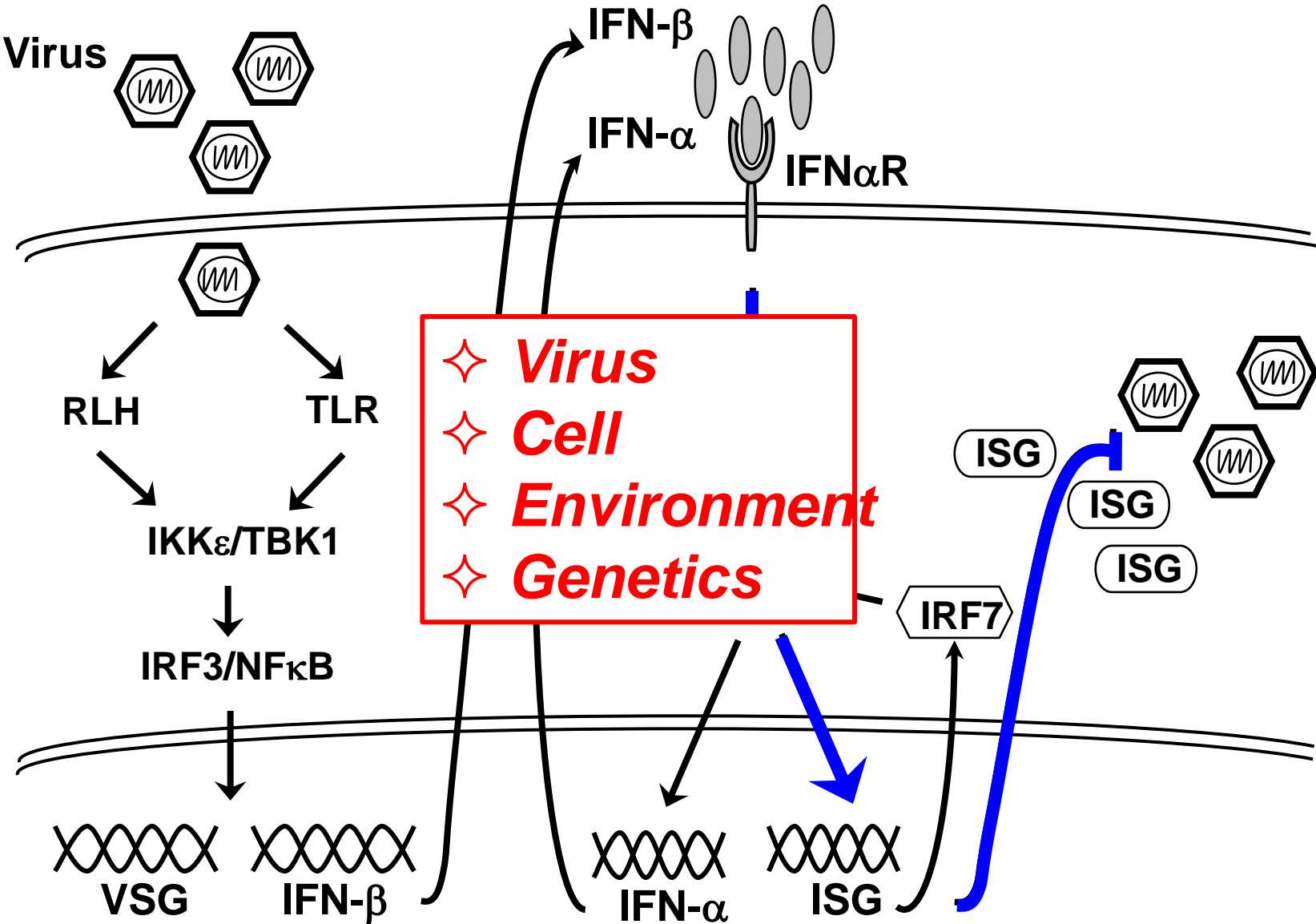


Intrinsic Innate Immunity and Hepatitis C Virus Infection

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Branch
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Interferon Response to Viral Infection



Interferon- λ : The Coming of Age

doi:10.1038/nature08309

nature

LETTERS

Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance

Dongliang Ge¹, Jacques Fellay¹, Alexander J. Thompson², Jason S. Simon³, Kevin V. Shianna¹, Thomas J. Urban¹, Erin L. Heinzen¹, Ping Qiu³, Arthur H. Bertelsen³, Andrew J. Muir², Mark Sulikowski⁴, John G. McHutchison² & David B. Goldstein¹

LETTERS

nature
genetics

Genome-wide association of *IL28B* with response to pegylated interferon- α and ribavirin therapy for chronic hepatitis C

Yasuhito Tanaka^{1,18}, Nao Nishida^{2,18}, Masaya Sugiyama¹, Masayuki Kurosaki³, Kentaro Matsuura¹, Naoya Sakamoto⁴, Mina Nakagawa⁴, Masaaki Korenaga⁵, Keisuke Hino⁵, Shuhei Hige⁶, Yoshito Ito⁷, Eiji Mita⁸, Eiji Tanaka⁹, Satoshi Mochida¹⁰, Yoshikazu Murawaki¹¹, Masao Honda¹², Akito Sakai¹², Yoichi Hiasa¹³, Shuhei Nishiguchi¹⁴, Asako Koike¹⁵, Isao Sakaida¹⁶, Masatoshi Imamura¹⁷, Kiyooki Ito¹⁷, Koji Yano¹⁷, Naohiko Masaki¹⁷, Fuminaka Sugauchi¹, Namiki Izumi³, Katsushi Tokunaga² & Masashi Mizokami^{1,17}

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nature
genetics

IL28B is associated with response to chronic hepatitis C interferon- α and ribavirin therapy

Vijayaprakash Suppiah^{1,2}, Max Moldovan³, Golo Ahlenstiel⁴, Thomas Berg⁵, Martin Weltman⁶, Maria Lorena Abate⁷, Margaret Bassendine⁸, Ulrich Spengler⁴, Gregory J Dore^{9,10}, Elizabeth Powell^{11,12}, Stephen Riordan¹³, David Sheridan⁸, Antonina Smedile⁷, Vincenzo Fragomeli⁶, Tobias Müller⁵, Melanie Bahlo³, Graeme J Stewart², David R Booth² & Jacob George¹, for the Hepatitis C Study¹⁴

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nature

LETTERS

Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus

David L. Thomas^{1*}, Chloe L. Thio^{1*}, Maureen P. Martin^{2*}, Ying Qi², Dongliang Ge³, Colm O'hUigin², Judith Kidd⁴, Kenneth Kidd⁴, Salim I. Khakoo⁵, Graeme Alexander⁶, James J. Goedert⁷, Gregory D. Kirk⁸, Sharyne M. Donfield⁹, Hugo R. Rosen¹⁰, Leslie H. Tobler¹¹, Michael P. Busch¹¹, John G. McHutchison¹², David B. Goldstein³ & Mary Carrington^{2,13}

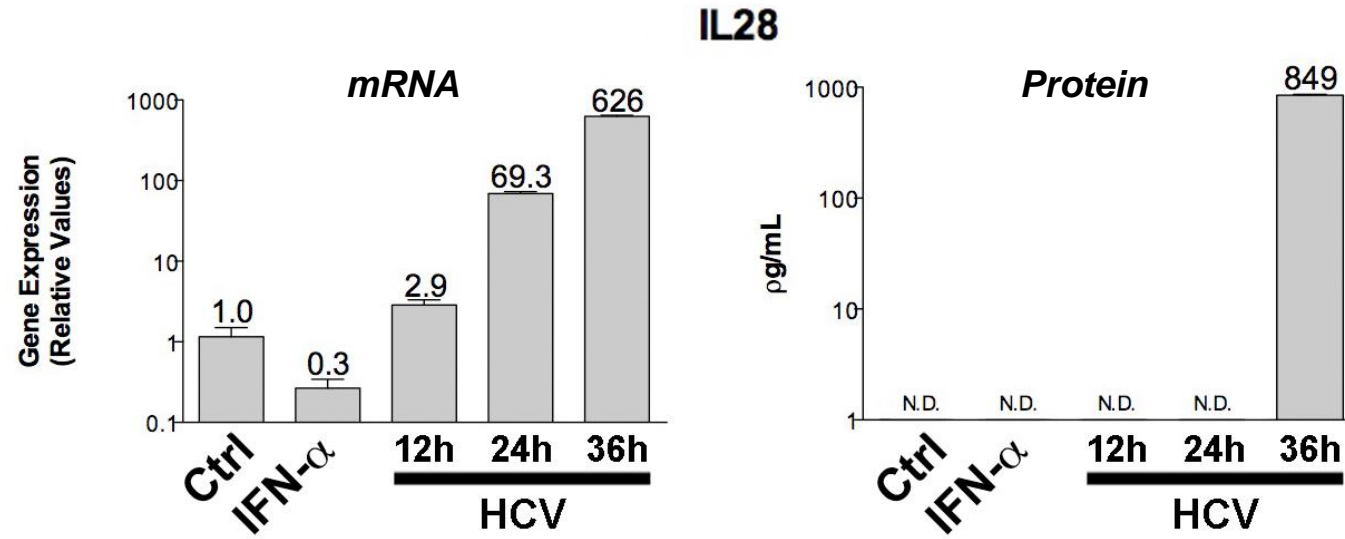
The Interferon- λ Family

- **Discovered in 2003**
- **Three members (Type III IFN): IFN λ 1, 2 and 3 corresponding to IL29, IL28A and IL28B**
- **Have similar antiviral activities and effects on gene expression as type I interferon**
- **Signal through distinct receptors IL28RA and IL10RB but the same JAK/STAT pathway**

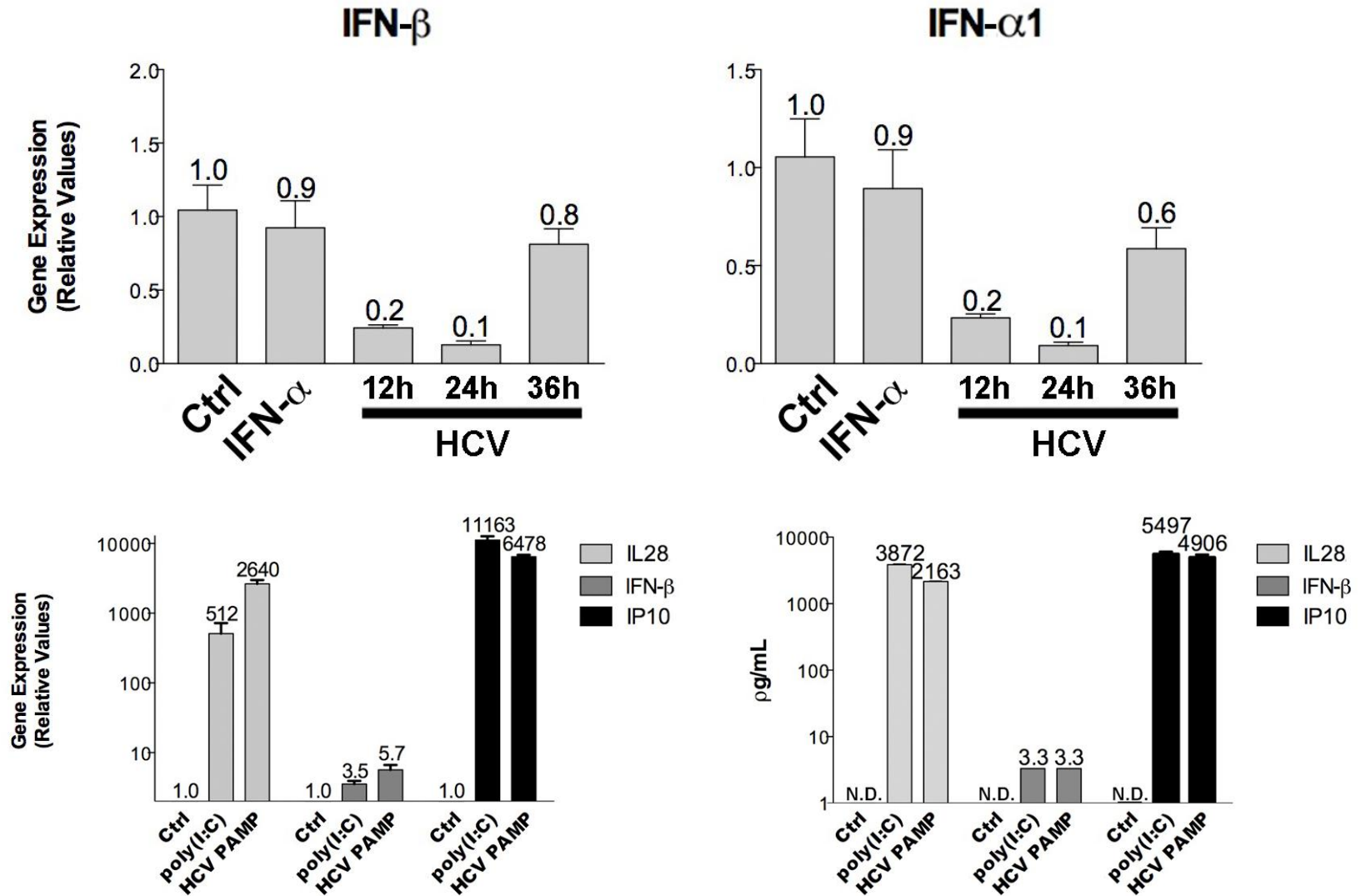
Challenges in Studying IFN Response to HCV

- **Numerous studies have reported activation of the IFN system in HCV infected chimpanzees and humans that demonstrate upregulated levels of ISG**
- **However, these findings have been difficult to reproduce in cell culture models of HCV infection: little or no induction of interferons and ISGs**

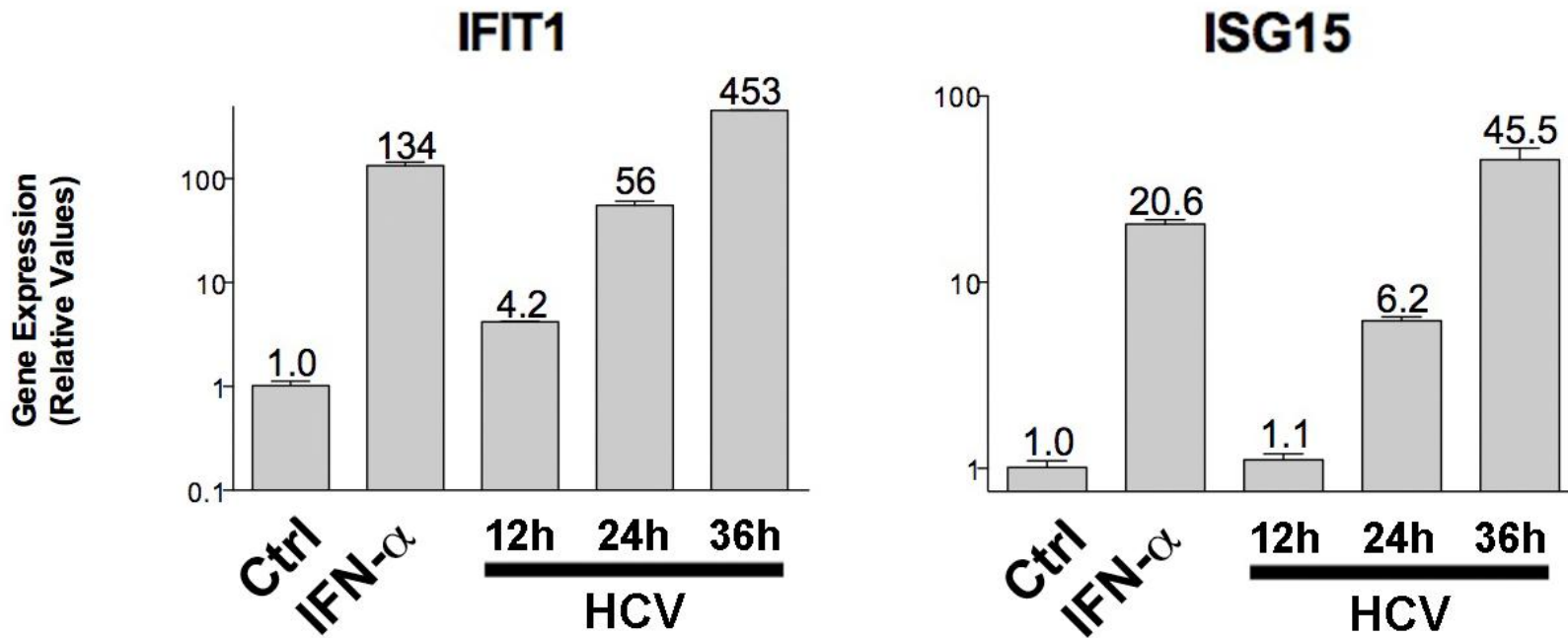
IL28 Induction by HCV Infection in Primary Human Hepatocytes (PHHs)



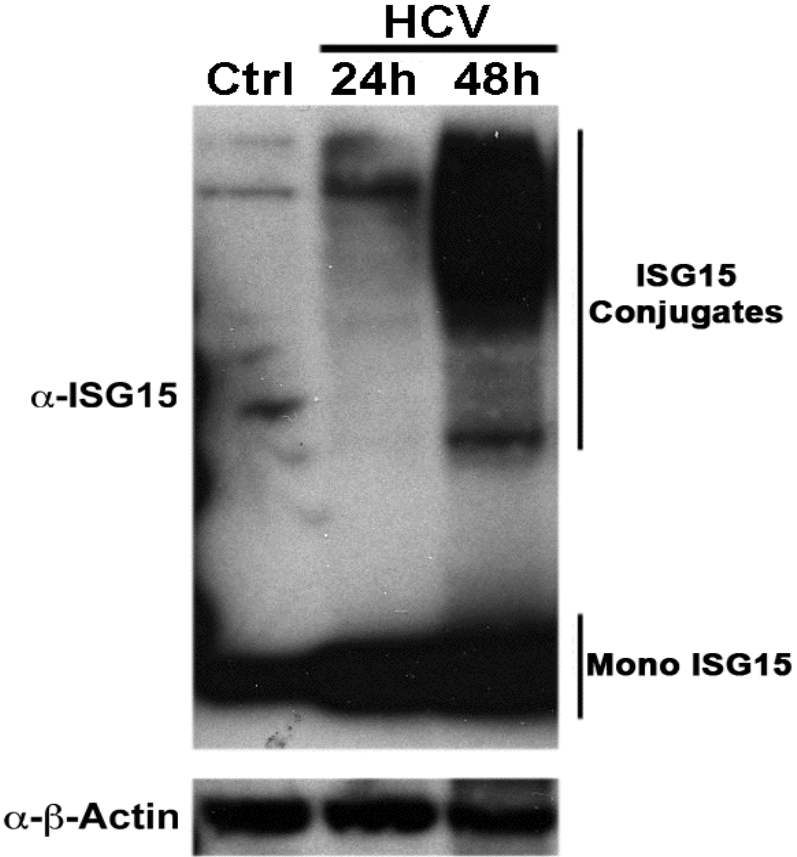
Lack of Type I IFN Induction by HCV Infection in PHHs



ISG Induction by HCV Infection in PHHs



Protein ISGylation Induced by HCV Infection in PHHs

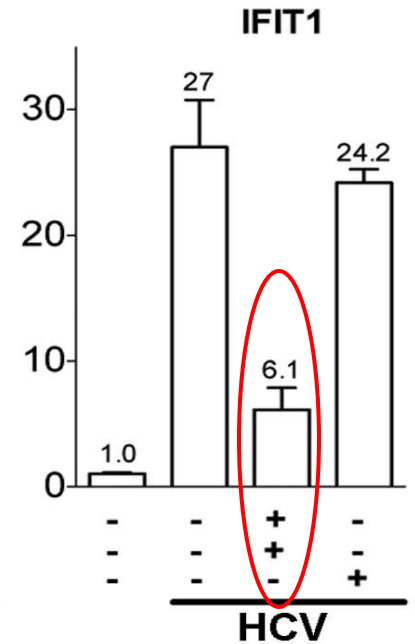
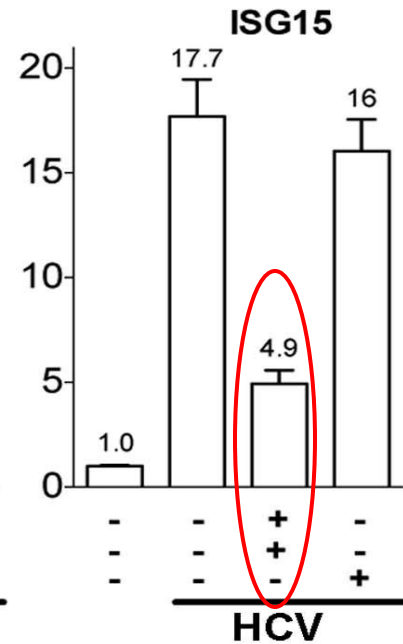
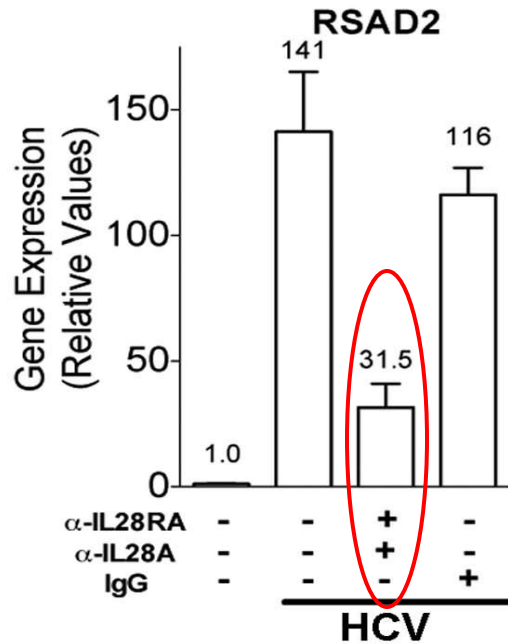
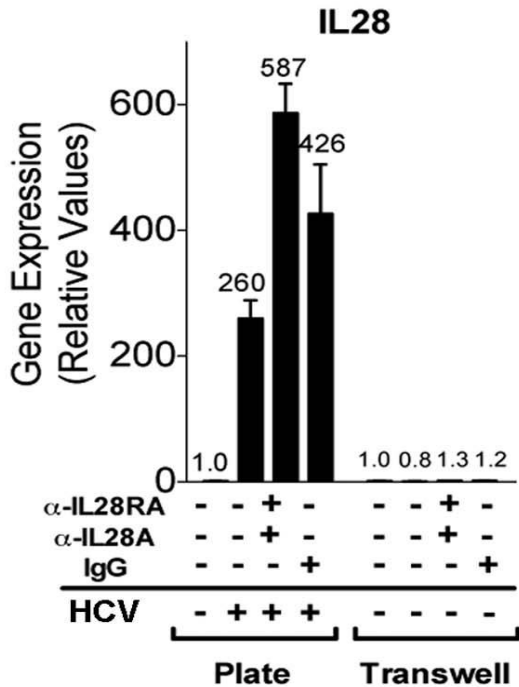
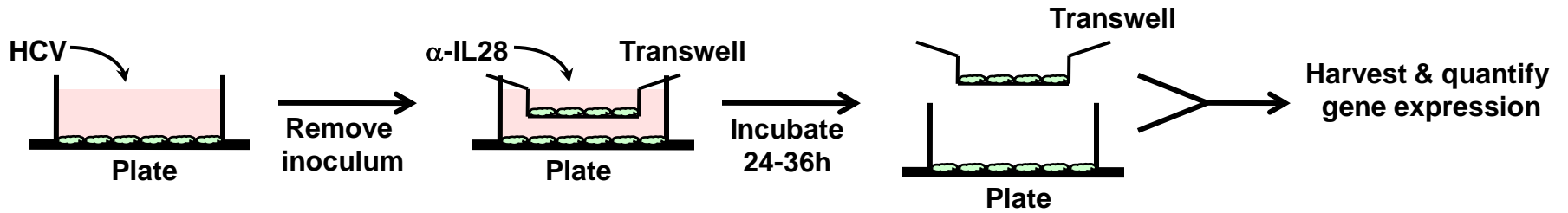


Summary I

- **Human hepatocytes produce predominantly Type III IFNs in response to HCV infection**
- **Induction of type III IFNs by HCV depends on active viral infection and replication**
- **IFN- λ , similar to IFN- β , is induced by viral stimuli via the IRF3 and NF- κ B signaling pathways**
- **ISGs and chemokines are also induced to**

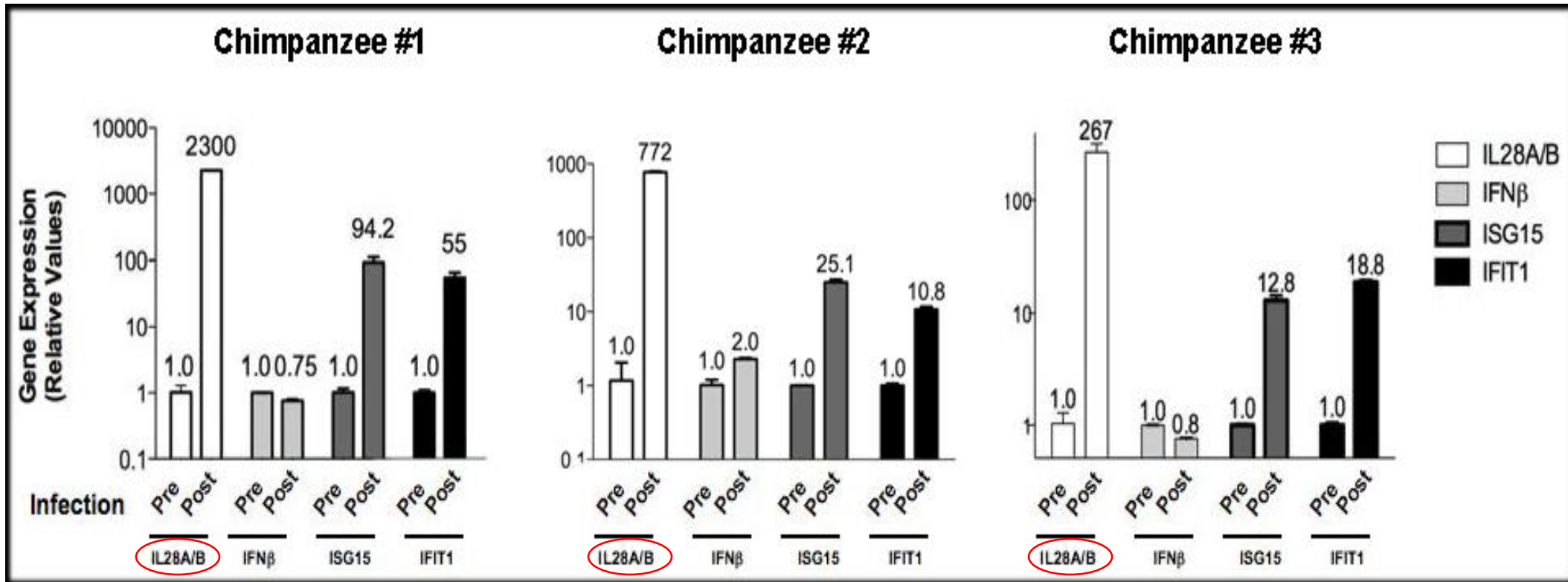
Is IL28 induction by HCV responsible for the up-regulation of ISGs in infected liver?

Role of IL28 Induction by HCV in ISG Expression

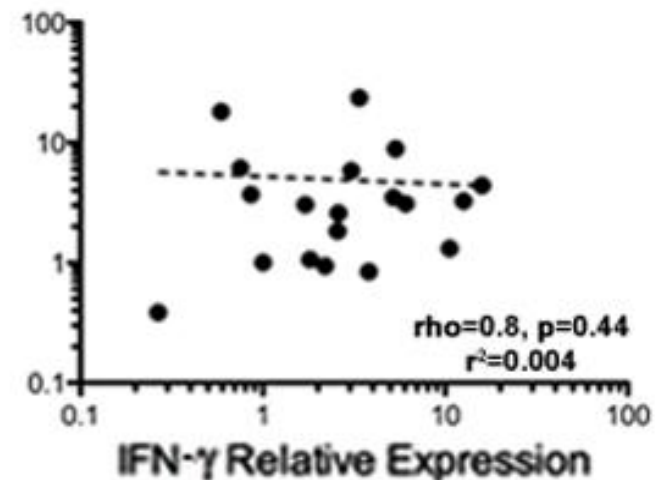
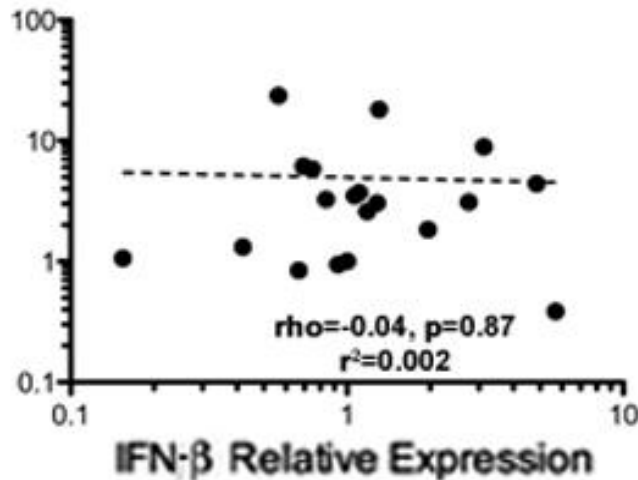
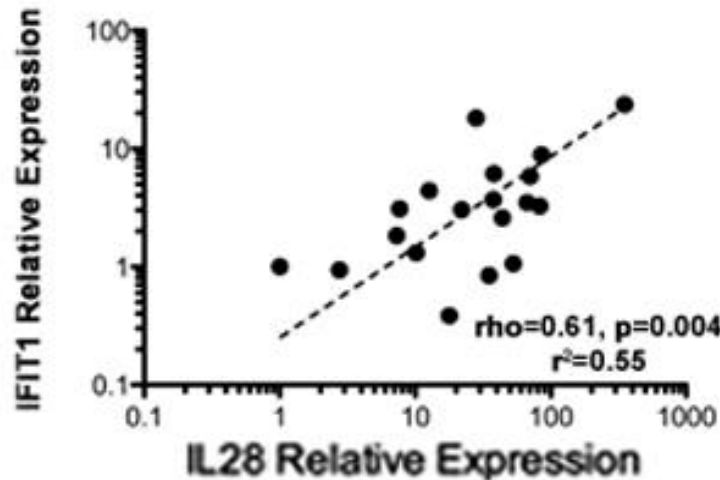
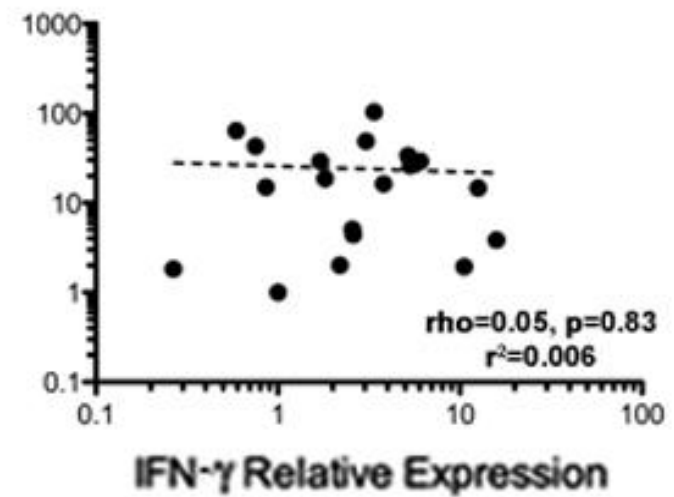
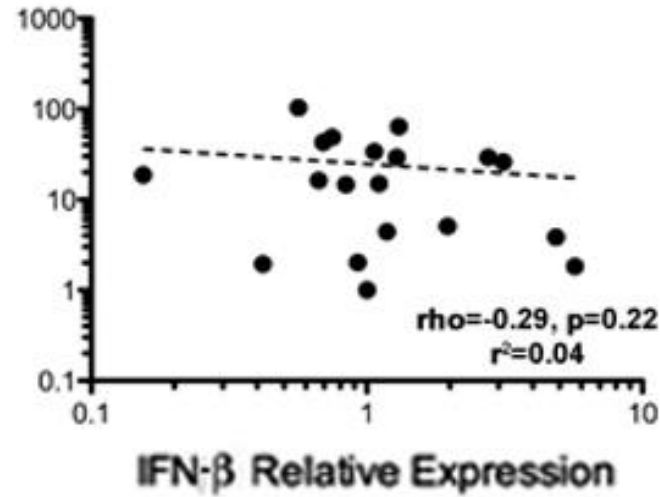
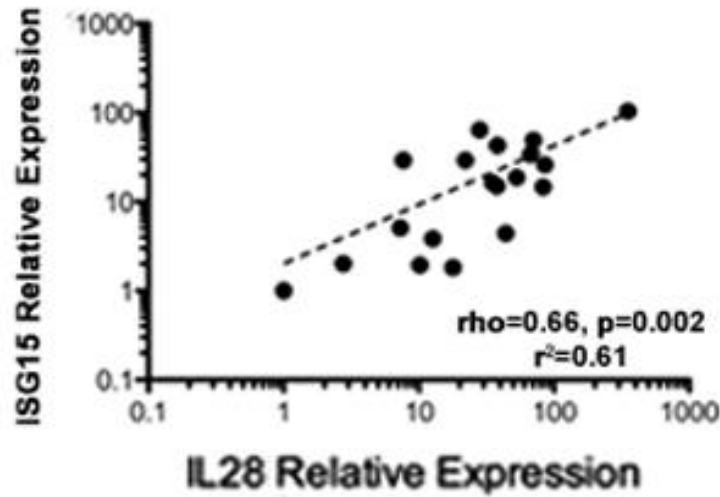


Transwell

IL28 Induction by HCV Infection in Vivo



IL28 Expression Correlates with ISG Expression in Liver of HCV Infected Patients

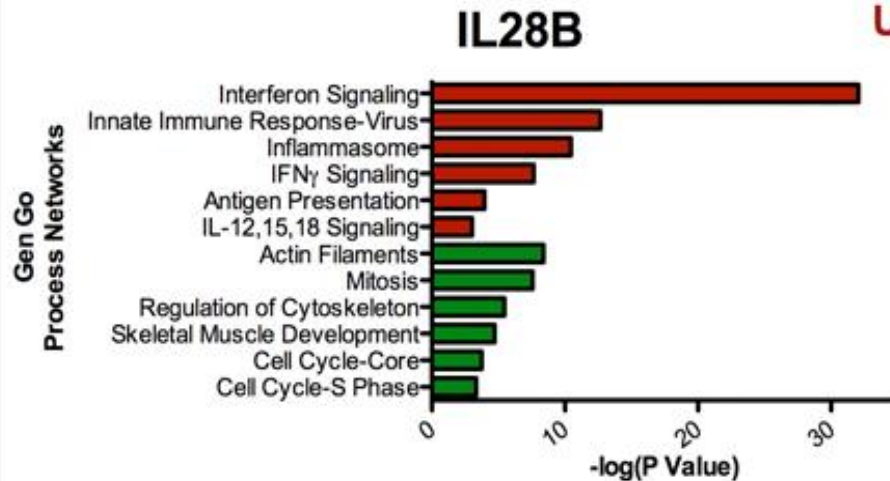
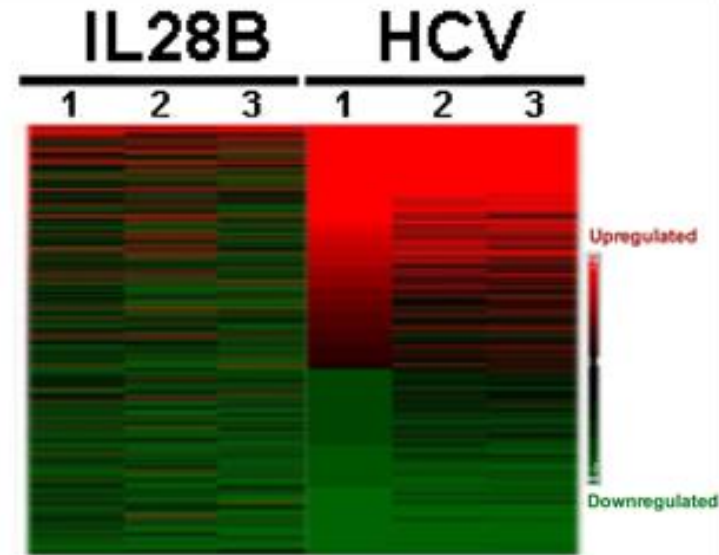


Does HCV infection modulate expression of genes other than IL28-induced ISGs?

Gene Expression of PHH by HCV vs IL28

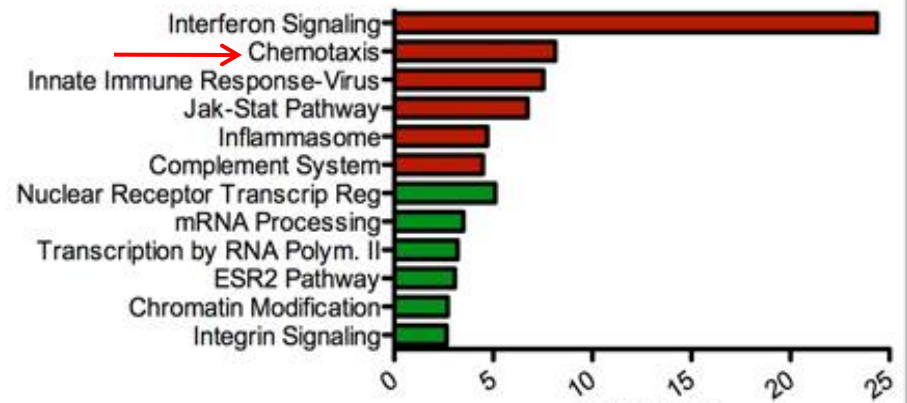
Upregulated

Downregulated



Upregulated

HCV



Downregulated

Microarray Analysis of PHHs Infected with JFH1

IL28B

Gene Symbol	p-value	Fold-Change
CXCL10	2.16E-16	503.432
RSAD2	4.07E-16	329.374
IFI44L	6.93E-14	260.279
CXCL11	3.70E-11	51.0818
IFITM1	1.69E-13	44.0105
EPSTI1	8.63E-13	40.5517
ETV7	4.80E-12	37.9481
IFIT1	3.52E-14	37.5133
IFI44	3.27E-12	35.8547
OASL	1.95E-10	32.7209

HCV

Gene Symbol	p-value	Fold-Change
CXCL10	6.95E-12	528.383
CXCL11	3.14E-08	394.524
IFI44L	1.50E-14	193.725
IFIT1	3.10E-11	181.853
IL28A	2.54E-11	169.535
CCL8	5.09E-11	131.151
IFITM1	1.86E-10	86.36
OASL	1.34E-08	81.9936
RTP4	2.49E-09	79.9043
IFIT3	1.61E-11	78.9209

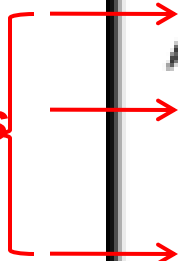
Microarray Analysis of PHHs Infected with JFH1

IL28B			HCV		
Gene Symbol	p-value	Fold-Change	Gene Symbol	p-value	Fold-Change
CXCL10	2.16E-16	503.432	CXCL10	6.95E-12	528.383
RSAD2	4.07E-16	329.374	CXCL11	3.14E-08	394.524
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EPSTI1	8.63E-13	40.5517	CCL8	5.09E-11	131.151
ETV7	4.80E-12	37.9481	IFITM1	1.86E-10	86.36
IFIT1	3.52E-14	37.5133	OASL	1.34E-08	81.9936
IFI44	3.27E-12	35.8547	RTR1	2.10E-09	79.9043
OASL					78.9209

HCV > IL28B

Gene Symbol	p-value	Fold-Change
IL28A	1.69E-14	169.534
CCL8	6.61E-14	131.154
ALDH8A1	2.99E-16	75.4206
CXCL9	7.22E-13	72.4083
WDR72	6.46E-14	47.9669
GLYAT	1.51E-13	47.083
CCL5	7.59E-14	44.65
SLC23A1	4.22E-12	44.3769
IL29	4.48E-11	44.3549
ACOT12	2.74E-17	42.6156

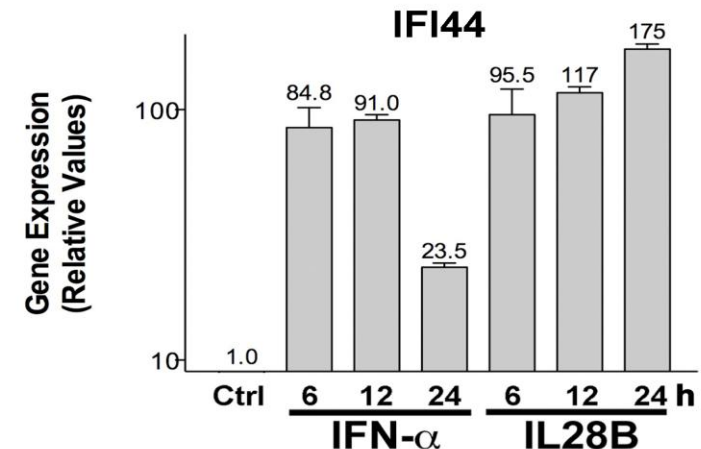
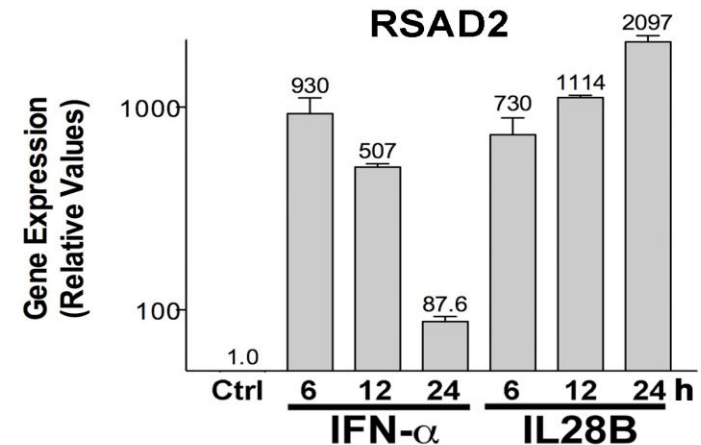
Chemokines



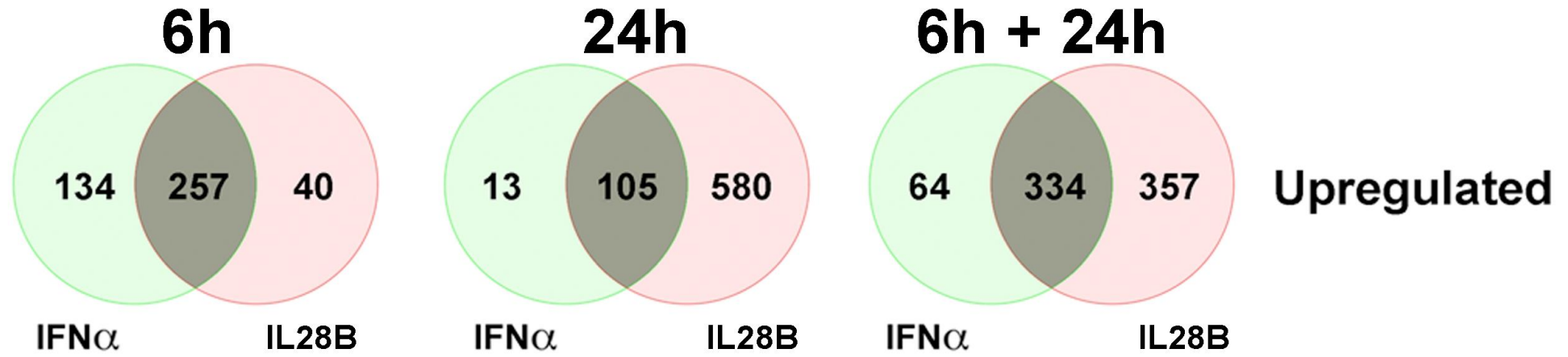
Do IL28 and IFN- α have the same functional effect on the induction of ISGs?

Microarray Analysis of IFN- α and IL28B Regulated Genes in PHHs

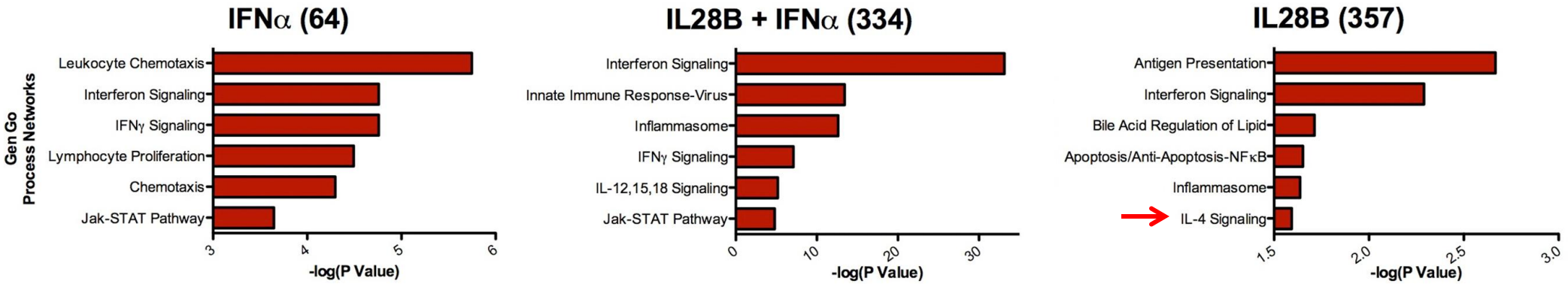
GENE SYMBOL	IFN α 6 Hr	IFN α 24 Hr	IL28B 6 Hr	IL28B 24 Hr
CXCL10	5461.191	102.821	992.552	5457.407
IFI44L	519.867	154.879	393.44	2045.162
RSAD2	142.913	22.1	129.696	201.97
CXCL11	42.194	3.267	14.36	167.497
MX2	107.039	45.317	18.025	161.68
GBP5	44.078	11.345	24.1	93.961
MGC40069	2.775	47.868	1.737	67.415
ETV7	74.646	10.389	64.445	63.69
FBXL14	5.888	1.629	16.2	61.777
OASL	30.442	6.713	32.649	60.338
HERC6	10.41	15.487	24.184	57.162
OR52K3P	21.288	2.421	15.67	56.649
SOCS1	21.781	1.065	30.867	54.455
EPST11	28.227	6.918	29.774	50.843
RBM43	24.967	1.903	24.47	45.918
MAK	35.728	2.112	14.006	44.047
OASL	32.876	7.706	34.083	43.231
TGM4	26.778	2.236	13.919	41.816
EPAG	27.55	5.038	13.473	41.041
SAMD9L	20.749	4.746	9.075	40.168
LOC339260	3.321	2.489	16.529	39.506
UBQLNL	26.263	14.221	12.058	39.451
SYNPQ2	20.492	8.117	8.117	38.532
IL1RN	29.527	26.118	11.337	38.399
BCL2L14	11.487	1.047	4.469	36.605
WDR44	1.655	7.862	27.455	36.58
EPB41L4A	7.26	37.091	21.082	35.26
SNTG2	7.13	1.875	4.41	35.26
ESPNL	25.333	4.21	23.703	34.751
ESYT2	11.672	4.293	28.168	34.13
DNAJA4	10.049	3.708	3.384	33.801
GZMA	10.324	10.475	-1.035	32.627
IFI27	7.387	16.982	6.788	31.977
TMEM144	60.757	3.391	1.317	31.69
IFI44	26.78	3.776	30.845	31.428
OAS2	16.29	5.105	9.613	31.428
FAM105A	16.865	2.867	38.054	31.276
GRIK2	1.952	11.471	1.481	31.081
MAGI1	93.636	10.231	7.007	31.06
STAT1	10.935	7.18	18.201	30.781



Microarray Analysis of PHHs Treated with Type I or III IFN

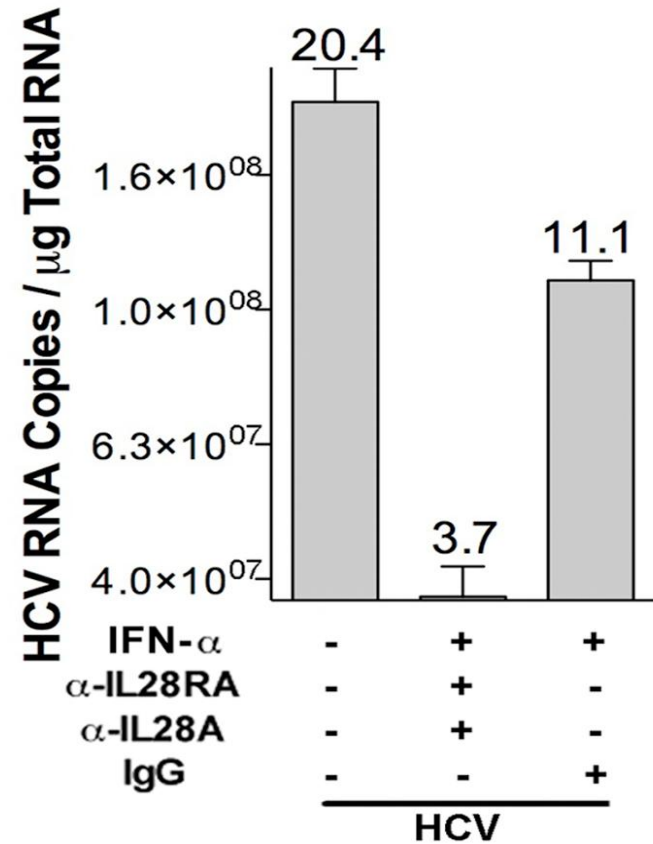
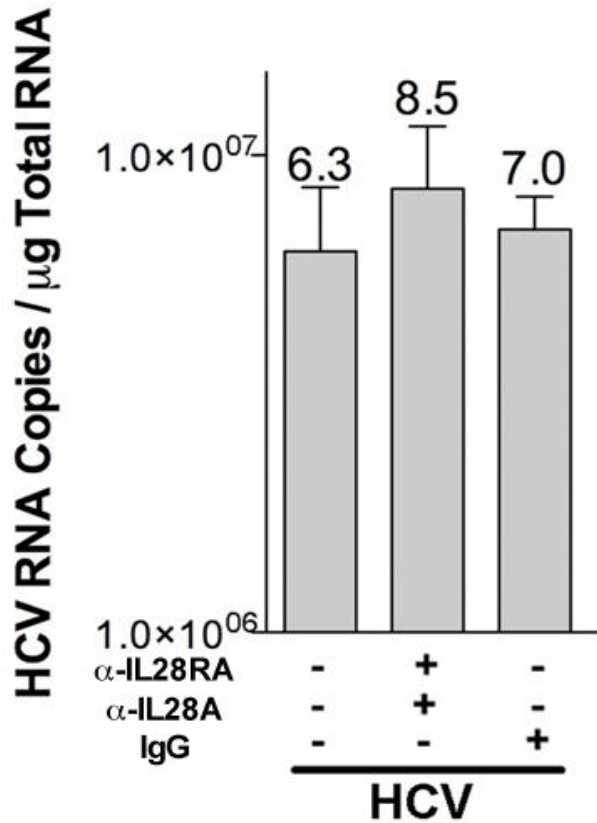


Upregulated (6h + 24h)

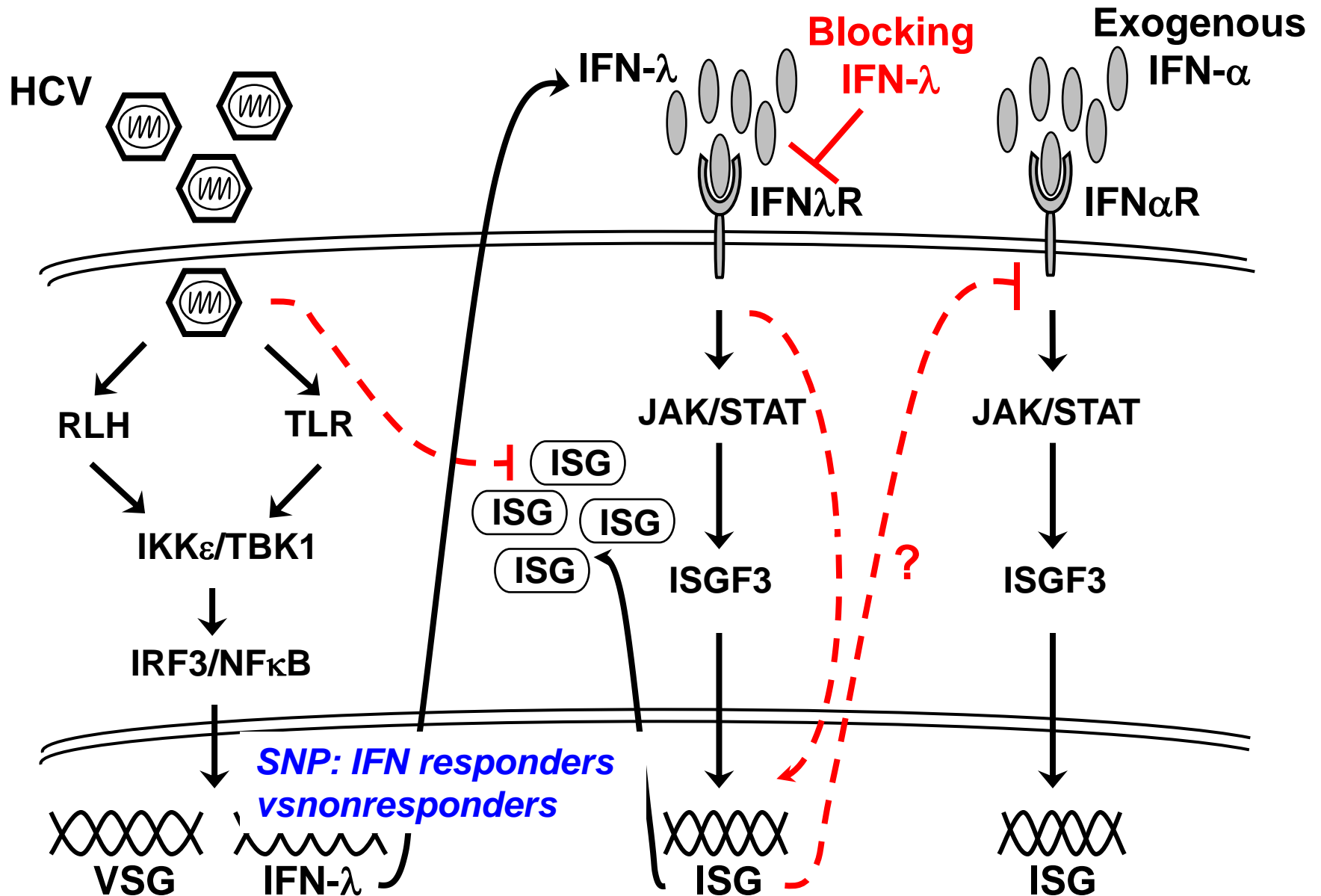


Does IL28 induction by HCV alter the antiviral effect of IFN- α ?

Effect of Blocking IL28 on the Antiviral Activities of Type I IFN in HCV-Infected PHHs



Model of Interferon Response in HCV Infected Liver



Summary & Conclusion

- **Type III interferon is the predominant interferon produced by hepatocytes in response to HCV infection**
- **Infected hepatocytes also produce high levels of chemokines, leading to subsequent inflammatory response and liver injury**
- **Type I and III IFNs may have distinct functional effects on hepatocytes**
- **IL28 genotype and induction by HCV may be functionally linked to HCV treatment response**

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