Analysis of viral RNA-host protein interactomes enables rapid antiviral drug discovery

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24 **Abstract**

25 **RNA viruses including SARS-CoV-2, Ebola virus (EBOV), and Zika virus (ZIKV)**
 26 **constitute a major threat to global public health and society. The interactions**
 27 **between viral genomes and host proteins are essential in the life cycle of RNA**
 28 **viruses and thus provide targets for drug development. However, viral RNA-host**
 29 **protein interactions have remained poorly characterized. Here we applied**
 30 **ChIRP-MS to profile the interactomes of human proteins and the RNA genomes**
 31 **of SARS-CoV-2, EBOV, and ZIKV in infected cells. Integrated interactome**
 32 **analyses revealed interaction patterns that reflect both common and**
 33 **virus-specific host responses, and enabled rapid drug screening to target the**
 34 **vulnerable host factors. We identified Enasidenib as a SARS-CoV-2 specific**
 35 **antiviral agent, and Trifluoperazine and Cepharanthine as broad spectrum**
 36 **antivirals against all three RNA viruses.**

37

38 **One Sentence Summary:**

39 Interactome analyses of host proteins and the SARS-CoV-2, EBOV, and ZIKV RNA
 40 genomes unveil viral biology and drug targets.

41

42 **Introduction**

43 It is notable that three of the most dangerous infectious diseases of recent times are all
 44 caused by RNA viruses: SARS-CoV-2, ZIKV and EBOV. The SARS-CoV-2
 45 coronavirus has caused the on-going Coronavirus Disease 2019 (COVID-19) pandemic,
 46 resulting in more than 20 million infections and 800 thousand deaths and global
 47 disruption of our society and economy(1, 2). Since its outbreak in 2012, ZIKV has
 48 infected millions of people(3), and has remained on the 2018 Blueprint list of priority
 49 diseases by the World Health Organization (WHO, February, 2018). EBOV has led to
 50 epidemics in Africa and has spread to Europe and North America; Ebola virus disease
 51 is severe with an average case fatality rate of ~80% in past outbreaks(4).

52 RNA viruses typically have a relatively small genome and proteome(5), and
 53 therefore rely heavily on interactions with host factors to complete their life cycles(6).
 54 RNA binding proteins function on many aspects of cellular and viral processing, e.g.,
 55 RNA translation, stabilization, modification and localization(7, 8), as well as
 56 anti-infection response against pathogens(9). Many studies have focused
 57 characterization of viral protein-host protein interactions(10-15). In contrast, the
 58 interactions between host proteins and viral RNA (vRNA) are much less well
 59 understood, despite the known importance of the viral RNA genome for multiple
 60 processes during infection, including viral genome translation and replication(16).

61 Recent years have seen an explosion of high-throughput methods that, in
 62 combination with diverse crosslinking reagents and data processing workflows, enable
 63 a global analysis of RNA-protein interactions (“the interactome”) in cells(17, 18).
 64 These approaches can substantially advance our understanding of the infection and
 65 pathology of RNA viruses and can inform diverse and effective therapeutic options, yet
 66 they have not yet been widely deployed for comparative analyses of cells infected with
 67 diverse RNA viruses or strains.

68 Here, we unveil the vRNA-host protein interactomes of SARS-CoV-2, EBOV,

and ZIKV based on ChIRP-MS analysis of infected human host cells. Our integrated interactome analysis identified interaction patterns that reflect both common and virus-specific host responses. We applied these insights to inform a targeted antiviral drug screening workflow based on standard and/or trans-complementation viral infection assay systems that ultimately identified the FDA approved drug Enasidenib, which inhibits the SARS-CoV-2 specific interacting protein IDH2, as a potent SARS-CoV-2 specific antiviral agent. Further, we found that the heat shock protein inhibitor Cepharanthine and the cytoskeleton disruptor Trifluoperazine are broad spectrum antivirals against all three of these pathogenic RNA viruses.

Results

ChIRP-MS reveals the SARS-CoV-2 vRNA-host protein interactome

To define host proteins associated with genomic RNA of SARS-CoV-2, we used the RNA-directed proteomic discovery method ChIRP-MS (Comprehensive identification of RNA-binding proteins by mass spectrometry)(19) ([Materials and Methods](#)). Briefly, human hepatocarcinoma Huh7.5.1 cells were infected with SARS-CoV-2 and crosslinked with formaldehyde to preserve vRNA-protein complexes. Biotinylated oligonucleotides tiling SARS-CoV-2 vRNA with specificity were used to enrich vRNA-host protein complexes from cell lysates ([Table S1](#)) and the co-purified proteins were identified by mass spectrometry ([Fig. 1A](#)).

ChIRP-MS analysis of SARS-CoV-2-infected Huh7.5.1 cells identified a total of 143 human proteins with a MiST score > 0.6 ([Fig. 1B](#) and [Table S2](#)). Note that the co-purification steps of the ChIRP-MS strategy depleted highly abundant host RNAs and enabled robust recovery of over 80% of the total viral RNA in infected cells ([Fig. S1A](#)). Correlation coefficients across three biological replicates were all above 0.9 ([Fig. 1C](#)). Our ChIRP-MS dataset underscored the extensive interactions between viral RNA and the SARS-CoV-2 nucleocapsid protein (NP), the viral M protein, the spike protein

96 and several non-structural proteins (NS) (Table S2. Also see Fig. 1B), consistent with
97 previous studies showing that NP directly binds and encases viral RNA during
98 infection.

99 To validate the vRNA-interacting host proteins from the mass spectrometry based
100 identification, we assessed the ChIRP samples using western blotting (ChIRP-WB)
101 (Fig. 1A. Materials and Methods). We examined 11 candidate proteins using antibodies
102 that were available in our lab (*e.g.*, HnRNPU and PPIA with a high MiST score 1, and
103 RACK1 with a lower MiST score 0.68), and confirmed that 9 of them as SARS-CoV-2
104 vRNA interacting proteins in infected Huh7.5.1 cells (Fig. 1D). Among them,
105 ELVAL1, hnRNPU, and PTBP1 were previously revealed to bind other coronavirus
106 vRNAs that function in viral replication(20, 21). PPIA (Cyclophilin A) has been widely
107 implicated in viral infection, and drugs targeting PPIA showed broad-spectrum
108 antiviral activity(22). MATR3 may associate with SFPQ/NONO heterodimers to
109 modulate the STING pathway(23). IGF2BP1 is a well-characterized RNA binding
110 protein involved in RNA stability and viral RNA translation(24). The THO complex
111 subunit protein (ALYREF) is a component of the TREX complex that is involved in
112 RNA processing and transport(25). Offering further support that our approach yielded
113 biologically relevant candidates rather than processing-related artefacts, we noted that
114 the vRNA-interacting candidate host proteins were enriched for canonical
115 RNA-binding domains (*e.g.*, RNA recognition motif, RNA secondary structure related
116 DEAD/DEAH box helicase domain, and RNA-binding KH domain) (Fig. 1E, Fig.
117 S1B).

118 We compared our 143 SARS-CoV-2 vRNA binding proteins with host factors
119 previously reported to bind coronaviruses, and found that 14 of the host factors in our
120 ChIRP-MS interactome are known to bind the viral RNA of other coronavirus and to
121 impact viral replication(20, 21) (Fig. 1B). In particular, we compared our dataset with
122 a recently reported host interactome for SARS-CoV-2 based on the “RNA antisense
123 purification and mass spectrometry” (RAP-MS) method(26), and found 17 host factors

common to both interactome datasets (Fig. S1C). In addition, of the 143 SARS-CoV-2 host RNA binding proteins that we identified, 69 (61%, Table S2) were previously reported as responsive to SARS-CoV-2 infection in a proteomics study(27). Collectively, these findings suggest that ChIRP-MS can reliably identify vRNA-interacting host factors to thus to catalog a high-confidence SARS-CoV-2 vRNA interactome.

SARS-CoV-2 infects multiple organs, leading to diverse symptoms such as fever, shortness of breath, severe acute pneumonia, haemoptysis, diarrhea, and neurological disorders(28). Indeed, we found that many of the SARS-CoV-2 vRNA-binding proteins were more highly expressed in organs known to host SARS-CoV-2 infection, such as lung(29), intestines(30) and brain(31) (Fig. 1F-G). We also performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses and found that the annotations of the interactome host factors were enriched for GO terms related to splicing, translation, and viral transcription (Fig. 1H), as well as KEGG pathways related to the spliceosome, ribosome and RNA metabolism (Fig. 1I). The aforementioned proteomics survey also reported that host proteins responsive to SARS-CoV-2 infection are similarly enriched for annotated functions relating to translation, RNA metabolism, and RNA transport related cellular processes(27).

142

143 **High-confidence vRNA-human protein interactomes for EBOV and ZIKV**

144 Seeking to delineate which host proteins are common to different RNA viruses vs.
145 virus-specific factors, we used ChIRP-MS to uncover the vRNA-host protein
146 interactomes for the ZIKV MR766 strain and a recombinant EBOVΔVP30-GFP virus
147 with the VP30 coding sequence of the Ebola virus (strain Zaire Mayinga) replaced by
148 the GFP sequence(32) (Materials and Methods). As above, we employed biotinylated
149 oligonucleotides tiling each viral RNA with specificity to enrich for viral RNA-host
150 protein complexes from lysates of infected cells (Table S1), and identified the
151 co-purified proteins by mass spectrometry (Fig. 1A).

152 These ChIRP-MS analyses identified a total 172 viral RNA binding proteins in
 153 MR766-infected Huh7 cells (MiST score > 0.7) and 223 viral RNA binding proteins in
 154 EBOVΔVP30-GFP infected Huh7.5.1-VP30 cells (MiST score > 0.6, [Fig. 2A-B](#) and
 155 [Table S2](#)). In each analysis, the co-purification steps of the ChIRP-MS strategy allowed
 156 for the depletion of highly abundant host RNAs, and, as with SARS-CoV-2, enabled
 157 robust recovery of more than 80% of the total viral RNA in infected cells ([Fig. S2A](#)).
 158 All data showed high correlations between the experimental replicates ($r > 0.7$ and 0.9
 159 respectively for MR766 and EBOV, [Fig. 2B-C](#)).

160 We again used ChIRP-WB to verify six of the host RNA binding proteins
 161 uncovered by ChIRP-MS for both ZIKV and EBOV (IGF2BP1, ALYREF, SFPQ,
 162 MATR3, PDIA6 and SND1) and validated their interactions with viral RNAs in
 163 ZIKV-infected Huh7 cells and in EBOV infected Huh7.5.1 cells ([Fig. 2C](#)). With only
 164 one exception (interaction between SND1 and EBOV vRNA), all validation results
 165 were consistent with our ChIRP-MS results. We then examined 12 host proteins that
 166 were shown previously to interact with various ZIKV strains, 8 of these 12 host
 167 proteins passed our scoring cutoff, suggesting a very high sensitivity for our
 168 ChIRP-MS experiments ([Table S2](#)). In addition, comparisons against previously
 169 reported ChIRP-MS based studies of host proteins that interact with another ZIKV
 170 strain(17) also revealed high level of overlap ([Fig. S2D](#)). Similarly, we also examined
 171 proteins that previously reported to interact with EBOV vRNA (DHX9, hnRNPR,
 172 hnRNPL, SYNCRIP, IGF2BP1)(33), and confirmed that all these interactions are
 173 covered in our EBOV ChIRP-MS result ([Table S2](#)). Overall, these analysis and
 174 observations suggest that our ChIRP-MS reliably captured host proteins that interact
 175 with the ZIKV and EBOV vRNAs in infected cells.

176

177 **An integrated analysis of the three RNA virus host factor interactomes identifies** 178 **common and virus-specific host cellular factors and responses**

179 Our interactome datasets highlight that the three RNA viruses interact with many
180 common host proteins, but also showcase their virus-specific host interaction partners.
181 In total, 33 human proteins interact with viral RNAs of all three viruses, and 88 proteins
182 interact with viral RNAs of two of the three viruses, results suggesting the potential
183 involvement of common host factors in conserved host responses to infection by RNA
184 viruses (Fig. 2D). Note that compared to the total set of all vRNA-interacting proteins,
185 these common interacting proteins exhibit stronger enrichment for canonical
186 RNA-binding domains (Fig. 2E), and consistently showed enrichment of cellular
187 functions such as splicing, translation, and metabolism related processes (Fig. 2F).

188 To further characterize conserved host responses to RNA virus infection, we
189 examined the common interacting protein complexes (and protein associations of the
190 same biological pathways) shared by the three RNA viruses. We searched for enriched
191 protein complexes and defined a complex as common one if it was present in at least
192 two of the three virus interactomes (Fig. 3. Table S3. Materials and Methods). The
193 analysis identified many common protein complexes, including the ribosome (and
194 related translation regulators such as eIF4A), the spliceosome (and also other RNA
195 processing proteins such as YBX1 and RBMX), the IGF2BP1-associated complex, the
196 large drosha and DGCR8 complexes, the TNF- α /NF- κ B signaling complex, the
197 microtubules and the cytoskeleton, the proteasome, and many stress-granule-related
198 proteins.

199 Many of these common complexes are known to function in the regulation of host
200 cell and/or viral RNA. For example, the IGF2BP1-associated complex regulates the
201 stability and translation of various cellular RNA molecules including *c-Myc*(34) and
202 also enhances IRES-mediated translation initiation of HCV RNA(24). The large drosha
203 and DGCR8 complexes, which are involved in miRNA biogenesis, are essential for
204 degrading exogenous double-stranded RNA, including viral RNAs(35). The

205 cytoskeleton is often rearranged by viral infection and is involved in the formation of
 206 virus-induced membranous replication factories and that chemicals targeting the
 207 cytoskeleton are known to inhibit the replication of RNA virus(36). Of particular
 208 interests, host factors that function as stress-granule-related proteins(37), were
 209 repeatedly observed in the interactomes of all three viruses (Fig. 3), supporting the idea
 210 that stress granules as a general host cell response to viral infection.

211 Of equally paramount interest, we also identified many virus-specific protein
 212 complexes and pathways (Fig. 3, Fig. 3A-F, Table S3). For example, the SARS-CoV-2
 213 vRNA interactome was specifically enriched for two metabolism pathways: the
 214 tricarboxylic acid (TCA)-cycle and the 2-Oxocarboxylic acid metabolism (*i.e.*, lipid
 215 metabolism). Indeed, previous proteomic investigation also observed that these
 216 pathways are disrupted by SARS-CoV-2 infection(27). We also discovered that ZIKV
 217 interacts with components of the TREX transcription/export complex (comprising
 218 THOCs, ALYREF, DDX39B and SARNP, Fig. 3). This protein complex is important
 219 for RNA transport and its disruption has been implicated in microcephaly(38, 39), a
 220 disease associated with ZIKV infection(40). The SARS-CoV-2 host factor interactome
 221 includes only two known THOC complex subunits, while the EBOV includes none. We
 222 also found that EBOV specifically interacts with a set of immune and inflammatory
 223 responses related proteins(41-43), including S100A9, ANXA1, and DDX3X, and
 224 especially the “IFN- γ -activated inhibitor of translation” (GAIT) complex (Fig. 3),
 225 which is known to repress the translation of inflammatory related mRNAs(44). At
 226 minimum, these findings warrant further investigation for a functional connection to
 227 the organ failure and death that arise from the dysregulated host inflammatory
 228 responses during EBOV infection(45).

229

230 **Interactome-informed drug repurposing discovered potent SARS-CoV-2** 231 **antiviral compounds**

232 The selective inhibition of host vRNA-binding proteins can attenuate infection and
233 inform the development of innovative therapies(12). Accordingly, existing drugs that
234 are known to target various host factor proteins can be exploited as potential treatments
235 strategies during epidemics and pandemics. Given the urgent need for COVID-19
236 therapies(46), we analyzed open-source chemical databases to uncover compounds that
237 target host factors within the SARS-CoV-2 vRNA interactome. Specifically, we
238 investigated the IUPHAR/BPS Guide to Pharmacology, Drugbank, Drugcentral, and
239 ChEMBL databases, and identified a total of 5309 compounds (Table S4) that are
240 known or predicted to interact with 56 SARS-CoV-2 vRNA-binding proteins. We
241 prioritized approved drugs and clinical phase agents, as well as those available at the
242 Center of Pharmaceutical Technology of Tsinghua University. Ultimately, we tested
243 the antiviral activities of 21 drugs (Fig. 4A, Table S5), including 20 FDA-approved
244 drugs and 1 agent (Silvestrol) being tested in preclinical trials.

245 To efficiently assess the potential antiviral effects on SARS-CoV-2 infection for
246 these drugs, we used a trans-complementation system based on human colorectal
247 adenocarcinoma epithelial Caco2 cells that supports infection and replication of
248 SARS-CoV-2 (Fig. 4B). Briefly, this system uses a SARS-CoV-2-GFPΔN genome
249 wherein the N protein-coding sequence is replaced with GFP; this replacement both
250 disrupts the packaging capacity of the virus and supports visualization.
251 SARS-CoV-2-GFPΔN vRNA was generated by *in vitro* transcription and transfected
252 into green monkey epithelial Vero cells that stably express the SARS-CoV-2 N protein.
253 These steps yielded packaged SARS-CoV-2-GFPΔN virions, which we used to infect
254 Caco2 cells that also stably express the SARS-CoV-2 N protein (Caco2-N). We used
255 GFP expression as a surrogate to monitor viral infection and replication in human cells.

256 We treated Caco2-N cells with each drug at the same time as the viral challenge.
257 Remdesivir, which targets the viral RdRp and is well known for its antiviral activity

258 against SARS-CoV-2, was used as a positive control, and vehicle (DMSO) was used as
259 a negative control. At 72 h post infection we quantified infected, GFP-positive cells by
260 FACS. Remdesivir (2 μ M) was found to strongly inhibit SARS-CoV-2-GFP Δ N
261 infection, with an infection about 3.7% relative to the negative control DMSO-treated
262 cells (Fig. 4C). Thus, our trans-complementation system reflects SARS-CoV-2
263 replication in cells and is suitable for initial drug-testing.

264 Our initial screen using 10 μ M of each selected compound uncovered activity
265 against SARS-CoV-2 for 15 of the 21 tested candidate drugs (Fig. 4C). Five exerted
266 strong antiviral effects (Fig. 4C): 1) Silvestrol, which targets the translation initiation
267 factor eIF4A(47) (preclinical on Chronic lymphocytic leukemia; 98.8% inhibitory
268 efficiency); 2), Cyclosporin A (CsA), which is an immunosuppression drug and targets
269 protein PPIA(48) (FDA-approved for immunosuppressant; 91.2% inhibitory
270 efficiency); 3), Trifluoperazine (FDA-approved for antipsychotic and an antiemetic;
271 87.5% inhibitory efficiency) disrupts cytoskeleton organization(49) and also inhibits
272 binding between HSP90 with its chaperons(50), and 4) Cepharanthine (Approved for
273 alopecia, 89.6% inhibitory efficiency), which is an inhibitor of TNF-alpha/NF- κ B, and
274 also an inhibitor of the heat shock proteins (HSP90s and HSPA8)(51, 52) and 5)
275 Enasidenib, which targets the mitochondrial energy related enzyme IDH2(53)
276 (FDA-approved for refractory acute myeloid leukemia; 58% inhibitory efficiency).

277 Despite the potential for inhibition of SARS-CoV-2 infection at the initial 10 μ M
278 concentrations, these five candidate drugs also showed varying levels of cytotoxicity
279 (Fig. S4A). We further used the trans-complementation assays to determine the
280 antiviral potency and cytotoxicity at different concentrations for the aforementioned
281 five candidates (Fig. 4D). For testing the drug effects, we infected Caco-2 cells with
282 SARS-CoV-2 virus (MOI 0.05) and treated cells with the compounds with different
283 concentrations. Remdesivir, as positive control, reduced the infection of SARS-CoV-2
284 virus with an IC50 value of 0.03 μ M (Fig. S4B). Silvestrol was previously reported as a
285 “broad-spectrum” antiviral agent that showed inhibition activity against the

coronavirus HCoV-229E(54). Although Silvestrol showed a dose-dependent effect on SARS-CoV-2 infection ($IC_{50}=0.32\mu M$), we found that the cell viabilities dropped dramatically at higher drug concentrations, and detected no inhibitory effects on infection of SARS-CoV-2 virus infection below a 100nM of Silvestrol concentration. CsA can inhibit infection of SARS-CoV-2 virus with an IC_{50} value of $3.8\mu M$, and a CC_{50} value $> 30\mu M$ in Caco-2 cells ($SI > 7.9$). Cepharanthine can inhibit SARS-CoV-2 infection with an IC_{50} value of $2.98\mu M$, and a CC_{50} value of $31.77\mu M$ ($SI=10.66$). Trifluoperazine also showed strong antiviral activities against SARS-CoV-2, with IC_{50} value of $3.15\mu M$, and CC_{50} value $> 30\mu M$ ($SI > 9.51$). Enasidenib, targeting the SARS-CoV-2 vRNA specific host factor IDH2, showed no antiviral effects. Thus, among the tested candidate drugs, Cepharanthine (approved in Japan) and Trifluoperazine (FDA-approved) are the most attractive for potential use for against SARS-CoV-2 infection.

299

300 **Identification of broad antiviral inhibitors against multiple RNA viruses by** 301 **targeting common host factors**

Our viral RNA interactome data indicated that both ZIKV and EBOV vRNAs interact with the translational machinery, heat shock proteins (*e.g.*, HSP90s, HSPA9, HSPA8 HSPD1, etc.), as well as cytoskeleton-related proteins (*e.g.*, TUBA1C, TUBB). This overlap amongst host factors for these RNA viruses motivated us to examine the antiviral effects of drugs targeting these host proteins, specifically using infection assays for ZIKV in Huh7 cells (MR766, MOI 0.5) and for EBOV using assays in in Huh7.5.1-VP30 cells based on an EBOV Δ VP30-GFP variant(32) (Fig. 4E-F).

The translation initiation inhibitor Silvestrol inhibited ZIKV infection with an IC_{50} value of $0.01\mu M$ and a CC_{50} value of $0.04\mu M$ ($SI=4$). CsA inhibited ZIKV infection with an IC_{50} value of $0.78\mu M$, and a CC_{50} value of $14.35\mu M$ ($SI=18.40$). And the cytoskeleton-disrupter Trifluoperazine inhibited ZIKV infection with an IC_{50} value of $0.96\mu M$, and a CC_{50} value of $7.79\mu M$ ($SI=8.11$). The most potent anti-ZIKV

effects we observed in these assays was from Cepharanthine that targets heat shock proteins, which inhibited ZIKV infection with an IC₅₀ value of 2.19 μM, and a CC₅₀ value of 24 μM (SI=10.97) (Fig. 4E). And Enasidenib targeting IDH2 showed low inhibitory effects on ZIKV infection (SI = 1.84).

As a negative-strand RNA virus, the infection and replication processes of EBOV are very distinct from those of positive-strand RNA virus such as SARS-CoV-2 and ZIKV(4). Consistently, our integrated viral RNA interactome analysis for the three RNA viruses indicated that SARS-CoV-2 and ZIKV share more overlapped host factors than that of any with EBOV, specifically, PPIA interacts with vRNAs of SARS-CoV-2 and ZIKV, while not with vRNA of EBOV. Indeed, the PPIA-inhibitor CsA showed antiviral activities against SARS-CoV-2 and ZIKV but not EBOVΔVP30-GFP viral infection (MOI 0.1), even at a high concentration (10 μM, Fig. 4D-F). This result is also consistent with a previous study reporting that another PPIA inhibitor (Alisporivir) has limited antiviral activity against EBOV(55). Again, Silvestrol showed obvious cell toxicity although it exerted dose-dependent inhibition to EBOVΔVP30-GFP infection (IC₅₀ = 0.02 μM), and Trifluoperazine (IC₅₀ = 1.79 μM, SI = 2.18) and Cepharanthine (IC₅₀ = 0.42 μM, SI = 12.10) showed antiviral activities to EBOVΔVP30-GFP virus infection. The IDH2 inhibitor Enasidenib also showed no effects on EBOVΔVP30-GFP virus infection. Note that in our study here, we used SARS-CoV-2 for initial test of the antivirals, which could potentially miss EBOV specific antivirals, given the distinct life cycle of positive-strand and negative-strand RNA viruses. It is worthy in the future to perform an EBOV host factor guided-large scale screening for discovery of more effective antivirals against EBOV.

Collectively, our results support that the FDA-approved cytoskeleton disrupter drug Trifluoperazine and the heat shock proteins inhibitor Cepharanthine have broad-spectrum antiviral activity against the three major RNA viruses.

341 **Conclusions**

342 Our ChIRP-MS profiling of the vRNA-host protein interactomes of human cells
 343 infected with the causal pathogenic viruses for COVID-19, Zika and Ebola virus
 344 diseases identified interaction patterns that reflect both common and virus-specific
 345 host responses. These interactome datasets can be viewed as a rich resource to help
 346 illuminate the infection biology for these three viral pathogens and potentially others as
 347 well. The RNA-centric view of ChIRP-MS was particularly informative in revealing
 348 molecular virology of all stages of the life cycle of RNA viruses in infected cells(17).
 349 Methods to analyze RNA-protein interactome datasets are not yet standardized, and we
 350 found that integrated comparisons offered a straightforward way to explore and
 351 visualize common and virus-specific interactions, which we arranged to highlight the
 352 functions of the various host proteins. Indeed, the analysis enabled the prediction and
 353 selection of small molecule inhibitors as either specific or likely broad-spectrum
 354 antiviral agents. And finally, we were able to combine the interactome-informed
 355 predictions and an innovative trans-complementation infection system to power a
 356 pipeline for rapid antiviral drug discovery.

357 Ultimately, these efforts demonstrated that the FDA-approved IDH2 inhibitor
 358 Enasidenib is a potent SARS-CoV-2 specific antiviral agent and that agents targeting
 359 the heat shock proteins and cytoskeleton components, Cepharanthine and
 360 Trifluoperazine, can exert broad-spectrum effects against all three RNA viruses we
 361 examined in our study. Having identified the activities of Enasidenib, Cepharanthine,
 362 and Trifluoperazine, next steps for their re-purposing and deployment as possible
 363 clinical antiviral agents would be *in vivo* infection model studies and pre-clinical
 364 safety evaluations. Moreover, deeper exploration of the antiviral modes of action for
 365 these drugs should reveal biological insights about how these viruses infect cells, how
 366 host cells respond, and which (if any) countermeasures the viruses may deploy to
 367 overcome host defense pathways. The strong current focus of the biomedical research
 368 community on the biology of RNA virus infection, especially the ongoing COVID-19

369 pandemic(56), has exposed how little we know about the infection and amplification
 370 mechanisms that these viruses employ. Our work illustrates how profiling datasets
 371 obtained with ChIRP-MS can be integrated to drive both basic discovery and
 372 therapeutic applications.

373

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511

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521 S.J. performed the ChIRP-MS and validation experiments assisted by L.S.. X.J.
522 developed the SARS-CoV-2 N trans-complementation system and screened the drugs.
523 L.G. screened the drugs on EBOV. W.H. analyzed the ChIRP-MS data with the
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526

527 **Supplementary Materials**

528 **Materials and Methods**

529 **Data reporting**

530 No statistical methods were used to predetermine sample size. The experiments
531 were not randomized and the investigators were not blinded to allocation during
532 experiments and outcome assessment.

533

534 **Cell line and antibodies**

535 Cell lines and antibodies used in this study are listed in (Table S6). All cell lines
536 were determined to be free of mycoplasma based on PCR and nuclear staining. All cell
537 lines mentioned in this study were cultured in DMEM (10% FBS,
538 1×Antibiotic-Antimycotic) under 37 °C, 5% CO₂.

539

540 **Virus strains and cell infection**

541 For SARS-CoV-2 infection, Huh7.5.1 cells were cultured in T-175 flasks (2×3×3
542 flasks, including 3 biological repeats, 3 flasks for each mock or infected ChIRP-MS), at
543 a density of 5×10⁶ cells. The cells were briefly washed with DMEM at 16 h after
544 seeding, and incubated with a clinical isolate of SARS-CoV-2/IPBCAMS-YL01/2020
545 for 1 h at a multiplicity of infection (MOI) of 0.05. Then the cells were supplemented
546 with DMEM maintenance medium containing 1% FBS and cultured at 37°C, 5% CO₂
547 for an additional 30 h. The cultured cells were washed twice with PBS and 4%
548 formaldehyde (Pierce, 28908) was added for crosslinking at room temperature for 4 h.
549 Live virus was inactivated by an additional 12 h incubation with 4% formaldehyde at
550 4 °C. The cells were then collected and washed with 0.125M Glycine at room
551 temperature, followed by centrifugation at 1000g, 4 °C for 5 min and 3 washes with
552 PBS. The cell pellets were used for ChIRP-MS experiments. Mock cells (no infection)

were cultured and treated the same as the infected cells. All experiments involving live SARS-CoV-2 in this study were performed in a biosafety level III facility.

For ZIKV infection, 7×10^6 Huh7 cells were cultured on a 15-cm dish for 20 h (2×3×3 plates, including 3 biological repeats, 3 plates for each mock or infected ChIRP-MS experiment), then infected with ZIKV (MR766, MOI 0.5). After 72 h, cells were collected using trypsin digestion and washed twice with cold PBS, followed by crosslinking in PBS containing 3% formaldehyde at room temperature for 30 min. Crosslinking was stopped by adding a 1/10 volume of room temperature 1.25M Glycine for 5 min. The cells were then washed with 3 times with PBS. After centrifugation, PBS was removed and the cell pellets were used for ChIRP-MS. Mock cells were cultured and treated like infected cells, but no virus was added.

EBOVΔVP30-GFP(32) (strain Zaire Mayinga) virions were generated using VP30 expressing Vero E6 cell lines, then infected Huh7.5.1 cells expressing VP30 (Huh7.5.1-VP30) at a MOI=0.1. For EBOV ChIRP-MS experiment, Huh7.5.1-VP30 cells were cultured on 15-cm plates (2×3×2 plates, including 2 biological repeats, 3 plates for each mock or infected ChIRP-MS) at a density of 6×10^6 cells, then infected with EBOVΔVP30-GFP virus. After 72 h, infected and mock cells were collected and crosslinked as in the ZIKV experiment.

571

572 **Identifying host proteins interacting with viral RNA by ChIRP-MS**

ChIRP-MS was performed according to the previous report(19) with some modifications. Briefly, crosslinked cells were resuspended in 1 mL lysis buffer (~100mg cells) containing 50 mM Tris-HCl, pH 7.0, 10 mM EDTA, 1% SDS, 0.1% sodium deoxycholate, 0.5% DDM and 0.1% NP-40) and sonicated. Probes (2 uL of 100 μM) tiling the whole genome of virus (Table S1) were used to capture vRNA-protein complexes. MyOne C1 beads (Invitrogen, 65001) were blocked with 3% BSA and yeast tRNA (Solarbio, T8630), and 200 uL of C1 beads was used for each ChIRP experiment. The captured materials were firstly washed with lysis buffer for 2 times × 5min, then

581 washed with ChIRP wash buffer (2×SSC, 0.5% SDS) for 4 times × 5min. The
 582 co-purified proteins were reverse crosslinked using 0.3M NaCl buffer (7.5 mM
 583 HEPES, pH 7.9, 12.5 mM D-biotin, 0.3M NaCl, 1.5 mM EDTA, 0.2% SDS, 5mM
 584 DTT, 0.075% sarkosyl and 0.02% sodium deoxycholate) at 70 °C with shaking for 1 h.
 585 Proteins were precipitated in 20% TCA, then the protein precipitate was resuspended in
 586 RIPA buffer (25mM Tris-HCl, pH 7.4, 1% NP-40, 0.5% DOC, 0.1% SDS). Protein
 587 samples were loaded onto an SDS-PAGE gel and visualized using silver staining.
 588 Protein bands were excised from the gel and de-stained following product instructions
 589 (Pierce Silver Stain for Mass Spectrometry, 24600). The excised gel bands were
 590 desalted, pH adjusted, and digested using Trypsin overnight. The digested peptides
 591 were extracted from the gel using 50% acetonitrile and 0.1% formic acid, and analyzed
 592 via mass spectrometry (Thermo Scientific Q Exactive). ChIRP-MS control
 593 experiments were performed on cells without viral infection (mock) by using the same
 594 set of probes.

595 To determine the viral RNA recovery rate for ChIRP-MS (%RNA retrieve), 10 µL
 596 of lysate (1%) was removed from 1 mL of sonicated cell lysate, and used as input
 597 sample. At the last wash, a 10 µL volume of wash buffer containing beads (1%) was
 598 removed as the eluate for RNA quantification. Samples (input and eluate) were
 599 suspended in 100 µL PK buffer (10 mM Tris-HCl pH 7.0, 100 mM NaCl, 0.5% SDS, 1
 600 mM EDTA) containing 20 µg/mL proteinase K (Roche, 3115879001). The input and
 601 eluate samples were incubated at 50 °C for 45 min and then 95 °C for 10 min with
 602 mixing. Finally, 300 µL of TRIzol LS reagent was added to extract RNA following the
 603 manufacturer's instructions. For both input and eluate, 2 µL of RNA was used for
 604 reverse transcription for cDNA synthesis using a PrimeScript RT reagent Kit
 605 (TAKARA, RR047A). For EBOV, sequence specific primers for the EBOV genome
 606 and *Gapdh* mRNA were used for reverse transcription. qPCR of viral RNA and *Gapdh*
 607 were performed using SYBR Green kits (TAKARA, RR420A) following the

608 manufacturer's instructions. The following primers were used for reverse transcription
 609 or *qPCR*:
 610 *Gapdh* Forward primer: ACACCCACTCCTCCACCTTTGAC;
 611 *Gapdh* Reverse primer: ACCCTGTTGCTGTAGCCAAATTC.
 612 ZIKV E Forward primer: CCGCTGCCCAACACAAGGTGAAG;
 613 ZIKV E Reverse primer: CCACTAACGTTCTTTTGCAGACAT.
 614 SARS-CoV-2 NP Forward primer: GGGGAACCTTCTCCTGCTAGAAT;
 615 SARS-CoV-2 NP Reverse primer: CAGACATTTTGCTCTCAAGCTG.
 616 EBOV NP Forward primer: CCGTTCAACAGGGGATTGTTTCG;
 617 EBOV NP Reverse primer: CTGCTGGCAGCAATTCCTCAAG.
 618 EBOV reverse transcription primer: CTCAGAAAATCTGGATGGCGCCGAGTCTC
 619 *Gapdh* reverse transcription prime: CTGAGTGTGGCAGGGACTCCCCAG

620

621 **ChIRP-WB**

622 For ChIRP-western blotting (ChIRP-WB), Huh7.5.1 (SARS-CoV-2),
 623 Huh7.5.1-VP30 (EBOV), or Huh7 (ZIKV) cells were cultured on ten 10-cm plates,
 624 infected with virus and crosslinked as above described. Then, ~150 mg mock or
 625 infected cells were resuspended in 1 mL lysis buffer and sonicated. ChIRP experiments
 626 were performed as described above. 10 uL of cell lysate (per 1 mL, 1%) was removed
 627 as input. After washing, MyOne C1 beads were resuspended using 50 uL of 0.3M NaCl
 628 elution buffer (7.5 mM HEPES, pH 7.9, 12.5 mM D-biotin, 0.3M NaCl, 1.5 mM
 629 EDTA, 0.2% SDS, 5mM DTT, 0.075% sarkosyl and 0.02% sodium deoxycholate),
 630 boiled at 70 °C for 1 h and then at 95 °C for 30 min to elute proteins. 10 uL of the
 631 protein eluate and input samples were separated on SDS-PAGE gels and
 632 immunoblotted using the specific antibodies listed in [Table S6](#).

633

634 **ChIRP-MS data analysis**

635 Raw mass spectrometry data were processed with Proteome Discover using the
636 built-in search engine to search against the human proteome (Uniprot database). Viral
637 proteins, including SARS-CoV-2 (NC_045512.2), ZIKV (NC_012532.1), and EBOV
638 (NC_002549.1) proteins were downloaded from NCBI and added into the database
639 manually. Proteomic data were filtered by applying a minimum Protein Score of 1.5.
640 Viral RNA interacting proteins were scored with the MiST scoring algorithm(57) using
641 default parameters. For SARS-CoV-2 and EBOV infected samples, proteins identified
642 with a MiST score > 0.6 were considered to interact with viral RNA. For ZIKV infected
643 samples, the cutoff MiST score was set as > 0.7. Every identified protein was assessed
644 with the same parameters as in MiST, *i.e.*, “Abundance” to represent protein abundance
645 in virus infected samples, defined as the mean of the bait-prey quantities (spectral
646 counts divided by protein length) over all replicates for virus-infected samples;
647 “Specificity” to measure the uniqueness of proteins identified in virus-infected samples
648 compared with non-infected samples (mock), defined as the proportion of the
649 abundance of virus infected samples compared to the abundance of non-infection
650 samples; “Reproducibility” to evaluate the variance of each identified protein
651 abundance among replicates, defined as the normalized entropy of the bait-prey
652 quantities over all replicates for virus-infected samples. The proteins interacting with at
653 least two viral RNAs were defined as common interacting proteins.

654

655 **Differential protein expression analysis**

656 To analyze differential protein expression of SARS-CoV-2 vRNA interacting
657 proteins (n=143) across human tissues, we obtained protein abundance values in 29
658 human tissues from a proteomics dataset(58). Then the abundances of interacting
659 proteins in each tissue were compared to their medians protein abundance among all 29
660 tissues. The significance *P* value of enrichment was calculated by Mann–Whitney U
661 test and adjusted by FDR.

662

663 **Protein domain analysis**

664 To analyze whether there were possible enriched protein domains among the
 665 identified proteins, we annotated the domains of identified proteins using the Uniprot
 666 database. For each protein domain, we counted the number of identified proteins
 667 containing this domain. The enrichment (odds ratio) and significance P value of each
 668 domain among identified proteins, compared to all human proteins, was calculated by
 669 Fisher's exact test.

$$\text{odds ratio} = \frac{\frac{\text{number of identified proteins containing the domain}}{\text{number of identified proteins not containing the domain}}}{\frac{\text{number of all proteins containing the domain}}{\text{number of all proteins not containing the domain}}}$$

670

671 **Protein complex analysis**

672 To analyze whether there were possible enriched protein complexes among the
 673 identified proteins, we performed a protein complex enrichment analysis using the
 674 CORUM database(59). For each protein complex, we counted the number of identified
 675 proteins in this complex. The enrichment (odd ratio) and significance P value of these
 676 proteins in each complex, compared to all human proteins, was calculated by Fisher's
 677 exact test.

$$\text{odds ratio} = \frac{\frac{\text{number of identified proteins in complex}}{\text{number of identified proteins not in complex}}}{\frac{\text{number of all proteins in complex}}{\text{number of all proteins not in complex}}}$$

678 We searched the significantly enriched complexes (P value < 0.05) for each of the
 679 three viral RNA interactomes. Protein complexes present in at least two of the three
 680 virus interactomes were defined as common interacting complexes. Complexes present
 681 in only a single virus interactome were defined as virus-specific interacting complexes.
 682

683 **GO and KEGG enrichment analyses**

684 We performed gene ontology (GO) and KEGG pathway enrichment analyses of
685 the identified proteins using The Database for Annotation, Visualization and Integrated
686 Discovery (DAVID) v6.8. The significance *P* values of GO terms and KEGG pathways
687 were calculated by Fisher's exact test. Top 10 enriched GO terms or KEGG pathways
688 (*P* value < 0.05) were shown.

689

690 **Interactome-informed drug discovery**

691 SARS-CoV-2 host factors were assessed looking for drugs known to impact
692 human proteins. Briefly, to identify drugs/compounds modulating 143 human
693 interacting proteins, protein uniprot IDs were searched against the IUPHAR/BPS
694 Guide to Pharmacology, Drugbank, Drugcentral, and ChEMBL databases. Then we
695 retrieved 5,309 compounds from these databases related to 56 host factors ([Table S4](#)).
696 Specifically, 7 compounds related with 5 interacting proteins from the IUPHAR/BPS
697 Guide to Pharmacology; 158 compounds related with 44 interacting proteins from the
698 Drugbank; 19 compounds related with 6 interacting proteins from the Drugcentral and
699 5125 compounds predicted to be related with 35 interacting proteins from the ChEMBL
700 ([Table S4](#)). Retrieved molecules were prioritized based on their FDA approval status
701 and commercial availability. FDA approved drugs were prioritized for testing its
702 antiviral activities; an exception is Silvestrol, which is an eIF4A specific inhibitor
703 currently under investigation in a registered preclinical trial.

704

705 **Antiviral drug screening**

706 Based on the SARS-CoV-2 reference sequence (Wuhan-Hu-1, NC_045512), we
707 cloned the SARS-CoV-2 genome as five fragments. These fragments were then
708 assembled using *in vitro* ligation but replacing the viral N gene with GFP, to generate
709 the SARS-CoV-2-GFPΔN genome. Genomic RNA of SARS-CoV-2-GFPΔN and
710 mRNA of N gene were *in vitro* transcribed using an mMESSAGE mMACHINE T7

Transcription Kit (Thermo Fisher Scientific, AM1344). Synthesized viral RNA was electroporated into Vero E6-N cells that were stably expressing the SARS-CoV-2 N protein generated by lentivirus transduction, to produce the P₀ virus. After 72 h, P₀ virus was collected. Caco2 cells stably expressing the N protein (Caco2-N) were generated using lentivirus infection; this Caco2-N cell line can support the infection and replication of the SARS-CoV-2-GFPΔN virus.

To screen compounds with antiviral effects, Caco2-N cells were cultured on 96-well plates at a density of 1×10^4 cells per well. After 24 h, cells were infected with SARS-CoV-2-GFPΔN virus at a MOI of 0.05 and analyte drugs were administered at the same time as the virus. After 72 h, FACS was performed to quantify GFP-expressing cells. Remdesivir was used as a positive antiviral control. The infection ratios resulting from treatment with various drug concentrations were normalized to the DMSO (0.2%) treated cells.

Compounds, known to target common interacting proteins and which showed antiviral effects against SARS-CoV-2-GFPΔN were also tested for EBOV and ZIKV infection. To assess the antiviral effects of the compounds on EBOV, Huh7.5.1-VP30 cells were cultured on 96 well plates with 1×10^4 cells per well. After 20 h, drugs were added and cells were infected at the same time with EBOVΔVP30-GFP virus (strain Zaire Mayinga) at a MOI of 0.1. After 72 h, GFP-expressing cells were counted using FACS. Remdesivir was used as a positive antiviral control. The infection ratios with various drug concentrations were normalized to the DMSO (0.4%) treated cells.

For ZIKV, Huh7 cells were cultured on 96 well plates (Corning, 3603) with 1×10^4 cells per well. To test the antiviral effects of drugs, Huh7 cells were infected with ZIKV (MR766) at a MOI of 0.5 and drugs were added at the same time. At 72 h, cells were fixed using 4% PFA, and blocked using blocking buffer (1×PBS, 5% FBS, 0.3% Triton X-100). The fixed cells were then incubated with Anti-Flavivirus Group Antigen Antibody (clone 4G2, MAB10216, 1:1000) in blocking buffer for 12 h at 4 °C. After washing 3 times with PBS, secondary antibody (Goat Anti-Mouse IgG H&L Alexa

Fluor 488, ab150113) was diluted in blocking buffer (1:1000) containing DAPI (5 ug/mL) and was added and incubated for 2 h at room temperature. The ZIKV infection ratio was then quantified using an Opera Phenix High-content System. Total cell numbers were quantified based on DAPI staining of nuclei, and infected cells were quantified according the Alexa Fluor 488 staining in cytoplasm. The infection ratio was calculated as the number of Alexa Fluor 488 stained cells divided by the number of DAPI stained nuclei. The infection ratios with various drug concentrations were normalized to the DMSO (0.4%) treated cells.

For cell viability assays, Caco2-N, Huh7.5.1-VP30, and Huh7 were seeded on 96 well plates with 1×10^4 cells per well, and treated using drugs with different concentrations. At 72 h, cell viability was measured using CellTiter-Glo Luminescent Cell Viability Assay kits (Promega, G7570). Cell viability values were normalized to DMSO treated cells (0.2% for Caco2-N, 0.4% for Huh7 and Huh7.5.1-VP30 cells).

Viral infection curves and cell viability curves were fitted using GraphPad Prism 8 (Nonlinear regression, Dose-response-Inhibition); the half maximal inhibitory concentration (IC50) and the half maximal cytotoxic concentration (CC50) values were calculated. The selective index (SI) was also calculated as the ratio of CC50/IC50.

Supplementary Tables

Table S1 Oligo probes used for ChIRP-MS

Table S2 Viral RNA interacting proteins identified by ChIRP-MS

Table S3 Enriched complexes of viral RNA interacting proteins

Table S4 The information of drugs targeting SARS-CoV-2 RNA interacting human proteins from drug database

Table S5 Information of the 21 selected drugs

Table S6 Regents, cell line, virus and antibodies

Figures and Figure Legends

Fig. 1. Identification of the human proteins interacting with the SARS-CoV-2 RNA genome in infected cells.

A, Schematic for identifying host proteins that interact with viral RNAs using ChIRP-MS. FA, formaldehyde. ZIKV, zika virus. EBOV, Ebola virus. Human cells were infected with SARS-CoV-2, ZIKV or EBOV. Cells without infection (Mock) were used for control. Infected and mock cells were crosslinked using formaldehyde, and then sonicated to release RNA-protein complexes. For each virus, the viral RNA (vRNA)-human protein complexes were purified using biotinylated oligos specifically tiling the viral RNA genome, and the co-purified human protein were identified using mass spectrometry.

B, Evaluation of the SARS-CoV-2 vRNA interacting proteins identified by ChIRP-MS. Peak intensities of peptides of a protein from the mass spectrum (Abundance), protein abundance over different replicates (Reproducibility), and uniqueness of an interacting protein across all replicated experiments (Specificity) were evaluated to define viral RNA interacting proteins (see [Materials and Methods, ChIRP-MS data analysis](#)).

C, Correlation of log₁₀ (PSMs) for proteins from three biological replicates.

In **B** and **C**, interacting human proteins are indicated as red dots if also known to interact with other coronavirus genome RNA, and otherwise as orange dots; SARS-CoV-2 viral proteins co-purified with viral RNA are blue dots.

D, Validation of the interactions between the SARS-CoV-2 vRNA and human proteins using ChIRP-Western blotting (ChIRP-WB). GAPDH protein, which is not known to bind SARS-CoV-2 vRNA, was used as a negative control for ChIRP-WB experiments. Mock, cells without infection. Infec., cells infected with SARS-CoV-2. *, indicates proteins that are not consistent between ChIRP-MS and ChIRP-WB.

794 **E**, Distribution of enriched protein domains among the interacting human proteins of
795 SARS-CoV-2 vRNA. Bold lines with different colors represent different protein
796 domains. Protein domains not known as RNA binding domains are indicated using
797 grey dashed boxes.

798 **F**, Human tissues or organs with significantly elevated expression levels for
799 SARS-CoV-2 vRNA interacting proteins (n=143). *P* value was calculated by
800 Mann–Whitney U test and adjusted by FDR. The 10 tissues with the most significant
801 *P* values are shown.

802 **G**, SARS-CoV-2 vRNA interacting proteins are more abundant than other
803 non-interacting proteins in lungs. *P* value was calculated by Mann–Whitney U test
804 and adjusted by FDR.

805 **H**, Gene Ontology enrichment analysis of the SARS-CoV-2 vRNA interacting
806 proteins. The top 10 enriched terms are shown.

807 **I**, Cellular pathway (KEGG) enrichment analysis of the SARS-CoV-2 vRNA
808 interacting proteins. The top 10 enriched terms are shown.

809

810 **Fig. 2. ChIRP-MS identified the human proteins interacting with the ZIKV and**
811 **EBOV RNA genomes in infected cells.**

812 **A, B**, Evaluation of the ZIKV (**a**) or EBOV (**b**) vRNA interacting proteins identified
813 by ChIRP-MS. Definitions of “specificity”, “abundance” and “reproducibility” are the
814 same as **Fig. 1B** (see [Materials and Methods, ChIRP-MS data analysis](#)). Host proteins
815 interacting with the ZIKV or EBOV vRNA are indicated using orange dots. ZIKV or
816 EBOV proteins co-purified with viral RNA are indicated as blue dots.

817 **C**, Validation of interactions between the indicated proteins with ZIKV or EBOV
818 vRNA using ChIRP-WB. HIST1H3A was used as a negative control. Mock, cells
819 without infection. ZIKV or EBOV, cells infected with indicated virus. *, indicates
820 proteins that are not consistent between ChIRP-MS and ChIRP-WB.

821 **D**, Comparison of common interacting proteins between the viral RNA interactomes
822 for SARS-CoV-2, EBOV, and ZIKV.

823 **E**, Distribution of enriched protein domains among the common (left) and total (right)
824 interacting human proteins of SARS-CoV-2, ZIKV, and EBOV vRNA. Bold lines
825 with different colors represent different protein domains. Protein domains not known
826 as RNA binding domains are indicated using grey dashed boxes.

827 **F**, Gene Ontology enrichment analysis of the common (left) and total (right)
828 interacting human proteins of SARS-CoV-2, ZIKV, and EBOV vRNA. The top 10
829 enriched terms are shown.

830

831 **Fig. 3. A compendium of viral RNA-human protein interactomes for**
832 **SARS-CoV-2, EBOV, and ZIKV.**

833 Central nodes represent viral RNAs. Circular nodes represent host proteins. Edges
834 indicate RNA-protein interactions (RPI, orange) and protein-protein interactions (PPI,
835 blue). Protein complexes based on the CORUM database and proteins associated with
836 the same biological processes according to functional annotation are enclosed with
837 dashed lines. Nodes are colored according to the MiST scores of proteins interacting
838 with SARS-CoV-2 (orange), ZIKV (blue), and EBOV (green) vRNA. Drugs targeting
839 or predicted to target the indicated proteins ([Table S5](#), [Materials and Methods](#),
840 [Interactome-informed drug discovery](#)) are highlighted with red circles.

841

842 **Fig. 4. Validation of antiviral activities of the FDA-approved and clinical-trial**
843 **drugs targeting viral RNA interacting proteins.**

844 **A**, FDA-approved (green) and clinical-trial (black) compounds modulating the
845 SARS-CoV-2 vRNA interacting proteins are presented. Nodes are colored by the
846 MiST scores of SARS-CoV-2 vRNA interacting proteins, and protein names are
847 colored by the up- (red) or down-regulation upon SARS-CoV-2 infection(27).

B, Antiviral activity test for the selected drugs using a SARS-CoV-2-GFPΔN trans-complementation system. For this validation system, the SARS-CoV-2 genome sequence segment encoding nucleocapsid (N) was replaced by GFP, thus the resulted SARS-CoV-2-GFPΔN only amplifies in N protein-expressing Caco-2 (Caco-2-N) cells. The infection ratio was then evaluated by quantifying the GFP positive cells. For initial testing of antiviral activities, drugs were added at the same time as the infection (MOI=0.05), at a concentration of 10μM. GFP ratios (indicating the infection ratio of SARS-CoV-2) were calculated based on FACS data after 72 h. Remdesivir was used as an antiviral positive control.

C, SARS-CoV-2-GFPΔN infection ratios in cells treated with the indicated drugs at a concentration of 10μM. Caco2-N cells were infected with SARS-CoV-2-GFPΔN virus for 72 h with drug treatment at day 0. Black bar, infection ratios of the SARS-CoV-2-GFPΔN. Grey bar, cell viabilities after treatment with the indicated compounds at 10μM. Data were normalized to DMSO treatment. Remdesivir as a positive control (2μM). Drugs selected for further validation were highlighted in red. Drugs with modest antiviral effects were highlighted in yellow. Drugs with no significant antiviral activities at a concentration of 10μM were in black. Data are means ± SD. n= 4 biologically independent samples. **<0.01, *<0.05, Student's *t*-test.

D-F, The antiviral activities of Silvestrol, CsA, Cepharanthine, Trifluoperazine, and Enasidenib against SARS-CoV-2 (**D**), ZIKV (**E**), and EBOVΔVP30-GFP (**F**) infections at different drug concentrations. Red line, cell viability; Black line, infection ratio relative to DMSO treatment. Data are means ± SD. n=3 biologically independent samples. IC50, CC50, and SI values are indicated.

872

873 **Supplementary Figures**

874 **Fig. S1. Identification of the human proteins interacting with SARS-CoV-2** 875 **vRNA in infected cells.**

876 **A**, SARS-CoV-2 vRNA recovery rates of ChIRP-MS experiments. *q*PCR of viral
877 RNA (NP gene) was performed in ChIRP input and elution samples, *Gapdh* was used
878 as a SARS-CoV-2 probe non-targeting control. Data are means $\pm$ SD, n= 3
879 biologically independent samples of ChIRP-MS.

880 **B**, Protein domain enrichment analysis of the interacting proteins of SARS-CoV-2
881 vRNA. The top 10 enriched terms are shown.

882 **C**, Comparison of SARS-CoV-2 vRNA interacting proteins identified in this study
883 with protein groups from two different RNA pull-down method (60, 61) (Fisher's
884 exact test).

885

886 **Fig. S2. Identification of human proteins interacting with ZIKV and EBOV** 887 **vRNAs in infected cells.**

888 **A**, Viral RNA retrieved by ChIRP-MS experiments. *q*PCR of viral RNA (ZIKV E
889 protein gene and EBOV NP gene) was performed in ChIRP-MS input and elution
890 samples, *Gapdh* mRNA was used as a non-targeting control for biotinylated oligo
891 probes. Data are means $\pm$ SD, n=3 biologically independent samples for ZIKV, n=2
892 biologically independent samples for EBOV ChIRP-MS, respectively.

893 **B, C**, Correlation of $\log_{10}$ (PSMs) for proteins identified in ZIKV infected Huh7 cells
894 (**B**) and EBOV Δ VP30-GFP infected Huh7.5.1-VP30 cells (**C**), respectively. Data
895 present Pearson correlation coefficient of $\log_{10}$ (PSMs) from biological replicates. The
896 viral RNA interacting proteins are indicated using red dots in **B** and **C**; Viral proteins
897 co-purified with viral RNA are in blue.

898 **D**, ZIKV vRNA interacting proteins identified by different RNA pull-down methods.
 899 Human protein groups identified in the present study was compared with the protein
 900 groups identified by other studies(17).

901 **Fig. S3. Functional analysis of the viral RNA-specific interacting proteins.**

902 **A, B**, Gene Ontology (**A**) and KEGG pathway (**B**) enrichment analysis of proteins
 903 specifically interacting with the SARS-CoV-2 vRNA. The top 10 enriched terms are
 904 shown.

905 **C, D**, Gene Ontology (**C**) and KEGG pathway (**D**) enrichment analysis of proteins
 906 specifically interacting with the ZIKV vRNA. The top 10 enriched terms are shown.

907 **E, F**, Gene Ontology (**E**) and KEGG pathway (**F**) enrichment analysis of proteins
 908 specifically interacting with the EBOV vRNA. The top 10 enriched terms are shown.

909

Fig. 1

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Fig. 2

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Fig. 3

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Fig. 4

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A

B

C

D

E

F

Extended Data Fig. 1

Extended Data Fig. 2

Extended Data Fig. 3