



**S7 Fig.** Upregulation of paired-like homeodomain transcription factor 2 (PITX2) in letrozole-resistant MDA-MB-415 cells. (A) After transfection with the human aromatase CYP19A1 gene (SinoBiological, Beijing, China) and subsequent Hygromycin selection (100  $\mu\text{g}/\text{mL}$ , Takara, Dalian, China), stably transfected MDA-MB-415 cells were inoculated at the flanks with 0.1 ml of cell suspension ( $2 \times 10^7$  cells/mL) in ovariectomized female nude mice. 4 weeks later, mice were injected with vehicle (control), or letrozole (10  $\mu\text{g}/\text{days}$ , Sigma-Aldrich, Shanghai, China), along with the aromatase substrate androstenedione ( $\Delta 4\text{A}$ , 100  $\mu\text{g}/\text{days}$ , Sigma-Aldrich), for the duration of the experiment. At the end of 56 weeks of letrozole injection, MCF7/LR cells were isolated and maintained in phenol red-free IMEM supplemented with 5% charcoal/dextran-treated fetal bovin serum, 1% penicillin/streptomycin, 100  $\mu\text{g}/\text{mL}$  hygromycin, and 1  $\mu\text{mol}/\text{L}$  of letrozole. Validation of MDA-MB-415/LR cells was carried out using trypan blue staining along with hemocytometer count (fold change was determined for each treatment relative to the untreated control cells, \* $p < 0.05$  and \*\* $p < 0.01$ ). (C) Characterization of PITX2 expression in different BCa cells using quantitative reverse transcription polymerase chain reaction. The results were presented as the mean  $\pm$  standard error of mean of the triplicate samples. (D) Western blotting analysis of PITX2 expression in different breast cancer cells. Actin served as the loading control.