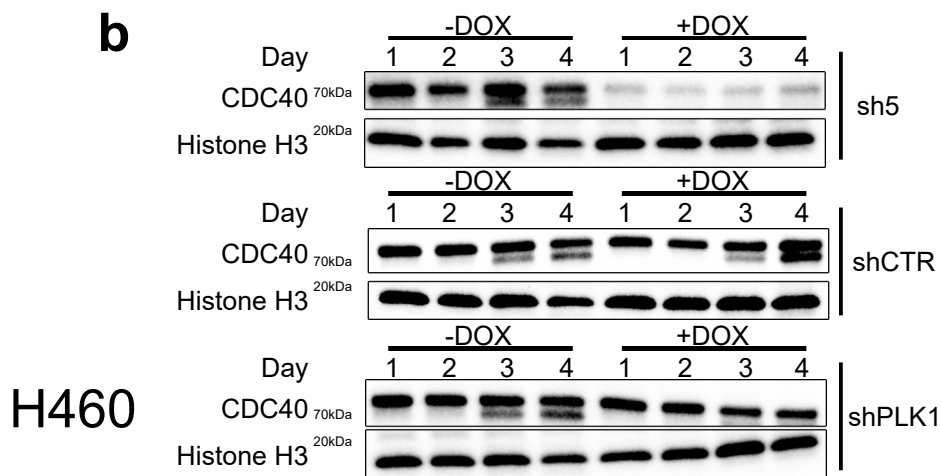
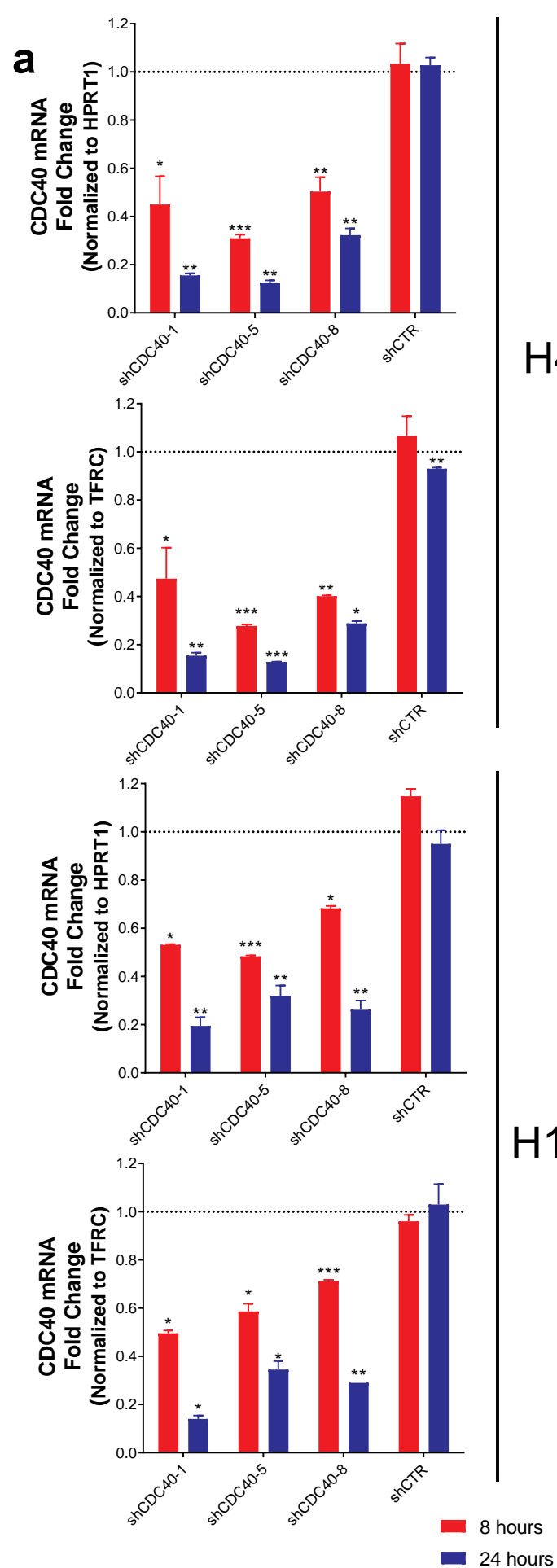
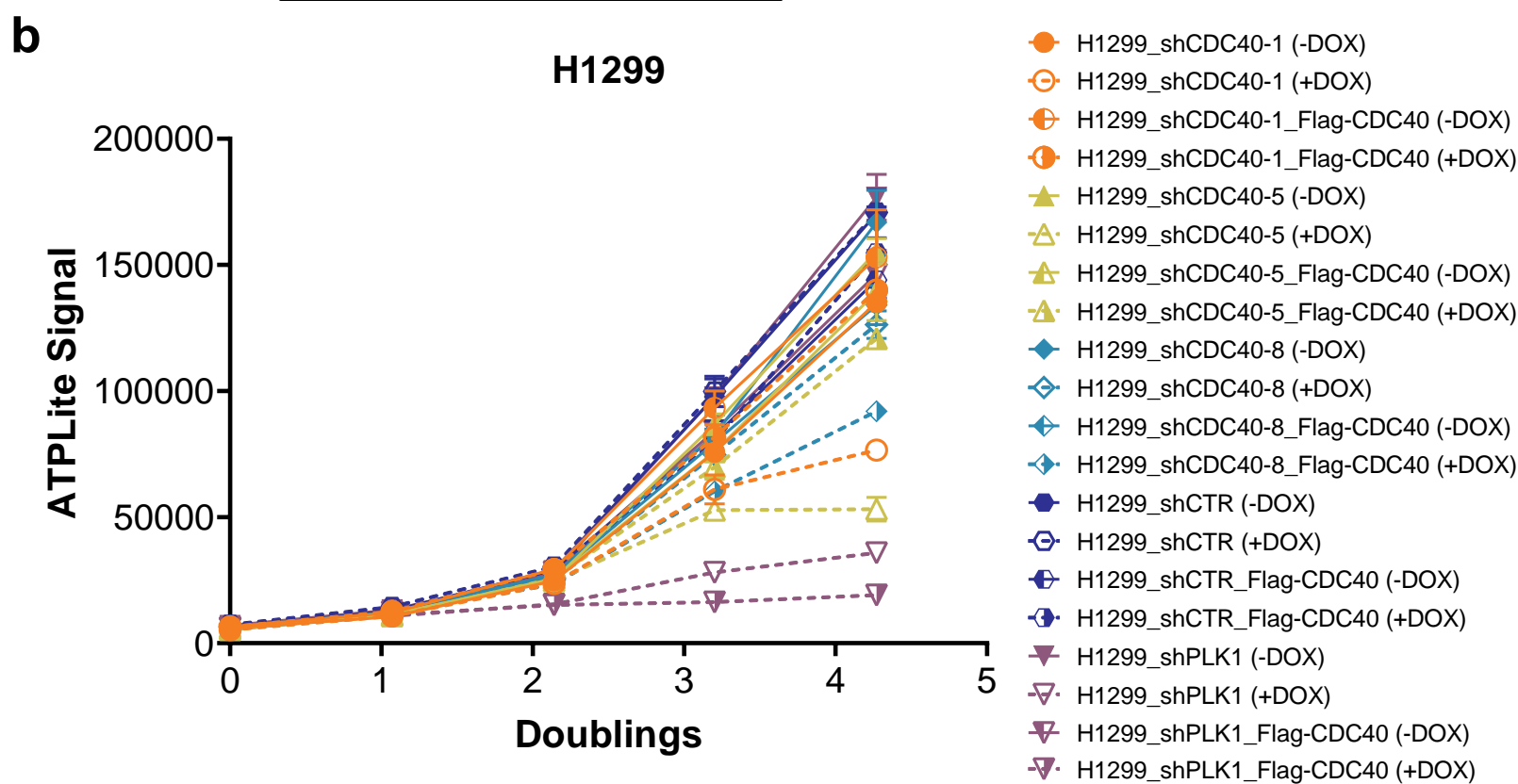
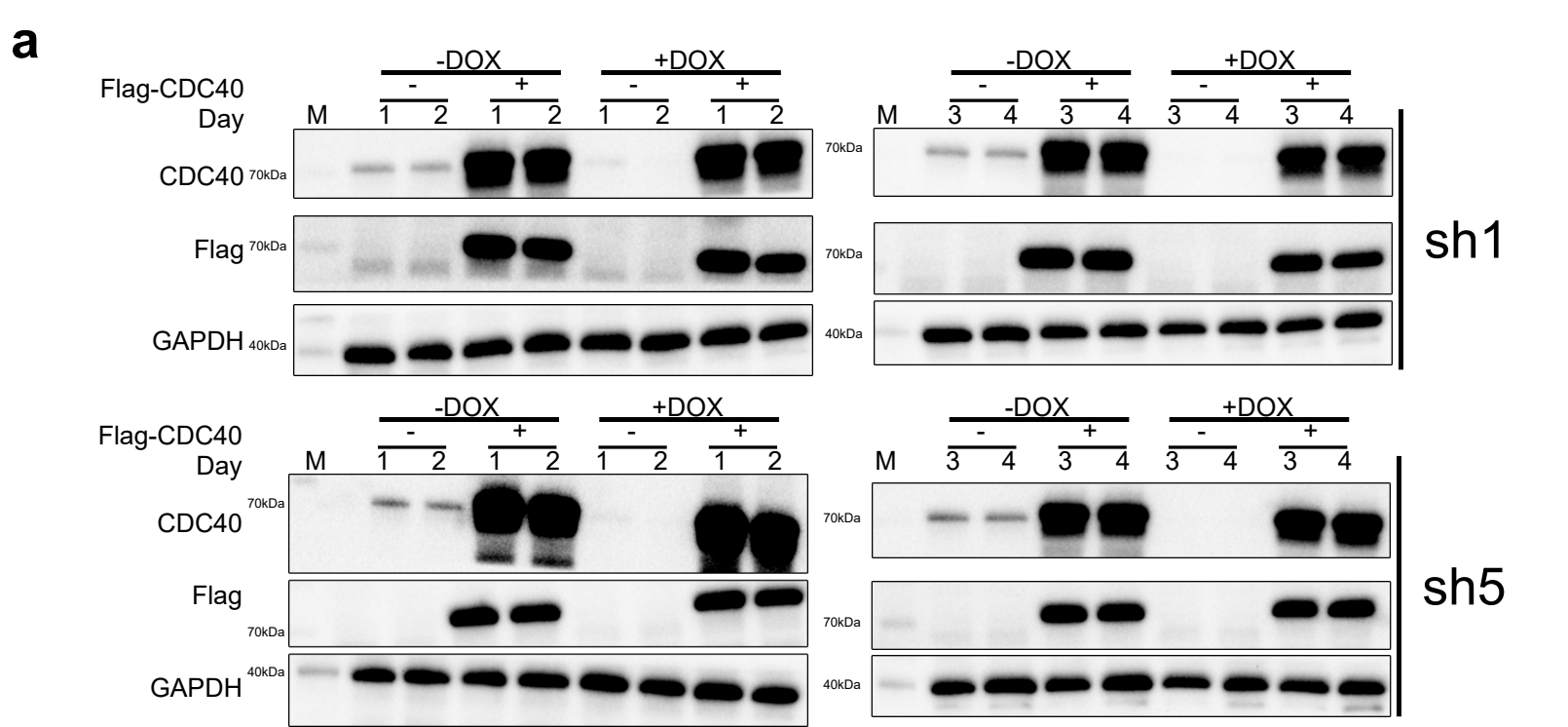


**CDC40 suppression induces CDCA5 splicing defects and anti-proliferative effects
in lung cancer cells**

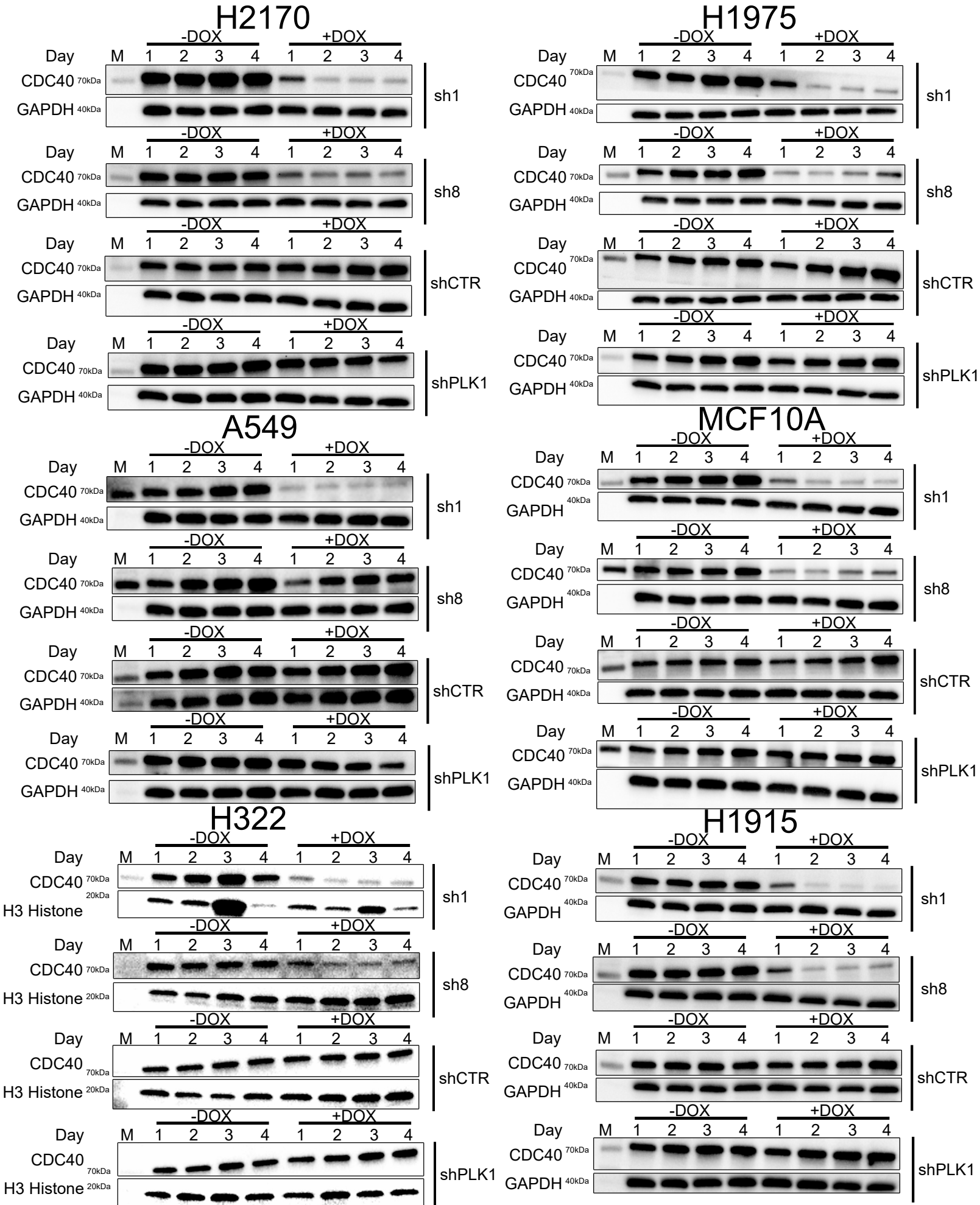
Die Hu^{1,2}, Brigitte L Thériault^{1#}, Vida Talebian¹, Laurent Hoffer¹, Julie Owen^{1%}, Justin Lim^{3,4}, Benjamin J. Blencowe^{3,4}, Evelyne Lima-Fernandes¹, Punit Saraon^{1&}, Richard Marcellus¹, Rima Al-awar^{1,2,5*}



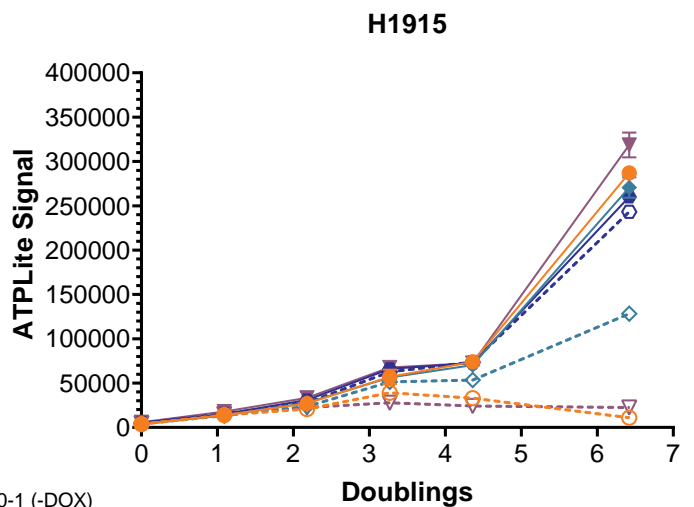
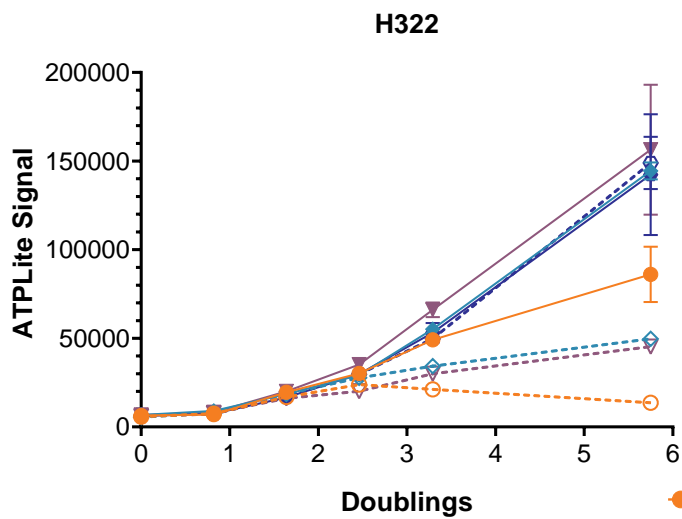
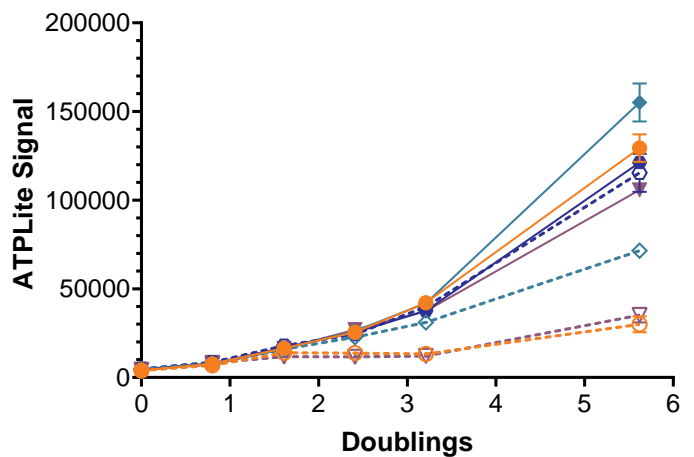
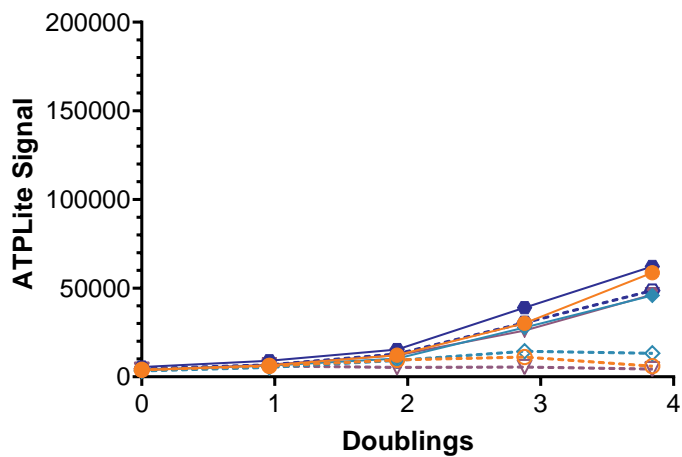
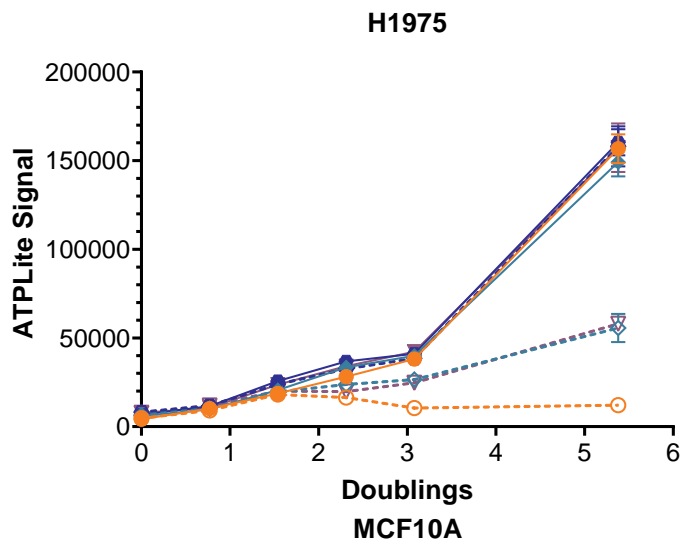
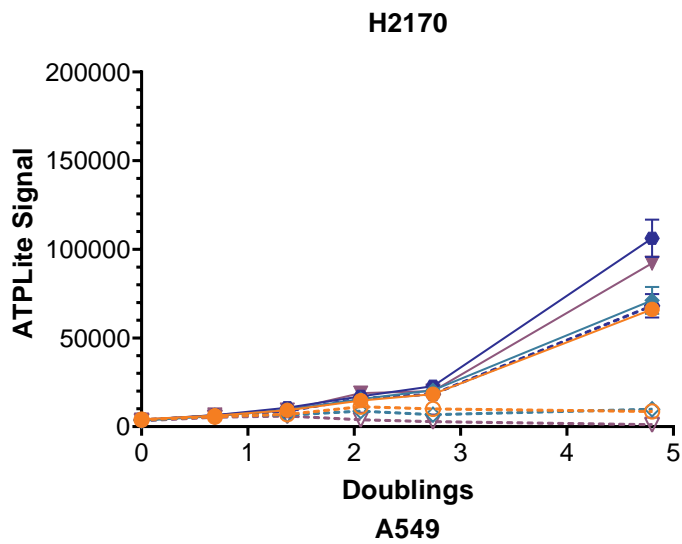
Supplementary Figure 1. Knockdown of CDC40 reduces CDC40 both mRNA and protein level. (a) RT-qPCR analysis showing the effect of CDC40 knockdown using three different shCDC40 constructs in comparison to control (shCTR) on CDC40 mRNA expression after 8 and 24 hours DOX induction in H460 and H1299 cells (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; $n=2$, \pm SD, Student's T-test). **(b)** Western blot analysis showing the effect of CDC40 knockdown using shCDC40-5 (representative) in comparison to negative (shCTR) and toxic (shPLK1) controls on CDC40 expression after 4 days of DOX induction. (see Supplementary Figure 12 for source blot images). Results are representative of at least two



Supplementary Figure 2. Knockdown of CDC40 reduces proliferation in H1299 lung cancer cells. (a) Western blot analysis showing the effect of Flag-CDC40 overexpression on the expression of Flag and CDC40 after 4 days DOX induction. Results are representative of at least two independent experiments. **(b)** Assessing the effect of Flag-CDC40 overexpression on the proliferation of H1299 cells after 4 days shCDC40 induction. Results were normalized to the doubling time of H1299 cells. (n=3, ±SD)



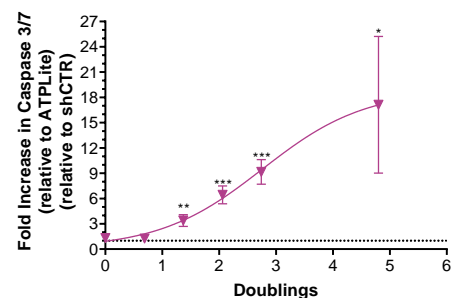
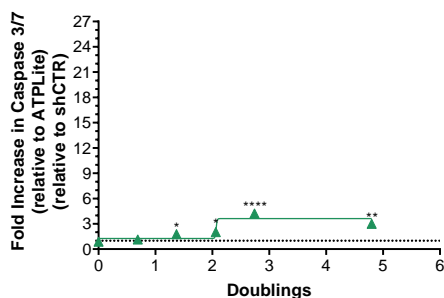
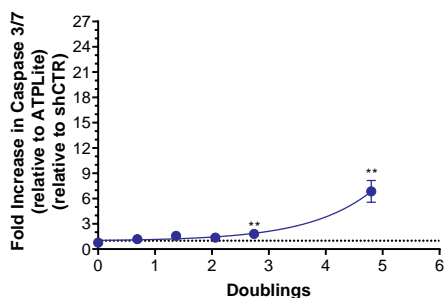
Supplementary Figure 3. Knockdown of CDC40 reduces CDC40 proteins in various lung cancer cells. Western blot analysis showing the effect of CDC40 knockdown using shCDC40 constructs (sh1 and sh8) in comparison to control (shCTR), and toxic control (shPLK1) on CDC40 expression after 4 days DOX induction.



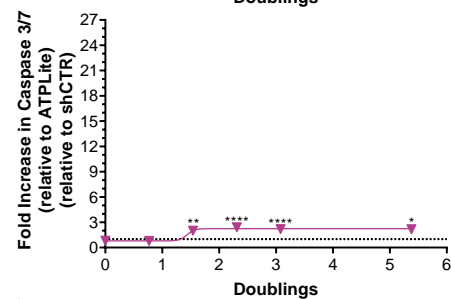
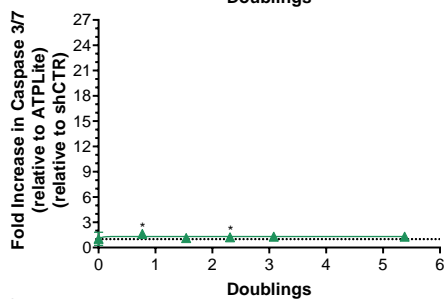
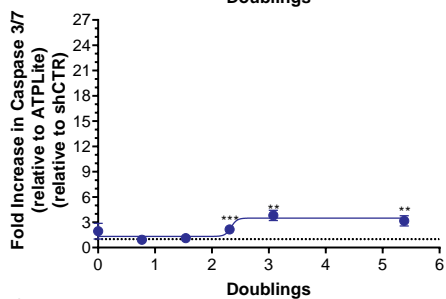
- shCDC40-1 (-DOX)
- shCDC40-1 (+DOX)
- ◆ shCDC40-8 (-DOX)
- ◇ shCDC40-8 (+DOX)
- shCTR (-DOX)
- shCTR (+DOX)
- ▼ shPLK1 (-DOX)
- ▽ shPLK1 (+DOX)

Supplementary Figure 4. Knockdown of CDC40 reduces proliferation in various lung cancer cells. Assessing the effect of CDC40 knockdown on the proliferation of H2170, H1975, A549, MCF10A, H322 and H1915 cells after 4 days of shCDC40-1, shCDC40-8, shCTR and shPLK1 induction. Results were normalized to the doubling time of corresponding cell lines. (n=3, ±SD)

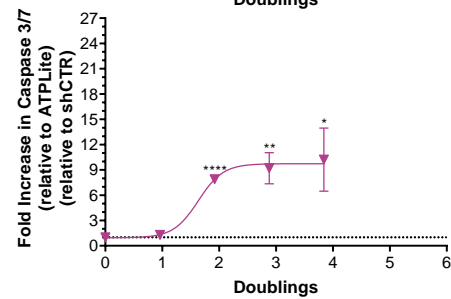
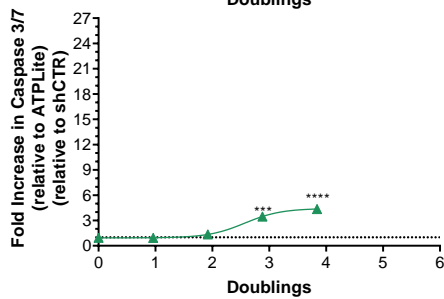
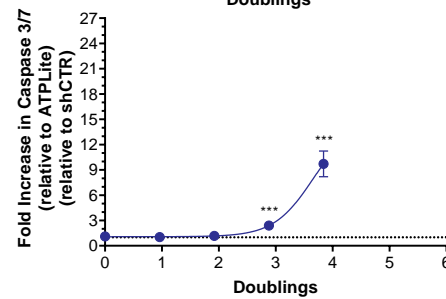
H2170



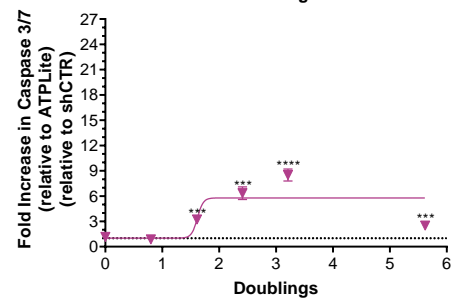
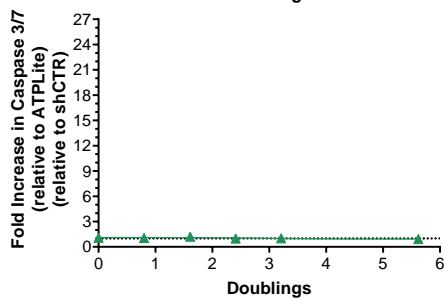
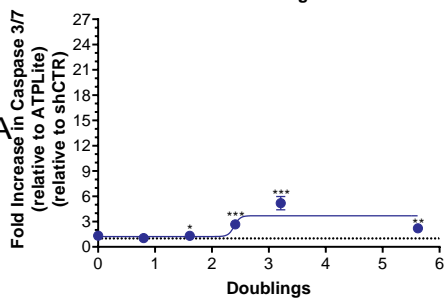
H1975



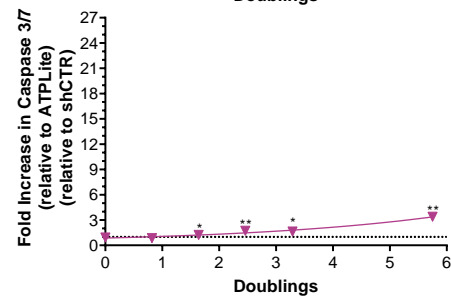
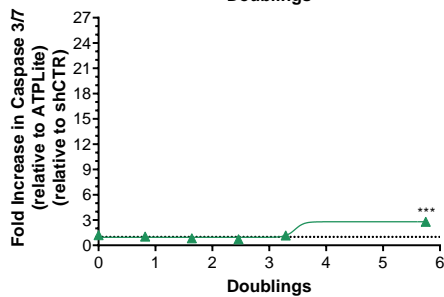
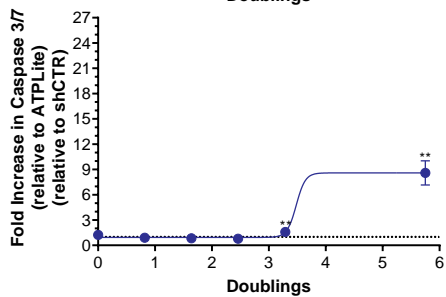
A549



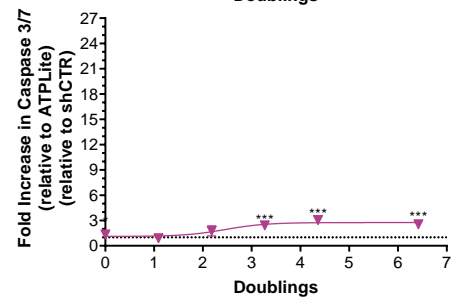
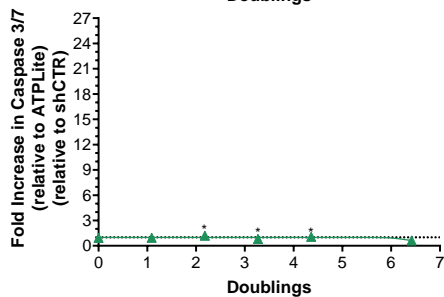
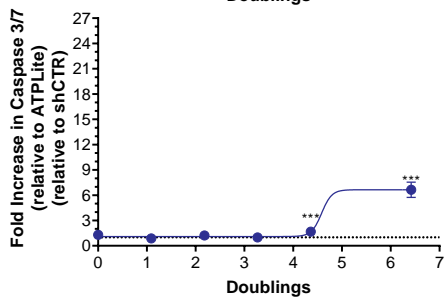
MCF10A



H322



H1915

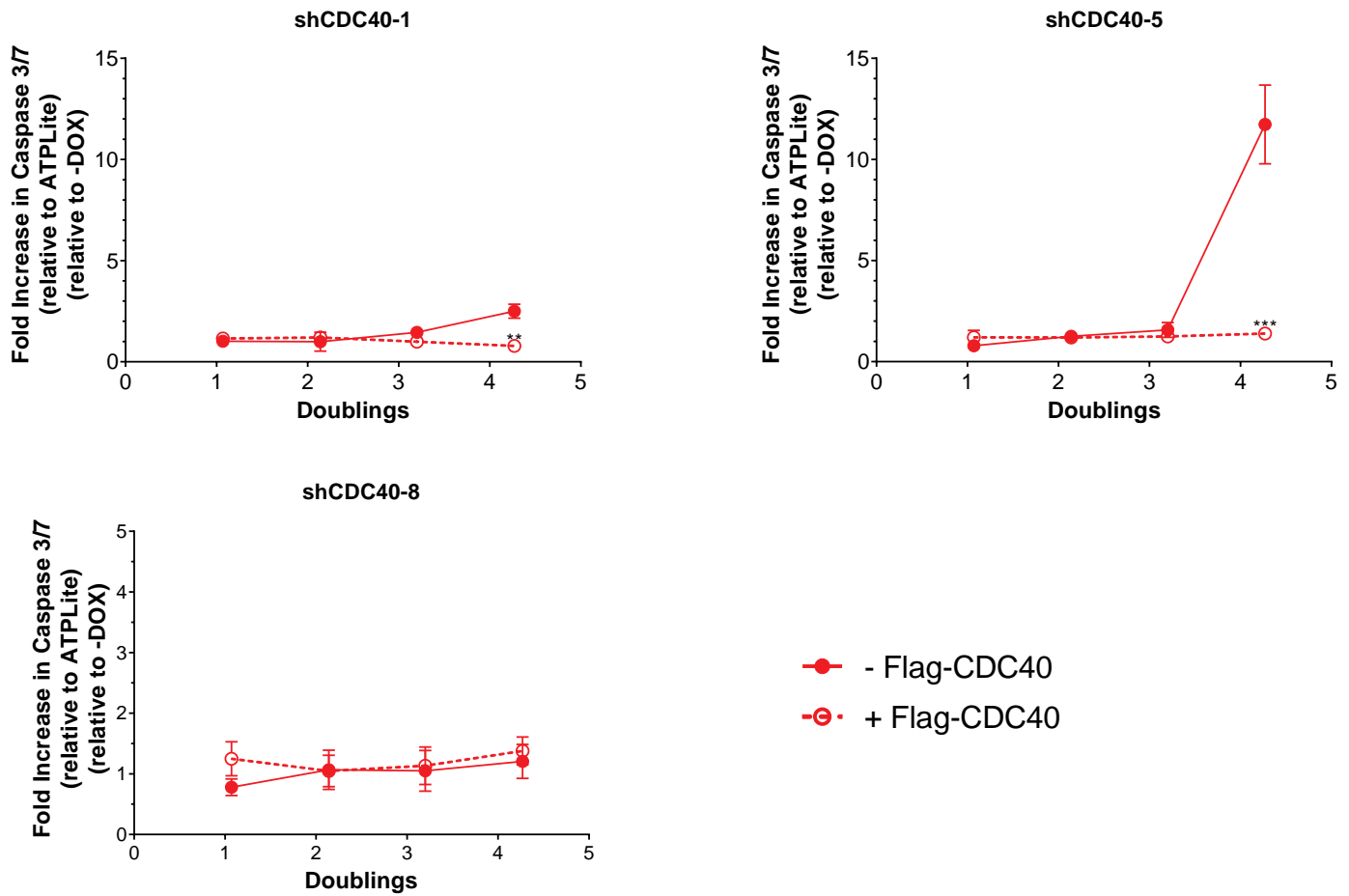


● shCDC40-1

▲ shCDC40-8

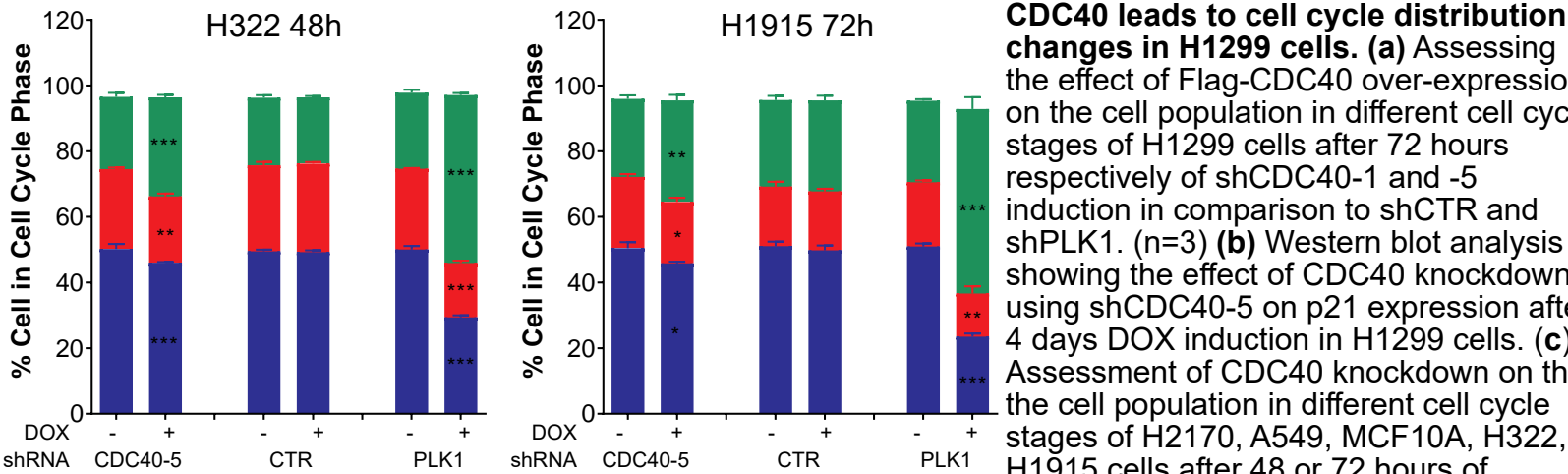
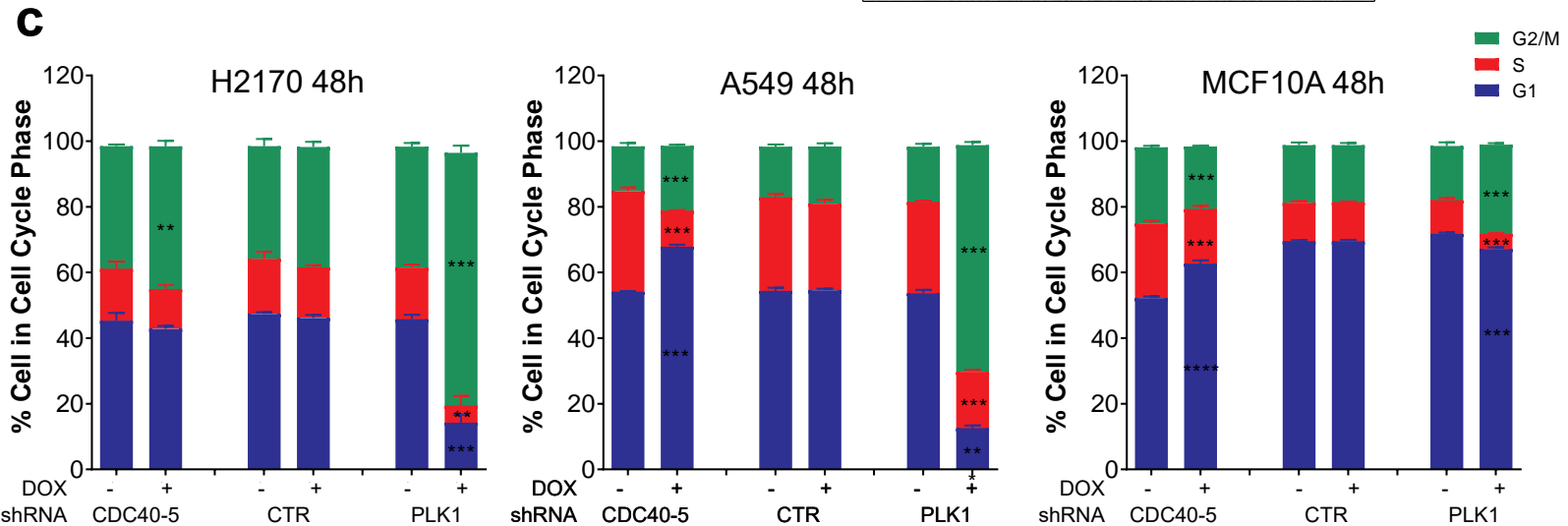
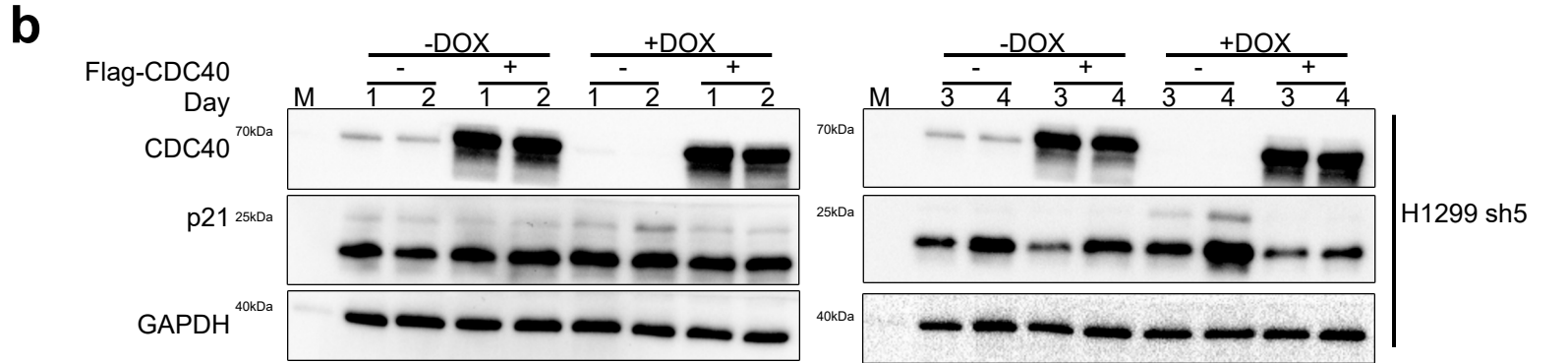
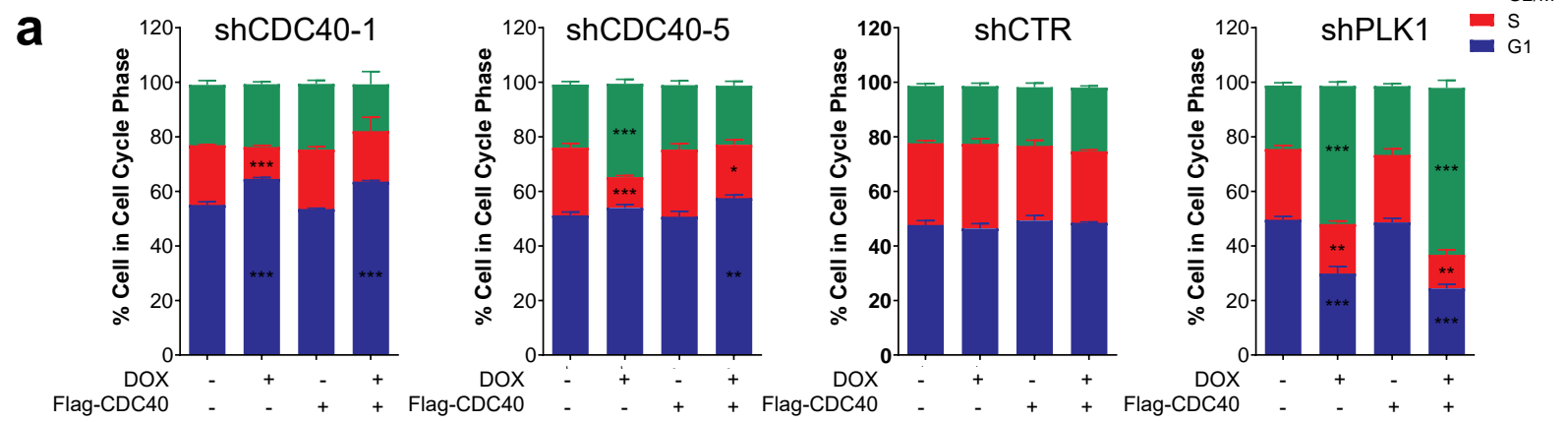
▼ shPLK1

Supplementary Figure 5. Knockdown of CDC40 induces late apoptosis marker level increase in various lung cancer cells. Assessment of CDC40 knockdown on late apoptotic markers (caspase 3 and 7) of H2170, H1975, A549, MCF10A, H322 and H1915 cells after 4 days of shCDC40-1, shCDC40-8 and shPLK1 (representative) induction. Results were normalized to the doubling time of corresponding cell lines. (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; $n=3$, \pm SD, Student's T-test)

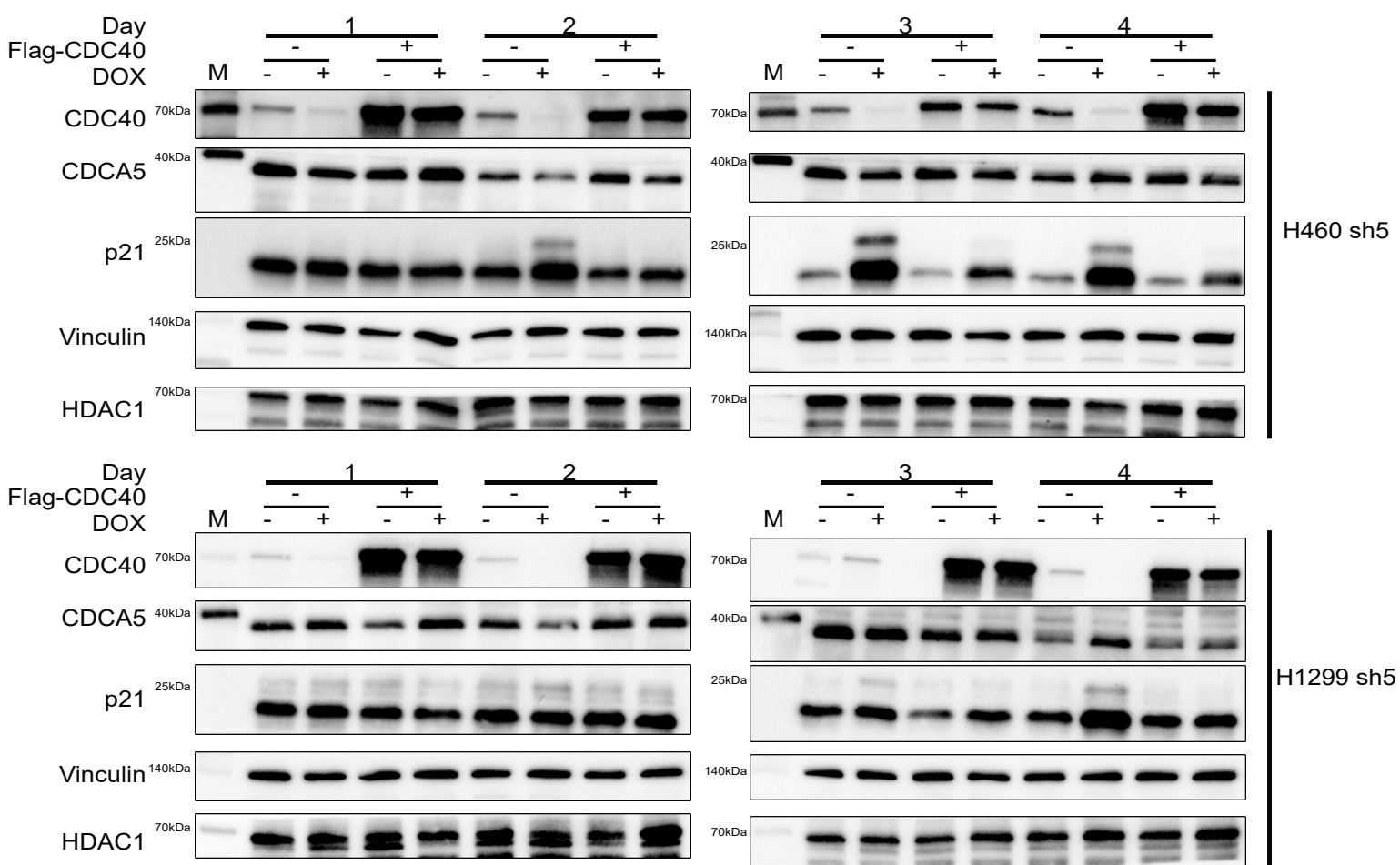
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Supplementary Figure 6. Knockdown of CDC40 induces late apoptosis marker level increase in H1299 cells. (a) Assessment of Flag-CDC40 overexpression on the late apoptotic markers (caspase 3 and 7) of H1299 cells after 4 days shCDC40 induction. Results were normalized to the doubling time of H1299 cells. (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; $n=3$, \pm SD, Student's T-test)

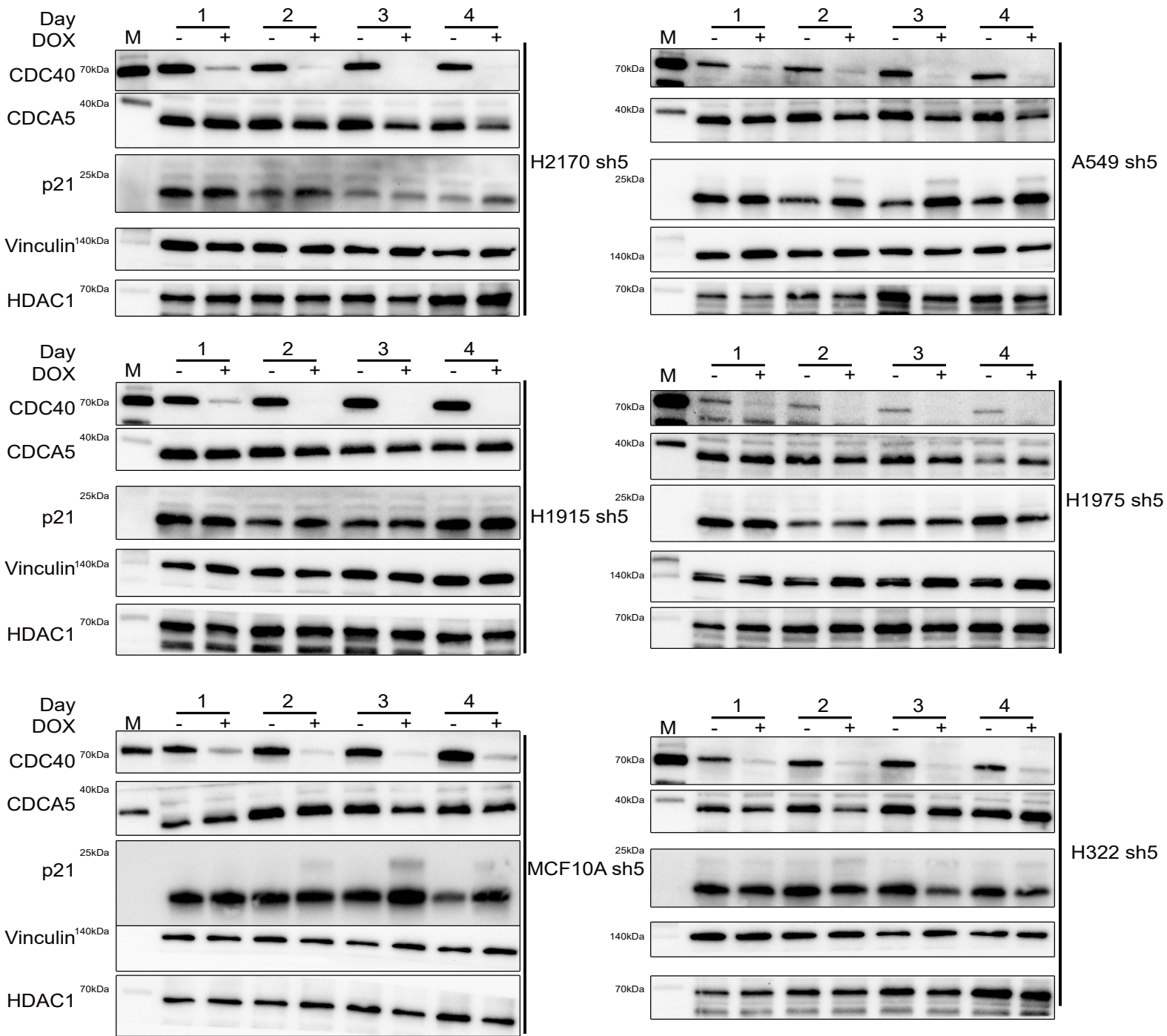
H1299 72h



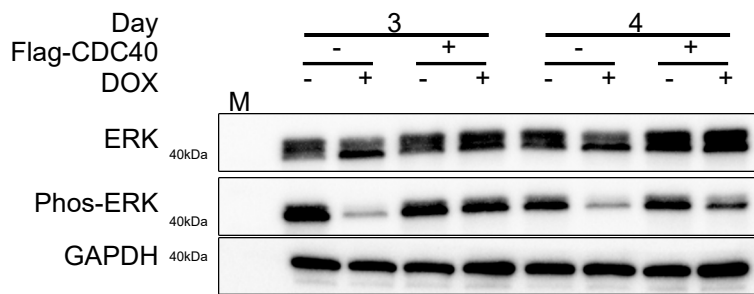
Supplementary Figure 7. Knockdown of CDC40 leads to cell cycle distribution changes in H1299 cells. (a) Assessing the effect of Flag-CDC40 over-expression on the cell population in different cell cycle stages of H1299 cells after 72 hours respectively of shCDC40-1 and -5 induction in comparison to shCTR and shPLK1. (n=3) **(b)** Western blot analysis showing the effect of CDC40 knockdown using shCDC40-5 on p21 expression after 4 days DOX induction in H1299 cells. **(c)** Assessment of CDC40 knockdown on the cell population in different cell cycle stages of H2170, A549, MCF10A, H322, H1915 cells after 48 or 72 hours of shCDC40-5 (representative) induction in comparison to shCTR and shPLK1. (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; n=3, \pm SD, Student's T-test)



Supplementary Figure 9. Knockdown of CDC40 reduces CDCA5 levels and increases p21 levels in various cell lines. Western blot analysis shows the effect of CDC40 knockdown using shCDC40-5 on CDCA5 and p21 expression after 4-day DOX induction in H460 and H1299 cells.



Supplementary Figure 10. Knockdown of CDC40 reduces CDCA5 levels and increases p21 levels in various cell lines. Western blot analysis shows the effect of CDC40 knockdown using shCDC40-5 on CDCA5 and p21 expression after 4-day DOX induction in H2170, A549, H1915, H1975, MCF10A and H322 cells.



Supplementary Figure 11. Knockdown of CDC40 leads to changes in pERK protein levels. Western blot analysis showing the effect of CDC40 knockdown using shCDC40-5 on ERK and pERK (Thr202Thy204) expression after 3 and 4 days DOX induction in H460 cells.

Supplementary Figure 1b, 20

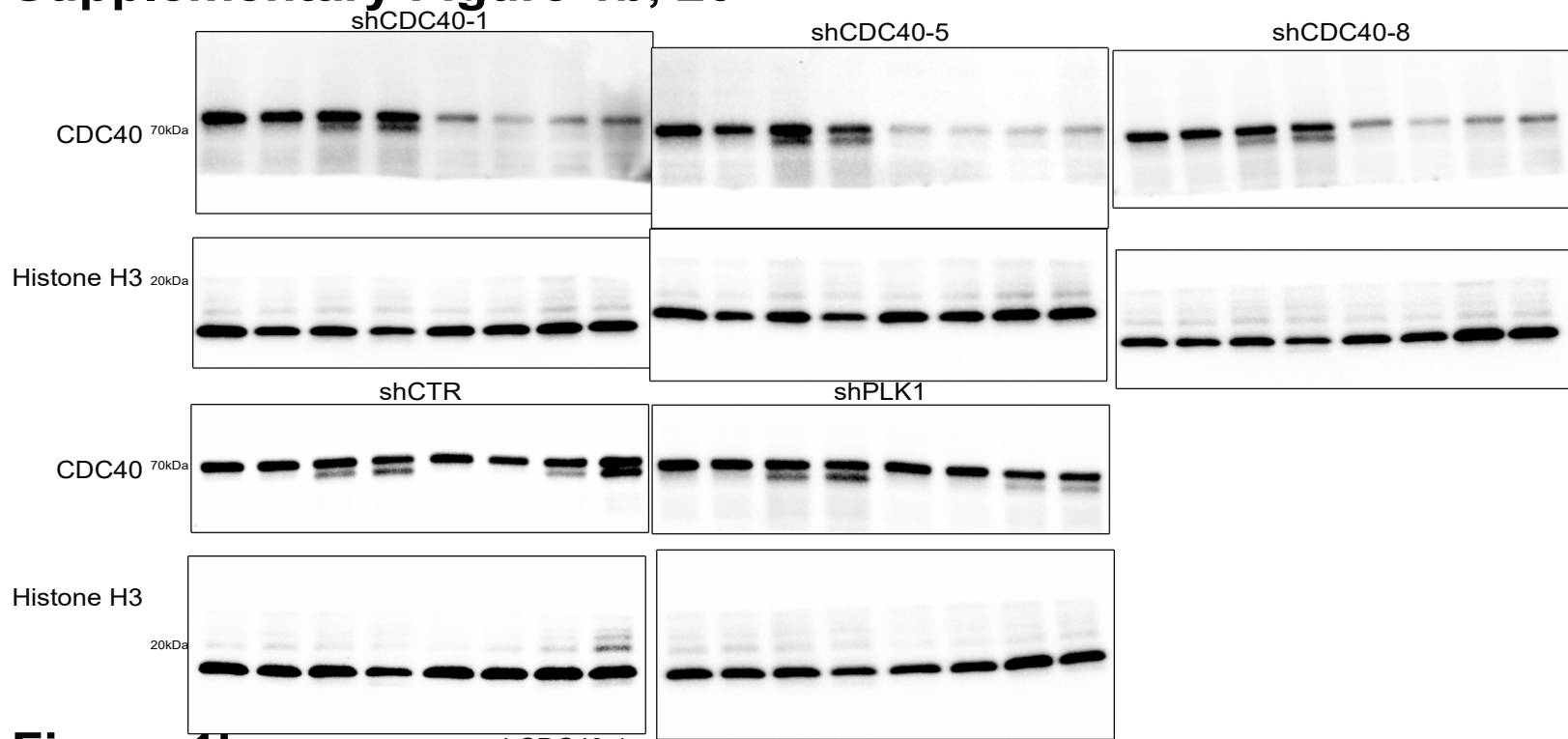
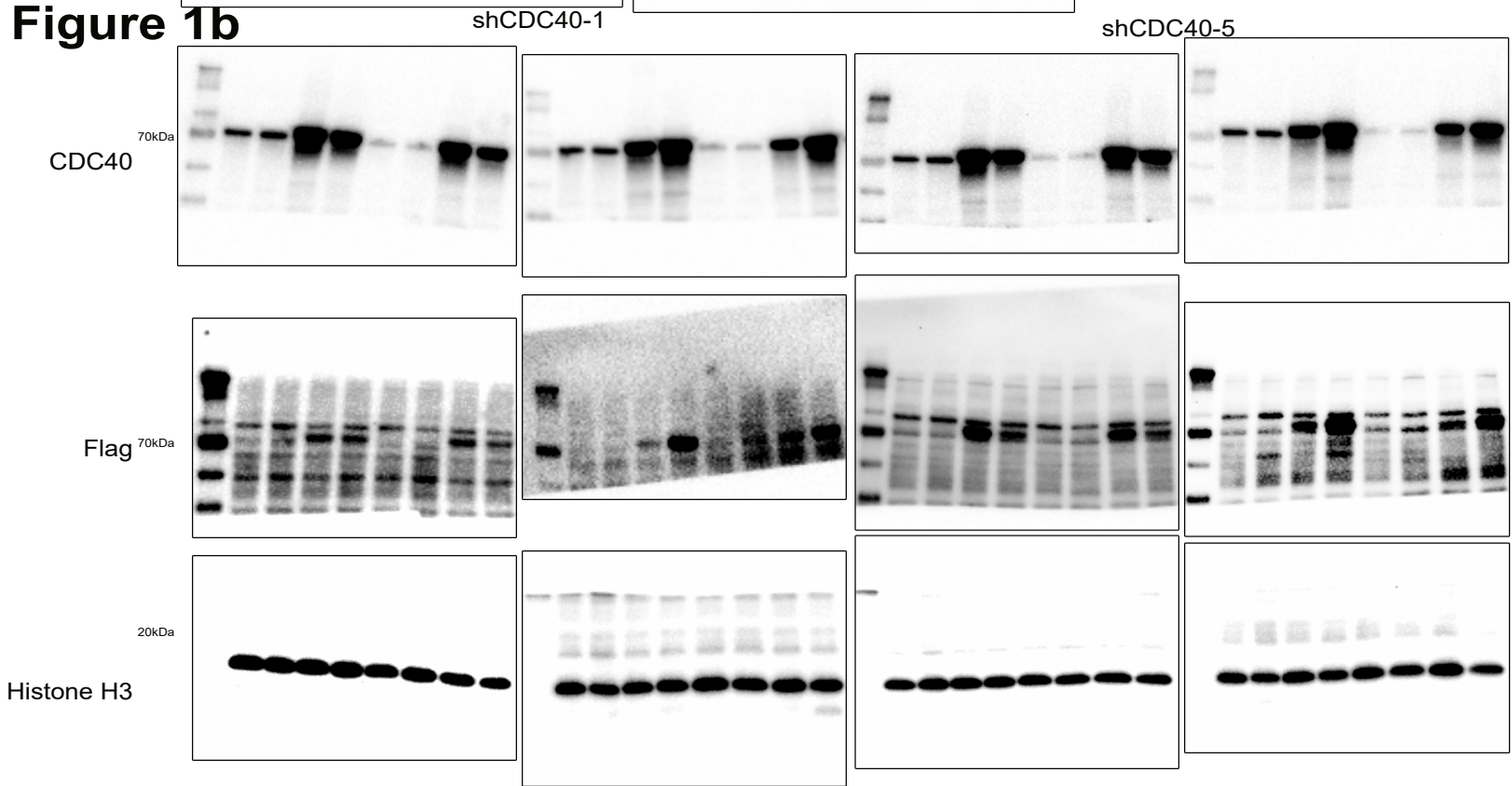


Figure 1b



Supplementary Figure 12. Source blot images for Supplementary Supplementary Figure 1, 20 and Figure 1.

Supplementary Figure 24

H2170

H1975

A549

CDC40

70kDa

GAPDH

40kDa

MCF10A

H1915

CDC40

70kDa

GAPDH

40kDa

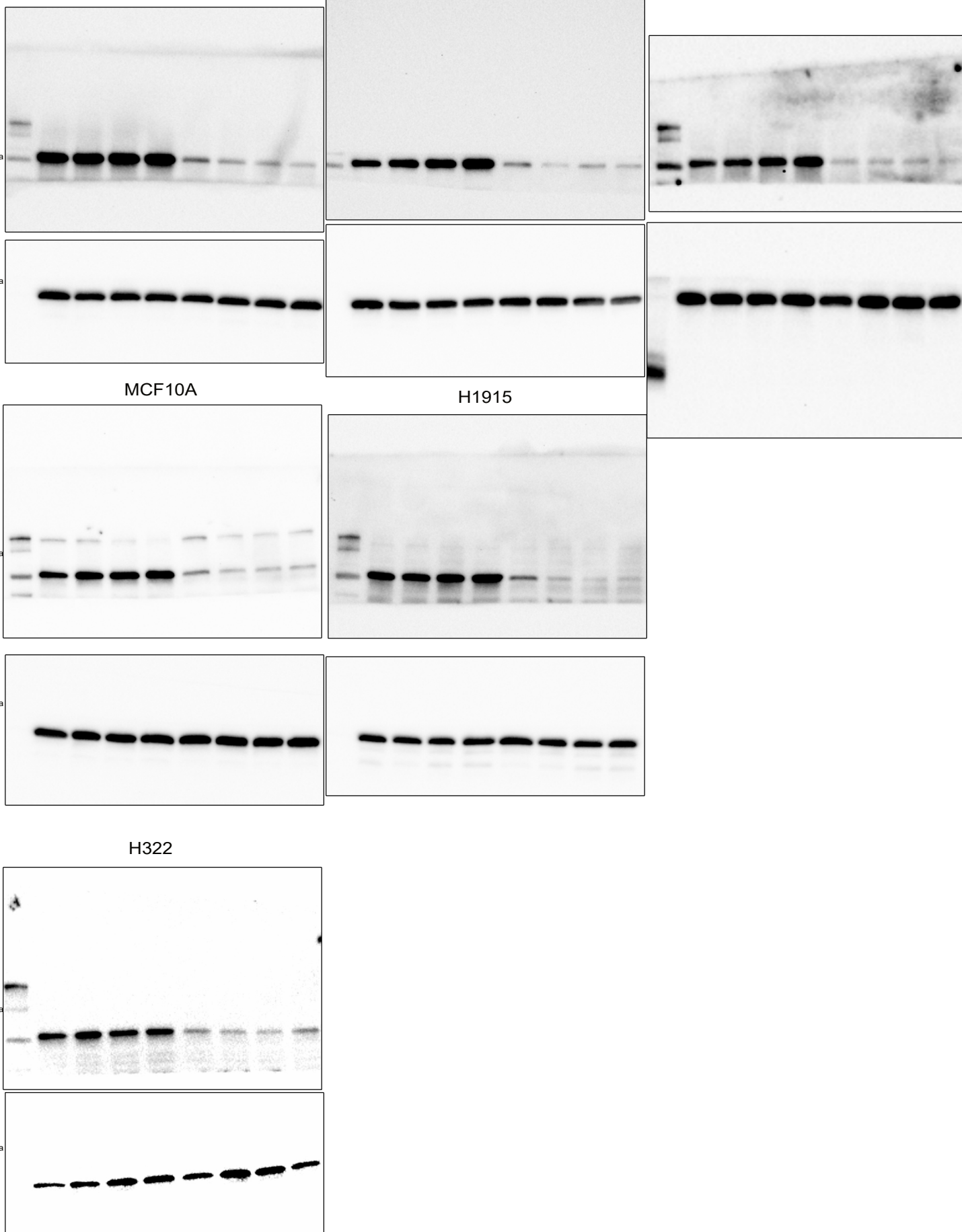
H322

CDC40

70kDa

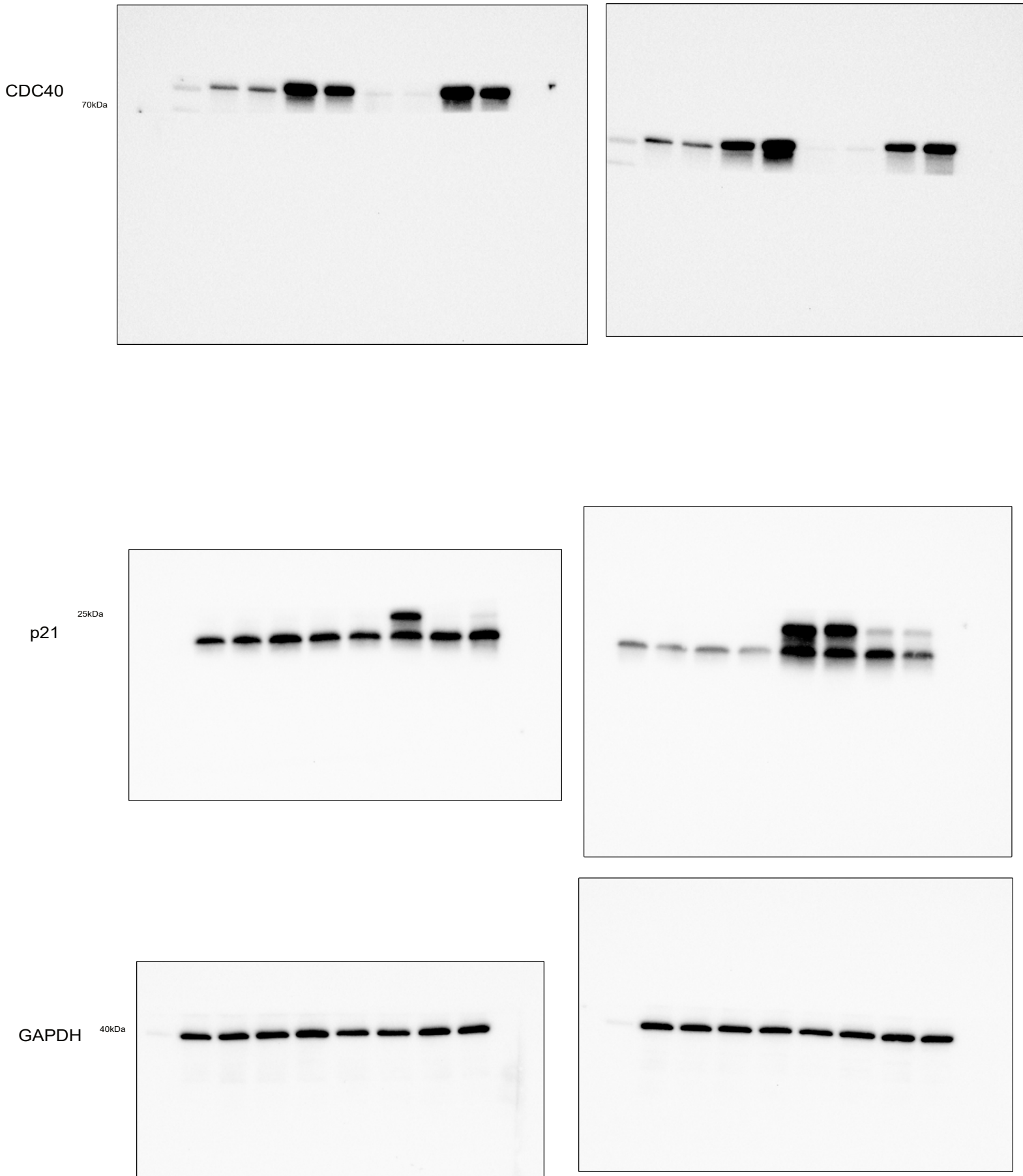
Histone H3

20kDa

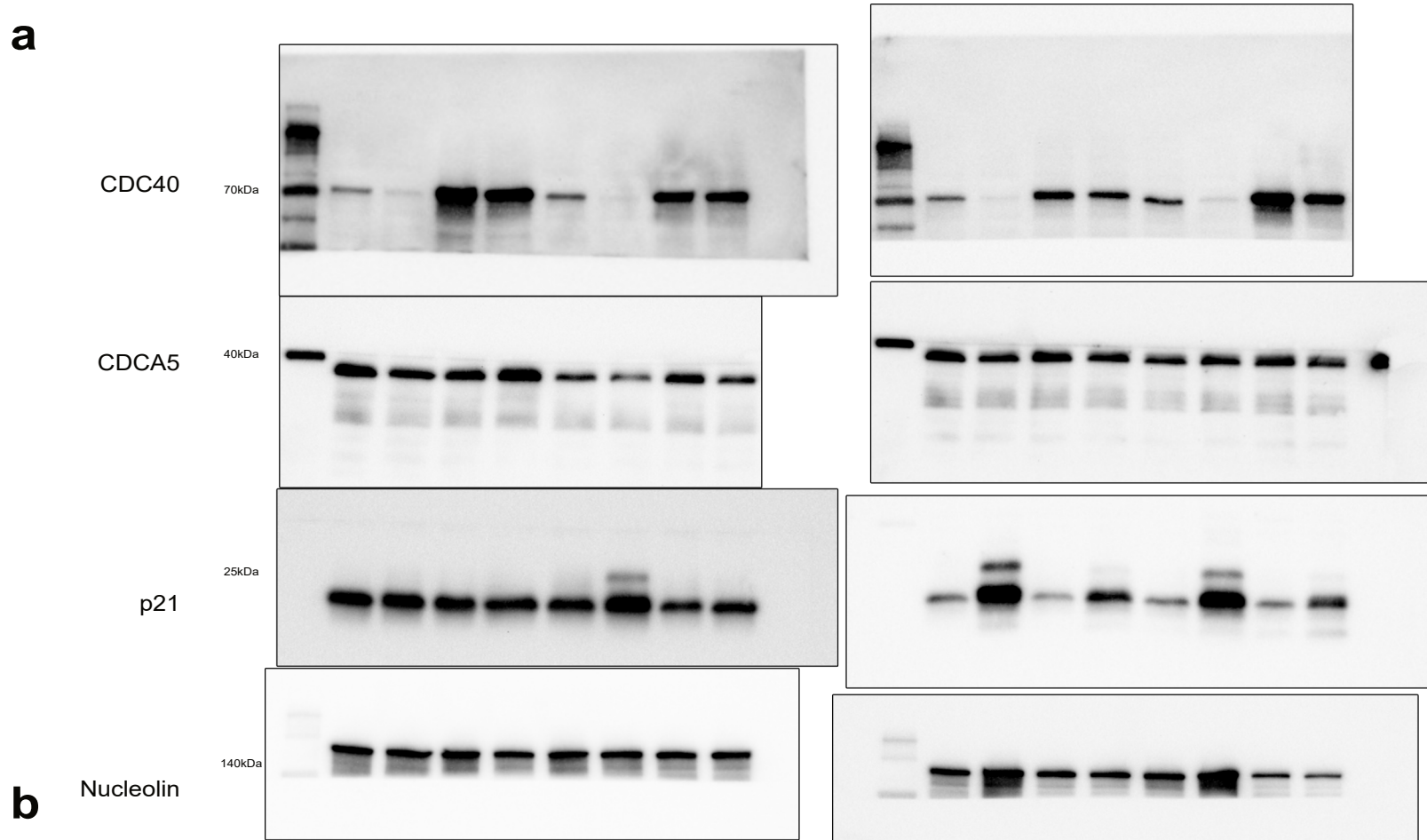


Supplementary Figure 13. Knockdown of CDC40 reduces proliferation in H460 lung cancer cells.
Source blot images for Supplementary Figure 24.

Figure 2b



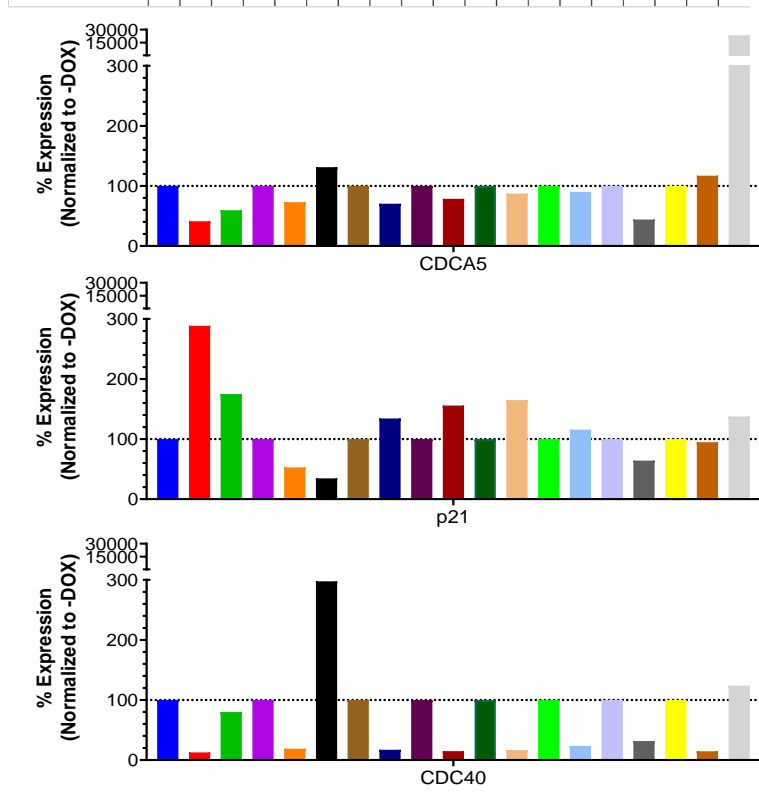
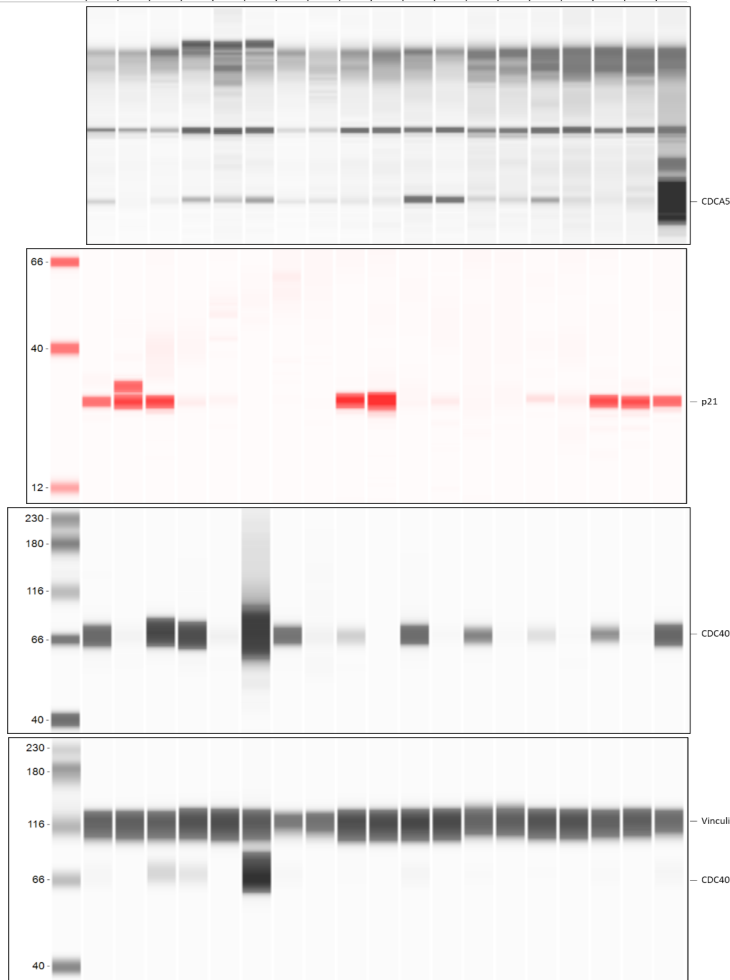
Supplementary Figure 14. Source blot images for Figure 2.



b

Cell Line	H460		H1299		H2170	A549	H1915	H1975	H322	MCF10A	H460
Flag-CDC40	-	-	-	-	-	-	-	-	-	-	-
CDCA5-Myc-DDK	-	-	-	-	-	-	-	-	-	-	+
DOX	-	+	+	-	+	+	-	+	-	+	-

Cell Line	H460	H1299	H2170	A549	H1915	H1975	H322	MCF10A	H460
Flag-CDC40	-	-	+	-	-	+	-	-	-
CDCA5-Myc-DDK	-	-	-	-	-	-	-	-	+
DOX	-	+	+	-	+	+	-	+	-

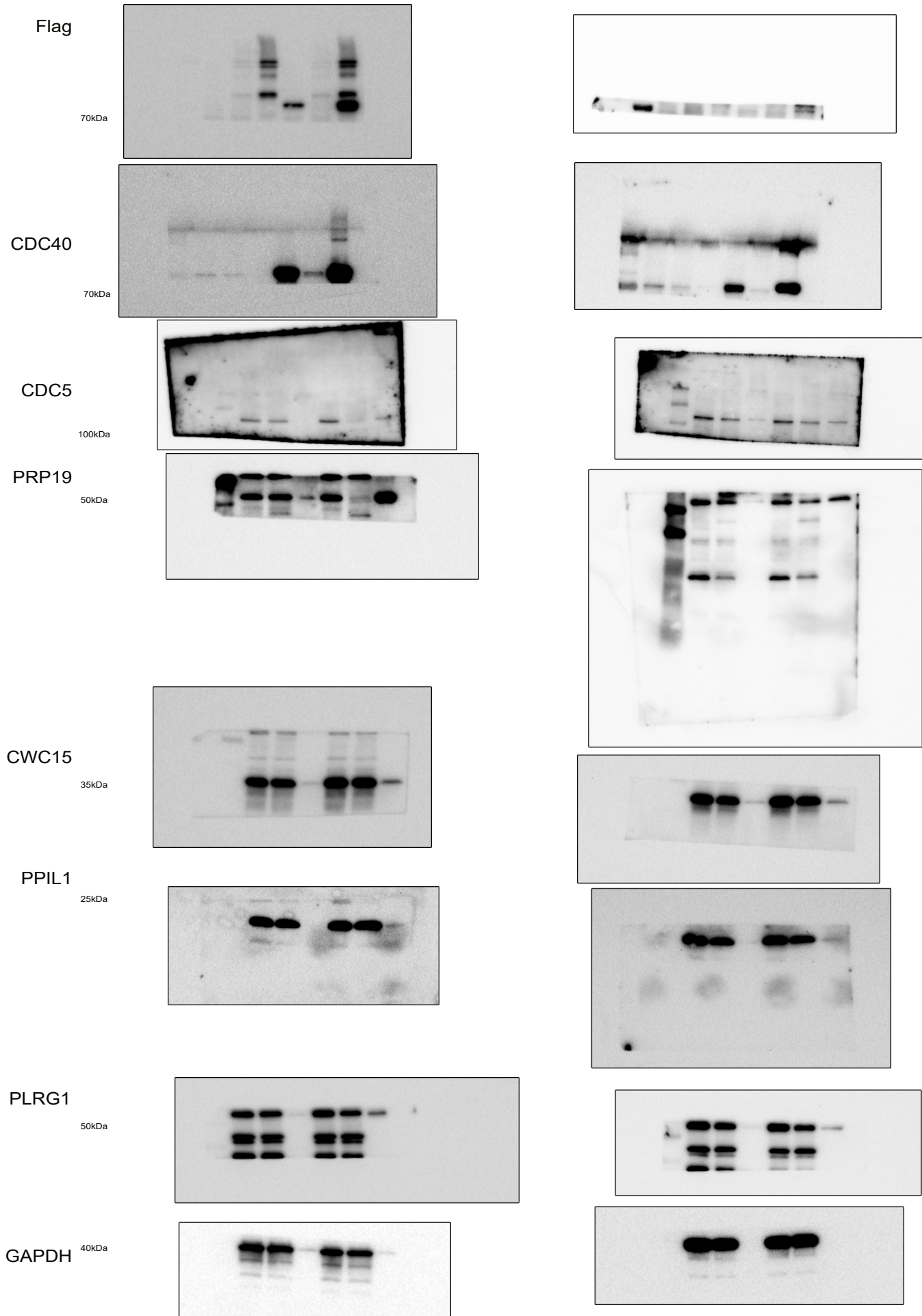


Supplementary Figure 15. (a) Source blot images for Figure 4d. (b) Left: Source blot images for Figure 4e. Right: Normalized band intensity to vinculin and -DOX.

Figure 5b and Supplementary Figure 26

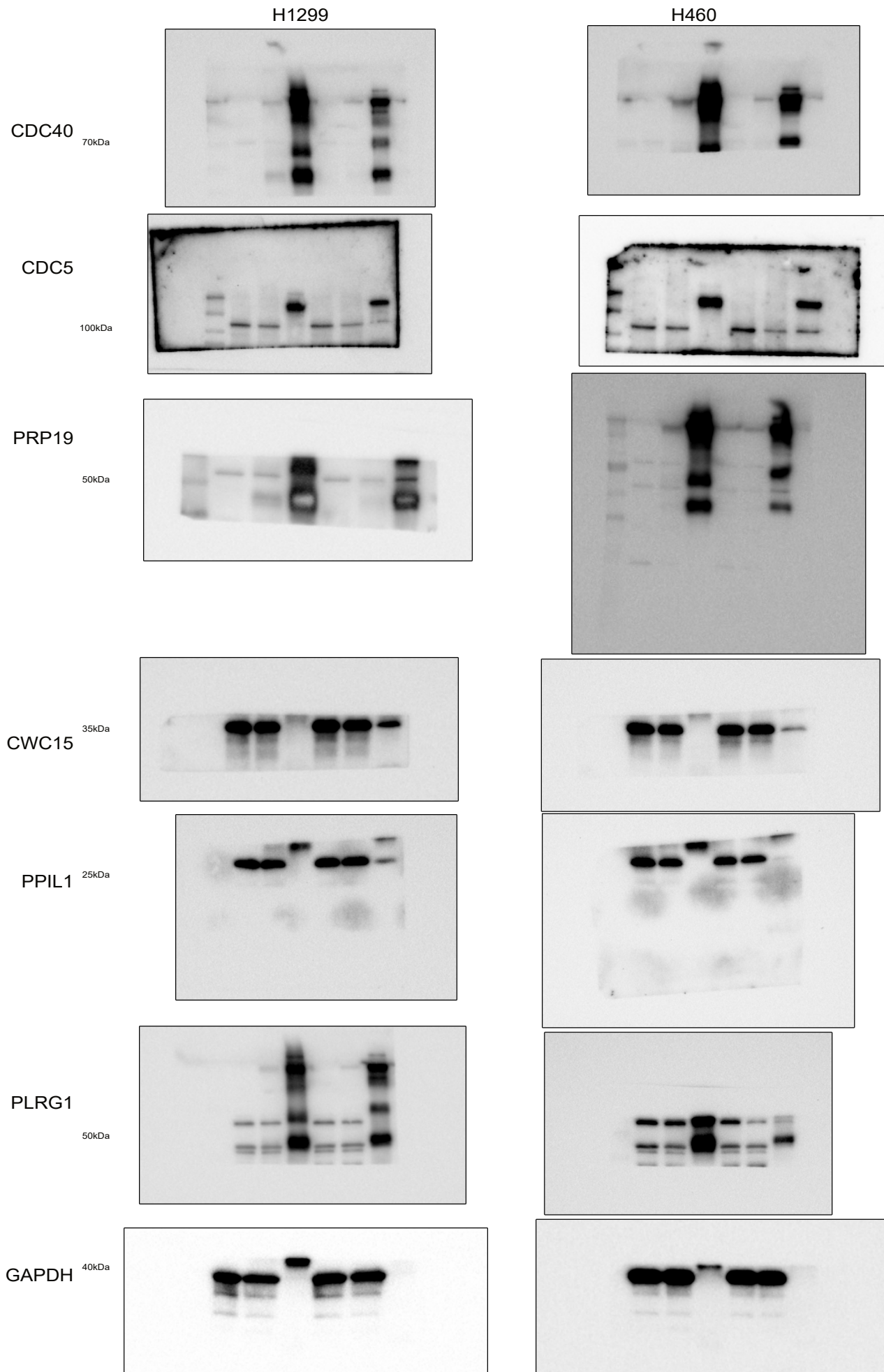
H1299

H460

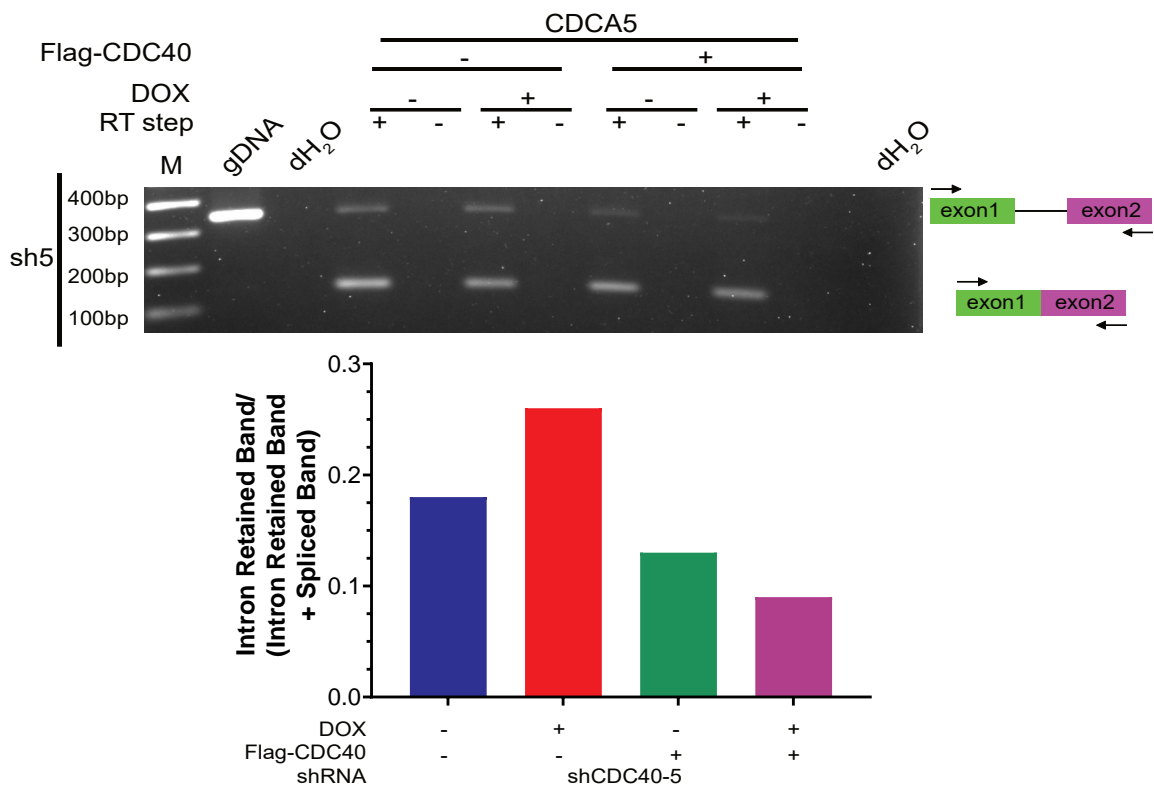


Supplementary Figure 16. Source blot images for Figure 5b and Supplementary Figure 26.

Figure 5b and Supplementary Figure 26



Supplementary Figure 17. Source blot images for Figure 5b and Supplementary Figure 26.



Supplementary Figure 18. Knockdown of CDC40 results in increased intron retention events. Top: DNA gels of RT-PCR products amplified from designed CDCA5 primers in H1299 cells. Bottom: Intron retention ratio of CDCA5 intron 1 after 36h of shCDC40-5 induction.

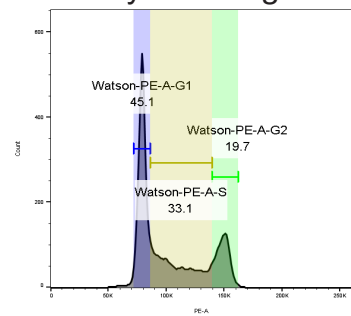
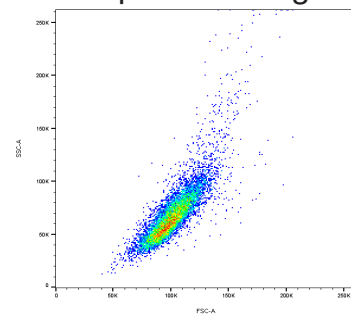
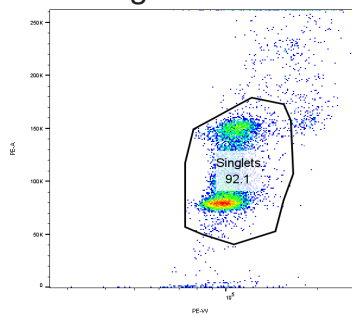
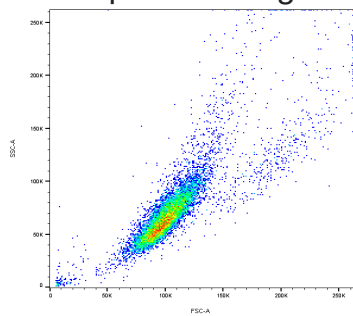
Cell Population-Ungated

Singlets Gate

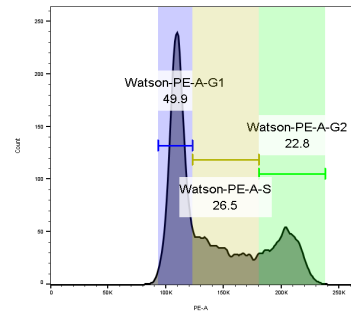
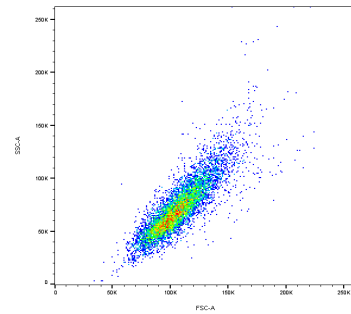
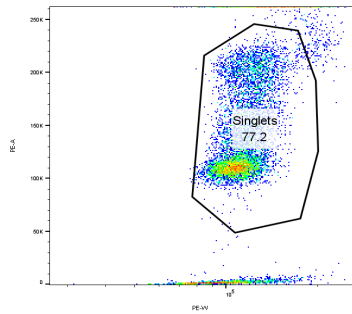
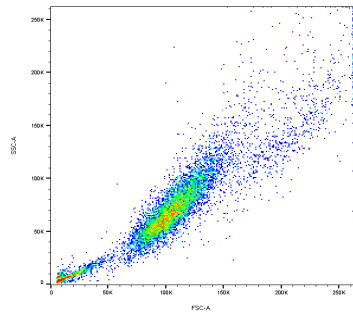
Cell Population-Singlets

Cell Cycle - Singlets

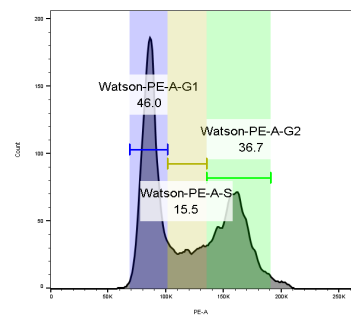
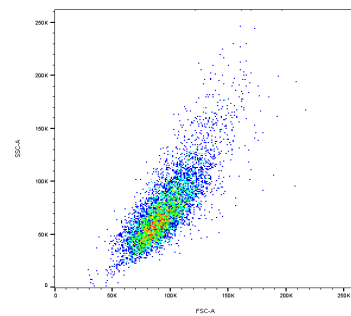
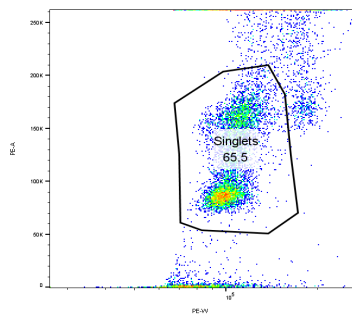
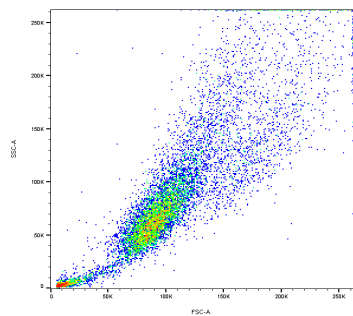
H460 48h



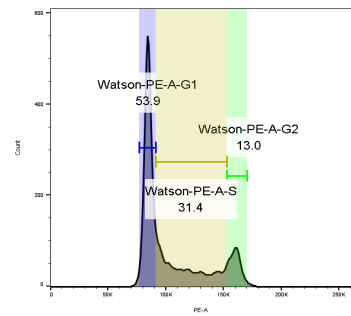
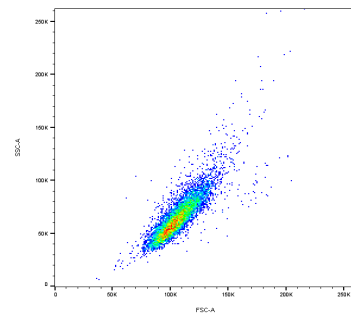
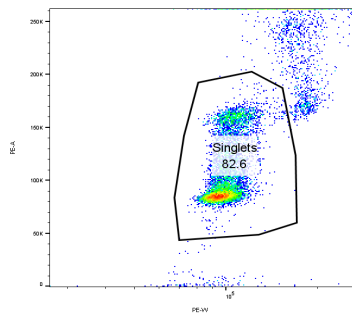
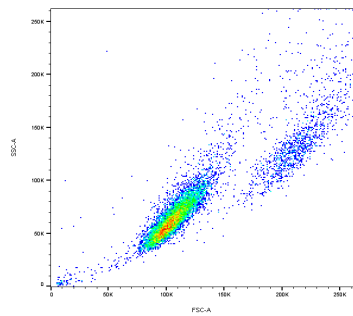
H1299 72h



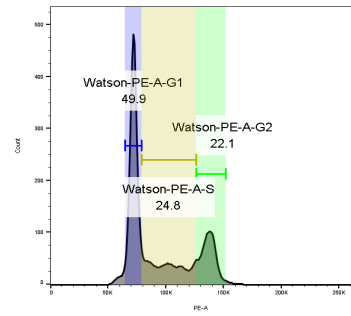
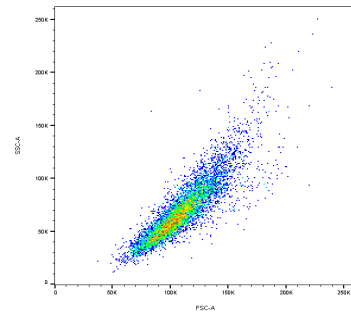
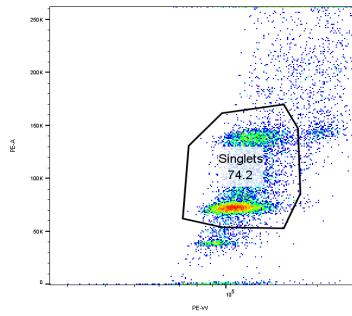
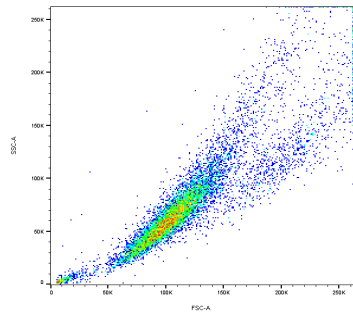
H2170 48h



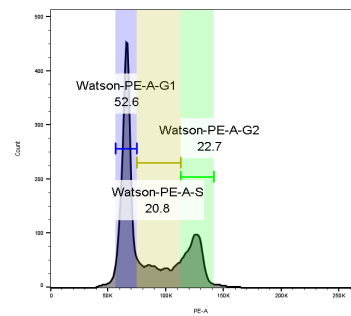
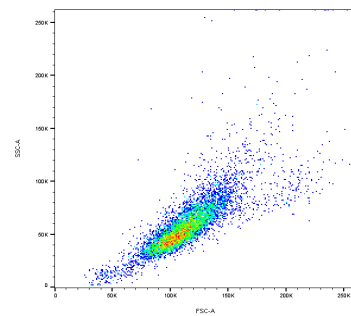
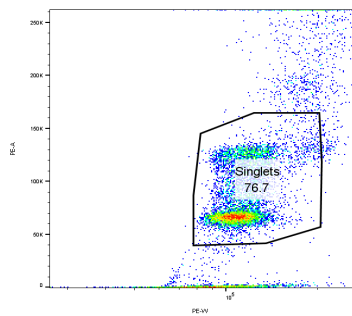
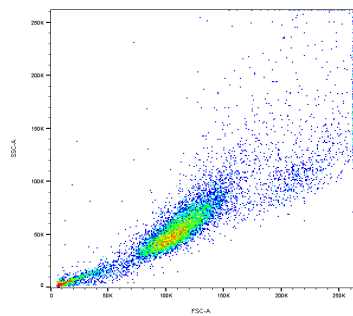
A549 48h



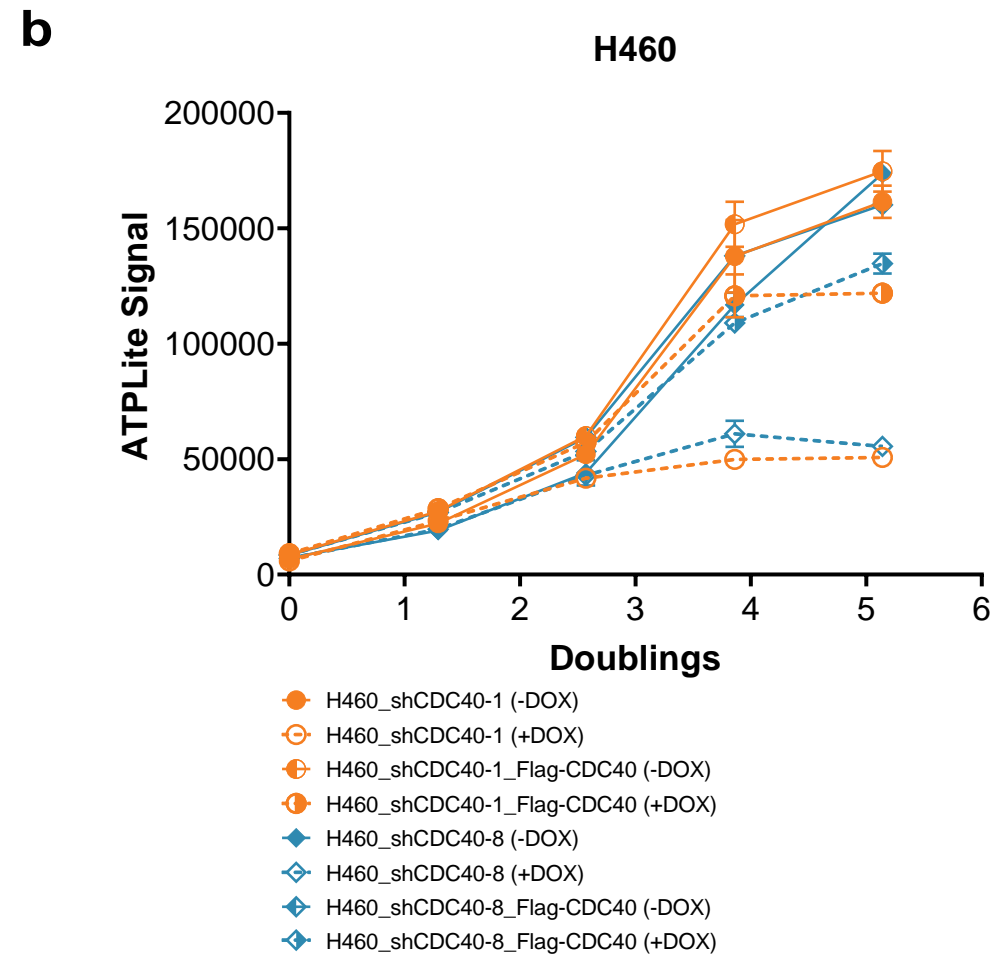
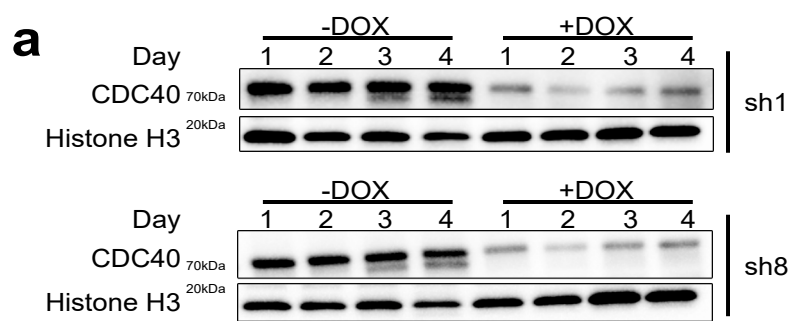
H322 48h



H1915 72h

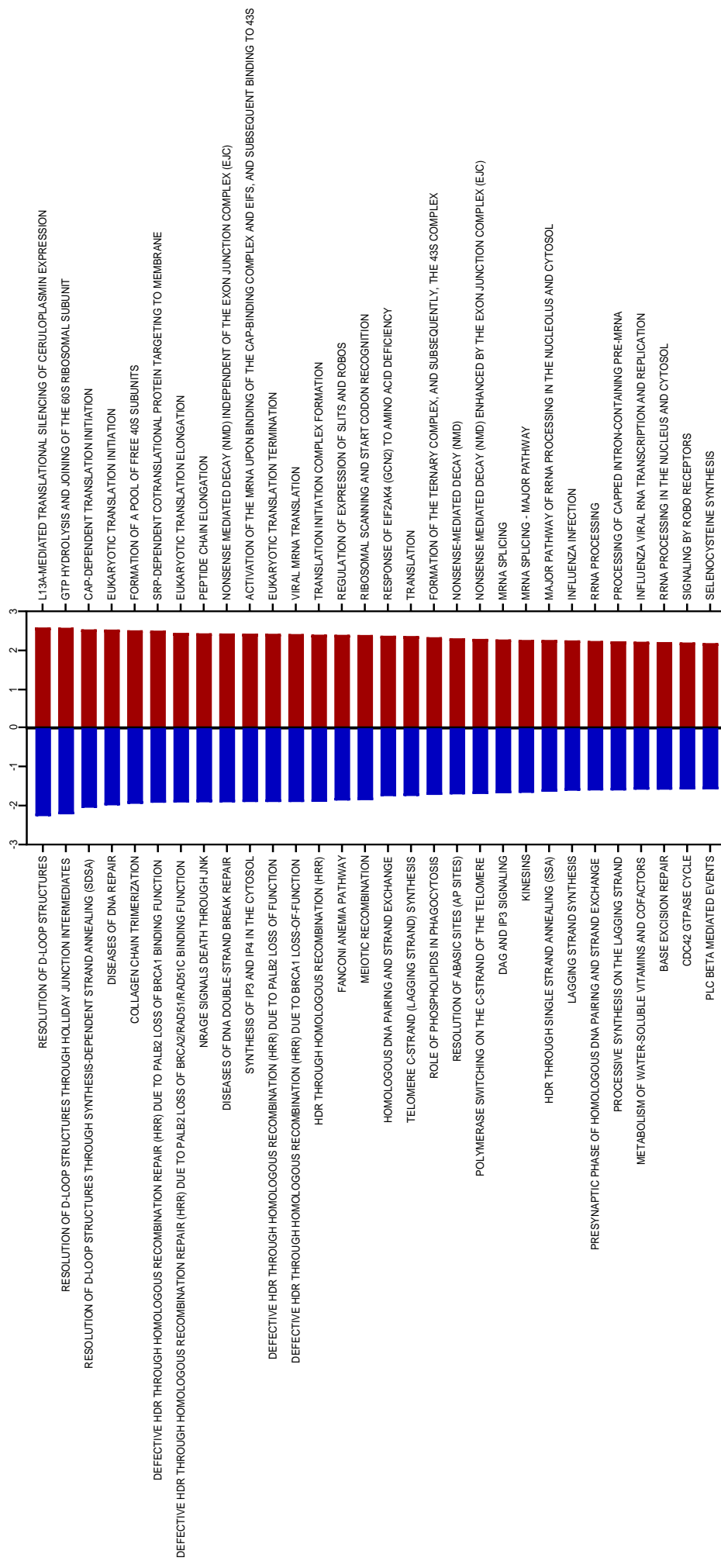


Supplementary Figure 19. Representative raw FACS data of various lung cancer cell lines. Raw FACS analysis (representative) of cell cycle distribution on H460, H1299, H2170, A549, H322 and H1915 cells after 48h or 72h of shCDC40-5 induction.

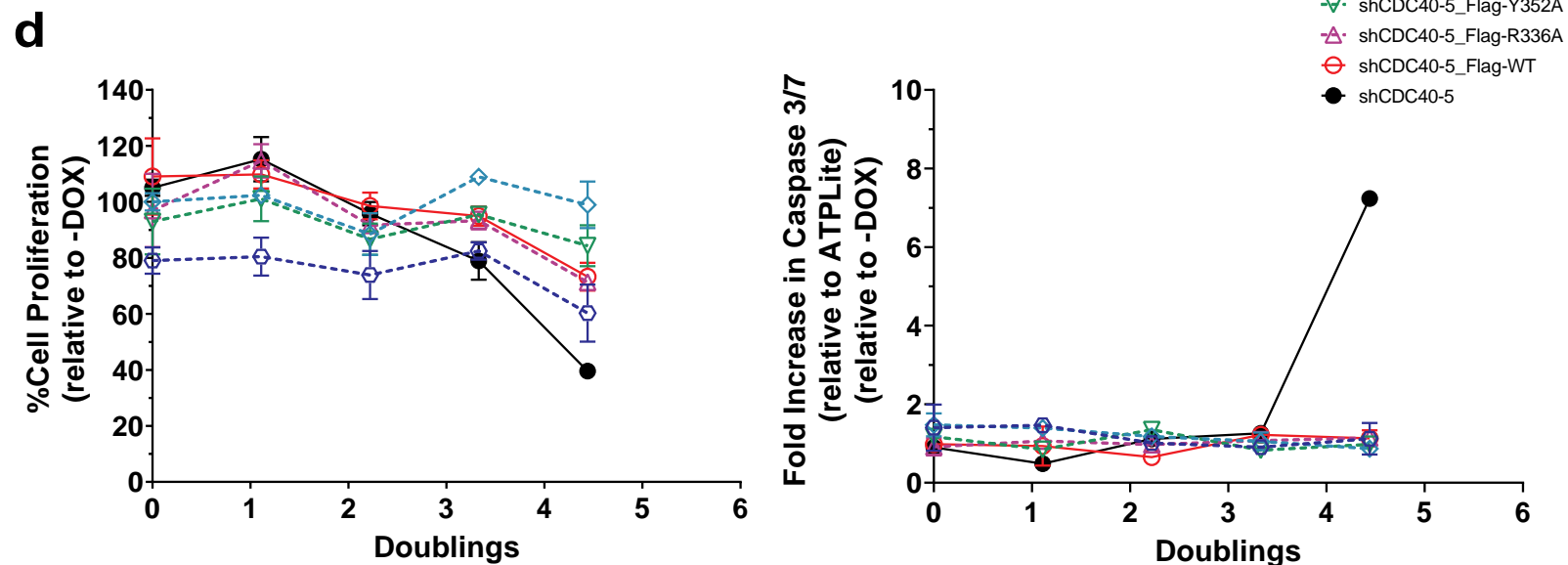
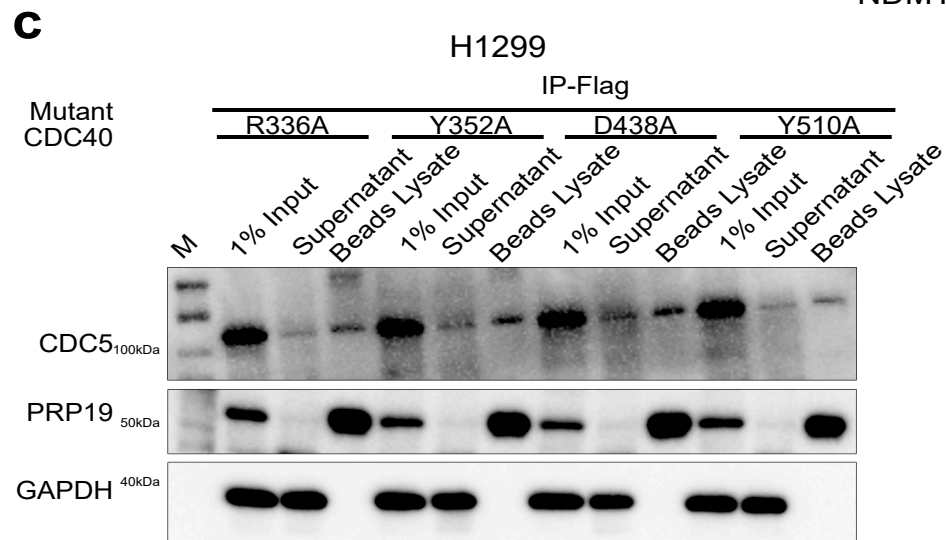
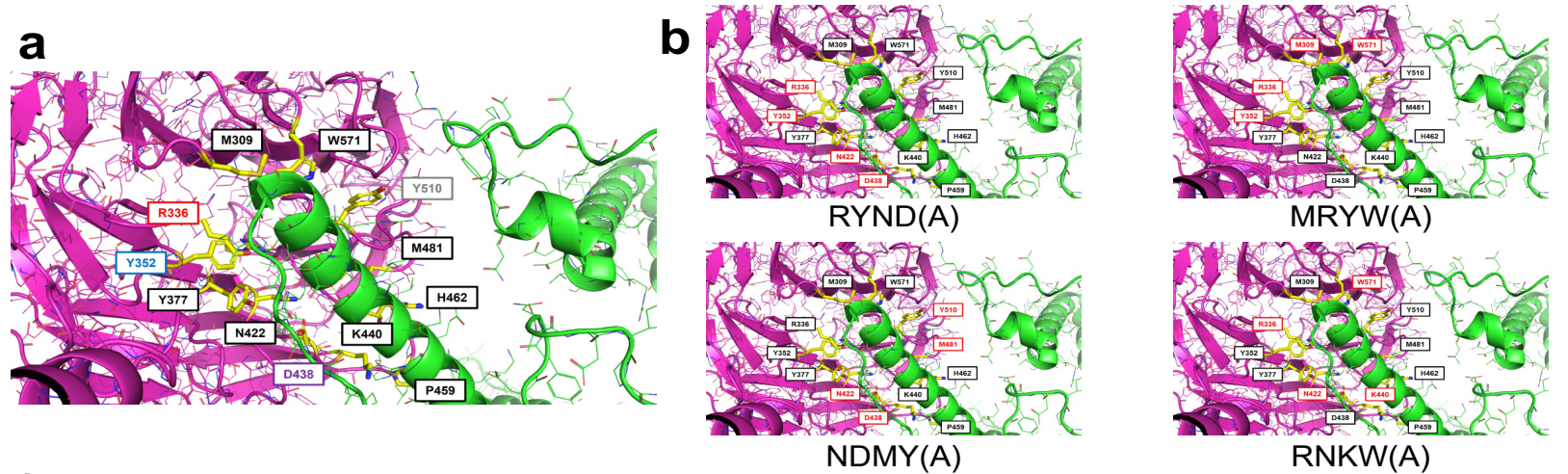


Supplementary Figure 20. Knockdown of CDC40 reduces proliferation in H460 lung cancer cells. (a) Western blot analysis showing the effect of CDC40 knockdown using shCDC40-1 and shCDC40-8 on CDC40 expression after 4 days DOX induction. (see Supplementary Figure 12 for source blot images). **(b)** Assessment of CDC40 knockdown on the proliferation of H460 cells after 4 days shCDC40 induction. (n=3, \pm SD)

sh1sh5 vs shCTR 36h



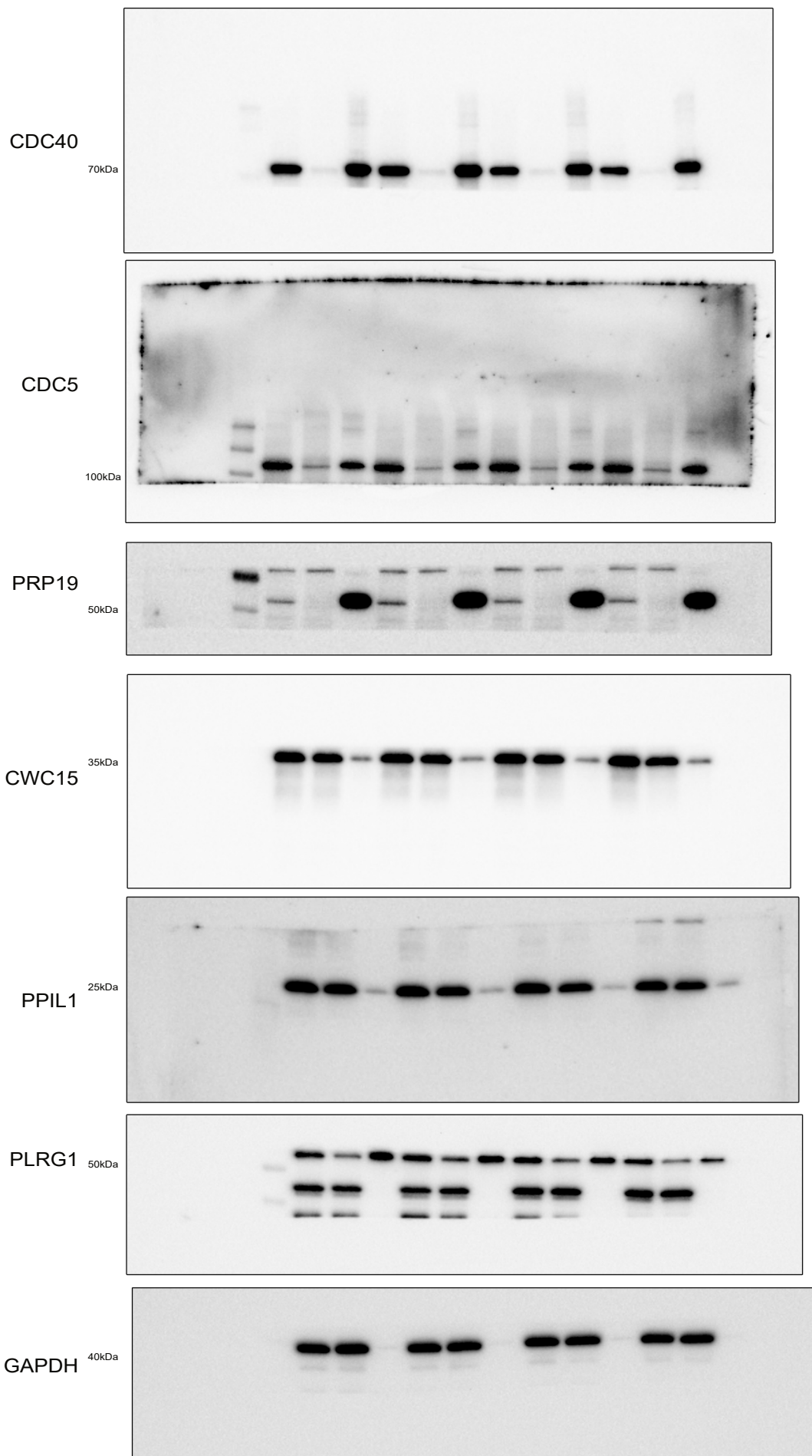
Supplementary Figure 21. Knockdown of CDC40 results in up-regulation of splicing and translation related genes as well as down-regulation of DNA damage response and proliferation related genes. Top 30 upregulated (red) and downregulated (blue) pathways ranked with NES commonly identified after 36h of shCDC40-1 and shCDC40-5 induction in comparison to shCTR.



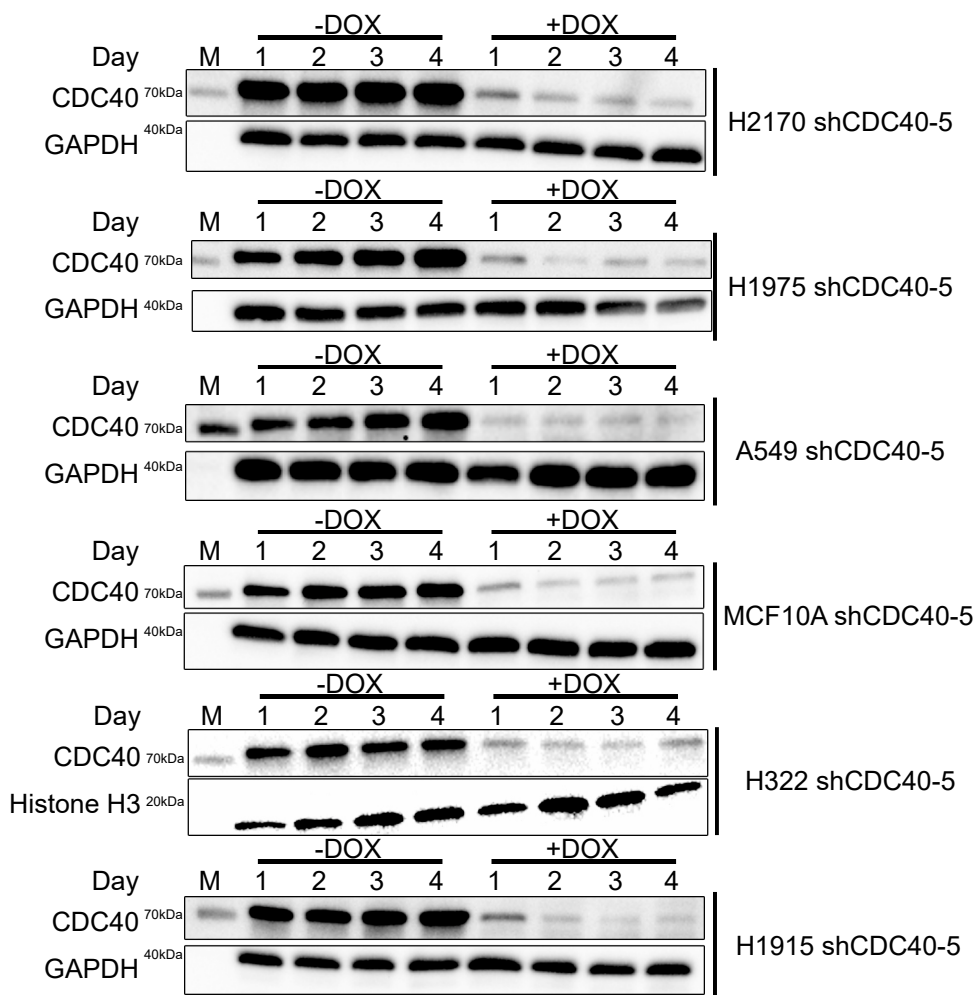
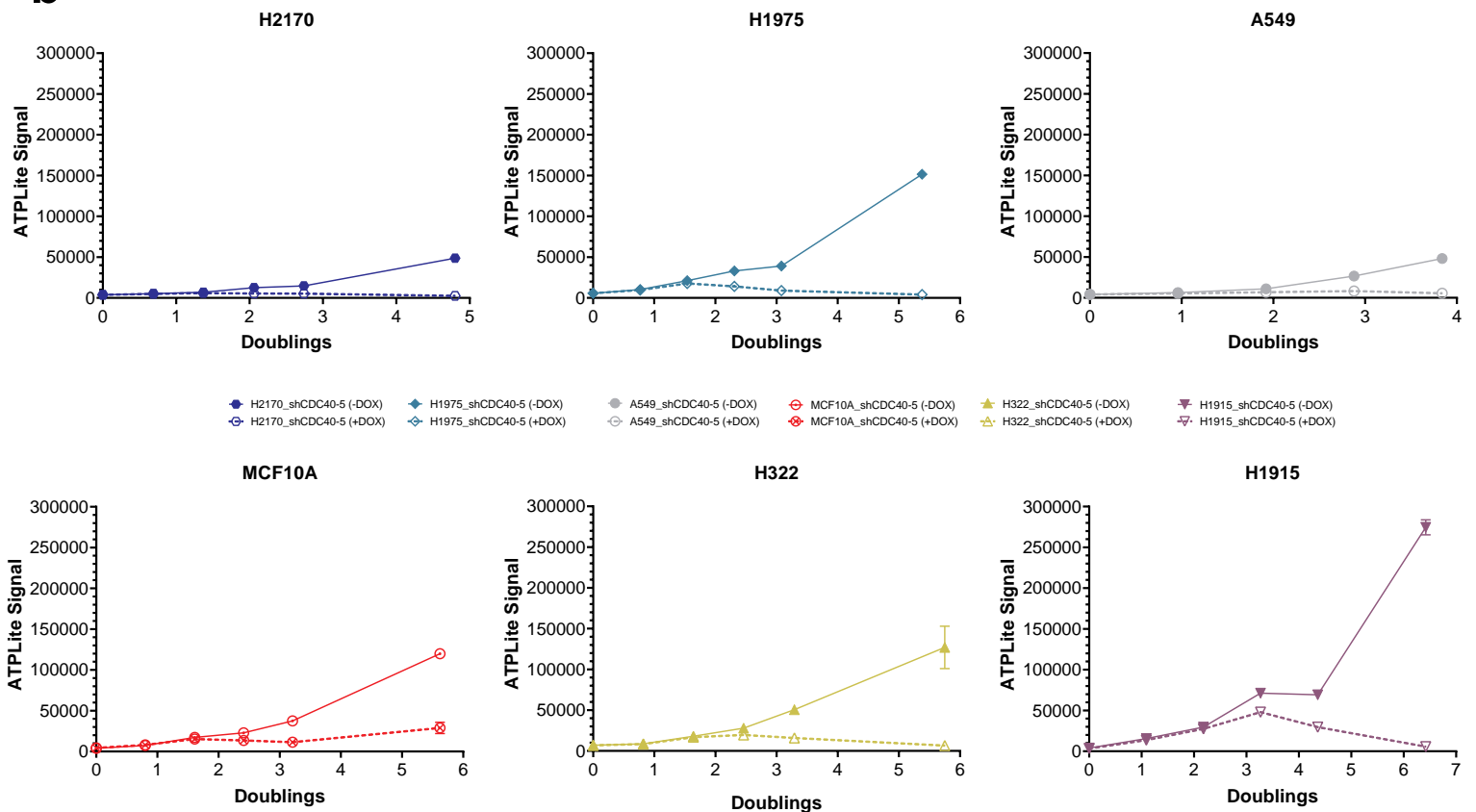
Supplementary Figure 22. Single-point and quadruple-point mutation of CDC40 do not disrupt CDC40 protein-protein interactions with CDC5 and PRP19. (a) Four essential amino acids for CDC40-CDC5 interaction were selected from twelve essential interaction residues identified *in silico* to individually mutate to alanine(A). (b) Four sets of four essential amino acid for CDC40-CDC5 interaction were selected from twelve essential interaction residues identified *in silico* to mutate to alanine(A). (c) colP-WB with anti-Flag beads in shCTR H1299 cells transduced with Flag-CDC40 single-point mutants. (d,) Assessing the effects of overexpressing Flag-CDC40 single-point mutants on the proliferation of H1299 cells after 4 days shCDC40-5 induction. Results were normalized to the doubling time of H1299 cells. (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; $n=3$, \pm SD, Student's T-test).

Figure 5d

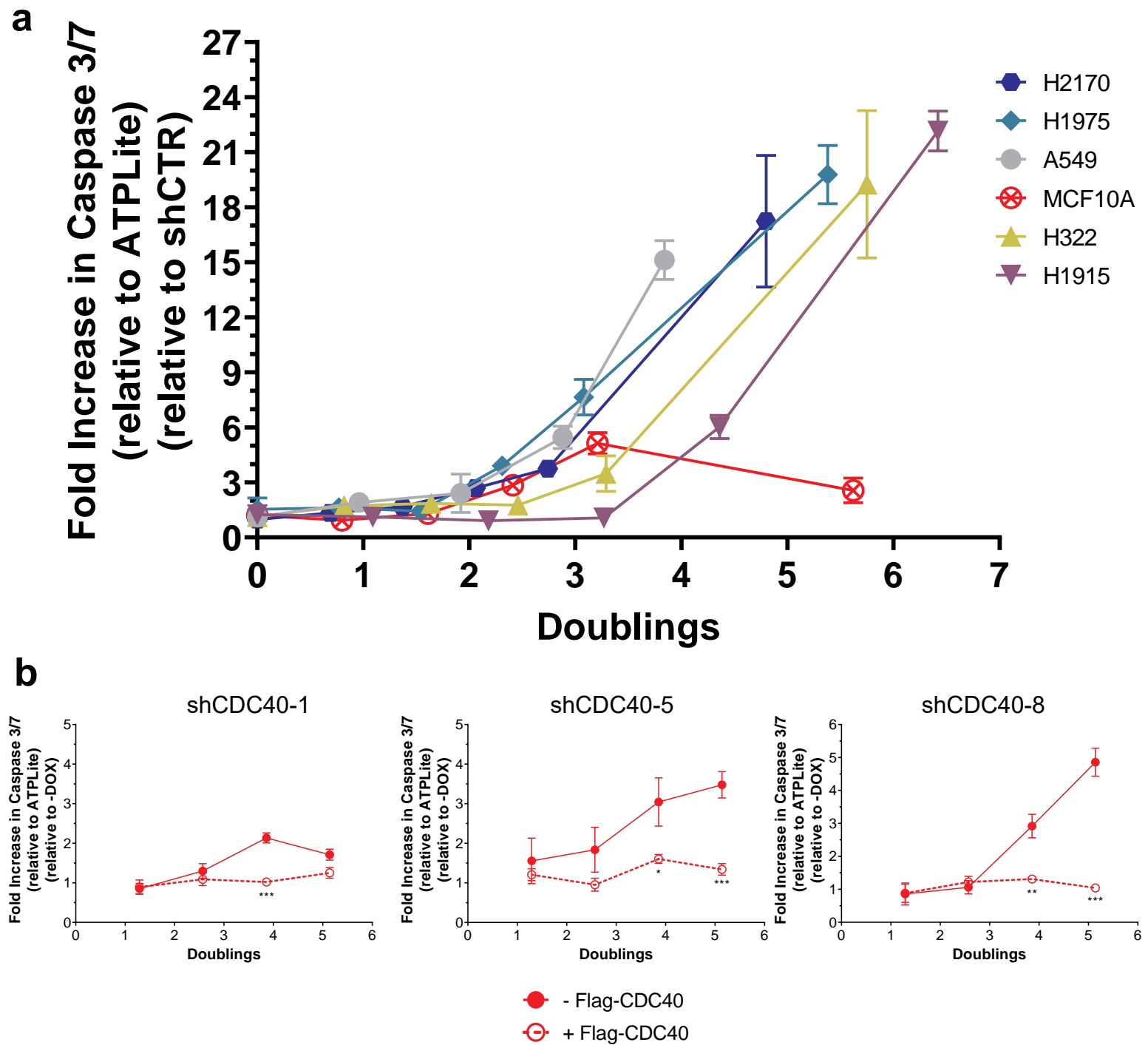
H1299



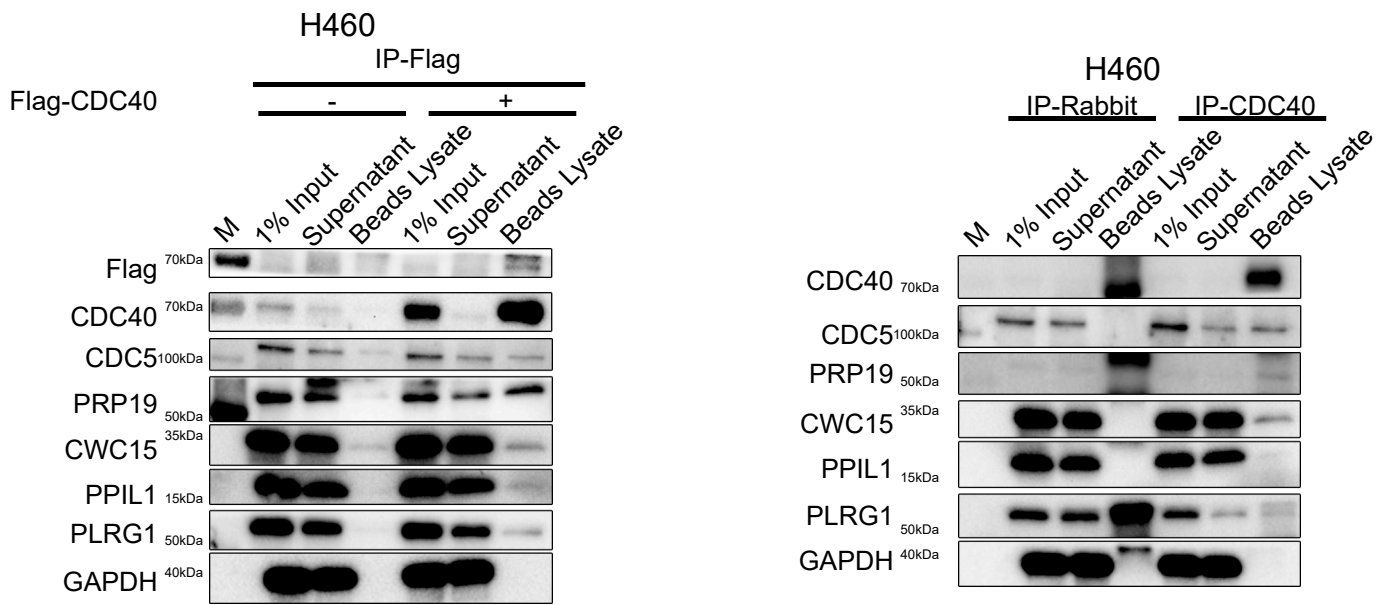
Supplementary Figure 23. Source blot images for Figure 5d.

a**b**

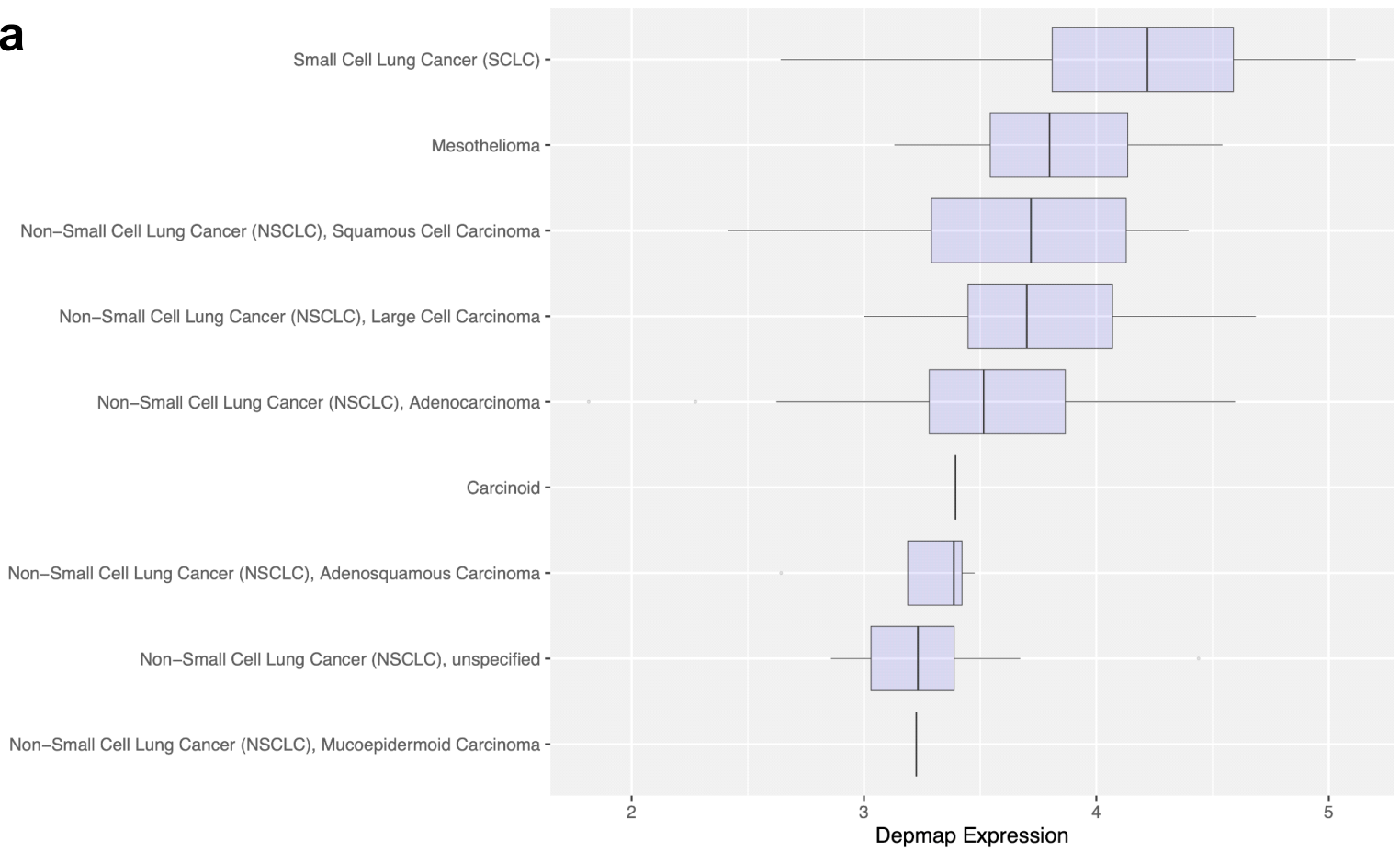
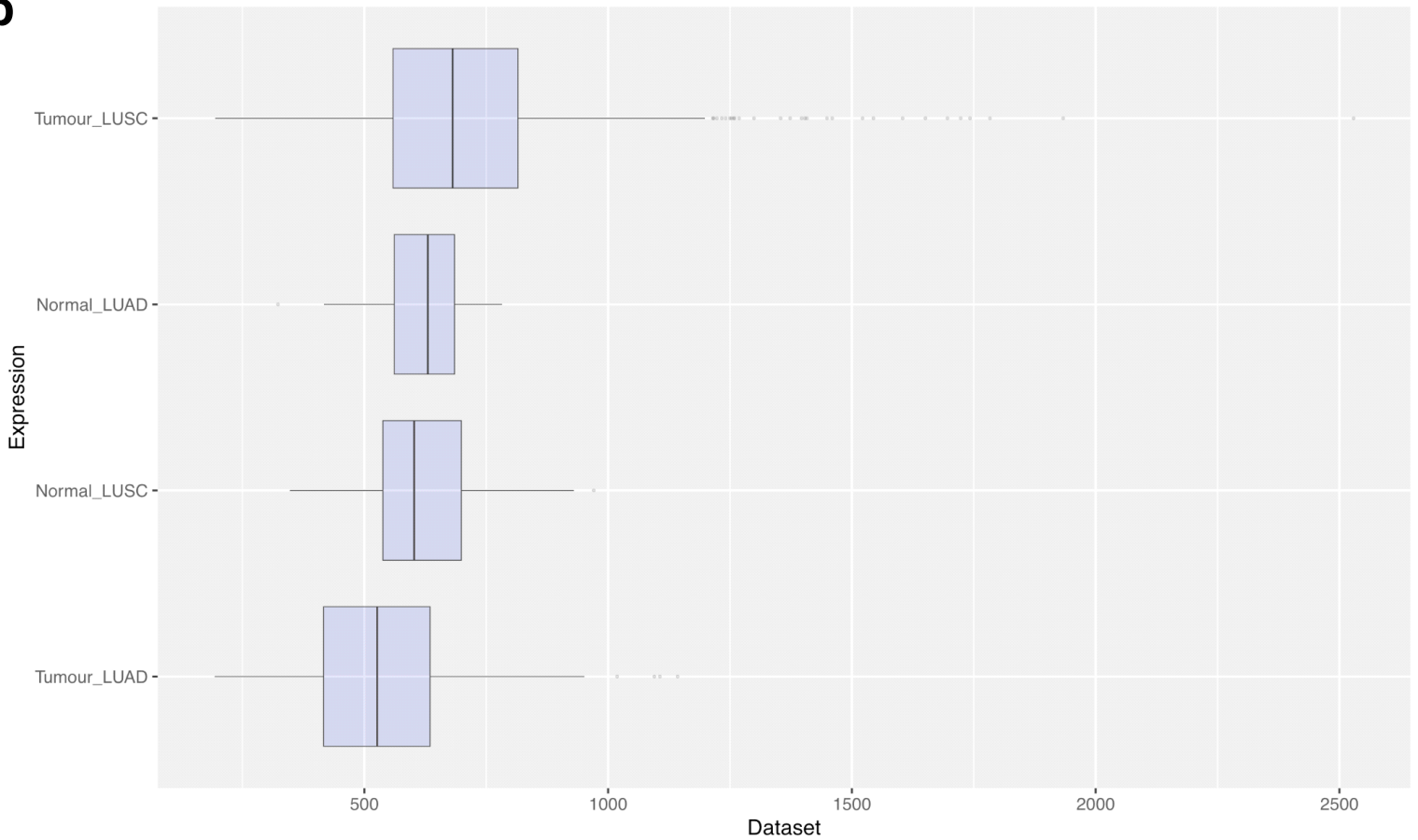
Supplementary Figure 24. CDC40 knockdown reduces proliferation of multiple lung cancer cell lines. (a) Western blot analysis showing CDC40 expression after 4 days of CDC40 knockdown using shCDC40-5 (representative) in H2170, H1975, A549, MCF10A, H322 and H1915 cells. (see Supplementary Figure 13 for source blot images). Results are representative of at least two independent experiments. (b) CDC40 knockdown effect on the proliferation of H2170, H1975, A549, MCF10A, H322, and H1915, cells after 4 or 7 days of shCDC40-5 (representative) induction. Results were individually normalized to the doubling time of each cell lines. (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; $n=3$, \pm SD, Student's T-test)



Supplementary Figure 25. CDC40 knockdown induces late apoptosis markers in multiple lung cancer cell lines. (a) CDC40 knockdown effect on caspase 3/7 activity in H2170, H1975, A549, MCF10A, H322, and H1915 cells after 4 or 7 days shCDC40-5 (representative) induction. Results were individually normalized to the doubling time of each cell line. (n=3, \pm SD) **(b)** The effect of Flag-CDC40 overexpression on activation of caspase 3/7 activity in H460 cells after 4 days of shCDC40 induction. Results were normalized to the doubling time of H460 cells. (n=3, \pm SD)



Supplementary Figure 26. CDC40 primarily interacts with spliceosome components. co-IP validation validation of the IP-MS results using western blot post pull-down with anti-Flag beads in shCTR containing H1299 cells expressing Flag-CDC40 (Left) and with anti-CDC40 (Right) beads in H460 cells. (see Supplementary Figure 16, 17 for source blot images)

a**b**

Supplementary Figure 27. CDC40 expression in lung cancer with differential pathological tissue types. (a) CDC40 expression among lung cancer types based on DepMap database. (b) CDC40 expression among lung cancer types based on TCGA database.

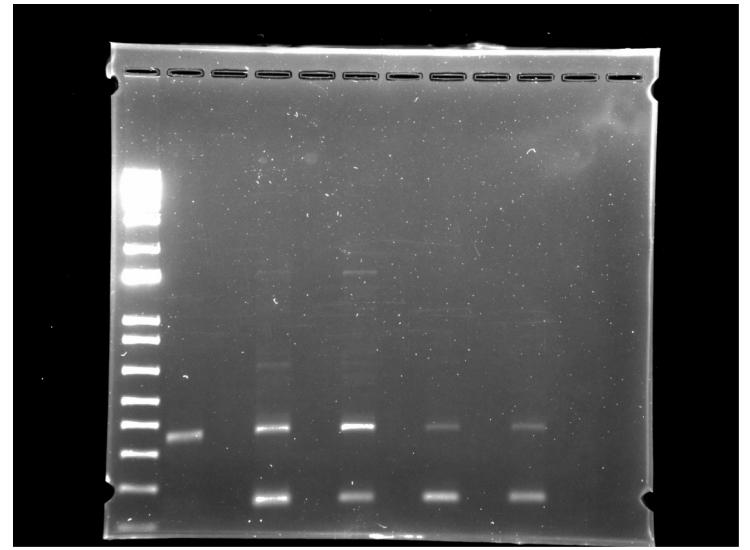
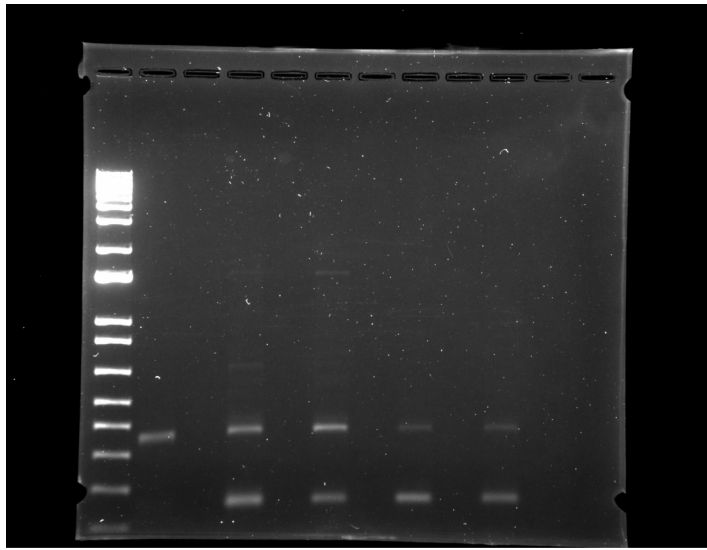
Figure 4c

H460

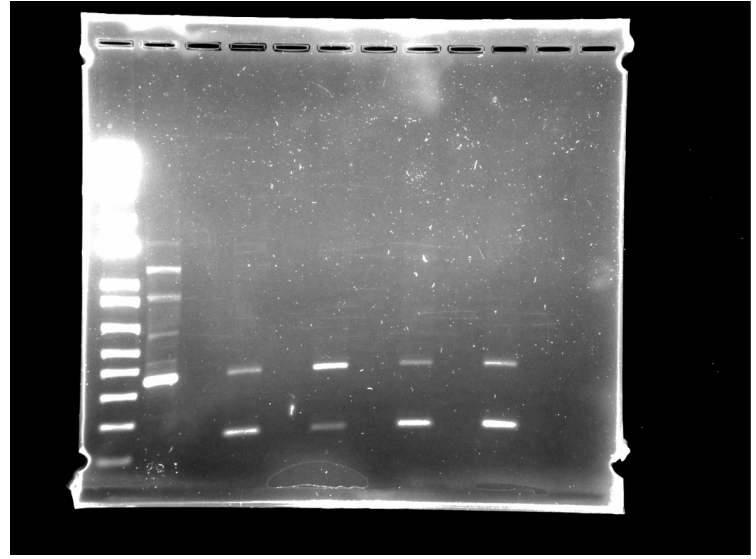
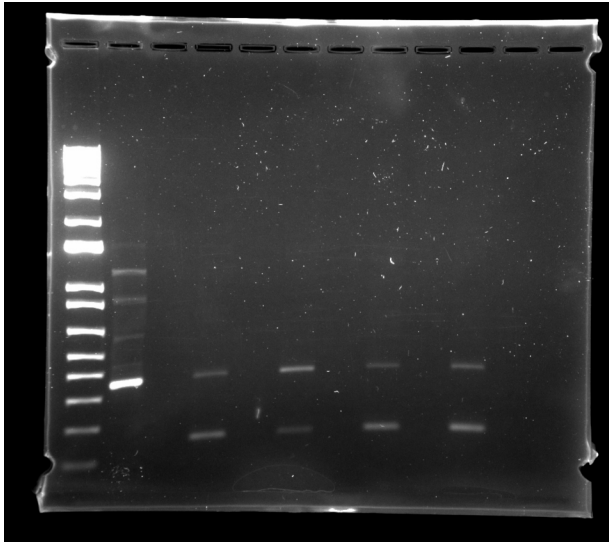
3s

5s

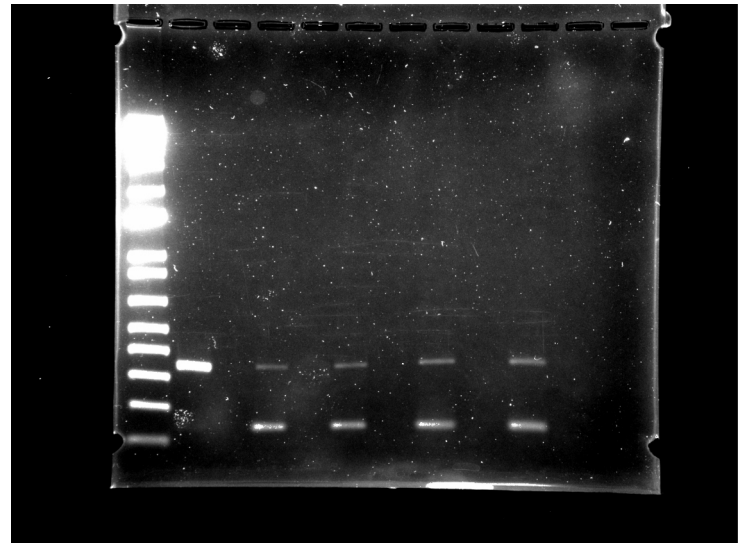
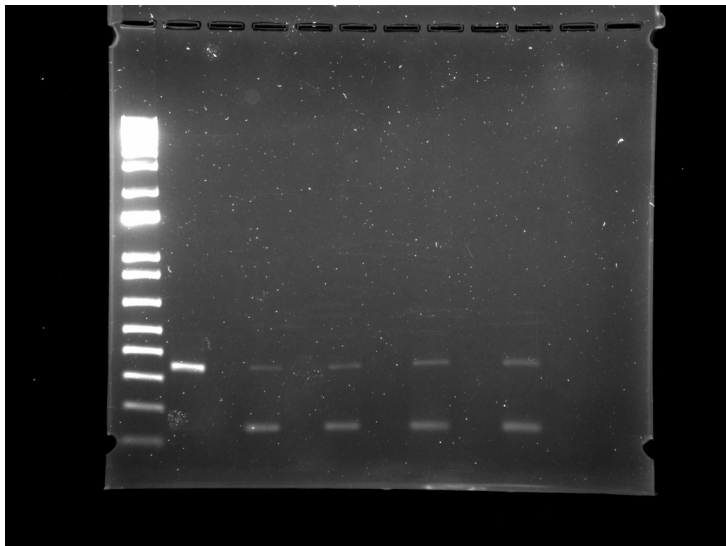
sh1



sh5



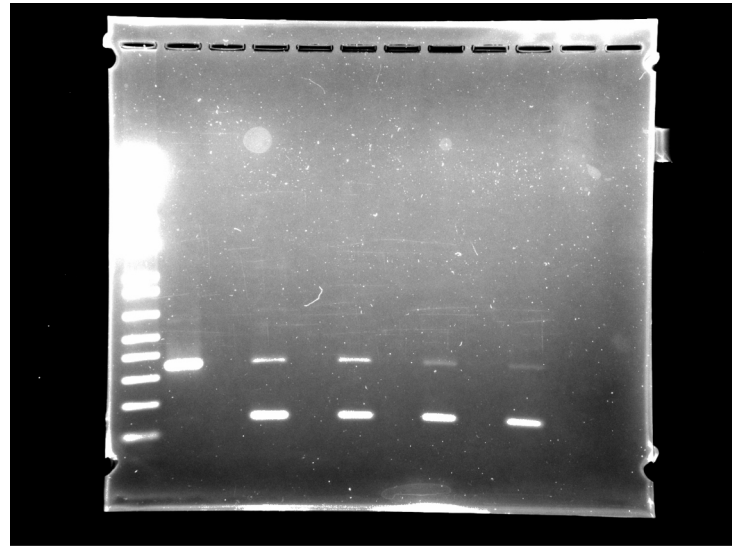
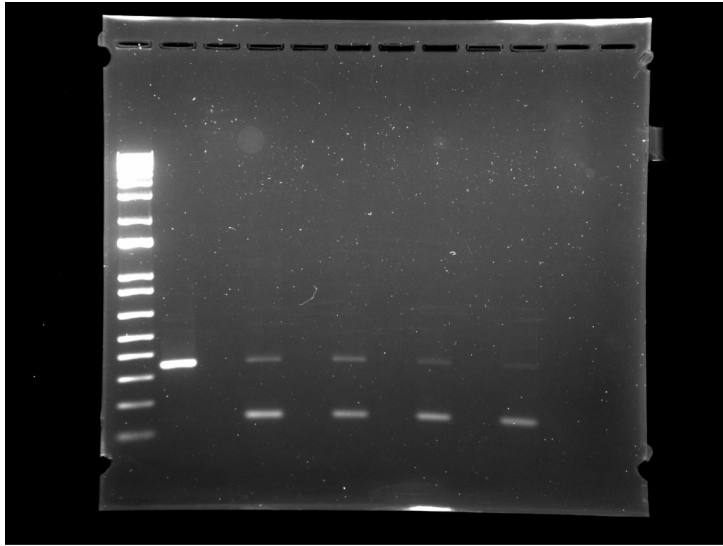
shCTR



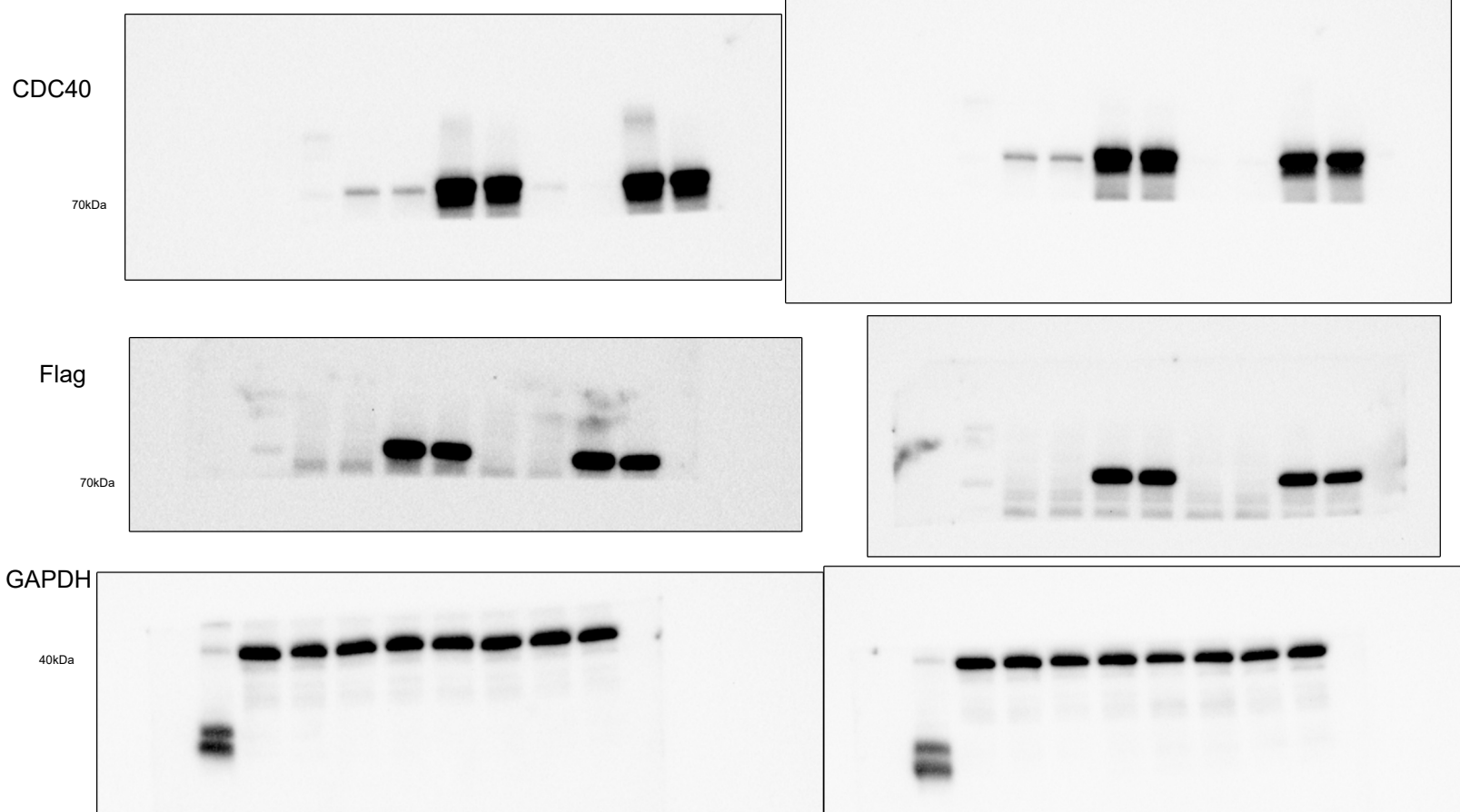
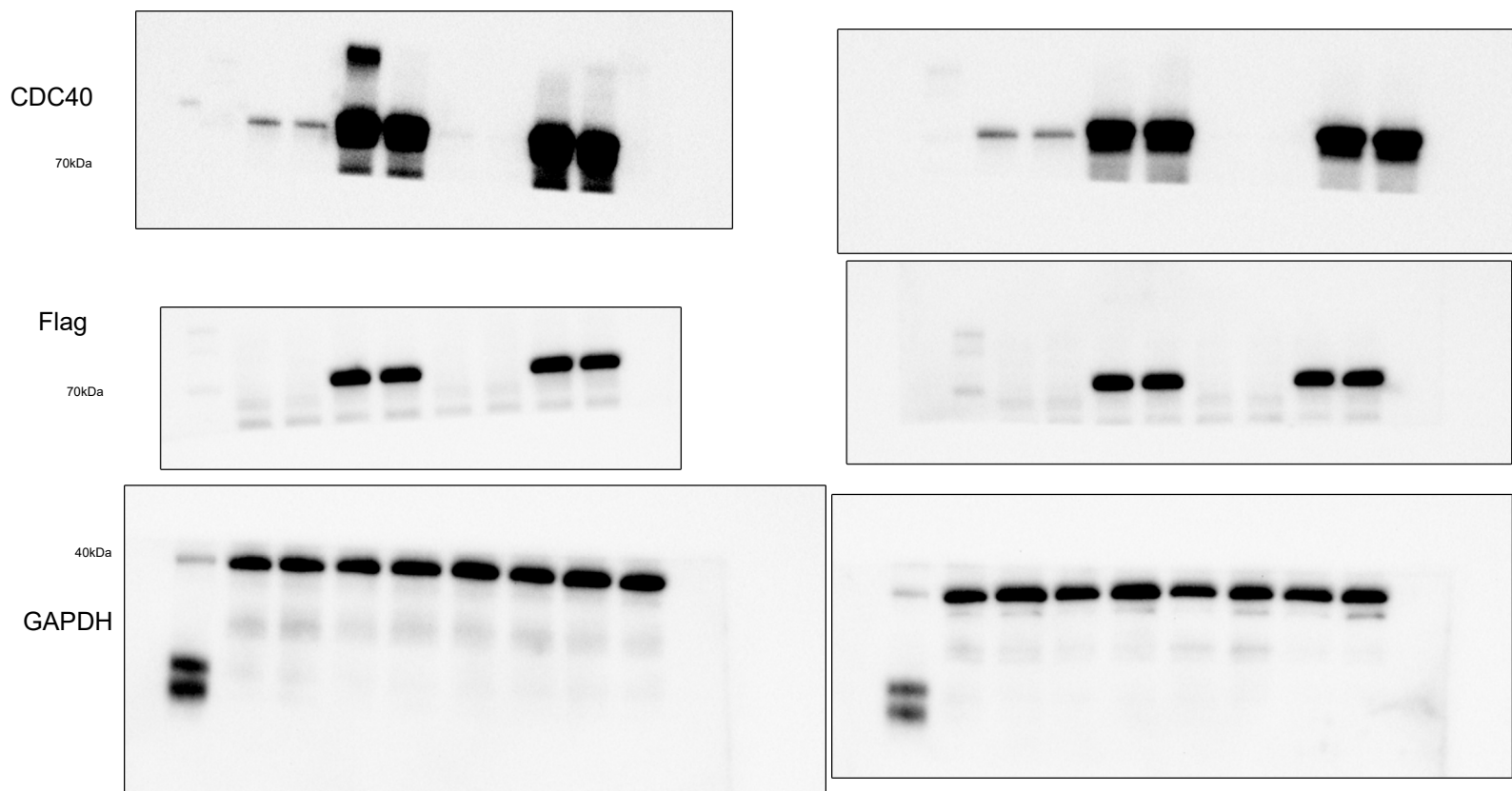
3s

5s

sh5



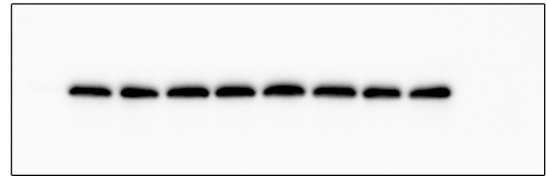
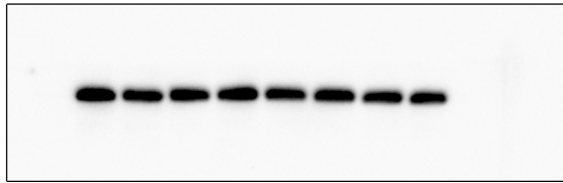
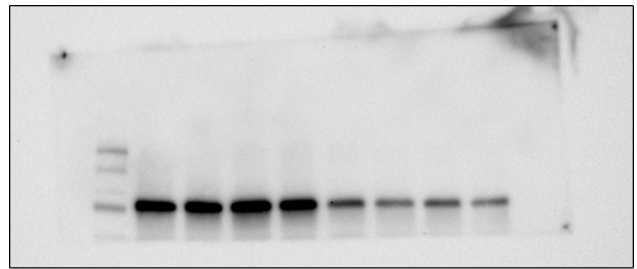
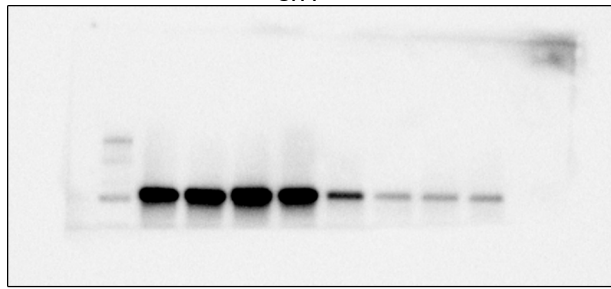
Supplementary Figure 29. Source blot images for Supplementary Figure 18.

a**sh1****sh5**

H2170

sh1

sh8

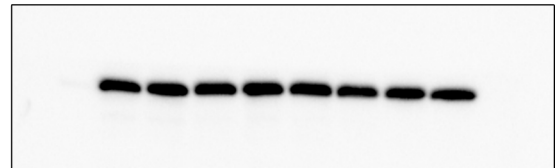
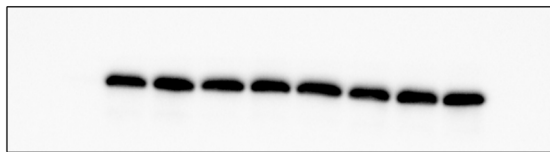
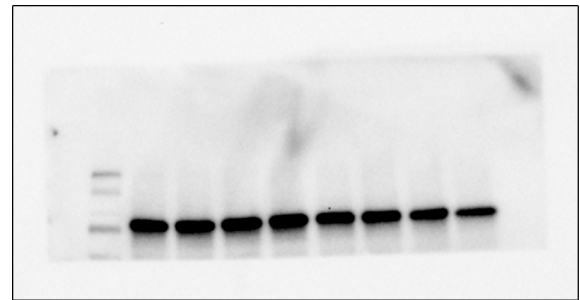
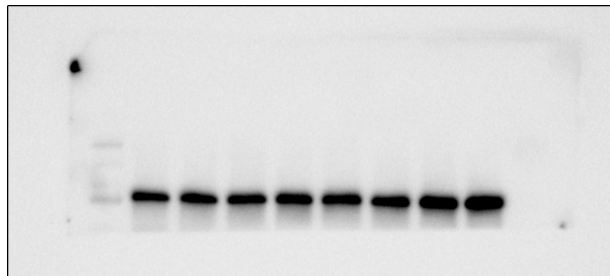


shCTR

shPLK1

CDC40

GAPDH



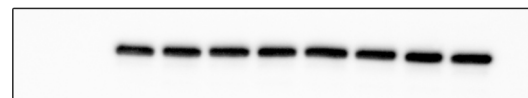
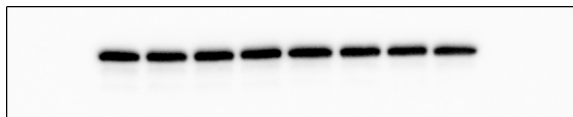
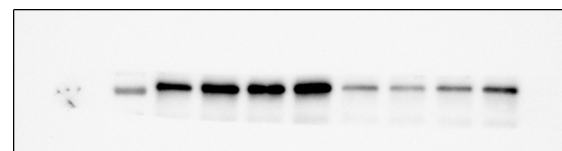
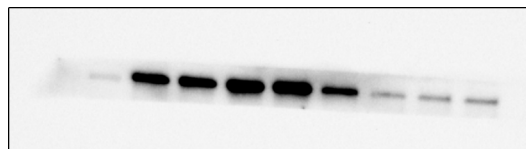
H1975

sh1

sh8

CDC40

GAPDH

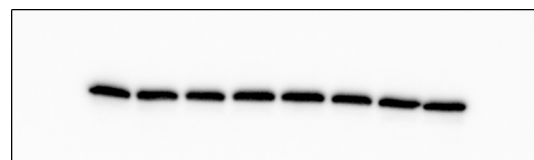
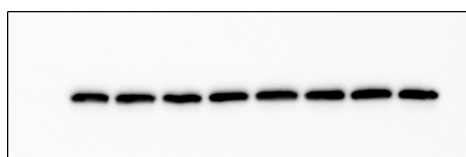
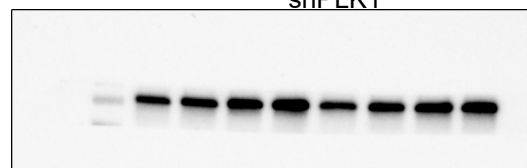
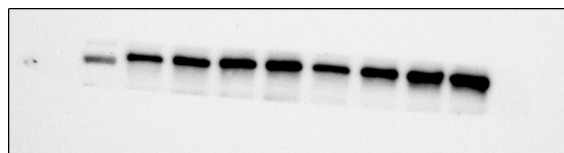


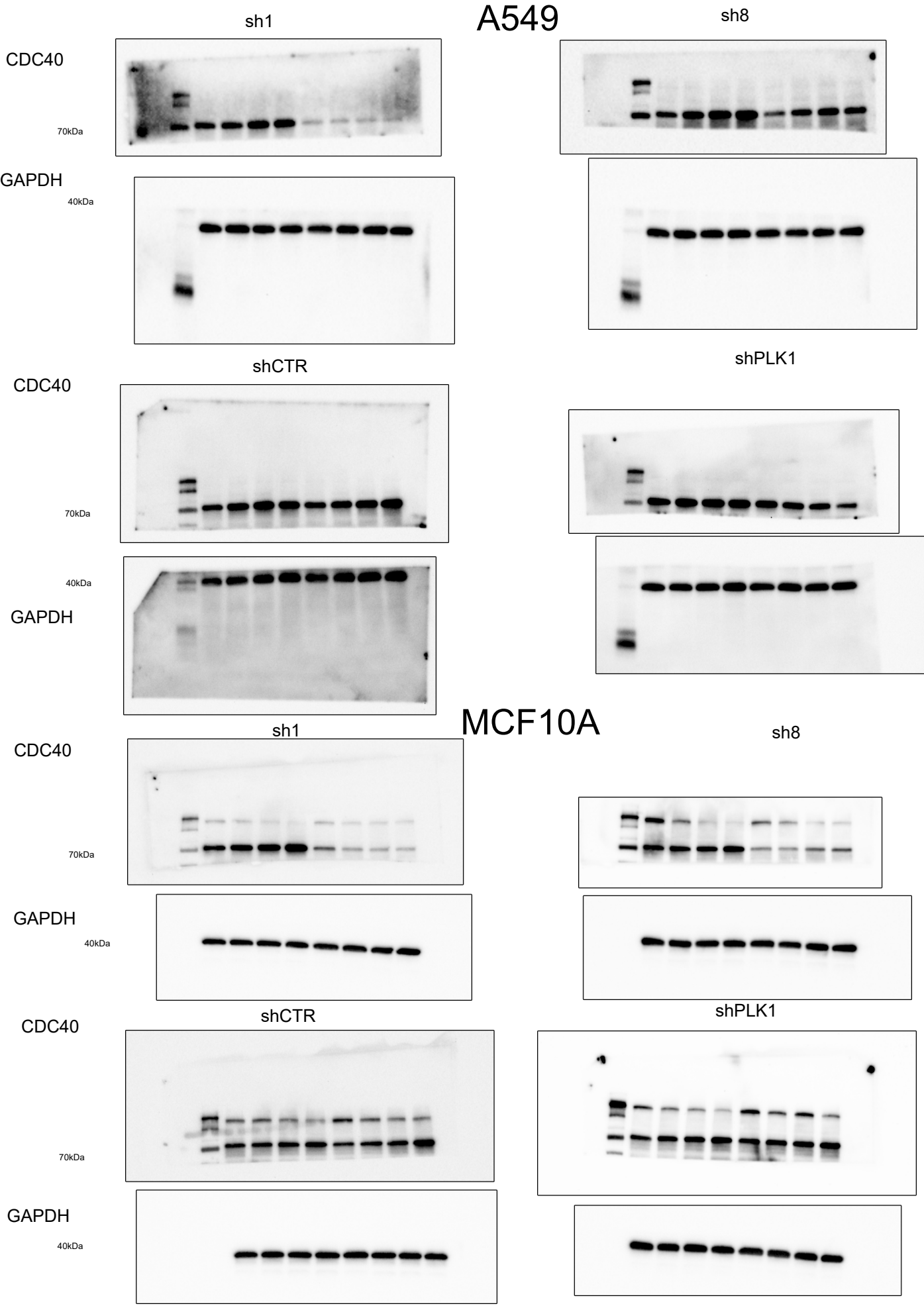
shCTR

shPLK1

CDC40

GAPDH





Supplementary Figure 32. Source image blots for Supplementary Figure 3 continued.

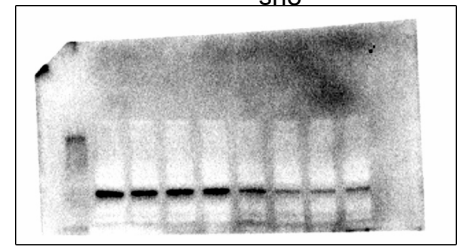
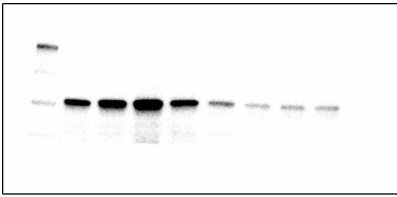
sh1

H322

sh8

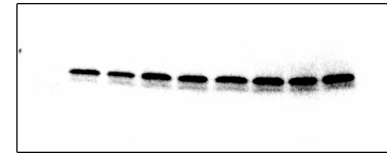
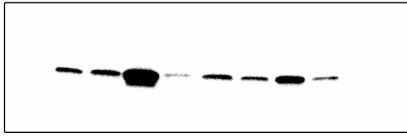
CDC40

70kDa



H3 Histone

20kDa

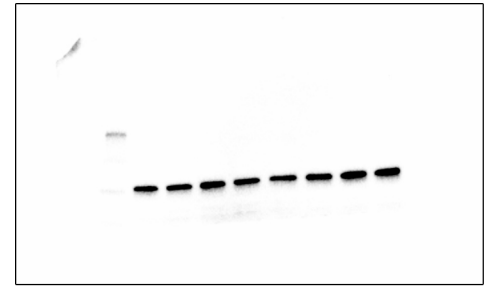
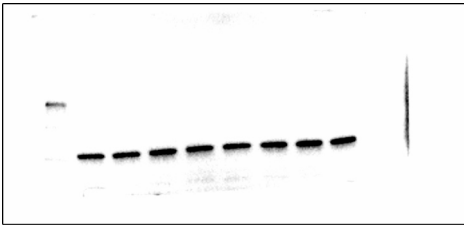


shCTR

shPLK1

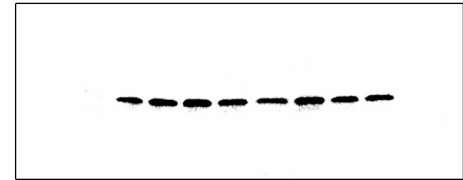
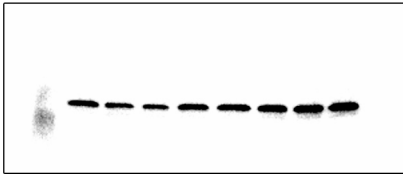
CDC40

70kDa



20kDa

H3 Histone



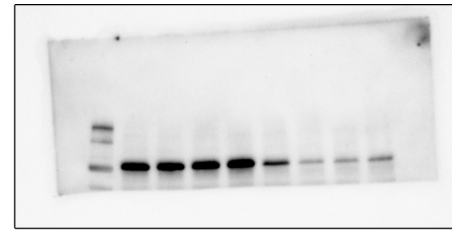
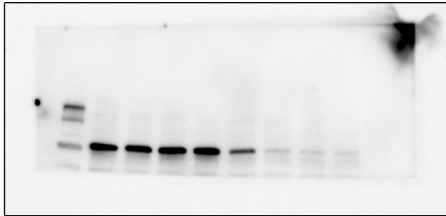
sh1

H1915

sh8

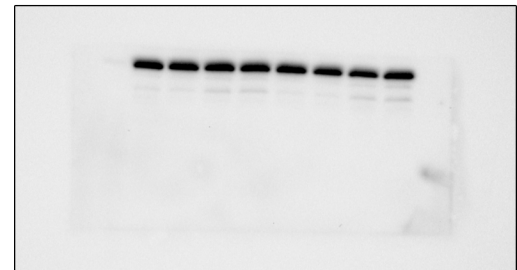
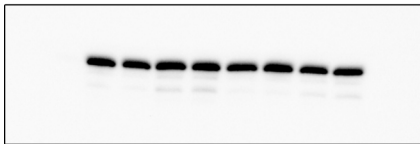
CDC40

70kDa



GAPDH

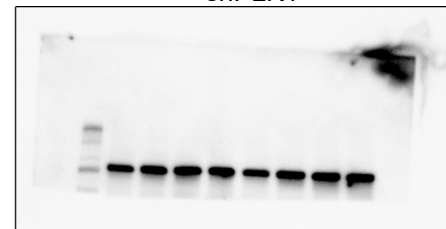
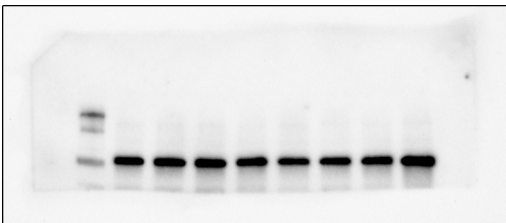
40kDa



shCTR

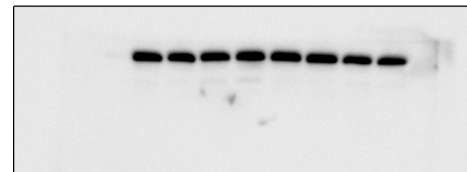
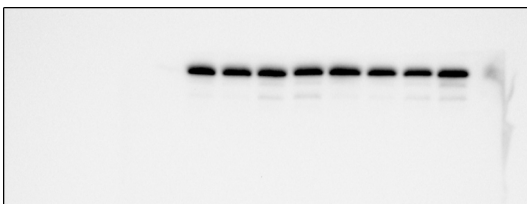
shPLK1

CDC40 70kDa



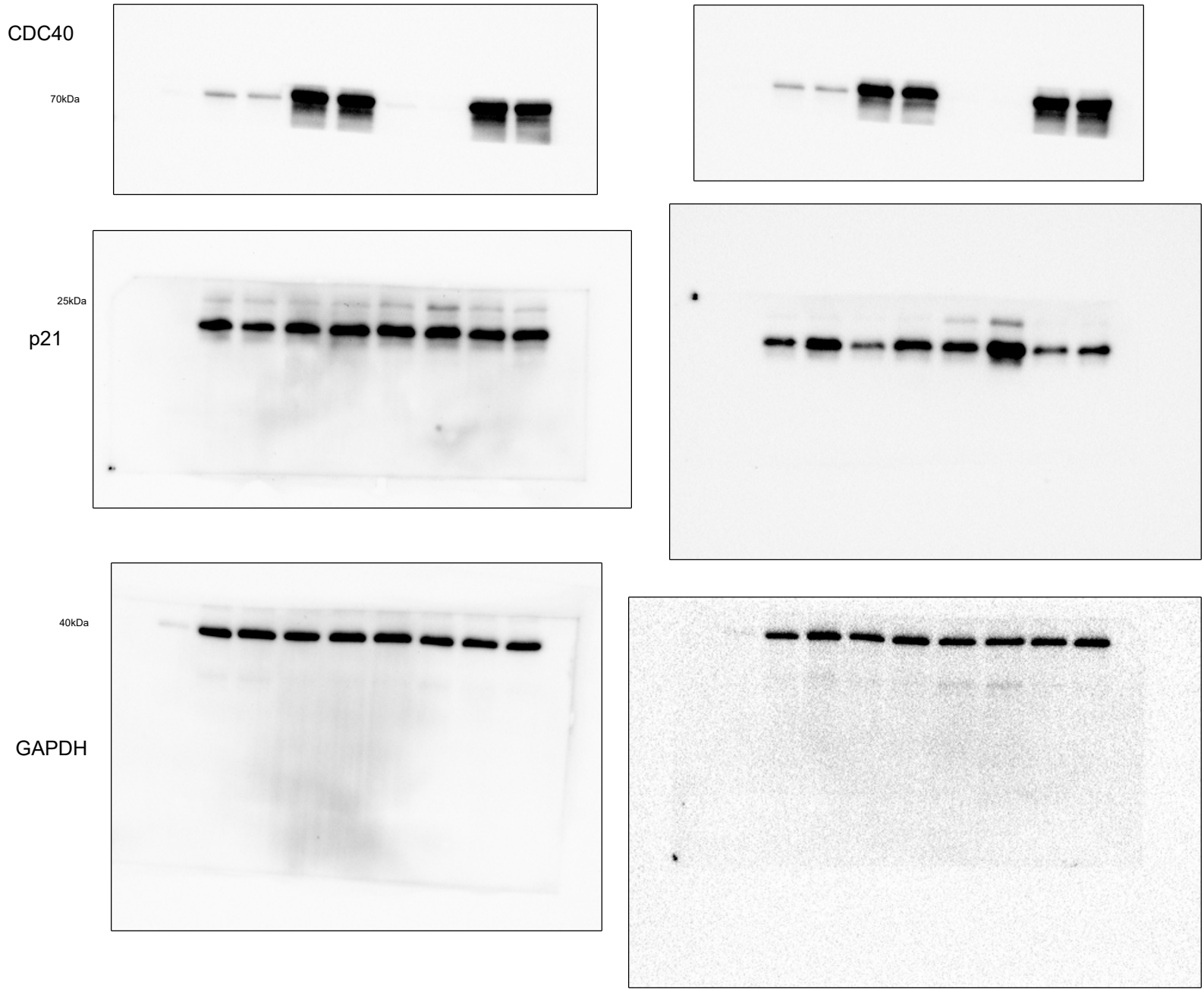
40kDa

GAPDH

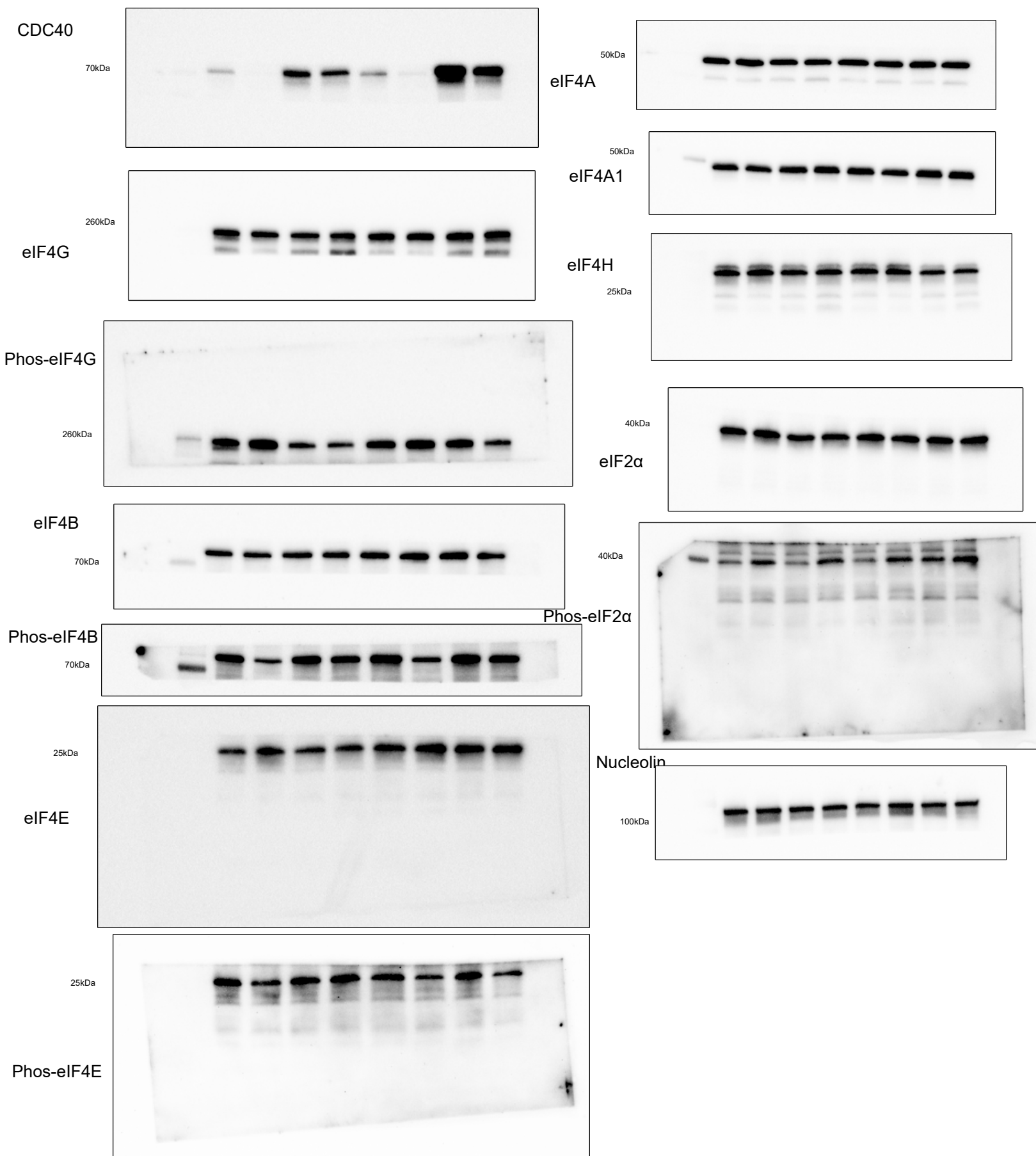


Supplementary Figure 33. Knockdown of CDC40 reduces CDC40 proteins in various lung cancer cells.
 Source image blots for Supplementary Figure 3 continued.

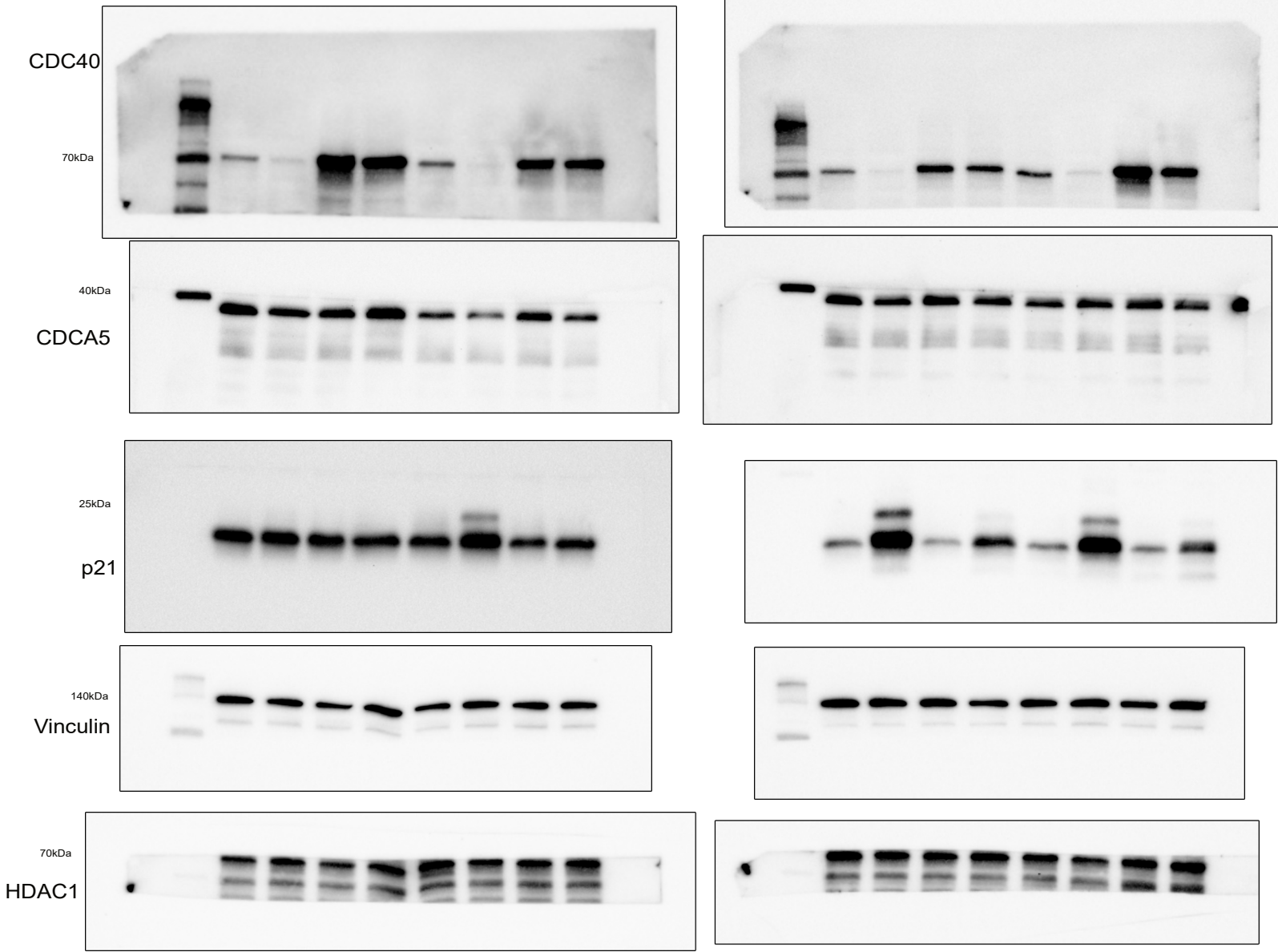
H1299 sh5



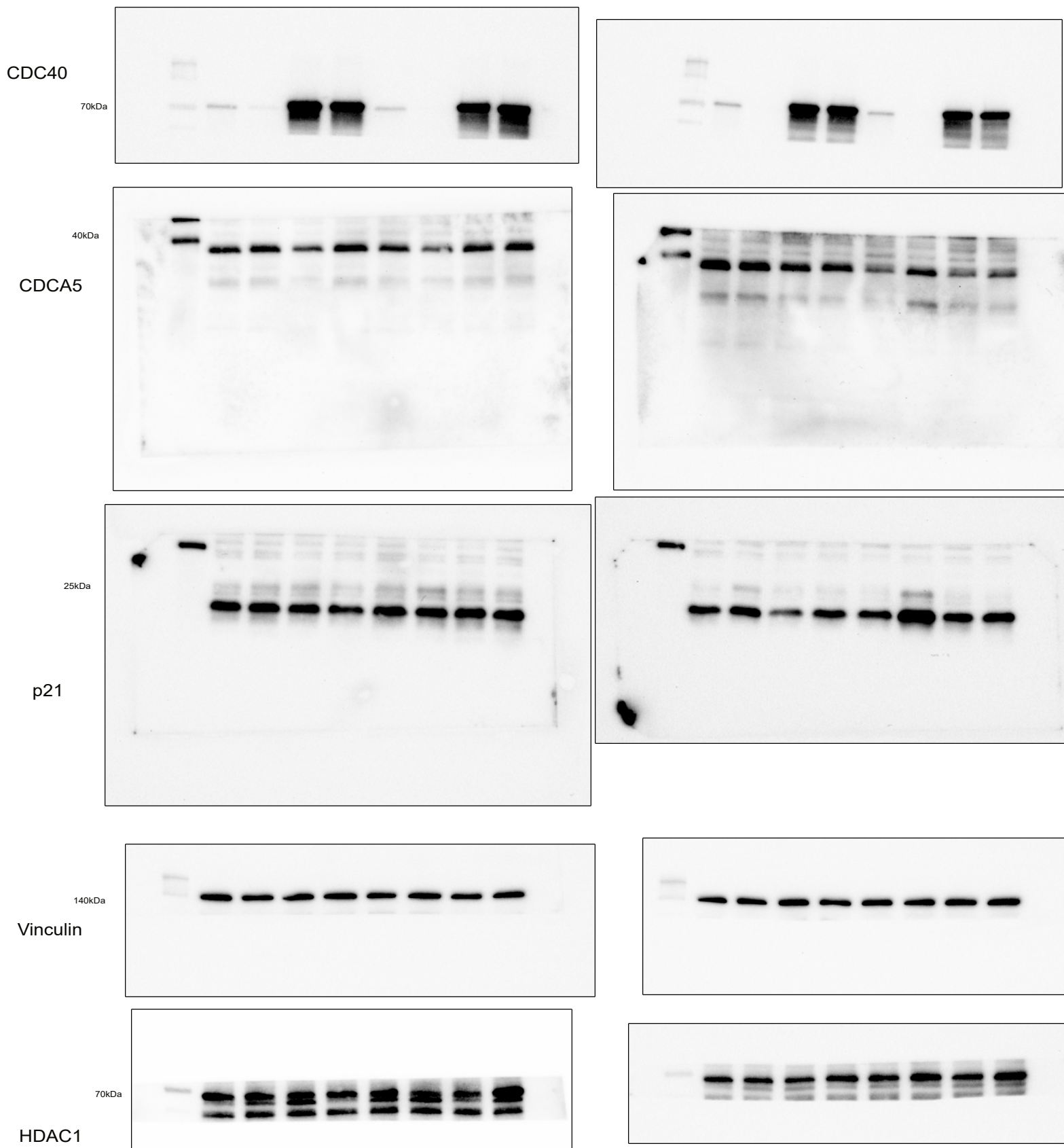
Supplementary Figure 34. Source image blots for Supplementary Figure 7b.



Supplementary Figure 35. Source image blots for Supplementary Figure 8a.



Supplementary Figure 36. Source image blots for Supplementary Figure 9.



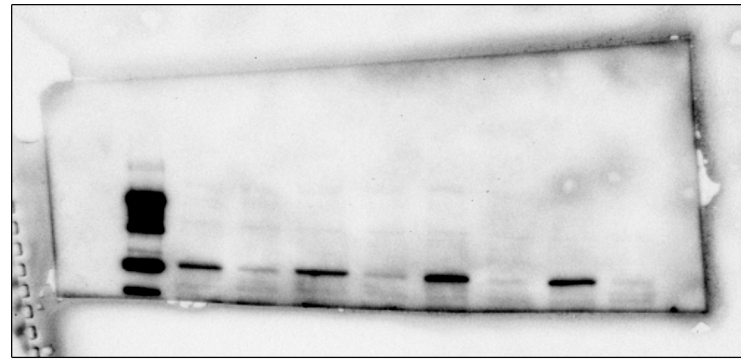
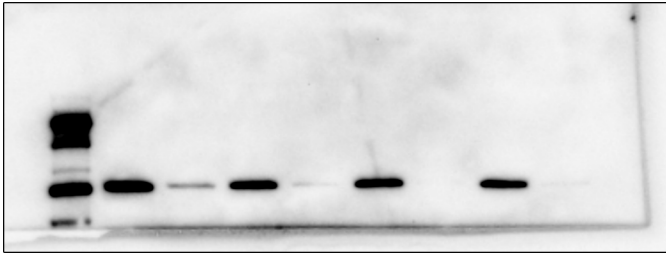
Supplementary Figure 37. Source image blots for Supplementary Figure 9 continued.

H2170 sh5

A549 sh5

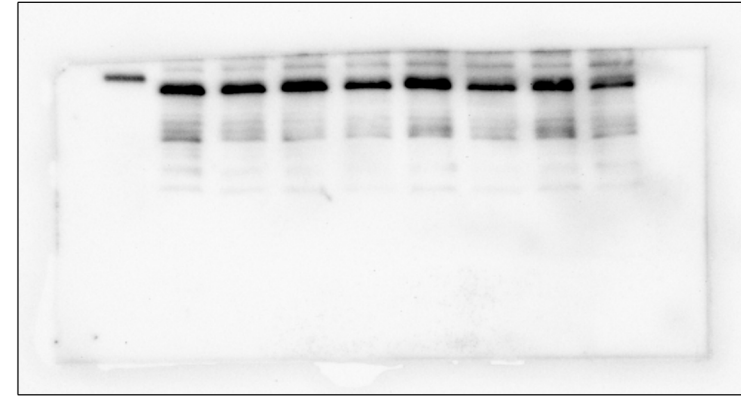
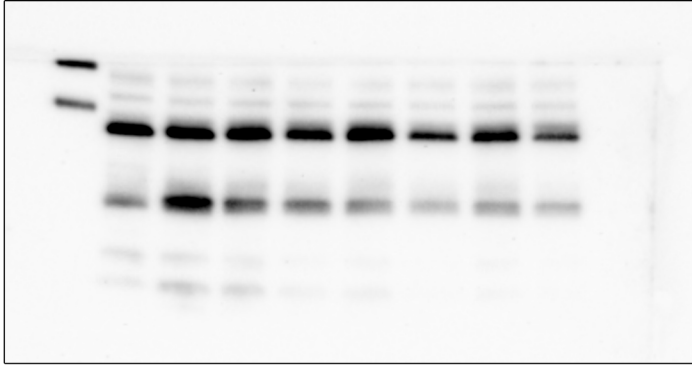
CDC40

70kDa



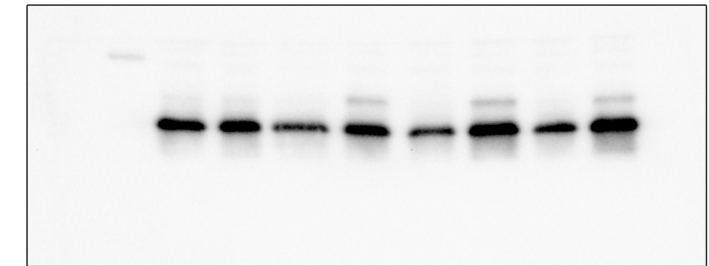
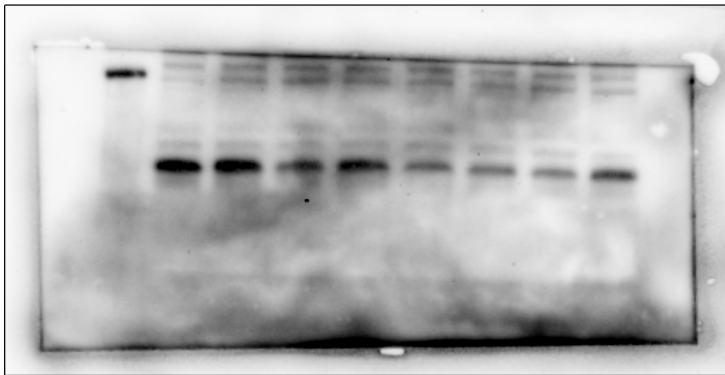
40kDa

CDCA5



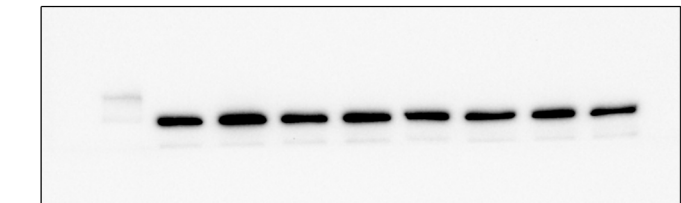
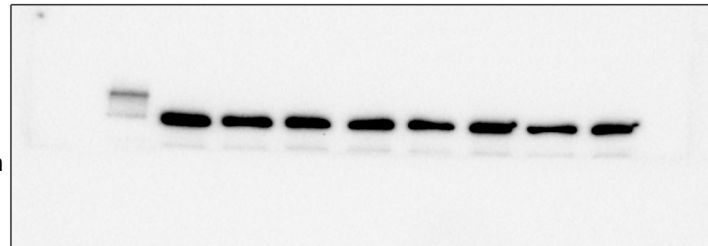
p21

25kDa



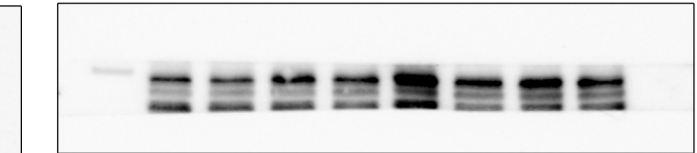
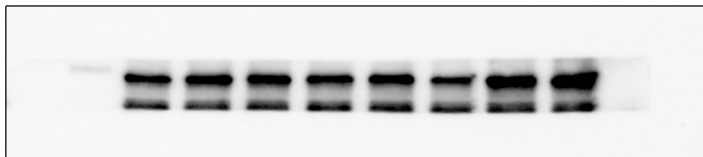
140kDa

Vinculin



70kDa

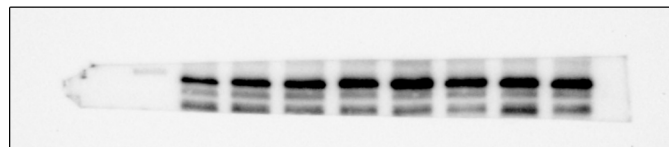
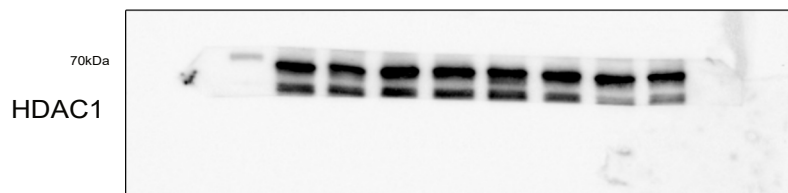
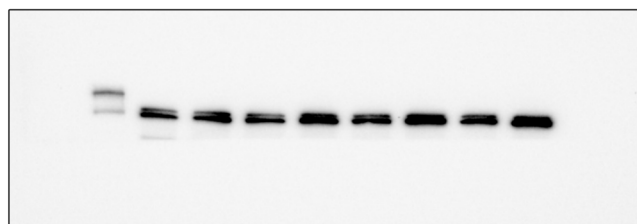
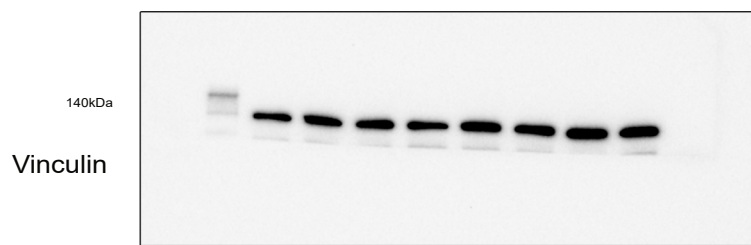
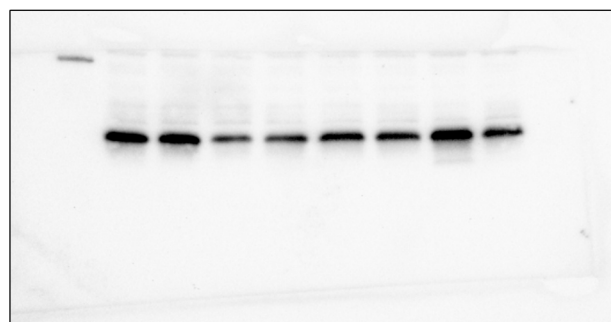
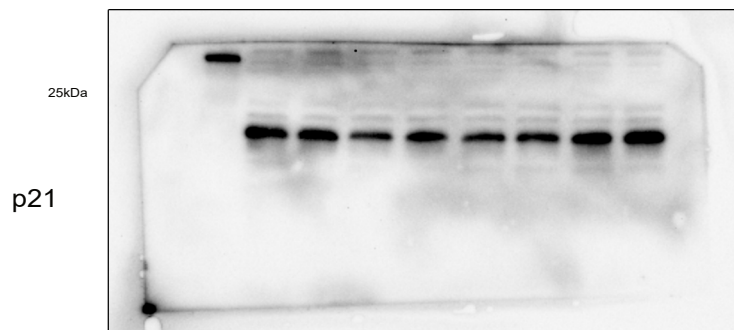
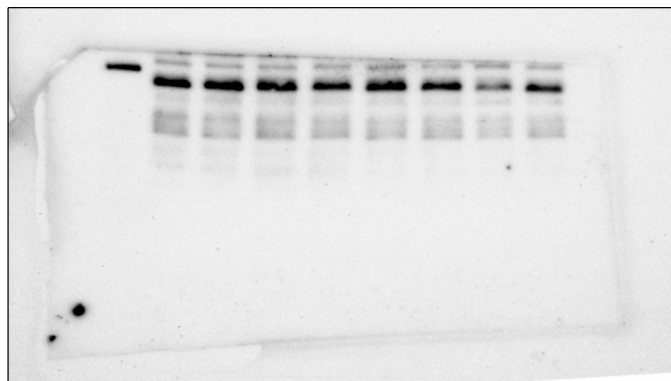
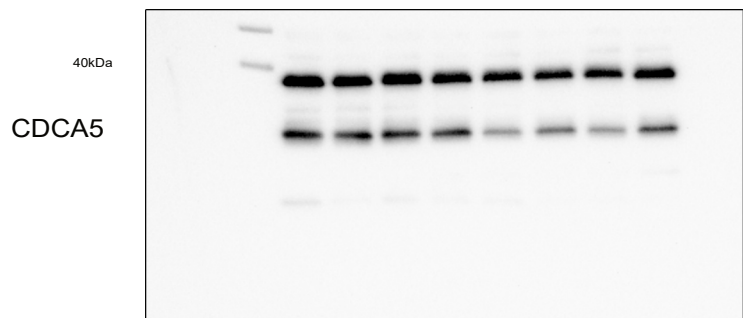
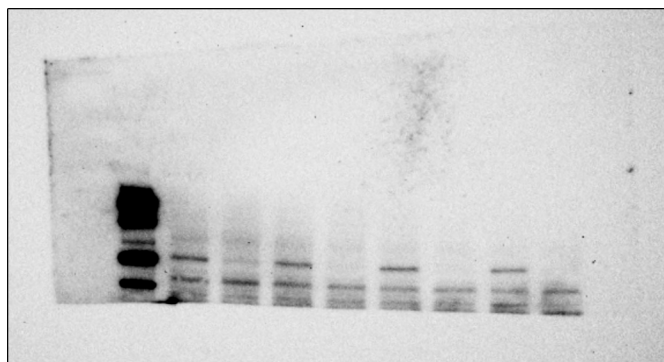
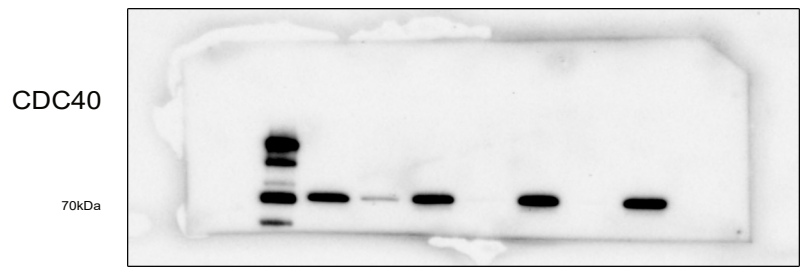
HDAC1



Supplementary Figure 38. Source image blots for Supplementary Figure 10.

H1915 sh5

H1975 sh5



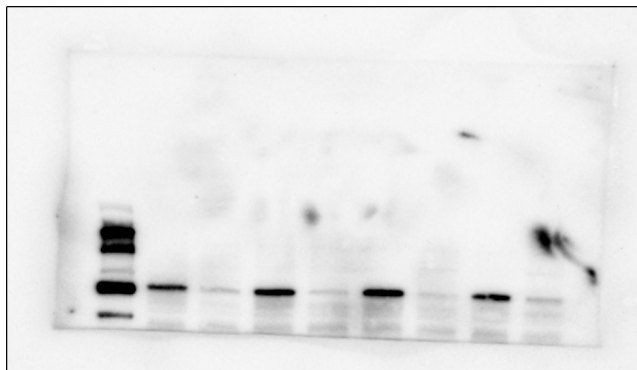
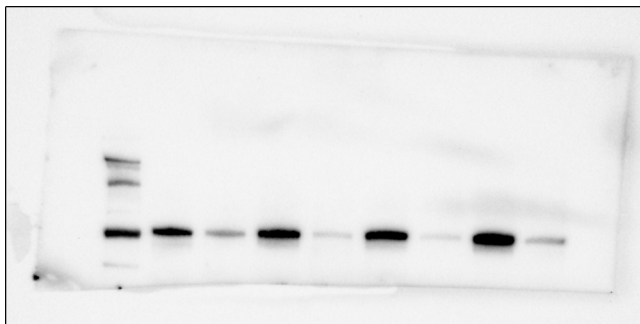
Supplementary Figure 39. Source image blots for Supplementary Figure 10 continued.

MCF10A sh5

H322 sh5

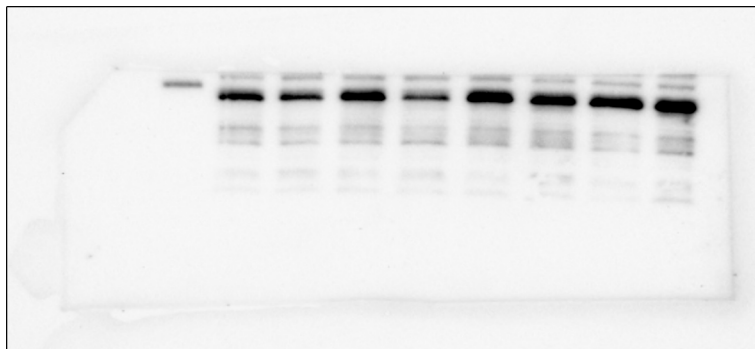
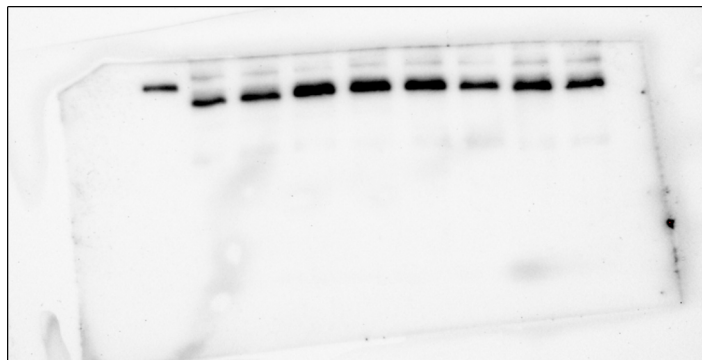
CDC40

70kDa



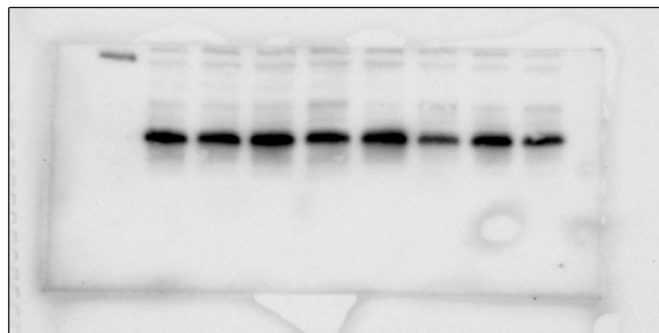
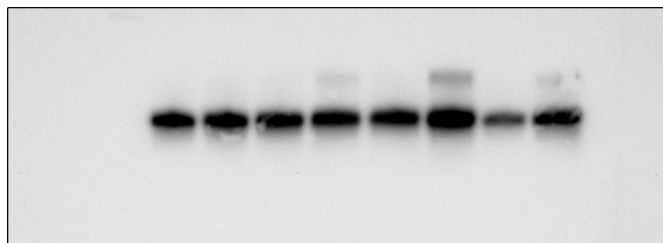
CDCA5

40kDa



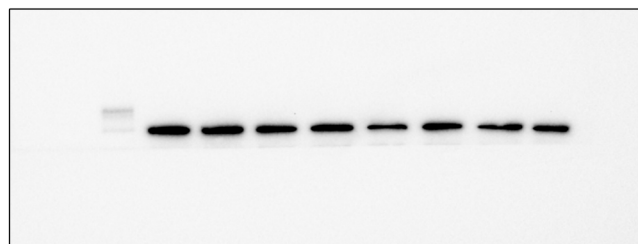
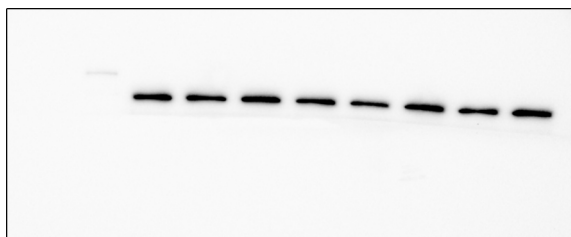
p21

25kDa



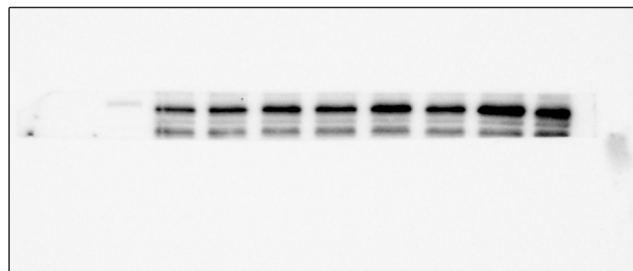
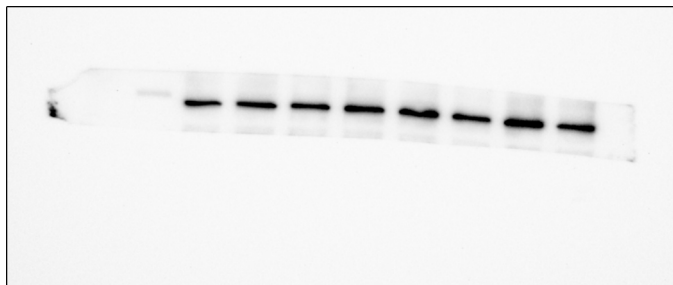
Vinculin

140kDa



HDAC1

70kDa



Supplementary Figure 40. Source image blots for Supplementary Figure 10 continued.

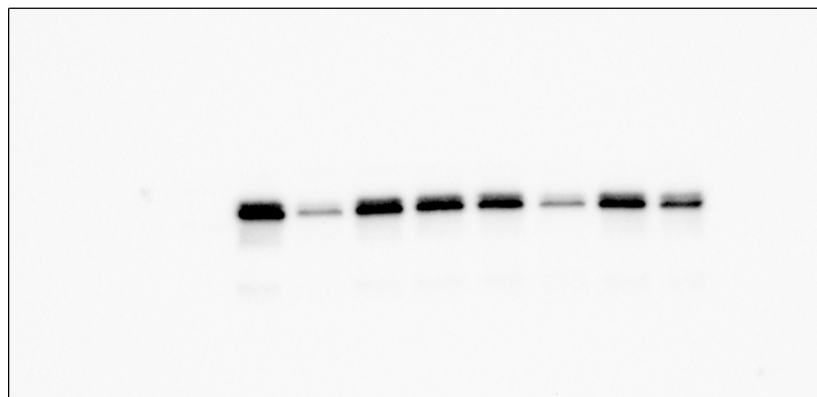
ERK

40kDa



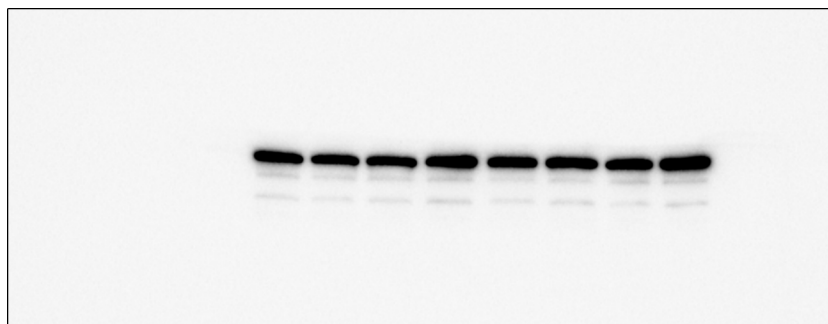
Phos-ERK

40kDa



GAPDH

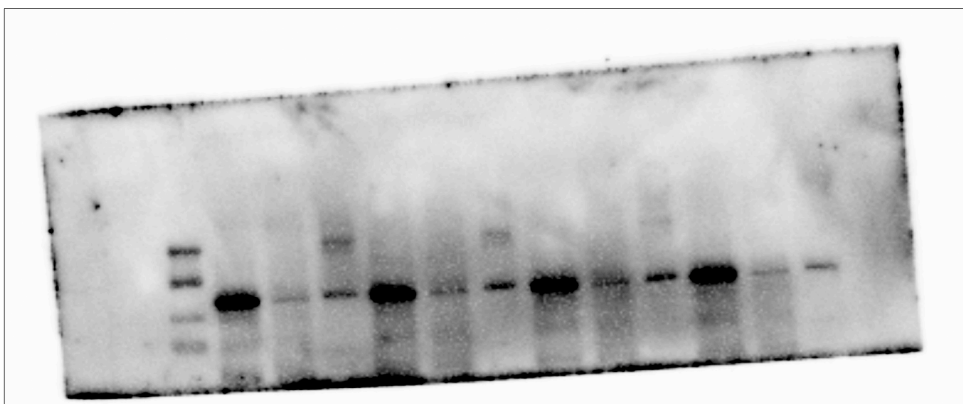
40kDa



Supplementary Figure 41. Source image blots for Supplementary Figure 11.

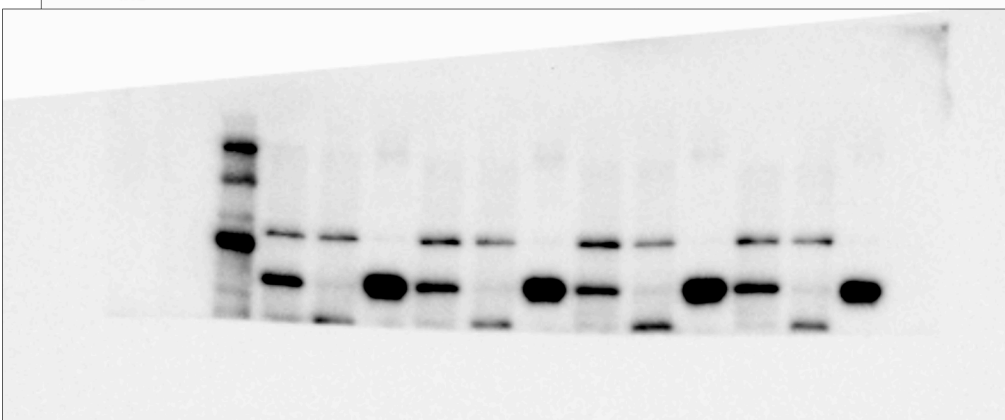
CDC5

100kDa

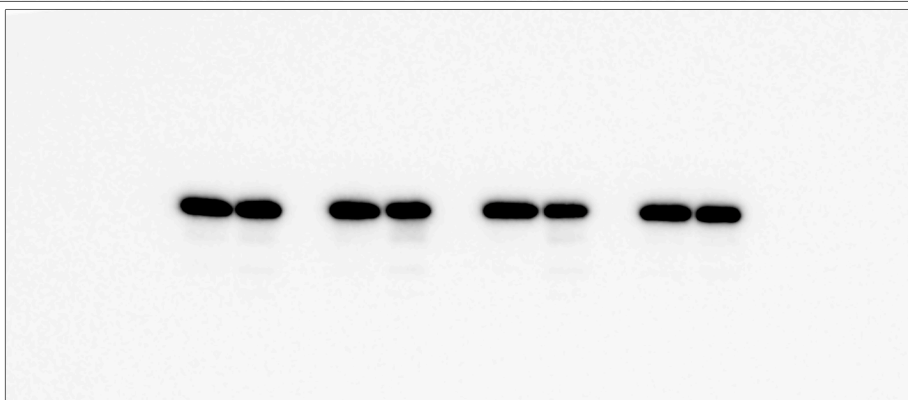


PRP19

50kDa



GAPDH 40kDa



Supplementary Figure 42. Source image blots for Supplementary Figure 22c.