
Supplementary information

Targeting G1–S-checkpoint-compromised cancers with cyclin A/B RxL inhibitors

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authors and unedited

Supplementary Information Guide

Targeting G1–S Checkpoint–Compromised Cancers with Cyclin A/B RxL Inhibitors

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Supplementary Figure 2: Quality Control Data for the Base Editor Screens that Identified Mutations that Confer Resistance to Cyclin A/B RxL Inhibitors. **a**, Replicate reproducibility plot of the base editor screen from **Fig. 3a-b** showing Log Fold Change (LFC) of each individual sgRNA at day 26 in cells treated with CIRc-005 (inactive enantiomer of CIRc-004) relative to plasmid DNA (pDNA). Top panels show replicate 1 vs. replicate 2 and bottom panels show replicate 3 vs. replicate 4 for the indicated Cas9 base-editor cell lines. Pearson correlation coefficients (r) are indicated. **b**, Dot plot of Log Fold Change (LFC) of cells from **a** at day 26 treated with CIRc-005 relative to pDNA showing sgRNA depletion/enrichment of positive control sgRNAs targeting essential genes relative to negative control sgRNAs that are either non-targeting or targeting intergenic genomic DNA (negative control sgRNAs). $n=4$ biological replicates. **c-e**, Dot plot of average z-scored log-fold change (LFC) for each sgRNA tiling *CDC20* (**c**), *CDK2* (**d**), and *CCNA2* (**e**) in cells treated with CIRc-004 vs. CIRc-005 at day 26. The x-axis indicates the position of each sgRNA along each protein coding sequence. Variants of interest are labeled with the predicted amino acid position and mutational change. A>G base-editor is shown on left and C>T base-editor is shown on right. $n=4$ biological replicates.

Supplementary Figure 3: Gating Strategy for Flow Cytometry Experiments. Gating strategy for cleaved PARP FACS analysis (top), PI FACS analysis (middle), and phospho-histone serine 10 H3 FACS analysis (middle), and EdU (bottom). The boundaries to call positive cells for cleaved PARP and phospho-histone serine 10 H3 were based on unstained cells.

Supplementary Figure 1

Figure 2i

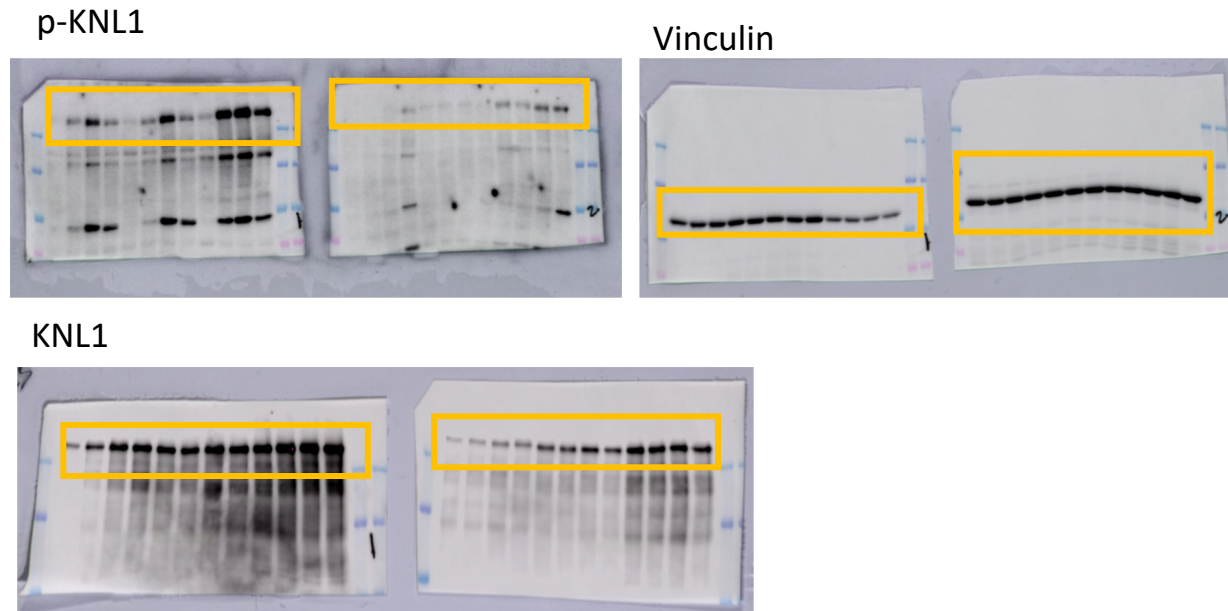


Figure 2k

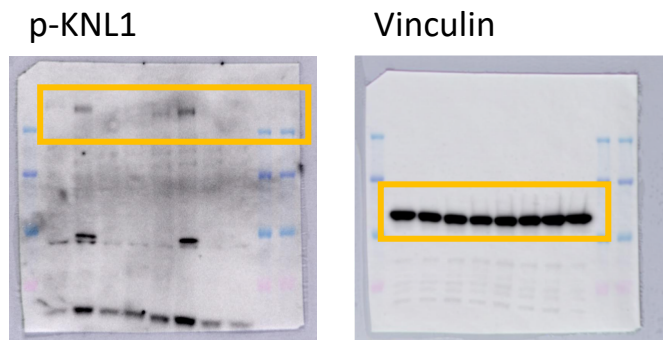
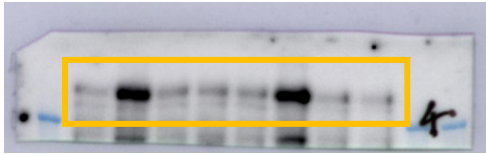
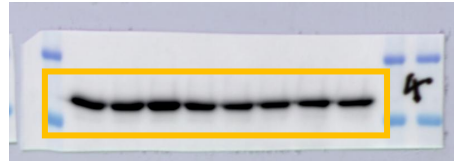


Figure 2n

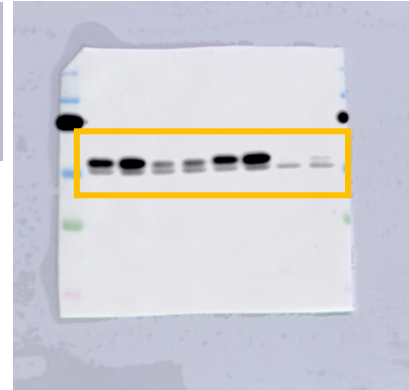
p-KNL1



Vinculin



cyclin B1



Actin

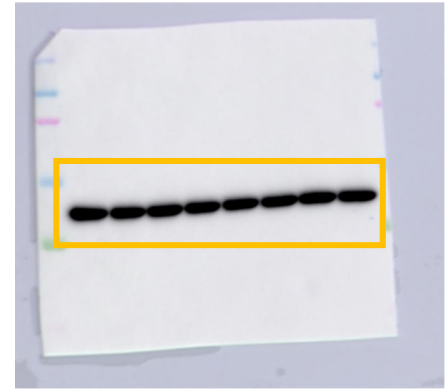
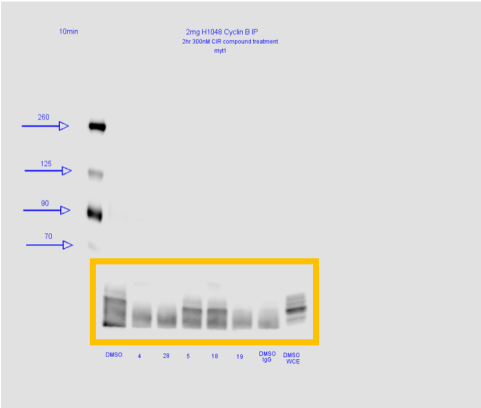
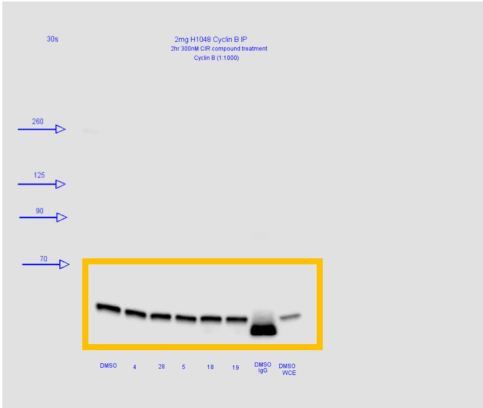


Figure 2o

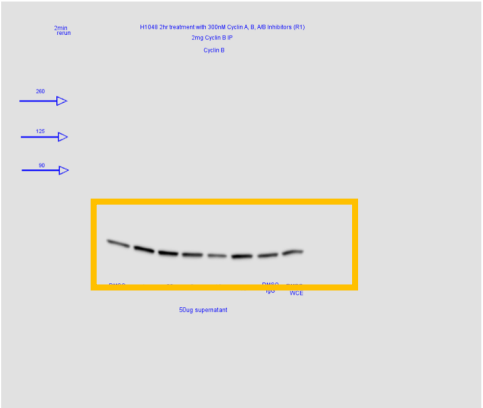
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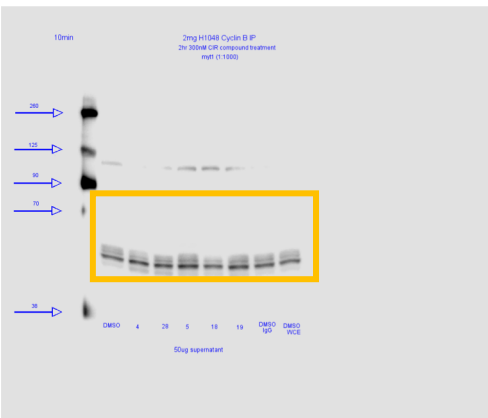
cyclin B1 (IP)



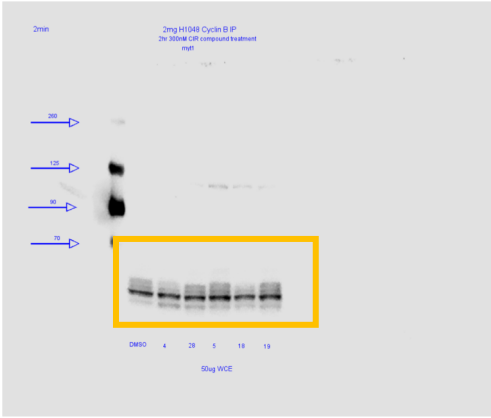
cyclin B1 (supernatant)



Myt1 (supernatant)



Myt1 (WCE)



cyclin B1 (WCE)

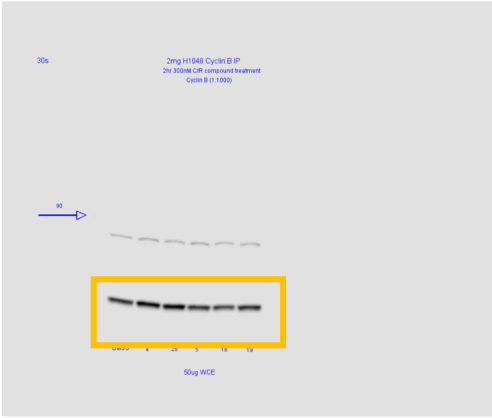


Figure 3h

Cdk2



cyclin B1



Cdk1

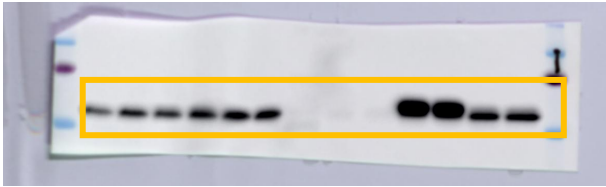


cyclin B1

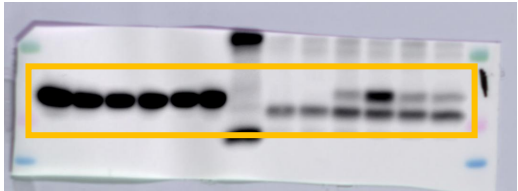


Figure 3i

cyclin B1



Cdk2



Cdk1

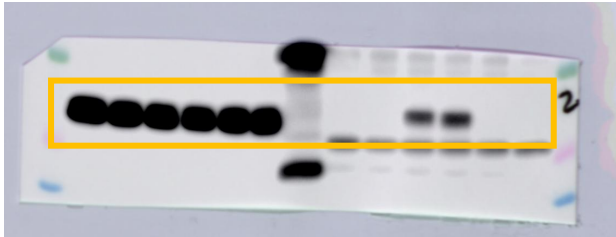
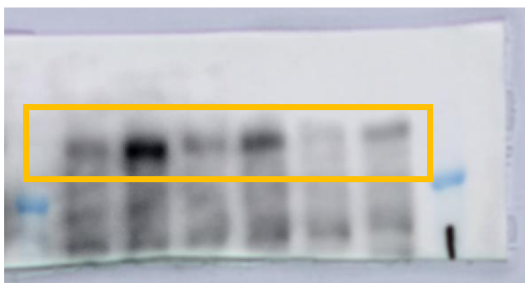
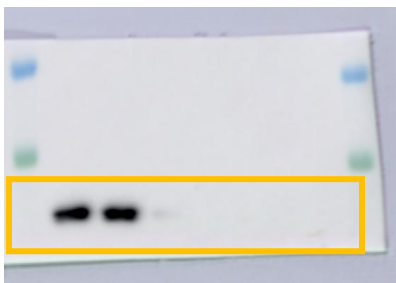


Figure 3j

p-KNL1



Cdk2



Vinculin

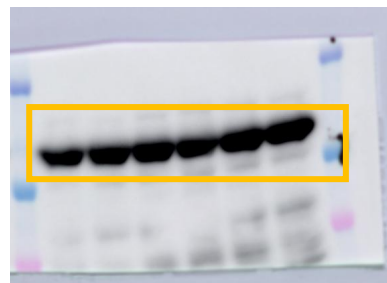
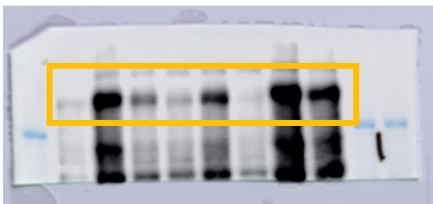


Figure 3k

p-KNL1



Vinculin



Figure 5b

γ H2AX

H3

γ H2AX

H3

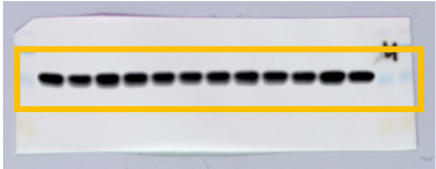
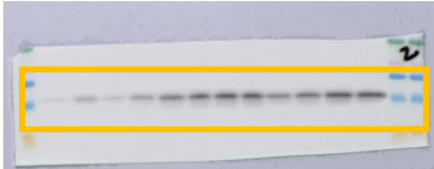
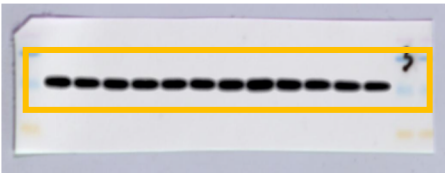
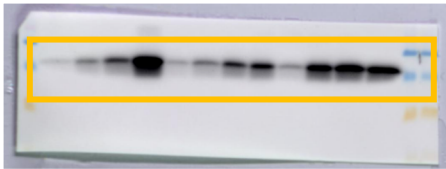
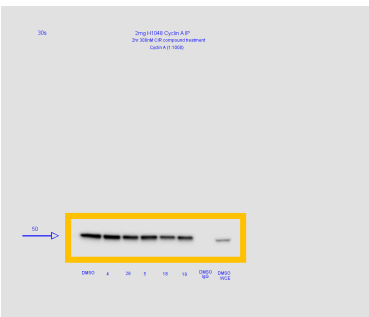


Figure 5c

E2F1 (IP)

cyclin A (IP)



E2F1 (WCE)

cyclin A (WCE)

E2F1 (supernatant)

cyclin A (supernatant)

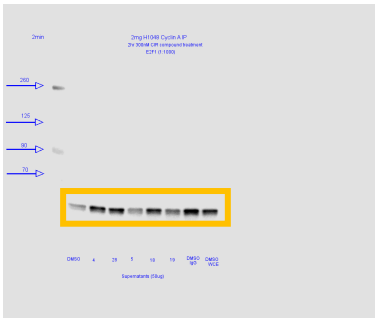
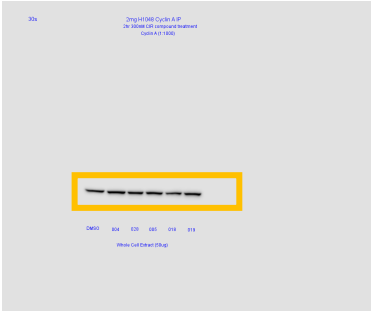
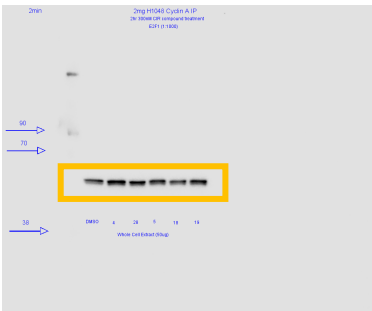


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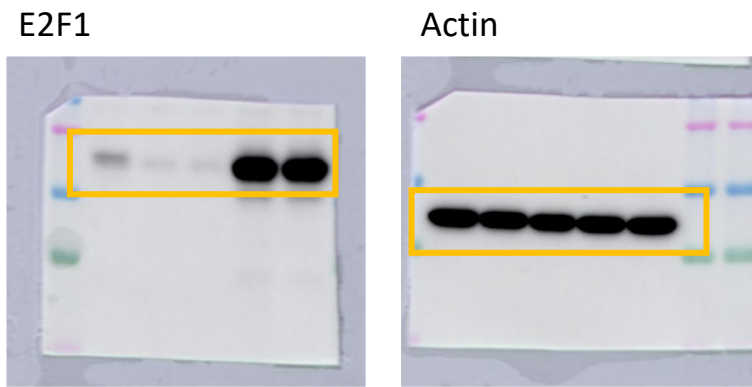


Figure 5e

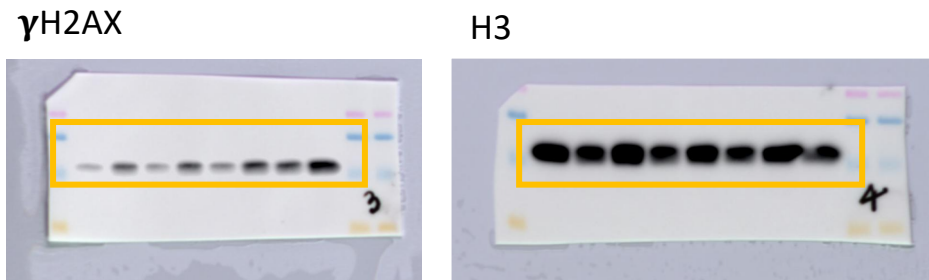
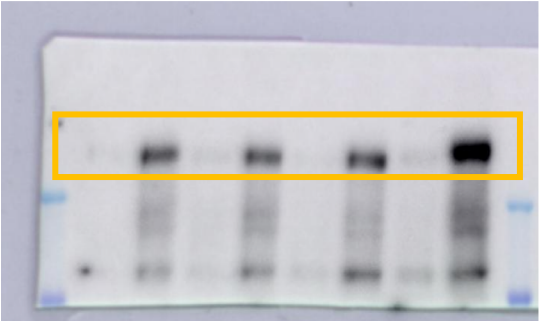
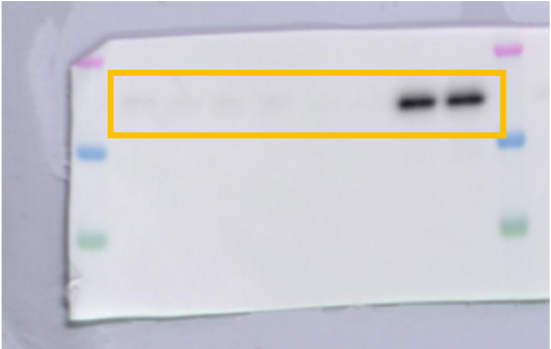


Figure 5g

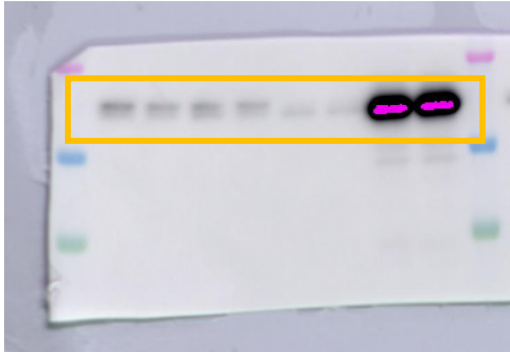
Phospho-KNL1



E2F1 (short exposure)



E2F1 (long exposure)



KNL1



Vinculin

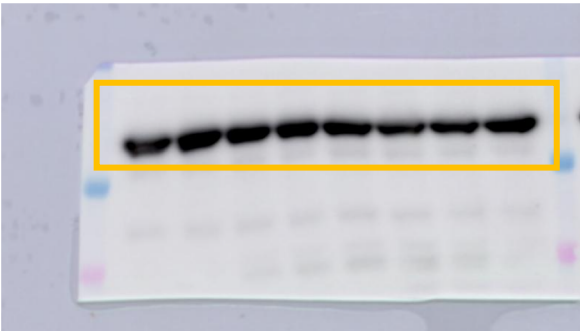


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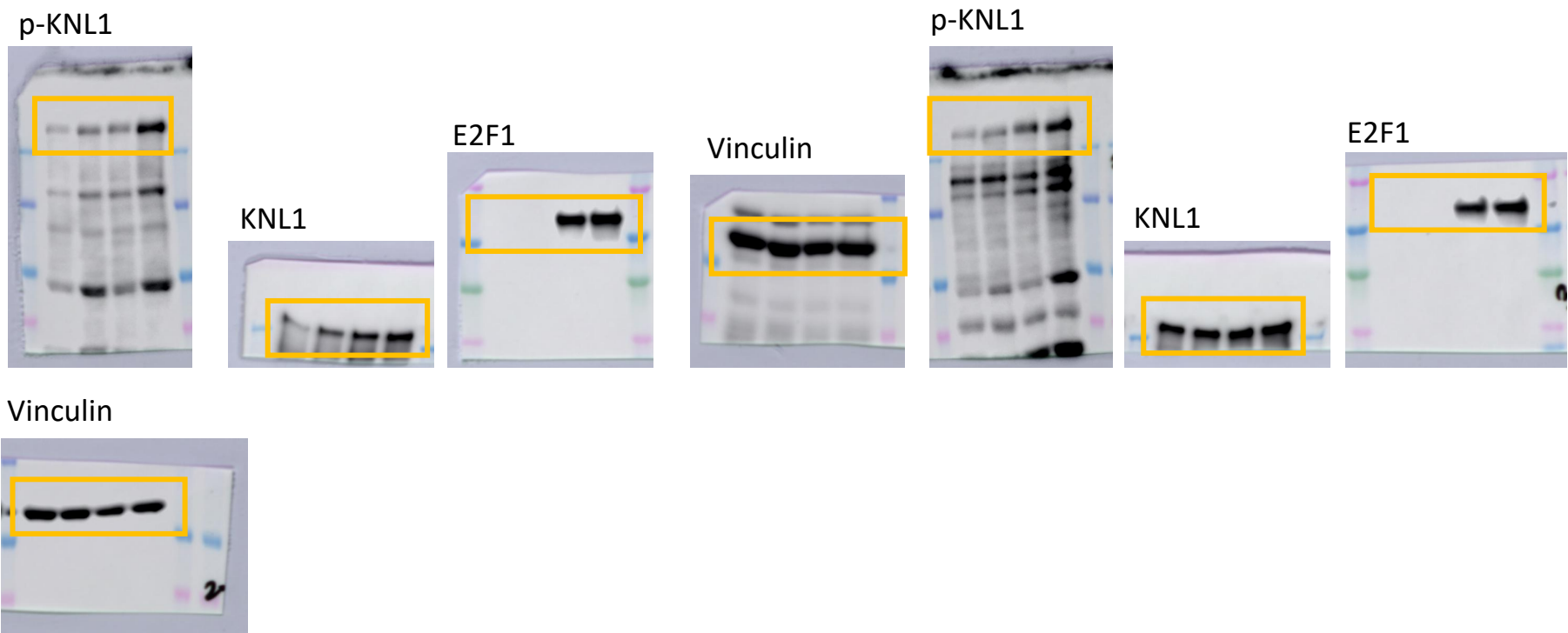


Figure 5k

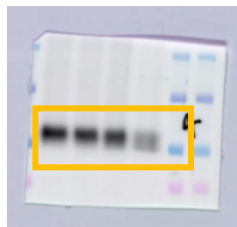


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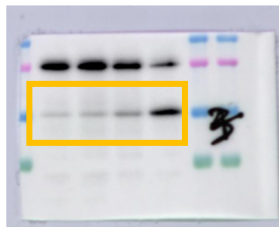
p-Rb



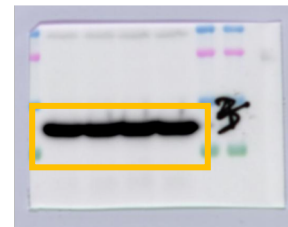
Rb



p53

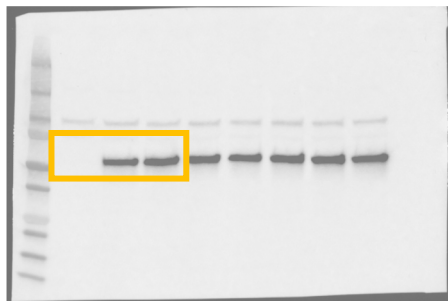


Actin

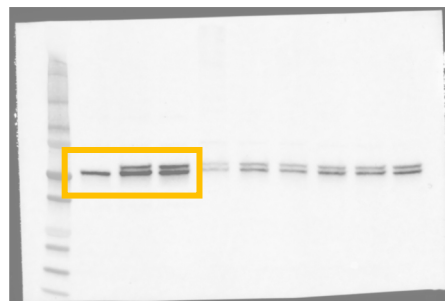


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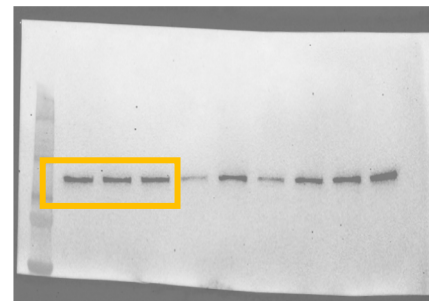
Flag



CDC20

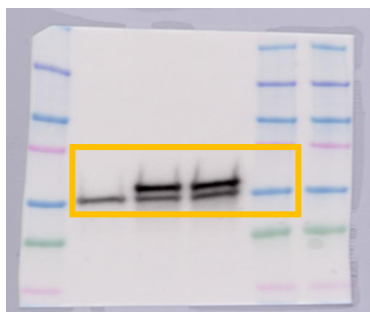


BUB1B

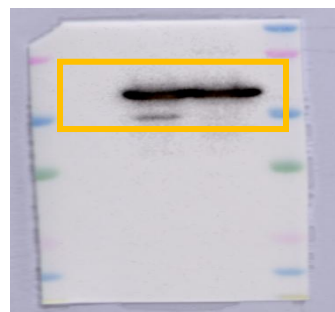


Extended Data Fig. 5j

CDC20



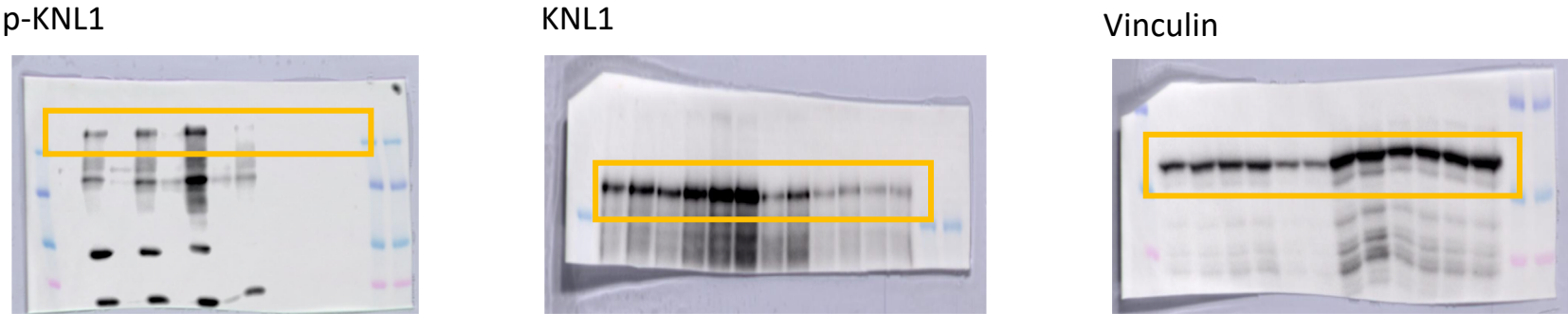
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Actin



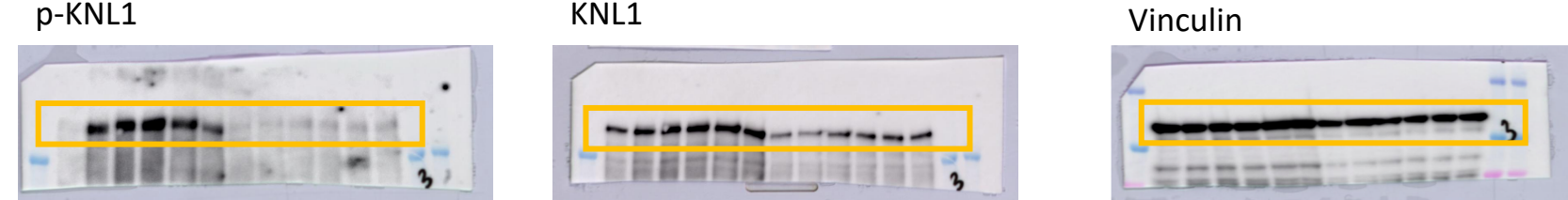
Extended Data Fig. 6f



Extended Data Fig. 6g

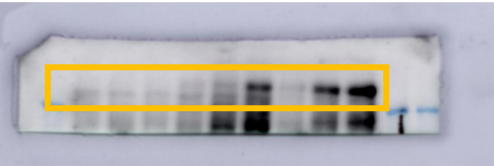


Extended Data Fig. 6h



Extended Data Fig. 6l

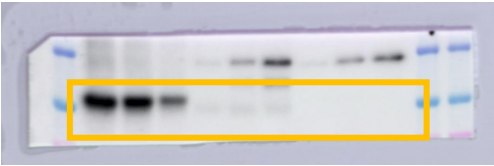
p-KNL1



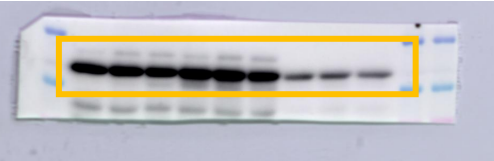
KNL1



p-Rb



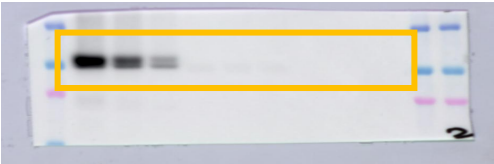
Vinculin



p53

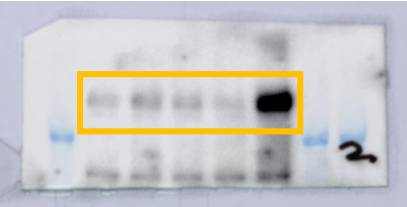


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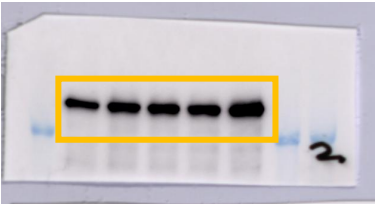


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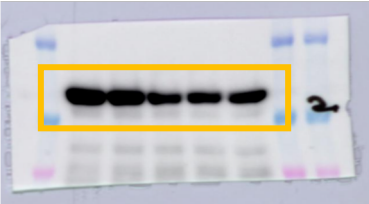
p-KNL1



KNL1

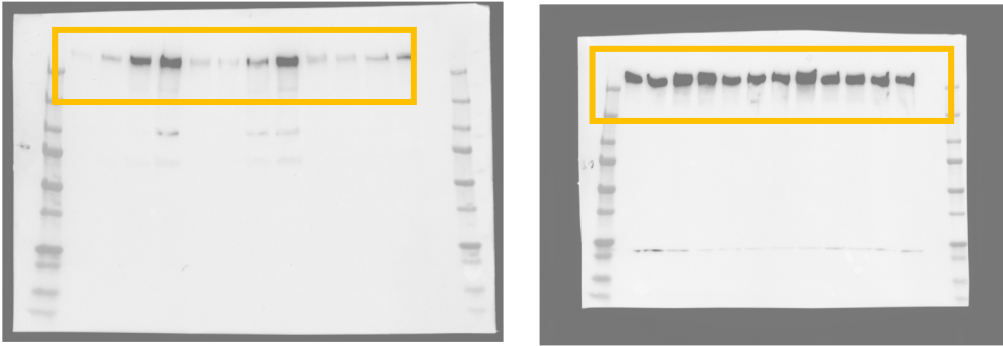


Vinculin



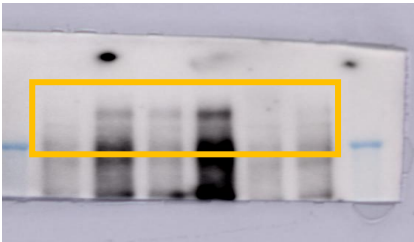
Extended Data Fig. 8g

p-KNL1

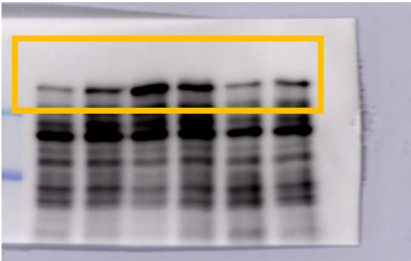


Extended Data Fig. 8h

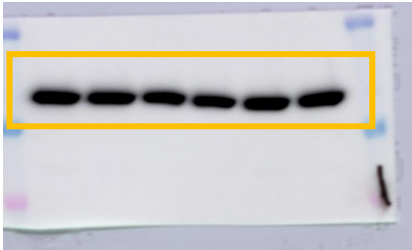
p-KNL1



KNL1

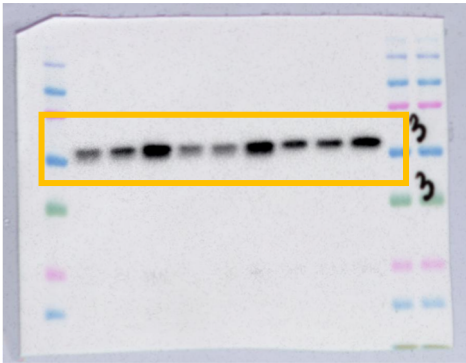


Vinculin

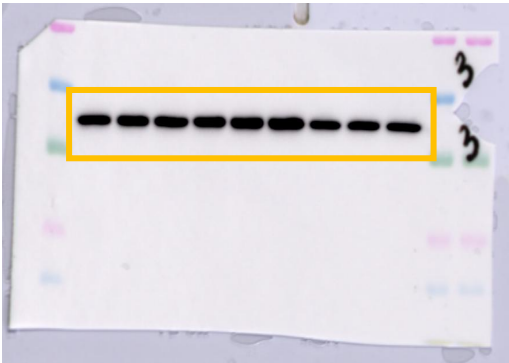


Extended Data Fig. 8i

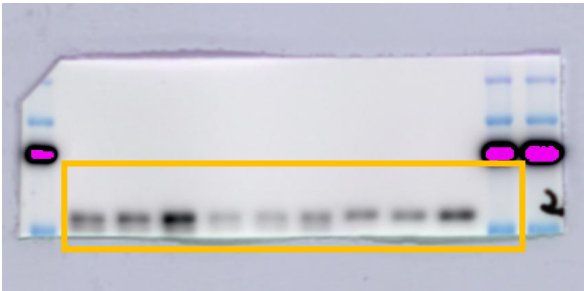
P126-cyclin B1



Actin

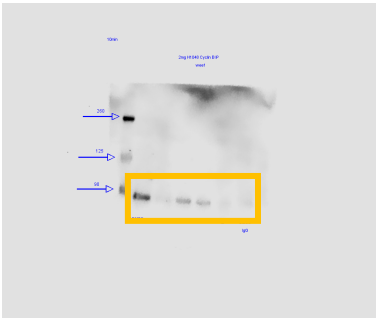


cyclin B1

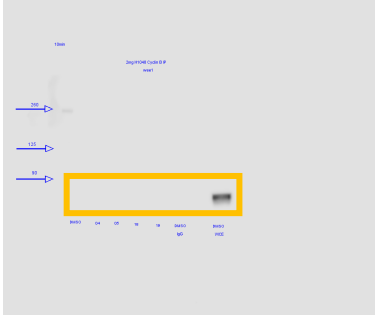


Extended Data Fig. 8u

Wee1 (L.E.)



Wee1 (S.E.)



Myt1

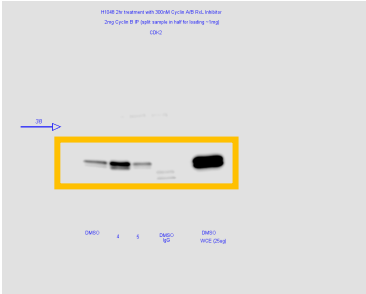


cyclin B1

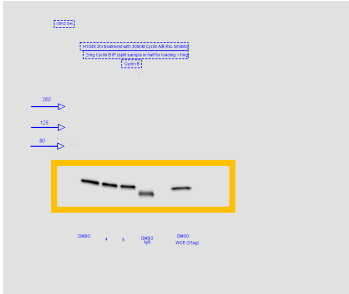


Extended Data Fig. 9d

Cdk2 (IP)



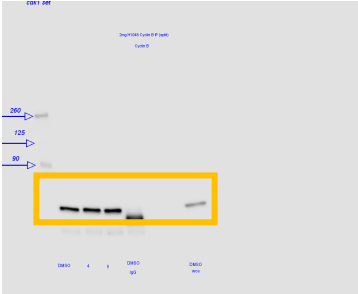
cyclin B1 (IP)



Cdk1 (IP)



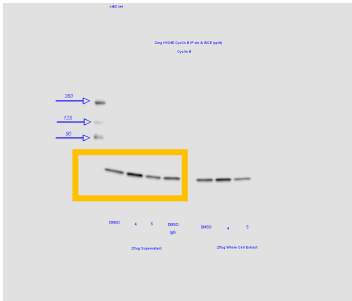
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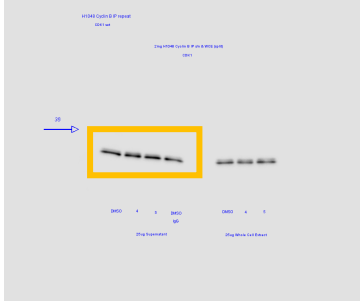
Cdk2 (Supernatant)



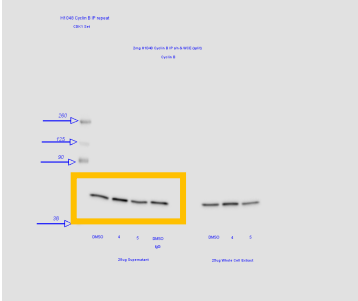
cyclin B1 (Supernatant)



Cdk1 (Supernatant)

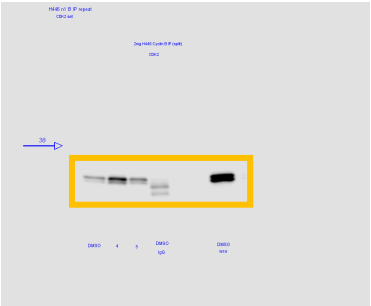


cyclin B1 (Supernatant)



Extended Data Fig. 9e

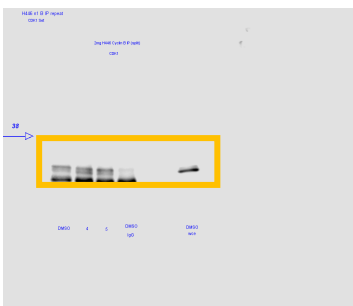
Cdk2 (IP)



cyclin B1 (IP)



Cdk1 (IP)



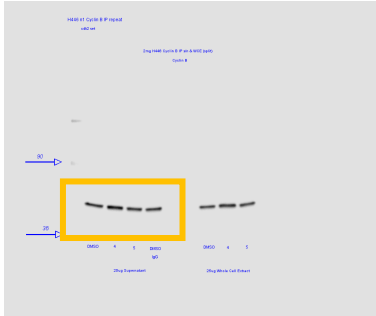
cyclin B1 (IP)



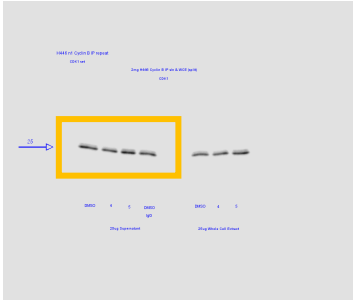
Cdk2 (Supernatant)



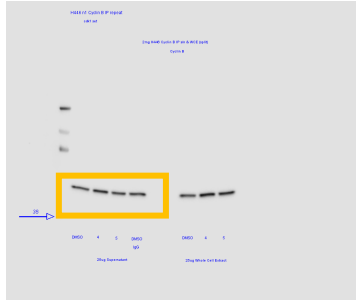
cyclin B1 (Supernatant)



Cdk1 (Supernatant)

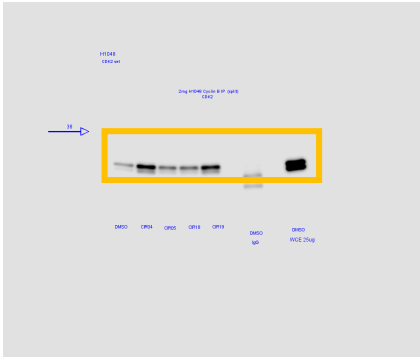


cyclin B1 (Supernatant)

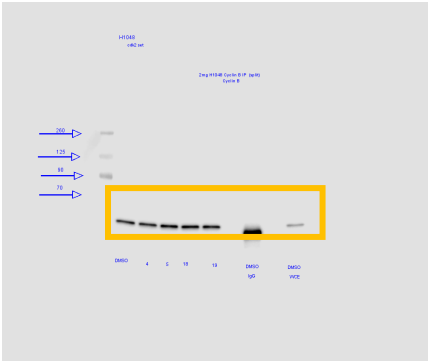


Extended Data Fig. 9f

Cdk2 (IP)



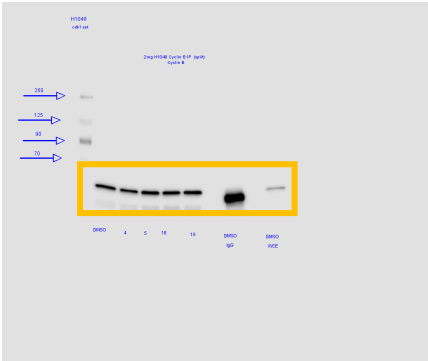
cyclin B1 (IP)



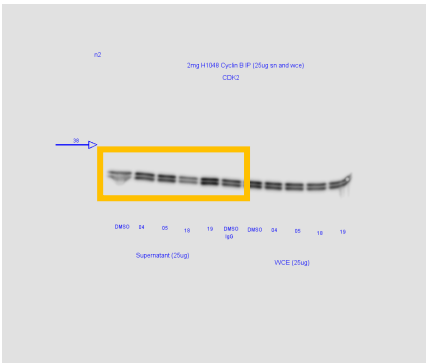
Cdk1 (IP)



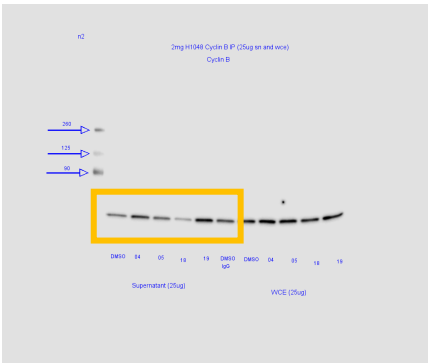
cyclin B1 (IP)



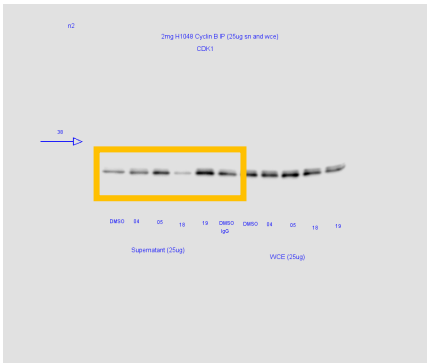
Cdk2 (Supernatant)



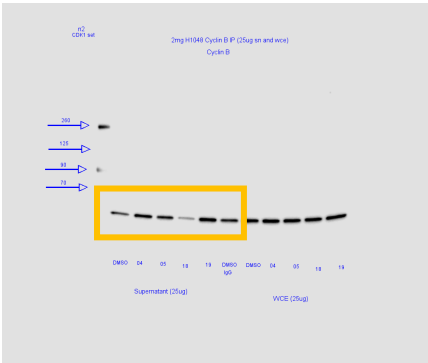
cyclin B1 (Supernatant)



Cdk1 (Supernatant)

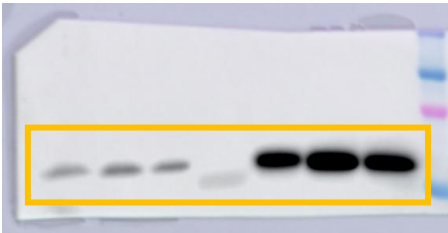


cyclin B1 (Supernatant)

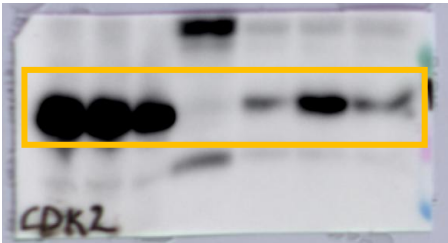


Extended Data Fig. 9g

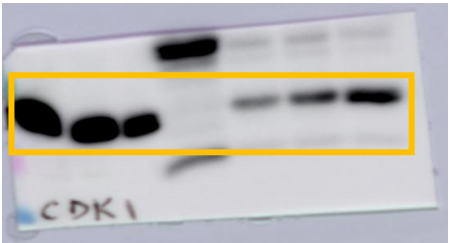
cyclin B1



Cdk2

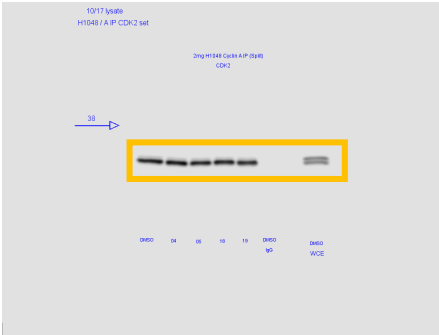


Cdk1

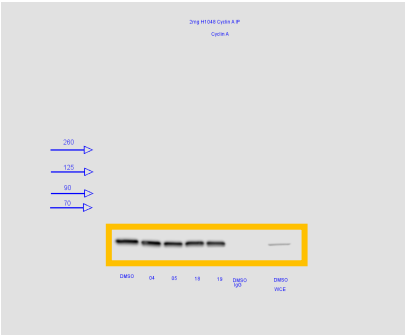


Extended Data Fig. 9h

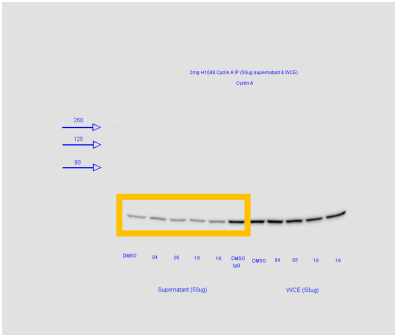
cyclin A



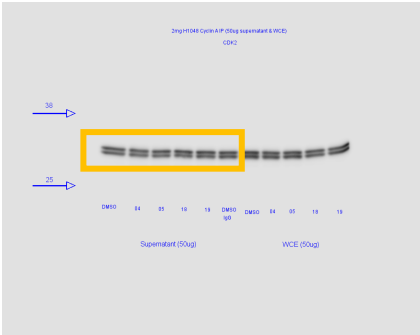
Cdk2



cyclin A (supernatant)

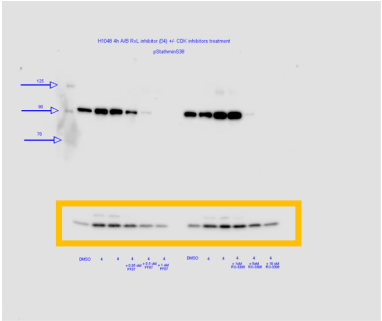


Cdk2 (supernatant)

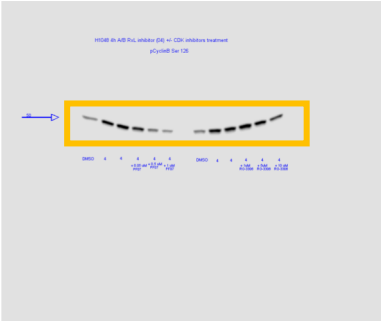


Extended Data Fig. 9i

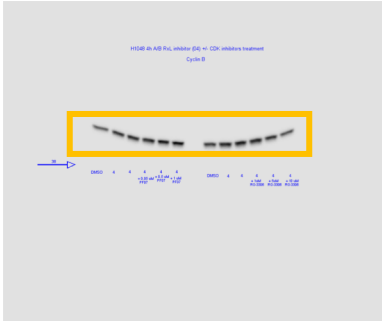
p-Stathmin S38



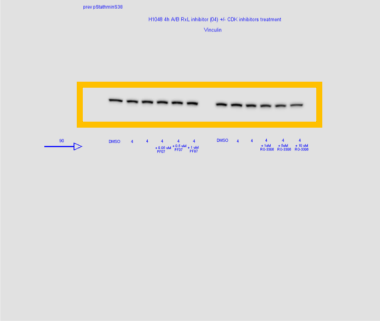
p-Cyclin B1 S126



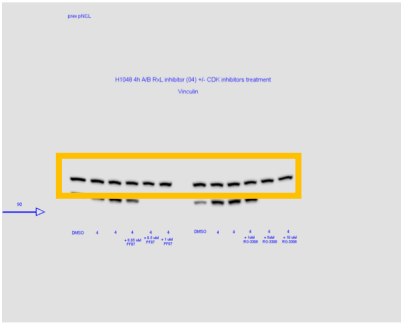
cyclin B1



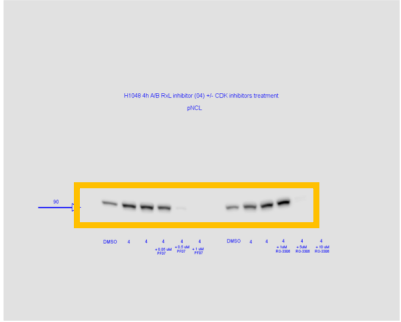
Vinculin



Vinculin



p-Nucleolin T84

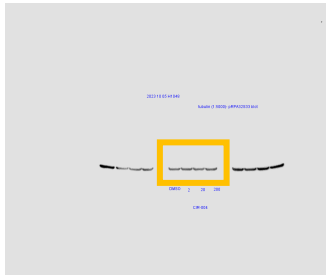


Extended Data Fig. 10b

p-RPA2 s33

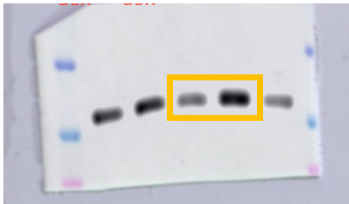


Tubulin

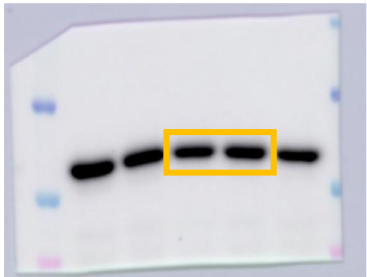


Extended Data Fig. 10c

p-KAP1



Vinculin



Extended Data Fig. 10d

γ H2AX

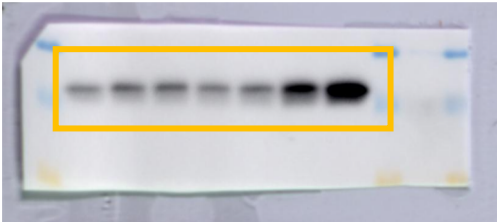


H3

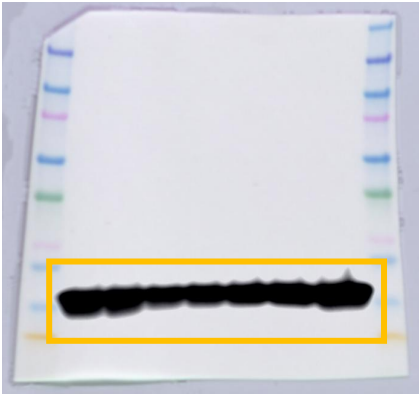


Extended Data Fig. 10e

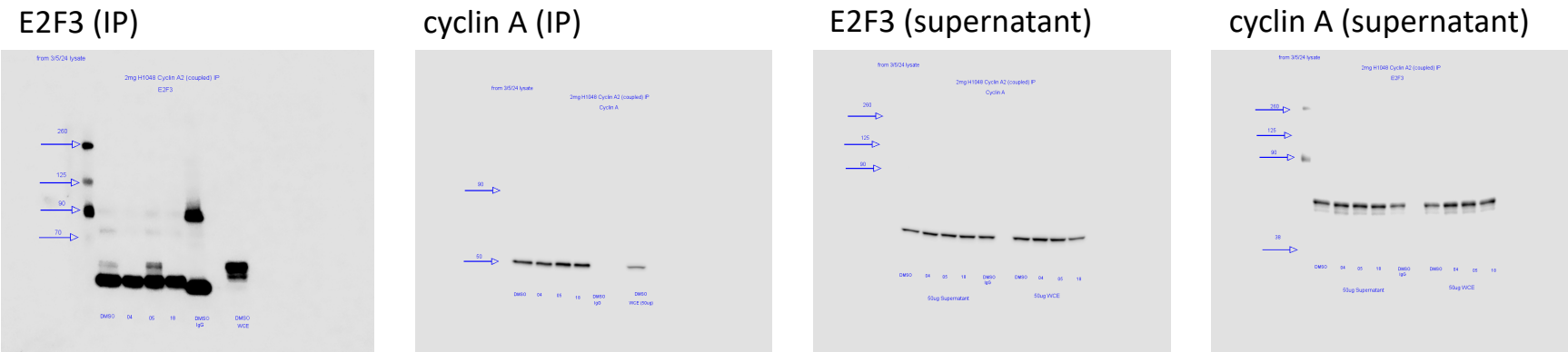
γ H2AX



H3



Extended Data Fig. 10f

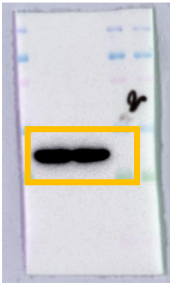


Extended Data Fig. 10g

E2F1

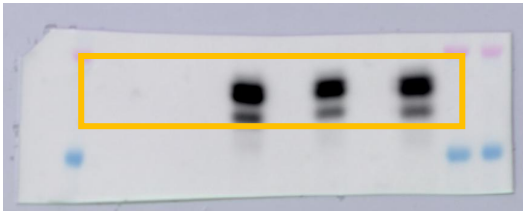


Actin

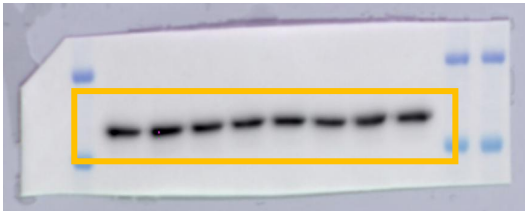


Extended Data Fig. 10i

Flag

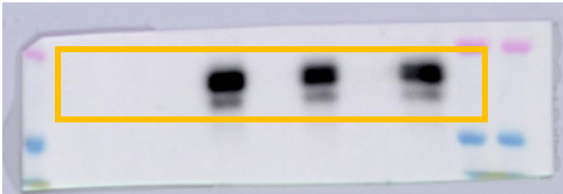


Vinculin

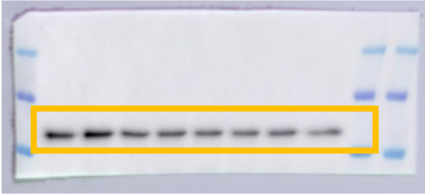


Extended Data Fig. 10k

Flag

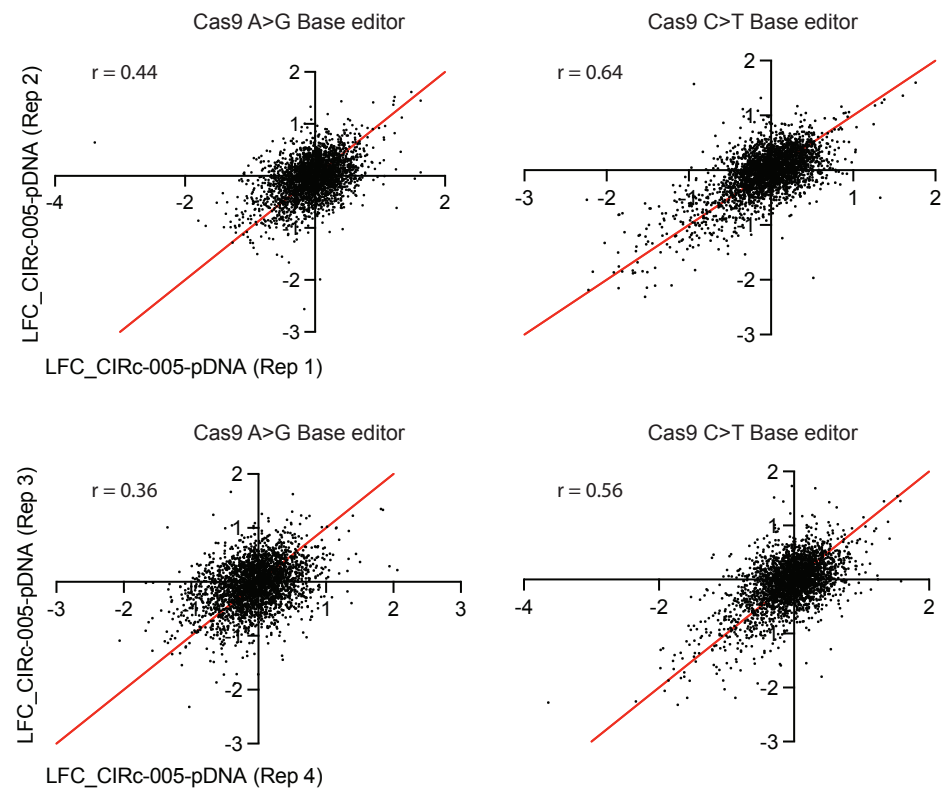


Vinculin

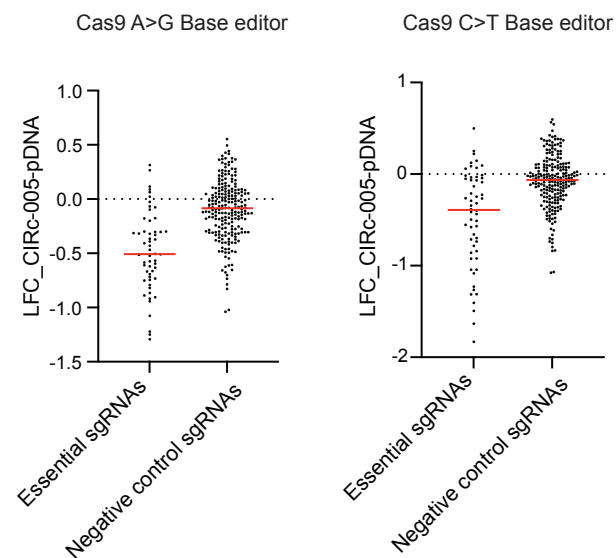


Supplementary Figure 2

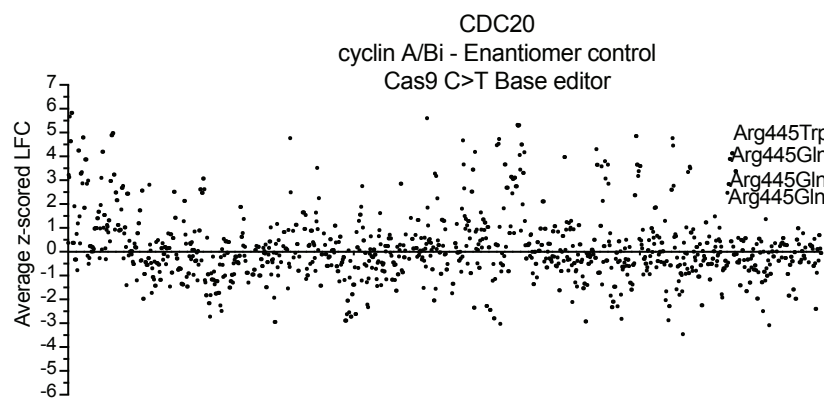
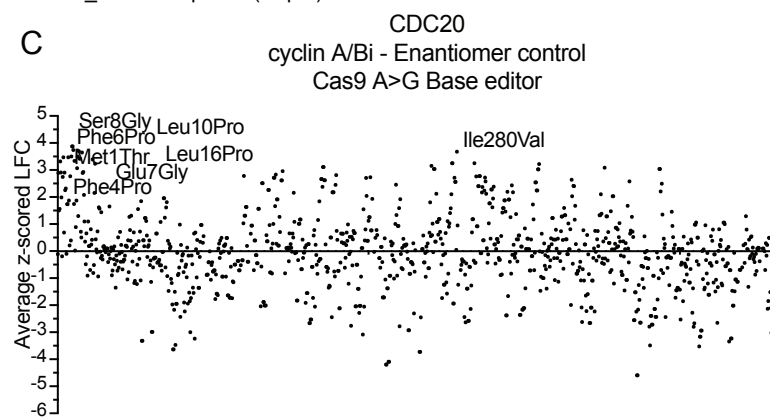
A



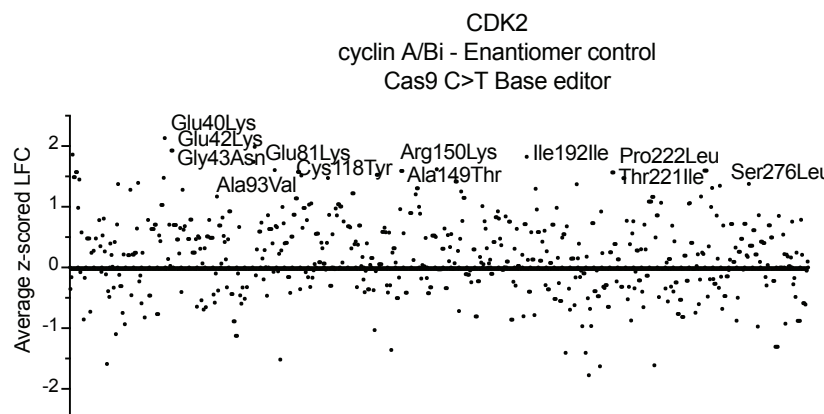
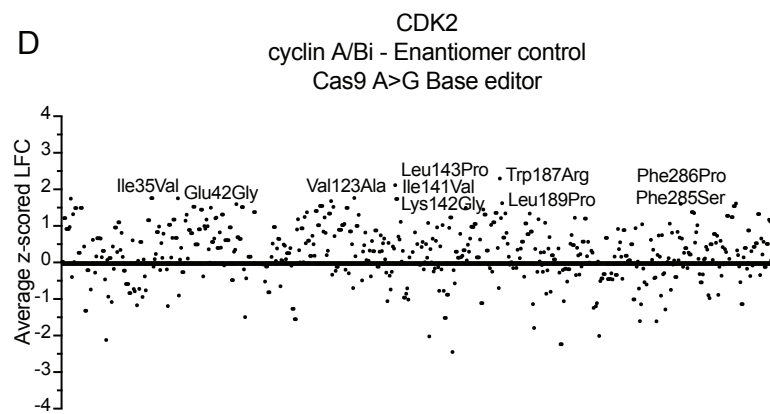
B



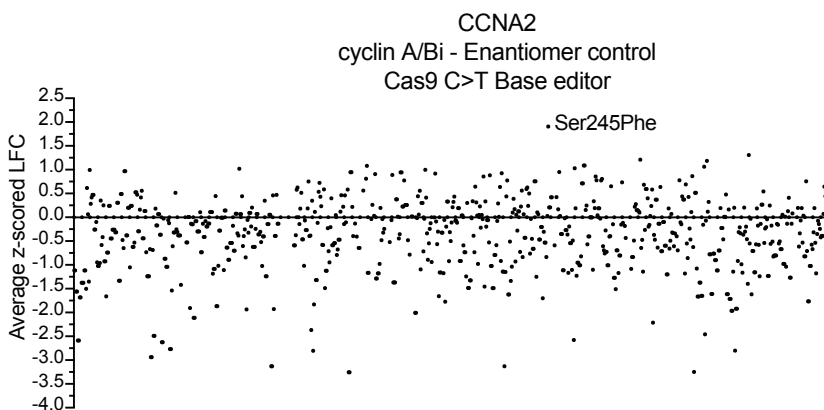
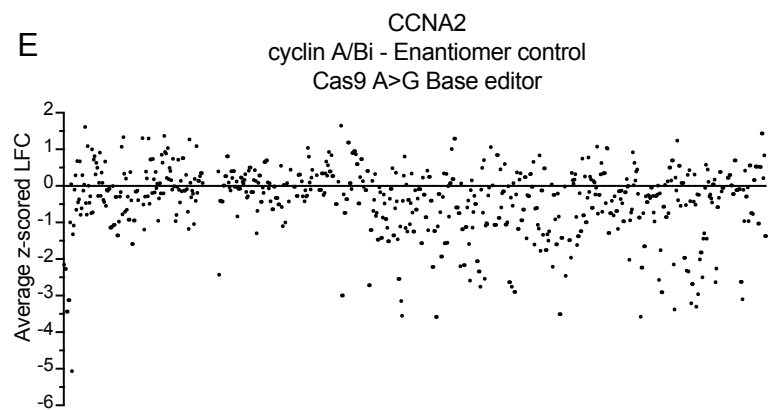
C



D

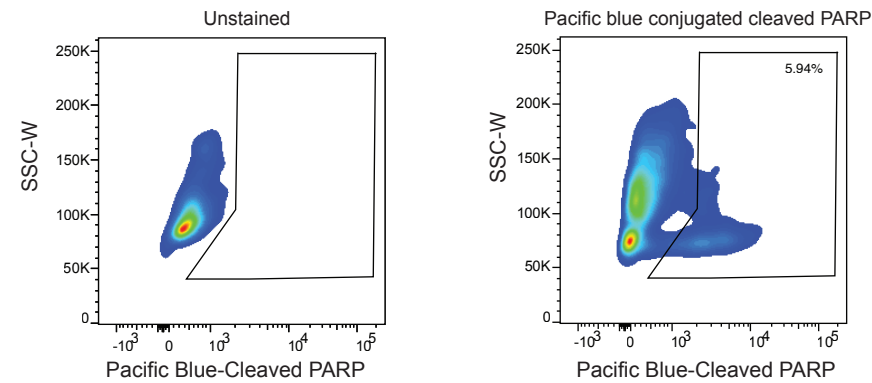


E

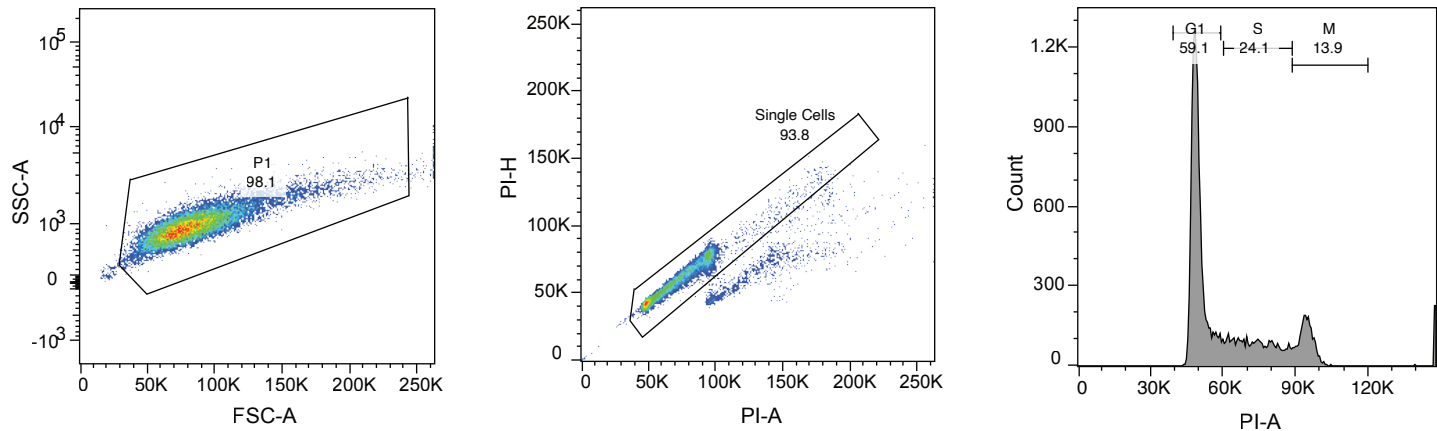


Supplementary Figure 3

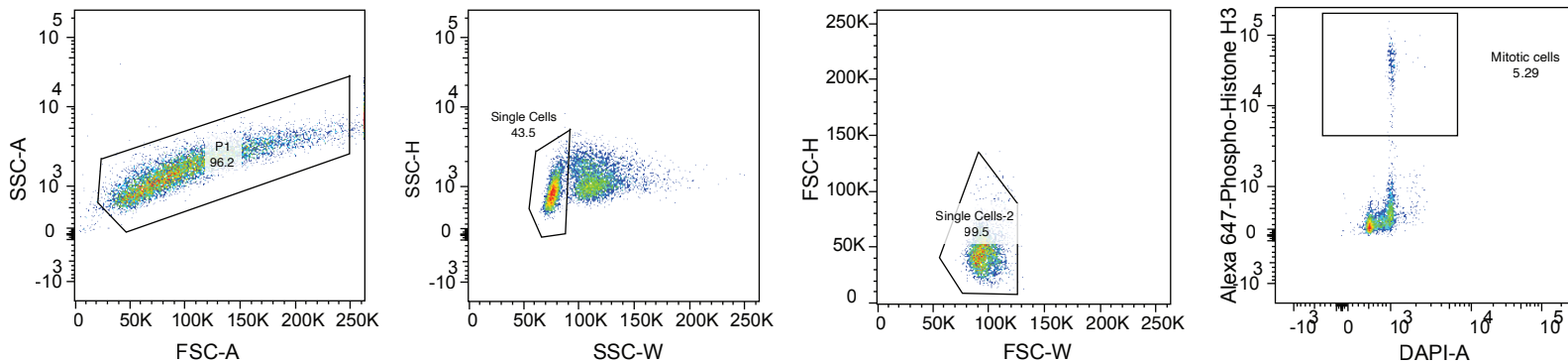
Gating strategy for Figure 1g, 2h, 3m, Extended Data Fig. 2g, 4l, 5l, 8d, 8f (Cleaved PARP FACS Analysis)



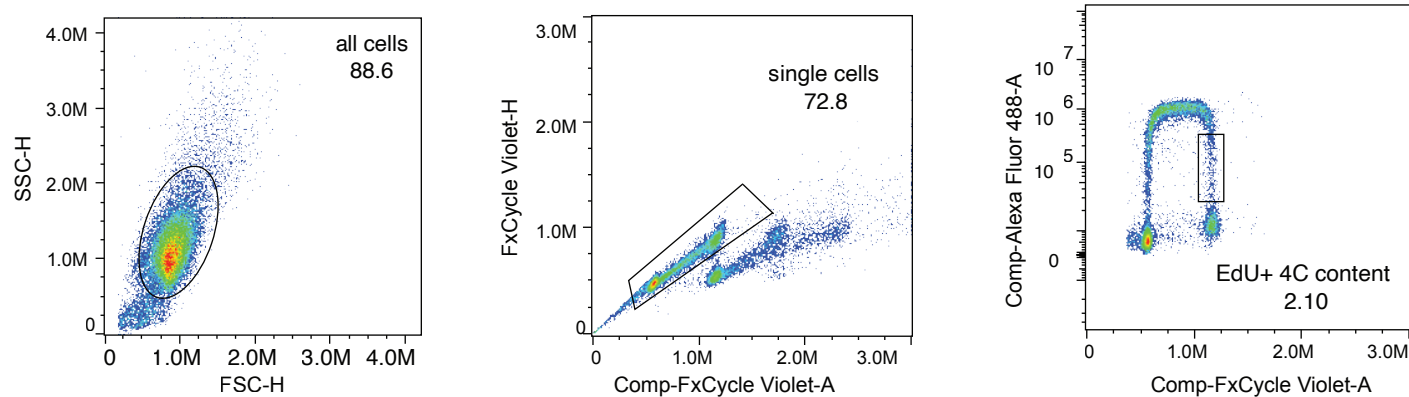
Gating strategy for Figure 1i, Extended Data Fig. 2e, 2h, 2m, 4k, 6k (Propidium Iodide FACS Analysis)



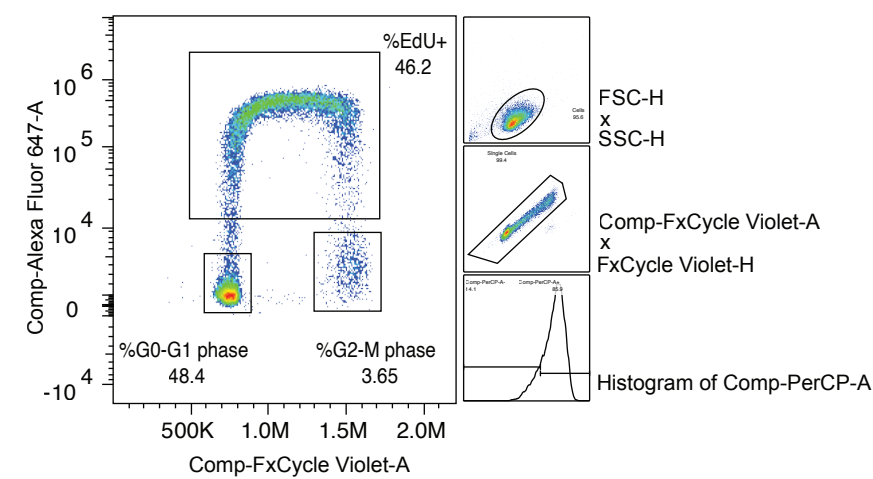
Gating strategy for Extended Data Fig. 4i, 5m (phospho-Histone H3 FACS Analysis)



Gating strategy for Figure 4a, 4l, Extended Data fig. 7b (EdU and FxCycle/DAPI FACS Analysis)



Gating strategy for Extended Data Fig. 2i (EdU and FxCycle FACS Analysis)



Gating strategy for Extended Data Fig. 2j (Cleaved PARP and FxCycle FACS Analysis)

