Figure S1 Ono H., *et al*.

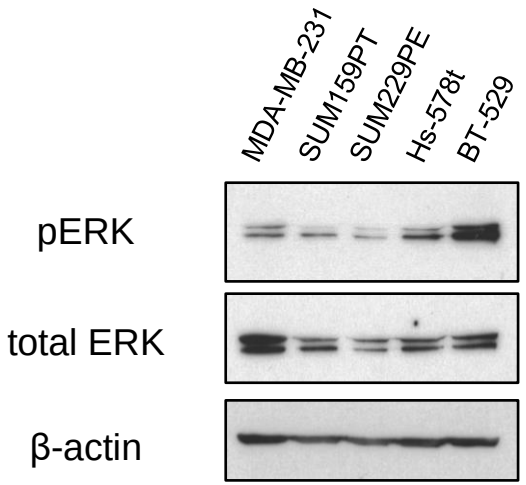


Figure S1. The activity of MAPK pathway in TNBC cells.

Comparison of the phosphorylation status in MAPK pathway among the five TNBC cells; MDA-MB-231, SUM159PT, SUM229PE, Hs-578t, and BT-549 cells.

Figure S2 Ono H., *et al*.

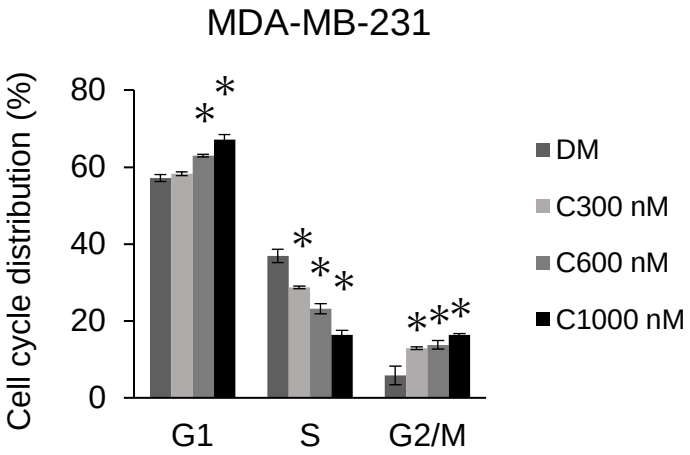


Figure S2. CH5126766 inhibits cell growth with G1- and G2/M-phase arrest.

MDA-MB-231 cells were treated with the indicated concentrations of CH5126766 for 72 h. The cell cycle populations treated with CH5126766 were analyzed by FACSCalibur. The cell cycle populations at the indicated concentrations of CH5126766 were compared with DMSO samples.

DM, treated with DMSO, C, treated with CH5126766.

Columns, mean values of triplicate data; bars, SD. *$P < 0.05$

Figure S3 Ono H., *et al*.

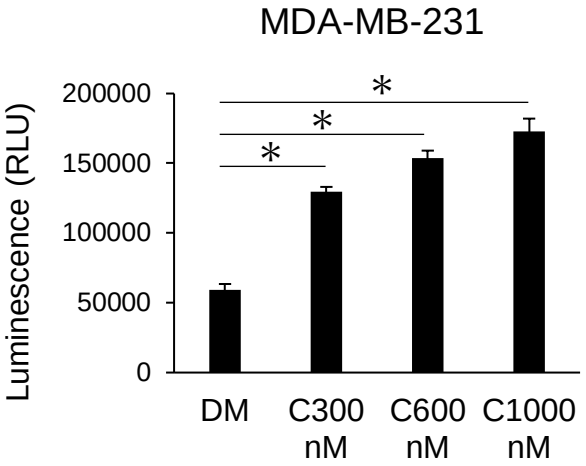


Figure S3. Apoptosis induced by combination treatment depends on caspase.

Luminescence in MDA-MB-231 cells treated with the indicated concentrations of CH5126766 was measured by the Caspase-Glo 3/7 assay. Luminescence shown in the figure was subtracted from the background, and compared with DMSO samples.

DM, treated with DMSO, C, treated with CH5126766.

Columns, mean values of triplicate data; bars, SD. *$P < 0.05$

Figure S4 Ono H., *et al*.

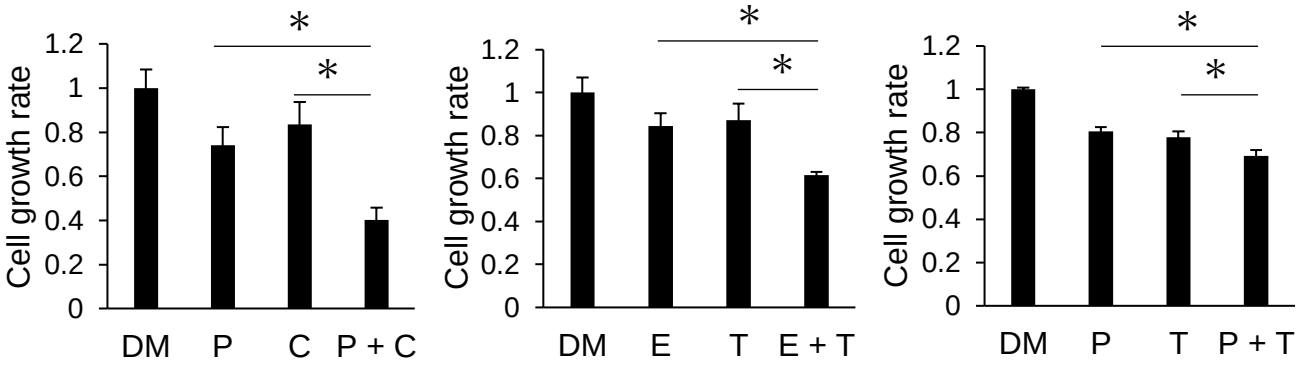
	E/C (nM)	Combination index
MDA-MB-231	0.5/300	0.382
SUM159PT	3.5/500	0.705

Figure S4. Combination treatment with eribulin and CH5126766 exerts synergistic effects against TNBC cells.

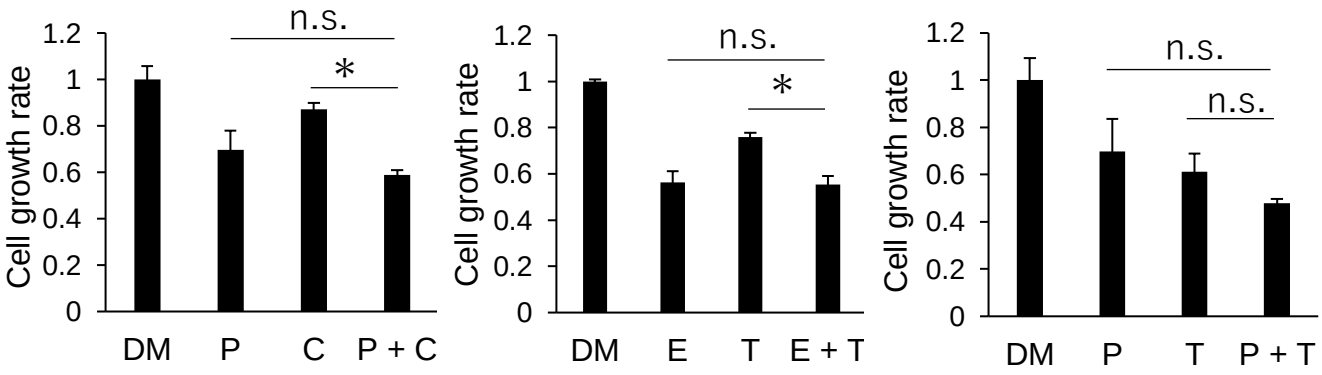
CIs for the treatment with eribulin and CH5126766 in each TNBC cell line were shown. CIs were calculated using CalcuSyn.

Figure S5 Ono H., *et al*.

a



b



c

	P/C (nM)	Combination index
MDA-MB-231	2.5/300	0.496
SUM159PT	2.5/500	Not additive
	E/T (nM)	Combination index
MDA-MB-231	0.5/2.5	0.659
SUM159PT	3.5/30	Not additive
	P/T (nM)	Combination index
MDA-MB-231	2.5/2.5	Not additive
SUM159PT	2.5/30	Not additive

Figure S5 Ono H., *et al*.

Figure S5. Combination treatment of paclitaxel and CH5126766, and eribulin/paclitaxel and trametinib in the TNBC cells.

(a) MDA-MB-231 cells were treated with 2.5 nM paclitaxel and 300 nM CH5126766, or 0.5 nM eribulin/2.5 nM paclitaxel and 2.5 nM trametinib for 72 h.

(b) SUM159PT cells were treated with 2.5 nM paclitaxel and 500 nM CH5126766, or 3.5 nM eribulin/2.5 nM paclitaxel and 30 nM trametinib for 72 h.

DM, treated with DMSO, P, treated with paclitaxel, E, treated with eribulin, C, treated with CH5126766, T, treated with trametinib, P+C, treated with combination of paclitaxel and CH5126766, E+T, treated with eribulin and trametinib, and P+T, treated with paclitaxel and trametinib. The inhibition of the cell growth was evaluated using CCK-8. The cell growth rate of the combination was calculated by comparing each single treatment.

Columns, mean values of triplicate data; bars, SD. $*P < 0.05$

(c) The synergism was showed in each combination treatment.

Figure S6 Ono H., *et al*.

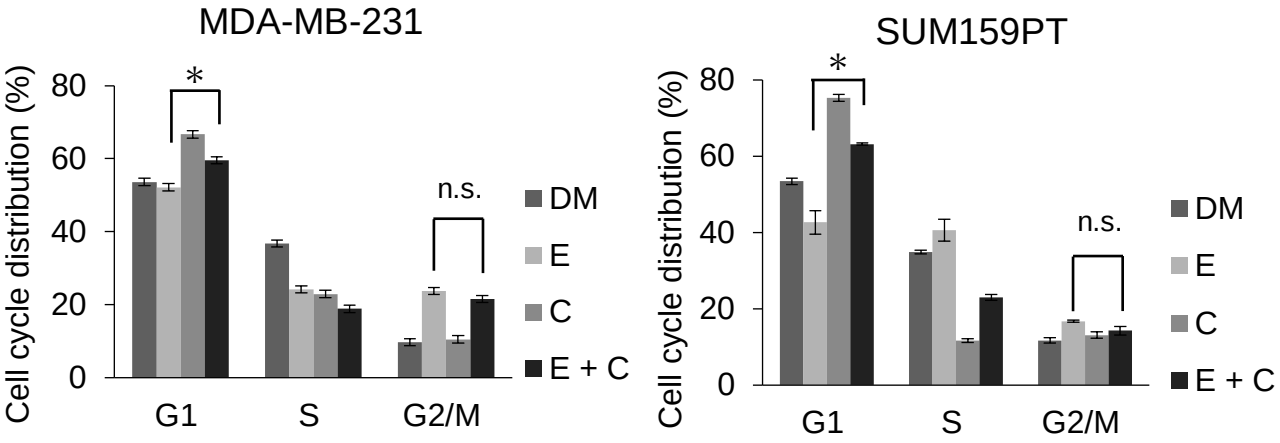


Figure S6. Combination treatment does not enhance cell cycle phase arrest more than single treatment.

Cell cycle populations in TNBC cell lines treated with each drug for 48 h were analyzed by FACSCalibur. Compared with eribulin treatment, combination treatment significantly enhanced G1-phase arrest, but not G2/M-phase arrest, while compared with CH5126766 treatment, combination treatment did not significantly enhance G1-phase arrest.

DM, treated with DMSO, E, treated with eribulin, C, treated with CH5126766, and E+C, treated with eribulin and CH5126766.

Columns, mean values of triplicate data; bars, SD. *$P < 0.05$

Figure S7 Ono H., *et al*.

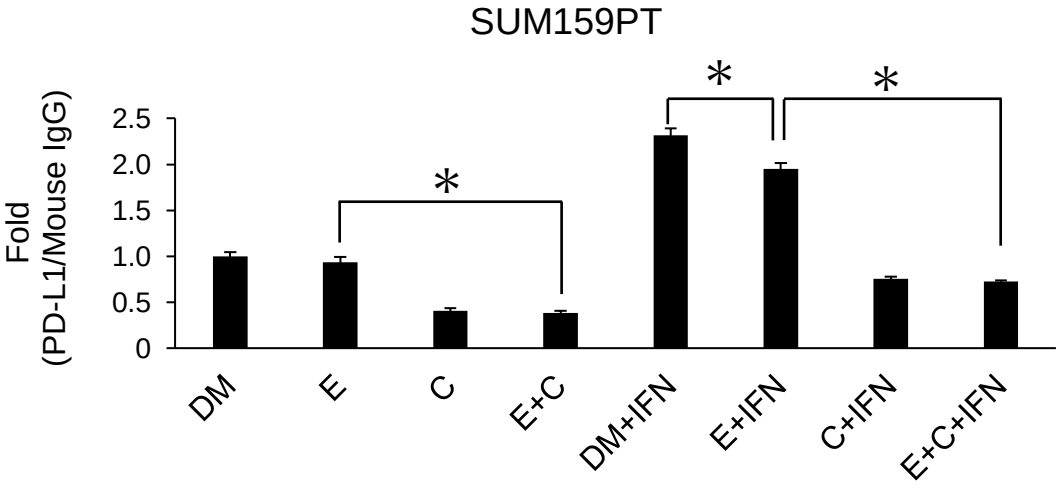


Figure S7. CH5126766 suppresses PD-L1 expression in SUM159PT cells under an IFN-γ stimulation.

The expression of PD-L1 on the surface of SUM159PT cells with or without 20 ng/ml IFN-γ was analyzed by FACSCalibur.

Drug concentrations were the same as those in Figures 2, 3, and 4.

DM, treated with DMSO, E, treated with eribulin, C, treated with CH5126766, and E+C, treated with eribulin and CH5126766.

Columns, mean values of triplicate data; bars, SD. *$P < 0.05$

Figure S8 Ono H., *et al*.

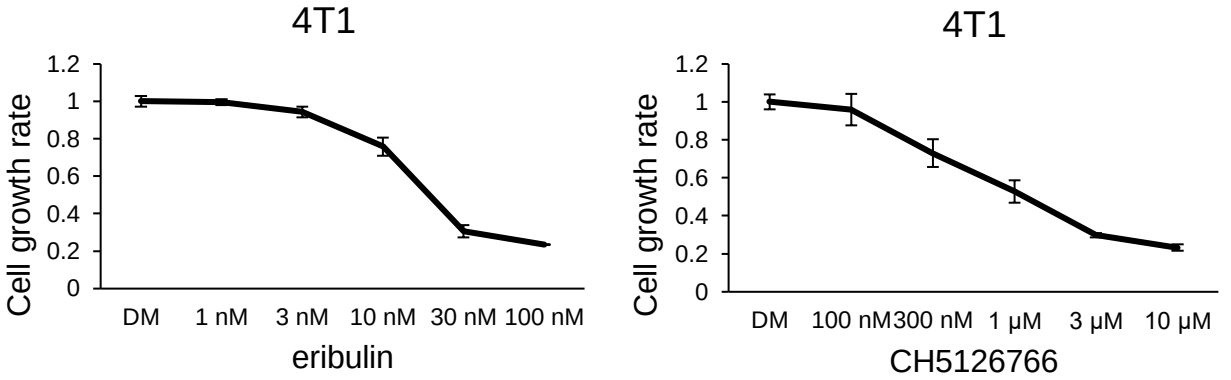


Figure S8. Effects of eribulin or CH5126766 on 4T1 cells.

Murine 4T1 cells were treated with the indicated concentrations of eribulin or CH5126766 for 48 h. DM, treated with DMSO. The inhibition of cell growth was evaluated using CCK-8. The cell growth rate of each treatment was calculated by comparisons with DMSO samples.