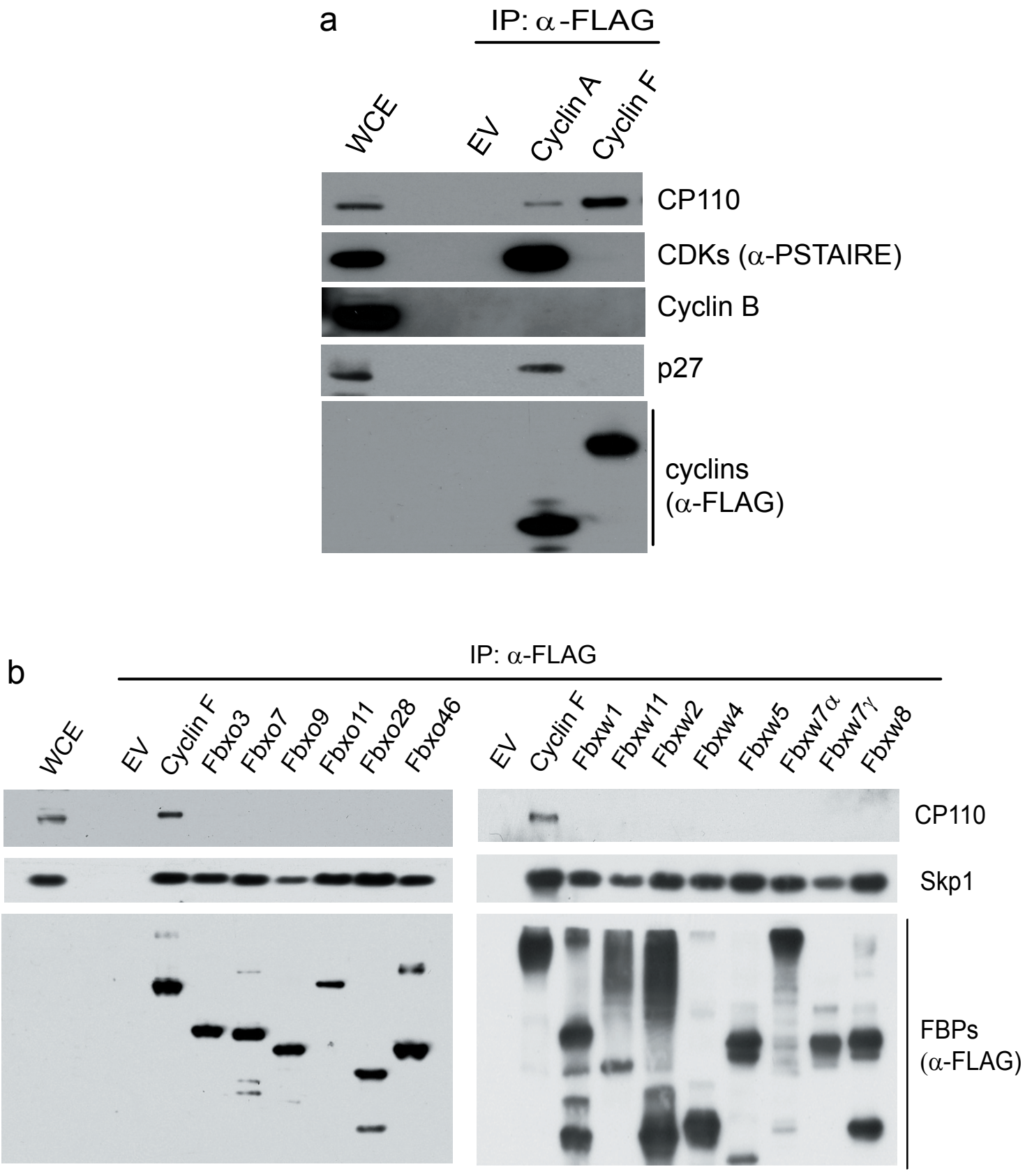
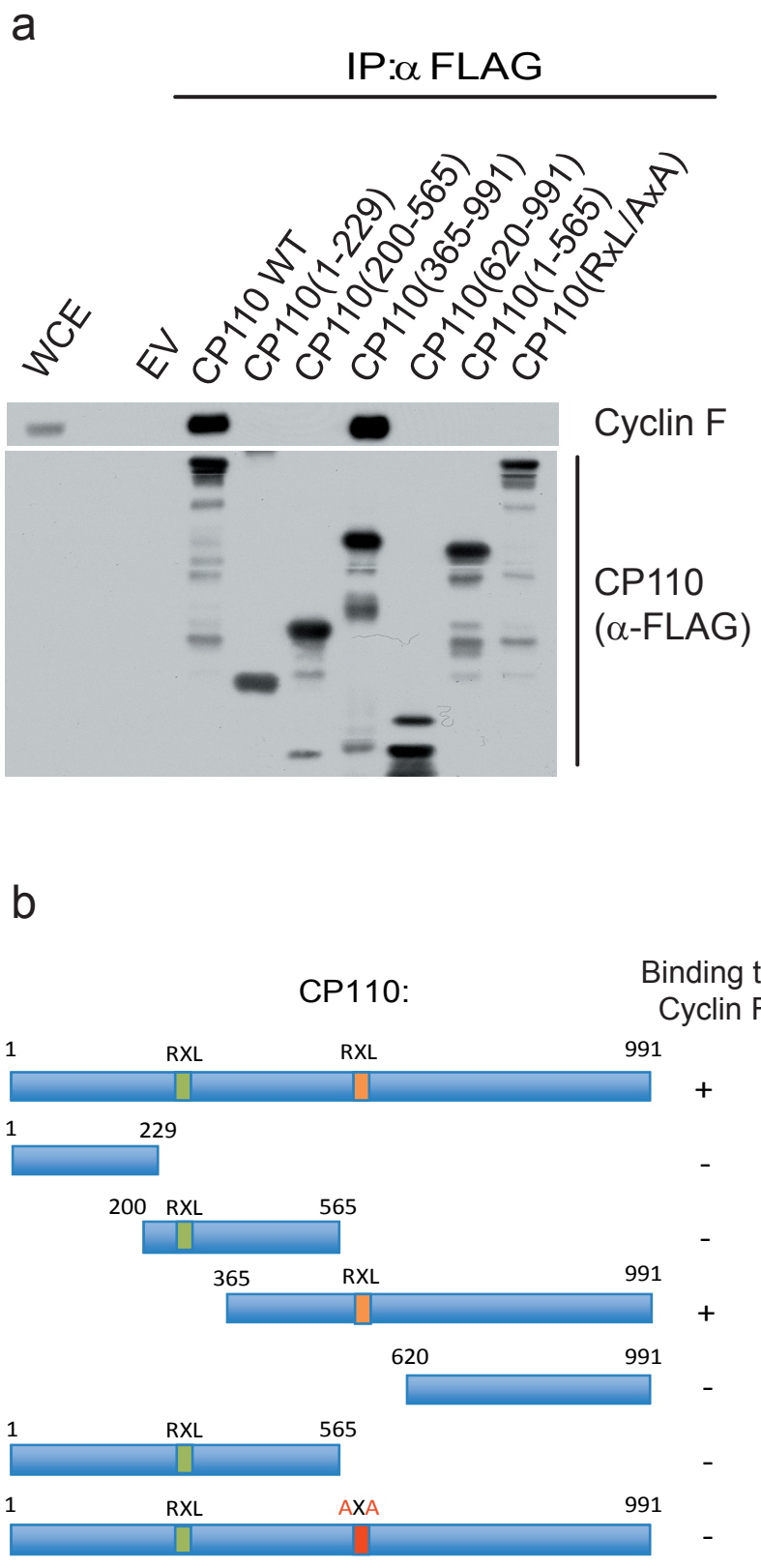


Supplementary Figure 1. D'Angiolella *et al.*

Supplementary Figure 1. Cyclin F interacts with CP110 but not CDKs

a, Cyclin F does not bind CDKs. HEK-293T cells were transfected with an empty vector (EV), FLAG-tagged Cyclin A, or FLAG-tagged Cylin F. Whole cell extracts (WCE) were immunoprecipitated (IP) with anti-FLAG resin, and immunoprecipitates were probed with antibodies to the indicated proteins. The anti-PSTAIRE antibody was generated against the sequence EGVPSTAIREISLLKE, a 16 amino acid sequence present in Cdk1, Cdk2, Cdk3, and other CDKs (ref. 33).

b, Cyclin F specifically interacts with CP110 in cultured cells. HEK-293T cells were transfected with empty vector (EV) or the indicated FLAG-tagged F-box protein constructs (FBPs). Whole cell extracts were immunoprecipitated (IP) with anti-FLAG resin, and immunoprecipitates were probed with antibodies to the indicated proteins.

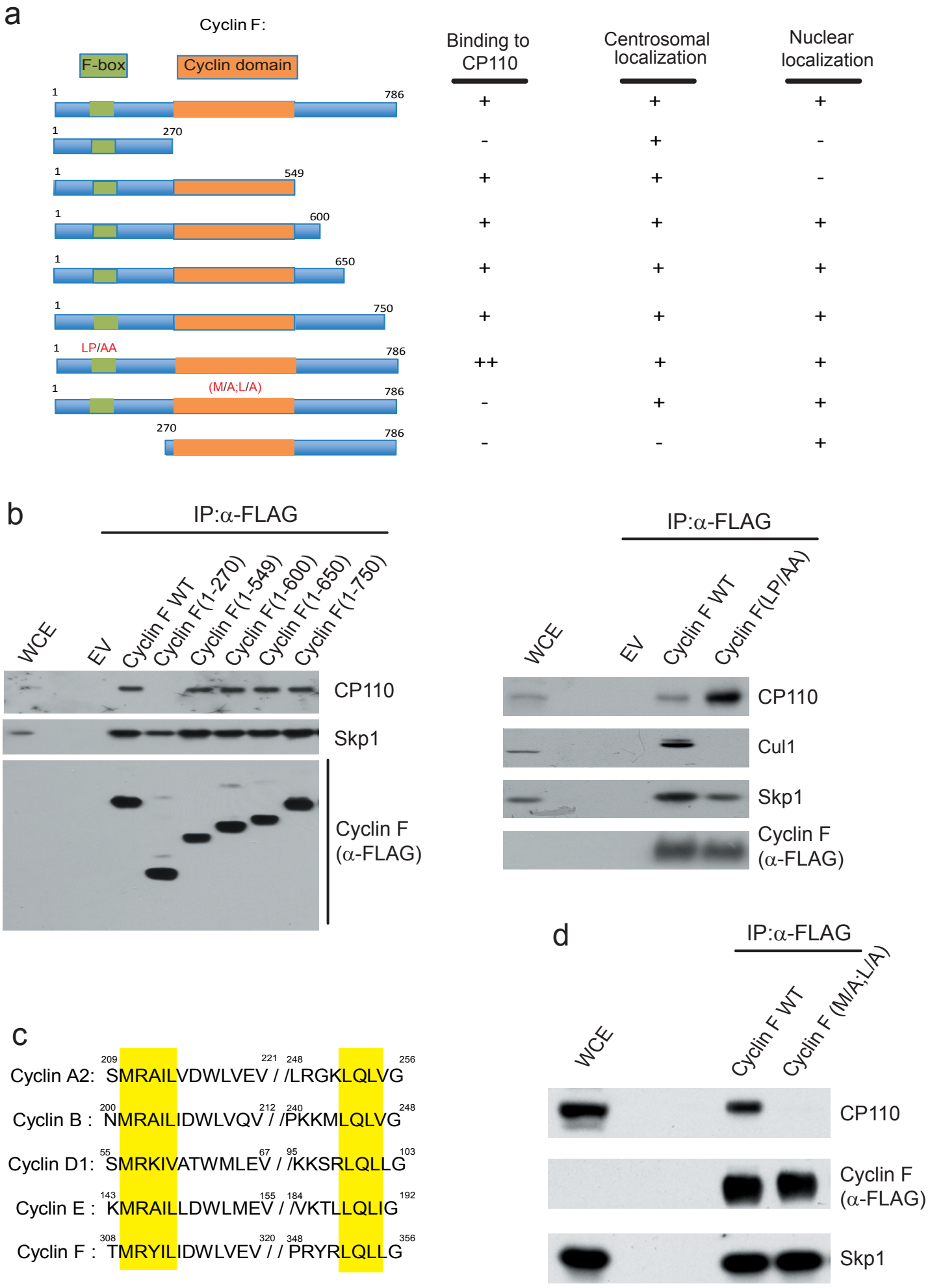


Supplementary Figure 2. D'Angiolella *et al.*

Supplementary Figure 2. The RXL cyclin binding motif of CP110 is necessary for Cyclin F binding

a, HEK-293T cells were transfected with an empty vector (EV), FLAG-tagged wild type CP110, or the indicated FLAG-tagged CP110 mutants. Whole cell extracts (WCE) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins.

b, Schematic representation of CP110 mutants. CP110 mutants that interacted with endogenous Cyclin F are designated with the symbol (+).



Supplementary Figure 3. D'Angiolella *et al.*

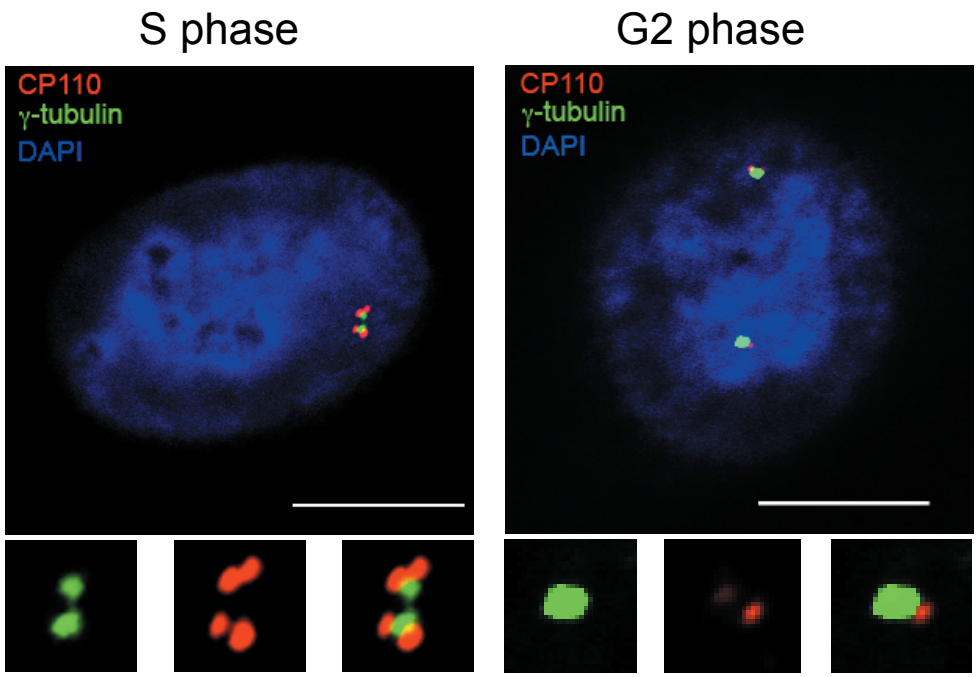
Supplementary Figure 3. Cyclin F binding to CP110 requires the hydrophobic patch of Cyclin F

a, Schematic representation of Cyclin F mutants. Binding to endogenous CP110, centrosomal localization, and nuclear localization of wild type Cyclin F and Cyclin F mutants are designated with the symbol (+).

b, HEK-293T cells were transfected with an empty vector (EV) or the indicated FLAG-tagged Cyclin F mutants. Whole cell extracts (WCE) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins.

c,. Alignment of the amino acid regions corresponding to the hydrophobic patch in human Cyclin F and the previously reported hydrophobic patches (which are responsible for the binding to the RXL motif of substrates) in other cyclins (highlighted in yellow).

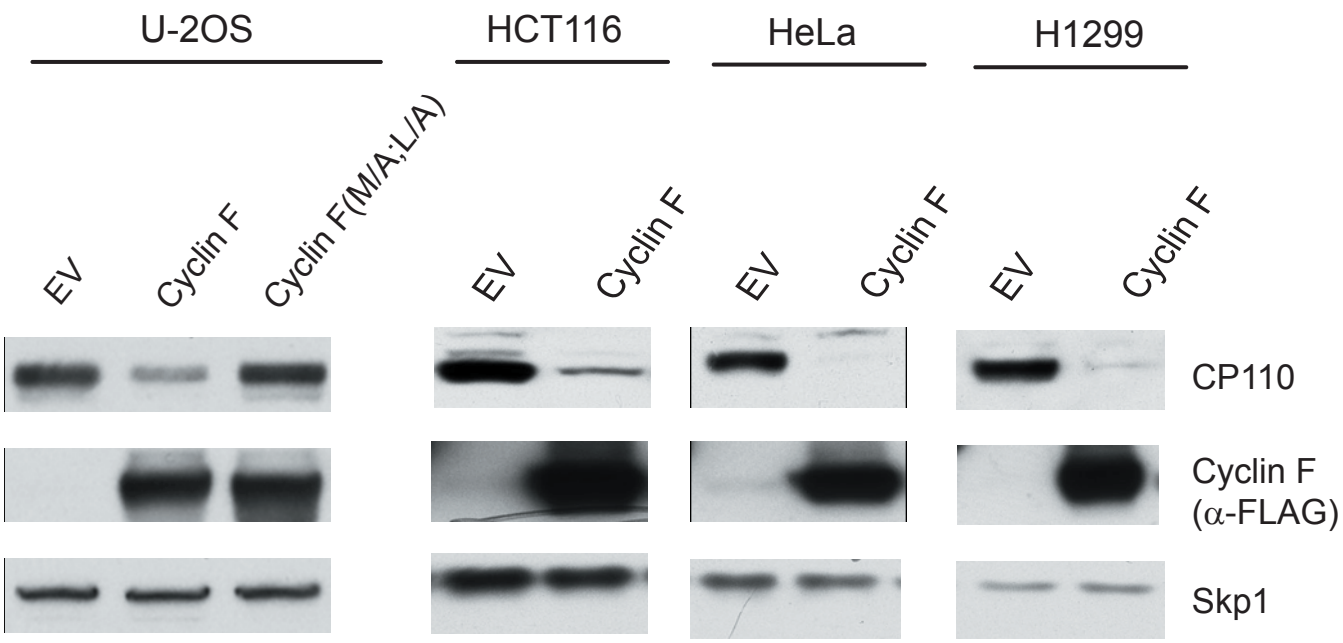
d, HEK-293T cells were transfected with either wild type, FLAG-tagged Cyclin F, or FLAG-tagged Cyclin F(M/A;L/A), a mutant in the hydrophobic patch. Whole cell extracts (WCE) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins.



Supplementary Figure 4. D'Angiolella *et al.*

Supplementary Figure 4. CP110 levels on the centrioles decrease in G2 cells

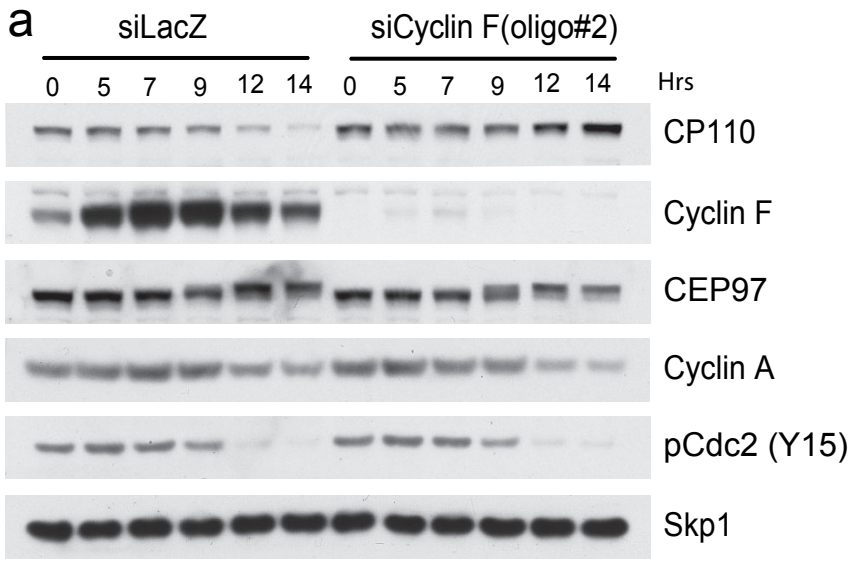
U-2OS cells were synchronized at G1/S using a double-thymidine block before release into fresh medium. Cells were then fixed and incubated with an anti-CP110 antibody (red) and anti-γ-tubulin antibody (green). DNA was stained with DAPI. Insets show magnified views of the two centrosomes (arrows). Scale bar = 10 μM. Confocal microscopy was used to visualize the cells. The figure shows representative cells in S and G2. In S phase, the two γ-tubulin dots are distinct but adjacent. In G2, they are clearly separated by at least 2 μM.

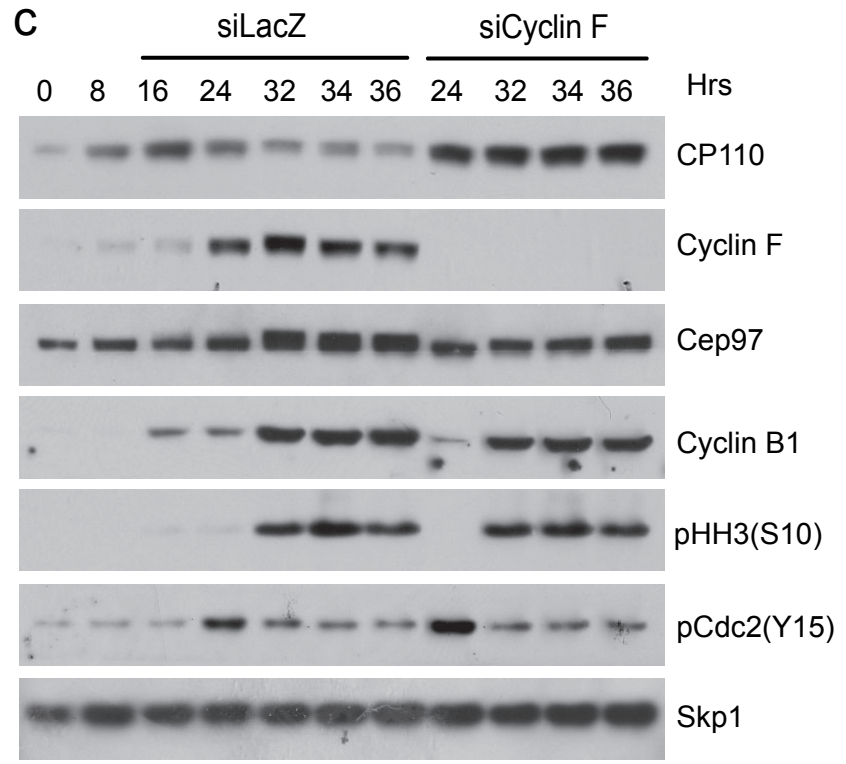


Supplementary Figure 5. D'Angiolella *et al.*

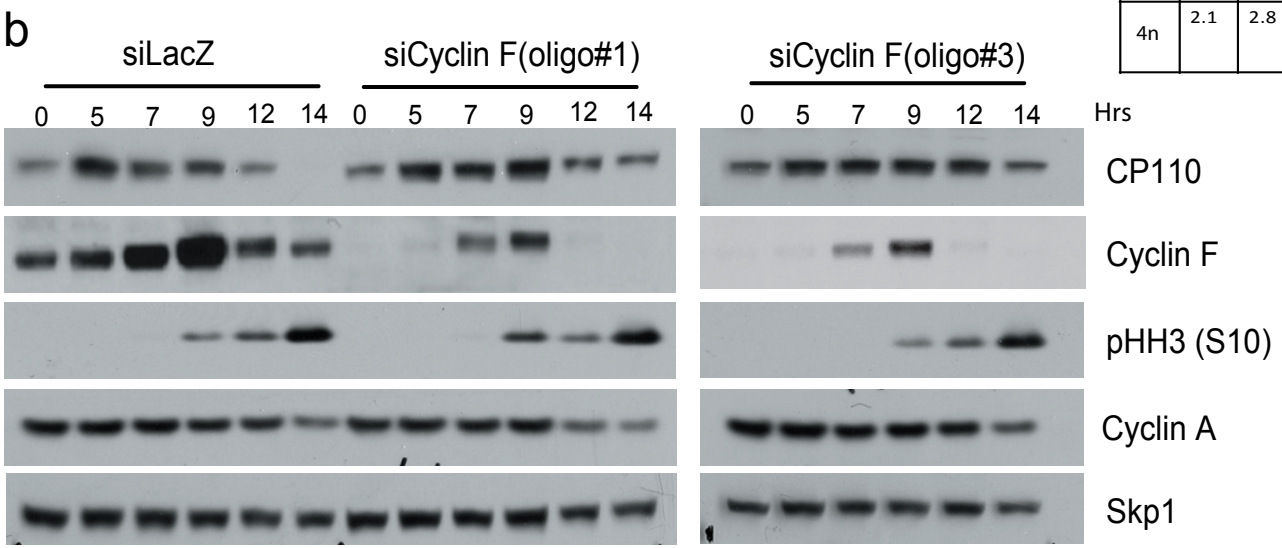
Supplementary Figure 5. Forced expression of Cyclin F induces a reduction in CP110 levels

U-2OS, HeLa, HCT116, and H1299 cells were transfected with an empty vector (EV) or constructs encoding FLAG-tagged, wild type Cyclin F, or FLAG-tagged Cyclin F(M/A;L/A), as indicated. Forty-eight hours after transfection, cells were collected, lysed, and immunoblotted with antibodies to the indicated proteins.





2n	97.1	95.1	18.2	12.3	6.8	6.5	6.5	13.4	6.4	9.38	7.42
>2n <4n	0.8	2.1	63.7	30.2	14.8	10.8	4.8	28.2	11.1	9.45	4.05
4n	2.1	2.8	18.1	57.5	78.4	82.7	88.7	58.4	82.5	81.1	88.5



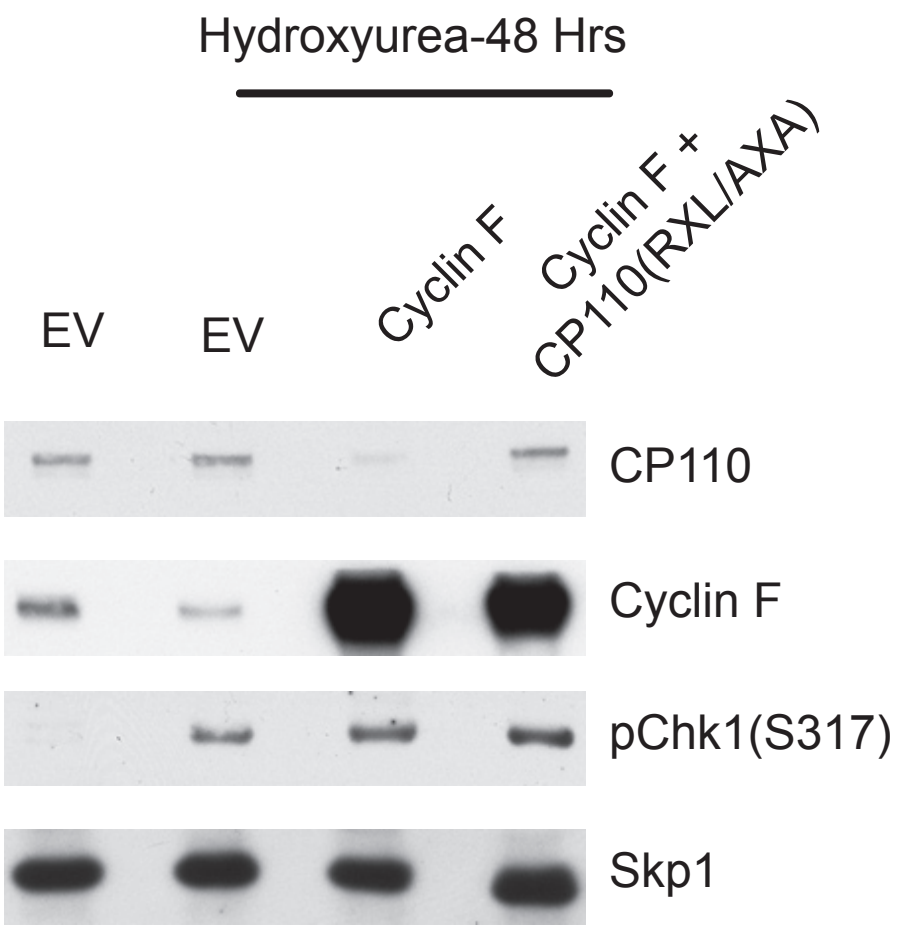
Supplementary Figure 6. D'Angiolella *et al.*

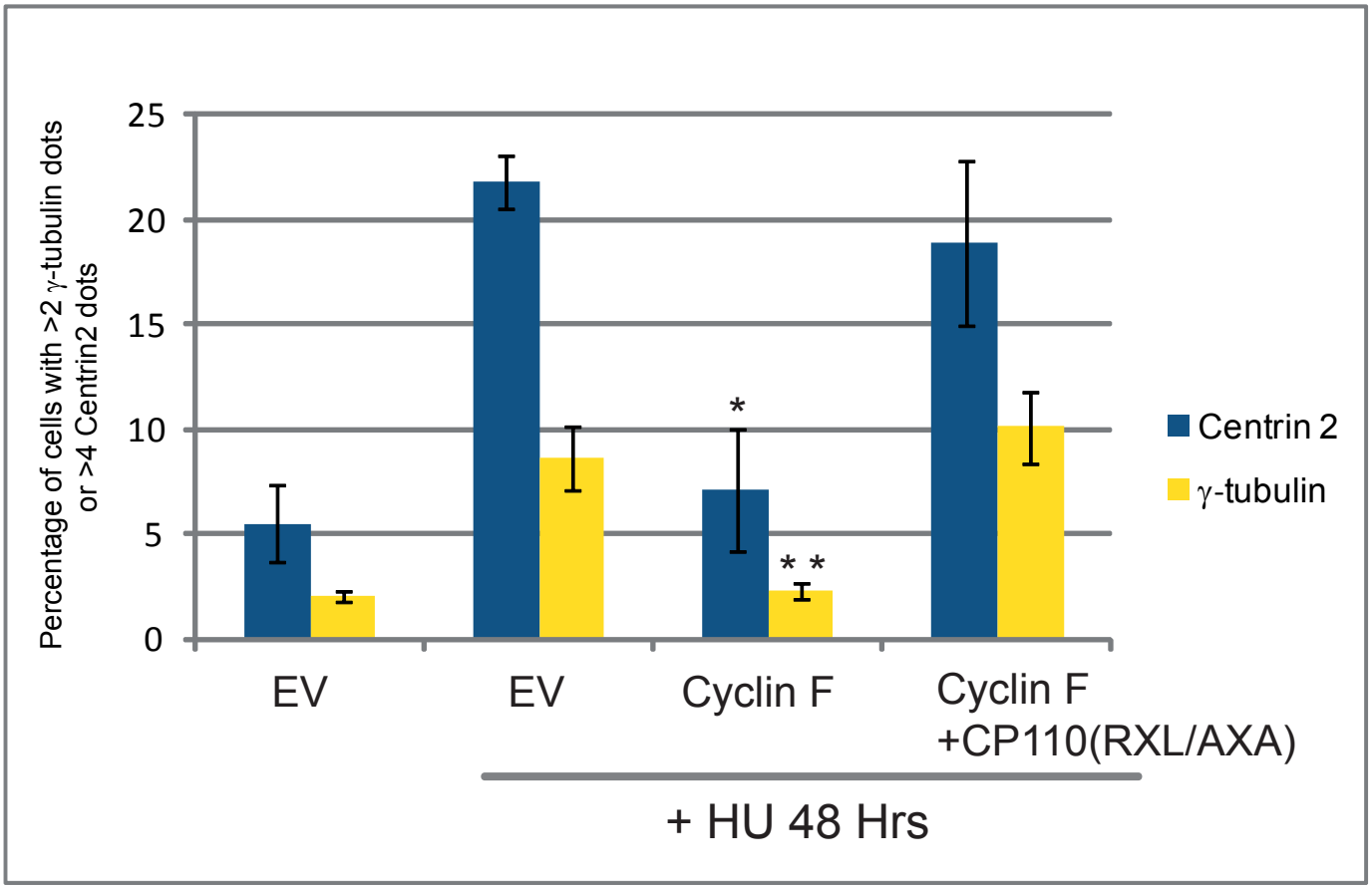
Supplementary Figure 6. Silencing of Cyclin F in G2 results in CP110 stabilization

a, U-2OS cells were transfected with short interfering RNAs (siRNAs) to either a non-relevant mRNA (LacZ) or Cyclin F mRNA (oligo #2). Cells were then synchronized at G1/S using a double-thymidine block before release into fresh medium. Cells were then collected at the indicated times, lysed, and immunoblotted with antibodies to the indicated proteins.

b, HeLa cells were transfected with siRNAs to either a non-relevant mRNA (LacZ) or Cyclin F mRNA (oligos # 1 and 3, as indicated). Cells were then synchronized at G1/S using a double-thymidine block before release into fresh medium. Cells were then collected at the indicated times, lysed, and immunoblotted with antibodies to the indicated proteins.

c, RPE1-hTERT cells were transfected with siRNAs to either a non-relevant mRNA (LacZ) or Cyclin F mRNA (oligo #2). Cells were then synchronized in G0/G1 by serum starvation for 72 hours before release into fresh medium containing serum. Cells were then collected at the indicated times, lysed, and immunoblotted with antibodies to the indicated proteins. DNA content was monitored by flow cytometry.

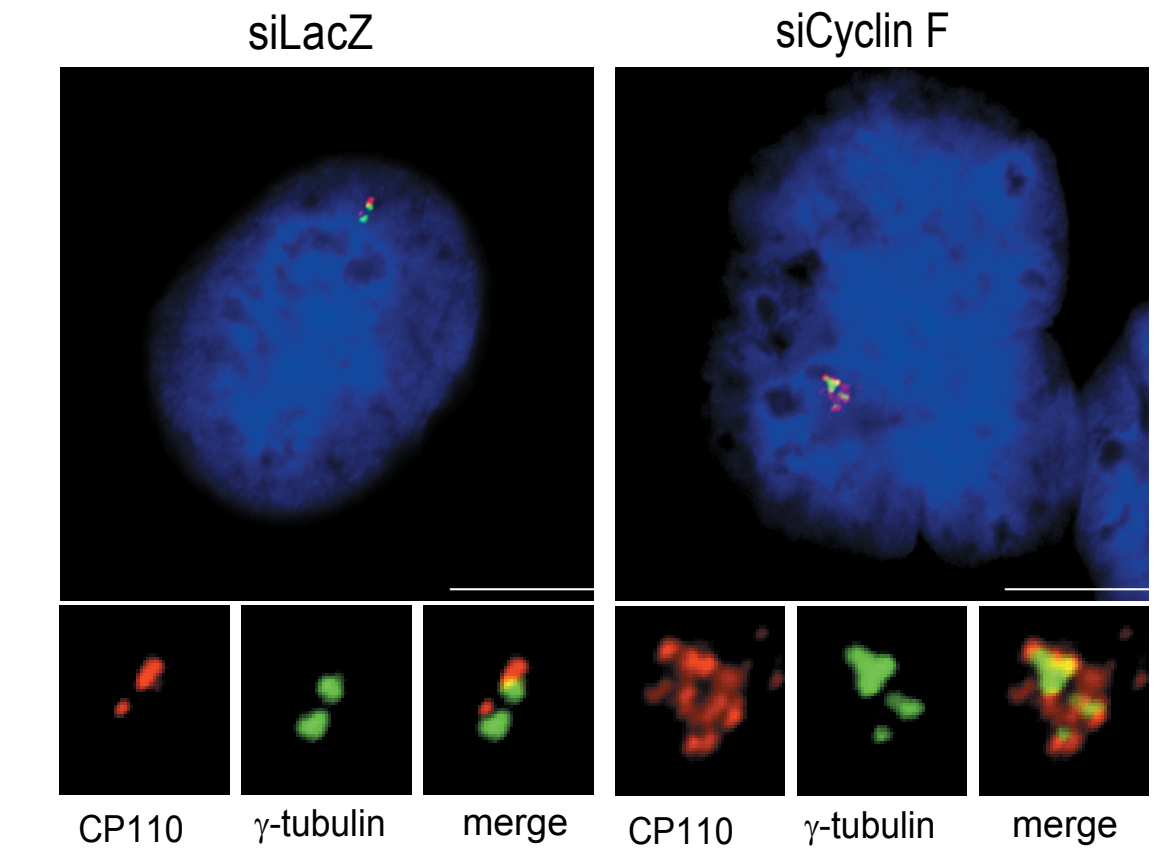


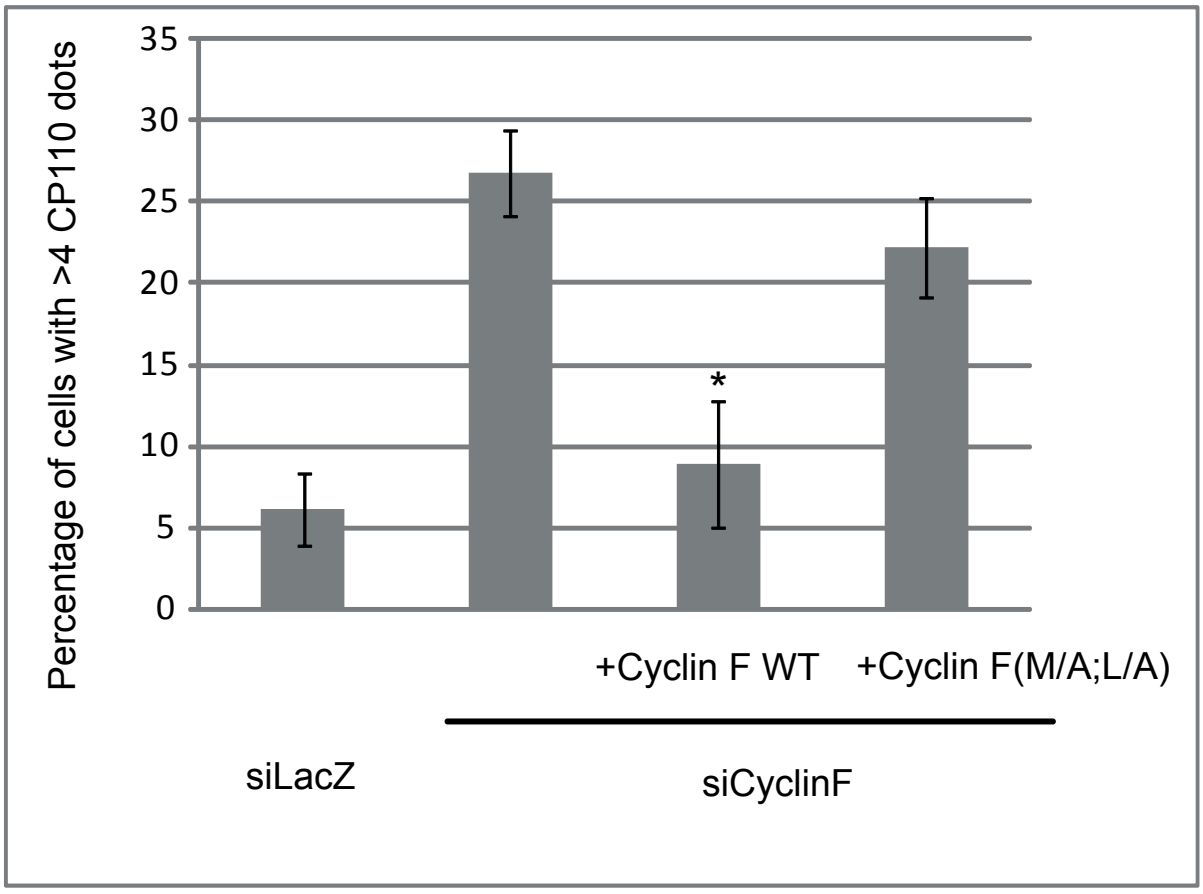


Supplementary Figure 7. D'Angiolella *et al.*

Supplementary Figure 7. Cyclin F overexpression inhibits centrosome reduplication in S phase-arrested cells

U-2OS cells were transfected with an empty vector (EV) or a construct encoding FLAG-tagged Cyclin F in the presence or absence of CP110(RxL/AxA), a mutant that does not interact with Cyclin F. Cells were then treated for 48 hours with hydroxyurea, to induce a block in S phase, before harvesting, lysis, and immunoblotting with antibodies to the indicated proteins. The graph shows the percentages of cells with excess Centrin 2 foci (more than four per cell) and excess γ-tubulin dots (more than two per cell). The data represent the average from three independent experiments, with at least 100 cells counted per experiment. Error bars indicate +/-SD. *= p=0.002; **= p=0.001 (calculated by ANOVA).

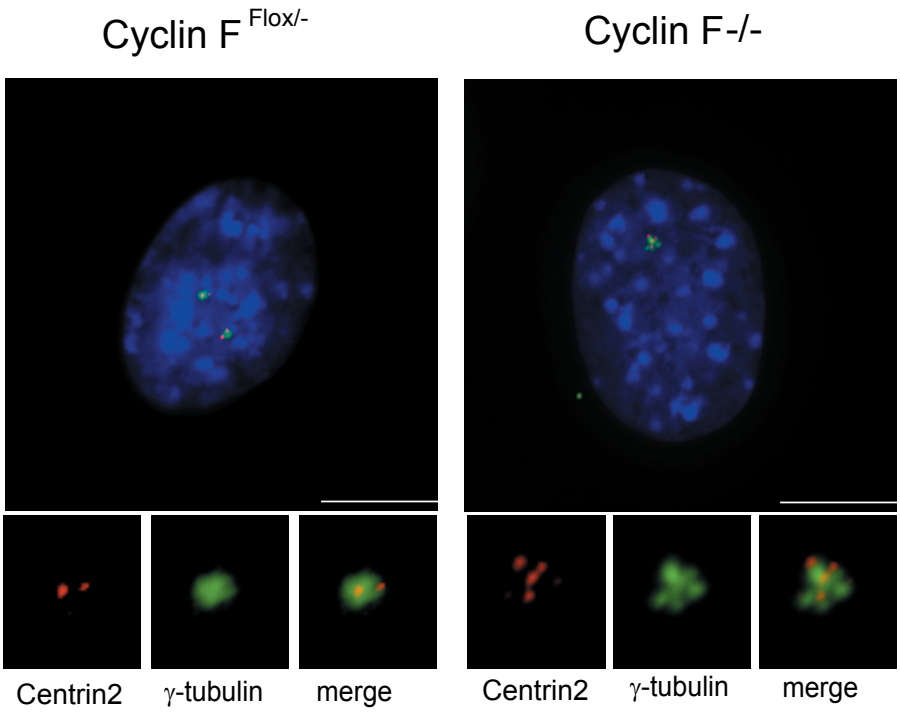


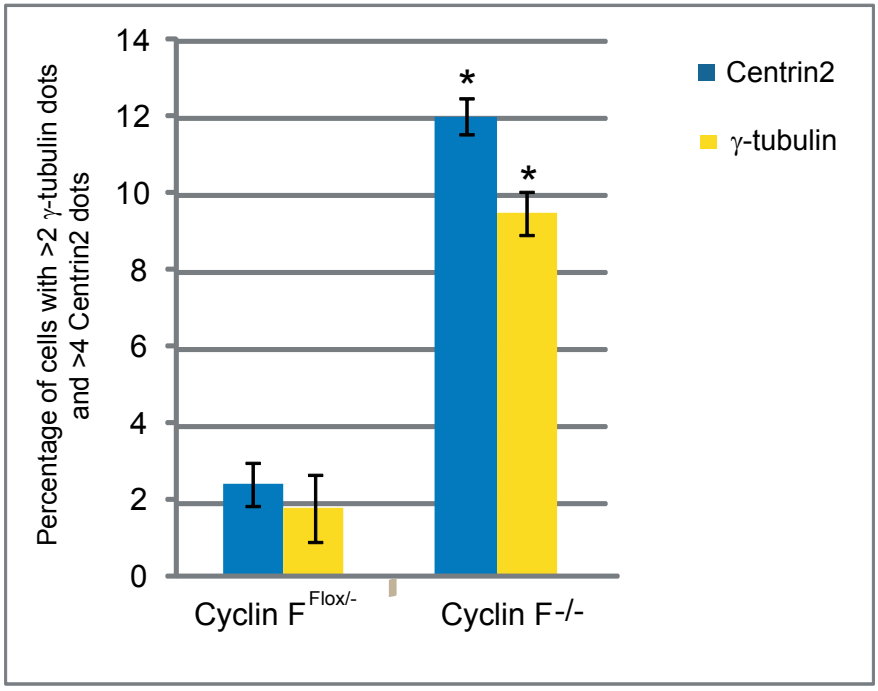


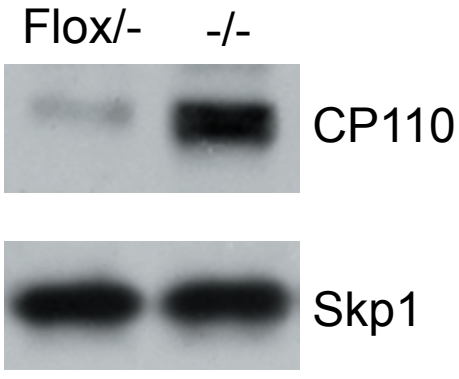
Supplementary Figure 8. D'Angiolella *et al.*

Supplementary Figure 8. Silencing of Cyclin F induces an increase in the number of CP110 foci

U-2OS cells were transfected with siRNAs to a non-relevant mRNA (LacZ) or to Cyclin F mRNA and synchronized by a double-thymidine block. Cells were fixed at nine hours after release from the block and incubated with an anti-CP110 antibody (red) and an anti-γ-tubulin antibody (green). DNA was stained with DAPI. Insets show magnified views of centrosomes. Scale bar = 10 μM. In parallel experiments, cells treated with siRNA oligos to Cyclin F were also transfected with constructs encoding either a wild type, but siRNA-insensitive, Cyclin F or an siRNA-resistant Cyclin F mutant unable to bind CP110 [Cyclin F(M/A;L/A)]. The graph shows the percentages of cells with excess CP110 dots (more than four per cell). The data represent the average from three independent experiments, with at least 100 cells counted per experiment. Error bars indicate +/-SD. *= p=0.001 (calculated with ANOVA).



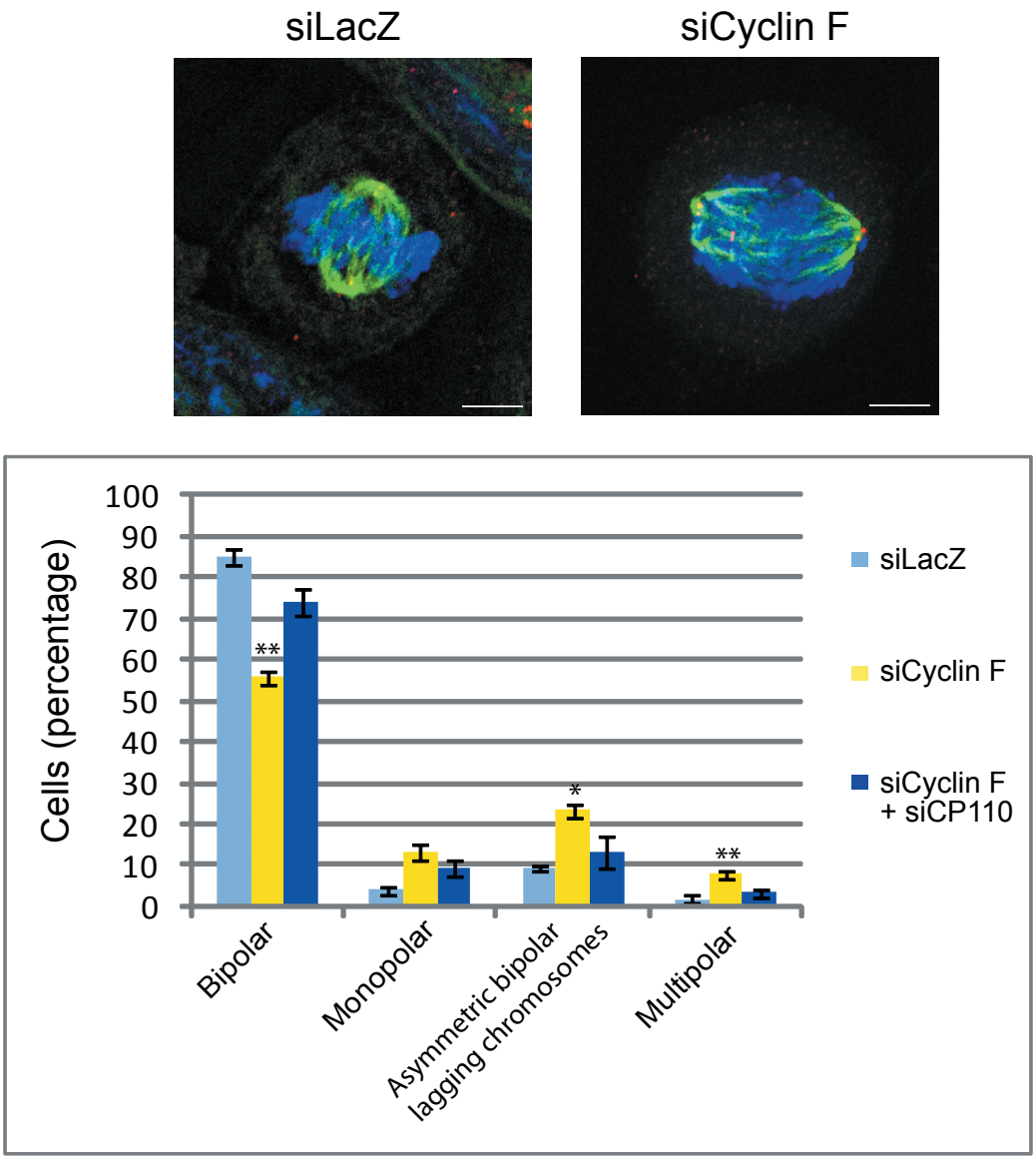




Supplementary Figure 9. D'Angiolella *et al.*

Supplementary Figure 9. *Cyclin F* null mouse embryonic fibroblasts (MEFs) display CP110 accumulation and increased centrosome number

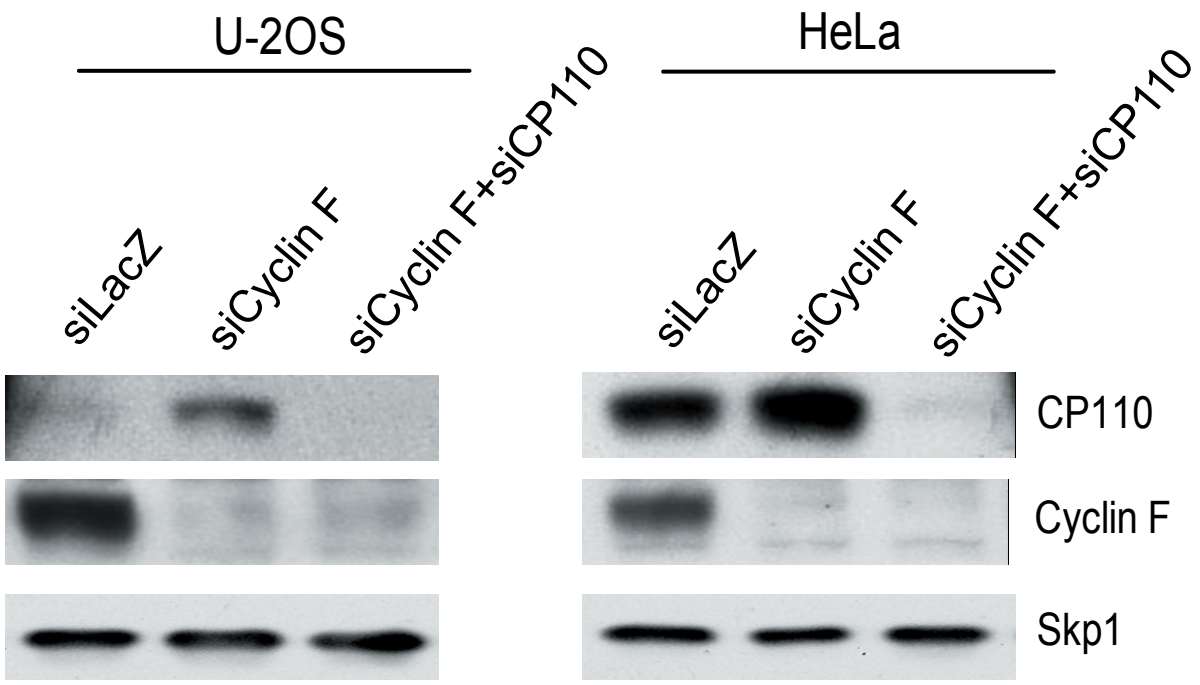
Cyclin F-/- and parental *Cyclin F*$^{Flox/-}$ MEFs were fixed and incubated with an anti-Centrin 2 antibody (red) and an anti-γ-tubulin antibody (green). DNA was stained with DAPI. Insets show magnified views of centrosomes. Scale bar = 10 μM. The graph shows the percentages of cells with excess Centrin 2 foci (more than four per cell) and excess γ-tubulin dots (more than two per cell). The data represent the average from three independent experiments, with at least 100 cells counted per experiment. Error bars indicate +/-SD. *= $p<0.0001$ (calculated by double tailed t-test). Bottom panels show *Cyclin F*-/- and parental *Cyclin F*$^{Flox/-}$ MEFs that were lysed and immunoblotted with antibodies to the indicated proteins.



Supplementary Figure 10. D'Angiolella *et al.*

Supplementary Figure 10. Silencing of Cyclin F induces mitotic aberrations

HeLa cells were transfected with siRNAs to a non-relevant mRNA (LacZ) or Cyclin F mRNA and synchronized by a double-thymidine block. Cells were fixed nine hours after release from the block and stained with an anti-Centrin 2 antibody (red) and an anti-α-tubulin antibody (green). DNA was stained with DAPI. The yellow color in merged images shows colocalization of Centrin 2 and α-tubulin. Scale bar = 5 μM. The graph shows the percentages of cells with various abnormal mitotic phenotypes. The data represent the average from three independent experiments, with at least 100 cells counted per experiment. Error bars indicate +/-SD. *= $p<0.003$; **= $p=0.001$ (calculated by ANOVA)



Supplemntary Figure 11. D'Angiolella *et al.*

Supplementary Figure 11. Cyclin F knockdown results in increased levels of CP110 in asynchronous cells

U-2OS (left panels) and HeLa cells (right panels) were transfected with siRNAs to either a non-relevant mRNA (LacZ), Cyclin F mRNA, or both. Cells were collected after 48 hours, lysed, and immunoblotted with antibodies to the indicated proteins.

Supplementary Table 1. MudPIT analysis of two Cyclin F immunopurifications, listing normalized spectral abundance factors (NSAFs; ref. 32) for the indicated proteins.

	Cyclin F WT		Cyclin F(1-270)		Control IP	
Protein	dNSAF AVG	Detected # Out of 2	dNSAF AVG	Detected # Out of 2	dNSAF AVG	Detected # Out of 4
Cyclin F	0.127895	2	0.040986	2	0	0
Skp1	0.258895	2	0.120016	2	0.000714	1
Cul1	0.010134	2	0.000216	1	0	0
CP110	0.00037	2	0	0	0	0