

**Appendix file – Schweiggert *et al.***

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**Appendix Table S1: Plasmids.**

Plasmid	Template for PCR/Method	Oligonucleotides	Source
pET22B-Uba1-His			24
pGEX6P3-GST-Cdc34			24
pGEX6P3-GST-UbcH5b			24
pGEX6P3-GST-UbcH5c			A. Pichler
pGEX6P3-GST-Ube2G1	cDNA of RPE-1 cells (Gibson)	5'-CCTGGGATCCCCGAATTCCATGACGGAGCTGCAGTC-3' 5'-TGCGGCCGCTCGAGTCGACTCACTCAAAGCAGTCTCTTGG-3'	This work
pGEX-GST-Nedd8			B. Schulman
pGEX-GST-Ubc12			B. Schulman
pGEX-GST-APPBP1-rbs-Uba3			B. Schulman
pGEX-4T-GST-PreScission			A. Dickmanns
pET28a-His-Ubiquitin			24
pET3a-Ubiquitin			S. Raasi <sup>69</sup>
pET3a-Ubiquitin(K48R)	pET3a-Ubiquitin (mutagenesis)	5'-TTGATCTTTGCCGGTA <sub>g</sub> GCAGCTCGAGGACGGTA-3' 5'-TACCGTCCTCGAGCTGC <sub>c</sub> TACCGGCAAAGATCAA-3'	This work
pET3a-Ubiquitin(K63R)	pET3a-Ubiquitin		S. Raasi <sup>69</sup>
pET3a-Ubiquitin(K48R/K63R)	pET3a-Ubiquitin(K63R) (mutagenesis)	5'-TTGATCTTTGCCGGTA <sub>g</sub> GCAGCTCGAGGACGGTA-3' 5'-TACCGTCCTCGAGCTGC <sub>c</sub> TACCGGCAAAGATCAA-3'	This work
pENTR221-GSTP1 (clone ID 182399431)			DKFZ ORF Collection
pENTR221-CSRP2 (clone ID 176815824)			DKFZ ORF Collection
pENTR221-Sec23B (clone ID 128836251)			DKFZ ORF Collection
pENTR221-HGS (clone ID 158422822)			DKFZ ORF Collection
pENTR223-CSPP1 (clone ID 148371129)			DKFZ ORF Collection
pENTR221-ODF2 (clone ID: 142932633)			DKFZ ORF Collection

pENTR221-Kif2C (clone ID 199650980)			DKFZ ORF Collection
pENTR221-Kif2A (clone ID 140305080)			DKFZ ORF Collection
pENTR223-Kif2B (clone ID 123156095)			DKFZ ORF Collection
pcDNA3.1-HA-GSTP1	pENTR221-GSTP1 (XhoI/KpnI)	5'-CGGCTCGAGATGCCGCCCTACACCGTG-3' 5'-CGGGGTACCCTACTGTTTCCCGTTGCC-3'	This work
pcDNA3.1-HA-CSRP2	pENTR221-CSRP2 (XhoI/KpnI)	5'-CGGCTCGAGATGCCTGTCTGGGGAGGTG-3' 5'-CGGGGTACCTTACTGGGCATGAACAAGAGC-3'	This work
pcDNA3.1-HA-Sec23b	pENTR221-Sec23B (XhoI/KpnI)	5'-CGGCTCGAGATGGCGACATACCTGGAG-3' 5'-CGCGGTACCTTAACAGGCACTGGAGACAGC-3'	This work
pcDNA3.1-HA-HGS	pENTR221-HGS (XhoI/KpnI)	5'-CGGCTCGAGATGGGGCGAGGCAGCGGC-3' 5'-CGGGGTACCTCAGTCGAATGAAATGAGCTG-3'	This work
pcDNA3.1-HA-CSPP1	pENTR223-CSPP1 (XhoI/HindIII)	5'-CGGCTCGAGATGAGACAGCCTTCTCCTATAG-3' 5'-CGGAAGCTTTTAACCATGTGCAGTCGACAG-3'	This work
pcGS5-HA-DMTN			P. Sauer
pcDNA3.1-HA-ODF2	pENTR221-ODF2 (BamHI/XbaI)	5'-CGGGGATCCATGAAGGACCGCTCTTCAACTC-3' 5'-CGGTCTAGATCAGGCAGGAGGAGATCGGGAG-3'	This work
pcDNA3.1-HA-MCAK	pENTR221-Kif2C (BamHI/NotI)	5'-ATATGGATCCATGGCCATGGACTCGTCCG-3' 5'-TATAGCGGCCCGCCTACTGGGGCCGTTTCTTG-3'	This work
pcDNA3.1-HA-Kif2A	pENTR221-Kif2A (BamHI/NotI)	5'-TATAGGATCCATGGTAACATCTTTAAATG-3' 5'-TATAGCGGCCCGCTTAAAGGGCACGGGGTC-3'	This work
pcDNA3.1-HA-Kif2B	pENTR223-Kif2B (EcoRI/NotI)	5'-TATAGAATTCATGGCCAGCCAGTTC-3' 5'-TATAGCGGCCGCTCACTCTACCTTGCTC-3'	This work
pcDNA3.1-Flag			24
pcDNA3.1-Flag-Fbxw5			24
pcDNA3.1-Flag-Fbxw5-ΔF-box			24
pCMV-3xFLAG-Fbxw7			I. Hoffmann <sup>77</sup>
pET28a-His-MCAK	pcDNA3.1-HA-MCAK (BamHI/NotI)	5'-ATATGGATCCATGGCCATGGACTCGTCCG-3' 5'-TATAGCGGCCCGCCTACTGGGGCCGTTTCTTG-3'	This work
pET28a-His-Kif2A	pcDNA3.1-HA-Kif2A (BamHI/NotI)	Cloned by restriction digestion	This work
pET28a-His-Kif2B	pcDNA3.1-HA-Kif2B	Cloned by restriction digestion	This work

	(EcoRI/NotI)		
pFastBacHT-B-His-Fbxw5			24
pFastBac1-Skp1ΔΔ			24,43
pFastBacHT-B-Eps8	pCDNA3.1-HA-Eps8 <sup>24</sup>	5'-GTATTTTCAGGGCGCCATGGATGAATGGTCATATTTCTAATCATC-3' 5'-CAGGCTCTAGATTCGAAAGCTTAGTGACTGCTTCCTTC-3'	This work
pFastBacHT-B-MCAK	pcDNA3.1-HA-MCAK (BamHI/NotI)	5'-ATATGGATCCATGGCCATGGACTCGTCCG-3' 5'-TATAGCGGCCGCCTACTGGGGCCGTTTCTTG-3'	This work
pFastBacHT-B-His-Kif2A	pcDNA3.1-HA-Kif2A (BamHI/NotI)	Cloned by restriction digestion	This work
pEGFP-PLK1-T210D			S. Lev
pFastBacHT-B-His-Plk1-T210D	pEGFP-PLK1-T210D (Gibson)	5'-AACCTGTATTTTCAGGGCGCAATGAGTGCTGCAGTGACTG-3' 5'-CAGGCTCTAGATTCGAAAGCTTAGGAGGCCTTGAGACG-3'	This work
pcDNA3.1-Flag-Fbxl2			L. Hengst
pFastBacHT-B-His-Fbxl2	pcDNA3.1-Flag-Fbxl2	5'-GTATTTTCAGGGCGCCATGGTTTTCTCAAACAATGATGAAGG-3' 5'-CTCTAGATTCGAAAGCGGCCTCAGAGAATGACACAGCAC-3'	This work
pFastBacHT-B-His-Fbxw7	pCMV-3xFLAG-Fbxw7		This work
pET11D-hRanGAP			A. Flotho
pBIG1B-His-Cul4A/His-DDB1/His-mmRbx1			E. Fischer
pBIG1B-His-Cul4A/His-DDB1/mmRbx1	pBigBac-His-Cul4A/His-DDB1/His-mmRbx1 (Gibson, 4 fragments)	5'-ATCTACCATGGGCACCAACAGC-3' 5'-TGACTCGCTGCGCTCGGTCGTTTCGGCTGCGGCGAGCGG-3' 5'-CGACCGAGCGCAGCGAGT-3' 5'-ATGGGCTTGTAGGCGAGCAG-3' 5'-TGCTCGCCTACAAGCCCATGG-3' 5'-GTCCGGGGGTACCTCATCAGGCCACGTAGTGGTAC-3' 5'-CTGATGAGGTACCCCGGAC-3' 5'-TGTTGGTGCCCATGGTAGATCCGTATTTATAG-3'	This work
pBIG1B-HisCul1/mmRbx1	cDNA of RPE-1 (Gibson) destination vector: pBigBac-His-Cul4A/His-DDB1/mmRbx1 cut with Sall/PvuII	5'-CCATCATCATCATCACTCCGCGGTCGACGAGAACCCTCTACTTC CAGGGAGGCGGCCGCTCGTCAACCCGGAGCCAG-3' 5'-GGCTTTACTGGAAATTCAGTTAAGCCAAGTAACTGTAGGTGTCC-3'	This work
pRetroX-TRE3G-mNG-MCAK			S. Hata

pRetroX-TRE3G-mNG-Fbxw5	mNG tag from pRetroX-TRE3G-mNG-MCAK via Gibson into pcDNA3.1-Flag-Fbxw5 cut with HindIII and BamHI. mNG-Fbxw5 then subcloned via BamHI/NotI into pRetroX	5'-ACTCACTATAGGGAGACCCAATGGTGAGCAAGGGCGAGG-3' 5'-GCGTGCCGCCCTCGTCCATGCCGCCTGCACCAGCTCCT-3'	This work
pRetroX-TRE3G-mNG-Kif2B	pcDNA3.1-HA-Kif2B (Gibson) destination vector: pRetro-TRE3G-mNG-MCAK cut with EcoRI/NotI	5'-GTGCAGGAGCTGGTGCAGGCATGGCCAGCCAGTTCTGC-3' 5'-GCGCCTCCCCTACCCGGTAGTCACTCTACCTTGCTCTTCACG-3'	This work
pQCXIZ-H2B-mRuby2			S. Hata
pRetroX-Tet3G			Clontech®
pVSV-G			Clontech®
p53-HaloTag® Fusion Vector			Promega
NanoLuc®-MDM2 Fusion Vector			Promega
pFN21A-HT-Fbxw5			Promega
pFC32K-MCAK-NLuc	pcDNA3.1-HA-MCAK	5'-GCGTGCGATCGCCATGGCCATGGACTCGTCGCT-3' 5'-GGCGGTTTAAACCTGGGGCCGTTTCTTGCTGCT-3'	This work
pFN31K-NLuc-MCAK	pcDNA3.1-HA-MCAK	5'-GCGTGCGATCGCCATGGCCATGGACTCGTCGCT-3' 5'-GGCGGTTTAAACCTGGGGCCGTTTCTTGCTGCT-3'	This work

**Appendix Table S2: Reagents**

<b>Antibodies</b>			
<b>Antibody</b>	<b>Species</b>	<b>Source</b>	<b>Identifier</b>
Monoclonal anti HA.11	Mouse	Covance	MMS-101R
Monoclonal anti-Flag	Mouse	Sigma Aldrich	F1804
Monoclonal anti-Flag	Rabbit	Sigma Aldrich	F7425
Monoclonal FK2 (ubiquitin)	Mouse	Enzo Life Sciences	BWL-PW8810
Monoclonal anti-Eps8	Mouse	BD Bioscience	610144
Monoclonal anti- $\gamma$ -Tubulin	Mouse	Sigma Aldrich	T6557
Monoclonal anti-HGS	Rabbit	Cell Signaling Technology	15087
Monoclonal anti-GSTP1	Mouse	Cell Signaling Technology	3369
Monoclonal anti- $\beta$ -actin	Mouse	Sigma Aldrich	A1978
Monoclonal anti-Plk1	Rabbit	Cell Signaling Technology	4513
Monoclonal anti-CyclinB1	Mouse	Santa Cruz	sc-245
Polyclonal anti-Arl13b	Rabbit	Proteintech	17711-1-AP
Polyclonal anti-CSR2	Rabbit	Proteintech	10892-2-AP
Polyclonal anti-Sec23b	Rabbit	Sigma Aldrich	HPA008216
Polyclonal anti-ODF2	Rabbit	Proteintech	12058-1-AP
Polyclonal anti-Fbxw5	Rabbit	<sup>24</sup>	n/a
Polyclonal anti-RanGAP1	Goat	<sup>78</sup>	n/a
Polyclonal anti-Uba2	Goat	<sup>79</sup>	n/a
Anti-Kif2c	Mouse	Santa Cruz	sc-81305
Anti-Kif2c*	Rabbit	Novus Biologicals	NB100-2588
Anti-Kif2a	Rabbit	Abnova	PAB-12407
Anti-Kif2b	Rabbit	Sigma Aldrich	HPA024795
Anti-Mouse-488	Donkey	Invitrogen	A21202
Anti-Mouse-594	Donkey	Invitrogen	A21203
Anti-Rabbit-488	Donkey	Invitrogen	A21206
Anti-Rabbit-594	Donkey	Invitrogen	A21207
<b>Chemicals, reagents, enzymes &amp; Media</b>			
<b>Name</b>	<b>Source</b>		<b>Identifier</b>
0.45 $\mu$ m pore size polyvinylidene difluoride membranes (PVDF)	Amersham		10600021/10600023
aprotinin	Roth		A162.3

ATP	Sigma Aldrich	n/a
Cellfectin-II	Invitrogen (Thermo Fisher Scientific)	10362100
DMEM	Gibco (Thermo Fisher Scientific)	41965-039
DMEM Glutamax™	Gibco (Thermo Fisher Scientific)	10566016
DMEM-F12	Gibco (Thermo Fisher Scientific)	21331-020
Doxycyclin	Sigma Aldrich	24390-14-5
DYKDDDDK	Pantecs	N/A
Energy regeneration system	Boston Biochem	B-10
ExCell® 420 Serum-Free Medium	Sigma Aldrich	14420C
ExCell® 420 Serum-Free Medium	Sigma Aldrich	14420C
Fetal Bovine Serum (FBS), Qualified	Gibco (Thermo Fisher Scientific)	10270-106
Fluorescence mounting medium	Dako	S3023
HA-peptide (YPYDVPDYA)	Pepscan Therapeutics or Peptides & Elephants	N/A
HiFi assembly mix	New England Biolabs	E2621
Hoechst 33342	Sigma Aldrich	23491-52-3
Human ProtoArray® Microarray (v5.0)	ThermoFischer Scientific/Invitrogen	PAH0525101
Hydroxyurea	Sigma Aldrich	127-07-1
Immobilon Western chemiluminescent HRP substrate	Merck Millipore	WBKLS0500
L-Glutamine 200 mM	Gibco (Thermo Fisher Scientific)	25030-024
Leupeptin Hemisulfat	Roth	CN33.4
Lipofectamin RNAi MAX	Invitrogen (Thermo Fisher Scientific)	13778150
Monoclonal anti-Flag-agarose	Sigma Aldrich	A2220
Monoclonal anti-HA-agarose	Sigma Aldrich	A2095
Ni-NTA-Agarose	Qiagen	30250
Nocodazole	Sigma Aldrich	31430-18-9
OptiMEM	Gibco (Thermo Fisher Scientific)	31985-047
PefaBloc® SC	Roche	11 585 916 001
PepstatinA	Roth	2936.3
Polyethyleneimide (PEI)	PolySciences	23966
Protein A agarose	Roche	11 134 515 001
Protino Glutathione Agarose 4B	Machery & Nagel	745500
Q5 Polymerase	New England Biolabs	M0491
Rabbit IgG isotype control	ThermoFischer Scientific/Invitrogen	10500C

Restriction enzymes	New England Biolabs	-	
RO-3306	Sigma Aldrich	SLM0569	
Rotiphorese® 30	Roth	3029.1	
Sf-900™ II SFM medium	Gibco (Thermo Fisher Scientific)	10902096	
Sf-900™ II SFM medium	Gibco (Thermo Fisher Scientific)	# 10902096	
Synthetic Block (10x)	ThermoFischer Scientific/Invitrogen	PA017	
TEMED	Roth	2367.3	
Thymidine	Sigma Aldrich	T9250-1G	
β-mercaptoethanol	Applichem	A1108,0025	
<b><i>E. coli</i> strains</b>			
DH5α:	F-φ80lacZ ΔM15 (lacZYA-argF) U169 deoR recA1 endA1 hsdR7 (rk-, mk+) phoA supE44 thi-1 gyrA96 relA1 λ-		
Rosetta:	F- ompT hsdSB (rB- mB-) gal dcm (DE3) pRARE (CmR)		
<b>Recombinant proteins (expressed and purified from <i>E.coli</i>)</b>			
Name	Tag	Expression plasmid	Source
APPBP1-Uba3	Cleaved	pGEX-GST-APPBP1-rbs-Uba3	This work
Cdc34	Cleaved	pGEX6P3-GST-Cdc34	This work
Kif2a	6xHis	pET28a-His-Kif2A	This work
Kif2b	6xHis	pET28a-His-Kif2B	This work
MCAK	6xHis	pET28a-His-MCAK	This work
Nedd8	Cleaved	pGEX-GST-Nedd8	This work
Uba1	6xHis	pET22B-Uba1-His	This work
Ubc12	Cleaved	pGEX-GST-Ubc12	This work
UbcH5b	Cleaved	pGEX6P3-GST-UbcH5b	This work
UbcH5c	Cleaved	pGEX6P3-GST-UbcH5c	This work
Ube2G1	Cleaved	pGEX6P3-GST-Ube2G1	This work
Ubiquitin	6xHis	pET28a-His-Ubiquitin	This work
Ubiquitin	-	pET3a-Ubiquitin	This work
Ubiquitin (K48R)	-	pET3a-Ubiquitin(K48R)	This work
Ubiquitin (K48R/K63R)	-	pET3a-Ubiquitin(K48R/K63R)	This work
Ubiquitin (K63R)	-	pET3a-Ubiquitin(K63R)	This work
Ubiquitin (methylated)	-	-	Boston Biochem (U-501)
Ubiquitin (FITC-labelled)		Thermo Fisher Scientific	PV4378
Cul1A-Cul1B/Rbx1		-	B. Schulmann
TEV(tobacco etch virus) protease	6xHis	His-TEV	This work

PreScission protease	GST	pGEX-4T-GST-PreScission	This work
<b>Recombinant proteins (expressed and purified from Sf21 cells)</b>			
<b>Name</b>	<b>Tag</b>	<b>Expression plasmid</b>	<b>Source</b>
Cul1/Rbx1 complex	TEV cleaved	pBIG1B-HisCul1/mmRbx1	This work
Cul4A/DDB1/Rbx1 complex	TEV cleaved	pBIG1B-His-Cul4A/His-DDB1/His-mmRbx1	This work
Eps8	6xHis	pFastBacHT-B-Eps8	This work
Fbxl2/Skp1ΔΔ subcomplex	6xHis	pFastBacHT-B-His-Fbxl2 pFastBac1-Skp1ΔΔ	This work
Fbxw5/Skp1ΔΔ subcomplex	6xHis	pFastBacHT-B-His-Fbxw5 pFastBac1-Skp1ΔΔ	This work
Fbxw7/Skp1ΔΔ subcomplex	6xHis	pFastBacHT-B-His-Fbxw7 pFastBac1-Skp1ΔΔ	This work
Kif2a	TEV cleaved	pFastBacHT-B-His-Kif2A	This work
MCAK	TEV cleaved	pFastBacHT-B-MCAK	This work
Plk1-T210D	6xHis	pFastBacHT-B-His-Plk1-T210D	This work
<b>siRNAs</b>			
<b>Target</b>	<b>Sequence</b>	<b>Source</b>	<b>Reference</b>
non-targeting (NT)	5'-UAGCGACUAAACACAUCAAUUdTdT-3'	Ambion/Qiagen	24
Fbxw5#1	5'-CCACAGGCGCCAAGAGCAAAdTdT-3'	Ambion / Sigma Aldrich	24
Fbxw5#2	5'-CGGGAGAGGUGGAGAUdGCUdTdT-3'	Ambion	24
Fbxw5#3	5'-CCCUACAACUGGAGCUACAdTdT-3'	Dharmacon / Sigma Aldrich	24
MCAK	5'-GAUCCAACGCAGUAAUdGdUdTdT-3'	Sigma Aldrich	61
Kif2a	5'-GGAAUGGCAUCCUGUGAAA-3'	Sigma Aldrich	83
Kif2b	5'-GGACCUdGGAUUAUCAUCACC-3'	Sigma Aldrich	49

\* MCAK antibody NB100-2588 was used for Figure 6B, Figure 7B, EV3A, EV4A,B and F. For all other western blots and all IF images, MCAK antibody sc-81305 was used.