Correlated Gene	Spearman's Correlation	p-Value	q-Value
PLK1	0.48	5.48E-13	7.64E-10
BUB1	0.458170533	7.97E-12	6.59E-09
CCNB1	0.42152357	4.61E-10	1.92E-07
BRCA1	0.419	5.86E-10	2.32E-07
XRCC4	0.414	9.71E-10	3.48E-07
RAD51	0.412	1.21E-09	4.20E-07
MAD2L1	0.405	2.52E-09	7.42E-07
MCM10	0.402795921	3.07E-09	8.44E-07
PLK4	0.383	2.03E-08	4.19E-06
CHEK2	0.371	6.08E-08	9.87E-06
CDC25C	0.362924486	1.19E-07	1.8E-05
BUB1B	0.362484114	1.24E-07	1.84E-05
CDC20	0.352021575	2.98E-07	3.78E-05
EXO1	0.331058076	1.58E-06	0.00015
RAD54L	0.328	1.98E-06	1.75E-04
FANCD2	0.322	3.22E-06	2.56E-04
FC3	0.32	3.58E-06	2.79E-04
CDC6	0.313958918	5.66E-06	0.000405
CDK1	0.312513669	6.28E-06	0.000426
DBF4	0.311987587	6.52E-06	0.00044
BRCA2	0.303	1.21E-05	7.31E-04
RAD51C	0.3	1.49E-05	8.47E-04
DBF4B	0.297784838	1.76E-05	0.000951
XRCC2	0.288	3.49E-05	1.51E-03
ORC6	0.271471849	9.69E-05	0.003159
MCM8	0.268619772	0.000115	0.003498
DNA2	0.263830353	0.000154	0.004249
MCM5	0.260254175	0.000191	0.004921
RAD51AP1	0.249	3.70E-04	7.58E-03
MKRN3	0.229259993	0.001061	0.015427
BLM	0.22890498	0.001081	0.015639
RPA2	0.225	1.31E-03	0.0178
GEN1	0.215608098	0.002112	0.024086
RAD17	0.215	2.16E-03	0.0244
HUS1B	0.214	2.23E-03	0.025
RFC4	0.213	2.36E-03	0.0258
HORMAD1	0.213	2.36E-03	0.0259
CDC7	0.209995567	0.002771	0.028978
PCNA	0.204260381	0.003631	0.034803
ORC1	0.202096941	0.004014	0.037158
PALB2	0.196140092	0.005261	0.044112
RAD1	0.177	0.0121	0.0747
RPA3	0.167	0.0181	0.0962
RFC2	0.166	0.0184	0.097
MAD2L2	0.155	0.0278	0.126

Supplemental Table 1. HR and checkpoint pathway genes significantly co-expressed with CCNE1 mRNA expression in Serous ovarian cancer samples from TCGA ovarian cancer data.

Correlated Protein	Spearman's Correlation	p-Value	q-Value
CCNB1	3.65E-01	4.99E-11	1.03E-08
CHEK2	3.46E-01	5.96E-10	6.17E-08
CDK1	0.307588481	4.39551E-08	2.27468E-06
FOXM1	0.260350401	4.22006E-06	6.43555E-05
BCL2L11	0.260140297	4.29867E-06	6.43555E-05
TP53	0.251881957	8.76872E-06	0.000121008
PIK3CA	0.231992414	4.42445E-05	0.000538741
CHEK2_PT68	0.223011497	8.78818E-05	0.000909577
CDKN1B	0.218056706	0.000126845	0.001250331
RPS6KA1	0.207954638	0.000261356	0.002352207
mTOR	0.204344818	0.000335649	0.002894972
ESR1	0.198539625	0.000497455	0.004118928
RPS6	0.1754389	0.002139684	0.012552436
EEF2K	0.175101632	0.002183032	0.012552436
EIF4G1	0.158309912	0.00566925	0.024448642
GSK3A	0.153544794	0.007317504	0.029700457
GSK3B	0.153544794	0.007317504	0.029700457
BRAF	0.149252514	0.009155311	0.033841954
TSC1	0.142804483	0.012687921	0.044028364
BIRC2	0.133323794	0.020050907	0.065881553
BCL2L1	0.12752031	0.02619441	0.079738866
GAB2	0.117231494	0.041089203	0.103725184

Supplemental Table 2. Protein expression of the mTOR and PI3K signaling pathways that were significantly co-expressed with CCNE1 protein expression in Serous ovarian cancer specimens from TCGA ovarian cancer data. by comparison of CCNE1-amplified and/or overexpressed (CCNE1:AMP EXP Z score>2) with the rest of the sample using the TCGA ovarian dataset.

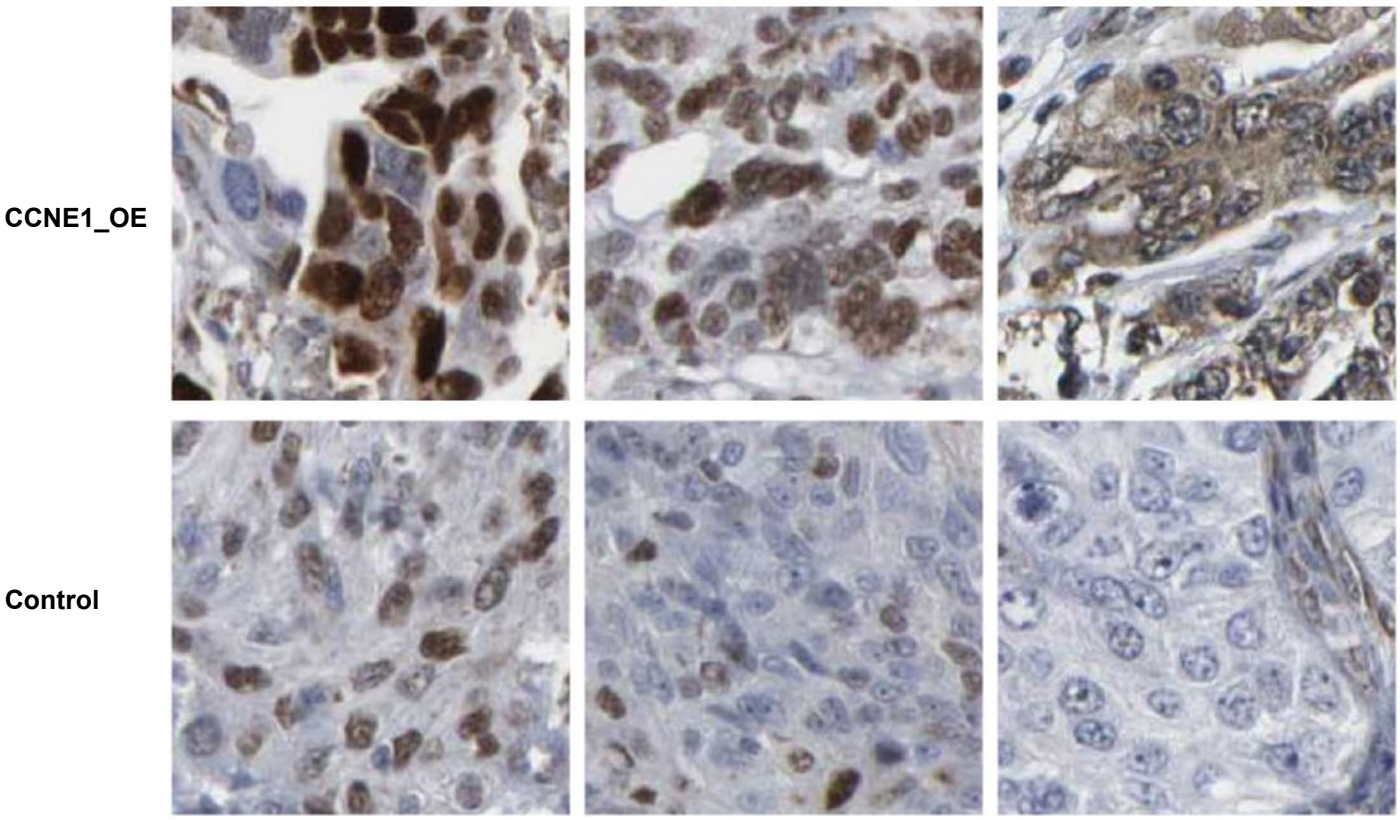
Primers	Sequences
HPRT	ATGCTGAGGATTTGGAAAGG
	CAGAGGGCTACAATGTGATGG
RAD51	GCTGGGAACTGCAACTCATCT
	TTCTTTGGCGCATAGGCAAC
ATR	ACATTTGTGACTGGAGTAGAAGA
	TCCACAATTGGTGACCTGGG
CHK2	ACTCTGCTGGCTGAGGCT
	GACTCCCGAGACATCACGAC
ATM	GTGGCCGCTCTCTACTGTC
	ATGTTCTAGTTGACGGCAGCA
CHK1	TGGAGAATTGCCATGGGACC
	TGGGAGACTCTGACACACCA
CCNE1	GCCAGCCTTGGGACAATAATG
	CTTGCACGTTGAGTTTGGGT
CCNE1 siRNA target	C6:ACCGGGTTTACCCAAACTCAA
	C8:CAAGATTTCTTTGACCGGTAT
RAD51 siRNA target	AAGGGAATTAGTGAAGCCAAA

Supplemental Table 3. Gene primers and siRNA sequences used in this study

Antibodies	Vendor	Catalog number	Dilution	
			Western Blotting	IF/IHC
CHK2	Cell Signaling	2197	1:1000	
pCHK2	Cell Signaling	2662	1:1000	1:200
ATR	Cell Signaling	2790	1:1000	
pATR	Cell Signaling	2853	1:1000	
RAD51	Cell Signaling	8875	1:1000	1:200
KU70	Cell Signaling	4588	1:1000	
KU80	Cell Signaling	2180	1:1000	
p-4EBP1	Cell Signaling	2855	1:1000	
p-p70S6 Kinase	Cell Signaling	9208	1:1000	
ALDH1A1	Gen Tex	GTA123973		1:200
GAPDH	Proteintech	60004	1:10000	
CCNE1	Sigma	HPA018169	1:2000	1:100-500
ABC-HRP Kit	Vector Laboratory	PK-4001		
a-tubulin	Sigma	T6199		1:2000
γH2AX	Millipore	05-636		1:200
Anti-rabbit IgG-594	Fisher	PIA32754		1:500
Anti-mouse IgG-488	Fisher	PIA32766TR		1:500

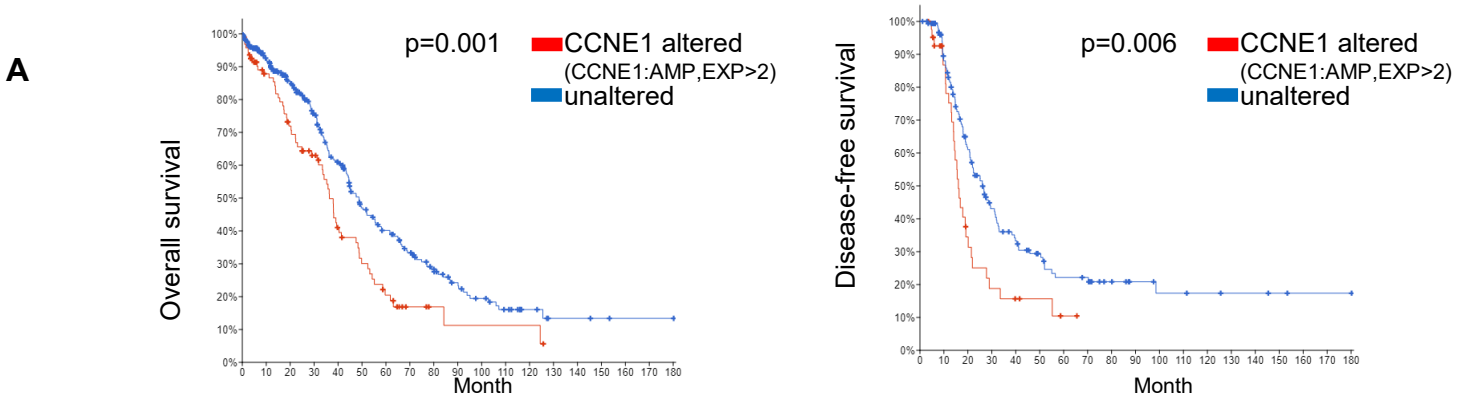
Supplemental Table 4. Antibodies used in this study.

Supplemental Figure 1



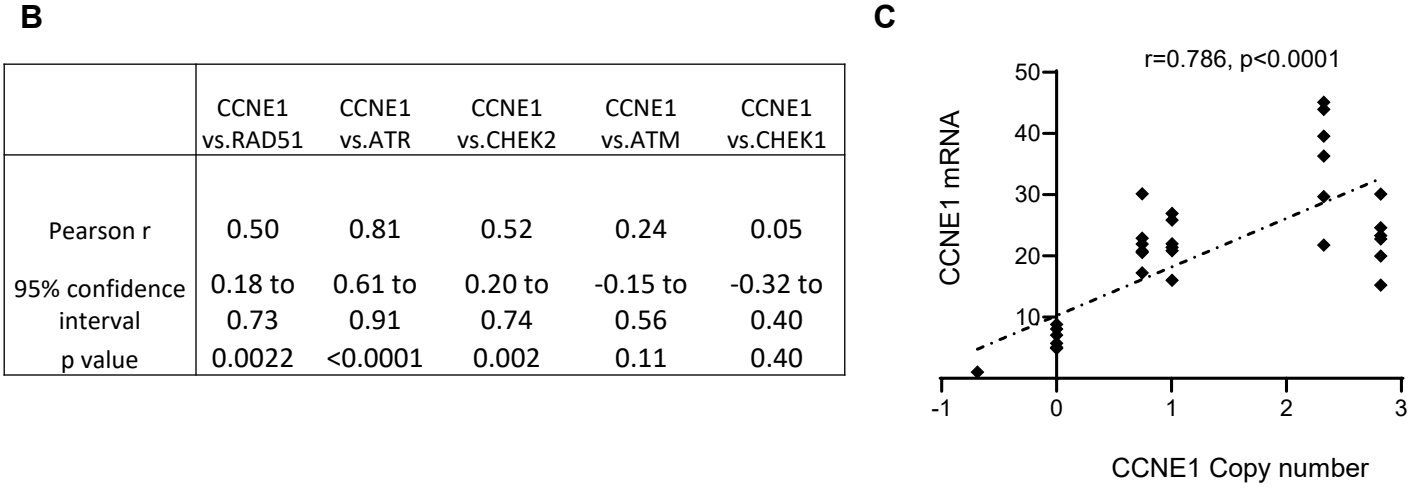
Supplemental Figure 1. CCNE1 overexpression (CCNE1_OE) is associated with increased PGCCs in ovarian cancer TMA. Ovarian cancer TMA and CCNE1 expressing data were obtained from protein atlas (https://www.proteinatlas.org/ENSG00000105173-CCNE1/pathology/ovarian+cancer#).

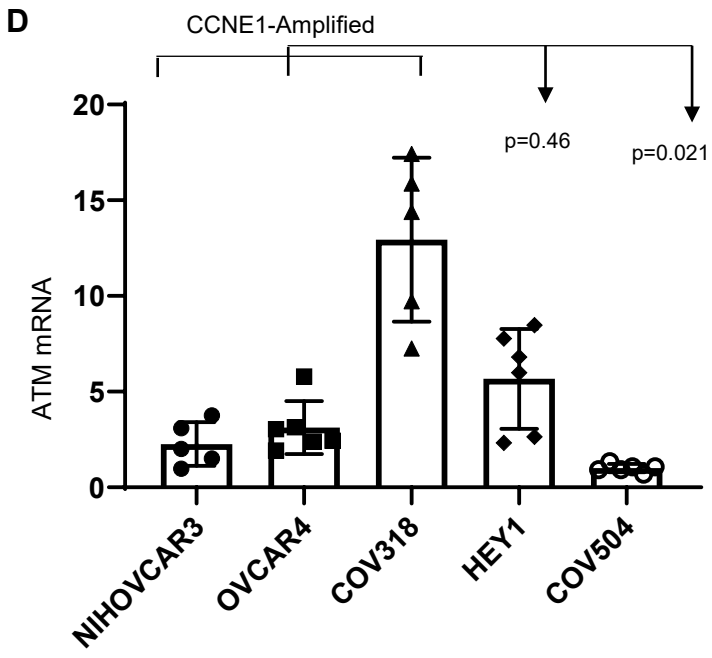
Supplemental Figure 2

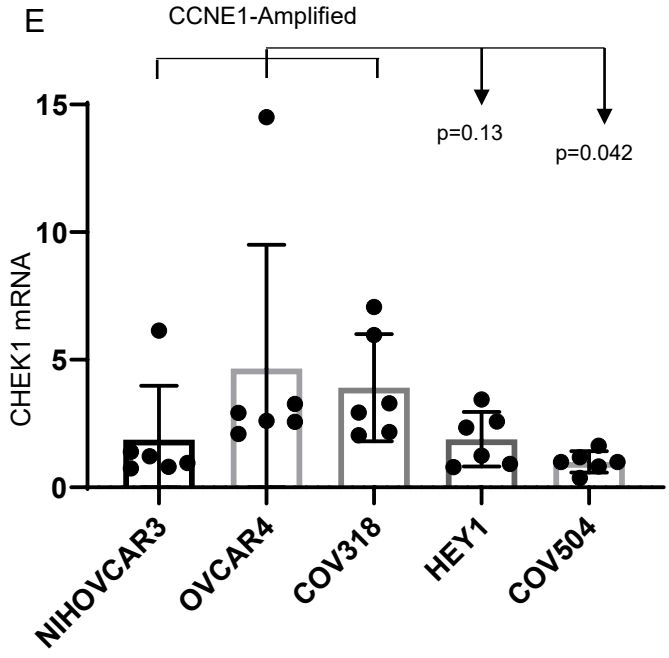


B

	CCNE1 vs.RAD51	CCNE1 vs.ATR	CCNE1 vs.CHEK2	CCNE1 vs.ATM	CCNE1 vs.CHEK1
Pearson r	0.50	0.81	0.52	0.24	0.05
95% confidence interval	0.18 to 0.73	0.61 to 0.91	0.20 to 0.74	-0.15 to 0.56	-0.32 to 0.40
p value	0.0022	<0.0001	0.002	0.11	0.40

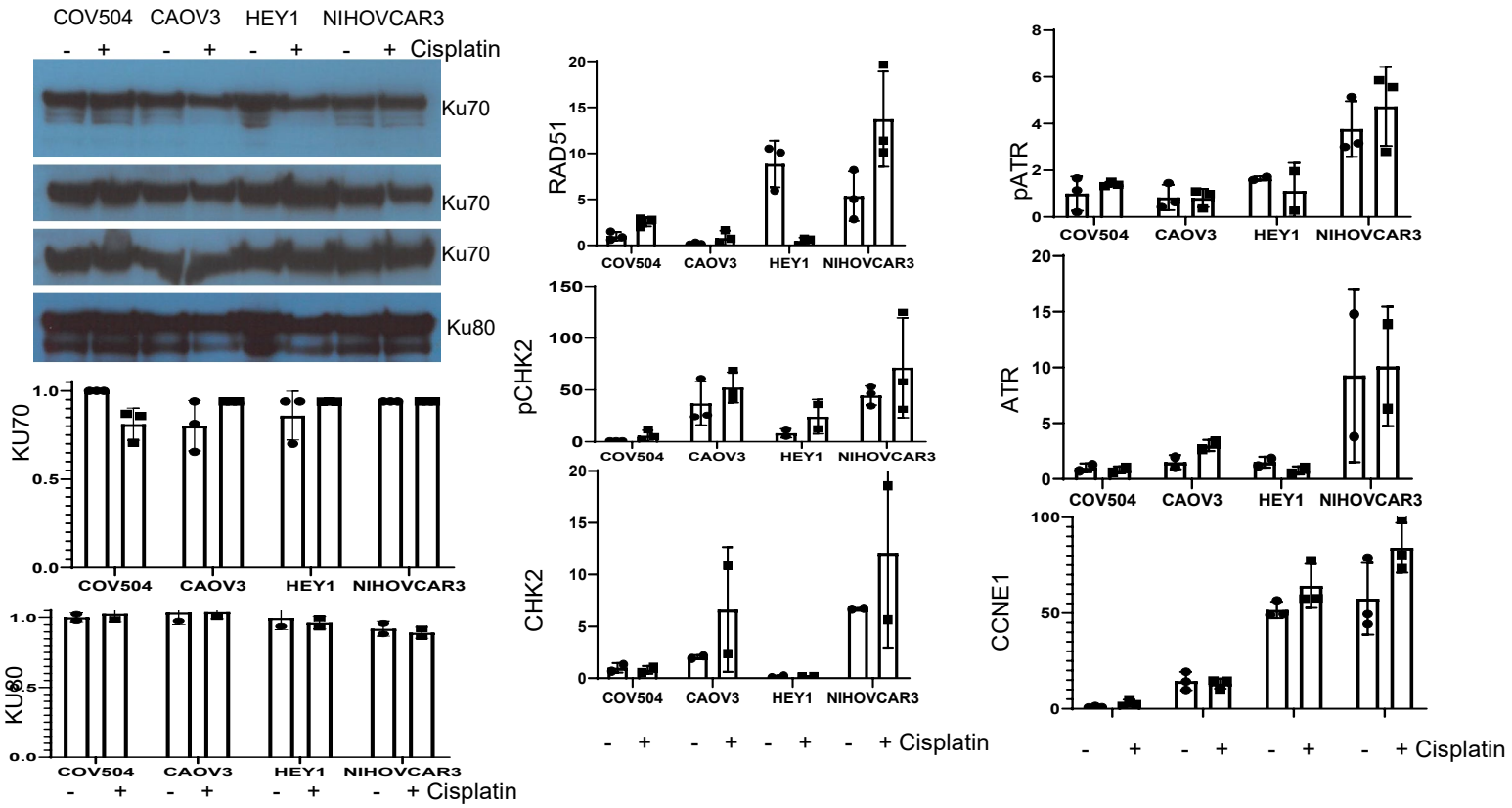






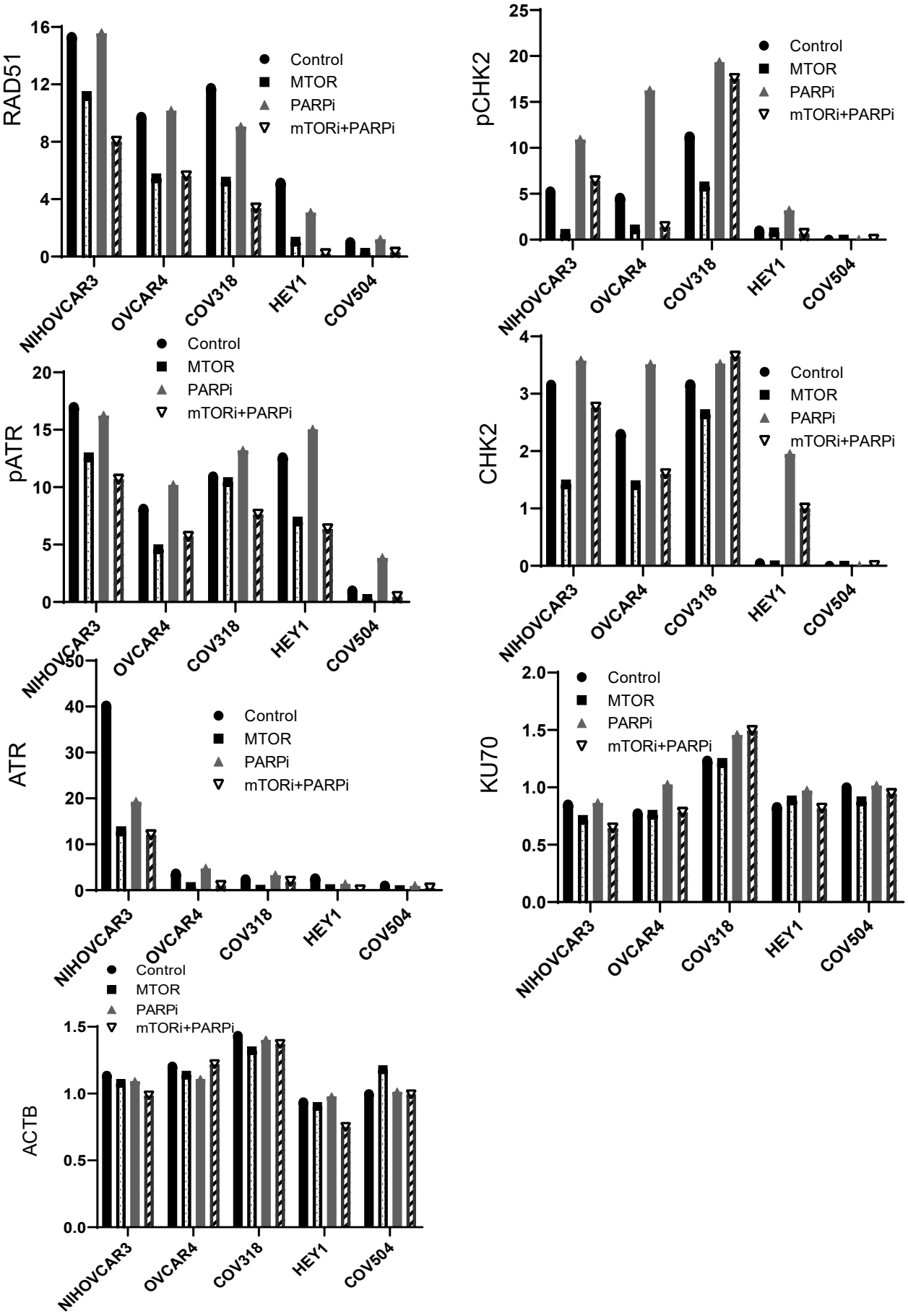
Supplemental Figure 2. CCNE1-amplified ovarian cancer correlates with poor survival and activation of key genes of the HR and checkpoint pathways. A. CCNE1-amplified ovarian cancer correlates with poor overall survival and disease-free survival. TCGA ovarian dataset were selected and analyzed in cbioportal (https://www.cbioportal.org/). B. Expression co-efficient analysis HR and checkpoint genes in ovarian cancer cell lines. C. mRNA level vs copy number of CCNE1 in CCNE1 amplified HGSOC lines and control lines. D-E. mRNA expression of ATM and CHK1 in CCNE1-amplified , WT and Hetdel cell lines.

Supplemental Figure 3



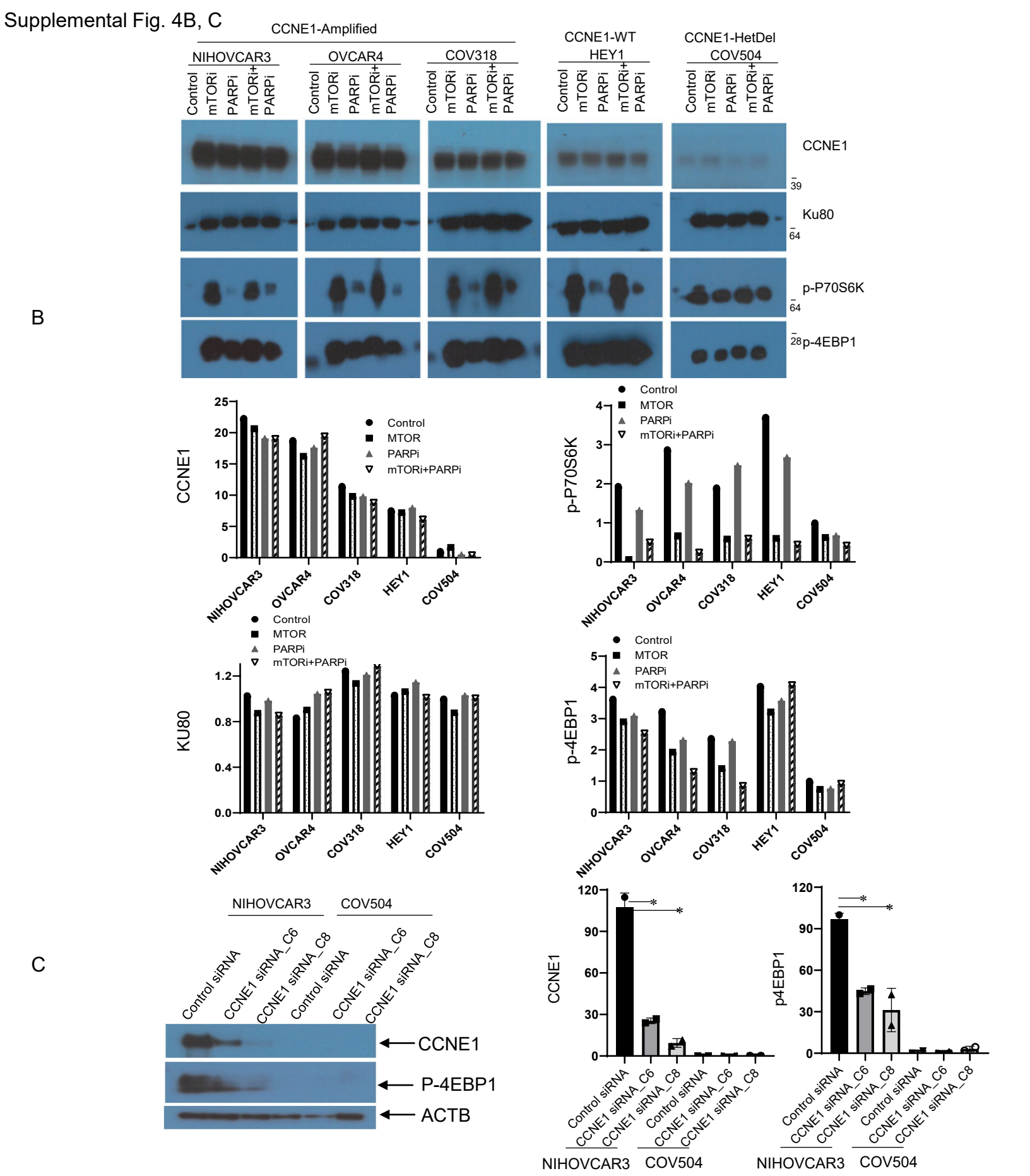
Supplemental Figure 3. Western blotting images and quantification of protein expression in cells in response to cisplatin. Ovarian cancer cells were treated with cisplatin (1μg/ml) for 2 days. The level of each protein in untreated COV504 was set as 1 for all proteins except that of CHK2 and p-CHK2 in untreated HEY1 was set as 1. KU70 western blot Images from three separate experiments are shown.

Supplemental Figure 4A



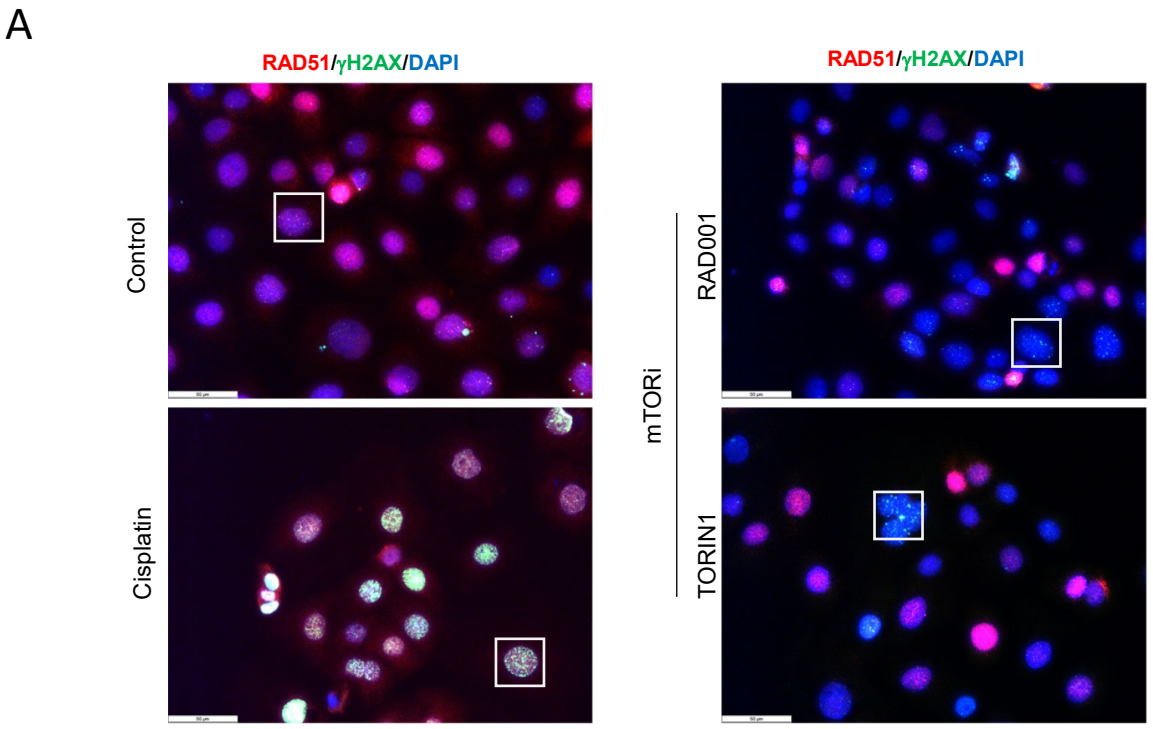
Supplemental Figure 4A. Quantification of protein expression in cells in response to mTORi and PARPi as demonstrated in Figure 2C . Ovarian cancer cells were treated with cisplatin mTORi(RAD001, 20nM), PARPi(Olaparib, 10μM), or in combination for 2 days. Experiments were repeated at least 2 times. The level of each protein in untreated COV504 was set as 1 for all proteins except that the level CHK2 or pCHK2 in untreated HEY1 was set as 1.

Supplemental Fig. 4B, C



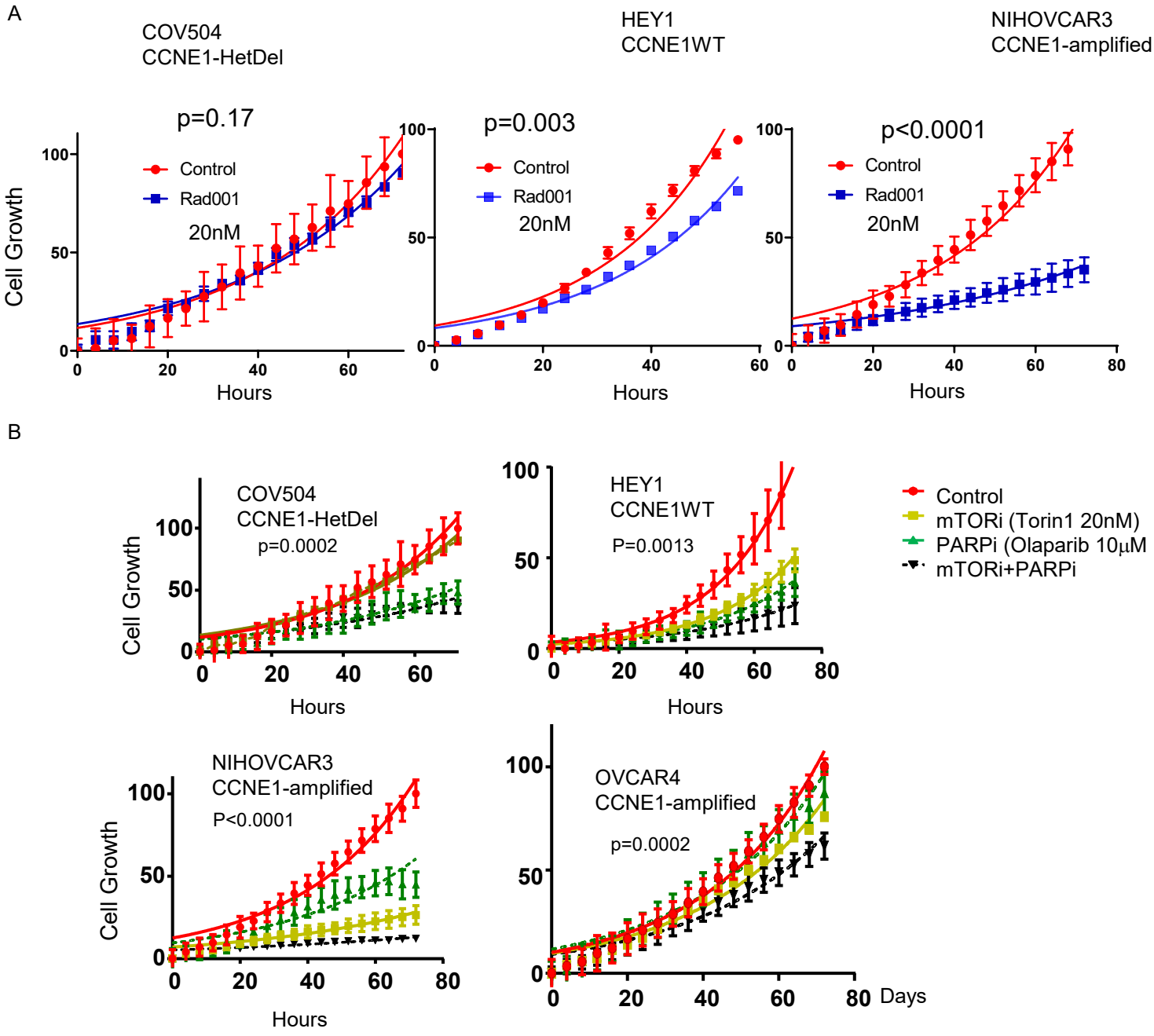
Supplemental Figure 4B. Representative Western blotting images and quantification of protein expression in cells in response to mTORi and PARPi . Ovarian cancer cells were treated with cisplatin mTORi(RAD001, 20nM), PARPi(Olaparib, 10μM), or in combination for 2 days. Experiments were repeated at least 2 times. The level of each protein in untreated COV504 was set as 1. **C.** Inhibition of CCNE1 expression on phosphorylated 4EBP1. Both CCNE1-amplified NIHOVCAR3 and Hetdel COV504 were transfected with two CCNE1 siRNA for 2 days. Cell lysates were collected and subjected to Western Blotting with CCNE1, p4EBP1 and ACTB. p value was calculated using Unpaired t-test.

Supplemental Figure 5



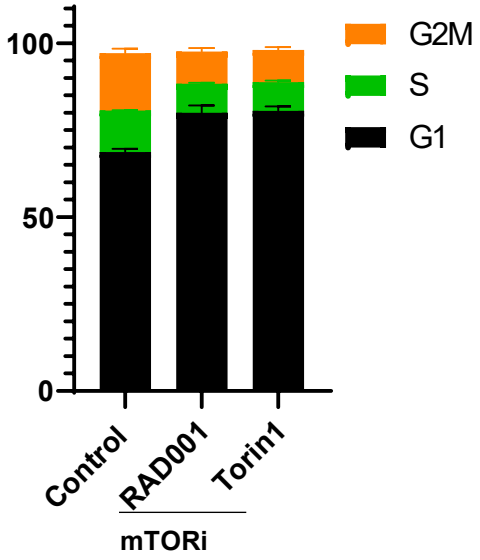
Supplemental Figure 5. A. Representative images of RAD51 and gH2AX staining in NIHOVCAR3 treated with mTORi RAD001 (10nM), or Torin1 (10nM) for 3 days. Cisplatin (300ng/ml) was used as positive control. White box indicates HPF images presented in Fig.2D. B. Quantification of RAD51 upon RAD51 knockdown. Cells were treated transfected with scrambled control or RAD51siRNA for 48 hours and Western blotting analysis was as described in Supplemental Methods.

Supplemental Figure 6



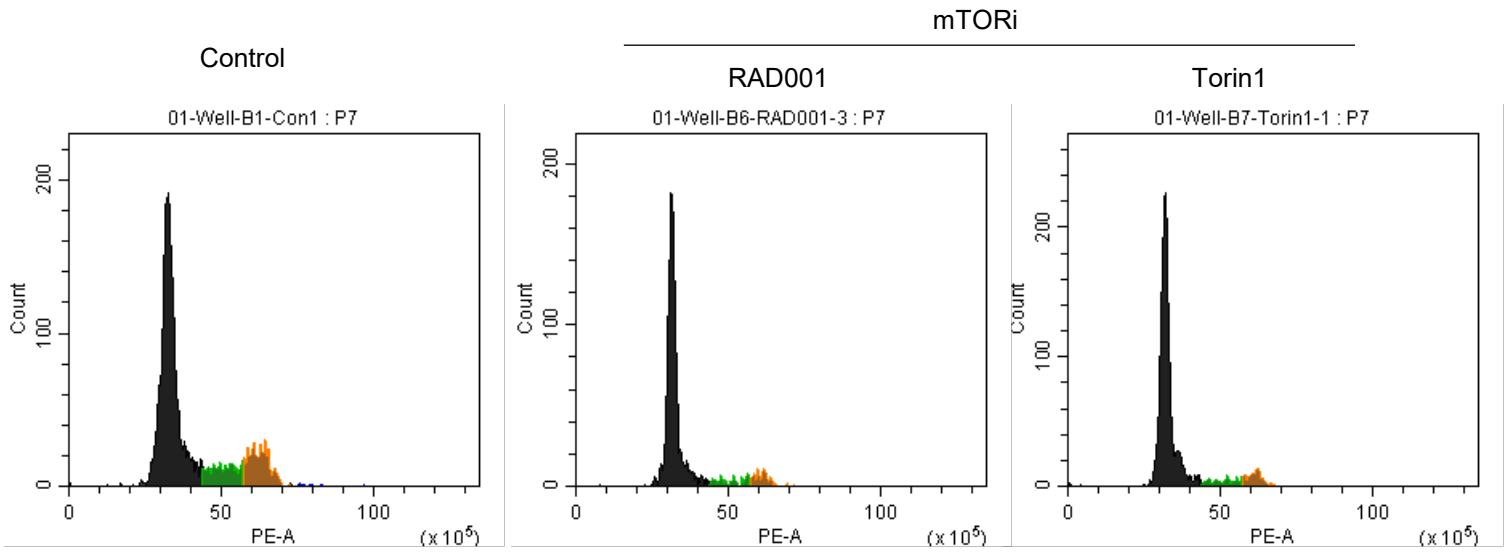
Supplemental Figure 6. CCNE1-amplified cells are preferentially responsive to mTOR inhibitors in an HR/checkpoint protein-dependent manner. **A.** Live cell growth of CCNE1-amplified NIHOVCAR3 and control lines (HEY1 and COV504) in response to mTORi (RAD001, 20nM) for up to 72 hours. Mean and standard deviation were calculated using four images each of three repeats, the experiments were repeated twice. **B.** CCNE1-amplified cells are relatively resistant to PARP inhibitors compared to CCNE1 WT and Hetdel lines. CCNE1-amplified NIHOVCAR3 and OVCAR4 and control lines (HEY1 and COV504) were treated with mTORi (Torin1, 20nM), PARPi(Olaparib, 10μM), or in combination for up to 72 hours.

Supplemental Figure 7



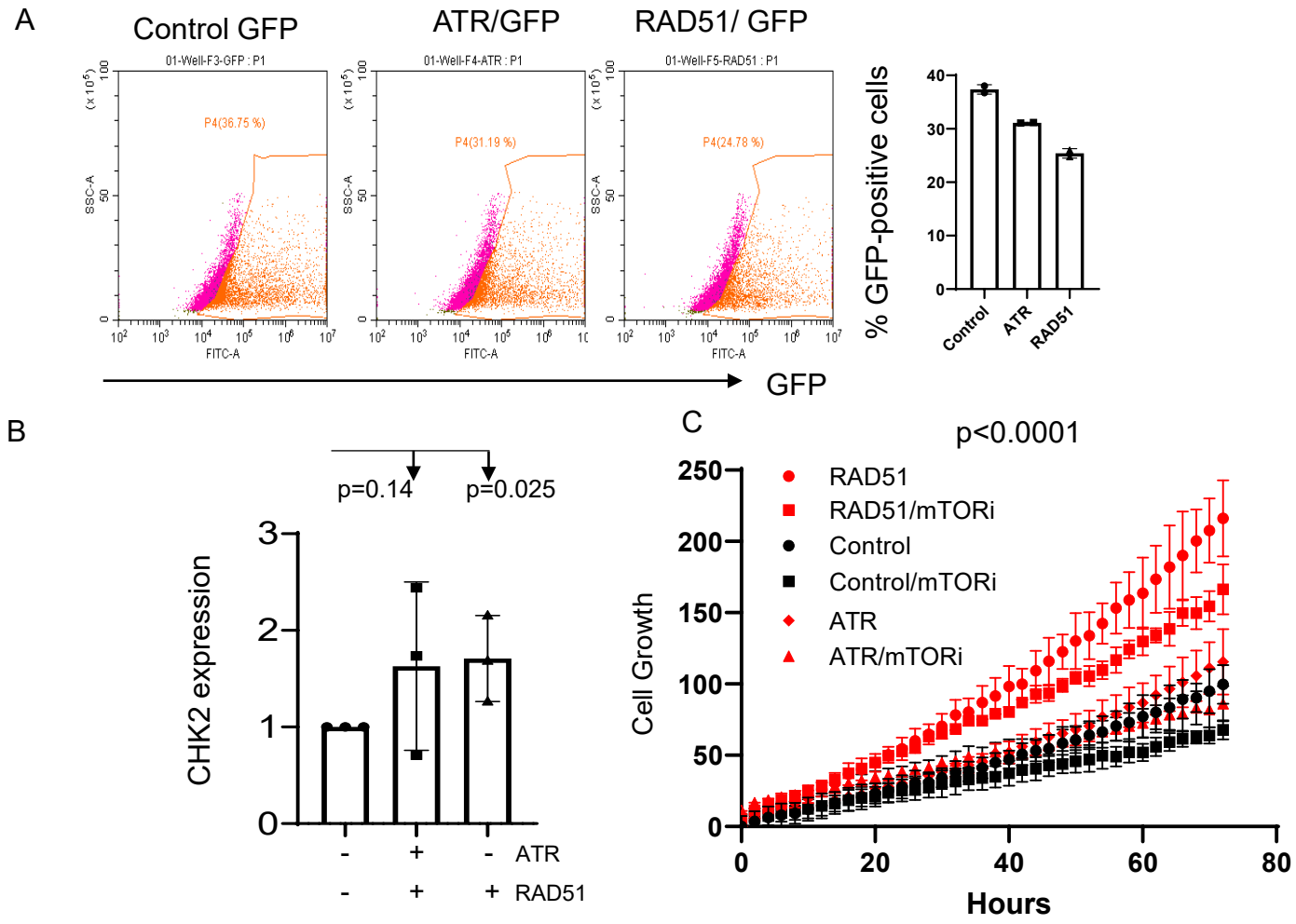
	mTORi		
	Control	RAD001	Torin1
G1	68.69	79.98	80.57
S	11.96	8.33	8.18
G2M	16.52	9.25	9.33

p Value	Con vs RAD001	Con vs TORIN1
G1	0.00112	0.00019
S	0.00003	0.00026
G2M	0.00147	0.00107



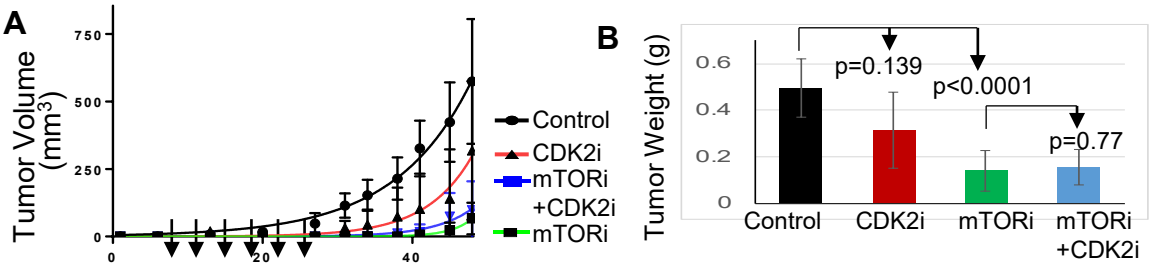
Supplemental Figure 7. Effect of mTORi inhibitors on cell cycle progression of CCNE1-amplified NIHOVCAR3 cells. Equally number of cells treated with either Torin1 (10nM) or RAD001 (RAD001) or control for 3 days were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton-X100 stained with propidium iodine(20µg/ml), followed by FACS analysis. Experiments were repeated 3 times. P value was calculated using t-test.

Supplemental Figure 8



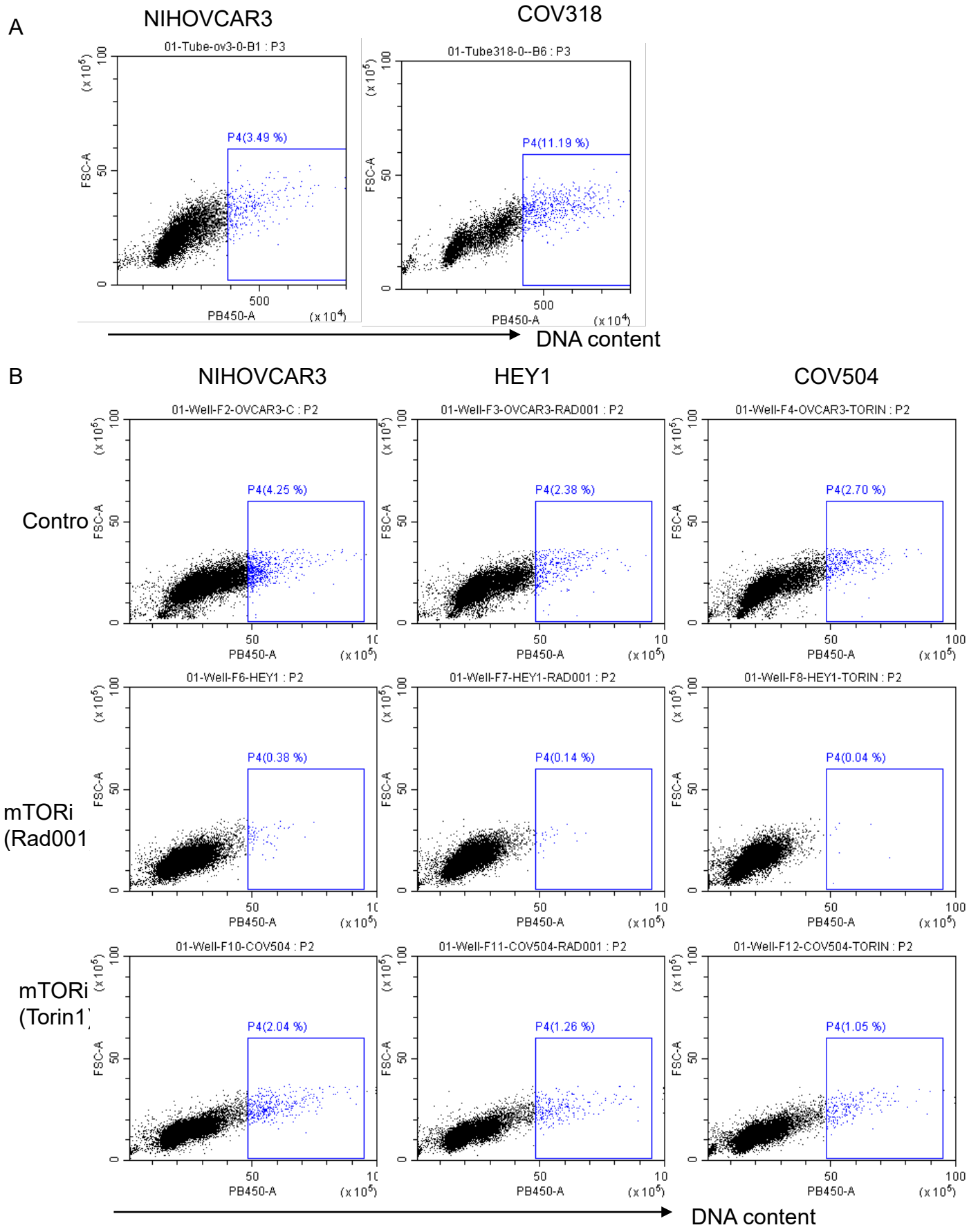
Supplemental Figure 8. Modulation of HR and checkpoint pathway proteins on mTOR inhibitor response in CCNE1-Amplified tumor cells. A. FACS analysis of NIHOVCAR3 cells transfected with GFP, or co-transfection of GFP with ATR, or RAD51. Densitometry of Western blot demonstrating fold changes of ATR and RAD51 expression compared to control. C. Live cell growth of CCNE1-amplified HGSOC lines overexpressing ATR or RAD51 compared to control in response to mTORi (10nM). Mean and standard deviation were calculated using four images each of three replicates.

Supplemental Figure 9

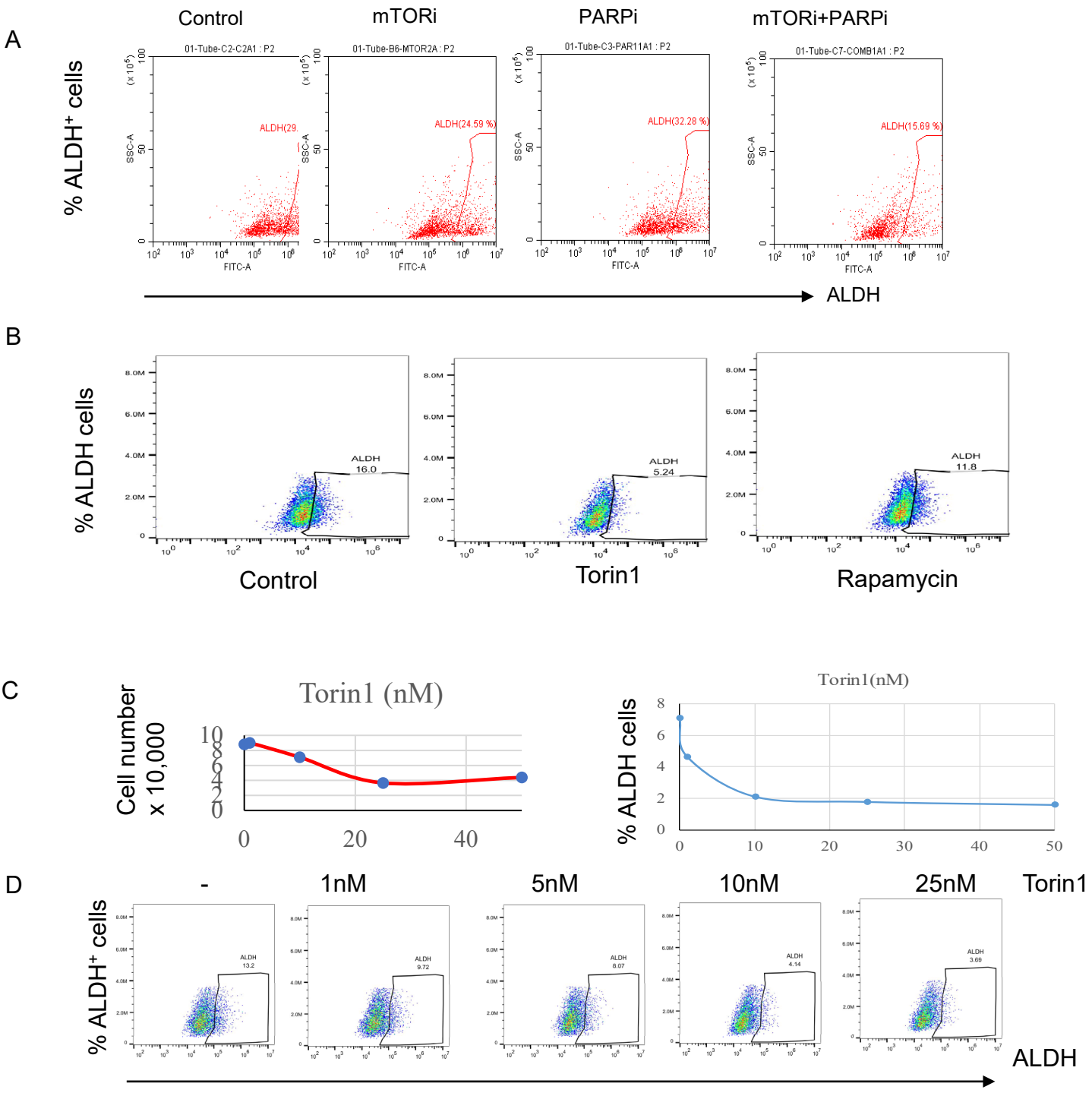


Supplemental Figure 9. Impact of mTOR or CDK2 inhibition on CCNE1-amplified ovarian tumor growth. mTOR inhibition (mTORi, Rad001, 10mg/kg, twice weekly) significantly impaired tumor volume (A) and tumor weight (B) whereas no effect of CDK2i (Dinaciclib, 20mg/kg, twice weekly) was observed when combined with mTORi.

Supplemental Figure 10



Supplemental Figure 10. Impact of mTORi on percentage of PCGGs. A. Percentage of PCGGs in CCNE1-amplified HGSOCs was measured by Hoechst staining (DNA content) followed by FACS analysis. B. Impact of mTOR inhibition on percentage of PCGGs in CCNE1 amplified NIHOVCAR3 and control lines. DNA content was measured by Hoechst staining . Cells were treated with two mTOR inhibitors Rad001(10nM) or Torin1 (10nM) for 3 days before collection for FACS analysis.



Supplemental Figure 11. mTOR inhibition preferentially killing of ALDH+ cell population in CCNE1-amplified NIHOVCAR3 cells. **A.** Representative FACS plots demonstrating percentage of ALDH+ CCNE1-amplified NIHOVCAR3 tumors of control, PARPi (Olaparib), mTORi (Rad001), and combined treatment as described in Fig. 6C. **B.** mTOR pathway targeting ALDH+ population on CCNE1-amplified NIHOVCAR3. Torin1 (10nM) or Rapamycin (50nM) was used for the treatment. Cells were treated 3 days before collection for cell count and ALDEFLUOR assay. **C.** mTOR inhibitor Torin1 dose dependently inhibits ALDH+ NIHOVCAR3 cells. **D.** Representative FACS plots demonstrating reduction of ALDH-positive cells using various concentration of Torin1 (1-25nM).