Supplemental Material

- **Supplementary Figure 1**: Isoforms of Cep152
- **Supplementary Figure 2**: Isoforms of Cep192 – identification of N-terminal extension

(Supplementary Figures 1 and 2 provide analyses of potential Cep152 and Cep192 isoforms including their expression in selected human cell lines.)

- **Supplementary Figure 3**: Cell cycle-dependent centrosome localization of Cep152 and Cep192.

(Supplementary Figure 3 shows a side-by-side comparison of cell cycle-dependent localizations and cellular protein levels of Cep152 and Cep192, related to Figure 1.)

- **Supplementary Figure 4**: Dependency of centriolar localization of Cep63 on Cep152 and Cep192.

(Supplementary Figure 4 provides another example for the dependency of centriolar proteins on Cep152 and Cep192.)

- **Supplementary Figure 5**: Cep152 and Cep192 co-operate to recruit Plk4 to centrosomes.

(In Supplementary Figure 5 Pericentrin depletion is shown as a negative control for the role of Cep192 and Cep152 in the centrosome recruitment of Plk4. In addition co-operation of Cep192 and Cep152 in centriolar recruitment of newly synthesized Plk4 is illustrated.)

- **Supplementary Figure 6:** Cep152 and Cep192 interact with Plk4 through similar acidic patches within their Plk4-binding domains.

(Supplementary Figure 6 shows the mapping of the Plk4-binding domain within Cep152 as well as alignments of Plk4-binding domains shared between Cep152 and Cep192 homologs, illustrating evolutionary conservation of acidic stretches.)

Supplementary Figure 1

Isoforms of Cep152.

A. Four potential Cep152 isoforms, termed Cep152-1 to Cep152-4, are generated by alternative splicing. They are illustrated schematically, together with their predicted molecular weights (MW) and lengths in amino acids (AA). Based on the presence of an extended C-terminus long (Cep152-1 and/or -2) and short forms of Cep152 (Cep152-3 and/or -4) can be distinguished. Two antibodies, Cep152-N and Cep152-C, were raised against the indicated regions. Databases provide full length transcript support for human Cep152-1, Cep152-2 and Cep152-3 (BX648822.1, BC117182.1, AB020719.1); using primers specific for the long Cep152 isoforms Cep152-2 was amplified from a HeLa cDNA library (data not shown).

B. HEK293T cells were transfected with the indicated plasmids and lysates were analysed by SDS PAGE and Western Blotting (WB), using antibodies against Flag, Cep152-N, Cep152-C and α-Tubulin. Note that Cep152-C recognizes only the isoform Cep152-2, whereas Cep152-N recognizes both Cep152-2 and Cep152-4. The asterisk denotes a non-specific band.

C. HEK293T cells were transfected with the indicated plasmids for 18 h. After lysis immunoprecipitations were performed using Cep152-N and Cep152-C antibodies and rabbit IgG as control. Immunoprecipitates were analyzed by Western Blotting (WB) using the indicated antibodies. Note that Cep152-N immunoprecipitates all versions of Cep152 analyzed, whereas Cep152-C precipitates only the version with the extended C terminus, i.e. full length Cep152-2.

D. HeLa S3 and U2OS were transfected with the indicated siRNA oligonucleotides for 48 h. After lysis the samples were analyzed by Western Blotting (WB) using the indicated antibodies.

Supplementary Figure 2

Isoforms of Cep192: identification of N-terminal extension.

A. Four Cep192 isoforms generated by alternative splicing are potentially expressed in human cells. These were termed Cep192-1 to Cep192-4. The isoforms together with their predicted molecular weights (MW) and lengths in amino acids (AA) are illustrated schematically. Also shown is the region against which the Cep192 antibody was generated. Green colouring illustrates alternative translation start sites.

B. HeLa S3 and U2OS cells were transfected with the indicated siRNA oligonucleotides for 72 h. After lysis the samples were analysed by Western Blotting (WB). α-Tubulin was analysed for loading control. Note that a prominent band above 250 kDa was strongly diminished upon depletion of Cep192 from both cell lysates.

Supplementary Figure 3

Cell cycle-dependent centrosome localization of Cep152 and Cep192.

A. U2OS cells were fixed and stained with the indicated antibodies. DAPI was used to visualize DNA. Cells harbouring centrioles before and after duplication were identified based on the number of CP110 dots. In the upper panel representative images are shown. Scale bar represents 1 μm (5 μm in the overview images to the right). In the lower panel Cep152 levels at Cep164-positive and Cep164-negative centrioles are quantified (15 cells, error bars denote standard deviation).

B. HeLa S3 cells were released from a double thymidine arrest and samples taken every two hours. After lysis the samples were analyzed by Western Blotting using the indicated antibodies.

C,D. U2OS cells were fixed and stained with the indicated antibodies. DNA was stained with DAPI. Different cell cycle stages were identified based on the number of CP110 dots per cell as well as the morphology of DNA staining. Representative images for Cep152 localization (**C**) and Cep192 localization (**D**) are shown. Scale bars represent 1 μm (5 μm in the overview images to the right).

E. U2OS cells were transfected for 48 h with Flag-Cep192-1 or Flag-Cep192-2 plasmids or vector control. After fixation cells were stained with the indicated antibodies and analysed by immunofluorescence microscopy. DAPI was used to visualize DNA. Scale bars represent 1 μm (5 μm in the overviews to the right).

Supplementary Figure 4

Dependency of centriolar localization of Cep63 on Cep152 and Cep192.

To investigate the dependency of Cep63 localization on Cep152 (**A**) and Cep192 (**B**), U2OS cells were transfected with the indicated siRNA oligonucleotides for 72 h. After fixation the cells were stained with the indicated antibodies. PCNA was used identify cells in S phase. Scale bars represent 1 μm (5 μm in the overview images to the right).

Supplementary Figure 5

Cep192 and Cep152 co-operate to recruit Plk4 to centrosomes.

A. Pericentrin depletion does not impair recruitment of Plk4 to centrosomes. U2OS cells were transfected with the indicated siRNA oligonucleotides for 72 h. After fixation the cells were stained with the indicated antibodies and analyzed by immunofluorescence microscopy. Anti-PCNA antibodies were used to visualize cells in S phase. Scale bars 1 μm or 5 μm (overview images).

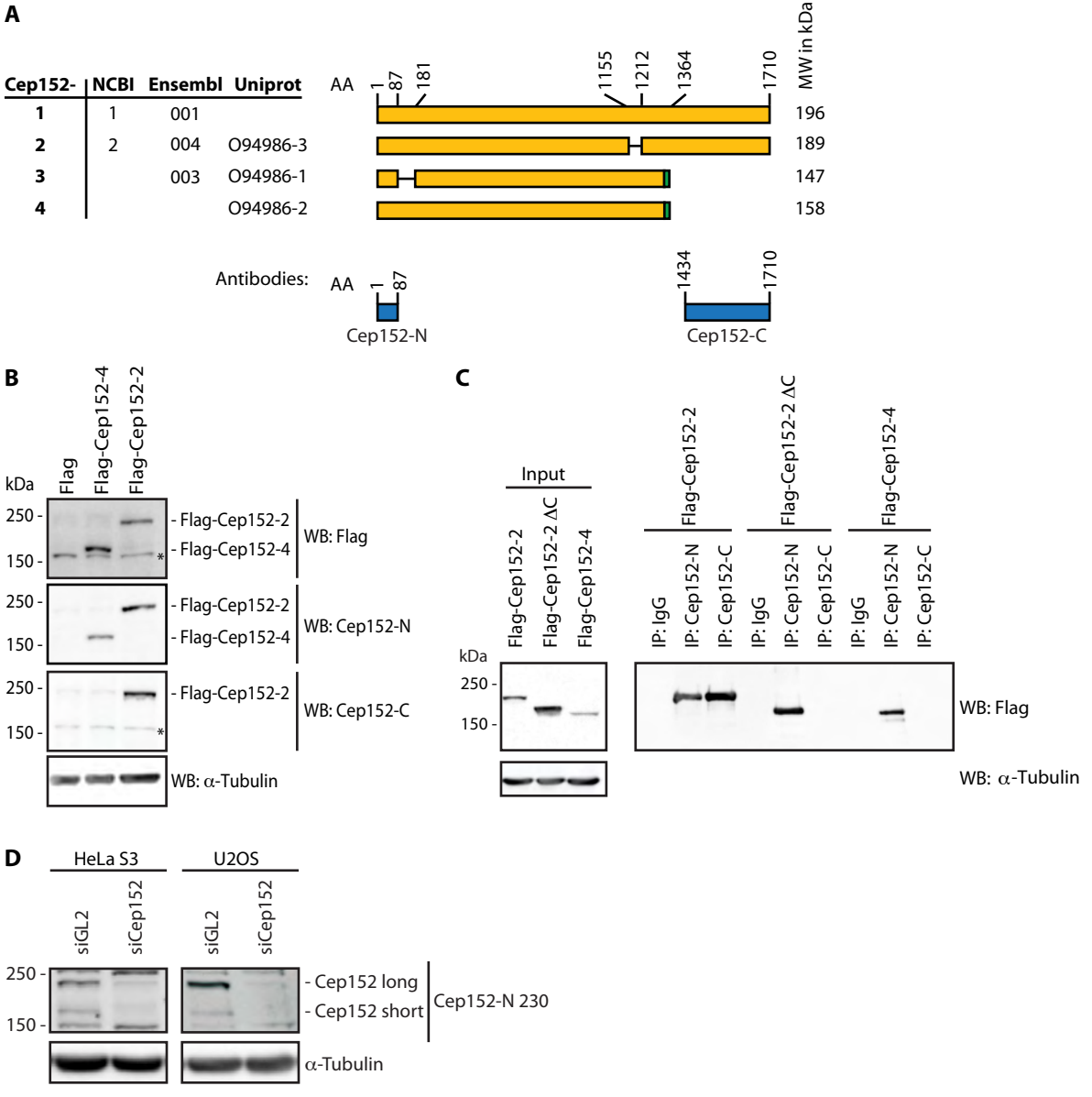
B. Cep152 and Cep192 co-operate in recruitment of over-expressed Plk4 to centrosomes. U2OS:myc-Plk4 cells were transfected with the indicated siRNA oligonucleotides for 48 h and then myc-Plk4 expression was induced for 16 h. After fixation the cells were stained with the indicated antibodies for immunofluorescence microscopy. DNA was stained with DAPI. Fluorescence intensities were not quantified due to considerable variations of Plk4 expression within a cell population. Scale bars represent 1 μm or 5 μm (overview images, right panels).

Supplementary Figure 6

Conserved acidic patches within Cep152 and Cep192 are required for interaction with Plk4.

A. Cep152 residues 1 – 46 are sufficient for interaction with Plk4 (region corresponds to conserved region CR1 identified by Hatch et al. [1]). HEK293T cells were transfected with the indicated plasmids for 18 h. Lysates were subjected to anti-GFP immunoprecipitations and analyzed by Western blotting (WB).

B,C. Alignments of Plk4 interaction domains within Cep192 (**B**) and Cep152 (**C**) homologs from different species. Yellow shading indicates Plk4 binding domains mapped in this study for the human proteins. Amino acid residues are color-coded: red = acidic, blue = basic, green = polar, black = non-polar).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Cep152-
NCBI
Ensembl
Uniprot
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001
2
2
004
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3
003
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4
O94986-2
AA
1
87
181
1155
1212
1364
1710
MW in kDa
196
189
147
158
Antibodies:
AA
1
87
1434
1710
Cep152-N
Cep152-C
B
kDa
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Flag-Cep152-2
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150 -
- Flag-Cep152-2
- Flag-Cep152-4
WB: Flag
WB: Cep152-N
WB: Cep152-C
WB: α-Tubulin
C
Input
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- Cep152 short
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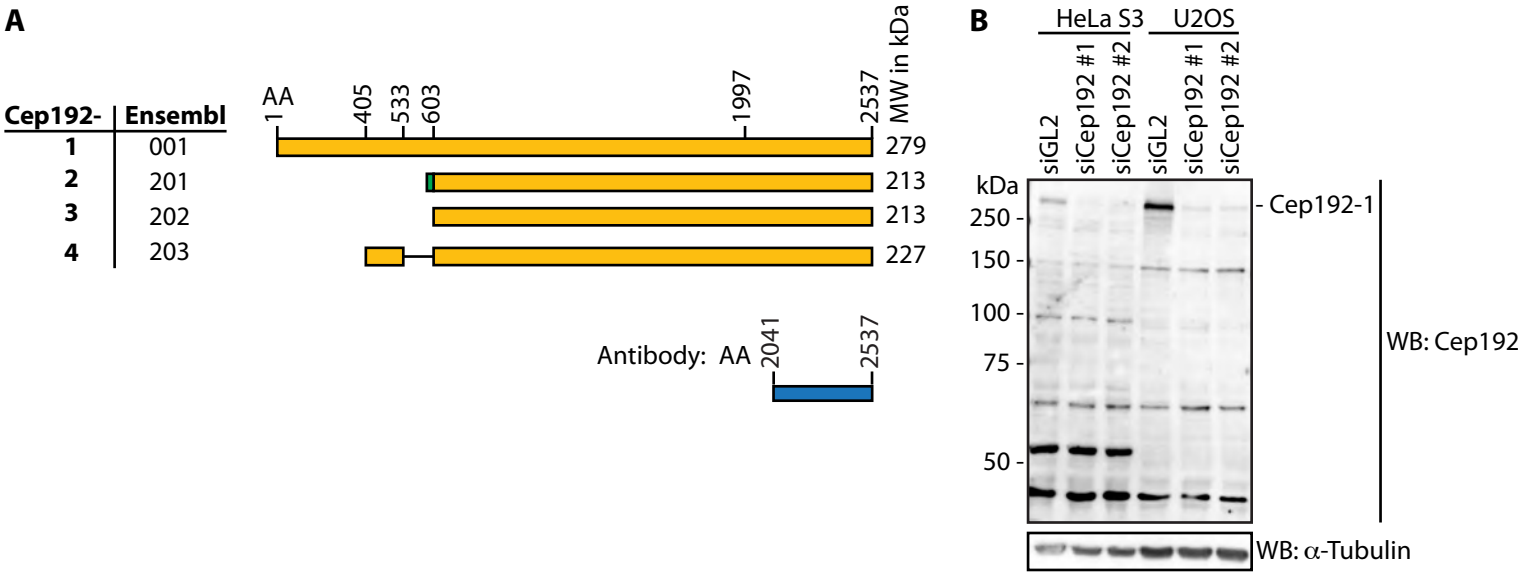
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3
202
4
203
AA
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213
213
227
Antibody: AA
2041
2537
B
HeLa S3
U2OS
siGL2
siCep192 #1
siCep192 #2
siGL2
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Cep192-1
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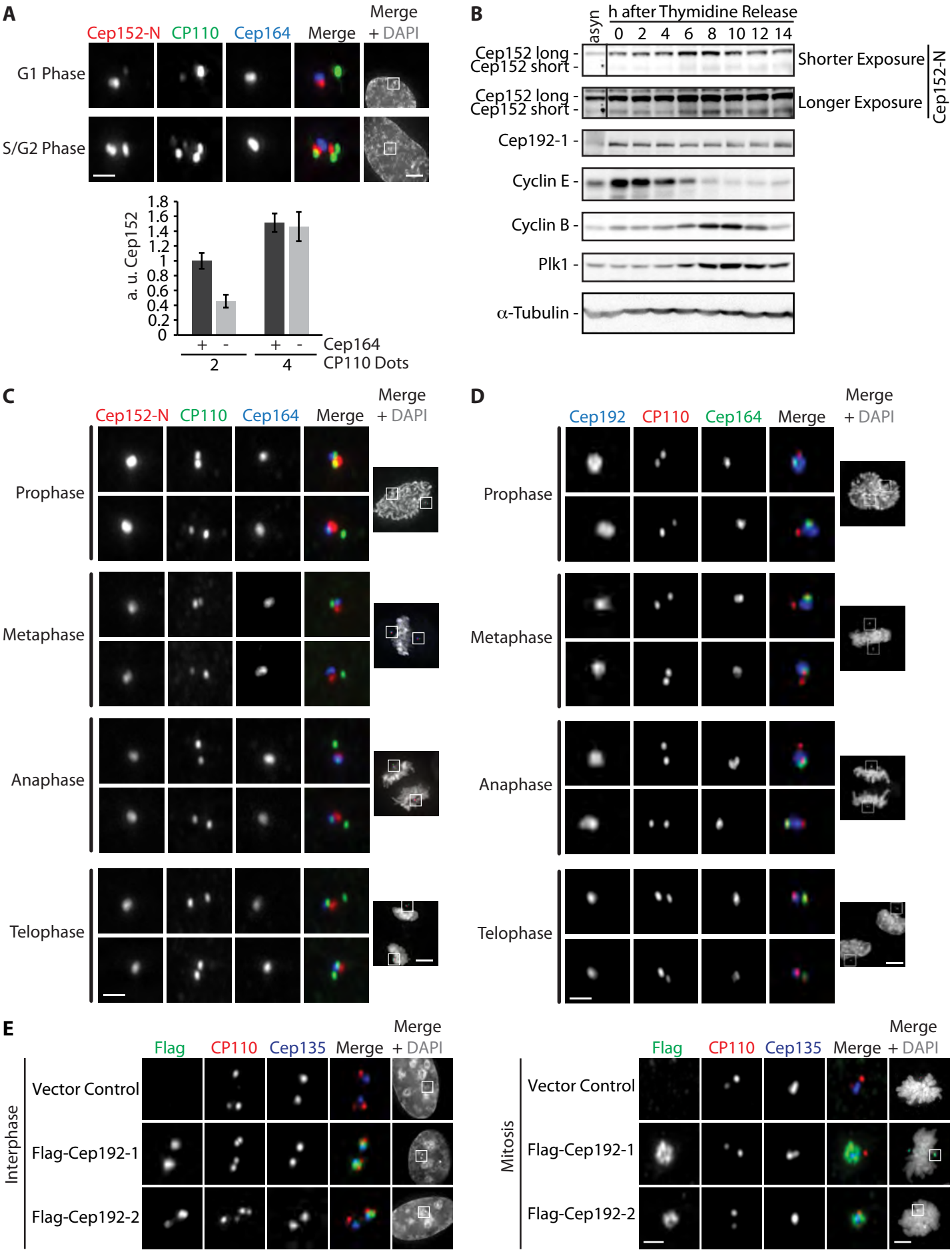
WB: α-Tubulin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Cep152-N
CP110
Cep164
Merge
Merge + DAPI
G1 Phase
S/G2 Phase
a. u. Cep152
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
+ -
+ -
2
4
Cep164
CP110 Dots
B
asyn
h after Thymidine Release
0 2 4 6 8 10 12 14
Cep152 long -
Cep152 short -
Shorter Exposure
Cep152 long -
Cep152 short -
Longer Exposure
Cep152-N
Cep192-1 -
Cyclin E -
Cyclin B -
Plk1 -
α-Tubulin -
C
Cep152-N
CP110
Cep164
Merge
Merge + DAPI
Prophase
Metaphase
Anaphase
Telophase
D
Cep192
CP110
Cep164
Merge
Merge + DAPI
Prophase
Metaphase
Anaphase
Telophase
E
Flag
CP110
Cep135
Merge
Merge + DAPI
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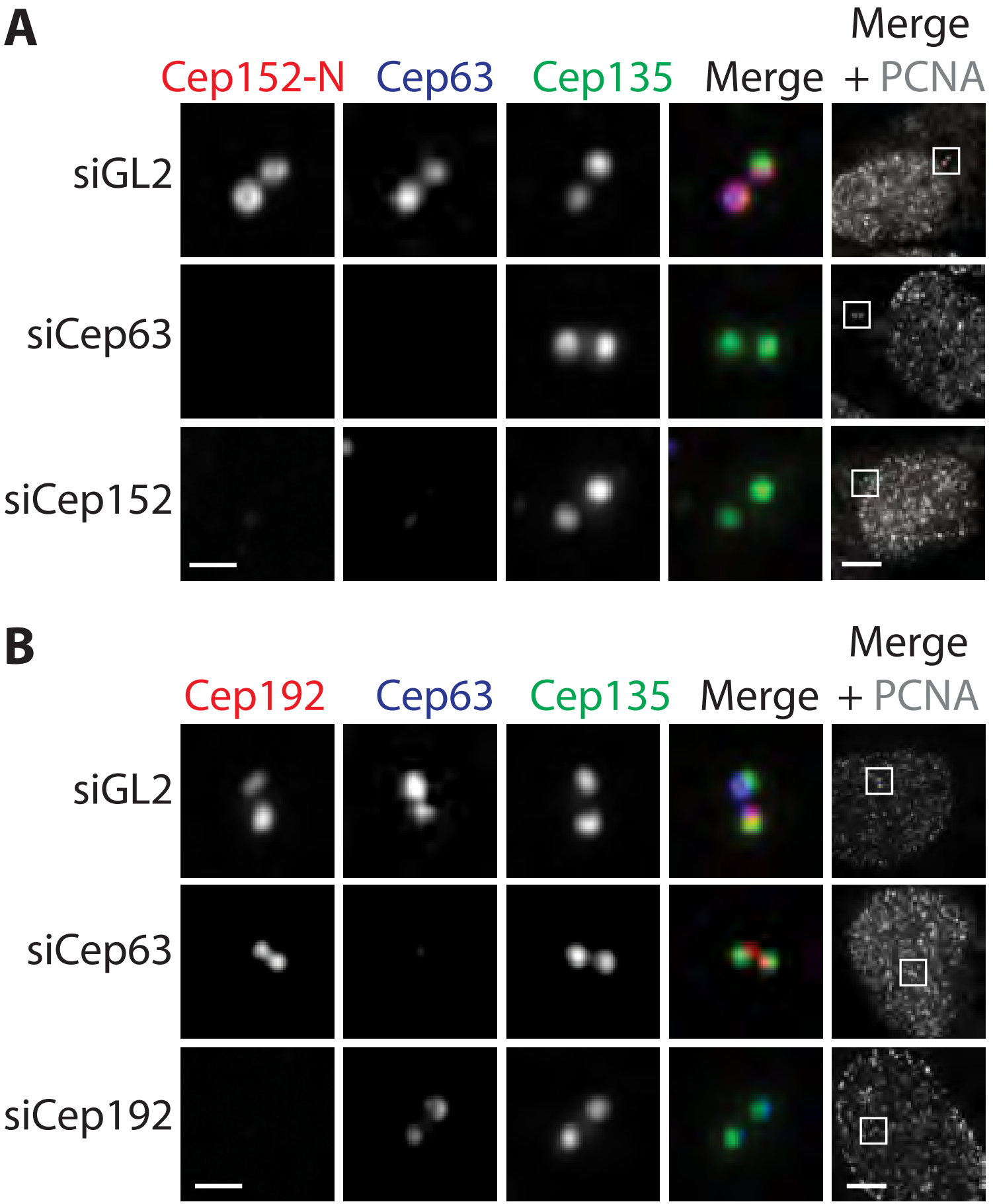
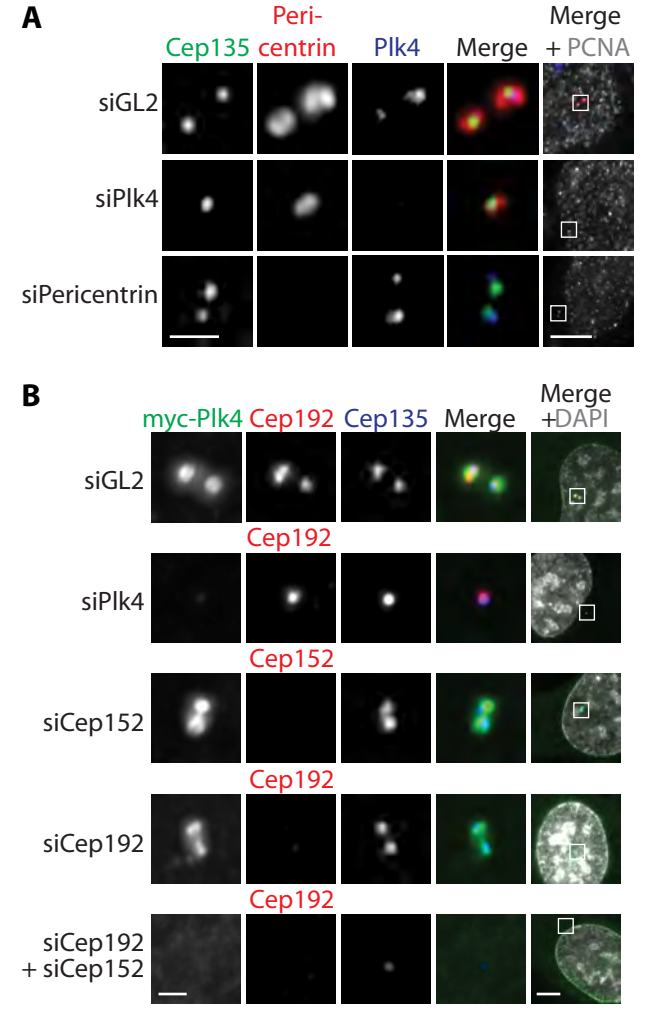
Sonnen_FigS4

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Plk4
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Cep135
Merge
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Cep152
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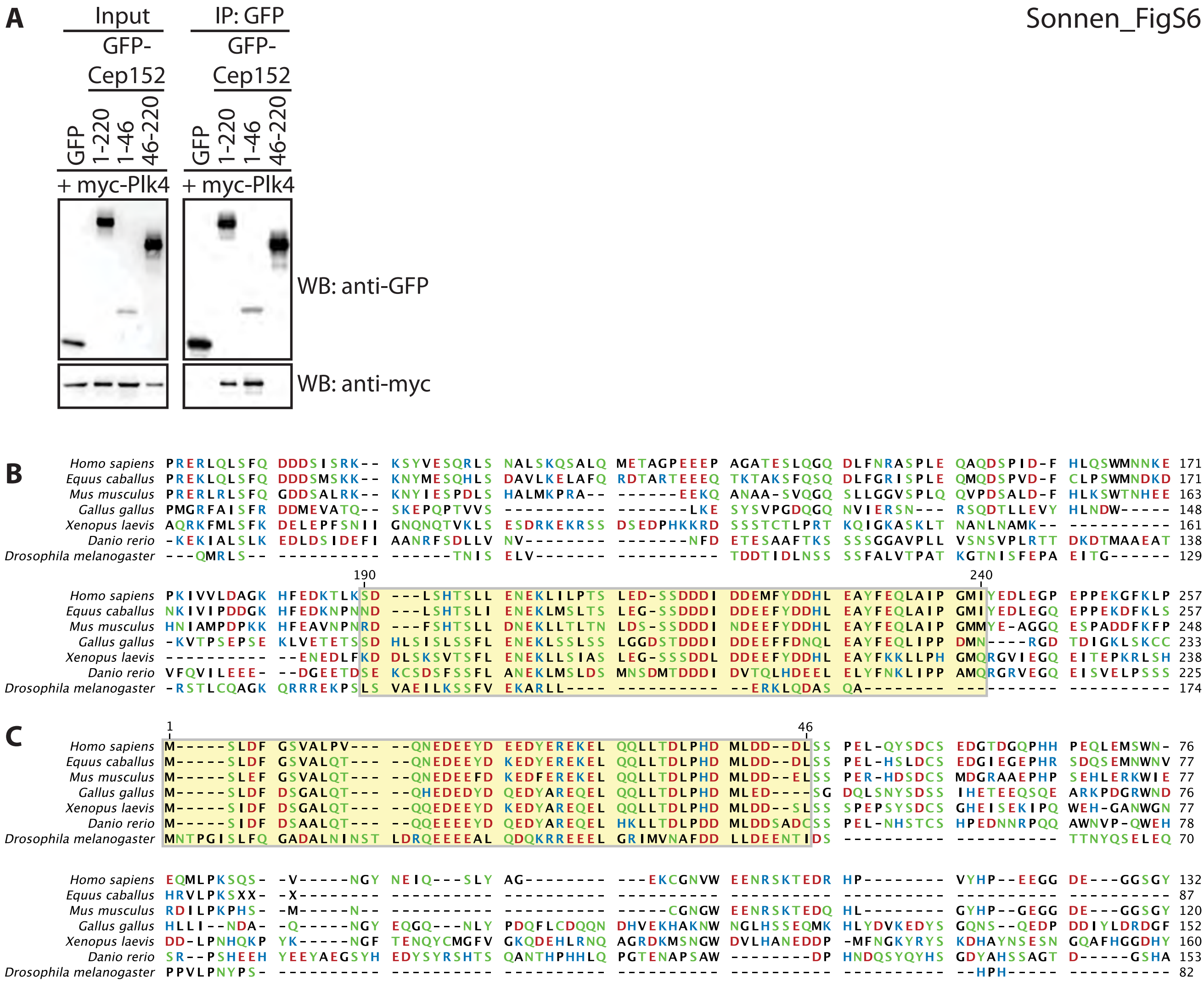
Cep192
siCep192 + siCep152


Sonnen_FigS6
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Cep152
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GFP-
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GFP
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1-46
46-220
+ myc-Plk4
WB: anti-GFP
WB: anti-myc
B
190
240
Homo sapiens
Equus caballus
Mus musculus
Gallus gallus
Xenopus laevis
Danio rerio
Drosophila melanogaster
C
1
46