A genetic mosaic screen identifies genes modulating Notch signaling in *Drosophila*

Luming Ren☯, Dongqing Mo☯, Yunlong Li☯, Tong Liu, Huan Yin, Na Jiang, Junzheng Zhang☯

Department of Entomology and MOA Key Lab of Pest Monitoring and Green Management, College of Plant Protection, China Agricultural University, Beijing, China

☯ These authors contributed equally to this work.

* zhangjz@cau.edu.cn

Abstract

Notch signaling is conserved in most multicellular organisms and plays critical roles during animal development. The core components and major signal transduction mechanism of Notch signaling have been extensively studied. However, our understanding of how Notch signaling activity is regulated in diverse developmental processes still remains incomplete. Here, we report a genetic mosaic screen in *Drosophila melanogaster* that leads to identification of Notch signaling modulators during wing development. We discovered a group of genes required for the formation of the fly wing margin, a developmental process that is strictly dependent on the balanced Notch signaling activity. These genes encode transcription factors, protein phosphatases, vacuolar ATPases and factors required for RNA transport, stability, and translation. Our data support the view that Notch signaling is controlled through a wide range of molecular processes. These results also provide foundations for further study by showing that Me31B and Wdr62 function as two novel modulators of Notch signaling activity.

Introduction

First identified in *Drosophila melanogaster*, the highly conserved Notch signaling pathway is required for cell fate specification in most, if not all, tissues during animal development [1]. Furthermore, Notch signaling also regulates stem cell maintenance and tissue homeostasis in adult life [2]. In keeping with its pleiotropic function in many cell types, dysregulation of Notch signaling in human leads to birth defects as well as tumor formation in various organs [3].

Despite its broad function in many spatially and temporally distinct developmental contexts, the Notch signaling pathway contains only a small number of core components [4]. The *Notch* gene encodes a transmembrane receptor protein which is trans-activated by its ligands, Delta (Dl) and/or Serrate, from neighboring cells. Activation of the Notch receptor triggers a sequence of proteolytic events that releases the Notch intracellular domain (NICD). The NICD subsequently translocates into the nucleus, where it forms an active transcription complex with Suppressor of Hairless [Su(H)] and Mastermind proteins and turns-on the expression of downstream target genes. In the absence of NICD, Su(H) recruits co-repressors to suppress the transcription of Notch target genes [5].
In addition to the components in the core pathway, numerous genes are found to fine-tune the Notch pathway in a context specific manner [4]. These genes encode auxiliary proteins that regulate the amount of Notch receptor and ligands, the proteolytic processing to generate active NICD, the formation of transcriptional active or repressive complexes on the chromatin, as well as trafficking of both the receptor and ligands [4, 5]. Thus, the functional diversity of Notch pathway is generated at different signal transduction steps by the modulatory factors.

The patterning of the adult fly wing blade represents the historical system for studying Notch signaling in vivo [6]. Notch signaling is crucial for several major developmental events in the wing, including vein differentiation, wing margin formation and sensory neuronal cell fate determination. We hypothesized that additional regulators may act to support the delicate roles of Notch signaling in these distinct developmental events. Therefore, we performed a genetic mosaic screen to identify genes that modulate Notch signaling activity during fly wing margin formation. We identified nine genes that exert regulatory function at various steps of Notch signal transduction. Other than several well-known components, four of them were poorly studied for regulation of Notch signaling during wing development. More importantly, we discovered two novel modulators of Notch signaling pathway.

Materials and methods

Fly genetics

Flies were maintained in standard medium and stocks were kept at room temperature (21–23°C). Crosses were performed at 25°C. Mutant alleles on FRT-containing chromosome were obtained from the Kyoto Drosophila Stock Center (BruinFly collection) [7]. The Ubx-FLP;Ubi-mRFP, FRT40A stock was used to generate mosaic clones in the developing wing. The hs-Flp;M(2)24F, arm-LacZ, FRT40A/Cyo stock was used to generate large clones in the Minute background [8]. Three days old larval were heat-shocked at 37°C for 1 hour to induce mosaic clones. Molecularly defined Deficiency stocks (7818, 9503, 7778, 7779, 8000) and RNAi line against me31B (33675) were obtained from the Bloomington Stock Center. Additional Deficiency stocks (150096, 150067) were obtained from the Kyoto Stock Center. RNAi line for Wdr62 (7337R-I) was provided by the National Institute of Genetics (NIG) in Japan. The Hh-Gal4, UAS-GFP/TM6B and dpp-Gal4, UAS-GFP/TM6B stocks were used to drive transgenic RNAi flies.

Screen design and phenotypes scoring

Males of each mutant allele from the BruinFly collection were crossed with the Ubx-FLP;Ubi-mRFP, FRT40A virgins. For the primary screen, at least 100 F1 progenies of each cross were examined for wing developmental defects. Phenotypes with penetration rates higher than 5% were recorded. Mutants that displayed wing margin nicking phenotypes were selected for secondary screen, during which Notch signaling activity was monitored in third-instar larval wing discs. The expression level of Notch signaling target genes Cut and Wingless (Wg) were visualized by immunostaining. Mutants affecting Cut or Wg expression were considered as Notch signaling modulators, and their effects on Notch and Delta were further examined. For mutants that led to disc distortion, clones were generated in the Minute background. Wild-type distribution pattern of Cut, Wg, Notch and Delta proteins are shown as control.

Wing imaginal disc immunostaining and microscopy

Wing discs from third-instar larvae were fixed in 4% paraformaldehyde and labeled with the following primary antibodies: mouse anti-Cut (1:100; 2B10; DSHB), mouse anti-Wingless (1:200; 4D4; DSHB), mouse anti-Notch intracellular domain NICD (1:200; C17.9C6; DSHB), mouse
anti-Delta (1:200; C594.9B; DSHB) and rabbit anti-LacZ (1:400; Cappel). Alexa fluor-conjugated secondary antibodies (1:400; Invitrogen) were used. The fluorescence images were acquired with an Olympus FV1000 or Leica SP8 confocal microscope and processed in Photoshop. Minor adjustments (brightness and/or contrast) were done in Photoshop. Representing single focal plane pictures were shown in Figs 1, 2, 3A–3C, 7A, 9C, 9D, 10A–10C and 11B–11D. Projection of z-stacks were generated by ImageJ and shown in Figs 3D, 4, 5, 6, 7B–7D, 8, 9A, 9B, 10D and 11A.

Adult wings were dissected and mounted as described previously [9]. The images of adult wings were acquired with a Leica DMIL inverted microscope equipped with a QImaging QICAM Fast 1394 digital camera.

Results and discussion

Genetic mosaic screen identifies genes modulating Notch signaling during fly wing development

Many Notch signaling modulators have been identified through genome-wide mutagenesis screens, RNAi screens and modifier screens. Therefore, it is unlikely to find new Notch

Fig 1. Putative Notch signaling modulators regulate wing margin formation. (A) The adult wing of parental Ubx-FLP;Ubi- mRFP, FRT40A stock is used as wild type control. Induction of Su(H) (B), Hrb27C (C, D, E), elf3h (F), Vha68-2 (G) VhaSFD (H), Pp2A-29B (I), mts (J), mc31B (K) and Wdr62 (L) mutant clones in the wing blade led to defective wing margin formation, with different degrees of severity. Specific BruinFly allele and corresponding DGRC stock number are labeled for each mutation.

https://doi.org/10.1371/journal.pone.0203781.g001
Fig 2. Reduced expression of Hrb27C leads to defective Notch signaling. Expression of Notch signaling targets Cut (A) and Wg (B) are abolished in Hrb27C<sup>EY12571</sup> homozygous mutant clones (marked by absence of RFP). (C) Notch protein level is attenuated in Hrb27C<sup>EY12571</sup> clones. (D) Reduction of DI expression is evident in Hrb27C<sup>EY12571</sup> mutant cells located at the DV boundary. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g002
Fig 3. eIF3h positively regulates Notch signaling in the wing disc. Expression of Notch signaling targets Cut (A) and Wg (B) are eliminated in eIF3h<sup>K09003</sup> homozygous mutant clones (marked by absence of RFP). Notch protein level (C) is substantially reduced in eIF3h<sup>K09003</sup> clones. Dl expression (D) is reduced in clones of eIF3h<sup>K09003</sup> mutant cells located at the DV boundary. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g003

regulators using similar strategies [2–5]. We designed a small scale screen using the Flippase (FLP)/FLP Recognition Target (FRT) genetic mosaic technique [10]. We screened a collection of lethal mutations, based on the knowledge that most Notch regulators are essential for larval development in Drosophila. We took advantage of a modified Ubx-promoter driven FLP transgene that expresses the FLP recombinase in the developing wing imaginal disc, and thus mediates mitotic recombination in a labor-free manner [11].
We screened 451 BruinFly lines with lethal mutations in genes located on the left arm of chromosome II [7]. While the initial focus of our screen was to find specific regulators for wing margin formation, we observed that many of these lines led to developmental defects in the wing. In total, 60 BruinFly lines displayed wing phenotypes when homozygous clones were

Fig 4. V-ATPase subunit Vha68-2 is a modulator of Notch signaling in the wing. Expression of Notch signaling targets Cut (A) and Wg (B) are reduced in Vha68-2^{s4214} homozygous mutant clones (marked by absence of LacZ staining). Both Notch (C) and Dl (D) proteins are accumulated in a subset of Vha68-2^{s4214} mutant cells. Representative mutant clones are indicated by arrows. Panels C and D are higher magnification of a small portion of S4 Fig. Clones presented here are generated in the Minute background. Phenotype shown in panels A (7 of 8 discs) and B (8 of 11 discs) are consistent, while accumulation of Notch and Dl shows variation among clones.

https://doi.org/10.1371/journal.pone.0203781.g004
The mutants were categorized into six classes based on similarity of resulting phenotypes: nicked wing margin, abnormal vein pattern, tissue necrosis, blistered wing, folded wing and club-like wing (S1 Fig). Mutants causing "nicked wing margin" were further analyzed as this represents the classical phenotype associated with dysregulation of Notch signaling.

Fig 5. VhaSFD is involved in Notch signaling regulation. Expression of Cut (A) and Wg (B) are reduced in VhaSFD_EY04644 homozygous mutant clones (marked by absence of LacZ staining). Notch (C) and Dl (D) proteins are accumulated in a subset of VhaSFD_EY04644 mutant cells. Representative mutant clones are indicated by arrows. Panels C and D are higher magnification of a small portion of S4 Fig. Clones presented here are generated in the Minute background. Phenotype shown in panels A (9 of 12 discs) and B (6 of 9 discs) are consistent, while accumulation of Notch and Dl shows variation among clones.

https://doi.org/10.1371/journal.pone.0203781.g005

present (S1 Table). The mutants were categorized into six classes based on similarity of resulting phenotypes: nicked wing margin, abnormal vein pattern, tissue necrosis, blistered wing, folded wing and club-like wing (S1 Fig). Mutants causing "nicked wing margin" were further analyzed as this represents the classical phenotype associated with dysregulation of Notch signaling.
Among the 30 mutants that showing notched wing phenotypes, five of them represent mutations of genes that are involved in Decapentaplegic (Dpp) and Epidermal Growth Factor Receptor (EGFR) signaling pathways. During fly wing development, Dpp protein functions as a morphogen that controls organ growth and vein differentiation. The type I receptor

Fig 6. PP2A activity is essential for proper Notch signaling output. Cells in the Pp2A-29B<sup>EP2332</sup> homozygous mutant clones lack expression of Cut (A) and Wg (B) at the DV boundary (marked by absence of LacZ staining). Reduced expression of Notch (C) proteins are observed in a subset of Pp2A-29B<sup>EP2332</sup> mutant cells. Dl proteins are largely unaffected in Pp2A-29B<sup>EP2332</sup> mutant clones (D). Clones presented here are generated in the Minute background. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g006
Fig 7. Mts regulates Notch signaling in the wing imaginal disc. Expression of Notch signaling target Cut (A) and Wg (B) are significantly decreased in mts<sup>s5286</sup> homozygous mutant clones (marked by absence of RFP or LacZ staining). Reduction of Notch (C) proteins are found in a subset of mts<sup>s5286</sup> mutant cells, while DI (D) proteins pattern remains unchanged. Clones presented here are generated in the Minute background except for panel A. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g007
Thickveins (Tkv) and downstream transcription factor Mothers against Dpp (Mad) are essential for Dpp signaling transduction [12]. We found that mutations of Tkv and Mad caused vein thickening as well as margin notches in the wing (S1 Fig). Vein thickening is a stereotypical developmental defect resulted from reduction of Dpp signaling [12]. And the notched wing

Fig 8. Me31B is a novel regulator of Notch signaling. Expression of Notch signaling targets Wg (A) and Cut (B) are abolished in me31B\textsuperscript{K06607} homozygous mutant clones (marked by absence of LacZ staining). In me31B\textsuperscript{K06607} mutant cells, Notch (C) and Dl (D) proteins are accumulated as cellular puncta. Panels C and D are higher magnification of a small portion of S5 Fig. Clones presented here are generated in the Minute background. Note that reduction of Cut and Wg are fully penetrating (N>10). Formation of Notch and Dl puncta are consistent among discs (N>15), but the degree of accumulation varies for cells inside the same clone. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g008
phenotypes might reflect the ability of Dpp signaling to regulate Wingless (Wg) signaling activity during wing margin formation [13, 14]. Star (S) encodes a transmembrane protein that facilitates processing and secretion of EGFR ligands [15]. In wings bearing S mutant

**Fig 9. Me31B is required for Notch signaling activation.** Expression of Notch signaling reporter NRE-GFP (A) is reduced in me31B<sup>k06607</sup> homozygous mutant clones (marked by absence of LacZ staining). In me31B<sup>k06607</sup> mutant clones (marked by absence of RFP), Neur-LacZ expressing cells are ectopically induced (B). RNAi knock-down of me31B in the posterior compartment (marked by GFP) leads to downregulation of Cut (C) and Wg (D). Clones presented here are generated in the Minute background except for panel B. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g009
clones, we observed missing of veins, and occasionally wing margin notches (S1 Fig). The vein missing phenotype resembles S loss-of-function alleles [16]. Again, EGFR pathway interacts with Wg signaling to regulate wing margin formation [17, 18]. Thus, these lines were not scored as specific Notch regulators.

Fig 10. Wdr62 regulates Notch signaling in the wing imaginal disc. In Wdr62<sup>EY09575</sup> mutant cells (marked by absence of LacZ staining), expression of Notch signaling target Cut (A) is abolished. Both Wg (B) and Dl (C) protein are accumulated as puncta in Wdr62<sup>EY09575</sup> mutant cells. Notch (D) proteins are mildly up-regulated in Wdr62<sup>EY09575</sup> homozygous cells. Clones presented here are generated in the Minute background. Representative mutant clones are indicated by arrows. Panels B, C and D are higher magnification of a small portion of S6 Fig.

https://doi.org/10.1371/journal.pone.0203781.g010
Directly controlled by Notch signaling activity, both Cut and Wg are expressed in cells located at the dorsal-ventral (DV) boundary of the wing disc (S2 Fig). Therefore, expression of Cut and Wg were examined in the mitotic clones for the rest 25 mutant alleles. We found that 5 of these mutants gave rise to very tiny clones which are much smaller than their twin-spot

Fig 11. Wdr62 is a positive regulator of Notch signaling activity. Expression of Notch signaling reporter NRE-GFP (A) is reduced in Wdr62EY09575 homozygous mutant cells (marked by absence of LacZ staining). Ectopic induction of Neur-LacZ expressing cells (B) are observed in Wdr62EY09575 mutant clones (marked by absence of RFP). RNAi knock-down of Wdr62 at the anterior-posterior boundary region (marked by GFP) leads to down-regulation of Cut (C), but not Wg (D). Clones presented here are generated in the Minute background except for panel B. Representative mutant clones are indicated by arrows.

https://doi.org/10.1371/journal.pone.0203781.g011
sister clones, suggesting that these genes are crucial for cell viability during wing development (S2 Table). Another 9 mutants resulted in no obvious changes in the expression of neither Cut nor Wg (S2 Table). These genes likely regulate wing margin formation through other signaling pathways or cellular events [6, 19].

At last, we isolated 9 genes from 11 mutant lines that were qualified as Notch signaling regulators during fly wing development (Fig 1). Given the variety of genes uncovered in our screen, we categorized them into three groups based on previous studies and importance (Table 1). Three of these genes are well-known regulators of the Notch signaling. Four of them were related with Notch signaling in other tissues, but their roles for regulation of Notch activity during wing development were poorly studied. More importantly, we identified two novel modulators of the Notch signaling pathway.

**Group I: Notch signaling components involved in wing development.** Gene encoding Su(H) was identified by the screen, which was expected as Su(H) is a core component required for Notch target gene activation [1, 5]. We observed a very high rate of marginal notches in mosaic wings harboring the P-element insertion allele Su(H)\(^{k07904}\) (Fig 1B). To confirm the effect of Su(H)\(^{k07904}\) in Notch signaling transduction, we generated homozygous Su(H)\(^{k07904}\) mutant clones in the wing discs and analyzed the expression of two Notch target genes, namely Cut and Wg as a readout. The expression of Cut and Wg were completely absent in cells homozygous for Su(H)\(^{k07904}\), which are in agreement with previous findings that Su(H) facilitates Notch target genes transcription (S3 Fig). We found that Notch itself was not regulated by Su(H) (S3 Fig). Interestingly, DI expression was modestly reduced in Su(H)\(^{k07904}\) mutant clones located at the DV boundary (S3 Fig). Our results suggest that Su(H) may play additional role in DI expression regulation during fly wing development.

We also found wing margin nicking phenotypes in three BruinFly alleles corresponding to *Hrb27C/Hrp48* (Fig 1C–1E). The *Hrb27C\(^{EY12571}\) allele was chosen for further characterization because it gave the highest percentage of wing notch phenotypes among three alleles. Reductions of Notch signaling targets were evident in *Hrb27C\(^{EY12571}\)* mutant clones (Fig 2A and 2B). Hrb27C is an abundant, essential RNA binding protein that functions in RNA splicing, localization and translation control [20–22]. During fly wing development, Hrb27C was shown to promote Notch expression [23]. Accordingly, Notch levels were reduced in *Hrb27C\(^{EY12571}\)* mutant clones (Fig 2C). The exact biochemical mode of action of Hrb27C in Notch signaling regulation is still elusive. It was proposed that Hrb27C regulates Notch expression through the female determinant Sex-lethal (Sxl), but neither the splicing nor stability of Sxl mRNAs were modulated by Hrb27C [23, 24]. Recently, Hrb27C was identified as an interacting partner of

| Gene Symbol | BruinFly Allele | Wing\(^{*}\) | Other tissues\(^{*}\) | Group |
|-------------|-----------------|-------------|-----------------|------|
| Su(H)       | Su(H)\(^{EY12571}\) | Yes [1, 5] | Yes [1, 5] | I    |
| Hrb27C      | Hrb27C\(^{EY12571}\) | Yes [23–25] | Yes [24] | I    |
| elF3h       | elF3h\(^{E09003}\) | Yes [9] | No | I    |
| Vha68-2     | Vha68-2\(^{E2174}\) | No | Yes [28] | II   |
| VhaSFD      | VhaSFD\(^{EY04644}\) | No | Yes [28] | II   |
| Pp2A-29B    | Pp2A-29B\(^{EFP2332}\) | No | Yes [28] | II   |
| mts         | mts\(^{E5286}\) | No | Yes [39, 40] | III  |
| me31B       | me31B\(^{E06607}\) | No | No | III  |
| Wdr62       | Wdr62\(^{EY09575}\) | No | No | III  |

\(^{*}\) Previous studies of each gene for their roles in Notch signaling regulation in the wing or other tissues are summarized.

https://doi.org/10.1371/journal.pone.0203781.t001

Table 1. Summary of Notch signaling regulators revealed by screen.
Deltex and they regulate Notch protein independent of Sxl [25]. Whether other core components of Notch pathway were influenced by Hrb27C was unknown. We found that Dl expression was also slightly reduced in cells homozygous of Hrb27C_EY12571 when clones are located at the DV boundary (Fig 2D). Thus, the role of Hrb27C in Notch signaling regulation appears to be more complex than expected and awaits further investigation.

The Drosophila eIF-3p40/eIF3h protein is a subunit of the eukaryotic translation initiation factor 3 (eIF3) complex [26]. A regulatory role of eIF3 complex in Notch pathway was discovered in our previous RNAi screen [9]. Here we report that mosaic clones of the BruinFly allele eIF3h_K09003 led to marginal defects in the wing (Fig 1F). Consistent with the adult phenotype, Notch signaling activity was diminished in eIF3h_K09003 clones as revealed by lack of the expression of Cut and Wg (Fig 3A and 3B). We found that Notch expression was substantially reduced upon loss of eIF3h (Fig 3C). These phenotypes are in agreement with the reported RNAi knock-down results [9]. Cells near the DV boundary express highest levels of Dl due to the positive feedback loop of the Notch signaling [1, 4, 6]. We found that Dl expression were dampened in eIF3h_K09003 mutant cells only when clones were located at the DV boundary, likely resulting from reduced Notch signaling activity (Fig 3D). Taken together, our results suggest that eIF3h is a bona fide Notch signaling regulator. It is possible that eIF3h is required for translation initiation of Notch protein. Alternatively, eIF3h may possess activities other than translation initiation, as is the case for eIF3f, another subunit of the eIF3 complex which regulates ubiquitination of Notch protein [27].

Group II Notch signaling modulators studied in other tissues. Genes in this group encode subunits of two protein complexes with crucial enzyme activities, and their roles in Notch signaling during wing development were not thoroughly studied.

Our screen isolated two vacuolar ATPase (V-ATPase) genes, Vha68-2 and VhaSFD as Notch signaling regulators in wing development. Mutant clones of Vha68-2s4214 and VhaSFD_EY04644 caused notching of the wing margin (Fig 1G and 1H) and thickening of the wing veins (Fig 1H). In agreement with the adult phenotype, we observed loss of Cut and Wg expression in Vha68-2s4214 and VhaSFD_EY04644 mutant clones (Fig 4A and 4B and Fig 5A and 5B). Clones of Vha68-2s4214 and VhaSFD_EY04644 mutant cells also displayed various degrees of Notch and Dl accumulation in intracellular puncta (Fig 4C and 4D, Fig 5C and 5D and S4 Fig). We noticed that the Vha68-2 and VhaSFD mutant phenotypes were also variably penetrant in the eye disc, with some cells showing very mild defects [28]. This could be explained by hypomorphy of the alleles or functional redundancy with other V-ATPase subunits. Despite the phenotypic variability, these results support the view that V-ATPase activity is positively required for Notch signaling. The V-ATPases are conserved multi-subunit ATP-driven proton pumps that present in the endo-membranes of all cells [29]. As demonstrated in Drosophila, V-ATPases play a multiplicity of roles during animal development [30–33]. Although some V-ATPase subunits also fulfill specialized roles [34], the similarity of loss-of-function phenotypes suggest that Vha68-2 and VhaSFD likely function in the same complex to regulate Notch signaling in various epithelia tissues. In general, the V-ATPase controls endosomal acidification and is a major regulator of membrane protein localization [28, 31, 35]. Membrane bound signaling molecules such as Dl are transported by the endocytic pathway [36]. Therefore, it is reasonable to predict that localization of DI is also regulated by Vha68-2 and VhaSFD.

Two subunits of the serine/threonine protein phosphatase 2A (PP2A) holoenzyme were identified in our screen. PP2A consists of a scaffolding A subunit, a regulatory B subunit, and a catalytic C subunit [37]. We found that the A (CG17291/PP2A-29B) and C (Mts) subunits were required for wing margin formation, mutant clones of either subunit resulted in wing notches (Fig 1I and 1J). As these phenotypes are reminiscent of the loss of Notch function, we examined the activity of Notch signaling in the wing discs. We observed that cells in the Pp2A-
$29^B_{EP2332}$ and $mts^{s5286}$ homozygous mutant clones lack expression of Cut and Wg at the DV boundary (Fig 6A and 6B and Fig 7A and 7B). We observed mild down-regulation of Notch protein in $Pp2A-29^B_{EP2332}$ and $mts^{s5286}$ mutant cells (Fig 6C and Fig 7C). DI proteins were maintained at normal levels in $Pp2A$ mutant clones (Fig 6D and Fig 7D). It has been shown that removing either the A or the C subunit destabilizes PP2A and reduces its activity [38]. Thus, we conclude that PP2A activity is required for proper Notch signaling output during wing development. The A and C subunits are essential for the activity of PP2A, but the substrate specificity are determined by the variable regulatory B subunits [37]. The fly genome encodes one A, one C, and four B subunits. One of the B subunits, Wdb, was shown to inhibit Notch signaling activity by targeting Enhancer of split M8, an effector of Notch signaling during eye development [39]. Over-expression of Wdb and mts in the developing wing disc led to wing margin loss and ectopic macrochaetes on the notum, also suggesting an inhibitory role for Wdb/PP2A in Notch signaling [39, 40]. However, a recent RNAi screen suggested that PP2A-29B, mts and Wdb act to promote of Notch signaling activity in the wing [41]. Further studies are needed to clarify the exact role of PP2A in wing development. PP2A might display tissue specific roles in Notch signaling. It has been demonstrated that PP2A regulates several substrate proteins through distinct B subunits in the Hh pathway [42, 43] and MAPK pathway [44, 45]. It is highly possible that similar strategy is taken by PP2A to regulate Notch signaling pathway.

**Group III Novel Notch signaling modulators.** The BruinFly allele $me31B^{k06607}$ disrupts function of a RNA binding protein named as Maternal expression at 31B (Me31B) [46]. Me31B is a putative DEAD-box containing RNA helicase that is involved in transport and translational control of oocyte-localizing maternal RNAs [47, 48] as well as neuronal RNAs [49, 50]. During our screen, we found that $me31B$ is required for fly wing development. Wing margin notches were observed in flies containing $me31B^{k06607}$ homozygous clones (Fig 1K). In the larval wing discs, expression of Notch signaling targets Cut and Wg were abolished in $me31B^{k06607}$ mutant clones (Fig 8A and 8B). Reduction in the levels of Notch targets could result from the ability of Me31B to regulate upstream signaling molecules such as Notch or Dl. To test this, we examined the effect of $me31B^{k06607}$ mutation on N and Dl protein levels. We found that upon loss of Me31B, both N and DI proteins were accumulated in intracellular puncta in a subset of mutant cells (Fig 8C and 8D, S5 Fig). These results suggest that the mislocalized Notch and DI proteins in $me31B^{k06607}$ cells were incompetent for signaling activity. It has been demonstrated that cellular localization of Notch and DI proteins are major determinants for their activity [51]. Accumulation of Notch or DI proteins in different cellular compartments can lead to gain- or loss-of-function defects in a context-dependent manner [4]. For example, when Notch proteins were trapped in the ER, server Notch signaling loss-of-function phenotypes were observed, despite that Notch protein level is highly elevated [52].

We further validated the requirement of Me31B in Notch signaling activation using two independent reporters. The GFP expression in the NRE-GFP transgenic line marks the cells with active Notch signaling along the DV boundary of the larval wing disc [53]. In $me31B^{k06607}$ mutant cells, the GFP expression was obviously reduced (Fig 9A, c.f. S5 Fig). The $neur-lacZ$ enhancer-trap insertion labels the sensory organ precursor (SOP) cells, whose fates are repressed by Notch signaling [54]. We found that the number of $neur-lacZ$ positive SOPs in $me31B^{k06607}$ clones was significantly increased (Fig 9B, c.f. S5 Fig). Interestingly, the lacZ proteins are expressed at lower level and exhibit disrupted nuclear localization in $me31B^{k06607}$ clones. Me31B is known to regulate mRNA stability and protein translational [48, 49]. Therefore, we speculate that Me31B might be required for efficient translation and nuclear transporting of the engineered LacZ protein. Taken together, we conclude that Notch signaling activity is indeed dampened in $me31B^{k06607}$ mutant cells.
To further demonstrate that these phenotypes are directly caused by me31B malfunction, we knocked-down me31B expression using transgenic RNAi line in the wing disc. We found that expression of both Cut and Wg were down-regulated in me31B RNAi cells (Fig 9C and 9D). Complementation tests were also performed to rule out potential secondary mutations in the me31Bk06607 stock. We linked the lethality associated with me31Bk06607 to chromosomal region 31B1 using deficiency stocks bearing deletions of different genomic regions (S3 Table). Finally, we obtained white eyed flies carrying the chromosome produced by precise excision of the transposon and found that they no longer produce the wing margin phenotypes. To ensure that the reversion was due to removing of the P-element but not FRT40A itself, we examined the ability of excision stocks to generate mosaic clones in the wing discs. We found that large clones were formed with unaltered expression of Cut at the DV boundary (S5 Fig). Collectively, we provided evidence for a direct relationship between defective Notch signaling and Me31B mutation.

Me31B has emerged as a central player in translational repression and mRNA decay [48, 49]. Our preliminary results indicate that both Notch and Dl mRNAs could be directly targeted by Me31B. Alternatively, Me31B might regulate genes of Notch pathway through microRNA-mediated translational repression in wing discs [49, 55]. In the follicle cells, RNAi knock-down of Me31B was reported to cause up-regulation of Cut during mid-oogenesis [56]. Taken together, we believe that Me31B might regulate Notch signaling in various developmental processes and may function in a tissue specific fashion. Genetic and molecular studies are underway to determine the underlying mechanism of Me31B in Notch signaling regulation.

The second novel Notch signaling modulator gene identified in our screen is wd40-repeat protein 62 (Wdr62). We observed marginal defects in mosaic wings harboring the Wdr62EY09575 mutant allele (Fig 1L). In Wdr62EY09575 mutant cells, expression of Cut was abolished (Fig 10A). The other target of Notch signaling, Wg, responded differently to Wdr62 mutation. We found that Wg protein accumulated as puncta inside Wdr62 mutant cells (Fig 10B). The Wg containing puncta were majorly observed at the apical focal plane of wing disc cells (S6 Fig). Our observations suggest that Wdr62 might be involved in regulation of differential Notch targets expression at the wing margin [57]. Aberrant accumulation of Dl proteins were evident in Wdr62EY09575 mutant cells (Fig 10C), and the effect was not limited to the apical side of wing disc cells as shown by projection of z-stacks (S6 Fig). We also found that Notch expression were mildly up-regulated in Wdr62EY09575 mutant cells (Fig 10D and S7 Fig). In Wdr62EY09575 mutant cells, the NRE-GFP expression was diminished (Fig 11A), while the neur-lacZ expression was expanded (Fig 11B). Consistently, expression of Cut, but not Wg, was inhibited when Wdr62 expression was knocked-down by RNAi (Fig 11C and 11D). Using complementation tests, we mapped the lethality associated with Wdr62EY09575 to chromosomal region 22B, matching to the genomic loci of Wdr62 (S3 Table). Finally, we found that the wing margin and Cut expression defects were reverted by precise excision of the transposon from Wdr62EY09575 (S7 Fig). Taken together, our results suggest that Wdr62 is involved in Notch signaling regulation during fly wing development.

The Wdr62 gene was identified, relatively recently, as the second most commonly mutated gene in primary microcephaly patients [58–60]. It has been revealed that Wdr62 functions in the regulation of spindle organization, mitotic progression and the duplication and biased inheritance of centrosomes during neural system development [61]. The Drosophila ortholog of Wdr62 was essential for larval brain growth, microcephaly defects similar to human patients were observed in Wdr62 mutants [62, 63]. Notch signaling also plays prominent roles during neural development [64]. Therefore, it is tempting to presume that Wdr62 interacts with Notch signaling to regulate the development of neural as well as somatic tissues. In particular, a functional link between Wdr62 and Notch signaling might be established through asymmetrical segregation of centrosomes. Wdr62 is required to maintain centrosome asymmetry in
both *Drosophila* and vertebrate neural stem cells [62, 65, 66]. At the same time, several Notch pathway regulators were found to interact with centrosomes and distribute asymmetrically after mitotic division of neuron progenitor cells [67, 68]. Further studies are needed to clarify whether Wdr62 is capable of regulating Notch signaling during asymmetry cell divisions.

**Conclusions and potential for future studies**

The first *Notch* mutant was isolated by T.H. Morgan at 1917, and the *Notch* gene was named after the wing margin loss phenotype [69]. Over the past century, phenotypic studies combined with subsequent genetic and molecular analysis of wing development have been extensively used to identify components of Notch signaling pathway [1–5]. We are encouraged by the fact that new regulators of Notch signaling are discovered by the genetic mosaic system in the wing. Future studies aimed at more deeply characterizing the molecular function of each of the identified targets, particularly the two novel regulators, will broaden our understanding of how Notch signaling is regulated in diverse developmental processes. We believe that somatic mosaic screens will continue to provide valuable insights for understanding Notch signaling regulation [11, 52].

It has been noted that about 5% of the Bruinfly FRT40A stocks might contain second-site mutation alleles of *lethal (2) giant larvae* [*l(2)gl*] [70]. Such mutational genetic background has raised concerns for interpreting genetic studies using these stocks [71]. Importantly, *l(2)gl* is involved in Notch signaling regulation in the developing eye and neuronal cells [72–76]. Therefore, we are obliged to clarify to which extent would the *l(2)gl* mutations confound our screen results. We believe that the presence of *l(2)gl* mutations had little, if any, impact for our screen. This conclusion is based on several important observations. Firstly, *l(2)gl* mutation is unlikely to generate recognizable phenotypes under our experimental conditions. The adult wing blade proper is derived from the pouch region of larval wing imaginal disc. In the pouch area, *l(2)gl* mutant cells growing in mosaic larvae were usually eliminated due to cell death [77, 78]. Therefore, the overall growth and patterning were largely normal in *l(2)gl* mosaic wings [77]. These results suggest that *l(2)gl* mutations would not be recovered in our mosaic screen system. Indeed, we were unable to detect consistent developmental defects for the five Bruinfly FRT40A lines which were shown to contain *lgl* alleles [70]. Secondly, after going through the literatures, we found lack of evidence to support a physiological role of *l(2)gl* in Notch signaling during wing development. When cell competition were alleviated in a Minute surrounding background, *l(2)gl* cells were permissive for survival and clonal growth [78, 79]. Adult wings containing large *l(2)gl* mutant clones displayed a diverse array of developmental defects, but none of them resembled Notch signaling activity disruptions [79]. Similarly, when *l(2)gl* expression was knocked-down by RNAi in the wing disc, the adult tissue displayed significant over-growth but wing margin formation and vein differentiation remained normal [80]. Genome-wide transcriptional profiling found misregulation of several signaling pathways in *l(2)gl* mutant wing disc cells, but failed to detect any significant change in Notch pathway genes [78]. Furthermore, the expression of Notch signaling target gene Wg was unaffected in *l(2)gl* mutant clones generated in the Minute background [78]. Collectively, current findings do not support a direct role of *l(2)gl* in Notch regulation during wing development. It has been shown that Lgl is not involved in regulating Notch signaling in the ovarian follicle cells, strongly suggesting a tissue specific role of Lgl in Notch regulation [81, 82]. Thirdly, among the 11 BruinFly FRT40A lines that identified as Notch regulators in our screen, none of them displayed the neoplastic phenotypes as reported in the *l(2)gl* mutants. Meanwhile, for the two novel regulators identified in our screen, complementation tests and excision experiments established a causal relationship between the mutants and the phenotypes. Taken together, we are confident that the *l(2)gl* mutation background has minimal impact for our study reported here.
Supporting information

S1 Table. Summary of mutant wing phenotypes revealed by screen.
(DOCX)

S2 Table. Analysis of potential Notch signaling regulators.
(DOCX)

S3 Table. Complementation analysis of \textit{me31B^{k06607}} and \textit{Wdr62^{EY09575}} stock.
(DOCX)

S1 Fig. Various wing phenotypes are discovered in the screen.
(TIF)

S2 Fig. Notch signaling components display stereotypical distribution patterns.
(TIF)

S3 Fig. Su(H) is required for Notch signaling activity in the developing wing.
(TIF)

S4 Fig. V-ATPases regulate cellular localization of Notch and Dl.
(TIF)

S5 Fig. Me31B regulates cellular localization of Notch and Dl.
(TIF)

S6 Fig. Wdr62 regulates cellular localization of Wg and Dl.
(TIF)

S7 Fig. \textit{Wdr62^{EY09575}} precise excision lines are restored to wild-type.
(TIF)

Acknowledgments

We thank Drs. Alan Jian Zhu, Renjie Jiao, Jie Shen, Wei Wu, the Bloomington Stock Center, the Kyoto Fly Stock Center, the National Institute of Genetics (NIG) in Japan and the Developmental Studies Hybridoma Bank (DSHB) for fly stocks and antibodies. This work was supported by National Natural Science Foundation of China (31471382 and 31772526 to J.Z.), and Chinese Universities Scientific Fund (2017ZB002 to J.Z).

Author Contributions

Conceptualization: Junzheng Zhang.
Funding acquisition: Junzheng Zhang.
Investigation: Luming Ren, Dongqing Mo, Yunlong Li, Tong Liu, Huan Yin.
Methodology: Luming Ren, Dongqing Mo, Yunlong Li, Na Jiang.
Project administration: Luming Ren, Junzheng Zhang.
Supervision: Junzheng Zhang.
Validation: Huan Yin.
Writing – original draft: Luming Ren, Dongqing Mo, Yunlong Li, Tong Liu, Na Jiang.
Writing – review & editing: Junzheng Zhang.
References

1. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science. 1999; 284:770–776. PMID: 10221902
2. Maše k J, Andersson ER. The developmental biology of genetic Notch disorders. Development. 2017; 144:1743–1763. https://doi.org/10.1242/dev.148007 PMID: 28512196
3. Nowell CS, Radtke F. Notch as a tumour suppressor. Nat Rev Cancer. 2017; 17:145–159. https://doi.org/10.1038/nrc.2016.145 PMID: 28154375
4. Bray SJ. Notch signalling in context. Nat Rev Mol Cell Biol. 2016; 17:722–735. https://doi.org/10.1038/nrm.2016.94 PMID: 27507209
5. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. Dev. Cell. 2009; 16:633–647. https://doi.org/10.1016/j.devcel.2009.03.010 PMID: 19460341
6. Blair SS. Wing vein patterning in Drosophila and the analysis of intercellular signaling. Annu. Rev. Cell Dev. Biol. 2007; 23:293–319. https://doi.org/10.1146/annurev.cellbio.23.090506.123606 PMID: 17506700
7. Chen J, Call GB, Beyer E, Bui C, Cespedes A, Chan A, et al. Discovery-Based Science Education: Functional Genomic Dissection in Drosophila by Undergraduate Researchers. PLoS Biol. 2005; 3:e59. https://doi.org/10.1371/journal.pbio.0030059 PMID: 15719063
8. Maurel-Zaffran C, Treisman JE. pannier acts upstream of wingless to direct dorsal eye disc development in Drosophila. Development. 2000; 127:1007–1016. PMID: 10662640
9. Zhang J, Liu M, Su Y, Du J, Zhu A.J. A targeted in vivo RNAi screen reveals deubiquitinas e as new regulators of Notch signaling. G3 (Bethesda). 2012; 2:1563–1575.
10. Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult Drosophila tissues. Development. 1993; 117:1223–1237. PMID: 8404527
11. Jafar-Nejad H, Andrews HK, Acar M, Bayat V, Wirtz-Peitz F, Mehta SQ, et al. Sec15, a component of the exocyst, promotes notch signaling during the asymmetric division of Drosophila sensory organ precursors. Dev Cell. 2005; 9:351–363. https://doi.org/10.1016/j.devcel.2005.06.010 PMID: 16137928
12. Affolter M, Basler K. The Decapentaplegic morphogen gradient: from pattern formation to growth regulation. Nat Rev Genet. 2007; 8:663–674. https://doi.org/10.1038/nrg2166 PMID: 17703237
13. Guichard A, Biehs B, Sturtevant MA, Wickline L, Chacko J, Howard K, et al. rhomboid and Star interact synergistically to promote EGFR/MAPK signaling during Drosophila wing vein development. Development. 1999; 126:2663–2676. PMID: 10331978
14. Giraldez AJ, Cohen SM. Wingless and Notch signaling provide cell survival cues and control cell proliferation during wing development. Development. 2003; 130:6533–6543. https://doi.org/10.1242/dev.00904 PMID: 14660542
15. Hammond LE, Rudner DZ, Kanaar R, Rio DC. Mutations in the hrp48 gene, which encodes a Drosophila heterogeneous nuclear ribonucleoprotein particle protein, cause lethality and developmental defects and affect P-element third-intron splicing in vivo. Mol Cell Biol. 1997; 17:7260–7267. PMID: 9372958
16. Huynh JR, Munro TP, Smith-Liërie K, Lepesant JA, Johnston D. The Drosophila hnrNPA/B homolog, Hrp48, is specifically required for a distinct step in osk mRNA localization. Dev Cell. 2004; 6:625–635. PMID: 15130488
17. Yano T, López de Quinto S, Matsui Y, Shevchenko A, Shevchenko A, Ephrussi A. Hrp48, a Drosophila hnrNPA/B homolog, binds and regulates translation of oskar mRNA. Dev Cell. 2004; 6:637–648. PMID: 15130489
23. Suissa Y, Kalifa Y, Dinur T, Graham P, Deshpande G, Schedl P, et al. Hrp48 attenuates Sxl expression to allow for proper notch expression and signaling in wing development. Proc Natl Acad Sci U S A. 2010; 107:6930–6935. https://doi.org/10.1073/pnas.0910570107 PMID: 20351283

24. Penn JK, Schedl P. The master switch gene sex-lethal promotes female development by negatively regulating the N-signaling pathway. Dev Cell. 2007; 12:275–286. https://doi.org/10.1016/j.devcel.2007.01.009 PMID: 17276344

25. Dutta D, Paul MS, Singh A, Mutsuddi M, Mukherjee A. Regulation of Notch Signaling by the Heterogeneous Nuclear Ribonucleoprotein Hrp48 and Deltex in Drosophila melanogaster. Genetics. 2017; 206:905–918. https://doi.org/10.1534/genetics.116.198879 PMID: 28396507

26. Lasko P. The Drosophila melanogaster genome: translation factors and RNA binding proteins. J. Cell Biol. 2000; 150:F51–F56. PMID: 10908586

27. Moretti J, Chastagner P, Gastaldello S, Heuss SF, Dirac AM, Bernards R, et al. The translation initiation factor 3f (eIF3f) exhibits a deubiquitina se activity regulating Notch activation. PLoS. Biol. 2010; 8: e1000545. https://doi.org/10.1371/journal.pbio.1000545 PMID: 21124883

28. Vaccari T, Duchu S, Cortese K, Tacchetti C, Bilder D. The vacuolar ATPase is required for physiological as well as pathological activation of the Notch receptor. Development. 2010; 137:1825–1832. https://doi.org/10.1242/dev.045484 PMID: 20460366

29. Yan Y, Denef N, Schubbach T. The vacuolar proton pump, V-ATPase, is required for notch signaling and endosomal trafficking in Drosophila. Dev Cell. 2009; 17:387–402. https://doi.org/10.1016/j.devcel.2009.07.001 PMID: 19758563

30. Hermete MC, Beck S, Helmstädt S, Simons M. Drosophila ATP6AP2/VhaPRR functions both as a novel planar cell polarity core protein and a regulator of endosomal trafficking. EMBO J. 2013; 32:245–259. https://doi.org/10.1038/emboj.2012.323 PMID: 23292348

31. Zhang T, Zhou Q, Ogmundsdottir MH, Möller K, Siddaway R, Larue L, et al. Mitf is a master regulator of the v-ATPase, forming a control module for cellular homeostasis with v-ATPase and TORC1. J Cell Sci. 2015; 128:2938–2950. https://doi.org/10.1242/jcs.173807 PMID: 26092939

32. Hiesinger PR, Fayyazuddin A, Heisenberg CP, Eaton S. Planar cell polarization requires Widerborst, a B’ regulatory subunit of protein phosphatase 2A. Development. 2002; 129:3493–3503. PMID: 12091318

33. Kunttas-Tatli E, Bose A, Kahali B, Bishop CP, Bidwai AP. Functional dissection of Timekeeper (Tik) implicates opposite roles for CK2 and PP2A during Drosophila neurogenesis. Genesis. 2009; 47:647–658. https://doi.org/10.1002/dvg.20543 PMID: 19536808

34. Jia H, Liu Y, Yan W, Jia J. PP4 and PP2A regulate Hedgehog signaling by controlling Smo and Ci phosphorylation. Dev Cell. 2009; 16:307–316. https://doi.org/10.1016/j.devcel.2009.03.012 PMID: 19088085

35. Su Y, Ospina JK, Zhang J, Michelson AP, Schoen AM, Zhu AJ. Sequential phosphorylation of Smoothened transduces graded Hedgehog signaling. Sci. Signal. 2011; 4:ra34. https://doi.org/10.1126/scisignal.2001747 PMID: 21730325
44. Wassarman DA, Solomon NM, Chang HC, Karim FD, Therrien M, Rubin GM. Protein phosphatase 2A positively and negatively regulates Ras1-mediated photoreceptor development in Drosophila. Genes Dev. 1996; 10:272–278. PMID: 8595878

45. Silverstein AM, Barrow CA, Davis AJ, Mumby MC. Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits. Proc Natl Acad Sci U S A. 2002; 99:4221–4226. https://doi.org/10.1073/pnas.072071899 PMID: 11904383

46. De Valoir T, Tucker MA, Belikoff EJ, Camp LA, Bolduc C, Beckingham K, et al. A second maternally expressed Drosophila gene encodes a putative RNA helicase of the ‘DEAD box’ family. Proc. Natl. Acad. Sci USA. 1991; 88:2113–2117. PMID: 1900936

47. Nakamura A, Amikura R, Hanyu K, Kobayashi S. Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development. 2001; 128:3233–3242. PMID: 11546740

48. Wang M, Ly M, Lugowski A, Laver JD, Lipshitz HD, Smibert CA, et al. ME31B globally represses maternal mRNAs by two distinct mechanisms during the Drosophila maternal-to-zygotic transition. eLife. 2017; 6:e27891. https://doi.org/10.7554/eLife.27891 PMID: 28875934

49. Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luederman RA, Collier JM, et al. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. Neuron. 2006; 52:997–1009. https://doi.org/10.1016/j.neuron.2006.05.028 PMID: 17178403

50. Lee J, Yoo E, Lee H, Park K, Hur JH, Lim C. LSM12 and ME31B/DDX6 Define Distinct Modes of Post-transcriptional Regulation by ATAXIN-2 Protein Complex in Drosophila Circadian Pacemaker Neurons. Mol Cell. 2017; 66:129–140. https://doi.org/10.1016/j.molcel.2017.03.004 PMID: 28388438

51. Yamamoto S, Charng WL, Bellin HJ. Endocytosis and intracellular trafficking of Notch and its ligands. Curr Top Dev Biol. 2010; 92:165–200. https://doi.org/10.1016/S0070-2153(10)92005-X PMID: 20816569

52. Tien AC, Rajan A, Schulze KL, Ryoo HD, Acar M, Steller H, et al. Enz handwritten ois, an essential regulator for Notch signaling through cysteine bridge formation of the Lin12-Notch repeats in Drosophila melanogaster. J Cell Biol. 2008; 182:1113–1125. https://doi.org/10.1083/jcb.200805001 PMID: 18809725

53. Saj A, Arziman Z, Stempfle D, van Belle W, Sauder U, Horn T, et al. A combined ex vivo and in vivo RNAi screen for notch regulators in Drosophila reveals an extensive notch interaction network. Dev Cell. 2010; 18:862–876. https://doi.org/10.1016/j.devcel.2010.03.013 PMID: 20493818

54. Huang F, Dambly-Chaudière C, Ghysen A. The emergence of sense organs in the wing disc of Drosophila. Development. 1991; 111:1087–1095. PMID: 1879352

55. McCann C, Holohan EE, Das S, Dervan A, Larkin A, Lee JA, et al. The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. Proc Natl Acad Sci U S A. 2011; 108:E655–E662. https://doi.org/10.1073/pnas.1008050108 PMID: 21795609

56. Jia D, Soylemez M, Calvin G, Bornmann R, Bryant J, Hanna C, et al. A large-scale in vivo RNAi screen to identify genes involved in Notch-mediated follicle cell differentiation and cell cycle switches. Sci Rep. 2015; 5:12328. https://doi.org/10.1038/srep12328 PMID: 26205122

57. Janody F, Treisman JE. Requirements for mediator complex subunits distinguish three classes of notch target genes at the Drosophila wing margin. Dev Dyn. 2011; 240:2051–2059. https://doi.org/10.1002/dvdy.22705 PMID: 21793099

58. Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, et al. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. Nature. 2010; 467:207–210. https://doi.org/10.1038/nature09327 PMID: 20729831

59. Nicholas AK, Khorshid M, Désir J, Carvalho OP, Cox JJ, Thornton G, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nat. Genet. 2010; 42:1010–1014. https://doi.org/10.1038/ng.683 PMID: 20890279

60. Yu TW, Mochida GH, Tischfeld DJ, Sgaiër SK, Flores-Sarnat L, Sergi CM, et al. Mutations in WDR62, encoding a centrosome-associated protein, cause microcephaly with simplified gyri and abnormal cortical architecture. Nat Genet. 2010; 42:1015–1020. https://doi.org/10.1038/ng.683 PMID: 20890278

61. Shohayeb B, Lim NR, Ho U, Xu Z, Dottori M, Quinn L, et al. The Role of WD40-Repeat Protein 62 (MCPH2) in Brain Growth: Diverse Molecular and Cellular Mechanisms Required for Cortical Development. Mol Neurobiol. 2017; https://doi.org/10.1007/s12035-017-0778-x PMID: 28940170

62. Ramdas Nair A, Singh P, Salvador Garcia D, Rodríguez-Crespo D, Egger B, Cabenard C, et al. The Microcephaly-Associated Protein Wdr62/Cg7337 Is Required to Maintain Centrosome Asymmetry in Drosophila Neuroblasts. Cell Rep. 2016; 14:1100–1113. https://doi.org/10.1016/j.celrep.2015.12.097 PMID: 26804909

63. Lim NR, Shohayeb B, Zaitseva O, Mitchell N, Millard SS, et al. Glial-Specific Functions of Microcephaly Protein WDR62 and Interaction with the Mitotic Kinase AURKA Are Essential for Drosophila Brain Development. Mol Neurobiol. 2017; https://doi.org/10.1007/s12035-017-0778-x PMID: 28940170
64. Pierfelice T, Alberi L, Gaiano N. Notch in the vertebrate nervous system: an old dog with new tricks. Neuron. 2011; 69:840–855. https://doi.org/10.1016/j.neuron.2011.02.031 PMID: 21382546

65. Jayaraman D, Kodani A, Gonzalez DM, Mancias JD, Mochida GH, Vagnoni C, et al. Microcephaly Proteins Wdr62 and Aspm Define a Mother Centriole Complex Regulating Centriole Biogenesis, Apical Complex, and Cell Fate. Neuron. 2016; 92:813–828. https://doi.org/10.1016/j.neuron.2016.09.056 PMID: 27974163

66. Sgourdou P, Mishra-Gorur K, Saotome I, Henagariu O, Tuysuz B, Campos C, et al. Disruptions in asymmetric centrosome inheritance and WDR62-Aurora kinase B interactions in primary microcephaly. Sci Rep. 2017; 7:43708. https://doi.org/10.1038/srep43708 PMID: 28272472

67. Jauffred B, Llense F, Sommer B, Wang Z, Martin C, Bellasche Y, et al. Regulation of centrosome movements by numb and the collapsin response mediator protein during Drosophila sensory progenitor asymmetric division. Development. 2013; 140:2657–2668. https://doi.org/10.1242/dev.087338 PMID: 23720047

68. Tozer S, Baek C, Fischer E, Goiame R, Morin X. Differential Routing of Mindbomb1 via Centrosomal Satellites Regulates Asymmetric Divisions of Neural Progenitors. Neuron. 2017; 93:542–551. https://doi.org/10.1016/j.neuron.2016.12.042 PMID: 28132826

69. Morgan TH. “The theory of the gene”. The American Naturalist. 1917; 51:513–544.

70. Forsers F, Kavaler J, Tolwinski N, Chou YT, Duan H, Bejarano F, et al. Frequent unanticipated alleles of lethal giant larvae in Drosophila second chromosome stocks. Genetics. 2009; 182:407–410. https://doi.org/10.1534/genetics.109.101808 PMID: 19279324

71. Gilb ET, Hwang GH, Finger DS, Hinnant TD, Drummond-Barbosa D. A genetic mosaic screen reveals ec dysome-responsive genes regulating Drosophila oogenesis. G3 (Bethesda). 2016; 6:2629–2642.

72. Justice N, Roegers F, Jan LY, Jan YN. Lethal giant larvae acts together with numb in notch inhibition and cell fate specification in the Drosophila adult sensory organ precursor lineage. Curr Biol. 2003; 13:778–783. PMID: 12725738

73. Langevin J, Le Borgne R, Rosenfeld F, Gho M, Schweisguth F, et al. Lethal giant larvae controls the localization of notch-signaling regulators numb, neutralized, and Sanpodo in Drosophila sensory-organ precursor cells. Curr Biol. 2005; 15:955–962. https://doi.org/10.1016/j.cub.2005.04.054 PMID: 15916953

74. Roegers F, Jan LY, Jan YN. Regulation of membrane localization of Sanpodo by lethal giant larvae and neutralized in asymmetrically dividing cells of Drosophila sensory organs. Mol Biol Cell. 2005; 16:3480–3487. https://doi.org/10.1091/mbc.E05-03-0177 PMID: 15901829

75. Parsons LM, Portela M, Grzeschik NA, Richardson HE. Lgl regulates Notch signaling via endocytosis, independently of the apical aPKC-Par6-Baz polarity complex. Curr Biol. 2014; 24:2073–2084. https://doi.org/10.1016/j.cub.2014.07.075 PMID: 25220057

76. Portela M, Parsons LM, Grzeschik NA, Richardson HE. Regulation of Notch signaling and endocytosis by the Lgl neoplastic tumor suppressor. Cell Cycle. 2015; 14:1496–1506. https://doi.org/10.1080/15384101.2015.1026515 PMID: 25789795

77. Froldi F, Ziosi M, Garoia F, Pession A, Grzeschik NA, Bellosta P, et al. The lethal giant larvae tumour suppressor mutation requires dMyc oncoprotein to promote clonal malignancy. BMC Biol. 2010; 8:33. https://doi.org/10.1186/1741-7007-8-33 PMID: 20374622

78. Khan SJ, Bajpai A, Alam MA, Gupta RP, Harsh S, Pandey RK, et al. Epithelial neoplasia in Drosophila entails switch to primitive cell states. Proc Natl Acad Sci U S A. 2013; 110:E2163–E2172. https://doi.org/10.1073/pnas.1215231110 PMID: 23708122

79. Agrawal N, Kango M, Mishra A, Sinha P. Neoplastic transformation and aberrant cell-cell interactions in genetic mosaics of lethal(2)giant larvae (lgl), a tumor suppressor gene of Drosophila. Dev Biol. 1995; 172:218–229. https://doi.org/10.1006/dbio.1995.0017 PMID: 7589802

80. Parsons LM, Grzeschik NA, Amarantunga K, Burke P, Quinn LM, Richardson HE, et al. A Kinome RNAi Screen in Drosophila Identifies Novel Genes Interacting with Lgl, aPKC, and Crb Cell Polarity Genes in Epithelial Tissues. G3 (Bethesda). 2017; 7:2497–2509.

81. Vaccari T, Lu H, Kanwar R, Fortini ME, Bilder D. Endosomal entry regulates Notch receptor activation in Drosophila melanogaster. J Cell Biol. 2008; 180:755–762 https://doi.org/10.1083/jcb.200708127 PMID: 18299346

82. Tian AG, Deng WM. Lgl and its phosphorylation by aPKC regulate oocyte polarity formation in Drosophila. Development. 2008; 135:463–471. https://doi.org/10.1242/dev.016253 PMID: 18094021