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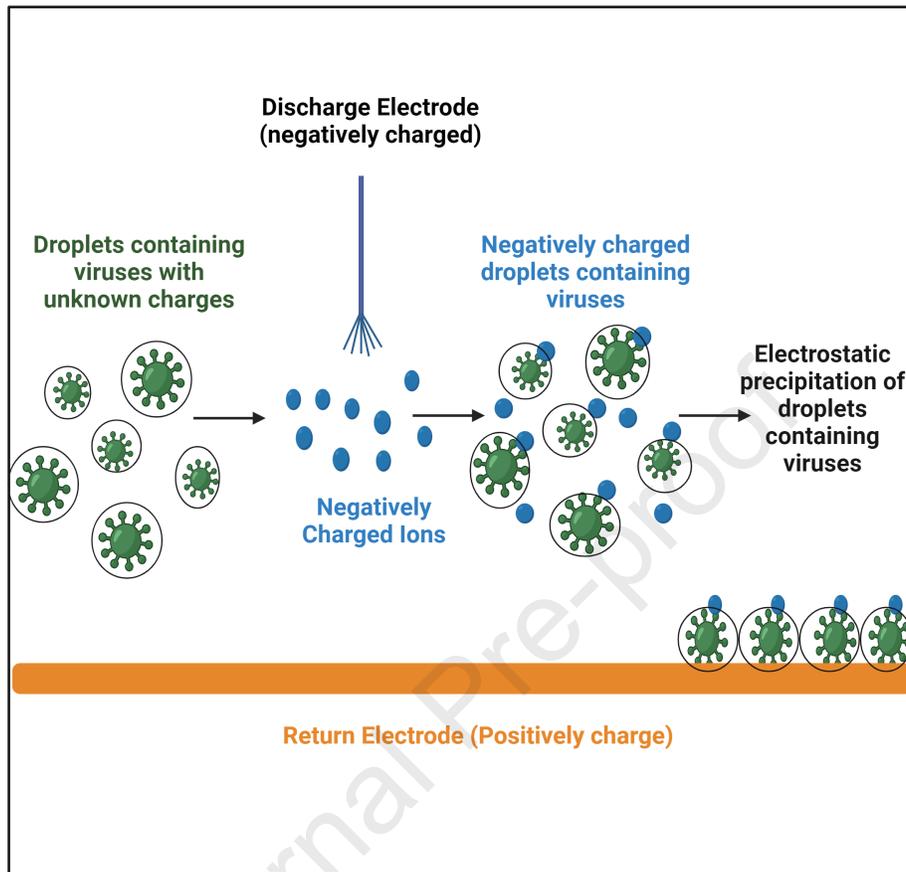
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# Capture and Inactivation of Viral Particles from Bioaerosols by Electrostatic Precipitation.

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28 **Summary:**

29 Infectious viral particles in bioaerosols generated during laparoscopic surgery place staff and patients  
30 at significant risk of infection and contributed to the postponement of countless surgical procedures  
31 during the COVID-19 pandemic causing excess deaths. The implementation of devices that inactivate  
32 viral particles from bioaerosols aid in preventing nosocomial viral spread. We evaluated whether  
33 electrostatic precipitation (EP) is effective in capturing and inactivating aerosolised enveloped and  
34 non-enveloped viruses. Using a closed-system model mimicking release of bioaerosols during  
35 laparoscopic surgery, known concentrations of each virus were aerosolised, exposed to EP and  
36 collected for analysis. We demonstrate that both enveloped and non-enveloped viral particles were  
37 efficiently captured and inactivated by EP, which was enhanced by increasing the voltage to 10kV or  
38 using two discharge electrodes together at 8kV. This study highlights EP as an effective means for  
39 capturing and inactivating viral particles in bioaerosols, which may enable continued surgical  
40 procedures during future pandemics.

41

## 42 Introduction

43 Acute respiratory viruses are the fourth leading cause of mortality worldwide [1]. Although respiratory  
44 viruses can be spread by physical contact, contaminated fomites, and large droplets, key transmission  
45 occurs via the dispersion of bioaerosols from an infectious individual [2]. Additionally, previous studies  
46 have shown that wildtype non-respiratory viruses, such as Human Immunodeficiency Virus (HIV) and  
47 Human Papillomavirus (HPV) can also be released in bioaerosols, during aerosol-generating medical  
48 procedures, enabling viral transmission [3, 4].

49 With particular focus on the 2019 SARS-CoV-2 pandemic, >640 million cases and >6.5 million directly  
50 related deaths were reported worldwide in December 2022 [5]. Regarding the indirect consequences  
51 of the pandemic, it is estimated that hundreds of thousands of surgeries were delayed or cancelled as  
52 a result. Bioaerosol-generating procedures, including laparoscopy, tracheostomy, open suctioning,  
53 and administration of nebulised treatments were at the highest risk of cancellation, due to the  
54 likelihood of airborne transmission to staff and other patients [6]. This has left patients untreated and  
55 undiagnosed, creating enormous backlogs of waitlisted surgeries, thereby increasing the demand for  
56 private health care [7].

57 Mitigation strategies such as mask wearing, personal protective equipment (PPE), social distancing,  
58 isolation of infected patients and mass vaccinations were enforced and encouraged by the health  
59 authorities to reduce the spread of SARS-CoV-2 [8]. However, cases of SARS-CoV-2 infection continued  
60 to fluctuate at high levels, due to the evolution of new viral strains, easing of government-enforced  
61 restrictions and a lack in vaccine confidence by the general public [9, 10]. Therefore, the population  
62 remains at risk, emphasising the need for novel non-pharmaceutical interventions (NPIs).

63 Commonly used NPIs for reducing the spread of disease in hospitals are Ultra-Low or High-Efficiency  
64 Particulate Airfilters (ULPA, HEPA), Ultraviolet (UV) light sterilisation and aerosolized hydrogen  
65 peroxide (AHP) sprays [11, 12]. Although these NPIs are somewhat capable of purifying indoor air and  
66 decontaminating surfaces, each system is hindered by limitations. ULPA/HEPA filters are non-  
67 economical and labour intensive, as they use high levels of energy to run and require regular filter  
68 changes. Viruses that are trapped via a filter can remain live and active, adding an additional risk to  
69 their use within hospitals and requiring appropriate treatment as a biohazard during disposal [13]. UV  
70 light is capable of inactivating viruses, however its efficiency is limited to its alignment with and  
71 distance from the virus itself [14]. As well as this, the exposure time and irradiance doses of UV light  
72 used to decontaminate indoor environments has not been well standardised, and incorrect usage of  
73 UV light can be hazardous [14]. AHP sprays consist of 6% hydrogen peroxide mixed with 50ppm silver  
74 ions and have been shown to eliminate SARS CoV-2 in nosocomial environments [12]. Although AHP

75 sprays are cost effective and have displayed efficacy as dry aerosol disinfectants, hydrogen peroxide  
76 is an irritant to Human skin and eyes, and if inhaled can be toxic [15].

77 As nosocomial virus transmission occurs most commonly by the release of bioaerosols from infectious  
78 patients, it would be beneficial to develop a NPI that efficiently captures and inactivates viral particles  
79 from bioaerosols in hospital environments. Electrostatic precipitation (EP) technology has been  
80 developed to be used during key-hole surgeries, such as abdominal laparoscopies, to eliminate  
81 surgical smoke [16, 17]. Surgical smoke is produced by the thermal destruction of tissue by  
82 electrosurgical instruments during medical procedures and can obstruct the surgeons field of vision,  
83 resulting in safety implications [18]. Surgical smoke consists of 95% water vapor and 5% cellular debris,  
84 of which can contain live bacterial and viral particles [18]. EP clears surgical smoke via the generation  
85 of an electric field which precipitates particles out of aerosolised dispersion and onto a charged  
86 collection surface [19]. This occurs by a discharge electrode emitting negatively charged ions into a  
87 neutrally charged space, creating a corona discharge [20]. The current produced from a negatively  
88 charged discharge electrode results in the creation of low-energy gas ions and subsequent transient  
89 electrostatic charging of aerosolised matter within a local atmosphere. A return electrode carrying a  
90 positive charge is connected to a collector plate and located at a distance from the discharge electrode  
91 enabling the precipitation of negatively charged particles onto the positively charged collector plate  
92 via electrostatic attraction. This mechanism is exploited during key-hole surgery to clear surgical  
93 smoke, whereby aerosolised particles are ionised by a discharge electrode and precipitated onto the  
94 patient's abdominal tissue, which is connected to a positively charged return electrode pad [21].  
95 Therefore, it was rational to assume that EP could also eliminate virus particles from surgical smoke,  
96 as bioaerosols released from patients consist of micrometre sized droplets, which can contain virus  
97 particles if the patient is infected. Subjecting virally contaminated aerosolised droplets to the negative  
98 charge emitted from the discharge electrode would thereby precipitate virus particles onto the  
99 positively charged return electrode, resulting in viral capture. Additionally, it was likely that EP could  
100 also inactivate virus particles from bioaerosols following contact with negatively charged air ions and  
101 formed radicals, as this has been previously suggested in other studies [22-25].

102

103 It has been suggested that EP could be used in point-of-care systems as a method of aerosol sampling,  
104 to diagnose patients rapidly and accurately for respiratory viral infections, reducing the need to  
105 perform invasive and uncomfortable diagnostic procedures such as bronchoscopy [26]. Furthermore,  
106 EP has been incorporated into a microfluidic lab-on-chip device, for immediate pathogenic detection  
107 from aerosol droplets released in the exhaled breath of patients [26]. Custom bioaerosol samplers,  
108 employing EP mechanisms have also been developed and demonstrated to detect airborne Influenza

109 Virus particles; of which studies have claimed may reduce sampling times down from hours to  
110 minutes, thus inhibiting viral transmission faster than currently existing approaches [27]. EP is thereby  
111 capable of efficiently capturing airborne virus particles. Besides medical applications, EP has been used  
112 for decades in aerosol science to collect aerosol particles onto substrates for subsequent  
113 morphological analysis by scanning electron microscopy (SEM) and total reflection x-ray fluorescence  
114 (TXRF) [28, 29].

115

116 Since EP is capable of efficiently clearing surgical smoke and has the capacity to capture airborne virus  
117 particles, it was rational to evaluate the ability of EP to capture and inactivate aerosolised viral  
118 particles from bioaerosols. Furthermore, EP has already been cleared by regulators as safe and  
119 effective in use [30, 31], thereby serving as a practical, multi-modal device to use during medical  
120 procedures to prevent the spread of aerosolised viral particles. In addition, EP is capable of  
121 precipitating particles at a minimum diameter of 7nm [17], thus improving the efficiency of particle  
122 capture and filtration compared to other established and commonly used ventilation and filtration  
123 systems, providing an alternative NPI for reducing disease transmission in hospitals.

124 The objective of our study was to evaluate the capture and inactivation of bioaerosol-containing viral  
125 particles by EP. Non-enveloped (Ad5) and enveloped (SARS-CoV-2 Pseudotyped Lentivirus) viral  
126 particles were aerosolised into a closed-system model, that was representative of key-hole surgery,  
127 and exposed to EP. Recovered samples were analysed for viral presence by real-time quantitative  
128 polymerase chain reaction (qPCR) of viral genomes and for biological activity by transduction and  
129 plaque assays in target cell lines. We hypothesised that viral exposure to EP would result in significant  
130 viral capture and inactivation.

131

132 Reducing viral transmission is not limited to SARS-CoV-2, but accounts for all viral outbreaks that may  
133 lead to future pandemics. It is therefore important that novel NPI's are evaluated and developed, to  
134 increase our preparation, improve safety within hospitals and prevent the need to cancel surgeries  
135 and medical procedures in the case of future pandemics.

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**142 Methods & materials****143 Key resources table**

144 Submitted as a separate file.

**145 Resource availability****146 Lead contact**

147 Further information and any related requests should be directed to and will be fulfilled by the lead  
148 contact, Professor Alan Parker ([ParkerAL@cardiff.ac.uk](mailto:ParkerAL@cardiff.ac.uk)).

**149 Materials availability**

150 This study did not generate new unique reagents.

**151 Data and code availability**

- 152 • All flow cytometry data presented in this study are deposited in the Mendeley data repository  
153 (FCS files) and are publicly available as of the date of publication. All qPCR data presented in  
154 this study are deposited in the Mendeley data repository (EDS/EDT files) and are publicly  
155 available as of the date of publication. Accession numbers are listed in the key resources table.
- 156 • This paper does not report original code.
- 157 • Any additional information required to reanalyse the data reported in this paper is available  
158 from the lead contact upon request.

**159 Experimental model details****160 Cell lines**

161 T-REx-293 (Tetracycline Repressor Protein expression cells, Invitrogen™, R71007) and HEK-293T cells  
162 (Human Embryonic Kidney cells, ATCC, CRL-1573) were used to produce Ad5 and SARS-2 PV virus  
163 stocks, respectively. Original CHO cell lines were obtained from ATCC (CCL-61). The CHO-CAR (Chinese  
164 Hamster Ovarian cells, transfected to express Human CAR) [32] and CHO-ACE2-TMPRSS2 (Chinese  
165 Hamster Ovarian cells, expressing Human ACE2 and TMPRSS2) stable cell lines were used in  
166 transduction assays with Ad5.GFP and SARS-2 PV, respectively. The CHO-ACE2-TMPRSS2 stable cell  
167 line was generated using the MT126 pRRL-SFFV-ACE2-IRES (AddGene, 145839) and MT131 pRRL-  
168 SFFV-TMPRSS2.v1-IRES (AddGene, 145843) plasmids [33]. T-REx-293 and HEK-293T cells were cultured  
169 in DMEM media (Dulbecco's Modified Eagle's Medium; Sigma-Aldrich, Gillingham, UK #D5796), whilst  
170 CHO-CAR and CHO-ACE2-TMPRSS2 cells were cultured in DMEM-F12 media (Dulbecco's Modified  
171 Eagle's Medium/Nutrient Mixture F-12 Ham; Sigma-Aldrich, Gillingham, UK #D0697). All media were

172 supplemented with 10% FBS (Foetal Bovine Serum; Gibco, Paisley, UK #10500-064), 2% Penicillin and  
173 Streptomycin (Gibco, Paisley, UK #15070-063) and 1% L-Glutamine (stock 200 mM; Gibco, Paisley, UK  
174 #25030-024). CHO-ACE2-TMPRSS2 cells were also passaged with 2µg/mL Puromycin and 100µg/mL  
175 Hygromycin once a week. Cells were grown at 37°C with 5% CO<sub>2</sub>. Dulbecco's Phosphate Buffered Saline  
176 (PBS, Gibco™, #10010023) and 0.05% Trypsin (Gibco™, #11590626) were used for subculture.

## 177 **Method details**

### 178 **Virus production**

179 Ad5 was modified to express Green Fluorescent Protein (GFP) [34] and was propagated in T-REx-293  
180 cells expressing E1 gene products and purified using Caesium Chloride gradient ultracentrifugation as  
181 previously described [35]. Stock titres were determined by Micro-BCA assay (Pierce, Thermo Fisher,  
182 Loughborough, #23235), assuming that 1µg protein was equal to 4 x 10<sup>9</sup> virus particles (vp) and  
183 monodispersity was confirmed by Nanoparticle Tracking Analysis (NanoSight NS300, Malvern, UK),  
184 which identified the mean diameter of particles in the stock solutions. Infectious titres were quantified  
185 by end-point dilution plaque assay, performed in T-REx-293 cells, determining plaque forming units  
186 per millilitre (PFU/ml).

187 The SARS-CoV-2 Pseudotyped Lentivirus (SARS-2 PV) contained a HIV core and expressed Wuhan strain  
188 SARS-CoV-2 Spike Proteins (GenBank accession: 43740568) on their viral envelope. SARS-2 PV are  
189 replication deficient and express GFP under the control of a spleen focus-forming virus (SFFV)  
190 promoter post transduction [36, 37]. SARS-2 PV were produced in HEK-293T/17 cells (ATCC CRL11268)  
191 that were pre-seeded in a T175 flask (Thermo) with approximately 5 x 10<sup>6</sup> cells the day before  
192 transfection. Cells were then co-transfected with 2 µg of packaging lentiviral core p8.91 [38], 3 µg of  
193 pCSGW encoding Green Fluorescent Protein [38], and 2 µg of the spike SARS2 (D614G)-pCAGGS  
194 (Medicines & Healthcare Products Regulatory Agency, #CFAR100985) using FuGENE HD (Promega, UK,  
195 #E2311) transfection reagent at a ratio of 1:3 DNA:Fugene in optiMEM (Gibco, Thermo, UK,  
196 #31985062). SARS-2 PV were harvested at 48h post transfection and supernatant filtered through a  
197 0.45 µm acetate cellulose filter (Starlab, Milton Keynes, #E4780-1453) [39] [40]. Functional titres were  
198 determined by plaque assay.

### 199 **Experimental setup of the closed-system model**

200 The standard closed-system model (**Error! Reference source not found.**) was optimised and altered  
201 for some experiments, however the general setup remained consistent in each run. A medical grade  
202 nebuliser (Aerogen® Solo Starter Kit, Aerogen Ltd, Galway, AG-A53000-XX) was used to aerosolise  
203 10ml of each sample into a 3L reaction kettle (QuickFit™ Wide Neck Flask Reaction 3L, Scientific

204 Laboratory Supplies Ltd, UK, QFR3LF). The nebuliser emitted droplet sizes of  $4.47 \pm 0.05 \mu\text{m}$ , at an  
205 aerosol output rate of  $0.536 \pm 0.01 \text{ ml/min}$ , as determined by laser diffraction (Spraytec; Malvern  
206 Panalytical Instruments) [41]. Aerosolised samples containing virus therefore consisted of  $4.47$   
207  $\pm 0.05 \mu\text{m}$  sized media droplets, each containing a dispersion of virus particles (each approximately  
208  $90\text{-}100\text{nm}$  in diameter). The reaction kettle was fitted with a lid containing multiple culture vessels  
209 (QuickFit™ Borosilicate Glass Flange Lid, Fisher Scientific, Leicestershire, MAF3/52), enabling the  
210 insertion of samples and materials, whilst maintaining an air-tight system. Ultravision™ technology  
211 was used to induce electrostatic precipitation. The power supply (Ultravision™ Generator, BOWA  
212 Medial UK, Newton Abbot, DAD-001-015) was stationed outside of the closed system. The discharge  
213 electrode (Ionwand™, BOWA Medial UK, Newton Abbot, DAD-001-003) was inserted into the reaction  
214 kettle through a Suba-Seal®, 15cm from the bottom of the reaction kettle and 7cm from either side of  
215 the reaction kettle. The power supply was attached to copper tape that covered the inside of the  
216 reaction kettle via a modified patient return electrode cable, functioning as a positively charged  
217 collector-plate. It is important to note that copper ions are virucidal, and therefore may affect viral  
218 viability. As a countercheck, an experimental run was performed using biologically inert stainless-steel  
219 as the positively charged collector-plate, to determine whether copper affected the viability of  
220 electrostatically precipitated viral particles. Stopcock adapters (QuickFit™ Borosilicate Glass Stopcock  
221 Adaptors with Sockets, Fisher Scientific, Leicestershire, MF14/3/SC) were placed throughout the  
222 system, ensuring unidirectional flow of the aerosol. A vacuum unit (Duet Flat- Back Aspirator, SSCOR,  
223 US, 2314B) was used, at maximum flow rate ( $>30\text{LPM}$ ), to suction the aerosol through the reaction  
224 kettle and into a sampling system (BioSampler®, SKC Ltd, Dorset, 225-9595). The sampling system  
225 (assembled as per manufacturer's instructions) contained 2ml sterile serum-free media (DMEM) to  
226 recover the captured aerosol samples. To prevent viral contamination, a cold-trap (QuickFit™ Cold-  
227 trap, VWR, Pennsylvania, 201-3052) was fitted between the sampling system and the vacuum unit. All  
228 experimentation was conducted in a Class II laminar flow hood, and all materials were autoclaved or  
229 sterilised with 70% Industrialised Methylated Spirit (IMS) (Thermo Fisher, #15950957, Leicestershire)  
230 before and after use.

### 231 **Experimental procedure**

232 To mimic the release of bioaerosols that occurs during key-hole surgery, we developed a closed-  
233 system model representing laparoscopy within a peritoneal cavity. A 3L reaction kettle was used to  
234 resemble the peritoneal cavity, which is sufflated to approximately 3L with  $\text{CO}_2$  during laparoscopy  
235 [32]. The discharge electrode was positioned within the reaction kettle, directly above the region of  
236 bioaerosol release, as it would be during laparoscopy. Quick-fit® glassware was used to ensure that  
237 the entire model was air-tight, preventing the release of virally contaminated aerosols.

238 In each experimental run, 10ml samples were aerosolised into the reaction kettle, which was heated  
239 to 37°C to avoid sample condensation and to resemble the average Human body temperature. Closed  
240 surgeries using electrocautery devices produce particle sizes of 0.07µm, whilst Ultrasonic scalpels  
241 produce particle sizes between 0.35-6.5µm[42, 43]. Particles produced by the nebuliser were  
242 approximately 4.5µm in size, and virus particles (90-100nm diameter) were dispersed within each  
243 particle, thus resembling aerosol particles that are released during surgery. The samples were exposed  
244 to inactivate/active EP, until the entire sample had been completely aerosolised (1 hour/sample).  
245 Samples aerosolised through the system included: Serum-free media (negative control), Ad5.GFP  
246 diluted to  $1 \times 10^{10}$ vp/ml in media and SARS-2 PV diluted to  $1 \times 10^7$ pfu/ml in media. Both viruses  
247 expressed GFP for detection in experimental assays. Additionally, 2ml of each sample was not  
248 aerosolised through the system ('non-exposed') and was immediately stored at -80°C to be used as  
249 'untreated' controls. A vacuum unit was employed to suction the aerosol through the closed-system  
250 model in a unidirectional flow into the sampling system for sample recovery, to assess viral presence  
251 within the aerosol following exposure to EP. Recovered samples were analysed for viral presence by  
252 qPCR and for viral activity via transduction and plaque assays. Immediately after complete sample  
253 aerosolisation, the collected samples were stored at -80°C. Physical parameters thought to affect the  
254 efficiency of EP were altered, in an attempt to determine optimal EP settings. Such parameters  
255 included temperature, voltage, the number of discharge electrodes within the reaction kettle and the  
256 material of the collector plate attached to the positively charged return electrode.

#### 257 **Quantification of viral genomes by qPCR**

258 DNA was extracted using the QIAamp MinElute Virus Kit (Qiagen, USA, #57704). Purified DNA was  
259 eluted in 50µl of Ultra-Pure Water (UltraPure™ DNase/RNase-Free Distilled Water, Invitrogen™,  
260 Thermo Fisher, #11538646) and stored at -20°C. DNA extracted from the virus stocks were used as  
261 standards (Serial dilution: undiluted (200ng/µl),  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ). DNA extracted from  
262 experimental samples remained undiluted. Primers (Ad5 Hexon Forward:  
263 CCTGCTTACCCCAACGAGTTTGA, Ad5 Hexon Reverse: GGAGTACATGCGGTCCTTGAGCTC; P24 Capsid:  
264 Forward: GGCTTTCAGCCCAGAAGTGATACC, P24 Capsid Reverse: GGGTCCTCCTACTCCCTGACATG)  
265 were used at 10Mm. qPCR for viral DNA was performed using the SYBR Green Master Mix (PowerUp™  
266 SYBR™ Green Master Mix, Applied Biosystems™, Thermo Fisher, #A25741) (per reaction: 15µl Master  
267 Mix and 5µl DNA). Reactions were performed in triplicate (for both samples and standards).  
268 QuantStudio™ software was used to set the thermal cycling conditions of the qPCR (Pharmaceutical  
269 Analytics QuantStudio™ 5 Real-Time PCR System, Applied Biosystems™, Thermo Fisher, #A31670).  
270 Samples were held at 50°C for 2 min, followed by 95°C for 2 min. Samples were then cycled at 95°C  
271 for 15 sec and 60°C for 1 min for 40 cycles.

272 **Transduction assays**

273 CHO-CAR/CHO-ACE2-TMPRSS2 cells were seeded into a 96-well plate at a density of  $2 \times 10^4$  cells/well  
 274 in 200  $\mu$ l complete media and cultured overnight. The following day, complete media was removed,  
 275 cells were washed briefly in PBS, and experimental samples were added to the cells (100  $\mu$ l, undiluted)  
 276 and incubated at 37°C for 3 hours. The media was then removed and discarded, and the cells were  
 277 washed twice with 100  $\mu$ l PBS, prior to replenishing the cells with 200  $\mu$ l total media and culturing for  
 278 an additional 48 hours. Cells were visualised for GFP expression using a microscopic imaging system  
 279 (EVOS M7000, Invitrogen™, Thermo Fisher Scientific, #AMF7000), then harvested in FACS buffer and  
 280 fixed with 4% Paraformaldehyde. Flow Cytometry was performed, using the Accuri (Accuri C6  
 281 v.1.0.264.21, BD Biosciences) and the FL1-A channel, to detect virally transduced cells. FlowJo™v10  
 282 software was used to analyse all Flow Cytometry data.

283 **Plaque assays**

284 T-REx-293/HEK-293T cells were seeded in 12-well plates in complete media, at a density of  $1 \times 10^5$   
 285 cells/well in triplicate. Cells were cultured for 24 hours, prior to the experiments. Medium was  
 286 removed, and the cells were washed with 1ml PBS. Experimental samples were added to the wells  
 287 (1ml, undiluted) in duplicate. The cells were incubated at 37°C for 2 hours, then the medium was  
 288 removed and replaced with 1ml complete media. The cells were cultured for a further 48 hours, before  
 289 analysis. Microscopy (EVOS M7000, Invitrogen™, Thermo Fisher Scientific, #AMF7000) was used to  
 290 image the cells (Objective Lens X20). Transduced cells fluoresced green light under the GFP light  
 291 source, enabling manual counting of infected cells. The PFU/ml of each sample was calculated using  
 292 the formula:

$$293 \left( \frac{\text{(Average number of fluorescent cells/well} \times 594 \text{ (Fields/well))}}{\text{(Volume of viral sample } \mu\text{l} \times \text{dilution factor)}} \right) \times 1000 = \text{PFU/ml}$$

294

295 **Quantification and statistical analysis**

296 All data presented show the mean  $\pm$  SD. GraphPad Prism v4.03 (GraphPad Software Inc., La Jolla, CA)  
 297 was used to produce all bar chart figures. The GraphPad Quickcalcs t-test calculator was used to  
 298 perform the two-tailed paired t-test. p-Values of \* =  $p < 0.05$ , \*\* =  $p < 0.005$ , \*\*\* =  $p < 0.0005$ , ns = not  
 299 statistically significant,  $p > 0.05$ . All statistical details of the experiments can be found in the figures and  
 300 figure legends of the results section. The n value is equal to the number of technical repeats.

## 301 Results

302 **Ad5 particles were successfully captured and inactivated by electrostatic precipitation when**  
303 **aerosolised at 37°C.**

304 First, we sought to evaluate whether EP could capture and inactivate aerosolised non-enveloped Ad5  
305 particles using our standard closed-system model. The number of recovered Ad5 genomes  
306 significantly decreased following Ad5 exposure to inactive EP as gauged by qPCR for viral genomes,  
307 indicating viral loss as a result of sample aerosolization alone (**Figure 2.A**). A significant 6.8-fold  
308 reduction in the number of recovered Ad5 genomes was observed following Ad5 exposure to active  
309 EP (**Figure 2.A**). Ad5 viability was not affected following exposure to inactive EP, as displayed by  
310 transduction and plaque assays (**Figure 2***Error! Reference source not found.***B & C**), indicating that sample  
311 aerosolization at 37°C was not detrimental to Ad5. The transduction assay demonstrated a 13.6-fold  
312 reduction in the percentage of transduction, in cells that were treated with Ad5 that had been exposed  
313 to active EP (**Figure 2.B**). Mirroring this, the plaque assay displayed a  $4 \times 10^3$ -fold reduction in active Ad5  
314 particles, in the sample exposed to active EP (**Figure 2.C & D**). These results indicated that EP  
315 successfully captured and inactivated aerosolised Ad5 particles within our standard closed-system  
316 model.

317 **Capture and inactivation of Ad5.GFP was most efficient when exposing viral particles to 10kV.**

318 Multiple parameters may impact the efficiency of EP. We assessed the impact of increasing voltages  
319 on the ability of EP to capture and inactivate aerosolised Ad5. EP is currently used at 8kV to clear  
320 surgical smoke during laparoscopies. We exposed aerosolised samples of Ad5 to EP active at 6kV, 8kV  
321 and 10kV, to determine whether decreasing or increasing the standard voltage impacted its ability to  
322 capture and inactivate viral particles. By increasing the voltage of EP, the region of corona discharge  
323 was expanded, thus reaching a larger surface area and contacting more aerosolised virus particles. As  
324 10kV is the maximum voltage that is medically approved for EP use during surgery, voltages above this  
325 were not evaluated.

326 qPCR analysis of treated samples indicated significant viral capture by EP, following sample exposure  
327 to 6kV, 8kV and 10kV (**Figure 3.A**). The number of viral genomes were reduced by 21.8-fold and 16.8-  
328 fold, following Ad5 exposure to 6kV and 8kV, respectively. However, Ad5 capture was enhanced when  
329 exposing the viral particles to 10kV, as shown by a  $7.4 \times 10^3$ -fold reduction in the number of viral  
330 genomes (**Figure 3.A**). Increasing the voltage to 10kV also improved viral inactivation, demonstrated  
331 by transduction and plaque assay (**Figure 3.B & C**). The percentage of transduced cells infected with  
332 Ad5 samples that had been exposed to 6kV and 8kV was significantly reduced by 6.6-fold and 25.6-  
333 fold, respectively (**Figure 3.B**). Cells treated with Ad5 that had been exposed to 10kV displayed a 529.4-

334 fold reduction in viral transduction (**Figure 3.B**). Mirroring this, plaque assays of treated samples  
335 demonstrated a significant decrease in the number of viable Ad5 particles in samples that were  
336 exposed to 6kV, 8kV and 10kV (**Figure 3.C & D**). Imaging of GFP highlighted a complete absence of  
337 viable Ad5 particles in cells infected with Ad5 samples that had been exposed to 10kV, indicating that  
338 10kV is the optimal voltage to elicit efficient EP of bioaerosols during surgery, to completely prevent  
339 the transmission of infectious aerosolised virus particles (**Figure 3.C**). Whilst 6kV significantly reduced  
340 the number of viable virus particles, EP by 8kV and 10kV resulted in log reductions of >3.5, suggesting  
341 a decrease within a clinically significant range.

#### 342 **Using 2 discharge electrodes enhanced adenoviral capture and inactivation.**

343 We next evaluated whether enhanced viral inactivation was possible when exposing aerosolised Ad5  
344 particles to 2, rather than a single discharge electrode. Both discharge electrodes were used at 8kV,  
345 maintaining the voltage setting that is currently used during laparoscopic surgery. Separate Ad5  
346 samples were exposed to either 1 or 2 discharge electrodes, to evaluate whether combining 2  
347 discharge electrodes improved viral capture and inactivation.

348 qPCR results displayed a significant decrease in the number of viral genomes in Ad5 samples that were  
349 exposed to either 1 or 2 active discharge electrodes. A 125-fold reduction in the number of Ad5  
350 genomes was observed in the sample exposed to 1 active discharge electrode, whereas exposure of  
351 Ad5 to 2 discharge electrodes resulted in an increased  $1.25 \times 10^3$ -fold reduction in the number of Ad5  
352 genomes detected (**Figure 4.A**). This indicated that using 2 discharge electrodes, both active at 8kV,  
353 enhanced viral capture by a further 10-fold. Similarly, Ad5 samples exposed to 1 or 2 discharge  
354 electrodes were both significantly inactivated. Cells treated with the Ad5 sample that had been  
355 exposed to a single active discharge electrode displayed a 31.6-fold reduction in the percentage of  
356 virally transduced cells (**Figure 4.B**). In comparison, cells treated with the Ad5 sample that had been  
357 exposed to 2 active discharge electrodes displayed a 215.2-fold reduction in the percentage of  
358 transduced cells, indicating that using 2 discharge electrodes enhanced viral capture (**Figure 4.B**).  
359 Plaque assay confirmed these findings, as shown by an 800-fold decrease in the number of active Ad5  
360 particles, post exposure to a single discharge electrode, in comparison to a complete elimination of  
361 active Ad5 particles, post exposure to 2 discharge electrodes (**Figure 4.C & D**). This experimental run  
362 highlighted that using 2 discharge electrodes enhanced viral capture and inactivation in a synergistic  
363 manner.

#### 364 **Replacing the copper return electrode with a stainless-steel electrode indicated that electrostatic 365 precipitation was the sole cause of viral inactivation.**

366 In previous runs, copper tape was attached to the positively charged return electrode, functioning as  
367 a collector plate for the precipitation of ionised virus particles. However, copper is a naturally virucidal

368 metal and studies have shown direct contact between copper and viral particles resulting in viral  
369 inactivation [44]. Therefore, we hypothesised that direct contact between the aerosolised viral  
370 particles and the copper tape may have been causing the viral inactivation observed in previous runs.  
371 To determine whether EP or the copper tape was causing viral inactivation, stainless-steel sheets were  
372 used to replace the copper tape. Stainless-steel is a biologically inert, non-toxic metal [45], and should  
373 not inactivate Ad5 particles upon direct contact. Ad5 samples that were not aerosolised, nor exposed  
374 to EP, were exposed to the stainless-steel sheets (direct contact for 2 minutes) and analysed for viral  
375 activity in the same way as the collected experimental samples.

376 There was no significant difference between the number of Ad5 viral genomes in the non-exposed  
377 Ad5 sample and the Ad5 sample that was exposed to stainless-steel (**Figure 5.A**). This indicated that  
378 stainless-steel did not alter the integrity of the viral DNA. The number of Ad5 genomes was  
379 significantly decreased in the Ad5 sample exposed to inactive EP, indicating that aerosolization alone  
380 resulted in a reduction in viral DNA collected within the sampling system, or potentially highlighting a  
381 size-specific particle loss phenomenon. However, the number of viral genomes was further  
382 significantly reduced in Ad5 samples following exposure to active EP at 8kV and 10kV (**Figure 5.A**). This  
383 indicated that EP successfully captured the aerosolised Ad5 particles. Cells treated with non-exposed  
384 Ad5 and the Ad5 sample that was non-exposed to the closed-system but exposed to stainless-steel  
385 showed no significant difference in the percentage of virally transduced cells (**Figure 5.B**). Plaque assay  
386 results mirrored this result, showing no visible differences between TReX-293T cells infected with  
387 either sample (**Figure 5. C**). This indicated that direct contact between Ad5 particles and stainless-steel  
388 did not affect viral viability. In addition, CHO-CAR cells infected with Ad5 samples exposed to active  
389 EP at 8kV and 10kV displayed 11.32-fold and 86.9-fold reductions in the percentage of virally  
390 transduced cells, indicating successful inactivation of Ad5 particles by EP (**Figure 5.B**). Confirming this,  
391 TReX-293T cells infected with Ad5 samples that had been exposed to active EP at 8kV and 10kV showed  
392 visibly reduced levels of fluorescence, indicating successful inactivation (**Figure 5.C**).

### 393 **Electrostatic precipitation successfully captured and inactivated enveloped viral particles (SARS-2** 394 **PV).**

395 Finally, we sought to evaluate the ability of EP to capture and inactivate enveloped viral particles, such  
396 as SARS-CoV-2. As Ad5 is a non-enveloped virus, we used a SARS-CoV-2 Pseudotyped Lentivirus (SARS-  
397 2 PV), as its core and genetic material is enclosed by a lipid envelope which expresses the Wuhan Spike  
398 protein on its surface, thereby resembling the external structure of wildtype SARS-CoV-2. Neat  
399 samples of SARS-2 PV were aerosolised and exposed to EP in the same way as Ad5 in **Error! Reference**  
400 **source not found..**

401 SARS-2 PV was significantly captured by EP, as quantified by qPCR (**Figure 6.A**). A 2.6-fold reduction in  
402 the number of viral genomes was observed in the SARS-2 PV sample that had been exposed to active  
403 EP, indicating successful virus capture (**Figure 6.A**). In addition, transduction and plaque assays using  
404 the collected samples showed that EP significantly inactivated aerosolised SARS-2 PV particles (**Figure**  
405 **6.B, C & D**). CHO-ACE2-TMPRSS2 cells infected with the SARS-2 PV sample that had been exposed to  
406 active EP displayed a 27.7-fold reduction in the percentage of viral transduction (**Figure 6.B**). Likewise,  
407 HEK-293T cells infected with SARS-2 PV that had been exposed to active EP displayed a visually  
408 decreased number of fluorescent cells, compared to the non-exposed sample and the SARS-2 PV  
409 sample exposed to inactive EP (**Figure 6.C**). However, the number of viral genomes, as well as viral  
410 viability, was significantly reduced in the SARS-2 PV samples that were aerosolised and exposed to  
411 inactive EP (**Figure 6**). This indicated that aerosolised SARS-2 PV was less stable than aerosolised Ad5,  
412 and that the sample was more susceptible to inactivation or degradation by aerosolization alone.

413

**414 Discussion**

415

416 Existing methods of purifying indoor air are limited by their inability to capture aerosolised particles  
417 smaller than  $0.15\mu\text{m}$  and failure to inactivate live pathogens upon successful capture [13]. These  
418 limitations facilitate disease transmission. During periods of viral outbreaks, such as the 2019 SARS-  
419 CoV-2 pandemic, bioaerosol-generating medical procedures are at risk of cancellation and delay, due  
420 to the likelihood of viral spread [6]. It is therefore crucial that novel non-pharmaceutical interventions  
421 (NPI's) are developed to prevent airborne viral transmission in hospital settings, enabling medical  
422 procedures to continue safely and as normal. Established EP systems are currently used to sample and  
423 filter indoor air, as well as to clear surgical smoke during key-hole surgeries. Here we have  
424 demonstrated additional modalities of EP, in its ability to efficiently capture and inactivate aerosolised  
425 viral particles.

426 Significant capture and inactivation of aerosolised Ad5 and SARS-2 PV particles by EP was observed in  
427 our standardised closed-system model. Viral capture was displayed by a reduction in the number of  
428 viral genomes collected within the sampling system, following sample exposure to active EP,  
429 compared to recovered samples exposed to inactive EP. Similarly, viral inactivation was shown by a  
430 reduction in biological activity of viral particles, as gauged by the percentage of transduced cells that  
431 were treated with recovered samples post exposure to active EP, compared to samples exposed to  
432 inactive EP. Interestingly, it appeared that viral inactivation by EP was more successful than viral  
433 capture. Although the copper collector plate used within our closed-system model was naturally  
434 virucidal, our findings show that EP was the major cause of viral inactivation. However, using a  
435 virucidal collector plate, such as copper, may provide additional safety benefits for the removal of  
436 viable pathogens from bioaerosols by EP, thereby outperforming existing devices like HEPA filters.

437 Viral inactivation by EP was highly efficient, at approximately 90-95% efficiency when using EP at 8kV,  
438 and at >99% efficiency when using EP at 10kV or when using 2 discharge electrodes (both at 8kV).  
439 Arguably, viral inactivation is more important than viral capture, as this can prevent the spread of  
440 disease. Previous studies evaluating the ability of EP to inactivate viruses suggest that the corona  
441 discharge, produced by the discharge electrode, generates air ions and reactive species ( $\text{O}_3$  and  
442 various radicals, such as  $\text{O}\cdot$ ,  $\text{N}\cdot$ ,  $\text{OH}\cdot$ , and  $\text{HO}_2\cdot$ ) capable of degrading and inactivating viral particles  
443 [22-25]. Although this mechanism has not been explicitly investigated here, our results indicate that  
444 this could be the cause of viral inactivation. In agreement, degradation of viral particles would result  
445 in the release of viral DNA/RNA, explaining the collection of viral genomes in the sampling system  
446 following sample exposure to active EP. As isolated viral DNA is biochemically inert and requires an

447 intact capsid to bind and enter target cells, the degradation of aerosolised viral particles seems a  
448 practical way of inactivating viruses and reducing their transmission [46, 47].

449 We have demonstrated that EP can efficiently capture and inactivate both non-enveloped (Ad5) and  
450 enveloped (SARS-2 PV) viral particles. However, aerosolization alone significantly reduced SARS-2 PV  
451 viability and the integrity of its capsid, causing the release of its viral genome. This was not surprising  
452 as SARS-2 PV is not a respiratory virus and is therefore not transmissible via airborne routes. However,  
453 other non-respiratory viruses, such as wildtype HIV and HPV, have been identified in surgical  
454 bioaerosols with the ability to infect healthcare staff. Therefore, it is important that EP can capture  
455 and inactivate a variety of viral particles [3, 4]. Future studies will focus on evaluating the ability of EP  
456 to capture and inactivate respiratory enveloped viruses, as well as non-respiratory non-enveloped  
457 viruses. In addition, other physical parameters govern viral spread and stability, including  
458 temperature, humidity, droplet size and air-space volume [48]. Evaluating changes to viral capture  
459 and inactivation, following the alteration of such parameters, as well as parameters effecting the  
460 efficiency of EP, such as voltage, flow rate, geometric design of the EP system and size and  
461 concentration of the ionised particles [49], will be important to optimise in future studies, prior to  
462 implementing EP in hospitals as a method of reducing viral spread.

463 In addition, EP may play a role beyond clearing surgical smoke and eliminating viral particles during  
464 key-hole surgery. Due to recent advances in EP technology, it is likely that EP will be employed during  
465 open surgeries in the near future to clear surgical smoke. It is therefore possible that EP could be  
466 manipulated to capture and inactivate viral particles in 'open' systems. For example, EP could be used  
467 to filter the release of CO<sub>2</sub> upon patient deflation following laparoscopic surgery, as well as during  
468 open surgery, to filter bioaerosols released into the surgical environment in an attempt to protect  
469 healthcare professionals within close proximity. This could provide an alternative and intriguing means  
470 of replacing HEPA filters, which are currently used to filter bioaerosols in open environments.  
471 However, this would of course require adaptations to the device itself to enable sufficient exposure  
472 of the corona discharge to bioaerosols covering a much larger surface area succeeding release from  
473 the patient. As well as this, EP could be implemented when delivering aerosolised medications or  
474 advanced therapy medicinal products (ATMPs) to patients. For example, pressurised intraperitoneal  
475 aerosol chemotherapy (PIPAC) has recently been developed as a method of treating unresectable  
476 metastatic peritoneal tumours [50, 51]. PIPAC is an emerging technology and may be useful for more  
477 novel therapeutic deliveries, such as oncolytic virotherapies. Moving forwards, use of these  
478 technologies will require efficient means of controlling their emission during delivery. EP could be  
479 implemented during this type of therapeutic delivery to prevent the escape of oncolytic viruses into  
480 operating theatres, whilst simultaneously ensuring and directing efficient delivery of drugs to the

481 tumour site. PIPAC has been developed for use during key-hole closed surgery, therefore EP could be  
482 placed within the patient's abdomen for the duration of drug delivery, as it already is during abdominal  
483 laparoscopies that use EP to clear surgical smoke.

484 In summary, our findings indicate that EP could be used during surgery to capture and inactivate viral  
485 particles released in bioaerosols, as well as potentially during other medical procedures, to enhance  
486 efficacy and safety. Employing EP as a NPI to reduce viral spread in hospitals may resolve issues  
487 experienced with existing air-purification systems, which in turn could reduce pressures on the NHS  
488 by preventing indirect morbidities and mortalities. For example, recent outbreaks of the Highly  
489 Pathogenic Avian Influenza A (H5N1) in wild birds and poultry has the capacity to spread to human  
490 hosts, which if unprevented, could result in the next human global pandemic [52]. Using data obtained  
491 from this study, we predict that it is possible to use EP to minimise viral spread thus preventing future  
492 viral pandemics.

493

494 **Competing interest statement/Declaration of interest:**

495 J.B. is an employee of Alesi Surgical Ltd. A.L.P. is Chief Scientific Officer of Trocept Therapeutics Ltd.

496

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506 with input from R.B., J.B. and A.L.P. Formal analysis, H.E.P., R.B. and A.L.P. Resources, M.M.N. and N.T.  
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635 [2023/nearing-record-number-avian-](https://www.cdc.gov/flu/avianflu/spotlights/2022-2023/nearing-record-number-avian-influenza.htm#:~:text=Since%20early%202022%2C%20more%20than,outbreak%20that%20occurred%20in%202015)  
636 [influenza.htm#:~:text=Since%20early%202022%2C%20more%20than,outbreak%20that%20o](https://www.cdc.gov/flu/avianflu/spotlights/2022-2023/nearing-record-number-avian-influenza.htm#:~:text=Since%20early%202022%2C%20more%20than,outbreak%20that%20occurred%20in%202015)  
637 [ccurred%20in%202015](https://www.cdc.gov/flu/avianflu/spotlights/2022-2023/nearing-record-number-avian-influenza.htm#:~:text=Since%20early%202022%2C%20more%20than,outbreak%20that%20occurred%20in%202015).

638

639

640

641 **Figure Legends:**

642 **Figure 1. Schematic of the experimental setup of the refined closed-system model.** All samples were  
643 aerosolised into the air-tight reaction kettle, exposed to EP (active/inactive) and suctioned into the  
644 BioSampler® for recovery and collection. Collected samples were stored at -80°C immediately after  
645 each experimental run, prior to experimental analysis.

646 **Figure 2. Capture and inactivation of Ad5 by electrostatic precipitation.** ‘EP OFF’ signifies sample  
647 exposure to inactive<sup>EP</sup> and ‘EP ON’ signifies sample exposure to active EP. ‘Non-Exposed’ signifies  
648 samples that were not aerosolised through the model system, nor exposed to EP. **(A)** Viral capture  
649 quantified by qPCR. **(B)** Viral inactivation demonstrated by transduction assay. **(C & D)** Viral  
650 inactivation displayed by plaque assay in TReX-293 cells. TReX-293 cells treated with samples and  
651 analysed for GFP fluorescence. TRANS = Brightfield transmitted light, GFP = GFP light source. Error  
652 bars represent the  $\pm$ SD (n = 3). Plaque assay functional titres represent the mean (n = 5).

653 **Figure 3. Increasing the voltage of EP to 10kV enhances viral capture and inactivation.** ‘EP OFF’  
654 signifies sample exposure to inactive EP and ‘EP ON’ signifies sample exposure to active EP. ‘Non-  
655 Exposed’ signifies samples that were not aerosolised through the model system, nor exposed to EP.  
656 **(A)** Viral capture demonstrated by qPCR. **(B)** Viral inactivation determined by transduction assay. **(C &**  
657 **D)** Viral inactivation displayed by plaque assay in TReX-293 cells. TReX-293 cells treated with samples  
658 and analysed for GFP fluorescence. TRANS = Brightfield transmitted light, GFP = GFP light source. Error  
659 bars represent the  $\pm$ SD (n = 3). Plaque assay functional titres represent the mean (n = 5).

660 **Figure 4. Exposing Ad5 particles to 2 discharge electrodes, opposed to 1, enhances viral capture and**  
661 **inactivation.** ‘EP OFF’ signifies sample exposure to inactive EP and ‘EP ON’ signifies sample exposure  
662 to active EP. ‘Non-Exposed’ signifies samples that were not aerosolised through the model system,  
663 nor exposed to EP. **(A)** Viral capture demonstrated by qPCR. **(B)** Viral inactivation determined by  
664 transduction assay. **(C & D)** Viral inactivation displayed by plaque assay in TReX-293 cells. TReX-293  
665 cells treated with samples and analysed for GFP fluorescence. TRANS = Brightfield transmitted light,  
666 GFP = GFP light source. Error bars represent the  $\pm$ SD (n = 3). Plaque assay functional titres represent  
667 the mean (n = 5).

668 **Figure 5. Evidencing EP as the sole cause of viral inactivation.** ‘EP OFF’ signifies sample exposure to  
669 inactive EP and ‘EP ON’ signifies sample exposure to active EP. ‘Non-Exposed’ signifies samples that  
670 were not aerosolised through the model system, nor exposed to EP. ‘Steel’ signifies samples that were  
671 exposed (direct contact) to stainless-steel for 2 minutes. **(A)** Viral capture demonstrated by qPCR. **(B)**  
672 Viral inactivation determined by transduction assay. **(C & D)** Viral inactivation displayed by plaque  
673 assay in TReX-293 cells. TReX-293 cells treated with samples and analysed for GFP fluorescence. TRANS  
674 = Brightfield transmitted light, GFP = GFP light source. Error bars represent the  $\pm$ SD (n = 3). Plaque  
675 assay functional titres represent the mean (n = 5).

676 **Figure 6. Capture and inactivation of SARS-2 PV by EP.** ‘EP OFF’ signifies sample exposure to inactive  
677 EP and ‘EPON’ signifies sample exposure to active EP. ‘Non-Exposed’ signifies samples that were not  
678 aerosolised through the model system, nor exposed to EP. **(A)** Viral capture determined by qPCR. **(B)**  
679 Viral inactivation demonstrated by transduction assay. **(C & D)** Viral inactivation displayed by plaque  
680 assay in HEK-293T cells. HEK-293T cells treated with samples and analysed for GFP fluorescence.  
681 TRANS = Brightfield transmitted light, GFP = GFP light source. Error bars represent the  $\pm$ SD (n = 3).  
682 Plaque assay functional titres represent the mean (n = 5).

683

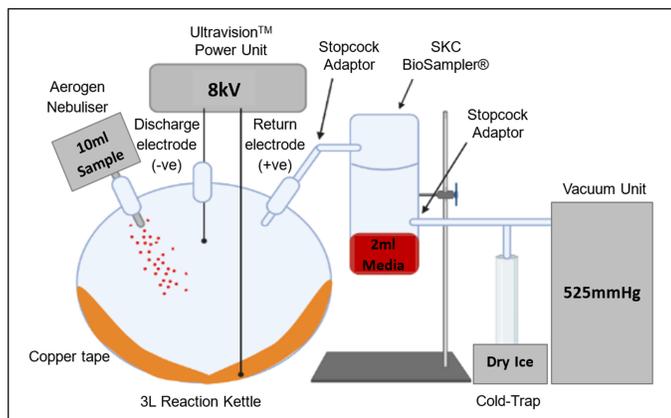


Figure 1

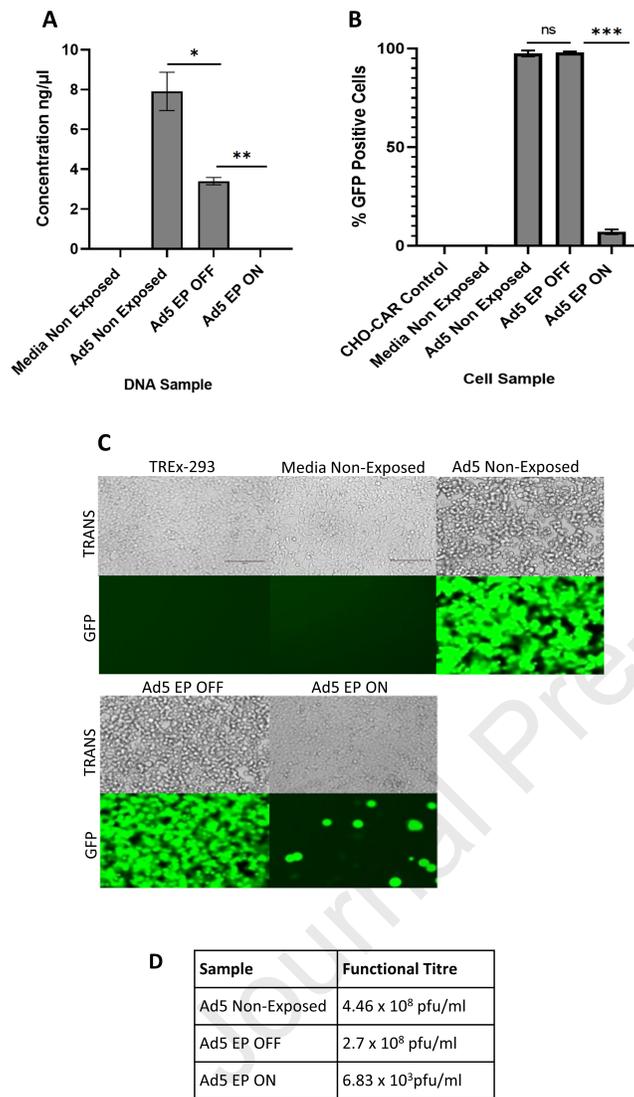


Figure 2

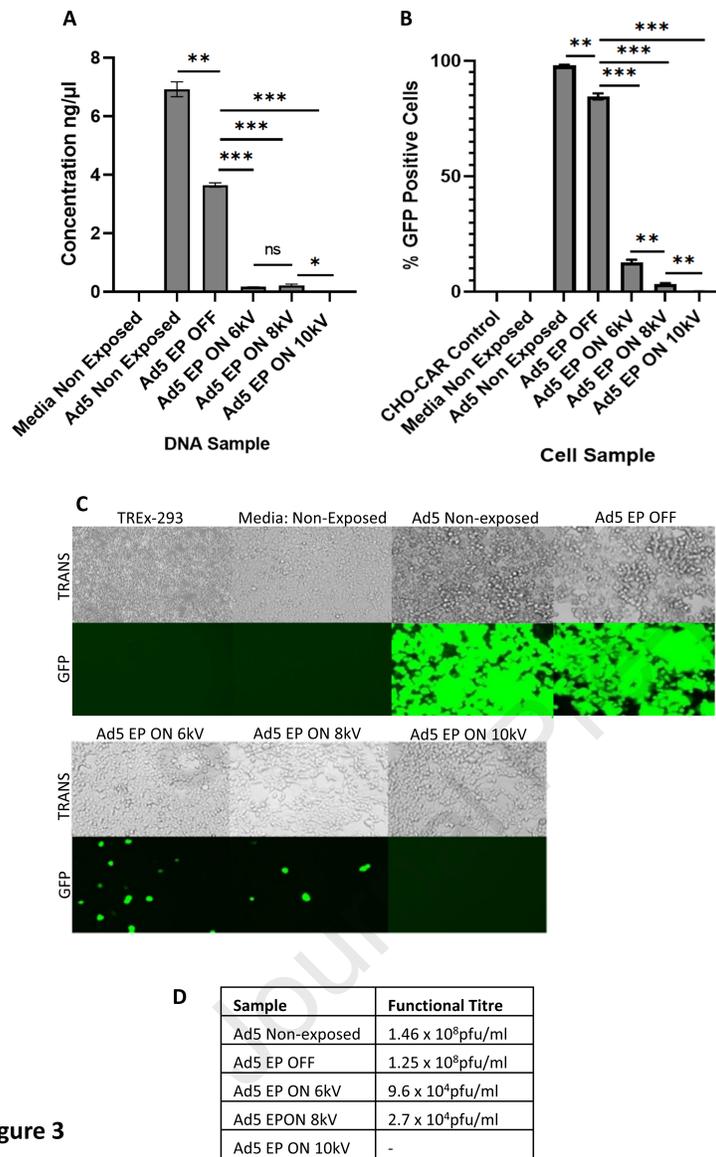


Figure 3

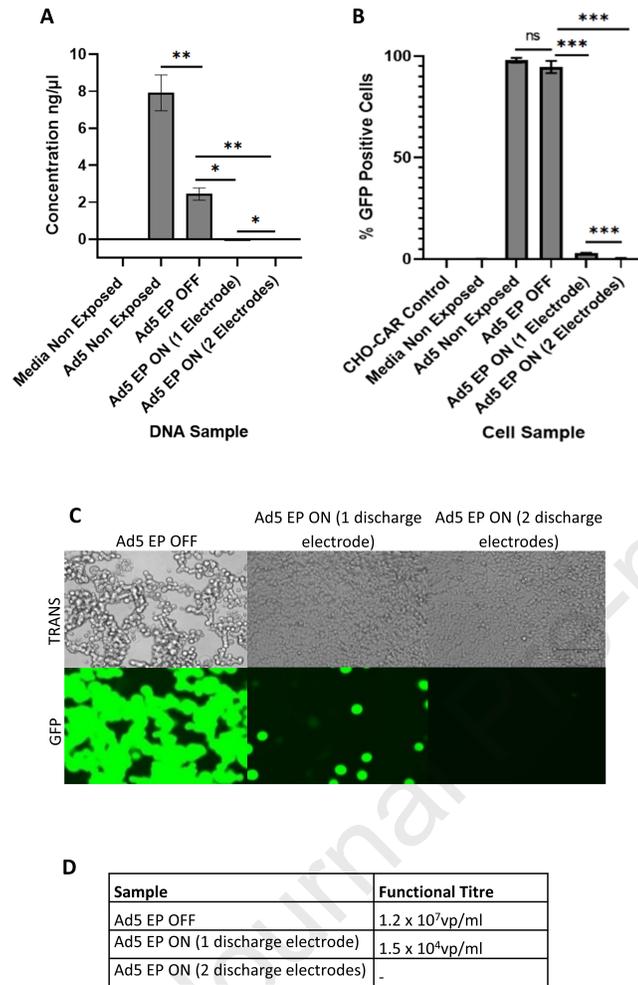


Figure 4

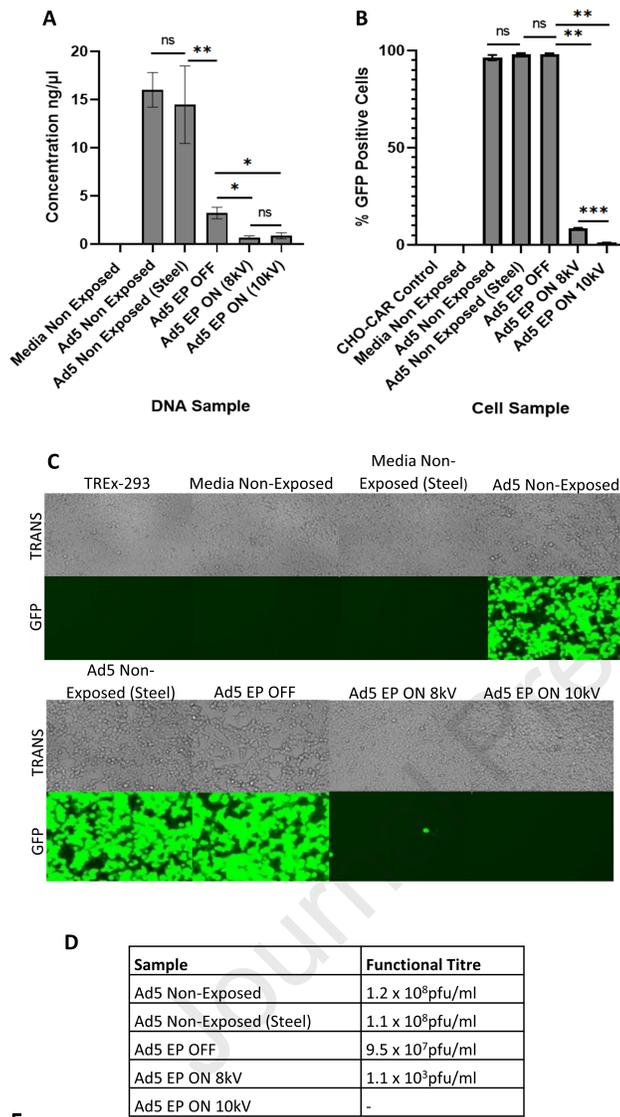


Figure 5

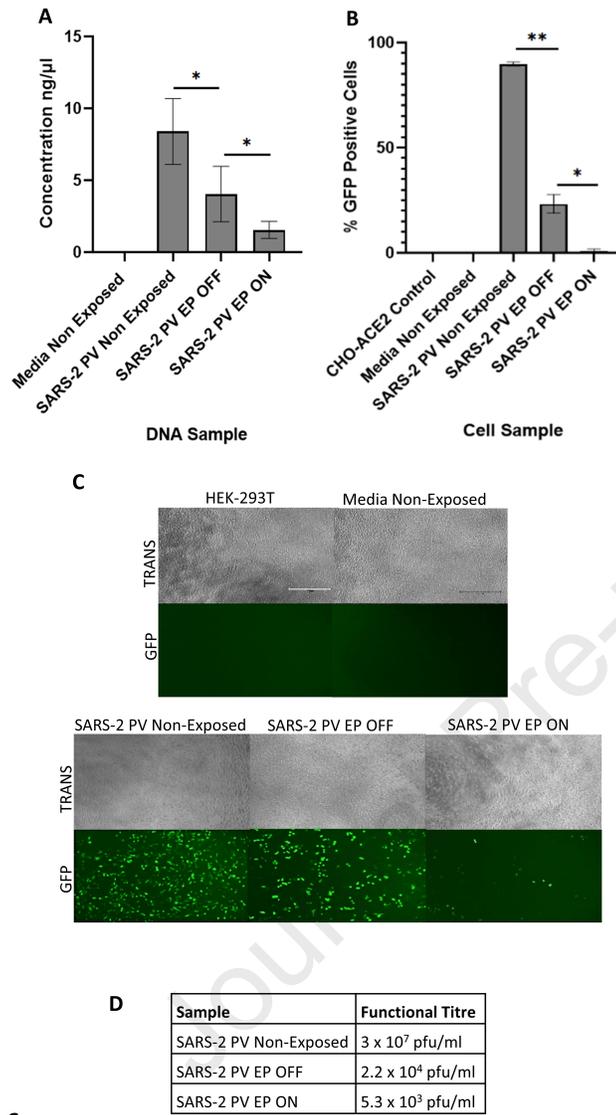


Figure 6

**Highlights**

- Bioaerosols released from patients during surgery can facilitate viral spread.
- Electrostatic precipitation captures and inactivates viral particles preventing spread.
- Electrostatic precipitation is effective against enveloped and non-enveloped viruses.
- Electrostatic precipitation represents a viable means to reduce nosocomial infections.

Journal Pre-proof

## KEY RESOURCES TABLE

The table highlights the reagents, genetically modified organisms and strains, cell lines, software, instrumentation, and source data **essential** to reproduce results presented in the manuscript. Depending on the nature of the study, this may include standard laboratory materials (i.e., food chow for metabolism studies, support material for catalysis studies), but the table is **not** meant to be a comprehensive list of all materials and resources used (e.g., essential chemicals such as standard solvents, SDS, sucrose, or standard culture media do not need to be listed in the table). **Items in the table must also be reported in the method details section within the context of their use.** To maximize readability, the number of **oligonucleotides and RNA sequences** that may be listed in the table is restricted to no more than 10 each. If there are more than 10 oligonucleotides or RNA sequences to report, please provide this information as a supplementary document and reference the file (e.g., See Table S1 for XX) in the key resources table.

**Please note that ALL references cited in the key resources table must be included in the main references list.** Please report the information as follows:

- **REAGENT or RESOURCE:** Provide the full descriptive name of the item so that it can be identified and linked with its description in the manuscript (e.g., provide version number for software, host source for antibody, strain name). In the experimental models section (applicable only to experimental life science studies), please include all models used in the paper and describe each line/strain as: model organism: name used for strain/line in paper: genotype. (i.e., Mouse: OXTR<sup>fl/fl</sup>; B6.129(SJL)-Oxtr<sup>tm1.1Wsy/J</sup>). In the biological samples section (applicable only to experimental life science studies), please list all samples obtained from commercial sources or biological repositories. Please note that software mentioned in the methods details or data and code availability section needs to also be included in the table. See the sample tables at the end of this document for examples of how to report reagents.
- **SOURCE:** Report the company, manufacturer, or individual that provided the item or where the item can be obtained (e.g., stock center or repository). For materials distributed by Addgene, please cite the article describing the plasmid and include “Addgene” as part of the identifier. If an item is from another lab, please include the name of the principal investigator and a citation if it has been previously published. If the material is being reported for the first time in the current paper, please indicate as “this paper.” For software, please provide the company name if it is commercially available or cite the paper in which it has been initially described.
- **IDENTIFIER:** Include catalog numbers (entered in the column as “Cat#” followed by the number, e.g., Cat#3879S). Where available, please include unique entities such as RRIDs, Model Organism Database numbers, accession numbers, and PDB, CAS, or CCDC IDs. For antibodies, if applicable and available, please also include the lot number or clone identity. For software or data resources, please include the URL where the resource can be downloaded. Please ensure accuracy of the identifiers, as they are essential for generation of hyperlinks to external sources when available. Please see the Elsevier [list of data repositories](#) with automated bidirectional linking for details. When listing more than one identifier for the same item, use semicolons to separate them (e.g., Cat#3879S; RRID: AB\_2255011). If an identifier is not available, please enter “N/A” in the column.
  - **A NOTE ABOUT RRIDs:** We highly recommend using RRIDs as the identifier (in particular for antibodies and organisms but also for software tools and databases). For more details on how to obtain or generate an RRID for existing or newly generated resources, please [visit the RII or search for RRIDs](#).

Please use the empty table that follows to organize the information in the sections defined by the subheading, skipping sections not relevant to your study. Please do not add subheadings. To add a row, place the cursor at the end of the row above where you would like to add the row, just outside the right border of the table. Then press the ENTER key to add the row. Please delete empty rows. Each entry must be on a separate row; do not list multiple items in a single table cell. Please see the sample tables at the end of this document for relevant examples in the life and physical sciences of how reagents and instrumentation should be cited.

**TABLE FOR AUTHOR TO COMPLETE**

Please upload the completed table as a separate document. **Please do not add subheadings to the key resources table.** If you wish to make an entry that does not fall into one of the subheadings below, please contact your handling editor. **Any subheadings not relevant to your study can be skipped.** (NOTE: References within the KRT should be in numbered style rather than Harvard.)

**Key resources table**

| REAGENT or RESOURCE                                  | SOURCE                          | IDENTIFIER              |
|--|---------------------------------|-------------------------|
| <b>Antibodies</b>                                    |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
| <b>Bacterial and virus strains</b>                   |                                 |                         |
| Ad5.GFP  | In-house (Stanton, et al. 2008) | N/A                     |
| SARS-2 PV  | (Di Genova, et al. 2021)        | N/A                     |
|  |                                 |                         |
|  |                                 |                         |
| <b>Biological samples</b>                            |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |
| <b>Chemicals, peptides, and recombinant proteins</b> |                                 |                         |
| Caesium Chloride                                     | Invitrogen™                     | 15507-023               |
| 0.45 µm acetate cellulose filter                     | StarLab                         | E4780-1453              |
| FuGene® HD Transfection reagent                      | Promega                         | E2311                   |
|  |                                 |                         |
|  |                                 |                         |
| <b>Critical commercial assays</b>                    |                                 |                         |
| Micro BCA™ Protein Assay Kit                         | Thermo Fisher                   | 23235                   |
| QIAamp MinElute Virus Kit                            | Qiagen                          | 57704                   |
| PowerUp SYBR Green Master Mix                        | Thermo Fisher                   | A25741                  |
|  |                                 |                         |
|  |                                 |                         |
| <b>Deposited data</b>                                |                                 |                         |
| Raw and analyzed data                                | Mendeley Data Repository        | Access numbers required |
|  |                                 |                         |
|  |                                 |                         |
|  |                                 |                         |

|   |                                    |   |
|---|------------------------------------|---|
|   |                                    |   |
| <b>Experimental models: Cell lines</b>  |                                    |   |
| Human T-REx-293   | Invitrogen™                        | R71007  |
| Human HEK-293T/17 cells   | ATCC                               | CRL-1573  |
| Hamster CHO   | ATCC                               |   |
| Hamster CHO-CAR   | (Uusi-Kerttula, et al. 2016)       | N/A   |
| Hamster CHO-ACE2-TMPRSS2  | (Rebendenne, et al. 2021)          | N/A   |
|   |                                    |   |
| <b>Experimental models: Organisms/strains</b>   |                                    |   |
|   |                                    |   |
|   |                                    |   |
|   |                                    |   |
|   |                                    |   |
|   |                                    |   |
| <b>Oligonucleotides</b>   |                                    |   |
| Primers Ad5 Hexon - Forward:<br>CCTGCTTACCCCAACGAGTTTGA. Reverse:<br>GGAGTACATGCGGTCCTTGTAGCTC. | Thermo Fisher                      | N/A   |
| Primers P24 Capsid – Forward:<br>GGCTTTCAGCCCAGAAGTGATACC. Reverse:<br>GGTCTCCTACTCCCTGACATG.   | Thermo Fisher                      | N/A   |
| <b>Recombinant DNA</b>  |                                    |   |
| Spike SARS2 (D614G)-pCAGGS  | NIBSC                              | CFAR100985  |
| pCSGW encoding Green Fluorescent Protein  | (Carnell, et al. 2015)             | N/A   |
| Lentiviral Core p8.91   | (Carnell, et al. 2015)             | N/A   |
| MT126 pRRL- SFFV-ACE2-IRES plasmid  | AddGene                            | 145839  |
| MT131 pRRL- SFFV-TMPRSS2.v1-IRES plasmid  | AddGene                            | 145843  |
| <b>Software and algorithms</b>  |                                    |   |
| QuantStudio™ 5 Real-Time PCR  | Thermo Fisher                      | <a href="https://www.thermofisher.com/uk/en/home/global/forms/life-science/quantstudio-3-5-software.html">https://www.thermofisher.com/uk/en/home/global/forms/life-science/quantstudio-3-5-software.html</a> |
| FlowJo™v10  | BD Biosciences                     | <a href="https://www.flowjo.com/solutions/flowjo/downloads">https://www.flowjo.com/solutions/flowjo/downloads</a>   |
| Prism v4.03   | GraphPad                           | <a href="https://www.graphpad.com/scientific-software/prism">https://www.graphpad.com/scientific-software/prism</a>   |
|   |                                    |   |
|   |                                    |   |
| <b>Other</b>  |                                    |   |
| Aerogen® Solo Nebuliser   | Aerogen Ltd                        | AG-A53000-XX  |
| QuickFit™ Wide Neck Flask Reaction 3L   | Scientific Laboratory Supplies Ltd | QFR3LF  |
| QuickFit™ Borosilicate Glass Flange Lid   | Fisher Scientific                  | MAF3/52   |
| Ultravision™ Generator  | BOWA Medial UK                     | DAD-001-015   |

|  |                     |             |
|--|---------------------|-------------|
| Ionwand™                                       | BOWA Medial UK      | DAD-001-003 |
| Suba-Seal®                                     | Sigma-Aldrich       | Z124621     |
| QuickFit™ Borosilicate Glass Stopcock Adaptors | Fisher Scientific   | MF14/3/SC   |
| Duet Flat- Back Aspirator                      | SSCOR               | 2314B       |
| BioSampler®                                    | SKC Ltd             | 225-9595    |
| QuickFit™ Cold-trap                            | VWR                 | 201-3052    |
| NanoSight NS300                                | Malvern Panalytical | N/A         |
| EVOS M7000                                     | Invitrogen™         | AMF7000     |
| Accuri C6 v.1.0.264.21                         | BD Biosciences      | N/A         |

**LIFE SCIENCE TABLE WITH EXAMPLES FOR AUTHOR REFERENCE**

| REAGENT or RESOURCE  | SOURCE  | IDENTIFIER  |
|--|---|---|
| <b>Antibodies</b>  |   |   |
| Rabbit monoclonal anti-Snail   | Cell Signaling Technology   | Cat#3879S; RRID: AB_2255011   |
| Mouse monoclonal anti-Tubulin (clone DM1A)                               | Sigma-Aldrich   | Cat#T9026; RRID: AB_477593  |
| Rabbit polyclonal anti-BMAL1   | This paper  | N/A   |
| <b>Bacterial and virus strains</b>                                       |   |   |
| pAAV-hSyn-DIO-hM3D(Gq)-mCherry   | Krashes et al. <sup>1</sup>   | Addgene AAV5; 44361-AAV5  |
| AAV5-EF1a-DIO-hChr2(H134R)-EYFP  | Hope Center Viral Vectors Core  | N/A   |
| Cowpox virus Brighton Red  | BEI Resources   | NR-88   |
| Zika-SMGC-1, GENBANK: KX266255   | Isolated from patient (Wang et al. <sup>2</sup> )   | N/A   |
| <i>Staphylococcus aureus</i>   | ATCC  | ATCC 29213  |
| <i>Streptococcus pyogenes</i> : M1 serotype strain: strain SF370; M1 GAS | ATCC  | ATCC 700294   |
| <b>Biological samples</b>  |   |   |
| Healthy adult BA9 brain tissue   | University of Maryland Brain & Tissue Bank; <a href="http://medschool.umaryland.edu/btbank/">http://medschool.umaryland.edu/btbank/</a> | Cat#UMB1455   |
| Human hippocampal brain blocks   | New York Brain Bank   | <a href="http://nybb.hs.columbia.edu/">http://nybb.hs.columbia.edu/</a> |
| Patient-derived xenografts (PDX)   | Children's Oncology Group Cell Culture and Xenograft Repository   | <a href="http://cogcell.org/">http://cogcell.org/</a>                   |
| <b>Chemicals, peptides, and recombinant proteins</b>                     |   |   |
| MK-2206 AKT inhibitor  | Selleck Chemicals   | S1078; CAS: 1032350-13-2  |
| SB-505124  | Sigma-Aldrich   | S4696; CAS: 694433-59-5 (free base)                                     |
| Picrotoxin   | Sigma-Aldrich   | P1675; CAS: 124-87-8  |
| Human TGF- $\beta$   | R&D   | 240-B; GenPept: P01137  |
| Activated S6K1   | Millipore   | Cat#14-486  |
| GST-BMAL1  | Novus   | Cat#H00000406-P01   |
| <b>Critical commercial assays</b>  |   |   |
| EasyTag EXPRESS 35S Protein Labeling Kit                                 | PerkinElmer   | NEG772014MC   |
| CaspaseGlo 3/7   | Promega   | G8090   |
| TruSeq ChIP Sample Prep Kit  | Illumina  | IP-202-1012   |
| <b>Deposited data</b>  |   |   |
| Raw and analyzed data  | This paper  | GEO: GSE63473   |
| B-RAF RBD (apo) structure  | This paper  | PDB: 5J17   |

|   |   |   |
|---|---|---|
| Human reference genome NCBI build 37, GRCh37  | Genome Reference Consortium               | <a href="http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/">http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/</a> |
| Nanog STILT inference   | This paper; Mendeley Data                 | <a href="http://dx.doi.org/10.17632/wx6s4mj7s8.2">http://dx.doi.org/10.17632/wx6s4mj7s8.2</a>   |
| Affinity-based mass spectrometry performed with 57 genes  | This paper; Mendeley Data                 | Table S8;<br><a href="http://dx.doi.org/10.17632/5hvpvpsw82.1">http://dx.doi.org/10.17632/5hvpvpsw82.1</a>                                    |
| Experimental models: Cell lines   |   |   |
| Hamster: CHO cells  | ATCC                                      | CRL-11268   |
| <i>D. melanogaster</i> : Cell line S2: S2-DRSC  | Laboratory of Norbert Perrimon            | FlyBase: FBtc0000181  |
| Human: Passage 40 H9 ES cells   | MSKCC stem cell core facility             | N/A   |
| Human: HUES 8 hESC line (NIH approval number NIHhESC-09-0021)                                     | HSCI iPS Core                             | hES Cell Line: HUES-8   |
| Experimental models: Organisms/strains  |   |   |
| <i>C. elegans</i> : Strain BC4011: srl-1(s2500) II; dpy-18(e364) III; unc-46(e177)rol-3(s1040) V. | Caenorhabditis Genetics Center            | WB Strain: BC4011; WormBase: WBVar00241916  |
| <i>D. melanogaster</i> : RNAi of Sxl: y[1] sc[*] v[1]; P{TRiP.HMS00609}attP2                      | Bloomington Drosophila Stock Center       | BDSC:34393; FlyBase: FBtp0064874  |
| <i>S. cerevisiae</i> : Strain background: W303  | ATCC                                      | ATTC: 208353  |
| Mouse: R6/2: B6CBA-Tg(HDexon1)62Gpb/3J  | The Jackson Laboratory                    | JAX: 006494   |
| Mouse: OXTRfl/fl: B6.129(SJL)-Oxtr <sup>tm1.1Wsy/J</sup>  | The Jackson Laboratory                    | RRID: IMSR_JAX:008471   |
| Zebrafish: Tg(Shha:GFP)t10: t10Tg   | Neumann and Nüsslein-Volhard <sup>3</sup> | ZFIN: ZDB-GENO-060207-1   |
| <i>Arabidopsis</i> : 35S::PIF4-YFP, BZR1-CFP  | Wang et al. <sup>4</sup>                  | N/A   |
| <i>Arabidopsis</i> : JYB1021.2: pS24(AT5G58010)::cS24:GFP(-G):NOS #1                              | NASC                                      | NASC ID: N70450   |
| Oligonucleotides  |   |   |
| siRNA targeting sequence: PIP5K I alpha #1: ACACAGUACUCAGUUGAUA                                   | This paper                                | N/A   |
| Primers for XX, see Table SX  | This paper                                | N/A   |
| Primer: GFP/YFP/CFP Forward: GCACGACTTCTTCAAGTCCGCCATGCC  | This paper                                | N/A   |
| Morpholino: MO-pax2a GGTCTGCTTTGCAGTGAATATCCAT  | Gene Tools                                | ZFIN: ZDB-MRPHLNO-061106-5  |
| ACTB (hs01060665_g1)  | Life Technologies                         | Cat#4331182   |
| RNA sequence: hnRNPA1_ligand: UAGGGACUUAGGGUUCUCUCUAGGGACUUAG GGUUCUCUCUAGGGA                     | This paper                                | N/A   |
| Recombinant DNA   |   |   |
| pLVX-Tight-Puro (TetOn)   | Clontech                                  | Cat#632162  |
| Plasmid: GFP-Nito   | This paper                                | N/A   |

|   |                                     |   |
|---|-------------------------------------|---|
| cDNA GH111110   | Drosophila Genomics Resource Center | DGRC:5666;<br>FlyBase:FBcl0130415   |
| AAV2/1-hsyn-GCaMP6- WPRE  | Chen et al. <sup>5</sup>            | N/A   |
| Mouse raptor: pLKO mouse shRNA 1 raptor   | Thoreen et al. <sup>6</sup>         | Addgene Plasmid #21339  |
| Software and algorithms   |                                     |   |
| ImageJ  | Schneider et al. <sup>7</sup>       | <a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>   |
| Bowtie2   | Langmead and Salzberg <sup>8</sup>  | <a href="http://bowtie-bio.sourceforge.net/bowtie2/index.shtml">http://bowtie-bio.sourceforge.net/bowtie2/index.shtml</a> |
| Samtools  | Li et al. <sup>9</sup>              | <a href="http://samtools.sourceforge.net/">http://samtools.sourceforge.net/</a>   |
| Weighted Maximal Information Component Analysis v0.9  | Rau et al. <sup>10</sup>            | <a href="https://github.com/ChristophRau/wMICA">https://github.com/ChristophRau/wMICA</a>                                 |
| ICS algorithm   | This paper; Mendeley Data           | <a href="http://dx.doi.org/10.17632/5hvpvpspw82.1">http://dx.doi.org/10.17632/5hvpvpspw82.1</a>                           |
| Other   |                                     |   |
| Sequence data, analyses, and resources related to the ultra-deep sequencing of the AML31 tumor, relapse, and matched normal | This paper                          | <a href="http://aml31.genome.wustl.edu">http://aml31.genome.wustl.edu</a>   |
| Resource website for the AML31 publication  | This paper                          | <a href="https://github.com/chrismiller/aml31SuppSite">https://github.com/chrismiller/aml31SuppSite</a>                   |

**PHYSICAL SCIENCE TABLE WITH EXAMPLES FOR AUTHOR REFERENCE**

| REAGENT or RESOURCE  | SOURCE   | IDENTIFIER  |
|--|--|---|
| Chemicals, peptides, and recombinant proteins                                  |  |   |
| QD605 streptavidin conjugated quantum dot                                      | Thermo Fisher Scientific                           | Cat#Q10101MP  |
| Platinum black   | Sigma-Aldrich                                      | Cat#205915  |
| Sodium formate BioUltra, ≥99.0% (NT)   | Sigma-Aldrich                                      | Cat#71359   |
| Chloramphenicol  | Sigma-Aldrich                                      | Cat#C0378   |
| Carbon dioxide ( <sup>13</sup> C, 99%) (<2% <sup>18</sup> O)                   | Cambridge Isotope Laboratories                     | CLM-185-5   |
| Poly(vinylidene fluoride-co-hexafluoropropylene)                               | Sigma-Aldrich                                      | 427179  |
| PTFE Hydrophilic Membrane Filters, 0.22 μm, 90 mm                              | Scientificfilters.com/Tisch Scientific             | SF13842   |
| Critical commercial assays   |  |   |
| Folic Acid (FA) ELISA kit  | Alpha Diagnostic International                     | Cat# 0365-0B9   |
| TMT10plex Isobaric Label Reagent Set   | Thermo Fisher                                      | A37725  |
| Surface Plasmon Resonance CM5 kit  | GE Healthcare                                      | Cat#29104988  |
| NanoBRET Target Engagement K-5 kit   | Promega  | Cat#N2500   |
| Deposited data   |  |   |
| B-RAF RBD (apo) structure  | This paper   | PDB: 5J17   |
| Structure of compound 5  | This paper; Cambridge Crystallographic Data Center | CCDC: 2016466   |
| Code for constraints-based modeling and analysis of autotrophic <i>E. coli</i> | This paper   | <a href="https://gitlab.com/elad.noor/sloppy/tree/master/rubisco">https://gitlab.com/elad.noor/sloppy/tree/master/rubisco</a>       |
| Software and algorithms  |  |   |
| Gaussian09   | Frish et al. <sup>1</sup>                          | <a href="https://gaussian.com">https://gaussian.com</a>   |
| Python version 2.7   | Python Software Foundation                         | <a href="https://www.python.org">https://www.python.org</a>   |
| ChemDraw Professional 18.0   | PerkinElmer  | <a href="https://www.perkinelmer.com/category/chemdraw">https://www.perkinelmer.com/category/chemdraw</a>                           |
| Weighted Maximal Information Component Analysis v0.9                           | Rau et al. <sup>2</sup>                            | <a href="https://github.com/ChristophRau/wMICA">https://github.com/ChristophRau/wMICA</a>   |
| Other  |  |   |
| DASGIP MX4/4 Gas Mixing Module for 4 Vessels with a Mass Flow Controller       | Eppendorf  | Cat#76DGMX44  |
| Agilent 1200 series HPLC   | Agilent Technologies                               | <a href="https://www.agilent.com/en/products/liquid-chromatography">https://www.agilent.com/en/products/liquid-chromatography</a>   |
| PHI Quantera II XPS  | ULVAC-PHI, Inc.                                    | <a href="https://www.ulvac-phi.com/en/products/xps/phi-quantera-ii/">https://www.ulvac-phi.com/en/products/xps/phi-quantera-ii/</a> |