

Supplementary Information

Y-box binding protein 1/cyclin A1 axis specifically promotes cell cycle progression at G₂/M phase in ovarian cancer

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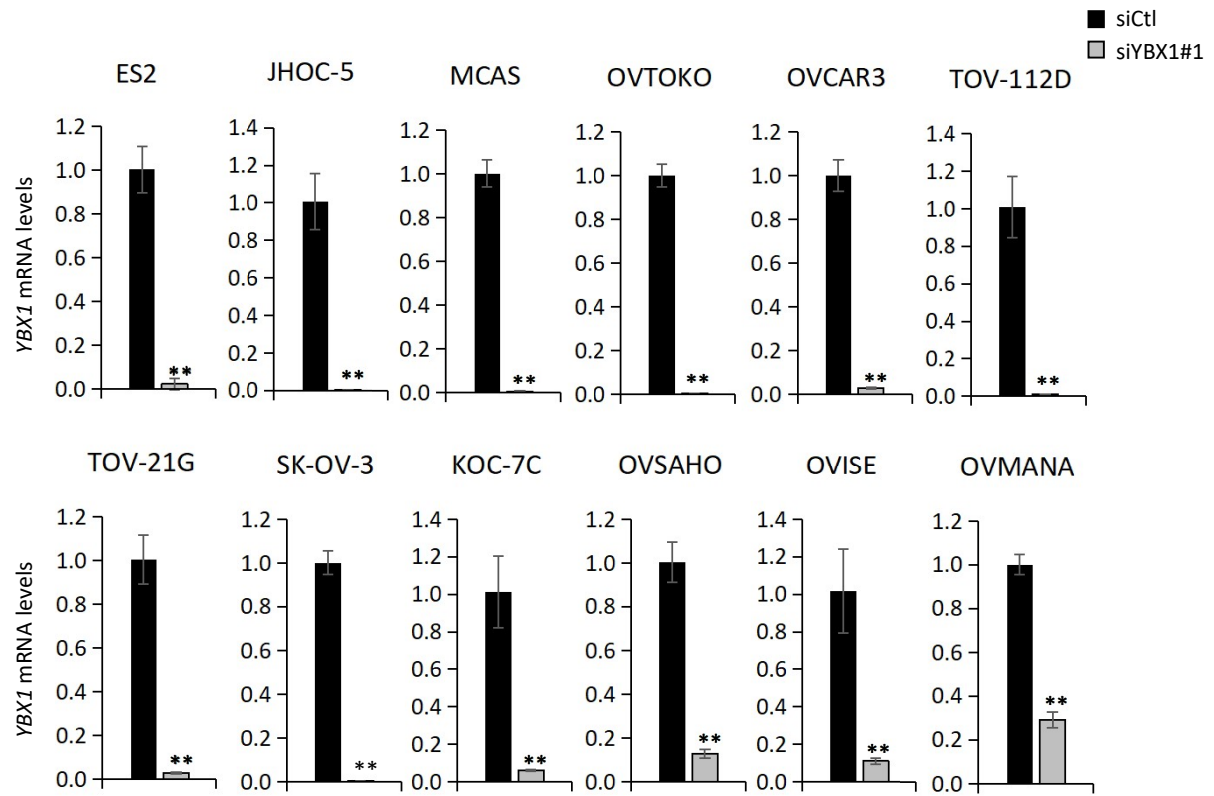
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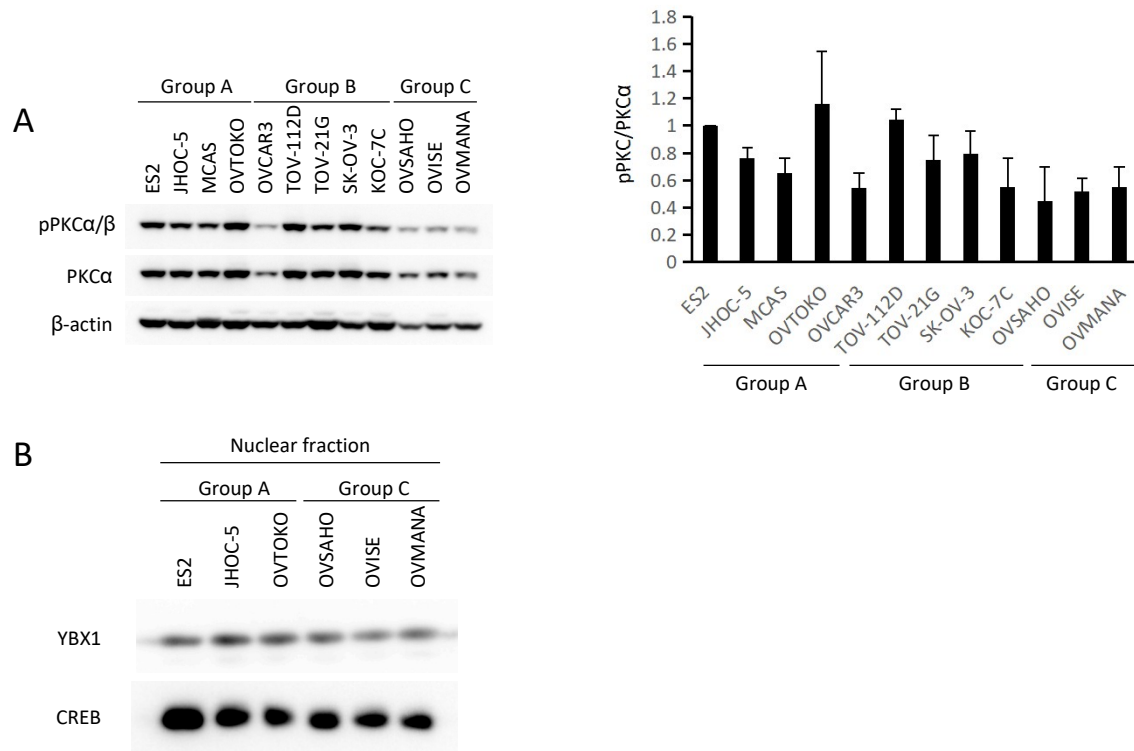
	Mutations ¹⁾									Doubling time ³⁾	Tumor Type ⁴⁾
	PIK3CA	PTEN	KRAS	BRAF	Cyclin E ²⁾	ARID1A	TP53	BRCA1	BRCA2		
ES2										18.8	HGSC
JHOC-5										26.7	OCCC
MCAS										17.5	MCA
OVTOKO										31.2	OCCC
OVCAR3										50.6	HGSC
TOV-112D										24.6	EA
TOV-21G										22.4	OCCC
SK-OV-3										37.9	HGSC
KOC-7C										29.1	OCCC
OVSCHO										59.9	HGSC
OVISE										28.5	OCCC
OVMANA										33.4	OCCC

- 1) Black represents mutations found
- 2) Cyclin E gene amplification
- 3) Doubling time (h) is determined during exponential proliferation of each cell line when followed for up to 5 days.
- 4) Abbreviations: OCCC, ovarian clear cell carcinoma; HGSC, high-grade serous carcinoma; EA, endometrioid adenocarcinoma; MCA, mucinous cystadenocarcinoma

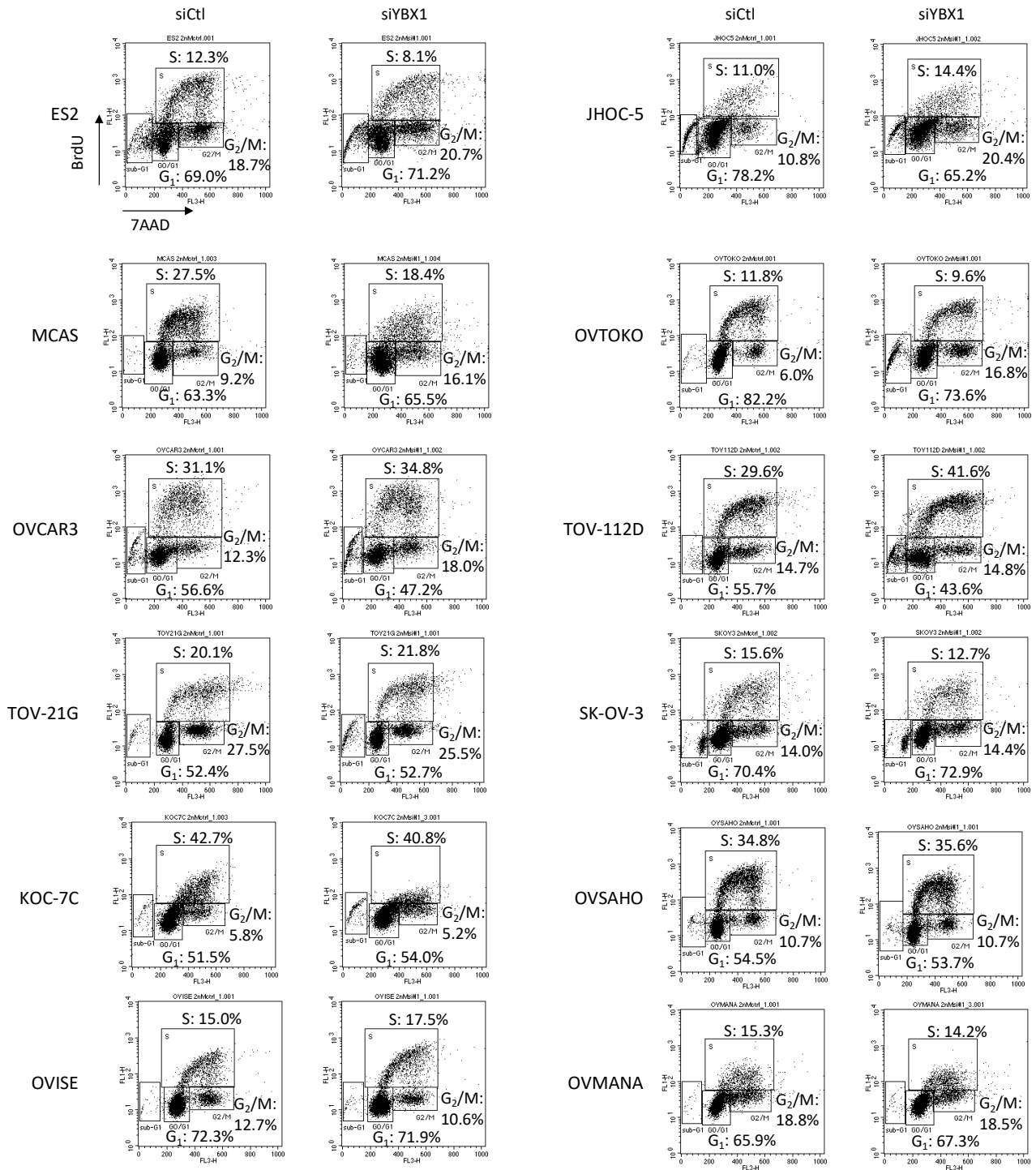
Supplementary Figure S1. List of the 12 ovarian cancer cell lines used in this study. The absence or presence of mutations in *PIK3CA*, *PTEN*, *KRAS*, *BRAF*, *ARID1A*, *TP53*, *BRCA1*, and *BRCA2* genes and amplification of the *cyclin E* gene is indicated. Doubling times (h) were determined during exponential proliferation for each cell line when followed for up to 5 days. Abbreviations: OCCC, ovarian clear cell carcinoma; HGSC, high-grade serous adenocarcinoma; EA, endometrioid adenocarcinoma; MCA, mucinous cystadenocarcinoma.



Supplementary Figure S2. Suppression of YBX1 mRNA expression by YBX1 siRNA#1. RT-qPCR analysis showed that treatment with YBX1 siRNA#1 for 3 days inhibited the expression of YBX1 mRNA in all cell lines. Error bars indicate standard deviations for 3 independent experiments. * $p < 0.05$; ** $p < 0.01$, 2-sided Student's t-test.



Supplementary Figure S3. Expression of pPKC α , PKC α , pAKT, and AKT, and nuclear expression of YBX1
 (A) Expression levels of pPKC α and PKC α in 12 cell lines, as assessed by western blot analysis and densitometric analysis of the western blot showing pPKC/PKC α ratio. Protein bands corresponding to pPKC were normalized to PKC α . Error bars indicate standard deviations for 3 independent experiments.
 (B) Western blot analysis revealed nuclear expression of YBX1 in group A and group C cell lines.



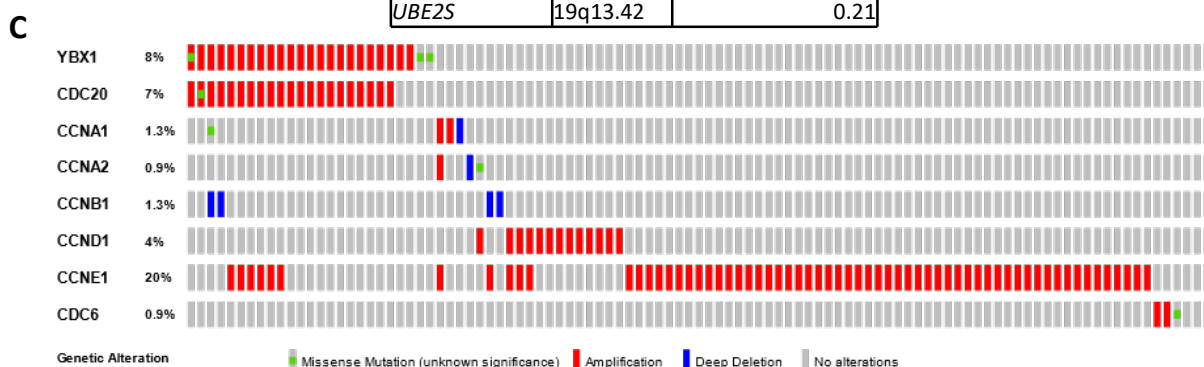
Supplementary Figure S4. Effects of YBX1 siRNA#1 on the cell cycle distribution. FACS analysis of the cell cycle distribution in ovarian cancer cell lines following transfection with YBX1 siRNA #1 for 3 days. Cell fractions in G₀/G₁, G₂/M, and S phases are indicated by boxes.

A

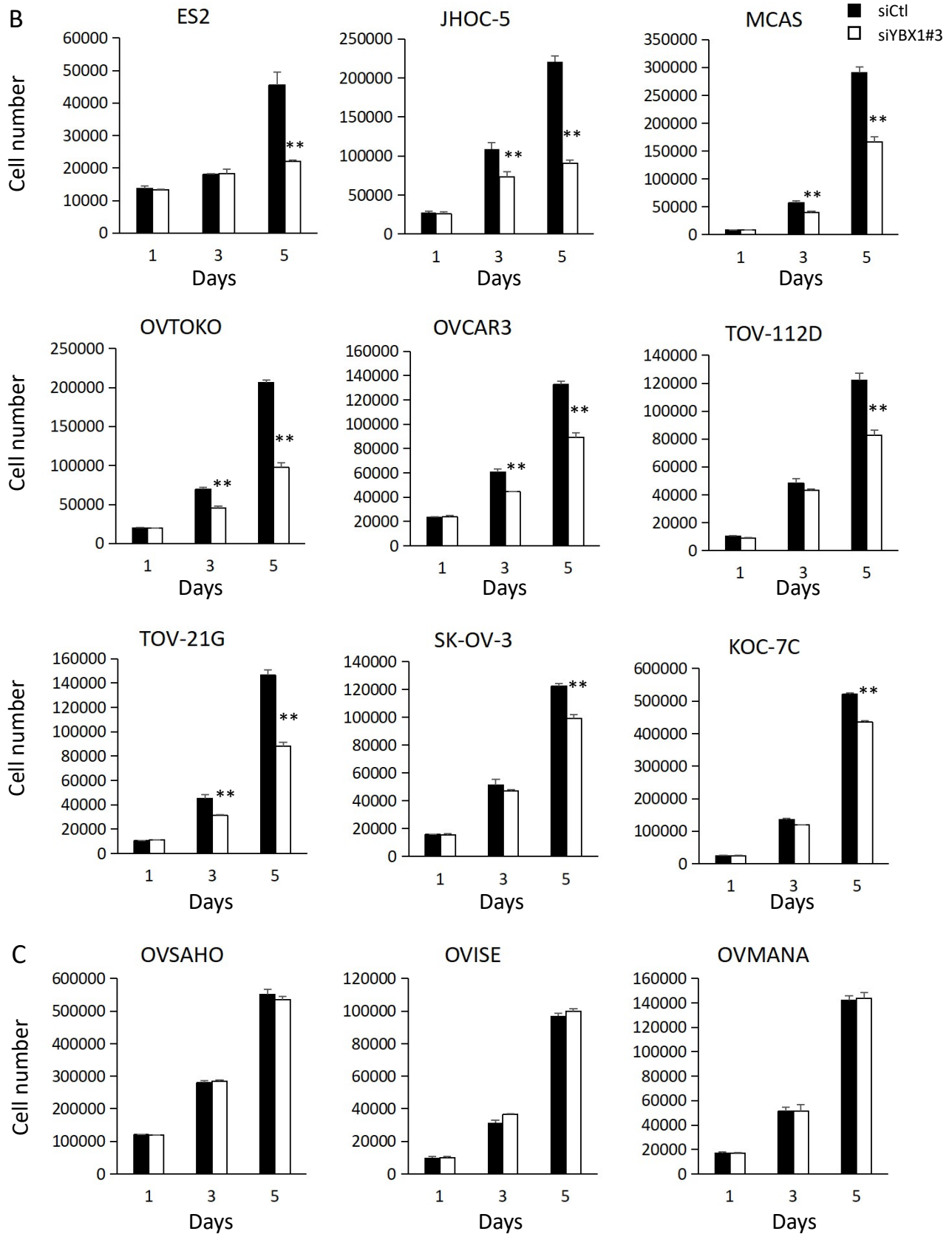
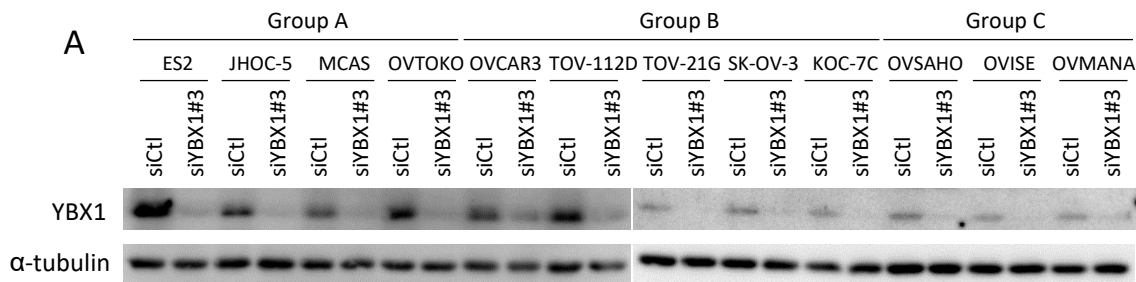
GO Term	GO id	P value
Cell proliferation	GO:0008283	8.50×10^{-9}
Cell division	GO:0051301	2.06×10^{-7}
DNA replication	GO:0006260	2.74×10^{-7}
Sister chromatid cohesion	GO:0007062	2.40×10^{-6}
G1/S transition of mitotic cell cycle	GO:0000082	1.34×10^{-5}
Mitotic nuclear division	GO:0007067	3.17×10^{-5}
Chromosome organization	GO:0051276	4.27×10^{-5}
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DNA replication initiation	GO:0006270	5.69×10^{-5}
DNA repair	GO:0006281	5.86×10^{-5}

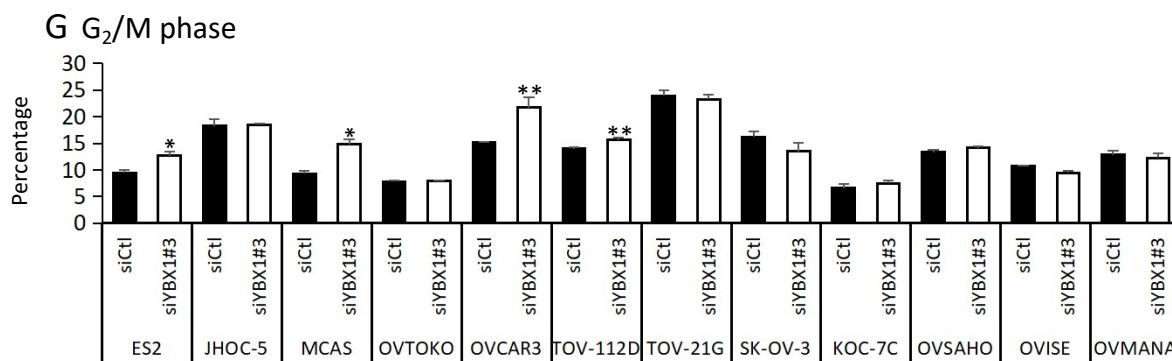
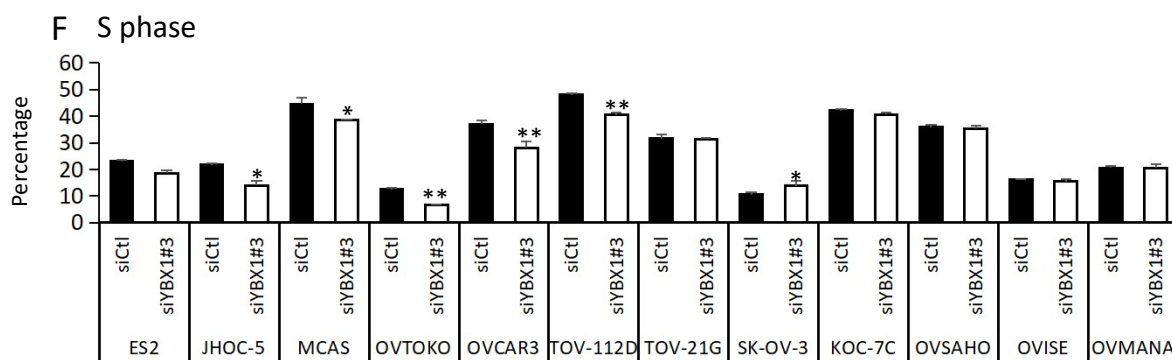
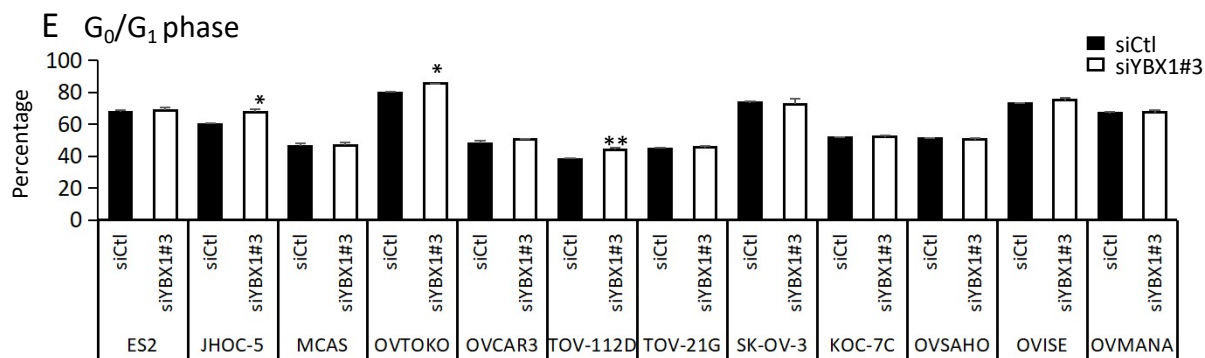
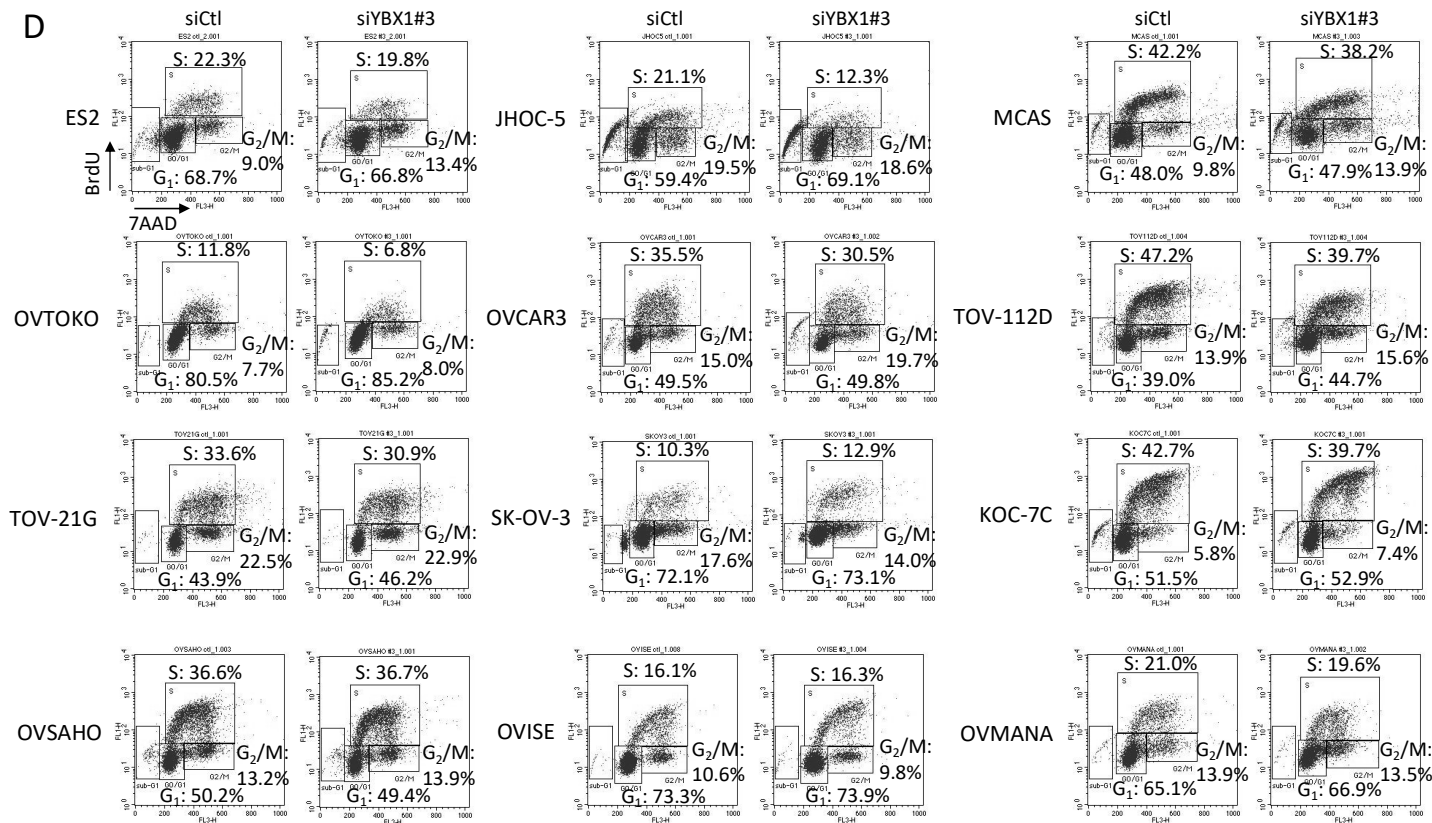
B

Gene Symbol	Cytoband	Spearman Score
<i>CDC20</i>	1p34.2	0.49
<i>CDCA8</i>	1p34.3	0.44
<i>KIF2C</i>	1p34.1	0.38
<i>TUBB</i>	6p21.33	0.31
<i>TUBA1C</i>	12q13.12	0.28
<i>CDC25A</i>	3p21.31	0.27
<i>ZWILCH</i>	15q22.31	0.26
<i>CDCA3</i>	12p13.31	0.24
<i>CCNB2</i>	15q22.2	0.24
<i>SPC25</i>	2q24.3	0.23
<i>CDCA5</i>	11q13.1	0.23
<i>ANAPC1</i>	2q13	0.23
<i>CDCA7</i>	2q31.1	0.22
<i>TUBA1B</i>	12q13.12	0.22
<i>KMT5A</i>	12q24.31	0.22
<i>NCAPD2</i>	12p13.31	0.22
<i>NCAPH</i>	2q11.2	0.21
<i>MIS18A</i>	21q22.11	0.21
<i>BUB1</i>	2q13	0.21
<i>UBE2S</i>	19q13.42	0.21



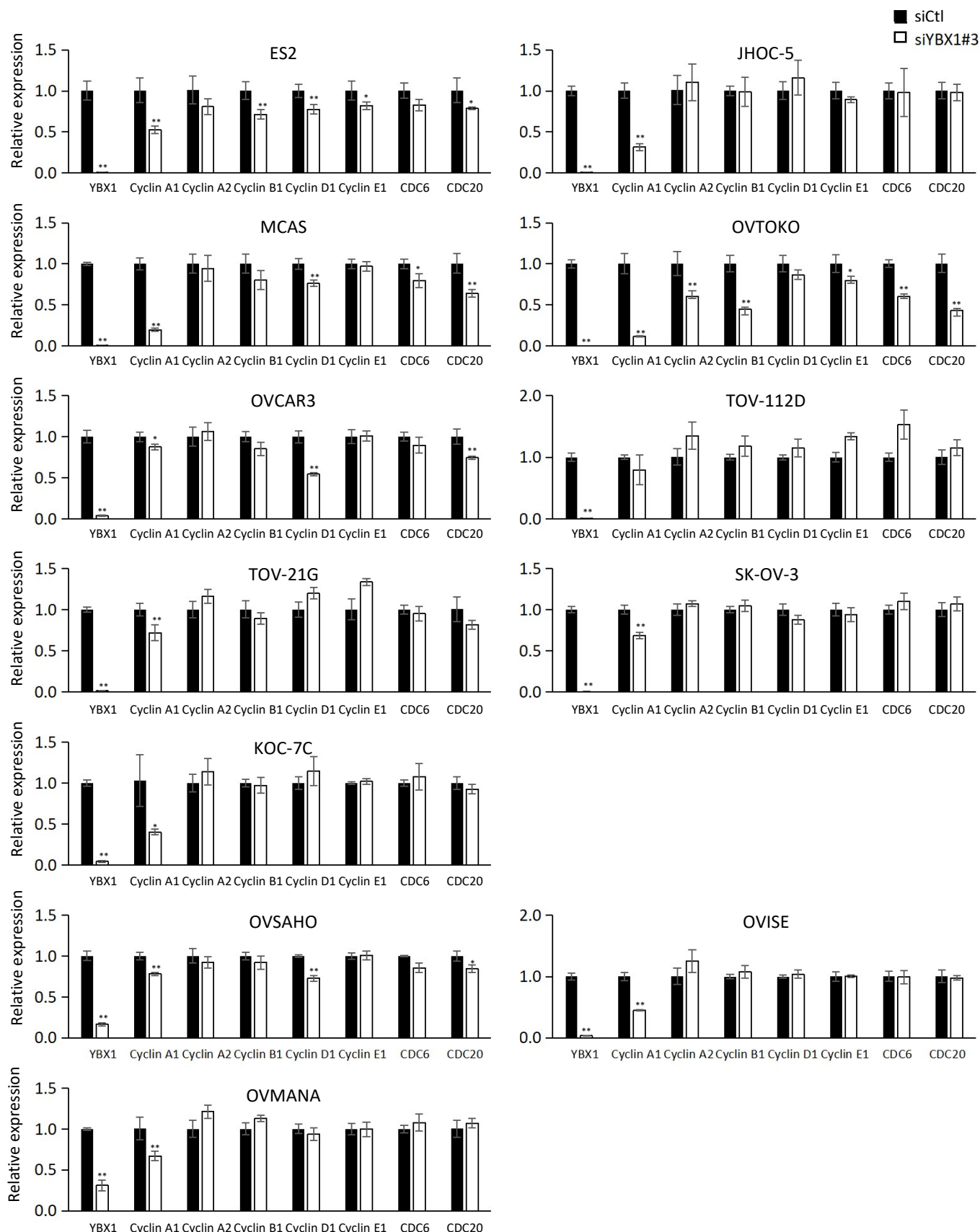
Supplementary Figure S5. Selection of cell cycle-related candidate genes closely associated with YBX1 in ovarian cancer. (A) Top 10 gene ontology terms for mRNAs showing positive correlations with YBX1. (B) Selection of the *CDC20* gene as a top cell division-related gene that is closely associated with YBX1, based on gene ontology analysis (TCGA database, n = 316) of HGSC. (C) The *CDC20* gene was co-amplified with the YBX1 gene on 1p34.2 in 7–8% of HGSC tumors (TCGA database, n = 316). *Cyclin A1*, *cyclin A2*, *cyclin B1*, and *CDC6* genes were rarely amplified, whereas *cyclin D1* and *cyclin E1* genes were amplified in approximately 10–20% of HGSC tumors exhibiting YBX1 amplification.



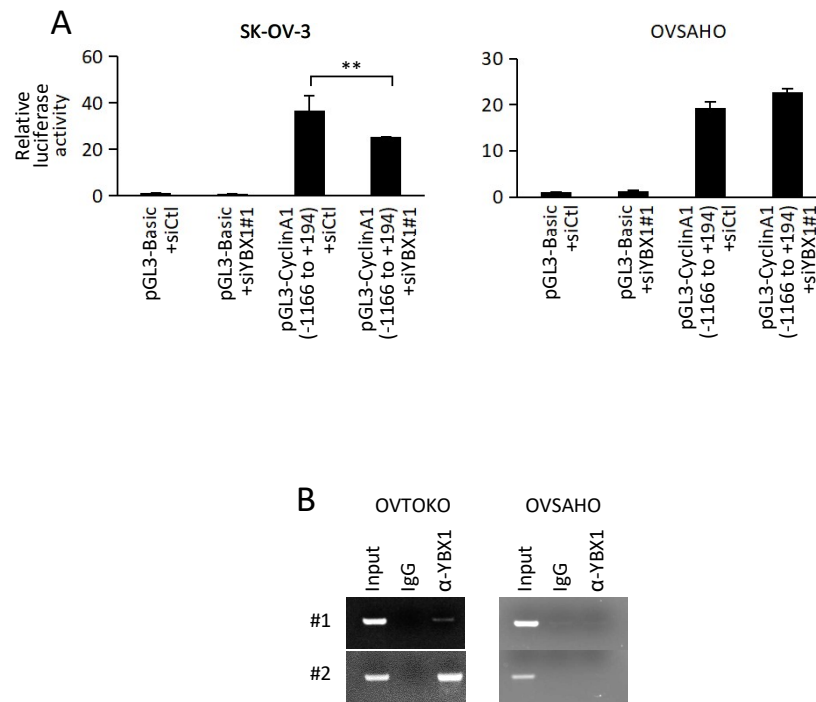


Supplementary Figure S6. Effects of YBX1 knockdown by YBX1 siRNA #3 on cell growth and the cell cycle.

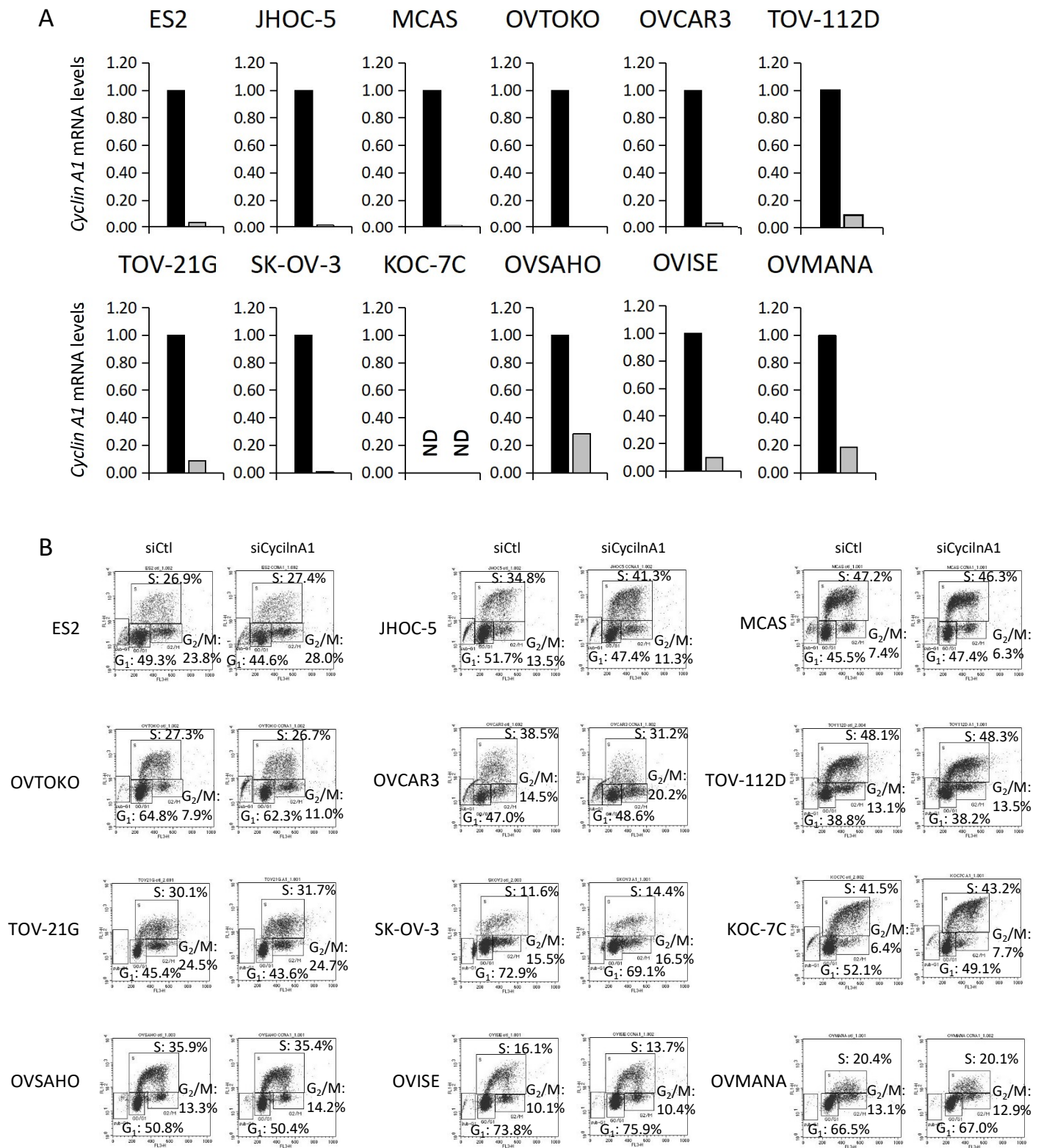
(A) Western blot analysis showed that treatment with *YBX1* siRNA#3 for 3 days decreased the expression of YBX1 in all cell lines. (B and C) The growth of 12 ovarian cancer cell lines was examined on days 1, 3, and 5 after transfection with siYBX1#3. Student's t-tests were used to compare differences between groups. Black, control siRNA; white, *YBX1* siRNA#3. * $p < 0.05$; ** $p < 0.01$, 2-sided Student's t-test. (D) FACS analysis of the cell cycle distribution in ovarian cancer cell lines following transfection with *YBX1* siRNA #3 for 3 days. Cell fractions in G₀/G₁, G₂/M, and S phases are indicated by boxes. (E–G) Percentages of cells in G₀/G₁ (E), S (F), and G₂/M (G) phases following transfection with *YBX1* siRNA#3 for 3 days. Error bars indicate standard deviations for 3 independent experiments. Black, control siRNA; white, *YBX1* siRNA#3. * $p < 0.05$; ** $p < 0.01$, 2-sided Student's t-test.



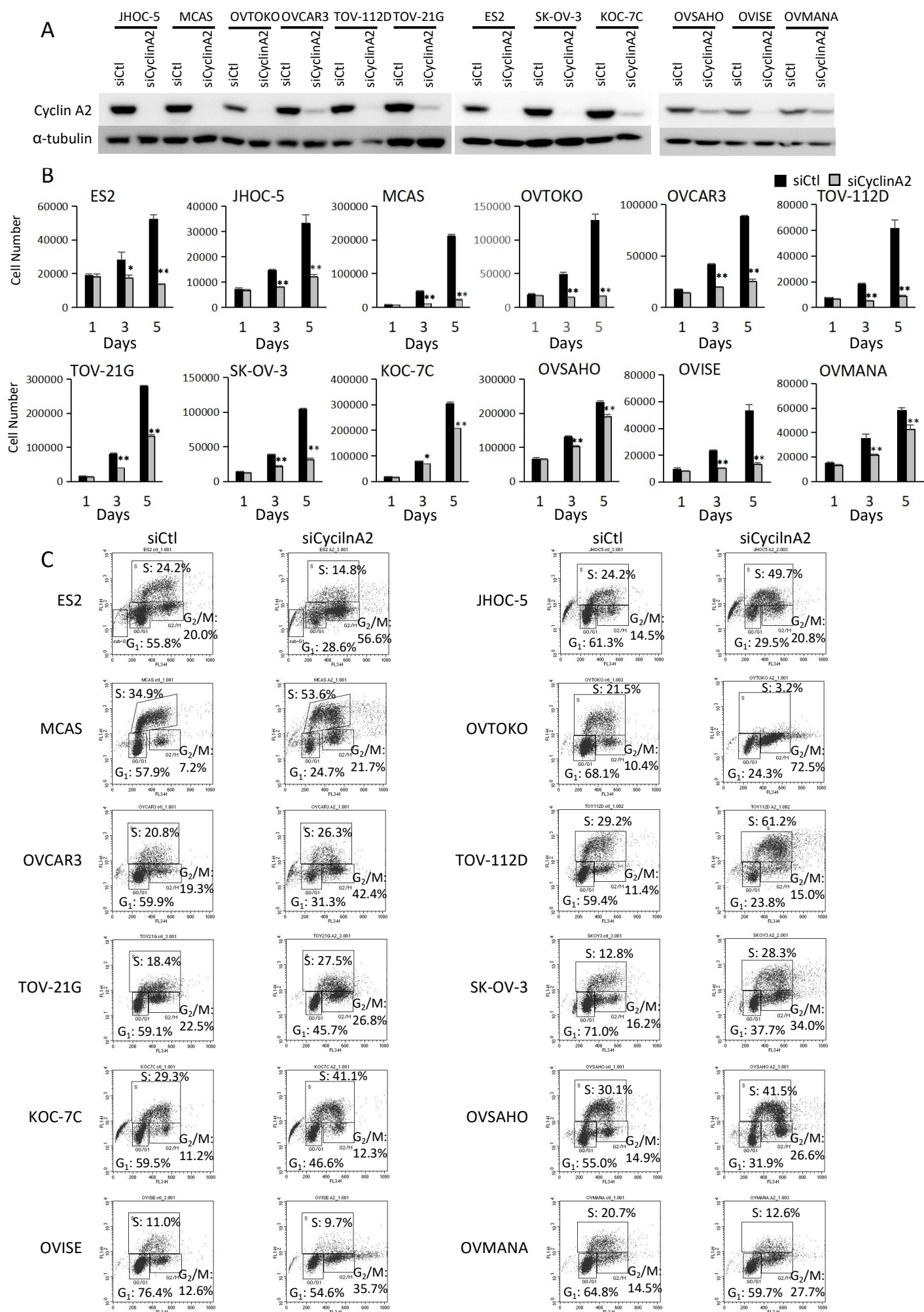
Supplementary Figure S7. Effects of YBX1 siRNA#3 on the mRNA expression of cell cycle-related genes. RT-qPCR analysis showing the mRNA expression levels of 8 genes in group A, group B and group C cell lines following transfection with YBX1 siRNA#3. Expression levels are presented as relative ratios based on 3 independent assays. Student's t-tests were used to compare differences between YBX1 siRNA#3-transfected cells and control cells. * $p < 0.05$; ** $p < 0.01$. Error bars indicate standard deviations.

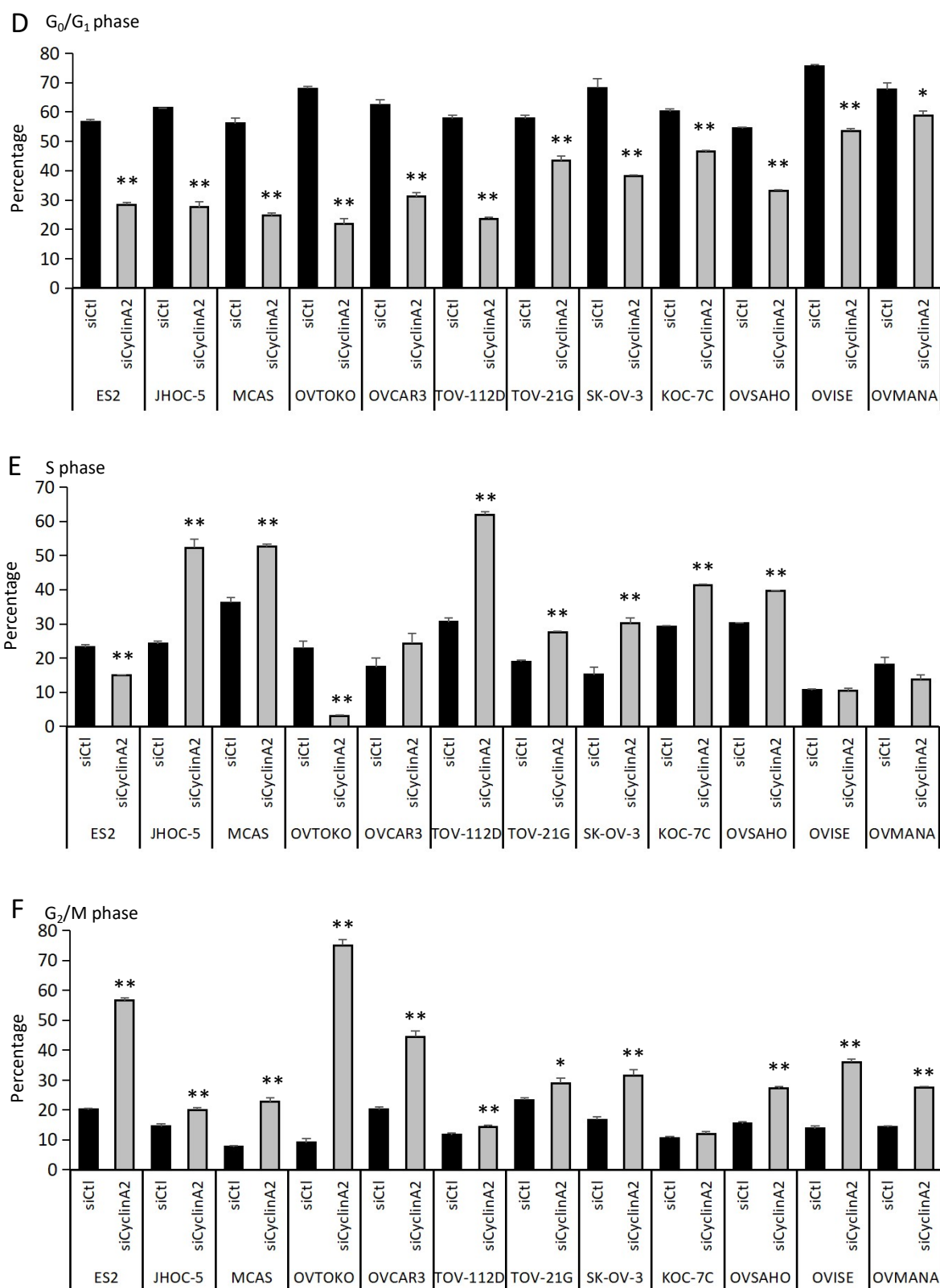


Supplementary Figure S8. Transcriptional activity of YBX1 on Cyclin A1 and binding of YBX1 to the Cyclin A1 promoter. (A) SK-OV-3 and OVSCHO cells were transfected with luciferase reporter constructs containing the 1166 bp 5'-flanking region of the cyclin A1 gene with YBX1 siRNA#1 for 2 days. The firefly luciferase activity was normalized to the Renilla luciferase activity. Student's t tests were used to compare differences between YBX1 siRNA#1-transfection groups and the control group. ** $p < 0.01$. Error bars indicate standard deviations for 3 independent experiments. (B) The YBX1 binding to the Cyclin A1 promoter by ChIP assay in OVTOKO and OVSCHO cells.



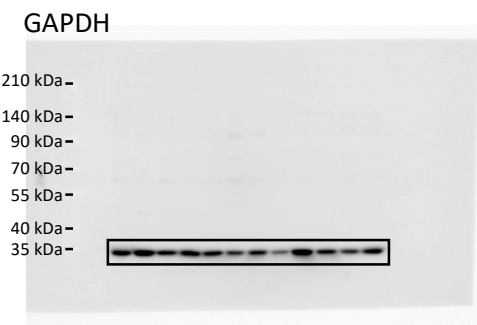
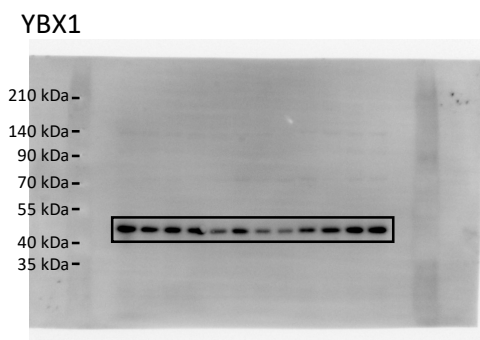
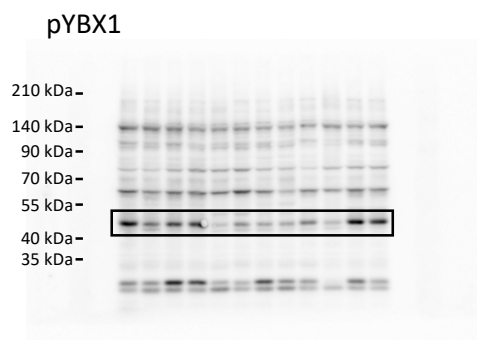
Supplementary Figure S9. Effects of *cyclin A1* siRNA on cell cycle distribution. (A) RT-qPCR analysis showed that treatment with *cyclin A1* siRNA for 3 days decreased the expression of *cyclin A1* mRNA in all cell lines. ND, not detected. (B) FACS analysis of the cell cycle distribution in ovarian cancer cell lines following transfection with *cyclin A1* siRNA for 3 days. Cell fractions in G₀/G₁, G₂/M, and S phases are indicated by boxes.





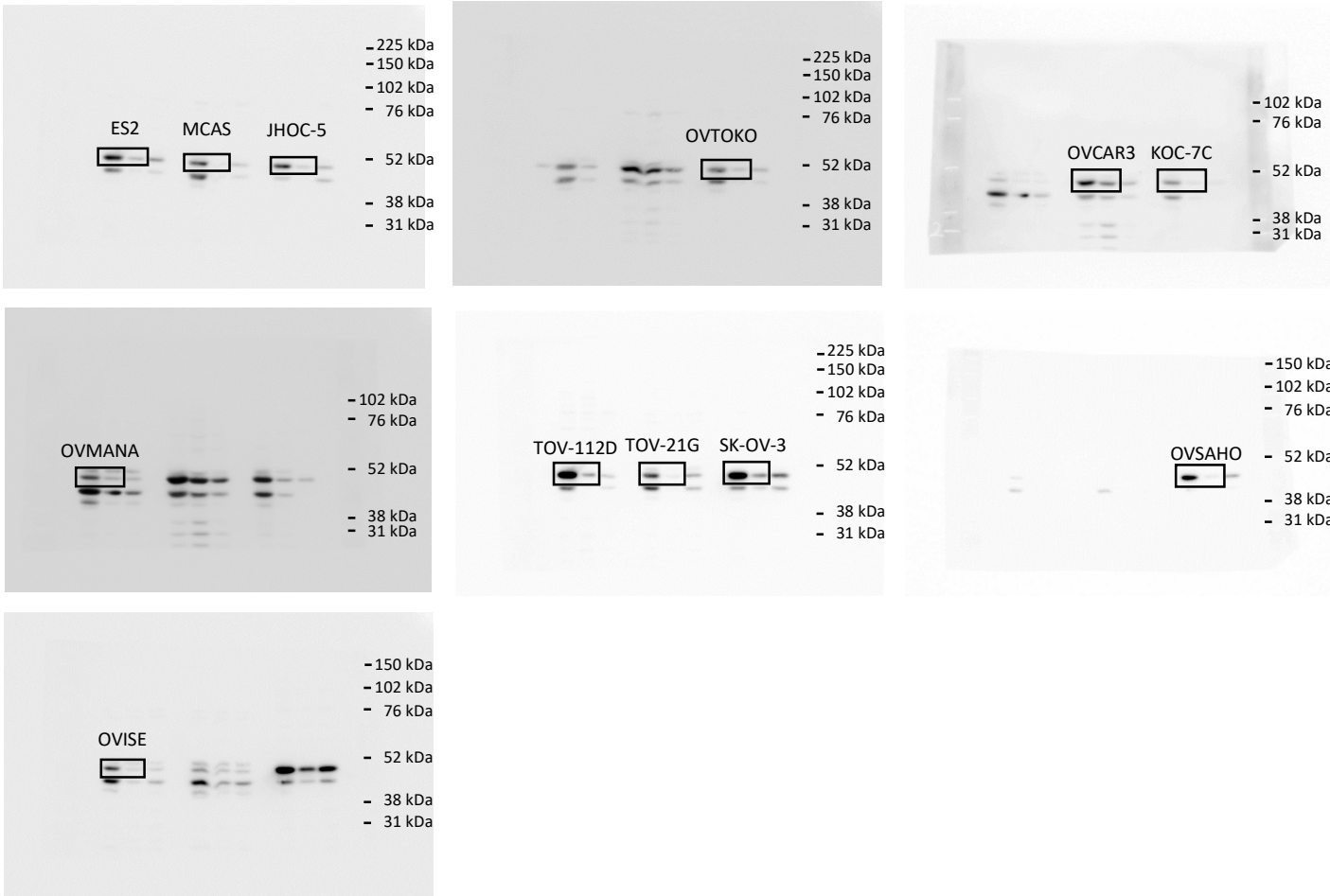
Supplementary Figure S10. Effects of cyclin A2 knockdown by *cyclin A2* siRNA on cell growth and cell cycle. (A) Western blot analysis showed that treatment with *cyclin A2* siRNA for 3 days decreased the expression of cyclin A2 in all cell lines. (B) Cell growth of ovarian cancer cell lines on days 1, 3, and 5 after transfection with *cyclin A2* siRNA. Student's t-tests were used to compare differences between control- and *cyclin A2* siRNA-transfected cells. * $p < 0.05$; ** $p < 0.01$. Error bars indicate standard deviations for 3 independent experiments. (C) FACS analysis of the cell cycle distribution in ovarian cancer cell lines following transfection with *cyclin A2* siRNA for 3 days. Cell fractions in G_0/G_1 , G_2/M , and S phases are indicated by boxes. (D–F) Percentages of cells in G_0/G_1 (D), S (E), and G_2/M (F) phases following transfection with *cyclin A2* siRNA for 3 days. Student's t-tests were used to compare differences between control siRNA- and *cyclin A2* siRNA-transfected cells. * $p < 0.05$; ** $p < 0.01$. Error bars indicate standard deviations for 3 independent experiments.

Uncropped blots for Figure 1C

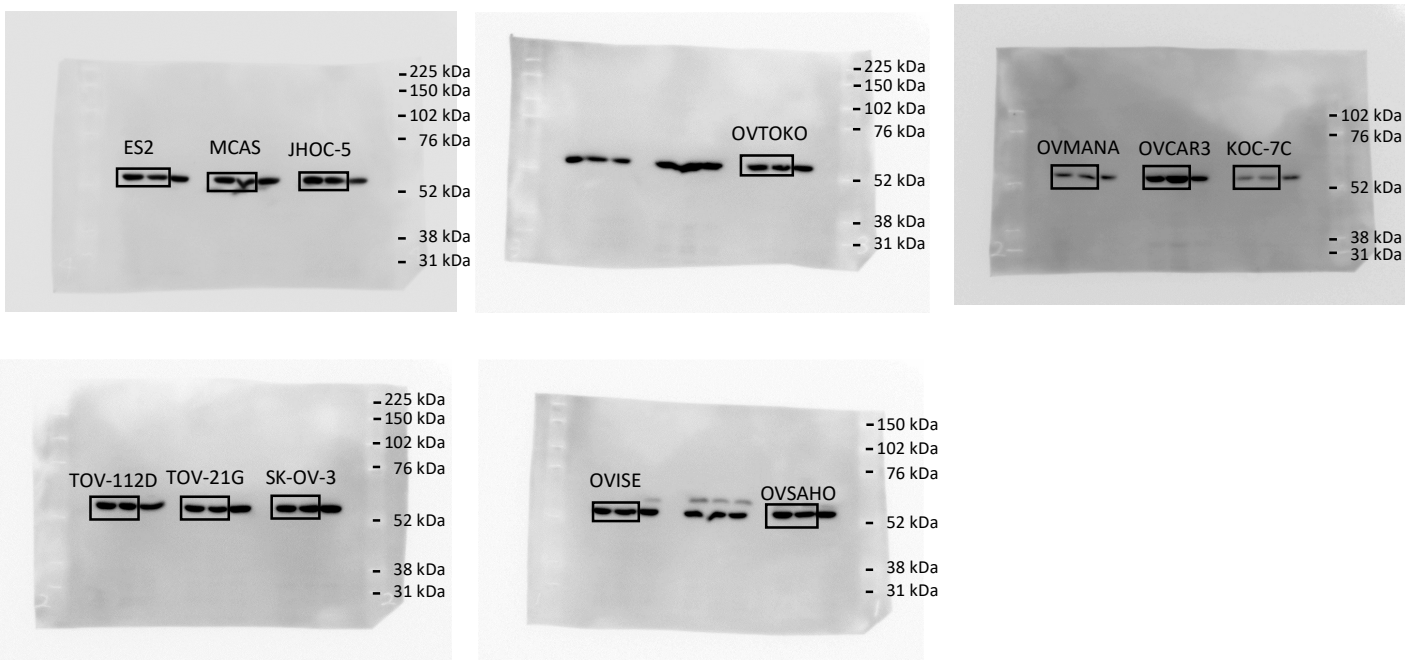


Uncropped blots for Figure 1D

YBX1

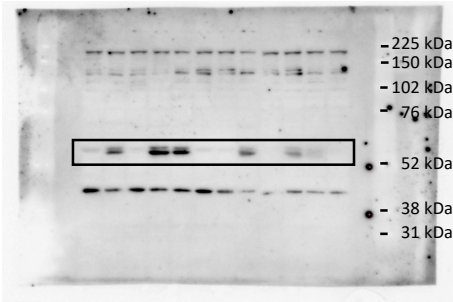


α -tubulin

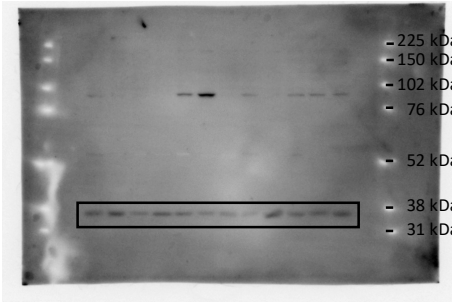


Uncropped blots for Figure 4A

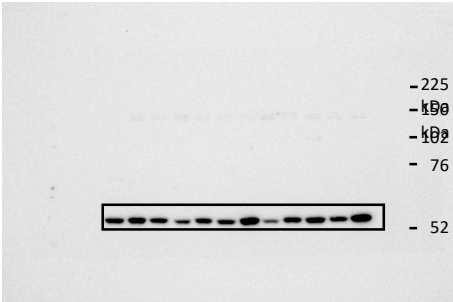
Cyclin A1



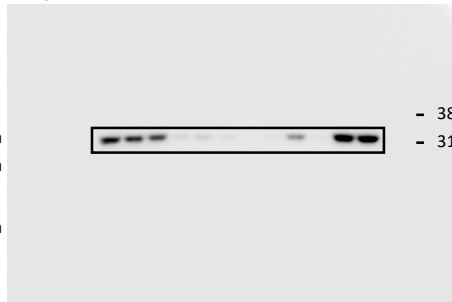
GAPDH



Cyclin B1



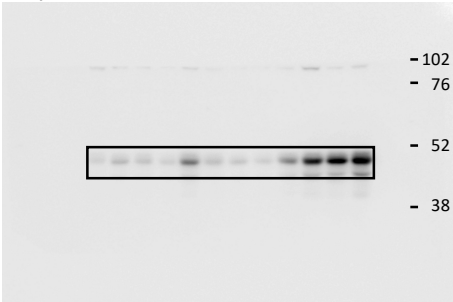
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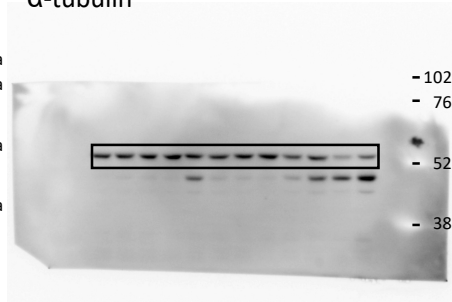
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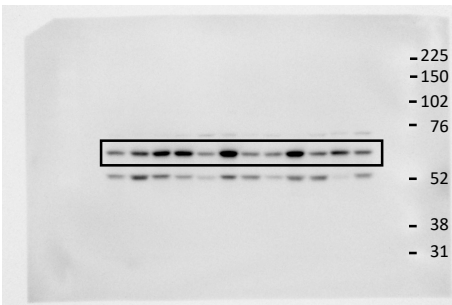
Cyclin E1



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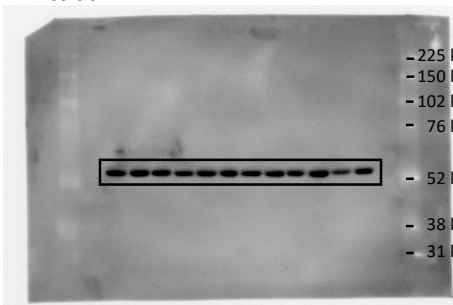
CDC6



CDC20

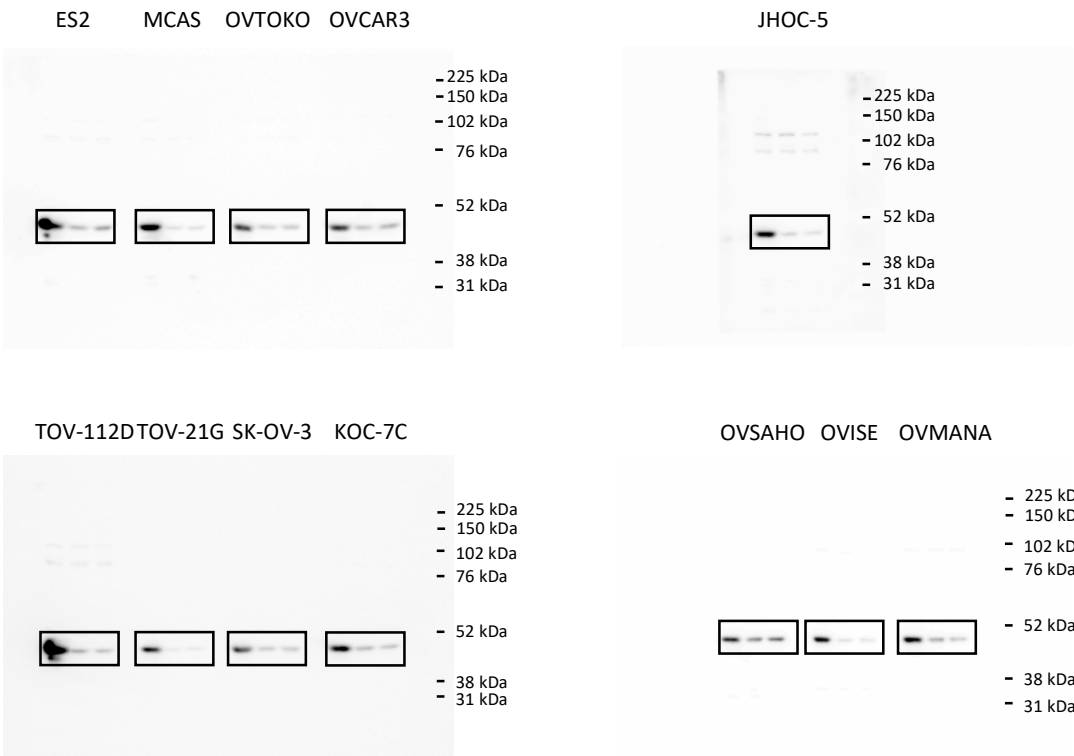


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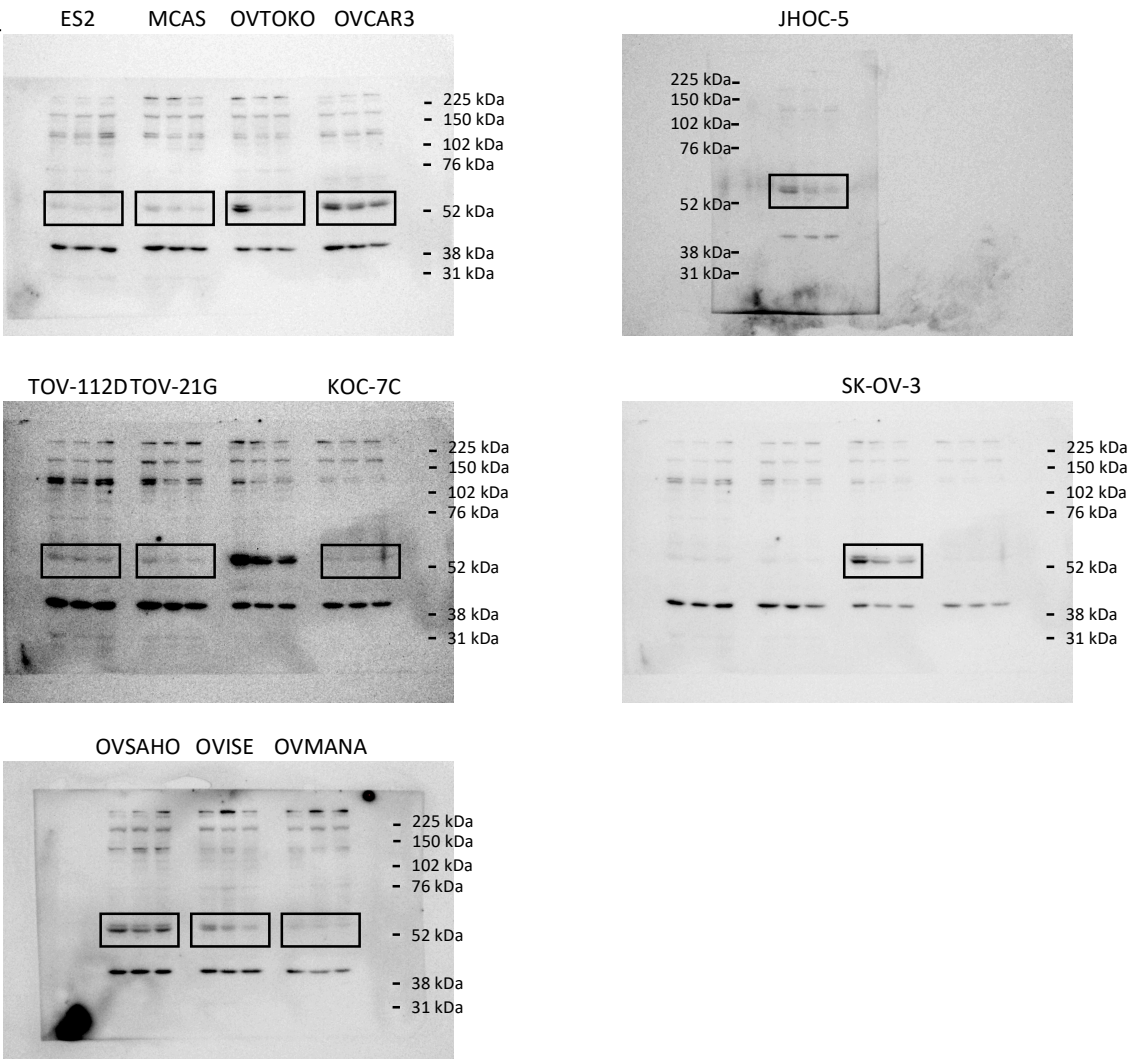


Uncropped blots for Figure 4B

YBX1

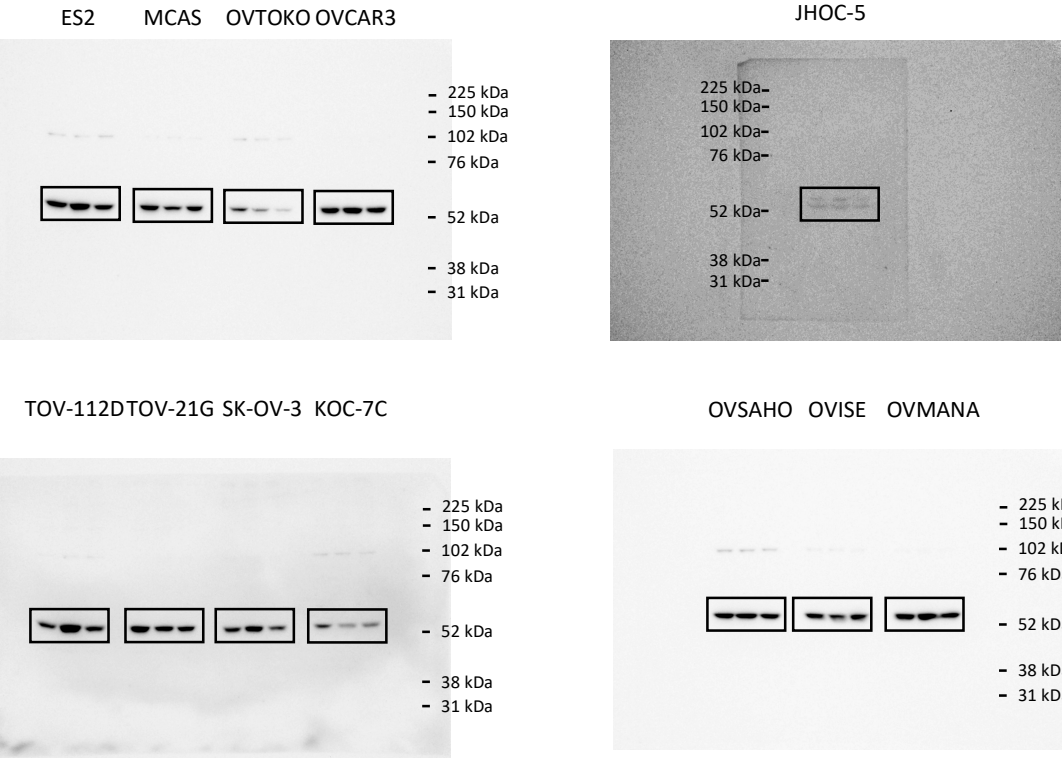


Cyclin A1

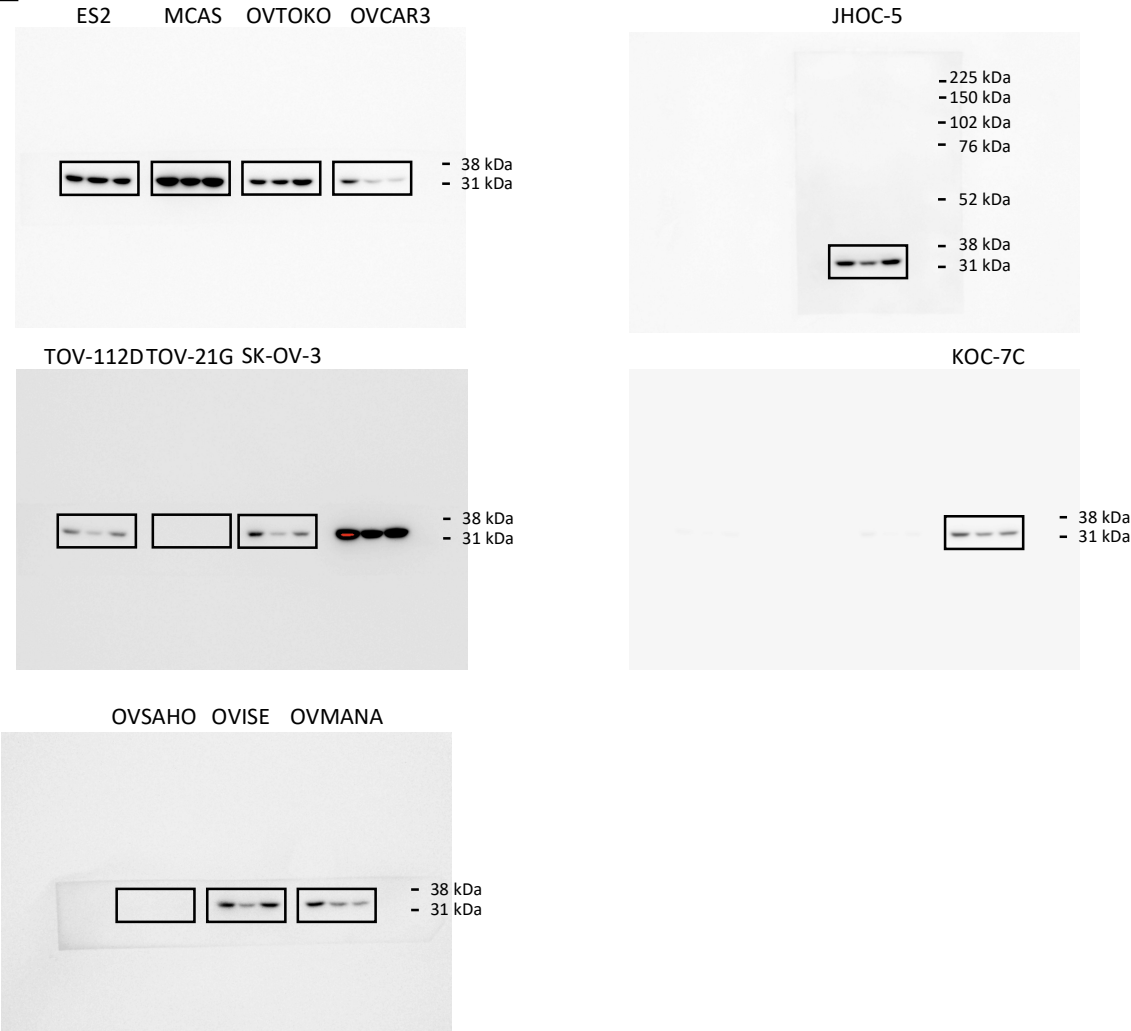


Uncropped blots for Figure 4B

Cyclin B1

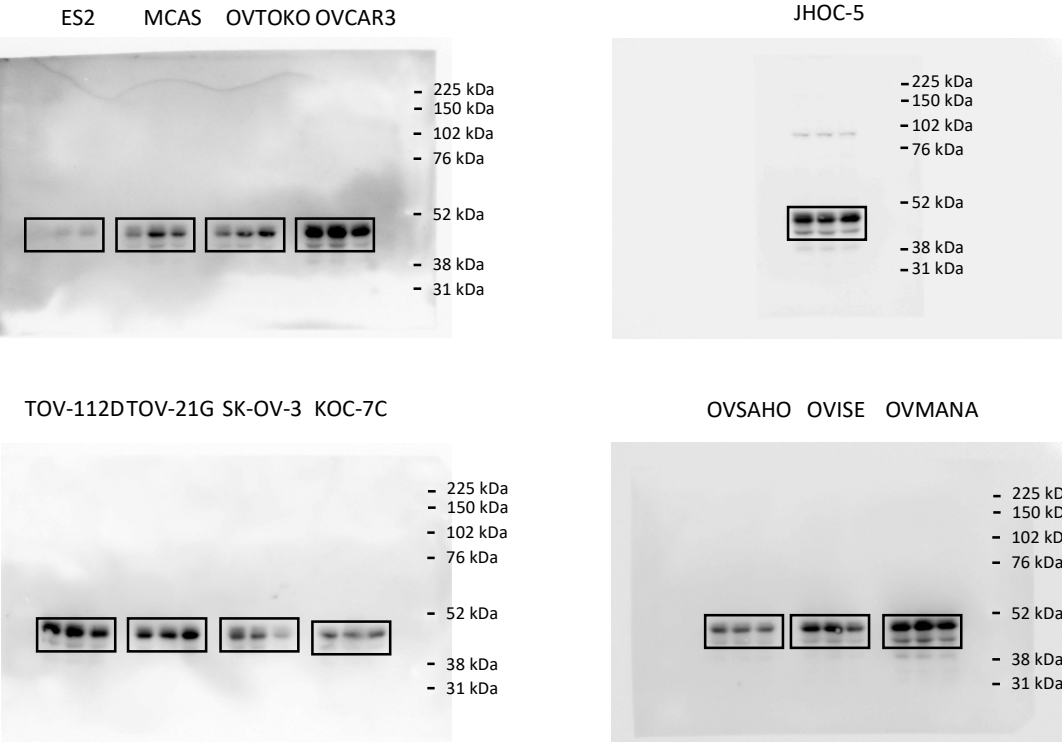


Cyclin D1

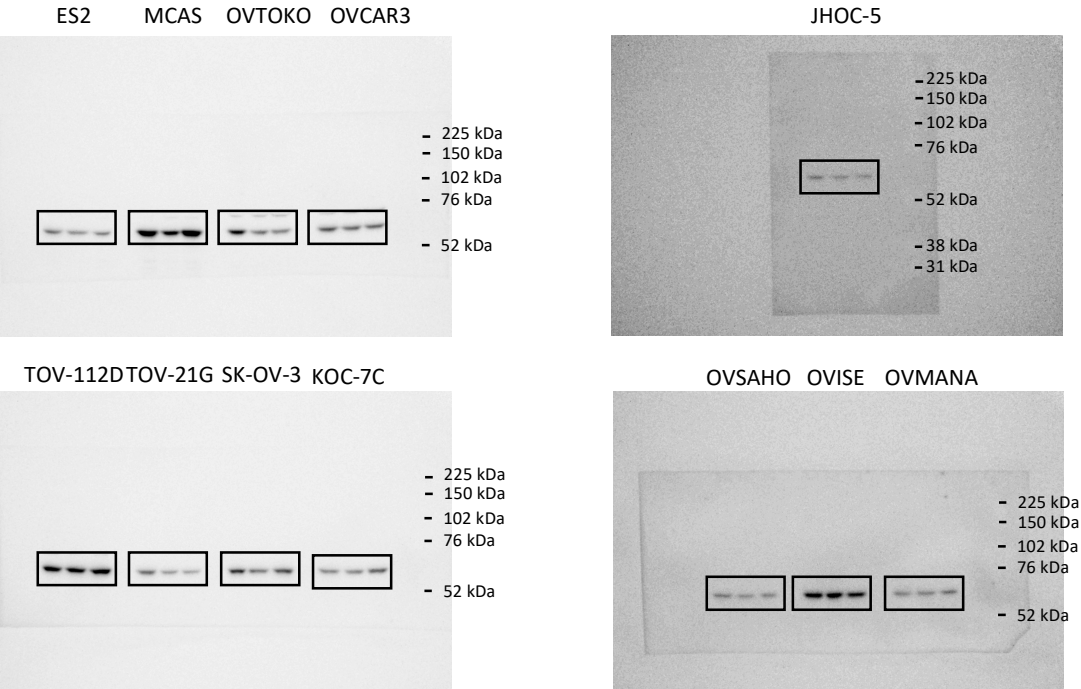


Uncropped blots for Figure 4B

Cyclin E1

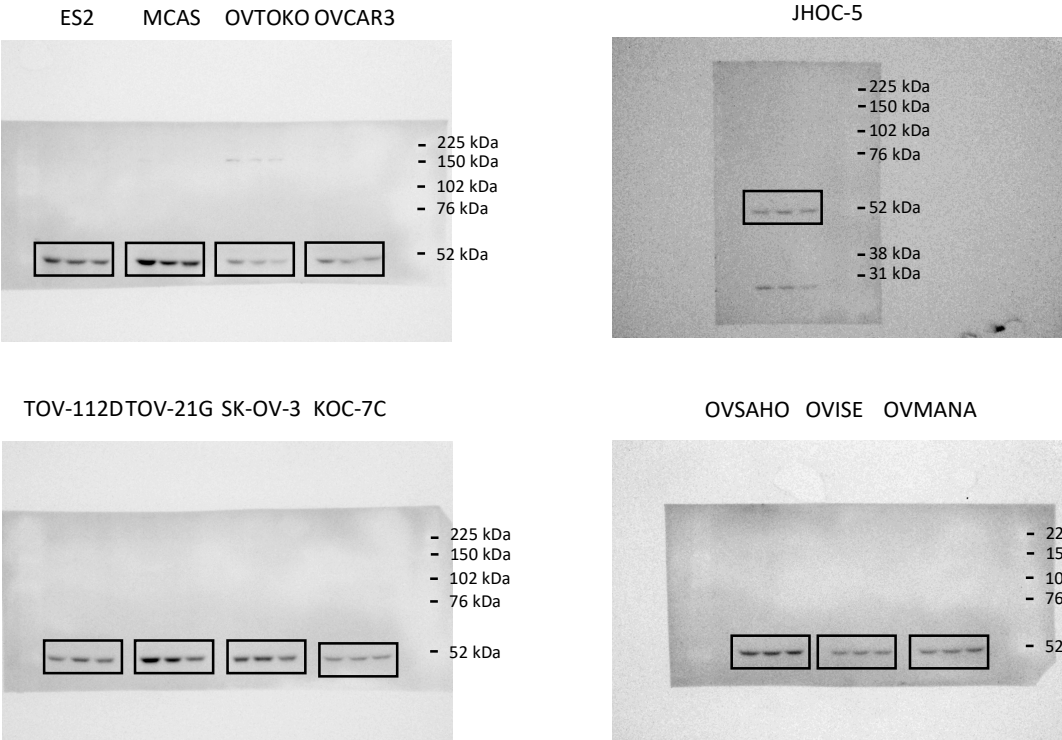


CDC6

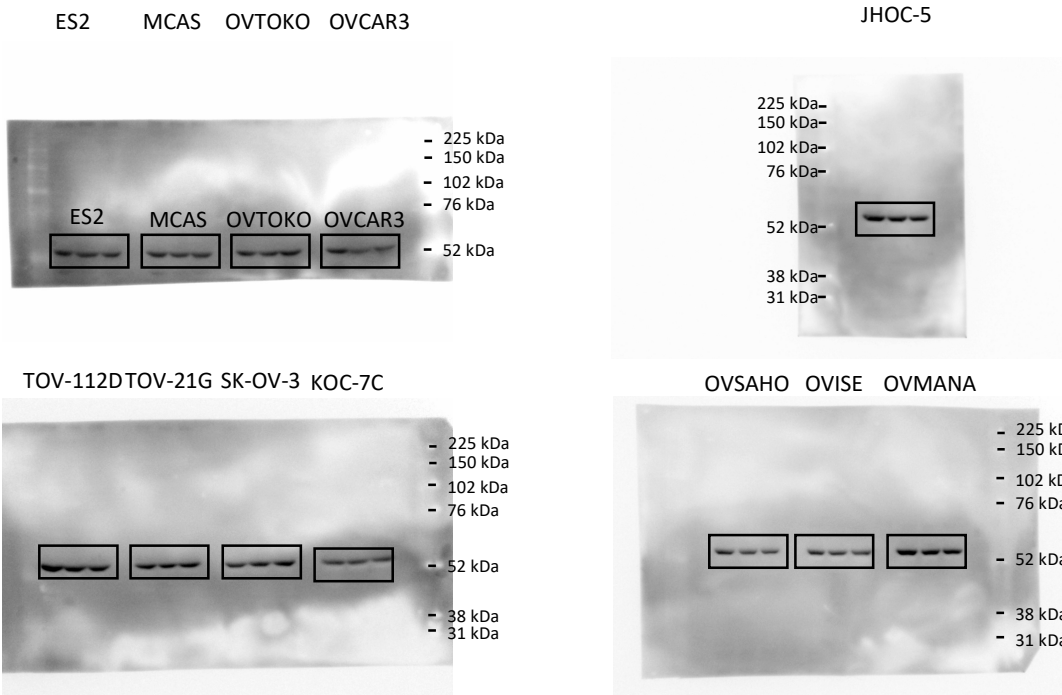


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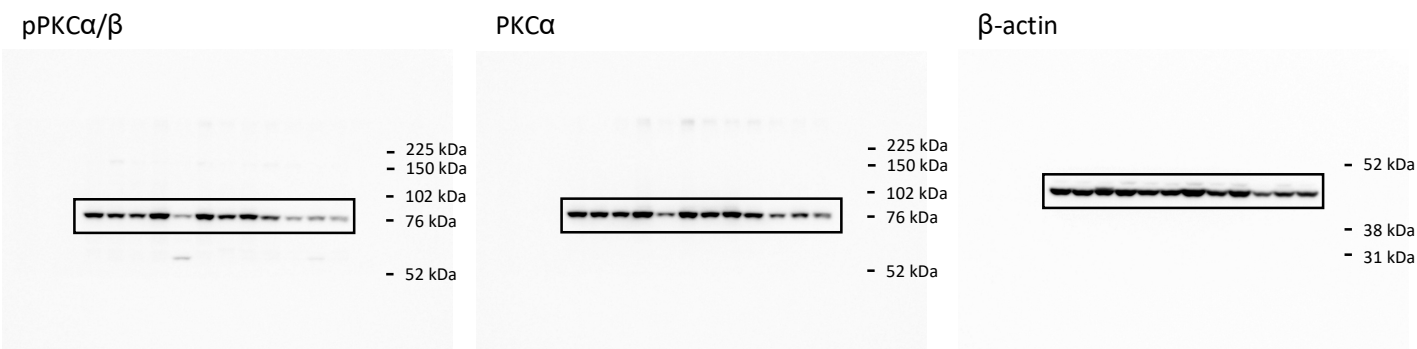
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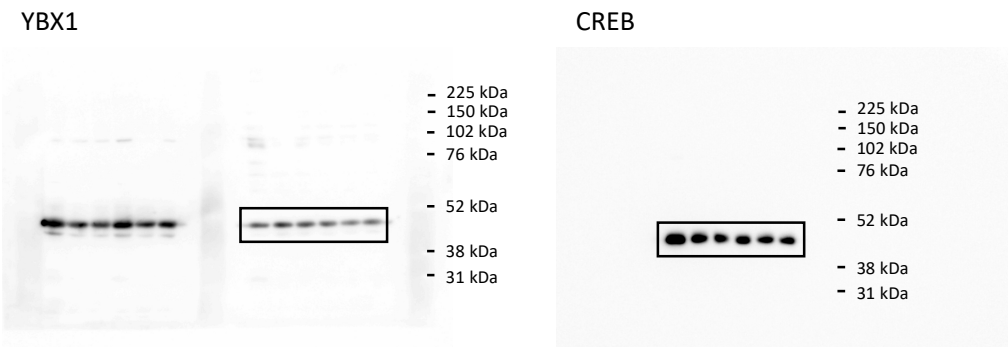
α -tubulin



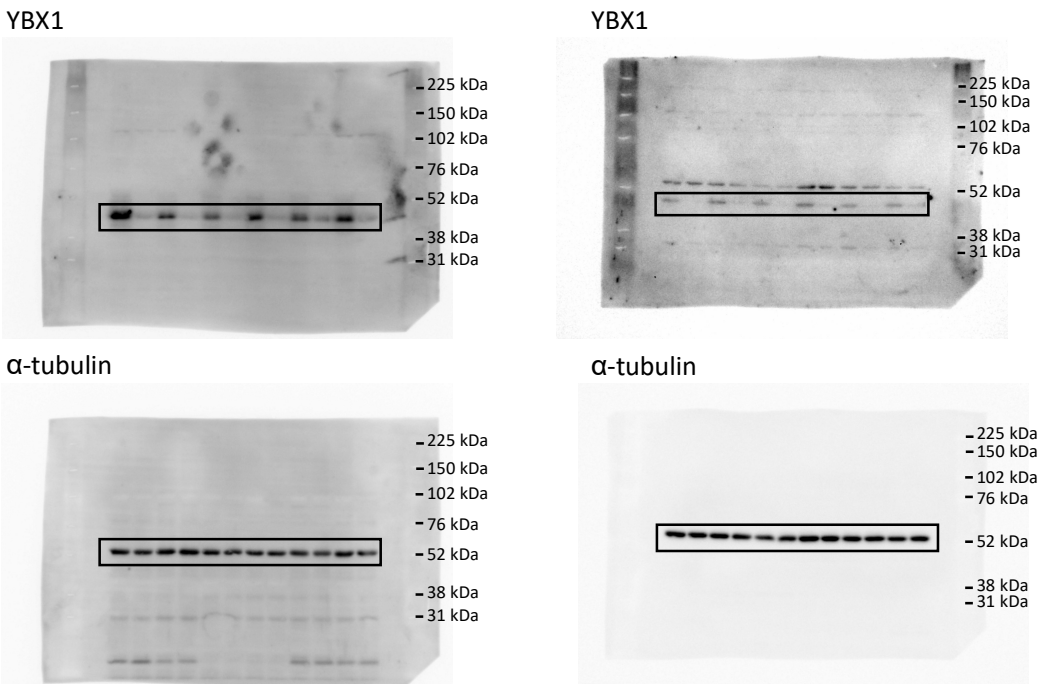
Uncropped blots for Supplementary Figure S3A



Uncropped blots for Supplementary Figure S3B

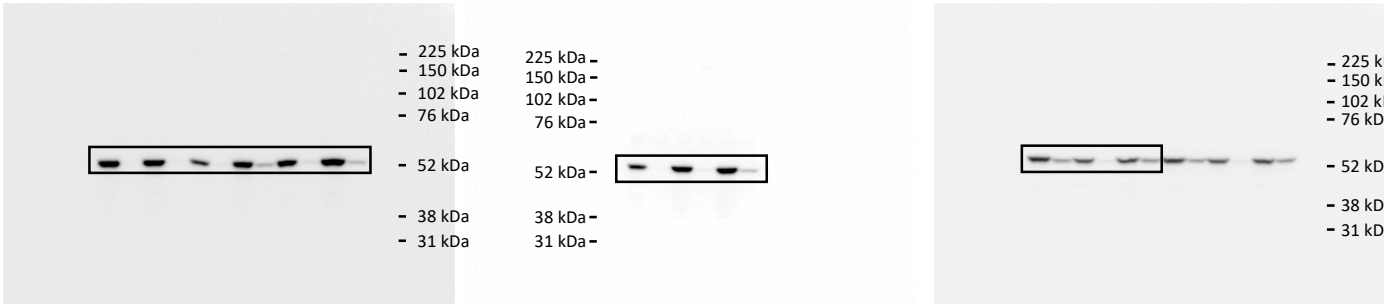


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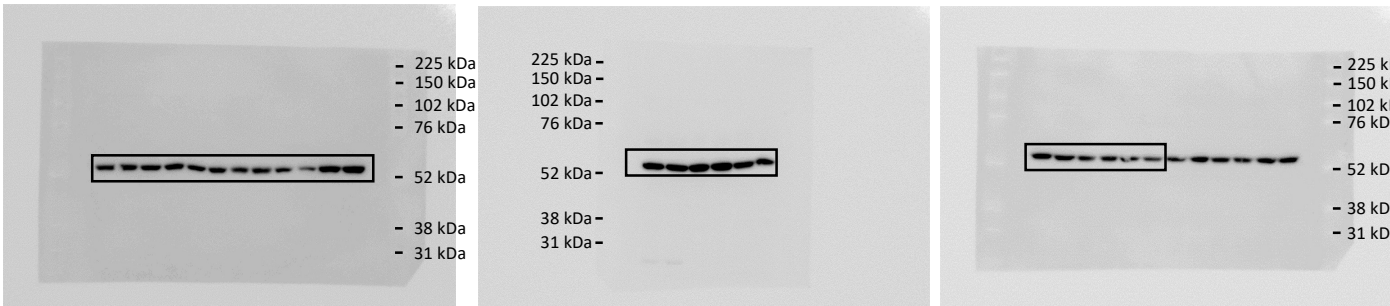


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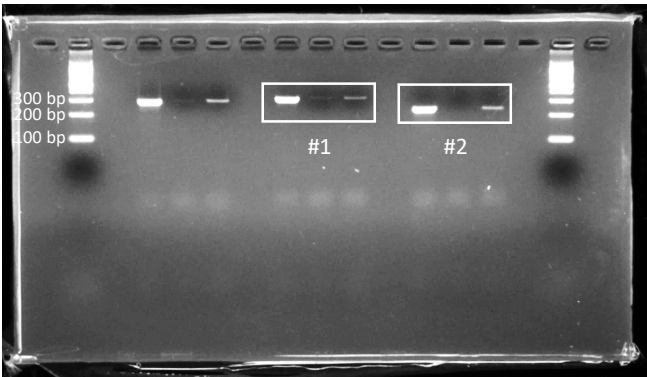
Cyclin A2



α -tubulin



Uncropped Images for Figure 3F



Uncropped Images for Supplementary Figure S8

