Genetic Modifier Screens Reveal New Components that Interact with the Drosophila Dystroglycan-Dystrophin Complex

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Abstract

The Dystroglycan-Dystrophin (Dg-Dys) complex has a capacity to transmit information from the extracellular matrix to the cytoskeleton inside the cell. It is proposed that this interaction is under tight regulation; however the signaling/regulatory components of Dg-Dys complex remain elusive. Understanding the regulation of the complex is critical since defects in this complex cause muscular dystrophy in humans. To reveal new regulators of the Dg-Dys complex, we used a model organism *Drosophila melanogaster* and performed genetic interaction screens to identify modifiers of Dg and Dys mutants in *Drosophila* wing veins. These mutant screens revealed that the Dg-Dys complex interacts with genes involved in muscle function and components of Notch, TGF-β and EGFR signaling pathways. In addition, components of pathways that are required for cellular and/or axonal migration through cytoskeletal regulation, such as Semaphorin-Plexin, Frazzled-Neurexin and Slit-Robos pathways show interactions with Dys and/or Dg. These data suggest that the Dg-Dys complex and the other pathways regulating extracellular information transfer to the cytoskeletal dynamics are more intercalated than previously thought.

Introduction

Muscular dystrophies are a group of inherited neuromuscular disorders that share the same basic phenotype of progressive loss of muscle integrity. Many muscular dystrophies are caused by defects in a specialized cell adhesion complex called the Dystrophin Glycoprotein Complex (DGC). It has become evident that this complex plays a central role in muscle integrity and forms a mechanical link from the actin cytoskeleton to the extracellular matrix (ECM). The core DGC is composed of a transmembrane component, Dystroglycan (Dg), which associates with the ECM protein, Laminin and the cytoplasmic protein Dystrophin which binds Actin (reviewed in [1,2]).

Many lines of evidence confirm that maintaining the structural link from the extracellular matrix to the actin cytoskeleton is crucial in preventing many forms of muscular dystrophy. Mutations that disrupt any component of this structural link results in a variety of muscular dystrophies like Duchenne’s, Becker’s, Muscle-eye-brain disease, Walker-Warburg syndrome, congenital muscular dystrophies 1C and 1D as well as limb girdle muscular dystrophy 2I. These diseases share the common symptoms of skeletal muscle degeneration, cardiomyopathy, as well as a reduced life span for afflicted individuals [3].

Additionally, alterations which reduce the affinity of components of the DGC lead to congenital muscular dystrophies like Fukuyama’s which, aside from muscular defects, also are associated with aberrant neuronal migrations that lead to mental retardation, epilepsy, as well as abnormal eye development. The use of animal model systems has led to the clarification of the roles of specific gene products in maintaining muscle integrity and function (reviewed in [4]), however, the regulation of this complex is largely unknown.

Initial characterization of the DGC in *Drosophila* has determined that components studied so far possess similar roles in muscle integrity and neuronal migration in flies as in humans (Figure 1, [5,6,7,8]). These abnormalities include age dependent muscle degeneration, reduced mobility, defects in eye development as manifested by altered photoreceptor axon pathfinding, and a shorter life span. Additionally, mutations in Dys and Dg affect cell polarity in the *Drosophila* germ line as both the follicular cell epithelium and the oocyte are disrupted [6,9,10]. Recently, a reduced lifespan in *Drosophila*, as well as heart and muscle abnormalities, have been reported in mutants of another component of the DGC, δ-sarcoglycan [5]. In addition, heart and eye phenotypes have been observed in *Drosophila* Dys and Dg mutants [11,12].

The similar defects in both flies and humans make *Drosophila* an attractive model for further studies on clarifying the role of the DGC. Such studies may reveal novel components that may likely have counterparts in humans. Additionally, since very little is
known about how the DGC is regulated insights may be gained on this heretofore unknown process. More recently, transmembrane signaling has been implicated in the function of the DGC. The C-terminus of Dystroglycan, in addition to having, EF and WW domain binding sites, also possesses SH2 and SH3 domain binding sites. These known protein-protein interaction motifs support the idea that Dystroglycan is a signaling receptor in addition to its known role as a conduit between the ECM and the cytoskeleton. Changes in MAPK kinase and GTPase signaling have also been observed when the DGC is perturbed [2,13]. Recent work has shown that specific sets of domains are critical in the function of Dystroglycan [8].

In the present work, we have used the genetic tractability of Drosophila to search for novel components of the DGC, as well as components that may be involved in its signaling and regulation. Such a search is straightforward because in addition to the muscle degeneration and photoreceptor axon pathfinding defects, mutations in dystrophin and Dystroglycan cause a visible phenotype manifested as alterations in the fly wing, particularly the posterior crossvein (Figure 2, [14]). Since this is an easily score-able, highly penetrant phenotype we undertook a dominant modifier screen approach and looked for flies that showed either a suppression of the crossvein phenotype or a noticeably altered crossvein. Importantly, crossvein development has been previously shown to require EGFR, TGF-β and Notch pathway activities and is therefore a sensitive place to observe potential interactions of the DGC with these signaling pathways [15,16,17]. In addition, hemocyte migration is shown to correlate with the crossvein development [14]. Therefore genes involved in correct migration processes might also be obtained by this approach. We screened P-element lethal as well as deficiency collections for interactors in addition to performing a classical ethylmethanesulfonate (EMS) screen for dominant modifiers.

Here we report that in using these screening strategies we have found modifiers that belong to six different functional groups. We have found genes involved in muscle development, neuronal/cell migration and motor function as well as cytoskeletal components and components of the TGF-β, EGFR and Notch pathways. A common theme among many of these interactors and Dg-Dys complex is their involvement in the cytoskeletal rearrangements controlled by extracellular cues.

Results

To identify genes that interact with Dys and Dg, we used the chromosomal lesion hypomorph Dys\(^{\text{ES}}\) as well as the RNAi knockdown mutants Dys\(^{\text{N-RNAi}}\), Dys\(^{\text{C-RNAi}}\) and Dys\(^{\text{A-RNAi}}\). These mutants exhibit age dependent muscle degeneration of indirect flight muscles (IFM, Figure 1B and 1C) as shown before for other Dys alleles [6].

In addition to the muscle phenotype, a visible wing vein phenotype was observed in these and previously analyzed alleles. In particular, the posterior crossvein (PCV) is defective in both Dys and Dg mutants, a disrupted anterior crossvein (ACV) and a partial duplication of L2, a longitudinal wing vein, can also be observed at a lower frequency (Figure 2). These phenotypes are present in the RNAi knockdown mutants, Dys\(^{\text{N-RNAi}}\), Dys\(^{\text{C-RNAi}}\) and Dys\(^{\text{A-RNAi}}\) (Figure 2C, 2D and 2E, respectively) as well as the chromosomal lesion hypomorphs Dys\(^{\text{ES}}\) (Figure 2B), Dys\(^{\text{R}}\), Dys\(^{\text{E6}}\), Dys\(^{\text{E7}}\) and Dys\(^{\text{G006}}\) [14]. Dys and Dg are expressed in third instar larval wing imaginal discs (Figure S1) and interact in the wing vein since the double mutant shows a new phenotype – a duplication of L3 longitudinal wing vein (Figure 2F and 2F’).

We used the visual wing vein phenotypes in P-element lethal, EMS induced and deficiency screens to find dystrophin and Dystroglycan interactors in Drosophila that either increase or decrease the wing vein phenotype. Additional studies reveal that many of the interactors identified in the screens are required for proper function in other tissues that require Dys and Dg, such as muscle, brain and ovary.

Modifier Screens

To identify Dys and Dg interactors, we screened three different kinds of mutants; a collection of 800 FRT-P-element lethal lines, a deficiency collection of 216 lines and 43,000 EMS induced mutants. From these analyses, we identified 37 interacting genes that can be clustered into six different functional groups (Table 1).

P-element screen. We screened FRT-P-element lethal lines from the Kyoto Stock Collection [Japan]. The homozygous lethality of the lines allows us to infer that the transposon is inserted in or near an essential gene. We set up crosses to search for modifiers of the RNAi mutants Dys\(^{\text{C-RNAi}}\), Dys\(^{\text{N-RNAi}}\) and Dys\(^{\text{A-RNAi}}\) as well as Dys\(^{\text{ES}}\), a hypomorph that removes DLP2, a specific long
Dys and Dg are Required for Proper Wing Vein Formation and Interact in the Process. In a wild type like wing (A, genotype Dys\(^{+/+}\)) there are 5 longitudinal veins (L1–L5). The anterior cross vein (ACV) forms between L3 and L4 and the posterior cross vein (PCV) forms between L4 and L5. (A) Higher magnification of the region of the wing that shows both cross veins, ACV and PCV. Dys\(^{+/+}\)/Dys\(^{+/+}\) homozygotes show defects in cross vein formation. The RNAi construct that knocks down long forms of dystrophin (act-Gal4:UAS-Dys\(^{RNAi}\)/+) also shows a PCV mutant phenotype where the cross vein fails to attach to L4 and L5 (B). (B') Higher magnification of the image in B where arrows indicate the altered ACV and PCV. Arrows indicate altered cross veins. The RNAi mutant that knocks down long forms of dystrophin (act-Gal4:UAS-Dys\(^{RNAi}\)/+) also shows a PCV mutant phenotype where the cross vein fails to attach to L4 and L5 (C). (C') Higher magnification of the image in (C) where the arrow indicates the altered PCV. (D) Shows the wing vein phenotype of the RNAi dystrophin mutant (tub-Gal4:UAS-Dys\(^{RNAi}\)/+) that reduces the protein levels of all isoforms. Here the PCV is drastically affected and there is extra vein material above L2. (D') Higher magnification of the image in (D) where the arrows indicate the alterations. The upper arrow shows extra wing vein material above L2. The lower arrow indicates an altered PCV. The RNAi Dystroglycan mutant (tub-Gal4:UAS-Dg\(^{RNAi}\)/+) also shows a wing vein phenotype (E). In this case we see a branch off the PCV as well as extra material above L2. (E') Higher magnification of (E) where the upper arrow shows extra wing vein material above L2 and the lower arrow shows branching from the PCV. Finally, Dys and Dg interact in the Drosophila wing as the double mutant (tub-Gal4:UAS-Dys\(^{RNAi}\)/UAS-Dg\(^{RNAi}\)) shows a novel phenotype (F, F'). The box indicates a thickened L3 vein. (F') Higher magnification of the box is shown in (F). Arrows indicate extra L3 longitudinal vein material. (G) Wild type L3 vein from the same region as shown in (F).

doi:10.1371/journal.pone.0002418.g002

isof orm of dystrophin [7]. Modifiers were divided into phenotypic classes of enhancers, suppressors and suppressors with extra wing vein material (En, Su or SuV; Figure 3). From the P-element screen we obtained 33 modifiers of the Dys phenotypes (Table 1), 25 of which also showed interaction with Dg (Figure 4). Since the identity of these genes is known, we were able to organize these modifiers into 6 different functional categories (Table 1).

We were excited to find genes involved in both muscle and motor/cytoskeletal function as these are processes in which Dys and Dg are known to act (Table 1, functional group I; Figure 4). Of the muscle category genes, muscleblind (mb) was the strongest suppressor in the wing vein. Weak suppressors of this group included Calmodulin (Cam) and the nicotinic acetylcholine receptor a 30D (nAChR30D) (Table 1, functional group I), though nAChR30D strongly interacted with Dg (Figure 4). Cytoskeletal and motor function genes (Lissencephaly-1 (Lis-1), Kinesin heavy chain (Khc), Dynamin (Dnm) and Flus) showed moderate to weak suppression with dystrophin mutants (Table 1, functional group I).

Another intriguing functional group that interacts with Dg-Dys complex includes genes known to be involved in neuronal migration (Table 1, functional group II). This group is composed of the genes which belong to Semaphorin-Plexin (Sema-1a, Sema2a, Slit-Robo (slit, sli), roundabout (robo), leak (lea) – a robo2 homolog, Syndecan (Sde) and Netrin-Frazzled (fraz, fra) pathways. In the wing vein most of these genes were scored as moderate to weak suppressors of the Dys RNAi mutants. Importantly, Sema-2a and fra modify Dys\(^{EG}\) (Table 1, functional group II; Figure S3). Sema-1a was found to moderately enhance the Dys wing vein phenotype. Using our Dys\(^{RNAi}\) mutant we found that robo and Sema-2a enhanced the Dg phenotype and Sema-1a and lea suppressed the Dg phenotype (Table 1; Figure 4 and Figure S4). We have previously shown that Dys and Dg function in the Drosophila Photoreceptor axon pathfinding [6] in processes similar to Semaphorin-Plexin, Slit-Robo and Netrin-Frazzled pathways.

Functional groups IV and V (Table 1) contain interactors that belong to the TGF-β signaling pathway: Daughters against dpp (Dad), decapentaplegic (dpp), thickveins (tv) and EGFR signaling pathway: kkkon-1 (kkk1) and avgd. Dad, dpp and kkk1 were strong modifiers of the Dg\(^{RNAi}\) mutant (Table 1; Figure 4 and S4) and Dad and tv of Dys\(^{EG}\) allele as well (Table 1, functional group IV; Figure S3). It has been known for some time that TGF-β, Notch and EGFR pathways are necessary for proper wing vein development [18]; reviewed in [19,20]). In fact, many novel factors of these pathways have been identified through the analysis of wing vein mutants. The longitudinal wing veins that begin to form during late larval stages of development require EGFR and TGF-β signaling pathways for proper fating of cells in the region [15,21]. Crossveins appear in late pupal stages of development and require TGF-β signaling for formation and EGFR and Notch signaling for final patterning [17]. The posterior crossvein is particularly sensitive to different levels of TGF-β signaling and forms only after the proper formation of the longitudinal veins.

The last functional group (Table 1, functional group VI) contains a group of 12 genes with disparate functions. One enhancer, glial cells missing (gcm), was identified. Three strong suppressors of this group also interacted with Dys\(^{EG}\), the Drosophila homolog of the homeodomain-interacting protein kinase (HIPK), kismet (kis) and l(3)S4092, which contains a zinc-finger motif, kis, deadlock (de), gcm, POSH and wuman (wun) also strongly modified Dg\(^{RNAi}\) (Figure 4).
| Functional groups | Gene name | Allele(s) | Function | act-Gal4: UAS-Dys<sup>E</sup> <sub>max</sub>/+ | tub-Gal4: UAS-Dys<sup>E</sup> <sub>max</sub>/+ | Dys<sup>E</sup> <sub>int</sub>-<br>interactors<sup>2</sup> |
|------------------|-----------|-----------|----------|------------------|------------------|-----------------|
| I. Muscle, motor<sup>1</sup> and cyto-skeleton function | Dys<sup>EMS-ModE10</sup> | | Dyson-Dys complex | - | - | En (S) + |
| Cam<sup>1</sup> | Cam[k04213] | Calmodulin, muscle contraction | Su (W) | - | - |
| mbl<sup>1</sup> | l(2)k04222b<sup>+</sup> | muscle development; splicing; RNA binding | Su (S) Su+ (S) | - | - |
| nAcR<sup>30D</sup> | l(2)k14204<sup>+</sup> | acetylcholine receptor; muscle contraction | Su+ (W) Su+ (W) | - | - |
| Lis-1<sup>1</sup> | Lis[1]k13209<sup>+</sup> | Dynein binding, WD repeats | - | Su+ (M) - | - |
| Khc<sup>1</sup> | Khc[k13219] | Kinesin, microtubule motor | - | Su+ (M) - | - |
| Dmn<sup>1</sup> | Dmn[k16109] | Subunit in Dynactin complex | Su+ (M) | | |
| Fhos<sup>1</sup> | l(3)SB6<sup>+</sup> | Actin organizing protein | Su+ (M) Su+ (M) | Mod (M) | + |
| II. Neuro-nal migration or planar cell polarity genes | sema-2a<sup>1</sup> | sema-2a[k13416]<sup>+</sup> | ligand of PlexinB, axon guidance | Su (W) Su+ (W) Mod (M) + |
| sema-1a<sup>1</sup> | sema-1a[k13702]<sup>+</sup> | axon guidance | En (M) En (M) - | + |
| fra<sup>1</sup> | l(2)k03003<sup>+</sup> | receptor of Netrin | Su+ (M) Su+ (M) Mod (W) |
| sl<sup>1</sup> | l(2)k02205<sup>+</sup> | ligand (interacts with Robo and Sdc) | Su (W) Su+ (W) Mod (W) |
| robo<sup>2</sup> | robo[k13219]<sup>+</sup> | receptor | Su+ (W) Su+ (W) - | + |
| Sdc<sup>1</sup> | l(2)k10317<sup>+</sup> | Heparan sulphate proteoglycan (interacts with Sli and Robo) | Su+ (S) Su+ (M) - |
| stan<sup>1</sup> | stan[k13702]<sup>+</sup> | Receptor, Flamingo | Su+ (M) Su+ (M) Mod (W) |
| wg<sup>1</sup> | wg[k10317]<sup>+</sup> | ligand | Su+ (S) Su+ (S) Mod (M) + |
| grh<sup>1</sup> | grh[k12140]<sup>+</sup> | transcription factor | Su+ (S) Su+ (S) Mod (W) + |
| III. Notch signaling | DI<sup>1</sup> | DI[EMS-Mod130]<sup>+</sup> | ligand of Notch | Su+ (S) Su+ (M) Mod (S) + |
| DI[EMS-Mod140]<sup>+</sup> | Su+ (S) Su+ (M) Mod (S) + |
| IV. TGF-β signaling | Dad<sup>1</sup> | Dad[k16109]<sup>+</sup> | negative regulator | Su+ (S) Su+ (S) Mod (M) + |
| dpp<sup>1</sup> | dpp[kG08191]<sup>+</sup> | TGF-β homolog | Su+ (M) Su+ (W) - | + |
| tkv<sup>1</sup> | tkv[k16713]<sup>+</sup> | type I receptor | - | - | Mod (M) |
| msk<sup>1</sup> | msk[EMS-Mod90]<sup>+</sup> | Importin | En (S) En (S) En (S) + |
| msk[k5]<sup>1</sup> | msk[k5]<sup>+</sup> | Su (W) Su+ (M) + |
| V. EGFR signaling | kek1<sup>1</sup> | kek1[k07322]<sup>+</sup> | repressor of EGFR signaling | Su+ (S) Su+ (M) - | + |
| argos<sup>1</sup> | l(3)10B8<sup>+</sup> | repressor of EGFR signaling; ligand | Su+ (W) - | - |
| argos[Delta7]<sup>1</sup> | argos[Delta7]<sup>+</sup> | Su+ (S) - | - |
| Vi. Other | Nrk<sup>1</sup> | Nrk[k14301]<sup>+</sup> | receptor tyrosine kinase | Su (W) Su+ (M) - | - |
| HIPK<sup>1</sup> | CG17090 [B0G0855]<sup>+</sup> | serine/threonine kinase, death | Su+ (S) Su+ (S) Mod (S) - |
| kis<sup>1</sup> | kis[k13416]<sup>+</sup> | ATP helicase activity; chromatin binding | Su+ (S) Su+ (S) Mod (W) + |
| gc<sup>1</sup> | gc[kG0117]<sup>+</sup> | transcription factor activity | En (S) En (M) - | + |
| CG4496<sup>1</sup> | CG4496[kG10365]<sup>+</sup> | zinc ion binding, nucleic acid binding | Su (S) Su+ (S) - |
| wun<sup>1</sup> | wun[k10201]<sup>+</sup> | lipid phosphate phosphatases | - | Su+ (W) - | + |
| l(2)k11120<sup>+</sup> | l(2)k11120a<sup>+</sup> | Su+ (W) - | - |
| POSH<sup>1</sup> | POSH[15815]<sup>+</sup> | SH3 adaptor protein, JNK signaling | Su (W) Su+ (M) - | + |
| vimal<sup>1</sup> | vimal[k16722]<sup>+</sup> | Ral GTPase binding | Su (W) Su+ (M) - | + |
| del<sup>1</sup> | del[kG10262]<sup>+</sup> | oogenesis | - | Su+ (S) + |
| SP1070<sup>1</sup> | Poly-EGF [EMS-Mod29]<sup>+</sup> | Notch binding (predicted) | Su+ (M) Su+ (M) - |
Deficiency screen. In addition to the P-element lethal collection, we also screened for the Dys interactors using a collection of deficiency lines, which covers about 30% of the 1st, 40% of the 2nd and 80% of the 3rd chromosome and found 10 regions on the 2nd and the 3rd chromosomes that interacted with $D_{6}^{N,RNAi}$ in the wing vein (Figure 5A). Nine of these deficiencies suppressed the Dys PCV phenotype with formation of extra wing vein material and belonged to the Su+ class (Figure 3C and 5B) and one to the En class (Figure 3D and 5B). The $cv$ and $stan$ (Flamingo, Fl) genes, which belong to the planar cell polarity pathway (Table 1), were identified as $D_{6}^{N,RNAi}$ interactors in [27D1-27F2] and [47A7-47C6] cytological regions respectively (Figure 5B). Both of these genes modify $D_{6}^{N,RNAi}$ phenotype in wing vein as well (Figure 4). A third component of the planar cell polarity pathway, grainy head (grh), was identified from the P-element screen. Furthermore, dystrophin and $Dl$ genes located in region [91F12-92A11], as well as poly-EGF and $CG4496$ from [27D1-27F2] region were also independently identified in the P-element and EMS screens (Figure 5B and Table 1).

EMS mutagenesis screen. We analyzed ~37,000 chromosomes for modifiers of $D_{6}^{N,RNAi}$ and $D_{6}^{N2,RNAi}$ and over 6000 chromosomes for enhancers of $D_{6}^{R}$ and isolated 27 modifiers (Table S1). Eighteen of these were localized to the 2nd chromosome and nine were localized to the 3rd chromosome. The genes defective in eight of these mutants were identified and shown to correspond to five genes (Table 1). The modifier screens using EMS produced similar phenotypes to those observed in the P-element screen (Figure 4) and additionally produced a “posterior crossveinless” class (14/27 modifiers; Figure S2). These modifiers may be alleles of the crossveinless genes on the second and third chromosomes. There are eight previously identified crossveinless loci, $cv$, $cv-2$, $cv-3$, $cv-b$, $cv-c$, $cv-d$, $cvl-5$ and $cvl-6$. Two, $cv$ and $cvl-6$, are on the X chromosome. One, $cv-3$ is on the second chromosome and the remaining five are on the third chromosome. Modulators of the TGF-$\beta$ pathway are encoded by two of the eight crossveinless loci, $cv$ and $cv-2$. Of the remaining six one other, $cv-c$ has been molecularly characterized and encodes a Drosophila Rho GTPase Activating Protein. The others may also encode effectors of TGF-$\beta$ signaling. Nevertheless, when $cv$ and $cv-2$ are lost the resultant aberrant TGF-$\beta$ signaling results in the loss of both crossveins. Of our crossveinless like modifiers, one, Mod90 (Table 1 and Table S1), was mapped to cytological location 66B, two map units distal to the hairy gene, and so does not appear to be an allele of any of the crossveinless genes on the third chromosome. An attractive candidate for this gene is moleskin ($msk$) which encodes Drosophila Importin-7, a protein involved in nuclear translocation that has been shown to regulate the TGF-$\beta$ pathway by controlling Mad localization. We further confirmed that moleskin ($msk^{5}$) interacts with $Dg$ and $Dys$ (Table 1).

The next largest class of modifiers (9/27 EMS induced modifiers; Table S1) belonged to the Suppressor+ (Su+) class with extra wing vein material (Figure 3) and showed a more global effect on the wing. Four of these modifier mutants, Mod59, Mod111, Mod130 and Mod140, had phenotypes in the absence of

![Figure 3. Wing Vein Phenotypes in Dys Modifier Classes.](image-url)

The posterior cross vein (PCV) was used in the screening process. The Dys mutant is depicted schematically in (A) with an actual fly wing (act-Gal4: UAS-$Dys^{N,RNAi}$) shown to the right. Among the modifiers from the original mutant phenotype was the Su class or the completely suppressed class (B) where the PCV reverted to the wild type cross vein. Another class of interactors suppressed the detached PCV phenotype but also produced extra vein material, either as a branch or as an extra L2 vein (arrows, C). This group was classified as suppressor-plus (Su+). Finally, a group of modifiers showed a complete loss of the PCV (D, arrow) and this group was classified as enhanced (En).

Table 1. cont.

| Functional groups | Gene name | Allele(s) | Function | act-Gal4: UAS-$Dys^{N,RNAi}$/+ | tub-Gal4: UAS-$Dys^{N,RNAi}$/+ | $Dys^{N2}$/+ | $Dg$ interactors |
|-------------------|-----------|-----------|----------|-----------------------------|-----------------------------|----------------|------------------|
| CG7845            | CG7845[EMS-Mod4] | WD40 domain protein | Su+ (M) | Su+ (M) | - | |
| I(3)L4092         | I(3)L4092 | Zn-finger protein | Su+ [S] | Su+ [S] | Mod [S] | |
**Figure 4. Dg Mutant Modifiers.** The RNAi Dystroglycan mutant modifiers were scored either as an increase in penetrance of the Dg phenotype, or as an addition of extra vein material. These are represented in a bar graph. The ordinate indicates the percent penetrance of the wing vein phenotype. The abscissa indicates the genes that interact with tub-Gal4:UAS-DgRNAi/+ as heterozygotes. The unmodified Dg mutant phenotype is at the far left (control) and shows that nearly 30% of DgRNAi/+ flies show the Dg mutant phenotype (orange color) with the rest having wild type wing veins (yellow color). Modifiers that show an extra vein material are indicated in brown. Wing vein phenotypes are shown in Figure S4.

doi:10.1371/journal.pone.0002418.g004

**Figure 5. Deficiency Screen.** (A) Cytological map of 2nd and 3rd chromosomes. Red bars represent cytological regions that were screened for interaction with Dys in wing vein. Blue bars represent deficiencies tested in the screen. Deficiencies that showed interactions are circled in red. Numbers next to the blue bars indicate deficiencies used to narrow down the Dys interacting region. (B) Numbers in column 1 describe the deficiencies used to narrow down region and respond to the following deficiencies: (1) w'; Df(2L)spdj2, wg^{en-l2}, (2) w^{en-l2}, Df(2L)ade3, (3) Df(2L)ED1473, (4) Df(2R)ED2098, (5) Df(2R)ren-B, b^{pr-l}, (6) Df(2R)ren-A, (7) Df(2R)PC4, (8) y^{en-1}/Dp(1;2)Jy': Df(2R)P34, (9) Df(3L)ED4978, (10) Df(3R)ED5612, (11) Df(3R)ED5942, (12) Df(3R)ED6025, (13) Df(3R)ED6069, (14) Df(3R)ED6076, (15) Df(3R)ED6265, (16) Df(3R)Tl-P, e^{ca-l}. Class of wing vein phenotype modifiers is listed in second column. Cytology of 10 Dys interacting regions found in the screen is shown in column 3. Column 4 indicates Dys interacting genes found in deficiency, EMS (*) and P-element (**) screens.

doi:10.1371/journal.pone.0002418.g005
the dystrophin mutant. We mapped them to cytological location 92A and showed that they are tightly linked to the Delta gene (DI, Table 1). Subsequent crosses to different DI alleles yielded lethal phenotypes, suggesting that these mutants are alleles of DI. Two of the modifiers ModI30 and ModI40 suppressed the posterior crossovein phenotype of DysN-RNAi, i.e. formed a complete crossovein from longitudinal vein L4 to longitudinal vein L5, in addition to generating extra wing vein material. We also found modifiers that belonged to the Enhancer as well as the Suppressor classes (Text S1).

Two other members of the class of mutants with extra wing vein material (Table S1 and Figure S2B), ModI4 and ModI29 were mapped to specific locations on the 2nd chromosome. ModI4 was mapped by following its lethality phenotype in crosses with deficiency lines. It was lethal in a cross with the Df(2R)Map9/Dys(2)BG, In(2L)Gia line and was not lethal in a cross with the Df(2R)ST1, Adh[ns] pr[1] en[*]/CyO line. The available lethal mutants from cytological region 42A1-42B3 were crossed to ModI4 and using a P-element insertion line, PBac{XP5.WH5}Exl7029/CyO we determined that the mutation was in gene G77845. This gene codes for a WD40 containing protein whose function is not known (Table 1). The underlying common function of all WD-repeat proteins is coordinating multi-protein complex assemblies, where the repeating units serve as a rigid scaffold for protein interactions. Such a scaffolding protein may be utilized in the formation or stabilization of the DGC.

Following the wing vein phenotype seen in homozygous flies as well as its semi-lethality, ModI29 was mapped to cytological region 27D1-27D4. This region was independently identified to contain a Dys interactors through the Deficiency screen (Figure 5B). We further fine-mapped the region by showing that two smaller deficiency lines, Df(2L)ade3/CyO, P{J/::-}/CyO E3 and Df(2L)Exel7029,P{Bac(XP5.WH5)}Exel7029/CyO phenocopy the semi-lethality/wing vein phenotypes seen with ModI29. Ultimately, ModI29 was determined to be a poly-EGF gene mutant. Other genes in the region were eliminated by complementation analysis. In addition to the wing vein phenotype, ModI29 also shows defects in muscles, in oogenesis and in the brain (photoreceptor axon termination; Figure S5). Additionally, these mutant animals have a very short lifespan. Since Poly-EGF is predicted to bind the Notch receptor further functional analysis will be very interesting.

As discussed above, the DysE6 mutant does not show a wing vein phenotype as a heterozygote (Figure 2A). But as a homozygote it displays a disrupted posterior crossovein similar to DysN-RNAi (Figure 2B and 2C). We reasoned that this heterozygote background was “sensitized” and therefore excellent for identifying enhancers. We screened 6000 chromosomes and found four modifiers for DysE6/+ . Three of those modifiers showed some extra wing vein material in the posterior crossovein (Figure S2B). Two of the modifiers, on the third chromosome ModE21 and ModE26 showed a branched crossovein phenotype in the absence of DysE6 though this dominant phenotype was not completely penetrant. The third of this group, ModE11 on the second chromosome, showed no wing vein phenotype in the absence of DysE6, however as a homozygote ModE11 flies displayed a globular disrupted wing vein characterized by an abundance of extra vein material (data not shown). Modifier E10 was the only modifier that showed an enhanced phenotype (Figure S3A). ModE10/DysE6 flies phenocopied the DysE6 homozygous phenotype which is a reduction in wing vein material (Figure 2B). Moreover, ModE10/Df[3R]Exel6184 flies also gave a similar wing vein phenotype to DysE6/Df[3R]Exel6184 flies strongly arguing that ModE10 is a dystrophin allele. Finding an additional allele of Dys confirms that the screening approach was successful and analysis of the EMS mutant will allow us to further characterize Dystrophin protein function. Furthermore, the newly identified dystrophin allele ModEl0 interacted strongly with the Dys RNAi mutant in the wing; instead of 27% penetrance, the phenotype was 96% penetrant in heterozygous Dys[Mod-E10] background (Figure 4). This further confirms that molecules acting closely with Dys and Dg can be found in this assay.

mbl Interacts with Dys in Muscles

These modifier screens led to the identification of genes that belong to different functional groups. Importantly, genes with known muscle function were identified in this screen, including dystrophin itself. In addition, the screen also identified muscle genes previously shown to interact with the Dg-Dys complex, Cnm, mbl and nkdRa-3D [22,23,24]. muscleblind encodes a RNA binding protein that has shown to function in splicing [25]. As part of a secondary screen in muscle tissue, we tested whether mbl, a strong interactor with Dys in wing vein, also interacts with Dys in the indirect flight muscles.

Animals that have lost one copy of mbl appear to have normal muscle structure (Figure 6A), while act-Gal4,UAS-DysN-RNAi mutants show moderate muscle degeneration (10 days old: 10%, n = 68; 18 days: 25%, n = 159; Figure 6B and 6D). However, the extreme muscle degeneration phenotype was not observed in 10 days old and at a low frequency in 18 day old DysN-RNAi mutants (Figure 6B and 6D). In contrast, when mbl was reduced by one copy in a DysN-RNAi mutant background, increase in muscles defects was observed. Furthermore, around 50% of the phenotypes observed were classified “extreme” both in 10 and 18 day timepoints (Figure 6C and 6D). These data suggest that mbl and Dys interact in the muscles and reduction of muscleblind level enhances the abnormal muscle phenotype in DysN-RNAi mutants. These results show that the screen successfully identified genes that interact with dystrophin to establish normal muscle function.

Interactors Show Germline Phenotypes Similar to Dys and Dg Mutants

The Dys and Dg genes are required in the germ line for the establishment of oocyte polarity [6,8,9]. The oogenesis defects of Dys and Dg serve as an excellent test to identify whether the new modifiers might interact with Dg-Dys complex in other tissues as well as in wing development. The ovariole contains a progression of egg chambers at different stages of development (Figure 7A). An early oocyte polarity marker, Orb, is localized to the anterior side in early stages and then migrates to the posterior side of oocyte by stage 3 of oogenesis. Between stages 3 and 6, Orb is clearly localized to the posterior of the oocyte, making it an excellent marker to analyze the polarity of the oocyte (Figure 7A-B). Dy loss-of-function germ line clones and homozygous point-mutants are arrested at early stages of oogenesis [9]; Figure 7B) and show mislocalization of the Orb marker that is usually missing or diffused [9]; Figure 7D). The oocyte polarity phenotype was also observed for viable Dys and Dg alleles (Figure 7B, 7D and 7E; 74%, n = 50 for Dys008/Dys055 82%, n = 34 for Dys043/Dys055 and 68%, n = 48 for Dys008/Dys043; the ovaries were dissected from 12 days old mutants).

Interestingly, many of the identified modifiers are genes previously shown to be required in germ line development. For example a scaffolding protein, Rack1 that contains multiple WD-domains and interacts with atypical protein kinase C (aPKC) has recently been shown to function during Drosophila oogenesis [26]. We now analyzed potential Rack1 function in germ line for posterior Orb localization. Importantly, this analysis revealed that Rack1 is required in the early oocyte polarity: lack of Rack1 in
early oocytes resulted in Orb mislocalization in the majority of the eggchambers (76%, n = 13, Figure 8H). Consistent with these findings aPKC is required for early oocyte development in Drosophila [27]. Similarly, another Dys and Dg interacting protein, Deadlock has previously shown to be required for Orb localization [28]. It is, therefore, likely that other identified Dys and Dg interactors are required in early oocyte polarity, possibly in a process closely related to Dg-Dys function in oogenesis.

Utilizing the FRT sites in the P-lethal lines we made germ line clones to determine how many of the selected modifiers had a role in the establishment of early oocyte polarity. We analyzed germ line clones of 14 loss-of-function mutants and found 8 genes that showed a similar phenotype to that seen in Dg loss-of-function clones. One of these genes, POSH, is an SH3 adapter protein. Loss-of-function clones of POSH (Figure 8A, white arrow) were arrested prior to stage 3–4 of oogenesis and appeared to lack Orb entirely. Orb mislocalization was also found in Sema-2a, kek1, kis, Lis-1, chif and Khc. Sema-2a loss-of-function clones showed clear developmental arrest prior to stage 4–6 with diffused Orb staining (Figure 8B). kek1, a negative regulator of the EGFR pathway, also showed developmental arrest in loss-of-function clones (Figure 8C). Orb in kek1 clones surround the oocyte in an irregular pattern. kis loss-of-function germine clones have shown developmental arrest and diffused Orb staining (Figure 8D). Similarly, the majority of Lis-1, chif and Khc germine clones showed abnormal Orb localization (Figure 8E–8G). Lis-1 and Khc have previously shown to be involved in oocyte microtubular motor activities and to interact in this process [29,30].

These data indicate that many of the genes that showed interactions with Dys and/or Dg in the wing vein assay, also showed early oocyte polarity phenotypes similar to Dys and Dg and potentially play a role in the establishment of early oocyte polarity.

**Discussion**

In mammalian systems the DGC is composed of Dystrophin, Dystroglycan, the Sarcoglycan complex (α, β, γ, δ, ε, and ζ), Sarcospan, the Syntrophins (α and β), the Dystrobrevins (α and β) and Caveolin-3 [2]. Presently, the regulation of Dg-Dys complex and its involvement in signaling are poorly understood. In this work, we have addressed these unknowns by using dominant modifier screens to find genes that may shed light on both of these processes. Our screens have revealed groups of modifiers that are components of canonical signaling pathways (TGF-β, EGFR, Wnt and Notch) as well as components involved in cell/neuronal migration, cytoskeletal rearrangements and most importantly muscle development.

**New Components of the Dg-Dys Pathway?**

The screens described in this paper revealed some expected interactors, Dys, Cam and Khc. Calmodulin, a calcium binding protein required for muscle and neuronal functions has previously been shown to interact with mammalian the Dg-Dys complex. However, whether reduction of Cam activities suppresses or enhances the muscular dystrophy phenotype is not totally clear. Targeted inhibition of Cam signaling exacerbates the dystrophic
The Neuronal/Cell Migration Pathways Interact with the DGC

There are only a few examples of signaling pathways that have been shown to transmit information from outside the cell that results in cytoskeletal rearrangements inside the cell. Slit-Robo, Netrin-Frazzled and Semaphorin-Plexin pathways are examples of such activity. Dg-Dys complex appears also regulate the cytoskeleton based on extracellular information. Interestingly, the interaction screens described in this paper show that these aforementioned pathways are much more intricately connected than previously thought. The Robo and Netrin Receptor (DCC) pathways have previously been shown to interact [36], now we report that Dg-Dys complex interact with these pathways as well.

The interactions that we see in wing development involving the Drosophila DGC and the genes that affect neuronal guidance (sli, robo, fra, sema-2a, sema-1a, Sdc) might be explained by their possible role in hemocyte (insect blood cell) migration. Analysis done in Drosophila shows that known axon guidance genes (sli, robo) are also implicated in hemocyte migration during development of the central nervous system [37]. Similar findings have been reported in mammals, where blood vessel migration is linked to the same molecular processes as axon guidance [38]. Both sli and robo have been implicated in the vascularization system in vertebrates [39]. A recent study demonstrated that proper hemocyte localization is dependent upon Dys and Dg function in pupa wings. Mutations in these genes result in hemocyte migration defects during development of the posterior crossvein [14]. Hence, we speculate that the neuronal guidance genes that we have found may interact with the DGC in wing veins by having a role in the migration process.

Similar to sli and robo [40], the Dys and Dg mutants also affect photoreceptor axon pathfinding in Drosophila larvae [6]. It is therefore possible that this group of modifiers will interact with the DGC in axon pathfinding and other processes. Supportive of that notion is the fact that mammalian Syndecan-3 and Syndecan-4 have been implicated in the migration process [41]. In addition sli-Dg interaction has previously been observed in cardiomyocyte alignment [42]. Sequence analysis of sli reveals that it possesses a laminin G-like domain at its C-terminus. Dystroglycan's extracellular domain has laminin G domain binding sites and has been shown to bind 2 of the five laminin G domains in laminin. It is therefore possible that sli, through its laminin G-like domain, binds to Dystroglycan and that Dystroglycan is a slit phosphorylating p33 [34]. Recently Drosophila HIPK has shown to be involved in a communal form of cell death, sudden, coordinated death among a community of cells without final engulfment step [35]. It remains to be seen whether this HIPK communal death pathway will utilize p33 phosphorylation. However, it is tempting to speculate that the cell death observed in muscular dystrophies use the newly described HIPK dependent communal death pathway. HIPK is shown to interact with a WD40-protein in mammalian system [35]. Since three WD40 proteins were identified in our screens, it will be interesting to test whether any of these interact with Drosophila HIPK.

Another interactor that might shed light in the pathways utilized by the Dg-Dys complex is an SH3-domain adapter-protein, POSH. Structure-function analysis of Dg protein has revealed that a potential SH3-domain binding site in Dg C-terminus is essential for Dg function [8]. However, the critical SH3-domain protein in this complex is still at large. The present screen revealed that POSH can interact with the Dg-Dys complex in the wing vein assay. It will now be interesting to determine whether POSH is the missing critical SH3-domain protein that interacts with Dg-Dys complex in Drosophila.

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receptor. It will be informative to reveal the mechanisms and nature of these interactions.

**Oocyte Phenotypes**

The establishment and formation of oocyte polarity during development is a complex multistep process (reviewed in [43]). In the anterior part of the germarium each stem cell undergoes asymmetric cell division to give rise to another stem cell and a cystoblast. The cystoblasts divide four times with incomplete cytokinesis to form a 16-cell cyst. The oocyte fate is determined when the cyst reaches the end of the germarium. At this point, BicD protein, Orb protein, the microtubule organizing center (MTOC) and the centrioles move from the anterior to the posterior of the oocyte (Figure 7A). These events mark the first sign of polarity in the oocyte. Subsequent Gurken signaling induces posterior follicle cells to signal back to the oocyte which repolarizes the microtubule cytoskeleton. This signal appears to require an intact extracellular matrix since Laminin A mutants do not undergo repolarization [44]. The outcome of the repolarization results in the disassembly of the MTOC at the posterior, nucleation of microtubules anteriorly and subsequent migration of the oocyte nucleus to an antero-lateral position.

Germ line clones that lack Dg show developmental arrest and mislocalization of the oocyte polarity marker Orb which is usually diffused or absent in the oocyte [9]. This phenotype might be due to Dg affecting the localization of the MTOC. But how exactly Dg is involved in this process is not clear. One possible explanation is that Dg is required for extracellular matrix (ECM) integrity since it is known to bind Laminin. Such a structural conduit may be necessary for proper signaling from the posterior follicle cells to the oocyte. This is supported by the fact that Dg loss-of-function mutants show defects in Actin accumulation. Another possibility is that Dg may be involved in microtubule organization. Since the regulation of actin- and microtubule-cytoskeleton are connected, these two models are not mutually exclusive.

Interestingly, in our screens we found several genes that showed phenotypes in oocyte development. One of these genes is kek1, a transmembrane protein of the Drosophila Kekkon family that has been reported to be a negative regulator of the EGFR receptor [45]. It has been previously shown that EGFR signaling regulates...
the expression pattern of Dystroglycan to establish anterior-posterior polarity of oocyte [10]. Further study is required to determine if \( \text{kohl} \), as an EGFR regulator controls \( \text{Dg} \) expression in the germ line.

Another interesting gene found in our screens is \( \text{POSH} \) (Plenty of SH3 domains), a \( \text{Drosophila} \) homologue of human \( \text{SH3MD2} \) protein. Interestingly \( \text{POSH} \) is a multidiomain scaffold protein that can interact with \( \text{Rho} \) related \( \text{GTPase - Rac1} \) and promotes the activation of the \( \text{JNK} \) pathway [46] \( \text{POSH} \) has also shown to regulate \( \text{POSH-MLK-MKK-JNK} \) complex [47]. A defect in this complex can affect brain function. Furthermore, \( \text{POSH} \) and \( \text{JNK} \)-mediated cell death pathway is thought to play an important role in Parkinson’s disease [48]. With many \( \text{SH3} \) domains, \( \text{POSH} \) has the potential to bind \( \text{Dg} \) that has a predicted \( \text{SH3-domain} \) binding site and has been shown to be necessary for the establishment of oocyte polarity [8].

In addition, we have found interactions with \( \text{Khc} \), \( \text{Liv-1} \) and \( \text{Dmn} \), three genes known to be part of the Dynenein-Dynactin complex which in addition to \( \text{Kinesin} \) microtubule motor activity have been shown to be necessary for establishment of intracellular polarity within the \( \text{Drosophila} \) oocyte [49,50]. In mid-oogenesis dynenein, dynactin and kinesin are thought to act cooperatively in cargo transport [49,51,52]. Since these genes interact with \( \text{Dys} \) (Table 1) and show similar phenotypes in Orb localization (Figure 7), it will be interesting to dissect their potential functional interactions with \( \text{Dys} \) in early oocyte development. Furthermore, since mammalian Dystrobrevin physically interacts with \( \text{Khc} \), it is plausible, that the Dynenein-, Dynactin-, Kinesin-complex will utilize localization cues set-up by \( \text{Dg-Dys} \) Complex.

In addition to the interactions with microtubular motor-complexes, we also found interactions with a \( \text{Drosophila} \) Formin homologue, \( \text{FHOS} \). Mammalian \( \text{FHOS} \) directly binds to \( \text{F-actin} \) and promotes actin fiber formation [53]. Recently \( \text{Drosophila} \) actin nucleators, Capu and Spire have shown to assemble a cytoplasmic actin mesh that maintains microtubular organization in the middle of oogenesis [54,55,56,57,58]. Therefore, it will be important to determine whether the actin nucleator, \( \text{FHOS} \) is also involved in actin nucleation that regulates microtubular activity in early oocyte development. Further study of these cytoskeletal genes will allow us to gain a more detailed understanding of how \( \text{Dg} \) and \( \text{Dys} \) function to ensure proper oocyte polarity during oogenesis.

Similar to microtubule and actin interplay in the regulation of oocyte polarity, the dynamic actin-microtubule interactions regulate growth cone steering at the growing axons [59]. It is therefore possible that similar mode of function for \( \text{Dg-Dys} \) interactions with these cytoskeletal modules is used in various cell types. Furthermore the axon pathfinding and oocyte polarity formation processes are similar in that they are controlled by extracellular information which is transmitted to the cell resulting in cytoskeletal rearrangement.

**Planar Cell Polarity Genes**

At the basal side of follicle epithelium, actin filaments exhibit a planar cell polarity that is perpendicular to the long axis, the AP axis, of the egg chamber. In \( \text{Dg} \) follicle cell clones the basal actin array is disrupted non-cell-autonomously [9]. Integrins and the receptor tyrosine phosphatase \( \text{Lar} \) are also involved in basal actin orientation [60]. It is unclear whether \( \text{Dg} \) and the other genes involved in basal actin polarity act together with the canonical planar cell polarity pathway or function independently of this pathway. Interestingly, we now report strong interactions between the DGC and \( \text{granny head} \) (\( \text{gh} \)) a transcription factor which is required for several different processes during the differentiation including the function of the \( \text{frizzled} \) dependent tissue polarity pathway, epidermal hair morphogenesis and wing vein specification [61]. In the absence of \( \text{gh} \) function the \( \text{Fz}, \text{Dsh} \) and \( \text{Vang} \) proteins fail to accumulate apically and the levels of \( \text{Stan} \) (or Flamingo) protein are dramatically decreased. The interactions seen with \( \text{stan} \) (\( \text{Flh} \)) and \( \text{ag} \) in wing veins supports the hypothesis that \( \text{Dg} \) might act together with the \( \text{frizzled} \)-dependent tissue polarity pathway in coordinating the polarity of cells in epithelial sheets.

### Conclusions

For screening alterations of a dominant wing vein phenotype we have found modifiers of the DGC that are involved in cytoskeletal organization. Initial characterization of some of these genes revealed that they have phenotypes also in other tissues, in which the DGC is known to function. These tissue/cell types include the oocyte, the brain and the indirect flight muscles. This argues strongly that the identified interactors may be involved globally in DGC function. Further study is required to determine mechanistically how these modifiers work in the context of the \( \text{Dg-Dys} \) complex. However a common theme, already arising is that the identified interactors appear to regulate cytoskeletal rearrangement. Mechanistic understanding of how the new interactors might regulate \( \text{Dg-Dys} \) communication with cytoskeleton of muscle cells may serve as a basis for the development of novel therapeutic approaches that might improve the quality of life of individuals afflicted with muscular dystrophy.

### Materials and Methods

#### Fly Strains and Genetics

The fly-strains used is in this study are: \( \text{Dys}^{ \text{del}}/\text{TM3}, \) \( \text{UAS-Dys}^{ \text{N-RNAi}}/\text{CyO} \) [both kindly provided by L.Fradkin and described previously as \( \text{dys}^{ \text{del}}/\text{E} \) and \( \text{RNAi-dysNH}_2 \) [62]], \( \text{UAS-Dys}^{ \text{N2-RNAi}}/\text{TM3}, \) \( \text{UAS-Dys}^{ \text{C-RNAi}}/\text{TM3}, \) \( \text{UAS-Dys}^{ \text{del}}/\text{TM3} \) (described previously as \( \text{UASd/Dys}^{ \text{N-term}}, \text{UASd/Dys}^{ \text{C-term}} \) and \( \text{UASd/Dys} \), respectively [6]), \( \text{Dg}^{ \text{del}1}\text{CyO}, \text{Dg}^{ \text{del}2}\text{CyO}, \text{Dg}^{ \text{del}3}\text{CyO} \) (kindly provided by R. Ray [14]), \( \text{KX}^{ \text{del}3}/\text{TM6C}, \) \( \text{Dsh}^{ \text{B2}}/\text{TM3} \) [6], act-Gal4/\text{CyO}, tab-Gal4/\text{TM3}, Ubi-GFP \( \text{FRT42D} \) \( \text{Dg}^{ \text{del}2}\text{CyO}, \) \( \text{hsFLP} \), \( \text{FRT40A} \) Ubi-GFP/\text{CyO}, \( \text{hsFLP} \), \( \text{FRT42D} \) Ubi-GFP/\text{CyO} and \( \text{hsFLP} \), \( \text{FRT82B} \) \( \text{TM3} \). Alleles used in this study \( \text{w[1118]}, \) \( \text{mbk}^{ \text{5}}, \) \( \text{n克斯z-30D/EY13897}, \) \( \text{foa}^{ \text{4}}, \) \( \text{mb[227]}, \) \( \text{sli}^{ \text{2}}, \) \( \text{ubvl}^{ \text{2}}, \) \( \text{rsc}^{ \text{2}}, \) \( \text{Scd[10608]}, \) \( \text{stan}[ \text{129}], \) \( \text{wg[spd-1]}, \) \( \text{argos[Delta7]} \) and \( \text{Df(3R)Exel6184}, \) the deficiency for \( \text{dystrophin} \) were obtained from Bloomington Stock Center.

The following \( \text{Dsh} \) and \( \text{Dm} \) mutants were used to screen for modifiers of the wing vein phenotype: \( \text{Dys}^{ \text{del}} \), an hypomorphic allele of \( \text{dystrophin} \), act-Gal4/\text{UAS-Dys}^{ \text{N-RNAi}}/\text{CyO}, tab-Gal4/\text{UAS-Dys}^{ \text{N2-RNAi}}/\text{TM3}, \) tab-Gal4/\text{UAS-Dys}^{ \text{C-RNAi}}/\text{TM3} which are three \( \text{Dys} \) RNAi mutants recombined onto chromosomes with actin and tubulin Gal4 drivers, respectively, and tab-Gal4/\text{UAS-Dys}^{ \text{del}}/\text{TM3}, a \( \text{Dg} \) RNAi mutant recombined onto a chromosome with the tubulin Gal4 driver. \( \text{Dys}^{ \text{del}}/\text{RNAi} \) is a transgene on the second chromosome that encodes an inverted repeat that forms a double stranded RNA hairpin complementary to the corresponding N-terminal of the protein. It reduces the protein levels of all the known long isoforms. \( \text{Dys}^{ \text{del}}/\text{RNAi} \) is a transgene on the third chromosome that encodes a different inverted repeat than \( \text{Dys}^{ \text{del}}/\text{RNAi} \) but still reduces the protein levels of the known long isoforms. All \( \text{Dys} \) RNAi mutants exhibited 100% penetrance of the wing vein phenotype. The \( \text{Dg} \) RNAi mutant exhibited ~30% penetration of the wing vein phenotype.
**P-element screen.** The FRT P-element insertion lethal lines used in this study were obtained from the Kyoto Stock Center (Japan). From this collection, 800 lines were screened. Two-to-four day-old males carrying P-element insertional over balances (or virgin females if the P-element was on the X chromosome) were mated to Df(2R)en-B, b1 pr1/TM3; act-Gal4;UAS-DysE6 N-RNAi/+ or Cyo; tub-Gal4;UAS-DysE6 N-RNAi/TM3 and tub-Gal4;UAS-DgRNAiN02 N-RNAi/+ or TM3 virgin females (or males). Non-balancer F1 progeny were screened for dominant modification of the wing vein phenotype. Modifiers were divided into phenotypic classes based on the alterations of their wing veins as compared to wing veins of act-Gal4;UAS-DyE6 N-RNAi/+ or Cyo, tub-Gal4;UAS-DysE6 N-RNAi/+ or TM3 and tub-Gal4;UAS-DgRNAiN02 N-RNAi/+ or TM3 sibling flies. Suppressors were identified when wing veins of F1; flies phenocopied the wing veins seen in wild type flies. Since DysE6/TM3) sibling flies. Suppressors were identified when wing veins of F1 flies from this cross were heat-shocked at 37°C for 2 hours. After eclosion, they were placed in vials with fresh yeast paste for 4–5 days before dissection.

**EMS screen.** For the EMS mutagenesis 2–4 day old w1118 males were placed in yeasted bottles overnight. Twenty males were placed in each vial and starved for 6–8 hours. The flies were then provided access to a solution of 22–25 mM EMS (ethyl methansulfonate; Sigma) in a 5% sucrose solution for 18 hrs. The flies were placed in new vials and allowed to recover for 1–3 hrs. Each vial of flies was transferred to yeasted bottles with 40 virgin females of the desired genotype per bottle. The females were allowed to lay eggs for two days then transferred to new bottles daily for two more days. Mutagenized w1118 males were crossed to act-Gal4;UAS-DyE6 N-RNAi/Cyo or tub-Gal4;UAS-DysE6 N-RNAi/TM3 females. Males of these crosses were scored for a modification of the wing vein phenotype, either a novel alteration or suppression. These were picked and backcrossed to the parental Dystrophin mutant females to determine whether the modification was on the second or third chromosomes. Once this was done these modifiers showed a dominant posterior wing vein phenotype. None modifiers showed a dominant posterior wing vein phenotype. Since P-element insertion lethal lines were on chromosomes with FRT sites, we made mosaics and subsequently mapped using those phenotypes. The F1 flies phenocopied the wing veins seen in wild type flies. Since DysE6/TM3) sibling flies. Suppressors were identified when wing veins of F1 flies from this cross were heat-shocked at 37°C for 2 hours. After eclosion, they were placed in vials with fresh yeast paste for 4–5 days before dissection.

### Loss of Function Mosaic Analysis

To obtain germine clones of the modifiers found in the FRT P-element lethal screen, they were crossed to corresponding chromosomal FRT stocks. Third instar larval and early pupar from this cross were heat-shocked at 37°C for 2 hours. After eclosion, they were placed in vials with fresh yeast paste for 4–5 days before dissection.

### Dissections and Immunohistochemistry

*Drosophila* ovaries and wing imaginal discs were dissected rapidly in phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde (PFA) for 10 minutes at room temperature. Adult’s brains were dissected in PBS and fixed in PFA for 30 minutes. All antibody stainings were performed as described previously [6], except with the Dystrophin antibody with which a protocol provided by L. Fradkin was used [62]. Ovaries, brains and wing imaginal discs were mounted in 70% glycerol in PBS for analysis using a Leica TSC SPE confocal microscope. The following antibodies were used: rabbit anti-Dg (1:5000; [9]), anti-Dys-CO-H (1:3000; [62]), mouse anti-Orb (1:20; Developmental Studies Hybridoma Bank), mouse anti-24B10 (1:20; Developmental Studies Hybridoma Bank), Alexa 488, 568, or 633 goat anti-rabbit, mouse or anti-DysC CO-H (1:3000; [62]), mouse anti-Orb (1:20; Developmental Studies Hybridoma Bank), Alexa 488, 568, or 633 goat anti-mouse, Alexa 488, 568 goat anti-rabbit. To mount adult wings, flies were pre-incubated in 80% ethanol and 20% glycerol solution, then dissected and mounted in 70% glycerol before analysis using a Leica light microscope.

### Muscle Analysis

Histological sections of muscle were prepared from wax-embedded material as described previously [6], stained with hematoxyline and eosin (H&E staining) and analyzed using light microscopy.

### Supporting Information

**Text S1**

Found at: doi:10.1371/journal.pone.0002418.s001 (0.03 MB DOC)

**Table S1**

Found at: doi:10.1371/journal.pone.0002418.s002 (0.14 MB DOC)

**Figure S1**  
**Dys and Dg Expression in Wild Type and Mutant Wing Discs.** In wild-type larvae, Dys is expressed in all cells of the wing disc (A) and is strongly reduced in the wing disc of the DysC-999 mutant (B; tubGal4/UAS-DysC-RNAi/+). Dystroglycan localization in wild type imaginal discs is enriched at the basal surface of the epithelial cells. Dg expression is more intense in folds formed from the contact of basal surfaces and is less visible in the folds made from apical surfaces of wing disc cells (C). The DgRNAi mutant also shows a reduction of Dystroglycan protein in the wing disc (D; tubGal4/UAS-DgRNAi/+). (A’–D’). Enlarged images of framed areas on (A–D). (A”–B”) Enlarged images of framed areas on (A–B) show Dystrophin single channel staining. (C”–D”). Enlarged images of framed areas on (C–D) show Dystroglycan single channel staining.

**Figure S2**  
**Subclasses of Dys(RNAi) Modifiers.** (A) Example of enhancers that lack the posterior cross vein (PCV) and belong to phenotypic class En. Arrow indicates where the PCV should be. (B) Wing representing Sn+ class phenotype that shows the PCV attached to L4 and L5 and extra wing vein material can be seen below L5.

Found at: doi:10.1371/journal.pone.0002418.s004 (0.94 MB TIF)
Figure S3  DysE6/+ Modifiers. DysE6/+ flies have normal posterior cross veins. (A) Represents an enhancer (ModE10) of this phenotype which phenocopies the wing veins from DysE6/DysE6 flies. (B) Shows an extra vein modification of the DysE6/+ posterior cross vein. Arrows indicate altered cross veins.

Found at: doi:10.1371/journal.pone.0002418.s005 (1.94 MB TIF)

Figure S4  DgRNAi Modifiers. (A) Wild type fly wing with normal posterior cross vein (PCV) indicated by the arrow. (B) DgRNAi mutant PCV (arrow) with a branch. (C–D) represent modifiers that produce extra vein material (indicated by arrows). In one case (C) the branch is elongated with extra material also seen above L2 (upper arrow). (D) Shows extra material below L5 (lower arrow).

Found at: doi:10.1371/journal.pone.0002418.s006 (1.77 MB TIF)

Figure S5  Phenotypes Observed in poly-EGF Mutants. (A) Fly wing from Mod29/Df flies. Arrows indicate extra vein material. (B) Transverse histological section of the indirect flight muscle in Mod29 homozygotes. (B') Higher magnification of the region indicated by the box which indicates the separation of the muscle into individual fibers. (C) Ovariole from a Mod29 homozygote. Anterior to the left. The gerarium is at the anterior most tip of the ovariole with developing egg chambers progressing to the right (posteriorly). The egg chambers undergo developmental arrest in later stages. Actin-Green, Adducin-Red, DAPI-purple. (D) 24B10 antibody staining of the adult wild type brain. Arrows indicate photoreceptor axon termination sites where the R8 photoreceptor axon (left arrow) terminates before the K7 axon (right arrow). (E) Mod29/Df adult brain. Termination of the R8 and K7 axons are indicated by the red arrows. White arrows indicate non termination of two axons that protrude deeper into the brain. (F) Dg323/Dg323 clone in the adult brain. Red arrows indicate where the R8 and K7 axons should terminate. Upper white arrows indicate a general disruption of axon termination in the R8/K7 termination region. Lower right arrow indicates a non terminating axon that proceeds deeper into the brain.

Found at: doi:10.1371/journal.pone.0002418.s007 (3.58 MB TIF)

Acknowledgments
We thank Drs. L.Fradkin, R.Ray, C.Berg and B.Edgar for the fly lines and advice. We also thank undergraduate students P.Siswara, K.Park and B.Lasley for help with genetic interaction screens and muscle’s histology and Dr. E.Ward for critical reading and helpful comments on the manuscript.

Author Contributions
Conceived and designed the experiments: HR MK MY HS. Performed the experiments: HR MK MY HS KD HM. Analyzed the data: HR MK MY HS. Contributed reagents/materials/analysis tools: HR MK MP AY HS KF DM. Wrote the paper: HR MK MY HS.

References
1. Haenggi T, Fritschy JM (2006) Role of dystrophin and utrophin for assembly and function of the dystrophin glycoprotein complex in non-muscle tissue. Cell Mol Life Sci 63: 1614–1631.
2. Lapidos KA, Kakkar R, McNally EM (2004) The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. Circ Res 94: 1023–1031.
3. Davies KE, Nowak KJ (2005) Molecular mechanisms of muscular dystrophies: old and new players. Nat Rev Mol Cell Biol 7: 762–773.
4. Kanagawa M, Toda T (2006) The genetic and molecular basis of muscular dystrophy: roles of cell-matrix linkage in the pathogenesis. J Hum Genet 51: 915–926.
5. Allikson M, Blahna G, Doospy P, Heydemaan A, Ryder P, et al. (2007) Reduced life span with heart and muscle dysfunction in Drosophila sarcoglycan mutants. Hum Mol Genet 16: 2933–2943.
6. Shcherbata HR, Yatsenko AS, Patterson L, Sood VD, Nudel U, et al. (2007) Dissecting muscle and neuronal disorders in a Drosophila model of muscular dystrophy. Emb J 26: 481–493.
7. van der Plas MC, Pilgram GS, de Jong AW, Bansraj MR, Fradkin LG, et al. (2007) Dystrophin Dystroglycan in required for integrity of the musculature. Mech Dev 124: 617–630.
8. Yatsenko AS, Gray EE, Shcherbata HR, Patterson LB, Sood VD, et al. (2007) A putative Src homology 3 domain binding motif but not the C-terminal dystrophin WW domain binding motif is required for dystrophin function in cellular polarity in Drosophila. J Biol Chem 282: 15159–15169.
9. Deng WM, Schneider M, Froock R, Castillejo-Lopez C, Gaman EA, et al. (2007) Dystroglycan is required for polarizing the epithelial cells and the oocyte in Drosophila. Development 130: 173–184.
10. Poultou JS, Deng WM (2006) Dystroglycan down-regulation links EGF receptor signaling and anterior-posterior polarity formation in the Drosophila oocyte. Proc Natl Acad Sci U S A 103: 12775–12780.
11. Taghdi-Lamallem O, Akasaka T, Hogg G, Nudel U, Yaffe D, et al. (2008) Dystrophin deficiency in Drosophila reduces lifespan and causes a diluted cardiomyopathy phenotype. Aging Cell 7: 237–249.
12. Carboettino S, personal communication.
13. Batchelor CL, Winder SJ (2006) Sparks, signals and shock absorbers: how dystrophin loss causes muscular dystrophy. Trends Cell Biol 16: 198–205.
14. Christoforou CP, Greer CE, Challoner BR, Charizanos D, Ray RP (2008) The putative Src homology 3 domain binding motif but not the C-terminal dystrophin Associated Protein Complex to influence intercellular signaling in developing wing veins. Dev Biol 315: 519–532.
15. Guichard A, Biels B, Struevant MA, Wickline L, Chacko J, et al. (1999) Rhomboid and Star interact synergistically to promote EGF/R MAPK signaling during Drosophila wing vein development. Development 126: 2603–2676.
16. Johannes B, Preiss A (2002) Wing vein formation in Drosophila melanogaster: hairless is involved in the cross-talk between Notch and EGF signaling pathways. Mech Dev 115: 3–14.
17. Rafton A, Blair SS (2005) Long-range Dpp signaling is regulated to restrict BMP signaling to a crossvein competent zone. Dev Biol 280: 187–200.
18. Artavasiss-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284: 770–776.
19. Bier E (2000) Drawing lines in the Drosophila wing: initiation of wing vein development. Curr Opin Genet Dev 10: 393–398.
20. de Celis JF (1993) Pattern formation in the Drosophila wing: The development of the veins. Bioessays 25: 443–451.
21. de Celis JF (1997) Expression and function of decapentaplegic and thick veins during the differentiation of the veins in the Drosophila wing. Development 124: 1007–1018.
22. Kalamida D, Pouls K, Avramopoulos D, Fostieri E, Lagueimintzis G, et al. (2007) Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. Feb J 274: 3599–3615.
23. Madhavan R, Massom LR, Jarrett HW (1992) Calmodulin specifically binds three proteins of the dystrophin-glycoprotein complex. Biochem Biophys Res Commun 185: 733–739.
24. Newboll B, Anderson JT, Jarrett HW (1997) Ca2+-calmodulin binding to mouse alpha 1-syntrophin: syntrophin is also a Ca2+-binding protein. Biochemistry 36: 1295–1305.
25. Pascual M, Vicente M, Monferrer I, Artero R (2006) The Muscl ebinder family of proteins: an emerging class of regulators of developmentally programmed alternative splicing. Differentiation 74: 65–80.
26. Kadrmas JL, Smith MA, Pronovost SM, Beerke MC (2007) Characterization of RACK1 function in Drosophila development. Dev Dyn 236: 2207–2215.
27. Cox DN, Seyfried SA, Jan LY, Jan VN (2001) Bazooka and atypical protein kinase C are regulated to regulate oocyte differentiation in the Drosophila ovary. Proc Natl Acad Sci U S A 98: 14475–14480.
28. Wehr K, Swan A, Schupbach T (2006) Deadlock, a novel protein of Drosophila, is required for germline maintenance, fusome morphogenesis and axial patterning in oogenesis and associates with centrosomes in the early embryo. Dev Biol 294: 406–417.
29. Swan A, Nguyen T, Suter B (1999) Drosophila Lasseynephalophy-1 functions with BicD and dynin in oocyte determination and nuclear positioning. Nat Cell Biol 1: 444–449.
30. Taya S, Shinoda T, Tsuboi D, Asaki J, Nagai K, et al. (2007) DISC1 regulates reduced life span with heart and muscle dysfunction in Drosophila sarcoglycan mutants. Hum Mol Genet 16: 2933–2943.
31. Chakkalakal JV, Michel SA, Chin ER, Michel RN, Jasmin BJ (2006) Targeted inhibition of Ca2+/calmodulin signaling exacerbates the dystrophic phenotype in mdx mouse muscle. Hum Mol Genet 15: 1423–1435.
32. Parsons SA, Millyan DP, Sargeant MA, Naya EJ, McNally EM, et al. (2007) Genetic disruption of calcium improves skeletal muscle pathology and cardiac disease in a mouse model of limb-girdle muscular dystrophy. J Biol Chem 282: 10068–10078.
33. Macioce P, Cambra G, Bernasola M, Gaddini L, Torreri P, et al. (2003) Beta-dystrobrevin interacts directly with kinesin heavy chain in brain. J Cell Sci 116: 4847–4856.
34. Rinaldo C, Prodosmo A, Siepi F, Sossu S (2007) HIPK2: a multitalented partner for transcription factors in DNA damage response and development. Biochem Cell Biol 85: 411–418.
35. Link N, Chen P, Lu WJ, Pogue K, Chuong A, et al. (2007) A collective form of cell death requires homeodomain interacting protein kinase. J Cell Biol 176: 567–574.
36. Furrer MP, Kim S, Wolf B, Chiba A (2003) Robo and Frazzled/DCC mediate dendritic guidance at the CNS midline. Nat Neurosci 6: 223–230.
37. Magalhaes TR, Palmer J, Tomancak P, Pollard KS (2007) Transcriptional control in embryonic Drosophila midline guidance assessed through a whole genome approach. BMC Neurosci 8: 59.
38. Carmeliet P, Tessier-Lavigne M (2005) Common mechanisms of nerve and blood vessel wiring. Nature 436: 193–200.
39. Park KW, Morrison CM, Sorensen LK, Jones CA, Rao Y, et al. (2003) Robo4 is required for oocyte signaling that leads to establishment of the anterior-posterior axis in Drosophila oocyte. Dev Biol 261: 251–267.
40. Taylor TD, Robichaux MB, Garrity PA (2004) Compartmentalization of visual centers in the Drosophila brain requires Slit and Robo proteins. Development 131: 5935–5945.
41. Cornelison DD, Wilcox-Adelman SA, Goetinck PF, Rapraeger AC, et al. (2004) Essential and separable roles for Syndecan-3 and Syndecan-4 in skeletal muscle development and regeneration. Genes Dev 18: 2231–2236.
42. Qian L, Liu J, Bodmer R (2005) Slit and Robo control cardiac cell polarity and morphogenesis. Curr Biol 15: 2271–2278.
43. Swinhaur J, Kalderon D (2006) Microtubule polarity and axis formation in the Drosophila oocyte. Dev Dyn 235: 1455–1468.
44. Deng WM, Rucshola-Baker H (2000) Lamin A is required for follicle cell-oocyte signaling that leads to establishment of the anterior-posterior axis in Drosophila. Curr Biol 10: 683–686.
45. Alvarado D, Rice AH, Duffy JB (2004) Bipartite inhibition of Drosophila epidermal growth factor receptor by the extracellular and transmembrane domains of Kekkon1. Genetics 167: 107–202.
46. Kulekov NV, Xu Z, Greene LA (2006) Direct interaction of the molecular scaffolds POSH and JIP is required for apoptotic activation of JNKs. J Biol Chem 281: 15517–15524.
47. Figueroa C, Tarras S, Taylor J, Vojtek AB (2003) Akt2 negatively regulates assembly of the POSH-MLK-JNK signaling complex. J Biol Chem 278: 47922–47927.
48. Zhou W, Zhu M, Wilson MA, Petsko GA, Fink AL (2006) The oxidation state of DJ-1 regulates its chaperone activity toward alpha-synuclein. J Mol Biol 356: 1036–1048.
49. Januschke J, Gervais L, Dass S, Kaltschmidt JA, Lopez-Schier H, et al. (2002) Polar transport in the Drosophila oocyte requires Dynein and Kinesin I cooperation. Curr Biol 12: 1971–1981.
50. Lei Y, Warrior R (2000) The Drosophila Lisencephaly1 (DLi1) gene is required for nuclear migration. Dev Biol 226: 57–72.
51. Brenda R, Serbus LR, Saxton WM, Dufny JB (2002) Posterior localization of dynein and dorsal-ventral axis formation depend on kinesin in Drosophila oocytes. Curr Biol 12: 1541–1545.
52. Duncan JE, Warrior R (2003) The cytoplasmic dynein and kinesin motors have interdependent roles in patterning the Drosophila oocyte. Curr Biol 12: 1982–1991.
53. Takeya R, Sumimoto H (2003) Fhos, a mammalian formin, directly binds to F-actin via a region N-terminal to the FH1 domain and forms a homotypic complex via the FH2 domain to promote actin fiber formation. J Cell Sci 116: 4567–4575.
54. Bosch M, Le KH, Baggi B, Correa JF, Renault L, et al. (2007) Analysis of the function of Spare in actin assembly and its synergy with formin and profilin. Mol Cell 28: 555–560.
55. Dahlgaard K, Ropoas AA, Niccol T, St Johnston D (2007) Capu and Spire assemble a cytoplasmic actin mesh that maintains microtubule organization in the Drosophila oocyte. Dev Cell 13: 539–553.
56. Qinian ME, Higelt S, Bedrossian A, Mullins RD, Kerkhoff E (2007) Regulatory interactions between two actin nucleators, Spare and Cappuccino. J Cell Biol 179: 117–128.
57. Rosales-Nieves AE, Jhondrow JE, Keller LC, Magic CR, Pinto-Santini DM, et al. (2006) Coordination of microtubule and microfilament dynamics by Drosophila Rhos1, Spare and Cappuccino. Nat Cell Biol 8: 367–376.
58. Wang Y, Riehmann V (2007) The role of the actomyosin cytoskeleton in coordination of tissue growth during Drosophila oogenesis. Curr Biol 17: 1349–1355.
59. Kalil K, Dent EW (2005) Touch and go: guidance cues signal to the growth cone cytoskeleton. Curr Opin Neurobiol 15: 521–526.
60. Bateman J, Reddy RS, Saito H, Van Vactor D (2001) The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the Drosophila follicular epithelium. Curr Biol 11: 1317–1327.
61. Lee E, Sali A, Kruger R, Heinrich R, Kirschner MW (2003) The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. PLoS Biol 1: E10.
62. van der Plas MC, Pilgrum GS, Plomp JJ, de Jong A, Fradkin LG, et al. (2006) Dystrophin is required for appropriate retrograde control of neurotransmitter release at the Drosophila neuromuscular junction. J Neurosci 26: 333–344.