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MDPI

Supplementary materials

Table S1. Specific Real-time RT-qPCR primer sequences containing.*Nanog, CD44, Oct4, c-myc, Sox2, GLI1, YAP1,* and *β-actin* genes.

Genes	Primers
Nanog	Forward: 5′-ATGCCTCACACGGAGACTGT-3′ Reverse: 5′-AAGTGGGTTGTTTGCCTTTG-3′
CD44	Forward: 5′-AGAAGGTGTGGGCAGAAGAA-3′ Reverse: 5′-AAATGCACCATTTCCTGAGA-3′
Oct4	Forward: 5′-AGCAAAACCCGGAGGAGT-3′ Reverse: 5′-CCACATCGGCCTGTGTATATC-3′
c-myc	Forward: 5′-AATGAAAAGGCCCCCAAGGTAGTTATCC-3′ Reverse: 5′-AGCAAAACCCGGAGGAGT-3′
Sox2	Forward: 5′-TTGCTGCCTCTTTAAGACTAGGA-3′ Reverse: 5′-CTGGGGCTCAAACTTCTCTC-3′
GLI1	Forward: 5′-CAGGGAAGAGAGCAGACTGAC-3′ Reverse: 5′-CAGGAGGATTGTGCTCCA-3′
YAP1	Forward: 5′-GAACCCCAGATGACTTCCTG-3′ Reverse: 5′-CTCCTTCCAGTGTTCCAAGG-3′
β-actin	Forward: 5′-TGTTACCAACTGGGACGACA-3′ Reverse: 5′-GGGGTGTTGAAGGTCTCAAA-3

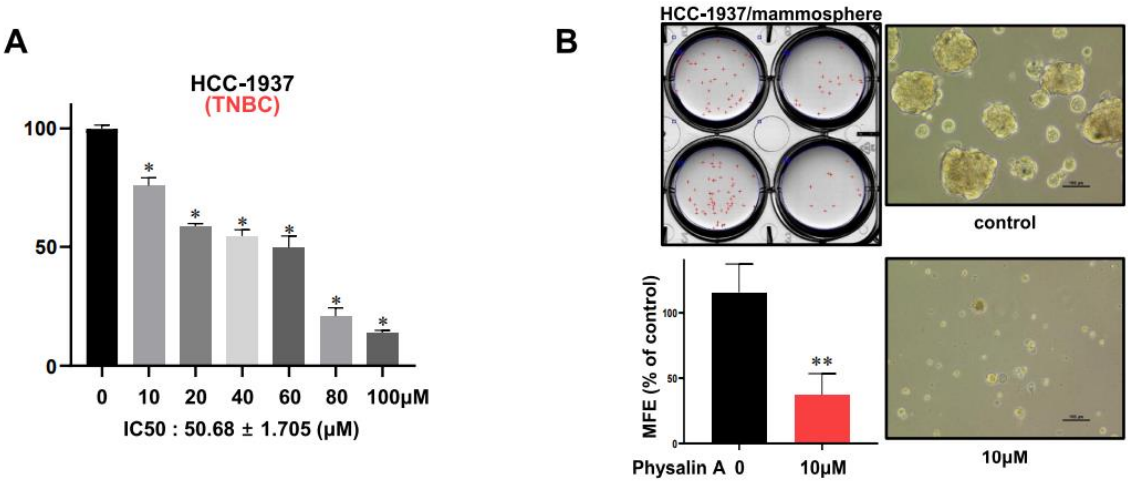

Figure S1. Physalin A inhibits breast cancer cell viability and mammosphere formation efficiency. (**A**) Breast cancer cell line, HCC-1937 (TNBC), was cultured with increasing concentration of physalin A (0, 10, 20, 40, 60, 80, 100 μM) for 24 h. The cytotoxic effect of physalin A was measured using the MTS assay. (**B**) Physalin A inhibits mammosphere-forming ability. HCC-1937 cells (4×10^4 per well) was cultured in 6-well ultralow attachment plates with/without physalin A. Representative mammospheres in the photos were captured by inverted light microscopy (scale bar: 100 μm). The mammosphere formation efficiency (MFE) was determined as shown in the graph. Mean ± SD values from three independent experiments are presented. *$p < 0.05$, **$p < 0.01$.

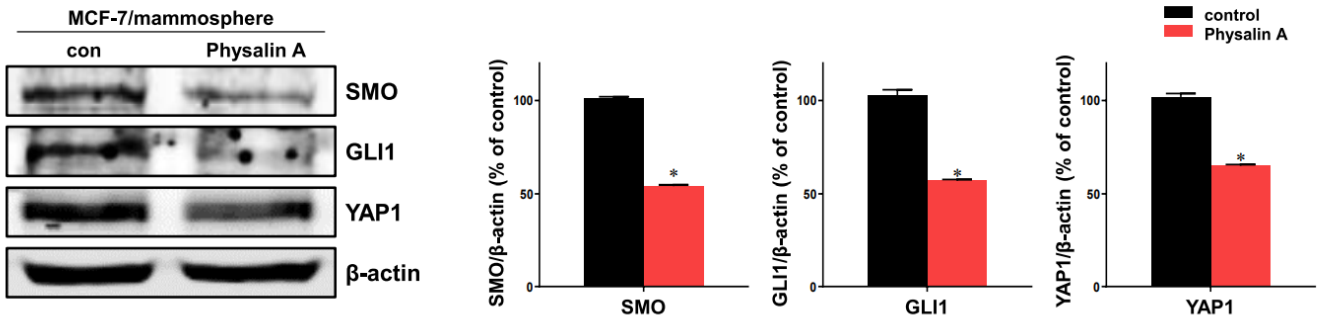

Figure S2. Effect of physalin A on Hedgehog and Hippo signaling pathway of MCF-7 cell line. Physalin A decreased the total protein level of SMO, GLI1, and YAP1 in breast mammospheres. Anti-SMO, anti-GLI1, and anti-GLI1 YAP1 antibody were used for immunoblotting. β-actin was used as the internal control. Western blot images of triplicate experiments are shown as mean ± SD. *$p < 0.05$ vs. DMSO-treated control.