Supporting Information

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SI Methods

Cloning and Recombinant Protein Expression. The tail and cargo-binding domain of *Drosophila* M6 (tail-CBD) was amplified from total mRNA isolated from fly embryos using the FastTrack 2.0 kit (Invitrogen). The sequence (Gene ID 42889, amino acids 833–1,256) was reverse-transcribed with specific primers and cloned into a modified pGEX vector with a PreScission protease recognition sequence (gift of Dr. Aaron Straight, Stanford University). The globular tail domain of *Drosophila* M5 (GTD) was similarly amplified and cloned (Gene ID 35680, amino acids 1,392–1,792). Rosetta 2 (DE3) cells (Novagen) expressing either construct were grown in autoinduction media (1) supplemented with 100 μg/mL carbenicillin and 34 μg/mL chloramphenicol until growth reached saturation. The cells were then centrifuged for 20 min at 2,500 × *g*, resuspended in lysis buffer [20 mM Tris, pH 8.0, 1 M NaCl, 1 mM EDTA, 10 mM DTT, and Complete EDTA-free protease inhibitors (Roche Applied Science)], frozen in liquid nitrogen, and stored at −80 °C. Cornetto 5 was amplified and cloned as above (Gene ID 38786, amino acids 770–978), and Rosetta 2 (DE3) cells were grown in LB supplemented with antibiotics at 37 °C and induced with 0.5 mM IPTG at 25 °C according to standard procedures. To express GST protein, empty pGEX vector was transformed into Rosetta 2 (DE3) cells that were grown in LB and induced as described above.

Recombinant Protein Purification. For the preparation of all GST-tagged proteins, frozen bacterial cells were thawed and lysed by sonication on ice. All subsequent steps were performed at 4 °C. The lysate was clarified at 113,000 × *g* for 30 min and then combined with 5 mL of washed glutathione-agarose. This slurry was mixed for 1 h and then washed with 20 bed volumes of wash buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT). Bound protein was either eluted in wash buffer supplemented with 10 mM reduced glutathione or cleaved from the GST-tag by incubation with 0.2 mg GST-HRV 3C protease overnight. Eluted or cleaved proteins were then further purified by ion exchange chromatography (M6 tail-CBD on a HiTrap SP column, at pH 7.5, with a gradient of 0–1 M NaCl; M5 GTD and Cornetto 5 on a HiTrap Q column, at pH 7.5, with a gradient of 0–1 M NaCl). The concentration of the purified proteins was determined by denaturing in guanidine-HCl and measuring their absorbance at 280 nm using the extinction coefficient calculated from their amino acid sequences.

Preparation of Drosophila Embryonic Extracts. Approximately 20 g of wild-type *Drosophila* embryos (Stanford University Developmental Biology Fly Center) aged 0–18 h were gathered by spraying collection plates with a buffer containing 5 mM HEPES, pH 7.5, and 1 mM EGTA. After the embryos were washed in collection buffer, an equal volume of lysis buffer (5 mM HEPES, pH 7.5, 1 mM EGTA, 0.5% Igepal CA-630, 2 mM sodium orthovanadate, 1 mM ATP, protease inhibitors) was added. The embryos were then subjected to a cold Dounce homogenizer, and the crude lysate was centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was diluted with an equal volume of buffer containing 100 mM HEPES, pH 7.5, 100 mM KCl, 20 mM $MgCl_2$, 2 mM DTT, 2 mM ATP, and protease inhibitors. After mixing, the sample was centrifuged at 200,000 × *g* for 90 min at 4 °C, and the supernatant from this step was applied to the columns as described below.

Column Construction. To construct two preclearing columns, 100 mg of BSA (Sigma) or GST (purified from bacteria as described above) were each combined with 10 mL of Affi-gel 10 activated resin (BioRad) in a buffer containing 100 mM HEPES, pH 6.0, 150 mM NaCl, 2 mM BME, and 1 mM EDTA. The coupling reaction was allowed to proceed on a tilting shaker for 2 h at 4 °C, after which the remaining active groups were quenched with 200 μL of 1 M ethanolamine for 1 h at 4 °C. The GST-M6 tail-CBD and GST-M5 GTD columns were made in a similar manner, except 1 mL of Affi-gel 10 was used for the coupling of 10 mg of each protein at pH 7.5. After quenching, all columns were washed with 10 column volumes of buffer containing 50 mM HEPES, pH 7.5, 1 M KCl, 0.5 mM EGTA, and 10 mM $MgCl_2$. A similar buffer, but with 50 mM KCl, was used to equilibrate and store the columns. All steps were performed at 4 °C.

Affinity Chromatography. All steps before SDS-PAGE were performed at 4 °C. The embryonic extract was passed over the BSA column twice and was then applied to the GST column twice, each by gravity flow. The precleared material was then divided in half, and each half was passed over either the GST-M6 or GST-M5 column using a peristaltic pump. After these first applications were complete, the two samples of extract were recombined, split in half, and again applied to the myosin columns. All wash and elution buffers contained 50 mM HEPES, pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 10% glycerol, and the indicated amount of salt. Twenty column volumes of a 100 mM KCl buffer were used to wash both myosin columns. One 3 mL elution was then performed for each salt concentration of 250 mM, 500 mM, and 1 M KCl. Each 3 mL fraction was split in half, and 170 μL of 100% trichloroacetic acid (TCA) was added to each and incubated overnight on ice. The TCA precipitates were centrifuged at 13,000 × *g* for 20 min, and the supernatants removed. The pellets were centrifuged again at 11,000 × *g* for 5 min, and any remaining supernatant was discarded. After air-drying, one pellet from each elution was frozen at −20 °C. To the other, 30 μL of 2X SDS-PAGE sample buffer was added, and each tube was boiled for 5 minutes. A 3 mL sample of the collected wash was treated in the same manner, except each was resuspended in 150 μL of buffer. With standard SDS-PAGE using 4–20% gradient gels (BioRad), the samples were inspected for differences after Coommassie-G250 or silver staining.

Mass Spectrometry. Mass spectrometry sample handling and protein identification were performed as a paid service by NextGen, Inc. on TCA-precipitated pellets from 1 M elutions with an LTQ Orbitrap XL mass spectrometer and Mascot search engine; data were provided as a searchable Scaffold file. Further details of their methods are available upon request.

In Vitro Transcription and Translation and In Vitro Binding Assays. Candidate cDNAs were cloned into a vector containing an SP6 promoter that encoded for N-terminal 6X myc-tag fusion proteins with AscI and PacI restriction sites (gift of Dr. Aaron Straight, stanford University). Listed are forward and reverse primer sequences for each cDNA:

Miranda (5′-GCGGGCGCGCCAGGATGTCTTTCTCCAAGGCCAAG and

5′-GCGTTAATTAACTAGATGTTGCGCGCCTTGAG),

Cornetto (5′-GCGGGCGCGCCAGGATGACCAGCTTGGAGCTGAGC and

5′-GCGTTAATTAATTAAAACCTTACGTCGATCTC),
Lava lamp N-terminus (5′-GCGGGCGCGCCAGGATGGC-GGAAGATTCGGGTGCT and
5′-GCGTTAATTAAATGCACTTGCTTCTGCTCCTG),
Cbs (5′-GCGGGCGCGCCAGGATGTTTGCTACACTGA-AAAAC and
5′-GCGTTAATTAATTAAGTTGGTTTCATGCCAAA),
CG31357 (5′-GCGGGCGCGCCAGGATGTCCGAGATCA-AGGCGTTG and
5′-GCGTTAATTAATTACATCACAAAGACCTTTTC),
CG7611 (5′-GCGGGCGCGCCAGGATGCAGAGCACCA-GTTCCACG and
5′-GCGTTAATTAATTAAGTCATATTCCAGGAGGA),
CG3295 (5′-GCGGGCGCGCCAGGATGGACAGCGAGT-CGTTGGCC and
5′-GCGTTAATTAATTAAAAGTAGATGCGAACAGC),
Nup107 (5′-GCGGGCGCGCCAGGATGGCCGACAGCCC-GTTCCCG and
5′-GCGTTAATTAACTAAGTTGTTATAGGATAGCC),
Kermit (5′-GCGGGCGCGCCAGGATGCCGCTCTTCACA-AGAAAA and
5′-GCGTTAATTAATTACTTGGGACTGGTCGTGGA),
CG3529 (5′-GCGGGCGCGCCAGGATGGCTTCATTTTT-CAACGTT and
5′-GCGTTAATTAATCAGAGCGCGAGCAGATCCTC),
CG17494 (5′-GCGGGCGCGCCAGGATGGTCTTGGTGT-GCAATGAG and
5′-GCGTTAATTAATTAAATTCGACTGGAAAGAAA),
CG7600 (5′-GCGGGCGCGCCAGGATGACGGAGCAAG-AGGATTTG and
5′-GCGTTAATTAATCAACGACCATTCCAATGGCA),
CG11092 (5′-GCGGGCGCGCCAGGATGGATCTGATGG-AACTGCTG and
5′-GCGTTAATTAATTAGTGCATAAGGATCTCCAG),
CG2774 (5′-GCGGGCGCGCCAGGATGGAGGTGGAAA-GCCCGGAA and
5′-GCGTTAATTAATTAAACGATTTCGCGGGCAAA), and
M5 coiled-coil and GTD (5′-GCGGGCGCGCCAGGATGA-CTCTGGATATCAATCGG and
5′-GCGTTAATTAATTAAATCTTTGTAAGGAACTCGTC).

Transcription and translation were performed in vitro using a rabbit reticulocyte lysate system according to manufacturer's instructions (Promega). The translation products were added to comparable amounts of purified GST-M6 and GST-M5 on glutathione-agarose beads in binding buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 3 mM DTT, 1 mM EDTA, and 0.5% Igepal CA-630) and incubated on ice for 1 h with frequent mixing. The resin was washed quickly three times in wash buffer (binding buffer with 300 mM NaCl), and was then resuspended in 15 μL of 2X SDS-PAGE sample buffer and boiled for 5 min. The samples were subjected to SDS-PAGE and Western blotting according to standard techniques, using an antimyc primary antibody (gift of Dr. Aaron Straight, Stanford University), a sheep antimouse HRP-conjugated secondary antibody (GE Healthsciences), and an ECL detection kit (Pierce). Translation of candidates, observed by Western blot, was of varying efficiency. For in vitro binding assays with recombinant Cornetto 5, the GST-tag was cleaved off both proteins before further purification steps, and M6 tail-CBD was coupled to NHS-Sepharose as described above. A final concentration of approximately 5 μM of M6 was added to each sample, with a range of Cornetto 5 from 0 to 90 μM. Volumes were equalized with binding buffer and samples were incubated at 4 °C for 1 h with mixing. The Sepharose was washed with 30 bed volumes of binding buffer and then resuspended in 15 μL of 2X SDS-PAGE sample buffer. Samples were run on 15% SDS-PAGE and proteins were stained with Coomassie-G250. Gels were dried, scanned, and bands were quantitated using ImageJ software. Values were plotted in Matlab, and the curve was fit to a rectangular hyperbola.

Immunofluorescence. Mixed-stage wild-type (Stanford University Developmental Biology Fly Center) and w^{1118}; *PBac{PB}CG31357*^{c01438} (Bloomington Drosophila Stock Center, BDSC) embryos were collected at 25 °C. Progeny of crosses between *Act5c-GAL4/CyO*, *P{ActGFP}JMR*1 virgin females (line generated from BDSC stocks) and w^{1118}; *UAS-cornetto RNAi* (Vienna Drosophila RNAi Center, VDRC) or w^{1118}; *UAS-lva RNAi/CyO*; *UAS-lva RNAi/TM6b*, *Tb*[1] (gift of Dr. Yuh-Nung Jan, University of California, San Francisco) males were collected for 3 h and allowed to develop for an additional 10.5 h, all at 25 °C. All embryos were dechorionated in 50% bleach for 5 min, washed with water, and then fixed by vigorously shaking for 20 min in a 1:1 mixture of heptane and 4% paraformaldehyde in PBS. The aqueous phase was removed, methanol was added, and the tubes were shaken gently to remove vitelline membranes. After several washes, the embryos were stored at −20 °C in methanol. For staining, all steps were performed at room temperature unless otherwise noted. Embryos were rehydrated with five washes of PBS + 0.05% Tween-20 (PBST) and blocked for 1 h with 10% heat-inactivated fetal bovine serum in PBST. The anti-M6 antibody was diluted 1:5,000 in PBST or blocking solution and applied to embryos, with gentle shaking, overnight at 4 °C. Next, the embryos were washed five times in PBST followed by secondary antibody (Alexa 488-labeled goat antirabbit or Alexa 555-labeled goat antirabbit at 1:500 in blocking solution; Invitrogen) for 2 h. After more washes, DAPI (0.2 mg/mL in blocking solution) was applied for 5 min, and another five washes in PBST were performed. Embryos were then mounted on slides with DABCO/Mowiol permanent mount. Images were collected on a Leica confocal microscope using the manufacturer's software.

Antibody Production/Purification. M6 tail-CBD, M5 GTD, and Cornetto 5 were expressed as GST fusion proteins and purified as described above. Aliquots of the myosin truncations were stored on ice for repeated injection into rabbits by Cocalico Biologicals, Inc. Aliquots of Cornetto 5 were snap-frozen in liquid nitrogen and thawed immediately before injection into chickens by Josman Labs. Injections, test bleeds, and boosts were performed according to standard techniques. Chicken eggs were collected during the course of the injections. At the end of the immunization period, each animal was exsanguinated in the production bleed. The rabbit sera were diluted in an equal volume of Tris-buffered saline containing 0.02% sodium azide, and whole IgY was purified from egg yolks using a kit from Gallus Immunotech, Inc. and then diluted in the same buffer. Antibodies were then purified over columns constructed from purified proteins coupled to 1 mL of Affi-gel resin or NHS-Sepharose, prepared as described above. The diluted rabbit sera or whole IgY fractions were continuously passed over TBS-equilibrated columns overnight, which were then washed with 80 column volumes of buffer (TBS, 0.5 M NaCl, 0.1% Tween-20) and placed in a 0.2X TBS solution. Antibodies were eluted as 0.5 mL fractions with a 0.2 M glycine, pH 2, 150 mM NaCl buffer and immediately neutralized with 1.8 M Tris, pH 8.5. The protein content of each was determined by measuring the absorbance at 280 nm on a spectrophotometer. The purification process was repeated over several days until most of the high-affinity products had been depleted. Those fractions with high protein content were combined and dialyzed into a PBS buffer containing 50% glycerol and stored at −20 °C.

CoImmunoprecipitations. Semiconfluent plates each containing 1×10^7 adherent S2R+ cells were detached with trypsin, washed with PBS, and lysed in 20 mM Tris, pH 7.4. 150 mM NaCl, 1 mM

EDTA, 3 mM DTT, and 0.5% Igepal CA-630. The lysates were solubilized on ice for 30 min and then centrifuged for 10 min at 7,800 × *g* at 4 °C. Supernatants were added to 30 μL IgY coupled to NHS-Sepharose (corresponding to about 10 μg antibody) or 10 μg rabbit IgG with 30 μL protein A-sepharose, and incubated at 4 °C with gentle agitation for 1.5 h. Beads were washed three times with 500 μL buffer (same as above), resuspended in 15 μL 2X SDS-PAGE sample buffer, boiled for 5 min, and subjected to separation by SDS-PAGE on 4–20% gradient gels (BioRad) and subsequent Western blotting with anti-M6 antibody and antirabbit secondary antibody according to standard techniques.

Hedgehog Secretion Assays in S2R+ Cells. dsRNAs targeted against *YFP*, *dispatched*, *jaguar* (M6), and *cornetto*, all containing T7 sequences, were designed using the SnapDragon tool (DRSC, Harvard University). The following primer sequences were used to amplify cDNAs from total fly embryonic mRNA as described above:

YFP (5′-TAATACGACTCACTATAGGGATGGTGAGCAAGGGCGAG and
5′-TAATACGACTCACTATAGGGGAAGTTCACCTTGATGCC)

Dispatched (5′-TAATACGACTCACTATAGGGTTGCCACAAAGCATATCCAA and
5′-TAATACGACTCACTATAGGGGAAAGGGTGTCCTGCACATT)

M6-1 (5′-TAATACGACTCACTATAGGGCACGGAGCAGTTCATTGAGA and
5′-TAATACGACTCACTATAGGGTGATTCGATTACGCAGCTTG)

M6-2 (5′-TAATACGACTCACTATAGGGCAACGATAATATATTGAAAAACGA and
5′-TAATACGACTCACTATAGGGGCTCCAGTTTCTCCATTAGC)

Cornetto-1 (5′-TAATACGACTCACTATAGGGTGTAGCTGGAGCATCCACAG and
5′-TAATACGACTCACTATAGGGAATTGCTGCAGGGAATCATC)

Cornetto-2 (5′-TAATACGACTCACTATAGGGCAAAGGGAATTCGAGTTGGA and
5′-TAATACGACTCACTATAGGGGTCTGGAGCAGACGGTTCTC).

dsRNAs were transcribed using the MEGAscript T7 kit (Ambion) and checked on agarose gels. S2R+ cells were maintained in Schneider's media (Invitrogen) with heat-inactivated fetal bovine serum (Invitrogen) supplemented with penicillin, streptomycin, and glutamine. On day 1 of each experiment, cells were seeded into 12-well plates at 1 million cells/well. On day 2, 1 μg of each dsRNA was transfected per well using Fugene HD (Roche), and fresh media was added. On day 3, 1.5 μg of Hedgehog (Hh) or HhN DNA (in pActSV vector, gift of Dr. Phil Beachy, Stanford University) was transfected per well using Fugene HD, and fresh media was added. On day 5, conditioned media was collected, and SDS-PAGE sample buffer was added before boiling and separating proteins on 4–20% gradient gels. To generate total cell lysates, cells were washed and lysed (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Igepal CA-630, protease inhibitors), and protein concentrations were measured using BioRad Protein Assay reagent. Equal amounts of total protein were added to SDS-PAGE sample buffer, boiled, and samples were run on 4–20% gradient gels and transferred to nitrocellulose membranes and probed by Western blotting with antiHedgehog antibody (d-300, Santa Cruz Biotechnology) or anti-M6 antibody. For each individual experiment, bands were quantified in ImageJ and normalized to the sample transfected with the YFP dsRNA. Multiple experiments could then be averaged and the data graphed in Excel.

Cuticle Preparations. Virgin *engrailed-GAL4* (BDSC) females were mated with y^1w^{67c23} (gift of Dr. Roel Nusse, Stanford University), w^{1118}; *UAS-jaguar tail-CBD* (see below), or w^{1118}; *UAS-cornetto RNAi* (VDRC) males at 28–29 °C. Embryos were collected overnight on grape juice plates, removed from the crossing cages, allowed to develop for an additional 6 h, and then fixed as described above. To prepare the cuticles, the embryos were placed in a 1:4 mixture of glycerol and actetic acid at 60 °C overnight and placed on slides. The excess solution was wiped away, and Hoyer's medium was added before coverslips were placed. The slides were left under weight at 65 °C overnight, then viewed on a darkfield microscope. Images were taken using Axiovision software. Embryos were scored as having normal or abnormal segmentation after examination of denticle belts, and the percentage in each category were recorded in Excel. To generate the dominant negative M6 line, the construct used for column generation (tail-CBD) was cloned into a modified pWALIUM10-moe vector (gift of Harvard Transgenic RNAi Project) for site-specific integration. The plasmid was injected into *yw*; *attp40* embryos by Genetic Services, Inc., and the resulting transgenics were used to generate the homozygous stock (w^{1118}; *UAS-jaguar tail-CBD*).

Cuticle Preparations for Counting Assays. Virgin females from w^{1118}; *UAS-jaguar RNAi* and w^{1118}; *UAS-cornetto RNAi* stocks (VDRC) were mated with y^1w^{67c23} (gift of Dr. Roel Nusse, Stanford University) or *engrailed-GAL4* (BDSC) males at 29–30 °C. Embryos were collected on grape juice plates for 8 h, removed from the crossing cages, allowed to develop for an additional 19 h, and then fixed and prepared as described above, though without the devitellinization step. The number of intact, empty membranes was compared to the number of embryos and larvae that had clear cuticular material, and the percentage with cuticles in each of the four genotypes was calculated and plotted in Excel.

1. Studier FW (2005) Protein production by auto-induction in high density shaking cultures. *Protein Expres Purif* 41:207–234.

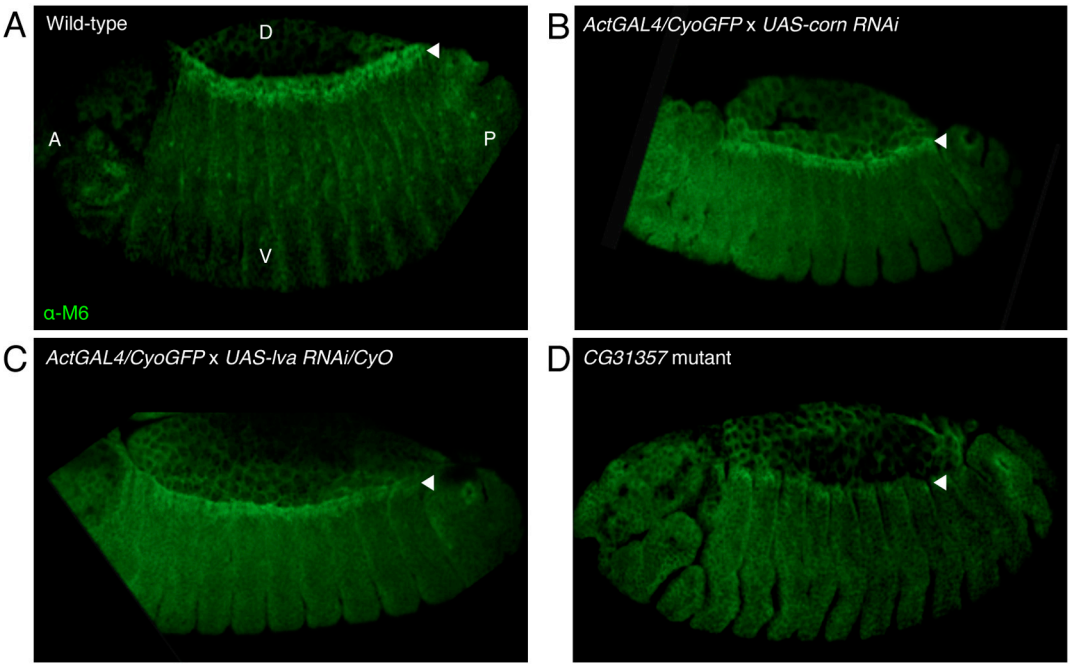

Fig. S1. Immunofluorescence of M6 in stage 13 embryos. (*A*) The anterior (A), posterior (P), dorsal (D), and ventral (V) sides are marked. The intense band of M6 (green) at the leading edge of cells undergoing dorsal closure (arrowheads) is apparent in wild-type (*A*), as observed previously (1, 2), and appears normal in embryos from crosses designed to deplete Cornetto by RNAi (*B*). The staining in these cells is somewhat reduced in embryos from crosses designed to deplete Lava lamp (Lva) by RNAi (*C*) and appears less prominent in the *CG31357* mutant (*CG31357*^{c01438}) (*D*).

1. Kellerman KA, Miller KG (1992) An unconventional myosin heavy chain gene from Drosophila melanogaster. *J Cell Biol* 119:823–834.
2. Millo H, Leaper K, Lazou V, Bownes M (2004) Myosin VI plays a role in cell-cell adhesion during epithelial morphogenesis. *Mech Dev* 121:1335–1351.

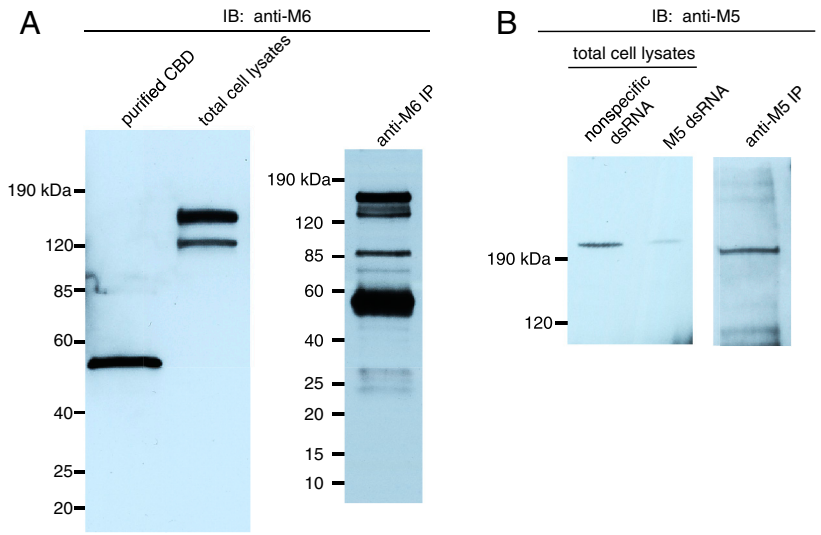

Fig. S2. Anti-M6 and anti-M5 antibodies specifically detect proteins in Western blots and immunoprecipitations. (*A*) Purified recombinant cargo-binding domain (CBD) of M6 and 10 μg high-speed supernatant from fly embryonic extracts were loaded on a gel and probed with the affinity-purified anti-M6 antibody. A doublet is observed in embryonic extracts, as previously reported (1). Anti-M6 antibody was also used for an immunoprecipitation from embryonic extracts followed by Western blotting. Two bands can again be observed, as can the heavy and light chains of the antibody, as well as some nonspecific bands. (*B*) Total cell lysates from S2R+ cells transfected with dsRNA targeting either a nonspecific sequence (YFP) or M5 were probed with anti-M5. Anti-M5 was also used to immunoprecipitate and detect protein from S2R+ cells by Western blotting, and the band at approximately 200 kDa corresponds to M5.

1. Kellerman KA, Miller KG (1992) An unconventional myosin heavy chain gene from Drosophila melanogaster. *J Cell Biol* 119:823–834.

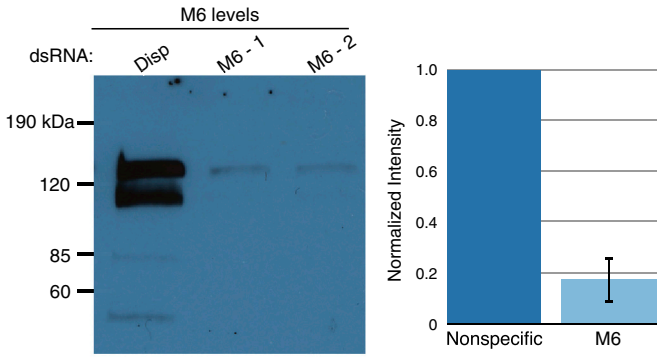

Fig. S3. Levels of M6 protein after dsRNA-mediated knockdown in S2R+ cells. After collecting conditioned media for analysis of Hedgehog secretion, total cell lysates were prepared to measure the level of knockdown of M6 protein. Two different dsRNA sequences targeting different regions of M6 were transfected and are shown on the *Left*, compared to cells transfected with Disp dsRNA. On the *Right*, levels of M6 from several independent experiments using both dsRNAs are quantified and normalized to levels of M6 in cells transfected with nonspecific dsRNAs (data are mean ± SEM, $n = 11$).

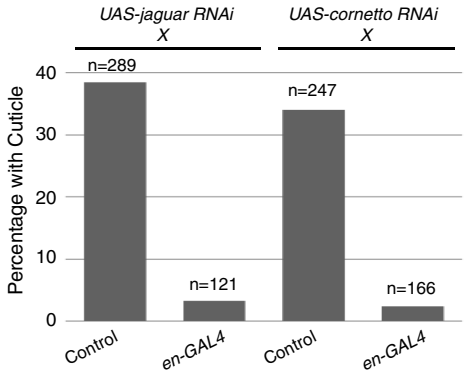

Fig. S4. Cuticles of embryos and larvae expressing M6 or Cornetto RNAi. Large-scale crosses were performed between M6 (*UAS-jaguar RNAi*) or Cornetto (*UAS-cornetto RNAi*) RNAi lines and control (y^1w^{67c23}) or *engrailed-GAL4* lines. Embryos developed at 29–30 °C for at least 19 h and were then fixed without devitellinization, and cuticles were prepared. Darkfield microscopy was used to count the number of cuticles compared to the number of empty vitelline membranes present on each slide. The *n* values indicated represent the number of embryos counted for each sample.

Table S1. Mass spectrometry identification of column-associated proteins

Protein	Identifier	M6 unique peptides	M5 unique peptides	Predicted molecular weight, kDa	M6 UPR	Functional terms*
Lava lamp	CG6450	257	4	316	0.81	Golgi
CLIP-190[†]	CG5020	139	12	189	0.74	microtubule binding
Didum (M5)	CG2146	112	5	208	0.54	myosin motor
CG17494	CG17494	47	0	104	0.45	—
CG31357	CG31357	20	3	45	0.44	—
CG7611	CG7611	28	4	70	0.40	—
CG7600	CG7600	38	0	104	0.37	heme and oxygen binding
Cornetto	CG42278	35	0	102	0.34	microtubule binding
Kermit[‡]	CG11546	12	0	37	0.32	signal transduction
Cbs	CG4840	22	0	71	0.31	centrosome
CG7671	CG7671	12	0	40	0.30	possible Nup43 orthologue
CG3295	CG3295	14	0	49	0.29	—
Miranda[§]	CG12249	26	0	93	0.28	neuroblast divison
Mob4	CG3403	7	1	26	0.27	centrosome
Nup75	CG5733	20	0	77	0.26	nuclear pore
CG10158	CG10158	9	0	35	0.26	—
Nup107	CG6743	24	2	97	0.25	nuclear pore
CG3529	CG3529	14	0	58	0.24	protein transport
βTub60D	CG3401	12	0	51	0.24	tubulin complex
Dhc64C	CG7507	123	0	530	0.23	dynein motor
Embargoed	CG13387	28	0	123	0.23	nuclear export
Faf	CG10372	10	0	53	0.19	—
Rab6	CG6601	4	0	23	0.17	exocytosis
Nup160	CG4738	25	0	160	0.16	nuclear pore
Nup133	CG6958	20	0	135	0.15	nuclear pore
Fs(2)Ket	CG2637	14	0	99	0.14	nuclear import
Sec31	CG8266	18	3	136	0.13	endoplasmic reticulum to Golgi transport
p115	CG1422	11	0	92	0.12	Golgi
CG16892	CG16892	5	0	52	0.10	—
Nup98	CG10198	11	0	135	0.08	nuclear pore
Pp1α-96A	CG6593	3	0	37	0.08	protein phosphatase
Mit(1)15	CG9900	5	0	82	0.06	kinetochore
Hippo	CG11228	4	0	75	0.05	signal transduction
Klp10A	CG1453	4	0	89	0.04	kinesin motor
Rod	CG1569	7	0	240	0.03	mitotic checkpoint
Chb	CG32435	3	0	166	0.02	mitotic spindle
LRP1	CG33087	9	0	525	0.02	LDL receptor

Proteins retained specifically by M6 column. Candidates relevant to the binding data in Fig. 2 are presented. Listed are their FlyBase names or symbols and their identifiers, which are assigned to each *Drosophila* gene as CG (computed gene or Celera gene) followed by a unique number. Proteins only referred to by their identifiers have not yet been formally named. The number of unique peptides that could be assigned with over 80% confidence and the predicted molecular weight of each full-length protein are displayed. The ratio of these two numbers is presented as the unique peptide ratio (UPR), and the table order is sorted from highest to lowest UPR.

*Functional terms are from FlyBase; some Nup proteins have been assigned as nuclear pore based on data from their mammalian orthologues.

[†]CLIP-190 has been shown to coimmunoprecipitate with M6 (1).

[‡]Kermit has been shown to interact genetically with M6 (2).

[§]Miranda has been shown to bind M6 directly (3).

1. Lantz VA, Miller KG (1998) A class VI unconventional myosin is associated with a homologue of a microtubule-binding protein, cytoplasmic linker protein-170, in neurons and at the posterior pole of Drosophila embryos. *J Cell Biol* 140:897–910.
2. Djiane A, Mlodzik M (2010) The Drosophila GIPC homologue can modulate myosin based processes and planar cell polarity but is not essential for development. *PLoS One* 5:e11228.
3. Petritsch C, Tavosanis G, Turck CW, Jan LY, Jan YN (2003) The Drosophila myosin VI Jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts. *Dev Cell* 4:273–281.