Supplementary methods

RNA purification, reverse transcription and qPCR: Intracellular RNA was extracted using Extract-All (Eurobio), extracellular RNA was extracted from 150 µl of supernatant using NucleoSpin RNA Virus kit (Macherey-Nagel). Intracellular RNA (1 µg) was treated with RQ1 DNAse (Promega) and reverse transcribed using M-MLV reverse transcriptase (Life technologies). Biopsies were extracted with Nucleospin RNA/Protein kit (Macherey Nagel). 400 ng of RNA were reverse transcribed with Vilo superscript enzyme (Life technologies) using random hexamers. Relative quantification of RNA was performed by quantitative PCR with SYBR Green I Master mix (Roche) using specific primers on a LightCycler480 Detection System (Roche). All results were standardized using β-glucuronidase (GUS) as housekeeping gene. For biopsy analysis, phosphomannomutase 1 (PMM1) was used as housekeeping gene.

Primer sequences were (5' to 3') GPx1 fwd TCTCCGGTGTGTCGCAACGA, rev ACAGCAGCACTGCAACTGCCA; GPx2 fwd CCCTTGCAACCAATTTGGAC, rev TCCTTCAGGTAGGCGAAGAC; GPx3 fwd TCGGTCTGGTCATTCTGGGCT, rev CGAGGTGGGAGGACAGGAGTTCTT; GPx4 fwd TACCGGGGCTTCGTGTGCAT, rev TAGCCCGCGGCGAACTCTTT; SOD1 fwd AAACACGGTGGGCCAAAGGAT, rev AAAGACAGGAAACGCGGAAAGTCG; SOD2 fwd GCTGACGGCTGCATCTGTTGG, rev AGGCCTGTTGTTCCTTGCAGTGG; CAT fwd TCGACCCAAGCAACATGCCA, rev AGGCAAAAAGGCGGCCCTGA; GS fwd CCCTGGCTGAGGGAGTATTG, rev TGCACAGCATAGGCTTGCTC; GR fwd CTGATCGCCGGTGGTATGC, rev ACAGCAATGTATGCACCAACAA; GUS fwd CGTGGTTGGAGAGCTCATTTGGAA,

rev ATTCCCCAGCACTCTCGTCGG; PMM1 fwd GGACAAGCGCTACTGCCTGGAT, rev ATCGCTGCACCGTGTCCTGA.

For intrahepatic HCV RNA quantification, 10 ng cDNA were applied to a TaqMan based Real Time PCR using the 5-prime master mix (Thermo Scientific). The 5´-UTR region was used as a target for the following primer set and probe: HCV sense CTA GCC GAG TAG CGT TGG GT, HCV rev TGC ACG GTC TAC GAG ACC TC and the HCV probe Fam-CAA GCA CCC TAT CAG GCA GTA CCA CAA G-Tamra. All values were normalized using GUS as housekeeping gene.

Inducible lentiviral vectors: The open reading frames for core, NS3/4A, NS5A and NS5B were amplified by PCR to add a V5-tag to the C-terminus and subcloned into the pCR Topo 2.1 Vector (Invitrogen). Fragments containing the HCV ORFs were then removed from pCR Topo 2.1 vectors by EcoRI digestion and subcloned into the EcoRI sites located downstream of a CMV TRE in the shuttle vector pENTTmcs (addgene plasmid 25751, [1]). The CMV TRE – HCV ORF cassettes were then transferred from the pENTTmcs backbones into the lentiviral vector pSLIK (addgene plasmid 25735, [w1]) by Gateway recombination (Invitrogen) to result into lentiviral vectors encoding multicistronic insert consisting of the CMV TRE driving the HCV ORFs, followed by a ubiquiting promoter driving a transactivator, followed by an IRES and the neomycine selection gene. Plasmid infos are available upon request. Packaging of lentiviral vectors was performed as previously described [w2].

Huh7.5 cells expressing GFP or the HCV proteins in a doxycyclin inducible manner were kept under selection using 500µg/mL G418 sulfate.

Western blotting and immunofluorescence: After quantification using BCA Protein Assay Reagent (Thermo Scientific), cell lysates (50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.1% SDS, 1% NP40, 1% protease inhibitors) were denatured 5min at

95°C in Laemmli buffer and proteins were separated using SDS-PAGE. After blotting onto nitrocellulose membrane, proteins were revealed using a rabbit polyclonal anti-GPx1 antibody [w3], a rabbit monoclonal anti-GPx4 (Abcam), a monoclonal anti-GFP and phospho-Akt (Santa Cruz), a mouse anti-V5 tag (Invitrogen) and a mouse monoclonal anti-β-actin (Sigma), respectively. Anti-rabbit and anti-mouse antibodies coupled to horseradish peroxidase (Dako) were used to detect proteins by enhanced chemiluminescence (Thermo Pierce). For immunofluorescence cells were fixed using a 1:1 v/v mixture of MeOH and acetone and stained with an HCV-specific human serum derived from a patient.

Supplementary Bibliography

w1 Shin KJ, Wall EA, Zavzavadjian JR, Santat LA, Liu J, Hwang JI*, et al.* A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression. Proc Natl Acad Sci U S A 2006;**103**:13759-64.

w2 Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. J Exp Med 2003;**197**:633-42.

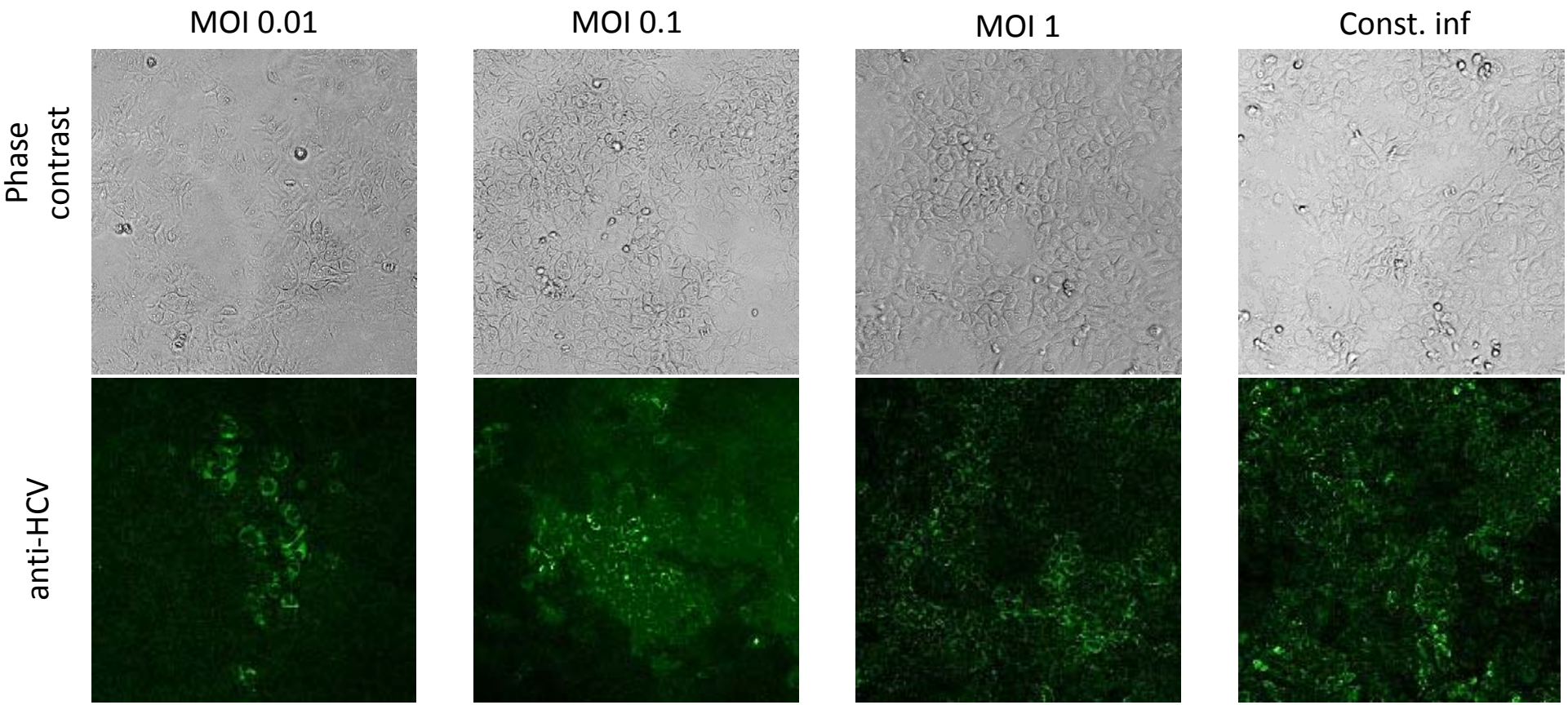
w3 Januel C, El Hentati FZ, Carreras M, Arthur JR, Calzada C, Lagarde M*, et al.* Phospholipid-hydroperoxide glutathione peroxidase (GPx-4) localization in resting platelets, and compartmental change during platelet activation. Biochim Biophys Acta 2006;**1761**:1228-34.

Supplementary Figure Legends

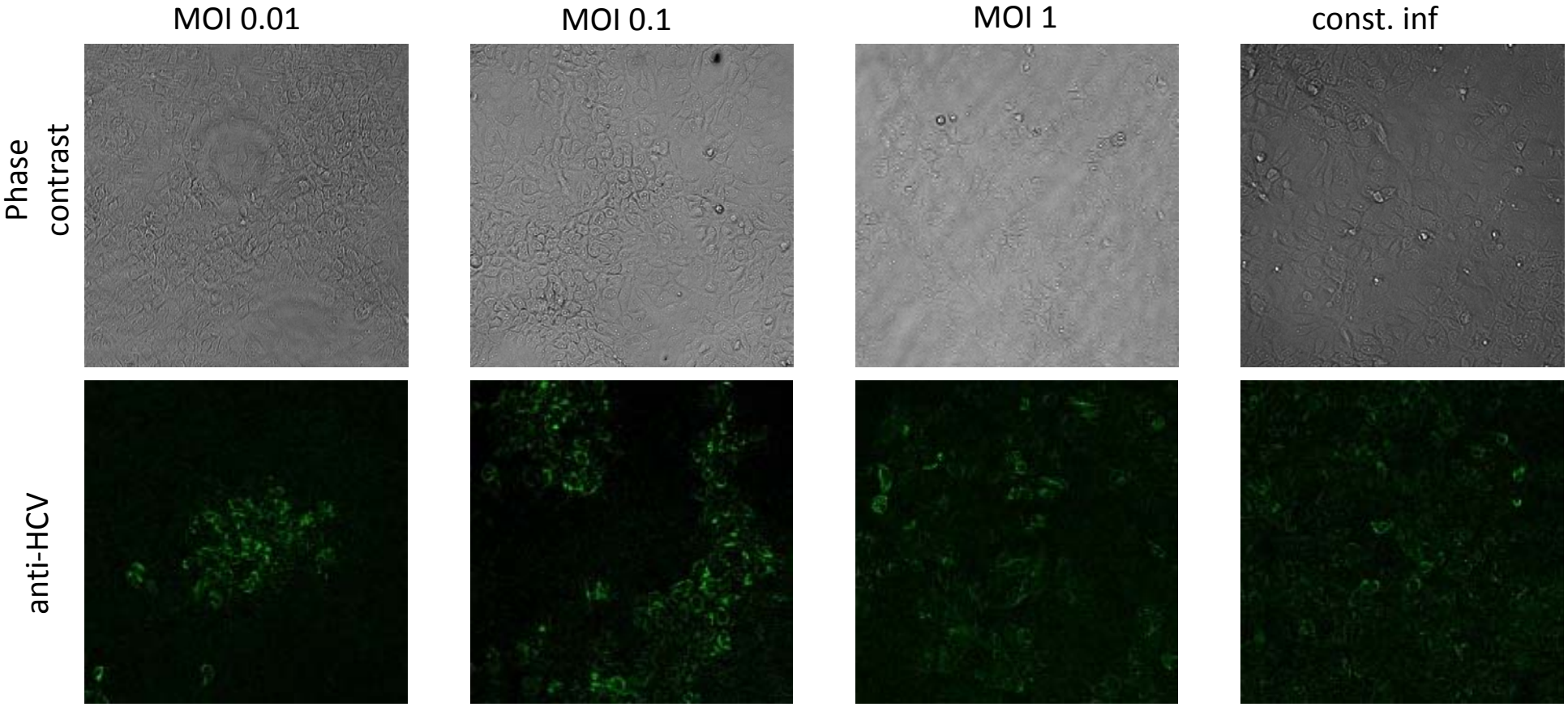
Supplemental FIG 1. HCV protein expression at the time of ROS quantification. Huh7.5 cells were infected with HCVcc at the indicated MOIs. Three or 4 dpi cells were used to assess ROS levels (see Fig 1) and in parallel stained with an anti HCV-serum. **(A)** HCV expression levels in cells stained with DHE. Phase contrast and anti-HCV staining are shown. **(B)** HCV expression levels in cells stained with DCFDA. Phase contrast and anti-HCV staining are shown. **(C)** HCV expression levels in cells used for quantification of the GSSG/GSH ratio. Dapi and anti-HCV staining are shown. **(A-C)** A representative result is shown (n>3).

Supplemental FIG 2. Kinetics of GPx4 downregulation. Huh7.5 cells were transfected with the indicated amounts (nM) of control or GPx4 targeting siRNAs. **(A, B)** Cell lysates of control or GPx4 specific siRNA treated Huh7.5 cells were analyzed by western blot 24, 48 and 72 hrs post transfection using GPx4 specific antibodies or an anti-beta actin antibody as control (n=2 for 24 hrs, n=3 for 48,72 hrs). A representative blot is shown. **(B)**. GPx4 levels at 48 and 72 hrs post transfection were quantified (b-actin normalized) using ImageJ (B) (n=3). **(C)** Quantification of GPx4 mRNA levels by RTqPCR (GUS normalized) relative to mock siRNA transfected cells 3 and 5 d post transfection (50nM), (n=3).

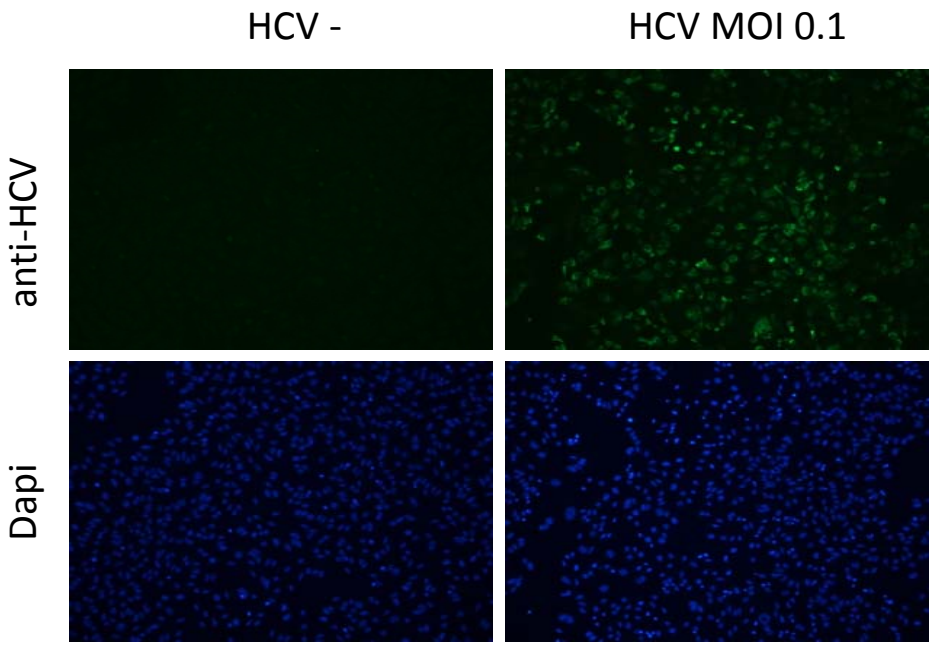
Supplementary Figure 1A



Supplementary Figure 1B



Supplementary Figure 1C



Supplementary Figure 2