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Electronic Supplementary Material

Phosphatidylserine-Specific Phospholipase A1 is the Critical Bridge for Hepatitis C Virus Assembly

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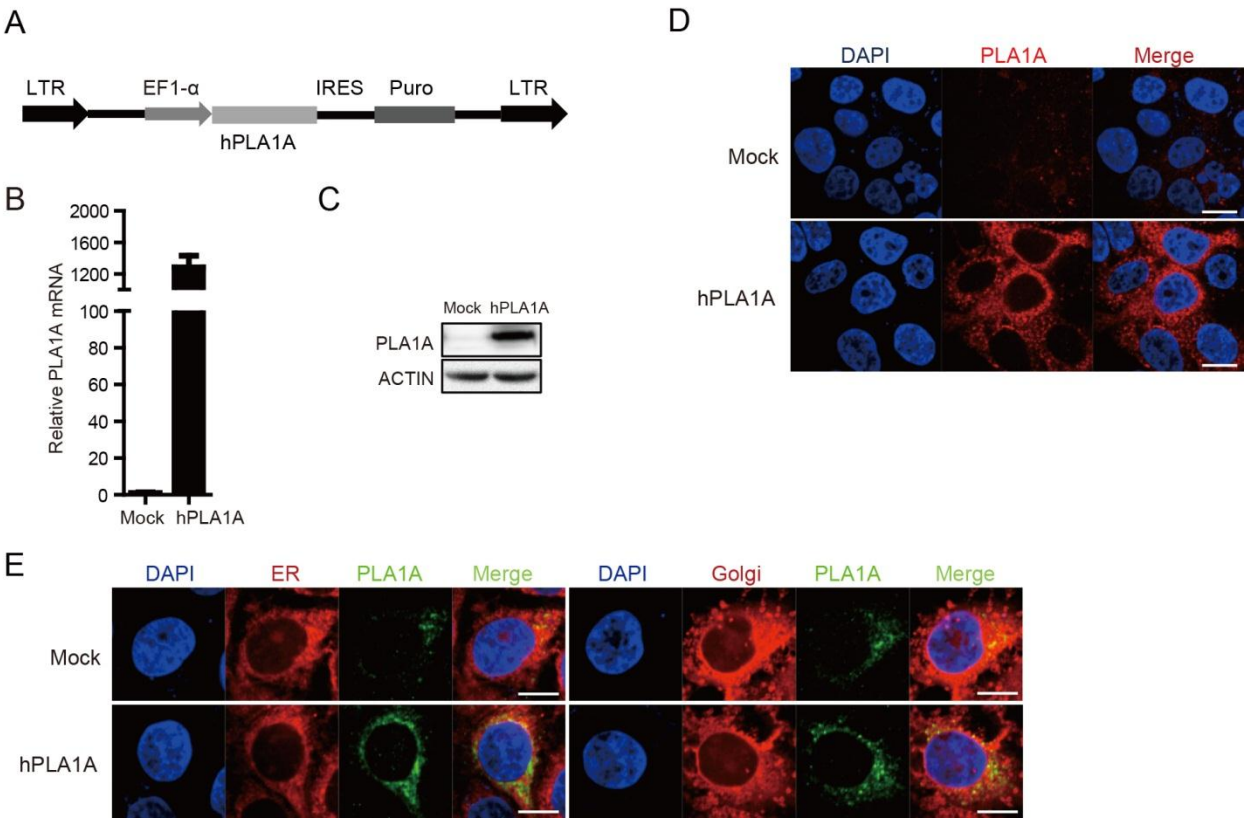

Figure S1. Establishment of Huh7.5.1 cells stably overexpressing PLA1A. (A) A schematic representation of stably overexpressing PLA1A plasmid. LTR, long terminal repeat; EF1-α promoter; Puro, puromycin resistance gene; IRES, EMCV internal ribosome entry site. (B-C) Huh-7.5.1 cells were infected with packaged PLA1A genes lentivirus or an irrelevant nontargeting control, and cell pools with stable expression were established. Amounts of PLA1A mRNA and protein levels in either cell pool were quantified by RT-qPCR and western blot, respectively. The graph represents the mean values from three independent experiments and standard deviations of the means. (D) PLA1A-specific stably overexpressing was determined at the single-cell level by immunofluorescence. Scale bars represent 10 μm. (E) Subcellular distribution and colocalization of PLA1A in Huh7.5.1 cells stably overexpressing PLA1A. Immunofluorescence was performed using antibodies against PLA1A and cellular organelle dyes. Blue, DAPI; Red, ER or Golgi tracker; Green, PLA1A and merge. Scale bars represent 8 μm.

Table S1. The Primers

Primers used to amplify the different deletions of PLA1A

PLA1A deletions	Orientation	Sequence (5′–3′)
PLA1A	F/R	AAGCTTATGCCCCCAGGTCCCTGG/ GGTACCCTACTACACACAGGCTATC
81-456	F81/R	AAGCTTCACTCTGGGAACCAAACT/GGTACCCTACTACACACAGGCTATC
Δ 81-217	OCF	AAACTCTGGGTTCAATGCACAGACACCGACAATTTG
Δ 81-217	OCR	CAAATTGTCGGTGTCTGTAGTTTGGTTCCCAGAGTG
Δ 218-258	OCF	TTCGTGGAAGCCATCCACGATCACATGAGGGCTGTG
Δ 218-258	OCR	CACAGCCCTCATGTGATCGTGGATGGCTTCCACGAA
1-258	F/R258	AAGCTTATGCCCCCAGGTCCCTGG/GGTACCCTAACAGCCCTCATGTGATCA
1-376	F/R376	AAGCTTATGCCCCCAGGTCCCTGG/GGTACCCTACTTAGGTATGGTGATCTT

F indicate forward primers; R indicate reverse primers; OCF and OCR indicate overlapping complementation forward or reverse primers, respectively. Red color denote *Hin*d III(forward) or *Kpn* I (reverse) restriction site. Blue color denote termination codon.

Primers used to amplify the different deletions of NS2

NS2 deletions	Orientation	Sequence (5′–3′)
NS2	F/R	AAGCTTTATGACGCACCTGTGCACG/CTGCAGCTAAAGGAGCTTCCACCCC
1-197	F/R197	AAGCTTTATGACGCACCTGTGCACG/CTGCAGCTAGAGTCGGGCGGACACGG
1-137	F/R137	AAGCTTTATGACGCACCTGTGCACG/CTGCAGCTAGCCAGTCCACCTGCC
1-93	F/R93	AAGCTTTATGACGCACCTGTGCACG/CTGCAGCTATAAGAGGTAAGCAGGCC
25-217	F25/R	AAGCTTGGGTATAAGACCCTCCTCGG/CTGCAGCTAAAGGAGCTTCCACCCC
49-217	F49/R	AAGCTTCAGGAGTGGGTACCACCC/CTGCAGCTAAAGGAGCTTCCACCCC
74-217	F74/R	AAGCTTGGTGTGGTGTTTGACATTACCAAATGGC
Δ49-73	OCF	CCCTGGGGGAAGCCATGATTGGTGTGGTGTTTGACATTACCAAATG
Δ49-73	OCR	GGTAATGTCAAACACCACACCAATCATGGCTTCCCCCAG
Δ74-93	OCF	CGTCACTATATTCTGCCCGAGGGCCGCTTTGACACATG
Δ74-93	OCR	CATGTGTCAAAGCGGCCCTCGGGCAGAATATAGTGACGGCC

F indicate forward primers; R indicate reverse primers; OCF and OCR indicate overlapping complementation forward or reverse primers, respectively. Red color denote *Hin*d III (forward) or *Pst* I (reverse) restriction site. Blue color denote termination codon.

Primers used to amplify the different deletions of NS5A

NS5A deletions	Orientation	Sequence (5′–3′)
NS5A	F/R	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTAGCAGCACACGG TGGTATC
1-352	F/R352	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTACCTTGGGGGAG GCGTCGG
1-338	F/R338	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTAACAACCAGCAA CGGTGGG
1-250	F/R250	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTAATAGGTGTTGCT GTGG
1-213	F/R213	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTAAGTCTCCGCCGT GATGTG
339-466	F339/R	AAGCTTGCTCTCCCCCCCCCCAAG/GGTACCCTAGCAGCACACGG TGGTATC
214-466	F214/R	AAGCTTGCGGCGCGGCGCTTGGC/GGTACCCTAGCAGCACACGGT GGTATC
1-213-Δ5-11	F11	AAGCTTTCCGGATCCTGGGTTTGCACCATC
1-213-Δ5-11	R213	GGTACCCTAAGTCTCCGCCGTGATGTG
Δ28-100	OCF	TGGCTGACCTCTAAATTGAAACCCCCCACGAACTAC
Δ28-100	OCR	GTAGTTCGTGGGGGGTTTCAATTTAGAGGTCAGCCAATTTTTG
Δ101-213	OCF	GAGGGCCAGTGCGCGCCGGCGGCGCGGCGCTTGGCACG
Δ101-213	OCR	GCCAAGCGCCGCGCCGCCGGCGCGCACTGGCCCTCCGTG
28-466	F28/R	AAGCTTTTCCCCAAGCTGCCCGGC/GGTACCCTAGCAGCACACGG TGGTATC
Δ101-128	OCF	GCCAGTGCGCGCCGTATGTAACAGGACTGACCACTGAC
Δ101-128	OCR	GGTCAGTCCTGTTACATACGGCGCGCACTGGCCCTCC
Δ129-163	OCF	CAGCATGGGTCGTACTCCACACCAAAGCCGTTTTTC
Δ129-163	OCR	GAAAAACGGCTTTGGTGTGGAGTACGACCCATGCTGCG
Δ164-183	OCF	ATCCATAGGTTTGCACCCGTCGGGTCCCAGCTTCCC
Δ164-183	OCR	GGGAAGCTGGGACCCGACGGGTGCAAACCTATGGATCTG
Δ 184-213	OCF	GGCTTAATTCCTATGCTGCGGCGCGGCGCTTG
Δ 184-213	OCR	AGCGCCGCGCCGCAGCATAGGAATTAAGCCCAAC
101-466	F101/R	AAGCTTAAACCCCCCACGAACTAC/GGTACCCTAGCAGCACACGG TGGTATC
1-100	F/R100	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTACGGCGCGCACT GGCCCTCC
60-466	F60/R	AAGCTTGGCGCCAACATCTCTGGC/GGTACCCTAGCAGCACACGG TGGTATC

F indicate forward primers; R indicate reverse primers; OCF and OCR indicate overlapping complementation forward or reverse primers, respectively. Red color denote *Hin*d III (forward) or *Kpn* I(reverse) restriction site. Blue color denote termination codon.

Site-directed mutagenesis of NS5A

NS5A deletions	Orientation	Sequence (5′–3′)
NS5A	F/R	AAGCTTTCCGGATCCTGGCTCCGC/GGTACCCTAGCAGCACACGG TGGTATC
m184-188	OCF	TAATTCCTATGCTGCCGCGGCCGCGGCTCCCTGTGAACCTG
m184-188	OCR	TCAGGTTCACAGGGAGCCGCGGCCGCGGCAGCATAGGAATTAAG
m189-193	OCF	GGGTCCCAGCTTGCCGCTGCAGCTGCGCCCGACGCAGACG
m189-193	OCR	GTCTGCGTCGGGCGCAGCTGCAGCGGCAAGCTGGGACCCGAC
m194-198	OCF	TGTGAACCTGAGGCCGCCGCAGCCGCATTGAGGTCCATGCTAAC
m194-198	OCR	CATGGACCTCAATGCGGCTGCGGCGGCCTCAGGTTCACAGGG

m199-203	OCF	GACGCAGACGTAGCGGCGGCCGCGGCAACAGATCCGCCCCAC
m199-203	OCR	TGGGGCGGATCTGTTGCCGCGGCCGCCGCTACGTCTGCGTCGGG
m204-208	OCF	AGGTCCATGCTAGCAGCTGCGGCCGCCATCACGGCGGAGACTG
m204-208	OCR	TCTCCGCCGTGATGGCGGCCGCAGCTGCTAGCATGGACCTC
m209-213	OCF	GATCCGCCCCACGCCGCGGCGGCGGCTGCGGCGCGGCGCTTG
m209-213	OCR	AGCGCCGCGCCGCAGCCGCCGCCGCGGCGTGGGGCGGATCTG

F indicate forward primers; R indicate reverse primers; OCF and OCR indicate overlapping complementation forward or reverse primers, respectively. Red color denote *Hin*dⅢ(forward) or *Kpn* Ⅰ (reverse) restriction site. Blue color denote termination codon.

Primers used to amplify the different deletions of E2

E2 deletions	Orientation	Sequence (5′–3′)
E2	F/R	AAGCTTGGCACCACCACCGTTGGAG/GGTACCCTATGCTTCGGCC TGGCCC
29-367	F29/R	AAGCTTCAGCTCATTAACACCAACGG/GGTACCCTATGCTTCGGC CTGGCCC
61-367	F61/R	AAGCTTACCAACCGCTTTAACTCGTC/GGTACCCTATGCTTCGGCC TGGCCC
144-367	F144/R	AAGCTTCCCACCTACACATGGGGAG/GGTACCCTATGCTTCGGCC TGGCCC
189-367	F189/R	AAGCTTCGCACCAGAGCTGACTTC/GGTACCCTATGCTTCGGCCT GGCCC
202-367	F202/R	AAGCTTTGCCCTACGGATTGTTTTAGG/GGTACCCTATGCTTCGGC CTGGCCC
273-367	F273/R	AAGCTTTGCGACTTGGAGGACAGGG/GGTACCCTATGCTTCGGCC TGGCCC
1-272	F/R272	AAGCTTGGCACCACCACCGTTGGAG/GGTACCCTATCGAACGACG TATTTTG
1-336	F/R336	AAGCTTGGCACCACCACCGTTGGAG/GGTACCCTAGCGATCCCCA CGAGTG

F indicate forward primers; R indicate reverse primers; Red color denote *Hin*d III (forward) or *Kpn* Ⅰ (reverse) restriction site, respectively. Blue color denote termination codon.

Primers used to construct stably overexpressing PLA1A plasmid

E2 deletions	Orientation	Sequence (5′–3′)
Pme1- PLA1A	F	GTTTAAACGGAATGCCCCCAGGTCCCTGGGAGAGCTGCTTCTGGG
Spe1-PLA1A	R	ACTAGTCTACTACACACAGGCTATCTTCAGGTCACAGGAAAC

F indicate forward primers; R indicate reverse primers; Red color denote *Pme* I (forward) or *Spe* I (reverse) restriction site, respectively. Blue color denote termination codon

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Table S2. Patients and liver biopsy data

Gender	BMI	Blood IU/mL (Log10)	Liver IU/mg (Log10)	PLA1A (fold)
F	23.48	N	N	1
F	30.86	N	N	1.67
F	20.73	N	N	1.13
F	35.06	N	N	2.35
F	26.68	N	N	1.24
M	29.02	N	N	1.56
M	34.2	N	N	2.1
M	24.24	N	N	0.57
M	22.18	N	N	0.81
M	22.68	N	N	0.97
F	24.24	6.53	8.12	19.73
F	27.06	6.23	8.23	19.63
F	24.22	5.75	8.39	22.98
F	28.89	5.87	8.65	25.25
F	26.95	6.08	8.04	21.26
F	23.53	6.59	8.19	22.95
M	24.26	5.63	8.63	29.62
M	20.28	5.39	8.42	23.53
M	24.22	6.42	8	21.2
M	20.83	6.03	8.14	32.29
M	25.61	5.81	8.38	20.72
M	24.8	6.68	8.19	24.64
F	24.49	6.41	8.16	19.2
M	24.49	6.58	8.1	23.56
M	21.13	6.61	8.33	26.47
F	17.96	7	8.64	25.66
F	23.81	6.67	8.75	34.93
F	23.44	6.41	8.09	23.14
M	18.37	6.51	8.43	24.46
F	23.26	6.65	7.8	21.95
M	18.37	6.53	8.13	21.94
M	23.74	5.75	8.37	24.98
F	20.55	5.87	8.81	32.33
F	22.89	4.83	7.04	15.68
M	22.49	6.68	8.3	19.92
M	31.11	6.99	8.37	22.87
M	27.34	6.89	8.17	23.46
M	22.15	6.37	8.28	22.36
M	20.76	5.08	8.14	27.22
M	20.01	4.99	7.87	19.62
M	25.39	6.74	8.93	31.99
M	29.41	4.89	6.3	18.21
M	19.38	4.94	6.37	15.98
M	27.43	5.08	7.39	18.33
M	18.34	5.03	7.65	17.23
M	27.68	4.98	6.14	14.68
M	19.84	5.16	7.26	19.97
M	24.49	5.2	6.38	16.9