SUPPLEMENTARY DATA

Lifelong Consumption of Freeze-Dried *Parabacteroides Distasonis* Improves Gut Barrier Integrity and Extends Lifespan and Health Span in Drosophila Melanogaster.

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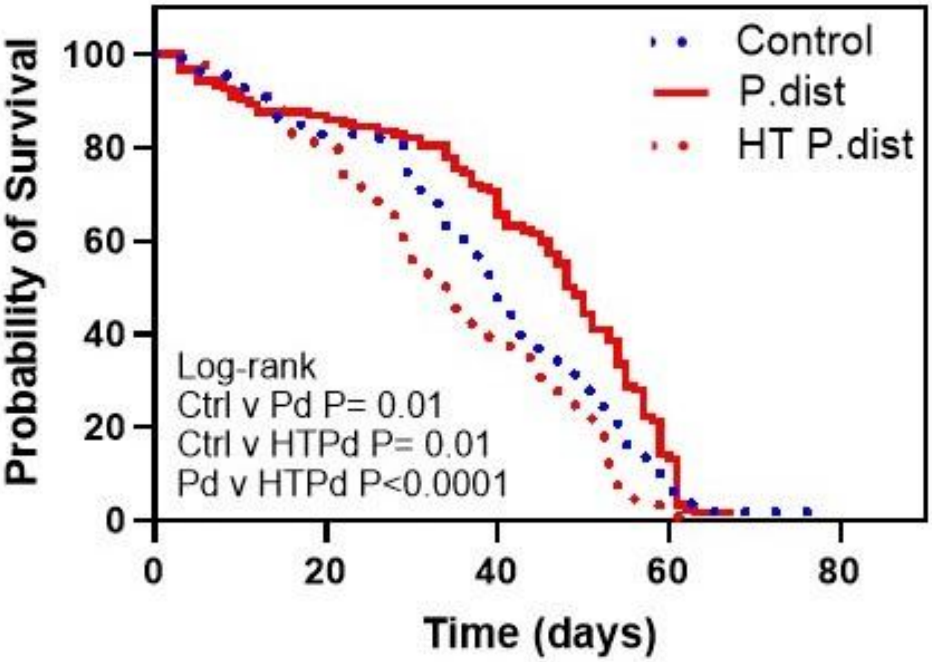

Figure S1. Heat treatment destroys the lifespan-extending effect of Pd Female w^{1118} flies raised on standard food with an overlay of water or water containing 0.1% freeze-dried Pd or 0.1% heat treated Pd starting 8 hours after adult eclosion. n = 111, 123 and 124 respectively. Survival curves were compared by log-rank test and significance accepted when P< 0.05.

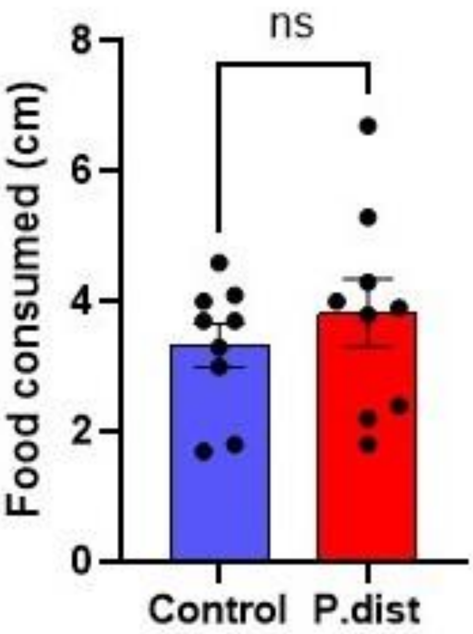

Figure S2. Pd supplementation does not affect food intake. Female flies were placed into CAFE apparatuses with capillary tubes containing 5% sucrose or 5% sucrose + 0.1% Pd. Food consumption was measured over a 4-hour period. N=9 vials/gp. Groups compared by t test. P> 0.05.

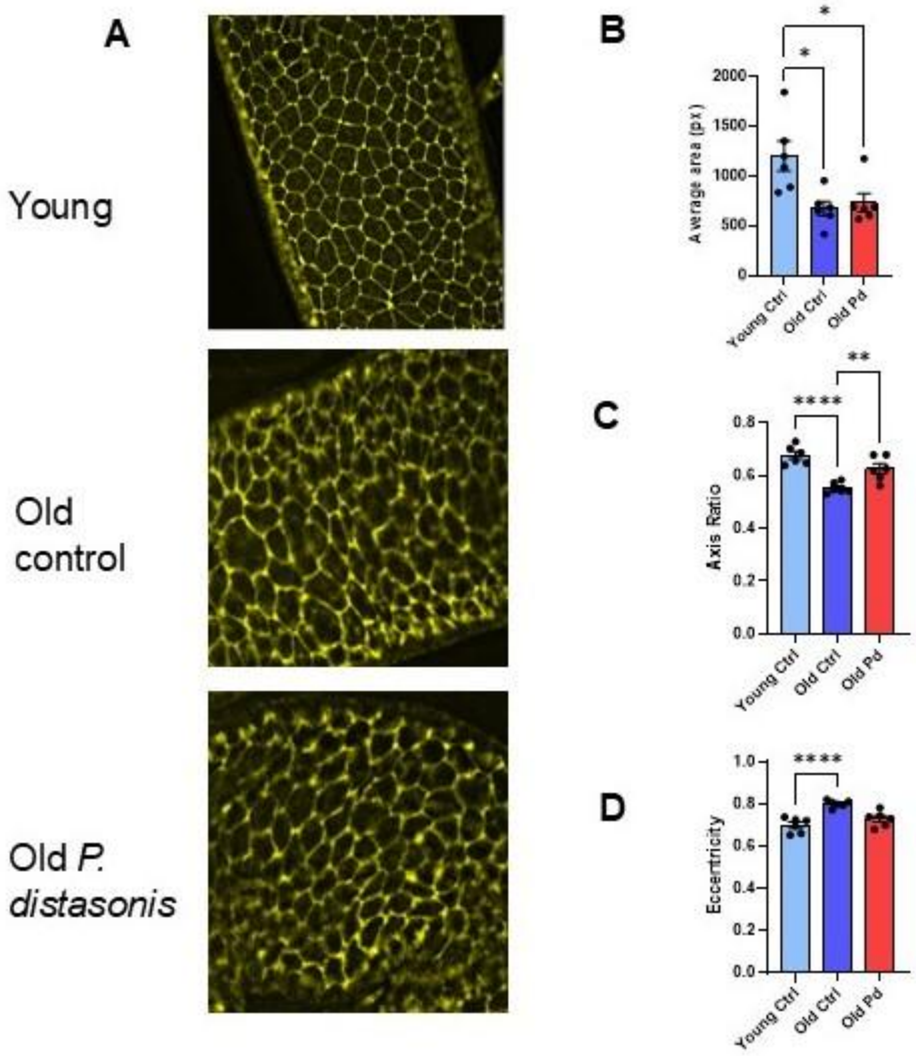

Figure S3. Pd supplementation promotes young cell morphologies in old guts. Guts were dissected from young adults (3 days post-eclosion) and old adults (40 days post-eclosion) maintained on food overlaid with water (control) or Pd. (A) Immunostaining of dlg. Shown are the L4 midgut regions at a magnification of 40X. Images randomly selected. (B) Area (size) of cells in the L4 region. (C) Axis ratio of cells in the L4 region. (D) Eccentricity of cells in the L4 region. n=7 guts for all treatments. Groups compared by Kruskal-Wallis test (B) or one-way ANOVA (C, D). * $P < 0.05$.

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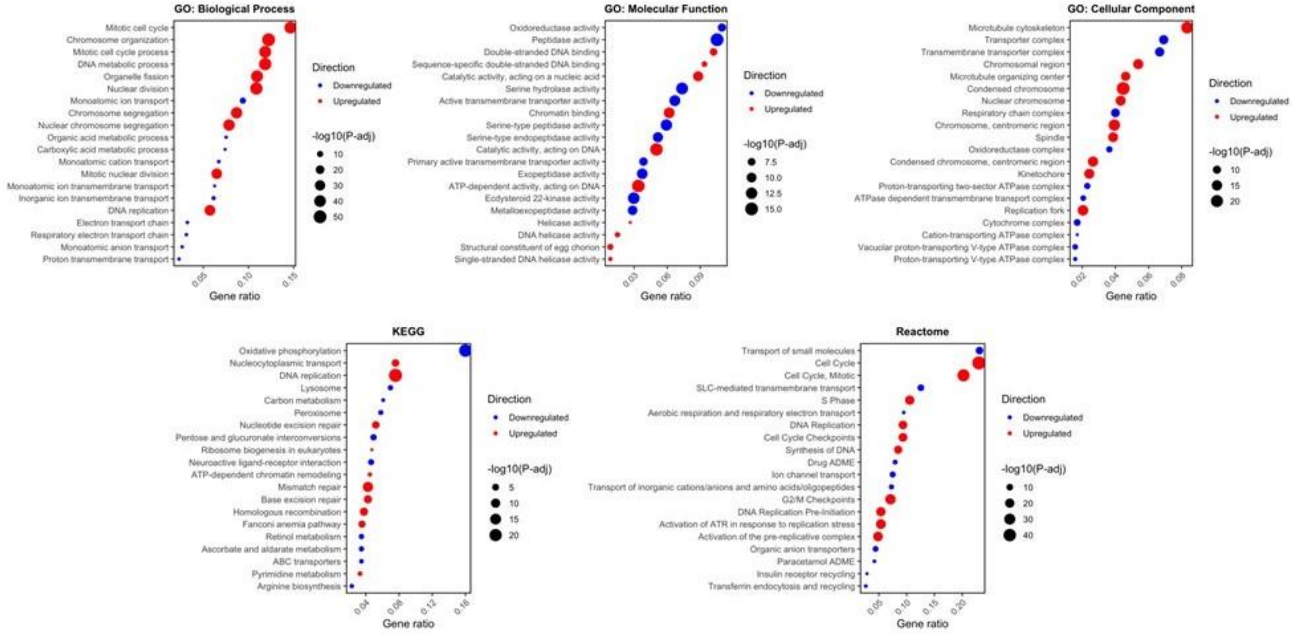

Figure S4. Pathway enrichment analysis of gene expression changes Pathway enrichment analysis showing the top 20 most significant Gene Ontology (Biological Process, Molecular Function, Cellular Component), KEGG, and Reactome pathways for upregulated in Pd (red) and downregulated in Pd (blue) genes. Dot size represents -log10(P-adj), and gene ratio indicates the proportion of genes in each pathway that are differentially expressed. Analysis performed on genes with |log2FC| > 1.0 and P-adj < 0.01.

Table S1. PCR Primer sequences

Gene Symbol	Gene name	NCBI Gene ID	Fwd	Rvs
AMPKa	AMP kinase alpha	43904	CCGTCAAGATACTCAATCGTCAG	GGATGGCGGAAGAGCTTTAG
βTub56D	beta-Tubulin at 56D	37238	ACATCCCGCCCCGTGGTC	AGAAAGCCTTGCGCCTGAACATAG
Blm	Bloom syndrome helicase	41366	CGCAGTCTACAATCCCATCTT	GCTGGACTTCTTCGTAGCAATA
Brca2	BRCA2, DNA repair associated	37916	CGCCGTGGAGGATCTTATTT	CGTATGTTGGAGACGAGCAA
Cat	catalase	40048	GTGAACTTCCTGGATGAGATGTC	CACCTCAAAGTAACCAAAAGCAC
Cdk1	Cyclin-dependent kinase 1	34411	CGGTGGCTTGCAAAGAAATAG	TTGCGACCCTTATACACCAC
cold	coiled	33237	TCTGGAGTGCTATGTCTGCTC	CTCTTGGACACGTAGTATTGCTT
crok	crooked	34646	ACGGTGAATGGCGCTACTTT	AGCAGTGTGCCAGTTAGGAC
CycE	Cyclin E	34924	TTTGAGCCTCCATCAGCTAAG	GAGGCAACCGATGACAGATT
Duox	Dual oxidase	33477	CATCAGAGGGCAGAAAGCA	CAGCTCCAGACCGTAACTAATC
Fancd2	Fancd2	2768674	GGCAAGCGAAGACAGTAAGA	ACACTAGCAGATGACCTTTGG
fz	frizzled	45307	CTCGATGCGAGTCCGTATTATC	CGATATGGTGATGGGTTCACA
kune	Kune-kune	35504	GCGTTTTCCACGCCTTATTGG	GGCATCCGTTAAAGGAATTGTCA
Mlh1	Mlh1	35796	CTTCGAGGATTTGTCCCAGATAG	GTCTTGGTCTGGATGCTTAGG
Msh6	Msh6	39654	GTGACCAACACGTCCAATCT	GCAAGGTGCTCTTATCGTAGTC
N	Notch	31293	GTGTGTGAGAGTGAGACGAAAG	GAGGATCGAGCCACAAATTCA
pyd	poylchaetoid	41062	TGATCGAACCACATGGGAGTA	GATTATCACGTCCACCGGAAAC
Sirt6	Sirtuin6	41254	AACTACGCGGATGGATTGTC	ACAACGTGTCCCGATTTCTT
spz	spatzle	43256	CTGCGATCAGCTACTTGGATAG	GTTCGATTTGGTTGCTCTGATG
Tl	Toll	43222	GTGCAGTGAGATGAGCATAGA	TCTTTGGGCTGGATGGTTAG
Toll-9	Toll-9	40245	GGAGAGAACGGACCTCAAATC	TGGGAAACTGCCACCATATC

Xrcc2	X-ray repair cross complementing 2	32837	AACCTAATGGGCTCACATCAA	GAGATCTCCACCAGCGTATC
*Pdist set 1** (FQ-PCR)	Parabacteroides distasonis complete genome	CP000140.1	TGCCTATCAGAGGGGGATAAC	ATCCCGCATGGGAATATTTGC
Pdist set 2* (conventional PCR)	Parabacteroides distasonis complete genome	CP000140.1	TGATCCCTTGTGCTGCT	TCCGAATGAGGGGGAT
16SrRNA*	16SrRNA	N/A	ACTCCTACGGGAGGCAGCAG	CCAGCAGCCGCGGTAAT

*For confirming *Pd* identity during culture. All others for RT-PCR.