1 **Macrophage-derived insulin/IGF antagonist Impl2 regulates systemic metabolism for** 2 **mounting an effective acute immune response in *Drosophila***

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10 **Abstract**

11 In response to invading pathogens, macrophages metabolically polarize toward Hif1α-induced
12 aerobic glycolysis, requiring increased supply of nutrients. Here, we show that in order to obtain
13 sufficient resources, *Drosophila* macrophages release the insulin/IGF antagonist Impl2 in a Hif1α-
14 dependent manner as a reflection of adopted polarization. Impl2 remotely induces Foxo-mediated
15 lipid mobilization and the release of lipoproteins and carbohydrates from adipose tissue to be
16 utilized by the activated immune system. Although these Impl2 effects are essential in acute
17 streptococcal infection, they become maladaptive during chronic intracellular *Listeria* infection. The
18 relevance of our model to mammalian immuno-metabolism is documented by the increased
19 expression of the Impl2 homolog IGFBP7 in human macrophages exposed to *Streptococcus*.
20 Therefore, we postulate that Impl2/IGFBP7 induce resource mobilization, which is beneficial during
21 the acute phase of infection but may act as cachectic factors in patients with cancer, sepsis, or
22 obesity.

24 **Keywords:**

25 Macrophage, immuno-metabolism, *Drosophila*, immunity, bacterial infection, *Streptococcus*, Impl2,
26 IGFBP7, energy mobilization, Hif1α, aerobic glycolysis, macrophage polarization, insulin resistance,
27 selfish immune system, adipose tissue remodeling, Foxo, infection-induced insulin resistance,
28 *Listeria*, Insulin/IGF antagonist, wasting, cachexia

30 **Introduction**

31 Macrophages represent the frontline in defence against invading pathogens. Although the
32 effectiveness of the immune response correlates with the number of immune cells (Nicholson and
33 Nicholson 2008), their maintenance requires energy and excessive activation can lead to a myriad of
34 pathologies and metabolic disorders (Shattuck-Heidorn et al. 2016; Zmora et al. 2017). Animals have
35 therefore developed a strategy that allows them to maintain a sufficient number of quiescent
36 immune cells that can be rapidly activated in response to the detection of pathogen- or danger-
37 associated molecular patterns (Kelly and O'Neill 2015). Numerous populations of sentinel
38 macrophages wait for activation stimuli without being an energy burden. As a consequence,
39 macrophages depend on rapid and sufficient supplementation from external sources, making the
40 acute phase of the immune response challenging for the whole organism (Newsholme et al. 1986).

41 In response to recognition of the invading pathogen, macrophages must rapidly alter their
42 metabolism to generate enough energy and precursors to support their bactericidal function.
43 Therefore, bactericidal (M1) macrophages substantially increase the rate of glycolysis and the
44 pentose phosphate pathway and rewire their mitochondrial metabolism in a Hif1α-dependent

manner (Van den Bossche, O'Neill, and Menon 2017). Such a metabolic setup resembles the Warburg effect originally described as a unique metabolic program for cancer cells (Warburg 1925). We have recently shown that the metabolic polarization of macrophages is an ancient and evolutionarily conserved process, since even *Drosophila* macrophages undergo a metabolic switch triggered by Hif1 α , which is essential for their bactericidal function (Krejčová et al. 2019).

An adverse aspect of M1 polarization is that these cells require more energy and become functionally dependent on external sources of glucose, glutamine, and lipids. Therefore, in response to their metabolic activation, macrophages release signals that regulate systemic metabolism and thus ensure their supply of nutrients at the expense of other organs (Straub 2014). Such privileged behavior, in which macrophages usurp nutrients from other processes, is crucial for an effective immune response (Bajgar and Dolezal 2018). One of the factors mediating such behavior is extracellular adenosine, which links the current metabolic state of activated immune cells to the systemic mobilization of carbohydrates, which serve as a source for immune defense (Bajgar et al. 2015; Bajgar and Dolezal 2018). However, the response is very complex and we assume the existence of other signaling factors with an analogous function.

To discover other signaling factors that are released by activated immune cells and regulate systemic metabolism, we sought inspiration in neoplastic tumor research. This idea is based on the notion that both tumors and activated immune cells share common features of their cellular metabolism because both use Hif1 α -triggered aerobic glycolysis as the predominant metabolic program (Biswas and Mantovani 2012; Nagao et al. 2019). They also share the impact on systemic metabolism, as both cancer patients and septic patients exhibit a wasting-like phenotype caused by cytokine-induced insulin resistance (Dev, Bruera, and Dalal 2018). Although insulin resistance is mostly studied as a pathological condition, its evolutionary conservation indicates that it must carry an important physiological function (Soeters and Soeters 2012; Odegaard and Chawla 2013). Hence, we hypothesize that activated immune cells may release the same factors as cancer cells, but with a beneficial role for the acute response as opposed to cancer-induced cachexia.

In this study, we focus on the insulin/IGF antagonist Impl2 (Imaginal morphogenesis protein-Late 2) as a potential candidate for such a role since it is released by neoplastic cancer cells to suppress insulin signaling via binding to *Drosophila* insulin-like peptides thus causing energy wasting (Alee 2011; Arquier et al. 2006; Honegger et al. 2008; Kwon et al. 2015; Figueroa-Clarevega and Bilder 2015). Besides the production by neoplastic tumors, Impl2 is known to be released by lipid-overloaded macrophages (Morgantini et al. 2019), as well as other cells employing metabolic programs dependent on the activity of Hif1 α in *Drosophila* (Alee 2011; Owusu-Ansah, Song, and Perrimon 2013; Kwon et al. 2015; Figueroa-Clarevega and Bilder 2015). Therefore, we decided to test the role of Impl2 as a macrophage-derived signaling factor responsible for nutrient mobilization during the acute immune response to bacterial infection.

We show here that activated macrophages produce Impl2 in the acute phase of infection, resulting in Foxo-mediated changes in adipose tissue metabolism. As an outcome of the Impl2 action, we observed an increased titer of circulating carbohydrates and lipids as well as their accumulation in macrophages. The role of Impl2 is necessary for effective resistance to streptococcal infection and effective phagocytosis. In contrast to this beneficial role in the fight against extracellular pathogens, the effects of Impl2 are maladaptive in response to intracellularly growing *Listeria*, and its chronic activation leads to developmental defects and excessive melanization. Conservation of Impl2 function between insects and mammals is indicated by increased expression of the Impl2 homolog IGFBP7 in streptococcus-treated human macrophages.

Results

Impl2 expression increases in immune-activated macrophages in a Hif1 α -dependent manner

To test the potential role of Impl2 during infection, we first monitored its expression profile during the acute phase of *Streptococcus pneumoniae* infection. Impl2 expression increased significantly in infected flies compared to PBS-injected controls, starting as early as 3 hours post-infection (hpi) and reaching up to a 3-fold increase in expression 21 hpi at the whole organism level (Figure 1A). We employed a transcript-specific Impl2-RA Gal4 driver (Bader et al. 2013) to drive the fluorescent marker UAS-mCherry to identify tissues responsible for infection-induced rise in Impl2 expression. The pattern of Impl2-positive cells resembled the characteristic distribution of macrophages in adult flies (Figure 1B). Moreover, the number of these mCherry-expressing cells increased substantially upon infection (Figure 1B and E). To verify that the cells expressing Impl2-mCherry are phagocytes, we injected the flies with the phagocytic marker *S. aureus*-pHrodo-Green and it indeed co-localized with these cells (Figure 1C). In addition, cells expressing Impl2 also actively recognized and engulfed *S. pneumoniae* bacteria *ex vivo* (Figure 1D). The fact that Impl2 production induced by infection is specific for macrophages is supported by its increased expression in FACS-sorted CrqGal4>UAS-GFP-labeled macrophages (Figure 1F).

Since there are three alternative transcriptional start sites for Impl2, we decided to analyze the expression pattern of each isoform 24 hpi to better understand the regulation of their expression. Consistent with the expression pattern of the *Impl2-RA* transcript-specific Gal4 driver (Figure 1B), *Impl2-RA* transcript expression was the highest of all transcript variants in macrophages and was hardly detectable in fat body or muscles (Figure 1G). While the *Impl2-RB+D* forms were the most abundant variants in adipose tissue, their expression was five-times weaker than *Impl2-RA* in macrophages (check the different y-axis scales in Figure 1G). In addition, the expression of the *Impl2-RA* isoform increased eightfold in macrophages, indicating the most significant increase in expression compared to other transcripts in response to infection (Figure 1G). The *Impl2-RA* transcript variant expressed in macrophages is therefore a major contributor to the overall increase in Impl2 expression observed at the onset of infection.

The previously characterized metabolic switch of activated macrophages is regulated by the transcriptional factor Hif1 α (Krejčová et al. 2019), which is known to be a potent regulator of Impl2 expression in cells relying on anaerobic metabolism (Allee 2011, Li et al. 2013; Owusu-Ansah, Song, and Perrimon 2013). We, therefore, hypothesized that the same regulation could underlie the infection-induced rise in Impl2 in activated macrophages. Macrophage-specific knockdown of Hif1 α during infection (see Figure 1-figure supplement 1A for efficiency) resulted in the inability of these cells to trigger characteristic infection-induced Impl2 expression, and this effect was particularly evident in the *Impl2-RA* isoform (Figure 1H). We identified a cluster of four Hif1 α binding sites upstream of the macrophage-specific transcription start site of *Impl2-RA* (Figure 1I). In addition, this enhancer region shows the strongest hypoxic induction of any sequence surrounding the Impl2 gene (Kamps-Hughes et al. 2015 and Figure 1I), suggesting that Hif1 α may directly drive the transcription of Impl2-RA in macrophages. In-depth analysis of this 500-bp region revealed the presence of eight immune and stress response elements that cluster together (four hypoxia response elements, two Relish bindings sites, and two heat-shock factor binding sites; Figure 1I and Figure 1-figure supplement 2).

These experiments identified activated macrophages as the predominant producers of Impl2 in infected adult flies. The increase of Impl2 production is associated with the metabolic switch induced by Hif1 α in macrophages, presumably by direct binding of Hif1 α and other stress-related transcription factors to the regulatory sequence of the *Impl2-RA* isoform.

Macrophage-derived Impl2 is required for mobilization of reserves to be utilized by macrophages

Activated macrophages release signaling factors to mobilize reserves to provide sufficient nutrients for the activated immune system. We show here that macrophages increase Impl2 production during infection, and because Impl2 was previously associated with reserve mobilization that led to wasting in flies with cancer, we further tested the impact of macrophage-derived Impl2 on systemic metabolism.

For this purpose, we employed Drosophila genetic tools for either macrophage-specific (CrqGal4) or macrophage-specific and time-restricted (HmlGal4, Gal80^{TS}) manipulation of Impl2 expression. Using conventional constructs for Impl2 knockdown (Impl2^{RNAi}) and overexpression (Impl2^{CD5}), we achieved significant changes in Impl2 expression (see Figure 1-figure supplement 1B for efficiency). Such experimental manipulations allowed us, on the one hand, to prevent upregulation of Impl2 in macrophages induced by infection and, on the other hand, to simulate an increase of Impl2 in uninfected individuals.

Both infection and overexpression of Impl2 in macrophages significantly reduced triglyceride content in whole flies (Figure 2A), accompanied by dramatic changes in adipose tissue morphology (Figure 2B-E). The average size of lipid droplets decreased with increasing number (Figure 2B, C, and E). The overall area of adipose tissue occupied by lipid droplets was markedly smaller (Figure 2D). It should be emphasized that these infection-induced effects were suppressed by macrophage-specific Impl2 knockdown (Figure 2B-E). Detailed lipidomic analysis of adipose tissue by mass spectrometry revealed that both infection and macrophage-specific overexpression of Impl2 cause a proportional shift of lipid content from storage lipid forms (triglycerides) to polar lipid species (phosphatidylethanolamine, phosphatidylinositol) participating in lipid mobilization and transport (Figure 2F). These effects were hampered by Impl2 knockdown (Figure 2F). Similar effects were also observed for glycogen stores when both infection and macrophage-specific overexpression of Impl2 significantly reduced glycogen content in whole flies, and knockdown of Impl2 suppressed this effect (Figure 2G). These data thus indicate that macrophage-derived Impl2 may serve as a mediator of crosstalk between the immune system and adipose tissue.

Besides substantially increased lipolytic and glycogenolytic programs in the adipose tissue of infected individuals, we also observed enhanced autophagy in the adipose tissue of Atg8a-mCherry-bearing flies (Figure 3A), further implying an increase in catabolic metabolism. These metabolic changes induced by infection are consistent with the expression pattern of many metabolic genes in adipose tissue. The *MTP*, *apoLPP*, *apoLTP*, and *Bmm* genes associated with lipid mobilization, as well as the autophagy-associated *Atg1* and *Atg6* genes, all increased in adipose tissue during infection (Figure 3B and Figure 3-figure supplement 1) and were under the control of macrophage-derived Impl2. Indeed, Impl2 knockdown suppressed this effect, while Impl2 overexpression mimicked the infection-induced response (Figure 3B and Figure 3-figure supplement 1). A remarkable number of these genes are known to be Foxo targets. The transcriptional activity of Foxo is regulated by insulin signaling and because Impl2 is an antagonist of insulin signaling, we analyzed the impact of macrophage-derived Impl2 on the subcellular localization of Foxo in adipose tissue. We found that Foxo was clearly localized to the nucleus upon infection, while remaining mostly in the cytoplasm of adipocytes of uninfected flies (Figure 4). Macrophage-specific overexpression of Impl2 recapitulated the infection-induced pattern and, conversely, knockdown reversed the effect of infection on Foxo localization (Figure 4); these effects of Impl2 on Foxo localization are in agreement with the expression of Foxo target genes (Figure 3B and Figure 3-figure supplement 1).

Stimulated catabolism of glycogen and triglycerides is manifested by hyperglycemia and hyperlipidemia in the circulation of infected flies when increased titers of glucose, trehalose, and cholesteryl-ester were detected in their hemolymph (Figure 5A-D). Accordingly, infection-activated macrophages increased the accumulation of these energy-rich compounds and cholesterol as a cholesteryl-ester derivative (Figure 5E-H). These results are supported by the increased occurrence of

lipid droplets in the cytosol of macrophages activated by infection and stained with the neutral lipid dye OilRedO (Figure 5I and J). The ability of activated macrophages to recognize and uptake lipoproteins was verified by injection of labeled human lipoproteins (LDL-pHrodo; Figure 5K) that can be endocytosed by the activity of the scavenger receptor croquemort (fly homolog of CD36), which is abundantly expressed in *Drosophila* macrophages. The mobilization of reserves, which leads to an increase in the amount of nutrients in the circulation and subsequently to their increased uptake by macrophages, depends on the production of Impl2 by macrophages. Indeed, macrophage-specific Impl2 knockdown suppresses all observed effects stimulated by infection, while Impl2 overexpression mimics these effects even in uninfected individuals (Figure 5A-I).

Combining all these results, we can conclude that Impl2, produced by macrophages during infection, triggers a Foxo-mediated transcriptional program in adipose tissue that provides macrophages with the necessary nutrients.

Impl2 is required for an effective immune response but can be detrimental during chronic infection

The efficiency of the immune response depends on the supply of sufficient energy and the necessary precursors to the activated immune cells. Therefore, we decided to investigate whether the Impl2-mediated metabolic supplementation for macrophages is essential for mounting an adequate immune response. The lack of Impl2 produced by macrophages significantly reduced the survival of *S. pneumoniae*-infected flies (Figure 6A), which was reflected in the elevated pathogen load (Figure 6B), both indicating a decreased resistance in these individuals. This notion was further supported by the reduced phagocytic rate in these flies (Figure 6E and F). Interestingly, overexpression of Impl2 24 hours prior to infection improved the resistance of these individuals to streptococcal infection, as evidenced by lower pathogen load and improved survival (Figure 6C and D). These observations suggest that Impl2, produced by metabolically activated acute phase macrophages, is essential for systemic metabolic changes that support their efficient bactericidal function.

Although our results demonstrate a beneficial role of Impl2 during acute infection, previous work showing the pathological role of Impl2 in cancer-induced cachexia (Kwon et al. 2015) suggests that the beneficial role of Impl2 may be restricted to a short-time window of acute response. Therefore, we tested the effects of Impl2 overexpression in macrophages over the lifespan of developing individuals and also challenged flies with chronic infection caused by the intracellular pathogen *Listeria monocytogenes*. Interestingly, the mere transient overexpression of Impl2 in macrophages led to a frequent occurrence of melanized spots (Figure 7A); even more severe outcomes were achieved by overexpression of Impl2 during development, resulting in a smaller adult size (data not shown; similar result to Honegger et al. 2008) accompanied by impaired dorsal closure in roughly half of the individuals (Figure 7A). In addition, overexpression of Impl2 reduced resistance to *L. monocytogenes* infection with chronically increased pathogen burden (Figure 7D and E), suggesting that the enhanced effects of Impl2, in contrast to the acute response, are detrimental in chronic infection. Consistent with this result, suppression of Impl2 production improved survival and significantly reduced bacterial load during this chronic type of infection (Figure 7B and C).

In conclusion, Impl2-mediated reallocation of resources to macrophages is essential for an adequate antibacterial immune response and resistance to infection. On the other hand, chronic or excessive production of Impl2 can cause developmental defects and rather enhance the deleterious effects of chronic infections.

Gene expression of *Impl2* mammalian homolog *IGFBP7* is increased in immune-activated THP-1 cells

We have previously shown that IGFBP7, the mammalian homologue of Impl2, is produced by lipid-loaded liver macrophages in response to feeding a high fat diet, with consequent effects on systemic metabolism through the regulation of insulin signaling in hepatocytes. Notably, Impl2 released from lipid-loaded macrophages appears to play an analogous role in obese *Drosophila* (Morgantini et al. 2019). To test the relevance of the role of Impl2 in humans during infection, we measured the expression of IGFBPs in PMA-activated THP-1 human monocytic cell line 24 hours after exposure to *S. pneumoniae* bacteria (Figure 8A). The expression of *IGFBP1*, *IGFBP2*, *IGFBP5*, and *IGFBP6* was not detected, indicating that these genes are not expressed in these cells (data not shown). Interestingly, *IGFBP7* gene expression was significantly increased in inoculated culture compared to PBS-treated controls (Figure 8B). In addition, gene expression of anti-inflammatory *IGFBP3* was reduced four-fold while *IGFBP4* showed no significant change (Figure 8B). **These data therefore indicate that the increase in Impl2 / IGFBP7 production by macrophages in response to infection is evolutionarily conserved in mammals. These results not only validate the importance of the acute bacterial infection model in *Drosophila* but also suggest that investigating the role of IGFBP7 in systemic metabolic regulation and inter-organ communication during infection would be of interest in humans.**

Discussion

In this work, we show that infection-driven Hif1 α transcriptional activity connects intracellular metabolic switch with Impl2 production in activated macrophages. Impl2 subsequently induces the mobilization of adipose tissue resources to be provided for activated immune cells. This metabolic rearrangement of adipose tissue is mediated by the transcription factor Foxo, which results in the acceleration of lipolysis and assembly of lipoproteins. The increased titer of circulating lipids facilitates the utilization of lipids by macrophages and thus supports their bactericidal function (Figure 9). Although such Impl2-induced metabolic adaptation is essential for an acute immune response, it can become maladaptive in cases of its chronic activation or infection by intracellular pathogens that metabolically exploit the host cell. This can be considered a remarkable example of how a beneficial metabolic program can become maladaptive in chronic diseases. That this mechanism might not be limited to insects is indicated by an experiment showing that human macrophages activated by the same bacteria increase the expression of IGFBP7, the human homologue of *Drosophila* Impl2.

We have previously shown that *Drosophila* macrophages undergo evolutionarily conserved polarization to the M1 phenotype, thus adjusting their metabolism toward Hif1 α -induced aerobic glycolysis in response to bacterial challenge. This cellular metabolic shift is accompanied by systemic metabolic changes necessary to supplement the sudden needs of macrophages (Krejčová et al. 2019). Coordination of cellular metabolism in macrophages with systemic metabolism is crucial for resistance to infection and anticipates the existence of circulating factors mediating this inter-organ crosstalk (Dolezal et al. 2019). Inspired by research on neoplastic cancer cells (Kwon et al. 2015; Figueroa-Clarevega and Bilder 2015; Bunker et al. 2015), we focus in this study on the cachectic factor Impl2 as a candidate previously linked to Hif1 α transcriptional activity while having the potential to regulate systemic metabolism (Alee 2011; Li et al. 2013). To test whether the interconnection between Hif1 α activity and Impl2 expression during hypoxia also applies to infection-activated macrophages, we performed a genomic *in silico* meta-analysis of hypoxic enhancer activity and Hif1 α binding sites (Kamps-Hughes et al. 2015). This approach revealed the induction of a hypoxic enhancer in close vicinity to the transcription start site of the *Impl2-RA* transcript variant, which is further supported by the presence of four hypoxia response elements at this locus. *Impl2-RA* is the most strongly expressed transcriptional variant in macrophages and its expression increases even more after infection. **This infection-induced increase depends on Hif1 α ,**

indicating a connection of Impl2 expression with an internal metabolic switch in infection-activated macrophages.

We have previously identified extracellular adenosine as an immune cell-derived systemic factor with an analogous function to Impl2 in mobilizing carbohydrate stores to be available to the immune system (Bajgar et al. 2015; Bajgar and Dolezal 2018). In addition to regulating carbohydrate metabolism, a prominent effect of Impl2 is the regulation of systemic lipid metabolism. As documented in this work by various approaches, **macrophage-derived Impl2 mediates infection-induced catabolic processes in adipose tissue resulting in the assembly and release of lipoproteins for the redistribution of resources to metabolically demanding tissues. Thus, infection-induced Impl2 production results in an increased titer of circulating lipids, which are then used by macrophages to effectively fight invading pathogens.** The observed metabolic changes in infection-challenged flies resemble lipemia as a characteristic symptom of sepsis in severely ill patients (Harris, Gosnell, and Kumwenda 2000). In sepsis-induced lipemia, hepatocytes release low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), providing lipids to be preferentially utilized by bactericidal macrophages in the periphery (Khovidhunkit et al. 2004; Aspichueta et al. 2012). Scavenger receptor-mediated endocytosis of serum lipoproteins, together with attenuated reverse cholesterol transport, both contribute substantially to cholesterol accumulation in bactericidal immune cells (Podrez et al. 2000). There are many immune-related functions in macrophages dependent on sufficient delivery of lipids, from phagocytosis and maturation of phagolysosome to catecholamine production or immune memory formation via the mevalonate pathway (summarized in Remmerie and Scott 2018). Although providing substantial amounts of lipids to macrophages is essential for their proper function during the acute phase of infection, chronic exposure of macrophages to excessive lipids may lead to the adoption of a foam-cell phenotype promoting metabolic syndrome and atherosclerosis (Chistiakov et al. 2017; Febbraio, Guy, and Silverstein 2004).

Although the mechanism of infection-induced lipid catabolism and lipoprotein assembly has not been fully elucidated, we revealed that nuclear translocation of Foxo, followed by increased expression of several Foxo-target genes involved in this process, was regulated by macrophage-derived Impl2. Since Foxo-driven mobilization of lipid stores requires suppression of insulin signaling in adipose tissue (Molaei, Vandehoef, and Karpac 2019; Luong et al. 2006), the effect of Impl2 can be explained by its well-documented ability to bind *Drosophila* insulin-like peptides (DILPs) and thus reduce insulin sensitivity in adipose tissue (Alee 2011; Alic et al. 2011; Arquier et al. 2006; Figueroa-Claresvega and Bilder 2015; Honegger et al. 2008; Kwon et al. 2015; Okamoto et al. 2013). Metabolic adjustments and the switch from anabolism to catabolism in adipose tissue during infection is regulated by multiple mechanisms (reviewed in Dolezal et al. 2019). In particular, Foxo has previously been associated with wasting in flies with chronic infection (Dionne et al. 2006), indicating rather its pathological effects during the prolonged immune response. Based on the aforementioned, the simplest explanation for the effect of Impl2 on the mobilization of adipose tissue sources is the suppression of insulin signaling to allow a metabolic program triggered by Foxo. **The remote Impl2 effect on reserve mobilization described in this work therefore represents a beneficial role of Impl2 during the acute immune response.** Suppression of insulin signaling is often associated with chronic inflammation and may become harmful to the organism by disrupting the metabolic balance. However, the theoretical concept of selfish immunity (Straub 2014) sees insulin resistance as an evolutionary adaptive mechanism for rerouting nutrients toward immunity during the acute immune response. Our results showing that Impl2 is required for an effective immune response through changes in lipid metabolism can be considered as experimental evidence of insulin resistance as an adaptive mechanism for mobilization of sources for acute phase of immune response.

Neoplastic tumors share a characteristic cellular metabolism (Warburg 1925) with bactericidal macrophages (Andrejeva and Rathmell 2017). **Here, we show that they may also share the production of Impl2, originally perceived as a cancer-derived cachectic factor** (Kwon et al. 2015;

Figuerola-Clavevega and Bilder 2015), **to regulate systemic metabolism**. Both tumor cells and activated immune cells produce Impl2 and thus subsequently silence systemic insulin signaling as a result of the adoption of Hif1 α -triggered aerobic glycolysis. The perception of Impl2 expression as a consequence of the cellular metabolic state is further supported by other conditions of mitochondria-independent generation of ATP, such as experimentally induced mitochondrial dysfunction, hypoxia, and neoplastic tumor growth (Alee 2011; Figuerola-Clavevega and Bilder 2015; Kwon et al. 2015; Li et al. 2013). Although this mechanism is beneficial to the organism in acute infection, it becomes harmful in the case of tumor growth or chronic infection, as demonstrated here by the negative effect of Impl2 on survival during chronic infection caused by the intracellular pathogen *L. monocytogenes*. This can be explained by the known behavior of various intracellular bacteria that use the supply of host cells and hijack their metabolic cascades to literally nourish themselves (Péan et al. 2017; Teng, Ang, and Guan 2017).

Our results revealed that human macrophages, activated by *S. pneumoniae*, increase the expression of IGFBP7, the human homolog of *Drosophila* Impl2, suggesting that a similar mechanism may function in humans. Notably, not only infection but also experimentally induced metabolic syndrome in both *Drosophila* and mammals led to increased expression of Impl2/IGFBP7 in macrophages (Morgantini et al. 2019). While Impl2-producing macrophages affect metabolism in the *Drosophila* fat body (integrating functions of both mammalian adipose tissue and liver), IGFBP7-producing Kupffer cells act directly on hepatocytes in order to induce systemic metabolic changes (Morgantini et al. 2019; Akiel et al. 2017). The ability of both Impl2 and IGFBP7 to bind extracellular insulin, thereby reducing systemic insulin signaling, further documents their functional homology, suggesting that the mechanism of their action may also be conserved (Honegger et al. 2008; Arquier et al. 2006; Oh et al. 1996). Accordingly, LPS-administered Kupffer cells have been shown to produce cytokines to affect Foxo-mediated release of LDL and VLDL from hepatocytes (Kamagate et al. 2008; Sparks and Dong 2009; Aspichueta et al. 2012). Although this mechanism of resource mobilization may be important for the acute phase response, when prolonged, it contributes to the progression of metabolic syndrome and atherosclerosis (Tomkin 2012). It is therefore not surprising that the levels of circulating IGFBP7 are considered a marker of systemic metabolic imbalances accompanying several human diseases, such as obesity, acute kidney injury, liver fibrosis, and chronic obstructive pulmonary disease (Liu et al. 2015; Gunnerson et al. 2016; Martínez-Castillo et al. 2020; Ruan et al. 2017).

Taking our data together with previous work, we can conclude that the insulin/IGF antagonist Impl2, released from M1 macrophages as a reflection of their metabolic polarization, mediates the mobilization of nutrients from adipose tissue by inducing insulin resistance to provide sufficient resources for the immune system. Despite the fact that both Impl2 and insulin resistance are mostly studied in the context of pathological conditions such as cancer-induced cachexia, obesity and chronic inflammatory conditions, our data revealed that they may play a beneficial role in the acute phase of bacterial infection. In addition, the relevance of our model is supported by the production of IGFBP7, the Impl2 homolog, in response to streptococcal infection in human macrophages. This leads us to the hypothesis that analogous mechanisms may also apply to macrophage-induced nutrient mobilization from hepatocytes during the acute phase of the immune response, which may become detrimental under chronic conditions.

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MATERIALS AND METHODS

Drosophila melanogaster strains

The flies were raised on a diet containing cornmeal (80 g/l), sucrose (50 g/l), yeast (40 g/l), agar (10 g/l), and 10%-methylparaben (16.7 mL/l) and maintained in a humidity-controlled environment with a natural 12 h/12 h light/dark cycle at 25°C. Flies carrying *Gal80* protein were raised at 18°C and transferred to 29°C 24 h prior to infection in order to degrade temperature-sensitive *Gal80*. Prior to the experiments, flies were kept in plastic vials on a sucrose-free cornmeal diet (cornmeal 53.5 g/l, yeast 28.2 g/l, agar 6.2 g/l and 10%-methylparaben 16.7 mL/l) for 7 days. Flies infected with *S. pneumoniae* were kept on a sucrose-free cornmeal diet in incubators at 29°C due to the temperature sensitivity of *S. pneumoniae*. They were transferred to fresh vials every other day without the use of CO₂ to ensure good food condition. Flies infected with *L. monocytogenes* were kept on a sucrose-free cornmeal diet at 25°C. The *Drosophila* Stock Center in Bloomington provided *ImpL2^{RNAi}* (*y1 sc* v1; P{TRiP.HMC03863}attP40; FBst0055855*) *TRiP^{control}* (*y[1] v[1] ; P{y[+t7.7]=CaryP}attP40; FBst0036304*) and *20xUAS-6xmCherry* (*P{20XUAS-6XmCherry-HA}attP2; FBtp0094992*) flies. *ImpL2-Gal4* (*ImpL2-RA-Gal4; FBal0290965*) and *UAS-ImpL2^{CDS}* (*UAS-s.ImpL2; FBal0249386*) were kind gifts from Hugo Stocker. *CrqGal4>2xeGFP* were obtained from Marc Dionne. The *Atg8a-mCherry* strain was kindly provided by Gabor Juhasz. The *w¹¹¹⁸* strain has a genetic background based on *CantonS*.

Genotypes of experimental models

Figure 1

ImpL2Gal4>UASmCherry corresponds to *+/+*; *20xUAS-6xmCherry/+*; *ImpL2-Gal4/+*

HmlGal4>GFP refers to *HmlΔ-Gal4 UAS-2xeGFP/HmlΔ-Gal4 UAS-2xeGFP; +/+*

HmlGal4>GFP; Hif1α^{RNAi} corresponds to *HmlΔ-Gal4 UAS-2xeGFP/UAS-Hif1α^{RNAi}; +/+*

Figure 2

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Figure 3

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Atg8a-mCherry refers to gen *+/+; Atg8a-mCherry/Atg8a-mCherry*

Figure 4

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Figure 5

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Crq>Gal4; UAS2xGFP x ImpL2^{RNAi} refers to *+/+; UAS-ImpL2^{RNAi}/+; Crq-Gal4, UAS-2xeGFP/+*

Crq>Gal4; UAS2xGFP x ImpL2^{cds} corresponds to *+/+; +/+; Crq-Gal4, UAS-2xeGFP/UAS-ImpL2^{cds}*

Crq>Gal4; UAS2xGFP x TRiP^{control} refers to *+/+; +/+; Crq-Gal4, UAS-2xeGFP/TRiP^{control}*

Crq>Gal4; UAS2xGFP x W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; Crq-Gal4, UAS-2xeGFP/+*

CrqGal4>GFP refers to *+/+; Crq-Gal4, UAS-2xeGFP/Crq-Gal4, UAS-2xeGFP*

Figure 6

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Crq>Gal4; UAS2xGFP x ImpL2^{RNAi} refers to *+/+; UAS-ImpL2^{RNAi}/+; Crq-Gal4, UAS-2xeGFP/+*

Crq>Gal4; UAS2xGFP x ImpL2^{cds} corresponds to *+/+; +/+; Crq-Gal4, UAS-2xeGFP/UAS-ImpL2^{cds}*

Crq>Gal4; UAS2xGFP x TRiP^{control} refers to *+/+; +/+; Crq-Gal4, UAS-2xeGFP/TRiP^{control}*

Crq>Gal4; UAS2xGFP x W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; Crq-Gal4, UAS-2xeGFP/+*

CrqGal4>GFP refers to *+/+; Crq-Gal4, UAS-2xeGFP/Crq-Gal4, UAS-2xeGFP*

Figure 7

ImpL2^{RNAi} refers to *+/+; HmlΔ-Gal4/ UAS-ImpL2^{RNAi}; P{tubPGal80ts}/+*

ImpL2^{cds} corresponds to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/UAS-ImpL2^{cds}*

TRiP^{control} refers to *+/+; HmlΔ-Gal4/+; P{tubPGal80ts}/TRiP^{control}*

W¹¹¹⁸ corresponds to *w¹¹¹⁸/+; HmlΔ-Gal4/+; P{tubPGal80ts}/+*

Crq>Gal4 x ImpL2^{cds} corresponds to *+/+; +/+; Crq-Gal4, UAS-2xeGFP/UAS-ImpL2^{cds}*

Hypoxic enhancer activity analysis

Genome-wide experimental data characterizing hypoxia-induced transcriptional enhancer activity (Kamps-Hughes et al. 2015) was used to analyze the ImpL2 region for hypoxic enhancers. Hypoxic transcriptional induction is defined as the ratio of expressed randomer tag counts in hypoxic versus normoxic conditions and was binned by 500-bp regions across the *ImpL2* locus. Each 500-bp bin was then analyzed for transcription factor binding sites corresponding to hypoxic (Hif1α), immune (Rel) and stress (HSF) response transcription factors. Transcription factor position frequency matrices were downloaded from the JASPAR database (https://academic.oup.com/nar/article/32/suppl_1/D91/2505159) and queried against the 500-bp bin sequences using BOBRO software (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3074163/>). BOBRO generates a p-value of enrichment for the position frequency matrix within the search sequence and also identifies the location of the predicted binding sites.

Gene expression analysis

Gene expression analyses were performed on several types of samples: six whole flies, six thoraxes, six fat bodies, or 20,000 isolated macrophages. Macrophages were isolated by a cell sorter as described in (Krejčová et al. 2019) while dissections were made in PBS, transferred to TRIzol Reagent (Invitrogen) and homogenized using a DEPC-treated pestle. Subsequently, RNA was extracted by TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Superscript III Reverse Transcriptase (Invitrogen) primed by oligo(dT)20 primer was used for reverse transcription. Relative expression rates for particular genes were quantified on a 384CFX 1000 Touch Real-Time Cycler (Bio-

Rad) using the TP 2x SYBR Master Mix (Top-Bio) in three technical replicates with the following protocol: initial denaturation - 3 min at 95°C, amplification – 15 s at 94°C, 20 s at 56°C, 25 s at 72°C for 40 cycles. Melting curve analysis was performed at 65–85°C/step 0.5°C. The primer sequences are listed in the Key Resources Table. The qPCR data were analyzed using double delta Ct analysis, and the expressions of specific genes were normalized to the expression of Ribosomal protein 49 (Rp49) in the corresponding sample. The relative values (fold change) to control are shown in the graphs. Samples for gene expression analysis were collected from three independent experiments. Data were compared with ANOVA followed by Tukey's multiple comparisons test in Graphpad Prism software. Sidak's multiple comparison correction was performed.

Metabolite measurement

To measure metabolite concentration, isolated macrophages, whole flies or hemolymph were used. Macrophages were isolated from 25 adult males by centrifugation (14,000 RPM, 5 min) through a silicagel filter into 50 µL of PBS. For measurement of metabolites from whole flies, five flies were homogenized in 200 µL of PBS and centrifuged (3 min, 4°C, 8,000 RPM) to discard insoluble debris. 50,000 macrophages were isolated by cell sorter as described above. Half of all samples were used for the quantification of proteins. Samples for glucose, glycogen, trehalose, and triglyceride measurement were denatured at 75°C for 10 min, whereas samples for protein quantification were frozen immediately in -80°C. Glucose was measured using a Glucose (GO) Assay (GAGO-20) Kit (Sigma) according to the manufacturer's protocol. Colorimetric reaction was measured at 540 nm. For glycogen quantification, sample was mixed with amyloglucosidase (Sigma) and incubated at 37°C for 30 min. A Bicinchoninic Acid Assay (BCA) Kit (Sigma) was used for protein quantification according to the supplier's protocol and the absorbance was measured at 595 nm. Cholesterol and cholesteryl esters were measured on isolated lipid fraction by using Cholesterol/Cholesteryl Ester Quantitation Kit (Sigma) according to the supplier's protocol. Triglycerides were measured using Triglyceride quantification Colorimetric/Fluorometric Kit (Sigma). For trehalose quantification, sample was mixed with trehalase (Sigma) and incubated at 37°C for 30 min. Samples for metabolite concentration were collected from three independent experiments. Measured data were compared in Graphpad Prism using ANOVA followed by Tukey's multiple comparisons test. Sidak's multiple comparison correction was performed.

Staining of lipid droplets

Flies were dissected in Grace's Insect Medium (Sigma) and subsequently stained with DAPI and Cell Brite Fix Membrane Stain 488 (Biotium) diluted with Grace's Insect Medium according to the manufacturer's protocol at 37°C. Tissues were washed in PBS and then fixed with 4% PFA (Polysciences). After 20 min, the tissues were washed in PBS and pre-washed with 60% isopropanol. Dissected abdomens were then stained with OilRedO dissolved in 60% isopropanol for 10 min. The tissues were then washed with 60% isopropanol and mounted in an Aqua Polymount (Polysciences). Tissues were imaged using an Olympus FluoView 3000 confocal microscope (Olympus). Content of lipids in adipose tissue and size of lipid droplets were analyzed using Fiji software. Flies for the analysis of LDs in the fat body were collected from three independent experiments and representative images are shown.

Lipidomic analysis

Adipose tissues from six flies from each group were dissected in ice-cold PBS for lipidomics analysis. The removed tissues were stored in PBS buffer in Eppendorf tubes at – 80 °C. Immediately after thawing, they were extracted by 500 µl of cold chloroform: methanol solution (v/v; 1:1). The samples were then homogenized by a Tissue Lyser II (Qiagen, Prague, Czech Republic) at 50 Hz, -18°C for 5

min and kept further in an ultrasonic bath (0°C, 5 min). Further, the mixture was centrifuged at 10,000 RPM at 4°C for 10 min followed by the removal of the supernatant. The extraction step was repeated at the same conditions. The lower layer of pooled supernatant was evaporated to dryness under a gentle stream of Argon. The dry total lipid extract was re-dissolved in 500 µl of chloroform: methanol solution (v/v; 1:1) and directly measured using previously described methods (Bayley et al. 2020). Briefly, high performance liquid chromatography (Accela 600 pump, Accela AS autosampler) combined with mass spectrometry LTQ-XL (all Thermo Fisher Scientific, San Jose, CA, USA) were used. The chromatographic conditions were as follows: Injection volume 5 µl; column Gemini 3 µM C18 HPLC column (150 × 2 mm ID, Phenomenex, Torrance, CA, USA) at 35°C; the mobile phase (A) 5 mM ammonium acetate in methanol with ammonia (0.025%), (B) water and (C) isopropanol: MeOH (8:2); gradient change of A:B:C as follows: 0 min: 92:8:0, 7 min: 97:3:0, 12 min: 100:0:0, 19 min: 93:0:7, 20-29 min: 90:0:10, 40-45 min: 40:0:60, 48 min: 100:0:0, and 50-65 min: 92:8:0 with flow rate 200 µl/min. The mass spectrometry condition: positive (3 kV) and negative (-2,5 kV) ion detection mode; capillary temperature 200°C. Eluted ions were detected with full scan mode from 200 to 1000 Da with the collisionally induced MS2 fragmentation (NCE 35). Data were acquired and processed by means of XCalibur 4.0 software (Thermo Fisher). The corrected areas under individual analytical peaks were expressed in percentages assuming that the total area of all detected is 100%.

Autophagy visualization

To visualize autophagy in adipose tissue, Atg8a-mCherry-bearing flies were dissected in PBS, fixed with 4% PFA, and then washed with PBS and stained with DAPI. Fat bodies were imaged using an Olympus FluoView 3000 confocal microscope (Olympus).

Foxo subcellular localization

Adipose tissues for immunolocalization of Foxo were dissected in ice-cold PBS. Dissection was followed by 20-min fixation in 4% PFA in PBS. After three washing steps in PBS-Tween (0.1%), unspecific binding was blocked with 10% NGS in PBS for 1 h at RT. The tissues were then incubated with primary antibody diluted in ratio 1:1,000 (Rabbit Drosophila anti-Foxo, CosmoBio) at 4°C overnight. After washing the unbound primary antibody (three times for 10 min in PBST), the secondary antibody was applied in dilution 1:250 for 2 h at RT (Cy2 Goat anti-Rabbit, Jackson-ImmunoResearch). The nuclei were stained with DAPI. Tissues were mounted with Aqua-Polymount (Polysciences), DAPI was used for nuclear staining. Foxo localization was detected by Plot-Profile analysis in FIJI.

Isolation of macrophages

GFP-labeled macrophages were isolated from *Crq-Gal4, UAS-eGFP* male flies using fluorescence-activated cell sorting (FACS). Approximately 200 flies were anaesthetized with CO₂, washed in PBS and homogenized in 600 mL of PBS using a pestle. The homogenate was sieved through a nylon cell strainer (40 µm). This strainer was then additionally washed with 200 µL of PBS, which was added to the homogenate subsequently. The samples were centrifuged (3 min, 4°C, 3,500 RPM) and the supernatant was washed with ice-cold PBS after each centrifugation (three times). Prior to sorting, samples were transferred to FACS polystyrene tubes using a disposable bacterial filter (50 µm, Sysmex) and macrophages were sorted into 100 µL of PBS using a S3™ Cell Sorter (BioRad). Isolated cells were verified by fluorescence microscopy and differential interference contrast.

Phagocytic activity

To visualize phagocytic activity, flies were injected with 50 nl of pHrodo™ Green *S. aureus* (Thermo Fischer Scientific) using an Eppendorf Femtojet microinjector. After 1 h, the flies were fixed in 4% PFA for 45 min, washed in PBS and subsequently mounted on imaging dishes using an Aqua Polymount (Polysciences). Thoraxes were imaged using an Olympus FluoView 3000 confocal microscope. To analyze phagocytic rate, flies were infected with 20,000 of *S. pneumoniae* and after 24 h, they were injected with 50 nl of pHrodo™ Red *S. aureus* (Thermo Fischer Scientific). After 1 h, abdomens of flies were opened in PBS and the macrophages were allowed to attach to a microscopic imaging dish for 40 min. Subsequently, the PBS was removed and the cells were fixed for 20 min with 4% PFA. Aqua Polymount (Polysciences) was used to mount the sample. Macrophages were imaged using an Olympus FluoView 3000 confocal microscope and red dots depicting phagocytic events were manually counted per cell.

Lipoprotein uptake

To document the uptake of lipoproteins by *Drosophila* macrophages, flies were injected with 50 nl of pHrodo™ Red LDL (Thermo Fischer Scientific) using an Eppendorf Femtojet microinjector. After 1 h, abdomens of flies were opened in PBS and the macrophages were allowed to attach to a microscopic imaging dish for 40 min. Subsequently, the PBS was removed and cells were fixed for 20 min with 4% PFA. Aqua Polymount (Polysciences) was used to mount the sample. Macrophages were imaged using an Olympus FluoView 3000 confocal microscope.

Bacterial strain and fly injection

The *Streptococcus pneumoniae* strain EJ1 was stored at -80°C in Tryptic Soy Broth (TSB) media containing 10% glycerol. For the experiments, bacteria were streaked onto agar plates containing 3% TSB and 100 mg/mL streptomycin and subsequently incubated at 37°C in 5% CO₂ overnight. Single colonies were inoculated into 3 mL of TSB liquid media with 100 mg/mL of streptomycin and 100,000 units of catalase and incubated at 37°C + 5% CO₂ overnight. Bacterial density was measured after an additional 4 h so that it reached an approximate 0.4 OD₆₀₀. Final bacterial cultures were centrifuged and dissolved in PBS so the final OD reached 2.4. The *S. pneumoniae* culture was kept on ice prior to injection and during the injection itself. Seven-day-old males were anaesthetized with CO₂ and injected with 50 nL culture containing 20,000 *S. pneumoniae* bacteria or 50 nL of mock buffer (PBS) into the ventrolateral side of the abdomen using an Eppendorf Femtojet microinjector. The *Listeria monocytogenes* strain 10403S was stored at -80°C in brain and heart infusion (BHI) broth containing 25% glycerol. For the experiments bacteria were streaked onto Luria Bertani (LB) agar plates containing 100 µg/mL streptomycin and incubated at 37°C overnight; plates were stored at 4°C and used for inoculation for a period of two weeks. Single colonies were used to inoculate 3 mL of BHI and incubated overnight at 37°C without shaking to obtain a morning 600 nm optical density (OD₆₀₀) of approx. 0.4. Further, *L. monocytogenes* cultures were diluted to OD₆₀₀ 0.01 in phosphate buffered saline (PBS) and stored on ice prior to loading into an injection needle. Approximately 1,000 *Listeria* per fly were injected.

Survival analysis

Streptococcus-injected flies were kept at 29°C in vials with approximately 30 individuals per vial and were transferred to fresh food every other day. *Listeria*-injected flies were kept at 25°C. Dead flies were counted daily. At least three independent experiments were performed and combined into a single survival curve generated in Graphpad Prism software; individual experiments showed comparable results. The average number of individuals was more than 500 for each genotype and replicate. Data were analyzed by Log-rank and Grehan-Breslow Wilcoxon tests (which gave more weight to deaths at early time points).

621 Pathogen load measurement

622 Single flies were homogenized in PBS using a motorized plastic pestle in 1.5 mL tubes. Bacteria were
 623 plated in spots onto LB (*L. monocytogenes*) or TSB (*S. pneumoniae*) agar plates containing
 624 streptomycin in serial dilutions and incubated overnight at 37°C before manual counting. To
 625 determine intracellular *L. monocytogenes* loads, flies were injected with 50 nL of gentamycin solution
 626 (1 mg/mL in PBS) 3 h prior to fly homogenization. Pathogen loads of 16 flies were determined for
 627 each genotype and treatment in each experiment; at least three independent infection experiments
 628 were conducted and the results were combined into one graph (in all presented cases, individual
 629 experiments showed comparable results).

630 THP-1 cell lines

631 THP-1 cells were cultured at 37°C, 5% CO₂, in RPMI-1640 medium (Sigma), supplemented with 2 mM
 632 glutamine (Applichem), 2 g/L sodium bicarbonate (J&K Scientific), 10% FBS (Biosera) and 100 mg/L
 633 streptomycin (Sigma). Prior to the experiment, cells were transferred to 24-well plates at 10⁵
 634 cells/well in four biological replicates. THP-1 cells were activated with phorbol-12-myristate-13-
 635 acetate (200 ng/mL, MedChemExpress). After 24 hours, *S. pneumoniae* bacteria were added (MOI 50
 636 bacteria/macrophage) and the plate was centrifuged briefly (2 min, 200xg). Following 6-h incubation,
 637 the cells were washed with RPMI-1640 medium, and fresh RPMI-1640 supplemented with
 638 gentamycin (0.1 mg/mL, Sigma) was added. After 1 h incubation, the medium was replaced with
 639 RPMI-1640 supplemented with penicillin-streptomycin (1%, Biosera). After another 17 h, the cells
 640 were harvested into TRIzol Reagent (Invitrogen) followed by RNA isolation.

643 Key resources table

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Strain, strain background (<i>Streptococcus pneumoniae</i>)	EJ1 strain	Provided by David Schneider		Dilution 20,000 units
Chemical compound, drug	TRIzol Reagent	Invitrogen	Cat# 15-596-018	
Chemical compound, drug	Superscript III Reversease Transcriptase	Invitrogen	Cat# 18080044	
Chemical compound, drug	2x SYBR Master Mix	Top-Bio	Cat# T607	

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Chemical compound, drug	Grace's Insect Medium	Sigma	Cat# G9771-1L	
Chemical compound, drug	Oil Red O	Sigma	Cat# O0625-25G	
Chemical compound, drug	DAPI	Sigma	Cat# D9542	
Chemical compound, drug	Cell Brite Fix Membrane Stain 488	Biotium	Cat# 30090-T	
Chemical compound, drug	Phalloidin blue	ThermoFisher	Cat# A30104	
Chemical compound, drug	pHrodo™ Red S. aureus Bioparticles™ Conjugate for Phagocytosis	ThermoFisher	Cat# A10010	
Chemical compound, drug	Phorbol-12-myristate-13-acetate	MedChemExpress	Cat# HY-18739	
Chemical compound, drug	RPMI-1640 Medium	Sigma	Cat# R7755	
Chemical compound, drug	Glutamine	Appllichem	Cat# A3704	
Chemical compound, drug	Gentamycin	Sigma	Cat# 1405-41-0	
Chemical compound, drug	FBS	Biosera	Cat# FB-1001	
Chemical	Penicillin-Streptomycin	Biosera	Cat#	

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
compound, drug			XC-A4122/100	
Chemical compound, drug	Protease inhibitor mixture	Sigma	Cat# SRE0055	
Chemical compound, drug	Phosphatase inhibitor	Thermo-Fisher	Cat# A32957	
Antibody	anti-Akt	Cell Signaling Technology	Cat# 9272	
Antibody	anti-phospho Ser505-Akt	Cell Signaling Technology	Cat# 4054	
Antibody	Alpha-Catenin	Development Studies Hybridoma Bank	Cat# DCAT-1	
Chemical compound, drug	SuperSignal ECL substrate	Thermo-Fisher	Cat# 34075	
Antibody	Rabbit Drosophila anti-Foxo	CosmoBio	Cat# THU-A-DFoxo	
Antibody	Cy2 Goat anti-Rabbit	Jackson-ImmunoResearch	AB_2338021	
Commercial assay, kit	Glucose (GO) Assay Kit	Sigma	Cat# GAGO20-1KT	
Commercial assay, kit	Bicinchoninic Acid Assay Kit	Sigma	Cat# BCA1	
Commercial assay, kit	Cholesterol/Cholesteryl Ester Quantitation Kit	Sigma	Cat# 428901-1KIT	
Commercial assay, kit	Triglyceride quantification Colorimetric/Fluorometric Kit	Sigma	Cat# MAK266-	

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
			1KT	
Chemical compound, drug	Trehalase from porcine kidney	Sigma	Cat# T8778-1UN	
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>HmlG4G80: w*</i> ; <i>HmlΔ-Gal4*</i> ; <i>P{tubPGal80ts}*</i>	Cross made in our laboratory by Tomas Dolezal		
Genetic reagent (<i>Drosophila melanogaster</i>)	ImpL2 ^{RNAi}	Bloomington Drosophila Stock Center		
Genetic reagent (<i>Drosophila melanogaster</i>)	ImpL2 Gal4	Provided by Hugo Stocker		
Genetic reagent (<i>Drosophila melanogaster</i>)	Atg8a-mCherry	Provided by Gabor Juhasz		
Genetic reagent (<i>Drosophila melanogaster</i>)	ImpL2 ^{cds}	Provided by Hugo Stocker		
Genetic reagent (<i>Drosophila melanogaster</i>)	20xUAS-6xmCherry	Bloomington Drosophila Stock Center		
Genetic reagent (<i>Drosophila melanogaster</i>)	CrqGal4>2xeGFP	Provided by Marc C. Dionne		
Genetic reagent (<i>D. melanogaster</i>)	<i>Hif1α[RNAi]: P{KK110834}VIE-260B</i>	Vienna Drosophila Resource Center	VDRC: v106504	FBst0478328
Genetic reagent (<i>D. melanogaster</i>)	<i>TRiP^{control}: y(1) v(1); P{y[+t7.7]=CaryP}attP2</i>	Bloomington Drosophila Stock Center	BDSC: 36303	FBst0036303
Genetic reagent (<i>D. melanogaster</i>)	<i>w: w¹¹¹⁸</i>	Genetic background based on CantonS		

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Sequence-based reagent	Foxo forward: CAATGCAAGAGATGGTCTCTC	KRD	CG3143	FBgn0038197
Sequence-based reagent	Foxo reverse: TGACCAGGTTCGTGTTGACC	KRD	CG3143	FBgn0038197
Sequence-based reagent	4EBP Forward: CCATGATCACCAGGAAGGTTGTC A	KRD	CG8846	FBgn0261560
Sequence-based reagent	4EBP Reverse: AGCCCGCTCGTAGATAAGTTTGG T	KRD	CG8846	FBgn0261560
Sequence-based reagent	EIF-4E Forward: AAGAAGAACATTCGCCCATG T	KRD	CG4035	FBgn0015218
Sequence-based reagent	EIF-4E Reverse: GGACTGCCGACGGAAACAAC	KRD	CG4035	FBgn0015218
Sequence-based reagent	Glut-CoA deh Forward: CTGATTGGTGCCTTTGGCC	KRD	CG9547	FBgn0031824
Sequence-based reagent	Glut-CoA deh Reverse: ATAGCGAGCGTTGTTTCAGAC	KRD	CG9547	FBgn0031824
Sequence-based reagent	Lip4 Forward: CCACTTGGGTGATGATGGGAC	KRD	CG6113	FBgn0032264
Sequence-based reagent	Lip4 Reverse: ACGTATTGCCACGAACATTGG	KRD	CG6113	FBgn0032264
Sequence-based reagent	MTP Forward: GAATCGAAATGCCCAGACG	KRD	CG9342	FBgn0266369
Sequence-based reagent	MTP Reverse: AACGTTGGTTTGTGTTGAGAAGC	KRD	CG9342	FBgn0266369
Sequence-based reagent	HSL Forward: CAGTCCTACGAGATTCACGG	KRD	CG11055	FBgn0034491
Sequence-based reagent	HSL Reverse: GGCTTCGTTGGATAACATTGTG	KRD	CG11055	FBgn0034491
Sequence-based reagent	Bmm Forward: CACCGCGCCGCAATGAATGTATA	KRD	CG5295	FBgn0036449

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
reagent	A			
Sequence-based reagent	Bmm Reverse: TTCAATCACTGTTTGTCGGTCGGC	KRD	CG5295	FBgn0036449
Sequence-based reagent	GlyP Forward: TCCACCCTGAGGGACTACTAC	KRD	CG7254	FBgn0004507
Sequence-based reagent	GlyP Reverse: GGTGTGGTCAGTGAGCGAC	KRD	CG7254	FBgn0004507
Sequence-based reagent	GlyS Forward: CGATGAAGAAGTACAGGGTC	KRD	CG6904	FBgn0266064
Sequence-based reagent	GlyS Reverse: TATCATCGTTATTGCCTGGAG	KRD	CG6904	FBgn0266064
Sequence-based reagent	apoLTP Forward: TGGGTGGATTGAAGCCACAG	KRD	CG15828	FBgn0032136
Sequence-based reagent	apoLTP Reverse: TGAGTGGATTTTCTCTCACTGC	KRD	CG15828	FBgn0032136
Sequence-based reagent	apoLPP Forward: TTGGAATCCTAGCTTCTGTGCT	KRD	CG11064	FBgn0087002
Sequence-based reagent	apoLPP Reverse: AGTCATAGTAGTTGCCGGGTAT	KRD	CG11064	FBgn0087002
Sequence-based reagent	Atg1 Forward: CTAAAGCCGTCGTCCAATGT	KRD	CG10967	FBgn0260945
Sequence-based reagent	Atg1 Reverse: GAACAGCATGCTCCGGTATT	KRD	CG10967	FBgn0260945
Sequence-based reagent	Atg6 Forward: CGGAGTTATCTTTGCCCATCT	KRD	CG5429	FBgn0264325
Sequence-based reagent	Atg6 Reverse: GTGCTACACGCTGTTGCTC	KRD	CG5429	FBgn0264325
Sequence-based reagent	Rp49 Forward: AAGCTGTCGCACAAATGGCG	KRD	CG7939	FBgn0002626

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Sequence-based reagent	Rp49 Reverse: GCACGTTGTGCACCAAGAAC	KRD	CG7939	FBgn0002626
Sequence-based reagent	Impl2 Forward: TTCGCGGTTTCTGGGCACCC	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2 Reverse: GCGCGTCCGATCGTCGCATA	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RA Forward: GTGCCAACGAAGCTTCGAGTG	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RA Reverse: GCGTGGCTTCTCCTCCTCC	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RB, D Forward: GTCGTCGGAAAGGATACCGC	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RB, D Reverse: TTCCGTCGTCAATCCAATAGCC	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RC Forward: ATAGACTCGGAGACCTCCCC	KRD	CG15009	FBgn0001257
Sequence-based reagent	Impl2-RC Reverse: GCGTGGCTTCTCCTCCTCC	KRD	CG15009	FBgn0001257
Sequence-based reagent	IGFBP7 Forward: GCCATCACCCAGGTCAGCAAG	KRD	GC04M057030	
Sequence-based reagent	IGFBP7 Reverse: GGATTCCGATGACCTCACAGCT	KRD	GC04M057030	
Sequence-based reagent	ACTB Forward: ATTGCCGACAGGATGCAGAA	KRD	GC07M005527	
Sequence-based reagent	ACTB Reverse: GCTGATCCACATCTGCTGGAA	KRD	GC07M005527	

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Sequence-based reagent	IGFBP1 Forward: TCCTTTGGGACGCCATCAGTAC	KRD	GC07P046552	
Sequence-based reagent	IGFBP1 Reverse: GATGTCTCCTGTGCCTTGGCTA	KRD	GC07P046552	
Sequence-based reagent	IGFBP2 Forward: CGAGGGCACTTGTGAGAAGCG	KRD	GC02P216632	
Sequence-based reagent	IGFBP2 Reverse: TGTTTCATGGTGCTGTCCACGTG	KRD	GC02P216632	
Sequence-based reagent	IGFBP3 Forward: CGCTACAAAGTTGACTACGAGTC	KRD	GC07M045912	
Sequence-based reagent	IGFBP3 Reverse: GTCTTCCATTCTCTACGGCAGG	KRD	GC07M045912	
Sequence-based reagent	IGFBP4 Forward: ACCCACGAGGACCTCTACATCA	KRD	GC17P040443	
Sequence-based reagent	IGFBP4 Reverse: CACACCAGCACTTGCCACGCT	KRD	GC17P040443	
Sequence-based reagent	IGFBP5 Forward: CGTGCTGTGTACCTGCCCAATT	KRD	GC02M216672	
Sequence-based reagent	IGFBP5 Reverse: ACTTGTCACGCACCAGCAGAT	KRD	GC02M216672	
Sequence-based reagent	IGFBP6 Forward: CACAGGATGTGAACCGCAGAGA	KRD	GC12P053097	
Sequence-based reagent	IGFBP6 Reverse: CACTGAGTCCAGATGTCTACGG	KRD	GC12P053097	
Software, algorithm	Graphpad Prism	https://www.graphpad.com/	Graphpad Prism	RRID:SCR_002798

Reagent type (species) or resource	Designation	Source reference	Identifier	Additional information
Software, algorithm	Microsoft Excel	https://www.microsoft.com/	Microsoft Excel	
Software, algorithm	Fiji	ImageJ - https://fiji.sc	ImageJ	RRID: SCR_002285
Other	S3e Cell Sorter	BioRad - http://www.biorad.com/	BioRad	
Other	Olympus FluoView 3000	Olympus - https://www.olympus-global.com/	Olympus	RRID: SCR_017015 RRID: SCR_014215
Other	Olympus IX71	Olympus - https://www.olympus-global.com/	Olympus	

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Figure legends

Figure 1. Streptococcal infection increases Impl2 expression in a Hif1 α -dependent manner in activated macrophages

(A) Expression of Impl2 mRNA at whole-body level in flies (Crq>GFP) infected with *S. pneumoniae* (Sp. Infection) and control flies (Buff. Injection) at 0, 3, 6, 9, 12, 15, 18 and 21 hpi. (B) Representative confocal microscopy images of control (left) and infected (right) Impl2Gal4>UASmCherry individuals imaged at 24 hpi, from Z stack of 7 layers, autofluorescence in the green channel was used to visualize the fly's body. (C) Confocal microscopy images of Impl2Gal4>UASmCherry hemocyte depicting its phagocytic ability visualized by *S. aureus*-pHrodo-green. The scale bar represents 10 μ m. (D) Confocal microscopy image of Impl2Gal4>UASmCherry hemocyte actively interacting with GFP-labeled *S. pneumoniae* (Sp-GFP) *ex vivo*. The scale bar represents 13 μ m. (E) Number of Impl2-positive macrophages in the thorax of control and infected adult flies (Impl2Gal4>UASmCherry) at 24hpi. (F) Expression of Impl2 mRNA (using primers targeting all transcript variants) in hemocytes isolated from control and infected Crq>GFP flies at 24hpi. (G) Gene expression of Impl2-RA (left), Impl2-RB and Impl2-RD (middle) and Impl2-RC (right) transcript variants in either hemocytes, fat body or thorax muscles dissected from control and infected Crq>GFP flies at 24 hpi. (H) Gene expression of Impl2 (using primers targeting all transcript variants) and Impl2-RA in hemocytes isolated from control and infected Crq>GFP flies with and without hemocyte-specific Hif1 α knockdown at 24 hpi. (I) In silico analysis of hypoxic enhancer activity by 500 bp bins at the Impl2 locus and visualization of Impl2 transcript variants (Flybase.org). Each black dot plots a log2 fold change (y-axis) of the difference in the random tag counts mapped to the 500-bp bin between normoxic and hypoxic conditions. The triangles show the position and negative log10 p-value (y-axis) of multiple hypoxic (Hif1 α), immune (Rel) and stress (HSF) response transcription factor binding sites. The close up shows distribution and clustering of individual response elements for the most significant bin. In (A, F, H), expression levels normalized against rp49 are reported as fold change relative to levels of Impl2 (using primers targeting all transcript variants) in PBS-injected Crq>GFP controls, which were arbitrarily set to 1. In (G), expression levels normalized against rp49 are reported as fold change relative to levels of Impl2-RA in hemocytes of PBS-injected Crq>GFP controls for all three graphs. The individual dots represent biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001). Hif1 α , hypoxia-inducible factor 1 α ; Rel, relish; HSF, heat shock factor.

Figure 1-figure supplement 1. Efficiency of genetic manipulations of Hif1 α and Impl2 in *Drosophila* macrophages

(A) Gene expression of Hif1 α in hemocytes of Buff-injected flies and *S. pneumoniae*-infected flies with or without macrophage-specific Hif1 α knockdown. (B) Impl2 gene expression measured at the whole organism level in flies with macrophage-specific knockdown and overexpression of Impl2 and their respective controls, either with Impl2 constructs only or with Gal4 drivers only. Expression levels normalized against rp49 are presented as a fold change relative to levels of Impl2 in *w*; Impl2^{RNAi} control and Hif1 α in uninfected *Hml>Gal4, UAS-GFP x TRiP^{control}*, respectively, which were arbitrarily set to 1. Individual dots represent biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001).

Figure 1-figure supplement 2. **Impl2-RA regulatory sequence with transcription factor binding sites**

500-bp long sequence upstream of Impl2-RA transcriptional start site (as shown on map) with marked binding sites for Hif1 α , Rel and HSF1. Hif1 α , hypoxia-inducible factor 1 α ; Rel, relish; HSF1, heat shock factor 1.

Figure 2. **Macrophage-derived Impl2 regulates changes in adipose tissue metabolism induced by infection**

(A) Triglyceride concentrations in PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at whole-body level at 24 hpi. (B-D) Average size (B) and number (C) of lipid droplets and percentage of area occupied (D) in the fat body of PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at 24 hpi. (E) Representative confocal microscopy images of dissected fat body of PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at 24 hpi, stained with OilRedO (red), DAPI (cyan) and phalloidin (white). The scale bar represents 20 μ m. (F) Relative proportions of different lipid species in the fat body of PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at 24 hpi. (G) Glycogen concentration in PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at whole-body level at 24 hpi. Metabolite concentrations were normalized to the amount of proteins in each sample and measured in three biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001). (B-D) were quantified from 8 confocal microscopy images for each genotype and treatment, (F) lipidomic analysis was performed in three biological replicates for each genotype and treatment. PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglycerides; DG, diglycerides; PG, phosphatidylglycerol; PI, phosphatidylinositol; LysoPC, Lyso-phosphatidylcholine; LysoPE, Lyso-phosphatidylethanolamine.

Figure 3. **Infection-induced autophagy and changes in gene expression in adipose tissue**

(A) Representative confocal microscopy images of fat body of control and infected flies with Atg8a-mCherry autophagy reporter (Atg8a, red; DAPI, cyan). The scale bar represents 20 μ m. (B) Graphical representation of log2-fold change in mRNA expression of genes involved in insulin signaling, lipoprotein and lipid metabolism, glycogenolysis and autophagy in the fat body of PBS-injected and S.p.-infected flies with macrophage-specific Impl2 knockdown (Impl2^{RNAi}), overexpression (Impl2^{CD5}) and their respective controls (TRiP^{control}, w¹¹¹⁸) at 24hpi. The first column (Infection) shows the log2-fold change in mRNA expression in S.p.-infected flies compared to Buff.-injected control flies (average change for TRiP^{control} and w¹¹¹⁸). The second to fourth columns represent the log2-fold change in mRNA expression compared to the corresponding control for knockdown (Impl^{RNAi}/TRiP^{control}) and overexpression (Impl2^{CD5}/w¹¹¹⁸) in either uninfected (Buff.) or S.p. infected flies. Foxo, forkhead box O; 4EBP, Thor; EIF-4E1, eukaryotic translation initiation factor 4E1; MTP, microsomal triacylglycerol transfer protein; apoLPP, apolipoprotein; apoLTP, apolipoprotein lipid transfer particle; Bmm, brummer; Lip4, lipase 4; Hsl, hormone sensitive lipase; Glutaryl-CoA deh., Glutaryl-CoA dehydrogenase; GlyS, glycogen synthase; GlyP, glycogen phosphorylase; Atg1, autophagy-related gene 1; Atg6, autophagy-related gene 6.

Figure 3-figure supplement 1. Expression levels of genes regulating metabolism in adipose tissue

Gene expression of *Foxo*, *4EBP*, *EIF4E1*, *GlutarylCoA deh.*, *Lip4*, *apoLTP*, *apoLPP*, *MTP*, *Hsl*, *Bmm*, *GlyS*, *GlyP*, *Atg1*, and *Atg6* genes in adipose tissue of flies injected with PBS (black bars) and infected with *S. pneumoniae* (grey bars) with macrophage-specific *Impl2* knockdown, overexpression and their respective controls; all genotypes depicted below the x-axis were crossed with *Hml>Gal4; TubGal80^{TS}*. Expression levels normalized against *rp49* are present as a fold-change relative to the levels in the PBS-injected *TRiP^{control}*, which was arbitrarily set to 1. The individual dots represent biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 4. Macrophage-derived *Impl2* affects adipose tissue metabolism via *Foxo* activity

Representative confocal microscopy images of *Foxo* immunolocalization in the fat body of PBS-injected and *S.p.*-infected flies with macrophage-specific *Impl2* knockdown (*Impl2^{RNAi}*), overexpression (*Impl2^{CD5}*) and their respective controls (*TRiP^{control}*, *w¹¹¹⁸*) at 24 hpi; anti-d*Foxo* antibody, green; DAPI, cyan. Histograms of *Foxo* cellular localization represent *Foxo* and DAPI signal intensity in sections indicated by a white freehand line. The scale bar represents 20 μ m.

Figure 5. Macrophage-derived *Impl2* increases circulating carbohydrates and cholesterol to be utilized by activated macrophages

(A-D) Concentrations of circulating glucose (A), trehalose (B), cholesterol (C) and cholesteryl-ester (D) in the hemolymph of PBS-injected and *S.p.*-infected flies with macrophage-specific *Impl2* knockdown (*Impl2^{RNAi}*), overexpression (*Impl2^{CD5}*) and their respective controls (*TRiP^{control}*, *w¹¹¹⁸*) at 24 hpi. (E-H) Concentrations of glucose (E), trehalose (F), cholesterol (G) and cholesteryl-ester (H) in hemocytes of PBS-injected and *S.p.*-infected flies with macrophage-specific *Impl2* knockdown (*Impl2^{RNAi}*), overexpression (*Impl2^{CD5}*), and their respective controls (*TRiP^{control}*, *w¹¹¹⁸*) at 24 hpi. (I) Proportional occurrence of macrophages containing at least one lipid droplet (stained by OilRed O), counted in at least fifty of *Crq>GFP* positive cells. (J) Representative confocal microscopy image of macrophages (*Crq>GFP*) containing lipid droplets; neutral lipids were stained with OilRed O (red). The scale bar represents 10 μ m. The image represents a Z-stack consisting of a maximum projection of 5 layers. (K) Representative confocal microscopy image depicting the ability of macrophage (*Crq>GFP*) to endocytose lipoproteins (human LDL-pHrodo, red). The scale bar represents 13 μ m. (A-H) Metabolite concentrations were normalized to the amount of proteins in each sample. Metabolic measurements were performed in three biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 6. Macrophage-derived *Impl2* is important for resistance to bacterial infection

(A, C) Survival rate of *S.p.*-infected and PBS-injected flies with macrophage-specific *Impl2* knockdown (*Impl2^{RNAi}*) (A), overexpression (*Impl2^{CD5}*) (C) and their respective controls (*TRiP^{control}*, *w¹¹¹⁸*). Three independent experiments were performed and combined into one survival curve; the number of individuals per replicate was at least 500 for each genotype. (B, D) Pathogen load in *S.p.*-infected and PBS-injected flies with knockdown (*Impl2^{RNAi}*) (B), overexpression (*Impl2^{CD5}*) (D) and their respective controls (*TRiP^{control}*, *w¹¹¹⁸*) at 24 and 48 hpi. The individual dots in the plot represent the number of

bacteria (colony forming units-CFUs) in one fly. The data show results combined from three independent biological replicates. (E) Confocal microscopy image depicting the ability of a macrophage (Crq>GFP) to phagocytose an invading pathogen (*S. aureus*-pHrodo-red). The scale bar represents 10 μ m. The image represents a Z-stack consisting of a maximum projection of 5 layers. (F) Phagocytic rate calculated for *S.p.*-infected flies with knockdown (ImpL2^{RNAi}), overexpression (ImpL2^{CDS}), and their respective controls (TRiP^{control}, w¹¹¹⁸); each dot in the plot represents the number of phagocytic events per macrophage (Crq>GFP). The results are combined from three biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001).

Figure 7. Maladaptive effects of ImpL2 when overexpressed during development and in chronic infection

(A) Representative images of a developmental defect of the dorsal closure in the abdomen of a fly with macrophage-specific ImpL2 overexpression throughout development (Crq>Gal4>ImpL2^{CDS}) (left), and melanized spots in a fly with temporal (48 hours), macrophage-specific ImpL2 overexpression (Hml>Gal4; Gal80^{TS}>ImpL2^{CDS}) (middle), with the aortic region (upper right with arrows pointing to melanized spots) and melanized spot (lower right) at higher magnification. (B, D) Survival rate of *L. monocytogenes*-infected and PBS-injected flies with macrophage-specific ImpL2 knockdown (ImpL2^{RNAi}) (B), overexpression (ImpL2^{CDS}) (D) and their respective controls (TRiP^{control}, w¹¹¹⁸). Three independent experiments were performed and combined into each survival curve; the number of individuals per replicate was at least 500 for each genotype. (C, E) Pathogen load of *L. monocytogenes* in flies with macrophage-specific ImpL2 knockdown (ImpL2^{RNAi}) (C), overexpression (ImpL2^{CDS}) (E) and their respective controls (TRiP^{control}, w¹¹¹⁸) shown as either total load or only intracellular *Listeria* subpopulation at 24 hpi and at 12 days post-infection. The individual dots in the plot represent the number of bacteria (colony forming units-CFUs) in one fly. The data show results combined from three independent biological replicates. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001).

Figure 8. IGFBP7, the mammalian homolog of ImpL2, is produced in human macrophages in response to *S. pneumoniae* in vitro

(A) Confocal microscopy images of PMA-activated control THP-1 cells (left) and PMA-activated THP-1 cells actively interacting with GFP-labeled *S. pneumoniae* (green) in vitro (right). The scale bar represents 10 μ m. The image on the right is a maximum projection of 3 layers. (B) Gene expression of IGFBP7, IGFBP3 and IGFBP4 in PMA-activated THP-1 cells 24 hours after exposure to *S. pneumoniae* in vitro. Expression levels normalized against ACTB are shown as a fold change relative to levels of IGFBP7 in controls that were arbitrarily set to 1. Values are mean $\pm$ SD, asterisks mark statistically significant differences (*p<0.05; **p<0.01; ***p<0.001).

Figure 9. Schematic representation of the proposed model of ImpL2 function during infection

Upon recognition of the bacteria, the macrophages switch to Hif1 α -driven aerobic glycolysis. Hif1 α also induces the expression of the ImpL2-RA isoform by binding to hypoxia response elements in the promotor of the ImpL2 gene. Macrophage-derived ImpL2 then remotely activates Foxo re-localization to the nucleus in the fat body, leading to the expression of metabolic Foxo-target genes.

983 Mobilized sources, manifested by hyperlipidemia and hyperglycemia in the hemolymph, are
 984 subsequently used by activated macrophages. These transient changes in Impl2-regulated
 985 metabolism are essential for efficient bacterial killing and resistance to infection. However, if
 986 prolonged, for example during chronic infection, macrophage-derived Impl2 can harm the host by
 987 potential wasting. Hif1 α , hypoxia-inducible factor 1 α ; HREs, hypoxia response elements; Impl2,
 988 imaginal morphogenesis protein-late 2; Foxo, forkhead box O.

AEfficiency of Hif1 α manipulations **B**

Efficiency of ImpL2 manipulations

ImpL2-RA regulatory sequence:

sequence:

GACCTCGCCAGGACCTGCCCCCTCAGCACCCGCCACGCAGTGGGCTGTGTG

TGGCCACATCCAGCCATCCATCTATGTGCCACACATCCACATCCGTAAG

CCCATCTGCAATCAGTCGGCAGCATGGCGTAATTAAACCCGCCGCTGTCAC

AGTTGTCGTCTCGTGGCGTTTGACATGTCCGCCTCGCGTTTATTGGCAACA

TTGTTCACTTAATCATCCACATTTACCATATTTATTAGCAACATCGATCT

ATCTGCTCGTATCCGAATGCACGGAATGGGGAGCCGTGGAAAATCCCTGGA

TCCGAAGGGAAATTGTACTCAACAAGCTTGGTGGAAAGACCCCTAATTTAC

TATTGTTATTATTTTCATCAGAAATCGAAATTTATATAAATTACATGTTTA

ATTTTAATTGAAATATCTGGAACCTTTTTCATGATATAATCTTAAAAGAGT

TTAATTTAACTTGAATATCTTTATCTATTTCTGAAGTAGCTA

Hif1- α binding site

Rel binding site

HSF binding site

A**Atg8a-mCherry** **DAPI**

Buff. injected

S.p. infected

B

Log2 change in expression

A**ImpL2 knockdown - *S. pneumoniae* infection** **B****Pathogen load of *S. pneumoniae*** **C****ImpL2 overexpression - *S.pneumoniae* infection** **D****Pathogen load of *S. pneumoniae*** **E** **F****Phagocytic rate**

A**ImpL2^{CDS} x****Crq>Gal4****Hml>Gal4; TubG80^{TS} (48 hours)** **B****ImpL2 knockdown - *L. monocytogenes* infection** **C****ImpL2 knockdown - pathogen load of *L. monocytogenes*** **D****ImpL2 overexpression - *L. monocytogenes* infection** **E****ImpL2 overexpression - pathogen load of *L. monocytogenes***

A **B**

Expression of IGFBPs in THP-1 cells