Figure S1. Protein expression of TRIM35 after TRIM35 overexpression. *P<0.05 vs. NC. NC, negative control; Control, blank control; Oe, overexpression; TRIM35, tripartite motif-containing 35.

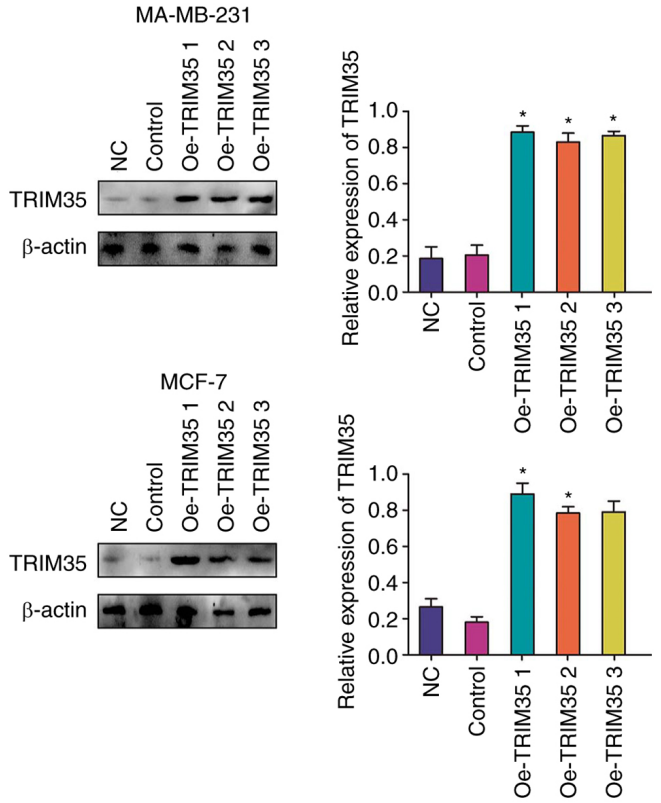

Figure S2. TRIM35 protein expression after TRIM35 silencing. *P<0.05 vs. NC. TRIM35, tripartite motif-containing 35; NC, negative control; Control, blank control; sh, short hairpin RNA.

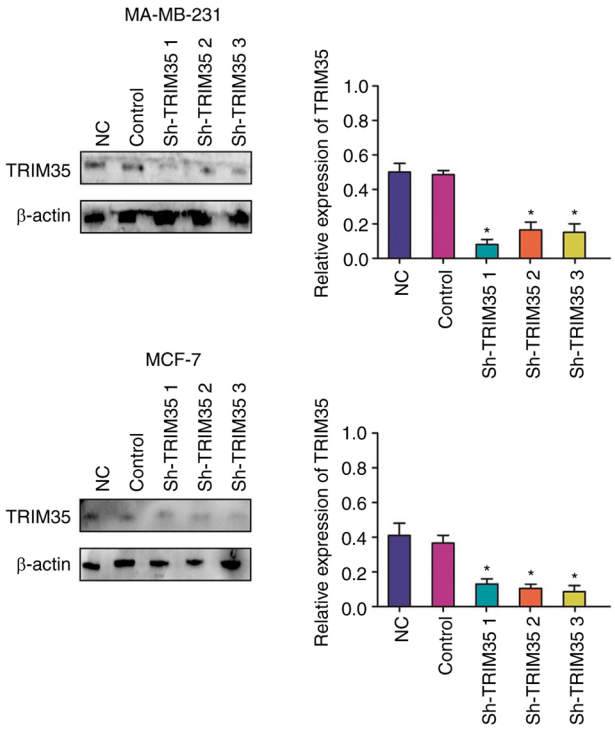

Figure S3. Reducing TRIM35 expression promotes the malignant biological behavior of breast cancer cells. (A) CCK-8 assay to detect proliferation of MD-MBA-231 cells. (B) CCK-8 assay to detect proliferation of MCF-7 cells. (C-F) Transwell assay to detect (C) MD-MBA-231 cell migration, (D) MCF-7 cell migration, (E) MD-MBA-231 cell invasion and (F) MCF-7 cell invasion (magnification, x200). *P<0.05. TRIM35, tripartite motif-containing 35; CCK-8, Cell Counting Kit-8; NC, negative control; sh, short hairpin RNA.

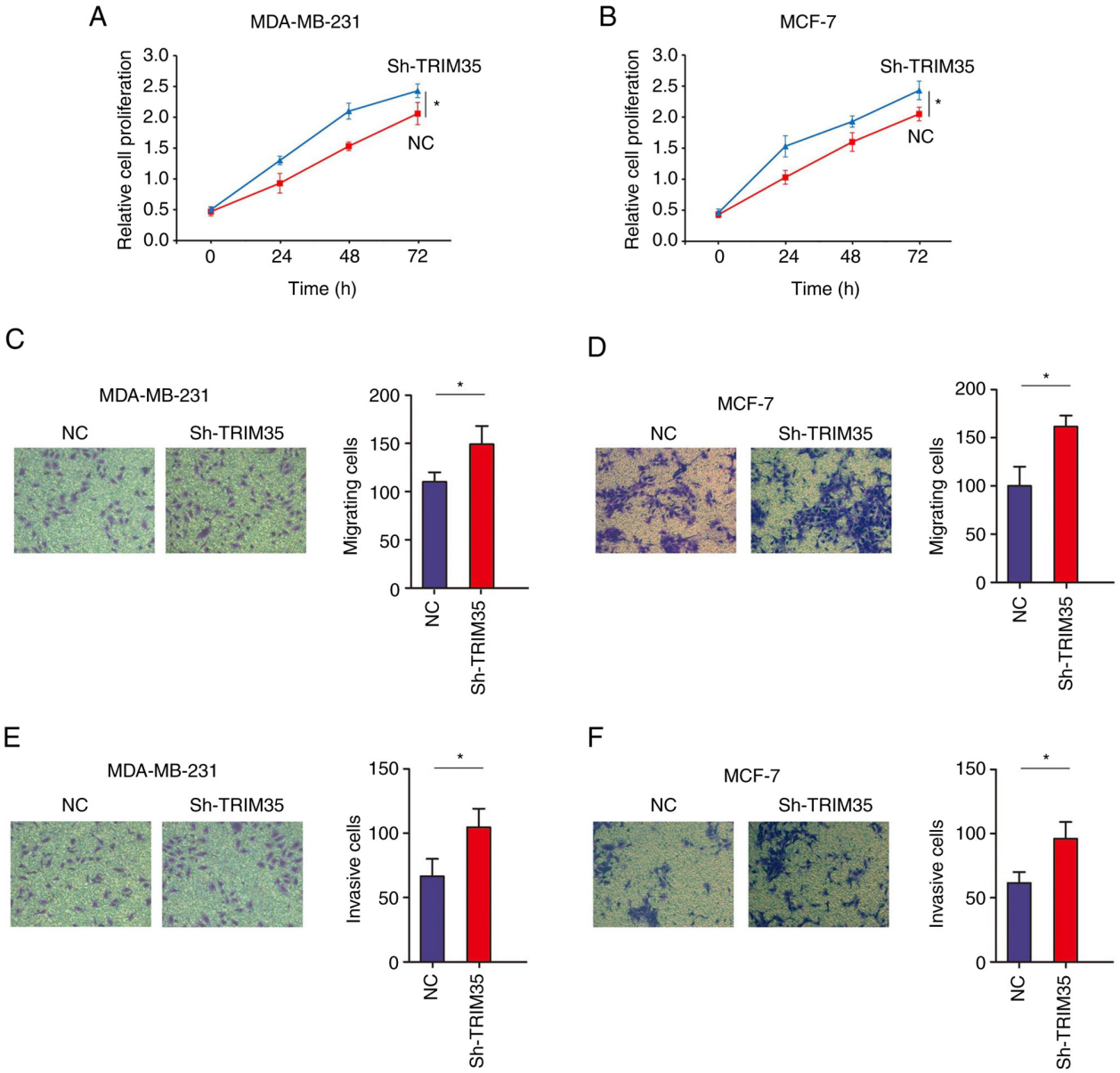

Figure S4. Expression of indices related to proliferation, apoptosis and epithelial-to-mesenchymal transition components detected by immunohistochemistry. (A) The protein expression of E-cadherin, N-cadherin, twist1, Bax, Bcl-2 and Ki-67 in MDA-MB-231 cells after increasing TRIM35 expression was detected by immunohistochemistry. (B) The protein expression of E-cadherin, N-cadherin, twist1, Bax, Bcl-2 and Ki-67 in MCF-7 cells after increasing TRIM35 expression were detected by immunohistochemistry (scale bars, 25 μm). TRIM35, tripartite motif-containing 35; NC, negative control; Oe, overexpression.

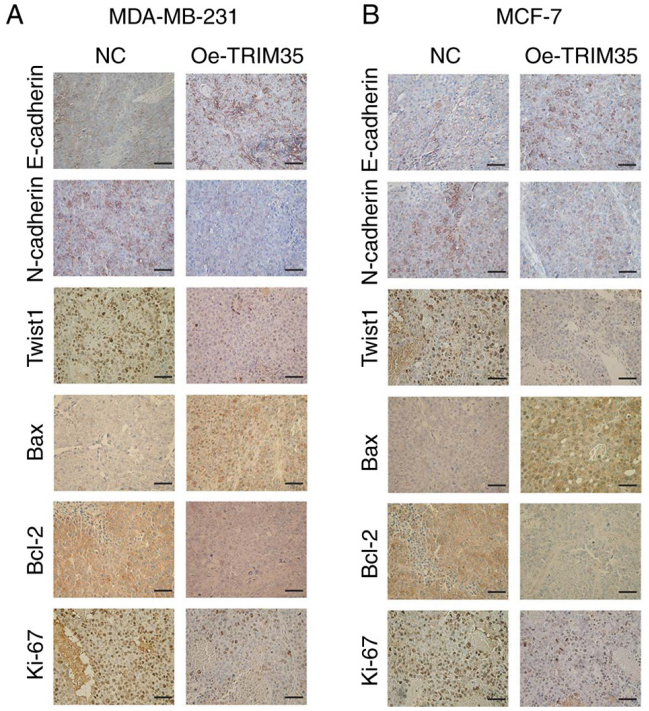

Figure S5. Network analysis of the interaction between TRIM35 and PKM2. TRIM35, tripartite motif-containing 35; PKM2, pyruvate kinase M2.

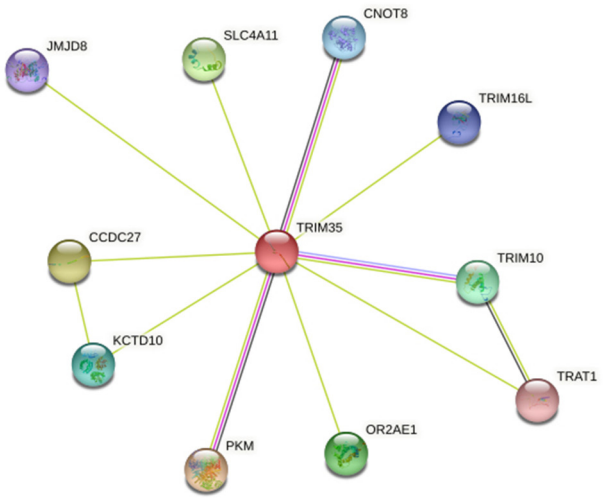

Figure S6. Expression of PKM2 tetramer and dimer in breast cancer cell lines. (A) Expression of PKM2 dimer and tetramer in breast cancer cell lines. The expression of (B) PKM2 tetramer and (C) PKM2 dimer in breast cancer cell lines was analyzed statistically. (D) Proportion of PKM2 tetramers and PKM2 dimers in breast cancer cell lines. *P<0.05, **P<0.01. PKM2, pyruvate kinase M2; WB, western blot.

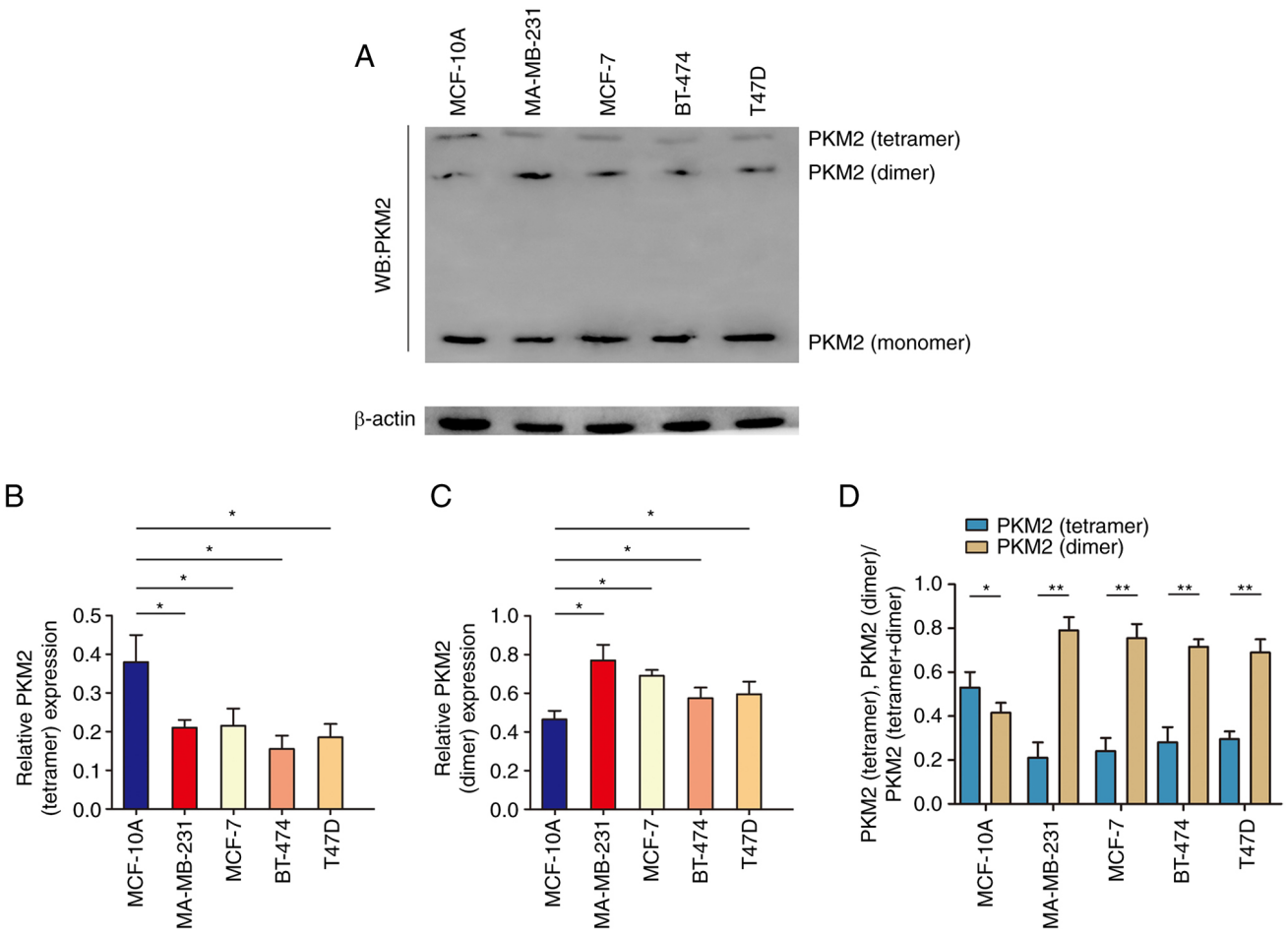

Figure S7. Effect of reducing the expression of TRIM35 on the expression of PKM2 tetramer and dimer. (A) Western blot analysis of the protein expression levels of monomeric, dimeric and tetrameric PKM2 in MDA-MB-231 cells. (B) Relative expression of tetrameric PKM2 in MDA-MB-231 cells. (C) Relative expression of dimeric PKM2 in MDA-MB-231 cells. (D) Proportion of PKM2 tetramers and PKM2 dimers in MDA-MB-231 cells. (E) Western blot analysis of the protein expression levels of monomeric, dimeric and tetrameric PKM2 in MCF-7 cells. (F) Relative expression of tetrameric PKM2 in MCF-7 cells. (G) Relative expression of dimeric PKM2 in MCF-7 cells. (H) Proportion of PKM2 tetramers and PKM2 dimers in MCF-7 cells. *P<0.05, **P<0.01. TRIM35, tripartite motif-containing 35; PKM2, pyruvate kinase M2; WB, western blot; NC, negative control; sh, short hairpin RNA.

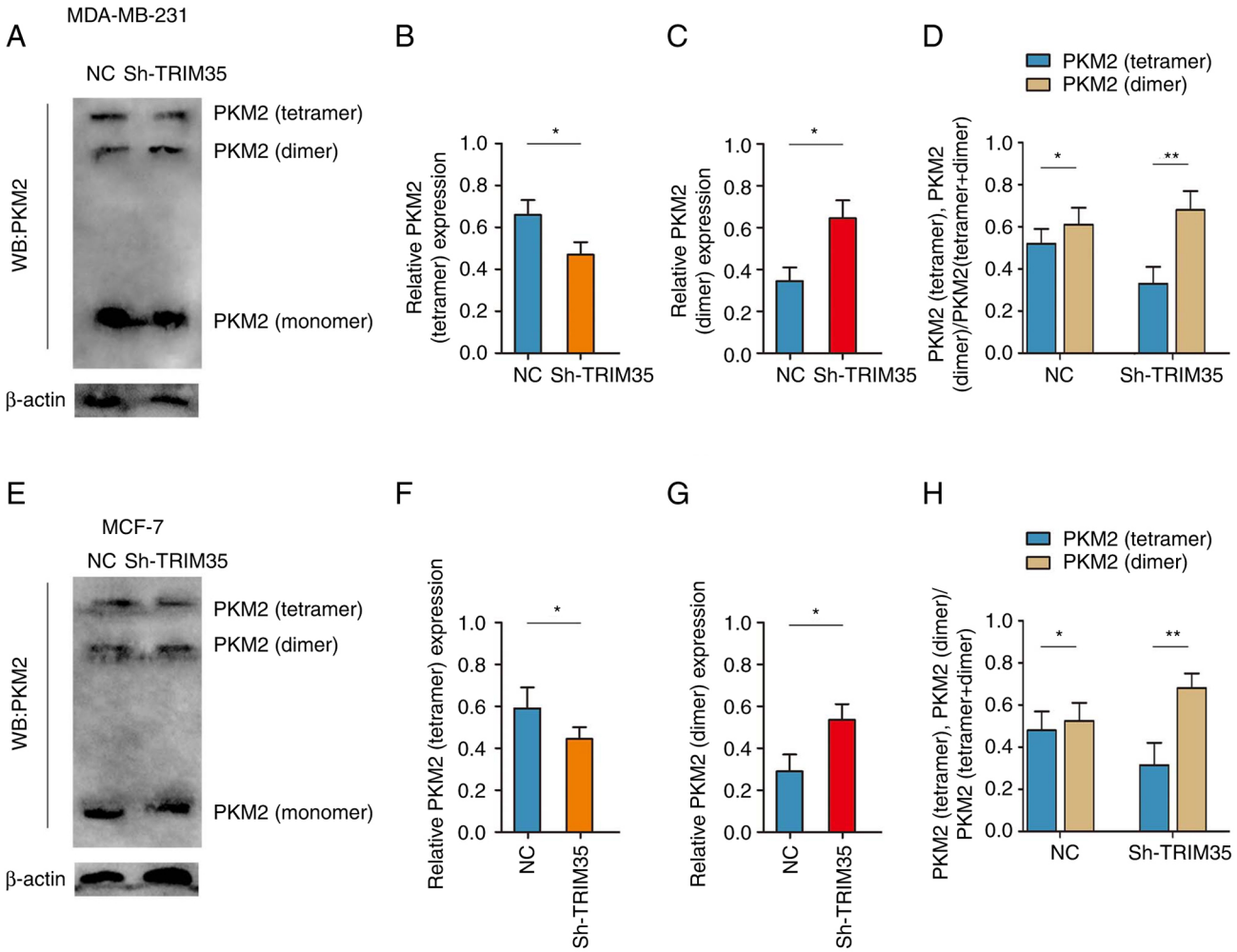

Figure S8. Effect of TRIM35 overexpression on PKM2 nuclear expression. (A) Expression of PKM2 nucleoprotein in MDA-MB-231 cells. (B) Expression of PKM2 nucleoprotein in MCF-7 cells. Expression of PKM2 nucleoprotein detected by immunofluorescence in (C) MDA-MB-231 and (D) MCF-7 cells (magnification, x200). *P<0.05. TRIM35, tripartite motif-containing 35; PKM2, pyruvate kinase M2; NC, negative control; Oe, overexpression.

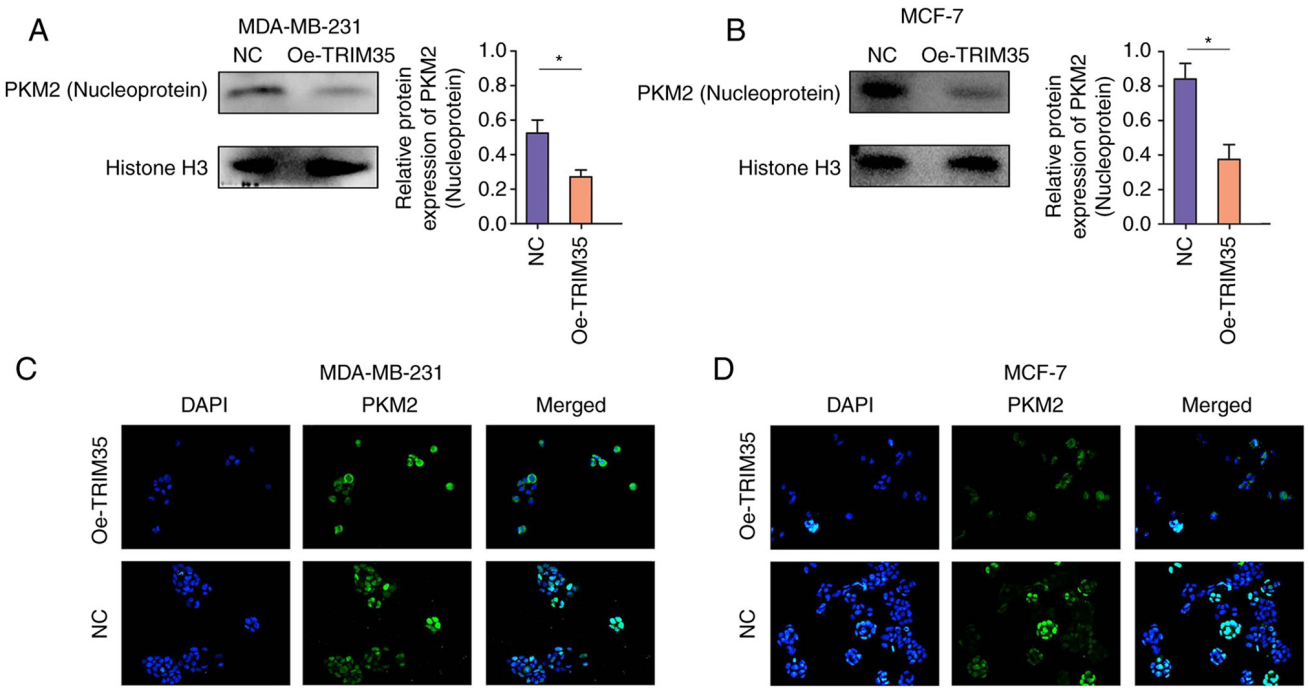

Figure S9. Effect of TEPP-46 treatment on the malignant biological behaviour of breast cancer cells. (A) CCK-8 assay to detect the proliferation of MD-MBA-231 cells. (B) CCK-8 assay to detect the proliferation of MCF-7 cells. Transwell assays to detect (C) MD-MBA-231 cell migration, (D) MCF-7 cell migration, (E) MD-MBA-231 cell invasion and (F) MCF-7 cell invasion (magnification, x200). (G) Flow cytometry was used to examine the effects of TEPP-46 on MD-MBA-231 cell apoptosis. (H) Flow cytometry was used to examine the effects of TEPP-46 on MCF-7 cell apoptosis. *P<0.05. CCK-8, Cell Counting Kit-8; NC, negative control; PI, propidium iodide.