Supplemental Data

Supplemental Figure Legends

Supplemental Figure 1. Correlation between tumoral mRNA expression of ERCC1 and ATR or Chek1 in multiple TCGA data sets. Accessed from cBIOportal.org. Waterfall plot depicting the spearman correlation between ERCC1 and ATR or CHEK1 expression in the indicated tumor types.

Supplemental Figure 2. Effects of ATR inhibition on sensitivity to mitomycin C in H460 and H1299 ERCC1 knockout cell lines. Clonogenic survival of **A.** H1299 (p53 null) or **B.** H460 (p53 WT) ERCC1 knockout cells treated with mitomycin C or mitomycin C + M6620. **C.** Effect of concentration of M6620 utilized for sensitization studies on plating efficiency relative to untreated control. Data were compared by two-sided t-test. * $p<0.05$. **D.** Table depicting IC_{50} values estimated utilizing Sigma Plot software from experiments performed in A. and B. Data are presented as the average of three independent experiments ± S.D. **E.** Western bot of ERCC1 and loading control β-actin in lung PDX tumor tissue. **F.** Western blot of ERCC1 and β-actin in resected lung tumor tissue. **G.** Clonogenic survival assay of H1299 (p53 null) WT and ERCC1 knockout following siControl or siERCC1. Plots are utilizing cisplatin alone or in combination with M6620. Data were compared by two-sided t-test. * $p<0.05$. **H.** Western blot of ERCC1 in siControl, siERCC1 and ERCC1 knockout cells.

Supplemental Figure 3. Effects of ATR inhibition or siATR on sensitivity to cisplatin in H1650 WT and ERCC1 knockout cells and H1299 WT and ERCC1 knockout cells. A. Clonogenic survival assay of H1650 cells treated with cisplatin alone or in combination with M6620. Data are presented as the average of three independent experiments ± S.D. **B.** Clonogenic survival assay of H1299 cells treated with cisplatin alone or in combination with a second ATR inhibitor, VX-803. Data are presented as the average of three independent experiments ± S.D. **C.** Clonogenic survival assay of H1299 WT or ERCC1 knockout cells treated with cisplatin ± siATR. Data are presented as the average of three independent experiments ± S.D. **D.** Western blot of ATR in control and siATR knockdown cells. This is a representative blot of ATR knockdown in these studies. Statistical analysis of clonogenic assays were performed by comparing IC50 values using two-sided t-test; **, $p<0.01$; ****, $p<0.0001$.

Supplemental Figure 4. Lack of sensitization of H1299 ERCC1 knockout cells to cisplatin by ATM, PARP, Chk1, or Wee1 kinase inhibition. Clonogenic survival of H1299 ERCC1 knockout cells to cisplatin in combination with **A.** the ATM inhibitor KU-55933, **C.** the PARP inhibitor, BMN-673, **E.** the Chk1 inhibitor, CHIR-124, and **G.** the Wee1 kinase inhibitor, MK-1775. All results are presented as the average of three independent experiments ± S.D. Statistical comparisons of IC50 values performed with two-sided t-test; n.s., not significant, **, $p<0.01$. **B, D, F, H.** Concentrations of each drug utilized for sensitization studies and the impact of each inhibitor on plating efficiency relative to untreated control. n=3 ± S.D. ** $p<0.01$.

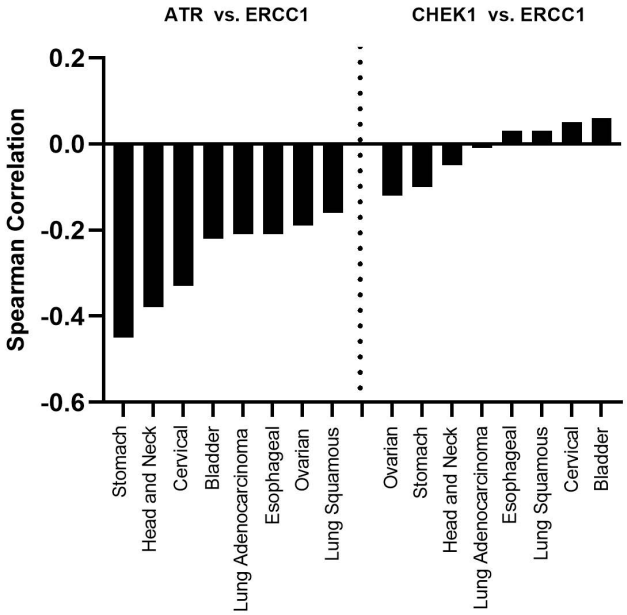
Supplemental Figure 5. ATR inhibition promotes premature entry into mitosis following platinum treatment. Cell cycle profiles over time after thymidine block in **A,** H1299 wildtype and **B,** H1299 ERCC1 knockout cells treated with cisplatin, ATR inhibitor, or combination. One experiment is presented. All cell cycle experiments were performed twice. **C.** Quantification of chromosome pulverization in H1299 wildtype and ERCC1 knockout cells following treatment with VX-803. **D.** Quantification of chromosome pulverization in H1650 wildtype and ERCC1 knockout cells following treatment with M6620. **E.** Quantification of chromosome pulverization in H1299 wildtype and ERCC1 knockout cells following siControl, siATR and drug treatments as depicted.

All metaphase spread experiment data are presented as the average of three independent experiments ± S.D. Data were compared by two-sided t-test; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

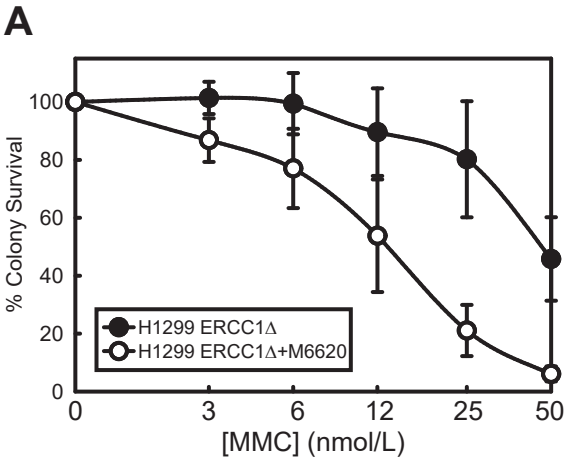
Supplemental Figure 6. Detection of micronuclei following treatment in H1299 wildtype and ERCC1 knockout cell lines. A, Quantification of the total percent of micronucleated cells ~48 hours after treatment. Data presented as the average of three independent experiments ± S.D. **B,** Quantification of the number of cells positive for >2 micronuclei ~48 hours after treatment. Data presented as the average of three independent experiments ± S.D. **C,** Immunofluorescence depicting colocalization of micronuclei with γH2AX in H1299 ERCC1 knockout cells following combination treatment. Data are representative from two independent experiments. **D,** Detection of γH2AX and cGAS colocalization with micronuclei in H1299 ERCC1 knockout cells treated with cisplatin and M6620. Data are representative from two independent experiments.

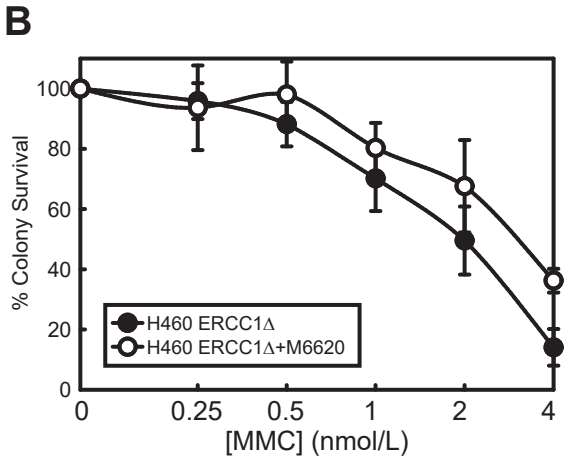
Supplemental Figure 1

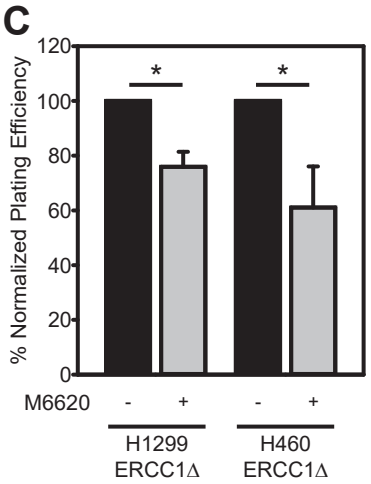
Cancer Type	*n*	ATR vs ERCC1 mRNA		Chek1 vs ERCC1 mRNA	
		Spearman	*p*	Spearman	*p*
Head and Neck Squamous Cell Carcinoma	520	-0.38	**1.39e-19**	-0.05	0.244
Bladder Urothelial Carcinoma	408	-0.22	**7.718e-6**	0.06	0.250
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	308	-0.33	**5.610e-9**	0.05	0.387
Esophageal Carcinoma	184	-0.21	**5.872e-3**	0.03	0.684
Lung Adenocarcinoma	515	-0.21	**9.910e-7**	-0.01	0.758
Lung Squamous Cell Carcinoma	501	-0.16	**4.894e-4**	0.03	0.456
Ovarian Serous Cystadenocarcinoma	304	-0.19	**6.635e-4**	-0.12	**0.0415**
Stomach Adenocarcinoma	415	-0.45	**9.940e-22**	-0.10	**0.0350**



Supplemental Figure 2

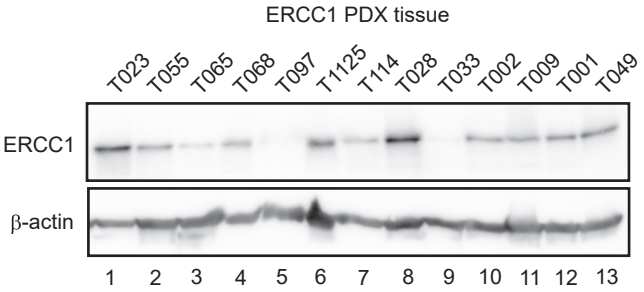


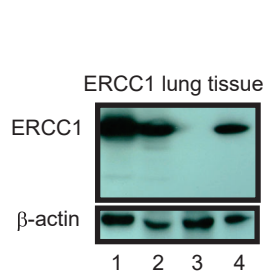


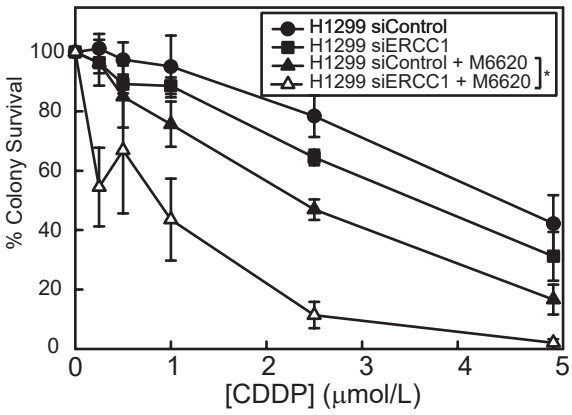


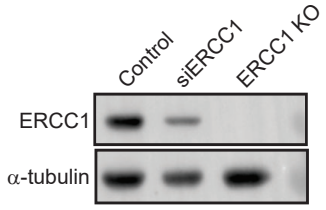
D

	Treatment	MMC IC_{50} Range
H1299 ERCC1Δ	MMC	31.6 - >50 nmol/L
	MMC+M6620	8.2 - 15.9 nmol/L
H460 ERCC1Δ	MMC	1.4 - 2.6 nmol/L
	MMC+M6620	2.4 - 3.6 nmol/L

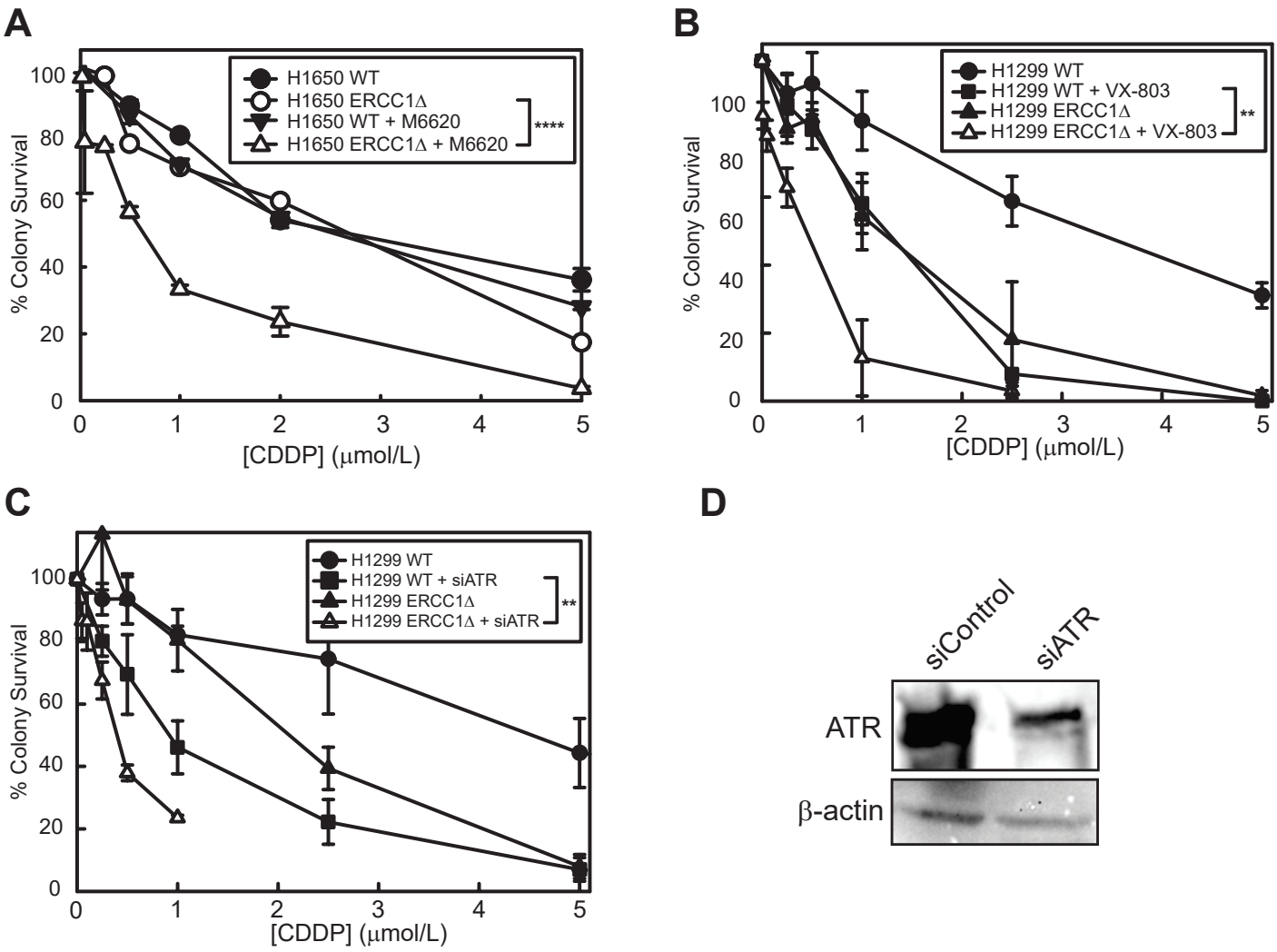




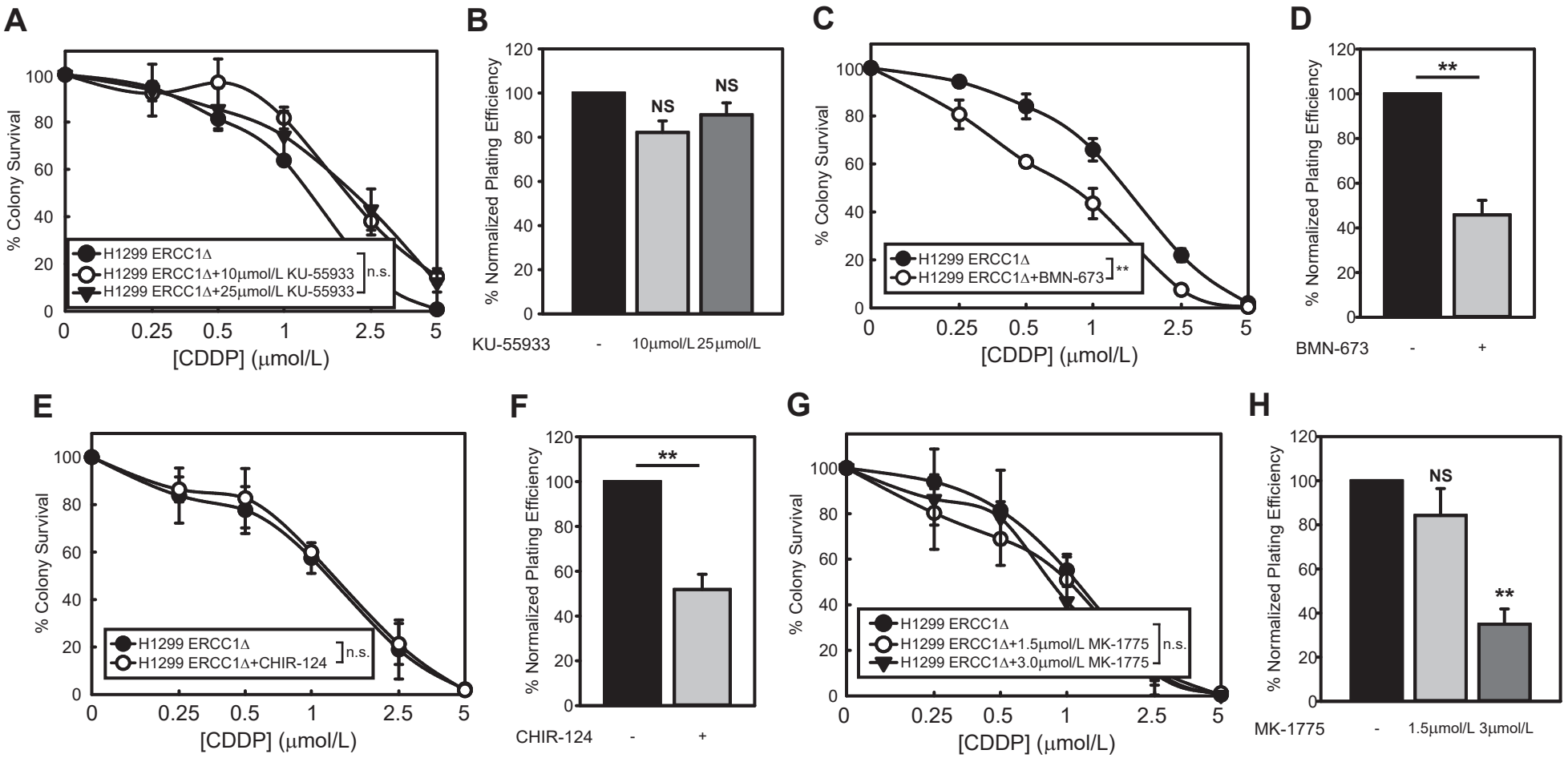




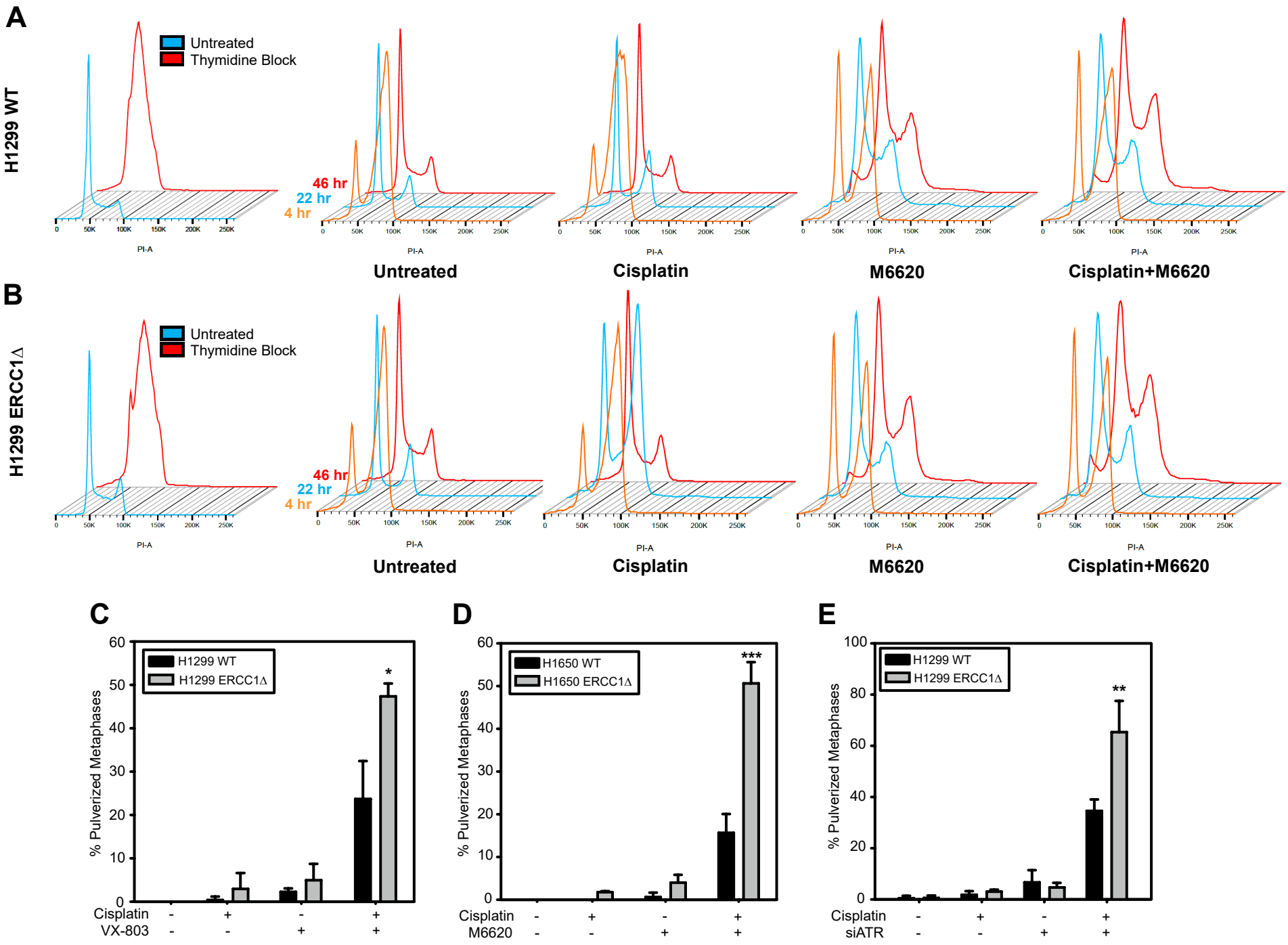
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6