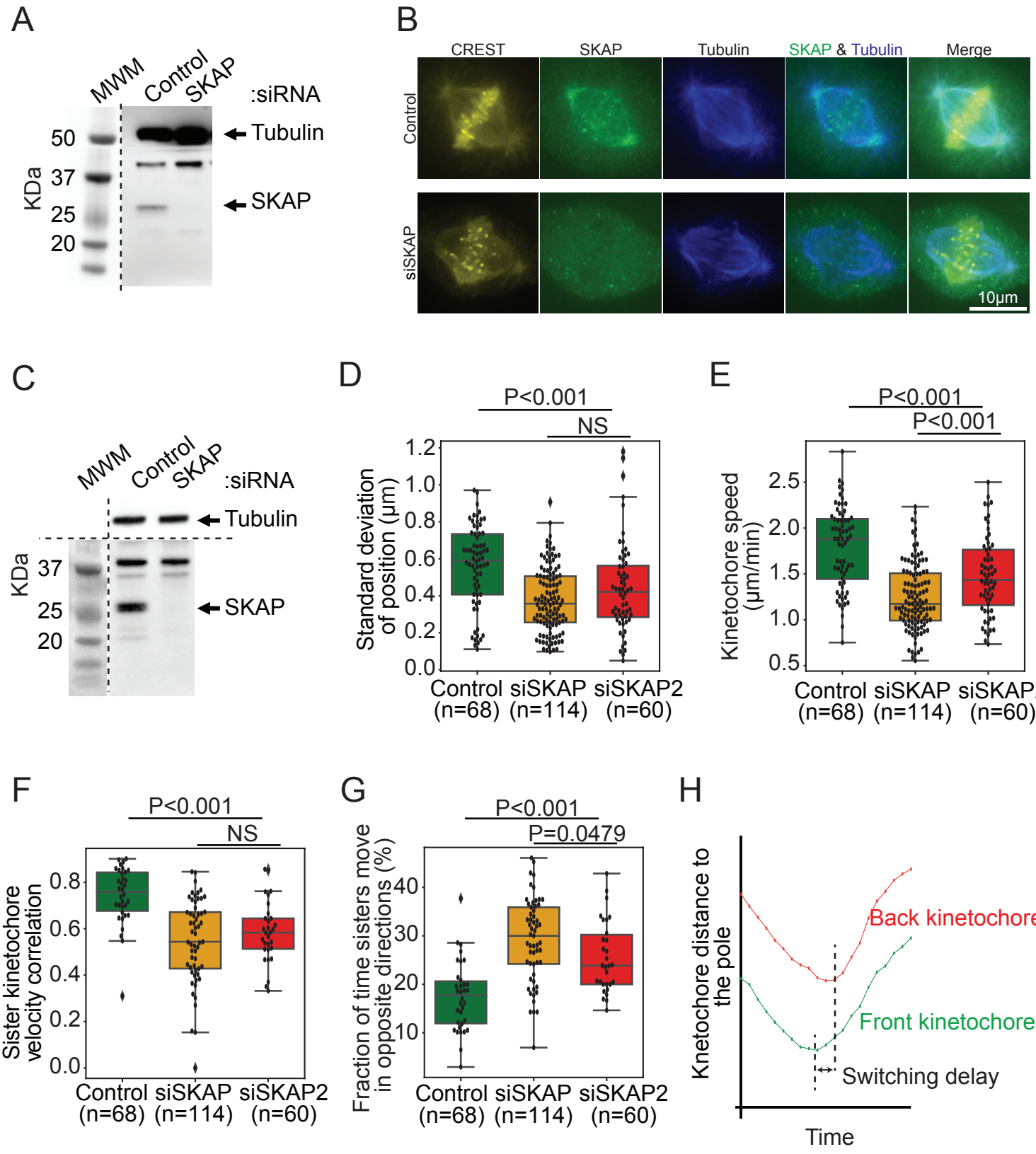
A
MWM
Control
SKAP
:siRNA
50
37
25
20
KDa
Tubulin
SKAP
B
CREST
SKAP
Tubulin
SKAP & Tubulin
Merge
Control
siSKAP
10μm
C
MWM
Control
SKAP
:siRNA
Tubulin
37
25
20
KDa
SKAP
D
P<0.001
NS
Standard deviation of position (μm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control (n=68)
siSKAP (n=114)
siSKAP2 (n=60)
E
P<0.001
P<0.001
Kinetochore speed (μm/min)
2.5
2.0
1.5
1.0
0.5
Control (n=68)
siSKAP (n=114)
siSKAP2 (n=60)
F
P<0.001
NS
Sister kinetochore velocity correlation
0.8
0.6
0.4
0.2
0.0
Control (n=68)
siSKAP (n=114)
siSKAP2 (n=60)
G
P<0.001
P=0.0479
Fraction of time sisters move in opposite directions (%)
40
30
20
10
Control (n=68)
siSKAP (n=114)
siSKAP2 (n=60)
H
Knetochore distance to the pole
Back kinetochore
Front kinetochore
Switching delay
Time

Figure S1. SKAP silencing oscillation defects do not depend on the specific siRNA sequence used. Related to Figure 1. (A) Representative western blot of SKAP expression in control and siSKAP in Rpe1-GFP cells at 24 h post-transfection (80-95% silencing), with tubulin as a loading control and with molecular weight marker (MWM). (B) Representative immunofluorescence images in control and siSKAP Rpe1-GFP cells (24 h post-transfection) stained for CREST (kinetochore marker, yellow), tubulin (blue) and SKAP (green). (C) Representative western blot of SKAP expression in control and siSKAP2 (alternative SKAP siRNA) in Rpe1-GFP cells at 48 h post-transfection (89% silencing), with tubulin as a loading control and with molecular weight marker (MWM). (D) Standard deviation of the position of individual control and siSKAP metaphase kinetochores over time (Mann-Whitney test). (E) Average speed of individual control and siSKAP metaphase kinetochores (Mann-Whitney test). (F) Velocity correlation between metaphase sister kinetochores (Mann-Whitney test). (G) Fraction of time individual metaphase sister kinetochores move in opposite directions (Mann-Whitney test). (H) Schematic representation of how sister kinetochores typically switch direction during metaphase oscillations[S1].

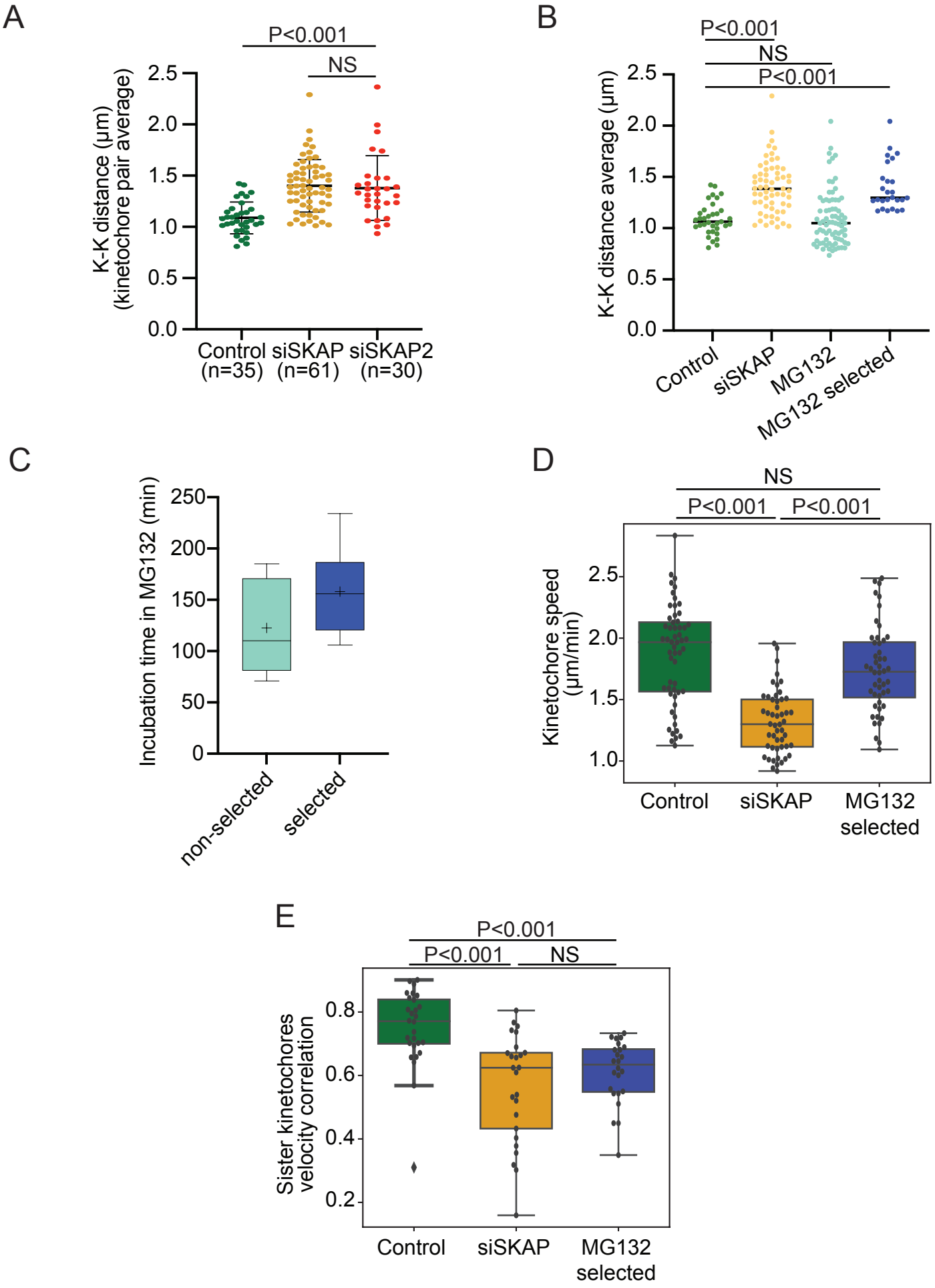
A
P<0.001
NS
K-K distance (µm)
(kinetochore pair average)
2.5
2.0
1.5
1.0
0.5
0.0
Control (n=35)
siSKAP (n=61)
siSKAP2 (n=30)
B
P<0.001
NS
P<0.001
K-K distance average (µm)
2.5
2.0
1.5
1.0
0.5
0.0
Control
siSKAP
MG132
MG132 selected
C
Incubation time in MG132 (min)
250
200
150
100
50
0
non-selected
selected
D
NS
P<0.001
P<0.001
Kinetochore speed
(µm/min)
2.5
2.0
1.5
1.0
Control
siSKAP
MG132
selected
E
P<0.001
P<0.001
NS
Sister kinetochores
velocity correlation
0.8
0.6
0.4
0.2
Control
siSKAP
MG132
selected

Figure S2. SKAP depletion is not consistent with a decrease in centromere stiffness. Related to Figure 2. (A) K-K distance average over time for individual kinetochore pairs in control, siSKAP and siSAP2 Rpe1-GFP cells from the dataset in Figure 1 and S1 (student´s t-test) (n=number of kinetochore pairs, 1-4 kinetochore pairs per analyzed cell from 18 control, 20 siSKAP and 11 siSKAP2 cells). (B) Average K-K distance for individual sister kinetochore pairs over time in live control (n=34 pairs from 18 cells), siSKAP (n=61 from 20 cells), MG132-treated (n=77 pairs from 18 cells), and MG132-treated "selected" (n=29 pairs from 6 cells) Rpe1-GFP cells (1-4 pairs per analyzed cell). The latter "selected" pairs only include those with mean K-K distances higher than two standard deviations over average control pairs. (C) Time of MG132 incubation of selected vs non-selected cells in (B). (D) Individual kinetochore speed during metaphase oscillations in control, siSKAP or MG132-treated selected cells (Mann-Whitney test) for the cells from (B). (E) Sister kinetochore velocity correlation during metaphase oscillations in control, siSKAP and MG132-treated selected cells from (B) (Mann-Whitney test).

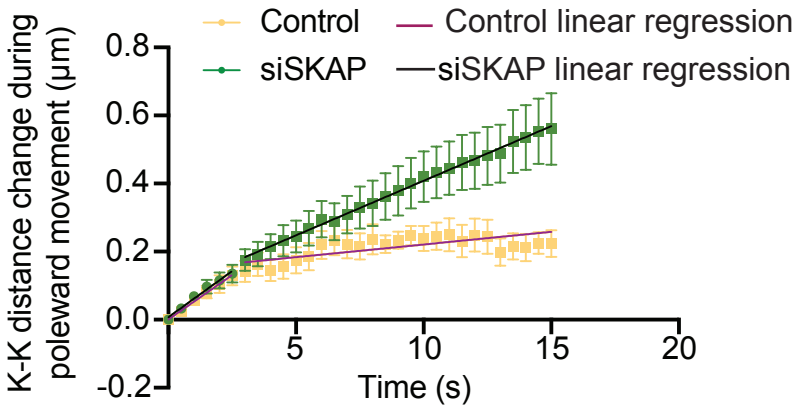
Control
Control linear regression
siSKAP
siSKAP linear regression
K-K distance change during poleward movement (µm)
0.8
0.6
0.4
0.2
0.0
-0.2
5
10
15
20
Time (s)

Figure S3. Differential velocity between front and back siSKAP sister kinetochores leads to increasing K-K distance after k-fiber ablation. Related to Figure 3. K-K distance during front kinetochore poleward movement after k-fiber ablation in Rpe1-GFP control and siSKAP cells, with t=0 at the start of front kinetochore poleward movement. Linear regression slope is similar in control and siSKAP cells during the first 2.5 s, but not at later timepoints (analysis of covariance test, ANCOVA). Tracks from Figure 2B dataset.

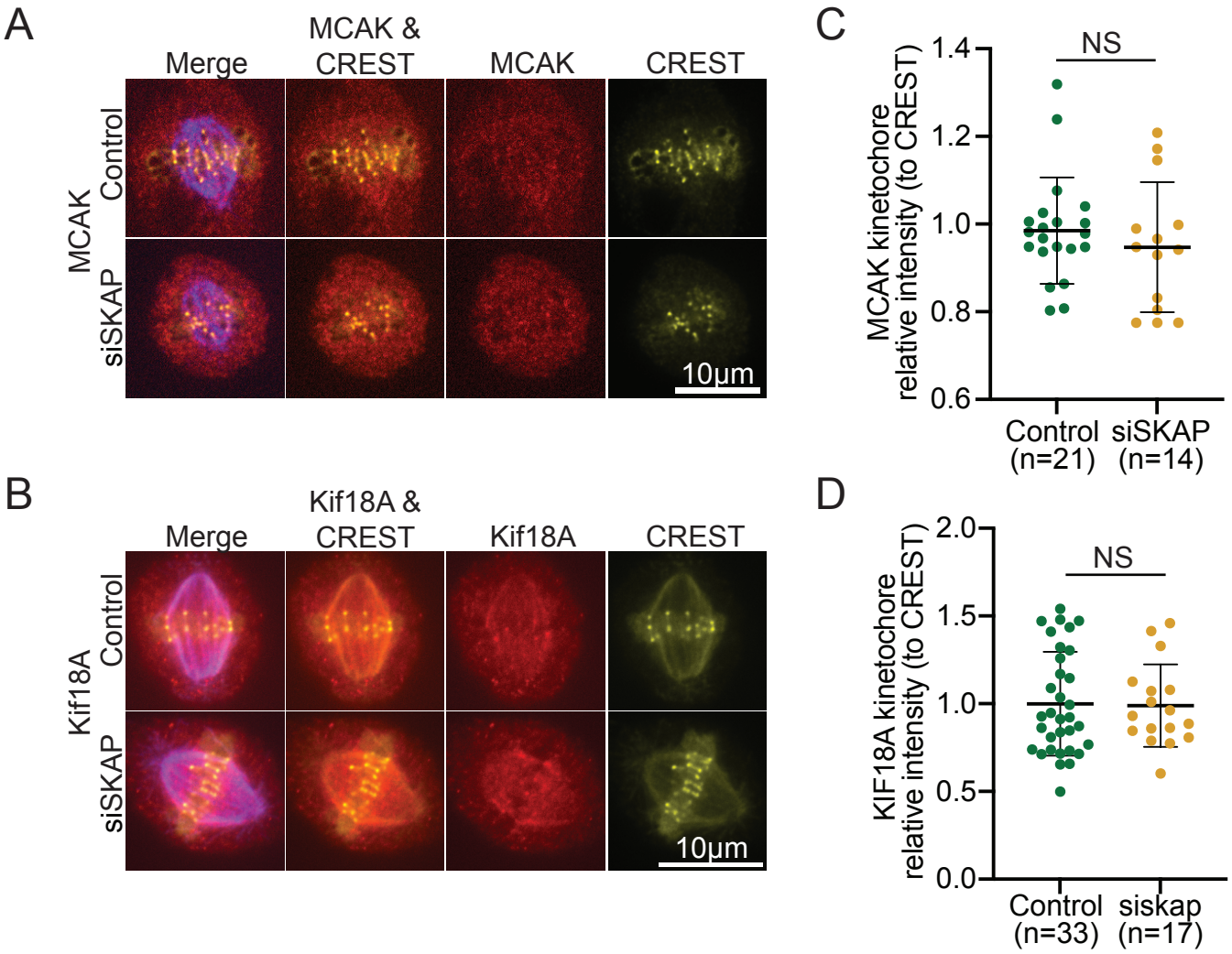
A
Merge
MCAK &
CREST
MCAK
CREST
MCAK
Control
siSKAP
10μm
B
Merge
Kif18A &
CREST
Kif18A
CREST
Kif18A
Control
siSKAP
10μm
C
NS
MCAK kinetochore
relative intensity (to CREST)
1.4
1.2
1.0
0.8
0.6
Control
(n=21)
siSKAP
(n=14)
D
NS
KIF18A kinetochore
relative intensity (to CREST)
2.0
1.5
1.0
0.5
0.0
Control
(n=33)
siskap
(n=17)

Figure S4. SKAP does not affect kinetochore recruitment of two proteins (MCAK and Kif18A) regulating microtubule dynamics. Related to Figure 4. Representative immunofluorescence images in control and siSKAP Rpe1-GFP metaphase cells with staining for tubulin (purple), CREST (yellow), and A) MCAK (red) or B) Kif18A (red). Kinetochore intensity of (C) MCAK and (D) Kif18A in control and siSKAP cells relative to CREST kinetochore intensity (student´s t-test; n=number of cells), obtained from immunofluorescence images as in (A) and (B), respectively.

SUPPLEMENTAL REFERENCE

S1. Wan, X., Cimini, D., Cameron, L.A., and Salmon, E.D. (2012). The coupling between sister kinetochore directional instability and oscillations in centromere stretch in metaphase PtK1 cells. Mol. Biol. Cell *23*, 1035–1046.