SUPPLEMENTARY FIGURE LEGENDS

Figure S1

Design and expression testing of various CLASP fragments.

(A) Prediction of coiled-coil regions in CLASP1 using COILS (Lupas et al. 1991). Arrows indicate beginning and end of constructs in relationship to predicted coiled-coil regions (top). (B) Table of protein fragments generated and tested for expression/purification (bottom). (C, D) Sequence Alignments of TOG domains (C) and SxIP motifs (D) using (Simossis and Heringa 2005). The degree of sequence homology is indicated by color (blues – unconserved; green/yellow moderately conserved; reds – conserved).

Figure S2

MT binding and bundling for different CLASP constructs

(A) EM micrographs showing MT bundling in the presence of CLASP1-662, which is not nearly as prominent with CLASP662-1473 and CLASP662-1171 constructs. (B) Cold-pelleting assay with CLASP1-662. For experiments 2 (tubulin only control), half the amount of tubulin was used as compared to experiment 3, reflected in the different intensity of the tubulin bands. Samples were pelleted and analyzed via SDS-PAGE (10% Bis Tris) and stained with Sypro-Ruby).

Figure S3

MT ends tracking assays for CLASP constructs lacking TOG domains

Representative time-lapse images of CLASP662-1171 and CLASP662-805 that contain the SxIP motif but lack TOG1 and crTOG2 domains. Arrows indicate individual MTs or bundles. Images were taken at 2.5 sec intervals. See movies S4 and S5 in supplemental material.

Figure S4

Dominant negative phenotypes caused by CLASP construct containing the Nterminal TOG domains

(A) Representative images of spindle phenotypes upon addition of 0.2 μ M CLASP1-662 to spindle assembly reaction upon entry into mitosis. Scale bar = 10 μ m. (B) Quantification of phenotype distribution comparing buffer control. Greater than 50 spindles were counted for each condition in 3 separate experiments.

SUPPLEMENTARY MOVIES

Movie S1. GFP-tagged Full-length CLASP (1-1463) associates with MT plus ends but strongly bundles MTs when added to centrosomes incubated in metaphase-arrested Xenopus egg extracts.

Movie S2. GFP-tagged CLASP1-662 fails to track MT plus ends in the assay, although it does bind MTs and promotes bundling.

Movie S3. GFP-tagged CLASP662-1463, containing the SxIP EB1 binding domain, but not N-terminal TOG domains, tracks MT plus ends in the assay.

Movie S4. GFP-tagged CLASP662-1171, containing the SxIP domain but not N or C terminal domains, tracks MTs plus ends, and binds weakly along centrosome MTs.

Movie S5. GFP-tagged CLASP662-805, containing a minimal SxIP EB1 binding domain is able to track MT plus ends in the assay.

Movie S6. GFP- tagged CLASP662-1463 does not track MT plus ends in EB1-depleted egg extract but does associate along the MT lattice

Movie S7. GFP-tagged CLASP662-1463 MT plus end tracking behavior is restored in EB1-depleted egg extract to which endogenous levels of EB1 were added back.

3



С					
CLASP TOG1	-CLAQVLQKD	120 VCKBLQVCQE)	0.140 Sadlerdotm) 150 LDK <mark>L</mark> VDCLAT
CLASP crTOG3	-VLNECASSN PIDQKCERKV	WSERKECLLC WKARLNCYEE	LONLLKSORT AVKLFOKIVD	LSRVE EKSPE	LKRLCEIFTR WSKYLCLIKR
XMAP215 TOG2	KIEAKK	WODRXEALEV	VETLVKN-PK	IEACD	YADLVKALKK
XMAP215 TOG4 XMAP215 TOG3	SCMOQLDSSN	WEERLASMEE	FORAVES-ME	RNDIP	CQALVEMLAK
CLASP cr10G2 XMAP215 TOG5	LODELFRAD	FORBIKCLAV	MTERLES	EKECAISC	LDLILKWFTL
Consistency	16	653 6 555544	5345645023	0 100	4337 3 4 3 65 3 200
CLASP TOG1	SXY-NSS	- <mark>NYKVVLLCM</mark>	DILSALVTRL	QDRFKAQ	- <mark>ICTVLPS<mark>L</mark>I</mark>
CLASP crTOG3 XMAP215 TOG1	MP ADPHSK	-NAVAOLKCL	VDFIIIRKD-	-DLODWL	- FVLLTQLLX - CEVVNCVVN
XMAP215 TOG2	V-VCKDN	-NVMLVALAA	KCIACLAACL	REEFCSY	ACRVVPTIL
XMAP215 TOG4 XMAP215 TOG3	KPCZKET	-NFOVMONKL	RIVALIAOKC	-NFSKTS	AYVVLDCLV
CLASP cr10G2 XMAP215 TOG5	RFFDT	-RSOVVREAC -NTSVLMKCL	EYLKLLSSVL	SQEEYELTEN	ECTSFLPYLM
Consistency	3100474000	0634654436	33 <mark>6</mark> 33 <mark>7</mark> 4434	1333433000	0433744384
CLASP TOG1	DRLCDAKDSV	0	IMDOAANPOY	0 240)
CLASP crTOG3 XMAP215 TOG1	KMCADLLCSV	QAKVOKALDV	TRDSFPFDQQ	FNILMRFI	VDQTQTPNLX
XMAP215 TOG2	EKFKEKKPOV	VOALGEAIDA	VFLTTTLONV	SEDILAVM	DNKNPA
XMAP215 TOG3	DKVCDVKC	CCNAKEALSC	IAEACNLPWT	AE-QVVSLAF	AQKNPK
XMAP215 TOG5	NLIPNSAKIM LKVC <mark>E</mark> PKDIV	ATSCVVAVRL RKDVRAILTK	MCQVYPASKM	IPVITSNC FNFVMECT	TSKSVA KSKNSK
Consistency	3444 <mark>6</mark> 2433 <mark>6</mark>	443343 <mark>57</mark> 33	4343334433	3 <mark>0053</mark> 75334	4470000835
) 300
CLASP crTOG3	VEVAILEY	SLAROMDETD		LAVSRITTWT	TEPNSSOVRK
XMAP215 TOG1 XMAP215 TOG2	IKQQASLFLA	RSFROCTAST	LPESLLEPPC	AALLKOINDS	APEVRD
XMAP215 TOG4 XMAP215 TOG3	NOSETLNWLS	NAIKEFCFT-	GINVKAFI	SNVKTALAAT	NCOVER
CLASP crTOG2 XMAP215 TOG5	VRRRCFEFLD QRAECLEELC	LLLQEWQTH- CLVESYCMNV	C-OPTPAKAL	SVLAETIK KETAIRICDR	BTTVRN
Consistency	4 <mark>64445</mark> 33 <mark>8</mark> 3	3464534430	00134 <mark>55</mark> 335	43 <mark>8</mark> 343 <mark>7</mark> 333	0000344985
	AAINSLVEIY	REVCE			
CLASP crTOG3	AAQIVLISLE EAKLLAVEIY	ELNT-			
XMAP215 TOGT XMAP215 TOG2	AAFEALCTAI	KVVCE Und	conserved 👩	234567891	Conserved
XMAP215 TOG4 XMAP215 TOG3	SAITLLCVM	LYMC-			•
CLASP crTOG2 XMAP215 TOG5	ADS	NVHCE			
Consistency	<mark>68</mark> 323 <mark>5</mark> 3344	32341			
-					
D		50 26	502	.70	30
APC TESSER REALEREDG CSRETSPART GLDR					
CLASP1	TPSSEK	<mark>RSKIPRSQ(</mark> <mark>RSKIPRSQ(</mark>	CSRETSPARI	GLDR	
CLASP1 CLASP2 MACF1	TPSSEK TPSSEK SASAQK MPSSPATPA	<mark>RSKIPRSQ(</mark> RSKIPRSQ(RSKIPRSQ(<mark>- RSKIPRSQ(</mark> - G TKVIPSSG)	CSRETSPNRI CSRETSPNRI CSREASPSRI CSREASPSRI KLKRPTPTFF	GLDR GLDR SVARSSRIPP SSR <mark>T</mark> SLAGDT	P-SVSQGCSR SNSSSPASTG

В

	AA	Expected	Predicted
Construct	length	Protein size	PI
CLASP1 (fl)	1463	168.52	10.21
CLASP			
1-1171	1171	128.44	9.42
CLASP			
1-662	662	72.77	9.22
CLASP			
662-1463	801	88.50	8.36
CLASP			
662-1171	509	55.70	9.15
CLASP			
662-850	188	19.26	10.86

Unconserved 012345678910 Conserved



+CLASP1-662





+CLASP662-1171



2:Tubulin (polymerized at 37° C for 1 hour then placed on ice) 3:CLASP1-662 + Tubulin (polymerized at 37° C for 1 hour then placed on ice) 4:CLASP1-662 AT 37° C for 1 hour Figure S3





Figure S4



