

**Gut microbiota-derived propionate mediates the neuroprotective effect of
osteocalcin in a mouse model of Parkinson's disease**

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Supplemental Table S1 The comparison of the behavioral tests in 6-OHDA-induced PD mice after the administration of OCN.

Behavioral Tests	control	6-OHDA	6-OHDA+	6-OHDA+	Total	P value		
	(A, n = 12)	(B, n = 8)	4 μ g/kg OCN (C, n = 7)	40 μ g/kg OCN (D, n = 6)		A vs B	B vs C	B vs D
Open Field Test								
Distance (m)	10.32 \pm 0.56	6.52 \pm 0.75	9.99 \pm 1.08	9.88 \pm 1.06	0.0068	0.0061	0.0343	0.0539
Number of rearing	34.08 \pm 1.82	22.25 \pm 2.09	28.86 \pm 2.11	28.50 \pm 4.16	0.0085	0.0042	0.2737	0.3561
Cylinder test								
Left/right forelimb touches	1.61 \pm 0.21	0.19 \pm 0.05	1.08 \pm 0.17	0.40 \pm 0.17	< 0.0001	< 0.0001	0.0122	0.8800
Rotarod test								
Time (s)	133.10 \pm 12.44	59.63 \pm 4.82	92.90 \pm 13.81	73.94 \pm 16.16	0.0006	0.0006	0.2999	0.8815

Data represented mean \pm SEM, $p < 0.05$ was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Table S2 The comparisons of the behavioral tests in 6-OHDA-induced PD mice treated with OCN after gut microbiota-depletion.

Behavioral Tests	control	6-OHDA	6-OHDA+OCN	Abx+control	Abx+6-OHDA	Abx+6-OHDA+OCN	P value			
	(A, n = 18)	(B, n = 11)	(C, n = 14)	(D, n = 11)	(E, n = 20)	(F, n = 12)	Total	A vs D	B vs C	E vs F
Open Field Test										
Distance (m)	7.57 ± 0.58	3.79 ± 0.35	6.35 ± 0.51	10.60 ± 0.77	6.69 ± 0.79	7.18 ± 0.42	<0.0001	0.0018	0.0218	0.8932
Number of rearing	15.50 ± 1.33	6.27 ± 0.90	13.93 ± 1.46	23.82 ± 1.21	10.00 ± 1.64	14.00 ± 1.65	<0.0001	0.0009	0.0053	0.8842
Cylinder test										
Left/right forelimb touches	1.08 ± 0.09	0.23 ± 0.11	1.57 ± 0.32	1.16 ± 0.14	0.25 ± 0.11	0.46 ± 0.15	<0.0001	0.9995	<0.0001	0.9568
Rotarod test										
Time (s)	129.30 ± 10.65	65.36 ± 7.51	121.30 ± 11.32	157.00 ± 13.44	93.47 ± 15.56	129.6 ± 20.98	0.0002	0.6426	0.0471	0.3015

The dose of OCN was 4 µg/kg. Data represented mean ± SEM, p < 0.05 was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Table S3 The comparisons of the behavioral tests after fecal microbiota transplantation.

Behavioral Tests	FMT _{control}	FMT _{6-OHDA}	FMT _{6-OHDA+OCN}	Total	P value	
	(A, n = 10)	(B, n = 10)	(C, n = 10)		A vs B	B vs C
Open Field Test						
Distance (m)	7.85 ± 0.46	6.55 ± 0.53	7.81 ± 0.33	0.0854	0.1217	0.1361
Number of rearing	17.70 ± 1.39	14.90 ± 1.36	17.40 ± 1.36	0.3005	0.3331	0.4131
Pole Test						
Time (s)	5.92 ± 0.33	7.29 ± 0.43	5.08 ± 0.32	0.0008	0.0336	0.0006
Rotarod test						
Time (s)	180.80 ± 18.30	120.20 ± 12.12	208.60 ± 16.06	0.0016	0.0288	0.0013

Data represented mean ± SEM, $p < 0.05$ was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Table S4 The overall OTU number and annotation levels.

	Annotated OTUs(n)	Annotation rate		
		Order	Family	Genus
control	669.1 ± 137.3	100%	77.06%	15.81%
6-OHDA	732.4 ± 80.82	100%	65.65%	16.13%
6-OHDA+OCN	509.3 ± 75.55	100%	87.63%	13.97%
Total	646.4 ± 136.50	100%	75.39%	15.53%

Data represented mean ± SD.

Supplemental Table S5 The comparison of taxonomy RAs of gut microbiota in 6-OHDA-induced mice after the administration of OCN.

Bacteria	control	6-OHDA	6-OHDA+OCN	Total	P value	
	(A, n = 12)	(B, n = 8)	(C, n = 7)		A vs B	B vs C
Phylum						
<i>Bacteroidetes</i>	0.7429 ± 0.0284	0.6286 ± 0.0215	0.8160 ± 0.0302	0.0009	0.0176	0.0007
<i>Firmicutes</i>	0.2219 ± 0.0324	0.3513 ± 0.0258	0.1597 ± 0.0298	0.0018	0.0162	0.0018
Family						
<i>S24-7</i>	0.7323 ± 0.0290	0.6286 ± 0.0215	0.8030 ± 0.0295	0.0021	0.0346	0.0016
<i>unclassified Clostridiales</i>	0.1188 ± 0.0316	0.2292 ± 0.0329	0.0669 ± 0.0244	0.0084	0.0455	0.0083
<i>Lachnospiraceae</i>	0.0148 ± 0.0041	0.0472 ± 0.0086	0.0152 ± 0.0057	0.0014	0.0022	0.0062
<i>Rikenellaceae</i>	0.0081 ± 0.0017	6.338e-006 ± 6.338e-006	0.0130 ± 0.0020	< 0.0001	0.0025	< 0.0001
<i>Erysipelotrichaceae</i>	0.0045 ± 0.0007	0.0017 ± 0.0004	0.0038 ± 0.0006	0.0199	0.0160	0.1432
Genus						
<i>unclassified S24-7</i>	0.7323 ± 0.0290	0.6286 ± 0.0215	0.8030 ± 0.0295	0.0021	0.0346	0.0016
<i>unclassified Clostridiales</i>	0.1188 ± 0.0316	0.2292 ± 0.0329	0.0669 ± 0.0244	0.0084	0.0455	0.0083
<i>unclassified Lachnospiraceae</i>	0.0113 ± 0.0034	0.0396 ± 0.0079	0.0107 ± 0.0049	0.0013	0.0023	0.0052
<i>unclassified Rikenellaceae</i>	0.0081 ± 0.0017	6.338e-006 ± 6.338e-006	0.0130 ± 0.0020	< 0.0001	0.0025	< 0.0001

Data represented mean ± SEM, p < 0.05 was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons. RAs = relative abundances.

Supplemental Table S6 The comparison of RAs of KOs regulating SCFA metabolism in 6-OHDA-induced mice after the administration of OCN.

KO	control	6-OHDA	6-OHDA+OCN	Total	P value	
	(A, n = 12)	(B, n = 8)	(C, n = 7)		A vs B	B vs C
K00656	0.001183 ± 5.093e-005	0.001332 ± 4.769e-005	0.001095 ± 4.546e-005	0.0184	0.1011	0.0161
K13788	1.459e-005 ± 2.445e-006	1.111e-005 ± 4.989e-006	8.693e-006 ± 1.472e-006	0.0436	0.7163	0.8815
K00925	0.0009388 ± 6.245e-006	0.0009353 ± 7.379e-006	0.0009581 ± 7.176e-006	0.0908	0.9246	0.1051
K01847	0.001214 ± 8.205e-005	0.0009171 ± 5.667e-005	0.001445 ± 8.812e-005	0.0011	0.0325	0.0008
K11264	1.028e-005 ± 1.717e-006	9.405e-006 ± 4.641e-006	5.917e-006 ± 9.615e-007	0.5355	0.9705	0.6964
K13923	1.050e-005 ± 1.751e-006	9.521e-006 ± 4.690e-006	5.937e-006 ± 9.542e-007	0.5166	0.9645	0.6891
K00932	9.578e-006 ± 1.991e-006	4.385e-006 ± 1.496e-006	5.551e-006 ± 3.432e-007	0.0849	0.0951	0.9027
K00626	0.0002503 ± 2.372e-005	0.0003044 ± 8.150e-006	0.0001684 ± 2.307e-005	0.0018	0.1802	0.0013
K00074	0.0002078 ± 2.159e-005	0.0002846 ± 1.425e-005	0.0001422 ± 2.332e-005	0.0009	0.0348	0.0006
K01692	0.0001377 ± 3.431e-005	0.0001150 ± 2.087e-005	0.0001240 ± 2.330e-005	0.8592	0.8521	0.9804
K00634	0.0007249 ± 2.221e-005	0.0006325 ± 1.705e-005	0.0008077 ± 3.053e-005	0.0003	0.0228	0.0002
K00929	0.0007458 ± 1.887e-005	0.0006698 ± 1.339e-005	0.0008219 ± 2.657e-005	0.0002	0.0277	0.0002
K01034	4.903e-005 ± 9.392e-006	5.426e-005 ± 1.323e-005	4.274e-005 ± 6.266e-006	0.7751	0.9278	0.7556
K01035	6.993e-006 ± 1.388e-006	3.380e-006 ± 1.175e-006	3.389e-006 ± 6.149e-006	0.0689	0.1154	>0.9999

Data represented mean \pm SEM, $p < 0.05$ was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons. RAs = relative abundances.

Supplemental Table S7 The comparison of the behavioral tests in 6-OHDA-induced mice after the administration of propionate.

Behavioral Tests	control	control+PPA	6-OHDA	6-OHDA+PPA	Total	P value	
	(A, n = 12)	(B, n = 12)	(C, n = 9)	(D, n = 7)		A vs C	C vs D
Open Field Test							
Distance (m)	10.05 ± 0.27	9.23 ± 0.63	6.77 ± 0.65	9.64 ± 0.88	0.0022	0.0017	0.0208
Number of rearing	17.42 ± 1.17	17.33 ± 1.21	8.22 ± 1.15	14.14 ± 1.82	< 0.0001	< 0.0001	0.0339
Cylinder test							
Left/right forelimb touches	1.15 ± 0.17	0.80 ± 0.10	0.33 ± 0.06	1.25 ± 0.22	0.0004	0.0011	0.0012
Rotarod test							
Time (s)	175.60 ± 9.88	160.70 ± 9.28	69.93 ± 7.35	122.3 ± 22.5	0.0001	< 0.0001	0.5025

PPA = propionate, Data represented mean ± SEM, $p < 0.05$ was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Table S8 The comparison of the behavioral tests in 6-OHDA-induced mice after the administration of FFAR3 agonist.

Behavioral Tests	control	control+DMSO	control	6-OHDA	6-OHDA+	6-OHDA+	P value		
	(A, n = 6)	(B, n = 6)	+AR420626 (C, n = 6)	(D, n = 6)	DMSO (E, n = 6)	AR420626 (F, n = 6)	Total	B vs E	E vs F
Open Field Test									
Distance (m)	11.98 ± 0.83	8.69 ± 0.98	11.48 ± 0.59	7.74 ± 0.27	6.09 ± 0.33	8.28 ± 0.92	<0.0001	0.1299	0.2751
Number of rearing	26.67 ± 2.25	23.83 ± 1.58	23.83 ± 2.74	9.17 ± 1.14	10.67 ± 0.76	18.67 ± 1.87	<0.0001	0.0003	0.0472
Cylinder test									
Left/right forelimb touches	1.42 ± 0.15	1.22 ± 0.24	1.61 ± 0.26	0.43 ± 0.07	0.26 ± 0.06	0.94 ± 0.16	<0.0001	0.0063	0.0899
Rotarod test									
Time (s)	183.3 ± 17.74	214.3 ± 12.06	208.7 ± 18.82	85 ± 8.11	99.17 ± 15.3	169.2 ± 21.71	<0.0001	0.0003	0.0496
Pole test									
Descent time (s)	4.40 ± 0.20	5.01 ± 0.25	5.79 ± 0.77	8.68 ± 1.23	10.32 ± 1.12	6.84 ± 0.40	<0.0001	0.0005	0.0386

Data represented mean ± SEM, $p < 0.05$ was set as the threshold for significance by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Figure legend

Supplemental Figure. 1 OCN administration had no effect on motor function, dopaminergic neurons, gut microbiota and SCFAs. (A) Bar plots of performance in the behavioral tests, including the open field test, pole test, cylinder test and rotarod test. n = 10 per group. (B) (Upper panel) Representative immunostaining showing TH-positive neurons in the SN. (Lower panel) The average number of TH-positive neurons in the ST. n = 3 per group, 3 sections per mouse. (C) (Left panel) Representative immunostaining (upper) and western blotting (lower) showing TH-positive fibers and TH protein levels in the striatum. (Right panel) The quantitation of TH-positive fibers (upper) and TH protein levels (lower) in the striatum. Immunostaining: n = 3 per group, 3 sections per mouse; western blotting: n = 3 per group. (D) (Upper panel) The plots of alpha and beta diversity. n=10. (Lower panel) Bar plots of the RAs of *f_S24-7*, *f_Rikenellaceae*, *f_Lachnospiraceae*, *f_unclassified Clostridiales* at the family level. n=10. (E) Bar plots of the RAs of K01847, K00634, K00929 and K00074, n = 10 per group. (F) Bar plots of comparing the fecal levels of acetate, propionate and butyrate among groups. n=10.

Supplemental Figure. 2 Antibiotic treatment depleted the gut microbiota. (A) The concentration of stool-extracted DNA between control group and Abx group. n=4. (B) The comparison of cecal size between control group and Abx group. (C) The comparison of Stool cultures between control group and Abx group.

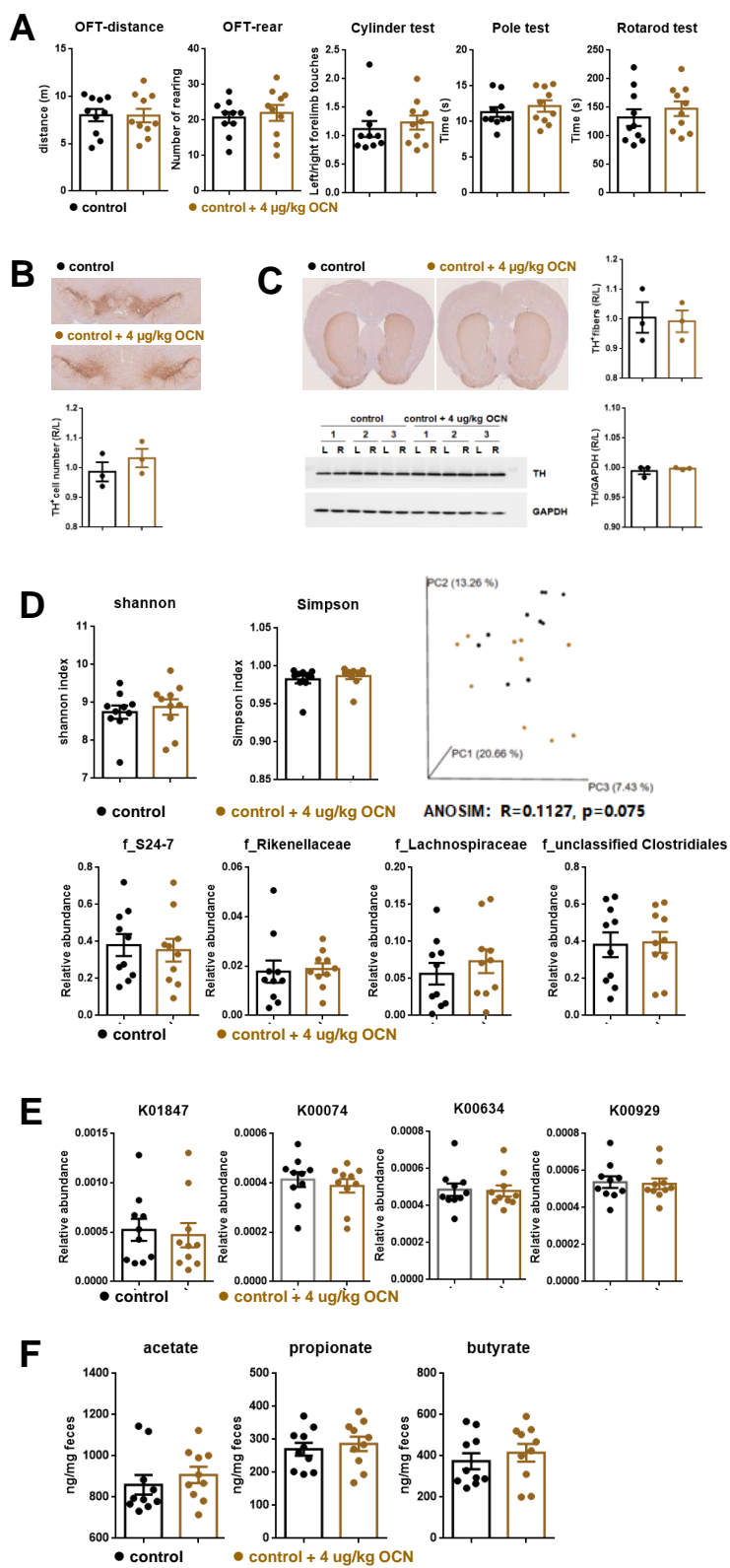
Supplemental Figure. 3 The comparison of alpha diversity of gut microbiota in 6-OHDA-induced PD mice. (Left panel) Bar plot of the Shannon index. (Right panel) Bar plot of the Simpson index. n=7-12, The data represent the mean \pm SEM and was analyzed by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Figure. 4 Cisplatin ablated the enteric neurons. (Left panel) The Representative immunostaining showing PGP9.5-positive neurons in the ileum between control group and cisplatin-treated group. (Right panel) The average number of PGP9.5-positive neurons in the ileum. n=3-5, The data represent the mean \pm SEM

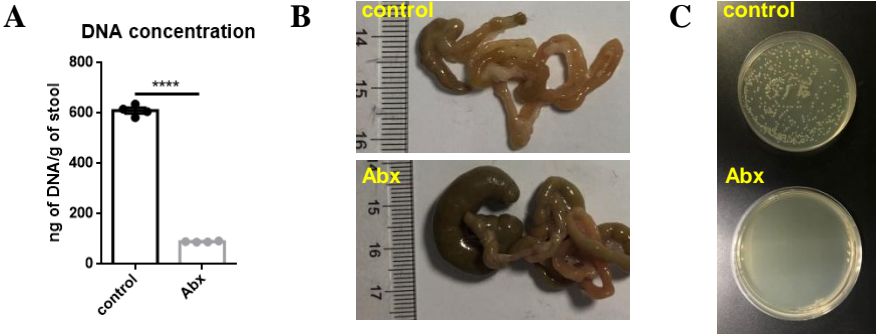
and was analyzed by one-way ANOVA followed by post hoc comparisons using Tukey's test for multiple groups' comparisons.

Supplemental Fig. 5 Correlation analysis between the altered KO and gut microbial taxa. Spearman correlation analysis of RAs of K01847 and that of p_*Bacteroidetes*, A; f_*S24-7*, B; f_*Rikenellaceae*, C; p_*Firmicutes*, D; f_*Lachnospiraceae*, E and f_*unclassified Clostridiales*, F. $p < 0.05$ was set as the threshold for significance.

Supplemental Fig. 1

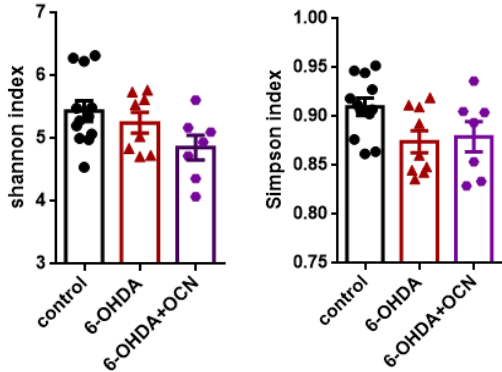


Supplemental Fig. 2



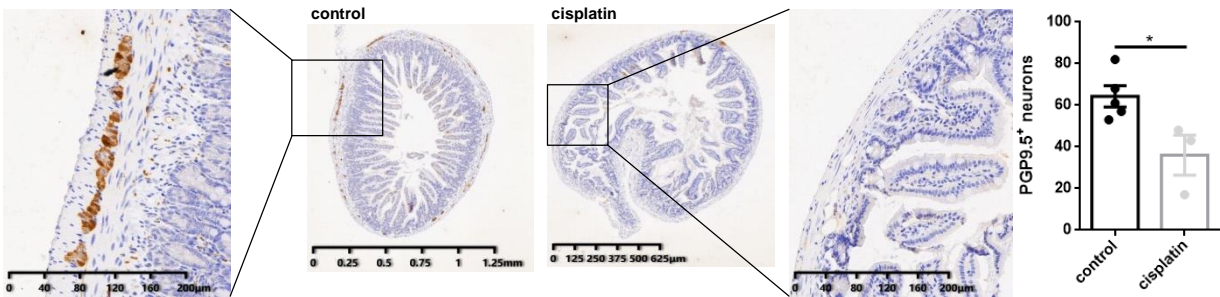
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Supplemental Fig. 3



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Supplemental Fig. 4



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Supplemental Fig. 5

