insomniac links the development and function of a sleep-regulatory circuit

Qiuling Li¹, Hyunsoo Jang†, Kayla Y Lim‡, Alexie Lessing¹, Nicholas Stavropoulos¹,2*

¹Neuroscience Institute, Department of Neuroscience and Physiology, New York University School of Medicine, New York, United States; ²Waksman Institute, Rutgers University, Piscataway, United States

Abstract Although many genes are known to influence sleep, when and how they impact sleep-regulatory circuits remain ill-defined. Here, we show that insomniac (inc), a conserved adaptor for the autism-associated Cul3 ubiquitin ligase, acts in a restricted period of neuronal development to impact sleep in adult Drosophila. The loss of inc causes structural and functional alterations within the mushroom body (MB), a center for sensory integration, associative learning, and sleep regulation. In inc mutants, MB neurons are produced in excess, develop anatomical defects that impede circuit assembly, and are unable to promote sleep when activated in adulthood. Our findings link neurogenesis and postmitotic development of sleep-regulatory neurons to their adult function and suggest that developmental perturbations of circuits that couple sensory inputs and sleep may underlie sleep dysfunction in neurodevelopmental disorders.

Editor’s evaluation This is an interesting study showing that the short sleep phenotype of inc mutants in Drosophila depends on the loss of the gene at a specific developmental time, and in a specific region, the mushroom bodies (MB). There are very few studies assessing the effects of sleep during development, in any animal species, and thus this paper is a very welcomed addition. The experiments are carefully done, and the conclusions are warranted.

Introduction A central goal of sleep research has been elucidating the mechanisms by which genes shape normal sleep patterns and cause sleep disorders. While numerous genes that strongly impact sleep have been identified in humans and in animals ranging from mammals to invertebrates (Chemelli et al., 1999; Chiu et al., 2016; Cirelli et al., 2005; Funato et al., 2016; He et al., 2009; Lin et al., 1999; Raizen et al., 2008), when these genes act to influence sleep is in many cases unresolved. Genes that act in the adult brain to modulate the activity of sleep-regulatory circuits in an ongoing manner have been intensively investigated (e.g. Chemelli et al., 1999; Lin et al., 1999), including with conditional gain-of-function, loss-of-function, and rescue in adult animals (Chiu et al., 2016; Clasadonte et al., 2017; Foltenyi et al., 2007; Guo et al., 2011; Ishimoto and Kitamoto, 2010; Joiner et al., 2006; Van Buskirk and Sternberg, 2007). In contrast, despite great progress in understanding neuronal development (Doe, 2008; Jessell and Sanes, 2000; Sanes and Zipursky, 2020; Tessier-Lavigne and Goodman, 1996; Weinstein and Hemmati-Brivanlou, 1999), developmental mechanisms by which genes influence sleep remain poorly explored, despite the likely relevance of such mechanisms to sleep disturbances in autism and other neurodevelopmental disorders (Angriman et al., 2015; Souders et al., 2017). Notably, the temporal contributions of genes that impact sleep are rarely
assessed in a comprehensive manner, and a further challenge has been linking particular genes to developmental processes that control the structure and function of discrete sleep-regulatory circuits.

Here, we assess the temporal contributions of insomniac (inc), a gene whose mutation sharply curtails sleep in Drosophila (Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011). Pan-neuronal depletion of inc causes short sleep, while restoring inc solely to neurons is largely sufficient to rescue the sleep deficits of inc mutants, indicating that inc impacts sleep chiefly through neurons (Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011). inc is expressed in the larval, pupal, and adult brain (Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011), but when inc acts to influence sleep remains uncertain (Li and Stavropoulos, 2016; Pfeiffenberger and Allada, 2012). inc encodes an adaptor for the Cul3 ubiquitin ligase (Li et al., 2019), which, like inc, is required in neurons for normal sleep (Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011). Both inc and Cul3 are highly conserved, and mammalian inc orthologs restore sleep to inc mutants (Li et al., 2017), suggesting that functions and substrates of inc are conserved in mammals. Human Cul3 mutations are implicated as a cause of autism and its associated sleep dysfunction (Codina-Solà et al., 2015; Kong et al., 2012; O’Roak et al., 2012), but the underlying mechanisms are unknown. Studies of inc may thus reveal fundamental and conserved mechanisms underlying sleep regulation which are altered in sleep disorders.

Using conditional genetic manipulations of inc, we show that inc acts transiently in developing neurons to impact sleep in adulthood. We furthermore identify developmental defects in inc mutants within the mushroom body (MB), a brain structure that integrates sensory stimuli and regulates sleep. Loss of inc alters MB neurogenesis, causing the overproduction of late-born neurons and changes

Figure 1. Expression of 3×FLAG-inc driven by inc-Gal4 in the larval, pupal, and adult brain. Maximal projections are shown for male inc-Gal4; UAS-3×FLAG-inc/+ brains stained with anti-FLAG. For larval brain, projection from a partial z-stack is shown to allow visualization of signal in mushroom body projections (arrowheads). In pupae and adults, signal is prominent in the mushroom body, pars intercerebralis, fan-shaped body, and ellipsoid body. Scale bars, 100 μm.
in postmitotic development that impair the assembly of MB circuits. These developmental alterations persist into adulthood and are associated with specific deficits in the ability of MB neurons to promote sleep in inc adults, in contrast to the anatomy and function of other sleep-regulatory circuits which remain intact. Together, these results elucidate an unexpected mechanism by which inc shapes the development and function of sleep-regulatory neurons to exert a lasting impact on sleep–wake behavior. Our findings additionally suggest that developmental alterations of neurogenesis and within brain centers that integrate sensory inputs may contribute to sleep dysfunction in autism and other neurodevelopmental disorders.

**Results**

**inc acts transiently during a restricted developmental period to impact sleep in adulthood**

inc impacts sleep through neurons and is expressed in the developing and adult brain (Figure 1; Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011). To assess the temporal mechanisms by which inc impacts sleep, we manipulated inc expression in neurons using the ligand-inducible Q-system (Potter et al., 2010; Riabinina et al., 2015). The Q-system circumvents nonspecific perturbations of sleep caused by other inducible systems and allows constitutive, developmental, and adult manipulations of sleep (Li and Stavropoulos, 2016). We performed a series of conditional rescue experiments in short-sleeping inc1 null mutants bearing a UAS-inc-HA transgene whose expression is induced in neurons by the Q-system upon exposure to quinic acid (Figure 2A). Animals exposed to vehicle throughout development and adulthood slept indistinguishably from inc1 mutants, while animals exposed constitutively to quinic acid exhibited strongly rescued sleep (Figure 2B, C; Figure 2—figure supplement 1), consistent with the rescue conferred by constitutive neuronal expression of inc (Pfeiffenberger and Allada, 2012; Stavropoulos and Young, 2011). Anti-HA staining of brains confirmed that the Q-system controlled inc expression as expected: vehicle-fed animals lacked inc-HA signal, while those exposed constitutively to quinic acid expressed inc-HA in the larval, pupal, and adult brain (Figure 2D). We next asked whether inc influences sleep through adult-specific or developmental mechanisms. Animals fed quinic acid in adulthood expressed inc-HA in the adult brain but exhibited no rescue of their sleep deficits (Figure 2B–D; Figure 2—figure supplement 1). In stark contrast, developmental induction of inc-HA from embryonic through pupal stages restored sleep to near wild-type levels (Figure 2B–D; Figure 2—figure supplement 1). These findings indicate that inc is dispensable in adult neurons and acts instead during neuronal development to ultimately impact sleep–wake behavior.

We further defined the developmental period in which inc functions, using more precise temporal manipulations. Neuronal induction of inc-HA from the late third instar larval stage through adulthood strongly rescued the inc sleep phenotype (Figure 2B–D; Figure 2—figure supplement 1), indicating that inc is dispensable in embryonic and early larval neurons. Induction of inc activity solely in late third instar larval and pupal neurons, using a pulse of quinic acid exposure (Figure 2D), restored sleep indistinguishably from constitutive neuronal induction (Figure 2B, C; Figure 2—figure supplement 1). The sleep deficits of inc2 animals, which bear an independent inc null allele that can be reverted by Gal4 (Stavropoulos and Young, 2011), were similarly rescued by this pulse of quinic acid (Figure 3A–C; Figure 3—figure supplement 1), confirming that inc activity in this developmental period is sufficient to restore sleep to inc mutants. We next assessed whether inc is required in late third instar larval and pupal neurons for normal sleep in adulthood, by using the Q-system to induce a pulse of inc RNAi. This manipulation markedly decreased sleep (Figure 3D, E; Figure 3—figure supplement 2). Together, these findings indicate that inc acts transiently in neurons of late third instar larvae and pupae to influence adult sleep–wake behavior. During these developmental stages, many neurons of the adult brain are born and assemble into circuits (Truman and Bate, 1988; White and Kankel, 1978).

**inc has a critical function in the MB that impacts sleep**

To identify neurons that might underlie the developmental impact of inc on sleep, we performed a rescue screen in inc2 mutants. We screened 277 Gal4 lines expressed in sleep-regulatory circuits or randomly selected populations of cells in the brain and identified two drivers, c253-Gal4 and c309-Gal4, that rescued sleep similarly to the pan-neuronal nsyb-Gal4 driver (Figure 4A). After backcrossing
Research article

Developmental Biology | Neuroscience

Li et al. eLife 2021;0:e65437. DOI: https://doi.org/10.7554/eLife.65437 4 of 42

Figure 2. inc acts in a restricted period of neuronal development to impact sleep in adulthood. (A) Conditional rescue of inc mutants using the ligand-inducible Q-system. Quinic acid relieves QS suppression of the pan-neuronally expressed Gal4QF transcriptional activator, inducing UAS-inc-HA in neurons. (B) Total sleep duration of controls (gray) and inc; UAS-inc-HA/tub-QS; nSyb-Gal4QF/+ animals exposed to quinic acid (+) or vehicle (−) at indicated life stages; embryos (E), larval stages (1–3), pupae (P), and adults (A). Bars represent mean ± standard error of the mean (SEM). n = 11–86. One-way analysis of variance (ANOVA) (F(7,397) = 86.73, p < 0.0001) and Tukey post hoc tests, *p < 0.01 for comparisons to inc; UAS-inc-HA/+.

(C) Average sleep profiles of flies in (B), with induction regimens indicated below. Shading indicates ± SEM. (D) Anti-HA staining of inc; UAS-inc-HA/tub-QS; nSyb-Gal4QF/+ brains from indicated induction regimens. Scale bars, 100 μm.

The online version of this article includes the following figure supplement(s) for figure 2:

Figure supplement 1. Additional sleep parameters for conditional rescue of inc mutants using the Q-system.

to an isogenic background, both drivers retained their ability to rescue most of the sleep phenotypes of inc mutants (Figure 4B, C; Figure 4—figure supplement 1). In late third instar larvae and adults, c253-Gal4 and c309-Gal4 are strongly expressed in the MB (Figure 4D), a structure important for sensory integration, associative learning, and sleep regulation (Heisenberg, 2003; Joiner et al., 2006; Pitman et al., 2006). Because c253-Gal4 and c309-Gal4 are also expressed outside of the MB, we used independent genetic manipulations to confirm that inc acts in the MB to influence sleep. inc-Gal4, a driver that bears inc regulatory sequences and fully rescues inc mutants when used to restore inc activity (Li et al., 2017; Stavropoulos and Young, 2011), is expressed in the larval, pupal, and adult MB (Figure 1). We tested whether the rescue conferred by inc-Gal4 was altered by MB-Gal80, a Gal4 suppressor expressed in MB neurons during development and adulthood (Krashes et al., 2007;
Figure 3. Conditional rescue of inc2 mutants and conditional inc RNAi in larval and pupal neurons. (A) Conditional neuronal rescue of inc2 mutants using the ligand-inducible Q-system. inc2 mutants contain a transposon insertion in the inc 5′UTR immediately upstream of the endogenous start codon. A UAS/TATA element within the transposon terminus permits Gal4-dependent restoration of inc expression (Stavropoulos and Young, 2011). (B) Total sleep duration in inc2; tub-QS/+; nysb-Gal4QF/+ animals exposed to vehicle or quinic acid at the late third instar larval and pupal stages. n = 20–83. One-way analysis of variance (ANOVA) (F3, 170) = 70.66, p > 0.0001) and Tukey post hoc tests, *p < 0.01 for comparisons to inc2. (C) Average sleep profiles of indicated genotypes from (B). (D) Total sleep duration in tub-QS/UAS-inc-RNAi; nysb-Gal4QF/UAS-dcr2 animals exposed to vehicle or quinic acid at the late third instar larval and pupal stages. n = 16–24. Student’s t-test, *p < 0.01 for comparison to vehicle-treated control. (E) Average sleep profiles of animals from (D). For (B) and (D), bars represent mean ± SEM. For (C) and (E), shading represents ± SEM.

The online version of this article includes the following figure supplement(s) for figure 3:

Figure supplement 1. Additional sleep parameters for conditional inc2 rescue in third instar larval and pupal neurons.

Figure supplement 2. Additional sleep parameters for conditional inc RNAi in larval and pupal neurons.
MB-Gal80 partially suppressed the ability of inc-Gal4 to restore sleep to inc̅ mutants, indicating that while inc does not influence sleep solely through the MB, inc is required in MB neurons for normal sleep regulation (Figure 4E, F; Figure 4—figure supplement 2).

Loss of inc abolishes the sleep-promoting functions of MB neurons but spares the functions of other sleep-regulatory circuits

While different circuits within the MB can promote or inhibit sleep upon activation (Joiner et al., 2006; Pitman et al., 2006; Sitaraman et al., 2015a), ablation of the MB strongly reduces sleep (Joiner et al., 2006; Pitman et al., 2006), suggesting that the integrated activity of the MB is sleep-promoting. To assess whether the sleep-regulatory functions of the MB are altered in inc mutants, we activated MB neurons in adult wild-type and inc̅ flies using the dTrpA1 heat-activated cation channel (Hamada et al., 2008). Wild-type control flies lacking Gal4 drivers exhibited no change in total sleep when shifted to 28.5°C for 24 hr, while inc̅ flies lacking Gal4 drivers exhibited decreased sleep at this temperature (Figure 5A, B), suggesting that inc mutants are hyperarousable by thermal stimuli, as for mechanical stimuli (Pfeiffenberger and Allada, 2012). Activation of neurons expressing TrpA1 under
the control of c253-Gal4 or c309-Gal4 strongly increased sleep in wild-type animals (Figure 5A, B; Figure 5—figure supplement 1), consistent with observations that inactivating synaptic output using the same drivers promotes wakefulness (Pitman et al., 2006). Because c253-Gal4 and c309-Gal4 are expressed in some cells outside of the MB, we also assessed a split-Gal4 driver expressed specifically in MB neurons (Figure 5—figure supplement 2). Using this driver to express TrpA1 and activate MB neurons increased sleep in wild-type animals (Figure 5A, B, 'pan-MB'). Strikingly, using the same three drivers to activate neurons in inc1 mutants elicited no significant changes in sleep compared to inc1; UAS-TrpA1/+ controls (Figure 5A, B; Figure 5—figure supplement 1), indicating that the sleep-promoting effects of MB activation are abolished in inc mutants.

To test whether the loss of inc specifically impairs the sleep-regulatory functions of MB neurons or causes more general deficits in sleep regulation, we assessed other neuronal populations that influence sleep. Activation of sleep-promoting populations that include ellipsoid body R5 (EB) (Liu et al., 2016) or Dorsal Paired Medial (DPM) neurons (Haynes et al., 2015) increased sleep similarly in
wild-type and inc' animals (Figure 5A, B; Figure 5—figure supplement 1). Conversely, activation of sleep-inhibiting populations that include Helicon (Donlea et al., 2018), l-LN v (Sheeba et al., 2008), or pars intercerebralis and dopaminergic PPM3 neurons (PI, PPM3) (Dubowy et al., 2016) strongly decreased sleep in wild-type and inc' animals (Figure 5A, B; Figure 5—figure supplement 1). The functions of these populations thus appear to be intact in inc mutants, suggesting that the loss of inc specifically impairs the sleep-regulatory functions of MB neurons. These findings, together with the developmental time-of-action of inc and its requirement within the MB for normal sleep, suggest that inc acts developmentally in MB neurons to have a lasting impact on their sleep-regulatory functions in adulthood.

**inc regulates the production and anatomy of late-born MB neurons**

During the critical developmental period through which inc impacts sleep, MB neurons are born and assemble into adult circuits (Ito and Hotta, 1992; Lee et al., 1999). In each brain hemisphere, four MB neuroblasts proliferate to yield ~2000 neurons comprising seven sequentially born subtypes (γd, γm, α/βw, α/βm, α/βr, α/βp, and α/βc) that project axons into distinct lobes (γ, α/βw, and α/β) (Aso et al., 2014a; Ito et al., 1997; Ito and Hotta, 1992; Kurusu et al., 2002; Lee and Luo, 1999; Tanaka et al., 2008; Truman and Bate, 1988; Zhu et al., 2003). Chemical ablation of the MB by exposing first instar larvae to hydroxyurea, an inhibitor of DNA replication, causes sleep deficits in adulthood (Joiner et al., 2006; Pitman et al., 2006). The sleep deficits caused by MB ablation are similar to but less severe than those of inc mutants, including reductions in sleep across the day and decreased sleep consolidation (Figure 6A–F). These findings and the partial suppression of inc rescue by MB-Gal80
DH44\(^+\) neurons, were unchanged in numbers of other sleep-regulatory neurons, including those of the dorsal fan-shaped body (dFB) and dendrites of other sleep-regulatory circuits, including those of the dFB, CRZ\(^+\) neurons, and PDF\(^+\) and this variation was greatest for \(\beta/\beta^\prime\) neurons, the last-born in the MB (\(\alpha^\prime\) mutants exhibited an average of nearly seven clusters (control, 3.7 ± 0.2; \(\alpha^\prime\)). Mutations expanded dendritic volume for \(\alpha^\prime/\beta^\prime\) neurons occupied enlarged territories in \(\alpha^\prime\) brains, axons of last-born \(\alpha/\beta\) neurons were present from control animals, reflecting their birth from four MB neuroblasts (Ito et al., 1997; Ito and Hotta, 1992; Truman and Bate, 1988), whereas \(\alpha^1\) mutants exhibited an average of nearly seven clusters (control, 3.7 ± 0.2; \(\alpha^1\), 6.8 ± 0.6) (Figure 7A, D; Figure 7—figure supplement 1), suggesting an origin from aberrant or excess neuroblasts. The numbers of other sleep-regulatory neurons, including those of the dorsal fan-shaped body (dFB) and DH44\(^+\) neurons, were unchanged in \(\alpha^1\) mutants (Figure 7A, I), indicating that neuronal overproduction in \(\alpha^1\) is specific to the MB or manifests preferentially within this neuronal lineage. These findings indicate that \(\alpha^1\) regulates neurogenesis, a fundamental process regulated by proteins conserved from flies to mammals (Doe, 2008; Knoblich, 2008), and suggest that alterations in early nervous system development can exert a lasting impact on sleep.

To further assess MB anatomy in \(\alpha^1\) mutants, we examined axons marked by myr-GFP and separately examined dendrites by expressing DenMark (Nicolaï et al., 2010). Axons of embryonic-born \(\gamma_d\) neurons exhibited no obvious changes in \(\alpha^1\) mutants (Figure 7A, H). In contrast, axons of larval- and pupal-born MB neurons exhibited morphological defects whose severity correlated with neuronal overproduction and birth order (Figure 7A, H). While \(\alpha/\beta\) axons were absent from MB lobes in a minority (10%) of \(\alpha^1\) brains, axons of \(\alpha/\beta\) neurons, the penultimate to be born, were missing from MB lobes in 53% of \(\alpha^1\) brains (1.07 ± 0.33 missing lobes per brain) (Figure 7H). Axons of last-born \(\alpha/\beta\) neurons showed the most severe defects; they failed to project into lobes in 86% of \(\alpha^1\) brains (2.23 ± 0.3 missing lobes per brain), fasciculated from ectopic neuronal clusters, and often aggregated near the peduncle (Figure 7A, H; Figure 7—figure supplement 1). The dendrites of \(\gamma_d\), \(\alpha/\beta\), and \(\alpha/\beta^\prime\) neurons occupied enlarged territories in \(\alpha^1\) mutants but otherwise appeared normal (Figure 7F, G). Expansions in dendritic volume for \(\alpha/\beta\) and \(\alpha/\beta^\prime\) subtypes paralleled increases in the numbers of these neurons (Figure 7A, B), while increases for \(\gamma_d\) dendrites occurred independently of neuron number, consistent with functions of \(\alpha^1\) in postmitotic \(\gamma_d\) neurons or non-cell autonomous mechanisms. Axons and dendrites of other sleep-regulatory circuits, including those of the dFB, CRZ\(^+\) neurons, and PDF\(^+\) circadian pacemaker neurons, exhibited no obvious changes in \(\alpha^1\) mutants (Figure 7I; Figure 7—figure supplement 2), suggesting that alterations of neuronal anatomy in \(\alpha^1\) mutants are specific to the MB. These findings indicate that increases in the numbers of late-born MB neurons in \(\alpha^1\) mutants are associated with changes in postmitotic development expected to perturb circuit assembly and function. In particular, the altered axons of multiple MB neuron subtypes are unlikely to form normal circuits with their targets that influence sleep, including dopaminergic neurons, MB output neurons, and recurrent connections to the MB (Aso et al., 2014b; Sitaraman et al., 2015a; Sitaraman et al., 2015b).

Discussion

Here, we have used temporally restricted genetic manipulations to show that \(\alpha^1\) acts during neuronal development to ultimately impact sleep in adulthood. While many genes are known to act in adults to impact sleep, developmental mechanisms underlying sleep regulation have only recently gained attention (Chakravarti Dilley et al., 2020; Gong et al., 2021; Iwasaki et al., 2021; Xie et al., 2019).
Figure 7. inc regulates neurogenesis and anatomy of late-born mushroom body (MB) neurons. (A) Adult control and inc brains expressing UAS-MyrGFP-2A-RedStinger in indicated MB neuron subtypes, stained with anti-GFP (cyan) and anti-dsRed (yellow). (B) MB neuron number per hemisphere. γd, n = 10–11; α/β′, n = 7–10; α/βc, n = 16–18. *p < 0.01, Welch’s t-test. (C) Absolute difference in MB neuron number between left and right brain hemispheres; γd, n = 5–6; α/β′, n = 3–5; α/βc, n = 8–9. *p < 0.01, Welch’s t-test. (D) Number of α/βc, neuron clusters per hemisphere. n = 16–18. *p < 0.01, Welch’s t-test. (E) Numbers of dorsal fan-shaped body (dFB) and DH44+ neurons. dFB, n = 26; DH44+, n = 6–8. ns, p > 0.01, Welch’s t-test. (F) Adult control and inc brains expressing UAS-DenMark-smGdP-V5 in indicated MB neuron subtypes, stained with anti-GFP. (G) Dendrite volume per hemisphere. γd, n = 16–17; α/β′, n = 19; α/βc, n = 14–16. *p < 0.01, Welch’s t-test. (H) Quantification of axonal projection defects for MB neuron subtypes. Colored bars represent the number of MB lobes in each brain entirely lacking axonal myrGFP signal. See also panel (A). n = 10–25. (I) Adult control and inc brains expressing UAS-MyrGFP-2A-RedStinger in dFB neurons. All scale bars represent 100 μm. For (B–E) and (G), bars represent mean ± SEM.

The online version of this article includes the following figure supplement(s) for figure 7:

**Figure supplement 1.** Analysis of α/β neuron clusters and projections.

**Figure supplement 2.** Analysis of dendrites in additional sleep-regulatory circuits.
Our results underscore the importance of unbiased temporal genetic manipulations to define critical periods through which genes impact sleep, and suggest that genes may influence sleep through unappreciated developmental mechanisms. A clear implication of these findings is that variations in human sleep patterns, including pathological disruptions of sleep, may have a developmental origin.

Reciprocal conditional manipulations have been critical in revealing surprising developmental and adult contributions of genes to neuronal function and behavior. In one notable example, anxiety-like behaviors in mice caused by mutations of the 5-HT1A serotonin receptor were found to be rescued by developmental expression of the receptor (Gross et al., 2002). Withdrawal of receptor expression in adulthood had no measurable consequences on anxiety-like behavior, and adult-specific receptor expression failed to provide rescue, indicating the necessity and sufficiency of the receptor during development (Gross et al., 2002). A second noteworthy example is provided by a mouse model of Rett syndrome, a neurodevelopmental disorder caused by mutation of MECP2, a transcriptional regulator. Conditional MeCP2 expression solely in adulthood was found to be sufficient to rescue mutant phenotypes, indicating a critical period for MeCP2 function in adults rather than during brain development (Guy et al., 2007; Guy et al., 2012). Inactivation of MECP2 specifically in adulthood causes MECP2 mutant phenotypes (McGraw et al., 2011), confirming its adult requirement. By analogy, various genes that influence sleep might act developmentally or in adulthood in a manner that cannot be anticipated in the absence of conditional manipulations.

inc activity is required in neurons for normal sleep, and conversely, restoring inc solely to neurons is largely sufficient to rescue the short sleep of inc mutants (Pfeifferberger and Allada, 2012; Stavropoulos and Young, 2011). Our conditional neuronal manipulations of inc span embryonic development through adulthood and indicate that inc expression in neurons of late third instar larvae and pupae is sufficient to rescue sleep in inc mutants to near wild-type levels, indistinguishable from the rescue provided by constitutive neuronal inc expression (Pfeifferberger and Allada, 2012; Stavropoulos and Young, 2011). Extending this developmental pulse of neuronal inc expression into adulthood does not augment the rescue of inc sleep phenotypes, nor does expressing inc only in adult neurons restore sleep to inc animals. inc expression in embryonic, early larval, and adult neurons thus appears dispensable for normal sleep. Instead, inc is required at a time coincident with the birth and development of many adult neurons, including those of the MB (Ito and Hotta, 1992; Lee et al., 1999; White and Kankel, 1978). While our findings suggest that the MB is not the sole brain structure through which inc impacts sleep, they establish a vital role for inc in regulating MB development and its sleep-regulatory functions.

Our findings reveal that inc governs neurogenesis, a fundamental process regulated by genes and pathways conserved from flies to mammals (Doe, 2008; Knoblich, 2008), and suggest that alterations of neurogenesis can cause lasting changes in sleep–wake behavior. The cellular and molecular mechanisms underlying altered neurogenesis in inc mutants, including the stochastic nature of these phenotypes and their apparent restriction to the MB, are of particular interest. inc null mutations are viable (Stavropoulos and Young, 2011), in contrast to the lethality of mutations that globally alter neurogenesis (Betschinger et al., 2006; Lee et al., 2006a; Lee et al., 2006b; Rolls et al., 2003; Vaessin et al., 1991), consistent with the notion that altered neurogenesis in inc mutants manifests preferentially or specifically within the MB. The stochastic nature of neurogenic defects in inc mutants and the overproduction of neurons with projection defects are reminiscent of phenotypes of mushroom body defect (mud) mutants (Guan et al., 2000; Hovhanyan and Raabe, 2009; Prokop and Technau, 1994). In mud mutants, infrequent errors in asymmetric neuroblast division give rise to excess neuroblasts and MB neurons (Bowman et al., 2006; Siller et al., 2006). Similar alterations in neuroblast proliferation in inc mutants may account for the stochastic and cumulative defects in the production of late-born MB neurons; a subtle defect in neuroblast proliferation would be expected to manifest particularly in the MB lineage, the longest in the fly brain. Our results do not yet distinguish the cellular populations through which inc regulates neurogenesis. One possibility is that inc acts in neurons to promote their differentiation, analogous to lola and midlife crisis, genes whose absence causes neurons to dedifferentiate and acquire the proliferative character of neuroblasts (Carney et al., 2013; Southall et al., 2014). Another possibility is that inc functions in neuroblasts, like mud, to govern their asymmetric division.

Our studies and recent findings (Gong et al., 2021) suggest that proper regulation of neurogenesis is essential for normal sleep and that altered neurogenesis in discrete circuits can cause lifelong sleep...
dysfunction. Intriguing but fragmentary evidence suggests that other genes whose mutation impacts sleep might similarly alter neurogenesis. wide awake (wake), whose mutation causes short sleep in Drosophila (Liu et al., 2014; Zhang et al., 2015), was characterized in an independent study as banderuola (bnd) and shown to regulate the asymmetric division of neuroblasts (Mauri et al., 2014). An interesting possibility yet to be assessed is whether sleep phenotypes of wake/bnd mutants might arise developmentally or through neuroblasts. Similarly, while short sleep phenotypes caused by mutations in the potassium channel subunits encoded by Shaker and Hyperkinetic (Bushey et al., 2007; Cirelli et al., 2005) are thought to reflect their role in regulating excitability in specific adult neurons (Kempf et al., 2019; Pimentel et al., 2016), developmental functions that could contribute to their impact on sleep remain unexplored. Notably, mutations in the Shaker ortholog Kv1.1 analogous to those that strongly reduce sleep in Drosophila (Cirelli et al., 2005; Gisselmann et al., 1989) cause megencephaly and neuronal overproduction in mammals, implicating Kv1.1 in regulating neurogenesis (Chou et al., 2021; Donahue et al., 1996; Petersson et al., 2003; Yang et al., 2012). Explicit tests of whether wake/bnd and Shaker impact sleep through adult or developmental mechanisms, or through a combination of the two, await conditional temporal analysis.

While further manipulations of inc are required to elucidate the precise developmental mechanisms by which it impacts sleep, Cul3 is known to regulate various aspects of neuronal development. Clonal analysis of Cul3 mutations in Drosophila indicates that Cul3 is required for normal axonal arborization and dendritic elaboration within the MB, as well as axonal fasciculation (Zhu et al., 2005). These phenotypes overlap those of inc mutants, although direct comparisons are complicated by the pleiotropic nature of Cul3 mutations, which dysregulate multiple adaptor and substrate pathways. Mosaic analysis of inc is required to discern its developmental functions in postmitotic neurons, to compare its phenotypes with Cul3, and to distinguish cell autonomous and non-cell autonomous mechanisms. In mammals, Cul3 mutations alter neurogenesis, cortical lamination, neuronal migration, synaptic development, and cause behavioral deficits (Amar et al., 2021; Dong et al., 2020; Fischer et al., 2020; Rapanelli et al., 2021). inc and Cul3 are present at synapses in flies and mammals (Kikuma et al., 2019; Li et al., 2017) and are required at the Drosophila larval neuromuscular junction for synaptic homeostasis (Kikuma et al., 2019), a process proposed to be a core function of sleep (Tononi and Cirelli, 2003). The impact of inc on the development and function of central synapses has yet to be assessed, and whether such functions contribute to inc sleep phenotypes remains unknown. As a Cul3 adaptor, inc may engage multiple molecular targets and cellular pathways. Identifying and manipulating inc substrates are thus important goals in elucidating the mechanisms through which inc impacts neuronal development and sleep–wake behavior.

The loss of inc causes enduring developmental and functional impairments in the MB, a structure important for sensory integration, learning, and sleep regulation. The MB integrates olfactory (de Belle and Heisenberg, 1994; Heisenberg et al., 1985), gustatory (Keene and Masek, 2012; Masek and Scott, 2010), visual (Li et al., 2020; Vogt et al., 2016), and thermal inputs (Frank et al., 2015; Hong et al., 2008; Shih et al., 2015), and its activity is altered by sleep pressure (Bushey et al., 2015; Sitaraman et al., 2015a). The MB may thus integrate and filter sensory stimuli to promote sleep in appropriate environmental conditions, in a manner modulated by learning and sleep history. The anatomical defects in inc mutants may render the MB hypersensitive to sensory stimuli, alter functions of the MB that link learning and sleep (Berry et al., 2015; Cervantes-Sandoval et al., 2017; Haynes et al., 2015; Seugnet et al., 2011; Seugnet et al., 2008), or impair the relay of sensory input from MB neurons to downstream sleep-promoting circuits (Aso et al., 2014b; Sitaraman et al., 2015a). While MB circuits and genetic pathways that act in the MB to influence sleep have been manipulated with increasing precision (Aso et al., 2014b; Cavanaugh et al., 2016; Guo et al., 2011; Joiner et al., 2006; Pitman et al., 2006; Sitaraman et al., 2015a; Sitaraman et al., 2015b; Yi et al., 2013), much remains unknown about the function of the MB in sleep regulation, and additional analysis is required to elucidate how inc lesions might alter discrete circuits within the MB and signaling to their targets.

While sensory hypersensitivity and sleep dysfunction are hallmarks of autism and other neurodevelopmental disorders, the underlying mechanisms remain obscure. Given the conserved functions of Cul3–inc complexes and the associations of Cul3 lesions with autism (Kong et al., 2012; Li et al., 2017; O’Roak et al., 2012), elucidating inc substrates and their contributions to neurogenesis and neuronal anatomy may provide insights into brain development, tumorigenesis, and sleep disorders.
## Materials and methods

### Key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Antibody                          | α-HA (rat monoclonal) | Roche | Cat# 11867431001, RRID:AB_390919 | (1:100) |
| Antibody                          | α-Brp (mouse monoclonal) | DSHB | Cat# nc82, RRID:AB_2314866 | (1:20 and 1:50) |
| Antibody                          | α-FLAG (mouse monoclonal) | Sigma-Aldrich | Cat# F1804, RRID:AB_262044 | (1:100) |
| Antibody                          | α-GFP (mouse monoclonal) | DSHB | Cat# GFP-G1, RRID:AB_2619561 | (1:1000) |
| Antibody                          | α-GFP (rabbit polyclonal) | Thermo Fisher Scientific | Cat# A11122, RRID:AB_221569 | (1:2000) |
| Antibody                          | α-dsRed (rabbit polyclonal) | Takara Bio | Cat# 632496, RRID:AB_10013483 | (1:1000) |
| Antibody                          | α-FasII (mouse monoclonal) | DSHB | Cat# 8 C6, RRID:AB_2314391 | (1:50) |
| Antibody                          | α-mouse Alexa Fluor 488 (donkey polyclonal) | Thermo Fisher Scientific | Cat# A21202, RRID:AB_141607 | (1:1000) |
| Antibody                          | α-rabbit Alexa Fluor 488 (donkey polyclonal) | Thermo Fisher Scientific | Cat# A21206, RRID:AB_2535792 | (1:1000) |
| Antibody                          | α-rat Alexa Fluor 488 (donkey polyclonal) | Thermo Fisher Scientific | Cat# A21208, RRID:AB_2535794 | (1:1000) |
| Antibody                          | α-rabbit Alexa Fluor 568 (donkey polyclonal) | Thermo Fisher Scientific | Cat# A10042, RRID:AB_2534017 | (1:1000) |
| Antibody                          | α-mouse Alexa Fluor 647 (donkey polyclonal) | Thermo Fisher Scientific | Cat# A31571, RRID:AB_162542 | (1:1000) |
| Chemical compound, drug          | Hydroxyurea | Sigma-Aldrich | HB627 | |
| Genetic reagent (D. melanogaster) | w1118        | Bloomington Drosophila Stock Center | RRID:BDSC_5905 | Ryder et al., 2004 |
| Genetic reagent (D. melanogaster) | inc2         | Stavropoulos lab | FLYB:FBal0266013 | Stavropoulos and Young, 2011; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | inc2         | Stavropoulos lab | FLYB:FBal0162225 | Stavropoulos and Young, 2011; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | tub-QS; nsyb-Gal4QF | Christopher Potter | | Riabinina et al., 2015; Li and Stavropoulos, 2016; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | inc-Gal4     | Stavropoulos lab | | Stavropoulos and Young, 2011; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | inc’inc-Gal4 | Stavropoulos lab | | Li et al., 2017; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | nsyb-Gal4    | Julie Simpson | | Simpson, 2016; BDSC #5,905 background |
| Genetic reagent (D. melanogaster) | c253-Gal4 (MB) | Bloomington Drosophila Stock Center | RRID:BDSC_6980 | Pitman et al., 2006; BDSC #5,905 background; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | c309-Gal4 (MB) | Bloomington Drosophila Stock Center | RRID:BDSC_6906 | Connolly et al., 1996; Pitman et al., 2006; Joiner et al., 2006; Aso et al., 2009; BDSC #5,905 background; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | c929-Gal4 (I-LNv) | Amita Sehgal | | \(\text{Hewes et al., 2000; Hewes et al., 2003; Sheeba et al., 2008; Parisky et al., 2008; Shang et al., 2008; iso31 background; used in inc[2] rescue screen}\) |
| Genetic reagent (D. melanogaster) | c584-Gal4 (P1, PPM3) | Amita Sehgal | | \(\text{Martin et al., 1999; Dubowy et al., 2016; iso31 background; used in inc[2] rescue screen}\) |
| Genetic reagent (D. melanogaster) | R69F08-Gal4 (EB) | Mark Wu | | \(\text{Liu et al., 2016; used in inc[2] rescue screen}\) |
| Genetic reagent (D. melanogaster) | R24B11-Gal4 (Helicon) | Bloomington Drosophila Stock Center | RRID:BDSC.49070 | \(\text{Donlea et al., 2018}\) |
| Genetic reagent (D. melanogaster) | R23E10-Gal4 (dFB) | Bloomington Drosophila Stock Center | RRID:BDSC.49032 | \(\text{Donlea et al., 2014}\) |
| Genetic reagent (D. melanogaster) | NP2721-Gal4 (DPM) | Leslie Griffith | | \(\text{Wu et al., 2011; Haynes et al., 2015; used in inc[2] rescue screen}\) |
| Genetic reagent (D. melanogaster) | DH44-Gal4 | Bloomington Drosophila Stock Center | RRID:BDSC.39347 | \(\text{Cavanaugh et al., 2014}\) |
| Genetic reagent (D. melanogaster) | pdf-Gal4 | Stavropoulos lab | | \(\text{Renn et al., 1999}\) |
| Genetic reagent (D. melanogaster) | crz-Gal4 | Stavropoulos lab | | \(\text{Taylor et al., 2012}\) |
| Genetic reagent (D. melanogaster) | MB004B (pan-MB) | Yoshinori Aso | | \(\text{Sitaraman et al., 2015a}\) |
| Genetic reagent (D. melanogaster) | MB607B (\(\gamma d\)) | Yoshinori Aso | | \(\text{Sitaraman et al., 2015a}\) |
| Genetic reagent (D. melanogaster) | MB370B (\(\alpha'\beta', \alpha'\beta'\alpha\)) | Yoshinori Aso | | \(\text{Sitaraman et al., 2015a}\) |
| Genetic reagent (D. melanogaster) | MB185B (\(\alpha\beta\)) | Yoshinori Aso | | \(\text{Sitaraman et al., 2015a}\) |
| Genetic reagent (D. melanogaster) | MB594B (\(\alpha\beta\gamma\)) | Yoshinori Aso | | \(\text{Sitaraman et al., 2015a}\) |
| Genetic reagent (D. melanogaster) | MB-Gal80 | Michael Young | | \(\text{Krashes et al., 2007}\) |
| Genetic reagent (D. melanogaster) | UAS-3xFLAG-Inc | Stavropoulos lab | | \(\text{Li et al., 2017; BDSC #5,905 background}\) |
| Genetic reagent (D. melanogaster) | UAS-inc-HA | Stavropoulos lab | | \(\text{Li et al., 2017; BDSC #5,905 background}\) |
| Genetic reagent (D. melanogaster) | UAS-inc-RNAi | Vienna Drosophila Resource Center | FLYB:FBst0453067 | \(\text{Dietzl et al., 2007, Stavropoulos and Young, 2011}\) |
| Genetic reagent (D. melanogaster) | UAS-dcr2 | Bloomington Drosophila Stock Center | RRID:BDSC.24651 | \(\text{Dietzl et al., 2007; BDSC #5,905 background}\) |
| Genetic reagent (D. melanogaster) | UAS-TrpA1 | Stavropoulos lab | | \(\text{Hamada et al., 2008; BDSC #5,905 background}\) |
| Genetic reagent (D. melanogaster) | UAS-MyrGFP-2A-RedStinger | Barry Ganetzky | | \(\text{Daniels et al., 2014}\) |
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster)  | 5xUAS-DenMark::smGdP-V5 | Bloomington Drosophila Stock Center | RRID:BDSC_62138 | Nern et al., 2015 |
| Genetic reagent (D. melanogaster)  | 5xUAS-IVS-Syt1::smGdP-HA | Bloomington Drosophila Stock Center | RRID:BDSC_62142 | Nern et al., 2015 |
| Genetic reagent (D. melanogaster)  | 20xUAS-IVS-CDB-GFP | Bloomington Drosophila Stock Center | RRID:BDSC_32194 | Pfeiffer et al., 2010 |
| Genetic reagent (D. melanogaster)  | NP1227-Ga4 | Kathy Nagel |  | Okada et al., 2009; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | R2-Split Ga4 | Greg Suh |  | Liu et al., 2016; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | R72G06-Ga4 | Mark Wu |  | used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | VT64246-Ga4 | Leslie Griffith |  | used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | c305a-Ga4 | Leslie Griffith |  | used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR49E09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38692 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR49F01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38694 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR49F02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38695 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR49G06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38707 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR51G05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38797 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR53B06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38863 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR53C04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_38871 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR54F06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39081 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR55A03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39095 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR55B12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39103 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P(GMR55D01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39110 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster)  | P{GMR55D05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39112 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR55F07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39128 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR55G11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39132 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR56H02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39164 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR56H09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39166 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR58E10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39184 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR58H05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39198 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR59B10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39209 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR59E09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39220 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR59H05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39229 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR60C01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39240 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR60D05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39247 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR60H12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39268 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR64A11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39289 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR64F03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39309 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR64G05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39316 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster)  | P{GMR65B04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39336 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P{GMR65D06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39352 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR65D07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39353 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR67A04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39396 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR69C02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39483 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR71D01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39579 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR72H03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39799 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR74H01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39872 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR76F06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39937 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR77H03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39976 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR78A01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_39985 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR78G06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40013 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR79A01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40021 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR79B08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40029 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR83H01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40368 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR85C07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40422 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR87A08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40473 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR92G09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40629 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P{GMR93C06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40647 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR93G05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40662 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR93H07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40669 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR94D04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40681 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR94E07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40688 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR94F06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40694 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR95E08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40710 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR95F11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_40714 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR40B09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41235 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR40E08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41238 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR41G11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41244 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR42F06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41253 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR60D10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41284 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR65C03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41290 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR74B11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41301 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR87B02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41316 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR65B09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_41353 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|--------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P{GMR34C12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45219 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR45D10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45323 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR60G12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45360 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR23G07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45493 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR26C01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45518 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR48D06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45774 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR20E01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45837 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR25G01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_45851 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR53G07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46041 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR55G02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46070 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR35H03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46205 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR46H09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46275 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR58G05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46410 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR59H01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46423 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR64D08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46539 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR65C05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46554 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR65H08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46566 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|----------------------------------|-------------|---------------------|------------|------------------------|
| Genetic reagent (D. melanogaster) | P(GMR69H02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46620 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR70G11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46641 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR71E04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46658 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR72A04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46665 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR73D06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46692 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR56F05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46714 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR77A04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_46976 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR80C12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47059 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR81C04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47087 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR81D04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47094 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR91A08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47148 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR91G01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47175 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR92H11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47211 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR93B04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47215 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR93D01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47221 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR93D06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47224 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR93G11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47238 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P{GMR94H10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47268 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR16D12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47325 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR16H05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47327 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR10E03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47447 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR42E09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47589 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR52A01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47634 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR70A09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47720 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR72F10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47731 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR74G04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47742 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR10A11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47839 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR10A12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47840 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR13C06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47860 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR19G10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47887 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR21C11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47898 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR30F07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47911 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR44G12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47933 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR52F09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_47943 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P(GMR28F06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48083 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR33H11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48119 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR50A07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48179 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR51B08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48183 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR52C05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48190 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR54H12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48205 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR59B11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48213 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR59C12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48219 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR59E04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48221 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR10D10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48261 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR10H09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48277 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR67B06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48294 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR73H09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48318 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR87C01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48389 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR89C02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48404 | Jenett et al., 2012, used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR92A08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48414 | Jenett et al., 2012, used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) P{GMR93C08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48417 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR93F02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48422 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR95F03-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48433 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR10E07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48440 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR11C07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48448 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR12B10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48490 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR12D12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48506 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR12G09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48525 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR13B10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48548 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR13D09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48561 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR13E04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48565 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR13E06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48566 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR13F04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48573 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR14C08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48606 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR20F01-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48610 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR14E05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48642 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) P{GMR14E06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48643 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
### Reagent type (species) or resource

| Designation                     | Source or reference          | Identifiers       | Additional information                          |
|---------------------------------|-----------------------------|-------------------|------------------------------------------------|
| P{GMR14E09-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48645   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR14E12-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48647   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR14F11-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48653   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR14G08-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48661   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR14H02-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48664   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR15B07-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48678   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR15D11-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48690   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR15E09-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48696   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR16E03-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48727   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR17B12-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48752   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR17D02-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48764   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR17G05-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48782   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR18D04-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48811   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR18D07-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48813   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR18F04-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48820   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR18G06-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48826   | Jenett et al., 2012; used in inc2 rescue screen |
| P{GMR19F05-GAL4}attP2          | Bloomington Drosophila Stock Center | RRID:BDSC_48855   | Jenett et al., 2012; used in inc2 rescue screen |

*Continued on next page*
| Reagent type (species) | Designation | Source or reference | Identifiers | Additional information |
|------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P{GMR20F04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48904 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR21C09-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48936 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR21D02-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48939 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR21D06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48942 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR22C12-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48978 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR22E06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_48986 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR22H10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49005 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR23B04-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49016 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR23C06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49023 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR23C10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49032 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR23F05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49035 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24A08-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49058 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24B11-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49070 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24C06-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49073 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24C07-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49074 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24C10-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49075 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P{GMR24E05-GAL4}attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49081 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | (GMR24F03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49086 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR24H03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49098 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25A01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49102 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25A06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49105 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25C01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49115 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25C03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49117 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25E04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49125 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR25H06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49144 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26B04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49158 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26B11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49164 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26B12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49165 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26C11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49171 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26E02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49179 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26E07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49182 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR26F09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49194 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR27A02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49207 | Jenett et al., 2012; used in inc2 rescue screen |
| Genetic reagent (D. melanogaster) | (GMR10E06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49236 | Jenett et al., 2012; used in inc2 rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P(GMR14B11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49255 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR15B03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49261 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR18G02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49278 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR32D08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49357 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR35F09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49371 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR60F05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49405 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR28E01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49457 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR29A12-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49478 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR30B10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49522 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR43D09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49553 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR47E07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49568 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48D07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49572 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR52F11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49579 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR59A05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49593 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR65H10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49614 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR66A03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49615 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR30G03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49646 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P(GMR31F06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49684 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR31G04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49686 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR31H05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49692 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR32E04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49717 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR33H07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49740 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR34B11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49774 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR34C08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49780 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR35B08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49818 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR35D07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49908 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|----------------------------------|-------------|---------------------|-------------|------------------------|
| Genetic reagent (D. melanogaster) | P(GMR37E08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49958 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR37F05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49961 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR38A11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49980 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR38B06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_49986 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR38E08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50008 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR39C07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50039 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR39E10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50053 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR39G09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50064 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR40C07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50080 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR42D11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50156 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR44B03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50200 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR44B10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50202 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR44D02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50205 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR45D05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50227 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR45G01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50241 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR45G05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50243 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR45H11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50248 | Jenett et al., 2012; used in inc[2] rescue screen |

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|----------------------------------|-------------|-------------------|------------|-----------------------|
| Genetic reagent (D. melanogaster) | P(GMR46B05-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50253 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR47D07-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50304 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR47F04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50319 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR47G08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50328 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR47H01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50330 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48A03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50339 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48A08-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50341 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48B10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50352 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48C06-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50357 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48E02-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50367 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48G01-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50381 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48G04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50383 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48H04-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50392 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48H10-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50395 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR48H11-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50396 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR49A09-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50403 | Jenett et al., 2012; used in inc[2] rescue screen |
| Genetic reagent (D. melanogaster) | P(GMR49C03-GAL4)attP2 | Bloomington Drosophila Stock Center | RRID:BDSC_50414 | Jenett et al., 2012; used in inc[2] rescue screen |

**Fly food and culture**

Fly food was prepared in batches containing the following ingredients: 1800 g cornmeal (Labscientific, FLY-8010-20), 1800 ml molasses (Labscientific, FLY-8008-16), 744 g yeast (Labscientific, FLY-8040-20F),
Developmental Biology | Neuroscience

To prepare food for conditional induction of the Q-system, solid fly food was melted in a microwave oven and allowed to cool before addition of quinic acid or vehicle. Quinic acid solution was freshly prepared essentially as described (Riabinina et al., 2015). 10 g of quinic acid (Sigma, 138622) was dissolved in 30 ml of water and the pH was adjusted to 6.5 with 10 mM NaOH. A volume of quinic acid solution containing the equivalent of 0.66 g of quinic acid (~2.4 ml) was added for each 10 ml of melted fly food and mixed well; ~12.4 ml was distributed to each empty vial. Food was allowed to cool and subsequently stored at 4°C prior to use. Vehicle food was prepared similarly, substituting an equal volume of water.

**Conditional Q-system induction**

Three sets of conditional induction experiments were performed. The first set contained vehicle treatment and constitutive, developmental-specific, and adult-specific induction regimens. The second set included vehicle, constitutive induction, and induction from the late third instar larval stage through adulthood. The third set included vehicle, constitutive induction, and a pulse of induction from the late third instar larval stage through pupal stages. Initiation, maintenance, or termination of induction at desired developmental stages was achieved by transferring larvae, pupae, and/or adults to food containing quinic acid or vehicle as described below. Within each set of experiments, w^{1118} and inc1 controls were exposed to vehicle and quinic acid induction regimens, and all animals underwent the same physical transfers in parallel. Sleep of w^{1118} and inc1 animals was not altered by exposure to vehicle or quinic acid, as described previously (Li and Stavropoulos, 2016), nor by physical transfer at larval, pupal, or adult stages. Vehicle-treated w^{1118} and inc1 animals, pooled across all three sets of experiments, are shown in Figure 2B. Two to three independent biological replications were performed for all induction experiments.

In the first set of experiments, developmental-specific induction was achieved by setting crosses on food containing quinic acid, allowing animals to develop and pupate in the same vials, and transferring adult males within 2–3 hr of eclosion to fresh vials with vehicle-containing food to terminate Q-system induction. Adult animals were maintained in these vials for 3–4 days, anesthetized with CO2, and transferred to DAM tubes with vehicle-containing food for measurement of sleep. For adult-specific induction, crosses were set on vehicle food and animals developed in the same vials. Adult males eclosing from these cultures were transferred within 2–3 hr of eclosion to fresh vials with food containing quinic acid, maintained on this food for 3–4 days, and transferred to DAM tubes containing food with quinic acid for measurement of sleep. For constitutive induction and vehicle treatment, food containing quinic acid or vehicle, respectively, was used throughout, along with the same transfer procedure.

In the second set of experiments, induction from the late third instar larval stage through adulthood was achieved as follows: crosses were set on vehicle-containing food and wandering third instar larvae from these cultures were gently collected with blunt forceps and examined under brief phosphate-buffered saline (PBS) immersion to select males by visual identification of gonads as described (Kerkis, 1931). Larvae were transferred to recipient vials containing isogenic w^{1118} larvae and pre-churned quinic acid food; these recipient cultures were initiated in parallel with experimental crosses to allow food consistency to be maintained during Q-system induction. Adult animals bearing mini-white-marked transgenes were transferred within 2–3 hr of eclosion to fresh vials containing quinic acid food to maintain Q-system induction. Three- to four-day-old adults were subsequently transferred to DAM tubes with food containing quinic acid for measurement of sleep. Constitutive induction and vehicle treatment were performed similarly, using appropriate food and the same transfer procedure.

In the third set of experiments, a pulse of Q-system induction specific to late third instar larval and pupal stages was achieved as follows: crosses were set on vehicle food and male wandering third instar larval progeny were selected and transferred to w^{1118} recipient vials containing pre-churned quinic acid food as described above. To prevent adult exposure to quinic acid, pupae bearing mini-white-marked transgenes were identified at approximately the P13–P14 stage by pigmented eyes and
black wings (Ashburner et al., 2005; Bainbridge and Bownes, 1981) and gently dislodged from vial walls with a paintbrush and transferred to the walls of fresh vials containing vehicle food. Three- to four-day-old adults eclosing from these vials were transferred to DAM tubes containing vehicle food for measurements of sleep. Constitutive induction and vehicle treatment were performed similarly, using appropriate food and the same transfer procedure.

**inc2 rescue screen**

*inc2* virgins were crossed to male flies carrying Gal4 transgenes and a minimum of five male progeny were screened for each genotype. A total of 277 Gal4 lines were screened, including 266 randomly selected drivers and 11 drivers previously characterized for expression in sleep-regulatory circuits. To select random lines, 4088 lines from the FlyLight collection available from the Bloomington Drosophila Stock Center were assigned sample numbers. Using the *randperm* command in Matlab, 300 lines were randomly selected. Expression patterns for these lines in the Janelia Flylight database were examined; 84 lines were excluded due to very low levels of expression, very broad expression patterns unlikely to be useful for functional mapping, or because expression data were unavailable. Expression patterns for the remaining 216 lines ranged from broad to sparse. This procedure for random selection was applied iteratively to yield 266 lines. Top-ranking hits from the initial screen were rescreened in independent crosses. Rescreening of *c253-Gal4* and *c309-Gal4* was performed after backcrossing each line six generations to an isogenic *w1118* stock (BDSC #5905) (Ryder et al., 2004).

**MB ablation**

MB ablation was performed essentially as described previously (de Belle and Heisenberg, 1994). Egg collection was performed on grape juice agar plates containing a spot of rehydrated dry yeast. *w1118* larvae at the first instar stage were transferred to a well of a 24-well plate bearing a spot of rehydrated dry yeast paste, containing water vehicle or 50 mg/ml hydroxyurea (Sigma, H8627). After 4–5 hr, larvae were collected and washed briefly with distilled water on a Nitex mesh filter (Genesee Scientific, 57–102) to remove yeast and subsequently transferred to vials containing standard food. Vials were cultured at 25°C in LD cycles and adult animals eclosing from these cultures were assayed for sleep as described above. MB ablation was verified in adult brains in a separate cohort of animals by staining with anti-FasII primary antibody (1:50, DSHB) and Alexa 488-conjugated donkey anti-mouse secondary as described below. Vehicle-treated animals exhibited MB lobes demarcated with FasII signal (100%, n = 9), while hydroxyurea-treated animals exhibited complete MB ablation as indicated by the lack of residual FasII staining (100%, n = 12); FasII signal within the EB was observed in all brains, providing a control for staining of the MB.

**Immunohistochemistry**

All fixing, washing, and incubation steps for immunohistochemistry were performed on a nutator. To assess conditional induction of *inc-HA* using the Q-system, larval, pupal, and adult brains were dissected from *inc1*; *UAS-inc-HA/tub-QS; nsyb-Gal4QF/+* males. Wandering third instar male larvae were selected by visual identification of gonads as described above. Larval brains were dissected in ice-cold PBS, fixed with 4% paraformaldehyde in PBS for 30 min at room temperature, and washed 3× 15 min in PBS containing 0.2% Triton X-100 (PBST). Male pupae at stage P13–P14 were identified by the staging criteria described above and the presence of sex combs. Pupal brains were dissected in ice-cold PBST, fixed with 4% paraformaldehyde in PBST for 30 min at room temperature, and washed 3× 15 min in PBST. To prepare adult brains, 2- or 4-day-old whole male adults were fixed with 4% paraformaldehyde in PBST for 3 hr at 4°C and washed 3× 15 min in PBST at room temperature prior to brain dissection in PBST. After dissection, all brains were blocked with 5% normal donkey serum (NDS) (Lampire Biological, 7332500) in PBST at room temperature for 30–60 min. Samples were incubated overnight at 4°C in rat anti-HA (1:100; Sigma, 11867431001) and mouse anti-Brp (1:20, DSHB, nc82) antibodies prepared in 5% NDS in PBST. Brains were subsequently washed 3× 15 min in PBST at room temperature, incubated overnight at 4°C in Alexa 488 donkey anti-rat (1:1000; Life Technologies, A21208) and Alexa 647 donkey anti-mouse (1:1000, Life Technologies A31571) antibodies prepared in 5% NDS in PBST, washed 3× 15 min at room temperature in PBST, and mounted on microscope slides (Fisher, 1255015) in Vectashield (Vector Labs, H-1000).
For all other immunohistochemistry, adult brains of 4-day-old males were dissected, fixed with 4% paraformaldehyde in PBST, and washed 3× 20 min in PBST at room temperature. Brains of male wandering third instar larvae and stage P13–P14 pupae were dissected, fixed, and stained as described above for Q-system experiments. Primary antibodies were mouse anti-FLAG (1:100; Sigma, F1804), rabbit anti-GFP (1:2000; Fisher, A11122), mouse anti-GFP (1:1000, DSHB, GFP-G1), rabbit anti-dsRed (1:1000; Takara, 632496), and mouse anti-Brp (1:50, DSHB, nc82). Secondary antibodies were Alexa 488 donkey anti-rabbit (1:1000; Life Technologies, A21206), Alexa 488 donkey anti-mouse (1:1000; Life Technologies, A21202), and Alexa 568 donkey anti-rabbit (1:1000; Life Technologies, A10042).

Imaging and quantitation of neuron number and cluster number

All imaging was performed on a Zeiss LSM800 confocal microscope, using a 10X air objective to capture z-stacks at 512 × 512 pixel resolution with 1 μM z-slices, unless indicated otherwise. All imaging settings were identical for each experiment comprising control and experimental brains stained in parallel.

To quantify MB neuron numbers, wild-type and inc brains expressing UAS-MyrGFP-2A-RedStinger under the control of split-Gal4 drivers were imaged as described above. For each neuron subtype, wild-type and inc brains were assigned sample numbers and a subset, randomly selected using the randperm command in Matlab, was imaged at higher resolution with a 63X oil objective. Both hemispheres of brains were imaged, capturing dsRed and myr-GFP channels separately. Only a single hemisphere could be imaged for two wild-type brains, one each in the γα and αβ′ groups, due to sample compression by the objective. z-stacks encompassing nuclei were captured at 512 × 512 resolution for γα neurons and at 1024 × 1024 resolution for αβ′ and αβc neurons; 2 μM z-slices were used to ensure that all nuclei (diameter ~3 μM) were segmented in at least one optical section.

High resolution z-stacks were assigned a random letter code and neurons were counted in a single-blind manner by two independent experimenters. Nuclei of γα and αβ′ neurons exhibited minimal overlap along the z-axis, allowing nuclei to be counted in maximum intensity z-projections using the Cell Counter plug-in in ImageJ; visual inspection of z-stacks in parallel allowed overlapping nuclei to be differentiated. Dense distribution of αβ′ neurons prohibited accurate counting in single maximum intensity z-projections; maximum intensity z-projections were generated for every 10 z-slices, yielding three to four maximum intensity z-projections representing 20 μM each. To improve visualization of densely clustered αβ′ nuclei, background was subtracted using a rolling ball/sliding paraboloid algorithm (radius set to the size of the largest nucleus: 50 pixels) and image intensity display range was adjusted (minimum: 5; maximum: 175). Processed maximum intensity z-projections representing 20 μM each were then merged into a single z-stack for manual counting using the Cell Counter plug-in in ImageJ; to avoid double-counting of nuclei segmented in adjacent z-projections, the original unprocessed z-stack was examined in parallel. The variation in MB neuron counts between experimenters, calculated as the absolute difference between the two counts divided by their mean, was (mean ± SEM) 2.0% ± 0.2% for γα; 1.5% ± 0.3% for αβ′; and 2.6% ± 0.3% for αβc. Where neuron counts were different for a given hemisphere, the average was plotted. Numbers of γα neurons in wild-type animals were intermediate between those reported in prior studies (Aso et al., 2014a; Shih et al., 2019), while numbers of αβ′ and αβc neurons were lower, likely reflecting conservative assignment of nuclei in our study and the use of different antibodies and reporters (Aso et al., 2014a; Shih et al., 2019). αβ′ counts obtained using the MB370B driver were similar to previously reported numbers of αβc neurons; because MB370B labels αβc neurons strongly and αβc neurons weakly, the lower absolute numbers of αβ′ neurons in our studies may reflect detection sensitivity and correspond chiefly to αβc neurons.

To count the number of αβc neuron clusters in wild-type and inc brains, the same randomly selected samples used to quantify neuron numbers were assessed in a single-blind manner by two independent experimenters. Each z-stack was analyzed using a combination of visual inspection of z-sections and rotating the image stack in three dimensions using the 3D Viewer plug-in in ImageJ (threshold: 0; resampling factor: 2). A group of nuclei distributed continuously along all axes was classified as a cluster; a continuous gap at least one nuclear diameter in width across all axes was used to define cluster edges and discrete clusters. Cluster counts were identical for wild-type brains; total
cluster counts for inc^1 brains differed by 8.2% ± 0.2% (mean ± SEM) between experimenters. Where cluster counts were different for a given hemisphere, the average was plotted.

DH44 and dFB somata were counted in a single-blind manner by two independent experimenters as described for γ_d and α'/β' neurons, using DH44-Gal4 to drive UAS-MyrGFP-2A-RedStinger and 23E10-Gal4 to drive 5× UAS-IVS-Syt1::smGdP-HA. Numbers of dFB and DH44 neurons were identical between two independent experimenters.

Analysis of axonal projections and dendritic volume
To analyze axonal projections and dendritic volume, image stacks were captured using a 10X objective at 512 × 512 resolution with 1 μM z-slices. Axonal projection defects were assessed in maximum intensity z-projections. The number of horizontal and/or vertical lobes missing myr-GFP signal entirely was counted for each brain. To quantify dendritic volume, the Threshold command in ImageJ was applied to z-stacks to select dendrites based on DenMark immunofluorescence; high signal to noise allowed unambiguous demarcation of dendrites and clear separation from background. The same minimum and maximum threshold values were applied to all wild-type and inc^1 brains stained in parallel in an experiment and captured the entirety of dendritic signal for all samples. A single rectangular region of interest of minimal area encompassing dendritic signals from both brain hemispheres across all z-slices was drawn for each z-stack. Dendritic volume was quantified using the Voxel Counter plug-in in ImageJ.

Sleep analysis
Three- to four-day-old male flies eclosing from LD-entrained cultures raised at 25°C were loaded in glass tubes (5 mm diameter × 65 mm length) containing standard food or appropriate food for Q-system experiments as described above. Animals were monitored for 5–7 days at 25°C in LD cycles using DAM2 monitors (Trikinetics). Locomotor activity data were collected in 1 min bins. Inactive periods of 5 min or longer were classified as sleep. The first 36–48 hr of data were discarded to allow acclimation of animals to tubes, and 3–5 integral days of data were analyzed beginning with ZT0. Dead animals were excluded from analysis by a combination of automated filtering and visual inspection of locomotor traces. Matlab code used to analyze sleep is available in Source code 1.

Thermogenetic activation
Crosses were set on standard fly food as described above and cultured at 21.5°C. One- to four-day-old male flies eclosing from these cultures were assayed for 5 days in LD cycles. Animals were maintained at 21.5°C for the first 60–72 hr of the assay, including 36–48 hr of acclimation and the subsequent baseline day beginning at ZT0. Temperature was increased to 28.5°C for 24 hr to activate dTrpA1, followed by 24 hr of recovery at 21.5°C. The percent change in sleep was calculated for each animal by subtracting the amount of sleep on the baseline day from the amount of sleep on the activation day and dividing this difference by the amount of sleep on the baseline day. The percent change in sleep for individual animals was averaged for each genotype.

Gal4 drivers used to express TrpA1 were as follows: pan-MB, MB004B split-Gal4; MB, c253-Gal4 and c309-Gal4; EB, R69F08-Gal4; DPM, NP2721-Gal4; Helicon, R24B11-Gal4; l-LNV, c929-Gal4; PI, PPM3, c584-Gal4.

Statistics
One-way analysis of variance (ANOVA) and Tukey post hoc tests were used for comparisons between more than two groups of animals for total sleep, daytime sleep, nighttime sleep, and sleep bout number; for comparisons of these sleep parameters between two groups, unpaired two-sided Student’s t-tests were used. Kruskal–Wallis tests and Dunn’s post hoc tests were used for comparisons of sleep bout length between more than two groups of animals; for comparison between two groups, Mann–Whitney tests were used. One-way ANOVA and Dunnett’s post hoc tests were used for
comparisons of percent change in sleep. Unpaired two-sided Welch’s t-tests were used for pairwise comparisons of neuron number, cluster number, and dendrite volume.

Acknowledgements

We thank C Desplan, N Ringstad, M Shirasu-Hiza, and members of the Stavropoulos lab for comments on the manuscript; Y Aso, B Ganetzky, L Griffith, W Joiner, W Li, K Nagel, C Potter, A Sehgal, J Simpson, G Suh, M Wu, M Young, the Bloomington Drosophila Stock Center, the Vienna Drosophila Resource Center, and the Janelia Flylight collection for fly stocks; and DSHB for antibodies. This work was supported by an International Student Research Fellowship from the Howard Hughes Medical Institute (HHMI) to QL and by grants from the National Institutes of Health (R01NS112844 and R21NS111304), the Mathers Foundation, Whitehall Foundation grant 2013-05-78, fellowships from the Alfred P Sloan and Leon Levy Foundations, a NARSAD Young Investigator Award from the Brain and Behavior Foundation, the J Christian Gillin, M.D. Research Award from the Sleep Research Society Foundation, and a Career Scientist Award from the Irma T Hirschl/Weill-Caulier Trust to NS.
References

Amar M, Pramod AB, Herrera VM, Qiu LR, Moran-Losada P, Zhang P, Trujillo CA, Ellegood J, Urresti J, Chau K, Diedrich J, Chen J, Gutierrez J, Sebat J, Ramanathan D, Lerch JP, Muotri AR, Iakoucheva LM. 2021. Autism-linked Cullin3 germline haploinsufficiency impacts cytoskeletal dynamics and cortical neurogenesis through RhoA signaling. Molecular Psychiatry 26:3586–3613. DOI: https://doi.org/10.1038/s41380-021-01052-x, PMID: 33727673

Angriman M, Caravale B, Novelli L, Ferri R, Bruni O. 2015. Sleep in children with neurodevelopmental disabilities. Neuropediatrics 46:199–210. DOI: https://doi.org/10.1055/s-0035-1550151, PMID: 25918987

Ashburner M, Golic KG, Hawley RS. 2005. Drosophila: A Laboratory Handbook. 2nd ed. New York: Cold Spring Harbor Laboratory Press.

Aso Y, Grübel K, Busch S, Friedrich AB, Siwanowicz I, Tanimoto H. 2009. The mushroom body of adult Drosophila characterized by GAL4 drivers. Journal of Neurogenetics 23:156–172. DOI: https://doi.org/10.1080/01677060802471718, PMID: 19140035

Aso Y, Hattori D, Yu Y, Johnston RM, Ngo TTB, Dionne H, Abbott LF, Axel R, Tanimoto H, Rubin GM. 2014a. The neuronal architecture of the mushroom body provides a logic for associative learning. eLife 3:e04577. DOI: https://doi.org/10.7554/eLife.04577, PMID: 25353793

Aso Y, Sitaraman D, Ichinose T, Kaun KR, Vogt K, Belliart-Guérin G, Plaçais PY, Robie AA, Yamagata N, Schnaitmann C, Rowell WJ, Johnston RM, Ngo TTB, Chen N, Korff W, Nitabach MN, Heberlein U, Preat T, Branson KM, Tanimoto H, et al. 2014b. Mushroom body output neurons encode valence and guide memory-based action selection in Drosophila. eLife 3:e04580. DOI: https://doi.org/10.7554/eLife.04580, PMID: 25535794

Bainbridge SP, Bownes M. 1981. Staging the metamorphosis of Drosophila melanogaster. Journal of Embryology and Experimental Morphology 66:57–80. DOI: https://doi.org/10.1242/dev.66.1.57, PMID: 6802923

Berry JA, Cervantes-Sandoval I, Chakraborty M, Davis RL. 2015. Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting. Cell 161:1656–1667. DOI: https://doi.org/10.1016/j.cell.2015.05.027, PMID: 26073942

Betschinger J, Mechtler K, Knoblich JA. 2006. Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell 124:1241–1253. DOI: https://doi.org/10.1016/j.cell.2006.01.038, PMID: 16564014

Bowman SK, Neumüller RA, Novatchkova M, Du Q, Knoblich JA. 2006. The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Developmental Cell 10:731–742. DOI: https://doi.org/10.1016/j.devcel.2006.05.005, PMID: 16740476

Bushey D, Huber R, Tononi G, Cirelli C. 2007. Drosophila Hyperkinetic mutants have reduced sleep and impaired memory. The Journal of Neuroscience 27:5384–5393. DOI: https://doi.org/10.1523/JNEUROSCI.0108-07.2007, PMID: 17507560

Bushey D, Tononi G, Cirelli C. 2015. Sleep- and wake-dependent changes in neuronal activity and reactivity demonstrated in fly neurons using in vivo calcium imaging. PNAS 112:4785–4790. DOI: https://doi.org/10.1073/pnas.1419603112, PMID: 25825756

Carney TD, Struck AJ, Doe CQ. 2013. midlife crisis encodes a conserved zinc-finger protein required to maintain neuronal differentiation in Drosophila. Development 140:4155–4164. DOI: https://doi.org/10.1242/dev.1093781, PMID: 24026126

Cavanaugh DJ, Geratowski JD, Wooltorton JRA, Spaethling JM, Hector CE, Zheng X, Johnson EC, Eberwine JH, Sehgal A. 2014. Identification of a circadian output circuit for rest/activity rhythms in Drosophila. Cell 157:689–701. DOI: https://doi.org/10.1016/j.cell.2014.02.024, PMID: 24766812

Cavanaugh DJ, Vigderman AS, Dean T, Garbe DS, Sehgal A. 2016. The Drosophila Circadian Clock Gates Sleep through Time-of-Day Dependent Modulation of Sleep-Promoting Neurons. Sleep 39:345–356. DOI: https://doi.org/10.5665/sleep.5442, PMID: 26350473
Gisselmann G, Sewing S, Madsen BW, Mallart A, Angaut-Petit D, Müller-Holtkamp F, Ferrus A, Pongs O. 1989. The interference of truncated with normal potassium channel subunits leads to abnormal behaviour in transgenic Drosophila melanogaster. The EMBO Journal 8:2359–2364. DOI: https://doi.org/10.1002/j.1460-2075.1989.tb08364.x, PMID: 2551680

Gong NN, Dilley LC, Williams CE, Moscato EH, Szuperak M, Wang Q, Jensen M, Girirajan S, Tan TY, Deardorff MA, Li D, Song Y, Kayser MS. 2021. The chromatin remodeler ISWI acts during Drosophila development to regulate adult sleep. Science Advances 7:eabe2597. DOI: https://doi.org/10.1126/sciadv.abe2597, PMID: 33597246

Gress C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416:396–400. DOI: https://doi.org/10.1038/416396a, PMID: 1191622

Guan Z, Prado A, Melzig J, Heisenberg M, Nash HA, Raabe T. 2000. Mushroom body defect, a gene involved in the control of neuroblast proliferation in Drosophila, encodes a coiled-coil protein. PNAS 97:8122–8127. DOI: https://doi.org/10.1073/pnas.97.14.8122, PMID: 10884435

Guo F, Yi W, Zhou M, Guo A. 2011. Go Signaling in Mushroom Bodies Regulates Sleep in Drosophila. Sleep 34:273–281. DOI: https://doi.org/10.1093/sleep/34.3.273, PMID: 21358844

Guy J, Gan J, Selfridge J, Cobb S, Bird A. 2007. Reversal of neurological defects in a mouse model of Rett syndrome. Science 315:1143–1147. DOI: https://doi.org/10.1126/science.1138389, PMID: 17289941

Guy J, McKay L, Brockett E, Spike RC, Selfridge J, De Sousa D, Merusi C, Riedel G, Bird A. 2012. Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. Brain: A Journal of Neurology 135:2699–2710. DOI: https://doi.org/10.1093/brain/awx096, PMID: 22525157

Hamada FN, Rosenzweig M, Kang K, Pulver SR, Ghezzi A, Jegla TJ, Garrity PA. 2008. An internal thermal sensor controlling temperature preference in Drosophila. Nature 454:217–220. DOI: https://doi.org/10.1038/ nature07001, PMID: 18548007

Heisenberg M, Borst A, Wagner S, Byers D. 1985. Drosophila mushroom body mutants are deficient in olfactory learning. Journal of Neurogenetics 2:1–30. DOI: https://doi.org/10.3109/01677060850100140, PMID: 4020527

Heisenberg M. 2003. Mushroom body memoir: from maps to models. Nature Reviews. Neuroscience 4:266–275. DOI: https://doi.org/10.1038/nrn1074, PMID: 12671643

Hewes RS, Schaefer AM, Taghert PH. 2000. The cryptocephal gene (ATF4) encodes multiple basic-leucine zipper proteins controlling molting and metamorphosis in Drosophila. Genetics 155:1711–1723. DOI: https://doi.org/10.1093/genetics/155.4.1711, PMID: 10924469

Hewes RS, Park D, Gauthier SA, Schaefer AM, Taghert PH. 2003. The bHLH protein Dimmed controls neuroendocrine cell differentiation in Drosophila. Development 130:1771–1781. DOI: https://doi.org/10.1242/dev.00404, PMID: 12642483

Hong ST, Bang S, Hyun S, Kang J, Jeong K, Paik D, Chung J, Kim J. 2008. cAMP signalling in mushroom bodies modulates temperature preference behaviour in Drosophila. Nature 454:771–775. DOI: https://doi.org/10.1038/nature07090, PMID: 18594510

Hovhanyan A, Raabe T. 2009. Structural brain mutants: mushroom body defect (mdb): a case study. Journal of Neurogenetics 23:42–47. DOI: https://doi.org/10.1080/01677060802471700, PMID: 19107630

Ishimoto H, Kitamoto T. 2010. The steroid molting hormone Ecdysone regulates sleep in adult Drosophila melanogaster. Genetics 185:269–281. DOI: https://doi.org/10.1534 genetics.110.114587, PMID: 20215472

Ito K, Hotta Y. 1992. Proliferation pattern of postembryonic neuroblasts in the brain Drosophila melanogaster. Developmental Biology 149:134–148. DOI: https://doi.org/10.1016/0012-1606(92)90270-q, PMID: 31728583

Ito K, Awano W, Suzuki K, Hiromi Y, Yamamoto D. 1997. The Drosophila mushroom body is a quadrapule structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development 124:761–771. DOI: https://doi.org/10.1242/dev.124.4.761, PMID: 9043058

Iwasaki K, Fujiyama T, Nakata S, Park M, Miyoshi C, Hotta-Hirashima N, Iikkyu A, Kakizaki M, Sugiyama F, Mizuno S, Abe M, Sakimura K, Takahashi S, Funato H, Yanagisawa M. 2021. Induction of Mutant Sik3Sleepy Allele in Neurons in Late Infancy Increases Sleep Need. The Journal of Neuroscience 41:2733–2746. DOI: https://doi.org/10.1523/JNEUROSCI.1004-20.2020, PMID: 33558433

Jennett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, Pfeiffer BD, Cavallaro A, Hall D, Jeter J, Iyer N, Fetter D, Hausenfluck JH, Peng H, Trautman ET, Svirskas RR, Myers EW, Iwinski ZR, Aso Y, DePasquale GM, et al. 2012. A GAL4-driver line resource for Drosophila neurobiology. Cell Reports 2:991–1001. DOI: https://doi.org/10.1016/j.celrep.2012.09.011, PMID: 23063364

Jessell TM, Sanes JR. 2000. Development: The decade of the developing brain. Current Opinion in Neurobiology 10:599–611. DOI: https://doi.org/10.1016/s0959-4388(00)00136-7, PMID: 11084323

Joiner WJ, Crocker A, White BH, Sehgal A. 2006. Sleep in Drosophila is regulated by adult mushroom bodies. Nature 441:757–760. DOI: https://doi.org/10.1038/nature04811, PMID: 16760980

Keene AC, Masek P. 2012. Optogenetic induction of aversive taste memory. Neuroscience 222:173–180. DOI: https://doi.org/10.1016/j.neuroscience.2012.07.028, PMID: 22820051
Kempf A, Song SM, Talbot CB, Miesenböck G. 2019. A potassium channel β-subunit couples mitochondrial electron transport to sleep. Nature 568:230–234. DOI: https://doi.org/10.1038/s41586-019-1034-5, PMID: 30894743

Kerkis J. 1931. The Growth of the Gonads in Drosophila melanogaster. Genetics 16:212–224. DOI: https://doi.org/10.1093/genetics/16.3.212, PMID: 17246617

Kikuma K, Li X, Perry S, Li Q, Goel P, Chen C, Kim D, Stavropoulos N, Dickman D. 2019. Cul3 and insomniac are required for rapid ubiquitination of postsynaptic targets and retrograde homeostatic signaling. Nature Communications 10:2913–2998. DOI: https://doi.org/10.1038/s41467-019-10992-6, PMID: 31278365

Koblich JA. 2008. Mechanisms of asymmetric stem cell division. Cell 132:583–597. DOI: https://doi.org/10.1016/j.cell.2008.02.007, PMID: 18295577

Kong A, Friggle ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WSW, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT, Thorsteinsdottir U, et al. 2012. Rate of de novo mutations and the importance of father's age to disease risk. Nature 488:471–475. DOI: https://doi.org/10.1038/nature11396, PMID: 22914163

Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S. 2007. Sequential use of mushroom body neuron subsets during Drosophila odor memory processing. Neuron 53:103–115. DOI: https://doi.org/10.1016/j.neuron.2006.11.021, PMID: 17196534

Kurusu M, Awasaki T, Masuda-Nakagawa LM, Kawauchi H, Ito K, Furukubo-Tokunaga K. 2002. Embryonic and larval development of the Drosophila mushroom bodies: concentric layer subdivisions and the role of fasciclin II. Development 129:409–419. DOI: https://doi.org/10.1242/dev.129.2.409, PMID: 11807033

Lee T, Lee A, Luo L. 1999. Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126:4065–4076. DOI: https://doi.org/10.1242.dev.126.18.4065, PMID: 10457015

Lee T, Luo L. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461. DOI: https://doi.org/10.1016/s0896-6273(00)80701-1, PMID: 10197526

Lee CY, Robinson KJ, Doe CQ. 2006a. Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. Nature 439:594–598. DOI: https://doi.org/10.1038/nature04299, PMID: 16357871

Lee CY, Wilkinson BD, Siegrist SE, Wharton RP, Doe CQ. 2006b. Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal. Developmental Cell 10:441–449. DOI: https://doi.org/10.1016/j.devcel.2006.01.017, PMID: 16549393

Li Q, Stavropoulos N. 2016. Evaluation of Ligand-Inducible Expression Systems for Conditional Neuronal Manipulations of Sleep in Drosophila. G3: Genes, Genomes, Genetics 6:3351–3359. DOI: https://doi.org/10.1534/g3.116.034132, PMID: 27558667

Li Q, Kellner DA, Hatch HM, Yumita T, Sanchez S, Machold RP, Frank CA, Stavropoulos N. 2017. Conserved properties of Drosophila Insomniac link sleep regulation and synaptic function. PLOS Genetics 13:e1006815. DOI: https://doi.org/10.1371/journal.pgen.1006815, PMID: 28558011

Li Q, Lim KYY, Stavropoulos N. 2019. Structural and Behavioral Analysis Reveals That Insomniac Impacts Sleep by Functioning as a Cul3 Adaptor. [bioRxiv]. DOI: https://doi.org/10.1101/689471

Li J, Mahoney BD, Jacob MS, Caron SJC. 2020. Visual Input into the Drosophila melanogaster Mushroom Body. Cell Reports 32:108138. DOI: https://doi.org/10.1016/j.celrep.2020.108138, PMID: 32937130

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 99:365–376. DOI: https://doi.org/10.1016/s0092-8674(00)81965-0, PMID: 10458611

Li R, Lamaze A, Liu Q, Tabuchi M, Yang Y, Fowler M, Bharadwaj R, Zhang J, Bedont J, Blackshaw S, Lloyd TE, Montell C, Sehgal A, Koh K, Wu MN. 2014. WIDE AWAKE mediates the circadian timing of sleep onset. Neuron 82:151–166. DOI: https://doi.org/10.1016/j.neuron.2014.01.040, PMID: 24631345

Liu S, Liu Q, Tabuchi M, Wu MN. 2016. Sleep Drive Is Enabled by Neural Plastic Changes in a Dedicated Circuit. Cell 165:1347–1360. DOI: https://doi.org/10.1016/j.cell.2016.04.013, PMID: 27212227

Martin JR, Raabe T, Heisenberg M. 1999. Central complex substructures are required for the maintenance of locomotor activity in Drosophila melanogaster. Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology 185:277–288. DOI: https://doi.org/10.1007/s003590050387, PMID: 10573866

Masek P, Scott K. 2010. Limited taste discrimination in Drosophila. PNAS 107:14833–14838. DOI: https://doi.org/10.1073/pnas.1009318107

Mauri F, Rechardt I, Mummer-Widmer JL, Yamazaki M, Knoblich JA. 2014. The conserved discs-large binding partner Banderalula regulates asymmetric cell division in Drosophila. Current Biology 24:1811–1825. DOI: https://doi.org/10.1016/j.cub.2014.06.059, PMID: 25088559

McGraw CM, Samaco RC, Zoghbi HY. 2011. Adult neural function requires MeCP2. Science 333:184. DOI: https://doi.org/10.1126/science.1206593, PMID: 21636743

Nern A, Pfeiffer BD, Rubin GM. 2015. Optimized tools for multicolor stochastic labeling reveal diverse stereotyped cell arrangements in the fly visual system. PNAS 112:E2967–E2976. DOI: https://doi.org/10.1073/pnas.1507631112, PMID: 25964354

Nicolaï LJJ, Ramaekers A, Raemaekers T, Drozdzecki A, Mauss AS, Yan J, Landgraf M, Annaert W, Hassan BA. 2010. Genetically encoded dendritic marker sheds light on neuronal connectivity in Drosophila. PNAS 107:20553–20558. DOI: https://doi.org/10.1073/pnas.1019810107, PMID: 21059961
Okada R, Awasaki T, Ito K. 2009. Gamma-amino butyric acid (GABA)-mediated neural connections in the Drosophila antennal lobe. The Journal of Comparative Neurology 514:74–91. DOI: https://doi.org/10.1002/cne.21971, PMID: 19260068

O’Reak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernet B, Malig M, Baker C, Reilly B, Akemy JM, Borenstein E, Rieder MJ, Nickerson DA, et al. 2012. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 495:250. DOI: https://doi.org/10.1038/nature11989, PMID: 22945309

Parisky KM, Agosto J, Pulver SR, Shang Y, Kuklin E, Hodge JJL, Kang K, Kang K, Liu X, Garrity PA, Rosbash M, Griffith LC. 2008. PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Neuron 60:672–682. DOI: https://doi.org/10.1016/j.neuron.2008.10.042, PMID: 19038223

Pauls D, Selcho M, Gendre N, Stocker RF, Thum AS. 2010. Drosophila larvae establish appetitive olfactory memories via mushroom body neurons of embryonic origin. The Journal of Neuroscience 30:10655–10666. DOI: https://doi.org/10.1523/JNEUROSCI.1281-10.2010, PMID: 20702697

Petersson S, Persson AS, Johansen JE, Ingvar M, Nilsson J, Klement G, Arhem P, Schalling M, Lavebratt C. 2003. Drosophila larvae establish appetitive olfactory circuitry, and molecular aberrations by region-specific deficiency of the high-risk autism gene Cul3. Molecular Psychiatry 8:1491–1504. DOI: https://doi.org/10.1038/sj.mp.4001217, PMID: 12821039

Pimentel D, Donlema JM, Talbot CB, Song SM, Thurston AJF, Miesenböck G. 2016. Operation of a homeostatic sleep switch. Nature 536:333–337. DOI: https://doi.org/10.1038/nature19055, PMID: 27487216

Pitman JL, McGill JJ, Keegan KP, Allada R. 2006. A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature 441:753–756. DOI: https://doi.org/10.1038/nature04739, PMID: 16760979

Potter CJ, Tasic B, Russler EV, Liang L, Luo L. 2010. The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. Cell 141:536–548. DOI: https://doi.org/10.1016/j.cell.2010.02.025, PMID: 20434990

Prokop A, Technau GM. 1994. Normal function of the mushroom body defect gene of Drosophila is required for homeostasis and a dopamine arousal pathway in Drosophila. PLOS Genetics 8:e1003003. DOI: https://doi.org/10.1371/journal.pgen.1003003, PMID: 23055946

Pfeiffer BD, Ngo TTB, Hibbard KL, Murphy C, Jenett A, Truman JW, Rubin GM. 2010. Refinement of tools for reagents for genetic manipulations. Nature Methods 7:536–548. DOI: https://doi.org/10.1038/nmeth.1414, PMID: 20514860

Pfeiffer BB, Ngo TTB, Hibbard KL, Murphy C, Jenett A, Truman JW, Rubin GM. 2010. Refinement of tools for targeted gene expression in Drosophila. Genetics 186:735–755. DOI: https://doi.org/10.1534/genetics.110.119917, PMID: 20697129

Rapanelli M, Tan T, Wang W, Wang X, Wang ZJ, Zhong P, Frick L, Qin L, Ma K, Qu J, Yan Z. 2021. Behavioral, circuitry, and molecular aberrations by region-specific deficiency of the high-risk autism gene Cul3. Molecular Psychiatry 26:1491–1504. DOI: https://doi.org/10.1038/s41380-019-0498-x, PMID: 31455885

Renn SC, Park JH, Rosbash M, Hall JC, Taghert PH. 1999. A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in Drosophila. Cell 99:791–802. DOI: https://doi.org/10.1016/s0092-8674(00)81676-1, PMID: 10619432

Riabinina O, Maroy P, et al. 2004. The DrosDel collection: a set of P-element insertions for generating custom chromosomal aberrations in Drosophila. Genetics 167:797–813. DOI: https://doi.org/10.1534/genetics.104.026658, PMID: 15238529

Sanes JR, Zippursky SL. 2020. Synaptic Specificity, Recognition Molecules, and Assembly of Neural Circuits. Cell 181:536–556. DOI: https://doi.org/10.1016/j.cell.2020.04.008, PMID: 32359437

Seugnet L, Suzuki Y, Vine L, Gottschalk L, Shaw PJ. 2008. D1 receptor activation in the mushroom bodies rescues sleep-loss-induced learning impairments in Drosophila. Current Biology 18:1110–1117. DOI: https://doi.org/10.1016/j.cub.2008.07.028, PMID: 18674913

Seugnet L, Suzuki Y, Vinson L, Gottschalk L, Duntley SP, Shaw PJ. 2011. Notch signaling modulates sleep homeostasis and learning after sleep deprivation in Drosophila. Current Biology 21:835–840. DOI: https://doi.org/10.1016/j.cub.2011.04.001, PMID: 21549599

Shang Y, Griffith LC, Rosbash M. 2008. Light-arousal and circadian photoreception circuits interconnect at the large PDF cells of the Drosophila brain. PNAS 105:19587–19594. DOI: https://doi.org/10.1073/pnas.0809577105, PMID: 19060186

Sheeba V, Fogle KJ, Kaneko M, Rashid S, Chou YT, Sharma VK, Holmes TC. 2008. Large ventral lateral neurons modulate arousal and sleep in Drosophila. Current Biology 18:1537–1545. DOI: https://doi.org/10.1016/j.cub.2008.08.033, PMID: 18771923
Zhu S, Chiang AS, Lee T. 2003. Development of the Drosophila mushroom bodies: elaboration, remodeling and spatial organization of dendrites in the calyx. Development 130:2603–2610. DOI: https://doi.org/10.1242/dev.00466, PMID: 12736205

Zhu S, Perez R, Pan M, Lee T. 2005. Requirement of Cul3 for axonal arborization and dendritic elaboration in Drosophila mushroom body neurons. The Journal of Neuroscience 25:4189–4197. DOI: https://doi.org/10.1523/JNEUROSCI.0149-05.2005, PMID: 15843622