1 **Alpha-synuclein alters the faecal viromes of rats in a gut-initiated model of Parkinson's** 2 **disease**

3

4 S. R. Stockdale^{1,†}, L. A. Draper^{1,†}, S. M. O'Donovan^{1,4,5,†}, W. Barton^{1,2}, O. O'Sullivan^{1,2}, L.
5 A. Volpicelli-Daley³, A. M. Sullivan^{1,4}, C. O'Neill^{1,5,*}, C. Hill^{1,6,*}

6

7 ¹APC Microbiome Ireland, University College Cork, Co. Cork, Ireland

8 ²Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

9 ³Civitan International Research Center, University of Alabama, Birmingham, USA

10 ⁴Department of Anatomy and Neuroscience, University College Cork, Co. Cork, Ireland

11 ⁵School of Biochemistry, University College Cork, Co. Cork, Ireland

12 ⁶School of Microbiology, University College Cork, Co. Cork, Ireland

13

14 [†]These authors contributed equally to this work.

15 ^{*}Corresponding authors: c.oneill@ucc.ie and c.hill@ucc.ie

16

17 **Keywords:** Parkinson's disease, faecal virome, alpha-synuclein, faecal phage, rat
18 microbiome, rat virome, rat phage

19 Abstract

20 Parkinson's disease (PD) is a chronic neurological disorder associated with the
21 misfolding of alpha-synuclein (α -syn) into Lewy body aggregates within nerve cells that
22 contribute to their neurodegeneration. Recent evidence suggests α -syn aggregation may begin
23 in the gut and travel to the brain along the vagus nerve, with microbes a potential trigger
24 initiating the misfolding of α -syn. However, changes in the gut virome in response to α -syn
25 alterations have not been investigated. In this study, we show longitudinal changes in the
26 faecal virome of rats administered either monomeric or preformed fibrils (PFF) of α -syn
27 directly into their enteric nervous system. Differential changes in rat viromes were observed
28 when comparing monomeric and PFF α -syn. The virome β -diversity changes after α -syn
29 treatment were compounded by the addition of LPS as an adjunct. Changes in the diversity of
30 rat faecal viromes were observed after one month and did not resolve within the study's five
31 month observational period. Overall, these results suggest that microbiome alterations
32 associated with PD may, partially, be reactive to host α -syn associated changes.

33

34 Introduction

35 Parkinson's disease (PD) is the second most common progressive neurodegenerative
36 disorder, affecting 0.1-0.2% of the population at any given time. This frequency increases
37 with age, with more than 1% of individuals over 60 living with PD ¹. While the overall cost
38 of PD varies from country to country, in the UK conservative and liberal estimates calculated
39 the annual cost of the illness between £449 million and £3.3 billion, respectively ². As the
40 mean age of populations increase, the social and economic burden of PD will rise. In fact,
41 worldwide cases of PD are expected to double to 14 million by 2040 ³; therefore, a greater
42 understanding of the disease and new interventions are urgently required.

Genetic and epidemiological studies investigating PD have not demonstrated a specific aetiology that triggers PD. Instead, many hypothesise that PD is a multifactorial disease involving a complex combination of host genetics and environmental factors ⁴. The pathophysiological onset of PD is associated with the loss of dopamine-producing neurons in the pars compacta sub-region of the substantia nigra structure of the midbrain ⁵. However, patients typically only display Parkinsonism, the motor features associated with PD, after 50-80% of dopaminergic neurons are lost ⁶. The presynaptic neuronal protein alpha-synuclein (α -syn) is linked to the loss of dopaminergic neurons, whereby the monomeric form of α -syn aggregates within nerve cells into toxic Lewy body (LB) formations that disrupt cellular homeostasis ⁷.

Gastrointestinal (GI) comorbidities are frequently associated with PD, with cohorts from independent studies reporting that 70-78% of patients experience excessive saliva, 30-97% of individuals have difficulty swallowing, and 20-89% report constipation ⁸. The non-motor symptoms associated with PD may also involve α -syn, as its aggregation is detected earlier and more frequently in the enteric (i.e. gastrointestinal) nervous system of PD patients ^{9,10}. Recent animal models have also demonstrated the spread of pathologic α -syn along the gut-brain axis through the vagus nerve ¹¹⁻¹³. This has led researchers to investigate the GI tract and its community of microbes as a potential environmental trigger for the aggregation of α -syn that may contribute to the development of PD.

There are 10^{13} to 10^{14} bacteria, archaea, fungi, and viruses associated with the human body, known collectively as the microbiota. Non-culture-based approaches to study their genomic material (microbiome) have demonstrated the GI composition of PD patients differs from controls ¹⁴⁻²¹. In addition, transplantation of human faecal microbiotas from either PD patients and non-PD controls to mice resulted in a motor function decline amongst the mice humanized with a PD microbiota ²². Specific bacterial taxa, and even bacterial-infecting viruses (termed bacteriophages or phages), have been proposed as potential contributors to

69 PD^{17,23}. However, caution is often required when interpreting microbial changes associated
70 with specific conditions, as alterations observed in the microbiome may be causal, or
71 reactive, to human host-associated changes²⁴.

72 In this study, we investigated if the viral component of the rat microbiome (virome)
73 changes in response to α -syn injected into rat enteric nervous systems. This study was
74 specifically performed in young rats to distinguish if α -syn could be associated with virome
75 changes independent of microbial changes that would occur during PD development in older
76 rats. Both the monomeric and aggregated fibrillar forms of α -syn were injected directly into
77 the myenteric plexus of surgically-prepared rat duodenal walls, with and without
78 intraperitoneal injection of bacterial lipopolysaccharide (LPS) as a potential inflammatory
79 adjunct. Virome diversity changes were observed between rats receiving specific α -syn
80 treatments, and also longitudinally across individual rats. Specific viruses and VCs were
81 noted as differentially abundant across treatment groups, with microbiome changes reacting
82 to α -syn and potentially PD-inducing conditions.

83

84 **Results**

85 **Defining the rat faecal virome.**

86 Viruses of the rat microbiome were identified using database-dependent and -
87 independent approaches, applied to both the viral-enriched and whole-genome sequencing
88 (WGS) data (see Methods). Viral sequences identified in the viral-enriched and WGS data
89 were merged into a single database and made non-redundant by removing duplicate
90 sequences and retaining the larger of any two homologous sequences ($\geq 90\%$ identity and $\geq$
91 90% coverage). The majority of sequences in the final rat viral database originated from the
92 viral-enriched fraction of the microbiome (3,506/4,009 sequences). The largest viral contig

93 was 291,209bp in length, while the mean and median length of viral contigs is 4,123 and
94 1,621bp, respectively (Figure 1a).

95 The viral nucleic acid extraction and sequencing protocols used in this study did not
96 employ random DNA amplification, such as multiple displacement amplification (MDA),
97 known to bias the viral composition towards small circular genetic elements. Therefore, the
98 relative abundance of specific viral taxa such as small single-stranded DNA *Microviridae*
99 may be more accurate than previous faecal virome studies ²⁵. The average raw sequencing
100 reads per sample for rat faecal viromes was 2,926,309 (max. 3,616,794, min. 2,221,657). The
101 rat faecal viromes analysed in this study were dominated by bacterial-infecting phage.
102 However, the term virome (and not “phageome”) is used throughout this study, as this study’s
103 viral-detecting protocol does not preclude the identification of rat-infecting viruses, and they
104 may be present amongst the ‘Unclassified’ viruses.

105 Of the tailed *Caudovirales* phages assigned a putative familial taxonomic rank,
106 *Siphoviridae* phages were the most abundant, with *Myoviridae* and *Podoviridae* phages
107 detected at decreasing frequencies (Figure 1b). All viral sequences identified within this
108 study were clustered into pseudogenera termed viral clusters (VCs) using the vContact2 gene
109 sharing network-based algorithm. A total of 920 VCs were generated, with the largest cluster
110 containing 20 sequences.

111 The majority of phage contigs identified in this study are linear (99.45%; Figure 1c).
112 While the rat faecal virome might have relatively few circular genomes, it is more likely that
113 greater sequencing depth is required to complete and circularize more viral genome
114 sequences. The viral sequences identified in this study were queried for the presence of phage
115 integrases and/or recombinases. Only 8.4% of phages possessed genes encoding for lysogenic
116 conversion that would facilitate integration and replication as a prophage (Figure 1d).

117 Using a database-dependent and -independent approach to identify viruses, the
118 average relative abundance of the identified rat faecal viruses accounts for 58.9% of the

119 virome sequencing reads generated (Figure 1e; min. 47.6%, max. 70.2%). Therefore, a
120 significant proportion of the rat faecal virome is not analysed and could be considered as
121 “viral dark matter”²⁶. While significantly lower than within the whole shotgun sequencing,
122 there are instances where the bacterial housekeeping gene, *cpn60*, is detected within the
123 virome sequencing data (Figure 1f). Therefore, potential bacterial contamination may have
124 been inadvertently categorised as viral, and a greater understanding of the rat microbiome
125 will be required to distinguish the origin of sequences.

126

127 **The rat microbiome changes over time.**

128 The male Sprague-Dawley rats employed in this study were approx. two months of
129 age at the study’s onset and seven months old at its conclusion. Therefore, the potential
130 impact of time in shaping the rat faecal microbiome was investigated. During the
131 MetaPhlAn2 analysis of the WGS data, an average of 99.7 and 0.3% of the microbiome
132 sequencing reads that could be assigned to microbial taxa were classified as bacterial and
133 viral, respectively. A longitudinal axis to the rat faecal virome was obtained by aligning the
134 WGS sequencing data onto the final rat viral database. An average of 44,431 WGS
135 sequencing reads aligned to viral sequences (min. 572; max. 256,910; median 33,298).

136 There was a statistically significant change in the faecal virome and total microbiome
137 composition of the rats over the study’s duration, with time explaining 14.6% and 13.3% of
138 their variances, respectively (Figures 2a & b). The two-dimensional separation of faecal
139 samples by dissimilarity (β -diversity) was not driven by intra-sample composition (α -
140 diversity). No statistical difference in α -diversities after Bonferroni correction are observed in
141 rat faecal viromes by treatment group, examining either virome or WGS data (Supplementary
142 Table 1). The PCoA axes 1 and 2 for the microbiome data explain more of the variation than
143 for the virome data (28.5 and 15.5% versus 21.1 and 9.4%, respectively).

144

145 α -syn alters the rat faecal virome.

146 As it is established that the rat faecal virome changed over the course of the study,
 147 any alterations occurring in α -syn injected rats need to be examined in light of the control
 148 treatments, Sham and LPS. The Sham and LPS controls experienced 22.6 and 19.5%
 149 variance, respectively, in their viromes over the study's duration (Figures 3a & b). Less than
 150 a 1% difference in the variance of the rat faecal viral compositions are observed in the rats
 151 that received the α -syn monomer or α -syn monomer with LPS (28.0 and 27.6% variances
 152 explained, respectively; Figures 3c & d). However, the effect of the α -syn monomer on rat
 153 viromes is far greater than differences attributable to either the Sham or LPS control
 154 treatments. The PFF form of α -syn appears to have had only a small effect on the rat virome
 155 relative to the Sham and LPS controls; however, the PFF plus LPS treatments caused the
 156 greatest virome alteration (variances explained 23.9% and 30.8%, respectively; Figures 3e &
 157 f).

158

159 Changes in individual rat viromes.

160 In order to investigate if changes in the rat faecal virome were directional, whereby a
 161 sample is not merely more or less similar to its baseline through random fluctuations, each
 162 individual rat's faecal virome was investigated relative to its baseline state. Using Bray-
 163 Curtis index values that indicate the dissimilarity of two given virome samples, it was
 164 observed that the greatest variation in the rat faecal virome occurs between Base and T1,
 165 compared to T1 and T5 (Figure 4a). There was no statistical difference in the dissimilarity
 166 values between the 'Base vs T1' and 'Base vs T5'. Additionally, the virome at five months
 167 was more frequently dissimilar to the baseline compared to the one-month time point (Figure
 168 4b).

For the individual rat viromes segregated by treatment conditions (Figures 4c-h), the least variation was observed between the average Sham, LPS, and α -syn monomer treatment baselines versus T1 time points (Figures 4c-e). Whereas within the first month, the α -syn monomer plus LPS, the PFF α -syn, and the PFF α -syn plus LPS treatments had the least similarity when averaged across rats (Figures 4f-h). The viromes of rats receiving the α -syn monomer plus LPS are the most dissimilar to their baseline after the study's five months (Figure 4f).

Additionally, we investigated if virome changes associated with specific α -syn treatments were the result of cage effects. Each rat was assigned a numerical value and a code based on its cage. For example, code C21-R2 specifies cage number 21 and rat 2. Two cages, with 2-3 rats per cage, were used for each treatment. Looking at each individual rat, no specific trend across any of the treatment conditions was observed, such as all the rats within a specific cage dramatically differing at T1 or T5 relative to their baseline (Figures 4c-h). Also, the average Bray-Curtis dissimilarity values were similar for each specific treatment across the two corresponding cages.

Viral changes caused by α -syn treatments.

If the α -syn treatments investigated in this study altered the rat microbiome and/or virome, it is expected that the abundance of viruses would be significantly altered in one or more time points (p-value < 1E-05). The Sham and LPS treatment arms of this study resulted in the fewest differential viral and VC changes (Figure 5a & b). There were only two viruses increased at the one- and five-month time points of the Sham-administered rats, while no viruses were consistently altered amongst LPS treated rats.

For rats receiving the monomeric form of α -syn, 16 and 6 viruses had decreased and increased differential abundance at the five-month time point (Figure 5c). The α -syn

monomer treatment did not induce a change in a virus or virus cluster across the time-points investigated. The α -syn monomer plus LPS, the PFF, and the PFF plus LPS treatment arms of the study all induced significant abundance changes in viruses or VCs consistent across T1 and T5 time points. These treatments caused decreased differential abundance in six, two, and five viruses and viral clusters, respectively (Figures 5d-f). While the α -syn monomer plus LPS and the PFF treatments both increased two viral groups across both time points (Figures 5d & e).

201

202 **Common viral changes across PD inducing treatments.**

In order to discern if a common pattern of changes occurs across different treatment conditions, all viruses and viral clusters that were noted to have a differential abundance at any time point were compared across the treatments tested. A total of 78 viruses and viral clusters, predicted from the virome sequencing and the WGS sequencing data, were recorded as differentially abundant at one or more time points relative to their baseline (Figure 6). Of these, the majority (48 of 78, 61.5%) were only altered in one treatment condition. These are most probably the result of random changes that have occurred in the rat microbiome over the course of the study.

Nine viruses or VCs were altered across both the T1 and T5 time points analysed, supporting the hypothesis that these are permanent microbiome changes. Only four viruses are concordantly changed across the Sham and LPS controls, seven in the α -syn monomer and α -syn monomer plus LPS treatments, and finally, nine in the α -syn PFF and α -syn PFF plus LPS treated rats. There was one virus with an increased differential abundance at T5 across all treatment groups (contig Rat_15_NODE_1829_length_1188_cov_3.973522). Whereas a second viral contig (C22-R1-T5_NODE_102_length_3426_cov_2.959953) has an increased differential abundance detected in all but the α -syn PFF treatment group.

219 Therefore, these two contigs may be fragments of the same altered virus, with the latter
220 sequence putative annotated as a *Siphoviridae* phage.

221

222 Discussion

223 There are a limited number of viral metagenomic analyses conducted on wild rats as
224 they are a potential source of zoonotic infections ^{27–29}. However, despite their frequent use as
225 models of human diseases, to our knowledge, the virome of lab rats has not been investigated
226 (see Methods). In this study, the majority of faecal viruses analysed are putatively annotated
227 as tailed phages belonging to the *Siphoviridae*, *Myoviridae*, and *Podoviridae* families, with
228 few *Microviridae* observed. A similar trend is observed in the accumulative relative
229 abundance of viral taxa within microbiome sequencing data (Supplementary figure 1). These
230 top-level hierarchical viral taxa are similarly observed in the faeces of mice and humans ^{30,31}.

231 Unlike many mammalian faecal virome analyses, advances in sequencing
232 technologies have enabled us to conduct our analysis without a nucleic acid amplification
233 step. Therefore, the relative abundance of viral taxa and overall diversity of viromes
234 presented here is likely to be more accurate than previous studies. With a sequencing depth of
235 approximately 3M reads per sample, fewer genomes than expected were circular. Without the
236 random amplification bias towards small circular viral elements, and an overall better
237 representation of the virome diversity within the microbiome, an even greater sequencing
238 depth may be required to complete metagenomic viral genomes. On average, there were more
239 lytic than lysogenic phages noted in the rat virome, and this did not statistically differ over
240 the study's duration (Supplementary figure 2). However, the relative abundance of lytic
241 phages decreased (p-value < 0.05) in the monomeric α -syn plus LPS and PFF treatment
242 groups, relative to the study's Sham treatment group. This was investigated because changes
243 in the ratio of lytic to lysogenic phages were observed in a comparison of healthy and
244 inflammatory bowel disease human viromes ³².

245 The majority of the rat faecal virome can be considered as ‘dark matter’, whereby
246 assembled sequences are unlike those deposited in reference databases. This is similar to
247 studies of the human virome, where 40-90% of sequences were unidentifiable ³³. In this
248 study, an average of 58.9% of the rat virome sequencing data could be aligned back to the
249 viral database generated from the sequencing of their faecal viromes. Therefore, while a
250 proportion of the virome sequencing data was not analysed, the remaining uncharacterised
251 data was a cautionary measure to prevent unintentional interpretation of bacterial-
252 contaminating sequences as viral.

253 The diversity of rat faecal viromes changed over the study’s five-month duration. As
254 we are not aware of any published studies on the longitudinal rat faecal virome, it is difficult
255 to ascertain the cause(s) for this variation. Interestingly, the magnitude of change observed
256 for the whole microbiome and virome was similar using the same methodological procedures
257 (variances of 14.6% and 13.3%, respectively). While this result may be coincidental, it would
258 be logical that changes in bacterial populations would have a concomitant effect on the
259 abundance of their viruses. One potential explanation for the shifts observed in the
260 microbiome and virome of rats over five months would be their accelerated aging relative to
261 humans. When comparing their lifespans, every 13.8 rat days approx. equates to a human
262 year ³⁴. Therefore, rats were potentially first analysed in this study during puberty and
263 reached social maturity at its conclusion. The virome changes associated with these
264 developmental periods are poorly described across all mammalian hosts.

265 Knowing that the rat faecal virome changes over time, all fluctuations associated with
266 inter-sample diversity must be interpreted cautiously. The variance (R^2) explained by viral β -
267 diversities did not differ dramatically between the Sham, LPS, or α -syn PFF treatments
268 (22.6%, 19.5%, and 23.9%, respectively). However, large β -diversity differences occurred
269 over the study’s duration between control groups and rats administered the α -syn monomer,
270 α -syn monomer plus LPS, and α -syn PFF plus LPS treatments (28.0%, 27.6%, and 30.8%,

271 respectively). Therefore, specific α -syn treatments affected the overall longitudinal β -
 272 diversity of rats differently, with the fibril form of α -syn with LPS injection as an
 273 inflammatory adjunct resulting in the greater variance.

274 When broken down into an investigation of individual rat viromes, the greatest
 275 variation was observed between the baseline and the first month, T1. However, the viromes
 276 of rats continued to change between T1 and T5, and more frequently the viromes were more
 277 dissimilar from their basal state at the five-month time point, T5. The magnitude by which
 278 individual rat viromes transformed over the study was smallest for the Sham, LPS, and α -syn
 279 monomer treatments. Once more, the greatest changes observed in individual rats occurred in
 280 those receiving the α -syn monomer plus LPS, the α -syn PFF, and α -syn PFF plus LPS
 281 treatments.

282 The individual viruses and VCs responsible for the rat faecal virome changes were
 283 investigated. A similar trend to the β -diversity analysis was observed across all of the study's
 284 treatment groups. More viruses were differentially altered after five months, compared to one
 285 month. Amongst the Sham rats investigated, only two viruses or VCs were differentially
 286 altered at one month with the change maintained until the five-month time point. The LPS
 287 treated control rats had no virus or VC altered across both time points. The abundance and
 288 magnitude of viral changes are amplified in rats treated with the α -syn monomer plus LPS,
 289 the α -syn PFF, and the α -syn PFF plus LPS treated rats.

290 Changes in rat faecal viromes were examined for consistent patterns across treatment
 291 groups. Ideally, specific viruses would be altered across the various α -syn treated rats
 292 indicating a consistent effect on viral composition. However, few viruses and viral clusters
 293 were differentially abundant across α -syn treatments, examining either the monomeric or
 294 fibrillar form of α -syn with and without LPS (Supplementary figure 3). Taken together, viral
 295 changes in the rat faecal virome may be indirectly associated with inflammatory or microbial
 296 changes associated with α -syn in the gut. However, no specific alteration in the virome could

be definitively linked to the native or misfolded α -syn, despite several viruses differentially abundant across different treatments.

In conclusion, we present the first analysis of the lab rat faecal virome. The study focused on the effect caused by the addition of α -syn on the viral composition over five months. There is clear evidence that the virome of rats changed following the injection of α -syn into the enteric nervous system. The magnitude of change observed across faecal viromes was consistently greater amongst rats administer α -syn monomer plus LPS, α -syn PFF, or α -syn PFF plus LPS. However, additional studies are required to clearly establish if α -syn induced virome alterations are associated with the pathogenesis or gastrointestinal comorbidities of PD.

Methods

Generation of α -syn PFFs.

Recombinant monomeric mouse α -syn was purified and used to generate α -syn PFFs, as previously described³⁵. A Pierce LAL high capacity endotoxin removal resin was used to minimize endotoxin. Endotoxin levels were 0.017 Unit/ μ g of protein as determined using a Pierce LAL endotoxin quantitation kit. Briefly, α -syn monomer, kindly provided by Laura Volpicelli-Daley, was thawed rapidly at 37°C in a 1.5 ml microcentrifuge tube, then centrifuged at 100,000g at 4°C for 60 min. The supernatant was removed and its protein concentration was determined using a Nanodrop spectrophotometer, using the mouse α -syn coefficient 7450 M⁻¹cm⁻¹ and molecular weight approximately 15 kDa. The supernatant was diluted in 50 mM Tris pH 7.5, 150 mM KCL to a final protein concentration of 5 mg/ml. A proportion of the solution was retained for subsequent ENS injection of α -syn monomer. The remainder was placed in a thermomixer at 37°C and shaken at 1000 r.p.m for 7 days. Following 7 days, the clear solution became turbid, which is indicative of PFF formation. The

formation of PFFs was validated using the sedimentation assay and transmission electron microscopy as previously described³⁵. Immediately prior to surgery, PFFs were sonicated at 30% power on the Misonix Sonicator 3000, 1s on, 1s off for 120s total, in two 30s sessions, in order to break the fibrils into small fragments. Transmission electron micrographs showed an aggregated protein structure, with filaments that are typical of PFFs. Measurement of fibril length after sonication revealed that > 60% of the fibrils were less than 60 nm in diameter. A sedimentation assay showed that there were approximately equal amounts of α -syn PFFs in the supernatant and pellet, indicating efficient PFF formation³⁵.

330

331 **Animal housing and husbandry.**

Adult male Sprague-Dawley rats were purchased from Envigo, UK and maintained on a 12h:12h light:dark cycle (lights on at 08:00h) at $21 \pm 2^\circ\text{C}$ and humidity (30 - 50%), with access to standard rat chow and water ad libitum. All experiments were conducted in accordance with the European Directive 2010/63/EU, and under a project authorization issued by the Health Products Regulatory Authority, Ireland (AE19130/P036). Animals were randomly divided into six treatment groups, as follows: (a) Sham: bovine serum albumin (BSA) injected into duodenal wall (n=12), (b) LPS: intraperitoneal (i.p.) injection of LPS (n=12), (c) monomer: α -syn monomer injected into duodenal wall (n=12), (d) monomer plus LPS: α -syn monomer injected into duodenal wall plus an immediate i.p injection of LPS (n=12), (e) PFF: PFF injected into duodenal wall (n=12), and (f) PFF plus LPS: PFF injected into duodenal wall plus an immediate i.p injection of LPS (n=12). Animals were housed three per cage, according to their treatment group. After one month, six animals from each group were culled and both gut and brain samples were retrieved for further analysis, the other six were culled at five months.

346

347 **Administration of α -syn into the duodenal wall.**

348 Animals were anaesthetised using gaseous isoflurane (4-5% to induce anaesthesia; 2-
349 3% to maintain anaesthesia). On the day before surgery, animals were pre-treated
350 systemically with the non-steroidal anti-inflammatory analgesic, carprofen (Rimadyl®, 1 ml/
351 500 ml drinking water). Animals were maintained at a constant body temperature by the use
352 of a heating pad. Each animal's abdomen was shaved and sterilised using betadine. The
353 laparotomy procedure was conducted as follows. A 2.5 cm-incision was made vertically
354 along the abdomen using a sterile blade, then a scissors was used to cut through the muscle
355 layer. Approximately 3 cm in length of the stomach and the duodenum was partially exposed
356 to aid visualisation of the injection site. A Harvard automatic pump connected by tubing to a
357 50 μ l Hamilton syringe with a 25-gauge needle was used to perform the injections. In order to
358 validate the injection procedure and the transmission from duodenal myenteric neurons to the
359 CNS, 2 μ l of 1 % GFP-non-toxic fluorescent cholera toxin (Thermofisher, catalogue number
360 C34775) was injected as described previously ^{36,37} into three sites in the duodenal wall.
361 Following this validation, 2 μ l of either mouse α -syn monomer (5 μ g/ μ l) or mouse PFF (5
362 μ g/ μ l) was injected into each of these three sites. Animals in the LPS groups also received an
363 i.p. injection of LPS (2.5 mg/kg, in 0.9 % w/v sterile saline) immediately after the laparotomy
364 (LPS: Sigma, catalogue number L2630, 0111:B4) ³⁸. Sham animals received an injection of
365 BSA (2 μ l per site, 5 μ g/ μ l) into the duodenum, as previously described ^{12,39-41} and LPS
366 animals only received 2.5 mg/kg LPS as i.p injection. All animals were treated for two days
367 following surgery with the non-steroidal anti-inflammatory analgesic, carprofen (Rimadyl®,
368 1 ml/500 ml drinking water) to ensure pain relief.

369

370 **Faecal collection and virome shotgun sequencing.**

371 Faecal pellets were directly extracted from animals at three time points; at the pre-
 372 surgical baseline, termed: baseline, one month post-surgery (T1) and five months post-
 373 surgery (T5). Samples were stored on dry ice and then frozen at -80°C. Nucleic
 374 corresponding to the viromes of each group of rats was purified from faecal samples, with
 375 approximately half a pellet per rat included in the extraction. Faecal samples were
 376 homogenised in 10 ml SM buffer followed by centrifugation twice at 5,000g at 10°C for 10
 377 mins and filtration through a 0.45 µm syringe filter to remove particulates and bacterial cells.
 378 NaCl (0.5 M final concentration; Sigma) and 10% w/v polyethylene glycol (PEG-8000;
 379 Sigma) were added to the resulting filtrate and incubated at 4°C overnight. Following
 380 centrifugation at 5,000g at 4°C for 20 mins, the pellet was resuspended in 400 µl SM buffer.
 381 An equal volume of chloroform (Fisher) was added and following 30 sec of vortexing the
 382 sample was centrifuged at 2,500g for 5 mins at RT. The aqueous top layer was retained and
 383 subjected to RNase I (10 U final concentration; Ambion) and DNase (20 U final
 384 concentration; TURBO DNA-free™ Kit, Invitrogen) treatment in accordance with the
 385 manufacturer's guidelines.

386 To isolate the nucleic acids, virus like particles were incubated with 20 µL of 10%
 387 SDS and 2 µL of proteinase K (Sigma, 20 mg/mL) for 20 min at 56°C, prior to lysis by the
 388 addition of 100 µL of Phage Lysis Buffer (4.5 M guanidine thiocyanate; 45 mM sodium
 389 citrate; 250 mM sodium lauroyl sarcosinate; 562.5 mM β-mercaptoethanol; pH 7.0) with
 390 incubation at 65°C for 10 min. Viral nucleic acid was purified by two treatments with an
 391 equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), finally passing it through a
 392 QIAGEN Blood and Tissue Purification Kit and eluting samples in 30 µL of AE Buffer. In
 393 order to include any RNA viruses in the downstream analysis, 22 µl of the eluent underwent
 394 reverse transcriptase (RT) treatment (Superscript IV, Thermo Fisher) as per the
 395 manufacturer's instructions, albeit doubling all volumes to increase the volume of template
 396 used. Prior to library preparation this RT product was sonicated following adjustment of the

397 volume to 52.5 µl with low-EDTA TE buffer. Shearing of unamplified DNA/cDNA mixture
398 was performed on M220 Focused-Ultrasonicator (Covaris) with the following settings: peak
399 power of 50 W, duty factor of 20%, 200 cycles per burst, total duration of 35 sec. All steps
400 were performed in accordance with the manufacturer's protocol.

401 In order to concentrate the DNA/cDNA fragments entering the library, it was passed
402 through the Zymo Genomic DNA Clean & Concentrator™ Kit, eluting in 17 µl DNA Elution
403 Buffer supplied in the kit. Library preparation was carried out using Accel-NGS 1S Plus kit
404 (Swift Biosciences) according to manufacturer's instructions, with the addition of a final
405 purification step using a 1:1 DNA/AMPure beads ratio to eliminate any small fragment
406 contamination prior to sequencing. A single-indexed pooled library was sequenced using
407 2×150 nt paired-end sequencing run on an Illumina Novaseq platform at Genewiz, Germany.

408

409 **Virome data processing.**

410 Illumina sequencing reads were downloaded and processed as follows. Read quality
411 was assessed pre- and post-processing using FastQC version 0.11.3 ⁴². Adaptors and
412 sequenced nucleotides demonstrating low quality were pruned using Trimmomatic version
413 0.36 in paired-end mode, when the quality dropped below a Phred score of 30 for a 4bp
414 sliding window ⁴³. While processed reads less than 70bp were dumped, the surviving paired
415 and unpaired reads were used in all subsequent analyses. The level of bacterial contamination
416 within the virome sequencing data was estimated by aligning reads against a *cpn60* database
417 ⁴⁴.

418 Reads were assembled into contigs using metaSPAdes version 3.11.1. All contigs less
419 than 1,000bp were discarded. Within the virome sequencing, viruses were predicted through
420 several different procedures. The assembled sequences were queried against the NCBI viral
421 RefSeq database version 95 (E-value 1E-05) using BLAST version 2.6.0+ ⁴⁵. All circular

contigs detected in the virome sequencing were considered as viral. Assembled nucleotide sequences were run through the VirSorter pipeline, where category 1 and 2 viruses were examined further. Viral dark matter was detected by a BLASTn search against NCBI's NT database, with assembled sequences from the viral-enriched shotgun data with no representative in the NT database included in downstream analyses. Viral proteins were predicted using Prodigal version 2.6.3⁴⁶ with the 'meta' option enabled and Shine-Dalgarno training bypassed. Contig-encoded proteins were queried against the Prokaryotic Viral Orthologous Groups database (pVOGs) using HMMER version 3.1.b2⁴⁷. The following cutoffs were employed to detect sequences rich in viral proteins: contigs < 5kb needed ≥ 3 pVOG hits; ≥ 5 and < 10kb, 4 pVOGs; ≥ 10 and < 20kb, 5 pVOGs; ≥ 20 and < 40kb, 6 pVOGs; ≥ 40 and < 60kb, 7 pVOGs; and ≥ 60kb, 8 pVOGs. Sequences identified through the different approaches were pooled together and made non-redundant, keeping the larger of two sequences when the BLAST identity and coverage between sequences exceeded 90%.

Subsequent to the detection of putative viruses in the rat faecal virome sequencing data, potential contaminant sequences were removed as follows. The encoded proteins of all putative viruses were queried against a database of ribosomal proteins. All contigs encoding ribosomal proteins were removed from further analysis. Additionally, proteins were queried for similarity to the Pfam plasmid replication proteins: PF01051, PF01446, PF01719, PF04796, PF05732, and PF06970. Contigs containing a HMMER hit with a score ≥ 15 were discarded.

Virome sequencing reads were aligned to the final viral contigs database using the WGS sequencing data (kindly supplied by Teagasc, Moorepark). While the virome sequencing data was generated on pooled rat faecal samples, the WGS sequencing was performed on multiple time points. Reads were aligned using Bowtie2 version 2.3.4.1 in end-to-end mode⁴⁸. The breadth of coverage of reads mapping across each viral contig was calculated using the BEDTools version 2.26.0 coverage function⁴⁹. In order to determine if a

448 viral sequence was truly present, and not the result of multiple reads stacking onto a single
449 conserved element, the following breadth of coverage cut-offs were applied: minimum 3
450 sequencing reads; for contigs < 5kb, 50% of the sequence needed to be covered in aligned
451 reads; contigs ≥ 5 and < 20kb, 30% of the sequence needed to be covered, and for contigs $\geq$
452 20kb, 10% of the sequence needs to be covered. If the breadth of coverage filter was not met
453 for a specific contig within a sample, zero reads were recorded.

454 Viral nucleotide sequences were grouped into Viral Clusters (VCs), a pseudo-genera
455 taxonomic rank, using vContact2 version 0.9.8⁵⁰. VCs were generated using default settings
456 with the inclusion of known viruses from the Bacterial and Archaeal Viral RefSeq version 85,
457 with ICTV and NCBI taxonomy. The sequencing reads of viruses belonging to specific VCs
458 were aggregated. Viruses grouped into ambiguous vContact2 clusters (i.e. outliers, overlap)
459 were treated as singletons. Viruses not grouping into VCs were nonetheless retained during
460 the analysis. The putative familial-level taxonomic composition of viral sequences were
461 predicted using the Demovir script (<https://github.com/feargalr/Demovir>) with default
462 settings. Integrases and recombinases were identified amongst viral-encoded proteins using a
463 probabilistic hidden Markov model (HMM) generated from Pfam protein families; PF00239,
464 PF00589, PF02899, PF07508, PF09003, PF09299, PF10136, PF13356, PFF14659, PF16795,
465 and PF18644.

466

467 **Virome analysis.**

468 Analyses were conducted in R version 3.6.1⁵¹, implemented through R studio. Images
469 were drawn using ‘ggplot2’⁵², utilising the ‘RColorBrewer’ and ‘jcolors’ packages for colour
470 schemes^{53,54}. Statistical comparisons were performed using the ‘ggpubr’ package⁵⁵, with two
471 and multiple group comparisons executed through Wilcoxon and Kruskal-Wallis tests,
472 respectively. α - and β -diversities of samples were calculated with the ‘vegan’ and ‘phyloseq’

473 packages, respectively ^{56,57}. Differences in sample β -diversities displayed in this study were
 474 calculated using the Bray-Curtis dissimilarity index with Principal Coordinate Analysis
 475 (PCoA) two-dimensional ordination. Permutational multivariate analysis of variance
 476 (PERMANOVA) statistical comparisons of PCoA-grouped samples was conducted with the
 477 adonis function from the vegan package. Differential abundance changes in viruses and VCs
 478 were calculated using the ‘DESeq2’ package ⁵⁸. Reported p-values were adjusted using
 479 Bonferroni correction. The heatmap annotation bar was generated using the
 480 ‘ComplexHeatmap’ package ⁵⁹. Literature searches were conducted in PubMed, February
 481 2020, using the search term: “rat” AND “phage” OR “rat” AND “phage”.

482

483 **Frequent abbreviations**

484 PD, Parkinson’s disease

485 α -syn, Alpha-synuclein

486 LB, Lewy body

487 GI, Gastrointestinal

488 LPS; Lipopolysaccharide

489 WGS, Whole genome shotgun

490 VCs, Viral clusters

491 PFF, Pre-formed (α -syn) fibrils

492

493 **Acknowledgements**

494 This publication has emanated from research conducted with the financial support of Science
495 Foundation Ireland under Grant numbers grant numbers SFI/12/RC/2273_P2 and SFI/14/SP
496 APC/B3032.

497

498 **Author contributions**

499 SMOD, AS and CON conceived the study. SMOD performed the animal study. AS and CON
500 supervised the animal study. LAD extracted and sequenced the faecal viromes. SRS
501 performed the analysis. WB and OOS conducted the WGS sequencing. LVD purified
502 monomeric α -syn. SRS, LAD, SMOD, CON, and CH interpreted the results and wrote the
503 manuscript. CON and CH secured the funding.

504

505 **Competing interests**

506 The authors declare no conflict of interest.

507

508 **Corresponding authors**

509 Correspondence should be addressed to: c.hill@ucc.ie or c.oneill@ucc.ie

510

511 **Data availability**

512 The supplementary data and scripts required to generate the images and interpret the results
513 of this study are openly available in Figshare at <http://doi.org/10.6084/m9.figshare.14332985>.

514

515 **References**

- 516 1. Tysnes, O.-B. & Storstein, A. Epidemiology of Parkinson's disease. *J Neural Transm* **124**, 901–905
517 (2017).
- 518 2. Findley, L. J. The economic impact of Parkinson's disease. *Parkinsonism & Related Disorders* **13**,
519 S8–S12 (2007).
- 520 3. Johnson, M. E., Stecher, B., Labrie, V., Brundin, L. & Brundin, P. Triggers, Facilitators, and
521 Aggravators: Redefining Parkinson's Disease Pathogenesis. *Trends in Neurosciences* **42**, 4–13
522 (2019).
- 523 4. Warner, T. T. & Schapira, A. H. V. Genetic and environmental factors in the cause of Parkinson's
524 disease. *Ann Neurol.* **53**, S16–S25 (2003).
- 525 5. Surmeier, D. J. Determinants of dopaminergic neuron loss in Parkinson's disease. *FEBS J* **285**,
526 3657–3668 (2018).
- 527 6. George DeMaagd & Ashok Philip. Parkinson's Disease and Its Management. *P T* **40**, 504–510
528 (2015).
- 529 7. Zhang, G. *et al.* New Perspectives on Roles of Alpha-Synuclein in Parkinson's Disease. *Front. Aging*
530 *Neurosci.* **10**, 370 (2018).
- 531 8. Pfeiffer, R. F. Gastrointestinal dysfunction in Parkinson's disease. *Parkinsonism & Related*
532 *Disorders* **17**, 10–15 (2011).
- 533 9. Yan, F. *et al.* Gastrointestinal nervous system α -synuclein as a potential biomarker of Parkinson
534 disease: *Medicine* **97**, e11337 (2018).
- 535 10. Minguéz-Castellanos, A. *et al.* Do α -synuclein aggregates in autonomic plexuses predate Lewy
536 body disorders?: A cohort study. *Neurology* **68**, 2012–2018 (2007).
- 537 11. Kim, S. *et al.* Transneuronal Propagation of Pathologic α -Synuclein from the Gut to the Brain
538 Models Parkinson's Disease. *Neuron* **103**, 627–641.e7 (2019).
- 539 12. Holmqvist, S. *et al.* Direct evidence of Parkinson pathology spread from the gastrointestinal
540 tract to the brain in rats. *Acta Neuropathol* **128**, 805–820 (2014).

- 541 13. Uemura, N. *et al.* Inoculation of α -synuclein preformed fibrils into the mouse gastrointestinal
542 tract induces Lewy body-like aggregates in the brainstem via the vagus nerve. *Mol*
543 *Neurodegeneration* **13**, 21 (2018).
- 544 14. Scheperjans, F. *et al.* Gut microbiota are related to Parkinson's disease and clinical phenotype.
545 *Mov Disord.* **30**, 350–358 (2015).
- 546 15. Keshavarzian, A. *et al.* Colonic bacterial composition in Parkinson's disease: COLONIC
547 MICROBIOTA IN PARKINSON'S DISEASE. *Mov Disord.* **30**, 1351–1360 (2015).
- 548 16. Bedarf, J. R. *et al.* Functional implications of microbial and viral gut metagenome changes in
549 early stage L-DOPA-naïve Parkinson's disease patients. *Genome Med* **9**, 39 (2017).
- 550 17. Hill-Burns, E. M. *et al.* Parkinson's disease and Parkinson's disease medications have distinct
551 signatures of the gut microbiome: PD, Medications, and Gut Microbiome. *Mov Disord.* **32**, 739–
552 749 (2017).
- 553 18. Heintz-Buschart, A. *et al.* The nasal and gut microbiome in Parkinson's disease and idiopathic
554 rapid eye movement sleep behavior disorder: Nose and Gut Microbiome in PD and iRBD. *Mov*
555 *Disord.* **33**, 88–98 (2018).
- 556 19. Lin, A. *et al.* Gut microbiota in patients with Parkinson's disease in southern China.
557 *Parkinsonism & Related Disorders* **53**, 82–88 (2018).
- 558 20. Unger, M. M. *et al.* Short chain fatty acids and gut microbiota differ between patients with
559 Parkinson's disease and age-matched controls. *Parkinsonism & Related Disorders* **32**, 66–72
560 (2016).
- 561 21. Li, W. *et al.* Structural changes of gut microbiota in Parkinson's disease and its correlation with
562 clinical features. *Sci. China Life Sci.* **60**, 1223–1233 (2017).
- 563 22. Sampson, T. R. *et al.* Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a
564 Model of Parkinson's Disease. *Cell* **167**, 1469-1480.e12 (2016).
- 565 23. Tetz, G., Brown, S. M., Hao, Y. & Tetz, V. Parkinson's disease and bacteriophages as its
566 overlooked contributors. *Sci Rep* **8**, 10812 (2018).

- 567 24. Hanage, W. P. Microbiology: Microbiome science needs a healthy dose of scepticism. *Nature*
568 **512**, 247–248 (2014).
- 569 25. Ann C. Gregory, Olivier Zablocki, Allison Howell, Benjamin Bolduc & Matthew B. Sullivan. The
570 human gut virome database. *bioRxiv* (2019) doi:10.1101/655910.
- 571 26. Roux, S., Hallam, S. J., Woyke, T. & Sullivan, M. B. Viral dark matter and virus–host interactions
572 resolved from publicly available microbial genomes. *eLife* **4**, e08490 (2015).
- 573 27. Tan, Z. *et al.* Virome profiling of rodents in Xinjiang Uygur Autonomous Region, China: Isolation
574 and characterization of a new strain of Wenzhou virus. *Virology* **529**, 122–134 (2019).
- 575 28. Hansen, T. A. *et al.* High diversity of picornaviruses in rats from different continents revealed
576 by deep sequencing. *Emerging Microbes & Infections* **5**, 1–8 (2016).
- 577 29. Phan, T. G. *et al.* The Fecal Viral Flora of Wild Rodents. *PLoS Pathog* **7**, e1002218 (2011).
- 578 30. Kim, M.-S. & Bae, J.-W. Spatial disturbances in altered mucosal and luminal gut viromes of diet-
579 induced obese mice: Mucosal and luminal gut viromes. *Environ Microbiol* **18**, 1498–1510 (2016).
- 580 31. Shkoporov, A. N. *et al.* The Human Gut Virome Is Highly Diverse, Stable, and Individual Specific.
581 *Cell Host & Microbe* **26**, 527–541.e5 (2019).
- 582 32. Clooney, A. G. *et al.* Whole-Virome Analysis Sheds Light on Viral Dark Matter in Inflammatory
583 Bowel Disease. *Cell Host & Microbe* **26**, 764–778.e5 (2019).
- 584 33. Krishnamurthy, S. R. & Wang, D. Origins and challenges of viral dark matter. *Virus Research*
585 **239**, 136–142 (2017).
- 586 34. Pallav Sengupta. The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med* **4**, 624–630
587 (2013).
- 588 35. Volpicelli-Daley, L. A. *et al.* Formation of α -synuclein Lewy neurite–like aggregates in axons
589 impedes the transport of distinct endosomes. *MBoC* **25**, 4010–4023 (2014).
- 590 36. Conte, W. L., Kamishina, H. & Reep, R. L. Multiple neuroanatomical tract-tracing using
591 fluorescent Alexa Fluor conjugates of cholera toxin subunit B in rats. *Nat Protoc* **4**, 1157–1166
592 (2009).

- 593 37. Hyland, N. P., Abrahams, T. P., Fuchs, K., Burmeister, M. A. & Hornby, P. J. Organization and
594 neurochemistry of vagal preganglionic neurons innervating the lower esophageal sphincter in
595 ferrets. *J. Comp. Neurol.* **430**, 222–234 (2001).
- 596 38. Kelly, L. P. *et al.* Progression of intestinal permeability changes and alpha-synuclein expression
597 in a mouse model of Parkinson's disease: GI Dysfunction in a Premotor Model of PD. *Mov Disord.*
598 **29**, 999–1009 (2014).
- 599 39. Couch, Y., Alvarez-Erviti, L., Sibson, N. R., Wood, M. J. & Anthony, D. C. The acute inflammatory
600 response to intranigral α -synuclein differs significantly from intranigral lipopolysaccharide and is
601 exacerbated by peripheral inflammation. *J Neuroinflammation* **8**, 166 (2011).
- 602 40. Freundt, E. C. *et al.* Neuron-to-neuron transmission of α -synuclein fibrils through axonal
603 transport. *Ann Neurol.* **72**, 517–524 (2012).
- 604 41. George, S., Rey, N. L., Reichenbach, N., Steiner, J. A. & Brundin, P. α -Synuclein: The Long
605 Distance Runner: α -Synuclein: The Long Distance Runner. *Brain Pathology* **23**, 350–357 (2013).
- 606 42. Andrews, S. FastQC: a quality control tool for high throughput sequence data. (2010).
- 607 43. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence
608 data. *Bioinformatics* **30**, 2114–2120 (2014).
- 609 44. Hill, J. E. cpnDB: A Chaperonin Sequence Database. *Genome Research* **14**, 1669–1675 (2004).
- 610 45. McGinnis, S. & Madden, T. L. BLAST: at the core of a powerful and diverse set of sequence
611 analysis tools. *Nucleic Acids Research* **32**, W20–W25 (2004).
- 612 46. Hyatt, D. *et al.* Prodigal: prokaryotic gene recognition and translation initiation site
613 identification. *BMC Bioinformatics* **11**, 119 (2010).
- 614 47. Grazziotin, A. L., Koonin, E. V. & Kristensen, D. M. Prokaryotic Virus Orthologous Groups
615 (pVOGs): a resource for comparative genomics and protein family annotation. *Nucleic Acids Res*
616 **45**, D491–D498 (2017).
- 617 48. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, 357–
618 359 (2012).

619 49. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic
620 features. *Bioinformatics* **26**, 841–842 (2010).

621 50. Bin Jang, H. *et al.* Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled
622 by gene-sharing networks. *Nat Biotechnol* **37**, 632–639 (2019).

623 51. R Core Team. *R: A Language and Environment for Statistical Computing*. [https://www.R-](https://www.R-project.org/)
624 [project.org/](https://www.R-project.org/) (2019).

625 52. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer-Verlag New York, 2016).

626 53. Erich Neuwirth. RColorBrewer: ColorBrewer Palettes. (2014).

627 54. Jared Huling. jcolors: Colors Palettes for R and ‘ggplot2’, Additional Themes for ‘ggplot2’.
628 (2019).

629 55. Alboukadel Kassambara. ggpubr: ‘ggplot2’ Based Publication Ready Plots. (2019).

630 56. Oksanen, J., Kindt, R., Legendre, P. & O’Hara, B. VEGAN: R package for community ecology.
631 (2006).

632 57. McMurdie, P. J. & Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and
633 Graphics of Microbiome Census Data. *PLoS ONE* **8**, e61217 (2013).

634 58. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for
635 RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014).

636 59. Zuguang Gu, Roland Eils & Matthias Schlesner. Complex heatmaps reveal patterns and
637 correlations in multidimensional genomic data. (2016).

638 Figure legends

639 **Figure 1. The rat faecal virome.** (a) Contig length (bp) versus sequencing coverage of
 640 identifiable viruses present in the faeces of male rats. The shape aesthetic was used to
 641 highlight the topology of assembled viral contigs, while colour represents proposed
 642 taxonomic assignment. (b) The breakdown of rat faecal viruses into their putative familial-
 643 level taxonomic assignments. (c) The topology of identified viral contigs, whereby circular
 644 sequences are most likely complete genomes. (d) Viral contigs encoding a phage integrase
 645 and/or recombinase were annotated as lysogenic phages. (e) The relative abundance of viral
 646 contigs within the virome sequencing data, aggregated by taxonomic assignment. Sequencing
 647 reads aligning to contigs not recognised as viral are omitted. (f) The relative abundance of the
 648 bacterial house-keeping gene *cpn60* detected in the virome sequencing data versus the WGS
 649 data.

650

651 **Figure 2. α - and β -diversity analysis of the rat faecal microbiome.** Study time points T1
 652 and T5 represent one and five months, respectively, and are grouped by ellipses. Two
 653 dimensional PCoA ordination of rat faecal samples, using the Bray-Curtis dissimilarity index,
 654 demonstrates the β -diversity between samples. The size of the sample points analysed
 655 represents their α -diversity Shannon index values. (a) WGS sequencing reads mapped onto
 656 the identified rat faecal virome, and (b) relative abundance of bacterial species in the WGS
 657 data.

658

659 **Figure 3. α - and β -diversity analysis of the rat faecal virome by treatment condition.**
 660 Ellipses group samples by the study time points investigated. The size of sample points
 661 represents their Shannon index α -diversity, while the two dimensional PCoA ordination of
 662 sample points represents their β -diversity dissimilarity using the Bray-Curtis index. The

663 treatment conditions investigated in this study are; **(a)** Sham, **(b)** LPS, **(c)** α -syn monomer,
664 **(d)** α -syn monomer and LPS, **(e)** α -syn PFF, and **(f)** α -syn PFF and LPS.

665

666 **Figure 4. Changes in the faecal virome of individual rats, separated by the treatment**
667 **conditions. (a)** The Bray-Curtis dissimilarity index values for all rat faecal viromes between
668 study time points, with Wilcoxon test p-values shown. Points represent the specific Bray-
669 Curtis index values. **(b)** Summary of whether faecal viromes analysed were more dissimilar
670 from the study's baseline (Base) using the Bray-Curtis index after 1 month (T1) or 5 months
671 (T5). The left-hand image of treatment panels **(c-h)** demonstrates the Bray-Curtis
672 dissimilarity for individual rat faecal viromes. A horizontal solid grey line connects the 'Base
673 vs T1' and 'Base vs T5' Bray-Curtis values, while a horizontal dashed grey line indicates the
674 Bray-Curtis change between T1 and T5. Vertical dashed lines and numerical insets are colour
675 coded to indicate the average Bray-Curtis index values at the respective time points. The
676 right-hand image of each treatment panel specifies if T1 or T5 Bray-Curtis values are more
677 dissimilar from the Base.

678

679 **Figure 5. Volcano plots showing differential abundance of rat faecal viruses and viral**
680 **clusters at one month (T1) and five months (T5) relative to the study's baseline.** The
681 vertical dashed lines of each panel indicate log2 fold changes equivalent to +2 or -2, while
682 the horizontal dashed line denotes a Bonferroni adjusted p-value significance of 1E-05. The
683 axis values of all panels are scaled equivalently.

684

685 **Figure 6. Viruses and viral clusters which were differentially abundant at statistical**
686 **significance in at least one of the study's time points, relative to the baseline.** Viruses or
687 viral clusters that were differentially abundant across two or more treatment groups are

688 connected using black dashed lines. The shape of each point indicates if the virus or viral
689 cluster had an increased or decreased differential abundance. The putative familial taxonomic
690 assignment of the various viruses is indicated to the right-hand side of the image.

691 Figures:

692 Figure 1.

693 **Figure 2.**

694 **Figure 3.**

695 **Figure 4.**

696 **Figure 5.**

697 **Figure 6.**

698 **Supplementary figure legends**

699 **Supplementary figure 1. Relative abundance of predicted viral taxa in WGS data.**

700 Aggregation of sequencing data for each rat faecal sample investigated by Demovir predicted
701 viral taxonomic assignment.

702

703 **Supplementary table 1. Alpha-diversity analysis of rat faecal viromes.** Wilcoxon-paired

704 statistical comparisons of rat faecal virome alpha-diversities, with Bonferroni correction,
705 using both viral and WGS sequencing data.

706

707 **Supplementary figure 2. Longitudinal changes in the relative abundance of rat faecal**

708 **lysogenic phages by treatment.** P-values for specific group comparisons were performed
709 using Wilcoxon-paired tests, while the Kruskal-Wallis test was performed across all groups.

710

711 **Supplementary figure 3. Overlapping rat faecal viruses differentially altered by alpha-**

712 **synuclein treatment.**

713 Supplementary figures and tables:

714 Supplementary figure 1.

715 Supplementary table 1.

716

	Data	Group 1	Group 2	P-value	Bonferroni P-value	Significant
1	Virome	Sham	LPS	0.394	1.000	ns
2	WGS	Sham	LPS	0.0177	0.270	*
3	Virome	Sham	Monomer	0.122	1.000	ns
4	WGS	Sham	Monomer	0.4745	1.000	ns
5	Virome	Sham	Monomer + LPS	0.083	1.000	ns
6	WGS	Sham	Monomer + LPS	0.2447	1.000	ns
7	Virome	Sham	PFF	0.062	0.920	ns
8	WGS	Sham	PFF	0.0380	0.570	*
9	Virome	Sham	PFF + LPS	0.525	1.000	ns
10	WGS	Sham	PFF + LPS	0.1837	1.000	ns
11	Virome	LPS	Monomer	0.357	1.000	ns
12	WGS	LPS	Monomer	0.0145	0.220	*
13	Virome	LPS	Monomer + LPS	0.083	1.000	ns
14	WGS	LPS	Monomer + LPS	0.3687	1.000	ns
15	Virome	LPS	PFF	0.219	1.000	ns
16	WGS	LPS	PFF	0.6598	1.000	ns
17	Virome	LPS	PFF + LPS	0.782	1.000	ns
18	WGS	LPS	PFF + LPS	0.3514	1.000	ns
19	Virome	Monomer	Monomer + LPS	0.483	1.000	ns
20	WGS	Monomer	Monomer + LPS	0.0773	1.000	ns
21	Virome	Monomer	PFF	0.636	1.000	ns
22	WGS	Monomer	PFF	0.0054	0.081	**
23	Virome	Monomer	PFF + LPS	0.386	1.000	ns
24	WGS	Monomer	PFF + LPS	0.1434	1.000	ns
25	Virome	Monomer + LPS	PFF	0.791	1.000	ns
26	WGS	Monomer + LPS	PFF	0.1038	1.000	ns
27	Virome	Monomer + LPS	PFF + LPS	0.097	1.000	ns
28	WGS	Monomer + LPS	PFF + LPS	0.8391	1.000	ns
29	Virome	PFF	PFF + LPS	0.192	1.000	ns
30	WGS	PFF	PFF + LPS	0.1427	1.000	ns

717 **Supplementary figure 2.**

718 **Supplementary figure 3.**