Figure S1. Thiostrepton inhibits MDA-MB-436 cell growth in dose- and time-dependent manners. Cells were treated with two-fold dilutions of thiostrepton at concentrations ranging from 0 to 30 μM for 24 (blue), 48 (green) and 72 (red) h. Cell viability was measured by an MTT assay. Representative data from three independent experiments are presented. Statistical analyses were performed using one-way ANOVA with Dunett's post hoc test and compared with the control (0 μM). *P<0.05 was considered as statistically significant.

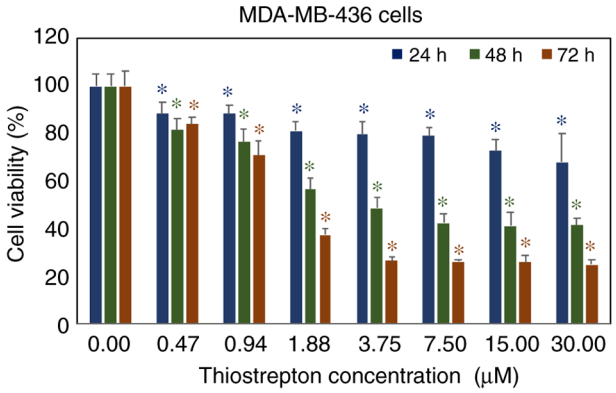

Figure S2. Triple-negative breast cancer MDA-MB-436 cells become senescent after exposure to thiostrepton. SA-β-gal staining of (A) untreated cells or cells treated with thiostrepton at concentrations of (B) 1 μM, (C) 2 μM and (D) 4 μM. The treated cells were stained for SA-β-gal activity (green color). Red arrows and blue asterisks indicate the morphological changes in senescent and dying cells. (E) The percentages of SA-β-gal-positive cells are presented as the mean ± standard deviation. Statistical analyses were performed using one-way ANOVA with Dunnett's post hoc test and compared with the control (0 μM). *P<0.05 (significant). SA-β-gal, senescence-associated β-galactosidase.

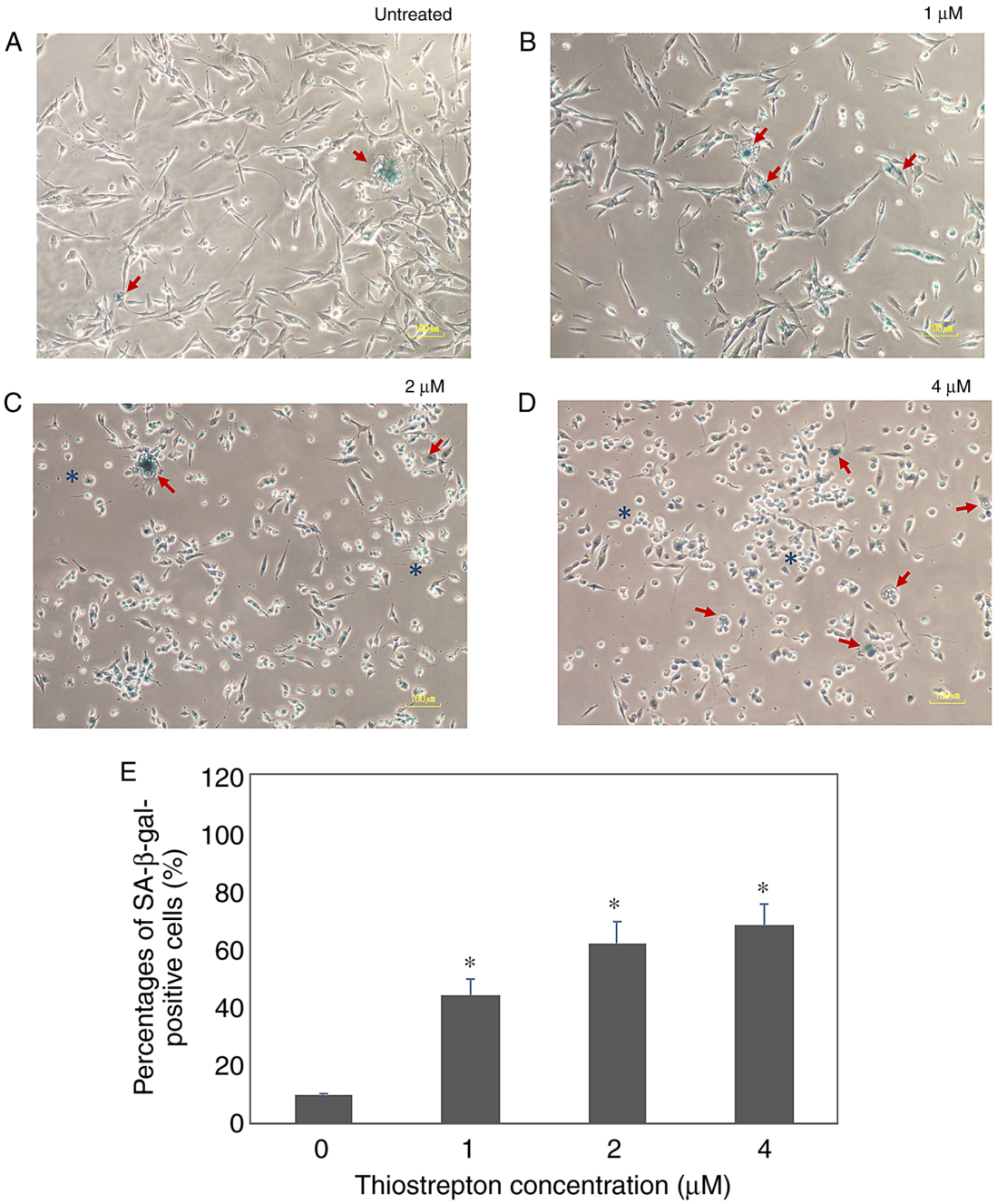

Figure S3. Thiostrepton downregulates the mRNA expression of FOXM1 downstream targets in a time-dependent manner. MCF-7 and MDA-MB-436 cells were treated with thiostrepton at a concentration of 7.5 μM for various lengths of time. RT-PCR analysis was performed to assess mRNA expression. FOXM1 and CCNB1 were the main downstream targets of the FOXM1 protein. β-actin served as the internal control. CCNB1, cyclin B1; FOXM1, Forkhead box M1.

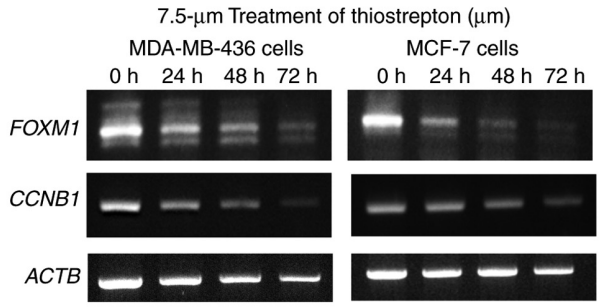

Figure S4. Triple-negative breast cancer MDA-MB-436 cells exhibit decreased mRNA expression of FOXM1 downstream targets following treatment with increasing concentrations of thiostrepton. *CCNB1* was the main downstream target of FOXM1, and β-actin served as the internal control. The RT-PCR results are representative of three independent experiments. *CCNB1,* cyclin B1; *ACTB,* β-actin; FOXM1, Forkhead box M1.