Appendix file – Schweiggert et al.

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### Appendix Table S1: Plasmids.

| Plasmid                  | Template for PCR/Method | Oligonucleotides                                                                 | Source |
|--------------------------|-------------------------|---------------------------------------------------------------------------------|--------|
| pET22B-Uba1-His          |                         |                                                                                 |        |
| pGEX6P3-GST-Cdc34        |                         |                                                                                 |        |
| pGEX6P3-GST-UbcH5b       |                         |                                                                                 |        |
| pGEX6P3-GST-UbcH5c       |                         |                                                                                 |        |
| pGEX6P3-GST-Ube2G1       | cDNA of RPE-1 cells (Gibson) | 5’-CCTGGGATCCCCGAATTCCATGACGGAGCTGCAGTC-3’<br>5’-TGCAGGCCGCTCGAGTCACTCAAAAGCAGTCTCTTG-3’ |        |
| pGEX-GST-Nedd8           |                         |                                                                                 |        |
| pGEX-GST-Ubc12           |                         |                                                                                 |        |
| pGEX-GST-APPBP1-rbs-Uba3 |                         |                                                                                 |        |
| pGEX-4T-GST-PreScission  |                         |                                                                                 |        |
| pET28a-His-Ubiquitin     |                         |                                                                                 |        |
| pET3a-Ubiquitin          |                         |                                                                                 |        |
| pET3a-Ubiquitin(K48R)    | pET3a-Ubiquitin (mutagenesis) | 5’-TTGATCTTTTCCGCGTAgGCAGCTCGAGGACGGTA-3’<br>5’-TACCGTCTCTCGAGCTGcTACCGGCAAGATCAA-3’ | This work |
| pET3a-Ubiquitin(K63R)    | pET3a-Ubiquitin         |                                                                                 |        |
| pET3a-Ubiquitin(K48R/K63R) | pET3a-Ubiquitin(K63R) (mutagenesis) | 5’-TTGATCTTTTCGGCGTAgGCAGCTCGAGGACGGTA-3’<br>5’-TACCGTCTCTCGAGCTGcTACCGGCAAGATCAA-3’ | This work |
| pENTR221-GSTP1           |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID 182399431)     |                         |                                                                                 |        |
| pENTR221-CSRP2           |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID 176815824)     |                         |                                                                                 |        |
| pENTR221-Sec23B          |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID 128836251)     |                         |                                                                                 |        |
| pENTR221-HGS             |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID 158422822)     |                         |                                                                                 |        |
| pENTR223-CSPP1           |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID 1483771129)    |                         |                                                                                 |        |
| pENTR221-ODF2            |                         |                                                                                 | DKFZ ORF Collection |
| (clone ID: 142932633)    |                         |                                                                                 |        |
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  
(XhoI/KpnI)  
5'-CGGCTCGAGATGCCGCCCTACACCGTG-3'  
5'-CGGGGTACCCCTACTGTTCCTCCGGTGCC-3'  
This work

pcDNA3.1-HA-CSRP2  
(XhoI/KpnI)  
5'-CGGCTCGAGATGCCCTGCTCTGGGGAGGCTG-3'  
5'-CGGGGTACCTTTACACCGCTTGAGAAGACG-3'  
This work

pcDNA3.1-HA-Sec23b  
(XhoI/KpnI)  
5'-CGGCTCGAGATGCCCTGCTCTGGGGAGGCTG-3'  
5'-CGGGGTACCTTTACACCGCTTGAGAAGACG-3'  
This work

pcDNA3.1-HA-HGS  
(XhoI/KpnI)  
5'-CGGCTCGAGATGCCCTGCTCTGGGGAGGCTG-3'  
5'-CGGGGTACCTTTACACCGCTTGAGAAGACG-3'  
This work

pcDNA3.1-HA-CSPP1  
(XhoI/HindIII)  
5'-CGGCTCGAGATGCCCTGCTCTGGGGAGGCTG-3'  
5'-CGGGGTACCTTTACACCGCTTGAGAAGACG-3'  
This work

pcGS5-HA-DMTN  
P. Sauer

pcDNA3.1-HA-ODF2  
(BamHI/XbaI)  
5'-CGGCGATCCATGAAGGACCGCTCTTCACACTC-3'  
5'-CGGCGATCCATGAAGGACCGCTCTTCACACTG-3'  
This work

pcDNA3.1-HA-MCAK  
(BamHI/NotI)  
5'-ATATGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
5'-ATATGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
This work

pcDNA3.1-HA-Kif2A  
(BamHI/NotI)  
5'-TATAGGTATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
5'-TATAGGTATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
This work

pcDNA3.1-HA-Kif2B  
(EcoRI/NotI)  
5'-TATAGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
5'-TATAGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
This work

pcDNA3.1-Flag  
24

cDNA3.1-Flag-Fbxw5  
24

cDNA3.1-Flag-Fbxw5-ΔF-box  
24

pCMV-3xFLAG-Fbxw7  
I. Hoffmann

pET28a-His-MCAK  
(BamHI/NotI)  
5'-ATATGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
5'-TATAGGATCCATGCCAGCAGTGCTCTGGGGAGGAG-3'  
This work

pET28a-His-Kif2A  
(BamHI/NotI)  
Cloned by restriction digestion  
This work

pET28a-His-Kif2B  
Cloned by restriction digestion  
This work
| Destination Vector | Source | Description |
|---------------------|--------|-------------|
| pFastBacHT-B-His-Fbxw5 | (EcoRI/NotI) |  |
| pFastBac1-Skp1ΔΔ |  |  |
| pFastBacHT-B-Eps8 | pCDNA3.1-HA-Eps8<sup>24</sup> |  |
| | 5' - GTATTTTCAGGCGCCATGGATGAATGGTCATATTTCTATTATCATC-3' | This work |
| | 5' - CAGGCTCTAGATTCGAAAGCTTAGTGACTGCTTCTCTTCC-3' |  |
| pFastBacHT-B-MCAK | pcDNA3.1-HA-MCAK (BamHI/NotI) |  |
| | 5' - ATATGGATCCATGGCCATGGACTGTCG-3' | This work |
| | 5' - TATAGCGGCCGCTACTGGGGGCCGTTTCTTG-3' |  |
| pFastBacHT-B-His-Kif2A | pcDNA3.1-HA-Kif2A (BamHI/NotI) | Cloned by restriction digestion |
| pEGFP-PLK1-T210D |  | S. Lev |
| pFastBacHT-B-His-Plk1-T210D | pEGFP-PLK1-T210D (Gibson) |  |
| | 5' - AACCTGTATTTTCAGGCGCAATGAGTGCTGCAGTGACTG-3' | This work |
| | 5' - CAGGCTCTAGATTCGAAAGCTTAGGAGGCCCTTGAGACG-3' |  |
| pcDNA3.1-Flag-Fbxl2 |  | L. Hengst |
| pFastBacHT-B-His-Fbxl2 | pcDNA3.1-Flag-Fbxl2 |  |
| | 5' - GTATTTTCAGGCGCCATGGCTTTTCTCAAAACATGATGAGG-3' | This work |
| | 5' - CTCTAGATTCGAAAGCGGCCCTCAGAGAATGACACACAC-3' |  |
| 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' | This work |
| | 5' - TGACTCGCTGGCGCGTGCTGGCTGGCTGGCGCGAGCG-3' |  |
| | 5' - CGACCCGAGCGCAAGG-3' |  |
| | 5' - ATGGGCTTTGTAGGGCGACG-3' |  |
| | 5' - TGCTCGCCTACAAGGCCATGG-3' |  |
| | 5' - GTCCGGGGGTCACCAGCAAGCAGTGATGCTTAC-3' |  |
| | 5' - CTGATAGAGTTACCCCGGAC-3' |  |
| | 5' - GTTGGTGCCATGTGATCCTGTATTTATAG-3' |  |
| pBIG1B-HisCul1/mmRbx1 | cDNA of RPE-1 (Gibson) | This work |
| | destination vector: pBigBac-His-Cul4A/His-DDB1/mmRbx1 cut with Sall/PvuII |  |
| | 5' - CCACTCATCATCATCATCCTCCGCCTGCAGAGAACCCTCTACACTTCGAGGCGCGCGCGCTGCTCAACCAGCGAGCAG-3' |  |
| | 5' - GGTTTCTACTGAAATTTCAGTTAAGCCCAAGTAACTGAGGGTGTCG-3' | This work |
| pRetroX-TRE3G-mNG-MCAK |  | S. Hata |
| Vector Description                                      | Sequence                                                                 | Reference/Note                  |
|---------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------|
| 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'-ACTCACTATAAGGAGACCCAATGGTAGCAAGGGCGAGGG-3' 5'-GGTGCCGAGAGTCGTCTCCCTTCAGGAGGAGGGG-3' | This work                       |
| pRetroX-TRE3G-mNG-Kif2B                                  | pcDNA3.1-HA-Kif2B (Gibson) destination vector: pRetro-TRE3G-mNG-MCAK cut with EcoRI/NotI. 5'-GTGCAGGAGCTGGCAGGCATGGCCAGCCAGTTCTGC-3' 5'-GGCGCCCTCCCCTACCCGGTAGTCACTCTACCTTGCTCCTACG-3' | This work                       |
| pQCXlZ-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'-GGCGACGGATGCGCCATGGCCATGGCACTCGTGCC-3' 5'-GGCGGTTTAAAACCTTGGGGCCGTTCTTGCTGCTG-3' | This work                       |
| pFN31K-NLuc-MCAK                                        | pcDNA3.1-HA-MCAK 5'-GGCGACGGATGCGCCATGGCCATGGCACTCGTGCC-3' 5'-GGCGGTTTAAAACCTTGGGGCCGTTCTTGCTGCTG-3' | This work                       |
### Antibodies

| Antibody                          | Species | Source           | Identifier       |
|-----------------------------------|---------|------------------|------------------|
| 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-y-Tubulin         | Mouse   | Sigma Aldrich    | T6557            |
| Monoclonal anti-HGS               | Rabbit  | Cell Signaling Technology | 15087          |
| Monoclonal anti-GSTP1             | Mouse   | Cell Signaling Technology | 3369          |
| Monoclonal anti-β-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-CSRP2             | Rabbit  | Proteintech      | 10892-2-AP       |
| Polyclonal anti-Sec23b            | Rabbit  | Sigma Aldrich    | HPA008216        |
| Polyclonal anti-ODF2              | Rabbit  | Proteintech      | 12058-1-AP       |
| Polyclonal anti-Fbxw5             | Rabbit  | n/a              | n/a              |
| Polyclonal anti-RanGAP1           | Goat    | n/a              | n/a              |
| Polyclonal anti-Uba2              | Goat    | n/a              | 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           |

### Chemicals, reagents, enzymes & Media

| Name                               | Source   | Identifier       |
|------------------------------------|----------|------------------|
| 0.45 µm pore size polyvinylidene difluoride membranes (PVDF) | Amersham | 106000021/10600023 |
| aprotinin                          | Roth     | A162.3           |
| Item                                                      | Supplier                        | Catalog Number  |
|----------------------------------------------------------|---------------------------------|-----------------|
| 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)                                   | Pepsan 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          |
| Lipojectamin 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

| Name                  | Source               | Catalog Number |
|-----------------------|----------------------|----------------|
| RO-3306               | Sigma Aldrich        | SLM0569        |
| Rotipholase® 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     |

## E. coli strains

- **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)

### Recombinant proteins (expressed and purified from E.coli)

| Name                  | Tag                     | Expression plasmid | Source       |
|-----------------------|-------------------------|--------------------|--------------|
| APPBP1-Uba3           | Cleaved                 | pGEX-GST-APPBP1-rbs-Uba3 | This work    |
| Cdc34                 | Cleaved                 | pGEX6P3-GST-Cdc34  | This work    |
| Klf2a                 | 6xHis                   | pET28a-His-Klf2A   | This work    |
| Klf2b                 | 6xHis                   | pET28a-His-Klf2B   | 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 (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    |
| Name                        | Tag         | Expression plasmid                              | Source            |
|-----------------------------|-------------|------------------------------------------------|-------------------|
| Cul1/Rbx1 complex           | TEV cleaved | pBIG1B-HisCul1/mmRbx1                           | This work         |
| Cu4A/DDB1/Rbx1 complex      | TEV cleaved | pBIG1B-His-Cu4A/His-DDB1/His-mmRbx1             | This work         |
| Eps8                        | 6xHis       | pFastBacHT-B-Eps8                               | This work         |
| Fbxl2/Skp1ΔΔ subcomplex     | 6xHis       | pFastBacHT-B-His-Fbxl2                           | This work         |
| Fbxw5/Skp1ΔΔ subcomplex     | 6xHis       | pFastBacHT-B-His-Fbxw5                           | This work         |
| Fbxw7/Skp1ΔΔ subcomplex     | 6xHis       | pFastBacHT-B-His-Fbxw7                           | 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         |

### siRNAs

| Target                  | Sequence                  | Source                  | Reference |
|-------------------------|---------------------------|-------------------------|-----------|
| non-targeting (NT)      | 5'-UAGCGACUAACACAUCAUUAdTdT-3' | Ambion/Qiagen          | 24        |
| Fbxw5#1                 | 5'-CCACAGGGCGCAAGAGCAAdTdT-3' | Ambion / Sigma Aldrich  | 24        |
| Fbxw5#2                 | 5'-CGGGAGAGGGAGGAUGCGUdTdT-3' | Ambion                  | 24        |
| Fbxw5#3                 | 5'-CCCUACACUGGAGCUACAdTdT-3' | Dharmacon / Sigma Aldrich | 24        |
| MCAK                    | 5'-GAUCCAACGGCAUAAUGGUdTdT-3' | Sigma Aldrich           | 61        |
| Kif2a                   | 5'-GGAUUGGCAUCCUGUGAA-3'   | Sigma Aldrich           | 83        |
| Kif2b                   | 5'-GGACCUGGAUCAUCACCC-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.