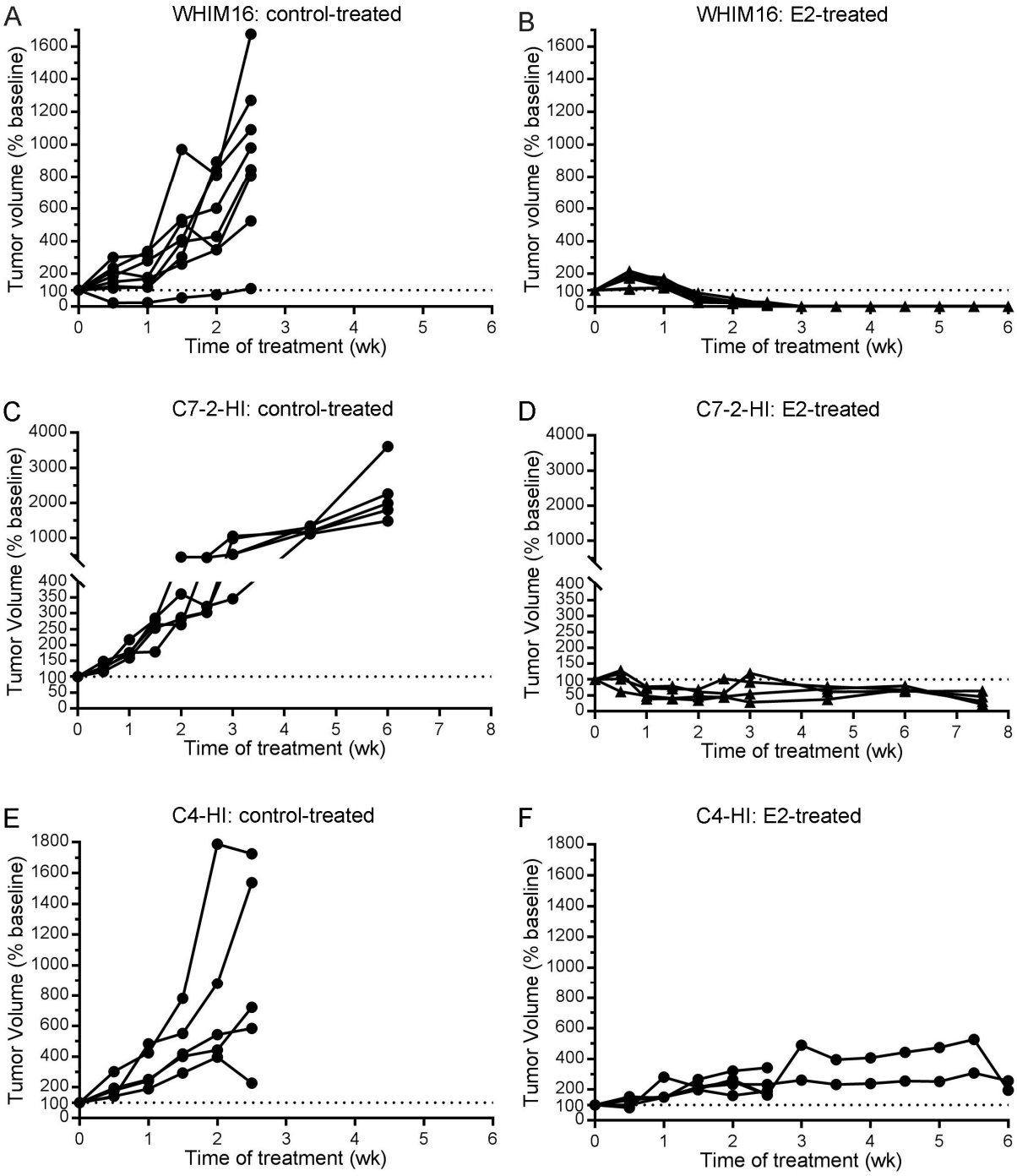
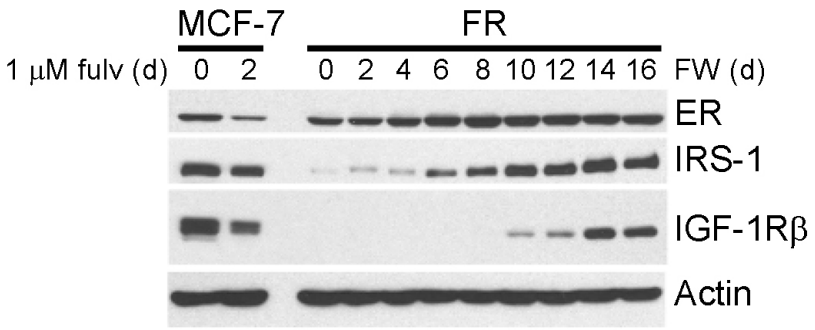
Supplemental Data for Hosford *et al.*

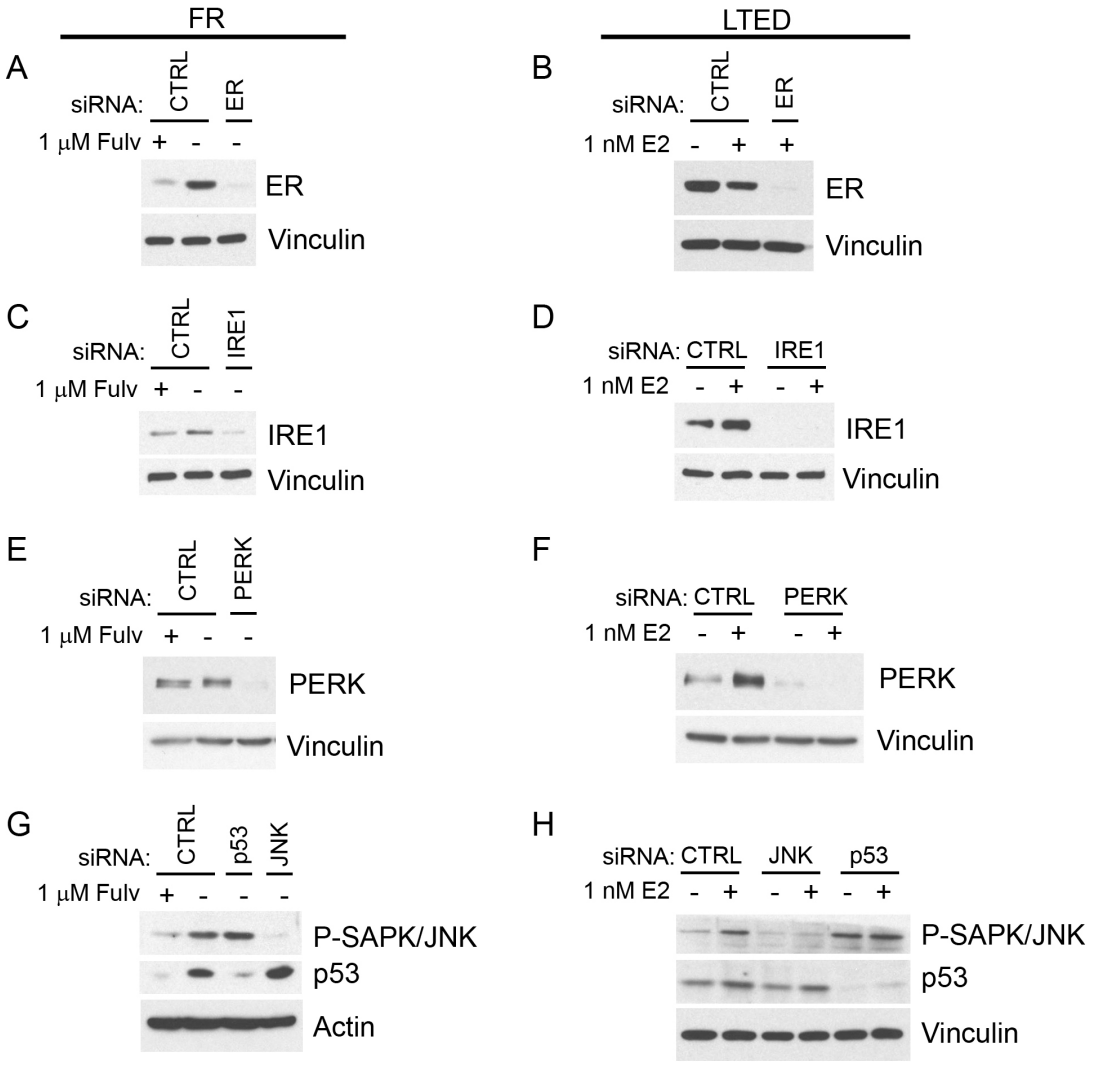
Supplemental Figure 1 – Estrogen-independent tumors exhibit therapeutic sensitivity to E2 treatment. Ovariectomized NSG (A/B) or Balb/c (B-F) mice were implanted s.c. with ~8-mm^3 fragments of serially transplanted WHIM16 patient-derived xenografts (A/B), C7-2-HI murine allografts (C/D), or C4-HI murine allografts (E/F). When tumors reached ~400 mm^3, mice were randomized to sham surgery or s.c. implantation with a 17β-estradiol (E2) pellet. Tumor volume is presented as % change from baseline at the day of E2 supplementation (Day 0). Each line represents one tumor. Summarized data are shown in Fig. 1A-C.



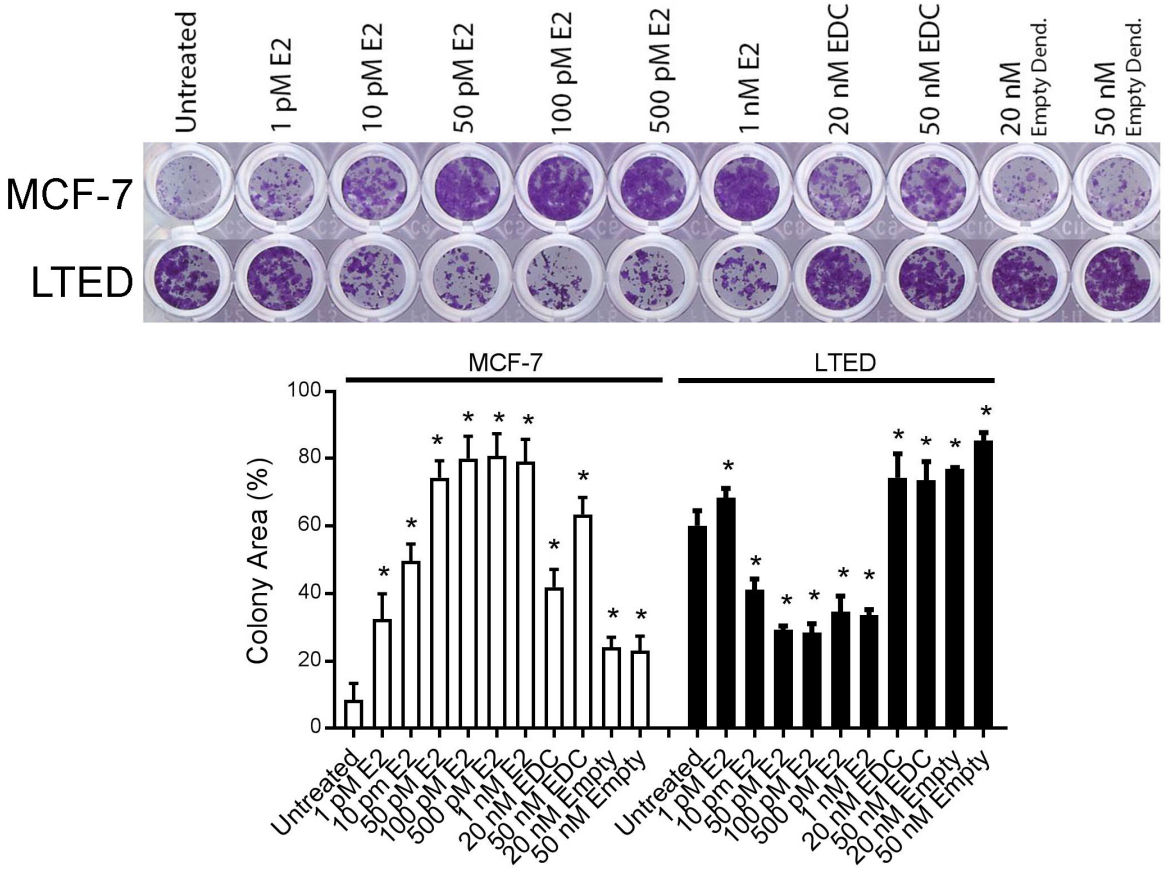
Supplemental Figure 2 - MCF-7/FR cells recover ER signaling following fulv withdrawal. Lysates from MCF-7 and FR cells treated ± fulv for 0-16 d were analyzed by immunoblot.



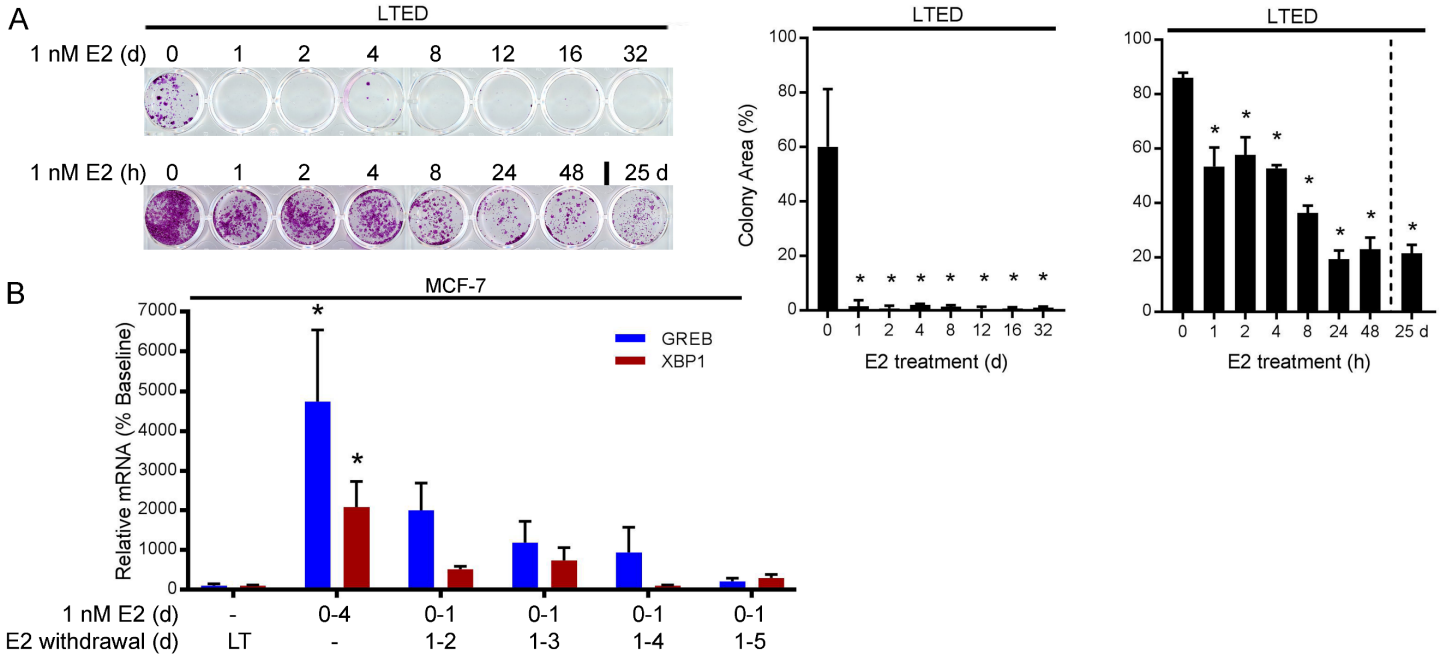
Supplemental Figure 3 – Confirmation of siRNA knockdown in FR and LTED cells. Cells were transfected with siRNA as indicated, and lysates harvested 4 d later were analyzed by immunoblot.



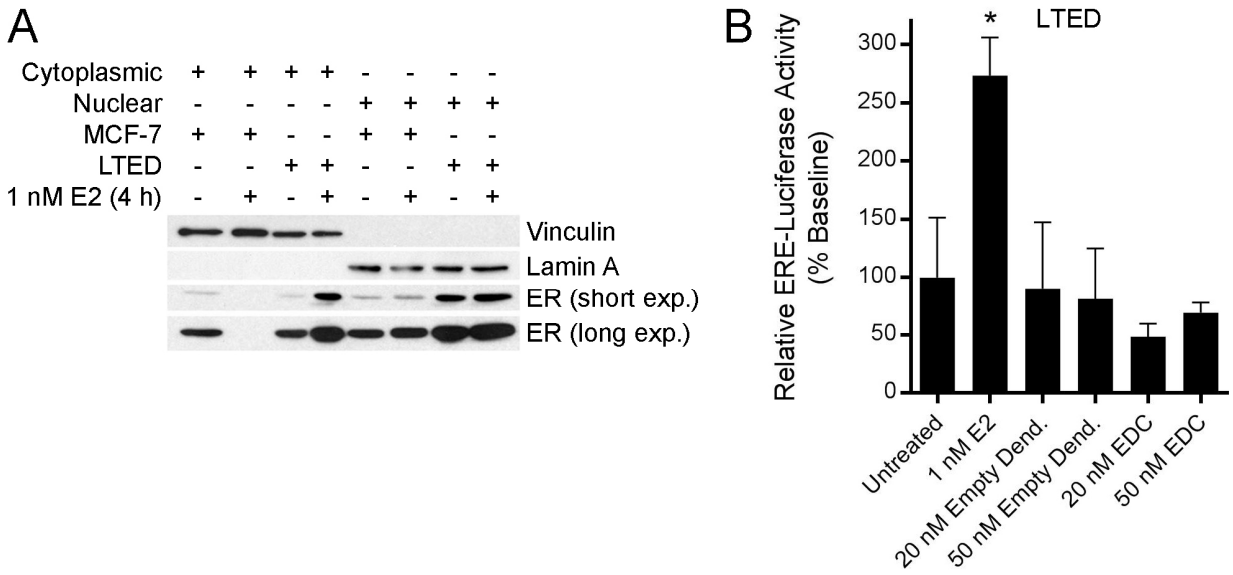
Supplemental Figure 4 - LTED cells are hypersensitive to E2 treatment. MCF-7 and LTED cells were treated ± E2, estrogen-dendrimer conjugate (EDC), or empty dendrimer as indicated for 3 wk, then cells were fixed and stained with crystal violet for colony quantification using the ColonyArea plugin for ImageJ. Data are shown as mean of triplicates ± SD. *$p \leq 0.05$ by Bonferroni multiple comparison-adjusted posthoc test compared to untreated (hormone-deprived) control within each cell line. Representative images are shown.



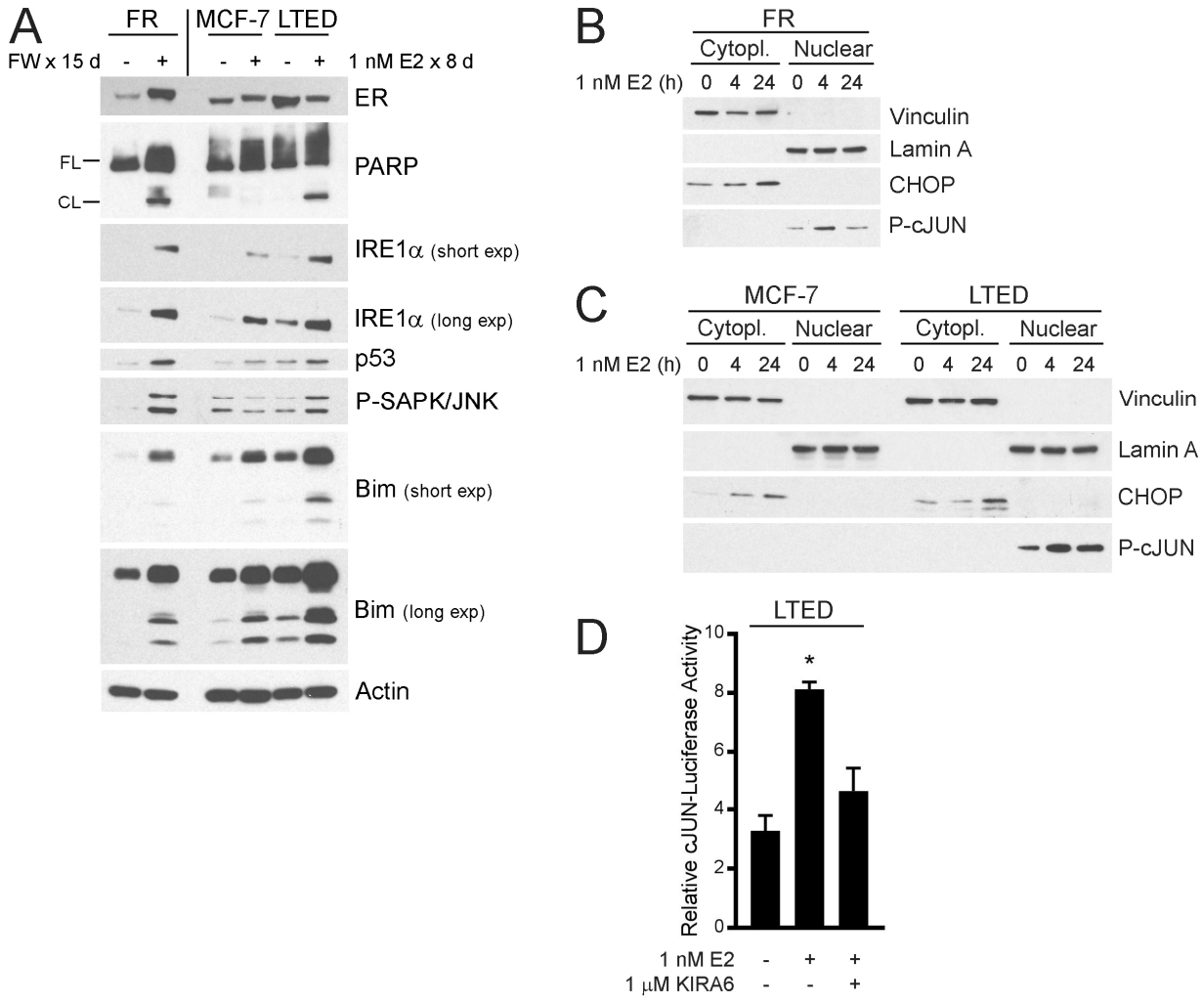
Supplemental Figure 5 – Growth-suppressive effect of E2 on LTED cells is acute. (A) LTED cells were seeded at low density and treated ± E2. Following the indicated time period, E2 was washed out, and cells were allowed to grow in hormone-depleted medium for 4 wk (*top*) or 25 d (*bottom*). Cells were then fixed and stained with crystal violet for colony quantification using the ColonyArea plugin for ImageJ. Data are shown as mean of triplicates ± SD. *$p \leq 0.05$ by Bonferroni multiple comparison-adjusted posthoc test compared to untreated (Day 0) control. (B) To estimate the length of time require for E2-induced ER transcriptional activity to subside following E2 withdrawal, MCF-7 cells were hormone-deprived for 4 d, then treated with ± E2 for 4 d, or for 1 d followed by washout and 1, 2, 3, or 4 d of hormone depletion. On Day 5, RNA was harvested. RT-qPCR was performed using primers targeting *GREB1*, *XBP1,* and *36B4* (control). Ratio of *GREB1/36B4* and *XBP1/36B4* (ΔΔCt) was normalized to baseline. LT- long-term hormone-deprivation for duration of study. *$p \leq 0.05$ by Bonferroni multiple comparison-adjusted posthoc test compared to LT control.



Supplemental Figure 6 - Anti-cancer effect of E2 treatment is dependent on nuclear ERα activation. A) MCF-7 and LTED cells were hormone-starved for 5 d, then treated ± E2 for 4 h. Cells were lysed, and cytoplasmic and nuclear fractions were harvested for analysis by immunoblot. Vinculin and lamin A served as purity/loading controls for cytoplasmic and nuclear fractions, respectively. (B) LTED cells were transfected with plasmids encoding estrogen response element (ERE)-driven firefly luciferase activity and CMV-Renilla. The next day, cells were treated ± E2, estrogen-dendrimer conjugate (EDC), or empty dendrimer. Luciferase activities were measured 2 d later, and firefly was normalized to Renilla. Data are shown as mean of triplicates ± SD. * $p \leq 0.05$ by Bonferroni multiple comparison-adjusted posthoc test compared to untreated control.



Supplemental Figure 7 – ER reactivation induces nuclear localization of phospho-cJUN and activation of pro-apoptotic CHOP. (A) Immunoblot analysis of lysates from FR cells treated ± FW, or MCF-7 and LTED cells treated ± E2. FL- full length; CL- cleaved. Summarized data are shown in Fig. 2E. FL- full-length; CL- cleaved. (B/C) Immunoblot analysis of cytoplasmic and nuclear fractions harvested from FR cells treated ± FW for 4 d (B), or MCF-7 and LTED cells treated ± E2 for 4 or 24 h (C). Vinculin and lamin A served as purity/loading controls for cytoplasmic and nuclear fractions, respectively. (D) LTED cells were transfected with cJUN-driven firefly luciferase and CMV-Renilla plasmids. The next day, cells were treated ± E2 and the IRE1α inhibitor KIRA6. Luciferase activities were measured 4 d later. Firefly was normalized to Renilla. *$p \leq 0.05$ by Bonferroni multiple comparison-adjusted posthoc test compared to control.



Supplemental Figure 8 - NFκB and mTORC1 signaling are not significantly altered following ER reactivation in FR or LTED cells. (A) MCF-7 and LTED cells were treated with hormone-depleted medium ± E2 for 0-8 d. Lysates were analyzed by immunoblot. (B) Cells were transfected with plasmids encoding NFκB-driven firefly luciferase and CMV-Renilla. Cells were then treated with hormone-depleted medium ± E2. Luciferase activities were measured 3 d later, and firefly was normalized to Renilla. Data are shown as mean of triplicates + SD. Groups were compared by *t*-test. (C) FR cells were treated with FW for 0-16 d. Lysates were analyzed by immunoblot. The same lysates were analyzed in Supplemental Figure 2; actin blot is reproduced here.