A furoviral replicase recruits host HSP70 to membranes for viral RNA replication

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Running title: A furoviral replicase recruits HSP70 to membranes for replication

Supplementary materials

Supplementary Table

Table S1 Primers used in this study.

Supplementary Figures

Figure S1 Western blotting assays showing inhibition of NbHSP70 by quercetin treatment (A) and over-expression of TaHSP70 by Agrobacterium infiltration of 35S:TaHSP70 (B) in *N. benthamiana* plants.

Figure S2 Sequence alignment of TaHSP70 and NbHSP70 showing that they are highly conserved.

Figure S3 Recombinant expression of GST-tagged Rep$^{1\text{-}333}$ (GST-Rep$^{1\text{-}333}$) and 6 x His-tagged TaHSP70 (His-TaHSP70) in *E. coli* BL21DE3 for ELISA-base binding assays. M, protein Marker; Lane 1, Protein sample extracted from *E. coli* BL21DE3 expressing GST-Rep$^{1\text{-}333}$; Lane 2, Protein sample extracted from *E. coli* BL21DE3; Lane 3, Protein sample extracted from *E. coli* BL21DE3 expressing His-TaHSP70. White arrows point to the recombinant proteins GST-Rep$^{1\text{-}333}$ and His-TaHSP70, respectively.

Table S1 Primers used in this study.

Primers	Sequences (5'-3')	Restriction sites (underlined)	The purpose of the primers
Nb70F	CTAGTCTAGATATGCATACAACATGAGG	XbaI	Vector TRV:HSP70
Nb70R	CGGGGTACCTTAGTCGACCTCCTCAAT	KpnI	
BDTa70N	GGAATTCCATATGATGGCCAAGGGCGAGGGG	NdeI	Bait plasmid BD-TaHSP70
BDTa70C	CGCGGATCCGTCCACCTCC TCGATCTT	BamHI	
BDNb70N	GGAATTCCATATGATGGCAGGAAAAGGTGAA	NdeI	Bait plasmid BD-NbHSP70
BDNb70C	CGCGGATCCGTCGACCTCC TCAATCTT	BamHI	
ADRep$^{1-670}$N	TCCCCACCCGGGATGCCGATCGATAGCAGT	XmaI	Prey plasmid AD- Rep$^{1-670}$
ADRep$^{1-670}$C	CGCGGATCCTCCTTTCATTCACATGGAC	BamHI	
ADRep$^{670-1430}$N	TCCCCACCCGGGAGGAGAAATACTTCTGAT	XmaI	Prey plasmid ADRep$^{670-1430}$
ADRep$^{670-1430}$C	CGCGGATCCCCAAACCCCGCTTGTCTTG	BamHI	
ADRep$^{1430-1858}$N	TCCCCACCCGGGTTGGTACCGGTGATGCGG	XmaI	Prey plasmid ADRep$^{1430-1858}$
ADRep$^{1430-1858}$C	CGCGGATCCCCCATTCAAAGTTCCTATC	BamHI	
ADRep$^{1-167}$N	TCCCCACCCGGGATGCCGATCGATAGCAGT	XmaI	Prey plasmid ADRep$^{1-167}$
ADRep$^{1-167}$C	CGCGGATCCTGGGTATCTCGTCCTTCTC	BamHI	
ADRep$^{167-333}$N	TCCCCACCCGGGCCCACCGAAGTCGGTGAA	XmaI	Prey plasmid ADRep$^{167-333}$
ADRep$^{167-333}$C	CGCGGATCCCGTCCGTCATTGTGAAGAA	BamHI	
ADRep$^{333-670}$N	TCCCCACCCGGGGACGCAACGGAAGCGAGG	XmaI	Prey plasmid ADRep$^{333-670}$
ADRep$^{333-670}$C	CGCGGATCCCTTCATTCACATGGACCAT	BamHI	
pETNb70N	CGCGGATCCATGGCAGGAAAAGGTGAA	BamHI	Plasmid pET28(a)-Nb70
pETNb70C	ATTTGCGGCCGCAGTCGACCTCCTCAATCTT	NotI	
pETTa70N	CGCGGATCCATGGCCAAGGGCGAGGGG	BamHI	Plasmid pET28(a)-Ta70
pETTa70C	ATTTGCGGCCGCAGTCCACCTC CTCGATCTT	NotI	
p6P1Rep$^{1-670}$ N	CGCGGATCCATGCCGATCGATAGCAGT	BamHI	Plasmid pGEX-Rep$^{1-670}$
p6P1Rep$^{1-670}$ C	CCGGAATTCTTAATTTCTCCTTTCATTCAC	EcoRI	
Rep$^{1-1350}$-GFPN	AAAAAGCAGGCTCCATGCCGATCGATAGCAGT		Plasmids Rep$^{1-1350}$-GFP
Rep$^{1-1350}$-GFPC	AGAAAGCTGGGTCTTTGTCGAATTCGGGACA		
Rep$^{1-1350}$-mCherryN	AAAAAGCAGGCTCCATGCCGATCGATAGCAGT		Plasmids Rep$^{1-1350}$-mCherry
Rep$^{1-1350}$-mCherryC	AGAAAGCTGGGTCTTTGTCGAATTCGGGACA		
Nb70-GFPN	AA AAAGCAGGCTCCATGGCAGGAAAAGGT		Plasmids NbHSP70-GFP
Nb70-GFPC	AGAAAGCTGGGTCGTCGACCTC CTCAATCTT		
Ta70-GFPN	AA AAAGCAGGCTCCATGGCCAAGGGCGAG		Plasmids TaHSP70-GFP
Ta70-GFPC	AGAAAGCTGGGTCGTCCACCTC CTCGATCTT		
attB1	ACAAGTTTGTACAAAAAAGCAGGCTCC		Gateway vector
attB2	ACCACTTTGTACAAGAAAGCTGGGTC		
BRepF	CGCGGATCCATGCCGATCGATAGCAGT	BamHI	Plasmids pCV-nYFP-Rep$^{1-670}$
BRepR	CGAGCTCCCTTTCATTCACATGGAC	SacI	
BTa70F	CGCGGATCCATGGCCAAGGGCGAGGGG	BamHI	Plasmids pCV-cYFP-TaHSP70
BTa70R	CGAGCTCGTCCACCTC CTCGATCTT	SacI	
BNb70F	CGCGGATCCATGGCAGGAAAAGGTGAA	BamHI	Plasmids pCV-cYFP-NbHSP70
BNb70R	ATTTGCGGCCGCGTCGACCTCCTCAATCTT	NotI	

Figure S1

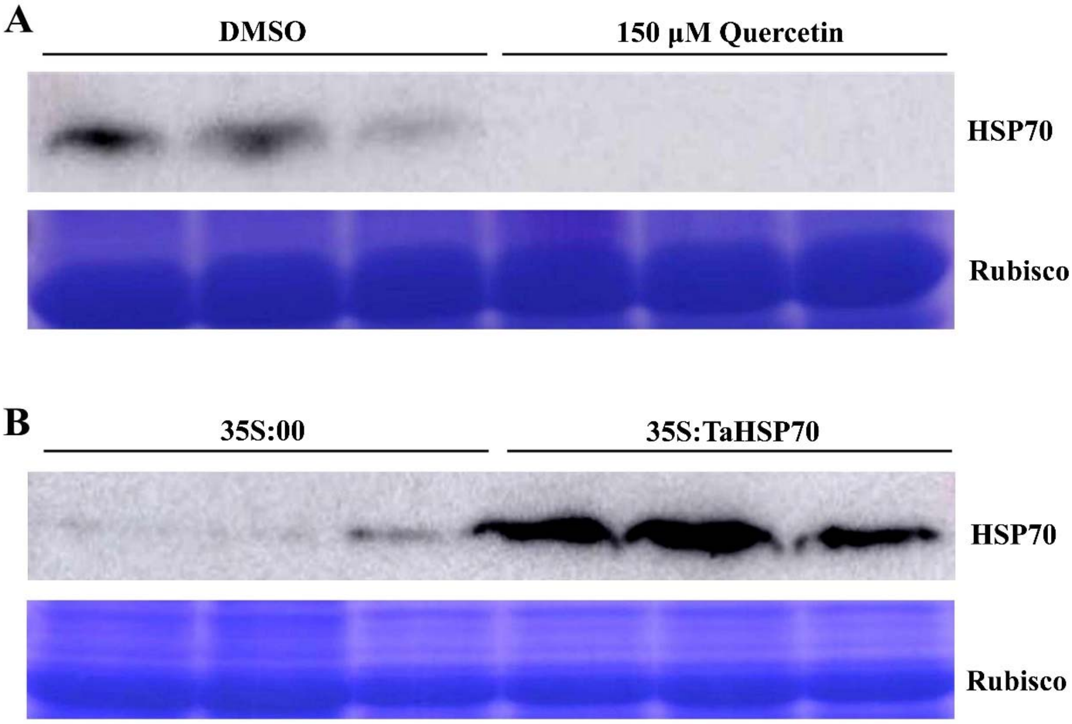
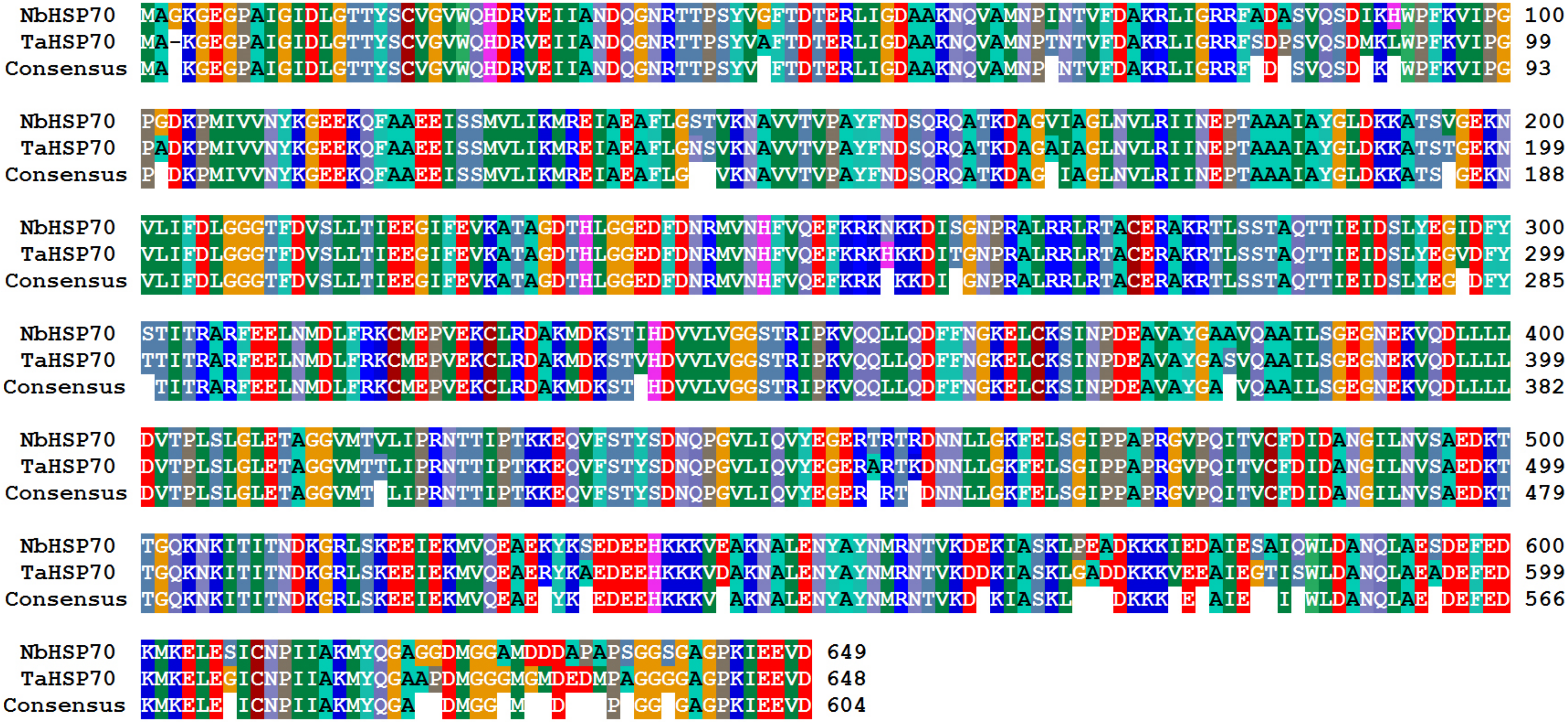

A
DMSO
150 µM Quercetin
HSP70
Rubisco
B
35S:00
35S:TaHSP70
HSP70
Rubisco

Figure S2



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NbHSP70   MAGKGEGPAIGIDLGTTYSCVGVWQHDRVEIIANDQGNRTTPSYVGFTDTERLIGDAAKNQVAMNPINTVFDAKRLIGRRFADASVQSDIKHWPFKVIPG  100
TaHSP70   MA-KGEGPAIGIDLGTTYSCVGVWQHDRVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPTNTVFDAKRLIGRRFSDPSVQSDMKLWPFKVIPG  99
Consensus MA KGEGPAIGIDLGTTYSCVGVWQHDRVEIIANDQGNRTTPSYV FTDTERLIGDAAKNQVAMNP NTVFDAKRLIGRRF D SVQSD K WPFKVIPG  93

NbHSP70   PGDKPMIVVNYKGEEKQFAAEEISSMVLIKMREIAEAFLGSTVKNAVVTVPAYFNDSQRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDKKATSVGEKN  200
TaHSP70   PADKPMIVVNYKGEEKQFAAEEISSMVLIKMREIAEAFLGNSVKNAVVTVPAYFNDSQRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDKKATSTGEKN  199
Consensus P DKPMIVVNYKGEEKQFAAEEISSMVLIKMREIAEAFLG  VKNAVVTVPAYFNDSQRQATKDAG IAGLNVLRIINEPTAAAIAYGLDKKATS GEKN  188

NbHSP70   VLIFDLGGGTFDVSLLTIEEGIFEVKATAGDTHLGGEDFDNRMVNHFVQEFKRKNKKDISGNPRALRRLRTACERAKRTLSSTAQTTIEIDSLYEGIDFY  300
TaHSP70   VLIFDLGGGTFDVSLLTIEEGIFEVKATAGDTHLGGEDFDNRMVNHFVQEFKRKHKKDITGNPRALRRLRTACERAKRTLSSTAQTTIEIDSLYEGVDFY  299
Consensus VLIFDLGGGTFDVSLLTIEEGIFEVKATAGDTHLGGEDFDNRMVNHFVQEFKRK KKDI GNPRALRRLRTACERAKRTLSSTAQTTIEIDSLYEG DFY  285

NbHSP70   STITRARFEELNMDLFRKCMEPVEKCLRDAKMDKSTIHDVVLVGGSTRIPKVQQLLQDFFNGKELCKSINPDEAVAYGAAVQAAILSGEGNEKVQDLLLL  400
TaHSP70   TTITRARFEELNMDLFRKCMEPVEKCLRDAKMDKSTVHDVVLVGGSTRIPKVQQLLQDFFNGKELCKSINPDEAVAYGASVQAAILSGEGNEKVQDLLLL  399
Consensus  TITRARFEELNMDLFRKCMEPVEKCLRDAKMDKST HDVVLVGGSTRIPKVQQLLQDFFNGKELCKSINPDEAVAYGA VQAAILSGEGNEKVQDLLLL  382

NbHSP70   DVTPLSLGLETAGGVMTVLIPRNTTIPTKKEQVFSTYSDNQPGVLIQVYEGERTRTRDNNLLGKFELSGIPPAPRGVPQITVCFDIDANGILNVSAEDKT  500
TaHSP70   DVTPLSLGLETAGGVMTTLIPRNTTIPTKKEQVFSTYSDNQPGVLIQVYEGERARTKDNNLLGKFELSGIPPAPRGVPQITVCFDIDANGILNVSAEDKT  499
Consensus DVTPLSLGLETAGGVMT LIPRNTTIPTKKEQVFSTYSDNQPGVLIQVYEGER RT DNNLLGKFELSGIPPAPRGVPQITVCFDIDANGILNVSAEDKT  479

NbHSP70   TGQKNKITITNDKGRLSKEEIEKMVQEAEKYKSEDEEHKKKVEAKNALENYAYNMRNTVKDEKIASKLPEADKKKIEDAIESAIQWLDANQLAESDEFED  600
TaHSP70   TGQKNKITITNDKGRLSKEEIEKMVQEAERYKAEDEEHKKKVDAKNALENYAYNMRNTVKDDKIASKLGADDKKKVEEAIEGTISWLDANQLAEADEFED  599
Consensus TGQKNKITITNDKGRLSKEEIEKMVQEAE YK EDEEHKKKV AKNALENYAYNMRNTVKD KIASKL   DKKK E AIE  I WLDANQLAE DEFED  566

NbHSP70   KMKELESICNPIIAKMYQGAGGDMGGAMDDDAPAPSGGSGAGPKIEEVD  649
TaHSP70   KMKELEGICNPIIAKMYQGAAPDMGGGMGMDEDMPAGGGGAGPKIEEVD  648
Consensus KMKELE ICNPIIAKMYQGA  DMGG M  D   P GG GAGPKIEEVD  604
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Figure S3