



S4 Fig. Verification of the promoting effects of paired-like homeodomain transcription factor 2 (PITX2) on anchorage-dependent and -independent growth of MCF7 or MCF/LR cells upon letrozole challenge. (A) MCF7 cells stably transfected with pPM-His-PITX2 or pPM-His vector were seeded at the density of 1.0×10^4 cells in the basement membrane extract (BME) culture medium (R&D Systems, Minneapolis, MN) containing 10^{-6} M of letrozole and 25 nM of androstenedione, and were cultured for another 16 days. The representative images of tumor cell clusters were shown. (B) The diameters of tumor clusters were finally documented after 16 days at $10\times$ magnification using spacial calibration of images (Image J software). The results were presented as the mean \pm standard error of mean (SEM) of the triplicate samples. (C) About 5,000 cells (MCF7/His-PITX2 or MCF7/His-vector cells)/60-mm plastic dish were cultured at 37°C for 48 hours. 10^{-6} M of letrozole was then added to cultures. After 3 days of treatment, the letrozole-containing media was replaced with fresh media and all cultures were incubated for an additional 14 days until colonies were large enough to be clearly discerned. Colonies were stained for 5 minutes with a solution containing 0.5% crystal violet and 25% methanol, followed by colonies measurement using Colony Counter software (Syngene, Frederick, MD). The results were presented as the mean \pm SEM of the triplicate samples. (D) MCF7/LR cells stably transfected with PITX2 shRNA or Scramble shRNA were seeded at the density of 1.0×10^4 cells in the BME culture medium containing 10^{-5} M of letrozole and 25 nM of androstenedione, and were cultured for another 16 days. The representative images of tumor cell clusters were shown. (E) The diameters of tumor clusters were finally documented after 16 days at $10\times$ magnification using spacial calibration of images (Image J software). The results were presented as the mean \pm SEM of the triplicate samples. (F) About 1,000 cells (MCF7/LR/PITX2 shRNA or MCF7/LR/Scramble shRNA cells)/60-mm plastic dish were cultured at 37°C for 48 hours. 10^{-5} M of letrozole was then added to cultures. After 3 days of treatment, the letrozole-containing media was replaced with fresh media and all cultures were incubated for an additional 9 days until colonies were large enough to be clearly discerned. Colonies were stained for 5 minutes with a solution containing 0.5% crystal violet and 25% methanol, followed by colonies measurement using Colony Counter software (Syngene, Frederick, MD).