

MethoD?S????????????????????????????????????????????????
 ???????????????????????????????????????????????????????12
 19-4093?????331kDa?Dyn????????N????S.
 cerevisiae????????DYN1?????????????????????????????????
 ?????????????DNA????URA3?????????????????????????????
 ?????????Extended Data Fig.2??S. cerevisiae??R. Vale
 (UCSF)????????????????????????????????ZZ???????T
 EV????????N????????DHA??N????C?????4?Extended
 Data Table
 1b????????IgG????????Tev?????????????????????????????
 ??4????IgG????????????????????10?M????????????????
 Tev????????????????????????????????????????MES-MT??
 ???30 mM MES pH 6.5, 70 mM NaCl, 1 mM MgCl2, 1 mM DTT???10
 mg ml-1????????????6mM
 GTP?Sigma????MES-MT?????2?????37???90?????????????2
 0?M????????MES-MT?????2????????????????????Dyn??
 DynRK+7hep??BRB10?10 mM PIPES pH7.0, 1 mM EGTA, 1 mM
 MgCl2, 1 mM DTT, 0.1% Tween-20??5????Amicon 100 MWCO 0.5
 ml?????????????????2????????????????????????125??
 ??????20,000r.c.f.?10????????BRB10?????????3?????
 ?M????150nM????????BRB10?????????4?l?????
 00%?22????FEI Vitrobot III????????Quantifoil Au300 R1./2/1.3
 ?????????4.5????????????????????????????????????
 ????.????????????????Gatan 626????????Falcon
 II???????200 kV????FEI F20
 TEM????????????????????EPU?????????????-4?m??
 ???50e Å-2 s-1???1.5????????2.06
 Å2????????????????14.????????????GCT
 F????????Relion????????FIJl27????????5c-
 g????????????????????????????????????
 ?????????????????Relion????????????
 ?????????????????????????????????
 ??????2????????1????????Dyn?DynRK+7hep????????
 ?????????????????PDB????????3VKG10
 ??????4AI611????????ADP????????EMAN?????pdb2mrc??
 ?????????????????????bfilter????30Å??500Å???
 ?????????Relion????????????????Nikon
 Ti-Eclipse ?????????????1.49 NA 100x
 ?????????????TIRF????????488
 nm?GFP?QDs??561nm?TMR?Cy3??633nm?Cy5????????
 ?????106nm?EM-CCD????Ixon?Andor????????1Hz????
 ?????????Optosplit
 II?Cairn????????CCD????2????????Microtubule-bri
 dge
 assays.????????????????19????????2?m???
 ?????EDC-NHS????GFP???Covance?????????0.5?
 M
 SRS85:82-GFP23????????????????????
 ?????????????1mg
 ml-1????30?l?10????????

DLBC?DLB?????30mM HEPES pH7.4?1mM EGTA?2mM
MgCl2??Cy3???????0.015mg
ml-1????????????????10????????????30?I?DLBC????????GFP??
?????????0.5?m??5-10nM
GFP-dynein????10????????????????????????????????????Niko
n Ti-E Eclipse?????Nikon 100x1.49 NA?????????Nikon 1.4
NA?????????LED????????Sutter????????????????????????10?
m?????2?????????????????????????????????????????????
????????????????CMOS????????????10Hz?????57nm??????
??????MATLAB????????????????????????????????????????
????????????x-y????????????19????????????????????????
????????????????r?27nm????????????tan-1(2?r/?)?????????
z????????????????Physik Instrumente????????????z???±250
nm?25
nm????????????????0.5?m????????????????????????????
????????N-????????NEM?????????BRB80?80 mM PIPES pH
6.8, 1 mM EGTA, 2 mM MgCl2???10 mg
ml-1????????????28?????1 mM NEM???0.5 mM
GTP???10????????????30???8 mM
?-????????ME?????????BRB80 ?? 0.4 mg ml-1 Cy3
????????0.5 mg ml-1 ?????????1 mM GMP-CPP (Jena
BioSciences) ??? 1 mM DTT ?37
??15????????????????????????????1.5?I?????BRB80???0.1 mg
ml-1 Cy3-tubulin?1 mg ml-1 unlabelled tubulin?1 mg ml-1
NEM-modified tubulin?1 mM GTP???1 mM
DTT????????37?????????????????2?M?20?M???200?M????
???10????????2?I?????37????15????????????????300?I?30??
????????65,000g?10????????????????20?M????????1mM
DTT???BRB80????????????????????????????4?????????
??GFP????0.4 mg
ml-1?Covance????????????5????????????????60?I?????
DLBCT?20?M????????DLBC????????DLBCT??20nM
GFP????????10?I?I????????3????????????30?I?DLBCT???
????????????????10?I?200nM????????????????????2
????????????????100?I?DLBCT????????????KCI????30?I??
????????2.5mM PCA????????50nM
PCD????????-3?4-????????1mM
ATP????DLBCT????????????1????????????????????
????????????????50?I?DLBC????GFP?????????0.2
nM????TMR?????????0.2
nM??DLBC????????????3????????????100?I?DLBC?20
?I????????????????????????????655nm?????QD?Invit
rogen????????SMCC?sulfosuccinimidyl 4-(N-maleimidomethyl)
cyclohexane-1-carboxylate
????GFP????????QD?100nM??25?M???SMCC??250?????
????1????????????SMCC??30k
MWCO????????????DLB?3????????GFP???0.4 mg
ml-1??4 mM
TCEP????2-????????????30?????QDs?????1????25mM??
????pH8.0?3????????????????5?M?????????G
FP?????QD?5 mM??100 nM
GFP????????1?1????????15????????????BSA?????
????????????????????????????????????-QD????100????
????????????3????????????????100?I?DLBC?5-10?M?ATP?
??20?I?I????????????Cy3?????QD?2????????????
????????