Supplementary Information

The human microcephaly protein STIL interacts with CPAP and is required for procentriole formation

Chieh-Ju C. Tang[1], Shin-Yi Lin[1], Wen-Bin Hsu[1], Yi-Nan Lin[1], Chien-Ting Wu[1], Yu-Chih Lin[1], Ching-Wen Chang[1], Kuo-Sheng Wu[2], and Tang K. Tang[1,#]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan
[2]Institute of Biochemistry and Molecular Biology, National Yang Ming University, Taipei 11221, Taiwan

Supplementary Information includes supplementary figure legends, 14 supplementary figures and 3 supplementary movies.

Supplementary figure legends

Figure S1. Model showing the role of STIL involved in the early event of procentriole assembly in human cells. See text for detail. For simplicity, only one parent centriole is shown.

Figure S2. Transiently expressed GFP-STIL (A, C, E) or Flag-STIL (B, D) induces centriole amplification in HEK293T (A, B), HeLa (C, D), and bovine aortic endothelial cells (E). Forty-eight hours after transfection, cells were fixed and stained with indicated antibodies. The GFP-STIL proteins were directly observed by confocal fluorescent microscopy. DNA was counterstained with DAPI.

Figure S3. No direct interaction between STIL and hSAS6 was evidenced by a yeast two-hybrid assay. (A, B) Testing the direct interaction of hSAS6-N (bait, residues 1-198, A) and hSAS6-C (bait, residues 172-657, B) with various portions of STIL (prey) using a liquid assay for β-galactosidase activity in a yeast two-hybrid assay. (C, D) Testing the direct interaction of STIL-N (bait, residues 1-634, C) and STIL-C (bait, residues 866-1288, D) with various portions of hSAS6 (prey) using a liquid assay for β-galactosidase activity.

Figure S4. (A) No direct interaction between STIL and hSAS6 was demonstrated by GST pulldown assay. The full-length coding sequences derived from hSAS6 and STIL

were *in vitro* transcribed and translated into each corresponding ^{35}S-methionine-labeled protein as described in Methods. (A-a) Various GST-tagged recombinant proteins used for GST pull-down assays were affinity purified and stained with Coomassie blue (C.B.). The ^{35}S-methionine-labeled proteins were then incubated with GST (negative control), GST-STIL (1-628), GST-STIL (620-1288), or full length GST-hSAS6 on beads and analyzed by SDS-PAGE and autoradiography (A-b). (B) *Drosophila* Ana2 directly interacts with dSAS4, an orthologue of human CPAP. GST-Ana2 was affinity purified (B-a) and incubated with various ^{35}S-Methionine-labeled proteins (dSAS4, dSAS6, and Ana2) and analyzed by SDS-PAGE and autoradiography (B-b).

Figure S5. Verification and mapping of the interaction domains between STIL and CPAP. GST-CPAP-CP1 (residues 1-450), GST-CPAP-CP4-10 (residues 423-1070), and GST-CPAP-CP3 (residues 895-1338) were affinity purified and stained with Coomassie blue (A). HEK 293T cells were transfected with each specific GFP-tagged construct (Plk4, STIL, Cep135, and Cep152). Twenty-four hours after transfection, equal amounts of various GST-CPAP truncated proteins were used to pull down the indicated transfected proteins (B). (C) To map the CPAP-interacting domain in STIL, GST-CPAP-CP3 was used to pull down the interacting STILs prepared from the lysates of various Flag-STIL-transfected cells.

Figure S6. A direct protein-protein interaction between STIL and CPAP was demonstrated by GST pulldown assay (A) and yeast two-hybrid assay (B). (A) The full-length coding sequences derived from Cep135, hSAS6 and STIL were *in vitro* transcribed and translated into each corresponding ^{35}S-methionine-labeled protein as described in Methods. The ^{35}S-methionine-labeled proteins were then incubated with either GST (negative control) or GST-CPAP-CP3 on beads and analyzed by SDS-PAGE and autoradiography. (B) Interaction between CPAP-CP3 (bait) with various portions of STIL (prey) using a liquid assay for β-galactosidase activity in a yeast two-hybrid assay. The interaction between CPAP and STIL was evidenced by the mating colonies growing on QDO plates (SD minimal medium, -Trp, -Leu, -Ade, and -His) and the activation of β-galactosidase activity. p53 (bait) and SV40-large T-Ag (prey) were used as a positive control.

Figure S7. Exogenous GFP-STIL forms a complex with endogenous CPAP and hSAS6 *in vivo*. HEK293T cells were transfected with GFP-STIL construct. Twenty-four hours after transfection, the cell lysates were immunoprecipitated with a non-relevant normal IgG (Mock), anti-hSAS6 (A), anti-CPAP (B), or anti-GFP

antibodies (C) and analyzed by western blotting using indicated antibodies.

Figure S8. Testing the interaction of truncated CPAP-CP3 (wild type) or CPAP-CP3 (E1235V) (bait) with various portions of STIL (prey) using a liquid assay for β-galactosidase activity in a yeast two-hybrid assay. The positive interaction was evidenced by the mating colonies growing on QDO plates (SD minimal medium, -Trp, -Leu, -Ade, and -His) and the activation of β-galactosidase activity. p53 (bait) and SV40-large T-Ag (prey) were used as a positive control.

Figure S9. STIL siRNAs-treated cells analyzed with anti-STIL-441a (A and B) or anti-STIL-442a (C) antibody. U2OS cells were transfected with control siRNA (siControl) or STIL siRNAs (siSTIL-1, siSTIL-2, and siSTIL-3) as described in Figure 5. After transfection, cells were analyzed by immunofluorescence confocal microscopy using indicated antibodies (A and C) or by immunoblotting (B). DNA was counterstained by DAPI. β-actin was used as a loading control (B).

Figure S10. The effects of siSTIL-treated cells in a 6-days follow-up analysis. (A) FACS analysis of siSTIL-treated cells. U2OS cells were transfected with siControl or siSTIL duplexes and analyzed by FACS at different time points. (B) Mitotic index of siControl and siSTIL-treated cells. The mitotic cells, labeled positive by phospho-histone H3 (P-H3) antibody, were counted and plotted on B. Quantification of centrioles (C) and growth curve analysis (D) in siControl and siSTIL-treated cells.

Figure S11. Live mCherry-α-tubulin expressing cells were transfected with either siControl (A) or siSTIL (B). 60 h after transfection, live cell images were taken with a Lecia DMI6000 inverted fluorescence microscope at the indicated times (minutes). In siSTIL-treated experiments, a mitotic cell divides to produce two separate cells (a), a large polyploid cell (b), or three daughter cells (c).

Figure S12. STIL depletion does not perturb the localization of endogenous Plk4 (A) and Cep152 (B) to the centriole. U2OS cells were transfected with siRNAs against STIL. Two days after transfection, cells were treated with aphidicolin for another day, followed by immunofluorescence staining using indicated antibodies (A, B). Quantification of centriolar signals of Plk4 (A, right panel) and Cep152 (B, right panel) in siControl and siSTIL treated cells were shown. Values are means of three experiments with s.d. bars (n = 100 cells).

Figure S13. Plk4 depletion impairs centriolar localization of STIL (A) and inhibits

GFP-STIL-induced centriole amplification (B). (A) U2OS cells were transfected with siRNA duplexes against Plk4 for two days, followed by aphidicolin treatment for 18 hours. After treatment, the cells were collected 6 hours after aphidicolin release and then processed for immunofluorescence staining using indicated antibodies. A quantification of the centriolar signals of STIL in siControl and siPlk4-treated cells is shown in A (right panel). Values are means of three experiments with s.d. bars (*n* = 100 cells). (B) GFP-STIL-inducible cells were transfected with siPlk4 duplexes for 1 day before GFP-STIL expression was induced for another day. These cells were then treated with aphidicolin and processed for immunofluorescence staining as described above. Histogram illustrating as percentages the centriole numbers in siPlk4-treated GFP-STIL-inducible cells (B, right panel). Error bars represent mean ± s.d. of 100 cells from three independent experiments.

Figure S14. GFP-STIL overexpression appears not to affect Plk4 kinase activity. Plk4-myc was cotransfected with GFP or GFP-STIL into HEK293T cells. 24 hours after transfection, Plk4-myc was immunoprecipitated by anti-myc antibody and the immunoprecipitated Plk4 was used to carry out an *in vitro* kinase assay as described in Materials and methods.

Supplementary Movies

Live mCherry-α-tubulin expressing cells were transfected with siSTIL. Sixty hours after transfection, live cell images were taken using a Lecia DMI6000 inverted fluorescence microscope at the indicated times (minutes). A siSTIL-treated mitotic cell divides to produce two separate cells (Supplementary Movie S1), a large polyploid cell (Supplementary Movie S2), or three daughter cells (Supplementary Movie S3).

Fig. S1

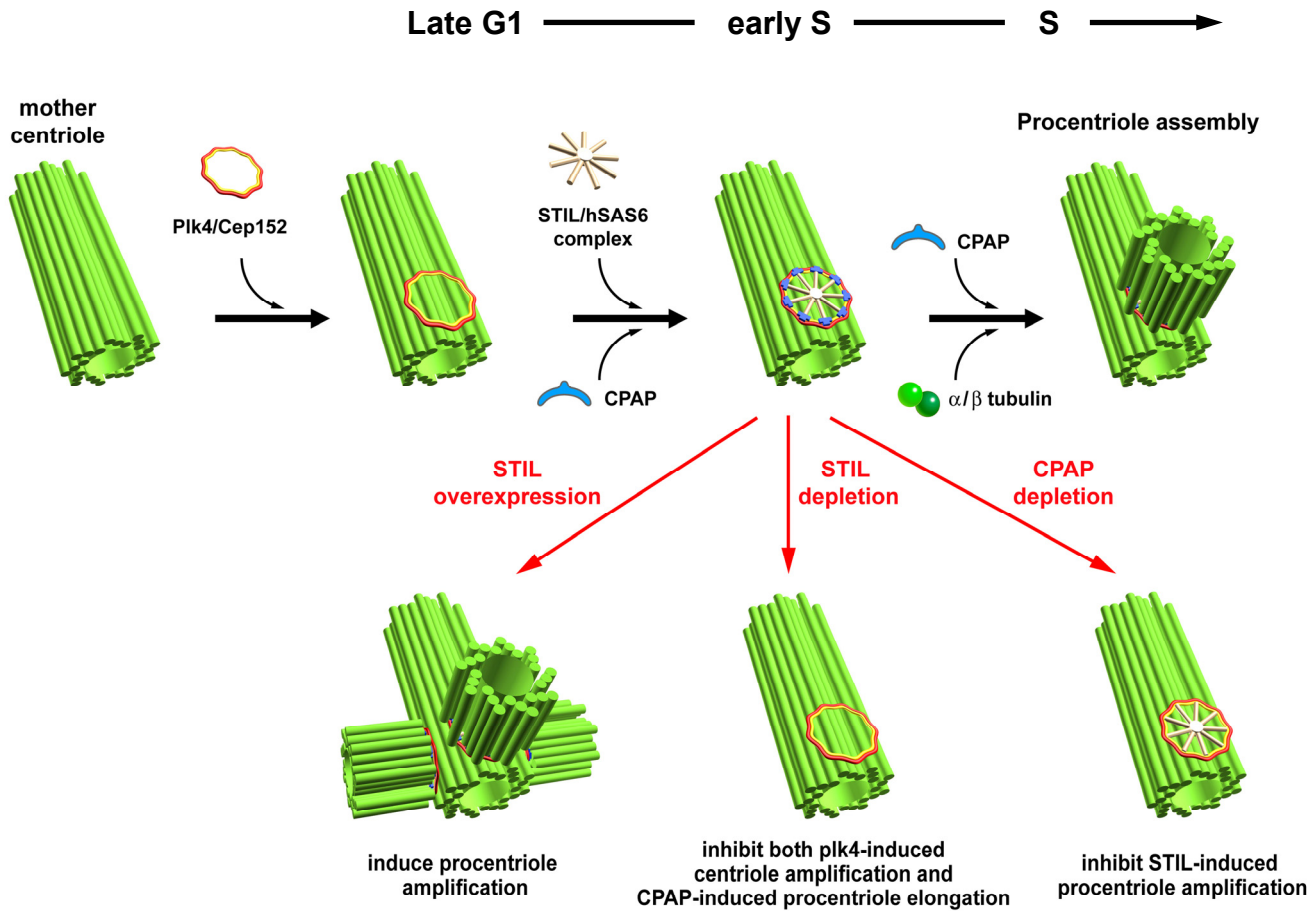

Late G1
early S
S
mother centriole
Plk4/Cep152
STIL/hSAS6 complex
CPAP
CPAP
α/β tubulin
Procentriole assembly
STIL overexpression
STIL depletion
CPAP depletion
induce procentriole amplification
inhibit both plk4-induced centriole amplification and CPAP-induced procentriole elongation
inhibit STIL-induced procentriole amplification

Fig. S2

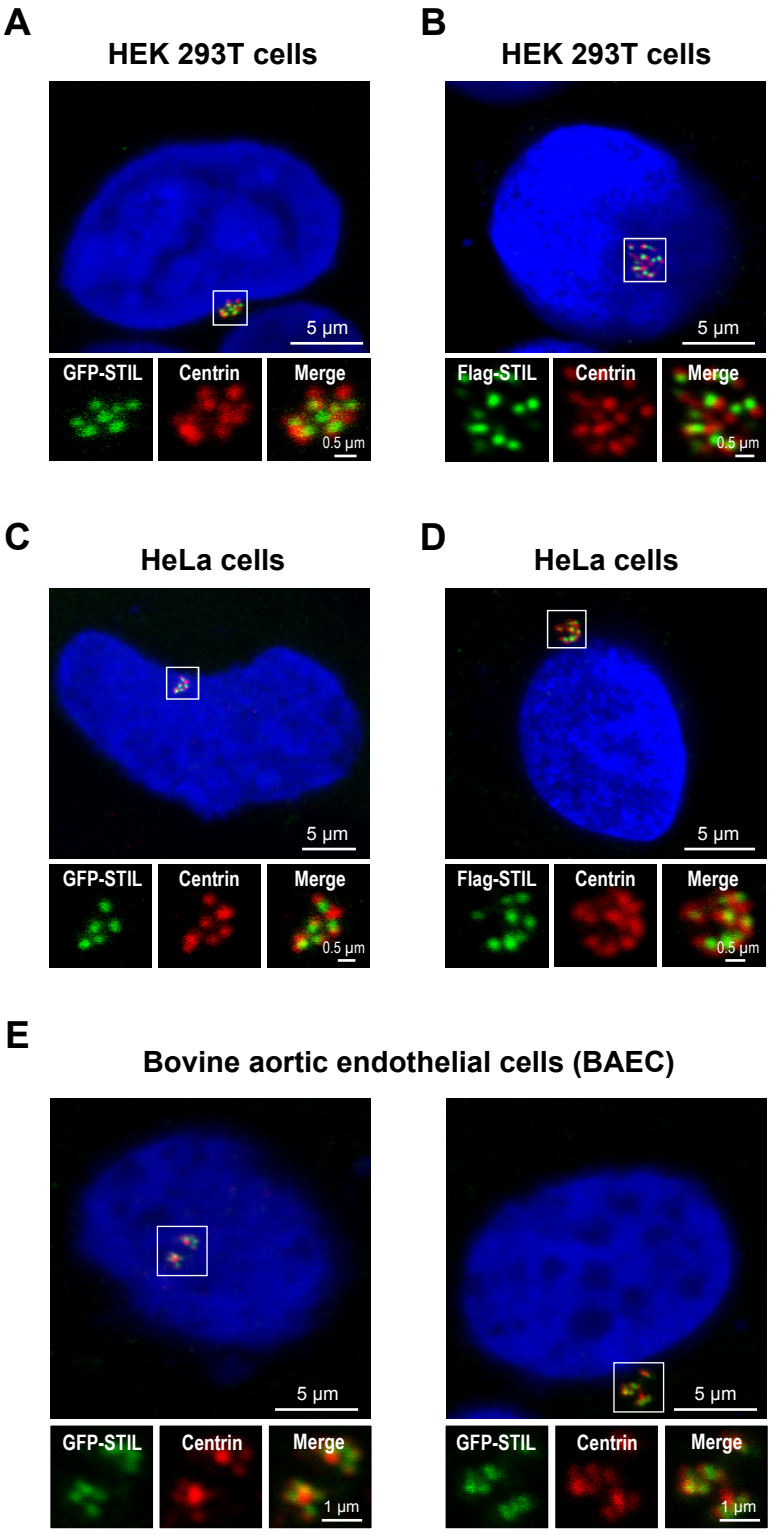

A
HEK 293T cells
5 μm
GFP-STIL
Centrin
Merge
0.5 μm
B
HEK 293T cells
5 μm
Flag-STIL
Centrin
Merge
0.5 μm
C
HeLa cells
5 μm
GFP-STIL
Centrin
Merge
0.5 μm
D
HeLa cells
5 μm
Flag-STIL
Centrin
Merge
0.5 μm
E
Bovine aortic endothelial cells (BAEC)
5 μm
GFP-STIL
Centrin
Merge
1 μm
5 μm
GFP-STIL
Centrin
Merge
1 μm

Fig. S3

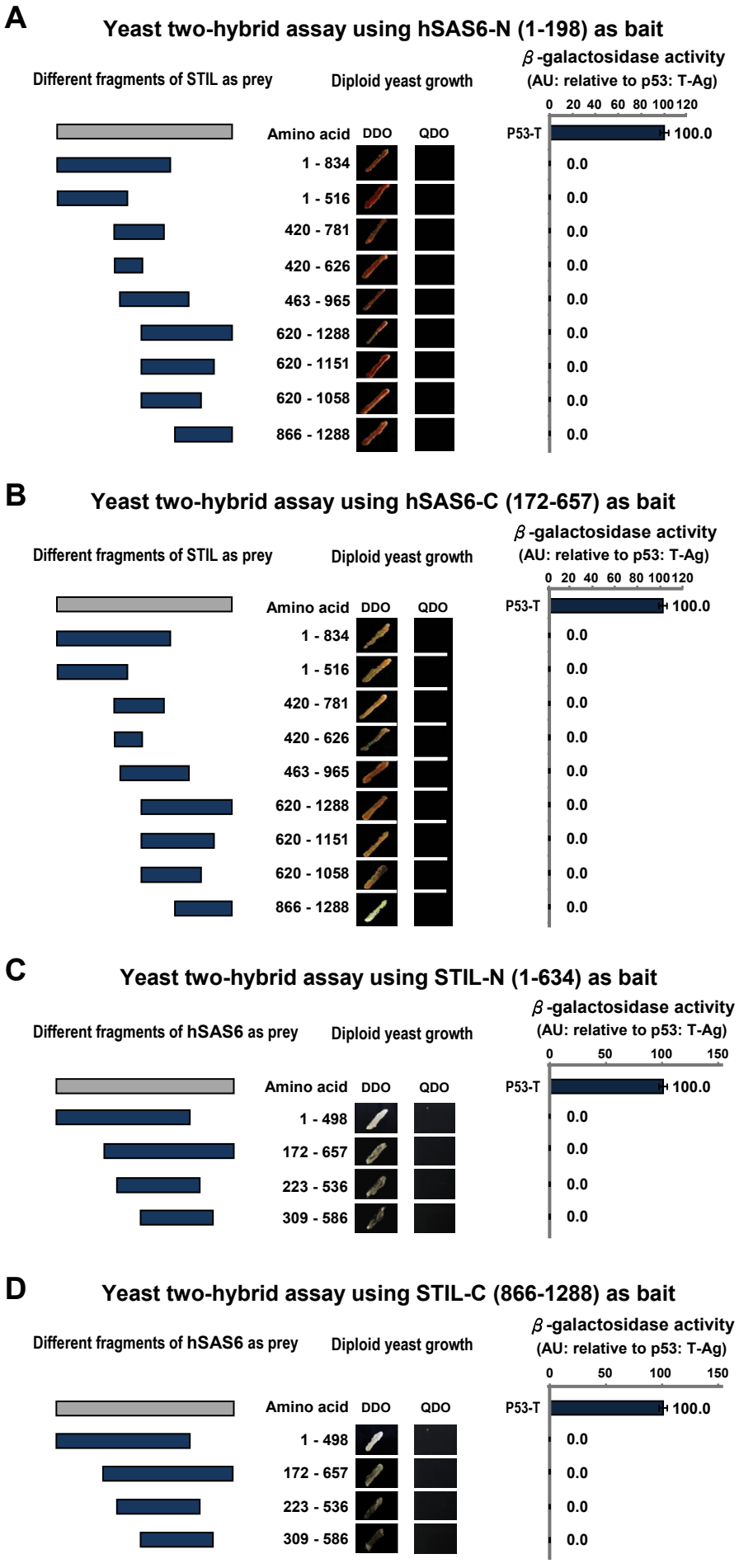

A
Yeast two-hybrid assay using hSAS6-N (1-198) as bait
Different fragments of STIL as prey
Diploid yeast growth
β-galactosidase activity
(AU: relative to p53: T-Ag)
0 20 40 60 80 100 120
Amino acid
DDO
QDO
P53-T
100.0
1 - 834
1 - 516
420 - 781
420 - 626
463 - 965
620 - 1288
620 - 1151
620 - 1058
866 - 1288
0.0
B
Yeast two-hybrid assay using hSAS6-C (172-657) as bait
Different fragments of STIL as prey
Diploid yeast growth
β-galactosidase activity
(AU: relative to p53: T-Ag)
0 20 40 60 80 100 120
Amino acid
DDO
QDO
P53-T
100.0
1 - 834
1 - 516
420 - 781
420 - 626
463 - 965
620 - 1288
620 - 1151
620 - 1058
866 - 1288
0.0
C
Yeast two-hybrid assay using STIL-N (1-634) as bait
Different fragments of hSAS6 as prey
Diploid yeast growth
β-galactosidase activity
(AU: relative to p53: T-Ag)
0 50 100 150
Amino acid
DDO
QDO
P53-T
100.0
1 - 498
172 - 657
223 - 536
309 - 586
0.0
D
Yeast two-hybrid assay using STIL-C (866-1288) as bait
Different fragments of hSAS6 as prey
Diploid yeast growth
β-galactosidase activity
(AU: relative to p53: T-Ag)
0 50 100 150
Amino acid
DDO
QDO
P53-T
100.0
1 - 498
172 - 657
223 - 536
309 - 586
0.0

Fig. S4

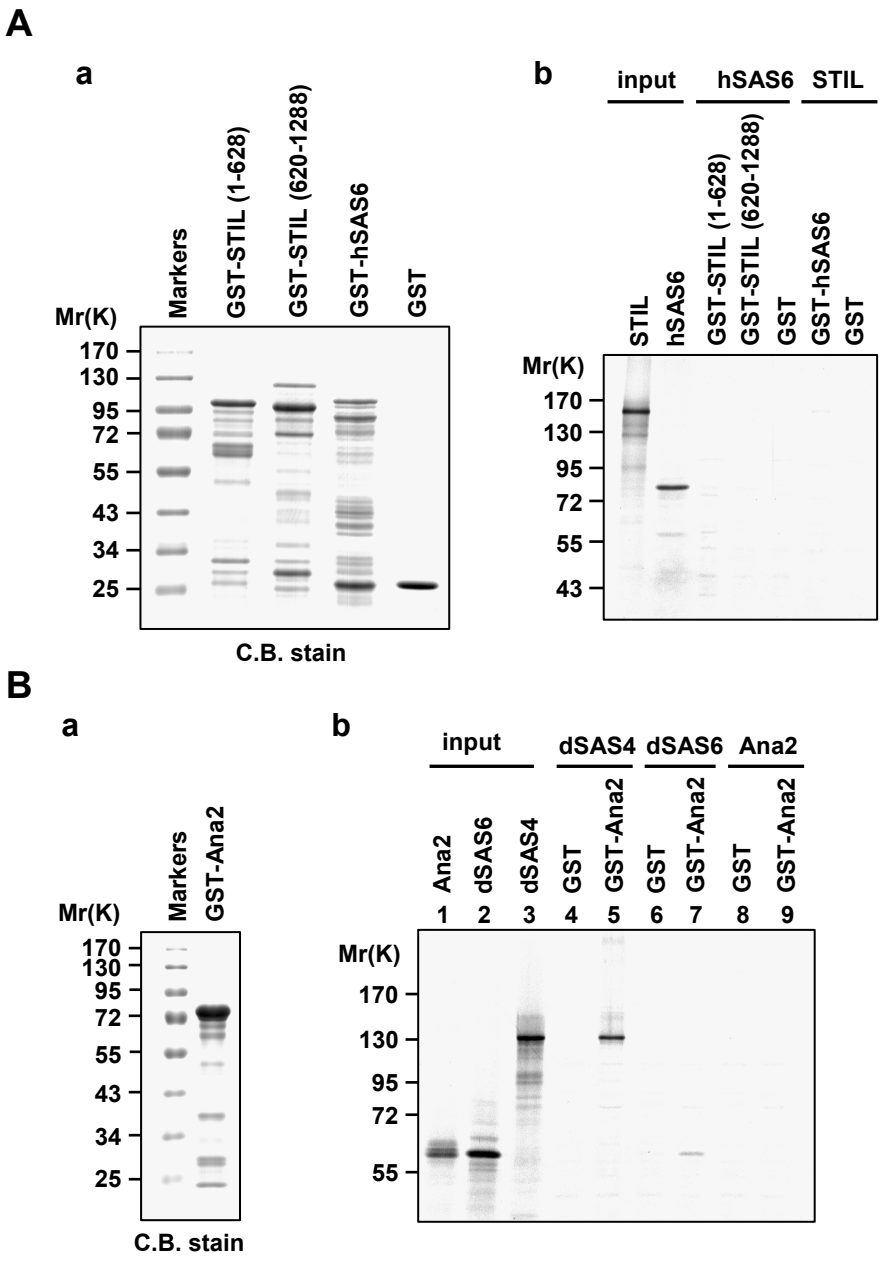

A
a
Markers
GST-STIL (1-628)
GST-STIL (620-1288)
GST-hSAS6
GST
Mr(K)
170
130
95
72
55
43
34
25
C.B. stain
b
input
hSAS6
STIL
STIL
hSAS6
GST-STIL (1-628)
GST-STIL (620-1288)
GST
GST-hSAS6
GST
Mr(K)
170
130
95
72
55
43
B
a
Markers
GST-Ana2
Mr(K)
170
130
95
72
55
43
34
25
C.B. stain
b
input
dSAS4
dSAS6
Ana2
Ana2
dSAS6
dSAS4
GST
GST-Ana2
GST
GST-Ana2
GST
GST-Ana2
1 2 3 4 5 6 7 8 9
Mr(K)
170
130
95
72
55

Fig. S5

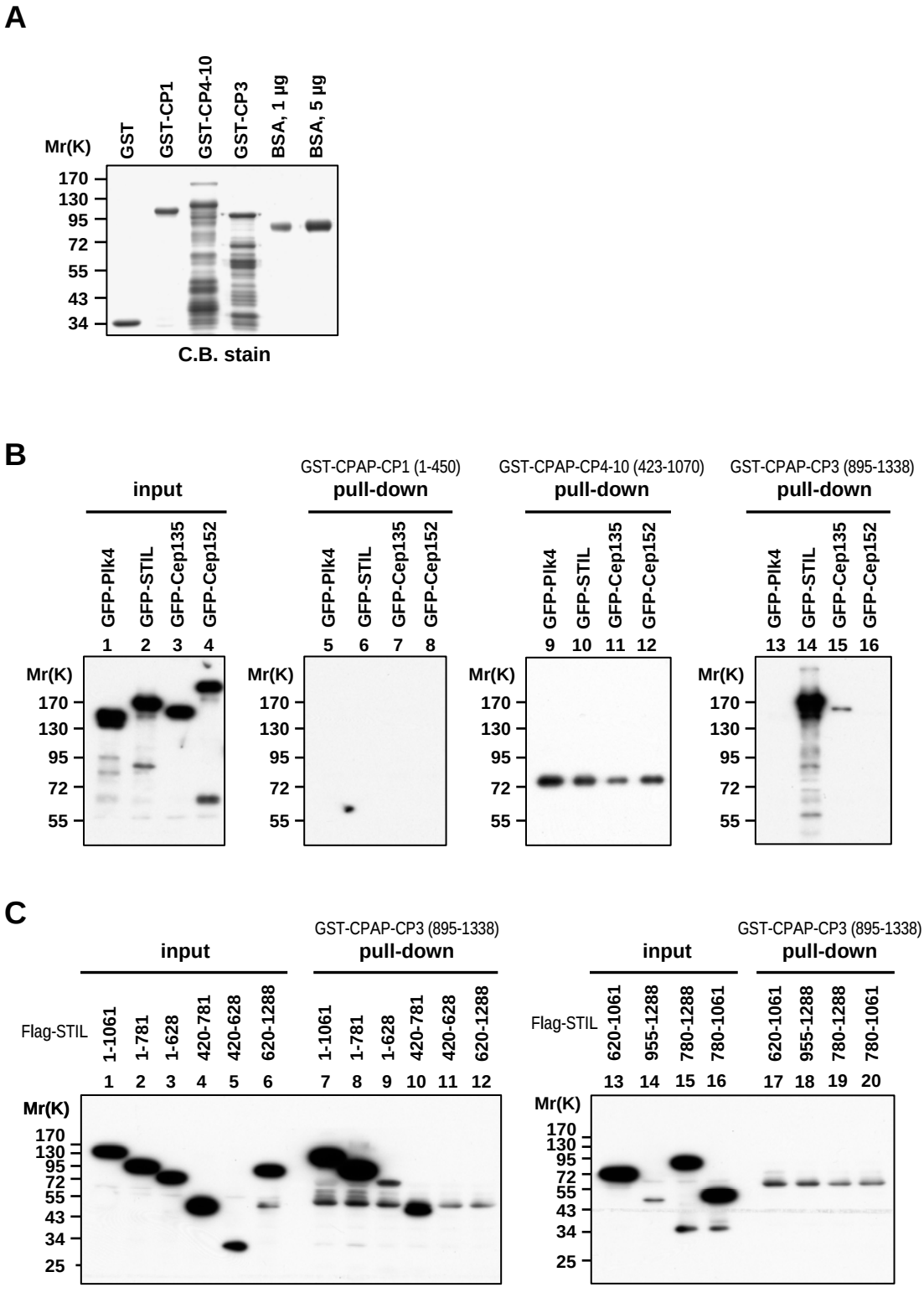
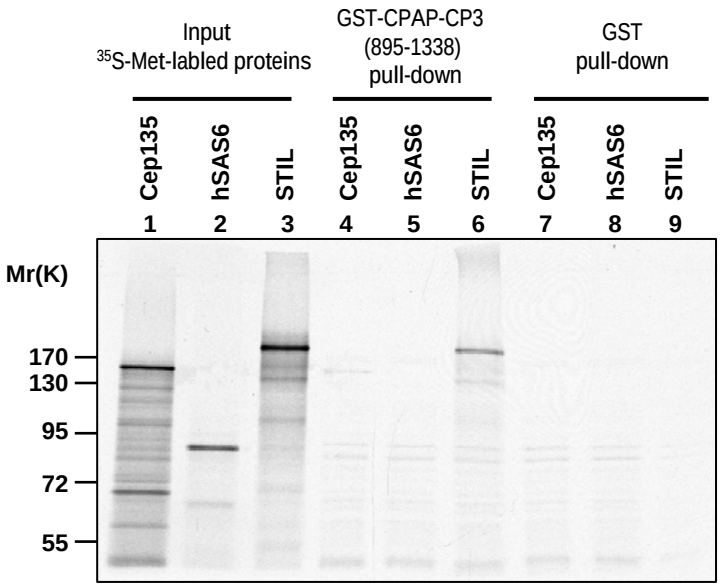

A
Mr(K)
GST
GST-CP1
GST-CP4-10
GST-CP3
BSA, 1 µg
BSA, 5 µg
170
130
95
72
55
43
34
C.B. stain
B
input
GST-CPAP-CP1 (1-450)
pull-down
GST-CPAP-CP4-10 (423-1070)
pull-down
GST-CPAP-CP3 (895-1338)
pull-down
GFP-Plk4
GFP-STIL
GFP-Cep135
GFP-Cep152
1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16
Mr(K)
170
130
95
72
55
C
input
GST-CPAP-CP3 (895-1338)
pull-down
Flag-STIL
1-1061
1-781
1-628
420-781
420-628
620-1288
1 2 3 4 5 6
7 8 9 10 11 12
620-1061
955-1288
780-1288
780-1061
13 14 15 16
17 18 19 20
Mr(K)
170
130
95
72
55
43
34
25

Fig. S6

A



B

Yeast two-hybrid assay using CPAP-CP3 (895-1338) as bait

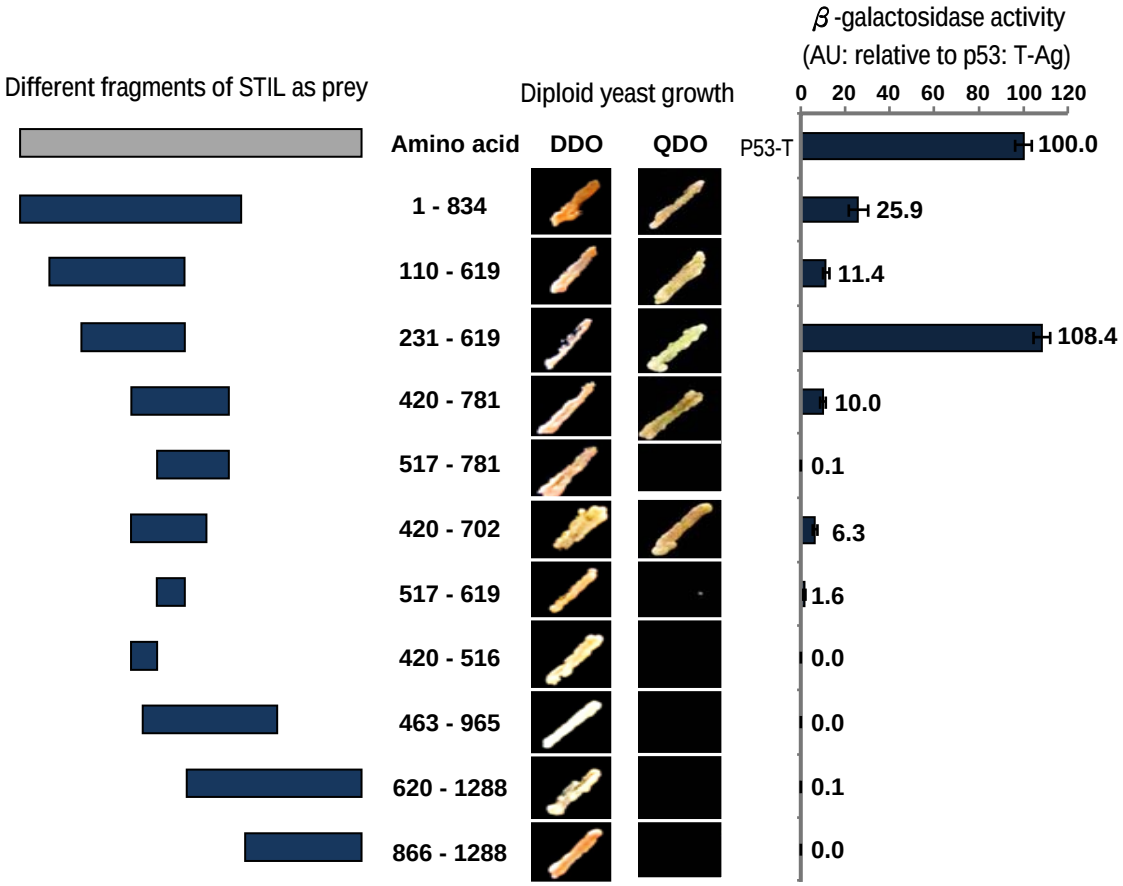


Fig. S7

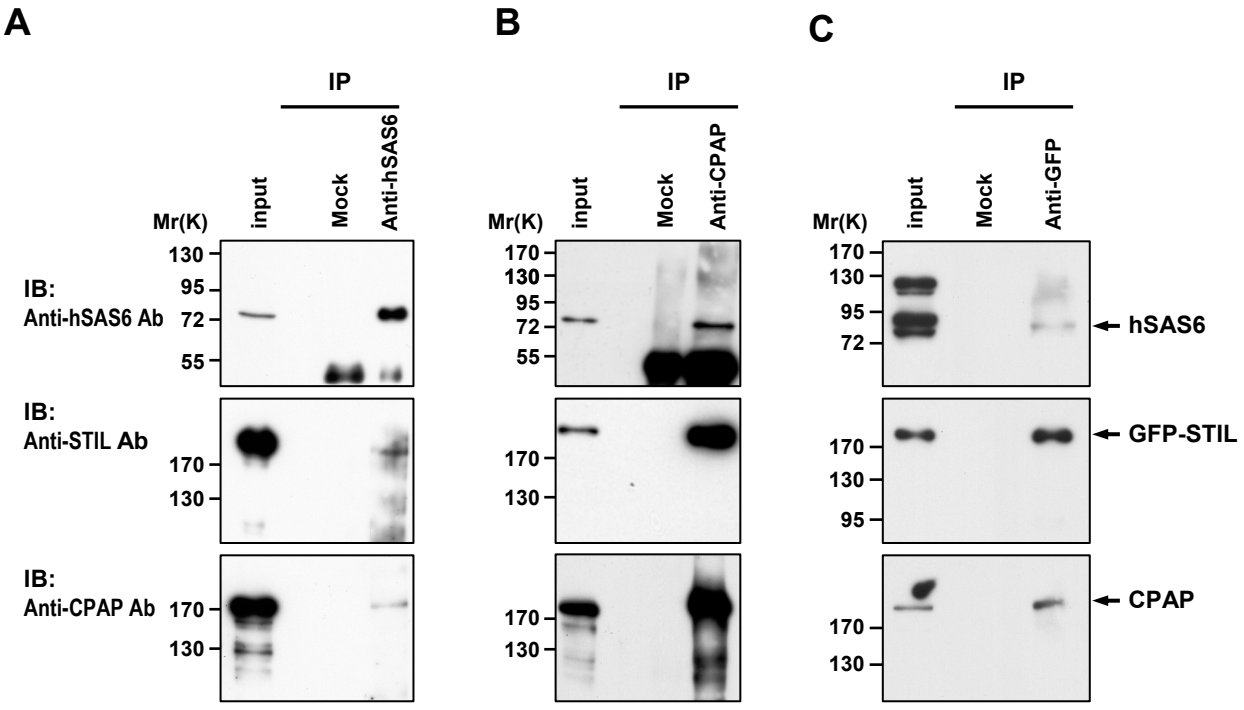

A
IP
input
Mock
Anti-hSAS6
Mr(K)
130
95
72
55
IB:
Anti-hSAS6 Ab
IB:
Anti-STIL Ab
170
130
IB:
Anti-CPAP Ab
170
130
B
IP
input
Mock
Anti-CPAP
Mr(K)
170
130
95
72
55
170
130
170
130
C
IP
input
Mock
Anti-GFP
Mr(K)
170
130
95
72
hSAS6
170
130
95
GFP-STIL
170
130
CPAP

Fig. S8

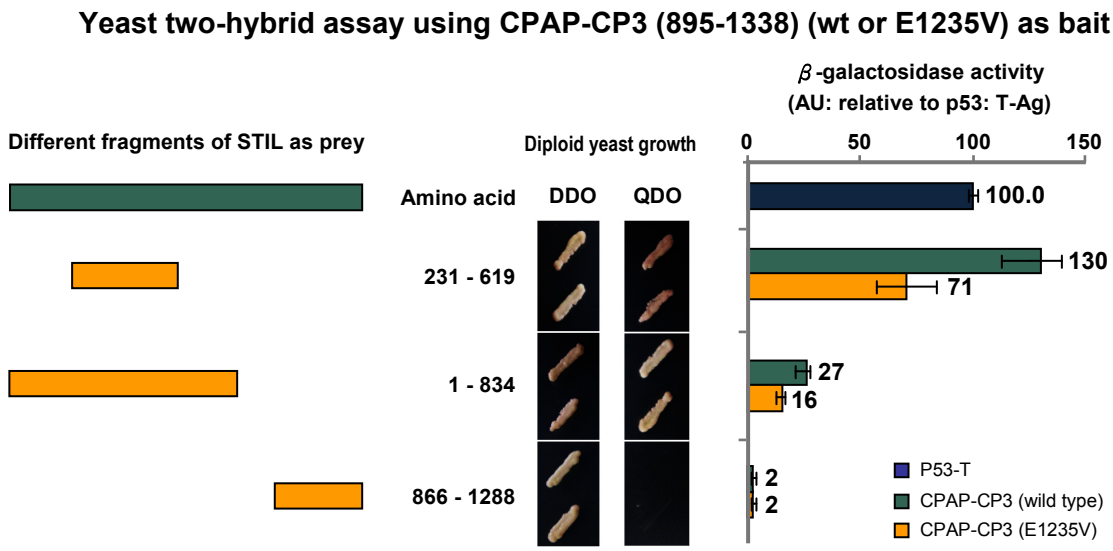

Yeast two-hybrid assay using CPAP-CP3 (895-1338) (wt or E1235V) as bait
β-galactosidase activity
(AU: relative to p53: T-Ag)
Different fragments of STIL as prey
Diploid yeast growth
Amino acid
DDO
QDO
231 - 619
1 - 834
866 - 1288
0
50
100
150
100.0
130
71
27
16
2
2
P53-T
CPAP-CP3 (wild type)
CPAP-CP3 (E1235V)

Fig. S9

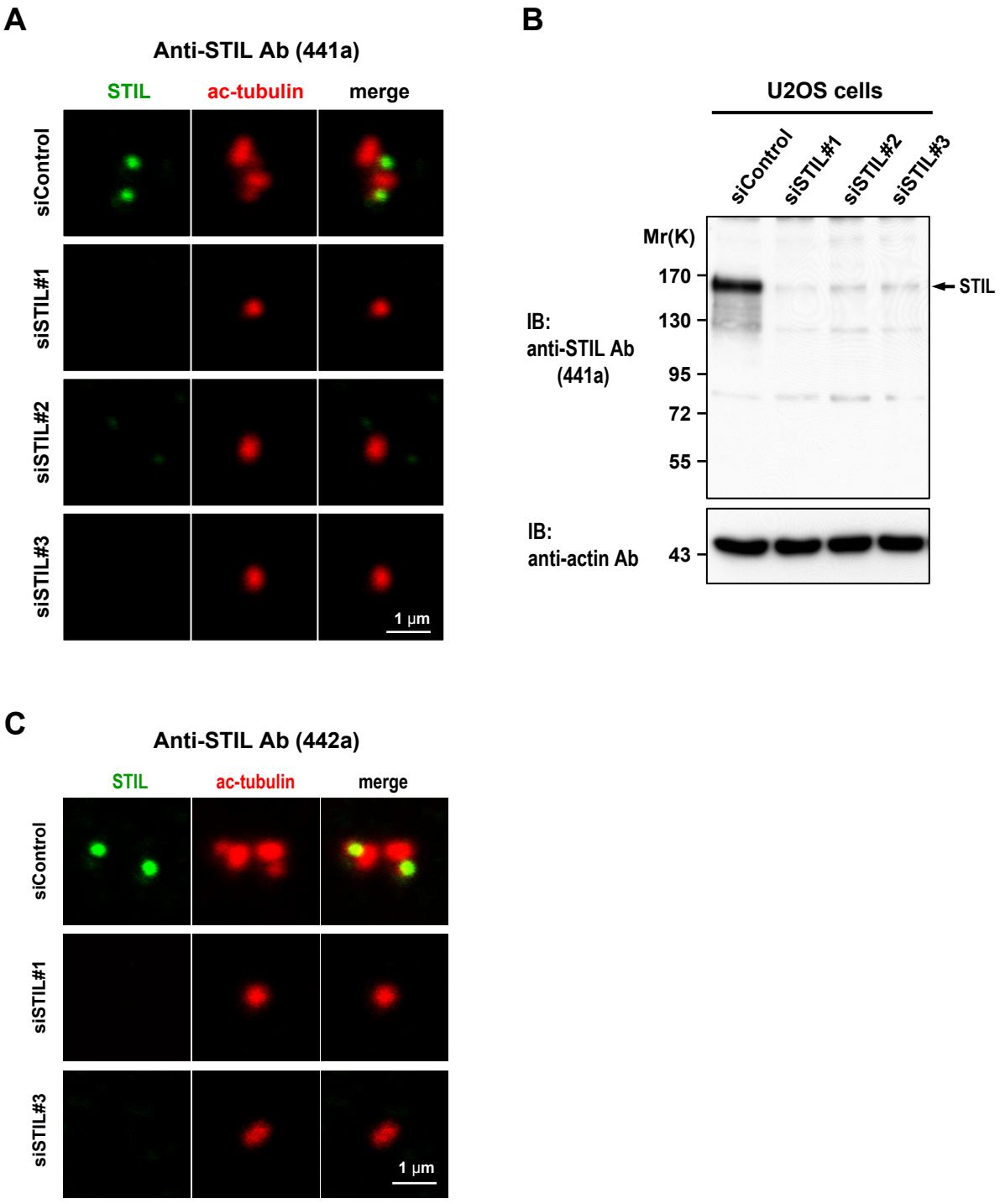

A
Anti-STIL Ab (441a)
STIL
ac-tubulin
merge
siControl
siSTIL#1
siSTIL#2
siSTIL#3
1 μm
B
U2OS cells
siControl
siSTIL#1
siSTIL#2
siSTIL#3
Mr(K)
170
130
95
72
55
43
STIL
IB:
anti-STIL Ab
(441a)
IB:
anti-actin Ab
C
Anti-STIL Ab (442a)
STIL
ac-tubulin
merge
siControl
siSTIL#1
siSTIL#3
1 μm

Fig. S10

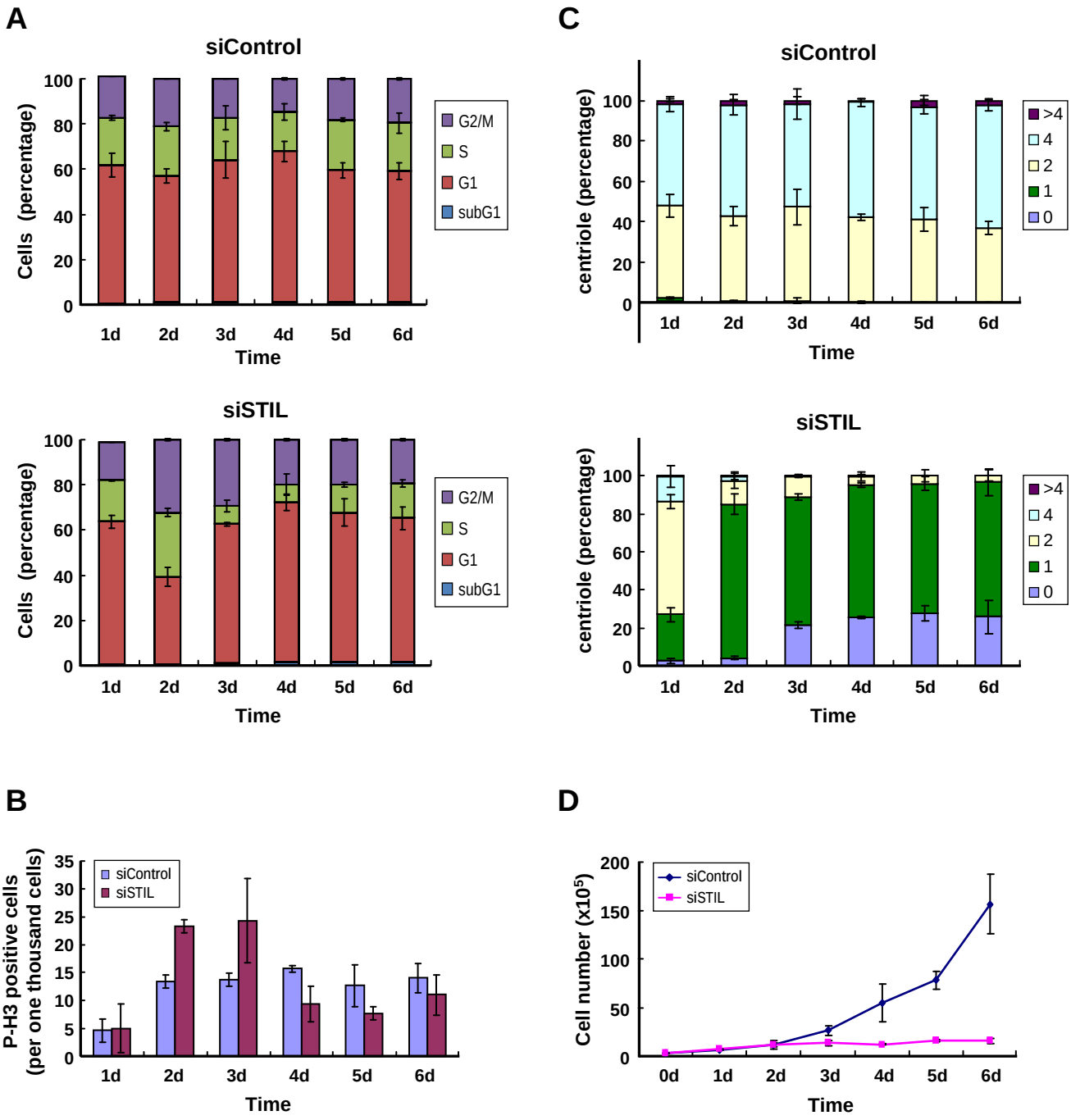

A
siControl
Cells (percentage)
100
80
60
40
20
0
1d
2d
3d
4d
5d
6d
Time
G2/M
S
G1
subG1
siSTIL
Cells (percentage)
Time
C
siControl
centriole (percentage)
Time
>4
4
2
1
0
siSTIL
centriole (percentage)
Time
B
P-H3 positive cells
(per one thousand cells)
35
30
25
20
15
10
5
0
siControl
siSTIL
Time
D
Cell number (x10⁵)
200
150
100
50
0
0d
siControl
siSTIL
Time

Fig. S11

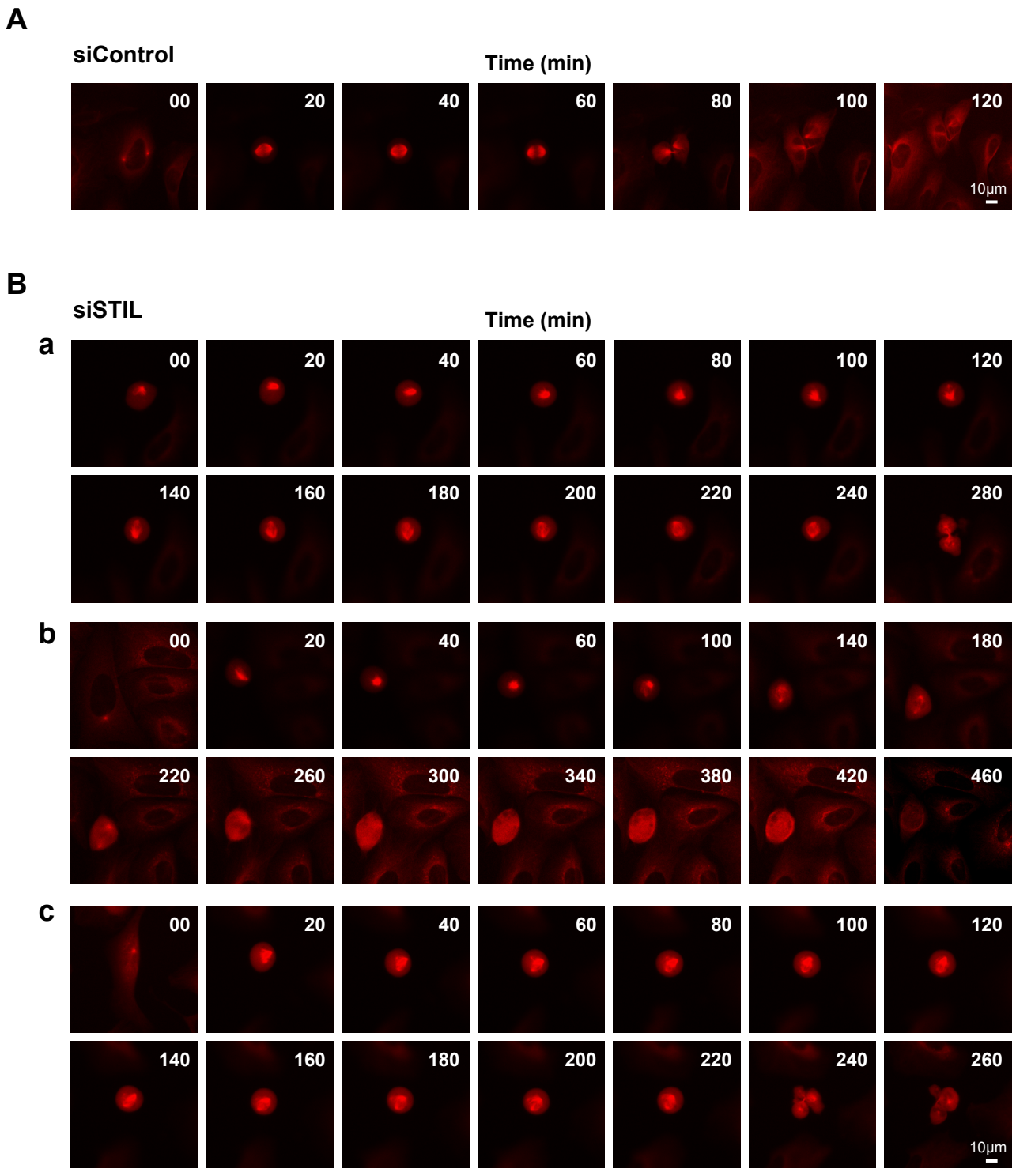
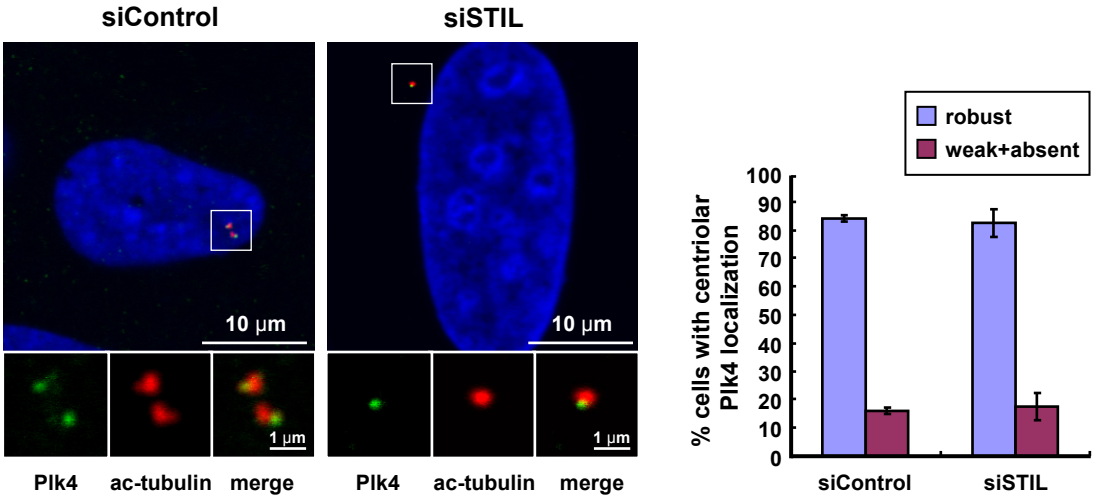

A
siControl
Time (min)
00
20
40
60
80
100
120
10μm
B
siSTIL
Time (min)
a
00
20
40
60
80
100
120
140
160
180
200
220
240
280
b
00
20
40
60
100
140
180
220
260
300
340
380
420
460
c
00
20
40
60
80
100
120
140
160
180
200
220
240
260
10μm

Fig. S12

A


siControl
siSTIL
10 μm
1 μm
Plk4
ac-tubulin
merge
robust
weak+absent
% cells with centriolar Plk4 localization
100
90
80
70
60
50
40
30
20
10
0
siControl
siSTIL

B

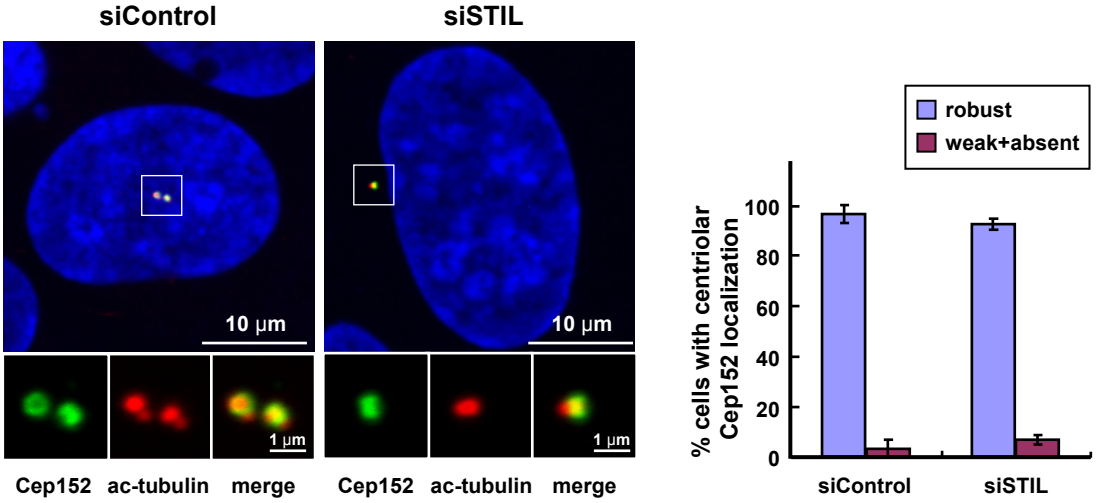

siControl
siSTIL
10 μm
1 μm
Cep152
ac-tubulin
merge
robust
weak+absent
% cells with centriolar Cep152 localization
100
80
60
40
20
0
siControl
siSTIL

Fig. S13

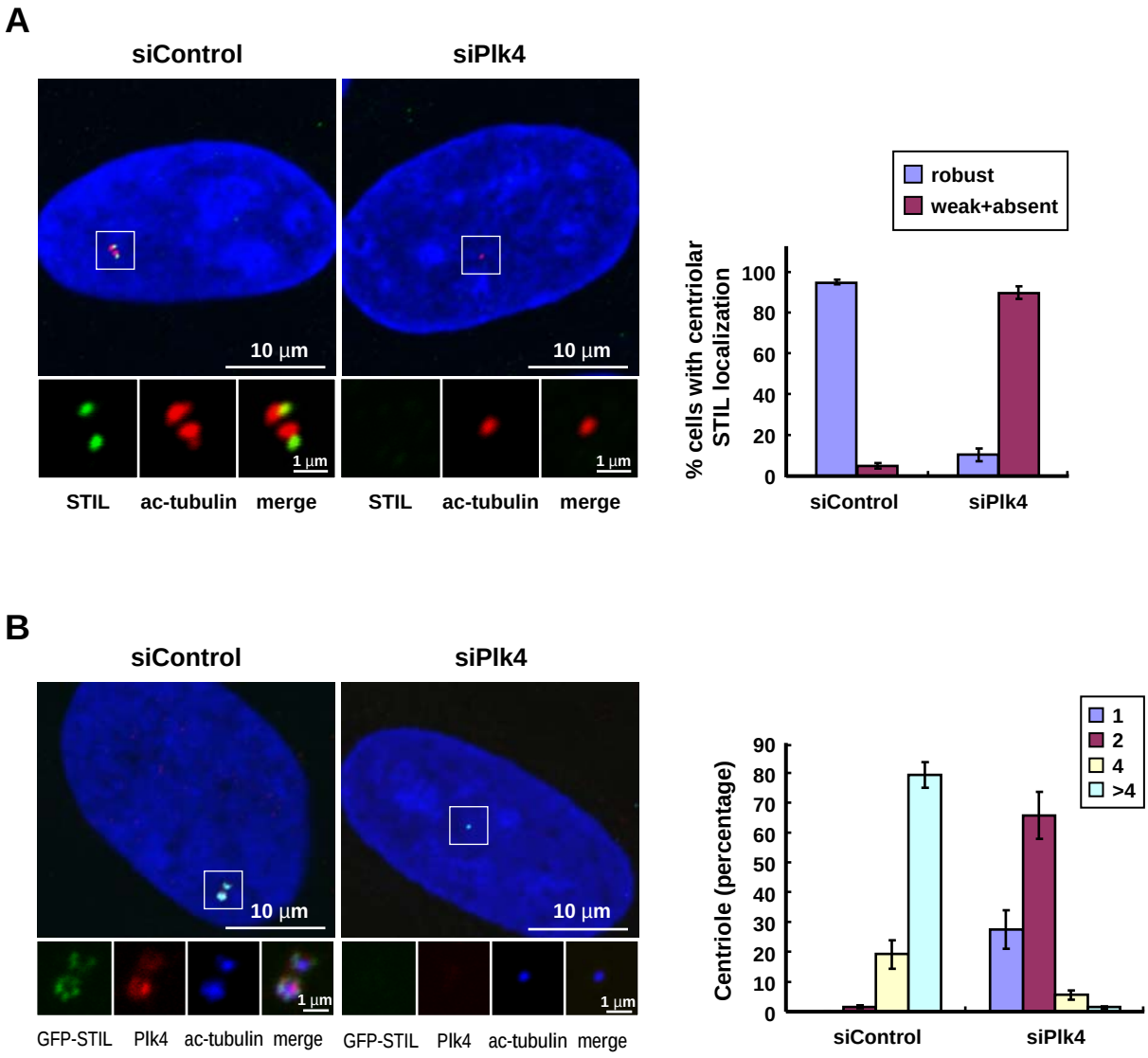
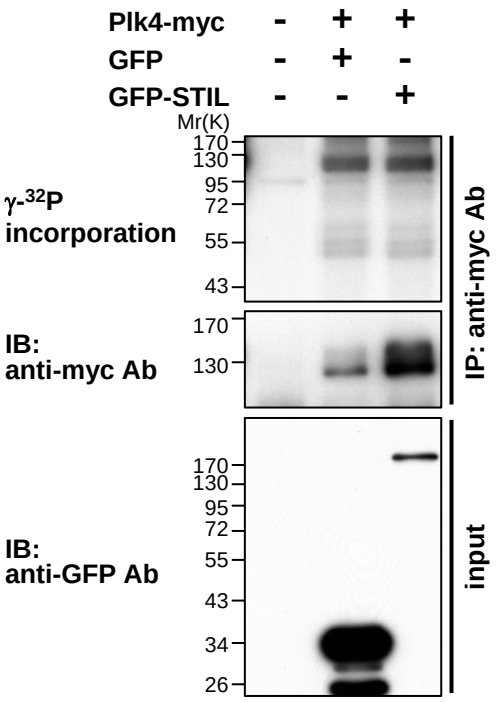

A
siControl
siPlk4
10 μm
10 μm
1 μm
1 μm
STIL
ac-tubulin
merge
STIL
ac-tubulin
merge
robust
weak+absent
% cells with centriolar STIL localization
100
80
60
40
20
0
siControl
siPlk4
B
siControl
siPlk4
10 μm
10 μm
1 μm
1 μm
GFP-STIL
Plk4
ac-tubulin
merge
GFP-STIL
Plk4
ac-tubulin
merge
1
2
4
>4
Centriole (percentage)
90
80
70
60
50
40
30
20
10
0
siControl
siPlk4

Fig. S14


Plk4-myc - + +
GFP - + -
GFP-STIL - - +
Mr(K)
170
130
95
72
55
43
γ-32P incorporation
IB: anti-myc Ab
IP: anti-myc Ab
input
IB: anti-GFP Ab
34
26