

1 Analysis of SARS-CoV-2-controlled autophagy reveals spermidine, MK-2206, and niclosamide as
2 putative antiviral therapeutics

3

4 **Authors/Affiliation**

5 Nils C. Gassen^{1§}, Jan Papias^{2,3}, Thomas Bajaj¹, Frederik Dethloff⁴, Jackson Emanuel^{2,3}, Katja Weckmann¹,
6 Daniel E. Heinz¹, Nicolas Heinemann^{2,3}, Martina Lennarz¹, Anja Richter^{2,3}, Daniela Niemeyer^{2,3}, Victor
7 M. Corman^{2,3}, Patrick Giavalisco⁴, Christian Drosten^{2,3}, Marcel A. Müller^{2,3,5§}

8

9 ¹Department of Psychiatry and Psychotherapy, University of Bonn, 53127 Bonn, Germany

10 ²Institute of Virology, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität
11 Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 10117 Berlin, Germany

12 ³German Centre for Infection Research (DZIF), partner site Charité, 10117 Berlin, Germany

13 ⁴Max Planck Institute for Aging, 50931 Cologne, Germany

14 ⁵Martsinovskiy Institute of Medical Parasitology, Tropical and Vector Borne Diseases, Sechenov
15 University, 119991 Moscow, Russia

16

17 Contact: [§]Correspondence should be addressed to Nils C. Gassen (nils.gassen@ukbonn.de) or Marcel
18 A. Müller (marcel.mueller@charite.de)

19

20

21 **Abstract**

22 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses an acute threat to public health
23 and the world economy, especially because no approved specific drugs or vaccines are available.
24 Pharmacological modulation of metabolism-dependent cellular pathways such as autophagy reduced
25 propagation of highly pathogenic Middle East respiratory syndrome (MERS)-CoV.

26 Here we show that SARS-CoV-2 infection limits autophagy by interfering with multiple metabolic
27 pathways and that compound-driven interventions aimed at autophagy induction reduce SARS-CoV-2
28 propagation *in vitro*. In-depth analyses of autophagy signaling and metabolomics indicate that SARS-
29 CoV-2 reduces glycolysis and protein translation by limiting activation of AMP-protein activated kinase
30 (AMPK) and mammalian target of rapamycin complex 1 (mTORC1). Infection also downregulates
31 autophagy-inducing spermidine, and facilitates AKT1/SKP2-dependent degradation of autophagy-
32 initiating Beclin-1 (BECN1). Targeting of these pathways by exogenous administration of spermidine,
33 AKT inhibitor MK-2206, and the Beclin-1 stabilizing, antihelminthic drug niclosamide inhibited SARS-
34 CoV-2 propagation by 85, 88, and >99%, respectively. In sum, SARS-CoV-2 infection causally diminishes
35 autophagy. A clinically approved and well-tolerated autophagy-inducing compound shows potential
36 for evaluation as a treatment against SARS-CoV-2.

37

38

39 The current pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses an
40 imminent threat to global health. As of 15 April 2020, 1,878,489 individuals were infected in >200
41 countries, with >119,000 fatalities (1). SARS-CoV-2 infections cause CoV-associated disease 19 (COVID-
42 19), which can lead to severe atypical pneumonia in humans (2). Currently, there are no approved
43 therapeutics or vaccines available. The development and licensing of new FDA-approved drugs takes
44 years, which is problematic given the urgent need for effective therapies against novel, rapidly
45 emergent diseases like COVID-19. Antiviral drug screenings are commonly based on testing FDA-
46 approved compound libraries against cellular and viral components (3). However, these undirected
47 approaches lack functional insights into how the drugs affect virus propagation. Risk evaluations for
48 drug repurposing and development of new therapeutics would benefit from rational drug design
49 founded on known SARS-CoV-2-host interactions.

50 Compound-based targeting of cellular proteins that are essential for the virus life cycle has led to the
51 discovery of broadly reactive drugs against a range of CoVs (3-6). As virus propagation strongly
52 depends on energy and catabolic substrates of host cells, drug target identification should consider
53 the metabolism of infected cells (3). Autophagy, a highly conserved cytosolic degradation process of
54 long-lived proteins, lipids, and organelles in eukaryotic cells, is tightly controlled by metabolism (7, 8).
55 During autophagy, intracellular macromolecules are recycled by incorporation into LC3B-lipidated
56 autophagosomes (AP) and degradation into their monomers, such as fatty and amino acids, after
57 fusion with low pH lysosomes (9). In the case of highly pathogenic Middle East respiratory syndrome
58 (MERS)-CoV, we recently showed that autophagy is limited by a virus-induced AKT1-dependent
59 activation of the E3-ligase S-phase kinase-associated protein 2 (SKP2), which targets the key autophagy
60 initiating protein Beclin-1 (BECN1) for proteasomal degradation (10). Congruently, inhibition of SKP2
61 by different compounds, including clinically approved drugs, stabilized BECN1 and limited MERS-CoV
62 propagation, indicating that autophagy-inducing compounds hold promise for evaluation as antiviral
63 drugs. This paper investigates the impact of SARS-CoV-2 infection on cell metabolism and the
64 downstream effects on autophagy, thereby identifying multiple targets for the application of approved
65 drugs and the development of new antiviral therapies.

66 We aimed to characterize the effect of SARS-CoV-2 infection on autophagy by applying previously
67 established assays (10) and additionally including detailed analyses of upstream autophagy regulators
68 according to expert-curated guidelines for detecting autophagy (11). First, we explored whether SARS-
69 CoV-2 reduces autophagic flux, a measure of autophagic degradation activity. We infected human
70 bronchial epithelial cells NCI-H1299 and monkey kidney cells (VeroFM) with SARS-CoV-2 strain Munich
71 (multiplicity of infection (MOI) of 0.0005) or heat-inactivated virus (mock-infected cells) and tested
72 autophagic flux by co-treatment with bafilomycin A1 (BafA1). BafA1 is a specific inhibitor of vacuolar
73 H⁺-ATPase interfering with lysosome acidification and degradation of AP cargo and preventing the

74 fusion of APs with lysosomes to autophagy-active autophagolysosomes (AL) (11). Based on our
75 previous experience (10), we chose a low MOI of 0.0005 and time points 8, 24, and 48 hours post
76 infection to monitor autophagy during the exponential growth of SARS-CoV-2 reaching maximum titers
77 of 10^7 genome equivalents per ml (GE/ml; NCI-H1299) and 10^{10} GE/ml (VeroFM) at 48 hours post
78 infection (**Figure S1a**). We found that 100 nM of BafA1 was sufficient to induce maximal LC3B lipidation
79 levels in both cell cultures (**Figure S1b-d**). Immunoblotting of LC3B-II/I in the presence of 100 nM BafA1
80 indicated that mock-infected compared to SARS-CoV-2-infected cells showed a significant increase of
81 LC3B-II over LC3B-I due to the BafA1-induced inhibition of autophagic flux (**Figures 1a-b**). At 8 hours
82 post infection, LC3B-II levels were slightly elevated in SARS-CoV-2-infected cells but could still be
83 enhanced by BafA1 treatment. At 24 and 48 hours, (vehicle- or) BafA1-treated and SARS-CoV-2
84 infected cells showed equally limited autophagic flux (**Figures 1a-b**). A reduction of autophagic flux
85 was further corroborated by elevated levels of the autophagy receptor SQSTM1/P62 in SARS-CoV-2-
86 infected cells (**Figures 1c-d**). Furthermore, we used a well-established autophagy reporter plasmid,
87 ptfLC3, to assess lysosomal-autophagosomal fusion, a hallmark of autophagic flux (12). The number of
88 ALs (in red) in mRFP and EGFP dual-tagged LC3B-expressing and SARS-CoV-2 infected NCI-H1299 cells
89 was clearly reduced compared to mock-infected cells (**Figures 1e-f**), indicating virus-induced reduction
90 of AP and AL fusion. Quantification showed that the total number of ALs was reduced from a mean of
91 88 to 62 vesicles per cell, whereas the number of APs per cell was comparable (Mock = 56 vesicles/cell;
92 SARS-CoV-2 = 51 vesicles/cell). The ratio of APs to ALs shifted in SARS-CoV-2-infected cells, indicating
93 strong AP accumulation (Mock= 0.707 ± 0.053 ; SARS-CoV-2= 0.921 ± 0.058) (**Figure 1e**). Taken together,
94 SARS-CoV-2 strongly reduced the autophagic flux in both cell lines, in a fashion similar to MERS-CoV
95 (10).

96 The initiation of autophagy is a cumulative result of multiple protein and signaling cascades that are
97 involved in the energy and nutrient sensing of cells (7, 11). The AMP- activated protein kinase (AMPK)
98 and the mammalian target of rapamycin complex 1 (mTORC1) kinase are in continuous crosstalk with
99 glucose and protein homeostasis respectively (13, 14). We monitored key proteins of the AMPK and
100 mTORC1 pathways and their regulation/degradation by Western blot analyses after infection with
101 SARS-CoV-2 (**Figure 2**). SARS-CoV-2 infection influenced most of the analyzed components of the
102 AMPK/mTORC1 pathway. Phosphorylated, active forms of AMPK, AMPK substrates (LXRXX), AMPK
103 downstream targets (TSC2 and ULK1), and mTORC1 were downregulated upon SARS-CoV-2 infection,
104 suggesting a virus-induced reduction of cellular glycolysis, protein translation, and cell growth. In
105 addition, we found increased levels of phosphorylated AKT1, which activates the negative regulator of
106 BECN1, SKP2 (10). Low BECN1 levels and subsequently reduced ATG14 phosphorylation and
107 oligomerization (**Figure 2, lower left panel**) explain the lack of fusion of APs with lysosomes observed
108 in **Figure 1e-f** as ATG14 oligomers facilitate fusion via SNARE-proteins, SNAP29 and STX17 (10). In

109 summary, SARS-CoV-2 alters autophagy-relevant signaling and at the same time appears to hamper
110 AP/lysosome fusion efficiency via autophagy.

111 To further explore the direct impact of SARS-CoV-2 infection on AMPK/mTORC1, we performed a
112 metabolomics profiling analysis in VeroFM cells 24 hours post infection using an elevated MOI of 0.1
113 to guarantee ubiquitous infection of cells (**Figure 3**). The metabolite profiles were analyzed by
114 multivariate principal component analysis (PCA), an unsupervised statistical method suitable for
115 analyzing and classifying metabolomics datasets (15). The profiles clearly separated into SARS-CoV-2
116 and control group (**Figure S2a**). Altogether, the levels of 25 metabolites were significantly altered by
117 SARS-CoV-2 infection (**Supplementary Table 1**). We performed pathway analyses in order to gain
118 insights into the cellular and mechanistic consequences of a SARS-CoV-2 infection. We retrieved eight
119 affected pathways using the significantly altered metabolites from **Supplementary Table 1 (Figure 3a)**.
120 Glutathione (GSH) metabolism, pyrimidine metabolism, and aminoacyl-tRNA biosynthesis had the
121 most pronounced changes. Metabolites of the butanoate metabolism as well as the alanine, aspartate
122 and glutamate metabolism were also elevated. In contrast, the tricarboxylic acid (TCA) cycle, glutamine
123 and glutamate metabolism, and glyoxylate and dicarboxylate metabolism were reduced by SARS-CoV-
124 2. The volcano plot visualizes the metabolites with large magnitude fold changes. We identified seven
125 altered metabolites: Cys-glycine, N-acetylputrescine, adenosine, putrescine, homocysteine and
126 cysteine levels were elevated whereas fructose-6P was strongly depleted (**Figure 3b**). Putative links
127 between the different pathways during SARS-CoV-2 infection are shown in **Figure 3c**. We observed
128 elevated ATP levels that might facilitate energy-dependent processes such as virus replication and
129 entry (**Figure 3c, blue**). High lactic acid and low fructose-6P levels indicate exhaustive glycolysis activity
130 (**Figure 3c, green**). Decreased levels of TCA metabolites (**Figure 3c, purple**) further support a switch
131 from (mitochondria-dependent) oxidative phosphorylation to lactic acid fermentation, to provide
132 NAD⁺ for glycolytic ATP production. Consequently, we observed a reduced AMP/ATP ratio (**Figure 3c,**
133 **blue**) limiting AMPK activation (16) and subsequently autophagy (see **Figure 2**). Limited autophagy
134 might prevent degradation of viral products and might enhance the availability of non-autophagic
135 membrane material to generate double-membrane vesicles that are generated during coronavirus
136 replication (17, 18). Interestingly, despite reduction of protein-degrading autophagy, we observed
137 strong upregulation of amino acids, especially those linked to glutathione metabolism, which is
138 responsible for elimination of reactive oxygen species (ROS). This might be essential to limit the
139 mitochondrion-derived ROS that are generated during excessive ATP production (19) and should be
140 investigated in future studies. Further investigations should also clarify why the observed upregulated
141 amino acid levels do not activate mTORC1 (see **Figure 2**, pmTOR^{S2448}/mTOR and pULK1^{S757}/ULK1).
142 However, spatially distinct pools of mTORC1 proteins might serve as an explanation (20).

143 Interestingly, putrescine (**Figure 3c, red**) was increased whereas its downstream products of the
144 polyamine biosynthesis pathway, spermidine and spermine, were strongly reduced. The putrescine
145 increase might be explained by a SARS-CoV-2-induced inhibition of spermidine synthase, the enzyme
146 that synthesizes spermidine from putrescine (**Figure 3c, red**). Spermidine is an important polyamine
147 capable of inducing autophagy (21) and responsible for hypusination of the eukaryotic translation
148 initiation factor eIF5A (22). eIF5A activates the autophagy transcription factor TFEB and regulates
149 proteins responsible for mitochondrial respiration (23).

150 Next, we targeted different components of the metabolic/autophagic pathway by exogenous
151 administration of selective inhibitors, approved drugs, or cell metabolites, and explored the effect on
152 SARS-CoV-2 propagation (**Figure 4**). Cell viability tests were performed for inhibitors and drugs to
153 exclude toxicity (**Figure S3a**). VeroFM cells were infected with an MOI of 0.0005 and treated 1 hour
154 post infection with different concentrations of each substance according to the manual instructions or
155 previous publications. Virus growth was monitored by real-time RT-PCR in cell culture supernatants 24
156 and 48 hours post infection (**Figure S3b-d**). DFMO (500 μ M) blocks ornithine-to-putrescine synthesis
157 (24) and was previously described as an RNA virus inhibitor upon pretreatment (25) but had minor
158 effects on SARS-CoV-2 growth upon post-treatment, whereas spermidine (100 μ M) and spermine (100
159 μ M) inhibited SARS-CoV-2 propagation by up to 66% (**Figure 4a, upper left panel, Figure S3b,c**). AICAR
160 (25 μ M), a known AMPK activator (26), slightly induced virus growth at 24 hours post infection (**Figure**
161 **4a, upper right panel**), which seems contradictory, as AMPK is downregulated upon SARS-CoV-2
162 infection (see **Figure 2**), but could be explained by AMPK-induced mTORC1 inhibition or AMPK-
163 unrelated functions of AICAR (27). Alternatively, AICAR-dependent AMPK activation might be
164 insufficient to restore autophagic flux (see **Figure 1**). In addition, mTORC1 inhibition by rapamycin (0.3
165 μ M) clearly induced virus growth (**Figure 4a, right panel, Figure S3d,e**). Rapamycin, which has
166 previously been proposed as a treatment option for COVID-19 (28), possibly enhances the inhibitory
167 effects of SARS-CoV-2 on mTORC1 (see **Figure 2**) to reduce cellular protein translation, which would
168 serve as an additional explanation for the above-mentioned elevated amino acid levels (see **Figure 3c,**
169 **orange**). These results suggest that detailed molecular and functional analyses should be performed
170 for rapamycin before considering its use in clinical trials. The AKT1 inhibitor MK-2206 (1 μ M), which is
171 currently being tested in a clinical phase 2 study against breast cancer (29), reduced SARS-CoV-2
172 propagation up to 50% (**Figure 4a, lower right, Figure S3d,e**). AKT1 blocks mTORC1 inhibitor TSC2 (30)
173 and further supports the suggestion that up-regulation of mTORC1 components has antiviral effects.
174 As AKT1 inhibition results in BECN1 up-regulation and autophagy induction (10, 31), SARS-CoV-2
175 growth inhibition was expected. Direct blocking of the negative BECN1 regulator SPK2 by previously
176 described inhibitors SMIP004, SMIP004-7, valinomycin, and niclosamide (10) showed SARS-CoV-2
177 growth inhibition from 50 (SMIP004, SMIP004-7) to over 99% in case of valinomycin and niclosamide

178 **(Figure 4a, lower panel, Figure S3d,e)**. We further confirmed that the dominant intervention of
179 niclosamide during SARS-CoV-2 infection acts on autophagy induction, as adding BafA1 after
180 niclosamide treatment showed an enhancing effect on the lipidation of LC3B as reflected by
181 comparable LC3B-II/I ratios between mock- and SARS-CoV-2-infected cells **(Figure 4b)**. However, we
182 cannot exclude that the activity of niclosamide as a hydrogen ionophore has additional inhibitory
183 functions, e.g. by blocking endosomal acidification (32), which is important for SARS-CoV-2 entry (6).
184 We further used our in vitro model to explore the possibility of prophylactic treatment with spermidine
185 and niclosamide, which are well-tolerated, clinically applied (33) or FDA-approved (34) compounds,
186 respectively. To assess the efficacy of pretreatment, VeroFM cells were preincubated with spermidine
187 (100 μ M) or niclosamide (5 μ M) for 24 hours. Cells were infected with SARS-CoV-2 using an MOI of
188 0.05 without further compound treatment and virus growth was monitored in supernatants for 24
189 hours **(Figure 4c, Figure S3f)**. In both cases, SARS-CoV-2 growth was reduced by 70%, suggesting that
190 both compounds exhibit long-lasting antiviral effects, and supporting further investigation into
191 prophylactic use of these compounds. For clinical use, half-maximal inhibitory concentration (IC₅₀) of
192 compounds should be in a non-toxic range and reach adequate plasma levels (35). The IC₅₀ was 149
193 μ M ($R^2 = 0.71$) for spermidine, 0.09 μ M ($R^2 = 0.95$) for MK-2206, and 0.17 μ M ($R^2 = 0.63$) for niclosamide,
194 based on plaque-forming infectious units **(Figure 4c)**. Maximal inhibition of infectious virus at non-
195 toxic concentrations was identified at 333.3 μ M for spermidine (85%), at 3.7 μ M for MK-2206 (88%),
196 and at 1.24 μ M for niclosamide (>99%). Whereas plasma levels and pharmacokinetics of spermidine
197 are yet to be evaluated, peak plasma concentrations were 0,176 μ g/ml (0.43 μ M) for MK-2206 (36)
198 and 0.25 to 6.0 μ g/ml (0.76-18.35 μ M) for niclosamide (37), encouraging use in clinical trials.
199 In summary, our data show that highly pathogenic SARS-CoV-2 reprograms the metabolism of cells
200 and limits AMPK/mTORC1 activation and autophagy. Our mechanistic approach, including metabolism,
201 protein/phosphoprotein analyses and targeted SARS-CoV-2 inhibition assays, identified multiple
202 cellular targets for the development of new and application of already available antiviral compounds.

203

204 **Acknowledgments**

205 We thank Patricia Tscheak and Antje Richter (Charité) for excellent technical assistance and Terry Jones
206 for editing of the manuscript. **Funding:** CD was supported by BMBF-RAPID 01KI1723A. **Author**
207 **contributions:** N.C.G. and M.A.M. designed and conceived the work. N.C.G., J.P., T.B., F.D., J.E., N.H.,
208 D.E.H., M.L., A.R., M.A.M. carried out experiments. N.C.G., J.P., T.B., J.E., K.W. V.M.C., D.N., P.G., C.D.,
209 M.A.M. analyzed data or contributed essential material. N.C.G., M.A.M. wrote the main paper text.
210 N.C.G., T.B. prepared all figures. All authors reviewed the paper. **Competing interests:** None of the
211 authors declares a competing interest.

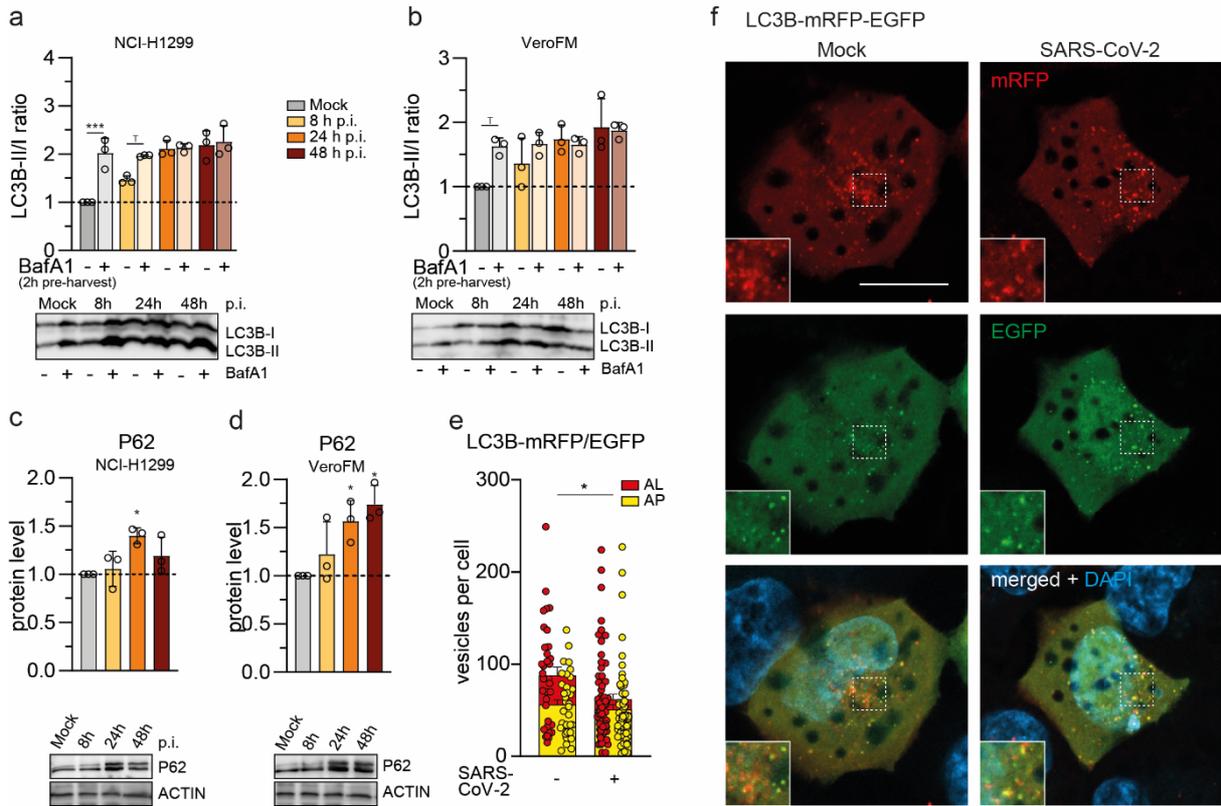
212

213 References

- 214 1. WHO, <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>. accessed
215 15 April 2020, (2020).
- 216 2. W. J. Guan *et al.*, Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl*
217 *J Med*, (2020).
- 218 3. J. Kindrachuk *et al.*, Antiviral potential of ERK/MAPK and PI3K/AKT/mTOR signaling
219 modulation for Middle East respiratory syndrome coronavirus infection as identified
220 by temporal kinome analysis. *Antimicrob Agents Chemother* **59**, 1088-1099 (2015).
- 221 4. S. Pfefferle *et al.*, The SARS-coronavirus-host interactome: identification of cyclophilins
222 as target for pan-coronavirus inhibitors. *PLoS Pathog* **7**, e1002331 (2011).
- 223 5. A. Lundin *et al.*, Targeting membrane-bound viral RNA synthesis reveals potent
224 inhibition of diverse coronaviruses including the middle East respiratory syndrome
225 virus. *PLoS Pathog* **10**, e1004166 (2014).
- 226 6. M. Hoffmann *et al.*, SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is
227 Blocked by a Clinically Proven Protease Inhibitor. *Cell*, (2020).
- 228 7. Y. Ohsumi, Historical landmarks of autophagy research. *Cell Res* **24**, 9-23 (2014).
- 229 8. K. Weckmann *et al.*, Metabolomics profiling reveals differential adaptation of major
230 energy metabolism pathways associated with autophagy upon oxygen and glucose
231 reduction. *Sci Rep* **8**, 2337 (2018).
- 232 9. T. Kirisako *et al.*, The reversible modification regulates the membrane-binding state of
233 Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J*
234 *Cell Biol* **151**, 263-276 (2000).
- 235 10. N. C. Gassen *et al.*, SKP2 attenuates autophagy through Beclin1-ubiquitination and its
236 inhibition reduces MERS-Coronavirus infection. *Nat Commun* **10**, 5770 (2019).
- 237 11. D. J. Klionsky *et al.*, Guidelines for the use and interpretation of assays for monitoring
238 autophagy (3rd edition). *Autophagy* **12**, 1-222 (2016).
- 239 12. S. Kimura, T. Noda, T. Yoshimori, Dissection of the autophagosome maturation process
240 by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy* **3**, 452-460
241 (2007).
- 242 13. I. Tamargo-Gomez, G. Marino, AMPK: Regulation of Metabolic Dynamics in the Context
243 of Autophagy. *Int J Mol Sci* **19**, (2018).
- 244 14. K. Inoki, J. Kim, K. L. Guan, AMPK and mTOR in cellular energy homeostasis and drug
245 targets. *Annu Rev Pharmacol Toxicol* **52**, 381-400 (2012).
- 246 15. B. Worley, R. Powers, Multivariate Analysis in Metabolomics. *Curr Metabolomics* **1**, 92-
247 107 (2013).
- 248 16. D. G. Hardie, AMPK--sensing energy while talking to other signaling pathways. *Cell*
249 *Metab* **20**, 939-952 (2014).
- 250 17. K. Knoops *et al.*, SARS-coronavirus replication is supported by a reticulovesicular
251 network of modified endoplasmic reticulum. *PLoS Biol* **6**, e226 (2008).
- 252 18. Y. Choi, J. W. Bowman, J. U. Jung, Autophagy during viral infection - a double-edged
253 sword. *Nat Rev Microbiol* **16**, 341-354 (2018).
- 254 19. M. P. Murphy, How mitochondria produce reactive oxygen species. *Biochem J* **417**, 1-
255 13 (2009).
- 256 20. R. Hatakeyama *et al.*, Spatially Distinct Pools of TORC1 Balance Protein Homeostasis.
257 *Mol Cell* **73**, 325-338 e328 (2019).
- 258 21. T. Eisenberg *et al.*, Induction of autophagy by spermidine promotes longevity. *Nat Cell*
259 *Biol* **11**, 1305-1314 (2009).

- 260 22. D. J. Puleston *et al.*, Polyamines and eIF5A Hypusination Modulate Mitochondrial
261 Respiration and Macrophage Activation. *Cell Metab* **30**, 352-363 e358 (2019).
- 262 23. H. Zhang *et al.*, Polyamines Control eIF5A Hypusination, TFEB Translation, and
263 Autophagy to Reverse B Cell Senescence. *Mol Cell* **76**, 110-125 e119 (2019).
- 264 24. H. M. Wallace, A. V. Fraser, Inhibitors of polyamine metabolism: review article. *Amino*
265 *Acids* **26**, 353-365 (2004).
- 266 25. B. C. Mounce *et al.*, Inhibition of Polyamine Biosynthesis Is a Broad-Spectrum Strategy
267 against RNA Viruses. *J Virol* **90**, 9683-9692 (2016).
- 268 26. J. E. Sullivan *et al.*, Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with
269 AICAR, a cell-permeable activator of AMP-activated protein kinase. *FEBS Lett* **353**, 33-
270 36 (1994).
- 271 27. J. Kirchner, B. Brune, D. Namgaladze, AICAR inhibits NFkappaB DNA binding
272 independently of AMPK to attenuate LPS-triggered inflammatory responses in human
273 macrophages. *Sci Rep* **8**, 7801 (2018).
- 274 28. Y. Zhou *et al.*, Network-based drug repurposing for novel coronavirus 2019-
275 nCoV/SARS-CoV-2. *Cell Discov* **6**, 14 (2020).
- 276 29. Y. Xing *et al.*, Phase II trial of AKT inhibitor MK-2206 in patients with advanced breast
277 cancer who have tumors with PIK3CA or AKT mutations, and/or PTEN loss/PTEN
278 mutation. *Breast Cancer Res* **21**, 78 (2019).
- 279 30. K. Inoki, Y. Li, T. Zhu, J. Wu, K. L. Guan, TSC2 is phosphorylated and inhibited by Akt and
280 suppresses mTOR signalling. *Nat Cell Biol* **4**, 648-657 (2002).
- 281 31. R. C. Wang *et al.*, Akt-mediated regulation of autophagy and tumorigenesis through
282 Beclin 1 phosphorylation. *Science* **338**, 956-959 (2012).
- 283 32. A. Jurgeit *et al.*, Niclosamide is a proton carrier and targets acidic endosomes with
284 broad antiviral effects. *PLoS Pathog* **8**, e1002976 (2012).
- 285 33. M. Wirth *et al.*, The effect of spermidine on memory performance in older adults at
286 risk for dementia: A randomized controlled trial. *Cortex* **109**, 181-188 (2018).
- 287 34. FDA,
288 [https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.proces](https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.proces&AppNo=018669)
289 [s&AppNo=018669](https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.proces&AppNo=018669). (accessed 01 April 2020).
- 290 35. C. Schwarz *et al.*, Safety and tolerability of spermidine supplementation in mice and
291 older adults with subjective cognitive decline. *Aging (Albany NY)* **10**, 19-33 (2018).
- 292 36. D. H. Ahn *et al.*, Results of an abbreviated phase-II study with the Akt Inhibitor MK-
293 2206 in Patients with Advanced Biliary Cancer. *Sci Rep* **5**, 12122 (2015).
- 294 37. W. E. Jones, Niclosamide as a treatment for *Hymenolepis diminuta* and *Dipylidium*
295 *caninum* infection in man. *Am J Trop Med Hyg* **28**, 300-302 (1979).
- 296
- 297

298 **Figure 1**
299

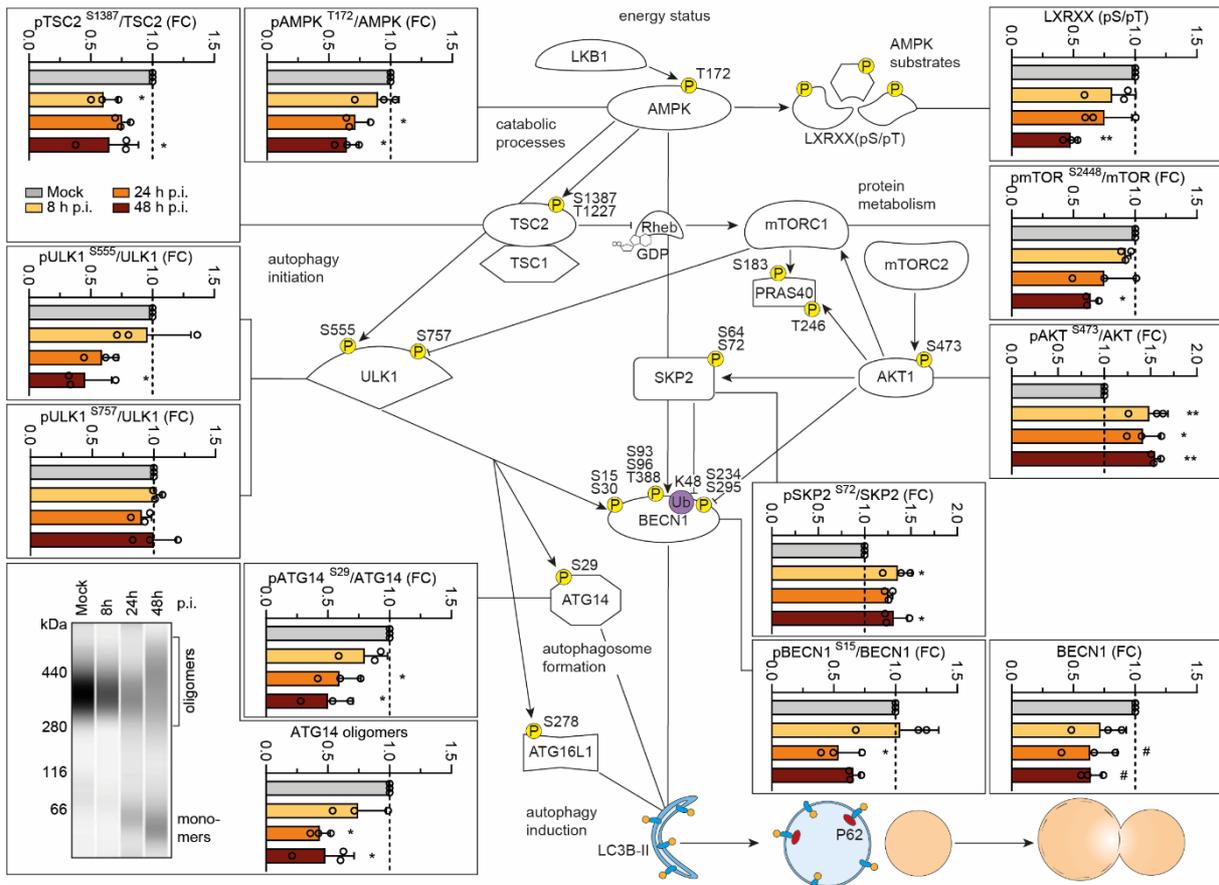


300
301

Fig. 1: SARS-CoV-2 decreases lysosomal-autophagosomal fusion. (a,b) SARS-CoV-2 blocks autophagic flux. NCI-H1299 cells (a) and VeroFM cells (b) were infected with SARS-CoV-2 (MOI = 0.0005) and incubated with bafilomycin A1 (BafA1) or vehicle (DMSO) for 2 h before samples were harvested at 8 h, 24 h, or 48 h post infection (h p.i.). The ratios of LC3B-II/I were determined by Western blotting. (c-d) SARS-CoV-2 stabilizes autophagy receptor P62 protein levels. Cells were infected analogously to (a-b) and harvested at indicated time points p.i. without additional treatment. P62 protein levels were determined by Western blotting. (e,f) SARS-CoV-2 blocks fusion of autophagosomes (AP) with lysosomes. NCI-H1299 cells were transfected with tandem fluorescently-tagged LC3B (mRFP and EGFP) and infected with SARS-CoV-2 (MOI = 0.0005). Twenty-four hours later, cells were fixed and analyzed by fluorescence microscopy. Vesicles with both green and red fluorescence (APs) and with red fluorescence only (autolysosomes, AL) were counted. In all panels error bars denote standard error of mean derived from n = 3 biologically independent experiments. $T_p < 0.1$, $*p < 0.05$, $***p < 0.001$ (two-way ANOVA in a,b, one-way ANOVA in c,d, t-test in e. Abbreviations: LC3B, microtubule-associated protein 1A/1B light chain 3B; mRFP, monomeric red fluorescent protein; EGFP, enhanced green fluorescent protein.

302
303
304
305
306
307
308
309
310
311
312
313
314
315
316

317 **Figure 2**



318

319 **Fig.2: SARS-CoV-2 hijacks autophagy signaling on multiple regulatory levels.** VeroFM cells were infected with SARS-CoV-2
 320 (MOI = 0.0005) and harvested at 8 h, 24 h, or 48 h post infection (h p.i.). Protein levels and phosphorylation status of selected
 321 autophagy-relevant proteins were determined by Western blotting. For analysis of ATG14 oligomers (bottom panel, left) cells
 322 were incubated with a chemical crosslinker (DSS) 2 h prior to cell harvest (for detailed protocol see methods section). Cell
 323 extracts were separated and immunoblotted using Wes (ProteinSimple) capillary electrophoresis. Error bars in all panels
 324 denote standard error of mean derived from n = 3 biologically independent experiments. $Tp < 0.1$, * $p < 0.05$, *** $p < 0.001$
 325 (one-way ANOVA; Bonferroni correction (post hoc)). Abbreviations: AMPK, AMP-activated protein kinase; TSC1/2, tuberous
 326 sclerosis 1/2; mTORC1/2, mammalian target of rapamycin complex 1/2; PRAS40, proline-rich AKT1 substrate 1; SKP2, S-phase
 327 kinase-associated protein 2; BECN1, Beclin-1; ATG, autophagy-related; Rheb, Ras homolog enriched in brain; ULK1, Unc-51-
 328 like kinase 1;

329

330

331

332

333

334

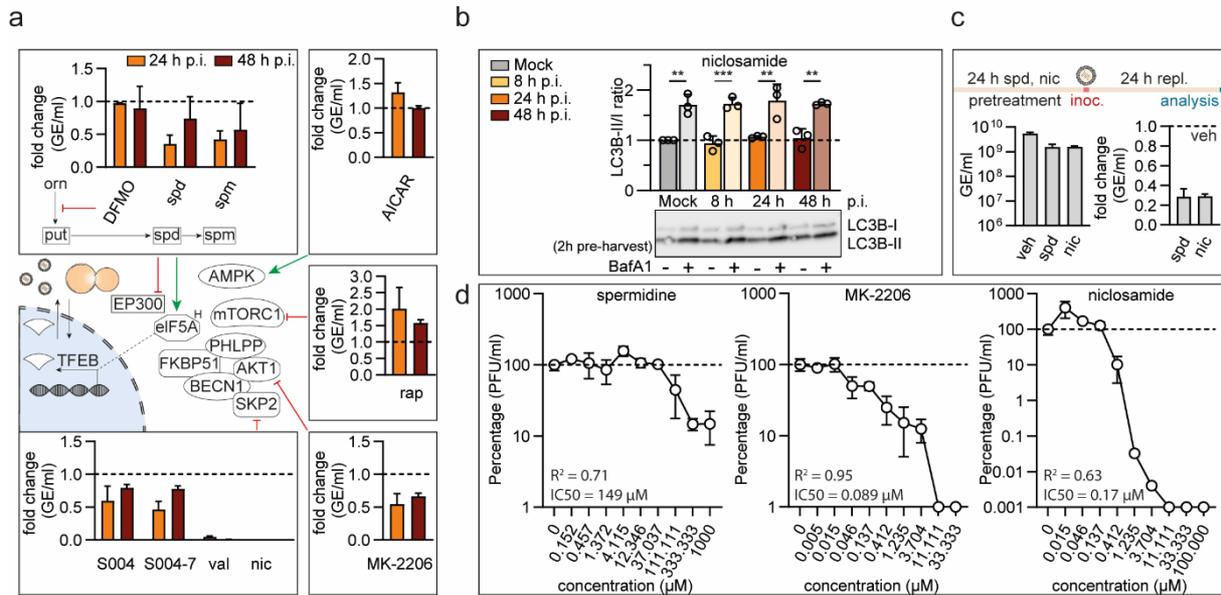
335

336

337

338

360 **Figure 4**



361
 362 **Fig.4: BECN1-stabilizing compounds and polyamines inhibit SARS-CoV-2 growth in cell cultures.** (a) Schematic
 363 representation of autophagy signaling indicating site of action of small-molecule inhibitors used for pathway modulation,
 364 tested in SARS-CoV-2 growth assays. VeroFM cells were infected with SARS-CoV-2 (MOI = 0.0005) and treated with (top, left)
 365 DFMO (500 μ M), spd (spermidine, 100 μ M), spm (spermine, 100 μ M), (top right) AICAR (25 μ M), (middle, right) rap
 366 (rapamycin, 300 nM), (bottom, left) SKP2 inhibitors S004 (SMIP004, 10 μ M), S004-7 (SMIP004-7, 10 μ M), val (valinomycin, 5
 367 μ M), nic (niclosamide, 10 μ M), (bottom, right) MK-2206 (1 μ M) or DMSO (vehicle, dashed lines). SARS-CoV-2 genome
 368 equivalents per mL (GE) were determined by real-time RT-PCR at 24 h and 48 h p.i., data are presented as fold difference. (b)
 369 Niclosamide treated (10 μ M) VeroFM cells were infected with SARS-CoV-2 (MOI = 0.0005) and incubated with bafilomycin A1
 370 (BafA1, 100 nM) or vehicle (DMSO) for 2 h before samples were harvested at 8 h, 24 h, or 48 h post infection (h p.i.). The
 371 ratios of LC3B-II/I were determined by Western blotting. (c) VeroFM cells were treated with spd (100 μ M), nic (5 μ M) or veh
 372 (vehicle) 24 h prior to infection with SARS-CoV-2 (MOI = 0.05). SARS-CoV-2 genome equivalents per ml (GE) were determined
 373 by real-time RT-PCR at 24 h p.i., data are presented GE/ml (left) or as fold difference (right). (d) Concentration-dependent
 374 inhibition of SARS-CoV-2 growth by spermidine, MK-2206, and niclosamide. VeroFM cells were infected with SARS-CoV-2
 375 (MOI = 0.0005) and treated with different concentrations of spermidine, MK-2206, and niclosamide as shown in the figure.
 376 SARS-CoV-2 plaque forming units (PFU) were determined at 24 h (spermidine, MK-2206) and 48 h (niclosamide) p.i. by plaque
 377 assay. Data are presented as virus growth in percent. In all panels error bars denote standard error of mean derived from n
 378 = 3 biologically independent experiments. Abbreviations: orn, ornithine; DFMO, difluoromethylornithine; eIF5AH,
 379 hypusinated eIF5A; PFU, plaque-forming unit.

380

381

382

383