

Supplementary Information for

Self-assembly of CIP4 drives actin-mediated asymmetric pit-closing in clathrin-mediated endocytosis

Yiming Yu and Shige H. Yoshimura*

Graduate School of Biostudies, Kyoto University, Kyoto 606-8501, Japan

*Corresponding author

Shige H. Yoshimura

Graduate School of Biostudies, Kyoto University, Kyoto 606-8501, Japan

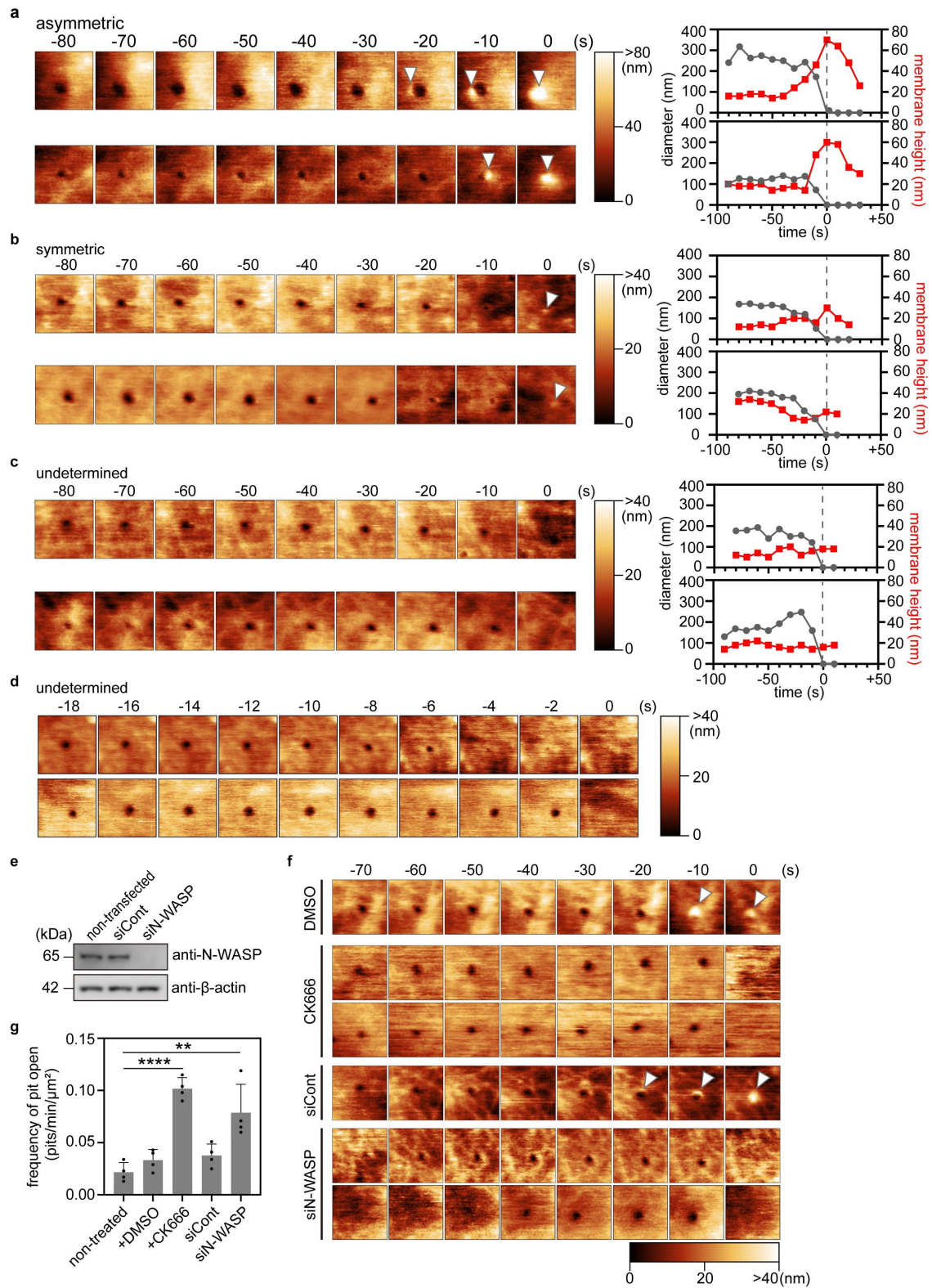
E-mail: yoshimura@lif.kyoto-u.ac.jp

Tel & Fax: +81-75-753-7906

ORCID: 0000-0001-6033-1301

This PDF file includes:

Supplementary Figures 1 to 6 and Supplementary Table 1



Supplementary Figure. 1 Asymmetric closing pattern of the CCP.

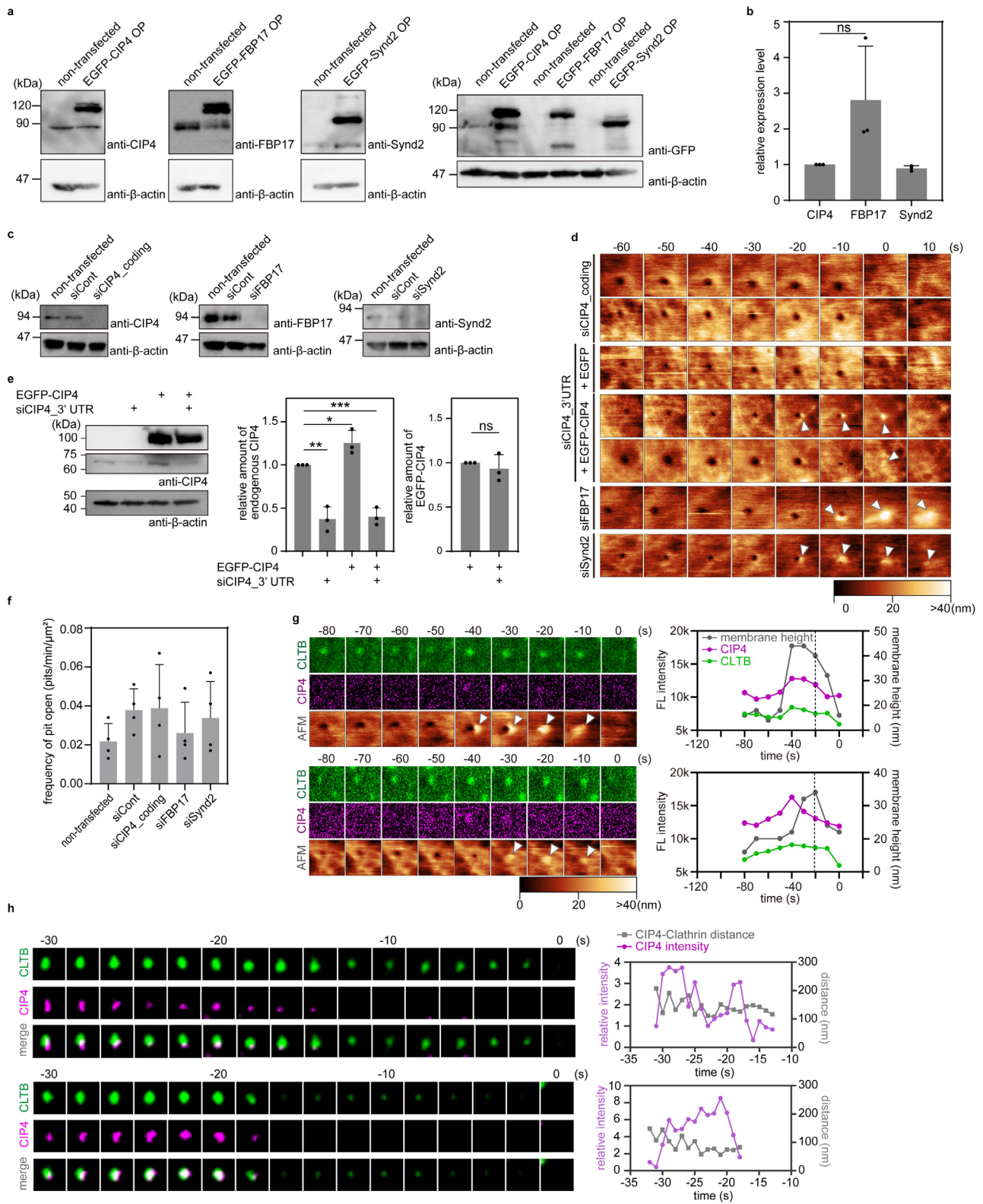
a–c, Additional examples of the time-lapse HS-AFM images of asymmetric (a), symmetric (b), and undetermined (c) closing patterns obtained from Cos7 cells. Details of the experimental conditions and data presentations are the same as those in Figs. 1a, 1c.

d, Time-lapse HA-AFM images of CCPs with the undetermined closing pattern at a scanning rate of 2 s/frame. Time 0 was when the pit completely closed on the HS-AFM image. The height information of the AFM image is presented using a colour bar. The image size was $1.0 \times 1.0 \mu\text{m}^2$.

e, Knockdown efficiency of N-WASP. The total cell lysate of non-transfected Cos7 cells and Cos7 cells transfected with siRNA for the control (luciferase) or N-WASP was prepared 48 h after the transfection and then subjected to the western blot analysis using an anti-N-WASP antibody. β -actin was used as an internal control. The experiment was repeated three times and similar results were obtained.

f, Time-lapse HS-AFM images obtained from Cos7 cells treated with DMSO or CK666 and cells transfected with siRNA for the control (luciferase) or N-WASP. Images were taken every 10 s. Time 0 was when the pit completely closed on the HS-AFM image. Arrowheads indicate the membrane bulge, and the height information of the AFM image is presented using a colour bar. Image size: $1.0 \times 1.0 \mu\text{m}^2$.

g, The frequency of CCP formation in non-treated Cos7 cells, the cells treated with DMSO or CK666, and the cells transfected with siRNA for the control (luciferase) or N-WASP were quantified and summarised. Data are presented as mean $\pm$ standard deviation from four independent experiments. All data points are shown. P values were calculated using the two-tailed Student's t -test, **: $P < 0.01$; ****: $P < 0.0001$, $\alpha = 0.05$. The exact P values are provided in Source Data.



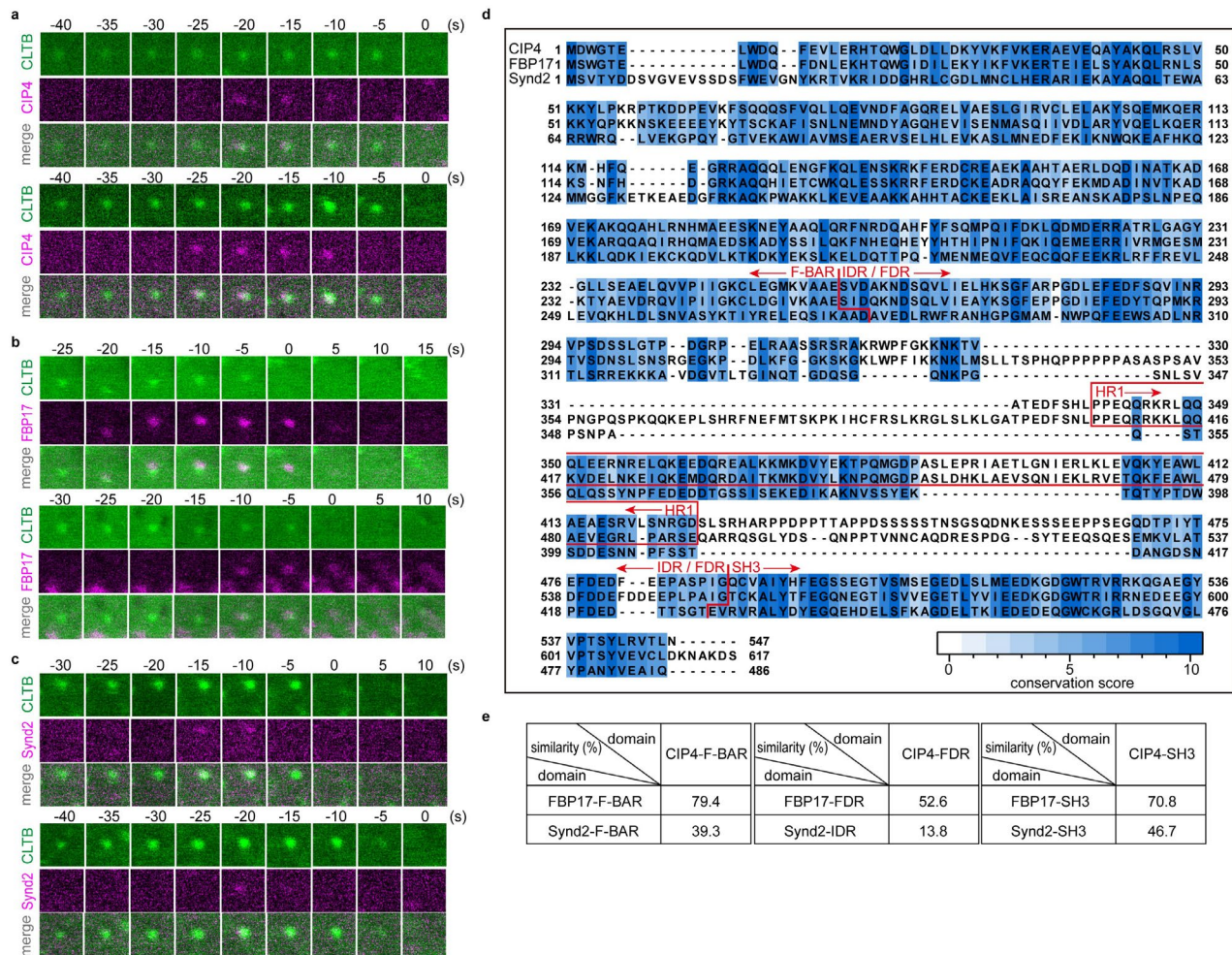
Supplementary Figure. 2 CIP4 is necessary for the asymmetric bulge.

- a, The endogenous amount of CIP4, FBP17, and Synd2 in Cos7 cells. The total cell lysate of Cos7 cells expressing EGFP-fused CIP4, FBP17, or Synd2 was subjected to western blotting using anti-CIP4, -FBP17, or -Synd2 antibodies, respectively. The same lysate was also blotted with an anti-GFP antibody for reference. The amount of β -actin was detected with an anti- β -actin antibody as an internal control.
- b, The endogenous amount of CIP4, FBP17, and Synd2 obtained from western blotting is indicated in (a). Data from the three independent experiments are summarised. *P* values were calculated using the two-tailed Student's *t*-test, ns: not statistically significant, $\alpha = 0.05$. The exact *P* values are provided in Source Data. Data are presented as mean $\pm$ standard deviation from three independent experiments as a relative value to that of CIP4. All data points are shown.
- c, Knockdown efficiency of CIP4, FBP17, and Synd2 in Cos7 cells. Total cell lysate of non-transfected Cos7 cells and Cos7 cells transfected with siRNA for the control (luciferase), CIP4, FBP17, or Synd2 was prepared 48 h after the transfection and then subjected to the western blot analysis using anti-CIP4, FBP17, or Synd2 antibodies. β -actin was detected as an internal control. The experiment was repeated once.
- d, Additional examples of the time-lapse HS-AFM images obtained from Cos7 cells transfected with siRNA against CIP4 (siCIP4_coding), Synd2, or FBP17 and Cos7 cells transfected with siRNA targeting the 3'UTR sequence of CIP4 (siCIP4_3'UTR), followed by the overexpression of EGFP or EGFP-fused CIP4. Details of the experimental conditions and data presentations are the same as those in Fig. 2c.
- e. Knockdown efficiency of CIP4 using siRNA targeting the 3'UTR sequence of CIP4 (siCIP4_3'UTR). Total cell lysate of non-transfected Cos7 cells, cells transfected with siCIP4_3'UTR, cells transfected with siCIP4_3'UTR followed by the transfection of plasmids encoding EGFP or EGFP-fused CIP4 was prepared 48 h after the transfection and then subjected to the western blot analysis using anti-CIP4 antibodies. β -actin was used as an internal control. The relative amount of endogenous CIP4 and overexpressed EGFP-CIP4 is summarised in the right panels. Data from the three independent experiments are summarised and presented as mean $\pm$ standard deviation. All data points are shown. *P* values were calculated using the two-tailed Student's *t*-test, *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not statistically significant, $\alpha = 0.05$. The exact *P* values are provided in Source Data.

f, The frequency of CCP formation in non-transfected Cos7 cells and Cos7 cells transfected with siRNA for the control (luciferase), CIP4, FBP17, or Synd2 was quantified and is summarised. *P* values were calculated using the two-tailed Student's *t*-test, $\alpha = 0.05$. The exact *P* values are provided in Source Data. No significant differences existed between any two groups. Data are presented as mean $\pm$ standard deviation from four independent experiments. All data points are shown.

g, Additional examples of the correlative imaging of HS-AFM and confocal laser-scanning microscopy (CLSM). The fluorescent (FL) intensity of EGFP-fused CLTB and mCherry-fused CIP4 and the maximum membrane height at the CCP area were plotted against time. Time 0 was when the pit completely closed and is indicated with a black dotted line. Other details of the experimental conditions and data presentations are the same as those in Fig. 2d.

h, Additional examples of the time-lapse super-resolution SIM images obtained from Cos7 cells expressing EGFP-fused CLTB and mCherry-fused CIP4. Details of the experimental conditions and data presentations are the same as those in Fig. 2e.

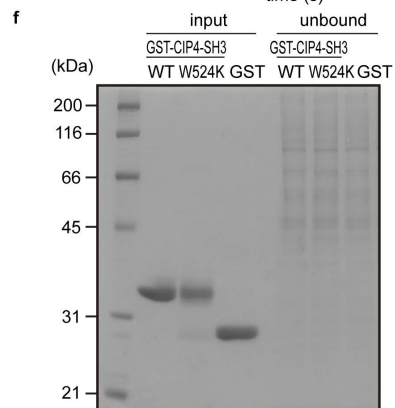
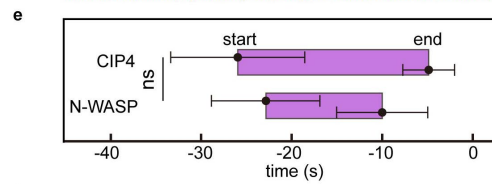
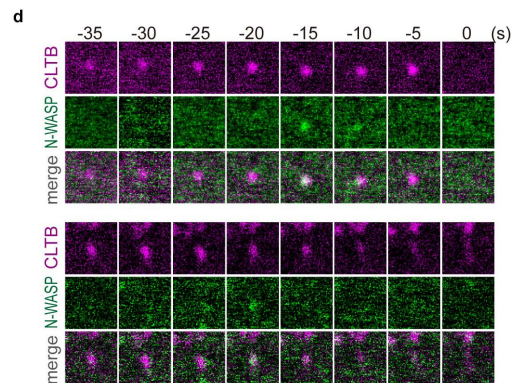
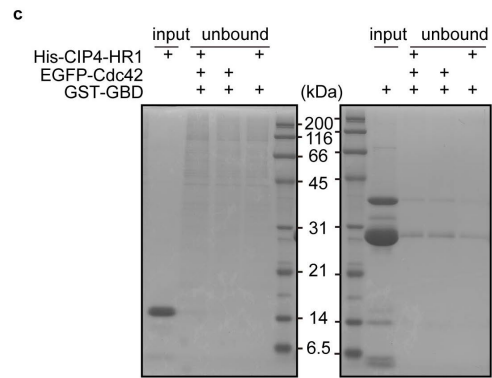
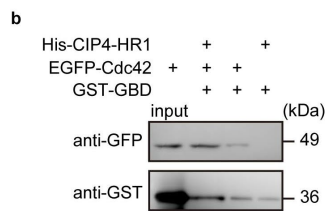
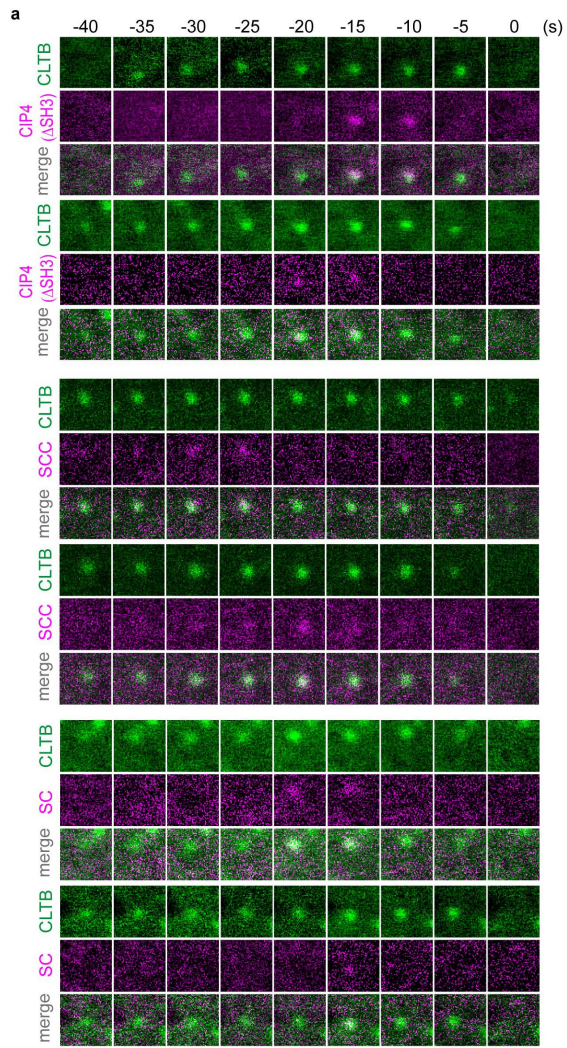


Supplementary Figure. 3 Assembly of F-BAR proteins CIP4, FBP17, and Synd2 to CCP.

a–c, Additional examples of the time-lapse fluorescence images obtained from Cos7 cells expressing EGFP-fused CLTB and mCherry-fused CIP4 (a), FBP17 (b), or Synd2 (c). Details of the experimental conditions and data presentations are the same as those in Fig. 2a.

d, Amino acid sequence alignment of *Mus musculus* CIP4 (Uniprot, Q8CJ53-3), *Homo sapiens* FBP17 (Uniprot, Q96RU3), and *Mus musculus* Synd2 (Uniprot, Q9WVE8). Alignment was performed using T-Coffee. The conservation was scored and presented as indicated in a colour bar. The F-BAR, FDR/IDR, HR1, and SH3 domains are indicated.

e, Pairwise-domain similarity among CIP4, FBP17, and Synd2. The analysis was performed using the EMBOSS Needle.



Supplementary Figure. 4 FDR and SH3 domain are necessary for asymmetric bulge formation.

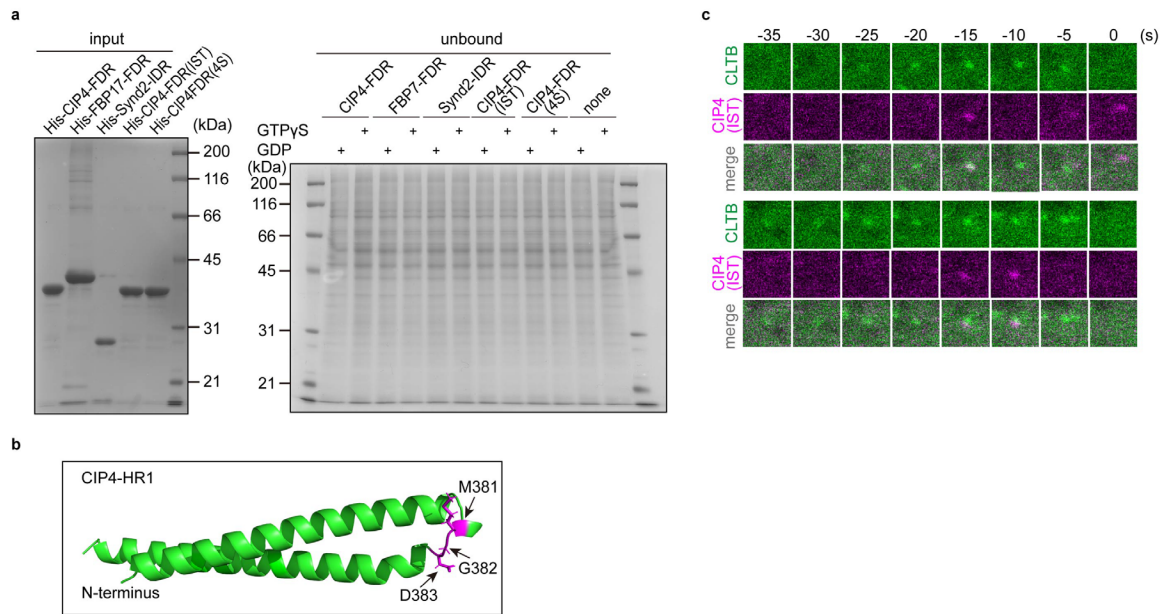
a, Additional examples of the time-lapse fluorescence images obtained from Cos7 cells expressing EGFP-fused CLTB and mCherry-fused chimeric CIP4 molecules. Details of the experimental conditions and data presentations are the same as those in Fig. 3b.

b, c, Pull-down assay among CIP4-HR1, Cdc42, and the GBD of N-WASP. GST-tagged GBD was incubated with or without hexahistidine-tagged CIP4-HR1 domain and the cell lysate containing EGFP-fused Cdc42. The eluted fractions were subjected to western blot analysis using an anti-GFP antibody (b). The input amount of CIP4-HR1 and the unbound fraction were analysed using SDS-PAGE (c). The experiment was repeated twice and similar results were obtained.

d, Time-lapse fluorescence images of EGFP-fused N-WASP and mCherry-fused CLTB expressed in Cos7 cells. Time 0 was when the clathrin signal disappeared. The image size is $1.0 \times 1.0 \mu\text{m}^2$.

e, A summary of the assembly profile of CIP4 and N-WASP at CCPs. Time 0 was when the clathrin signal disappeared. The timing when the fluorescence signal appeared and disappeared at the CCP area was defined as 'start' and 'end', respectively, and are plotted as mean $\pm$ standard deviation (N = 20 pits examined over three independent experiments, for each condition). *P* values were calculated using the two-tailed Student's *t*-test, ns: not statistically significant, $\alpha = 0.05$. The exact *P* values are provided in Source Data.

f, Input amount of GST-tagged CIP4-SH3(WT), CIP4-SH3(W524K), empty GST, and the pull-down assay's unbound fraction in Fig. 3e was analysed using SDS-PAGE. The experiment was repeated three times and similar results were obtained.

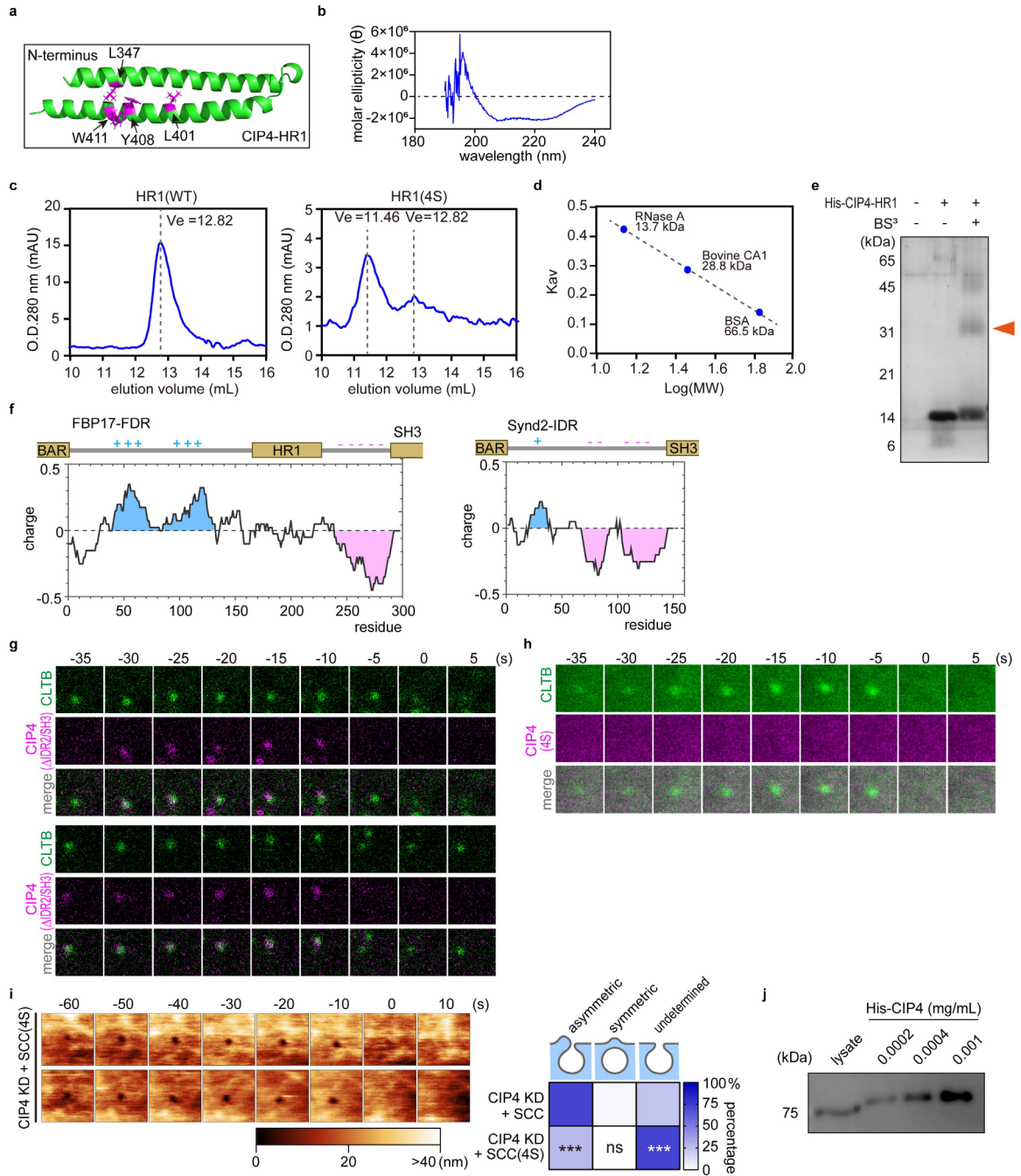


Supplementary Figure. 5 Cdc42 recruits CIP4 to the CCP site.

a, SDS-PAGE analysis of the input and the unbound fraction of the pull-down assay presented in Fig. 4a.

b, A three-dimensional structure of CIP4-HR1 predicted using AlphaFold2. Three mutated residues in CIP4(IST) (M381, G382, D383) are indicated in magenta.

c, Additional examples of the time-lapse fluorescence images of EGFP-fused CLTB and mCherry-fused CIP4(IST) expressed in Cos7 cells. Details of the experimental conditions and data presentations are the same as those in Fig. 4b.



Supplementary Figure. 6 Structural and functional properties of FDRs.

a, Three-dimensional structure of the CIP4-HR1 domain predicted using AlphaFold2. The mutated residues in the CIP4-HR1(4S) are indicated in magenta.

b, Circular dichroism spectrum of purified CIP4-HR1.

c, Gel-filtration chromatography of CIP4-HR1(WT), and CIP4-HR1(4S). The absorbance at 280 nm (mAU) is plotted against the elution volume (mL). The peak position (V_e) is indicated with a dotted line.

d, A size calibration of gel-filtration chromatography shown in b. RNase A (13.7 kDa), bovine carbonic anhydrase 1 (bovine CA-1, 28.8 kDa), and bovine serum albumin (BSA, 66.5 kDa) were analysed. The partition coefficient (K_{av}) obtained from the chromatogram is plotted against the molecular weight.

e, Crosslinking assay of CIP4-HR1. Non-treated and bis(sulfosuccinimidyl)suberate (BS^3)-treated CIP4-HR1(WT) were subjected to SDS-PAGE, followed by silver staining. Band of speculated HR1 dimer is indicated with an arrowhead. The experiment was repeated three times and similar results were obtained.

f, Charge plot of Synd2-IDR and FBP17-FDR. Positively and negatively charged regions are marked in blue and red, respectively.

g, Additional examples of the time-lapse fluorescence images of EGFP-fused CLTB and mCherry-fused CIP4 (Δ IDR2/SH3) expressed in Cos7 cells. Details of the experimental conditions and data presentations are the same as those in Fig. 5f.

h, Time-lapse fluorescence images of EGFP-fused CLTB and mCherry-fused CIP4(4S) expressed in Cos7 cells. The image size was $1.0 \times 1.0 \mu\text{m}^2$. The time point of the disappearance of the clathrin signal was defined as '0 s'.

i, Time-lapse HS-AFM images obtained from Cos7 cells expressing EGFP-fused SCC(4S) under the CIP4-KD background. Images were taken every 10 s. Time 0 was when the pit completely closed on the HS-AFM image. The height information of the AFM image is presented using a colour bar. The image size was $1.0 \times 1.0 \mu\text{m}^2$. The frequency of three different closing patterns was measured and summarised in the right panel ($N = 4$ biologically independent cells, for each condition). P values were calculated by comparing the ratio of the same closing pattern between the non-treated and treated group using a two-tailed Student's t -test and

indicated in each block, ***: $P < 0.001$; ns: not statistically significant, $\alpha = 0.05$. The exact P values are provided in Source Data.

j, Endogenous amount of CIP4 in Cos7 cells. The amount of CIP4 in the total cell lysate of Cos7 cells was detected using western blotting and compared with *in vitro* purified Hexa-histidine-tagged CIP4 with known concentration. The experiment was repeated three times and similar results were obtained.

Insert	Vector/Template	Enzyme/Polymerase	Forward (5'-3')	Reverse (3'-5')
CIP4	pEGFP-C1	Xho I/Kpn I	AAACTCGAGACATGGATTGGGGTACCGAGT	AAAGGTACCTCAGTTGAGTGTGACTCGGAG
CIP4	pmCherry-C1	Xho I/Kpn I	AAACTCGAGACATGGATTGGGGTACCGAGT	AAAGGTACCTCAGTTGAGTGTGACTCGGAG
CIP4	pET-28a(+)	EcoR I/Sal I	AAAGAATTTCATGGATTGGGGTACCGAGTTG	AAAGTCGACTCAGTTGAGTGTGACTCGGAG
CIP4(IST)	pmCherry-C1-CIP4	PrimeSTAR [®] Max DNA Polymerase	CCACAAATCAGCACCCCTGCCAGCTTAGAGCCC	GGCAGGGGTGCTGATTGTGGTGTCTTCATA
CIP4(ΔSH3/IDR2)	pmCherry-C1	Xho I/EcoR I	AAACTCGAGACATGGATTGGGGTACCGAGT	AAGAATTCAGTGTCCCTCGGTTACTGAGG
CIP4(L347S)	pmCherry-C1-CIP4	PrimeSTAR [®] Max DNA Polymerase	AAGCGAAGTCAGCAACAGCTGGAAGAG	TTGCTGACTTCGCTTTCTCTGCTGCTC
CIP4(L401S)	pmCherry-C1-CIP4	PrimeSTAR [®] Max DNA Polymerase	GAGAGGAGTAAGTTGGAAGTGCAGAAG	CAACTTACTCCTCTCAATGTTGCCAC
CIP4(Y408S)	pmCherry-C1-CIP4	PrimeSTAR [®] Max DNA Polymerase	CAGAAGAGTGAGGCTTGGTTGGCAGAA	AGCCTCACTCTTCTGCACTTCCAACCT
CIP4(W411S)	pmCherry-C1-CIP4	PrimeSTAR [®] Max DNA Polymerase	GAGGCTAGTTTGGCAGAAGCTGAAAGC	TGCCAAACTAGCCTCATAGTTCTGCAC
FBP17	pEGFP-C1	EcoR I/Sal I	AAAGAATTCAATGAGCTGGGGCACCAGCT	AAGTCGACCTAGGAATCTTGGCATTTTTG
FBP17	pmCherry-C1	EcoR I/Sal I	AAAGAATTCAATGAGCTGGGGCACCAGCT	AAGTCGACCTAGGAATCTTGGCATTTTTG
FBP17-FDR	pET-28a(+)	EcoR I/Sal I	AAAGAATTCTCAATTGATCAGAAAAATGAT	AAAGTCGACCTATATGGCAGGGAGGGGCTC
Syndapin2	pEGFP-C1	Bgl II/Sac I	AAAGATCTCCGATGTCTGTCACTACGATG	AAAGTCGACTCACTGGATAGCCTCGACATA
Syndapin2	pmCherry-C1	Bgl II/Sac I	AAAGATCTCCGATGTCTGTCACTACGATG	AAAGTCGACTCACTGGATAGCCTCGACATA
Syndapin2-IDR	pET-28a(+)	EcoR I/Sal I	AAAGAATTCCGACAGATGCGGTAGAGGAC	AGTCGACCTATTCTGTCTCTGAGTGTGCTG
CIP4-ΔSH3	pmCherry-C1	Xho I/EcoR I	AAACTCGAGACATGGATTGGGGTACCGAGT	ACAAGAATTCTCAGCCGATAGGGGATGCGAG
CIP4-F-BAR	pmCherry-C1	Xho I/EcoR I	AAACTCGAGACATGGATTGGGGTACCGAGT	AAGAATTCTCACTCGCGGCCACCTTCATG
CIP4-FDR	pmCherry-C1	Bgl II/Hind III	AAAAGATCTTCTGTGGATGCTAAGAACGAC	ACCGAAGCTTTCAGCCGATAGGGGATGCGAG
CIP4-FDR	pET-28a(+)	EcoR I/Sal I	AAAGAATTCGGCTGCTCTGTGGATGCTAAGAACGACTC	AAGTCGACTCAACACTGGCCGATAGGGGAT
CIP4-FDR(IST)	pET-28a(+)-CIP4-FDR	PrimeSTAR [®] Max DNA Polymerase	CCACAAATCAGCACCCCTGCCAGCTTAGAGCCC	GGCAGGGGTGCTGATTGTGGTGTCTTCATA
CIP4-FDR(L347S)	pET-28a(+)-CIP4-FDR	PrimeSTAR [®] Max DNA Polymerase	AAGCGAAGTCAGCAACAGCTGGAAGAG	TTGCTGACTTCGCTTTCTCTGCTGCTC
CIP4-FDR(L401S)	pET-28a(+)-CIP4-FDR	PrimeSTAR [®] Max DNA Polymerase	GAGAGGAGTAAGTTGGAAGTGCAGAAG	CAACTTACTCCTCTCAATGTTGCCAC
CIP4-FDR(Y408S)	pET-28a(+)-CIP4-FDR	PrimeSTAR [®] Max DNA Polymerase	CAGAAGAGTGAGGCTTGGTTGGCAGAA	AGCCTCACTCTTCTGCACTTCCAACCT
CIP4-FDR(W411S)	pET-28a(+)-CIP4-FDR	PrimeSTAR [®] Max DNA Polymerase	GAGGCTAGTTTGGCAGAAGCTGAAAGC	TGCCAAACTAGCCTCATACTTCTGCAC
CIP4-FDR(ΔHR1)	pET-28a(+)	EcoR I/Sal I	TCCGGGATCCGAATTCGGCTGCTCTGTGGATGCTA	CTAGCGTGACGGCTTAGGCTCAAGTGACTGAAATCTTCGG
			CCGAAGATTTCACTGCTTGAAGCTAAGCCGTACCGCTAG	GCCCGAAGCTTGTGCTGCTCAGCCGATAGGGGATGCA
CIP4-FDR(ΔIDR1)	pET-28a(+)	EcoR I/Sal I	AAAGAATTCGGCTGCCCCCGGAGCAGCAGAGAAA	AAAGTCGACTCAGCCGATAGGGGATGCGAG
CIP4-FDR(ΔIDR2)	pET-28a(+)	EcoR I/Sal I	AAGAATTCGGCTGCTCTGTGGATGCTAAGAACGAC	AAGAATTCAGTGTCCCTCGGTTACTGAGG
CIP4-SH3	pGEX-6P-1	EcoR I/Sal I	AAAGAATTCGAATGTGTGGCTATCTACCAT	AAAGTCGACTCAGTTGAGTGTGACTCGGAG
CIP4-SH3(W524K)	pGEX-6P-1-CIP4-SH3	PrimeSTAR [®] Max DNA Polymerase	GATGGAAAGACGCGGGTCAGGAGGAAA	CCGCGTCTTTCCATCACCCTTGCTCTC
CIP4-HR1	pET-28a(+)	EcoR I/Sal I	AAAAGAATTCGCCCGGAGCAGCAGAGAAA	AAGTCGACTCAGTCCCTCGGTTACTGAGG
CIP4-HR1(L347S)	pET-28a(+)-CIP4-HR1	PrimeSTAR [®] Max DNA Polymerase	AAGCGAAGTCAGCAACAGCTGGAAGAG	TTGCTGACTTCGCTTTCTCTGCTGCTC
CIP4-HR1(L401S)	pET-28a(+)-CIP4-HR1	PrimeSTAR [®] Max DNA Polymerase	GAGAGGAGTAAGTTGGAAGTGCAGAAG	CAACTTACTCCTCTCAATGTTGCCAC
CIP4-HR1(Y408S)	pET-28a(+)-CIP4-HR1	PrimeSTAR [®] Max DNA Polymerase	CAGAAGAGTGAGGCTTGGTTGGCAGAA	AGCCTCACTCTTCTGCACTTCCAACCT
CIP4-HR1(W411S)	pET-28a(+)-CIP4-HR1	PrimeSTAR [®] Max DNA Polymerase	GAGGCTAGTTTGGCAGAAGCTGAAAGC	TGCCAAACTAGCCTCATAGTTCTGCAC
SCC	pEGFP-C1	Hind III I/Kpn I	AAAGCTTAAATGTCTGTCACTACGATGAC	GGCCACCTTCAATGCCCTCGAGCTCCCGGTAATGGTTTTA
			TAAACCATTTTACCGGGAGCTCGAGGGCATGAAGGTGGCC	AAAGGTACCTCAGTTGAGTGTGACTCGGAG
SCC	pmCherry-C1	Hind III I/Kpn I	AAAGCTTAAATGTCTGTCACTACGATGAC	AAAGGTACCTCAGTTGAGTGTGACTCGGAG
SCC(W524K)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	GATGGAAAGACGCGGGTCAGGAGGAAA	CCGCGTCTTTCCATCACCCTTGCTCTC
SCC(IST)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	CCACAAATCAGCACCCCTGCCAGCTTAGAGCCC	GGCAGGGGTGCTGATTGTGGTGTCTTCATA
SCC(L347S)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	AAGCGAAGTCAGCAACAGCTGGAAGAG	TTGCTGACTTCGCTTTCTCTGCTGCTC
SCC(L401S)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	GAGAGGAGTAAGTTGGAAGTGCAGAAG	CAACTTACTCCTCTCAATGTTGCCAC
SCC(Y408S)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	CAGAAGAGTGAGGCTTGGTTGGCAGAA	AGCCTCACTCTTCTGCACTTCCAACCT
SCC(W411S)	pEGFP-C1-SCC	PrimeSTAR [®] Max DNA Polymerase	GAGGCTAGTTTGGCAGAAGCTGAAAGC	TGCCAAACTAGCCTCATACTTCTGCAC
SC	pEGFP-C1	Hind III I/Kpn I	AAAGCTTAAATGTCTGTCACTACGATGAC	AAGGTACCTCAGCCGATAGGGGATGCAGGC
SC	pmCherry-C1	Hind III I/Kpn I	AAAGCTTAAATGTCTGTCACTACGATGAC	AAGGTACCTCAGCCGATAGGGGATGCAGGC
CS	pmCherry-C1	Hind III I/Sal I	AAAAGAGTTCGATGGATTGGGGTACCGAGT	TGCTTTGATGCTCTGCTCGAGGCAATTTGCCAATAATGGGA
			TCCCATTTATGGCAATGCCTCGAGCAGCATCAAAGCA	AAAGTCGACTTCTGTTCTGAGGTCGTGTG
Cdc42	pEGFP-C1	Xho I/EcoR I	AGCCTCGAGAAATGCAGACAATTAAGTGTG	AAAGAATTCAATCATAGCAGCACACACCTG
Cdc42	pET-28a(+)	EcoR I/Sal I	AAAGAATTTCATGCAGACAATTAAGTGTGTT	AAAGTCGACTCATAGCAGCACACACCTGCG
Cdc42(Q61L)	pET-28a(+)-Cdc42	PrimeSTAR [®] Max DNA Polymerase	GCAGGGCTAGAGGATTATGACAGATTA	ATCCTCTAGCCCTGCAGTATCAAAAAG
N-WASP	pEGFP-C1	Xho I/EcoR I	AACTCGAGAAATGAGCTCCGTCACGACGCA	AGAATTTCTCAGTCTTCCCACTCATCATCAT
N-WASP	pET-28a(+)	EcoR I/Hind III	AAAAGAATTCATGAGCTCCGTCACGACGCA	AAAAGCTTTCAGTCTTCCCACTCATCATCA
N-WASP-GBD	pGEX-6P-1	EcoR I/Xho I	AAGAATTCGATATAGGAACACCAAGCAATT	AGCGGCCGCTCAAATAAAGTCATATATAACT
CLTB	pEGFP-N1	Sac I/Kpn I	AAGAGCTCAAAATGGCTGATGACTTTGGCT	AAAGGTACCGAGCGGGACAGTGGCGTCTGC
CLTB	pmCherry-N1	Sac I/Kpn I	AAGAGCTCAAAATGGCTGATGACTTTGGCT	AAAGGTACCGAGCGGGACAGTGGCGTCTGC

Supplementary Table. 1 Primers used in the study.

A summary of the oligonucleotide primers used in this study. Primers were used to either amplify fragments of interest by PCR or generate point mutations by using PrimerSTAR[®] Max DNA Polymerase. Plasmids encoding SCC and CS chimeric molecules and CIP4-FDR(Δ HR1) were obtained by separately amplifying two different fragments, ligating, and subcloning into desired vectors (Methods), therefore, two pairs of primers were listed.