Supplementary figure legends

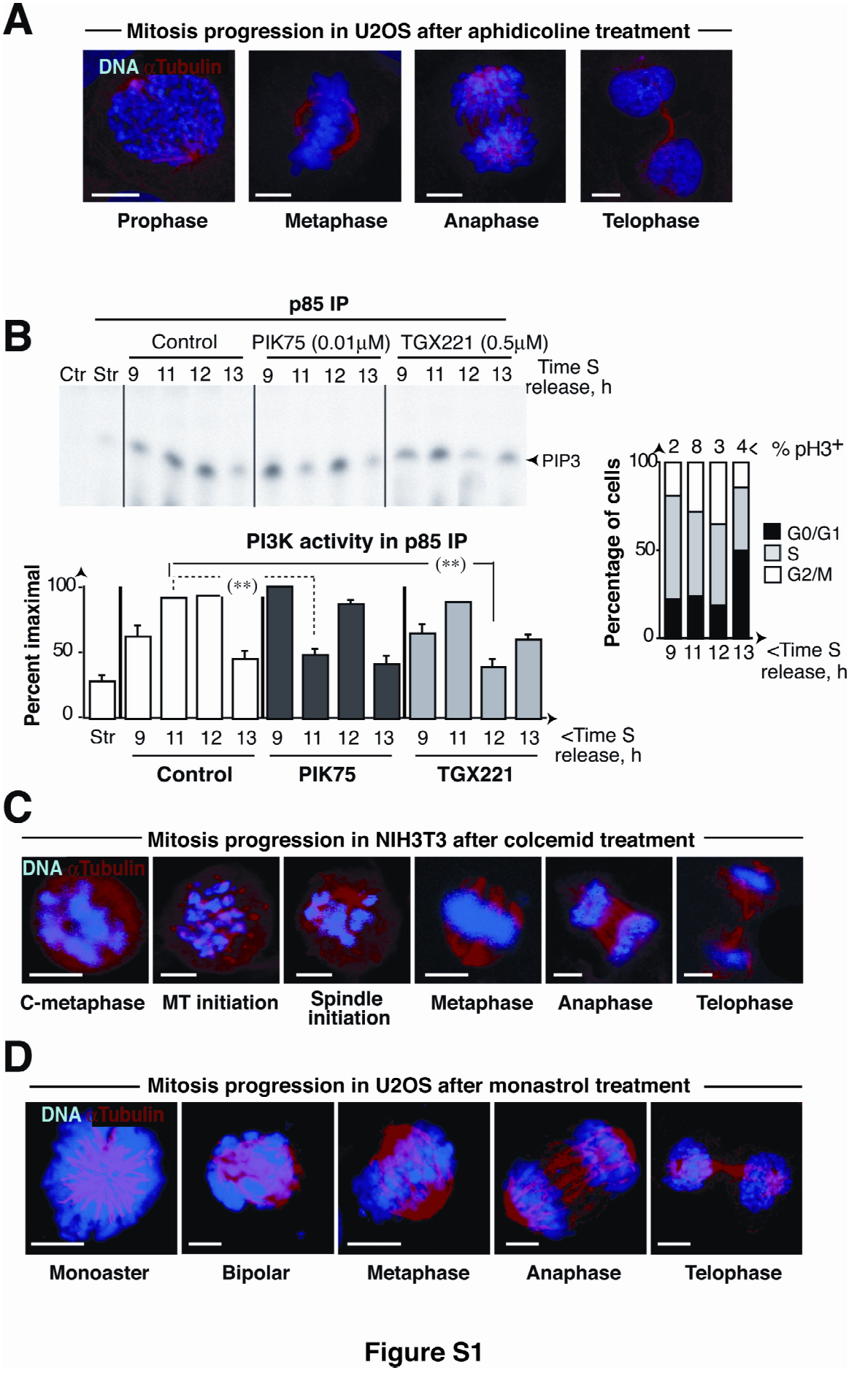

Figure S1

Figure S1. **Mitotic progression after different cell treatments**. (**A,C,D**) Cells were synchronized by various treatments (indicated), then released in fresh medium for different times and examined by IF using anti-α-Tubulin (red) and Hoechst 33258 to stain DNA (blue). Representative images of cell progression through mitosis after aphidicolin (A), colcemid (C), or monastrol (D) treatment. Bar = 5 μm. (**B**) U2OS cells were also synchronized in S phase using aphidicolin and released for different times; cell extracts (250 μg) were immunoprecipitated using pan-p85 Ab. PI3K kinase assays using phosphatidylinositol (4,5)P_2 as substrate were performed alone or with the indicated doses of PIK75 or TGX221. The graph (bottom) shows the percent inhibition at each time point. Bar graph (right) shows the percentage of cells in distinct cell cycle phases; the percentage of phospho-histone H3-positive ($pH3^+$) cells is indicated. Student's *t*-test (**) $P < 0.01$.

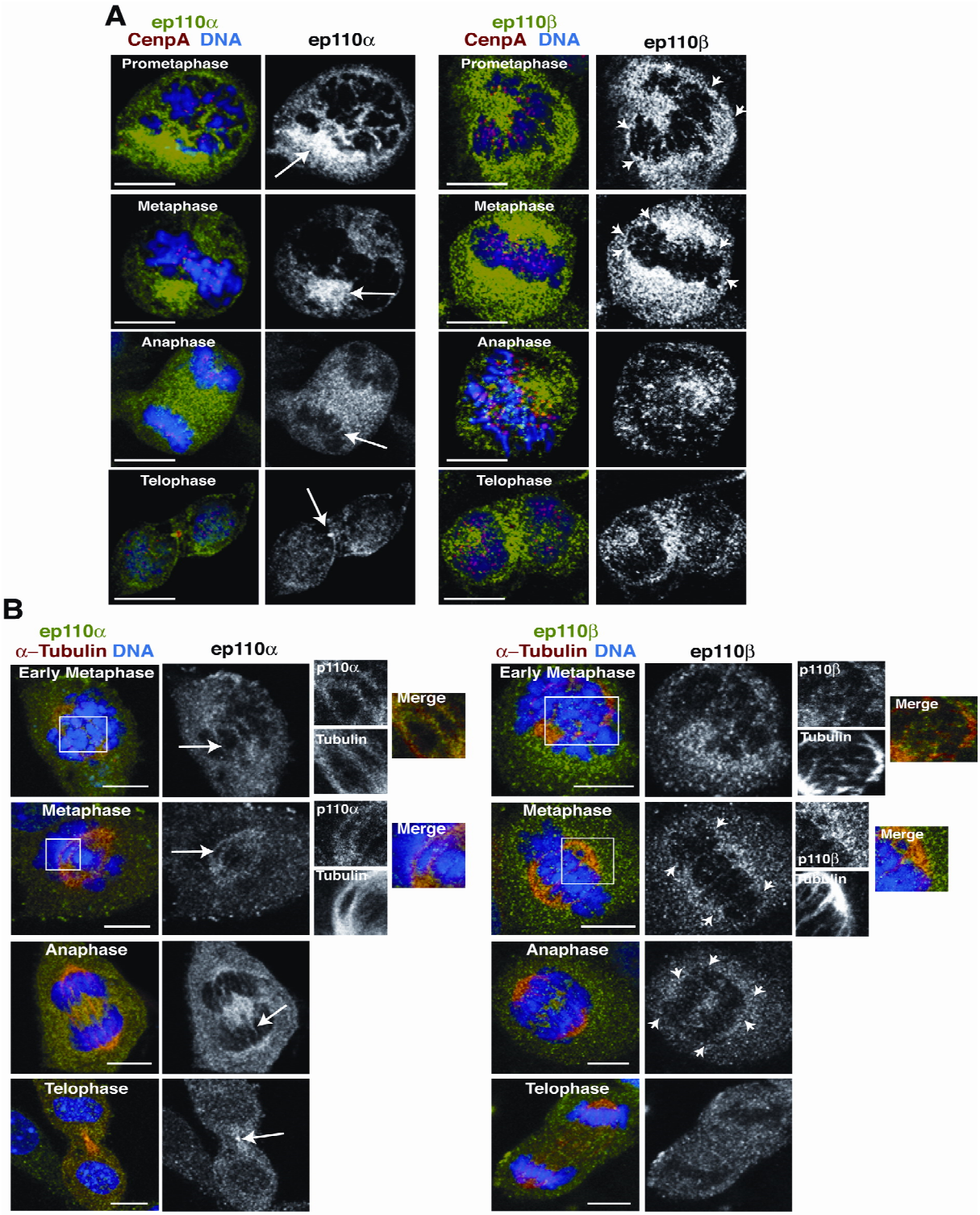

Figure S2

Figure S2. Endogenous p110α and p110β localization in mitosis. (**A**) Representative images of U2OS cells stained with anti-p110α- or p110β-specific antibodies (green) and anti-CenpA (red). (**B**) Representative IF images of mitotic U2OS cells stained with anti-α-Tubulin (red) and -p110α or -p110β (green). Arrows indicate p110α localization in the mitotic spindle, spindle pole or midbody; arrowheads indicate p110β localization near DNA.

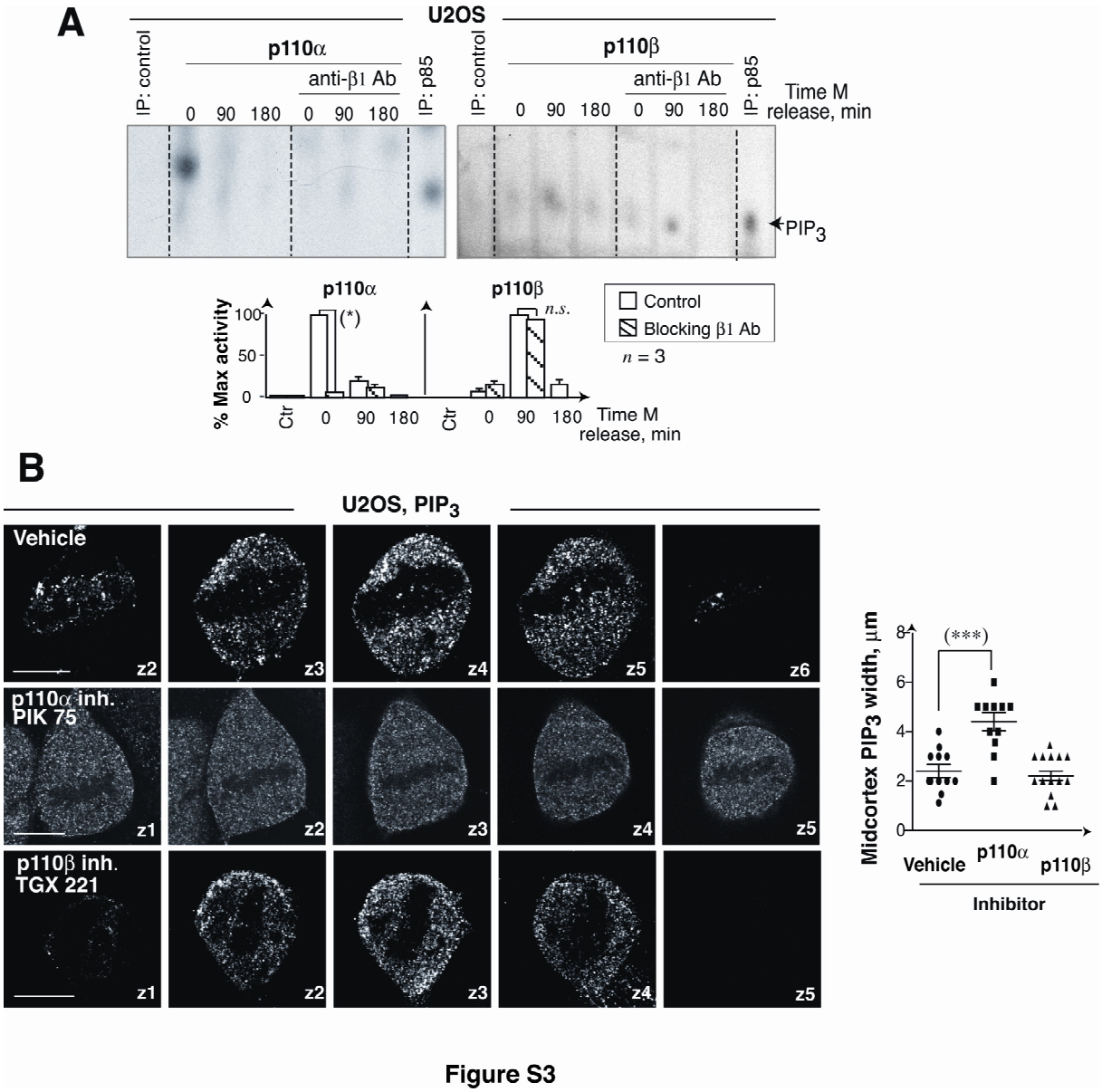

Figure S3

Figure S3. **Inhibition of p110α reduces midcortex PIP_3.** (**A**) U2OS cells were colcemid-arrested (75 ng/ml, 12 h), and released in medium or in the presence of Lia1/2 mAb (100 μg/ml). p110α and p110β were immunopurified from extracts and tested in a kinase assay. Graph shows PIP_3 signal intensity relative to maximum activity (100%; n = 3). (**B**) U2OS were cultured with p110α (PIK75, 0.5 μM) or p110β (TGX221, 30 μM) inhibitors (2 h) and stained with anti-PIP_3 Ab. Representative z-sections (1 μm). Graph shows the percentage of the width (μm, right) of midcortex PIP_3. Bar = 5 μm. Student's t-test (*) P <0.05, (***) P <0.001.

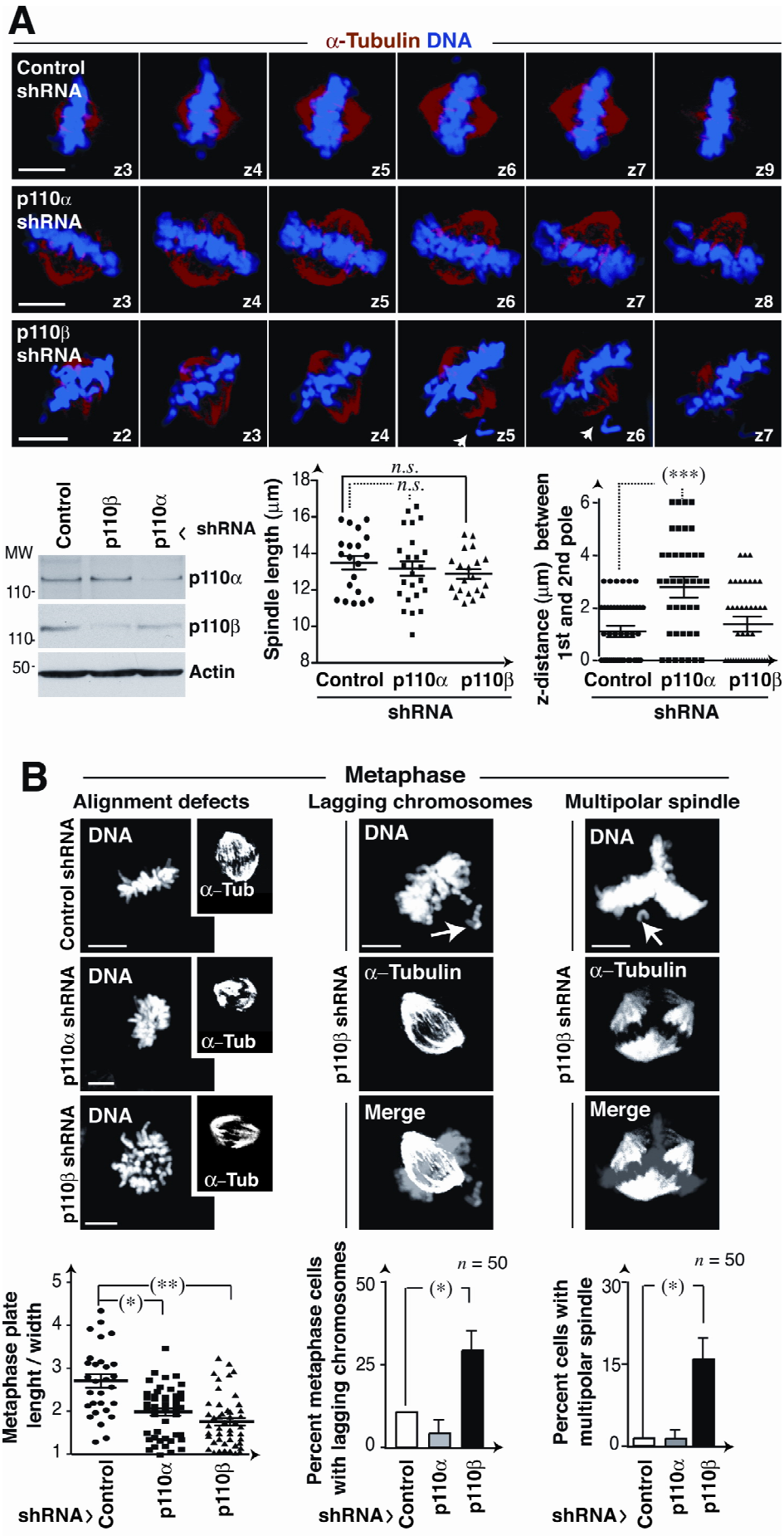
A
α-Tubulin DNA
Control shRNA
p110α shRNA
p110β shRNA
z3
z4
z5
z6
z7
z9
z8
z2
MW
Control
p110β
p110α
shRNA
110-
p110α
110-
p110β
50-
Actin
Spindle length (μm)
n.s.
n.s.
Control p110α p110β
shRNA
z-distance (μm) between 1st and 2nd pole
(***)
Control p110α p110β
shRNA
B
Metaphase
Alignment defects
Lagging chromosomes
Multipolar spindle
Control shRNA
p110α shRNA
p110β shRNA
DNA
α–Tub
α–Tubulin
Merge
Metaphase plate lenght / width
(**)
(*)
shRNA Control p110α p110β
Percent metaphase cells with lagging chromosomes
n = 50
(*)
shRNA Control p110α p110β
Percent cells with multipolar spindle
n = 50
(*)
shRNA Control p110α p110β

Figure S4

Figure S4. Spindle rotation control by p110α. (**A**) U2OS cells transfected with control, p110α- or β-specific shRNA (48 h) were analyzed by IF using anti-α-Tubulin antibody and Hoechst 33258. Representative metaphase serial z-sections (1 μm). Arrow indicates a misaligned chromosome after p110β shRNA transfection. WB shows p110α and p110β levels. Graphs show spindle length (μm; left) and z-distance between poles (μm; right). (**B**) U2OS cells in exponential growth were treated and tested as in (A). Images show metaphase DNA (Hoechst 33258) and α-Tubulin-labeled mitotic spindle. Graph indicates length/width ratio of metaphase plates (left); each dot represents an individual cell. Bar graphs show the percentage of cells with the indicated phenotypes (center and right; n = 50 cells in 3 assays). Bar = 5 μm. Student's t-test (*) P <0.05; (**) P <0.01; (***) P <0.001; n.s. = not significant.

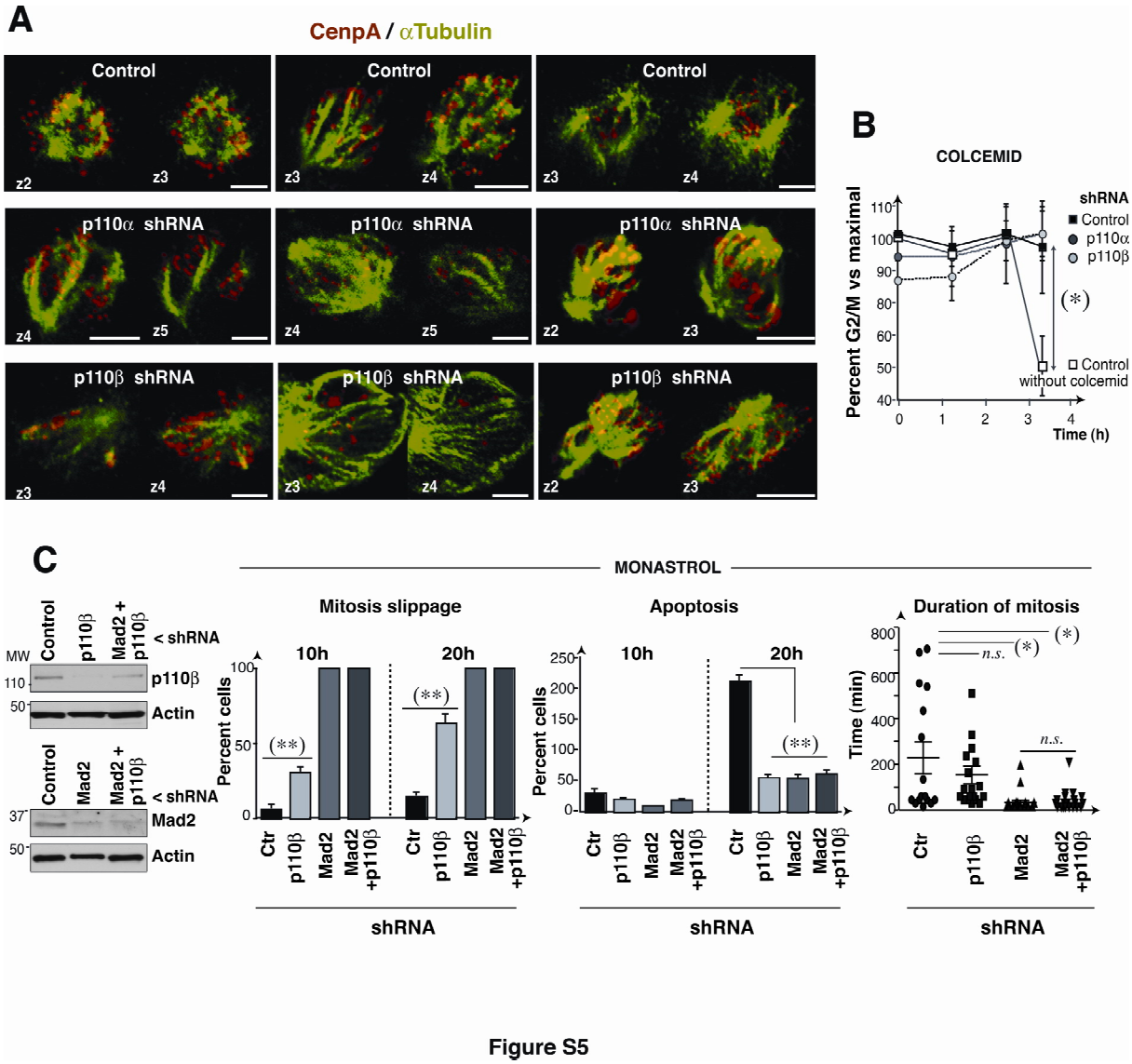

Figure S5

Figure S5. p110β depletion does not impair colcemid-induced SAC. (**A**) Representative z-sections of U2OS cells transfected with control, p110α or p110β shRNA (48 h) and stained for α-Tubulin and -CenpA. (**B**) SAC efficacy was determined in U2OS cells transfected with control, p110α or p110β shRNA (48 h) by counting cells that remained in G2/M after preincubation with colcemid (100 ng/ml, 16 h), and an additional incubation with fresh colcemid (1 to 4 h).Colcemid deprivation in control cells was used to permit mitosis exit. Relative percentage of G2/M cells (mean ± SD; $n = 3$). (**C**) U2OS cells transfected with shRNA control or shRNA for p110β, Mad2 or both (48 h) and maintained in monastrol (4 h). The reduction of protein expression was tested in WB. Graphs (left and centre) show the percentage of cells after 10 or 20h additional incubation in monastrol with indicated phenotypes compared to the number of mitotic cells. Graph (right) shows the time of mitosis. Depletion of Mad2, but not of p110β, reduced mitosis time. Student's *t*-test (**) $P < 0.01$.

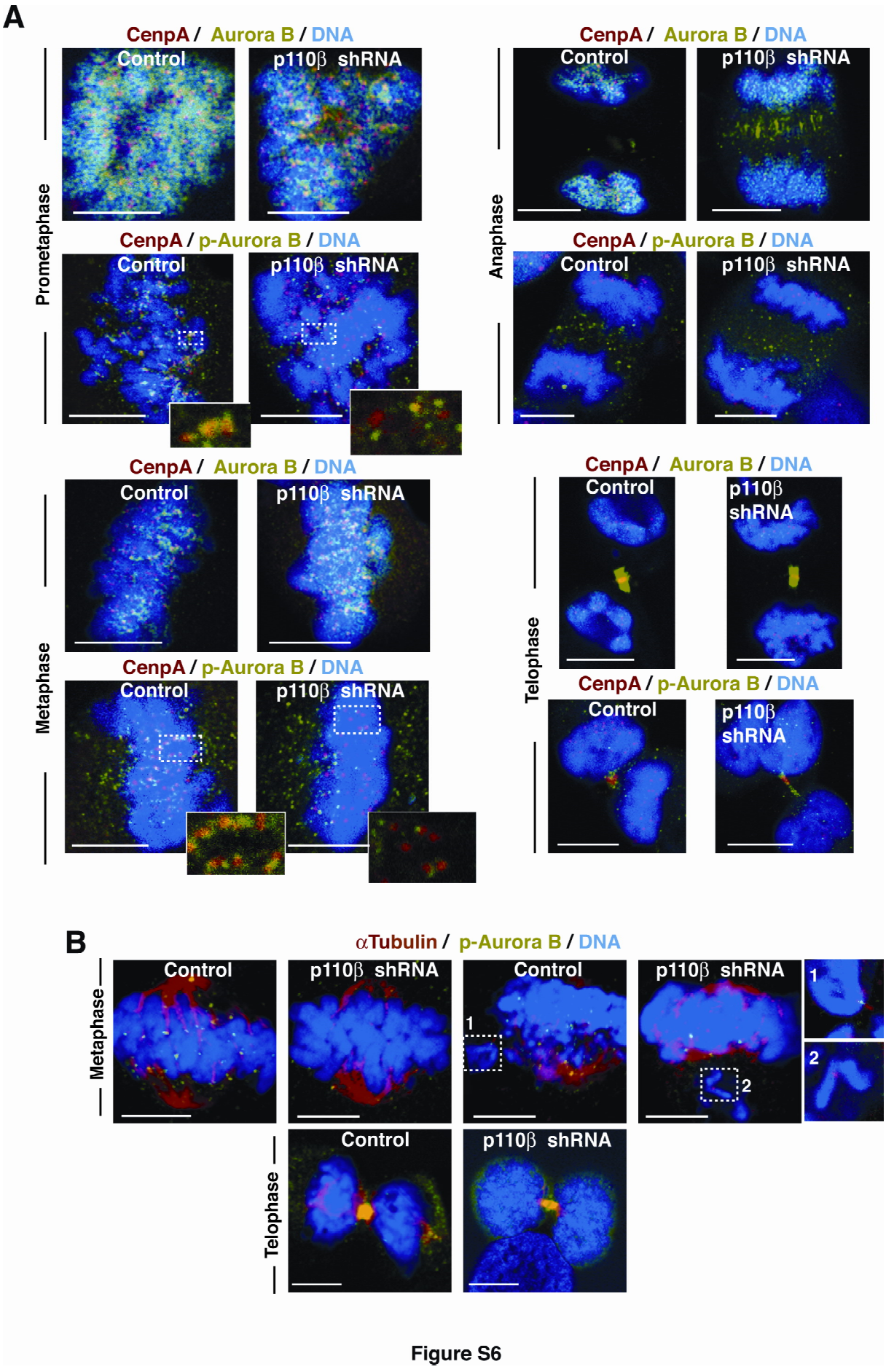
A
CenpA / Aurora B / DNA
Control
p110β shRNA
Prometaphase
CenpA / p-Aurora B / DNA
Control
p110β shRNA
CenpA / Aurora B / DNA
Control
p110β shRNA
Anaphase
CenpA / p-Aurora B / DNA
Control
p110β shRNA
CenpA / Aurora B / DNA
Control
p110β shRNA
Metaphase
CenpA / p-Aurora B / DNA
Control
p110β shRNA
CenpA / Aurora B / DNA
Control
p110β shRNA
Telophase
CenpA / p-Aurora B / DNA
Control
p110β shRNA
B
αTubulin / p-Aurora B / DNA
Metaphase
Control
p110β shRNA
Control
p110β shRNA
1
2
Telophase
Control
p110β shRNA

Figure S6

Figure S6. p110β regulates p-Aurora B in kinetochores. (**A,B**) z-sections of control or p110β knocked-down U2OS cells (48 h) stained with anti-Aurora B or -p-Aurora B (green) and anti-CenpA (A) or -α-Tubulin (B) (red), and Hoechst 33258 (blue). Bar = 5 μm.

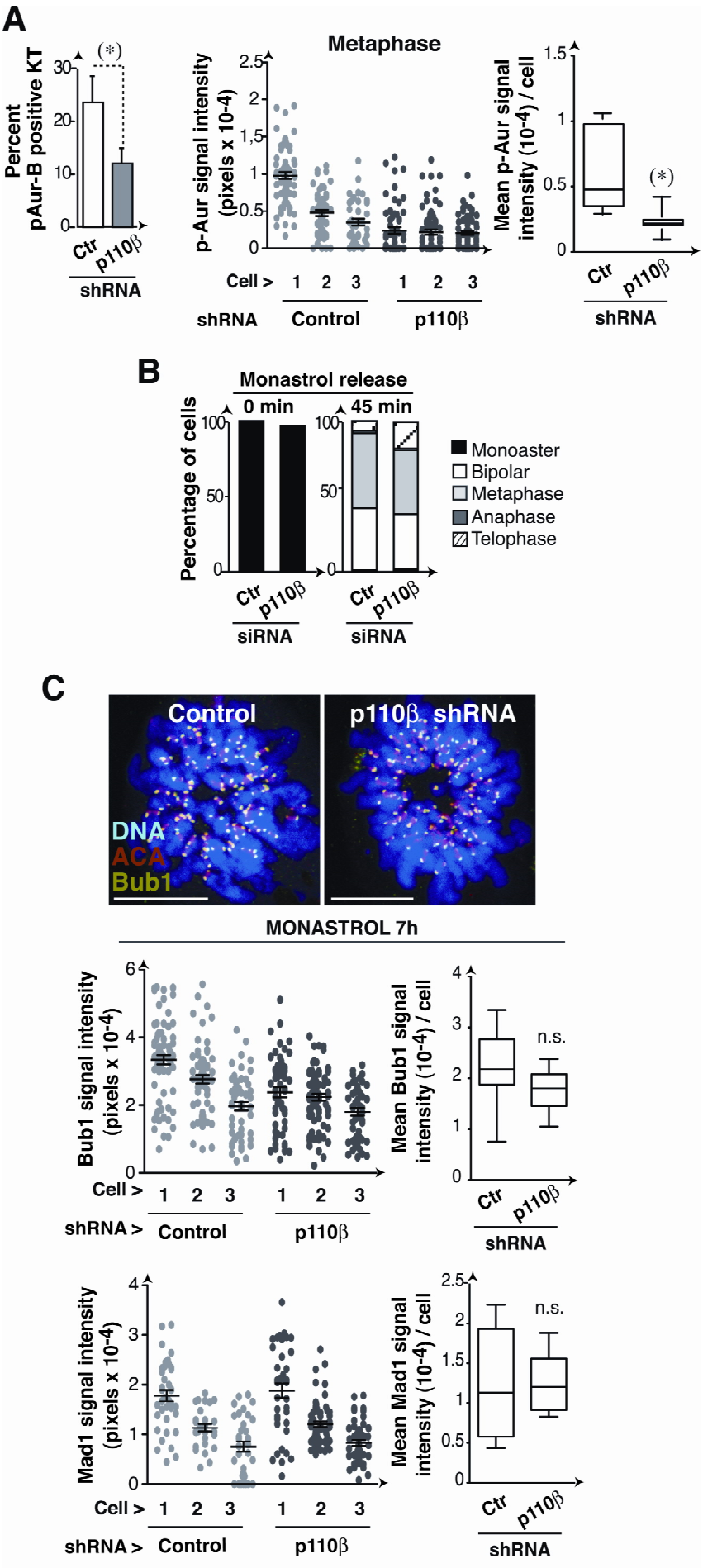
A
Percent pAur-B positive KT
(*)
Ctr
p110β
shRNA
Metaphase
p-Aur signal intensity (pixels x 10-4)
Cell >
shRNA
Control
p110β
Mean p-Aur signal intensity (10-4) / cell
B
Monastrol release
0 min
45 min
Percentage of cells
Monoaster
Bipolar
Metaphase
Anaphase
Telophase
siRNA
C
Control
p110β shRNA
DNA
ACA
Bub1
MONASTROL 7h
Bub1 signal intensity (pixels x 10-4)
Mean Bub1 signal intensity (10-4) / cell
n.s.
Mad1 signal intensity (pixels x 10-4)
Mean Mad1 signal intensity (10-4) / cell

Figure S7

Figure S7. p110β regulates Bub1 signal in kinetochores. (**A**) Control, p110α or p110β knocked-down U2OS cells (48 h) were monastrol-treated (100 μM, 4 h), released in medium (60 min) and stained with CenpA, Aurora B or p-Aurora B Ab and Hoechst 33258. Graphs show the percentage of p-Aurora B positive KT in metaphase. (**B**) U2OS cells were transfected with control or p110β siRNA, synchronized with monastrol (100 μM, 4 h), and collected at different times after monastrol deprivation. Graph shows the percentage of cells in the different mitotic phases at 0 or 45 min after monastrol release. (**C**) Control or p110β knocked-down U2OS cells (48 h) were maintained in monastrol for 7 h. Representative confocal z-stacks of IF performed with Bub1 or Mad1 Ab and Hoechst 33258. We measured the fluorescence intensity in arbitrary units (AU, pixels) for each KT (each dot represents a single KT, n = 3 cells of each type). We also calculated the mean fluorescence intensity per cell and represented (right) the mean ± SD of these values for each cell type (n = 15 cells). Bar = 5 μm.) (*) Student's t-test with Welch´s correction P <0.05.