Supplemental Information
Supplemental Figures

Figure S1

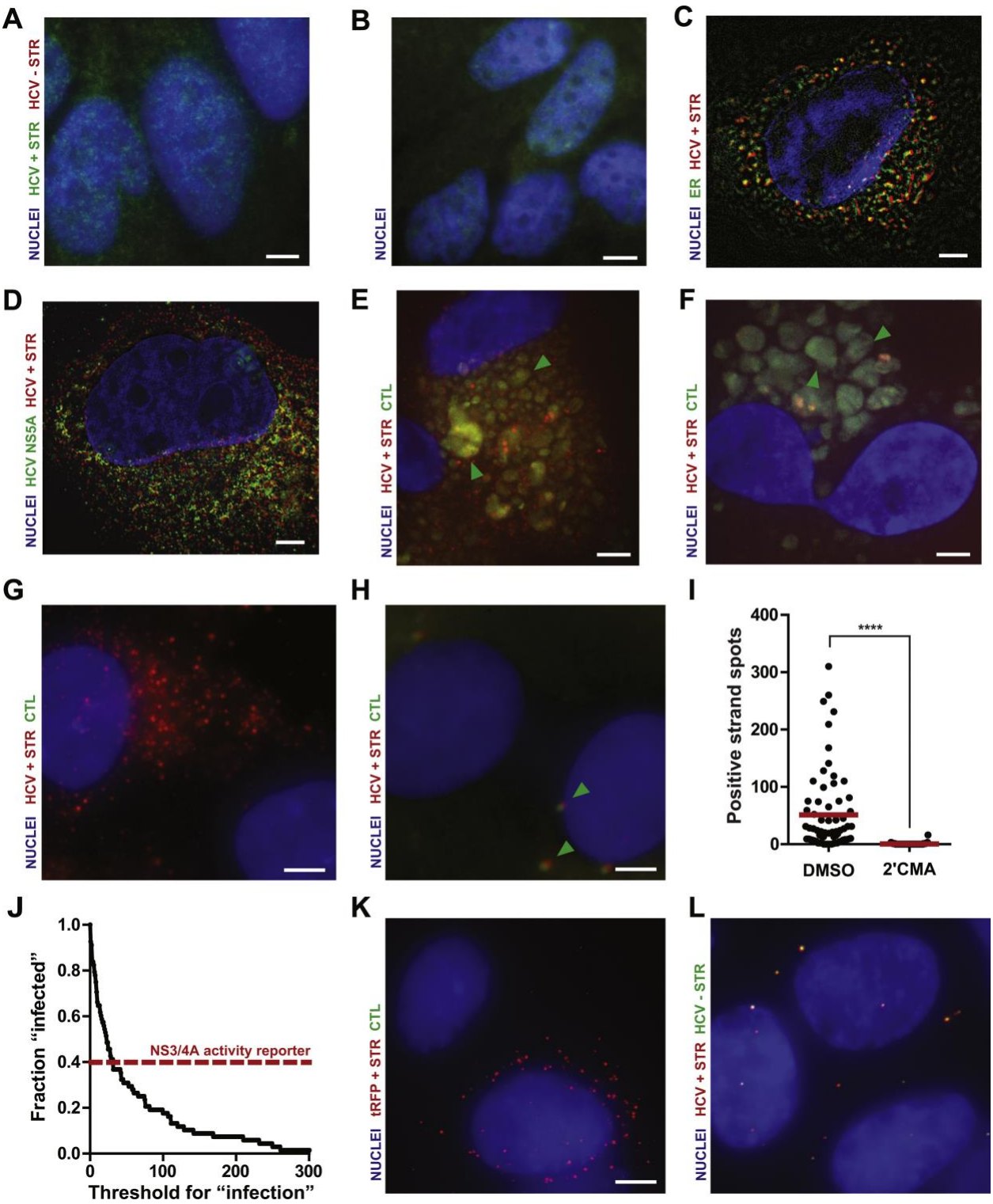


Figure S2

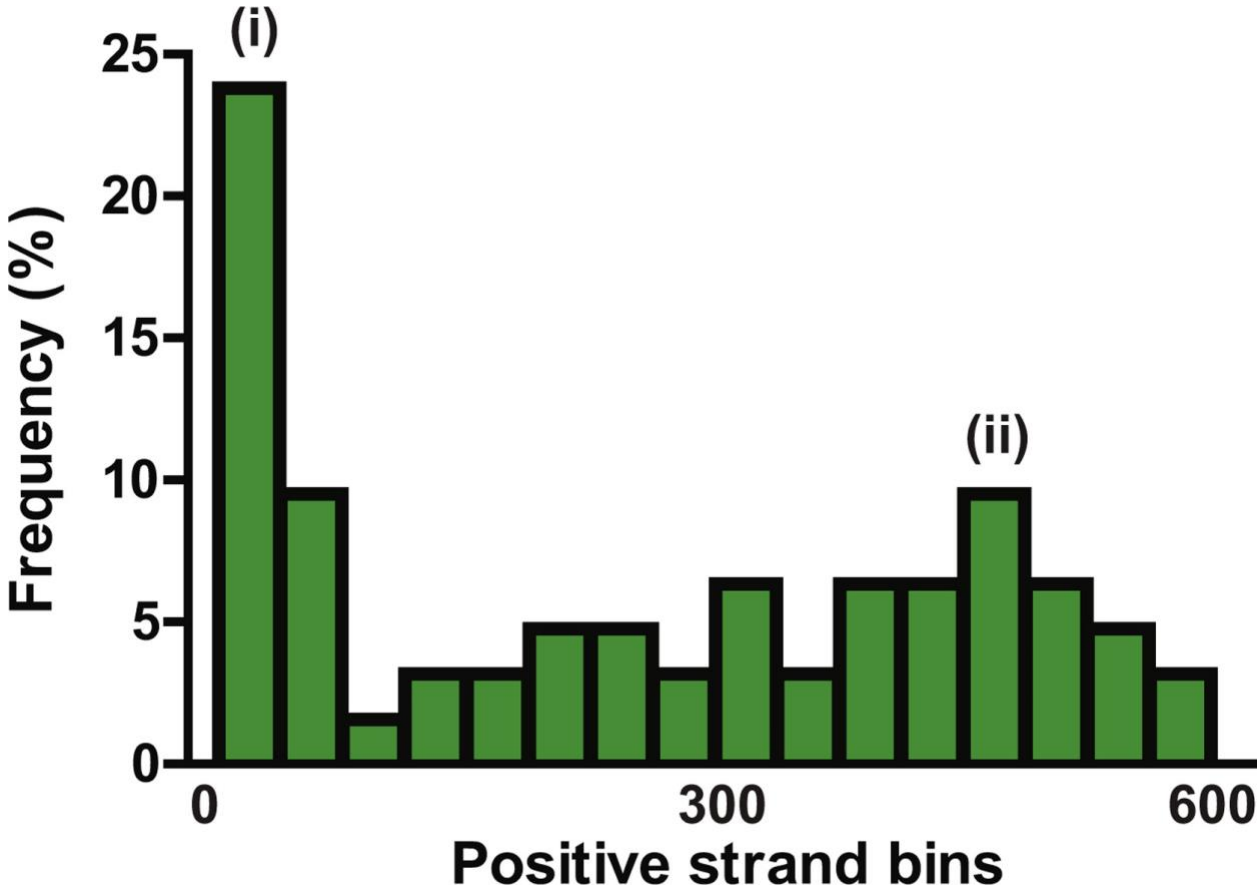


Figure S3

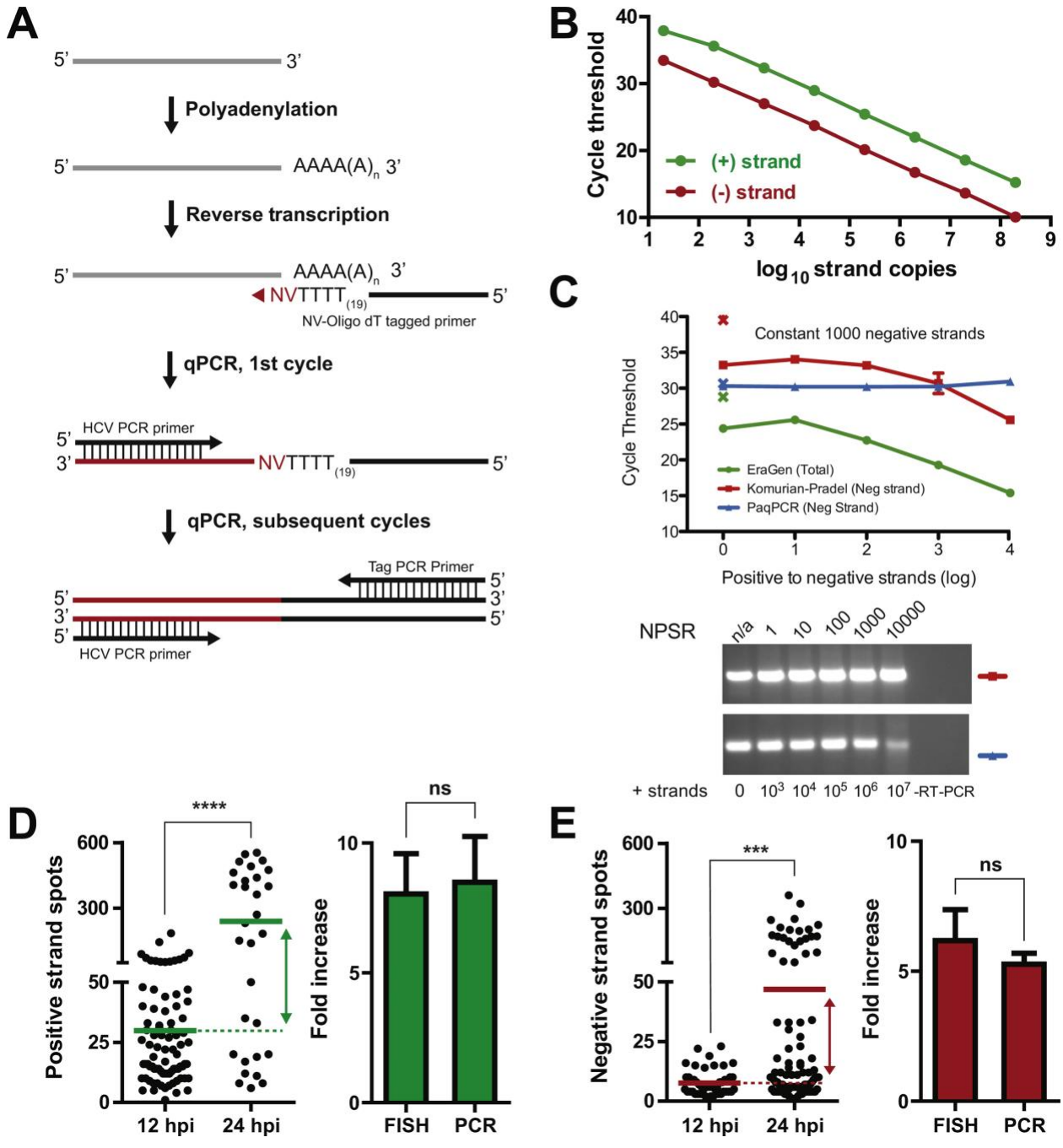
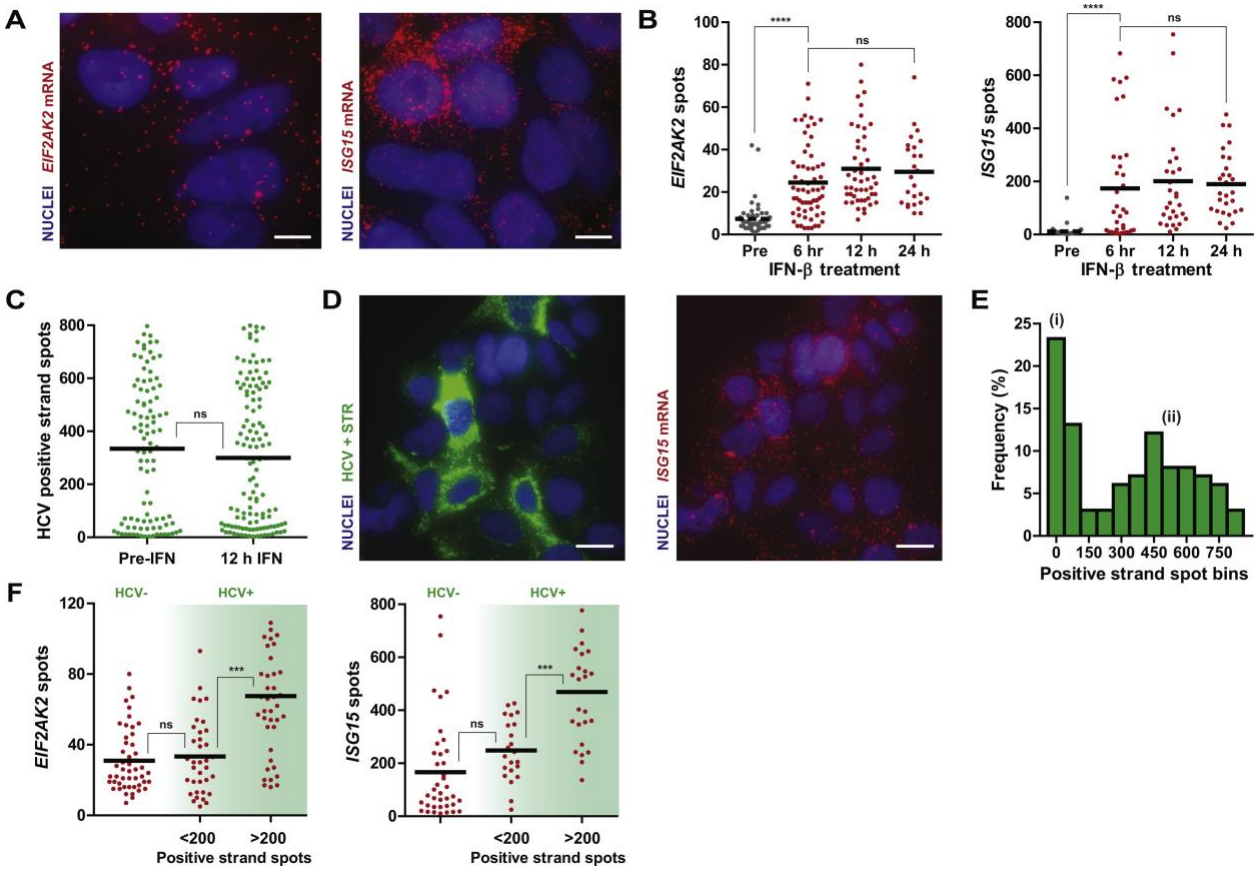

A
5' 3'
Polyadenylation
5' AAAA(A)n 3'
Reverse transcription
5' AAAA(A)n 3'
NVTTTT(19) 5'
NV-Oligo dT tagged primer
qPCR, 1st cycle
HCV PCR primer
5'
3' NVTTTT(19) 5'
qPCR, subsequent cycles
Tag PCR Primer
5'
3'
5'
5'
3'
5'
HCV PCR primer
B
Cycle threshold
40
30
20
10
1 2 3 4 5 6 7 8 9
log10 strand copies
(+) strand
(-) strand
C
Constant 1000 negative strands
Cycle Threshold
40
35
30
25
20
15
10
0 1 2 3 4
Positive to negative strands (log)
EraGen (Total)
Komurian-Pradel (Neg strand)
PaqPCR (Neg Strand)
NPSR
n/a 1 10 100 1000 10000
+ strands 0 10^3 10^4 10^5 10^6 10^7 -RT-PCR
D
****
ns
Positive strand spots
600
300
50
25
0
12 hpi 24 hpi
Fold increase
10
5
0
FISH PCR
E
***
ns
Negative strand spots
600
300
50
25
0
12 hpi 24 hpi
Fold increase
10
5
0
FISH PCR

Figure S4


A
NUCLEI EIF2AK2 mRNA
NUCLEI ISG15 mRNA
B
EIF2AK2 spots
ISG15 spots
****
ns
Pre
6 hr
12 h
24 h
IFN-β treatment
C
HCV positive strand spots
ns
Pre-IFN
12 h IFN
D
NUCLEI HCV + STR
NUCLEI ISG15 mRNA
E
Frequency (%)
(i)
(ii)
0 150 300 450 600 750
Positive strand spot bins
F
HCV-
HCV+
EIF2AK2 spots
ISG15 spots
***
<200
>200
Positive strand spots

Supplemental Figure Legends

Supplemental Figure 1. Specific and sensitive imaging of viral RNA in hepatoma and primary hepatocyte culture models. (A) Uninfected Huh-7.5 hepatoma cells imaged with probe sets for both the positive and negative strand (Z-stack projection, scale bar ≈ 4.9 μm). (B) Infected Huh7.5 cells imaged 24 hours post infection (hpi) on DAPI, Cy5, and Alexa594 channels but without FISH probe sets (Z-stack projection, scale bar ≈ 6.2 μm). (C) Structured illumination microscopy (SIM) of HCV (Cy5) and ER (Alexa594) (scale bar ≈ 3.0 μm) in infected Huh-7.5 cells at 24 hpi. (D) SIM of HCV positive strand RNA (Cy5) and HCV NS5A RNA (Alexa594) (scale bar ≈ 3.7 μm) in infected Huh-7.5 cells at 48 hpi. (E) Primary induced pluripotent stem-cell (iPSC)-derived hepatocyte-like cells imaged 1 week post infection (40) using confocal microscopy (scale bar ≈ 4.7 μm). Alexa594 (green) imaging performed without probe sets as a control to aid in the identification of frequent lipid-like autofluorescent foci (green arrows) distinct from diffraction-limited spots specific to the delivered probe set. (F) Uninfected iPSC-derived hepatocyte-like cells show only autofluorescent foci as indicated (scale bar ≈ 3.2 μm). (G) Primary human fetal liver cells (HFLCs) imaged at 48 hpi with the J6/JFH Clone 2 strain of HCV (Z-stack projection, scale bar ≈ 4.2 μm). Alexa594 (green) imaging performed without probe sets also to identify occasional autofluorescent foci (none in this field of view); autofluorescent foci are larger than smFISH spots, and fluoresce on both channels. (H) Uninfected HFLCs (Z-stack projection, scale bar ≈ 3.2 μm) imaged with HCV positive strand RNA (Cy5) probes. Several autofluorescent foci as indicated. (I) Number of positive strand spots in individual HFLCs infected in the presence of DMSO or the HCV NS5B polymerase

inhibitor 2'CMA at 48 hpi (red line = mean); ****$p < 0.0001$ (two-tailed *t* test). (J) Fraction of HFLCs "infected" at 48 hpi depending on the minimum threshold number of genomes for a cell to be deemed infected. NS3-4A activity reporter (38) infection rate estimate on replicate samples provided as a comparison. (K) Huh-7.5 hepatoma cells were infected with a J6/JFH hybrid HCV strain with a tagRFP reporter inserted into the viral genome. 22 Alexa 594-labeled oligo probes specific to the tagRFP sequence were designed and constructed, and used for detection of the tagRFP sequence-containing viral genomes (Z-stack projection, scale bar ≈ 5.0 μm). (L) Huh-7.5 cells transfected with pre-complexed HCV dsRNA and imaged 3 hours post-transfection show colocalized positive- and negative-strand spots, indicative of dsRNA accessibility by both positive- and negative-strand probes under hybridization conditions.

Supplemental Figure 2. Bimodal distribution of infection. The frequency histogram depicts the distribution of cells with a given number of positive strand spots within an infected population at 48 hpi, after binning individual cells into groups (bin width = 50 spots). The observed bimodal distribution has been previously observed in cases with, (i) a poorly infected mode and a (ii) highly infected mode.

Supplementary Figure 3. Single-molecule RNA FISH (smFISH) is a quantitative assay as verified by a novel bulk assay for quantifying genomic viral RNA (vRNA). **(A)** Schematic of polyadenylated quantitative polymerase chain reaction (PAqPCR) assay for measuring vRNA strands. Briefly, HCV is polyadenylated before undergoing an RT reaction with an NV anchored oligo-dT tagged primer. The resulting cDNA is used for qPCR using the exogenous tag primer and a strand-specific HCV primer directed towards the RNA 3' end. **(B)** Linear relationship between known strand copies

and PCR cycle thresholds demonstrates a sensitivity of 8 orders of magnitude for positive and negative HCV RNA strands. **(C)** Comparison of three separate strand-specific HCV assays (top) in their capacity to detect 1000 negative strands present within a background of the given number of positive HCV strands. Notably, the PAqPCR method (blue) yields a primarily flat line (i.e. a consistent detection threshold) across this range of background positive strands. Komurian-Pradel refers to the technique in (61). The 'x' depicted for each assay is the cycle threshold for negative strand detection with no positive strand contamination. Gel images (bottom) for the Komurian-Pradel method and the PAqPCR depict negative strand detection at varying degrees of positive strand contamination. **(D)** qPCR comparison with smFISH-based quantification of HCV positive strand spots in infected Huh-7.5 hepatoma cells. Cultures were inoculated with HCV and replicate samples were fixed for smFISH or lysed for qPCR at 12 and 24 hpi. The number of positive strand spots detected by smFISH in individual cells are shown for 12 hpi and 24 hpi (green line = mean), with the fold increase occurring between these two timepoints indicated with a green arrow (*Left*). A comparison of the fold increase in infection, as measured by both assays is also presented (*Right*). Data plotted as mean ± standard error of the mean (SEM); $p > 0.05$ by two-tailed *t* test. **(E)** A parallel analysis to that presented in (D) is repeated for the HCV negative strand. The number of negative strand spots detected by smFISH in individual cells at 12 hpi and 24 hpi is shown (red line = mean), with the fold increase between timepoints indicated by red arrow (*Left*). A comparison of the fold increase in infection, as measured by both assays is also presented (*Right*). Data plotted as mean ± SEM; $p > 0.05$ by two-tailed *t* test. For **(D-E)**, the differences in normalization methods used (e.g. per-cell in smFISH,

versus per ng RNA in PAqPCR) make a comparison of absolute RNA counts difficult. However, estimating a total of 20 ug RNA/1e6 Huh-7.5 cells, we estimate absolute counts of 27 and 228 positive-strand vRNAs at 12 and 24 hpi, respectively, by PAqPCR, compared to 29 and 240 positive-strand vRNAs by smFISH. We estimate absolute counts of 13 and 80 negative-strand vRNAs at 12 and 24 hpi, respectively, by PAqPCR, compared to 8 and 48 negative-strand vRNAs by smFISH; this slight undercounting may be due to lower accessibility of negative-strand vRNAs.

Supplemental Figure 4. Association between viral infection and interferon-stimulated gene (ISG) expression. (A) Typical smFISH images of *EIF2AK2* (*Left*, Z-stack projection, scale bar ≈ 9.5 μm) and *ISG15* (*Right*, Z-stack projection, scale bar ≈ 9.5 μm) post IFN-β-treatment (100 U/mL, 48 hours). **(B)** Number of ISG mRNA transcripts in individual cells pre-IFN (gray) or at various times post-IFN-β treatment (10 U/mL) (red) for *EIF2AK2* (*Left*) and *ISG15* (*Right*). Means are shown as dashed or solid lines for pre- and post-IFN treatment, respectively. Differences between pre-IFN and 6 hours post-IFN are statistically significant for both *EIF2AK2* (****$p < 0.0001$) and *ISG15* (****$p < 0.0001$). All differences between post-treatment IFN time-points are non-significant (n.s.) for both *EIF2AK2* and *ISG15* ($p > 0.05$) as determined by one-way ANOVA with Tukey's post-test. **(C)** Number of HCV positive strand spots in individual cells pre-IFN-β and post-IFN-β (10 U/mL) for 12 h (means in black). Difference was n.s. by two-tailed *t* test ($p > 0.05$). **(D)** Multiplexed images showing the same field of view 12 hours post dosing of IFN-β in terms of HCV positive strand (*Left,* scale bar ≈ 17.0 μm) and *EIF2AK2* mRNA (*Right,* scale bar ≈ 17.0 μm). **(E)** Single-cell distribution of the number of positive strand spots visualized as a frequency histogram reveals bimodality

with (i) poorly infected (< 200) and (ii) highly infected (> 200) groups. **(F)** Number of ISG transcripts in individual cells for both *EIF2AK2* (*Left*) and *ISG15* (*Right*) (means in black). On each plot, the number of transcripts in uninfected cells is shown (HCV-), and on the right (HCV+), the number of transcripts is presented for each cell after splitting cells into a poorly and highly infected bin based on the number of positive strand spots (using 200 strand spots as a threshold). The difference in ISG expression between uninfected cells and poorly infected cells is n.s. for both *EIF2AK2* and *ISG15* ($p > 0.05$), and the difference in ISG expression between poorly and highly infected cells is highly significant for both *EIF2AK2* (***$p < 0.001$) and *ISG15* (***$p < 0.001$) by one-way ANOVA with Tukey's post-hoc test.

Supplemental Information: Probe sets

HCV NEGATIVE STRAND 5' HALF

Probe #	Sequence: Set A (5'->3')	Sequence: Set B (5'->3')
1	CTTGACGCCTTTTCTATGCA	GGCCAGTGAATTCTAATACG
2	CCAGCCATAATTGAGAGGTT	ATTAAGTTGGGTAACGCCAG
3	TACTCCGTGAATCCTTTGGA	AGGAGAAAATACCGCATCAG
4	GCATGGTCCTAATGACACAC	TTAACTATGCGGCATCAGAG
5	GTATGCTCCAACCATATGGG	AAAACCTCTGACACATGCAG
6	CCCTATCAATTCATGGCTGG	CCTGACGTCTAAGAAACCAT
7	GCAGATACTACCTGACCAGA	GTCTCATGAGCGGATACATA
8	TTCCTCAAATGTGTCTGTGG	GCAAAAAAGGGAATAAGGGC
9	GGCGATGACCTAGTAGTCAT	CCAACTGATCTTCAGCATCT
10	ACACCATCACATGCTATGTG	ATTGGAAAACGTTCTTCGGG
11	TCGCTGACTGAGAGACTTTA	TCTGAGAATAGTGTATGCGG
12	CGAGGAGTCCATATACCAGG	GCAGTGTTATCACTCATGGT
13	TCACTGAGAGAGACATCAGG	TGGTATGGCTTCATTCAGCT
14	CCCATGGGTTTTTCGTATGA	TAATTGTTGCCGGGAAGCTA
15	TATCTCTTGAAAGCATGGGC	AGATAACTACGATACGGGAG
16	TTCCTATGGCTTCCAGTACT	ATGCTTAATCAGTGAGGCAC
17	CGAGAAAATGGCCCTCTATG	CACGTTAAGGGATTTTGGTC

18	CAAAAATGAGGTGTTCTGCG	GCGGTGGTTTTTTTGTTTGC
19	GTTAACCACATCAAGTCCGT	AACTACGGCTACACTAGAAG
20	CCATTCTGCAAGATCCAAGT	ACTATCGTCTTGAGTCCAAC
21	CTCACAGAGGGCTAAAAAGG	TTCTCAATGCTCACGCTGTA
22	GCTGTTGCGATACCATAACA	TAAAGATACCAGGCGTTTCC
23	GCCAATCAACCCTTTGAGTA	AAGGCCAGGAACCGTAAAAA
24	CTCCATGTCATACTCCTGGA	CGGTAATACGGTTATCCACA
25	GAGTCTGATCAGGTAGAGCT	ATTAATGAATCGGCCAACGC
26	GAGAGCACCATATCAGAAGC	ATAAAGTGTAAAGCCTGGGG
27	CAGACGTATTGAGGTCCATG	TTCTTGGTGGCTCCATCTTA
28	GCGTTGGGCTTAATTCCTAT	CTCCATAGCTAACTGTTCCT
29	GTGTGCAGATCCATAGGTTT	TTATCCAGTTGGTTCACCGT
30	CAGAGTTTTTCTCCTGGGTG	TTCAGCCCTCAGAAAACTTG
31	ATGTAACAGGACTGACCACT	GTATGGATCAGTATACTCCG
32	AGGGGACCTTTCCTATCAAT	CACACTTCTTCTCCATTCTC
33	CATCTCTTGTCAAAAGGGGT	AGATACTACCTGACCAGAGA
34	TCAAAAATTGGCTGACCTCT	TAGTCATCTCAGAAAGCCAG
35	GTTTGCACCATCTTGACAGA	ACTAGCATGGGTAACACCAT
36	TAACCAGCCTACTCAGAAGA	TATGATACCCGATGCTTCGA
37	CTACTCACTACGTGACGGAG	TACACAAAAGCTTCCTCAGG
38	ATGAACAGGCTTATTGCCTT	AAAATGAGGTGTTCTGCGTG
39	AGAAGCCCTCTATGGAAGAT	AGATCCAAGTATGGATTCGG
40	CTCGTCGCATTCAAGATCAT	CACAGAGGGCTAAAAAGGTA
41	CCTTCTCAACATCATGGGAG	CGAAGAGGAAAAGTTGCCAA
42	CAATACCTCGCAGGATTGTC	ATCAGGTAGAGCTTCAACCT
43	ATGTGGAACTTCATTAGCGG	AACTGGCCATCAAGACCTTT
44	GCCCAAAGTGGAACAATTTT	TCAATACCATCGGAGTGCAT
45	CGGATAAGGAGGTCCTGTAT	AACACCTATGACGTGGACAT
46	TTTGGGCCCTATTACCAATG	TAATTCCTATGCTGTCGGGT
47	TACCTAGTAGCCTACCAAGC	TTCCTTGCCAACTACCTTCT
48	TAGAGCGTATTTCAACACGC	TATCAATTGCTACACGGAGG

HCV NEGATIVE STRAND 3' HALF

Probe #	Sequence: Set A (5'->3')	Sequence: Set B (5'->3')
1	TATGTTTCCACTGGTGAACG	TAACCAGCCTACTCAGAAGA
2	CGTGATCGACTGCAATGTAG	AGAAGCCCTCTATGGAAGAT
3	TGGACGTCTCCATAATACCA	TTCCATGATGGCATTCAGTG

4	GGGAGACACCTGATTTTCTG	ATAAGGAGGTCCTGTATGAG
5	CCTTGATCAAGCAGAGACAG	ACTGGATGCGTTTCCATCAT
6	ATCATATGCGATGAATGCCA	AAGTACATCGCCACATGCAT
7	CGTACTCCACATATGGCAAA	AGAACTTCGCGTACCTAGTA
8	CATTAGGACTGGAGTCAGGA	GTGTGTCAAGACCATCTTGA
9	GTACCTATCCAAGGCACATG	CTCAGGAATGTTTGACAGTG
10	AGTACTAGTGCTTAACCCCT	TACACTGGAGACTTTGACTC
11	GACGTTGTTACAAGGTCTCC	AGAAAAAGTGTGACGAGCTC
12	TCGACCTATATCTGGTCACG	TCCTTGATCAAGCAGAGACA
13	TTTGTGGACTGTTTACCACG	CATCACGTACTCCACATATG
14	GAAGTCCAAATCCTGTCCAC	AGTACTAGTGCTTAACCCCT
15	CATTCTACATGGACTTCCCG	AGACCCATTTCGACCTTGAA
16	GTGGAACCCATCATCTTCAG	TTTGTGGACTGTTTACCACG
17	TCACGCTCTGATAAGGGTAT	TTGCTCCCATCACTGCTTAT
18	GCTCTGCTGGTAATGATCAC	ATGGAGAAGAAGGTCATCGT
19	GTTCTGACCTGCTCAAGAAG	TATGTTCAGGTGGCGCTATT
20	GCCCAAGATGAAGAAGTTCA	AATGGCTTTTGGCGTTGCTT
21	CTCAAAGAGATGGAAGCCAA	TCACTCTCTTTACTCTCACC
22	ACGAAGACTTCAACATCGTG	TTTGACCTTCTCAAGTTGGC
23	CCCAACAGGCTTATGCTTAT	TTCTGACCTGCTCAAGAAGT
24	GCTGCAATGGCTTCCTATAT	TCGACATTCCTGAGATTCCT
25	ACTAGAGAAGCTGGTCATCT	TGAAGAAGTTCATCCCAGGA
26	CTTATTCCTGCTCTTAGCGG	AAAGAGATGGAAGCCAATGC
27	AAAATACATCGTCCGATGGG	TAGCTACCTATTCCCTCACT
28	CGTGGACGTACAATTCATGT	GCTGCAATGGCTTCCTATAT
29	TTACCTTGCTCTTACTCGGA	ACTAGAGAAGCTGGTCATCT
30	AGGACAGAGACAGAAGTCAA	TGCCCTCACAAAATACATCG
31	CCACGGACTGTTTTAGGAAG	TTACCTTGCTCTTACTCGGA
32	CCGTACTAGAGCTGACTTCA	TACCACTTACCTCAAATGCG
33	CACGTGGATGAACTCTTCTG	TATTGAACAGCACTCGACCA
34	CTATTGAACAGCACTCGACC	TGTCACCAATCCAGAGGATA
35	ATCCAGAGGATATGAGACCC	TATCGCGTCTCTGTTCTACA
36	CTTCAACTCGTCAGGATGTC	ACCAGCTTATTTGACATGGG
37	TATCGCGTCTCTGTTCTACA	TCGTTGTCATCCTTCTGTTG
38	CTGAACTGCAATGACTCCTT	ACATGATGATGAACTGGTCG
39	GCAGAAAATCCAGCTCGTTA	AAATGTTCATTGTCTCGCCG
40	ACCAGCTTATTTGACATGGG	AAAGTGGGGAATGCATCTCA

41	CGAAAGTCGTTGTCATCCTT	AATGACAGCATTACCTGGCA
42	CCTACTTCTCTATGCAGGGA	AGTGAAGAACATCAGTACCG
43	TCCCCGAGGTCATTATAGAC	TACCCGGTTGCTCCTTTTCT
44	GACTGCAATTGCTCCATCTA	ATACCCTAACGTGCGGCTTT
45	ATCGACATGGTTGTGATGTC	CTGGCAAATCCTGGGGAAAA
46	GTGAAGAACATCAGTACCGG	GTGCACCATGAGCACAAATC
47	TTTCTATCTTCTTGCTGGCC	GAAGACTGGGTCCTTTCTTG
48	CGGGGTTAATTTTGCAACAG	GAGGAACTACTGTCTTCACG

ISG15 mRNA

Probe #	Sequence (5'->3')
1	CAGGCAGCACCGGCCCTATT
2	GCCTCTCAGCCGCCGGCTTC
3	TACTGGCAAAGATGAGTTCG
4	TGGGCCACGGCACAAGCTCC
5	CATGGCTGTGGGCTGTGGGC
6	TCTTCACCGTCAGGTCCCAG
7	TGGAATTCGTTGCCCGCCAG
8	CATGGAGCTGCTCAGGGACA
9	GCGCCTTCAGCTCTGACACC
10	ACGCCGATCTTCTGGGTGAT
11	CAGACGCTGCTGGAAGGCGT
12	CCACACCGCTCGGGTGGACA
13	AGGGGGACCCTGTCCTGCAG
14	GGGGCCCAGGCCCTGGCTGG
15	CCACCAGCAGGACCGTGCTG
16	AGAGGTTCGTCGCATTTGTC
17	GTTATTCCTCACCAGGATGC
18	CGTAGGTGCTGCTGCGGCCC
19	ACGGTCTGCGTCAGCCGTAC
20	CACTTGCTGCTTCAGGTGGG
21	CCTGCACACCCTCCAGCCCG
22	AAGGTCAGCCAGAACAGGTC
23	GTCCTCCAGGGGCTTCCCCT
24	CGTACTCCCCCAGCGGGAGC
25	ACGGTGCTCAGGGGCTTGAG
26	CCGCAGGCGCAGATTCATGA
27	CGCCAGGCTCTGTGCCGCCT
28	GGTGGAGGCCCTTAGCTCCG
29	CCCTTGATCCTGCTCGGATG
30	TTACAACAGCCTTTATTTCC

EIF2AK2 mRNA

Probe #	Sequence (5'->3')
1	TTTCGTTTTCCCCTTGGACT
2	AAAGTATGAGCAAACTGCGC
3	TACCAAGTGTGGAGCTGAAT
4	TTTCTATGCTTGTCGTGCCT
5	GATGCCTCGATGAAGATTGT
6	CCAGGGTCTCCTGATTTTTA
7	GCGAAGACTAAGGTCTATGA
8	GTTCAGACAGACGAGTGATA
9	GCTCCAGAAACTGGTAAAAG
10	ATCCAGGAAGGCAAACTGAA
11	GTCAGATGGAAGAACTGCTA
12	ATGTTGATTCTGAAGACCGC
13	ACGCAGATAATCACGGAAGT
14	GGTTGGAAGCTTTGTCCAAA
15	GCCATTTCTTCTTCCCGTAT
16	CCATGAAGAAACCTGCTGAA
17	CTGCTTCTGACGGTATGTAT
18	CCTGAATTAGGCAGTTCTTG
19	GTAAACCTCCTATCATGTGG
20	CCTTCTGGAAATTCTCTTCC
21	GCTTCCTTCTTTGATCTACC
22	CTAATTTGGCTGCGGCATTT
23	GACTAACTGCCTTCTTTTCC
24	CCCATGGATAATCCTTCTGA
25	GTTAGTCTTTTCTTCTGGGC
26	CCGATGCACACTGTTCATAA
27	ATGAAATCCTTCTGGCCCAT
28	GCTTCCTGTTTAGTAGAACC
29	CAAGTTTAGCGGCCAATTGT
30	GTAGTCAGATTTCACTGAGG
31	GTAGCAAAAGAACCAGAGGA
32	AAGAGTTGCTTTGGGACTCA
33	ATGATTCAGAAGCGAGTGTG
34	ATCTGCTGAGAAGTCACCTT
35	GAGACCATTCATAAGCAACG
36	GTGCCAAAGATCTTTTTGCC
37	CATGTCAGGAAGGTCAAATC
38	CCTCTTGTCCACAGTATACT
39	CCACCTGAGCCAATTAATTC
40	CCGTCAATTCTGTGTTTTGC

41	CTCCGCCTTCTCGTTATTAT
42	CAACAGCCATTGTAGTGAAC
43	CTGGTCTCAGGATCATAATC
44	CACTGCTCTCAAGAGAATCA
45	GCACTTAGTCTTTGACCTTG
46	CAAGGTCCCTTTATCACAGA
47	CTAGTTTCTCGCCTCTTCTT
48	GTTCAAAGAGTTCCAAAGCC

tagRFP portion of tRFP-HCV mRNA

Probe #	Sequence (5'->3')
1	AATCAGCTCTTCGCCCTTAG
2	TACAGCTTCATGTGCATGTT
3	TGGTTGTTCACGGTGCCCTC
4	CTCGGATGTGCACTTGAAGT
5	CCTCGTAGGGCTTGCCTTCG
6	CCACCTTGATTCTCATGGTC
7	GTTGATGAAGGTTCTGCTGC
8	GACTGCTTAAAGAAGTCGGG
9	CCATGTGAAGCCCTCAGGGA
10	TCTTCGTATGTGGTGACTCT
11	TCCTGGGTAGCGGTCAGCAC
12	CTTGACGTTGTAGATGAGGC
13	TTTTCTTCTGCATCACAGGG
14	GGTACAGCATCTCGGTGTTG
15	ATGTCGCTTCTGCCTTCCAG
16	AAGTTGCAGATCAGGTGGCC
17	CTTGGATCTGTATGTGGTCT
18	CATCTTGAGGTTCTTAGCGG
19	TGTGGTCCACATAGTAGACG
20	GCCTCCTTGATTCTTTCCAG
21	CTCGACGTAGGTCTCTTTGT
22	ATTAAGTTTGTGCCCCAGTT