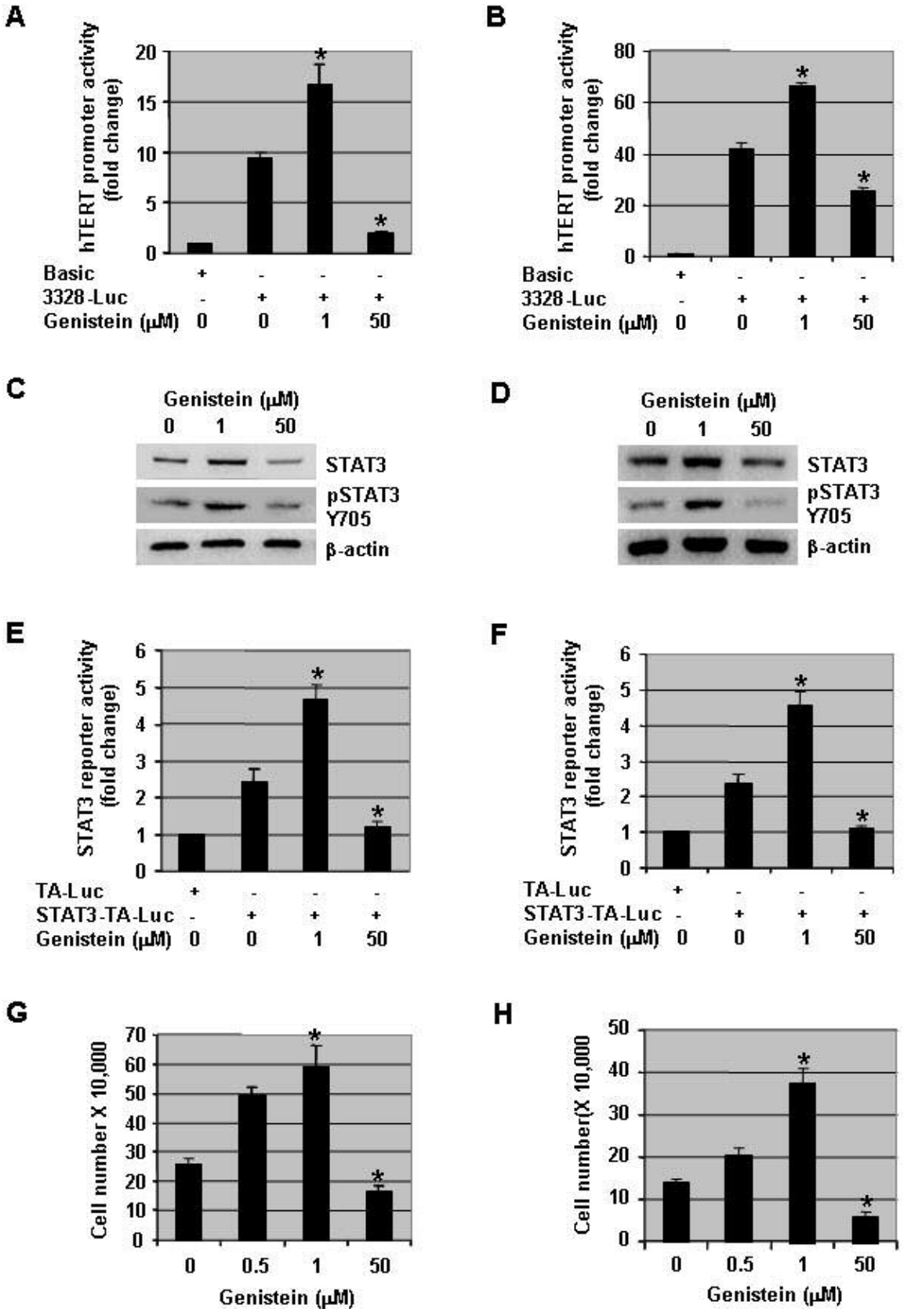
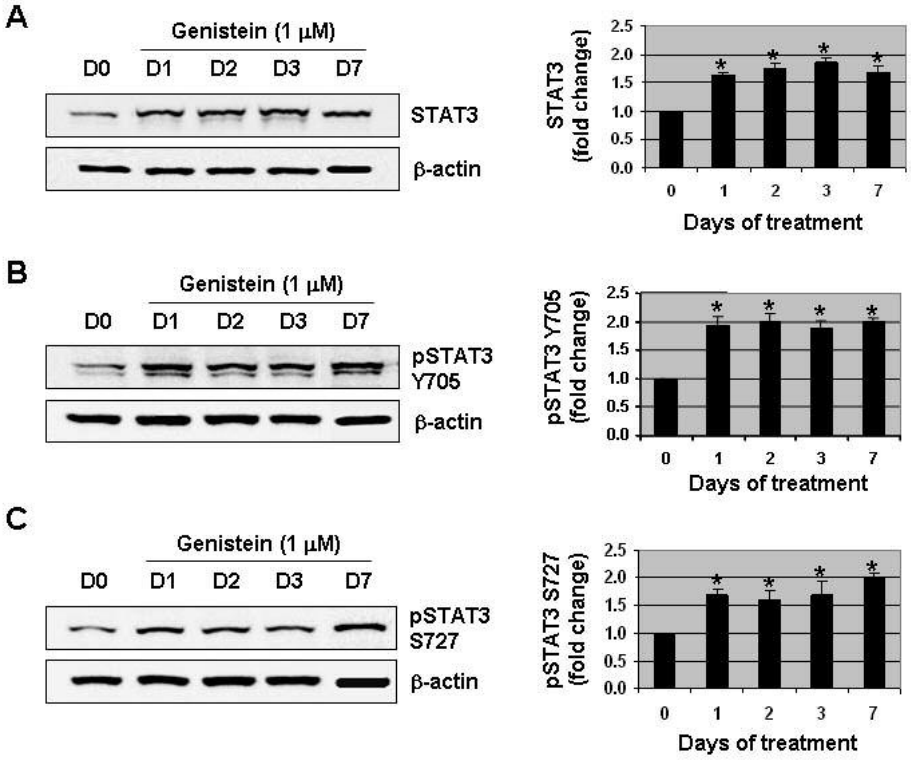
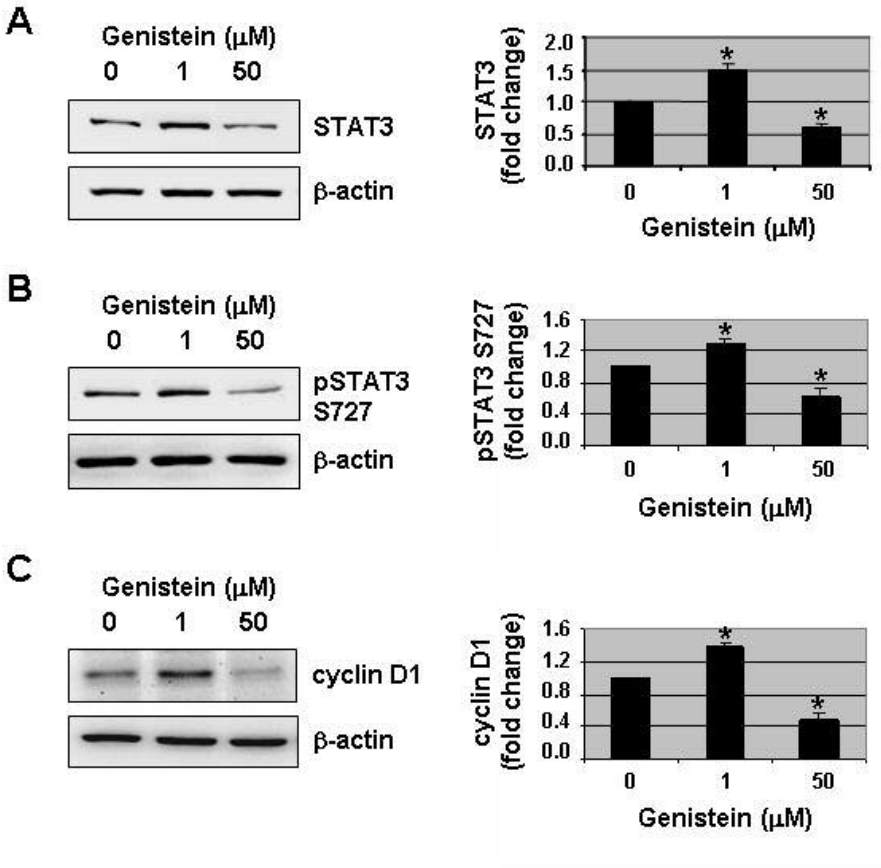


Supplementary Figure 1. Physiological concentrations of genistein enhance hTERT promoter activity, STAT3 reporter activity, and proliferation of other cancer cell lines. Data for MCF-7 cells are on the left and data for SKOV-3 cells are on the right. (**A, B**)

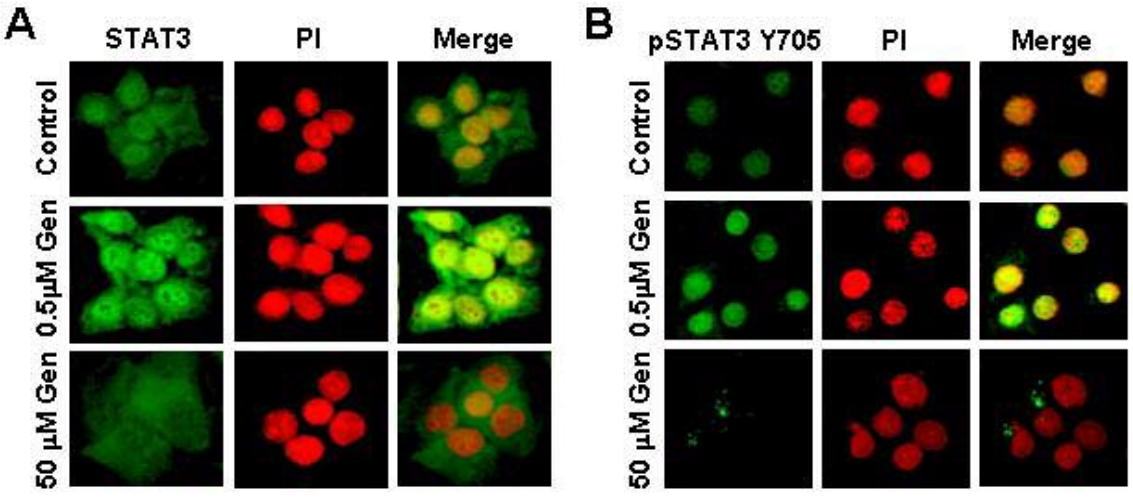
MCF-7 and SKOV-3 cells were transfected with full-length hTERT promoter luciferase plasmid (pGL3-3328-Luc) and Renilla luciferase (pRL-TK-Luc) plasmid and then treated with 0, 1, and 50 μM of genistein for 2 days. Cells were harvested and promoter assays were performed. hTERT promoter activity was normalized to Renilla luciferase activity. Promoter activity in control samples (with pGL3-basic) was considered as 1.0. (**C, D**) Protein lysates (50 μg) from MCF-7 and SKOV3 cells treated with 1 and 50 μM of genistein for 3 days were resolved on 12% SDS-PAGE and immunoblots were probed with antibodies to total STAT3 and pSTAT3 Y705. Immunoblots were reprobed with β-actin antibody to ensure equal loading. Representative photographs from an experiment that was repeated twice. (**E, F**) MCF-7 and SKOV-3 cells were transfected with STAT3-TA luciferase plasmid and Renilla luciferase (pRL-TK-Luc) plasmid and then exposed to 0, 1, and 50 μM of genistein for 2 days. Cells were harvested and reporter assays were performed. STAT3 reporter activity was normalized to Renilla luciferase activity. Reporter activity in control samples (with TA-Luc) was considered as 1.0. (**G, H**) MCF-7 and SKOV-3 cells were treated with various concentrations of genistein (0, 0.5, 1, and 50 μM) for 3 days and then viable cells (as assessed by trypan blue exclusion) were counted using a hemocytometer. Columns, mean of three independent experiments; bars, SE. *, $p<0.01$, significantly different from control.



Supplementary Figure 2. Sustained activation of STAT3 in DU-145 cells by physiologically achievable concentrations of genistein. (**A-C**) Protein lysates (50 μg) from DU-145 cells treated with 1 μM of genistein for various time points (0, 1, 2, 3, and 7 days) were resolved on 12% SDS-PAGE and immunoblots were probed with antibodies to total STAT3 (A), pSTAT3 Y705 (B), and pSTAT3 S727 (C). All immunoblots were reprobed with β-actin antibody to ensure equal loading. Representative photographs from an experiment that was repeated thrice. Quantitative analyses of relative levels of total STAT3 and pSTAT3 (Y705 and S727) are shown on the right panels. Columns, mean; bars, SE. *, $p<0.01$, significantly different from control.



Supplementary Figure 3. Genistein-induced activation of STAT3 is not cell line specific. (**A-C**) Protein lysates (50 μg) from LNCaP cells treated with 0, 1, and 50 μM of genistein for 3 days were resolved on 12% SDS-PAGE and immunoblots were probed with antibodies to total STAT3 (A), pSTAT3 S727 (B), and cyclin D1 (C). All immunoblots were reprobed with β-actin antibody to ensure equal loading. Representative photographs from an experiment that was repeated thrice. Quantitative analyses of relative levels of total STAT3, pSTAT3 S727, and cyclin D1 are shown on the right panels. Columns, mean of three independent experiments; bars, SE. *, $p<0.01$, significantly different from control.



Supplementary Figure 4. Genistein increases the nuclear location of total and activated STAT3. DU-145 cells were plated on chamber slides and exposed to 0, 0.5, and 50 μM of genistein for 1 day. Then the cells were fixed in methanol, incubated with total STAT3 (**A**) or pSTAT3 Y705 (**B**) antibodies, and counterstained with propidium iodide (PI). Slides were mounted and examined using a fluorescence microscope. Photographs were taken at the same magnification (20x) and then imported to Adobe Photoshop.