Akap200 suppresses the effects of Dv-cbl expression in the *Drosophila* eye

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Abstract:

The *Drosophila melanogaster* orthologue of the c-Cbl proto-oncogene acts to down-regulate signalling from receptor tyrosine kinases by enhancing endocytosis of activated receptors. Expression of an analogue of the C-terminally truncated v-Cbl oncogene, *Dv-cbl*, in the developing *Drosophila* eye conversely leads to excess signalling and disruption to the well ordered adult compound eye. Co-expression of activated Ras with *Dv-cbl* leads to a severe disruption of eye development. We have used a transposon-based inducible expression system to screen for molecules that can suppress the *Dv-cbl* phenotype and have identified an allele that upregulates the A-kinase anchoring protein, Akap200. Overexpression of Akap200 not only suppresses the phenotype caused by *Dv-cbl* expression but also the severe disruption to eye development caused by the combined expression of *Dv-cbl* and activated Ras. Akap200 is also endogenously expressed in the developing *Drosophila* eye at a level that modulates the effects of excessive signalling caused by expression of *Dv-cbl*.

Introduction

Receptor tyrosine kinase (RTK) signalling must be precisely modulated to allow transduction of appropriate levels and ensure appropriate timing of intracellular signals. One of the key mechanisms to downregulate receptor activity after ligand binding is endocytic removal of the activated receptor from the plasma membrane [1]. The Cbl family of proteins act as E2-dependent ubiquitin ligase to ubiquitinate activated receptors, thereby stimulating receptor endocytosis [2, 3]. The first characterised member of the family, murine c-Cbl, was originally identified as the protein produced by the cellular counterpart of v-*cbl*, the transforming gene of the Cas NS-1 retrovirus [4]. Both the human and mouse genomes contain three *Cbl* family genes, *c-Cbl*, *Cbl-b* and *Cbl-3*. *c-Cbl* and *Cbl-b* have been shown to encode similar proteins containing an N-terminal SH2-like tyrosine kinase-binding (TKB) domain, a RING finger, an SH3-binding region and a C-terminal UBA domain that act to down regulate activated receptors [5-7]. The TKB domain corresponds to the truncated protein expressed from the v-*Cbl* oncogene. The *C. elegans* and *Drosophila* genomes each contain a single *cbl* orthologue and mutations in each have shown that Cbl proteins also negatively regulate signalling from the EGF receptor in these two organisms [8-12].

v-Cbl can bind to activated RTKs and interfere with the ability of endogenous Cbl proteins to downregulate receptor activity. Genetic studies of a *Drosophila* analogue of v-Cbl have shown that it acts as a dominant-negative protein, enhancing signalling from the *Drosophila* Epidermal Growth Factor Receptor (Egfr) [11]. Phenotypes associated with v-Cbl expression can be readily investigated in the *Drosophila* eye as all cells of the retina are dependent upon Egfr signalling for correct differentiation and survival [13]. The adult *Drosophila* compound eye is composed of approximately 800 individual units, or ommatidia, arranged in a regular array. Each ommatidium consists of 8 light sensing photoreceptors, 4 lens-secreting cone cells, 8 optically insulating pigment cells, and a mechanosensory bristle cell. Perturbations to the array caused by aberrations in cell number or differentiation can be easily observed by light or scanning electron microscopy [14].

c-Cbl has been shown to interact with a wide range of receptors and cytoplasmic proteins including non-receptor tyrosine kinases, phosphatases, cytoskeletal proteins and signal transduction adaptors suggesting that the oncogenic capacity of v-Cbl could be mediated by multiple signal transduction pathways [5]. The ability to conduct unbiased genetic screens in

Drosophila allows the capacity to identify genes or mutations that can modulate the activity of *Drosophila* v-Cbl (Dv-Cbl).

The use of screens for modifying mutations in *Drosophila*, that is, mutations that enhance or suppress the effect of known mutations, is illustrative of how a genetic analysis of a signalling pathway in flies can be of direct value to mammalian studies. The mechanism of signal transduction from Ras to the nucleus has been elucidated by a combination of biochemical studies in mammalian cells and genetic analysis in *C. elegans*, and the *Drosophila* eye [15]. A screen conducted for enhancers and suppressors of an oncogenic Ras protein that was expressed in the *Drosophila* eye uncovered alleles of all the genes known at that time to be in the Ras pathway - including MEK, MAP kinase, Raf, and Ras prenylation enzymes - together with about a dozen previously uncharacterised genes [16]. Included amongst the uncharacterised proteins was *ksr* (kinase suppressor of ras), a component of the system that was not detected in biochemical studies. Subsequent identification and analysis of mammalian homologues of *ksr* has shown that it modulates Ras signalling by augmenting Raf activity [17]. Systematic genetic screens such as this are therefore an extremely powerful way of characterising conserved signalling pathways and complementing mammalian studies.

We have used the Gene Search System whereby random insertion of transposable elements containing an inducible promoter into the genome allows overexpression of genes neighbouring the insertion sites [18]. We have utilised this method to screen for genes that when overexpressed can suppress the effects of Dv-Cbl expression in the *Drosophila* eye. This class of gene is of particular interest as there is the potential for an oncogene suppressor to suppress malignancies associated with oncogene activation. One of the genes that we identified was *Akap200*, a gene that was previously identified in the screen for suppressors of *ras*^{V12} [20]. Little is known about *Akap200*, except that it is a member of the A-kinase anchoring protein family that interact with the regulatory subunits of protein kinase A to anchor kinase activity to specific subcellular compartments. Studies in the *Drosophila* ovary showed that mutations in *Akap200* suppressed a phenotype associated with loss of *Src* function [26]. Thus, it was known that *Akap200* can suppress alleles of three different oncogenes, *ras*^{V12}, *Src64B* and *Dv-cbl*, making it an attractive candidate for further analysis.

In this paper we show that ectopic expression of the *Akap200* suppresses the phenotype induced by v-Cbl and that endogenous *AKAP200* expression modulates v-Cbl

activity. We also show that AKAP200 suppressed the cooperative phenotype produced by co-expression of Dv-Cbl with ras^{V12} .

Materials and methods

Drosophila strains and culture

Genetic experiments were performed at 25°C on semolina molasses media supplemented with live yeast. *Drosophila* alleles used were: w^{1118} , *GMR-GAL4*, *sev-GAL4*, *sev-Ras1^{V12}*, *GS2208*, *EP2254*, *UAS-Dv-cbl*, *UAS-p35*, *Akap200^{k07118a}* (*Akap200-lacZ*), *P{TRiP.HM05018}attP2* (*Akap200RNAi*), *P{KK111598}VIE-260B* (*Akap200RNAi*) and *P{GD1207}v5647* (*Akap200RNAi*). All stocks were obtained from the Bloomington Stock Center, Kyoto *Drosophila* Genetic Resource Center, Vienna *Drosophila* RNAi Center or generated in our laboratory.

Scanning electron microscopy.

Adult female flies were dehydrated through an acetone series (30%, 50%, 70% 100% 100%) then air dried in a fume hood, mounted on carbon stubs and sputter coated with an Edwards S150B Gold Sputter Coater. The eyes were then imaged on a Philips XL30 FEG Field Emission Scanning Electron Microscope.

mRNA in situ hybridisation

DIG labelled mRNA probes for in situ hybridisation to sense and antisense strands were generated using the Megascript T7 kit (Ambion), from a template formed from the 3'UTR of *Akap200* using the following primer sets (T7 polymerase site underlined). Antisense: GATCTCGCCAAGGATCTGAA and GGATCCTAATACGACTCACTATAGGG TTGCGTTCTTTTGTGTTGTC, Sense: TTGCGTTCTTTTGTGTTGTC and GGATCCTAATACGACTCACTATAGGGGATCTCGCCAAGGATCTGAA.

3rd instar imaginal eye discs were dissected in cold PBS then fixed in 4% paraformaldehyde on ice for 20 minutes. The discs were fixed at room temperature for 20 min in 4% paraformaldehyde in PBS containing 0.6% Triton X 100 and then washed three times with 0.6% Triton X 100 in PBS. Tissue was pre hybridised for 1 hour at 55°C, then hybridised

overnight at 55C in hybridisation buffer (pre warmed) which contained 5µg/ml of sense or antisense DIG labelled RNA probe. Tissue was washed 4x in wash buffer at 55C, where the washes were changed every 2-3 hours, and left in wash buffer overnight at 55C. The next day the tissue was washed in PBS with 0.1% Tween -20 PBS (PBS/Tween) for 30 min, which was followed by 2 hours incubation with anti DIG antibody 1:2000 in PBS/Tween with 5% goat serum at room temperature, then washed 4x 20 min in PBS/Tween, and then the PBS/Tween was removed and replaced with AP buffer. The signal was detected in AP buffer containing NBT/BCIP at 37C for approximately 45 minutes. Then the tissues were mounted in 80% glycerol in PBS.

Immunostaining

Imaginal discs were dissected in PBS and fixed for 30 minutes at room temperature in 4% paraformaldehyde, then washed 3 x 10 minutes each in PBT. Samples were incubated in primary antibody diluted in PBT plus 5% goat serum overnight at 4C, washed 3 x 10 minutes and incubated in secondary antibody for 2 hours at room temperature. Antibodies used were: mouse anti-Elav (Developmental Studies Hybridoma Bank) and chicken anti-β-galactosidase (Abcam).

Quantitative real-time PCR

RNA was extracted from adult female heads using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was produced using Superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions and qRT-PCR was performed in triplicate on a Corbett Rotor-Gene 3000, using Sybr Green (Fisher) and Platinum Taq Polymerase (Invitrogen). The primers used were Akap200- forward GATCTCGCCAAGGATCTGAA, Akap200- reverse TTGCGTTCTTTTGTTGTTGC, internal control GAPDH2A-forward AGCCATCACAGTCGATTCC, GAPDH 2A – reverse GGTGCCCTTAAAACGTCCGTG. Mean mRNA levels were calculated using the Standard curve method. The concentration of Akap200 mRNA in each of the samples was calculated relative to the amount of GAPDH2A mRNA. Plots show standard deviation.

Results

Overexpression of Akap200 suppresses the rough eye phenotype exhibited by *Dv-cbl* transgenic flies

The *Drosophila* compound eye exhibits an ordered array of uniformly sized ommatidia when viewed by scanning electron microscopy (Fig. 1a). Previous work by our laboratory [11] has shown that using the GAL4-UAS system to drive expression of the *Dv-cbl* transgene results in a disordered ommatidial array and variation in size of individual ommatidial units (also Fig. 1b). In this experiment GAL4 expression has been driven by the GMR promoter which is active posterior to the morphogenetic furrow, a late stage of development of the third instar larval eye imaginal disc during which there is limited mitosis and cells are being specified to take on their adult fates. Expression of GAL4 alone at this stage of development does not result in an ommatidial phenotype (Fig. 1a) but when this strain is crossed to a strain carrying a *Dv-cbl* cDNA fused to the UAS promoter the presence of GAL4 will drive expression of Dv-Cbl in the GMR expression domain. This double transgenic animal is referred to as *GMR>Dv-cbl* in this study and the heterozygote is characterised by a mildly rough eye in which ommatidia vary somewhat in size and shape (Fig. 1b). We utilised this bipartite expression system to screen a set of transgenic lines, known as the Gene Search System (GSS), in which the UAS promoter had been placed on a P-transposable element that had been mobilised to generate a collection of lines with insertion sites throughout the genome. Hence each strain had the potential to drive UAS-dependent expression of a gene adjacent to the transposon when crossed to a strain expressing GAL4 under the control of an endogenous promoter [18]. We crossed these strains individually to *GMR>Dv-cbl* in order to identify GSS lines that could suppress the ommatidial defects exhibited by *GMR>Dv-cbl* eyes. Crosses to *GMR-GAL4* were used as controls. Strain *GS2208* exhibited a mild ommatidial disorganisation when crossed to *GMR-GAL4* (Fig. 1c) but partially restored order to the ommatidial array when crossed to *GMR>Dv-cbl* (Fig. 1d). The GSS set of strains have been developed using several transgenic constructs, some with bi-directional UAS promoters such that genes could be expressed either side of the transposon insertion site [18]. *GS2208* carries such a bi-directional element that has inserted in the 5' UTR of the longest isoform of the *Akap200* gene (Fig. 1g). Thus, the genetic suppression we observed could be due to co-expression of Akap200, the adjacent gene *CG13398*, or possibly an antisense effect from the *Akap200* 5'UTR. We therefore repeated the experiment using another transposable element strain containing a single UAS promoter driving expression of Akap200. This strain, *EP2254*,

was generated as part of the EP misexpression screen of Rorth (1998)[19] using a different vector to the GSS strains. *EP2254* is inserted 42 basepairs 5' of the *Akap200* transcriptional start site and had previously been identified in a screen for suppressors of activated Ras[20]. GMR driven expression of *EP2254* had no effect on the ommatidial array (Fig. 1e) and when crossed to *GMR>Dv-cbl* the ommatidial variation in size was suppressed and the order of the array somewhat restored, (Fig. 1f) suggesting that *Akap200* suppressed *Dv-cbl*.

EP2254 results in upregulation of *Akap200* expression

EP2254 has previously been reported to suppress the rough eye phenotype resulting from *sevenless* (*sev*) driven expression of activated *Ras* (*Ras^{V12}*) [20]. We used mRNA *in situ* hybridisation with a probe synthesised from the common *Akap200* 3'UTR to determine whether this strain overexpresses *Akap200* when crossed to GMR-GAL4. Discs expressing GMR-GAL4 alone showed a low level of expression of *Akap200* within the 3rd instar imaginal eye disc (Fig. 2a) that was not altered in the *GMR>Dv-cbl* strain (Fig. 2b). Eye imaginal discs derived from animals that carry *GMR-GAL4* and *EP2254* showed a strong upregulation of *Akap200* in a stripe within and posterior to the morphogenetic furrow, in the region of the disc that correlated with strong GMR promoter activity (Fig. 2c). Quantification of this expression by qRT-PCR demonstrated an approximately 5-fold upregulation of *Akap200* expression in tissue derived from *GMR-GAL4*, *EP2254* animals compared to *GMR-GAL4* animals (Fig. 2d). Thus, *EP2254* contains a UAS insertion capable of driving overexpression of *Akap200* when crossed to a Gal4 driver line.

Overexpression of *Akap200* also suppresses the phenotype due to activated Ras

A previous study [20] reported that *EP2254* suppresses the effects of constitutively activated Ras, but did not present images of the suppression of *sev*-driven *Ras^{V12}* (*sev-Ras^{V12}*). We repeated this experiment to determine whether *Akap200* could suppress the *Ras^{V12}* phenotype more effectively than the *Dv-cbl* phenotype. *sev-GAL4* is expressed in a more restricted pattern in differentiating photoreceptors than *GMR-GAL4* and also does not affect ommatidial development on its own (Fig. 3a). *sev-Ras^{V12}* results in a disorganised ommatidial lattice that is more severe than the *GMR>Dv-cbl* phenotype (Fig. 3b). *sev-GAL4*-driven *Akap200* does

not result in any morphological abnormalities (Fig. 3c) but when co-expressed with *Ras*^{VI2} it moderately suppresses the ommatidial disorganisation caused by *Ras*^{VI2} (Fig. 3d).

The *Akap200* suppression of *Dv-cbl/Ras*^{VI2} is independent of apoptosis

We have previously reported that *Dv-cbl* can co-operate with *Ras*^{VI2} to cause severe disruption to eye development [11]. The adult eyes of *GMR-GAL4; GMR-Dv-cbl, sev-Ras*^{VI2} flies have significant tissue overgrowth, ommatidial fusion and melanotic lesions (Fig. 4a and [11]). When *EP2254* is introduced into this strain it suppresses this phenotype to a remarkable degree (Fig. 4b). While the eye does not revert to a wildtype appearance, ommatidial fusion is much reduced and the ommatidia are much more ordered.

The disordered phenotype of eyes co-expressing *Dv-cbl* and *Ras*^{VI2} is not a simple hyperplastic overgrowth but is suggestive of an increase in both mitosis and apoptosis. To determine whether part of the phenotype associated with *GMR-GAL4; GMR-Dv-cbl, sev-Ras*^{VI2} is due to induction of apoptosis, we co-expressed the viral apoptosis inhibitor, p35, with *Dv-cbl* and *Ras*^{VI2}. This moderated the phenotype, however fusion and overgrowth were still present (Fig. 4c). Furthermore, the strong suppression of the *Dv-cbl Ras*^{VI2} co-operative phenotype by ectopic expression of *Akap200* is not simply due to a suppression of apoptosis, as *Akap200* expression is able to further suppress the *GMR-GAL4; GMR-Dv-cbl, sev-Ras*^{VI2}/*UAS-p35* phenotype (Fig. 4d) producing a much more ordered eye than observed in Fig. 4c. *Akap200* is therefore a good suppressor of the *Dv-cbl, Ras*^{VI2} co-operative phenotype in a manner that is partially independent of blocking apoptosis.

Endogenous *Akap200* moderates *Dv-cbl* activity

The above experiments indicate that ectopic *Akap200* can suppress phenotypes associated with expression of *Dv-cbl* but do not indicate that *Akap200* has an endogenous function in eye development. The *Akap200* mRNA *in situ* hybridisation (Fig. 2a) suggests that *Akap200* is expressed at a low level throughout the third instar larval eye disc. Antibodies directed against *Akap200* were not available so we used an *Akap200-lacZ* enhancer trap inserted in the first intron to examine more closely *Akap200* expression within the eye disc. β -galactosidase expression from this strain was detected using immunofluorescence and found to be present at a low level throughout the disc, with a concentration within a subset of developing

photoreceptor neurons (Fig. 5a) as observed by colocalisation with the neuron-specific marker Elav (Fig. 5b,c, e). Lack of colocalisation with Prospero indicated that Akap200 was not expressed in cone cells or photoreceptor R7 (Fig. 5d). The low level of endogenous Akap200 expression within the eye disc could be having a moderating effect on the phenotype of the *Dv-cbl* transgene so we used three independent shRNA constructs to downregulate endogenous levels of *Akap200* by crossing strains containing *UAS-Akap200 RNA interference (RNAi)* transgenes to *GMR-GAL4*. In each case we saw very little effect of lowering levels of *Akap200* on eye development (Fig. 6a, c, e). When each of these strains was crossed to *GMR>Dv-cbl* the level of eye disruption was more severe (Fig.b, d, e) than *GMR>Dv-cbl* alone (Fig. 1b) suggesting that endogenous Akap200 does act to reduce excessive signal transduction caused by expression of *GMR>Dv-cbl*.

Discussion

In this study we have demonstrated that overexpression of *Akap200* is capable of suppressing phenotypes generated by expression of *Dv-cbl* or activated Ras in the developing *Drosophila* eye. The suppression is most clear when *Dv-cbl* and *Ras^{VI2}* are co-expressed and produce a severe disruption to eye development. *Akap200* is endogenously expressed in the third instar eye disc, being concentrated in developing photoreceptor neurons, and this endogenous level of *Akap200* appears to moderate the severity of phenotypes generated from *Dv-cbl* expression.

What is the function of Akap200?

A-kinase anchoring proteins (AKAPs) are a structurally diverse family of proteins that have been grouped based on their ability to bind to the Ser/Thr kinase, protein kinase A (PKA, or cAMP-dependent protein kinase). PKA is a tetrameric protein composed of two regulatory (of RI or RII subtypes) and two catalytic subunits. Most AKAPs bind to the regulatory subunits but they do not play a role in regulating enzymatic activity. Instead, they act as scaffolding proteins that co-localise components of a signalling pathway. This can localise signalling to a specific region of the cell, modulate propagation of signal transduction responses, or assist in generation of feedback (or feed forward) regulatory loops, reviewed in [21-23].

Akap200 encodes a PKA-RII binding site and a myristoylated alanine-rich C-kinase substrate (MARCKS) domain [24] and has been observed to bind to actin filaments, suggesting that it localises specific PKA complexes to the actin cytoskeleton [25]. Akap200 mutant *Drosophila* exhibit slightly reduced viability and some bristle patterning defects. Akap200 localises PKA-RII to germ cell membranes and Akap200 mutants have multinucleate germ cells and structural defects within the actin-rich ring canals that connect ovarian nurse cells and the oocyte [26]. Akap200 also mildly suppresses the eye phenotype associated with *sev*-driven RasV12 and mildly enhances the phenotype of *sev*-driven dominant negative Kinase Suppressor of Ras (KSR) [20]. KSR is a scaffolding protein that facilitates transduction through the RAF-MEK-MAPK cascade [17]. This study is consistent with our findings that *Akap200* overexpression can suppress the phenotypes derived from expression of *Dv-cbl* and activated Ras although we find that it is a very effective suppressor of the severe eye defects generated by co-expressing *Dv-cbl* and activated Ras. We have also shown that this suppression ability is not simply a result of the high level ectopic expression of *Akap200* from *EP2254* but that endogenous *Akap200* moderates signalling from the *Dv-cbl* transgene. A recent study of the mammalian AKAP member, AKAP-Lbc, has shown that it interacts with KSR-1 and PKA to enhance RAF signal transduction and thereby regulate MAPK signalling. AKAP-Lbc acts as scaffold to promote a close association between RAF and MEK1 thereby facilitating activation of MEK1 by RAF. It also enhances PKA-mediated phosphorylation of KSR-1, which also facilitates MAPK activation [27]. Although AKAP-Lbc is not a direct orthologue of Akap200, and indeed Akap200 does not appear to have a closely related vertebrate orthologue, our data suggest that Akap200 could play a similar role in modulating signalling via the Ras-MAPK cascade. This requires further investigation and raises the possibility that regulation of AKAP activity may have therapeutic potential for ameliorating the excessive Ras-MAPK activity exhibited by a wide range of human tumours.

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Figure Legends

Figure 1. Scanning electron microscopy of adult eyes. (a) Eyes from flies of genotype *GMR-GAL4* have a normal appearance. (b) *GMR>Dv-cbl* results in disruption to the ommatidial lattice and fusion of individual ommatidia. (c) *GMR-GAL4/GS2208* causes a mild disruption to the ommatidial lattice but (d) suppresses the severity of the *GMR>Dv-cbl* phenotype. (e) *GMR-GAL4/EP2254* eyes have a normal appearance but also (f) mildly suppress the severity of *GMR>Dv-cbl*. (g) The *Akap200* gene generates four transcripts. The positions of the insertions of *EP2254* and *GS2208* are indicated (arrowheads show direction of transcription from the transposon encoded UAS promoters). (g) is modified from <http://flybase.org/cgi-bin/gbrowse/dmel/?Search=1;name=FBgn0027932>.

Figure 2. mRNA *in situ* hybridisation of a probe specific to *Akap200* to third instar eye imaginal discs. (aa) and (as) antisense and sense probe hybridisation to *GMR-GAL4* eyes shows a low level of *Akap200* expression across the eye disc that is also observed in *GMR>Dv-cbl* discs (ba) and (bs). *GMR-GAL4/EP2254* discs (ca) and (cs) show strong upregulation of *Akap200* expression posterior to the morphogenetic furrow. (d) qRT-PCR quantification of *Akap200* expression shows an approximately 5-fold upregulation in *GMR-GAL4/EP2254*, compared to the GAPDH2A normalised *Akap200* mRNA expression levels in *GMR-GAL4/+*. The error bars represent $\pm$ one standard deviation.

Figure 3. Scanning electron microscopy of adult eyes. (a) *sev-GAL4* has a wildtype morphology but (b) *sev-GAL4, sev-Ras^{v12}* has a rough eye. (c) *sev-GAL4/EP2254* has wildtype appearance but can suppress the roughness of *sev-GAL4, sev-Ras^{v12}*.

Figure 4. Scanning electron microscopy of adult eyes. (a) *GMR-GAL4; GMR-Dv-cbl, sev-Ras^{v12}* eyes have severely disrupted morphology that can partially restored by suppression of apoptosis via *UAS-p35* (b). (c) *EP2254* effectively suppresses the disruption of *GMR-GAL4; GMR-Dv-cbl, sev-Ras^{v12}* eyes but this is not simply due to suppression of apoptosis as further suppression also occurs (d) in the presence of *UAS-p35*.

Figure 5. Immunofluorescence of third instar eye imaginal discs. (a) β -galactosidase expression from *Akap-lacZ* is concentrated in photoreceptor neurons indicated by expression of the neural-specific RNA-binding protein Elav (b). A merged image is shown in (c). Higher magnification images of *Akap200-lacZ* colocalisation with Prospero (d-d'') and Elav (e-e'').

Figure 6. Scanning electron microscopy of adult eyes. *GMR-GAL4* crossed to transgenic strains carrying one of three independent *Akap200* shRNA transgenes (a) P{GD1207}v5647, (c) P{KK111598}VIE-260B or (e) P{TRiP.HM05018}attP2 all display wildtype phenotypes but each RNAi line (b), (d), (f) enhances the phenotype of *GMR>Dv-cbl*.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6