

1 **Dietary fibre deprivation and bacterial curli exposure shift gut microbiome**  
2 **and exacerbate Parkinson's disease-like pathologies in an alpha-synuclein-**  
3 **overexpressing mouse**

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41 **Abstract**

42 The microbiome-gut-brain axis has been proposed as a pathogenic path in Parkinson's disease  
43 (PD). Dietary driven dysbiosis and reduced gut barrier function could facilitate the interaction  
44 of toxic external or internal factors with the enteric nervous system, where PD could start.  
45 Amyloid bacterial protein such as curli can act as seed to corrupt enteric  $\alpha$ -synuclein and lead  
46 to its aggregation. Misfolded  $\alpha$ -synuclein can propagate to and throughout the brain. Here, we  
47 aimed at understanding if fibre deprivation and amyloidogenic protein curli could, individually  
48 or together, exacerbate the phenotype in both enteric and central nervous systems of a  
49 transgenic mouse overexpressing wild-type human  $\alpha$ -synuclein. We analysed the gut  
50 microbiome, motor behaviour, gastrointestinal and brain pathologies in these mice. Our  
51 findings show that external interventions, akin to unhealthy life habits in humans, can  
52 exacerbate PD-like pathologies in mice. We believe that our results shed light on how lifestyle  
53 affects PD progression.

## 54 **Introduction**

55 Lifestyle and environmental factors contribute to a variety of chronic, degenerative diseases  
56 burdening socio-economic structures in an expanding and ever-ageing population (Nations n.d.;  
57 Council (US) et al. 2013). The incidence rate of Parkinson’s disease (PD), the second most  
58 common neurodegenerative disease, has consistently increased over the last three decades  
59 (Dorsey et al. 2018) and is predicted to further increase (Yang et al. 2020). Of all current cases,  
60 only 5-10% can be attributed to heritable genetic factors alone (Poewe et al. 2017). For most  
61 cases, PD is a complex multi-factorial disease with genetic and environmental/lifestyle risk  
62 factors contributing to its onset and progression (reviewed in (Gorell et al. 2004)).

63 Environmental and lifestyle factors associated with PD are for example exposure to  
64 different chemicals (e.g. pesticides), head trauma, physical activity, stress, smoking and diet.  
65 Except for smoking and caffeine consumption, all other factors have been associated with  
66 increased risk of PD (reviewed in (Marras, Canning, and Goldman 2019)). In recent years, a  
67 growing body of evidence has put forward the importance of diet and its implications in PD  
68 progression. It has been proposed that a Mediterranean diet rich in fresh unprocessed foods,  
69 especially vegetables, reduces the risk for PD (Maraki et al. 2019). On the other hand, a  
70 “western” diet with low amounts of fibre and high amounts of saturated fats and simple  
71 carbohydrates has been associated, amongst a variety of other diseases, with neurodegenerative  
72 diseases such as PD (reviewed in (Hirschberg et al. 2019; Martínez Leo and Segura Campos  
73 2020)) and is a strong modulator of the gut microbiome. Functional comparative metagenomics  
74 analysis has shown that such a diet is associated with reduced gene expression related to  
75 complex carbohydrate fermentation (Rampelli et al. 2015). In different mouse models, low to  
76 fibre-free diets led to lower abundance of fibre fermenting bacteria (Schroeder et al. 2018) and  
77 higher abundance of mucus foraging species like *Akkermansia muciniphila* (Desai et al. 2016).

78 Desai and colleagues, amongst others (Martens, Chiang, and Gordon 2008), showed that this  
79 consequently led to increased mucus erosion and susceptibility to pathogens (Desai et al. 2016).

80 While such pathogens rather come from infection (Nerius, Doblhammer, and Tamgüney  
81 2020), there is evidence that such pathogens can also originate from commensal bacteria of the  
82 resident gut microbiome and contribute to the disease phenotype (Miller et al. 2021).  
83 Commensal bacteria occupy the outer mucus layer of the colon and can form biofilms  
84 (Johansson, Larsson, and Hansson 2011; Johansson, Sjövall, and Hansson 2013). Even though  
85 there is no consensus on biofilm formation in the healthy gut (Tytgat et al. 2019; De Vos and  
86 M 2015), it has been observed in a variety of gastrointestinal disease scenarios. One biofilm  
87 forming microbial family, *Enterobacteriaceae*, has been associated with severity of a specific  
88 subtype of PD (Scheperjans et al. 2015). Amongst the most prominent biofilm forming species  
89 are *Escherichia coli* (*E. coli*) and *Salmonella* (Miller et al. 2021). Both express curli, a major  
90 biofilm component (Miller et al. 2021) and amyloidogenic protein, which has structural and  
91 physiological similarities to  $\beta$ -amyloid and  $\alpha$ -synuclein ( $\alpha$ Syn) (Chapman et al. 2002; Barnhart  
92 and Chapman 2006). It has been shown to act as a seed for  $\alpha$ Syn aggregation *in vitro* (Sampson  
93 et al. 2020). When injected intramuscularly into the intestinal wall,  $\alpha$ Syn aggregation was  
94 accelerated and led to motor deficits and GI dysfunction (Sampson et al. 2020). Further,  
95 colonization with curli expressing *E. coli* by oral gavage of young germ-free human wild-type  
96  $\alpha$ Syn overexpressing mice (Sampson et al. 2020) and microbiota-depleted Fischer 344 rats (S.  
97 G. Chen et al. 2016) did also replicate different aspects of PD including neuroinflammation,  
98 exacerbated motor deficits, and abnormal  $\alpha$ Syn accumulations in gut and brain.

99 Alpha-synuclein accumulations in the gut have been observed in many PD patients  
100 (Wakabayashi et al. 1988; 1993; 1990; Qualman et al. 1984). Braak and colleagues proposed  
101 that  $\alpha$ Syn accumulations in the ENS preceded those in the lower brainstem regions of the CNS  
102 and subsequently  $\alpha$ Syn would propagate retrogradely in a “prion-like” manner via the vagus

103 nerve to the brain (Braak et al. 2006). Interestingly, truncal vagotomy, the cutting of the vagus  
104 nerve near the gastroesophageal junction, has been associated with reduced risk for PD  
105 (Svensson et al. 2015; Liu et al. 2017). Under normal conditions, however,  $\alpha$ Syn would further  
106 spread to the dorsal motor nucleus of the vagus and then follow Braak's proposed trajectory  
107 leading to the common pathological hallmarks of abnormal accumulation of  $\alpha$ Syn (Lewy  
108 bodies) and loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) and  
109 its projections in the dorsal striatum.

110         In this study we wanted to investigate the exacerbating effect of dietary fibre deprivation  
111 and bacterial curli exposure, individually or combined, on a human  $\alpha$ Syn overexpressing  
112 transgenic mouse. Our subsequent treatment strategy, was to first prime the naïve untreated  
113 microbiome with a "westernized" fibre deprived diet (Desai et al. 2016), followed by the  
114 exposure to curli producing bacteria (S. G. Chen et al. 2016). We analysed the mice at gut  
115 microbial, behavioural, gastrointestinal, and neuropathological levels. Overall, transgenic, but  
116 not wild-type mice, were susceptible to the different challenges. In transgenic mice, our  
117 findings suggest that even though  $\alpha$ Syn overexpression is greatly responsible for the observed  
118 behavioural impairments, the fibre deprived caused dysbiosis led to increased mucus erosion  
119 and pathogen susceptibility, consequently resulting in PD-like pathologies, such as  $\alpha$ Syn  
120 accumulation in the enteric nervous system (ENS) and nigro-striatal degeneration and  $\alpha$ Syn  
121 accumulations in the central nervous system (CNS) further exacerbating coordinative skills of  
122 our transgenic mice. We believe that our study sheds light on how a combination of internal  
123 and external pathogenic factors can differentially contribute to PD-like pathologies in the CNS  
124 and ENS. Therefore, our findings may have important implications for lifestyle adjustments  
125 that could mitigate PD.

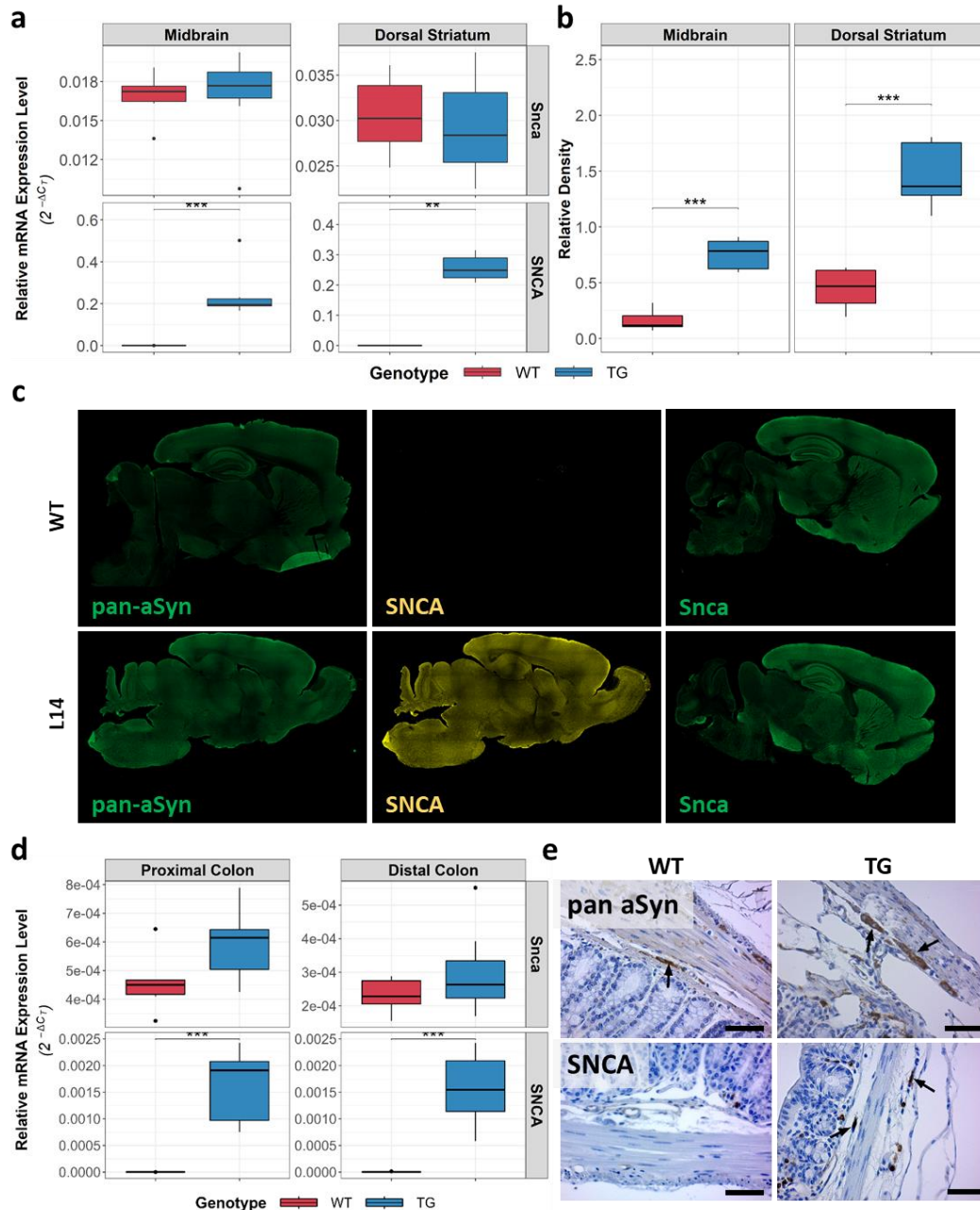
## 126 **Results**

127

### 128 **Thy1-Syn14 overexpress alpha-synuclein in brain and gut with regional differences**

129 The mouse model used in this study was first mentioned in (P. J. Kahle et al. 2001). It  
130 overexpresses human wild-type  $\alpha$ Syn under the transcriptional regulation of the neuron specific  
131 Thy1 promotor and carries 13 copies of the transgene (P. J. Kahle et al. 2001). The model has  
132 so far not been fully described in literature, and only protein levels in bulk brain tissue have  
133 been reported (P. J. Kahle et al. 2001). Thus, determining baseline expression and protein levels  
134 of  $\alpha$ Syn in different CNS regions and in the gut was crucial for the subsequent interpretation  
135 of data generated in our study.

136 In the CNS, we focused on ventral midbrain and dorsal striatal structures using RT-  
137 qPCR for gene expression and Western blot for protein level quantifications. We focused on  
138 both the differences between genotypes and the regions. First, we saw that gene expression  
139 levels for the murine  $\alpha$ Syn (*Snca*) gene (**Fig. 1a**, top row) did no change between genotypes,  
140 but were significantly different ( $p < 0.0001$ ) between the ventral midbrain and dorsal striatum.  
141 On the other hand, for the human transgene of  $\alpha$ Syn (**Fig. 1a**, bottom row), we could only  
142 detect signal in transgenic mice and those levels did not differ significantly between regions.  
143 We saw similar protein profile changes for total  $\alpha$ Syn protein levels. To measure total  $\alpha$ Syn  
144 protein levels, we used a pan- $\alpha$ Syn antibody. This antibody detects both murine and human  
145  $\alpha$ Syn. We observed significant differences between genotype for each region and between  
146 regions (WT:  $p = 1.52E-2$ ; TG:  $p = 1.55E-4$ ; **Fig. 1b**). For transgenic mice, we measured a  
147 2.91-fold increase ( $p = 6.67E-4$ ) in the dorsal striatum and a 6.67-fold increase ( $p = 6.67E-4$ )  
148 in the ventral midbrain compared to their wild-type littermates (**Fig. 1b**). The observed  
149 distribution profile showed us that the expression of murine  $\alpha$ Syn (*Snca*, **Fig. 1c**, right column)  
150 varied between the regions of interest, no matter the genotype, while human  $\alpha$ Syn (SNCA, **Fig.**



**Fig. 1 Thy1-Syn14 present high alpha-synuclein gene expression and protein levels with regional differences**

a) Boxplots showing relative mRNA expression levels of *Snca* (bottom panel) and *SNCA* (top panel) of 9 months old *Thy1-Syn14* TG (blue) mice and wild-type littermates (WT, red) in midbrain and dorsal striatum. Only TG mice express human  $\alpha$ Syn. There were no differences for *Snca* between WT and TG animals per region. b) Boxplots illustrating the calculated relative density from Western blots for pan- $\alpha$ Syn in the ventral midbrain and dorsal striatum. Alpha-tubulin was used as reference protein. c) Representative immunofluorescent stainings for pan- $\alpha$ Syn, mouse  $\alpha$ Syn (*Snca*) and human  $\alpha$ Syn (*SNCA*). Human  $\alpha$ Syn is absent in WT and homogeneously expressed in TG animals (middle panel). Murine  $\alpha$ Syn, is expressed uniformly, except for the ventral midbrain, and there are no differences between genotypes (right panel). Qualitatively, pan- $\alpha$ Syn is more readily expressed in TG mice. d) Boxplots showing relative mRNA expression levels of *Snca* (top panel) and *SNCA* (bottom panel) for 9 months old TG (blue) and WT (red) mice in proximal and distal colon. Only TG animals express human  $\alpha$ Syn (*SNCA*) without regional differences. Murine  $\alpha$ Syn appears to be higher expressed in proximal colon samples and more so in TG animals. e) Representative images (Scale bar: 50  $\mu$ m) from immunohistochemistry stainings showing that *SNCA* in enteric neurons (arrow, right lower panel) is only expressed in TG animals, while both WT and TG are positive for pan- $\alpha$ Syn (top row panel, black arrows).

Stats: Kruskal-Wallis test; \*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.0001$

151 WT, wild-type littermates, TG, *Thy1-Syn14* animals, pan- $\alpha$ Syn, total alpha-synuclein protein



152 **1c**, middle column), aside from only present TG mice, was expressed homogeneously in all  
153 regions. Finally, the total  $\alpha$ Syn expression profile was identical to what we observed for SNCA.  
154 This reflected the high protein level differences observed by Western blot.

155

156 Next, we checked the  $\alpha$ Syn expression profile in the colon. We split the colon into  
157 proximal and distal parts and measured both mouse (*Snca*) and human (SNCA)  $\alpha$ Syn  
158 expression levels via RT-qPCR. It was apparent that overall the levels for *Snca* and SNCA are  
159 much lower when comparing to the observed CNS levels (**Fig. 1d**). This is most likely due to  
160 the much lower density of neurons in whole colon compared to e.g. ventral midbrain samples.  
161 In colon, only about 1% of all cells are neurons (Drokhlyansky et al. 2020), whereas e.g. ventral  
162 structures of the midbrain, comprising several populations of dopaminergic neurons (e.g.  
163 Substantia Nigra and Ventral Tegmental Area), have a roughly estimated proportion of 15-20%  
164 neurons (Keller, Erö, and Markram 2018; Murakami et al. 2018; J. Zhang et al. 2007; Y. Zhang  
165 et al. 2012). Nevertheless, we saw that SNCA was only expressed in transgenic mice, while  
166 there were regional but no genotype differences for *Snca*. Additionally we stained for human  
167  $\alpha$ Syn and total  $\alpha$ Syn (**Fig. 1e**). Latter was expressed in both genotypes, while only transgenic  
168 mice expressed human  $\alpha$ Syn (**Fig. 1e**, left bottom panel).

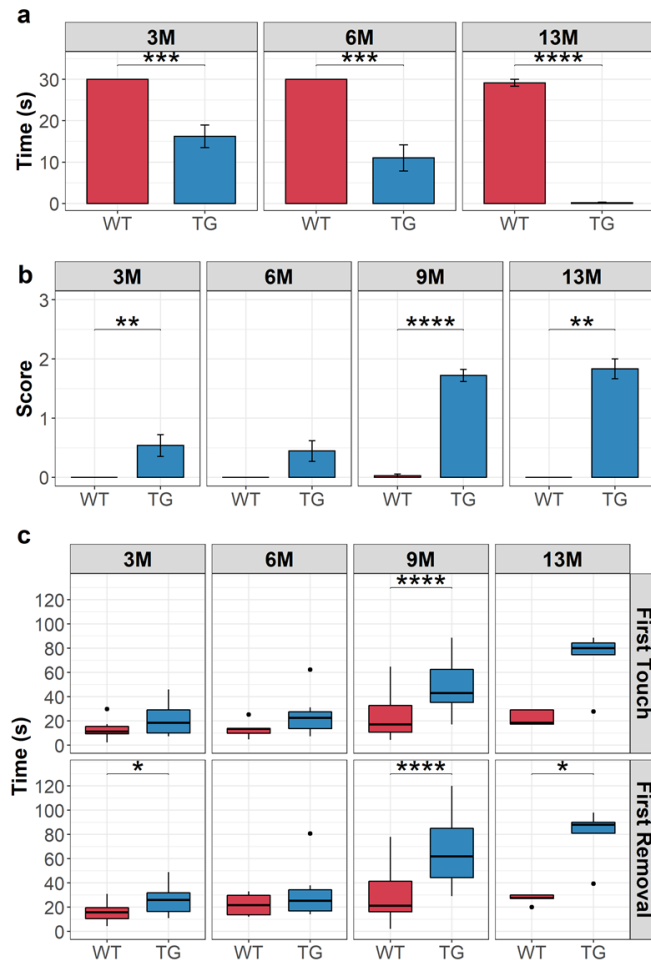
169 The data shown here, indicates that the Thy1-Syn14 model presents an appropriate  
170 system to investigate  $\alpha$ Syn related gut-brain interactions.

171

### 172 **Thy1-Syn14 mice exhibit progressive motor deficits**

173 PD-like motor deficits have been reported in a variety of mouse models of the disease (reviewed  
174 in (Barber Janer, Vonck, and Baekelandt 2021)). Here we assessed for grip strength, hind limb  
175 reflexes and coordination/movement.





**Fig. 2 Thy1-Syn14 showed a progressive decline in behavioral motor skills.**

a-c) Animals were tested at different ages (3M, 6M, 9M, 13M) for gross and fine motor skills. Results are depicted in boxplots indicating median values with interquartile ranges. a) Inverted grid indicates that animals gradually lose grip strength. Muscle weakening is already significantly reduced at three months of age. At 6 months of age, grip strength declines further, and at 13 months of age, transgenic animals cannot hold on to the grid anymore at all. Data for 9 month old animals is missing. b) Hind-limb clasping appears also as early as three months in some animals. When specifically looking into age dependant responses, animals slightly older than three month were more likely to show single hind-limb clasping (score = 1). In the three and 6 month cohorts there was no clasping of both hind-paws (score = 2). Only at 9 months and to a greater extend at 13 months animals showed clasping of both hind-paws. Wild-type animals did not show (with one exception at 9 months) hind-limb clasping at any age on average. c) Adhesive removal measure for fine sensory and motor coordination skills. Results indicated that these skills are age dependant and differed significantly between wild-type and transgenic animals in the 9 and 13 months cohorts.

Stats: Mann-Whitney U, corrected for FDR; \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$ , \*\*\*\*,  $p < 0.0001$

WT, wild-type littermates; TG, Thy1-Syn14 animals, 3M, 3 months old; 6M, 6 months old; 9M, 9 months old; 13M, 13 months old

176 To assess grip strength, indicative of striatal dysfunction and neurotransmitter loss  
 177 (Tillerson et al. 2002a), we used a simplified version of the inverted grid test (Tillerson et al.  
 178 2002b; Tillerson and Miller 2003). Here, we only measured the hanging time and therefore  
 179 assessed the simultaneous 4-limb grip strength. Grip strength gradually decreased as the TG  
 180 mice aged (3M:  $16.2 \pm 9.88s$ ,  $p = 3E-4$ ; 6M:  $11 \pm 9.39s$ ,  $p = 3E-4$ ; 13M:  $0.17 \pm 0.41s$ ,  $p =$   
 181  $4.86E-7$ ; Fig. 2a). Grip strength in wild-type littermates remained unchanged.

182 The hindlimb clasping or reflex test, an additional test assessing striatal dysfunction  
 183 (Fernagut et al. 2004; Lieu et al. 2013), confirmed that the performance of TG mice decreased  
 184 with age (3M:  $p = 9.33E-3$ ; 6M:  $p = 5.7E-2$ ; 9M:  $p < 0.0001$ ; 13M:  $p = 4E-3$ ; Fig. 2b), while  
 185 we did not observe an age-dependent motor impairment in the wild-type littermates.

186 Finally, we tested mice for coordination and fine motor skills with the adhesive removal  
187 test. Deficits in time of removal have been associated with loss of dopaminergic neurons  
188 (Fleming, Ekhtor, and Ghisays 2013). We did not observe relevant motor deficits in young  
189 mice (3M and 6M; **Fig. 2c**). At 9 and 13 months, we saw that both sensitivity (Time at touch,  
190 upper strip), as well as coordination (Time at removal, lower strip) were significantly delayed  
191 in TG mice (Time at touch – 9M:  $p = 47.57E-7$ , 13M:  $p = 0.057$ ; Time at Removal – 3M:  $p =$   
192  $0.016$ , 9M:  $p = 1.12E-6$ , 13M:  $p = 0.016$ ).

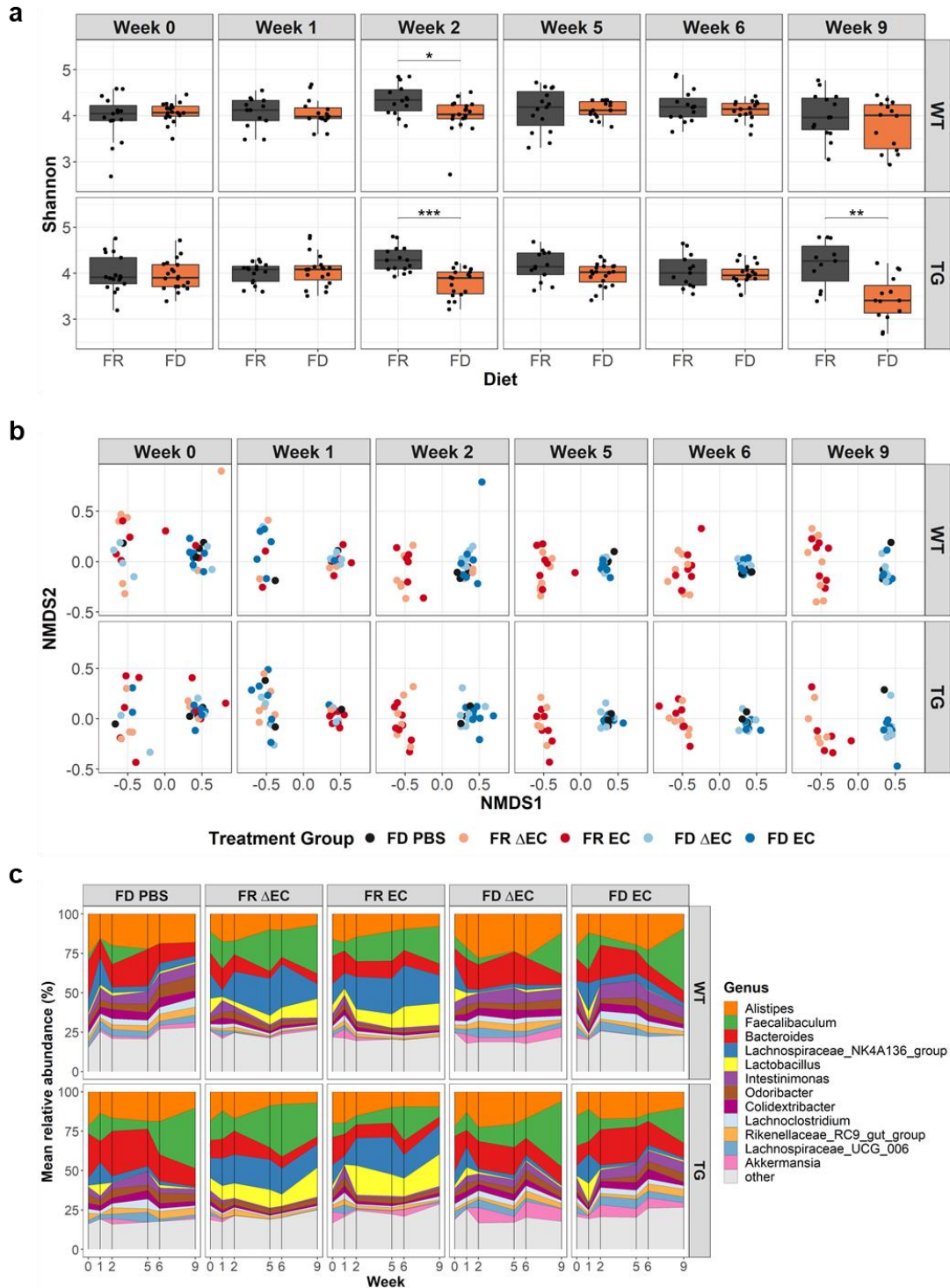
193 Taken together, these data indicate that overexpression of human wild-type  $\alpha$ Syn drives  
194 progressive motor dysfunction in the Thy1-Syn14 mice.

195

#### 196 **Translationally relevant PD-like microbial changes induced after fibre deprivation**

197 Onset and progression of PD, especially idiopathic PD, has been linked to the exposure of  
198 different environmental factors (H. Chen and Ritz, n.d.; Di Monte, Lavasani, and Manning-  
199 Bog 2002; Dick et al. 2007; Warner and Schapira 2003), of which some, e.g. diet, impact the  
200 gut microbiome (Bernardo-Cravo et al. 2020; Singh et al. 2019). Changes in gut microbial  
201 composition in PD has been described extensively in humans (Boertien et al. 2019; Gerhardt  
202 and Mohajeri 2018; Heintz-Buschart et al. 2018; Keshavarzian et al. 2015; Scheperjans et al.  
203 2015; Shen et al. 2021; Unger et al. 2016), but also more and more in different animal  
204 models (Gorecki et al. 2019; Sampson et al. 2016; Yan et al. 2021). Using 16S rRNA amplicon  
205 sequencing, our goal was to understand how the disease challenges affected, independently or  
206 in combination, the microbial phenotype in our mice.

207 First, we looked on a large how the challenges affected microbial diversity. Overall, we  
208 identified the FD diet and TG challenges to be implicated in reduced inner-group diversity  
209 (alpha diversity), while no changes were observed for the gavage challenges (**Supplementary**  
210 **Fig. 2**). Alpha diversity was lowest at weeks 2 (WT:  $p = 0.045$ ; TG:  $p = 6.1E-4$ ) and 9 (TG:  $p$



**Fig. 3 Longitudinal changes in microbial diversity and composition shifts are dietary driven**

a) Boxplots illustrating alpha diversity for the two diet challenges at different time points and both genotypes separately. The absence of dietary fibre (FD) reduced microbial diversity significantly at week 2 in WT (FDR < 0.05) and TG (FDR < 0.001) animals. At week 2 we started the gavages. In TG animals microbial diversity is again significantly ( $p < 0.01$ ) reduced at week 9. Stats: Mann-Whitney U corrected for FDR; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . b) Non-metric multi-dimensional scaling (NMDS) representations for beta diversity showing the different treatment groups faceted by genotype (row) and the different time points (Week, column). We observe a composition shift leading to two homogeneous clusters by week 2. This separation is driven by the dietary challenges. c) Temporal distribution of relative abundance for the 12 most abundant and relevant taxa on the genus level for weeks 0 (Baseline), 1, 2, 5, 6 and 9. Both WT (top row) and TG (bottom row) mice show similar changes in relative abundance for the different taxa. Differences were observed between the diet challenge groups. WT, wild-type littermates; TG, Thy1-Syn14 mice; FD, fibre deprived; FR, fibre rich; PBS, phosphate buffered saline solution;  $\Delta$ EC, curli-KO *E.coli*; EC, wild-type curli expressing *E.coli*

212 = 9.7E-3) in FD challenged and more prominently so in TG mice (**Fig. 3a**). We made similar  
213 observations for beta diversity, where the diet challenge was the main driver (adonis  $p = 0.001$ )  
214 of dissimilarities (**Supplementary Fig. 2a**, middle panel). However, different to alpha  
215 diversity, the gavage challenges (adonis  $p = 0.001$ ) appeared to contribute as well  
216 (**Supplementary Fig. 2b**, right panel). This observation might however be due to the PBS  
217 gavaged mice having all been FD challenged. Hence, solely the FD challenge drove the  
218 observed rapid microbial shift (**Fig. 3b**). Already at week 2, the FD and FR challenged groups,  
219 independent of the other challenges, formed two homogeneous clustered until the end of the  
220 experimental in-life phase. Such a shift is indicative of dysbiosis, the functional imbalance of  
221 the microbiome. Changes in the *Firmicutes* to *Bacteroidetes* ratio, the two most abundant phyla  
222 in the gut, can hint to an overall microbial and functional imbalance (Magne et al. 2020; Mariat  
223 et al. 2009). Our data showed significant increases in the *Firmicutes* to *Bacteroidetes* ratio in  
224 FD challenged mice (**Supplementary Fig. 3**). Next, we focused our analysis on taxa abundance  
225 changes at the genus level.

226 We first subdivided the taxa according to their abundance into three separate groups  
227 (high, mid, and low; **Supplementary Fig. 4**). Analogous to what has been seen before, the diet  
228 challenge was the main driver of microbial abundance changes (**Supplementary Fig. 4**). Next,  
229 we focused our analysis on the most relevant genera in our data, which were *Alistipes*,  
230 *Faecalibaculum*, *Bacteroides*, *Lachnospiraceae* NK4A136 group, *Lactobacillus*,  
231 *Intestinimonas*, *Odoribacter*, *Colidextribacter*, *Lachnoclostridium*, *Lachnospiraceae* UCG  
232 006, *Rikenellaceae* RC9 gut group and *Akkermansia* (**Fig. 3c**). FD challenged mice had  
233 increasing or constantly higher levels in *Faecalibaculum*, *Intestinimonas*, *Odoribacter*,  
234 *Colidextribacter*, *Rikenellaceae* RC9 gut group and *Akkermansia*, and decreasing levels in  
235 *Lachnospiraceae* NK4A136 group and *Lactobacillus* over time (**Fig. 3c, Supplementary Fig.**  
236 **4**). *Alistipes*, *Bacteroides*, *Lachnoclostridium* and *Lachnospiraceae* UCG\_006 on the other

237 hand saw fluctuations over time. They first increased in abundance before dropping back to  
238 initial levels in FD challenged mice.

239 Next, when compared to data from PD patients (Table 3 in Boertien et al., 2019(Boertien  
240 et al. 2019)), FD challenged mice showed similar changes in *Akkermansia* (and its  
241 corresponding Family and Phylum), *Lachnospiraceae*, *Roseburia* and *Prevotellaceae*, while  
242 *Lactobacillaceae* and its genus *Lactobacillus* were inversely altered (**Supplementary Table**  
243 **1**). Other taxa that are often reported to be dysregulated in human PD stool samples were not  
244 detected, e.g. *Bifidobacteriaceae*, *Faecalibacterium* (*Clostridiaceae*) or *Enterobacteriaceae*  
245 (**Supplementary Table 1**). When we checked for the relative abundance of *Escherichia coli*,  
246 the *Enterobacteriaceae* species that we gavaged, it was barely detected in our 16S rRNA  
247 amplicon sequencing data (**Supplementary Fig. 5**).

248 In summary, the FD challenge caused reduced gut microbial diversity and similar shifts  
249 as seen in PD patients. To note are reduced levels of *Lactobacillus*, a known probiotic(Heeney,  
250 Gareau, and Marco 2018; Martín et al. 2013) with possible neuroprotective effects(Wang et al.  
251 2021) and associated with gut barrier integrity(Blackwood et al. 2017), and *Lachnospiraceae*  
252 *NK4A136* group, which inversely correlates with risk for PD or dementia(Stadlbauer et al.  
253 2020). Additionally, *Lachnospiraceae* *NK4A136* group and *Roseburia* are important butyrate  
254 producers, thus associated with gut barrier function(Plöger et al. 2012). Consequently, even  
255 though plasma endotoxin levels were not significantly increased in FD challenged mice  
256 (**Supplementary Fig. 6**), it is reasonable to assume that FD challenged mice had reduced gut  
257 barrier function. Together with higher levels of the known mucin-foraging genera *Akkermansia*  
258 and *Bacteroides*(Desai et al. 2016; Tailford et al. 2015), the susceptibility for pathogenic factors  
259 was most likely increased.

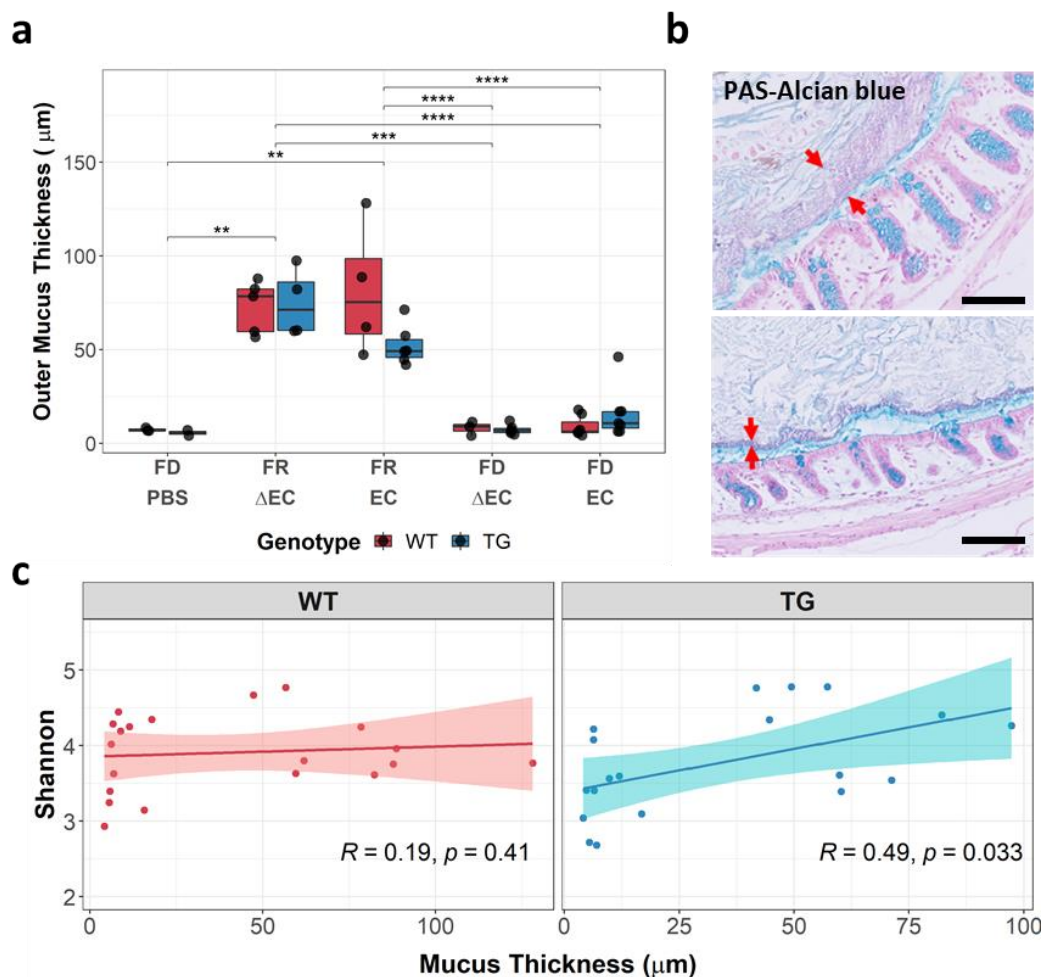
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262 **Microbial driven mucus erosion of the colon in fibre deprived challenged mice**

263 The mucus layers of the colon have two basic functions: the inner layer acts as physical barrier  
 264 to prevent pathogens of reaching the gut epithelium and the outer layer harbours commensal  
 265 bacteria interacting with the host(Johansson, Sjövall, and Hansson 2013). Increasing levels of  
 266 mucin degrading bacteria, such as *Akkermansia muciniphila* and certain *Bacteroides* spp.(Desai  
 267 et al. 2016), have been shown to cause mucus thinning consequently facilitating epithelial  
 268 access for potential pathogens.



**Fig. 4 Microbial mediated outer mucus erosion associated to reduced diversity**

a) Boxplot of outer mucus thickness measurements. The FD challenge causes a vast reduction in outer mucus thickness. Stats: Mann-Whitney U test, corrected for FDR; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ . b) Representative images illustrating the differences in outer mucus erosion between FR (top) and FD (bottom) challenged mice. The red arrows delimit the outer mucus layer. c) Scatterplots of Spearman rank tests comparing alpha diversity (y-axis) and mucus thickness (x-axis) in both genotypes separately. There is a significant positive correlation between microbial diversity and mucus thickness in *Thy1-Syn14* animals independent of the other challenges. Stats: Spearman rank test.

WT, wild-type littermates; TG, *Thy1-Syn14*; FD, fibre deprived; FR, fibre rich; PBS, phosphate buffered saline solution;  $\Delta\text{EC}$ , curli-KO *E.coli*; EC, wild-type curli expressing *E.coli*

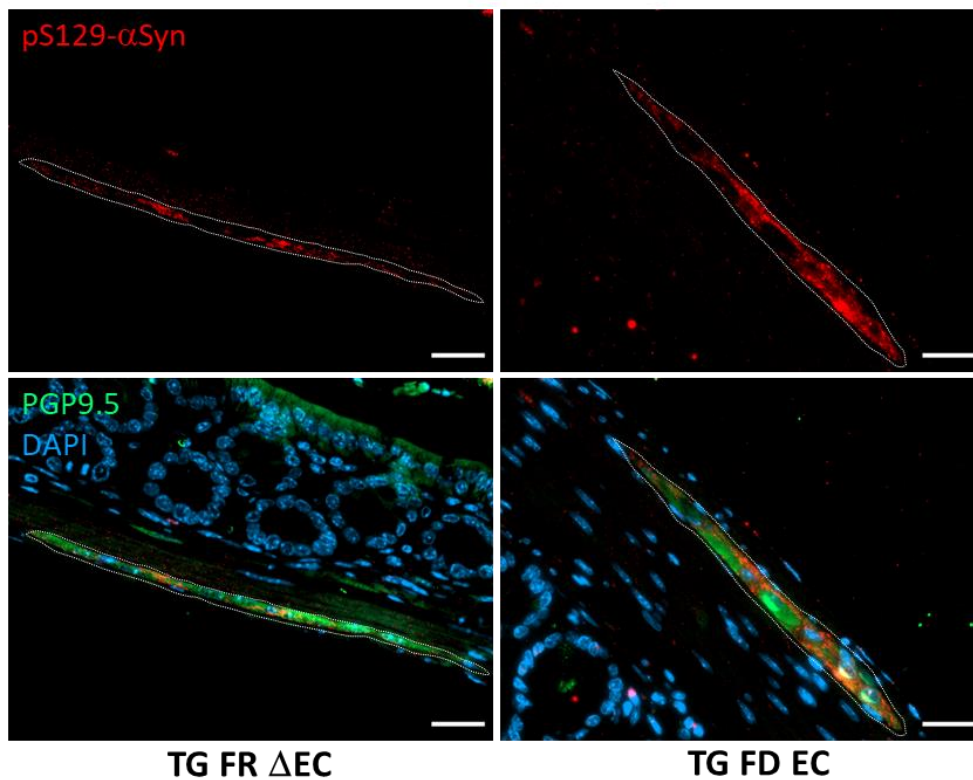
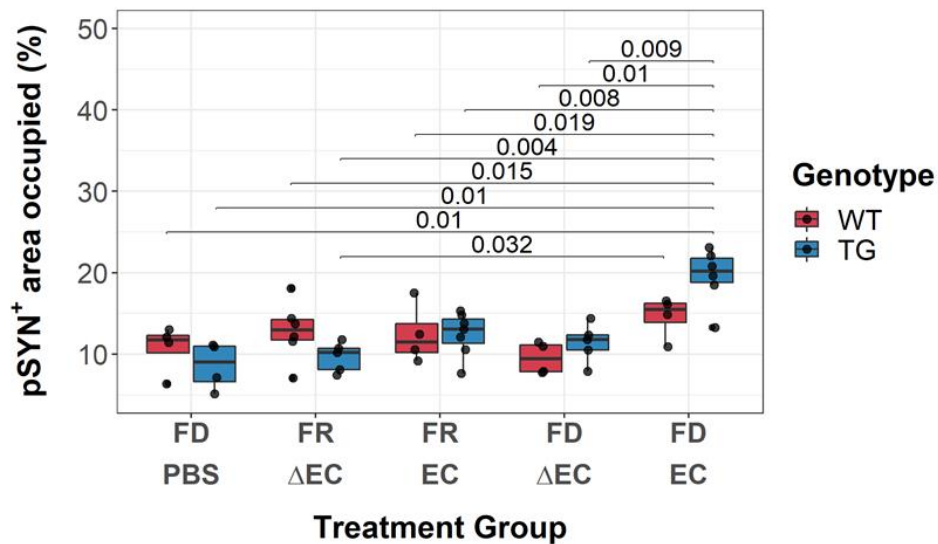
269 For this study we focused on the outer mucus layer. Our results clearly showed that the  
270 FD challenge caused significant ( $p = 1.15E-12$ ) thinning of the outer mucus layer (**Fig. 4a**).  
271 The layer thickness decreased extensively by 49.1% to 92.9% due to the diet challenge (**Fig.**  
272 **4b**). Hence, the habitat for gut bacteria was reduced having direct consequences on microbial  
273 diversity. Therefore, we compared alpha diversity and mucus thickness using the Spearman's  
274 rank test. We were specifically interested in dietary or transgene driven associations. While we  
275 only saw moderate dietary driven correlations (FR:  $r=-0.47$ ,  $p=0.051$ ; FD:  $r=0.38$ ,  $p=0.08$ ;  
276 **Supplementary Fig. 7**), there was a significant positive correlation between alpha diversity  
277 and mucus thickness in TG mice (**Fig. 4c**) pointing to an  $\alpha$ Syn-related increased susceptibility  
278 to microbial changes.

279

### 280 **Bacterial curli drives alpha-synuclein accumulation in the colonic myenteric plexus in** 281 **fibre deprived challenged Thy1-Syn14 mice**

282 Alpha-synuclein accumulation in the gut has been observed in a substantial number of PD  
283 patients(Braak et al. 2006; Del Tredici and Braak 2012; Del Tredici and Duda 2011). To test  
284 for increased levels of  $\alpha$ Syn levels in the gut of our mice, we used an antibody directed against  
285 phosphorylated S129  $\alpha$ Syn (pS129- $\alpha$ Syn). This kind of antibody is commonly used to detect  
286  $\alpha$ Syn accumulation in both the murine and human central nervous system (CNS)(Vaikath et al.  
287 2019) and enteric nervous system (ENS)(Shannon et al. 2012; Stockholm et al. 2016). We  
288 quantified the pS129- $\alpha$ Syn positive accumulations in protein gene product 9.5 (PGP9.5), a  
289 neuronal cytoplasmic marker(Sidebotham et al. 2001), positive ganglions of the myenteric (or  
290 Auerbach) plexus. Other pS129- $\alpha$ Syn positive accumulations in the in the submucosal plexus  
291 and submucosa were irregular and mainly detected in TG mice. This did however not differ  
292 between the different challenge group and we could not determine the cell type with our  
293 approach.





**Fig. 5 Curli-driven phospho-synuclein accumulation in fibre deprived challenged Thy1-Syn14 mice**

Boxplot illustrating the changes of area occupied by pS129- $\alpha$ Syn<sup>+</sup> forms in ganglia of the myenteric plexus of the colon. Only TG mice on the combined challenge have an increased area occupied for pS129- $\alpha$ Syn<sup>+</sup> forms. Stats: Mann-Whitney U test, not corrected for FDR. Representative images below, illustrate the average differences between FR  $\Delta$ EC and FD EC challenged Thy1-Syn14 mice. Besides the area occupied, the pS129- $\alpha$ Syn<sup>+</sup> particles are also enlarged in FD EC challenged mice. WT, wild-type littermates; TG, Thy1-Syn14; FD, fibre deprived; FR, fibre rich; PBS, phosphate buffered saline solution;  $\Delta$ EC, curli-KO *E.coli*; EC, wild-type curli expressing *E.coli*

294 In our analysis, we focused on the myenteric plexus. First, the stainings showed that  
 295 both WT and TG mice had pS129- $\alpha$ Syn positive accumulations in ganglia of the myenteric  
 296 plexus (Supplementary Fig. 8). The quantification of the area occupied showed that only TG

297 mice exposed to the combined challenge FD EC had increased levels of pS129- $\alpha$ Syn in PGP9.5  
298 positive ganglions (vs TG FD PBS:  $p = 0.019$ ; vs WT FR EC:  $p = 0.019$ ; vs TG FR EC:  $p =$   
299  $0.008$ ; vs WT FD  $\Delta$ EC:  $p = 0.019$ ; **Fig. 5**). Besides having a greater area occupied, the average  
300 particle size of the  $\alpha$ Syn accumulations appeared increased in TG FD EC challenged mice  
301 (representative images **Fig. 5**).

302 We can sum up that even though, as seen above, the FD challenge in general led to an  
303 increased pathogen/pathobiont susceptibility, only TG EC challenged mice showed  
304 significantly higher levels of pS129- $\alpha$ Syn positive accumulations in the myenteric plexus of  
305 the colon.

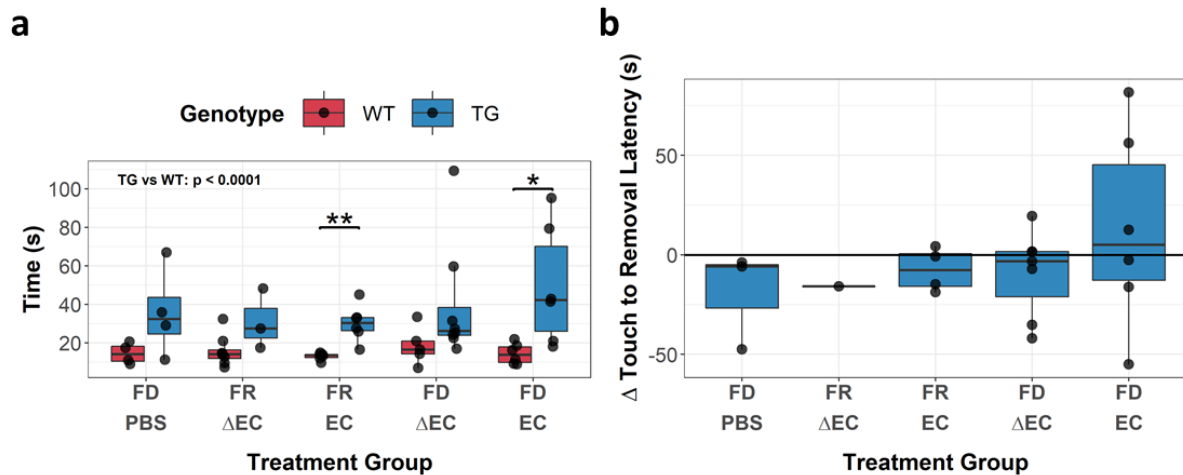
306

### 307 **Alpha-synuclein overexpression is the main but not sole driver of behavioural changes**

308 The exposure to curli, here and in previous studies (S. G. Chen et al. 2016; Sampson et al. 2020),  
309 has been shown to lead to increased accumulation of abnormal  $\alpha$ Syn in the gut. The subsequent  
310 spreading to and within the brain has been associated to motor impairment progression, as  
311 hypothesized by Braak and colleagues (Braak et al. 2003; 2006). In our aged TG mice, we did  
312 already observe motor performance deficits compared to their WT littermates. Therefore, we  
313 turned our interest to the exacerbation of motor performance deficits after the challenges,  
314 individually or in combination.

315 Three different tests were chosen to assess motor performance: hindlimb clasping, grip  
316 strength and adhesive removal. Hindlimb clasping and grip strength are so called basic or gross  
317 motor function tests and were used to monitor motor performance changes along the in-life  
318 phase of the study. Already at baseline, our aged mice differed significantly in their clasping  
319 and grip strength phenotype (**Supplementary Fig. 9a, 9b**). Over the course of the experiments  
320 only TG mice showed changes for both features (**Supplementary Fig. 9a, 9b**). The behavioural

321 changes in TG mice were, however, neither linked to the diet, nor the gavage challenges. Hence,  
 322 the progressive impairment of these features was driven by the overexpression of  $\alpha$ Syn.



**Fig. 6 Challenge effect on motor impairment in a subset of Thy1-Syn14 mice despite the strong age-related transgenic phenotype**

a) Boxplots illustrating the latency for removal in the different treatment groups (x-axis) for WT (red) and TG (blue) animals after 9 weeks. The results shown are for the first paw and from the first replicate. WT animals do not show any difference. TG mice on the other hand that have been FD EC challenges showed greater latency to remove the adhesive tape. There are significant in the FR EC and FD EC groups between WT and TG mice. Stats: Mann-Whitney U test, not corrected for FDR; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . b) Summary plot for TG animals illustrating how the adhesive removal performance changed from baseline to endpoint when focusing on the time difference from touch to removal. Most animals improved in performance from baseline to endpoint. Only the FD EC group shows for 3 out of 6 animals a performance drop. Note: for group 7 only one animal performed normally and so this group result can/should be neglected. (FD PBS:  $n=3$ , FR  $\Delta$ EC:  $n=1$ , FR EC:  $n=5$ , FD  $\Delta$ EC:  $n=6$ , FD EC:  $n=6$ ).

WT, wild-type littermates; TG, Thy1-Syn14; FD, fibre-deprived; FR, fibre-rich (normal chow); PBS, phosphate buffered saline;  $\Delta$ EC, curli-KO E.coli; EC, wild-type curli expressing E.coli

323 The adhesive removal test was used to assess changes in fine motor function (see  
 324 Material and Methods). The test consists in measuring the latencies of touch and removal,  
 325 which at baseline were significantly ( $p < 0.0001$ ) greater in TG mice compared to their WT  
 326 littermates (Fig. 2c, 9M). This was still the case at the end of the 9-week long experimental  
 327 phase ( $p < 0.0001$ ; Fig. 6a, Supplementary Fig. 10). Hence,  $\alpha$ Syn overexpression was again  
 328 key in driving motor impairment. For the external challenges, particularly TG EC challenged  
 329 mice, independent of the diet, showed increased times of touch and removal, respectively,  
 330 compared to the WT littermates (Fig. 6a, Supplementary Fig. 10). Further, we saw a much  
 331 greater increase in both measures for a subset of TG FD EC challenged mice. This raised

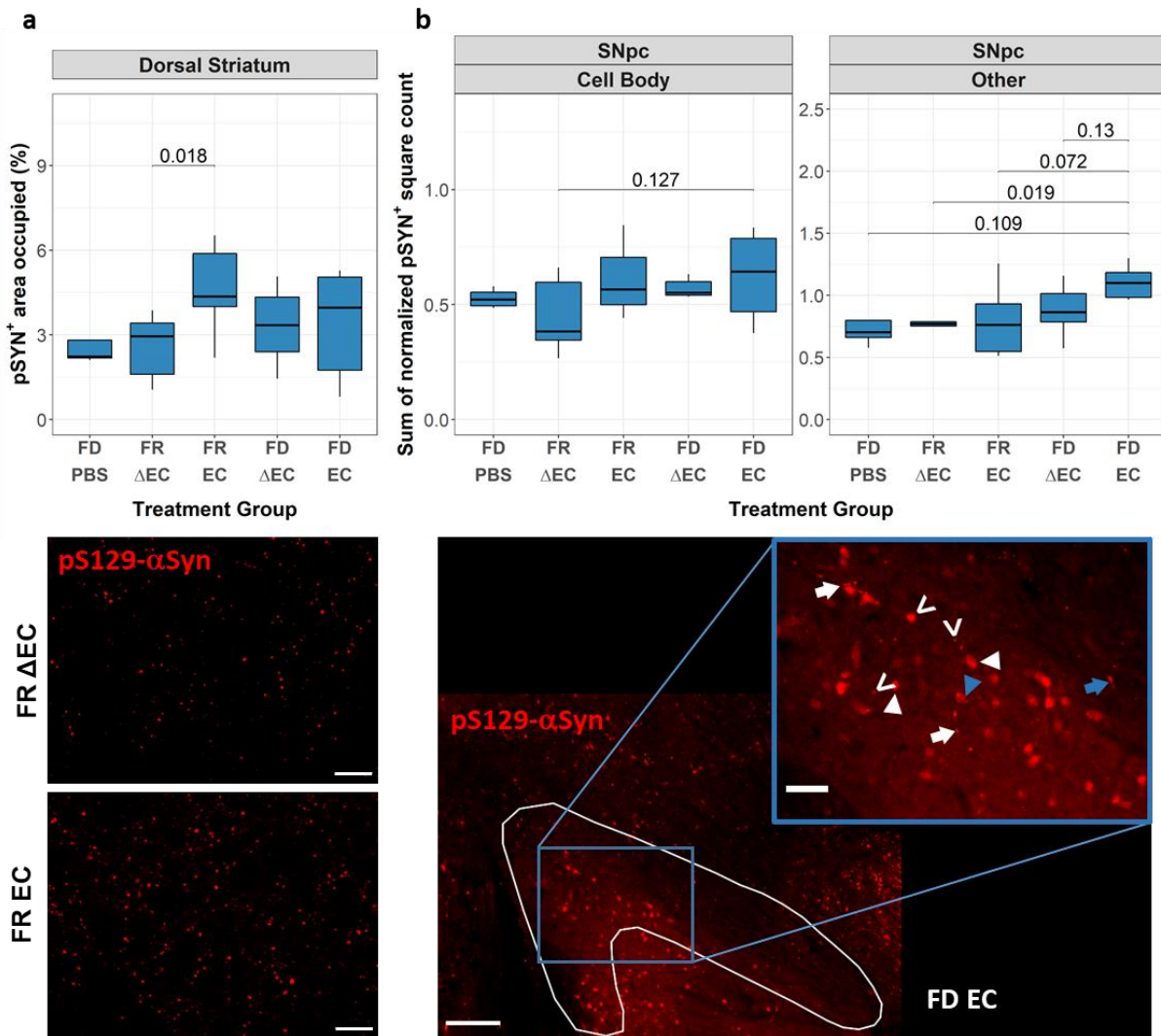
332 additional questions: how did the performance change over time for these TG mice? And what  
333 is the interval between the time of touch and time of removal? To answer these questions, we  
334 1) subtracted the time of touch from the time of removal and then 2) compared “Baseline” to  
335 “Endpoint” results. We found that for the TG FD EC group 3 out of 6 mice had reduced  
336 coordinative ability to remove the adhesive tape compared to their initial performance (**Fig.**  
337 **6b**). While statistically we did not get significant differences, the combination of the diet and  
338 curli challenges did appear to further exacerbate the motor phenotype in aged TG mice.

339

#### 340 **Bacterial curli mediated accumulation of alpha-synuclein in the nigrostriatal pathway**

341 All neuropathological analyses were limited to TG mice, since they have shown to be more  
342 susceptible to the challenges. In a first step, we wanted to determine abnormal  $\alpha$ Syn  
343 accumulations by immunofluorescence staining in the nigrostriatal pathway. To do so, we used  
344 again the pS129- $\alpha$ Syn antibody and quantified pS129- $\alpha$ Syn positive accumulations in the  
345 dorsal striatum and the substantia nigra pars compacta (SNpc). Overall, we observed that the  
346 EC challenge was the main driver of pS129- $\alpha$ Syn accumulation in both regions of interest (**Fig.**  
347 **7**).

348 In the dorsal striatum the FR EC challenged TG mice had the highest levels of pS129-  
349  $\alpha$ Syn positive accumulations, with even significant difference to the FR  $\Delta$ EC ( $p = 0.018$ ) group  
350 (**Fig. 7a**, top panel), as seen in the representative microscopy images (**Fig. 7a**, lower panels).  
351 In the SNpc, we saw that the impact of the FD challenge greater than seen in the dorsal striatum  
352 (**Fig. 7b**). Both pS129- $\alpha$ Syn positive cell body counts and all “other” pS129- $\alpha$ Syn positive  
353 accumulations were especially increased in FD EC challenged mice (“Cell Body”, vs FR  $\Delta$ EC:  
354  $p = 0.127$ ; “Other”, vs FD PBS:  $p = 0.109$ , vs FR  $\Delta$ EC:  $p = 0.019$ , vs FR EC:  $p = 0.072$ , vs FD  
355  $\Delta$ EC:  $p = 0.13$ , **Fig. 7b**, top panel). Qualitatively, the pS129- $\alpha$ Syn positive accumulations that  
356 we observed in cell bodies were usually either of a diffused when in the cytoplasm and more



**Fig. 7 Curli-driven nigrostriatal phospho-synuclein accumulations in Thy1-Syn14 mice**

a,b) Quantification and representative images of immunofluorescent pS129- $\alpha$ Syn stainings of the nigrostriatal pathway. a) Quantitative analysis of pS129- $\alpha$ Syn accumulations in the dorsal striatum by measuring the relative pS129- $\alpha$ Syn+ occupied area. The representative images (40X, scale bar: 20 $\mu$ m) below, illustrate the differences in pS129- $\alpha$ Syn accumulations between FR  $\Delta$ EC and FR EC challenged TG mice. b) Quantitative analysis of pS129- $\alpha$ Syn+ accumulations in the SNpc accounting for two different forms: accumulations cell bodies based on morphological attributes (Cell body) and other forms of accumulations (Other). The majority of accumulations could be attributed to the EC challenge. The combined challenge (FD EC) appears to have a slightly greater impact on pS129- $\alpha$ Syn accumulation. The representative images below illustrate the different observed forms of pS129- $\alpha$ Syn+ accumulations: see details in main text.

Stats: Mann-Whitney U test, not corrected for FDR

FD, fibre-deprived; FR, fibre-rich (normal chow); PBS, phosphate buffered saline;  $\Delta$ EC, curli-KO E.coli; EC, wild-type curli expressing E.coli

357 compact in the nuclei (**Fig. 7b**, bottom panel, white arrowhead). However, in FD EC challenged  
 358 mice we also found dense pS129- $\alpha$ Syn positive accumulations in cell bodies (**Fig. 7b**, bottom  
 359 panel, blue arrowheads). All other pS129- $\alpha$ Syn positive accumulations, not limited to cell  
 360 bodies, appeared generally more intensively immunopositive. We observed three different

361 forms of accumulations: bead like varicosities (**Fig. 7b**, bottom panel, white arrow), similar to  
362 what has been observed in other *in vivo* (Lauwers et al. 2003) and *in vitro* (Kouroupi et al. 2017)  
363 models, and human post-mortem brains (Del Tredici et al. 2002), spheroid shaped  
364 accumulations (**Fig. 7b**, bottom panel, open arrow) and, even though rarely and only in FD EC  
365 challenged mice, corkscrew-like spheroid accumulations (Fig. 9b, bottom panel, blue arrow).

366 In summary, the EC challenge drove pS129- $\alpha$ Syn positive accumulations and were  
367 exacerbated in FD challenged TG mice.

368

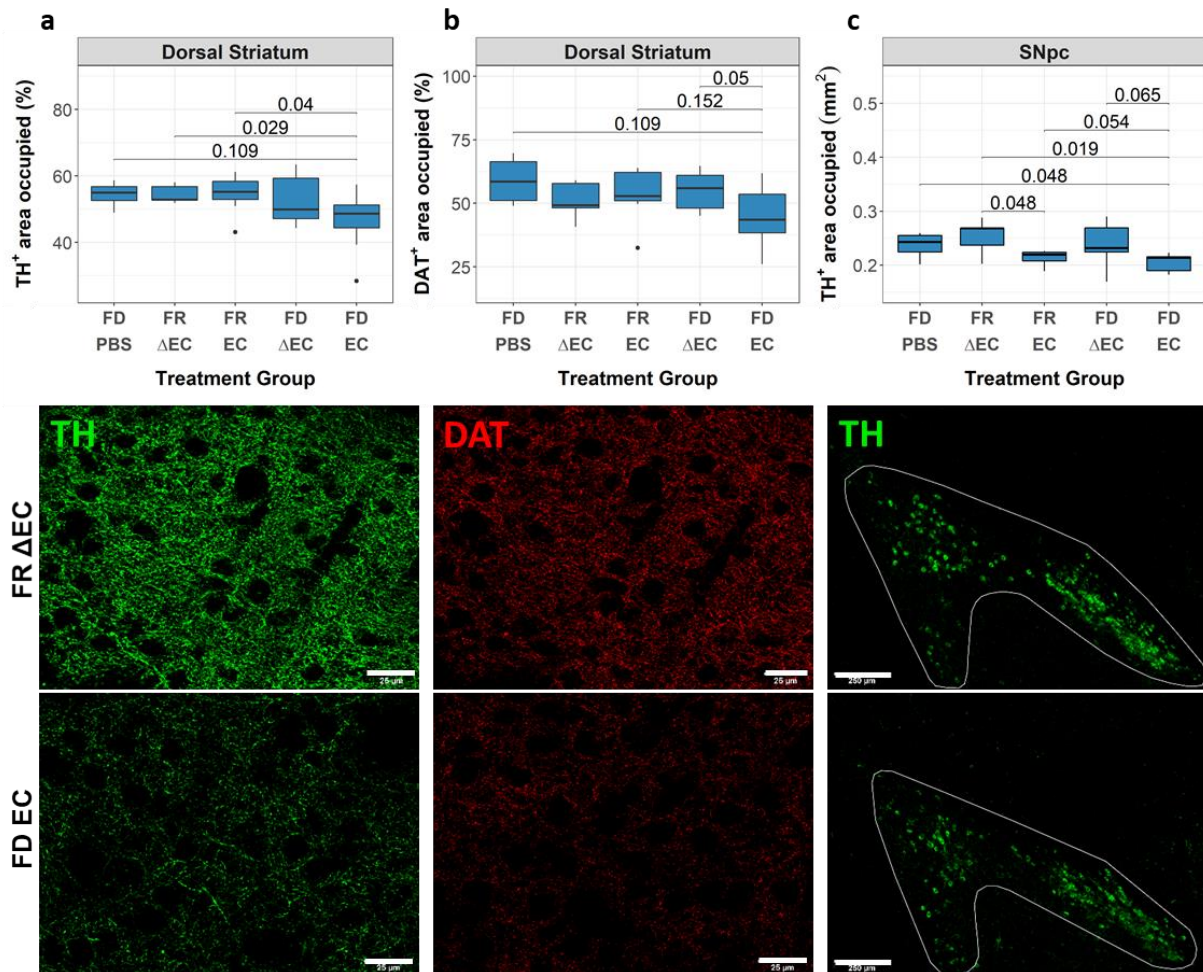
### 369 **Combined bacterial curli protein and dietary fibre deprivation challenges drive** 370 **neurodegeneration in Thy1-Syn14 mice**

371 The loss of neurons in the SNpc and their projections to the dorsal striatum is amongst the main  
372 pathological hallmarks of PD (Poewe et al. 2017). To detect neurodegeneration in our TG mice,  
373 we stained against tyrosine hydroxylase (TH), an enzyme involved in dopamine synthesis and  
374 a marker for dopaminergic neurons and their projections, in both the SNpc and dorsal striatum.  
375 Additionally, in the dorsal striatum, we stained for the dopamine transporter (DAT), a marker  
376 for dopamine-cycling synapses.

377 In the dorsal striatum, the combination of FD and EC challenges in TG mice resulted in  
378 a significantly reduced area occupied by TH positive projections when compared to the FR  
379  $\Delta$ EC ( $p = 0.029$ ) and FR EC ( $p = 0.04$ ) groups (**Fig. 8a**, top panel). For DAT we observed  
380 almost the exact same pattern (**Fig. 8b**, top panel). The FD EC challenged TG mice showed  
381 significantly reduced levels in DAT area occupied when compared to FD  $\Delta$ EC ( $p = 0.05$ ) and  
382 strong trends compared to the FD PBS and FR EC groups (**Fig. 8b**, top panel).

383 Tyrosine hydroxylase positive fibres in the dorsal striatum are the projections from  
384 dopaminergic neurons located in the SNpc. Quantitation of the area occupied by TH positive  
385 neurons (**Fig. 8c**, top panel) showed that the exposure to curli caused significant neuronal loss





**Fig. 8 Curli-driven neurodegeneration is exacerbated by fibre-deprivation in the nigrostriatal pathway of Thy1-Syn14 mice**

a-c) Boxplots and representative images from two different transgenic treatment groups (top: FR ΔEC; bottom: FD EC) from the dorsal striatum and the substantia nigra pars compacta. Boxplots exhibit the median differences of area occupied by specific neuronal or synaptic markers used to investigate neurodegeneration. a) Quantification and representative high magnification (40X, scale bar: 25μm) images of the percent area occupied by tyrosine hydroxylase-positive (TH<sup>+</sup>) fibres in the dorsal striatum. b) Quantification and representative high magnification (40X, scale bar: 25μm) images of the percent area occupied by the synaptic dopamine transporter (DAT) marker in the dorsal striatum. c) Quantification and representative images (10X; scale bar: 250μm) of the summed area occupied in square millimeters (mm<sup>2</sup>) by TH<sup>+</sup> dopaminergic neurons in the Substantia Nigra pars compacta.

Stats: Mann-Whitney U test, not corrected for FDR

SNpc, substantia nigra pars compacta; FD, fibre-deprived (diet); FR, fibre-rich (diet) (normal chow); PBS, phosphate buffered saline; ΔEC, curli-KO E.coli; EC, wild-type curli expressing E.coli

386 (EC-PBS: FDR = 0.041; EC-ΔEC: FDR = 1.68E-4). Fibre deprived challenged mice, showed  
 387 an exacerbated neuronal phenotype. The difference between the FR EC and FD EC groups does  
 388 show a strong (p = 0.054) trend. Hence, the FD challenge potentially leads to an increased  
 389 susceptibility to a curli-driven neurodegenerative process.

390

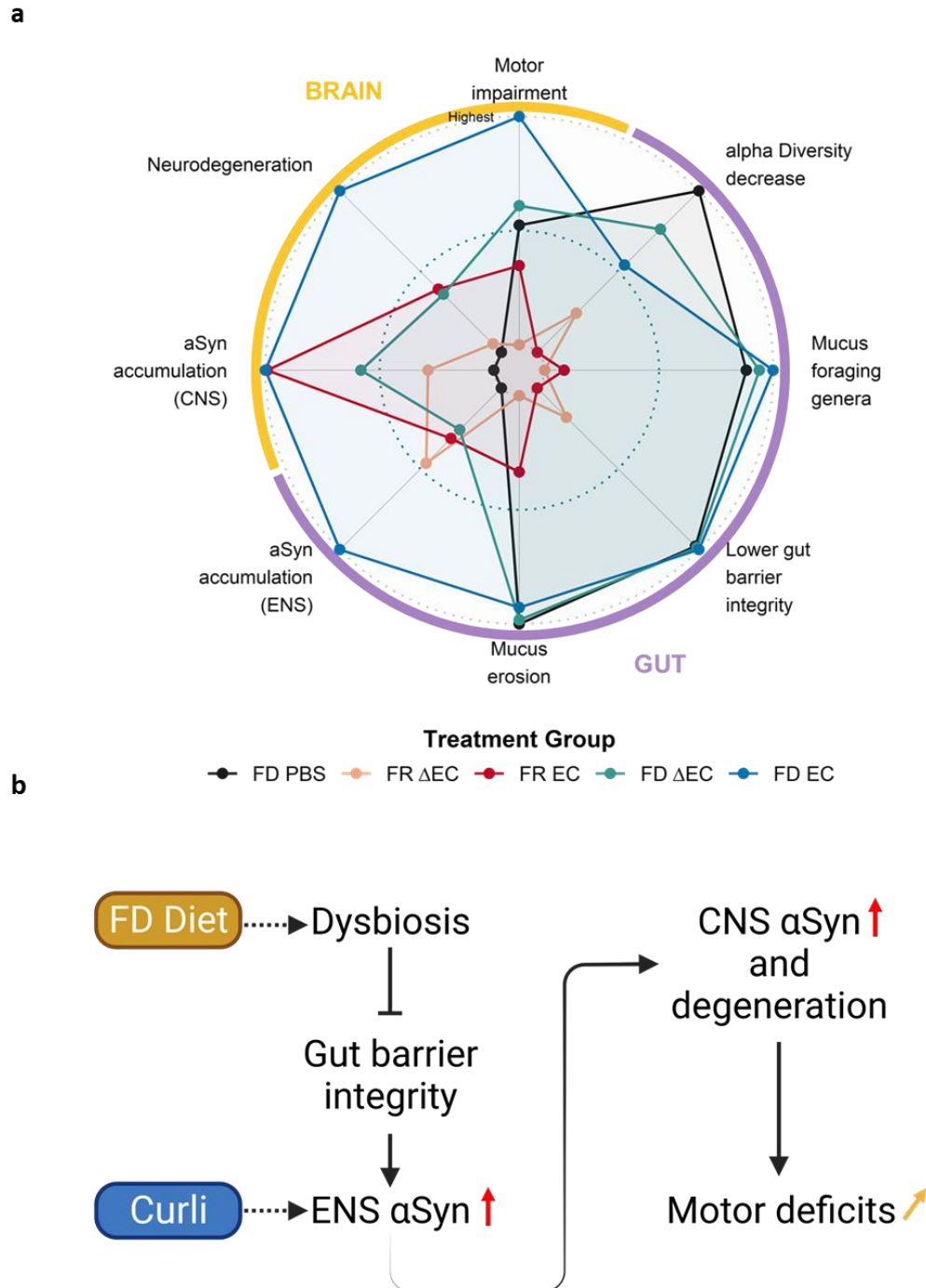


391 **Summary: The combination of fibre deprivation and bacterial curli exacerbates PD-like**  
392 **pathologies in Thy1-Syn14 mice**

393 To obtain a bird's-eye view, we generated a radar plot summarising the collection of our  
394 findings. We simplified the output by classifying the results of the different treatment groups  
395 (FD PBS, FR  $\Delta$ EC, FR EC, FD  $\Delta$ EC, FD EC) from lowest (centre of plot, **Fig. 9a**) to highest  
396 (plot outline, **Fig. 9a**). We further split our findings over two categories (brain and gut) and 8  
397 sub-categories (brain: Motor impairment, Neurodegeneration and  $\alpha$ Syn accumulation (CNS);  
398 gut: alpha diversity decrease, mucus foraging genera, lower gut barrier integrity, mucus erosion  
399 and  $\alpha$ Syn accumulation (ENS); **Fig. 9a**).

400 Under overexpressing  $\alpha$ Syn condition, we saw that the diet challenge impacted almost  
401 all aspects of the gut and curli drove  $\alpha$ Syn accumulation (**Fig. 10**). However, the combination  
402 of all challenges (TG FD EC) had the greatest effect, except for alpha diversity decrease, on all  
403 PD relevant pathologies.

404 Based on our results, we propose a sequence of events (**Fig. 9b**) where dietary fibre  
405 deprivation led to changes in microbial populations and enteric physiology. These changes  
406 cumulated in the increase of gut penetrability. The exposure to the bacterial protein curli caused  
407 increased  $\alpha$ Syn accumulations in the ENS and in the CNS accompanied by neurodegeneration  
408 in the nigrostriatal pathway. These changes finally resulted in the further exacerbation of  
409 already strong motor deficits in these TG mice.



**Fig. 9 A multi-challenged driven sequence of events for PD progression**

a) Radarplot of the total output for each challenge group in Thy1-Syn14 mice. The center of the plot defines the treatment with the lowest and the outline the one that showed the highest effect on Thy1-Syn14 mice. Overall, the combination of FD and EC has the greatest impact on  $\alpha$ Syn overexpressing mice. View main text for more details. b) Scheme of a potential sequence of events based on the results in this study. FD challenge leads to changes in the microbiome (dysbiosis), which in turn affects the gut barrier integrity and consequently facilitates the interaction of curli with the submucosa and the plexuses. As a results,  $\alpha$ Syn abnormally accumulates. This is further seen in the brain where we observed  $\alpha$ Syn accumulation, accompanied by neurodegeneration in the nigrostriatal pathway. these changes impact the locomotion and exacerbate the already strong motor deficits in Thy1-Syn14 mice.

CNS, central nervous system; ENS, enteric nervous system; FD, fibre-deprived; FR, fibre-rich (normal chow); PBS, phosphate buffered saline;  $\Delta$ EC, curli-KO E.coli; EC, wild-type curli expressing E.coli

## 411 Discussion

412 Most Parkinson's disease cases have a complex multi-factorial risk profile. There is only little  
413 known on how these varied factors come together to affect or exacerbate PD progression. In  
414 our study we investigated how genetically predisposed aged mice were affected by a fibre  
415 deprived diet and exposure to curli producing *E. coli*, individually or combined and uncovered  
416 a sequence of events that systematically led to the exacerbation of PD pathologies.

417 There is a great body of evidence on the impact of diet on microbial gut health and how  
418 it can influence the course of a disease. *In vivo* studies where rodents were fed a fibre deprived  
419 diet saw rapid shifts in microbial gut populations (Schroeder et al. 2018; Desai et al. 2016;  
420 Neumann et al. 2021; Riva et al. 2019). Accordingly our data showed decreased diversity,  
421 increased *Firmicutes/Bacteroidetes* ratios and altered abundance of many taxa. The lack of  
422 dietary fibre was shown to make specialized taxa switch to host glycans, which resulted in  
423 increased mucus erosion and increased susceptibility to pathogens (Desai et al. 2016; Martens,  
424 Chiang, and Gordon 2008). Longer absence of dietary fibre is thought to trigger a compensatory  
425 mechanism, increasing mucin production, and re-establishing the inner mucus  
426 thickness (Schroeder et al. 2018). Based on our results, however, the outer mucus layer does not  
427 recover. The thin outer mucus layer associated with reduced bacterial diversity and therefore it  
428 can be assumed that there are changes in host-microbe interactions. Recent studies showed the  
429 impact of microbial metabolite changes on gut barrier integrity. The metabolite butyrate for  
430 instance is essential in regulating energy metabolism, proliferation, and differentiation of gut  
431 epithelial cells, has anti-inflammatory properties, stimulates mucin production, and most  
432 importantly is involved in gut barrier protection by stimulating expression of ZO-1, ZO-2,  
433 cingulin and occludin (Plöger et al. 2012; Rivière et al. 2016). While we did not assess butyrate  
434 levels, we did observe reduced abundances of the butyrate producing genera *Lachnospiraceae*  
435 *NK4A136* and *Roseburia*. Additionally, *Lactobacillus*, which was also reduced in our fibre

436 deprived fed mice, has been proposed to stimulate butyrate production of such bacteria(Lin et  
437 al. 2020). There is still conflicting evidence on the effect of short-chain fatty acids, in particular  
438 butyrate, in PD. Most animal studies however report beneficial effects(Paiva et al. 2017; St.  
439 Laurent, O'Brien, and Ahmad 2013; Sharma, Taliyan, and Singh 2015). Taken together, even  
440 though we do not assess for gut barrier integrity, our observations of reduced outer mucus  
441 thickness, higher levels of particular mucus foraging taxa and reduced gut health relevant taxa  
442 under fibre deprived conditions did let us conclude that these mice were more susceptible to  
443 potential pathogenic factors such as curli.

444 Curli is a bacterial protein, mainly produced by *Enterobacteriaceae*. This bacterial  
445 family has been reported to be increased in PD patients and is associated with disease  
446 severity(Barichella et al. 2019; Li et al. 2017). The curli protein has amyloidogenic properties  
447 and has been shown to act as a seed for  $\alpha$ Syn aggregation *in vitro*(Sampson et al. 2020). In  
448 physiological conditions, either gavaged(S. G. Chen et al. 2016) or supplemented in a human  
449 faecal microbiota transplant(Sampson et al. 2020), curli presence led to different PD  
450 pathologies. Chen and colleagues observed increased  $\alpha$ Syn accumulation in the gut of exposed  
451 Fischer 344 rats(S. G. Chen et al. 2016). Interestingly, the deposits observed in the gut were  
452 soluble while they did find proteinase K resistant  $\alpha$ Syn, as one would find in Lewy bodies, in  
453 the brain(S. G. Chen et al. 2016). Analogously, we found increased levels of pS129- $\alpha$ Syn in  
454 the gut, more specifically in the myenteric plexus. The other study by Sampson and colleagues  
455 did not investigate  $\alpha$ Syn in the ENS, but they did show increased pS129- $\alpha$ Syn positive levels  
456 in the brain in transgenic mice exposed to curli producing *E. coli*. Neither these studies nor we  
457 elucidate on the spreading mechanism from the ENS to the CNS via the vagus nerve. The  
458 spreading hypothesis was postulated by Braak and colleagues after they discovered that in some  
459 patients,  $\alpha$ Syn deposits preceded CNS pathologies. The spreading process is described as  
460 “prion-like” since pathological forms of  $\alpha$ Syn act as seed for yet uncorrupted  $\alpha$ Syn (Jucker and

461 Walker 2018; Mezias et al. 2020), similar to what was proposed for curli (Chapman et al. 2002;  
462 Barnhart and Chapman 2006; Sampson et al. 2020). Based on post-mortem observations, the  
463 Braak hypothesis posits that  $\alpha$ Syn first accumulates in the lower regions of the brainstem  
464 namely the dorsal motor nucleus of the vagus. Subsequently,  $\alpha$ Syn deposits gradually move  
465 upwards in a “prion-like” manner, resulting in different PD symptoms from early non-motor to  
466 the typical motor symptoms as the disease progresses. Direct evidence for the “prion-like”  
467 spreading comes from both *in vitro* and *in vivo* studies(Rey et al. 2016; Vasili, Dominguez-  
468 Meijide, and Outeiro 2019). Animal models which were injected directly into the brain with  
469 different forms of  $\alpha$ Syn developed a variety of PD pathologies including spreading of  
470 pathological endogenous  $\alpha$ Syn(Rey et al. 2016; Luk et al. 2012; Garcia et al. 2022). To  
471 investigate whether the propagation via the vagus is a possible route, a team from Johns  
472 Hopkins University injected preformed  $\alpha$ Syn fibrils into the muscle layers of the GI tract of  
473 non-transgenic mice(Kim et al. 2019). They observed a progressive retrograde propagation  
474 originating in the dorsal motor nucleus of the vagus. After three months, the SNpc showed  
475 pS129- $\alpha$ Syn positive accumulations, followed by significant degeneration at month seven(Kim  
476 et al. 2019). After truncal vagotomy, both  $\alpha$ Syn deposits and neurodegeneration were  
477 absent(Kim et al. 2019). This is in accordance with epidemiological meta-analyses suggesting  
478 that truncal vagotomy reduces the risk to develop PD.

479 Taken together, this study supports the idea of the gut-brain-axis in PD and thus Braak’s  
480 spreading hypothesis. To our knowledge we are the first to propose a combinatorial mechanism  
481 of interdependent exogenous and endogenous factors contributing to the progression PD.  
482 Additionally, we cannot exclude that these events are also involved in the onset of PD. We  
483 further underlined the importance of a balanced healthy diet and its implications in disease  
484 progression. Hence, our results propose a translational PD-relevant sequence of events putting  
485 forth the idea for lifestyle adaptations to prevent or mitigate disease progression.

## 486 **Material and Methods**

487

### 488 **Animals and experimental design**

489 **Ethical Approval** All animal experimentations were approved by the Animal Experimentation  
490 Ethics Committee of the University of Luxembourg and the appropriate Luxembourg  
491 governmental agencies (Ministry of Health and Ministry of Agriculture) and registered under  
492 LUPA 2020/25. Additionally, all experiments were planned and executed following the 3R  
493 guidelines (<https://www.nc3rs.org.uk/the-3rs>) and the European Union directive 2010/63/EU.

494 **Mice** We used the transgenic line B6.D2-Tg(Thy1-SNCA)14Pjk(Philipp J. Kahle et al. 2000;  
495 P. J. Kahle et al. 2001), which we will refer to as Thy1-Syn14 or TG from here on forth. This  
496 line overexpresses wild-type human  $\alpha$ Syn under the transcriptional regulation of the neuron  
497 specific Thy1 promoter. As control animals we used the wild-type (WT) littermates. All mice  
498 used were male. For the characterization of the line we used four different cohorts. For the  
499 experimental challenge cohort, we used 72 male animals, 36 TG and 36 WT littermates. They  
500 were singly-caged to avoid coprophagy, had access *ad libitum* to food and water and were  
501 exposed to a regular 12h-day-night cycle. Animals were monitored twice a week. According to  
502 our welfare guidelines, we set a humane endpoint based on different physical parameters, e.g.  
503 weight loss/gain, body temperature and coat condition. At the end of the in-life phase, we  
504 anesthetized the mice with a mix of 150mg/kg ketamine + 1mg/kg medetomidine, collected  
505 blood from the right atrium and subsequently transcardially flush-perfused with 1X PBS.

506 During the in-life phase of the study, 10 mice were either found dead in their home cage  
507 or reached a humane endpoint (**Supplementary Fig. 1a**). This measure agrees with the animal  
508 welfare guidelines.

509 **Experimental Design** For the challenge study, mice were randomly assigned to 10 different  
510 treatment and respective control groups (**Supplementary Fig. 1a**) and treated for a total of 9

511 weeks; 1 week dietary priming of the colon and additional 8 weeks combined diet and bacterial  
512 challenges (**Supplementary Fig. 1b**). We provided fresh diet and gavaged the animals with the  
513 respective bacteria or sham solution weekly. Body weight and overall health was checked twice  
514 a week. We collected stool samples for microbiome analysis and monitored basis gross motor  
515 functions by assessing hind limb clasping and grip strength weekly (**Supplementary Fig. 1b**).  
516 After euthanasia we collected brains and colons for molecular biology and histology.

517

### 518 **Bacterial solution preparation and gavage**

519 The *E. coli* strains used for treatment were the C600 (EC) and its isogenic curli-operon knock-  
520 out ( $\Delta$ EC) strains(Chapman et al. 2002; S. G. Chen et al. 2016). Both strains were a kind gift  
521 from Matthew Chapman, University of Michigan. Expression/absence of curli operon was  
522 tested via PCR using the following primer pairs: *csgA*\_F-5'-  
523 GCGTGACACAACGTTAATTTC CA-3', *csgA*\_R-5'-  
524 CATATTCTTCTCCCGAAAAAAACAG-3'; *csgB*\_F-5'-  
525 CCATCGGATTGATTTAAAAGTCGAAT-3', *csgB*\_R-5'-  
526 AATTTCTTAAATGTACGACCAGGTCC-3'. Additionally, curli protein expression was  
527 confirmed by Congo red staining (not shown). Both strains were grown in Lennox broth under  
528 aerobic (5% CO<sub>2</sub>) conditions at 37°C agitating at 300rpm. Bacteria were resuspended in sterile  
529 PBS for oral administration at 10<sup>10</sup>CFUs/mL.

530 They were gavaged 100 $\mu$ L of bacterial solution, or PBS for the gavage control groups,  
531 at a total bacterial load of 10<sup>9</sup>CFUs. Reusable stainless steel 20G feeding needle (Fine Science  
532 Tools, 18060-20) were used. Prior and in-between gavages, the feeding needle was washed  
533 with filtered 70% ethanol and rinsed with sterile PBS. One group of feeding needles per  
534 treatment were used to avoid cross-contamination. Importantly, we did not pre-treat our mice  
535 with an antibiotic mix because 1) antibiotics have been shown to prevent  $\alpha$ Syn aggregation and



536 to be neuroprotective(Yadav et al., n.d.), and 2) we were interested in the impact of the fibre  
537 deprivation on a native microbiome.

538

### 539 **Tissue collection and preparation**

540 Prior to the transcardial perfusion, we collected up to 400 $\mu$ L of venous blood from the right  
541 atrium in EDTA K3 coated collection tubes (41.1504.005, Sarstedt), for blood-endotoxin  
542 measurements. The tubes were gently inverted and then kept on ice. The plasma was collected  
543 after centrifugation at 2000 x g for 10 mins, transferred to RNase-free tubes and stored at -  
544 80°C.

545 After perfusion, the brain was placed on ice and split along the longitudinal fissure into  
546 two hemibrains. For molecular biology analyses, one hemibrain was dissected into different  
547 regions of interest (striatum and ventral midbrain). The dissected regions were then put on dry  
548 ice and further stored at -80°C. The other hemibrains were fixed for immunohistochemistry in  
549 4% PBS-buffered paraformaldehyde (PFA) for 48h at 4°C and then stored in PBS-azide  
550 (0.02%) at 4°C. Subsequently, they were cut to generate 50 $\mu$ m thick free-floating sections using  
551 the Leica vibratome VT1000 (Wetzlar, Germany) and stored the sections in a 1% (w/v) PVPP  
552 + 1:1 (v/v) PBS/ethylene glycol anti-freeze mix at -20°C until staining.

553 Colon samples for mucus measurements and histopathology were fixed in methacarn  
554 (60% absolute methanol: 30% chloroform: 10% glacial acetic acid) solution for 2-4 hours, then  
555 transferred to 90% ethanol and kept at 4°C. Next, whole colon samples were first transversally  
556 cut by hand with a microtome blade into 4-5mm long sections. Those pre-cut sections were  
557 then put into a histology cassette while respecting the proximal to distal order. They were held  
558 in place in an ethanol soaked perforated sponge. After 24h post-fixation in 10% formalin, the  
559 samples were processed in a vacuum infiltration processor. Finally, all samples were embedded

560 in paraffin and cut at 3 $\mu$ m on a microtome. If not processed immediately, the slides were stored  
561 at 4°C before the stainings.

562

### 563 **RNA extraction and RT-qPCR**

564 RNA from dissected colon and different brain regions was extracted using the Qiagen RNeasy  
565 Plus Universal Mini Kit (Qiagen, 73404). Briefly, 900 $\mu$ L QIAzol lysis buffer (Qiagen, 79306)  
566 and three cold 5mm steel balls were added to each sample (previously stored at -80°C). In ice  
567 cooled racks, samples were homogenized at 20Hz for 2mins using the Retsch Mixer Mill  
568 MM400. Homogenates were transferred to new RNase clean 2mL tubes and left to rest for  
569 5mins at room temperature (RT). 100 $\mu$ L gDNA eliminator solution was added and the tubes  
570 were shaken vigorously for 15 seconds. Then 180 $\mu$ L of chloroform was added and another  
571 strong shake was applied for 15 seconds. Homogenates were left to incubate for 3mins at RT.  
572 Samples were then centrifuged at 12000 x g for 15 mins at 4°C. Five hundred  $\mu$ L of the upper  
573 aqueous phase was collected and transferred to new 2mL RNase free tubes. Five hundred  $\mu$ L  
574 of ethanol was added to the supernatant and mixed by inverting the tubes back and forth.  
575 RNeasy mini spin columns were then loaded with 500 $\mu$ L of the mix, centrifuged at 8000 x g  
576 for 30s at RT, followed by discarding the flow-through from the collection tube. This step was  
577 repeated once more. The spin columns were then washed with two different buffers in three  
578 steps: one time with 700 $\mu$ L of RWT buffer and twice with 500 $\mu$ L of RPE buffer. At each  
579 washing step the columns were centrifuged at 8000 x g for 30s at RT and the flow-through was  
580 discarded. Columns are then transferred to new collection tubes and spun at maximum speed  
581 for 1min. Finally, columns were transferred to an RNase free 1.5mL Eppendorf tube. Fifty  $\mu$ L  
582 of RNase-free water was added to the columns to elute total RNA. RNA purity and quantity  
583 were checked by spectrophotometry using the NanoDrop™ 2000 (ThermoFisher Scientific)  
584 and the Agilent 2100 Bioanalyzer, respectively. Finally, RNA samples were stored at -80°C.

585           The model used in this study, Thy1-Syn14, carries a transgene for wild-type human  
586  $\alpha$ Syn (*SNCA*). To determine the levels of transcript expression in comparison to endogenous  
587 murine  $\alpha$ Syn (*Snca*), quantitative RT-PCR was performed on a separate untreated age-matched  
588 male cohort (N=15), using the following primer pairs: *Snca* F 5'-GAT-CCT-GGC-AGT-GAG-  
589 GCT-TA-3', R 5'-CT-TCA-GGC-TCA-TAG-TCT-TGG-3', *SNCA* F 5'-AAG-AGG-GTG-  
590 TTC-TCT-ATG-TAG-GC-3', R 5'-GCT-CCT-CCA-ACA-TTT-GTC-ACT-T-3' and  
591 reference gene *Gapdh* F 5'-TGC-GAC-TTC-AAC-AGC-AAC-TC-3', R 5'-CTT-GCT-CAG-  
592 TGT-CCT-TGC-TG-3'. For the reverse transcription of RNA to cDNA we used the  
593 SuperScript™ III RT reverse transcriptase from Invitroge. Briefly, 1 $\mu$ L of oligo (dT) 20 (50  
594  $\mu$ M) and 1 $\mu$ L of 10mM dNTP mix was added to 1 $\mu$ g of total RNA. If needed, nuclease free  
595 water was added to obtain the final reaction volume of 13 $\mu$ L. The mixture was briefly  
596 centrifuged for 2-3s, heated at 65°C for 5 minutes, and again chilled on ice for at least 1 minute.  
597 Another mixture of 4 $\mu$ L 5 $\times$  first strand buffer, 1 $\mu$ L RNaseOUT (RNase inhibitor), 1 $\mu$ L of 0.1M  
598 DTT and 1 $\mu$ L of Superscript reverse transcriptase (200 U/ $\mu$ l) was added. The final mixture was  
599 briefly centrifuged and incubated at 50°C for 1h followed by 15mins at 70°C for enzyme  
600 deactivation. 80 $\mu$ L of RNase free water was added to the reaction mixture. The obtained cDNA  
601 was then placed on ice for immediate use or stored at -20°C for future use.

602           The qPCR reaction mix contained 2 $\mu$ L of cDNA, 10 $\mu$ M forward and reverse primers,  
603 1X iQ™ SYBR® Green Supermix (Bio-Rad) and PCR grade water up to a volume of 20 $\mu$ L.  
604 Each qPCR reaction was run in duplicates on a LightCycler® 480 II (Roche). The thermo  
605 cycling profile included an initial denaturation of 3 minutes at 95°C, followed by 40 Cycles at  
606 95°C for 30 seconds, 62°C (annealing) for 30 seconds and 72°C (elongation) for 30 seconds,  
607 with fluorescent data collection during the annealing step. Data acquisition was performed by  
608 LightCycler® 480 Software (version 1.5.0.39).

609

## 610 **Microbial DNA extraction and 16S rRNA Amplicon Sequencing**

611 For microbial DNA extraction from single faecal pellets we used an adapted version of the  
612 IHMS protocol H(Dore et al. 2015). Faecal samples were preserved in 200 $\mu$ L of a glycerol  
613 (20%) + PBS solution and stored at -80°C. Prior to the extraction, we slightly thawed the  
614 samples and added 250 $\mu$ L guanidine thiocyanate and 40 $\mu$ L N-lauryl sarcosine (10%). The  
615 samples were then left at RT to fully thaw. We then added 500 $\mu$ L N-lauryl sarcosine (5%)  
616 before the faecal pellet was scattered and vortexed to homogeneity. Samples were then shortly  
617 spun down and incubated at 70°C for 1h. Seven hundred fifty  $\mu$ L pasteurized zirconium beads  
618 were added to the tubes, then put in pre-cooled racks and horizontally shaken for 7.5mins at  
619 25Hz in a Retsch mixer mill MM400. Fifteen mg polyvinylpyrrolidone (PVPP) was added and  
620 vortexed until dissolved. Then the samples were centrifuged at 20814 x g for 3mins. The  
621 supernatants were transferred to new 2mL tubes and kept on ice. We then washed the pellet  
622 with 500 $\mu$ L TENP (Tris, EDTA, NaCl and PVPP) and centrifuged at 20814 x g for 3mins. This  
623 step was repeated three times in total and each supernatant was added to the previously new  
624 2mL tube. To minimize carryover, the tubes were centrifuged again at 20814 x g for 5mins and  
625 the supernatant was split equally in two new 2mL tubes. We then added 1mL isopropanol  
626 (Merck) to each tube and mixed them by inverting the tubes. After a 10min incubation at RT,  
627 the samples were centrifuged at 20814 x g for 15mins. The supernatant was discarded and the  
628 remaining pellet air dried under the fume hood for 10mins. The pellet was then resuspended in  
629 450 $\mu$ L phosphate buffer and 50 $\mu$ L potassium acetate by pipetting up and down, before the  
630 duplicates were pooled and incubated on ice for 90mins. Then the sample was centrifuged  
631 (20,814 x g) at 4 °C for 35mins, the supernatant transferred into a new tube. Next, 2 $\mu$ L of RNase  
632 (10 mg/ml) were added. Then, the tube was vortexed, briefly centrifuged and finally incubated  
633 at 37 °C for 30mins. We then added 50 $\mu$ L of sodium acetate, 1mL of ice cold 100% ethanol  
634 (Merck) and mixed the tube by inverting several times. The sample was again incubated at RT

635 for 5mins and centrifuged at 20814 x g for 7.5 min. The supernatant was discarded and the  
636 newly formed pellet was subsequently washed three times in total with 70% ethanol (Merck)  
637 and centrifuged at 20814 x g for 5mins. The supernatant was discarded each time. Finally, the  
638 clean pellet was dried at 37 °C for 15 min, then resuspended in 100µl TE Buffer and  
639 homogenized by pipetting. After incubation at 4°C over night, DNA quality and quantity were  
640 checked by Nanodrop™ 2000/2000c and Qubit 2.0 fluorometer (Thermo Fischer Scientific).  
641 Samples were stored at -80°C until sequencing.

642 Five ng of isolated gDNA were used for PCR amplification using primer (515F  
643 (GTGBCAGCMGCCGCGGTAA) and 805R (GACTACHVGGGTATCTAATCC)) specific  
644 to V4 region of 16S rRNA gene. For the first round of PCR, samples were amplified for 15  
645 cycles to avoid over-amplification. Additional 6 PCR amplification cycles were performed in  
646 the second round to introduce sample specific barcode information. All samples were pooled  
647 in equimolar concentration for sequencing. Sample preparation and sequencing were performed  
648 at LCSB Sequencing platform using v3 2x300 nucleotide paired end sequencing kit for MiSeq.

649

#### 650 **16S rRNA gene amplicon sequence analysis**

651 *Sequence analysis* Amplicon Sequence Variants (ASVs) were inferred from 16S rRNA gene  
652 amplicon reads using the dada2 package(Callahan et al. 2016) following the paired-end big data  
653 workflow ([https://benjjneb.github.io/dada2/bigdata\\_paired.html](https://benjjneb.github.io/dada2/bigdata_paired.html), accessed: September, 2020),  
654 with the following parameters: truncLen = 280 for forward, 250 for reverse reads, maxEE = 3,  
655 truncQ = 7, and trimLeft = 23 for forward, 21 for reverse reads. The reference used for  
656 taxonomic assignment was version 138 of the SILVA database ([https://www.arb-](https://www.arb-silva.de)  
657 [silva.de](https://www.arb-silva.de))(Quast et al. 2013).

658 *Microbial diversity and related statistics* Microbiome count data was managed using the  
659 phyloseq R package(McMurdie and Holmes 2013) this package was also used to calculate the

660 Shannon index for alpha diversity and non-metric multidimensional scaling (NMDS)  
661 ordination for beta diversity. Statistical significance of alpha diversity differences was  
662 evaluated with the Kruskal-Wallis test (overall comparison between all groups) and the  
663 Wilcoxon Rank Sum Test with false discovery rate correction for multiple comparisons  
664 (pairwise contrasts). For beta diversity comparisons, we used the adonis PERMANOVA test  
665 from the R package vegan (Oksanen et al. 2020). All diversity comparisons were performed  
666 using ASV count data rarefied to the lowest number of sequences in a sample. Taxon-specific  
667 plots (genus and family level) were made using relative abundances (% of taxa out of total).

668

#### 669 **Endotoxin plasma level measurement by ELISA**

670 Plasma samples were diluted 1:10 in 1X PBS. To measure the endotoxin plasma we used the  
671 EndoLISA® kit from BioVendor. We followed the supplier's protocol. Briefly, a serial dilution  
672 for the standard for the non-linear regression model was prepared. In duplicates, 100µL of well  
673 mixed standard and samples were applied on the supplied 96-well plate. Then, 20µL of 6X  
674 binding buffer was added to each well and the plate was sealed with a cover foil. The plate was  
675 incubated at 37°C for 90 mins on a shaker at 450rpm. The reaction buffer was removed by  
676 quickly inverting the plate. The excess buffer was removed as best as possible by tapping the  
677 plate gently on a paper towel. The wells were then washed twice with 150µL of wash buffer.  
678 Again, the plate was inverted quickly and the excess buffer was removed by tapping the plate  
679 gently on a paper towel. Finally, we added 120µL of Detection buffer and start measurements  
680 in a 37°C pre-heated plate reader. We measured from T=0min to T=90mins at 15mins intervals.

681

#### 682 **Alcian blue staining and outer mucus thickness measurements**

683 The Alcian blue stainings were performed at the National Center of Pathology (NCP) of the  
684 Laboratoire National de Santé in Dudelange (Luxembourg). The sections were stained for

685 Alcian blue (Artisan Link Pro Special Staining System, Dako, Glostrup, Denmark) according  
686 to manufacturer's instructions.

687 We took 5-10 images per section at 20X. This resulted in up to 24 images per animal.  
688 The criteria for the correct images were that the sections were cut at the correct plane level.  
689 This was determined by the orientation and definition of the crypts, which had to fully visible  
690 pointing towards the colonic lumen. We measured only outer mucus areas which could clearly  
691 be distinguished from the inner mucus layer and the colonic content. After the images were  
692 scaled in image using the imprinted scale bar as reference, an average of 6 measure points,  
693 spanning the outer mucus layer, per image were taken using Image J.

694

#### 695 **Immunofluorescent staining of colon sections**

696 We followed a standard protocol with minor adjustments('Immunofluorescent Staining of  
697 Paraffin-Embedded Tissue' n.d.). Briefly, sections were deparaffinised in xylene 3 x 5mins.  
698 Before proceeding to rehydration, we checked that all paraffin was removed. If not, the sections  
699 were treated another round with xylene. A three step rehydration step with 100%, 70% and  
700 50% ethanol followed deparaffinization. After washing with dH<sub>2</sub>O, we proceeded with a citrate  
701 buffer (0.1M, pH6.0, + 0.1% Tween 20) based antigen retrieval at 80°C for 30-35mins. After  
702 letting the sections cool down for 20mins, the slides were again washed with dH<sub>2</sub>O 2 x 5mins.  
703 We proceeded as described previously('Immunofluorescent Staining of Paraffin-Embedded  
704 Tissue' n.d.) for peroxidase inactivation, permeabilization and blocking. After washing again  
705 with dH<sub>2</sub>O, the tissue was circled with a hydrophobic Dako pen (S2002, DAKO) and the  
706 primary antibodies were added. They were incubated at room temperature (RT) for 2 hours  
707 (hrs) and then transferred to 4°C for overnight incubation in a humidified chamber. The  
708 following day, slides were washed briefly with dH<sub>2</sub>O and then washed with 1% BSA + PBS  
709 0.4% Triton X100 2 x 5mins and finally rinsed with dH<sub>2</sub>O. Tissues were circled again with the



710 hydrophobic pen and secondary antibodies were added. Slides were then incubated for 2 hrs at  
711 RT in the humidified chamber. Finally, they were washed 3 x 5mins with PBS 0.4% Triton  
712 X100 and rinsed with dH<sub>2</sub>O. Excess water was removed by gentle tapping and slides were  
713 coverslipped with DAPI Fluoromount-G® (0100-20, SouthernBiotech).

714 To detect phosphorylated  $\alpha$ Syn in the ENS we performed double staining using the  
715 following antibodies: polyclonal chicken anti-PGP9.5 (ab72910, Abcam; 1:1000), monoclonal  
716 rabbit anti-pS129- $\alpha$ Syn (ab51253, Abcam; 1:500).

717

## 718 **Behaviour**

719 ***Hindlimb Clasp*** The method was adapted from Bouet et al.(Guyenet et al. 2010). Brief,  
720 animals were taken by the tail near the base and suspended for 10 seconds. If both hindlimbs  
721 stayed stretched and did not touch the abdomen for more than 50% of the suspension time we  
722 scored it 0. A score of 1 or 2 was given if one respectively both hindlimbs were retracted for  
723 more than 50% of the suspension time. If they were retracted and touched the abdomen for the  
724 entire suspension time a score of 3 was given. In the most severe cases, the animals twisted  
725 around the vertical body axis or even rolled up to a so-called bat position. These cases were  
726 given a score of 4. This test was repeated weekly.

727 ***Grip Strength*** The grip strength test(Mao et al. 2016) was performed using Bioseb's grip  
728 strength meter (Vitrolles, France). Animals were gently placed on a grid, allowed to grab onto  
729 it with all four paws and then gently pulled off in a continuous backwards motion by their tail.  
730 Technical triplicates were taken for each mouse. Values were normalized to the weight of the  
731 respective mouse. The test was repeated weekly.

732 ***Adhesive Removal*** The test was adapted from Bouet and colleagues (Bouet et al. 2009). Brief,  
733 animals were placed in a round transparent arena for one minute as habituation. A piece of  
734 rectangular tape (3x5mm) was placed on each forepaw. The time was taken once the animals

735 touched the bottom of the arena. We measured the time of first touch and first removal. The  
736 test was performed in duplicates, and performed at baseline and at the end of the experimental  
737 phase.

738

### 739 **Immunofluorescent staining on free-floating brain sections**

740 Immunofluorescent stainings on free-floating sections were performed following a standard  
741 protocol(Ashrafi et al. 2017) with minor adaptations. Briefly, sections were washed in PBS +  
742 0.1% Triton X100 ( $T_{X100}$ ) to rinse off the anti-freeze solution. Then, they were treated with a  
743 permeabilization/peroxidase inactivation solution (PBS + 1.5%  $T_{X100}$  + 3%  $H_2O_2$ ) for 30mins  
744 followed by 2x5mins washing. To prevent unspecific antibody binding, the sections were  
745 incubated in 5% BSA + 0.02%  $T_{X100}$  for 1 hour. After a short washing step, sections were  
746 incubated with primary antibody(ies) diluted in antibody solution (PBS + 2% BSA) over night  
747 at room temperature (RT) on an orbital shaker. The next day, sections were washed with PBS  
748 + 0.1% TX100 to remove all excess first antibody. Sections were then incubated with secondary  
749 antibody (+ antibody solution) for 2 hours at RT on an orbital shaker under a light trap. Finally,  
750 sections were washed with simple PBS (at least three times for 10mins) and then mounted on  
751 Superfrost™ (ThermoFisher Scientific) slides, let to dry for up to 12h, and cover-slipped using  
752 the Fluoromount-G® (Invitrogen) mounting solution.

753 The following antibodies were used: monoclonal rabbit anti-pS129- $\alpha$ Syn (Abcam,  
754 ab51253; 1:1000), monoclonal mouse anti-pS129- $\alpha$ Syn (Prothena Biosciences Inc., 11A5;  
755 1:1000), polyclonal chicken anti-tyrosine hydroxylase (Abcam, ab76442; 1:1000), polyclonal  
756 rabbit anti-tyrosine hydroxylase (Merck (Sigma-Aldrich), AB152; 1:1000), polyclonal rat anti-  
757 dopamine transporter (MAB369, Merck (Sigma-Aldrich); 1:1000).

758

### 759 **Quantitative neuropathology**

760 We performed the imaging our sections using a Zeiss AxioImager Z1 upright microscope,  
761 equipped with a PRIOR motorized slide stage and coupled a “Colibri” LED system to generate  
762 fluorescence light of defined wavelengths, and a Zeiss Mrm3digital camera for image capture.  
763 The complete imaging system was controlled by Zeiss’ Blue Vision software. All histological  
764 analyses were performed blinded.

765 The quantification of TH-positive fibres and DAT-positive synaptic terminals was done  
766 as described before in Garcia *et al.*, 2022(Garcia et al. 2022). Briefly, two doubly labelled  
767 (rabbit anti-TH and rat anti-DAT) sections were used. A total of 6 (3/section) 40x  
768 ( $223.8 \times 167.7 \mu\text{m}^2$ ) pictures of the dorsal striatum were acquired using the optical sectioning  
769 system Apotome.2 (Zeiss). The percent area occupied of TH and DAT by intensity thresholding  
770 was determined using Image J software and averaged for each mouse.

771 The quantification of TH-positive neurons in the SNpc has been described and the  
772 obtained results have been correlated with stereological cell counts (see supplementary  
773 information in Ashrafi et al., 2017(Ashrafi et al. 2017)). Briefly, to estimate TH-positive  
774 neurons in the SNpc, anatomically distinguishable levels were identified and applied to 7-12  
775 fifty-micron sections/mouse. Then, 2x2 tiled pictures/section were taken at 10X objective and  
776 converted into single Tiff files for image analysis. Next, the region-of-interest (ROI) of the area  
777 occupied only by TH-positive neurons was outlined. After thresholding, the ROI occupied (in  
778 pixels) by TH-positive neurons was measured. For each anatomical levels of the SN, up to 2  
779 sections/level were measured. Single and/or averaged values/level were finally summed up to  
780 one single representative value, the “cumulative SN surface” and converted to  $\text{mm}^2$ .

781 To quantify pS129- $\alpha$ Syn in the dorsal striatum (Double label: area reference marker  
782 polyclonal rabbit anti-TH; pS129- $\alpha$ Syn marker monoclonal mouse 11A5), 20X tile images  
783 were converted to 8-bit, the ROI was determined and the threshold was automatically set to  
784 “MaxEntropy”. Next, the images were appropriately scaled from pixel to  $\mu\text{m}$ . This allowed us

785 to adjust our settings to exclude all particles surpassing the size of a synapse. These settings in  
786 “Analyze Particles” were *Size ( $\mu\text{m}^2$ ): 30.00-Infinity; Circularity: 0.25-1*. Each particle created  
787 an enumerated ROI and was added to the ROI Manager. All images were then manually curated  
788 to delete falsely selected or to add missed particles. Subsequently, the full picture frame and all  
789 ROIs to be excluded from quantification were selected and combined by “XOR”. Finally, the  
790 percentage area occupied and intensity after thresholding for pS129- $\alpha$ Syn positive synaptic  
791 areas were measured.

792 To estimate pS129- $\alpha$ Syn positive accumulations in the SNpc (monoclonal rabbit anti-  
793 pS129- $\alpha$ Syn), the same ROIs as chosen for TH quantification were used. In Image J, a virtual  
794 grid with a square area of  $2500\mu\text{m}^2$  was overlaid. Pictures were then manually analysed for  
795 pS129- $\alpha$ Syn positive accumulations. We counted separately, based on morphology, pS129-  
796  $\alpha$ Syn positive cell bodies (1 count = 1 cell body) and other pS129- $\alpha$ Syn positive particles  
797 (number of particles per square). Finally, counts were normalized per square (area of ROI/area  
798 of square) and summed up for all 4 zones (see TH quantification). Phosphorylated- $\alpha$ Syn  
799 intensities in the SNpc were not measured.

800

## 801 **Statistics**

802 For the characterisation of the Thy1-Syn14 to test for differences in gene expression levels,  
803 protein levels and motor behaviour performance we used the Kruskal-Wallis test and corrected  
804 for FDR. If needed we adjusted for multiple comparison using the Mann-Whitney U test and  
805 corrected for FDR. For the statistics of the 16S amplicon rRNA sequencing data see above. For  
806 all other measurements of the challenged mouse cohort, we performed the non-parametric  
807 Kruskal-Wallis test and post-hoc Mann-Whitney U test to adjust for multiple comparison. We  
808 additionally corrected for FDR. For all other experiments we used the Mann-Whitney U test to  
809 adjust for multiple comparison. If corrected or not for FDR is specified in the figure legends.

## 810 **Data Availability**

811 All original datasets are available upon reasonable request to the corresponding author  
812 ([kristopher.schmit@uni.lu](mailto:kristopher.schmit@uni.lu)).

813

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820 11A5 antibody and Matt Chapman (University of Michigan) for providing the two *E. coli*  
821 strains used in this study. The authors would like to thank the Animal Facility of the University  
822 of Luxembourg for their support throughout the animal experiments.

823

## 824 **Abbreviations**

825 PD, Parkinson's disease;  $\alpha$ Syn, alpha-synuclein; pS129- $\alpha$ Syn, phosphorylated S129 alpha-  
826 synuclein; TG, Thy1-Syn14 transgenic mice; WT, wild-type littermates; FR, fibre-rich or  
827 normal chow; FD, fibre-deprived diet; EC, wild-type *E. coli* expressing curli protein;  $\Delta$ EC,  
828 curli-operon KO *E. coli* strain

829

830

831

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833

834

835 **Additional information**

836 **Ethics approval**

837 Animal studies performed at the Luxembourg Centre for Systems Biomedicine were approved  
838 by the institutional Animal Experimentation Ethics Committee of the University of  
839 Luxembourg, and the responsible Luxembourg government authorities (Ministry of Health,  
840 Ministry of Agriculture), following EU directive 2010/63/EU.

841

842 **Consent for publication**

843 All authors have approved of the contents of this manuscript and provided consent for  
844 publication.

845

846 **Availability of materials**

847 The 11A5 monoclonal anti  $\alpha$ -synuclein antibody can be obtained, under an MTA, from  
848 Prothena Biosciences.

849

850 **Authors contributions**

851 K.J.S., M.B., E.C.M. and P.W. designed the study. K.J.S., A.S., B.P.R., P.G., M.H.T., J.J.G.,  
852 I.B.A., C.C., and T.H did the experiments (gavages, behavioural tests, tissue processing,  
853 stainings, imaging, DNA, RNA and protein extractions, Western blots). R.H. performed the  
854 16S rRNA amplicon sequencing. V.T.E.A. analysed the 16S rRNA amplicon sequencing data.  
855 K.J.S., A.S., V.T.E.A., P.G., I.O., M.M., M.B., and P.W. analyzed and interpreted the data.  
856 K.J.S. drafted the paper. All authors read and approved the final manuscript.

857



## 858 **References**

- 859 Ashrafi, Amer, Pierre Garcia, Heike Kollmus, Klaus Schughart, Antonio Del Sol, Manuel  
860 Buttini, and Enrico Glaab. 2017. ‘Absence of Regulator of G-Protein Signaling 4 Does  
861 Not Protect against Dopamine Neuron Dysfunction and Injury in the Mouse 6-  
862 Hydroxydopamine Lesion Model of Parkinson’s Disease’. *Neurobiology of Aging* 58  
863 (October): 30–33. <https://doi.org/10.1016/j.neurobiolaging.2017.06.008>.
- 864 Barber Janer, Anna, Eline Vonck, and Veerle Baekelandt. 2021. ‘Chapter Two - Modeling  
865 Synucleinopathies in Rodents’. In *International Review of Movement Disorders*, edited  
866 by Benjamin Dehay and Erwan Bezard, 2:65–154. Mechanisms of Cell Death and  
867 Approaches to Neuroprotection/Disease Modification in Parkinson’s Disease.  
868 Academic Press. <https://doi.org/10.1016/bs.irmvd.2021.09.001>.
- 869 Barichella, Michela, Marco Severgnini, Roberto Cilia, Erica Cassani, Carlotta Bolliri, Serena  
870 Caronni, Valentina Ferri, et al. 2019. ‘Unraveling Gut Microbiota in Parkinson’s  
871 Disease and Atypical Parkinsonism’. *Movement Disorders* 34 (3): 396–405.  
872 <https://doi.org/10.1002/mds.27581>.
- 873 Barnhart, Michelle M., and Matthew R. Chapman. 2006. ‘Curli Biogenesis and Function’.  
874 *Annual Review of Microbiology* 60: 131–47.  
875 <https://doi.org/10.1146/annurev.micro.60.080805.142106>.
- 876 Bernardo-Cravo, Adriana P., Dirk S. Schmeller, Antonis Chatzinotas, Vance T. Vredenburg,  
877 and Adeline Loyau. 2020. ‘Environmental Factors and Host Microbiomes Shape Host–  
878 Pathogen Dynamics’. *Trends in Parasitology* 36 (7): 616–33.  
879 <https://doi.org/10.1016/j.pt.2020.04.010>.
- 880 Blackwood, Brian P, Carrie Y Yuan, Douglas R Wood, Joseph D Nicolas, Justyna S Grothaus,  
881 and Catherine J Hunter. 2017. ‘Probiotic Lactobacillus Species Strengthen Intestinal  
882 Barrier Function and Tight Junction Integrity in Experimental Necrotizing  
883 Enterocolitis’. *Journal of Probiotics & Health* 5 (1): 159. [https://doi.org/10.4172/2329-  
884 8901.1000159](https://doi.org/10.4172/2329-8901.1000159).
- 885 Boertien, Jeffrey M., Pedro A. B. Pereira, Velma T. E. Aho, and Filip Scheperjans. 2019.  
886 ‘Increasing Comparability and Utility of Gut Microbiome Studies in Parkinson’s  
887 Disease: A Systematic Review’. *Journal of Parkinson’s Disease* 9 (s2): S297–312.  
888 <https://doi.org/10.3233/JPD-191711>.
- 889 Bouet, Valentine, Michel Boulouard, Jérôme Toutain, Didier Divoux, Myriam Bernaudin,  
890 Pascale Schumann-Bard, and Thomas Freret. 2009. ‘The Adhesive Removal Test: A  
891 Sensitive Method to Assess Sensorimotor Deficits in Mice’. *Nature Protocols* 4 (10):  
892 1560–64. <https://doi.org/10.1038/nprot.2009.125>.
- 893 Braak, Heiko, Kelly Del Tredici, Udo Rüb, Rob A. I de Vos, Ernst N. H Jansen Steur, and Eva  
894 Braak. 2003. ‘Staging of Brain Pathology Related to Sporadic Parkinson’s Disease’.  
895 *Neurobiology of Aging* 24 (2): 197–211. [https://doi.org/10.1016/S0197-  
896 4580\(02\)00065-9](https://doi.org/10.1016/S0197-4580(02)00065-9).
- 897 Braak, Heiko, Rob A. I. de Vos, Jürgen Bohl, and Kelly Del Tredici. 2006. ‘Gastric Alpha-  
898 Synuclein Immunoreactive Inclusions in Meissner’s and Auerbach’s Plexuses in Cases

- 899 Staged for Parkinson's Disease-Related Brain Pathology'. *Neuroscience Letters* 396  
900 (1): 67–72. <https://doi.org/10.1016/j.neulet.2005.11.012>.
- 901 Callahan, Benjamin J., Paul J. McMurdie, Michael J. Rosen, Andrew W. Han, Amy Jo A.  
902 Johnson, and Susan P. Holmes. 2016. 'DADA2: High-Resolution Sample Inference  
903 from Illumina Amplicon Data'. *Nature Methods* 13 (7): 581–83.  
904 <https://doi.org/10.1038/nmeth.3869>.
- 905 Chapman, Matthew R., Lloyd S. Robinson, Jerome S. Pinkner, Robyn Roth, John Heuser,  
906 Marten Hammar, Staffan Normark, and Scott J. Hultgren. 2002. 'Role of Escherichia  
907 Coli Curli Operons in Directing Amyloid Fiber Formation'. *Science (New York, N.Y.)*  
908 295 (5556): 851–55. <https://doi.org/10.1126/science.1067484>.
- 909 Chen, Honglei, and Beate Ritz. n.d. 'The Search for Environmental Causes of Parkinson's  
910 Disease: Moving Forward'. *Journal of Parkinson's Disease* 8 (Suppl 1): S9–17.  
911 <https://doi.org/10.3233/JPD-181493>.
- 912 Chen, Shu G., Vilius Stribinskis, Madhavi J. Rane, Donald R. Demuth, Evelyne Gozal, Andrew  
913 M. Roberts, Rekha Jagadapillai, et al. 2016. 'Exposure to the Functional Bacterial  
914 Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344  
915 Rats and Caenorhabditis Elegans'. *Scientific Reports* 6 (1): 1–10.  
916 <https://doi.org/10.1038/srep34477>.
- 917 Council (US), National Research, Institute of Medicine (US), Steven H. Woolf, and Laudan  
918 Aron. 2013. *Physical and Social Environmental Factors. U.S. Health in International  
919 Perspective: Shorter Lives, Poorer Health*. National Academies Press (US).  
920 <https://www.ncbi.nlm.nih.gov/books/NBK154491/>.
- 921 De Vos, and Willem M. 2015. 'Microbial Biofilms and the Human Intestinal Microbiome'. *Npj*  
922 *Biofilms and Microbiomes* 1 (1): 1–3. <https://doi.org/10.1038/npjbiofilms.2015.5>.
- 923 Del Tredici, Kelly, and Heiko Braak. 2012. 'Lewy Pathology and Neurodegeneration in  
924 Premotor Parkinson's Disease'. *Movement Disorders* 27 (5): 597–607.  
925 <https://doi.org/10.1002/mds.24921>.
- 926 Del Tredici, Kelly, and John E. Duda. 2011. 'Peripheral Lewy Body Pathology in Parkinson's  
927 Disease and Incidental Lewy Body Disease: Four Cases'. *Journal of the Neurological  
928 Sciences, Seventh Congress of Mental and other Non-motor Dysfunctions in  
929 Parkinson's Disease*, 310 (1): 100–106. <https://doi.org/10.1016/j.jns.2011.06.003>.
- 930 Del Tredici, Kelly, Udo Rüb, Rob A.I. de Vos, Jürgen R.E. Bohl, and Heiko Braak. 2002.  
931 'Where Does Parkinson Disease Pathology Begin in the Brain?' *Journal of  
932 Neuropathology & Experimental Neurology* 61 (5): 413–26.  
933 <https://doi.org/10.1093/jnen/61.5.413>.
- 934 Desai, Mahesh S., Anna M. Seekatz, Nicole M. Koropatkin, Nobuhiko Kamada, Christina A.  
935 Hickey, Mathis Wolter, Nicholas A. Pudlo, et al. 2016. 'A Dietary Fiber-Deprived Gut  
936 Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen  
937 Susceptibility'. *Cell* 167 (5): 1339–1353.e21.  
938 <https://doi.org/10.1016/j.cell.2016.10.043>.

- 939 Di Monte, Donato A., Mitra Lavasani, and Amy B. Manning-Bog. 2002. 'Environmental  
940 Factors in Parkinson's Disease'. *NeuroToxicology* 23 (4): 487–502.  
941 [https://doi.org/10.1016/S0161-813X\(02\)00099-2](https://doi.org/10.1016/S0161-813X(02)00099-2).
- 942 Dick, F D, G De Palma, A Ahmadi, N W Scott, G J Prescott, J Bennett, S Semple, et al. 2007.  
943 'Environmental Risk Factors for Parkinson's Disease and Parkinsonism: The  
944 Geoparkinson Study'. *Occupational and Environmental Medicine* 64 (10): 666–72.  
945 <https://doi.org/10.1136/oem.2006.027003>.
- 946 Dore, Joel, Stanislav Dusko Ehrlich, Florence Levenez, Maria Teresa Pellecchia, A. Alberti, L.  
947 Bertrand, P. Bork, et al. 2015. 'IHMS\_SOP 07 V1: Standard Operating Procedure for  
948 Fecal Samples DNA Extraction, Protocol H.' *International Human Microbiome*  
949 *Standards*. <http://www.microbiome-standards.org>.
- 950 Dorsey, E. Ray, Alexis Elbaz, Emma Nichols, Nooshin Abbasi, Foad Abd-Allah, Ahmed  
951 Abdelalim, Jose C. Adsuar, et al. 2018. 'Global, Regional, and National Burden of  
952 Parkinson's Disease, 1990–2016: A Systematic Analysis for the Global Burden of  
953 Disease Study 2016'. *The Lancet Neurology* 17 (11): 939–53.  
954 [https://doi.org/10.1016/S1474-4422\(18\)30295-3](https://doi.org/10.1016/S1474-4422(18)30295-3).
- 955 Drokhlyansky, Eugene, Christopher S. Smillie, Nicholas Van Wittenberghe, Maria Ericsson,  
956 Gabriel K. Griffin, Gokcen Eraslan, Danielle Dionne, et al. 2020. 'The Human and  
957 Mouse Enteric Nervous System at Single-Cell Resolution'. *Cell* 182 (6): 1606-  
958 1622.e23. <https://doi.org/10.1016/j.cell.2020.08.003>.
- 959 Fernagut, P. O., E. Diguët, B. Bioulac, and F. Tison. 2004. 'MPTP Potentiates 3-Nitropropionic  
960 Acid-Induced Striatal Damage in Mice: Reference to Striatonigral Degeneration'.  
961 *Experimental Neurology* 185 (1): 47–62.  
962 <https://doi.org/10.1016/j.expneurol.2003.09.014>.
- 963 Fleming, Sheila M., Osunde R. Ekhaton, and Valentins Ghisays. 2013. 'Assessment of  
964 Sensorimotor Function in Mouse Models of Parkinson's Disease'. *Journal of Visualized*  
965 *Experiments : JoVE*, no. 76 (June): 50303. <https://doi.org/10.3791/50303>.
- 966 Garcia, Pierre, Wiebke Jürgens-Wemheuer, Oihane Uriarte Huarte, Alessandro Michelucci,  
967 Annette Masuch, Simone Brioschi, Andreas Weihofen, et al. 2022. 'Neurodegeneration  
968 and Neuroinflammation Are Linked, but Independent of Alpha-Synuclein Inclusions,  
969 in a Seeding/Spreading Mouse Model of Parkinson's Disease'. *Glia*, January.  
970 <https://doi.org/10.1002/glia.24149>.
- 971 Gerhardt, Sara, and M. Hasan Mohajeri. 2018. 'Changes of Colonic Bacterial Composition in  
972 Parkinson's Disease and Other Neurodegenerative Diseases'. *Nutrients* 10 (6): 708.  
973 <https://doi.org/10.3390/nu10060708>.
- 974 Gorecki, Anastazja M., Leah Preskey, Megan C. Bakeberg, Jade E. Kenna, Christi Gildenhuis,  
975 Gabriella MacDougall, Sarah A. Dunlop, et al. 2019. 'Altered Gut Microbiome in  
976 Parkinson's Disease and the Influence of Lipopolysaccharide in a Human  $\alpha$ -Synuclein  
977 Over-Expressing Mouse Model'. *Frontiers in Neuroscience* 13 (August): 839.  
978 <https://doi.org/10.3389/fnins.2019.00839>.

- 979 Gorell, Jay M., Edward L. Peterson, Benjamin A. Rybicki, and Christine Cole Johnson. 2004.  
980 'Multiple Risk Factors for Parkinson's Disease'. *Journal of the Neurological Sciences*  
981 217 (2): 169–74. <https://doi.org/10.1016/j.jns.2003.09.014>.
- 982 Guyenet, Stephan J., Stephanie A. Furrer, Vincent M. Damian, Travis D. Baughan, Albert R.  
983 La Spada, and Gwenn A. Garden. 2010. 'A Simple Composite Phenotype Scoring  
984 System for Evaluating Mouse Models of Cerebellar Ataxia'. *Journal of Visualized*  
985 *Experiments : JoVE*, no. 39 (May). <https://doi.org/10.3791/1787>.
- 986 Heeney, Dustin D., Mélanie G. Gareau, and Maria L. Marco. 2018. 'Intestinal Lactobacillus in  
987 Health and Disease, a Driver or Just along for the Ride?' *Current Opinion in*  
988 *Biotechnology* 49 (February): 140–47. <https://doi.org/10.1016/j.copbio.2017.08.004>.
- 989 Heintz-Buschart, Anna, Urvashi Pandey, Tamara Wicke, Friederike Sixel-Döring, Annette  
990 Janzen, Elisabeth Sittig-Wiegand, Claudia Trenkwalder, Wolfgang H. Oertel, Brit  
991 Mollenhauer, and Paul Wilmes. 2018. 'The Nasal and Gut Microbiome in Parkinson's  
992 Disease and Idiopathic Rapid Eye Movement Sleep Behavior Disorder'. *Movement*  
993 *Disorders: Official Journal of the Movement Disorder Society* 33 (1): 88–98.  
994 <https://doi.org/10.1002/mds.27105>.
- 995 Hirschberg, Sarah, Barbara Gisevius, Alexander Duscha, and Aiden Haghikia. 2019.  
996 'Implications of Diet and The Gut Microbiome in Neuroinflammatory and  
997 Neurodegenerative Diseases'. *International Journal of Molecular Sciences* 20 (12):  
998 3109. <https://doi.org/10.3390/ijms20123109>.
- 999 'Immunofluorescent Staining of Paraffin-Embedded Tissue'. n.d. Novus Biologicals. Accessed  
1000 5 January 2022. [https://www.novusbio.com/support-by-application/Fluorescent-IHC-](https://www.novusbio.com/support-by-application/Fluorescent-IHC-Staining-of-FFPE-Tissue)  
1001 [Staining-of-FFPE-Tissue](https://www.novusbio.com/support-by-application/Fluorescent-IHC-Staining-of-FFPE-Tissue).
- 1002 Johansson, Malin E. V., Jessica M. Holmén Larsson, and Gunnar C. Hansson. 2011. 'The Two  
1003 Mucus Layers of Colon Are Organized by the MUC2 Mucin, Whereas the Outer Layer  
1004 Is a Legislator of Host–Microbial Interactions'. *Proceedings of the National Academy*  
1005 *of Sciences* 108 (Supplement 1): 4659–65. <https://doi.org/10.1073/pnas.1006451107>.
- 1006 Johansson, Malin E.V., Henrik Sjövall, and Gunnar C. Hansson. 2013. 'The Gastrointestinal  
1007 Mucus System in Health and Disease'. *Nature Reviews. Gastroenterology &*  
1008 *Hepatology* 10 (6): 352–61. <https://doi.org/10.1038/nrgastro.2013.35>.
- 1009 Jucker, Mathias, and Lary C. Walker. 2018. 'Propagation and Spread of Pathogenic Protein  
1010 Assemblies in Neurodegenerative Diseases'. *Nature Neuroscience* 21 (10): 1341–49.  
1011 <https://doi.org/10.1038/s41593-018-0238-6>.
- 1012 Kahle, P. J., M. Neumann, L. Ozmen, V. Müller, S. Odoy, N. Okamoto, H. Jacobsen, et al.  
1013 2001. 'Selective Insolubility of Alpha-Synuclein in Human Lewy Body Diseases Is  
1014 Recapitulated in a Transgenic Mouse Model'. *The American Journal of Pathology* 159  
1015 (6): 2215–25. [https://doi.org/10.1016/s0002-9440\(10\)63072-6](https://doi.org/10.1016/s0002-9440(10)63072-6).
- 1016 Kahle, Philipp J., Manuela Neumann, Laurence Ozmen, Veronika Müller, Helmut Jacobsen,  
1017 Alice Schindzielorz, Masayasu Okochi, et al. 2000. 'Subcellular Localization of Wild-  
1018 Type and Parkinson's Disease-Associated Mutant  $\alpha$ -Synuclein in Human and



- 1019 Transgenic Mouse Brain'. *Journal of Neuroscience* 20 (17): 6365–73.  
1020 <https://doi.org/10.1523/JNEUROSCI.20-17-06365.2000>.
- 1021 Keller, Daniel, Csaba Erö, and Henry Markram. 2018. 'Cell Densities in the Mouse Brain: A  
1022 Systematic Review'. *Frontiers in Neuroanatomy* 12: 83.  
1023 <https://doi.org/10.3389/fnana.2018.00083>.
- 1024 Keshavarzian, Ali, Stefan J. Green, Phillip A. Engen, Robin M. Voigt, Ankur Naqib,  
1025 Christopher B. Forsyth, Ece Mutlu, and Kathleen M. Shannon. 2015. 'Colonic Bacterial  
1026 Composition in Parkinson's Disease'. *Movement Disorders* 30 (10): 1351–60.  
1027 <https://doi.org/10.1002/mds.26307>.
- 1028 Kim, Sangjune, Seung-Hwan Kwon, Tae-In Kam, Nikhil Panicker, Senthilkumar S.  
1029 Karuppagounder, Saebom Lee, Jun Hee Lee, et al. 2019. 'Transneuronal Propagation  
1030 of Pathologic  $\alpha$ -Synuclein from the Gut to the Brain Models Parkinson's Disease'.  
1031 *Neuron* 103 (4): 627-641.e7. <https://doi.org/10.1016/j.neuron.2019.05.035>.
- 1032 Kouroupi, Georgia, Era Taoufik, Ioannis S. Vlachos, Konstantinos Tsioras, Nasia Antoniou,  
1033 Florentia Papastefanaki, Dafni Chroni-Tzartou, et al. 2017. 'Defective Synaptic  
1034 Connectivity and Axonal Neuropathology in a Human iPSC-Based Model of Familial  
1035 Parkinson's Disease'. *Proceedings of the National Academy of Sciences* 114 (18):  
1036 E3679–88. <https://doi.org/10.1073/pnas.1617259114>.
- 1037 Lauwers, Erwin, Zeger Debyser, Jo Van Dorpe, Bart De Strooper, Bart Nuttin, and Veerle  
1038 Baekelandt. 2003. 'Neuropathology and Neurodegeneration in Rodent Brain Induced  
1039 by Lentiviral Vectormediated Overexpression of  $\alpha$ -Synuclein'. *Brain Pathology* 13 (3):  
1040 364–72. <https://doi.org/10.1111/j.1750-3639.2003.tb00035.x>.
- 1041 Li, Wei, Xiaoli Wu, Xu Hu, Tao Wang, Shan Liang, Yunfeng Duan, Feng Jin, and Bin Qin.  
1042 2017. 'Structural Changes of Gut Microbiota in Parkinson's Disease and Its Correlation  
1043 with Clinical Features'. *Science China Life Sciences* 60 (11): 1223–33.  
1044 <https://doi.org/10.1007/s11427-016-9001-4>.
- 1045 Lieu, Christopher A., Shankar J. Chinta, Anand Rane, and Julie K. Andersen. 2013. 'Age-  
1046 Related Behavioral Phenotype of an Astrocytic Monoamine Oxidase-B Transgenic  
1047 Mouse Model of Parkinson's Disease'. *PLOS ONE* 8 (1): e54200.  
1048 <https://doi.org/10.1371/journal.pone.0054200>.
- 1049 Lin, Ruqin, Yu Sun, Peiqiang Mu, Ting Zheng, Haibin Mu, Fengru Deng, Yiqun Deng, and  
1050 Jikai Wen. 2020. 'Lactobacillus Rhamnosus GG Supplementation Modulates the Gut  
1051 Microbiota to Promote Butyrate Production, Protecting against Deoxynivalenol  
1052 Exposure in Nude Mice'. *Biochemical Pharmacology* 175 (May): 113868.  
1053 <https://doi.org/10.1016/j.bcp.2020.113868>.
- 1054 Liu, Bojing, Fang Fang, Nancy L. Pedersen, Annika Tillander, Jonas F. Ludvigsson, Anders  
1055 Ekblom, Per Svenningsson, Honglei Chen, and Karin Wirdefeldt. 2017. 'Vagotomy and  
1056 Parkinson Disease'. *Neurology* 88 (21): 1996–2002.  
1057 <https://doi.org/10.1212/WNL.0000000000003961>.
- 1058 Luk, Kelvin C., Victoria M. Kehm, Bin Zhang, Patrick O'Brien, John Q. Trojanowski, and  
1059 Virginia M.Y. Lee. 2012. 'Intracerebral Inoculation of Pathological  $\alpha$ -Synuclein

- 1060 Initiates a Rapidly Progressive Neurodegenerative  $\alpha$ -Synucleinopathy in Mice'. *The*  
1061 *Journal of Experimental Medicine* 209 (5): 975–86.  
1062 <https://doi.org/10.1084/jem.20112457>.
- 1063 Magne, Fabien, Martin Gotteland, Lea Gauthier, Alejandra Zazueta, Susana Pesoa, Paola  
1064 Navarrete, and Ramadass Balamurugan. 2020. 'The Firmicutes/Bacteroidetes Ratio: A  
1065 Relevant Marker of Gut Dysbiosis in Obese Patients?' *Nutrients* 12 (5).  
1066 <https://doi.org/10.3390/nu12051474>.
- 1067 Mao, Xiaobo, Michael Tianhao Ou, Senthilkumar S. Karuppagounder, Tae-In Kam, Xiling Yin,  
1068 Yulan Xiong, Preston Ge, et al. 2016. 'Pathological  $\alpha$ -Synuclein Transmission Initiated  
1069 by Binding Lymphocyte-Activation Gene 3'. *Science (New York, N.Y.)* 353 (6307).  
1070 <https://doi.org/10.1126/science.aah3374>.
- 1071 Maraki, Maria I., Mary Yannakoulia, Maria Stamelou, Leonidas Stefanis, Georgia  
1072 Xiromerisiou, Mary H. Kosmidis, Efthimios Dardiotis, et al. 2019. 'Mediterranean Diet  
1073 Adherence Is Related to Reduced Probability of Prodromal Parkinson's Disease'.  
1074 *Movement Disorders* 34 (1): 48–57. <https://doi.org/10.1002/mds.27489>.
- 1075 Mariat, D., O. Firmesse, F. Levenez, VD Guimarães, H. Sokol, J. Doré, G. Corthier, and J-P  
1076 Furet. 2009. 'The Firmicutes/Bacteroidetes Ratio of the Human Microbiota Changes  
1077 with Age'. *BMC Microbiology* 9 (1): 123. <https://doi.org/10.1186/1471-2180-9-123>.
- 1078 Marras, Connie, Colleen G. Canning, and Samuel M. Goldman. 2019. 'Environment, Lifestyle,  
1079 and Parkinson's Disease: Implications for Prevention in the next Decade'. *Movement*  
1080 *Disorders: Official Journal of the Movement Disorder Society* 34 (6): 801–11.  
1081 <https://doi.org/10.1002/mds.27720>.
- 1082 Martens, Eric C., Herbert C. Chiang, and Jeffrey I. Gordon. 2008. 'Mucosal Glycan Foraging  
1083 Enhances Fitness and Transmission of a Saccharolytic Human Gut Bacterial Symbiont'.  
1084 *Cell Host & Microbe* 4 (5): 447–57. <https://doi.org/10.1016/j.chom.2008.09.007>.
- 1085 Martín, Rebeca, Sylvie Miquel, Jonathan Ulmer, Noura Kechaou, Philippe Langella, and Luis  
1086 G. Bermúdez-Humarán. 2013. 'Role of Commensal and Probiotic Bacteria in Human  
1087 Health: A Focus on Inflammatory Bowel Disease'. *Microbial Cell Factories* 12 (1): 71.  
1088 <https://doi.org/10.1186/1475-2859-12-71>.
- 1089 Martínez Leo, Edwin E., and Maira R. Segura Campos. 2020. 'Effect of Ultra-Processed Diet  
1090 on Gut Microbiota and Thus Its Role in Neurodegenerative Diseases'. *Nutrition* 71  
1091 (March): 110609. <https://doi.org/10.1016/j.nut.2019.110609>.
- 1092 McMurdie, Paul J., and Susan Holmes. 2013. 'Phyloseq: An R Package for Reproducible  
1093 Interactive Analysis and Graphics of Microbiome Census Data'. *PLOS ONE* 8 (4):  
1094 e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- 1095 Mezas, Christopher, Nolwen Rey, Patrik Brundin, and Ashish Raj. 2020. 'Neural Connectivity  
1096 Predicts Spreading of Alpha-Synuclein Pathology in Fibril-Injected Mouse Models:  
1097 Involvement of Retrograde and Anterograde Axonal Propagation'. *Neurobiology of*  
1098 *Disease* 134 (February): 104623. <https://doi.org/10.1016/j.nbd.2019.104623>.



- 1099 Miller, Amanda L., Shingo Bessho, Kaitlyn Grando, and Çağla Tükel. 2021. ‘Microbiome or  
1100 Infections: Amyloid-Containing Biofilms as a Trigger for Complex Human Diseases’.  
1101 *Frontiers in Immunology* 12: 514. <https://doi.org/10.3389/fimmu.2021.638867>.
- 1102 Murakami, Tatsuya C., Tomoyuki Mano, Shu Saikawa, Shuhei A. Horiguchi, Daichi Shigeta,  
1103 Kousuke Baba, Hiroshi Sekiya, et al. 2018. ‘A Three-Dimensional Single-Cell-  
1104 Resolution Whole-Brain Atlas Using CUBIC-X Expansion Microscopy and Tissue  
1105 Clearing’. *Nature Neuroscience* 21 (4): 625–37. <https://doi.org/10.1038/s41593-018-0109-1>.
- 1107 Nations, United. n.d. ‘LIFESTYLE DISEASES: An Economic Burden on the Health Services’.  
1108 United Nations. United Nations. Accessed 16 February 2022.  
1109 [https://www.un.org/en/chronicle/article/lifestyle-diseases-economic-burden-health-](https://www.un.org/en/chronicle/article/lifestyle-diseases-economic-burden-health-services)  
1110 [services](https://www.un.org/en/chronicle/article/lifestyle-diseases-economic-burden-health-services).
- 1111 Nerius, Michael, Gabriele Doblhammer, and Gültekin Tamgüney. 2020. ‘GI Infections Are  
1112 Associated with an Increased Risk of Parkinson’s Disease’. *Gut* 69 (6): 1154–56.  
1113 <https://doi.org/10.1136/gutjnl-2019-318822>.
- 1114 Neumann, Mareike, Alex Steimle, Erica T. Grant, Mathis Wolter, Amy Parrish, Stéphanie  
1115 Willieme, Dirk Brenner, Eric C. Martens, and Mahesh S. Desai. 2021. ‘Deprivation of  
1116 Dietary Fiber in Specific-Pathogen-Free Mice Promotes Susceptibility to the Intestinal  
1117 Mucosal Pathogen *Citrobacter Rodentium*’. *Gut Microbes* 13 (1): 1966263.  
1118 <https://doi.org/10.1080/19490976.2021.1966263>.
- 1119 Oksanen, Jari, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan  
1120 McGlinn, Peter R. Minchin, et al. 2020. *Vegan: Community Ecology Package* (version  
1121 2.5-7). <https://CRAN.R-project.org/package=vegan>.
- 1122 Paiva, Isabel, Raquel Pinho, Maria Angeliki Pavlou, Magali Hennion, Pauline Wales, Anna-  
1123 Lena Schütz, Ashish Rajput, et al. 2017. ‘Sodium Butyrate Rescues Dopaminergic Cells  
1124 from Alpha-Synuclein-Induced Transcriptional Deregulation and DNA Damage’.  
1125 *Human Molecular Genetics* 26 (12): 2231–46. <https://doi.org/10.1093/hmg/ddx114>.
- 1126 Plöger, Svenja, Friederike Stumpff, Gregory B. Penner, Jörg-Dieter Schulzke, Gotthold Gäbel,  
1127 Holger Martens, Zanming Shen, Dorothee Günzel, and Joerg R. Aschenbach. 2012.  
1128 ‘Microbial Butyrate and Its Role for Barrier Function in the Gastrointestinal Tract’.  
1129 *Annals of the New York Academy of Sciences* 1258 (1): 52–59.  
1130 <https://doi.org/10.1111/j.1749-6632.2012.06553.x>.
- 1131 Poewe, Werner, Klaus Seppi, Caroline M. Tanner, Glenda M. Halliday, Patrik Brundin, Jens  
1132 Volkmann, Anette-Eleonore Schrag, and Anthony E. Lang. 2017. ‘Parkinson Disease’.  
1133 *Nature Reviews Disease Primers* 3 (1): 1–21. <https://doi.org/10.1038/nrdp.2017.13>.
- 1134 Qualman, S. J., H. M. Haupt, P. Yang, and S. R. Hamilton. 1984. ‘Esophageal Lewy Bodies  
1135 Associated with Ganglion Cell Loss in Achalasia. Similarity to Parkinson’s Disease’.  
1136 *Gastroenterology* 87 (4): 848–56.
- 1137 Quast, Christian, Elmar Pruesse, Pelin Yilmaz, Jan Gerken, Timmy Schweer, Pablo Yarza, Jörg  
1138 Peplies, and Frank Oliver Glöckner. 2013. ‘The SILVA Ribosomal RNA Gene

- 1139 Database Project: Improved Data Processing and Web-Based Tools'. *Nucleic Acids*  
1140 *Research* 41 (Database issue): D590–96. <https://doi.org/10.1093/nar/gks1219>.
- 1141 Rampelli, Simone, Stephanie L. Schnorr, Clarissa Consolandi, Silvia Turroni, Marco  
1142 Severgnini, Clelia Peano, Patrizia Brigidi, Alyssa N. Crittenden, Amanda G. Henry, and  
1143 Marco Candela. 2015. 'Metagenome Sequencing of the Hadza Hunter-Gatherer Gut  
1144 Microbiota'. *Current Biology* 25 (13): 1682–93.  
1145 <https://doi.org/10.1016/j.cub.2015.04.055>.
- 1146 Rey, Nolwen L., Jennifer A. Steiner, Nazia Maroof, Kelvin C. Luk, Zachary Madaj, John Q.  
1147 Trojanowski, Virginia M.-Y. Lee, and Patrik Brundin. 2016. 'Widespread  
1148 Transneuronal Propagation of  $\alpha$ -Synucleinopathy Triggered in Olfactory Bulb Mimics  
1149 Prodromal Parkinson's Disease'. *Journal of Experimental Medicine* 213 (9): 1759–78.  
1150 <https://doi.org/10.1084/jem.20160368>.
- 1151 Riva, Alessandra, Orest Kuzyk, Erica Forsberg, Gary Siuzdak, Carina Pfann, Craig Herbold,  
1152 Holger Daims, Alexander Loy, Benedikt Warth, and David Berry. 2019. 'A Fiber-  
1153 Deprived Diet Disturbs the Fine-Scale Spatial Architecture of the Murine Colon  
1154 Microbiome'. *Nature Communications* 10 (1): 4366. [https://doi.org/10.1038/s41467-  
1155 019-12413-0](https://doi.org/10.1038/s41467-019-12413-0).
- 1156 Rivière, Audrey, Marija Selak, David Lantin, Frédéric Leroy, and Luc De Vuyst. 2016.  
1157 'Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for  
1158 Their Stimulation in the Human Gut'. *Frontiers in Microbiology* 7 (June): 979.  
1159 <https://doi.org/10.3389/fmicb.2016.00979>.
- 1160 Sampson, Timothy R, Collin Challis, Neha Jain, Anastasiya Moiseyenko, Mark S Ladinsky,  
1161 Gauri G Shastri, Taren Thron, et al. 2020. 'A Gut Bacterial Amyloid Promotes  $\alpha$ -  
1162 Synuclein Aggregation and Motor Impairment in Mice'. Edited by Isaac M Chiu,  
1163 Wendy S Garrett, and Michel Desjardins. *ELife* 9 (February): e53111.  
1164 <https://doi.org/10.7554/eLife.53111>.
- 1165 Sampson, Timothy R., Justine W. Debelius, Taren Thron, Stefan Janssen, Gauri G. Shastri,  
1166 Zehra Esra Ilhan, Collin Challis, et al. 2016. 'Gut Microbiota Regulate Motor Deficits  
1167 and Neuroinflammation in a Model of Parkinson's Disease'. *Cell* 167 (6): 1469-  
1168 1480.e12. <https://doi.org/10.1016/j.cell.2016.11.018>.
- 1169 Scheperjans, Filip, Velma Aho, Pedro A. B. Pereira, Kaisa Koskinen, Lars Paulin, Eero  
1170 Pekkonen, Elena Haapaniemi, et al. 2015. 'Gut Microbiota Are Related to Parkinson's  
1171 Disease and Clinical Phenotype'. *Movement Disorders* 30 (3): 350–58.  
1172 <https://doi.org/10.1002/mds.26069>.
- 1173 Schroeder, Bjoern O., George M. H. Birchenough, Marcus Ståhlman, Liisa Arike, Malin E. V.  
1174 Johansson, Gunnar C. Hansson, and Fredrik Bäckhed. 2018. 'Bifidobacteria or Fiber  
1175 Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration'. *Cell*  
1176 *Host & Microbe* 23 (1): 27-40.e7. <https://doi.org/10.1016/j.chom.2017.11.004>.
- 1177 Shannon, Kathleen M., Ali Keshavarzian, Hemraj B. Dodiya, Shriram Jakate, and Jeffrey H.  
1178 Kordower. 2012. 'Is Alpha-Synuclein in the Colon a Biomarker for Premotor  
1179 Parkinson's Disease? Evidence from 3 Cases'. *Movement Disorders: Official Journal*  
1180 *of the Movement Disorder Society* 27 (6): 716–19. <https://doi.org/10.1002/mds.25020>.

- 1181 Sharma, Sorabh, Rajeev Taliyan, and Sumel Singh. 2015. 'Beneficial Effects of Sodium  
1182 Butyrate in 6-OHDA Induced Neurotoxicity and Behavioral Abnormalities:  
1183 Modulation of Histone Deacetylase Activity'. *Behavioural Brain Research* 291  
1184 (September): 306–14. <https://doi.org/10.1016/j.bbr.2015.05.052>.
- 1185 Shen, Ting, Yumei Yue, Tingting He, Cong Huang, Boyi Qu, Wen Lv, and Hsin-Yi Lai. 2021.  
1186 'The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-  
1187 Analysis'. *Frontiers in Aging Neuroscience* 13: 40.  
1188 <https://doi.org/10.3389/fnagi.2021.636545>.
- 1189 Sidebotham, E. L., M. N. Woodward, S. E. Kenny, D. A. Lloyd, C. R. Vaillant, and D. H.  
1190 Edgar. 2001. 'Assessment of Protein Gene Product 9.5 as a Marker of Neural Crest-  
1191 Derived Precursor Cells in the Developing Enteric Nervous System'. *Pediatric Surgery*  
1192 *International* 17 (4): 304–7. <https://doi.org/10.1007/s003830100599>.
- 1193 Singh, Yogesh, Mohamed El-Hadidi, Jakob Admard, Zinah Wassouf, Julia M. Schulze-  
1194 Hentrich, Ursula Kohlhofer, Leticia Quintanilla-Martinez, Daniel Huson, Olaf Riess,  
1195 and Nicolas Casadei. 2019. 'Enriched Environmental Conditions Modify the Gut  
1196 Microbiome Composition and Fecal Markers of Inflammation in Parkinson's Disease'.  
1197 *Frontiers in Neuroscience* 13: 1032. <https://doi.org/10.3389/fnins.2019.01032>.
- 1198 St. Laurent, R., L. M. O'Brien, and S. T. Ahmad. 2013. 'Sodium Butyrate Improves Locomotor  
1199 Impairment and Early Mortality in a Rotenone-Induced Drosophila Model of  
1200 Parkinson's Disease'. *Neuroscience* 246 (August): 382–90.  
1201 <https://doi.org/10.1016/j.neuroscience.2013.04.037>.
- 1202 Stadlbauer, Vanessa, Lara Engertsberger, Irina Komarova, Nicole Feldbacher, Bettina Leber,  
1203 Gerald Pichler, Nicole Fink, et al. 2020. 'Dysbiosis, Gut Barrier Dysfunction and  
1204 Inflammation in Dementia: A Pilot Study'. *BMC Geriatrics* 20 (1): 248.  
1205 <https://doi.org/10.1186/s12877-020-01644-2>.
- 1206 Stokholm, Morten Gersel, Erik Hvid Danielsen, Stephen Jacques Hamilton-Dutoit, and Per  
1207 Borghammer. 2016. 'Pathological  $\alpha$ -Synuclein in Gastrointestinal Tissues from  
1208 Prodromal Parkinson Disease Patients'. *Annals of Neurology* 79 (6): 940–49.  
1209 <https://doi.org/10.1002/ana.24648>.
- 1210 Svensson, Elisabeth, Erzsébet Horváth-Puhó, Reimar W. Thomsen, Jens Christian Djurhuus,  
1211 Lars Pedersen, Per Borghammer, and Henrik Toft Sørensen. 2015. 'Vagotomy and  
1212 Subsequent Risk of Parkinson's Disease'. *Annals of Neurology* 78 (4): 522–29.  
1213 <https://doi.org/10.1002/ana.24448>.
- 1214 Tailford, Louise E., Emmanuelle H. Crost, Devon Kavanaugh, and Nathalie Juge. 2015. 'Mucin  
1215 Glycan Foraging in the Human Gut Microbiome'. *Frontiers in Genetics* 6: 81.  
1216 <https://doi.org/10.3389/fgene.2015.00081>.
- 1217 Tillerson, Jennifer L., W. Michael Caudle, Maria E. Reverón, and Gary W. Miller. 2002a.  
1218 'Detection of Behavioral Impairments Correlated to Neurochemical Deficits in Mice  
1219 Treated with Moderate Doses of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine'.  
1220 *Experimental Neurology* 178 (1): 80–90. <https://doi.org/10.1006/exnr.2002.8021>.

- 1221 ———. 2002b. ‘Detection of Behavioral Impairments Correlated to Neurochemical Deficits in  
1222 Mice Treated with Moderate Doses of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine’.  
1223 *Experimental Neurology* 178 (1): 80–90. <https://doi.org/10.1006/exnr.2002.8021>.
- 1224 Tillerson, Jennifer L, and Gary W Miller. 2003. ‘Grid Performance Test to Measure Behavioral  
1225 Impairment in the MPTP-Treated-Mouse Model of Parkinsonism’. *Journal of*  
1226 *Neuroscience Methods* 123 (2): 189–200. [https://doi.org/10.1016/S0165-](https://doi.org/10.1016/S0165-0270(02)00360-6)  
1227 0270(02)00360-6.
- 1228 Tytgat, Hanne L. P., Franklin L. Nobrega, John van der Oost, and Willem M. de Vos. 2019.  
1229 ‘Bowel Biofilms: Tipping Points between a Healthy and Compromised Gut?’ *Trends in*  
1230 *Microbiology* 27 (1): 17–25. <https://doi.org/10.1016/j.tim.2018.08.009>.
- 1231 Unger, Marcus M., Jörg Spiegel, Klaus-Ulrich Dillmann, David Grundmann, Hannah  
1232 Philippeit, Jan Bürmann, Klaus Faßbender, Andreas Schwierz, and Karl-Herbert  
1233 Schäfer. 2016. ‘Short Chain Fatty Acids and Gut Microbiota Differ between Patients  
1234 with Parkinson’s Disease and Age-Matched Controls’. *Parkinsonism & Related*  
1235 *Disorders* 32 (November): 66–72. <https://doi.org/10.1016/j.parkreldis.2016.08.019>.
- 1236 Vaikath, Nishant N., Issam Hmila, Vijay Gupta, Daniel Erskine, Martin Ingelsson, and Omar  
1237 M. A. El-Agnaf. 2019. ‘Antibodies against Alpha-Synuclein: Tools and Therapies’.  
1238 *Journal of Neurochemistry* 150 (5): 612–25. <https://doi.org/10.1111/jnc.14713>.
- 1239 Vasili, Eftychia, Antonio Dominguez-Meijide, and Tiago Fleming Outeiro. 2019. ‘Spreading  
1240 of  $\alpha$ -Synuclein and Tau: A Systematic Comparison of the Mechanisms Involved’.  
1241 *Frontiers in Molecular Neuroscience* 12. <https://doi.org/10.3389/fnmol.2019.00107>.
- 1242 Wakabayashi, K., H. Takahashi, E. Ohama, and F. Ikuta. 1990. ‘Parkinson’s Disease: An  
1243 Immunohistochemical Study of Lewy Body-Containing Neurons in the Enteric Nervous  
1244 System’. *Acta Neuropathologica* 79 (6): 581–83. <https://doi.org/10.1007/BF00294234>.
- 1245 Wakabayashi, K., H. Takahashi, E. Ohama, S. Takeda, and F. Ikuta. 1993. ‘Lewy Bodies in the  
1246 Visceral Autonomic Nervous System in Parkinson’s Disease’. *Advances in Neurology*  
1247 60: 609–12.
- 1248 Wakabayashi, K., H. Takahashi, S. Takeda, E. Ohama, and F. Ikuta. 1988. ‘Parkinson’s  
1249 Disease: The Presence of Lewy Bodies in Auerbach’s and Meissner’s Plexuses’. *Acta*  
1250 *Neuropathologica* 76 (3): 217–21. <https://doi.org/10.1007/BF00687767>.
- 1251 Wang, Lei, Shengyu Li, Yu Jiang, Zijian Zhao, Yunjiao Shen, Junjie Zhang, and Lei Zhao.  
1252 2021. ‘Neuroprotective Effect of Lactobacillus Plantarum DP189 on MPTP-Induced  
1253 Parkinson’s Disease Model Mice’. *Journal of Functional Foods* 85 (October): 104635.  
1254 <https://doi.org/10.1016/j.jff.2021.104635>.
- 1255 Warner, Thomas T., and Anthony H. V. Schapira. 2003. ‘Genetic and Environmental Factors  
1256 in the Cause of Parkinson’s Disease’. *Annals of Neurology* 53 (S3): S16–25.  
1257 <https://doi.org/10.1002/ana.10487>.
- 1258 Yadav, Narayan, Ajit Kumar Thakur, Nikhila Shekhar, and Ayushi. n.d. ‘Potential of  
1259 Antibiotics for the Treatment and Management of Parkinson’s Disease: An Overview’.  
1260 *Current Drug Research Reviews* 13 (3): 166–71.

- 1261 Yan, Yaping, Shuchao Ren, Yanchao Duan, Chenyu Lu, Yuyu Niu, Zhengbo Wang, Briauna  
1262 Inglis, Weizhi Ji, Yun Zheng, and Wei Si. 2021. ‘Gut Microbiota and Metabolites of  $\alpha$ -  
1263 Synuclein Transgenic Monkey Models with Early Stage of Parkinson’s Disease’. *Npj*  
1264 *Biofilms and Microbiomes* 7 (1): 1–9. <https://doi.org/10.1038/s41522-021-00242-3>.
- 1265 Yang, Wenya, Jamie L. Hamilton, Catherine Kopil, James C. Beck, Caroline M. Tanner, Roger  
1266 L. Albin, E. Ray Dorsey, et al. 2020. ‘Current and Projected Future Economic Burden  
1267 of Parkinson’s Disease in the U.S.’ *Npj Parkinson’s Disease* 6 (1): 1–9.  
1268 <https://doi.org/10.1038/s41531-020-0117-1>.
- 1269 Zhang, Jiasheng, Vanee Pho, Stephen J. Bonasera, Jed Holtzman, Amy T. Tang, Joanna  
1270 Hellmuth, Siuwah Tang, Patricia H. Janak, Laurence H. Tecott, and Eric J. Huang.  
1271 2007. ‘Essential Function of HIPK2 in TGF $\beta$ -Dependent Survival of Midbrain  
1272 Dopamine Neurons’. *Nature Neuroscience* 10 (1): 77–86.  
1273 <https://doi.org/10.1038/nn1816>.
- 1274 Zhang, YaJun, Ann-Charlotte Granholm, Kyounghee Huh, Lufei Shan, Oscar Diaz-Ruiz, Nasir  
1275 Malik, Lars Olson, et al. 2012. ‘PTEN Deletion Enhances Survival, Neurite Outgrowth  
1276 and Function of Dopamine Neuron Grafts to MitoPark Mice’. *Brain* 135 (9): 2736–49.  
1277 <https://doi.org/10.1093/brain/aws196>.
- 1278