

APPENDIX

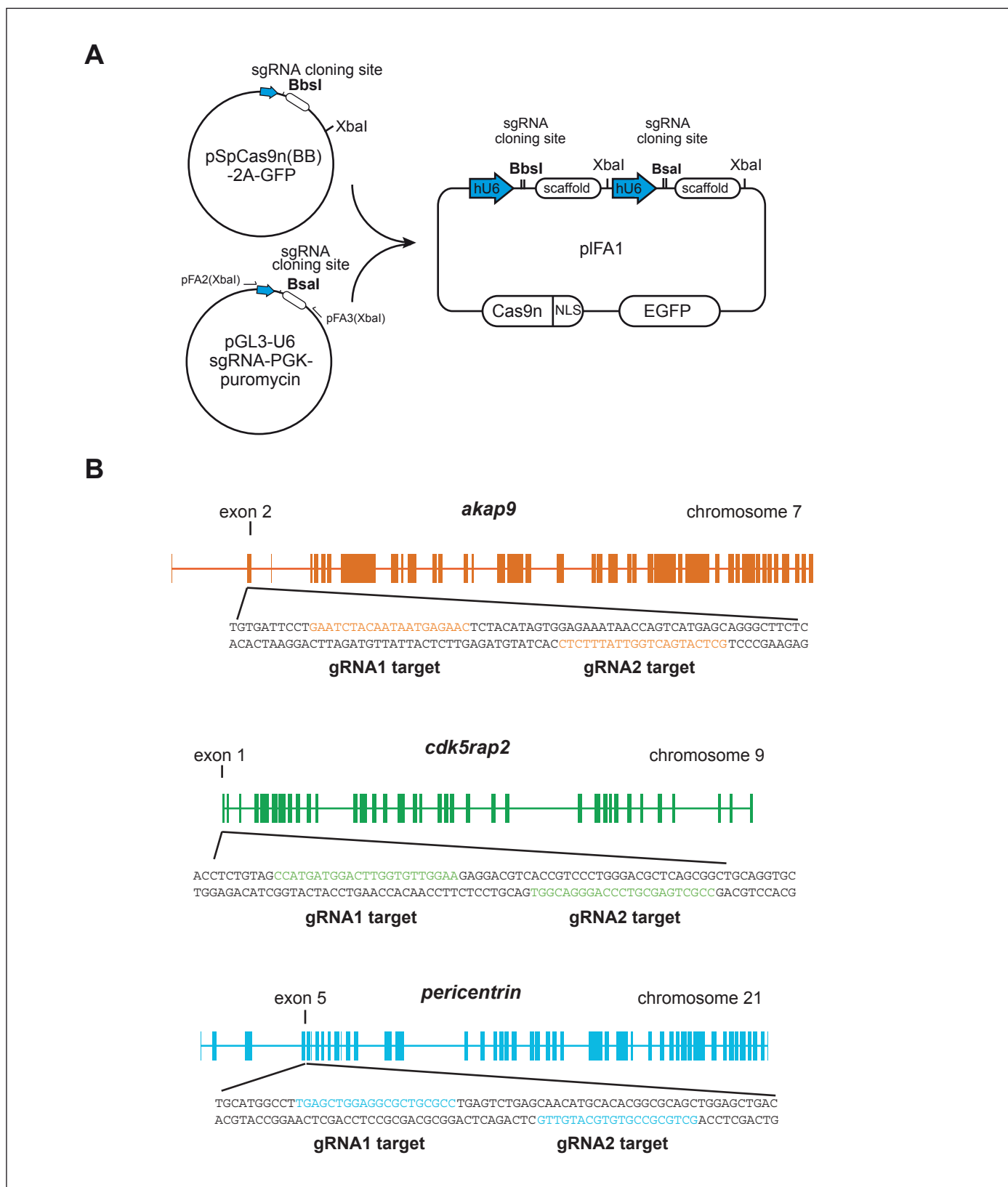
THE DUAL ROLE OF THE CENTROSOME IN ORGANIZING THE MICROTUBULE NETWORK IN INTERPHASE

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Appendix Figure S1



Appendix Figure S1. Generation of *akap9*, *cdk5rap2* and *pcnt* knock-out cell lines, related to Figure 1. (A) Strategy followed to generate the pIFA1 plasmid. A fragment of pGL3-U6-sgRNA-PGK-puromycin plasmid containing the hU6 promoter, Bsal cloning sites for a sgRNA, and a scaffold RNA coding sequence was PCR amplified and cloned into the XbaI site of pSpCas9n(BB)-2A-GFP. pFA2 and pFA3 represent the primers used to amplify this region by PCR (see Materials and Methods for details). (B) Exon composition of *akap450*, *cdk5rap2* and *pcnt* genes. Genomic regions targeted by sgRNAs used for Crispr/Cas9 mutagenesis of *akap450* (top), *cdk5rap2* (middle) and *PCNT* (bottom) are indicated.

Appendix Figure S2

A

AKAP450-wt

1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Arg Thr Leu - 87
88 - His Ser Gly Glu Ile Thr Ser His Glu Gln Gly Phe Ser Val Glu Leu Glu Ser Glu Ile ...

AKAP450-KO Clon 1

Allele A 1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Ser Arg Ala - 87
88 - Ser Leu Trp Asn Trp Lys Val Lys Phe Gln Pro Gln Gln Met Thr Ala Val Gln Arg End

Allele B 1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Arg Thr Leu - 87
88 - Val Tyr Cys End

B

CDK5RAP2-wt

1 - Met Met Asp Leu Val Leu Glu Glu Asp Val Thr Val Pro Gly Thr Leu Ser Gly Cys Ser Gly Leu Val Pro Ser Val Pro Asp Asp - 29
30 - Leu Asp Gly Ile Asn Pro Asn Ala Gly Leu Gly Asn Gly Leu Leu Pro Asn Val Ser Glu Glu Thr Val Ser Pro Thr Arg Ala Arg - 58
59 - Asn Met Lys Asp Phe Glu Asn Gln Ile Thr ...

CDK5RAP2-KO Clon 1

Allele A The mutation deleted the start codon. No putative protein.

Allele B 1 - Met Met Asp Leu Val Leu Asp Leu Val Leu Lys Gly Val Gly Leu Gly Val Gly Arg Gly Arg His Arg Pro Trp Asp Ala Gln Arg - 29
30 - Leu Gln Trp Pro Cys Ser Gln Cys Thr Arg End

C

PCNT-wt

204 - Pro Ala Glu Gln Arg Gly Met Phe Thr Lys Glu Cys Glu Gln Glu Cys Glu Leu Ala Ile Thr Asp Leu Glu Ser Gly Arg Glu Asp - 232
233 - Glu Ala Gly Leu His Gln Ser Gln Ala Val His Gly Leu Glu Leu Glu Ala Leu Arg Leu Ser Leu Ser Asn Met His Thr Ala Gln - 261
262 - Leu Glu Leu Thr Gln Ala Asn Leu Gln Lys Glu Lys Glu Thr Ala Leu Thr Glu Leu Arg Glu Met Leu Asn Ser Arg Arg Ala Gln - 290
291 - Glu Leu Ala Leu Leu Gln Ser Arg Gln Gln His Glu Leu Glu Leu Leu Arg Glu Gln His Ala Arg Glu Lys Glu Glu Val Val Leu - 319
320 - Arg Cys Gly Gln Glu Ala Ala Glu Leu Lys Glu Lys Leu Gln Ser Glu Met Glu Lys Asn Ala Gln Ile Val Lys Thr Leu Lys ... - 348

PCNT-KO Clon 1

Allele A 204 - ... Ala Glu Gln Arg Gly Met Phe Thr Lys Glu Cys Glu Gln Glu Cys Glu Leu Ala Ile Thr Asp Leu Glu Ser Gly Arg Glu Asp - 232
233 - Glu Ala Gly Leu His Gln Ser Gln Ala Val His Gly Leu Glu Leu Glu Ala Leu Arg Leu Ser Leu Gln His Ala His Gly Ala Ala - 261
262 - Gly Ala Asp Thr Gly Gln Pro Pro Glu Gly Glu Gly Asp Gly Ile Asp Gly Ala Ala Gly Asp Ala Gln Gln Pro Ala Cys Pro Gly - 290
291 - Ala Gly Pro Ala Thr Glu Gln Ala Ala Arg Ala Gly Ala Pro Gln Gly Ala Ala Arg Thr Gly Glu Gly Gly Gly Ala Gln - 319
320 - Val Trp Thr Gly Ser Ser End - 348

Allele B 204 - ... Ala Glu Gln Arg Gly Met Phe Thr Lys Glu Cys Glu Gln Glu Cys Glu Leu Ala Ile Thr Asp Leu Glu Ser Gly Arg Glu Asp - 232
233 - Glu Ala Gly Leu His Gln Ser Gln Ala Val His Gly Leu Glu Leu Glu Ala Leu Arg Leu Ser Leu Ser Asn Met His Thr Ala Arg - 261
262 - Trp Arg Arg Cys Ala End - 290

D

PCNT-KO AKAP450-KO Clon 1

AKAP450 aa sequence

Allele A 1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Arg Thr Leu - 87
88 - Leu Met Ser Arg Ala Ser Leu Trp Asn Trp Lys Val Lys Phe Gln Pro Gln Gln Met Thr Ala Val Gln Arg End ...

Allele B A deletion larger than 500 nucleotides covering most of exon 2.

E

PCNT-KO CDK5RAP2-KO Clon 1

CDK5RAP2 aa sequence

Allele A The mutation deleted the start codon. No putative protein.

Allele B The mutation deleted the start codon. No putative protein.

F

AKAP450-KO Clon 2

Allele A 1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Arg Thr Leu - 87
88 - Leu Tyr Asn Asn Glu Asn Ser Thr End

Allele B 1 - Met Glu Asp Glu Glu Arg Gln Lys Lys Leu Glu Ala Gly Lys Ala Lys Leu Ala Gln Phe Arg Gln Arg Lys Ala Gln Ser Asp Gly - 29
30 - Gln Ser Pro Ser Lys Lys Gln Lys Lys Lys Arg Lys Thr Ser Ser Ser Lys His Asp Val Ser Ala His His Asp Leu Asn Ile Asp - 58
59 - Gln Ser Gln Cys Asn Glu Met Tyr Ile Asn Ser Ser Gln Arg Val Glu Ser Thr Val Ile Pro Glu Ser Thr Ile Met Arg Thr Leu - 87
88 - His Ile Thr End

Appendix Figure S2 (cont.)

G

CDK5RAP2-KO Clon 2

Allele A 1 - Met Met Asp Leu Val Leu Glu Glu Asp Trp Thr Trp Cys Trp Lys Arg Thr Ser Pro Ser Leu Gly Arg Ser Ala Ala Ala Val Ala - 29
 30 - Leu Phe Pro Val Tyr Gln Met Thr Trp Met Ala Ser Thr Pro Met Leu Gly Trp Glu Met Val Cys Ser Gln Met Cys Gln Lys Lys - 58
 59 - Gln Cys Leu Pro Pro Glu His Gly Thr End

Allele B 1 - Met Met Asp Leu Val Leu Asp Leu Val Leu Val Gly Leu Gly Val Gly Arg Asp Leu Val Leu Val Gly Leu Gly Val Gly Arg Gly - 29
 30 - Arg His Arg Pro Trp Asp Ala Gln Arg Leu Gln Trp Pro Cys Ser Gln Cys Thr Arg End

H

PCNT-KO Clon 2

Allele A 204 - ... Ala Glu Gln Arg Gly Met Phe Thr Lys Glu Cys Glu Gln Glu Cys Glu Leu Ala Ile Thr Asp Leu Glu Ser Gly Arg Glu Asp - 232
 233 - Glu Ala Gly Leu His Gln Ser Gln Ala Val His Gly Leu Glu Leu Ala His Gly Ala Ala Gly Ala Asp Thr Gly Gln Pro Pro Glu - 261
 262 - Gly Glu Gly Asp Gly Ile Asp Gly Ala Ala Gly Asp Ala Gln Gln Pro Ala Cys Pro Gly Ala Gly Pro Ala Thr Glu Gln Ala Ala - 290
 291 - Ala Arg Ala Gly Ala Pro Arg Gly Ala Ala Arg Thr Gly Glu Gly Gly Gly Ala Gln Val Trp Thr Gly Ser Ser End - 319

Allele B 204 - ... Ala Glu Gln Arg Gly Met Phe Thr Lys Glu Cys Glu Gln Glu Cys Glu Leu Ala Ile Thr Asp Leu Glu Ser Gly Arg Glu Asp - 232
 233 - Glu Ala Gly Leu His Gln Ser Gln Ala Val His Gly Leu Glu Leu Glu Ala Leu Arg Leu Ser Leu Ser Val Cys Ala End - 261

Appendix Figure S2. Predicted amino acid sequence of putative AKAP450, CDK5Rap2 and PCNT truncated forms in Crispr/Cas9 mutant cell lines, related to Figure 1.

(A-C) Predicted AKAP450 (A), CDK5Rap2 (B) and PCNT (C) protein sequences expressed (if any) by the selected clones used in this work as inferred from the DNA sequence of mutated regions. Wild-type protein sequences are also shown for comparison. Text in red indicates the altered fragment of the protein expressed until the first in-frame stop codon. Allele A of CDK5Rap2 in c5rap2 KO Clone 1 and both alleles of CDK5Rap2 in pc-c5-2KO clone 1 contain a deletion affecting the start codon of exon 1. Other alleles contained either DNA insertions or deletions leading to the appearance of premature stop codons. (D-E) Putative AKAP450 (D) and CDK5Rap2 (E) protein sequences in pc-ak-2KO and pc-c5-2KO cell lines. In both cases, double KO cell lines were generated based on pcnt KO cells. (F-H) Same as in (A), (B) and (C) but for the second knock-out clone of each protein.