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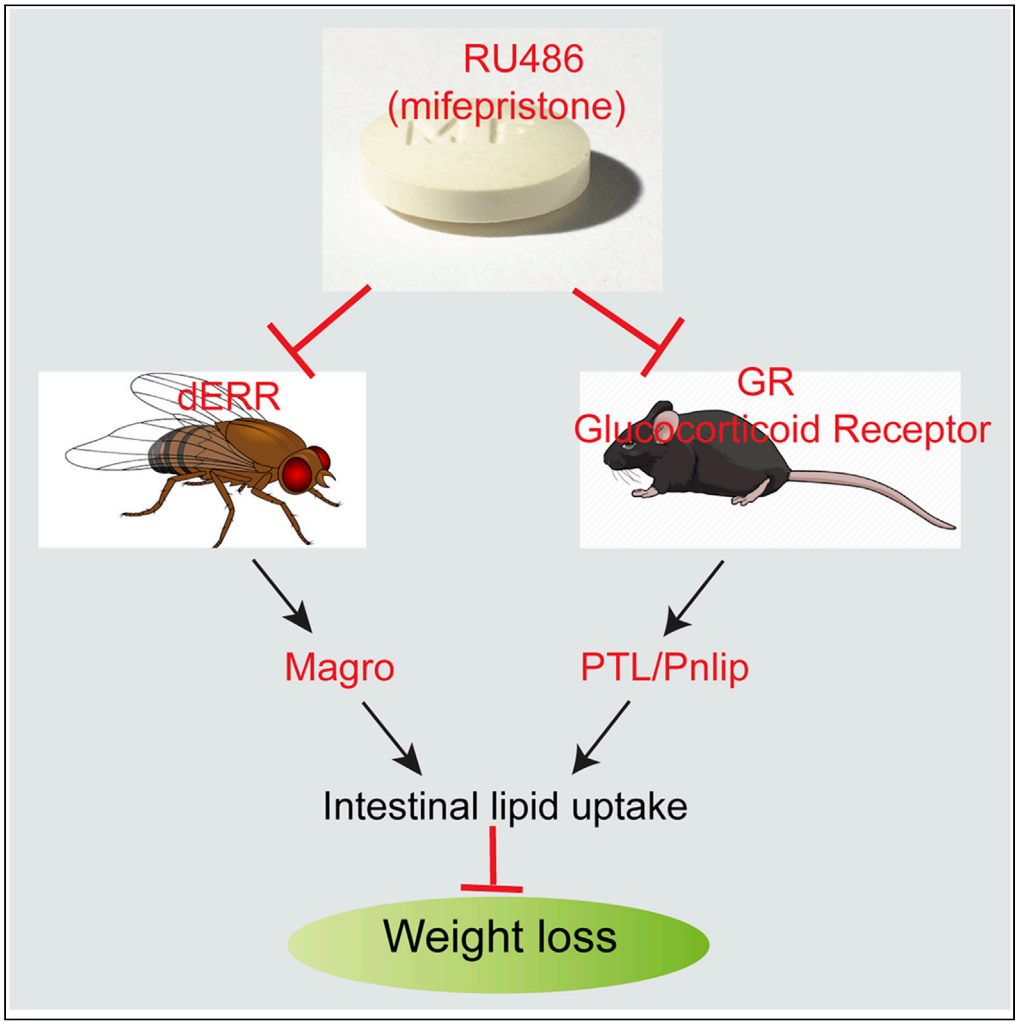

Peng Ma, Yao Zhang, Qiying Liang, ..., Ruolei Han, Chunyu Huo, Hansong Deng

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Highlights

RU486 suppresses lipid digestion both in mice and flies.

In flies, lipase *Magro* is transcriptionally suppressed by RU486 through dERR.

In mice, intestinal lipid digestion is inhibited by RU486 through (GR)/PTL pathway in pancreas.

RU486 alleviates high fat diet-induced obesity both in flies and mice.

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Mifepristone (RU486) inhibits dietary lipid digestion by antagonizing the role of glucocorticoid receptor on lipase transcription

Peng Ma,[1] Yao Zhang,[1] Qiying Liang,[1] Youjie Yin,[1] Saifei Wang,[1] Ruolei Han,[1] Chunyu Huo,[1] and Hansong Deng[1,2,*]

SUMMARY

Lipid digestion and absorption are tightly regulated to cope with metabolic demands among tissues. How these processes are coordinated is not well characterized. Here, we found that mifepristone (RU486) prevents lipid digestion both in flies and mice. In flies, RU486 administration suppresses lipid digestion by transcriptional downregulating *Magro* in guts. Similarly, intestinal lipid uptake in mice was also suppressed by RU486 through the glucocorticoid receptor (GR). Further studies showed that the pancreatic lipase *Pnlip* is a direct transcriptional target of GR in pancreas tissues. Glucocorticoid levels in mice fed a high fat diet (HFD) are significantly lower than those fed on a conventional diet, and RU486 administration inhibits HFD-induced obesity both in mice and flies. Our findings identified a novel mechanism of RU486 functions as a GR antagonist systematically regulating lipid metabolism, providing new insight on the role of Glucocorticoid/GR in Cushing disease, diabetes, and other related metabolic syndromes.

INTRODUCTION

Digestion and uptake of dietary fats in the gastrointestinal tract involves crosstalk among different tissues (Ko et al., 2020). For instance, lipases in the pancreatic juice are secreted into the duodenum via the pancreatic duct to facilitate hydrolysis, emulsification, and micellization of dietary fats. Exocrine pancreatic lipase (Pnlip) is crucial for intestinal hydrolysis of dietary triglyceride to fatty acids (Iqbal and Hussain, 2009). Recent studies indicated that polymorphism of *Pnlip* is associated with metabolic syndromes, such as obesity and pancreatitis (Hegele et al., 2001; Muramatsu et al., 2005). After uptake by enterocytes, fatty acids are re-esterified and transport as chylomicrons into other tissues. How systematic lipid metabolism is cooperated among different tissues, however, is not well characterized.

Steroid hormones, such as progesterone and glucocorticoids (GCs), play essential roles in reproduction, inflammation, and metabolism (Beato et al., 1995; Ozawa, 2005). They exert their effects by binding to the progesterone receptor (PR) NR3C3 and glucocorticoid receptor (GR) NR3C1, respectively. After ligand binding, GR and PR undergo dimerization and conformational change, followed by translocation to the nucleus, where they respectively interact with progesterone response elements (PRE) or glucocorticoid response elements (GRE) in the promoter region of target genes (Scarpin et al., 2009).

PR is highly expressed in reproductive tissues and deregulation of PR is associated with female reproductive tract cancer (Di Renzo et al., 2016; Ishikawa et al., 2010; Czyzyk et al., 2017). As stress hormones, GCs are critical for glucose homeostasis, adipocyte development, and inflammation (Ito et al., 2006). In terms of glucose homeostasis, GCs promote gluconeogenesis in liver, whereas in skeletal muscle, they decrease glucose uptake and utilization by antagonizing insulin response (Kuo et al., 2015). Further, the synthetic GC dexamethasone (DXMS) induces amylase transcription in pancreas, aiding starch digestion in the gastrointestinal tract (Logsdon et al., 1987). Excessive GC level is associated with Cushing syndrome, which is characterized by insulin resistance and abdominal obesity, suggesting that GC plays pivotal roles in the development of obesity and pathogenesis of obesity related diseases (Geer et al., 2014; Ito et al., 2006). However, the effects of GCs on lipid metabolism are quite complex and inconsistent. For instance, in

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adipose tissues, previous studies found that subcutaneously administered GC-treated rats exhibit higher rates of lipolysis in primary adipocyte culture by increasing transcription of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (Xu et al., 2009; Campbell et al., 2011), whereas other studies reported no significant effect of DXMS on lipolysis in primary cultures of newly differentiated human adipocytes or explanted ones (Lee and Fried, 2009; Lee et al., 2014).

RU486, also known as mifepristone, was first identified as a GR antagonist by drug screening (Moguilewsky and Philibert, 1984), and subsequently found to have a higher affinity for PR. Since its initial characterization, it was widely used as a contraceptive drug (Cadepond et al., 1997), and more recently, there is clinical evidence that RU486 is a potential therapeutic drug target for breast cancer and Cushing disease (Chen et al., 2014; Basina et al., 2012). Recent studies also indicated that RU486 can reverse metabolic defects associated with diet-induced obesity, such as insulin resistance and lipid abnormalities(Priyadarshini and Anuradha, 2017; Kusunoki et al., 1995). Whether RU486 can influence intestinal lipid digestion and uptake, however, remains elusive.

Because there are no clear homologs of PR in *Drosophila*, RU486 is used to activate a drug-inducible gene expression system in *Drosophila* (Roman et al., 2001; Osterwalder et al., 2001). A binary system of UAS/GAL4 is commonly used for temporal and spatial control of transgene expression in *Drosophila* (Brand and Perrimon, 1993). One way to achieve temporal control is by using a drug-inducible Gal4 system, such as GeneSwitch, in which GAL4 is fused with the ligand-binding domain of human PR and activation domain of human p65 (Wang et al., 1994; Burcin et al., 1999). In the presence of RU486, the chimeric molecule binds to the UAS sequence and activates transcription. Interestingly, recent studies showed that RU486 may affect aging, mating behavior, and other side effects in flies (Ford et al., 2007; Poirier et al., 2008; Landis et al., 2015; Robles-Murguia et al., 2019), however, the underlying mechanism is unknown. It was recently discovered that *Drosophila* estrogen related receptor (*dERR*), the sole member of the steroid receptor family, shares functional ortholog with GR (Bartolo et al., 2020). In fact, dERR is an essential regulator of carbohydrate metabolism during larval stages (Tennessen et al., 2011). Whether RU486 exerts these effects through dERR remains elusive.

In the current study, we unexpectedly found RU486 suppresses lipid uptake in flies, and further demonstrated that DXMS/RU486 play a conservative role on intestinal lipid metabolism by regulating lipase transcription through the nuclear receptor ERR in fly gut and GR in mice pancreatic acinar cells. These results indicated that GC/GR coordinates lipid digestion among tissues and systematically regulates lipid metabolism.

RESULTS

RU486 negatively regulates lipid level in *Drosophila*

In *Drosophila*, RU486 (final concentration 10–200 μg/mL) was dissolved in ethanol and mixed in regular cornmeal food to turn on the GeneSwitch Gal4 system (Osterwalder et al., 2001; Roman et al., 2001). Unexpectedly, we found using LipidTOX neutral lipid dye staining that 5966-Gal4^{GS} flies, characterized by RU486-inducible Gal4 expression in enterocytes (Mathur et al., 2010), have fewer intestinal lipids when fed for 7 days on RU486-supplemented food (final concentration 75 μg/mL) compared with flies fed on control food (Figure 1A). We and others have previously shown that gut lipid level is regionally distributed with strong enrichment in intestinal segments R2–R4 (Luis et al., 2016; Buchon et al., 2013). For simplicity, we focused on these segments in this study. Decreased lipid levels were also observed by Oil Red O (ORO) staining and by measurement of triacylglycerols (TAGs) in gut (Figures 1B and 1C). Dietary lipids are digested and absorbed by enterocytes, and then transported to other tissues as lipoproteins (Abumrad and Davidson, 2012; Carey et al., 1983). As shown in Figure 1C, whole-body TAG level is also decreased. Lipids are the main energy storage form for flies, and are responsible for survival under conditions of long-term starvation (Gronke et al., 2005; Gutierrez et al., 2007). As expected, we found that RU486-fed flies were sensitive to starvation, although food intake was largely normal (Figures S1A and S1B). Similarly, wildtype W^{1118} flies fed RU486 (100 μg/mL) for 7 days also showed significantly lower lipid levels in gut (Figure 1D), indicating that the effect of RU486 on lipid level was not because of genetic background. In addition, the effect of RU486 is dosage-dependent because animals fed with 100 μg/mL RU486 show a greater lipid-decreasing effect compared with those fed with 10 μg/mL RU486 (Figure 1D). These results indicate that RU486-supplemented feeding decreases lipid level in *Drosophila*.

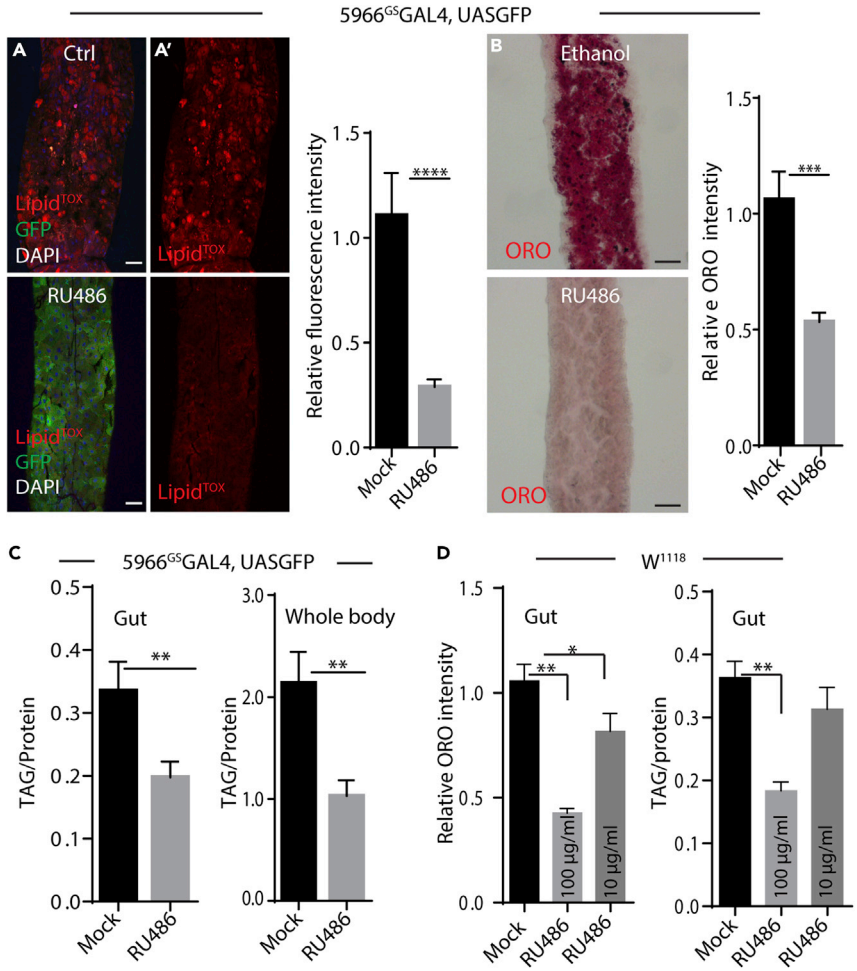

Figure 1. RU486 negatively regulates lipid level in Drosophila

(A) Effect of RU486 (final concentration: 75ug/ml) on lipid level in fly guts. Lipid was measured by lipophilic dye LipidTox in red. RU486 induce GFP expression (in green) in 5966^{GS}Gal4; UASGFP. Fluorescence intensity was quantified on right. n = 12 for each condition.

(B) Effect of RU486 on intestinal lipid was measured by Oil Red O (ORO) staining. Intensity was quantified on right. n = 10.

(C) RU486 suppresses lipid level both in gut and whole body as indicated by TAG assay kit. n = 12 for each genotype.

(D) W^{1118} flies fed on RU486 also contain less lipid level in a dosage-dependent manner. ORO stains gut lipid in red. n = 12 for each condition, RU486 at 100ug/ml or 10ug/ml were mixed with the conventional food. t-test was performed for statistical analysis. **:$p < 0.01$, ***:$p < 0.001$,****: $p < 0.0001$. Scale bar: 10µm.

RU486 decreases lipid level by negatively regulating *magro* transcription in *Drosophila*

To identify the transcription targets of RU486 on lipid metabolism, transcriptional profiling was performed in fly intestine by RNA sequencing (RNA-seq). As shown in Figure 2A and Table S1, genes involved in proteasome composition, metabolism, stress response, and immune function are highly enriched among differentially expressed genes (DEGs) in the gut of RU486-fed flies compared with those found in control conditions. Intriguingly, a subset of genes involved in lipid metabolism is significantly reduced in fly intestine after RU486 treatment (Figure 2A).

Particularly, we found transcription values (fragments per kilobase of transcript per million mapped reads (FPKM)) of *magro* (CG5932), which encodes a lysosomal acid lipase (LipA) homolog involved in cholesterol and triglyceride hydrolysis (Sieber and Thummel, 2009; Karpac et al., 2013), was significantly reduced in the gut (Table S1). This reduction was confirmed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis (Figure 2B). Consistently, RU486 administration reduces lipase activity in the guts

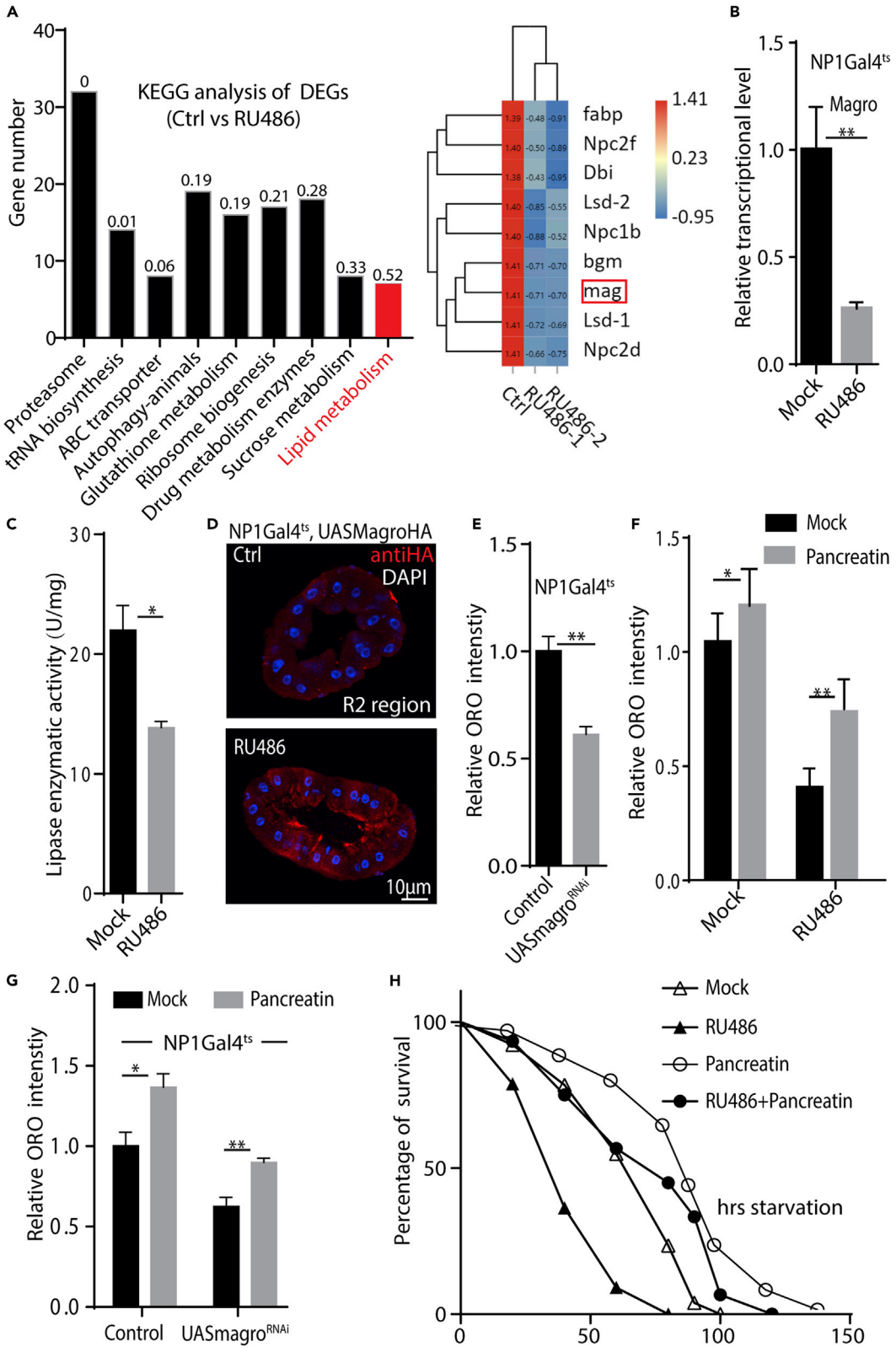
A
KEGG analysis of DEGs
(Ctrl vs RU486)
Gene number
0
0.01
0.06
0.19
0.19
0.21
0.28
0.33
0.52
Proteasome
tRNA biosynthesis
ABC transporter
Autophagy-animals
Glutathione metabolism
Ribosome biogenesis
Drug metabolism enzymes
Sucrose metabolism
Lipid metabolism
fabp
Npc2f
Dbi
Lsd-2
Npc1b
bgm
mag
Lsd-1
Npc2d
Ctrl
RU486-1
RU486-2
1.41
0.23
-0.95
B
NP1Gal4ts
Magro
Relative transcriptional level
Mock
RU486
C
Lipase enzymatic activity (U/mg)
Mock
RU486
D
NP1Gal4ts, UASMagroHA
Ctrl
antiHA
DAPI
R2 region
RU486
10μm
E
NP1Gal4ts
Relative ORO intenstiy
Control
UASmagroRNAi
F
Mock
Pancreatin
Relative ORO intenstiy
Mock
RU486
G
Mock
Pancreatin
NP1Gal4ts
Relative ORO intenstiy
Control
UASmagroRNAi
H
Mock
RU486
Pancreatin
RU486+Pancreatin
Percentage of survival
hrs starvation

Figure 2. RU486 blocks lipid level by negatively regulating *Magro* transcription

(A) Left, KEGG analysis of differential expressed genes (DEGs) by RU486 treatment. p values were shown on top of each column. Y-axis indicates the number of genes changes in each category. Right, genes involve in lipid metabolism with more than two-fold changes by RNAseq were presented as a heatmap.

(B)*Magro* transcriptional level in gut was analyzed by RT-q-PCR. Genotype: NP1Gal4, UASGal80^{ts}.

(C) Enzymatic activity of lipase in fly guts was measured after 7 days treatment with or without RU486(75μg/ml). n = 20 for each condition, Student t-test was performed for statistical analysis, *: $p < 0.05$. Accumulation of Magro protein in cytosol of enterocytes in R2 region of adult fly gut.

(D) Frozen section of enterocytes in R2 region of adult fly gut. *Margo* protein signals in guts were detected by anti-HA staining (in red). Genotype: NP1GAL4^{ts}; UAS*Magro*^{HA}.

(E) Lipid level in intestine was quantified by ORO staining when *magro* was specifically knocked down in gut. Genotype: NP1Gal4^{ts}; UAS*magro*^{RNAi}.

(F) Pancreatin supplementation (2mg/ml) restores intestinal lipid level in flies fed with RU486(75μg/ml, 7days). Lipid level was quantified based on ORO signaling intensity from flies of each condition (n = 10).

(G) Pancreatin supplementation (2mg/ml) also restores gut lipid in flies with *magro* knocked down in gut at 29°C for 4 days. Genotypes: NP1Gal4^{ts}; UAS*magro*^{RNAi}(n = 10).

(H) Pancreatin supplementation suppress starvation sensitivity induced by RU486 feeding. Flies at each condition(n = 20) were starved in ddH2O for indicated time point. Percentage of survival was quantified as shown in Y-axis.

(Figure 2C). However, overexpressing *Magro* in enterocytes using the enterocyte-specific driver NP1Gal4 (Li et al., 2016; Karpac et al., 2013) failed to restore TAG level in RU486 treated flies (Figure S2B). Secretion into the gut lumen is critical for *Magro* to exert its function(Sieber and Thummel, 2012). Intriguingly, *Magro* protein was found accumulated inside enterocytes after RU486 administration, while total *Magro* protein level is decreased in NP1Gal4; UASMagro guts (Figures 2D and S2C). Meanwhile, GFP expression under the control of UAS-GAL4 system is largely unchanged by RU486 (Figure S2D), suggesting RU486 did not interfere with GAL4 activity itself. These results indicated that RU486 can also regulate *Magro* at post-transcriptional level.

In contrast, *Magro* knockdown specifically in the gut of adult *Drosophila* by NP1GAL4^{ts}> UAS*Magro*^{RNAi} causes significant reduction of lipid levels in the gut and the whole body compared with controls after a 5-day induction at a restrictive temperature (29°C) (Figures 2E and S2A). To determine whether RU486 reduces lipid level through *magro*, RU486-containing food was supplemented with pancreatin, which is a mixture of exogenous lipases (Lohr et al., 2009). As shown in Figure 2F, the intestinal lipid level of flies fed with RU486 was significantly restored by pancreatin. Similarly, the intestinal lipid level of NP1GAL4^{ts}> UAS*Magro*^{RNAi} flies was also significantly restored by pancreatin supplementation (Figures 2G and S3). Furthermore, animals fed with RU486 plus pancreatin were resistant to starvation compared with those fed only with RU486 (Figure 2H). Collectively, these results indicate that RU486 decreases lipid level by transcriptionally suppressing *magro* in *Drosophila* intestine.

RU486 downregulates magro by antagonizing ERR activity in *Drosophila*

Studies have shown that nuclear receptors are important regulators of lipid metabolism in *Drosophila* (King-Jones and Thummel, 2005). *Drosophila* dHR96 regulates TAG and cholesterol metabolism by promoting *Magro* transcription (Sieber and Thummel, 2009; King-Jones and Thummel, 2005; Buchon et al., 2013). HNF4 was also shown to manipulate lipid mobilization and β-oxidation in larvae gut (Palanker et al., 2009; Barry and Thummel, 2016). Recently, studies have reported that dERR has a similar function with GR in mammals in terms of inflammation and glucose metabolism(Bartolo et al., 2020). Thus, we next explored whether RU486 regulates lipid uptake through nuclear receptors in *Drosophila*.

Ligand sensors for all 18 nuclear receptors in *Drosophila* have been generated to monitor their activity (Palanker et al., 2006). In brief, the ligand binding domain (LBD) was fused with Gal4 under the control of heat shock promoters. After heat shock, a UAS-fused reporter is expressed under the control of hsGal4NRLBD in response to ligand (Palanker et al., 2006). A UAS-luciferase construct was used as a sensitive activity reporter (Figure 3A). Relative luciferase activity was measured in the intestine after treatment with or without RU486. We found that only flies possessing hsERR^{LBD}Gal4 showed a significant decrease of luciferase activity in response to RU486, whereas sensors of hsDHR96^{LBD}Gal4 and hsHNF4^{LBD}Gal4 did not show strong changes in expression after RU486 feeding (Figure 3B). These results suggest that RU486 antagonizes ERR activity in fly gut. Consistently, we found that ERR knockdown in gut significantly decreased lipid level and *magro* transcription (Figure 3C), whereas ERR overexpression by NP1GAL4^{ts}> UAS::*ERR* promotes *magro* transcription (Figure 3D) and increases lipid level in a *magro*-dependent manner (Figures 3E and S2A). dERR transcription in the guts is largely unchanged by RU486 treatment (Figure S2A). Together, these

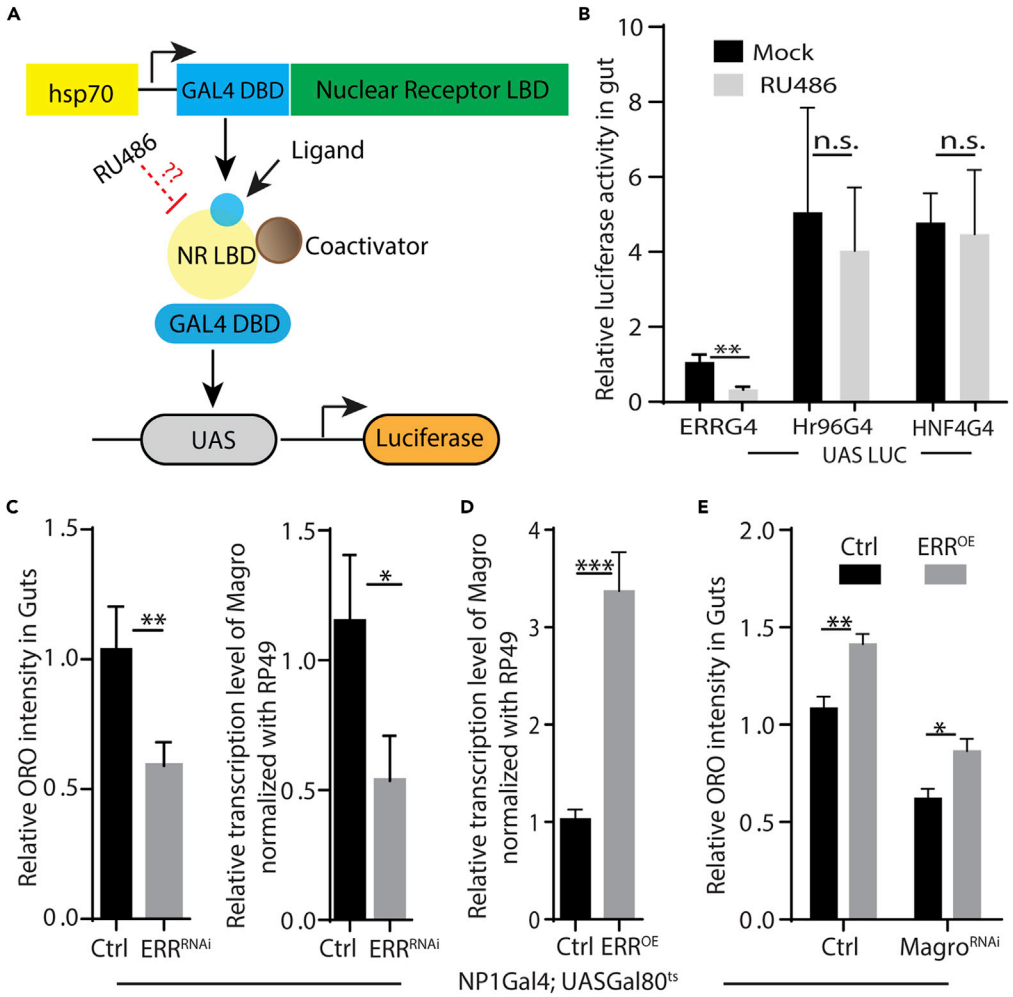

Figure 3. RU486 downregulates Magro by antagonizing ERR activity

(A) Schematic of a luciferase sensor for nuclear receptors in Drosophila. The scheme is modified based on previous publication (Palanker et al., 2006).

(B) Effect of RU486 on different nuclear receptor sensors were quantified by luciferase reporter was measured by luminescence activity kit. Genotypes: hs-ERRLBDGal4; UASRen-LUC, hs-dHR96LBDGal4; UASRen-LUC, HNF4Gal4; UASRen-LUC.

(C) ERR knocking-down in gut reduces lipid level and magro transcription. Lipid level was quantified by ORO staining and *magro* transcript in gut was normalized with RP49. Genotypes: NP1Gal4, UASGal80^{ts}, and UASERR^{RNAi}.

(D) ERR overexpression increase *magro* transcription in gut. Three independent replicates were measured.

(E) ERR overexpression in gut lead to elevated lipid level in a *magro*-dependent manner. Genotype for (D and E) NP1Gal4^{ts}, UAS::ERR^{OE} or NP1Gal4^{ts}, UAS::ERR^{OE}; UAS:Magro^{RNAi}.

results indicated that RU486 suppresses lipid uptake and inhibits *magro* transcription by blocking ERR activity in *Drosophila*.

RU486 blocks lipid uptake by inhibiting transcription of *Pnlip* in pancreas

Next, we investigated whether RU486 regulates lipid metabolism in mice. Since *magro* is a lipase A homolog in mice, we then determined whether the transcription of lipases in mice was regulated by RU486. There are more than a dozen lipases encoded by the mice genome(Warden et al., 1993). These lipases often exhibit a clear tissue-specific expression pattern. For instance, pancreatic lipases (PTL, also known as *Pnlip*) and gastric lipases are the main enzymes responsible for digestion of dietary triglycerides in the gastrointestinal tract. They function synergistically to further digest fat in the duodenum via the branching ductal

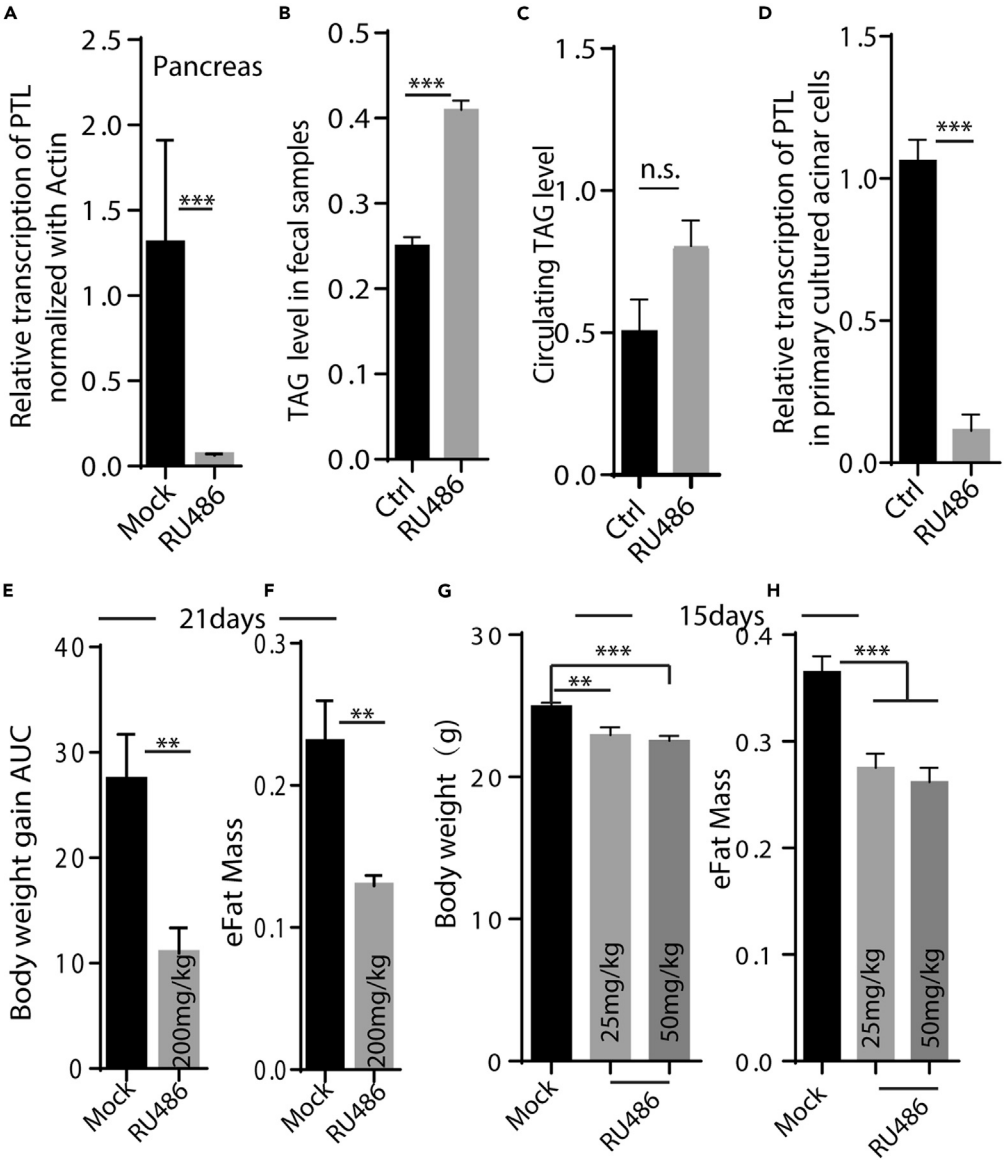

Figure 4. RU486 blocks lipid uptake by negatively regulating transcription of Pancreatic lipase (PTL) in pancreatic acinar cells

(A) PTL transcription in pancreas tissue was significantly reduced after oral administration of RU486 for 7 days at 200 mg/kg.

(B and C) TAG level in fecal(B) and in blood samples (C) was measured under indicated conditions.

(D) PTL transcriptional level in *ex vivo* cultured acinar cells was quantified by RT-qPCR. n = 3 for each condition. Concentration of RU486:0.05mg/ml.

(E) Body weight gain was measured at different time point. 5–6 male mice for each condition were measured. Concentration of RU486:200mg/ml.

(F) Epididymal fat (eFat) weight decreased significantly after fed with RU486. n = 6 for each condition. Student t-test was performed for statistical analysis.

(G and H) Body weight (G) and eFat mass(H) were measured after RU486 administration. Concentrations were as indicated in columns. n = 5 to 6. Student t-test was performed for statistical analysis.

system (Carey et al., 1983; Hamosh et al., 1975; Carriere et al., 1993) (Figure S4A). As shown in Figure 4A, we found a drastic reduction of *PTL* transcripts in pancreas tissue of mice after oral administration of RU486 (200 mg/kg body weight) for 10 days. There were no significant changes in the transcription level of other

lipases following RU486 supplementation (Figure S4B). Because PTL is required for dietary fat absorption, we also examined lipid levels in feces and in blood, and as shown in Figures 4B and 4C, fecal lipid levels were significantly higher while circulating lipids were largely normal in RU486-fed animals compared with mice fed on control food.

Acinar cells are the main exocrine cell type in the pancreas. Immunofluorescence analysis of pancreas cryosections showed strong PTL staining in rosette-like acinar cells (Figure S5A). To determine whether RU486 directly regulates *PTL* transcription in acinar cells, *PTL* transcription was compared in *ex vivo* cultured acinar cells. As shown in Figure 4D, *PTL* transcription was significantly reduced by RU486 administration (0.1 μM, 8 h), while no obvious morphological changes were observed in these primary cells (Figure S5B). Similar to the effect of RU486 in flies, chronic oral administration of RU486 (200 mg/kg, 5 weeks) caused significant weight loss (Figures 4E and S4C) and decrease of adipose tissue mass in treated mice (Figure 4F), despite having largely normal food intake (Figure S5C). RU486 at lower concentrations (50 mg/Kg or 25 mg/Kg), also showed robust reduction of body weight and epididymal fat (efat) mass (Figures 4G and 4H). Intriguingly, transcription of *ATGL*, a lipolytic enzyme, is upregulated by RU486 in fat tissues (Figure S4D). These results indicate that RU486 regulates mice lipid homeostasis by suppressing transcription of pancreatic *PTL* lipase and promoting lipolysis of fat tissue via *ATGL*.

RU486 directly regulates *PTL* transcription through the nuclear receptor GR

As RU486 is an antagonist of steroid hormone receptors, we next determined the steroid hormone receptor responsible for the effect of RU486 on *PTL* regulation in pancreatic acinar cells. PR is a nuclear receptor that responds to progesterone, and it is highly expressed in sex glands, such as mammary glands and ovaries (Di Renzo et al., 2016; Diep et al., 2015; Scarpin et al., 2009). We found no evidence of PR expression in pancreas as indicated by immunofluorescence staining, Western blot, and RT-qPCR analysis, while showing a high expression in the uterus (Figures 5A, S5D, and S5E and data not shown). High concentration of RU486 was shown to suppress Androgen receptor activity(Tovar et al., 1997). However, transcription of AR and its known transcriptional targets, such as *Cyp1a, HSPa5, TgfBeta5,* and *Vegfa*, are not detectable by qPCR in the pancreas (Figure S5F). In contrast, GR and PTL were highly expressed in the pancreas as shown by immunofluorescence analysis of cryosections and by western blot (Figures 5A and S5G). Considering the complex crosstalk of these steroid hormones in distal tissues(De Bosscher et al., 2020), the role of AR on PTL transcription in pancreas, however, cannot be excluded.

Intriguingly, in addition to amylase, acute DXMS administration (intraperitoneal injection, 10 mg/kg, 8 h) causes increased *PTL* transcription in the pancreas (Figure 5B). Because DXMS is a GR agonist, these results suggest that GR can regulate *PTL* transcription. There are two typical consensus GRE motifs in the *PTL* promoter region of both mice and rats (Figure 5C). To determine whether GR directly regulates *PTL* transcription, we performed chromatin immunoprecipitation (ChIP) using a GR antibody as bait in the pancreas extract. Using qPCR, we found that the GRE containing promoter region in *PTL* was nearly 2000-fold enriched in anti-GR samples compared with anti-IgG samples, indicating that PTL is a target of GR in the pancreas (Figure 5D).

The molecular link between GR and Pnlip was further analyzed in rat pancreatic tumor cell line AR42J. We found that DXMS incubation (100nM, 24 h) significantly increases transcription of *Pnlip* and amylase in AR42J cells, and that this effect was blocked by RU486 (1μM, 24 h) (Figure 5E). To further test if GR regulates *Pnlip* transcription via the two conserved GRE motifs, we generated luciferase reporters in which luciferase was fused with either a 2 kb promoter region of rat *Pnlip* with wild type (GRE^{wt}) or a GRE-deleted version (GRE^{del}). A promoterless Renilla luciferase vector was co-transfected as an internal control (Figure 5C). Relative luciferase activity with GRE^{wt} was found significantly higher than that with GRE^{del} after acute DXMS treatment (Figure 5F). These findings demonstrate that GR regulates *Pnlip* transcription by directly binding to the GRE motif in the promoter region.

RU486 inhibits obesity in HFD models of *Drosophila* and mice

Because aberrant lipid metabolism is associated with obesity and other metabolic syndromes(Grundy, 2004; Weiss et al., 2004), we next explored whether RU486 affects obesity. Consistent with previous studies, we found wild type flies fed with high fat diet (HFD, conventional food supplemented with 30% coconut oil) are enriched in gut lipids (Galikova and Klepsatel, 2018) (Figures 6Aand 6B), whereas administration of RU486 (75 μg/mL) leads to a significant decrease of lipid levels both in the gut and the whole body (Figures

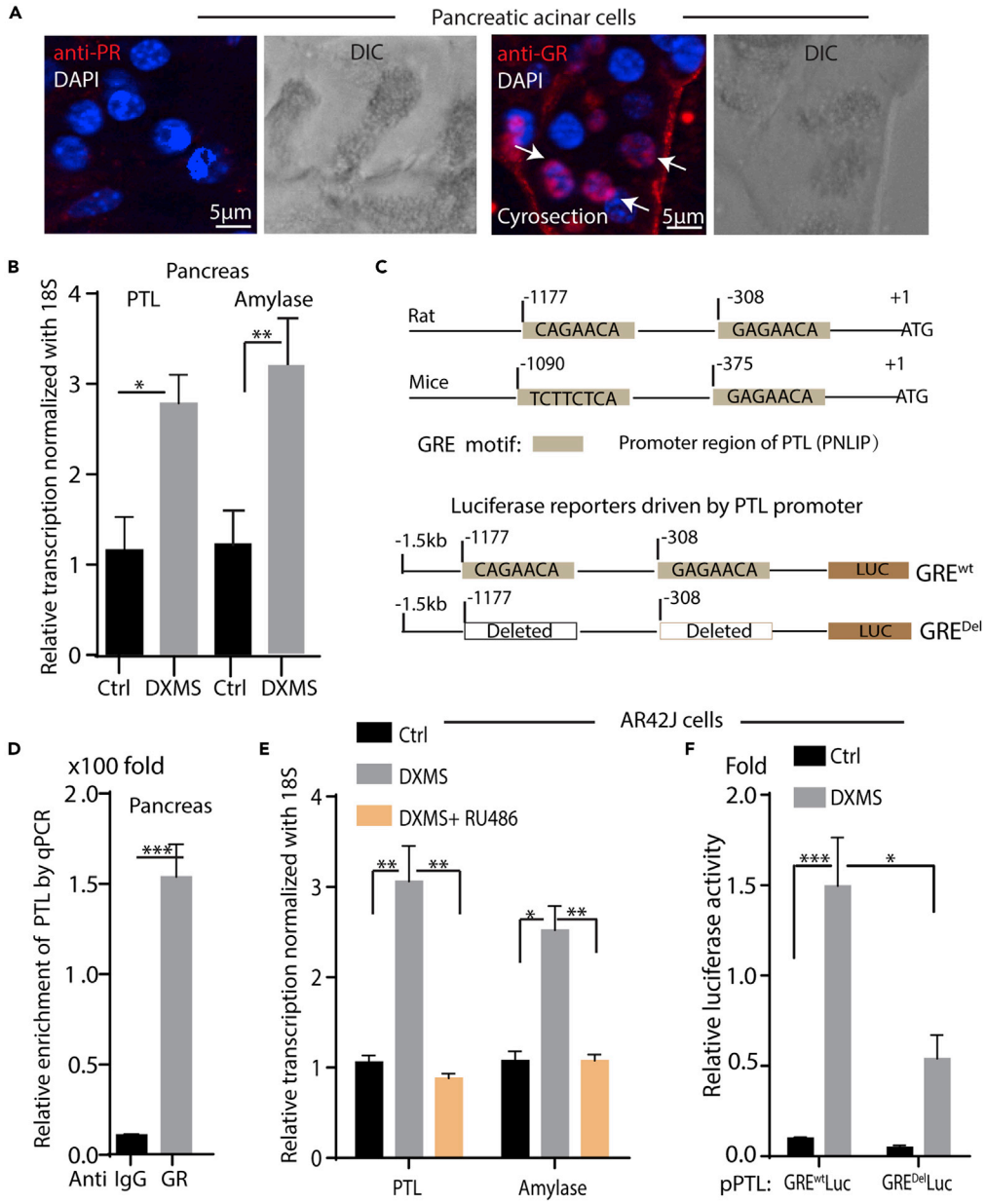

Figure 5. RU486 directly regulate PTL transcription through nuclear receptor GR

(A) Expression of Progesterone Receptor (PR) and glucocorticoid receptor (GR) in pancreas was examined by immuno-staining in frozen samples after cryosection. Differential Interference Contrast (DIC) microscope image from same field was shown on right. Arrows points to the positive staining of GR in the nucleus (in red). Scale bars for A and (B) 5um.

(B) Acute DXMS administration (i.p.injected at 100mg/kg, 8 h) in mice promotes transcription of PTL and amylase in pancreas. n = 4 to 5 for each condition.

(C) Schematics, top: two conserved GRE (GR Response Element) motif in promoter region of PTL (both in mice and rat) was shown. Bottom: Strategy of luciferase reporter assay to evaluate the GRE motif in PTL promoter region.

(D) qPCR experiments shown that PTL promoter region are highly enriched in anti-GR ChIP samples from mice pancreas.

(E) Incubation of DXMS (100nM, 24 h) promotes PTL and amylase transcription in AR42J cell lines, and this effect can be suppressed by coincubation of RU486.

(F) The role of GRE motif in PTL promoter in response to DXMS was monitored in AR42J cell lines transfected with indicated luciferase reporters. These reporters contain luciferase fused with PTL promoter bearing wild type GRE(GRE^{WT}luc) or deleted GRE (GRE^{Del} luc).

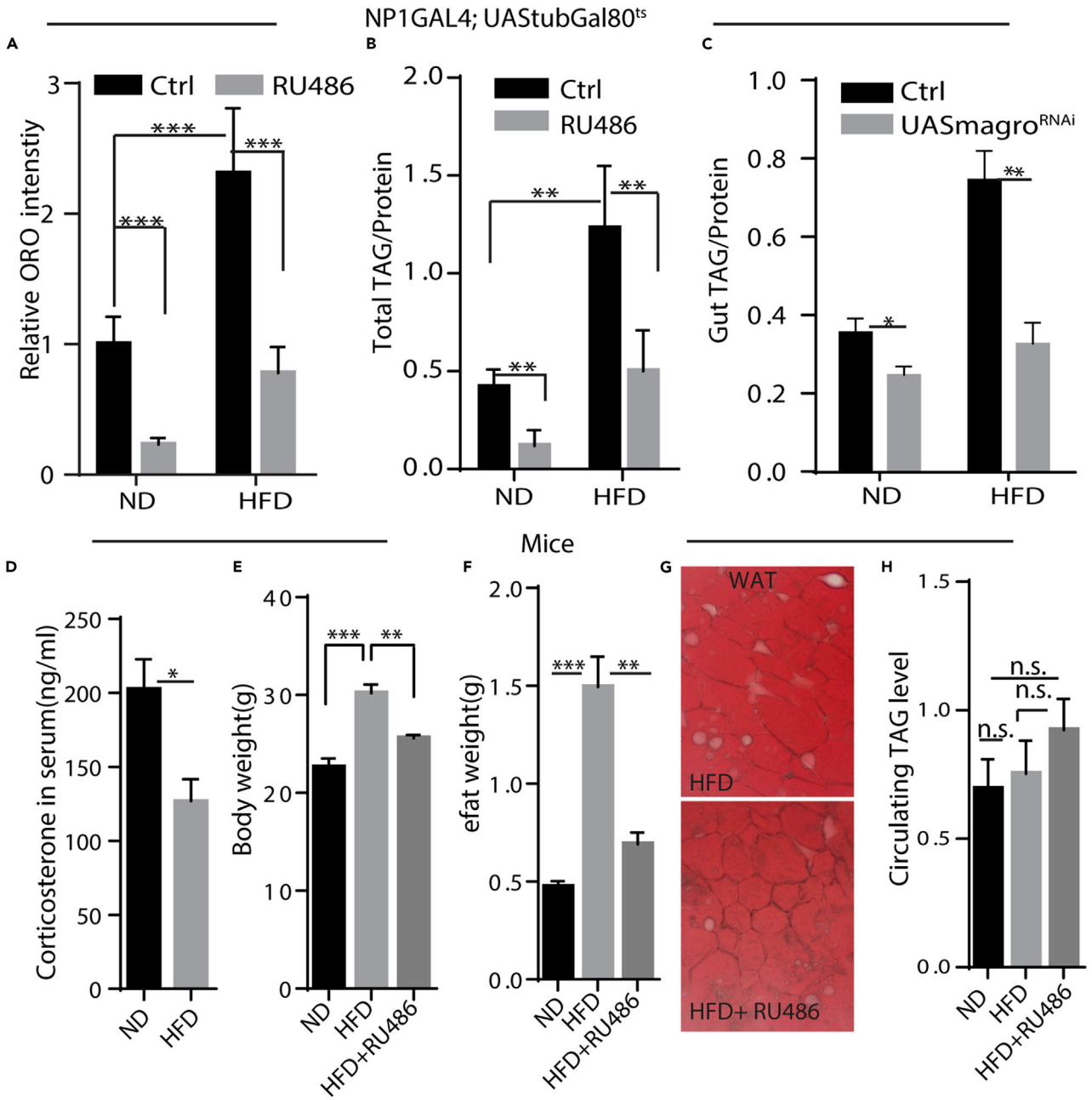

Figure 6. RU486 inhibits obesity in HFD models of Drosophila and mouse

(A) Neutral lipid level in R2 region of fly guts were quantified based on the intensity of Oil Red O staining. Triplicates were analyzed for each condition. ND: Normal Diet, HFD: High Fat Diet. Student t-test performed for statistics.

(B) Lipid level in whole fly were quantified by TAG assay kit. TAG levels were normalized with protein level.

(C) Gut specific knockdown of *Magro* decrease lipid level in flies fed with high fat diet (HFD). Genotype: NP1Gal4, tubGal80^{ts}, UASMagro^{RNAi}.

(D) Corticosterone level in serum was examined in mice fed with *ad libitum* diet (ND) or with high fat diet (HFD). 4–5 animals for each condition were quantified.

(E and F) RU486 administration reduces body weight and efat mass in HFD male mice. n = 5 for each condition.

(G) Representative images of frozen sectioned samples of white adipocyte tissues (WAT) after Oil Red O staining. Note: Significant smaller adipocytes size was observed after 2-week RU486 treatment.

(H) Circulating TAG levels were quantified by TAG kit normalized with protein level. One-way ANOVA analysis was performed for A to C. Student t-test was performed for statistics for (D, E, F, and H).

6A and 6B, and Figure S6A). Similar effects were obtained in NP1GAL4^{ts}> UAS::*Magro*^{RNAi} flies, in which *magro* was specifically knocked down in the gut in *ad libitum* (AL) food for 4 days before shifting to coconut oil-supplemented food for another 5 days (Figure 6C). On the other hand, *Magro* transcripts were reduced in fly guts after they were fed with HFD (Figure S6B). These results indicate that RU486 and *Magro* suppress HFD-induced obesity in flies.

HFD-induced obese mice were generated by feeding 4-week-old male mice an HFD for three months (Buettner et al., 2007; Winzell and Ahren, 2004). As expected, body weight and fat tissues, such as efat and perirenal fat (pfat), were significantly increased in these animals compared with those mice fed on AL food. Intriguingly, corticosterone levels in the serum were significantly reduced in HFD mice (Figures 6D and S6C), while RU486 supplementation (200mg/kg, 14 days) significantly reduced body weight, adipose tissues mass, and adipocyte cell size in HFD-fed animals (Figures 6E–6G,S6D, and S6E). Circulating TAG levels were largely unchanged in obese mice after RU486 administration (Figure 6H).

These results indicate that glucocorticoid level is decreased in diet-induced obese mice and RU486 administration suppresses lipid accumulation in obese animals, both in flies and mice.

DISCUSSION

In our study, we found that administration of RU486 (mifepristone) blocks intestinal lipid uptake in both mice and flies. Further, we showed that RU486 directly inhibits *Pnlip* transcription via GR in pancreatic acinar cells, and that it negatively regulates fly *Magro* transcription by inhibiting dERR. Collectively, these findings revealed an unexpected role of GR signaling on lipid digestion that is conserved in flies and mice (Figure 7).

RU486 is used as a drug inducer for the GeneSwitch system in *Drosophila*. Recent studies indicated that RU486 has some context-dependent side effects (Ford et al., 2007; Poirier et al., 2008; Landis et al., 2015; Robles-Murguia et al., 2019), although the underlying mechanism remains unexplored. In the current study, we found that RU486 globally regulates gene expression in *Drosophila* intestine; metabolic processes and detoxify pathways were all significantly upregulated. As genes involved in stress response and metabolism are important regulators of multiple pathophysiological processes, such as aging, immunity, and stem cell activity (Karpac et al., 2013; Zou et al., 2000; Hochmuth et al., 2011), findings derived from long-term high-dose RU486 feeding need to be well controlled and interpreted with caution.

Dietary TAGs are hydrolyzed into free fatty acids by lipases for intestinal absorption. Thus, preventing intestinal uptake is a strategy for weight loss. One such example of this strategy is Orlistat, which is a lipase inhibitor approved by the Food and Drug Administration of the United States as a weight loss drug (Heck et al., 2000). The lipid-lowering effect of RU486 demonstrated in our study is similar to that found with Orlistat. In fact, circulating glucocorticoid level is decreased in HFD-induced mice, and administration of RU486 suppresses HFD-induced obesity in both flies and mice. However, since RU486 is pleiotropic, whether it is suitable to treat obesity in humans requires further characterization.

As a stress hormone, glucocorticoids catabolize energy substrates to cope with the increased metabolic demand (Vegiopoulos and Herzig, 2007; Wang et al., 2012). Unlike the effects of GCs on glucose metabolism, its effect on lipid metabolism is quite complex (Macfarlane et al., 2008; Vegiopoulos and Herzig, 2007). Studies on the role of GCs in individual metabolic organs, such as adipose tissue and liver, often present conflicting results (Vegiopoulos and Herzig, 2007) (Gregoire et al., 1998; Campbell et al., 2011) (Slavin et al., 1994; Samra et al., 1998; Rhee et al., 2003). Here we found GC level can regulate PTL transcription from exocrine acinar cells and regulate dietary fat digestion in intestine. Intriguingly, we found that corticosterone level was downregulated in HFD-fed mice (Figures 6D and S6C), suggesting a compensatory mechanism that exists to prevent excessive lipid uptake. Further reduction of PTL by RU486 treatment can ameliorate HFD-induced obesity. In addition to suppressing intestinal lipid uptake, RU486 can also promote lipolysis in fat tissues by upregulating transcription of ATGL. These results indicated that RU486/GR play a systematic role on lipid metabolism, which might explain the conflicting results observed on its role in individual tissues.

Metabolic demand between tissues has to be coordinated to maintain lipid homeostasis. Here we found RU486/DXMS regulates intestinal lipid digestion through GR in pancreatic acinar cells. Our study identified

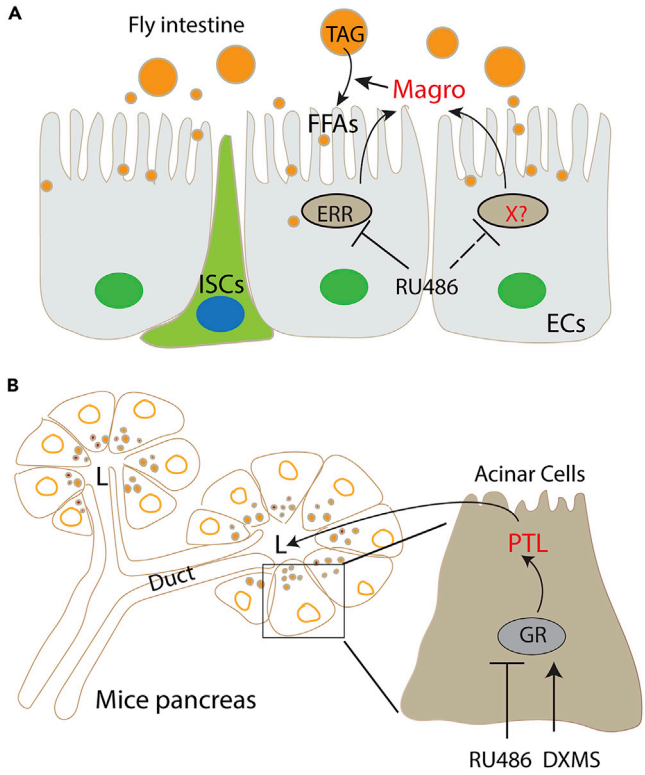

Figure 7. RU486 suppress intestinal lipid uptake through a conserved pathway

In Drosophila (A), dietary fat (large solid orange circles) was digested into free fatty acids (FFAs, small solid circle) by LipA homolog, Magro, in gut lumen. RU486, a GR/PR antagonist, can suppress *magro* transcription via negatively regulated nuclear receptor ERR, a functional homolog of GR in Drosophila. In addition to ERR, RU486 might regulate *Magro* transcription through other unknown factors (shown as a question mark). Cell types in fly gut were also shown: ISCs (intestinal stem cells) and ECs (Enterocytes).

In mice (B), lipase PTL and amylase from pancreatic acinar cells are secreted into the lumen (L) and transported into the intestine via the branching ductal system. Similarly, RU486 can suppress PTL transcription via GR to regulate dietary fat uptake, whereas DXMS can promote GR in acinar cells. L: Lumen, Duct: Pancreatic duct system.

a novel conserved role of GC/GR on lipid metabolism, and provided new insights on the role of GCs in Cushing disease, obesity, and other related metabolic syndromes.

Limitation of study

In this study, we found that *Pnlip* in pancreatic acinar cells is a direct transcriptional target of the glucocorticoid receptor (GR). However, conclusions are mainly based on studies with antagonist and agonist. Since RU486 and DXMS are quite pleiotropic, future studies in GR conditional knock-out mice would help to consolidate the role of GR on lipid digestion and uptake.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102507.

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AUTHOR CONTRIBUTIONS

H. D. and P. M. conceived and designed the study. H. D. wrote the paper. P. M., Y. Z., Q. L., Y. Y., R. H., S. W., C. H. contributed to the experimental work. H. D. and P. M. conducted the statistical analysis of the data.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

Abumrad, N.A., and Davidson, N.O. (2012). Role of the gut in lipid homeostasis. Physiol. Rev. *92*, 1061–1085.

Barry, W.E., and Thummel, C.S. (2016). The Drosophila HNF4 nuclear receptor promotes glucose-stimulated insulin secretion and mitochondrial function in adults. Elife *5*, e11183.

Bartolo, G., Gonzalez, L.O., Alameh, S., Valencia, C.A., and Martchenko Shilman, M. (2020). Identification of glucocorticoid receptor in Drosophila melanogaster. BMC Microbiol. *20*, 161.

Basina, M., Liu, H., Hoffman, A.R., and Feldman, D. (2012). Successful long-term treatment of Cushing disease with mifepristone (RU486). Endocr. Pract. *18*, e114–120.

Beato, M., Herrlich, P., and Schutz, G. (1995). Steroid hormone receptors: many actors in search of a plot. Cell *83*, 851–857.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.

Buchon, N., Osman, D., David, F.P., Fang, H.Y., Boquete, J.P., Deplancke, B., and Lemaitre, B. (2013). Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. Cell Rep. *3*, 1725–1738.

Buettner, R., Scholmerich, J., and Bollheimer, L.C. (2007). High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity (Silver Spring) *15*, 798–808.

Burcin, M.M., Schiedner, G., Kochanek, S., Tsai, S.Y., and O'malley, B.W. (1999). Adenovirus-mediated regulable target gene expression in vivo. Proc. Natl. Acad. Sci. U S A *96*, 355–360.

Cadepond, F., Ulmann, A., and Baulieu, E.E. (1997). RU486 (mifepristone): mechanisms of action and clinical uses. Annu. Rev. Med. *48*, 129–156.

Campbell, J.E., Peckett, A.J., D'souza A, M., Hawke, T.J., and Riddell, M.C. (2011). Adipogenic

and lipolytic effects of chronic glucocorticoid exposure. Am. J. Physiol.Cell. Physiol. *300*, C198–C209.

Carey, M.C., Small, D.M., and Bliss, C.M. (1983). Lipid digestion and absorption. Annu. Rev. Physiol. *45*, 651–677.

Carriere, F., Barrowman, J.A., Verger, R., and Laugier, R. (1993). Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. Gastroenterology *105*, 876–888.

Chen, J., Wang, J., Shao, J., Gao, Y., Xu, J., Yu, S., Liu, Z., and Jia, L. (2014). The unique pharmacological characteristics of mifepristone (RU486): from terminating pregnancy to preventing cancer metastasis. Med. Res. Rev. *34*, 979–1000.

Czyzyk, A., Podfigurna, A., Genazzani, A.R., and Meczekalski, B. (2017). The role of progesterone therapy in early pregnancy: from physiological role to therapeutic utility. Gynecol. Endocrinol. *33*, 421–424.

De Bosscher, K., Desmet, S.J., Clarisse, D., Estebanez-Perpina, E., and Brunsveld, L. (2020). Nuclear receptor crosstalk - defining the mechanisms for therapeutic innovation. Nat. Rev. Endocrinol. *16*, 363–377.

Di Renzo, G.C., Giardina, I., Clerici, G., Brillo, E., and Gerli, S. (2016). Progesterone in normal and pathological pregnancy. Horm. Mol. Biol. Clin. Invest. *27*, 35–48.

Diep, C.H., Daniel, A.R., Mauro, L.J., Knutson, T.P., and Lange, C.A. (2015). Progesterone action in breast, uterine, and ovarian cancers. J. Mol. Endocrinol. *54*, R31–R53.

Dutta, D., Dobson, A.J., Houtz, P.L., Glaber, C., Revah, J., Korzelius, J., Patel, P., Edgar, B.A., Buchon, N., et al. (2015). Regional Cell-Specific Transcriptome Mapping Reveals Regulatory Complexity in the Adult Drosophila Midgut. Cell Reports *12*, 346–358.

Dutta, D., Xiang, J., and Edgar, B.A. (2013). RNA expression profiling from FACS-isolated cells of the Drosophila intestine. Curr. Protoc. Stem Cell Biol *27*, 2F.2.1–F.2.12.

Ford, D., Hoe, N., Landis, G.N., Tozer, K., Luu, A., Bhole, D., Badrinath, A., and Tower, J. (2007). Alteration of Drosophila life span using conditional, tissue-specific expression of transgenes triggered by doxycyline or RU486/ Mifepristone. Exp. Gerontol. *42*, 483–497.

Galikova, M., and Klepsatel, P. (2018). Obesity and aging in the Drosophila model. Int. J. Mol. Sci. *19*, 1896.

Geer, E.B., Islam, J., and Buettner, C. (2014). Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. Endocrinol. Metab. Clin.North Am. *43*, 75–102.

Gout, J., Pommier, R.M., Vincent, D.F., Kaniewski, B., Martel, S., Valcourt, U., and Bartholin, L. (2013). Isolation and culture of mouse primary pancreatic acinar cells. J. Vis. Exp. *78*, 50514.

Gregoire, F.M., Smas, C.M., and Sul, H.S. (1998). Understanding adipocyte differentiation. Physiol. Rev. *78*, 783–809.

Gronke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Muller, G., Jackle, H., and Kuhnlein, R.P. (2005). Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab. *1*, 323–330.

Grundy, S.M. (2004). Obesity, metabolic syndrome, and cardiovascular disease. J. Clin. Endocrinol. Metab. *89*, 2595–2600.

Guo, L., Karpac, J., Tran, S., and Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell *156*, 109–122.

Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature *445*, 275–280.

Hamosh, M., Klaeveman, H.L., Wolf, R.O., and Scow, R.O. (1975). Pharyngeal lipase and digestion of dietary triglyceride in man. J. Clin. Invest. *55*, 908–913.

Heck, A.M., Yanovski, J.A., and Calis, K.A. (2000). Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy *20*, 270–279.

Hegele, R.A., Ramdath, D.D., Ban, M.R., Carruthers, M.N., Carrington, C.V., and Cao, H. (2001). Polymorphisms in PNLIP, encoding pancreatic lipase, and associations with metabolic traits. J. Hum. Genet. *46*, 320–324.

Hochmuth, C.E., Biteau, B., Bohmann, D., and Jasper, H. (2011). Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. Cell Stem Cell *8*, 188–199.

Iqbal, J., and Hussain, M.M. (2009). Intestinal lipid absorption. Am. J. Physiol. Endocrinol. Metab. *296*, E1183–E1194.

Ishikawa, H., Ishi, K., Serna, V.A., Kakazu, R., Bulun, S.E., and Kurita, T. (2010). Progesterone is essential for maintenance and growth of uterine leiomyoma. Endocrinology *151*, 2433–2442.

Ito, K., Chung, K.F., and Adcock, I.M. (2006). Update on glucocorticoid action and resistance. J. Allergy Clin. Immunol. *117*, 522–543.

Ja, W.W., Carvalho, G.B., Mak, E.M., De La Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T., and Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. Proc. Natl. Acad. Sci. U S A *104*, 8253–8256.

Karpac, J., Biteau, B., and Jasper, H. (2013). Misregulation of an adaptive metabolic response contributes to the age-related disruption of lipid homeostasis in Drosophila. Cell Rep. *4*, 1250–1261.

King-Jones, K., and Thummel, C.S. (2005). Nuclear receptors–a perspective from Drosophila. Nat. Rev. Genet. *6*, 311–323.

Ko, C.W., Qu, J., Black, D.D., and Tso, P. (2020). Regulation of intestinal lipid metabolism: current concepts and relevance to disease. Nat. Rev. Gastroenterol. Hepatol. *17*, 169–183.

Kuo, T., Mcqueen, A., Chen, T.C., and Wang, J.C. (2015). Regulation of glucose homeostasis by glucocorticoids. Adv. Exp. Med. Biol. *872*, 99–126.

Kusunoki, M., Cooney, G.J., Hara, T., and Storlien, L.H. (1995). Amelioration of high-fat feeding-induced insulin resistance in skeletal muscle with the antiglucocorticoid RU486. Diabetes *44*, 718–720.

Landis, G.N., Salomon, M.P., Keroles, D., Brookes, N., Sekimura, T., and Tower, J. (2015). The progesterone antagonist mifepristone/ RU486 blocks the negative effect on life span caused by mating in female Drosophila. Aging (Albany NY) *7*, 53–69.

Lee, M.J., and Fried, S.K. (2009). Integration of hormonal and nutrient signals that regulate leptin synthesis and secretion. Am. J. Physiol. Endocrinol. Metab. *296*, E1230–E1238.

Lee, M.J., Pramyothin, P., Karastergiou, K., and Fried, S.K. (2014). Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity. Biochim. Biophys. Acta *1842*, 473–481.

Li, H., Qi, Y., and Jasper, H. (2016). Preventing age-related decline of gut compartmentalization limits microbiota dysbiosis and extends lifespan. Cell Host Microbe *19*, 240–253.

Liang, Q., Ma, P., Zhang, Q., Yin, Y., Wang, P., Wang, S., Zhang, Y., Han, R., and Deng, H. (2020). A gum Arabic assisted sustainable drug delivery system for adult Drosophila. Biol. Open *9*, bio052241.

Logsdon, C.D., Perot, K.J., and Mcdonald, A.R. (1987). Mechanism of glucocorticoid-induced increase in pancreatic amylase gene transcription. J. Biol. Chem. *262*, 15765–15769.

Lohr, J.M., Hummel, F.M., Pirilis, K.T., Steinkamp, G., Korner, A., and Henniges, F. (2009). Properties of different pancreatin preparations used in pancreatic exocrine insufficiency. Eur. J. Gastroenterol. Hepatol. *21*, 1024–1031.

Luis, N.M., Wang, L., Ortega, M., Deng, H., Katewa, S.D., Li, P.W., Karpac, J., Jasper, H., and Kapahi, P. (2016). Intestinal IRE1 is required for increased triglyceride metabolism and longer lifespan under dietary restriction. Cell Rep. *17*, 1207–1216.

Ma, P., Yun, J., Deng, H., and Guo, M. (2018). Atg1-mediated autophagy suppresses tissue degeneration in pink1/parkin mutants by promoting mitochondrial fission in Drosophila. Mol. Biol.Cell *29*, 3082–3092.

Macfarlane, D.P., Forbes, S., and Walker, B.R. (2008). Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. J. Endocrinol. *197*, 189–204.

Mathur, D., Bost, A., Driver, I., and Ohlstein, B. (2010). A transient niche regulates the specification of Drosophila intestinal stem cells. Science *327*, 210–213.

Moguilewsky, M., and Philibert, D. (1984). RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J. Steroid Biochem. *20*, 271–276.

Muramatsu, Y., Yamada, T., Taniguchi, Y., Ogino, T., Kose, H., Matsumoto, K., and Sasaki, Y. (2005). Pnlip encoding pancreatic lipase is possible candidate for obesity QTL in the OLETF rat. Biochem. Biophys. Res. Commun. *331*, 1270–1276.

Osterwalder, T., Yoon, K.S., White, B.H., and Keshishian, H. (2001). A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. U S A *98*, 12596–12601.

Ozawa, H. (2005). Steroid Hormones, their receptors and neuroendocrine system. J. Nippon Med. Sch. *72*, 316–325.

Palanker, L., Necakov, A.S., Sampson, H.M., Ni, R., Hu, C., Thummel, C.S., and Krause, H.M. (2006). Dynamic regulation of Drosophila nuclear receptor activity in vivo. Development *133*, 3549–3562.

Palanker, L., Tennessen, J.M., Lam, G., and Thummel, C.S. (2009). Drosophila HNF4 regulates lipid mobilization and beta-oxidation. Cell Metab. *9*, 228–239.

Poirier, L., Shane, A., Zheng, J., and Seroude, L. (2008). Characterization of the Drosophila gene-switch system in aging studies: a cautionary tale. Aging Cell *7*, 758–770.

Priyadarshini, E., and Anuradha, C.V. (2017). Glucocorticoid antagonism reduces insulin resistance and associated lipid abnormalities in high-Fructose-fed mice. Can. J. Diabetes *41*, 41–51.

Rhee, J., Inoue, Y., Yoon, J.C., Puigserver, P., Fan, M., Gonzalez, F.J., and Spiegelman, B.M. (2003). Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. Proc. Natl. Acad. Sci. U S A *100*, 4012–4017.

Robles-Murguia, M., Hunt, L.C., Finkelstein, D., Fan, Y., and Demontis, F. (2019). Tissue-specific alteration of gene expression and function by RU486 and the GeneSwitch system. NPJ Aging Mech. Dis. *5*, 6.

Roman, G., Endo, K., Zong, L., and Davis, R.L. (2001). P[Switch], a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc. Natl. Acad. Sci. U S A *98*, 12602–12607.

Samra, J.S., Clark, M.L., Humphreys, S.M., Macdonald, I.A., Bannister, P.A., and Frayn, K.N. (1998). Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. J. Clin. Endocrinol. Metab. *83*, 626–631.

Scarpin, K.M., Graham, J.D., Mote, P.A., and Clarke, C.L. (2009). Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. Nucl. Recept.Signal. *7*, e009.

Sieber, M.H., and Thummel, C.S. (2009). The DHR96 nuclear receptor controls triacylglycerol homeostasis in Drosophila. Cell Metab. *10*, 481–490.

Sieber, M.H., and Thummel, C.S. (2012). Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. Cell Metab. *15*, 122–127.

Slavin, B.G., Ong, J.M., and Kern, P.A. (1994). Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J. Lipid Res. *35*, 1535–1541.

Tennessen, J.M., Baker, K.D., Lam, G., Evans, J., and Thummel, C.S. (2011). The Drosophila estrogen-related receptor directs a metabolic switch that supports developmental growth. Cell Metab. *13*, 139–148.

Tovar, A., Sanchez-Capelo, A., Galindo, J.D., Cremades, A., and Penafiel, R. (1997). Antiandrogenic effect of RU-486 in the mouse kidney. Int. J. Biochem.Cell Biol. *29*, 361–366.

Vegiopoulos, A., and Herzig, S. (2007). Glucocorticoids, metabolism and metabolic diseases. Mol.Cell. Endocrinol. *275*, 43–61.

Wang, J.C., Gray, N.E., Kuo, T., and Harris, C.A. (2012). Regulation of triglyceride metabolism by glucocorticoid receptor. Cell Biosci. *2*, 19.

Wang, Y., O'malley, B.W., Jr., Tsai, S.Y., and O'malley, B.W. (1994). A regulatory system for use in gene transfer. Proc. Natl. Acad. Sci. U S A *91*, 8180–8184.

Warden, C.H., Davis, R.C., Yoon, M.Y., Hui, D.Y., Svenson, K., Xia, Y.R., Diep, A., He, K.Y., and Lusis, A.J. (1993). Chromosomal localization of lipolytic enzymes in the mouse: pancreatic lipase, colipase, hormone-sensitive lipase, hepatic lipase, and carboxyl ester lipase. J. Lipid Res. *34*, 1451–1455.

Weiss, R., Dziura, J., Burgert, T.S., Tamborlane, W.V., Taksali, S.E., Yeckel, C.W., Allen, K., Lopes, M., Savoye, M., Morrison, J., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. N. Engl. J. Med. *350*, 2362–2374.

Winzell, M.S., and Ahren, B. (2004). The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes *53*, S215–S219.

Xu, C., He, J., Jiang, H., Zu, L., Zhai, W., Pu, S., and Xu, G. (2009). Direct effect of glucocorticoids on lipolysis in adipocytes. Mol. Endocrinol. *23*, 1161–1170.

Zou, S., Meadows, S., Sharp, L., Jan, L.Y., and Jan, Y.N. (2000). Genome-wide study of aging and oxidative stress response in Drosophila melanogaster. Proc. Natl. Acad. Sci. U S A *97*, 13726–13731.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
PNLIP Rabbit pAb-Polyclonal	ABClonal	A6396
Rabbit progesterone receptor antibody	Affinity	AF6106
Rabbit Glucocorticoid receptor polyclonal antibody	Invitrogen	PA1-511A
Rabbit anti-HA antibody	Sigma-Aldrich	H6908
Rabbit anti-Actin antibody	Sigma-Aldrich	A2066
Chemicals, peptides, and recombinant proteins		
DXMS	Sigma-Aldrich	D4902
RU486/mifepristone	Macklin	M830038
RU486/mifepristone	Calbiochem	#475838
High fat diet	Xietong.Organism	D12492
Oil red O	Sigma-Aldrich	O0625
Critical commercial assays		
Direct-zol RNA MiniPrep kit	ZYMO Research	R2061
One-step EvaGreen qRT-PCR kit	Applied Biological Materials Inc	G891
Triglyceride kit	Nanjing Jiangcheng Bioengineering institute	A110-1-1
Mouse Corticosterone (CORT) ELISA Kit	Nanjing Jiangcheng Bioengineering institute	H205
LipidTOXTM kit	Thermo Fisher	Cat #H34476
LipofectAMINE™ reagent	Invitrogen	#18324012
Deposited data		
RNAseq processed data	Github	https://handeng1.github.io/Processed-RNAseq-data-for-RU486-in-fly-guts/
Experimental models: cell lines		
AR42J	ATCC	ATCC® CRL1492™
HEK293T	ATCC	ATTC Cat# CRL-3216
Experimental models: organisms/strains		
C57BL/6	Jackson Laboratory	#000664
W1118	Bloomington Drosophila Stock Center	3605
hs-Gal4-ERRLBD	Bloomington Drosophila Stock Center	83690
hsGAL4-DHR96LBD	Bloomington Drosophila Stock Center	29648
hsGal4-Hnf4LBD	Bloomington Drosophila Stock Center	28856
UASERR^{RNAi}	Vienna Drosophila RNAi Center	V108349
UASmagro^{RNAi}	Vienna Drosophila RNAi Center	v109706
UASMagroHA	FlyORF	F003039
NP1-GAL4	Henri Jasper lab	Guo et al., 2014
5966GS-GAL4	Henri Jasper lab	Guo et al., 2014

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
PNLIPforChIPseq 5′AAAGATG GCGGCTCTGG3′	Invitrogen DNA Technologies	N/A
PNLPforChIPseq 5′GTCCGTG GGAATCTGAGAAAG3′	Invitrogen DNA Technologies	N/A
F1:5′CTCCGCCATGCAACAC TGCTTTAAGTTGCAGGCCC ACACTTATGC-3′	Invitrogen DNA Technologies	N/A
R1:5′TGAGAAAGTGCTAGAT TTGGCAGACCCGTGTGCTG TGTGCTGCTCA-3′.	Invitrogen DNA Technologies	N/A
F2:5′TGAGCAGCACACAGCA CACGGGTCTGCCAAATCTA GCACTTTCTCA-3′	Invitrogen DNA Technologies	N/A
R2:5′ATCTTTCTGAGCAGTG TCATGGAAAAGCGGCTCGA GAAGAGAGAGGCTCCA-3′	Invitrogen DNA Technologies	N/A
F: 5′-ACGGGTCTAGTCCTTA AAACTGC-3′	Invitrogen DNA Technologies	N/A
R: 5′-CTGCACAGAGGCAAAA ACATGG-3′	Invitrogen DNA Technologies	N/A
Recombinant DNA		
PNLIP-GREWT-LUC	This paper	N/A
PNLIP-GRE^{Del}-LUC	This paper	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Hansong Deng, Tongji University, Shanghai, 20092 China (hdeng@tongji.edu.cn).

Materials availability

This study did not generate new unique materials.

Data and code availability

All relevant data are available from the lead contact upon request. Processed RNAseq data is shown in Table S1 and is also available in https://handeng1.github.io/Processed-RNAseq-data-for-RU486-in-fly-guts/

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Drosophila stocks and genetics

The fly stocks used in this study are: W1118, UASERR (83690), hs-Gal4-ERRLBD (28867), hsGAL4-DHR96LBD (29648), hsGal4-Hnf4LBD (28856), from Bloomington Drosophila Stock Center, UASERR^{RNAi}(V108349) and UASmagro^{RNAi}(v109706) from Vienna Drosophila RNAi Center. UASMagroHA(F003039) from FlyORF, 5966GS-GAL4 and NP1-GAL4 (Guo et al., 2014) from Dr. Henri Jasper lab.

For fly culture, normal diet (ND) recipe is: 1L ddH2O, 13.8 g agar, 22 g molasses, 80 g malt extract, 18 g Brewer's yeast, 80 g corn flour, 10 g soy flour, 6.25 mL propionic acid, 2 g methyl-pbenzoate, 7.2 mL of Nipagin (20% in EtOH). Flies were maintained at 25°C and 60-70% humidity, on a 12 h light/dark cycle unless otherwise indicated. For RU486 food supplementation, 100 to 200 μl of a 5 mg/ml solution of RU486 or

vehicle (ethanol 80%) was deposited on top of the food (5-10ml in total) and dried overnight to ensure complete evaporation, resulting in indicated concentration of RU486 in the food accessible to flies. For HFD models in Drosophila, high fat diet (HFD) 30% coconut oil was mixed in normal diet. Kimwipe paper was used to get rid of extra oil on the food surface. Pancreatin was purchased from Macklin Inc, CAS #8049-47-6, which derived from porcine pancreas.

Mice stock and maintenance

C57BL/6 male mice and the standard chow were supplied by the laboratory animal center, Tongji University, China. Mice were housed in the Specific Pathogen Free (SPF) facility (21 ± 1°C, 55 ± 5% relative humidity, a 12-h light/dark cycle). Animals were allowed free access to water and the standard chow for at least 1 week prior to starting the experiments.

All experimental procedures were carried out in accordance with the internationally accepted principles for laboratory animal use and care, and approved by the Animal Ethics Committee, Tongji University, China (TJAB05320101).

Cell culture

AR42J cells, a rat pancreatic acinar cell line, were maintained in F-12K Medium (Kaighn's modification of Ham's F-12 Medium containing 2 mM L-glutamine and 1500 mg/L sodium bicarbonate; ATCC, Manassas, VA, USA) supplemented with 20% FBS. HEK293T cells were maintained in DMEM supplemented with 10% FBS.

Primary acinar cells culture

Dissection and culture of acinar cells were based on previous studies with modifications (Gout et al., 2013). In brief, mice were first sterilized with 70% ethanol, and a V-shaped incision made at the genital area to completely open the abdominal cavity. Pancreas were removed and rinsed in 1x Hank's Balanced Salt Solution (HBSS) twice, and then minced into pieces of 1 to 3 mm^3. After centrifuge for 2 min at 1000 rpm and 4 °C, supernatant was aspirated. After being dissociated with collagenase IA solution (HBSS 1x containing 10 mM HEPES, 200 U/ml of collagenase IA, and 0.25 mg/mL of trypsin inhibitor) for 20–30 min at 37°C, the enzymatic reaction was stopped by adding 10 mL of cold buffered washing solution (HBSS 1x containing 5% Fetal Bovine Serum (FBS) and 10 mM HEPES). It was transferred into a sterile 50 mL polypropylene tube and centrifuged for 2 min at 1000rpm and 4°C. The pellet was resuspended and washed with 10 mL of buffered washing solution. After centrifuge for 3 min at 1000rpm and 4°C, the supernatant was discarded. We repeated this step two more times. We resuspended the cell pellet in 7 mL of DMEM medium containing 5% FBS, 0.1% penicillin-streptomycin mixture (PS), 0.25 mg/mL of trypsin inhibitor and filtrated the cell mixture by allowing it to pass through a 100μm filter to retain the non-digested fragments (ducts, blood vessels, and Langerhans islets). The pancreatic acinar structures (acinus of 10–15 cells) passed through. We rinsed the filter with 6 mL of DMEM medium containing FBS, PS, trypsin inhibitor and seeded the isolated acini in a 6-well culture dish (2 mL per well). We added ethanol (1:1000) or 50ug/ml RU486 (dissolved in ethanol) and cultured them at 37°C under 5% (v/v) CO2 atmosphere for 24 h.

METHOD DETAILS

Survivorship assay

Studies of flies' survival upon starvation in ddH2O were carried out and analyzed as described previously(Liang et al., 2020). Flies were scored every 12 h for the presence of deceased adults. 20 flies of each condition were carried out with three independent replicates. Adults were considered dead when they did not display movement upon agitation. Results were analyzed using the GraphPad Prism software.

Lipid analysis

Measurement of triglyceride (TAG) by kit. Triglycerides were measured using a commercially available kit (A110-1-1, Nanjing Jiangcheng Bioengineering Institute). For fly experiments, flies with heads removed or guts dissected in fresh PBS were snap frozen in liquid nitrogen. Frozen samples were then homogenized in PBS for measurement of triglyceride and free fatty acid at 510nm as per the instructions of the manufacturer. Total protein was measured by the Bradford assay for normalization.

Oil Red O (ORO) staining and quantification. For fly experiments, guts were dissected in PBS and fixed in 4% formaldehyde/PBS for 20 min. Guts were then washed twice in PBS and incubated with fresh Oil Red O solution (6 mL of 0.5% Oil Red O in isopropanol and 4 mL of demineralized water, passed by a 0.45um filter) for 30 min, without agitation. Guts were then rinsed twice in distilled water and mounted in mounting media (60% glycerol in ddH2O). Quantification of the ORO signal was performed in the R2-R3 region of the midguts as described in (Luis et al., 2016; Buchon et al., 2013), using ImageJ. Briefly, images were converted to 8bit and a constant minimum threshold applied to the entire image for the red channel. The area above the threshold was then measured in the anterior midgut, to estimate the amount of local neutral lipids.

For Oil Red O staining in mice, mice tissues (eFat) were frozen sectioned at 20 μm thickness. The slides were air-dried for 30–60 min at room temperature and rinsed immediately in 3 changes of distilled water. We incubated the fresh Oil Red O solution (120mL of 0.5% Oil Red O in isopropanol and 80 mL of ddH2O) in a shaker for 30 min and filtered it in 60% isopropanol for 1–2 min. After being rinsed in 3 changes of distilled water, samples were then mounted with Glycerin (60% glycerol in ddH2O) for immediate imaging.

For LipidTOX^{TM} staining, fixed guts were washed briefly in washing buffer (PBS, 0.5% BSA, and 0.1% Triton X-100) for 3X5mins, then directly stained with LipidTOX^{TM} solution (1:200) diluted in washing buffer. LipidTOX^{TM} kit was purchased from Thermo Fisher (Cat # H34476).

Lipase assay of fly gut

Flies were dissected in chilled 1xPBS and 20 guts were collected per replicate and three independent samples were used for each replicate. Each sample was placed immediately 50ul in cooled 1xPBS Wwith mechanical homogenization under ice water bath conditions, centrifuged at 2500rpm for 10 min and the supernatant removed. The lipase assay kit (A054-2-1, Nanjing Jiancheng Bioengineering Institute) was used to test the enzyme activity of the samples. The BCA kit (B1001231, YESEN) tested the protein concentration of the sample. The protein concentration of the sample was corrected for lipase activity.

Frozen section of fly guts

Flies were dissected in chilled 1XPBS and 4% paraformaldehyde fixation for 30 min, 1XPBS wash 3 times, 15 min each time. The guts were embedded with OCT. The samples were sectioned (10um thick) using a freezer slicer (Leica CM3050S). Transverse cryosections from the guts were allowed to dry at room temperature. The sections were blocked with normal goat serum for 30 min and incubated with rabbit anti-HA (H6908, Sigma-Aldrich) in blocking buffer at 4°C overnight. Sections were rinsed with 1xPBS three times and incubated with CY3-labeled goat anti-rabbit IgG as secondary antibody in blocking buffer for 30 min. Sections were rinsed with 1xPBS three times again and nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Finally, sections were mounted and analyzed. Images were taken by Zeiss-Axio Imager M2 with ApoTome system, and processed by Photoshop and ImageJ.

Mice body weight analysis

C57BL/6 male mice of two groups were fed with regular chow diet (Control) or with high fat diet (HFD) ad libitum for 12 weeks. The Control and HFD groups were oral administrations of 5% Gum Arabic or 200mg/kg body weight RU486 (suspended in 5% Gum Arabic) at week 12. High fat diet (D12492) and chow diet was purchased from Xietong Organism.

The consumed chow and body weight were measured daily. Animals were weighed and euthanized after being fasted overnight at the endpoint of the treatments. Epididymal and peri-renal fats and pancreas were collected. These tissues were individually snap-frozen in liquid nitrogen and stored at −80°C for subsequent determination of the content of triglyceride and/or gene expression.

Corticosterone Measurement

Mice were anesthetized with isoflurane before being fasted overnight, and blood was collected from the orbital sinus by capillary. Supernatant of blood samples were obtained after centrifuge for 15 min, 3000rpm at 4°C. Serum corticosterone was measured using a mouse corticosterone (CORT) ELISA Kit (H205,Nanjing Jiangcheng Bioengineering Institute).

AR42J cell transfection and luciferase activity

Plasmid Construction and Luciferase Reporter Assays.

The rat PNLIP promoter region (−2000 to +1bp) was amplified by PCR using rat genomic DNA as a template and subcloned into the MCS sites of pGL3-Basic vector (PNLIP-GREWT-LUC). The PCR primers were as follows:

F: 5′-ACGGGTCTAGTCCTTAAAACTGC-3′,

R: 5′-CTGCACAGAGGCAAAAACATGG-3′.

Site-directed mutagenesis PCR was performed to generate GRE deleted form PNLIP-GRE^{Del}-LUC using the PNLIP-GRE^{WT}-LUC template. The PCR primers were synthesized from Genewiz, Inc, and the primer sequences were as follows:

F1: 5′- CTCCGCCATGCAACACTGCTTTAAGTTGCAGGCCCACACTTATGC-3′,

R1: 5′-TGAGAAAGTGCTAGATTTGGCAGACCCGTGTGCTGTGTGCTGCTCA-3′.

F2: 5′-TGAGCAGCACACAGCACACGGGTCTGCCAAATCTAGCACTTTCTCA-3′

R2:5′ATCTTTCTGAGCAGTGTCATGGAAAAGCGGCTCGAGAAGAGAGAGGCTCCA-3′

All constructs were verified by DNA sequencing.

AR42J cells (ATCC # CRL1492™) were transfected with the indicated plasmids using LipofectAMINE™ reagent (Invitrogen, Carlsbad, CA). In each transfection, 4ug of PNLIP-GREWT-LUC or PNLIP-GREMT-LUC and the Renilla luciferase plasmid (40ng) were used as indicated. After 8 h, the supernatant was replaced by new media in the presence or absence of DXMS (100nM) for 24 h and luciferase activity was measured using the Dual-Glo luciferase assay kit according to the manufacturer's instructions (Promega; Madison, WI, USA). After 24 h, cells were incubated in the presence or absence of isoproterenol for 24 h and luciferase activity was measured using the Dual-Glo luciferase assay kit according to the manufacturer's instructions. The relative luciferase activity was calculated after normalizing the transfection efficiency by Renilla luciferase activity.

Immunohistochemistry

For flies, intact guts were fixed at room temperature for 45 min in 100 mM glutamic acid, 25 mM KCl, 20 mM MgSO4, 4 mM sodium phosphate, 1 mM MgCl2, and 4% formaldehyde. All subsequent incubations were done in washing buffer (PBS, 0.5% BSA, and 0.1% Triton X-100) at 4oC. The following primary antibodies were used in overnight incubations: rabbit anti-HA (H6908, Sigma-Aldrich). Fluorescent secondary antibodies were obtained from Jackson ImmunoResearch. DAPI was used to stain DNA.

For mice, frozen sections of the pancreas or ovary (5um thick) were immune-stained against PNLIP (1:50, ABClonal, A6396), PR (1:50, Affinity, AF6106), or GR (1:20, Invitrogen, PA1-511A) using conventional protocol. Images were taken by Zeiss-Axio Imager M2 with Apotome system, and processed by Photoshop and ImageJ.

Fecal fat analysis

Fecal samples were collected at week 13 (48 h collection) to measure the levels of the fecal triglycerides. The amount of fat in the fecal samples was assessed with the Triglyceride Kit (A110-1-1, Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

mRNA preparation and RT-qPCR

For fly experiments, flies were dissected in chilled PBS and guts were placed immediately in cooled Trizol (Invitrogen). 10–12 guts or 3–4 whole flies were collected per replicate and three independent samples were used for each replicate. Total RNA was extracted using Direct-zol RNA MiniPrep kit (R2061, ZYMO Research). 2 ug of total RNA was used per sample and cDNA was synthesized using One-Step EvaGreen qRT-PCR Kit for RT-qPCR (Applied Biological Materials Inc). The qPCR reactions were performed with at least 3 independent biological replicates. Rp49 was utilized as an internal control.

For mice experiments, tissues were freshly dissected in cold PBS and mRNA was extracted by TRizol. After cDNA synthesis, qPCR reactions were performed with at least 4 animals of each condition as indicated. Primers were as follows:

For cell culture experiments, AR42J cells (ATCC# CRL1492) seeded at 10^5/mL (Countstar cell counter) were further cultured for 24 h, cells were dissociated form the culture dishes. After centrifuge, the cells were lysed in Trizol. qPCR experiments were performed with at least 3 independent biological replicates. For mice and AR42J experiments, 18S was used as an internal control. To study the role of GR on PNLIP transcription, cells were treated with the following reagents: DXMS (Sigma-Aldrich, D4902) and RU486 (Macklin, Shanghai, M830038 or mifepristone; Calbiochem #475838).

Food intake assay in drosophila and mice

In Drosophila, food intake was measured by the capillary feeder assay (CAFE) as previously described(Ja et al., 2007). In brief, 10 flies were presented with liquid food using two 5-μL calibrated capillaries per chamber. Changes in liquid meniscus height were measured every 12 h over 2–3 days at each capillary change. Consumption volume was calculated after background subtraction of measurements from control chambers without flies.

Acute administration of DXMS in mice

Male mice were injected with intraperitoneal injection of either DXMS phosphate (Sigma-Aldrich, D4902) in 1x PBS at 10 mg/kg body weight or mock treated with 1x PBS. The animals were sacrificed 8 h thereafter. Pancreas were then minced and homogenized for RT-PCR.

Chromatin immunoprecipitation (ChIP)

Pancreas tissues were minced into fine pieces and sonification by Covaris system (Gene Co, M220) plus system. Cross-linked DNA fragments were then immunoprecipitated with the GR antibody or equivalent concentrations of normal rabbit IgG as a negative control. The DNA was then eluted from the immune complexes and subjected to PCR amplification of the region of the mouse PNLIP gene promoter containing the GRE fragments using the following primer sets: AAAGATGGCGGCTCTGG and GTCCGT GGGAATCTGAGAAAG.

RNAseq

For RNAseq in fly gut, around 20 guts of each sample were dissected in RNase-free PBS and placed in Trizol. Extracted RNA and cDNA library was generated as described previously (Dutta et al., 2013, Dutta et al., 2015). Sequencing was performed using an Illumina HiSeq2000 machine. Data were analyzed with OmicShare software.

Western Blot analysis

For protein detection, samples were collected and western blot analysis was conducted as previously described(Ma et al., 2018). Primary antibodies PTL, GR, PR were incubated at 1:1000, and actin antibody for 1:10000. Predicted molecular weight: PR 120kD; GR 94kD; PTL 55kD; actin42kD.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data was analyzed using GraphPad Prism 5 (GraphPad, San Diego, CA, (USA). Unless otherwise indicated, Student t-test was performed for statistics. p values <0.05 were considered significant; significance values are indicated as *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. All pooled data are presented as mean $\pm$ standard error of the mean (SEM). Please see individual figure legends for details regarding number of technical and biological replicates.