1 # Supplementary materials and methods

2 ## Cells and virus

3 HEK293 (ATCC, CRL-1573) and Vero E6 cells (ATCC, CRL-1586) were maintained
4 in Eagle's minimum essential medium supplemented with 10% fetal bovine serum
5 (FBS) at 37 °C in a humidified atmosphere with 5% CO_2. HEK293T (ATCC,
6 CRL-11268) cells were cultured in Dulbecco's modified Eagle's medium.
7 HEK293-ACE2 cell line was generated by transduction of lentiviral vector expressing
8 ACE2 and subsequent monoclonal screening as described below. SARS-CoV-2
9 (nCoV-2019BetaCoV/Wuhan/WIV04/2019) was isolated from the clinical sample [1].
10 The propagation and titer determination of SARS-CoV-2 were performed in Vero E6
11 cells in a biosafety level-3 (BSL-3) laboratory of National Biosafety Laboratory,
12 Wuhan, Chinese Academy of Sciences [1].
13

14 ## Plasmids

15 Open reading frame (ORF) encoding NSP13 was amplified by RT-PCR from
16 SARS-CoV-2 genomic RNA and cloned into the pCAGGSP7 vector without or with
17 HA or S tag (Stag) [2-4]. Truncated NSP13 and SOCS1 expression plasmids were
18 constructed into pCAGGSP7 vector with C-terminal HA tag by standard molecular
19 biology methods. S or HA-tagged STAT1 expression plasmids were constructed as
20 previously described [3, 5-7]. Human angiotensin I-converting enzyme-2 (ACE2)
21 expression plasmid (pLVX-IRES-PURO-ACE2) was generated by cloning the ORF
22 sequence of ACE2 into lentiviral vector pLVX-IRES-PURO. Luciferase reporter
23 plasmids for type I, II, or III IFN-responsive promoters and the *Renilla* luciferase
24 control plasmid (pRL-TK) were kindly provided by Prof. Hong-Bing Shu (Wuhan
25 University) and were described previously [3, 6, 8-11].
26

27 ## Antibodies and Reagents

28 Primary antibodies include mouse antibody to HA tag (Sigma-Aldrich, 05–904) and
29 rabbit antibodies to S tag (Abcam, ab18588), β-actin (ABclonal, AC026), NSP13

30 (AtaGenix, ATP238), STAT1 (Abcam, ab109320), pSTAT1 (Cell Signaling
31 Technology, 9167S), IRAK1 (proteintech, 10478-2-AP), HAX1 (proteintech,
32 11266-1-AP), DNAJA2 (ABclonal, A7059), HSPD1 (ABclonal, A0564), CCT4
33 (ABclonal, A6548), CCT8 (ABclonal, A4449), and TCP1 (ABclonal, A1950). For
34 Immunofluorescence assay (IFA), secondary antibodies include Alexa Fluor
35 647-conjugated goat anti-rabbit IgG (H+L) (Abcam, ab150079) and Alexa Fluor
36 488-conjugated goat anti-mouse IgG (H+L) (Abcam, ab150113). For WB analysis,
37 secondary antibodies include horseradish peroxidase (HRP)-conjugated goat
38 anti-mouse or anti-rabbit IgG antibodies (Abcam, ab6789 and ab6721). Recombinant
39 human IFN-α (300-02AA), IFN-γ (300-02), and IFN-λ (300-02K) were all purchased
40 from Peprotech.
41

42 **Real-time qPCR**

43 Total RNA was extracted from the treated HEK293T cells with TRIzol reagent (NZK
44 biotech, China). Reverse transcription of RNA was performed with a cDNA reverse
45 transcription kit (TaKaRa, RR047A), followed by real-time quantitative PCR with an
46 SYBR Green real-time PCR kit (TaKaRa, RR820A). The relative mRNA levels of the
47 indicated genes were calculated using the $2^{-\Delta\Delta CT}$ method with *GAPDH* mRNA levels
48 as an internal control. Primers used have been described in previous studies [12-15].
49

50 **Dual-luciferase reporter (DLR) gene assay**

51 HEK293T cells cultured in 96-well plates were cotransfected with 80 ng NSP13
52 expression plasmid or the control vector, together with 20 ng ISRE (for type I and III
53 IFN signaling analyses) or GAF reporter plasmid (for type II IFN signaling analysis)
54 and 0.5 ng pRL-TK, using lipofectamine 3000 (Invitrogen) by following the
55 manufacturer’s instructions. At 24 h posttransfection, cells were mock stimulated or
56 stimulated with IFN-α (type I IFN; 2000 u/ml), IFN-γ (type II IFN; 100 ng/ml), or
57 IFN-λ (type III IFN; 200 ng/ml) for 16 h before measurements of luciferase activities
58 with a DLR assay kit (Promega, E2940). The relative luciferase activities (Rel. Luc.
59 Act) were exhibited by normalizing firefly luciferase activity to *Renilla* luciferase

60 activity.

61

62 **Generation of HEK293-ACE2 cell line**

63 HEK293T cells were co-transfected with the human ACE2 expression plasmid
64 pLVX-IRES-PURO-ACE2 and packaging plasmids provided by Prof. Xinwen Chen
65 (Wuhan Institute of Virology) for 48 h [14, 16]. Packaged lentiviral particles were
66 then harvested from culture medium and used for transduction of HEK293 cells,
67 followed by 3-day selection with 2 μg/mL puromycin. Then, cells were diluted and
68 seeded into 96-well plates for further screening of single colony stably
69 over-expressing ACE2.

70

71 **Protein interaction, silver staining, and WB analyses**

72 S-pulldown assays utilizing the high-specific and strong affinity of S tag with
73 S-protein coupled on agarose beads were performed as described previously to
74 analyze protein interactions [3, 5, 14, 17-19]. In cotransfection assays before
75 S-pulldown, HEK293T cells were transfected with the S-tagged protein expression
76 plasmids together with the plasmids encoding other indicated proteins for 36 h. For
77 protein interaction analysis in the contexts of infection combined with transfection,
78 HEK293-ACE2 cells were firstly transfected with the plasmid encoding STAT1-Stag
79 or the control vector and at 12 h posttransfection, the cells were infected with
80 SARS-CoV-2 (MOI = 1) for 24 h. Then, the transfected or SARS-CoV-2-infected
81 cells expressing S-tagged proteins were lysed with the immunoprecipitation lysis
82 buffer (Beyotime) and after centrifugation, supernatants of cell lysates were mixed
83 with S-protein agarose (Millipore) by rotating incubation. The beads were next
84 washed with the lysis buffer and phosphate-buffered saline (PBS) successively,
85 followed by silver staining, WB, or quantitative MS analyses of the bound protein
86 samples. For silver staining analysis, protein samples were eluted with 1×SDS
87 sample buffer by boiling for 10min and subjected to 10% SDS polyacrylamide gel
88 electrophoresis (SDS-PAGE). Then, the gel was fixed, sensitized, and stained by
89 Pierce Silver Stain Kit (Thermo Fisher Scientific) according to the manufacturer's

instructions. For WB analysis, protein samples separated by SDS-PAGE were transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% BSA in Tris-buffered saline-Tween 20 (TBS-T), the membranes were incubated successively with primary antibodies and HRP-conjugated secondary antibodies. Protein bands were detected with an enhanced chemiluminescence kit (Thermo Fisher Scientific). In nuclease treatment assays, cell lysates (supplemented with 2 mM MgCl2) were divided into halves and then subjected to excessive treatment with Benzonase (100 U, Millipore, Cat#70746) or left untreated at 4˚C for 4 h before S-pulldown as described previously [14].

Quantitative MS analysis

For MS quantification analysis, bead-bound proteins (in total of 6 samples from three biological replicates for both NSP13 pulldown and control groups) were denatured, reduced, alkylated, and eluted by one-step treatment in the reaction buffer (1% SDC/100 mM Tris-HCl, pH 8.5/10 mM TCEP/40 mM CAA) at 95 °C for 10 min. The eluates were diluted with equal volume of H_2O and then subjected to trypsin digestion at 37 °C overnight. Obtained peptides were desalted for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis using a Q Exactive HF-X system coupled with Easy-nLC 1200 (Thermo Fisher Scientific) [20]. MS raw tandem spectra were analyzed with MaxQuant (V1.6.6) (AB SCIEX) using the Andromeda database search algorithm [21]. Spectra files were searched against the Human Unified Protein Database (UniProt) [22]. The main parameters were as follows: LFQ mode was checked for quantification; Variable modifications, Oxidation (M) & Acetyl (Protein N-term); Fixed modifications, Carbamidomethyl (C); Digestion, Trypsin/P; Match between runs was used for identification transfer. Search results were filtered with 1% FDR at both protein and peptide levels. Significantly changed proteins were screened out according to the fold changes and p-values of the proteins. In this study, criteria with a p-value < 0.05 and fold change > 2 were taken for selection of NSP13-interacting proteins.

Bioinformatic analysis of Gene Ontology (GO) and protein-protein interaction (PPI) network and data mining of druggable targets and host-directing drugs

STRING database (Version 11.0) was used to evaluate the potential interactions of the identified cellular proteins and to group proteins into similar functional classes [23]. The protein-protein interaction (PPI) network was then visualized using Cytoscape software (Version 3.8) [24]. By STRING analysis, 830 interactions (edges) are represented in the protein-protein interaction network of the present study. Gene ontology (GO) analysis was used to determine the cellular component (CC), molecular function (MF) and biological process (BP) attributes of the identified NSP13-interacting cellular proteins by using the enricher function of clusterProfiler package in R with default parameters [25]. Significant GO terms (adjusted $p < 0.05$) were identified and further refined by the simplify method (cutoff = 0.6, by="*p*.adjust") to cut redundant gene sets.

Cellular NSP13-interacting proteins may positively or negatively regulate NSP13 function (hence bolstering or inhibiting viral infection), or in turn, may be affected by NSP13, leading to viral fitness or cellular dysfunction. Thus, the NSP13-cell interaction interface could be a promising target to intervene viral infection and pathogenesis. To explore potential druggable targets, the identified NSP13-interacting proteins were searched against DrugBank database [26]. Of the 172 cellular proteins interacting with NSP13 with high significance, 45 were returned with 95 targeting drugs (50 approved, 33 experimental, 10 investigational, and 2 nutraceutical). The cellular target-drugs network was then visualized with Cytoscape software [24].

Immunofluorescence and confocal microscopy

Cells were fixed with 4% paraformaldehyde in PBS for 10 min and permeabilized with 0.5% Triton X-100 in PBS. After blocking with 2.5% BSA and 2.5% normal goat serum in PBS, cells were incubated with primary antibodies overnight at 4 °C and then stained with secondary antibodies for 1 h at room temperature (RT). To visualize the nuclei, cells were treated with Hoechst 33258 (Beyotime) for 10 min at RT. Image acquisition and analyses were performed with a Nikon Ti confocal microscopy with

150 the Volocity software (PerkinElmer).

151

152 **Statistical analysis**

153 Statistical analyses were performed by GraphPad Prism 8 using unpaired two-tailed *t*
154 test or Welch's *t* test. Data were expressed as mean ± SD, $n = 3$ or 4 biological
155 replicates as indicated. $p < 0.05$ was considered statistically significant.

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