**Phosphatidic acid as a limiting host metabolite for the proliferation of the
microsporidium *Tubulinosema ratisbonensis* in *Drosophila* flies**

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Microsporidia are located at the base of the fungal evolutionary tree. They are obligate intracellular parasites and harness host metabolism to fuel their growth and proliferation. However, how the infestation of cells impacts the whole organism and how the organism contributes to parasite proliferation remain poorly understood. Here, we have developed a *Tubulinosema ratisbonensis* systemic infection model in the genetically-amenable *Drosophila melanogaster* host in which parasite spores obtained in a mammalian cell culture infection system are injected into adult flies. The parasites proliferate within flies and ultimately kill their hosts. As commonly observed for microsporidia infecting insects, *T. ratisbonensis* preferentially grows in the fat body and ultimately depletes the host metabolic stores. We find that supplementing the fly diet with yeast does not benefit the host but the parasite, which increases its proliferation. Unexpectedly, fatty acids and not carbohydrates nor amino-acids are the critical components responsible for this phenomenon. Our genetic dissection of host lipid metabolism identifies a crucial compound hijacked by *T. ratisbonensis*: phosphatidic acid. We propose that phosphatidic acid is a limiting precursor for the synthesis of the parasite membranes and hence of its proliferation.

With more than 1,300 species described, microsporidia infect most animal species¹. They are highly adapted to an intracellular parasitic lifestyle characterized by a strong reduction of their genomes². The parasite invades a host cell by discharging directly or indirectly the spore contents into the cytoplasm of the host cell through a polar tube³. The extruded sporoplasm first forms meronts that intensely proliferate, thereby filling the host cell prior to becoming sporonts and ultimately developing into mature spores. Microsporidiosis affects the fitness of its host resulting in shortened lifespan, reduced fertility, loss of vigor, and stunted juvenile growth⁴. Some microsporidia induce cellular hypertrophy by forming specialized structures known as syncytial or neoplastic xenomas⁵⁻⁷. The former results from the fusion of infected cells into plasmodia while the latter involves hyperplasia of infected host cells. Several

biochemical pathways are absent in microsporidia, including the tricarboxylic cycle⁸. Indeed, microsporidia lack mitochondria and rely on a remnant, the mitosome, to ensure the assembly of Fe-S clusters⁹. Glycolysis is essentially functional in spores and may not take place during intracellular life stages during which the parasite relies on ATP transporters to steal host energy¹⁰. Some species initiate host glycolysis by secreting a hexokinase and thus may drive host biosynthesis to their own benefit¹¹. Microsporidian genomes lack genes for fatty acid (FA) metabolism such as Acetyl-CoA carboxylase (ACC) or fatty acid synthase (FASN) as regards anabolism, or acyl-CoA-oxidase or enzymes required for FA β -oxidation, in keeping with a lack of mitochondria⁸.

Several parasites hijack host lipid metabolism. For instance, lipid synthesis genes have been lost in a majority of parasitoid wasp species¹². Specific lipids may be required for parasite growth, like cardiolipins for *Spiroplasma poulsonii*¹³, phosphatidylcholine for *Plasmodium berghei* growing in hepatocytes¹⁴, or phosphatidylthreonine for *Toxoplasma gondii*¹⁵. These lipids are directly acquired from the host^{14,16,17} or are derived from host lipidic precursors^{13,15}. Lipids represent important structural compounds, *e.g.*, for membranes; they also constitute a rich resource for energy through β -oxidation. Indeed, it has been proposed that FA degradation by this process is important for *Trypanosoma cruzi* proliferation¹⁸.

Lipids ingested by the host are digested by intestinal lipases into monoacylglycerols (MAGs) and free FAs in the gut lumen. After absorption by enterocytes, FAs and MAGs are transformed into diacylglycerol (DAG) by MAG acyltransferases, which in *Drosophila*, complexes with acyl carrier proteins such as ApoLPP for transport to the fat body, the main metabolic organ of insects¹⁹. The adult fat body is formed by lobules of few adipocytes. There, DAGs are transformed into triacylglycerides (TAGs) and stored into lipid droplets (LDs). LDs are complex dynamic structures with a core of neutral lipids and cholesterol esters and a monolayer of phospholipids at the surface that harbors proteins such as the perilipins LSD-1

and LSD-2²⁰. When needed, LDs can be hydrolyzed by lipases such as Brummer or the Hormone-sensitive lipase HSL²¹. In *Drosophila*, HSL might be mobilized by the adipokinetic hormone AKH through its receptor AKHR. Lipids are also synthesized *de novo* from sugars through glycolysis and the tricarboxylic cycle that produces the basic FA building stone, citrate. Several key enzymes such as ACC, FASN1, AcetylCoA synthetase (AcCoAS) will then synthesize FA prior to coupling to a glycerol moiety. The intermediate product phosphatidic acid (PA) can either be used to synthesize phosphatidylinositols (PIs) or be converted to TAG or precursors of other phospholipids through DAG synthesis by the PA phosphatase Lipin.

It is unknown at present whether specific metabolites critically limit the proliferation of microsporidia. We present here the characterization of a microsporidium that parasitizes the genetic model organism *Drosophila melanogaster*.

Results

Tubulinosema are natural pathogens of *D. melanogaster* and *T. ratisbonensis* is sometimes found in laboratory stocks²². *Tubulinosema* appear to contaminate the insect through the digestive tract only at the larval/pupal stage²³. As we also failed to infect adult flies by feeding them with a *T. ratisbonensis* spore solution, we resorted to injecting spores grown in cultured cells directly in the thorax (injection in the abdomen yielded similar results; Supplementary Fig. 1a,b). We observed that flies were ultimately killed in a dose and temperature-dependent manner, which correlated with parasite proliferation measured by qPCR (Fig. 1a,b, Supplementary Fig. 1c,d). Interestingly, temperature-shift experiments from 18°C to 29°C or the reverse illustrated the dependence of the parasite on higher temperatures for virulence, as monitored using death as an endpoint (Supplementary Fig. 1e,f). As reported earlier, the progression of infection in this injection paradigm resembles that observed in

naturally infested flies, with variable titers measured in the latter²⁴. The infection progressively decreased the fitness of the fly in terms of motility, feeding, egg maturation in the ovaries, and egg-laying (Fig. 1c-f).

We analyzed the progression of the infection through histology on whole flies fixed by direct injection of paraformaldehyde solution in the hemocoel. The small nuclei of meronts were discriminated from large host cell nuclei using a simple DNA stain whereas spores were detected using an antiserum raised against them that also binds to extruded polar tubes. The parasite appeared to preferentially proliferate in the fat body. The cardiac vessel, oenocytes, some visceral muscles, ovarian, and intersegmental muscles were infected, yet the flight muscles never harbored *T. ratisbonensis* spores (Fig. 2a,b, Supplementary Fig. 2a,b). These results agree with previous data on flies infested at the larval stage by feeding, except that the flight muscles and neural tissues of naturally-infested flies were also harboring microsporidia²². The infected organs were inflated. Interestingly, Malpighian tubules were also affected: the duct cells were dilated and obstructed the lumen of the tubules. We therefore tested their excretory function by injecting a dye in the hemocoel in a novel assay we call FRUMS. Whereas uninfected control flies cleared the dye within six hours through excretion, infected flies still displayed a light blue body up to 18 hours after injection, which correlated with diminished defecation (Supplementary Fig. 2c-f). The impaired excretory function of Malpighian tubules likely accounts for the increased water retention measured in infected flies and hence for their enlarged size²⁴.

Upon closer inspection of fat body lobules, we observed a correlation between the loss of LDs and the appearance of the meronts. The lobules ultimately filled up with the parasite, the spores differentiating at the cortex of the lobule (Fig. 2c,d; Supplementary Figs. 3a-d & 4e,f). At late stages of the infection, mitochondria and peroxisomes were no longer detected and the nuclei of fat body cells were confined to the periphery of lobules (Supplementary Fig. 4).

114 Unexpectedly, the basal and lateral intra-lobular cytoplasmic membranes of fat body cells
115 labeled by reporter transgenes vanished whereas the cortical membrane of the lobule was
116 preserved (Fig. 2 e,f, Supplementary Fig. 4e,f). The infected fat body lobules therefore appear
117 to form syncytial xenoma. Functionally, microsporidia-infected flies challenged with a
118 mixture of nonpathogenic bacteria to trigger the systemic immune response progressively lost
119 the ability to induce antimicrobial peptide gene expression in fat body cells (Supplementary
120 Fig. 3e-j). This likely explains why naturally-infested flies appeared to succumb to a variety
121 of microbial infections in our laboratory, which experienced a severe bout of contamination
122 some years ago. While trying to identify the microbe at the origin of the infection, we
123 retrieved several bacterial strains from the hemolymph, including *Staphylococcus xylosus*²⁵.
124 To confirm experimentally these observations, we have re-infested a wild-type stock by
125 feeding it on medium contaminated by the cadavers of *T. ratisbonensis*-injected flies that are
126 eaten by foraging larvae. Next, we collected hemolymph from infested flies and plated it on
127 different media to identify bacteria that may have escaped from the digestive tract. We
128 retrieved a total of 4840 colonies from the infested strain and 360 from the control
129 noninfested stock. We sequenced an equal number of colonies originating from each stock.
130 We found 39 colonies to correspond to *Lactobacillus plantarum*, a major constituent of our
131 wild-type strain microbiota, and one to *S. xylosus*, which was originating from the infested
132 stock. These data also suggest that the intestinal barrier is impaired in infested flies. In
133 keeping with the results obtained with “naturally” infested flies, we found that the overall
134 bacterial content of *T. ratisbonensis*-injected flies increased (Supplementary Fig. 1g). Death
135 was however not caused by the unhampered bacterial proliferation of the host microbiota, as
136 axenically-grown flies succumbed earlier to the injection of spores (Supplementary Fig. 1h).
137 The enhanced death rate after injection of *T. ratisbonensis* of sterile flies may be related to
138 their increased lipid stores (see below)^{26,27}.

Since the fat body plays a central role in fly physiology, we measured several metabolic parameters and found that glucose, free fatty acids, glycogen and triglyceride stores were strongly reduced in infected flies (Fig. 3a,b). This may however reflect the decreased food intake (Fig. 1d). We therefore asked whether providing a nutrient (yeast)-rich food would improve the survival of infected flies. Strikingly, insects succumbed faster to the infection when dead or live yeasts were added to our low-yeast fly food (Fig. 3c). This enhanced mortality was likely caused by the increased proliferation of the parasite measured in those flies (Fig. 3d). To identify the biochemical compounds that mediate this effect, we added amino-acids, sucrose, sterols or free fatty acids to a sucrose solution the flies were feeding on. Interestingly, amino-acid supplementation or an increased sucrose concentration favored a slightly increased survival of infected flies without however altering the parasite burden (Fig. 3e-h). Whereas the addition of sterols mildly benefited the host, oleate or palmitate supplementation recapitulated the survival and parasite burden effects observed when adding yeast to the diet (Fig. 3i-l). This result is in keeping with our histologic observations in which the parasite seems to proliferate in fat body cells at the expense of LDs (Fig. 2c,d, Supplementary Fig. 3a-d). To corroborate these data, we raised flies under different nutrient conditions for five days prior to the injection of *T. ratisbonensis*, which led to differing amounts of stored triglycerides. Flies kept on yeast poor medium displayed survival curves and parasite load similar to those shown in Fig. 1a (Fig. 4a-c). In contrast, flies kept on 100 mM sucrose succumbed faster to infection even though the parasite proliferated poorly, possibly as a result of the low triglyceride reserves of those flies that get depleted faster (Fig. 4a-c). As expected, flies kept on a yeast-rich diet succumbed also faster, likely due to an almost doubled parasite load that correlated with initially increased triglyceride stores (Fig. 4a-c). Females contain twice as much triglyceride as males and allowed a parasite proliferation that was twice as high as in males, which nevertheless died sooner than females,

with exhausted TAG stores (Fig. 4d-f). Thus, infected flies appear to succumb either to the depletion of their FA reserves or to the generalized infection, depending on their nourishment status.

To determine which host lipid metabolic processes are required for parasite proliferation, we performed a small-scale genetic screen using RNAi transgenes expressed ubiquitously only at the adult stage. While the genome contains at least 600 genes related to lipid metabolism, we limited ourselves to testing some 100 genes involved in FA synthesis, transport, lipid droplet biogenesis and degradation, β -oxidation, and regulation of lipid metabolism (Supplementary Table 1)^{21,28,29}. Knocking-down a gene required to synthesize a product indispensable for parasite growth would be expected to alleviate the infection burden of the host, and possibly enhance its survival to infection. Conversely, decreasing the expression of a host gene that governs the consumption by the host of a key parasite nutrient should lead to an increased supply of that nutrient and thereby of the microsporidian load, thus decreasing the survival of the host. This strategy allows mapping the host metabolic pathways essential for parasite growth.

Lipids in the fat body are gained either through digestion of nutrients in the gut or directly by *de novo* FA and triglyceride synthesis. Whereas targeting the intestinal lipase *magro* gene by RNAi failed to yield a *T. ratisbonensis* infection phenotype, reducing the expression of *apolpp* but not of the two accessory lipid transporter genes *ApoLTP* or *MTP* led to an improved survival as compared to controls, and a reduced parasite load (Fig. 5a,b, Supplementary Table 1)^{19,30}. These data suggest that the dietary intake of lipids contributes to the virulence of the infection, thereby confirming our previous results with the manipulated diet composition.

Lipids are stored in the fat body as LDs. We found that depleting endogenous LSD-2 through RNAi led to the filling of both noninfected or infected fat body cells by lipids, suggesting they are not accessible to the parasite. Accordingly, the flies survived longer to the infection whereas the microsporidian load was decreased (Supplementary Fig. 5). Interestingly, the *Hsl* and *Lipase 3 (Lip3)* but not the Brummer lipase genes yielded survival and parasite burden phenotypes similar to those of *Lsd-2* when knocked-down by RNAi (Fig. 5a,b, Table S1)²⁰. The finding that lipid droplet-lipases and LSD-2 are required for *T. ratisbonensis* growth suggests that the disappearance of LDs during *T. ratisbonensis* infection does not merely result from an impaired formation of LDs due to the parasite scavenging FAs prior to their incorporation into them but rather that lipids stored in droplets constitute an indirect supply of lipids accessible to the parasite through the host cell machinery.

FAs stored in LDs represent an important energy resource through β -oxidation in peroxisomes or in mitochondria. As the parasite lacks mitochondria, a tricarboxylic acid cycle, and β -oxidation⁸, it likely should not be able to directly generate ATP nor carbohydrates from FAs but could use the host machinery for this purpose. We therefore asked whether host β -oxidation was required for *T. ratisbonensis* proliferation. Blocking acyl-CoA oxidation in the peroxisome through targeting by RNAi either *CG5009* or *CG9527*, the first step for long chain FA shortening, improved the survival of the parasitized host, whereas the microsporidian load decreased (Supplementary Table 1). In contrast, β -oxidation in mitochondria appears to be largely dispensable for parasite growth (*e.g.*, *colt* and *CRAT* encode enzymes required for the import of FAs into mitochondria; they display a RNAi-silenced phenotype opposite to that of *CG5009* or *CG9527*; Fig. 5c,d,g Supplementary Table 1). These data suggest on the one hand that the parasite is unable to directly use long chain FAs that need first being shortened by the peroxisome and on the other hand that host FAs are

not used to generate ATP that would then have been hijacked by the parasite through its ATP transporters.

We next investigated the role of *de novo* FA synthesis and found that targeting by RNAi the key enzymes required for the synthesis of PA, from FA synthesis to its direct glycerol phosphate precursors (*ACC*, *FASN1*, *AcCoAS*, *GPAT4*, and *ful2*), decreased parasitic growth and enhanced host survival while decreasing LDs in the fat body (Fig. 5a,b,g, Supplementary Table 1). This phenotype was observed when *ACC* and *FASN* were silenced in the adult fat body, but not in the larval fat body nor in muscles (Supplementary Fig. 6). The FA synthesis deficiency but not the *ApoLpp* phenotype was rescued by supplementing the food with fatty acids (Supplementary Fig. 7). In contrast, preventing the use of PA by the host by blocking phosphatidylethanolamine, phosphatidylcholine (*bbc*) or triacylglyceride synthesis (*Lpin* and *mdy*) favored the proliferation of *T. ratisbonensis* while accelerating the demise of the host (Fig. 5c,d,g, Supplementary Table 1). Thus, our data indicate that PA may represent the compound hijacked by the parasite (Fig. 5g, Fig. 6). While enriching the diet with PA did not alter the survival of the host or the proliferation of the parasite (Supplementary Fig. 8), possibly because dietary PA may be converted to DAG in enterocytes, the direct injection of PA into flies led to a strong susceptibility to *T. ratisbonensis* infection that correlated with an almost four-fold increase in the parasite load (Fig. 5e,f). We conclude that PA is a critical limiting factor for *T. ratisbonensis* proliferation in *Drosophila*.

Discussion

Several lines of evidence underscore the importance of *Drosophila* lipids for the proliferation of *T. ratisbonensis*. It infects the fat body and its intense proliferation there correlates with a loss-of-lipids. The parasite burden is limited by the quantity of FAs stored in the fat body,

235 depending on sex or nutrient-quality of the food. Importantly, the supplementation of the diet
236 with FAs but not with amino-acids nor with increased sugar concentrations benefits the
237 parasite and not the host. Finally, interfering genetically with FA synthesis or supply also
238 alters the proliferation of the parasite and its virulence. The fat body uses four interconnected
239 pathways to generate FAs, which are shown in Fig. 6. FAs extracted from the food reach the
240 fat body as DAGs, which are transformed into TAGs prior to their incorporation in LDs.
241 Alternatively, imported DAG may also be directly used to synthesize host phospholipids.
242 When needed, FAs stored either in LDs or as long chain FAs can be mobilized in the cell
243 through the use of dedicated enzymes such as lipases or through partial β -oxidation in the
244 peroxisome and become available. FAs can also be neo-synthesized thanks to the anabolic
245 role of the tricarboxylic acid cycle. PA constitutes a nexus in lipid metabolism as it can either
246 be used to synthesize membrane phosphoinositol or alternatively can further be transformed
247 into DAG for storage as TAGs or the synthesis of other membrane phospholipids. We have
248 shown here that knocking down the expression of the genes involved in any of the pathways
249 that leads to the synthesis of PA results in a decreased parasite burden that correlates with its
250 decreased virulence (green arrows in Fig. 6). In contrast, targeting genes encoding enzymes
251 that use up PA to make directly or indirectly phospholipids or TAGs leads to the opposite
252 phenotype (red arrows). As a result of interference with the latter, more PA would become
253 available for the parasite to proliferate more intensely, a point confirmed by the direct
254 injection of PA into flies that also promotes the parasite virulence. As *Lpin* is also required for
255 the synthesis of TAGs, one might expect therefore that LDs would be affected and thereby
256 indirectly limit the supply of FAs to make PAs. However, we interfere with the expression of
257 the genes encoding these enzymes only at the adult stage, once LDs are formed. Thus, the
258 flux of FAs originating from LDs would not be impaired, at least initially, and later be
259 compensated by the direct use of PA by the parasite. Taken together, our data indicate that PA

is a critical metabolite for the proliferation of *T. ratibonensis* in *Drosophila*. They do not however exclude the possibility that other metabolites may also be important for the parasite. Indeed, it is not clear at this stage how the parasite is able to scavenge host phospholipids coming from the different membranes of the host cell, for instance those of mitochondria or basal/lateral cytoplasmic membranes.

How the parasite imports host PA present in the endoplasmic reticulum (ER), LDs, or mitochondria remains to be established and may involve a process akin to that used for transferring PA from the ER to the inner mitochondrial membrane³¹, although we did not find any predicted protein encoding homologs to known PA transporters in the microsporidiaDB database that yields access to 27 microsporidian genomes³². Contacts between the meront membranes and host organelles are likely to be required³³⁻³⁵. The scavenging of PA might represent a passive mechanism for depleting PA from the host and ultimately then of its lipid reserves that would be driven by host cellular homeostatic processes. Active parasite-driven processes can also not be discounted at this stage³⁶. For instance, a possibility would be that the parasite induces at the adipocyte level the observed increased expression of the lipase LIP3, resulting in lipid droplet depletion, or decreases at the organismal level that of ImpL2, an antagonist of insulin-like peptides that promotes wasting of metabolic stores, thereby promoting anabolism in the fat body (Supplementary Fig. 9).

Future studies will determine whether this dependence on lipids is widespread in microsporidia as has been described for several other intracellular parasites¹⁴⁻¹⁸. Here, we propose that the fusion of infected fat body cells with neighboring noninfected cells within a lobule is mediated by the parasite through the fusion of the intralobular cytoplasmic membranes. This strategy minimizes the amount of host membrane required to preserve the structure of the lobule, maximizes the phospholipids indirectly scavenged by the parasite through the production of PA, and thereby optimizes its efficient proliferation. Microsporidia

285 that produce syncytial xenomas⁵⁻⁷ may similarly be highly dependent on host PA. The
286 findings we describe here echo the recent discovery that plants transfer lipids that sustain
287 colonization by mutualistic mycorrhizal and parasitic fungi^{37,38}. We note also that *T. cruzi*
288 requires the activity of β -oxidation for long chain but not short chain fatty acids, thus opening
289 the possibility that shortened FAs might also be pumped by the parasite¹⁸. These FAs may
290 however be obtained directly from LDs as TAGs³⁹. Genetically obese mice are more
291 susceptible to *T. cruzi* parasitization both in terms of survival and parasitic load⁴⁰, a finding in
292 keeping with our own results in *Drosophila* (Fig. 4).

293

294 **Methods**

295 **Parasite culture** The microsporidia *Tubulosema ratisbonensis* was propagated and
296 harvested as described^{24,41}. The Human lung fibroblasts (MRC-5) cells used for this purpose
297 (a kind gift of Pr. Caspar Franzen⁴¹) were grown in Dulbecco's modified eagle medium
298 (DMEM+ GlutaMAX: Gibco), supplemented with 10% (v/v) fetal calf serum (FCS) and 1%
299 (v/v) PenStrep (Invitrogen) in a tissue culture incubator under 5% CO₂ at 37°C. The cell
300 culture was intermittently tested for the presence of mycoplasma. The MRC-5 cell line was
301 not authenticated and does not appear in the databases of commonly misidentified cell lines
302 maintained by ICLAC and NCBI Biosample.

303 **Fly strains** Fly lines were raised on two different media at 25°C with 65% humidity. For 25 L
304 of nutrient "poor" medium (standard medium), 1.2 kg of cornmeal (Priméal), 1.2 kg of crystal
305 sugar (Erstein), 300 g of yeast (Bio Springer), 100 g of moldex (Millipore) diluted into 400
306 mL of milliQ water, 20 g of agar-agar (Sobigel), and water qsp were used. For 25 L of
307 nutrient "rich" medium 1.2 kg of cornmeal (Priméal), 1.2 kg of glucose (Tereos Syral), 1.5 kg
308 of yeast (Bio Springer), 90 g of nipagin (VWR Chemicals) diluted into 350 mL of ethanol
309 (Sigma-Aldrich), 120 g of agar-agar (Sobigel) and water qsp were employed.

310 *w*^{A5001} flies were used as wild type control⁴². For RNAi experiments, virgin females *Ubi-*
311 *Gal4-Gal80*, *yolk-Gal4*, *C₅₆₄-Gal4*, *ppl-Gal4*, *how-Gal4* were crossed with males carrying
312 *UAS-RNAi* transgenes from the Vienna *Drosophila* RNAi Center (VDRC) or from the
313 Transgenic RNAi Project (TRiP) at Harvard Medical School^{43,44}. For the VDRC RNAi flies,
314 control *w*¹¹¹⁸ (#60000) were used for the GD construct and *y,w*¹¹¹⁸; *P{attP,y⁺,w^{3'}}* (#60100)
315 for the KK construct. For TRiP RNAi flies, the control flies were *mCherry VALIUM20*
316 (#35785). Crosses were performed at 18°C with the driver *Ubi-Gal4-Gal80^{ts}*, then the
317 progeny was transferred one week at 29°C with 70% humidity to allow Gal4-mediated
318 transcription.

319 For fluorescence labeling of organelles and membranes, the lines from the Bloomington stock
320 Center used were: (a) mitochondria *w*¹¹¹⁸; *P{w⁺mC=UAS-mito-HA-GFP.AP}3,e¹*; (b)
321 peroxisomal activity *w*^{*}; *P{UAS-GFP.SK1}2*; (c) cytoplasmic membrane *y¹w^{*}*;
322 *L¹/CyO;P{UAS-PLCδ-PH-EGFP}3/TM6B,Tb¹* and *w*^{*}; *P{UAS-mCD8.ChRFP}2*; All crosses
323 involving flies without RNAi expression were performed at 25°C with 65% humidity.

To obtain axenic flies, eggs were collected, washed with water, and then 70% ethanol prior to dechoriation by pipetting up and down eggs in a solution of 50% bleach until the chorion disappeared. Eggs were transferred into sterile vials containing media and a mix of antibiotics: ampicillin, chloramphenicol, erythromycin, and tetracycline. Once emerged, adult flies were crushed and tested on Lysogeny broth-, Bacitracin-heated blood-, deMan Rogosa and Sharpe- (MRS), and Yeast peptone dextrose-agar plate to observe any contamination by bacteria, fungi, or yeast. Of note, no anaerobic microorganisms have been detected in the *Drosophila* microbiota.

To obtain “naturally” infested flies, one hundred *w*^{A5001} flies were injected with 10,000 spores in 9.2 nL and kept at 29°C. The larval progeny fed on the medium containing the microsporidia-killed flies. After three generation, the presence of the spores was checked by qPCR and the infested fly stock was maintained at 25°C.

Treatment of stocks infected by Drosophila C virus (DCV), Wolbachia, microsporidia, or Nora virus All fly stocks used in this study were DCV, *Microsporidia*, *Wolbachia*, and *Nora* virus free. Contaminated flies were placed in cages containing apple juice with yeast paste to allow females to lay eggs. Eggs were collected, washed with water and dechorionated with a solution of bleach 50% by up and down pipetting until the chorion disappeared. The eggs were abundantly rinsed with water and placed on normal medium and covered with mineral oil to cover them. *Wolbachia*-positive strains were further grown on tetracyclin-containing fly media for two generations. Once flies emerged after treatments, they were tested for the absence of DCV, *Microsporidia*, *Wolbachia*, and *Nora* virus by qRT-PCR using specific primer couples (Table S2).

Microsporidia infection For microsporidia infection, spores were stored in PBS at 4°C. Unless otherwise stated, microsporidia spores were injected into the thorax, precisely into the mesopleuron on adult flies at the concentration of 2,000 spores in 9.2 nL PBS containing 0.01% Tween20 using a micro-capillary connected to a Nanoject II auto-nanoliter injector (Drummond). The same volume of PBS-0.01% Tween20 was injected for control experiments. All experiments were performed at 29°C unless otherwise indicated.

Survival tests They were performed using 20 flies per vial in biological triplicates. Adult flies used for survival tests were five to seven days old from 25°C stock. For survival tests using RNAi-silencing genes, flies were kept seven days more at 29°C to express the RNAi prior to

the experiment. Flies were counted every day. Each experiment shown is representative of at least three independent experiments.

Parasite quantification Parasite quantification was determined using five adult flies per condition. Flies were transferred into 2 mL microtubes (Starstedt) containing five 1.4 mm ceramic beads (Dominique Dutcher) in 200 μ L of Proteinase K solution and crushed using the Precellys 24 Tissue homogenizer (Bertin Technologies). Total gDNA was extracted using the NucleoSpin 96 Tissue kit (Macherey-Nagel) according to the instructions of the manufacturer. Samples of gDNA were diluted to 1/50 into milliQ water. Quantification was performed by qPCR using SYBR Green (BioRad) as described previously²⁴. Only experiments with over 90% of efficiency were validated. Each qPCR analysis was performed with *Drosophila* ubiquitous gene *RpL32* (coding the ribosomal protein 49) for normalization. Of note, results were essentially similar when the normalization was performed instead per number of infected flies.

Negative geotaxis assay To perform the Rapid iterative negative geotaxis (RING) or climbing assay⁴⁵, 10 flies were transferred in empty tubes, in biological triplicates. Flies were kept in the tube for 15 minutes to get acclimated to the new environment. The tube was banged on the bench to place all the flies at the bottom of the tube. A picture was taken after five seconds. The process was reiterated five times, with a lay period of five minutes between experiments.

Adapted CAFE assay Because infected flies have difficulties climbing, we adapted the standard Capillary Feeder (CAFE) assay⁴⁶. Five flies were transferred into an empty tube containing an Eppendorf cap (Cap Feeder assay) with 200 μ L of 100 mM sucrose with 5% Blue food dye (Brilliant Blue FCF E133) (Vahiné). The liquid on the cap was collected and measured every three days and the difference of initial volume to the collected volume corresponds to the volume of ingested liquid. Tubes were placed in a box saturated with water to reduce evaporation. The experiment was performed at 25°C.

FRUMS assay The SMURF assay was developed to observe the integrity of the intestinal barrier⁴⁷. The reverse SMURF assay we call FRUMS was designed to test the filtration/excretory capacity of the Malpighian tubules into the gut lumen. Blue food dye (Brilliant Blue FCF E133) (Vahiné) was injected into the thorax, precisely into the mesopluron of adult flies, resulting in blue-bodied flies. The injected volume was 69 nL using a micro-capillary connected to the Nanoject II auto-nanoliter injector (Drummond). After transfer of 20 flies per tube, the disappearance of the blue phenotype was monitored every 30

minutes. For the feces assay, each fly was placed in a single Eppendorf tube and blue fecal spots counted every 30 minutes.

Immunostainings Flies were first rinsed with 70% ethanol then injected with approximately 100 nL of 8% Paraformaldehyde (PFA) into the thorax and incubated for two hours in 4% PFA. Flies were washed with 1X PBS and manually cut in half longitudinally with a scalpel in the case of whole fly preparations or cut transversally to observe the fat body in the tergite moiety of the abdomen. Flies were permeabilized for 15 minutes with 1X PBS, 0.1% Triton X-100. Samples were blocked for two hours in 1X PBS, 0.1% Triton X-100, 2% BSA (PTB). Flies were incubated during two hours in PTB plus the primary rabbit antibody anti-*T. ratisbonensis* spores (1/500). Samples were washed for five minutes with PTB. Flies sections were incubated for two hours on PTB plus the secondary goat antibody anti-rabbit coupled to Cy5 (1/250) (Life Technologies Alexa Fluor 647) and phalloidin-FITC (1/100) (Sigma-Aldrich). Samples were washed for five minutes with PTB. Flies were incubated for five minutes on PTB plus 1 μ M Nile Red. Samples were washed for five minutes with 1X PBS. Sections were mounted on diagnostic microscope slides (Thermo Fisher Scientific) with Vectashield plus DAPI (Vector Laboratories). All the incubation steps were performed at room temperature. Samples were observed using a LSM 780 confocal microscope (Zeiss).

AMP gene expression measurements For each condition, flies were pricked into the thorax with a sharpened needle previously dipped into pellets obtained by centrifugation of a bacterial mix of *Escherichia coli* and *Micrococcus luteus* saturated overnight cultures placed in a 1.5 ml Eppendorf tube. Clean sharpened needles were used as control. Anti-microbial peptide (AMP) gene expression was quantified on samples of five adult flies per condition. Flies were transferred into 2 mL microtubes (Starstedt) containing five 1.4 mm ceramic beads (Dominique Dutcher) and mixed using the Precellys 24 Tissue homogenizer (Bertin Technologies). Total RNA was extracted using the NucleoSpin 96 RNA kit (Macherey-Nagel) according to the instructions of the manufacturer. Reverse transcription was performed using iScript cDNA Synthesis Kit (BioRad) according to the instructions of the manufacturer. Samples of cDNA were diluted to 1/20 into milliQ water. Quantification was performed by qPCR using SYBR Green (BioRad) as described previously²⁴, using the primer couples shown in Table S2. Only experiments with qPCR efficiency over 90% were validated. Each qPCR analysis was performed with *Drosophila* ubiquitous gene *RpL32* normalization. Of note, results were essentially similar when the normalization was performed per number of infected flies.

Fluorescence quantification Fluorescence quantification was achieved using the ImageJ program⁴⁸. Channels were separated and analyzed by measuring the fluorescent signal intensity for each channel.

Metabolite measurements Sextuplicates of five flies per condition were used to measure the metabolite levels. Glycogen, glucose, triglycerides (TAG) and free fatty acids were respectively measured using the Glycogen Assay Kit (#700480), the Glucose Colorimetric Assay Kit (#10009582), the Triglyceride Colorimetric Assay kit (#10010303), and the Free Fatty Acid Fluorometric Assay kit (#700310) (Cayman Chemical). Standard curves were obtained according to the instructions of the manufacturer. Each metabolite measurement was normalized to protein levels of the extract. Of note, results were essentially similar when the normalization was performed per weight of flies. The selected flies for each experiment had approximately the same body weight.

Food supplementation Sucrose (Euromedex) was added to milliQ water to obtain solutions at the following concentrations: 100 mM, and 200 mM. Food supplementation experiments were performed using 100 mM sucrose solution. MEM essential amino acids and non-essential amino acids solutions (Thermo Fisher Scientific) were mixed to a 1x final concentration containing: L-arginine hydrochloride 126.4 mg/L, L-cysteine 24 mg/L, L-histidine hydrochloride 42 mg/L, L-isoleucine 52.4 mg/L, L-leucine 52.4 mg/L, L-lysine hydrochloride 72.5 mg/L, L-methionine 15.1 mg/L, L-phenylalanine 33 mg/L, L-threonine 47.6 mg/L, L-tryptophan 10.2 mg/L, L-tyrosine 36 mg/L and L-valine 46.8 mg/L, whereas glycine, L-Alanine, L-asparagine, L-aspartate, L-glutamate, L-proline and L-serine were at 0.1 mM. Water-soluble cholesterol (Sigma-Aldrich) was used at 100 μ M concentration. Ergosterol (Sigma-Aldrich) was initially diluted into DMSO and used at 100 μ M concentration. Soluble palmitic acid was obtained by conjugation of sodium palmitate to BSA, following the protocol of preparation of BSA-conjugated palmitate (Seahorse Bioscience). Palmitic acid coupled to BSA was used at a 100 μ M concentration. Water-soluble Oleic acid (Sigma-Aldrich) was used at a 100 μ M concentration.

Phosphatidic acid supplementation Phosphatidic acid (Sigma-Aldrich) was initially solubilized into chloroform and then diluted to 100 μ M in either 100 mM sucrose solution (ingestion experiments) or PBS (injection solution); both injected solutions contained a final concentration of 0.1% chloroform. Flies raised on rich medium were injected with 69 nL at 0, 3, and 6 days post infection.

452 **Quantification of the fly microbiota** It was measured on five adult flies per condition. Flies
453 were transferred into 2 mL microtubes (Starstedt) containing five 1.4 mm ceramic beads
454 (Dominique Dutcher) in 500 μ L of 1X PBS and mixed using a Precellys 24 Tissue
455 homogenizer (Bertin Technologies). The dilution series was performed from pure sample to
456 1/100,000 dilution. Samples were deposited with 5 μ L of each condition in duplicate on Luria-
457 Bertoni (LB) agar (15 g/L) plates. The plate was incubated at 37°C overnight. The number of
458 colony forming unity (CFU) was counted, according to the level of dilution.

459 **Identification of bacteria present in the hemolymph** Hemolymph samples were collected
460 from batches of 20 flies, with five samples for infested and 5 samples for uninfested
461 *w^{A5001}* flies. The samples were plated on five media as described⁴⁹. 40 colonies obtained on LB
462 plates from infested or noninfested samples (20 each) were separately put in a tube containing
463 a PCR mix containing primers D1&D2 (Table S2) in order to amplify the 16S DNA of each
464 colony. Sequencing was performed by GATC[®] company. Sequences obtained were blasted
465 using standard Nucleotide BLAST from NCBI website.

466 **Small-scale genetic screen using RNAi transgenes** We selected 100 genes involved in lipid
467 metabolism to obtain an overview of the major pathways involved in *Drosophila* lipid
468 metabolism. Several criteria were applied for the selection of these genes: genes with a
469 documented function (an emphasis was placed on validated RNAi lines in the scientific
470 literature), evolutionarily conserved genes, and genes identified through interactome studies
471 (STRING website).

472 **Antibody production** Polyclonal rabbit antiserum raised against *T. ratisbonensis* spores was
473 produced as described⁵⁰.

474 **Statistical analysis and reproducibility** All statistical analyses were performed using Prism 7
475 (GraphPad Software). The Mann-Whitney and/or Kruskal-Wallis tests were used unless
476 otherwise indicated. For survival experiments, the time it takes for 50% of the flies to
477 succumb (LT50) was determined for each curve being compared. An unpaired t-test was used
478 on the LT50s from biological triplicates was used to assess the significance between two
479 survival curves. When using parametric tests (ANOVA, t-test), a Gaussian distribution of data
480 was checked using either D'Agostino-Pearson omnibus or Shapiro-Wilk normality tests. All
481 experiments have been performed at least three times. Significance values: $p < 0.05 = *$ / $p <$
482 $0.01 = **$ / $p < 0.001 = ***$ / $p < 0.0001 = ****$.

483 ***Data availability***

484 The datasets, including the 16S rRNA DNA sequences of bacteria retrieved in the hemolymph
485 of control or infected flies, generated during and/or analyzed during the current study are
486 available in the “***Phosphatidic acid as a limiting host metabolite for the proliferation of the***
487 ***microsporidium Tubulinosema ratisbonensis in Drosophila flies*** ” repository:
488 <https://figshare.com/s/c6ef12b7c46e81e58bb1>
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Acknowledgments

We are grateful to Miriam Yamba and Marie-Céline Lacombe for expert technical assistance. We would like to thank Drs. Frédéric Delbac, Alex Gould, Bruno Lemaitre, and Pierre Léopold for informative discussions and suggestions, and Dr. Samuel Liégeois for critical reading of the manuscript. Transgenic fly stocks were obtained from the Vienna Drosophila Resource Center (VDRC, www.vdrc.at). Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. This work has been funded by CNRS, University of Strasbourg, Fondation pour la Recherche Médicale (Equipe FRM to D.F. and FDT20140930952 to A.F.), Agence Nationale de la Recherche (BEELOSS, ANR-11-EQPX-0022), and the Institut d'Etude Avancée de l'Université de Strasbourg.

Author Contributions

AF performed most of the experimental work described in this study, with assistance from GC for the natural infestation experiment and the ingestion/injection of PA into control or microsporidia-infected flies. SN developed the infection model and all techniques required to maintain parasite cultures as well as those for infection of flies; he has participated in all aspects of the early stages of this study. AF and DF designed the experiments, analyzed the data, and wrote the manuscript.

Competing Interests statement

The authors report no conflict of interest.

Figure legends

Figure 1: *Tubulinosema ratisbonensis* infection alters *Drosophila melanogaster* fitness.

a, Survival curves of flies infected by *T. ratisbonensis* and uninfected control flies. Mean and standard deviation of the survival of triplicates of each 20 flies are displayed. **b**, Kinetics of the parasite load measured by qPCR in six samples of five flies each. **c**, Climbing activity of five batches of ten flies each infected by *T. ratisbonensis* relative to uninfected control flies..

654 **d**, Volume of nutrient solution ingested by five batches of ten *T. ratisbonensis*-infected flies
655 or uninfected control flies. **e**, Ovary from uninfected female (left panel) and infected female
656 (right panel); scale bar: 100 μ m. **f**, Fertility of ten batches of ten flies each seven days after
657 infection with *T. ratisbonensis* expressed as the percentage of eggs laid relative to uninfected
658 control flies. All flies were infected by injection of 2,000 *T. ratisbonensis* spores at 29°C on
659 standard medium. Each graphic is representative of three independent experiments. **b-d, f**: the
660 middle bar of box plots represents the median and the upper and lower limits of boxes
661 indicate respectively the first and third quartiles; the whiskers define the minima and maxima.
662 **c, d, f**: data were analyzed using the Mann-Whitney statistical test.

663 **Figure 2: *T. ratisbonensis* proliferates in the fat body at the expense of host lipids.**

664 **a-f**, Uninfected (**a**) or infected (**b-d**) *Drosophila* fixed and stained for DNA (DAPI, blue), for
665 actin (FITC, green), for lipid droplets (Nile Red, red), and for *T. ratisbonensis* spores (Cy5,
666 yellow). **a**, Uninfected abdomen; the blue signal originates from the cross-reaction of DAPI
667 with the tracheal system (°: air sacs); the dorsal vessel appears green (outlined); fat body
668 lobules are stained in red. **b**, Infected abdomen at 9d post infection; the blue signal
669 corresponds essentially to the nuclei of the proliferating meronts. Spores differentiate at the
670 cortex of the hypertrophied fat body lobules (asterisks) as well as pericardial cells, which line
671 the dorsal vessel (outlined). **c**, Infected fat body at 3d post infection; arrowhead = uninfected
672 cell in a partially infected fat body lobule; arrow = infected area of the lobule; asterisks: fully
673 infected lobules filled with meronts. **d**, Fat body lobules at 9d post-infection: most of them are
674 filled by the parasite (asterisks), which has initiated spore formation (yellow), arrow.
675 Uninfected fat body lobule: arrowhead. **e, f**, Uninfected (arrowheads) and infected (white
676 arrows) fat body lobules of flies carrying a mCD8-mCherry (**e**) or a PLC δ -GFP (**f**) membrane
677 reporter transgene. Note that the cortical membrane of the lobule (grey arrows) is preserved
678 whereas baso-lateral membranes of the cells that form the lobule have vanished, thereby

forming a syncytium. *Drosophila* females were infected by injection of 2,000 *T. ratisbonensis* spores and kept at 29°C. Each picture is representative of at least three independent experiments.

Figure 3: Fatty acid as the limiting factor for the proliferation of *T. ratisbonensis*.

a-b, Relative quantification of metabolic stores of whole flies uninfected (a) and infected with *T. ratisbonensis* (b) after 9 days; the stores have been set to 1 in uninfected flies. The p-values in (b) obtained after comparison to uninfected flies analyzed in parallel (a) are shown for each biochemical category. **c-d**, Survival (c) and parasite load (d) of flies infected with *T. ratisbonensis* and fed on a yeast-poor food supplemented either with live or killed yeasts; controls were fed on nonsupplemented yeast-poor food. Kruskal-Wallis analysis of the multiple comparisons demonstrated a global significance of yeast supplementation, $p=0.0002$ (d). **e-f**, Survival (e) and parasite load (f) of flies infected with *T. ratisbonensis* and fed on a 100 mM sucrose solution supplemented with an amino acids solution; controls were fed on nonsupplemented sucrose solution. **g-h**, Survival (g) and parasite load (h) of flies infected with *T. ratisbonensis* and fed on a 300 mM sucrose solution; controls were fed on a 100 mM sucrose solution. **i-j**, Survival (i) and parasite load (j) of flies infected with *T. ratisbonensis* and fed on a sucrose solution supplemented either with cholesterol or ergosterol; controls were fed on nonsupplemented sucrose solution. Kruskal-Wallis analysis of the multiple comparisons failed to reveal a global significance, $p=0.77$ (j) of sterol supplementation to the diet; **k-l**, Survival (k) and parasite load (l) of flies infected with *T. ratisbonensis* and fed on a sucrose solution supplemented either with palmitate or oleate; controls were fed on nonsupplemented sucrose solution. Kruskal-Wallis analysis of the multiple comparisons demonstrated a global significance, $p=0.0005$ (l) of FA supplementation. Survival

experiments c, e, g, i, and k: mean and SD of three batches of 20 flies are displayed; an unpaired t-test on LT50s of the three biological replicates of the indicated curve(s) with respect to controls (blue curve) was used to assess statistical significance. a-b, d, f, h, j, l: the data obtained on six batches of five flies each are shown; data on simple or pairwise comparisons after Kruskal-Wallis tests were analyzed using the Mann-Whitney statistical test. The middle bar of box plots represents the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers define the minima and maxima. *Drosophila* females were infected by injection of 2,000 *T. ratisbonensis* spores and kept at 29°C. c, e, g, i, k: Each graphic is representative of three independent experiments.

Figure 4: Fatty acid availability in the diet determines the proliferation of *T. ratisbonensis*.

a-c, Survival (a), triglyceride quantification (b), and parasite load (c) of flies infected or not with *T. ratisbonensis* and fed on a 100 mM sucrose solution, yeast-poor food, or yeast-rich food. **d-f**, Survival (d), triglyceride quantification (e), and parasite load (f) of female or male flies infected or not with *T. ratisbonensis* and kept on yeast-rich food. a, d: survival experiments were performed on batches of 20 flies in triplicates, with mean and SD displayed on the graph; an unpaired t-test on LT50s of the three biological replicates of the indicated curve(s) with respect to controls (blue curve) was used to assess statistical significance. b, c, e, f: six batches of five flies each were analyzed; the middle bar of box plots represents the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers define the minima and maxima. Data were analyzed using two-way ANOVA. *Drosophila* flies were infected by injection of 2,000 *T. ratisbonensis* spores and kept at 29°C.

Figure 5: Phosphatidic acid is a compound scavenged by *T. ratisbonensis* that limits its proliferation in *Drosophila*.

a-d, Survival (a,c) and parasite load (b,d) of *T. ratisbonensis*-infected flies in which the expression of specific genes is reduced by transgenic RNAi driven by a ubiquitin-Gal4-Gal80^{ts} driver; a, c inset graphs display the survival of control noninfected flies in batches of 20 flies; b-d: Kruskal-Wallis analysis of the multiple comparisons demonstrated a global significance, $p=0.0023$ (b) and $p=0.0035$ (d). **e-f**, Survival (e) and parasite load (f) of flies infected with *T. ratisbonensis* and injected with a phosphatidic acid-containing solution at zero, three, and six days post-infection; controls were injected with PBS. **g**, Simplified scheme of the synthesis of fatty acids and downstream metabolites used for storage or synthesis of membrane phospholipids. All of the enzymes shown in this scheme have been tested by the RNAi approach: down-regulating their expression led either to an improved host survival to *T. ratisbonensis* challenge and a decreased parasite load (green area) or the opposite phenotype (red area). In the latter case, the decreased expression of these enzymes that use up phosphatidic acid is expected to increase the availability of this compound for the parasite. ACC: Acetyl-CoA Carboxylase; FASN1: Fatty Acid Synthase 1; GPAT4: Glycerol-3-Phosphate Acyltransferase 4; apolpp (apolipoprotein): apolipoprotein; Hsl: Hormone-sensitive lipase; Lip3: Lipase 3; AcCoAS: Acetyl Coenzyme A Synthase; Mino (Minotaur): glycerol-3-phosphate O-acyltransferase; fu12: 1-acylglycerol 3-phosphate O-acyltransferase; CdsA: CDP-diacylglycerol synthase; Lpin (Lipin): phosphatidate phosphatase; bbc (bb in a boxcar): choline/ethanolamine phosphotransferase; mdy (midway): diacylglycerol acyltransferase; colt (congested-like trachea): carnitine/acyl carnitine antiporter. TAG: triacylglycerol; DAG: diacylglycerol; FA-CoA: fatty acid CoA; LPA: lysophosphatidic acid (1-Acyl-sn-glycerol-3-phosphate). a, c, e: survival experiments were performed on batches of 20 flies in triplicates, with mean and SD displayed on the graph; an unpaired t-test on LT50s

of the three biological replicates of the indicated curve(s) with respect to controls (blue curve) was used to assess statistical significance. b, d, f: the data obtained on six batches (F: eight batches) of five flies each are shown; data on simple or pairwise comparisons after Kruskal-Wallis tests were analyzed using the Mann-Whitney statistical test. The middle bar of box plots represents the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers define the minima and maxima. *Drosophila* females were infected by injection of 2,000 *T. ratisbonensis* spores and kept at 29°C. Each graphic is representative of three independent experiments.

Figure 6: Synthetic view of the pathways beneficial or detrimental to the proliferation of *T. ratisbonensis* in *D. melanogaster*.

This scheme summarizes the findings presented in this study and highlights the central requirement of PA for parasite growth. Gene products are represented within cartouches. Cartouche and arrows in green represent the pathways used by the parasite to hijack the host of PA. When these pathways are compromised, the infection is hampered both in terms of survival and parasite burden. When the cartouche is not highlighted (Apoltp, GlyP), only the survival of infected mutant flies was enhanced whereas no significant change in parasitic titer could be measured. Cartouches and arrows in red represent host pathways with which the parasite is directly or indirectly competing. When these pathways are impaired, the flies succumb earlier to infection and display a higher parasitic burden. See also Table S1. Of note, the *CG1941* gene has not been studied in *Drosophila* so far and may fulfill a function distinct from that predicted here, in as much as two other MOGAT family members exist (*CG1942* and *CG1946*) and might play a redundant role in the gut.

CG1941: human monoacylglycerol-O-acyltransferase 2 (MOGAT2) potential homolog;
apolpp (apolipophorin): apolipoprotein; Apoltp (apolipoprotein lipid transfer particle):

775 apolipoprotein; bbc (bb in a boxcar): choline/ethanolamine phosphotransferase; Lpin (Lipin):
 776 phosphatidate phosphatase; mdy (midway) diacylglycerol acyltransferase; Lsd-2 (Lipid
 777 storage droplet-2): perilipin; Hsl: Hormone-sensitive lipase; Lip3: Lipase 3; fu12: 1-
 778 acylglycerol 3-phosphate O-acyltransferase; ACC; Acetyl-CoA Carboxylase; FASN1: Fatty
 779 Acid Synthase 1; AcCoAS: Acetyl Coenzyme A Synthase; CG5009: acyl-CoA oxidase;
 780 CG9527: acyl-CoA oxidase; colt (congested-like trachea): carnitine/acyl carnitine antiporter;
 781 CRAT: Carnitine O-Acetyl-Transferase; GlyP: Glycogen Phosphorylase; GPAT4: Glycerol-
 782 3-Phosphate Acyltransferase 4. TAG: triacylglycerol; DAG: diacylglycerol; MAG:
 783 monoacylglycerol; VLCFA: very long chain fatty acid; FA-CoA: fatty acid CoA; LPA:
 784 lysophosphatidic acid (1-Acyl-sn-glycerol-3-phosphate); PA: phosphatidic acid; PI:
 785 Phosphatidyl Inositol; PC: Phosphatidyl Choline; PE: Phosphatidyl Ethanolamine; PS:
 786 Phosphatidyl Serine.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Supplementary Information to the *Nature Microbiology* article

“Phosphatidic acid as a limiting host metabolite for the proliferation of the microsporidium *Tubulinosema ratisbonensis* in *Drosophila* flies”

by Adrien Franchet, Sebastian Niehus, Gaëtan Caravello, and Dominique Ferrandon

Supplementary Figures 1 to 9 and supplementary Tables 1 & 2

Supplementary Figure 1 | *T. ratisbonensis* is killing in a dose and temperature-dependent manner. **a, b**, Survival curves of flies infected by *T. ratisbonensis* and uninfected control flies by injection in the abdomen or the thorax (**a**) and relative parasite load measured by qPCR on six batches of five flies each (**b**). **c, d**, Survival curves of flies infected by different concentrations of *T. ratisbonensis* at 18°C (**c**) or at 25°C (**d**). **e, f**, Survival curves of *T. ratisbonensis*-infected flies transferred from 18°C to 29°C (**e**) or from 29°C to 18°C (**f**). **g**, Microbiota load monitored on ten batches of five flies each at 6 days post-infection. Data were analyzed using the Mann-Whitney statistical test. The middle bar of box plots (**b, g**) represents the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers define the minima and maxima. **h**, Survival test performed to compare normal or axenic infected and uninfected flies +/- yeast supplementation. For all survival experiments, mean and SD of three batches of 20 flies are displayed. All flies were infected by injection of 2,000 *T. ratisbonensis* spores at 29°C (except **c, e, f** as indicated; **d&h**: 25°C) and kept on standard medium. Inset graphs show the survival of PBS-injected control flies. Each graphic is representative of three independent experiments.

Supplementary Figure 2 | Physiology of flies infected by *T. ratisbonensis*.

a, b Confocal pictures of an uninfected female (**a**) and of a *T. ratisbonensis*-infected female (**b**) at 9 days post-infection. Each female is about 3 mm-long. Arrow: indirect flight muscles; arrowheads: uninfected fat body lobules (grey: cephalic, white: abdominal); °: ovaries; 4-branch star: intestine; asterisks: infected fat body lobules producing spores. **c**, Confocal pictures of infected Malpighian tubule at 6 days post-infection at 29°C. a-c: blue channel = nuclei; green channel = muscles; red channel = lipid droplets; yellow channel = parasite spores.

d, FRUMS assay: pictures of flies injected with blue dye either just after injection of the dye (top panels) or 6h thereafter (bottom panels); left panels: uninfected fly controls, right panels: 6 day-*T. ratisbonensis*-infected flies. **e**, Quantification of FRUMS assay: three batches of twenty flies were monitored every 30 minutes. **f**, Defecation assay monitored every 30 minutes on three batches of five flies. **e, f**: the mean and SD are displayed. Each graphic/picture is representative of three independent experiments.

Supplementary Figure 3 | *T. ratisonensis* alters the functions of the host fat body. **a**, Uninfected (left panel) and infected (right panel) abdominal fat body (scale bar is 200 μ m). **b**, Quantification of Nile Red signal on five samples. **c**, Infected fat body lobules (scale bar is 50 μ m (left panel) or 100 μ m (right panel)). **d**, Quantification of DAPI signal. Blue channel = nuclei; green channel = muscles; red channel = lipid droplets; yellow channel = spores. **b**, **d**: the middle bars of the box plots represent the median and the upper and lower limits of boxes indicate respectively the first and third quartiles. Data were analyzed using two-way ANOVA. **e**, Measurements of Drosomycin; **f**, Cecropin; **g**, Diptericin; **h**, Metchnikowin; **i**, Attacin; **j**, Drosocin expression by RTqPCR 24 hours after bacterial challenge on infected and uninfected flies; “clean injury” control injection of sterile PBS. x-axis represents the number of days after the initial injection of *T. ratisonensis* spores when the bacterial challenge was made. Error bars represent the standard error of the mean (SEM) of biological triplicates. AMP expression measurements were performed on 3x5 flies per condition (biological triplicates). Experiments were performed at 29°C on initially 5-7 day old female flies injected with 2,000 *T. ratisonensis* spores and are representative of three independent experiments.

Supplementary Figure 4 | *T. ratisbonensis* alters the host organelles. **a**, Confocal picture of fat body with GFP-labeled mitochondria: uninfected (left panel) and infected fly (right panel) at 9 days post-infection. **b**, Quantification of the fluorescence of the fat body tissue DAPI and GFP signals on biological quintuplicates. **c**, Confocal picture of fat body with peroxisome activity-labeled: uninfected fly at 9 days post-infection (left panel) and infected fly (right panel) at 9 days post-infection. **a**, **c**, arrowheads point to the GFP signal associated respectively to mitochondria and peroxisomes. Arrows indicate lobules in which the GFP signal has been lost. **d**, Quantification of the fluorescence of the fat body tissue DAPI and GFP signals in biological quintuplicates. **a**, **c**: scale bar 50 μ m, except right panel of (**a**): 20 μ m. **e**, Confocal picture of PLC δ -GFP-labeled membranes in infected fat body at 9 days post-infection. **f**, Confocal picture of mCD8-RFP-labeled membranes in infected fat body at 9 days post-infection. **e**, **f**: arrowheads point to host nuclei displaced to the periphery of the syncytial lobule. Blue channel = nuclei; yellow channel = spores. Experiments were performed at 29°C on 5-7 days old female flies injected with 2,000 *T. ratisbonensis* spores. Uninfected control flies were challenged with 1X PBS injection. **b**, **d**: the middle bar of box plots represent the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers represent the minima and maxima. Two-way ANOVA was used to assess the significance of the data. Each graphic/picture is representative of three independent experiments.

Supplementary Figure 5 | Access to host lipid droplets is required for *T. ratibonensis* proliferation. **a**, Survival of *T. ratibonensis*-infected flies in which the expression of *Lsd-2* is reduced by transgenic RNAi driven by a ubiquitin- Gal4- Gal80^{ts} driver. Mean and SD of three batches of 20 flies for each survival curve are shown; an unpaired t-test on LT50s of the three biological replicates of the indicated curve(s) with respect to controls (blue curve) was used to assess statistical significance. Inset shows the survival of PBS-injected control flies. **b**, Parasite load on six batches of five flies; a Mann-Whitney test was used to assess significance. **c**, Quantification of the fluorescence signal of Nile Red signal on five samples of uninfected fat body lobules (two-way ANOVA statistical analysis), as shown in **d** control and *Lsd-2* uninfected (left panels) and infected (right panels); scale bar is 15 μ m (left and right panels were taken at the same magnification). Note that lipid droplets are still detected in the infected areas of the *Lsd-2* fat bodies, as revealed by the blue DNA stain that reveals the small nuclei of meronts, whereas they have vanished in the wild-type. Each graph is representative of three independent experiments. **b**, **c**: bars represent the median and the upper and lower limits of boxes indicate respectively the first and third quartiles. Experiments were performed at 29°C on initially 5-7 day old female flies injected with 2,000 *T. ratibonensis* spores.

Supplementary Figure 6 | Lipogenesis is required in the fat body for *T. ratisbonensis* proliferation. **a**, Survival of *T. ratisbonensis*-infected flies in which the expression of *ACC* or *FASN1* is silenced by transgenic RNAi driven by a *ubiquitin*-Gal4-Gal80^{ts} driver. **b**, by a *ppl*-Gal4 driver (larval fat body). **c**, by a *yolk*-Gal4 driver (female adult fat body). **d**, by a *C564*-Gal4 driver (fat body and hemocytes). **e**, by a *how*-Gal4 driver (muscles); mean and SD of three batches of 20 flies each are displayed. Insets show the survival of PBS-injected control flies. All flies were infected by injection of 2.000 *T. ratisbonensis* spores at 29°C and kept on standard medium. Each graphic is representative of three independent experiments.

Supplementary Figure 7 | Rescue of *T. ratisthonensis* proliferation of lipogenesis-impaired mutants by fatty acid supplementation. **a**, Survival test comparing *mCherry* (control) and *ACC* silenced genes flies fed on sucrose solution supplemented or not with 100 μ M oleic acid. **b**, Relative parasite load at 6 days post-infection (flies from the experiment shown in **a**). **c**, Survival test comparing *mCherry* and *ApoLpp* silenced flies fed on a sucrose solution supplemented or not with 100 μ M oleic acid. **d**, Relative parasite load at 6 days post-infection (flies from the experiment shown in **c**). Whereas oleate can rescue the *ACC* mutant phenotype by providing dietary lipids to the fat body and thereby alleviating a requirement for fatty acid neosynthesis, it fails to rescue the *ApoLpp* mutant phenotype, consistent with an impaired transport of dietary lipids from the gut to the fat body in this mutant background. **a**, **c**: mean and SD of three batches of 20 flies are displayed. Insets show uninfected, PBS-injected, control survival experiments. Quantification of parasite loads was performed on 3x5 flies, mean and SD are shown for each condition (**b**,**d**). Experiments were performed at 29°C on initially 5-7 day old female flies injected with 2,000 *T. ratisthonensis* spores. Each graph is representative of three independent experiments.

Supplementary Figure 8 | Ingestion of phosphatidic acid does not affect *T. ratisonensis* proliferation and virulence. **a**, Survival curves of flies infected by *T. ratisonensis* fed on sucrose solution supplemented with 100 μ M phosphatidic acid; mean and SD of three batches of 20 flies are displayed. **b**, Relative parasite load measured by qPCR on eight samples of five flies each (two independent experiments with four samples each combined). The middle bar of box plots represents the median and the upper and lower limits of boxes indicate respectively the first and third quartiles; the whiskers define the minima and maxima. Experiments were performed at 29°C on initially 5-7 day old female flies injected with 2,000 *T. ratisonensis* spores. The survival experiment and parasite burden measurements were repeated with the injection of 1,500 spores and yielded similar results.

Supplementary Figure 9 | Expression of *ImpL2* and *Lip3* after *T. ratisbonensis* infection. **a, b**, Expression of *ImpL2* (**a**) or *Lipase3* (**b**) in uninfected flies or *T. ratisbonensis*-infected flies. *ImpL2* inhibits insulin-signaling by binding to insulin-like peptides. A decreased expression is therefore expected to promote anabolism by favoring insulin signaling. Gene expression was measured using RTqPCR on 4x5 flies. Data were analyzed using two-way ANOVA. Bars represent the median and the upper and lower limits of boxes indicate respectively the first and third quartiles. Experiments were performed at 29°C on initially 5-7 day old female flies injected with 2,000 *T. ratisbonensis* spores. Each graphic is representative of three independent experiments.

	#	Symbol	Annotation	Gene description	Survival test	Parasite titer	Statistical p value	Parasitic disease	Human ortholog
de novo FA synthesis	1	ACC	CG11198	Acetyl-CoA Carboxylase Alpha	+	-	**	+	ACACA
	2	AcCoAS	CG9390	Acyl-CoA Synthetase Short-Chain Family Member	+	-	**	+	ACSS2
	3	AcsI	CG8732	Acyl-CoA Synthetase Long-Chain Family Member	+	-	**	+	ACSL3
	4	FASN1	CG3523	Fatty Acid Synthase	+	-	**	+	FASN
	5	FASN2	CG3524	Fatty Acid Synthase	Ø	Ø	Ø	Ø	FASN
	6	SREBP	CG8522	Sterol Regulatory Element Binding Transcription Factor	+	Ø	Ø	Ø	SREBF2
	7	CG3961	CG3961	Acyl-CoA Synthetase Long-Chain Family Member	+	-	**	+	ACSL5
	8	CG12170	CG12170	3-Oxoacyl-ACP Synthase	+	-	**	+	OXSM
Phospholipids	9	bbc	CG6016	Choline/Ethanolamine Phosphotransferase	-	+	**	-	CEPT1
	10	bgm	CG4501	Acyl-CoA Synthetase Bubblegum Family Member	Ø	Ø	Ø	Ø	ACSBG2
	11	Dhap-at	CG4625	Glyceronephosphate O-Acyltransferase	-	Ø	Ø	Ø	GNPAT
	12	eas	CG3525	Ethanolamine Kinase	Ø	Ø	Ø	Ø	ETNK1
	13	fu12	CG17608	1-Acylglycerol-3-Phosphate O-Acyltransferase	+	-	**	+	AGPAT2
	14	Gllspla2	CG1583	Phospholipase A2 Group III	+	Ø	Ø	Ø	PLA2G3
	15	GXIVsPLA2	CG17035	Phospholipase A2 Group XIA	+	Ø	Ø	Ø	PLA2G12A
	16	Gpat4	CG3209	Glycerol-3-Phosphate Acyltransferase	+	-	*	+	GPAT4
	17	Lpin	CG8709	Phosphatidate Phosphatase	-	+	**	-	LPIN3
	18	mdy	CG31991	Diacylglycerol O-Acyltransferase	-	+	**	-	DGAT1
	19	Pect	CG5547	Phosphate Cytidyltransferase 2, Ethanolamine	Ø	-	*	Ø	PCYT2
	20	sPLA2	CG11124	Phospholipase A2 Group III	Ø	Ø	Ø	Ø	PLA2G3
	21	CG3812	CG3812	1-Acylglycerol-3-Phosphate O-Acyltransferase	Ø	-	*	Ø	AGPAT1
	22	CG4729	CG4729	1-Acylglycerol-3-Phosphate O-Acyltransferase	Ø	-	*	Ø	AGPAT3
	23	CG4753	CG4753	1-Acylglycerol-3-Phosphate O-Acyltransferase	Ø	Ø	Ø	Ø	AGPAT3
	24	CG14507	CG14507	Phospholipase A2 Group IB	+	Ø	Ø	Ø	PLA2G1B
	25	CG15450	CG15450	Glycerol-3-Phosphate Acyltransferase	Ø	Ø	Ø	Ø	GPAT3
Lipases	26	bmm	CG5295	Adipose Triglyceride Lipase	+	Ø	Ø	Ø	PNPLA2
	27	Hsl	CG11055	Lipase E, Hormone Sensitive Type	+	-	**	+	LIPE
	28	Lip1	CG7279	Lipase F, Gastric Type	Ø	Ø	Ø	Ø	LIPF
	29	Lip2	CG17116	Lipase Family Member N	Ø	Ø	Ø	Ø	LIPF
	30	Lip3	CG8823	Lipase A, Lysosomal Acid Type	+	-	**	+	LIPA
	31	Lip4	CG6113	Lipase Family Member M	Ø	Ø	Ø	Ø	LIPM
	32	CG2772	CG2772	Lipase Family Member N	+	Ø	Ø	Ø	LIPF
	33	CG6295	CG6295	Lipase I	Ø	Ø	Ø	Ø	LIPG
	34	CG10163	CG10163	Pancreatic Lipase Related Protein	-	-	*	Ø	LIPG
	35	CG31871	CG31871	Lipase Family Member M	+	Ø	Ø	Ø	LIPM
β-oxidation	36	Acox57D-d	CG9709	Acyl-CoA Oxidase	-	Ø	Ø	Ø	ACOX1
	37	Arc42	CG4703	Acyl-CoA Dehydrogenase, C-2 To C-3 Short Chain	Ø	Ø	Ø	Ø	ACADS
	38	colt	CG3057	Solute Carrier Family 25 Member 20	-	+	**	-	SLC25A20
	39	CRAT	CG1041	Carnitine O-Acetyltransferase	-	+	*	-	CRAT
	40	Mtpa	CG4389	Hydroxyacyl-CoA Dehydrogenase, Alpha Subunit	+	Ø	Ø	Ø	HADHA
	41	scu	CG7113	Hydroxysteroid 17-Beta Dehydrogenase	+	Ø	Ø	Ø	HSD17B10
	42	Thiolase	CG4581	Hydroxyacyl-CoA Dehydrogenase, Beta Subunit	Ø	Ø	Ø	Ø	HADHB
	43	whd	CG12891	Carnitine Palmitoyltransferase 1A	Ø	Ø	Ø	Ø	CPT1A
	44	yip2	CG4600	Acetyl-CoA Acyltransferase	+	Ø	Ø	Ø	ACAA2
	45	CG4335	CG4335	Trimethyllysine Hydroxylase, Epsilon	-	-	*	Ø	TMLHE
	46	CG4586	CG4586	Acyl-CoA Oxidase	Ø	Ø	Ø	Ø	ACOX1
	47	CG5009	CG5009	Acyl-CoA Oxidase	+	-	*	+	ACOX1
	48	CG5321	CG5321	Gamma-Butyrobetaine Hydroxylase	+	Ø	Ø	Ø	BBOX1
	49	CG6543	CG6543	Enoyl-CoA Hydratase, Short Chain	-	Ø	Ø	Ø	ECHS1
	50	CG7461	CG7461	Acyl-CoA Dehydrogenase, Very Long Chain	-	-	*	Ø	ACADVL
	51	CG8778	CG8778	Enoyl-CoA Hydratase Domain Containing 2	-	Ø	Ø	Ø	AUH
	52	CG9527	CG9527	Acyl-CoA Oxidase	+	-	*	+	ACOX3
	53	CG9577	CG9577	Enoyl-CoA Hydratase, Short Chain 1	+	Ø	Ø	Ø	ECH1
	54	CG10814	CG10814	Gamma-Butyrobetaine Hydroxylase	+	Ø	Ø	Ø	BBOX1
	55	CG10932	CG10932	Acetyl-CoA Acetyltransferase	Ø	Ø	Ø	Ø	ACAT1
	56	CG12262	CG12262	Acyl-CoA Dehydrogenase, C-4 To C-12 Straight Chain	+	Ø	Ø	Ø	ACADM
	57	CG17544	CG17544	Acyl-CoA Oxidase	Ø	Ø	Ø	Ø	ACOX3

Supplementary Table 1 (1/2) | List and phenotypes of the genes inhibited by RNAi for the *in vivo* screen

	#	Symbol	Annotation	Gene description	Survival test	Parasite titer	Statistical p value	Parasitic disease	Human ortholog
Fatty acids transporters	58	<i>Apolpp</i>	CG11064	Apolipoprotein B	+	-	**	+	APOB
	59	<i>Apoltp</i>	CG15828	Vitellogenin	+	Ø	Ø	Ø	APOB
	60	<i>cv-d</i>	CG31150	Vitellogenin	+	Ø	Ø	Ø	no ortholog
	61	<i>Fatp</i>	CG7400	Solute Carrier Family 27 Member	Ø	-	*	Ø	SLC27A1
	62	<i>LpR1</i>	CG31094	Very Low Density Lipoprotein Receptor	Ø	Ø	Ø	Ø	VLDLR
	63	<i>LpR2</i>	CG31092	Very Low Density Lipoprotein Receptor	Ø	Ø	Ø	Ø	VLDLR
	64	<i>LRP1</i>	CG33087	LDL Receptor Related Protein	Ø	Ø	Ø	Ø	LRP1
	65	<i>Lsd-1</i>	CG10374	Perilipin	Ø	Ø	Ø	Ø	PLIN3
	66	<i>Lsd-2</i>	CG9057	Perilipin	+	-	**	+	PLIN2
	67	<i>MTP</i>	CG9342	Microsomal Triglyceride Transfer Protein	Ø	Ø	Ø	Ø	MTTP
	68	<i>slif</i>	CG11128	Solute Carrier Family 7 Member	-	Ø	Ø	Ø	SLC7A1
	69	<i>CG1941</i>	CG1941	Monoacylglycerol O-Acyltransferase	-	+	*	-	MOGAT2
TCA cycle	70	<i>CG1942</i>	CG1942	Monoacylglycerol O-Acyltransferase	Ø	+	*	Ø	MOGAT2
	71	<i>CG1946</i>	CG1946	Monoacylglycerol O-Acyltransferase	Ø	Ø	Ø	Ø	MOGAT2
	72	<i>ATPCL</i>	CG8322	ATP Citrate Lyase	Ø	Ø	Ø	Ø	ACLY
	73	<i>Acon</i>	CG9244	Aconitase	+	-	*	+	ACO2
Glucose	74	<i>dmGlut</i>	CG5304	Solute Carrier Family 17 Member	+	Ø	Ø	Ø	SLC17A3
	75	<i>Gdh</i>	CG5320	Glutamate Dehydrogenase	Ø	-	*	Ø	GLUD1
	76	<i>ldh</i>	CG7176	Isocitrate Dehydrogenase (NADP(+))	-	-	*	Ø	IDH1
	77	<i>GlyP</i>	CG7254	Glycogen Phosphorylase	+	Ø	Ø	Ø	PYGM
Hormones	78	<i>Pgi</i>	CG8251	Glucose-6-Phosphate Isomerase	-	Ø	Ø	Ø	GPI
	79	<i>sgl</i>	CG10072	UDP-Glucose 6-Dehydrogenase	-	Ø	Ø	Ø	UGDH
	80	<i>Tpi</i>	CG2171	Triosephosphate Isomerase	-	Ø	Ø	Ø	TP11
	81	<i>CG10672</i>	CG10672	Dehydrogenase/Reductase	+	Ø	Ø	Ø	DHRS4
Hormones	82	<i>Akh</i>	CG1171	Gonadotropin Releasing Hormone	+	Ø	Ø	Ø	no ortholog
	83	<i>AkhR</i>	CG11325	Gonadotropin Releasing Hormone Receptor	-	+	*	-	GNRHR
	84	<i>conv</i>	CG8561	Leucine-Rich Repeat Protein	Ø	Ø	Ø	Ø	IGFALS
	85	<i>daw</i>	CG16987	Transforming Growth Factor Beta	+	Ø	Ø	Ø	TGFB1
	86	<i>Hr38</i>	CG1864	Nuclear Receptor Subfamily 4 Group A Member	Ø	-	*	Ø	NR4A2
	87	<i>Hr96</i>	CG11783	Nuclear Receptor Subfamily 1 Group I Member	+	Ø	Ø	Ø	NR1I2
	88	<i>lnR</i>	CG18402	Insulin Like Growth Factor 1 Receptor	-	+	*	+	IGF1R
	89	<i>Orct</i>	CG6331	Solute Carrier Family 22 Member	+	Ø	Ø	Ø	SLC22A4
Others	90	<i>AMPKα</i>	CG3051	Protein Kinase AMP-Activated Catalytic Subunit Alpha	+	Ø	Ø	Ø	PRKAA2
	91	<i>blw</i>	CG3612	ATP Synthase, Mitochondrial F1 Complex, Alpha Subunit	Ø	Ø	Ø	Ø	ATP5F1A
	92	<i>boss</i>	CG8285	G Protein-Coupled Receptor Class C Group 5 Member	+	Ø	Ø	Ø	GPRC5B
	93	<i>Desat2</i>	CG5925	Stearoyl-CoA Desaturase	+	Ø	Ø	Ø	SCD
	94	<i>Fad2</i>	CG7923	Stearoyl-CoA Desaturase	+	Ø	Ø	Ø	SCD5
	95	<i>fat-spondin</i>	CG6953	Spondin	+	Ø	Ø	Ø	SPON1
	96	<i>Pi3k59F</i>	CG5373	Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3	-	-	*	Ø	PIK3C3
	97	<i>sesB</i>	CG16944	Solute Carrier Family 25 Member	+	Ø	Ø	Ø	SLC25A4
	98	<i>CG8630</i>	CG8630	Stearoyl-CoA Desaturase	-	Ø	Ø	Ø	SCD
	99	<i>CG9743</i>	CG9743	Stearoyl-CoA Desaturase	+	-	**	+	SCD
	100	<i>CG16935</i>	CG16935	Mitochondrial Trans-2-Enoyl-CoA Reductase	+	Ø	Ø	Ø	MECR

Supplementary Table 1 (2/2) | List and phenotypes of the genes inhibited by RNAi for the *in vivo* screen. +, respectively -, in the survival test column indicates that the mutant host survives better, respectively less well, the *T. ratisbonensis* infection than the control. Ø: no significant phenotype was detected. +, respectively -, in the parasite titer indicates that the parasite burden is higher, respectively lower, in the mutant than in the control. The p-value for the parasite titer is given in the next column. The parasitic disease column highlights the function of the wild-type genes in promoting infection (green +) as the gene function favors the metabolic supplies of the parasite; red -: wild-type gene products that tend to deprive the parasite of its metabolic supplies. The genes marked with + or - in this column correspond to genes that yielded consistent mutant phenotypes between survival and parasite burden, respectively enhanced survival and decreased parasite load (+) or conversely reduced survival and increased parasite burden (-).

<i>Rpl32</i>	FW	GACGCTTCAAGGGACAGTATCTG
<i>Rpl32</i>	RV	AAACGCGGTTCTGCATGAG
<i>T. ratisbonensis</i>	FW	TCTCACAGTAGTGGCGAATG
<i>T. ratisbonensis</i>	RV	AACACCGTATTGGAATACAG
<i>Wolbachia</i>	FW	ATAGCTGGTGGTGGTGCATT
<i>Wolbachia</i>	RV	CTCCTTCAACATCAACCCTGA
<i>Nora</i>	FW	AACCTCGTAGCAATCCTCTCAAG
<i>Nora</i>	RV	TTCTTGTCCGGTGTATCCTGTATC
<i>DCV</i>	FW	TCATCGGTATGCACATTGCT
<i>DCV</i>	RV	CGCATAACCATGCTCTTCTG
<i>Drosomycin</i>	FW	CGTGAGAACCTTTTCCAATATGATG
<i>Drosomycin</i>	RV	TCCCAGGACCACCAGCAT
<i>Metchnikowin</i>	FW	CGTCACCAGGGACCCATTT
<i>Metchnikowin</i>	RV	CCGGTCTTGGTTGGTTAGGA
<i>Attacin</i>	FW	GGCCCATGCCAATTTATTCA
<i>Attacin</i>	RV	AGCAAAGACCTTGGCATCCA
<i>Cecropin</i>	FW	ACGCGTTGGTCAGCACACT
<i>Cecropin</i>	RV	ACATTGGCGGCTTGTTGAG
<i>Drosocin</i>	FW	TGAAGTTCACCATCGTTTTCTG
<i>Drosocin</i>	RV	CACCCATGGCAAAAACGC
<i>Diptericin</i>	FW	GCTGCGCAATCGCTTCTACT
<i>Diptericin</i>	RV	TGGTGGAGTGGGCTTCATG
<i>Lip3</i>	FW	GTGCCGTTTCGAGAAGTGGGCC
<i>Lip3</i>	RV	GGATTGCATCTGCTTCAAGACCTCGTC
<i>ImpL2</i>	FW	AAGAGCCGTGGACCTGGTA
<i>ImpL2</i>	RV	TTGGTGAACCTTGAGCCAGTCG
<i>D1</i>	FW	AGAGTTTGATCCTGGCTCAG
<i>D2</i>	RV	AAGCTTAAGGAGGTGATCCAGCC

Supplementary Table 2 | List of primers.