SUPPLEMENTARY INFORMATION

Exploration of Panviral Proteome: High-Throughput Cloning and Functional Implications in Virus-host Interactions

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Supplementary methods

Western blot

The plasmid DNA of HCMV expression clones was prepared using our HT DNA preparation procedure as previously described [29]. Their concentrations were normalized to 1 μg/μL using H_2O. The expression of HCMV proteins in pANT7_cGST vector with a C-terminal GST tag was performed by adding 1μg cDNA plasmid into 10 μL 2-fold diluted human HeLa lysate-based cell-free expression system (Thermo scientific, Rockford, IL) with the incubation of 2 hours (hrs) at 30^{o}C. The expressed proteins were then mixed with 10 μL 2×SDS loading buffer containing 10% 2-mercaptoethanol, and loaded into a 4-15% Tris-Criterion™ Precast Gel (Bio-rad, Hercules, CA). The protein electrophoresis was performed at 200V for 35 minutes (min). The proteins in gel were transferred to a nitrocellulose membrane using Trans-Blot® SD Semi-Dry Transfer Cell (Bio-rad, Hercules, CA) for 1 hr at 20 V. After blocking with 5% milk for 1 hr, the membrane was incubated with 1:3000 dilution of mouse anti-GST antibody overnight. After washing with PBST, the membrane was incubated with 1:5000 dilution of HRP conjugated sheep anti-mouse IgG antibody (Jackson Immunotech, West Grove, PA) for 1 hr at room temperature. The detection was performed using Chemiluminescence with SuperSignal West Femto Luminol/Enhancer Solution (Pierce, Rockford, IL. The expression of proteins was visually inspected by the band showed at their expected sizes on nitro-cellular membrane.

In-gel fluorescence

The expression of rubella viral proteins containing a C-terminal Halo tag and coxsackievirus proteins containing an N-terminal Halo tag was examined using in-gel fluorescence. Protein expression of viral ORF clones with Halo tag was performed by mixing 1 µL 1µg/µL cDNA plasmid and 9 µL diluted human HeLa expression solution, and incubating at 30^{o}C for 2 hrs. The detection was performed by adding 10 µL of Alexa 660 conjugated Halo-ligand (Promega, Madison, WI) (6 µM) and following incubation for 1hr at room temperature. After protein electrophoresis, the protein gel was rinsed three times with H_2O and scanned with an Amersham Bioscience Typhoon 9400 variable mode imager at 635 nm. All image adjustments were performed using Adobe® Photoshop® CS4 software (Adobe Systems, San Jose, CA).

Bead based pull-down assays

Coupling of bait protein to the magnetic beads

The Protein G coated Dynabeads (Life Technology, Carlsbad, CA) were completely re-suspended and transferred into a 1.5 mL centrifuge tube. The magnetic beads were separated from the solution using a magnet stand. After removal of the supernatant, the coupling of Dynabeads was performed by incubation with 100 µg/mL rabbit anti-GST polyclonal antibody (GE Healthcare Biosciences, Piscataway, NJ) at room temperature for 2 hrs on a Eppendorf Thermomixer® R mixer incubator (Eppendorf, Hauppauge, NY) at 1400 rpm. After the reaction, the beads were washed three times with PBST and stored at 4^{o}C. In parallel, the bait proteins were expressed by using 50 ng/µL plasmid cDNA in human cell-free expression system with the incubation of 2 hrs

at 30°C. The bait proteins were purified using anti-GST antibody coupled magnetic beads incubated for 2 hrs at 4°C.

Pull-down assays

The Halo tagged rubella viral proteins were prepared by the same means as described above. To perform pull-down assay, the supernatant of the purified bait proteins coated magnetic beads was removed with the magnet. The resulting bait protein-beads were incubated with 25 µL of the C-terminal Halo tagged rubella viral proteins overnight at 4°C in the Eppendorf Thermomixer® R mixer incubator at 1400 rpm. After washing three times with PPI wash buffer, the detection was performed by adding 25 µL of Alex660 labeled Halo-ligand (6 µM) and incubating for 2 hrs at 4°C. Lastly, the resulting beads were washed three times with PBST and the supernatant was removed with the magnet.

To dissociate the query and bait proteins from the beads, 20 µL 1×SDS loading buffer containing 10% 2-mercaptoethanol was added to the beads, and incubated for 5 min at 95°C. The dissociated proteins were ran on a 4-15% Tris-Criterion™ Precast Gel (Bio-rad, Hercules, CA) at 200 V for 35 min. After rinsing three times with diH_2O, the protein gels were scanned in an Amersham Bioscience Typhoon 9400 variable mode imager at 635 nm. All image adjustments were performed using Adobe® Photoshop® CS4 software (Adobe Systems, San Jose, CA). Subsequent to in-gel fluorescence scanning, the bait protein with C-terminal GST tag in the same gel was examined with

western blot using mouse anti-GST antibody and HRP conjugated sheep anti-mouse IgG antibody.

Supplementary figures

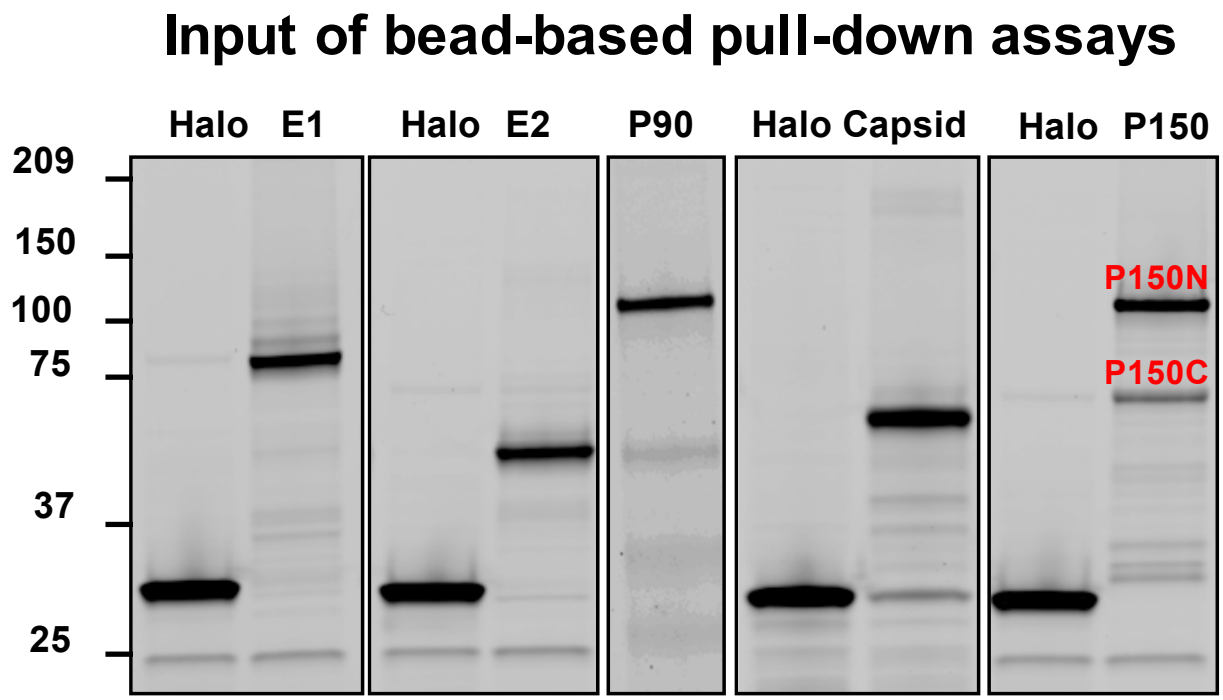

Figure S1 The input of Halo and rubella viral proteins used for the bead-based pull-down assays. Each protein has a C-terminal Halo tag and was detected using Alexa660 labeled Halo ligand.

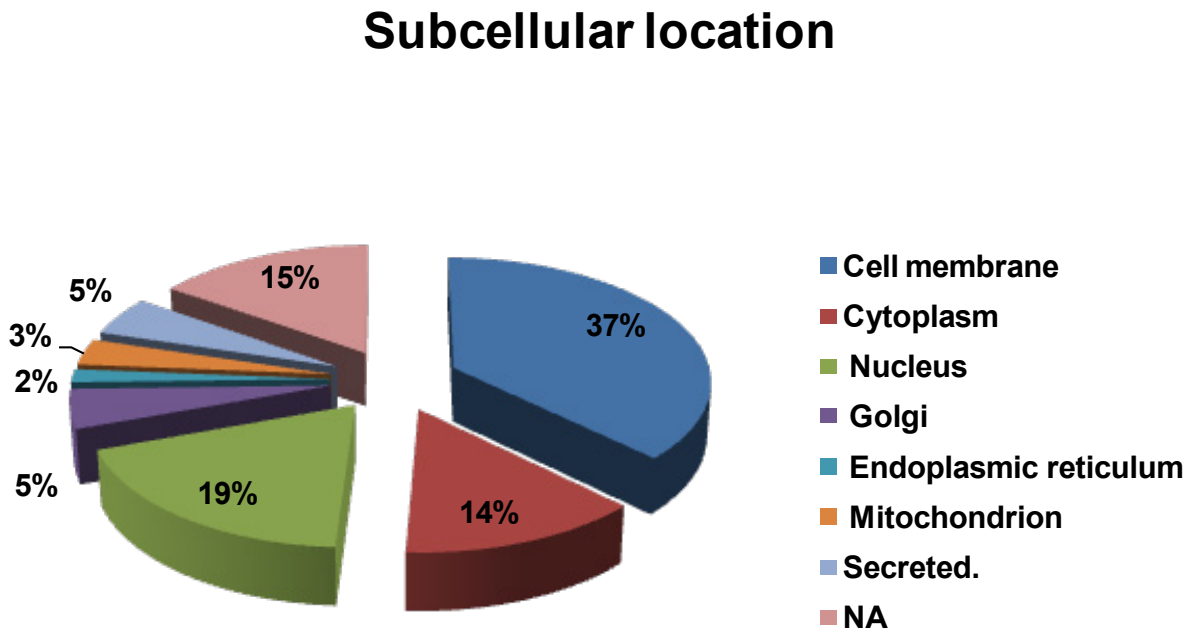

Figure S2 The subcellular location of host target candidates for rubella virus. The annotation was performed using UniProt (Universal Protein Resource) database (www.uniprot.org/).

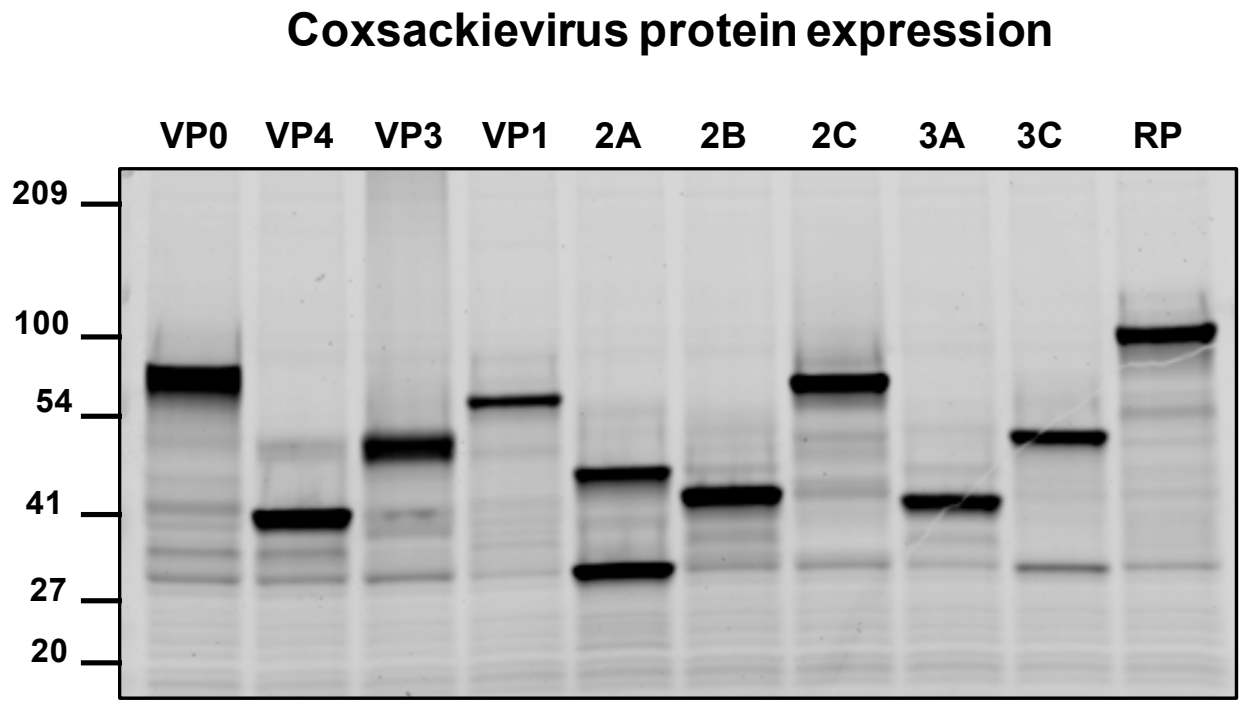

Figure S3 Examination of coxsackievirus protein expression using in-gel fluorescence. Each protein has an N-terminal Halo tag and was detected using Alexa660 labeled Halo ligand.

Supplementary Tables

Table S1 Visualized inspection of HCMV ORFeome expression using western blot analysis.

No.	Gene	Class	Protein	Visualized inspection
1	UL104	capsid	capsid portal protein	No
2	UL46	capsid	capsid triplex subunit 1	Yes
3	UL48A	capsid	small capsid protein	Yes
4	UL80	capsid	capsid maturation protease	Yes
5	UL80.5	capsid	capsid scaffold protein	Yes
6	UL85	capsid	capsid triplex subunit 2	Yes
7	UL86	capsid	major capsid protein	Yes
8	UL49	core	protein UL49	Yes
9	UL76	core	nuclear protein UL24	Yes
10	UL79	core	protein UL79	Yes
11	UL92	core	protein UL92	Yes
12	UL95	core	protein UL95	Yes
13	RL10	envelop	envelope glycoprotein RL10	Yes
14	UL100	envelop	envelope glycoprotein M	Yes
15	UL115	envelop	envelope glycoprotein L	Yes
16	UL128	envelop	envelope protein UL128	Yes
17	UL130	envelop	envelope glycoprotein UL130	No
18	UL131A	envelop	envelope protein UL131A	Yes
19	UL132	envelop	envelope glycoprotein UL132	Yes
20	UL33	envelop	envelope glycoprotein UL33	Yes
21	UL37	envelop	envelope glycoprotein UL37	Yes
22	UL4	envelop	envelope glycoprotein UL4	Yes
23	UL55	envelop	envelope glycoprotein B	Yes
24	UL73	envelop	envelope glycoprotein N	Yes
25	UL74	envelop	envelope glycoprotein O	Yes
26	UL74A	envelop	envelope glycoprotein 24	Yes
27	UL75	envelop	envelope glycoprotein H	Yes
28	UL78	envelop	envelope protein UL78	Yes
29	US27	envelop	envelope glycoprotein US27	Yes
30	US28	envelop	envelope protein US28	Yes
31	RL11	membrane	membrane glycoprotein RL11	Yes
32	RL13	membrane	membrane protein RL13	Yes
33	UL1	membrane	membrane protein UL1	Yes
34	UL10	membrane	membrane protein UL10	Yes
35	UL11	membrane	membrane glycoprotein UL11	Yes
36	UL119	membrane	membrane glycoprotein UL119	Yes
37	UL120	membrane	membrane protein UL120	Yes
38	UL121	membrane	membrane protein UL121	Yes
39	UL124	membrane	membrane protein UL124	Yes
40	UL133	membrane	protein UL133	Yes
41	UL136	membrane	protein UL136	Yes

42	UL138	membrane	protein UL138	Yes
43	UL139	membrane	membrane glycoprotein UL139	Yes
44	UL14	membrane	membrane protein UL14	Yes
45	UL140	membrane	protein UL140	Yes
46	UL141	membrane	membrane glycoprotein UL141	Yes
47	UL142	membrane	membrane glycoprotein UL142	No
48	UL144	membrane	membrane glycoprotein UL144	Yes
49	UL147A	membrane	membrane protein UL147A	Yes
50	UL148	membrane	membrane protein UL148	Yes
51	UL148A	membrane	protein UL148A	Yes
52	UL148B	membrane	protein UL148B	Yes
53	UL148C	membrane	protein UL148C	Yes
54	UL148D	membrane	protein UL148D	No
55	UL15A	membrane	protein UL15A	Yes
56	UL16	membrane	membrane glycoprotein UL16	Yes
57	UL16	membrane	membrane glycoprotein UL16	Yes
58	UL18	membrane	membrane glycoprotein UL18	Yes
59	UL2	membrane	protein UL2	Yes
60	UL20	membrane	membrane protein UL20	Yes
61	UL40	membrane	membrane glycoprotein UL40	Yes
62	UL41A	membrane	protein UL41A	Yes
63	UL42	membrane	protein UL42	Yes
64	UL5	membrane	protein UL5	Yes
65	UL50	membrane	nuclear egress membrane protein	Yes
66	UL6	membrane	membrane protein UL6	Yes
67	UL7	membrane	membrane protein UL7	Yes
68	UL8	membrane	protein UL8	Yes
69	UL9	membrane	membrane glycoprotein UL9	Yes
70	US10	membrane	membrane glycoprotein US10	Yes
71	US11	membrane	membrane glycoprotein US11	Yes
72	US12	membrane	membrane protein US12	Yes
73	US13	membrane	membrane protein US13	Yes
74	US14	membrane	membrane protein US14	Yes
75	US15	membrane	membrane protein US15	Yes
76	US16	membrane	membrane protein US16	Yes
77	US17	membrane	membrane protein US17	Yes
78	US18	membrane	membrane protein US18	Yes
79	US19	membrane	membrane protein US19	Yes
80	US2	membrane	membrane glycoprotein US2	Yes
81	US20	membrane	membrane protein US20	Yes
82	US21	membrane	membrane protein US21	Yes
83	US29	membrane	protein US29	Yes
84	US3	membrane	membrane glycoprotein US3	Yes
85	US30	membrane	membrane protein US30	Yes
86	US34A	membrane	protein US34A	Yes
87	US6	membrane	membrane glycoprotein US6	Yes
88	US7	membrane	membrane glycoprotein US7	Yes
89	US8	membrane	membrane glycoprotein US8	Yes
90	US9	membrane	membrane glycoprotein US9	Yes

91	RL1	other	protein RL1	Yes
92	RL5A	other	protein RL5A	Yes
93	RL6	other	protein RL6	Yes
94	UL102	other	helicase-primase subunit	Yes
95	UL105	other	helicase-primase helicase subunit	Yes
96	UL112	other	protein UL112	Yes
97	UL114	other	uracil-DNA glycosylase	Yes
98	UL117	other	protein UL117	Yes
99	UL122	other	regulatory protein IE2	Yes
100	UL123	other	regulatory protein IE1	Yes
101	UL145	other	protein UL145	Yes
102	UL154	other	protein UL154	Yes
103	UL17	other	protein UL17	Yes
104	UL19	other	protein UL19	Yes
105	UL21A	other	protein UL21A	Yes
106	UL27	other	protein UL27	Yes
107	UL29	other	protein UL29	No
108	UL30	other	protein UL30	Yes
109	UL31	other	protein UL31	Yes
110	UL34	other	protein UL34	Yes
111	UL38	other	protein UL38	Yes
112	UL44	other	DNA polymerase processivity subunit	Yes
113	UL51	other	DNA packaging protein UL33	Yes
114	UL52	other	DNA packaging protein UL32	Yes
115	UL53	other	nuclear egress lamina protein	Yes
116	UL54	other	DNA polymerase catalytic subunit	Yes
117	UL56	other	DNA packaging terminase subunit 2	Yes
118	UL57	other	single-stranded DNA-binding protein	Yes
119	UL69	other	multifunctional expression regulator	Yes
120	UL70	other	helicase-primase primase subunit	Yes
121	UL72	other	deoxyuridine triphosphatase	Yes
122	UL84	other	protein UL84	Yes
123	UL87	other	protein UL87	Yes
124	UL89	other	DNA packaging terminase subunit 1	Yes
125	UL91	other	protein UL91	Yes
126	UL98	other	deoxyribonuclease	Yes
127	US1	other	protein US1	Yes
128	US23	other	protein US23	Yes
129	US26	other	protein US26	Yes
130	US31	other	protein US31	Yes
131	US32	other	protein US32	Yes
132	UL111A	secreted	interleukin-10	Yes
133	UL116	secreted	protein UL116	Yes
134	UL13	secreted	protein UL13	Yes
135	UL135	secreted	protein UL135	Yes
136	UL146	secreted	chemokine vCXCL1	Yes
137	UL147	secreted	chemokine vCXCL2	Yes
138	UL150	secreted	protein UL150	Yes
139	UL22A	secreted	glycoprotein UL22A	No

140	US34	secreted	protein US34	Yes
141	TRS1	tegument	tegument protein TRS1	Yes
142	UL103	tegument	tegument protein UL7	Yes
143	IRS1	tegument	tegument protein IRS1	Yes
144	UL23	tegument	tegument protein UL23	Yes
145	UL24	tegument	tegument protein UL24	Yes
146	UL25	tegument	tegument protein UL25	Yes
147	UL26	tegument	tegument protein UL26	Yes
148	UL32	tegument	tegument protein pp150	Yes
149	UL35	tegument	tegument protein UL35	Yes
150	UL36	tegument	tegument protein vICA	Yes
151	UL43	tegument	tegument protein UL43	Yes
152	UL45	tegument	ribonucleotide reductase subunit 1	Yes
153	UL47	tegument	tegument protein UL37	Yes
154	UL48	tegument	large tegument protein	Yes
155	UL71	tegument	tegument protein UL51	Yes
156	UL77	tegument	DNA packaging tegument protein UL25	Yes
157	UL82	tegument	tegument protein pp71	Yes
158	UL83	tegument	tegument protein pp65	Yes
159	UL88	tegument	tegument protein UL88	Yes
160	UL93	tegument	DNA packaging tegument protein UL17	Yes
161	UL96	tegument	tegument protein UL14	Yes
162	UL97	tegument	tegument serine/threonine protein kinase	Yes
163	UL99	tegument	myristylated tegument protein	Yes
164	US22	tegument	tegument protein US22	Yes
165	US24	tegument	tegument protein US24	Yes