Expanded View Figures

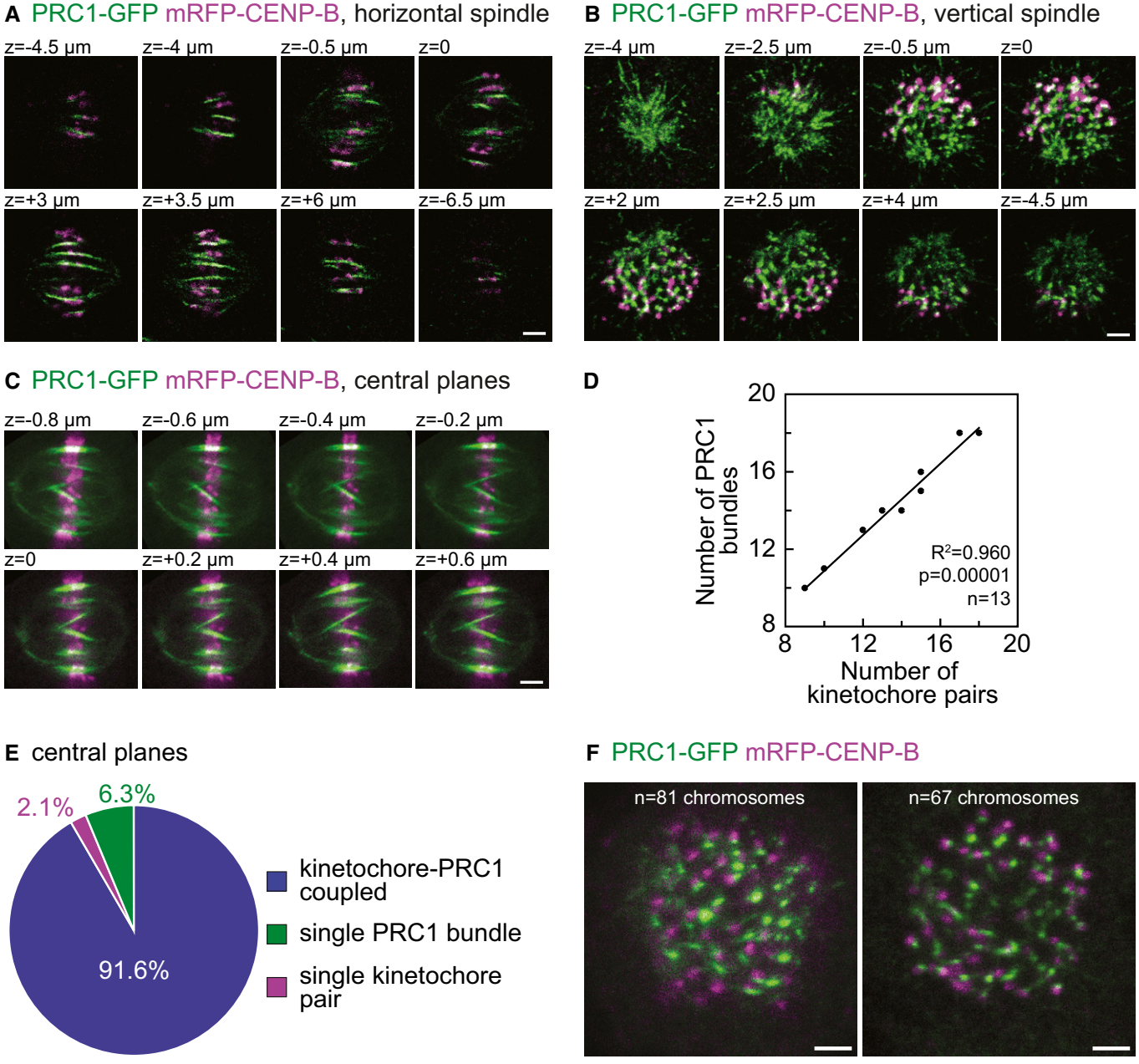

Figure EV1. Additional characterization of kinetochore-PRC1 pairs.

A–C Individual *z*-stack images acquired in fixed HeLa cells stably expressing PRC1-GFP (green) and transiently mRFP-CENP-B (magenta). *Z*-stack images of (A) horizontal spindle (same spindle shown in Fig 1A), (B) vertical spindle (same spindle shown in Fig 1B), and (C) spindle imaged with different settings (line averaged 16 times with 200 nm spacing between *z*-slices). Regardless of depicted approaches, images reveal majority of sister kinetochores positioned in close proximity of PRC1-labeled fibers.

D Graph shows correlation between the number of sister kinetochores and PRC1 signals counted in the central *z*-plane of fixed HeLa cells in metaphase.

E Pie chart shows what fraction of PRC1-labeled fibers and sister kinetochores are linked (blue) in the central *z*-plane of fixed HeLa cells in metaphase. Percentage of single kinetochores and single PRC1 signals are shown in magenta and green, respectively.

F A cross section of vertical spindles with 81 (left) and 67 (right) chromosomes.

Data information: Scale bars, 2 µm; *n*, number of cells; R^2, coefficient of determination; *P*, *P*-value from a *t*-test.

Figure EV2. Dynamics of PRC1-labeled bundles and kinetochores in the spindle cross section.

A Dynamics of PRC1-labeled bundles in live HeLa cells expressing PRC1-GFP and mRFP-CENP-B. The distance between PRC1-labeled bundles and center of the mass of the spindle (CM) is plotted over t = 150 s. n, number of pairs.

B Dynamics between PRC1-labeled bundles and corresponding kinetochores in live HeLa cells expressing PRC1-GFP and mRFP-CENP-B. The distance between them is plotted over t = 150 s. n, number of pairs.

C Examples of trajectories of a kinetochore pair (magenta), its coupled PRC1-labeled bundle (green), nearest PRC1 neighbors (blue), next nearest PRC1 neighbors (black), and randomly chosen PRC1 bundles (gray) with respect to the spindle's center of the mass (CM) over t = 200 s in three cells. n, number of PRC1-labeled bundles.

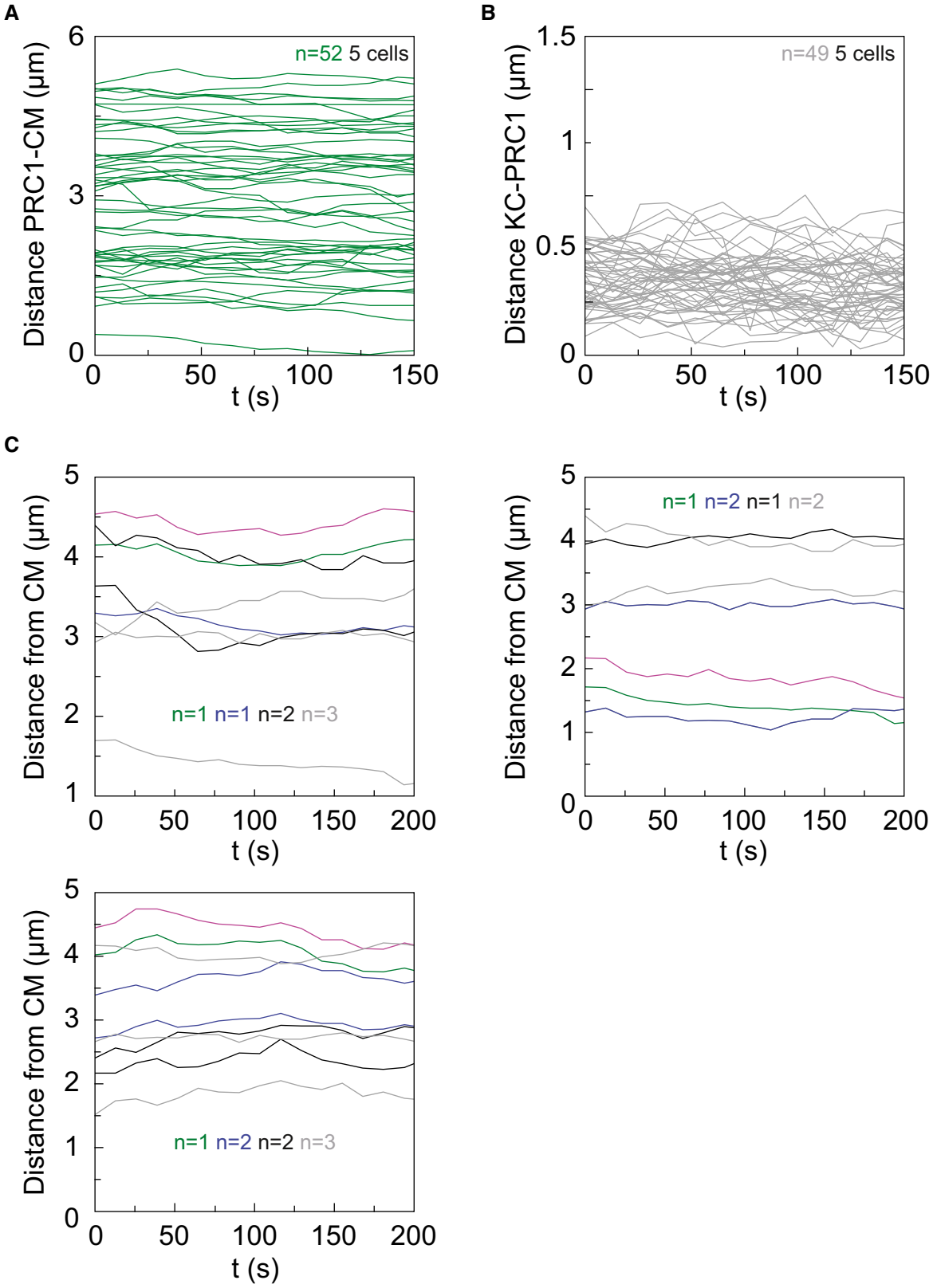
A
Distance PRC1-CM (μm)
n=52 5 cells
t (s)
B
Distance KC-PRC1 (μm)
n=49 5 cells
t (s)
C
Distance from CM (μm)
n=1 n=1 n=2 n=3
t (s)
Distance from CM (μm)
n=1 n=2 n=1 n=2
t (s)
Distance from CM (μm)
n=1 n=2 n=2 n=3
t (s)

Figure EV2.

Figure EV3. Additional characterization of PRC1-labeled bundles.

A, B Western blot for PRC1 in various HeLa cell lines and conditions, as denoted in the figure. PRC1 expression in PRC1-GFP HeLa cells was 60.97 ± 10.01% higher than in unlabeled cells. Synchronized cells were lysed in lysis buffer 29 h after transfection. Cell lysates were subjected to SDS-PAGE (12% polyacrylamide), transferred on to a nitrocellulose membrane and immunoblotted with anti-PRC1 and anti-GAPDH antibodies used as a loading control. Intensities of the Western blot bands of endogenous PRC1 isoforms (61–71 kDa) and PRC1-GFP (98 kDa) were quantified as described in Materials and Methods. *P*-value was 0.0004.

C From left to right: image of the immunostained PRC1 in the spindle of a HeLa cell expressing tubulin-GFP and immunostained for PRC1; dependence of the length of immunostained PRC1 signal in HeLa cells expressing tubulin-GFP and immunostained for PRC1 on the distance from the spindle long axis; dependence of the signal intensity *I* of immunostained PRC1 signal in HeLa cells expressing tubulin-GFP and immunostained for PRC1 on the distance from the spindle long axis; dependence of I_{cross} of immunostained PRC1 signal in HeLa cells expressing tubulin-GFP and immunostained for PRC1 on the distance from the spindle long axis.

D From left to right: image of PRC1-GFP in the spindle of a fixed HeLa cell expressing PRC1-GFP and mRFP-CENP-B; dependence of the length of PRC1-GFP signal in fixed HeLa cells expressing PRC1-GFP on the distance from the spindle long axis; dependence of the signal intensity *I* of PRC1-GFP signal in fixed HeLa cells expressing PRC1-GFP on the distance from the spindle long axis; dependence of I_{cross} of PRC1-GFP signal in fixed HeLa cells expressing PRC1-GFP and on the distance from the spindle long axis.

E From left to right: image of PRC1-GFP in the spindle of a live HeLa cell expressing PRC1-GFP and mRFP-CENP-B; dependence of the length of PRC1-GFP signal in live HeLa cells expressing PRC1-GFP on the distance from the spindle long axis; dependence of the signal intensity *I* of PRC1-GFP signal in live HeLa cells expressing PRC1-GFP on the distance from the spindle long axis; dependence of I_{cross} of PRC1-GFP signal in live HeLa cells expressing PRC1-GFP and on the distance from the spindle long axis.

Data information: Scale bars, 2 μm; R^2, coefficient of determination; *P*, *P*-value from a *t*-test; *n*, number of bridging fibers; error bars, s.e.m.
Source data are available online for this figure.

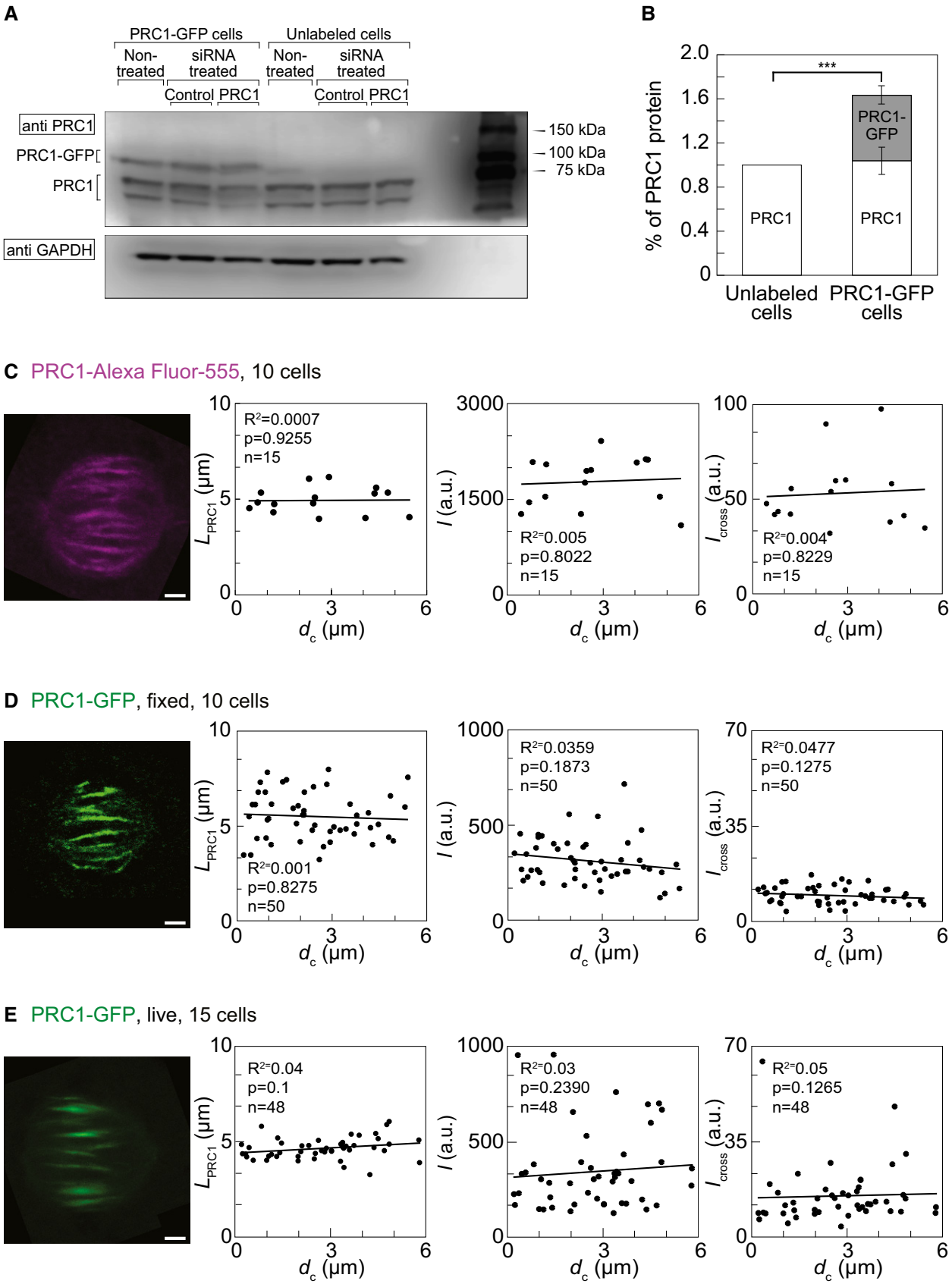

Figure EV3.

Figure EV4. Additional characterization of PRC1 silencing effect.

A Western blot for PRC1 in various HeLa cell lines and conditions, as denoted in the figure. Graphs show quantification of Western blots. Please note a decrease of the amount of PRC1 after treatment with PRC1 siRNA. HeLa cells grown on 6-well plates were transfected with 200 nM control or PRC1 siRNA. Synchronized cells were lysed in lysis buffer 29 h after transfection. Cell lysates were subjected to SDS-PAGE (12% polyacrylamide), transferred on to a nitrocellulose membrane and immunoblotted with anti-PRC1 and anti-GAPDH antibodies. Percent of PRC1 protein was calculated from Western blot band intensities measured in Image Studio Lite program after normalizing to the corresponding GAPDH band intensity. The data were acquired from three to six independent experiments. Please note that the tubulin-GFP HeLa was the least viable of the cell lines and thus the data varied. *P*-values are given in the graphs.

B Left: Image of tubulin-GFP in the spindle of a HeLa cell expressing tubulin-GFP and mRFP-CENP-B, treated with PRC1 siRNA. Right: Dependence of the ratio of signal intensities of the bridging fiber and the sum of the bridging and k-fiber, I_b/I_{bk} measured in control cells (gray) and PRC1 siRNA-treated (black) HeLa cells expressing tubulin-GFP and mRFP-CENP-B on the distance from the spindle long axis.

C Dependence of the distance between sister kinetochores in control cells (gray) and PRC1 siRNA-treated (black) cells expressing tubulin-GFP and mRFP-CENP-B on the distance from the spindle long axis.

D From left to right: image of PRC1-Alexa Fluor-555 in the spindle of a HeLa cell expressing tubulin-GFP and immunostained for PRC1, treated with PRC1 siRNA; dependence of the length of immunostained PRC1 signal in HeLa cells expressing tubulin-GFP and immunostained for PRC1 on the distance from the spindle long axis; dependence of the signal intensity *I* of immunostained PRC1 signal in HeLa cells expressing tubulin-GFP and immunostained for PRC1 on the distance from the spindle long axis. Control cells (gray) and PRC1 siRNA-treated cells (black) are shown.

E From left to right: image of PRC1-Alexa Fluor-555 in the spindle of a HeLa cell expressing PRC1-GFP and immunostained for PRC1, treated with PRC1 siRNA; dependence of the length, signal intensity *I*, and I_{cross} of immunostained PRC1 signal in HeLa cells expressing PRC1-GFP and immunostained for PRC1, measured in control cells (gray) and PRC1 siRNA-treated cells (black) on the distance from the spindle long axis.

F From left to right: image of PRC1-GFP in the spindle of a HeLa cell expressing PRC1-GFP and immunostained for PRC1, treated with PRC1 siRNA; dependence of the length, signal intensity *I*, and I_{cross} of PRC1-GFP signal in HeLa cells expressing PRC1-GFP and immunostained for PRC1, measured in control cells (gray) and PRC1 siRNA-treated cells (black) on the distance from the spindle long axis.

Data information: Scale bars, 2 μm; *n*, number of bridging fibers; error bars, s.e.m.; R^2, coefficient of determination; *P*, *P*-value from a *t*-test.
Source data are available online for this figure.

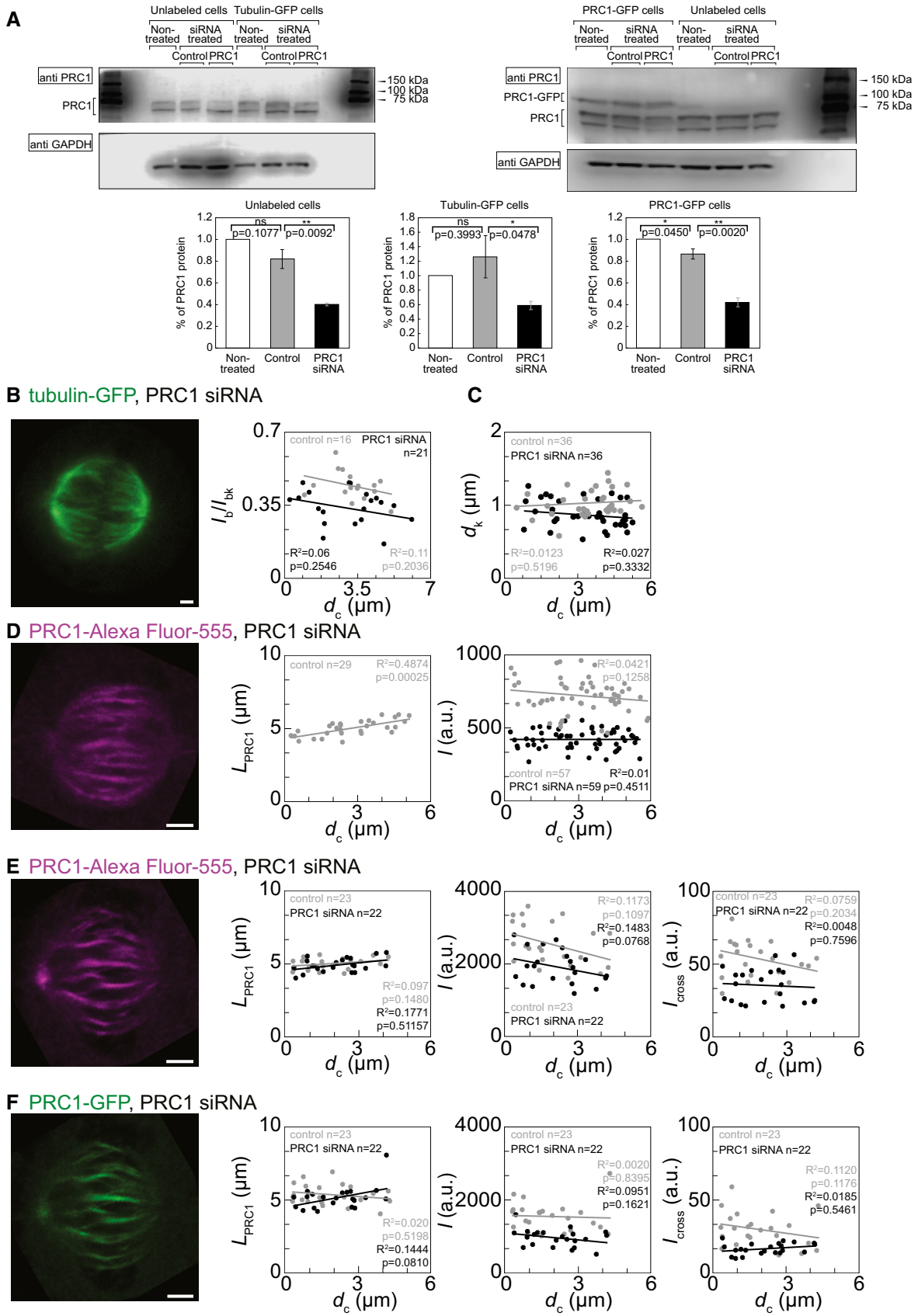
A
Unlabeled cells
Tubulin-GFP cells
PRC1-GFP cells
Unlabeled cells
Non-treated
siRNA treated
Control
PRC1
anti PRC1
PRC1
PRC1-GFP
anti GAPDH
150 kDa
100 kDa
75 kDa
% of PRC1 protein
ns
p=0.1077
**
p=0.0092
p=0.3993
*
p=0.0478
p=0.0450
p=0.0020
Non-treated
Control
PRC1 siRNA
B tubulin-GFP, PRC1 siRNA
C
control n=16
PRC1 siRNA n=21
control n=36
PRC1 siRNA n=36
R²=0.06
p=0.2546
R²=0.11
p=0.2036
R²=0.0123
p=0.5196
R²=0.027
p=0.3332
D PRC1-Alexa Fluor-555, PRC1 siRNA
control n=29
R²=0.4874
p=0.00025
R²=0.0421
p=0.1258
control n=57
PRC1 siRNA n=59
R²=0.01
p=0.4511
E PRC1-Alexa Fluor-555, PRC1 siRNA
control n=23
PRC1 siRNA n=22
R²=0.097
p=0.1480
R²=0.1771
p=0.51157
R²=0.1173
p=0.1097
R²=0.1483
p=0.0768
R²=0.0759
p=0.2034
R²=0.0048
p=0.7596
F PRC1-GFP, PRC1 siRNA
R²=0.020
p=0.5198
R²=0.1444
p=0.0810
R²=0.0020
p=0.8395
R²=0.0951
p=0.1621
R²=0.1120
p=0.1176
R²=0.0185
p=0.5461
d_c (μm)
L_PRC1 (μm)
I_b/I_bk
d_k (μm)
I (a.u.)
I_cross (a.u.)

Figure EV4.