Appendix 1

Supplementary methods

Cell culture

MCF-7, MDA-MB-231, and T47D cell lines were cultured as previously described in the text method.

Plasmid construction

The *SUMO1* promoter region was amplified from genomic DNA by PCR and cloned into the plasmid pGL3-basic. Two truncated versions of the *SUMO1* promoter were constructed, and the promoter was fused with the luciferase reporter gene. SUMO1-WT-Luc (2884/+992) was amplified by sense 5'-GATCGGTACCCCAGTAGAAGCACTGAAATG-3' and antisense 59'-GATCCTCGAGTCGCTGGAGTCAGACGCTAAT-3'; the truncated SUMO1-M1-Luc (2297/+63) was amplified by sense 5'- GATCGGTACCAAAGCCAAAGAGCCTCC-3' and antisense 5'-GATCCTCGAGTTTTAAACCGGCAGCC-3'; the truncated SUMO1-M2-Luc (+552/+992) was amplified by sense 5'-GTACGGTACCGAGCTGCGGCCGATTCC-3' and antisense 5'-GATCCTCGAGTCGCTGGAGTCAGACGCTAAT-3'. The ERE½ and ERE in SUMO1 WT-Luc were mutated using a site-directed mutagenesis kit. The mutant SUMO1-M3-Luc contained base substitutions in the ERE½ of SUMO1 (+2/+6), but the mutant SUMO1-M4-Luc contained base substitutions in the ERE of SUMO1 (+753/+764), while the mutant SUMO1-M5-Luc contained base substitutions at ERE½ and ERE. The mutants were generated by primers: SUMO1M3-Luc (sense 5'-CCGCGGGGTCGCTTGCGACGCATGCGCCGG-3', and antisense 5'-CCGGCGCATGCGTCGCAAGCGACCCCGCGG-3'), SUMO1-M4-Luc (sense 5'-CTGGGGACCCGCTAGGCAATGTTGCGCACTTTATTCCTGTCA-3', antisense 5'-TGACAGGAATAAAGTGCGCAACATTGCCTAGCGGGTCCCCAG-3'). The ERα shRNA-expression vector was constructed by DNA vector-based shRNA synthesis using the vector pRNATU6.1 (GenScript). ERα: 5'-GCTACTGTTTGCTCCTAAC-3', and the sequences for silencing the expression of SUMO1 were 5'-AGTTTGTGTGCCTCAAATC-3'; the control shRNA: 5'-GACGCTTACCGATTCAGAA-3'.

Luciferase reporter assay

After transfection for 24 h, cells were washed with phosphate-buffered saline (PBS), and the luciferase activity was measured. In brief, cells were lysed in a cold buffer, and assay buffer and luciferin potassium salt were added to the cell lysate. Then, the luciferase activity was detected. Transfection efficiency was examined by transfecting the cells with a β-galactosidase construct. Finally, cell lysate was added to the β-galactosidase buffer and the absorbance was measured at 450 nm.

RNA extraction and RT-qPCR

Total RNA was extracted from cells by Trizol (Invitrogen). Total RNA was reverse transcribed into cDNA by Omniscript RT kit (Qiagen). RT-qPCR was applied by Mastercycler Ep Realplex (Eppendorf 2S) with these primers: SUMO1 sense: 5'-AAGTTAGGGCTGAAAGACGACGA-3' and antisense 5'-GAACTCCGAGAAGAGGCAGAAG-3'; GAPDH sense: 5'-GAAGGTGAAGGTCGGAGTC-3' and antisense 5'-GAAGATGGTGATGGGATTTC-3'. RT-qPCR analysis followed the instructions of the Maxima SYBR Green RT-qPCR Master Mix (Thermo Scientific). 10-fold serial dilutions of cDNA produced from cells were used for RT-qPCR assay to generate a set of standard curve data. To evaluate the quality of RT-qPCR products, a melting curve analysis was applied. Relative expression was calculated by the ΔCt method with GAPDH (internal reference).

Western blot

The preparation of cell extracts and subsequent western blot analysis wereperformed as previously described in the text method.

ChIP assay

Cells were cultured for 2 days in phenol red-free DMEM containing 5% charcoal-dextran-stripped FBS. Next, they

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were treated with or without 1 mM E2 for 1 h and cross-linked with 1% formaldehyde in PBS at room temperature for 15 min. Cell lysates were sonicated to DNA fragments of 300–1500 bp, which were diluted at 1:10 in dilution buffer. Protein A and anti-ERα or rabbit IgG were added to the diluted sheared chromatin, and the mixture was cultured at 4℃ overnight. Immunoprecipitated chromatin was purified from the chromatin–antibody mixture and eluted in the elution buffer. PCR was performed on the isolated DNA to amplify the region using specific primers for SUMO1 (+672/+805): 5'-GAGCTGCGGCCGATTCC-3' (sense) and 5'-GCTGCTCCAAACGTGC-3' (antisense); 5'-AAAGCCAAAGAGCCTCC-3' (sense) and 5'-TTTTAAACCGGCAGCC-3' (antisense) for SUMO1 (–297/+63); and 5'-TGAAAGAGGGAGGAGTCAAAGAT-3' (sense) and 5'-AGCAAGACGGAGGCAAAGTTATT-3' (antisense) for SUMO1 (–1866/–1626). Total input DNA was used as a positive control. An anti-IgG antibody was used as a non-specific control. The product of RT-qPCR was displayed by 1.5% agarose gel electrophoresis.

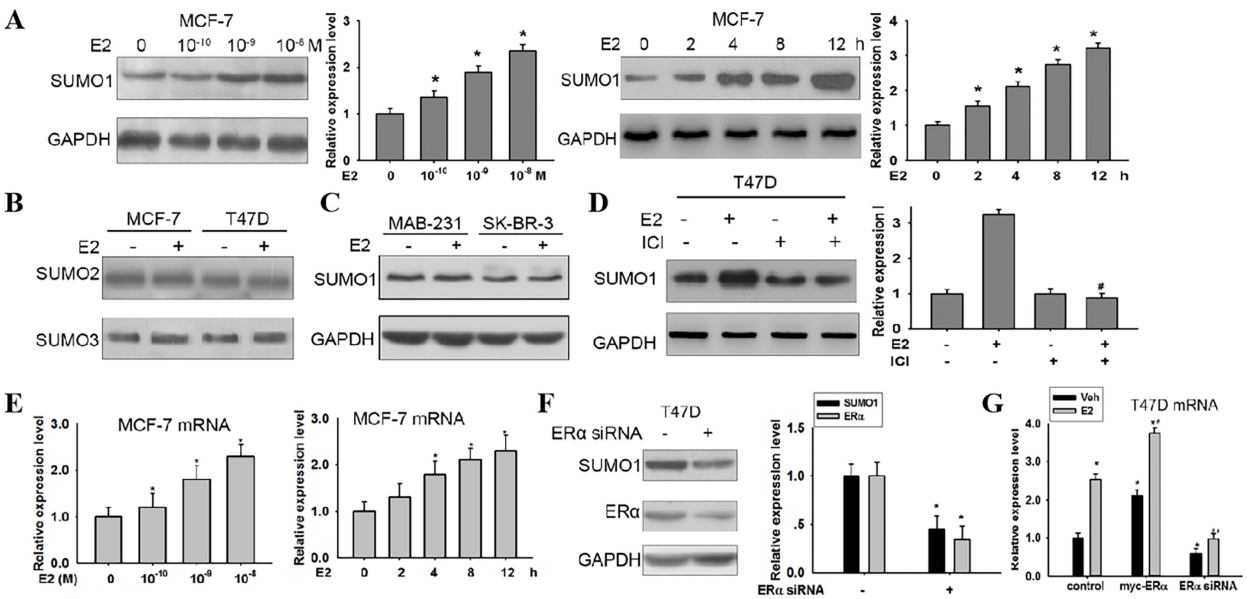

Figure S1 ERα regulates the expression of *SUMO*1 in breast cancer cells. (A,B) MCF-7 cells were treated with different concentrations of E2 for 12 h or 10 mM E2 for a different time (0, 2, 4, 8, 12 h), and *SUMO1* expression was confirmed by western blot. *, compared with control group, P<0.05. (B) MCF-7 and T47D cells were treated with vehicle (control) or 1 mM E2 for 24 h, and the SUMO2 and SUMO3 expressions were detected by western blot. (C) T47D cells were treated with vehicle, 1 mM E2 or 0.1 mM ICI alone or combined for 24 h, and SUMO1 expression was examined by western blot. (D,E) RT-qPCR was applied to evaluate the *SUMO1* expression in cells were treated with E2 or ICI alone or in combination for 24 h. *, compared with the untreated group, P<0.05; #, compared with the E2 treated group, P<0.05. (F) T47D cells transfected with control siRNA or ERα siRNA for 48 h and treated with 1 mM E2 for the expression of SUMO1 and ERα. *, compared with the untransfected group, P<0.05. (G) T47D cells transfected with ERα overexpression or ERα siRNA vector for detection of the level of SUMO1 mRNA by RT-qPCR. *, compared with the untransfected group, P<0.05; #, compared with the E2 treated group, P<0.05; ##, compared with the E2 treated ERα overexpression group, P<0.05.

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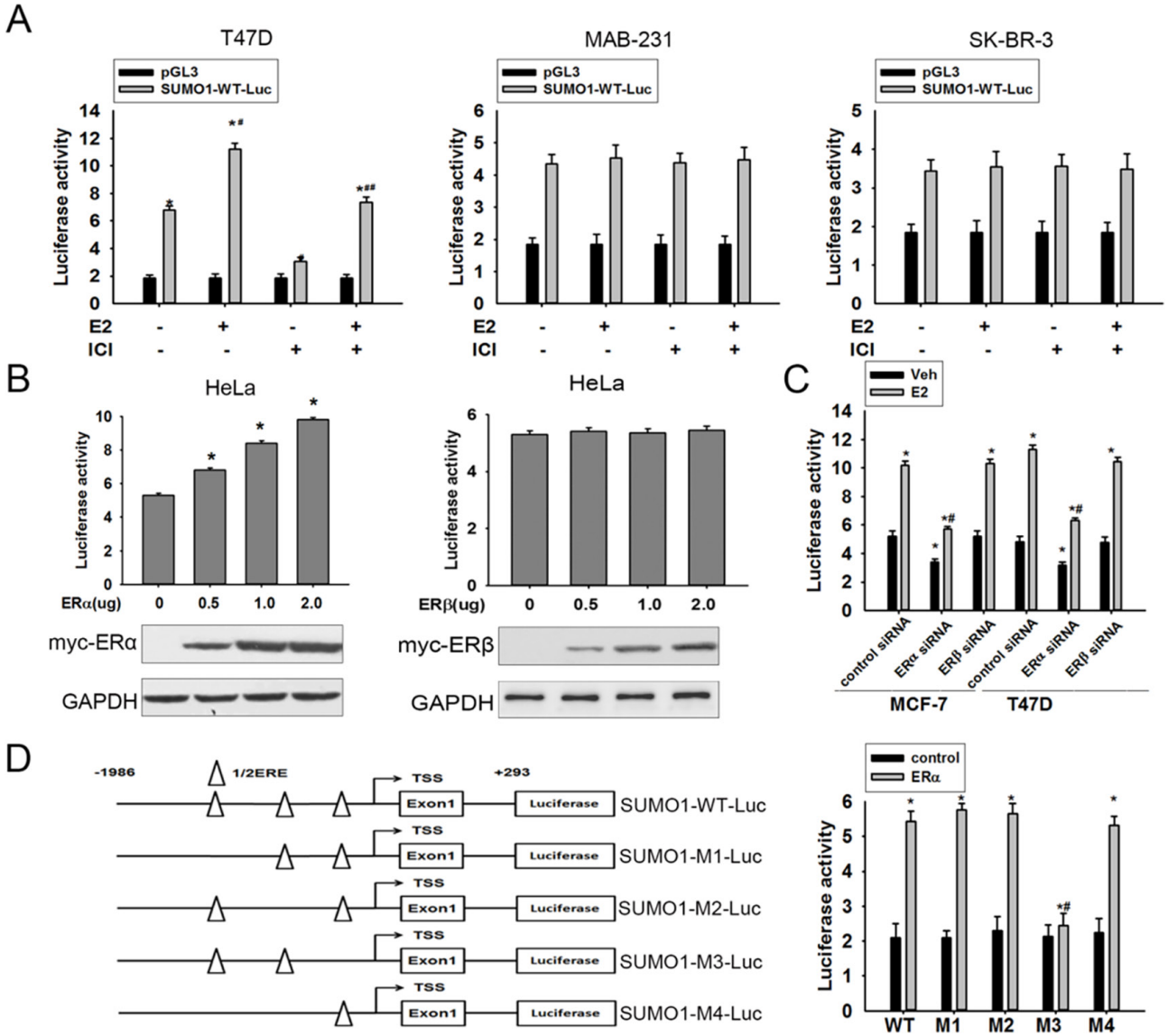

Figure S2 ERα binds to *SUMO1* promoter regions, and regulates *SUMO1* promoter activity. (A) Cells transfected with SUMO1-WT-Luc and subsequent treatment with E2 or ICI alone or in combination. *, compared with untreated group, P<0.05; #, compared with untreated SUMO-WT-Luc group, P<0.05; ##, compared with E2-treated SUMO1-WT-Luc group, P<0.05. (B) HeLa cells transfected with different doses of ERs vectors for detection of luciferase activity. *, compared with the untransfected group. (C) MCF-7 and T47D cells transfected with different siRNA vectors for detection of luciferase activity. *, compared with the untreated SUMO-WT-Luc group, P<0.05; #, compared with E2 treated SUMO1-WT-Luc group, P<0.05. (D) HeLa cells transfected with different luciferase vectors combined with ERα for detection of luciferase activity. *, compared with untreated SUMO1-WT-Luc group, P<0.05; #, compared with ERα co-transfected SUMO1-WT-Luc group, P<0.05.

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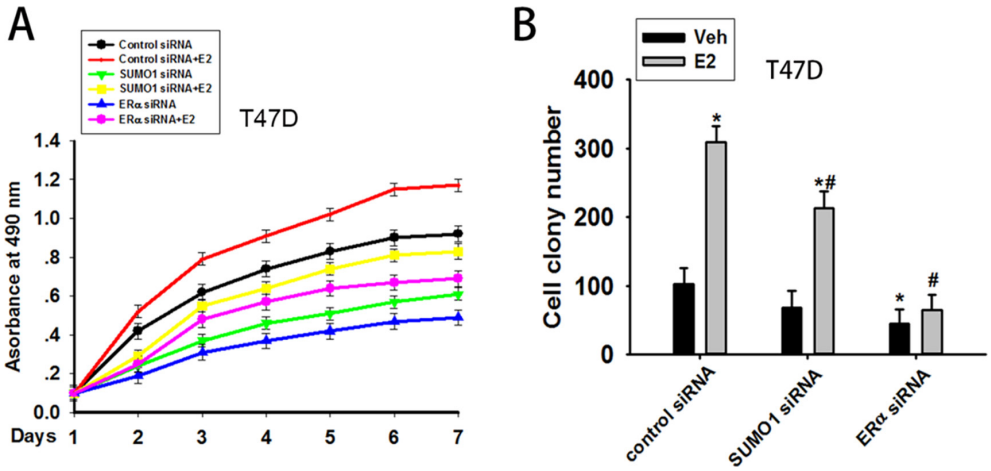

Figure S3 ERα-induced *SUMO1* expression promotes BC cells proliferation. (A) In the presence or absence of E2 for 6 or 7 days, T47D cells were transfected with control siRNA, *SUMO1* siRNA, or ERα siRNA followed by MTT assay. (B) T47D cells transfected with indicated vectors in the presence or absence of E2 for 7 days and colonies were stained with crystal violet and counted. *, compared with untreated group, P<0.05; #, compared with E2 treated group, P<0.05.

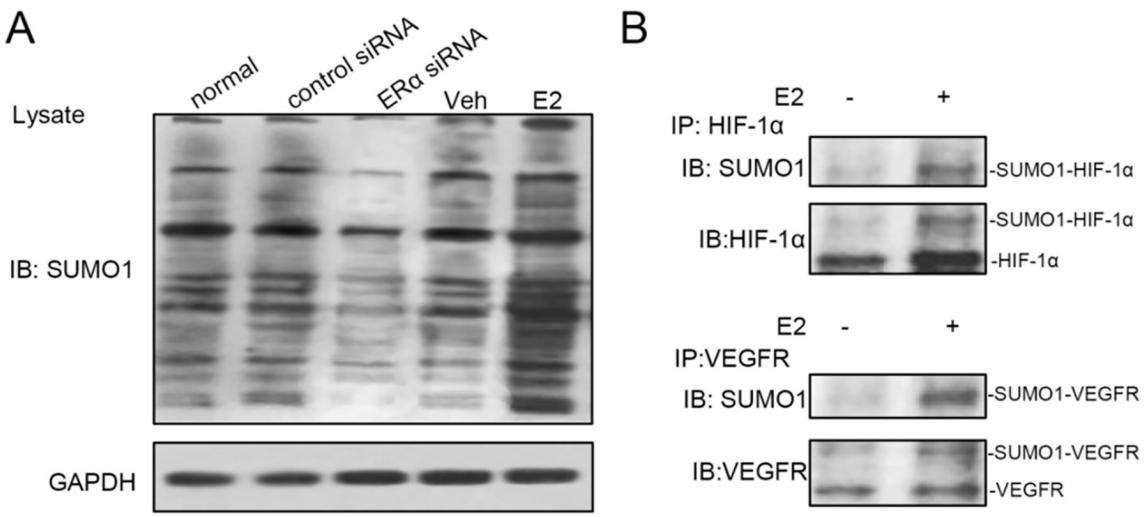

Figure S4 ERα induced SUMO1-mediated protein SUMOylation in breast cancer cells. (A) MCF-7 cells transfected with ERα siRNA or treated with E2 for detection of SUMO1-mediated protein SUMOylation by western blot. (B) MCF-7 cells pretreated with E2, then lysed to detect the binding between HIF1α and VEGFR with SUMO1 by immunoprecipitation.

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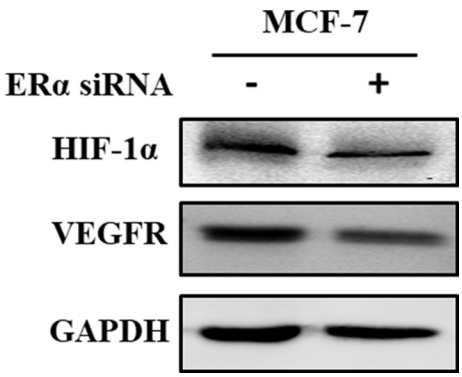

Figure S5 Expression of VEFGR and HIF1, after SUMOylation detected by western blot.

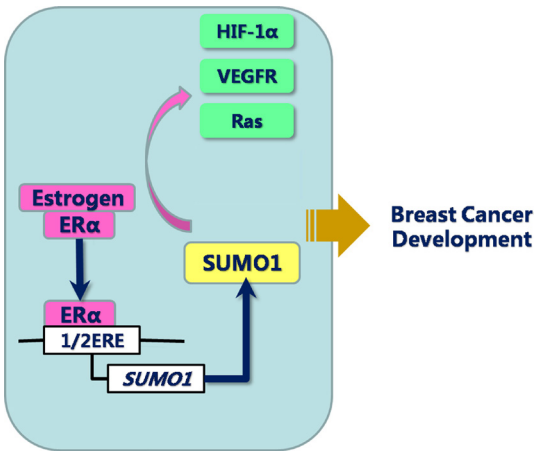

Figure S6 Schematic diagram of crosstalk between E2-ERα signaling and SUMO1-mediated protein SUMOylation.

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