Gut Microbiota as Modulators and Therapeutic Targets in Parkinson's Disease

Thesis by

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ABSTRACT

The gastrointestinal (GI) tract is a unique junction of the nervous system, immune system, and the gut microbiome. The gut microbiome, a complex community of bacteria, fungi, and viruses, is able to regulate host development, behavior, immunity, and disease. In Parkinson's disease (PD), a neurodegenerative disorder characterized by motor dysfunction, α-synuclein (αSyn) pathology, and common GI symptoms, the gut bacterial composition is significantly altered, with depletions in beneficial, anti-inflammatory taxa compared to healthy controls. This thesis explores whether specific gut bacteria may be disease-protective. We first assembled a consortium of taxa that are reduced in individuals with PD across multiple cohorts and geographies. We find that both therapeutic and prophylactic oral administration of this consortium to Thy-1-αSyn overexpressing (Thy1-ASO) mice, a preclinical model of PD, improves select motor and GI deficits and reduces αSyn pathology in the brain. We next identified three taxa that independently drive motor function improvements, with *Faecalibacterium prausnitzii* producing the most pronounced effects. Further characterization of treatment with *F. prausnitzii* revealed improvements in GI symptoms, reduced αSyn aggregates in the brain, remodeling of the gut microbiome, and induction of anti-inflammatory and tissue-regenerative pathways in the colon. Collectively, these findings provide a foundation for developing specific bacterial species as novel therapeutics for PD and highlight the broader potential of the gut microbiome to transform the way we understand and treat human health and disease.

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A.M. conceived the project, designed and performed experiments, led analyses, created figures, and wrote the manuscript.

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ABBREVIATIONS

ACME Average Causal Mediation Effect

AD Alzheimer's Disease

ADE Average Direct Effect

A. hadrus *Anaerostipes hadrus*

A. muciniphila *Akkermansia muciniphila*

ANOVA Analysis of Variance

ASD Autism Spectrum Disorder

αSyn Alpha-Synuclein

AVNM Ampicillin, Vancomycin, Neomycin, Metronidazole

BBB Blood-Brain Barrier

benCom-PD Beneficial Commensals for PD

B. fragilis *Bacteroides fragilis*

BHIS Brain Heart Infusion - Supplemented

B. ovatus *Bacteroides ovatus*

BSA Bovine Serum Albumin

B. thetaiotaomicron *Bacteroides thetaiotaomicron*

CCL5 C-C motif Chemokine Ligand 5

CD4 Cluster of Differentiation 4

CD8 Cluster of Differentiation 8

CD25 Cluster of Differentiation 25

cfu Colony Forming Unit

CMC Chopped Meat with Carbohydrates

CNS Central Nervous System

COVID-19 Coronavirus Disease of 2019

CSF Cerebrospinal Fluid

Edn3 Endothelin-3

EEC Enteroendocrine Cell

EOS Extremely Oxygen Sensitive

E. rectale *Eubacterium rectale*

FDR False Discovery Rate

F. prausnitzii *Faecalibacterium prausnitzii*

FMT Fecal Microbiota Transplant

FoxP3 Forkhead box P3

F. saccharivorans *Fusicatenibacter saccharivorans*

GABA Gamma-Aminobutyric Acid

GF Germ-Free

GI Gastrointestinal

GO Gene Ontology

GVHD Graft Versus Host Disease

hCom2 human Commensal 2

hCom2-Fp human Commensal 2 without *Faecalibacterium prausnitzii*

HDAC Histone Deacetylase

Hif1a Hypoxia inducible factor-1α

HPLC High-Performance Liquid Chromatography

IBD Inflammatory Bowel Disease

IBS Irritable Bowel Syndrome

IFNγ Interferon gamma

IL-10 Interleukin-10

IL-17 Interleukin-17

L-DOPA Levodopa

L. plantarum *Lactobacillus plantarum*

Ly6C^{hi} Lymphocyte Antigen 6 Complex high

MAM Microbial Anti-inflammatory Molecule

MHCII Major Histocompatibility Complex 2

MLN Mesenteric Lymph Nodes

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MS Multiple Sclerosis

NaHCO3 Sodium Bicarbonate

NCX3 Sodium (Na)/Calcium (Ca) exchanger 3

ns Not Significant

PBS Phosphate Buffered Saline

PCoA Principal Coordinate Analysis

PD Parkinson's Disease

PERMANOVA Permutational Multivariate Analysis of Variance

PFA Paraformaldehyde

PFF Pre-Formed Fibril

P. histicola *Prevotella histicola*

pS129 Phosphorylated-Serine-129

PYG Peptone Yeast Extract Broth with Glucose

RANTES Regulated upon activation, normal T cell expressed and secreted

R. faecis *Roseburia faecis*

R. intestinalis *Roseburia intestinalis*

RNA Ribonucleic Acid

ROS Reactive Oxygen Species

SCFA Short-Chain Fatty Acid

sCOD Soluble Chemical Oxygen Demand

SELENOS Selenoprotein S

SGB Species-level Genome Bins

Slc8A3 Solute Carrier Family 8 Member 3

SNpc Substantia Nigra pars compacta

SPF Specific Pathogen-Free

TBS-T Tris-Buffered Saline with Tween-20

TF Transferrin

TFRC Transferrin Receptor

Thy1-ASO Thy1-Alpha Synuclein Overexpressing

TLR Toll-Like Receptor

TNFα Tumor Necrosis Factor alpha

Tnfrsf12a Tumor necrosis factor superfamily member 12a

T_{REG} Regulatory T cell

TTR Transthyretin

TWEAK TNFα-like Weak inducer of Apoptosis

UPDRS Unified Parkinson's Disease Rating Scale

VFA Volatile Fatty Acid

WT Wildtype

YCFAC Yeast Casitone Fatty Acids with Carbohydrate

Chapter 1

INTRODUCTION

In the early 1900s, Ilya Metchnikov theorized on the contributions of toxic bacteria in the human gut to aging and suggested the use of lactic acid 'orthobiosis' – what is now referred to as probiotics – as a means to prolong life (Metchnikov, 1907; Podolsky, 2012). His theories, largely overlooked until the mid-1990s, have since been widely studied and accepted. Indeed, over the past decade alone, there have been over 4,400 publications on the microbiome (Yuan et al., 2021). These studies have revealed the central role of the gut microbiome in human health and disease, with broad implications across gastrointestinal, immune, and neurological conditions. Today, probiotics composed of lactic acid-producing bacteria are commonly used as dietary supplements. However, the development of personalized or condition-specific probiotics is an area that is only beginning to be explored. This thesis will examine the importance of the gut microbiome overall and as it pertains to Parkinson's disease, and evaluate the use of defined bacterial consortiums and single bacterial strains as potential therapeutics in a preclinical mouse model of disease.

The gut microbiome

The human gastrointestinal (GI) tract is home to trillions of microbial organisms, including bacteria, fungi, archaea, and viruses that have evolved to live in symbiosis with the host and are collectively called the gut microbiome (Hou et al., 2022). The gut is colonized by microbes immediately at birth, and these early microbial interactions play a critical role in shaping brain and immune system development through adolescence (Derrien, Alvarez & Vos, 2019). The composition of the microbiome is unique to each individual and is influenced by a combination of genetic, environmental, and lifestyle factors. By approximately three years of age, the gut microbiome is fully established and remains stable throughout most of the lifespan, before undergoing a decline in diversity and beneficial species during old age (Ghosh, Shanahan, & O'Toole, 2022).

Commensal bacteria residing in the gut can exert both beneficial and detrimental effects on host physiology through metabolism of gut luminal contents as well as direct interaction with immune and epithelial cells. The gut microbiome provides numerous benefits to the host, including regulation of the immune system, breakdown of complex dietary

components, production of metabolites, and importantly, colonization resistance against pathogenic microorganisms (Jandhyala et al., 2015). In a healthy state, the gut microbial community exists in a homeostasis, in which beneficial commensal bacteria exert anti-inflammatory effects to maintain immune tolerance and restrict the expansion of potentially pathogenic species. In addition to direct interactions of bacterial components with epithelial and immune cells, anti-inflammatory effects are also mediated through the microbial metabolism of indigestible carbohydrates into short-chain fatty acids (SCFAs), which exert downstream immunoregulatory effects by modulating gene expression and signaling pathways (Mann, Lam & Uhlig; Al Bander et al., 2020). Disruption of this balance, a state known as dysbiosis, can arise from factors such as antibiotic use, poor diet, stress, or exposure to toxins, ultimately compromising the gut microbiome's protective functions (Belizario & Faintuch, 2018; Hou et al., 2022). Dysbiosis is associated with impaired epithelial barrier integrity, metabolic dysfunction, and chronic inflammation, thereby contributing to diseases such as inflammatory bowel disease (IBD), obesity, diabetes, and cancer (Belizario & Faintuch, 2018; Zhang et al., 2015).

The gut microbiome and inflammation

During dysbiosis, populations of beneficial anti-inflammatory commensal species decline, enabling the expansion of potentially pathogenic bacteria and subsequent activation of gut-resident immune cells. The resulting inflammatory processes increase levels of reactive oxygen species (ROS), which contribute to tissue damage and further perturb the gut environment (Zhao et al., 2023). Loss of epithelial barrier integrity – commonly referred to as 'leaky gut'— allows gut luminal contents, including bacterial components, to translocate into the bloodstream. These microbial products are recognized as foreign by the host immune system, triggering systemic inflammation and promoting inflammatory responses in distal tissues, while further exacerbating inflammation in the gut (Camilleri, 2019). Pathogenic bacteria often possess mechanisms that allow survival under elevated ROS conditions, whereas obligate anaerobic commensals lack such adaptations, thereby reinforcing gut microbial imbalance (Zeng, Inohara, & Nunez, 2016; Baumler &

Sperandio, 2016). Together, these processes establish a self-perpetuating cycle of local and systemic inflammation, thus contributing to both the onset and progression of underlying disease. Importantly, systemic inflammation can also compromise the integrity of the blood–brain barrier (BBB), extending inflammatory processes to the central nervous system (CNS) (Wardill et al., 2016; Elahy et al., 2015).

The gut-brain axis

There is continuous bi-directional communication between the gastrointestinal tract and the brain. The gut and the gut microbiome contribute substantially to the host neurochemical environment through the production of neurotransmitters and neuropeptides; for instance, approximately 90% of the body's serotonin is synthesized in the gut by enterochromaffin cells (Gershon & Tack, 2007; Yano et al., 2015) and microbes such as *Bacteroides* and *Escherichia* can produce γ-aminobutyric acid (GABA) (Strandwitz et al., 2019; Morais, Schreiber, & Mazmanian, 2020). Physically, this connection is mediated by the vagus nerve, which provides direct signaling between the gut and the brain. Studies have revealed a direct connection from the gut lumen to the brain via specialized enteroendocrine cells (EECs) located throughout the gut epithelium (Kaelberer et al., 2018). EECs sample luminal contents on their apical side and form synapse-like connections with vagal afferent neurons on the basal side, thereby enabling signaling and also transport of bacterial-derived molecules, such as amyloid proteins (which are implicated in Alzheimer's and Parkinson's disease pathology), to the brain (Chandra et al., 2016). This connection highlights the capacity of the gut microbiome to directly influence host brain development, behavior, and cognition in health and disease.

Several neurological conditions have been found to be associated with changes in the gut microbial community. Patients with autism spectrum disorder (ASD), multiple sclerosis (MS), Alzheimer's and Parkinson's disease have all been shown to have an altered microbiome compared to healthy controls. Children diagnosed with ASD often experience gastrointestinal symptoms such as diarrhea and abdominal pain (Babinska et al., 2016; Taniya et al., 2022) and have been found to have increased abundance of potentially

pathogenic *Clostridium* and *Desulfovibrio* species and reduced beneficial taxa such as *Bifidobacteria* and *Prevotella* (Anaclerio et al., 2024; Peralta-Marzal et al., 2024). MS patients lack the immune-regulating microbes *Prevotella, Faecalibacterium prausnitzii,* and *Bacteroides*, with elevated levels of *Akkermansia muciniphila* and *Methanobrevibacter* species, which also fluctuate with severity of disease (Zhou et al., 2022; Mirza et al., 2020). The Alzheimer's disease (AD) gut microbiome exhibits reduced diversity, with decreased abundances of the beneficial *Lachnospiraceae* and *Ruminococcaceae* and increased pro-inflammatory *Proteobacteria* (Murray et al., 2022). In Parkinson's disease (PD), patients often experience constipation up to 20 years prior to any motor dysfunction onset and also have decreased diversity and depleted SCFA-producing anti-inflammatory commensals, such as *Faecalibacterium prausnitzii, Roseburia,* and *Blautia* species (Kalia & Lang, 2015; Sampson, 2020). Each condition is characterized by a distinct microbial signature, but a trend towards the reduction of beneficial commensal microbes and increases in pro-inflammatory species is consistent across the board. Whether these changes are the cause or consequence of the underlying condition is a question that continues to be explored. Nonetheless, it is evident that the gut bacteria, at least in part, are able to modulate symptoms across a range of neurological disorders.

Microbiome interventions for neurological disease

With increasing evidence implicating the gut microbiome in a wide range of diseases, research exploring strategies to modulate the microbiome as potential therapeutics has expanded considerably. One such approach is fecal microbiota transplant (FMT), in which stool from a healthy donor is transferred to a patient with the aim of restoring microbial homeostasis and ameliorating symptoms. Studies using preclinical models demonstrate benefits with the use of FMTs across neurological conditions such as ASD, MS, AD, and PD (reviewed in Li, Wang, & Zhang, 2022; Yang, Chen, & Cai, 2023; Matheson & Holsinger, 2023), with broad effects associated with restored microbial diversity and reduced inflammation. In clinical trials, FMTs have been implemented with some success in children with ASD, improving core symptoms and GI disturbances (Li et al., 2024;

Maniscalco et al., 2025) and in Parkinson's disease, where some patients experienced improved intestinal motility and reduced motor symptoms (Bruggeman et al., 2024; Cheng et al., 2023; DuPont et al., 2023; Scheperjans et al., 2024). However, this intervention brings inherent risks, is costly and challenging to administer, and its beneficial effects are often variable or diminish over time.

Probiotics are a more targeted approach to gut microbiome modulation. Most currently available probiotic formulations consist of *Lactobacillus* and *Bifidobacterium* species. These microbes are relatively easy to culture and possess long shelf-life, making them practical for commercial development and over-the-counter use. These probiotic species have been shown to improve gut motility and barrier integrity, and reduce inflammation, thus indirectly benefiting potential disease symptoms (Mitelmao et al., 2022; Camilleri, 2021; Faghfouri et al., 2023). *Lactobacillus reuteri*, a species commonly found in probiotics, is shown to improve social behavior in ASD (Sgritta et al., 2020; Mazzone et al., 2024). *Lactobacillus plantarum*, a recently commercialized probiotic, mildly improves symptoms in preclinical mouse models, as well as Parkinson's disease patients (Liao et al., 2020; Lee et al., 2023; Lu et al., 2021). Paradoxically, *Lactobacillus* and *Bifidobacterium* species are the most elevated microbes in the Parkinson's disease gut microbiome (Sampson, 2020), raising questions about their therapeutic potential in this condition.

Anaerobic commensal bacteria are only beginning to be used as probiotics, despite evidence of their therapeutic potential in preclinical mouse models. For example, *Prevotella histicola* has been shown to be as effective as prescribed medication for the treatment of MS through increases in regulatory T cells in the gut and reduction of pro-inflammatory T cells in the CNS (Shahi et al. 2019). In an animal model of ASD, autism-like behaviors were corrected by the administration of *Bacteroides fragilis* (Hsiao et al., 2013). Both *Clostridium butyricum* and *Akkermansia muciniphila* induce improvements in motor deficits and dopaminergic neuron loss in a mouse model of Parkinson's disease (Sun et al., 2021; Qiao et al., 2024). Although *A. muciniphila* is not yet used for the treatment of neurological conditions, it is the first commensal bacterium to be commercialized as a

probiotic supplement, likely owing to its efficacy even in pasteurized form, and has been shown to be beneficial for the management of obesity and type 2 diabetes (Everard et al., 2013; Depommier et al., 2020; Pendelum® 2025). Therefore, targeted probiotic use as a therapeutic strategy for neurological diseases remains an ongoing area of research, requiring further validation in preclinical models and expansion into clinical studies.

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C h a p t e r 2

A HUMAN COMMENSAL CONSORTIUM IMPROVES PARKINSON'S-ASSOCIATED OUTCOMES IN A PRECLINICAL MOUSE MODEL

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"*Faecalibacterium prausnitzii,* depleted in the Parkinson's disease microbiome, improves motor deficits in α-synuclein overexpressing mice"
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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor dysfunction and accumulation of α-synuclein (αSyn) aggregates in the substantia nigra, and is frequently accompanied by gastrointestinal (GI) symptoms. The gut microbiome composition is significantly altered in PD patients, with depletions in microbial taxa with anti-inflammatory properties compared to healthy controls. To explore whether specific gut bacteria may be disease-protective, we assembled a microbial consortium ("benCom-PD") of 8 human-associated taxa that are reduced in individuals with PD across multiple cohorts and geographies. Therapeutic (short-term) oral administration of benCom-PD to α-synuclein overexpressing (Thy1-ASO) mice, an animal model of PD, improved select motor and GI deficits. Extended, or prophylactic, supplementation with benCom-PD further ameliorated motor and GI dysfunction. Accordingly, animals receiving treatment exhibited reduced αSyn in the striatum. Immune profiling revealed trends towards reduction of inflammatory cytokines and significantly reduced interleukin-10 (IL-10) in colon tissue of short-term treated animals; however, flow cytometric analysis of multiple tissues from long-term treated mice did not reveal any changes. Additionally, in mice receiving prophylactic administration, gut microbiome composition and volatile fatty acid levels remained unaffected by treatment, suggesting an involvement of alternate mechanisms. These findings expand our current knowledge on the effects of gut microbes in PD and embolden future uses of targeted bacterial interventions as therapeutics.

Introduction

Parkinson's disease is the second most common neurodegenerative disorder, affecting over 10 million people globally and 1% of individuals over the age of 60 (Tysnes et al., 2017; Poewe et al., 2017). The diagnosis of PD is rapidly rising due to increased lifespan and potentially to certain environmental exposures (Dorsey et al., 2018). Synucleinopathies such as PD, Lewy body dementia, and multiple system atrophy, among other disorders, are characterized by aggregation of the neuronal protein α-synuclein (αSyn) (Koga et al., 2021). αSyn pathology can lead to inflammation, neuronal dysfunction, and ultimately to the death of dopaminergic neurons in the nigrostriatal pathway of the substantia nigra pars compacta (SNpc), resulting in motor symptoms such as tremors, rigidity, gait impairment, and bradykinesia (Hawkes et al., 2010; Tysnes et al., 2017; Poewe et al., 2017). Current treatments provide symptomatic relief, but can have considerable side effects, present dosing challenges, and often lose efficacy over time. At present, there are no disease-modifying drugs for PD (Poewe et al., 2017; Pardo-Moreno et al., 2023).

Although PD is predominantly classified as a brain disorder, a role for non-motor symptoms and peripheral organs dates back over 200 years. In the seminal 1817 "Essay on the Shaking Palsy", Parkinson observed that many patients had symptoms of gastrointestinal distress, particularly constipation. In a case of early onset of the disease, the patient was prescribed calomel and Epsom salts to clear the bowels, after which all symptoms, including tremors, were eliminated (Parkinson, 2002). Indeed, 60-80% of PD patients experience GI symptoms – primarily constipation, but also deficits in gastric emptying, abdominal pain, and increased intestinal permeability – that appear up to 20 years prior to a PD diagnosis (Forsyth et al., 2011; Yang et al., 2019). Other non-motor symptoms, including hyposmia, depression, and REM sleep behavior disorder, also manifest in the prodromal phase (Pellicano et al., 2007; Galbiati et al., 2019; Postuma et al., 2016). Braak's hypothesis, and more recently the "brain-first vs. body-first" hypothesis (Dorsey et al., 2024; Berg et al., 2021), postulates that αSyn aggregation may start in peripheral tissues such as the GI tract, spread via the vagus nerve, and eventually

reach the brainstem, substantia nigra, and neocortex (Braak et al., 2003). Indeed, injection of αSyn aggregates (as pre-formed fibrils [PFFs] or other pathogenic αSyn species) into the intestines of mice and rats results first in GI symptoms, and eventually leads to neurodegeneration in the brain and motor deficits (Kim et al., 2019; Liu et al., 2017; Svensson et al., 2015; Challis et al., 2020). Severing the vagus nerve in animals halts propagation of gut-induced αSyn pathology into the brain (Kim et al., 2019) and epidemiologic data suggest that vagotomies are protective against development of PD in humans (Liu et al., 2017; Svensson et al., 2015). Whether PD can originate outside the brain, and potentially in the GI tract, is a timely yet still unresolved question.

Gut microbiome analyses of stool samples have revealed that individuals with PD harbor a distinct microbial profile compared to non-PD controls. PD patients show increased abundance of potential pathobionts and decreased levels of beneficial bacteria including *Blautia, Roseburia*, and *Faecalibacterium* species (Sampson, 2020; Hill-Burns et al., 2017; Aho et al., 2019; Bedarf et al., 2017; Wallen, et al., 2022). A recurring feature observed across numerous cohorts is loss of short-chain fatty acid (SCFA)-producing bacteria, many of which promote anti-inflammatory pathways (Elford et al., 2024; Dalile et al., 2019; Fernandez et al., 2016). Indeed, the gut microbiome plays a unique role in modulating host immune responses, particularly by inducing regulatory T cells (T_{REGS}) which maintain immune tolerance and suppress inflammation (Sakaguchi et al., 2008). Treatment with T_{REG}-inducing commensals shows therapeutic efficacy in preclinical models of inflammatory bowel disease (IBD) (Qiu et al., 2013; Zhou et al., 2018) and multiple sclerosis (MS) (Shahi et al., 2019), among others. In PD, studies have demonstrated the protective effects of *Lactobacillus planetarium* (Liao 2020, Ma 2023), *Lacticaseibacillus rhamnosus* (Xie & Prasad, 2020; Liu et al., 2022), as well as individual or multistrain probiotics containing Lactobacilli and Bifidobacteria (Ibrahim et al., 2020; Perez Visnuk et al., 2020; Srivastav et al., 2019). Paradoxically, while treatments with Lactobacilli and Bifidobacteria have shown mild clinical improvements (Hamilton et al., 2024; Tan et al., 2021; Ibrahim et al., 2020; Barichella et al., 2016), these taxa are among the most enriched in the PD patient microbiome (Sampson, 2020),

raising questions about their therapeutic potential in the context of PD. Exploring the efficacy of microbes that are depleted in PD is a more intuitive therapeutic approach, yet remains largely unexplored.

Results

A human gut microbial consortium is therapeutic in an aSyn-based model of PD

To investigate the potential therapeutic benefits of gut microbes in PD, we used transgenic Thy1-ASO mice that overexpress the native human αSyn gene under a modified Thy1 promoter, driving protein expression in many neuronal subtypes throughout the body and brain (Chesselet et al., 2012). Thy1-ASO mice exhibit clinical features of synucleinopathies, including human PD, such as αSyn pathology in the gut and brain, neuroinflammation, mitochondrial deficits, and progressive age-dependent deficits in motor and GI function (Chasselet et al., 2012; Richter et al., 2023).

We assembled a defined microbial consortium composed of eight culturable type strains that represent the closest relatives of bacterial taxa depleted in stool samples across multiple human PD cohorts, which we termed "benCom-PD" for *ben*eficial *com*mensals for *PD* (**Fig. 1A**). BenCom-PD was intragastrically administered to specific pathogen-free (SPF; standard laboratory microbiome) Thy1-ASO mice twice weekly for approximately 8 weeks, beginning at the mature age of 12 weeks, to model a therapeutic intervention (**Fig. 1B**). Correspondingly, wildtype (WT) and Thy1-ASO control animals received a vehicle gavage. At 20 weeks of age, motor and GI function was evaluated using a battery of standard behavioral tests. Mice receiving benCom-PD exhibited improvements in motor performance tasks compared to vehicle-treated controls, with reduced errors per step on the beam traversal test, indicating improved gross motor function, and significantly less pronounced hindlimb clasping, which reflects reduced striatal dysfunction (Zhang et al., 2014) (**Fig. 1C-G**). We did not observe significant improvements in time to traverse the beam, wire hang, pole descent, or adhesive removal tasks, although trends towards reduced latency to descend the pole and remove the adhesive are evident. GI symptoms were mildly improved; namely, percentage of water

content of fecal pellets was increased in treated animals, indicating that benCom-PD ameliorates stool consistency and constipation (**Fig. 1H-J**). However, no significant changes in fecal output, fecal score, total gut transit, and bead expulsion assays were found.

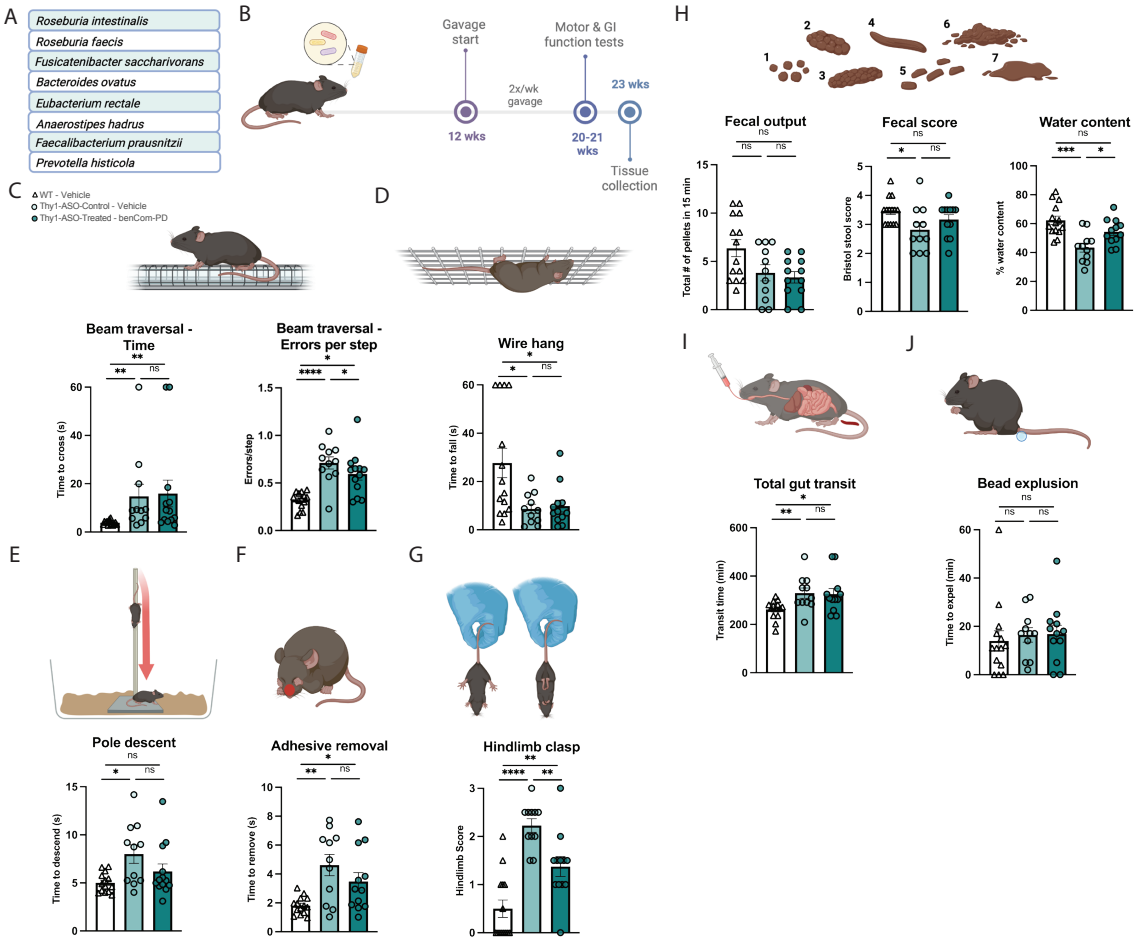

Figure 1. Short-term treatment with a commensal consortium ameliorates select measures of motor and GI dysfunction in Thy1-ASO mice. (**A**) Composition of the benCom-PD consortium consisting of 8 taxa that are reduced in the PD microbiome. (**B**) Animal treatment and testing timeline. (**C-G**) Assays used to assess motor function after benCom-PD treatment of Thy1-ASO mice compared to controls. BenCom-PD-treated animals showed improvements on beam traversal – errors/step and hindlimb clasp. (C) Beam traversal assay, an assessment of balance and coordination, measured by time to cross the beam and errors per step during a trial. (D) Wire hang assay, an assessment of grip strength, as measured by time taken to fall from the wire mesh. (E) Pole descent test, a measure of gross motor function determined by time required to descend a pole. (F) Adhesive removal, an assessment of fine motor function, measured by time taken to remove adhesive from nose bridge. (G) Hindlimb clasp, an assessment of striatal

function, determined by an assigned clasping score, where 0 indicates no clasping and 3 indicates severe clasping. (**H-J**) Assays used to assess GI function with benCom-PD treatment of Thy1-ASO mice compared to controls. BenCom-PD-treated animals showed improvements on the water content assay. (H) Tests of general constipation – fecal output, a measure of fecal pellets produced within 15 minutes; fecal score, a measure of stool consistency using the Bristol Stool Scale; water content, the percentage of water found in fecal pellets. (I) Total gut transit time, a measure of whole gut motility determined by time taken for a red dye to transit. (J) Bead expulsion, a measure of rectal function determined by time taken to expel a glass bead. For more detailed descriptions of behavior tasks, refer to **Chapter 5 - Methods**. N = 11-14. Points represent individual animals; bars represent standard error of the mean. Data analyzed by Kruskal-Wallis test followed by the Conover-Iman post-hoc test with Benjamini-Hochberg false discovery rate (FDR) correction. Images created using BioRender (Moiseyenko, A., 2025, https://BioRender.com/zvkyy6g). ns – not significant; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001

Accordingly, motor and GI function improvements in mice treated with benCom-PD for 8 weeks were accompanied by a reduction of phosphorylated-S129 α-synuclein (pS129-αSyn), a pathogenic modification that correlates with disease measures (Anderson et al., 2006), and aggregated αSyn in the striatum, but not in the substantia nigra (**Fig. 2A-H)**. An assessment of select cytokines in colon tissue revealed a significant reduction in interleukin-10 (IL-10) levels, suggesting a decreased need for immunomodulatory signaling in benCom-PD-treated animals compared to Thy1-ASO controls (**Fig. 2I**). Pro-inflammatory cytokines also trended towards a decrease in treated colon tissue, indicating overall reduced inflammatory processes in treated animals. Together, these findings demonstrate that a therapeutic supplementation with specific commensal microbes commonly depleted in human PD improves some symptoms and pathology in the Thy1-ASO model.

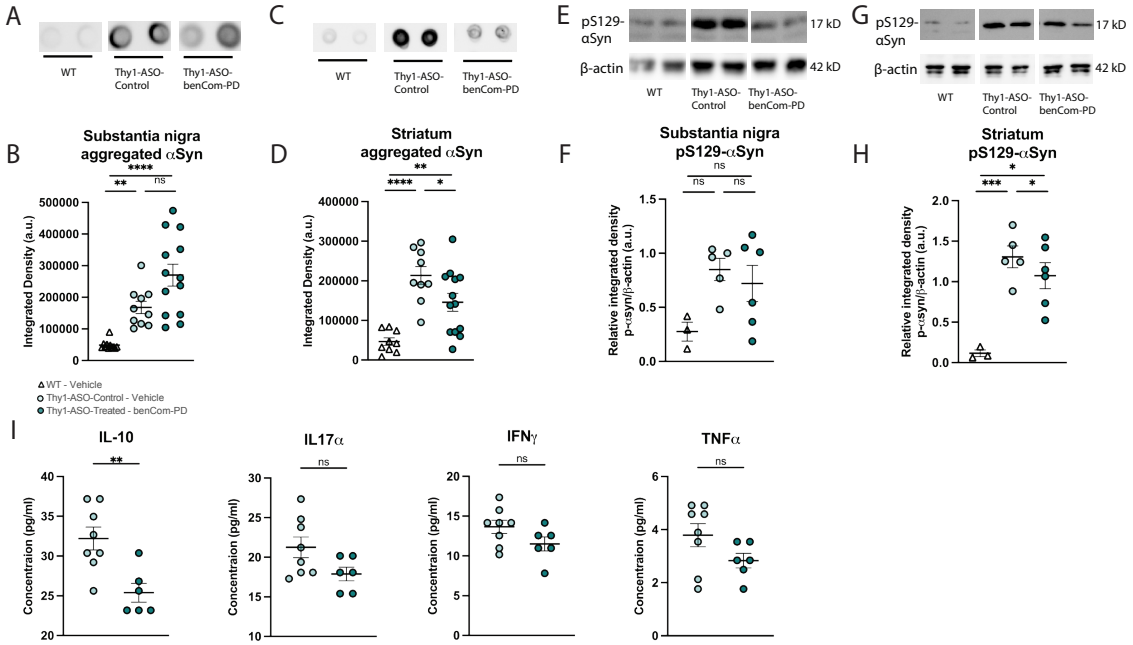

Figure 2. Short-term treatment with benCom-PD partially rescues αSyn pathology in the striatum and reduces IL-10 levels in gut tissue. (**A-D**) Representative images (A, C) and quantification (B, D) of dot blots for aggregated α-synuclein in the substantia nigra and striatum, N = 10-13. (**E-H**) Representative images (E, G) and quantification (F, H) of Western blots for phosphorylated-S129-α-synuclein in nigral and striatal tissue, N = 3-6. (**I**) Interleukin-10, interleukin-17, interferon-γ, and tumor necrosis factor α concentrations in colon tissue as measured by multiplex bead assay, N = 6-8. Points represent individual animals, bars represent standard error of the mean. Data analyzed by one-way ANOVA with Tukey post-hoc test. ns – not significant; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001

Prophylactic administration of benCom-PD further ameliorates symptoms in Thy1-ASO mice

To determine whether prolonged benCom-PD administration could enhance improvements observed with therapeutic intervention, SPF Thy1-ASO mice were gavaged twice weekly for approximately 15 weeks, beginning at 5 weeks of age, as a prophylactic treatment (**Fig. 3A**). At 20 weeks of age, motor and GI function was evaluated using the same battery of behavioral tests (see **Fig. 1)**. Animals receiving long-term benCom-PD supplementation exhibited further improvements in motor performance

compared to short-term treated animals. Latency in the adhesive removal test was reduced, indicating improved fine motor coordination, accompanied by significantly less pronounced hindlimb clasping, signifying reduced striatal dysfunction (**Fig. 3B**). Interestingly, here we did not observe significant effects on the beam traversal test, nor on the pole descent and wire hang assays. GI function was also further improved; notably, benCom-PD-treated mice expelled a rectally-inserted glass bead more rapidly than controls and produced fecal pellets with higher scores on the Bristol stool scale, suggesting alleviation of constipation-like symptoms (**Fig. 3C**). Significant changes in fecal output, water content, and total gut transit time were not observed. Amelioration of motor and GI dysfunction in mice treated with benCom-PD was again accompanied by a reduction in pS129-αSyn in the striatum (**Fig. 3F, G)**, but not in the substantia nigra (**Fig. 3D, E**), and no differences were detected in abundance of aggregated αSyn (**Fig. 3H-K**). Taken together, these findings indicate that prophylactic supplementation with the commensal microbes in benCom-PD further alleviates PD-related symptoms and pathology in this mouse model, although the pattern of improvement differs slightly from that observed with therapeutic intervention.

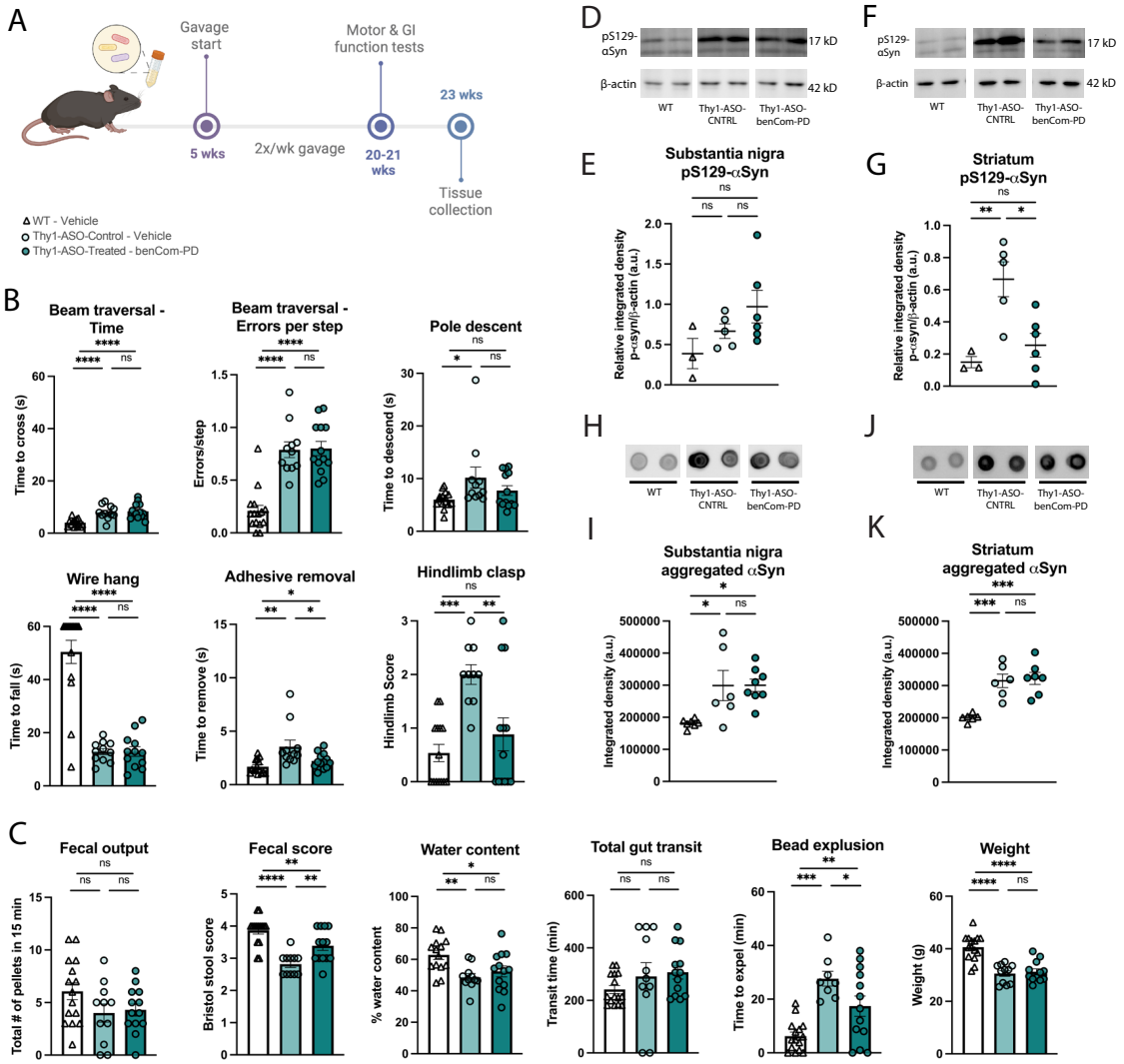

Figure 3. Long-term treatment with benCom-PD further ameliorates motor and GI dysfunction and reduces pS129-αSyn pathology in the striatum. (**A**) Animal treatment and testing timeline. (**B**) Motor function assays used to assess effects of benCom-PD treatment of Thy1-ASO mice compared to controls. BenCom-PD-treated animals showed improvements on adhesive removal and hindlimb clasp assays. (**C**) GI function assays used to evaluate effects of benCom-PD treatment. BenCom-PD-treated mice showed improvements in fecal score and bead expulsion assays. N = 11-15. (**D-G**) Representative images (D, F) and quantification (E, G) of Western blots for phosphorylated-S129-α-synuclein in the substantia nigra and striatum, N = 3-6. (**H-K**) Representative images (H, J) and quantification (I, K) of dot blots for aggregated α-synuclein in the substantia nigra and striatum, N = 6-8. Points represent individual animals; bars represent standard error of the mean. Behavior data in (B, C) analyzed by Kruskal-Wallis test followed by the Conover-Iman post-hoc test with Benjamini-Hochberg false discovery rate (FDR)

correction; protein data in (D-K) analyzed by one-way ANOVA with Tukey post-hoc test. ns – not significant; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001

Thy1-ASO microbiome remains largely unchanged following long-term administration of benCom-PD

The fecal microbiome is altered in Thy1-ASO mice and other PD models compared to WT mice (Sampson et al., 2025, Abdel-Haq et al., 2022; Aktas et al., 2023; Yang et al., 2018). Metagenomic analysis revealed that benCom-PD treatment did not significantly alter the gut microbiome composition in Thy1-ASO mice (**Fig 4**). On the phylum level, relative abundance profiles were consistent across groups, with no notable differences across conditions (**Fig. 4A**). At the species genome bin (SGB) level, Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarity and pairwise comparisons indicated some compositional changes between WT and Thy1-ASO-control animals, with the benCom-PD treated microbiome more closely resembling the WT profile; however, differences did not reach statistical significance (**Fig. 4B, C**). Differential abundance analysis of benCom-PD treated mice relative to Thy1-ASO controls revealed only trends towards increases in specific taxa (**Fig. 4D**), and no significant changes were observed in community diversity as measured by observed richness, Shannon diversity, or the Firmicutes/Bacteroidetes ratio (**Fig. 4E**). Interestingly, the presence of benCom-PD community members was not detected in the samples, likely owing to the limited ability of human commensal species to colonize the mouse gut. These results suggest that improvements in PD-like pathology in the Thy1-ASO animals upon benCom-PD treatment are independent of changes to the gut microbiome.

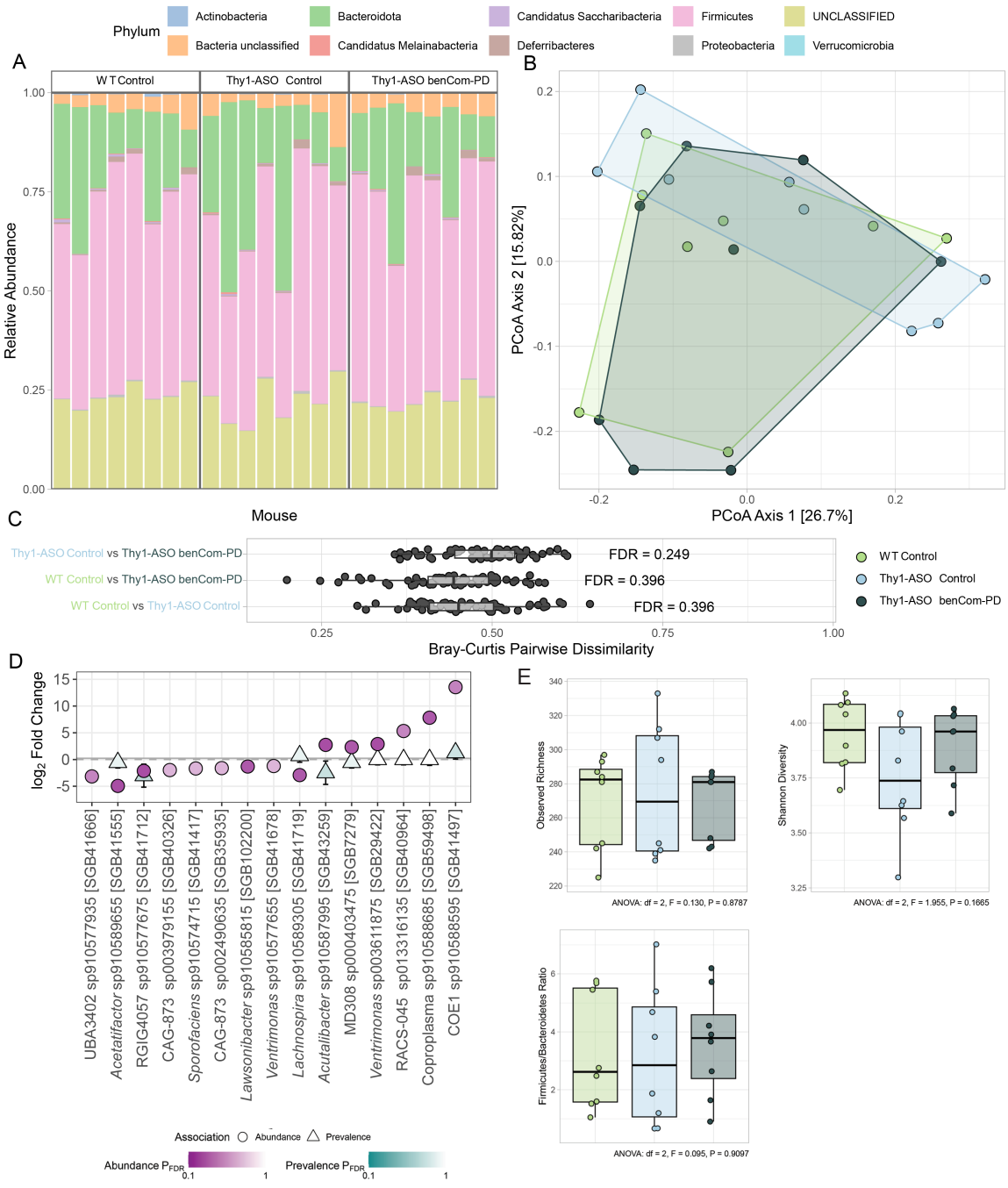

Figure 4. The gut microbiome of Thy1-ASO mice is minimally affected by benCom-PD treatment. (**A**) Stacked bar plot of relative abundance of bacterial phyla in the gut microbiome, including unclassified reads. Each bar represents a single mouse. (**B**) Principal Coordinate Analysis (PCoA) of the Bray-Curtis dissimilarity of fecal microbiome composition of each group. Brackets represent variance explained by each axis. (**C**) Boxplot of pairwise dissimilarity. Statistical significance was calculated using pairwise permutational multivariate analysis of variance (PERMANOVA) with

Benjamini-Hochberg adjusted false discovery rate (FDR). An FDR greater than 0.05 suggests high similarity between the two compared groups. (**D**) Differential abundance and/or prevalence of the top 15 species-level genome bins (SGBs) in benCom-PD-treated Thy1-ASO mice compared to vehicle-treated Thy1-ASO controls. Analysis was performed in MaAsLin3 on log2-transformed relative abundance values scaled between 0 and 1 with no further adjustments. The SGB nomenclature was enriched using the Genome Taxonomy Database provided in MetaPhlAn v4.1.1. (**E**) Distributions of observed fecal SGB richness, Shannon diversity, and Firmicutes/Bacteroidetes ratio. Data were analyzed using a one-way ANOVA. Refer to the **Supplementary Data** file for all additional values and complete taxonomy.

benCom-PD supplementation does not alter the immune landscape or SCFA profiles in Thy1-ASO mice

Nearly all species compiled in benCom-PD have been attributed immunomodulatory properties and are major SCFA producers (Han et al., 2024; Liu et al., 2024; Ihekweazu et al., 2019; Lu et al., 2022; Marietta et al., 2016). To determine if immunomodulatory effects may be contributing to improvements seen in benCom-PD-treated mice, we performed flow cytometric analysis of adaptive and innate immune profiles in the spleen, mesenteric lymph nodes (MLNs), superficial cervical lymph nodes, and meninges, with a focus on potential shifts in pro- and anti-inflammatory processes. Immune profiles remained unchanged across all tissues and conditions. A representative analysis in the spleen is shown in **Fig. 5**.

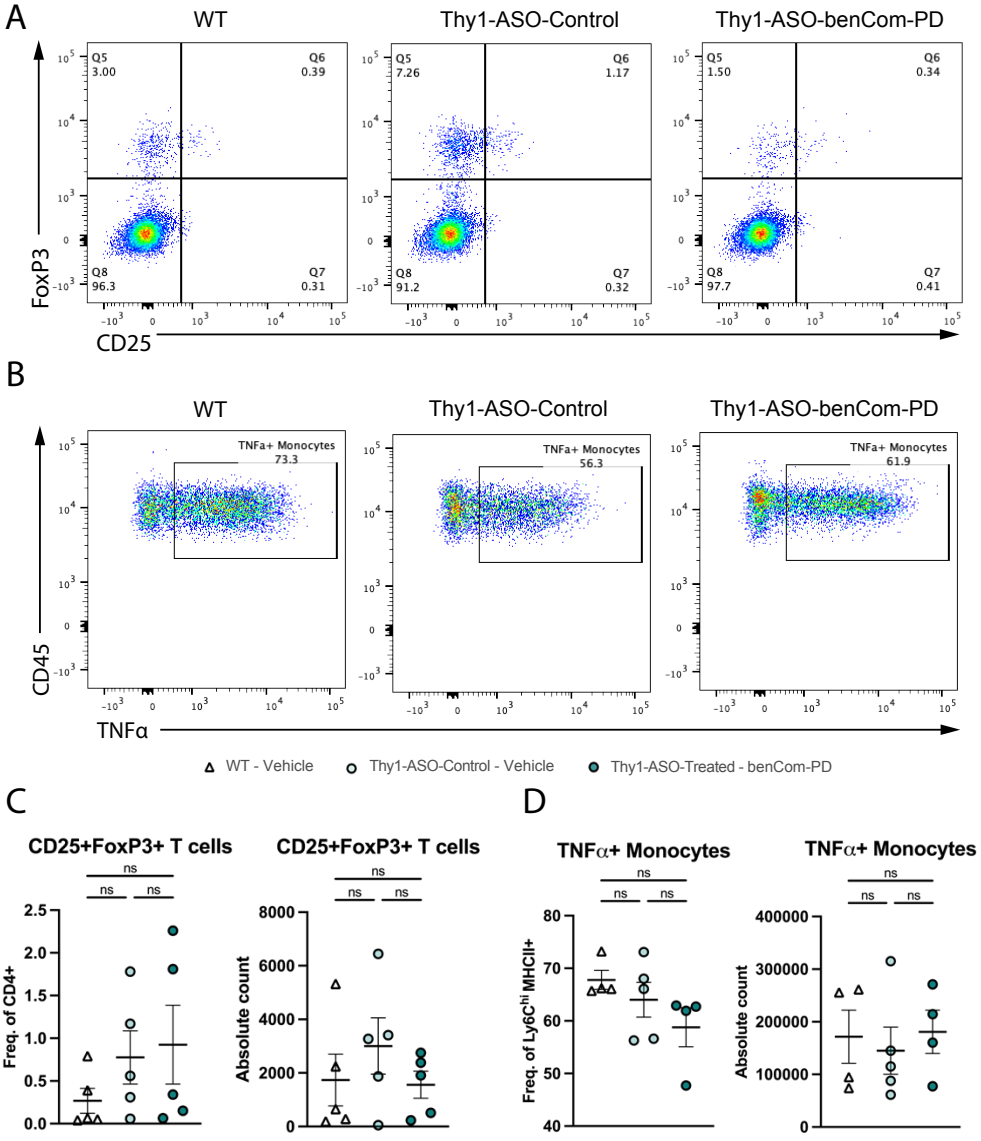

Figure 5. benCom-PD supplementation does not influence the immune landscape in Thy1-ASO animals. (**A, C**) Representative flow cytometry plots (A) and quantification (C) of activated CD25+FoxP3+ regulatory T cells in the spleen, N = 4-5. (**B, D**) Representative flow cytometry plots (B) and quantification (D) of Ly6C^{hi} MHCII+ monocytes producing TNFα in the spleen, N = 4-5. Points represent individual animals, bars represent standard error of the mean. Data analyzed by one-way ANOVA with Tukey post-hoc test. ns – not significant

Consistent with the absence of immunological changes, no differences were detected in volatile fatty acid profiles, including SCFAs, in fecal, cecal, or serum samples (**Fig. 6 A-C**). This finding was further confirmed by fermentation pathway analysis of

metagenomic data, indicating no changes in abundance of genes associated with SCFA metabolite production pathways across treatment groups (**Fig. 6D**). Although the therapeutic effects of benCom-PD do not appear to be mediated by the immune system or SCFAs in this study, further investigation into immune profiles and SCFA pathways is warranted before these mechanisms can be excluded.

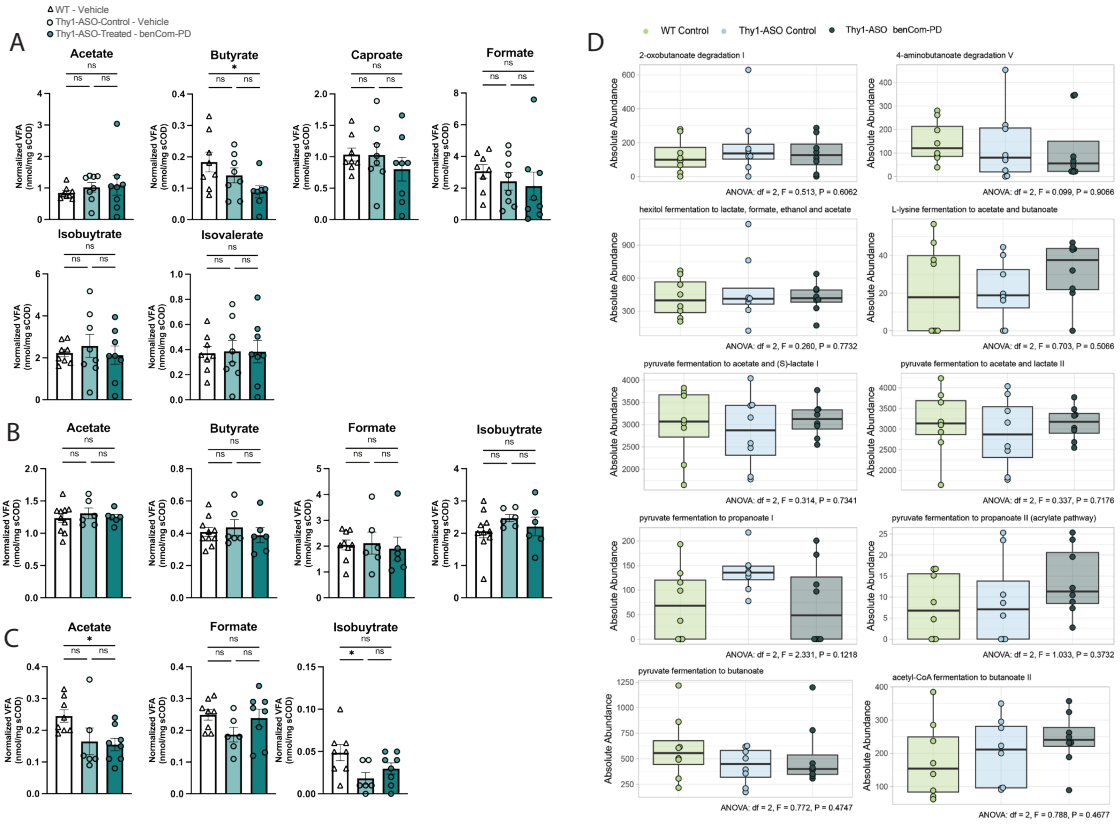

Figure 6. Volatile fatty acid and fermentation pathway analyses did not reveal changes in SCFA levels with benCom-PD treatment. (**A**) Fecal, (**B**) cecal, and (**C**) serum detectable volatile fatty acid measurements, normalized by soluble chemical oxygen demand (sCOD), from Thy1-ASO animals treated with benCom-PD and controls. N = 6-9. Points represent individual animals, bars represent standard error of the mean. Data analyzed by one-way ANOVA with Tukey post-hoc test. (**D**) Abundances of predicted fermentation pathways inferred from metagenomic sequencing data using HUMAnN3. Boxes indicate the median and interquartile range. Data were analyzed using a one-way ANOVA. No significant changes were detected between benCom-PD treated animals and Thy1-ASO controls. ns – not significant

Discussion

Fewer than 5% of PD cases are caused by monogenic risk, and only about 20% can be attributed to any genetic factor, suggesting that most PD likely arises from environmental exposures or gene–environment interactions. Indeed, pesticides, heavy metals, solvents, air pollution, and other environmental toxicants have been implicated in PD (De Miranda et al., 2021). Concordant studies have uncovered differences in gut microbiome composition between PD patients and household or population controls (Keshavarzian et al., 2015; Scheperjans et al., 2015; Tan et al., 2014; Boktor et al., 2023), representing a potential environmental risk. Indeed, the microbiome has profound effects on disease outcomes in several animal models of PD. For example, we and others have demonstrated that pathogenic gut bacterial species can accelerate motor symptoms and brain pathology in PD mouse models (Sampson et al., 2016; Choi et al., 2018; Matheoud et al., 2019). A high-fiber diet corrects microbiome alterations in Thy1-ASO mice and prophylactically improves motor deficits via modulation of microglial activation (Abdel-Haq et al., 2022). Furthermore, fecal microbiome transplants (FMT) from human PD donors into Thy1-ASO mice worsen motor performance compared to FMT from healthy donors (Sampson et al., 2016). In addition to animal studies (Zhao et al., 2021; Sun et al., 2018; Zhong et al., 2021; Xie et al., 2023), several clinical trials have assessed the safety and efficacy of FMTs in PD, yielding encouraging but inconsistent results that suggest further optimization of treatment strategies is needed (Bruggeman et al., 2024; Cheng et al., 2023; DuPont et al., 2023; Scheperjans et al., 2024).

Here, we investigated, for the first time to our knowledge, whether the reintroduction of microbes depleted in PD could restore physiological balance. Oral administration of a defined microbial consortium (benCom-PD) composed of taxa consistently depleted in the gut microbiome of multiple PD cohorts improved select features of disease in the Thy1-ASO mouse model. A therapeutic intervention with benCom-PD modestly ameliorated motor and GI dysfunction and reduced pS129-αSyn and αSyn aggregates in the striatum of Thy1-ASO mice. The anti-inflammatory cytokine IL-10 was reduced in colon tissue following short-term treatment, accompanied by trends towards decreased

pro-inflammatory cytokines, suggesting an overall dampening of inflammation in the colon. A prophylactic, or long-term, treatment with benCom-PD further improved motor and GI function in the Thy1-ASO animals and reduced pS129-αSyn in the striatum. Analysis of gut microbiome composition, immune landscape, and SCFA profiles, however, revealed no detectable changes in animals that received benCom-PD. Nonetheless, our findings demonstrate that supplementation with specific commensal microbes depleted in the PD microbiome is beneficial in a preclinical model. While further studies are needed to begin to understand potential mechanisms of action, the current work suggests translational potential for replenishing microbial populations in PD patients as a therapeutic strategy.

Many of the strains included in the benCom-PD consortium have therapeutic or anti-inflammatory effects in other contexts. *Faecalibacterium prausnitzii* has been well studied for its ability to induce regulatory T cells, IL-10 production, and improve outcomes in preclinical models of IBD (Qiu et al., 2013; Zhou et al., 2018). *Roseburia intestinalis* has been shown to inhibit pro-inflammatory interleukin-17 (IL-17) production, promote T_{REG} expansion, and also ameliorate colitis in mice (Nie et al., 2021; Zhu et al., 2018; Han et al., 2024). A related but less studied bacterium, *Roseburia faecis,* improves symptoms related to irritable bowel syndrome (IBS) in a stress-induced rat model (Choi et al., 2023). *Fusicatenibacter saccharivorans* and *Anaerostipes hadrus* are both depleted in patients with colorectal cancer and IBD (He et al., 2021; Ai et al., 2019; Chen et al., 2024; Liu et al., 2024), while *Bacteroides ovatus* reduces colitis severity in animals through modulation of immunity (Ihekweazu et al., 2019; 2021) and improves immunotherapy response in graft versus host disease models (Hayase et al., 2023). Treatment with *Eubacterium rectale* has also been shown to improve response to anti-PD1 therapy (Liu et al., 2023), and mitigates intestinal lymphoma in tumor-bearing mice (Lu et al., 2022). *Prevotella histicola* promotes T_{REG} function, and suppresses arthritis (Marietta et al., 2016) and multiple sclerosis symptoms in animal models (Shahi et al., 2019).

Prior studies of multi-strain probiotics containing *Lactobacillus* and *Bifidobacteria* species in PD animal models report improvements in motor function and rescue of dopaminergic neurons upon treatment (Perez Visnuk et al., 2020; Srivastav et al., 2019). In early clinical work, similar multi-strain probiotics have been shown to ameliorate constipation in PD patients (Ibrahim et al., 2020; Barichella et al., 2016). Interestingly, unlike members of the benCom-PD consortium, *Lactobacillus* and *Bifidobacteria* species have not been found to be reproducibly depleted in PD patients; instead, they are commonly found at higher abundances, with Lactobacilli specifically positively correlating with disease severity (Sampson, 2020). The enrichment of these bacteria in PD patients, despite their typically beneficial role, may be driven by factors such as medication effects (e.g., altered luminal pH, L-DOPA metabolism) or slower gastric motility, creating conditions that favor the persistence and proliferation of these taxa (Khedr et al., 2021; Gerhardt & Mohajeri, 2018; Wallen et al., 2020), and bring their therapeutic potential for PD into question. Supplementation with beneficial taxa found to be reduced in the PD gut microbiome as compared to healthy, age-matched controls appears to be a more intuitive approach.

Here, we explored two temporal interventions with benCom-PD: a short-term therapeutic treatment started at a mature age, and a long-term prophylactic treatment, given to young animals prior to onset of most disease symptoms. While benCom-PD ameliorated motor and GI function deficits in Thy1-ASO mice in both experiments, there were several differences in outcomes. Short-term benCom-PD treatment resulted in significant improvements in errors/step on the beam traversal assay, hindlimb clasping scores, and percentage of fecal water content, with a trend towards improvement on the pole descent. With long-term treatment, improvements were observed in adhesive removal task, hindlimb clasping, fecal scores, and bead expulsion, yet beam traversal outcomes remained unchanged and only trends were noted for fecal water content and pole descent. Ameliorated fine motor and rectal dysfunction, as demonstrated by adhesive removal and bead expulsion tests, indicate that prolonged supplementation with benCom-PD is beneficial. However, differences in beam traversal results are more difficult to reconcile.

Additionally, in colon tissue of treated mice, we observed a significant reduction in IL-10, along with trends towards decreased IL-17 and TNFα; however, no corresponding changes in immune system profiles were detected in long-term treated animals. αSyn aggregates in this mouse model are a direct result of αSyn overexpression, and may be cleared upon induction of autophagy or proteasome processes (Deleidi & Maetzler, 2012); whereas pS129-αSyn is largely driven by cell stress responses and kinase activation (Kawahata et al., 2022; Dzamako et al., 2014). αSyn reduction appeared more pronounced in short-term treated animals, with decreases in both pS129-αSyn and αSyn aggregates in the striatum, whereas prophylactic intervention reduced only αSyn aggregates. These findings suggest that while both treatment regimens confer benefits, an acute response to benCom-PD administration may be occurring, which may not be captured by week 20 in animals receiving long-term treatment. Additional experiments are needed to determine the exact temporal effects of benCom-PD supplementation.

Despite functional improvements, metagenomic analysis indicated no significant differences in gut microbiome composition across treatment groups. Given that microbiome alterations in Thy1-ASO mice compared to WT have been previously documented (Sampson et al., 2025; Abdel-Haq et al., 2022), the absence of significant changes in this study was unexpected. Stress has been shown to substantially alter the gut microbiome (Hantsoo & Zemel, 2021). The repeated stress of twice-weekly gavages throughout the experiments may explain the absence of strong genotype-driven microbiome differences, potentially leading to a convergence of microbial profiles across treatment groups. Additionally, we did not detect residual presence of benCom-PD microbes in metagenomic analyses. This is not unexpected, since all consortium members are obligately anaerobic human commensals, making stable engraftment in the mouse gut unlikely. However, the lack of significant compositional changes with benCom-PD treatment is surprising, perhaps owing to the relatively small amount of each microbial strain administered per gavage. Within the benCom-PD consortium, *F. prausnitzii, E. rectale, A. hadrus, R. intestinalis,* and *R. faecis* are established producers of butyrate (Lu et al., 2022; Allen-Vercoe et al., 2012; Duncan, 2002; Duncan et al., 2006), while *F.*

saccharivorans, B. ovatus, and *P. histicola* primarily produce acetate (Takada et al., 2013; Horvath et al., 2022; Downes et al., 2008). Given that several members are major butyrate producers, we expected to detect increases in butyrate, and potentially in acetate, in fecal or cecal samples. However, as butyrate is a primary energy source for colonocytes (Hodgkinson et al., 2023), it is possible that it was rapidly absorbed during the probiotic's transit. Nonetheless, further investigation into the possible contributions of the gut microbiome, bacterial metabolites, and immunomodulatory effects is necessary to rule out these aspects as potential mechanisms of action.

Other possible mechanisms of action should be further explored through *in vitro* experiments to understand inter-community interactions and metabolite production, as well as host tissue interactions. This can be achieved by systematically removing one strain at a time from bacterial community culture, as well as co-culturing benCom-PD with colon epithelial cell lines, enteroendocrine cell lines, or gut organoids. *In vivo* colonization experiments using antibiotic pre-treatments or germ-free mice would show if engraftment in the mouse gut is possible, and if benCom-PD colonization enhances its beneficial effects on the host. Ultimately, this treatment should be tested in various mouse models of both sporadic and familial PD, and using animals colonized with human PD stool samples, for optimal translatability to future human studies.

Collectively, these findings contribute to the rapidly expanding understanding of the gut microbiome's connection with neurological disease. While we hypothesized that these beneficial commensal microbes exert their effects by restoring gut homeostasis and modulating the immune system, our results suggested otherwise, pointing to potentially unexplored mechanisms and highlighting opportunities for further advancements in the gut-brain axis field. We hope that this work will spearhead more exploration into the use of non-traditional, specific probiotics for the treatment of PD and other conditions, and may even one day become the new standard of care, as we gain more knowledge on the importance of the gut microbiome in human health and disease.

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C h a p t e r 3

SPECIFIC COMMENSAL SPECIES DEPLETED IN THE PARKINSON'S DISEASE MICROBIOME AMELIORATE SYMPTOMS IN THE THY1-ASO MODEL

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Portions of this chapter have been submitted for review at *npj-Parkinson's disease* as part of the manuscript:

"*Faecalibacterium prausnitzii,* depleted in the Parkinson's disease microbiome, improves motor deficits in α-synuclein overexpressing mice"
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Abstract

Individuals with Parkinson's disease (PD) harbor a significantly altered gut microbiome, with decreased abundances of anti-inflammatory, short chain fatty acid (SCFA)-producing microbes compared to healthy individuals. To explore whether these taxa may be individually therapeutic in an animal model of PD, we orally administered cultures to α-synuclein overexpressing (Thy1-ASO) mice. Of the nine bacteria tested, *Bacteroides fragilis, Prevotella histicola,* and *Faecalibacterium prausnitzii* improved outcomes on behavioral tasks. *F. prausnitzii,* the most consistently depleted microbe in the PD microbiome, produced the most robust results and was chosen for further study. Colonization experiments with *F. prausnitzii* indicated that this fastidious human anaerobe is unable to engraft in the established mouse gut microbiome, thereby necessitating continuous administration. Assessment of a broad defined community lacking *F. prausnitzii* also did not reveal significant changes in animal behavior. These findings highlight the involvement of specific microbial species in PD and provide a foundation for further exploration into their use as novel therapeutics for PD.

Introduction

Parkinson's disease (PD) patients often experience gastrointestinal (GI) symptoms, particularly constipation, up to 20 years prior to motor dysfunction onset (Kalia & Lang, 2015). Indeed, the PD gut microbiome is significantly altered compared to healthy age-matched household and population controls, a pattern that is consistent across geographic locations and cohorts (Boktor et al., 2023). Reductions in SCFA-producing, anti-inflammatory commensal microbes are a recurrent finding in the PD microbiome (Sampson, 2020), and several of these taxa have been shown to independently improve outcomes in models of other diseases by exerting anti-inflammatory effects. For example, *Bacteroides fragilis* has been shown to ameliorate symptoms in a model of ASD (Hsiao et al., 2013), *Prevotella histicola* improves rheumatoid arthritis and multiple sclerosis in mice (Shahi et al., 2019; Marietta et al., 2016), and *Faecalibacterium prausnitzii* benefits colitis models (Martin et al., 2018). Across published studies of PD patients curated in the BugSigDB database (Geistlinger et al., 2024), *F. prausnitzii* emerges as the most consistently reduced taxon, reported as decreased in 16 independent studies and never as increased (**Table 1**).

Taxon Name	Taxonomic Level	Total signatures	Increased signatures	Decreased signatures
Akkermansia	genus	37	37	0
Faecalibacterium	genus	46	4	42
Roseburia	genus	45	4	41
Bifidobacterium	genus	33	30	3
Blautia	genus	25	2	23
Faecalibacterium prausnitzii	species	16	0	16
Fusicatenibacter	genus	19	1	18
Roseburia intestinalis	species	12	0	12
Peptoniphilus	genus	12	12	0
Alistipes	genus	12	12	0

Table 1. **Summary of the top 10 microbial signatures across human Parkinson's disease studies.** Top 10 taxa found to be altered at the genus and species levels as of September 11, 2025, using BugSigDB (https://bugsigdb.org/; Geistlinger et al., 2024), a comprehensive database of published microbial signatures. Refer to the **Supplementary Data** file for an extended list of signatures.

Faecalibacterium prausnitzii is one of the most abundant microbes in the healthy human gut, accounting for approximately 5% of total microbiota (Martin et al., 2018). As an extremely oxygen sensitive (EOS) bacterium, *F. prausnitzii* colonizes the ileum and colon, where intestinal oxygen levels are low (Duncan, 2002). Although *F. prausnitzii* is a diverse species, with new strains continuing to be identified and characterized, the A2-165 strain of phylogroup II, which produces high levels of butyrate and is non-biofilm-forming, is the most extensively studied (Lopez-Siles et al., 2012; Rossi et al., 2015; Benevides et al., 2017). This strain has been well characterized for its ability to ameliorate experimental colitis, with studies demonstrating increases in IL-10 levels and the expansion of regulatory T cells (T_{REGS}) in the colon and systemically, potentially through interactions with resident dendritic cells and signaling via toll-like receptors 2 or 4 (Martin et al., 2014; Rossi et al., 2015; Alameddine et al., 2019; Zhang et al., 2021). Treatment with *F. prausnitzii* A2-165 improves gut barrier integrity and dysbiosis in mouse models of inflammatory bowel disease (IBD) (Sokol et al., 2008; Martin et al., 2015). Notably, individuals with IBD also show decreased *F. prausnitzii* in the gut microbiome, and there is a strong positive correlation between IBD and developing PD later in life (Li et al., 2023; Zhu et al., 2022).

Several studies have found that the supernatant from *F. prausnitzii* cultures is as effective as, or in some cases more effective than, treatment with live bacteria (Qui et al., 2013; Martin et al., 2014 and 2015; Huang et al., 2016), suggesting the involvement of butyrate, which is able to promote anti-inflammatory responses (Chriett et al., 2019). However, further attempts to recapitulate beneficial outcomes with butyrate treatment yielded variable results (Sokol et al., 2008; Zhou et al., 2018; Martin et al., 2018; Lenoir et al., 2020), indicating that not all of *F. prausnitzii's* effects can be attributed to butyrate production. Indeed, Quevrian et al. (2015) isolated a 15 kDa anti-inflammatory protein ("MAM" – microbial anti-inflammatory molecule) from the supernatant of *F. prausnitzii* cultures that, when expressed in Lactobacilli and administered to animals, reproduced

beneficial effects observed with *F. prausnitzii* treatment by ameliorating colitis in an animal model and reducing IL-17 and IFNγ in mesenteric lymph nodes.

Beyond individual effects, *F. prausnitzii* also emerges as a central organism in gut microbial community dynamics. In the largest PD metagenomic study to date, a network analysis revealed *F. prausnitzii* as a central node within a cluster, showing 59 abundance correlations, and thus presumably interactions, with other community members, ranking it as the third most correlated taxon in the PD gut microbiome (Wallen et al., 2022). Thus, given the recent findings of *F. prausnitzii's* consistent depletion in the PD microbiome, immunomodulatory effects, and IBD – PD correlation, this microbe is a compelling candidate for further study as a potential probiotic treatment for PD.

Using the Thy1- α-synuclein overexpressing mouse model (Thy1-ASO) (Chesselet et al., 2012), we investigated the individual therapeutic potential of several taxa that are found to be reduced in the PD patient gut. Among the bacteria tested, *F. prausnitzii* treatment markedly improved motor dysfunction and was thus selected as the focus for further investigation.

Results

Multiple human PD study cohorts report reduced abundances of specific taxa in the patient gut microbiome compared to healthy individuals (Sampson, 2020; Keshavarzian et al., 2015; Hill-Burns et al., 2017; Aho et al., 2019; Bedarf et al., 2017; Wallen, et al., 2022; Boktor et al., 2023), suggesting that replenishing these bacteria may provide therapeutic benefit, as we demonstrated with benCom-PD treatment (see **Chapter 2**). To further dissect the contribution of individual microbes, we next tested whether treatment with single taxa could also confer therapeutic effects. Thus, cultured type strains representing taxa found to be depleted in PD (**Table 2**) were individually administered to SPF Thy1-ASO mice twice weekly, beginning at 5 weeks of age. Throughout treatment, motor and GI dysfunction were evaluated every 3-5 weeks. We found that several tested microbes are therapeutic in the Thy1-ASO mouse model of PD,

two of which (*P. histicola* and *F. prausnitzii*) are also members of the benCom-PD consortium investigated in Chapter 2.

Individual taxa improve motor dysfunction in Thy1-ASO mice

Taxon	Strain	Therapeutic?
Alistipes shahii	WAL 8301	No
Bacteroides coprocola	M16	No
Bacteroides fragilis	NCTC 9343	Yes
Bacteroides massiliensis	B84634	No
Bacteroides plebeius	M12	No
Blautia obeum	ATCC 29174	No
Clostridium hylemonae	TN-271	No
Faecalibacterium prausnitzii	A2-165	Yes
Prevotella histicola	T05-04	Yes

Table 2. Strains tested in Thy1-ASO mice for therapeutic potential. Representative type strains of taxa found to be depleted in the PD gut microbiome, individually administered and tested in Thy1-ASO mice. Microbes that improved any aspect of tested motor or GI function are indicated as therapeutic.

Of the nine species individually tested, three produced some therapeutic effects in Thy1-ASO mice (**Table 2, Fig. 7**). Animals that received *B. fragilis* exhibited significant motor improvements compared to controls in their ability to traverse the challenging beam and descend the pole – measures of balance, coordination, and overall gross motor function (**Fig. 7A**). *P. histicola* supplementation also resulted in marked improvements on the beam traversal and pole descent tasks (**Fig. 7B**). However, performance on the wire hang assay was significantly impaired in treated animals, suggesting *P. histicola* may selectively influence aspects of movement. Administration of *F. prausnitzii* robustly ameliorated motor dysfunction. Treated Thy1-ASO mice demonstrated improvements on the beam traversal task when assessed for time to cross and errors per step; whereas control Thy1-ASO mice declined in beam crossing performance over time, *F. prausnitzii*-treated animals remained stable (**Fig. 7C**). Mice receiving *F. prausnitzii* also exhibited improved grip strength on the wire hang task, and reduced latency to remove an adhesive from the nose, indicating ameliorated fine motor dysfunction. Hindlimb clasping, a marker of striatal dysfunction, was also significantly reduced in *F. prausnitzii*-treated

mice, with effects evident after just three weeks of supplementation. Taken together, these findings demonstrate that specific microbial taxa can independently drive improvement of PD-associated symptoms in a preclinical model.

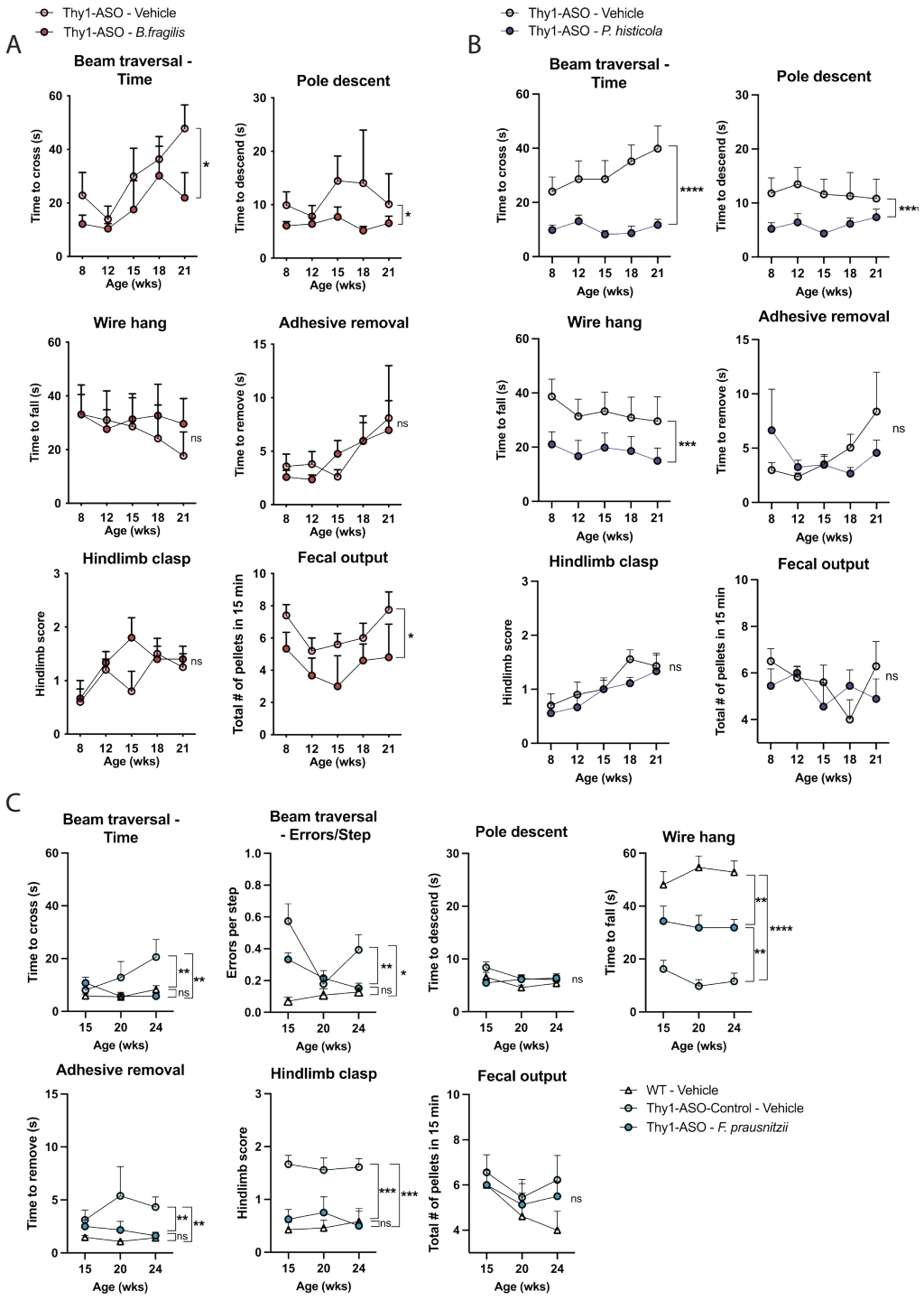

Figure 7. Administration of several individual commensal microbes drives motor function improvements over time. Thy1-ASO animals were treated with **(A)** *Bacteroides fragilis* (N = 5-6), (**B**) *Prevotella histicola* (N = 8-10), or (**C**) *Faecalibacterium prausnitzii* (N = 8-12) 2x/week starting at 5 weeks of age and tested at

time points throughout for motor and GI function. Points represent means; bars represent standard error. Data analyzed by 2way ANOVA; group effects are indicated at the end of each plot. ns – not significant; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001
Abbreviations: wks – weeks

Ability of F. prausnitzii to engraft in the mouse gut

The pronounced benefits of *F. prausnitzii* supplementation, along with its consistent depletion in PD patients, compelled our further investigation of this microbe. As an EOS anaerobe, culturing and administering *F. prausnitzii* presents significant challenges. Furthermore, continuous repeated gavage over time is highly stressful to animals and can confound behavior and tissue analyses. Therefore, we investigated *F. prausnitzii's* ability to colonize the mouse gut. In SPF animals, *F. prausnitzii* established only modestly following administration (~10^4 cfu/g feces) and declined rapidly thereafter, with levels dropping by 12 hours post-gavage and little remaining after 1 week or 1 month (**Fig. 8A, B**). To create a niche for *F. prausnitzii* engraftment in the mouse gut, animals were pre-treated with a broad-spectrum antibiotic cocktail prior to microbe administration. This yielded markedly higher levels of *F. prausnitzii* (~10^5-10^7 cfu/g feces) one day post-gavage (**Fig. 8C**). However, at one week post-gavage, *F. prausnitzii* levels declined below those observed in SPF animals without antibiotic pre-treatment, suggesting that inter-bacterial competition with mouse gut species may limit *F. prausnitzii's* sustained colonization. Notably, *F. prausnitzii* failed to engraft in the germ-free (GF) mouse gut when administered alone (**Fig. 8D**), consistent with findings from other studies (Wrzosek et al., 2013; Miquel et al., 2015; Kim et al., 2020). However, alongside *Bacteroides thetaiotaomicron*, *F. prausnitzii* colonized the GF gut robustly and was stable for a week following administration (**Fig. 8E**). We hypothesized that once *F. prausnitzii* was established in the mouse gut, it may engraft more effectively in the SPF microbiome. However, following the administration of cecal contents from *B. thetaiotaomicron* and *F. prausnitzii* dual-colonized animals to SPF mice, *F. prausnitzii* levels remained very low (~10^1 cfu/g feces) (**Fig. 8F**). Taken together, these findings illustrate the inherent sensitivity of this bacterium and necessitate further investigations into oxygen-resistant

formulations that would allow routine administration to animals, and eventually, human patients, without complex preparations or stressful intragastric gavage.

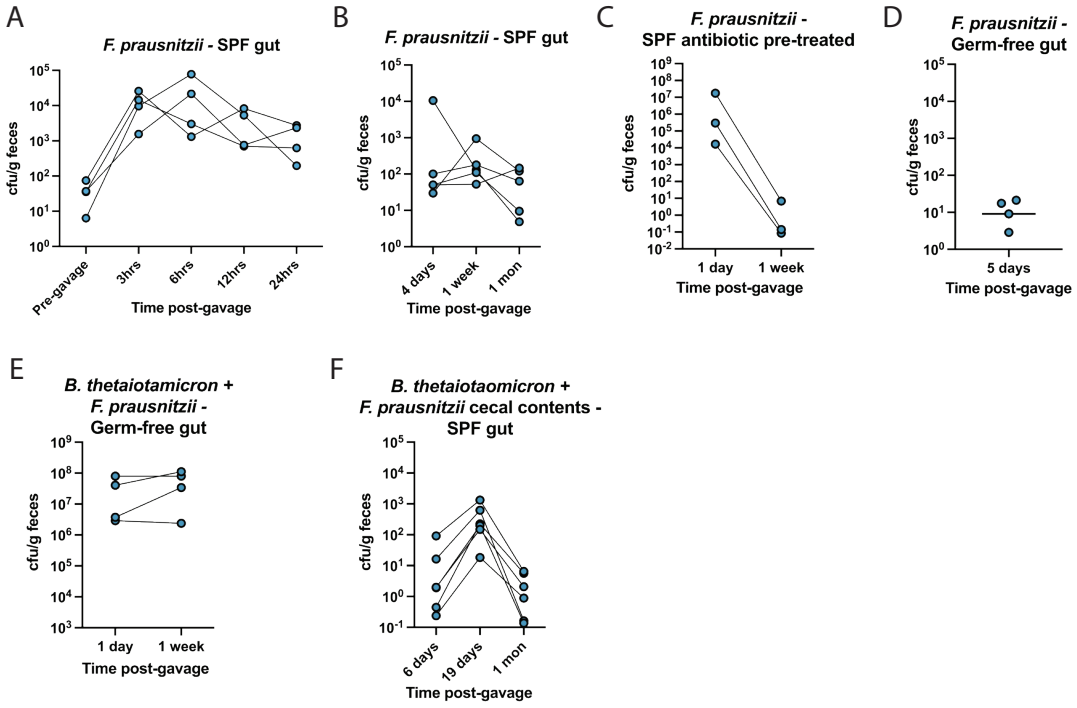

Figure 8. *Faecalibacterium prausnitzii* ability to colonize the mouse gut. (**A**) *F. prausnitzii* colonization of Thy1-ASO SPF (specific pathogen-free; standard laboratory mouse microbiome) mice, measured over 24 hours post-administration, N = 3. (**B**) *F. prausnitzii* colonization of SPF gut up to 1 month after gavage, N = 4 (**C**) *F. prausnitzii* colonization following SPF mouse AVNM antibiotic cocktail pre-treatment, up to 1 week after gavage, N = 3 (**D**) *F. prausnitzii* colonization of germ-free Thy1-ASO mice, measured 5 days after gavage, N = 4. (**E**) Colonization of the germ-free gut with *F. prausnitzii* and *Bacteroides thetaiotaomicron* together, measured at 1 day and 1 week post-gavage, N = 3. (**F**) Cecal contents from germ-free mice colonized with *F. prausnitzii* and *B. thetaiotaomicron* administered to SPF animals, tracked up to 1 month post-administration, N = 5. Points represent individual animals.
Abbreviations: AVNM – ampicillin, vancomycin, neomycin, metronidazole; cfu – colony forming unit; hrs – hours; mon – month

Thy1-ASO mice colonized with a complex synthetic community with and without F. prausnitzii

In Chapter 2, we found that a small consortium containing *F. prausnitzii* is beneficial in the Thy1-ASO mouse model of PD. Here, we determined that *F. prausnitzii* alone can drive motor function improvements. To further assess *F. prausnitzii's* ability to modulate

behavioral outcomes and assess the potential keystone role of *F. prausnitzii* broadly within the microbiome, we implemented the hCom2 (human commensal 2) complex synthetic community, provided by the Fischbach laboratory. hCom2 is a defined consortium of 119 commensal bacteria most commonly found in the healthy human gut microbiome (Cheng et al., 2022). Thy1-ASO breeders were colonized with full hCom2 or hCom2 without *F. prausnitzii* and offspring were tested for differences in motor and GI function (**Fig. 9**). We hypothesized that Thy1-ASO animals lacking *F. prausnitzii* in the hCom2 microbiome would display exacerbated motor and GI dysfunction in comparison to Thy1-ASO mice harboring the full hCom2 community. Both groups, however, demonstrated comparable outcomes across motor (**Fig. 9A**) and GI (**Fig. 9B**) function tasks, with no significant differences between Thy1-ASO animals with the two microbiomes. Yet, trends towards decreased performance on the wire hang, adhesive removal, and fecal score tests were observed, with significant genotype differences emerging in the absence of *F. prausnitzii*, but not in animals harboring the full hCom2 microbiome. Through these experiments, we determined that several alternative strategies to studying *F. prausnitzii* were ineffective and confirmed that continuous supplementation into a standard mouse microbiome is required for effective treatment.

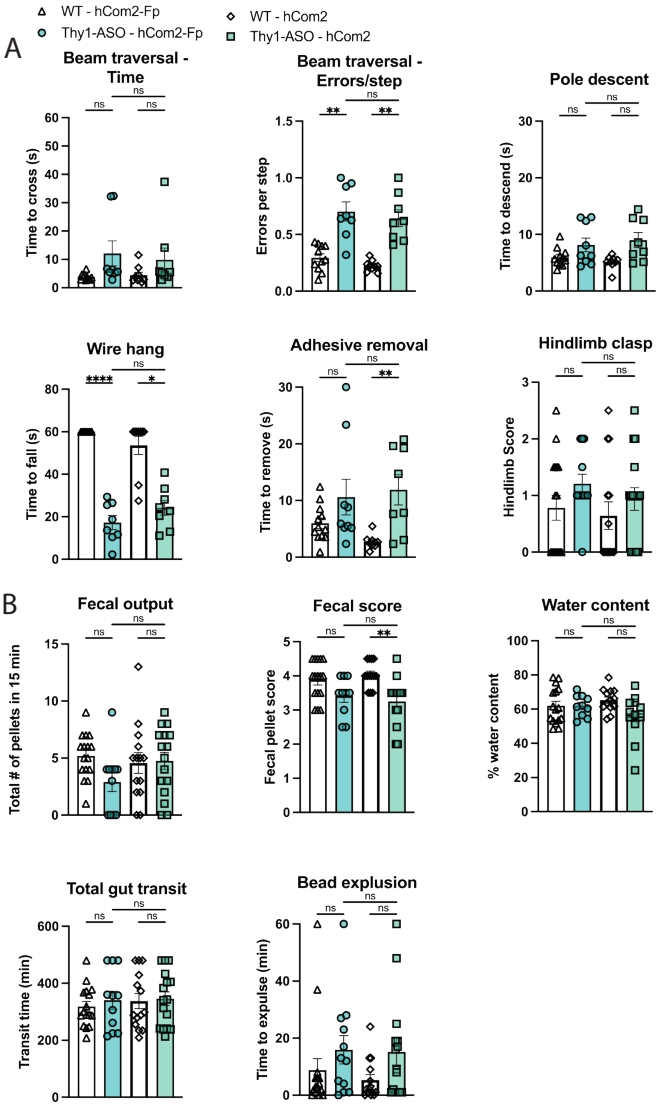

Figure 9. hCom2 microbiome with or without *F. prausnitzii* does not significantly alter Thy1-ASO performance in motor and GI function tests. (**A, B**) WT and Thy1-ASO animals colonized from birth with full hCom2 or hCom2 without *F. prausnitzii* were tested for changes in (**A**) motor and (**B**) GI function at 20 weeks of age, N = 8-11. Points represent individual animals; bars represent standard error of the mean. Data were analyzed by Kruskal-Wallis test followed by Dunn's post-hoc test. ns – not significant; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001

Abbreviations: hCom2 – full human commensal 2 community; hCom2-Fp – human commensal 2 community without *F. prausnitzii*

Discussion

Here, we investigated the capacity of individual taxa depleted in the PD microbiome to influence motor and GI function in Thy1-ASO mice. Several microbes conferred therapeutic effects, with *F. prausnitzii* yielding the most pronounced improvements across behavioral tasks. Further, we investigated *F. prausnitzii*'s ability to engraft in the mouse gut and assessed motor and GI outcomes in the Thy1-ASO mouse model colonized with a complex synthetic community with and without *F. prausnitzii*.

B. fragilis, P. histicola, and *F. prausnitzii* all ameliorated aspects of motor dysfunction in the Thy1-ASO mice, demonstrating, for the first time to our knowledge, that commensal microbes found to be depleted in the PD microbiome are individually therapeutic. Whereas previously considered strictly a neurological disease, PD studies over the past decade have confirmed a strong inflammatory component, both systemically and particularly in the trafficking of T cells to the brain (Tansey et al., 2022; Yan et al., 2021; Wang et al., 2021; Galiano-Landeria et al., 2020). T_{REG} deficiencies have also been implicated in PD and its associated mouse models (Park et al., 2023; Reynolds et al., 2007; Arce-Sillas et al., 2024). Interestingly, *B. fragilis, P. histicola,* and *F. prausnitzii* have all been shown to induce T_{REGS} in other models of disease (Hsiao et al., 2013; Marietta et al., 2016; Martin et al., 2014). *B. fragilis* polysaccharide A induces conversion of effector T cells into T_{REGS} in the gut (Round & Mazmanian, 2010). *P. histicola* dampens inflammation through T_{REG} induction and subsequent reduction in Th17 responses in arthritis-susceptible mice and a model of MS (Marietta et al., 2016; Shahi et al., 2019). In animal models of colitis, *F. prausnitzii* increases levels of IL-10 and induces T_{REGS} in the colon and systemically (Sokol et al., 2008; Martin et al., 2014; Qui et al., 2013).

The anti-inflammatory effects of these taxa may be mediated in part by SCFA production. *B. fragilis* and *P. histicola* both produce acetate, which our lab has shown to ameliorate motor deficits and neuroinflammation in the Thy1-ASO mice (Abdel-Haq et al., 2022). Butyrate, largely produced by *F. prausnitzii*, inhibits histone deacetylases

(HDACs) and thus reduces downstream pro-inflammatory gene expression and induces T_{REGS} (Chriett et al., 2019; Arpaia et al., 2013; Furusawa et al., 2013). Several studies have shown the therapeutic effects of *F. prausnitzii* supernatant in colitis mouse models (Qui et al., 2013; Martin et al., 2014 and 2015; Huang et al., 2016) and even find that supernatant from *F. prausnitzii* strains directly inhibits HDAC1 *in vitro* (Zhou et al., 2018). However, Martin et al. (2018) demonstrated that using the supernatant from another high butyrate producer, *Roseburia intestinalis*, had no effect on IBD outcomes, in comparison to improvements with *F. prausnitzii* supernatant administration, suggesting additional mechanisms beyond butyrate may be involved. Thus, these immunomodulatory avenues warrant investigation as potential mechanisms underlying *B. fragilis, P. histicola,* and *F. prausnitzii* effects on motor outcomes in the Thy1-ASO model.

Colonization of the mouse gut with *F. prausnitzii* proved to be challenging. Considering that *F. prausnitzii* is a fastidious human taxon introduced into an established mouse microbiome, its rapid decline in the SPF microbiome is unsurprising. Antibiotic pre-treatment led to initially high *F. prausnitzii* levels, but these declined by one week post-administration, again suggesting that *F. prausnitzii* may be ill-adapted to engrafting in the mouse gut compared to native species. *F. prausnitzii* has been shown several times in literature to require a companion microbe to colonize the GF gut (Wrozsek et al., 2013; Miquel et al., 2015; Kim et al., 2020). This is likely due to its EOS nature and the elevated oxygen levels in the GF colon, which necessitates a less oxygen-sensitive bacterium to pre-reduce the environment. Indeed, *B. thetaiotaomicron* and *F. prausnitzii* successfully colonized GF Thy1-ASO mice together. However, transferring these cecal contents containing pre-conditioned *F. prausnitzii* failed to improve engraftment in SPF mice. These results indicate that continuous administration of *F. prausnitzii* is necessary for effective treatment in mice.

To assess the contribution of *F. prausnitzii* within a broad community, we utilized the hCom2 defined synthetic consortium, a representation of the healthy human gut

microbiome that includes *F. prausnitzii*. To circumvent developmental changes in GF offspring, Thy1-ASO breeders were colonized with hCom2 with and without *F. prausnitzii* and offspring harboring the communities were later tested for motor and GI function differences. Thy1-ASO animals not exposed to *F. prausnitzii* performed comparably to those harboring the full hCom2 community, showing no significant differences in motor or GI function tasks across genotype and microbiome. However, several trends were evident, indicating that larger sample sizes and testing at a later age may reveal more pronounced differences. It is possible that *F. prausnitzii* is not essential within the hCom2 community, given the presence of other anti-inflammatory, butyrate-producing taxa such as Roseburia and Eubacteria, that may functionally compensate for its absence. Metagenomic analysis of hCom2 lacking *F. prausnitzii* would reveal its specific contributions to community interactions.

Together, these findings provide evidence that several beneficial commensal microbes are able to independently improve motor symptoms in a preclinical model of PD. Further investigations repeating these results and exploring formulations and potential mechanisms of action are warranted. For instance, immunomodulatory effects following the administrations of *B. fragilis, P. histicola,* and *F. prausnitzii* in the Thy1-ASO mice should be assessed, and additional experiments pooling all three taxa together to determine if motor function is enhanced would be valuable. *F. prausnitzii*, the most consistently depleted taxon in the PD microbiome, produced the most distinct effects on PD-associated symptoms in our mouse model. Furthering our understanding of its benefits in this context and beyond may open opportunities for the future use of *F. prausnitzii* as a therapeutic treatment for PD.

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Chapter 4

FAECALIBACTERIUM PRAUSNITZII, DEPLETED IN THE PARKINSON'S DISEASE MICROBIOME, IMPROVES MOTOR DEFICITS IN A-SYNUCLEIN OVEREXPRESSING MICE

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This chapter has been submitted for review at *npj-Parkinson's disease*.

Abstract

The gut microbiome composition is altered in Parkinson's disease (PD), a progressive neurodegenerative disorder leading to motor dysfunction and gastrointestinal (GI) symptoms. Notably, microbial taxa with anti-inflammatory properties are depleted in PD patients compared to controls, with *Faecalibacterium prausnitzii* emerging as the most consistently reduced. To explore the therapeutic effects of *F. prausnitzii*, we orally administered cultures to α-synuclein overexpressing (Thy1-ASO) mice, an animal model of PD. Treatment with *F. prausnitzii* robustly ameliorated motor and GI symptoms and reduced α-synuclein aggregates in the brain. *F. prausnitzii* was also sufficient to correct gut microbiome deviations**,** induce anti-inflammatory immune responses, and promote protective colonic gene expression profiles in Thy1-ASO mice. These findings support the emerging hypothesis of functional contributions by the microbiome to PD and embolden development of potential probiotic therapies.

Introduction

The Parkinson disease (PD) microbiome is significantly altered, with individuals often experiencing gastrointestinal (GI) distress long before motor symptom onset. Multiple metagenomic analyses have found *Faecalibacterium prausnitzii* to be consistently depleted from the gut microbiome of PD patients (see **Chapter 3, Table 2**) (Sampson, 2020; Keshavarzian et al., 2015; Unger et al., 2016; Hill-Burns et al., 2017; Li et al., 2017; Petrov et al., 2017; Wallen et al., 2022; Boktor et al., 2023). *F. prausnitzii*, a fastidiously anaerobic Gram-positive species, is highly abundant in the ileum and colon of the healthy human gut and is a major producer of the SCFA butyrate (Sokol et al., 2008). Its potent anti-inflammatory effects have been extensively documented in models of inflammatory bowel disease (IBD) (Quevrain et al., 2016; Sokol et al., 2008; Martin et al., 2018; Zhou et al., 2018).

Using the transgenic Thy1 α-synuclein overexpressing (Thy1-ASO) mouse model of PD (Chesselet et al., 2012), we previously demonstrated that treatment with a small consortium (benCom-PD) of taxa depleted in the PD microbiome ameliorated PD-associated symptoms (see **Chapter 2**). Moreover, treatment with select independent taxa was sufficient to drive motor function improvements (see **Chapter 3**). In this chapter, we corroborate these findings and further examine the therapeutic potential of *F. prausnitzii*. We find that *F. prausnitzii* alone was able to robustly ameliorate motor and GI dysfunction, as well as reduce αSyn accumulation in the brain. Additionally, *F. prausnitzii* treatment mildly remodeled the gut microbiome of Thy1-ASO mice to more closely resemble that of wild-type (WT) animals. T_{REG} numbers and production of the anti-inflammatory cytokine IL-10 were increased in *F. prausnitzii*-treated intestinal tissues. Furthermore, *F. prausnitzii* supplementation increased colonic expression of gene pathways associated with tissue repair and regeneration. Our findings demonstrate that treatment of Thy1-ASO mice with a commensal bacterium that is depleted in PD patients improves molecular and behavioral outcomes, providing conceptual validation for the development of microbial strategies to treat PD.

Results

F. prausnitzii has robust therapeutic effects on motor symptoms and synucleinopathy

To confirm preliminary findings (see **Chapter 3**), we treated animals with *F. prausnitzii* mono-cultures for 15 weeks, beginning at 5 weeks of age, and assessed motor and GI function (**Fig. 10A**). *F. prausnitzii*-treated mice showed significant improvements in the adhesive removal and hindlimb clasping tests compared to vehicle-treated Thy1-ASO mice (**Fig. 10B**), similar to the effects observed with benCom-PD treatment (see **Chapter 2**). Animals treated with *F. prausnitzii* alone further exhibited an improved ability to cross a challenging beam, a test of motor coordination, as reflected by reduced crossing time and fewer errors per step (paw slips) (**Fig. 10B**). GI function was also improved following *F. prausnitzii* treatment, marked by decreased time required to expel a rectally-inserted glass bead and improved Bristol stool scale scores following treatment (**Fig. 10C**). While we did not detect changes in levels of pS129-αSyn in brain tissues (**Fig. 10D-E**), supplementation with *F. prausnitzii* reduced aggregation of αSyn in the substantia nigra (**Fig. 10H, I**), but not in the striatum (**Fig. 10J, K**), of Thy1-ASO mice relative to vehicle-control Thy1-ASO mice. These findings demonstrate that treatment with *F. prausnitzii,* a single microbe found to be depleted in the PD patient gut microbiome, is sufficient to markedly improve disease-associated outcomes in the Thy1-ASO mouse model.

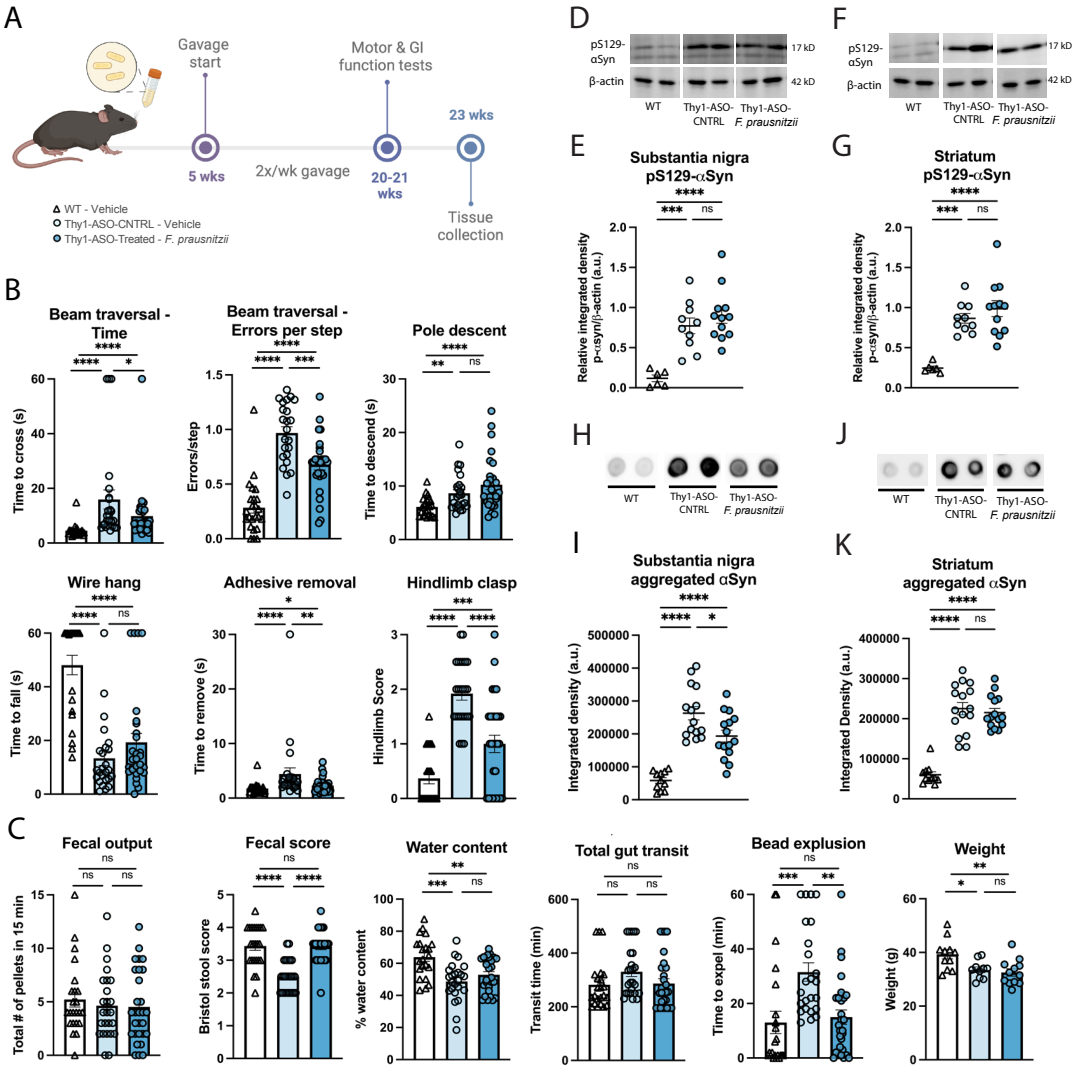


Figure 10. Treatment with *F. prausnitzii* mono-culture ameliorates motor and GI symptoms and reduces αSyn aggregates in the substantia nigra. (**A**) Animal treatment and testing timeline. (**B, C**) Improvements in (B) motor and (C) GI function assays with *F. prausnitzii* treatment in Thy1-ASO animals compared to controls, tested at 20 weeks, N = 23-29. Points represent individual animals, compiled from two independent cohorts; bars represent standard error of the mean. (**D-G**) Representative images (D, F) and quantification (E, G) of Western blots for phosphorylated-S129-α-synuclein in (D, E) substantia nigra and (F, G) striatum, N = 6-12. (**H-K**) Representative images (H, J) and quantification (I, K) of dot blots for aggregated α-synuclein in (H, I) substantia nigra and (J, K) striatum, N = 11-15. Points represent individual animals, compiled from two independent cohorts; bars represent standard error of the mean. Behavior data in (B, C) analyzed by Kruskal-Wallis test followed by the Conover-Iman post-hoc test with Benjamini-Hochberg false discovery rate (FDR) correction; protein data in (D-K)

analyzed by one-way ANOVA with Tukey post-hoc test. *p≤0.05; **p≤0.01; ***p≤ 0.001; ****p≤0.0001.
Abbreviations: pS129-αSyn – phosphorylated-S129-α-synuclein

F. prausnitzii treatment aligns the Thy1-ASO microbiome to more closely resemble a WT profile

The fecal microbiome is altered in Thy1-ASO mice and other PD models compared to WT mice (Sampson et al., 2025, Abdel-Haq et al., 2022; Aktas et al., 2023; Yang et al., 2018; Yan et al., 2021). Metagenomic analysis revealed that *F. prausnitzii* treatment remodeled or maintained the gut microbiome of Thy1-ASO mice to more closely resemble that of WT animals, while the composition in untreated Thy1-ASO mice deviated from the other two groups (**Fig. 11**). At the phylum level, relative abundance profiles appeared largely stable across groups, with no major shifts observed between conditions (**Fig. 11A**). However, at the species genome bin (SGB) level, Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarity revealed a separation of the microbiomes of WT and vehicle-treated control Thy1-ASO animals (**Fig. 11B**), reflecting genotype-associated differences that align with previous findings (Sampson et al., 2025; Abdel-Haq et al., 2022). Notably, supplementation with *F. prausnitzii* partially prevented compositional changes in the Thy1-ASO mice (**Fig. 11B**). Pairwise comparisons of Bray-Curtis dissimilarity showed significant differences between WT and control Thy1-ASO mice (pairwise PERMANOVA FDR = 0.011), as well as between *F. prausnitzii*-treated Thy1-ASO and control Thy1-ASO animals (pairwise PERMANOVA FDR = 0.020), while microbial compositions of WT and treated Thy1-ASO mice showed less dissimilarity (pairwise PERMANOVA FDR = 0.250) (**Fig. 11C**). Differential abundance and prevalence analyses identified subtle shifts in multiple taxa, suggesting that a community-wide alteration may underlie the Thy1-ASO microbiome's increased similarity to the WT profile, rather than pronounced alterations in specific taxa (**Fig. 11D**). However, measures of observed richness, Shannon diversity, and the Firmicutes/Bacteroidetes ratio did not differ significantly between groups (**Fig. 11E-G**). Interestingly, presence of *F. prausnitzii* was not identified in the metagenomes, likely due

to its weak ability to individually colonize the mouse gut (see **Chapter 3**) (Wrzosek et al., 2013; Miquel et al., 2015; Kim et al., 2020).

To determine whether the microbiome changes we observed play a role in the motor and GI symptom improvements seen with treatment, we conducted mediation analysis with *F. prausnitzii* treatment as the predictor and altered microbiome composition as a potential mediator (**Fig. 11H**). As functional outcomes, we selected errors/step in beam traversal and time to bead expulsion, as these were the most robust and disease-relevant effects among the motor and GI function assays. Mediation analysis revealed that *F. prausnitzii* administration had a significant direct influence on both behavioral outcomes, with an insignificant indirect effect, reflected by an average causal mediation effect (ACME) close to zero. These findings suggest that the physiological outcomes were not mediated by overall microbiome alterations, as approximated by the first three principal coordinates of Bray-Curtis dissimilarity, but rather influenced directly by *F. prausnitzii*. Together, our results demonstrate that *F. prausnitzii* is sufficient to drive behavioral improvements and contributes to a mild remodeling or maintenance of the gut microbiome profile in Thy1-ASO mice to more closely resemble that of WT animals.

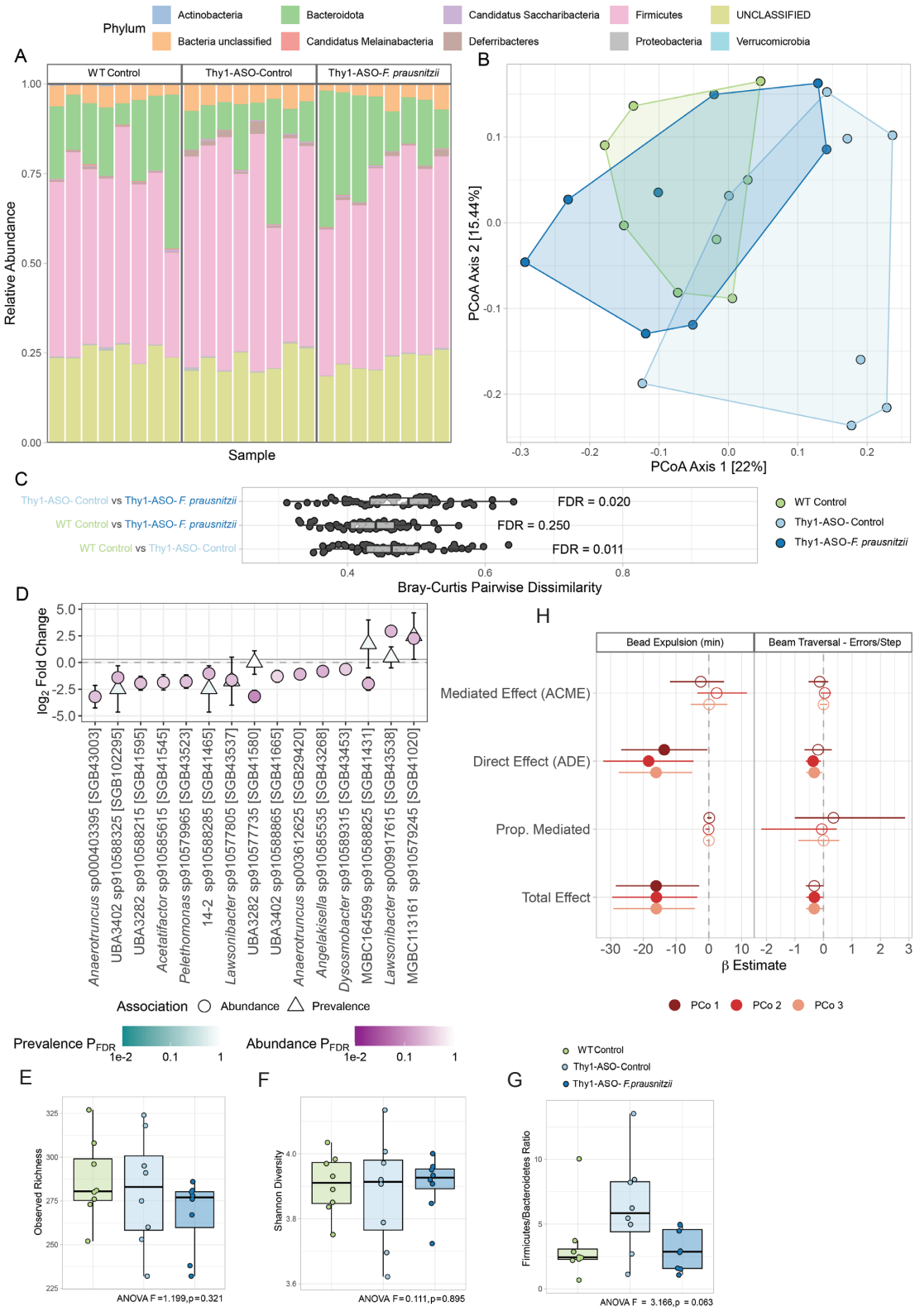


Figure 11. The gut microbiome of Thy1-ASO mice is mildly remodeled upon

treatment with *F. prausnitzii*. (**A**) Stacked bar plot of relative abundance of bacterial phyla in the gut microbiome, including unclassified reads. Each bar represents a single mouse. (**B**) Principal Coordinate Analysis (PCoA) of the Bray-Curtis dissimilarity of fecal microbiome composition of each group. Percentages in brackets represent variance explained by each axis. (**C**) Boxplot of pairwise dissimilarity. Statistical significance was calculated using pairwise permutational multivariate analysis of variance (PERMANOVA) with Benjamini-Hochberg adjusted false discovery rate (FDR) across multiple pairwise comparisons. (**D**) Differential abundance and/or prevalence of the top 15 species-level genome bins (SGBs) in *F. prausnitzii*-treated Thy1-ASO mice compared to vehicle-treated Thy1-ASO controls. Analysis was performed in MaAsLin3 on log2-transformed relative abundance values scaled between 0 and 1 with no further adjustments. SGB nomenclature was enriched using the Genome Taxonomy Database provided in MetaPhlAn v4.1.1 See **Supplementary Data file** for full taxonomy and values. (**E-G**) Distributions of (E) observed fecal SGB richness, (F) Shannon diversity, and (G) Firmicutes/Bacteroidetes ratio. Data were analyzed using a one-way ANOVA. (**H**) Mediation analysis of the first three microbiome principal coordinates as mediators of the effect of *F. prausnitzii* treatment on the beam traversal errors/step and bead expulsion assays. Effect sizes (β) and 95% confidence intervals are shown. Full circles indicate a p-value ≤0.05, empty circles indicate p>0.05. Refer to **Supplementary Data 10 and 11** for full mediation analysis data.
Abbreviations: ACME – Average Causal Mediation Effect; ADE – Average Direct Effect; Prop. – Proportion

F. prausnitzii alters the immune landscape in treated animals

Probiotic treatment with *F. prausnitzii* suppresses intestinal and systemic inflammation in mouse models of colitis, arthritis, and during testing of anti-cancer drugs (Martin et al., 2023; Moon et al., 2023; Bredon et al., 2024; Shi et al., 2025). Here, flow cytometric analysis of Thy1-ASO mice receiving *F. prausnitzii* revealed that treatment led to a significant increase in CD4+CD25+FoxP3+ T_{REG} cell numbers in the mesenteric lymph nodes (MLNs) (**Fig. 12A, C, D**). T_{REG} deficiencies have been implicated in PD and its associated mouse models (Park et al., 2023; Reynolds et al., 2007; Arce-Sillas et al., 2024). T_{REG} expansion was accompanied by elevated levels of the anti-inflammatory cytokine IL-10 in proximal large intestinal tissue, as measured by multiplex bead assay (**Fig. 12E**). Interestingly, we also observed increased frequency of tumor necrosis factor α (TNFα)-producing $Ly6C^{hi}$ MHCII+ monocytes in the spleens of *F. prausnitzii*-treated animals (**Fig. 12B, G, H**). In colonic tissue, TNFα concentration trended towards an increase in treated Thy1-ASO mice (**Fig. 12F**). While classically thought of as a pro-

inflammatory cytokine, TNFα can be required for T_{REG} survival, differentiation, and effector function (Chen & Oppenheim 2010; Chen et al., 2013) and is involved in tissue repair (Ritsu et al., 2017; Choi et al., 2023). *F. prausnitzii* has also been shown to induce TNFα production in macrophage cell lines *in vitro* (Brown et al., 2021), suggesting this response may be part of its broader immune regulatory profile. Overall, these findings demonstrate that *F. prausnitzii* exerts immunomodulatory effects on innate and adaptive immune cell populations in Thy1-ASO mice.

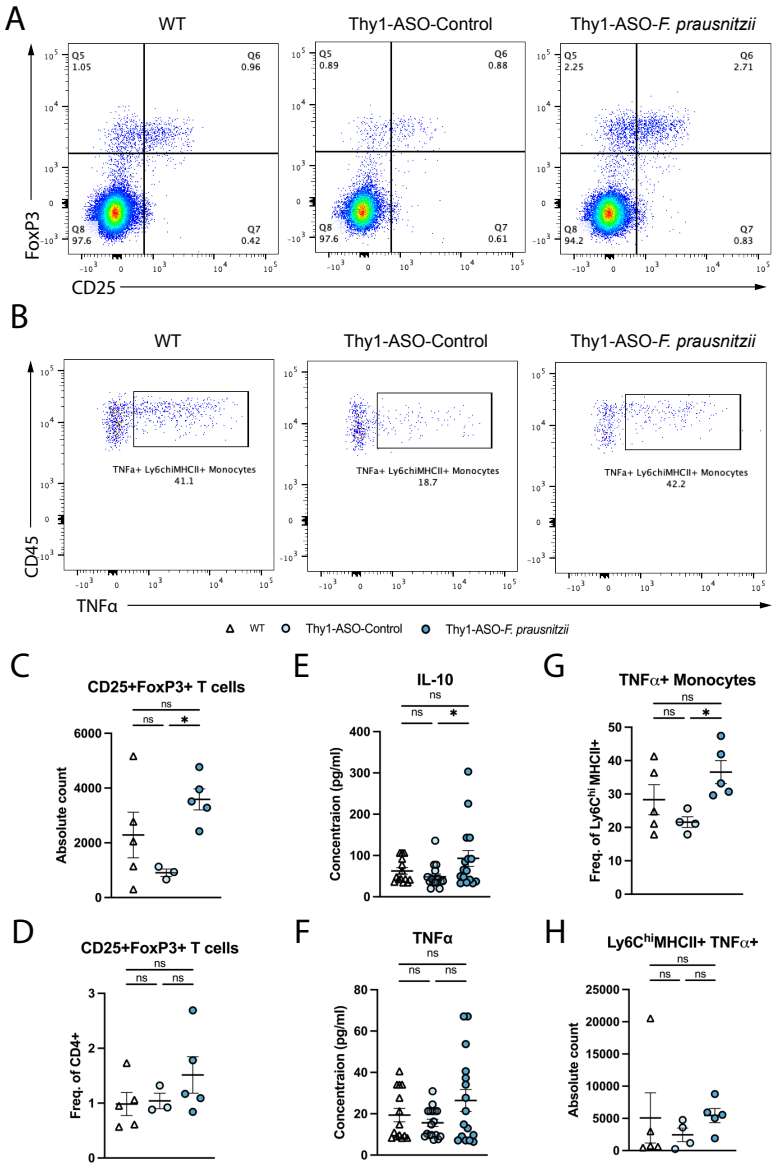


Figure 12. *F. prausnitzii* supplementation impacts gut and systemic immune compartments.

(**A, C, D**) Representative (A) flow cytometry plots and (C, D) quantification, expressed as absolute count (C) and percentage (D), of activated CD25+FoxP3+ regulatory T cells in mesenteric lymph nodes, N = 3-5. (**B, G, H**) Representative (B) flow cytometry plots and (G, H) quantification, expressed as percentage (H) and absolute count (H), of $Ly6C^{hi}$ MHCII+ monocytes producing TNFα in spleen, N = 4-5. (**E, F**) (E) IL-10 and (F) TNFα concentration in colon tissue as measured by multiplex bead assay. Data are compiled from two independent cohorts, N= 14-17. Points represent individual animals, bars represent standard error of the mean. Data analyzed by one-way ANOVA with Tukey post-hoc test. For gating strategies, see **Supplementary Figures 1 & 2**. ns – not significant; *p≤0.05
Abbreviations: IL-10 – interleukin-10; TNFα – tumor necrosis factor α

Despite the alterations in the immune profile and shifts in the gut microbiome (see **Fig. 11**) observed in *F. prausnitzii*-treated animals, we did not detect differences in the levels of volatile fatty acids, including measurable SCFAs, in fecal, cecal, or serum samples from Thy1-ASO-treated mice (**Fig. 13A-C**). Fermentation pathway analysis of metagenomic data confirmed no changes in abundance of genes associated with SCFA metabolite production pathways across experimental conditions (**Fig. 13D**). This outcome is in contrast to previous studies reporting increases in SCFAs following *F. prausnitzii* supplementation (Miquel et al., 2014; Lenoir et al., 2020; Moon et al., 2023), though at least one other study similarly found no effect (Mohebali et al., 2023). While the therapeutic effects of *F. prausnitzii* in Thy1-ASO mice do not appear to be mediated by SCFAs, further mechanistic studies with knockouts of bacterial (butyrate production) or mouse (SCFA receptors) genes are needed.

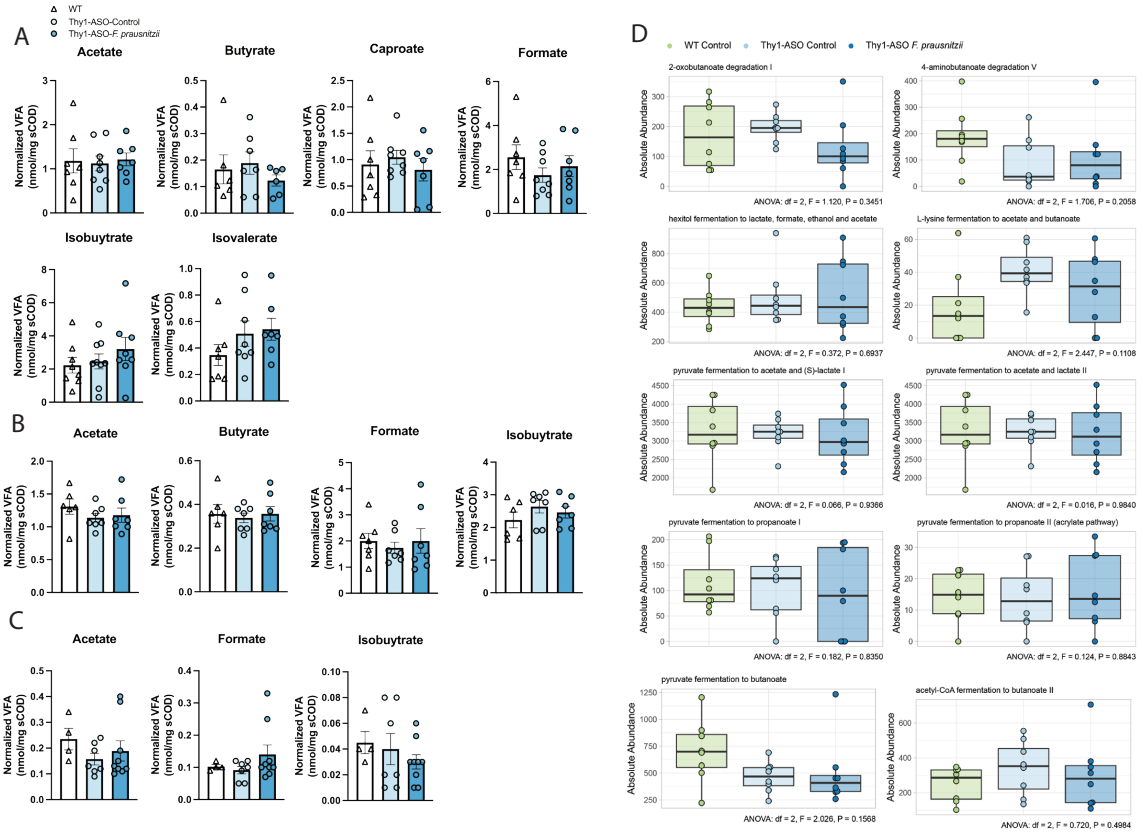


Figure 13. Volatile fatty acid and fermentation pathway analyses. (**A**) Fecal, (**B**) cecal, and (**C**) serum detectable volatile fatty acid measurements, normalized by soluble chemical oxygen demand (sCOD), from Thy1-ASO animals treated with *F. prausnitzii* and controls. N = 4-9. Points represent individual animals, bars represent standard error of the mean. Data analyzed by one-way ANOVA with Tukey post-hoc test. No significant changes were detected. Points represent individual animals, bars represent standard error of the mean. (**D**) Boxplots of the abundance of predicted fermentation pathways inferred from metagenomic sequencing data using HUMAnN3. Boxes indicate the median and interquartile range. Data were analyzed using a one-way ANOVA.

F. prausnitzii supplementation remodels the gene expression profile of the large intestine

To gain insight into potential molecular mechanisms of *F. prausnitzii*-mediated protection, we performed bulk RNA sequencing of the proximal large intestine, which revealed significant (p = 2.00E-04) gene expression differences between experimental groups (**Fig. 14A**). Compared to control (vehicle-treated) Thy1-ASO mice, *F. prausnitzii* supplementation altered expression of several immune-related genes linked to PD (**Fig 14B, E**). The gene encoding transferrin receptor (*Tfrc*), whose transcription was

upregulated in colon tissue from *F. prausnitzii*-treated animals, is essential for iron uptake and supports T and B cell proliferation (Jabara et al., 2016). TFRC and transferrin (TF) are reduced in the temporal cortex and substantia nigra of PD patients, and TF injections ameliorate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model outcomes (Bolen et al., 2025; Sabbir et al., 2024; Ayton et al., 2016). CCL5, or RANTES, is a chemokine that is elevated in the serum of PD patients (Rentzos et al., 2007; Tang et al., 2014), and its gene (*Ccl5*) was downregulated in mice treated with *F. prausnitzii*. *Cd8a,* which encodes a protein expressed on cytotoxic T cells, was also downregulated following *F. prausnitzii* supplementation. CD8+ cytotoxic T cells have been found to infiltrate the brain in PD mouse models and are elevated in the peripheral blood of patients (Yan et al., 2021; Galiano-Landeira et al., 2020; Williams et al., 2021). Thus, in the gut tissue of *F. prausnitzii*-treated Thy1-ASO mice, we observed normalized expression of several genes (or their products) previously reported to be altered in the blood and brain of PD patients.

Comparative analysis of differentially expressed genes revealed a strong positive correlation between differences observed in WT versus Thy1-ASO control mice and those seen in Thy1-ASO *F. prausnitzii*-treated versus Thy1-ASO control mice ($\rho = 0.37$, $p < 2.2E{-}16$) (**Fig. 14C**), suggesting that probiotic treatment shifts the transcriptional profile of Thy1-ASO animals towards that of WT mice. Of note, among the genes that were upregulated in colonic tissue of both WT and Thy1-ASO *F. prausnitzii* mice compared to Thy1-ASO control animals, was transthyretin (*Ttr*), a gene relevant to PD. *Ttr* encodes a transporter of thyroid hormones and retinol, and mutations in this gene cause transthyretin amyloidosis, which is characterized by amyloid deposits (Sekijima et al., 2014). TTR is reduced in the cerebrospinal fluid (CSF) of Lewy body disease and Alzheimer's disease patients (Maetzler et al., 2012), and is able to cleave free αSyn *in vitro*, suggesting a possible neuroprotective role (Sarkany et al., 2023). Selenoprotein S, or SELENOS, supports protein folding, limits oxidative stress, and localizes to Lewy bodies in PD (Cardoso et al., 2015). SELENOS activity is dependent on selenium, a trace element whose deficiency has been linked to cognitive decline, and selenium

supplementation has shown therapeutic effects in several PD models (Xu et al., 2023; Cardoso et al., 2015). *Selenos* gene expression was slightly downregulated in both WT and *F. prausnitzii*-treated Thy1-ASO mice, potentially indicating a reduced need for its activity in these conditions.

Gene ontology (GO) analysis indicated significant enrichment in pathways associated with tissue repair and cytoskeletal remodeling in *F. prausnitzii*-treated Thy1-ASO animals compared to Thy1-ASO control mice (**Fig. 14D, E**). Enrichment of *Actin filament*-related, *wound healing*, and *muscle contraction* pathways suggests activation of cellular mechanisms involved in restoring intestinal barrier integrity and repairing gut function. Hypoxia inducible factor-1α (*Hif1a*), which is part of the *wound healing* and *tissue remodeling* pathways, encodes a transcription factor that regulates cellular response to low oxygen and is also an inducer of FoxP3+ regulatory T cells in inflamed intestinal tissue (Clambey et al., 2012). Additionally, HIF-1α has been shown to be involved in mechanisms related to several drug treatments in PD animal models and has been proposed as a potential therapeutic target (Guo et al., 2016; Kandil et al., 2019; Wang et al., 2024). Endothelin-3 (*Edn3*), involved in the *regulation of metal ion transport* and *muscle contraction* pathways, promotes T_{REG} proliferation *in vitro* (Freitas et al., 2021). Additionally, GO pathways containing TNFα-related genes were impacted by *F. prausnitzii* treatment. While a pro-inflammatory cytokine, TNFα stimulates T_{REG} function (Chen et al., 2010, 2013) and is involved in tissue repair (Ritsu et al., 2017; Choi et al., 2023). Tumor necrosis factor superfamily member 12a (*Tnfrsf12a*), upregulated in the *wound healing* pathway, encodes a receptor of TNFα-like weak inducer of apoptosis (TWEAK/Tnfrsf12), which plays a role in tissue regeneration (Winkles et al., 2008; Mendez-Barbero et al., 2019). *Slc8A3* (NCX3) encodes a sodium/calcium exchanger protein that regulates TNFα release from macrophages and monocytes (Staiano et al., 2013), and was upregulated in the *muscle contraction* pathway by *F. prausnitzii*. Interestingly, calcium homeostasis disruption is believed to contribute to PD pathology, with Na^+/Ca^{2+} exchanger proteins implicated in this dysregulation (Bastioli et al., 2024). The upregulation of genes involved in immune homeostasis and tissue repair suggest that

F. prausnitzii has therapeutic effects in the intestine of Thy1-ASO animals, though potential gut-to-brain pathways remain to be explored. Collectively, our findings highlight the therapeutic potential of *F. prausnitzii* in a preclinical model of PD, demonstrating its capacity to ameliorate both behavioral and neuropathological features, while also modulating gut microbiota composition, immune responses, and host gene expression in the Thy1-ASO mouse model.

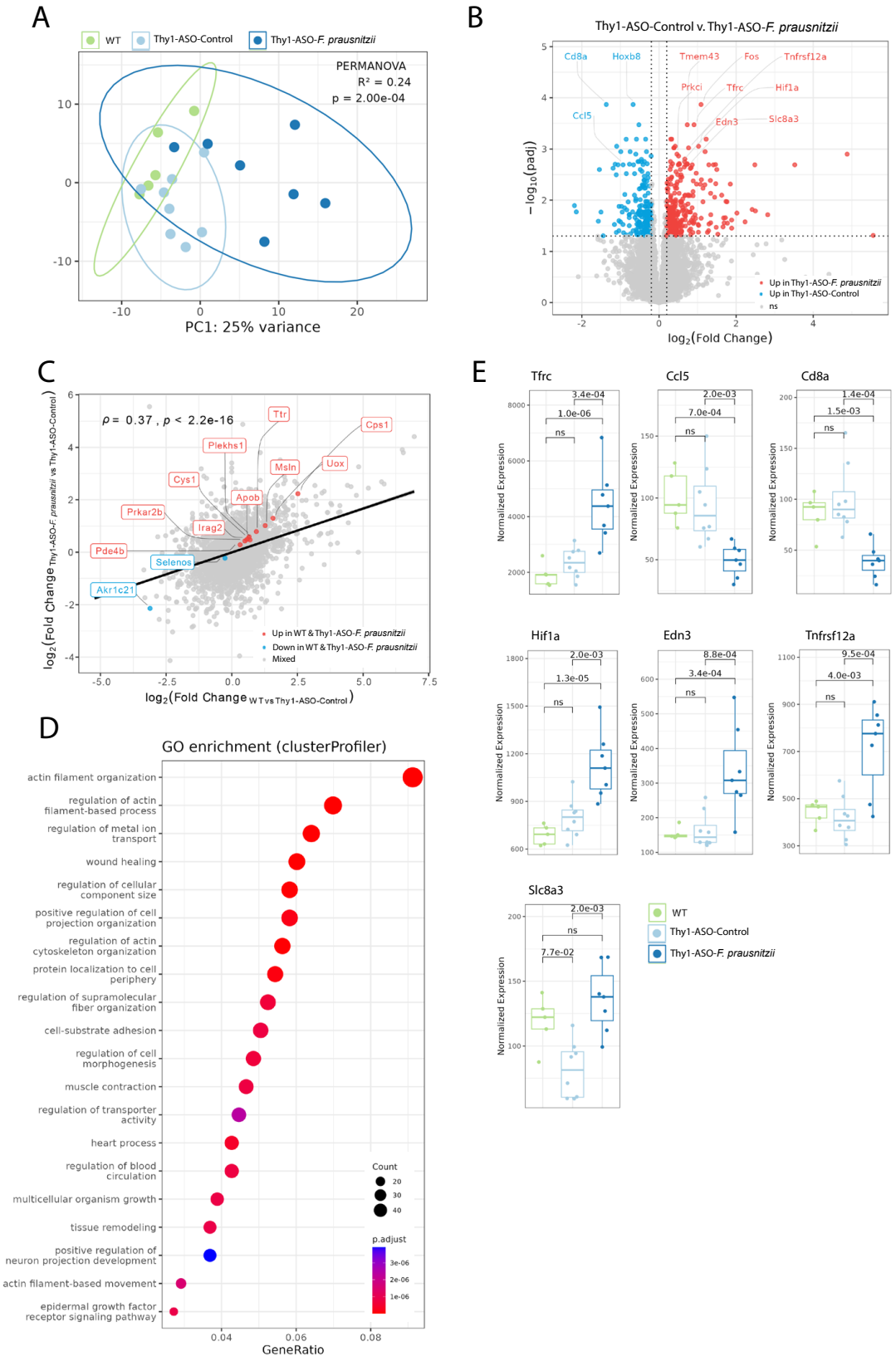


Figure 14. Host gene expression profiles in the large intestine are modulated by *F.*

***prausnitzii* supplementation**. (**A**) Principal component analysis of gene expression profiles in large intestine. Ellipses indicate 95% confidence intervals. R^2 and p-value PERMANOVA statistics are displayed for the experimental condition category using 9,999 permutations. (**B**) Volcano plot of genes differentially expressed between Thy1-ASO-Control and Thy1-ASO-*F. prausnitzii* groups. (**C**) Cross-condition scatter plot of genes differentially expressed between WT and Thy1-ASO-Control on the x-axis and Thy1-ASO- *F. prausnitzii* and Thy1-ASO-Control on the y-axis. Pearson correlation coefficient (ρ) and p-value of log2 fold-change of all genes tested are shown. (**D**) Gene ontology (GO) enrichment analysis showing pathways upregulated in Thy1-ASO-*F. prausnitzii* tissue v. Thy1-ASO-Control. (**E**) Expression levels of differentially regulated genes of interest. Box plots show normalized gene expression values (size-factor scaled counts) with DESeq2 adjusted p-values between comparisons. ns – not significant

Discussion

Herein, we demonstrate that oral administration of a single taxon found to be depleted in the PD microbiome, *F. prausnitzii*, is sufficient to ameliorate both motor and GI function deficits and reduce αSyn aggregation in the brain in a mouse model of disease. *F. prausnitzii* treatment shifted the microbiome in Thy1-ASO mice closer to a WT profile, exerted immunomodulatory effects, and enhanced intestinal transcriptional programs associated with tissue regeneration. Our findings demonstrate that targeted microbial supplementation can elicit functional improvements in preclinical models, and may translate to replenishing microbes that are depleted in PD patients.

Metagenomic sequencing revealed that treatment with *F. prausnitzii* modestly, but significantly, remodeled or maintained the fecal microbiome of Thy1-ASO mice, with treatment-associated shifts in overall community structure. Measures of alpha diversity remained largely unchanged across groups, consistent with a selective, rather than global, change in the microbial community by *F. prausnitzii*. Differential abundance analysis indicated subtle directed ecological interactions with endogenous microbial populations, but lacked strong associations, likely reflecting *F. prausnitzii*'s instability in the mouse gut. Importantly, and consistent with the finding that the effects of *F. prausnitzii* on microbiome composition appear targeted, mediation analysis suggested that the therapeutic effects of *F. prausnitzii* are likely to be largely direct rather than mediated by remodeling of the broader microbiome.

Following treatment with *F. prausnitzii*, we observed increased intestinal T_{REG} populations and elevated levels of the anti-inflammatory cytokine IL-10, consistent with prior findings of its capacity to induce immune tolerance (Qiu et al., 2013; Martín et al., 2014; Zhou et al., 2018). We also found increases in TNFα-producing monocyte populations in the spleen. While this finding is in contrast to observations in a mouse model of chronic colitis treated with *F. prausnitzii* (Martin et al., 2014), TNFα has been shown to be induced by *F. prausnitzii* (Brown et al., 2021) and is implicated in tissue repair (Ritsu et al., 2017; Choi et al., 2023), suggesting this may be part of its broader immune regulatory profile. Despite being a major SCFA produced by *F. prausnitzii* and inducer of T_{REGS} (Arpaia et al., 2013), butyrate was not found to be increased in animals receiving treatment. This finding indicates that the bacterium's therapeutic effects in Thy1-ASO mice may be independent of SCFAs and perhaps driven by secreted immunomodulatory molecules such as the Microbial Anti-inflammatory Molecule (MAM) (Quévrain et al., 2016).

Transcriptomic profiling of colonic tissue revealed that *F. prausnitzii* shifted the gene expression landscape of PD mice toward that of WT control animals, with enrichment of genes and pathways involved in immune regulation, epithelial repair and regeneration. Notably, *F. prausnitzii* treatment normalized the expression of several genes associated with PD. Thus, *F. prausnitzii* is able to alter the gene expression profile in this PD mouse model, potentially contributing to its immunomodulatory and therapeutic effects, a hypothesis that remains to be tested.

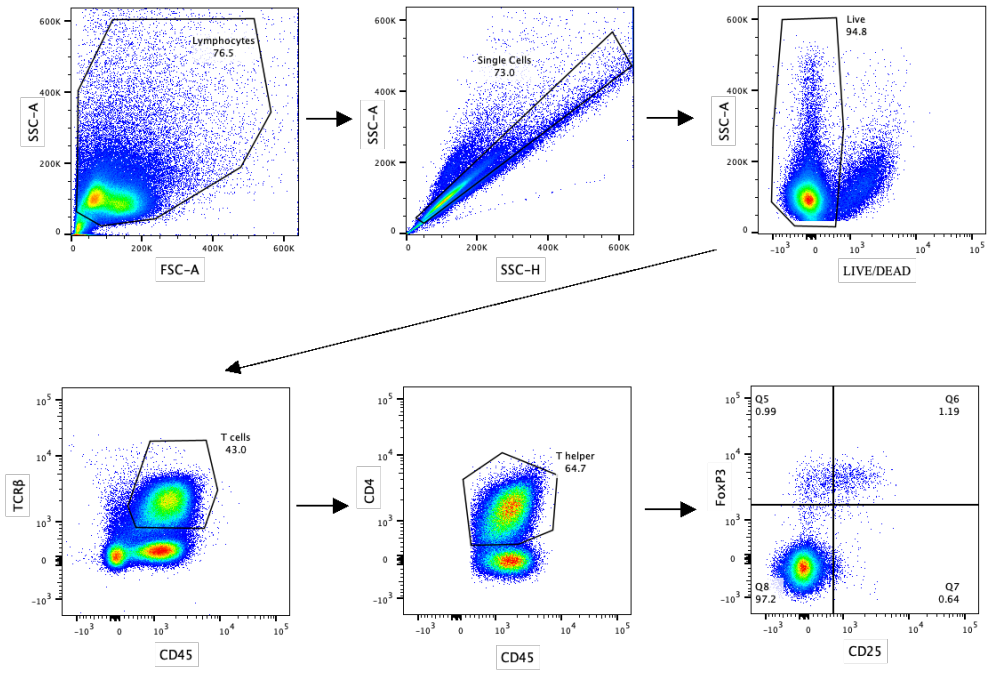
In prior probiotic treatment studies using PD animal models, *Lacticaseibacillus rhamnosus* has shown neuroprotective effects in MPTP-treated mice (Aktas et al., 2024; Liu et al., 2020). Similarly, *Lactobacillus plantarum* PS128 reduces microglial activation and motor deficits in MPTP-treated (Liao et al., 2020) and rotenone-exposed mice (Lee et al., 2023). Another study reported the ability of *Akkermansia muciniphila* to ameliorate PD-like symptoms in the MPTP model (Qiao et al., 2024). In early clinical work, multi-

strain probiotics containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* have demonstrated improvements in constipation (Ibrahim et al., 2020; Barichella et al., 2016). *L. plantarum* PS128 elicits modest improvements in Unified Parkinson's Disease Rating Scale (UPDRS) scores, likely mediated by increased plasma dopamine metabolites (Lu et al., 2021). However, meta-analyses emphasize that while commercial probiotics modestly improve GI and motor outcomes, the quality of evidence remains low due to small sample sizes, short treatment durations, and lack of mechanistic biomarkers (Chu et al., 2023). Further, unlike *F. prausnitzii*, these species have not been shown to be reproducibly reduced in PD patients.

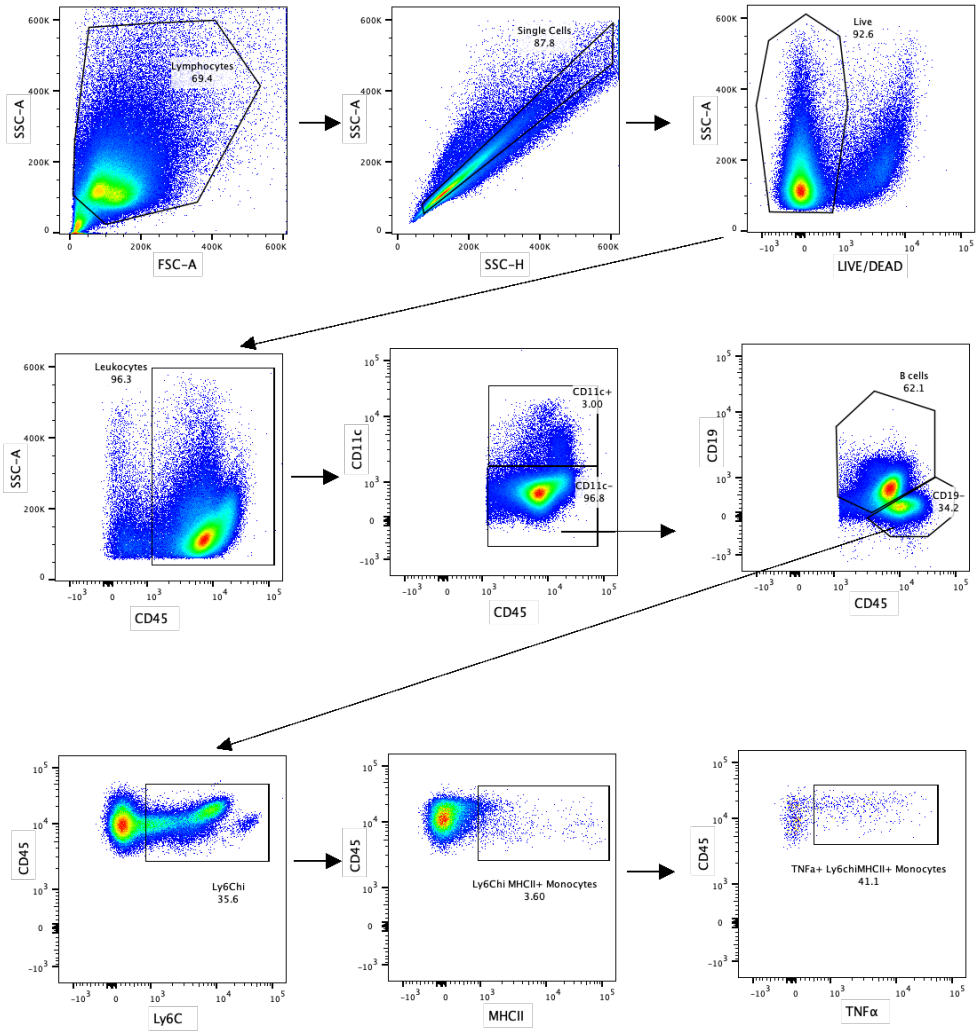
There are several limitations to this study. First, we only assessed *F. prausnitzii* in a single preclinical model – the Thy1-ASO mouse – which captures several key aspects of PD, including αSyn accumulation and progressive behavioral deficits (Chesselet et al., 2012), but lacks others, such as overt dopaminergic neuron loss at the ages tested. Additionally, integration of the αSyn transgene on the X chromosome makes expression inconsistent in female mice due to X inactivation, so only male mice were studied. Future studies employing complementary models and including aging (the primary risk factor for PD) will help validate and extend the observations of the current work. Second, although *F. prausnitzii* improved PD-associated behaviors and corrected certain molecular changes caused by αSyn overexpression, the precise mechanisms underlying its effects remain untested. Whether these improvements are mediated primarily through peripheral immune modulation, direct gut-brain signaling (i.e., vagal or spinal neurons), production of beneficial microbial metabolites, microbiome restoration, or a combination thereof requires further investigation. Finally, our treatment strategy tested a prophylactic approach prior to disease symptom onset; thus, translation to human studies would require identification of at-risk or prodromal PD populations, which the PD research community is advancing, but is not yet reliably achievable.

Our findings provides proof-of-concept that targeted restoration of bacterial species that are depleted in PD patients can improve disease-relevant outcomes, supporting the emerging hypothesis that the gut microbiome is a modifiable contributor to PD. Addressing environmental risk factors of PD, such as the gut microbiome, compared to correcting genetic predispositions, offers a likely more tractable therapeutic route. Furthermore, development of PD-specific, rather than generic, probiotics represents a promising avenue for advancing treatment options.

Supplementary figures



Supplementary Figure 1. Adaptive immune system gating strategy for flow cytometry analysis. *(Associated with Figures 5 & 12)*



Supplementary Figure 2. Innate immune system gating strategy for flow cytometry analysis. *(Associated with Figures 5 & 12)*

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C h a p t e r 5

METHODS

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"*Faecalibacterium prausnitzii,* depleted in the Parkinson's disease microbiome, improves motor deficits in α-synuclein overexpressing mice"
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Bacterial cultures

Strains were purchased from DSMZ and ATCC and grown and prepared inside an anaerobic chamber (Coy Laboratories) (80% nitrogen, 10% carbon dioxide, and 10% hydrogen) at 37℃. *Roseburia intestinalis* (DSMZ14610), *Roseburia faecis* (DSMZ16840), and *Anaerostipes hadrus* (ATCC29173) were grown in Yeast Casitone Fatty Acids with Carbohydrates (YCFAC) media (Anaerobe Systems). *Fusicatenibacter saccharivorans* (DSMZ26063) and *Prevotella histicola* (DSMZ19854) were grown in Peptone Yeast Extract Broth with Glucose (PYG) media (Anaerobe Systems). *Bacteroides fragilis* (ATCC25285) and *Bacteroides thetaiotaomicron* (ATCC29148) were grown in Brain Heart Infusion - Supplemented (BHIS) media (ATCC medium: 1293). *Bacteroides ovatus* (ATCC8483) was grown in Chopped Meat Broth (Anaerobe Systems) and *Eubacterium rectale* (ATCC33656) was grown in Mega Medium (Cheng 2022, Han 2021). *Faecalibacterium prausnitzii* (DSMZ17677) was grown in Chopped Meat with Carbohydrates (CMC) (Anaerobe Systems). For preparing aliquots for intragastric administration (gavage), cultures were grown to an approximate optical density (600nm) of 1, then spun down and resuspended in sterile 50% glycerol to an approximate Colony-Forming Unit (CFU) density of 10^8/mL. For benCom-PD, all bacterial cultures were pooled in equal quantities. Aliquots were frozen at -20℃ for short-term storage in cryotubes wrapped with parafilm to protect from oxygen exposure. On the day of gavage, an aliquot was thawed and resuspended in a sterile solution of phosphate buffered saline (PBS) with 1.5% $NaHCO_3$. Gavage tubes were set up inside the anaerobic chamber to reduce oxygen exposure and placed into a GasPak box (BD) with an AnaeroPack (MGC) for transportation to the animal facility. Two times a week, animals received a gavage of 100µL of vehicle (PBS + 1.5% $NaHCO_3$) (WT and Thy1-ASO-Control groups) or bacterial culture (benCom-PD or individual microbe - Thy1-ASO-Treated groups).

Mice

All animal husbandry and experiments were approved by the Caltech Institutional Animal Care and Use Committee. Alpha-synuclein overexpressing (Thy1-ASO) male

mice were bred by crossing wildtype (WT) BDF1 males with Thy1-ASO females of the BDF1 background that were heterozygous for the Thy1 promoter driving the human alpha-synuclein transgene located on the X chromosome (Chesselet et al., 2012; Rockenstein et al., 2002). Conventional mice had a specific pathogen-free (SPF) microbiome and were housed in microisolator cages with HEPA-filtered ventilation. All SPF animals received irradiated chow (LabDiet 5053) and water *ad libitum*, and were maintained on a 12 hour light-dark cycle.

For hCom2 experiments, germ-free WT BDF1 males and Thy1-ASO females were colonized (2 gavages, 1 day apart) with full hCom2 or hCom2 without *F. prausnitzii*, as formulated by the Fischbach lab. Colonized breeders and offspring were kept in the same room as SPF mice but housed in sterile cages with sterile bedding and water, and received autoclaved chow (LabDiet 5010).

Motor and gastrointestinal function testing

Motor function test methods were similar to those previously described (Fleming et al., 2004; Sampson et al., 2016). All motor and GI function tests were performed between hours 7 and 9 of the light phase, at the same time every day, inside a biosafety cabinet in the same facility. Animals were habituated to the behavior testing room for 1 hour prior to the start of motor and GI function experiments. Beam traversal and pole descent training and testing were done together on two consecutive days, with approximately one hour of rest time between each task. After one rest day, testing for wire hang was conducted. Lastly, adhesive removal and hindlimb clasping scoring were performed on the final day. GI function tests were performed the following week, with bead expulsion assayed last.

Beam traversal

Four 0.25m plexiglass beam sections, starting at 3.5cm width and narrowing to 0.5cm width, were placed together to form a 1m beam. The beam was supported by 3 standard size mouse cages at the beginning, middle, and end. Animals were placed onto the widest

section of the beam and trained to run across the beam towards their home cage, which was placed sideways at the end of the narrowest section, with the open side facing the animal. Mice were given 3 trials on the training day. The first trial was guided, with the home cage being brought close and moved along with the animal to encourage forward progress. The second trial was performed similarly but with less guidance and encouragement. For the third trial, the home cage was returned to the end of the beam and animals traversed the beam with limited assistance. On the next day, animals were tested for their ability to traverse the beam. Timing began once the mouse crossed onto the second (2.5cm) section of the beam and stopped once one of the forelimbs was inside the home cage. The maximum time of 60 seconds was recorded for animals that did not finish the assay within the allotted time or fell off the beam on the last (0.5cm) section. Test day trials were videotaped and later assessed in slow motion for number of steps and slips (errors) that occurred during the traversal. Slips were counted if at least ¾ of any limb left the beam. Errors per step were not recorded for animals that did not complete the task. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.kxygx4znzl8j/v1.

Pole descent

A 0.5m rod, 1cm in diameter, attached to a base and wrapped in shelf liner to increase grip, was used to assess the animal's ability to climb down a pole. Mice were given 1 day of training prior to the test day, on the same day as beam training and testing, with about an hour of rest time between assays. The training day consisted of 3 trials. On the first trial, cagemates were removed and the pole was placed into the home cage. The animal was placed at the bottom of the home cage with the pole for approximately 1 minute to allow exploration. Then the animal was placed approximately ⅓ of the way up the pole, facing head down, and allowed to descend. For the second trial, the animal was placed ⅔ of the way up the pole, and for the third trial, at the top of the pole. On the consecutive day, animals were tested for their ability to descend from the top of the pole. Timing started at the moment the animal's tail was released and ended when one of the hindlimbs touched the base of the pole. The maximum time of 60 seconds was recorded for animals

that slid or fell off the pole apparatus. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.8epv5k9mjv1b/v1.

Wire hang

A clean cage with bedding was placed 40cm underneath a 30cm x 30cm wire mesh screen. Animals were placed at the center of the screen and gently flipped head-first until upside down. Timing started once the animal was hanging from the wire screen horizontally and stopped when the animal fell to the bedding underneath or remained hanging for 60 seconds. For detailed protocol, see dx.doi.org/10.17504/protocols.io.6qpvrw62plmk/v1.

Adhesive removal

Cagemates were removed from the home cage and a circular ⅜ in sticker (Tough-Spots - Diversified Biotech/USA Scientific) was placed on the nose bridge of the animal being tested using tweezers. The mouse was released into the home cage and timing was started. Timing was stopped when the mouse successfully removed the sticker from its nose, with a maximum time of 30 seconds. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.4r3l21objg1y/v1.

Hindlimb clasping

Mice were picked up by the tail, with the underside facing the observer, for approximately 10 seconds. Rigidity of the movement of hindlimbs was assessed on a scale of 0 to 3. A score of 0 was given if the animal was able to extend its hindlimbs outward freely, with flexibility and no clasping. A score of 1 was given if the animal had some tendency to clasp its hindlimbs inward, either one or both, but for the majority of the restraint the limbs were still spread outward and flexible. A score of 2 was given if hindlimbs were clasped inward for the majority of the time, but some movement and flexibility was still present. A score of 3 was given if the hindlimbs were clasped together entirely, with no movement or flexibility (Zhang et al., 2014). For a detailed protocol, see dx.doi.org/10.17504/protocols.io.eq2ly4n2mlx9/v1.

Constipation assays

For fecal output measurements, mice were individually placed into empty, bedding-free, standard mouse cages. The number of fecal pellets produced was counted after 15 minutes.

Following the fecal output assay, remaining fecal pellets were assessed according to the Bristol stool scale: a score of 1 was given for fecal pellets that were small, dry, hard lumps, indicating severe constipation. A score of 2 was given for lumpy, dry, but sausage-like fecal pellets, indicating mild constipation. A score of 3 was given for mildly dry sausage-like pellets with cracks in the surface. A score of 4 was given for smooth, soft, sausage or snake-shaped pellets. Both scores of 3 and 4 are considered normal. A score of 5 was given for soft blobs with clear-cut edges. A score of 6 was given for fluffy pieces with ragged edges, indicating mild diarrhea. Lastly, a score of 7 was given for an entirely liquid consistency, indicating severe diarrhea (Lewis et al., 1997). An average score was taken when animals produced fecal pellets of various scores during one fecal output session.

Following fecal output and score assays, 2-3 fecal pellets were collected in pre-weighed tubes. Tube and wet fecal pellet weights were measured and then pellets were incubated at 65℃ for 24 hours. Tubes and dry fecal pellets were re-weighed and percentage of water content calculated. For detailed protocols of constipation assays, see dx.doi.org/10.17504/protocols.io.6qpvrw6qplmk/v1.

Whole gut transit time

Animals were gavaged with 150µl of autoclaved 6% (w/v) Carmine red (Millipore Sigma) and 0.5% methylcellulose (Sigma-Aldrich) solution in water. After 3 hours, animals were transferred to empty, bedding-free, standard mouse cages and observed every 30 minutes up to 8 hours for the first red fecal pellet (Nagakura et al., 1996;

Camilleri et al., 2016). For a detailed protocol, see dx.doi.org/10.17504/protocols.io.rm7vz9yq8gx1/v1.

Bead expulsion

Animals were placed into an isoflurane induction chamber on a heating pad in 1 L/min oxygen and a concentration of 3-5% isoflurane until the respiratory rate reached one breath per minute. Animals were then transferred to a nose cone to maintain anesthesia and a 2mm glass bead inserted into the colon 2 cm proximal to the anus using a metal gavage tube with lubricating jelly. Once the animal emerged from anesthesia, it was transferred to an individual empty, bedding-free, standard mouse cage and timed until the bead was expelled, up to 60 minutes (Koslo et al., 1986; Camilleri et al., 2016). For a detailed protocol, see dx.doi.org/10.17504/protocols.io.n92ld6zn7g5b/v1.

Motor and gastrointestinal function datasets in Chapters 2 and 4 were analyzed using the Kruskal-Wallis test followed by a Conover-Iman post-hoc test of multiple comparisons with Benjamini-Hochberg false discovery rate (FDR) (Benjamini and Hochberg, 1995) to correct for multiple testing. Motor function datasets in Chapter 3 were analyzed using a 2-way ANOVA and Kruskal-Wallis test followed by a Dunn's post-hoc test of multiple comparisons. Graphs were generated using GraphPad Prism 10 software (http://www.graphpad.com, RRID:SCR_002798). P-values, n values, and standard errors are indicated in figures and legends.

Colonization experiments

Experiments were conducted using mature animals 6-8 weeks old. For *F. prausnitzii* engraftment into SPF mice, a 10^{10}cfu/mL culture gavage was given for 5 days consecutively (see **Bacterial cultures** section for gavage preparation). For antibiotic pre-treatment, 1g/L ampicillin (Methapharm), 1g/L neomycin sulfate (Sigma-Aldrich), 0.5g/L vancomycin hydrochloride (Mylan), 0.5g/L metronidazole (Acros Organics), and 10g/L sucrose (1%) was mixed in water and filter sterilized using a 0.22µ vacuum filtration system. Animals received antibiotic water for 5 days, followed by regular water for 1 day

prior to initiation of colonization gavages. *F. prausnitzii* was subsequently administered for 5 days consecutively at a concentration of 10^{10}cfu/mL. For germ-free engraftment experiments, animals were gavaged with *F. prausnitzii* or *B. thetaiotaomicron* with *F. prausnitzii* every other day for 3 days to facilitate gradual deoxygenation of the mouse gut. For cecal content transfer, germ-free mice colonized with *B. thetaiotaomicron* and *F. prausnitzii* were euthanized and ceca were dissected inside an anaerobic chamber (Coy Laboratories). Extracted cecal contents were resuspended in pre-reduced sterile solution of phosphate buffered saline (PBS) with 1.5% $NaHCO_3$. Solid contents were filtered out using a sterile 100µm cell strainer (Corning) and 100µL of slurry was gavaged to SPF animals every other day for 3 days.

qPCR

Fresh fecal pellets were sterilely collected in pre-weighed tubes at time points following the last day of a colonization gavage and stored at -80°C until further processing. Standards and positive control for assays were created by adding serially diluted pure *F. prausnitzii* culture to a germ-free fecal pellet prior to extraction. DNA was extracted following manufacturer's protocol using the Qiagen QIAamp PowerFecal Pro kit (Cat. #51804) and quantified using a NanoDrop spectrophotometer and the Quant-iT™ 1X dsDNA Assay Kit, BR (Life Technologies). Samples were combined with SYBR™ Green PCR Mastermix (Applied Biosystems) and run on a CFX Opus 384 Real-Time PCR System (Bio-Rad) using species specific primers for *F. prausnitzii* Fwd: 5'-GGAGGAAGAAGGTCTTCGG-3' Rev: 5'-AATTCCGCCTACCTCTGCACT-3' (Fitzgerald et al., 2018). Average starting quantity results were used to normalize to mass of feces.

Protein assays

Following motor and GI function testing, animals were sacrificed and tissues collected.

Western blots

2mm sections of the substantia nigra and striatum were dissected, flash frozen, and stored at –80°C until further processing. Brain tissues were lysed using a handheld homogenizer (BT LabSystems) and the Bio-Plex Cell Lysis Kit according to manufacturer's instructions (Bio-Rad). Protein concentrations were normalized using the Pierce BCA Protein Assay Kit (Thermo Scientific). Samples were separated on a 4-20% Tris-Glycine gel (Invitrogen) and transferred onto a 0.45µm PVDF membrane (Immobilon-P - Merck Millipore Ltd.). To enhance detection of αSyn, membranes were placed in 4% paraformaldehyde (PFA) solution for 30 minutes (Lee et al., 2011). Blocking was performed using 5% bovine serum albumin (BSA) in Tris-buffered saline with 0.1% Tween-20 (TBS-T) for phosphorylated αSyn antibody or 5% nonfat milk in TBS-T for β-actin antibody. Membranes were cut at 25kDa and incubated overnight at 4°C with primary antibodies: Anti-phosphorylated-S129 α-Synuclein Rabbit (Abcam Cat# ab51253, RRID:AB_869973, 1:1000) or Anti-β-actin Mouse (Abcam Cat# ab8226, RRID:AB_306371**,** 1:1000) in the respective blocking buffers. After washing, membranes were incubated with Anti-IgG HRP rabbit or mouse antibodies (Cell Signaling Technology Cat# 7074, RRID:AB_2099233 and Cat# 7076, RRID:AB_330924, 1:1000) in TBS-T for 1.5 hours at room temperature. Signal was detected using the Clarity Western ECL Substrate (Bio-Rad) on a Bio-Rad ChemiDoc imaging system. Densitometry analysis was performed using ImageJ. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.x54v92z2ql3e/v1.

Dot blots

Homogenized brain tissue samples were diluted to 500ng/µL of protein and directly spotted onto a 0.45um nitrocellulose membrane (Thermo Scientific). Membranes were blocked using 5% nonfat milk in TBS-T and incubated for 1.5 hours with Anti-Aggregated α–Synuclein Rabbit (Abcam Cat# ab209538, RRID:AB_2714215, 1:1000) at room temperature in blocking buffer. Subsequent steps followed the same procedure as

described for Western blot analysis. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.261gen2xdg47/v1.

Multiplex bead assay

Proximal large intestine segments (~2cm) were homogenized in lysis buffer (Bio-Plex Cell Lysis Kit, Bio-Rad) using Lysing Matrix D tubes and a bead beater (MP Biomedicals). Total protein concentrations were quantified and normalized using the Pierce BCA Protein Assay Kit (Thermo Scientific). Cytokine levels were then measured on the BioPlex 200 platform using the Bio-Plex Pro Mouse Th17 6-plex Assay (Bio-Rad), following the manufacturer's protocol.

Flow cytometry

Spleen and mesenteric lymph nodes were collected and processed for flow cytometric analysis. Tissues were passed through 70µm cell strainers, and spleen cells were treated with red blood cell lysing buffer (Hybri-Max - Sigma Aldrich) for 5 minutes at room temperature. For a detailed isolation protocol, see dx.doi.org/10.17504/protocols.io.nm2dc8e. For later intracellular staining, isolated cells were stimulated with phorbol myristate acetate (PMA) (50 ng/ml, Sigma Aldrich) and ionomycin (750ng/ml, Sigma Aldrich) with GolgiPlug™ (1:1000, BD Biosciences) for 4 hours at 37°C and 5% CO_2. All cells were then blocked using anti-Fcy receptor (anti-CD16/CD32) (clone 93, eBioscience) and labelled with the following fluorescent-conjugated antibodies: CD45 (clone 30-F11, eBioscience and BioLegend), TCRB (clone H57-597, eBioscience), CD4 (clone RM4-5, eBioscience), and CD25 (clone PC61.5, eBioscience) for adaptive immune system analysis and CD11c (clone N418, eBioscience), CD19 (clone 1D3, BioLegend), MHCII (clone M5, BioLegend), and Ly6C (clone HK1.4, BioLegend) for innate immune system analysis (all at 1:500 concentration). All cells were labelled with Fixable Near-IR LIVE/DEAD Stain Kit (Invitrogen) to distinguish live cells from dead/debris. For intracellular staining, cells were treated using the Foxp3/Transcription Factor Fixation/Permeabilization Kit (eBioscience) according to the manufacturer's instructions and stained with fluorescent-

conjugated antibodies against FoxP3 (clone FJK-16s, eBioscience, RRID:AB_763537) and TNFα (clone MP6-XT22, eBioscience) at 1:100 concentration. Data were acquired on a CytoFLEX S Analyzer (Beckman Coulter) and analyzed with FlowJo software (BD, Tree Star, https://www.flowjo.com/solutions/flowjo, RRID:SCR_008520).

Data generated from protein and cellular assays were analyzed using a one-way ANOVA with Tukey post-hoc test and graphs generated with GraphPad Prism 10 software. P–values, n values, and standard errors are indicated in figures and legends.

RNA sequencing

Approximately 1cm of proximal large intestine was collected, immediately flash-frozen in TRIzol reagent (Zymo Research), and stored at –80°C. Total RNA was extracted using the Direct-zol RNA MiniPrep Plus Kit (Zymo Research) per the manufacturer's protocol, including an on-column DNase I treatment. RNA quality and concentration were assessed using a NanoDrop spectrophotometer and an Agilent 2100 Bioanalyzer (Agilent Technologies). Libraries were prepared using the ABclonal FAST RNA-seq Library Prep Kit. Pooled libraries underwent 50 base pair, paired-end sequencing at a target depth of 30 million reads per sample on the Illumina NovaSeq X platform.

Raw sequencing reads were aligned to the *Mus musculus* GRCm36 Primary Assembly reference genome (Release M36 from GENCODE) using STAR aligner v2.7.1 (https://github.com/alexdobin/STAR, RRID:SCR_004463) (Dobin et al., 2013) with default parameters. Gene-level read counts were quantified using featureCounts (Liao et al., 2014). Only protein coding genes with a minimum sum of at least 10 counts across all samples were retained. Normalization and differential gene expression analysis was performed using the DESeq2 package (https://bioconductor.org/packages/release/bioc/html/DESeq2.html, RRID:SCR_015687) (Love et al., 2014) in R (v4.3.0, http://www.r-project.org, RRID:SCR_001905). Genes with an adjusted p-value < 0.05 and log2 fold change were considered significantly differentially expressed. Gene ontology and pathway enrichment analyses for

differentially expressed genes were conducted using the clusterProfiler package (http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html, RRID:SCR_016884) (Yu et al., 2012). Gene sets with an adjusted p-value < 0.1 and $|\log_2$ fold-change$| > 0.2$ were analyzed for up and down regulated genes separately for each comparison of interest. Data visualization, including volcano plots and principal component analysis (PCA), was performed using ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html, RRID:SCR_014601) (Wickham et al., 2016).

Volatile fatty acid measurement

Fecal pellets, cecal contents, and serum were collected and flash frozen on dry ice for later processing. Fecal pellets were collected fresh from live animals at 18 weeks of age. Cecal contents were collected at 23 weeks during tissue collection. For serum isolation, blood was drawn via cardiac puncture or from the trunk into microtubes containing serum gel (Z-gel, Sarstedt AG & Co.), allowed to clot on ice for 30 minutes, then centrifuged at 10,000xg for 5 minutes. The resulting serum was collected for downstream analysis.

Fecal, cecal, and blood serum volatile fatty acid (VFA) concentrations were quantified by high-performance liquid chromatography (HPLC) using sample extracts. For fecal and cecal extracts, deionized water was added to the samples at a ratio of 1 mL of water to 100 mg of sample. For serum extracts, in order to precipitate any proteins in the serum, 2.5 mM H_2SO_4 was added to samples at a 1:1 ratio. Serum samples were then vortexed briefly and placed at 4°C for 20 minutes. All extracts were vortexed at 3,200 rpm for 5 minutes and then centrifuged at 13,000 rpm for 15 minutes at 4°C. The supernatants were filter-sterilized through a 0.22 μm syringe filter. The filtrates were used for HPLC analysis and soluble chemical oxygen demand (sCOD) measurements. Analysis of VFAs was performed on an Agilent 1260 Infinity II (Agilent, Santa Clara, California, USA) diode array detector at 210 nm with a Bio-Rad Aminex HPX-87H column (Cat. #: 1250095) as previously described (Reynolds et al., 2022; Mohana Rangan et al., 2023). Samples were analyzed at a column temperature of 65°C using 5 mM H_2SO_4 as a mobile

phase at a flow rate of 0.6 mL/min for 80 minutes. A standard curve was generated using a 10 mM VFA mixed standard (Supelco Volatile Free Acid Mix, Cat. #: CRM46975) containing acetate, formate, butyrate, isobutyrate, valerate, isovalerate, propionate, caproate, and heptanoate. Dilutions were done in triplicate to achieve the following concentrations: 0.25 mM, 2 mM, 5 mM, 7.5 mM, and 10 mM. To account for differences in sample consistency, all VFA measurements were normalized against the sCOD measured from the filtrate, as previously reported (Sampson et al., 2016; Davis et al., 2021). To measure sCOD, a HACH high-range (20 – 1500 mg COD/L) COD kit (Cat. #: 2565115) was used (Davis et al., 2021; Dirks et al., 2025; Corbin et al., 2023). 100 µL of fecal or cecal sample filtrate, or 25 µL of serum sample filtrate was transferred to a HACH tube and adjusted to a final volume of 2 mL with deionized water. Samples were digested on a HACH DRB200 Digital Reactor Block (Cat. #: LTV082.53.44001) according to the manufacturer's recommendations. A HACH DR6000 spectrophotometer (Cat. #: LPV441.99.00012) was used to measure sCOD. Resultant data were analyzed using a one-way ANOVA with Tukey post-hoc test and graphs generated with GraphPad Prism 10 software. P-values, n values, and standard errors are indicated in figures and legends.

Fecal microbiome analysis with shotgun metagenomics

Fecal pellets for metagenomics were collected fresh from live animals at 18 weeks of age and DNA was isolated from one mouse stool sample pellet using the Qiagen QIAamp PowerFecal Pro kit following the manufacturer's instructions. Aliquots were shipped to Prebiomics S.r.l. (Trento, Italy) for library preparation and sequencing.

DNA was quantified using the Quant-iT™ 1X dsDNA Assay Kits, BR (Life Technologies) in combination with the Varioskan LUX Microplate Reader (Thermo Fisher Scientific). Sequencing libraries were prepared with the Illumina DNA Prep (M) Tagmentation (96 Samples, IPB) kit in combination with Illumina DNA/RNA UD Indexes. Amplified libraries were purified using the double-sided bead purification procedure, as described in the Illumina protocol. Then, library concentrations (ng/µL)

were quantified and base pair lengths (bp) evaluated using the D5000 ScreenTape Assay in combination with a TapeStation 4150 (Agilent Technologies) to establish library pooling volumes. Pooling was then quantified with the Qubit 1x dsDNA HS kit (Life Technologies) and a Qubit 3.0 Fluorometer (Life Technologies). Libraries were sequenced using the Novaseq X Plus platform (Illumina) at an average depth of 15 billion base pairs per sample. For a detailed protocol, see dx.doi.org/10.17504/protocols.io.e6nvw15dwlmk/v1.

FASTQ files were uniformly processed using the curated curatedMetagenomicsNextflow pipeline (https://github.com/seandavi/curatedMetagenomicsNextflow) (commit 4cde6fb). Mouse genomic reads were removed in the KneadData step using the C57BL mouse reference genome. The core output of the pipeline includes quality control (FastQC and Trimmomatic via KneadData) and MetaPhlAn v4.1.1 (https://huttenhower.sph.harvard.edu/metaphlan/, RRID:SCR_004915) (Blanco-Miguez et al., 2023) and HUMANnN v3.9 (https://huttenhower.sph.harvard.edu/humann, RRID:SCR_014620) (Beghini et al., 2021) abundance profiles, which were used for statistical analyses. All analyses were performed in R 4.5.1. System, R, and package versions were reported with *sessionInfo()* at the end of all analysis scripts (see Data and Materials Availability).

Data were downloaded and formatted for analyses using the R package parkinsonsMetagenomicData (https://github.com/ASAP-MAC/parkinsonsMetagenomicData). Samples were analyzed at the species genome bin (SGB) level except for relative abundance stackplots (Fig. 11A), which were aggregated at the phylum level to aid legend readability.

The mia package (https://bioconductor.org/packages/release/bioc/html/mia.html, RRID:SCR_023619) was used to perform alpha and beta diversity analyses. Observed

richness and Shannon diversity were calculated with addAlpha. ANOVA was used to test for differences in means between genotype-treatment groups. The Bray-Curtis dissimilarity matrix was calculated with addDissimilarity on relative abundance data ranging from 0 to 1. The function addMDS coloring by genotype-treatment was used to plot the first two axes of the principal coordinate analysis (PCoA) on the Bray-Curtis dissimilarity matrix. To test for genotype-treatment effect on the overall microbiome composition, a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2017) followed by permutational test for group homogeneity (permutest) was used, both implemented in addPERMANOVA. To further test for non-overlap between groups, a pairwise PERMANOVA with pairwiseAdonis was used, a custom function documented at https://github.com/pmartinezarbizu/pairwiseAdonis. False Discovery Rate (FDR) was calculated for all pairwise tests on beta-diversity (e.g., 3 pairwise tests for 3 groups) using the Benjamini-Hochberg method.

The effect of probiotic treatment on the relative abundance and prevalence of each SGB was determined using MaAsLin3 (Nickols et al., 2024). SGB relative abundances in each sample were scaled from 0 to 1, followed by a log2 transformation. Thy-1-ASO treated mice were compared to Thy-1-ASO control mice. False discovery rate across all SGBs was calculated using the Benjamini-Hochberg method as implemented in MaAsLin3.

For mediation analysis, behavior data from only one *F. prausnitzii* cohort was used to match sequencing data. The first three principal coordinates were used as continuous mediators using the Baron and Kenny approach (Baron & Kenny, 1986), with the mediate function implemented in the "mediation" R package (https://cran.r-project.org/web/packages/mediation/index.html, RRID:SCR_026984). Confidence intervals were estimated using quasi-Bayesian simulations (n = 1000) (King et al., 2000).

Data and Materials Availability

Microbiome analysis data and raw FASTQ files were deposited in the SRA (PRJNA1259538 and PRJNA1309735) along with sample metadata, and code to generate data, figures, and statistics is available at https://doi.org/10.5281/zenodo.17128213. For

RNA sequencing analysis data, raw FASTQ files were deposited in the SRA (PRJNA1308739) along with sample metadata, and code to generate data, figures, and statistics is available at https://github.com/jboktor/fprausnitzii-treatment-rnaseq. Raw *F. prausnitzii* flow cytometry data were deposited to Zenodo (https://doi.org/10.5281/zenodo.16929959) along with sample metadata. All other raw data and analyses for chapter 4 can be found at https://doi.org/10.5281/zenodo.17137678. Data pertaining to chapters 2 and 3 can be found at https://doi.org/10.22002/ys0m5-1bb85.

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Chapter 6

CONCLUSION

Microbiome treatments for PD

In Chapter 2, we establish that a consortium composed of beneficial taxa consistently found to be depleted in the PD gut microbiome improves motor and GI function in a preclinical mouse model, providing the first evidence that disease-specific microbes can mitigate PD-associated deficits. In Chapter 3, we identify several individual species that independently drive motor improvements, and in Chapter 4, we further characterize the beneficial effects of one such species, *F. prausnitzii*, the most consistently reduced taxon in the PD gut microbiome. These findings demonstrate that a single bacterium is capable of ameliorating motor and GI function deficits, as well as reducing α−synuclein aggregates in the substantia nigra, remodeling the gut microbiome towards a healthier composition, and promoting immunomodulatory and tissue regenerative pathways in the colon. This work provides a foundation for further mechanistic studies into *F. prausnitzii* and its therapeutic uses.

Within the context of the PD microbiome field today, this thesis contributes a significant advancement towards developing microbiota-based treatments for PD. Replenishing taxa reduced in the PD patient microbiome may restore microbial homeostasis, potentially attenuating gut-brain inflammatory processes, and thereby improve disease state. Current treatments for PD rely primarily on dopamine replacement therapy with levodopa (L-DOPA), an orally administered dopamine precursor. While currently the most effective treatment for alleviating motor symptoms, L-DOPA causes numerous side effects including nausea, vomiting, hallucinations, compulsive behaviors, and dyskinesia, and its benefits often diminish over time (Tambasco, Romoli, & Calabresi, 2018). Moreover, L-DOPA does not address GI symptoms, which many patients experience and often find more debilitating than motor symptoms (Pilipovich et al., 2022). A therapeutic that, either alone or alongside standard treatment, can improve both motor and GI symptoms while targeting underlying inflammatory processes with minimal side effects, would be invaluable. Future clinical trials assessing the safety, tolerability, and efficacy of this probiotic will be a key step in evaluating its applicability for PD patients.

Gut microbiota as therapeutics in human health and disease

The use of targeted, or 'next-generation', probiotics is an essential step towards personalized medicine. Research is continuing to reveal the central role of the gut microbiome in regulating whole-body function. Whereas a decade ago the brain was thought to exert primary control over all bodily functions, it is now recognized that the gut, enteric nervous system, and the microbiome – often collectively referred to as the 'second brain' – can influence development, immunity, behavior, and disease (Avetisyan et al., 2015; Ullah et al., 2023). Already, companies such as Viome and DayTwo provide personalized nutrition and lifestyle recommendations based on an individual's microbiome composition, assessed through stool sampling kits. While these assessments remain limited, they represent a path towards using stool analyses to identify depletions in beneficial microbes, replenishment of which may prove important for maintaining health. Although our understanding of how specific microbes influence host function remains in its early stages, the potential applications are far-reaching. Beyond CNS disorders, gut microbiota analyses can provide insights into how individuals respond to standard drug treatments, common illnesses like influenza or COVID-19, or eating certain foods. Such knowledge could prove valuable not only in everyday life, but also in the treatment of serious conditions like cancer and in extreme environments where maintaining health is critical, such as space missions.

Several companies are already leveraging our growing understanding of the human microbiome to develop microbial therapeutics. Seres Therapeutics, Vedanta Biosciences, and Finch Therapeutics are each pursuing live biotherapeutic consortia for the treatment of conditions such as ulcerative colitis and recurrent *Clostridium difficile* infection, with Finch also advancing treatments for ASD (Seres, 2025; Vedanta, 2025; Finch, 2025). Evelo Biosciences focused on single non-live microbial therapeutics, but was forced to shut down when several clinical trials did not meet endpoints (Taylor, 2023). Notably, all three consortia-based companies are taking distinct approaches to developing treatments. For ulcerative colitis, Seres employs a mix of 8-10 bacterial spores and non-spore-forming strains (Seres, 2021), Finch utilizes either FMTs enriched with beneficial

microbes or a targeted consortium (BioSpace, 2022), and Vedanta has formulated a 16-microbe human commensal consortium selected for its ability to induce immune tolerance (Vedanta, 2023), with all strategies aiming to reduce inflammation and restore gut integrity. The progress of these companies highlights the promise of microbial therapies and emboldens continued exploration in this rapidly growing field.

Limitations and challenges

Many unresolved questions for microbiome-based treatment approaches remain. A central challenge is the definition of a 'healthy' microbiome, as each individual's community differs based on genetics, environment, and lifestyle. Several large-scale efforts aim to establish baseline profiles and examine how conditions, genetics, and geography influence microbial communities. The largest of these studies include the American Gut Project, which analyzed over 10,000 gut microbiome samples from citizen-scientists across the United States, United Kingdom, and Australia (McDonald et al,, 2018), and the NIH Human Microbiome Project, which sequenced microbiomes from 242 U.S. individuals sampled across 18 body sites (Huttenhower et al., 2012). While these studies provide valuable insights, they remain geographically restricted. Given the high variability between individuals, the definition of a 'healthy' microbiome may ultimately remain fluid, underscoring the need for personalized rather than universal approaches to microbiome-based interventions.

Another significant challenge in administering next-generation probiotics is preserving the viability of beneficial commensal microbes, many of which are obligate anaerobes. Difficulties in formulation and scaling are the main reasons why many current probiotic companies opt to develop therapeutics based on bacterial small molecules rather than the microbes themselves, or implement non-obligate anaerobes instead. *F. prausnitzii,* which is classified as an extremely oxygen-sensitive bacterium, presents a particular challenge. However, several studies have demonstrated that the addition of antioxidants to formulations of *F. prausnitzii* strains may aid in protection from oxygen exposure (Khan et al., 2014; 2023). Indeed, the company Exeliom Biosciences has successfully overcome

this challenge in developing an *F. prausnitzii* live biotherapeutic for cancer and Crohn's disease (Exeliom, 2025). While different approaches must be tailored for each microbe or consortium, making a universal solution unlikely, ongoing research and technological advancements are already actively addressing these challenges.

Lastly, and perhaps most importantly, societal recognition of the influence of microbial organisms on human health is still in its early stages. As Ilya Metchnikov's ideas were initially overlooked, many today continue to view the microbiome as 'complementary' or 'integrative' and secondary to the fundamental branches of medicine. Yet, there is clear evidence for the significant role of the gut microbiome in disease treatment. For instance, patients receiving allogenic stem cell transplantation therapy for cancer are at high risk of developing graft versus host disease (GVHD). Studies have shown that patients harboring microbiomes enriched in pathogenic taxa and depleted in beneficial anti-inflammatory taxa are significantly more susceptible to GVHD (Koyama et al., 2023), and replenishing beneficial microbes alleviates this risk in clinical trials (Seres, 2024). Indeed, microbiome interventions are gaining medical acceptance, particularly for cancer and *Clostridium difficile* infection treatments. As research continues to elucidate the contributions of the microbiome to human health and advance microbiome-treatment options, broader societal and clinical acceptance is likely to follow.

This thesis builds on existing studies outlining the PD gut microbiome and demonstrates the potential of targeted microbial interventions for PD. More broadly, these findings provide further evidence of the significance of the gut microbiome in human health and disease. As the last decade has seen the reinvigoration of microbiome research, the coming decade will be pivotal for translating these discoveries into clinical and societal applications, with the potential to transform our approach to human health.

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