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virtual liver
network

Cross-talk of HGF and IL-6 in liver regeneration

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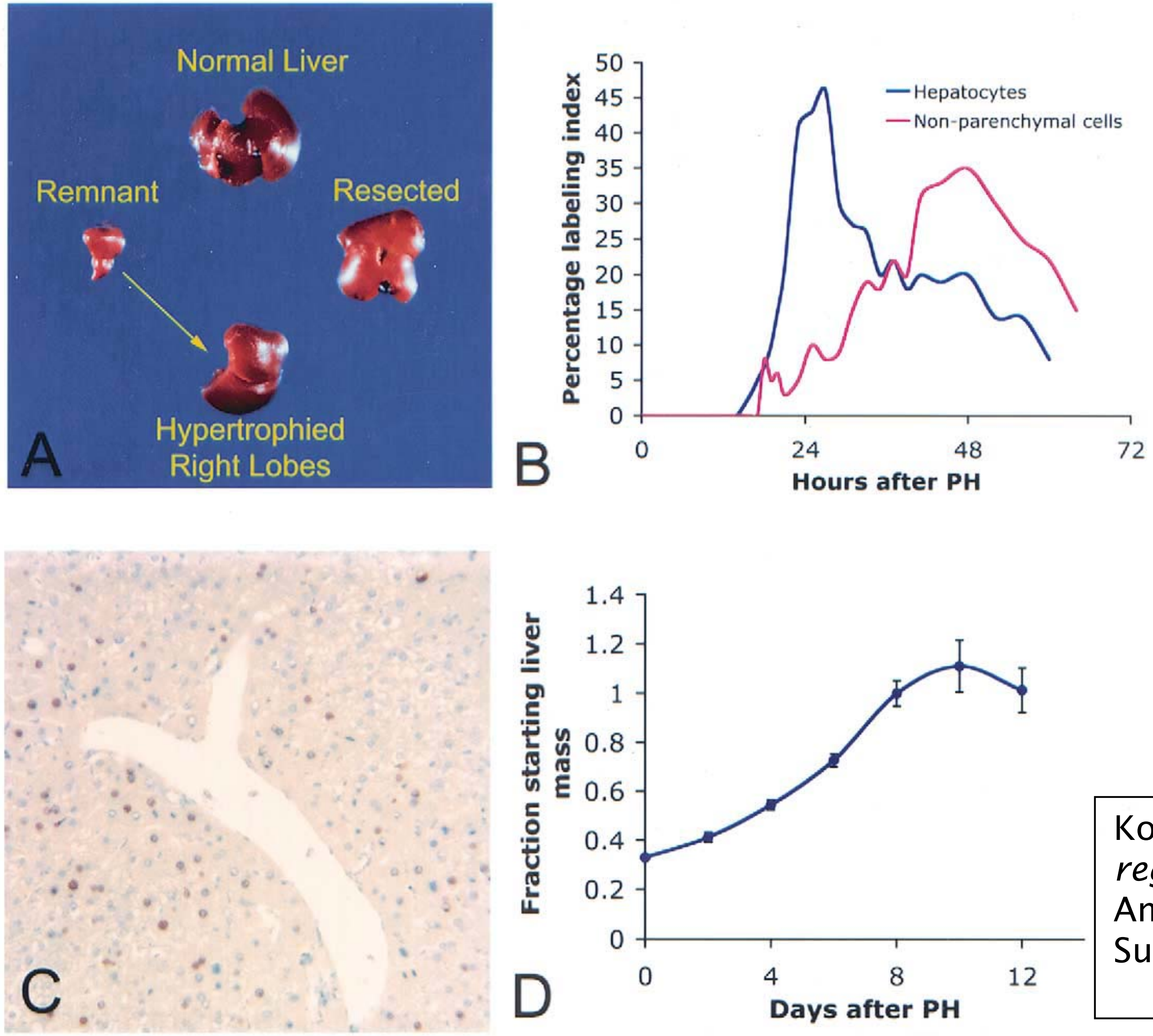
COMPUTATIONAL SYSTEMS BIOCHEMISTRY

Outline

- Role of HGF and IL-6 in liver regeneration
    - liver regeneration
    - HGF as regeneration driver
    - IL-6 as regeneration unlocker
- Cross-talk categorized and measured by indicators
- ExTILAR
- Hepcidin story - example of a highlighted gene

Liver regeneration after 2/3 hepatectomy

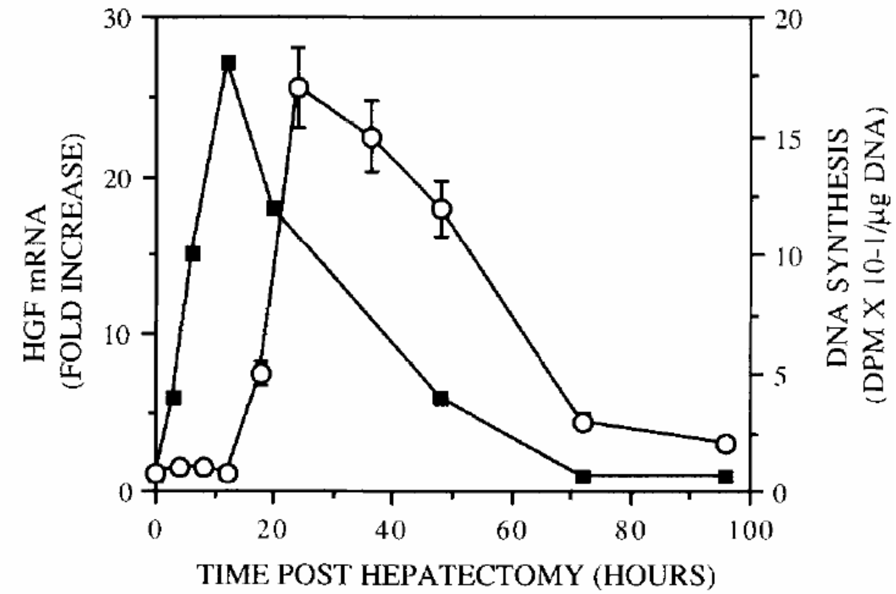


(B) Percentage of 3H-thymidine labeled liver cells at timepoints after two-thirds PH.

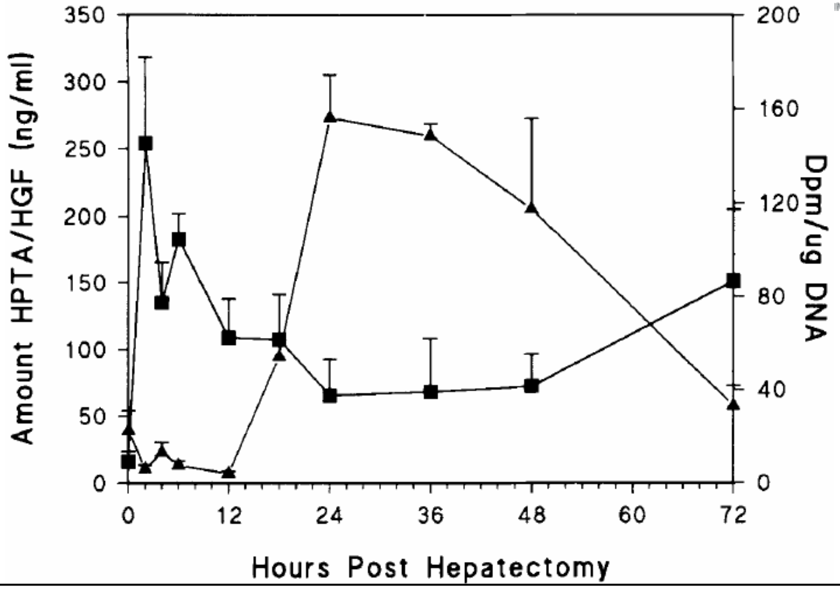
(C) BrdU incorporation into proliferating rat hepatocytes after CCl4 treatment

Koniaris et al. (2003) *Liver regeneration.* Journal of the American College of Surgeons, 197(4), 634-659.

HGF hepatocellular growth factor=hepatopoietin A=scatter factor released and induced in regeneration



Zarnegar et al. (1991) *Expression of Hepatocyte Growth Factor mRNA in regenerating rat liver after partial hepatectomy.* Biochem. Biophys. Res. Comm. 25(7) : 585-592.

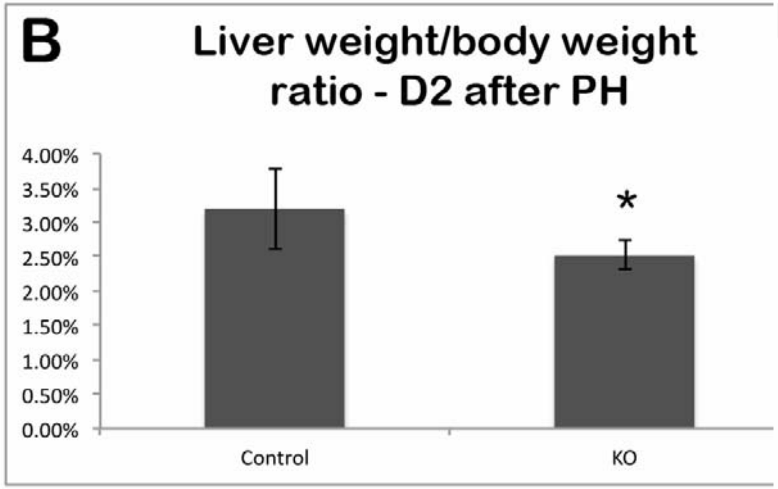


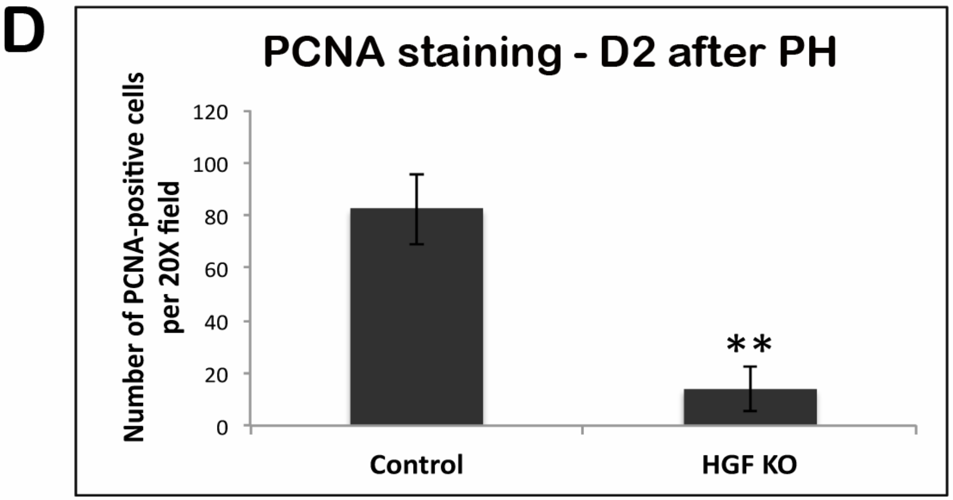
Lindroos et al. (1991) *Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration.* Hepatology 13:743-749.

- HGF is deposited in the extracellular matrix
- It is released by any type of injury – and by partial hepatectomy
- It is induced in hepatocytes and stellate cells
- Excreted from stellate and sinusoidal endothelial cells

Michalopoulus (2007) *Liver Regeneration.* J. Cell. Physiol. 213: 286-300.

HGF essential for proliferation





Liver regeneration is compromised in HGF KO mice after CCl4 and PH due to a decrease in HGF

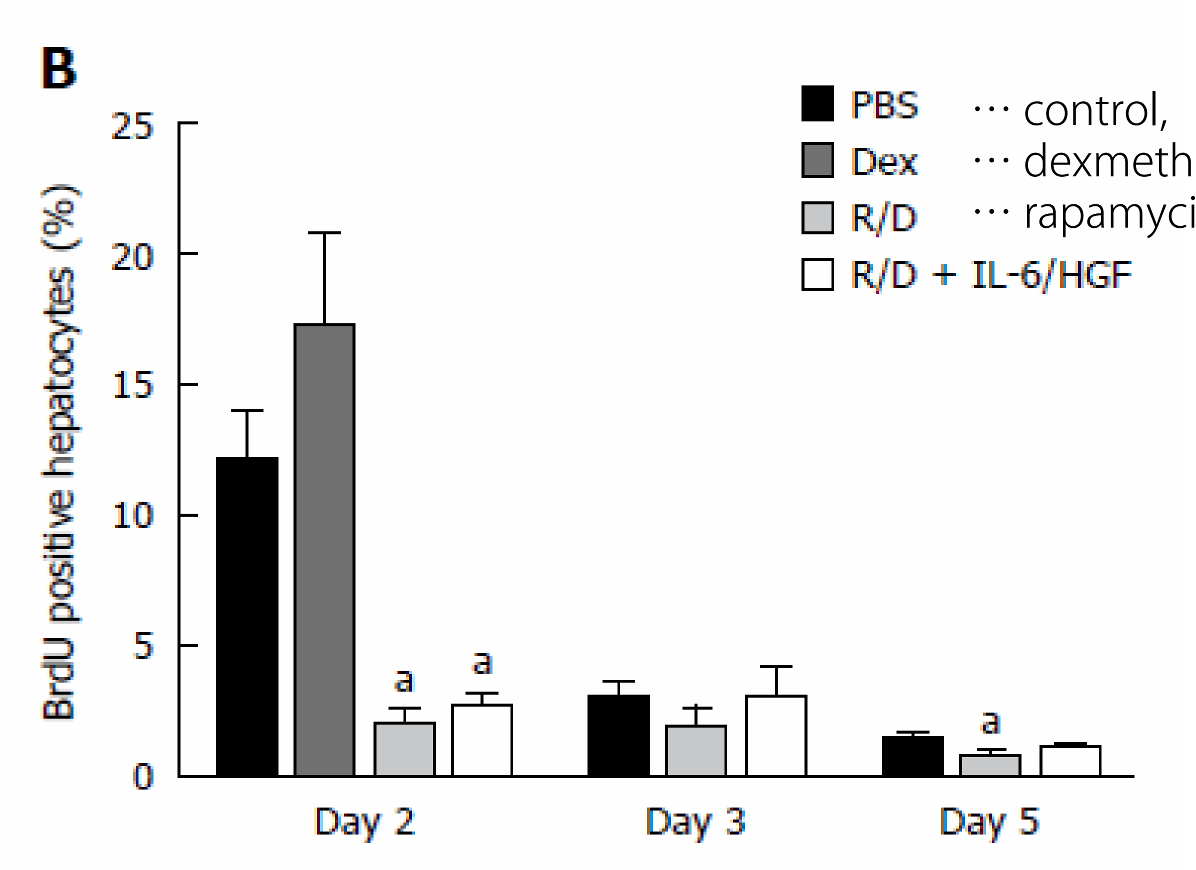
(B) Graph of liver weight to body weight ratios after CCl4/PH shows a significant decrease in HGF KOs at D2. (*P,0.05)

(D) PCNA IHC on livers harvested at D2 after PH in control and HGF KO animals treated with CCl4

- PCNA ··· Proliferating-Cell-Nuclear-Antigen

Nejak-Bowen et al. (2013) *Conditional Genetic Elimination of Hepatocyte Growth Factor in Mice Compromises Liver. Regeneration after Partial Hepatectomy.* PLoS ONE 8(3): e59836.

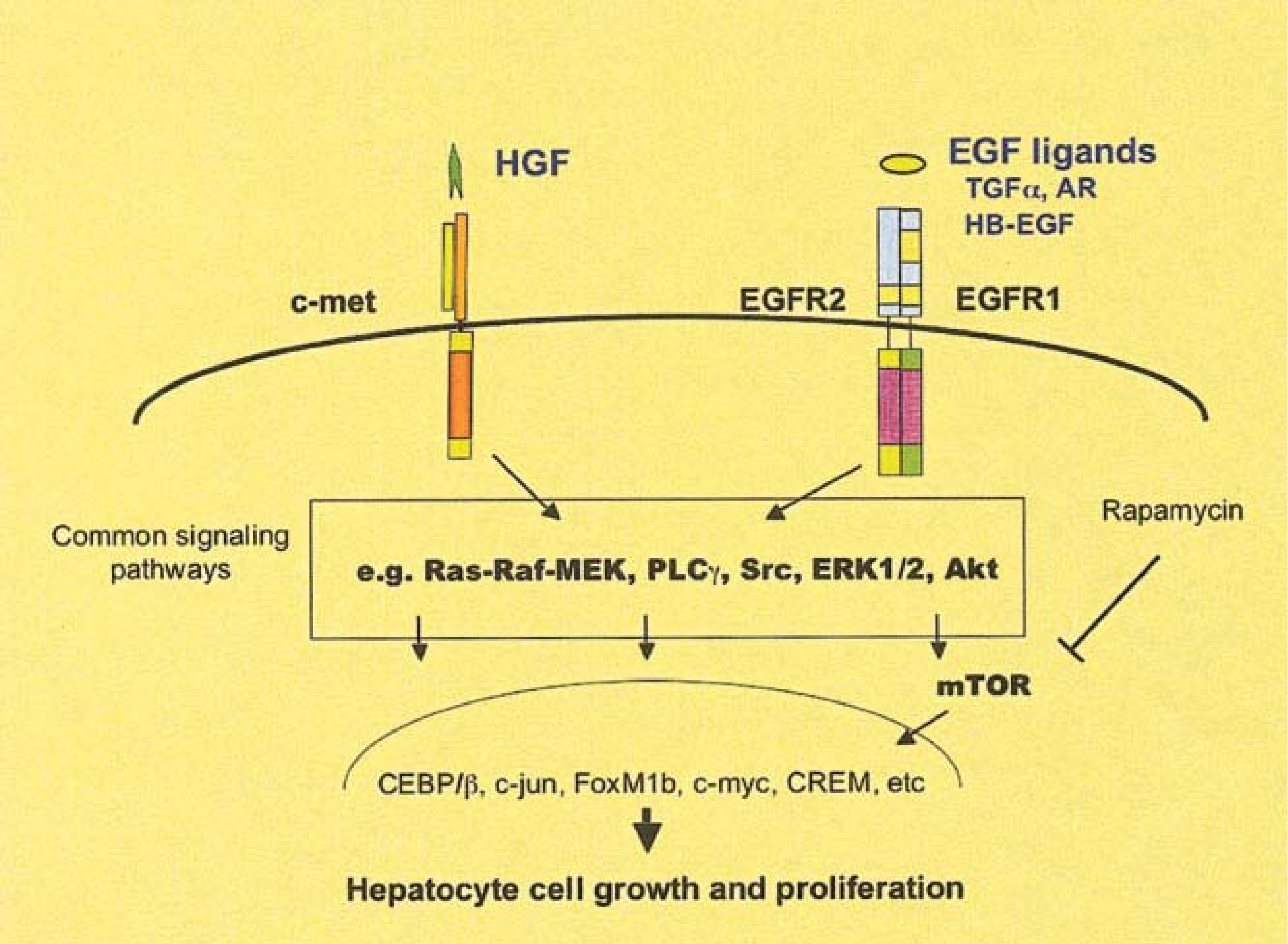
mTOR signaling essential for proliferation



··· control,
··· dexmethasone,
··· rapamycin+ dexmethasone

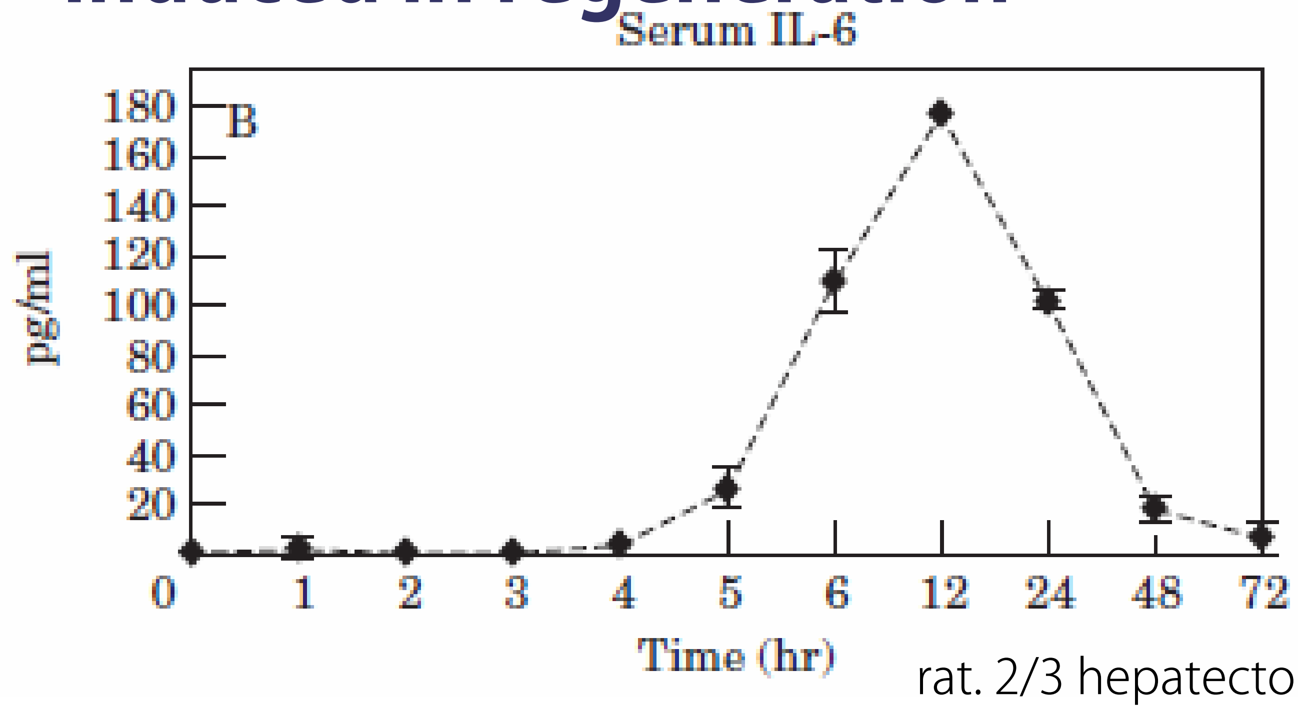
Fouraschen et al (2013) *mTOR signaling in liver regeneration: Rapamycin combined with growth factor treatment.* World J Transplant; 3(3): 36-47.

HGF signaling



Fausto et al. (2006) Liver Regeneration. Hepatology 2006;43:S45-S53.

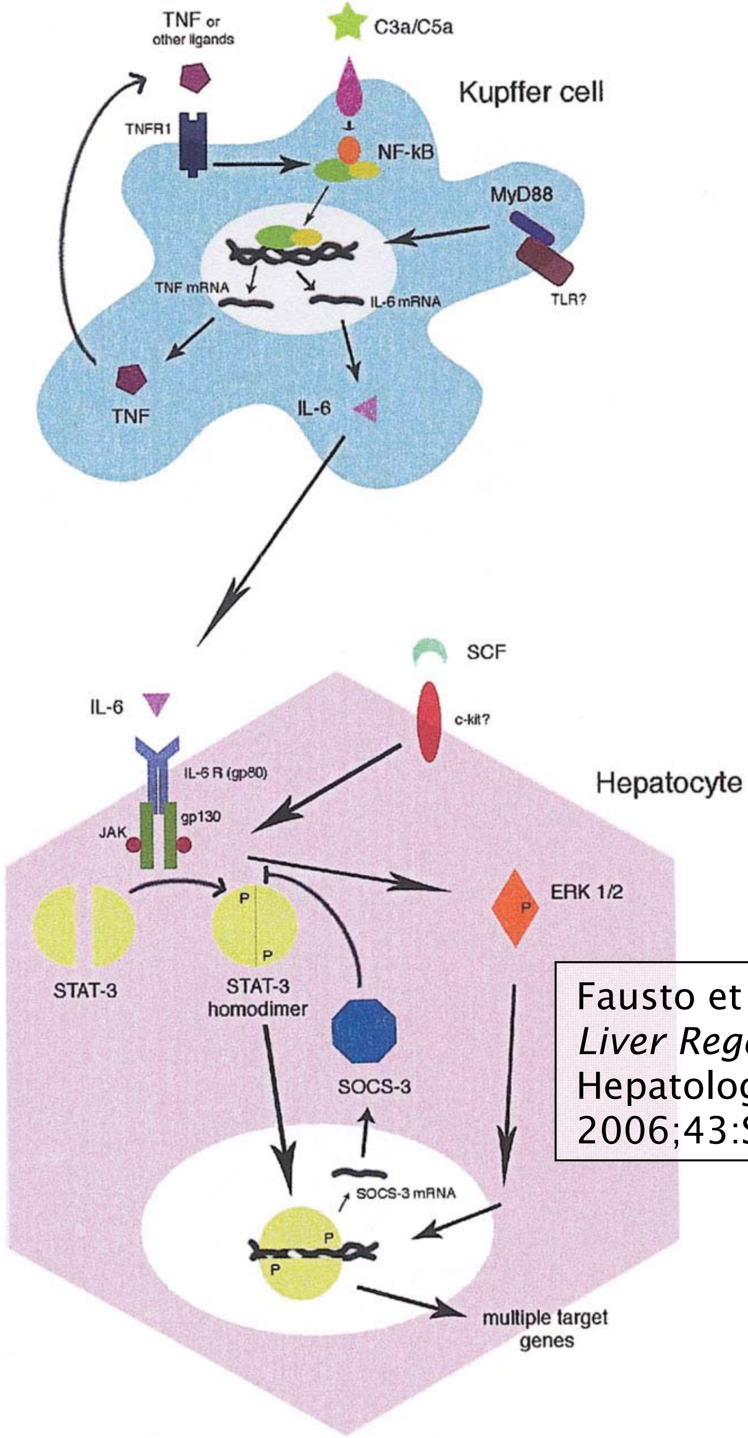
IL-6 (interleukin 6) induced in regeneration



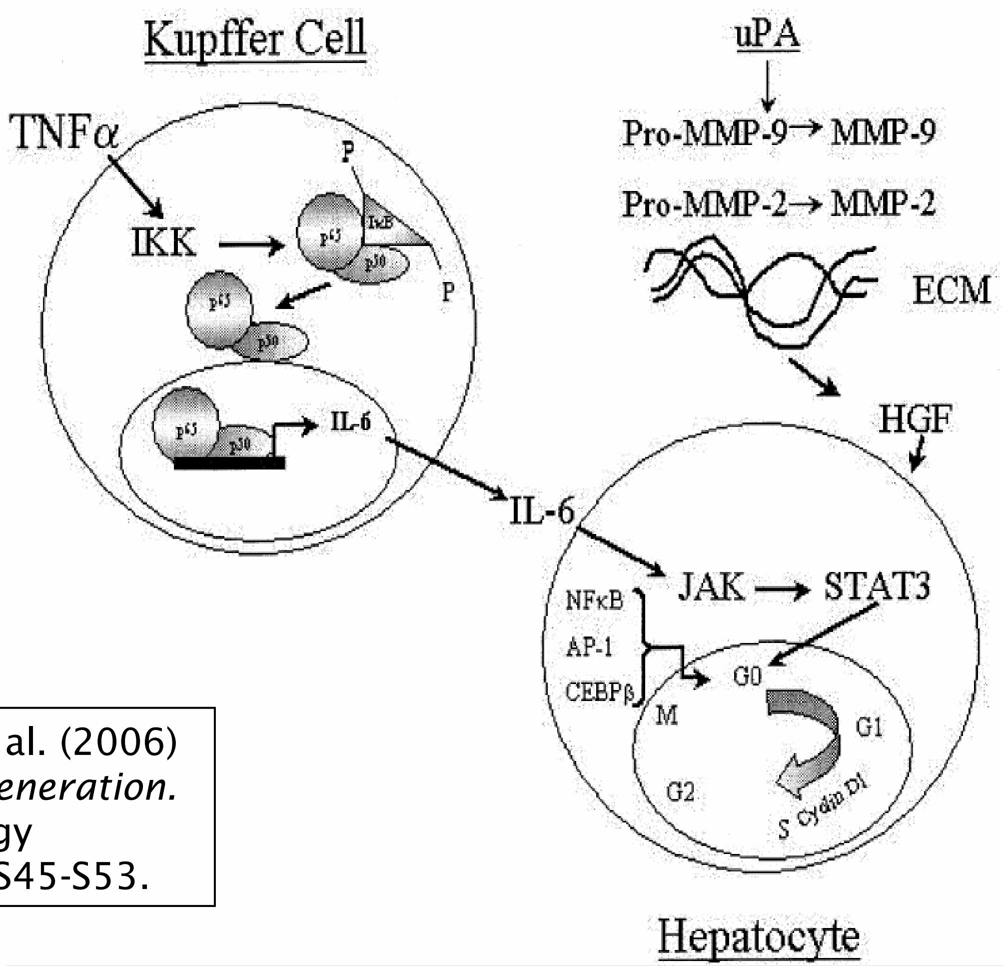
rat. 2/3 hepatectomy

Fulop et al. (2001) *Hepatic regeneration induces transient acute phase reaction: systemic elevation of acute phase reactants and soluble cytokine receptors.* Cell biology international 25(7) : 585-592.

IL-6 signaling



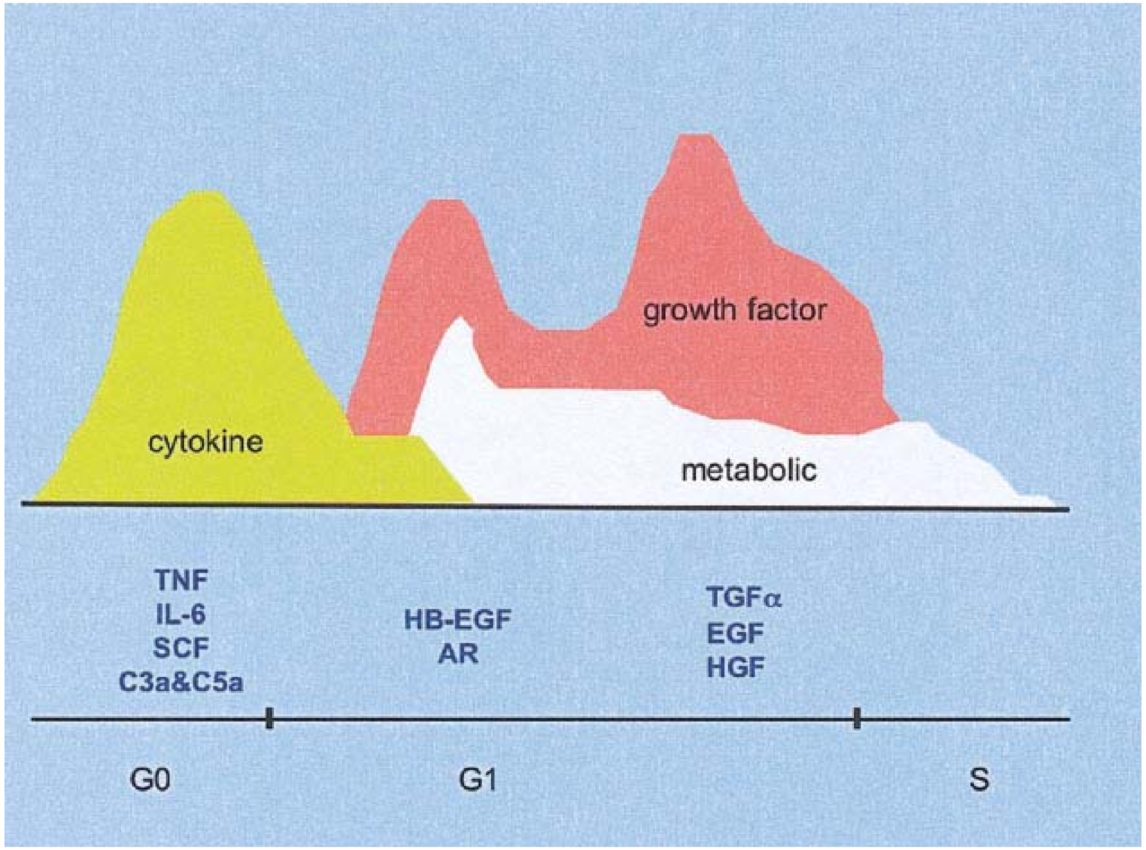
Fausto et al. (2006) *Liver Regeneration.* Hepatology 2006;43:S45-S53.



Black et al. (2004) *Molecular and Cellular Features of Hepatic Regeneration.* Journal of Surgical Research 117, 306–315.

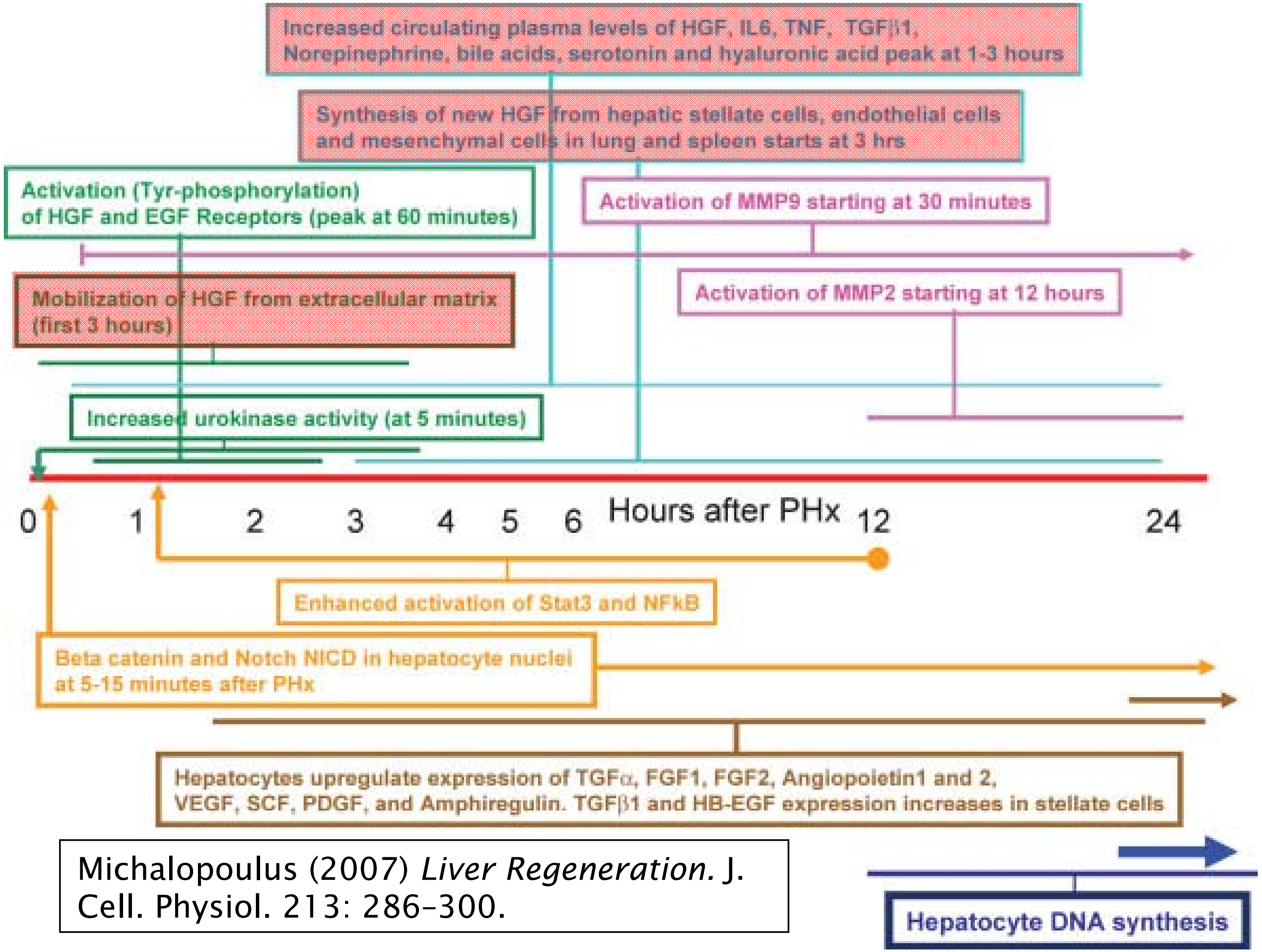
Fausto model of regeneration control

- Scheduled protocol of cytokine signals



Fausto et al. (2006) *Liver Regeneration.* Hepatology 2006;43:S45-S53.

Cytokine signaling program in regeneration



Increased circulating plasma levels of HGF, IL6, TNF, TGFβ1, Norepinephrine, bile acids, serotonin and hyaluronic acid peak at 1-3 hours

Synthesis of new HGF from hepatic stellate cells, endothelial cells and mesenchymal cells in lung and spleen starts at 3 hrs

Activation (Tyr-phosphorylation) of HGF and EGF Receptors (peak at 60 minutes)

Activation of MMP9 starting at 30 minutes

Mobilization of HGF from extracellular matrix (first 3 hours)

Activation of MMP2 starting at 12 hours

Increased urokinase activity (at 5 minutes)

Enhanced activation of Stat3 and NFkB

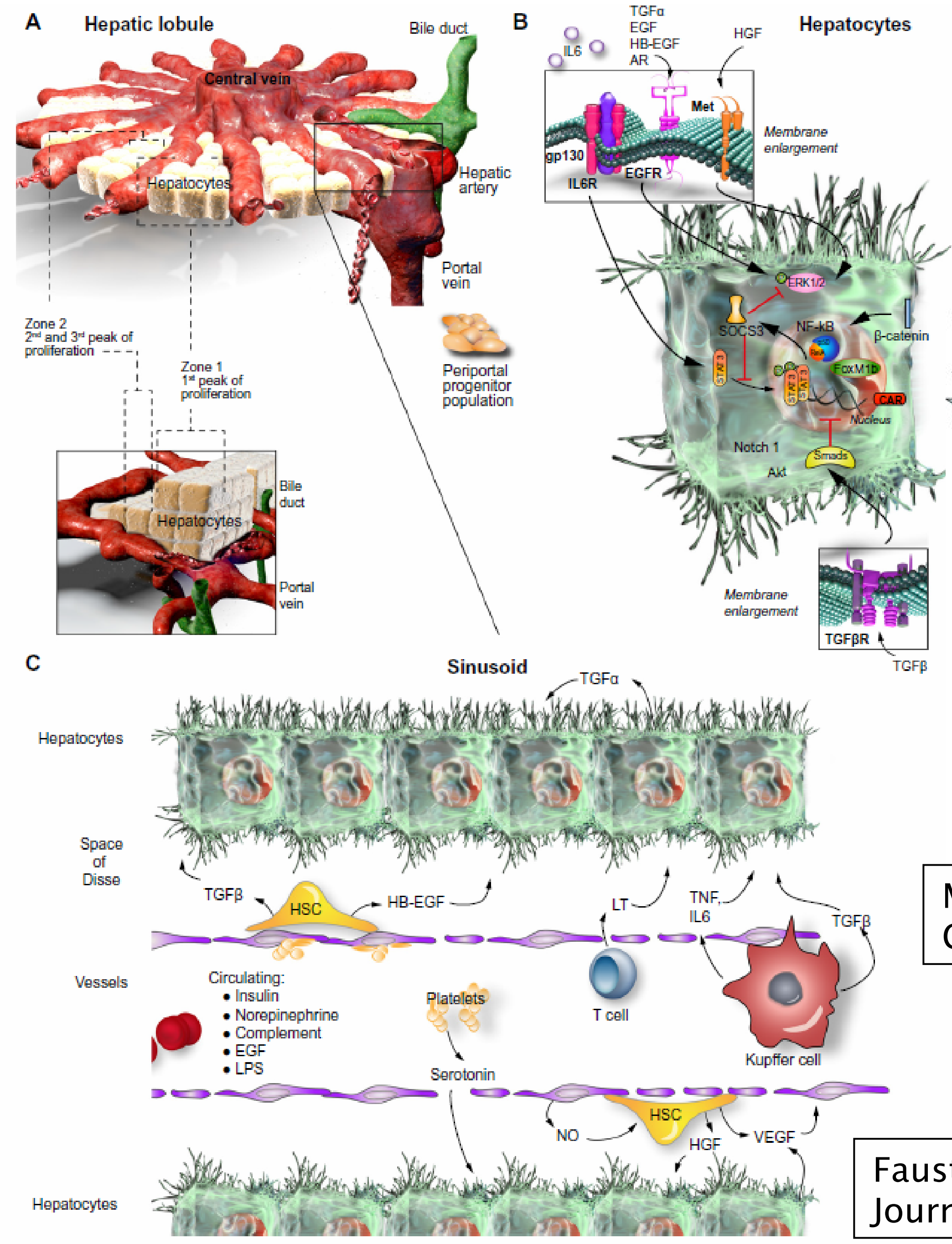
Beta catenin and Notch NICD in hepatocyte nuclei at 5-15 minutes after PHx

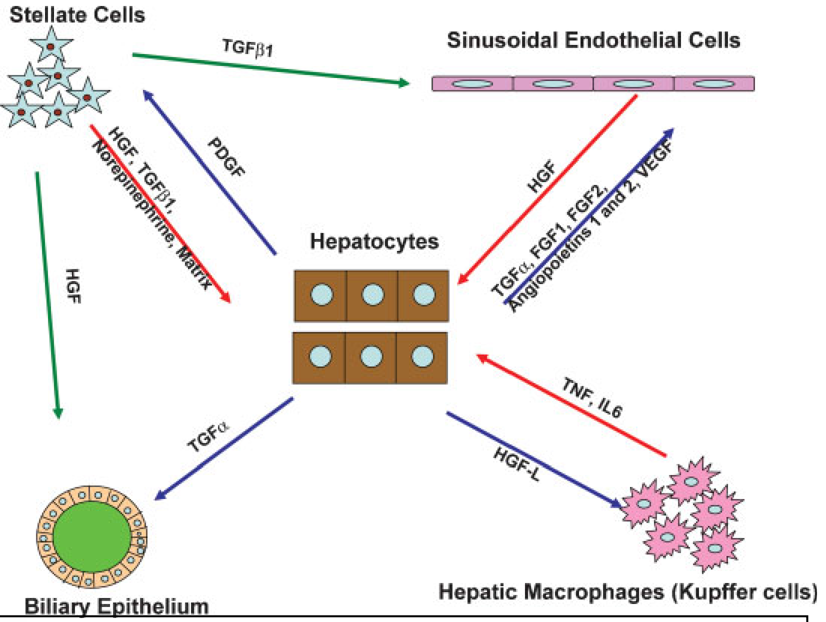
Hepatocytes upregulate expression of TGFα, FGF1, FGF2, Angiopoietin1 and 2, VEGF, SCF, PDGF, and Amphiregulin. TGFβ1 and HB-EGF expression increases in stellate cells

Michalopoulus (2007) *Liver Regeneration.* J. Cell. Physiol. 213: 286-300.

Hepatocyte DNA synthesis

Tissue level





Michalopoulus (2007) Liver Regeneration. J. Cell. Physiol. 213: 286-300.

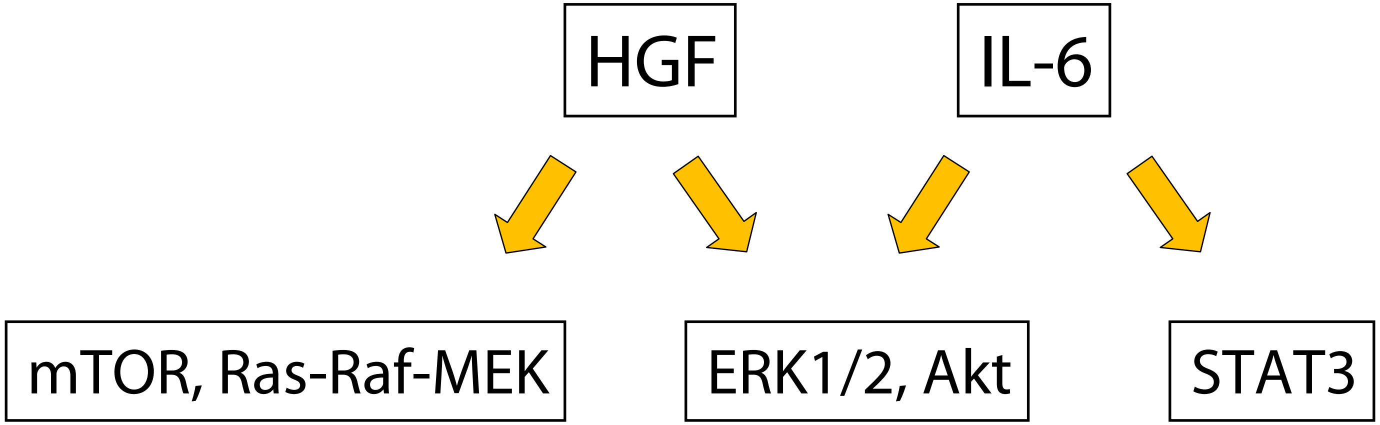
Fausto et al. (2012) *Liver Regeneration.* Journal of Hepatology 57: 692-4.

Problems - Question

Michalopoulus (2007) *Liver Regeneration.* J. Cell. Physiol. 213: 286–300.

- In hepatocyte cell culture experiments:
  - HGF alone more or less mitogenic
  - IL-6 alone not mitogenic
  - IL-6 and HGF combined strongly mitogenic
- Both HGF and IL-6 polyspecific
  - IL-6
    - pro-inflammatory cytokine
    - anti-inflammatory myokine
  - HGF
    - growth signal for epithelial, endothelial cells, haemopoietic progenitor cells.
    - cell motility
    - morphogenic factor, in myogenesis, wound healing
- What specific effects occur if HGF and IL-6 acting at the same time?

Disjoint vs. common signaling



- For which genes
  - do they support each other? *Synergy*
  - does one block the other? *Attenuation*
  - do they diverge? *Spread*
  - Which dominates for diverging genes? *Dominance*

Ranked list

- Genes filtered
  - at least one time point >5.5
  - at least one treatment effect >0.5, $p<0.05$
- Indicators defined for each category:
  - Synergy
  - Attenuation
  - Spread
  - Dominance
- Purposes
  - rank
  - measure, compatible $\log_2$-fold change

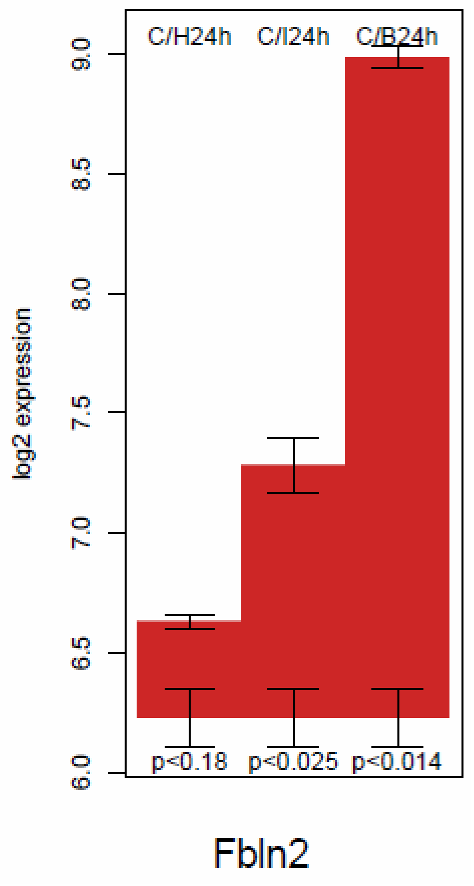
Synergy

··· creation of a whole that is greater than the simple sum of its parts.

- Same direction of regulation of HGF and IL-6
- Combined treatment larger effect than single

$$\begin{aligned} \sigma_t^+ &= c_t - \max\ (a_t, b_t) \quad a_t > 0, b_t > 0 \\ \sigma_t^- &= -c_t + \min\ (a_t, b_t) \quad a_t < 0, b_t < 0 \end{aligned}$$

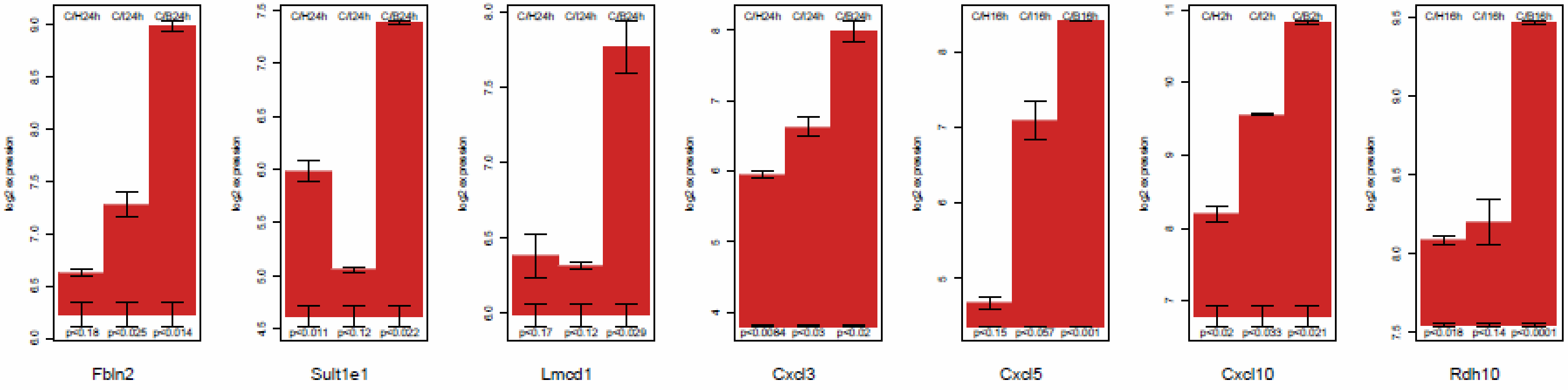
a_t … expression change induced by HGF
b_t … expression change induced by IL-6
c_t … expression change induced by HGF+IL-6



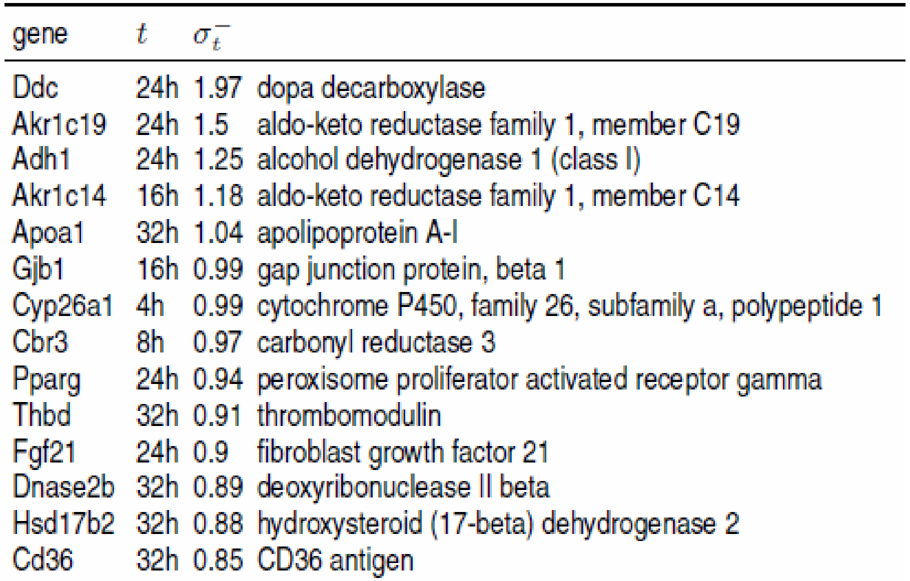
Highest synergy in up-regulation

gene	t	σ_t^+	
Fbln2	24h	1.7	fibulin 2
Sult1e1	24h	1.39	sulfotransferase family 1E, member 1
Lmcd1	24h	1.38	LIM and cysteine-rich domains 1
Cxcl3	24h	1.36	chemokine (C-X-C motif) ligand 3
Cxcl5	16h	1.31	chemokine (C-X-C motif) ligand 5
Cxcl10	2h	1.26	chemokine (C-X-C motif) ligand 10
Rdh10	16h	1.26	retinol dehydrogenase 10 (all-trans)
Arg2	16h	1.24	arginase type II
Spred3	16h	1.22	sprouty-related, EVH1 domain containing 3
Irx3	24h	1.21	Iroquois related homeobox 3 (Drosophila)
Fos	2h	1.05	FBJ osteosarcoma oncogene
P2ry14	24h	1.05	purinergic receptor P2Y, G-protein coupled, 14
Tgm1	4h	1.04	transglutaminase 1, K polypeptide
Havcr1	16h	0.99	hepatitis A virus cellular receptor 1

- Fibulin 2, marker for myofibroblasts, shows fibrosis
- C-X-C ligands, intracellular communication

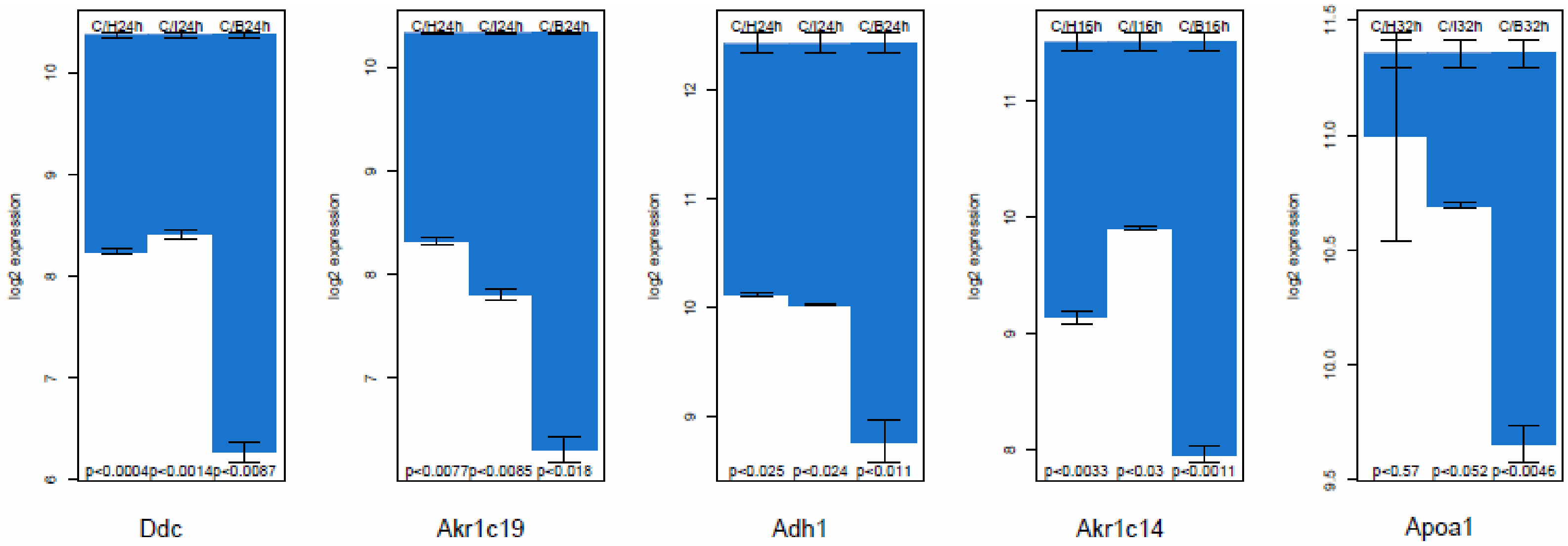


Highest synergy in down-regulation



gene	t	σ_t^-	
Ddc	24h	1.97	dopa decarboxylase
Akr1c19	24h	1.5	aldo-keto reductase family 1, member C19
Adh1	24h	1.25	alcohol dehydrogenase 1 (class I)
Akr1c14	16h	1.18	aldo-keto reductase family 1, member C14
Apoa1	32h	1.04	apolipoprotein A-I
Gjb1	16h	0.99	gap junction protein, beta 1
Cyp26a1	4h	0.99	cytochrome P450, family 26, subfamily a, polypeptide 1
Cbr3	8h	0.97	carbonyl reductase 3
Pparg	24h	0.94	peroxisome proliferator activated receptor gamma
Thbd	32h	0.91	thrombomodulin
Fgf21	24h	0.9	fibroblast growth factor 21
Dnase2b	32h	0.89	deoxyribonuclease II beta
Hsd17b2	32h	0.88	hydroxysteroid (17-beta) dehydrogenase 2
Cd36	32h	0.85	CD36 antigen

- Ddc, also degrades cytokines
- Akr 1c14/1c19 embryonal isoforms

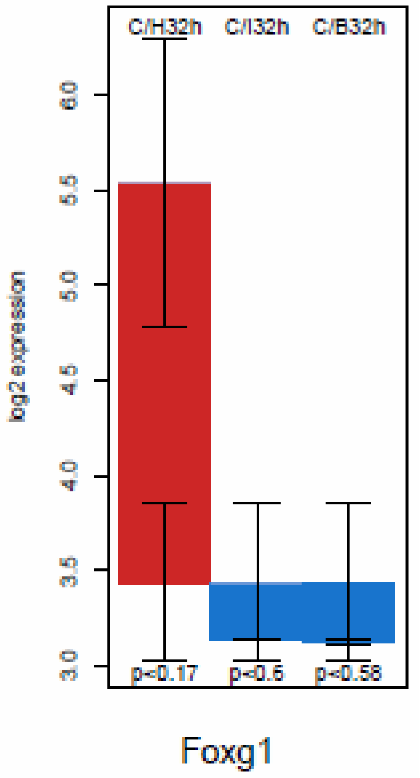


Attenuation

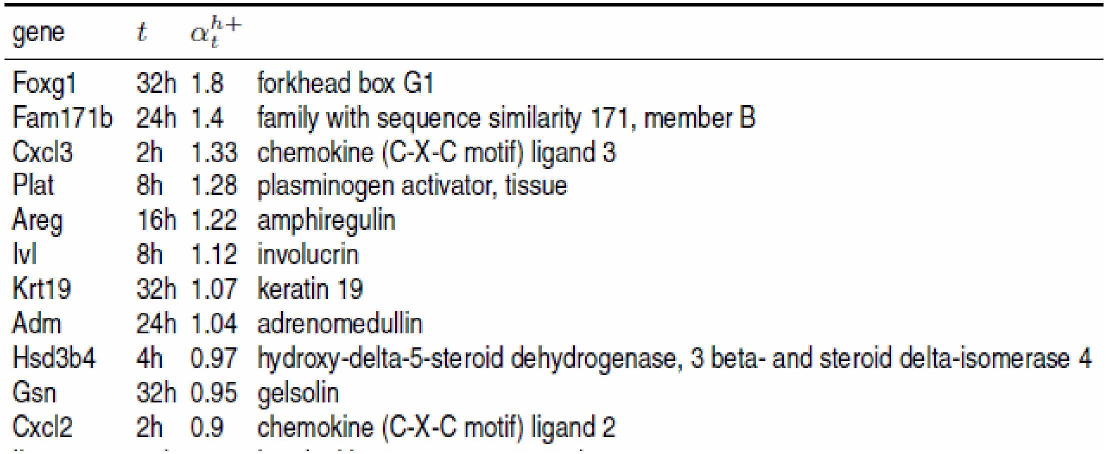
- Significant effect to one factor
- Little effect to the other
- Little effect to combined treatment

$$\begin{aligned}
\alpha_t^{h+} &= a_t - \mathrm{pos}(c_t) - \mathrm{neg}(b_t) && \text{HGF up}\\
\alpha_t^{h-} &= -a_t - \mathrm{neg}(c_t) - \mathrm{pos}(b_t) && \text{HGF down}\\
\alpha_t^{i+} &= b_t - \mathrm{pos}(c_t) - \mathrm{neg}(a_t) && \text{IL-6 up}\\
\alpha_t^{i-} &= -b_t - \mathrm{neg}(c_t) - \mathrm{pos}(a_t) && \text{IL-6 down}
\end{aligned}$$

a_t … expression change induced by HGF
b_t … expression change induced by IL-6
c_t … expression change induced by HGF+IL-6

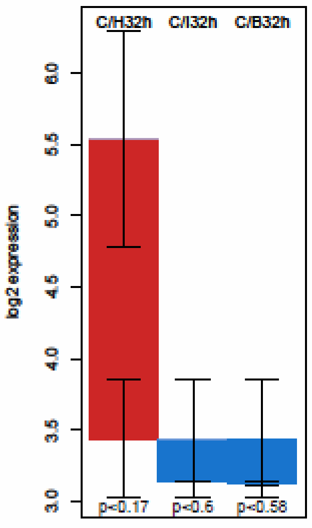


Highest attenuation of HGF up-regulation

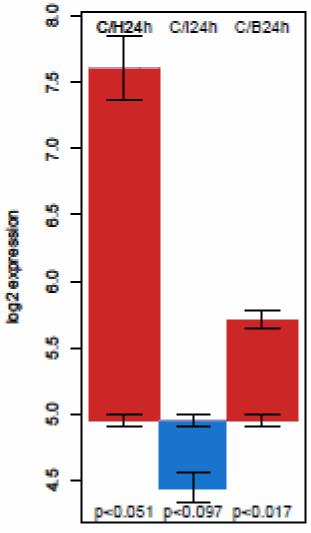


gene	t	α_t^{h+}	
Foxg1	32h	1.8	forkhead box G1
Fam171b	24h	1.4	family with sequence similarity 171, member B
Cxcl3	2h	1.33	chemokine (C-X-C motif) ligand 3
Plat	8h	1.28	plasminogen activator, tissue
Areg	16h	1.22	amphiregulin
Ivl	8h	1.12	involucrin
Krt19	32h	1.07	keratin 19
Adm	24h	1.04	adrenomedullin
Hsd3b4	4h	0.97	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 4
Gsn	32h	0.95	gelsolin
Cxcl2	2h	0.9	chemokine (C-X-C motif) ligand 2

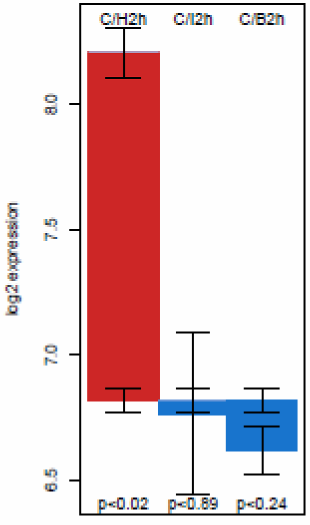
- Foxg1 cancer marker, unknown function



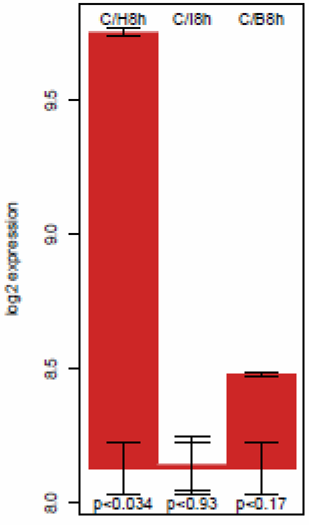
Foxg1



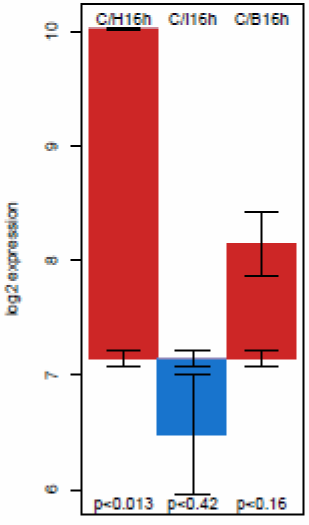
Fam171b



Cxcl3



Plat

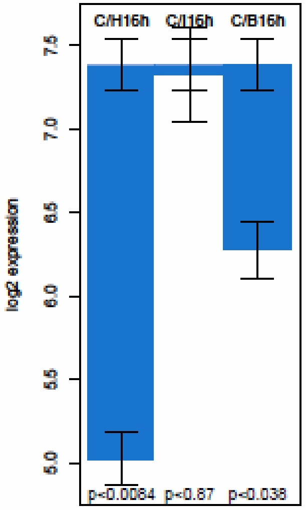


Areg

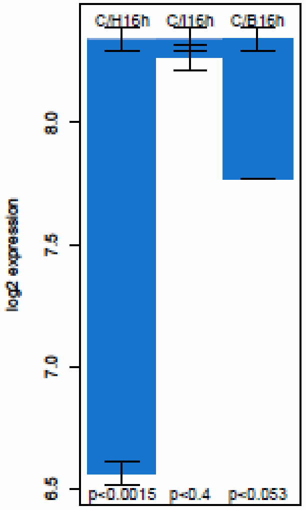
Highest attenuation of HGF down-regulation

gene	t	α_t^{h-}	
Cpn2	16h	1.25	carboxypeptidase N, polypeptide 2
Nedd9	16h	1.21	neural precursor cell expressed, developmentally down-regulated gene 9
Crp	16h	1.13	C-reactive protein, pentraxin-related
Hamp	4h	1.08	hepcidin antimicrobial peptide
Acnat2	24h	1.08	acyl-coenzyme A amino acid N-acyltransferase 2
Ccl9	24h	1.03	chemokine (C-C motif) ligand 9
St5	8h	0.99	suppression of tumorigenicity 5
Apon	16h	0.99	apolipoprotein N

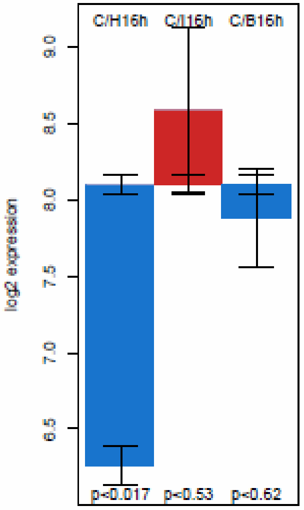
- Nedd9 metastasis marker



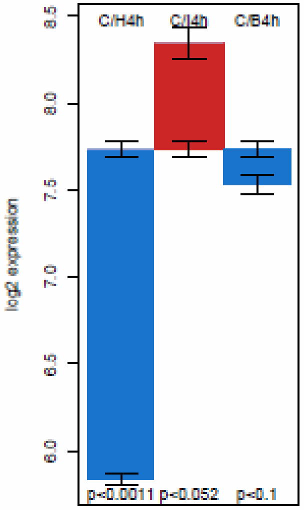
Cpn2



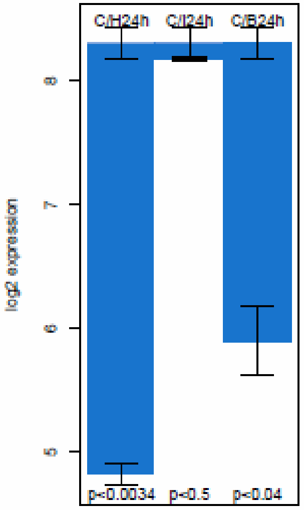
Nedd9



Crp



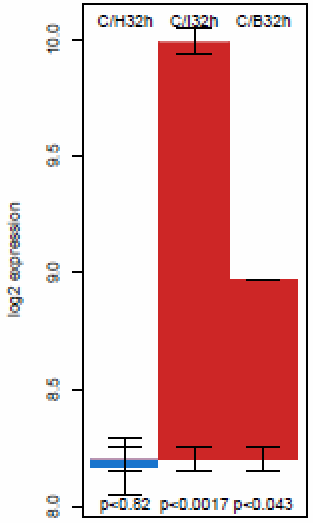
Hamp



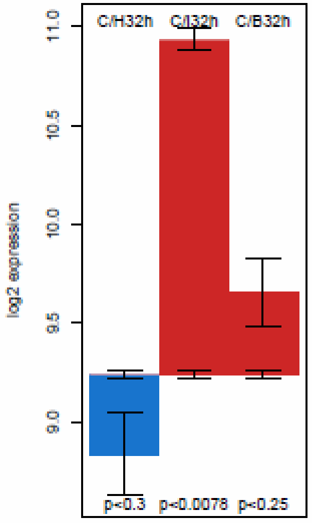
Acnat2

Highest attenuation of IL-6 up-regulation

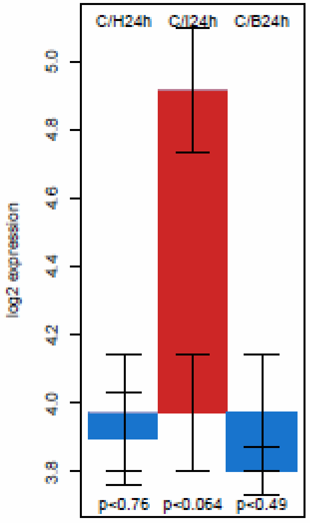
gene	t	α_t^{i+}	
Serpina3n	32h	0.99	serine (or cysteine) peptidase inhibitor, clade A, member 3N
1100001G20Rik	32h	0.88	RIKEN cDNA 1100001G20 gene
Ugt2b38	24h	0.87	UDP glucuronosyltransferase 2 family, polypeptide B38
Tifa	16h	0.76	TRAF-interacting protein with forkhead-associated domain
Ivl	24h	0.73	involucrin



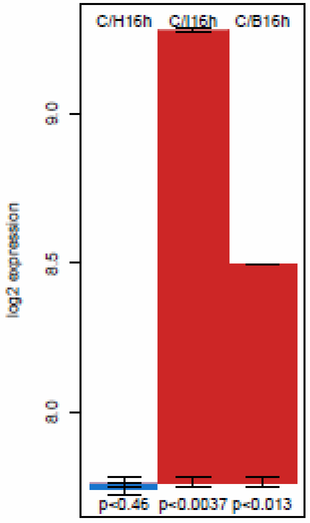
Serpina3n



1100001G20Rik

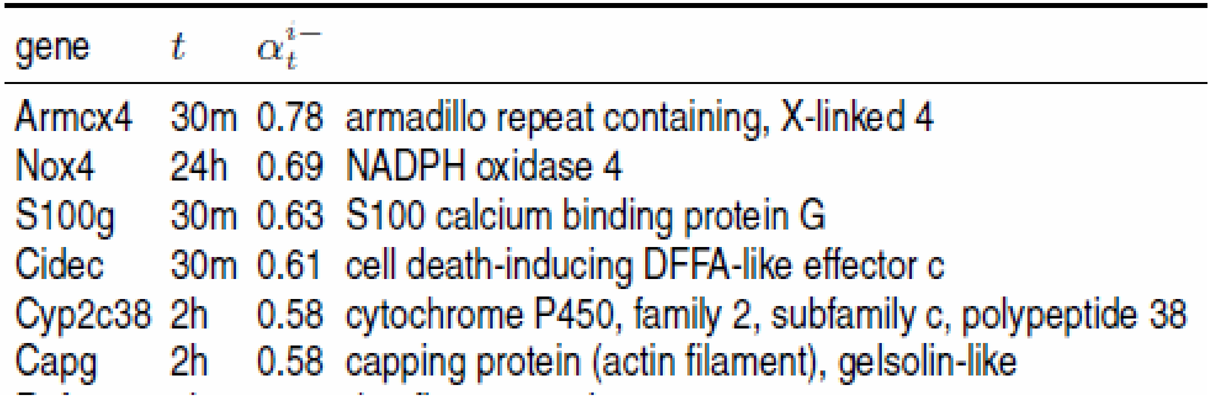


Ugt2b38

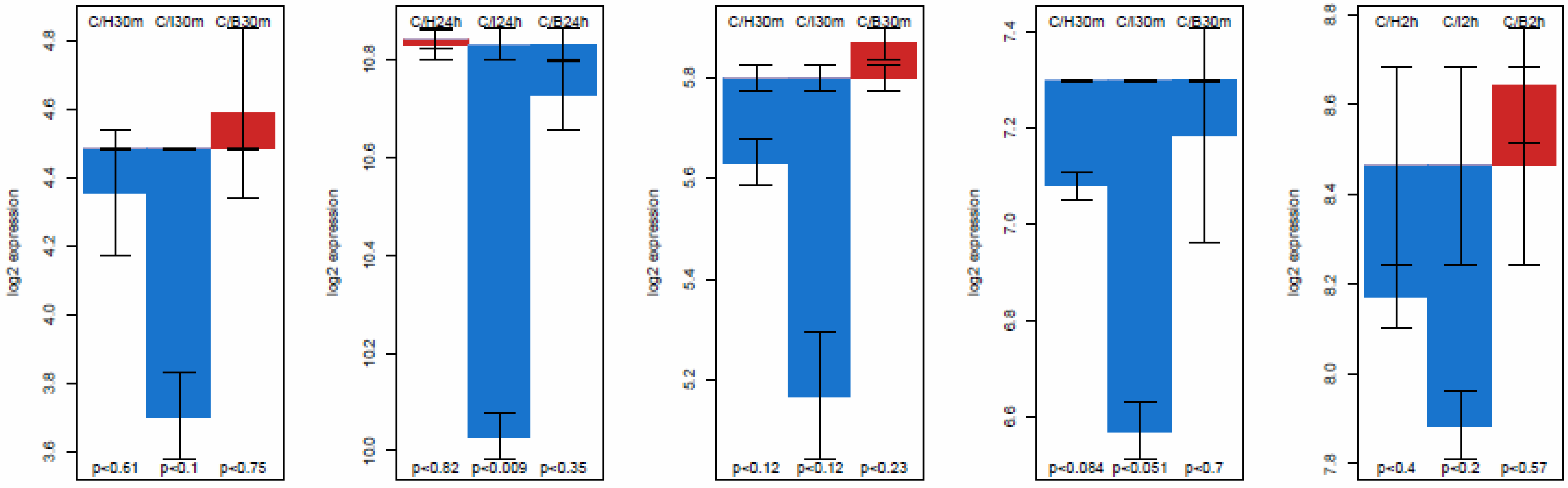


Tifa

Highest attenuation of IL-6 down-regulation



gene	t	α_t^{i-}	
Armcx4	30m	0.78	armadillo repeat containing, X-linked 4
Nox4	24h	0.69	NADPH oxidase 4
S100g	30m	0.63	S100 calcium binding protein G
Cidec	30m	0.61	cell death-inducing DFFA-like effector c
Cyp2c38	2h	0.58	cytochrome P450, family 2, subfamily c, polypeptide 38
Capg	2h	0.58	capping protein (actin filament), gelsolin-like



Armcx4 Nox4 S100g Cidec Cyp2c38

Divergent regulation

$$\psi_t^h = \frac{3}{4}\min\,(a_t,-b_t)+\frac{1}{4}\max\,(a_t,-b_t) \quad a_t>0, b_t<0$$

HGF up/ IL-6 down

$$\psi_t^i = \frac{3}{4}\min\,(-a_t,b_t)+\frac{1}{4}\max\,(-a_t,b_t) \quad a_t<0, b_t>0$$

HGF down/IL-6 up

a_t … expression change induced by HGF
b_t … expression change induced by IL-6
c_t … expression change induced by HGF+IL-6

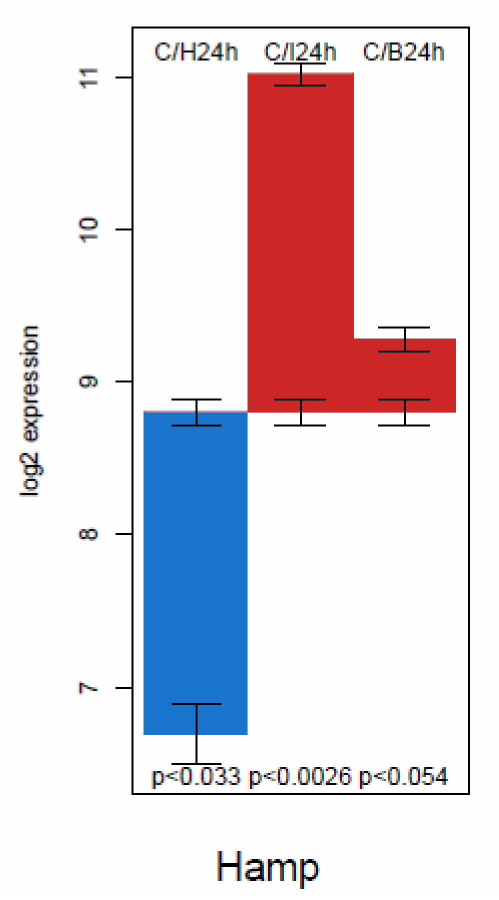
- What the effect of combined treatment?
- Cancel out, HGF dominates, IL-6 dominates.

$$\delta_t^h = \begin{cases} \mathrm{pos}(c_t) & \text{if } a_t>0, b_t<0 \\ \mathrm{neg}(c_t) & \text{if } a_t<0, b_t>0 \end{cases}$$

HGF dominates

$$\delta_t^i = \begin{cases} \mathrm{pos}(c_t) & \text{if } b_t>0, a_t<0 \\ \mathrm{neg}(c_t) & \text{if } b_t<0, a_t>0 \end{cases}$$

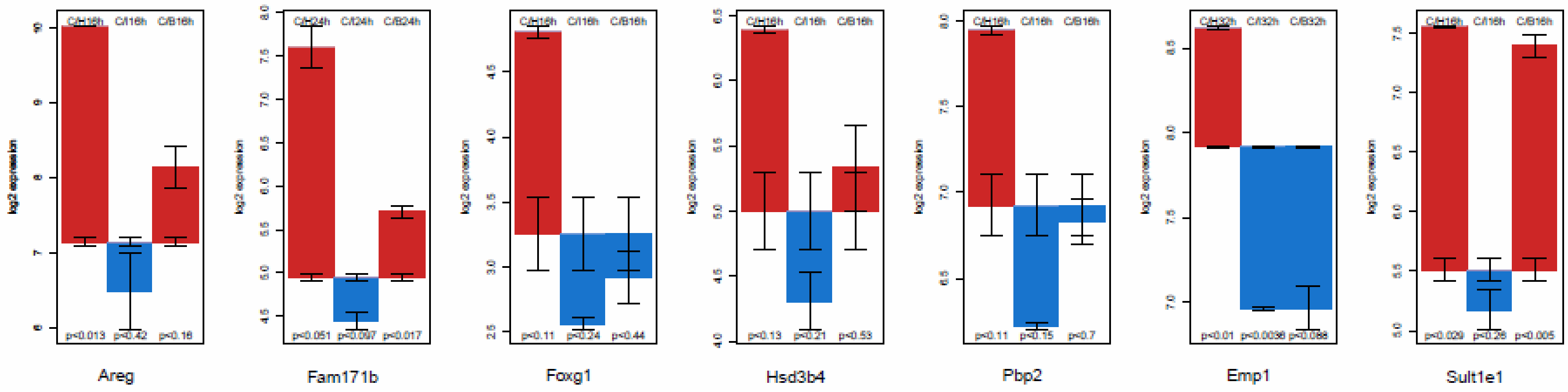
IL-6 dominates



Highest spread of HGF up, IL-6 down

gene	t	ϕ_t^h	δ_t^h	δ_t^i	
Areg	16h	1.22	0.34		amphiregulin
Fam171b	24h	1.04	0.29		family with sequence similarity 171, member B
Foxg1	16h	0.91		0.49	forkhead box G1
Hsd3b4	16h	0.87	0.24		hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid de
Pbp2	16h	0.79		0.14	phosphatidylethanolamine binding protein 2
Emp1	32h	0.77		0.99	epithelial membrane protein 1
Sult1e1	16h	0.76	0.92		sulfotransferase family 1E, member 1

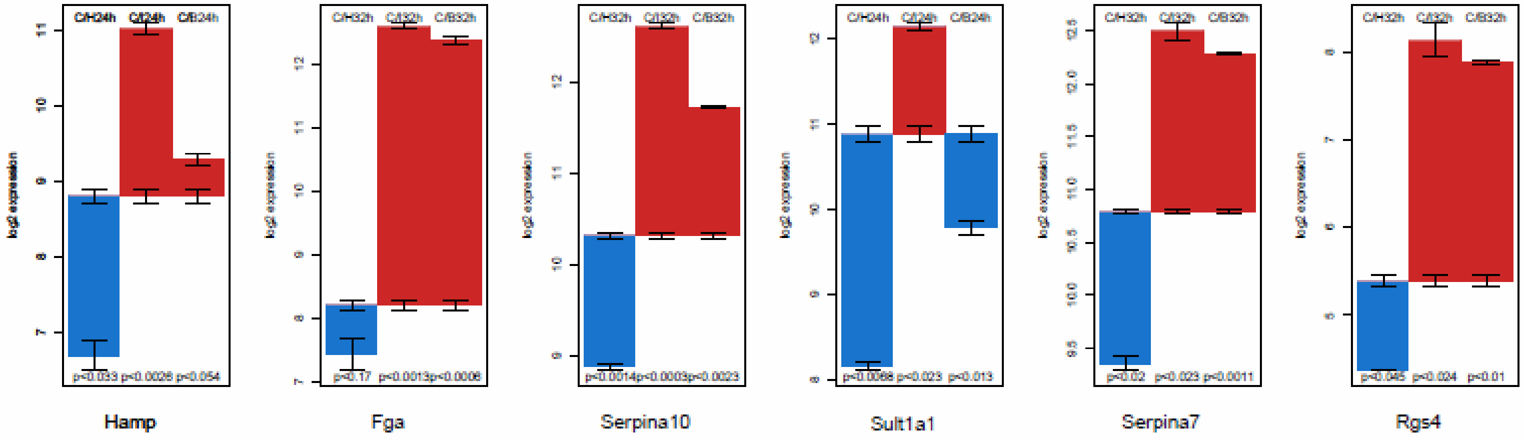
- Amphiregulin, part of wound healing, damage response program
- Hsd3b4 hormone degradation



Highest spread of HGF down, IL-6 up

gene	t	ϕ_t^i	δ_t^h	δ_t^i	
Hamp	24h	2.13		0.21	hepcidin antimicrobial peptide
Fga	32h	1.68		0.95	fibrinogen alpha chain
Serpina10	32h	1.65		0.61	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10
Sult1a1	24h	1.62	0.41		sulfotransferase family 1A, phenol-preferring, member 1
Serpina7	32h	1.5		0.87	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7
Rgs4	32h	1.45		0.91	regulator of G-protein signaling 4
Casp12	16h	1.44		0.22	caspase 12
Nrg4	24h	1.35		0.5	neuregulin 4
Mpeg1	24h	1.34		0.52	macrophage expressed gene 1
Crp	24h	1.33		0.01	C-reactive protein, pentraxin-related
Prg4	24h	1.3		0.57	proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)
Fgg	32h	1.3		0.91	fibrinogen gamma chain
Steap4	24h	1.28		0.6	STEAP family member 4

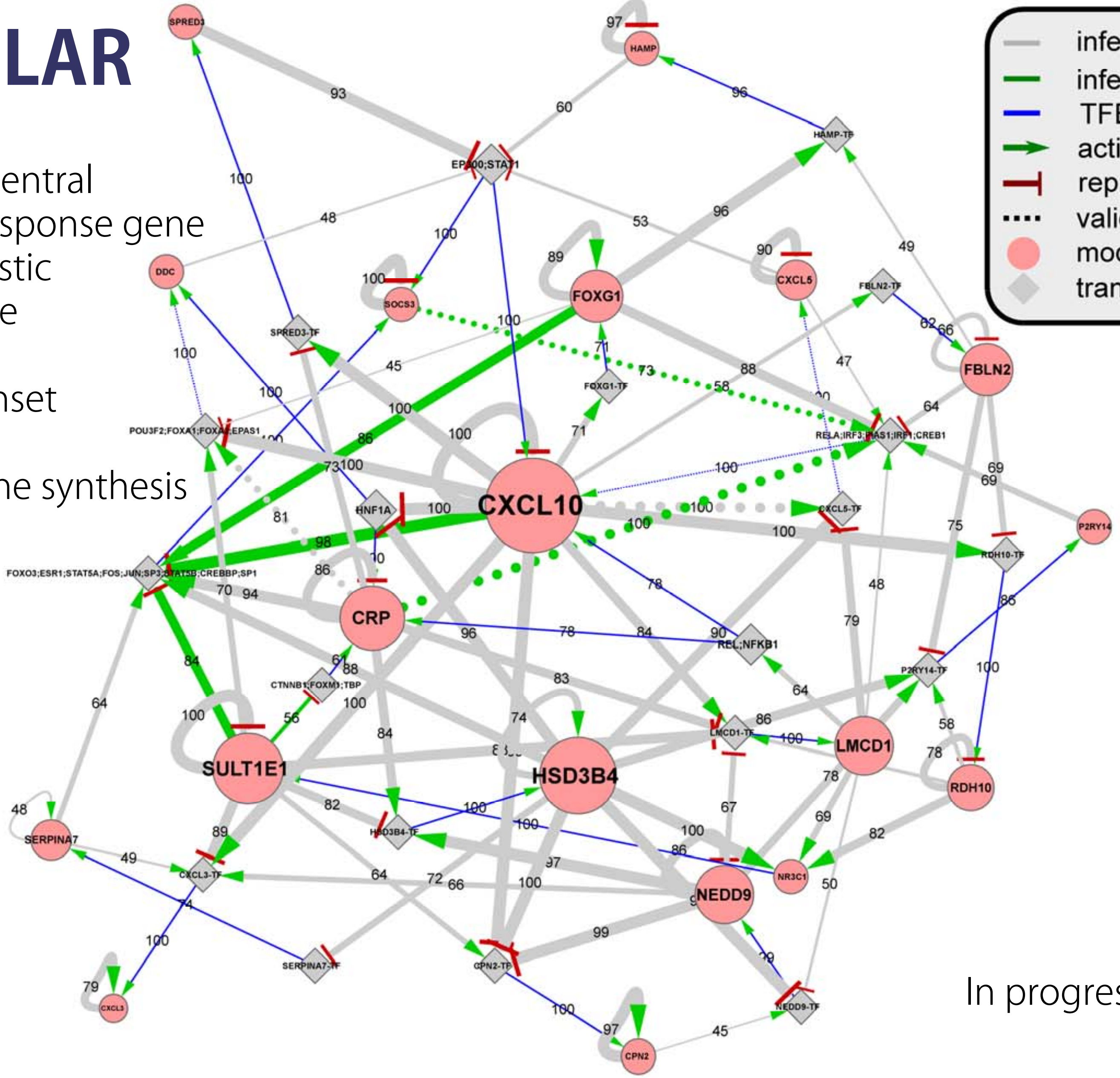
Mostly IL-6 dominates



ExTILAR

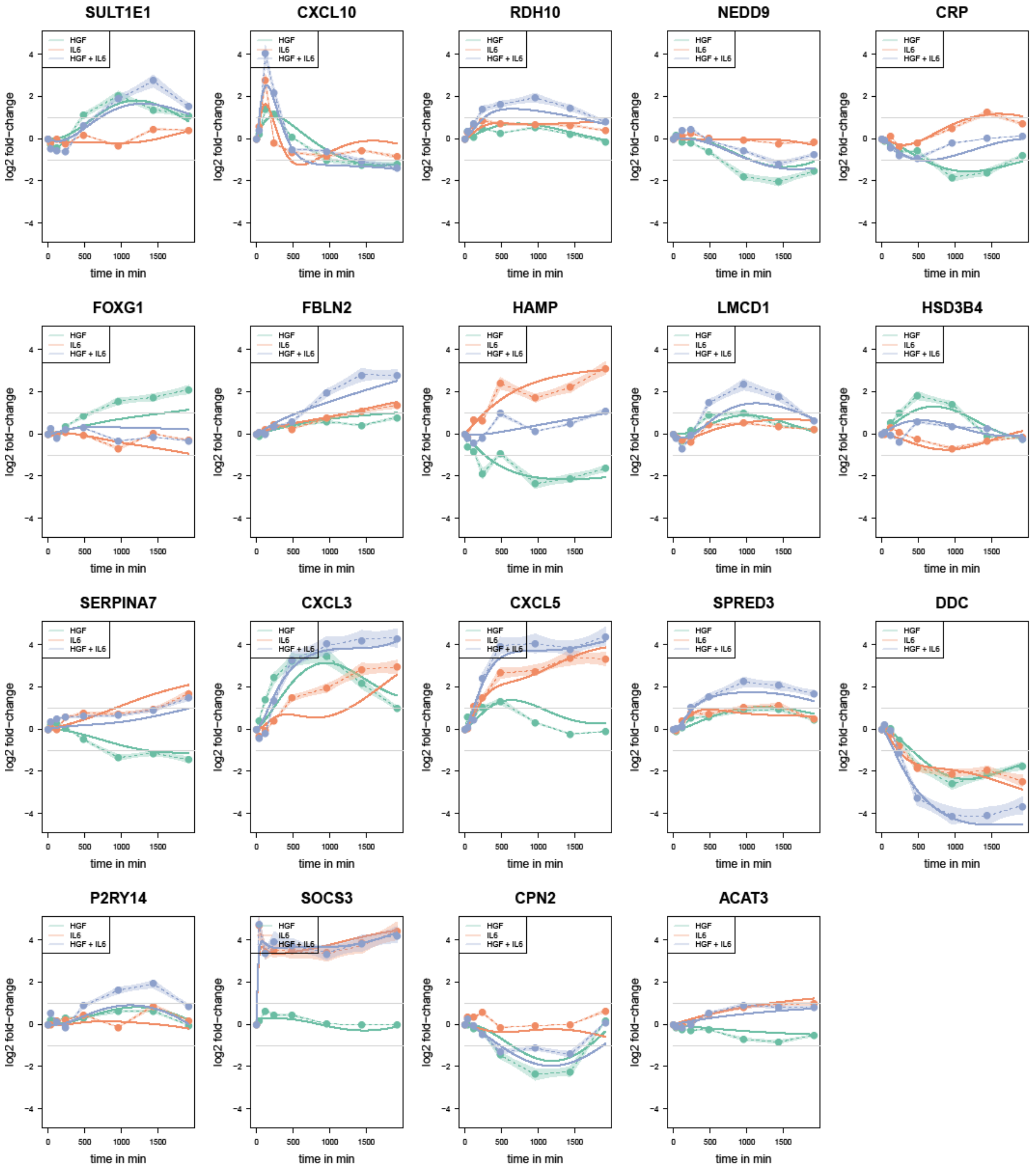
CXCL10 central
- early response gene
- synergistic
- cytokine

HSD3B4
- early onset
- spread
- hormone synthesis



— inferred - new
— inferred - prior-knowledge
— TFBS-knowledge
→ activating
⊣ repressing
···· validation knowledge
● modelled gene
◆ transcription factor

In progress



virtual liver network

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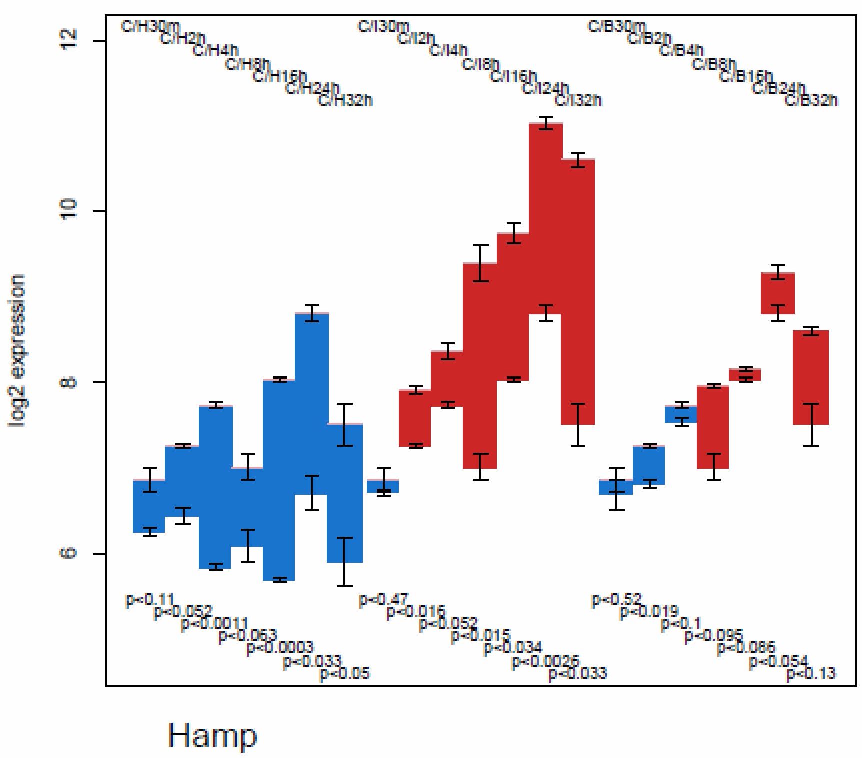
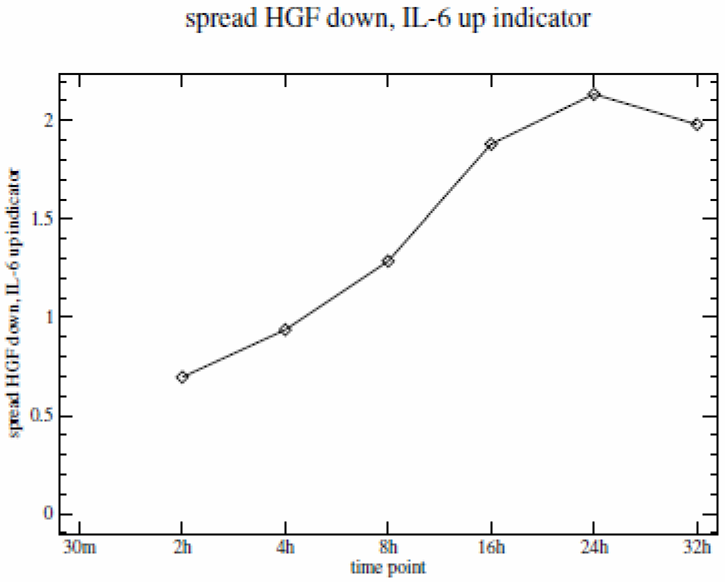
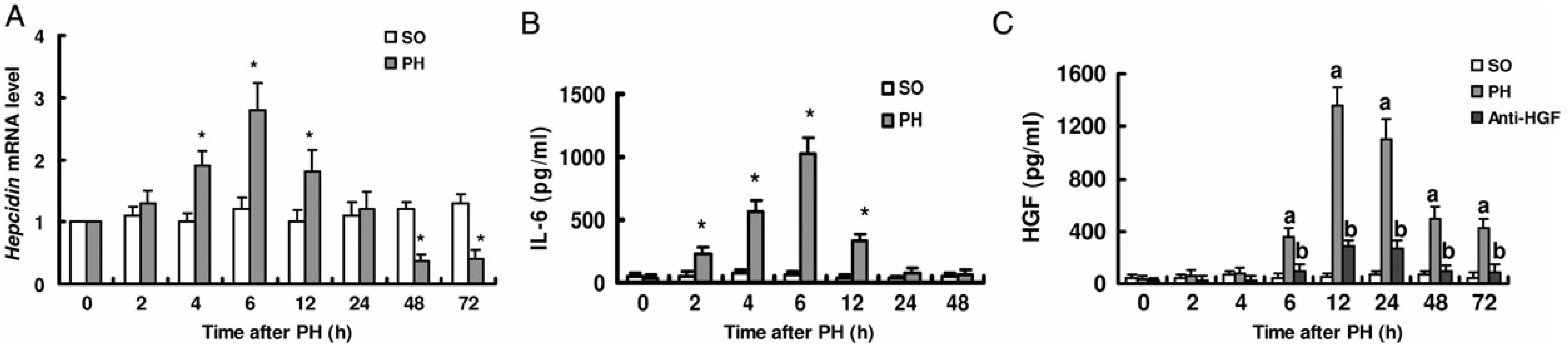
Hepcidin


log2 expression
6
8
10
12
C/H30m
C/H2h
C/H4h
C/H8h
C/H16h
C/H24h
C/H32h
C/I30m
C/I2h
C/I4h
C/I8h
C/I16h
C/I24h
C/I32h
C/B30m
C/B2h
C/B4h
C/B8h
C/B16h
C/B24h
C/B32h
p<0.11
p<0.052
p<0.0011
p<0.063
p<0.0003
p<0.033
p<0.05
p<0.47
p<0.016
p<0.052
p<0.015
p<0.034
p<0.0026
p<0.033
p<0.52
p<0.019
p<0.1
p<0.095
p<0.066
p<0.054
p<0.13
Hamp


spread HGF down, IL-6 up indicator
spread HGF down, IL-6 up indicator
0
0.5
1
1.5
2
30m
2h
4h
8h
16h
24h
32h
time point

Hepcidin in liver regeneration



SO ··· sham operation

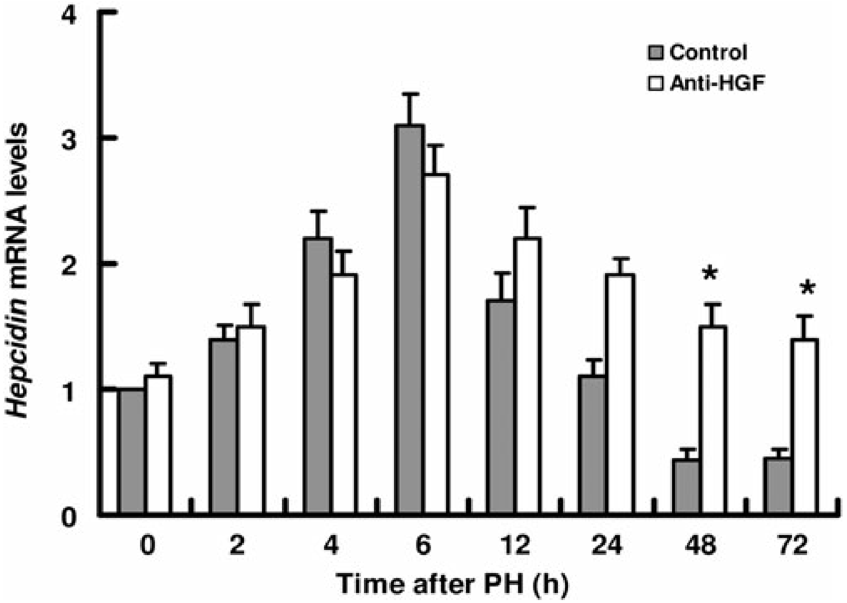
PH ··· partial 2/3 hepatectomy

(B) Time course of serum IL-6.

(C) Mice were treated with anti-HGF neutralizing antibodies or an equal dose of normal IgG antibodies.

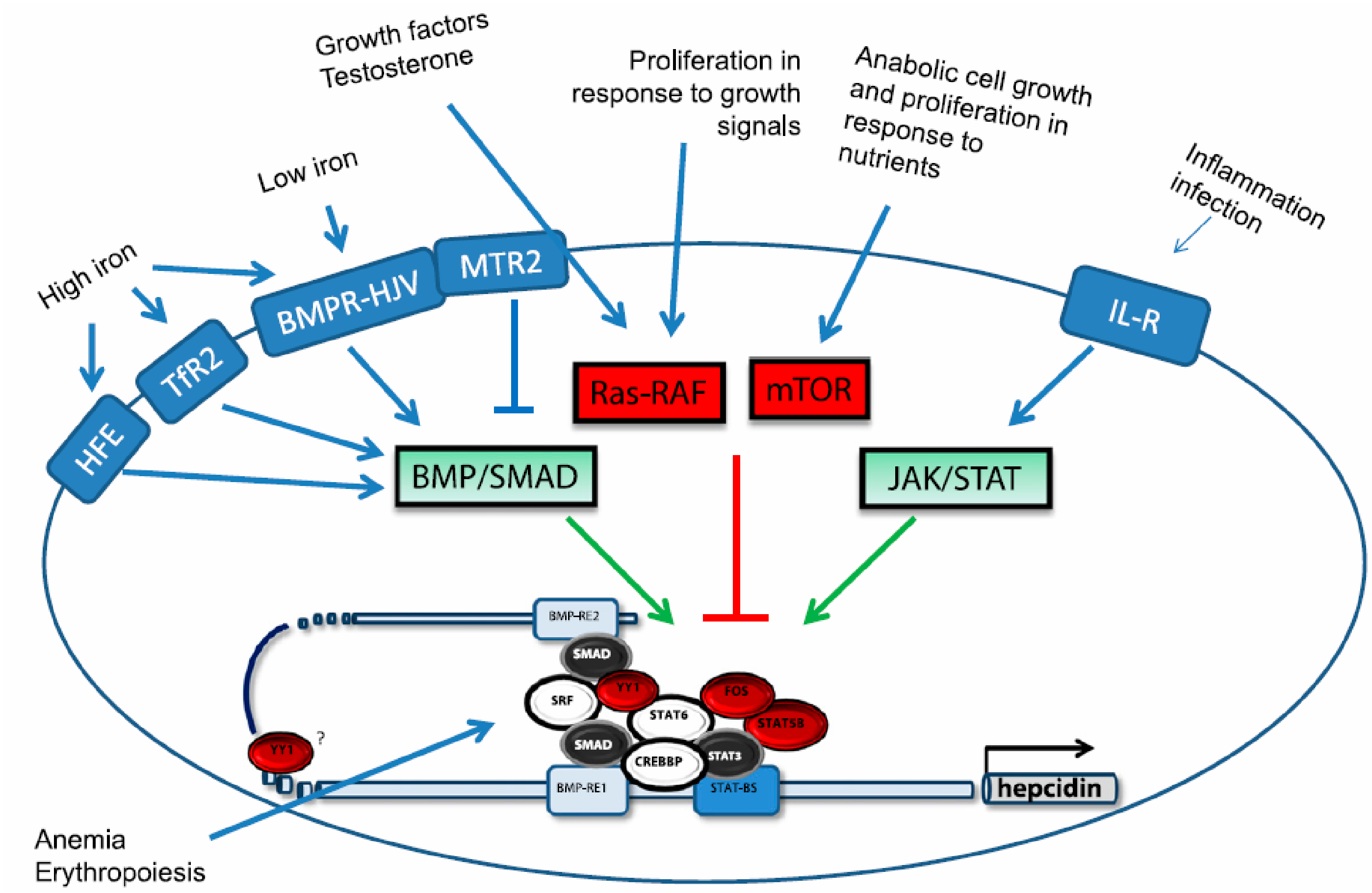
Wang et al. (2013) Hepcidin plays a negative role in liver regeneration. Acta Biochim Biophys Sin 45(12), 1049-54.

HGF inhibition increases hepcidin



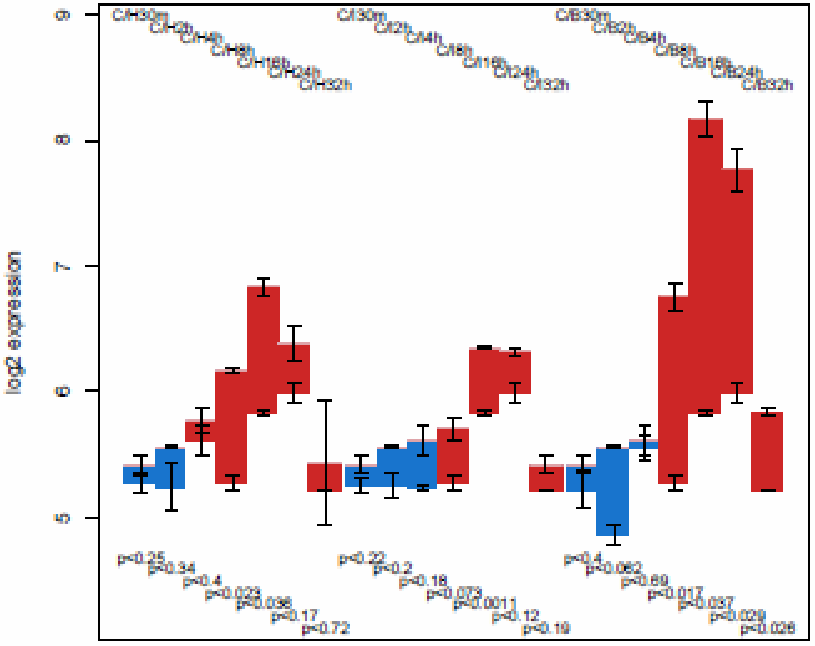
Wang et al. (2013) Hepcidin plays a negative role in liver regeneration. Acta Biochim Biophys Sin 45(12), 1049-54.

Hepcidin regulation

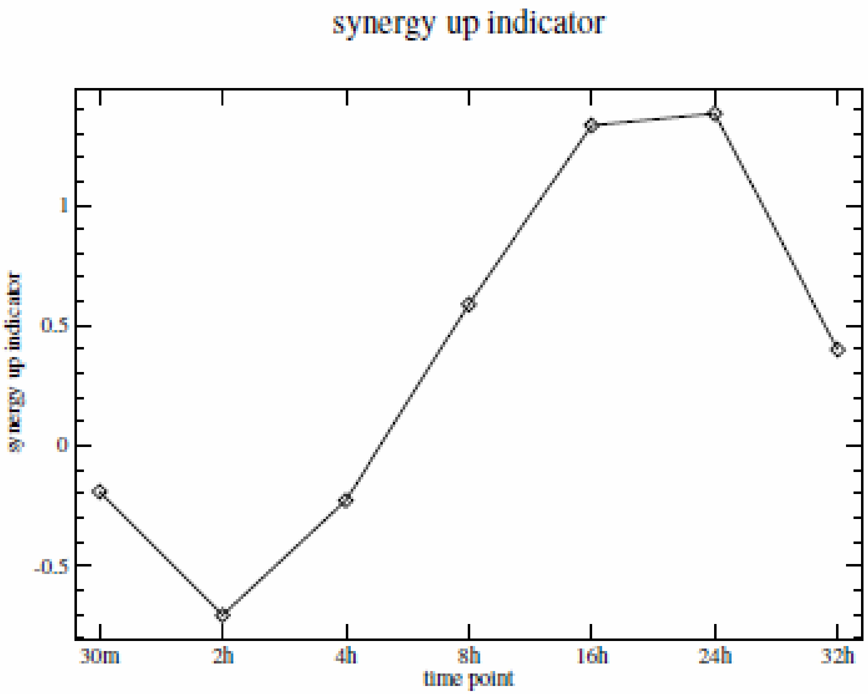


Arosio (2014) New signaling pathways for hepcidin regulation. Blood 123: 1433-1434.

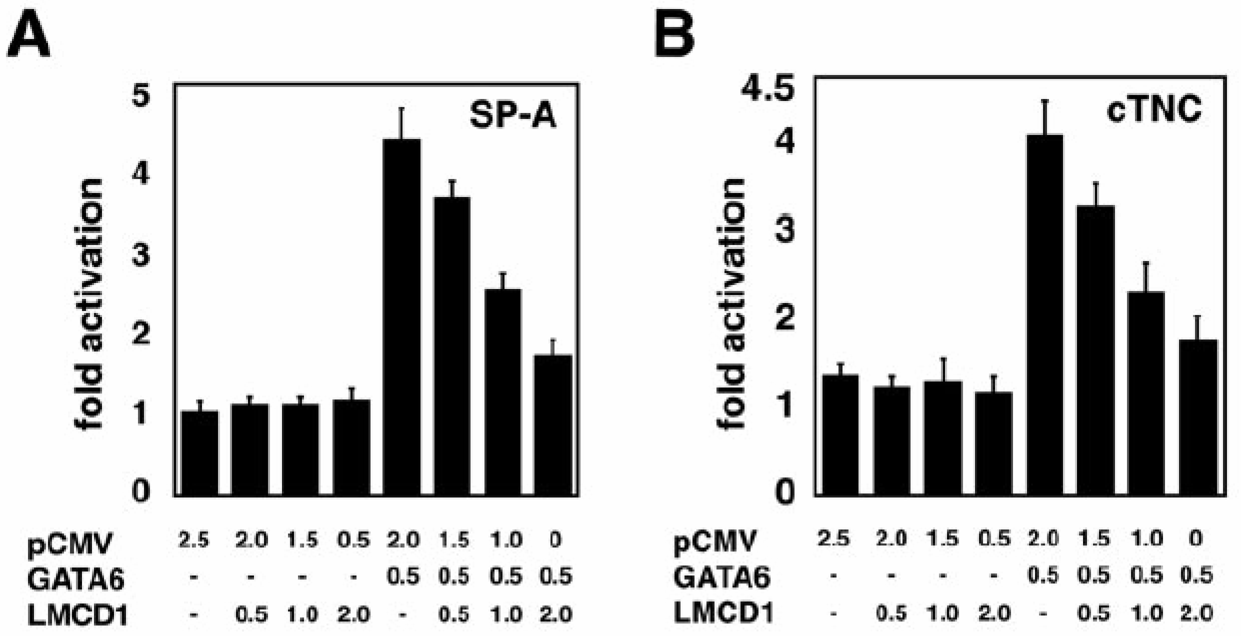
Dyxin, synergistic gene



Lmcd1



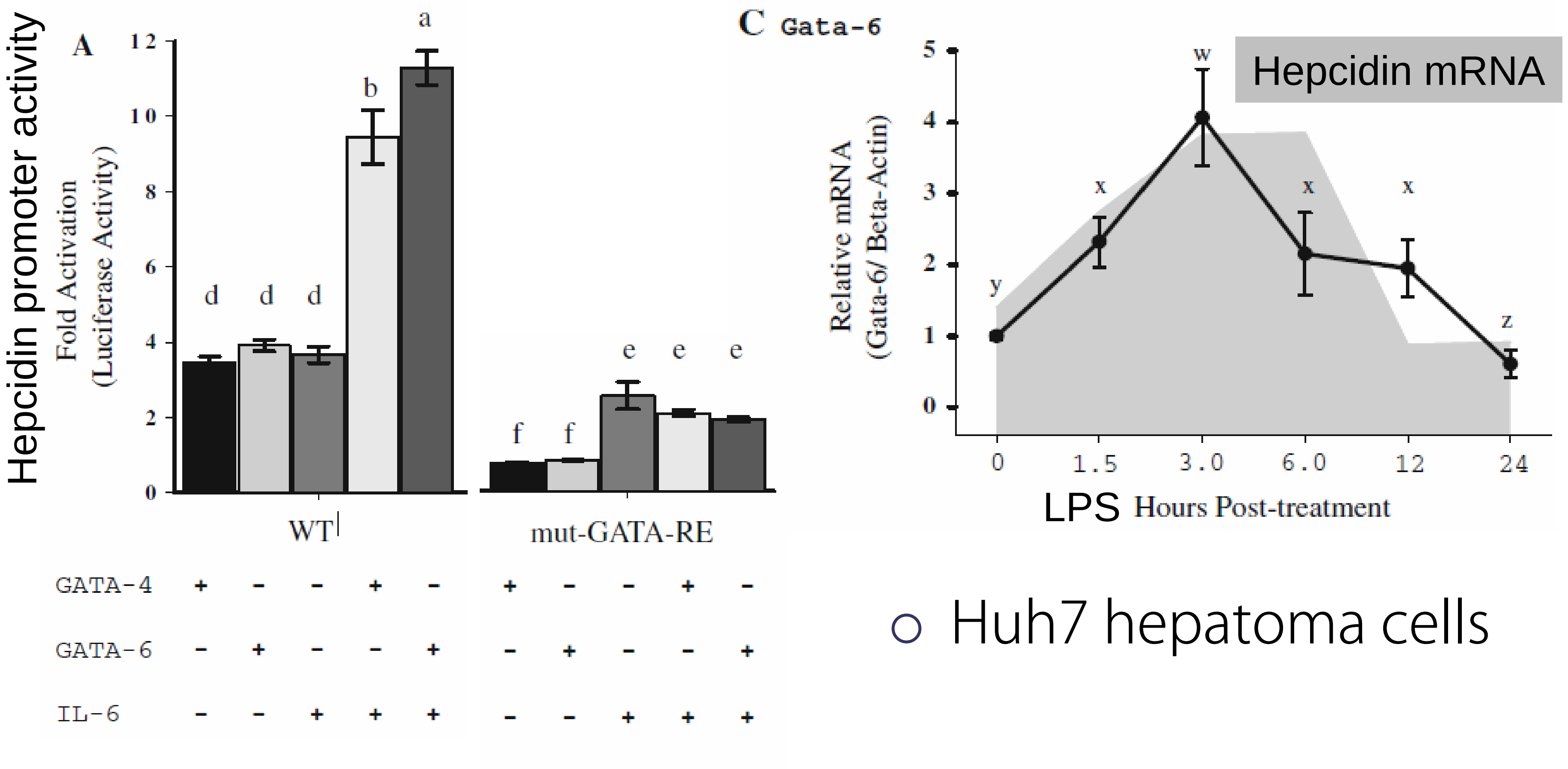
Dyxin binds to GATA6 and inhibits the signal



- Target promoters of GATA6
  - SP-A … mouse surfactant protein A
  - cTNC … cardiac troponin C

Rath et al. (2005) *LMCD1/Dyxin Is a Novel Transcriptional Cofactor That Restricts GATA6 Function by Inhibiting DNA Binding.* Mol. and Cell Biol. 25(20) : 8864-8873.

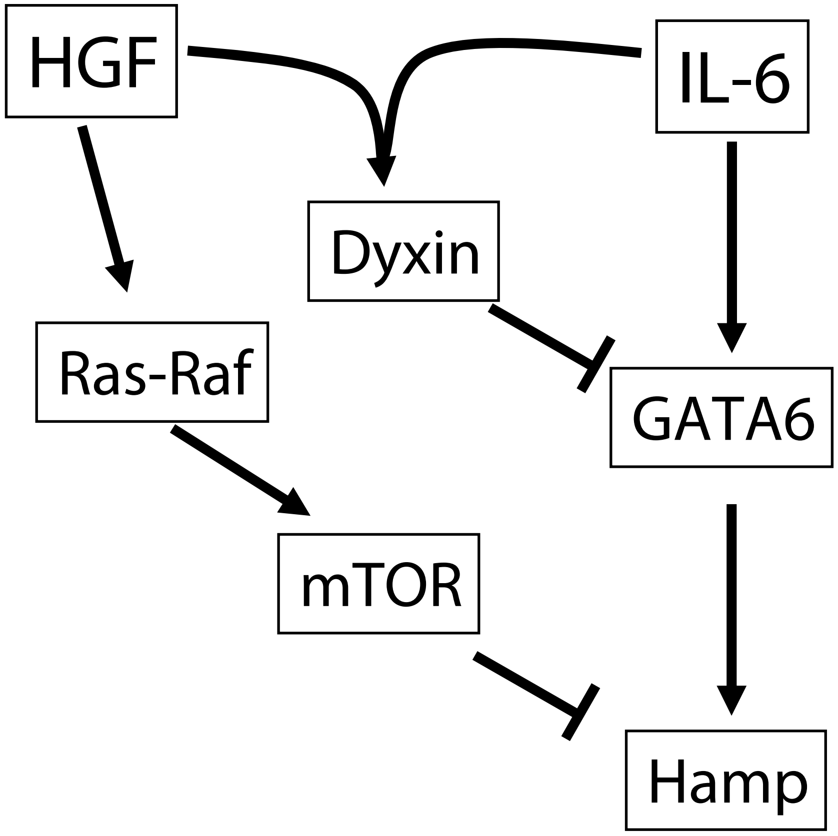
GATA6 facilitates Hepcidin induction by IL-6



- ○ Huh7 hepatoma cells

Bagu et al. (2013) *Friend of GATA and GATA-6 modulate the transcriptional up-regulation of hepcidin in hepatocytes during inflammation.* Biometals 26:1051-1065.

Hypothesis: role of dyxin in hepcidin regulation



HGF

IL-6

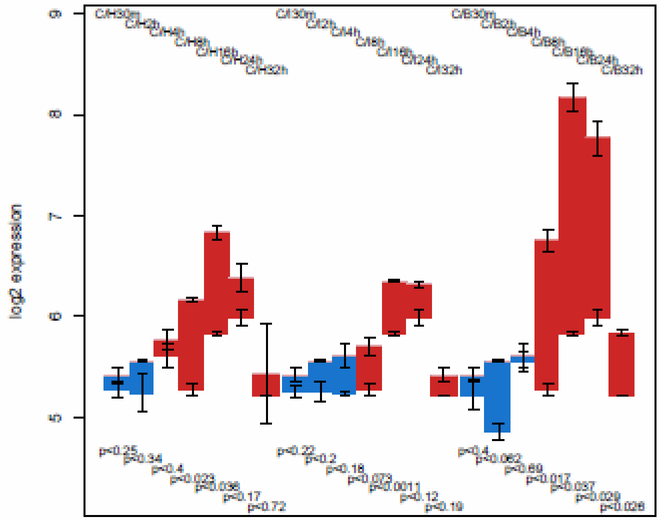
Dyxin

Ras-Raf

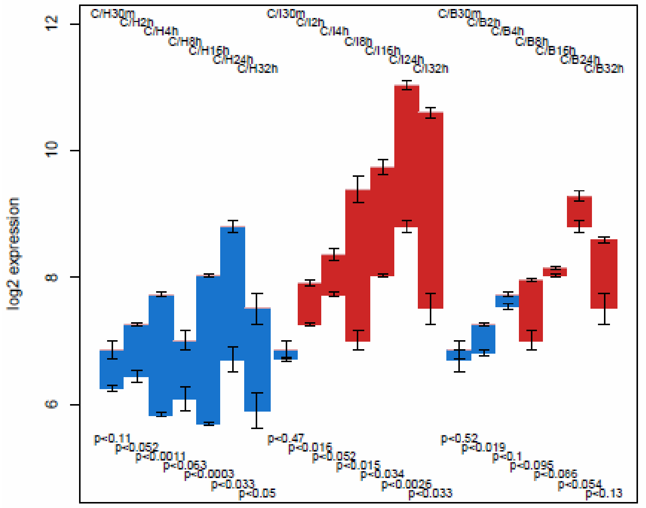
GATA6

mTOR

Hamp



Lmcd1



Hamp

Take-home message

- HGF and IL-6 important complementary roles in liver regneration
- Top ranked genes for synergy, attenuation, spread are often associated to growth, cancer, (de)differentiation, fibrosis
- Dyxin, 3rd most synergistic, and Hepcidin, most divergent IL-6 induced have known transcriptional connection, putative role in growth control

Acknowledgements

- Sebastian Vlaic
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