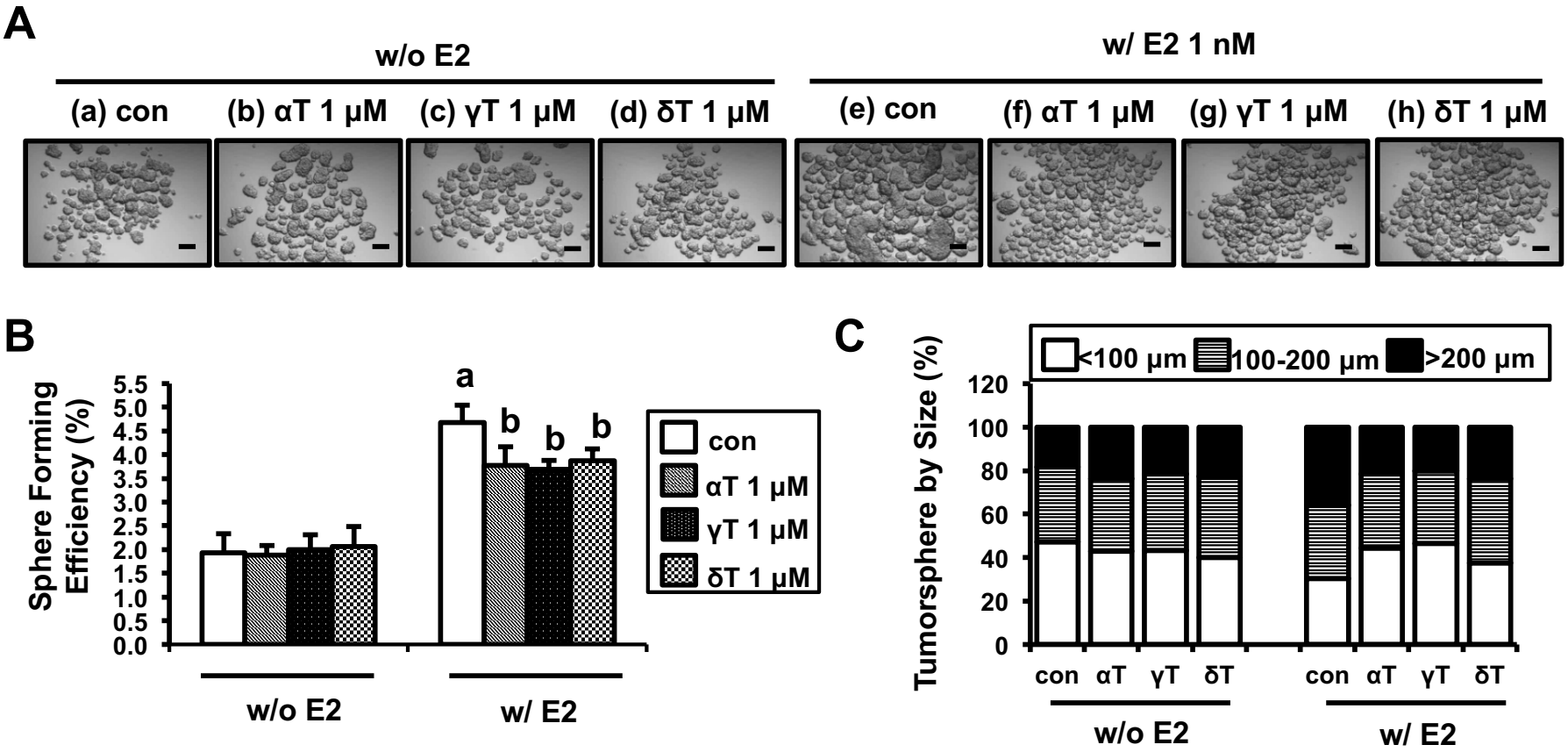
Supplementary Data 1

Tocopherols inhibit estrogen-induced cancer stemness and OCT4 signaling in breast cancer

Min Ji Bak[1], Philip Furmanski[1], Naing Lin Shan[1], Hong Jin Lee[2], Cheng Bao[2], Yong Lin [3,4], Weichung Joe Shih [3,4], Chung S, Yang[1,4], and Nanjoo Suh[1,4,*]



Supplementary Figure S1. Effects of tocopherols on formation and size of T47D tumorspheres. (A) T47D cells were plated at a density of 10,000 cells/mL in ultra-low attachment 6-well plates and grown for 4 days in the presence of estrogen (E2, 1 nM) or/and α-, γ-, δ-tocopherol (1 μM). Representative pictures of T47D tumorspheres are shown for phenotypic comparison, scale bar 100 μm. (B) Sphere forming efficiency (SFE) of T47D tumorspheres is shown. SFE was calculated by dividing the number of tumorspheres (>100 μm) formed by the number of cells seeded presenting this as a percentage. The data are represented as mean ± SD. n=3 independent experiments in triplicate, [a,b] Significantly different from the control and E2, respectively ($p<0.05$). (C) The size of tumorspheres was divided into three ranges (<100, 100-200 and >200 μm). Average number of tumorspheres in each size range is shown in the graph.

Supplementary Data 2

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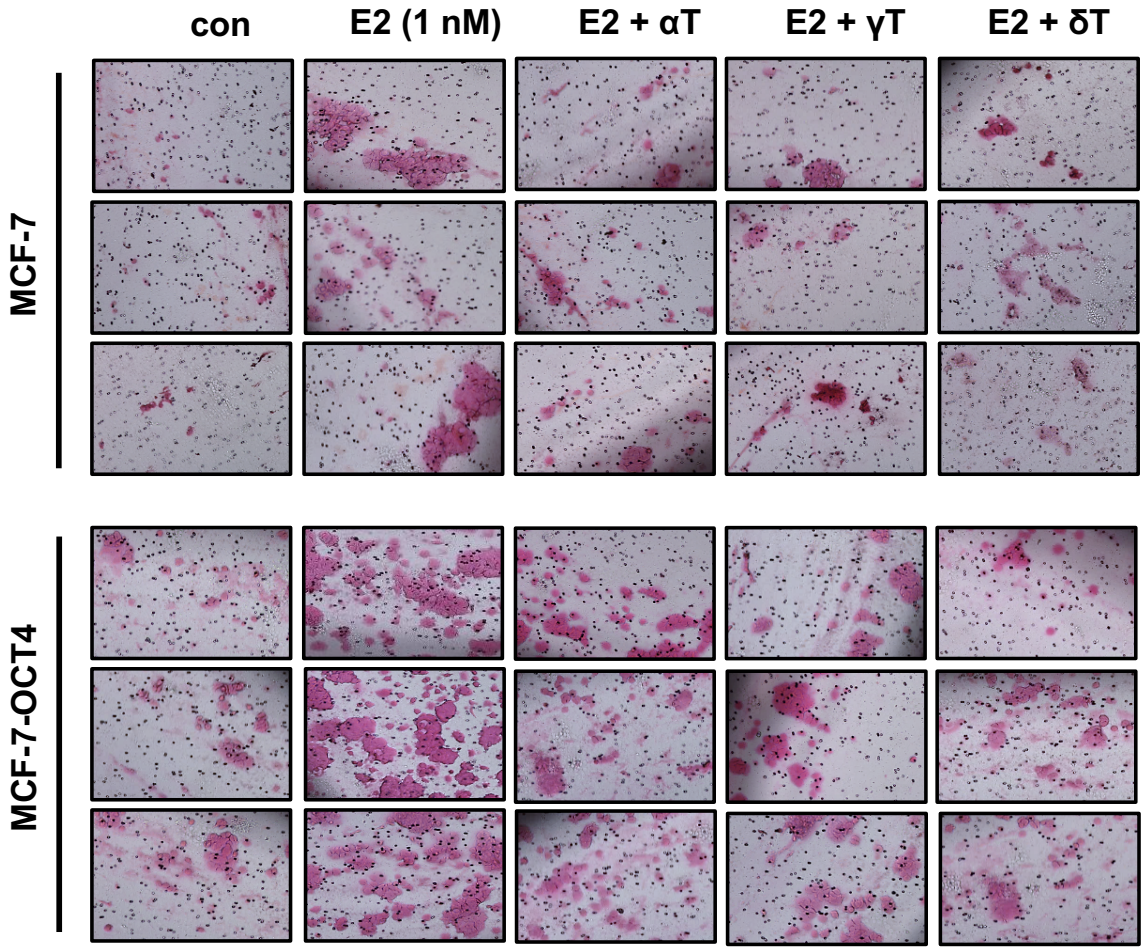

Supplementary Figure S2. Tocopherols inhibit cell invasion induced by estrogen and OCT4. Cells were transfected with control vector (MCF-7) and pSin-EF2-OCT4-Pur vector (MCF-7-OCT4) for 24 h and then cells were treated with estrogen (E2, 1 nM) and/or α-, γ-, δ-tocopherol (1 μM) for an additional 24 h. Cells ($8x10^4$ cells/mL) were loaded onto transwells coated with matrigel. After 22 h incubation, the transwells were stained with H&E stain. Representative pictures are shown, 10X magnification.

Supplementary Data 3

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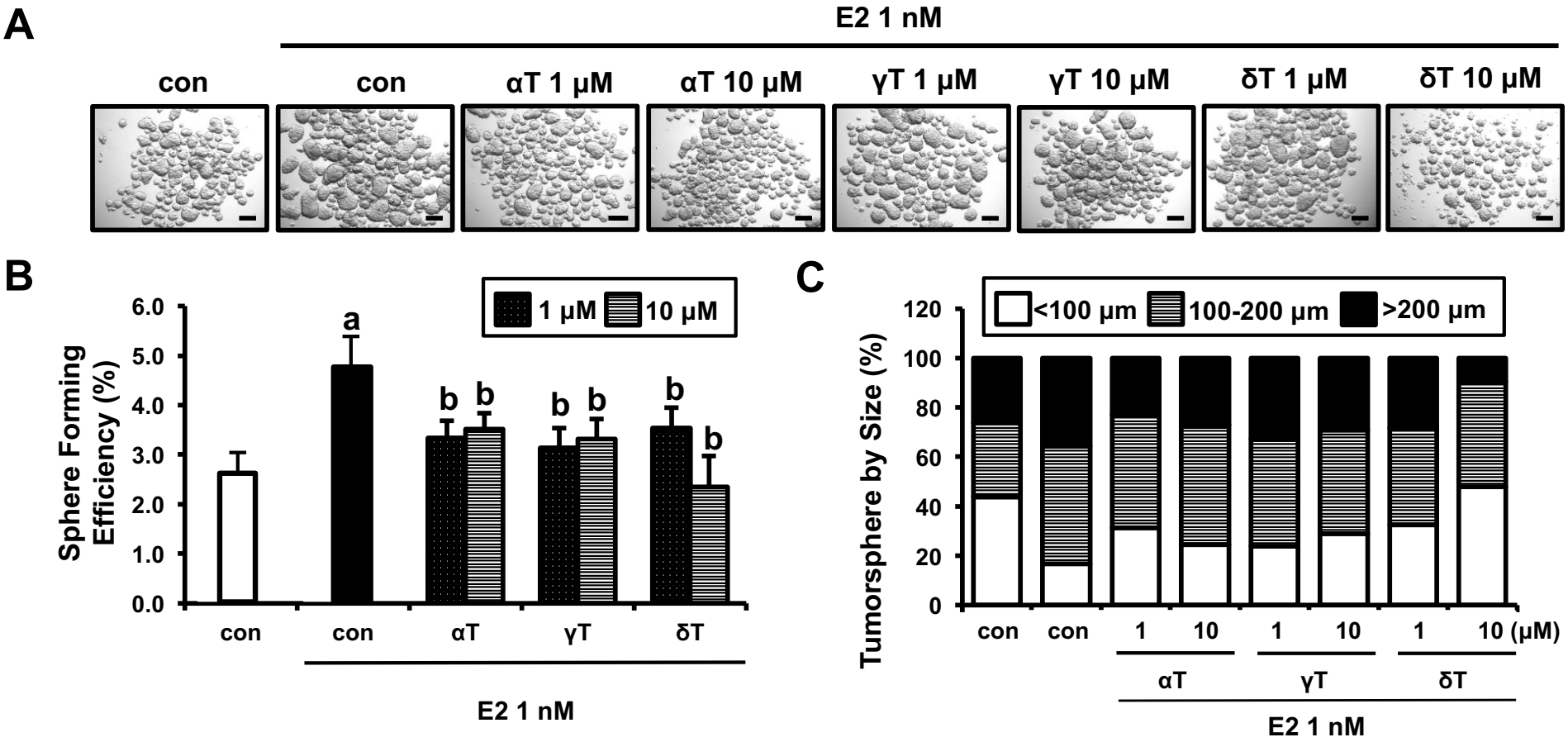

Supplementary Figure S3. Effects of tocopherols on formation and size of MCF-7 tumorspheres. (A) MCF-7 cells were plated at a density of 10,000 cells/mL in ultra-low attachment 6-well plates and grown for 4 days in the presence of estrogen (E2, 1 nM) or/and α-, γ-, δ-tocopherol (1 and 10 μM). Representative pictures of MCF-7 tumorspheres are shown for phenotypic comparison, scale bar 100 μm. (B) Sphere forming efficiency (SFE) of MCF-7 tumorspheres is shown. SFE was calculated by dividing the number of tumorspheres (>100 μm) formed by the number of cells seeded presenting this as a percentage. The data are represented as mean ± SD. n=2 independent experiments in triplicate, [a,b] Significantly different from the control and E2, respectively ($p<0.05$). (C) The size of tumorspheres was divided into three ranges (<100, 100-200 and >200 μm). Average number of tumorspheres in each size range is shown in the graph.

Supplementary Data 4

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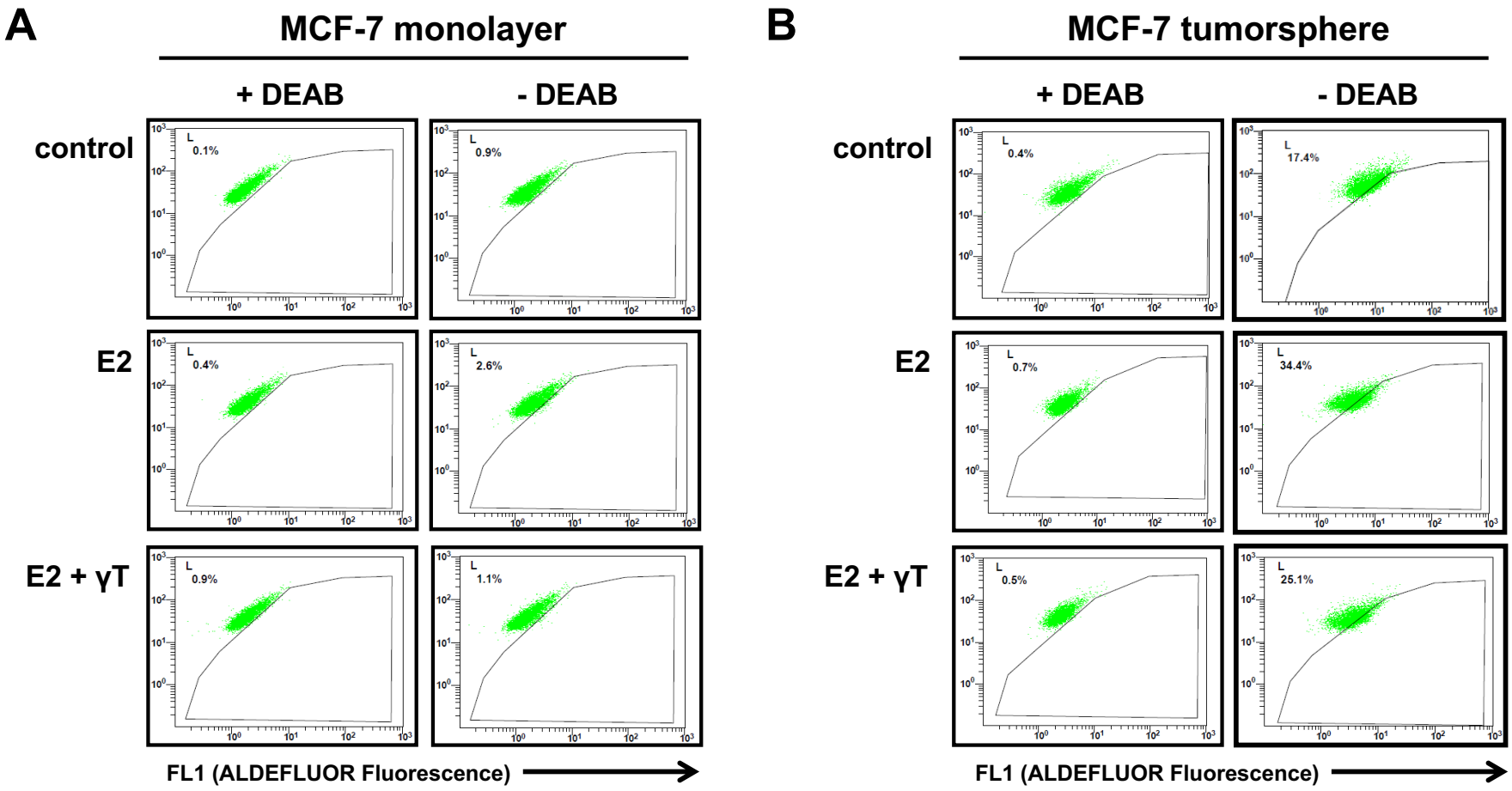

Supplementary Figure S4. Effects of γ-tocopherol on ALDH activity in MCF-7 monolayer and tumorspheres. (A) MCF-7 monolayer cells were treated with estrogen (E2, 1 nM) and γ-tocopherol (1 μM) for 24 h. (B) MCF-7 tumorspheres were formed by plating 10,000 cells/mL in ultra-low attachment 6-well plates and treated with E2 (1 nM) and γ-tocopherol (1 μM) for 4 days. Cells were assayed with ALDEFLUOR assay kit as per the manufacturer's guidelines. As a negative control for all experiments, cells were incubated with diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor. Experiments were performed in triplicate and representative histograms from flow cytometry are shown.

Supplementary Data 5

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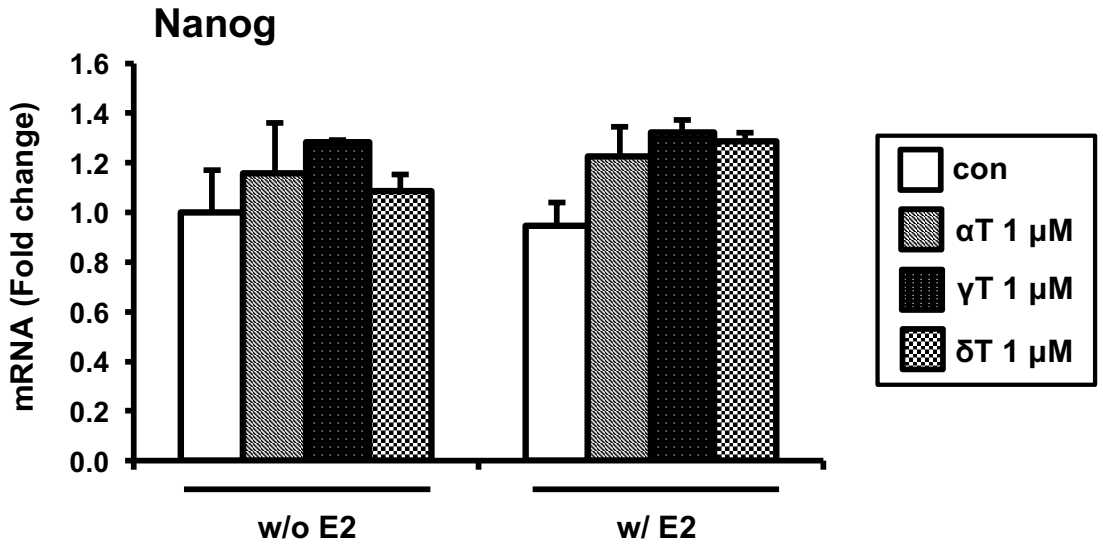

Supplementary Figure S5. Effects of estrogen and/or tocopherols on the mRNA level of Nanog in MCF-7 tumorspheres. qPCR analysis was performed on MCF-7 tumorspheres collected from 4 days of treatment with estrogen (E2, 1 nM) and/or tocopherols (1 μM), and analyzed for Nanog. Cycle numbers for control group were 30. The data are represented as mean ± SD. n=3 independent experiments.

Supplementary Data 6

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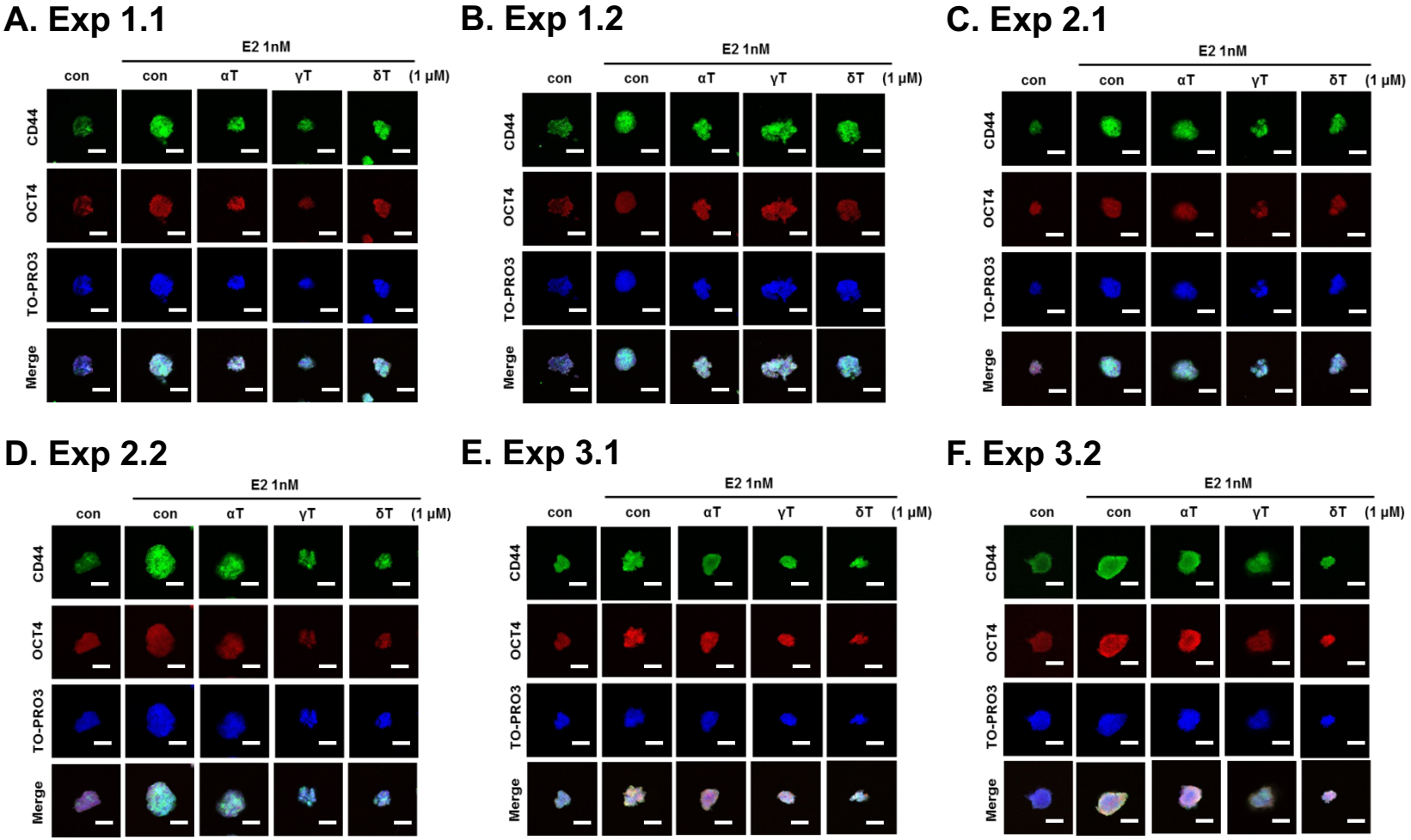

Supplementary Figure S6. Effects of tocopherols on expression of CD44 and OCT4 in estrogen-treated MCF-7 tumorspheres. Immunofluorescence analysis was performed on MCF-7 tumorspheres collected from 4 days of treatment with control, estrogen (E2, 1 nM) or tocopherols (1 μM). MCF-7 tumorspheres were fixed using 4% paraformaldehyde and stained with antibodies against CD44 (green) and OCT4 (red). Nuclei were stained with TO-PRO3 (blue). Three independent experiments in duplicate were performed and shown as A, B, C, D, E and F. Scale bars: 200 μm.

Supplementary Data 7

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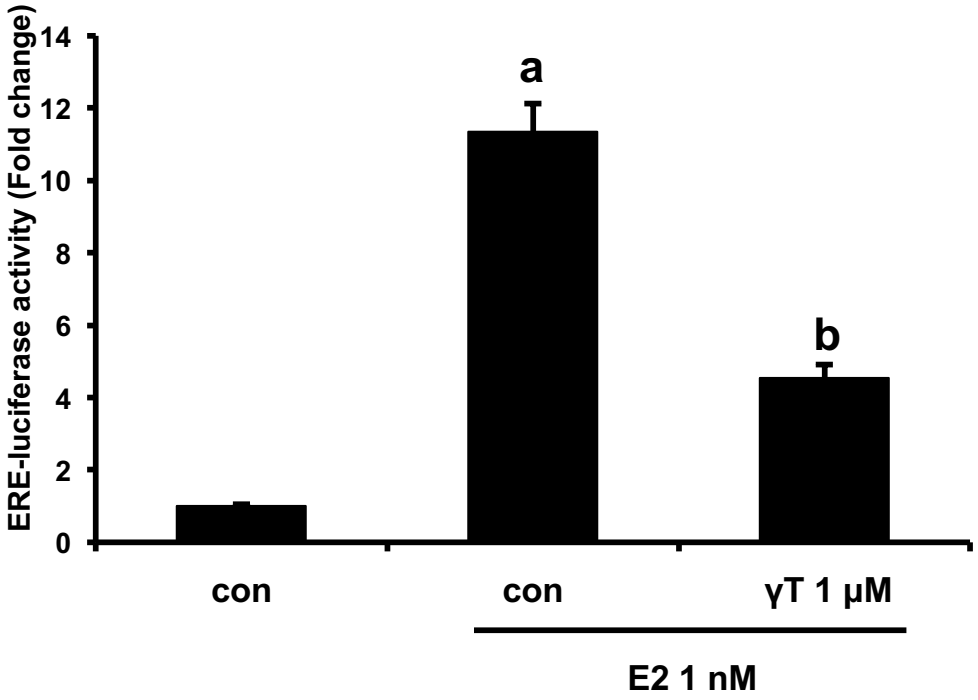

Supplementary Figure S7. Effects of estrogen and/or γ-tocopherol on ERE-luciferase activity in MCF-7 cells. Cells were transfected with the ERE-TATA-luc reporter plasmid for 24 h and then cells were treated with estrogen (E2, 1 nM) and/or γ-tocopherol (1 μM) for an additional 12 h. The data are represented as mean ± standard deviation (SD). n=3 independent experiments, [a,b] significantly different from the control and E2, respectively ($p<0.05$).