Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system

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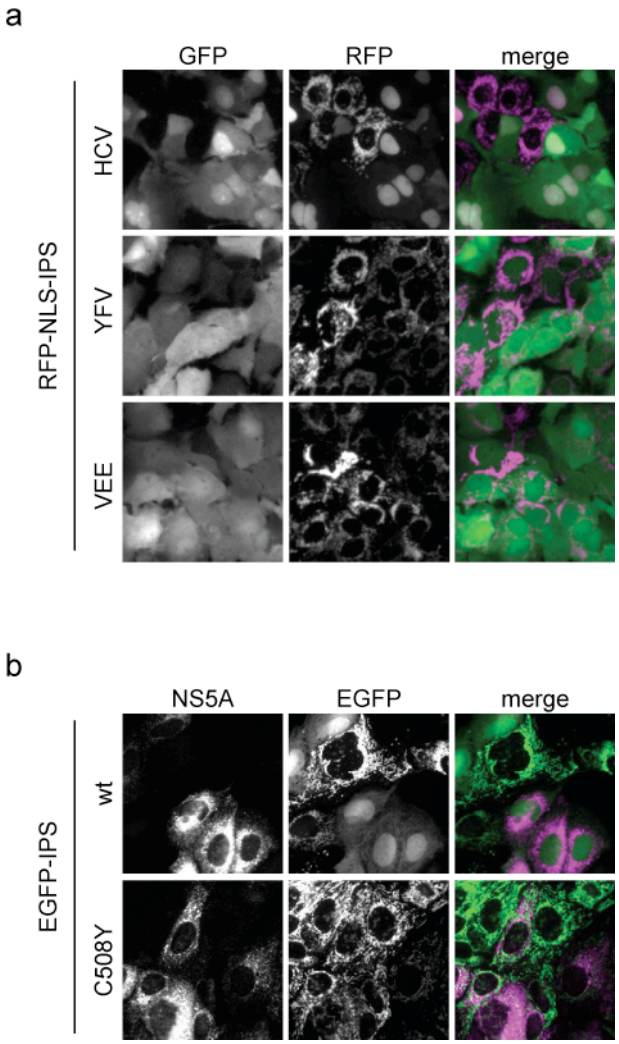
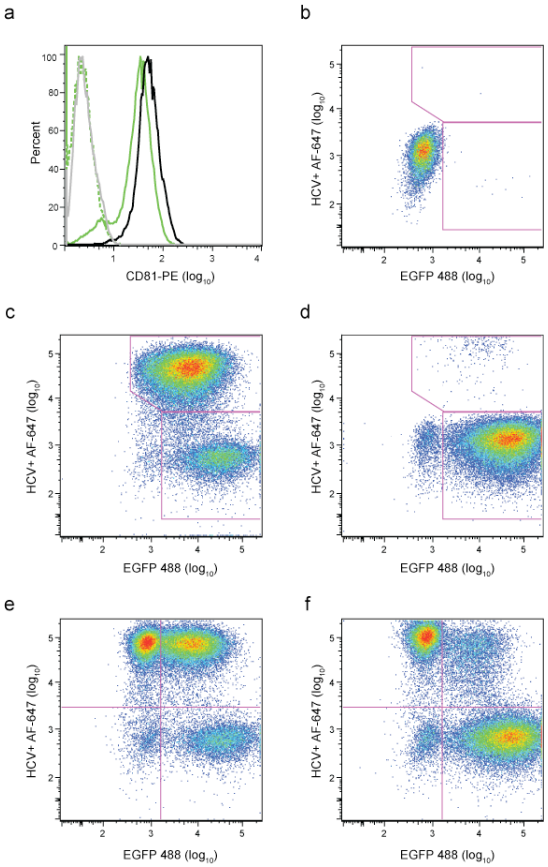
Supplementary material

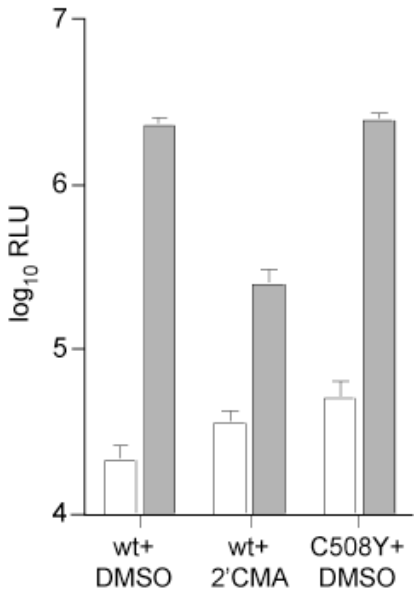


Supplementary Figure 1. HCV-specificity of the IPS-1-based reporter system (**a**) RFP-NLS-IPS relocalization is not a pan-viral phenotype. Huh-7.5 cells harboring RFP-NLS-IPS were infected with RNA viruses expressing green fluorescent proteins: an HCVcc reporter genome expressing Ypet (HCV, 48 h post infection), yellow fever virus expressing Venus (YFV, 24 h post infection), or Venezuelan equine encephalitis encoding EGFP (VEE, 8 h post infection)**.** Grayscale images show virally-encoded green fluorescence (GFP) and RFP-NLS-IPS localization (RFP). Merge images indicate an association between HCV, but not YFV or VEE, infection and nuclear RFP-IPS fluorescence. Wide-field fluorescence images of unfixed cells are shown. (**b**) EGFP-IPS relocalizes in HCV-infected cells**.** Huh-7.5 cells expressing wild type (wt) or mutant (C508Y) EGFP-IPS were infected with HCVcc virus, Jc1. Grayscale images show EGFP-IPS fluorescence (EGFP) and immunostaining for an HCV replicase protein (NS5A) in fixed cells at 48 h post-infection. Merged images show correlation of NS5A-positivity and diffuse EGFP in wt, but not C508Y, reporter cells.



	Cultures: (% HCV+ cells)	
	Mono-	Co-
RFP-NLS-IPS	92.4	93
EGFP-IPS/IRR	78.5	79.2
EGFP-IPS/CD81$^-$	0.64	13.7

Supplementary Figure 2. FACS analysis of HCVcc-infected reporter cell mono- and co-cultures. (**a**) CD81 expression in uninfected wild type (wt) and shRNA-expressing Huh-7.5 cell lines, as detected by staining with PE-conjugated anti-CD81 antibody. Huh-7.5 cells, solid black line; EGFP-IPS/IRR cells, solid green line; EGFP-IPS/CD81$^-$ cells, dashed green line and grey line (unstained). NS5A, detected by staining with 9E10 conjugated to AlexFluor-647, and EGFP signals of uninfected Huh-7.5 cells (**b**) and J6/JFH clone 2-infected monocultures of EGFP-IPS/IRR (**c**) or EGFP-IPS/CD81$^-$ cells (**d**). NS5A and EGFP levels in co-cultures of J6/JFH clone 2-infected RFP-NLS-IPS cells with EGFP-IPS/IRR (**e**) or EGFP-IPS/CD81$^-$ (**f**) cells. Table shows percent NS5A-positive cells for each cell population in mono- or co-culture.



Supplementary Figure 3. Infection of primary hepatocyte MPCC. MPCC transduced with lentiviruses expressing wild type (wt) or mutant (C508Y) RFP-NLS-IPS were infected with Jc1FLAG2(p7-nsGluc2A). At 12 h post-infection virus was removed, cells were washed three times, and media was replaced with MPCC media containing DMSO or 2'CMA. Levels of secreted *Gaussia* luciferase activity were measured immediately after media replacement (white bars) and at 48 h post-infection (grey bars). Average values of three independent infections are shown; error bars represent standard error of the mean. Graph generated using Prism 4 software.

Supplementary Video 1. Time-lapse live cell imaging of HCVcc infection. Huh-7.5 cells expressing mito-EGFP and RFP-NLS-IPS were infected with Jc1FLAG2(p7-nsGluc2A) in the presence of DMSO or 2'CMA. At 5 h post infection, virus was removed and replaced with imaging media containing either DMSO (**a**) or 2'CMA (**b**). Images were captured every 30 min, starting at 6 h post-infection. In a second experiment, cells were infected for 24 h prior replacement of complete media with imaging media containing DMSO (**c**) or VX-950 (**d**). Image acquisition was initiated 0.5 h after the addition of imaging media. Images were assembled and processed using ImageJ64 software. For clarity only RFP images (displayed as grayscale) are shown.

Supplementary Video 2. Time-lapse live cell imaging of HCV-induced stress response. Huh-7 cells expressing RFP-NLS-IPS and EGFP-G3BP were infected with Jc1FLAG2(p7-nsGluc2A). At 5 h post-infection virus was removed and replaced with imaging media containing DMSO (**a** and **b**) or 2'CMA (**c**). Images were captured every 30 min, starting at 6 h post-infection. Images were assembled and processed using ImageJ64 software. RFP-NLS-IPS (left), EGFP-G3BP (middle), color merge (right).