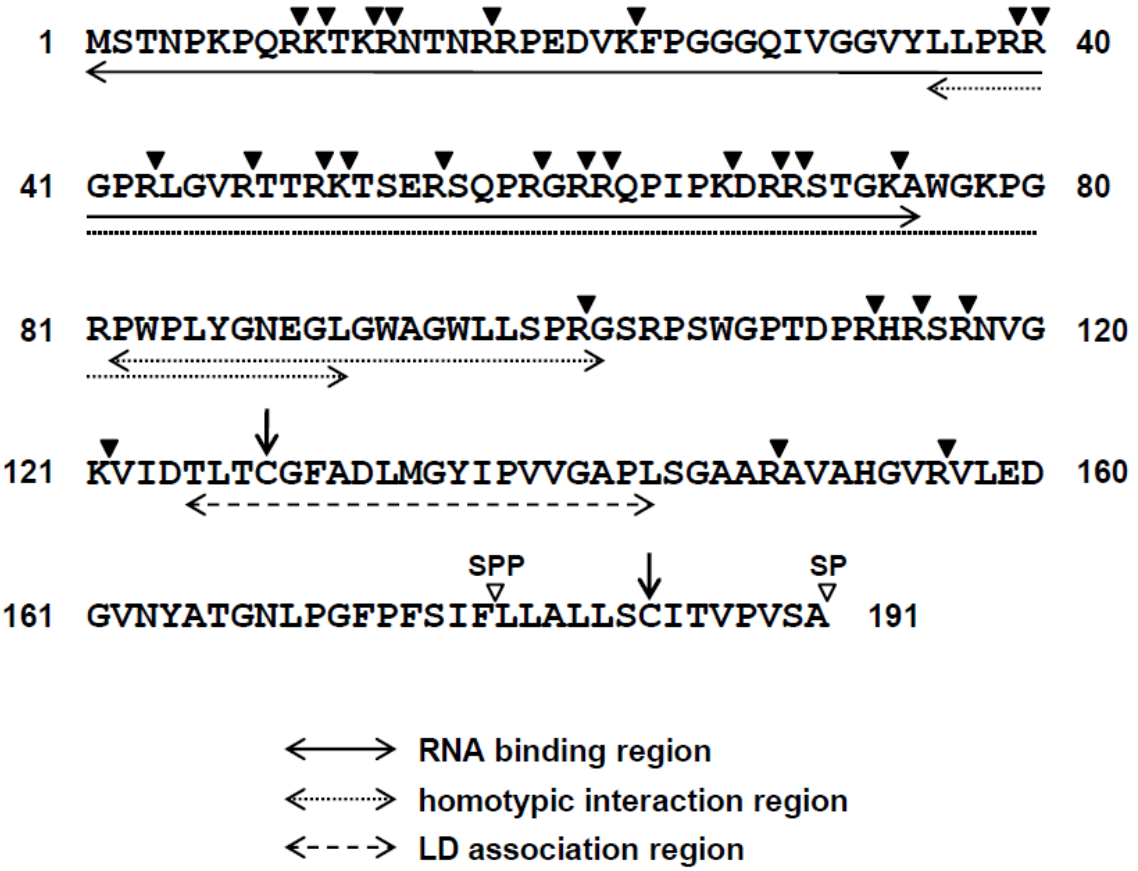
Supplementary imformation for:

A DISULFIDE-BONDED DIMER OF THE CORE PROTEIN OF HEPATITIS C VIRUS IS IMPORTANT FOR VIRUS-LIKE PARTICLE PRODUCTION

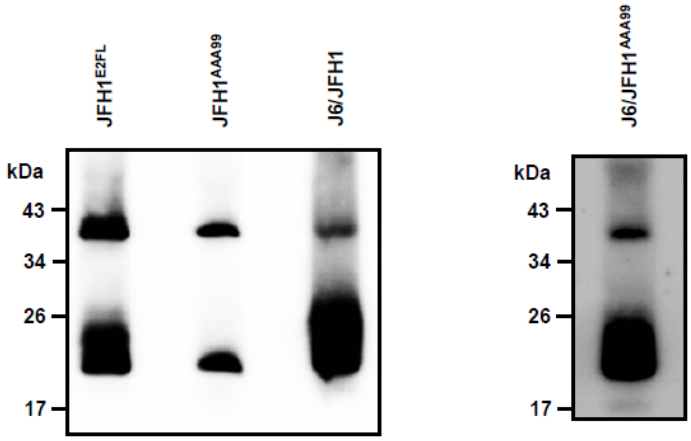
Yukihiro Kushima,[1, 2] Takaji Wakita,[3] Makoto Hijikata[1, 2]

1. **Department of Viral Oncology, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan**
2. **Graduate School of Biostudies, Kyoto University, Kyoto 606-8507, Japan**
3. **Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640, Japan**

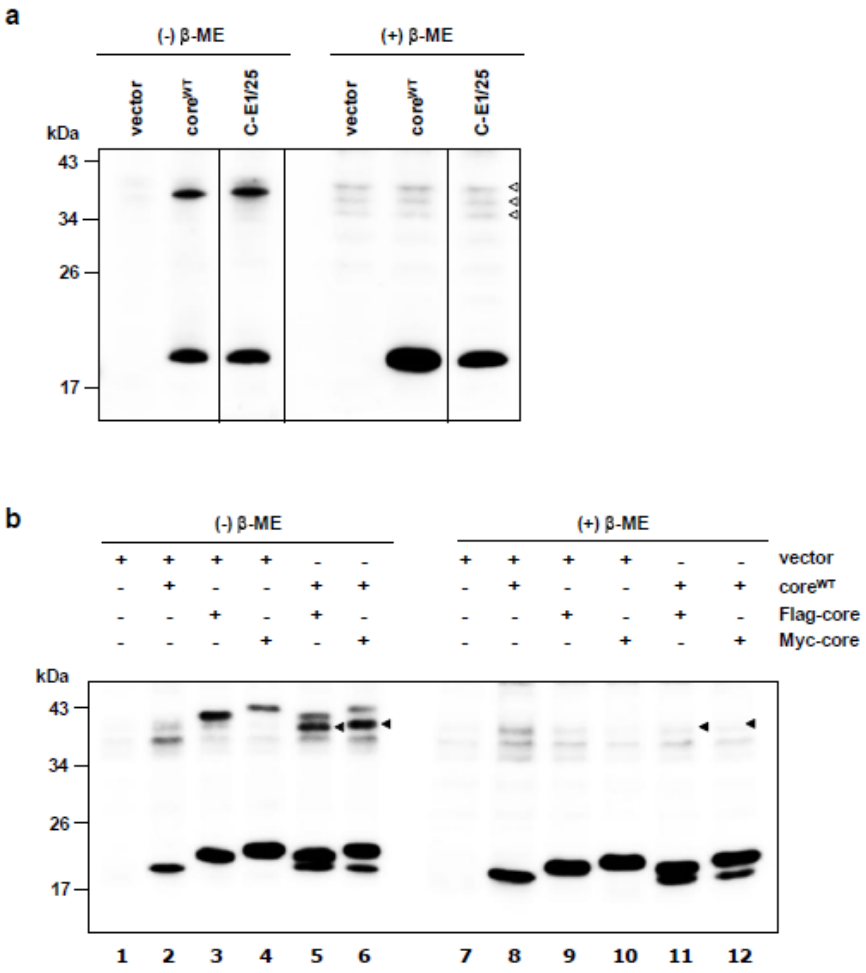
Supplementary Figures and legends



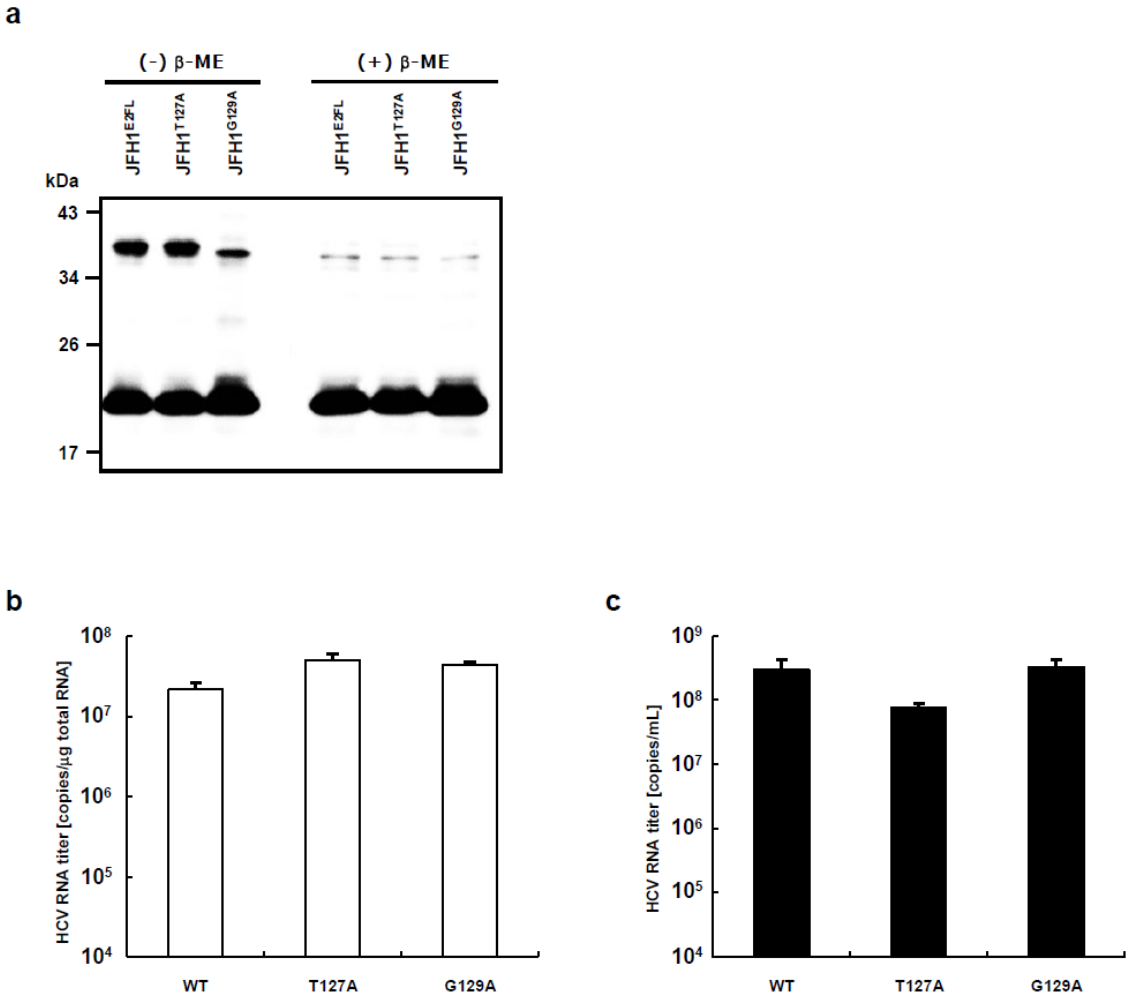
Supplementary Figure 1. JFH1^{E2FL} core protein. Map of the reported functional regions of the core protein from residues 1 to 191 is shown as indicated in figure. The white arrowheads indicate signal peptidase (SP) and proposed signal peptide peptidase (SPP) cleavage site by Okamoto et al. (37). The filled arrowheads represents potential trypsin cleavage sites. Cystein residues of the core are indicated by arrows.



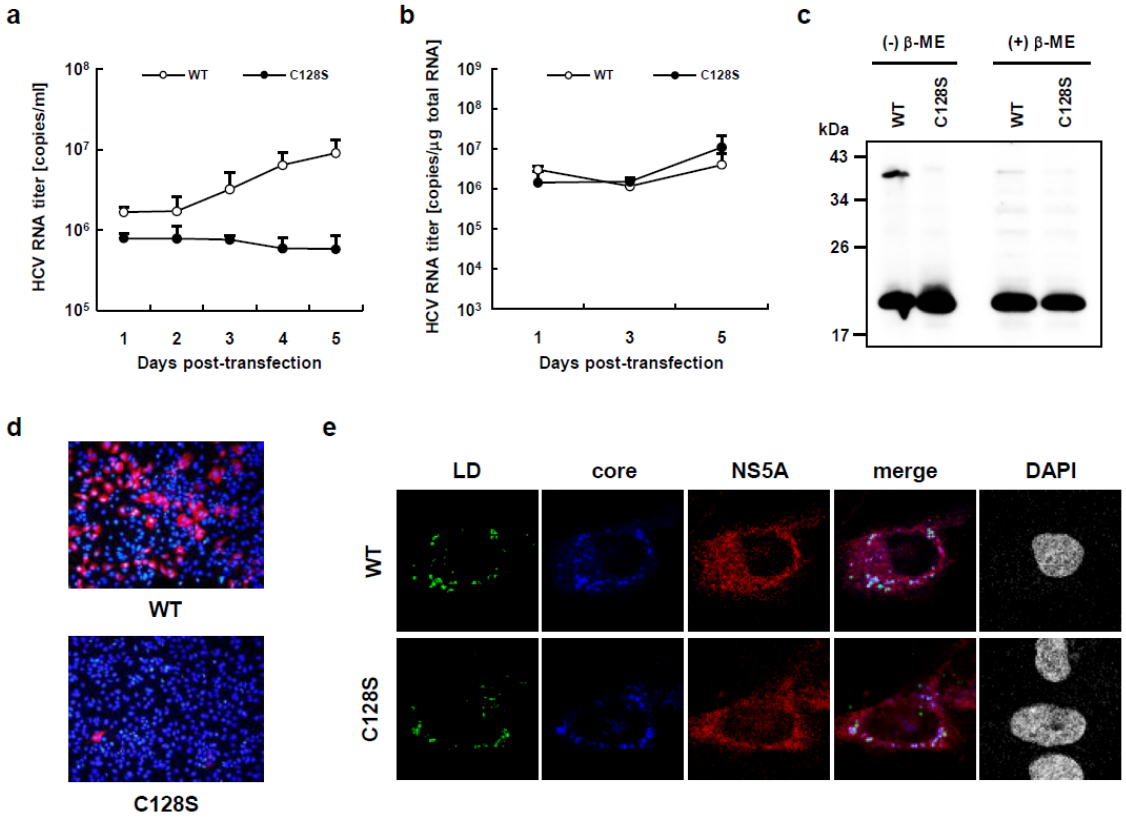
Supplementary Figure 2. Core complexes from various HCV strains. Immunoblot analysis of core from pellets containing HCV virus particles collected following ultracentrifugation of the concentrated culture medium from JFH1^{E2FL}, JFH1^{AAA99}, J6/JFH1, or J6/JFH1^{AAA99} RNA–transfected HuH-7 or HuH7.5 cells under non-reducing conditions. Data are representative of three independent experiments.



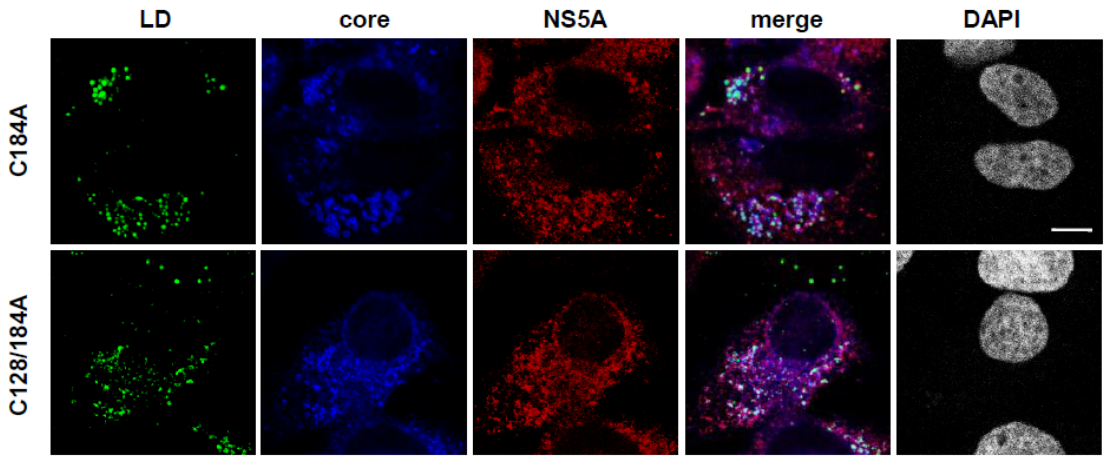
Supplementary Figure 3. Analysis of core complex in microsomal membrane fractions (MMF) of core expressing cells. (**a**) MMF of HuH-7 cells transfected with pcDNA3 (vector), pcDNA3-core^{WT} (core^{WT}), or pcDNA3-C-E1/25 (C-E1/25), bearing full length core and the N-terminal 25 amino acid sequence of E1, were subjected to non-reducing ((-) β-ME) and reducing ((+) β-ME) SDS-PAGE and analyzed by immunoblotting against core. Open arrowheads indicate the non-specific bands observed in MMF samples in reducing condition which positions are close to the core dimers detected in non-reducing condition. (**b**) Immunoblot analysis of core in the MMF collected from HuH-7 cells transfected with pcDNA3 (vector) and/or core expression plasmids (core191, FLAG-core, and Myc-core) as indicated. Samples were treated with or without 5% β-mercaptoethanol (β-ME). Filled arrowheads indicate the positions of the intermediate core complexes formed by core^{WT} and tagged core. Data are representative of two (**a**) or three (**b**) independent experiments.



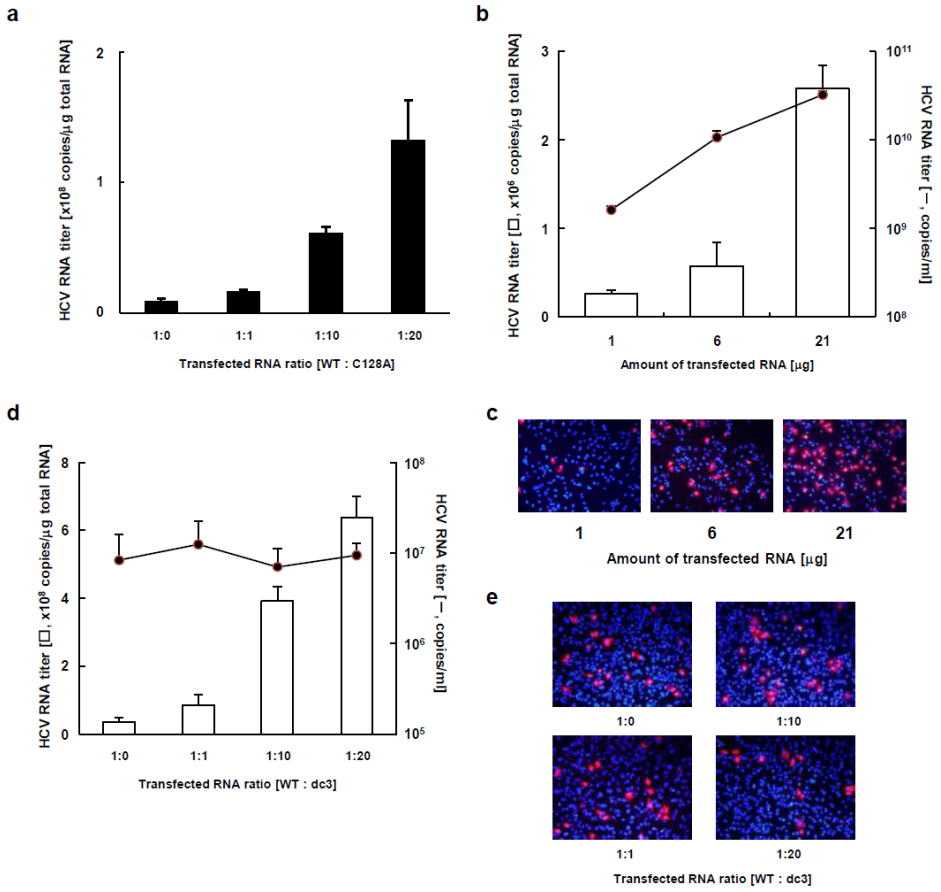
Supplementary Figure 4. Site-directed mutagenesis of amino-acid position 127 or 129 had no effect on HCV replication or the production of HCV particles. (**a**) Immunoblot analysis of core in microsomal membrane fractions collected on day 3 post-transfection from cells transfected with JFH1^{E2FL} (WT), JFH1^{T127A} (T127A), or JFH1^{G129A} RNA. Samples were treated with or without 5% β-mercaptoethanol (β-ME). (**b**, **c**) Real-time qRT-PCR analysis of HCV RNA titers in total cellular RNA (**b**) or culture medium (**c**) collected on day 5 post-transfection. Data are representative of three independent experiments (**a**) or are the means ± s.d. from three independent experiments (**b**, **c**).



Supplementary Figure 5. Analysis of core C128S mutant. (**a**) Real-time qRT-PCR analysis of HCV RNA titers in culture medium collected at the indicated time points from HuH-7 cells transfected with JFH1^{E2FL} (WT, open circles) or JFH1^{C128S} (C128S, filled circles) RNA. (**b**) Real-time qRT-PCR analysis of the HCV RNA titer using total cellular RNA collected at the indicated time points from cells transfected with WT (open circles) or (C128S) (filled circles). (**c**) Immunoblot analysis of core in microsomal membrane fraction collected on day 3 post-transfection from cells transfected with JFH1^{E2FL} (WT) or JFH1^{C128S} RNA (C128S). (**d**) Infectivity of culture medium collected and concentrated on day 5 post-transfection from HuH-7 cells transfected with WT or C128S RNA. (**e**) Confocal microscopy of the subcellular localization of the LD (green), core (blue), NS5A (red), and nucleus (DAPI) (grey) in cells transfected with JFH1^{E2FL} (WT) or JFH1^{C128S} RNA (C128S) on day 3 post-transfection. Data are the means ± s.d. from three independent experiments (**c**, **b**) or are representative of three independent experiments (**c, d, e**).



Supplementary Figure 6. Subcellular localization of HCV proteins. Confocal microscopy of the subcellular localizations of the lipid droplet (LD), core, NS5A, and the nucleus (DAPI) three days post-transfection with $JFH1^{C184A}$ (C184A) or $JFH1^{C128/184A}$ (C128/184A). Scale bar indicates 10 μm. Data are representative of three independent experiments.



Supplementary Figure 7. Transfection of various amounts of HCV RNA had no effect on HCV replication. (**a**) Real-time qRT-PCR analysis of the HCV RNA titer in total cellular RNA collected on day 3 post-transfection from HuH-7 cells transfected with the indicated RNA ratio of JFH1^{E2FL} (WT) or JFH1^{C128A} (C128A) RNA. (**b**) Real-time qRT-PCR analysis of the HCV RNA titer in total cellular RNA (open bars) or culture medium (filled circles) collected on day 3 post-transfection from HuH-7 cells transfected with the indicated amount of JFH1^{E2FL} RNA. (**d**)Real-time qRT-PCR analysis of the HCV RNA titer in total cellular RNA (open bars) or culture medium (filled circles) collected on day 3 post-transfection from HuH-7 cells transfected with the indicated ratio of WT and JFH1^{dc3} (dc3) RNA. (**c, e**) The infectivity of culture medium collected from HuH-7 cells transfected with the indicated amount of JFH1^{E2FL} RNA (**c**) and culture medium collected from HuH-7 cells transfected with the indicated ratio of WT and JFH1^{dc3} (dc3) RNA (**e**) were analyzed as described in the Materials and Methods. Data are the means ± s.d. from three independent experiments (**a, b, d**) or are representative of three independent experiments (**c, e**).

Plasmid name	Primer sequences (5'-3')	Template for PCR	Restriction enzyme site	Original plasmid
pJFH1^{T127A}	CACGACGTTGTAAAACGACG	pJFH1^{E2FL}	EcoRI / BsiWI	pJFH1^{E2FL}
	ATCGACACCCTAGCGTGTGGCTT			
	ATGTCTATGATGACCTCGGG			
pJFH1^{C128A}	CACGACGTTGTAAAACGACG	pJFH1^{E2FL}	EcoRI / BsiWI	pJFH1^{E2FL}
	ACCCTAACGGCTGGCTTTGCC			
	ATGTCTATGATGACCTCGGG			
pJFH1^{C128S}	CACGACGTTGTAAAACGACG	pJFH1^{E2FL}	EcoRI / BsiWI	pJFH1^{E2FL}
	ACCCTAACGTCTGGCTTTGCC			
	ATGTCTATGATGACCTCGGG			
pJFH1^{G129A}	CACGACGTTGTAAAACGACG	pJFH1^{E2FL}	EcoRI / BsiWI	pJFH1^{E2FL}
	ACCCTAACGTGTGCCTTTGCCGACCTC			
	ATGTCTATGATGACCTCGGG			
pJFH1^{C184A}	CACGACGTTGTAAAACGACG	pJFH1^{E2FL}	EcoRI / BsiWI	pJFH1^{E2FL}
	CCTGTTGTCCGCCATCACCGTTC			
	ATGTCTATGATGACCTCGGG			
pJFH1$^{C128/184A}$	CACGACGTTGTAAAACGACG	pJFH1^{C184A}	EcoRI / BsiWI	pJFH1^{E2FL}
	ACCCTAACGGCTGGCTTTGCC			
	ATGTCTATGATGACCTCGGG			
pcDNA3-C-E1/25	tgataAAGCTTCACCATGAGCACAAATCC	pJFH1^{E2FL}	HindIII / EcoRI	pcDNA3
	taataGAATTCTCACGGGGACGTGGAGAACCG			
pcDNA3-FLAG-core	tgataAAGCTTACCATGGACTACAAGGATGAC GATGACAAGATGAGCACAAATCCTAAAC	pJFH1^{E2FL}	HindIII / EcoRI	pcDNA3
	taataGAATTCTCAAGCAGAGACCGGAACG			
pcDNA3-Myc-core	tgataAAGCTTACCATGGAACAAAAACTCATC TCAGAAGAGGATCTGATGAGCACAAATCC TAAAC	pJFH1^{E2FL}	HindIII / EcoRI	pcDNA3
	taataGAATTCTCAAGCAGAGACCGGAACG			
pcDNA3-core^{C128A}	tgataAAGCTTCACCATGAGCACAAATCC	pJFH1^{C128A}	HindIII / EcoRI	pcDNA3
	taataGAATTCTCAAGCAGAGACCGGAACG			

Supplementary Table. The sets of primers used to amplify the target genes, template plasmids used in the PCRs, restriction sites, and plasmids into which the amplified DNA fragments were inserted are shown.