

N-chlorotaurine, a novel inhaled virucidal antiseptic is highly active against respiratory viruses including SARS-CoV-2 (COVID-19)

Michaela Lackner

Medical University of Innsbruck <https://orcid.org/0000-0001-9073-2886>

Annika Rössler

Medical University Innsbruck

André Volland

Institute of Virology, Department of Hygiene, Microbiology and Public Health, Medical University of Innsbruck

Marlena Stadtmüller

Robert Koch-Institute / Unit 17-Influenza and Other Respiratory Viruses <https://orcid.org/0000-0001-8893-5326>

Brigitte Müllauer

Medical University of Innsbruck / Institute of Virology

Zoltan Banki

Medical University of Innsbruck / Institute of Virology

Johannes Ströhle

Medical University of Innsbruck / Institute of Hygiene and Medical Microbiology

Angela Luttick

360biolabs Pty Ltd

Jennifer Fenner

360biolabs Pty Ltd

Heribert Stoiber

Medical University of Innsbruck / Institute of Virology

Dorothee von Laer

Institute of Virology, Department of Hygiene, Microbiology and Public Health, Medical University of Innsbruck

Thorsten Wolff

Robert Koch Institute <https://orcid.org/0000-0001-7688-236X>

Carsten Schwarz

Christiane Herzog Zentrum Berlin/Charité-Universitätsmedizin Berlin

Markus Nagl (✉ m.nagl@i-med.ac.at)

Medical University of Innsbruck <https://orcid.org/0000-0002-1225-9349>

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NCT against respiratory viruses

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Authors:

Michaela Lackner¹, Annika Rössler², André Volland², Marlena Stadtmüller³, Brigitte Müllauer², Zoltan Banki², Johannes Ströhle¹, Angela Luttick⁴, Jennifer Fenner⁴, Heribert Stoiber², Dorothee von Laer², Thorsten Wolff³, Carsten Schwarz⁵, Markus Nagl^{1*}

¹Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

²Institute of Virology, Medical University of Innsbruck, Innsbruck, Austria

³Unit 17-Influenza and Other Respiratory Viruses, Robert Koch-Institute, Berlin, Germany.

⁴360biolabs Pty Ltd, Melbourne, Australia

⁵Christiane Herzog Zentrum Berlin/Charité-Universitätsmedizin Berlin, Berlin, Germany

Co-senior-authors DvL, TW, CS, MN

*Corresponding author:

Markus Nagl, Assoc.Prof., MD

Institute of Hygiene and Medical Microbiology

Medical University of Innsbruck

Schöpfstr. 41

A-6020 Innsbruck / Austria

29 Tel. +43-(0)512-9003-70708

30 Fax +43-(0)512-9003-73700

31 E-mail: m.nagl@i-med.ac.at

32 ORCID: 0000-0002-1225-9349

33 Abstract

34 *N*-chlorotaurine (NCT) is a long-lived oxidant generated in activated cells of the innate
35 immune system, namely neutrophilic and eosinophilic granulocytes and monocytes. NCT
36 acts as an antiseptic agent that can be synthesized chemically and applied topically on
37 different infected body sites. Even treatment of the lower respiratory tract via inhalation,
38 which has been in development in the last years, was well tolerated in a recent phase I
39 clinical trial. In this study, we demonstrate the activity of NCT against viruses causing acute
40 respiratory tract infections, in fact severe acute respiratory syndrome coronavirus 2 (SARS-
41 CoV-2), influenza viruses, and respiratory syncytial virus.

42 NCT revealed broad virucidal activity against all viruses tested. In the presence of organic
43 proteinaceous material simulating body fluids, this activity was enhanced by transchlorination
44 mechanisms so that significant inactivation of viruses could be achieved within 1 – 10
45 minutes.

46 Inhalation of 1.0% NCT as a prophylactic and therapeutic strategy against acute viral
47 respiratory tract infections deserves comprehensive clinical investigation.

48

49 Introductory paragraph*50 Background*

51 The coronavirus disease 2019 (COVID-19) pandemic is the major challenge of humanity
52 presently. No highly effective treatment or a vaccine is available so far. Application of *N*-
53 chlorotaurine (Cl-NH-CH₂-CH₂-SO₃Na, NCT), a safe, well tolerated, endogenous, mild
54 antiseptic with anti-inflammatory properties may be a significant step forward to combat
55 COVID-19 and other viral respiratory tract infections. NCT as an inhaled anti-infective has
56 already demonstrated broad-spectrum microbicidal activity against bacteria, fungi, viruses
57 and protozoa. Here, we aimed to establish the virucidal activity of NCT against three main
58 viruses responsible for lower respiratory tract infections, namely severe acute respiratory
59 syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, and respiratory syncytial viruses
60 (RSV).

61

62 The COVID-19 pandemic is caused by SARS-CoV-2. The pandemic is affecting individuals,
63 populations, and health systems far beyond infection. The virus might persist globally and
64 become a prolonged or permanent threat ^{1,2}. Up to date, there is no breakthrough regarding
65 a highly sufficient and well tolerated prophylaxis, vaccine or therapy. The race for a cure is a
66 global effort and different approaches have been developed and are currently studied ^{1,3}.

67 Another major public concern is posed by influenza viruses, which annually cause 3 – 5
68 million cases of severe illness and about 290 000 to 650 000 of death worldwide ⁴. Protection
69 by the yearly influenza virus vaccine is unsatisfactory and resistance against existing antiviral
70 drugs develops rapidly ⁵. Therefore, new tools to combat influenza viruses are urgently
71 needed.

72 One less known intervention is inhalation therapy with antiviral agents. An appeal for the
73 inhaled route of administration has been published recently ⁶. A first advantage is direct
74 delivery of a high concentration of the medication to the lung, where the virus causes most of
75 the severe problems ⁷. Furthermore, topically applied therapies that are not systemically
76 distributed avoid interactions with systemic medications, which are frequently necessary in

77 elderly or multimorbid patients who are particularly at risk for severe COVID-19 complications
78 ⁸. An ideal inhaled drug should have broad-spectrum antimicrobial activity to cover not only
79 SARS-CoV-2, but also co-infections and superinfections with other respiratory viruses and
80 microorganisms (bacteria and fungi) ⁹⁻¹¹. Antiviral drugs are often specific to distinct viruses,
81 but identifying the virus causing an infection requires logistic and diagnostic efforts, which in
82 the case of SARS-CoV-2 amounts to at best one to two days for a diagnosis¹². Such an ideal
83 inhaled broad-spectrum drug mentioned above could be applied instantly regardless of the
84 pathogen causing the respiratory illness and would thus eliminate the need for time-
85 consuming diagnostics. Another key requirement is anti-inflammatory activity of the
86 compound to downregulate the 'cytokine storm', particularly for SARS-CoV-2, which causes
87 hyper-inflammation in severe cases ¹³.

88 One molecule that fulfils the criteria of broad-spectrum antimicrobial (virucidal,
89 bactericidal, fungicidal, protozoocidal) and anti-inflammatory activity ^{14,15}, and good
90 tolerability upon inhalation is *N*-chlorotaurine (Cl-NH-CH₂-CH₂-SO₃⁻) ¹⁶. It is known since the
91 1970's as a product of activated human granulocytes and monocytes and belongs to the
92 long-lived oxidants and chloramines formed by the myeloperoxidase via hypochlorous acid to
93 combat invading pathogens ¹⁷⁻¹⁹ (Fig. 1). Moreover, *N*-chlorotaurine is thought to be involved
94 in the control of inflammation by downregulating of nuclear factor kappaB activation,
95 chemokines and proinflammatory cytokines such as tumor necrosis factor alpha, some
96 prostaglandins and interleukins like IL-6 ^{15,20,21}. The synthesis of the sodium salt of *N*-
97 chlorotaurine (Cl-NH-CH₂-CH₂-SO₃Na, NCT) was successful in our laboratory ²², which
98 enabled its development as an endogenous anti-infective and mild antiseptic in human
99 medicine. As an active chlorine compound belonging to the class of chloramines, it has the
100 typical broad-spectrum microbicidal activity without development of resistance against Gram-
101 positive and Gram-negative bacteria including multi-resistant strains, yeasts and moulds,
102 protozoa, and worm larvae (for review see ^{14,23,24}). Broad-spectrum activity was found against
103 adenoviruses ²⁵⁻²⁷, herpes viruses 1 and 2 ^{26,27}, human immunodeficiency virus ²⁸, and it was
104 shown *in vivo* against adeno and herpes viruses in epidemic keratoconjunctivitis up to a

105 phase II study as well as in herpes zoster in a case report, respectively ²⁹⁻³¹. Activity against
106 coxsackievirus A24 and enterovirus 70 was found by the NCT-derivative *N,N*-dichloro-
107 dimethyltaurine *in vitro* ³².

108 In the last years, inhalation of NCT has been investigated and developed in detail.
109 Enhanced bactericidal and fungicidal activity has been found in the presence of lung
110 epithelial cells ³³. Tolerability of repeatedly inhaled NCT has been confirmed in the normal
111 lung and in a streptococcal inflammation model each in pigs, and in the normal lung of mice
112 ³⁴⁻³⁶. In humans, tolerability was confirmed in a placebo-controlled phase I clinical study ¹⁶.
113 Only minor and transient adverse effects were found, i.e. chlorine taste and occasional tickle
114 in the throat ¹⁶. NCT is not distributed systemically, which explains the absence of systemic
115 adverse effects.

116 A safe, well tolerated, endogenous, inhaled substance with broad-spectrum activity
117 against pathogens supported by anti-inflammatory properties may be a significant step
118 forward for treatment of COVID-19 and other viral infections of the lower airways without the
119 need of further diagnostics to discriminate between the infectious agents. In this regard, the
120 aim of the present study was to establish and characterize the virucidal activity of NCT
121 against three major viruses responsible for respiratory infections in humans, namely SARS-
122 CoV-2, influenza viruses, and RSV *in vitro*.

123

124 **Results**

125

126 NCT was incubated with SARS-CoV-2, influenza A virus or RSV, followed by assessment of
127 virus inactivation using various readouts. NCT at a clinically relevant concentration of 0.1% -
128 1.0% demonstrated virucidal activity against SARS-CoV-2 (SARS-CoV-2 BavPat1, hCoV-
129 19/Australia/VIC01/2020, clinical isolate 1.2 Innsbruck), influenza A virus, and RSV (RSV
130 long strain). Longer NCT-exposure periods were required to inactivate SARS-CoV-2 than to
131 inactivate influenza viruses or RSV. In the presence of organic matter, inactivation of viruses
132 was even enhanced so that a significant reduction of plaque forming units and infected cells,

133 respectively, could be observed already after 5 min with SARS-CoV-2 by 1.0% NCT.
134 Controls without NCT and specific inactivation controls showed full viral replication in all
135 cases to warrant valid results. Detailed results are presented in the following paragraphs.

136

137 **Virucidal activity of NCT against SARS-CoV-2**

138

139 Inactivation of SARS-CoV-2 was assessed by incubating stock virus with NCT for indicated
140 time periods at 37°C and then determining the remaining infectious particles using plaque
141 assay or immunostaining as well as determining virus inactivation via RT-qPCR or TCID50.
142 Exact incubation times of virus with NCT were ensured by adding met/his at the end of the
143 incubation period, which inactivates NCT. All assays demonstrated a significant inactivation
144 of SARS-CoV-2 with slight differences according to the individual test method and strain
145 used. With plaque assay readout, a significant reduction in infectious particles was detected
146 after 15 min of incubation, when incubating SARS-CoV-2 with NCT in a buffered aqueous
147 solution (Fig. 2a). The mild oxidizing activity of the test antiseptic may explain why it took as
148 long as 15 min to reduce infectious particles. In the presence of Vero cells (Fig. 2b) or
149 particularly 5.0% peptone (Fig. 2a), however, a significant reduction of infectious virus
150 particles occurred already after 5 min of incubation with 1.0% NCT. This remarkable
151 enhancement of activity by organic load is typical for NCT and shown for viruses for the first
152 time here and is explained most likely by transhalogenation (see discussion).

153 Virus inactivation assays with immunostaining readout showed a 50% reduction of infected
154 cells after 1 min (not significant, $p = 0.085$), 20 – 80% reduction after 5 min ($p = 0.0102$), 81 –
155 91% after 7 min ($p < 0.01$), 81 – 97% after 10 min, 96 – 99% after 20 min, and > 99% after
156 30 min ($p < 0.0001$ for these values). A logarithmic scale with respective statistics is provided
157 in Fig. 2c. The results found by RT-qPCR assay were similar with a highly significant
158 reduction of genome copies (Fig. 2d). This was further confirmed by the TCID50 readout
159 (Fig. 2e).

160 The antiviral activity was concentration-dependent. Inactivation controls demonstrated full
161 inactivation of 1.0% NCT by 1.0% methionine / 1.0% histidine (met/his). This was valid for all
162 tests and viruses in this study. Absence of cytotoxicity of inactivated NCT to the inoculated
163 cell culture was proved by MTT testing with values of MTT reduction of 94.1 ± 8.5 (0.1% NCT
164 plus 0.1% met/his) and 100.3 ± 5.6 (PBS control) ($p = 0.12$ by Student's unpaired t-test).

165

166 **Virucidal activity of NCT against influenza viruses**

167

168 Inactivation of influenza viruses was assessed like inactivation of SARS-CoV-2 by incubating
169 stock virus with NCT for indicated time periods at 37°C and then determining the remaining
170 infectious particles using plaque assay.

171 Virus inactivation as determined by plaque assay readout demonstrated an even faster
172 inactivation of influenza viruses by NCT compared to SARS-CoV-2. All tested virus strains
173 were inactivated rapidly with a 2 \log_{10} reduction of the H3N2 virus within 5 min (Fig. 3a) and a
174 6 \log_{10} reduction of H1N1 and H1N1pdm viruses within 1 min by 1.0% NCT (Fig. 3b and 3c).

175 In general, H1N1 and H1N1pdm viruses were more susceptible than the H3N2 virus.

176 Addition of ammonium chloride (NH_4Cl) to NCT significantly enhanced its activity against
177 influenza viruses (Fig. 3d). Ammonium chloride alone and the inactivation control with 0.1%
178 NCT plus 0.1% ammonium chloride showed no antiviral effect at least up to 10 min
179 incubation time.

180

181 **Virucidal activity of NCT against RSV**

182 Inactivation of RSV was assessed like inactivation of SARS-CoV-2 and influenza A viruses.

183 As with SARS-CoV-2 and influenza A virus, inactivation of RSV as determined by plaque
184 assay readout demonstrated a significant reduction of PFU/ml by NCT compared to mock
185 treated controls. The incubation of RSV with 1.0% NCT resulted in a rapid drop of infectious
186 virus titre with 4 \log_{10} decrease within 5 minutes (Fig. 4). Almost no detectable amount of
187 infectious RSV was measurable after 15 minutes. In the presence of 0.1% NCT, RSV titres

188 dropped in a time- and concentration-dependent manner reaching significant titre reduction
189 after 15 minutes (Fig. 4).

190

191 **Discussion**

192 Safe, well tolerated, affordable, and effective medications are urgently needed against
193 COVID-19 and would be beneficial for treatment of viral bronchopneumonia caused by other
194 viruses such as influenza and RSV. As an endogenous mild long-lived oxidant ¹⁷, inhaled
195 NCT has been demonstrated to be well tolerated and safe in animals (pigs and mice) and in
196 a clinical phase I study in humans ^{16,34-36}. As an active chlorine compound belonging to the
197 class of chloramines, it has broad-spectrum activity against pathogens without occurrence of
198 resistance because of the oxidizing mechanism of activity with thio- and amino-groups as the
199 main targets ^{14,22,23}.

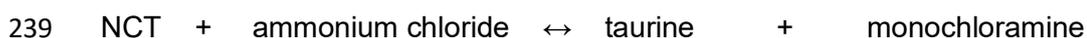
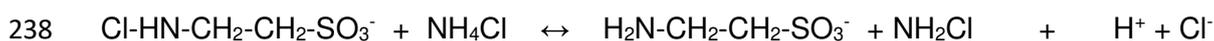
200 Actually, in the present study NCT had clear virucidal activity against three enveloped
201 RNA viruses highly relevant for infections of the bronchopulmonary system. Depending on
202 the NCT-concentration and test conditions, a rapid reduction of the number of infectious virus
203 particles by several powers of ten within 1 - 10 minutes is achieved. Influenza A viruses of
204 pre-pandemic and pandemic H1N1 subtype (H1N1 and H1N1pdm) were the most sensitive
205 ones with reduction to the detection limit by 1.0% NCT within 1 min, followed by RSV,
206 influenza (H3N2), and SARS-CoV-2. These differences can be explained by individual
207 dynamics of oxidation and chlorination of proteins of the viral surface, and of penetration of
208 NCT and attack on the viral nucleocapsid proteins. All these target sites have been shown
209 with the NCT analogue *N,N*-dichloro-2,2-dimethyltaurine in adenovirus type 5 ³⁷. Thereby,
210 chlorination of the surface proteins is the first step ³⁸, which can be assumed to impact their
211 function and therefore the attachment of viruses to body cells. Oxidation and chlorination of
212 virulence factors of different pathogens by NCT and analogue chloramines with the
213 consequence of their inactivation has been also shown for shigatoxin of *Escherichia coli* ³⁹,
214 several toxins of *Staphylococcus aureus* ⁴⁰, aspartyl proteinases of *Candida spp.* and
215 gliotoxin of *Aspergillus fumigatus* ^{41,42}. This indicates that inactivation of key proteins of all

216 kinds of pathogens is a central principle of the antimicrobial action of NCT and may underline
 217 such a function in innate immunity besides its anti-inflammatory one ^{15,20,23}.

218 Accordingly, NCT has not only virucidal activity against enveloped viruses (herpes
 219 virus type 1 and 2 ^{26,27}, human immunodeficiency virus 1 ²⁸, and the viruses of the present
 220 study), but also non-enveloped ones. From the latter, a panel of adenoviruses has been
 221 tested mainly due to their importance in epidemic keratoconjunctivitis ^{25,27,30}. Similar to other
 222 active halogen compounds and other antiseptics such as tensidic compounds ⁴³,
 223 adenoviruses are slightly less sensitive to NCT than the enveloped viruses ²⁷. Nevertheless,
 224 efficacy of NCT *in vivo* against adenoviruses in epidemic keratoconjunctivitis has been
 225 proven in the New Zealand White rabbit ocular model and in a phase II study in humans ^{30,31}.
 226 Application of NCT had a curative effect in a patient suffering from therapy-refractory herpes
 227 zoster infection in the upper thoracic area ²⁹.

228 It must be taken into account that organic substances are omnipresent *in vivo* (in all
 229 human body fluids and tissues), and therefore we performed a part of the inactivation assays
 230 in the presence of organic matter as well. The results of Fig. 2a clearly show an
 231 enhancement of the virucidal activity of NCT in the presence of 5.0% peptone, which in the
 232 first view appears surprising since active chlorine compounds underlie a decrease of their
 233 oxidation capacity by chlorine-reducing substances of such organic load ^{23,44,45}. With NCT as
 234 a low-reactive chloramine compound, however, transchlorination as one of the reaction
 235 mechanisms becomes important ^{14,23}. Thereby, amongst others, monochloramine (NH₂Cl) is
 236 formed in equilibrium from NCT and ammonium chloride ^{14,17}.

237



240

241 Monochloramine is more lipophilic than NCT and penetrates microorganisms more easily,
 242 which leads to enhanced inactivation by the reaction just mentioned ^{17,46}. The stronger
 243 activity of NCT in the presence of fluids containing proteinaceous material is a general

244 principle observed in different compositions, such as artificial sputum medium, different body
245 fluids, peptone, and plasma for bacteria and fungi (for review see ^{14,24,47}). In the present
246 study, it has been confirmed for viruses for the first time, too. The discrepancy between the
247 incubation time of 15 min (Fig. 2a) and of 10 min or less (Fig. 2b-e) needed for a significant
248 viral reduction in buffer solution in different tests may be explained by the presence of 1.0%
249 FCS in the tests depicted in Fig. 2c-e and organic matter in the presence of Vero cells in Fig.
250 2b. In agreement with these results, enhancement of the bactericidal and fungicidal activity of
251 NCT in the presence of different lung epithelial cells was observed recently ³³.

252 Enhancement of antimicrobial and antiviral activity by organic material is of practical
253 relevance for topical treatment of infections with NCT, for instance bronchopulmonary ones.
254 The concentration of active chlorine after the end of an inhalation of 1.0% NCT decreases to
255 traces within 1 min and vanishes completely after further 10 min ¹⁶. Inhalation for 10 min is
256 feasible and well tolerated, and within this time an impact on SARS-CoV-2 and on other
257 viruses can be expected *in vivo*, too, but remains to be evaluated in respective clinical
258 studies.

259 Also of practical relevance is the fact that NCT has broad-spectrum activity against
260 viruses, including important representatives relevant for bronchopulmonary infections
261 (SARS-CoV-2, influenza viruses, RSV). Topical treatment of all these virus infections by
262 inhaled NCT without the necessity of a diagnosis of the specific virus at hand is conceivable
263 and should urgently be investigated in clinical studies. Notably, the activity of NCT against
264 bacteria and fungi, including multi-resistant ones, may prevent super- and secondary
265 infections, which are a considerable problem in COVID-19 patients as well ⁹⁻¹¹. In addition,
266 the anti-inflammatory activity of NCT might have the potential to influence the aggressive
267 inflammatory response by downregulating the 'cytokine storm' and prevent airway damage in
268 severe ill patients with SARS-CoV-2 infection.

269 Further advantages of NCT would be high safety and high tolerability by human tissue ¹⁴,
270 absence of systemic absorption, of systemic adverse effects ¹⁶, of systemic interaction with

271 other medications, and of resistance development because of the oxidizing and chlorinating
272 mechanism of action ^{14,23}.

273

274 **Conclusions**

275

276 NCT demonstrated rapid activity against SARS-CoV-2, influenza A viruses, and RSV at a
277 therapeutic concentration of 1.0% that can be safely inhaled. The activity is enhanced by an
278 organic environment, which is omnipresent in human body fluids and tissues *in vivo*. Clinical
279 efficacy of NCT in viral bronchopulmonary infections should be investigated in respective
280 clinical studies.

281

282

283 **Methods**

284

285 **Reagents**

286

287 *N*-chlorotaurine sodium salt (NCT, molecular weight 181.57 g/l, lot 2020-03-17) was
288 prepared in pharmaceutical quality as established at our Department and frozen at minus
289 20°C for storage ²². For testing, it was freshly dissolved in phosphate-buffered saline (PBS)
290 at pH 7.1 (7.0 – 7.2) to desired stock concentrations between 1.0% (55.08 mM) and 10%.
291 As inactivation solution for NCT, a mixture of 1.0% methionine and 1.0% histidine (met/his, L-
292 methionine and L-histidine, both from Carl Roth GmbH, Karlsruhe, Germany) in distilled
293 water was used ⁴⁸. For tests in peptone, peptone enzymatic digest from Casein was applied
294 (Fluka no. 82303, Sigma-Aldrich GmbH, Buchs, Switzerland). RPMI-1640 medium and fetal
295 calf serum (FCS) were from Sigma-Aldrich GmbH, too.

296

297 **Viruses, virus cell culture and preparation of viral suspensions**

298

299 **SARS-CoV-2**

300

301 *Robert Koch-Institute, Berlin (RKI)*

302 SARS-CoV-2 BavPat1 strain was obtained from Christian Drosten's laboratory at the Institute
303 of Virology at Charité Universitätsmedizin Berlin. Vero E6 cells were maintained in DMEM
304 (supplemented with 10% FCS, 2 mM L-glutamine, non-essential amino acids, 1mM sodium
305 pyruvate, 100 mg/ml streptomycin and 100 units/ml penicillin). For virus stock preparation,
306 Vero E6 monolayer cultures grown in 75 cm² cell culture flasks were infected with a
307 multiplicity of infection (MOI) of 0.01 in PBS (supplemented with 0.3% BA) for 2 days at 37°C
308 and 5.0% CO₂. The supernatant was harvested and stored at minus 80°C until use.

309

310 *Biolabs, Melbourne (Biolabs)*

311 COVID-19 strain used was SARS-CoV-2 hCoV-19/Australia/VIC01/2020 (Melbourne's Peter
312 Doherty Institute for Infection and Immunity, Melbourne, Australia). Parent stock of the virus
313 was passaged twice in Vero cells. A working stock was generated at 360biolabs by two
314 further passages in Vero cells in virus growth media, which comprised Minimal Essential
315 Medium without L-glutamine supplemented with 1.0% (w/v) L-glutamine 1.0 µg/ml of TPCK-
316 Trypsin, 0.2% BSA, 1 x Pen/Strep, and 1.0% Insulin Transferrin Selenium (ITS), then a
317 further 2 passages in Vero E6 cells in growth media. This growth media comprised MEM
318 supplemented with 1.0% (w/v) L-glutamine, 4.0 µg/ml of TPCK-Trypsin and 2.0% (v/v) heat
319 inactivated FBS.

320 African Green Monkey Kidney (Vero E6) cells (ATCC-CRL1586) were sub-cultured to
321 generate cell bank stocks in cell growth medium, which comprised Minimal Essential Medium
322 without L-glutamine supplemented with 10% (v/v) heat-inactivated Fetal Bovine Serum and
323 1.0% (w/v) L-glutamine. Cell stocks were frozen at minus 80°C overnight and then
324 transferred to liquid nitrogen. Vero E6 cells were passaged for a maximum of 13 passages,
325 after which a new working cell bank stock was retrieved from liquid nitrogen for further use.
326 Vero E6 cells were seeded into 96-well plates at 2 x 10⁴ cells / well in 100 µl E6 seeding

327 media (Minimal Essential Medium supplemented with 1.0% (w/v) L-glutamine, 2.0% FBS).

328 Plates were incubated overnight at 37°C, 5.0% CO₂.

329

330 *Institute of Virology, Innsbruck*

331 SARS-CoV-2 Isolate 1.2 was a clinical isolate from a patients' respiratory swab sample in

332 Innsbruck, Austria. Virus stocks were produced on Vero/TMPRSS2 cells, kindly provided by

333 Dr. Markus Hoffmann and Prof. Stefan Pöhlmann, Leibniz Institute for Primate Research,

334 Göttingen, Germany ⁴⁹. Cells were cultured in DMEM plus 10% FCS and Pen/Strep. For

335 Virus stock production, 80% confluent Vero/TMPRSS2 cells were infected with a MOI of 0.01

336 in DMEM plus 2.0% FCS. The supernatant was harvested 60 h post infection. Virus aliquots

337 were stored at minus 80°C.

338

339 **Influenza**

340

341 *Robert Koch-Institute, Berlin (RKI)*

342 Influenza A/Panama/2007/1999 (H3N2) virus was grown in the allantois cavity of 11 day old

343 embryonated chicken eggs for 2 days. Virus was harvested, clarified by centrifugation

344 (300xg, 10 min) and stored at minus 80°C until use. Madin-Darby-Canine-Kidney (MDCK) II

345 cells (ATCC) were maintained in MEM (supplemented with 10% FCS, 2 mM L-glutamine,

346 100 mg/ml streptomycin and 100 units/ml penicillin) at 37°C and 5.0% CO₂.

347

348 *Institute of Hygiene and Medical Microbiology, Innsbruck*

349 Influenza A/Singapore/Hongkong/2339/2000 (H1N1) was kindly provided by H. Katinger,

350 Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences,

351 Vienna, Austria. Influenza A/Swine Origin Virus (S-OIV)/California/2009 (H1N1pdm) was a

352 clinical isolate from Innsbruck, Austria.

353 Influenza viruses were grown on MDCK cells (Collection of Cell Lines in Veterinary Medicine,

354 Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald,

355 Germany). MDCK cells were grown in 25 cm² cell culture flasks (Sarstedt, Inc. Newton, NC,
356 USA) in RPMI plus 10% FCS to a monolayer. The medium was replaced by 5 ml RPMI
357 without FCS, and 10 µl of 1 mg/ml trypsin (final concentration 0.002 mg/ml) was added to
358 activate neuraminidase. Viral suspension deep frozen at minus 80°C in RPMI (200 µl) was
359 added. After 60 h of incubation at 37°C, a cytopathic effect was seen in all cells, and the
360 supernatant was taken and centrifuged at 275 × g. The supernatant again was used as viral
361 suspension for the tests.

362

363 **RSV**

364 RSV long strain, (kindly provided by T. Grunwald, Fraunhofer Institute for Cell Therapy and
365 Immunology, Leipzig, Germany), was generated by infection of HEp2 cells at low MOI as
366 described previously⁵⁰. Virus titers were determined in plaque assays by infection of HEp2
367 with serial dilutions of the virus followed by immunocytochemical staining with polyclonal goat
368 antibody against RSV (Gt X RSV, Merck) and HRP-conjugated rabbit polyclonal anti-goat
369 IgG (Novusbio). 3-Amino-9-ethylcarbazole (AEC, Sigma) was used as a chromogen in
370 immunohistochemistry to visualize the RSV infected cells.

371

372 **Virus inactivation tests (quantitative killing assays) with NCT**

373

374 General overview of the test method

375

376 The viral suspension was mixed with NCT (final concentration 0.1% to 1.0%) and incubated
377 at 37°C for 1, 5, 7, 10, 15, 20, 30, 45, and 60 min. At the end of each incubation time,
378 aliquots were removed and diluted in 1.0% met/his to inactivate NCT and to warrant exact
379 incubation times. Virus inactivation was assessed by subsequent plaque assay,
380 immunostaining or RT-qPCR as detailed below.

381 Controls were done in PBS or PBS with 5.0% peptone without NCT in parallel as well as

382 inactivation controls. For the latter, 1.0% NCT was mixed with met/his before the addition of

383 the respective virus. The virus must survive in the inactivation solution to obtain reliable
384 results, which was the case in all tests.

385

386 **SARS-CoV-2**

387

388 Virus inactivation assay with plaque assay readout (RKI Berlin)

389 Three µl of concentrated virus suspension (4.76×10^9 PFU/ml) were added to 400 µl of NCT
390 (1.0% or 0.1% in PBS), NCT with peptone (1.0% NCT, 5.0% peptone in PBS) or PBS and
391 incubated at 37°C. After 5, 15, 30 and 60 min, 100 µl were removed and added to 100 µl of
392 met/his. As inactivation control, 100 µl of 1.0% NCT or 1.0% NCT with 5.0% peptone were
393 added to 100 µl of met/his and thereafter 0.75 µl of virus suspension were added. Infectious
394 virus particles in all suspensions were determined with plaque assay. Briefly, a serial tenfold
395 dilution of the virus suspension in PBS with 0.3% bovine albumin was added to confluent
396 Vero E6 cells in 12-well plates, which were washed with PBS immediately before. After
397 incubation at 37°C for 1h, the inoculum was removed followed by a washing step with PBS.
398 Avicel-overlay medium (double-strength DMEM supplemented with 10% FCS, 1.0% DEAE
399 dextran, 5.0% sodium bicarbonate and 1.25% Avicel) was added and plates incubated at
400 37°C and 5.0% CO₂ for 3 days before staining with crystal violet for visualization of plaques.
401 Counted plaques are expressed as plaque forming units per ml (PFU/ml).

402

403 Virus inactivation assay with plaque assay readout (GLP lab 360 biolabs, Melbourne)

404 Cell seeding media were removed from a pre-seeded plate (assay plate) and cell monolayers
405 were washed with PBS twice. A volume of 50 µl of non-supplemented MEM was added to all
406 wells except the isopropanol positive control wells. A volume of 50 µl of (4.0% or 0.4%) NCT
407 was added to NCT-treated wells, 50 µl of non-supplemented MEM was added to virus only
408 wells and 100 µl of isopropanol ($\geq 99.5\%$, Sigma-Aldrich) added to positive control wells. A
409 100 µl volume of SARS-CoV-2 (B3) that had been pre-diluted 1:10 in non-supplemented
410 MEM was added to all wells. Plates were incubated for 5, 10, 20, and 60 min at 37°C, 5.0%

411 CO₂. An additional 100 µl of virus growth media containing TPCK trypsin required for virus
412 growth (MEM supplemented with 1.0% (w/v) L-glutamine, 2.0% FBS and 8 µg/ml TPCK
413 trypsin) was added to plates pre-seeded with Vero E6 cells that samples were to be titrated
414 onto.

415 At each time point, 100 µl of either 1.0% or 0.1% NCT media was removed from the
416 assay plate and added to either 100 µl of met/his in distilled water to inactivate the NCT. The
417 positive control and virus only controls were also diluted 1:2 into distilled water. Each sample
418 was diluted a further 1:10 into virus growth media, MEM supplemented with 1.0% (w/v) L-
419 glutamine and 2.0% FBS containing 4.0 µg/ml of TPCK trypsin (i.e. 100 µl of inactivated
420 sample + 900 µl of virus growth media). The remaining virus after NCT inactivation was
421 quantified by addition of 100 µl volume of 1:10 diluted inactivated NCT to triplicate wells of
422 96-well plate pre-seeded with Vero E6 cells. Plates were incubated at 37°C, 5.0% CO₂ for 4
423 days. Virus-induced CPE was scored visually. The TCID₅₀ of the virus suspension was
424 determined using the method of Reed-Muench⁵¹. The virucidal effect was quantified as the
425 log₁₀ reduction in virus titre compared to the SARS-CoV-2 control. Isopropanol (≥ 99.5%) was
426 used as the assay positive control.

427 For inactivation controls, 100 µl of NCT at 4.0% and 0.4% was added to 100 µl of
428 4.0% met/his or 0.4% met/his prior to addition of virus. A volume of 100 µl of this inactivation
429 mix was added to wash pre-seeded Vero E6 cells. To this, 100 µl of virus pre-diluted 1:10 in
430 non-supplemented MEM was added and incubated at 37°C, 5.0% CO₂ for 10 min or 60
431 minutes. Following the incubations, 100 µl was diluted into 900 µl of virus growth media
432 containing 4 µg/ml of TPCK trypsin (1:10). The remaining virus was quantified as outlined
433 above.

434

435 Absence of cytotoxicity of inactivated NCT to the inoculated cell culture.

436 As a cytotoxicity control, the 1.0% NCT and 0.1% NCT was set up the same and inactivated
437 by met/his as outlined above but instead of 100 µl of virus being added, 100 µl of non-
438 supplemented MEM was added. These samples were treated exactly the same as above

439 and titrated across pre-seeded cells to ascertain any cytotoxicity observed by the NCT. For
440 cell viability staining, a 100 µl volume of a 3 mg/ml solution of 3-(4,5-dimethylthiazole-2-yl)-
441 2,5-diphenyl tetrazolium bromide (MTT) was added to cytotoxicity control plates and
442 incubated for 2 h at 37°C in a 5.0% CO₂ incubator. Wells were aspirated to dryness using a
443 multichannel manifold attached to a vacuum chamber and formazan crystals solubilised by
444 the addition of 200 µl 100% 2-Propanol at room temperature for 30 minutes. Absorbance was
445 measured at 540 – 650 nm on a plate reader. Absorbance values were averaged and
446 reported as % reduction of MTT to formazan.

447

448 Virus inactivation assay with immunostaining and RT-qPCR (Virology Innsbruck).
449 Each 150 µl of NCT (2.0% in PBS) and of virus suspension (in DMEM plus 2.0% FCS plus 2
450 mM glutamine, plus Pen/Strep) were mixed and incubated at 37°C. After each incubation
451 time, 50 µl were removed and transferred to an equal volume of met/his. Controls were done
452 in PBS without NCT. As inactivation controls, 75 µl of 2.0% NCT in PBS were added to 150
453 µl of met/his, followed by addition of 75 µl virus suspension. After serial tenfold dilution of this
454 suspension, 50 µl each were added to 90% confluent Vero/TMPRSS2 or
455 Vero/TMPRSS2/ACE2 cells in 96-well plates, from which the medium was removed
456 immediately before. After incubation of 1 h at 37°C, the supernatant was removed, and after
457 a washing step with 100 µl of medium, 100 µl of fresh medium was added. After further 9 h
458 incubation, cells were fixed for immunostaining or total RNA was extracted for RT-qPCR as
459 described below.

460

461 Immunostaining (detection by antibodies and peroxidase-marked second antibody)
462 After fixation for 5 min with 96% EtOH, cells were blocked for 15 min with PBS containing
463 0.1% FCS. Subsequently, cells were stained using serum from a SARS-CoV-2 recovered
464 patient and horse radish peroxidase (HRPO)-conjugated anti-human secondary antibody.
465 The signal was developed using a 3-amino-9-ethylcarbazole (AEC) substrate. Infected cells
466 were visible as red spots and the number of infected cells was counted using an

467 ImmunoSpot S6 Ultra-V reader and CTL analyser *BioSpot@* 5.0 software (CTL Europe
468 GmbH, Bonn, Germany).

469

470 RT-qPCR

471 For RNA extraction, the supernatant was removed, and the cell monolayer was washed twice
472 with PBS. The cells were lysed 5 min at room-temperature using 100 µl in-house direct lysis
473 buffer (10 mM Tris-HCL pH 7.4, 25 mM NaCl, 0.5% IGEPAL, 10 Units RiboLock RNase
474 Inhibitor in DEPC-treated water)⁵². Subsequently, 5 µl RNA was used in a one-step RT-
475 qPCR assay using the iTaq™ RT-PCR (BIO-RAD) kit and previously published primers and
476 probes specific for detection of the SARS-CoV-2 E Gene on a CFX96™ real-time system
477 (BIO-RAD)⁵³.

478

479 Virus titration by TCID₅₀

480 Virus titrations were performed by tenfold serial dilution and end-point titration on 10⁴
481 Vero/TMPRSS2/ACE2 cells per well in 96-well microtitre plates. Four days after inoculation,
482 the CPE was analysed and the TCID₅₀ titre was calculated.

483

484 **Influenza**

485

486 Virus inactivation assay with plaque assay readout (RKI)

487 Eight µl of virus suspension (A/Panama/2007/1999 (H3N2), 1.3 × 10⁸ PFU/ml) were added to
488 400 µl of NCT (1.0% or 0.1% in PBS) or PBS and incubated at 37°C. After 5, 15, 30 and 60
489 min, 100 µl were removed and added to 100 µl of met/his. As inactivation control, 100 µl of
490 1.0% NCT were added to 100 µl of met/his and thereafter 2 µl of virus suspension were
491 added. Infectious virus particles in all suspensions were determined with plaque assay.
492 Briefly, a serial tenfold dilution of the virus suspension in PBS with 0.3% bovine albumin was
493 added to confluent MDCK II cells in 12-well plates, which were washed with PBS
494 immediately before. After incubation at 37°C for 1 h, the inoculum was removed followed by

495 a washing step with PBS. Avicel-overlay medium (double-strength MEM supplemented with
496 0.2% BA, 1.0% DEAE dextran, 5.0% sodium bicarbonate, 1 mg/ml TPCK-trypsin and 1.25%
497 Avicel) was added and plates incubated at 37°C and 5.0% CO₂ for 2 days before staining
498 with crystal violet for visualization of plaques. Counted plaques are expressed as plaque
499 forming units per ml (PFU/ml).

500

501 Virus inactivation assay with plaque assay readout (Hygiene and Medical Microbiology
502 Innsbruck)

503 MDCK cells (2×10^4 / well) were grown in 96- well flat microtitre plates (Becton Dickinson
504 Labware and Company, Franklin Lakes, NJ USA) for 24 h in RPMI plus 10% FCS.

505 Subsequently, the medium was replaced by 100 µl of plain RPMI per well.

506 Each viral strain (H1N1 and H1N1pdm) was tested separately. Tenfold concentrated NCT
507 (10.0%, 5.0%, and 1.0%, respectively) in distilled water (50 µl; water without NCT for
508 controls) was added to 450 µl of virus suspension in RPMI (pH 7.2) to a final concentration of
509 1.0%, 0.5%, and 0.1%, respectively, and incubated for 1, 5, and 10 min at 22°C. A separate
510 series of experiments was done with a final concentration of 0.1% NCT (5.5 mM) plus 0.1%
511 ammonium chloride (18.7 mM) (Merck) and 1 min incubation time. At the end of the
512 incubation time, aliquots of 100 µl were removed and mixed with 100 µl of met/his to
513 inactivate NCT. Aliquots of 11 µl of this viral suspension in inactivated NCT were added to
514 the MDCK cells in 96-well microtitre plates containing 100 µl RPMI per well. A series of
515 tenfold dilutions in microtitre plates was performed. Inoculated plates were incubated at 37°C
516 and 5.0% CO₂ and evaluated for plaques after 5 days. As inactivation controls, 100 µl each
517 of 1.0% NCT and met/his were mixed. An aliquot of 50 µl was added to 450 µl of virus
518 suspension.

519

520 **RSV**

521 RSV was incubated in the presence or absence of NCT at a final concentration of 0.1% and
522 1.0% for 5, 10, 15, 30 and 60 minutes at 37°C. Virus (24 µl in DMEM plus 1.0% FCS and 2

523 mM L-glutamine) was mixed with 24 μ l of NCT in PBS. After indicated time-points, 48 μ l of
524 met/his was added to stop the reaction. As inactivation control, NCT was preincubated with
525 equal amount of met/his for 10 minutes at RT prior to incubation with RSV. Infectious virus
526 particles in all samples were titrated in plaque assay using HEp2 cells as described above.
527 Aliquots of 25 μ l were serially diluted in 100 μ l of medium (DMEM plus 10% FCS and 2 mM
528 L-glutamine) in microtitre plates, and 100 μ l of Hep2 cells were added.

529

530 **Statistics**

531 Results are presented of mean values and standard deviation (SD) of generally at least three
532 independent experiments each. Detection limits are indicated by dotted lines in the figures.
533 Student's unpaired t-test, in cases of two groups, and one-way analysis of variance (ANOVA)
534 and Dunnett's multiple-comparison test, in cases of more than two groups, were used to test
535 for differences between the test and control groups. P values of < 0.05 were considered
536 significant for all tests and indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.
537 Calculations were performed with GraphPad Prism 7.00 software (Graph- Pad, Inc., La Jolla,
538 CA, USA).

539

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692

693

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701

702 Author Contributions

703 A.R., A.V., M.S., T.W., A.L., J.F. and H.S. performed the assays against SARS-CoV-2, M.S.,
704 T.W., J.S. and M.N. the assays against influenza viruses, B.M. and Z.B. the assays against
705 RSV. M.L., M.N., C.S., D.v.L., planned the work, made the concept and guided the work.
706 M.N. and M.L. wrote the manuscript under contribution of all other authors. All authors edited
707 and approved the manuscript.

708

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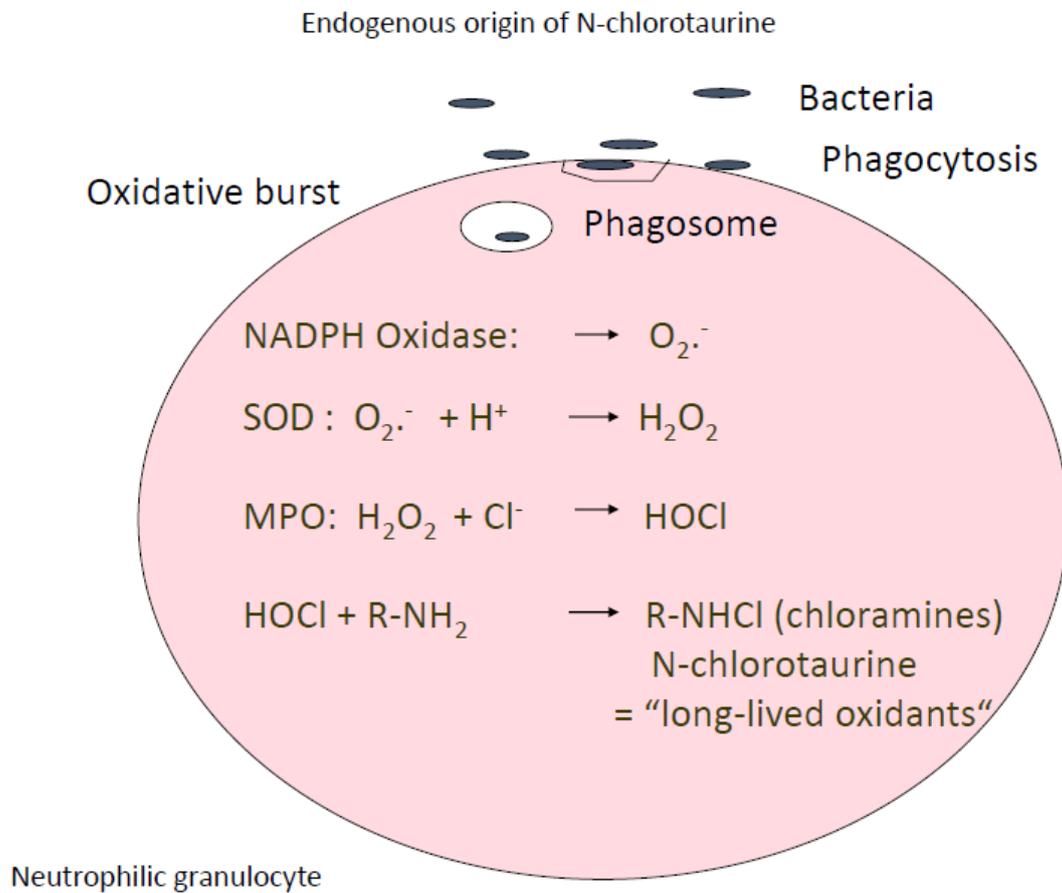
714 Competing Interests statement

715 M. Nagl is co-inventor of a patent on the application of NCT for inhalation (EP 2265267 B1).
716 All other authors declare no conflict of interest.

717

719 **Figures**

720



721

722

723 Fig. 1

724

725 **Fig. 1: Endogenous origin of NCT.**

726 NCT is formed in activated human granulocytes and monocytes via an enzymatic cascade,

727 the oxidative burst. Subsequent to superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), the

728 highly reactive hypochlorite (HOCl) is created by myeloperoxidase (MPO), which among

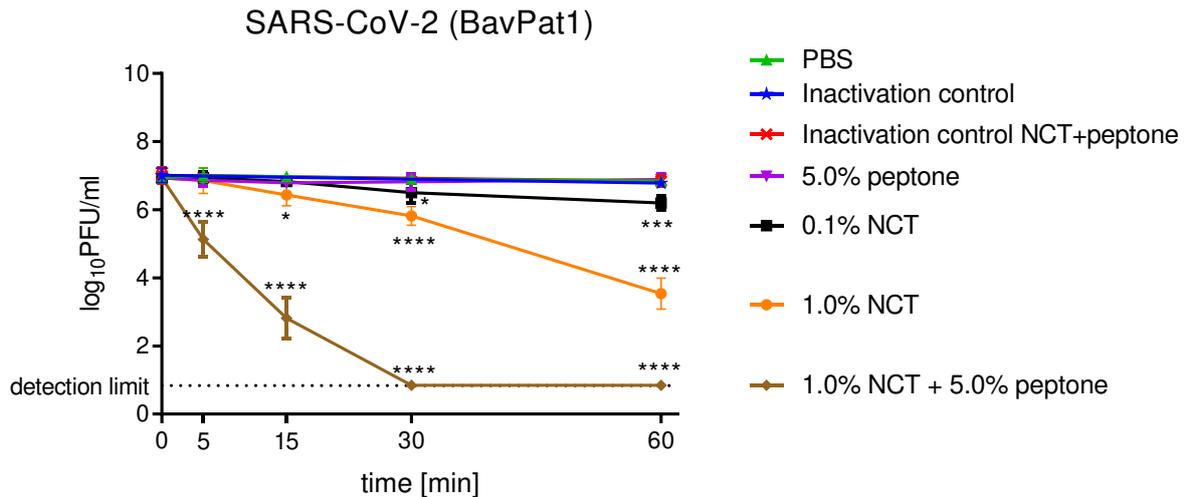
729 others reacts with amino compounds to form less reactive chloramines, also named long-

730 lived oxidants. NCT is the main representative of these chloramines. SOD superoxide

731 dismutase.

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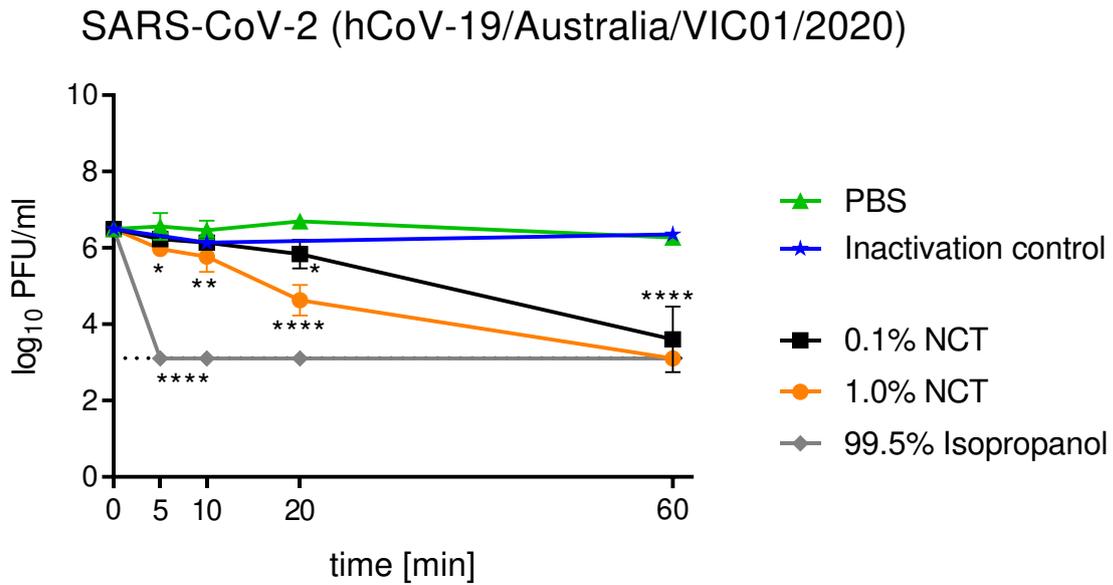
735 Fig. 2a

736

737 **Fig. 2: Inactivation of SARS-CoV-2 by NCT.**

738 a, Virus suspension (SARS-CoV-2 BavPat1) was incubated with 1.0% (55 mM) NCT, 0,1%
 739 (5.5 mM) NCT, 1.0% NCT with 5.0% peptone or PBS or 5.0% peptone for 5 min, 15 min, 30
 740 min, or 60 min at 37°C, after which samples were diluted 1:1 in met/his solution for
 741 inactivation of NCT. Remaining infectious virus particles were determined using plaque
 742 titration. To control for inactivation of NCT by met/his, virus was added after dilution of 1.0%
 743 NCT with or without peptone in met/his. Mean values \pm SD of three to eight independent
 744 experiments in duplicates. The dotted line indicates the detection limit (0.84 log₁₀). Data were
 745 statistically analysed using a two-way ANOVA including a Dunnett's multiple comparison test
 746 to PBS controls. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Of note, the
 747 inactivation of the virus by NCT was markedly enhanced in the presence of peptone.

748



749

750 Fig. 2b

751

752 **Fig. 2: Inactivation of SARS-CoV-2 by NCT.**

753 **b**, Virus suspension (SARS-CoV-2 h CoV-19/Australia/VIC01/2020) was incubated with NCT
 754 or PBS or isopropanol (positive control) for 5 min, 10 min, 20 min, and 60 min at 37°C and
 755 then diluted 1:1 in met/his for inactivation of NCT, followed by plaque titration. Mean values ±
 756 SD of three independent experiments. Detection limit 3.11 log₁₀ (dotted line).

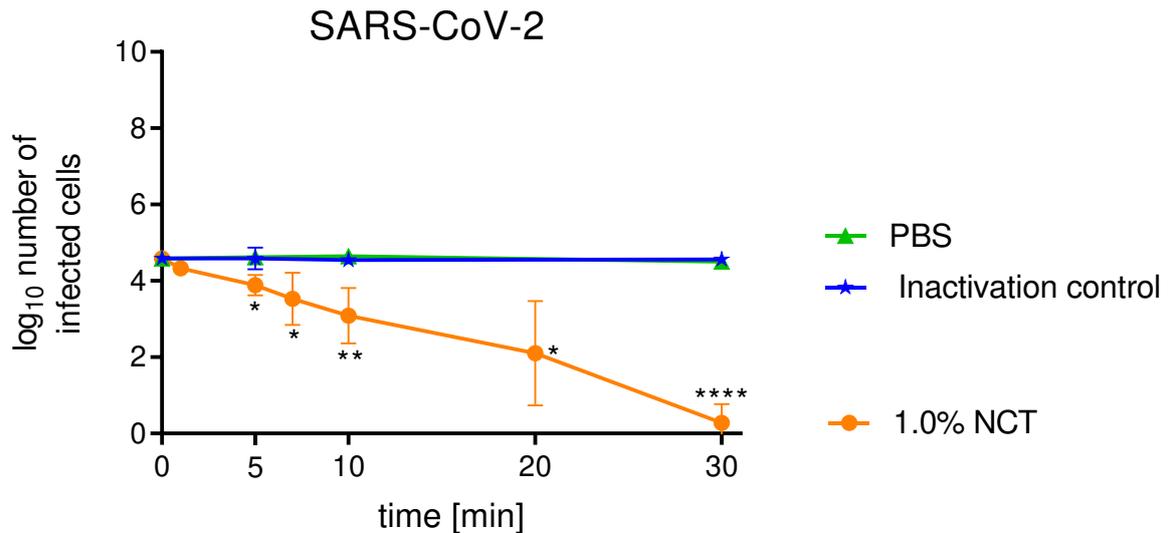
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764 Fig. 2c

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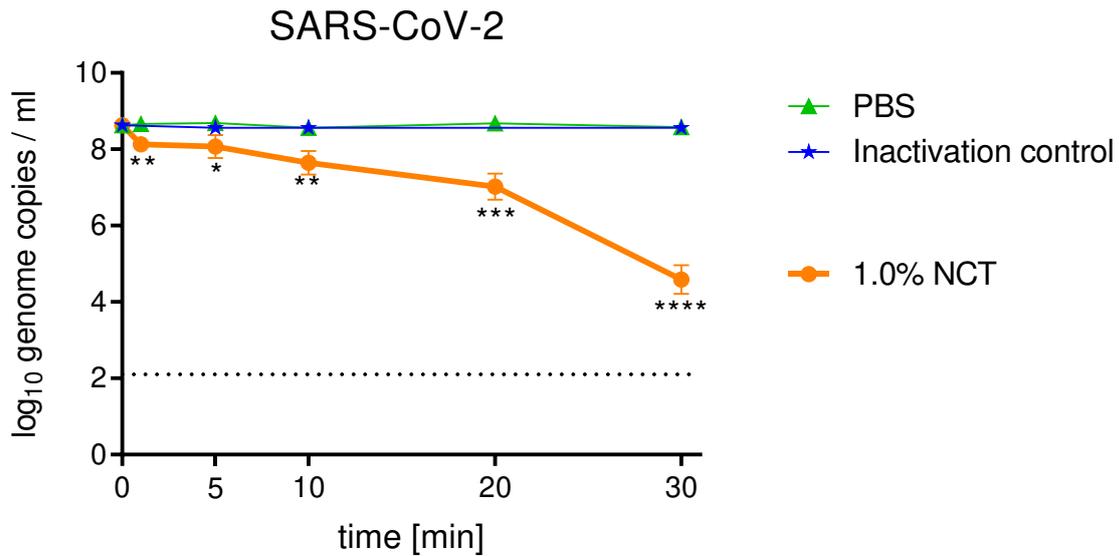
766 **Fig. 2: Inactivation of SARS-CoV-2 by NCT.**

767 **c**, Virus suspension (SARS-CoV-2, clinical isolate) was incubated with 1% NCT or PBS for 1
768 min, 5 min, 7 min, 10 min, 20 min, and 30 min at 37°C. After inactivation of NCT and serial
769 dilution, aliquots were added to Vero/TMPRSS2/ACE2 cells for 1 h in 96-well plates. Cells
770 were washed, incubated for further 9 h, and fixed for Immunostaining (**c**) or RT-qPCR (**d**). In
771 immunostaining, infected cells were visible as red spots and counted using an ImmunoSpot
772 S6 Ultra-V reader and CTL analyser *BioSpot@ 5.0* software. Mean values \pm SD of three
773 independent experiments.

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778

779 Fig. 2d

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781 **Fig. 2: Inactivation of SARS-CoV-2 by NCT.**782 **d**, After cell lysis and RNA extraction, one-step RT-qPCR assay was performed using the

783 iTaq™ RT-PCR (BIO-RAD) kit and previously published primers and probes specific for

784 detection of the SARS-CoV-2 E Gene on a CFX96™ real-time system (BIO-RAD). Mean

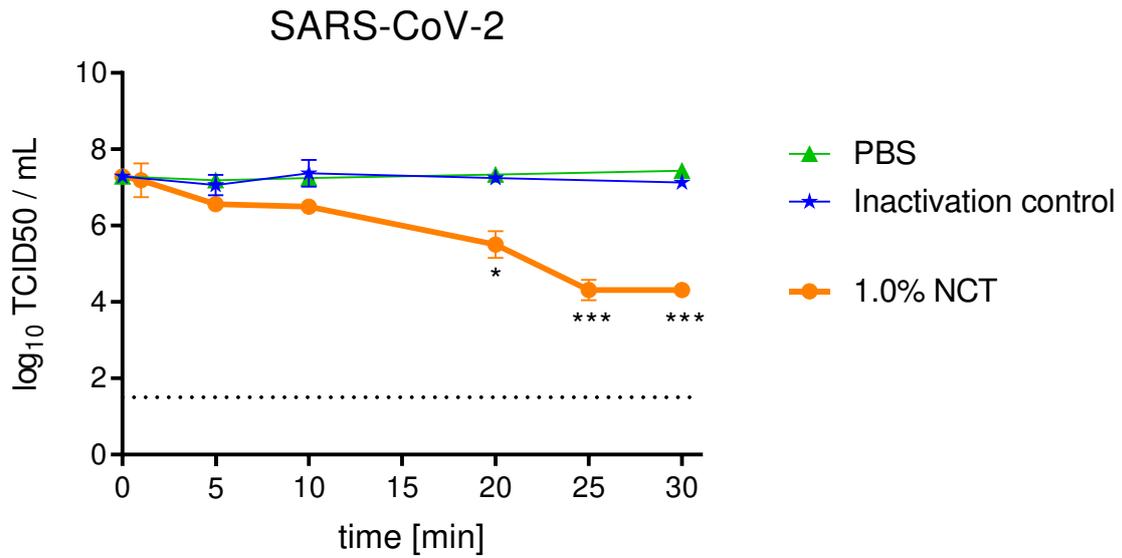
785 values ± SD of genome copies of three independent experiments. Detection limit 2.10 log₁₀

786 RNA copies/ml (dotted line).

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790

791 Fig. 2e

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793 **Fig. 2: Inactivation of SARS-CoV-2 by NCT.**794 e, Virus titration by TCID₅₀. Mean values ± SD of two independent experiments. Detection795 limit 1.50 log₁₀ (dotted line).

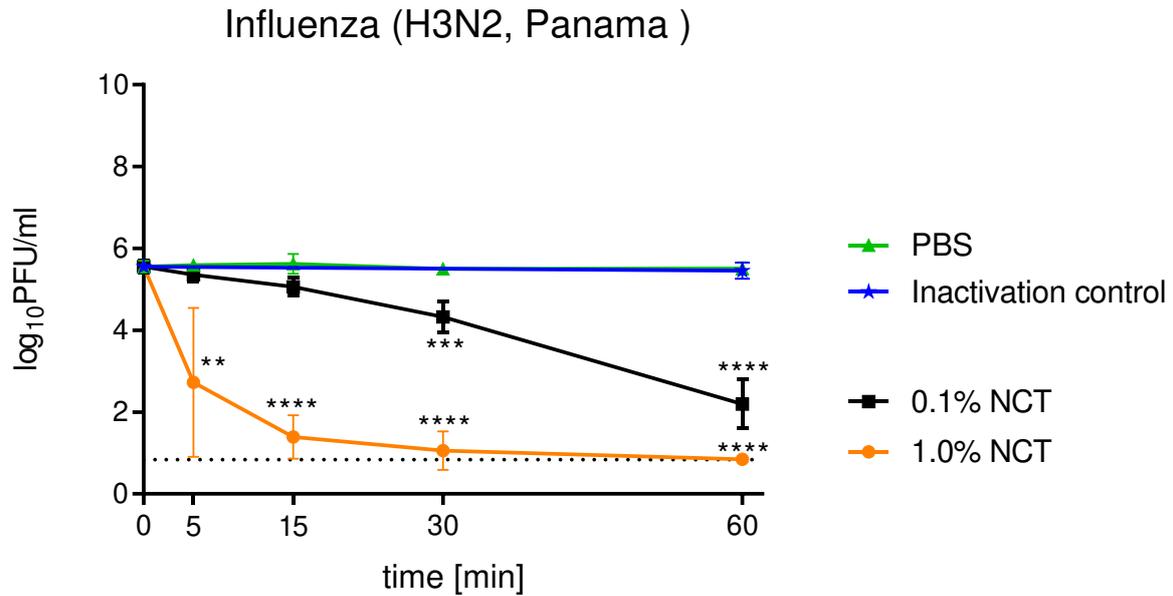
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803 Fig. 3a

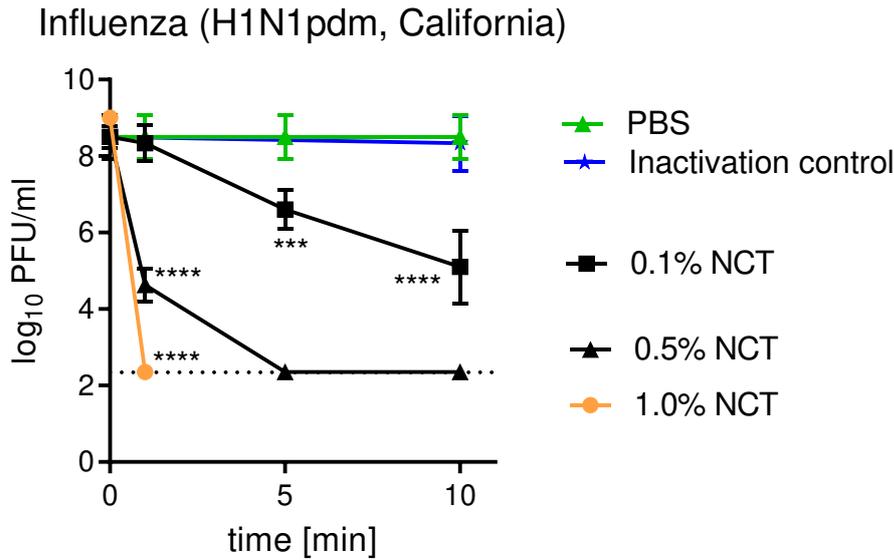
804

805 **Fig. 3: Inactivation of influenza viruses by NCT.**

806 **a**, Inactivation of Influenza A/Panama/2007/1999 (H3N2) by 1.0% (55 mM) and 0.1% (5.5
 807 mM) NCT. Virus suspension was incubated with NCT or PBS for 5 min, 15 min, 30 min, or 60
 808 min at 37°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT.
 809 Remaining infectious virus particles were determined using plaque titration. To control for
 810 inactivation of NCT by met/his, virus was added after dilution of 1.0% NCT in met/his in the
 811 inactivation control. Mean values \pm SD of five independent experiments. * $p < 0.05$, ** $p <$
 812 0.01 , *** $p < 0.001$, **** $p < 0.0001$ versus PBS control. Detection limit $0.84 \log_{10}$ (dotted
 813 line).

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817

818 Fig. 3b

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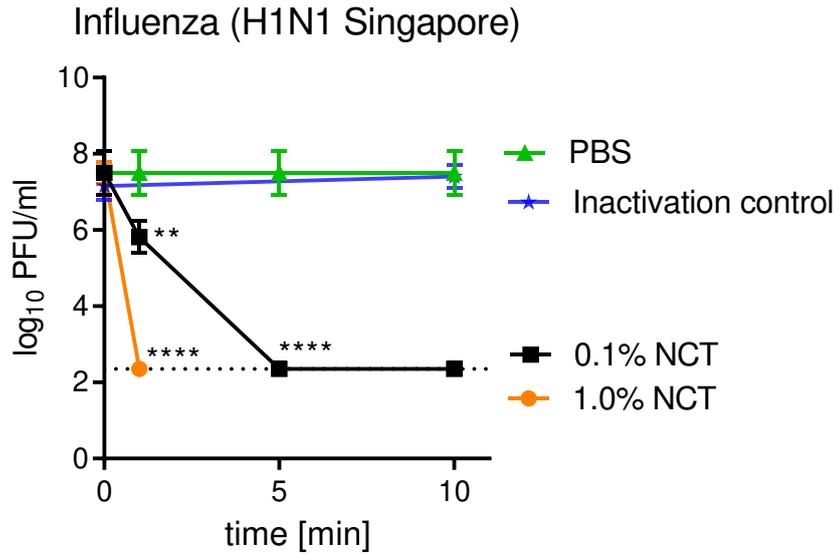
820 **Fig. 3: Inactivation of influenza viruses by NCT.**

821 **b**, Inactivation of Influenza A/California/ Swine Origin Virus/2009 (H1N1pdm) by 0.1%, 0.5%
 822 and 1.0% NCT.

823 Virus suspension was incubated with NCT in RPMI or plain RPMI for 1 min, 5 min, and 10
 824 min at 22°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT.
 825 Remaining infectious virus particles were determined using plaque titration. Mean values \pm
 826 SD of four independent experiments. Detection limit 2.35 log₁₀ (dotted line).

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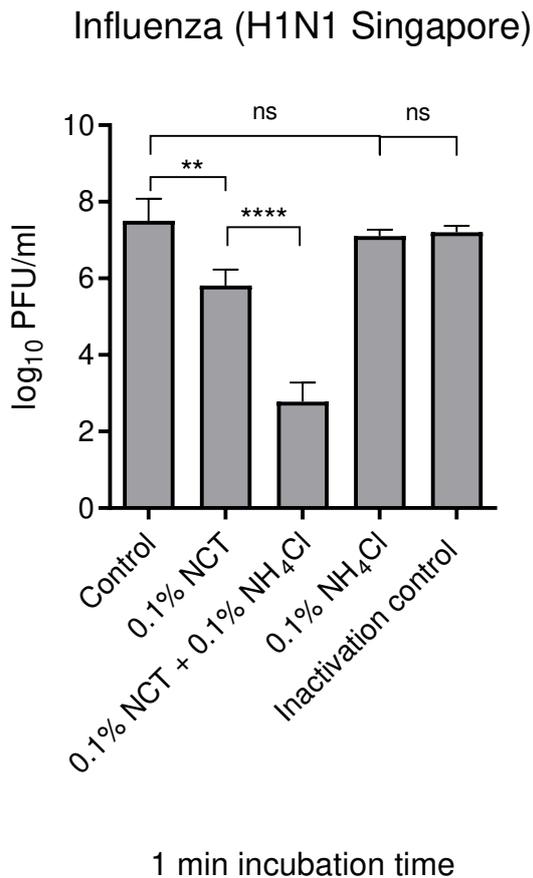
831 Fig. 3c

832

833 **Fig. 3: Inactivation of influenza viruses by NCT.**834 **c**, Inactivation of Influenza A/Singapore/Hongkong/2339/2000 (H1N1) by 0.1% and 1.0%835 NCT and **(d)** by 0.1% NCT and 0.1% (5.5 mM) NCT plus 0.1% (18.7 mM) ammonium

836 chloride compared. Test procedure and number of independent experiments as in Fig. 3b.

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838

839 Fig. 3d

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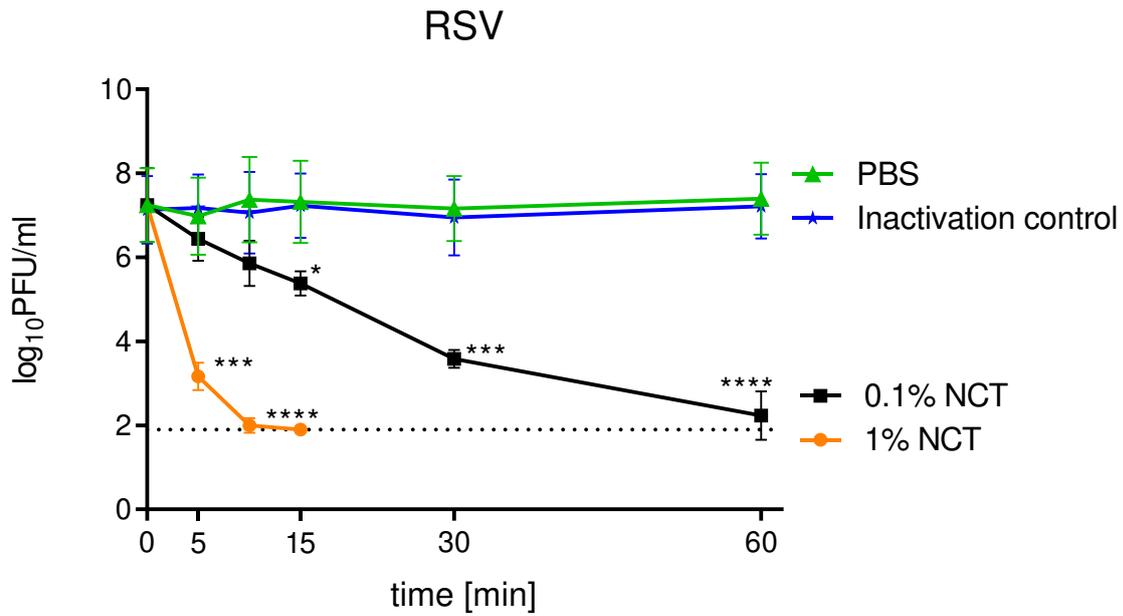
841 **Fig. 3: Inactivation of influenza viruses by NCT.**842 **c**, Inactivation of Influenza A/Singapore/Hongkong/2339/2000 (H1N1) by 0.1% and 1.0%843 NCT and **(d)** by 0.1% NCT and 0.1% (5.5 mM) NCT plus 0.1% (18.7 mM) ammonium

844 chloride compared. Test procedure and number of independent experiments as in Fig. 3b.

845 Inactivation control in **(d)** consisting of 0.1% NCT plus 0.1% NH₄Cl plus inactivator.

846

847



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849

850 Fig. 4

851

852 **Fig. 4: Inactivation of respiratory syncytial virus by NCT.**

853 Virus suspension was incubated with 1.0% or 0.1% NCT or PBS for 5 min, 10 min, 15 min,

854 30 min, or 60 min at 37°C, after which samples were diluted 1:1 in met/his solution for

855 inactivation of NCT. Remaining infectious virus particles were determined using plaque

856 titration. Mean values \pm SD of three independent experiments. Detection limit 1.90 log₁₀

857 (dotted line).

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Figures

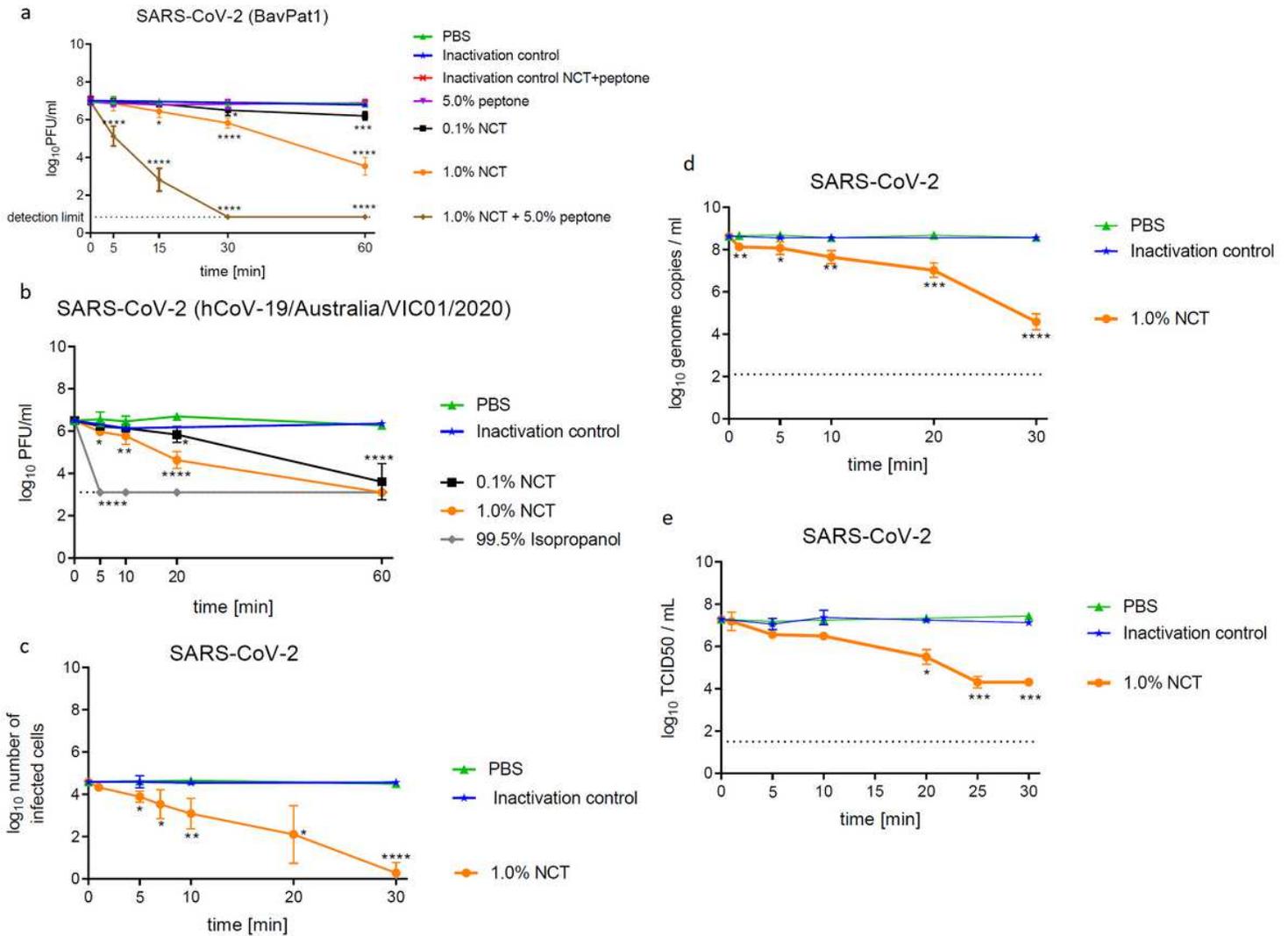


Figure 1

Inactivation of SARS-CoV-2 by NCT. a, Virus suspension (SARS-CoV-2 BavPat1) was incubated with 1.0% (55 mM) NCT, 0.1% (5.5 mM) NCT, 1.0% NCT with 5.0% peptone or PBS or 5.0% peptone for 5 min, 15 min, 30 min, or 60 min at 37°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT. Remaining infectious virus particles were determined using plaque titration. To control for inactivation of NCT by met/his, virus was added after dilution of 1.0% NCT with or without peptone in met/his. Mean values \pm SD of three to eight independent experiments in duplicates. The dotted line indicates the detection limit (0.84 log₁₀). Data were statistically analysed using a two-way ANOVA including a Dunnett's multiple comparison test to PBS controls. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. Of note, the inactivation of the virus by NCT was markedly enhanced in the presence of peptone. b, Virus suspension (SARS-CoV-2 hCoV-19/Australia/VIC01/2020) was incubated with NCT or PBS or isopropanol (positive control) for 5 min, 10 min, 20 min, and 60 min at 37°C and then diluted 1:1 in met/his for inactivation of NCT, followed by plaque titration. Mean values \pm SD of three independent

experiments. Detection limit 3.11 log₁₀ (dotted line). c, Virus suspension (SARS-CoV-2, clinical isolate) was incubated with 1% NCT or PBS for 1 min, 5 min, 7 min, 10 min, 20 min, and 30 min at 37°C. After inactivation of NCT and serial dilution, aliquots were added to Vero/TMPRSS2/ACE2 cells for 1 h in 96-well plates. Cells were washed, incubated for further 9 h, and fixed for Immunostaining (c) or RT-qPCR (d). In immunostaining, infected cells were visible as red spots and counted using an ImmunoSpot S6 Ultra-V reader and CTL analyser BioSpot® 5.0 software. Mean values ± SD of three independent experiments. d, After cell lysis and RNA extraction, one-step RT-qPCR assay was performed using the iTaq™ RT-PCR (BIO-RAD) kit and previously published primers and probes specific for detection of the SARS-CoV-2 E Gene on a CFX96™ real-time system (BIO-RAD). Mean values ± SD of genome copies of three independent experiments. Detection limit 2.10 log₁₀ RNA copies/ml (dotted line e, Virus titration by TCID₅₀. Mean values ± SD of two independent experiments. Detection limit 1.50 log₁₀ (dotted line).

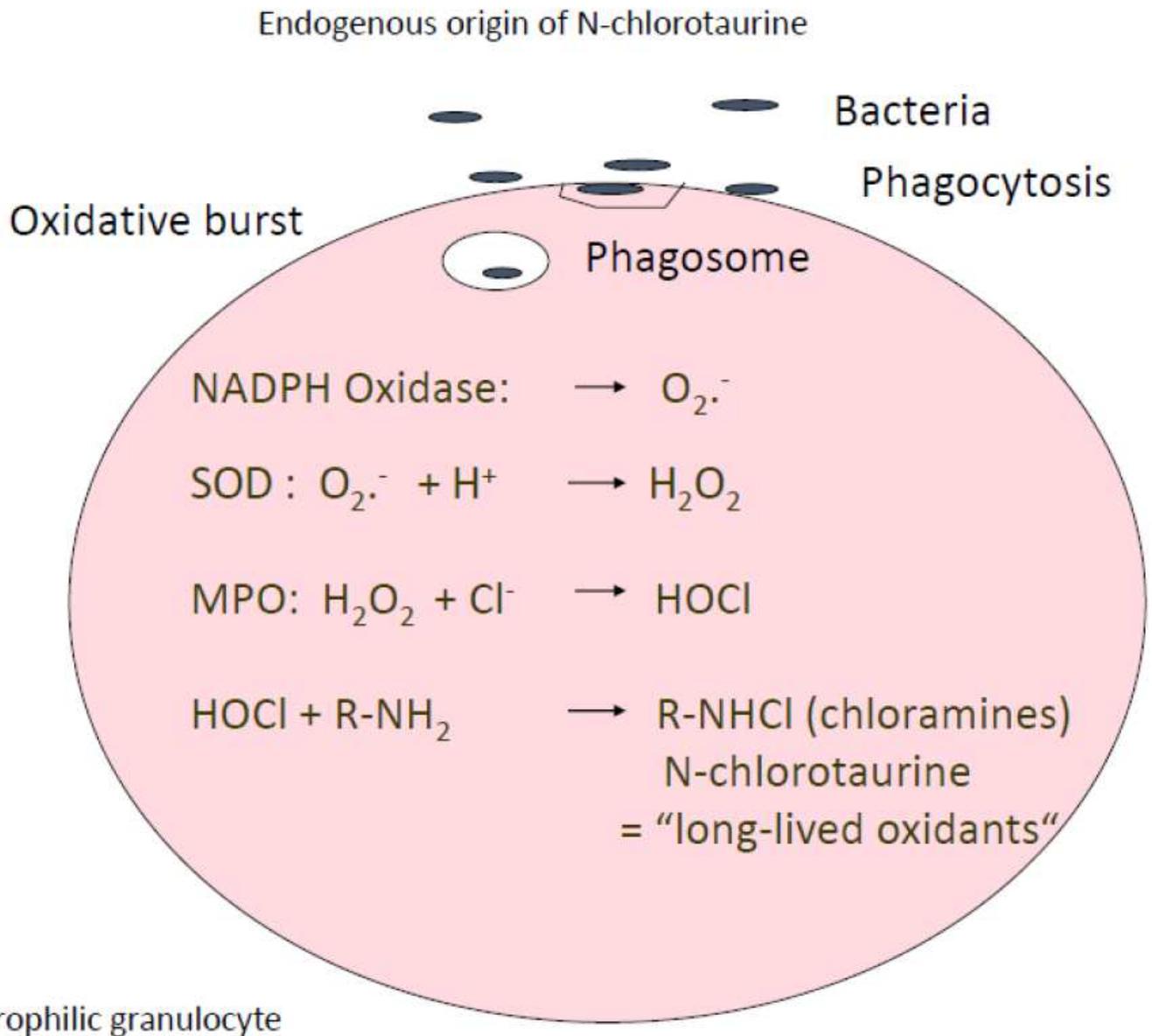


Figure 1

Endogenous origin of NCT. NCT is formed in activated human granulocytes and monocytes via an enzymatic cascade, the oxidative burst. Subsequent to superoxide (O_2^-) and hydrogen peroxide (H_2O_2), the highly reactive hypochlorite ($HOCl$) is created by myeloperoxidase (MPO), which among others reacts with amino compounds to form less reactive chloramines, also named long-lived oxidants. NCT is the main representative of these chloramines. SOD superoxide dismutase.

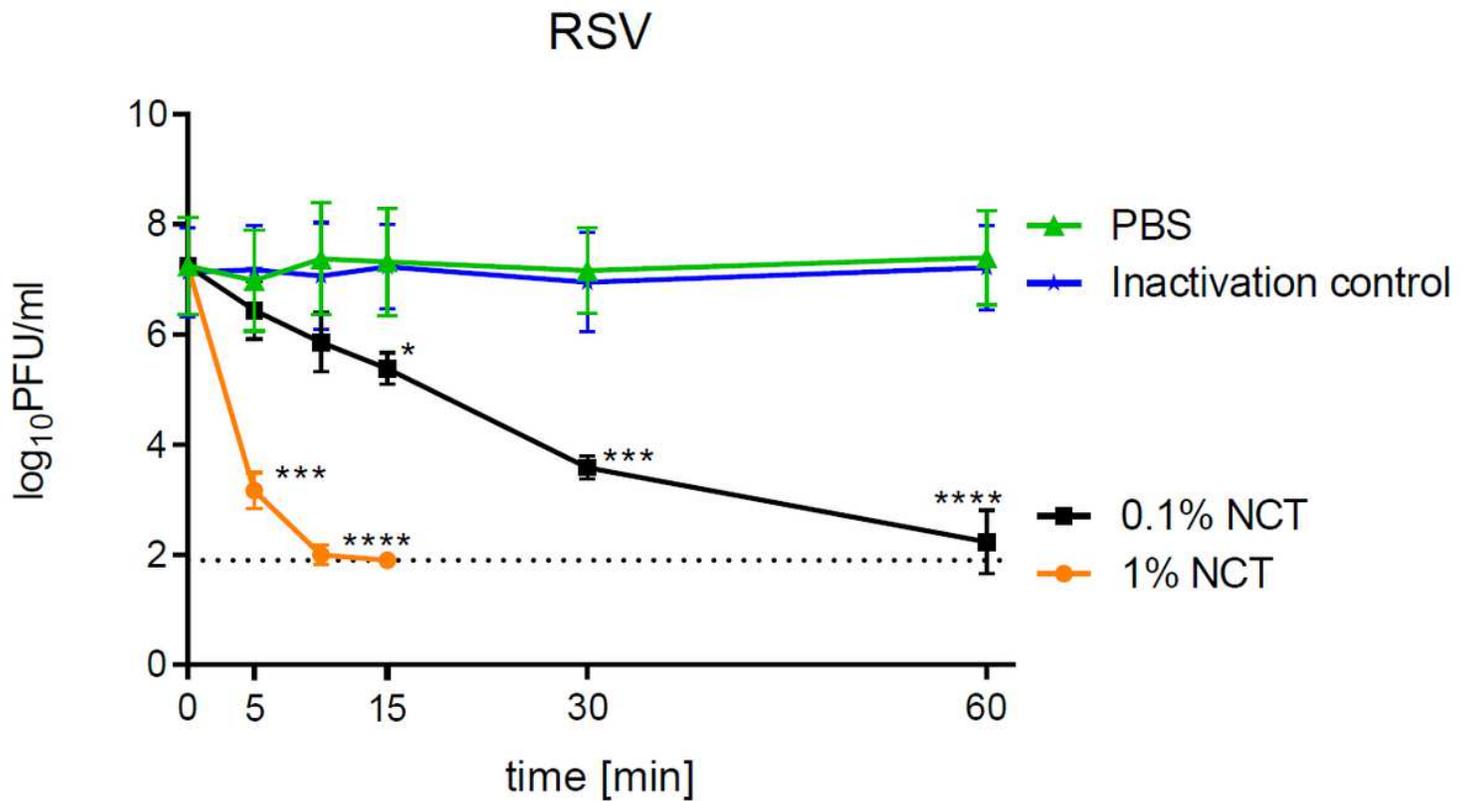


Figure 1

Inactivation of respiratory syncytial virus by NCT. Virus suspension was incubated with 1.0% or 0.1% NCT or PBS for 5 min, 10 min, 15 min, 30 min, or 60 min at 37°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT. Remaining infectious virus particles were determined using plaque titration. Mean values \pm SD of three independent experiments. Detection limit 1.90 log₁₀ (dotted line).

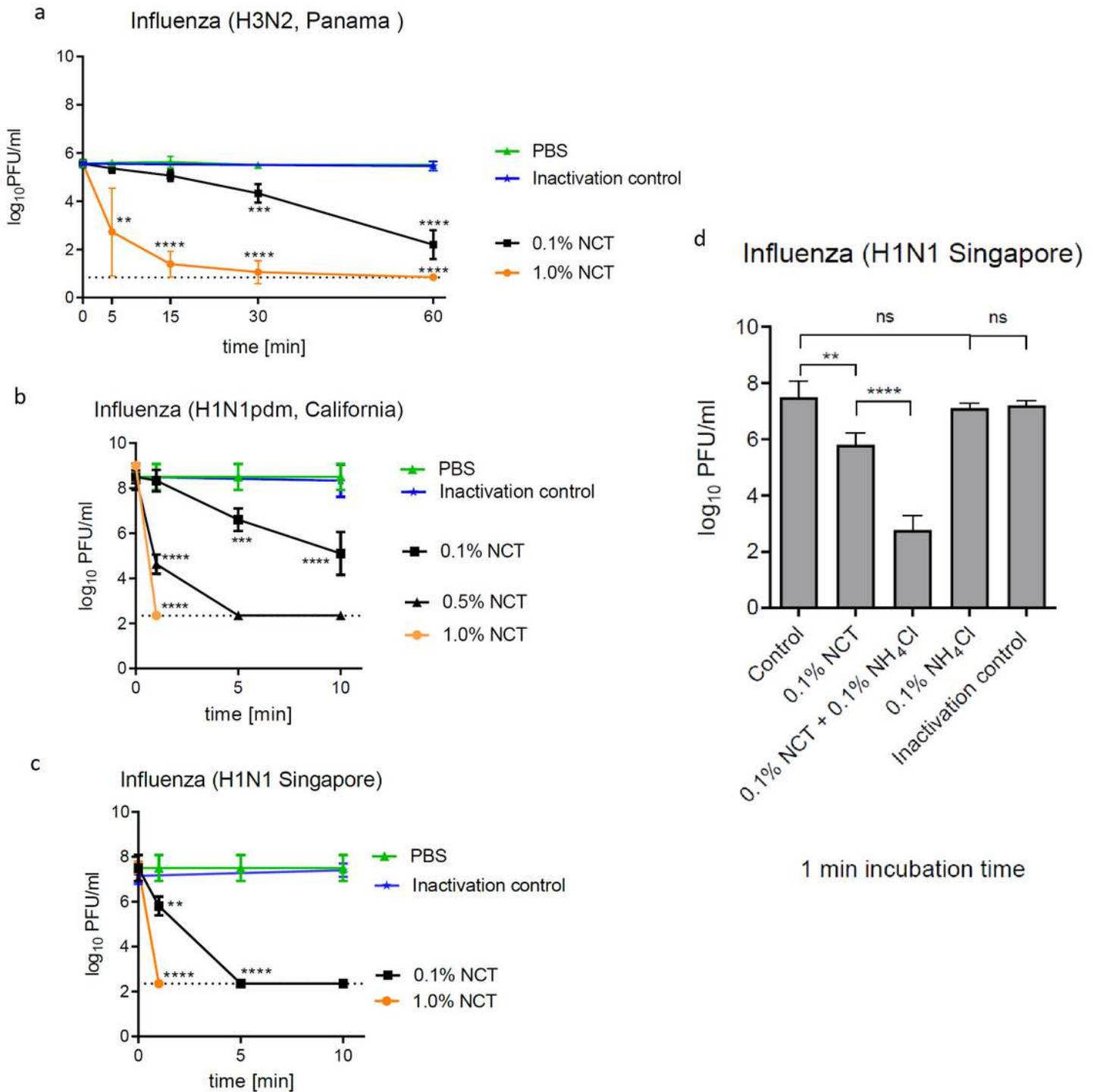


Figure 1

Inactivation of influenza viruses by NCT. a, Inactivation of Influenza A/Panama/2007/1999 (H3N2) by 1.0% (55 mM) and 0.1% (5.5 mM) NCT. Virus suspension was incubated with NCT or PBS for 5 min, 15 min, 30 min, or 60 min at 37°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT. Remaining infectious virus particles were determined using plaque titration. To control for inactivation of NCT by met/his, virus was added after dilution of 1.0% NCT in met/his in the inactivation

control. Mean values \pm SD of five independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ versus PBS control. Detection limit 0.84 log₁₀ (dotted line). b, Inactivation of Influenza A/California/ Swine Origin Virus/2009 (H1N1pdm) by 0.1%, 0.5% and 1.0% NCT. Virus suspension was incubated with NCT in RPMI or plain RPMI for 1 min, 5 min, and 10 min at 22°C, after which samples were diluted 1:1 in met/his solution for inactivation of NCT. Remaining infectious virus particles were determined using plaque titration. Mean values \pm SD of four independent experiments. Detection limit 2.35 log₁₀ (dotted line). c, Inactivation of Influenza A/Singapore/Hongkong/2339/2000 (H1N1) by 0.1% and 1.0% NCT and (d) by 0.1% NCT and 0.1% (5.5 mM) NCT plus 0.1% (18.7 mM) ammonium chloride compared. Test procedure and number of independent experiments as in Fig. 3b.