Adaptive modification of lipid droplets mediated by Plin1 functions in infection-induced pathogenesis in *Drosophila*

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ABSTRACT

Lipid droplets (LDs) are dynamic intracellular organelles critical for lipid metabolism. Alterations in the dynamics and functions of LDs during innate immune response to infections and the underlying mechanisms however, remain largely unknown. Herein, we describe the morphological dynamics of LDs in fat body of *Drosophila*, which vary between transient and sustained bacterial infections. Detailed analysis shows that *perilipin1* (*plin1*), a core gene regulating lipid metabolism of LDs is suppressed by IMD/Relish, an innate immune signaling pathway via MyD88 (MRT) /Putzig (PZG) complex. During transient immune activation, downregulated *plin1* promotes the formation of large LDs, which alleviates immune reaction-induced reactive oxygen species (ROS) stress. Thus, the growth of LDs is likely an active adaptation to maintain redox homeostasis in response to IMD activation. Whereas, under sustained inflammatory conditions, *plin1* deficiency accelerates excessive decomposition of large LDs through recruitment of Brummer/ATGL lipase resulting in energy wasting, severe lipotoxicity and then deteriorated pathogenesis. Taken together, our study provides evidence that *plin1* has a dual function on LDs' morphology in regulating infection-induced pathogenesis, and Plin1 might be a potential therapeutic target for coordinating inflammation resolution and lipid metabolism.

INTRODUCTION

Immune activation is essentially accompanied by metabolic reprogramming, which redistributes accessible energy to prioritize immune protection against pathogenic infections (Hotamisligil, 2017; O'Neill et al., 2016). Thus, stringent regulation of

metabolic machinery in response to immunoreaction is critical for the host fitness. Unlike transient infection, sustained infection is often associated with chronic pathogenesis, which subsequently contributes to metabolic disorders, a major threat to individual health. Besides carbohydrates, lipids provide another important bioenergetic and synthetic resource to the host. A number of lipid metabolites have been reported to play key roles in pro- or anti-inflammatory pathways (Arita, 2012; Serhan et al., 2014; Walpole GFW, 2018). However, the dynamics and differences between lipid metabolism under transient and sustained inflammatory conditions are barely described thus far.

Lipid droplets (LDs) are important intracellular organelles in all eukaryotic and some prokaryotic cells (Murphy, 2001), providing a major place for lipid synthesis, lysis, transfer and storage. These organelles contain a phospholipid monolayer surrounding neutral lipids, such as di/triacylglycerols or sterol esters (Tauchi-Sato et al., 2002). Since LDs function mainly through storing and providing energy (Walther and Farese, 2012), they are apparently involved in multiple physiological and pathological processes in the host. Emerging evidences show that LDs are responsive to ER stress (Fu et al., 2011), buffer starvation stress (Velazquez et al., 2016) and play key roles in diseases such as diabetes and cancer (Cohen JC, 2011; Greenberg et al., 2011). Most intriguingly, LDs also participate in immune regulation including modulation of myeloid cell functions (den Brok et al., 2018), combating pathogenic bacterial infections, being exploited by virus and so on (Saka and Valdivia, 2012; Vallochi et al.,

2018; Welte, 2015). The number, size and anchored proteins of LDs are dynamic and change during infection or inflammation (Harsh et al., 2019; Henne et al., 2018; Menon et al., 2019), making it difficult to predict LDs' role as pro- or anti-inflammatory modulators(Pereira-Dutra et al., 2019). It indicates that the status of LDs should be tightly controlled. Defects in the biogenesis and mobilization of LDs result in free fatty acid induced lipotoxicity(Ertunc and Hotamisligil, 2016; Listenberger et al., 2003) or even the accumulation of pro-inflammatory mediators(Feldstein et al., 2004; Summers, 2006), all of which lead to metaflammation accompanied by organelles dysfunction, secondary inflammatory responses and exacerbated metabolic dysbiosis (Ertunc and Hotamisligil, 2016). However, the role and dynamic pattern of LDs during immune activation still remains obscure. Especially, the factors mediating this immunometabolic switches of LDs have not been well identified.

Drosophila melanogaster has emerged as a productive organism to investigate immunometabolism, due to the advantages of powerful genetic manipulation and highly conserved mechanisms in both innate immunity and metabolism (Heier and Kuhnlein, 2018; Lemaitre and Hoffmann, 2007; Myllymaki et al., 2014). Especially, the fat body (analogous to human liver and adipose tissue) is an ideal place for studying the interaction between LDs' metabolism and inflammation, due to its richness in LDs and role as a major organ mediating systemic innate immunity(Arrese and Soulages, 2010; Kleino and Silverman, 2014). LDs are non-homogenous organelles, which accommodates hundreds of variable proteins (Beller M et al., 2006; Guo Y et al., 2008),

including Perilipins (Plins), a group of constitutive proteins that span the surface of LDs (Itabe et al., 2017; Kimmel and Sztalryd, 2016). There are two Plins in *Drosophila*, Lipid storage droplet-1 (*lsd1*, homologous to human PLIN1) and *lsd2* (homologous to human PLIN2) (Bickel et al., 2009). Plin2 acts to promote lipid storage and LDs' growth as a barrier for lipase (Fauny et al., 2005; Grönke et al., 2003; Teixeira et al., 2003), while Plin1 modulates protein flux on LDs (Beller et al., 2010; Bi et al., 2012). In human or mouse adipocyte tissue, PLIN1 deficiency leads to uncontrollable LDs lipolysis and infiltration of inflammatory cells (Gandotra S, 2011; Sohn et al., 2018; Tansey et al., 2001). Inhibition of lipases, such as adipose triglyceride lipase (ATGL) or hormone-sensitive lipase (HSL), can alleviate this metaflammation (Cani et al., 2007; Schweiger et al., 2017). It also indicates a link between immunometabolism and LDs. However, the function of LDs and the underlying mechanisms involving Plins in response to infection are still poorly understood.

In this study, the dynamic switches in morphology and number of LDs during transient and sustained inflammation, induced either by bacterial infection or genetic manipulation, were compared. Plin1 was found to play a dual role in regulating LDs' morphology under different inflammatory conditions. Our data reveal that adaptive modification of LDs acts as a potential modulator of infection-induced pathogenesis.

Results

Immune activation modulates lipid metabolism, and particularly alters morphology of lipid droplets (LDs) in the fat body.

113 In *Drosophila*, the fat body is not only a central organ mediating systemic immune
114 responses, but also the epicenter for lipid metabolism. Thus, to decipher the mechanistic
115 connections between innate immunity and lipid metabolism, triglycerides (TAGs)
116 kinetics was tested in the fat body of *Drosophila* after systemic infection. Two bacterial
117 species were used to perform nano-injection to infect adult fruit flies. One being the
118 Gram-negative bacterium, *Escherichia. Coli* (*E. coli*), which is non-pathogenic to flies
119 and its infection results in a transient innate immune response within 48 hour post
120 infection (hpi)(Vodovar et al., 2005). Whereas, the Gram-negative bacterium,
121 *Salmonella. Typhimurium* (*S. typhimurium*), which is a deadly pathogen for flies, was
122 used to induce sustained intracellular infection that triggers a prolonged inflammatory
123 response in *Drosophila*(Brandt et al., 2004). The immune deficiency (IMD) pathway is
124 a dominant innate immune singling against Gram-negative bacterial infections that
125 regulates Relish/NF-κB-dependent transcription of AMPs, such as *Diptericin* (*Dpt*)
126 (Hoffmann JA., 2003; JA., 2003; Kaneko T, 2004; Lemaitre and Hoffmann, 2007;
127 Lemaitre, 2007). Thus, by measuring the expression level of *Dpt*, IMD signaling
128 activity could be monitored (Leulier et al., 2003; Neyen et al., 2016). Infection by either
129 bacterial strains led to a gradient increase in IMD activity in the fly fat body from 0 hpi
130 to 12 hpi. However, the transient immune response induced by *E. coli* infection
131 subsided to the basal level after 48 hpi (**Fig.1A**), while activation of IMD continued to
132 increase in case of *Salmonella* infection (**Fig. 1B**). Interestingly, compared to mock
133 injection control (**Supplementary Fig. S1A**), the TAGs level in the fat body of flies
134 with *E.coli* infection steadily increased from 6 hpi to 16 hpi but then declined at 24 hpi

and almost recovered after 48 hpi (**Fig. 1A**). In contrast, after *S. typhimurium* infection, TAGs levels continuous decreased in the fat body, which was in negative correlation with the progressive increase in IMD activity (**Fig. 1B**). Moreover, a systemic decrease of TAGs levels was observed in the fly body after *E.coli* infection (**Supplementary Fig. S1B**), suggesting a preferred lipid import rather than *de novo* fatty acid synthesis in the fat body from 0 hpi to 12 hpi. This data is consistent with previous studies showing transcriptional levels of most triglyceride synthesis genes are suppressed during the initial phase of infection (Clark et al., 2013; Dionne et al., 2006). While in case of sustained infection by *S. typhimurium*, TAGs levels continuously decreased in the whole body (**Supplementary Fig. 1C**) similar to that observed in the fat body (**Fig. 1B**). Thus, these results indicate a link between lipid metabolism and IMD signaling activation.

LDs are the main site for lipid anabolism, catabolism and mobilization(Kühnlein, 2012), which prompted us to investigate whether the morphology of LDs in the fat body responds to bacterial infection. BODIPY staining of fat body cells revealed that compared to PBS injection group (**Fig. 1C and 1C1**), both *E. coli* and *S. typhimurium* infection increased the percentage of intracellular small LDs (diameter < 2 μ m) at 6 hpi (**Fig. 1C and 1C2- C3**). In case of *E. coli* infection, however, LDs grew bigger at 16 hpi as indicated by the decrease in the percentage small LDs and concurrent increase in large LDs (diameter > 4 μ m), and then, this size distribution of LDs was restored to basal levels at 24 hpi (**Fig. 1C and 1C2**). But after *S. typhimurium* infection, the

percentage of small LDs only kept on increasing (**Fig. 1C and 1C3**). Similar to the changes observed in TAGs levels, the average size of LDs in fat body cells increased till 16 h and then decreased at 24 h post *E. coli* infection, while a continuous decrease was noticed in *S. typhimurium* infection (**Supplementary Fig. S1D**). These results indicate that small LDs are prone to fuse into bigger ones during the initial 16 h of *E. coli* infection. On the contrary, large LDs tend to disintegrate during *S. typhimurium* infection (**Fig. 1C and Supplementary Fig. S1D**). Together, these results suggest that the morphology of LDs in the fat body changed during bacterial infections and were distinct for transient or sustained immune activation.

IMD signaling activation sufficiently modifies the morphology of LD.

To determine whether the IMD activation rather than the potential bacterial effects are responsible for the modification of LDs during infection, a fat body-specific RU486-inducible GAL4 driver (*GSI06-GAL4*) (Roman et al., 2001) was applied to detect the relationship between IMD signaling and LDs' patterns in the fat body. Ectopic expression of either different isoforms of *PGRP-LC* (*PGRP-LCx* or *PGRP-LCa*), the receptor for IMD signaling pathway (Choe et al., 2002; He et al., 2017; Yang et al., 2019), or the N-terminal of *Relish* (*Rel.68*), the *Drosophila* NF- κ B factor (Wiklund et al., 2009), resulted in constitutive activation of IMD signaling (**Supplementary Fig. S1E-G**). IMD activation by *GSI06-GAL4* in the fat body led to an increase in TAGs levels in the fat body at 12 h after RU486 treatment (**Fig. 1D and 1E**), simultaneously, the TAGs level of whole body didn't change within 12 hpi (**Supplementary Fig. S1H-I**). These results mimicked the phenotypes observed in the early stages (within 12 hours)

of transient infection by *E. coli*. However, the elevated levels of TAGs in fat bodies of WT flies disappeared in flies with mutation along IMD signaling pathway (*imd^l*, *PGRP-LC^{Δ5}* and *relish^{E20}*) at 12h post injection with heat-killed *E. coli* (These immune deficient flies died too quickly when primed by live bacteria.) (**Fig. 1F and Supplementary Fig. S1J**). Furthermore, IMD signaling deficiency also restricted the increase in LDs size at 16 hpi, compared to WT controls (**Supplementary Fig. S1K-L**). Whereas, prolonged hyper-activation of IMD signaling induced by RU486 (after 24 hpi) led to a dramatic decrease in TAGs levels in both fat body and the whole body (**Fig. 1D-E and Supplementary Fig. S1H-I**), accompanied by a decrease in both the percentage of large LDs (**Fig. 1G and 1G1-G3**) and the average LDs size in the fat body (**Fig. 1H**), as observed in sustained infection by *S. typhimurium*. Therefore, these results suggest that IMD activation is necessary and sufficient to modify LDs.

***plin1* is involved in LDs modification induced by IMD activation.**

To explore the underlying mechanisms of LDs' metabolism in response to immune activation, genes involved in the regulation of LDs (Beller et al., 2010; Grönke et al., 2003; Heier and Kuhnlein, 2018; Kuhnlein, 2012) were tested in the fat body by real-time PCR. After comprehensive analysis of mRNA expression, only four genes, *APGAT2*, *DGAT2*, *Rfabg* and *plin1* showed opposite transcriptional trends (from 4 hpi to 12 hpi) in transient infection by *E. coli* compared to sustained infection by *S. typhimurium* (**Supplementary Fig. S2A and S2B**). Furthermore, we noticed maximum reduction in the expression of *plin1* in the fat body, in which IMD was overactivated through overexpressing *Rel.68* by fat body-specific driver *ppl-GAL4* (**Supplementary**

Fig. S2C). These results indicate a potential role of *plin1* in the regulation of LDs' metabolism accompanied by IMD activation.

Interestingly, similar to the correlation between TAGs level and IMD activity observed in the fat body during bacterial infection of flies (**Fig. 1A**), transient infection of *E. coli* induced a significant downregulation of *plin1* mRNA levels at 4 hpi, which was then restored to basal levels at 24 hpi (**Fig. 2A**). While prolonged activation of IMD signaling either by sustained intracellular *S. typhimurium* infection (**Fig. 2B**), or by RU486 treatment in fat body-specific GS flies (**Fig. 2C**), led to continuous reduction in *plin1* expression. Martik (MRT) /Putzig (PZG) complex, a chromosome remodeling complex, has been reported to suppress *plin1* at transcriptional level (Yao et al., 2018). Intriguingly, mRNA levels of both *mrt* and *pzg* were upregulated in the fat body after bacterial infection (**Fig. 2D and Supplementary Fig. S3A**). Interestingly, homologous alignment showed that at least one conserved binding motif of Relish existed in the promoter region of both *mrt* and *pzg* genes across *Drosophila* species with different evolutionary ages (**Supplementary Fig. S3B and S3C**). This implies a potential regulation of these genes by IMD/Relish. Peptidoglycan (PGN) derived from gram-negative bacteria can activate IMD signaling in *Drosophila* S2* cells *in vitro* (Kaneko T, 2004). Indeed, luciferase activity controlled by the promoter of *mrt* or *pzg* was significantly enhanced in S2* cells upon PGN treatment, which was blocked by the knockdown of Relish using dsRNA (He et al., 2017) (**Fig. 2E and Supplementary Fig. S3D**). Additionally, two Relish binding motifs in truncated *mrt* promoter region (T-

mrt(Rel), -870 to +1bp) were critical for *mrt* transcription (**Fig. 2F and Supplementary Fig. S3E**), because PGN treatment didn't enhance T-*mrt*-Luc activity any further when these two sites were removed (**Fig. 2F**). Thus, these results suggest that suppression of *plin1* by IMD signaling might be through upregulation of *mrt*/p_zg. Consistent with previous studies (Beller et al., 2010; Bi et al., 2012), *plin1* deficiency by mutation (*plin1*³⁸) or fat body specific knockdown (*UAS-plin1 RNAi* driven by *ppl-GAL4*) promoted the formation of large LDs (**Fig. 2G**). In contrast, ectopic expression of *plin1* in the fat body enhanced lipid mobilization and inhibited LD coalescence (Yao et al., 2018), leading to the accumulation of small LDs (**Fig. 2G**). All together, these results provide explanation for LDs' growth in the early stages of transient IMD activation.

Sustained immune activation accelerates lipolysis of large LDs in a Bmm/ATGL-dependent manner.

Unlike transient IMD activation, which promoted LDs' growth (**Fig. 1C and Supplementary Fig. S1D**), sustained IMD hyper-activation in the fat body, either driven by *ppl-GAL4* (**Fig. 3A**) or RU486 inducible *GS106-GAL4* for 5 days (**Fig. 3B and 3B1**), accelerated large LDs breakdown and small LDs generation. Simultaneous knockout (**Fig. 3A**) or knockdown (**Fig. 3B**) of *plin1* in the fat body induced the formation of large LDs (**Fig. 3B1**), albeit the average size of these LDs was smaller than that of flies with *plin1* deficiency alone (**Fig 2G**), but bigger than RU486-untreated controls(**Fig. 3B and 3B1**). Although these results confirmed the role of Plin1 in reducing LDs' growth, we questioned, why IMD-induced autonomous downregulation

of *plin1* was unable to overcome LDs' breakdown in prolonged immune activation conditions. Thus, the long-time course of LDs' morphological change was traced in the fat body of flies with IMD overactivation (*ppl-Gal4>Rel.68*). Although *plin1* deficiency made *ppl-Gal4>Rel.68;plin1³⁸* flies develop much larger LDs than that of *ppl-gal4>Rel.68* at the first week after eclosion, these larger LDs experienced much faster disintegration rate (**Fig. 3C**). Intriguingly, irrespective of the distribution of LDs size (**Fig. 3C1-C2**) or the average LDs size (**Fig. 3D**), there were no significant differences in the fat body of *ppl-GAL4>Rel.68* and *ppl-GAL4>Rel68;plin1³⁸* flies since the second week. Therefore, these results suggest that decomposition of LDs occurs quickly and preferably on large LDs during sustained immune activation.

Persistent inflammation always over-consumes energy, most of which is produced by lipolysis. Indeed, sustained IMD activation in the fat body (*ppl-GAL4>Rel.68*) enhanced the decrease in TAGs levels in the whole body, which interestingly became much faster in the absence of *plin1* (*ppl-GAL4>Rel68;plin1³⁸*) (**Supplementary Fig. S4A**). For further confirmation, these flies were exposed to starvation, a much more sensitive condition. *plin1* mutation dramatically enhanced TAGs consumption rate in *ppl-GAL4>Rel.68;plin1³⁸* flies since the beginning of starvation, compared to *ppl-GAL4>Rel.68* flies (**Fig. 3E**). Consequently, flies with a higher TAGs consumption rate were more sensitive to starvation (**Fig. 3F**). A previous study had suggested that LDs lipolysis is predominately mediated by Brummer (Bmm), the *Drosophila* homolog of mammalian adipose TAG lipase (ATGL)(Grönke S, 2007; Gronke et al., 2005). Fat

body-specific knockdown of *Bmm* effectively blocked the quick decomposition of large LDs in the fat body of both *ppl-GAL4>Rel.68* and *ppl-GAL4>Rel68;plin1³⁸* flies (**Fig. 3G**), and further promoted the LDs growth in the fat body (**Fig. 3H**) as well as TAGs content of the whole body (**Supplementary Fig. S4B**). Next, *Bmm-GFP* knock-in allele was used to detect the localization of Bmm on LDs under normal and IMD-activated conditions. Compared to control, IMD activation by fat body-specific overexpression of *Rel.68* significantly enhanced the Bmm-GFP abundance on the surface of LDs. It's worthy to note that Bmm-GFP signals were largely concentrated in the contact site between LDs, a sign of overactive lipolysis (Gronke et al., 2005) (**Fig. 3I**). Importantly, further removing Plin1 led to a much more recruitment of Bmm-GFP (**Fig. 3I and 3J**). Together, these results suggest that sustained IMD activation enhanced Bmm/ATGL-mediated lipolysis of large LDs.

***plin1* compromises host protection against bacterial infection.**

Naturally, whether *plin1*-mediated LDs modification participated in the regulation of immune function was tested next. Compared to genetic controls, either *plin1* deficiency (*plin1³⁸*) (**Fig. 4A and 4B**) or fat body-specific knockdown of *plin1* (*ppl-GAL4>UAS-plni1RNAi*) (**Fig. 4C and 4D**) prolonged the survival rate and reduced bacterial loads (colony-forming units, CFUs) after *S. typhimurium* systemic infection, indicative of enhanced resistance against bacterial infection. Conversely, ectopic expression of *plin1* in the fat body (*ppl-GAL4>UAS-plin1*) led to a dramatic increase in mortality rate of flies infected with *S. typhimurium* (**Fig. 4E** , Reducing infection OD because O.E.*plin1* flies died too quickly.), or even by non-pathogenic *E. coli* (**Fig. 4G**), possibly due to

uncontrolled bacterial growth (**Fig. 4F and 4H**). To exclude the potential effects of *plin1* on the development of flies, RU486 induced fat body-specific GAL4 (*GS106-GAL4*) was used after fly eclosion. After RU486 treatment for 3 days, a significant decrease or increase in LDs size by overexpression or knockdown of *plin1* respectively, was clearly observed in the fat body (**Fig. 4I**). Subsequently, flies were challenged with *S. typhimurium* and as expected, downregulation of *plin1* prolonged the survival rate, while upregulation of *plin1* shortened the life span and elevated bacterial loads in the *S. typhimurium* infected flies (**Fig. 4J and 4K**). However, deficiency of *plin1* did not affect anti-microbial peptides (AMPs) (*Diptericin*, *Dpt*; *AttacinA*, *AttA*) response upon *E. coli* infection (**Supplementary Fig. S5A**), but specifically improved *Dpt* expression upon *S. typhimurium* infection (**Supplementary Fig. S5B**). Interestingly, overexpression of *plin1* dampened AMPs response in both *E. coli* and *S. typhimurium* infections (**Supplementary Fig. S5C and S5D**). Taken together, these results suggest that adaptive downregulation of *plin1* in response to IMD signaling activation protected the host against bacterial infections.

Large LDs relieves oxidative stress associated with immune activation.

Lipid metabolic disorder usually follows sustained inflammatory response hallmarked by excessive lipolysis-induced lipotoxicity (Blaser et al., 2016; Grisouard et al., 2012; Morgan and Liu, 2011; Zu et al., 2009), which is a causative factor for reactive oxygen species (ROS)-related tissue damage (Ertunc and Hotamisligil, 2016; Herms et al., 2013; Listenberger et al., 2003). Since LDs are major hubs for lipid metabolism in fat body cells, it promoted us to investigate whether *plin1*-mediated LDs modification during

immune response regulates intracellular redox homeostasis. A transgenic allele with a *gstD-GFP* insertion was utilized to monitor ROS activity *in vivo* by measuring GFP intensity (Sykietis and Bohmann, 2008). Transient infection by *E.coli* only induced a slight and temporary elevation of ROS levels in the fat body at 16 hpi. However, *S. typhimurium* infection resulted in a continuous and strong increase in intracellular ROS levels in fat bodies (**Fig. 5A and 5A1-A2**). Additionally, fluorescent probe 2',7'-dichlorofluorescein diacetate (DCFH-DA) was also used to evaluate intracellular ROS levels. DCFH-DA staining showed that sustained IMD activation induced by RU486 feeding for 5 days caused a significant increase in ROS levels in the fat body of *GS106-GAL4>PGRP-LC (LCa or LCx)* and *GS106-GAL4>Rel.68* flies (**Fig. 5B and 5B1-B3**). These results suggest a link between sustained IMD activation and accumulation of intracellular oxidative stress. To determine whether immune activation induced-oxidative stress affects the host against bacterial infections, N-acetylcysteine (NAC), a widely-used ROS scavenger, was fed to flies after *S. typhimurium* infection. Feeding flies with NAC specifically at 12 h, not 0h, post *S. typhimurium* infection, a time point when excessive ROS accumulation has already developed (**Fig. 5A**), significantly improved the survival of flies, compared to non-feeding controls (**Fig. 5C**). These results suggest that oxidative stress, which develops during sustained immune response is harmful for the fly after bacterial infection.

Strikingly, data correlation analysis seemed reveal a positive correlation between the continuously increasing percentages of small LDs (< 2 μ m) and the intensity of ROS

accumulation in fat body cells after *S. typhimurium* infection (**Fig. 5D**). Considering the phenotypes of low ROS intensity and large LDs formation in WT flies during transient infection by *E. coli* (**Fig. 5A and Fig. 1C**), whether large LDs have the ability to resist intracellular oxidative stress was determined next. *plin1* deficiency (*plin1*³⁸) or knockdown (*UAS-plin1RNAi* driven by *ppl-GAL4*), which promoted LDs growth, contained a lower level of ROS than that of control (**Fig. 5E and 5E1-E2**). In contrast, overexpression of *plin1* (*ppl-GAL4>plin1*), which transformed LDs into smaller ones, markedly increased ROS intensity (**Fig. 5E and 5E2**). Similar phenotypes were observed in fat body-specific *GS106-GAL4* flies after RU486 induction (**Fig. 5F and 5F1-F2**). Intriguingly, further removing *plin1* from *ppl-GAL4>Rel.68* flies significantly ameliorated ROS accumulation in the fat body in this sustained IMD activated condition (**Fig. 5G and 5G1**). Importantly, deficiency of *plin1* significantly prolonged the survival of *ppl-GAL4>UAS-Rel.68* flies after *S. typhimurium* infection, with the note that flies with IMD hyper-activation alone (*ppl-GAL4>UAS-Rel.68*) were more susceptible to *S. typhimurium* infection than its genetic controls (**Fig. 5H**). In addition, blocking the breakdown of large LDs associated with IMD overactivation by knockdown of *Bmm* in the fat body, strikingly benefited the survival of *ppl-GAL4>Rel.68* flies, even without infection (**Fig. 5I**). Therefore, these results suggest that large LDs formation contribute to alleviate the intracellular oxidative stress induced by IMD activation and Plin1 might serve as an important linker to respond to IMD signaling and modify LDs.

To further investigate the antioxidative role of LDs, the morphology of LDs in fat body cells was examined in flies with skewed ROS metabolism. In *Drosophila*, superoxide dismutase genes (*sod1/sod2*) or catalase gene (*cat*) encode enzymes for intracellular ROS clearance (C M Griswold et al., 1993; J P Phillips et al., 1989). Knockdown of either *sod1*, *sod2* or *cat* led to excessive ROS accumulation and significantly promoted LDs growth (**Fig. 6A and 6B**). Interestingly, once these flies were fed with NAC to scavenge intracellular ROS, the morphology and the average size of LDs almost reverted to control levels (**Fig. 6A and 6B**). Furthermore, simultaneous overexpression of *plin1* or *Bmm* in the fat body sufficiently blocked the formation of large LDs in *ppl-GAL4>UAS-sod1-RNAi*, *sod2-RNAi* or *cat-RNAi* genetic background (**Fig. 6C and 6D**), and as expected led to increased ROS accumulation than controls (**Fig. 6E and 6F**). Taken together, these results suggest that adaptive LDs growth may benefit flies to resist oxidative stress.

Discussion

Metabolic reprogramming of lipids has been widely reported to be associated with immune responses (Buck et al., 2017; Hotamisligil, 2017; O'Neill et al., 2016). As a major intracellular organelle for lipid metabolism and storage, LDs also seem to be involved in immune processes. Immune stimulation either by infection with bacteria (D'Avila et al., 2006; Peyron et al., 2008), virus (Barba G, 1997; Hope et al., 2002; Samsa et al., 2009), fungus (Sorgi et al., 2009) or protozoan parasites (Vallochi et al., 2018), or by cytokines inoculation (Bandeira-Melo et al., 2001; Pacheco P et al., 2002)

may promote the biogenesis of LDs in mammalian leukocytes. Recently, Hash et al also confirmed that LDs are infection-inducible organelles in the gut of *Drosophila* at a certain timepoint after infection (Harsh et al., 2019). However, the status and morphology of LDs change rapidly *in vivo*. This dynamic transformation of LDs during immune response, and their possible link to regulation of pathogenesis is rarely described.

In this study (**Fig.7**), we carefully traced the time-course morphogenesis of LDs in the fat body beside the dynamic curve of IMD signaling activity. We found that transient IMD activation (within 12hpi) by non-pathogenic bacterial infection promoted LDs' growth in the fat body. The LDs' size and TAGs levels in the fat body was maximum when IMD activity achieved its peak. However, the TAGs level in the whole body decreased around that period, suggesting that the substrates for LDs' biogenesis in the fat body were probably imported lipids rather than *de novo* synthesized fatty acids, and IMD signaling activation is required for this process. Detailed analysis showed that *plin1* downregulation is critical for LDs' growth in response to transient IMD activation, considering its expression was suppressed by IMD/Relish activated MRT/PZG complex. These findings indicate that LDs' biogenesis is likely an active host adaptation to immune challenges. To further support this hypothesis, we found that enlarged LDs benefit the host against intracellular ROS-mediated oxidative stress associated to IMD activation. Excessive ROS accumulation is often the main cause of inflammation/infection-induced cellular damages. A similar antioxidant function of

LDs were also reported in neuronal stem cell niche(Bailey et al., 2015) and in cancer cells (Bensaad et al., 2014). However, the detailed mechanisms how large LDs prevent ROS accumulation needs further investigation. One possibility is that biological processes such as cancer, neural activity, and inflammation are energy-intensive, rely on robust fat metabolism, which releases large amounts of free fatty acids. The oxidation of free fatty acids generates ROS and the growth of LDs efficiently sequester these excessive free lipids. Moreover, large LDs' formation can reduce the opportunity of pathogens to utilize free fatty acids for their own growth(Arena et al., 2011; LaRock et al., 2015; Narayanan and Edelman, 2014). This is possibly the reason why *plin1* deficient flies, owning bigger LDs, had lower bacterial loads after infection. In addition, larger LDs might contain more resident histones, a cationic protein, which has been reported to kill bacteria in a previous study(Anand P et al., 2012). In mammals, IFN- γ treatment of *M. tuberculosis* infected bone-marrow derived macrophage (BMDM) can induce the formation of LDs, in which neutral lipids serve as a source to produce eicosanoids for enhancing host defense (Knight et al., 2018). Thus, LDs' growth is beneficial for both redox homeostasis and to combat infection. The downregulation of Plin1 to promote the enlargement of LDs might be an effective host adaptation to resolve inflammation-associated stress in response to immune activation.

However, during sustained pathogenesis, induced either by pathogenic bacterial infections or hyper-activation of IMD signaling through genetic manipulation, progressively smaller LDs were observed. We speculate that LDs' growth by

426 autonomous downregulation of endogenous *plin1* in response to IMD activation was
 427 unable to overcome LDs' breakdown due to robust lipolysis in high energy-consuming
 428 hyperinflammation. Consistent with this idea, the levels of TAGs in the whole body
 429 was continuously decreased. Interestingly and impressively, even though the mutation
 430 of *plin1* in flies with IMD overactivation enlarged LDs initially, the disintegration of
 431 LDs in these flies was rather faster than flies with IMD hyper-activation alone. Protein
 432 translocation assay confirmed that *plin1* deficiency increased the recruitment of the key
 433 rate-limiting lipase, Bmm/ATGL, on the surface of LDs in sustained IMD
 434 overactivation condition. Furthermore, a recent finding showing a higher rate of ATGL-
 435 mediated lipolysis in the adipose tissue of *Plin1*^{-/-} mice (Sohn et al., 2018), supports our
 436 notion that Bmm/ATGL-mediated lipolysis preferentially occurs in *plin1* deficiency-
 437 induced large LDs. As we mentioned above, hyperinflammation leads to excess
 438 accumulation of free fatty acids in the cytoplasm, which promote lipotoxicity and ROS-
 439 induced oxidative stress(Aitken et al., 2006; Koppers et al., 2010; Song et al., 2014).
 440 The high levels of intracellular ROS can further promote lipolysis and free fatty acids
 441 release(Krawczyk SA et al., 2012). This vicious circle finally drives the host to enter a
 442 severe metaflammatory state during chronic hyperinflammation, and consequently
 443 shorten lifespan. A recent study showed that renal purge of hemolymphatic lipids can
 444 efficiently prevent ROS-mediated tissue damage during inflammation(Li et al., 2020).
 445 In our study, knockdown of Bmm, to prevent free lipids generation, also prolonged the
 446 lifespan of flies with hyper-inflammation. Thus, although the growth of LDs was driven
 447 by *plin1* downregulation is an active protection mechanism, high energy wasting under

severe pathological conditions is more powerful to consume these large LDs very quickly. Instead, *plin1* mutation seems to enhance the breakdown of large LDs in this case. However, the mechanism of lipase recruitment on large LDs in response to hyper-inflammation, and the involvement of Plin1 require further investigation.

Plin1 is an important protein factor on the surface of LDs. It has been reported to control the mobilization of lipids on LDs surface by recruiting kinds of enzymes (Gandotra et al., 2011; Sztalryd et al., 2003). In this study, we found that *plin1* expression is also regulated by innate immune signaling. This provokes us to conceive that Plin1 may serve as a bridge to link immunity and lipid metabolism through modification of LDs. In response to transient immune activation, adaptative enlarged LDs benefit the host against inflammation-induced stress; while under prolonged hyper-immune activation, large LDs decompose quickly and enhance lipolysis, thus aggravating pathogenesis. The dynamic morphogenesis of LDs under these two conditions are distinctly different, even though both scenarios involve Plin1. It is worthy in the future to trace and dissect the dynamic protein compositions on the surface of LDs along the different stages of inflammation, especially the ones that interact with Plin1. In summary, we found that the homeostasis of LDs' morphology is critical in regulating pathogenesis during infection. Thus, regulation of LDs may provide a potential therapeutic target for resolution of inflammation.

Materials and Methods

Detailed information on *Drosophila* stocks and bacterial strain; cloning and double-strand RNAs; infection and survival rate counting; lifespan counting; bacterial loads assay; cell culture, transfection and luciferase assay; qRT-PCR; lipid droplet staining and counting; TAG assay; RU486 treatment; ROS detection; starvation test; microscopy and software; statistical analyses are described in the Supplemental Experimental Procedures.

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MAIN FIGURES AND LEGENDS

Including seven main figures.

Figure 1

A

B

C

C1

C2

C3

D

E

F

G

G1

G2

G3

H

Fig. 1. IMD signaling activation switches lipid metabolism and LDs morphology in the fat body. **(A and B)** Relative *Dptericin(Dpt)* mRNA expression and TAGs level in the fat body of wild type flies at indicated time points post *E. coli* **(A)** and *S. typhimurium* **(B)** infection. The mean values of *Dpt* mRNA expression or TAGs level were connected by dash line. The fold change of mRNA expression was normalized to that of 0 h and four independent repeats (n =20 flies per repeat) were performed at each time point. Total TAGs level of 25 flies' fat body tissues was quantified in six biological replicates at each time point. **(C)** BODIPY staining (green) of LDs in the fat body of wild type flies at indicated time points post *E. coli* (upper panel) and *S. typhimurium* (lower panel) infection. Nuclei of fat body cells were stained with DAPI (blue). Scale bar: 10 μ m. The corresponding statistics of the distribution of LDs' size was shown in **(C1)** for *E. coli* infection and in **(C2)** for *S. typhimurium* infection (n =30 cells for each time point). Eight fat bodies were examined for each time point. **(D and E)** Relative changes of TAGs level in the fat body of *GS106-GAL4> PGRP-LCx* **(D)** and *GS106-GAL4> PGRP-LCa* **(E)** flies after RU486 treatment for indicated time. The change of TAG levels was normalized to that of 0h without RU486 treatment. Six independent repeats (n =25 flies per repeat) at each time point were performed. **(F)** Relative TAGs level in the fat body of wild type and IMD pathway mutants (*Relish*, *PGRP-LC* and *Imd*). Each value of TAGs level was normalized to that of 0h of wild type. Each data contains four independent repeats (25 flies' fat body tissues per repeat). **(G)** BODIPY staining (green) of LDs in the fat body of indicated flies after 5 days with (lower panel) or without (upper panel) RU486 treatment **(G)**. Nuclei of fat body cells were stained with DAPI (blue). Scale bar: 20 μ m. The corresponding statistics of the distribution of LDs' size was shown in **(G1)** for *GS106-GAL4> Rel.68* flies, in **(G2)** for *GS106-GAL4> PGRP-LCx* flies and in **(G3)** for *GS106-GAL4> PGRP-LCa* flies (n =30 cells in each genotype). Eight fat bodies were examined for each sample. **(H)** The statistics of LDs size (n =30 cells) in fat body cells of indicated flies after RU486 treatment for 5 days. Each scattering dot represents the data from one fat body cell. Error bars represent the mean $\pm$ s.d. **(A-B, D-F)** and mean with range **(H)**. Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test **(A-B, D, E)** and Multiple t-tests **(D-F, H)**. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, not significant. See also in Supplementary Figure 1.

Figure 2

Fig. 2. *plin1* responds to IMD activation through Mrt/Pzg complex, and regulates LDs' morphology. **(A and B)** Relative *plin1* mRNA levels in the fat body of wild type flies at the indicated time points post *E. coli* **(A)** or *S. typhimurium* **(B)** infection. Flies treated with sterile PBS were used as a control. The fold change of mRNA expression was normalized to that of 0 h. **(C)** Relative *plin1* mRNA levels in the fat body of *GS106-GAL4>PGRP-LCs* flies after RU486 treatment for indicated time. The fold change of mRNA expression was normalized to that of 0 h. **(D)** Relative *mrt* mRNA levels in the fat body of wild type flies post *E. coli* or *S. typhimurium* infection. Flies treated with sterile PBS were used as a control. The fold change of mRNA expression was normalized to that of 0 h. **(E)** Relative luciferase activities of F-*mrt*(Rel) (Full length promoter of -1.5kb to +1bp including all predicted Relish Binding motifs in Fig. S3) reporter in S2* cells after double strand RNA (dsRNA) and PGN (35 µg/ml) treatment. All data were normalized to dsGFP control group at 0 h. Three independent repeats were performed at each time point for each treatment. **(F)** Relative luciferase activities of T-*mrt*(Rel) (Truncated length promoter of -870 to +1bp with two key binding motifs) and T-*mrt* (Truncated length promoter with no binding motifs) reporter in S2* cells after PGN (35 µg/ml) treatment. All data were normalized to T-*mrt*(Rel) group at 0 h. Three independent repeats were performed at each time point for each group. **(G)** BODIPY staining (green) of LDs in the fat body of indicated flies. Nuclei of fat body cells were stained with DAPI (blue). Eight fat bodies were examined for each genotype. Scale bar: 20 µm. Error bars represent the mean ± s.d.. Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test **(A-D)**, Multiple t-tests **(A-B)** and Student's t test **(E-F)**. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, no significance. **See also in Supplementary Figure 2 and 3.**

Fig. 3. Sustained IMD activation prefers to enhance Bmm-mediated lipolysis on large LDs. **(A)** BODIPY staining (green) of LDs in the fat body of indicated flies. Nuclei of fat body cells were stained with DAPI (blue). Eight fat bodies were examined for each genotype. Scale bar: 20 μ m. **(B)** BODIPY staining (green) of LDs **(B)** and statistics of the size distribution of LDs **(B1)** in the fat body of indicated flies after RU486 treatment for 5 days. Nuclei of fat body cells were stained with DAPI (blue). Eight fat bodies were examined for each sample. Scale bar: 20 μ m. **(C and D)** BODIPY staining (green) of LDs in the fat body of *ppl-GAL4>Rel.68* and *ppl-GAL4>Rel.68,plin³⁸* adult flies (up panel, one-week age; middle panel, two-week age; low panel, three-week age). The corresponding statistics of the distribution of LDs' size was shown in **(C1)** for *ppl-GAL4>Rel.68* flies and in **(C2)** for *ppl-GAL4>Rel.68,plin³⁸* flies (n =30 cells), and the corresponding statistics of the average size of LDs **(D)**. Nuclei of fat body cells were stained with DAPI (blue). Eight fat bodies were examined for each sample. Scale bars: 20 μ m. **(E)** Relative TAGs level in the whole body of indicated flies before/after starvation. The change of TAG levels was normalized to that of 0 h for each genotype. Six independent replicates for each sample were performed (n =12 per repeat). **(F)** Survival curves of indicated flies (n = 60) with starvation. **(G and H)** BODIPY staining (green) of LDs **(G)** and the corresponding statistics of the average size of LDs (n =30 cells) **(H)** in the fat body of indicated adult flies (up panel, one-week age; low panel, two-week age). Eight fat bodies were examined for each sample. Each scattering dot represents the data from one fat body cell. Nuclei of fat body cells were stained with DAPI (blue). Scale bars: 20 μ m. **(I-J)** Nile red staining (red) of LDs and imaging of GFP signals (green) around LDs in the fat body of Bmm-GFP knock-in flies **(I)**, and quantification of GFP intensity on the surface of lipid droplet **(J)**. The GFP enrichment indicated by arrow is a sign of lipolysis. Eight fat bodies were examined for each genotype. Scale bars: 10 μ m. Values of plotted curves represent mean $\pm$ s.d. of at least three independent repeats **(E-F)**. Error bars represent the mean with range **(D, H)** and mean $\pm$ s.d. **(J)**. Data were analyzed by Student t-tests **(D-E, J)**, Multiple t-tests **(H)** and Kaplan - Meier **(F)**. ****p < 0.01; ***p < 0.001; ns, no significance. See also in Supplementary Figure 4.**

Figure 4

Fig. 4. *plin1* participates in the susceptibility of flies to bacterial infection. (**A** and **B**) Survival curves (**A**) and bacterial loads (CFUs) (**B**) of wild type and *plin1*³⁸ flies (n =60) post *S. typhimurium* infection. (**C** and **D**) Survival curves (**C**) and bacterial loads (CFUs) (**D**) of *ppl-GAL4>plin1 RNAi* and control flies (n =60) post *S. typhimurium* infection. (**E** and **F**) Survival curves (**E**) and bacterial loads (CFUs) (**F**) of *ppl-GAL4>plin1* and control flies (n =60) post *S. typhimurium* infection. (**G** and **H**) Survival curves (**G**) and bacterial loads (CFUs) (**H**) of *ppl-GAL4>plin1* and control flies (n =60) post *E. coli* infection. (**I**) BODIPY staining (green) of LDs in the fat body of *GS106-GAL4>plin1* and *GS106-GAL4>plin1 RNAi* flies after 3 days with (lower panel) or without (upper panel) RU486 treatment. Eight fat bodies were examined for each sample. Scale bar: 20 μ m. (**J** and **K**) Survival curves (**J**) and bacterial loads (CFUs) (**K**) of above flies (**I**) (n =60) post *S. typhimurium* infection. Values of plotted curves represent mean $\pm$ s.d. (**A**, **C**, **E**, **G**, **J**) of at least three independent repeats. Each scattering dot (CFUs) represents one technical replicate, line represents the mean of four independent repeats (**B**, **D**, **F**, **H**, **K**). Data were analyzed by Kaplan–Meier (**A**, **C**, **E**, **G**, **J**) and Multiple t-tests (**B**, **D**, **F**, **H**, **K**). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. See also in **Supplementary Figure 5**.

Figure 5

Fig. 5. The formation of large LDs ameliorates sustained inflammation induced oxidative stress. **(A)** ROS level indicated by GFP intensity (green) of *gstD-GFP* reporter in the fat body of wild type flies infected with *E. coli* (upper panel) or *S. typhimurium* (lower panel) at indicated time points. The statistics of GFP intensity was plotted in **(A1)** for *E. coli* infection and in **(A2)** for *S. typhimurium* infection. Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. **(B)** ROS level indicated by DCFH-DA staining (green) in the fat body of indicated flies with (lower panel) or without (Upper panel) RU486 treatment for 5 days. The statistics of fluorescence intensity was plotted in **(B1)** for *GS106-GAL4>Rel.68* flies, in **(B2)** for *GS106-GAL4>PGRP-LCx* flies and in **(B3)** for *GS106-GAL4>PGRP-LCa* flies. Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. **(C)** Survival curves of wild type flies (n=60 flies) with or without NAC treatment at indicated time post *S. typhimurium* infection. **(D)** Correlation analysis between the percentage of small LDs (< 2 μ m, green) and intracellular ROS level (red) in fat body cells at indicated time points post *S. typhimurium* infection. The regression analysis was performed, respectively. Red regression dash line presents the change of intracellular ROS level ($r^2 = 0.59$) and green regression dash line present the change of small LDs percentage ($r^2 = 0.87$). The Pearson's correlation of these two set of data was analyzed (Pearson's correlation coefficient R=0.74). **(E)** ROS level indicated by DCFH-DA staining (green) in the fat body of indicated flies. The statistics of fluorescence intensity was plotted in **(E1)** for *plin1³⁸* and in **(E2)** for *ppl-GAL4>plin1* and *ppl-GAL4>plin1-RNAi* flies and their genetic controls, respectively. Dashed circle indicated LDs. Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. **(F)** ROS level indicated by DCFH-DA staining (green) in the fat body of indicated flies with (lower panel) or without (upper panel) RU486 treatment for 5 days. The statistics of fluorescence intensity was plotted in **(F1)** for *GS106-GAL4>plin1* and in **(F2)** for *GS106-GAL4>plin1 RNAi* flies. Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. **(G)** ROS level indicated by DCFH-DA staining (green) in the fat body of *ppl-GAL4>Rel.68*, *ppl-GAL4>Rel.68, plin³⁸* flies and control flies. The corresponding fluorescence intensity was quantified in **(G1)**. Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. **(H)** Survival curves of *ppl-GAL4>Rel.68*, *ppl-GAL4>Rel.68, plin³⁸* one-week old adult flies and control flies (n =60) after *S. typhimurium* infection. PBS injection as a control. **(I)** The lifespan curves of indicated flies at normal condition (n =60 per genotype). Error bars represent the mean with range (**A1-2, B1-3, C, E1-2, F1-2, G1**). Values of plotted curves represent mean $\pm$ s.d. of at least three independent repeats (**C, H, I**). Data was analyzed by One-Way ANOVA with Tukey's multiple-comparison test (**A1-2, E2, G1**), Student's t test (**B1-3, E1-2, F1-2**) and Kaplan–Meier (**C, H, I**). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 6

A

B

C

D

E

F

Fig. 6. Adaptive large LDs formation contributes to eliminate excessive intracellular ROS. (A and B) BODIPY staining (green) of LDs (A) and the corresponding statistics of LDs' size (n=30 cells) (B) in the fat body of indicated flies after 5 days with (lower panel) or without (upper panel) N-acetyl-L-cysteine (NAC) treatment. Eight fat bodies were examined for each sample. Scale bar: 20 μ m. (C and D) BODIPY staining (green) of LDs (C) and the corresponding statistics of LDs' size (n=30 cells) (D) in the fat body of indicated one-week old adult flies. Eight fat bodies were examined for each sample. Scale bar: 20 μ m. (E and F) ROS levels indicated by DCFH-DA staining (green) in the fat body of indicated one-week old adult flies and control flies (E). The corresponding fluorescence intensity was quantified in (F). Eight fat bodies were examined for each sample and each scattering dot represents the data from one image. Scale bar: 20 μ m. Error bars represent the mean with range. Data was analyzed by One-Way ANOVA with Tukey's multiple-comparison test (B, D, F) and Student's t test (B, F). * p < 0.05; *** p < 0.001; ns, no significance.

Figure 7

Fig. 7. The schematic diagram of LDs' morphogenesis mediated by Plin1 during infection-induced pathogenesis. LD's growth induced by downregulation of *plin1* at early-stage of *E. coli* infection. And enlarged LDs provides antioxidant role and benefits the host for anti-infection. While, sustained hyper-inflammation induced either by *S. typhimurium* infection or genetic manipulation triggers progressively enhanced LDs' lipolysis. And *plin1* deficiency accelerates excessive decomposition of large LDs, which results in energy wasting and severe lipotoxicity, thus deteriorated pathogenesis.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, and one table.

Supplementary Figure Legends

Figure S1

Fig. S1. IMD signaling modifies lipid metabolism and LDs morphology. Related to Figure 1. **(A)** Relative *Dpteracin* (*Dpt*) mRNA expression (black) and TAGs level (gray) in the fat body of wild type flies at indicated time points after sterile PBS injection. The fold change of mRNA expression was normalized to that of 0 h and four independent repeats (n = 20 flies per repeat) were performed at each time point. Total TAGs level of 25 flies' fat body tissues was quantified in six biological replicates at each time point. **(B and C)** Relative TAGs levels in the whole body of wild type flies at indicated time points post *E. coli* **(B)** or *S. typhimurium* **(C)** infection. Sterile PBS injection as a control. The change of TAG levels was normalized to that of 0 h, respectively. Six independent replicates for each time point were performed (n = 12 flies per replicate). **(D)** The statistics of LDs' size (n = 30 cells) in the fat body of wild type flies at indicated time point post *E. coli* or *S. typhimurium* infection. Each scattering dot represents the data from one fat body cell. **(E-G)** Relative *Dpt* mRNA expression in the fat body of *GS106-GAL4>PGRP-LCx* **(E)** and *GS106-GAL4>PGRP-LCa* **(F)** and *GS106-GAL4>Rel.68* **(G)** flies at indicated time point after with or without RU486 feeding. The data was normalized to that of 0 h without RU486 treatment. Four independent repeats at each time point were performed (n = 20 per repeat). **(H and I)** TAGs level in the whole body of *GS106-GAL4>PGRP-LCx* **(H)** and *GS106-GAL4>PGRP-LCa* **(I)** flies at indicated time point after with or without RU486 feeding. Six independent replicates for each time point were performed (n = 12 flies in each replicate). **(J)** Relative *Dpteracin* (*Dpt*) mRNA expression (black) and TAGs level (Grey) in the fat body of wild type flies at indicated time points post heat-killed *E. coli* (HK-*E. coli*) infection. The sample were treated and analyzed in the same way as above **(A)** described. **(K-L)** BODIPY staining (green) of LDs **(K)** and the corresponding statistics of LDs' size (n = 30 cells) **(L)** in the fat body of IMD pathway mutant flies and corresponding genetic control flies. Eight fat bodies were examined for each sample. Scale bar: 20 μ m. Error bars represent mean $\pm$ s.d. **(A-C, E-J)** or mean with range **(D, L)**. Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test **(A-D, H-J)** and Multiple t-tests **(B-C, E-I, L)**. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, no significance.

Figure S2

A

B

C

Fig. S2. The expression profile of genes involved in the regulation of LDs metabolism during IMD activation. Related to Figure 2. **(A and B)** Relative mRNA expression of a set of genes related to LD metabolism in the fat body of wild type flies at the indicated time points post *E. coli* **(A)** or *S. typhimurium* **(B)** infection. All measurements were normalized to that of 0 h (red line). Four independent repeats at each time point were performed (n = 20 per repeat). **(C)** Relative mRNA expression of genes *APGAT2*, *DGAT2*, *Rfabg* and *Plin1* in the fat body of *ppl-GAL4>Rel.68* flies compared with its genetic control flies of *ppl-GAL4> +*. Four independent repeats at each time point were performed (n = 20 per repeat). Error bars represent mean $\pm$ s.d.. Data were analyzed by Multiple t-tests. *** $p < 0.001$.

Figure S3

Fig. S3. Relish/NF- κ B potentially regulates the transcription of *mrt* or *pzg* in *Drosophila* subgroups. Related to Figure 2. **(A)** Relative *pzg* mRNA levels in the fat body of wild type flies post *E. coli* or *S. typhimurium* infection. Flies treated with sterile PBS were used as a control. The fold change of mRNA expression was normalized to that of 0 h. Four independent repeats (n=20 flies fat body tissues per repeat) were performed at each time point for each group. **(B and C)** Predicted Relish/NF- κ B-binding motifs in the promoter locus of *mrt* **(B)** and *pzg* **(C)** genes of five *Drosophila* subgroups. Dmel, *Drosophila melanogaster*; Dsim, *D. simulans*; sec, *D. sechelia*; Dyak, *D. yakuba*; Dere, *D. erecta*. S1-S5 represent the location site (red color) of conserved binding motifs. Sequence alignment is analyzed by BLAST in flybase website. TSS: transcription start site. **(D)** Relative luciferase activities of *pzg* (1.5 KB upstream of ATG, all predicted Relish binding sites are covered) reporter in S2* cells after double strand RNA (dsRNA) and PGN (35 μ g/ml) treatment. All data were normalized to dsGFP control group at 0 h. Three independent repeats were performed at each time point for each treatment. **(E)** Schematic diagram of the *mrt* promoter locus and the plasmid constructs used for luciferase assay. The full length (F-*mrt*(Rel):-1.5k to +1bp), truncated length (T-*mrt*(Rel):-870 to +1bp) and mutant length (T-*mrt*: -870 to +1bp without binding motifs) of *mrt* promoter were indicated. Error bars represent the mean $\pm$ s.d.. Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test **(A)** and Student's t test **(D)**. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, no significance.

Figure S4

A

B

Fig. S4. *plin1* deficiency enhances Bmm/ATGL-dependent lipolysis in the condition of sustained IMD activation. Related to Figure 3. **(A)** TAGs level change in the whole body of indicated one-week or three-week old flies. The change of TAG levels at 3 w was normalized to that of 1w for each genotype. The number on the right of each line represents the percentage reduction of TAGs level at 3 w compared with 1 w for each genotype. Six independent replicates at each time point for each genotype were performed (n =12 flies per replicate). **(B)** Relative TAGs levels in the whole body of indicated one-week old adult flies. All values of TAGs level were normalized to that of 0h of *ppl-GAL4*>+ control flies. Six independent replicates for each genotype were performed (n =12 flies per replicate). Error bars represent the mean $\pm$ s.d.. Data were analyzed by One-way ANOVA with Student t-test. * $p < 0.05$; *** $p < 0.001$.

Figure S5

Fig. S5. Overexpression *plin1* compromises AMP responses. Related to Figure 4. (**A** and **B**) Relative *dipteracin* (*Dpt*) and *attacin-A* (*AttA*) mRNA expression in the fat body of *plin1*³⁸ mutant flies and wild type flies at indicated time points post *E. coli* (**A**) or *S. typhimurium* (**B**) infection. Four independent repeats were performed (n = 20 per repeat). (**C** and **D**) Relative *dipteracin* (*Dpt*) and *attacin-A* (*AttA*) mRNA expression of *ppl-GAL4*> *plin1* flies and *ppl-GAL4*> + control flies at indicated time points post *E. coli* (**C**) and *S. typhimurium* (**D**) infection. Four independent repeats were performed (n = 20 per repeat). Error bars represent mean ± s.d. Data was analyzed by Multiple t-tests. ***p* < 0.01; ****p* < 0.001.

1266 **Table S1: Primers used in this study.**

Genes	Forward primer	Reverse primer
qRT-PCR primers		
<i>Rp49</i>	AGATCGTGAAGAAGCGACCAAG	CACCAGGAACTTCTTGAATCCGG
<i>Dpt</i>	GGCTTATCCGATGCCCCGACG	TCTGTAGGTGTAGGTGCTTCCC
<i>AttA</i>	CACAACTGGCGGAACCTTTGG	AAACATCCTTCACTCCGGGC
<i>plin1</i>	CAGCGCATACCACTGGTCTAT	GCATTACCGATTGCTTGACAG
<i>plin2</i>	CGAGCGCCTCCTTGAATAC	AGAACTCTTGCCATTCTGCAC
<i>DGAT2/CG1942</i>	GATTACTTTCCCGTGAGCTGG	CCCATGTTAATGCCAATGCCTG
<i>DGAT1/mdy</i>	AAGACAGGCCTCTACTATTGT	CCCATCATGCCCCATAAATGCC
<i>DGAT/CG1941</i>	GTTGCTGCCGTGCTGTTCTAT	GTGGGTTCTCTTGATTCGC
<i>DGAT/CG1946</i>	CTTCTTTACGCTGCCCATCTCG	CCATTACCTTCCATAATGCCG
<i>GPAT1/CG5508</i>	CCTTTTGGCCTTCACTTCC	TCTCGATCTGCTTCGTGTTC
<i>APGAT4/CG4753</i>	CTTCACATGCGGATTCTTCGTC	ACCATTCCGCCACACAGACAAG
<i>APGAT3/CG4729</i>	CTCTGTCCTGCATTTTCTACTACG	CTTGCTGATCTGCGTCTTGTTG
<i>APGAT2/CG3812</i>	GCTGGCCTAATCTTCATCG	CCCACAGTTTGATACGTTGC
<i>APGAT1/CG17608</i>	CTGTGCGTGCCCTTTATGATTC	CAGACCACGAACCTCCATTG
<i>Lipin</i>	GACATCCGCGATCTGTTTCCC	GGACTGGAATGTTTGGGTCAGC
<i>Lip4</i>	GACCGAACAAGGGACTTGG	CGAGTACGTATTGCCACGAA
<i>ACC</i>	GGAACACTGAACTGGGGAAA	GCAATCACGCTGGAGTTGTA
<i>FASN</i>	CGAATCCATCAATATGCCCCG	ACATAGTGACCACCACCCAGGT
<i>ACSL</i>	TTCCGGCAAGATTATGCGTC	CAGTTGCTCCACAATTTGCTCA
<i>dHSL</i>	TTGCCCTCTTCAATGACAACTG	CTGGTGGGTGTCTTTCCA
<i>BMM</i>	AAGTATGCACCGCATCTGTTG	CAAATCGCAGAGGAGACAGC
<i>sws/CG2212</i>	CTGGATGGCGGCTATGTGAATAA	CCATTTCTTGAGAGCAGCCAC
<i>Cct1</i>	TCCACCTCGCAGCAATTCTTC	GTAGTCACATCTATCCCGCTCC
<i>Fatp</i>	GAAATCCTTCTCGGAATTCCT	GGAGATGAAAGCCATATCGCC
<i>Rfabg</i>	GGTTTCTGGCATATTGTTGA	TAGGGTGCGTATGCC ATCAA
<i>DUOX</i>	GCTGCACGCCAACCACAAGAGACT	CACGCGCAGCAGGATGTAAGGTTT
<i>mrt</i>	GGAGGATATTCTCGAGTGGAGC	GCTTCCTGCCTCGTAGTCGAAC
<i>pzg</i>	GCTGAGGAACCACAACCATCTGAC	GTAACCTCGCCTTCGCCAGATT
Primers for plasmid construction and dsRNA synthesis		
F- <i>mrt</i> (Rel)-luc	CGAGATCTGCGATCTAAGTATAACG TCGTAGCGCACACGCACACC	CAGTACCGGAATGCCAAGCTGGGT GCACCCTTTGATCAAGGTCTT
pzg-luc	CGAGATCTGCGATCTAAGTACACAG TAGCAGCACAACGGAGACG	CAGTACCGGAATGCCAAGCTCTGT AGCAGTTCTCGACGGACGCGT
T- <i>mrt</i> (Rel)-luc	CGAGATCTGCGATCTAAGTAGCCAC TTTGTCGCAGTGTATCTGT	CAACAGTACCGGAATGCCAGCTGG GTGCACCCTTTGATCAAG
T- <i>mrt</i> -luc	CGAGATCTGCGATCTAAGTACGTTA GCTTTTTGCTGTCATTCTGT	CCAACAGTACCGGAATGCCAGATG CTGTTGGAAAACAAGCAA
dsRelish	TAATACGACTCACTATAGGGAGATC AAACACGTGCCGC	TAATACGACTCACTATACCCAGACT CACGCTCTGTCTC
dsGFP	TAATACGACTCACTATAGGGAGAAT GGTGAGCAAGGGCGAGGA	TAATACGACTCACTATAGGGAGACT TGTACAGCTCGTCCATGC

Supplementary Information for

Adaptive modification of lipid droplets mediated by Plin1 functions in infection-induced pathogenesis in *Drosophila*

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This PDF file includes:
SI Materials and Methods
Fig. S1 to S5 legend.
Table S1.

SI Materials and Methods.

Drosophila stocks and bacterial strain.

All flies were propagated at 25°C on standard cornmeal food (1 L food contains 77.7 g cornmeal, 32.19 g yeast, 10.6 g agar, 0.726 g CaCl₂, 31.62 g sucrose, 63.2 g glucose, 2 g potassium sorbate and 15 ml 5% Tegosept), 30%-55% humidity with a 12h/12h light/dark cycle. Fly resources that were used in this study as follows: *w¹¹¹⁸* were used as wild-type controls if no additional indication. *plin1³⁸*, *UAS-plin1* mcherry, *UAS-plin1 RNAi*, *Bmm-GFP* knock-in and *ppl-GAL4* were kindly gifted from Dr. Xun Huang (Institute of Genetics and Developmental Biology, CAS). *UAS-gstD-GFP* was kindly gifted from Dr. ZhiWei Liu (Shanghai Ocean University). *w¹¹¹⁸; P{GD5139=UAS-Bmm RNAi}* (VDRC, V37877 and V37880) were kindly gifted from Dr. Yong Liu (College of Life Sciences, Wuhan University). *w¹¹¹⁸*, *w[1118]; P{w[+mW.hs]=Switch1}106*, *Bmm¹*, *w[*]; P{w[+mC]=UAS-FLAG-Rel.68}i21-B*; *TM2/TM6C*, *Sb[1]*, *y[1]* *w[*]; P{w[+mC]=UAS-PGRP-LC.x}1* and *y[1]* *w[*]; P{w[+mC]=UAS-PGRP-LC.a}3* were obtained from Bloomington stock center. All flies used in this study were male. Two bacterial strain, *E. coli* (*DH5a*) and *S. typhimurium* (*SR-11*) (a gift from Dr. ZhiHua Liu, Institute of Biophysics, CAS) were used in this study.

Cloning and double-strand RNAs

To construct the *mrt* and *pzg* reporter vector (*mrt-luc* and *pzg-luc*), the *mrt* and *pzg* promoter sequence (about -1500 bp or -1000 bp to 0 bp) was PCR amplified from *Drosophila* genomic DNA and introduced into *pGL3* vector (Progenia) at HindIII restriction site by using recombination technology (Hieff Clone® Plus One Step Cloning Kit, YEASEN). All the plasmid constructs were verified by nucleotide sequencing. *pAC5.1-renilla* plasmid as a normalized reporter. Double-stranded RNAs (dsRNAs) against relish or GFP used in the luciferase reporter assay were synthesized using MEGAscript T7 kit (Invitrogen). Primers used for PCR amplification are listed in Supplementary Table 1.

Infection and survival rate counting

Bacterial strains used in this study are *E. coli* (*DH5a*) and *Salmonella typhimurium* (*S. typhimurium*). Two days before infection, both bacteria from glycerol stocks were streaked onto Luria Broth (LB) agar plates and grown overnight at 37°C. The plate could be stored at 4 °C for up to 1 week. A single colony was inoculated to 6 ml fresh LB medium and grown at 37°C with shaking (200 rpm). Grow the bacteria to an OD₆₀₀ of 0.7 to 0.8 (about 3.5 hours). The bacterial culture was pelleted with sterile phosphate-buffered saline (PBS) to the desired concentration. We injected 50.6 nl of bacterial suspension into dorsal prothorax of each fly with Nanoject II injector (Drummond). All flies used were 1 week old after eclosion. The final optical density (O.D. / ml) at 600 nm for injection were *E. coli* (O.D. 10) and *S. typhimurium* (O.D. 6 or O.D. 3). For *E. coli* infection, each fly obtained about 1x10⁶ CFUs. For *S. typhimurium* infection, each fly obtained the lower dose (about 2x10⁵ CFUs) or the higher dose (about 1x10⁶ CFUs) according to the experiment design. Infected flies about 23 per vial were maintained at 25°C. Death was recorded at the indicated time point, and alive flies were

transferred to fresh food every day for the survival analysis and CFUs assay.

Lifespan counting

For lifespan analysis, male flies were collected within three days after adult emergence and raised in incubator (temperature: 25°C, humidity: 55%, 12:12 light: dark cycle). These flies were randomly divided into a separate vial in a number of 25 with at least three biological replicates. Dead flies were counted every three days and alive flies were flipped to fresh food.

Bacterial loads assay

To monitor bacterial loads of the flies during infection, the number of colony forming units (CFUs) grown on LB agar plate was determined as follow: 5 living flies were randomly collected in a 1.5 ml EP tube, rinsed with 70% ethanol two times by vertex for 10s to sterile the surface adherent bacteria, then rinsed with sterile deionized water two times by vertex for 10 s, and then homogenized in 200 µl of sterile PBS with three fly body volumes of ceramic beads (diameter: 0.5 mm) in the Minilys apparatus (Bertin TECHNOLOGIES) at highest speed for 30 s. The suspensions obtained were then serially diluted in PBS and plated on LB agar. Specially noted for *S. typhimurium* plating, PBS was substituted with PBS + 1% Triton X-100. For the bacterial load at zero time point, flies were allowed to rest for 10 min after bacterial injection before plating as described above. The agar plate was maintained at 37°C for 18 hours before CFUs counting. CFUs were log₁₀ transformed.

Cell culture, transfection and luciferase assay

S2* cells (a gift from Dahua Chen, Institute of Zoology, CAS) were maintained in Drosophila Schneider's Medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 units/ml of penicillin, and 100 mg/ml of streptomycin at 28°C. Transient transfection of various plasmids, dsRNA was performed with lipofectamine 3000 (Invitrogen), according to the manufacturer's manual. Luciferase reporter assays were carried out using a dual-luciferase reporter assay system (Promega). Where indicated, cells were treated with PGN (35 µg/µl, 6 h) purified from *Erwinia carotovora carotovora* 15 (*Ecc15*) referring to previous study(1).

qRT-PCR

For quantification of mRNA level, about 20 flies carcass/fat body tissue were dissected in sterile PBS buffer on ice at indicated time points post infection, immediately homogenized in 200 µl cold TRIzol with three fly body volumes of ceramic beads (diameter: 0.5 mm), then supplied additional 300 µl TRIzol to reach total 500 µl volume and samples were stored at -80°C. RNA extraction was referred to the manual of commercial kit (Magen, Hipure Total RAN Plus Micro Kit), this kit can effectively remove genomic DNA contamination. cDNA was synthesized by using the kit (abm, 5X All-In-One MasterMix) with total 1µg isolated RNA as template in a 20 µl reaction system. Quantitative real-time PCR (qRT-PCR) was performed using a SYBR green kit (abm, EvaGreen supermaster Mix) on an ABI 7500 or ViiATM 7 thermocycler (Life

Technology). Samples from at least four independent biological replicates per genotype were collected and analyzed. House-keeping gene *rp49* as the reference gene for data normalization. Primer data for qRT-PCR are provided in Supplementary Table 1.

Lipid droplet staining and counting

For lipid droplet staining, adult male carcass/fat body tissues were dissected and fixed in 4% fresh prepared paraformaldehyde (PH=7.5) in PBS for 10 min on ice. Tissues were then rinsed twice with PBS (3 min each time), then incubated in PBS containing 1 µg/ml of BODIPY 493/503 (Invitrogen) dye or 0.5 µg/ml Nile Red (Sigma) for 30 min on ice, DAPI (1 µg/µl, final concentration) was added to stain nuclei at last 5 mins of staining process. After staining, tissues were rinsed three times with PBS (3 mins each time), then mounted in mounting medium (Vector, H-1000) for microscopy analysis. To quantify the average lipid droplet size, the average diameter of the three largest lipid droplets per cell was measured generally, with the exception of *plin1* deficiency associated flies, we measured their biggest lipid droplets in one cell (2). 30 fat body cells of each genotype fly randomly selected from eight confocal images were used to analysis the lipid droplet size. To count the size distribution of lipid droplets, the average percentage of the indicated size range of lipid droplets per cell from 30 fat body cells were determined by using the “Analyze Particles” tool embedded in ImageJ software (<https://imagej.nih.gov/ij/>). To quantify the fluorescence intensity of GFP on the surface of lipid droplets, confocal images acquired from eight fat bodies were measured by ImageJ software.

TAG assay

TAG amounts were measured using a TG Quantification Kit (BIOSINO, TG kit). Briefly, for whole body TAG quantification, groups of 12 one-week old male flies were collected and weighted (about 10 mg) in a 1.5 ml EP tube, then immediately stored at -80°C for subsequent assay. Stored flies were homogenized in 200 µl lysis buffer (10mM KH₂PO₄, 1mM EDTA, PH=7.4) with three fly body volumes of ceramic beads, and inactivated in water bath at 75°C for 15 min. The inactivated homogenate was homogenized again for 30 s and kept on ice ready for assay. For each TAG measurement, 3 µl of homogenate was incubated with 250 µl reaction buffer at 37°C for 10 min. After removal debris by centrifugation (2000 rpm, 2 min), 150 µl of clear supernatant was used to perform a colorimetric assay in 96 well plate (Corning® Costar) for absorbance reading at 505 nm. TAGs level was normalized with fly weight in each homogenate (unit: nmol/mg.fly). For fat body TAG quantification, 25 fly’s carcass/fat body tissues were dissected and following assay as described above. TAGs level was normalized with per 25 flies (unit: nmol/25.fly).

RU486 treatment

RU486 induction was described as before (3). Briefly, A 10 mg/ml stock solution of RU486 (mifepristone; Sigma) was dissolved in DMSO. Appropriate volumes of RU486 stock solution was diluted with water containing 2% ethanol to final concentration of 50 µg/ml. 100 µl of the diluted RU486 solution was dipped onto the surface of fresh

food in vials (Diameter: 2 cm). The vials were then allowed to dry at room temperature for half day or 4°C for overnight. Flies were transferred to RU486-contained food and raised in 25°C and fresh food was changed every two days.

NAC Treatment

N-acetyl-L-cysteine (NAC) (Beyotime) fresh solution was prepared by dissolving 0.5 g of NAC powder in 10 ml distilled water, the solution could be aliquoted into 1 ml per EP tube and frozen or stored at -80 °C. 100 µl of NAC solution was dipped onto the surface of fresh food in vials (Diameter: 2 cm). The vials were then allowed to dry at room temperature for half day or 4°C for overnight. Flies were transferred to NAC-contained food and raised in 25°C and fresh food was changed every day.

ROS detection

We used two methods to detect ROS in fat body, which are *gstD-GFP* reporter flies and dichlorofluorescein diacetate (DCFH-DA) labeling. The oxidative stress reporter construct *gstD-GFP* for evaluating cellular ROS levels has been describe before (4). Briefly, the carcass/fat body of transgenic flies containing a *gstD-GFP* reporter construct were dissected in sterile PBS, fixed in 4% formaldehyde for 10 min on ice, rinsed twice with ice-chilled PBS (3 min each time), then the flaky fat body cells attached to the inner carcass shell were dissected out to mount and confocal image (Vector, H-1000). DCFH-DA (Beyotime, Reactive Oxygen Species Assay Kit) labeling of fresh dissected carcass/fat body tissues was performed according to the manufacturer's manual, which based on the ROS-dependent oxidation of DCFH-DA to fluorescent molecule 2'-7'dichlorofluorescein (DCF). In brief, the tissues were incubated with PBS containing 20 µM DCFH-DA for 30 min at 37°C, washed with sterile PBS for three times (3 min each) to remove free DCFH-DA that do not uptake by the cell, then the flaky fat body cells attached to the inner carcass shell immediately were dissected out to mount and confocal image (Vector, H-1000). It should be noted that the slices were confocal imaged using the exact same settings for control and experimental groups. The fluorescence intensity is proportional to the ROS levels, fluorescence intensity of GFP or DCF was quantified by using ImageJ software.

Starvation test

Adult male flies were collected within three days after eclosion and raised on standard fly food at 25°C. One week later, flies were randomly distributed in groups of 20 flies/vial and starved on 1% agar (dissolved in distilled water), new agar vial was changed every day. For the survival counting, the deaths were scored every two hours until all experimental files were dead. For the TAG assay, 12 alive flies were randomly collected at indicated time point, and delivered to TAG assay as describe above.

Microscopy and software

LSM700 (Leica) and Olympus FV-1200 confocal laser scanning microscopy were used for imaging. Captured images were analyzed by implemented soft respectively. ImageJ (<https://imagej.nih.gov/ij/>) was used for analysis of fluorescence intensity and lipid

droplets size.

Statistical analyses

All replicates are showed as the mean $\pm$ SD or mean with range. Statistical significance was determined using a paired Student's t-test for two measurements, one-way ANOVA (Tukey's HSD) with a multiple t-tests and Multiple t-tests for pairwise comparisons. Kaplan–Meier test for survival curves comparison. All data processing was used with GraphPad Prism 7.0.

Sample size choice. The sample size was determined according to the number of data points. Batches of experiment were carried out to ensure repeatability and the use of enough animals for each data point.

Randomization. Measures were taken to ensure randomization. Each experimental batch contained more animals than the number of data points, to ensure randomization and the accidental exclusion of animals. In vitro analyses were usually performed on a specimen from animals at each data point to ensure a minimum of three biological replicates.

Blinding. Data collection and data analysis were routinely performed by different people to blind potential bias. All measurement data are expressed as mean $\pm$ s.d. to maximally show derivations, unless otherwise specified.

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Supplementary Figure Legends

Fig. S1. IMD signaling modifies lipid metabolism and LDs morphology. Related to Figure 1. (A) Relative *Dpterisin* (*Dpt*) mRNA expression (black) and TAGs level (gray) in the fat body of wild type flies at indicated time points after sterile PBS injection. The fold change of mRNA expression was normalized to that of 0 h and four independent repeats (n =20 flies per repeat) were performed at each time point. Total TAGs level of

25 flies' fat body tissues was quantified in six biological replicates at each time point. (B and C) Relative TAGs levels in the whole body of wild type flies at indicated time points post *E. coli* (B) or *S. typhimurium* (C) infection. Sterile PBS injection as a control. The change of TAG levels was normalized to that of 0 h, respectively. Six independent replicates for each time point were performed (n=12 flies per replicate). (D) The statistics of LDs' size (n=30 cells) in the fat body of wild type flies at indicated time point post *E. coli* or *S. typhimurium* infection. Each scattering dot represents the data from one fat body cell. (E-G) Relative *Dpt* mRNA expression in the fat body of *GS106-GAL4> PGRP-LCx* (E) and *GS106-GAL4> PGRP-LCa* (F) and *GS106-GAL4>Rel.68* (G) flies at indicated time point after with or without RU486 feeding. The data was normalized to that of 0 h without RU486 treatment. Four independent repeats at each time point were performed (n = 20 per repeat). (H and I) TAGs level in the whole body of *GS106-GAL4> PGRP-LCx* (H) and *GS106-GAL4> PGRP-LCa* (I) flies at indicated time point after with or without RU486 feeding. Six independent replicates for each time point were performed (n =12 flies in each replicate). (J) Relative *Dpterisin* (*Dpt*) mRNA expression (black) and TAGs level (black) in the fat body of wild type flies at indicated time points post heat-killed *E. coli* (HK-*E. coli*) infection. The sample were treated and analyzed in the same way as above (A) described. (K-L) BODIPY staining (green) of LDs (K) and the corresponding statistics of LDs' size (n =30 cells) (L) in the fat body of IMD pathway mutant flies and corresponding genetic control flies. Eight fat bodies were examined for each sample. Scale bar: 20 μ m. Error bars represent mean $\pm$ s.d. (A-C, E-J) or mean with range (D, L). Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test (A-D, H-J) and Multiple t-tests (B-C, E-I, L). * $p < 0.05$; *** $p < 0.001$; ns, no significance.

Fig. S2. The expression profile of genes involved in the regulation of LDs metabolism during IMD activation. Related to Figure 2. (A and B) Relative mRNA expression of a set of genes related to LD metabolism in the fat body of wild type flies at the indicated time points post *E. coli* (A) or *S. typhimurium* (B) infection. All measurements were normalized to that of 0 h (red line). Four independent repeats at each time point were performed (n = 20 per repeat). (C) Relative mRNA expression of genes *APGAT2*, *DGAT2*, *Rfabg* and *Plin1* in the fat body of *ppl-GAL4>Rel.68* flies compared with its genetic control flies of *ppl-GAL4> +*. Four independent repeats at each time point were performed (n = 20 per repeat). Error bars represent mean $\pm$ s.d.. Data were analyzed by Multiple t-tests. *** $p < 0.001$.

Fig. S3. Relish/NF- κ B potentially regulates the transcription of *mrt* or *pzg* in *Drosophila* subgroups. Related to Figure 2. (A) Relative *pzg* mRNA levels in the fat body of wild type flies post *E. coli* or *S. typhimurium* infection. Flies treated with sterile PBS were used as a control. The fold change of mRNA expression was normalized to that of 0 h. Four independent repeats (n=20 flies fat body tissues per repeat) were performed at each time point for each group. (B and C) Predicted Relish/NF- κ B-binding motifs in the promoter locus of *mrt* (B) and *pzg* (C) genes of five *Drosophila*

subgroups. Dmel, *Drosophila melanogaster*; Dsim, *D. simulans*; sec, *D. sechelia*; Dyak, *D. yakuba*; Dere, *D. erecta*. S1-S5 represent the location site (red color) of conserved binding motifs. Sequence alignment is analyzed by BLAST in flybase website. TSS: transcription start site. (D) Relative luciferase activities of *pzg* (1.5 KB upstream of ATG, all predicted Relish binding sites are covered) reporter in S2* cells after double strand RNA (dsRNA) and PGN (35 µg/ml) treatment. All data were normalized to dsGFP control group at 0 h. Three independent repeats were performed at each time point for each treatment. (E) Schematic diagram of the *mrt* promoter locus and the plasmid constructs used for luciferase assay. The full length (F-*mrt*(RelB):-1.5k to +1bp), truncated length (T-*mrt*(RelB):-870 to +1bp) and mutant length (T-*mrt*:-870 to +1bp without binding motifs) of *mrt* promoter were indicated. Error bars represent the mean ± s.d.. Data were analyzed by One-way ANOVA with Tukey's multiple-comparison test (A) and Student's t test (D). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, no significance.

Fig. S4. *plin1* deficiency enhances Bmm/ATGL-dependent lipolysis in the condition of sustained IMD activation. Related to Figure 3. (A) TAGs level change in the whole body of indicated one-week or three-week old flies. The change of TAG levels at 3 w was normalized to that of 1w for each genotype. The number on the right of each line represents the percentage reduction of TAGs level at 3 w compared with 1 w for each genotype. Six independent replicates at each time point for each genotype were performed (n =12 flies per replicate). (B) Relative TAGs levels in the whole body of indicated one-week old adult flies. All values of TAGs level were normalized to that of 0h of *ppl-GAL4*>+ control flies. Six independent replicates for each genotype were performed (n =12 flies per replicate). Error bars represent the mean ± s.d.. Data were analyzed by One-way ANOVA with Student t-test. **p* < 0.05; ****p* < 0.001.

Fig. S5. Overexpression *plin1* compromises AMP responses. Related to Figure 4. (A and B) Relative *dipteracin* (*Dpt*) and *attacin-A* (*AttA*) mRNA expression in the fat body of *plin1*³⁸ mutant flies and wild type flies at indicated time points post *E. coli* (A) or *S. typhimurium* (B) infection. Four independent repeats were performed (n = 20 per repeat). (C and D) Relative *dipteracin* (*Dpt*) and *attacin-A* (*AttA*) mRNA expression of *ppl-GAL4*> *plin1* flies and *ppl-GAL4*> + control flies at indicated time points post *E. coli* (C) and *S. typhimurium* (D) infection. Four independent repeats were performed (n = 20 per repeat). Error bars represent mean ± s.d. Data was analyzed by Multiple t-tests. ***p* < 0.01; ****p* < 0.001.

Table S1: Primers used in this study.

Genes	Forward primer	Reverse primer
qRT-PCR primers		
<i>Rp49</i>	AGATCGTGAAGAAGCGCACCAAG	CACCAGGAACTTCTTGAATCCGG
<i>Dpt</i>	GGCTTATCCGATGCCCGACG	TCTGTAGGTGTAGGTGCTTCCC
<i>AttA</i>	CACAACTGGCGGAACCTTTGG	AAACATCCTTCACTCCGGGC
<i>plin1</i>	CAGCGCATACCACTGGTCTAT	GCATTACCGATTGCTTGACAG

<i>plin2</i>	CGAGCGCCTCCTTGAATAC	AGAACTCTTGCCATTCTGCAC
<i>DGAT2/CG1942</i>	GATTACTTTCCCGTGGAGCTGG	CCCATGTTAATGCCAATGCCTG
<i>DGAT1/mdy</i>	AAGACAGGCCTCTACTATTGT	CCCATCATGCCCATAAATGCC
<i>DGAT/CG1941</i>	GTTGCTGCCGTGCTGTTCTAT	GTGGGTCTCTTGTGATTTCGC
<i>DGAT/CG1946</i>	CTTCTTTACGCTGCCCATCTCG	CCATTACCTTCCATAATGCCG
<i>GPAT1/CG5508</i>	CCTTTTGGCCTTCACTTCC	TCTCGATCTGCTTCGTGTTC
<i>APGAT4/CG4753</i>	CTTCACATGCGGATTCTTCGTC	ACCATTCCGCCACACAGACAAG
<i>APGAT3/CG4729</i>	CTCTGTCCTGCATTTTCTACTACG	CTTGCTGATCTGCGTCTTGTTG
<i>APGAT2/CG3812</i>	GCTGGCCTAATCTTCATCG	CCCACAGTTTGATACGTTGC
<i>APGAT1/CG17608</i>	CTGTGCGTGCCCTTTATGATTC	CAGACCACGAACCTCCATTG
<i>Lipin</i>	GACATCCGCGATCTGTTTCCC	GGACTGGAATGTTTGGGTCAGC
<i>Lip4</i>	GACCGAACAAGGGACTTGG	CGAGTACGTATTGCCACGAA
<i>ACC</i>	GGAACACTGAACTGGGGAAA	GCAATCACGCTGGAGTTGTA
<i>FASN</i>	CGAATCCATCAATATGCCCCG	ACATAGTGACCACCACCCAGGT
<i>ACSL</i>	TTCCGGCAAGATTATGCGTC	CAGTTGCTCCACAATTTGCTCA
<i>dHSL</i>	TTGCCCTCTTCAATGACAATCTG	CTGGTGGGTGTCCTTTCCA
<i>BMM</i>	AAGTATGCACCGCATCTGTTG	CAAATCGCAGAGGAGACAGC
<i>sws/CG2212</i>	CTGGATGGCGGCTATGTGAATAA	CCATTTCTTGATAGAGCAGCCAC
<i>Cct1</i>	TCCACCTCGCAGCAATTCTTC	GTAGTCACATCTATCCCGCTCC
<i>Fatp</i>	GAAATCCTTCTCGCGAATTCCT	GGAGATGAAAGCCATATCGCC
<i>Rfabg</i>	GGTTTCTGGCATATTGTTGA	TAGGGTGCATATGCC ATCAA
<i>DUOX</i>	GCTGCACGCCAACCACAAGAGACT	CACGCGCAGCAGGATGTAAGGTTT
<i>mrt</i>	GGAGGATATTCTCGGAGTGGAGC	GCTTCCTGCCTCGTAGTCGAAC
<i>pzg</i>	GCTGAGGAACCACAACCATCTGAC	GTAACCTCGCCTTCGCCAGATT
Primers for plasmid construction and dsRNA synthesis		
<i>F-mrt(RelB)-luc</i>	CGAGATCTGCGATCTAAGTATAACG TCGTAGCGCACACGCACACC	CAGTACCGGAATGCCAAGCTGGGT GCACCCTTTGATCAAGGTCTT
<i>pzg-luc</i>	CGAGATCTGCGATCTAAGTACACAG TAGCAGCACAACGGAGACG	CAGTACCGGAATGCCAAGCTCTGT AGCAGTTCTCGACGGACGCGT
<i>T-mrt(RelB)-luc</i>	CGAGATCTGCGATCTAAGTAGCCAC TTTGTGCGAGTGTATCTGT	CAACAGTACCGGAATGCCAGCTGG GTGCACCCTTTGATCAAG
<i>T-mrt-luc</i>	CGAGATCTGCGATCTAAGTACGTTA GCTTTTGTGCTGTCATTCTG	CCAACAGTACCGGAATGCCAGATG CTGTTGGAAAACAAGCAA
<i>dsRelish</i>	TAATACGACTCACTATAGGGAGATC AAACACGTGCCGC	TAATACGACTCACTATACCCAGACT CACGCTCTGTCTC
<i>dsGFP</i>	TAATACGACTCACTATAGGGAGAAT GGTGAGCAAGGGCGAGGA	TAATACGACTCACTATAGGGAGACT TGTACAGCTCGTCCATGC

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