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Supplemental information

Compartmentalization-aided interaction

screening reveals extensive high-order

complexes within the SARS-CoV-2 proteome

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FigureS1. Characterization of scaffold candidates compartmentalizing and validating the direct interaction between p53 and MDM2 (Related to

Figure1)

(A-G) FRAP analysis of scaffold candidates fused with GFP-tag in HEK293 cells. Scale bar, 5 µm. All assays were performed in triplicates.

(H) Histogram of the circularity of compartments formed by testing seven protein scaffolds in cells. n (Nup98N) = 100; n (DDX4GFP) = 182; n (FUSN) = 73; n (TAF15N) = 179; n (FIB1N) = 58; n (TDP-43C) = 60; n (RBM14LCD (N)) = 120. Each data point was determined by three independent assays, and error bars represent standard deviations. Values are means \pm standard deviation.

(I) Histogram of the area ratio of phase-separated puncta to the nucleus, indicating the phase separation capability of the testing scaffolds. n = 6. Each data point was determined by three independent assays, and error bars represent standard deviations. Values are means \pm standard deviation. (J) FRAP analysis of DDX4GFP-MDM2 in HEK293 cells. Scale bars, 5 µm. All assays were performed in triplicates.

(K and L) Validation of the direct interaction between Hfq-GFP-FUSN-MDM2 and mCherry-p53 using CoPIC. The p53 fusion protein, as indicated by the mCherry signal, was recruited to the green compartment of Hfq-GFP-FUSN-MDM2 by the specific interaction (K). The co-expression of Hfq-GFP-FUSN and mCherry-p53 served as the control (L). Scale bars, 5 µm. All assays were performed in triplicates. (M) Plots of Pearson's correlation coefficient for the intensity of and mCherry-p53 versus GFP-Nup98N-MDM2 pre-treatment and after 46 s. Each data point was determined by three independent assays.



Figure S2. CoPIC analysis of the interaction patterns of SUZ12 with the binding factors and EZH2-mediated indirect interaction of SUZ12 with

EED (Related to Figure 2)

(A) Schematic diagram depicting the architecture of the PRC2 complex.

(B) CoPIC analysis of pair-wise interactions between GFP-Nup98N fused SUZ12 (full-length SUZ12) or SUZ12C (C-terminal region of SUZ12)

and mCherry-fused binding factors (EZH2, RBBP7, PHF1 and AEBP2). Scale bar, 5 µm. All assays were performed in triplicates.

(C) CoPIC analysis of the indirect interaction of SUZ12 with EED mediated by EZH2. Scale bars, 5 µm. All assays were performed in triplicates.



Anti-mCherry

Figure S3. Genome annotation and subcellular localization of SARS-CoV-2 RTC-related factors (Related to Table 1 and Figure 3)

(A) SARS-CoV-2 genome annotation on replication/transcription-related factors.

(B) Subcellular localization of SARS-CoV-2 Nsps and N protein used in CoPIC screening. Scale bar, 5 µm.

(**C**) Western blot validation of the constructs fused with an mCherry tag expressed in Vero E6 cells. The cell lysates were extracted and examined with the specific antibody against mCherry.

Merge	Nsp5	Nsp5		Merge	Nsp7	Nsp7	DAPI	Merge	Nsp8	Nsp8	
Merge	Nsp13	Nsp13	DAPI	Merge	Nsp16	Nsp16	DAPI —	Merge	N		DAPI

B

Mèrge	Nsp5	Nsp9		Merge	Nsp5	Nsp16	DAPI	Merge	Nsp9	Nsp12	DAPI
Merge	Nsp8	Nsp12	DAPI	Merge	Nsp16	Nsp5	DAPI	Merge	Nsp12	Nsp9	
Merge	Nsp9	Nsp13	DAPI	Merge	Nsp9	Nsp16	DAPI	Merge	Nsp10	Nsp14	DAPI
Merge	Nsp13	Nsp9 64 5	DAPI	Merge	Nsp16	Nsp9	DAPI	Merge	Nsp14	Nsp10	
Merge	Nsp10	Nsp16	DAPI	Merge	Nsp12	Nsp14	DAPI	Merge	Nsp13	Nsp14	DAPI
Merge	Nsp16	Nsp10	DAPI	Merge	Nsp14	Nsp12	DAPI —	Merge	Nsp14	Nsp13	
Merge	Nsp13	Nsp16	DAPI =	Merge	Nsp14	Nsp15		Merge	Nsp14	Nsp16 A Constant States and States and A	DAPI
Merge	Nsp16	Nsp13	DAPI	Merge	Nsp15	Nsp14	DAPI	Merge	Nsp16	Nsp14	DAPI

Figure S4. Positive pairwise (self- and bidirectional-) interactions of SARS-CoV-2 identified by CoPIC screening (Related to Figures 3, 4 and 5)

(A) Collections of positive self-interactions identified by CoPIC screening. All assays were performed in triplicates. Scale bar, 5 µm.

(B) Collections of positive bidirectional interactions identified by CoPIC screening. All assays were performed in triplicates. Scale bar, 5 µm.

Merge	Nsp5	Nsp11	DAPI	Merge	Nsp5	Nsp14	DAPI	Merge	Nsp5	Nsp15	DAPI
Merge	Nsp5	• N •	DAPI	Merge	Nsp7	Nsp11	DAPI	Merge	Nsp9	Nsp11	DAPI
Merge	Nsp9	Nsp15	DAPI	Merge	Nsp9	N	DAPI	Merge	Nsp15	Nsp10	DAPI
Merge	Nsp9	Nsp8	DAPI	Merge	Nsp12	Nsp11		Merge	Nsp12	N	DAPI
Merge	Nsp13	Nsp7		Merge	Nsp13	Nsp8	DAPI	Merge	Nsp14	Nsp9 	DAPI
Merge	Nsp16	Nsp15	DAPI	Merge	Nsp13	Nsp12		Merge	Nsp13	Nsp15	DAPI
Merge	Nsp14	Nsp11	DAPI	Merge	Nsp16	Nsp11	DAPI	Merge	Nsp16		DAPI
B Antibodies	Nsp5 + Nsp5 Lysate Input Co-IP GFP-Nsp5 - + + - + + mCherry-Nsp5 + - + + - + Anti-GFP Anti-mCherry Anti-mCherry			Nsp7 + Nsp7 Lysate Input Co-IP GFP-Nsp7 - + + - + + mCherry-Nsp7 + - + + - + Anti-GFP Anti-GFP Anti-mCherry Anti-mCherry			Nsp8 + Nsp8 Lysate Input Co-IP GFP-Nsp8 - + + - + + mCherry-Nsp8 + - + + - + Anti-GFP Anti-GFP Anti-mCherry Anti-mCherry Anti-mCherry				
Antibodies	GFP-N GFP-N MCherry-N Anti-GFP Anti-mCherry	+ N Lysate Input - + + + - +	Co-IP - + + + - + Anti-mCherry	mCh Antibodies An	Nsp8 + 1 GFP-Nsp8 herry-Nsp13 Anti-GFP	Nsp13 Lysate Input - + + + - +	Co-IP - + + + - + Anti-mCherry	mCh Antibodies An	Nsp14 + N GFP-Nsp16 - erry-Nsp14 + Anti-GFP - nti-mCherry -	Nsp16 ysate Input + + - +	Co-IP - + + + - + Anti-mCherry

Figure S5. Positive pairwise (unidirectional-) interactions of SARS-CoV-2 identified by CoPIC screening (Related to Figures 3, 4 and 5)

(A) Collections of positive unidirectional interactions identified by CoPIC screening. All assays were performed in triplicates. Scale bar, 5 µm.

(B) Co-IP analysis of selected pairwise interactions identified by CoPIC screening.

3	Merge	Nsp13	Nsp7	Nsp14
4	Merge	Nsp16	Nsp7	Nsp14
9	Merge ***	Nsp15	Nsp8	Nsp9
3	Merge	Nsp14	Nsp8	Nsp13
3 N	Merge	Nsp13	Nsp8	N _
15	Merge	Nsp10	Nsp9 ∘	Nsp15
2	Merge	Nsp12	Nsp16	Nsp9
2	Merge	Nsp12	Nsp10	Nsp14
3	Merge	Nsp12	Nsp15	Nsp13
4	Merge	Nsp12	Nsp16	Nsp14 —

Nsp7

Nsp16

Nsp8

Nsp8

Nsp8

Nsp9

Nsp15

Nsp10

Ν

Nsp16

0

Nsp9

Nsp7

Nsp9

Nsp12

Nsp16

Nsp14

Nsp9

Nsp13

Nsp16

Nsp13

7

7

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8

8

9

9

10-

12

12

Figure S6. Collections of positive tertiary complexes identified by CoPIC screening (Related to Figure 6)

Positive tertiary interactions identified by CoPIC screening. All assays were performed in triplicates. Scale bar, 5 µm.

Figure S7. Characterization of mutual interactions among Nsp7, Nsp8, and Nsp12 (Related to Figure 6)

(A) CoPIC analysis of binary and tertiary interactions among Nsp7, Nsp8, and NSp12. All assays were performed in triplicates. Scale bar, 5 µm.

(B) CoIP validation of pairwise interactions between Nsp7 and Nsp8, Nsp7 and Nsp12, Nsp8 and Nsp12. All assays were performed in triplicates.

(C) CoIP analysis of Nprotein and binding candidates under SARS-CoV-2 GFP/ N trVLP infection in Caco-2-N^{Flag} cells.

Figure S8. Higher-order complexes deduced from CoPIC analysis (Related to Figure 7)

- (A) List of quaternary complexes deduced from CoPIC analysis.
- (B) List of quinary complexes deduced from CoPIC analysis.
- (C) List of six-membered complexes deduced from CoPIC analysis.
- (D) List of seven-membered complexes deduced from CoPIC analysis.

Protein	Accession Number	Nucleotide positions	Position in polyprotein	Protein length (aa)
Nsp1	YP_009725297.1	266-805	M1-G180	180
Nsp2	YP_009725298.1	806-2719	A181-G818	638
Nsp5	YP_009725301.1	10055-10972	S3264-Q3569	306
Nsp7	YP_009725303.1	11843-12091	S3860-Q3942	83
Nsp8	YP_009725304.1	12092-12685	A3943-Q4140	198
Nsp9	YP_009725305.1	12686-13024	N4141-Q4253	113
Nsp10	YP_009725306.1	13025-13441	A4254-Q4392	139
Nsp11	YP_009725312.1	13442-13480	S4393-V4405	13
Nsp12	YP_009725307.1	12480-16236	V4406-Q5324	919
Nsp13	YP_009725308.1	16237-18039	A5325-Q5925	601
Nsp14	YP_009725309.1	18040-19620	A5926-6452	527
Nsp15	YP_009725310.1	19621-20658	S6453-Q6798	346
Nsp16	YP_009725311.1	20659-21555	S6799-N7096	298
N protein	YP_009724397.2	28274-29533		419

Table S1 Sequence information for RTC-related viral proteins of SARS-CoV-2 used in the study for plasmid construction.(Related to Figure 3)

Number	Positive interactions	References
1	Nsp2-Nsp2	(von Brunn et al., 2007)
2	Nsp2-Nsp7	(Pan et al., 2008)
3	Nsp2-Nsp8	(von Brunn et al., 2007)
4	Nsp2-Nsp11	(von Brunn et al., 2007)
5	Nsp2-Nsp15	(Pan et al., 2008)
6	Nsp2-Nsp16	(von Brunn et al., 2007)
7	Nsp5-Nsp5	(von Brunn et al., 2007)
8	Nsp5-Nsp7	(von Brunn et al., 2007)
9	Nsp5-Nsp8	(von Brunn et al., 2007)
10	Nsp5-Nsp12	(Pan et al., 2008)
11	Nsp5-Nsp14	(Pan et al., 2008)
12	Nsp7-Nsp7	(von Brunn et al., 2007)
13	Nsp7-Nsp8	(von Brunn et al., 2007)
14	Nsp7-Nsp9	(von Brunn et al., 2007)
15	Nsp7-Nsp13	(von Brunn et al., 2007)
16	Nsp8-Nsp8	(von Brunn et al., 2007)
17	Nsp8-Nsp9	(von Brunn et al., 2007)
18	Nsp8-Nsp12	(von Brunn et al., 2007)
19	Nsp8-Nsp13	(von Brunn et al., 2007)
20	Nsp8-Nsp14	(von Brunn et al., 2007)
21	Nsp9-Nsp9	(Sutton et al., 2004)
22	Nsp10-Nsp10	(Su et al., 2006)
23	Nsp10-Nsp14	(Pan et al., 2008)
24	Nsp10-Nsp16	(Pan et al., 2008)
25	Nsp12-Nsp13	(von Brunn et al., 2007)
26	Nsp13-Nsp13	(von Brunn et al., 2007)
27	Nsp15-Nsp15	(Joseph et al., 2007)
28	N protein-N protein	(Chen et al., 2007)

Table S2 Collections of reported interaction patterns of SARS-CoV. (Related to Figure 4)