Onionin A inhibits ovarian cancer progression by suppressing cancer cell proliferation and the protumour function of macrophages.

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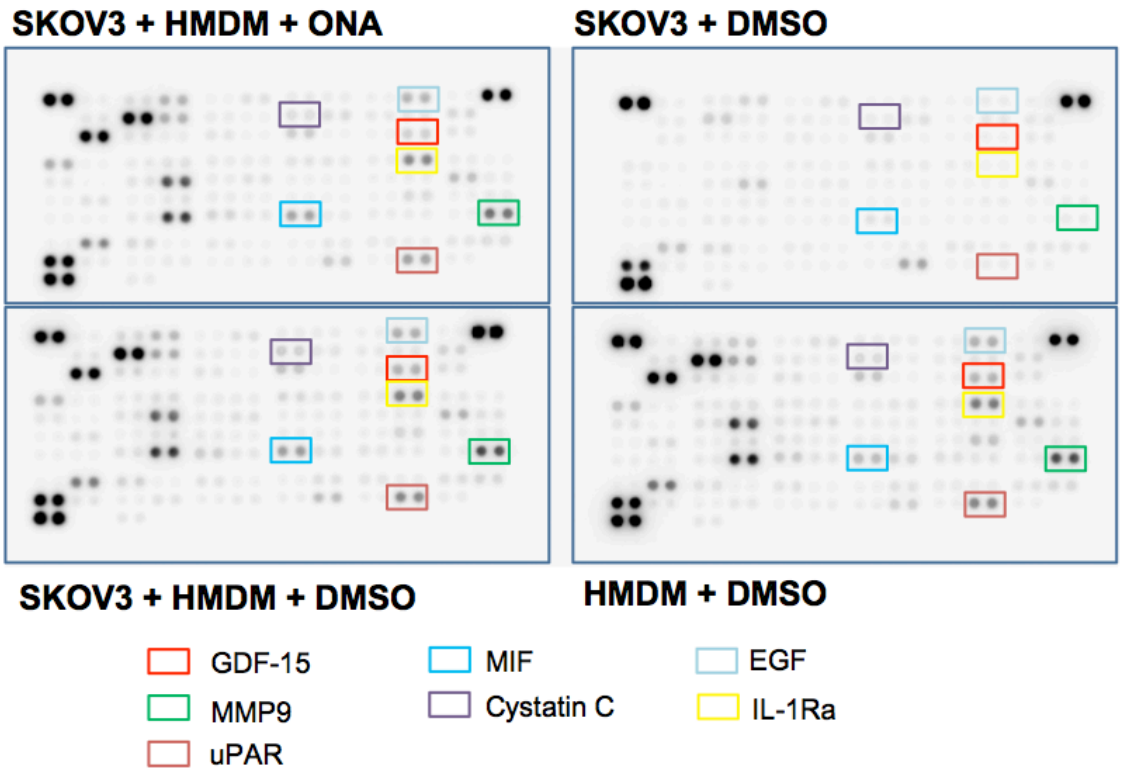
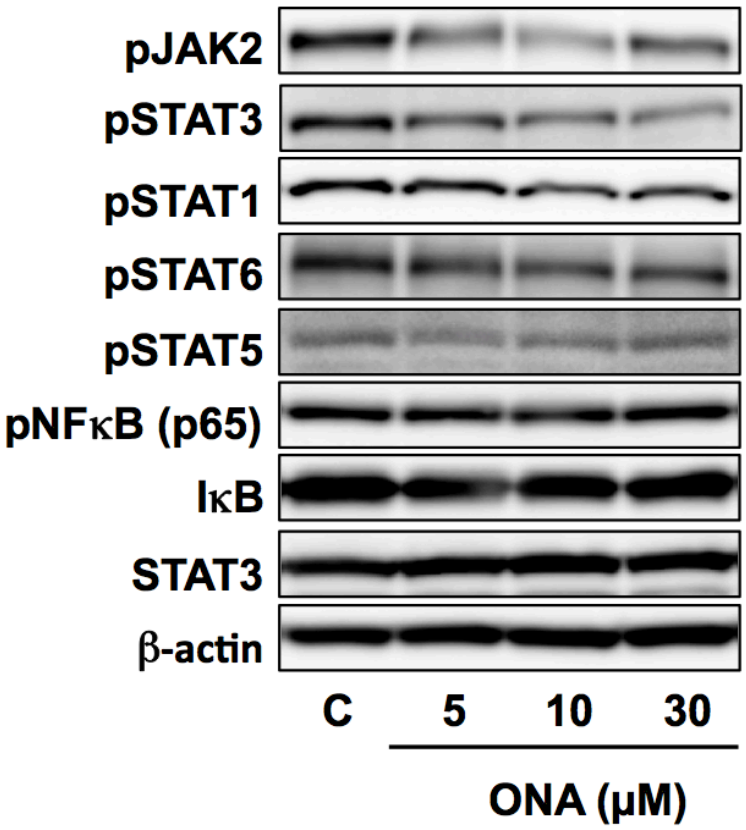
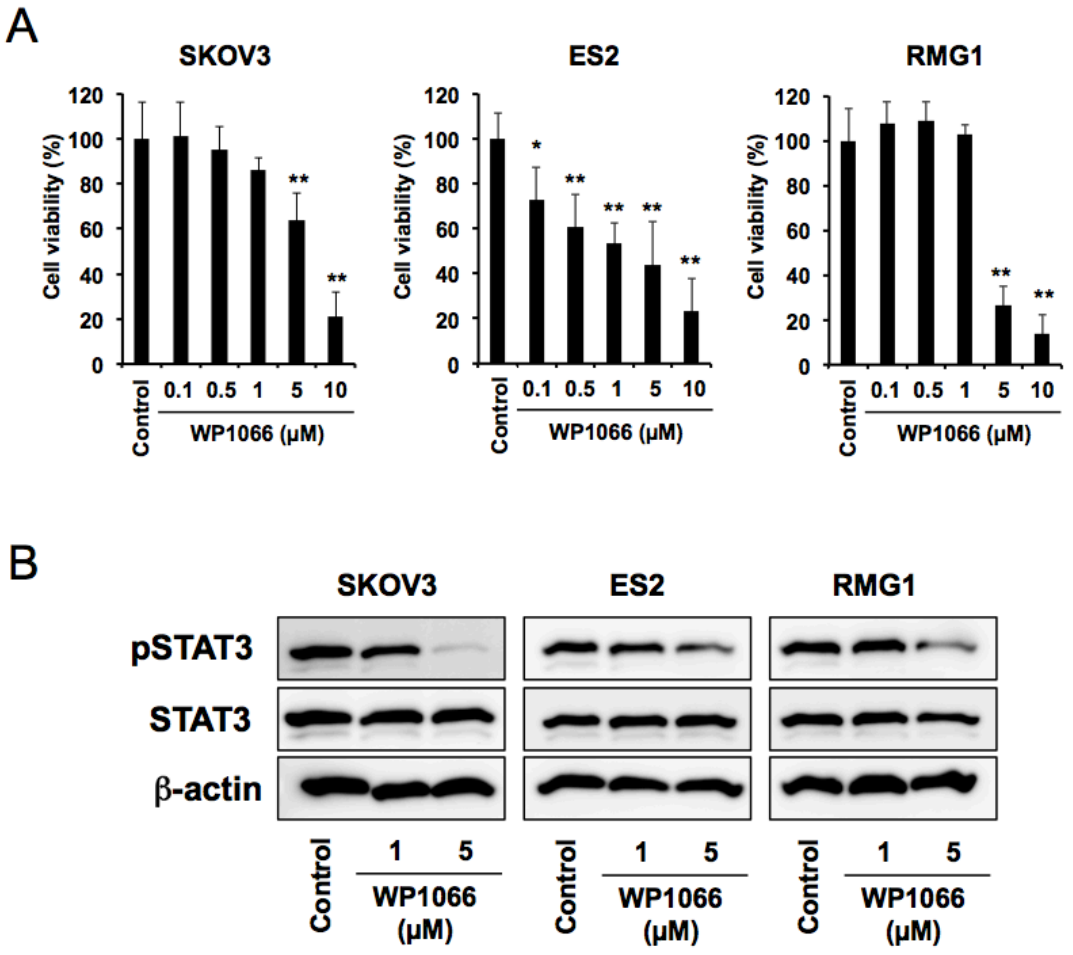


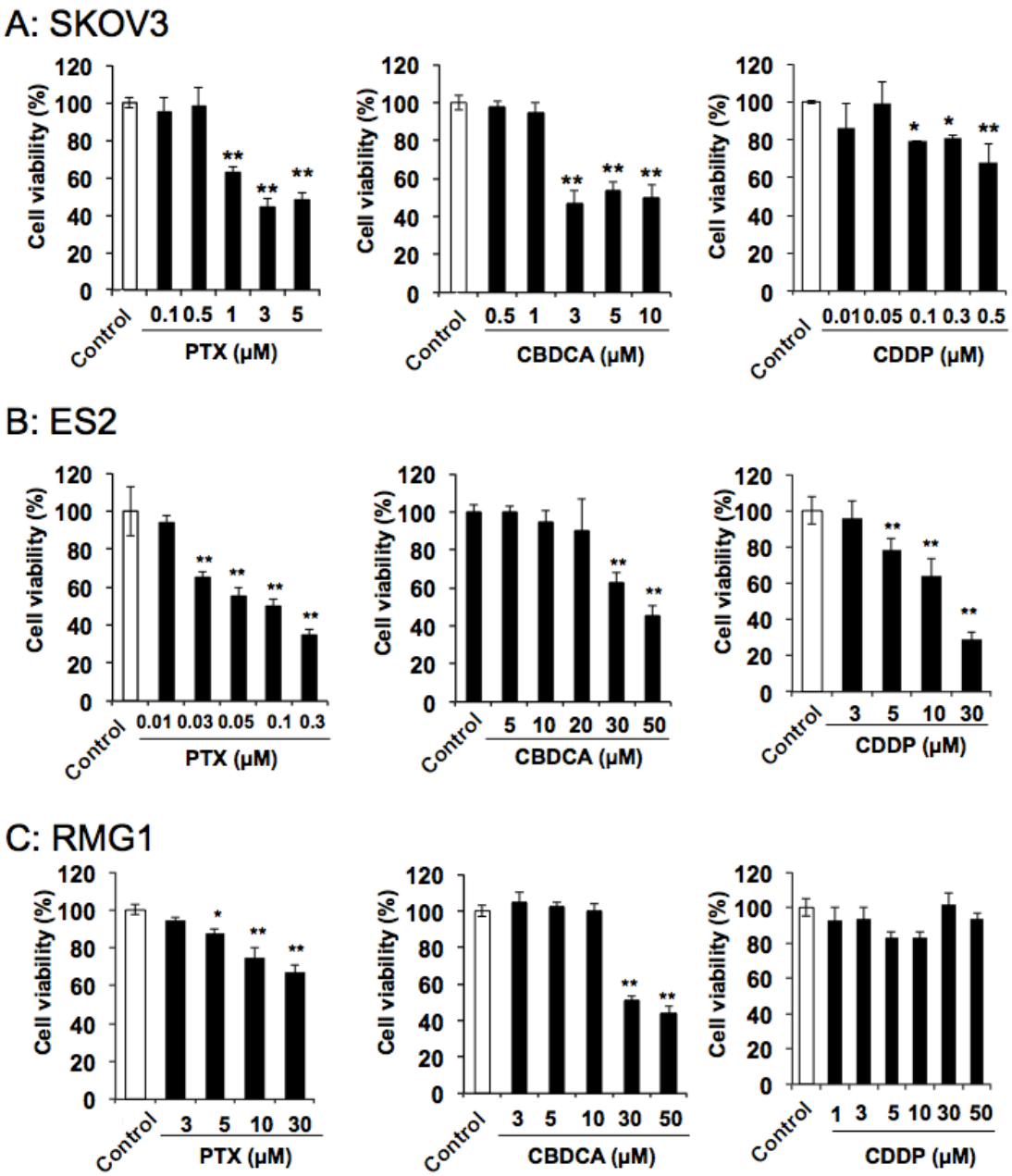
Supplementary Information 1. ***Cytokine array of culture supernatant.*** SKOV3 cells were incubated with HMDM during treatment of ONA (10 μM) for 24 hours, followed by the determination of cytokines production in culture supernatant by cytokine array kit.



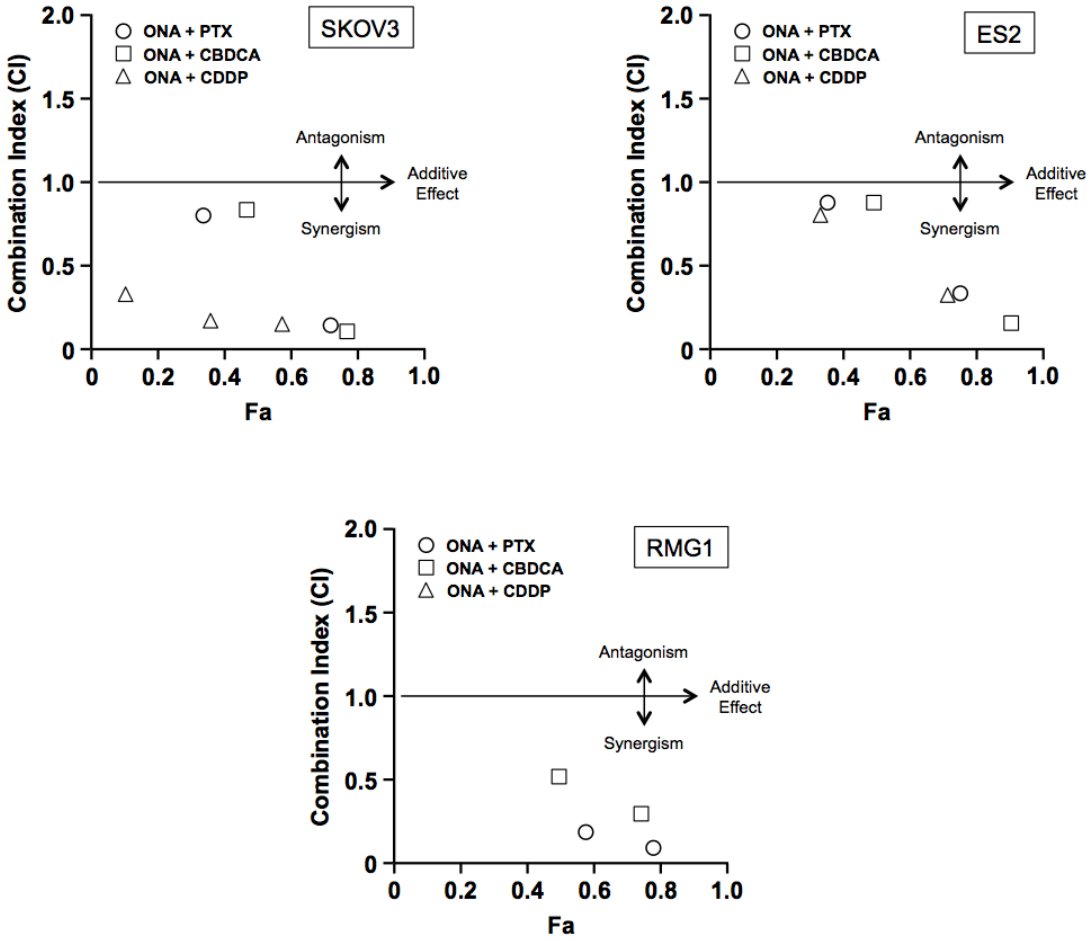
Supplementary Information 2. ***Effect of ONA on JAK, STAT and NF-kB activation in SKOV3 cells.*** SKOV3 cells were incubated with the indicated concentrations of ONA for 4 hours, followed by the determination of pJAK2, pSTAT1, pSTAT3, pSTAT5, STAT6, pNF-κB, IκB, STAT3 and β-actin by a Western blot analysis.



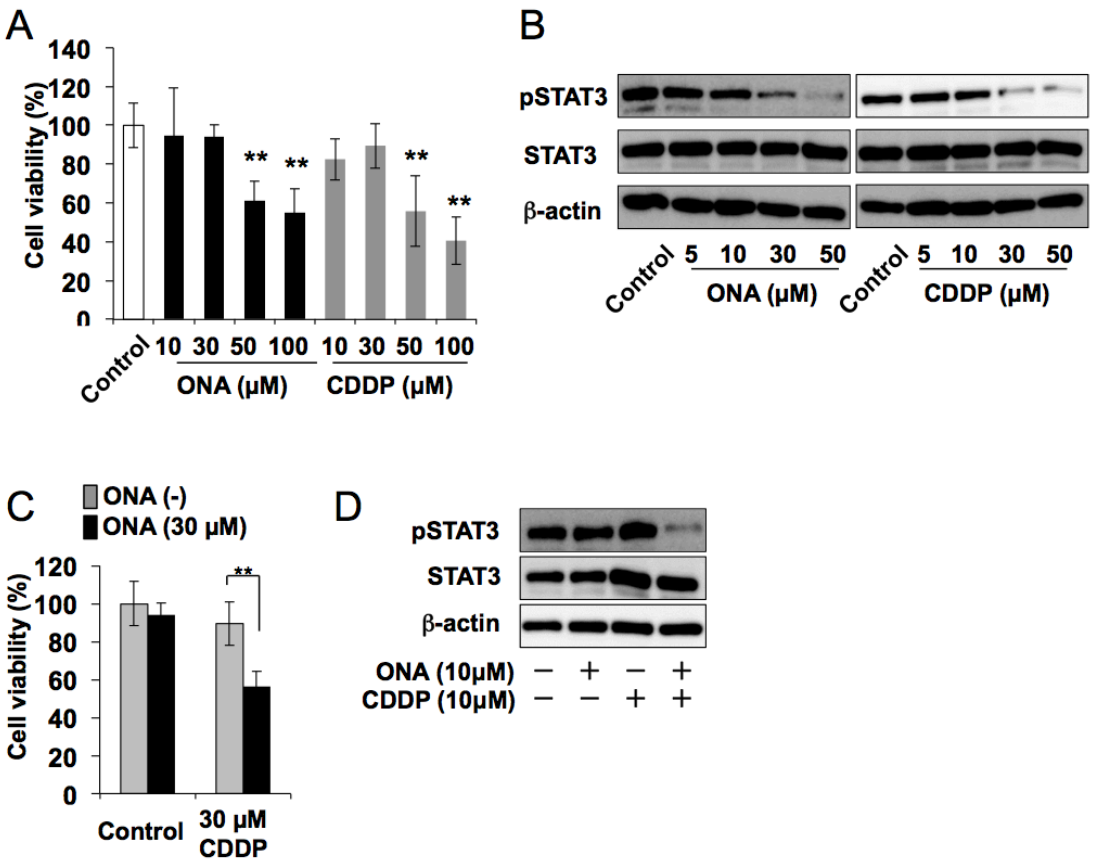
Supplementary Information 3. ***Effect of WP1066 on cell proliferation and STAT3 activation in EOC cells.*** EOC cells (SKOV3, ES2, and RMG1) were incubated with the indicated concentrations of WP1066 for 24 hours, followed by the determination of cell proliferation using the WST-8 assay (A). EOC cells were incubated with the indicated concentrations of ONA for 3 hours, followed by the determination of pSTAT3, STAT3 and β-actin by a Western blot analysis (B). The data are presented as the mean±SD. *: p-value <0.05, **: p-value <0.01 vs. control.



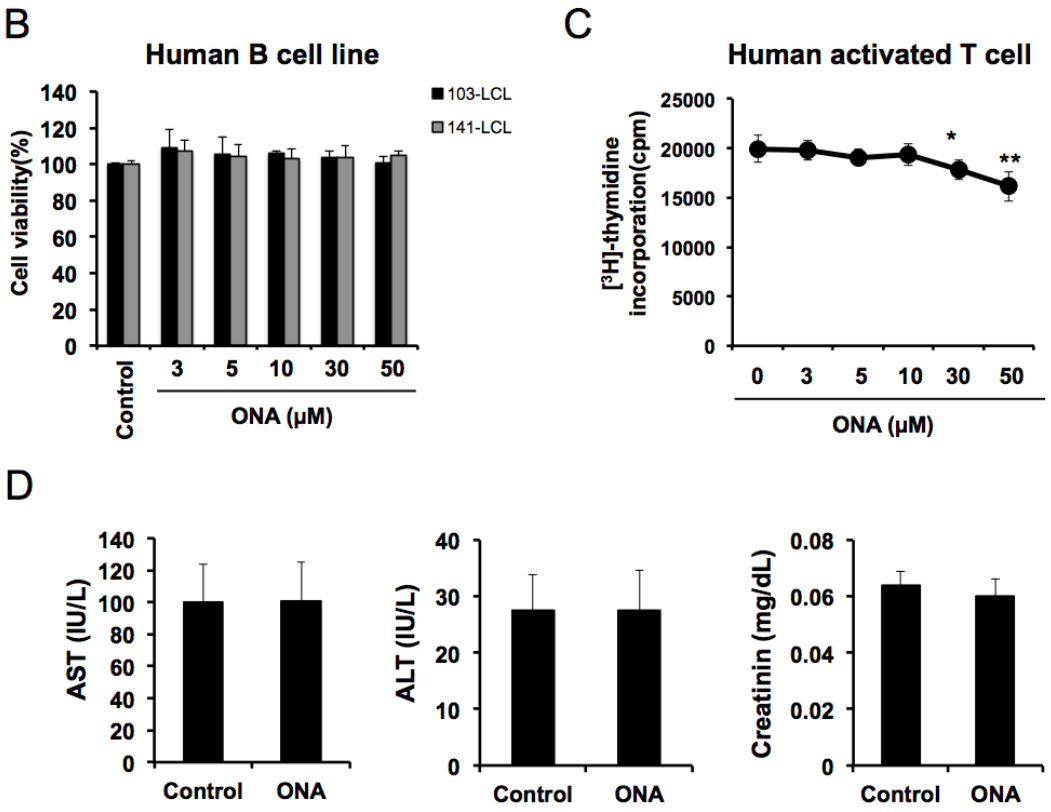
Supplementary Information 4. ***Ineffective concentration of ONA and anti-cancer drugs on cell proliferation.*** EOC cells (SKOV3, ES2, and RMG1) were incubated with the indicated concentrations of PTX, CBDCA, and CDDP for 24 hours, followed by the determination of cell proliferation using the WST-8 assay (SKOV3: A, ES2: B, RMG1: C), and the ineffective concentration of each anti-cancer drug on each cell line was determined. The data are presented as the mean±SD. *: p-value <0.05, **: p-value <0.01 vs. control.



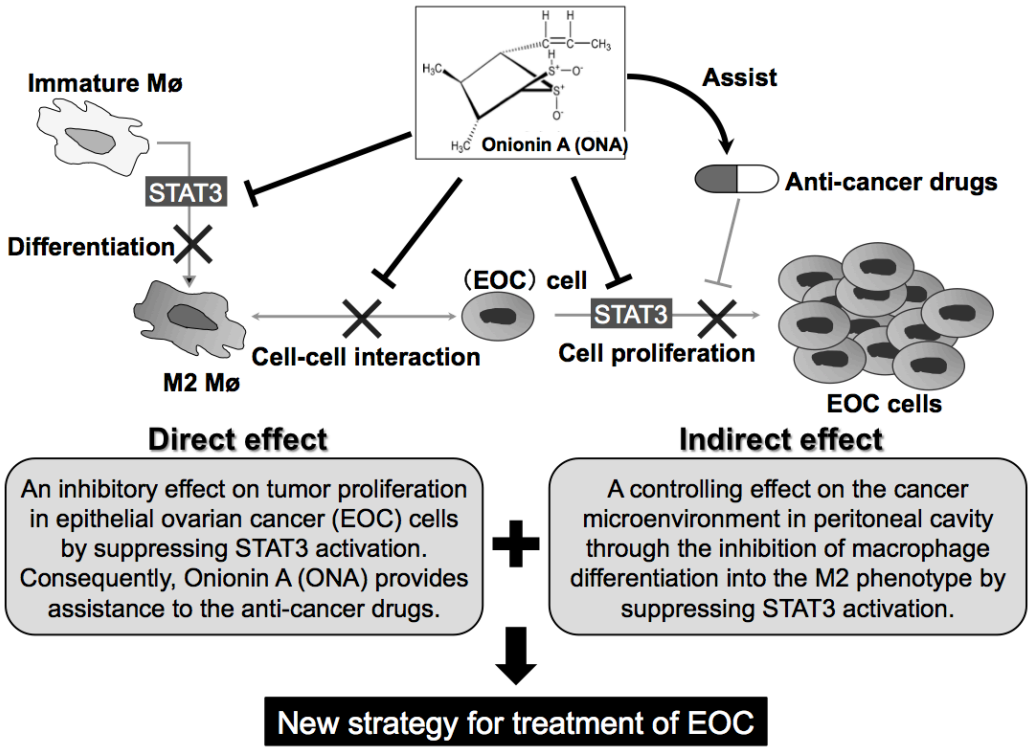
Supplementary Information 5. ***The fraction affected (Fa)-combination index (CI) plot (Chou-Talalay Plot).*** The effect relationships of ONA and anti-cancer drugs (PTX, CBDCA, or CDDP) and two-drug combinations on growth inhibition in EOC cells (SKOV3, ES2 and RMG1). The *f*a-CI plot with x=fraction affected (Fa) vs. y=combination index (CI) where CI <1, =1, and >1 indicates synergism, additive effect and antagonism, respectively.



Supplementary Information 6. ***Effect of ONA on murine ovarian cancer cell line.*** iMOC cells were incubated with the indicated concentrations of ONA or CDDP for 24 hours, followed by the determination of cell proliferation using the WST-8 assay (A). iMOC cells were incubated with the indicated concentrations of ONA and/or CDDP for 3 hours, followed by the determination of pSTAT3, STAT3 and β-actin by a Western blot analysis (B). iMOC cells were incubated with a combination of the ineffective concentration of anti-cancer drug and ONA, followed by the determination of cell proliferation using the WST-8 assay (C). iMOC cells were incubated with the ineffective concentration of each anti-cancer drug with or without ONA for 3 hours, followed by the determination of pSTAT3, STAT3 and β-actin by a Western blot analysis (D).



Supplementary Information 7. ***Effect of ONA on cytotoxicity in normal cells and mice.*** Human monocyte-derived macrophages (HMDMs) were treated with ONA for 24 hours and cell viability was tested by WST assay (A). EBV transformed B-lymphoblast cell line (103-LCL, 104-LCL) were treated with ONA for 24 hours and cell viability was tested by WST assay (B). PBMCs from two donors were mixed at 1:1 ratio following cultured for 5days with or without ONA, and the 3H-thymidine incorporation assay was performed (C). ONA (60 mg/kg) was administrated to mice fourth a week for 2 weeks, followed by determination of AST, ALT and creatinin concentration in blood (D). The data are presented as the mean±SD. *: p-value <0.05, **: p-value <0.01 vs. control.



Supplementary Information 8. ***Inhibitory mechanism of ONA on EOC progression.*** As shown in the schematic diagram, ONA has indirect and direct anti-cancer effects. Indirect effect is to control differentiation into M2-polarization of macrophages that support EOC progression. Direct effect includes to inhibit EOC cells' proliferation and to assist anti-cancer effect of anti-cancer drugs.