Bioactive isoprenoids guide migrating germ cells to the embryonic gonad

Barton, Lacy J¹, Sanny, Justina¹, Dawson, Emily P¹, Nouzova, Marcela^{2,3}, Noriega, Fernando Gabriel², Stadtfeld, Matthias^{1,4}, Lehmann, Ruth^{1,5}

¹ Department of Cell Biology, Skirball Institute of Biomolecular Medicine, NYU Grossman School of Medicine, NY, USA, 540 First Avenue, New York, NY 10016

² Department of Biological Sciences and Biomolecular Sciences Institute, Florida International University, 11200 SW 8th St, Miami, FL 33199

³ Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic

⁴ Present: Department of Medicine, Weill Cornell Medicine, 413 E 69th St, New York, NY 10021

⁵ Present: Department of Biology, Whitehead Institute Biology at MIT, 455 Mainstreet, Cambridge, MA 02142

Abstract

Germ cells are essential to sexual reproduction. Across the animal kingdom, extracellular isoprenoids, such as retinoic acids (RAs) in vertebrates and juvenile hormones (JHs) in insects, impact the germline lifecycle from meiosis to gametogenesis. Emerging evidence suggests that these bioactive isoprenoids broadly influence embryonic reproductive development, though the precise functions remain unclear. Here, we investigated the role of bioactive isoprenoids in early germ cell development using JHs in *Drosophila* as a model. With a newly generated *in vivo* reporter, we find that JH signaling is active in the vicinity of germ cells as they migrate to colonize the somatic gonad. Through a combination of *in vivo* and *in vitro* assays, we find that JHs are both necessary and sufficient for primordial germ cell migration through mechanisms independent of canonical nuclear receptor-mediated transcription. These findings reveal that JH is present during *Drosophila* embryogenesis and that bioactive isoprenoids impact germ cell development earlier than previously appreciated. Interestingly, we find that like JH in *Drosophila*, RA is sufficient for murine germ cell migration *in vitro*, suggesting that the impact of bioactive isoprenoids on embryonic germ cell development may be broadly conserved.

Main

Animal reproduction requires the precise specification, development, and protection of germ cells. In many species, a critical stage in germ cell development occurs within a narrow window of embryogenesis, during which primordial germ cells (PGCs) migrate long distances to colonize the developing somatic gonad¹, often while undergoing genome-wide epigenetic and transcriptional changes^{2,3} and overcoming selective survival barriers⁴. Shortly after colonizing the somatic gonad, PGCs commit to gametogenesis⁵, and together with somatic gonadal cells, undergo sex-specific differentiation. Failure to complete these processes causes diverse complications ranging from extragonadal germ cell tumors to infertility. Despite these important consequences, how PGCs complete and coordinate these temporally overlapping processes during early embryogenesis remains enigmatic and an active area of investigation.

Germ cell development is regulated by signals coming from the extracellular environment. One family of extracellular factors are isoprenoid lipids. These bioactive lipids are found across the animal kingdom, including retinoic acids (RAs) in vertebrates, methyl farnesoate in crustaceans and some insects, and juvenile hormones (JHs) in insects. In many species, isoprenoids are produced in and near the somatic gonad and exert powerful effects on reproduction. In crustaceans, isoprenoids are both necessary and sufficient for sex determination⁶. In mice, RAs have long been implicated as a meiosis-inducing factor, with the male-specific delay in meiotic entry coinciding with male-specific expression of the gene encoding the RA degradation enzyme, *CYP26B1*, in the somatic gonad^{7,8}. However, emerging evidence suggests that the impact of RA on embryonic germ cell development is multifaceted and extends beyond meiosis^{9,10}. The most recent studies of RAs demonstrate that the precise roles for secreted isoprenoids in embryonic reproductive development remains unclear¹¹⁻¹⁵.

Like RA in vertebrates, JHs impact multiple processes in the germline lifecycle. In fact, JHs are the central gonadotropic hormones in many insects, regulating late stage gametogenesis and reproductive diapause in adults¹⁶. Embryonic germ cell development in mice and *Drosophila* have striking similarities in expression of isoprenoid enzymes. Like *CYP26B1* in mice, expression of the gene encoding JH degradation enzyme, JH epoxide hydrolase 2 (*Jheh2*), is induced in the embryonic male gonad¹⁷. Expression of isoprenoid biosynthetic enzymes also overlap. In mice, genes encoding key RA enzymes, *Retinaldehyde dehydrogenases* (*RALDH*)1,2, and 3, are expressed in and near the genital ridge as primordial germ cells (PGCs)

colonize the mesodermal gonad^{13, 18}. In *Drosophila*, the key JH biosynthetic enzyme, JH acid methyltransferase (Jhamt), is expressed in the mesoderm during the same corresponding window of germ cell development in embryogenesis¹⁹. Yet, despite these striking similarities, whether JHs regulate PGC development is unknown.

Here we investigated the role of JHs in early *Drosophila* germ cell development. To determine whether mesodermal *jhamt* expression drives JH biosynthesis, we generated an *in vivo* JH reporter, finding that JH signaling is first active near PGCs as they migrate towards the developing somatic gonadal precursors. Using newly generated *jhamt* mutants, the first germline phenotype we observe is an increase in extragonadal PGCs after migration to the gonad is complete. Strikingly, this phenotype is shared with mutations in the rate-limiting enzyme of the mevalonate pathway upstream of JH biosynthesis, HMG Coenzyme A reductase (Hmgcr), an enzyme that is both necessary and sufficient for PGC migration in *Drosophila*²⁰. With a combination of *in vivo* and *in vitro* assays, we find JHs are sufficient for PGC migration. We provide evidence that the effect of JH on germ cell migration is independent of canonical transcription-dependent signaling, but instead involves the cytoplasmic calcium signaling factor, Phospholipase C at 21c (Plc21c). Together, these data reveal a new requirement for secreted isoprenoids in early germ cell development. Finally, we find that the vertebrate isoprenoid, RA, is sufficient to induce migration of both *Drosophila* and mouse embryonic germ cells, suggesting this newly identified role of isoprenoids on early germ cell development may be broadly shared across invertebrate and vertebrate species.

Results

Juvenile hormone signaling is active during early germ cell development in *Drosophila* embryos

Isoprenoid bioavailability is governed by dynamic spatiotemporal expression of biosynthetic and degradation enzymes. Consistently, the key JH biosynthetic enzyme, Jhamt, is highly expressed in the endocrine gland called the *corpus allatum*, which is the major source of JH in larval and adult insects²¹. However, *Drosophila* embryos also express *jhamt*¹⁹, though no function for JH in embryogenesis has been described. Given the important roles of isoprenoids in germline development and fertility, we determined where *jhamt* is expressed relative to germ cell development using dual-label fluorescent *in situ* hybridization for mRNAs encoding Jhamt and the germ cell factor, Nanos. Consistent with previous work¹⁹, we found *jhamt* expression is enriched in the somatic mesoderm starting at embryonic Stage 10 (~4 hours after fertilization), continuing through Stage 13 (~11 hours after fertilization) (**Fig 1a and b**). This analysis revealed *jhamt* expression in proximity to PGCs as they enter and migrate within the mesoderm to colonize the developing somatic gonad (**Fig 1b**).

Mesodermal *jhamt* expression occurs prior to development of the *corpus allatum*²² and thus whether mesodermal *jhamt* leads to *bona fide* JH production during *Drosophila* mid-embryogenesis was unknown. To detect JH signaling during embryogenesis, we generated an *in vivo* eGFP JH reporter by placing eight tandem copies of a JH response element (JHRE) from the *early trypsin* gene of *Aedes aegypti*^{23, 24}, which has been previously shown to robustly and specifically responds to JH *in vitro*²³. To establish JH dependency *in vivo*, we fed second instar larvae the JH analogue, methoprene, and measured GFP mRNA by RT-qPCR. GFP transcription was induced more than 4-fold in JHRE-GFP larvae fed methoprene (**Fig 1c**). Expression of GFP did not increase in methoprene fed JHRE-GFP larvae lacking both JH nuclear receptors (JHNRs), Methoprene tolerant (Met) and Germ cell expressed (Gce) (**Fig 1c and Supp Fig 1b**). These data demonstrate that this fluorescent reporter faithfully responds to JH dynamics *in vivo*.

To determine whether JH is present and signaling is active during embryonic germ cell development, we stained JHRE-GFP embryos for GFP and the germ cell-specific RNA helicase, Vasa. GFP signal was first detected in Stage 10 embryos in two locations: in bilateral groups within the anterior head region and in the posterior germband (**Fig 1d**). Unlike the GFP signal in

the anterior region, signal in the posterior region was not detected in embryos carrying a negative control reporter transgene, in which the Met/Gce binding motif of the JH response element was mutated. Further investigation revealed that the GFP signal in the anterior region was non-specific and localizes to hemocytes (**Supp Fig 1a-c**)²³. Analysis of specific reporter activity in the posterior germband revealed that JH signaling begins within the mesoderm at Stage 10, when PGCs have migrated from the endoderm into the mesoderm, and continues to localize near PGCs as they colonize the gonad (**Fig 1e, Supp 1c**). Together, these observations demonstrate that JH activity is first detected in mid-embryogenesis in *Drosophila* prior to *corpus allatum* development and is enriched in the mesoderm near PGCs that are migrating toward the developing somatic gonadal precursors.

Juvenile hormones are required for migrating germ cells to reach the somatic gonad

The proximity of JH activity to the migrating and developing PGCs suggested a previously unanticipated role for JH during *Drosophila* embryogenesis. To determine whether JH facilitates germ cell development, we generated new mutant *jhamt* alleles by CRISPR gene editing in which small, internal deletions induced frame-shifts at the start of the methyltransferase domain critical for *jhamt* function (**Fig 2b**). In total, fifteen *jhamt* alleles were generated, two carrying a stop codon at amino acid 46, while the remaining thirteen carry a stop codon at amino acid 54 (**Supp Fig 2a**). To avoid confounding effects from second site mutations, all analyses carried out using the newly generated *jhamt* lines were performed in heteroallelic combinations or *in trans* to a small deficiency lacking the entire *jhamt* locus. To verify loss of *jhamt* function, titers of two major JH species in *Drosophila*, JH III and JHB₃, were determined by liquid chromatography-tandem mass spectrometry²⁵. As expected²⁶, animals with stop codon mutation at the beginning of the methyltransferase domain have reduced JH III titers, undetectable JHB₃ titers (**Fig 2c**), and compromised female fecundity (**Fig 2d**).

To analyze germ cell development, wild type and *jhamt* mutant embryos from all stages of embryogenesis were stained for Vasa and DAPI. This analysis revealed that the first detectable germ cell defect in embryos zygotically mutant for *jhamt* was an increased number of germ cells remaining outside the gonad after Stage 14, when PGC colonization of the somatic gonad is complete (**Fig 2e,f**). Quantification of extragonadal PGCs revealed a significant increase in *jhamt* mutant animals, but not when a complementing, *jhamt*⁺ transgene gene was also

expressed in somatic cells (**Fig 2e,f**). These data indicate Jhamt facilitates PGC migration to the developing somatic gonad.

Germ cells in *jhamt* mutant embryos exit the endoderm but remain posterior to the gonad, a PGC migration phenotype similar to embryos lacking the key mevalonate pathway enzyme, Hmgcr (**Fig 2e and f**)²⁰. Hmgcr is both necessary and sufficient for PGC migration in *Drosophila* such that ectopic expression of Hmgcr mislocalizes PGCs to a variety of locations^{20, 27}. In larval development, Jhamt functions downstream of Hmgcr in the biosynthesis of JHs²⁶. To determine whether Jhamt functions downstream of Hmgcr in PGC migration, we tested for genetic interaction two ways. First, we measured the effect of genetic *hmgcr* reduction in sensitized *jhamt* heterozygous animals and found that significantly more PGCs failed to reach the gonad in *jhamt*^{+/-}; *hmgcr*^{+/-} heterozygous embryos than embryos heterozygous for *jhamt* alone (**Fig 2f**). Second, we tested whether genetic reduction of Jhamt can suppress the ability of ectopic Hmgcr expression to misdirect PGCs. Indeed, loss of one wild type *jhamt* copy reduced the number of PGCs misdirected by Hmgcr (**Fig 2g**). Together, these data suggest that *jhamt* functions downstream of *hmgcr* to direct PGC migration to the somatic gonad.

Juvenile hormone is sufficient for germ cell migration

Hmgcr is sufficient to direct migrating PGC to many tissues, even far from the normal path, such as to the central nervous system (**Fig 3a and Supp Fig 3a**)^{20, 27}. To determine whether, like Hmgcr, Jhamt is sufficient for germ cell migration *in vivo*, we ectopically expressed *jhamt* using the GAL4/UAS system. Indeed, PGCs localize near neuronal Elav-positive cells when UAS-*jhamt* is induced by *Elav-GAL4*, including to the central nervous system (**Fig 3a**). However, significantly fewer PGCs are misdirected by ectopic *jhamt* compared to ectopic *hmgcr* driven by the same *Elav-GAL4* (**Fig 3b**). Moreover, ectopic *jhamt* expression is not sufficient to drive PGCs to inappropriate locations when UAS-*jhamt* is induced by the endodermal driver, *48Y-GAL4*, or the pan-mesodermal drivers, *twist-GAL4* and *24B-GAL4*, even though ectopic *hmgcr* expression with these same GAL4 drivers mislocalizes PGCs (**Supp Fig 3a**). Thus, Jhamt is sufficient to guide germ cell migration *in vivo* in at least one, but not all, tissues.

Hmgcr is the rate-limiting enzyme in the mevalonate pathway upstream of JH biosynthesis²⁸. Therefore, tissue-specific differences in Hmgcr expression²⁰ may underlie the observed context-

dependent differences in the ability of ectopic *jhamt* to misdirect PGCs. To circumvent this biological limitation to assess necessity and sufficiency, we turned to an *in vitro* transwell migration assay. In this assay, *Drosophila* PGCs are enriched by fluorescence-activated cell sorting (FACS), placed on a porous filter across media. Previous work had shown that PGCs will translocate across the filter in response toward serum-free culture media conditioned with Kc167 cells²⁹ (**Fig 3c**). To determine the requirements of JH in this *in vitro* migration assay, we reduced *jhamt* mRNA levels in Kc167 cells using two, independent dsRNAs (Supp Table 3). Like Hmgcr depletion²⁹, significantly fewer PGCs translocated in response to media that was conditioned by Kc167 cells depleted Jhamt compared to control conditioned medium (**Fig 3d**). Reduced translocation of PGCs could be due to either reduced production in pro-migratory factors or reduced Kc167 cell numbers upon Jhamt knockdown. As such, we measured Kc167 number at the end of *jhamt* knockdown and before collection of conditioned media, finding Jhamt depletion had no effect on Kc167 cell number (**Supp Fig 3b**). These data indicate that Jhamt is required for somatic cells to induce PGC migration *in vitro*.

With a suitable *in vitro* migration system in place, we next sought to directly determine whether JH alone is sufficient for PGC migration. To this end, FACS-enriched PGCs were placed on the transwell membrane above serum-free, unconditioned culture media supplemented with commercially available JH III with 1% BSA as a non-specific carrier protein. We found that PGCs translocated in response to JH III in a dose-dependent manner, such that increased JH III increased PGC translocation except at the highest dose tested (**Fig 3e**). To test the specificity of this response, we subjected PGCs to the JH III analogue and insecticide, methoprene, and found that germ cells translocated in response to methoprene at levels comparable to JH III (**Fig 3f**). JH signaling is intertwined with the small steroid hormone, ecdysone, which has known roles in the migration of many cell types, including ovarian somatic cells^{30, 31}. Therefore, we tested whether PGCs translocated in response to the bioactive form of ecdysone, 20-hydroxyecdysone and found that PGCs did not translocate at any biologically active dose tested (**Fig 3e, Supp Fig 3c**). Together, these data demonstrate that JHs are sufficient to induce PGC migration.

Juvenile hormones impact germ cell migration beyond the canonical transcriptional pathway

Classical JH signaling involves direct transcriptional responses via nuclear JH receptors (JHNR) (**Fig 4a**)²³. To determine whether JHNRs are required for PGC migration, we analyzed embryos mutant for two functionally redundant JHNRs, Met and Gce. Existing *met*²⁷, *gce*^{2.5k} mutant animals die during larval development³². Therefore, to obtain embryos which lack maternal contributions of Met and Gce, we generated new *met*, *gce* double mutants that lack *gce* mRNA (**Supp Fig 4a,b**) and carry a point mutation in *met* that garners animals 33x more resistant to methoprene-mediated lethality³³. When this strain is crossed to *met*²⁷, *gce*^{2.5k} animals under non-crowding conditions, ~5% of the expected class of heteroallelic *met*, *gce* double mutant animals eclose. As expected³², we found that fecundity of these escaper *met*, *gce* females was significantly reduced (**Supp Fig 4c**) but sufficient to analyze the requirements of Met and Gce in embryonic germ cell development. Quantification revealed an increased number of extragonadal PGCs in maternally and zygotically mutant *met*, *gce* mutant embryos compared to wildtype embryos (**Fig 4b,c**). Analysis of embryos lacking either maternal or zygotic Gce and Met revealed a partial rescue in the number of extragonadal PGCs, which is consistent with an autonomous requirement in PGCs for gonad colonization. However, unlike in *jhamt* and *hmgcr* mutant embryos where germ cells are found posterior to the gonad, we observed that extragonadal PGCs in *met*, *gce* double mutant embryos were often central and anterior to the gonads, suggesting that PGC migration may be compromised earlier than in *hmgcr* or *jhamt* mutant embryos (**Fig 4b**).

To further interrogate autonomous requirements for JHNRs in PGCs, we took advantage of our observation that ectopic endodermal *Hmgcr* expression traps 50-80% of PGCs in the gut at Stage 10 (**Fig 4d**), when PGCs primarily rely on maternally-deposited mRNAs³. We thus tested whether maternal loss of Met and Gce suppressed the ability of ectopic *Hmgcr* expression to trap germ cells by crossing wildtype or *met*, *gce* double mutant females to males containing *48Y-GAL4* and *UAS-Hmgcr* transgenes. Quantification of the percentage of PGCs remaining in the gut after gonad colonization revealed a small, but statistically significant decrease when PGCs were maternally mutant for Met and Gce (**Fig 4d,e**). As many PGCs derived from *met*, *gce* mutant mothers were still trapped in the gut by ectopic *hmgcr* expression, these data suggest that either Met/Gce in PGCs is zygotically transcribed and translated in time to respond to ectopic *Hmgcr* or that the requirements for these JHNRs in PGC migration is non-autonomous.

Classical JH signaling culminates in the induction of transcription. To determine whether transcription is autonomously required in PGCs to respond to JH, we utilized the *in vitro* migration assay, which allows us to restrict transcriptional inhibition to PGCs. As expected³⁴, flavopiridol rapidly and robustly inhibited transcription in PGCs enriched by FACS (**Supp Fig 4d**). We then subjected control and transcriptionally-deficient PGCs to conditioned media, serum-free media, or serum-free media containing JH III and found that inhibition of transcription did not alter PGC translocation in response to any condition tested (**Fig 4f**). These data suggest that classical transcription-dependent signaling is not required for the migratory response of PGCs to JH.

JHs can induce rapid biological responses through signaling at the plasma membrane³⁵⁻³⁷. One class of plasma membrane signaling factors implicated downstream of JH in several pharmacological studies are phospholipase C proteins (PLCs)³⁸⁻⁴¹. The *Drosophila* genome encodes one gamma PLC, small wing, and two beta PLCs: Plc21c and no receptor potential A (norpA). All three *Drosophila* PLCs are not required for viability, allowing us to examine PGC migration in embryos lacking maternal and zygotic contributions of each PLC. Thus, we examined embryos carrying heteroallelic combinations of existing mutations in *small wing*, *norpA*, and *plc21c*. Somatic defects in *small wing* embryos precluded our investigation into its effect on PGC colonization, as defects in somatic patterning indirectly compromises PGC colonization⁴². Quantification of extragonadal PGCs revealed a mild migration defect in some *norpA* mutant embryos but a significantly stronger PGC migration phenotype in *plc21c* mutant embryos (**Fig 4g,h**). Similar to *jhamt* and *hmgcr* mutant embryos, extragonadal PGCs in *plc21c* mutant embryos were found within the mesoderm posterior to the gonad, suggesting a failure in the second step of PGC migration (**Fig 4g,h**). To test whether Plc21c functions within PGCs to respond to Hmgcr/JH, we crossed *plc21c* mutant females to males carrying *48Y-GAL4* and *UAS-hmgcr* transgenes and quantified the percentage of PGCs trapped in the endoderm. These experiments revealed that maternal loss of Plc21c suppress the number of PGCs trapped in the endoderm by ectopic Hmgcr expression (**Fig 4i,j**). Together, these results suggest that JH facilitates PGC migration to the gonad through non-transcriptional signaling that includes Plc21c

Role of bioactive isoprenoids in early germ cell development may be broadly conserved

Insect JHs and vertebrate retinoic acids (RAs) have pleiotropic functions throughout the germline lifecycle. However, a role in germ cell migration had not previously been described for either bioactive isoprenoid. JHs and RAs are capable of cross-activating respective transcriptional signaling cascades⁴³. To determine whether *in vitro* cross activation extends to PGC migration, we isolated *Drosophila* germ cells by FACS and subjected them to JH III or to two RA species, all-trans retinoic acid (ATRA) or 9-cis RA. Notably, 9-cis RA induced *Drosophila* germ cell translocation to levels comparable of JH III (**Fig 5a**). These data show that RAs are capable of inducing PGC migration in *Drosophila*, suggesting commonality in PGC response to bioactive isoprenoids.

Enzymes critical for biosynthesis of RAs are expressed in the developing bipotential somatic genital ridge of mouse embryos⁷. To determine whether RAs may have a similar role in vertebrate germ cell development, we adapted the *in vitro* transwell migration assay developed for *Drosophila* PGCs to mouse germ cells. To this end, we obtained mouse germ cells by FACS from embryonic day 10.5 embryos carrying a germ cell-specific transgene (*Oct4ΔPE::GFP*). We exposed germ cells to fetal bovine serum in PGC media as a positive control or serum-free PGC media as a negative control. Strikingly, both ATRA and 9-cis RA induced translocation of mouse embryonic germ cells to levels comparable to the known mouse PGC chemoattractant, C-X-C Motif Chemokine Ligand (CXCL12) (also called SDF1a) (**Fig 5b**)⁴⁴⁻⁴⁶. Importantly, while RAs can have a proliferative effect on mouse PGCs, this effect was reported over 24-72 hours⁴⁷. Consistently, we did not detect PGC proliferation in response to RAs during our two-hour *in vitro* migration assay, indicating the increased number of translocated PGCs was not due to RA-induced proliferation (**Supp Fig 5b**). Together, these data suggest that the newly uncovered role of secreted isoprenoids in early germ cell guidance may be broadly conserved among invertebrate and vertebrate species.

Discussion

The bioactive isoprenoids RAs and JHs impact the germline lifecycle in multiple and complex ways across the animal kingdom. Here we provide the first evidence that bioactive isoprenoids act earlier during germ cell development than previously described. Our results suggest that bioactive isoprenoids guide migrating PGCs to colonize the somatic gonad (**Fig 5c**). In contrast to established endocrine, transcription-dependent mechanisms, we show that JHs facilitate PGC migration locally within the mesoderm through transcription independent mechanism. Our studies using *Drosophila* significantly expand the scope and mechanisms by which JHs impact reproductive development. Our studies of RAs and mouse germ cells suggest that these new functions for bioactive isoprenoids may be broadly conserved.

JH functions locally in *Drosophila* embryogenesis

Investigations into JH biology have largely focused on systemic, endocrine-mediated effects on juvenile development and adult fertility. Our study uncovered an embryonic function for JH that precedes development of the endocrine gland thought to be the principal source of JH in insects^{19, 22}. Interestingly, instead of systemic JH activity throughout the embryo, we find that JH signaling in *Drosophila* mid-embryogenesis occurs within a thin mesodermal strip along the germband (**Fig 1e, Supp Fig 1c**). Although local JH signaling could reflect tissue-specific differences in transduction capability, both JHNRs and their co-factor Taiman are maternally provided^{48, 49}. Thus, we favor a model in which expression of the key JH biosynthetic gene, *jhamt* and JH reporter activity within the mesoderm reflects local production and paracrine signaling (**Fig 1b,e**). Evidence for local JH activity in adult *Drosophila* intestines⁵⁰ and germline stem cell niches of adult *Drosophila*⁵¹ has been described, with the caveat that many gonadal Gal4 drivers are also active in the nervous system⁵². Our data build on these recent findings and suggest that JH functions in a short range, paracrine manner, similar to the mammalian bioactive isoprene RA. To generate instructive spatiotemporal differences, RA gradients form through dynamic changes in expression of RA biosynthesis and degradation enzymes. Curiously, degradation enzymes for both RA and JH exhibit strikingly similar dynamic and sex-specific expression patterns¹⁷. Together, these finding suggest that spatial JH dynamics may be instructive to PGCs. Consistent with this postulate, we found that in addition to PGC migration defects, *met*, *gce* double mutant larvae have markedly increased titers of JH III and JHB₃ (**Supp**

Fig4e). It will be important to determine whether, like RA, spatial JH dynamics are instructive to PGCs during embryogenesis.

PGCs respond to JH through non-canonical signaling

Classic JH and RA signaling involves nuclear receptor-mediated transcription. Curiously, we find that PGCs respond to JHs even when transcription is globally inhibited (**Fig 4f**), suggesting that PGCs respond to JH through rapid, non-transcriptional mechanisms. Rapid, non-transcriptional responses to JH during ovarian follicle development have been postulated for decades^{36, 53-55}, however the precise mechanisms have been more elusive. Consistent with pharmacological-based studies that implicate plasma membrane receptors, Protein Kinase C and PLC³⁸⁻⁴¹, our data suggest that Plc21c is involved in JH-mediated PGC colonization of the gonad (**Fig 4g,h**). As a beta type PLC, Plc21c is linked to G protein-coupled receptors (GPCRs). GPCRs have a prominent role in cell migration and GPCR-mediated desensitization could account for our observation that *in vitro* migration is reduced in response to higher JH concentrations (**Fig 3e**). A GPCR that binds JH has not been identified in *Drosophila*, though putative JH GPCRs have been identified in other insects⁵⁶. Identification of the GPCR that mediates JH, or RA signaling, in early PGC development and other reproductive processes will be an exciting area of future exploration.

Bioactive isoprenoids in cell migration

The timing, modality, and directionality of cell migration is dictated by extracellular information. The regulation of such parameters is particularly important when cells are migrating within the context of a developing tissue or animal. Our investigations revealed that extracellular JH ensure migrating PGCs reach the embryonic somatic gonad. To our knowledge, such a role for JHs has not been previously described. Intriguingly, ecdysone, the insect hormone that is functionally opposed by JH signaling in larval development, governs the timing, motility, and organization of several ovarian somatic cells in *Drosophila*^{30, 31, 57}. Ecdysone signaling is not active in the *Drosophila* embryo until Stage 13, where it is first active in the amnioserosa far from developing gonads⁵⁸. Consistently, we did not find evidence that ecdysone impacts PGC migration *in vitro* (**Fig 3e**). However, given the antagonism between ecdysone and JHs in other

biological contexts and the coordinated nature of germ cell and somatic gonad development^{57, 59-61}, it will be important to determine whether JH dynamics impact the migration of somatic gonadal cells.

Results presented here suggest that JHs directly impact PGC migration. While JHs have not been implicated cell migration, the same is not true for retinoids. While most investigations into the role of RAs in cellular migration have focused on transcription-based mechanisms that upregulate receptors required for homing^{62, 63}, evidence for transcription-independent functions of RAs is also emerging⁶⁴. RAs facilitate axon guidance in *Xenopus laevis*⁶⁵, and in snails through non-transcriptional mechanisms involving calcium flux, Rho GTPases and G proteins⁶⁶⁻⁶⁹. Our studies also implicate G protein, calcium signaling and leave open the possibility that cytoplasmic signaling axis is broadly shared across bioactive isoprenoids. Consistently, we find that RAs are sufficient to induce mouse germ cell migration *in vitro* (**Fig 5b**). Unlike previous *in vitro* migration assays done on mouse embryonic germ cells^{46, 70, 71}, our transwell incubation period for mouse PGCs was only two hours. This short temporal window is suggestive of a rapid response by mouse PGCs downstream of RAs, but future work will be needed to determine whether the impact of RA on mouse germ cells requires transcription, RA nuclear receptors or PLC-based signaling.

A cohesive class of bioactive isoprenoids in reproductive development

In total, our findings support a hypothesis that JHs and RAs are functionally analogous molecules in reproductive development. Beyond similar roles in post-meiotic gametogenesis^{72, 73}, we provide evidence that, like RAs, JHs act in a paracrine manner to support embryonic reproductive development. Like JH, RA biosynthesis enzymes are expressed during PGC gonad colonization,⁷ and after PGC colonization, degradation enzymes are induced specifically in male gonads in *Drosophila* and mice. RA was recently implicated in sex-specific differentiation of the gonad⁹, which may be akin to the role of the bioactive isoprenoid, methyl farnesoate, in crustacean sex determination^{6, 74}. Given these similarities, we assert that *Drosophila* can serve as a powerful model to understand how bioactive isoprenoids impact embryonic reproductive development. Such additional genetic models are needed, as recent investigations have brought the role of RA in early reproductive development into question^{7, 8, 13, 11, 14, 15, 75}. *Drosophila* is a simplified and tractable model in which to tease apart primary

functions and molecular mechanisms to understand the full breadth of isoprenoid impact on reproductive development.

Acknowledgements

We thank Marek Jindra and Ryusuke Niwa for reagents and discussions, and Peter Nicholls and David Page for instructive discussions on mammalian reproductive development and assay development. We are grateful to Alexey A Soshnev for graphical designs, Cesar Ramirez and Francisco Fernandez-Lima for assistance with mass spectrometry. We are especially grateful to Rebecca Spokony, Jessica Treisman, Pamela K Geyer, and Toby Lieber for valuable advice and insightful discussions, as well as all members of the Lehmann laboratory for valuable input. Stocks obtained from the Bloomington Drosophila Stock Center (NIH P400D018537) were used in this study. In addition, several monoclonal antibodies used in this study were obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA. We also thank the NYULMC Core Cytometry Facility, which is partially funded by NIH/NCI P30CA016087. This work was supported by a Damon Runyon Cancer Research Foundation postdoctoral fellowship from the William Raveis Charitable Fund (DRG2235-15, L.J.B.), an NIH Pathway to Independence award (K99 HD097306, L.J.B.), an NIH-NIAID R01 award (R01AI04554, F.G.N), and NIH-NIAID R21 award (R21AI153689 to M.N.) and an NIH MERIT award (R37HD41900, R.L). RL was a HHMI investigator.

Author Contributions

Conceptualization: LJB, RL; Experimentation: LJB, JS, EPD MN, FGN, MS; Analysis: LJB, MN, FGN; Writing: LJB, RL; Funding Acquisition: LJB, RL; Supervision: LJB, MS, RL

Declaration of Interest

The authors declare no competing interest

Materials and Methods

Animal Maintenance

Drosophila melanogaster were raised on medium containing 1.5% yeast, 3.6% molasses, 3.6% cornmeal, 1.12g Tegosept, 1.12% alcohol and 0.38% propionic acid. For embryo collections, apple juice plates contained 25% apple juice, 2.5% sucrose, 2.25% bactoagar, 0.15% Tegosept. Animals were kept at 25°C for all experiments. Fly strains used in this study are listed in Supplementary Table 1.

Mice expressing a germ cell-specific *Oct4 Δ PE::GFP* transgene⁷⁶ (B6; CBA-Tg(Pou5f1-EGFP)2Mnn/J) were obtained from Jackson Laboratory (#004654, Bar Harbor, ME, USA). All protocols were approved by the NYU Institutional Animal Care and Use Committee.

Generation and validation of the P[JHRE-GFP, w+] transgenic reporter

Eight copies of the wildtype JH response element (JHRE) from the *early trypsin* gene from *Aedes aegypti*²⁴, or eight tandem copies of the mutant JHRE (Supp Fig 1), were excised from *pGL4.17* vectors provided by Jindra Marek. JHREs were inserted upstream of the minimal *hsp70b* promoter and eGFP coding sequence of pS3aG transformation vector (Addgene #31171) containing an *attb* for site-specific integration. JHRE reporter constructs were integrated into *y+*, *attP2* at cytological position 68A4 sites by Bestgene.

To validate the JHRE-GFP reporter, transgenes were placed in *met*¹, *gce*^M mutant background. Embryonic GFP signal was measured by immunohistochemistry (Supp Fig 1). To measure JH responsiveness, first instar larvae were placed in food containing 1.6ug of methoprene per gram of food⁷⁷, or ethanol as a control for 24 hours. *GFP* mRNA was measured in whole second instar larvae by qPCR using primers in Table 3 (Fig 1 and Supp Fig 1).

Generation of *jhamt* alleles

The crispr guide CCTGGATGTGGTTCAGGATCT was cloned into pU6-2 gRNA vector (DGRC #1363) and injected into embryos into a stock derived from BL51324 carrying *w*¹¹¹⁸;; *PBacy[mDint2]=vas-cas9VK00027* by Bestgene. Of 29 fertile founders, 87 F1 crosses were set up and screened for *jhamt* mutations by PCR amplification and Sanger sequencing using primers listed in Table 3. Of 48 F1 lines screened, 13 lines were obtained wherein small indels created a frame shift just before the methyltransferase domain, which starts at amino acid 40, leading to a stop codon just downstream, two lines at amino acid 46 (12F1 and 12F2) and the remaining lines at amino acid 54 (1F1, 1F2, 4F1, 4F3, 6F3, 7F2, 7F3, 8F1, 8F2, 12F3, 14M2, 14M3). Nonsense mediated decay was detected in all lines by measuring *jhamt* mRNA in whole adult animals by qPCR (Supp Fig 2a). The new alleles on JH III and JHB₃ titers were quantified in third instar larval hemolymph (See separate subsection in Methods).

Embryo Immunofluorescence

Prior to embryo collection, young parental strains (less than 5 days old) were mated in vials for one day before transferring to embryo collection cages containing apple juice plates and wet yeast. Embryos were dechorionated in 50% bleach for three minutes, rinsed with water and fixed in a scintillation vial containing 2ml of 4% formaldehyde in PBS and 8ml of heptane. Embryos were fixed for 20 minutes on a shaker. To remove the vitelline membrane, formaldehyde was removed from the scintillation vial and 10ml methanol was added, followed by one minute of vigorous shaking by hand. Embryos were washed with fresh methanol three times before storage at -20C or future processing.

For immunohistochemistry, embryos were sequentially rehydrated with increasing percentage of PBSTx (PBS with 0.1% TritonX100) and then washed six times with PBSTx before a one-hour block using 1% BSA in PBSTx at room temperature. Primary antibodies were incubated overnight at 4C and then washed six times in PBSTx. Secondary antibodies in 1% BSA in PBSTx were incubated for two hours at room temperature, washed six times in PBSTx. Embryos were then equilibrated in Vectashield (Vector Laboratories, H-1200) overnight at 4°C before mounting on coverslips.

Fluorescent *in situ* hybridization was done as previously shown⁷⁸. Probes were generated using *Drosophila* clones from DGRC (*jhamt* #11840, clone AT13581) and (*Nanos* #1124533, clone RE53469).

mRNA quantification by qPCR

RNA was obtained by TRIzol (Invitrogen #15596018) extraction. DNA was removed using RQ1 RNase-Free Dnase (Promega #M6101). cDNA was generated using SuperScript III Reverse Transcriptase (Fisher Scientific #18080-044) using both oligo dT and random hexamers. Primers used are listed in Table 3. qPCR was carried out using LightCycler 480 SYBR Green I Master 2X (Roche #04887352001) and a Roche LightCycler 480 machine. Results were calculated as previously described⁷⁹ from crossing points and normalized to one of three reference genes found to have invariant expression across tissues and developmental stages: *DCTN2-p50*, *und*, *DCTN5-p25*⁸⁰.

Evaluation of JH titers by quantitative mass spectrometry

The following genetic backgrounds were used to measure JH III and JHB₃ titers: *jhamt*^{8F2/12F1} paired with the *w*¹¹⁸; *CyO*, *ft-lacZ* genotype, which was the genetic background the crispr alleles were extensively crossed into and *gce*^{Mi}, *met*¹ paired with *w*¹¹⁸. Twenty-five third instar larvae per biological replicate were collected from sparsely populated vials, washed in PBS before placing in 50µl of pre-chilled PBS for hemolymph collection. To collect hemolymph, the cuticle was pinched and pulled apart down the length of the animal. Care was taken not to rupture fat body or intestines. Hemolymph was transferred to pre-chilled silanized glass vials (Fisher Scientific #C4011S5 and #C401154B) using silanized glass pipettes (Brain Bits #FPP). The glass collection well was rinsed with 50µl additional, pre-chilled PBS to obtain any

remaining hemolymph and additional PBS was added to silanized glass vial for a total volume of 150µl. Deuterated JH III (62.5pg) dissolved in acetonitrile (Thermo Fisher #51101) was added to each replicate, then 600µl hexane (Sigma Aldrich #1037011000). Samples were vortexed for 1 minute and centrifuged for 5 min at 2000g at 4C. Organic phase was transferred to new silanized vial for analysis by mass spectrometry.

For mass spectrometry analysis, the JH III and JH III bisepoxide (JHB₃) amounts present in hemolymph were quantified by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS)²⁵. The identification and quantification of JH III and JHB₃ in hemolymph samples was based on multiple reaction monitoring (MRM), using the two most abundant fragmentation transitions: for JH III: 267->235 (primary) and 267->147 (secondary), and for JHB₃: 283->233 (primary) and 283->251 (secondary)²⁵.

Fecundity measurements

Fecundity was measured as previously described⁸¹ and reported as the average number of eggs laid in three- and four-day old females at 25°C. In each biological replicate, 10-12 females were crossed to 5-6 *w¹¹⁸* males to mitigate male fertility defects upon *jhamt* loss.

Knockdown of *jhamt* in Kc167 Cells

To target *jhamt*, genomic DNA obtained from Kc167 cells using Qiagen's Blood and Tissue Kit (#69506) was used as a template. To target lacZ, pBluescript SK plasmid used as a template. dsDNA was generated by Choice Taq Mastermix (Denville Scientific #CB4070-8). Gradient PCR was used to obtain optimal dsDNA templates. dsRNAs were generated from dsDNA templates using T7 MEGAScript (Invitrogen #am1334) according to standard protocol. dsRNAs were DNase treated (Qiagen #79254) and purified using Qiagen RNAeasy Mini Kit (Qiagen #74104) according to instructions. For transfection, Kc167 cells were grown to confluency, resuspended in fresh Schneider's media (Fisher #21720-001) supplemented with Penn/Strep (Fisher, #15140122) and 10% heat inactivated FBS (Thermo Fisher #16140071) to 1x10⁶ cells/ml and allowed to settle overnight. 2µg of dsRNAs were used in conjunction with Effectene Transfection Reagent (Qiagen #301425) into each 6-well dish. After 48 hours at 25°C, media was removed, transfected Kc167 cells were washed in PBS and resuspended in serum-free Schneider's Media (with Penn/Strep) for three additional days at 25°C prior to collection of Kc167-conditioned media. To determine whether *jhamt* knockdown compromised Kc167 viability, the number of Kc167 cells was counted on the day conditioned media was collected. Knockdown efficiency was determined in Kc167 cells by qPCR when conditioned media was collected for the transwell assay. Primers were chosen outside of region targeted by dsRNAs.

Isolation of PGCs

Drosophila embryos carrying the *P[nos-Moe::eGFP.nos 3'UTR]* transgene from an overnight collection were dechorionated in 50% bleach for two minutes, rinsed and placed in filter-sterilized Chan and Gehring's Balanced Saline, PGC Sort Buffer (3.2g/L NaCl, 3.0g/L KCl, 0.69g/L CaCl₂·2H₂O, 3.7g/L MgSO₄·7H₂O, 1.79g/L Tricine buffer (pH 7), 3.6g/L glucose, 17.1g/L sucrose, 1g/L BSA, fraction V). Dechorionated embryos were manually homogenized in PGC Sort Buffer with Dounce homogenizer (Pyrex #7727-15) and passed through a 100µm filter and 20µm filter and placed in pre-chilled polypropylene falcon tubes (#352063). Live, GFP-positive *Drosophila* PGCs were sorted using a MoFlo XDP cell sorter fitted with a 70µm nozzle into Maxymum recovery microtubes (Axygen #311-05-051) containing 200µl Schneider's Media.

To enrich for mouse PGCs, B6; CBA-Tg(Pou5f1-EGFP)2Mnn/J (Jax #004654) mice were bred to produce embryos homozygous for the *Oct4PE::GFP* transgene. Embryonic day 0.5 (E0.5) was assumed to be noon of the day the vaginal plug was observed. Pregnant females were euthanized by carbon dioxide followed by cervical dislocation. Trunks were dissected from E10.5 embryos in chilled PBS. For each two embryos, tissue was dissociated in 300µl of 0.5% trypsin (0.25%, Life Technologies #25200056) for 15 min at 37°C, with occasional manual disruption by pipette. Trypsin was inactivated with 200µl of mouse PGC culture media [DMEM/F-12 (Thermo Fisher #11-320-033), 250µM sodium pyruvate (Thermo Fisher #11360070), 2mM L-glutamate (Gibco #25030081), 1X MEM Non-essential amino acids (Thermo Fisher #11-140-050) containing 25% Bovine Albumin Fraction V (Thermo Fisher #15260037). Dissociated tissue was centrifuged at 200g for 7 min and cells were resuspended in 2mL of PGC media. Cell suspension was filtered through a 35µm cell strainer into a 5 ml falcon round bottom tube (VWR #21008-948) for sorting using a MoFlo XDP cell sorter fitted with a 100µm nozzle. Live, GFP+ germ cells were sorted into Maxymum recovery microtubes (Axygen #311-05-051) containing mouse PGC media.

in vitro Migration Assays

For *in vitro* transwell assays, all plastic ware, tips, tubes and transwell plates used throughout the assay were pre-coated with PBS containing 1% BSA to prevent hydrophobic molecules from sticking to the surfaces. Assays with light sensitive isoprenoids were done in dark conditions as much as possible. For Kc167-conditioned media, serum-free media was added to washed, confluent Kc167 cells and collected three days later. Conditioned media was collected and centrifuged 4,000rpm for 20 min to remove all cellular debris and placed on ice. All isoprenoids (Supplementary Table 4) were prepared by dilution series in either serum-free Schneider's media for *Drosophila* PGC assays or mouse PGC media. Transwell assays were done using the 96-well ChemoTx Disposable Chemotaxis System (NeuroProbe #116-10) which had a standard PCTE filter membrane with 10µm pore size, 300µl well capacity and 5.7mm cell site diameter. Sorted germ cells recovered on ice for 30 min while the transwell system was set up. Sorted germ cells were then diluted 10-15-fold in serum-free Schneider's media for *Drosophila* PGC assays or mouse PGC media so that 60µl diluted PGCs were placed above each well. Transwell plates with *Drosophila* PGCs were incubated 25°C for 1.5 hours and those with mouse PGCs were incubated at 37°C for 2 hours. After the incubation period, PGCs were

removed from above membrane, the plate was centrifuged at 460rpm for 10min to dislodge cells from the well side of the membrane. Translocated germ cells were removed from the 96-well plate by pipetting up and down and transferring to Nunc Lab-Tek II Chambered Coverglass (Thermo Fisher #155409) for manual quantification by fluorescent microscopy.

The number of PGCs in each top well was calculated by setting aside 10% input (6μl) in duplicate into Lab-Tek wells, each with 300μl serum-free media at the beginning of the transwell assay and quantifying the number of germ cells when the experimental translocated germ cells were quantified. To compare biological replicates done on different days, Specific Migration was calculated by first subtracting the number of germ cells that translocated in the negative control wells (serum-free media) and then calculating the percentage of normalized experimental condition to the respective positive control. Each transwell assay is the result of two to four biological replicates, each consisting of two technical replicates.

Verification of Hormone Activity *in vitro*

To verify the activity of JH III and 20-hydroxecdysone (Supplemental Table 4), hormones were added to 50% confluent Schneider 2 (S2) cells at the concentrations indicated in Supp Fig 3d. After a six-hour incubation at 25°C, S2 cells were harvested and mRNA was extracted and analyzed using protocols described in the '*mRNA quantification by qPCR*' Methods subsection.

Transcription Inhibition in *Drosophila* PGCs

Efficient inhibition of transcription in *Drosophila* PGCs isolated by FACS was determined by testing different concentrations of Actinomycin D and Flavopiridol (See Supplemental Table 4). To visualize transcription by immunohistochemistry, Lab-Tek II 8-chambered coverglass were incubated with poly-L-Lysine solution (Sigma-Aldrich #P4707) for 30 min at room temperature and then dried for 1.5 hours. Sorted *Drosophila* PGCs were added to chambers and incubated with Actinomycin D or Flavopiridol for either 15 min or 3 hours, then fixed with 4% paraformaldehyde for 15 minutes, washed in 0.05% PBS with TritonX100, blocked in 1% BSA for 30 min and stained for Vasa, RNA Pol II Ser2CTD and RNA Pol II Ser5 CTD (See Supplemental Table 2 for antibody information). As expected³⁴, Flavopiridol rapidly inhibited transcription in *Drosophila* PGC while not compromising cell viability. For transwell assays, sorted *Drosophila* PGCs were first incubated with 1μM Flavopiridol for 30 min prior to placing on transwell membrane and solutions in the bottom well chambers also contained 1μM Flavopiridol to ensure PGC transcription was inhibited throughout the assay.

Microscopy and Image Processing

Most embryos and other samples were imaged with a Zeiss LSM800, an upright laser scanning confocal microscope using Zen Blue 2.3 software and a Plan-Apochromat 20x0.8 NA air objective and 40x1.3NA oil objectives with pinhole size for all image was set to 1AU. Images were processed using ImageJ.

Statistics

Statistical analysis was done as described in the Figure Legends. Briefly, for quantification of phenotypic data, such as extragonadal PGC quantification, data was not always normally distributed and thus median +/- IQR are reported and statistical significance was assessed by Mann-Whitney U test. For mRNA Fold Change and *in vitro* Specific Migration results, mean +/- standard deviation is reported and statistical significance was tested by unpaired t-test. Sample size per biological replicate was influenced by power analysis based on previously observed phenotypic variation.

Supplemental Table 1: Animal lines used in this study

Name	Species	Source	Notes
<i>P[JHREWt8x-eGFP]attp2</i>	<i>Drosophila</i>	This study	JH responsive reporter, with non-specific activity in hemocytes
<i>P[JHREMut8x-eGFP]attp2</i>	<i>Drosophila</i>	This study	Negative control reporter transgene with non-specific activity in hemocytes
<i>y¹, w⁺, gce^{mi02742}</i>	<i>Drosophila</i>	BL60189	Insertion that leads to RNA null
<i>met¹</i>	<i>Drosophila</i>	BL3472	EMS-generated from OregonR-C causing G1161736A, DNA mutation and G166D protein change, genetically amorphic allele ^{82 33}
<i>y⁺, w⁺, v⁺, met¹, gce^{mi02742}/Fm7ai</i>	<i>Drosophila</i>	This study	Recombinant between existing mutants listed above
<i>w⁻, v⁻, met²⁷, gce^{2.5k}/Fm7ai</i>	<i>Drosophila</i>	Rebecca Spokony and Lynn Riddiford ⁸³	Both <i>met²⁷</i> and <i>gce^{2.5k}</i> are large deletions that lead to full loss of RNA
<i>w¹¹¹⁸, jhamt^{8F2} or ^{12F1}/CyO, ft-lacZ</i>	<i>Drosophila</i>	This study	crispr generated small deletions
<i>jhamt^{Df(2L)BSC781}/CyO, ft-lacZ</i>	<i>Drosophila</i>	BL27353	2L:16325113;16417726, including <i>jhamt</i> and 13 other genes
<i>hmgcr^{26.31}/Tm3, Ubx-lacZ</i>	<i>Drosophila</i>	⁴²	Also known as <i>clb²</i>
<i>hmgcr^{11.54}/Tm3, Sb, Ubx-lacZ</i>	<i>Drosophila</i>	⁴²	Also known as <i>clb¹</i>
<i>hmgcr⁰¹¹⁵²/Tm3, Ubx-lacZ</i>	<i>Drosophila</i>	²⁰	L(3)01152 source: Transposon insertion 179 nucleotides into <i>hmgcr</i> locus
<i>y¹, w⁺; pPlc21c^{Mi}/Cyo, ft-lacZ</i>	<i>Drosophila</i>	BL60761	MI02818 gene trap insertion with a stop codon after amino acid 126 ⁸⁴
<i>plc21c^{Df(2L)BSC106}</i>	<i>Drosophila</i>	BL8672	
<i>w¹¹¹⁸, itpr^{P05616}</i>	<i>Drosophila</i>	BL11664	p-element insertion in first exon
<i>w⁺, norpA³⁶</i>	<i>Drosophila</i>	BL9048	Ems-induced amorphic, loss of function allele
<i>w¹¹¹⁸, norpA^{Df(1)BSC}</i>	<i>Drosophila</i>	BL26575	X:431805;X4533198,2 includes <i>norpA</i> and 14 other genes
<i>w, sl^{K08}</i>	<i>Drosophila</i>	⁸⁵	1 bp deletion in exon 1, leading to a premature stop codon at aa28
<i>Ln(1)bbDf, y1, sl²/Fm4</i>	<i>Drosophila</i>	BL724	x-ray induced loss of function allele
<i>UAS-hmgcr</i>	<i>Drosophila</i>	²⁰	pNB36C <i>hmgcr</i> cDNA downstream of UAS
<i>y, w; ; UAS-jhamt</i>	<i>Drosophila</i>	Ryusuke Niwa ¹⁹	RN286 and RN287, both on the third chromosome, containing two point mutations also found in w118 (F166Y and S182N)
<i>w; elav-GAL4</i>	<i>Drosophila</i>	J Treisman	Second chromosome
<i>w; 48Y-GAL4</i>	<i>Drosophila</i>		Second chromosome
<i>w; twi-GAL4; 24B-GAL4</i>	<i>Drosophila</i>	M. Akam	Second and third chromosome
<i>y, w;; Tub-GAL4</i>	<i>Drosophila</i>		Third chromosome
<i>w-, P[nos-moesin::egfp::nos 3'UTR]</i>	<i>Drosophila</i>	⁸⁶	
<i>Oct4ΔPE::GFP</i>	Mouse	Jax #004654	B6; CBA-Tg(Pou5f1-EGFP)2Mnn/J

Supplemental Table 2: Antibodies used in this study

Name	Source	Species	Concentration
Vasa	Lehmann lab	Rabbit	1:5,000
GFP	AVES #1020	Chicken	1:500
GFP	Thermo Fisher Scientific #A11122	Rabbit	1:1,000
Singed (fascin)	DSHB #sn 7c	Mouse	1:100
Elav	DSHB #9F8A9	Mouse	1:500
b-galactosidase	Abcam #ab9361	Chicken	1:500
Hindsight	DSHB #1G9	Mouse	1:20
RNA Pol II Ser5	Chromotek #3e8-1	Rat	1:100
RNA Pol II Ser2	Abcam #ab5095	Rabbit	1:500

Supplemental Table 3: Primers used in this study

Element	Purpose	Forward Primer	Reverse Primer
<i>jhamt</i>	Genotyping crispr deletions	AGGTGCAAAGGCACGAT	AGGTGCAAAGGCACGAT
<i>jhamt</i>	Crispr guide	CTTCGAGATCCTGAACCCACATCC	AAACGGATGTGGGTTCAAGATCTC
<i>jhamt</i>	<i>in situ</i> probe	GGCACGATGCCAAATTGAT	GATAATACGACTCACTATAGGGGATGTGTAGGCTC CAGGAAA
<i>Nanos</i>	<i>in situ</i> probe	AGATTGCGCGATCCTTGAA	TAATACGACTCACTATAGGGCTGGATTGCGCCTCT CTAAAC
<i>und</i>	reference gene for qPCR	GCAAGAAAAGCGGTCCAGACT	CGTGTTGATACGGTCCAGAG
<i>Dnm/DC TN2-p50</i>	Reference gene for qPCR	AGACGCCTGGAAGTAAGCAG	GTAAGGCGGCTCAACTTGTC
<i>DCN5- p25</i>	reference gene for qPCR	ACAAAGTTAGTCGCCACACG	ATGACGGAGTTCTTGCCAAT
<i>twinstar</i>	Detect DNA contamination for qPCR	TCAGAGGTGCCTTGACACTG	GATGTGGAGACTGTGGCAGA
<i>eGFP</i>	mRNA quantification	GAACCGCATCGAGCTGAA	TGCTTGTGCGCCATGATATAG
<i>gce</i>	Genotyping <i>gce</i> alleles	GGATGCCATCGATCGCAAGT	GCTTCGTCACCTACGCCGAAA
<i>met</i>	Genotyping <i>met^l</i> allele	CTGCTTCCTCACCTAACCT	CTGCTTCCTCACCTAACCT
<i>jhamt</i>	mRNA quantification	TGACCATGTACCTCGTTCTACTGC	G A A G T C A T C C A G G A A C T G T T C A T G C
<i>jhamt</i>	dsRNA 1 for knockdown in Kc167 cells	TAATACGACTCACTATAGGGCCTCACGCCT TCATAAACAA	TAATACGACTCACTATAGGGGGTAACGTGCTCATG GACT
<i>jhamt</i>	dsRNA 2 for knockdown in Kc167 cells	TAATACGACTCACTATAGGGCTTCGTAAAG CCACTGCTCC	TAATACGACTCACTATAGGGCCTCGCCAGGACTTA GACTG
<i>lacZ</i>	dsRNA knockdown DRSC42733	TAATACGACTCACTATAGGG CTGGCGTAATAGCGAAGAGG	TAATACGACTCACTATAGGG CATTAAAGCGAGTGGCAACA
<i>jhamt</i>	Knockdown efficiency	CAGTTCTGGATGACTTCATAGAC	CCA CCA CCA ATT TAT AGG GAG ATA G
<i>kr-h1</i>	JH responsiveness	G A A T A C G A C A T A A C A G C C	CGATTTCCGTGAATATGTTCT
<i>Eip74b</i>	20E responsiveness	GTACGCGTATTGGGACTATCAG	CACGCGAGATAGGGATTCTT
<i>Eip75</i>	20E responsiveness	GTCCATCAGCGAGTTGGTATC	CACCAGCAGCCATCTGAA
<i>plc21c</i>	Genotype <i>plc21c^{K03}</i>	TCGGATACCAACCAGGACTATG	TATCTCGGGCACGAACGTATAG
<i>sl</i>	Genotype <i>sl^{K08}</i>	CGGATGAGAACTGGATTGATAG	GTGCAGTATGACAAAGCACTTG

Table 4: Pharmacologics used in this study

Name	Source	Product Number	Stock solvent	Final Concentration
JH III	Sigma-Aldrich	J2000-10MG	Ethanol	0.25-25µM
Methoprene	Sigma-Aldrich	33375-100MG	Ethanol	2.5µM
20-hydroxecdysone	Sigma-Aldrich	H5142-5MG	Ethanol	0.25-25µM
ATRA	Sigma-Aldrich	R2625-50MG	DMSO	2.5µM
9cis-RA	Sigma-Aldrich	R4643	DMSO	2.5µM
Flavopiridol	Selleck	S1230	PBS with 0.1% BSA	1µM
Actinomycin D	Sigma-Aldrich	A1410-Smg	DMSO	1-5µg/mL
CXCL12/Sdf-1a	R&D Systems	460-SD-010	PBS with 0.1% BSA	50ng/mL

Figure Legends

Figure 1: Juvenile Hormone (JH) signaling is present during embryonic germ cell

development **a** Schematic of germ cell development during Stage 10-14 of *Drosophila* embryogenesis. SD stands for sex differentiation. **b** Lateral view images of a late Stage 12 embryo stained by Fluorescent *in situ* hybridization (FISH) for *jhamt* (green) and *nanos* (magenta) mRNA. Primers used to generate *in situ* probes are listed in Supplementary Table 3. **Bottom**: Magnified image of *jhamt* and *nanos* mRNA within the boxed area. **c** **Top**: schematic of the JH reporter transgene (JHRE-GFP) in which eight copies of the JH Response Element from the *early trypsin* gene from *A. aegypti* was placed upstream of eGFP (See Materials and Methods for construct design). **Bottom**: Induction of *kr-h1* mRNA, a known JH transcriptional target, and *GFP* mRNA. deltaCt was calculated relative to the average Ct of two housekeeping genes, *DCTN5-p25* and *Und*, and fold change was calculated by for whole second instar larvae fed cornmeal food containing 1.6µg/mg of the JH analogue, methoprene normalized to age and genotype-matched larvae fed ethanol. Animals were fed methoprene or ethanol as a control for 24 hours prior to RNA isolation. Bars and error bars represent average fold change and standard deviation from three biological replicates, each consisting of ten second instar larvae. **d** **Left**: Dorsal/Ventral (d/v) images of Stage 10 *JHRE-GFP* embryos that are wild type or mutant for *Met* and *Gce*. **Right**: Magnified images of the boxed area. **e** **Left**: d/v view images of a Stage 11 *JHRE-GFP* embryo stained for GFP (green), Vasa (magenta) protein, and DNA using DAPI (gray). **Right**: d/v view of a Stage 11 *JHRE-GFP* embryo. GFP staining only is shown below each merged image. For all images, genotypes are shown in the top left corner, embryo orientation in the top right corner. All embryo images are maximum projections of two to three 2µm confocal slices and scale bars represent 25µm in all images.

Figure 2: The key JH biosynthesis enzyme, Jhamt, facilitates germ cell migration to the somatic gonad. **a** Schematic of the JH biosynthetic pathway from Acetyl co-A upstream of Hmgcr activity to final steps involving Jhamt methyl transferase activity. **b** Schematic of the *jhamt* locus noting where the CRISPR-mediated editing created 15 stable animal lines containing indels to generate premature stop codons (denoted by Stop) within the methyltransferase domain (shown in green). Light gray denotes 5' and 3' UTR. **c** Titers of two JHs, JH III and JH bisepoxide (JH B3) in hemolymph of third instar *Drosophila* larvae measured by quantitative mass spectrometry using deuterated standards. Each dot represents a biological replicate of hemolymph bled from 25 larvae. For titers, *w*, *Sp/CyO,ftz-lacZ* was used as a wild type strain as the CRISPR-induced alleles in a *w* background were initially crossed into this background. **d** Fecundity of young females. Each dot represents the average number of eggs laid per female from cohorts of 10-12 females in a 24-hour period three- and four-days post eclosion. To avoid male reproductive effects, all virgin females were mated to wild type (*w*¹¹¹⁸) males. For **c-d**, bars and whiskers represent mean +/- standard deviation and statistical significance was tested by unpaired student t test. **e** Dorsal/Ventral (d/v) view images of Stage 14 *Drosophila* embryos stained for Vasa (magenta) and DNA (DAPI, gray). Zygotic genotypes are noted in the top left corner. To yield *jhamt*^{-/-} embryos, with heteroallelic combinations of independently-derived indels, *jhamt*^{8F1/CyO,ftz-lacZ} females were mated to *jhamt*^{12F1/CyO,ftz-lacZ}. To generate *jhamt*^{-/Def}, *jhamt*^{8F1/CyO,ftz-lacZ} animals were mated to *Df(2L)BSC781/CyO,ftz-lacZ animals. Gonads are circled with a yellow dashed circle. Arrowheads highlight extragonadal germ cells. All embryo images are maximum projections of two to three 2µm confocal slices and scale bars represent 25µm. **f** Quantification of extragonadal PGCs in Stage 14-16 embryos. Each dot represents one embryo. To the right of the dashed line, *jhamt*^{+/-} virgin females were either crossed to *w*¹¹¹⁸ or *hmgcr*^{+/-} males and unbalanced Stage 14+ progeny were scored. *jhamt* and *hmgcr* alleles used are described in Supplemental Table 1. **g** Quantification of the number of germ cells remaining outside the gonad of Stage 14-16 embryos when Hmgcr is ectopically expressed in the nervous system using *Elav-GAL4* and *UAS-hmgcr*. For panels **d-g**, bars and whiskers represent median +/- interquartile range (IQR), statistical significance was tested by Mann-Whitney U test, and *w*¹¹¹⁸ was used as a reference strain.*

Figure 3: Juvenile Hormone is sufficient for germ cell migration. **a** Dorsal/Ventral (d/v) view images of Stage 14 embryos stained for Vasa (magenta) and Elav (green). Genotypes are noted in the top left corner of each image. Arrowheads highlight germ cells in contact with Elav-positive central nervous system. Scale bars represent 25µm images. **b** Quantification of the number of PGCs in Stage 14-16 embryos contacting Elav-positive cells upon ectopic expression of either *Hmgcr* or *Jhamt* using the *Elav-GAL4* driver. Bar and whiskers represent mean +/- standard deviation and statistical significance was tested by unpaired t-test. **c** *Top Left:* Scatter plots from FACS of GFP-positive PGCs from *nos-moesin::GFP* transgenic embryos. Region 1 (R1) denotes collection cutoff. *Top Right:* Image of isolated GFP-positive germ cells. *Bottom:* Schematic of *in vitro* transwell assay in which FACS-enriched PGCs are placed on a 10µm filter above either serum-free media conditioned with Kc167 cells as a positive control, serum free unconditioned media as a negative control, or experimental conditions such as serum-free media with JH III. The number of PGCs that translocated to the bottom well was quantified manually after 1.5 hours. **d** Quantification of Specific PGC migration, which is the percentage of experimental PGCs that translocate relative to positive (serum-free Kc167-conditioned media) and negative (serum-free media) controls to allow for comparison across biological replicates done on different days. See Materials and Methods for further details on assay design and derivation of percent specific migration. Numbers shown in white within each *jhamt* knockdown bar represents the Fold Change (FC) of *jhamt* mRNA at the time conditioned media was collected. Bar height represents mean and error bars represent standard deviation from two to four biological replicates and statistical significance was tested by unpaired t-test. **e** Quantification of specific migration in transwell assays using isolated PGCs placed above serum-free media containing various concentrations of commercially available JH III (green bars) or 20-hydroxyecdysone (20E, magenta bars) with 1% BSA as a non-specific carrier protein. Bars represent means and error bars represent standard deviation from three biological replicates, which were each obtained by averaging two to three technical replicates. **f** Percentage specific migration wherein isolated PGCs were placed across either serum-free media conditioned (gray), serum-free media with 1% BSA (black) and either JH III (dark green, 2.5µM) or the JH analogue, methoprene, (light green, 2.5µM) with 1% BSA as a non-specific carrier protein. Bars represent means and error bars represent standard deviation from at two biological replicates, which were each obtained by averaging two to three technical replicates.

Figure 4: JHs impact germ cell migration through cytoplasmic signaling **a** Schematic of the nuclear and cytoplasmic proteins implicated in cellular responses to extracellular JH. **b** Lateral view images of Stage 14 embryos stained for Vasa (magenta) and DAPI (gray). Maternal and zygotic genotype are noted in the top left corner. To generate maternal and zygotic mutant embryos, mutant $met^{1/27}$, $gce^{Mi/2.5k}$ mothers were crossed to $met^1, gce^{Mi}/Y$ males. Arrowheads point to extragonadal germ cells. **c** Quantification of extragonadal PGCs from Stage 14-16 embryos. The maternal and paternal genotypes in relation to met , gce are noted as + or -. Arrowheads highlight extragonadal germ cells. To yield embryos in which maternal contributions of Met and Gce were mutant, $met^{1/27}$, $gce^{Mi/2.5k}$ females were used. For maternal mutant and zygotic mutant embryos, $met^{1/27}$, $gce^{Mi/2.5k}$ females were crossed to $met^1, gce^{Mi}/Y$ males. For maternal mutant, zygotic wildtype embryos, $met^{1/27}$, $gce^{Mi/2.5k}$ females were crossed to w^{1118} males. For maternal wildtype and zygotic mutant embryos, met^{27} , $gce^{2.5k}/Fm7a$ females were crossed to $met^1, gce^{Mi}/Y$ males. **d** Top panels: schematic of genetic suppression experiment. In otherwise wild type embryos, ectopic expression of Hmgcr in the endoderm (green) using the $48Y-GAL4$; $UAS-Hmgcr$ will trap ~75% of PGCs (pink) in the gut (left schematic). To test the role of Met and Gce downstream of Hmgcr, we crossed $met^{1/27}$, $gce^{Mi/2.5k}$ females to $48Y-GAL4$; $UAS-Hmgcr$ males (right schematic) and measured how many PGCs remained trapped in the endoderm after PGC colonization of the gonad is complete. Bottom panels: Lateral view images of Stage 14 embryos stained for Vasa (magenta), the endodermal marker, Hnt (green) and the DNA stain, DAPI (gray). Bold **M**: designates the maternal genotype that PGCs primarily rely on when exiting the endoderm. Females mutant for met and gce were heteroallelic $met^{1/27}$, $gce^{Mi/2.5k}$. Bold **P**: designates the paternal genotype in which $Hmgcr$ is ectopically expressed in the endoderm using the $48Y-GAL4$ driver. White brackets indicate germ cells that remain in gut. **e** Quantification of the percentage of PGCs remaining in the gut in Stage 14+ embryos upon expression of $UAS-Hmgcr$ using the $48Y-GAL4$ driver. For panels B-E, the wild type reference strain was obtained by crossing w^{1118} to OregonR, as met and gce alleles used were generated from both of these genetic backgrounds (**Supp Table 1**). **f** Quantification of specific *in vitro* migration when isolated PGCs were treated with 1 μ M flavopiridol to inhibit transcription (Txn) or vehicle control for 30 minutes before being placed in the transwell membrane across serum-free media alone (black) or serum-free media conditioned with Kc167 (gray) or containing 5 μ M JH III (green). Bars represent means and error bars represent standard deviation from two biological replicates, which were each obtained by averaging two technical replicates. **g** Left: d/v view images of Stage 14 embryos stained for Vasa (magenta) and DAPI (gray). Maternal and zygotic genotype is noted in the top left corner.

To generate embryos maternally and zygotically depleted of Plc21c, *plc21c^{Mi/Df(2L)BSC106}* females were crossed to *plc21c^{Mi}/CyO,ft-lacZ* males. Detailed descriptions of the mutant alleles are described in Supplemental Table 1. Wild type reference strain was *w¹¹¹⁸*. Arrowheads point to extragonadal germ cells. **h** Quantification of extragonadal PGCs in Stage 14-16 embryos. Maternal and zygotic genotypes are noted below each column. **i** *Top*: Schematic of experiment to test whether the maternal loss of Plc21c suppresses the ability of ectopic endodermal Hmgcr (green) to trap PGCs (pink) in the gut. *Bottom*: Dorsal/Ventral view images of Stage 14 embryos stained for Vasa (magenta), the endodermal marker, Hnt (green) and the DNA stain, DAPI (gray). Bold **M**: designates the maternal genotype that PGCs primarily rely on when exiting the endoderm. Maternal *plc21c* mutant genotype was heteroallelic *plc21c^{Mi/Df(2L)BSC106}*. Bold **P**: designates the paternal genotype in which Hmgcr is ectopically expressed in the endoderm using the *48Y-GAL4* driver. White brackets indicate germ cells that remain in gut. **j** Quantification of the percentage of PGCs remaining in the gut in Stage 14+ embryos when Hmgcr was ectopically expressed in the developing gut using *48Y-GAL4; UAS-Hmgcr* transgenes. Wild type control is *w¹¹¹⁸*. For **c**, **e**, **h** and **j**, each dot represents one embryo. Bars and whiskers represent median +/- IQR and statistical significance was assessed by Mann-Whitney U test. ns- not significant. Dashed yellow circle highlight the gonad and scale bars represent 25µm in all images.

Figure 5: Tests of conserved impact of, and model for, isoprenoids in germ cell migration. **a** Quantification of specific *in vitro* migration wherein isolated *Drosophila* PGCs are placed in a transwell migration system across Kc167-conditioned media (gray), serum-free media (black), JH III (green, 5 μ M), all trans retinoic acid (ATRA, dark blue, 5 μ M) or 9-cis RA (light blue 5 μ M). **b** Quantification of specific *in vitro* migration wherein mouse PGCs were FACS-enriched from trunk tissues dissected from E10.5 *Oct4-GFP* embryos and placed across two positive controls: Fetal Bovine Serum (FBS) or CXCL12 (50 ng/mL) (gray), serum-free media as a negative control (black) or ATRA (dark blue, 1 μ M) or 9cis-RA (light blue, 1 μ M). For **A** and **B**, bars and error bars represent mean $\pm$ standard deviation from at least two biological replicates, which were obtained by averaging two to three technical replicates. **c** Schematic of model for how the bioactive isoprenoids, such as JH, ensure migrating PGCs (pink) colonize the somatic gonad (green). Also included is a model for what is required within PGCs to respond to JH. Purple cylinders at the plasma membrane represent an unknown JH receptor thought to link JH to Plc21c activation. Signaling from Plc21c to JH nuclear receptors (JHNR, yellow circle), Met or Gce remains to be determined but a role for transcription is not required. Model JH transcriptional target is shown within the blue nucleus, wherein the target gene is shown as a gray rectangle, the JHNR is a yellow circle and the JHNR co-factor, Taiman is shown as a red circle.

Supplemental Data for Figure 1 a Sequence of the JH response element (JHRE) from the *early trypsin* gene of *A. aegypti* that contains the JH nuclear receptor (JHNR) binding motif highlighted in bold²⁴. The six base pair wild type JHRE sequence is on top and the JHRE in which the JHNR-binding motif was mutated is on the bottom. Eight copies of each sequence were provided by Marek Jindra²³ and placed upstream of *eGFP*. **b** Dorsal/ventral (d/v) view of Stage 14 JHRE Mut8x-GFP embryo stained for GFP (green) and the hemocyte marker, Fascin (gray). Right panel is the same image but with the Fascin signal alone in grayscale. **c** Lateral view of embryos stained for Vasa (magenta), GFP (green) and DAPI (gray). Genotype is noted above each early Stage 12 and Stage 14 pair. To the right of each whole-embryo image is the area within the white, dashed box magnified. Scale bars represent 25µm in all images.

Supplemental Data for Figure 2 Nature of the small deletions in *jhamt* created by CRISPR-mediated cutting. Seven founders generated fifteen lines which possessed small insertions or deletions at the cut site that created premature stop codons at the beginning of methyl transferase domain of *jhamt* (amino acids 40-137). Class 1 lines (12F1, 12F2) cause a premature stop codon at amino acid 46, while Class II mutations (1F1, 1F2, 4F1, 4F3, 6F2, 6F3, 7F2, 7F3, 8F1, 8F2, 12F3, 14M2, 14M3) cause a premature stop codon at amino acid 54.

Supplemental Data for Figure 3 a Left: Quantification of the number of PGCs in Stage 14-16 embryos that remained in the gut upon ectopic expression of either *Hmgcr* or *jhamt* using the *48Y-GAL4* driver. **Right:** Quantification of the number of germ cell in Stage 14-16 embryos that contact Zfh-1-positive cells upon the expression of either *Hmgcr* or *jhamt* using the *twist(twi)-GAL4* and *24B-GAL4* drivers. Each dot represents one embryo. Bars and whiskers represent median +/- IQR and statistical significance was analyzed by the Mann-Whitney U test. **b** Quantification of the number of Kc167 cells that were used to condition serum-free media after *jhamt* mRNA was knocked down relative to the number of Kc167 cells in the transfection control. Bars and error bars represent mean +/- standard deviation from two biological replicates. **c** Quantification of the fold change in mRNAs in Schneider 2 (S2) cells upon incubation with varying concentrations of 20-Ecdysone (20-E) or JH III for six hours prior to mRNA isolation. mRNAs from two verified ecdysone-responsive genes were measured: *Eip74b* (lighter purple) and *Eip75* (darker purple) and one JH-responsive gene was measured, *Kr-h1* (green) and normalized to the housekeeping gene, *DCTN2-p50*. Error bars represent standard deviation from two biological replicates.

Supplemental Data for Figure 4 **a** Schematics of *met* and *gce* loci indicating the mutations that were recombined to yield *met*¹, *gce*^{Mi} alleles. **b** Quantification of fold change of *gce* and *Kr-h1* mRNAs in *met*, *gce* mutant one-day old adult females relative to wildtype control animals. deltaCt was calculated relative to the average Ct of two housekeeping genes, *DCTN5-p25* and *Und*. Shown are females homoallelic for the newly recombined mutant alleles *met*¹, *gce*^{Mi} (dark blue) and heteroallelic combination with existing or *met*²⁷, *gce*^{2.5k} alleles (light blue). Bars and error bars represent mean +/- standard deviation from three biological replicates of five females each. **c** Fecundity of young females. Each dot represents the average number of eggs laid in a 24-hour period from a cohort of 10-12 three- and four-days post eclosion females per biological replicate. To avoid male reproductive effects, all virgin females were mated to wild type, *w*¹¹¹⁸, males. Bars and whiskers represent mean +/- standard deviation, which statistical significance analyzed by unpaired t-test. **d** Images of *Drosophila* PGCs enriched by FACS and stained for Vasa (magenta), RNA Pol II, Ser5p (green), RNA Pol II, Ser2p (yellow) and DAPI (gray). Bottom panel shows a germ cell treated with 1μM Flavopiridol for 3 hours. Scale bars represent 5μm. **e** JH titers in hemolymph of second instar larvae measured by quantitative mass spectrometry in wild type and *met*¹, *gce*^{Mi} mutant larvae. Each dot represents a biological replicate of hemolymph from 25 larvae. Error bars represent standard deviation and p values are from unpaired t-tests.

Supplemental Data for Figure 5 **a** Scatter plots from FACS of GFP-positive PGCs enriched from trunks of E10.5 mouse embryos carrying the *Oct4ΔPE::GFP* reporter. The number inside the gate lines denotes the percentage of collected events in a typical biological replicate. **b** Quantification of the percent change in the number of mouse germ cells before and two hours after application of 1μM ATRA or 9cis-RA. Two hours was chosen because this is the duration of the *in vitro* migration assay. Graphed is the mean and standard deviation from three biological replicated. Each dot represents the averaged technical replicates of each biological replicate.

References

1. Richardson, B.E. & Lehmann, R. Mechanisms guiding primordial germ cell migration: strategies from different organisms. *Nat Rev Mol Cell Biol* **11**, 37-49 (2010).
2. Saitou, M., Kagiwada, S. & Kurimoto, K. Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells. *Development* **139**, 15-31 (2012).
3. Siddiqui, N.U. *et al.* Genome-wide analysis of the maternal-to-zygotic transition in *Drosophila* primordial germ cells. *Genome Biol* **13**, R11 (2012).
4. Slaidina, M. & Lehmann, R. Quantitative Differences in a Single Maternal Factor Determine Survival Probabilities among *Drosophila* Germ Cells. *Current biology : CB* **27**, 291-297 (2017).
5. Nicholls, P.K. *et al.* Mammalian germ cells are determined after PGC colonization of the nascent gonad. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 25677-25687 (2019).
6. Olmstead, A.W. & Leblanc, G.A. Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean *Daphnia magna*. *J Exp Zool* **293**, 736-739 (2002).
7. Bowles, J. *et al.* Retinoid signaling determines germ cell fate in mice. *Science* **312**, 596-600 (2006).
8. Koubova, J. *et al.* Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 2474-2479 (2006).
9. Bowles, J. *et al.* Retinoic Acid Antagonizes Testis Development in Mice. *Cell Rep* **24**, 1330-1341 (2018).
10. Miyauchi, H. *et al.* Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. *EMBO J* **36**, 3100-3119 (2017).
11. Chassot, A.A. *et al.* Retinoic acid synthesis by ALDH1A proteins is dispensable for meiosis initiation in the mouse fetal ovary. *Sci Adv* **6**, eaaz1261 (2020).
12. Vernet, N. *et al.* Meiosis occurs normally in the fetal ovary of mice lacking all retinoic acid receptors. *Sci Adv* **6** (2020).
13. Bowles, J. *et al.* ALDH1A1 provides a source of meiosis-inducing retinoic acid in mouse fetal ovaries. *Nat Commun* **7**, 10845 (2016).
14. Kumar, S. *et al.* Sex-specific timing of meiotic initiation is regulated by Cyp26b1 independent of retinoic acid signalling. *Nat Commun* **2**, 151 (2011).
15. Minkina, A. *et al.* Retinoic acid signaling is dispensable for somatic development and function in the mammalian ovary. *Developmental biology* **424**, 208-220 (2017).
16. Hartfelder, K. Insect juvenile hormone: from "status quo" to high society. *Braz J Med Biol Res* **33**, 157-177 (2000).
17. Casper, A.L. & Van Doren, M. The establishment of sexual identity in the *Drosophila* germline. *Development* **136**, 3821-3830 (2009).
18. Niederreither, K., Fraulob, V., Garnier, J.M., Chambon, P. & Dolle, P. Differential expression of retinoic acid-synthesizing (RALDH) enzymes during fetal development and organ differentiation in the mouse. *Mech Dev* **110**, 165-171 (2002).
19. Niwa, R. *et al.* Juvenile hormone acid O-methyltransferase in *Drosophila melanogaster*. *Insect Biochem Mol Biol* **38**, 714-720 (2008).

- 1151 20. Van Doren, M., Broihier, H.T., Moore, L.A. & Lehmann, R. HMG-CoA reductase guides
1152 migrating primordial germ cells. *Nature* **396**, 466-469 (1998).
- 1153 21. Riddiford, L.M. Rhodnius, Golden Oil, and Met: A History of Juvenile Hormone Research.
1154 *Front Cell Dev Biol* **8**, 679 (2020).
- 1155 22. Sanchez-Higueras, C. & Hombria, J.C. Precise long-range migration results from short-
1156 range stepwise migration during ring gland organogenesis. *Developmental biology* **414**,
1157 45-57 (2016).
- 1158 23. Jindra, M., Uhlirova, M., Charles, J.P., Smykal, V. & Hill, R.J. Genetic Evidence for
1159 Function of the bHLH-PAS Protein Gce/Met As a Juvenile Hormone Receptor. *PLoS Genet*
1160 **11**, e1005394 (2015).
- 1161 24. Noriega, F.G., Shah, D.K. & Wells, M.A. Juvenile hormone controls early trypsin gene
1162 transcription in the midgut of *Aedes aegypti*. *Insect Mol Biol* **6**, 63-66 (1997).
- 1163 25. Ramirez, C.E., Nouzova, M., Michalkova, V., Fernandez-Lima, F. & Noriega, F.G. Common
1164 structural features facilitate the simultaneous identification and quantification of the
1165 five most common juvenile hormones by liquid chromatography-tandem mass
1166 spectrometry. *Insect Biochem Mol Biol* **116**, 103287 (2020).
- 1167 26. Wen, D. *et al.* Methyl farnesoate plays a dual role in regulating *Drosophila*
1168 metamorphosis. *PLoS Genet* **11**, e1005038 (2015).
- 1169 27. Kenwrick, K., Mukherjee, A. & Renault, A.D. Hmgcr promotes a long-range signal to
1170 attract *Drosophila* germ cells independently of Hedgehog. *J Cell Sci* **132** (2019).
- 1171 28. Goldstein, J.L. & Brown, M.S. Regulation of the mevalonate pathway. *Nature* **343**, 425-
1172 430 (1990).
- 1173 29. Ricardo, S. & Lehmann, R. An ABC transporter controls export of a *Drosophila* germ cell
1174 attractant. *Science* **323**, 943-946 (2009).
- 1175 30. Bai, J., Uehara, Y. & Montell, D.J. Regulation of invasive cell behavior by taiman, a
1176 *Drosophila* protein related to AIB1, a steroid receptor coactivator amplified in breast
1177 cancer. *Cell* **103**, 1047-1058 (2000).
- 1178 31. Banisch, T.U. *et al.* A transitory signaling center controls timing of primordial germ cell
1179 differentiation. *Dev Cell* **56**, 1742-1755 e1744 (2021).
- 1180 32. Abdou, M.A. *et al.* *Drosophila* Met and Gce are partially redundant in transducing
1181 juvenile hormone action. *Insect Biochem Mol Biol* **41**, 938-945 (2011).
- 1182 33. Wilson, T.G., Wang, S., Beno, M. & Farkas, R. Wide mutational spectrum of a gene
1183 involved in hormone action and insecticide resistance in *Drosophila melanogaster*. *Mol*
1184 *Genet Genomics* **276**, 294-303 (2006).
- 1185 34. Bensaude, O. Inhibiting eukaryotic transcription: Which compound to choose? How to
1186 evaluate its activity? *Transcription* **2**, 103-108 (2011).
- 1187 35. Yamamoto, K., Chadarevian, A. & Pellegrini, M. Juvenile hormone action mediated in
1188 male accessory glands of *Drosophila* by calcium and kinase C. *Science* **239**, 916-919
1189 (1988).
- 1190 36. Pszczolkowski, M.A., Peterson, A., Srinivasan, A. & Ramaswamy, S.B. Pharmacological
1191 analysis of ovarian patency in *Heliothis virescens*. *J Insect Physiol* **51**, 445-453 (2005).
- 1192 37. Pszczolkowski, M.A., Olson, E., Rhine, C. & Ramaswamy, S.B. Role for calcium in the
1193 development of ovarian patency in *Heliothis virescens*. *J Insect Physiol* **54**, 358-366
1194 (2008).

- 1195 38. Liu, P., Peng, H.J. & Zhu, J. Juvenile hormone-activated phospholipase C pathway
1196 enhances transcriptional activation by the methoprene-tolerant protein. *Proceedings of*
1197 *the National Academy of Sciences of the United States of America* **112**, E1871-1879
1198 (2015).
- 1199 39. Ojani, R., Liu, P., Fu, X. & Zhu, J. Protein kinase C modulates transcriptional activation by
1200 the juvenile hormone receptor methoprene-tolerant. *Insect Biochem Mol Biol* **70**, 44-52
1201 (2016).
- 1202 40. Cai, M.J. *et al.* Juvenile hormone prevents 20-hydroxyecdysone-induced metamorphosis
1203 by regulating the phosphorylation of a newly identified broad protein. *The Journal of*
1204 *biological chemistry* **289**, 26630-26641 (2014).
- 1205 41. Jing, Y.P., An, H., Zhang, S., Wang, N. & Zhou, S. Protein kinase C mediates juvenile
1206 hormone-dependent phosphorylation of Na(+)/K(+)-ATPase to induce ovarian follicular
1207 patency for yolk protein uptake. *The Journal of biological chemistry* **293**, 20112-20122
1208 (2018).
- 1209 42. Moore, L.A., Broihier, H.T., Van Doren, M., Lunsford, L.B. & Lehmann, R. Identification of
1210 genes controlling germ cell migration and embryonic gonad formation in *Drosophila*.
1211 *Development* **125**, 667-678 (1998).
- 1212 43. Harmon, M.A., Boehm, M.F., Heyman, R.A. & Mangelsdorf, D.J. Activation of mammalian
1213 retinoid X receptors by the insect growth regulator methoprene. *Proceedings of the*
1214 *National Academy of Sciences of the United States of America* **92**, 6157-6160 (1995).
- 1215 44. Knaut, H., Werz, C., Geisler, R., Nusslein-Volhard, C. & Tübingen Screen, C. A zebrafish
1216 homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. *Nature*
1217 **421**, 279-282 (2003).
- 1218 45. Doitsidou, M. *et al.* Guidance of primordial germ cell migration by the chemokine SDF-1.
1219 *Cell* **111**, 647-659 (2002).
- 1220 46. Molyneaux, K.A. *et al.* The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate
1221 mouse germ cell migration and survival. *Development* **130**, 4279-4286 (2003).
- 1222 47. Koshimizu, U., Watanabe, M. & Nakatsuji, N. Retinoic acid is a potent growth activator
1223 of mouse primordial germ cells in vitro. *Developmental biology* **168**, 683-685 (1995).
- 1224 48. Ashok, M., Turner, C. & Wilson, T.G. Insect juvenile hormone resistance gene homology
1225 with the bHLH-PAS family of transcriptional regulators. *Proceedings of the National*
1226 *Academy of Sciences of the United States of America* **95**, 2761-2766 (1998).
- 1227 49. Tomancak, P. *et al.* Systematic determination of patterns of gene expression during
1228 *Drosophila* embryogenesis. *Genome Biol* **3**, RESEARCH0088 (2002).
- 1229 50. Rahman, M.M., Franch-Marro, X., Maestro, J.L., Martin, D. & Casali, A. Local Juvenile
1230 Hormone activity regulates gut homeostasis and tumor growth in adult *Drosophila*. *Sci*
1231 *Rep* **7**, 11677 (2017).
- 1232 51. Luo, W. *et al.* Dual roles of juvenile hormone signaling during early oogenesis in
1233 *Drosophila*. *Insect Sci* **27**, 665-674 (2020).
- 1234 52. Weaver, L.N., Ma, T. & Drummond-Barbosa, D. Analysis of Gal4 Expression Patterns in
1235 Adult *Drosophila* Females. *G3 (Bethesda)* **10**, 4147-4158 (2020).
- 1236 53. Ilenchuk, T.T. & Davey, K.G. The Development of Responsiveness to Juvenile-Hormone
1237 in the Follicle Cells of *Rhodnius-Prolixus*. *Insect Biochemistry* **17**, 525-529 (1987).

- 1238 54. Yamamoto, K., Chadarevian, A. & Pellegrini, M. Juvenile-Hormone Action Mediated in
1239 Male Accessory-Glands of Drosophila by Calcium and Kinase-C. *Science* **239**, 916-919
1240 (1988).
- 1241 55. Sevala, V.L. & Davey, K.G. Action of Juvenile-Hormone on the Follicle Cells of Rhodnius-
1242 Prolixus - Evidence for a Novel Regulatory Mechanism Involving Protein Kinase-C.
1243 *Experientia* **45**, 355-356 (1989).
- 1244 56. Bai, H. & Palli, S.R. Identification of G protein-coupled receptors required for vitellogenin
1245 uptake into the oocytes of the red flour beetle, Tribolium castaneum. *Sci Rep* **6**, 27648
1246 (2016).
- 1247 57. Gancz, D., Lengil, T. & Gilboa, L. Coordinated regulation of niche and stem cell
1248 precursors by hormonal signaling. *PLoS biology* **9**, e1001202 (2011).
- 1249 58. Kozlova, T. & Thummel, C.S. Essential roles for ecdysone signaling during Drosophila
1250 mid-embryonic development. *Science* **301**, 1911-1914 (2003).
- 1251 59. Gilboa, L. & Lehmann, R. Soma-germline interactions coordinate homeostasis and
1252 growth in the Drosophila gonad. *Nature* **443**, 97-100 (2006).
- 1253 60. Arora, R. *et al.* Meiotic onset is reliant on spatial distribution but independent of germ
1254 cell number in the mouse ovary. *J Cell Sci* **129**, 2493-2499 (2016).
- 1255 61. Yao, H.H., DiNapoli, L. & Capel, B. Meiotic germ cells antagonize mesonephric cell
1256 migration and testis cord formation in mouse gonads. *Development* **130**, 5895-5902
1257 (2003).
- 1258 62. Iwata, M. *et al.* Retinoic acid imprints gut-homing specificity on T cells. *Immunity* **21**,
1259 527-538 (2004).
- 1260 63. Kim, M.H., Taparowsky, E.J. & Kim, C.H. Retinoic Acid Differentially Regulates the
1261 Migration of Innate Lymphoid Cell Subsets to the Gut. *Immunity* **43**, 107-119 (2015).
- 1262 64. Al Tanoury, Z., Piskunov, A. & Rochette-Egly, C. Vitamin A and retinoid signaling:
1263 genomic and nongenomic effects. *J Lipid Res* **54**, 1761-1775 (2013).
- 1264 65. Rand, C.D., Spencer, G.E. & Carlone, R.L. Retinoic acid as a chemoattractant for cultured
1265 embryonic spinal cord neurons of the African Clawed Frog, Xenopus laevis. *Can J Zool*
1266 **95**, 653-661 (2017).
- 1267 66. Farrar, N.R., Dmetrichuk, J.M., Carlone, R.L. & Spencer, G.E. A novel, nongenomic
1268 mechanism underlies retinoic acid-induced growth cone turning. *J Neurosci* **29**, 14136-
1269 14142 (2009).
- 1270 67. Vesprini, N.D., Dawson, T.F., Yuan, Y., Bruce, D. & Spencer, G.E. Retinoic acid affects
1271 calcium signaling in adult molluscan neurons. *J Neurophysiol* **113**, 172-181 (2015).
- 1272 68. Johnson, A., Nasser, T.I.N. & Spencer, G.E. Inhibition of Rho GTPases in Invertebrate
1273 Growth Cones Induces a Switch in Responsiveness to Retinoic Acid. *Biomolecules* **9**
1274 (2019).
- 1275 69. de Hoog, E., Lukewich, M.K. & Spencer, G.E. Retinoid receptor-based signaling plays a
1276 role in voltage-dependent inhibition of invertebrate voltage-gated Ca²⁺ channels.
1277 *Journal of Biological Chemistry* **294**, 10076-10093 (2019).
- 1278 70. Farini, D., La Sala, G., Tedesco, M. & De Felici, M. Chemoattractant action and molecular
1279 signaling pathways of Kit ligand on mouse primordial germ cells. *Developmental biology*
1280 **306**, 572-583 (2007).

71. Laird, D.J., Altshuler-Keylin, S., Kissner, M.D., Zhou, X. & Anderson, K.V. Ror2 enhances polarity and directional migration of primordial germ cells. *PLoS Genet* **7**, e1002428 (2011).
72. Damdimopoulou, P., Chiang, C. & Flaws, J.A. Retinoic acid signaling in ovarian folliculogenesis and steroidogenesis. *Reprod Toxicol* **87**, 32-41 (2019).
73. Santos, C.G., Humann, F.C. & Hartfelder, K. Juvenile hormone signaling in insect oogenesis. *Curr Opin Insect Sci* **31**, 43-48 (2019).
74. Toyota, K. *et al.* Methyl farnesoate synthesis is necessary for the environmental sex determination in the water flea *Daphnia pulex*. *Journal of Insect Physiology* **80**, 22-30 (2015).
75. Vernet, N. *et al.* Meiosis occurs normally in the fetal ovary of mice lacking all retinoic acid receptors. *Science Advances* **6** (2020).
76. Yoshimizu, T. *et al.* Germline-specific expression of the Oct-4/green fluorescent protein (GFP) transgene in mice. *Dev Growth Differ* **41**, 675-684 (1999).
77. Restifo, L.L. & Wilson, T.G. A juvenile hormone agonist reveals distinct developmental pathways mediated by ecdysone-inducible broad complex transcription factors. *Dev Genet* **22**, 141-159 (1998).
78. Lecuyer, E., Necakov, A.S., Caceres, L. & Krause, H.M. High-resolution fluorescent in situ hybridization of *Drosophila* embryos and tissues. *CSH Protoc* **2008**, pdb prot5019 (2008).
79. Livak, K.J. & Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408 (2001).
80. Celniker, S.E. *et al.* Unlocking the secrets of the genome. *Nature* **459**, 927-930 (2009).
81. Barton, L.J. *et al.* Nuclear lamina dysfunction triggers a germline stem cell checkpoint. *Nat Commun* **9**, 3960 (2018).
82. Wilson, T.G. & Fabian, J. A *Drosophila melanogaster* mutant resistant to a chemical analog of juvenile hormone. *Developmental biology* **118**, 190-201 (1986).
83. Baumann, A.A. *et al.* Genetic tools to study juvenile hormone action in *Drosophila*. *Sci Rep* **7**, 2132 (2017).
84. Venken, K.J. *et al.* MiMIC: a highly versatile transposon insertion resource for engineering *Drosophila melanogaster* genes. *Nat Methods* **8**, 737-743 (2011).
85. Hu, Q., Velez-Aviles, A.N. & Wolfner, M.F. *Drosophila* Plc21C is involved in calcium wave propagation during egg activation. *MicroPubl Biol* **2020** (2020).
86. Sano, H., Renault, A.D. & Lehmann, R. Control of lateral migration and germ cell elimination by the *Drosophila melanogaster* lipid phosphate phosphatases Wunen and Wunen 2. *The Journal of cell biology* **171**, 675-683 (2005).

Figure 1

Juvenile hormone signaling is present during embryonic germ cell development

Figure 2
Key JH biosynthesis enzyme, Jhamt, facilitates germ cell migration to the somatic gonad

Figure 3

Juvenile hormone is sufficient for germ cell migration

Figure 4

JHs impact germ cell migration through cytoplasmic signaling

Figure 5
Tests of conserved impact of, and model for, isoprenoids in germ cell migration

Supplementary Figure 1

a

JHRE Wt8x - 5' ccattccca**CACGCG**aagacgataaaaacca 3'
JHRE Mut8x - 5' ccattccca**GTGCGC**aagacgataaaaacca 3'

b

c

Supplementary Figure 2

Deletions in *jhamt* caused by guide: CCTGGA | TGTGGTTCAGGATCT

Class I: frameshift leading to premature stop codon at a.a. 46

Lines with 5 bp deletion to generate TC | TGT: 12F1, 12F2

Class II: frameshift leading to premature stop codon at a.a. 54

Lines with T deletion to generate GGA | GT: 1F1, 1F2, 4F3, 6F2, 6F3, 7F2, 7F3, 8F1, 8F2, 12F3

Lines with A deletion to generate GG | TGT: 14M2, 14M3

Lines with TCC insertion and G deletion to generate GGA | TTCCGT: 4F1

Supplementary Figure 3

Supplementary Figure 4

Supplementary Figure 5