

Gut microbial production of imidazole propionate drives Parkinson's pathologies

Corresponding Author: Professor Ara Koh

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Editorial Note: Parts of this peer review file have been redacted as indicated to maintain the confidentiality of unpublished data.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

We thank the authors for their responses and the additional experiments provided. Although the revisions have strengthened several aspects of the manuscript, a few concerns remain and need to be addressed to support the claims.

In figure 2, the authors demonstrate that *E. coli* expressing UrdA recapitulates the PD-like phenotypes. The rationale for extending the colonization protocol to four weeks and using bi-daily gavages in GF mice is unclear and needs to be discussed. Given the heightened risk of contamination in GF studies, additional details on contamination measures (e.g. 16s sequencing on stool samples) are needed to ensure the validity of the results.

For figures 1i, 2i and 3j, although the increase in animal numbers used for behavioral testing is appreciated, the pole test alone remains insufficient to support claims of motor dysfunction. Since rotarod testing was included in other models (e.g., Figures 4i and 5j), we strongly encourage the authors to include this test as a second behavioral assay in the *E. coli* (with or without UrdA) and *S. mutans* colonization conditions to confirm the motor deficits.

For the characterization of neuroinflammation (Figures 1g–h, 2g–h, 3i, 5h, the use of GFAP and Iba1 staining area alone does not distinguish between increased cell number, cell hypertrophy, or morphological transformation. A more robust characterization should include quantification of cell density (GFAP+, Iba1+), microglial morphology classification (e.g., ramified vs. amoeboid), and ideally, cytokine profiling (e.g., qPCR or ELISA). Without these, the claim of neuroinflammation remains only partially supported.

In figure 5, the body weight following chronic i.p injections of ImP should be mentioned as it can confound the behavioral readout. In addition, the brain levels of ImP are considerably (~100-200x) lower than in the colonization models (Extended Data Fig. 5a), yet the observed phenotype is worsened. Please provide plasma ImP concentrations to clarify systemic exposure and address this discrepancy.

Reviewer #2

(Remarks to the Author)

This is a revised manuscript. The authors have satisfactorily addressed my concerns.

Reviewer #3

(Remarks to the Author)

The authors have provided a fairly robust response to my, and the other reviewers' initial concerns. I do think the manuscript is largely improved, and the addition of urdA-expressing *E. coli* is a very strong experimental addition that supports the authors' interpretations. There are still some larger, outstanding concerns in this revised manuscript that limit some

enthusiasm, however it is overall improved.

Major

UrdA in other Streptococcus species

I appreciate the authors' responses in regards to my original concerns regarding the specificity and necessity of UrdA from *S. mutans*.

The authors show, as do prior data including the original description of the underlying human dataset used, that *S. mutans* is preferentially enriched in the PD-associated microbiome compared to other Strep species. This certainly supports the specificity of the association between the species and PD.

However, my original concern regarding urdA specificity still remains. I apologize since I may not have been clear in my prior comments. Given the authors observe similar relative abundances of other urdA from other species between those with and without PD, it further raises the question as to whether there is something unique about urdA from *S. mutans* or if urdA from these other organisms are also acting similarly.

The alignment the authors show in the reviewer response is not the most convincing that these urdA homologs would have different activity. Similar to the new data shown with expression of *S. mutans* urdA in *E. coli*, if this could be performed with a homologous urdA that is not differentially abundant in PD, but still prevalent (like that from *S. parasanguinis* or *S. mitis*) it would be exceedingly informative. If a homologous urdA performs similarly, then it would not be the presence of the enzyme itself leading to the altered pathology, but perhaps a species or strain-specific regulation of it.

It is surprising that only a small subset of urdA (Reviewer figure a, extended data Fig 1b) is encoded by the *S. mutans* detected in the dataset.

To investigate synaptic loss, the authors examined TH fiber density in the SNr. The striatal synapses, where SNpc projects to, is thought to be most vulnerable. So it is unclear why the striatum is not examined, and the SNr is.

Minor

The behavior performed with the PFF injected animals (extended data Fig 4l, m) is much too under powered given the variability seen. Further, if the authors wish to directly compare between antibiotic-treated and untreated mice, in their response to PFF injection, the experiment should be set-up and performed side-by-side, given technical variabilities in PFF preps and administration. The PFF data presented in the main figures is very rapid compared to prior works, and I appreciate the authors responses in why this may be. However, while I agree it may be true, a direct experimental comparison would be needed to support their claims.

Line 95- it might be more appropriate to state that the re-analysis "confirmed" the findings regarding Bifidobacterium and Streptococcus, since these taxa were indeed identified in the original analysis.

I disagree that the action of imidazole propionate in the small intestine is not physiologically relevant. Mice are coprophagic, and this metabolite, while produced in the colon, would be naturally ingested and absorbed in the SI. In addition, in humans, exposure to this molecule would be through intestinal adsorption.

In response to R1 and R2 concern re: the original low number of mice used for behavior analysis, the authors' responded that a prior study used similar N of 4-5 for behavior, calling out Figure 4 of "A gut-derived metabolite alters brain activity and anxiety behavior in mice" It should be noted that Figure 4 of this paper actually uses >20 mice for its behavior testing, and the lower N was for electron microscopy and diffusion tensor imaging. While the increased N in the revised manuscript is appreciated, the authors should be careful in their reading and interpretations of past literature.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors sufficiently addressed most comments. Regarding the repeated gavage of Ecoli-urdA into GF mice: the fact that the urdA plasmid is rapidly lost from the *E. coli* may suggest that it confers a fitness disadvantage and raises the question of whether the urdA plasmid (and production of ImP) may lead to downstream alterations in bacterial physiology that confound interpretation of results or mediate effects thought to be caused by ImP production. The authors should discuss this point as a potential limitation

Reviewer #3

(Remarks to the Author)

I appreciate the authors' thoughtful responses- this manuscript has been improved over the rounds of review. I have no further concerns that should preclude publication, and believe that this manuscript will be a worthwhile addition to the field.

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REVIEWER COMMENTS

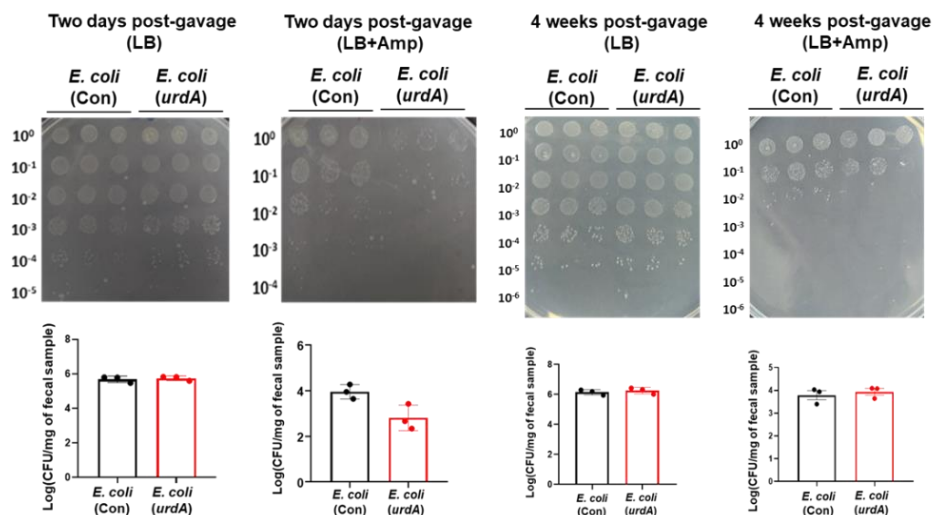
Reviewer #1 (Remarks to the Author):

We thank the authors for their responses and the additional experiments provided. Although the revisions have strengthened several aspects of the manuscript, a few concerns remain and need to be addressed to support the claims.

We sincerely thank again the reviewer for their insightful previous comments and thoughtful suggestions, which have helped to strengthen our manuscript.

In figure 2, the authors demonstrate that *E. coli* expressing UrdA recapitulates the PD-like phenotypes. The rationale for extending the colonization protocol to four weeks and using bi-daily gavages in GF mice is unclear and needs to be discussed. Given the heightened risk of contamination in GF studies, additional details on contamination measures (e.g. 16s sequencing on stool samples) are needed to ensure the validity of the results.

Response: We thank the reviewer for raising this important point. In our initial attempts to establish a colonization protocol using *E. coli* ectopically expressing UrdA, we found that the **UrdA-encoding plasmid was rapidly lost within two days post-gavage, in contrast to control plasmids, as shown by colony counts on ampicillin-selective plates (Reviewer Figure)**. We believe this plasmid loss is likely due to a lack of selective pressure in the gut environment, as *E. coli* is not adapted to rely on UrdA for survival or fitness *in vivo*. To overcome this limitation and achieve stable colonization, we implemented a **bi-daily gavage protocol** over a four-week period. This approach resulted in **consistent *E. coli* levels in fecal samples for both strains (UrdA-expressing and control), as confirmed by colony formation on ampicillin-selective plates (Reviewer Figure)**.



We also appreciate the reviewer's concern regarding the risk of contamination in GF studies. To ensure the integrity of our experiments, we routinely monitored contamination using 16S rRNA-based qPCR analysis of fecal samples. We employed **both universal 16S rRNA primers and taxa-specific primers, confirming that colonization was limited to *E. coli* (Enterobacteriaceae), with no detectable contamination from other bacterial taxa.** Water controls were included as negative controls in each qPCR run. **(Reviewer Figure).**

16S rRNA (V3-V4)		Enterobacteriaceae		Bacteroides		Firmicutes	
<i>E. coli</i> (Con)	<i>E. coli</i> (<i>urdA</i>)	<i>E. coli</i> (Con)	<i>E. coli</i> (<i>urdA</i>)	<i>E. coli</i> (Con)	<i>E. coli</i> (<i>urdA</i>)	<i>E. coli</i> (Con)	<i>E. coli</i> (<i>urdA</i>)
11.59	11.57	11.82	11.82	N.D.	N.D.	N.D.	N.D.
11.64	10.37	12.17	10.27	N.D.	N.D.	N.D.	N.D.
11.3	11.17	12.61	11.35	N.D.	N.D.	N.D.	N.D.
12.05	11.19	12.37	11.89	N.D.	N.D.	N.D.	N.D.
11.44	9.23	12.34	10.08	N.D.	N.D.	N.D.	N.D.
11.36	10.45	11.46	10.28	N.D.	N.D.	N.D.	N.D.
	10.94		11.29		N.D.		N.D.

N.D.: No Ct or Ct>Ct_{water control}

In response to the reviewer's suggestion, **we have now added the following sentence to the Methods section of the revised manuscript (Lines 1198–1204):** “For *E. coli* colonization, GF C57BL/6 mice maintained in sterile flexible film isolators were orally gavaged with *E. coli* (10⁹ CFU/mouse) every two days to enhance colonization efficiency and improve reproducibility, **due to the instability of ectopically expressed plasmids. This repetitive gavage approach was adapted from similar protocols used in previous studies⁴²⁻⁴⁴. Potential contamination was routinely monitored during and at the end of the colonization period using universal 16S rRNA primers and taxa-specific primers.**”

For figures 1i, 2i and 3j, although the increase in animal numbers use for behavioral testing is appreciated, the pole test alone remains insufficient to support claims of motor dysfunction. Since rotarod testing was included in other models (e.g., Figures 4i and 5j), we strongly encourage the authors to include this test as a second behavioral assay in the *E. coli* (with or without UrdA) and *S. mutans* colonization conditions to confirm the motor deficits.

Response: We thank the reviewer for this valuable suggestion. We fully agree that incorporating multiple behavioral assays strengthens the interpretation of motor deficits. The GF colonization model represents the gold standard for testing the direct impact of microbial colonization. However, due to **strict sterility requirements, behavioral testing in the GF**

isolator is currently limited to assays that can be reliably conducted within the confined and sterile environment. At present, only the pole test is feasible under these conditions, and we therefore used it to assess motor function in the GF setting.

To address this limitation and assess the reproducibility of our findings, **we performed complementary experiments using alternative models—such as antibiotic-treated or intraperitoneally injected mice—outside of the GF isolator.** In these settings, where more flexible behavioral testing is possible, we **included the rotarod test** in addition to the pole test. The results from these models consistently demonstrated motor deficits across both assays, thereby supporting the robustness of our findings.

Introducing a new behavioral assay such as rotarod within the GF isolator requires significant modifications to the sterile setup and institutional approval. We respectfully ask for the reviewer's understanding of this limitation and note that the *S. mutans* experiments in both GF and antibiotic-treated mice yielded comparable results, further supporting the reliability of our conclusions.

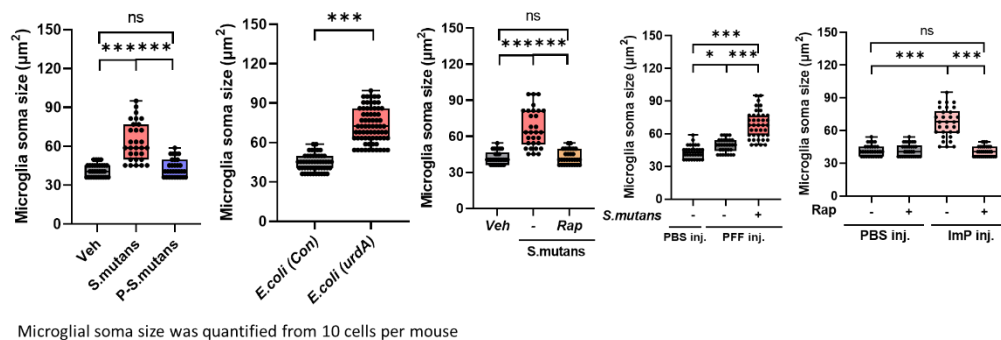
In line with the Reviewer's suggestion, we have expanded the discussion to reflect the importance of incorporating additional behavioral tests in future studies. Specifically, we added the following text:

“Indeed, we observed a synergistic interaction between imidazole propionate and α -Syn seeds in cortical neurons, suggesting that imidazole propionate may facilitate α -Syn pathology formation and propagation beyond the dopaminergic system, implicating it in non-dopaminergic neuronal populations. **Building on these findings, future studies should aim to further characterize both motor and non-motor consequences of imidazole propionate and *S. mutans* colonization. This includes incorporating additional motor assessments, such as the rotarod and narrow beam walking tests, to validate motor impairments. Furthermore, a broader range of behavioral paradigms should be employed to evaluate non-motor symptoms associated with PD (e.g., depression, olfactory deficits, cognitive impairment, anxiety, and constipation) following α -Syn PFF injections into relevant brain regions.”**

We hope these additions and clarifications address the Reviewer's concern and demonstrate our commitment to rigorously evaluating both motor and non-motor outcomes in future work.

For the characterization of neuroinflammation (Figures 1g–h, 2g–h, 3i, 5h, the use of GFAP and Iba1 staining area alone does not distinguish between increased cell number, cell hypertrophy, or morphological transformation. A more robust characterization should include quantification of cell density (GFAP+, Iba1+), microglial morphology classification (e.g., ramified vs. amoeboid), and ideally, cytokine profiling (e.g., qPCR or ELISA). Without these, the claim of neuroinflammation remains only partially supported.

Response: We sincerely thank the Reviewer for this insightful comment and for pointing out the limitations in our characterization of neuroinflammation. In response to the Reviewer’s suggestions, we have quantified microglial soma size, as increases in soma size are indicative of microglial activation (Reviewer Figure; Figs. 1h, 2h; Extended Data Figs. 3m, 4k, 5k). These additional data support aspects of neuroinflammation in our model. However, we acknowledge that our current dataset does not fully substantiate a definitive claim of “neuroinflammation.”



To reflect this limitation and in accordance with the Reviewer’s guidance, we have revised the manuscript terminology throughout (lines 41, 146, 153, 179, 182, 190, 245, 291, 315, 335, 336, 672, 944, 1009) to describe the observed glial changes as “astrogliosis” and “microgliosis” rather than broadly as “neuroinflammation.”

We appreciate the suggestion to include cytokine profiling such as qPCR or ELISA analyses; however, these are beyond the current study’s primary scope, which focuses on dopaminergic neurodegeneration in the midbrain. Nonetheless, we have incorporated a **discussion of this important point** (lines 382–388), stating:

“However, further research is needed to reveal how this metabolite specifically induces midbrain dopaminergic neurotoxicity and whether it contributes to neuroinflammation. While our current data demonstrate astrogliosis and microgliosis, they do not fully support a definitive claim of neuroinflammation. Additional analyses such as cytokine profiling will be necessary

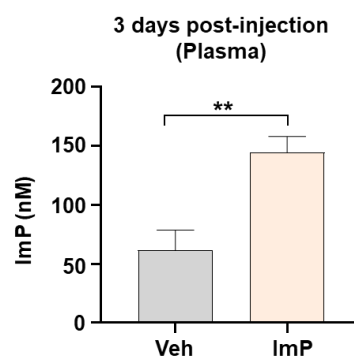
to determine whether the observed gliosis reflects a broader neuroinflammatory response. This represents an important direction for future investigation.”

We hope these revisions address the Reviewer’s concerns and strengthen the clarity and rigor of our manuscript.

In figure 5, the body weight following chronic i.p injections of ImP should be mentioned as it can confound the behavioral readout. In addition, the brain levels of ImP are considerably (~100-200x) lower than in the colonization models (Extended Data Fig. 5a), yet the observed phenotype is worsened. Please provide plasma ImP concentrations to clarify systemic exposure and address this discrepancy.

Response: We sincerely thank the reviewer for their thoughtful comments and careful review of our data.

We would also like to clarify the issue regarding brain ImP levels. The lower concentrations shown in Extended Data Fig. 5a were obtained following a **3-day i.p. injection** protocol, which was specifically **designed to assess the ability of ImP to penetrate the blood–brain barrier and distribute across various brain regions** (as described on pages 14–15, lines 296–302 in the **previous version** of the manuscript). Under these conditions, plasma ImP concentrations averaged approximately 133 nM after 3 days of treatment (see **Reviewer Figure**).

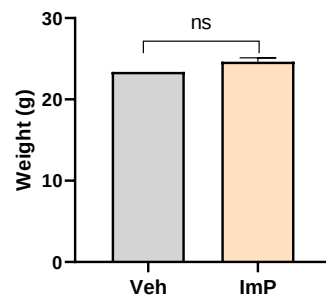


In contrast, the behavioral experiments were conducted following a **21-day i.p. injection** protocol. While this was **described in the Methods section**, we acknowledge that the distinction between the two protocols was not clearly stated in the main text. We have now **clarified this point in the revised manuscript (Line 304-313)**, with the following additions:

“To evaluate its ability to cross the blood–brain barrier and distribute across different brain regions, we first administered imidazole propionate systemically via intraperitoneal injection of either vehicle or imidazole propionate for three days...”

and “Consistent with the observations in *S. mutans*-colonized mice, systemic administration of imidazole propionate for 21 days led to mTORC1 activation.”

Regarding body weight, we **did not observe any significant differences** between the ImP-injected and control groups during the 21-day chronic i.p. treatment in our experiment to establish the treatment conditions (**Reviewer Figure**).



Reviewer #2 (Remarks to the Author):

This is a revised manuscript. The authors have satisfactorily addressed my concerns.

We sincerely thank the Reviewer for their positive feedback and thoughtful evaluation. We truly appreciate the time and effort dedicated to reviewing our manuscript.

Reviewer #3 (Remarks to the Author):

The authors have provided a fairly robust response to my, and the other reviewers initial concerns. I do think the manuscript is largely improved, and the addition of *urda*-expressing *E. coli* is a very strong experimental addition that supports the authors interpretations. There are still some larger, outstanding concerns in this revised manuscript that limit some enthusiasm, however it is overall improved.

We sincerely thank the Reviewer for the thoughtful evaluation and constructive feedback. We are grateful for the recognition of the improvements made in the revised manuscript, particularly regarding the addition of *urda*-expressing *E. coli*.

Major

UrdA in other Streptococcus species

I appreciate the authors' responses in regards to my original concerns regarding the specificity and necessity of UrdA from *S. mutans*.

The authors show, as do prior data including the original description of the underlying human dataset used, that *S. mutans* is preferentially enriched in the PD-associated microbiome compared to other Strep species. This certainly supports the specificity of the association between the species and PD.

However, my original concern regarding *urda* specificity still remains. I apologize since I may not have been clear in my prior comments. Given the authors observe similar relative abundances of other *urda* from other species between those with and without PD, it further raises the question as to whether there is something unique about *urda* from *S. mutans* or if *urda* from these other organisms are also acting similarly.

The alignment the authors show in the reviewer response is not the most convincing that these *urda* homologs would have different activity. Similar to the new data shown with expression of *S. mutans urda* in *E. coli*, if this could be performed with a homologous *urda* that is not differentially abundant in PD, but still prevalent (like that from *S. parasanguinis* or *S. mitis*) it

would be exceedingly informative. If a homologous *urdA* performs similarly, then it would not be the presence of the enzyme itself leading to the altered pathology, but perhaps a species or strain-specific regulation of it.

It is surprising that only a small subset of *urdA* (Reviewer figure a, extended data Fig 1b) is encoded by the *S. mutans* detected in the dataset.

Response: We are very grateful to the Reviewer for their continued thoughtful feedback and for raising this important point regarding the specificity of *urda* from *S. mutans*.

We apologize for any confusion in our previous response and now better understand the Reviewer's request—to clarify whether *UrdA* from other *Streptococcus* species, such as *S. parasanguinis* or *S. mitis*, may exert similar or different functional effects.

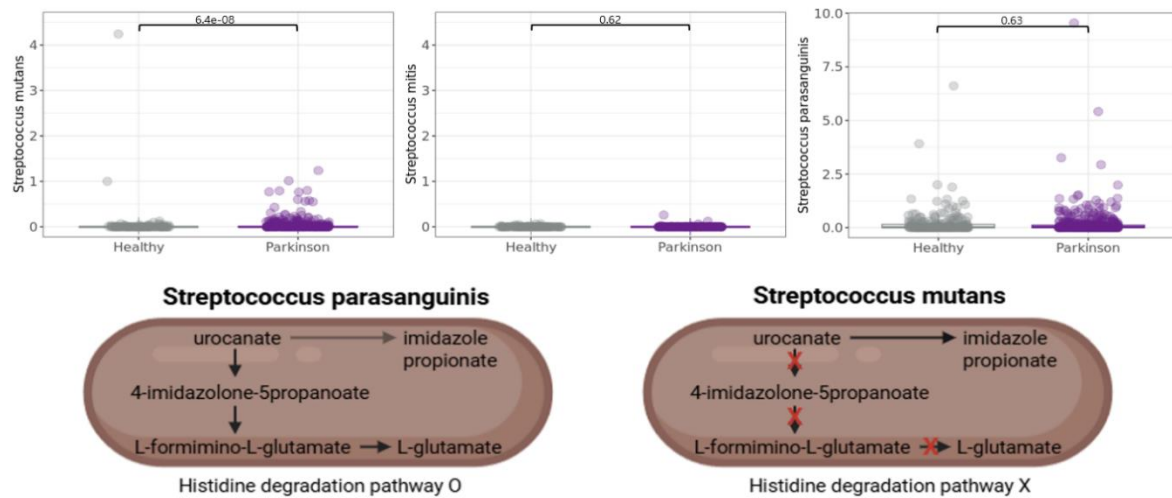
To address this, we have begun exploring *UrdA* structural predictions using AlphaFold3 for *S. mutans* and other *Streptococcus* species (**Reviewer Figure**). Thus far, **these analyses have not revealed major structural differences across homologs.**

[redacted]

However, we agree that structural similarity alone may not fully explain functional specificity. Importantly, we would like to emphasize that *S. parasanguinis* and *S. mitis* are **generally considered commensal species** in the oral microbiome, **unlike the cariogenic and pathogenic *S. mutans***. In fact, *S. parasanguinis* has been reported to competitively inhibit *S. mutans* in various niches.

Moreover, *S. mitis* **abundance was substantially lower than *S. mutans*** in both PD and control microbiomes in the dataset we used (**Reviewer Figure**), making it less likely to be a major contributor to the observed phenotype. *S. parasanguinis*, while more abundant, possesses a **broad urocanate metabolic capability (Reviewer Figure)**: it encodes enzymes that allow urocanate conversion into glutamate **via canonical histidine degradation pathways**. In contrast, *S. mutans* **lacks this pathway and primarily converts urocanate into imidazole**

propionate. This distinction suggests that even if *S. parasanguinis* encodes *urda*, its metabolic context may not favor ImP accumulation, potentially mitigating any pathogenic effect.



As the Reviewer insightfully noted, genomic DNA-based assessments alone do not confirm transcriptional activity. To resolve this, we believe that **future metatranscriptomic analyses will be essential** to determine **the active expression of *urda*** in different microbial contexts.

Importantly, we showed that the downstream functional product of *UrdA*, **ImP, is significantly elevated in the circulation of PD patients, supporting the relevance of the *UrdA*–*ImP* axis in disease pathology**. We hope the Reviewer agrees that this finding provides a meaningful link between microbial metabolism and host phenotype.

Finally, as noted, we are actively working to further characterize *UrdA* homologs from other bacteria species. Based on our preliminary analyses (see **Reviewer Figure**), we predict that there are likely two major classes of *UrdA*, and we are currently pursuing both **structural and functional studies** to investigate this distinction in detail. These ongoing efforts are aimed at directly addressing the reviewer's insightful question. We greatly appreciate this valuable suggestion and look forward to sharing these findings in future work.

[redacted]

To investigate synaptic loss, the authors examined TH fiber density in the SNr. The striatal synapses, where SNpc projects to, is thought to be most vulnerable. So it is unclear why the striatum is not examined, and the SNr is.

Response: We thank the Reviewer for this important comment and fully agree that the striatum, as the primary projection site of SNpc dopaminergic neurons, is a more direct and functionally relevant region to assess synaptic terminal loss in Parkinson's disease models. In response, we have **now quantified the density of TH-positive dopaminergic axon terminals in the striatum**. These data are included **in the revised manuscript as Extended Data Figures 1k, 2i, 3l, and 4i**, providing a more comprehensive evaluation of synaptic loss consistent with the Reviewer's suggestion.

Minor

The behavior performed with the PFF injected animals (extended data Fig 4l, m) is much too under powered given the variability seen. Further, if the authors wish to directly compare between antibiotic-treated and untreated mice, in their response to PFF injection, the experiment should be set-up and performed side-by-side, given technical variabilities in PFF preps and administration. The PFF data presented in the main figures is very rapid compared to prior works, and I appreciate the authors responses in why this may be. However, while I agree it may be true, a direct experimental comparison would be needed to support their claims.

Response: We thank the Reviewer for their thoughtful comments and for acknowledging our supporting experiment involving α -synuclein (aSyn) preformed fibril (PFF) injection into SPF mice. As noted, our goal was to demonstrate that, under our experimental conditions using rigorously quality-controlled aSyn PFF, dopaminergic neurodegeneration is not observed within three weeks in the SPF mice—consistent with prior reports using comparable timelines and protocols. We believe this finding sufficiently establishes that our PFF preparation yields outcomes in SPF mice that align with previously published work. Details of our PFF preparation and quality control, including validation in primary cultured cortical neurons, are provided in the revised Extended data Fig. 4a. That said, we fully agree with the Reviewer that the behavioral data presented in Extended Data Fig. 4l, m are underpowered and subject to high variability. Moreover, we acknowledge that these data were not generated from a study designed to directly compare antibiotic-treated and untreated groups, nor were the behavioral tests performed side-by-side. Considering these limitations, and in agreement with the Reviewer's suggestion, we have removed the behavioral data from the revised manuscript. We

believe that **the absence of dopaminergic neurodegeneration—despite the presence of mild α -synuclein pathology—following the same quality-controlled α -synuclein PFF injection under SPF conditions** provides sufficient evidence to support the conclusions drawn from this experiment.

Line 95- it might be more appropriate to state that the re-analysis “confirmed” the findings regarding Bifidobacterium and Streptococcus, since these taxa were indeed identified in the original analysis.

Response: We thank the Reviewer for careful reading, and we amended the text as requested.

I disagree that the action of imidazole propionate in the small intestine is not physiologically relevant. Mice are coprophagic, and this metabolite, while produced in the colon, would be naturally ingested and absorbed in the SI. In addition, in humans, exposure to this molecule would be through intestinal adsorption.

Response: We thank the Reviewer for this important clarification. We fully agree that exposure to ImP in the small intestine is physiologically relevant, particularly in coprophagic animals like mice. Our intention was not to imply that ImP action in the small intestine lacks physiological significance, but rather to emphasize that—given prolonged exposure—the colon may represent a major site of sustained ImP activity.

In response to R1 and R2 concern re: the original low number of mice used for behavior analysis, the authors’ responded that a prior study used similar N of 4-5 for behavior, calling out Figure 4 of “A gut-derived metabolite alters brain activity and anxiety behavior in mice” It should be noted that Figure 4 of this paper actually uses >20 mice for its behavior testing, and the lower N was for electron microscopy and diffusion tensor imaging. While the increased N in the revised manuscript is appreciated, the authors should be careful in their reading and interpretations of past literature.

Response: We sincerely apologize for the oversight and thank the Reviewer for the correction. Our intention was not to suggest that all experiments in the referenced Nature paper were performed with low sample sizes. We appreciate the Reviewer’s careful reading and will be more cautious in our interpretation and referencing of prior literature. We also thank the Reviewer for acknowledging the increased number of mice included in the revised manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors sufficiently addressed most comments. Regarding the repeated gavage of *E. coli*-*urdA* into GF mice: the fact that the *urdA* plasmid is rapidly lost from the *E. coli* may suggest that it confers a fitness disadvantage and raises the question of whether the *urdA* plasmid (and production of ImP) may lead to downstream alterations in bacterial physiology that confound interpretation of results or mediate effects thought to be caused by ImP production. The authors should discuss this point as a potential limitation

Response: We thank the reviewer for the careful and insightful comments. We have added the following sentence **in the discussion (lines 408-418)**:

“Furthermore, the ongoing development of a bacterial UrdA inhibitor presents a potential therapeutic avenue to limit gut production of the dopaminergic neurotoxic imidazole propionate, based on our observation that UrdA expression in a UrdA-deficient bacterial strain colonized in the gut was sufficient to induce PD-related pathology. However, because we expressed UrdA in an *E. coli* strain that does not naturally harbor this enzyme, we cannot exclude the possibility that plasmid carriage may impose a fitness cost and alter bacterial physiology independently of imidazole propionate production. Future studies using *S. mutans* deficient in UrdA will help disentangle these potential confounding effects. Nonetheless, administration of imidazole propionate alone recapitulated the phenotypes observed with either *S. mutans* or *E. coli* expressing UrdA from *S. mutans*, supporting its role as a key driver of the observed effects.”

Reviewer #3 (Remarks to the Author):

I appreciate the authors' thoughtful responses- this manuscript has been improved over the rounds of review. I have no further concerns that should preclude publication, and believe that this manuscript will be a worthwhile addition to the field.

We sincerely thank the reviewer for their thoughtful feedback and for helping us improve our manuscript.