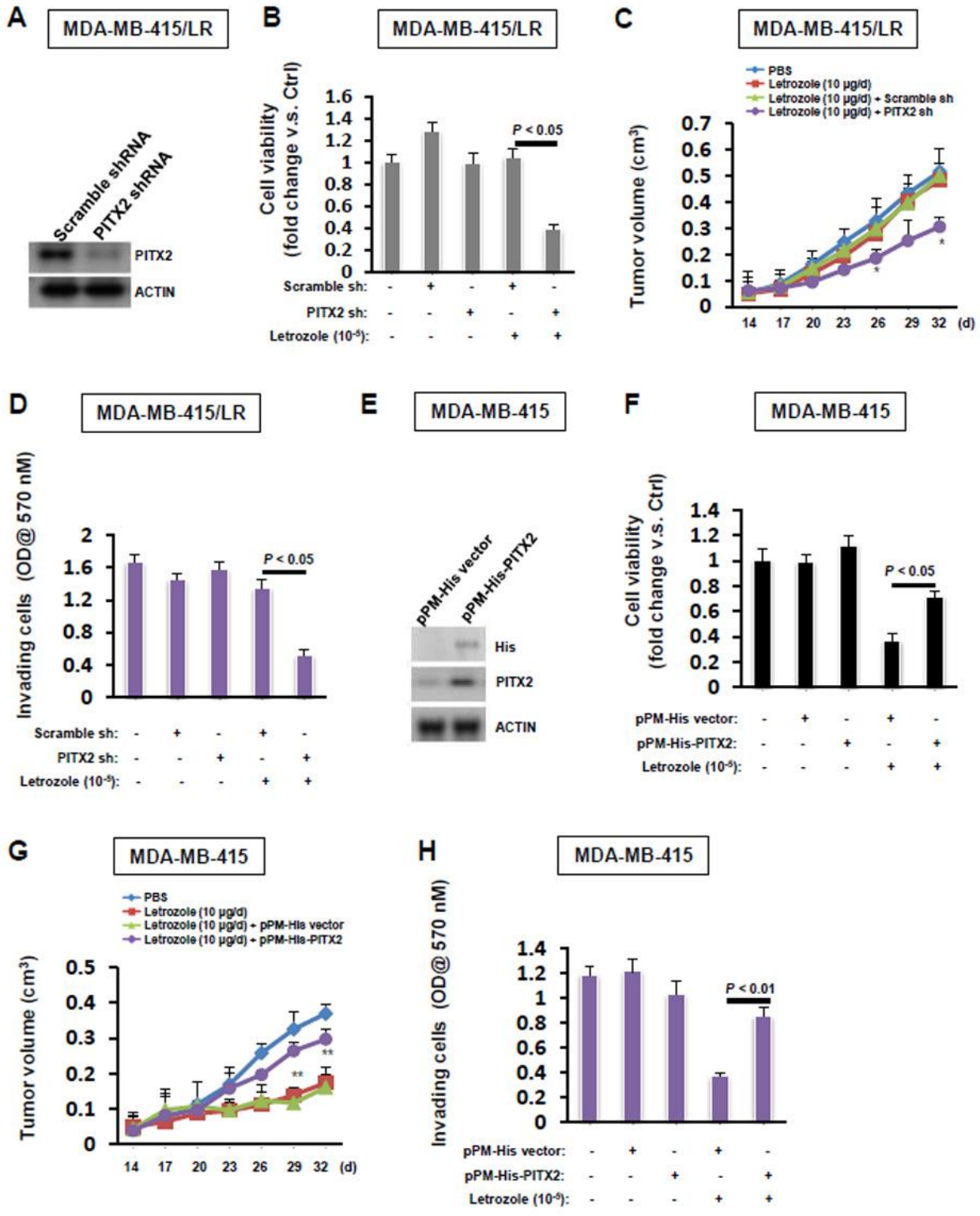

S8 Fig. Manipulation of paired-like homeodomain transcription factor 2 (PITX2) expression affects letrozole sensitivity in MDA-MB-415 and MDA-MB-415/LR cells. (A) Western blotting analysis of PITX2 level in MDA-MB-415/LR cells stably transfected with PITX2 shRNA or scramble shRNA. (B) After a 3-day culture and a following overnight starvation, MDA-MB-415/LR cells with different transfections were treated with 10^{-5} M of letrozole,

along with 25 nM of androstenedione, for another 4 days. Viable cell numbers were then determined using Trypan blue staining (fold change was determined for each treatment relative to the untreated control cells). The results were presented as the mean±standard error of mean of the triplicate samples. (C) Each nude mouse received subcutaneous injections at one site on each flank with 0.1 mL of suspension of different transfected MDA-MB-415/LR cells (1×10^7 cells/mL). Mice were then injected subcutaneously daily for 32 days with androstenedione (100 μg/day) plus letrozole (10 μg/day) from the day of inoculation. Tumor volumes were measured every 3 days. *$p < 0.05$ and **$p < 0.01$ when comparing letrozole (10 μg/day)+scramble shRNA group to letrozole (10 μg/day)+PITX2 shRNA group. (D) Cell invasiveness assay: MDA-MB-415/LR cells (1×10^4) were cultured in the upper chamber of Transwell with a membrane coated with Matrigel for 24 h, followed by treatment with 10^{-5} M of letrozole, along with 25 nM of Δ4A, or with DMSO (Sigma-Aldrich) for 48 hours. The remaining cells on the membrane were then stained for 10 minutes with 0.1% crystal violet solution and subsequent spectrophotometry was developed at 570 nM. (E) Western blotting analysis of PITX2 level in MDA-MB-415 cells stably transfected with pPM-His-PITX2 or pPM-His vector. (F) After a 3-day culture and a following overnight starvation, MDA-MB-415 cells with different transfections were treated with 10^{-5} M of letrozole, along with 25 nM of androstenedione, for another 4 days. Viable cell numbers were then determined using Trypan blue staining (fold change was determined for each treatment relative to the untreated control cells). (G) Tumor xenograft assay, as described above, was carried out to evaluate the effects of PITX2 overexpression in MDA-MB-415 cells on in vivo letrozole sensitivity. *$p < 0.05$ and **$p < 0.01$ when comparing Letrozole (10 μg/day)+His vector group to letrozole (10 μg/day)+His-PITX2 group. (H) Cell invasiveness assay as described above. Shown are the absorptions at 570 nm of MDA-MB-415 cells treated with different transfection plus 10^{-5} M of letrozole for 48 hours, in the lower chambers of transwells.