Identification of glucocorticoid receptor in Drosophila melanogaster

Gloria Bartolo, Leandra O. Gonzalez, Saleem Alameh, C. Alexander Valencia and Mikhail Martchenko Shilman

Abstract

Background: Vertebrate glucocorticoid receptor (GR) is an evolutionary-conserved cortisol-regulated nuclear receptor that controls key metabolic and developmental pathways. Upon binding to cortisol, GR acts as an immunosuppressive transcription factor. Drosophila melanogaster, a model organism to study innate immunity, can also be immunosuppressed by glucocorticoids. However, while the genome of fruit fly harbors 18 nuclear receptor genes, the functional homolog of vertebrate GR has not been identified.

Results: In this study, we demonstrated that while D. melanogaster is susceptible to Saccharomyces cerevisiae oral infection, the oral exposure to cortisol analogs, cortisone acetate or estrogen, increases fly sensitivity to yeast challenge. To understand the mechanism of this steroid-induced immunosuppression, we identified the closest genetic GR homolog as D. melanogaster Estrogen Related Receptor (ERR) gene. We discovered that Drosophila ERR is necessary for cortisone acetate- and estrogen-mediated increase in sensitivity to fungal infection: while ERR mutant flies are as sensitive to the fungal challenge as the wildtype flies, the yeast-sensitivity of ERR mutants is not increased by these steroids. Interestingly, the fungal cortisone analog, ergosterol, did not increase the susceptibility of Drosophila to yeast infection. The immunosuppressive effect of steroids on the sensitivity of flies to fungi is evolutionary conserved in insects, as we show that estrogen significantly increases the yeast-sensitivity of Culex quinquefasciatus mosquito, whose genome contains a close ortholog of the fly ERR gene.

Conclusions: This study identifies a D. melanogaster gene that structurally resembles vertebrate GR and is functionally necessary for the steroid-mediated immunosuppression to fungal infections.

Keywords: Glucocorticoid receptor, Drosophila melanogaster, Fruit fly, Estrogen receptor, Cortisol, Cortisone, Culex quinquefasciatus, Mosquitoes, Saccharomyces cerevisiae, Infection

Background

Glucocorticoids (GCs), steroid hormones produced in the adrenal cortex of the kidney [1], are important for regulating numerous physiological functions such as glucose metabolism and immune response [2]. Naturally occurring GCs in the human body are inactive precursor cortisone and its active metabolite, cortisol [1, 3, 4]. Cortisol, converted from cortisone via type 1 11ß-hydroxysteroid enzyme, functions by binding directly to the ligand binding domain (LBD) of glucocorticoid receptors (GRs) found within the cytosol of the target cell [1, 3, 4]. Once bound to cortisol, GR translocates from the cytosol to the nucleus where it homodimerizes [3, 4]. The cortisol-bound GR homodimer can act as a transcriptional activator of genes encoding anti-inflammatory proteins by allowing its DNA binding domain (DBD) to bind to glucocorticoid-responsive elements (GREs) [3, 4]. Concurrently, the cortisol-GR homodimer can bind to and inhibit the function of transcription factor Nuclear Factor κB (NF-κB), 

* Correspondence: mikhail_shilman@kgi.edu
1Henry E. Riggs School of Applied Life Sciences, Keck Graduate Institute, Claremont, CA 91711, USA
Full list of author information is available at the end of the article

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ultimately repressing the synthesis of NF-κB-dependent inflammatory proteins [3, 4] (Fig. 1a, left panel).

NF-κB is evolutionary-conserved and an integral part of the fruit fly Drosophila melanogaster innate immune response when challenged with entomopathogenic microbes. Upon the detection of microbial pathogens, Drosophila systemic humoral response activates two NF-κB-activating pathways, Imd and Toll, leading to the production of anti-microbial peptides (AMPs) [5, 6]. The Imd pathway is primarily induced by gram-negative bacteria by recognizing DAP-type peptidoglycan via PGRP-LC receptors located on the cell surface of enterocytes or fat body cells [5, 7]. Once the presence of bacteria is detected, the NF-κB transcription factor Relish activates the transcription of AMPs such as drosomycin [5] (Fig. 1a, right panel). Recent studies show that sensing of the type of the bacterial cell wall is less stringent than previously thought and that both fly pathways are capable of detecting lys- and dap-peptidoglycan based on the accessibility of bacterial cell wall [7].

Human GR is a member of a nuclear receptor (NR) class of proteins. NRs are a superfamily of ligand regulated transcription factors activated by steroid hormones and various other lipid-soluble signals responsible for regulating a variety of processes such as embryonic development and metabolism [8–10]. Additionally, NRs are evolutionary conserved and represented in all animal phyla, including humans and Drosophila [11]. Human NR superfamily includes 48 NRs, which could be divided into six subfamilies based on their sequence similarity. Although Drosophila has only 18 NRs, they represent all 6 sub-families found in humans [8, 12, 13]. A previous study demonstrated that orally administered corticosteroid increases the sensitivity of Drosophila to pathogenic fungus, Rhizopus oryzae [14], alluding to the existence of unidentified fly GR (Fig. 1a). In this study, we report the identification and function of the D. melanogaster GR ortholog by examining yeast infection susceptibility upon steroid treatments.

**Results**

**Genetic in silico search for GR homolog in the Drosophila melanogaster genome**

Mammalian NR members of the same group share at least 80% identity in DBDs and at least 40% identity in LBDs [10]. Human GR belongs to the NR subfamily 3 (also known as steroid NRs), which is comprised of a total of nine family members, such as Homo sapiens estrogen-related receptor and estrogen receptor (hsERR1 and hsER1, respectively). Interestingly, Drosophila harbors only one member in the subfamily 3, which was called D. melanogaster ERR (dmERR) for its sequence homology to hsERR1 [8, 10, 15, 16]. We aligned the sequence of dmERR with sequences of several members of human NR subfamily 3 (hsERR1, hsER1, and GR) and observed an overall amino acid homology between 47 to 58% (Fig. 1b), with high similarity in the DBD (70–96%) and the LBD (55–57%) (Additional file 1). Due to a comparable degree of sequence homology of dmERR to GR and to hsERR1 (Fig. 1b), as well as the ability of corticosteroids to immunosuppress flies and humans to fungi [14], we hypothesized that dmERR may function as a functional ortholog of GR.

**Susceptibility of Drosophila melanogaster to Saccharomyces cerevisiae oral infection**

Microbial pathogens, both bacterial and fungal, can infect Drosophila via different potential routes. A common route of access to microbial pathogens is the penetration of the gut, as Drosophila is naturally exposed to pathogens when foraging for food sources [17]. In addition to exposure to pathogens, Drosophila can also be exposed to non-pathogenic microbes such as Saccharomyces cerevisiae, a budding yeast that co-habitats with Drosophila in nature [18, 19]. Since the previous study demonstrated that a medically used GC decreases the survival of Drosophila to a human fungal pathogen [14], we set out to test whether naturally occurring GCs sensitize flies to non-pathogenic yeast, Saccharomyces cerevisiae.

Before the administration of GCs, we first assessed the sensitivity of Drosophila to Saccharomyces via oral exposure. Wildtype Oregon-R Drosophila flies were treated with sucrose solutions containing various concentrations of S. cerevisiae (Fig. 2a and Additional file 2), and compared to the pathogenicity of known entomopathogenic Gram-positive Bacillus cereus and Micrococcus luteus, as well as Gram-negative Escherichia coli and Serratia liquefaciens bacteria [7, 20–22] (Figs. 2b–e, respectively). Surprisingly, despite being known as natural symbionts [18, 19], we observed that S. cerevisiae is pathogenic to flies of both genders via continuous oral exposure. At 4.17 × 10⁷ yeast cells/ml in sucrose solution, fly median survival time (i.e. the time at which 50% of flies are dead) occurs after 58 h of exposure in both female and male flies (Fig. 2a). At 1.67 × 10⁷ yeast cells/ml, S. cerevisiae yields a median survival by 71 h in female flies (Fig. 2a) and 95 h in male flies (Additional file 2). A further half-fold decrease in fungal concentration (8.30 × 10⁶ cells/ml) leads to even longer median survival times: 101.5 h in female flies and 141 h in male flies (Fig. 2a and Additional file 2,
Fig. 1 (See legend on next page.)
In conjunction with exposure to S. cerevisiae dexamethasone [14]. Exposed to 20 mg/ml (50 mM) of the synthetic GC, zygomycotic systemic infection when flies are orally susceptible of wildtype flies to Rhizopus oryzae susceptibility of human NR subfamily 3 could also increase sensitivity of flies to fungal infection. Because dmERR is homologous to both hsERR1 and hsER1, we tested the ability of a mammalian ER ligand, the estrogen steroid hormone, 17β-oestradiol (17β-E) (Fig. 3a), to affect fly susceptibility to S. cerevisiae infection. 17β-E, a molecule with both anti- and pro-inflammatory effects [23], exhibits a steroid skeleton structure very similar to CA.

Since 17β-E is a female hormone in mammals, we utilized only female flies to determine the effect of the compound on the susceptibility of flies to S. cerevisiae infection. 17β-E was orally supplied within the concentration range as that of CA. At 75 mM, 17β-E was more effective than CA in increasing the susceptibility of female wildtype flies to S. cerevisiae infection, without causing toxicity to uninfected flies (Fig. 3c). The median survival of infected flies occurred 15 h sooner in the presence of 17β-E than without this compound. This data demonstrates that multiple steroid hormones could increase the sensitivity of flies to fungal challenge.
Fig. 2 (See legend on next page.)
Effect of estrogen on *Culex quinquefasciatus* mosquitoes during fungal infection

To determine whether steroids affect the sensitivity of other insects to yeast infections, we searched for ERR homologs in other species. The genome of *Culex quinquefasciatus* mosquitoes harbors a close homolog of fly ERR, with 63% amino acid identity and 75% similarity (Fig. 4a). The function of *C. quinquefasciatus* ERR homolog, locus EDS37237, is currently unknown and annotated as ERR based on its sequence similarity to other ERR genes. Because mosquitoes contain an ERR ortholog, we tested the ability of a steroid molecule to affect the sensitivity of yeast-infected *C. quinquefasciatus*, who just like *Drosophila*, is a member of the insect order Diptera [24].

Before the exposure to 17β-E, we determined $3.3 \times 10^7$ fungal cells/ml as the minimal lethal dose necessary to kill female adult *C. quinquefasciatus* mosquitoes, aged 4–5 days. Female mosquitoes were then orally exposed to 17β-E, the same concentration shown to immunosuppress flies (Fig. 4b). Like fly experiments, we observed that 75 mM of 17β-E

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**Fig. 2** Sensitivity of female wildtype flies to microbial infections. Female Oregon-R wild type (WT) flies were orally challenged with different amounts of (a) *S. cerevisiae*, (b) *Bacillus cereus*, (c) *Micrococcus luteus*, (d) *Escherichia coli*, and (e) *Serratia liquefaciens*. Flies were fed in vials with 50 mM sucrose solution containing various microbial concentrations. Each condition contains ten flies. Vials are incubated at 30 °C and checked a minimum of twice per day for fly survival.
increased the sensitivity of mosquitoes to *S. cerevisiae* and decreased median survival time by 73 h. This data shows the steroid hormone-mediated increased sensitivity to fungal infection is conserved in multiple insect species harboring the *ERR* gene.

**Drosophila** ERR is necessary for CA- and 17β-E-mediated increased sensitivity to fungal infection

To determine whether dmERR acts as a functional human GR ortholog, female *ERR* homozygous loss-of-function mutant flies were challenged with *S. cerevisiae* in the presence or absence of CA (Fig. 5a) or 17β-E (Fig. 5b). We observed that in the absence of steroids, *ERR* mutant flies exhibited similar sensitivity to yeast infection as wildtype *Drosophila* (median survival at 50–70 h) (Fig. 5), allowing for a parallel analysis between both fly strains. Interestingly, with the oral exposure of CA or 17β-E, dmERR mutant flies did not show increased susceptibility to *S. cerevisiae* infection at concentrations found to increase the susceptibility of wildtype flies (100 mM for CA and 75 mM for 17β-E). The median survival time of dmERR mutant flies exposed to *S. cerevisiae* + CA or *S. cerevisiae* + 17β-E was comparable to the median survival time of wildtype flies to *S. cerevisiae* alone, which illustrated the inability of dmERR mutant flies to become more susceptible to a fungal infection in the presence of CA or 17β-E. This data showed that *Drosophila* ERR is necessary for steroid-mediated increased sensitivity of flies to fungal infection.
Determination of the effect of fungal ergosterol on the susceptibility of Drosophila to yeast infection

While CA and 17ß-E are naturally occurring soluble and plasma-circulating mammalian steroids [3, 25], we investigated the effect of fungal steroids on the sensitivity of flies to yeast. Previously, S. cerevisiae has been shown not to generate extracellular soluble steroids [26] and instead only produces cell membrane-bound steroid-like ergosterol, which is important for maintaining membrane fluidity, permeability, and structure [27]. Like CA and 17ß-E, ergosterol exhibits the steroid skeleton but with a longer side chain on the 17th carbon atom (Fig. 3a). We explored the hypothesis that after ingestion of S. cerevisiae cells, fungal ergosterol may affect the sensitivity of Drosophila to S. cerevisiae. Ergosterol was exogenously provided to female wildtype flies at a range from 100 to 200 mM (Fig. 6). At concentrations tested previously for CA and 17ß-E, ergosterol yielded no increased susceptibility of flies to S. cerevisiae infection (Fig. 6a). Even at double the concentration (200 mM), no effect on fly sensitivity was also seen (Fig. 6b).

Discussion

Here, we presented dmERR is required for steroid-mediated increased fly sensitivity to fungal infections. Previous studies have reported roles for dmERR in carbohydrate metabolism and hypoxic responses [28–30]. Other research has focused on the possible role of dmERR in mitochondrial biogenesis [31], as hsERR1 has been shown to have a role in generating mitochondria [32]. In humans, the expression of medium-chain acyl coenzyme A dehydrogenase (MCAD), which is an enzyme that mediates the mitochondrial beta-oxidation of fat, is regulated by an ERR-a response element (ERRE) present in the 5′-flanking region. In COS-7 cells, hsERR1 interacts with the MCAD nuclear receptor response element 1 (NRRE-1). hsERR1 may regulate cellular energy balance by controlling the expression of MCAD through the NRRE1 [33]. Recent studies demonstrated that mitochondria are critical in stimulating innate immune signaling. Specifically, released mitochondrial DNA (mtDNA) and mitochondria-derived reactive oxygen species (mtROS) activate innate immune responses, such as inflammasome, sGAS-STING, and NF-κB signaling pathways [34]. In addition, changes in mitochondria and metabolic pathways (TCA cycle, oxidative phosphorylation, and fatty acid oxidation) induce transcription in immune cells. For example, M1 macrophages with an impaired TCA cycle have a pro-inflammatory response, and M2 macrophages perform β-oxidation to produce anti-inflammatory responses [35]. Additionally, mitochondria are known to induce the inflammatory response: mitochondrial antiviral signaling and NLRP3 can be activated by mitochondria [36]. Moreover, the mass and mobility of mitochondria are affected by fission and
fusion that affect the immune functions [37]. In immune cells, mitochondria are located close to the endoplasmic reticulum (ER), which allows cells to control metabolism that is essential for immune functions [37]. Our results are the first to allude the role of dmERR in Drosophila immunity, specifically in increasing the susceptibility of flies to fungal infections when orally exposed to steroid molecules. Specifically, we have shown the ability of synthetic GC, CA, to increase the susceptibility of flies to fungal infection when orally presented. This is consistent with dmERR being the only homolog for members of the human NR subfamily 3, in which GR is included. dmERR is necessary for steroid-induced immunosuppression of flies and is highly homologous to GR. Thus, we propose that dmERR is the functional ortholog of GR.

Not all molecules exhibiting the steroid backbone elicit the same effect on fly sensitivity to fungal infection. Tested concentrations of ergosterol did not increase fly sensitivity to S. cerevisiae infection. Unlike CA and 17β-E, which are transported to their target via the bloodstream in mammals, ergosterol is not soluble and/or mobile. Rather, it is integrated within the cell wall of S. cerevisiae and may not be readily bioavailable for fly ingestion. It is possible ergosterol elicits an effect at a higher concentration than tested, but such concentration may not be physiologically relevant. The inability of ergosterol to sensitize flies to S. cerevisiae could be either because ergosterol is not a ligand for dmERR or because the supplied ergosterol is soluble and out of the context of the fungal membrane.

Similarly to human ERR, the ligand for dmERR has yet to be discovered. Several studies have alluded to the existence of a ligand for dmERR [13, 38, 39], but a definitive ligand has not been reported. If dmERR is indeed the functional ortholog of human GR, there exists a greater possibility of the existence of a natural ligand for dmERR. If one is found to exist, Drosophila may be used as a model to identify a ligand for hsERR1 in humans, relieving the NR of its orphan status and providing a deeper understanding of the biological functions of dmERR and/or hsERR1. Additionally, D. melanogaster’s sophisticated innate immune system has largely evolved to combat bacterial and fungal pathogens relevant to the understanding of human inflammatory conditions [40]. In response to pathogenic challenges, AMPs are released through two primary pathways that involve evolutionarily conserved components, including Toll and Toll-like receptors, as well as NF-κB, tumor necrosis factor-α, and JAK/STAT signaling [41]. Thus, human mutations identified in hsERR1 can be studied by creating humanized dmERR to elucidate the innate immune effects of those genetic changes, as Drosophila lack adaptive immunity. Fruit flies have thus far only one identified steroid hormone, 20-hydroxyecdysone (20E). Ecdysone was shown to regulate both immunity and major developmental transitions in the fly, such as metamorphosis [42]. Two
**Drosophila** nuclear receptors are known to be receptors for 20E: ecdysone receptor (EcR) and ultraspiracle (USP). EcR binds to 20E, heterodimerizes with USP [43], and activates the expression of a large set of genes known to function in cell motility, cell shape, and phagocytosis [42]. Ecdysone-regulation in *Drosophila* was shown to be essential for hemocyte immune functions and survival after infection: 20E induces the phagocytosis in *Drosophila* hemocytes, and larvae lacking ecdysone-activated hemocytes are defective in bacterial phagocytosis and are susceptible to oral bacterial infections. In contrast, the results presented here show that the role of *Drosophila* ERR is to suppress the immunity of flies, which may be needed to counterbalance the positive effect of EcR on immunity.

While this study observes the necessity of fly ERR for the steroid-mediated immunosuppression, future studies will focus on the mechanism by which GC affects ERR, such as whether GC binds to ERR and induces its nuclear translocation, followed by its nuclear activity. Mammalian GRs are known to affect the immunity by two nuclear mechanisms: by binding directly to and repressing the transcriptional activity of NF-kB, as well as by binding directly to the promoters of immunity-related genes and regulating their transcription. Moreover, GRs affect immunity-related cytoplasmic proteins by a third non-nuclear mechanism via activating cytoplasmic phosphatidylinositol 3-kinase and protein kinase Akt, leading to the activation of secondary messengers nitric oxide [3]. Investigating whether fly ERR is affecting immunity-related genes and processes analogously to the three mammalian mechanisms will help us understand how GC influences ERR and innate immunity pathways.

**Conclusions**

This study identifies a *D. melanogaster* gene that structurally resembles vertebrate GR and is functionally necessary for the steroid-mediated immunosuppression to fungal infections.

**Methods**

**Drosophila rearing**

*Drosophila melanogaster* strains were housed at 25°C with 12-h light/dark cycles and fed on standard cornmeal-molasses-agar fly medium with yeast flakes. Wildtype experiments were conducted with Oregon-R, selected for their rapid egg-laying ability (Bloomington *Drosophila* Stock Center (BDSC) stock #2376), *Drosophila* aged 4–5 days. Experiments with homozygous ERR (BDSC stock #28467) *Drosophila* utilized unaged flies at the time of the experiments.

**Drosophila oral feeding survival assay**

*Saccharomyces cerevisiae* diploid strain YEF473, ATCC® 200970 [44] was used as the infective agent for all *Drosophila* survival assays. *S. cerevisiae* was incubated on YPD medium at 30°C. Overnight cultures were grown in YPD at 30°C at 180 rpm for 14–16 h.

*Bacillus cereus* (ATCC 10987), *Escherichia coli* (C600), *Micrococcus luteus* (ATCC 4698), and *Serratia liquefaciens* (ATCC 27592) were used. *B. cereus* and *E. coli* were cultured in Lysogeny broth (LB) at 37°C. *M. luteus* was grown in LB at 25°C. *S. liquefaciens* was incubated in Tryptic Soy Broth (TSB) at 30°C overnight.

Flies were infected according to the microbial intestinal infection methods described previously in Nehme, et al [45] with the following modifications. *Drosophila* vials were prepared by placing three 25 mm diameter circles of extra-thick Whatman blotting paper (Bio-Rad Laboratories, catalog #1703965) at the bottom of the vials and capping with a foam plug. *S. cerevisiae* overnight cultures were centrifuged, and the pellets resuspended in 50 mM sucrose solution to a final desired optical density (0.83 OD, 1.7 OD or 3.3 OD) at 600 nm (OD<sub>600</sub>). OD<sub>600</sub> values were converted to cells/ml (OD<sub>600</sub> of 1.0 corresponds to approximately 10<sup>9</sup> cells/ml) [46]. Bacterial infections were carried out analogously, except at higher cells/ml (converted from OD<sub>600</sub> values according to McFarland’s scale) [47, 48]. Depending on the experiment, steroid molecules were added to the fungal sucrose solution. 17ß-estradiol (catalog #10006315), cortisone acetate (catalog #23798), and ergosterol (catalog #19850) were all purchased from the Cayman Chemical Company. Each prepared *Drosophila* vial contained 2.5 ml of its respective solution, which was absorbed by the Whatman paper found at the bottom of the vial. Flies were anesthetized by CO<sub>2</sub>, separated by gender, and placed into the *Drosophila* vials, with ten flies in each vial. Vials were incubated at 30°C and checked a minimum of twice per day for fly survival.

**Mosquito rearing**

*C. quinquefasciatus* mosquitoes were obtained from a colony maintained by Benzon Research (Carlisle, PA, USA). Mosquitoes were reared and maintained at 28°C and 80% relative humidity in 30 × 30 × 30-cm cages with 12-h light/dark cycles. Adult mosquitoes were maintained on 10% sucrose ad libitum, while larvae were fed a 1:1:1 mixture of bovine liver powder (Carlisle, PA, USA). For experiments, adult female mosquitoes aged 4–5 days were used.

**Mosquito oral infection survival assay**

*S. cerevisiae* diploid strain YEF473, ATCC® 200,970 [44] was used as the infective agent for all *C. quinquefasciatus* survival assays.
C. quinquefasciatus were infected similar to the technique used to infect Drosophila, as described above, but include the following modifications. C. quinquefasciatus vials were prepared by placing 5 × 5-cm of extra-thick Whatman blotting paper (Bio-Rad Laboratories, catalog #1703965) in square-bottom, polypropylene Drosophila bottles and capped with a foam plug. S. cerevisiae overnight cultures were centrifuged, and the pellets resuspended in 50 mM sucrose solution to a final optical density at 600 nm (OD$_{600}$) of 3.3 OD. OD$_{600}$ values were converted to cells/ml (OD$_{600}$ of 1.0 corresponds to approximately 10$^7$ cells/ml) [46]. 17β-estradiol (catalog #10006315) steroid molecules, purchased from Cayman Chemical Company, were added to the fungal sucrose solutions. Each prepared C. quinquefasciatus bottle contained 10 ml of its respective solution and absorbed by the Whatman paper found at the bottom of each vial. Mosquitoes were anesthetized using CO$_2$ separated by gender, and placed in the prepared vials, with ten mosquitoes in each vial. Vials were incubated at 30°C and checked a minimum of once a day for mosquito survival.

Supplementary information
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Additional file 1. The amino acid sequence similarity between DNA and ligand binding domains of dmERR with hsERR1, hsER1, and hsGR. The amino acid sequences of DNA binding domain (DBD) and ligand binding domain (LBD) are compared between dmERR, hsERR1, hsER1, and hsGR. The extent (%) of the identity/similarity is shown for each domain.

Additional file 2. Sensitivity of male wildtype flies to Saccharomyces cerevisiae infection. Male Oregon-R wild type (WT) flies were orally challenged with different amounts of S. cerevisiae. Flies were fed in vials with 50 mM sucrose solution containing various yeast concentrations. Each condition contains ten flies. Vials are incubated at 30°C and checked a minimum of twice per day for fly survival.

Additional file 3. Cortisone acetate increases the sensitivity of male wildtype flies to Saccharomyces cerevisiae. Male wild type (WT) flies were orally challenged as in Fig. 1 with 1.67 × 10$^6$ S. cerevisiae cells/ml with and without various concentrations of cortisone acetate (CA). Uninfected flies exposed to 140 mM cortisone acetate were included to test the toxicity of this compound. P-value indicates statistical significance compared to the yeast-only condition (asterisks) on the basis of the Log-rank (Mantel-Cox) test.

Abbreviations
GC: Glucocorticoid; LBD: Ligand binding domain; GR: Glucocorticoid receptor; DBD: DNA binding domain; GRE: Glucocorticoid responsive elements; NF-kB: Nuclear factor κB; AMP: Anti-microbial peptide; NR: Nuclear Receptor; hsERR1: Human estrogen-related receptor 1; hsER1: Human estrogen receptor 1; dmERR: Drosophila estrogen-related receptor; CA: Cortisone acetate; 17β-E: 17β-estradiol

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Authors’ contributions
M.M.S. designed research; G.B., L.O.G., S.A., performed research; all analyzed data; and G.B., C.A.V., and M.M.S. wrote the paper, which was edited by all authors. All authors have read and approved the manuscript.

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Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Henry E. Riggs School of Applied Life Sciences, Keck Graduate Institute, Claremont, CA 91711, USA. 2Aperionics, Inc., Sterling, VA 20166, USA. 3Lake Erie College of Osteopathic Medicine, 1838 W Grandview Blvd, Erie, PA 16509, USA. 4Department of Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

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References
1. Ramamoorthy S, Cidlowski JA. Corticosteroids. Rheum Dis Clin N Am. 2016;42:15–31.
2. Escotet-Torres L, Caratti G, Mectidou A, Tuckermann J, UhlenhauN, Vettorazzi S. Fighting the fire: mechanisms of inflammatory gene regulation by the glucocorticoid receptor. Front Immunol. 2019;10. https://doi.org/10.3389/fimmu.2019.01859.
3. Rhen T, Cidlowska JA. Antiinflammatory action of glucocorticoids — new mechanisms for old drugs. N Engl J Med. 2005;353:1711–23.
4. Cain DW, Cidlowska JA. Immune regulation by glucocorticoids. Nat Rev Immunol. 2017;17:233–47.
5. Buchon N, Silverman N, Cherry S. Immunity in Drosophila melanogaster — from microbial recognition to whole-organism physiology. Nat Rev Immunol. 2014;14:796–810.
6. Troha K, Buchon N. Methods for the study of innate immunity in Drosophila melanogaster. Wiley Interdiscip Rev Dev Biol. 2019;8:e344.
7. Vaz F, Nounati1 I, Covas G, Parton RM, Harkiolaki M, Davis I, et al. Accessibility to Peptidoglycan Is Important for the Recognition of Gram-Positive Bacteria in Drosophila. Cell Reports. 2019;27:2480–2492.e6.
8. King-Jones K, Thummel CS. Nuclear receptors — a perspective from Drosophila. Nat Rev Genet. 2005;6:31–23.
9. Sever R, Glass CK. Signaling by nuclear receptors. Cold Spring Harb Perspect Biol. 2013;5:a016709.
10. A Unified Nomenclature System for the Nuclear Receptor Superfamily. Cell. 1999;97:161–3.
11. Bodefsky S, Koitz F, Whiteburg B. Conserved and Exapted Functions of Nuclear Receptors in Animal Development. Nuclear Receptor Research. 2017;4. doi:https://doi.org/10.11311/2017/101305.
12. Henrich VC, Beatty JM. Chapter 248 - nuclear receptors in Drosophila. In: Bradshaw RA, Dennis EA, editors. Handbook of cell signaling (Second Edition). San Diego: Academic Press; 2010. p. 2027–37. doi:https://doi.org/10.1016/B978-0-12-374145-5.00248-5.
13. Palanker L. Dynamic regulation of Drosophila nuclear receptor activity in vivo. Development. 2006;133:549–62.
14. Chamilos G, Lewis RE, Hu J, Xiao L, Zal T, Gilliet M, et al. Drosophila melanogaster as a model host to dissect the immunopathogenesis of zygomycosis. Proc Natl Acad Sci. 2008;105:9367–72.
15. Maglich JM, Sluder A, Guan X, Shi Y, McKee DD, Carrick K, et al. Comparison of complete nuclear receptor sets from the human, Caenorhabditis elegans and Drosophila genomes. -7.
16. Mazaira G, Zgajnjar NR, Lofuto CM, Daneri-Becerra C, Sivills JC, Soto OB, et al. The Nuclear Receptor Field: A Historical Overview and Future Challenges.
Nuclear Receptor Research. 2018;5. doi:https://doi.org/10.11131/2018/101320.

17. Mondotte JA, Saleh M-C. Antiviral immune response and the role of infection in Drosophila melanogaster: In Advances in Virus Research. Elsevier; 2018. p. 247–78. https://doi.org/10.1016/bs.avir.2017.10.006.

18. Madden AA, Epps MJ, Fukami T, Irwin RE, Sheppard J, Songer DM, et al. The ecology of insect–yeast relationships and its relevance to human industry. Proc R Soc B Biol Sci. 2018;285:20172733.

19. Quan AS, Eisen MB. The ecology of the Drosophila yeast mutualism in wineries. PLoS One. 2018;13:e0196440.

20. Nehme NT, Quintin J, Cho JH, Lee J, Lafarge B, Kocks C, et al. Relative roles of the cellular and Humoral responses in the Drosophila host defense against three Gram-positive bacterial infections. PLoS One. 2011;6:e14743.

21. Tracy C, Käraimer H. Escherichia coli infection of Drosophila. Bio Protoc. 2017;7. doi:https://doi.org/10.1017/BioProtoc.2256.

22. Corticosteroids. In: LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. http://www.ncbi.nlm.nih.gov/books/NBK593000/. Accessed 11 Nov 2019.

23. Bereshchenko O, Bruscoli S, Riccardi C. Glucocorticoids, sex hormones, and immunity. Front Immunol. 2018;9:1332.

24. Arensburger P, Megy K, Waterhouse RM, Abrudan J, Amedeo P, Antelo B, et al. Sequencing of Culex quinquefasciatus establishes a platform for mosquito comparative genomics. Science. 2010;330:86-5.

25. Jacobson MN, Lewis DFV. Steroid hormone receptors and dietary ligands: a selected review. Proc Nutr Soc. 2002;61:105–22.

26. Feldman D, Krishnan A. Estrogens in unexpected places: possible implications for researchers and consumers. Environ Health Perspect. 1995;103:5.

27. Bhattacharya S, Esquivel BD, White TC. Overexpression or Deletion of Ergosterol Biosynthesis Genes Alters Doubling Time, Response to Stress Agents, and Drug Susceptibility in Saccharomyces cerevisiae. mBio. 2018;9: e01291–18. /mBio/9/4/mBio.01291–18. atom.

28. Li Y, Padmanabha D, Gentile LB, Dumur CI, Beckstead RB, Baker KD. HIF- and non-HIF-regulated hypoxic responses require the estrogen-related receptor in Drosophila melanogaster. PLoS Genet. 2013;9:e1003230.

29. Li H, Chawla G, Hurlburt AJ, Sterrett MC, Zaslaver O, Cox J, et al. Drosophila larval sense amylose as a oncometabolite during normal developmental growth. Proc Natl Acad Sci U S A. 2011;108:13535–8.

30. Tennessen JM, Baker KD, Lam G, Evans J, Thummel CS. The Drosophila estrogen-related receptor directs a metabolic switch that supports normal developmental growth. Cell Metab. 2011;13:349–58.

31. Misra S, Pandey AK, Gupta S, Kumar A, Khanna P, Shankar J, et al. Estrogen related receptor is required for the testicular development and for the normal sperm axoneme/mitochondrial derivatives in Drosophila males. Scientific Reports. 2017;7. doi:https://doi.org/10.1038/srep40372.

32. Eichner LI, Giguere V. Estrogen related receptors ( ERRs): a new dawn in transcriptional control of mitochondrial gene networks. Mitochondrion. 2011;11:544–52.

33. Sladek R, Bader J-A. The orphan nuclear receptor estrogen-related receptor _ is a transcriptional regulator of the human medium-chain acyl coenzyme a dehydrogenase gene. Mol Cell Biol. 1991;17:1-0.

34. Chen Y, Zhou Z, Min W. Mitochondria, oxidative stress and innate immunity. Front Physiol. 2018;9. https://doi.org/10.3389/fphys.2018.01487.

35. O'Neill LAJ. A broken Krebs cycle in macrophages. Immunity. 2015;42:393–4.

36. Park S, Juliana C, Hong S, Datta P, Hwang I, Fernandes-Alnemri T, et al. The mitochondrial antiviral protein MAVS associates with NLRP3 and regulates its inflammasome activity. J Immunol. 2013;191:4358–66.

37. Angajala A, Lim S, Phillips JB, Kim J-H, Yates C, You Z, et al. Diverse roles of mitochondria in immune responses: novel insights into immune-metabolism. Front Immunol. 2018;9. https://doi.org/10.3389/fimmu.2018.01605.

38. Sun W, Valero MC, Seong KM, Steele LD, Huang J-T, Lee CH, et al. A Glycine insertion in the estrogen-related receptor (ERR) is associated with enhanced expression of three cytochrome P450 genes in transgenic Drosophila melanogaster. PLoS One. 2015;10:e0117879.

39. Osterberg T, Jacobsson M, Attersson A, Mata de Urquiza A, Jendeberg L. A Triple Mutant of the Drosophila ERR Confers Ligand-Induced Suppression of Activity. 1 Biochemistry. 2003;42:6427–35.

40. Ferrandon D, Imler J-L, Heitru C, Hoffmann JA. The Drosophila system immune response: sensing and signalling during bacterial and fungal infections. Nat Rev Immunol. 2007;7:862–74.

41. Wu L, Silverman N. Fighting infection. Fly. 2007;1:106–9.

42. Regan JC, Brandão AS, Leitão AB, Mantas Dias ÂR, Sucena E, Jacinto A, et al. Steroid hormone signaling is essential to regulate innate immune cells and fight bacterial infection in Drosophila. PLoS Pathog. 2013;9:e1003720.

43. Thummler CS. Molecular mechanisms of developmental timing in C. elegans and Drosophila. Dev Cell. 2001;1:453–65.

44. Bi E, Pingue JR. ZDS1 and ZDS2, genes whose products may regulate Coq42p in Saccharomyces cerevisiae. Mol Cell Biol. 1996;16:5264–75.

45. Nehme NT, Liégeois S, Kele B, Giammarinaro P, Pradel E, Hoffmann JA, et al. A model of bacterial intestinal infections in Drosophila melanogaster. PLoS Pathog. 2007;3:e173.

46. Groves JD, Felson P, le Maire M, Tanner MJ. Functional cell surface expression of the anion transport domain of human red cell band 3 (AE1) in the yeast Saccharomyces cerevisiae. Proc Natl Acad Sci. 1996;93:12245–50.

47. McFarland T. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. JAMA. 1907;XLIX:1176–8.

48. Hartmann S, Nusbaum DJ, Kim K, Alamleh S, Ho C-LC, Cruz RL, et al. Role of a small molecule in the modulation of cell death signal transduction pathways. ACS Infectious Diseases. 2018. https://doi.org/10.1021/acsinfecdis.8b00231.

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