Prokunina-Olsson *et al.*

A Genetic Variant Creating a Novel Interferon Analog (IFNAN) Protein is Associated with Impaired Clearance of Hepatitis C Virus

Supplementary Fig. 1. DNA and protein sequence analysis of the regions upstream of the *IL28A* and *IL28B* genes. Identical nucleotides and amino acids are shaded in black. The location of ss469415590 is marked by a red bar.

a. Alignment of DNA sequences shows multiple mismatches between regions upstream of *IL28A* and *IL28B* genes. Protein translation is based on sequences upstream of *IL28B* gene; M (Met) marks first amino acid of predicted proteins.

b. The region upstream of *IL28A* is predicted to generate a protein fragment of 49 aa invariably terminated by a stop codon and expected to be degraded by nonsense-mediated decay. The region upstream of *IL28B* gene is predicted to produce several full-length protein isoforms depending on alleles of ss469415590, ΔG and TT (ΔC and AA based on complementary DNA strand). The open reading frames and the identity of these proteins depend on ss469415590, a frame-shift variant at amino acid 22.

Supplementary Fig. 2. Sequence and genetic variants in *IFNAN* gene

Transcription start site

Gttgcccaggtggagacggctctggacgcctcccaggggacagtggacggcagcacctgctgcagcacgaggcacagagg

rs186768149 rs4803222

gtgcactgcagacaggagtgagggcagaggccaaggcgag**[G/A]**agggggccggctcccactctct**[C/G]**tcccactg

tgtgtgctgtgccttcacgctccgagcattgccttccctgggatcctaacccaaggcgggggctggacgcgctggaccc

tctctttggcttccctgacgtctctcgcctgctgcagaagcagag

Exon 1 rs150891559,Ala11Ala rs73555604,Cys17Tyr ss469415590

ATGCGGCCGAGTGTCTGGGCCGCAGTGGCC**GC[G/A]**GGGCTGTGGGTCCTG**T[G/A]C**ACGGTGATCGCA**GC[TT/-G]**GCCC

rs4803221,Ser30Ser

CCCGGCGCTGCCTGCT**CTC[G/C]**CACTACCGCTCGCTGGAGCCCCGGACGCTGGCGGCTGCCAAGGCGCTGAGGGACCGCTAC

Intron 1 rs117436747

gtaagtcaccgcccagcccctgtgccccctgggaccctggccccacc**[G/A]**ggttcccatacacccgttcctgtcccaaggg

gtcctgcgtcctagcgcccagcaggcgcctctcctatgtcagcgcccacaattcccaccacgagacccccgcagtccccgtcg

tcagcgcgaacgcaggctcagggtcaatcacagaagggagccctgccgggaggactcggctccaggtcggggcgaggggcttt

rs12979860

gctgggggagcgcggagtgcaattcaaccctggttc**[G/A]**cgccttcggggagctccctggttcagtacacgacaggcacga

rs181637919

c**[C/T]**gtgcgctgccagtacccatccacgtccaggaatcccagactgtgcagaggttaggggccctggcgaggggggcctagc

cgtatgcgataagcgccgcttgtcccgcag

Exon 2 (alternative) **rs142981501,Arg60Pro** **rs117648444,Pro70Ser**

GAGGAAGAGGCGCTGAGCTGGGGGCAG**C[G/C]C**AACTGCTCCTTCCGCCCCAGGAGGGAT**[C/T]CT**CCGCGGCCATCC

Intron 2

gtgaggcccgggagtgggcgggagaggcatggcccgggcgcggcccgctctaacgccctctcgtccccgcag

Exon 3(alternative)

TCCTGCGCTCGGCTCCGCCACGTGGCCCGGGGCATCGCGGACGCCCAGGCAGTGCTCAGCGGCCTGCACCGCTCGGAGCTGCT

CCCCGGCGCCGGCCCGATCCTGGAGCTGCTGGCGGCCGCGGGGAGGGATGTGGCGGCCTGC

Intron 3

rs112794654 **rs111531283**

gtgag**[T/C]**gacggccgcgccccgccgcccctc**[T/G]**cccccgccagcttctctgcatcctcaggcccacggcgagcccca

rs143958949

gcgctttgccaatctgtcctgcttagcggaaaaacccatccagac**[C/A]**ggagtcgggtcctctgggtgtcctgaaatccgg

gctcgagtctgcggctgggagggccacgggcagatgcagagaggggcttcgtccttcgccttttccatttgcctcatgtccca

cctccag

Exon 4

CTTGAGCTGGCACGGCCAGGCTCCTCCAGGAAGGTCCCCGGGGCCCAGAAGAGGCGTCACAAACCCCGGAGAGCG

Intron 4 **rs77811741**

gtgagtgcaacaggcaatacagg**[G/T]**ttagcccgcagggaggaccaggcgaggctgacaaggacgggactgaggctgcgag

cagcgggactggaggggggattccgggggcgggggggaagagcctggcttagccccgctgccctccctccctggctccag

Exon 5

rs12971396,Ser149Ser rs149445319,Leu171Leu

GAC**TC[G/C]**CCTCGGTGCCGCAAAGCCAGCGTGGTCTTCAACCTCCTGCGCCTGCTCACGTGGGAG**CT[C/T]**CGGCTGGCT

rs137902769,Ser175Ser

GCACAC**TC[T/A]**GGGCCTTGCCTC**TGA**

rs11672932 rs150308118

ccccgccccctctggcagcacggaaacctccacgccattggctgccgaaagcagct**[C/G]**ctgt**[C/G]**gtccattgggc

Supplementary Fig. 3 Analysis of *IFNAN* variants based on Sanger sequencing and TaqMan genotyping of HapMap samples.

variants	SNP ID	alleles	annotation	Allele 1	Allele 1 frequencies in HapMap samples		
					CEU	YRI	CHB/JPT
IFNAN, upstream	rs7248668	A/G	4,602 bp upstream of translation start	A	0.183	0.025	0.056
IFNAN, upstream	rs8099917	T/G	3,946 bp upstream of translation start	G	0.183	0.025	0.061
IFNAN, upstream	rs8109886	A/C	3,543 bp upstream of translation start	A	0.422	0.864	0.073
IFNAN, upstream	rs10853727	T/C	1,244 bp upstream of translation start	C	0.108	0.108	0.006
IFNAN, 5'UTR	rs4803222	G/C	5'UTR, 134 bp upstream of translation start	C	0.305	0.305	0.067
IFNAN, ex1	rs150891559	A/G	Ala11Ala	T	0.008	0	0
IFNAN, ex1	rs73555604	A/G	Cys17Tyr	T	0.017	0.275	0
IFNAN, ex1	ss469415590	ΔG/TT	Ala22/no IFNAN	ΔG	0.314	0.767	0.067
IFNAN, ex1	rs4803221	G/C	Ser30Ser	G	0.186	0.192	0.056
IFNAN, intron1	rs12979860	C/T	intron 1	T	0.317	0.700	0.067
IFNAN, ex2	rs142981501	C/G	Pro60Arg	C	0	0.042	0
IFNAN, ex2	rs117648444	T/C	Pro70Ser	T	0.102	0.100	0.001
IFNAN, ex5	rs12971396	C/G	Ser149Ser	C	0.205	0.192	0.062
IFNAN, ex5	rs149445319*	C/T	Leu171Leu	T	0	0	0
IFNAN, ex5	rs137902769	T/A	Ser175Ser	A	0	0.133	0
IL28B, 5'UTR	rs28416813	G/C	5'UTR	G	0.325	0.683	0.063
IL28B, ex2	rs8103142	C/T	Lys70Arg	C	0.325	0.711	0

* - reported in dbSNP based on 1000 Genomes but found monomorphic by Sanger sequencing.

Supplementary Fig. 4. Analysis of pair-wise linkage disequilibrium (r^2) within *IL28B/IFNAN* region in HapMap samples from Europeans (CEU), West-Africans (YRI) and Asians - Chinese (CHB) and Japanese (JPT). Monomorphic markers are highlighted in light-gray.

HapMap, CEU N=90	Markers	rs7248668	rs8099917	rs8109886	rs10853727	rs4803222	rs150891559	rs73555604	ss469415590	rs4803221	rs12979860	rs142981501	rs117648444	rs12971396	rs149445319	rs137902769	rs28416813	rs8103142
IFNAN, upstream	rs7248668																	
IFNAN, upstream	rs8099917	1.000																
IFNAN, upstream	rs8109886	0.217	0.320															
IFNAN, upstream	rs10853727	0.017	0.027	0.158														
IFNAN, 5'UTR	rs4803222	0.393	0.522	0.609	0.282													
IFNAN, ex1	rs150891559	0.001	0.002	0.012	0.001	0.004												
IFNAN, ex1	rs73555604	0.002	0.004	0.025	0.002	0.008	0.496											
IFNAN, ex1	ss469415590	0.356	0.435	0.635	0.271	0.885	0.019	0.038										
IFNAN, ex1	rs4803221	0.820	0.891	0.329	0.028	0.522	0.002	0.004	0.435									
IFNAN, intron1	rs12979860	0.341	0.420	0.640	0.262	0.885	0.019	0.038	0.923	0.435								
IFNAN, ex2	rs142981501																	
IFNAN, ex2	rs117648444	0.015	0.008	0.152	0.823	0.267	0.001	0.002	0.256	0.005	0.248							
IFNAN, ex5	rs12971396	0.913	0.946	0.394	0.028	0.593	0.002	0.005	0.497	0.946	0.497		0.007					
IFNAN, ex5	rs149445319																	
IFNAN, ex5	rs137902769																	
IL28B, 5'UTR	rs28416813	0.341	0.466	0.666	0.252	0.924	0.018	0.036	0.962	0.482	0.962		0.238	0.546				
IL28B, ex2	rs8103142	0.341	0.466	0.666	0.197	0.850	0.018	0.036	0.886	0.482	0.962		0.182	0.546			0.925	

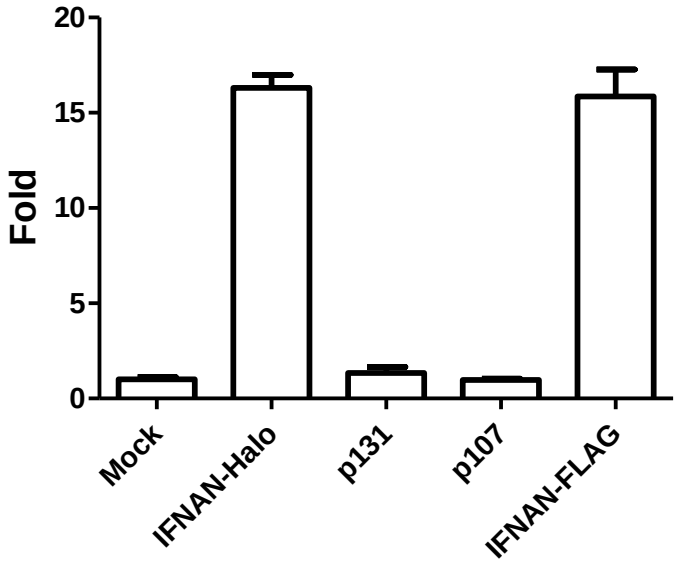
HapMap, YRI N=90	Markers	rs7248668	rs8099917	rs8109886	rs10853727	rs4803222	rs150891559	rs73555604	ss469415590	rs4803221	rs12979860	rs142981501	rs117648444	rs12971396	rs149445319	rs137902769	rs28416813	rs8103142
IFNAN, upstream	rs7248668																	
IFNAN, upstream	rs8099917	1.000																
IFNAN, upstream	rs8109886	0.003	0.004															
IFNAN, upstream	rs10853727	0.002	0.003	0.019														
IFNAN, 5'UTR	rs4803222	0.041	0.059	0.069	0.282													
IFNAN, ex1	rs150891559																	
IFNAN, ex1	rs73555604	0.000	0.010	0.058	0.046	0.170												
IFNAN, ex1	ss469415590	0.005	0.008	0.504	0.037	0.130		0.115										
IFNAN, ex1	rs4803221	0.075	0.108	0.036	0.029	0.551		0.090	0.072									
IFNAN, intron1	rs12979860	0.008	0.011	0.357	0.052	0.178		0.163	0.710	0.102								
IFNAN, ex2	rs142981501	0.001	0.001	0.007	0.005	0.101		0.016	0.013	0.100	0.019							
IFNAN, ex2	rs117648444	0.002	0.003	0.018	0.915	0.258		0.042	0.034	0.026	0.048	0.005						
IFNAN, ex5	rs12971396	0.075	0.108	0.036	0.029	0.551		0.090	0.072	1.000	0.102	0.100	0.026					
IFNAN, ex5	rs149445319																	
IFNAN, ex5	rs137902769	0.003	0.004	0.023	0.019	0.069		0.406	0.047	0.036	0.066	0.007	0.017	0.036				
IL28B, 5'UTR	rs28416813	0.008	0.011	0.276	0.030	0.142		0.172	0.628	0.101	0.880	0.019	0.050	0.101		0.069		
IL28B, ex2	rs8103142	0.007	0.011	0.301	0.023	0.133		0.158	0.610	0.093	0.800	0.018	0.046	0.093		0.064	0.920	

HapMap, CHB/JPT N=90	Markers	rs7248668	rs8099917	rs8109886	rs10853727	rs4803222	rs150891559	rs73555604	ss469415590	rs4803221	rs12979860	rs142981501	rs117648444	rs12971396	rs149445319	rs137902769	rs28416813	rs8103142
IFNAN, upstream	rs7248668																	
IFNAN, upstream	rs8099917	1.000																
IFNAN, upstream	rs8109886	0.823	0.836															
IFNAN, upstream	rs10853727																	
IFNAN, 5'UTR	rs4803222	0.904	0.911	0.918														
IFNAN, ex1	rs150891559																	
IFNAN, ex1	rs73555604																	
IFNAN, ex1	ss469415590	0.904	0.911	0.918		1.000												
IFNAN, ex1	rs4803221	0.895	0.904	0.755		0.824			0.824									
IFNAN, intron1	rs12979860	0.904	0.911	0.918		1.000			1.000	0.824								
IFNAN, ex2	rs142981501																	
IFNAN, ex2	rs117648444																	
IFNAN, ex5	rs12971396	1.000	1.000	0.836		0.911			0.911	0.904	0.911							
IFNAN, ex5	rs149445319																	
IFNAN, ex5	rs137902769																	
IL28B, 5'UTR	rs28416813	1.000	1.000	0.836		0.911			0.911	0.904	0.911			1.000				
IL28B, ex2	rs8103142	0.904	0.911	0.918		1.000			1.000	0.824	1.000			0.911			0.911	

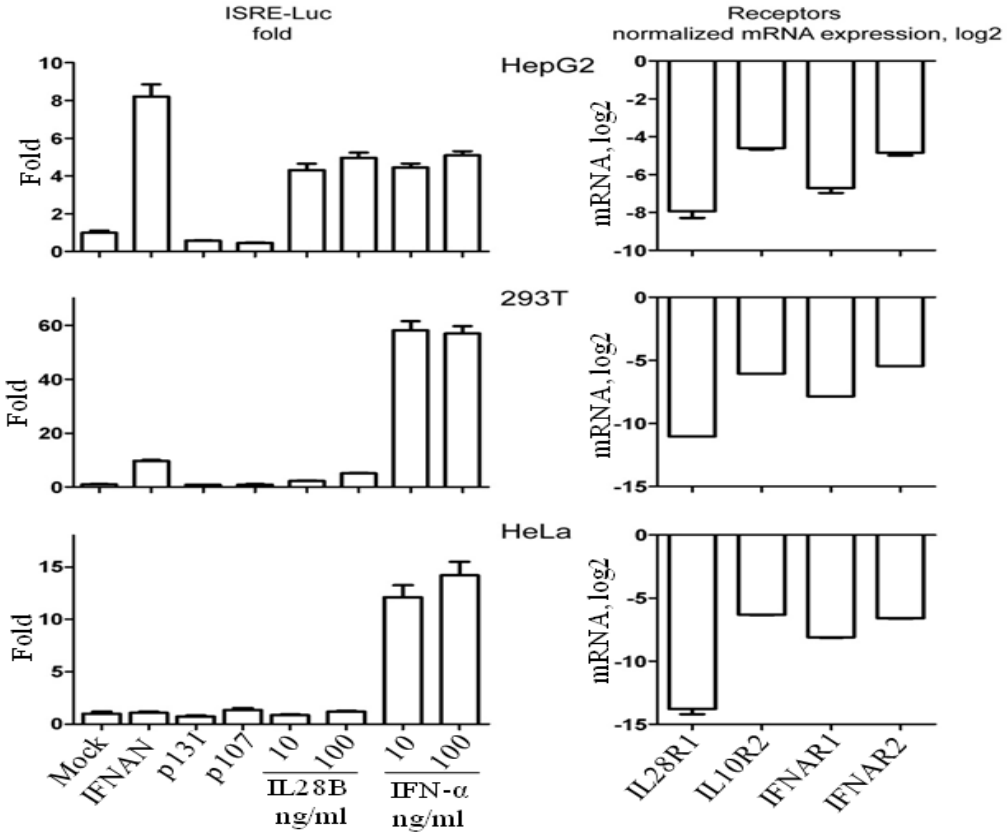
Supplementary Fig. 5. Haplotype analysis in the *IL28B/IFNAN* region in HapMap samples and participants of Virahep C study. Sustained virological response (SVR) was used as pegIFN-α/RBV therapy treatment outcome (responders/non-responders). Bold underlined are markers included in the haplotype analysis of SVR by Smith et. al, 2011., * - 8 markers used in the final haplotype analysis in HapMap and Virahep-C samples. In yellow – the unfavorable haplotypes based on studies in Europeans and Asians, and their extrapolation in Africans; in grey – a common favorable haplotype shared by all populations. Highlighted bold underlined are non-synonymous variants within *IFNAN* found on the background of unfavorable haplotypes. Genotypes of *IFNAN* markers were determined by Sanger sequencing; no other coding variants were identified in these samples. EA- European-Americans; AA- African-Americans. Haplotype frequencies in all groups (HapMap and responders/non-responders) are indicated as %.

location	IL28B			IFNAN									upstream of IFNAN				% in HapMap	Based on Smith et al, Genome Medicine, 2011, Table 2 n=819				Virahep-C EA: n=93 resp; n=85 non-resp; AA: n=43 resp; n=112 non-resp		
	intron 2	Lys70Arg	5'UTR	Ser175Ser, ex5	Ser149Ser, ex5	Pro70Ser, ex2	Arg60Pro, ex2	intron 1	Ser30Ser, ex1	Indel, ex1	Cys17Tyr, ex1	5'UTR	intergenic	intergenic	intergenic	intergenic								
variant	**rs11881222**	**rs8103142**	rs28416813	rs137902769	rs12971396*	rs11764844*	rs142981501*	**rs12979860***	**rs4803221 ***	ss469415590*	rs73555604*	rs4803222	**rs10853727**	**rs8109886**	**rs8099917***	**rs7248668**		resp, %	non-resp, %	p-value	OR, 95%CI	resp, %	non-resp, %	p-value
CEU n=90	G	C	G	T	**C**	C	G	T	**G**	**ΔG**	C	C	T	A	G	A	15.3	16.5	30.4	9.51E-11	2.20(1.72-2.80)	14.5	24.1	ref
	G	C	G	T	G	**T**	G	T	C	**ΔG**	C	C	**C**	A	T	G	9.3	10.1	10.6	0.77	1.04(0.75-1.43)	5.9	13.5	0.43
	A/G	C	G	T	G	C	G	T	C	**ΔG**	**T**	G	T	A	T	G	1.7	1.1	1.6	0.39	1.49 (0.62-3.56)	4.3	2.9	0.20
	A	T	C	T	G	C	G	C	C	**TT**	C	G	T	C	T	G	57.3	48.4	39.6	4.00E-04	0.70(0.57-0.85)	74.7	58.2	0.011
	A	T	C	T	G	C	G	C	C	**TT**	C	G	T	A	T	G	9.7	12.3	7.8	2.40E-03	0.60(0.43-0.83)			
YRI n=90	A	C	G	T/A	G	C	G	T	C	**ΔG**	**T**	G	T	A	T	G	24.8					14.0	22.3	
	G	C	G	T	**C**	C	G	T	**G**	**ΔG**	C	C	T	A	T	G	15.2					5.8	8.0	
	G	C	G	T	G	**T**	G	T	C	**ΔG**	C	C	**C**	A	T	G	10.0					9.3	4.9	
	A/G	C	G	T	G	C	G	T	C	**ΔG**	C	G	T	A	T	G	11.6					9.3	12.9	
	A	**T**	C	T	G	C	G	**C**	C	**ΔG**	C	G	T	A	T	G	6.7					1.1	7.6	
	G	C	G	T	**C**	C	**C**	T	**G**	**ΔG**	C	C	T	A	T	G	3.1					5.8	5.4	
	G	C	G	T	**C**	C	G	T	**G**	**ΔG**	C	C	T	A	G	A	2.5					9.3	6.7	
	A	C	G	T	G	C	**C**	T	C	**ΔG**	**T**	G	T	A	T	G	1.0					-	-	
	A	T	C	T	G	C	G	C	C	**TT**	C	G	T	C	T	G	12.5							
	A	T	C	T	G	C	G	C	C	**TT**	C	G	T	A	T	G	8.3					44.2	31.3	
	A	C	C	T	G	C	G	C	C	TT	C	G	T	A	T	G	1.7							
CHB, JPT, n=90	G	C	G	T	C	C	G	T	G	**ΔG**	C	C	T	A	G	A	5.5							
	A	T	C	T	G	C	G	C	C	TT	C	G	T	C	T	G	92.8							

Supplementary Fig. 6. Activation of the Interferon Stimulated Response Element (ISRE) - Luc reporter in HepG2 cells transiently co-transfected with IFNAN expression constructs carrying either the Halo-tag or a FLAG-tag. Alternatively, cells are transfected with expression constructs for non-functional forms p131 and p107, or an empty vector (mock). Similar activation of ISRE-Luc reporter is induced by IFNAN with both protein tags, while mock, p131 and p107 did not induce any activation. The results represent mean values of 8 biological replicates, with standard errors.

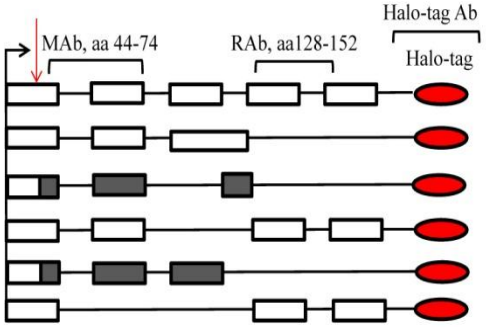


Supplementary Fig. 7. Activation of the interferon-stimulated response element (ISRE)-Luc reporter and mRNA expression of receptors for type–I IFNs (*IFNAR1* and *IFNAR2*) and type–III IFNs (*IL28R1* and *IL10R2*) in human cell lines HepG2, 293T and HeLa. Activation of ISRE-Luc reporter was analyzed after transient co-transfection with IFNAN, p131 or p107 expression constructs or after treatment with 10 and 100 ng/ml of IFN-α and IL28B. Results represent mean values of 8 biological replicates, with standard errors. mRNA expression of interferon receptors was evaluated by qRT-PCR assays in non-treated cells and normalized to expression of four endogenous controls; less negative values represent higher mRNA expression. Expression of *IFNAR1*, *IFNAR2* and *IL10R2* was found at comparable levels in all cell lines tested. However, these cell lines differ by the level of expression of *IL28R1*– the highest expression was detected in HepG2 cells, compared to which ~10 fold lower expression was detected in 293T cells and ~50 fold lower expression was detected in HeLa cell line.



Supplementary Fig. 8. Outline of expression constructs for the 6 protein isoforms. Epitopes recognized by the anti-IFNAN mouse and rabbit monoclonal antibodies and the Halo-tag ab are indicated. The mouse monoclonal antibody recognizes IFNAN (p179) and two non-functional proteins, p170 and p131; while the rabbit monoclonal antibody recognizes IFNAN (p179) and two non-functional proteins, p131 and p107. All Halo-tag protein isoforms are recognized with the Halo-tag antibody in cell lysates of transiently transfected hepatoma HepG2 cells.

a

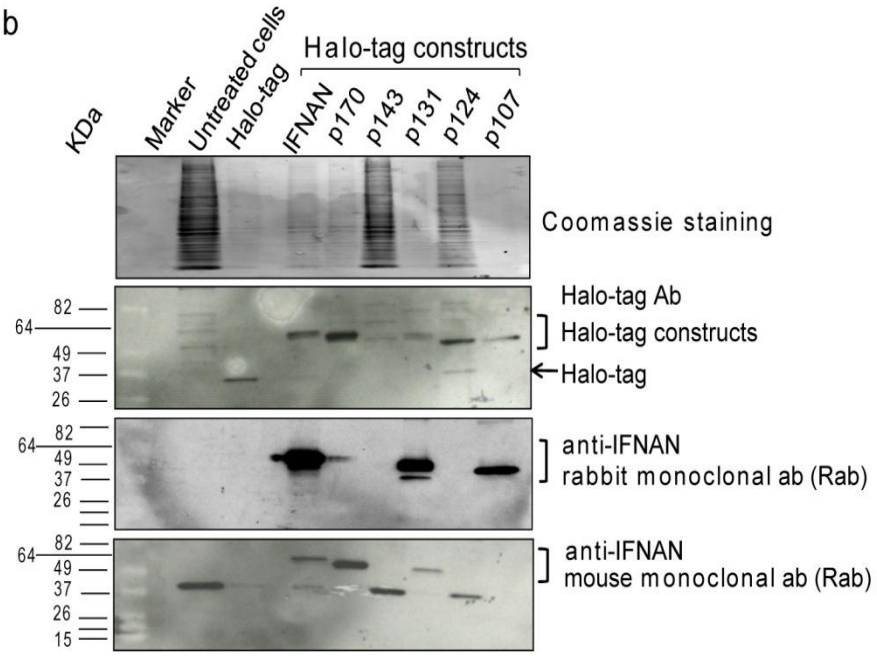


protein	Halo-tag Ab	IFNAN-ab	
		MAb	RAb
IFNAN p179 aa	Yes	**Yes**	**Yes**
p170 aa	Yes	**Yes**	No
p143 aa	Yes	No	No
p131aa	Yes	**Yes**	**Yes**
p124aa	Yes	No	No
p107aa	Yes	No	**Yes**

- transcripts with ΔG allele of ss469415590 (frame-shift), encode 4 protein isoforms, p179 (IFNAN) and 3 non-functional forms

- transcripts with TT allele of ss469415590, encode 2 protein isoforms unrelated to IFNAN

b



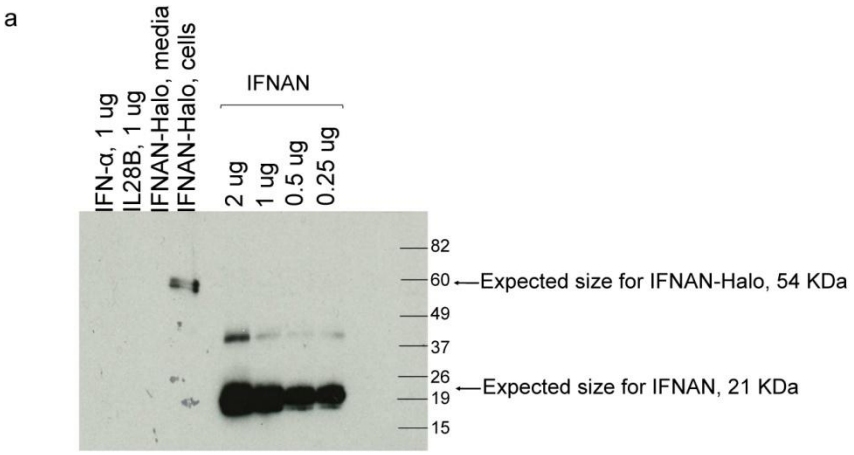
Supplementary Fig. 9. Analysis of IFNAN expression in HepG2 cells and primary human hepatocytes.

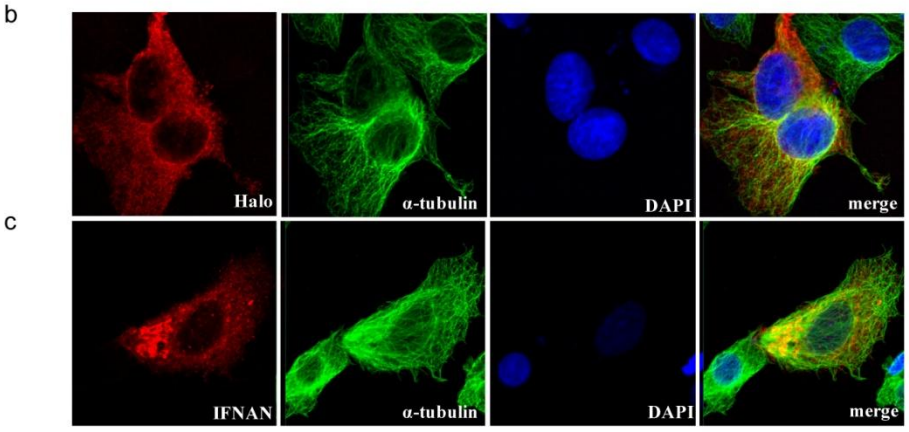
a. Western blot analysis of recombinant purified proteins (IFN-α, IL28B and IFNAN) and lysates of HepG2 cells transfected with IFNAN-Halo expression construct. Expression is detected with the mouse monoclonal anti-IFNAN antibody. IL28B and IFNAN are recombinant purified proteins generated in the sfs9 baculoviral system.

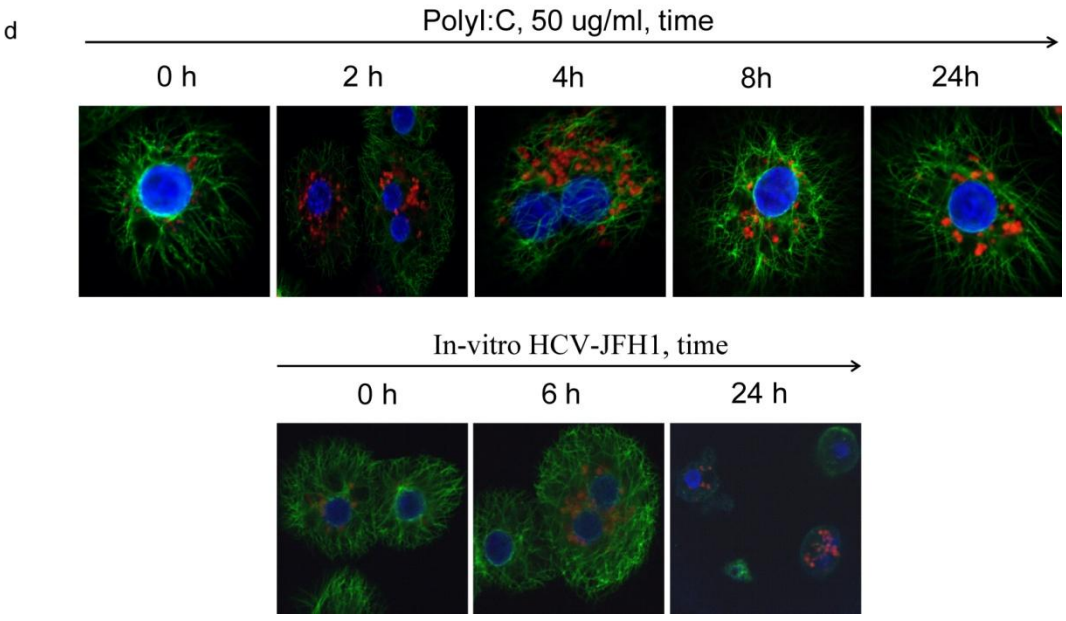
b. Confocal imaging with an anti-Halo-tag antibody in HepG2 cells transiently transfected with IFNAN-Halo construct;

c. Confocal imaging with a mouse monoclonal anti-IFNAN antibody in HepG2 cells transiently transfected with IFNAN-Halo construct;

d. Confocal imaging in primary human hepatocytes from a liver donor not infected with HCV, who was heterozygous for ss469415590 ΔG/TT genotype. The cells were treated with 50 ug/ml of PolyI:C (the same image as Fig. 5, ΔG/TT) or *in-vitro* infected with JFH1-HCV. The detection is performed with a mouse monoclonal anti-IFNAN antibody. Red – IFNAN; green – α-tubulin (cytoplasm), blue – nuclei. Weak IFNAN expression was detected even in samples not infected with JFH1-HCV (0 h). There was massive cell death in cells infected with JFH1-HCV at 24 h, but the consistency of this observation should be tested in additional samples.

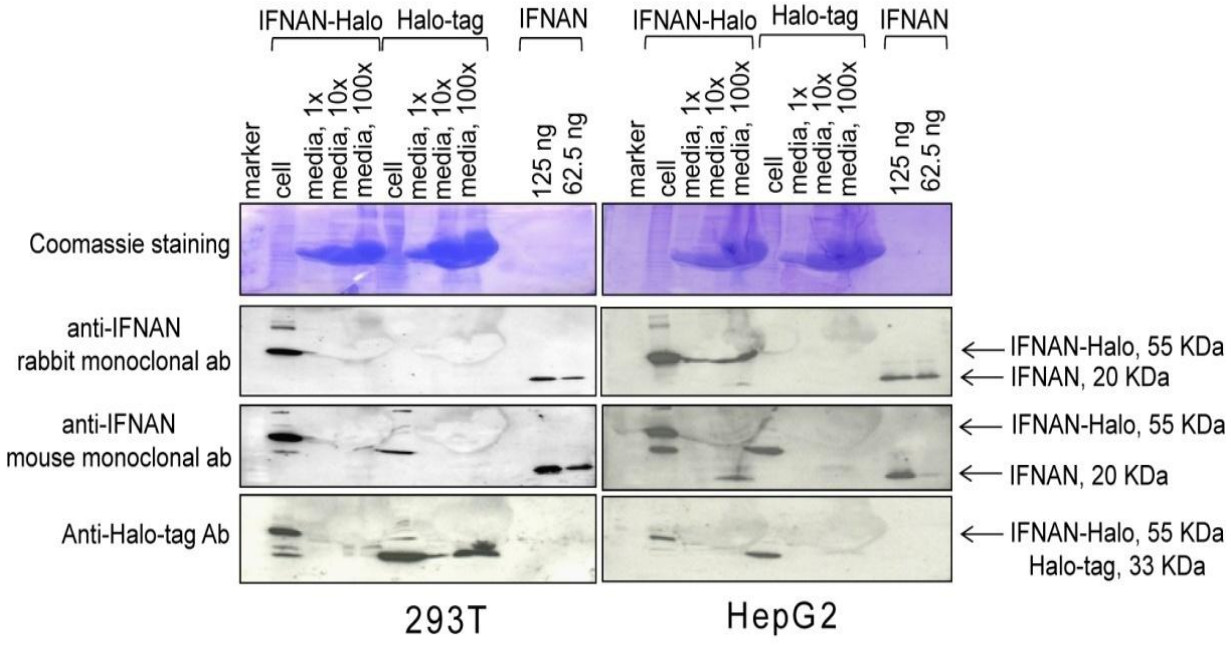

a
IFN-α, 1 ug
IL28B, 1 ug
IFNAN-Halo, media
IFNAN-Halo, cells
IFNAN
2 ug
1 ug
0.5 ug
0.25 ug
82
60
49
37
26
19
15
Expected size for IFNAN-Halo, 54 KDa
Expected size for IFNAN, 21 KDa


b
Halo
α-tubulin
DAPI
merge
c
IFNAN
α-tubulin
DAPI
merge

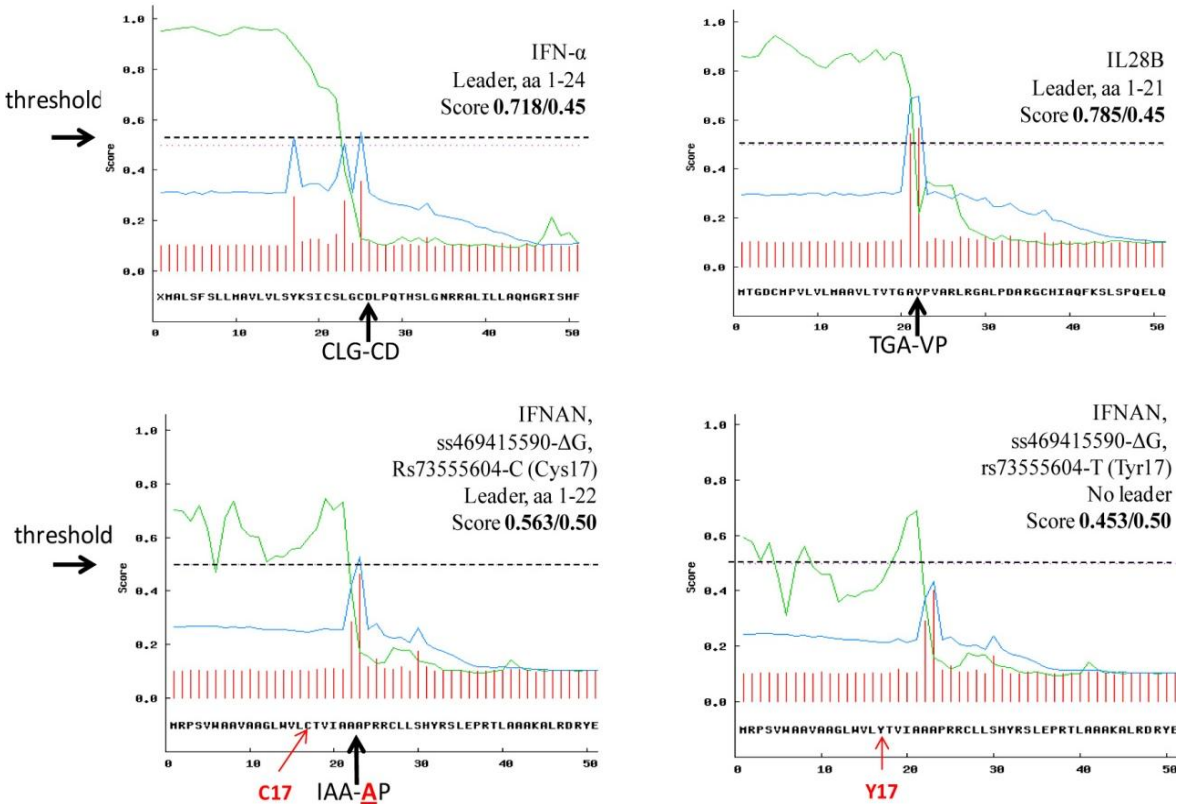

d
Polyl:C, 50 ug/ml, time
0 h
2 h
4h
8h
24h
In-vitro HCV-JFH1, time
0 h
6 h
24 h

Supplementary Fig. 10. Analysis of IFNAN expression in 293T and HepG2 cells transiently transfected with IFNAN-Halo construct or an empty Halo-tag construct.
a. The cells and the media were collected 72 hours post-transfection, using one well of 6-well plate per condition. Cells were lysed in 300 ul of RIPA buffer and ~10 ul of each lysate was used per gel lane; cell media was used un-concentrated or concentrated 10x or 100x. Weak secreted IFNAN expression was detected with the rabbit monoclonal anti-IFNAN antibody in HepG2 cells, but not in 293T cells. Very weak or no secreted IFNAN expression was detected with the mouse monoclonal anti-IFNAN or a Halo-tag antibodies.
b. Prediction of leader peptides with SignalP4.0 server for secreted proteins IFN-α and IL28B and two allelic forms of IFNAN that carry ss469415590-ΔG alleles but differ by rs73555604 (Cys17Tyr alleles). The weak leader peptide of IFNAN-Halo construct (with rs73555604, Cys17 variant) indicates that, compared to IFN-α and IL28B, IFNAN has lower secretability.

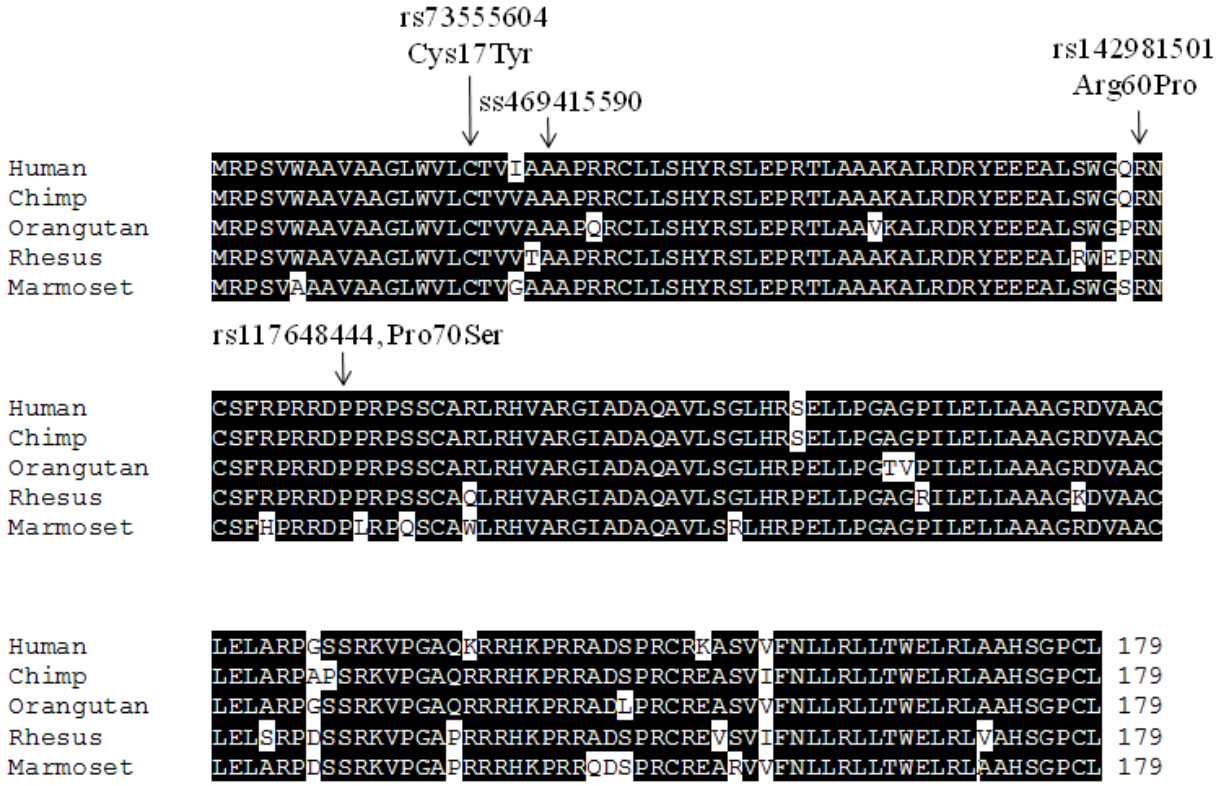
a



b


threshold
IFN-α
Leader, aa 1-24
Score 0.718/0.45
CLG-CD
IL28B
Leader, aa 1-21
Score 0.785/0.45
TGA-VP
threshold
IFNAN,
ss469415590-ΔG,
Rs73555604-C (Cys17)
Leader, aa 1-22
Score 0.563/0.50
C17
IAA-AP
IFNAN,
ss469415590-ΔG,
rs73555604-T (Tyr17)
No leader
Score 0.453/0.50
Y17

Supplementary Fig. 11. Prediction of IFNAN protein based on genomic sequences of 45 species available in UCSC genome browser (genome.uscs.edu). IFNAN protein is predicted only in the genomes of macaque (marmoset and rhesus), orangutan, chimpanzee and human. Identical amino acids are shaded in black and the positions of human genetic variants are indicated.



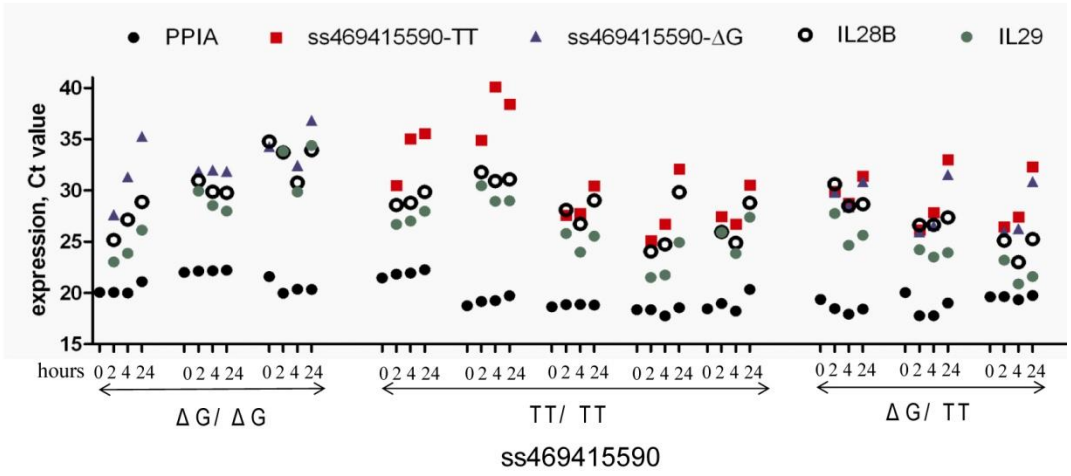
Supplementary Fig. 12. Quantitative reverse-transcriptase PCR (qRT-PCR) analysis of allele-specific expression of transcripts with ss469415590-TT and ΔG alleles and expression of *IL28B*, *IL29* and *PPIA* (endogenous control) in various samples. Expression is presented on log 2 scale as Ct values (PCR cycle at detection), with higher Ct values corresponding to lower mRNA expression. The fold difference between any two samples can be roughly calculated as fold=$2^{\wedge(\text{Ct sample1-Ct sample 2})}$. Equal amounts of DNase-treated RNA were used for all the assays. Graphs show activation of transcripts carrying both ss469415590 alleles (TT and ΔG). Transcripts carrying the risk ss469415590-ΔG allele generate the functional IFNAN/p179 protein, as well as non-functional proteins p170, p131, p107, while transcripts with the beneficial ss469415590-TT allele generate only non-functional proteins. All the samples were also genotyped for rs12979860 and ss469415590 alleles TT and ΔG correspond to rs12979860 alleles C and T. All primary human hepatocytes (PHH) are from liver donors not infected with HCV.

a. Expression analysis in PHH treated with 50 ug/ml of PolyI:C for 0, 2, 4 and 24 hours. PHH are from liver donors with ss469415590 ΔG/ΔG (n=3), TT/TT (n=5) and TT/ΔG (n=3) genotypes.

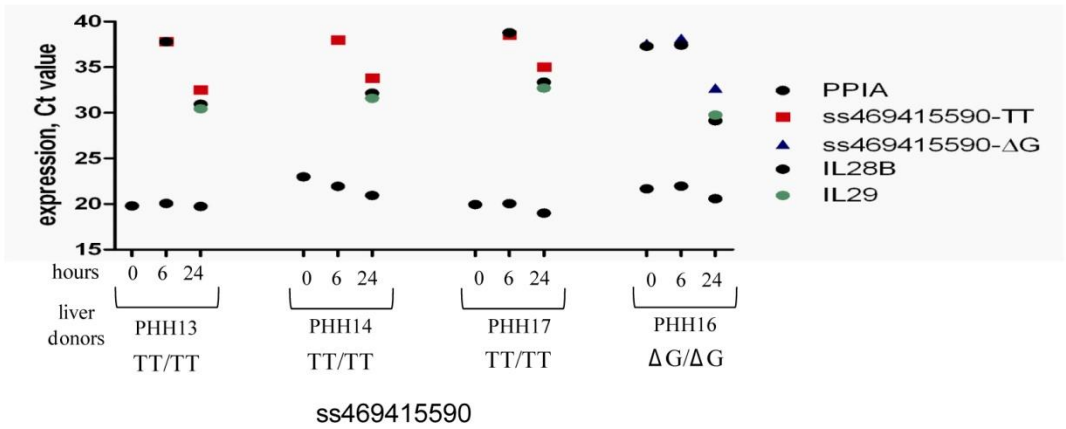
b. Expression analysis in PHH *in-vitro* infected with JFH1-HCV for 0, 6 and 24 hours. PHH are from liver donors with ss469415590 ΔG/ΔG (n=1) and TT/TT (n=3) genotypes.

c. Expression analysis in PHH or HepG2 cells. PHH are from a liver donor with ss469415590 ΔG/ΔG genotype. PHH were untreated or treated with 100 ng/ml of IFN-α or IFN-λ. Only IFN-α treatment induced low *IFNAN* expression. HepG2 cells with ss469415590 TT/ΔG genotype were treated with 50 ug/ml of PolyI:C for 0, 1, 2, 4, 8 and 24 hours, but no expression of transcripts with either TT or ΔG alleles of ss469415590 was induced.

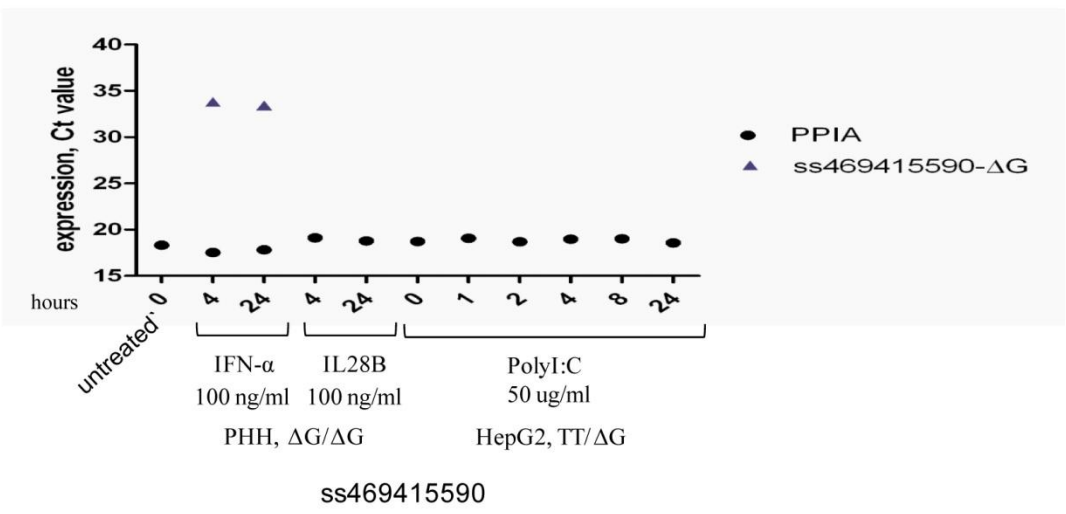
a


PPIA
ss469415590-TT
ss469415590-ΔG
IL28B
IL29
expression, Ct value
40
35
30
25
20
15
hours 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24 0 2 4 24
ΔG/ ΔG
TT/ TT
ΔG/ TT
ss469415590

b


expression, Ct value
40
35
30
25
20
15
hours 0 6 24 0 6 24 0 6 24 0 6 24
liver donors
PHH13
TT/TT
PHH14
TT/TT
PHH17
TT/TT
PHH16
ΔG/ΔG
PPIA
ss469415590-TT
ss469415590-ΔG
IL28B
IL29
ss469415590

c

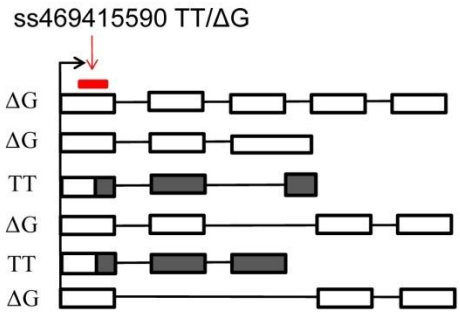

expression, Ct value
40
35
30
25
20
15
hours
0
4
24
4
24
0
1
2
4
8
24
untreated
IFN-α
100 ng/ml
IL28B
100 ng/ml
PolyI:C
50 ug/ml
PHH, ΔG/ΔG
HepG2, TT/ΔG
PPIA
ss469415590-ΔG
ss469415590

Supplementary Fig. 13. Outline of mRNA transcripts within *IFNAN* region and their detection by RNA-sequencing and an allele-specific expression assay.

a. Outline of all mRNA transcripts generating full-length protein isoforms. An allele-specific expression assay for ss469415590 TT/ΔG is located within the first exon of *IFNAN* and detects all transcripts with TT and ΔG alleles. Thus, this assay does not discriminate between the four transcripts with ΔG allele generating the functional IFNAN (p179) protein and non-functional forms p170, p131 and p107. RNA-seq results show fragments per kilobase of exon per million fragments mapped (FPKM) values which roughly correspond to relative levels of expression of each of the transcripts detected by RNA-seq analysis of PolyI:C-stimulated primary human hepatocytes from a liver donor heterozygous for ss469415590 (Fig. 1). Of all the transcripts carrying the ΔG allele, the transcript for the non-functional p170 is the most abundant isoform at 2, 4, 8 and 24 hours of PolyI:C treatment. The functional IFNAN (p179) transcript is the second common isoform at 4, 8 and 24 hours after PolyI:C treatment.

b. Outline of DNA/cDNA sequence and the primers used for an allele-specific genotyping and expression assay for ss469415590 TT/ΔG (corresponds to AA/-C in on a complementary strand). The full information about this assay is presented in Supplementary Table 11. The assay targets ss469415590 and avoids other variants in this amplicon. Since the assay is located within exonic sequence, it can be used both for genotyping and expression analysis, for which RNA has to be DNAse-treated to eliminate any possible signal from residual contaminating DNA.

a



	RNA-seq, FPKM			
transcript	2 h	4 h	8 h	24 h
IFNAN p179 aa	0	96.2	31.7	18.5
p170 aa	133.4	233.2	71.6	50.0
p143 aa	1.3	0	0	1.5
p131 aa	0	39.3	11.3	0
p124 aa				
p107 aa	71.7	0	0	8.9

- transcripts with ΔG allele of ss469415590 (frame-shift), encode 4 protein isoforms, p179 (IFNAN) and 3 non-functional forms

-transcripts with TT allele of ss469415590, encode 2 protein isoforms unrelated to IFNAN

b

forward primer
GCCTGCTGCAGAAGCAGAGATGCGGCCGAGTGTCTGGGCCGCAGTG
rs150891559 rs73555604 **ss469415590**
GCCGC[**G/c**]GGGCTGTGGGTCCTGT[**G/a**]CACGGTGATCGCAG**[AA/-C]**
rs4803221 reverse primer
GGCCCCCCGGCGCTGCCTGCTCTC[**G/c**]**CACTACCGCTCGCTGGAGC**