Figure S1. Lin28 is negatively associated with let-7 in breast cancer cells. (A) RT-qPCR analysis of the RNA expression levels of let-7 and Lin28 in T-47D cells transfected with let-7 mimic. (B) Western blot analysis of Lin28 expression in T-47D cells transfected with let-7 mimic. (C) RT-qPCR analysis of the RNA expression levels of let-7 and Lin28 in MDA-231 cells transfected with let-7 inhibitor. (D) Western blot analysis of Lin28 expression in MDA-231 cells transfected with let-7 inhibitor. (E) RT-qPCR analysis of the RNA expression levels of Lin28 and let-7 in S1 and S24 cells. Mock was used as a control. (F) RT-qPCR analysis of the RNA expression levels of Lin28 and let-7 in T-47D cells transfected with siLin28. Results represent the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. the corresponding control group. RT-qPCR, reverse transcription-quantitative PCR; S1/S24, SK-BR-3 clones infected with a Lin28 overexpression lentivirus.

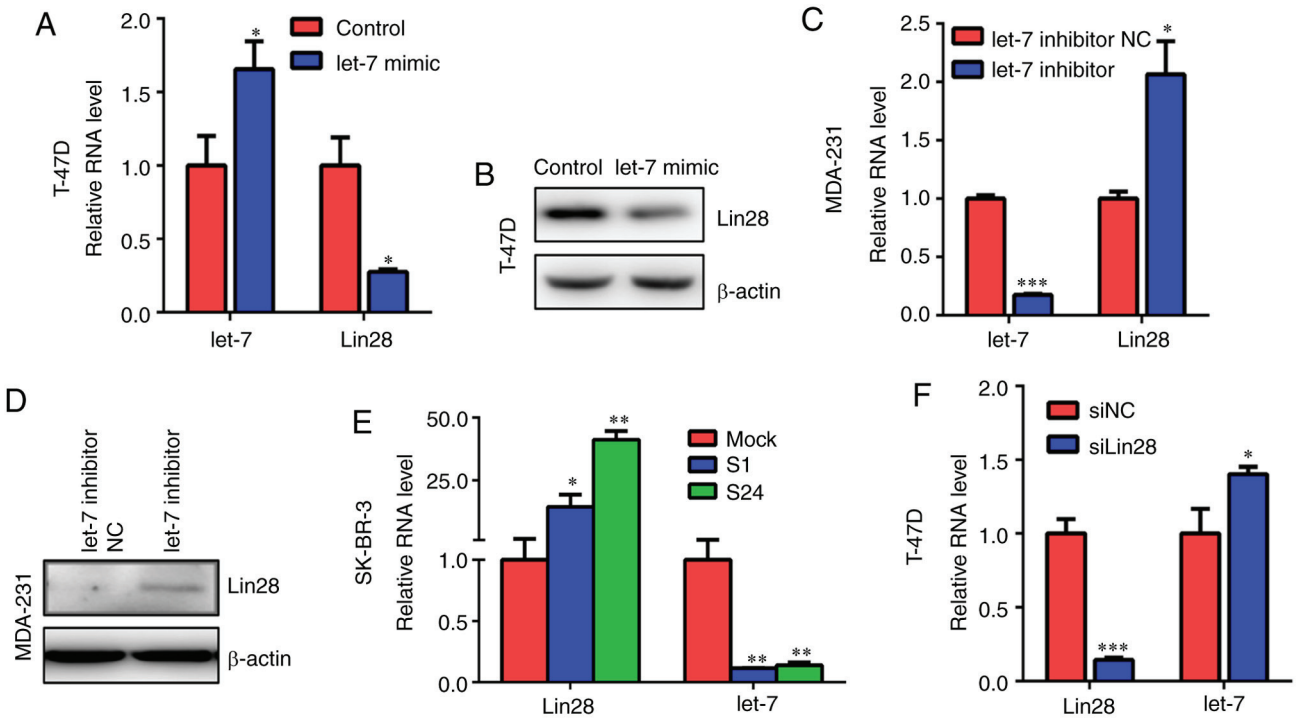

Figure S2. H19 and Lin28 promote the migration and invasion of breast cancer cells. (A) Wound healing assays for H19-overexpressing MDA-231 cells and H19-depleted T-47D cells. (B) Wound healing assays for Lin28-overexpressing S24 cells and Lin28-silenced T-47D cells. Scale bar=200 μm. si, small interfering RNA; pH19, pFlag-CMV-H19; Con, control; S24, SK-BR-3 clone infected with a Lin28 overexpression lentivirus.

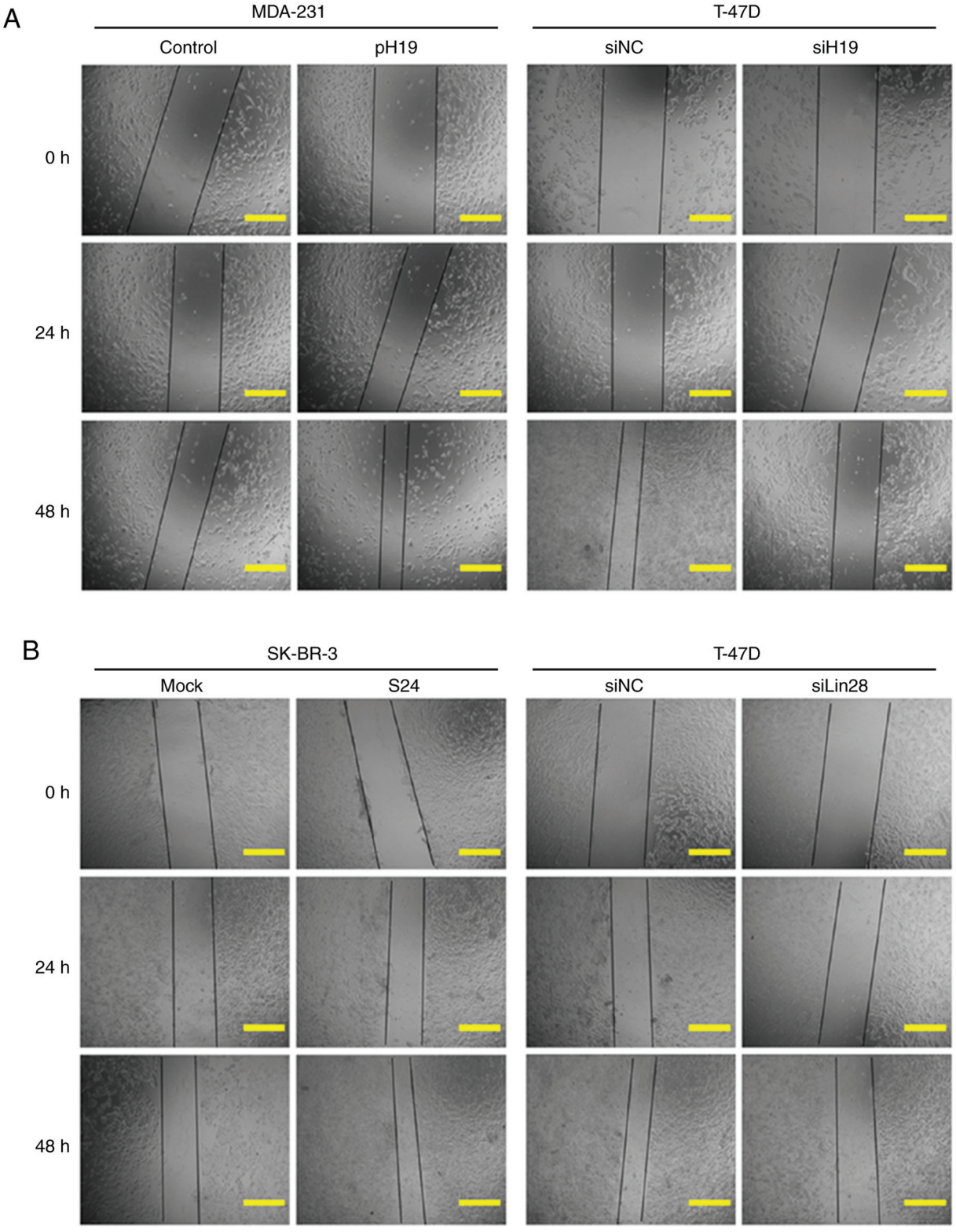

Figure S3. H19/let-7/Lin28 loop is involved in the EMT of breast cancer cells and regulates EMT-related genes. (A) T-47D cells were treated with siH19 and let-7 inhibitor, and cell migration and invasion were evaluated by Transwell assays. Scale bar=50 μm. (B) Quantification of migration and invasion rates of the various groups. (C) Western blot analysis of E-cadherin markers. (D) Reverse transcription-quantitative PCR analysis of EMT-inducing transcription factors in T-47D cells incubated with siH19 and let-7 inhibitor. Results represent the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. siNC + let-7 inhibitor NC unless otherwise indicated. EMT, epithelial-mesenchymal transition; si, small interfering RNA; NC, negative control.

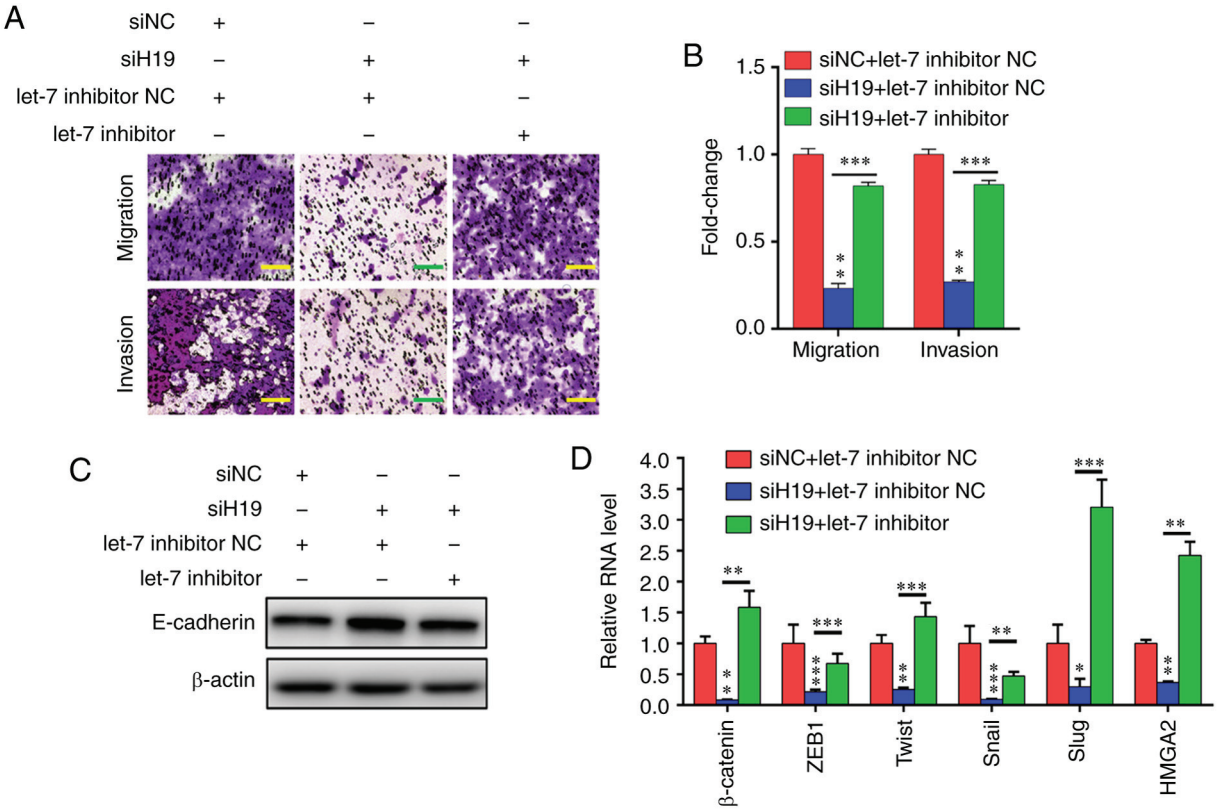

Figure S4. H19/let-7/Lin28 loop is involved in the epithelial-mesenchymal transition of breast cancer cells. (A) Morphological changes of SK-BR-3 cells treated with TGF-β, pH19 and let-7 inhibitor for 24 h. Scale bar=5 μm. H19 overexpression was detected by RT-qPCR in (B) SK-BR-3 and (E) T-47D cell lines. (D) Morphological changes of T-47D cells treated with TGF-β, pH19 and let-7 inhibitor for 24 h. Scale bar=5 μm. let-7 knockdown was detected by RT-qPCR in (C) SK-BR-3 and (F) T-47D cell lines. Results represent the mean ± SD of three independent experiments. **P<0.01, ***P<0.001 vs. the corresponding control group. TGF-β, transforming growth factor-β; pH19, pFlag-CMV-H19; NC, negative control.

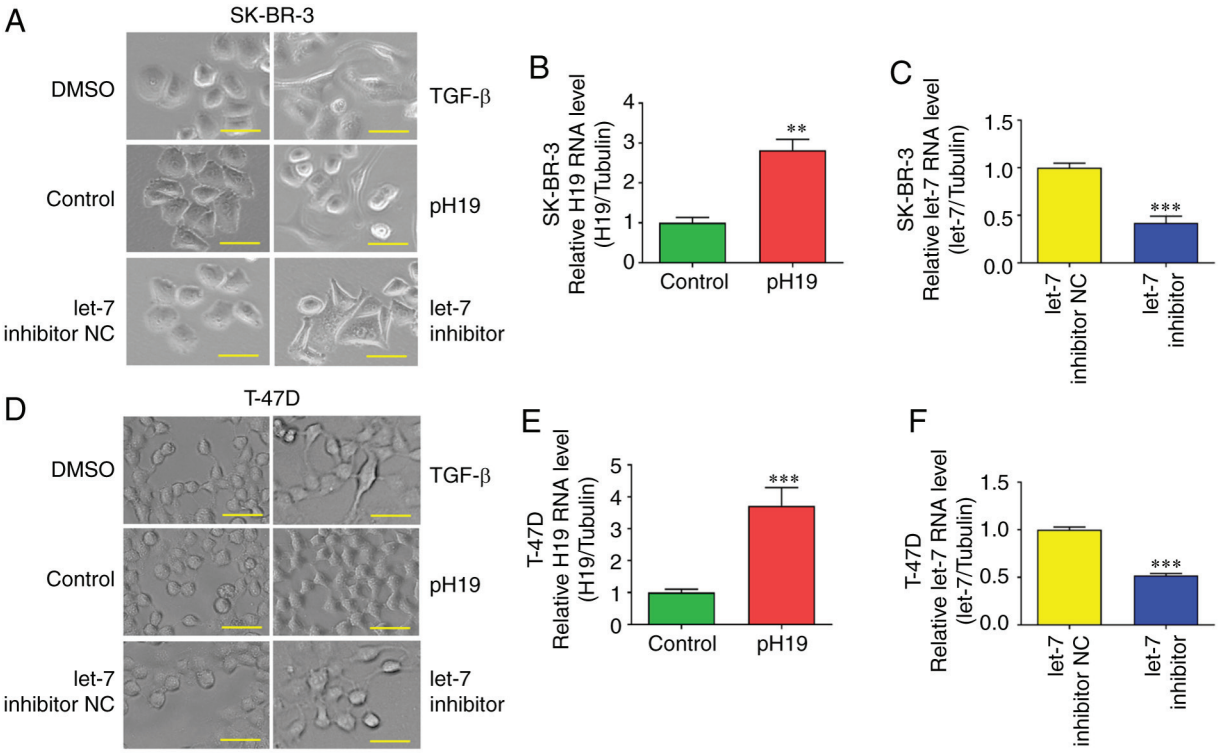

Figure S5. H19 and Lin28 have no significant effect on the viability of breast cancer cells. Cell viability was measured by MTT assays in (A) H19-overexpressing MDA-231 cells and (B) H19-depleted T-47D cells. Cell viability was measured by MTT assays in (C) S24 cells and (D) Lin28-depleted T-47D cells. Results represent the mean ± SD of three independent experiments. pFlag-CMV-H19; si, small interfering RNA; Con, control; S24, SK-BR-3 clone infected with a Lin28 overexpression lentivirus.

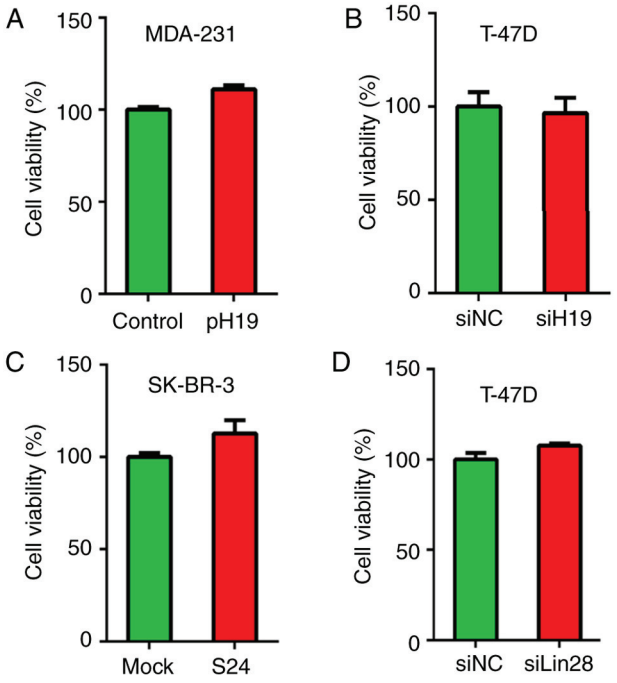

Figure S6. H19 affects Lin28 and metastasis by interacting with let-7 in breast cancer cells. (A) let-7 putative binding sites exist in the sequence of pH19, which were mutated in pH19mut cDNA. (B) RT-qPCR analysis of the RNA expression levels of H19 and Lin28 in MDA-231 cells transfected with pH19 or pH19mut. (C) Western blot analysis of Lin28 expression in MDA-231 cells transfected with pH19 or pH19mut. (D) MDA-231 cells were transfected with pH19mut, and cell migration and invasion were evaluated by Transwell assays. Scale bar=50 μm. Quantification of (E) migration and (F) invasion rates. Results represent the mean ± SD of three independent experiments. *P<0.05, ***P<0.001 vs. Control.

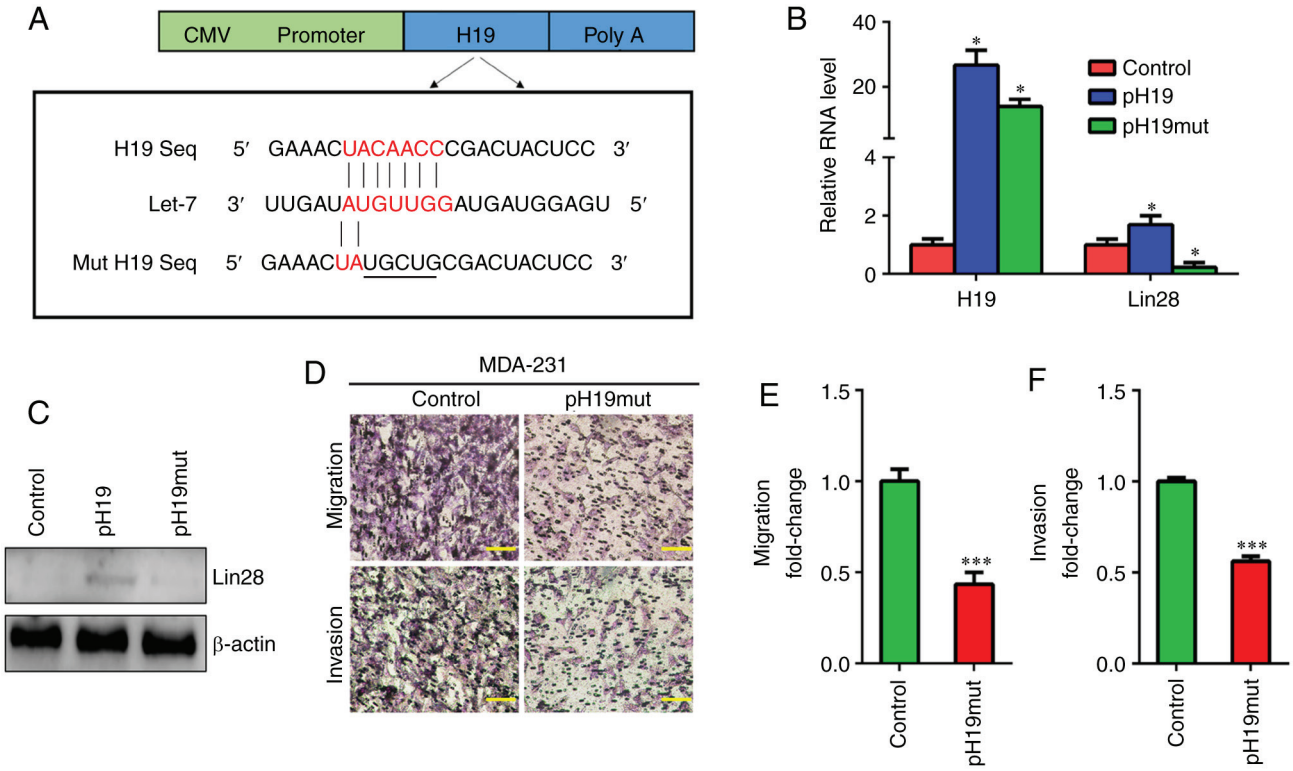

Figure S7. Impact of autophagic drugs on breast cancer cells. Western blot analysis of autophagic marker expression in T-47D cells incubated with 200 nM Rapa and 50 μM CQ. Con unless otherwise indicated. Rapa, rapamycin; CQ, chloroquine; LC3, light chain 3; Con, control.

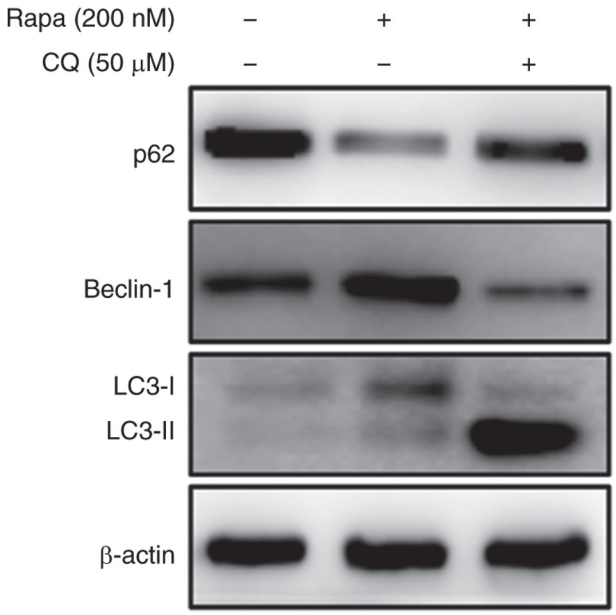

Figure S8. TGF-β negatively regulates autophagy in breast cancer cells. (A) Western blot analysis of autophagy marker expression in MDA-231 cells treated with TGF-β. (B) mRNA expression. (C) Western blot analysis of autophagy marker expression in T-47D cells treated with TGF-β. (D) mRNA expression. Results represent the mean ± SD of three independent experiments. **P<0.01, ***P<0.001, ****P<0.0001. TGF-β, transforming growth factor-β; LC3, light chain 3.