Supporting Information

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SI Materials and Methods

Gene Constructs. The *Drosophila merry-go-round* (Mgr), *Von Hippel Lindau* protein (Vhl), and *β1-tubulin* cDNAs were obtained from *Drosophila* Genomics Resource Center (DGRC) clone LD34406, RH61560, and LD43681, respectively (http://dgrc.cgb.indiana.edu/). These clones were cloned by recombination of PCR products into the pDONR221 vector (Gateway system; Invitrogen) (see primers list in Table S1). Bacterial expression constructs for Maltose binding protein (MBP) and GST fusions were made using the destination vectors: pKM596 (1) (Addgene; plasmid 8837) and pDEST24 (Invitrogen). These constructs were used for the expression and purification of GST- and MBP-tagged proteins in *Escherichia Coli*, for the synthesis of S^{35}-labeled proteins by coupled transcription and translation for in vitro binding assays, and for the production of dsRNA for use in RNAi experiments. The detailed protocols for these methods are given below. The *Vhl* sequence in the entry vector was recombined into the destination vectors pAWM (*Drosophila* Gateway vector collection) for expression in fusion with 6× myc at the C terminus under the control of the Actin 5C promoter.

Antibodies. The following antibodies were used: Mouse anti–γ-tubulin (GTU88; Sigma),Western blot 1/5,000; Rabbit anti–Spd-2 (2), immunofluorescence (IF) 1/2,000; Mgr antibody was raised in rabbit against the amino acids 171–185 (MARVYNWGVKKRQAA) of CG6719, Western blot 1/2,000, IF 1/1,000; Rabbit anti-H2A (Abcam), Western blot 1/1,000; Mouse anti–β-tubulin E7 (Hybridoma Bank), Western blot 1/2,000; Mouse anti-GFP (Roche); Western blot 1/1,000; Chicken anti-Dplp (3), IF 1/2,000; mouse anti–α-tubulin (DM1A; Sigma), IF 1/1,000, Western blot 1/5,000; Mouse anti-actin (Sigma), Western blot 1/1,000; Mouse anti-myc (Sigma), Western blot 1/1,000, IF 1/1,000; Rabbit anti–β1-tubulin, Western blot 1/1,000, and Rabbit anti–β2-tubulin antibodies, Western blot 1/50, were a generous gift of Renate Renkawitz-Pohl (Philipps-Universität Marburg, Marburg, Germany) (4). The secondary antibodies used were conjugated with Rhodamine Redex (Jackson Immunochemicals), Alexa 488 or Alexa 350 (Molecular Probes; 1/700) or peroxidase (Jackson Immunochemicals; 1/10,000).

Protocols used for immunofluorescence and Western blotting are described below.

Cell Culture. DMEL-2 cells were grown at 25 °C in Express Five SFM *Drosophila* media (Invitrogen) complemented with L-glutamine (2 mM; Gibco) and penicillin-streptomycin (50,000 units/L to 50 000 μg/L; Gibco).

dsRNA Experiments. dsRNA against *Mgr*, *Vhl*, and *GFP* or *GST* (as control) were made from an oligodT cDNA library or generated from plasmid DNA. A list of primer pairs is given in the primers list (Table S1). Cells were plated at 1.5×10^6 cells in six-wells plates a few hours before transfection with 25 μg of dsRNA in 10 μL of H_2O. For codepletions, 25 μg of each dsRNA against *Mgr* or *Vhl* were used and single dsRNAs were supplemented with 25 μg of control dsRNA. dsRNAs were incubated with 20 μL of Transfast (Promega) and 970 μL of media for 15 min before transfection (5). The dsRNA solute on (1-mL mix) was then incubated on the cells for 1 h before the addition of 3 mL of media. Cells were harvested after 3 d and resubmitted to the same transfection protocol where indicated.

Immunofluorescence and Microscopy. Testes from young pupae and the CNS of third-instar larvae were dissected in PBS, transferred in PBS-5% (vol/vol) glycerol before being squashed between slide and cover-slip. After snap-freezing in liquid nitrogen, testes on slides were fixed in methanol, quickly rehydrated in PBS-0.5% (vol/vol) Triton-X100, rinsed for 10 min in PBS, and used immediately for staining.

DMEL-2 cells were harvested and plated on 13-mm diameter glass cover-slips in 24-wells per plate at 3×10^5 cells per well 3 h before fixation. Cells were then pre-extracted 10 s in 0.1% (vol/vol) Nonidet P-40 in BRB80 buffer (80 mM K-Pipes pH 6.8, 1 mM $MgCl_2$, 1 mM Na-EDTA pH 8) and immediately fixed in BRB80 4% (wt/vol) paraformaldehyde for 20 min. The cells were then permeabilized in BRB80 0.1% (vol/vol) Triton-X100 for 10 min.

Staining of cells on cover-slips or tissues on slides was performed by incubating them for 1 h at room temperature or overnight at 4 °C, respectively, in PBS containing 0.1% (vol/vol) Tween 20, 3% (wt/vol) BSA and the primary antibodies and 45 min or 3 h, respectively, with the secondary antibodies in the same buffer at room temperature. Cells were mounted on slides in Vectashield (with or without DAPI) (Vector Laboratories).

Images were acquired using a Zeiss Axiovert 200M microscope using a 100 × 1.4 NA objective and a Coolsnap HQ2 camera controlled by Metamorph software (Universal Imaging). Figures shown are the maximum-intensity projections of optical sections acquired at 0.25-μm *z*-steps.

Electron microscopy was performed as previously described (2).

Preparation of Extracts and Western Blot. Protein extracts from cultured cells were prepared after homogenization in 1D buffer [50 mM Tris pH 8, 150 mM NaCl, and 1% (vol/vol) Nonidet P-40] and SDS/PAGE sample buffer, boiled 5 min, and incubated with benzonase (Novagen) on ice for 20 min to remove DNA. Extracts equivalent to 1.5×10^5 cells were processed for Western blot analysis.

Brains from third-instar larvae and testis from pharate adult were dissected in PBS. Tissues were pestle-homogenized in 1D lysis buffer, incubated on ice for 20 min, and centrifuged at 18,000 × *g* for 10 min. Soluble proteins fractions were quantified by Bradford assay (Bio-Rad Protein Assay; Bio-Rad) and 10 μg of soluble protein extracts were processed for Western blot analysis.

Cell or fly extracts were loaded onto SDS/PAGE (ProGel Tris Glycin 8–16%; Anamed) and transferred onto nitrocellulose membranes (Hybond ECL; Amersham Biosciences). Membranes were blocked with TBS, 0.2% (vol/vol) Tween20, 5% (wt/vol) Milk for 30 min at room temperature. Incubation with primary or secondary antibodies diluted into TBS, 0.2% (vol/vol) Tween20, 5% (wt/vol) Milk were performed at 4 °C overnight and 1 h at room temperature, respectively. Peroxidase activity was detected with Amersham ECL plus Western blotting detection system (GE Healthcare).

Microtubule pull-down assays were performed with DMEL-2 cell protein extracts, as previously described (6).

Expression and Purification of GST- and MBP-Tagged Proteins from *E. coli*. MBP and GST fusion proteins were expressed in BL21-AI *E. coli* cells (Invitrogen) by Arabinose induction at 37 °C for 3 h. Bacterial cells were lysed by sonication in PBS containing complete EDTA free antiprotease (Roche) and fusion proteins were purified onto Amylose resin (New England Biolabs) or Glutathione Sepharose 4b resin (GE Healthcare) according to the

manufacturers' instructions. Purity and protein concentrations were determined on SDS/PAGE gel stained by Coomassie (BioSafe Coomassie; Bio-Rad).

In Vitro Binding Assays. [^{35}S]methionine-labeled Mgr, Vhl, and β1-tubulin were produced by in vitro transcription-translation (IVT) using the TNT T7 Quick Coupled Transcription/Translation System (Promega) according to the manufacturer's instructions. Full-length cDNA-containing plasmids with an upstream T7 promoter were used. The IVT reactions were checked on Western blot and comparable levels of labeled proteins were used with 2 μg of GST- or MBP-tagged proteins on resins. One-hour binding and 5× 2-min washes were performed on ice in the following buffer: 20 mM Hepes pH 7.4, 1 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.1% (vol/vol) Triton X100, complete EDTA-free protease inhibitors (Roche). The beads were boiled in SDS/PAGE sample buffer, separated on an acrylamide gel, and transferred onto nitrocellulose membranes. Membranes were stained with Amido black to check the loading of the proteins and subjected to autoradiography.

For direct-interaction assays, 2 μg of MBP or MBP-Vhl on amylose resin were incubated on ice for 1 h with 10 μg of pure αβ-tubulin dimers (Cytoskeleton). Binding and washes were performed exactly as for the IVT-produced proteins. The analysis was done directly on the SDS/PAGE gel by Coomassie staining (BioSafe Coomassie; Bio-Rad).

For the competition assay, the interaction between MBP-Vhl and ^{35}S-Mgr was prepared as described above. The resin was then incubated with 10 μg of αβ-tubulin for 1 h on ice, washed 7× 2 min. The beads were boiled in SDS/PAGE sample buffer, separated on an acrylamide gel, and transferred onto nitrocellulose membranes. Membranes were stained with Amido black to check the loading of the proteins and subjected to autoradiography.

Fly Genetics, cDNA Rescue Construct. Initially *mgr* was mapped to the cytological interval 86E3: 87A9 (7). *Mgr* was cytologically localized to the region 86E3-10 based on its inclusion in *Df(3R)TE61* ($86^{E}3$–6 to 87A6-10) and its absence from *Df(3R)TE41* ($86^{E}12$–20 to 87C1-2) (7) and its absence from *Df(3R)P10* (86E6-9–87A9-B1). Cloning of *mgr* was first attempted in advance of the availability of the complete genome sequence by microdissecting this chromosomal region and using this to probe a bacteriophage-λ library for which a series of overlapping recombination phage were isolated corresponding to 76 kb of chromosomal DNA from the region 86E4-8 that did not include *mgr* (8). We then found that three deficiencies, *Df(3R)thoR1*, *Df(3R)pros640*, and *Df(3R)pros235* [kindly provided by Bill Engels (University of Wisconsin–Madison, Madison, WI) (9)] all uncovered *mgr* mutant alleles allowing us to place *mgr* within the limits of the smallest deficiency, *Df(3R)thoR1* (86E2 to -6). The endpoints of these breakpoints had been sequenced and indicated that the interval contained 14 predicted genes. Down-regulation of each of these genes was carried out by RNAi in cultured *Drosophila* cells, revealing mitotic defects with three of these genes (Fig. S2). The sequences of these genes were then determined in wild-type and *mgr* mutant chromosomal DNA, this identified CG6719 as the *mgr* locus. Next, we subcloned the CG6719 cDNA into the p-element vector pUAST, where the transgene is under the control of the UAS promoter. An Act5C-GAL4 driver was then able to drive expression of the trangene and rescue the lethality of mgr^{l4}/mgr^{1} mutants.

***mgr* Alleles.** Sequencing revealed a deletion affecting the promoter region and 5′UTR of mgr^{l4} and extending into the second exon of the adjacent gene, *CG5247*. This adjacent gene encodes a subunit of the Ku heterodimer, which binds to DNA double-strand break ends and is required for the nonhomologous end joining pathway of DNA repair. Its knockdown in cultured cells leads to some mitotic abnormalities. Sequencing also revealed a frameshift in the ORF of mgr^{1}, leading to the addition of a novel 42 amino acids to the C terminus of the protein, a translation termination codon in mgr^{5} replacing a tryptophan codon and truncating the protein by 17 amino acids, together with a 41-bp deletion in the 3′ UTR and the same translation termination mutation in $mgr^{DD1\text{-}9388}$ as in mgr^{5}. Mgr protein was not detectable using our antibody in larval brains of any of these mutants when homozygous with the exception of $mgr^{DD1\text{-}9388}$, where it could be detected in overexposed Western blots, indicating its hypomorphic nature. To establish whether mgr^{l4}, mgr^{1}, or mgr^{5} were hypomorphs or amorphs, we placed each over a deficiency for the locus, *Df(3R)pros235*. Homozygous mgr^{l4} animals showed the same lethal phase as hemizygotes, with the larvae dying as third-instar larvae/pupae. However, because this deletion mutation extends into the neighboring gene, we cannot exclude the possibility that this lethal stage reflects in part the loss of *CG5247* function and have not tested this. The mgr^{1} allele showed a less severe phenotype when placed over a deficiency or when placed against mgr^{l4}; these animals died as pharate adults compared with the third larval instar lethal phase of homozygous mgr^{1}. This finding suggests the presence of a second site mutation on the mgr^{1} chromosome. A small number of transheterozygous mgr^{5}/mgr^{l4} survive to give sterile adults; the majority of this progeny dies as pharate adults. The lethal stage is slightly earlier for mgr^{5}/*Df*. Taken together, these data allowed us to placed in the following tentative allelic series $mgr^{l4} > mgr^{1} = mgr^{5} > mgr^{DD1\text{-}9388}$, ranging from a potentially null-allele to hypomorphs of various strength.

Transient Transfections. Transient transfections of myc-Vhl were obtained as follow: 20×10^{6} cells were plated on a 75-cm^{2} flask; a few hours later, 20 μg of myc-Vhl or myc-control constructs in 800 μL of H_2O were mixed with 100 μL of Fugen-HD (Roche) and incubated at room temperature for 15 min. The mix was then added drop by drop to the cells. Twenty-four hours later, the cells were harvested and used for immunofluorescence or biochemical analysis.

Electron Microscopy. Cultured cells on coverslips or testes dissected in phosphate buffer, fixed in 4% (wt/vol) paraformaldehyde (EM grade) in PBS for 15 min, were immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at 4°C. They were then washed three times for 30 min in phosphate buffer, postfixed with 1% OsO4 for 1 h at 4°C, and washed once in phosphate buffer and then in distilled water. Samples were stained for 1 h in uranyl acetate. They were washed again and then dehydrated in a graded series of ethanols and flat-embedded in a mixture of Epon and Araldite. After polymerization for 2 d at 60°C, the coverslips were removed from the resin after a short immersion in liquid nitrogen. Ultrathin serial sections were obtained with a LKB ultratome, stained with uranyl acetate and lead citrate, and observed and photographed with a Philips CM10 electron microscope at 80 kV.

1. Fox JD, Routzahn KM, Bucher MH, Waugh DS (2003) Maltodextrin-binding proteins from diverse bacteria and archaea are potent solubility enhancers. *FEBS Lett* 537:53–57.
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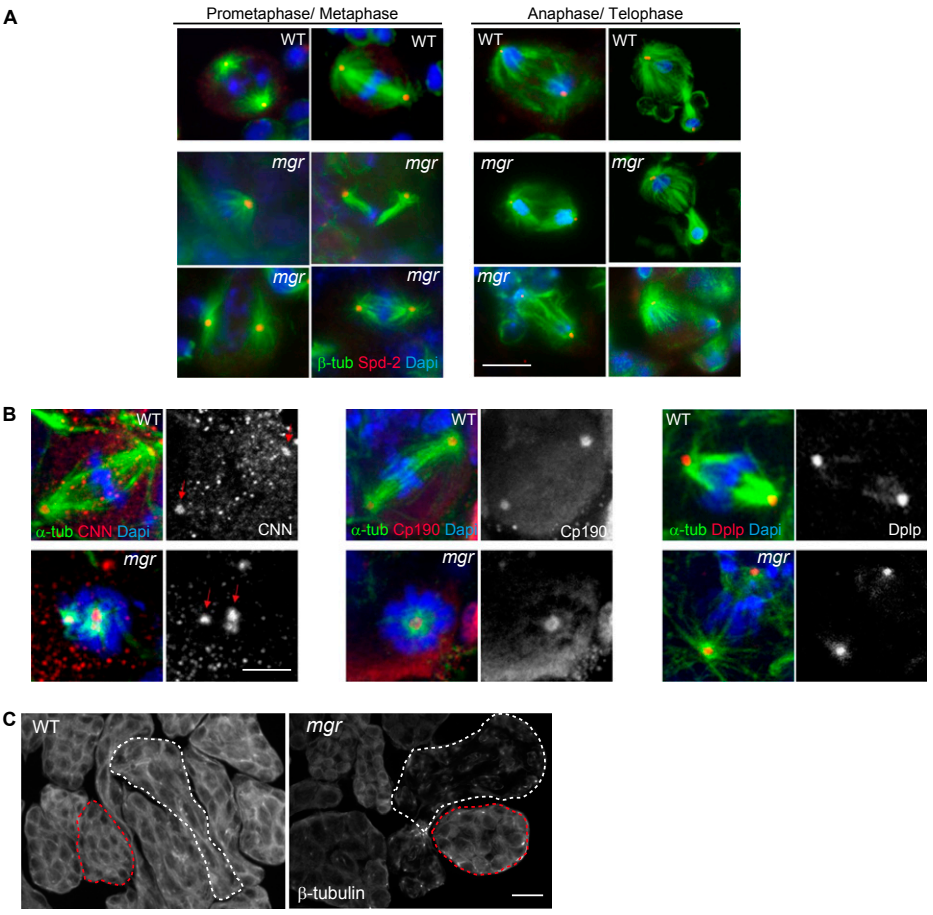

Fig. S1. Additional phenotypes of *mgr* mutant flies. (*A*) Representative mitotic figures from squashed preparations of wild-type and mgr^{l4}/mgr^{5} mutant CNS stained to reveal microtubules (β-tubulin, green), centrosome (Spd-2, red), and DNA (Dapi, blue). Whereas in wild-type most of the CNS prometaphase/metaphase cells present bipolar spindles, many (mgr, *Upper Left* and Fig. 1) but not all (remaining panels) *mgr* cells present monopolar spindles. Examples of the variety of bipolar spindle shapes are shown for *mgr* mutant metaphase-like cells. Bipolar spindle structures of equally varied appearance were also seen in anaphase/telophase figures from *mgr* mutant cells. Of the mitotic figures, 32% ± 1.4 were in prometaphase/metaphase in wild-type larval brains compared with 40% ± 0.9 in a prometaphase/metaphase-like state in *mgr* brains. Of the mitotic figures, 28% ± 1 were in anaphase/telophase in wild-type cells compared with 18% ± 1.6 in *mgr* cells. Differences between wild-type and *mgr* are significant (prometaphase/metaphase $P = 0.0001$, anaphase/ telophase $P < 0.0001$, P values from Student t test). (Scale bar, 5 μm.) (*B*) Comparisons of wild-type and mgr^{l4}/Df spindles stained to reveal α-tubulin (green), centrosomin (*Left*; Cnn, red, arrows indicate centrosomes above background staining), centrosomal protein 190 (*Center*, CP190; red), pericentrin-like protein (*Right*; Dplp, red), and DNA (Blue). (Scale bar, 10 μm.) (*C*) Immunofluorescence to reveal tubulin (β-tubulin) showing that mature primary spermatocytes (white outlined cysts) but not young primary spermatocytes (red outlined cysts) present decreased levels of tubulins. (Scale bar, 10 μm.)

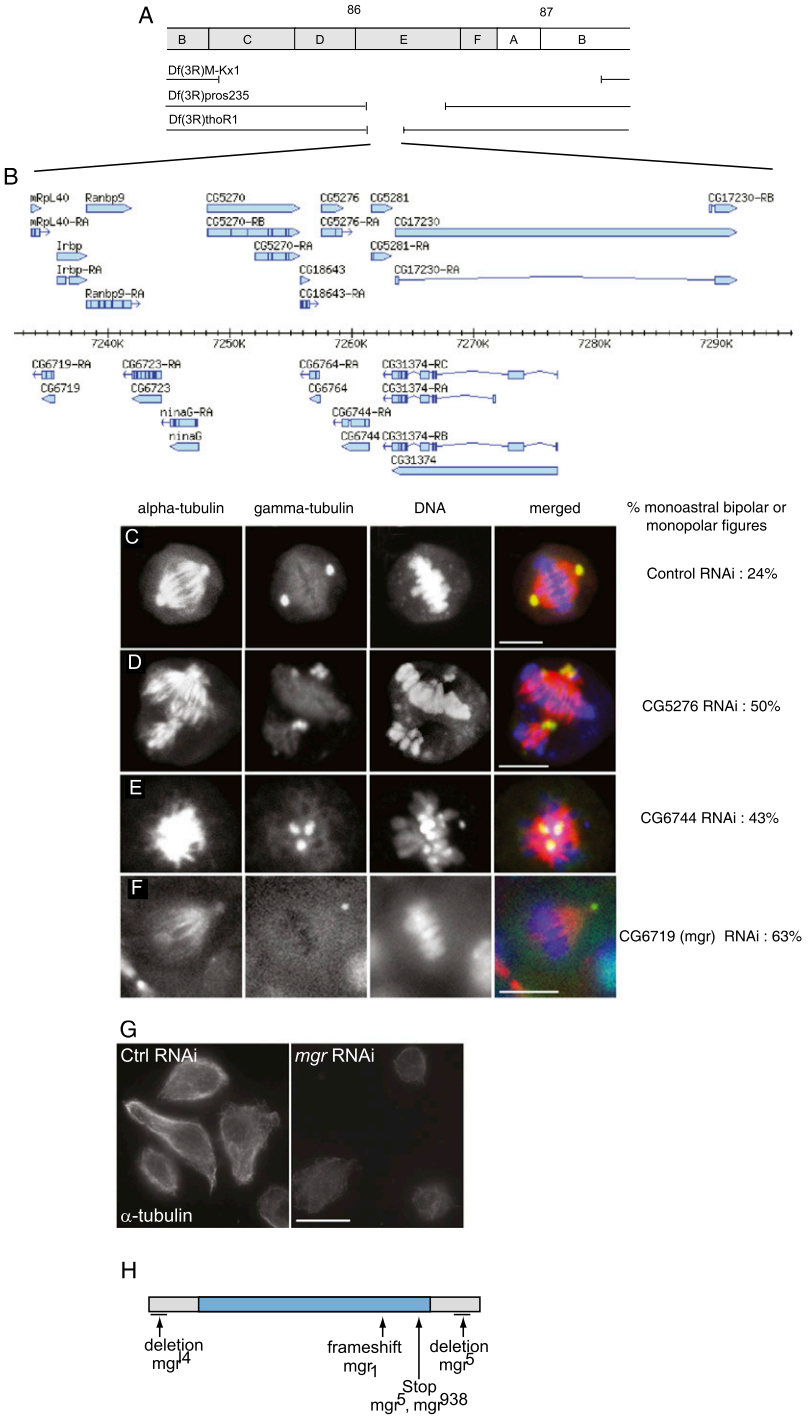

Fig. S2. Characterization of *mgr* gene. (*A*) Schematic layout of the cytological region 86E2 to 86F4 indicating chromosome deficiencies used in complementation tests with *mgr* alleles. (*B*) The 14 predicted genes in the region area 86E2-6 uncovered by the deficiency *Df(3R)thoR1*. (*C–F*) Mitotic DMEL-2 cells following treatment with dsRNAs indicated below and stained to reveal α-tubulin (red), γ-tubulin (green), and DNA (blue). (Scale bars, 10 μm.) The proportion of mitotic spindles that were either bipolar and monoastral or monopolar is indicated. (*C*) Treatment with control (*GFP*) dsRNA gives cells with bipolar spindles, two clearly focused poles and aligned chromosomes. (*D*) Mitotic defects in a DMEL-2 cell following treatment with *CG5276* dsRNA. FlyBase indicates that *CG5276* encodes a nucleoside triphosphatase/diphosphatase. (*E*) Mitotic defects in a DMEL-2 cell following treatment with *CG6744* dsRNA. FlyBase indicates that *CG6744* encodes a 3′–5′ exonulease corresponding to the mammalian Werner syndrome exonuclease. Spindle abnormalities of the kind we see here for knockdowns of *CG5276* and *CG6744* were not reported in a previous screen (1). (*F*) Mitotic defects in a DMEL-2 cell following treatment with *CG6719* (*mgr*). (*G*) Representative DMEL-2 cells in interphase immunostained to reveal β-tubulin after 6 d of treatment with control or *CG6719* (*mgr*) dsRNA showing loss of microtubules in absence of Mgr. (Scale bar, 10 μm.) (*H*) Representative diagram showing the mutations characterized in four different *mgr* mutant alleles.

1. Goshima G, et al. (2007) Genes required for mitotic spindle assembly in *Drosophila* S2 cells. *Science* 316:417–421.

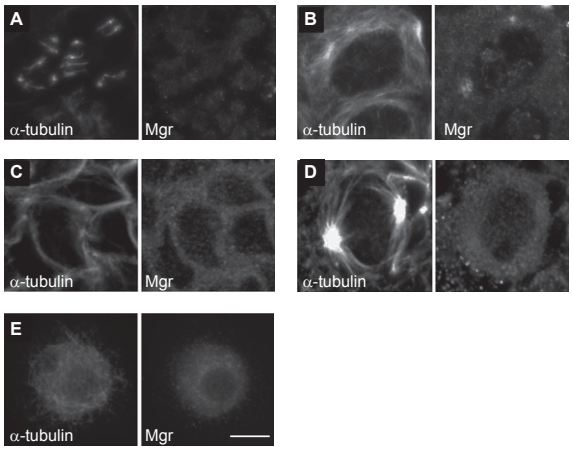

Fig. S3. Mgr localization. Immunofluorescence staining to reveal localization of Mgr (*Left*) and tubulin (α-tubulin, *Right*), in mitotic (*A*) and meiotic cells (*B*) from wild-type testes; in interphase (*C*) or mitotic (*D*) cells from the larval CNS, and in cultured cells in interphase (*E*). (Scale bar, 10 μm; applies to all panels.)

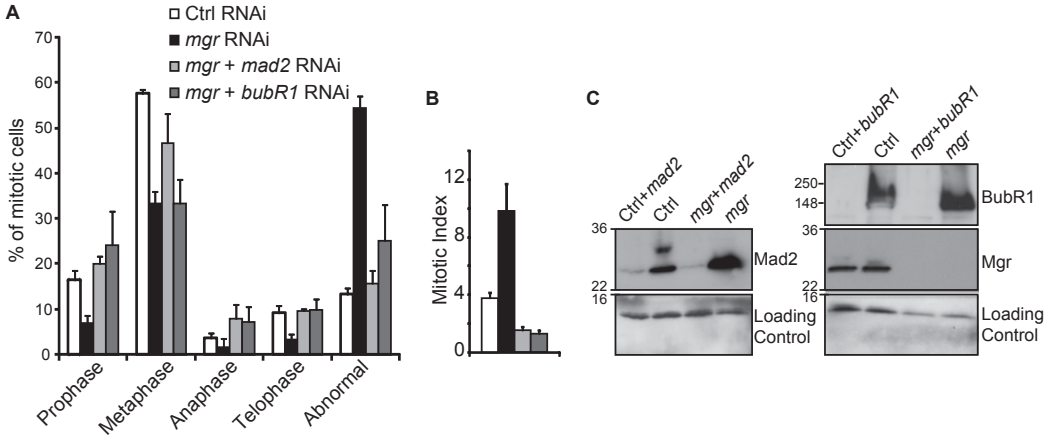

Fig. S4. Mitotic defects resulting from *mgr* RNAi are abrogated by inactivating the spindle assembly checkpoint. DMEL-2 cells were transfected with control (Ctrl) or *mgr* dsRNA for 3 d and then cotransfected with Ctrl or *mgr* dsRNA and *mad2*, *bubR1* or Ctrl dsRNA for an additional 3 d. (*A*) Proportions of cells in each phase of mitosis. Abnormal spindles represent monopolar or disorganized spindles. $n > 150$ cells, Error bars = SEM of three quantifications of one experiment. (*B*) Mitotic index in each indicated conditions, $n > 1{,}500$, Error bars = SEM of three quantifications of one experiment. (*C*) Western blot of a protein extract of DMEL-2 cells probed to reveal Mad2, BubR1, Mgr, and H2A (loading control) following depletion.

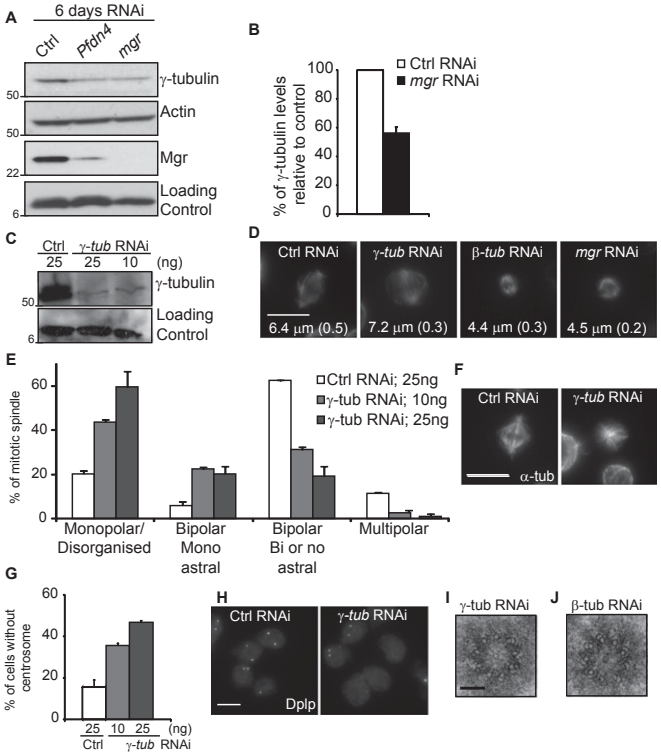

Fig. S5. Additional aspects of Mgr depletion phenotype in DMEL-2 cells. (*A*) Western blot of DMEL-2 cell extracts probed to reveal γ-tubulin, actin, Mgr, and H2A (loading control) levels after 6-d treatment with the indicated dsRNAs. Whereas no change of actin levels is observed, γ-tubulin levels are decreased following loss of the prefoldin complex. (*B*) Quantitation of the endogenous γ-tubulin levels relative to the wild-type control on Western blots. Error bars = SEMs for three independent experiments. DMEL-2 cells treated with a range of concentrations (10 and 25 ng/mL) of γ-tubulin dsRNA for 6 d (*C*–*I*). (*C*) Western blot of γ-tubulin and H2A (loading control) following such treatment. (*D*) Representative images of bipolar mitotic spindles after Ctrl, γ-*tubulin* (10 ng), β-*tubulin* (10 ng), or *mgr* RNAi, showing reduction in spindle size following β-tubulin or Mgr, but not γ-tubulin depletion. Numbers indicate average size of the spindle (SEM between 14 cells). (Scale bar, 10 μm.) (*E*) Proportion of prometaphase and metaphase cells with monopolar/disorganized spindles or bipolar monoastral or bipolar or multipolar spindles in relation to γ-*tubulin* dsRNA treatment. Error bars = SEMs of three quantifications of one experiment; $n > 100$ metaphase cells. (*F*) Cells labeled with an anti–α-tubulin to reveal spindle microtubules in control and γ-*tubulin* dsRNA-treated cells. (Scale bar, 10 μm.) (*G*) Percentage of cells without centrosomes in relation to γ-*tubulin* dsRNA treament. Error bars = SEMs of three quantifications of one experiment; $n > 200$ cells. (*H*) Cells labeled to reveal centrosomes (Dplp) following control dsRNA and γ-*tubulin* dsRNA treatment. (Scale bar, 10 μm.) (*I*) Electron micrographs of centrioles in cells following 6 d of γ-*tubulin* RNAi ($n = 10$). (Scale bar, 0.1 μm; also applies to *J*.) (*J*) Electron micrographs of centrioles in cells following 6 d of β-*tubulin* RNAi ($n = 10$).

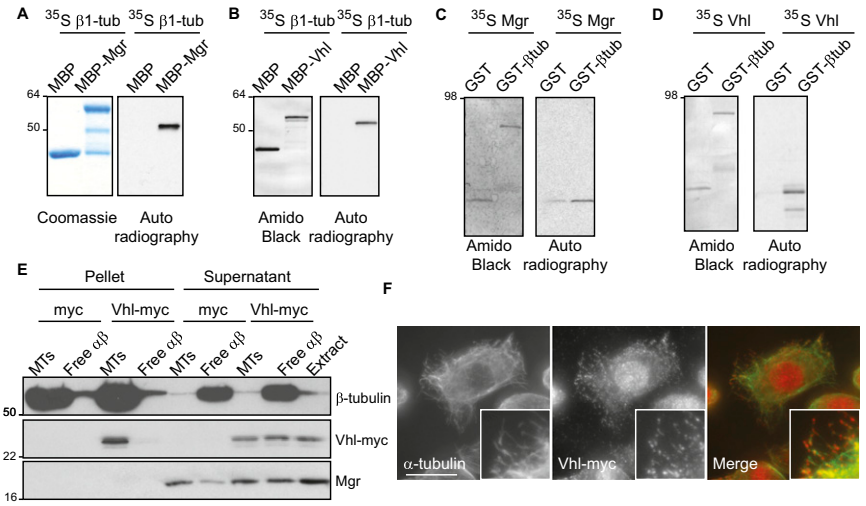

Fig. S6. Vhl interacts with microtubules. (*A*) MBP and MBP-Mgr, affinity purified from *E. coli* extracts (Coomassie stain) and tested for binding ^{35}S-Methionine-labeled β1-tubulin, produced by coupled transcription-translation in vitro (Autoradiography). (*B*) MBP and MBP-Vhl, affinity purified from *E. coli* extracts (Coomassie stain) and tested for binding ^{35}S-Methionine-labeled β1-tubulin, synthesized by coupled transcription-translation in vitro (Autoradiography). (*C*) GST and GST–β1-tubulin, affinity purified from *E. coli* extracts (Amido black stain) and tested for binding ^{35}S-Methionine-labeled Mgr, produced by coupled transcription-translation in vitro (Autoradiography). (*D*) GST and GST–β1-tubulin, affinity purified from *E. coli* extracts (Amido black stain) and tested for binding ^{35}S-Methionine-labeled Vhl, synthesized by coupled transcription-translation in vitro (Autoradiography). (*E*) In vitro MT-binding assays using whole cell extracts from cells expressing myc or Vhl-myc for 24 h. Extracts were incubated with taxol stabilized microtubules (MTs) or free tubulin in the presence of nocodazole (Free αβ) as a control. Following centrifugation through a 10% sucrose cushion, supernatant and pellet (MT) fractions were assayed by Western blot analysis for myc, β-tubulin, and Mgr. Vhl is associated with the microtubule pellet, whereas Mgr is not. (*F*) DMEL-2 cells transiently transfected with Vhl tagged with myc, and fixed after 24 h for staining to reveal MTs (β-tubulin, green) and Vhl-myc (Red). Note that dots of Vhl-myc follow the paths of MTs. (Scale bar, 10 μm.) *Inset* magnification is threefold greater.

Table S1. List of oligos used in this study

Oligo names	Sequences
T7b_tub (825 ex2)Fw	TAATACGACTCACTATAGGGAGAATGCCCGGCTTCG
T7b_tub (1335 ex2)Rev	TAATACGACTCACTATAGGGAGAGAACTCGGCGTCCTC
GST-RNAi-F	TAATACGACTCACTATAGGGAGATTTGTATGAGCGCGATGAAG
GST-RNAi-R	TAATACGACTCACTATAGGGAGAATCCGATTTTGGAGGATGGT
T7 VHL (23 ex1)Fw	TAATACGACTCACTATAGGGAGACAACCGCGACGGCCAG
T7 VHL (529 ex1)Rev	TAATACGACTCACTATAGGGAGAGCCACTCGGGAATAGG
attB1 VHL (ACC 1)Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGGCGCTCCAAATAGC
attB2 VHL (NoStop 534)Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCACGCCGACGTCGC
T7 CG6719 Fw(3′UTR)	TAATACGACTCACTATAGGGAGAGCATTTGTAGACGGCACGGCTGAA
T7 CG6719 Rev(ex2)	TAATACGACTCACTATAGGGAGAGTCTTGATGAACACCTGGTCGCTGAG
CG6302 F (ex1)	TAATACGACTCACTATAGGGAGAGCGAAGCCGGCACTCAGC
CG6302 R (ex2)	TAATACGACTCACTATAGGGAGAGGTTCTCCGCCTTGTCCTC
GFPT7F	TAATACGACTCACTATAGGGAGACTTCAGCCGCTACCCC
GFPT7R	TAATACGACTCACTATAGGGAGATGTCGGGCAGCACG
AB 6719 F	GCGACGAATTCGATGACAGGAATAATGGACTCG
AB 6719 R	GCGACAAGCTTTTAGGATGGGGTAGTGGCGGTG
attB1 VHL (1) Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGCTCCAAATAGC
attB2 VHL (stop) Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAACGCCGACGTCG