

25 cells by reverse transcriptase (RT) from LINE-1 elements or by HIV-1 RT, and that these DNA
26 sequences can be integrated into the cell genome and subsequently be transcribed. Human
27 endogenous LINE-1 expression was induced upon SARS-CoV-2 infection or by cytokine
28 exposure in cultured cells, suggesting a molecular mechanism for SARS-CoV-2 retro-integration
29 in patients. This novel feature of SARS-CoV-2 infection may explain why patients can continue
30 to produce viral RNA after recovery and suggests a new aspect of RNA virus replication.

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33 Introduction

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35 Continuous or recurrent positive SARS-CoV-2 PCR tests have been reported in patients
36 weeks or months after recovery from an initial infection¹⁻¹⁴. Although *bona fide* re-infection of
37 SARS-CoV-2 after recovery has been reported lately¹⁵, cohort-based studies with strict
38 quarantine on subjects recovered from COVID-19 suggested “re-positive” cases were not caused
39 by re-infection^{16,17}. Furthermore, no replication-competent virus was isolated or spread from
40 these PCR-positive patients^{1-3,5,6,12}. The cause for such prolonged and recurrent viral RNA
41 production is unknown. As positive-stranded RNA viruses, SARS-CoV-2 and other beta-
42 coronaviruses such as SARS-CoV-1 and MERS employ an RNA-dependent RNA polymerase to
43 replicate their genomic RNA and transcribe their sub-genomic RNAs¹⁸⁻²⁰. One possibility is that
44 SARS-CoV-2 RNAs could be reverse-transcribed and integrated into the human genome, and
45 transcription of the integrated DNA copies could be responsible for positive PCR tests.

46 Endogenous reverse transcriptase (RT) activity has been observed in human cells, and the
47 products of reverse transcription have been shown to become integrated into the genome^{21,22}. For
48 example, *APP* transcripts have been shown to be reverse-transcribed by endogenous RT, with

Prions -> APP

49 resultant APP fragments integrated into the genome of neurons and transcribed²². Human LINE-
50 1 elements (~17% of the human genome), a type of autonomous retrotransposons, are a potential
51 source of endogenous RT, able to retro-transpose themselves and other non-autonomous
52 elements such as Alu^{21,23}.

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55 **Results**

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57 **Expression of viral-cellular chimeric transcripts in infected cultured and in patient-derived** 58 **cells is consistent with genomic integration of viral sequences.**

59 To investigate the possibility of viral integration into virus infected cells we analyzed
60 published RNA-Seq data from SARS-CoV-2 -infected cells for evidence of chimeric transcripts,
61 which would be indicative of viral integration into the genome and expression. Examination of
62 these data sets²⁴⁻³⁰ (Fig. S1a-b) revealed a substantial number of host-viral chimeric reads (Fig.
63 1a-c, S1c). These occurred in multiple sample types, including cells and organoids from
64 lung/heart/brain/stomach tissues, as well as BALF cells directly isolated from COVID-19
65 patients (Fig. 1c). Chimeric read abundance was positively correlated with viral RNA level
66 across the sample types (Fig. 1c). Chimeric reads generally accounted for 0.004% - 0.14% of
67 total SARS-CoV-2 reads across the samples, with a 69.24% maximal number of reads in
68 bronchoalveolar lavage fluid cells derived from severe COVID19 patients and near no chimeric
69 reads from patient blood buffy coat cells (corresponding to almost no total SARS-CoV-2 reads).
70 A majority of chimeric junctions mapped to SARS-CoV-2 nucleocapsid (N) sequence (Fig. 1d-
71 e). This is consistent with the finding that nucleocapsid (N) RNA is the most abundant SARS-
72 CoV-2 sub-genomic RNA³¹, and thus is most likely to be a target for reverse transcription and

73 integration. These analyses support the hypothesis that SARS-CoV-2 RNA may retro-integrate
74 into the genome of infected cells resulting in the production of chimeric viral-cellular transcripts.

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76 **SARS-CoV-2 RNA can be reverse-transcribed and integrated into the human genome in**
77 **cells overexpressing a reverse transcriptase Platelets**

78 To provide experimental evidence for reverse-transcription and integration of SARS-
79 CoV-2 RNA, we overexpressed human LINE-1 or HIV-1 reverse transcriptase (RT) in
80 HEK293T cells and infected the transduced cells with SARS-CoV-2. The cells were tested 2
81 days after infection for viral sequences by PCR or fluorescence *in situ* hybridization (FISH) (Fig.
82 2a). Considering that the N RNA is the most abundant SARS-CoV-2 sub-genomic RNA³¹ and is
83 most likely to be retro-integrated (Fig. 1d-e), we chose four N – targeting PCR primer sets that
84 are used in COVID-19 tests (primer source from WHO³², Fig. 2a). PCR amplification of purified
85 cellular DNA showed positive gel-bands in cells with human LINE-1 or HIV-1 RT
86 overexpression (Fig. 2b) but not in non-transfected or non-infected cells. To test whether the
87 DNA copies of N sequences were integrated into the cellular genome, we gel-purified cell
88 genomic DNA (gDNA, >23 kb, Fig. S2a) and qPCR confirmed N sequences in gDNA of cells
89 with expression of all three types of RT (Fig. 2c). Cells with strong expression of LINE-1 driven
90 by a CMV promoter showed ~8-fold higher signals of N sequence detection suggesting a higher
91 copy-number of integrated N sequences than in cells expressing LINE-1 driven by its natural
92 promoter (5'UTR) or HIV-1 RT (Fig. 2c). We were able to clone full-length N DNA from gDNA
93 of cells overexpressing CMV-LINE-1 and confirmed its sequence by Sanger sequencing (Fig.
94 S2b). We did not detect the full-length N sequence from gDNA of cells transfected with 5'UTR-
95 LINE-1 or HIV-1 RT, which may be due to lower expression of RT in these cells (Fig. S2b). We

96 further confirmed that purified SARS-CoV-2 RNA from infected cells can be reverse-transcribed
97 *in vitro* by lysates of cells expressing either LINE-1 or HIV-1 RT (Fig. S2c-d).

98 We conducted single-molecule RNA-FISH (smRNA-FISH) using fluorophore-labeled
99 oligo-nucleotide probes targeting N (Fig. 2a) to confirm that viral N sequences were integrated
100 and detected their transcription in the nucleus. SARS-CoV-2 infected cells showed the expected
101 cytoplasmic FISH signals of N RNA (Fig. S3a). N RNA FISH signals were detected in cell
102 nuclei with cells overexpressing LINE-1 (Fig. 2d, S3b), indicating nascent transcription sites of
103 integrated N sequences. In the same cell population, a significantly higher fraction (~35%) of
104 infected cells overexpressing LINE-1, as indicated by LINE-1 ORF1p immunostaining, showed
105 nuclear N signals than cells not overexpressing LINE-1 (~12%) (Fig. 2e). A significantly higher
106 fraction of infected cells that were transfected with LINE-1 plasmid (~80% transfection
107 efficiency) showed positive nuclear N FISH signals (~30%) as compared to non-transfected cells
108 (13%; Fig. S3c). Infected but not transfected cells also exhibited nuclear N signals, albeit at a
109 lower frequency (~10%; Fig. 2e, S3c), implying integration of SARS-CoV-2 N RNA by cell
110 endogenous RT activity.

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112 **Human endogenous LINE-1 expression induced by SARS-CoV-2 infection and cytokines** 113 **correlates with retro-integration**

114 Human LINE-1 elements are autonomous retro-transposons with their encoded reverse
115 transcriptase (ORF2p) and supporting protein (ORF1p) also aiding non-autonomous elements to
116 retro-transpose, such as Alu and other cellular RNAs²¹. We found that expression of LINE-1
117 elements was significantly up-regulated in published RNA-Seq data of cells upon infection with
118 SARS-CoV-2 and correlated with chimeric read abundance (Fig. 3a-b, S4a-d, compare Calu3

119 cells that are efficiently infected versus NHBE cells that are resistant to infection). Although the
120 upregulation in Calu3 was not higher than that in NHBE, multiple LINE-1 elements were
121 upregulated as compared to just one in NHBE (Fig. 3a, S4b, d). Expression analysis using LINE-
122 1 specific primers^{33,34} showed a ~3-4-fold up-regulation of LINE-1 in Calu3 cells when infected
123 by SARS-CoV-2 (Fig. 3c). Moreover, PCR analysis on Calu3 cellular DNA showed retro-
124 integration of SARS-CoV-2 N sequences after infection (Fig. 3d-e), possibly by the activated
125 LINE-1 reverse transcriptase.

126 Patients infected with SARS-CoV-2 and other corona viruses show evidence of cytokine
127 induction associated with the immune response, and in severe cases experience a cytokine
128 storm³⁵⁻³⁷, prompting us to investigate whether cytokines alone can induce LINE-1 activation.
129 We treated cells with cytokine-containing conditioned media from Myeloid, Microglia, or CAR-
130 T cell cultures and found a ~2-3-fold upregulation of endogenous LINE-1 expression by PCR
131 analysis (Fig. 3f, S5b). Expressed LINE-1 protein (ORF1p) was also confirmed by
132 immunofluorescence staining (Fig. 3g-h, S5a). In summary, our results show induced LINE-1
133 expression in cells stressed by viral infection or exposed to cytokines, suggesting a molecular
134 mechanism for SARS-CoV-2 retro-integration in human cells.

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137 **Discussion**

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In this study, we showed evidence that SARS-CoV-2 RNAs can be reverse-transcribed and integrated into the human genome by several sources of reverse transcriptase such as activated human LINE-1 or co-infected retrovirus (HIV). We found LINE-1 expression can be induced upon SARS-CoV-2 infection or cytokine exposure, suggesting a molecular mechanism responsible for SARS-CoV-2 retro-integration in patients. Moreover, our results suggest that the integrated SARS-CoV-2 sequences can be transcribed, as shown by RNA-Seq and smRNA-FISH data, providing a possible explanation for the presence of viral sequences at later times after initial virus exposure and in the absence of detectable infectious virus¹⁻¹⁴. The retro-inserted SARS-CoV-2 sequences are most likely sub-genomic fragments, as the integration junctions are mostly enriched at the N sequence (Fig. 1d-e), excluding the production of infectious virus. Our data may also explain that patients, after recovery from disease symptoms, may become again positive for viral sequences as detected by PCR^{1,8-14}.

An important follow-up question is whether these integrated SARS-CoV-2 sequences can express viral antigens. If so, it will be of clinical interest to assess whether viral antigens expressed from integrated virus fragments could trigger an immune response in patients that could affect the course and treatment of the disease. It is possible that the clinical consequences of the integrated viral fragments may depend on their insertion sites in the human genome, and on epigenetic regulation which has been shown in HIV patients³⁸. Careful analysis on SARS-CoV-2 retro-integration sites in patient samples and correlation with disease severity will help to elucidate potential clinical consequences. Furthermore, immune response may vary depending on an individual's underlying conditions. More generally, our results suggest a novel aspect of infection possibly also for other common disease-causing RNA viruses such as Dengue, Zika or

161 Influenza virus, which could be subject to retro-integration and perhaps affect disease
162 progression.

163 Human LINE-1 accounts for ~17% of the human genome, ~100 out of 500,000 copies of
164 which are active^{21,23}. LINE-1 – encoded reverse-transcriptase (ORF2p) and supporting protein
165 (ORF1p) are known to retro-transpose not only LINE-1 transcripts (in *Cis*), but also other RNA
166 species such as Alu (SINE) and cellular mRNA (in *Trans*, creating processed pseudogenes), with
167 a “target-site – primed reverse transcription” mechanism²¹. LINE-1 proteins have been shown as
168 nucleic acid chaperones with high RNA binding affinity³⁹, therefore it is perhaps not surprising
169 that they can retro-integrate exogenous viral RNAs. From an evolutionarily perspective, retro-
170 integration of viral RNA by LINE-1 could be an adaptive response by the host to provide
171 sustaining antigen expression possibly enhancing protective immunity. Conversely, retro-
172 integration of viral RNAs could be detrimental and cause a more severe immune response in
173 patients such as a “cytokine storm” or auto-immune reactions.

174 Our results may also be relevant for current clinical trials of antiviral therapies⁴⁰. The
175 reliance of PCR tests to assess the effect of treatments on viral replication and viral load may not
176 reflect the efficacy of the treatment to suppress viral replication as the PCR assay may detect
177 viral transcripts from viral sequences stably integrated into the genome rather than infectious
178 virus.

179

180 **Methods**

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182 **Cell culture and plasmid transfection**

183 HEK293T cells were obtained from ATCC (CRL-3216) and cultured in DMEM
184 supplemented with 10% heat-inactivated FBS (Hyclone, SH30396.03) and 2mM L-glutamine
185 (MP Biomedicals, IC10180683) following ATCC's method. Calu3 cells were obtained from
186 ATCC (HTB-55) and cultured in EMEM (ATCC 30-2003) supplemented with 10% heat-
187 inactivated FBS (Hyclone, SH30396.03) following ATCC's method.

188 Plasmid for HIV-1 reverse transcriptase expression: pCMV-dR8.2 dvpr was a gift from
189 Bob Weinberg (Addgene plasmid # 8455 ; <http://n2t.net/addgene:8455> ;
190 RRID:Addgene_8455)⁴¹. Plasmids for human LINE-1 expression: pBS-L1PA1-CH-mneo
191 (CMV-LINE-1) was a gift from Astrid Roy-Engel (Addgene plasmid # 51288 ;
192 <http://n2t.net/addgene:51288> ; RRID:Addgene_51288)⁴²; EF06R (5'UTR-LINE-1) was a gift
193 from Eline Luning Prak (Addgene plasmid # 42940 ; <http://n2t.net/addgene:42940> ;
194 RRID:Addgene_42940)⁴³. Transfection was done with Lipofectamine™ 3000 (Invitrogen
195 L3000001) following manufacturer's protocol.

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197 **SARS-CoV-2 infection**

198 SARS-CoV-2 USA-WA1/2020 (Gen Bank: MN985325.1) was obtained from BEI
199 Resources and expanded and tittered on Vero cells. Cells were infected in DMEM +2% FBS for
200 48 hrs using multiplicity (MOI) of 0.5 for infection of HEK293T cells and an MOI of 2 for
201 Calu3 cells. All sample processing and harvest with infectious virus were done in the BSL3
202 facility at the Ragon Institute.

203

204 **Nucleic acids extraction, *in vitro* reverse transcription and PCR/qPCR**

205 DNA extraction was following a published protocol²². For purification of genomic DNA,
206 extracted total cellular DNA was run on 0.4% (w/v) agarose/1x TAE gel for 1.5 hrs with a
207 3V/cm voltage, with λ DNA-HindIII Digest (NEB N3012S) as size markers. Large fragment
208 bands (>23.13 kb) were cut off, frozen in -80 °C and then crushed by a pipette tip. 3 times of
209 volume (v/w) of high T-E buffer (10 mM Tris – 10 mM EDTA, pH 8.0) was added and then
210 NaCl was added to 200 mM. Gel solution was heated at 70 °C for 15 mins with constant mixing
211 and then extracted with Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) (Life Technologies
212 15593031) and Chloroform:Isoamyl alcohol 24:1 (Sigma C0549-1PT). DNA was then
213 precipitated by sodium acetate and isopropyl alcohol. For small amount of DNA, glycogen (Life
214 Technologies 10814010) was added as a carrier to aid precipitation.

215 RNA extraction was done with either TRIzol™ LS Reagent (Invitrogen 10296010) or
216 RNeasy Plus Micro Kit (Qiagen 74034) following manufacturers' protocols. RNA reverse
217 transcription was done with either SuperScript™ III First-Strand Synthesis SuperMix (oligo dT +
218 random hexamer, Invitrogen 18080400) or qScript cDNA SuperMix (QuantaBio 95048-500),
219 following manufacturers' protocols. *In vitro* reverse transcription assay for viral RNA by cell
220 lysates was done following a published protocol²².

221 PCR was done using AccuPrime Taq DNA Polymerase, high fidelity (Life Technologies
222 12346094). qPCR was done using SYBR™ Green PCR Master Mix (Applied Biosystems
223 4309155) or PowerUp™ SYBR™ Green Master Mix (Applied Biosystems A25742) in a
224 QuantStudio™ 6 system (Applied Biosystems). See **Supplementary Table 1** for primer
225 sequences used in this study. qPCR plots were generated with Prism 8 (Prism).

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227 **Immuno-fluorescence staining and single-molecule RNA-FISH**

228 Cells subject to SARS-CoV-2 infection were grown in μ -Slide 8 Well (#1.5 polymer,
229 Ibidi 80826) and fixed with 4% paraformaldehyde/CMF-PBS at room temperature (RT) for 30
230 mins. Otherwise, cells were grown on 12 mm round coverslips (#1.5, Warner Instruments 64-
231 0712) and fixed with 1.6% paraformaldehyde/CMF-PBS at room temperature (RT) for 15 mins.
232 Cells were permeabilized with 0.5% (v/v) Triton X-100/PBS, blocked with 4% (w/v) BSA/CMF-
233 PBS at RT for 1 hr, incubated with 1:200 diluted anti-LINE-1 ORF1p mouse monoclonal
234 antibody (clone 4H1, Sigma MABC1152, Lot 3493991), and then with 1:400 diluted Donkey-
235 anti-Mouse-Alexa Fluor 594 second antibody (Invitrogen 21203).

236 Single-molecule RNA-FISH probes (Stellaris®) were ordered from LGC Biosearch
237 Technologies with Quasar® 670 Dye labeling. See **Supplementary Table 2** for probe
238 sequences. FISH procedure combining with immuno-fluorescence staining was following
239 previous publications^{44,45}.

240 Cells in μ -Slide were mounted with Ibidi Mounting Medium With DAPI (Ibidi 50011).
241 Cells on coverslips were mounted with VECTASHIELD® HardSet™ Antifade Mounting
242 Medium with DAPI (Vector Laboratories H-1500-10).

243

244 **Microscopy and imaging analysis**

245 3D optical sections were acquired with 0.2- μ m z-steps using a DeltaVision Elite Imaging
246 System microscope system with a 100 \times oil objective (NA 1.4) and a pco.edge 5.5 camera and
247 DeltaVision SoftWoRx software (GE Healthcare). Image deconvolution was done using
248 SoftWoRx. All figure panel images were prepared using FIJI software (ImageJ, NIH) and Adobe
249 Illustrator 2020 (Adobe), showing deconvolved single z-slices.

250 To measure the LINE-1 ORF1p immuno-staining signal intensity, we projected cell
251 optical sections (sum, 42 slices) with the “z projection” function in FIJI. We measured the sum
252 of intensity of the entire cell area in the z-projected image as the signal intensity, subtracted the
253 background intensity outside of cells and then divided by the mean of the “Basal media
254 treatment” group to have the normalized signal intensity, as previously described^{44,45}. All images
255 from the same experiment were using the same exposure time and transmitted exciting light. All
256 intensity measurements were done with non-deconvolved raw images. Box plot was done in R
257 (version 4.0.3)⁴⁶.

258

259 **RNA-Seq data analysis**

260 RNA-Seq data were downloaded from GEO with the accession numbers GSE147507²⁴,
261 GSE153277²⁵, GSE156754²⁶, GSE157852²⁷, GSE153684²⁸, GSE145926²⁹, GSE154998³⁰
262 (summarized in **Supplementary Figure 1a**).

263 To identify human – SARS-CoV-2 chimeric reads, raw sequencing reads were aligned to
264 concatenated human and SARS-CoV-2 genomes plus transcriptomes by STAR (version
265 2.7.1a)⁴⁷. Human genome version hg38 with no alternative chromosomes and gene annotation
266 version GRCh38.97 were used. SARS-CoV-2 genome version NC_045512.2 and gene
267 annotation (<http://hgdownload.soe.ucsc.edu/goldenPath/wuhCor1/bigZips/genes/>) were used.
268 The following STAR parameters³¹ were used to call chimeric reads unless otherwise specified
269 (**Supplementary Figure 1a**): --chimOutType Junctions SeparateSAMold WithinBAM HardClip
270 \ --chimScoreJunctionNonGTAG 0 \ --alignSJstitchMismatchNmax -1 -1 -1 -1 \ --
271 chimSegmentMin 50 \ --chimJunctionOverhangMin 50.

272 To analyze human LINE-1 expression in RNA-Seq data, a published method,
273 RepEnrich²⁴⁸, was used to map RNA-Seq reads to human repeat annotations, using human
274 repeat masker (hg38). Differential expression was analyzed using EdgeR package (version
275 3.30.3)^{49,50} in R (version 4.0.3)⁴⁶.

276

277 **Conditioned media production and treatment**

278 As previously described⁵¹, myeloid precursors were derived from human pluripotent stem
279 cells. Briefly, human embryonic stem cells were cultured in StemFlex (ThermoFisher) feeder-
280 free medium on MatrigelTM- (Corning) coated tissue culture polystyrene. 24 hrs before single-
281 cell harvesting via TrypLE Express (ThermoFisher), cells were treated with 10 μ M ROCK
282 Inhibitor (Y-27632) (Stem Cell Technologies) in Essential 8 (E8) medium (ThermoFisher). After
283 harvesting, cells were centrifuged at 300 g for 3 mins in non-adherent U-bottom 96-well plates
284 (Corning) at 10,000 cells per 150 μ L/well of embryoid body (EB) medium consisting of 10 μ M
285 ROCK Inhibitor, 50 ng/mL BMP-4 (Peprotech), 20 ng/mL SCF (Peprotech), 50 ng/mL VEGF
286 (Peprotech), and 100 U/mL Penn/Strep (ThermoFisher) in E8 base medium. EBs were cultured
287 in the 96-well plates for 4 days with 150 μ L/well of EB medium added at day 2. After 4 days, 16
288 EBs/well were plated in a 6-well tissue culture polystyrene plated coated with MatrigelTM in
289 hematopoietic myeloid medium (HIM) consisting of 2mM GlutaMax (ThermoFisher), 55 μ M
290 beta-mercaptoethanol, 100 ng/mL M-CSF (Peprotech), and 25 ng/mL IL-3 (Peprotech) in X-
291 VIVO 15 base medium (Lonza). HIM media was changed every 3-4 days for 2-3 weeks until
292 floating CD14-positive myeloid precursors emerged. Myeloid conditioned media consisted of
293 floating myeloid cells cultured in HIM media for 7 days at a concentration of $0.5 \times 10^6 - 1 \times 10^6$
294 cells/mL. Cells in conditioned media were removed by centrifugation and filtration through 0.2

295 μ M filters. Calu3 cells were cultured in the myeloid conditioned media or HIM media (basal) for
296 two days with daily media change before harvest or fixation.

297 Microglia were differentiated from human induced pluripotent stem cells (hiPSCs) via
298 embryoid bodies and primitive macrophage precursors (PMPs)⁵¹. In brief, hiPSCs (cultured
299 feeder-free on matrigel in StemFlexTM (Gibco)) were dissociated with TrypLE Express (Gibco),
300 and 10,000 cells were plated per well in 96-well ultra-low attachment plates (Corning) in 100 μ L
301 embryoid body medium (10 μ M ROCK inhibitor, 50 ng/mL BMP-4, 20 ng/mL SCF, and 50
302 ng/mL VEGF-121 in StemFlex), before centrifugation at $300 \times g$ for 3 mins at 4 °C. Embryoid
303 bodies were cultured for 4 days, with adding 100 μ L embryoid body medium after 2 days. 12 to
304 16 embryoid bodies were plated per well of tissue culture-treated 6-well plates and cultured in 3
305 mL hematopoietic medium (2 mM GlutaMax, 100 U/mL penicillin, 100 μ g/mL streptomycin, 55
306 μ M β -mercaptoethanol, 100 ng/mL M-CSF, 25 ng/mL IL-3, 100 U/mL penicillin, 100 μ g/mL
307 streptomycin in X-VIVO 15 (Lonza, BW04418Q). From this point on, 2 mL medium was
308 exchanged every 4–7 days. PMPs were harvested from suspension during medium exchange and
309 plated in microglia differentiation media over 7-14 days to produce microglia like cell
310 monocultures (Neurobasal (Life Technologies 21103049) supplemented with Gem21 NeuroPlex
311 without Vitamin A (GeminiBio, 400-161), 2mM GlutaMAX (Gibco), 100 ng/mL IL-34, and 10
312 ng/mL GM-CSF, 100 U/mL penicillin, 100 μ g/mL streptomycin). For microglia stimulation,
313 microglia differentiation media was exchanged with HEK293T media (DMEM + 10% heat-
314 inactivated FBS + final 2mM L-Glutamine) and supplemented with 100 hg/ml
315 lipopolysaccharide (LPS, Sigma Aldrich L4391-1MG) or PBS. After 24 hrs, the microglia
316 conditioned media was collected, centrifugated (1000 rpm 10min) and the supernatant was

317 directly applied to HEK293T cells. HEK293T cells received microglia conditioned media or
318 basal HEK293T media on three constitutive days before fixation.

319 Human anti-CD19 CAR-T cells were generated by transduction of primary T cells
320 purified from human peripheral blood mononuclear cells (PBMC) with CD19-CAR expressing
321 retrovirus⁵². Anti-CD19 CAR-T cells were co-cultured with CD19-expressing beta-like cells⁵² or
322 WIBR3 cells with a luciferase-2A-CD19 expressing cassette integrated at the AAVS1 locus in
323 RPMI1640 medium with 10% human serum AB. Cells in the conditioned medium were removed
324 by filtration through 0.45 μ M filters. RPMI1640 medium with 10% human serum AB was used
325 as basal media control. Calu3 cells were cultured in the CAR-T conditioned media with indicated
326 dilutions or in the basal media for two days before harvest.

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329 **Data Availability**

330 The datasets generated during and/or analysed during the current study are available from
331 the corresponding author on reasonable request.

332

333

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345 **Author contributions**

346 Project design by R.J. and R.A.Y, execution of experiments and data analysis by L.Z.,
347 A.R, R.J and R.A.Y; E.W., A.K., and H.M. generated cells and reagents; Manuscript preparation
348 by L.Z. and R.J. with input from all authors.

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351 **Competing interests**

352 R.J. is an advisor/co-founder of Fate Therapeutics, Fulcrum Therapeutics, Omega
353 Therapeutics, and Dewpoint Therapeutics. R.A.Y. is a founder and shareholder of Syros
354 Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, and Dewpoint Therapeutics. All
355 other authors declare no competing interests.

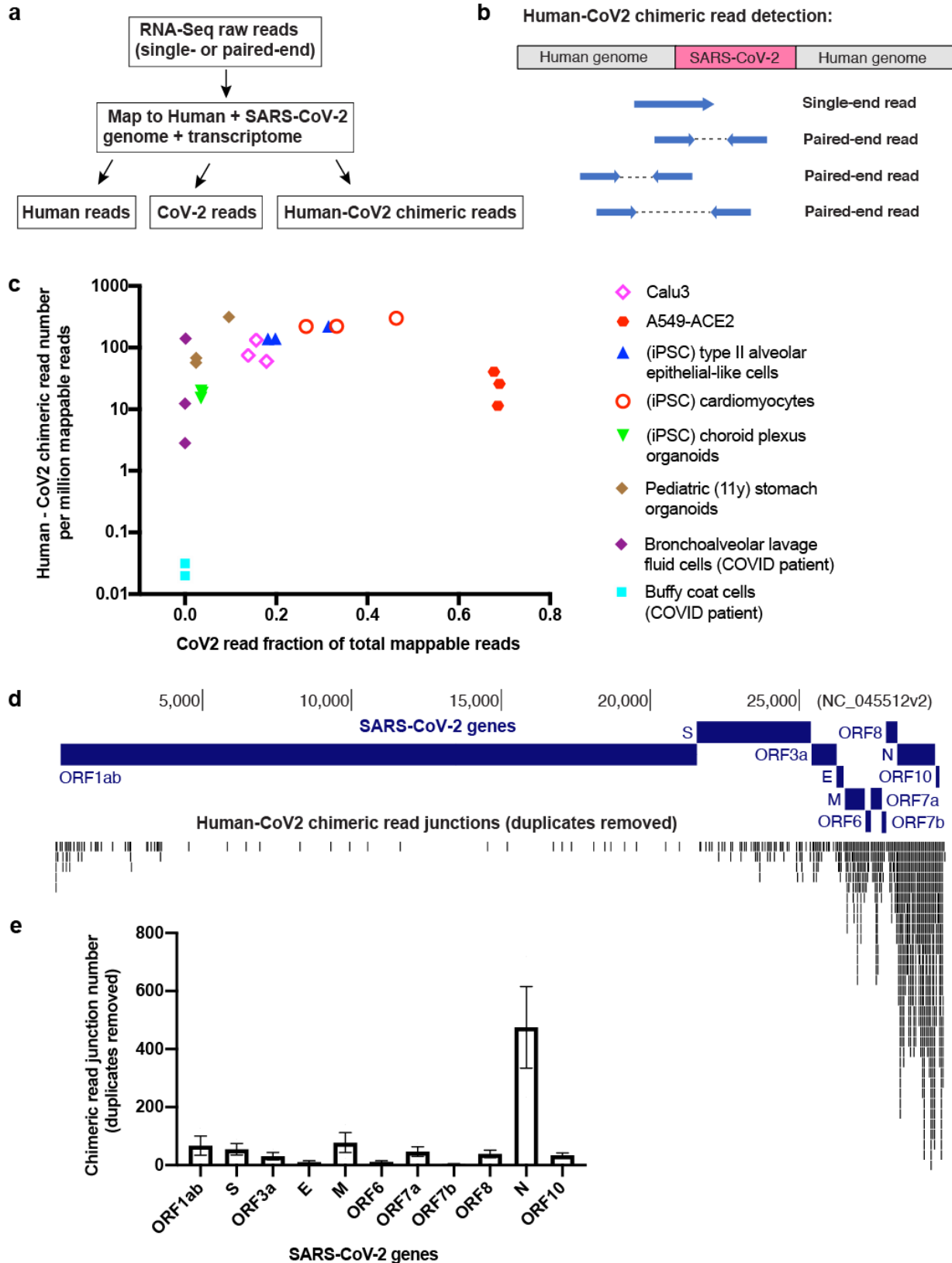
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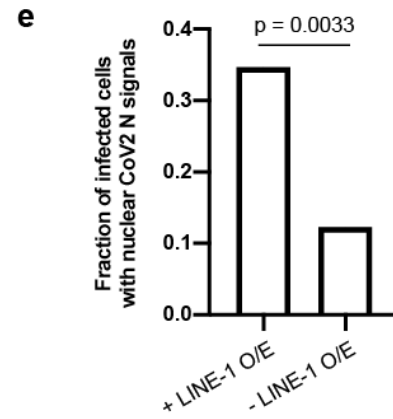
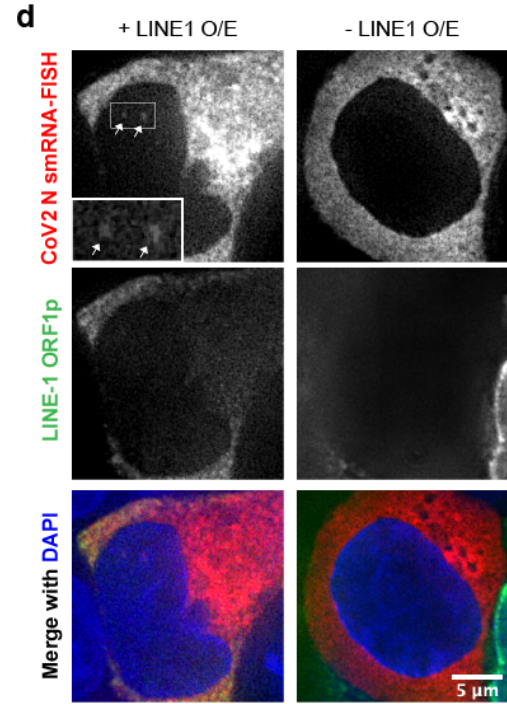
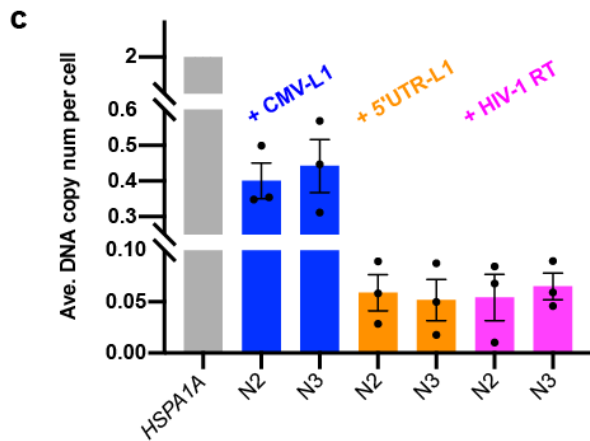
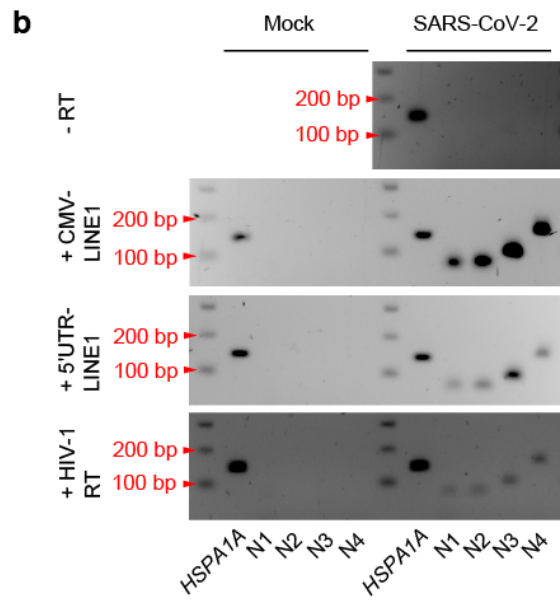
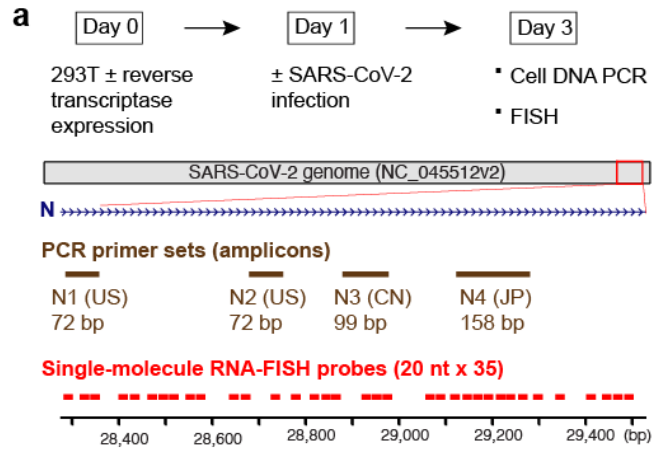
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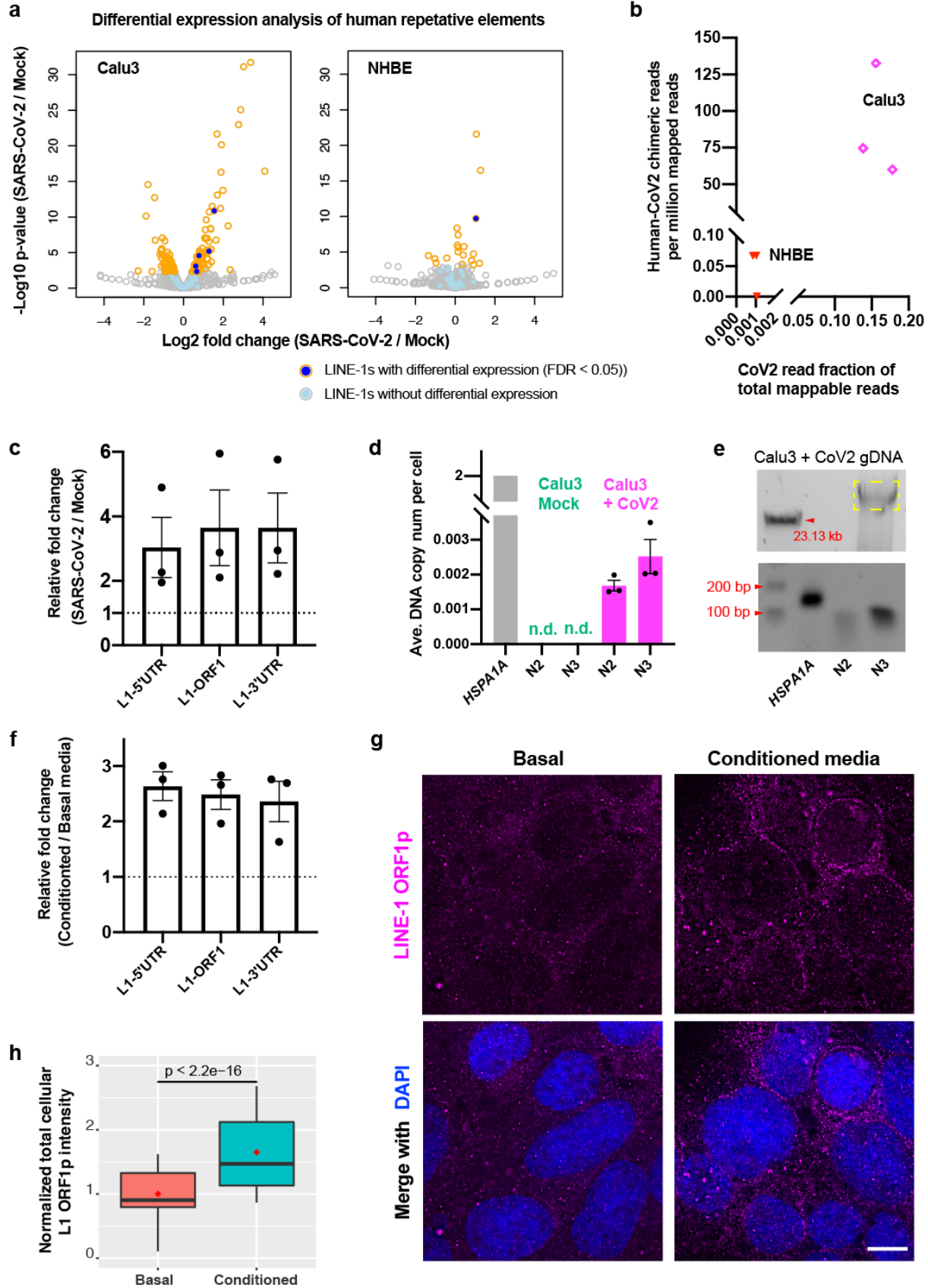
488 **Figures**



490 **Figure 1. Human – SARS-CoV-2 chimeric transcripts identified in published data sets of**
491 **infected cultured and patient-derived cells. a)** Pipeline to identify human-CoV2 chimeric
492 RNA-Seq reads. **b)** Diagram showing human-CoV2 chimeric reads mapped to potential SARS-
493 CoV-2 integration sites in the human genome from published RNA-Seq data. **c)** Scatter plot
494 showing human-CoV2 chimeric read number (per million total mappable reads, y-axis) versus
495 SARS-CoV-2 read fraction of total mappable reads (x-axis) in published RNA-Seq datasets
496 (summarized in **Supplementary Figure 1a**) from different bio-samples with SARS-CoV-2
497 infection. **d-e)** Human-CoV2 chimeric read junctions (duplicates removed) mapped to the SARS-
498 CoV-2 genome (**d**) and distribution among SARS-CoV-2 genes (**e**, three biological replicates;
499 mean \pm s.e.m.). RNA-Seq data is from SARS-CoV-2 infected Calu3 cells (see **Supplementary**
500 **Figure 1a**). Chimeric read junction is defined as the “breaking point” of sequences mapped to
501 human or SARS-CoV-2 genome/transcriptome in a given RNA-Seq read.



503 **Figure 2. SARS-CoV-2 RNA can be reverse-transcribed and integrated into the host**
504 **genome in cells with reverse transcriptase expression. a)** Experimental workflow (top), PCR
505 primer sets (shown as amplicons, brown) and single-molecule RNA-FISH probes (red) to detect
506 reverse-transcription and integration of SARS-CoV-2 nucleocapsid (N) sequence (middle, blue).
507 **b)** PCR detection of SARS-CoV-2 N sequences in cellular DNA purified from mock (left) or
508 SARS-CoV-2 (right) infected HEK293T cells without or with transfection of human LINE-1
509 (CMV-LINE1 or 5'UTR-LINE1) or HIV-1 RT. *HSPA1A*: human *HSPA1A* gene as control; N1 –
510 N4: SARS-CoV-2 N sequences as shown in **a**). **c)** qPCR detection and copy-number estimation
511 of SARS-CoV-2 N sequences on gel-purified HEK293T genomic DNA. *HSPA1A*: human
512 *HSPA1A* gene as a reference; N2, N3: SARS-CoV-2 N sequences as shown in **a**). Three
513 biological replicates; mean \pm standard error of the mean (s.e.m.). **d)** Single-molecule RNA-FISH
514 (red) targeting SARS-CoV-2 N sequence using probes shown in **a**) plus LINE-1 ORF1 protein
515 immuno-staining (green) and merged channels with DAPI (blue) in SARS-CoV-2 infected
516 HEK293T cells with (left) or without (right) transfected LINE-1. Insets: 2.5x enlargement of
517 region in white-box to show nuclear signals of SARS-CoV-2 N sequence (white arrows). Images
518 were single z-slices from 3D optical sections (0.2- μ m z-steps). **e)** Fraction of HEK293T cells
519 infected by SARS-CoV-2 (indicated by cytoplasmic FISH signals) showing nuclear FISH signals
520 of N sequence with (+ LINE-1 O/E, n = 75) or without (- LINE-1 O/E, n = 57) LINE-1
521 overexpression (indicated by LINE-1 ORF1 protein immuno-staining). Combination of two
522 independent cell samples; Chi-Square Test of Homogeneity.



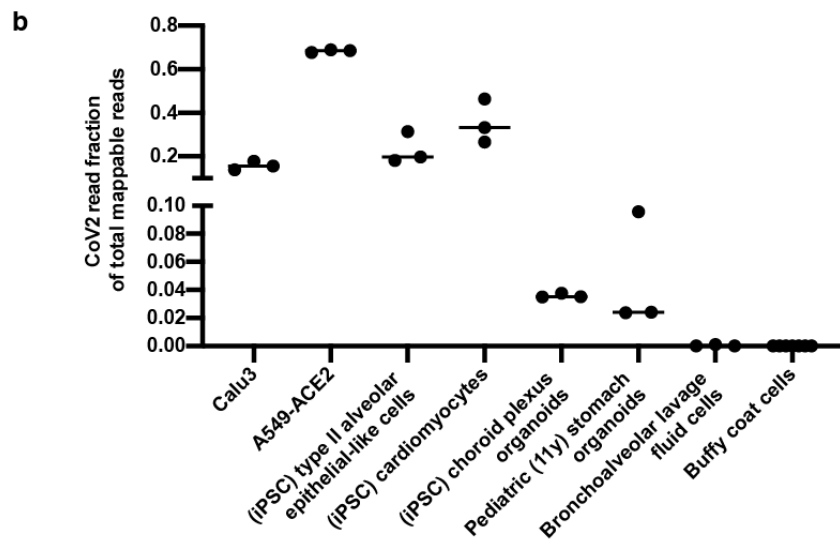
524 **Figure 3. LINE-1 expression as an endogenous reverse-transcriptase source in human cells**
525 **is induced by SARS-CoV-2 infection and cytokine-containing conditioned media treatment.**
526 **a)** RNA-Seq (GSE147507, see **Supplementary Figure 1a**) differential expression analysis for
527 all human repetitive elements in SARS-CoV-2 versus mock-infected Calu3 (left) or NHBE
528 (right) cells. Volcano plots showing $-\text{Log}_{10}$ p-values (y-axis) versus Log_2 fold-changes (x-axis)
529 for all human repetitive elements with (orange circle) or without (grey circle) significant
530 expression changes (SARS-CoV-2 versus mock-infected); dots: LINE-1 families with (dark
531 blue) or without (light blue) significant expression changes. **b)** Scatter plot showing human-
532 CoV2 chimeric read number (per million total mappable reads, y-axis) versus SARS-CoV-2 read
533 fraction of total mappable reads (x-axis) in published RNA-Seq (GSE147507, see
534 **Supplementary Figure 1a**) from infected Calu3 (magenta) or NHBE (red) cells. **c)** Endogenous
535 LINE-1 expression fold-changes between SARS-CoV-2 versus mock-infected Calu3 cells
536 measured by RT-qPCR with primers probing 5'UTR, ORF1, or 3'UTR regions of LINE-1.
537 Reference genes: *GAPDH* and *TUBB*. Three biological replicates; mean \pm s.e.m. **d)** qPCR
538 detection and copy-number estimation of SARS-CoV-2 N sequences in mock (green) or SARS-
539 CoV-2 infected (magenta) Calu3 cellular DNA. *HSPA1A*: human *HSPA1A* gene as a reference;
540 N2, N3: SARS-CoV-2 N sequences as shown in **Figure 1a**. Three biological replicates; mean \pm
541 s.e.m; n.d.: not detected. **e)** Gel purification of large-fragment genomic DNA (yellow box, top)
542 from SARS-CoV-2 infected Calu3 cells and PCR detection of SARS-CoV-2 N sequences in the
543 purified genomic DNA (bottom) with same primer sets as in **d)**. **f)** Endogenous LINE-1
544 expression fold-changes in Calu3 cells comparing Myeloid conditioned versus basal media
545 treatment measured by RT-qPCR with primers probing 5'UTR, ORF1, or 3'UTR regions of
546 LINE-1. Reference genes: *GAPDH* and *TUBB*. Three biological replicates; mean \pm s.e.m. **g)**

547 LINE-1 ORF1 protein immuno-staining (magenta, same exposure and intensity scaling) plus
548 merged channels with DAPI (blue) in Calu3 cells cultured in basal or myeloid conditioned
549 media. Scale bar: 10 μm . **h)** Normalized cellular total LINE-1 ORF1p immuno-staining signals
550 of Calu3 cells cultured in basal (n = 84, mean = 1.0, median = 0.9) or myeloid conditioned media
551 (n = 126, mean = 1.7, median = 1.5). Combination of two independent cell samples. Box plots
552 show median (inside line), means (red dot), interquartile range (IQR, box), and upper/lower
553 quartile \pm 1.5-times IQRs (whiskers). Welch's t-test.

554 Supplementary Figures

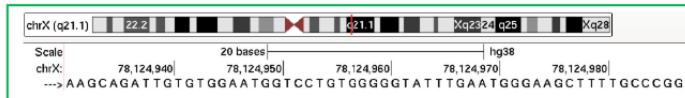
a

Data ID (GEO)	Sample	SARS-CoV-2 infection	RNA-Seq read length (nt)	Min overhang (threshold)
GSE147507	Calu3, A549-ACE2, NHBE	MOI 2, 24 hrs	150	50
GSE153277	iPSC-derived type II alveolar epithelial-like cells	MOI 5, 4 days	76+76	50
GSE156754	iPSC-derived cardiomyocytes	MOI 0.1, 48 hrs	76+76	50
GSE157852	iPSC-derived choroid plexus organoids	MOI 0.1-0.05, 72 hrs	72	20
GSE153684	Cultured organoid from pediatric stomach tissue (age 11 year)	MOI 1	51+51	20
GSE145926	Bronchoalveolar lavage fluid (BALF) cells	COVID19+ patient (severe)	26+100	20
GSE154998	Blood buffy coat cells	COVID19+ patient (ICU)	72	20



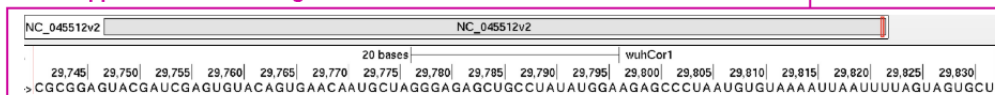
c Human - CoV2 chimeric read example from Calu3 (infected) RNA-Seq:

57 nt mapped to human Chromosome X

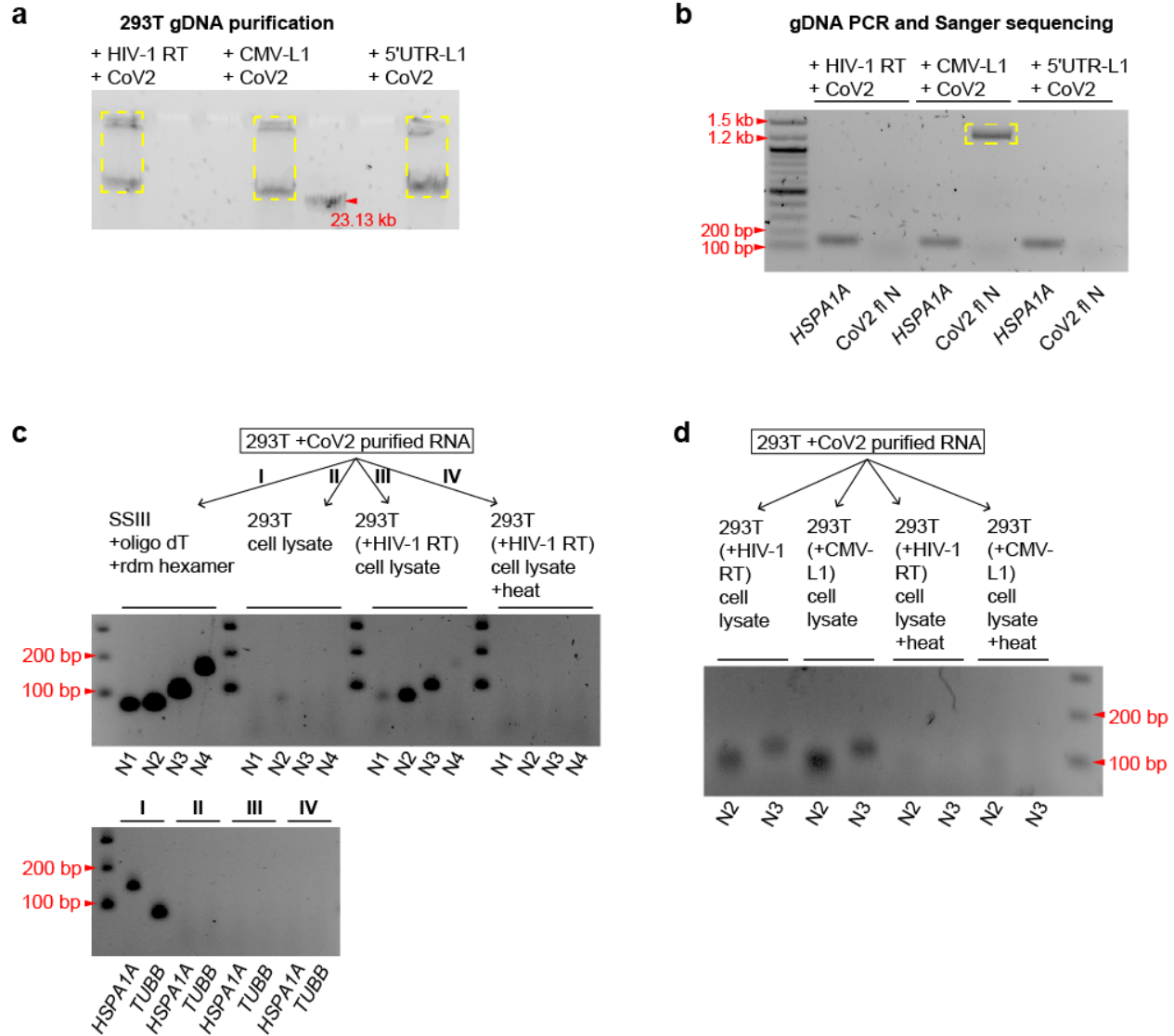


AAGCAGATTGTGTGGAATGGTCCTGTGGGGTATTTGAATGGGAAGCTTTT
 GCCCGGCGCGTAGTACGATCGAGTGTACAGTGAACAATGCTAGGGAGAGC
 TGCCATATGGAAGAGCCCTAATGTGTAATAATTTAGTAGTGCT

92 nt mapped to SARS-CoV-2 genome



556 **Supplementary Figure 1. Human – SARS-CoV-2 chimeric reads identified from published**
557 **RNA-Seq data. a)** Published data used to identify human – CoV2 chimeric reads summarizing
558 GEO accession number (data ID), sample type, infection method/type (MOI: Multiplicity Of
559 Infection), RNA-Seq format (single or paired-end with read length), and threshold to call
560 chimeric reads (Min overhang: minimum number of bases mapped to either human or SARS-
561 CoV-2 genome/transcriptome to call a chimeric reads). **b)** Comparison of SARS-CoV-2 read
562 fraction of total mappable reads in the published RNA-Seq datasets as shown in **a)**. **c)** One
563 chimeric read example (149 nt) from Calu3 (infected) RNA-Seq with 57 nt mapped to human
564 Chromosome X (green) and 92 nt (magenta) mapped to the SARS-CoV-2 genome.



565

566 **Supplementary Figure 2. SARS-CoV-2 RNA can be reverse-transcribed *in vivo* and *in vitro***

567 **by other sources of reverse transcriptase. a)** Gel purification of large-fragment genomic DNA

568 (yellow boxes) from SARS-CoV-2 infected HEK293T cells carrying transfected HIV-1 RT,

569 CMV-LINE1 or 5'UTR-LINE1. **b)** Cloning and Sanger sequencing of DNA copy of full-length

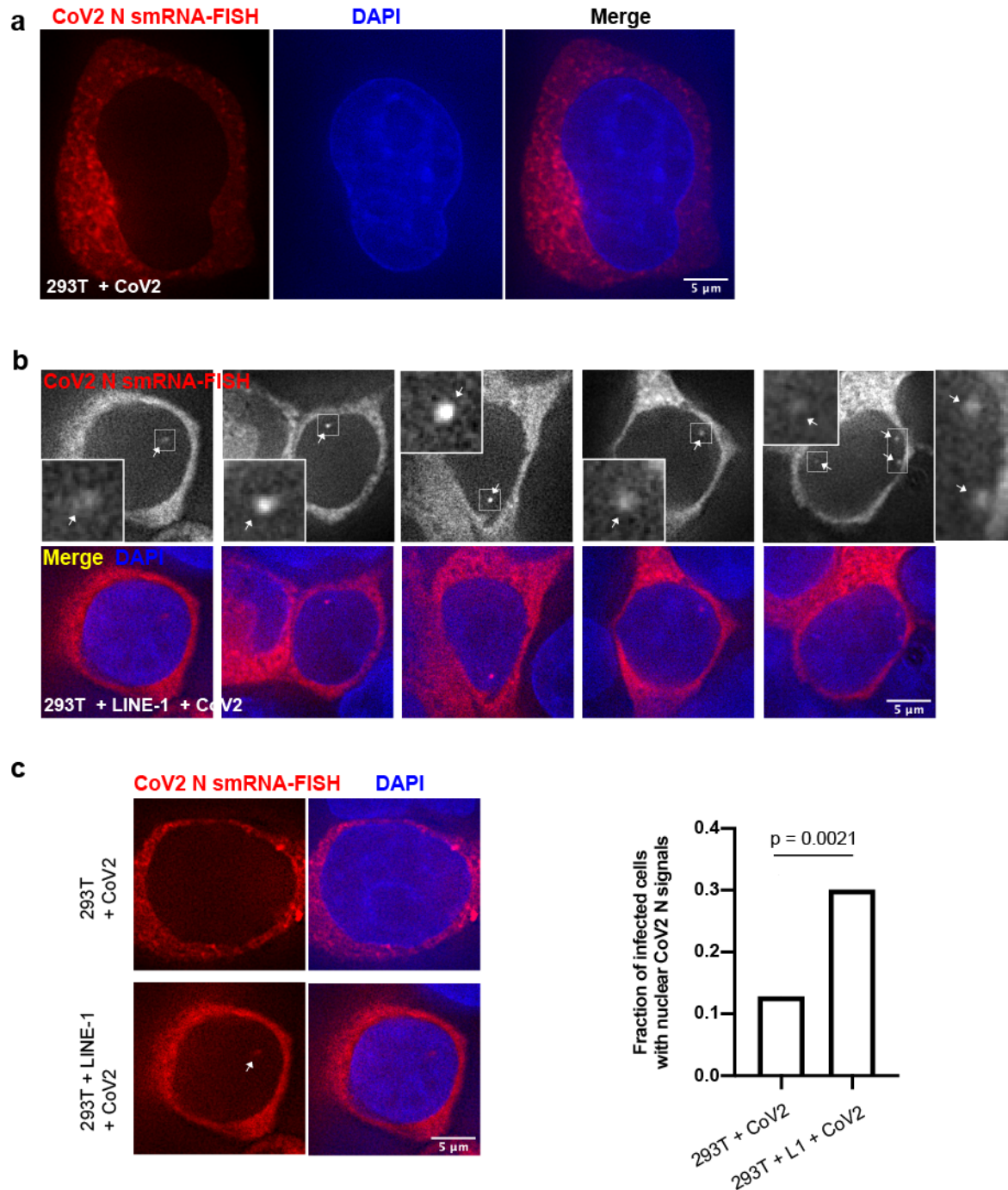
570 SARS-CoV-2 N sequence (CoV2 fl N, yellow box) from gel-purified HEK293T genomic DNA

571 as shown in **a**. CoV2 fl N: amplification of full-length N sequence (1.26 kb) by primers

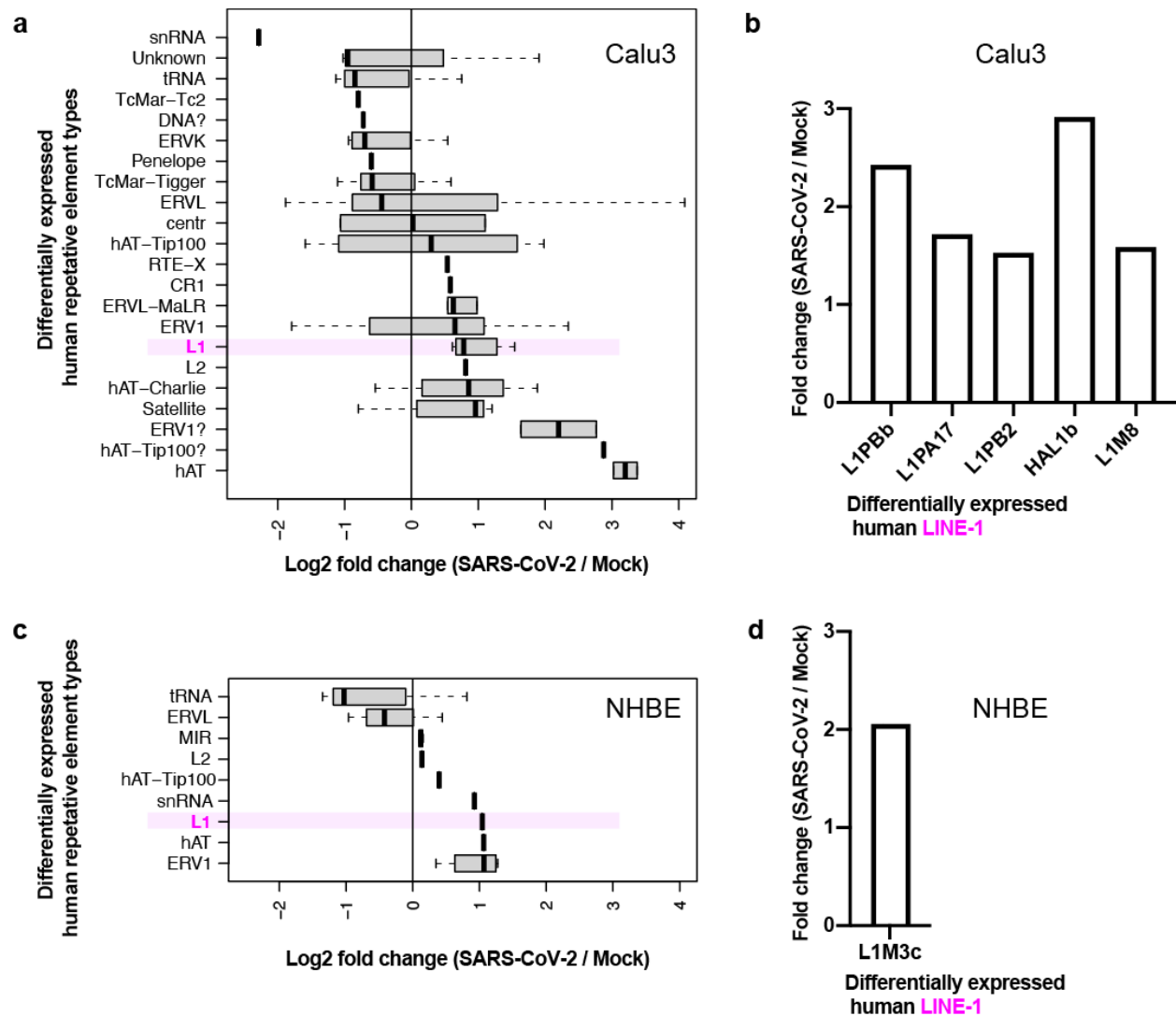
572 targeting the two ends of N. *HSPA1A*: human *HSPA1A* gene as a control. Note that we can only

573 detect full-length N sequence in gDNA from cells with CMV-LINE-1 expression, corresponding

574 to the high copy-number of integrated N sequences as shown in **Figure 2c**. **c)** *In vitro* reverse
575 transcription of SARS-CoV-2 RNA by adding RNA purified from SARS-Cov-2 infected
576 HEK293T cells to a commercial reverse transcriptase (I, SSIII, with oligo dT and random
577 hexamer primers, positive control), or HEK293T cell lysate (II), or lysates of HEK293T cells
578 expressing HIV-1 reverse transcriptase without (III) or with (IV) heat inactivation. Gel images
579 showing PCR detection of SARS-CoV-2 N sequences from the *in vitro* reverse transcription
580 products using primer sets (N1 – N4) as shown in **Figure 2a**. *HSPA1A* and *TUBB*: PCR primer
581 sets against human *HSPA1A* and *TUBB* genes as controls. **d)** Same *in vitro* reverse transcription
582 and PCR detection setup as in **c)** using lysates of HEK293T cells expressing HIV-1 reverse
583 transcriptase or human LINE-1.



589 transfection. Insets in **b**): 4x enlargement of regions in white-boxes to show nuclear signals of
590 SARS-CoV-2 N sequence (white arrows). **c**) Comparison of nuclear N RNA-FISH signals in
591 SARS-CoV-2 infected HEK293T cells without or with human LINE-1 transfection. Left:
592 example images as in **a**) and **b**); Right: fraction of HEK293T cells infected by SARS-CoV-2
593 (indicated by cytoplasmic FISH signals) showing nuclear N RNA-FISH signals in cell
594 populations without (left bar, n = 109) or with (right bar, n = 103) CMV-LINE-1 plasmid
595 transfection (~80% transfection efficiency). Combination of two independent cell samples; Chi-
596 Square Test of Homogeneity. All images shown were single z-slices from 3D optical sections
597 (0.2- μ m z-steps).



598

599 **Supplementary Figure 4. LINE-1 induction in human cells correlates with SARS-CoV-2**

600 **infection. a, c** Log2 fold-changes (x-axis) of different types of human repetitive elements (y-

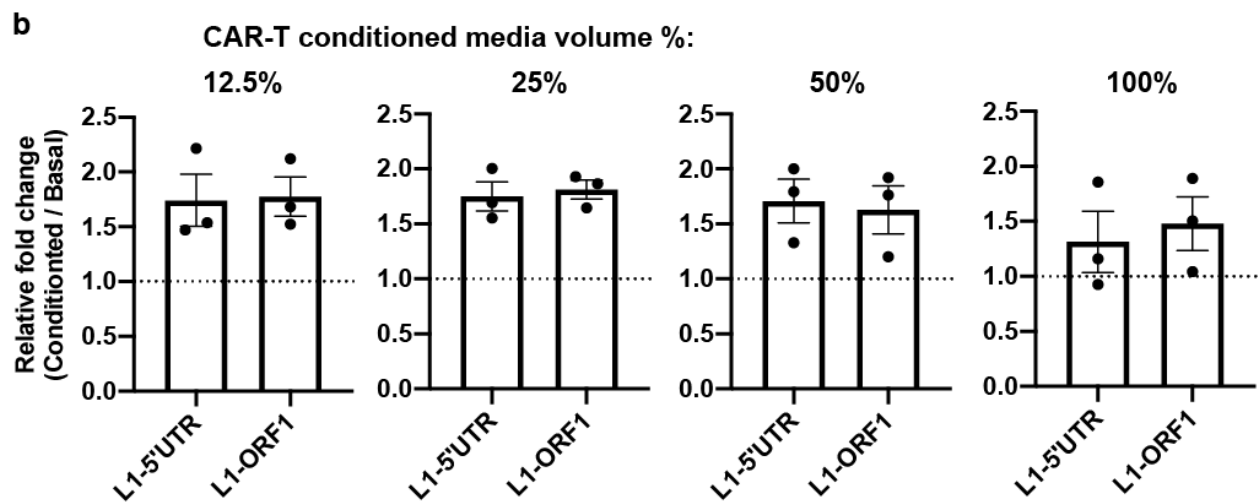
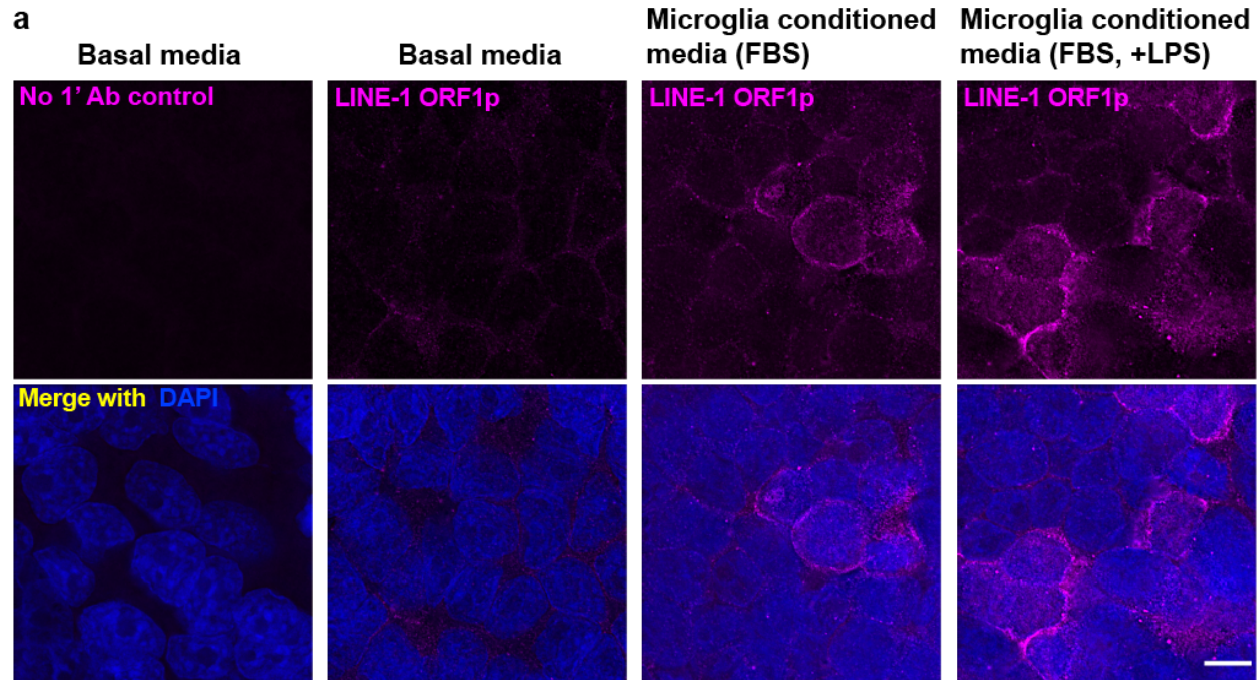
601 axis) with significant (FDR < 0.05) expression changes in SARS-CoV-2 versus mock infected

602 Calu3 (**a**) or NHBE (**c**) cells from published RNA-Seq data (GSE147507). **b, d** Fold changes (y-

603 axis) of different human LINE-1 families (x-axis) with significant (FDR < 0.05) expression

604 changes in SARS-CoV-2 versus mock infected Calu3 (**b**) or NHBE (**d**) cells from published

605 RNA-Seq data (GSE147507, see **Supplementary Figure 1a**).



606

607 **Supplementary Figure 5. Cytokine containing media treatment triggers LINE-1 expression**

608 **in human cells. a)** LINE-1 ORF1 protein immuno-staining (magenta, same exposure and

609 intensity scaling, 1st column: no primary antibody control) plus merged channels with DAPI

610 (blue) in HEK293T cells cultured in basal (1st and 2nd columns) or microglia conditioned media

611 (3rd column) or LPS-treated microglia conditioned media (4th column). Scale bar: 10 μ m. **b)**

612 Endogenous LINE-1 expression fold-changes in Calu3 cells between CAR-T conditioned

613 (diluted with basal media at indicated percentage in volume) versus basal media treatment
614 measured by RT-qPCR with primers probing 5'UTR, ORF1, or 3'UTR regions of LINE-1.
615 Reference genes: *GAPDH* and *TUBB*. Three independent cell samples treated with two batches
616 of media; mean \pm s.e.m.

617 **Supplementary Tables**

618

619 **Supplementary Table 1. Primer sequences used in this study**

Species	Name	Sequence
SARS-CoV-2	N1	Forward: GACCCCAAATCAGCGAAAT Reverse: TCTGGTACTGCCAGTTGAATCTG
SARS-CoV-2	N2	Forward: GGGAGCCTTGAATACACCAAAA Reverse: TGTAGCACGATTGCAGCATTG
SARS-CoV-2	N3	Forward: GGGGA ACTTCTCCTGCTAGAAT Reverse: CAGACATTTTGCTCTCAAGCTG
SARS-CoV-2	N4	Forward: AAATTTTGGGGACCAGGAAC Reverse: TGGCACCTGTGTAGGTCAAC
SARS-CoV-2	N (full length)	Forward: ATGTCTGATAATGGACCCCAAAT Reverse: TTAGGCCTGAGTTGAGTCAGC
Human	<i>HSPA1A</i>	Forward: ATCTCCACCTTGCCGTGTT Reverse: ATCCAGTGTTCCGTTTCCAG
Human	<i>TUBB</i>	Forward: TCCCTAAGCCTCCAGAAACG Reverse: CCAGAGTCAGGGGTGTTTCAT
Human	<i>GAPDH</i>	Forward: GTCTCCTCTGACTTCAACAGCG Reverse: ACCACCCTGTTGCTGTAGCCAA
Human	LINE-1-5'UTR	Forward: GACGCAGAAGACGGTGATTT Reverse: TCACCCTTTCTTTGACTCG
Human	LINE-1-ORF1	Forward: CTCGGCAGAAACCCTACAAG Reverse: CCATGTTTAGCGCTTCCTTC
Human	LINE-1-3'UTR	Forward: CATGGAATACTATGCAGCCATAAA Reverse: TCCCACCTATGAGTGAGAA

620

621 **Supplementary Table 2.** Single-molecule RNA FISH probe sequences used in this study

Name	Sequence
Cov2NC 1	tgattttgggtccattatc
Cov2NC 2	agggtccaccaaacgtaatg
Cov2NC 3	tggttactgccagttgaatc
Cov2NC 4	ttattgggtaaaccttgggg
Cov2NC 5	tgagagcgggtgaaccaagac
Cov2NC 6	cctcgagggaatttaaggtc
Cov2NC 7	ttggtgtaattggaacgcc
Cov2NC 8	aatttggatctctggactgc
Cov2NC 9	accacgaattcgtctggtag
Cov2NC 10	gatctttcattttaccgtca
Cov2NC 11	tttgtagcaccatagggaa
Cov2NC 12	cagttgcaacccatgatg
Cov2NC 13	gattgcagcattgtagcag
Cov2NC 14	tagaagccttttgcaatgt
Cov2NC 15	acgagaagaggcttgactgc
Cov2NC 16	actgttgcgactacgtgatg
Cov2NC 17	tgctggagttgaatttctt
Cov2NC 18	cagcaaagcaagagcagcat
Cov2NC 19	agctggttcaatctgtcaag
Cov2NC 20	tttaccagacattttgctct
Cov2NC 21	tatgctttagtggcagtacg
Cov2NC 22	gccgaaagcttgtgttacat
Cov2NC 23	aatttcttgggtttgttct
Cov2NC 24	cttgctctgattagttcctgg
Cov2NC 25	ggccaatgtttgtaatcagt
Cov2NC 26	tgggggcaaattgtgcaatt
Cov2NC 27	cgacattccgaagaacgctg
Cov2NC 28	gtgtgacttccatgccaatg
Cov2NC 29	tgtgtaggtcaaccacgttc
Cov2NC 30	atttggatctttgtcatcca
Cov2NC 31	ttgtatgcgtcaatatgctt
Cov2NC 32	ggtaaggcttgagtttcatc
Cov2NC 33	gaagagtcacagtttctgt
Cov2NC 34	atcatcacaatctgcagcag
Cov2NC 35	ggattgtgcaattgtttgg

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