

# Supplementary Material

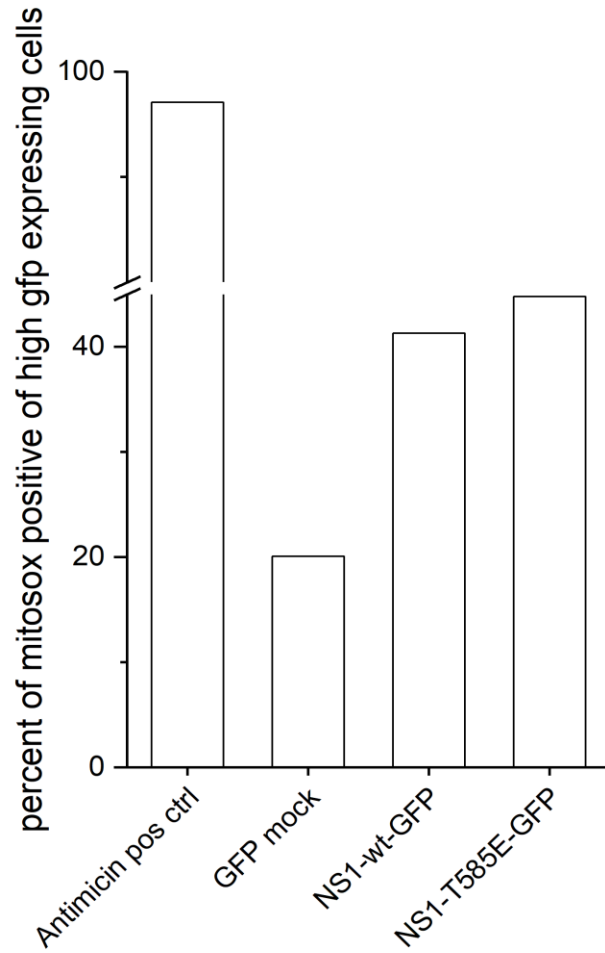
Table S1: List of PCR primers. Mutated amino acid codons are marked in bold.

#	Plasmid	Primer sequence
p1	BamHI-NS1-fwd	ATTGGATCCGCGATGGCTGGAAACGCTTACTCC
p2	NotI-NS1-rev	TATTAATGCGGCCGCTTAGTCCAAGGTCAGCTCCTCG
p3	BamHI-NS1-rev	AATGGATCCGCGTCCAAGGTCAGCTCCTCG
p4	AgeI-NS1-fwd	AGCACCGGTGACATGGCTGGAAACGCTTACTCC
p5	AgeI-NS1-rev2	AATACCGGTTGTCCAAGGTCAGCTCCTCG
p6	NS1-K85Q-fwd	AGCGCAGTGGATGACATGACCAAAC <b>AG</b> CAAGTATTTATT
p7	NS1-K85Q-rev	AATAAATACTTGCT <b>GT</b> TTTGGTCATGTCATCCACTGCGCT
p8	NS1-K257Q-fwd	CTGTATACTGATGAGATG <b>CA</b> ACCAGAAACGGTCGAGACC
p9	NS1-K257Q-rev	TCTCGACCGTTTCTGG <b>TT</b> GCATCTCATCAGTATACAG
p10	NS1-S283E-fwd	CAAAC TAGAAAGGAGGTC <b>GAG</b> ATTAAAACCACTCAAA
p11	NS1-S283E-rev	TTTGAGTGTGGTTTTAAT <b>CT</b> CGACCTCCTTTCTAGTTTG
p12	NS1-T435E-fwd	TTTCCATTTAATGACTGT <b>GAG</b> AACAAAACTTGATTTGG
p13	NS1-T435E-rev	CCAAATCAAGTTTTTGT <b>TCT</b> CACAGTCATTAAATGGAAA
p14	NS1-S473E-fwd	GATCAAAAAGGAAAAGG <b>CGAG</b> AAACAGATTGAACCAACAC
P15	NS1-S473E-rev	GTGTTGGTTCAATCTGTT <b>TCT</b> CGCCTTTTCCTTTTGGATC
p16	NS1-T585A-fwd	CGCTCACCATCTCTG <b>GCT</b> CCGAGAAGTACGCCTCTCAGC

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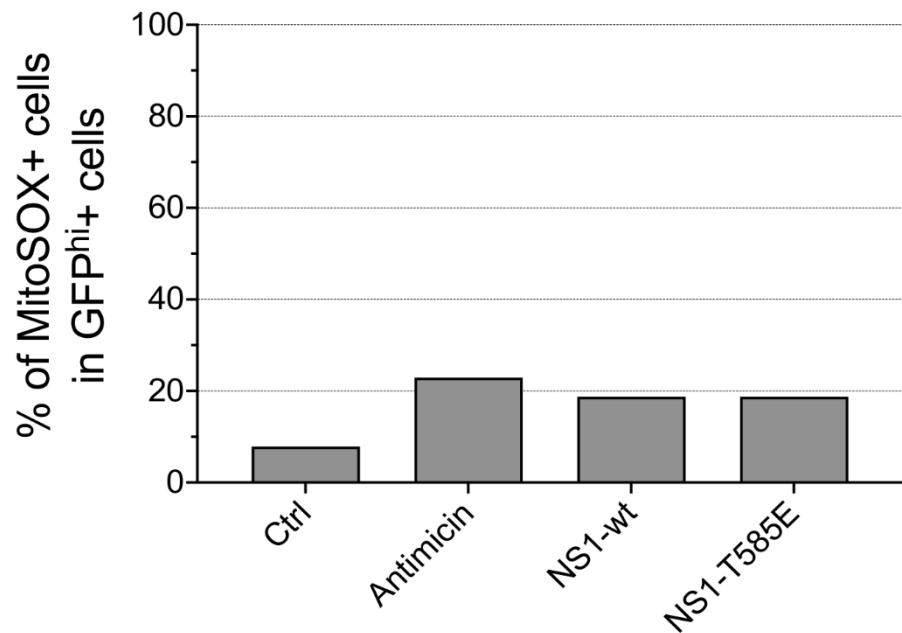
p17	NS1-T585A-rev	GCTGAGAGGCGTACTTCTCGG <b>A</b> GCCAGAGATGGTGAGC
p18	NS1-T585E-fwd	CTCACCATCTCTGG <b>A</b> GCCGAGAAGTACGCCT
p19	NS1-T585E-rev	AGGCGTACTTCTCGG <b>C</b> TCCAGAGATGGTGAG
p20	NS1-d114-rev	CAGCCTCCCCAGTGTTTTGGCTCGGAGTCAGAGATGGTGA
p21	NS1-d114-fwd	TCACCATCTCTGACTCCGAGCCAAAACACTGGGGAGGCTG
p22	AgeI-ATG-BioID2-fwd	ATACCAACCGGTGAC <b>A</b> TGTTCAAGAACCTGATCTGGCTGA
p23	HindIII-stop-HA-rev	TATACGTAAGCTTCTATGCGTAATCCGGTACATCGT

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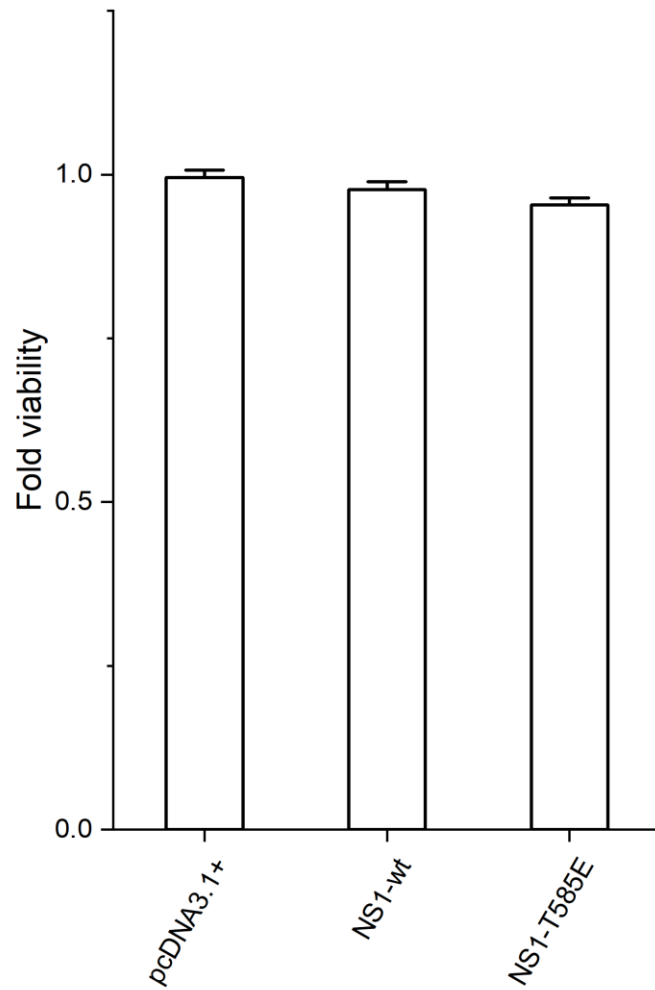


**Figure S1: Determination of reactive oxygen species (ROS) production.** Intracellular ROS levels were determined in responder Hep3B cells using the MitoSox assay (ThermoFischer) 72h post transfection. Cells were transfected with NS1-wt GFP or NS1-T585E GFP. Antimycin A treated cells were used as a positive control. GFP expressing cells were used as a negative control. >1400 high GFP expressing cells per group were counted.

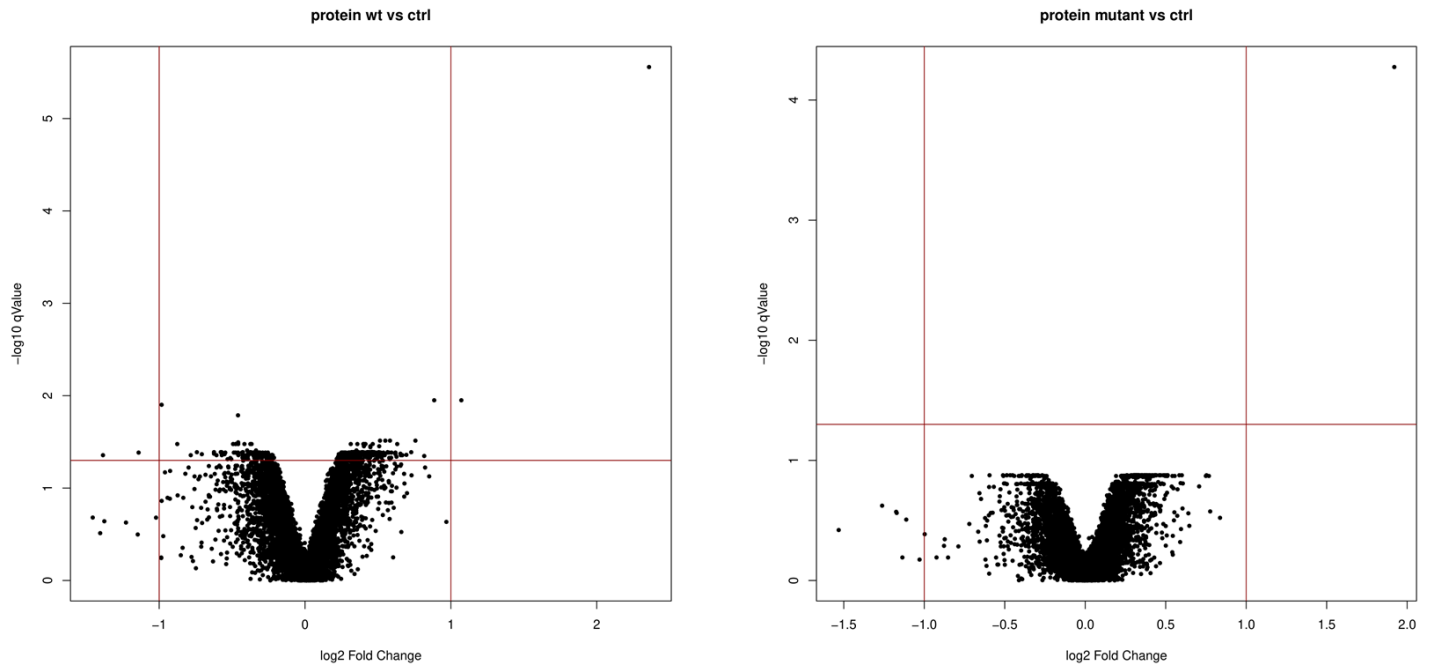
HepG2 cells - 72 h



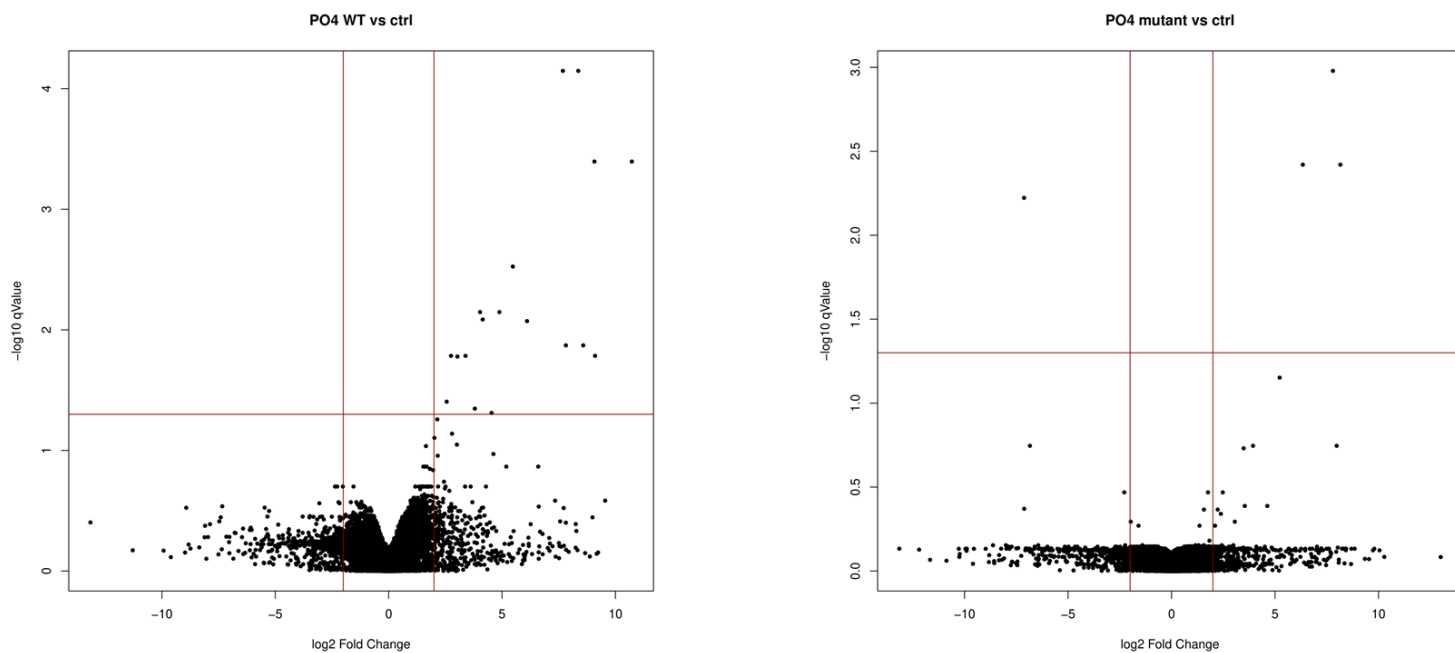
**Figure S2: Determination of reactive oxygen species (ROS) production.** Intracellular ROS levels were determined in non-responder HepG2 cells using the MitoSox assay (ThermoFischer) 72h post transfection. Cells were transfected with NS1-wt GFP or NS1-T585E GFP. Antimycin A treated cells were used as a positive control. GFP expressing cells were used as a negative control. >1400 high GFP expressing cells per group were counted.



**Figure S3: Viability of transfected primary mouse hepatocytes.** Viability determined by the MTT assays of non-responder primary hepatocytes transfected with empty pcDNA3.1+ vector (mock), pcDNA3.1-NS1-wt, or with pcDNA3.1-NS1-mutant. Relative viability was determined 72h post transfection and normalized to pcDNA3.1+ (mock) transfection. Values are means  $\pm$  SEM (n = 10 measurements).



**Figure S4: Volcano plot depicting the changes in the proteome after NS1-wt (left) and NS1-T585E (right) expression.** Log(2) of ratios of protein levels after NS1 (wt, left or mutant T585E, right) and empty plasmid (mock) transfection of Hep3B cells (x-axis) blotted against the neg. log(10) of corresponding q-values (y-axis).



**Figure S5: Volcano plot depicting the changes of protein phosphorylation after NS1-wt (left) and NS1-T585E (right) expression.** Log(2) of ratios of identified phosphorylated proteins after NS1 (wt, left or mutant T585E, right) and empty plasmid (mock) transfection of Hep3B cells (x-axis) blotted against the neg. log(10) of corresponding q-values (y-axis).