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Supplemental Information

The Negative Cross-Talk between SAG/RBX2/ROC2 and APC/C E3 Ligases in Regulation of Cell Cycle Progression and Drug Resistance

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SUPPLEMENTAL INFORMATION

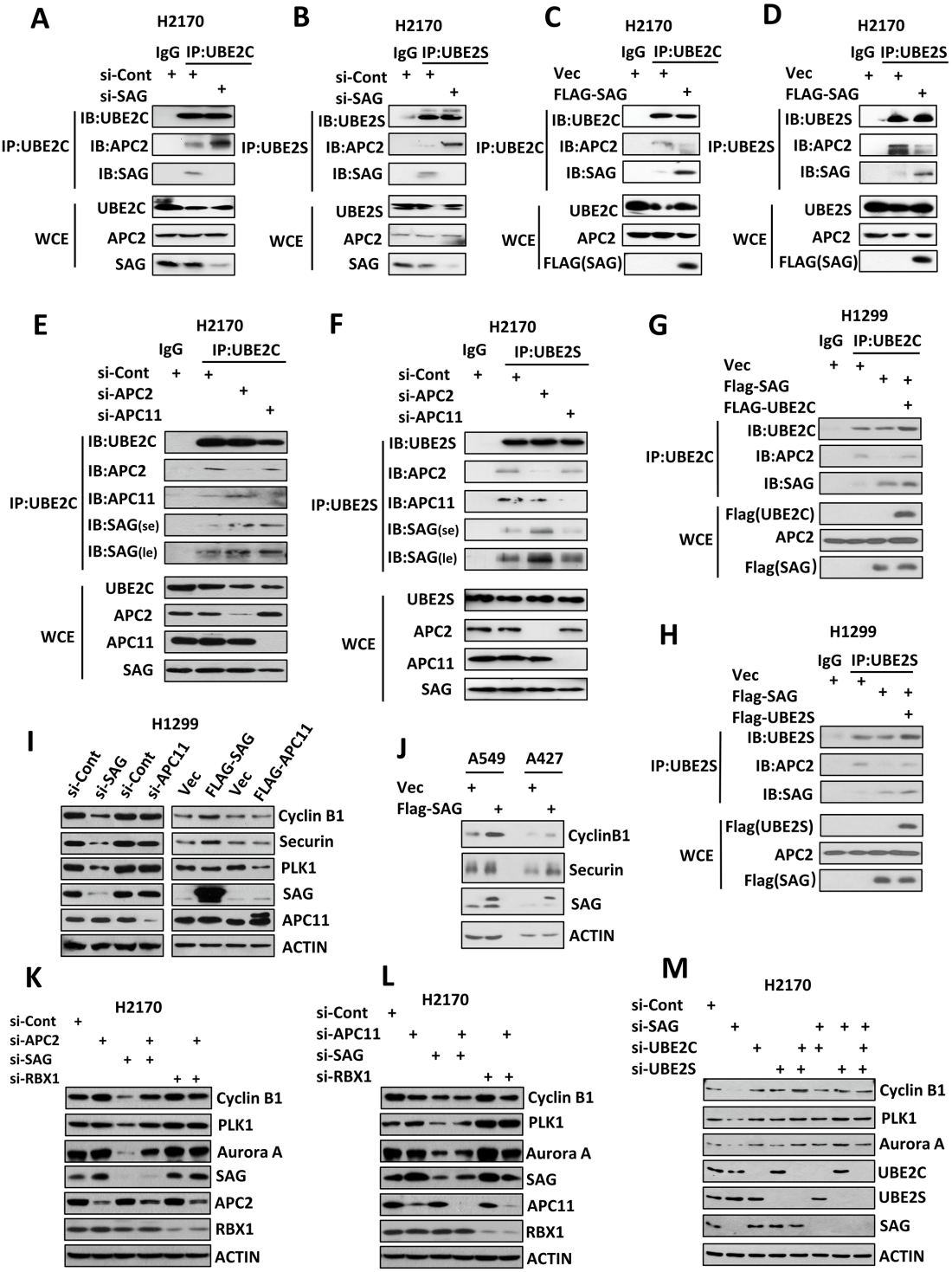


Figure S1: SAG competes with APC/C for binding with UBE2C or UBE2S to affect APC/C activity, related to Figure 1.

(A-D) H2170 cells were transfected with siRNA targeting *SAG* or scrambled control siCont (A&B) or a plasmid expressing FLAG-tagged SAG or vector control (C&D) for 48 hr with last 8 hr treatment of 10 μM MG132 before harvesting. Cell lysates were prepared for immunoprecipitation with IgG control or antibodies against UBE2C (A&C) or anti-UBE2S (B&D), followed by immunoblot (IB) with indicated Abs. WCE: whole-cell extract.

(E&F) H2170 cells were transfected with siRNA targeting *APC2* or *APC11* or scrambled control siCont for 48 hr with last 8 hr treatment of 10 μM MG132 before harvesting. Cell lysates were prepared for immunoprecipitation with IgG control or antibodies against UBE2C (E) or anti-UBE2S (F), followed by IB with indicated Abs. WCE: whole-cell extract.

(G&H) H1299 cells were transfected with plasmids expressing FLAG-tagged SAG or UBE2C/S, or vector control for 48 hr with last 8 hr treatment of 10 μM MG132 before harvesting. Cell lysates were prepared for immunoprecipitation with IgG control or antibodies against UBE2C (G) or anti-UBE2S (H), followed by IB with indicated Abs. WCE: whole-cell extract.

(I) H1299 cells were transfected with various siRNA targeting indicated mRNAs, along with scrambled control siCont or plasmid expressing FLAG-tagged SAG or FLAG-tagged APC11 or vector control for 48 hr. Cell lysates were prepared for IB analysis with indicated Abs.

(J) A549 and A427 cells were transfected with plasmid expressing FLAG-tagged SAG or vector control for 48 hr. Cell lysates were prepared for IB analysis with indicated Abs.

(K-M) H2170 cells were transfected with various siRNA targeting indicated mRNAs, along with scrambled control siCont for 48 hr. Cell lysates were prepared for IB analysis with indicated Abs.

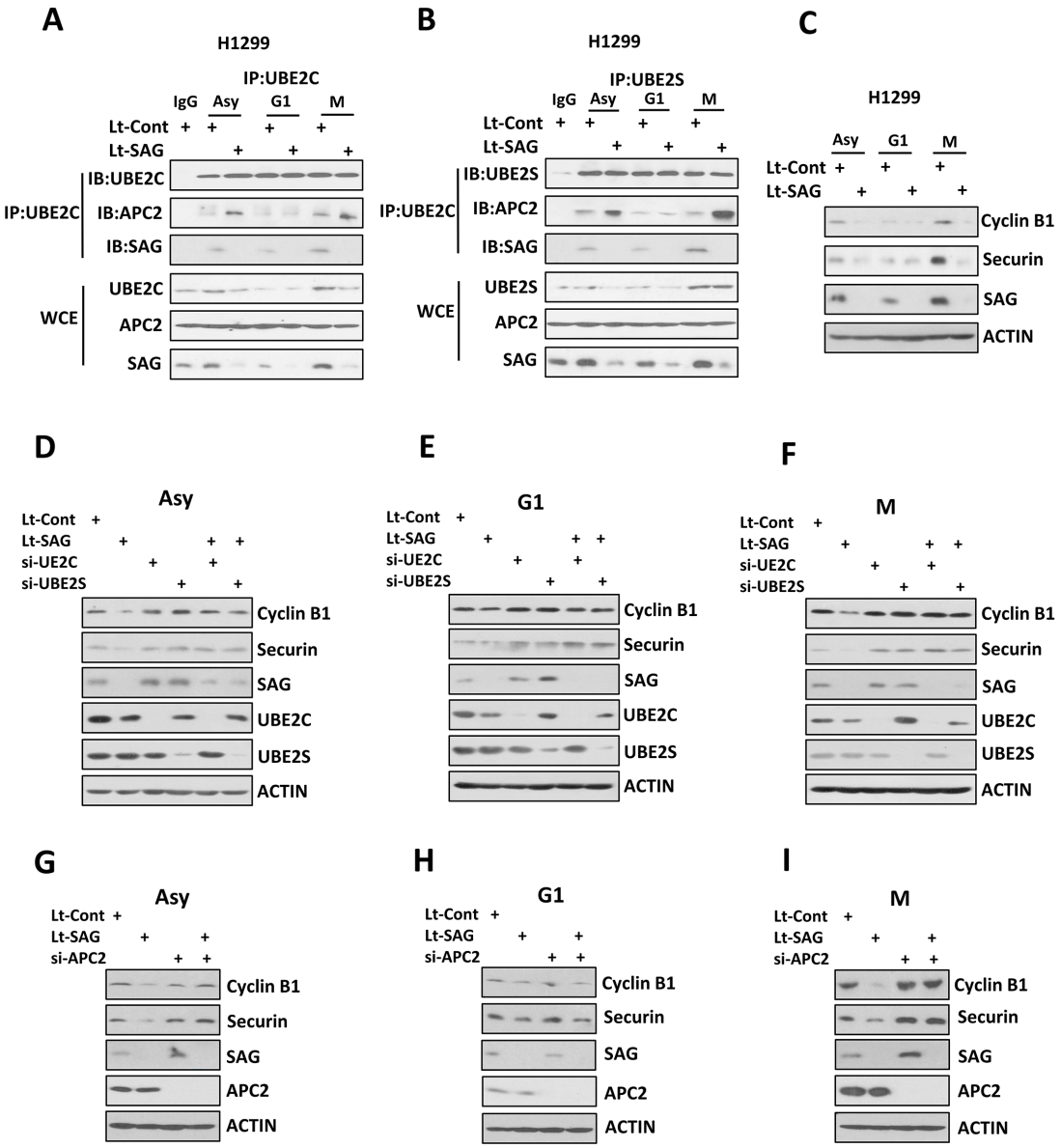


Figure S2: *SAG* knockdown activates APC/C activity at the M phase, related to Figure 1.

(A&B) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were arrested in G1 by serum starvation or in M phase by nocodazole treatment for 24 hr with last 8 hr treatment of 10 μM MG132 before harvesting. Cell lysates were prepared for immunoprecipitation with IgG control or antibodies against UBE2C (A) or anti-UBE2S (B), followed by IB with indicated Abs. WCE: whole-cell extract.

(C) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were arrested at the different phases of cell cycle before being

harvested for IB analysis with indicated Abs.

(D-I) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were transfected with various siRNA targeting indicated mRNAs, then cells were arrested in G1 by serum starvation or in M phase by nocodazole treatment for 24 hr. Cell lysates were prepared for IB analysis with indicated Abs.

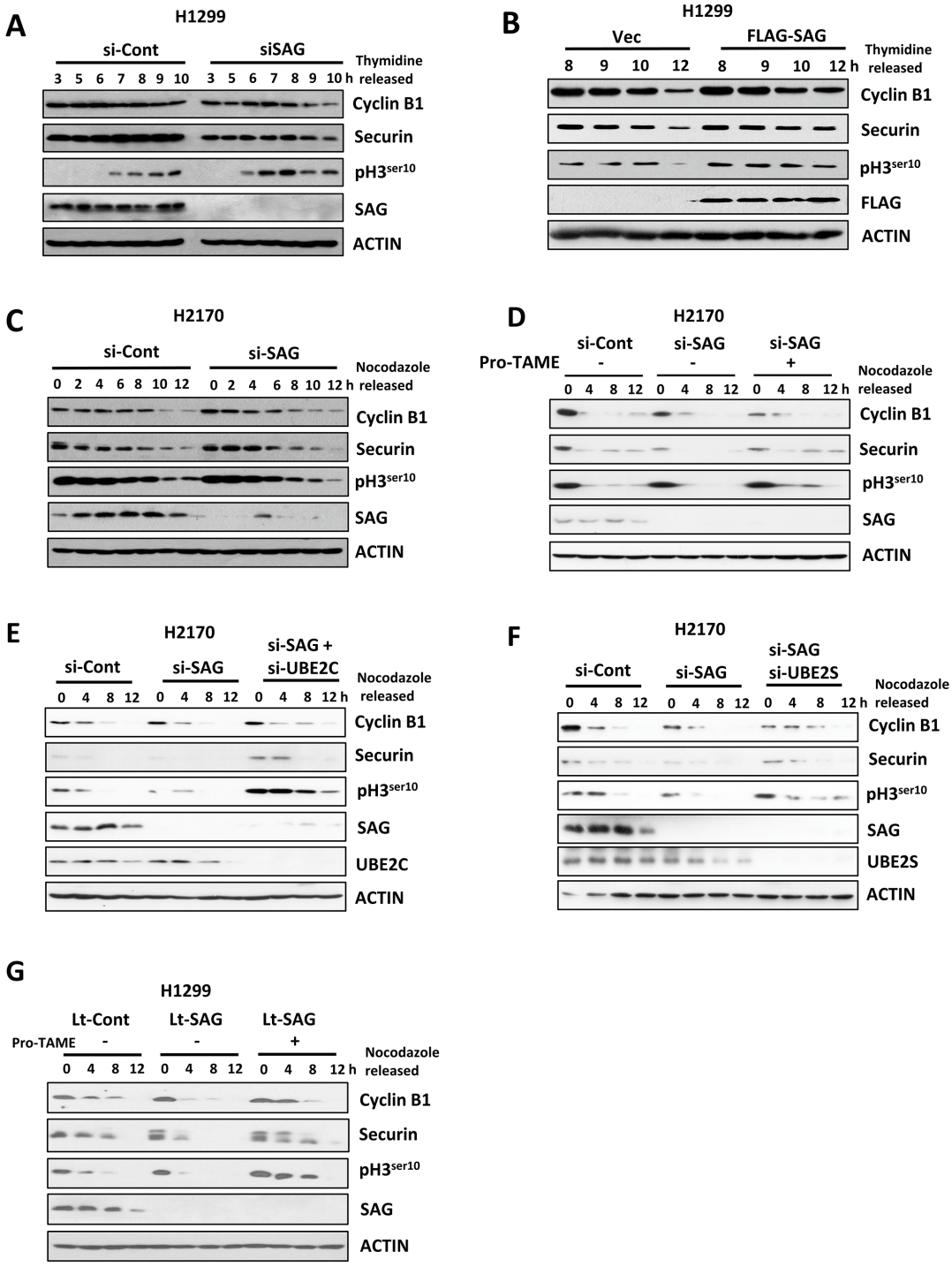


Figure S3: SAG affects APC/C activity in synchronized cells, related to Figure 2.

(A&B) H1299 cells were transfected with siRNA targeting *SAG* or scrambled control siCont (A) or a plasmid expressing FLAG-tagged SAG or vector control (B) for 48 hr and synchronized at S by thymidine block, then released for indicated time points. Cell lysates were prepared for IB analysis with indicated Abs.

(C) H2170 cells were transfected with various siRNA targeting indicated mRNAs,

along with scrambled control siCont for 24 hr and synchronized at M phase by nocodazole blockage, then released for indicated time points. Cell lysates were prepared for IB analysis with indicated Abs.

(D) H2170 cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 48 hr, and synchronized at M phase by nocodazole blockage. Cells were then released in the absence or presence of pro-TAME for indicated time points before harvesting for IB using indicated Abs.

(E&F) H2170 cells were transfected with various siRNA targeting indicated mRNAs, along with scrambled control siCont for 24 hr, and synchronized at M phase by nocodazole blockage. Cells were then released for indicated time points before harvesting for IB using indicated Abs.

(G) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were synchronized at M phase by nocodazole blockage and then released in the absence or presence of pro-TAME for indicated time points before harvesting for IB using indicated Abs.

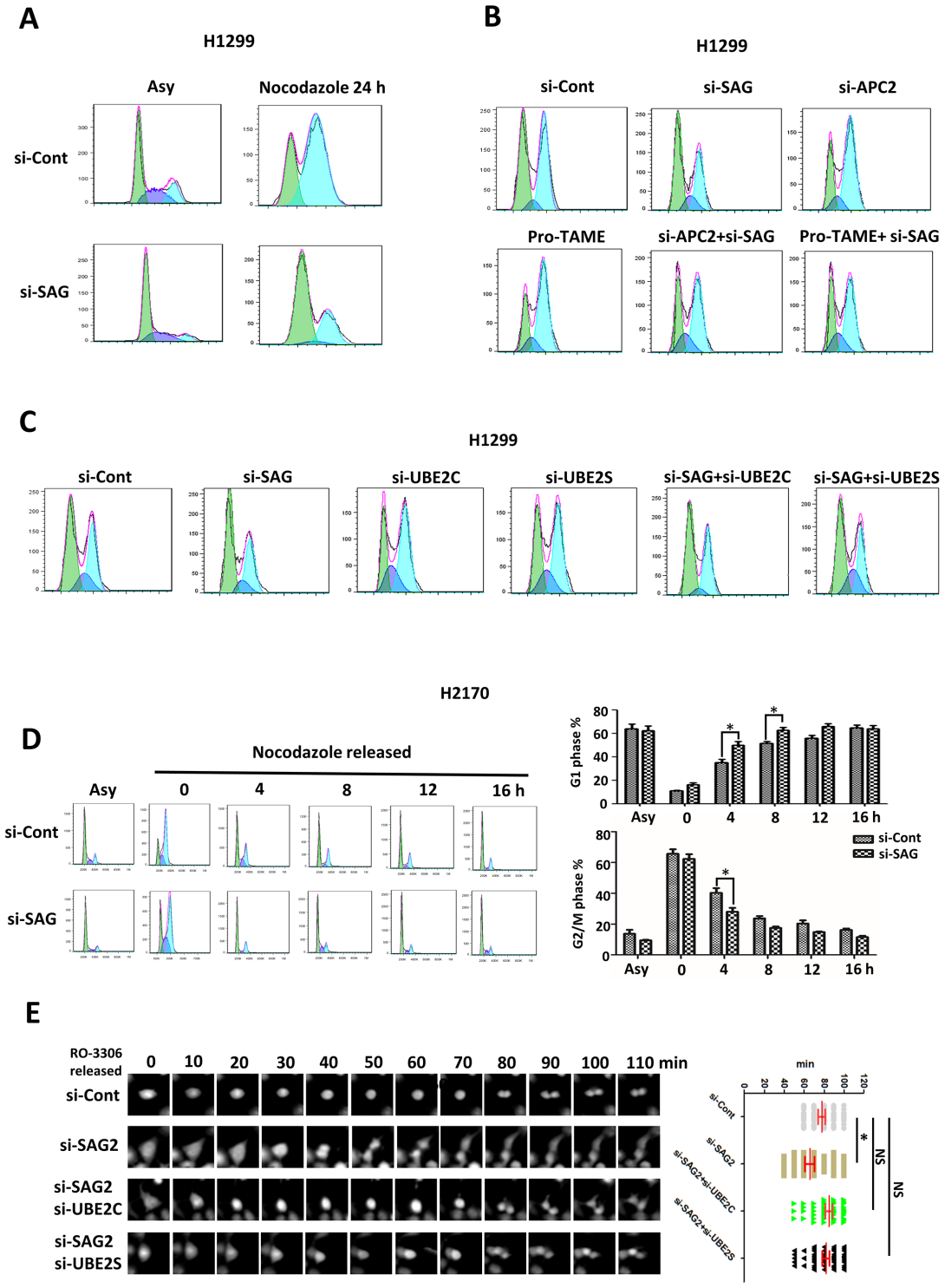


Figure S4. *SAG* knockdown promotes mitotic progression, related to Figure 3.

(A) Representative histograms of cell DNA content of cells in Figure 3A, as measured by FACS.

(B) Representative histograms of cell DNA content of cells in Figure 3B, as measured by FACS.

(C) Representative histograms of cell DNA content of cells in Figure 3C, as measured by FACS.

(D) H2170 cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 24 hr, cells were then blocked at the M phase by nocodazole treatment, then released from the M phase for indicated time points for FACS analysis. The percentage of cell populations at the G1 and G2/M were plotted. Shown are mean ± SEM from three independent experiments. *: $p<0.05$, **: $p<0.01$.

(E) H1299 cells were transfected with various siRNA targeting indicated mRNAs, along with scrambled control siCont for 24 hr, followed by G2 arrest by RO3306 for 24 hr. Cells were then released from G2/M at the indicated time points when the photos of individual cells were taken (left). The time required for the separation into two daughter cells (mitotic time) was recorded and plotted (right). The results were derived from three independent experiments. *: $p<0.01$, NS: not significant.

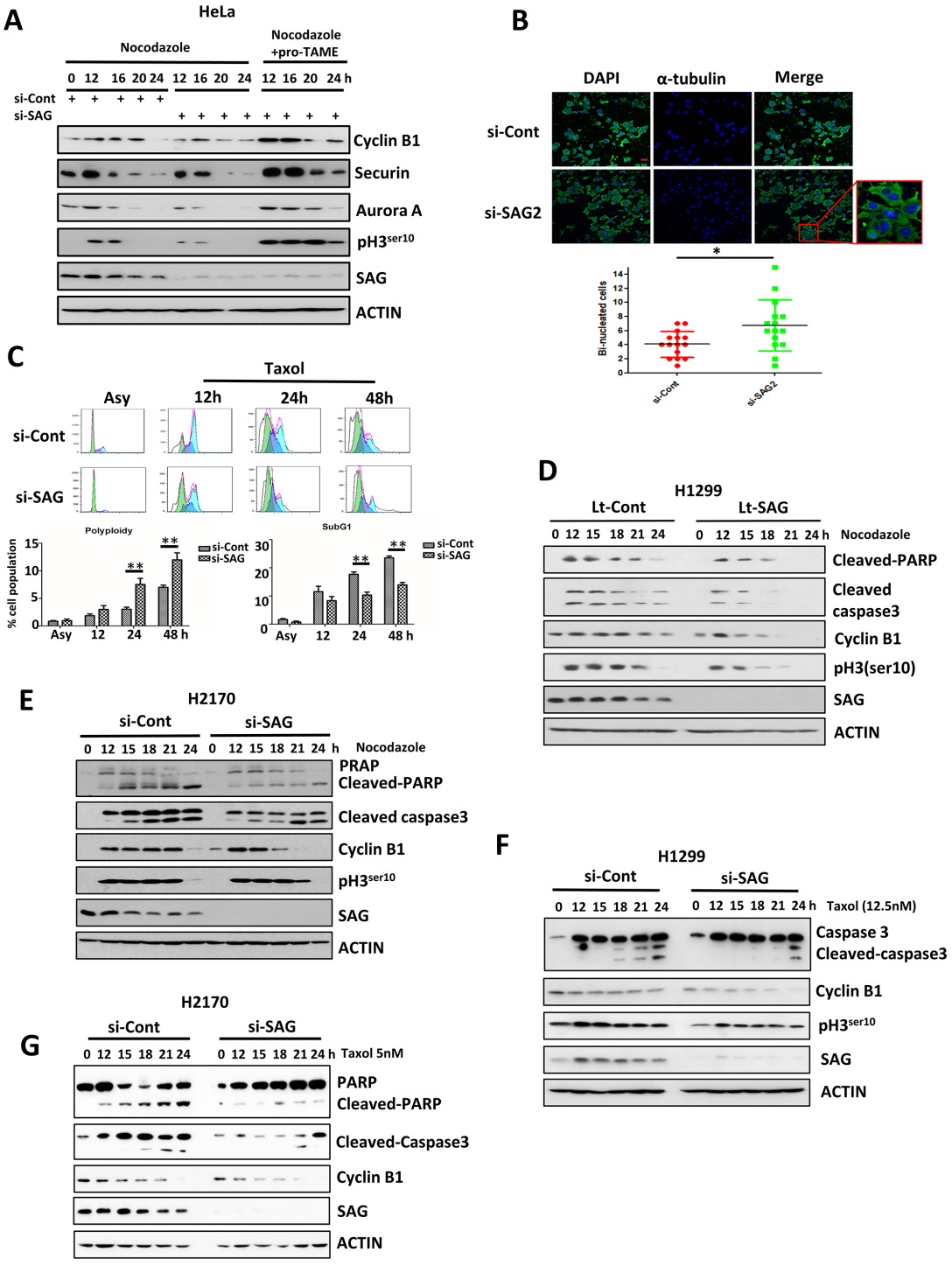


Figure S5. *SAG* knockdown triggers mitotic slippage and confers resistance to apoptosis, related to Figure 4.

(A) HeLa cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 24 hr. Cells were then exposed to nocodazole in the presence or absence of pro-TAME for indicated time points, then harvested for IB analysis with indicated Abs.

(B) H1299 cells were transfected with an independent siRNA targeting *SAG* (siSAG2) or scrambled control siCont for 48 hr, followed by nocodazole treatment for 24 hrs. Cells were then stained with α-tubulin or DAPI and examined under a confocal microscope for photos. Representative images of binucleated cells are shown (upper), and the percentage of binucleated cells were counted from at least 5 fields and plotted (lower). The results were derived from three independent experiments. *: $p<0.05$. Scale bar, 20 μm.

(C) H1299 cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 48 hr, then cells were treated with continuous exposure of taxol for up to 48 hr before being subjected to FACS analysis (up). The percentage of polyploidy and sub-G1 populations were recorded and plotted (bottom). Shown are mean ± SEM from three independent experiment. **: $p<0.01$

(D) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were treated by the continuous exposure of nocodazole for indicated time points. Cell lysates were prepared for IB analysis with indicated Abs.

(E) H2170 cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 48 hr, then cells were treated by the continuous exposure of nocodazole for indicated times. Cell lysates were prepared for IB analysis with indicated Abs.

(F&G) H1299 (F) and H2170 (G) cells were transfected with siRNA targeting SAG or scrambled control siCont for 48 hr, then cells were treated by the continuous exposure of taxol for indicated times. Cell lysates were prepared for IB analysis with indicated Abs.

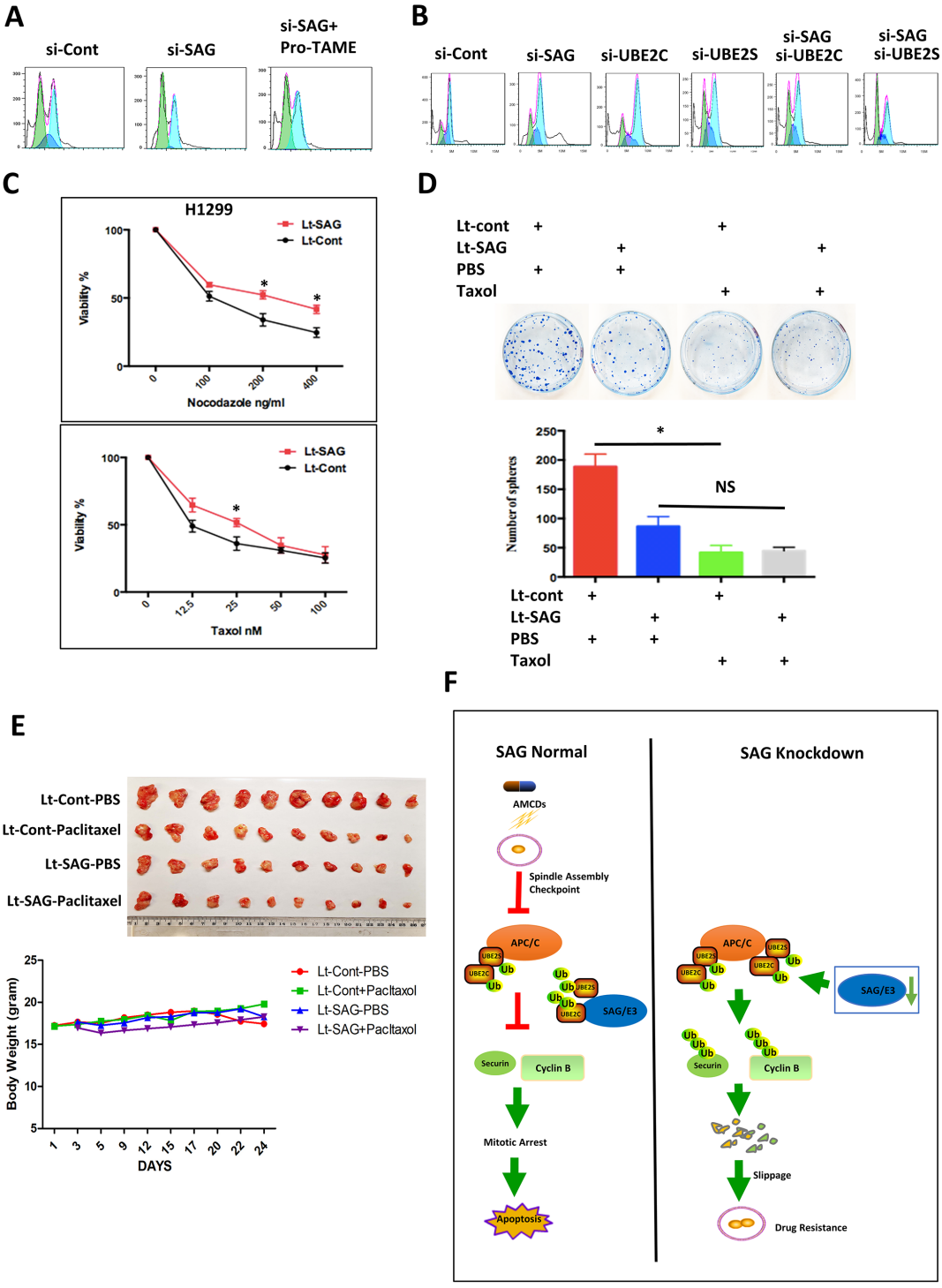


Figure S6. *SAG* knockdown confers better survival of cancer cells to anti-microtubule drugs, related to Figure 4.

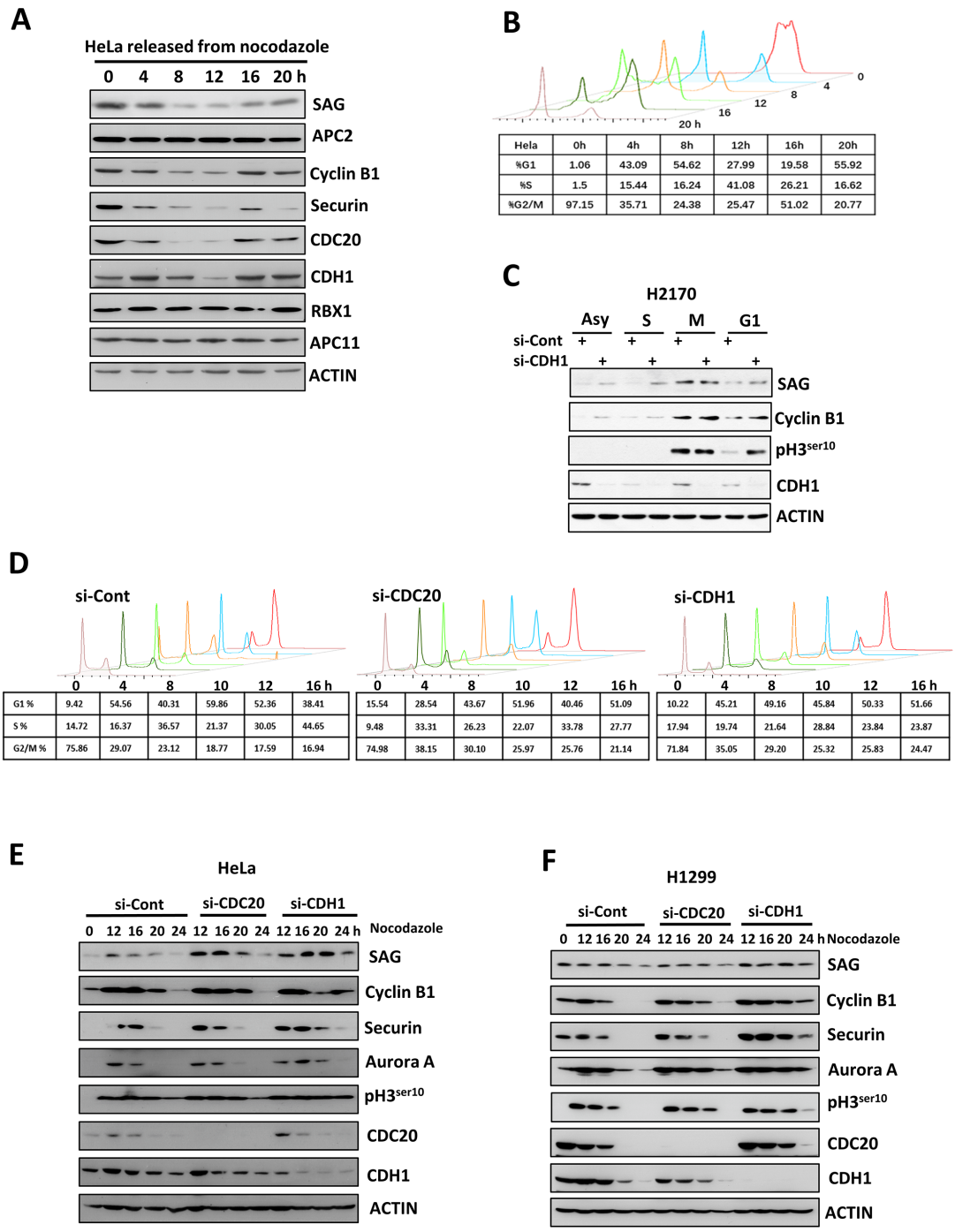
(A&B) Representative histograms of cell DNA content of cells in Figure 4E (A) and Figure 4F (B), as measured by FACS.

(C&D) H1299 cells with lentivirus-mediated *SAG* knockdown (Lt-SAG) and its vector control (Lt-Cont) were treated with indicated concentrations of nocodazole or

taxol, followed by ATP-lite assay (C) and clonogenic survival (D). Shown are mean ± SEM from three independent experiment. *: $p<0.05$, **: $p<0.01$, NC: not significant.

(E) At the end of the *in vivo* experiment, tumors were harvested and photographed (left). The weight of animals was monitored each time before administration and the results plotted (right).

(F) A schematic illustration of the proposed model that SAG knockdown triggers resistance to anti-microtubule drugs-induced apoptosis.



Hela	0h	4h	8h	12h	16h	20h
%G1	1.06	43.09	54.62	27.99	19.58	55.92
%S	1.5	15.44	16.24	41.08	26.21	16.62
%G2/M	97.15	35.71	24.38	25.47	51.02	20.77

si-Cont	0	4	8	10	12	16 h
G1 %	9.42	54.56	40.31	59.86	52.36	38.41
S %	14.72	16.37	36.57	21.37	30.05	44.65
G2/M %	75.86	29.07	23.12	18.77	17.59	16.94

si-CDC20	0	4	8	10	12	16 h
	15.54	28.54	43.67	51.96	40.46	51.09
	9.48	33.31	26.23	22.07	33.78	27.77
	74.98	38.15	30.10	25.97	25.76	21.14

si-CDH1	0	4	8	10	12	16 h
	10.22	45.21	49.16	45.84	50.33	51.66
	17.94	19.74	21.64	28.84	23.84	23.87
	71.84	35.05	29.20	25.32	25.83	24.47

Figure S7. SAG expression fluctuates during cell cycle and is stabilized in *CDH1*-depleted cells, related to Figure 5.

(A&B) HeLa cells were arrested at M phase by nocodazole blockage, cells were then released for indicated time points and subjected to IB analysis (A) or FACS analysis (B).

(C) H2170 cells were transfected with siRNA targeting *CDH1* or scrambled control siCont for 24 hr, and then arrested at the different phases of cell cycle before being harvested for IB analysis with indicated Abs.

(D) FACS analysis of cell-cycle changes for HeLa cells in Figure 5D.

(E&F) HeLa cells (E) or H1299 cells (F) were transfected with siRNA targeting *CDC20* or *CDH1*, along with scrambled control siCont for 24 hr, then cells were treated by continuous exposure to nocodazole for the indicated time points. Cells were harvested for IB analysis with indicated Abs.

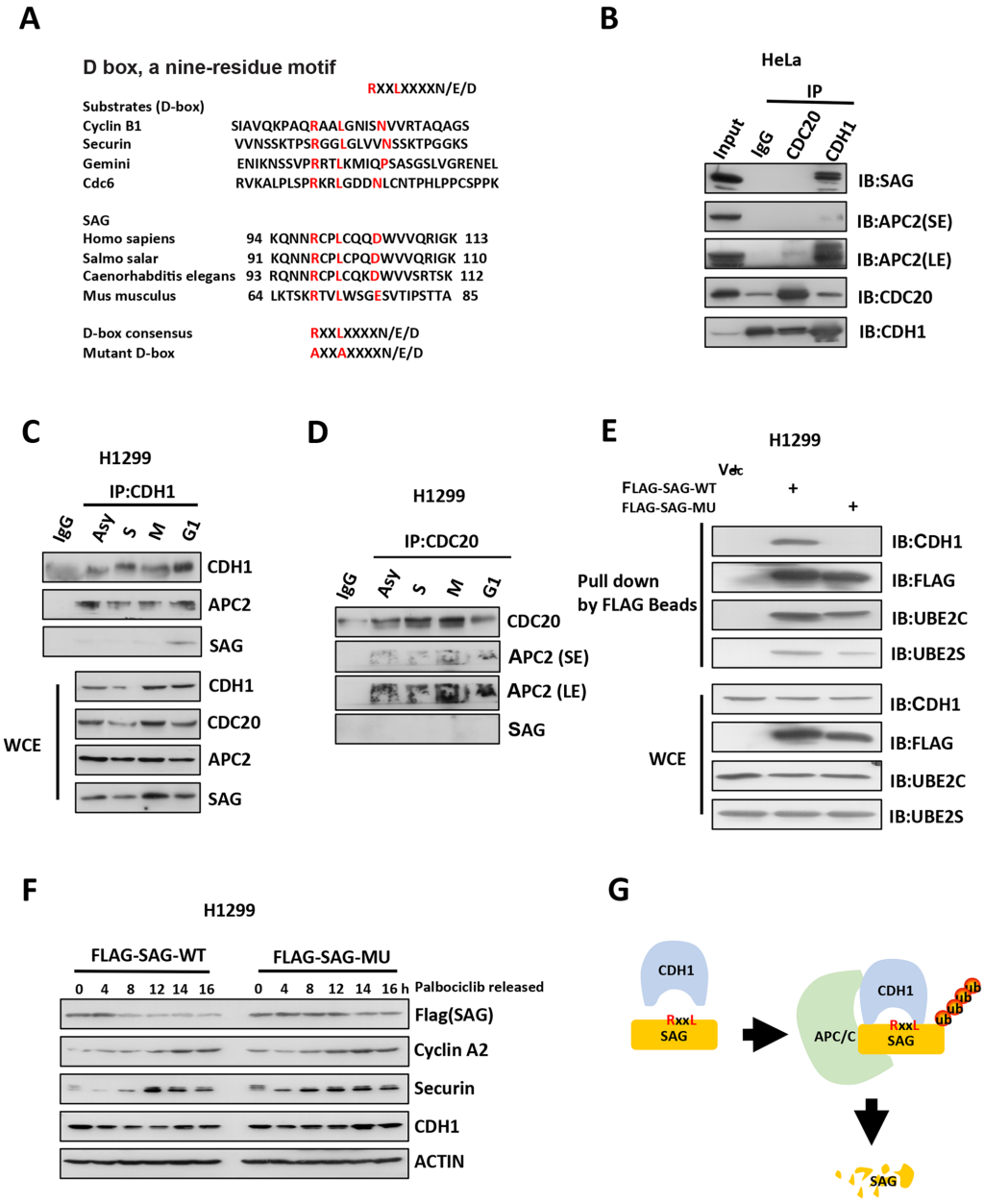


Figure S8. CDH1 promotes SAG ubiquitination and subsequent degradation in a D-Box-Dependent manner, related to Figure 5.

(A) Evolutionary conservation of the D-box motif on SAG (top); and the schematic representation of the D box mutant (SAG-MU) used in the following studies (bottom). **(B)** HeLa cells were pretreated with 10 μM MG132 for 8 hr before harvesting for immunoprecipitation assay using anti-CDC20 or anti-CDH1 Abs, along with IgG control, followed by IB analysis with indicated Abs.

(C&D) H1299 cells were arrested at different phases of cell cycle phases, followed by immunoprecipitation with anti-CDH1 Ab (C) or anti-CDC20 Ab (D), and IB analysis with indicated Abs. WCE: whole-cell extract.

(E) H1299 cells were transfected with a plasmid expressing FLAG-tagged SAG-WT or SAG-MU, along with the vector control for 48 hr with last 8 hr treatment of 10 μM MG132 before harvesting. Cell lysates were prepared for immunoprecipitation with IgG control or FLAG-beads, followed by IB analysis with indicated Abs.

(F) H1299 cells were transfected with a plasmid expressing FLAG-tagged SAG-WT or SAG-MU for 48 hr. Cells were arrested at the G1 by palbociclib treatment for 24 hr, then released for indicated time points, followed by IB with indicated Abs.

(G) A schematic illustration of the proposed model for APC/C^{CDH1} to interact with SAG and promote its ubiquitination and subsequent degradation through a D-box dependent manner.

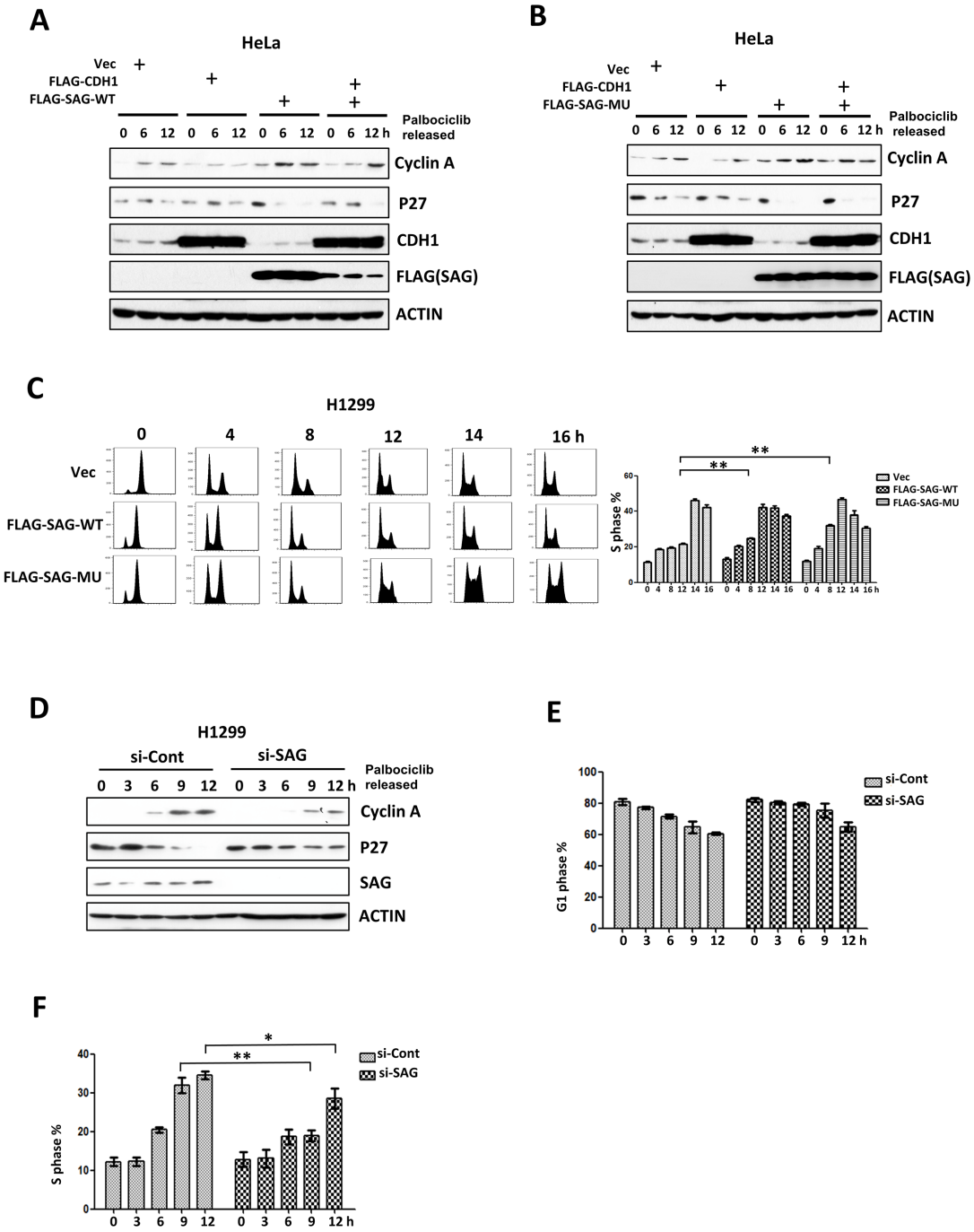


Figure S9. SAG degradation-resistant mutant promotes the G1 to S transition, related to Figure 6.

(A&B) HeLa cells were transfected with indicated plasmids for 24 hr, then cells were synchronized at G1 phase by Palbociclib block and released for indicated time points, followed by IB analysis with indicated Ab.

(C) H1299 cells were transfected with indicated plasmids for 24 hr. Cells were then arrested at the M by nocodazole, harvested at indicated hours post release for FACS

analysis (left). The percentage of cell population at S was plotted (right). Shown is mean ± SEM from three independent experiments. **: $p<0.01$.

(D) H1299 cells were transfected with siRNA targeting *SAG* or scrambled control siCont for 48 hr, then cells were blocked at G1 by Palbociclib and released for indicated time points, followed by IB analysis with indicated Ab.

(E&F) The percentage of cell population at G1 phase (E) and S phase (F) were plotted bases on FACS analysis of cells in (D). Shown is mean ± SEM from three independent experiments. *: $p<0.05$, **: $p<0.01$.