

Stem Cell Reports, Volume 3

Supplemental Information

Modulating Innate Immunity Improves

Hepatitis C Virus Infection and Replication

in Stem Cell-Derived Hepatocytes

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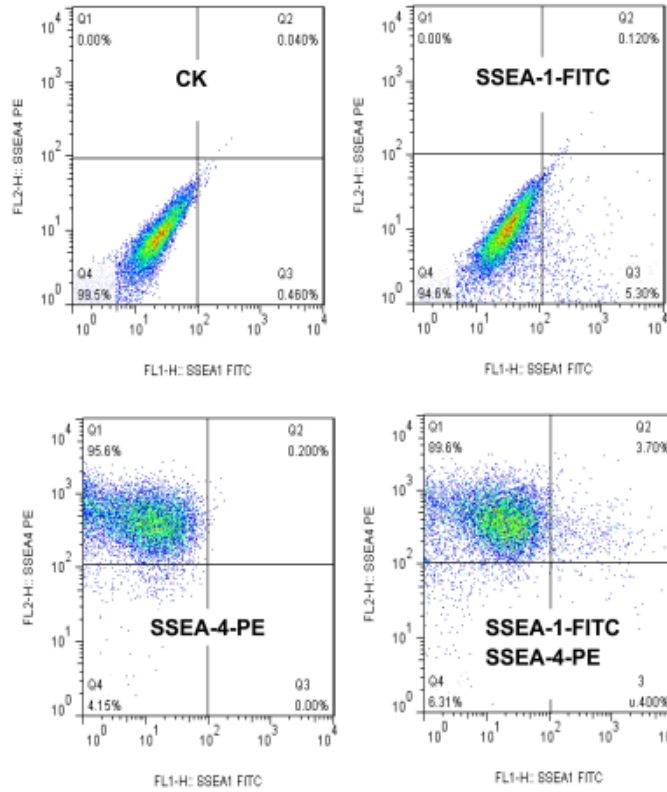


Figure S1. hESC characterization, Related to Figure 1

hESCs were maintained feeder-free prior to hepatic differentiation. hESCs cell surface expression was determined by flow cytometry. hESCs were labeled with control (CK), SSEA-1 and SSEA-4 antibodies and percentage positive cells were measured.

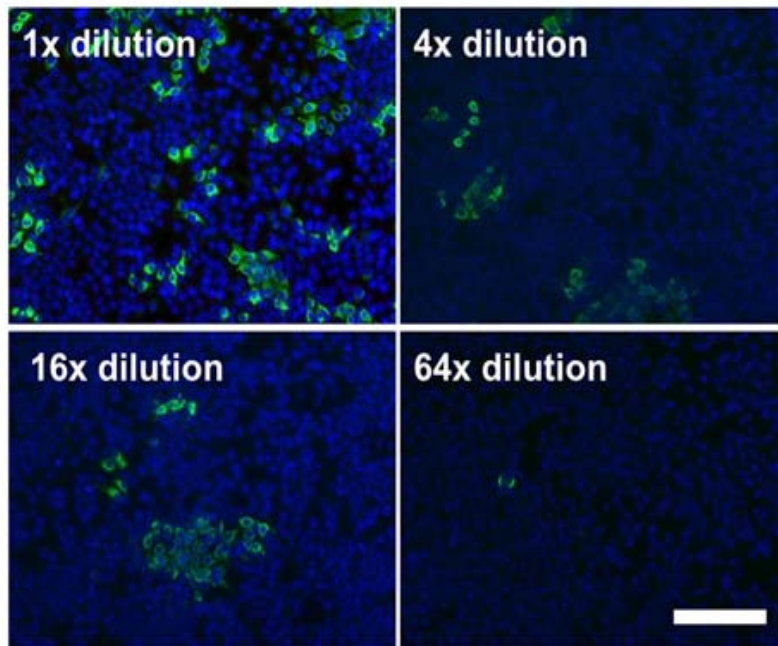


Figure S2. Focus forming assay of HCV-containing supernatant from hESC-Heps, Related to Figure 3

Supernatants were collected from hESC-Heps 5 days post-infection. Naive Huh7 cells seeded in 96 well plates were infected with 100 μ l of serially diluted supernatants collected from infected hESC-Heps. Following incubation at 37°C for 3 d, the infected Huh7 cells were fixed with methanol and stained for the viral NS5A protein (green) and counterstained by DAPI (blue). Scale bar = 100 μ m

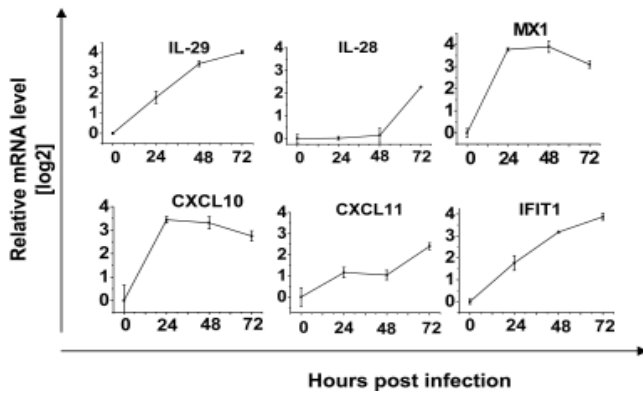


Figure S3. ISG gene expression in response to HCV infection

RNA was extracted from hESC-Heps at 0, 24, 48 or 72 h post-infection, and the mRNA levels of IFN (*IL-29*, *IL-28*) and ISGs (*MX1*, *CXCL10*, *CXCL11*, *IFIT1*) were determined by QPCR. n=3 biological replicates.

	<i>Type</i>	<i>Source</i>	<i>Dilution for Western blot</i>	Dilution for Immunostaining
Primary antibodies				
Oct4	Rb poly	Abcam	1/2000	1/500
Albumin	Mo mono	Sigma		1/500
HNF4 α	Rb poly	Santa Cruz		1/100
E-cadherin	Mo mono	Abcam		1/50
AFP	Mo mono	Sigma	1/2000	
β -Actin	Mo mono	Sigma	1/10000	
Occludin	Mo mono	Invitrogen	1/1000	1/200
CD81	Mo mono	Santa Cruz	1/200	1/50
SR-BI	Mo mono	BD	1/1500	1/200
Claudin-1	Mo mono	Invitrogen	1/1000	1/200
Secondary antibodies				
Anti Ra HRP	Go anti-Rb	DAKO	1/5000	
Anti Mo HRP	Go anti-Mo	DAKO	1/5000	
Anti-Mo 488	DN anti-Mo	Molecular Probes		1/500
Anti-Ra 488	DN anti-Mo	Molecular Probes		1/500
Anti-Ra 594	DN anti-Ra	Molecular Probes		1/500

Table S1. Antibodies used in this study, Related to Figures 1–5

<i>Gene name</i>	<i>Sequence (5' →3')</i>	Direction
<i>CD81</i>	TCTTCAAGGAGGACTGCCACC	Forward
	ATGATCACAGCGACCACGATG	Reverse
<i>CLDN1</i>	CCTCCTGGGAGTGATAGCAAT	Forward
	GGCAACTAAAATAGCCAGACCT	Reverse
<i>SRBI</i>	TGGGAAGATTGAGCCTGTGGT	Forward
	AGGACGTACTGGGCATAGTGC	Reverse
<i>OCN</i>	TTCCAATGGCAAAGTGAATGAC	Forward
	CAAAGTTACCACCGCTGCTGTA	Reverse
<i>IL-29</i>	GGCAGGTTCAAATCTCTGTCAC	Forward
	CTGCCACTCCAGTTTTTCAGCT	Reverse
<i>IL-28</i>	CTGCCACATAGCCCAGTTCA	Forward
	CAGTCCTTCAGCAGAAGCGA	Reverse
<i>IFIT1</i>	AGAAGCAGGCAATCACAGAAAA	Forward
	CTGAAACCGACCATAGTGGAAAT	Reverse
<i>MX1</i>	TCCGACACGAGTTCCACAAAT	Forward
	AAAGCCTGGCAGCTCTCTACC	Reverse
<i>ISG15</i>	CGCAGATCACCCAGAAGATCG	Forward
	TTCGTTCGATTTGTCCACCA	Reverse
<i>CXCL10</i>	CTGATTTGCTGCCTTATCTTTCT	Forward
	ATGCAGGTACAGCGTACAGTTCT	Reverse
<i>CXCL11</i>	GCCTTGCTGTGATATTGTGTG	Forward
	TGCCACTTTCAGTCTTTTACC	Reverse
<i>IRF9</i>	GCTCTTCAGAACCGCCTACTT	Forward
	GGCTCTCTCCCAGAAATTC	Reverse
<i>RIG-1</i>	AGTTGCTGATGAAGGCATTGAC	Forward
	GCACTTGCTACCTCTTGCTCTT	Reverse
<i>HCV</i>	TCTGCGGAACCGGTGAGTAC	Forward
	GCACTCGCAAGCGCCCTATC	Reverse
<i>GAPDH</i>	CACCATCTTCCAGGAGCGA	Forward
	TCAGCAGAGGGGCAGAGA	Reverse

Table S2. Primer sequences used in this study, Related to Figures 2–5

<i>Position</i>	<i>Unigene</i>	<i>GeneBank</i>	<i>Symbol</i>	<i>Fold change (IL29 test)</i>	<i>Fold change (HCV test)</i>
A01	Hs.12341	NM_001111	<i>ADAR</i>	4.81	1.62
A02	Hs.129966	NM_001842	<i>CNTFR</i>	3.69	4.48
A03	Hs.287729	NM_001012288	<i>CRLF2</i>	2.13	8.25
A04	Hs.520937	NM_006140	<i>CSF2RA</i>	1.36	3.57
A05	Hs.524517	NM_000760	<i>CSF3R</i>	1.1	3.71
A06	Hs.632586	NM_001565	<i>CXCL10</i>	110.63	4.32
A07	Hs.501452	NM_005755	<i>EBI3</i>	1.99	4.16
A08	Hs.62192	NM_001993	<i>F3</i>	6.9	1.16
A09	Hs.380250	NM_005531	<i>IFI16</i>	4.34	18.39
A10	Hs.532634	NM_005532	<i>IFI27</i>	927.55	5.91
A11	Hs.14623	NM_006332	<i>IFI30</i>	4.05	6.37
A12	Hs.632258	NM_005533	<i>IFI35</i>	11.27	5.65
B01	Hs.82316	NM_006417	<i>IFI44</i>	389.86	6.64
B02	Hs.389724	NM_006820	<i>IFI44L</i>	216.25	2.54
B03	Hs.730125	NM_002038	<i>IFI6</i>	111.52	5.12
B04	Hs.163173	NM_022168	<i>IFIH1</i>	51.89	4.19
B05	Hs.20315	NM_001548	<i>IFIT1</i>	2488.73	4.64
B06	Hs.437609	NM_001547	<i>IFIT2</i>	152.75	3.41
B07	Hs.714337	NM_001549	<i>IFIT3</i>	9.69	2.1
B08	Hs.458414	NM_003641	<i>IFITM1</i>	20.27	6.29
B09	Hs.709321	NM_006435	<i>IFITM2</i>	0.85	4.6
B10	Hs.37026	NM_024013	<i>IFNA1</i>	0.82	6.04
B11	Hs.93907	NM_002172	<i>IFNA14</i>	N/A	5.41
B12	Hs.56303	NM_002173	<i>IFNA16</i>	N/A	3.47
C01	Hs.211575	NM_000605	<i>IFNA2</i>	N/A	3.68
C02	Hs.113211	NM_002175	<i>IFNA21</i>	N/A	7.45
C03	Hs.1510	NM_021068	<i>IFNA4</i>	N/A	2.28
C04	Hs.37113	NM_002169	<i>IFNA5</i>	N/A	7.91
C05	Hs.533470	NM_021002	<i>IFNA6</i>	N/A	7.66
C06	Hs.282274	NM_021057	<i>IFNA7</i>	N/A	5.85
C07	Hs.73890	NM_002170	<i>IFNA8</i>	16.9	6.49
C08	Hs.529400	NM_000629	<i>IFNAR1</i>	1.24	4.33
C09	Hs.708195	NM_000874	<i>IFNAR2</i>	0.36	4.88
C10	Hs.93177	NM_002176	<i>IFNB1</i>	N/A	7.99
C11	Hs.682604	NM_176891	<i>IFNE</i>	0.65	8.25
C12	Hs.856	NM_000619	<i>IFNG</i>	N/A	7.77
D01	Hs.520414	NM_000416	<i>IFNGR1</i>	2.65	4.89
D02	Hs.634632	NM_005534	<i>IFNGR2</i>	1.14	4.21
D03	Hs.591083	NM_020124	<i>IFNK</i>	0.47	8
D04	Hs.73010	NM_002177	<i>IFNW1</i>	N/A	6.31
D05	Hs.7879	NM_001550	<i>IFRD1</i>	0.44	4.14
D06	Hs.315177	NM_006764	<i>IFRD2</i>	1.62	7.56
D07	Hs.504035	NM_001558	<i>IL10RA</i>	2.25	5.6
D08	Hs.654593	NM_000628	<i>IL10RB</i>	0.76	4.28
D09	Hs.591088	NM_004512	<i>IL11RA</i>	1.04	5.37
D10	Hs.674	NM_002187	<i>IL12B</i>	N/A	4.38

D11	Hs.496646	NM_001560	<i>IL13RA1</i>	0.58	4.28
D12	Hs.654378	NM_000585	<i>IL15</i>	2.29	5.5
E01	Hs.445868	NM_014432	<i>IL20RA</i>	5.54	9.52
E02	Hs.61232	NM_144717	<i>IL20RB</i>	2.29	4.52
E03	Hs.210546	NM_021798	<i>IL21R</i>	2.46	6.69
E04	Hs.126891	NM_052962	<i>IL22RA2</i>	1.06	4.71
E05	Hs.567792	NM_172138	<i>IL28A</i>	N/A	5.47
E06	Hs.221375	NM_173065	<i>IL28RA</i>	1.96	3.37
E07	Hs.406745	NM_172140	<i>IL29</i>	10.99	7.1
E08	Hs.474787	NM_000878	<i>IL2RB</i>	0.97	5.32
E09	Hs.84	NM_000206	<i>IL2RG</i>	1.17	1.37
E10	Hs.55378	NM_139017	<i>IL31RA</i>	0.37	5.52
E11	Hs.632790	NM_002183	<i>IL3RA</i>	0.62	5.88
E12	Hs.513457	NM_000418	<i>IL4R</i>	1.64	7
F01	Hs.68876	NM_000564	<i>IL5RA</i>	3.39	7.17
F02	Hs.654458	NM_000600	<i>IL6</i>	0.26	6.88
F03	Hs.709210	NM_000565	<i>IL6R</i>	1.25	3.92
F04	Hs.591742	NM_002185	<i>IL7R</i>	1.44	8.5
F05	Hs.406228	NM_002186	<i>IL9R</i>	N/A	5.67
F06	Hs.436061	NM_002198	<i>IRF1</i>	2.26	7.21
F07	Hs.654566	NM_002199	<i>IRF2</i>	2.22	3.86
F08	Hs.515477	NM_015649	<i>IRF2BP1</i>	0.05	15.31
F09	Hs.75254	NM_001571	<i>IRF3</i>	0.89	3.77
F10	Hs.401013	NM_002460	<i>IRF4</i>	0.46	3.13
F11	Hs.521181	NM_001098629	<i>IRF5</i>	1.43	6.53
F12	Hs.591415	NM_006147	<i>IRF6</i>	2.56	2.16
G01	Hs.166120	NM_001572	<i>IRF7</i>	18.56	12.51
G02	Hs.137427	NM_002163	<i>IRF8</i>	2.18	3.69
G03	Hs.519680	NM_001145805	<i>IRGM</i>	N/A	4.78
G04	Hs.458485	NM_005101	<i>ISG15</i>	52.36	4.35
G05	Hs.705413	NM_002303	<i>LEPR</i>	5.25	3.19
G06	Hs.82906	NM_005373	<i>MPL</i>	0.18	7.76
G07	Hs.517307	NM_002462	<i>MX1</i>	11.26	4.66
G08	Hs.524760	NM_002534	<i>OAS1</i>	88.16	5.73
G09	Hs.75348	NM_176783	<i>PSME1</i>	0.79	2.93
G10	Hs.710248	NM_152501	<i>PYHIN1</i>	N/A	4.54
G11	Hs.145150	NM_004509	<i>SP110</i>	6.59	6.31
G12	Hs.134602	NM_003319	<i>TTN</i>	0.14	2.51
H01	Hs.520640	NM_001101	<i>ACTB</i>	1.79	2.39
H02	Hs.534255	NM_004048	<i>B2M</i>	1.19	9.83
H03	Hs.592355	NM_002046	<i>GAPDH</i>	1	1
H04	Hs.412707	NM_000194	<i>HPRT1</i>	1.28	5.18
H05	Hs.546285	NM_001002	<i>RPLP0</i>	0.54	1.71

Table S3. RT² Profiler™ PCR Array Data with IL29 treatment or HCV treatment, Related to Table 1

<i>Position</i>	<i>Unigene</i>	<i>GeneBank</i>	<i>Symbol</i>	<i>Fold change (JAK inhibitor test)</i>
A01	Hs.12341	NM_001111	<i>ADAR</i>	0.89
A02	Hs.129966	NM_001842	<i>CNTFR</i>	14.96
A03	Hs.287729	NM_001012288	<i>CRLF2</i>	1.20
A04	Hs.520937	NM_006140	<i>CSF2RA</i>	1.34
A05	Hs.524517	NM_000760	<i>CSF3R</i>	1.51
A06	Hs.632586	NM_001565	<i>CXCL10</i>	6.01
A07	Hs.501452	NM_005755	<i>EBI3</i>	5.00
A08	Hs.62192	NM_001993	<i>F3</i>	1.32
A09	Hs.380250	NM_005531	<i>IFI16</i>	1.13
A10	Hs.532634	NM_005532	<i>IFI27</i>	2.27
A11	Hs.14623	NM_006332	<i>IFI30</i>	1.59
A12	Hs.632258	NM_005533	<i>IFI35</i>	0.76
B01	Hs.82316	NM_006417	<i>IFI44</i>	6.96
B02	Hs.389724	NM_006820	<i>IFI44L</i>	2.20
B03	Hs.730125	NM_002038	<i>IFI6</i>	1.59
B04	Hs.163173	NM_022168	<i>IFIH1</i>	2.17
B05	Hs.20315	NM_001548	<i>IFIT1</i>	1.41
B06	Hs.437609	NM_001547	<i>IFIT2</i>	6.66
B07	Hs.714337	NM_001549	<i>IFIT3</i>	12.18
B08	Hs.458414	NM_003641	<i>IFITM1</i>	8.29
B09	Hs.709321	NM_006435	<i>IFITM2</i>	10.60
B10	Hs.37026	NM_024013	<i>IFNA1</i>	8.86
B11	Hs.93907	NM_002172	<i>IFNA14</i>	5.85
B12	Hs.56303	NM_002173	<i>IFNA16</i>	3.20
C01	Hs.211575	NM_000605	<i>IFNA2</i>	5.23
C02	Hs.113211	NM_002175	<i>IFNA21</i>	1.83
C03	Hs.1510	NM_021068	<i>IFNA4</i>	3.23
C04	Hs.37113	NM_002169	<i>IFNA5</i>	1.10
C05	Hs.533470	NM_021002	<i>IFNA6</i>	1.07
C06	Hs.282274	NM_021057	<i>IFNA7</i>	2.88
C07	Hs.73890	NM_002170	<i>IFNA8</i>	4.97
C08	Hs.529400	NM_000629	<i>IFNAR1</i>	7.72
C09	Hs.708195	NM_000874	<i>IFNAR2</i>	1.94
C10	Hs.93177	NM_002176	<i>IFNB1</i>	2.96
C11	Hs.682604	NM_176891	<i>IFNE</i>	6.23
C12	Hs.856	NM_000619	<i>IFNG</i>	3.77
D01	Hs.520414	NM_000416	<i>IFNGR1</i>	1.27
D02	Hs.634632	NM_005534	<i>IFNGR2</i>	1.25
D03	Hs.591083	NM_020124	<i>IFNK</i>	2.34
D04	Hs.73010	NM_002177	<i>IFNW1</i>	1.17
D05	Hs.7879	NM_001550	<i>IFRD1</i>	1.35
D06	Hs.315177	NM_006764	<i>IFRD2</i>	2.51
D07	Hs.504035	NM_001558	<i>IL10RA</i>	5.56
D08	Hs.654593	NM_000628	<i>IL10RB</i>	1.25
D09	Hs.591088	NM_004512	<i>IL11RA</i>	1.18
D10	Hs.674	NM_002187	<i>IL12B</i>	1.58
D11	Hs.496646	NM_001560	<i>IL13RA1</i>	1.74

D12	Hs.654378	NM_000585	<i>IL15</i>	1.72
E01	Hs.445868	NM_014432	<i>IL20RA</i>	7.11
E02	Hs.61232	NM_144717	<i>IL20RB</i>	1.23
E03	Hs.210546	NM_021798	<i>IL21R</i>	5.12
E04	Hs.126891	NM_052962	<i>IL22RA2</i>	5.78
E05	Hs.567792	NM_172138	<i>IL28A</i>	1.14
E06	Hs.221375	NM_173065	<i>IL28RA</i>	1.70
E07	Hs.406745	NM_172140	<i>IL29</i>	2.21
E08	Hs.474787	NM_000878	<i>IL2RB</i>	1.91
E09	Hs.84	NM_000206	<i>IL2RG</i>	1.20
E10	Hs.55378	NM_139017	<i>IL31RA</i>	5.86
E11	Hs.632790	NM_002183	<i>IL3RA</i>	3.24
E12	Hs.513457	NM_000418	<i>IL4R</i>	5.32
F01	Hs.68876	NM_000564	<i>IL5RA</i>	1.62
F02	Hs.654458	NM_000600	<i>IL6</i>	1.09
F03	Hs.709210	NM_000565	<i>IL6R</i>	1.08
F04	Hs.591742	NM_002185	<i>IL7R</i>	1.82
F05	Hs.406228	NM_002186	<i>IL9R</i>	1.14
F06	Hs.436061	NM_002198	<i>IRF1</i>	3.43
F07	Hs.654566	NM_002199	<i>IRF2</i>	1.67
F08	Hs.515477	NM_015649	<i>IRF2BP1</i>	1.01
F09	Hs.75254	NM_001571	<i>IRF3</i>	1.18
F10	Hs.401013	NM_002460	<i>IRF4</i>	1.36
F11	Hs.521181	NM_001098629	<i>IRF5</i>	1.04
F12	Hs.591415	NM_006147	<i>IRF6</i>	2.29
G01	Hs.166120	NM_001572	<i>IRF7</i>	1.14
G02	Hs.137427	NM_002163	<i>IRF8</i>	1.93
G03	Hs.519680	NM_001145805	<i>IRGM</i>	2.76
G04	Hs.458485	NM_005101	<i>ISG15</i>	6.00
G05	Hs.705413	NM_002303	<i>LEPR</i>	1.16
G06	Hs.82906	NM_005373	<i>MPL</i>	1.71
G07	Hs.517307	NM_002462	<i>MX1</i>	1.82
G08	Hs.524760	NM_002534	<i>OAS1</i>	0.84
G09	Hs.75348	NM_176783	<i>PSME1</i>	0.88
G10	Hs.710248	NM_152501	<i>PYHIN1</i>	0.88
G11	Hs.145150	NM_004509	<i>SP110</i>	1.94
G12	Hs.134602	NM_003319	<i>TTN</i>	1.47
H01	Hs.520640	NM_001101	<i>ACTB</i>	1.22
H02	Hs.534255	NM_004048	<i>B2M</i>	9.37
H03	Hs.592355	NM_002046	<i>GAPDH</i>	1.03
H04	Hs.412707	NM_000194	<i>HPRT1</i>	0.87
H05	Hs.546285	NM_001002	<i>RPLP0</i>	0.91

Table S4. RT² Profiler™ PCR Array Data with Jak/STAT Inhibitor treatment