**Drosophila Tel2 Is Expressed as a Translational Fusion with EpsinR and Is a Regulator of Wingless Signaling**

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### Abstract

Tel2, a protein conserved from yeast to vertebrates, is an essential regulator of diverse cellular processes including telomere maintenance, DNA damage checkpoints, DNA repair, biological clocks, and cell signaling. The *Drosophila* Tel2 protein is produced as a translational fusion with EpsinR, a Clathrin adapter that facilitates vesicle trafficking between the Golgi and endosomes. EpsinR and Tel2 are encoded by a *Drosophila* gene called *lqfR*. *lqfR* is required for viability, and its specific roles include cell growth, proliferation, and planar cell polarity. We find that all of these functions of *lqfR* are attributed entirely to Tel2, not EpsinR. In addition, we find that *Drosophila* *lqfR/Tel2* is a component of one or more protein complexes that contain E-cadherin and Armadillo. Moreover, Tel2 modulates E-cadherin and Armadillo cellular dynamics. We propose that at least one of the functions of *Drosophila* Tel2 is regulation of Wingless signaling.

### Results and Discussion

**Exon 6 of *lqfRa* encodes the *Drosophila* Tel2 homolog**

The *lqfR* gene pre-mRNA is alternatively spliced to generate mRNAs with different C-terminal exons and thus two different proteins, LqfRa (1415 aa) and LqfRb (649 aa) (Fig. 1) [18,32]. Both LqfRa and LqfRb have structural elements characteristic of Golgi Epsin: the ENTH domain and binding motifs for AP-1 and Clathrin. The larger protein also contains a domain encoded by its LqfRa-specific C-terminal exon 6 (921 aa) that is homologous to Tel2. Tel2 is a Y-shaped protein in the HEAT repeat family of superhelical proteins, in which 32 interacting α-helices are packed to generate two α-helical segments that form the long (21 α-helices) and short (11 α-helices) lines of the Y. Human Tel2 and LqfRa exon6 are 19% identical and 13% similar in amino acid sequence throughout the length of their polypeptide chains (Fig. S1).

The evidence that exon 6 is not a separate gene - that the *lqfRa* splice form truly exists - is convincing. First, there is compelling evidence that exons 5 and 6 are joined in an mRNA; an RT-PCR amplification product containing exon 5 spliced to exon 6 has been generated [32]. Moreover, an exon 3–5 probe hybridizes not only to a species the size of *lqfRb*, but also to a larger mRNA corresponding in size to *lqfRb* that also hybridizes to an exon 6.
Epsin homology domain, AP-binding N-terminal homology domain, AP-1 = binding motif for the Clathrin-adapter AP-T, CBM = Clathrin binding motif.

DOI:10.1371/journal.pone.0046357.g001

Figure 1. lqfR gene products. At top is a diagram of the two lqfR mRNAs formed by alternative pre-mRNA splicing. Exons 1–7 are indicated by bars and introns by bent lines. The black region in each transcript is the open reading frame. The larger transcript, lqfRa, contains exon6 which encodes Tel2. At bottom are the protein products of each mRNA. ENTH = Epsin N-terminal homology domain, AP-1 = binding motif for the Clathrin-adapter AP-T, CBM = Clathrin binding motif.

We were curious to determine whether or not the fusion of the genes for Golgi Epsin and Tel2 was specific to Drosophila. We performed two BLAST searches (at www.uniprot.org), one using as a query the amino acid sequence of LqfR exons 1–5, and the other using exon 6. In some species that had clear homologs of both genes, both queries identified the same gene or adjacent genes, indicating that lqfR and tel2 are likely fused in that species. In other species each query identified distinct, non-adjacent genes. Although our analysis was not exhaustive, we did find that the lqfR and tel2 genes were likely fused in all queried Drosophila species and also in other insects in the database, but not in yeast, nematodes, nor any vertebrates (data not shown).

Exon 6 of lqfRa is necessary and sufficient for all lqfR/Tel2 gene functions tested

We found previously [32] that either full-length LqfRa fused at its C-terminus to GFP (LqfRa

GFP) or a version of the fusion protein that lacks the ENTH domain (LqfRa

ENTH-GFP), when expressed usingGal4/ UAS and the ubiquitous Actin5C-gal4 driver, is sufficient to rescue all of the obvious defects due to loss of lqfR gene activity: these include larval lethality and the absence of imaginal discs. The dispensability of the ENTH domain was not entirely surprising, as endocytic Epsin also functions well without its ENTH domain [33,34]. However, further structure/function experiments did yield results that were completely unexpected.

First, we generated five UAS transgenes in P element vectors, in which full-length (FL) lqfRa or four deletion derivatives were tagged with 6xmyc epitope coding sequences at their 5’ ends (Fig. 2A) and used them to transform Drosophila. Each transgene was tested for its ability when expressed with Actin5C-gal4 to substitute for the endogenous lqfR gene. The results obtained by expressing LqfRa

ENTH-GFP or LqfRa

ENTH-GFP described above were recapitulated by 6xmyc-LqfRa

ENTH and 6xmyc-LqfRa

ENTH; expression of either protein rescued lqfR null mutants to wild-type (Fig. 2A). In contrast, neither the ENTH domain alone (6xmyc-LqfR

ENTH) nor exons 1–5 alone (6xmyc-LqfR

ex1–5) had any rescuing activity (Fig. 2A). This was not due to a failure of transgene expression as the 6xmyc-LqfR

ex1–5 proteins accumulated in the flies to levels at least as high as 6xmyc-LqfRa

ENTH (Fig. 2B). The most remarkable result was that exon 6 alone (6xmyc-LqfR

ex6) rescued lqfR null mutants to wild-type (Fig. 2A). In summary, we found that expression of exon 6, which contains only the Tel2-like region of LqfRa, was sufficient to rescue the imaginal disc proliferation and patterning defects of lqfR null mutants and no other portions of LqfRa were able to provide any rescuing activity independently.

The results so far predict that LqfRb, which does not contain exon 6, would not have any rescuing activity. We could not test LqfRb in the assay described above because unexpectedly, expression of UAS-6xmyc-lqfRb with Actin5C-gal4 was lethal. To overcome this obstacle, we expressed 6xmyc-lqfRb in the eye only, and asked if the eye morphology defects in eyes with no LqfR protein in otherwise normal flies (lqfR

null mutants to wild-type (Fig. 2A). In summary, we found that expression of exon 6 alone of lqfRa is sufficient to rescue the imaginal disc proliferation and patterning defects of lqfR null mutants and no other portions of LqfRa were able to provide any rescuing activity independently.

The Tel2-like portion of LqfRa encoded by exon 6 expressed alone is mainly nuclear

Using either of two different polyclonal antibodies, one to LqfR exons 1–5 and the other to an ENTH-less LqfRb, LqfR was shown to colocalize with Golgi markers in the eye and elsewhere [18,32]. We were curious to know where the truncated protein consisting of LqfRa exon 6 alone (6xmyc-LqfR

ex6) accumulates in the cell. Full length 6xmyc-LqfRa

, monitored with anti-Myc had a cytoplasmic localization pattern similar to that of endogenous LqfR and other Golgi markers (Fig. 3) [18,32]. By contrast, 6xmyc-LqfR

ex6 rescued lqfR

null mutant flies (Fig. 2B). The inability of lqfRb to complement the lqfR mutant phenotype is consistent with the finding that exon 6 alone of lqfRa is sufficient to do so.

We conclude that Golgi Epsin and Tel2, although fused in LqfRa, are independent protein functions. Moreover, the external morphology and lethality aspects of the mutant phenotype described for lqfR null mutants reflects only the loss of Tel2 activity, and not the loss of Golgi Epsin. We therefore propose renaming the lqfR gene lqfR/tel2.
Figure 2. Rescue of lqfR null mutant phenotype by lqfRa exon 6. (A) At left, the table shows six epitope-tagged proteins expressed in Drosophila by a UAS transgene. The columns at right show the results when each transgene was expressed in a lqfR^{117} or lqfR^{117}/Df(3R)Exel6191 background with either an Actin5C-gal4 or an eyeless-gal4 driver. + : lethality and externally obvious morphological defects were rescued, − : no rescue. (B) A blot of electrophoresed adult fly protein extracts probed first with antibodies to the Myc tag (α-Myc) and reprobed with antibodies to β-tubulin (α-βtub) as a loading control. The flies contain the UAS construct indicated and an eyeless-gal4 driver. The genotypes of the flies used were: EGUF/UAS; FRT82B lqfR^{117}/TM6B. For each UAS construct, two different P element transformant lines were tested. Note that one of the UAS-lqfR^{117} lines expressed little or no protein and this line also failed to rescue the lqfR^{117} mutant phenotype. The numbers at the right of the blot indicate the positions of corresponding size markers (kD). (C) Light microscope images of the eyes of adult flies. The flies are lqfR^{117}/lqfR^{+} and their eyes are lqfR^{117}/lqfR^{+} homozygous clones. The fly at the very left has no UAS transgene and the others contain a copy of the UAS transgene indicated, expressed by eyeless-gal4. The genotypes of the flies were: EGUF/UAS; FRT82B lqfR^{117}/FRT 82B GMR-hid. scale bar: ~50 μm.
doi:10.1371/journal.pone.0046357.g002

Figure 3. Subcellular localization of Myc-tagged LqfR proteins. Confocal microscope images of third instar larval eye disc tissue from two different discs (each row is a single disc) are shown. The portion of the eye disc shown is the peripodial epithelium, a layer of cells that lies atop the cell layer that forms the retina. The peripodial cells are large and flat the nuclei and cytoplasm are distinguished more easily than in the retinal cells. The discs were immunostained with antibodies to the Myc epitope (green) and the DNA stain TOPRO3 (purple). The Myc-tagged proteins indicated were expressed by UAS transgenes using an Actin5C-gal4 driver. scale bar: ~10 μm.
doi:10.1371/journal.pone.0046357.g003

the N-terminus of the protein, so that the antibody to exons 1–5 does not detect exon 6-encoded protein. Yet another possibility is that LqfRa/Tel2 normally shuttles between the cytoplasm and the nucleus and the 6xmyc-Tel2 protein fusion is retained at the nuclear envelope abnormally. The generation of an antibody specific to the Tel2-like region of LqfRa might help to distinguish among these alternatives.

Wingless pathway genes interact strongly with lqfR/tel2

The specific cell growth and patterning defects in lqfR/Tel2 mutants are suggestive of defects in a variety of different signaling pathways [32]. Wingless signaling, for example, regulates both cell proliferation and patterning in the eye [35]. Wingless regulates initiation of the wave front of eye morphogenesis called the morphogenetic furrow. In addition, Wingless expressed at the lateral margins of the eye disc forms a gradient that results in formation of a dorsal/ventral midline called the equator about which the facets, or ommatidia, are mirror-image symmetrical. Separation of eye and head cuticle tissue also requires Wingless. As the lqfR/tel2 mutant phenotype includes defects in morphogenetic furrow movement and planar cell polarity in both the eye and wing [32], it seemed reasonable that the function of lqfR/tel2 could somehow relate to the Wingless pathway.

We tested two genes encoding core components of the Wingless pathway, wingless and armadillo, for interactions with lqfR/Tel2. Wingless ligand binds its receptor Frizzled which results in accumulation in the nucleus of the transcriptional regulator Armadillo [36]. We found strong genetic interactions between lqfR/Tel2 and each of the two Wingless pathway genes. Heterozygotes for a hypomorphic allele and a null allele of lqfR (lqfR^{F2}/lqfR^{117}) are semi-viable and the adult escapers may have normal, slightly roughened, or kidney-shaped eyes [32] (Fig. 4). Flies that were lqfR^{F2}/lqfR^{117} and also heterozygous for a loss-of-function allele of armadillo (arm^{0} or arm^{a}) died in their pupal cases. (arm^{-}/arm^{+} flies appear wild-type.) The pupae had small or absent eyes, small head capsules, and also had morphological defects in the head cuticle, wings, and legs (Fig. 4). wingless loss-of-function
modest decrease in Wingless signaling are amplified by down-
could see no effect). One possible explanation is that the effects of a
genetic interactions between
lqfR heterozygous in a lqfR+/lqfR<sup>117</sup> background; there were viable
adult escapers with severely defective eyes varying from kidney-
shaped to nearly absent, and also with defects in the head cuticle
(Fig. 4). (wg<sup>−</sup>/wg<sup>+</sup> animals appear wild-type.) These strong genetic
interactions suggest that lqfR/tel2 may function in the Wingless
signaling pathway.

Wingless target gene expression depends to some extent on lqfR/tel2 function
The dominant enhancement of lqfR/tel2 mutant phenotypes by loss of function mutations in Wingless pathway genes suggests that lqfR/tel2 facilitates Wingless pathway activation. To test this idea, we generated lqfR/tel2 null clones in eye discs and monitored expression of the Wingless target genes dachsous (ds) [36,37] and optomotor blind (omb) [38]. Weak effects on target gene expression were apparent in both cases. As the effects on ds expression was stronger, this data is shown below.

The dachsous gene encodes an atypical cadherin adhesion protein involved in cell polarity and cell growth and is a transcriptional target of Arm [37,38]. ds-<sup>lacZ</sup> enhancer trap lines express β-galactosidase in response to Wg pathway activation [38]. Wingless ligand is expressed in the dorsal- and ventral-most margins of the eye disc and the protein forms a gradient with its lowest point at the dorsal/ventral axis (the equator) [40]. β-galactosidase expression by ds-<sup>lacZ</sup> reflects the Wg gradient [39]. We found that ds-<sup>lacZ</sup> expression was reduced in lqfR/tel2 null clones (Fig. 5). Moreover, we found that a dachsous loss-of-function mutation, <sup>ds<sup>364</sup></sup>, is as strong a dominant enhancer of the lqfR<sup>+/lqfR<sup>117</sup></sup> mutant phenotype as are armadillo mutations (Fig. 5). These results suggest that in the absence of LqfR/Tel2, Wg signaling is less efficient than it is normally.

The effects of lqfR/tel2 loss of function on Wingless target gene expression are weaker than expected based on the dramatic genetic interactions between lqfR/tel2 mutations and mutations in arm, wg, or ds. (We also monitored expression of the Wingless target gene senseless (sens) [41] in wing disc lqfR/tel2 null clones but could see no effect). One possible explanation is that the effects of a modest decrease in Wingless signaling are amplified by downstream effects on other signaling pathways. Therefore, the

combined effect of losses in several signaling pathways in lqfR/tel2 mutants could account for the striking genetic interactions.

Plasma membrane levels of E-cadherin and Armadillo increase in the absence of lqfR/Tel2 activity
In a mutagenesis screen for dominant enhancers of the lqfR/tel2 mutant eye phenotype [Lee et al., manuscript preparation], we identified loss-of-function alleles of polychaetoid, which encodes the Drosophila homolog of vertebrate ZO-1 [42]. In ZO-1/Polychaetoid is present at tight junctions and adherens junctions, where it connects other proteins present there to the actin cytoskeleton [42–44]. Although the mechanism is unclear, loss of Polychaetoid in Drosophila results in increased accumulation of the cell adhesion protein E-cadherin at the plasma membrane [43].

The transmembrane protein E-cadherin is a central component of adherens junctions through homotypic interactions between E-cadherin extracellular domains on adjacent cells [45]. The intracellular domain of E-cadherin binds proteins, including Armadillo and α-catenin, which are essential for E-cadherin's
function as a cell adhesion protein [46,47] Because E-cadherin binds Armadillo, E-cadherin function effects Wingless signaling [40]. Notably, E-cadherin overexpression antagonizes Wingless signaling, presumably by preventing Armadillo from entering the nucleus [49,50].

We wondered whether the genetic interaction between polychaetoid and lqfR/tel2, which suggests that both genes facilitate Wingless signaling, could be explained by the effect of Polychaetoid on E-cadherin levels. To test this hypothesis, first we asked whether E-cadherin levels, which increase in the absence of Polychaetoid, were also elevated in eye disc clones lacking LqfR/Tel2. We found that E-cadherin levels do indeed increase in lqfR/tel2 null clones; this effect appears most dramatic near the morphogenetic furrow where the highest levels of E-cadherin accumulate normally (Fig. 6). In discs where either 6xmyc-LqfR [41] or 6xmyc-LqfR_6xENTH were overexpressed, lqfR/tel2 null clones had the same levels of E-cadherin as surrounding wild-type tissue, confirming that the effect on Cadherin is mediated by Tel2 (Fig. S2). Like E-cadherin levels, Armadillo levels at the plasma membrane also are higher than usual in the absence of LqfR/Tel2 (Fig. 6). We conclude that the Tel2-like portion of LqfRa modulates Wingless signaling through an effect, either direct or indirect, on E-Cadherin levels and Armadillo localization.

LqfR/Tel2 interacts physically with E-cadherin, Armadillo and a-catenin

To determine whether or not the effect of LqfR/Tel2 on E-cadherin and Armadillo is direct, we asked whether LqfR/Tel2 is present in a complex with either protein. We also tested for physical interactions between LqfR/Tel2 and the adherens junction protein a-catenin, which binds Armadillo. First, we used antibodies to GFP to immunoprecipitate LqfRa-GFP from fly embryos that overexpress it (Actin5C>lqfRa-gfp). Next, using antibodies to each of the three proteins on blots, we determined whether E-cadherin, Armadillo, or a-catenin were also present in the precipitate. We found that each of the three proteins co-immunoprecipitated with LqfRa-GFP (Fig. 7). The adherens junction proteins are not binding to GFP because we did the same experiment with embryos that overexpress the ENTH domain only fused to GFP (LqfRa-ENTH-GFP) and we found that LqfRa-ENTH-GFP did not coimmunoprecipitate with any of the three proteins (Fig. 7). Moreover, the correlation between rescue of the lqfR/tel2 mutant phenotype and binding to the adherens junction proteins (LqfRa-GFP both rescues and binds and LqfRa_6xENTH-GFP does neither) suggests that the interaction between LqfR/Tel2 and E-cadherin, Armadillo and a-catenin may be relevant to the lqfR/tel2 mutant phenotype and that the effect of LqfR/Tel2 on adherens junctions is direct. Further experiments are required to determine whether or not all four proteins are present in a single complex.

LqfR/Tel2 is not required for Wntless-mediated Wingless secretion

Wingless secretion requires the transmembrane protein Wntless, which binds to Wingless at the Golgi and guides it to the plasma membrane. After releasing Wingless, Wntless is endocytosed and trafficked back to the Golgi. Retrograde trafficking of Wingless from endosomes to Golgi is essential for Wingless secretion and requires the retromer complex. Cell clones lacking retromer complex proteins cannot secrete Wingless and instead Wingless accumulates inside the cells [51–53]. In human cultured cells, EpsinR is required for retromer complex function [54,55]. We have shown that loss of Tel2 activity, and not loss of Golgi Epsin, is the reason why Wingless signaling falters in lqfR/tel2 mutants. Nevertheless, as Golgi Epsin is expected to be required for Wingless secretion, we tested whether or not lqfR/tel2 activity is required. Port et al., 2008 showed that cell clones in the wing disc lacking the retromer protein Dvps35 accumulate Wingless and we were able to replicate this result (Fig. 8A,A’). In contrast, lqfR/tel2 null clones have wild-type Wingless levels (Fig. 8B,B’) and we infer that Wingless is secreted normally from the mutant cells. This result is consistent with the observation that Tel2 expression rescues the lqfR/tel2 null mutant phenotype. Moreover, we
conclude that in *Drosophila*, Golgi Epsin is not always required for retromer complex function.

**Conclusions**

Many of the results presented here were unexpected and raise questions that remain to be answered. First, we were surprised to find that in *Drosophila*, EpsinR and Tel2 proteins are fused. The gene fusion appears to have no consequence for the essential function of the locus in *Drosophila*. Whether or not the two parts of the protein function together in other contexts, for example during oogenesis or in redundant functions undetectable in our experiments, is unknown. It was also unexpected that only the Tel2 function is essential in *Drosophila*. EpsinR is not essential for viability in flies and EpsinR is not required for the function of the retromer complex in Wingless recycling. Whether retrograde trafficking of Wingless is the exception, or whether retromer complex function is generally EpsinR-independent in *Drosophila* remains to be determined.

At least one aspect of Tel2’s essential function in *Drosophila* is to promote Wingless signaling through modulation of adherens junction proteins. Whether or not the lqfR/tel2 mutant phenotype in its entirety reflects a failure of Wingless signaling is yet unclear.

There are mutant phenotypes of *lqfR* mutant flies that are not easily explained by a loss of Wingless signaling [32]. Moreover, recent results link the function of LqfRa/Tel2 to a PIKK complex [56]. Nevertheless, the results presented suggest that the association of Tel2 with adherens junction proteins prevents the accumulation of excess E-cadherin at the plasma membrane which would otherwise sequester Armadillo and prevent efficient Wingless signaling (Fig. 9). Further experiments are required to determine precisely how Tel2 activity affects E-cadherin levels. It is interesting that loss of Tel2 activity in *C. elegans* phenocopies, at least in part, loss of Wnt signaling proteins rather than loss of PIKK activities [7]. The results presented here pave the way for genetic experiments to determine whether or not Tel2 activity in *Drosophila* always involves PIKK complexes.

**Materials and Methods**

*Drosophila* strains

The following mutations were used: *lqfR* (FBal0230310), *lqfR* 

**Transgene construction and transformation**

DNA fragments for four of the *lqfR* P element constructs were generated as follows. First, the template p*UASt-lqfR-a-gfp* was used with the following primer pairs to amplify four different products:

- lqfR(a-a-gfp):
  - F: 5’-CACGGTCCGATAAAATTCATCAGACTGTAAGAG
  - R: 5’-TTAGGCGACCTGTCCATGGCCG

- lqfR(a-exons1-5):
  - F: 5’-CACGTGGGATAAATTCATCAGCATGTGGAAAG
  - R: 5’-TTAGGCAGCCTGTTCCATGGCG

- lqfR(a-exon6):
  - F: 5’-CACGTGGGATAAATTCATCAGCATGTGGAAAG
  - R: 5’-TTAGGCAGCCTGTTCCATGGCG

The following transgenic lines (on chromosome 2) were generated: UAS-lqfRaENTH-gfp [32], UAS-lqfRaENTH-gfp [57].

**Analysis of eyes**

For immunofluorescence, eye discs were fixed in PEMS and antibody incubations and washes were in PBST as described [58].

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For immunofluorescence, eye discs were fixed in PEMS and antibody incubations and washes were in PBST as described [58].
Primary antibodies used (DSHB = Developmental Studies Hybridoma Bank): rat anti-E-cadherin (DSHB:DCAD2, used 1:100), mouse anti-Armadillo (DSHB:N27A1, used 1:100), mouse anti-β-galactosidase (DSHB:40-1a, used 1:50), mouse anti-Myc (Santa Cruz Biotechnology:sc-40, used 1:20), mouse anti-Wingless (DSHB:4D4, used 1:100). Secondary antibodies were as in Lee et al., 2009. Confocal microscopy of eye discs, light microscopy of adult eyes, and image processing was as described [32].

Protein blot in Figure 2
Protein extracts of 2 adult flies containing one copy each of the transgene indicated and the ey-gal4 driver were made by homogenizing and boiling in 2× Laemmli Buffer. After SDS-PAGE, Western blotting and probing was performed as described [59]. Primary antibodies were mouse anti-β-tubulin (DSHB:E7, 1:100), and anti-Myc (Santa Cruz Biotechnology:sc-40, used 1:500) [32].

Immunoprecipitation
Protein extracts were prepared from Act>lqfRa-gfp and Act>lqfR<sup>E117H</sup>-gfp embryos: GFP-positive embryos were homogenized in 100 μl lysis buffer (1% NP40, 0.5% deoxycholate, 1 mM DTT, 150 mM NaCl, 50 mM Tris pH 8.0 with protease inhibitor cocktail [Roche, complete-mini, EDTA-free] and 2 mM PMSF). Lysis buffer (300 μl) was added followed by centrifugation at 12,000 rpm at 4°C. A 300 μl aliquot was removed and mixed with 20 μl of a 50% slurry of GFP-trapA (Chromotek) and a 10 μl aliquot was mixed with 2× SDS loading buffer as a loading control. After incubating 2 hrs. with mild shaking at 4°C, the 300 μl aliquot was spun down, the pellet collected and washed for 5 min. with shaking in 1 ml lysis buffer, and then washed again for 10 min. with shaking in 1 ml of 500 mM NaCl. The pellet was washed 4 times more in 1 ml of 500 mM NaCl and then mixed with 20 μl of 2× Laemmli Buffer. Each sample was boiled for 5 min, microfuged, and the supernatant subjected to SDS-PAGE in a 7.5% gel. Western blotting was performed as described [Chen et al., 2002]. Primary antibodies were: rat anti-E-cadherin (DSHB:DCAD2, used 1:1000), mouse anti-Armadillo (DSHB:N27A1, used 1:500), rat anti-α-catenin (DSHB:DCAT-1, used 1:100), rat anti-GFP (Chromotek:3H9, used 1:1000). Secondary antibodies were from Santa Cruz Biotechnology and used at 1:5000: goat anti-rat HRP, goat anti-mouse HRP, goat anti-rat HRP.

Supporting Information

Figure S1 Amino acid sequence alignment of human and yeast Tel2 and Drosophila LqfR-exon 6. The amino acid sequences of H. sapiens Tel2, D. melanogaster LqfR exon 6, and

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**Figure 8. Wg protein secretion in lqfR- clones.** Shown are confocal microscope images of two third instar larval wing discs immunostained with Wg antibodies (purple). Wingless is expressed and secreted by a stripe of cells at the dorsal/ventral boundary. Homozygous mutant clones are marked by the absence of GFP (green). (A,A’) A wing disc with vps35<sup>E42</sup>-FRT42D ubi-gfp. (B,B’) A wing disc with lqfR<sup>D117</sup> mutant clones, outlined in white in B’. The genotype is hs-flp; FRT42D vps35<sup>E42</sup>-FRT42D ubi-gfp. Scale bar: ~10 μm. doi:10.1371/journal.pone.0046357.g008

**Figure 9. The effect of Tel2 on Wingless signaling.** A model for how Wingless signaling is compromised in the absence of Tel2 is illustrated. We speculate that in the absence of Tel2, increased E-cadherin at the plasma membrane sequesters Armadillo (Arm) so that little remains free in the cytoplasm to enter the nucleus in response to Wingless signaling. doi:10.1371/journal.pone.0046357.g009

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Only Tel2 Portion of Fly EpsinR/Tel2 Is Essential

PLOS ONE | www.plosone.org 7 September 2012 | Volume 7 | Issue 9 | e46357
S. cerevisiae Tel2 were aligned using MacVector and the results are shown. H. sapiens vs. S. cerevisiae: aligned length = 950, gaps = 25, identities = 116 (13%), similarities = 102 (12%); H. sapiens vs. D. melanogaster: aligned length = 929, gaps = 15, identities = 101 (19%), similarities = 156 (17%); D. melanogaster vs. S. cerevisiae: aligned length = 924, gaps = 18, identities = 110 (11%), similarities = 121 (13%).

**Figure S2 Rescue of E-cadherin accumulation abnormality in lqfR clones by transgene expression.** Confocal microscope images of three third instar larval eye disc immunostained with antibodies to E-cadherin (red). lqfR clones are marked by the absence of GFP (green). The images at bottom are identical to the ones at the top except only the red layer is shown and the clone is outlined. (A-C) The discs express the transgenes indicated. The genotype is ey-fyb; FRT82B lqfR<sup><s>+/;</s></sup> FRT82B ubi-gfp in all panels, with the addition of Act5C-gal4, UAS-lqfR<sup>+/+</sup> and (B,B<sup>+</sup>) and Act5C-gal4, UAS-lqfR<sup>+/+</sup> (C,C<sup>+</sup>) on chromosome 2. scale bar: ~10 μm in A-B<sup>+</sup>; ~25 μm in C,C<sup>+</sup>.

**Acknowledgments**

We are grateful to Konrad Basler, Xinunna Lin, and the Bloomington Drosophila Stock Center for flies. We acknowledge the DNA sequencing and confocal microscope facilities of the ICMB at UT Austin, and we thank Paul Macdonald for the use of his confocal microscope.

**Author Contributions**

Conceived and designed the experiments: JHL JAF. Performed the experiments: JHL. Analyzed the data: JHL JAF. Contributed reagents/materials/analysis tools: JHL JAF. Wrote the paper: JHL JAF.

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