Cell Metabolism, Volume *27*

Supplemental Information

Drosophila Perpetuates Nutritional Mutualism by Promoting the Fitness of Its Intestinal Symbiont *Lactobacillus plantarum*

Gilles Storelli, Maura Strigini, Théodore Grenier, Loan Bozonnet, Martin Schwarzer, Catherine Daniel, Renata Matos, and François Leulier

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A farming mechanism sustains commensal bacteria fitness and nutritional mutualism upon chronic undernutrition

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Four Supplementary Figures

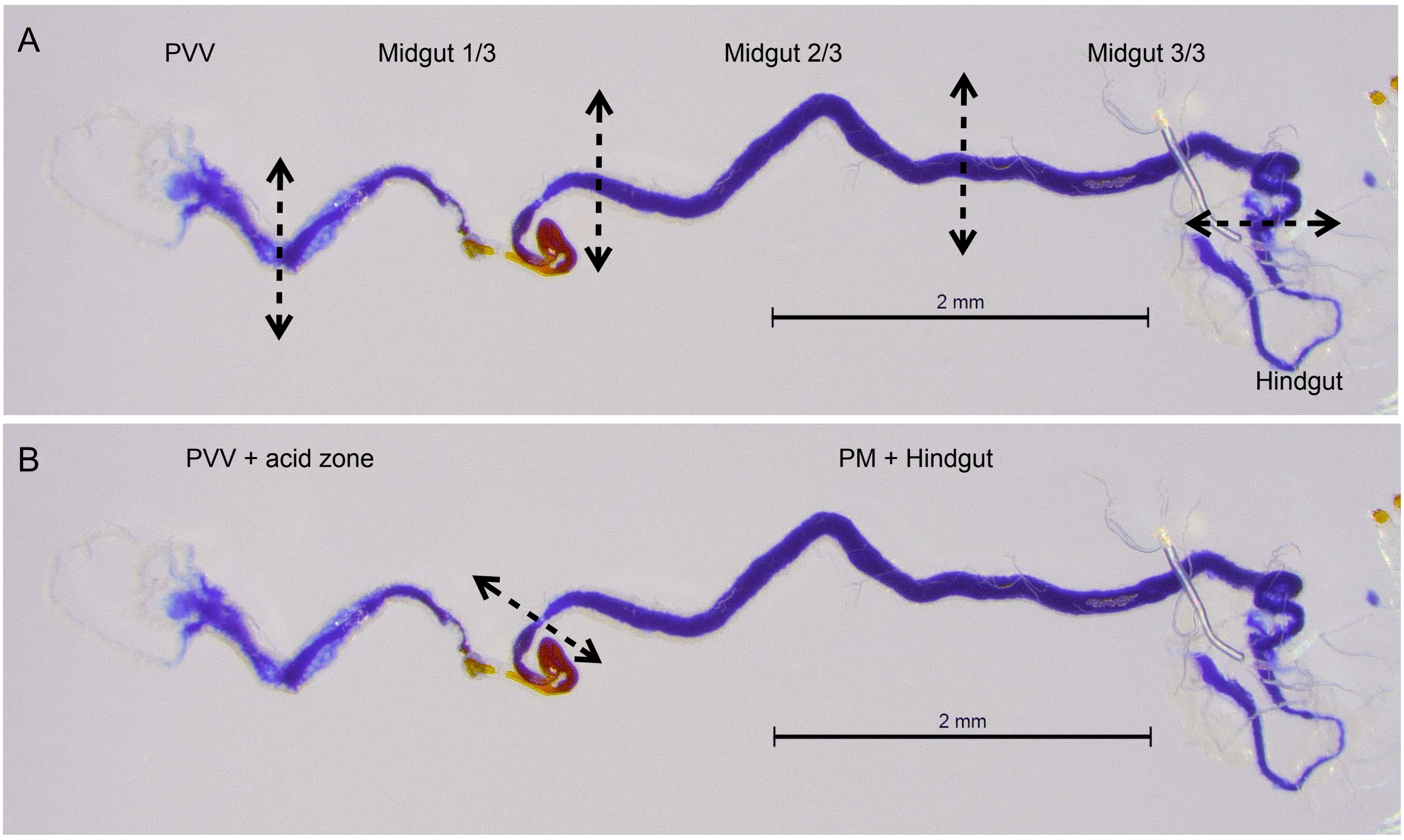

Figure S1
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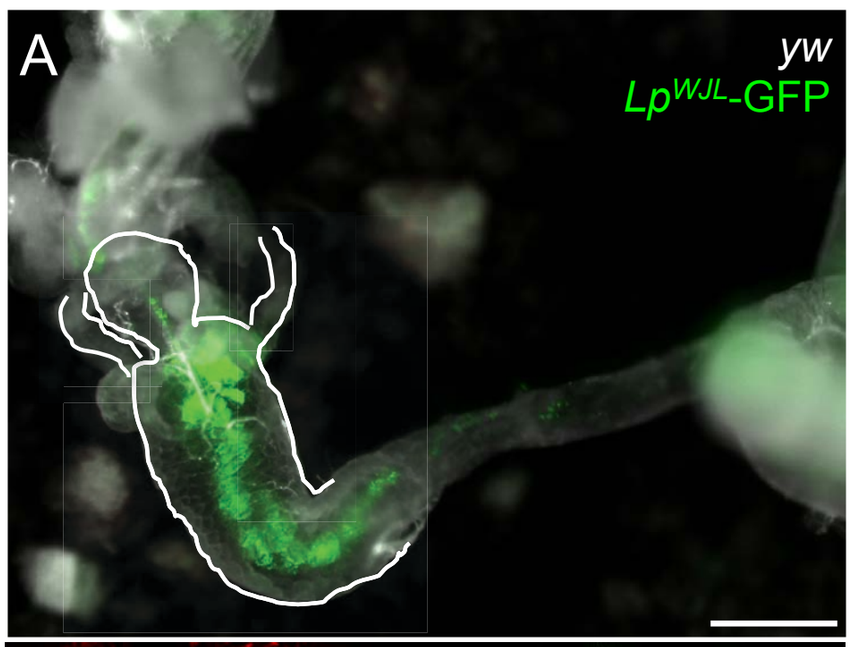
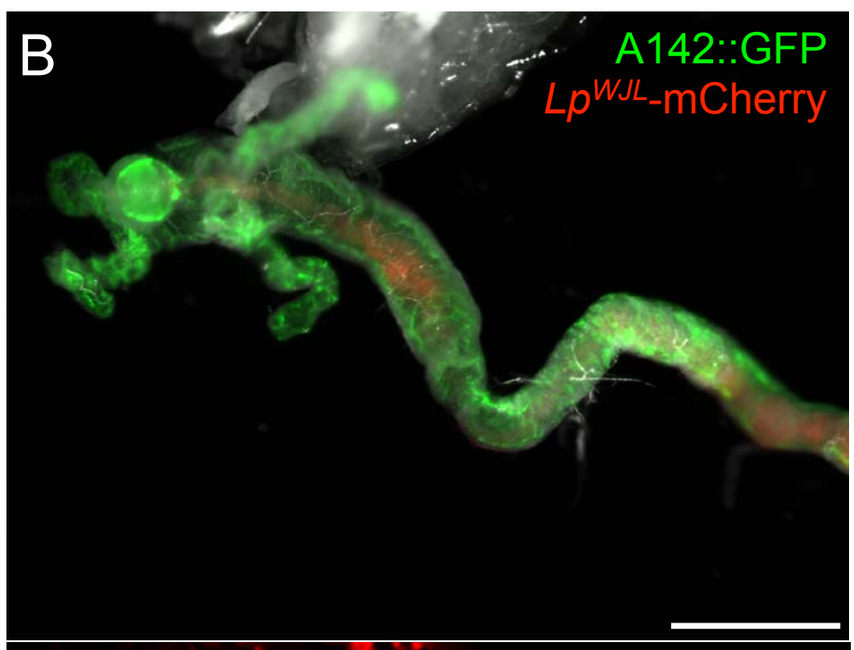
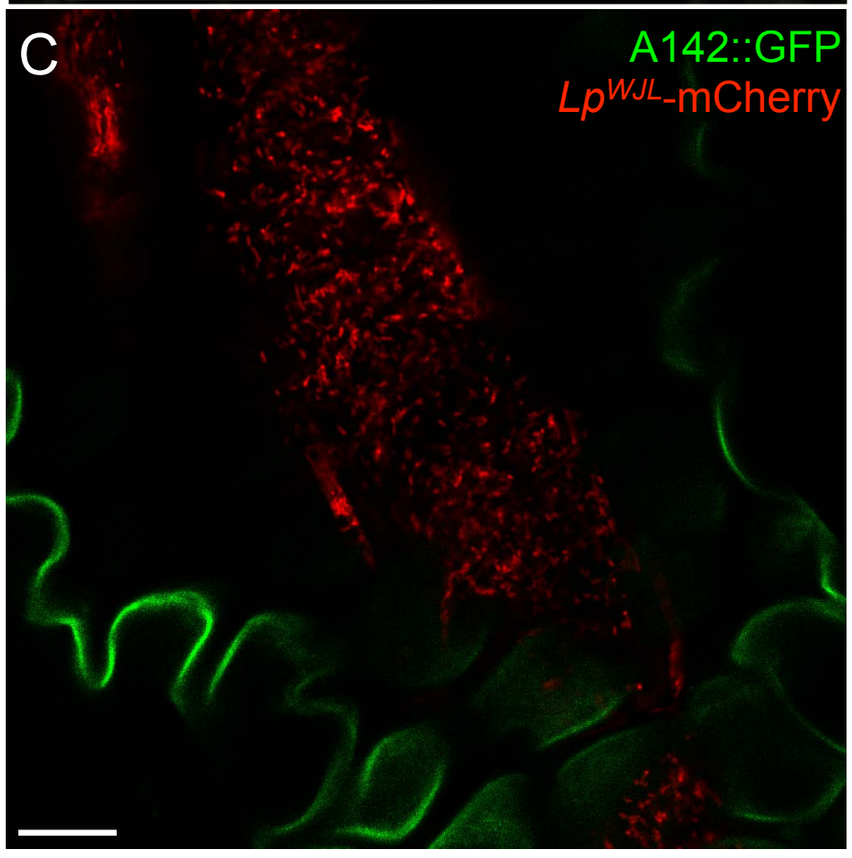
Supplementary Figure 1 (related to Fig.1): Representation of dissected gut portions used to define *Lactobacillus plantarum* localization in the larval gut.

(**A**) Dissection scheme for the experiment shown in Fig1C. Dotted lines represent where guts have been sectioned to isolate the different portions. PVV = Proventriculus and Ventriculus. The rest of the midgut (minus the ventriculus) was dissected in (approximately) 3 equal parts: Midgut 1/3: first third of the midgut, Midgut 2/3: second third of the midgut and Midgut 3/3: third third of the midgut. (**B**) Dissection scheme for the experiments shown in Fig1F and 1H (excepted for *mex*>*lab*-IR genotype). Larvae were previously reared on PYD-BB to visualize the gut acid zone and guts were sectioned accordingly to the dotted line: PVV + acid zone = dissected gut portion encompassing the Proventriculus, the Ventriculus and the acid zone. PM + Hindgut = dissected gut portion encompassing the posterior midgut and approximately half of the hindgut. (**C**) Dissection scheme for *mex*>*lab*-IR genotype in Fig.1H. In the case of loss of the acid zone in *mex*>*lab* RNAi larvae, guts were cut in two (approximately) equal parts, labeled “First half of the midgut” and “Second half of the midgut”.







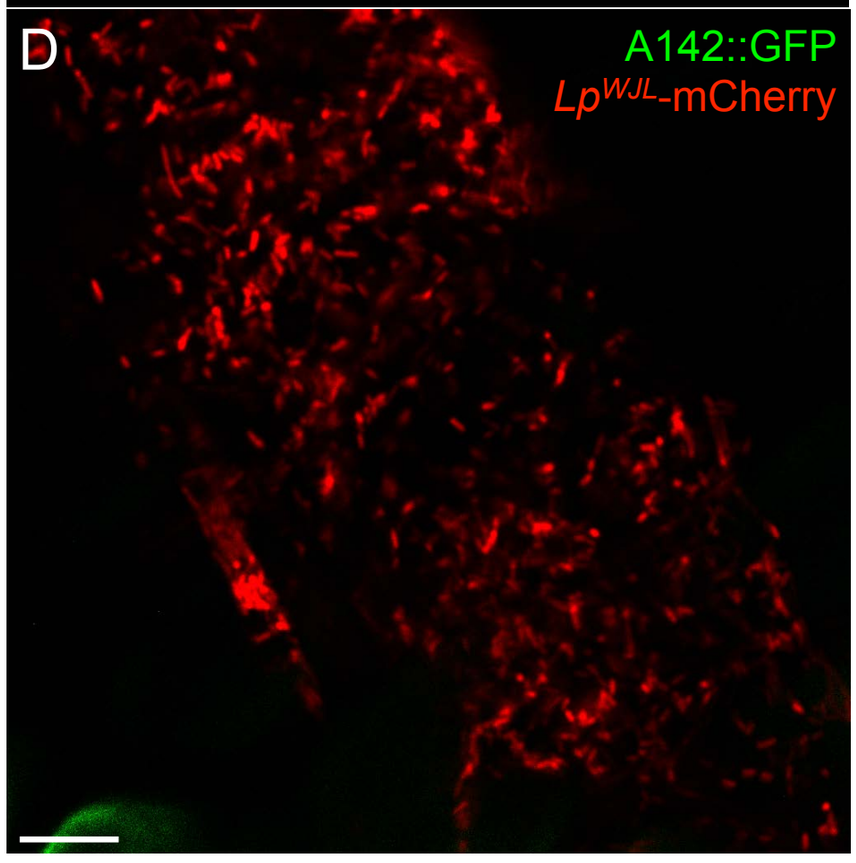


Figure S2
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Supplementary Figure 2 (related to Fig.2): Viable *Lactobacillus plantarum* cells accumulate in the endoperitrophic space of the anterior midgut

Guts dissected from larvae fed on food containing fluorescent bacteria: ingested bacteria occupy the central part of the gut lumen. Top panels **(A-B)**: unfixed tissue imaged at low magnification at the stereomicroscope. Unmarked larval tissue was imaged with bright light and appears as white-grey. **(A)**: gut of a *y,w* larva fed on Lp^{WJL}-GFP. The outline of the PV and the anterior part of the midgut has been traced for clarity. **(B)**: gut of an A142::GFP larva fed on Lp^{WJL}-mCherry. GFP highlights the brush border and thus the apical side of the enterocytes. **(C and D)**: confocal images of guts dissected from A142::GFP larvae. The two panels illustrate different magnification of same unfixed gut from larva fed on food containing fluorescent bacteria (Lp^{WJL}-mCherry). Scale bars: **(A)** 250 μm, **(B)** 500 μm, **(C)** 25 μm, **(D)** 12.5 μm.

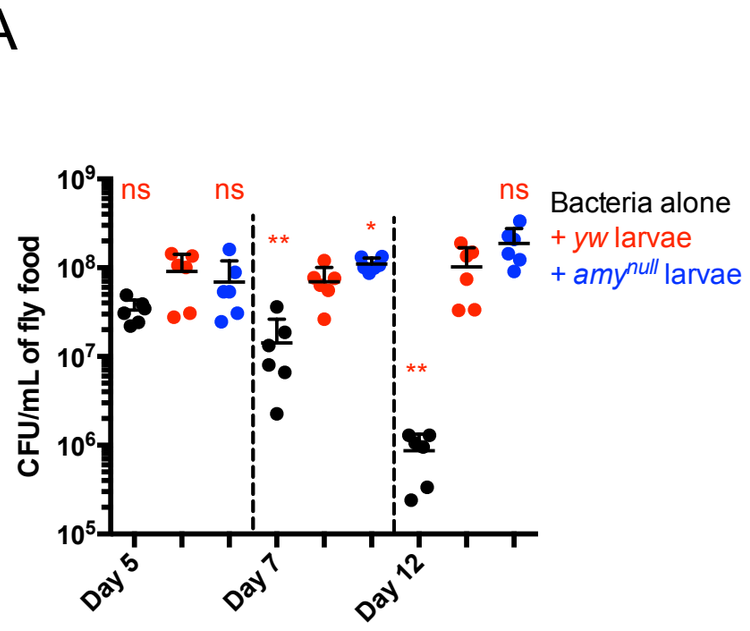

Figure S3
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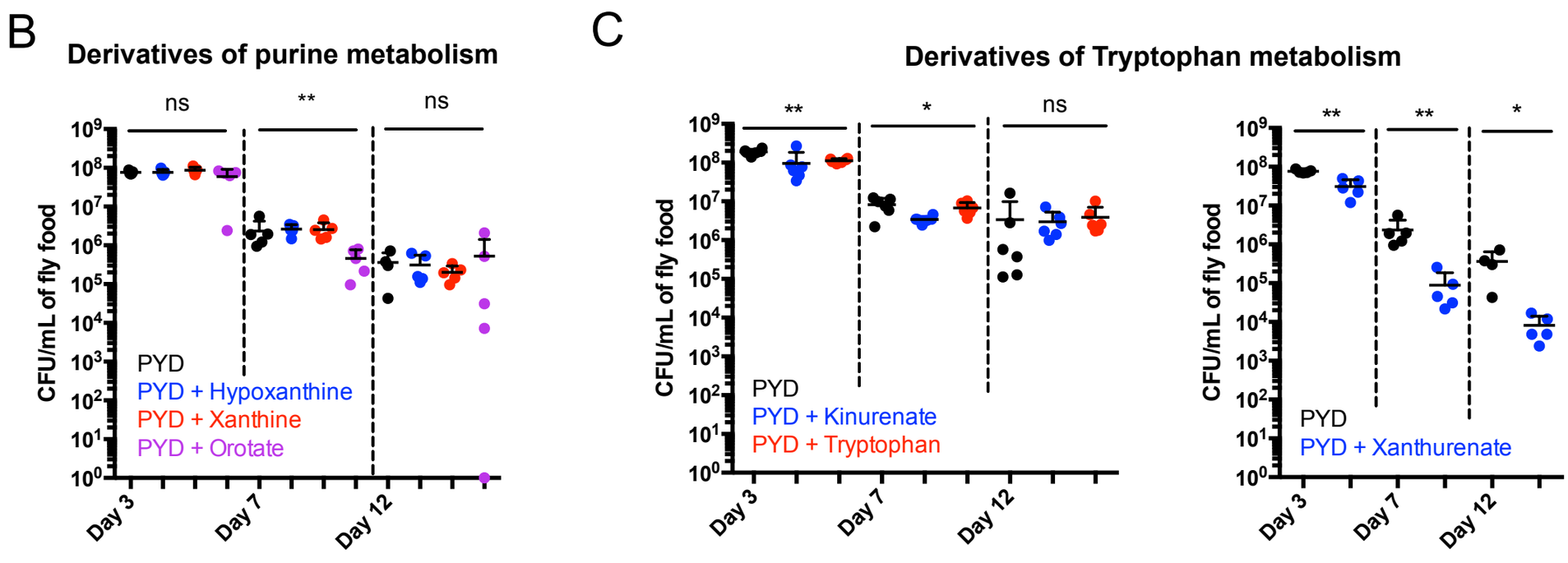
Supplementary Figure 3 (related to Fig.7): Processing of complex dietary nutrients by *Drosophila* larvae is not rate-limiting for *L.plantarum* long-term maintenance

(A): Bacterial load evolution in the food matrix (black dots) or in the niche, in presence of *y,w* (red dots) or amy^{null} larvae (blue dots). Each dot represents the quantification from a single food matrix or niche. The horizontal line in the dot plot represents the mean value. Whiskers represent standard deviation. Red asterisks just above dot plots illustrate statistically significant difference with the bacterial load of the niche with *y,w* larvae.**:0,001<p<0,01. **(B):** Effect of protease inhibitors on the larval length gain at 7DAEL, for Germ Free (grey dot plots) and Lp^{WJL} associated animals (black dot plots). GF embryos were inoculated with PBS or Lp^{WJL} bacteria on standard PYD, or on PYD containing a protease inhibitor cocktail (PYD + PIC), and larvae were measured at 7DAEL. Each single dot represents an individual larval measurement; the horizontal bar in the dot plot represents the mean value obtained from the pool of individual larval measurements. The whiskers represent the standard deviation. Grey asterisks illustrate statistically significant difference with GF larvae reared on PYD, black asterisks with monoassociated larvae reared on PYD: ****: p<0,0001. (**C**): Evolution of food matrix bacterial load (black dots) and niche bacterial load in the absence or presence of a protease inhibitor cocktail (respectively blue and red dots). Asterisks above horizontal bars represent statistically significant differences between conditions. **(D-F):** Larvae purge their intestinal content when bathed in PBS. **(D)** Rearing larvae on PYD supplemented with Erioglaucine Blue allows visualizing the alimentary bolus throughout the external cuticle (left panel). The ingested blue dye can be followed by spectrometry, as Erioglaucine blue absorbs at 625 nm. The graph represents the absorbance of homogenates of larvae fed PYD with Erioglaucine Blue (n=4 pools of n=5 larvae), relative to the absorbance of the homogenates of larvae fed standard PYD (n=6 pools of n=5 larvae) (right panel). **(E)** Larvae purge their intestinal content when bathed in PBS. The graph represents the absorbance of homogenates of larvae fed PYD with Erioglaucine Blue after an overnight bath in PBS ("after bath", n=6 pools of n=5 larvae), relative to the homogenates of age-matched larvae freshly sampled from PYD with Erioglaucine Blue ("before bath", n=4 pools of n=5 larvae). The decrease in absorbance indicates that larvae partly excrete their intestinal content during the bath. **(F)** Larval intestinal content is retrieved in PBS after bath. The PBS used to bathe larvae reared on

PYD with Erioglaucine blue remains tainted in blue after larvae removal (blue arrow), while the PBS used to bath larvae reared on standard PYD is unaffected (white arrow), indicating that larvae excrete their intestinal content in PBS (inner panel). The graph represents the absorbance of the PBS used to bath larvae fed PYD with Erioglaucine Blue (n=11), relative to the absorbance of the PBS used to bath larvae fed standard PYD (n=12)(right panel). **(G)**: Evolution of the food matrix bacterial load after bacteria co-inoculation with PBS, excreta from live larvae ("+ live larvae excreta"), or excreta from dead larvae ("+ Dead larvae excreta"). Briefly, starved larvae were bathed alive in PBS to collect "live larvae excreta", or were killed with a brief microwave pulse and then bathed overnight in PBS to collect "dead larvae excreta". Excreta were co-inoculated with *Lp*^{WJL} onto the food matrix, and the bacterial titre followed over time. Black dots: bacteria co-inoculated with sterile PBS, red dots: bacteria co-inoculated with the excreta from live larvae, blue dots: bacteria co-inoculated with the excreta from dead larvae. Red asterisks above dot plots illustrate statistically significant difference with the bacterial loads obtained for bacteria co-inoculated with "live larvae excreta". **(H)**: Evolution of the food matrix bacterial load after bacteria co-inoculation with PBS, larval excreta, or heat-treated larval excreta. Briefly, GF *y,w* larvae were reared on Rich diet + ATB, collected as late L3 and bathed overnight in PBS. Larvae were then removed, and the PBS used for the bath (containing "larval excreta") is kept "as is" ("larval excreta"), or submitted to heat treatment (70°C for 10mn, "Heat-treated excreta") to disrupt eventual enzymatic activities. Larval excreta and heat-treated larval excreta are then co-inoculated with $7x10^6$ CFUs of *Lp*^{WJL} on the food matrix, and the bacterial titre followed over time (black dots: bacteria co-inoculated with sterile PBS, red dots: bacteria co-inoculated with larval excreta and blue dots: bacteria co-inoculated with heat-treated larval excreta). **(D-F)**: The histograms represent the samples' mean relative absorbance at 625nm. The whiskers represent standard deviation. **(A, C, G-H)** Representation of food matrix bacterial loads. Each dot represents quantification from a single food matrix. The horizontal line in the dot plot represents mean value. Whiskers represent standard deviation. **(A-H):** Asterisks illustrate statistical significance between conditions: ****: $p<0,0001$, ***: $0,0001<p<0,001$, **: $0,001<p<0,01$, *: $p<0,05$. ns = not significant ($p>0,1$).

A

$p \leq 0.05$, group means fold of change ≥ 1.00					Fold of Change	Statistical Values	
					Welch's Two-Sample t-Test	Welch's Two-Sample t-Test	
Pathway	Biochemical Name	KEGG	HMDB	PubChem	Live / Dead	Live / Dead	
						p-value	*q*-value
Purine metabolism	hypoxanthine	C00262	HMDB00157	790	4,65	0,0449	0,0249
	xanthine	C00385	HMDB00292	1188	16,26	0,0001	0,0002
	orotate	C00295	HMDB00226	967	61,30	0,0000	0,0000
Tryptophan metabolism	kynurenine	C00328	HMDB00684	161166	3,80	0,0057	0,0060
	kynurenate	C01717	HMDB00715	3845	148,22	0,0000	0,0000
	3-hydroxykynurenine	C02794	HMDB00732	89	13,11	0,0000	0,0000
	xanthurenate	C02470	HMDB00881	5699	159,98	0,0000	0,0000
N-acetylated amino acids	N-acetylglycine		HMDB00532	10972	11,14	0,0000	0,0000
	N-acetylserine		HMDB02931	65249	2,45	0,0055	0,0060
	N-acetylalanine	C02847	HMDB00766	88064	2,61	0,0031	0,0043
	N-acetylasparagine		HMDB06028	99715	3,65	0,0013	0,0021
	N-acetylglutamate	C00624	HMDB01138	70914	3,51	0,0003	0,0007
	N-acetylglutamine	C02716	HMDB06029	182230	2,35	0,0041	0,0051
	N-acetylhistamine	C05135	HMDB13253	69602	46,96	0,0002	0,0004
	N-acetylvaline		HMDB11757	66789	2,18	0,0014	0,0023
	N-formylmethionine	C03145	HMDB01015	439750	2,08	0,0454	0,0249
	N-acetyltaurine			159864	19,57	0,0000	0,0001
	N-acetylarginine	C02562	HMDB04620	67427	10,24	0,0008	0,0014
Aminosugars	N-acetylglucosamine/N-acetylgalactosamine		HMDB00215	24139	7,91	0,0008	0,0014



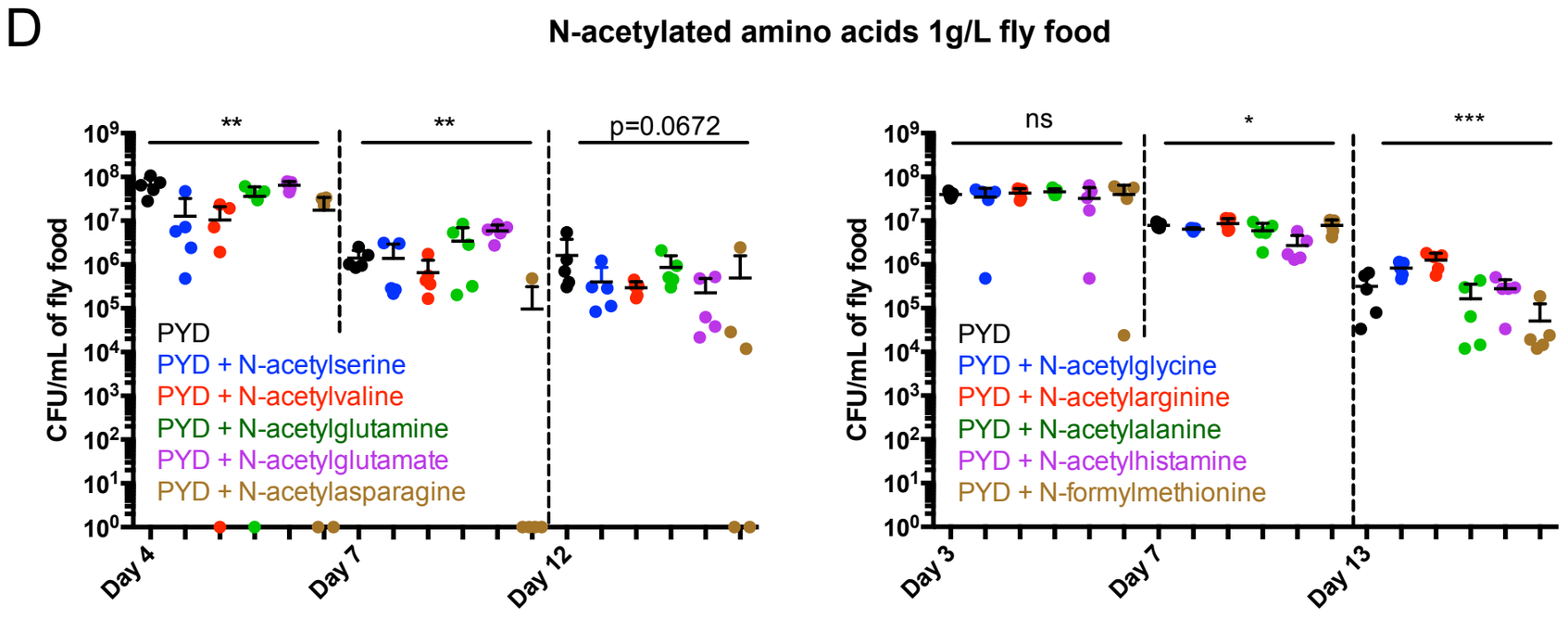


Figure S4
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Supplementary Figure 4 (related to Fig.7 and Supplementary Table 3): Derivatives of purine metabolism, tryptophan metabolism and N-acetyl amino acids are excreted by larvae but do not promote bacterial persistence when supplied individually.

(A) List of candidate maintenance factors based on metabolic profiling of larval excreta (**B**) Evolution of food matrix bacterial load on substrate supplemented with derivatives of Purine Metabolism. Hypoxantine and Orotate were added at a concentration of 2g/L fly food, Hypoxanthine at 1g/L fly food (**C**) Evolution of food matrix bacterial load on substrate supplemented with derivatives of Tryptophan Metabolism. Compounds were added at a concentration of 1g/L fly food. (**D-E**): Evolution of food matrix bacterial load on substrate supplemented with single N-acetyl amino acids and formyl-methionine. N-acetyl amino acids and formylmethionine were supplemented at 1g/L fly food (**D**) or 20g/L fly food (**E**). (**F-G**) High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) of N-acetyl-Glucosamine (NAG). Representative chromatogram obtained after separation of a mixture of pure NAG and chitobiose (dimer of NAG) (**F**) or carbohydrates contained in the larval excreta (**G**). Red arrowhead points towards N-acetyl-Glucosamine, with a retention time of approx. 16 minutes. (**B-E**): Each dot represents quantification from a single food matrix. The horizontal line in the dot plot represents the mean value. Whiskers represent standard deviation. For grouped analysis, significant difference in the distribution of samples at the same timing was assayed using Kruskal Wallis test (**B-E**). Asterisks illustrate statistical significance in the distribution of samples: ****: $p<0,0001$, ***: $0,0001<p<0,001$, **: $0,001<p<0,01$, *: $p<0,05$. ns = not significant ($p>0,1$). The exact p-value is indicated when approaching statistical significance ($0,05<p<0,1$).