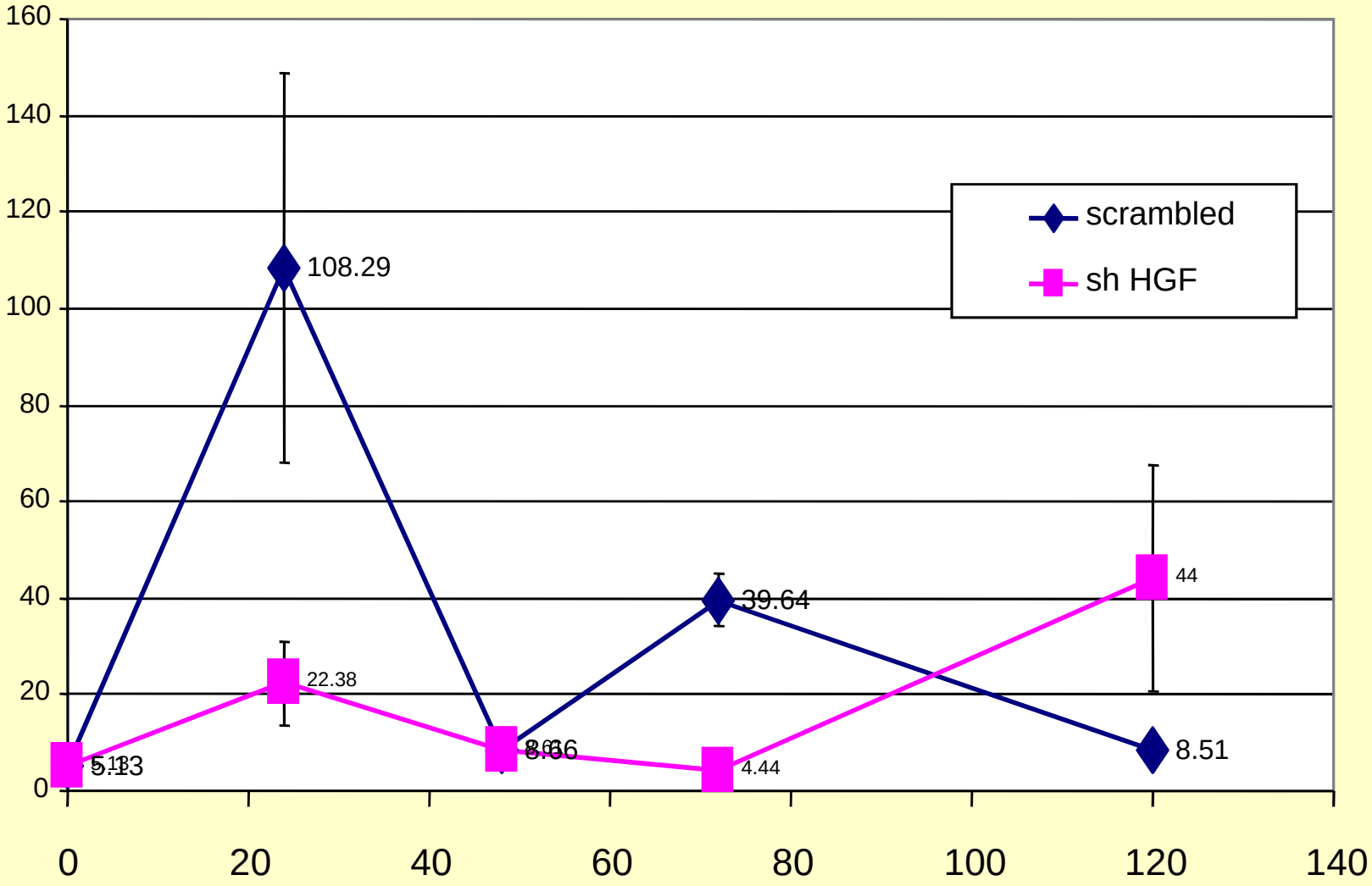
In both of the above protocols, mice had developed fatty changes and fibrosis and steatohepatitis. Not clear whether the defects in regeneration were due to absence of Met or to the secondary histopathology.

A new approach: Knock-down of HGF and c-Met expression by silencing RNA in normal rats immediately before and after partial hepatectomy.

Expression of GFP tag contained into the silencing DNA vector in hepatocytes and stellate cells.



Absolute quantization of HGF in ShHGF treated rats compared to Scrambled RNA treated rats



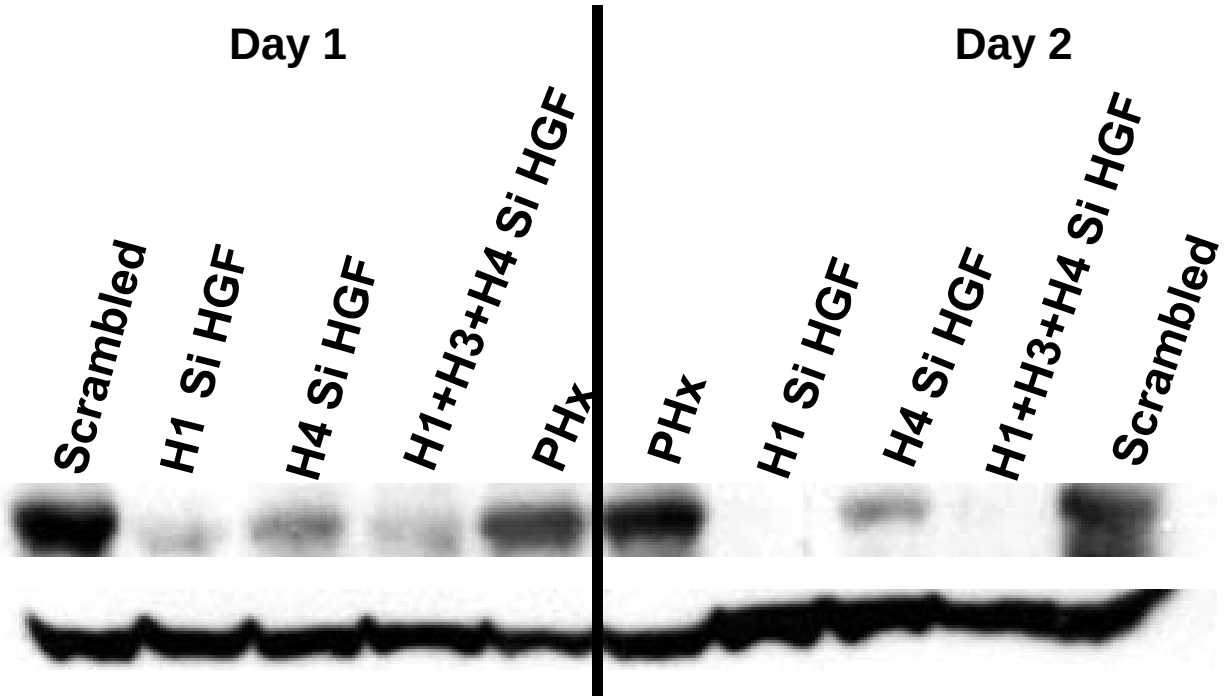
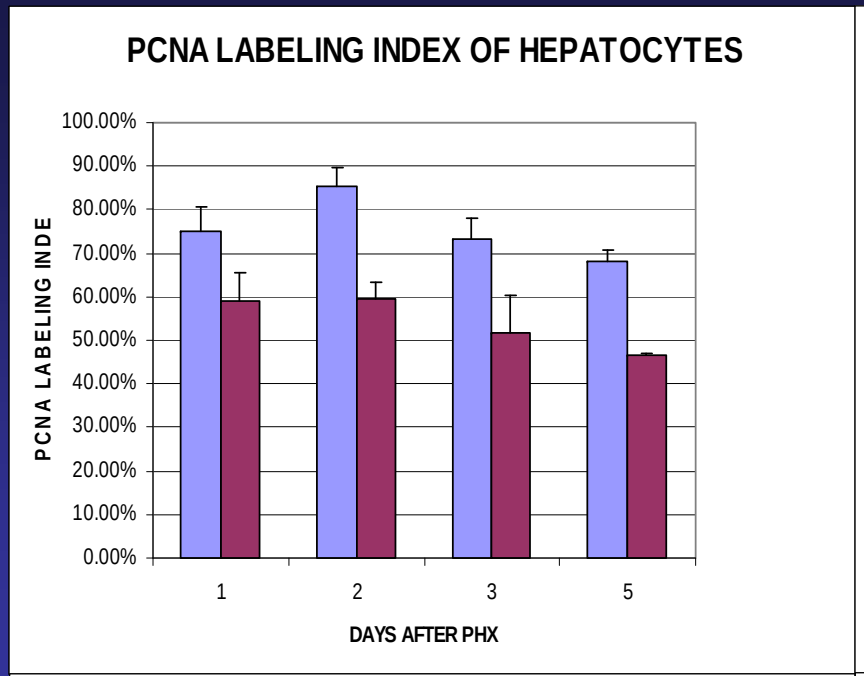
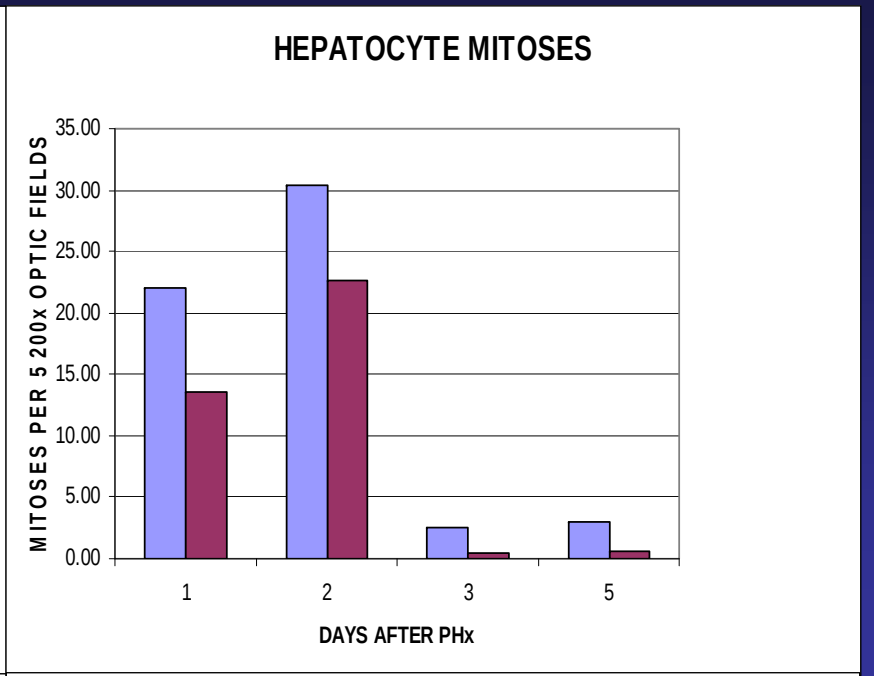
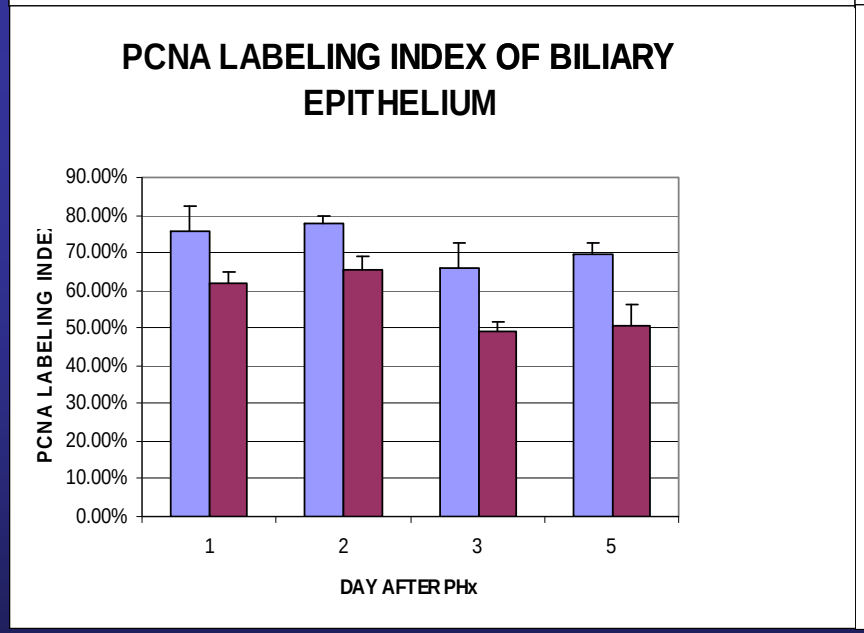
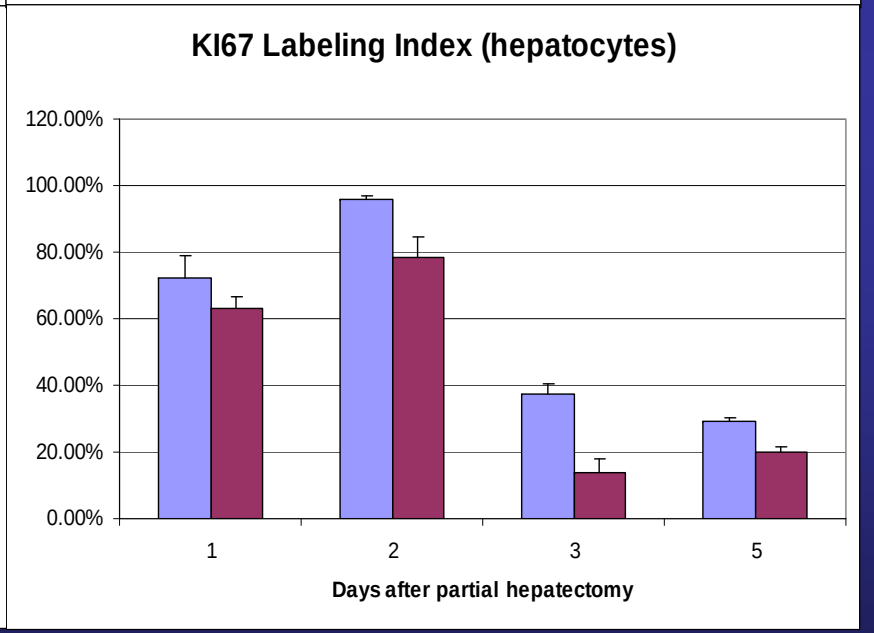


Fig.3B. Western blot analysis of HGF expression in rat liver treated with H1, H4, H1+H3+H4 cocktail and scrambled RNA construct at day 1 and day 2 following PHx. Actin served as loading control.





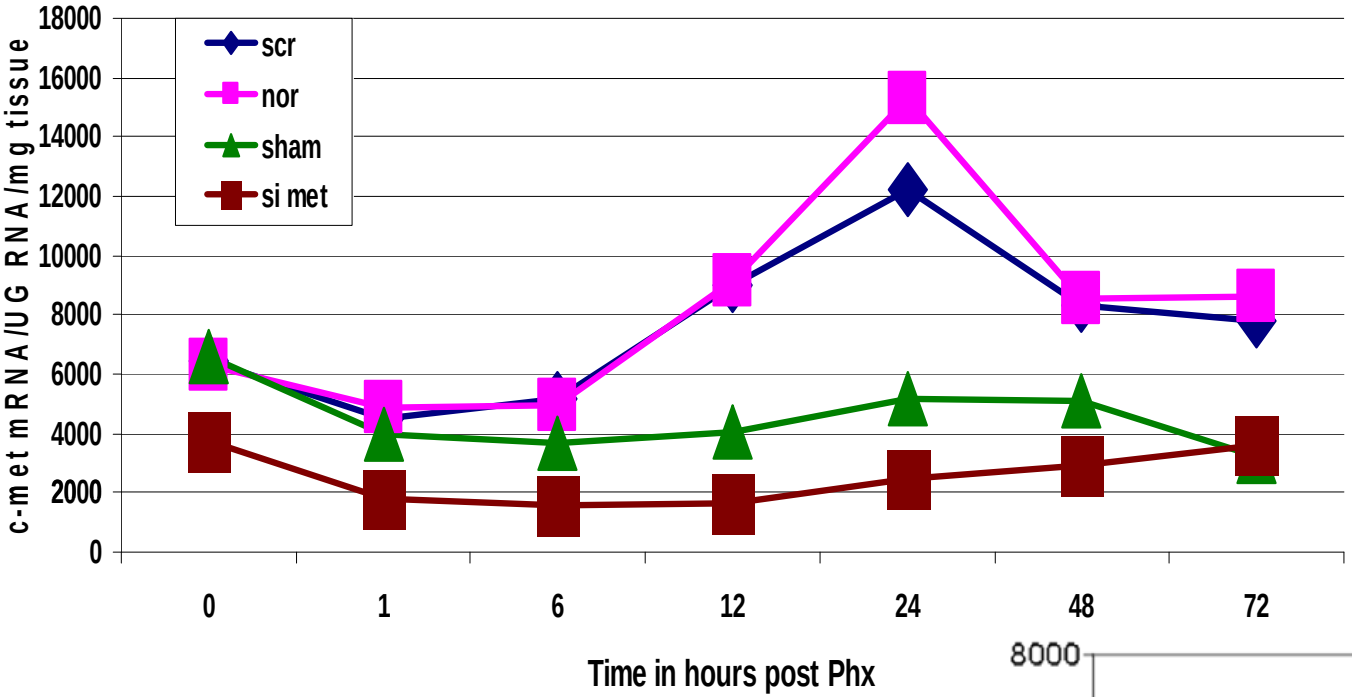




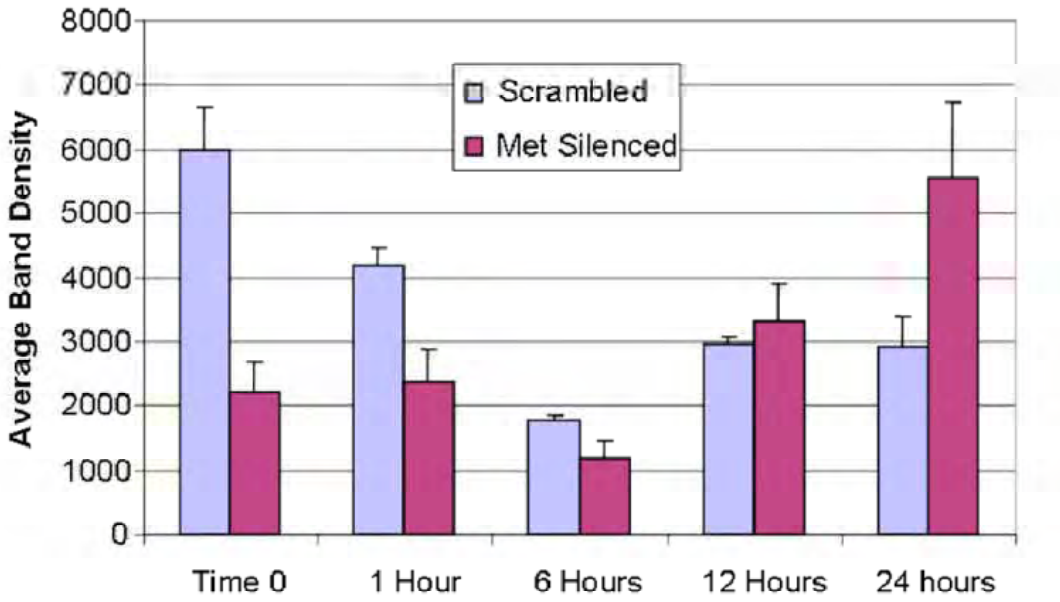
Cellular kinetics in livers of rats treated with either HGF ShRNA or scrambled RNA, followed by PHx

Decrease in c-met after ShMet Treatment

Real Time PCR

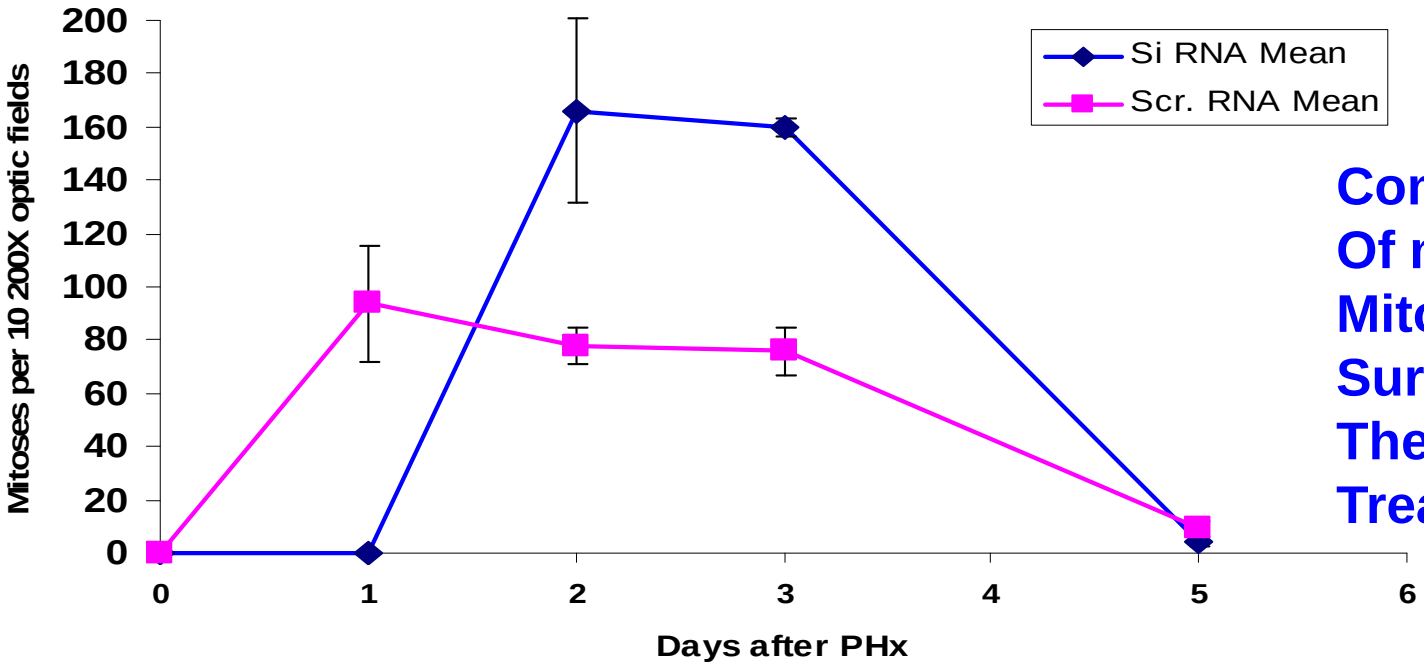


Phospho Met WB

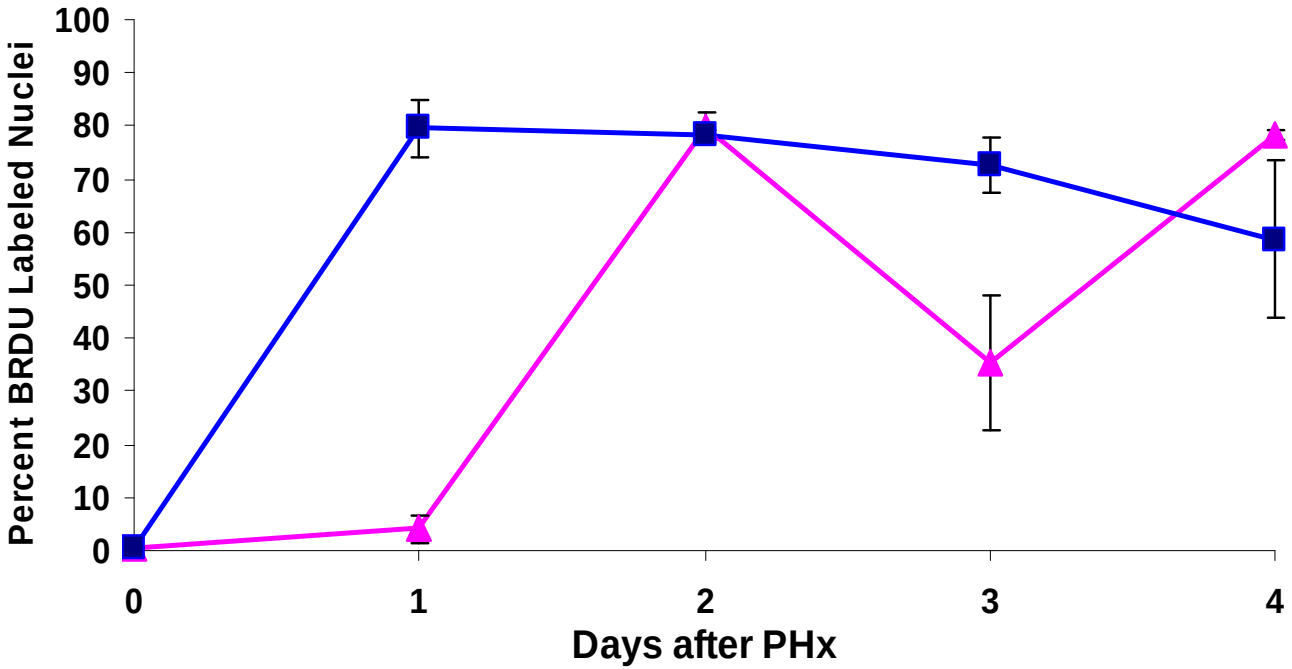


mRNA & protein levels in ShMet treatment

Suppression of Cell Division after shMet treatment



Complete absence
Of mitoses at 24 hours
Mitotic rate at 48 hrs
Surpassed the rate of
The scrambled RNA
Treated rats.



ShRNA: 4%.
Scr RNA: 80%.

Changes in cell cycle related genes following treatment with si-Met, Day 1 after PHx

Silenced Day 1	Scrambled Day 1	Ratio Si/Scr	Gene name
			Cyclins
167.50	733.30	**0.23**	**G1S-SPECIFIC CYCLIN E1**
339.00	760.10	**0.45**	**HUMAN CYCLIN T2**
1050.80	1977.20	**0.53**	**cyclin H**
108.00	115.80	**0.93**	**cyclin-dependent kinase 4**
1823.20	1886.30	**0.97**	**cyclin G-associated kinase**
577.80	523.60	**1.10**	**cyclin L**
235.30	210.90	**1.12**	**cyclin-dependent kinase p130-PITSLRE**
2030.00	1797.00	**1.13**	**cyclin G1**
1895.40	1573.60	**1.20**	**CYCLIN-DEPENDENT KINASES REGULATORY SUBUNIT 2**
1103.90	849.90	**1.30**	**Cyclin dependent kinase inhibitor 2C(p18, inhibits CDK4)**
874.20	668.60	**1.31**	**MOUSE CYCLIN-DEPENDENT KINASE 4 INHIBITOR D**
628.70	423.20	**1.49**	**cyclin-dependent kinase 7**
349.30	232.20	**1.50**	**cyclin-dependent kinase 5**
129.10	78.00	**1.66**	**cyclin-dependent kinase p130-PITSLRE**
2545.40	1356.40	**1.88**	**cyclin-dependent kinase 4**
261.00	136.80	**1.91**	**cyclin D2**
713.40	340.00	**2.10**	**CYCK_MOUSE CYCLIN K**
1474.10	699.50	**2.11**	**RAT G2MITOTIC-SPECIFIC CYCLIN B1**
2293.50	1067.40	**2.15**	**cyclin-dependent kinase regulatory subunit 1**
914.20	406.30	**2.25**	**cyclin D3**
299.60	129.30	**2.32**	**CYCLIN-DEPENDENT KINASE 4 INHIBITOR D**
1128.00	477.30	**2.36**	**CYCLIN D1**
240.10	90.10	**2.66**	**cyclin-dependent kinase p130-PITSLRE**
848.00	278.70	**3.04**	**MOUSE G2MITOTIC-SPECIFIC CYCLIN F**
170.00	38.40	**4.43**	**cyclin-dependent kinase inhibitor 2b**
280.90	58.90	**4.77**	**CYCLIN-DEPENDENT KINASE INHIBITOR 1C**
3205.90	538.60	**5.95**	**cyclin B**
948.70	133.70	**7.10**	**MOUSE CYCLIN I**
2291.40	252.00	**9.09**	**MITOTIC-SPECIFIC CYCLIN B1**
1961.80	106.70	**18.39**	**cyclin B**
4252.70	209.10	**20.34**	**cyclin-dependent kinase inhibitor 3**

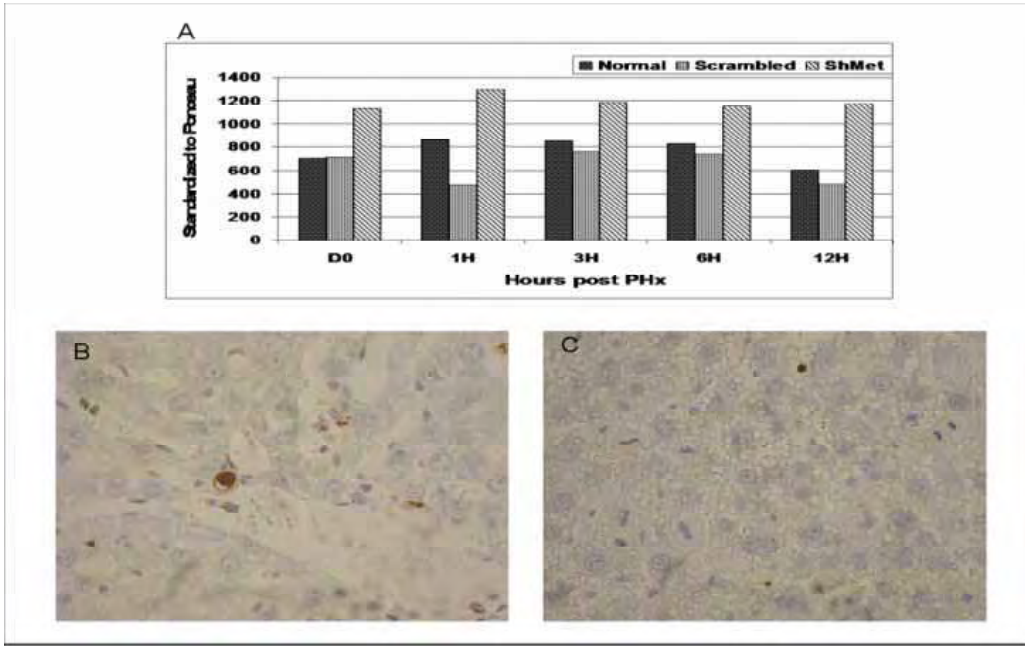
Changes in cell cycle related genes following treatment with si-Met, Day 1 after PHx

Silenced Day 1	Scrambled Day 1	Ratio Si/Scr	Gene name
			p53
507.90	658.50	**0.77**	**p53-activated gene 608**
385.70	393.00	**0.98**	**p53 tumor suppressor-binding protein 1**
339.00	184.50	**1.84**	**p53-binding protein**
272.80	12.10	**22.55**	**Sestrin 1 (p53-regulated protein PA26)**
634.60	16.10	**39.42**	**p53**
733.20	18.60	**39.42**	**p53**
			p21
690.00	1216.20	**0.57**	**p21 (CDKN1A)-activated kinase 1**
498.40	167.00	**2.98**	**p21**

Differentially Expressed Genes at 24 hr after PHx + ShMet

↑	↓
DAPK3	BCL-XL
fas	BCL-A
TGF-beta receptor II	SOD2
Bak 1	Galectin-3
bcl10	PCNA
Apaf-1	IGFBP1
PDCD4	C/EBP-B
DAXX	Cyclin E1
C/EBP-alpha	
caspase 8	
caspase 7	
caspase 12	
caspase 3	
p53	
Galectin 1	
p21	
stat 1	

Caspase 3 & TUNEL Assay in ShMet Treated & Control Rats

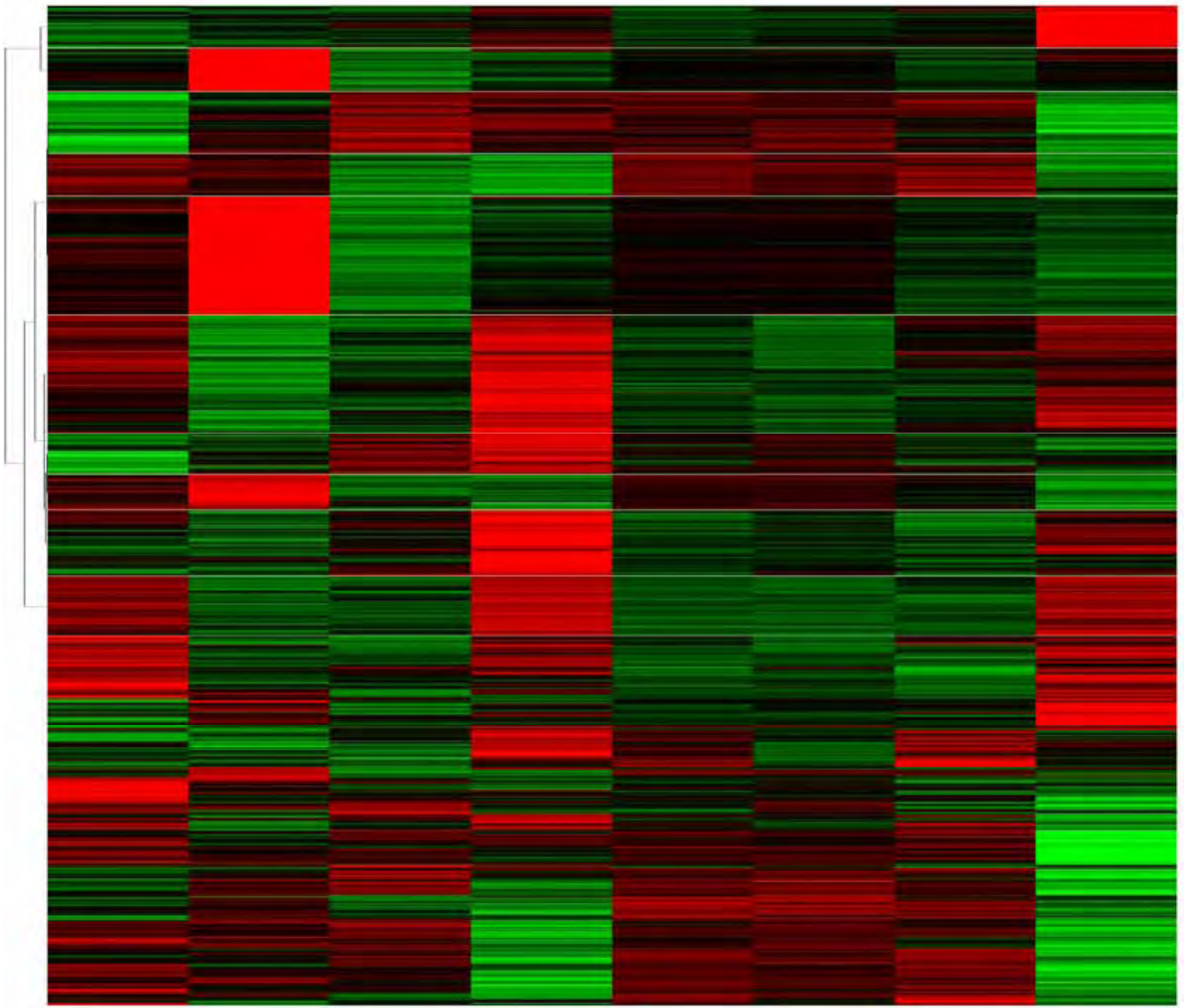


- Suppression of cyclin E1 associated with G1-S progression
- 2.5 fold increase in TUNEL + cells
- Absence of met results in aggregation of fas, activation of caspase 3 and induction of apoptotic pathway

Summary

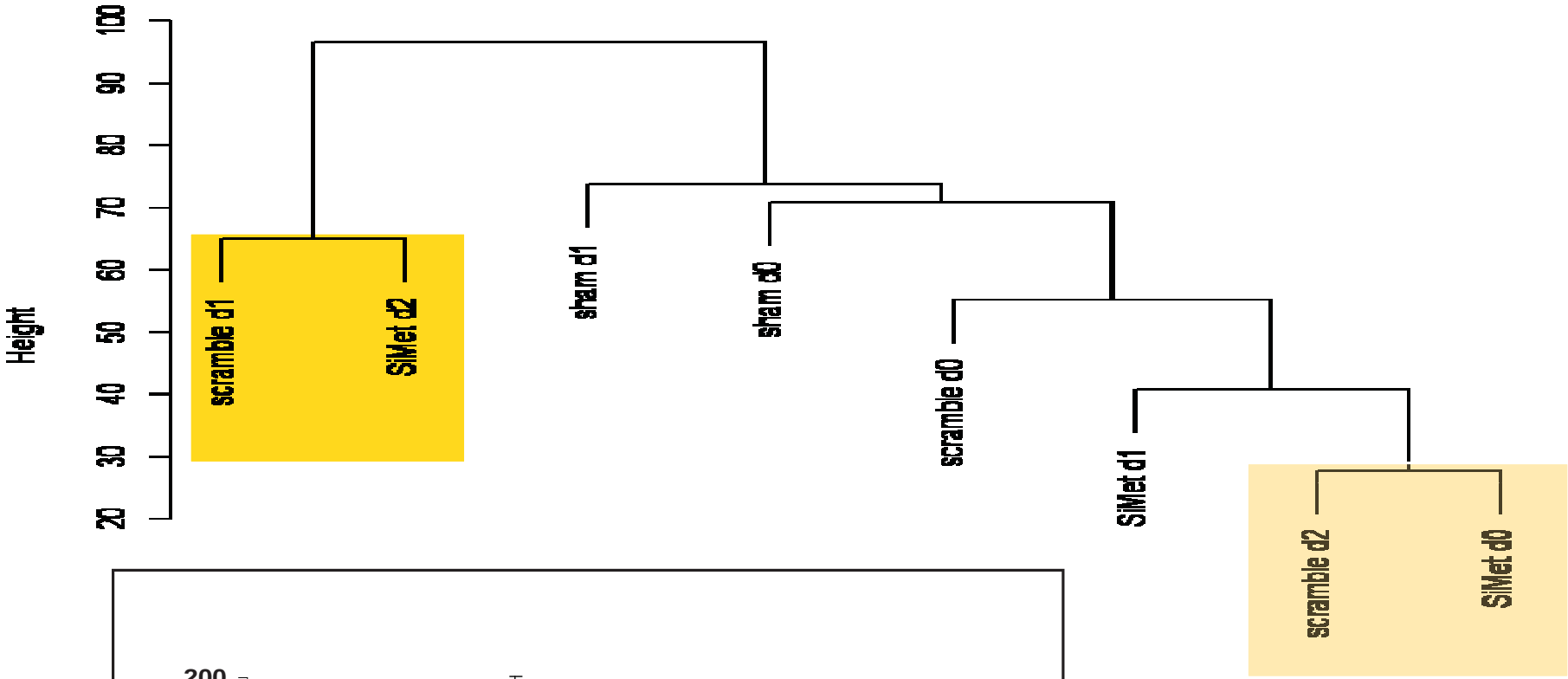
- Dysregulation of many genes involved in cell cycle, stress response & growth regulation after ShMet treatment
- Pro-apoptotic genes like Caspase 3, Apaf 1, Galectin 1, 2 and 9 and Fas were significantly upregulated
- Prosurvival genes like Bcl-xl, SOD 2 and galectin 3 were down regulated
- Some of the genes involved in cell cycle that were upregulated were p21, p53 and C/EBP-alpha while C/EBP-beta was down regulated
- The decrease in the ratio of C/EBP-alpha to C/EBP-beta known to occur after PHx was offset in the animals treated with ShMet RNA

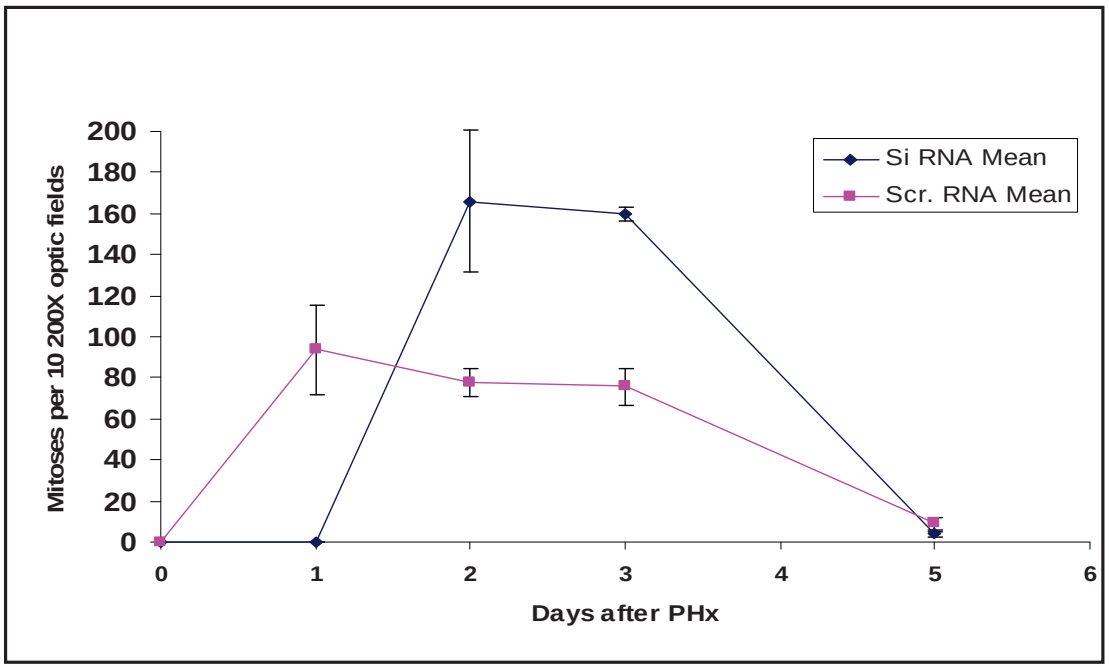
Heat Map



Dendrogram

hierarchical clustering





Genes with similar function cluster together

STANDARD AND UNUSUAL FUNCTIONS OF ACTIVATED HGF RECEPTOR (MET) IN LIVER (AND OTHER TISSUES?)

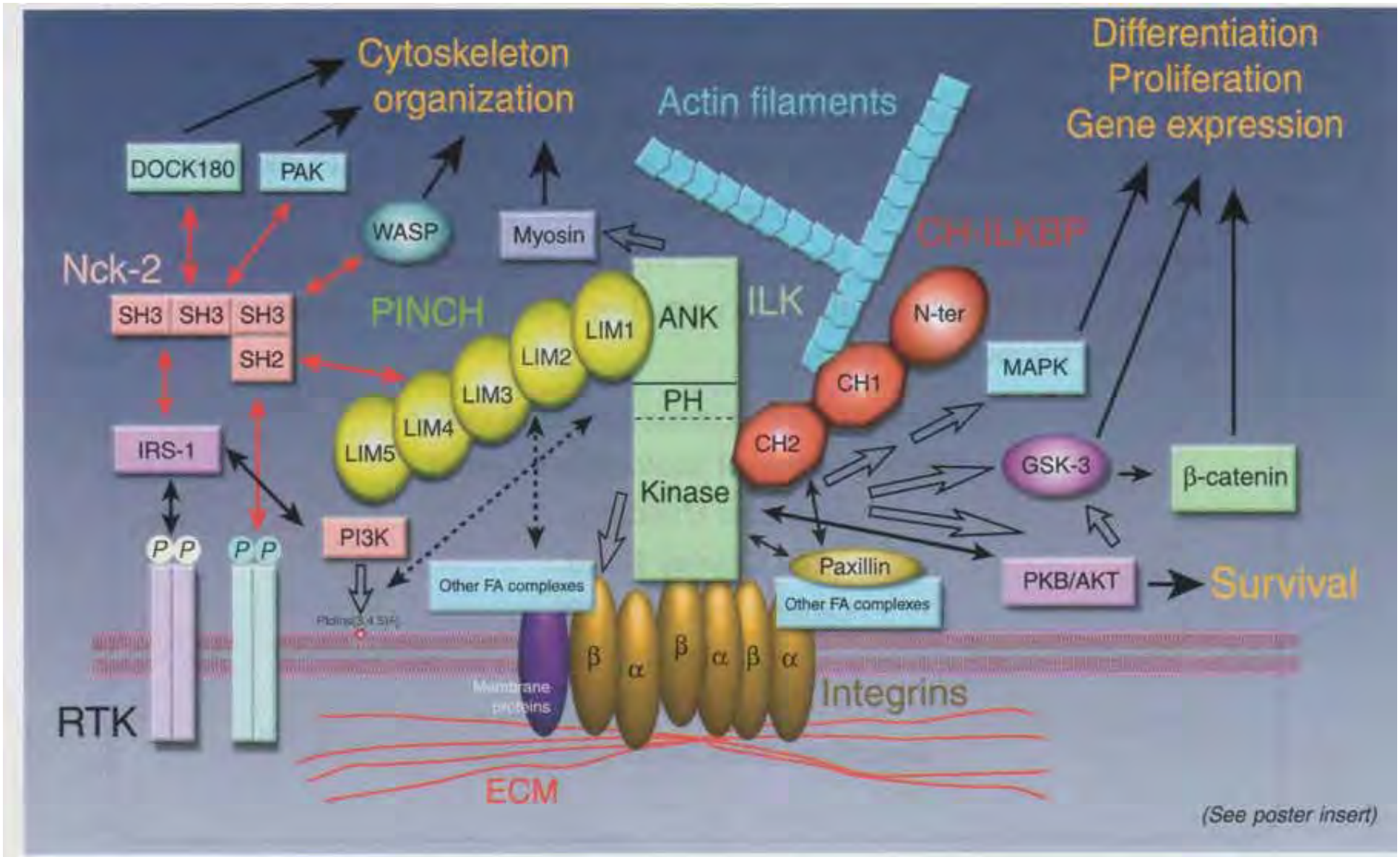
Transduction of signals related to mitogenesis and motogenesis (Ras/MAPK, etc.)
Mobilization of beta catenin to hepatocyte nuclei (in culture; same phenomenon occurs in vivo after PHx. Is Met responsible?)
Dimerization with Fas via YLGA peptide (antiapoptotic effects; SiMet caused activation of caspase 3).
Lung cancer: Dimerization between Met and Erb3 allows tumors to overcome inhibition of Erb1 (EGFR) by specific inhibitory agents.

Integrin Linked Kinase, Extracellular Matrix, Hepatocyte Differentiation, Proliferation and Death

- Interactions between cells and extracellular matrix are mediated by trans-membrane receptors known as integrins.
- Loss of contact with surrounding extracellular matrix often causes altered cell differentiation and/or cell death (anoikis).
- Integrin Linked Kinase (ILK) is a protein which becomes activated by binding to matrix-occupied integrins and transmits specific signaling affecting multiple cellular processes.
- Hepatocytes in culture maintained in the absence of matrix rapidly lose patterns of hepatocyte specific gene expression and characteristic cellular micro-architecture.
- Addition of hydrated complex matrix preparations (Matrigel, Collagen Gel) to un-differentiated hepatocytes restores differentiation and ultra-structure within 3 days.
- Matrix breakdown and reconstitution are essential components of the processes associated with liver regeneration after partial hepatectomy.
- STUDY: Eliminate matrix induced signaling from hepatocytes by liver-targeted genetic elimination of ILK, in mice carrying. These mice were either treated with Adenovirus-Cre or mated with mice expressing Cre recombinase under hepatocyte specific promoters (Albumin promoter, AFP enhancer/Albumin promoter) and endoderm-specific promoter (Foxa3).

- Mouse strains:
  - Promoter Cre mice were provided by Dr. Klaus Kaestner, University of Pennsylvania.
  - ILK $^{FloxP/FloxP}$ mice were provided by Dr. Shoukat Dedhar, University of British Columbia.

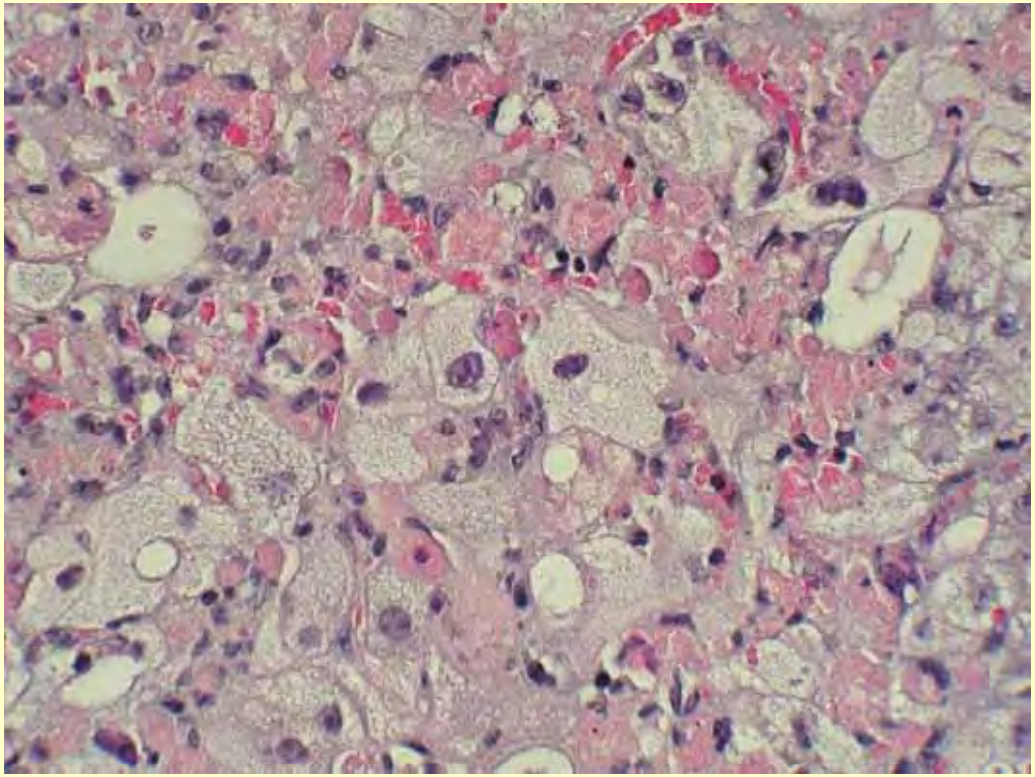
Integrin Signal transduction Pathways



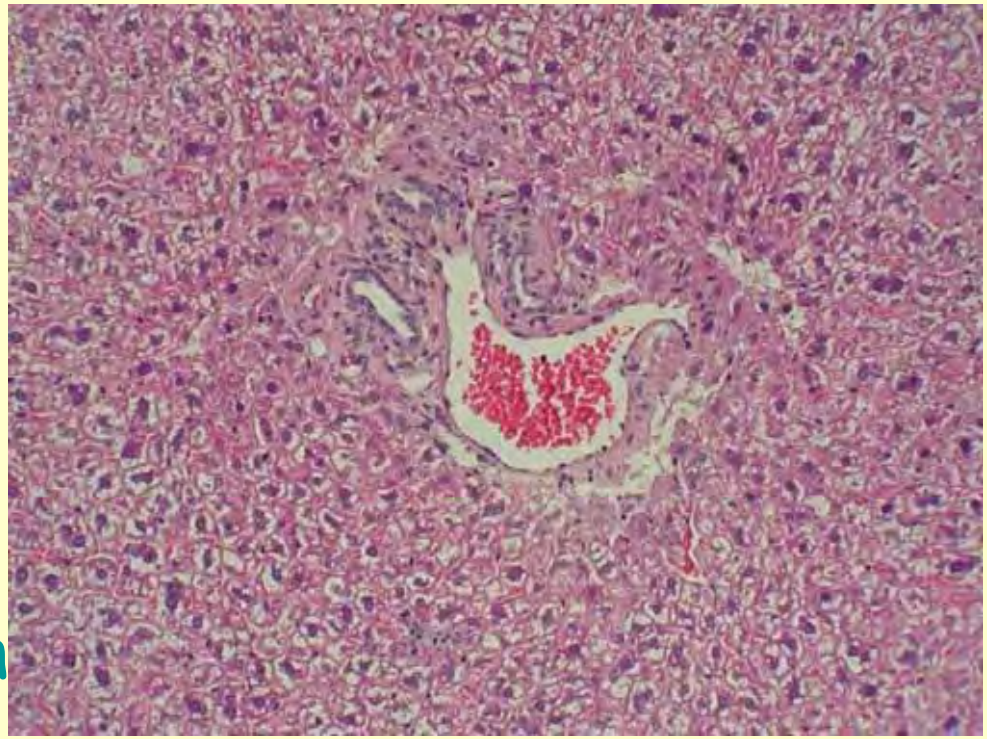
From: Cary Wu J. Cell Science 114:2549, 2001

Integrin Linked Kinase, Extracellular Matrix, Hepatocyte Differentiation, Proliferation and Death

- Adenovirus expressing Cre recombinase was added to **hepatocyte cultures** from control (ILK $^{FloxP+/FloxP-}$) and ILK $^{FloxP+/FloxP+}$ mice. Control hepatocytes were essentially unaffected, whereas hepatocytes from ILK $^{FloxP+/FloxP+}$ mice rapidly went into apoptosis.
- Adenovirus expressing Cre recombinase was **injected to control (ILK $^{FloxP+/FloxP-}$) and ILK $^{FloxP+/FloxP+}$ mice**. Control mice were essentially unaffected. Mice in which hepatocyte ILK was removed developed a clinical and histologic picture of massive fulminant hepatitis with large numbers of hepatocytes in apoptosis and compensatory proliferation.

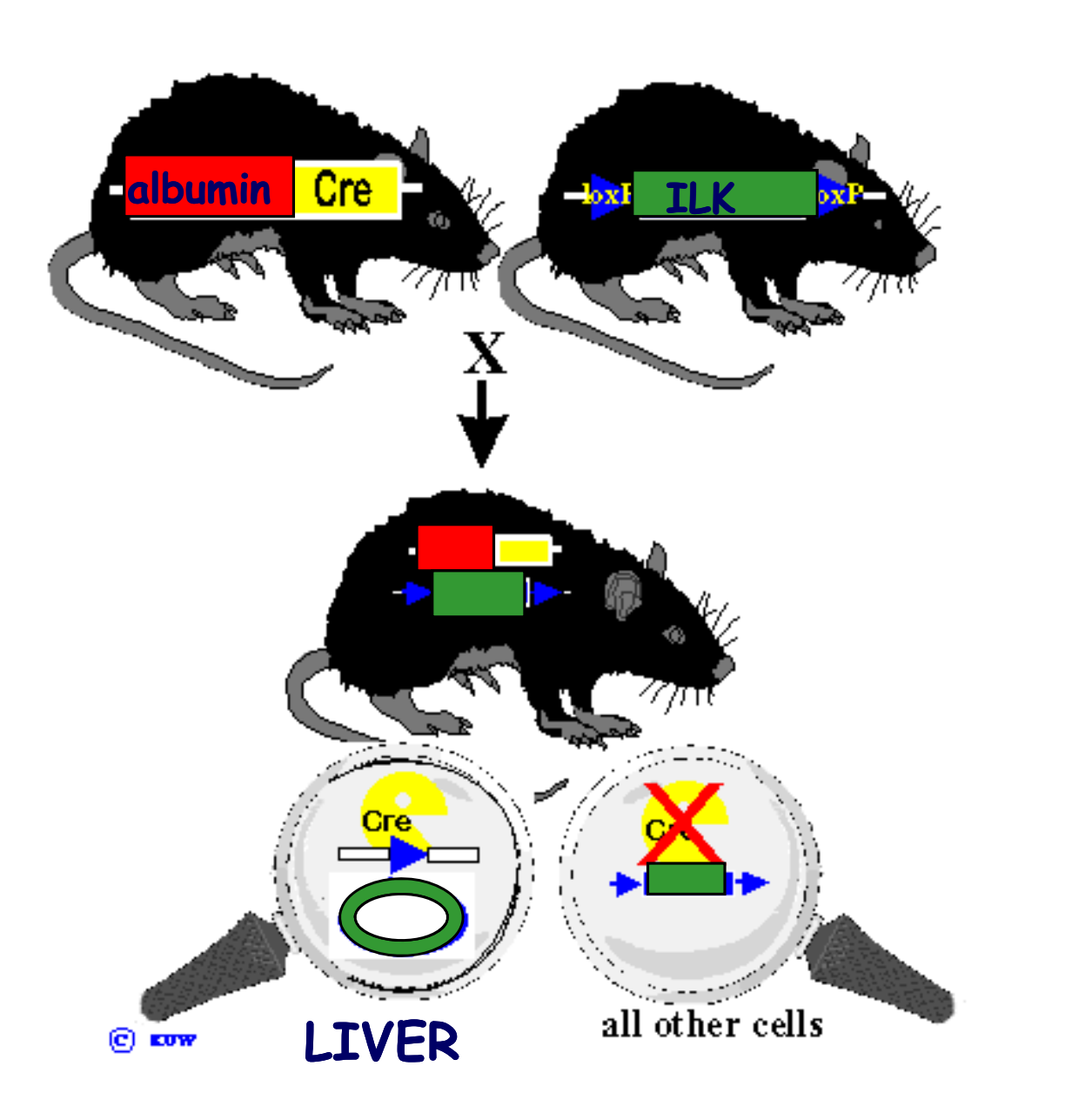


Adeno-Cre injection



Adeno-beta-Gal injection

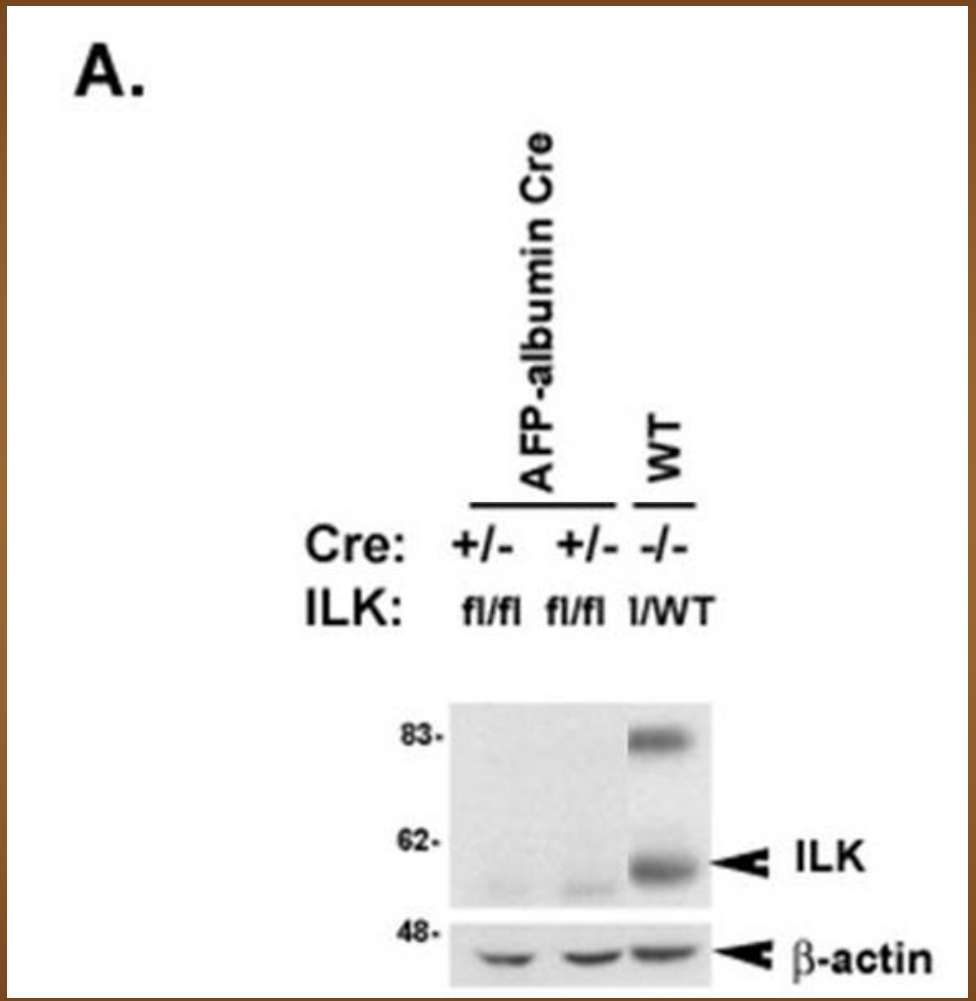
Crossing of the ILK-Floxed mice with the Foxa3 Cre, AFP-albumin Cre, albumin-Cre mice→ **conditional knock-out of ILK in the liver at different stages of development**

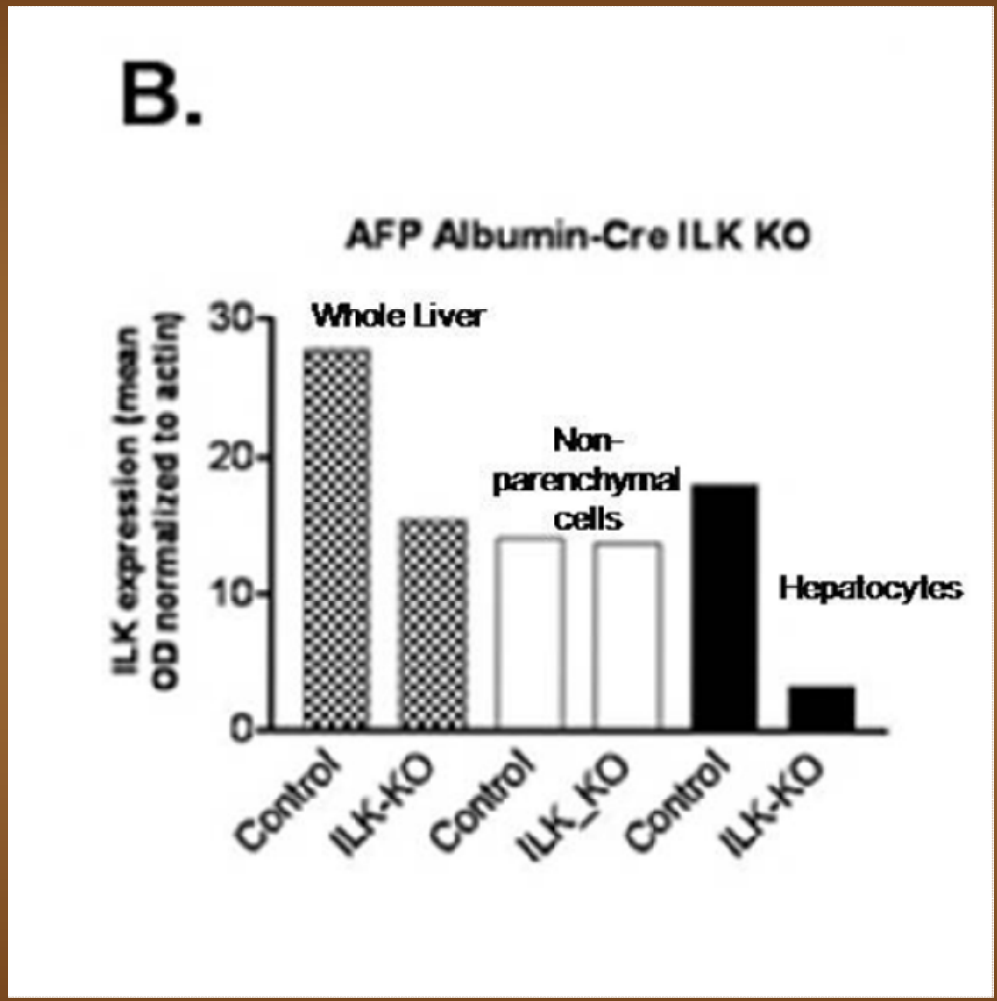


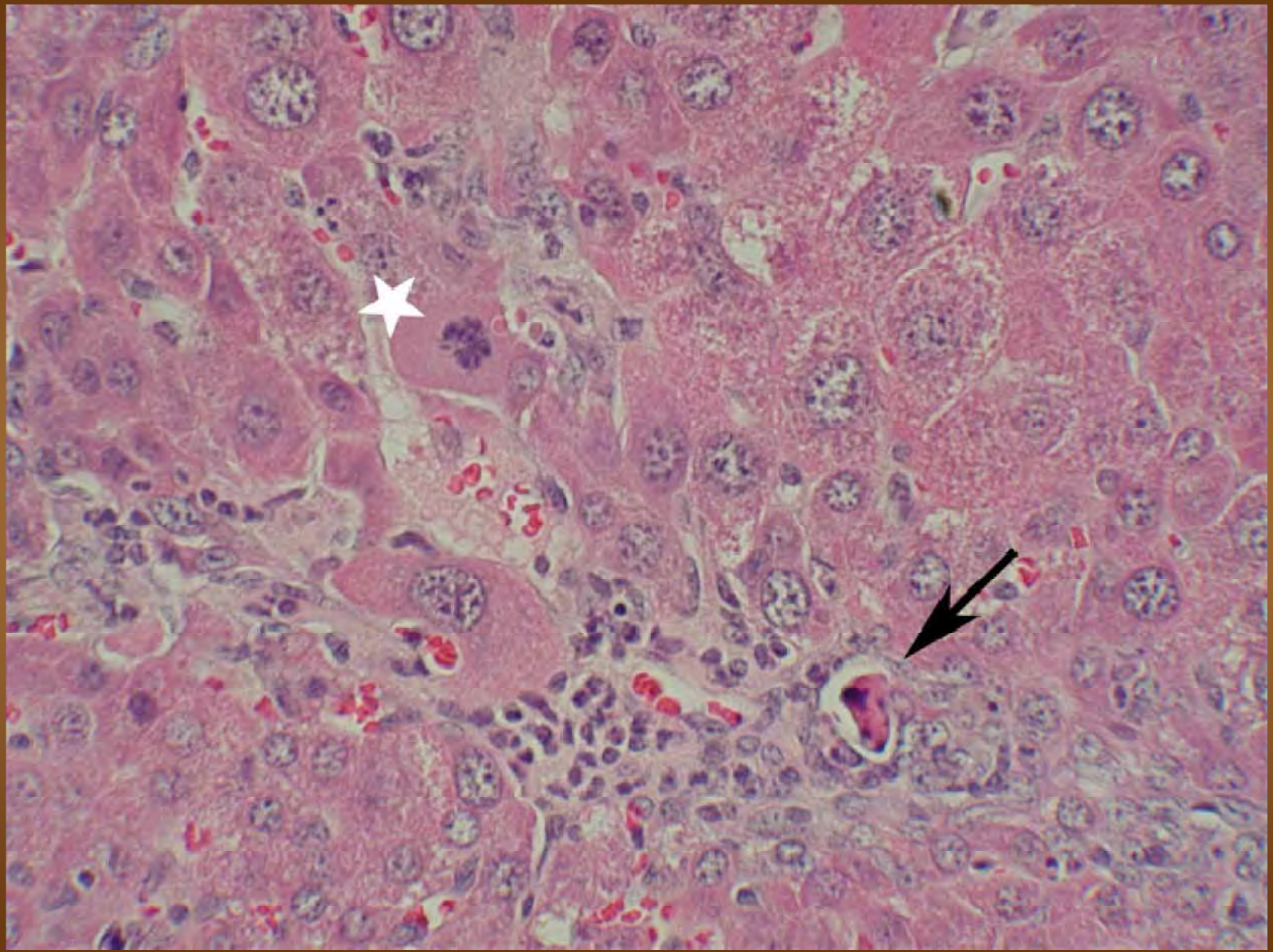
Common elements of the phenotype induced by hepatocyte-targeted elimination of ILK by three promoter-Cre arrangements:

- Mice are born viable. There is no evidence of embryonic mortality.
- At 2 weeks after birth, ILK-/- livers have irregular hepatocyte plates, and atrophic or absent bile ducts.
- At 4-6 weeks after birth, there are multiple hepatocytes in proliferation throughout the lobule, surrounded by inflammatory cells. Other hepatocytes are in apoptosis.
- There is increased proliferation of extraportal biliary cells and a decrease in the number of portal ductules (absent in Foxa3-Cre)
- In Foxa3-Cre mice, most hepatocytes are small and some have dysplastic changes.

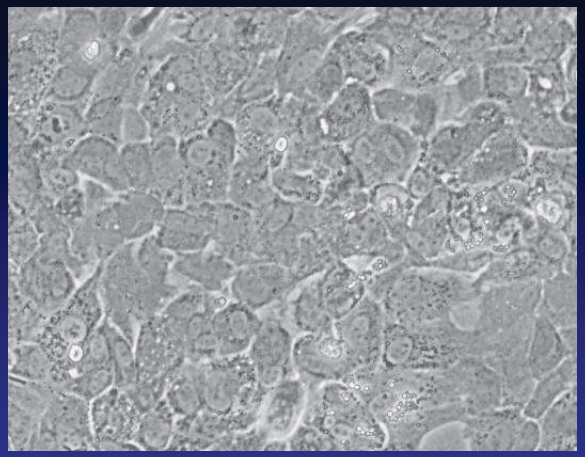
ILK is eliminated specifically from hepatocytes



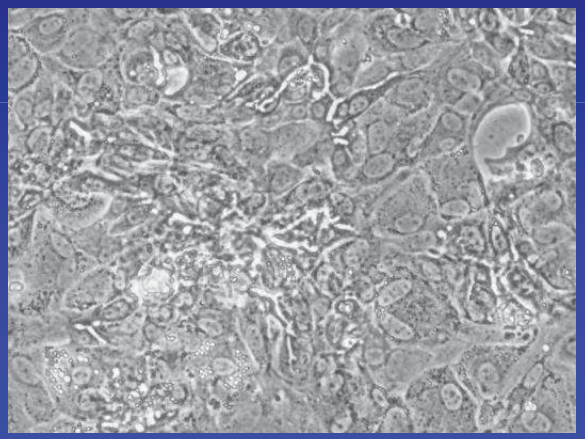




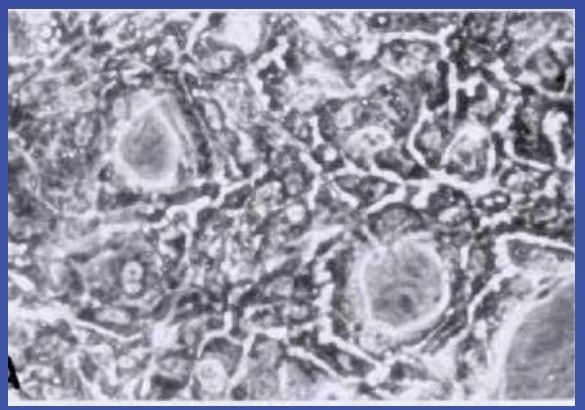
In-vitro Differentiation



Day-8 (control)
De-differentiated hepatocytes
- large flat shape
- cytoplasm
- poorly defined nucleus



Day-9 (24hr Matrigel)
Re-differentiating hepatocytes
- Less cytoplasm
- Prominent nuclei
- Bile canaliculi

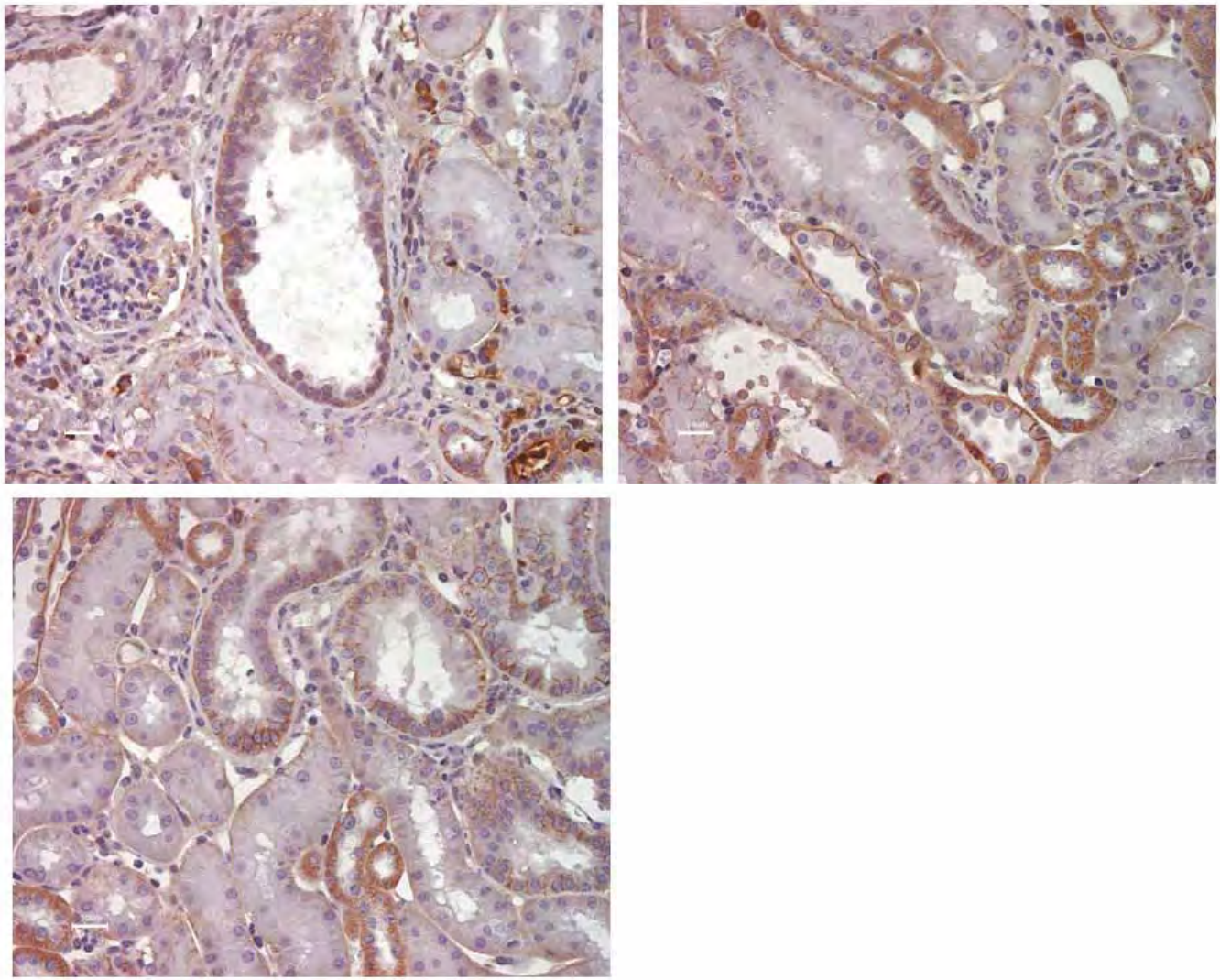


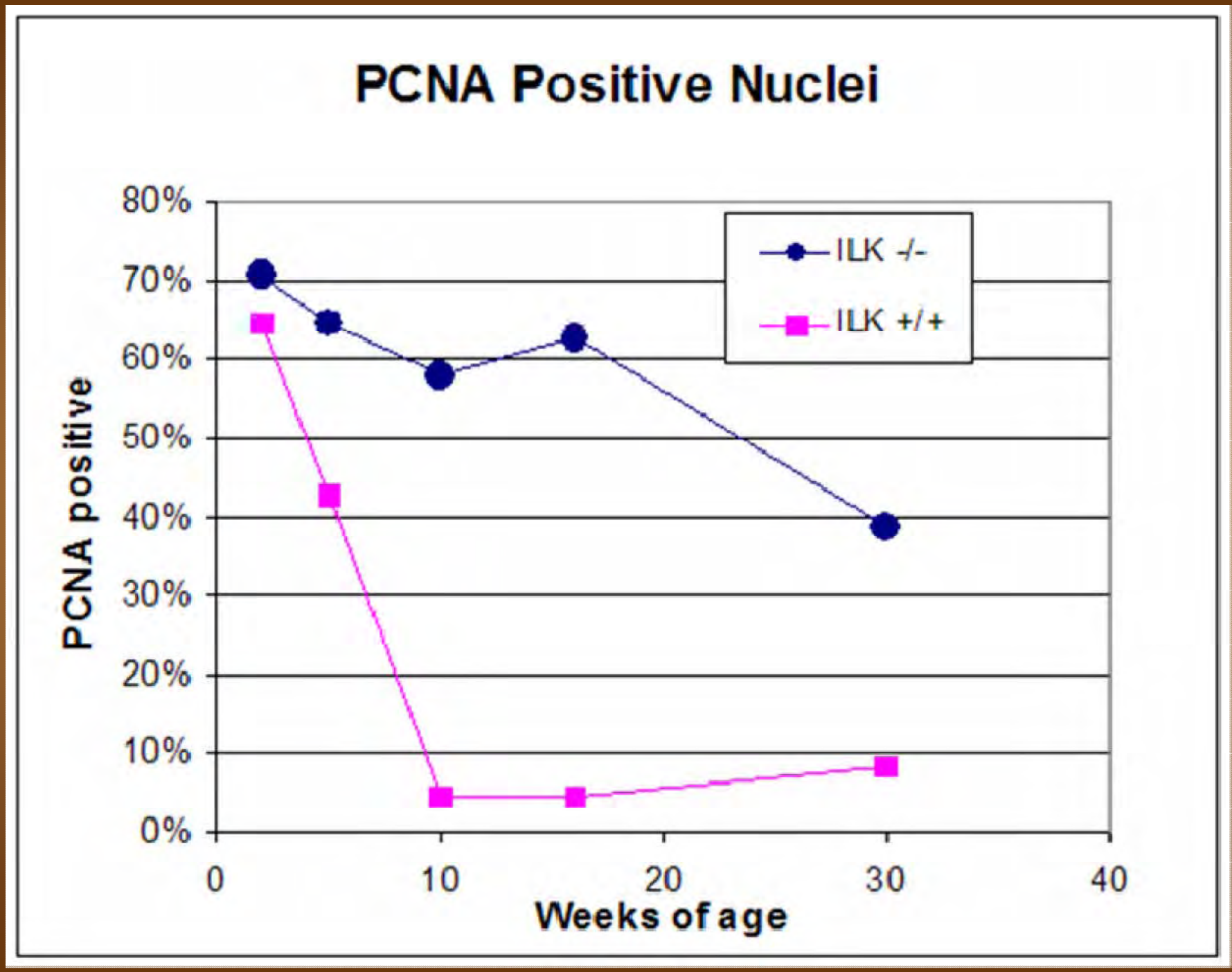
Day-14 (5 days Matrigel)
- Small 3D-shape
- Well defined canaliculi

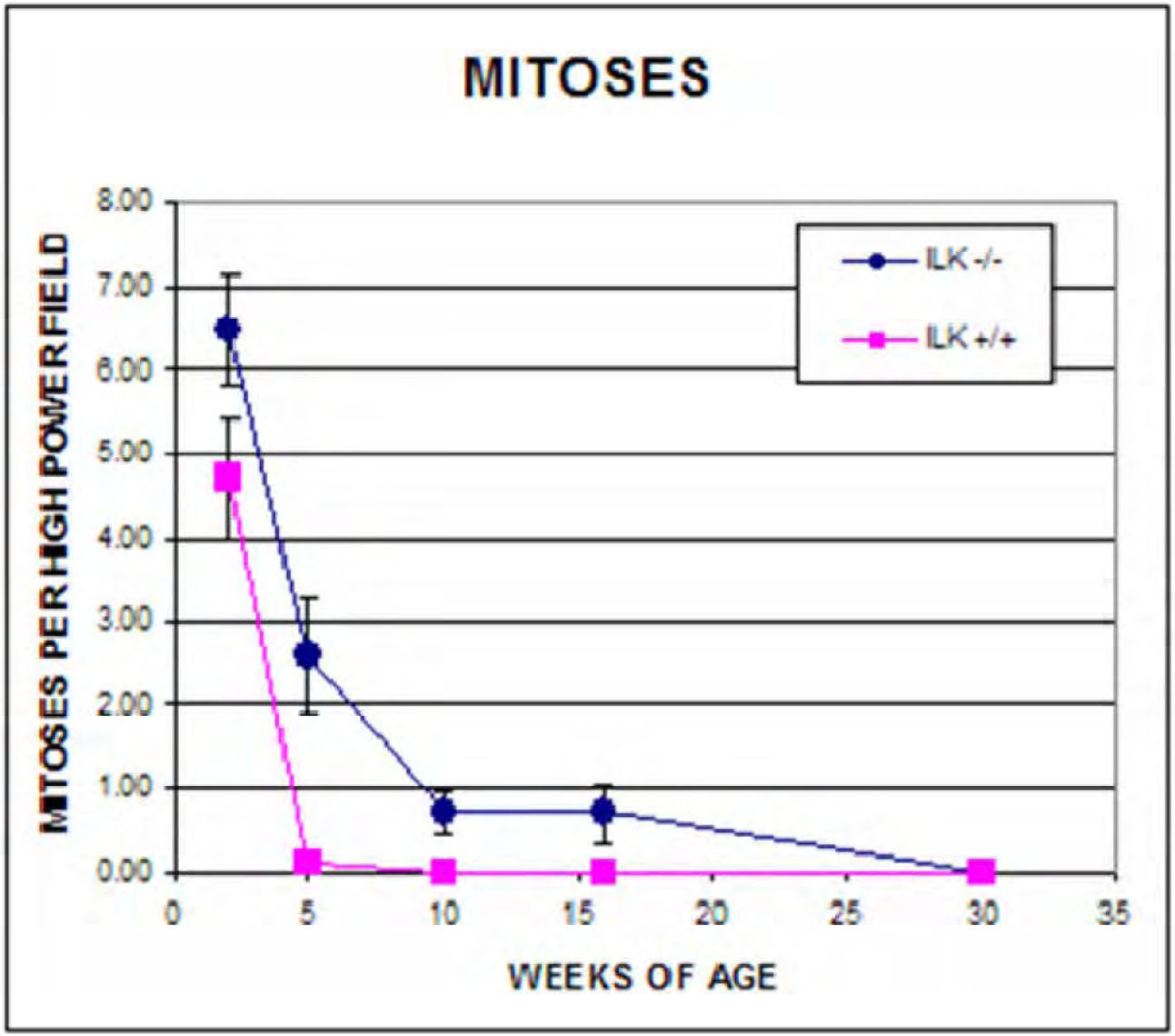
LIVER CONUNDRUM

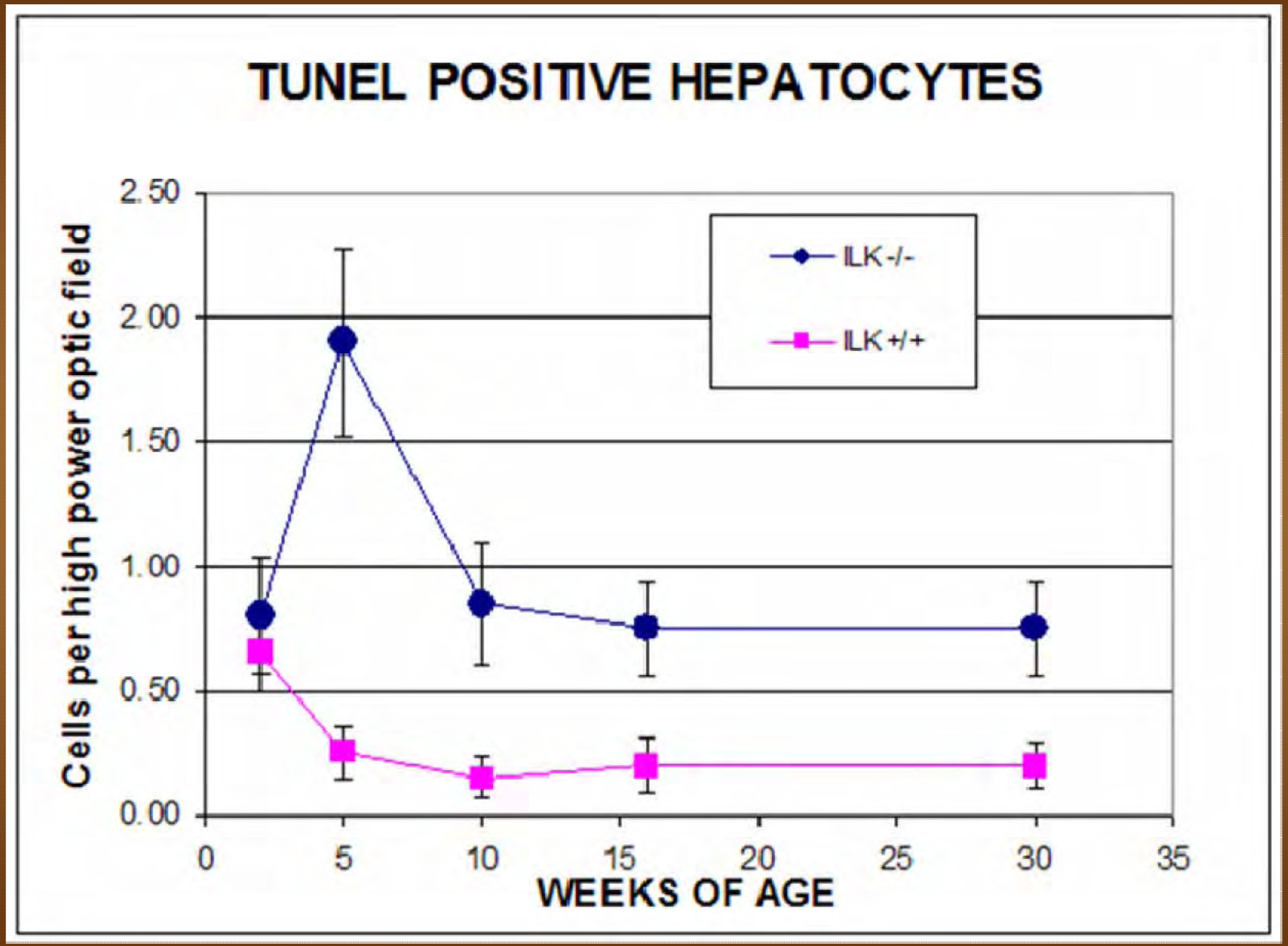
- Matrix signaling inhibits hepatocyte proliferation in culture.
- Acute removal of ILK causes massive apoptosis.
- In the absence of matrix signaling, there is both hepatocyte proliferation and apoptosis.
- Is proliferation or apoptosis the primary event?

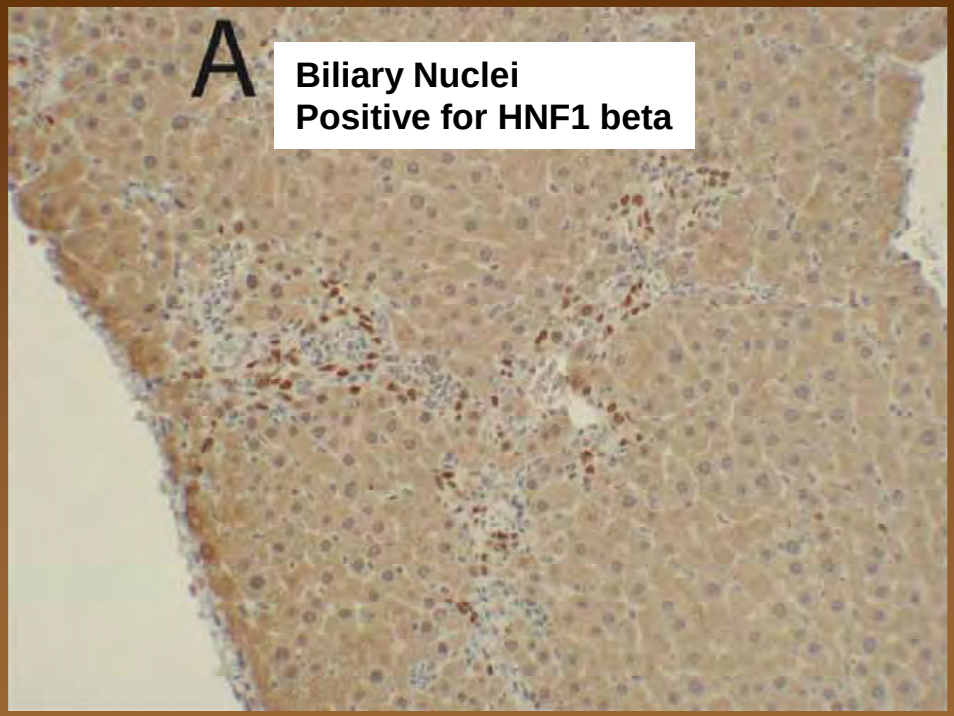
Hyper-proliferation of the kidney tubular epithelial cells in mice With kidney targeted knockout of ILK.

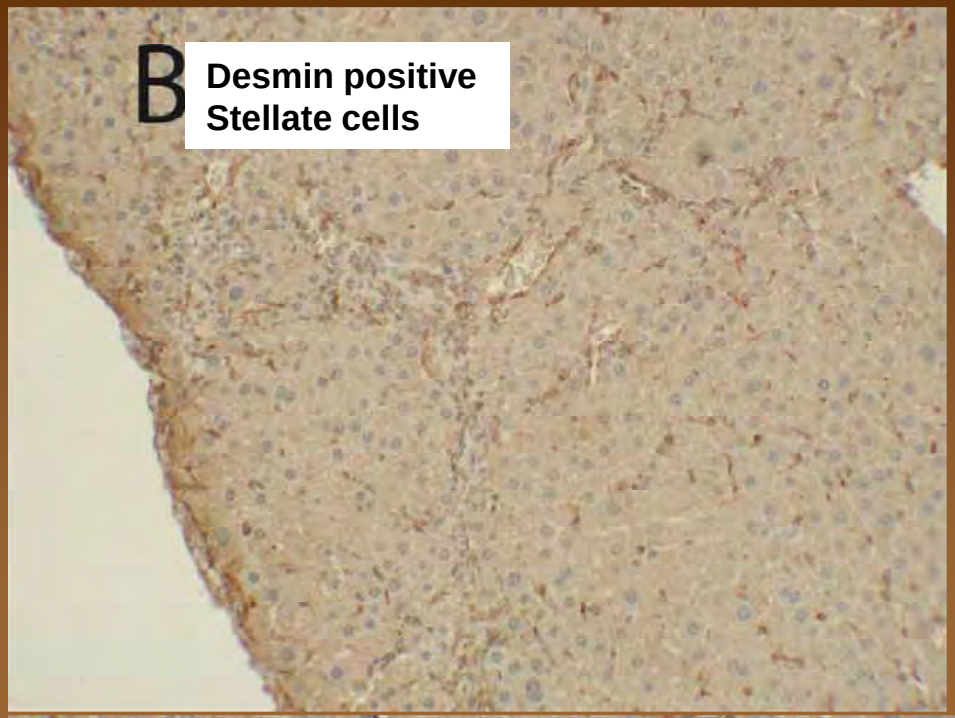


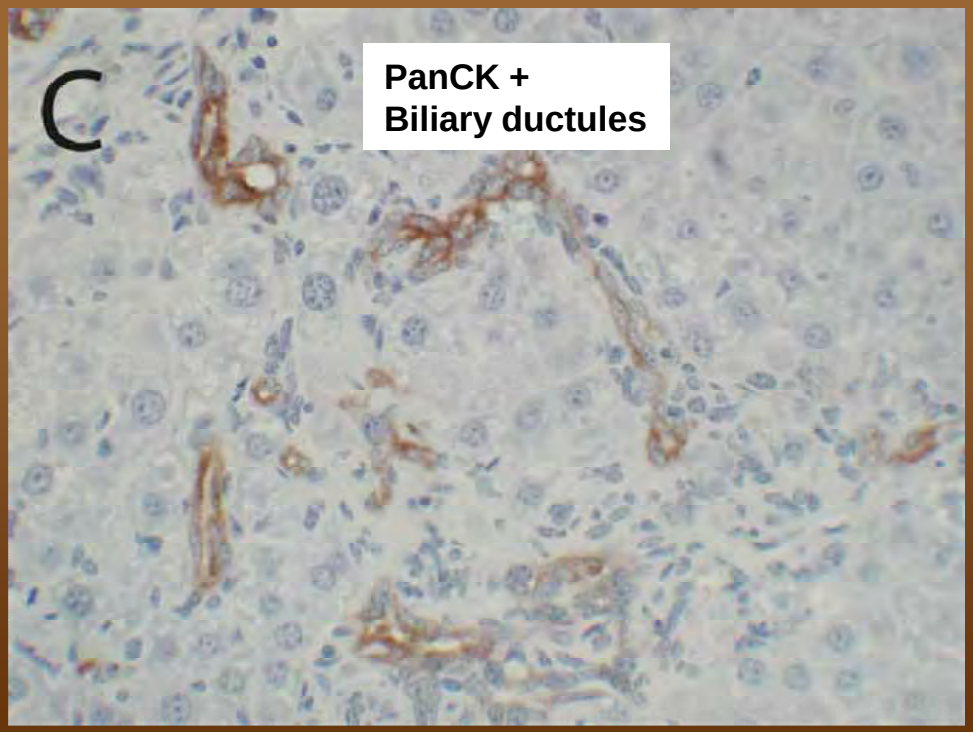

PCNA Positive Nuclei
80%
70%
60%
50%
40%
30%
20%
10%
0%
PCNA positive
0
10
20
30
40
Weeks of age
ILK -/-
ILK +/+

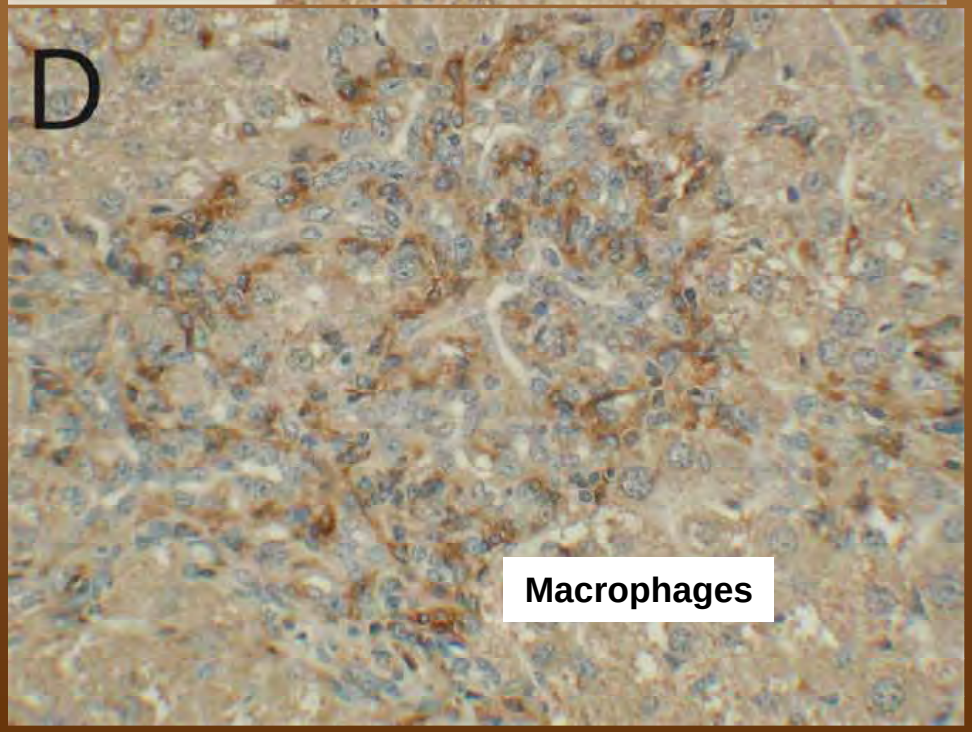

MITOSES
MITOSES PER HIGH POWER FIELD
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
5
10
15
20
25
30
35
WEEKS OF AGE
ILK -/-
ILK +/+

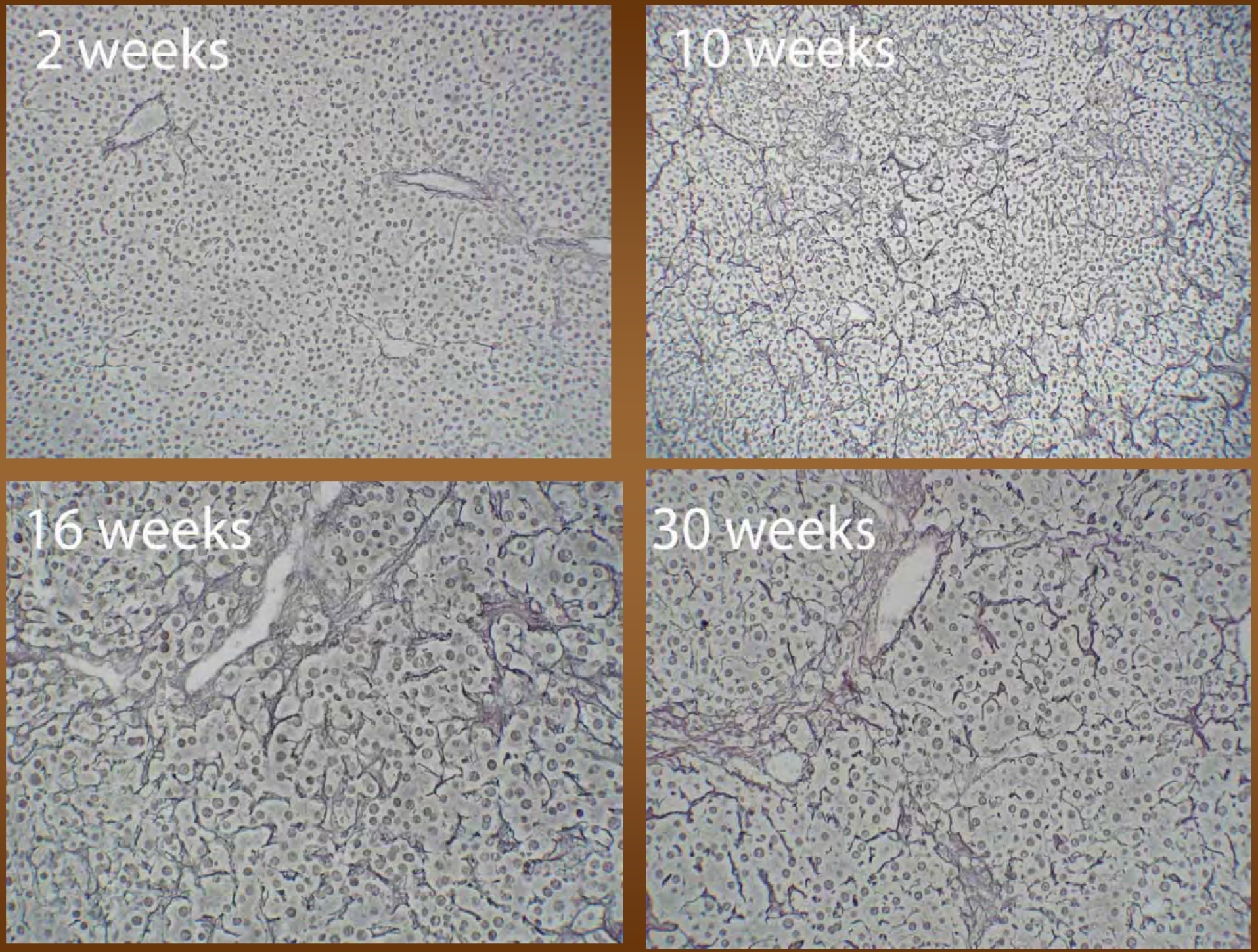

TUNEL POSITIVE HEPATOCYTES
Cells per high power optic field
2.50
2.00
1.50
1.00
0.50
0.00
0
5
10
15
20
25
30
35
WEEKS OF AGE
ILK -/-
ILK +/+

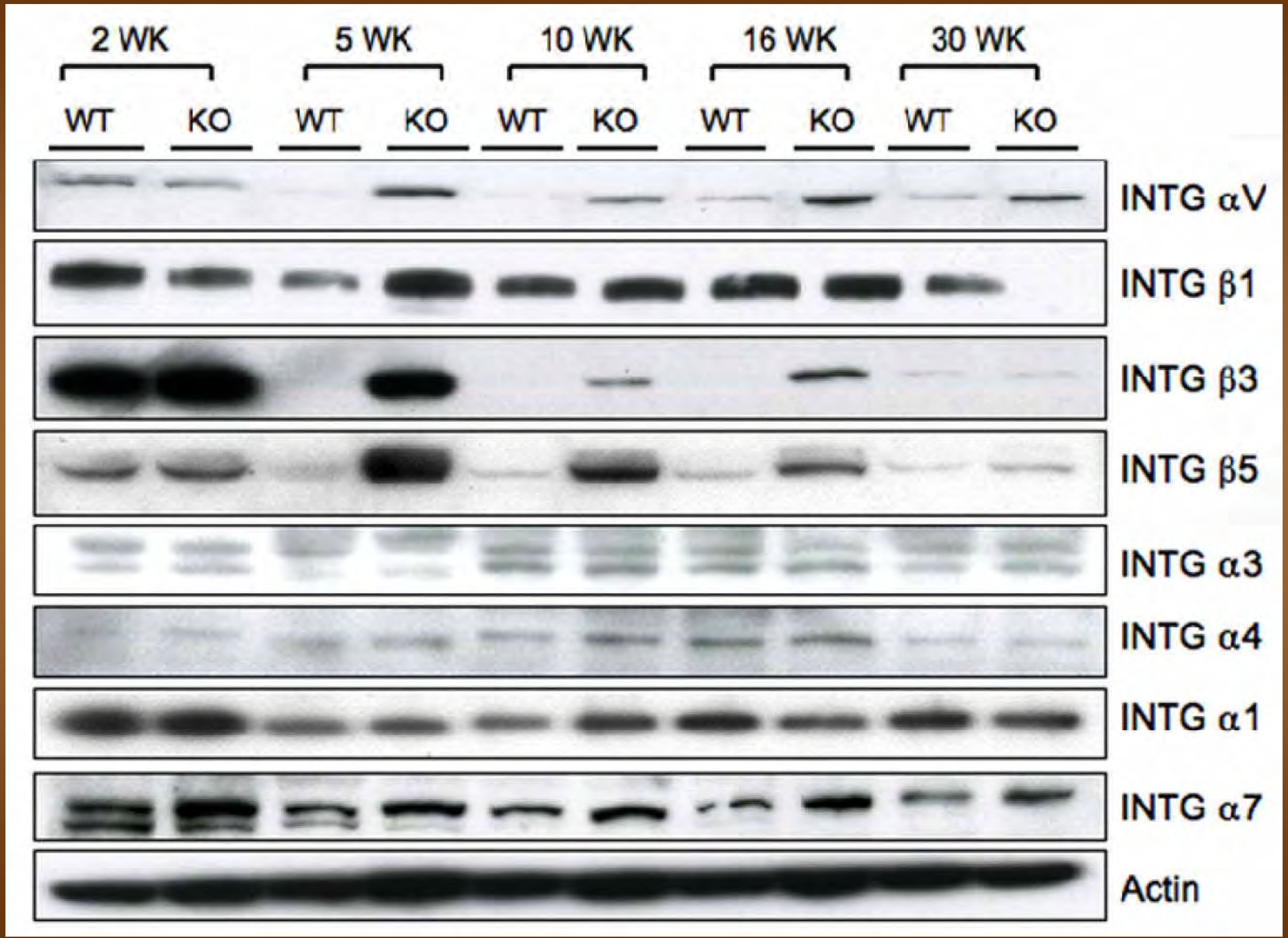

A
Biliary Nuclei
Positive for HNF1 beta

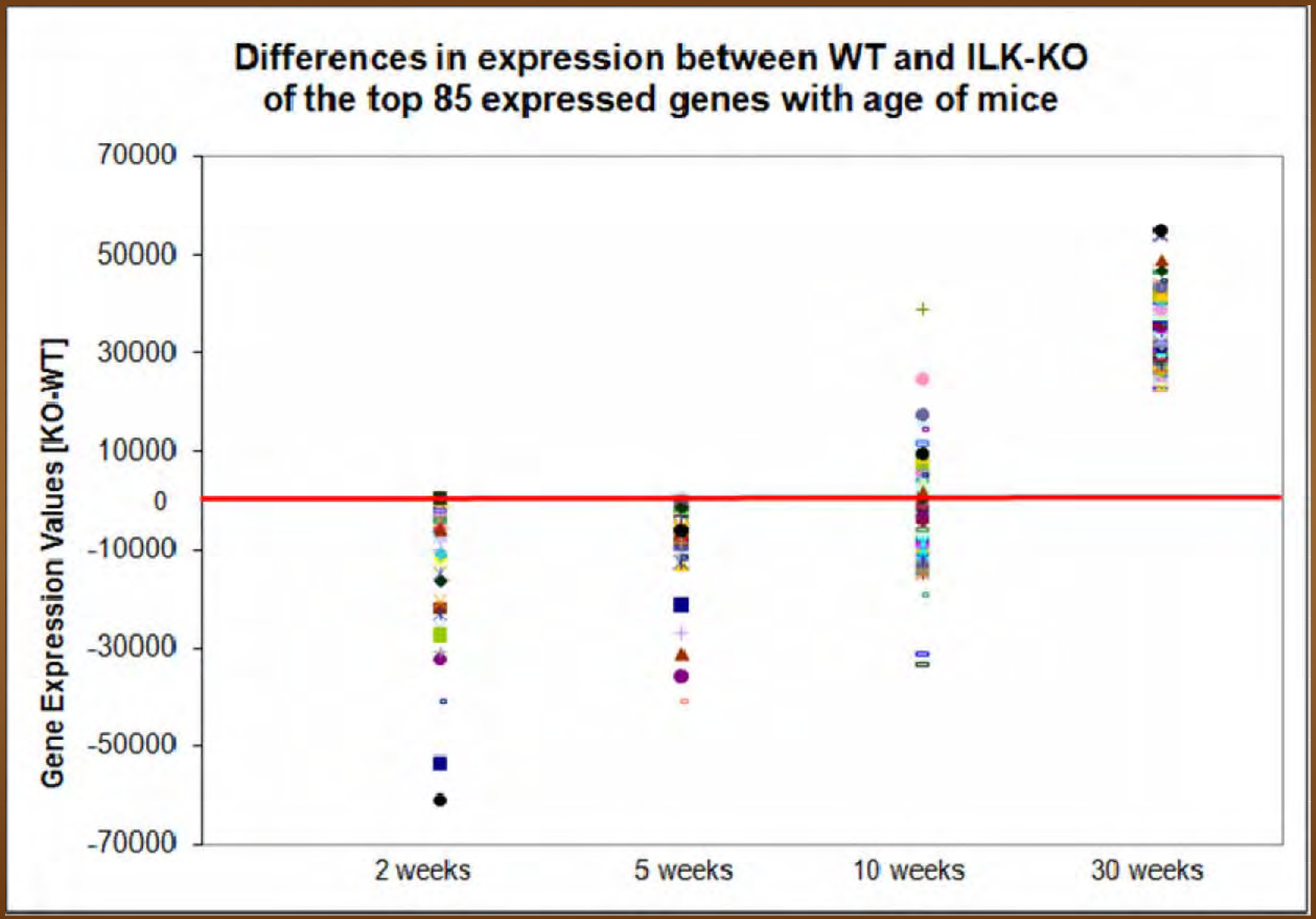

B
Desmin positive
Stellate cells

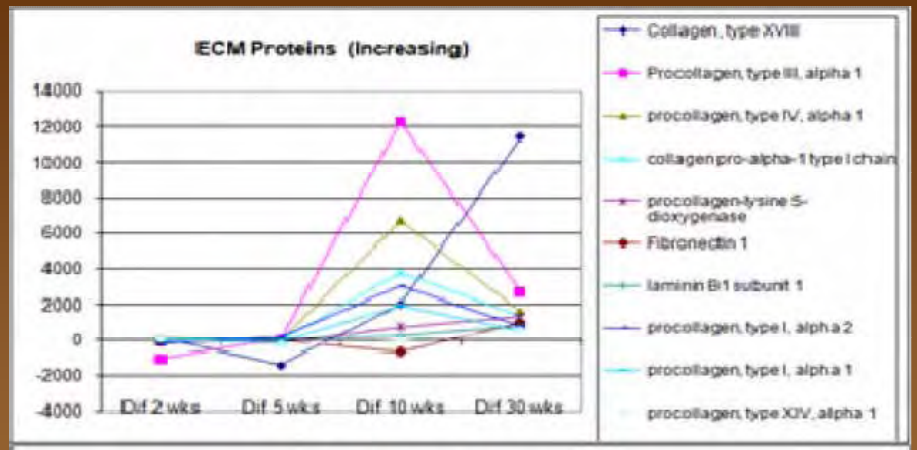

C
PanCK +
Biliary ductules

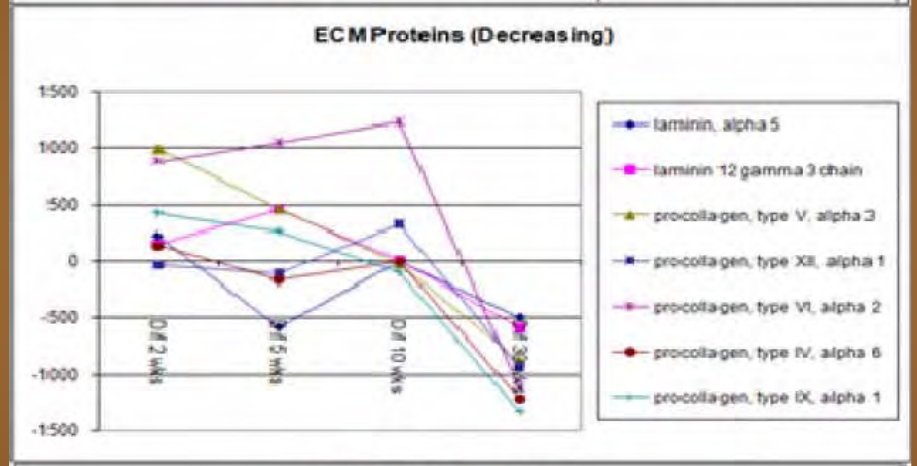

D
Macrophages

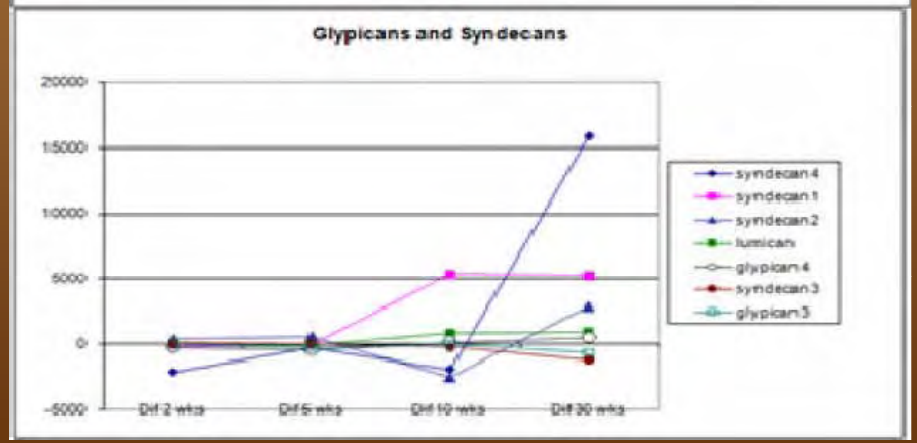

2 weeks
10 weeks
16 weeks
30 weeks

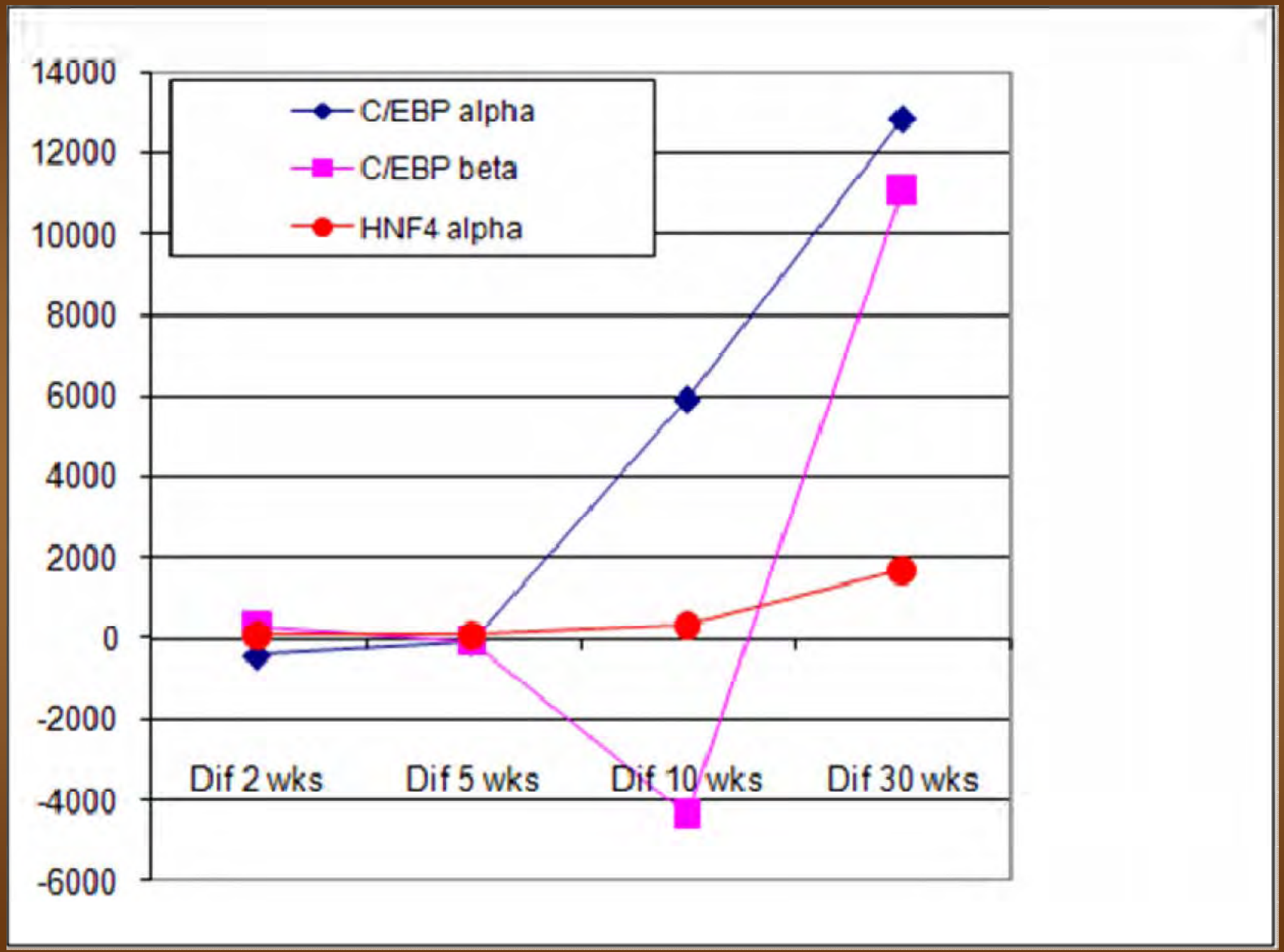

2 WK
5 WK
10 WK
16 WK
30 WK
WT
KO
INTG αV
INTG β1
INTG β3
INTG β5
INTG α3
INTG α4
INTG α1
INTG α7
Actin


Differences in expression between WT and ILK-KO
of the top 85 expressed genes with age of mice
Gene Expression Values [KO-WT]
70000
50000
30000
10000
0
-10000
-30000
-50000
-70000
2 weeks
5 weeks
10 weeks
30 weeks

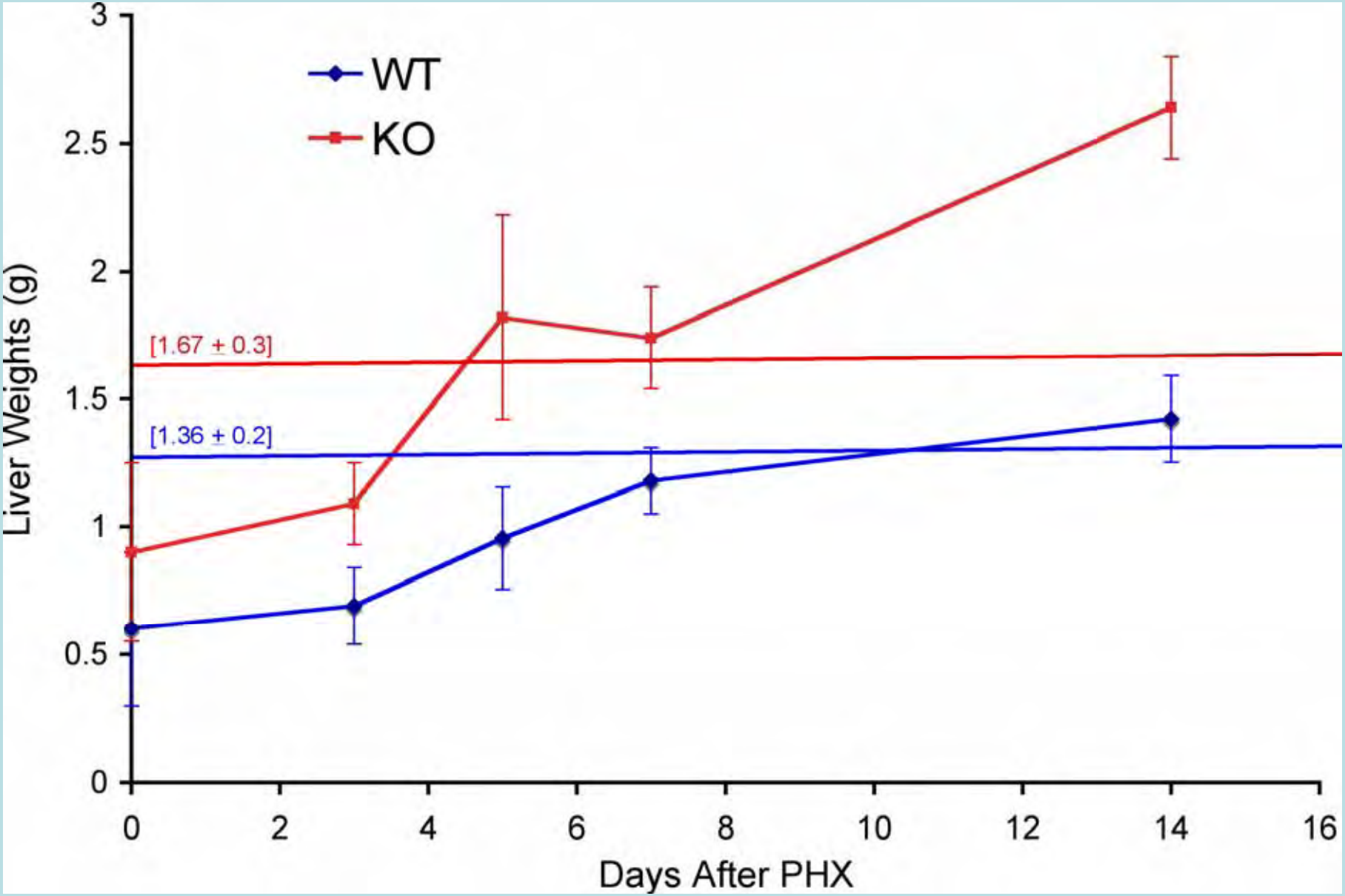

ECM Proteins (Increasing)
14000
12000
10000
8000
6000
4000
2000
0
-2000
-4000
Dif 2 wks
Dif 5 wks
Dif 10 wks
Dif 30 wks
Collagen, type XVIII
Procollagen, type III, alpha 1
procollagen, type IV, alpha 1
collagen pro-alpha-1 type I chain
procollagen-lysine 5-dioxygenase
Fibronectin 1
laminin B1 subunit 1
procollagen, type I, alpha 2
procollagen, type I, alpha 1
procollagen, type XIV, alpha 1

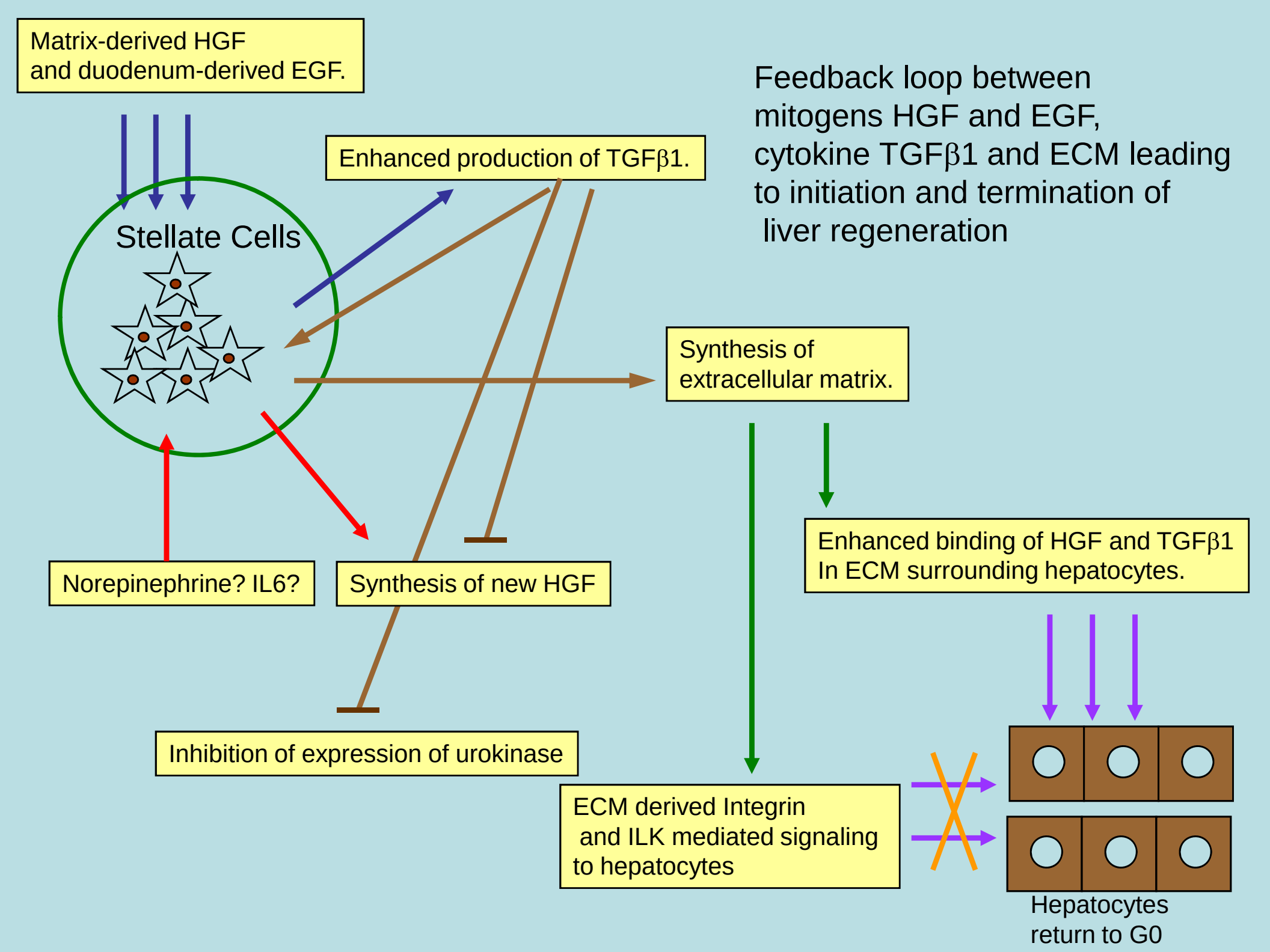

ECM Proteins (Decreasing)
1500
1000
500
0
-500
-1000
-1500
Dif 2 wks
Dif 5 wks
Dif 10 wks
laminin, alpha 5
laminin 12 gamma 3 chain
procollagen, type V, alpha 3
procollagen, type XII, alpha 1
procollagen, type VI, alpha 2
procollagen, type IV, alpha 6
procollagen, type IX, alpha 1


Glypicans and Syndecans
20000
15000
10000
5000
0
-5000
Dif 2 wks
Dif 5 wks
Dif 10 wks
Dif 30 wks
syndecan 4
syndecan 1
syndecan 2
lumican
glypican 4
syndecan 3
glypican 5

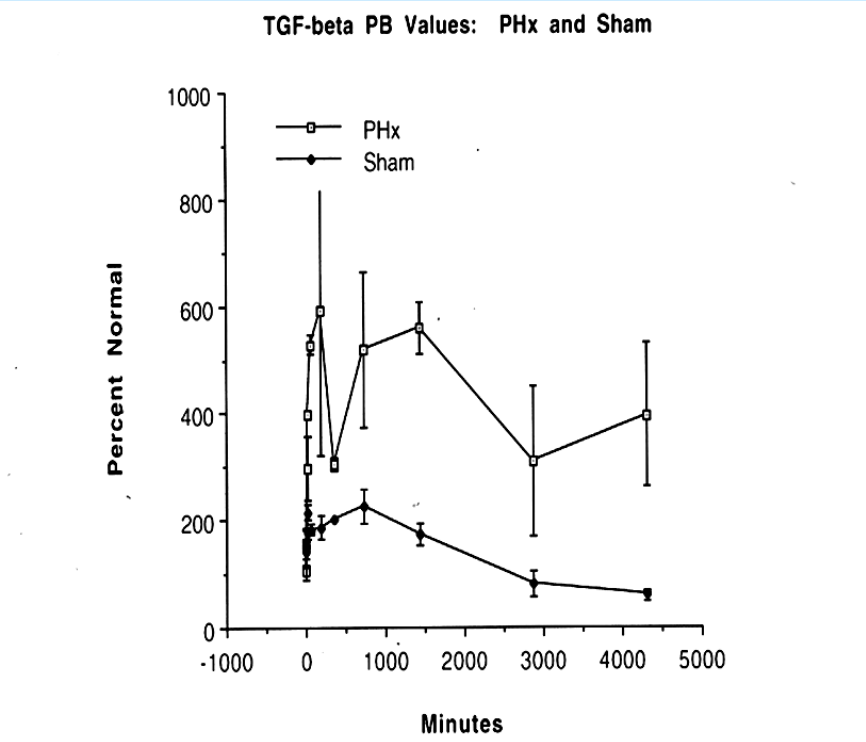

14000
12000
10000
8000
6000
4000
2000
0
-2000
-4000
-6000
C/EBP alpha
C/EBP beta
HNF4 alpha
Dif 2 wks
Dif 5 wks
Dif 10 wks
Dif 30 wks

ILK-KO livers return to a larger weight than the original following partial hepatectomy!



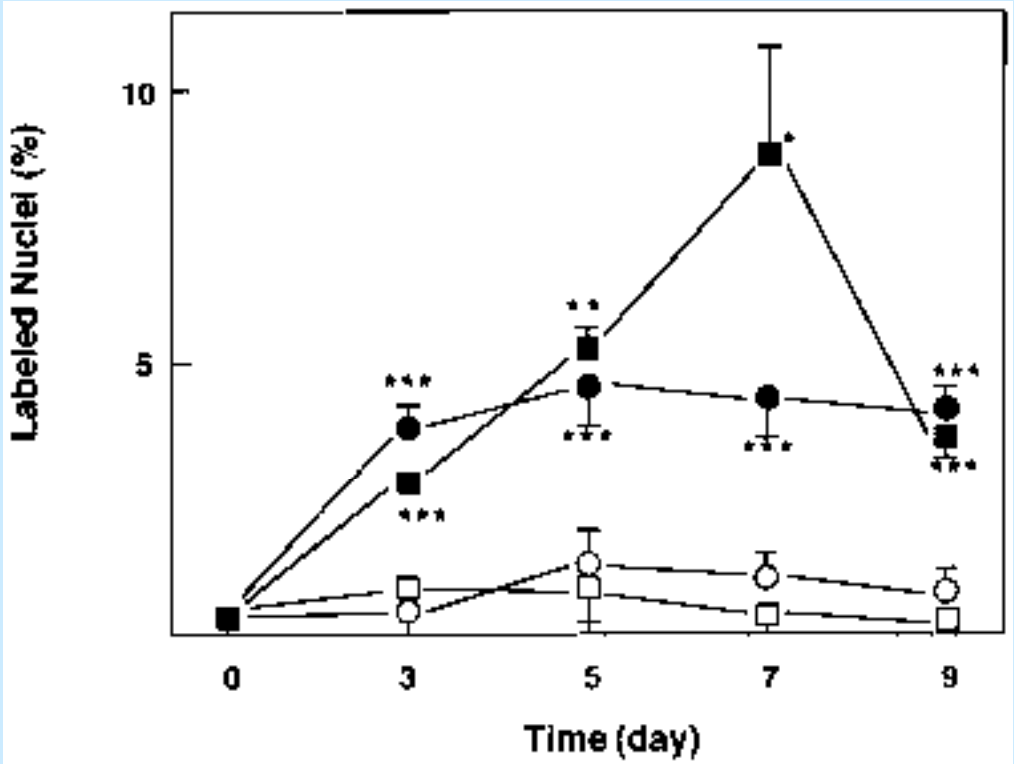

Matrix-derived HGF
and duodenum-derived EGF.
Feedback loop between
mitogens HGF and EGF,
cytokine TGFβ1 and ECM leading
to initiation and termination of
liver regeneration
Enhanced production of TGFβ1.
Stellate Cells
Synthesis of
extracellular matrix.
Enhanced binding of HGF and TGFβ1
In ECM surrounding hepatocytes.
Norepinephrine? IL6?
Synthesis of new HGF
Inhibition of expression of urokinase
ECM derived Integrin
and ILK mediated signaling
to hepatocytes
Hepatocytes
return to G0

Supporting evidence 1: When TGFb signaling is interfered in intact liver there is stimulation of hepatocyte DNA synthesis



TGFb1 levels in plasma after PHx.

Jirtle and Michalopoulos



Time course of BrdU labeling after gene transfer. AdextTR (●), AdextAR (■), AdexLacZ (○), or PBS (□) was infused into the portal vein, and nuclear BrdU-labeling in hepatocytes was counted at various time points (n = 4). **P* < .005 vs. AdexLacZ; ***P* < .01 vs. AdexLacZ; ****P* < .05 vs. AdexLacZ

Ichikawa T., Hepatology 2001 Nov;34(5):918-25

Supporting evidence 2:

HGF and EGF induce expression of TGFb1 in organoid cultures

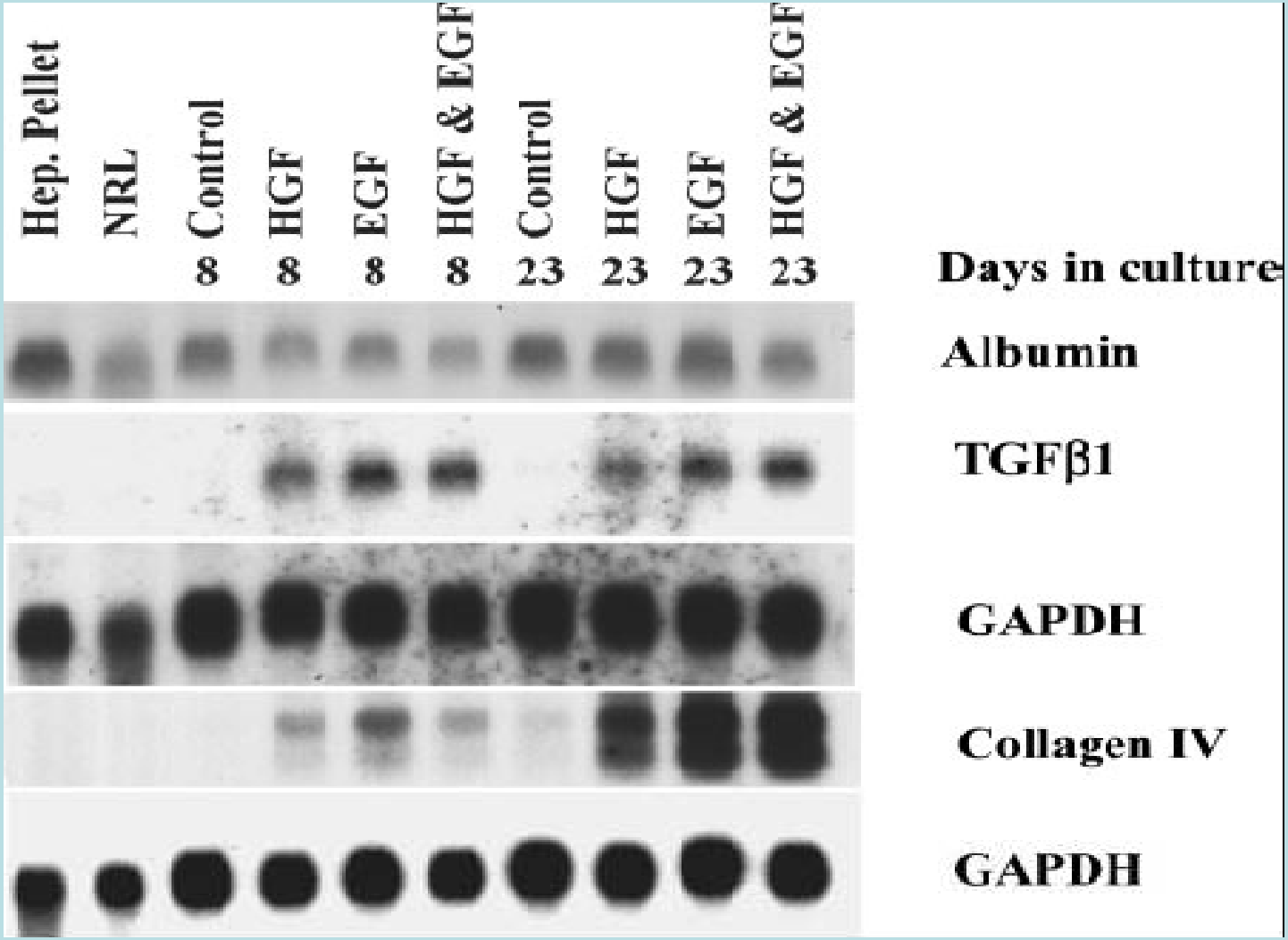


Figure 8. Expression of albumin, TGF-1 and collagen type IV in cultures at different days, maintained in the presence of either HGF or EGF or both. Control cultures had neither HGF nor EGF supplementation. Hepatocyte pellet isolated at the end of collagenase perfusion as well as whole normal rat liver tissue (NRL) were also examined for comparison. Analysis of extracted RNA was conducted by Northern gels. The upper GAPDH is used as a normalizing control for albumin and TGF-1 whereas the lower GAPDH was used for the normalization of the data on collagen type IV, because the corresponding RNA were run on two separate gels. EGF was a stronger inducer of both TGF-1 and collagen type IV at day 8, compared to HGF.

Supporting evidence 3:
Proteoglycan Gene Expression in Rat Liver after Partial Hepatectomy

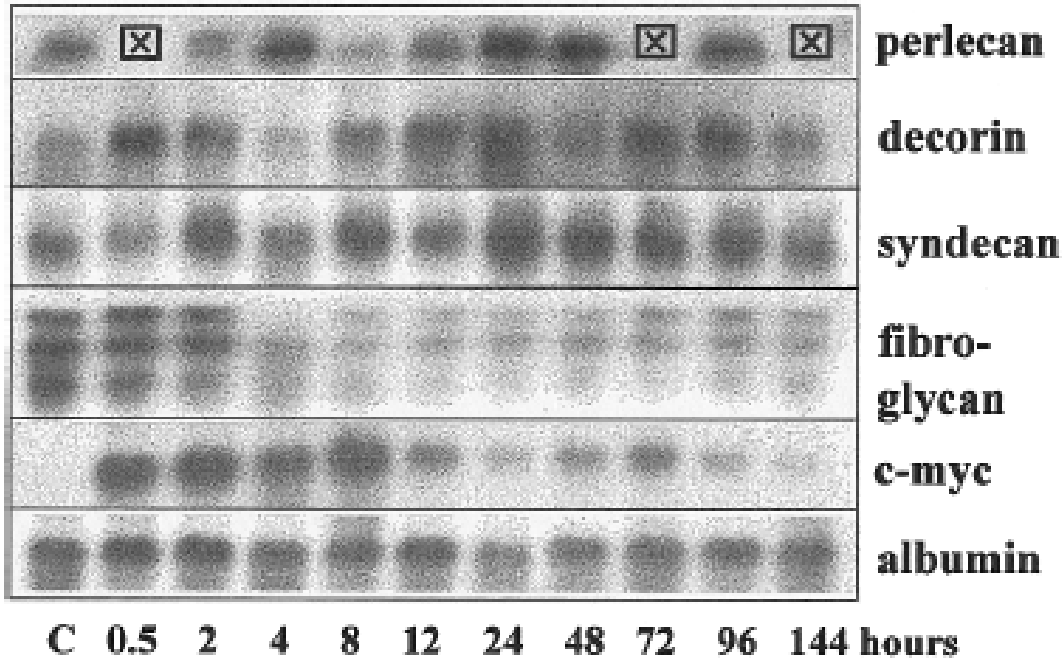
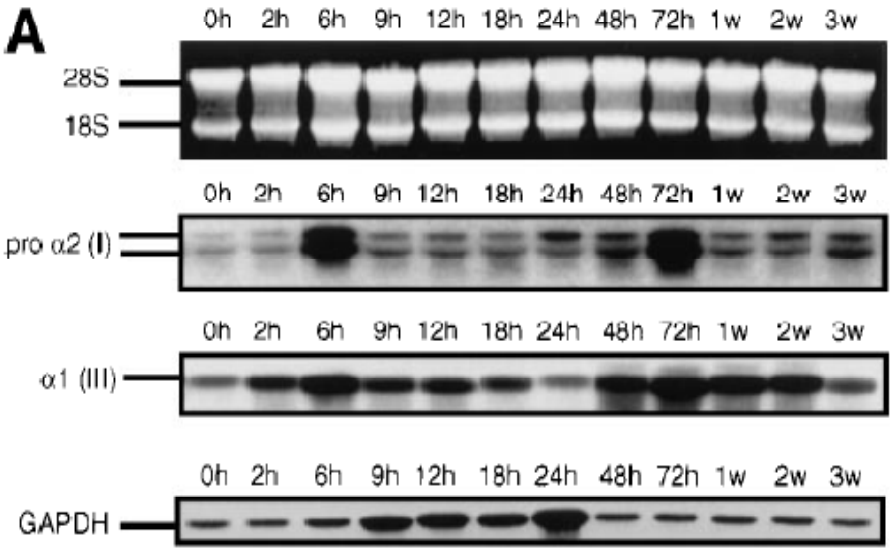
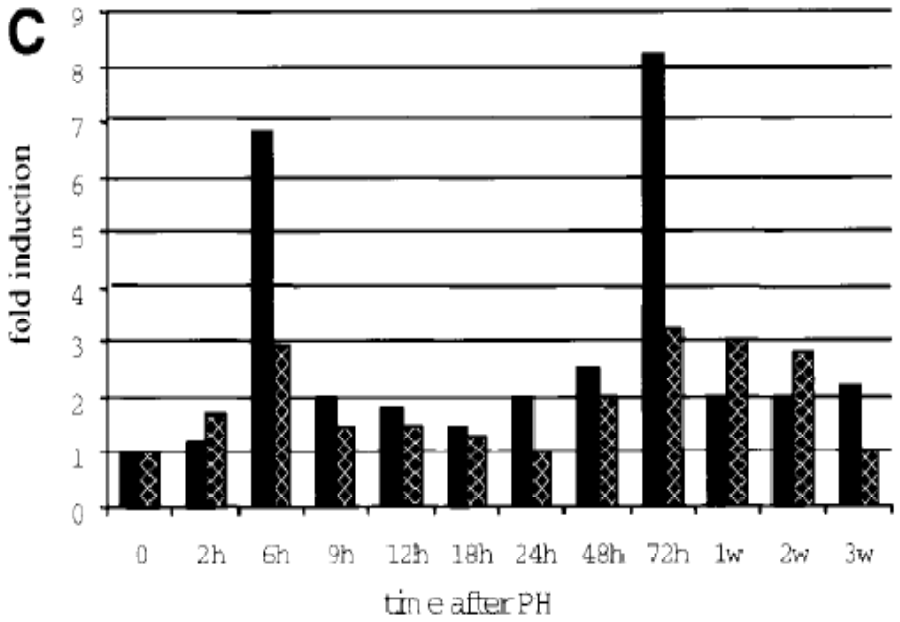


FIG. 1. Time course of the expression of perlecan (12 kb), decorin (1,6 and 1,9 kb), syndecan (2,6 and 3,4 kb), fibroglycan (3,4; 2,2; 1,1 kb), c-myc (2,3 kb), and albumin (2,4 kb) after partial hepatectomy. RNA was separated on formaldehyde-agarose gel, blotted to Hybond membrane and hybridized with specific cDNA probes labelled with [^{32}P]dCTP by random priming.

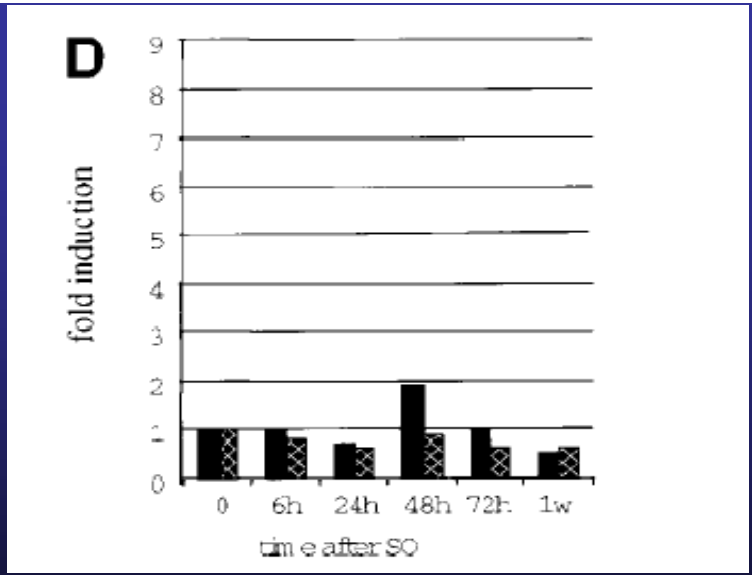
Monika Gallai,1 Anna Sebestyen, Peter Nagy, Ilona Kovalszky,
Tamas Onody, and **Snorri S. Thorgeirsson**.
Proteoglycan Gene Expression in Rat Liver after Partial Hepatectomy.
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **228,** 690–694 (1996)

Supporting evidence 4: Enhanced synthesis of procollagens at 48-72 hrs after hepatectomy



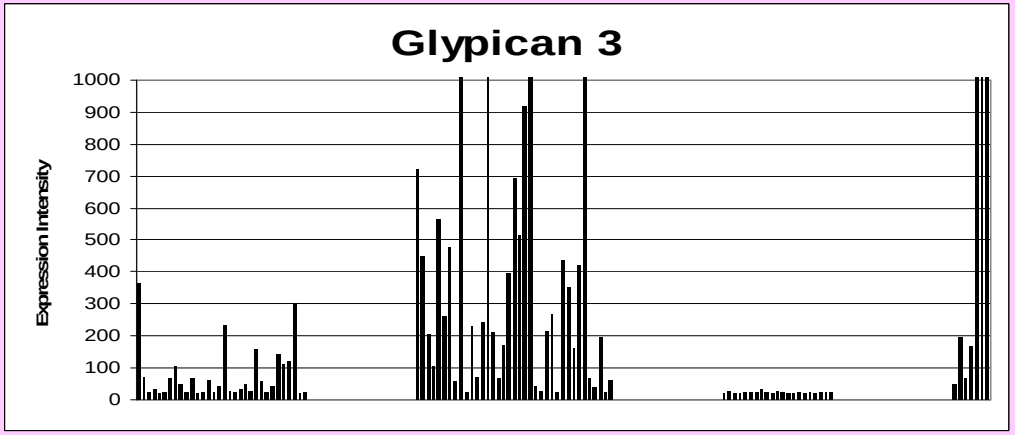


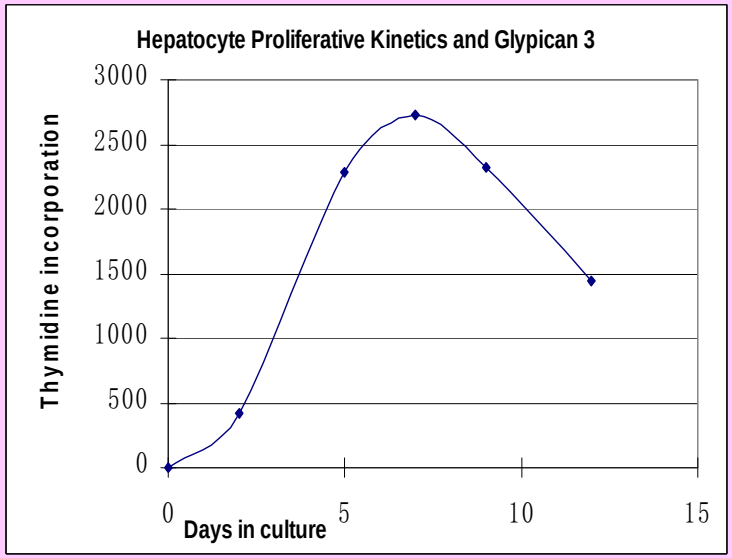
Increased mRNA expression of procollagen a2 (I) and a1 (III) after two-thirds partial hepatectomy. Northern blot analysis of procollagen a2 (I) and a1 (III) using 30 mg total RNA: (A) after partial hepatectomy and (B) after sham operation. Ethidium bromide staining monitored equal loading of the gels and hybridization with a GAPDH-probe was used as an internal standard. Quantification of the specific signal was performed after subtraction of the background signal and normalization with the GAPDH-signal. Procollagen a2 (I) showed a sharp upregulation 72 hours after partial hepatectomy, whereas a1(III) was upregulated from 72 hours to 2 weeks after partial hepatectomy (C). The level of induction was lower for a1 (III) (threefold induction) comparedwith procollagen a2 (I) (eightfold induction). After sham operation none of the collagens showed any significant regulation (D).



K. LENHARD RUDOLPH, CHRISTIAN TRAUTWEIN, STEFAN KUBICKA, TIM RAKEMANN, MATTHIAS J. BAHR, NICK SEDLACZEK, DETLEF SCHUPPAN, AND MICHAEL P. MANNS (HEPATOLOGY 1999;30:1159-1166.)

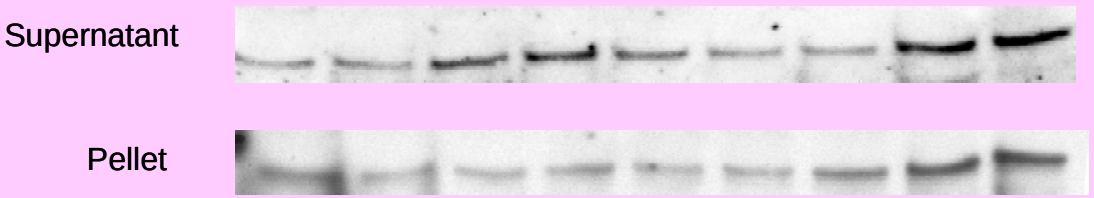
Is Glypican 3 involved?

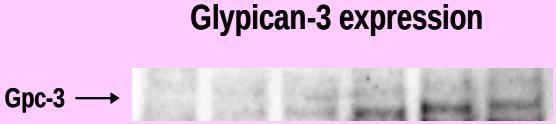




GPC3 expression in human hepatocellular carcinomas

Expression of Glypican 3 at different times after PHx

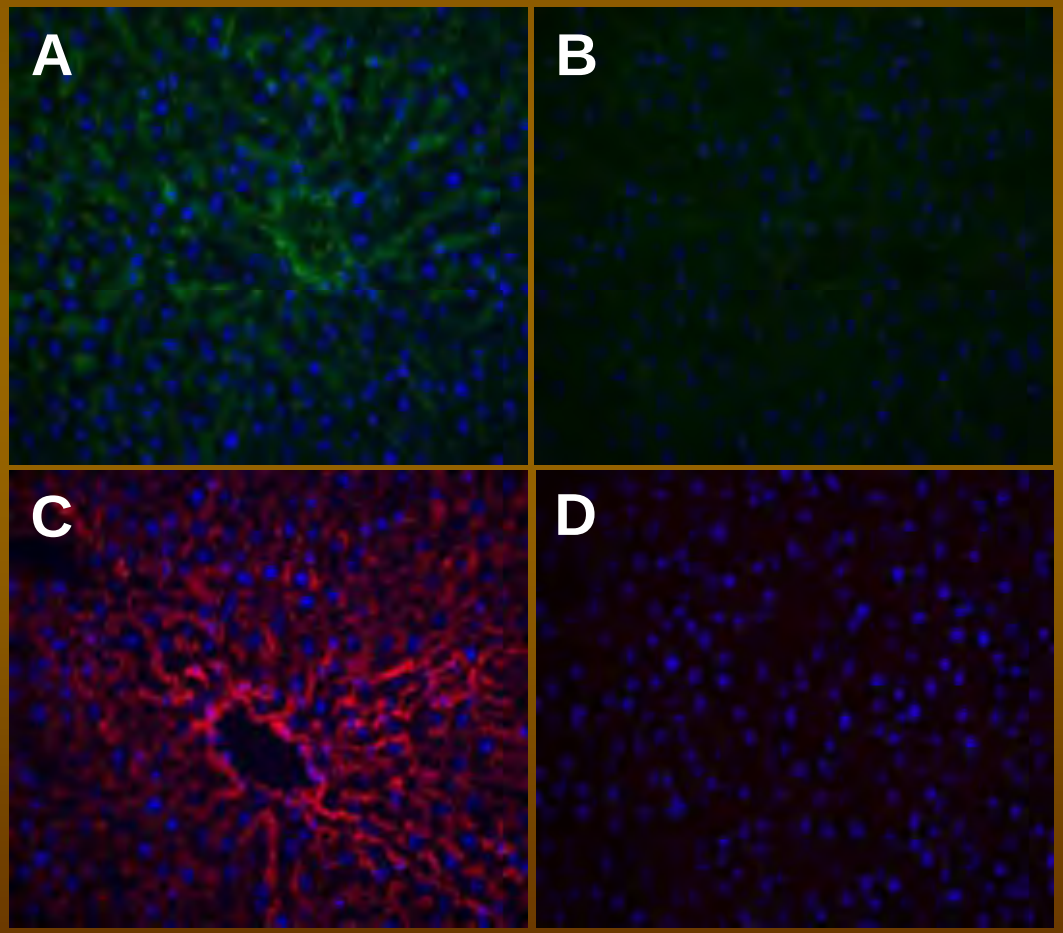




Glypican 3 over-expressed in human hepatocellular carcinomas:
A growth associated gene or a tumor suppressor? Deletions lead to Simpson Golabi Behmel syndrome: diffuse organomegaly including liver.
A candidate terminator for regeneration?

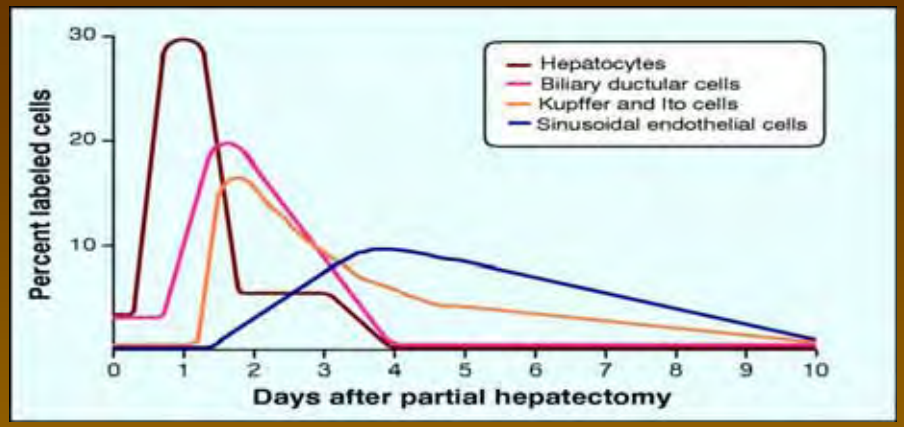
Glypican 3 was subjected to yeast-2 hybrid analysis to check for pairing partners.
Two main partners identified:

1. CD-81 (also known as TAPA-1: Target of Anti-Proliferative Antibody).
 It is considered to be the receptor of entry for HCV.
2. Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate).



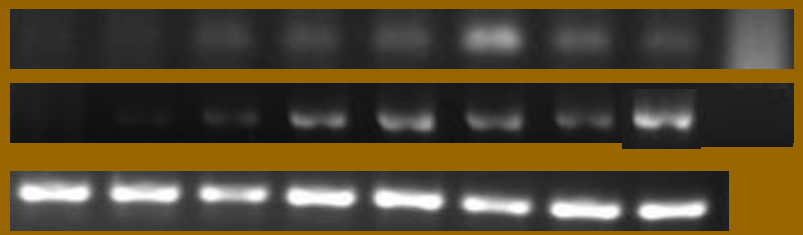
A and B: Glypican 3 immunofluorescence
C and D: CD81 immunofluorescence.
B and D: Negative controls.

A



B

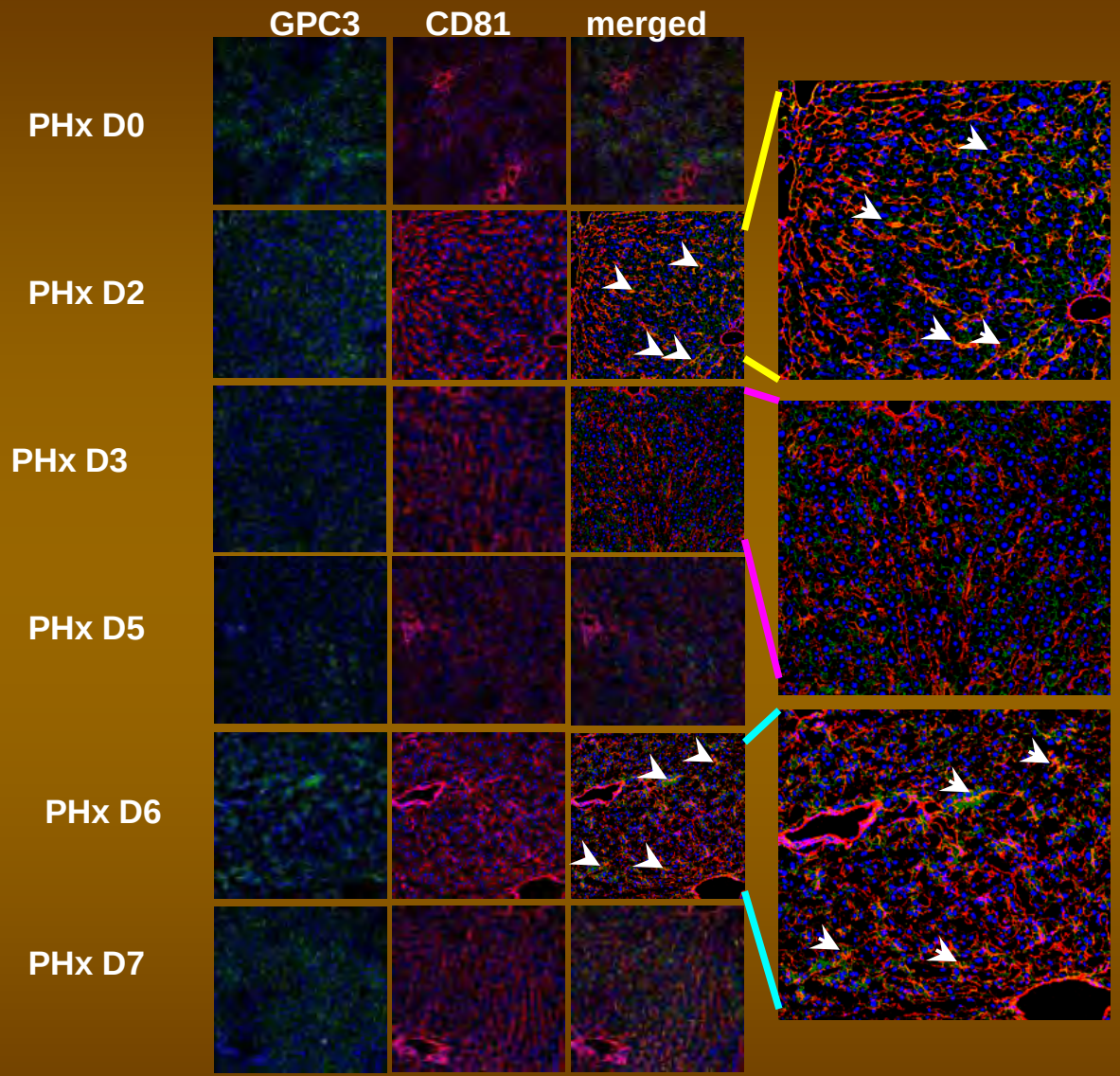
Time after PHx: D0 D1 D2 D3 D4 D5 D6 D7



C

Time after PHx: D0 D1 D2 D3 D4 D5 D6 D7

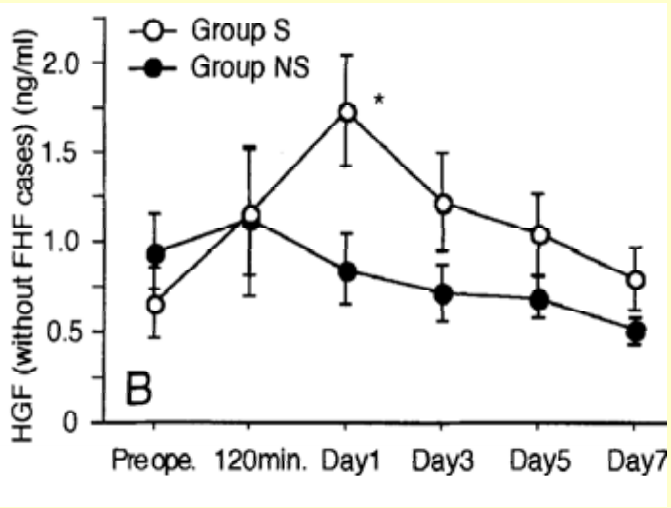
Colocalization of Glypican 3 and CD81 during liver regeneration

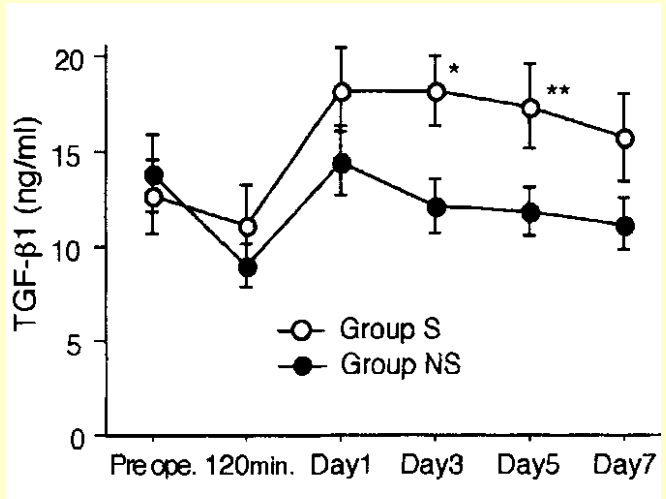


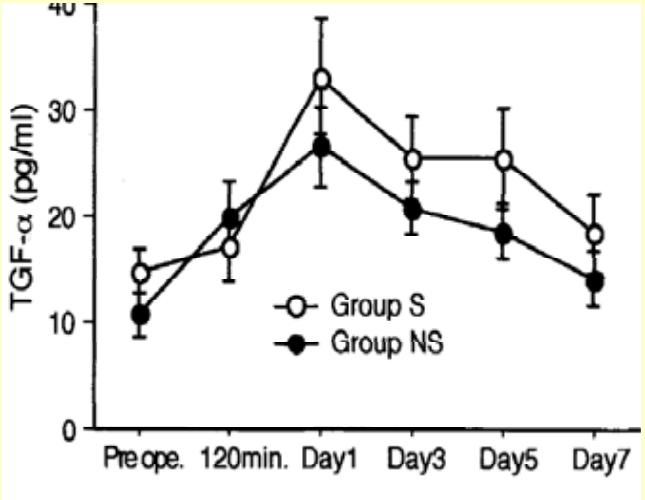
Hepatocyte growth factor and transforming growth factor β contribute to regeneration of small-for-size liver graft immediately after transplantation.

Human

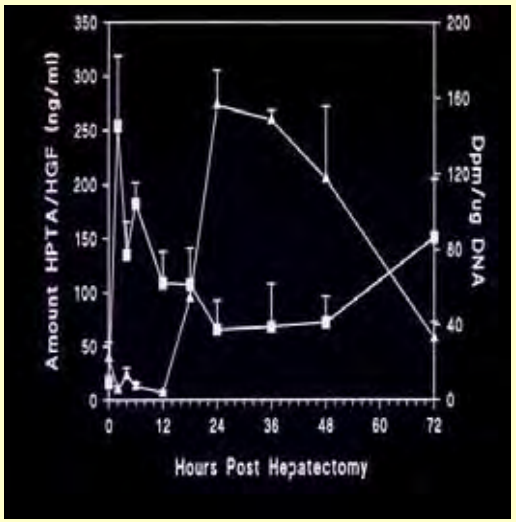
S: Small-for-size.
NS: Not small-for-size

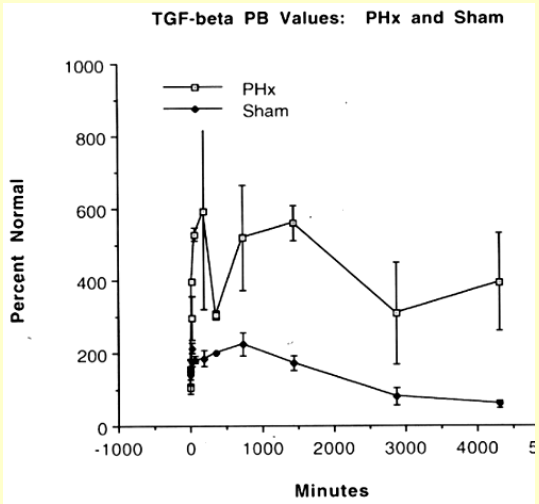




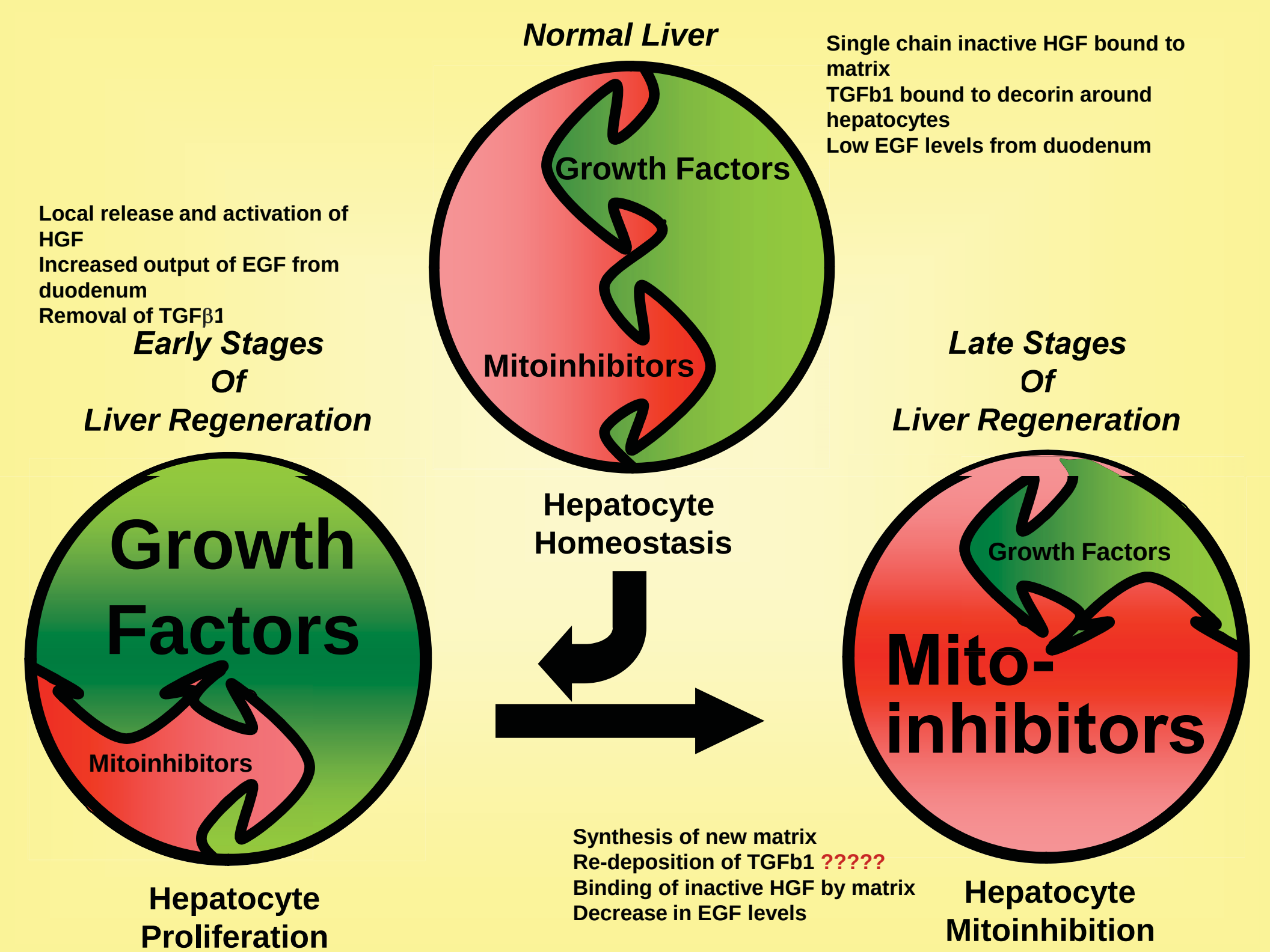


Ninomiya et. al., Transplantation International, 2003, 16:814-819.





Rat


Normal Liver
Single chain inactive HGF bound to matrix
TGFb1 bound to decorin around hepatocytes
Low EGF levels from duodenum
Growth Factors
Mitoinhibitors
Local release and activation of HGF
Increased output of EGF from duodenum
Removal of TGFβ1
Early Stages Of Liver Regeneration
Late Stages Of Liver Regeneration
Hepatocyte Homeostasis
Growth Factors
Mitoinhibitors
Growth Factors
Mito-inhibitors
Synthesis of new matrix
Re-deposition of TGFb1 ?????
Binding of inactive HGF by matrix
Decrease in EGF levels
Hepatocyte Proliferation
Hepatocyte Mitoinhibition

Lab Collaborators:

- William Bowen Senior Research Technician All good things in the lab
- Kari Nejak-Bowen Graduate Student Notch and Jagged, Homer 1c.
- Zahida Khan M.D., Ph.D student HIF, PHD and peroxisomes
- Pallavi Limaye Research Associate Hepatocyte transformation into bile ducts
- Shirish Paranjpe Research Associate HGF and Met silencing
- Bowen Liu Graduate Student Glypican 3

Independent Collaborators

• Wendy Mars	uPA and uPAR
• Paul Monga	beta Catenin
• Reza Zarnegar	Met and Fas
• Aaron Bell	Matrix, PB and HNF4
• Jianhua Luo	HCC Gene Expression and Genomic Analysis
• George Tseng	Biostatistics
• Marie DeFrances	Regeneration studies
• A. Jake Demetris,	Liver Pathology
• Mike Nalesnik and	
• Erin Ochoa	
• Steve Strom	Human Hepatocytes
• Yuhua Liu	HGF Plasmids