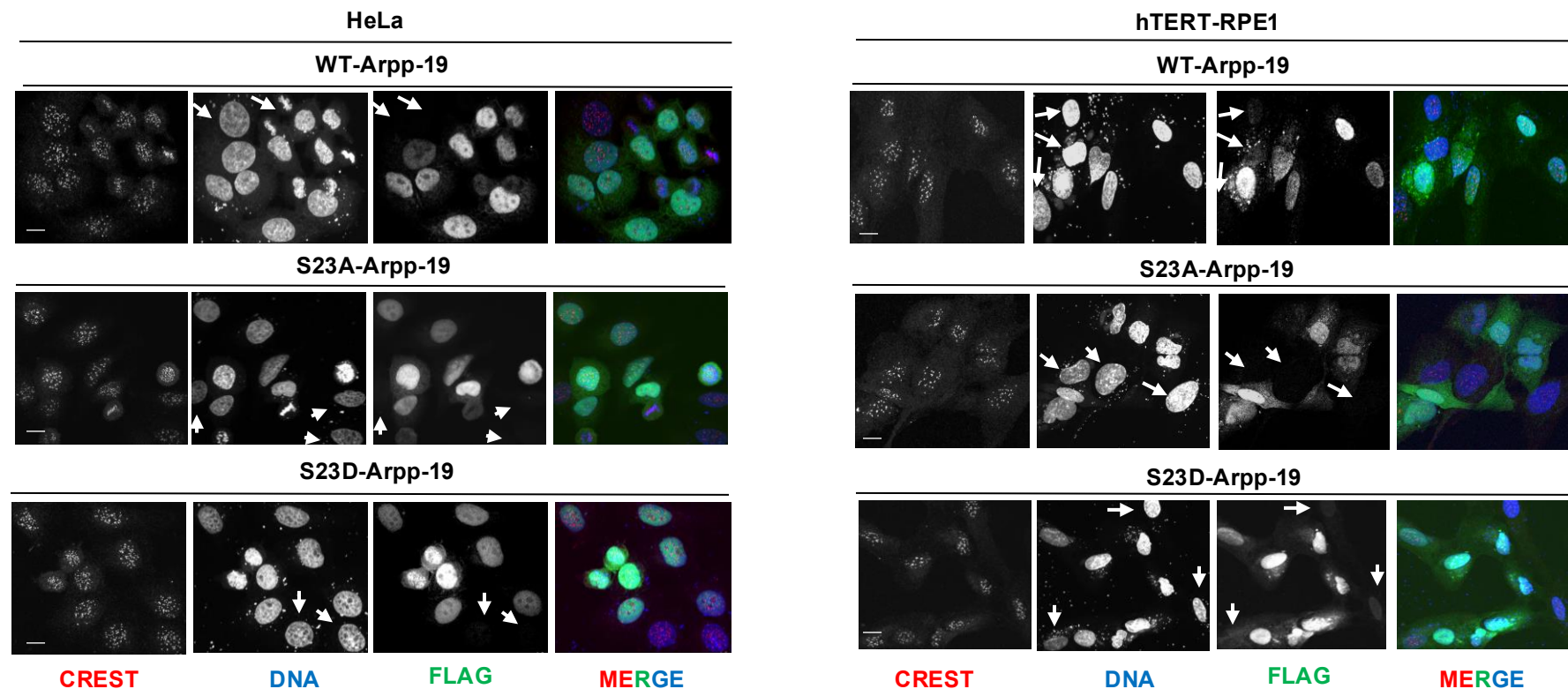


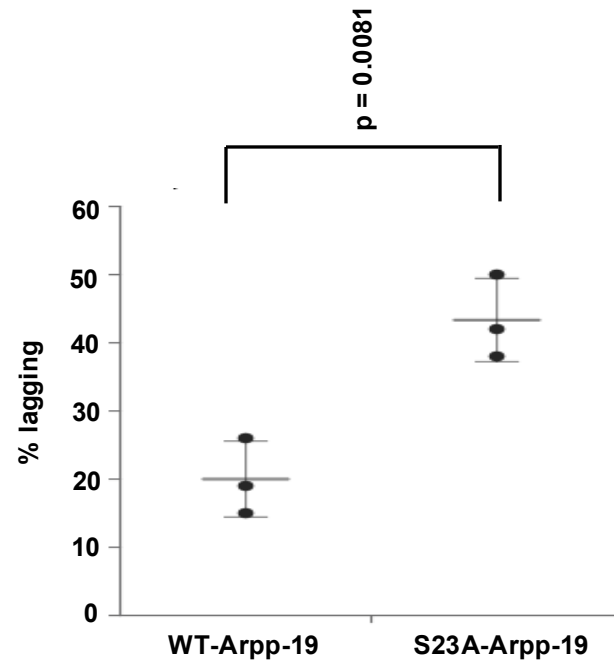
Supplementary Figure 1



Supplementary Figure 1. Flag-Arpp-19 vector transfection efficiency.

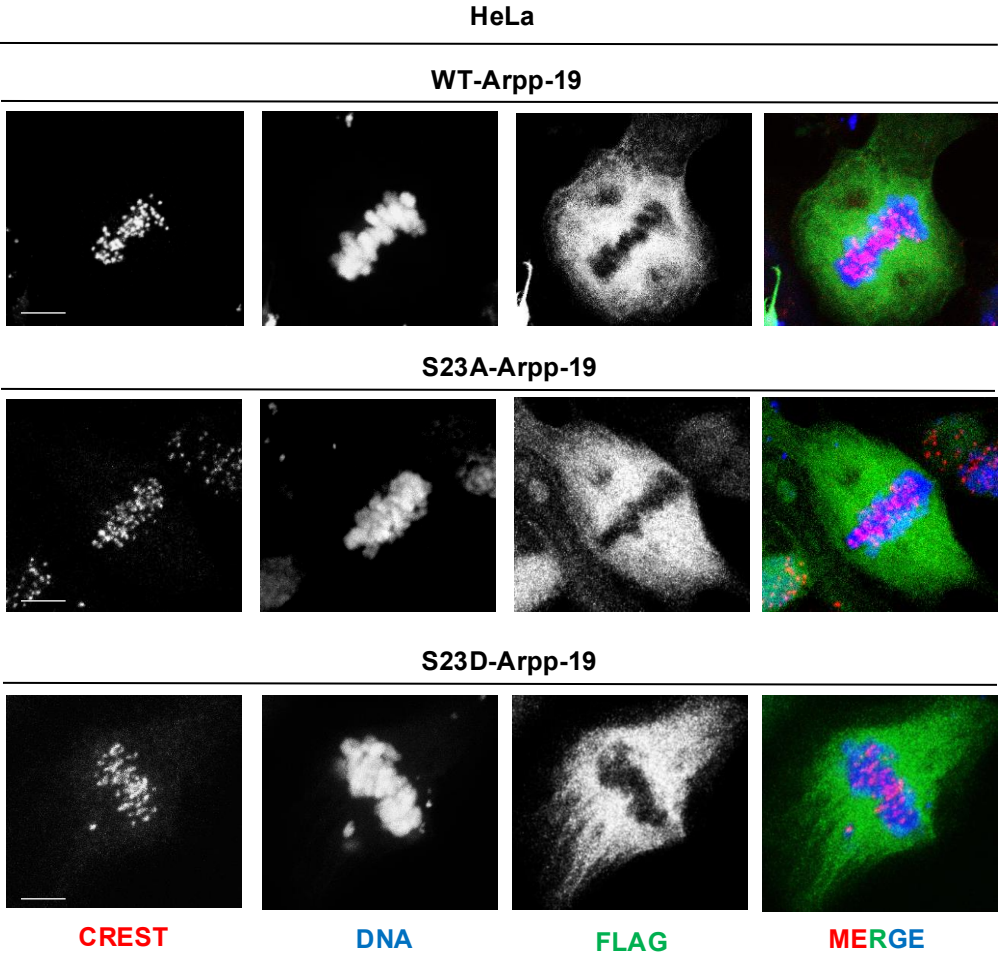
Representative images of HeLa and hTERT-RPE1 cells transfected with Flag-Arpp-19 vectors. Cells were stained for the indicated antigens; arrowheads indicate untransfected cells. The Flag-Arpp-19 vector transfection efficiency was comparable among the various (wild type and mutant) constructs and was for HeLa cells around 73% (+/- 5% in three independent experiments) and for hTERT-RPE1 cells around 63% (+/- 5% in three independent experiments). Scale bar: 10 μ m.

Supplementary Figure 2



Supplementary Figure 2. Lagging, centromere-positive, in WT-Arpp-19- and S23A-Arpp-19 expressing cells during transition through anaphase upon nocodazole wash out. HeLa cells were treated with Arpp-19-targeting siRNAs and transfected, 10 hours later, with siRNA-resistant wild type Arpp-19 (WT) or S23A-Arpp-19 expression vectors. Cells were further arrested at prometaphase by a 14-hour treatment with nocodazole. Upon nocodazole wash out, cells were sampled at 20 min intervals of further incubation in fresh medium up to 80 min. During this period the majority of cells passed through anaphase. Cells were fixed and stained for α -tubulin, CREST and DNA. The graph shows the cumulative percent of anaphase cells showing lagging, CREST-positive, chromosomes. Around 100 cells were analyzed per time point and cells in anaphase with CREST-positive lagging chromosome were annotated. Error bars refer to mean and standard deviation of the cumulative percent of cells in anaphase with CREST-positive lagging chromosome in three independent experiments performed under similar experimental conditions. The p-value was calculated by comparison of percent of anaphase cells with lagging chromosomes in WT-Arpp-19- with S23A-Arpp-19- expressing cells from three independent experiments using a two-tailed unpaired *t*-test.

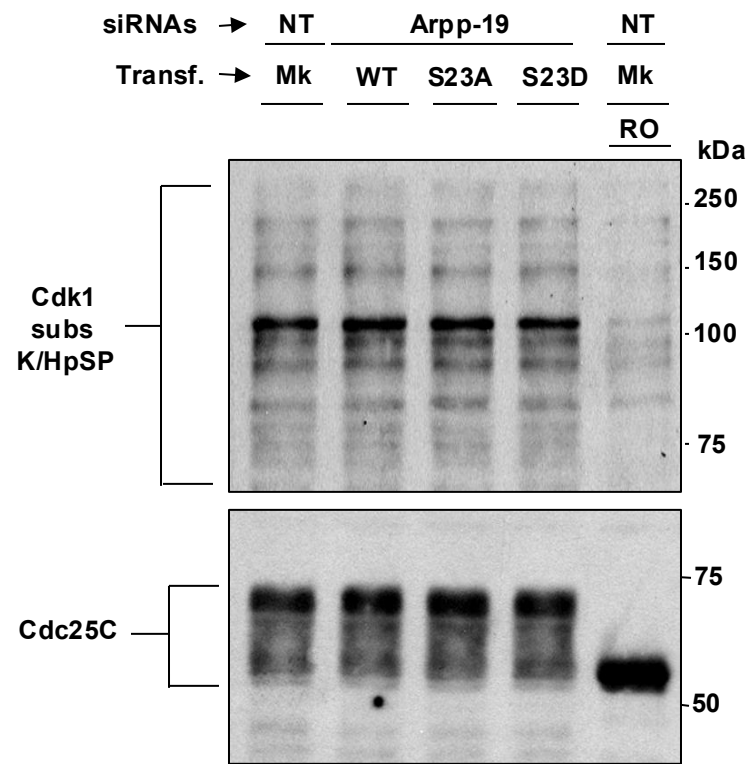
Supplementary Figure 3



Supplementary Figure 3. WT-, S23A- and S23D-Flag-Arpp-19 mitotic localization.

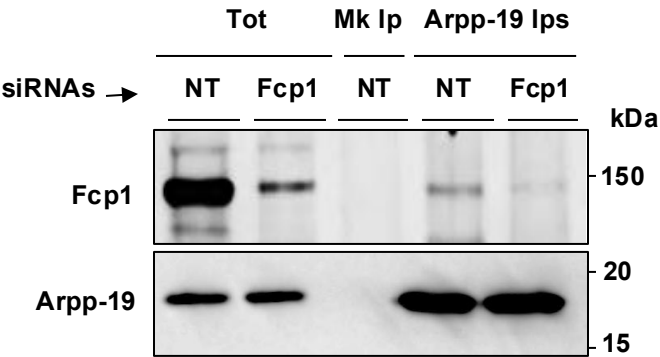
Representative images of mitotic HeLa cells transfected with Flag-tagged WT-, S23A- and S23D-Arpp-19 expression vectors. Cells were stained for the indicated antigens. Scale bar: 5 μ m.

Supplementary Figure 4



Supplementary Figure 4. S23-Arpp-19 phosphorylation mutants do not affect overall mitotic phosphorylation. HeLa cells were treated with non-targeting (NT) or Arpp-19-targeting siRNAs and transfected, 10 hours later, with an empty vector (mock; Mk) or with siRNA-resistant Flag-tagged wild type Arpp-19 (WT) or Flag-tagged S23A-Arpp-19 (S23A) or Flag-tagged S23D-Arpp-19 (S23D). Cells were arrested at prometaphase by a 14-hour treatment with nocodazole added 32 hours post siRNA treatments. Total cell lysates were probed for the indicated antigens.

Supplementary Figure 5



Supplementary Figure 5. Loss of Arpp-19-bound Fcp1 in mitotic Fcp1-downregulated HeLa cells.

HeLa cells were treated with non-targeting (NT) or Fcp1-targeting siRNAs. Cells were arrested at prometaphase by a 14-hour treatment with nocodazole added 32 hours post siRNA treatments. Total cell lysates (Tot), mock (Mk) and Arpp-19 Ips were probed for the indicated antigens.

Supplementary Figure 6 Uncropped and unedited blots

Figure 1b

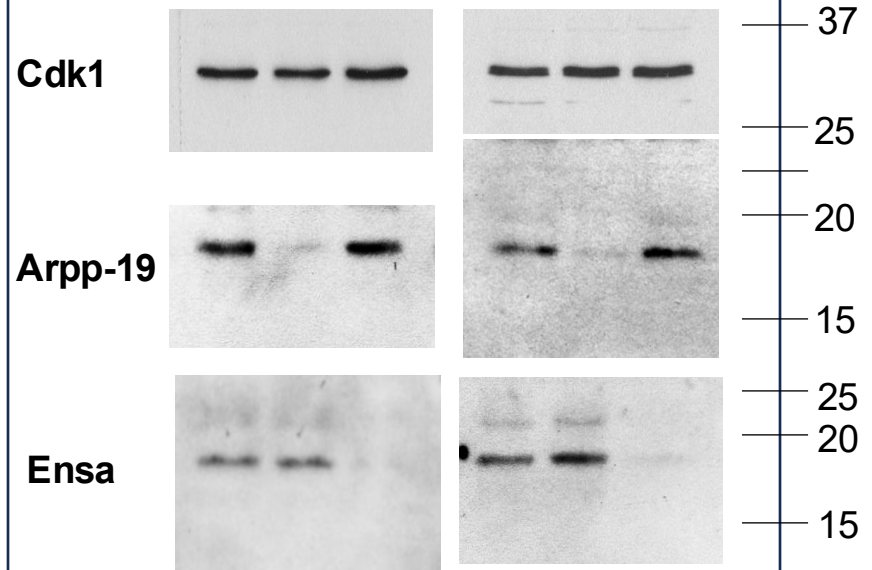


Figure 1d

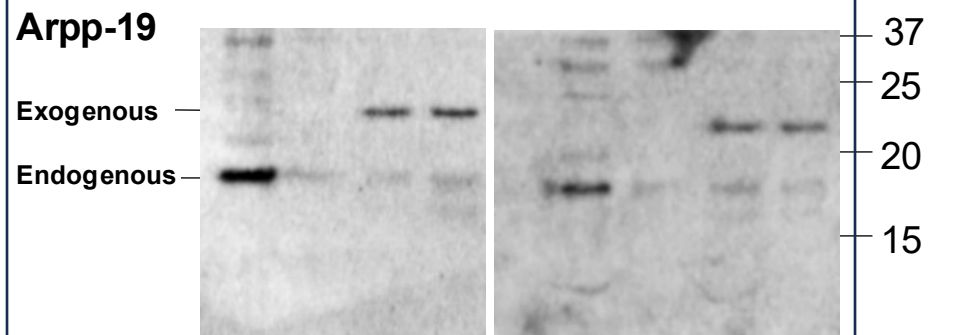


Figure 1f

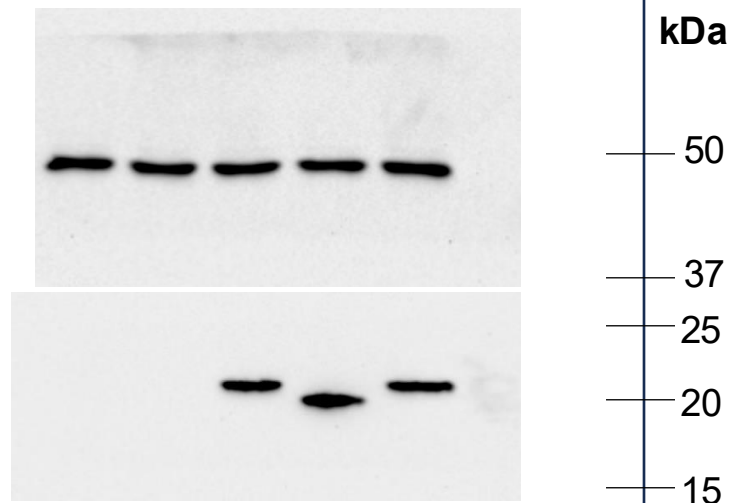
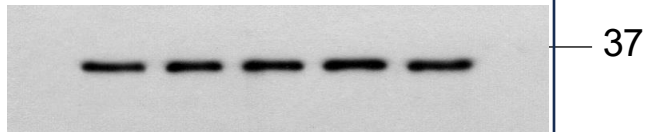


Figure 2a

Cdk1



Arpp-19

Exogenous

Endogenous

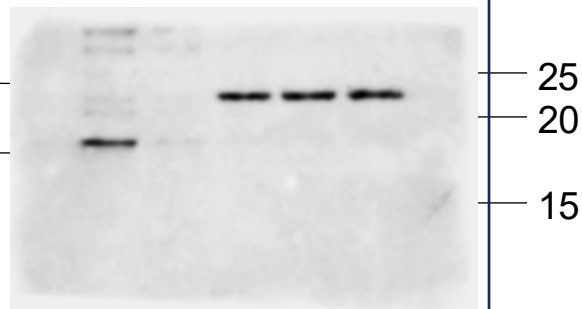
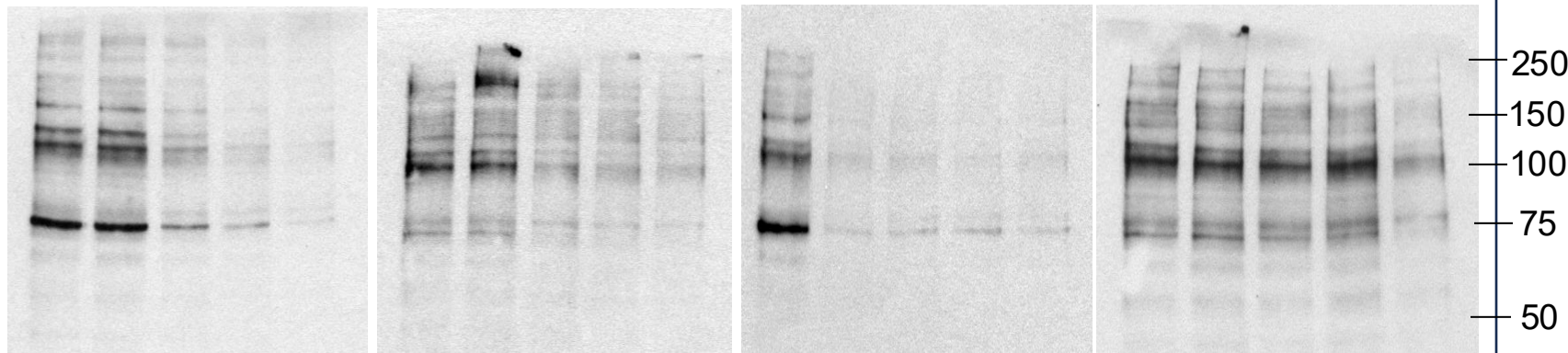
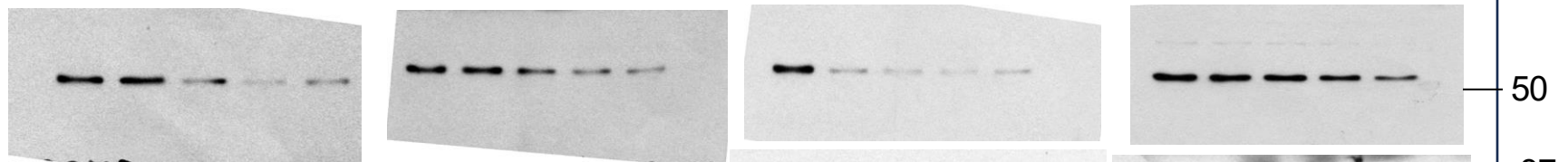


Figure 2b

**Cdk1
subs
K/HpSP**



Cyc B1



Cdk1

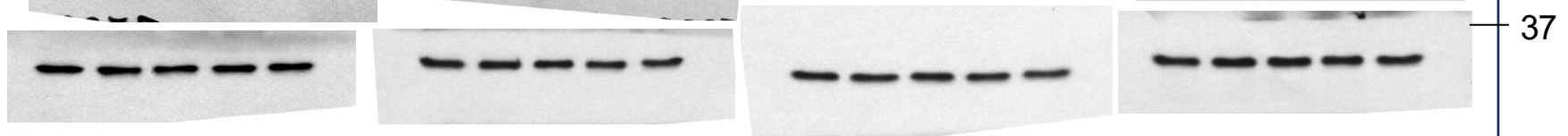
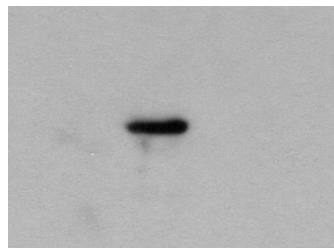


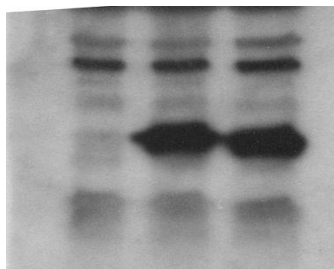
Figure 3a

**pS23-
Arpp-19**



— 25
— 20

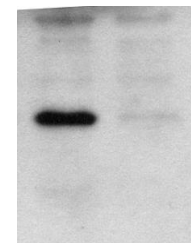
Arpp-19



— 25
— 20

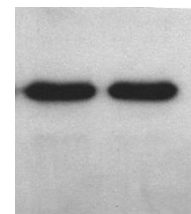
Figure 3b

**pS23-
Arpp-19**



— 20
— 15

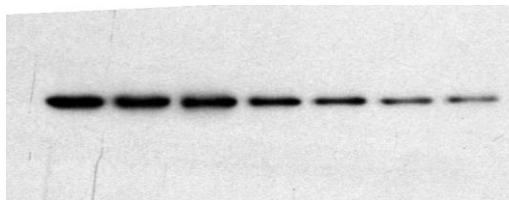
Arpp-19



— 20
— 15

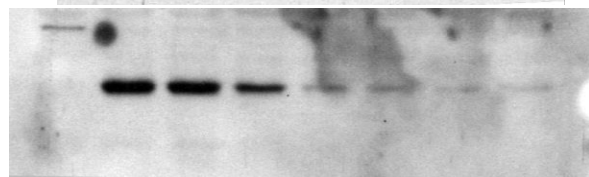
Figure 3c

Cyc B1



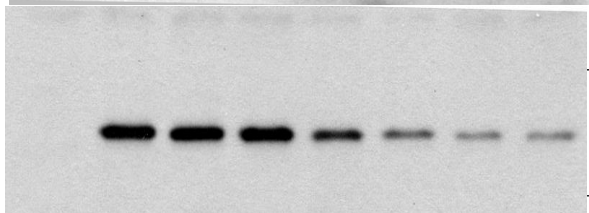
— 50

**pS23-
Arpp-19**



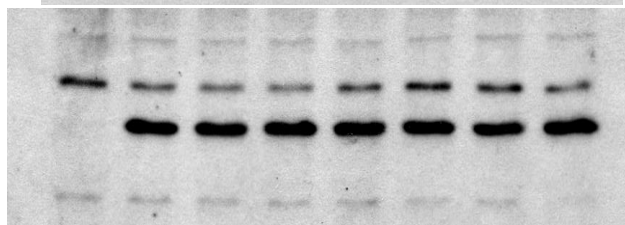
— 20
— 15

DpSG



— 20
— 15

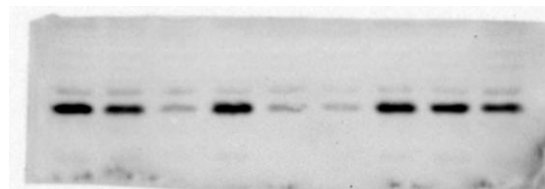
Arpp-19



— 20
— 15

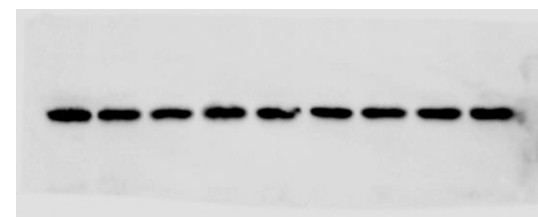
Figure 3d

DpSG



— 25
— 20

Arpp-19



— 25
— 20

Figure 4a

pS23-
Arpp-19

DpSG

Arpp-19

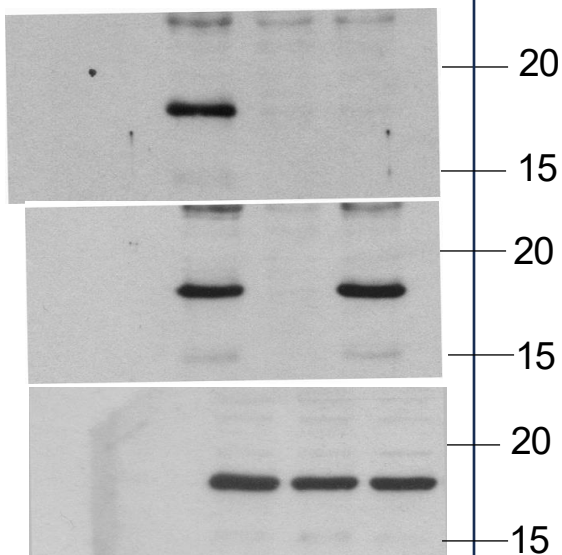


Figure 4b

Fcp1

pS23-
Arpp-19

DpSG

Arpp-19

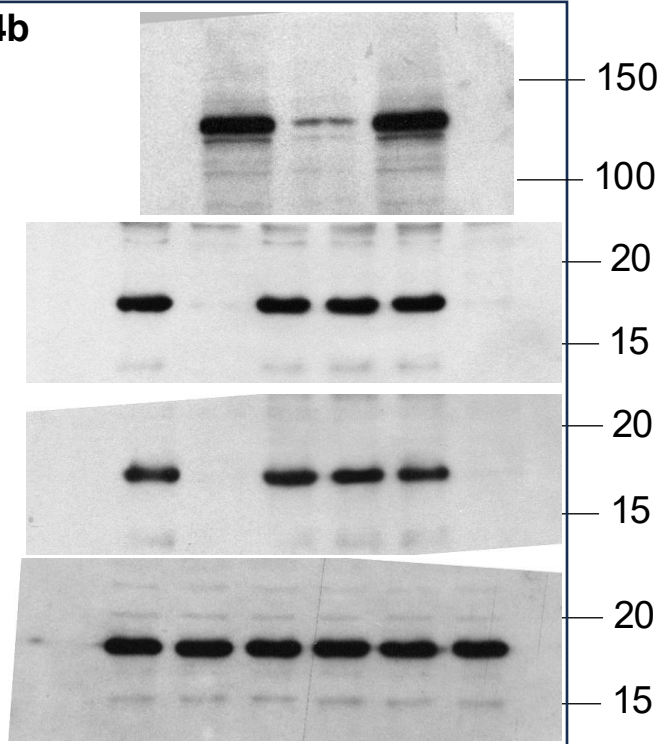


Figure 4c

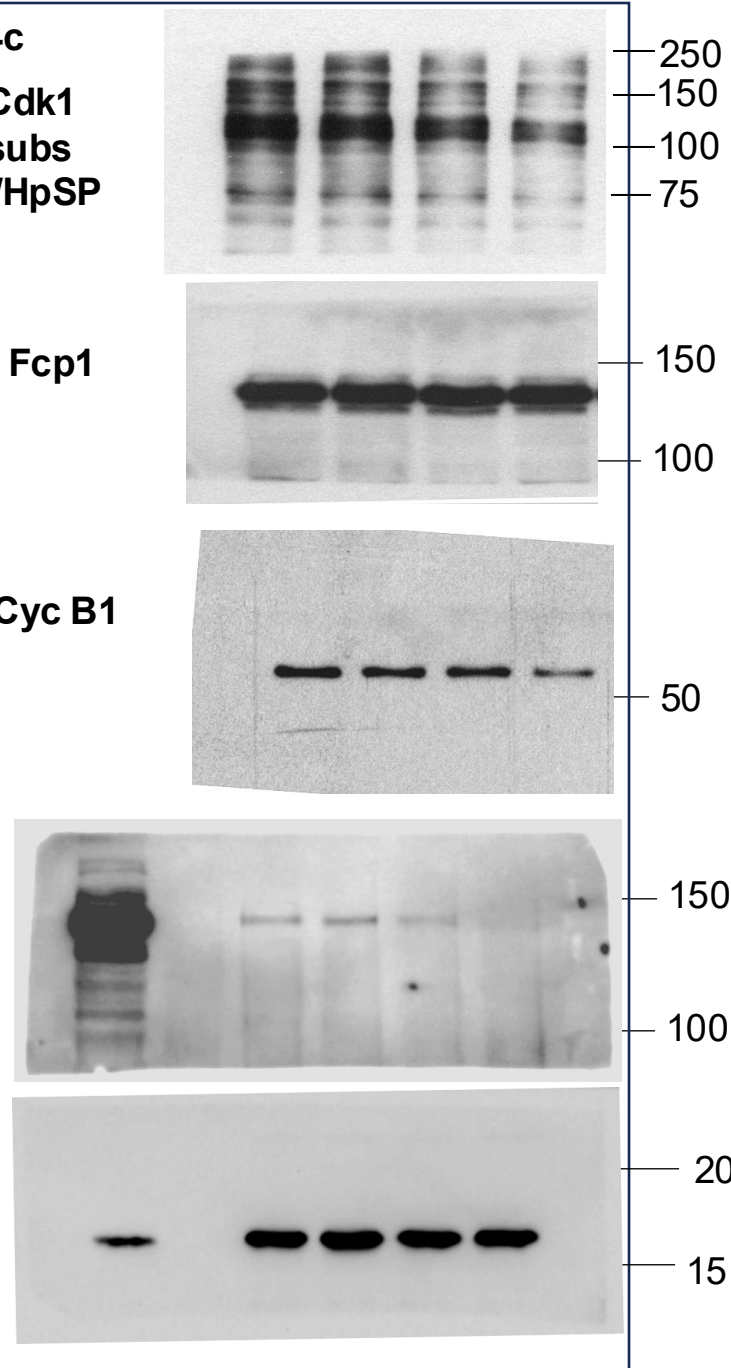
Cdk1
subs
K/HpSP

Fcp1

Cyc B1

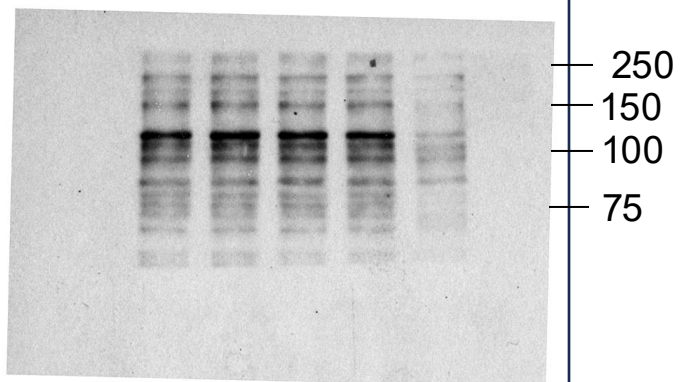
Fcp1

Arpp-19

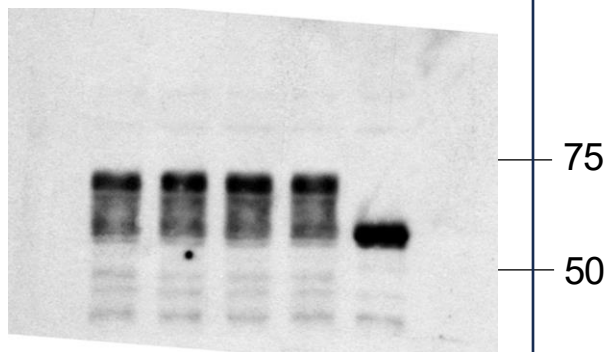


Supplementary Figure 4

**Cdk1
subs
K/HpSP**

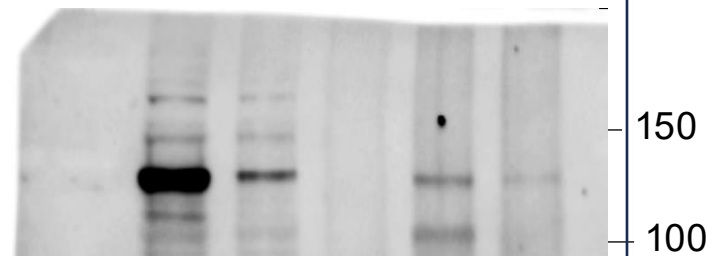


Cdc25C



Supplementary Figure 5

Fcp1



Arpp-19

