

MethoS??
??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????????37??90?????????????
0?M????????MES-MT????????????????????????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?4.5????????????????????????????
???.????????????????Gatan 626????????Falcon
II??????200 kV????FEI F20
TEM????????????????????EPU????????-4?m??
??50e Å s⁻¹??1.5??????2.06
Å2????????????????14.????????????GCT
F????????Relion????????Fiji27????????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?l?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?l????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?l?37????15????????300?l?30??
????????65,000g?10????????20?M??????1mM
DTT??BRB80????????????????4????????
??GFP????0.4 mg
ml-1?Covance????????5????????60?l????
DLBCT?20?M????????DLBC????????DLBCT??20nM
GFP????10?l?37????3????????30?l?DLBCT??
????????10?l?200nM????????????
2????????100?l?DLBCT?????????KCl????30?l?
????????2.5mM PCA????????50nM
PCD?????-3?4-????????1mM
ATP????DLBCT????????1????????
????????50?l?DLBC?????GFP????????0.2
nM????TMR????????0.2
nM??DLBC????????3????????100?l?DLBC?20
?l?????????????????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?l?DLBC?5-10?M?ATP?
??20?l?I????????Cy3?????QD?2????
???????