SUPLEMENTAL MATERIALS:

Experimental Procedures

Plasmid constructs

MalBP fusions of Eg5 fragments and Xl-Eg5[773-1067] were generated by PCR cloning into pMAL-c2x (NEB). GST-Eg5 was generated by PCR cloning full-length Eg5 into pEBG. To generate Eg5 mutants site directed mutagenesis was performed according to the manufacturers instructions (Stratagene) using specific primers (T927A 5'ATCCCAACAGGTACGGCTCCACAGAGGAAAAGT, S1033A 5'AACACACTGGAGAGGGCTAAAGTGGAAGAAACT, S1033D 5'AACACACTGGAGAGGGATAAAGTGGAAGAAACT with the appropriate reverse complement), while RNAi resistant Eg5 primers encoded silent mutations (5' GGAAACCTAACTGAAGATTTAAAGACAATAAAGCAGACC with reverse complement). All constructs were sequenced after generation.

Motor assays

Microtubule pelleting and gliding assays were performed as previously described (Vale et al., 1985; Saxton, 1994; Lockhart and Cross, 1996).

Supplementary references.

Lockhart, A. and Cross, R.A. Kinetics and motility of the Eg5 microtubule motor (1996). Biochemistry. 35, 2365-73.

Saxton WM (1994). Isolation and analysis of microtubule motor proteins. Methods Cell Biol. 44, 279-88.

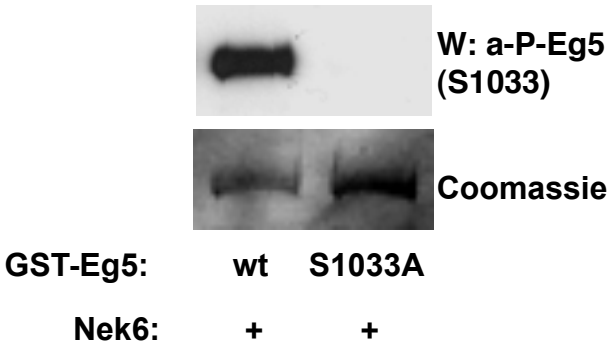
Vale, R.D., Reese, T.S. and Sheetz, M.P. (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42, 39-50.

Supplementary Figures

Supplementary Figure 1. Characterization of anti-HsEg5[Ser1033P] and anti-XlEg5[Ser1046P] antibodies. (A) Characterization of anti-HsEg5[Ser1033P] antibodies. Recombinant GST- Eg5 wildtype or [Ser1033Ala], purified by glutathione affinity from extracts of 293T cells, were incubated with FLAG-Nek6 in the presence of ATP/Mg^{2+}. After SDS-PAGE, immunoblot with affinity purified anti-Eg5[Ser1033P] antibody (top panel) was carried out; a Coomassie blue stain of the GST-fusion proteins is shown (bottom panel). (B) Bacterial recombinant -expressed wt and MalBP-Eg5[762-1057] and MalBP-Eg5[762-1057/ Ser1033Ala] were incubated with ATP/Mg^{2+} and either NIgG (-) or FLAG-Nek6 (+) immunoprecipitates. Immunoblot with affinity purified anti-Eg5[Ser1033P] antibody as in *A*. (C) Characterization of anti-XlEg5[Ser1046P] antibodies. Bacterial recombinant MalBP-XlEg5[773-1067] was incubated with ATP/Mg^{2+} and either NIgG (-) or FLAG-Nek6 (+) immunoprecipitates. XlEg5[Ser1046] phosphorylation was visualized by immunoblot with anti-XlEg5[Ser1046P] antibody (top panel); MalBP-fusion proteins were stained with Coomassie blue (bottom panel).

Supplementary Figure 2. Microtublue binding affinity of Eg5 wt, T926A, S1033A and S1033D. (A) Loading quantification of recombinant Eg5. His_6-Eg5 wildtpe, Thr926Ala, Ser1033Ala and Ser1033Asp were purified from Sf9 cells and equalized for protein content. (B) Microtubule binding assay. 5.7μg of recombinant Eg5 was incubated with polymerized microtubules and pelleted by centrifugation. Representative aliquots of the supernatants (S) and pellets (P) was analyzed by immunoblot of Eg5 (upper panel), whereas tubulin abundance was determined by Coomassie blue stain (lower).

A



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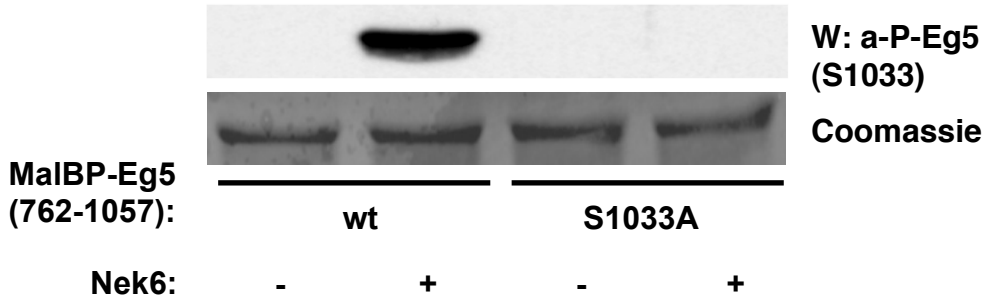

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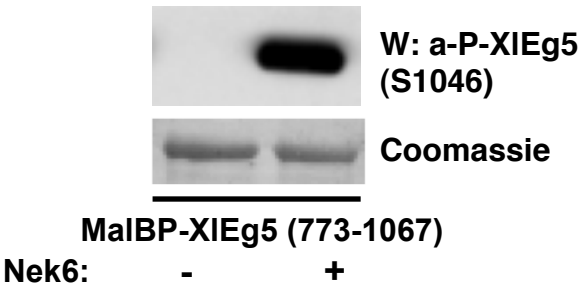
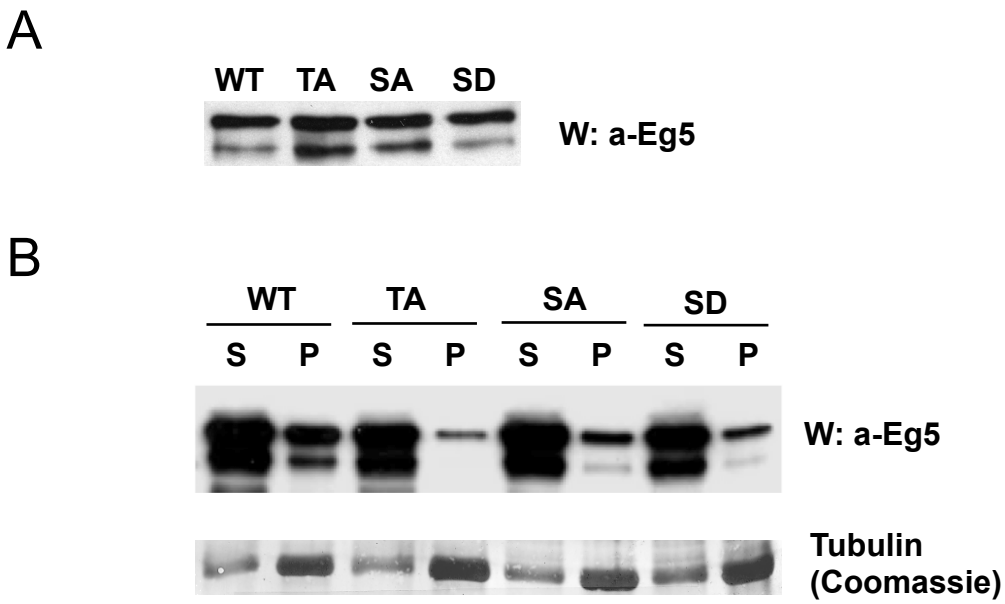


Supp. Figure 1



Supp. Figure 2