



Supplemental Material to:

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Three-dimensional culture sensitizes epithelial ovarian cancer cells to EZH2 methyltransferase inhibition

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Supplemental Figure 1. The EZH2 inhibitor GSK343 does not significantly affect the growth of human EOC cells in 2D monolayer culture. (A) OVCAR10 cells were treated with the indicated concentrations of GSK343 or vehicle control (0.1% DMSO). Expression of EZH2, H3K27Me3, H3K9Me3 and β -actin in these cells was determined by immunoblotting. (B) Same as (A) but for SKOV3 cells. (C-E) Cell growth curves for the indicated EOC cells treated with GSK343 (1 μ M) or vehicle control (0.1% DMSO) over 12 days. Media was changed and cells counted every 3 days, time points represent the mean of 3 independent experiments with SD. * $p > 0.05$. (F) Same as (C-E) but assayed for expression of H3K27Me3 and β -actin by immunoblotting.

Supplemental Figure 2. The EZH2 inhibitor GSK343 decreases the number of human EOC cells in 3D culture. Equal number of the indicated human EOC cells cultured in 3D treated with 1 μ M GSK343 or vehicle control (0.1% DMSO) for 4 days and the number of the cells in each of the indicated groups were counted. * $p < 0.01$.

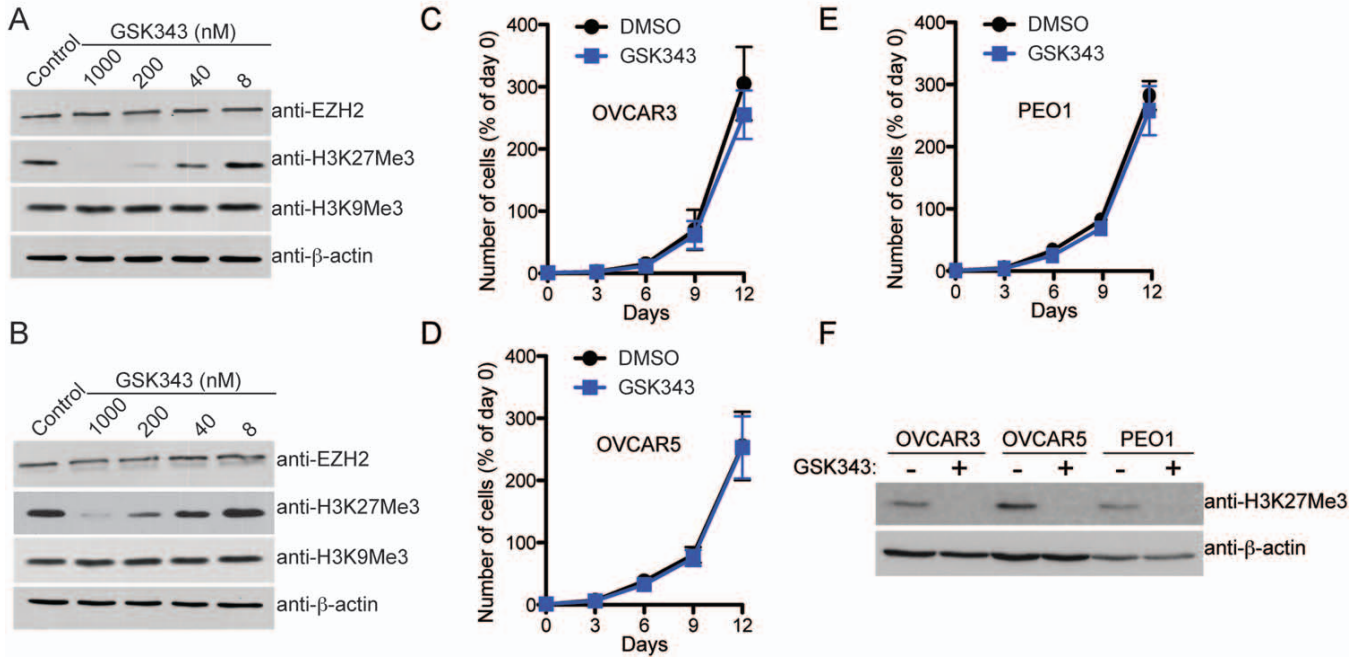
Supplemental Figure 3. The EZH2 inhibitor GSK343 suppresses invasion of human EOC cells.

(A) Equal number of OVCAR10 EOC cells treated with 1 μ M GSK343 or vehicle control (0.1% DMSO) were assayed for invasion through Matrigel-coated membrane. The cells invaded through Matrigel-coated membrane were stained with 1% crystal violet in PBS. (B) Quantification of (A). Number of SKOV3 cells migrated through control membrane or invaded through Matrigel-coated membrane. Numbers represent mean of 3 independent experiments. * $p < 0.05$. (C-D) Same as (A-B) but for UPN289 cells. * $p < 0.05$.

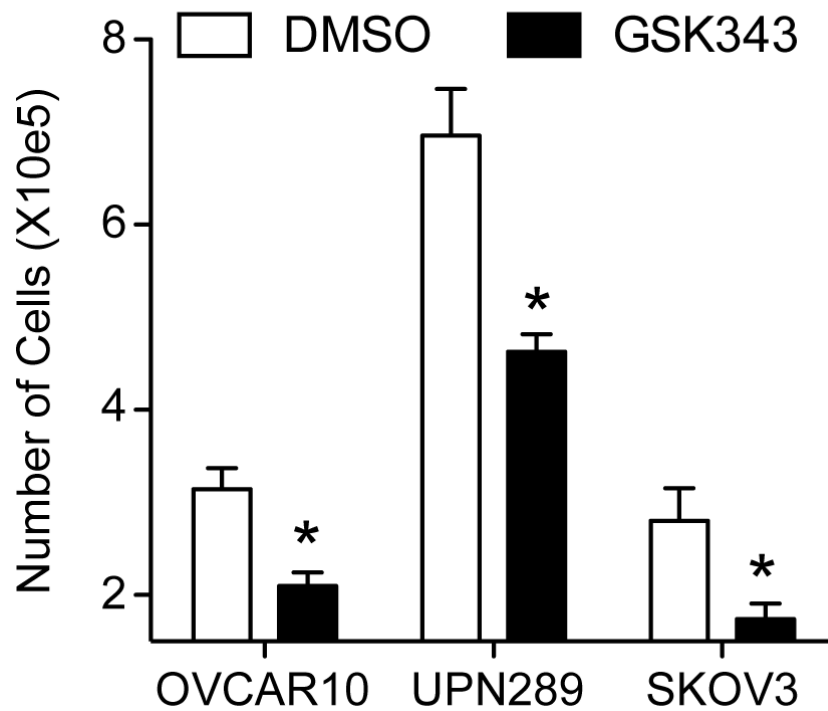
Supplemental Figure 4. The EZH2 inhibitor GSK343 induces apoptosis and decreases the G2/M population of EOC cells cultured in 3D conditions.

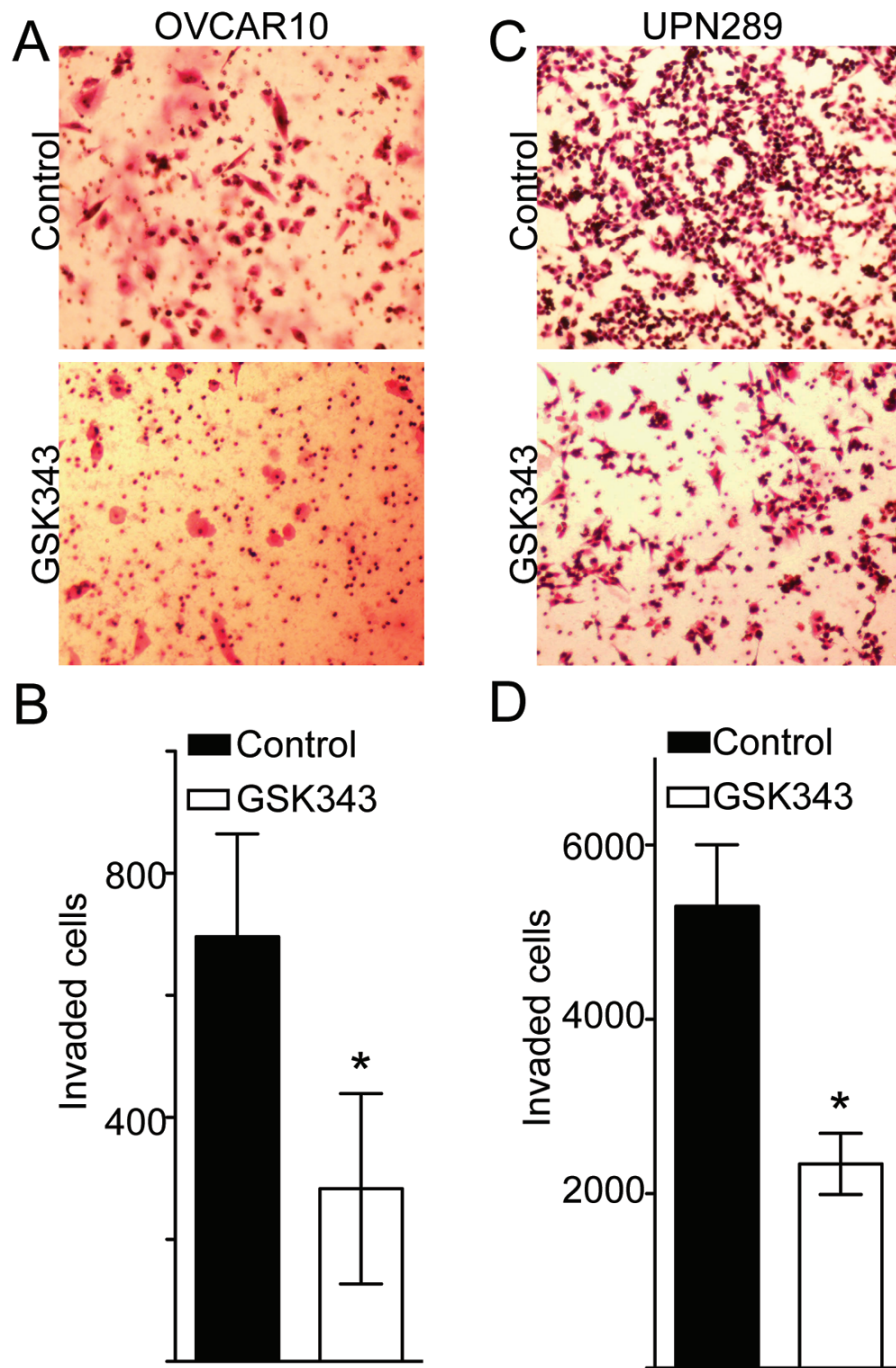
(A) The percentage of sub-G1, G1, S, and G2/M of OVCAR10 cells treated with 1 μ M GSK343 (black bars) or 0.1% DMSO vehicle control (white bars) after 4 days of growth in 2D monolayer culture. Mean of 3 independent experiments. (B) Same as (A) but for OVCAR10 cells cultured in 3D in Matrigel. Mean of three independent experiment with SD. * $p < 0.05$. (C-D) Same as (A-B) but for UPN289 cells. Mean of 3 independent experiments with SD. * $p < 0.05$.

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