Miller *et al.*- Supplemental Materials

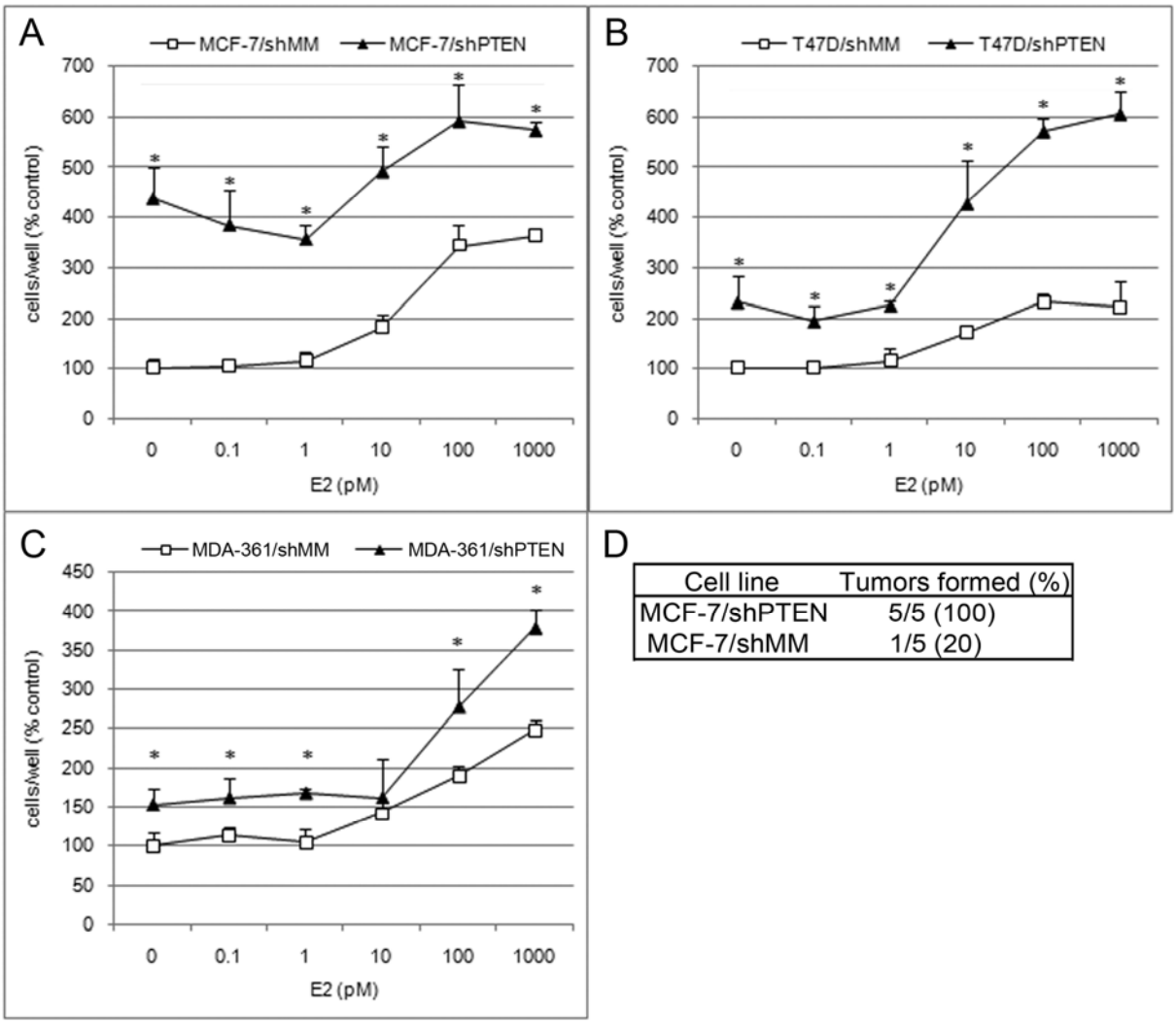

Fig. S1- PTEN loss confers increased hormone-independent and E2-induced growth. A-C) MCF-7 (A), T47D (B), and 361 (C) lines were treated as indicated. Media were refreshed every 2-3 days. Cells were trypsinized and counted after 5-8 days. Data are presented as % untreated shMM control, mean of triplicates +/- SD. * $p<0.05$ by *t*-test comparing shPTEN to shMM control under each condition. D) MCF-7/shPTEN and /shMM xenografts in E2-supplemented, nude female mice (5 per cell line) were evaluated for tumor appearance over eight wks by palpataion and visualization (>3 mm in diameter).

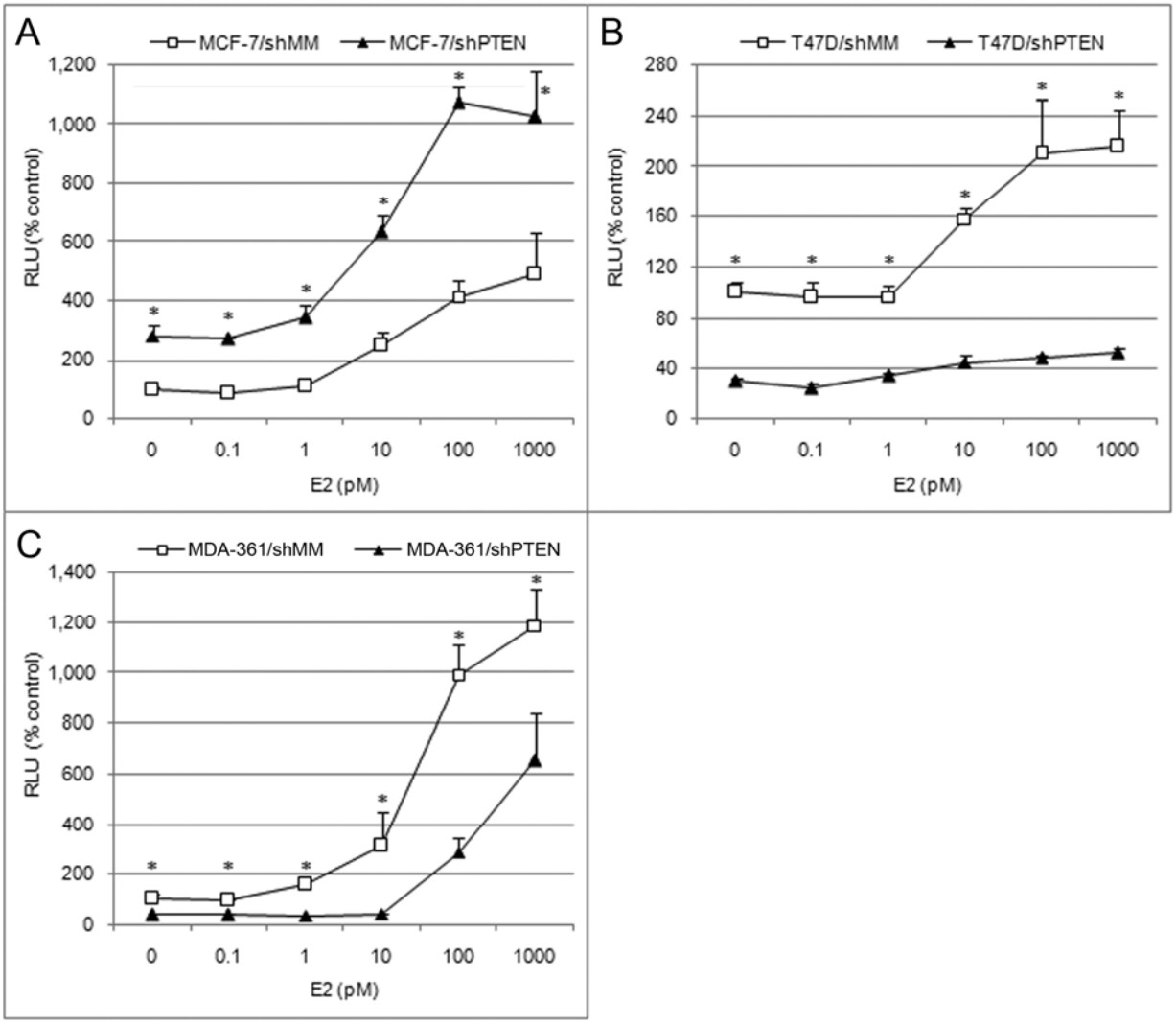

Fig. S2- PTEN loss alters ER transcriptional activity. MCF-7 (A), T47D (B), and 361 (C) lines were transfected with ERE-driven firefly and control *Renilla* luciferase reporter plasmids. Cells were treated as indicated, and luciferase activity was measured 16-20 hrs later. RLU- relative light units (firefly/*Renilla*). Data are presented as % untreated shMM control, mean of triplicates +/- SD. * $p<0.05$ by *t*-test comparing shPTEN to shMM under each condition.

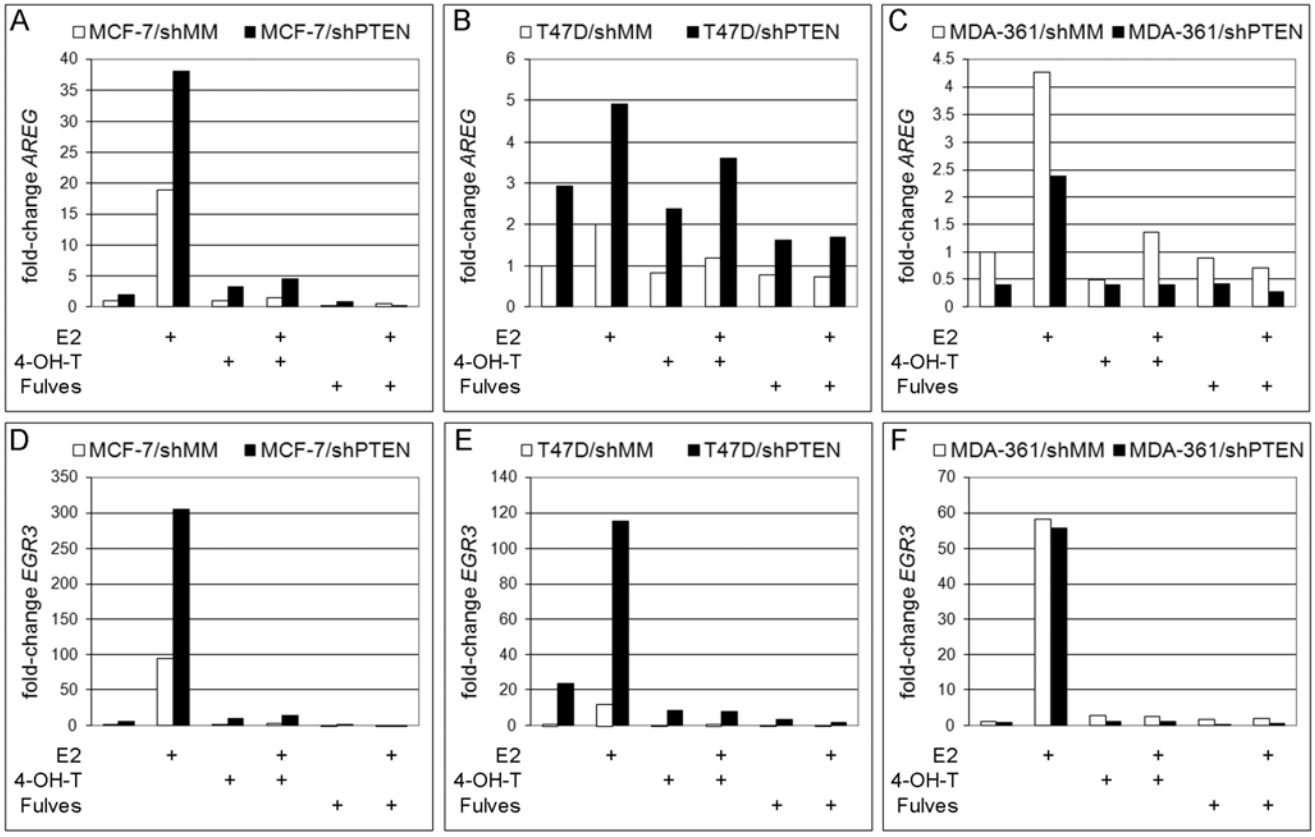

Fig. S3. PTEN loss alters the expression of ER-regulated genes. MCF-7 (A,D), T47D (B, E), and 361 (C,F) lines were hormone-deprived with IMEM + DCC-FBS (MCF-7: 2%; T47D, MDA-361: 0.5%) x 2-3 days. Cells were then treated with the same medium +/- E2 (1 nM), 4-OH-T (1 μM), or fulvestrant (1 μM) x 16-20 hrs, then RNA was harvested. RNA was reverse-transcribed and used for real-time PCR with primers for *AREG*, *EGR3*, and *36B4* (ribosomal protein). *AREG* (A-C) and *EGR3* (D-F) Ct values were normalized against *36B4* Ct values. Data are presented as mean of duplicates, fold-change vs. each shMM control.

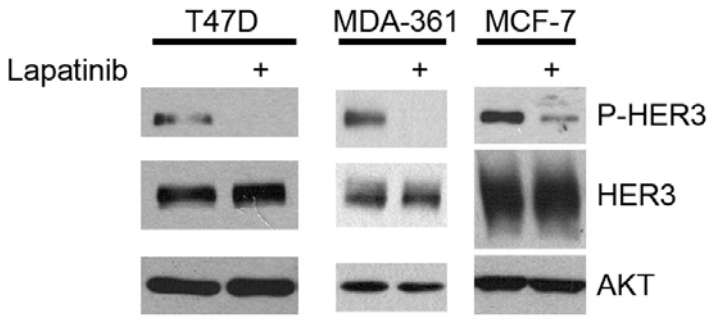

Fig. S4- Phosphorylation of HER3 is blocked by the EGFR/HER2 inhibitor lapatinib. Lysates from shMM lines of MCF-7, T47D, and 361 cells that had been treated overnight +/- 1 μM lapatinib were separated by SDS-PAGE followed by Western blotting with P-HER3$_{Y1289}$, HER3, and AKT antibodies. AKT was used as a loading control.

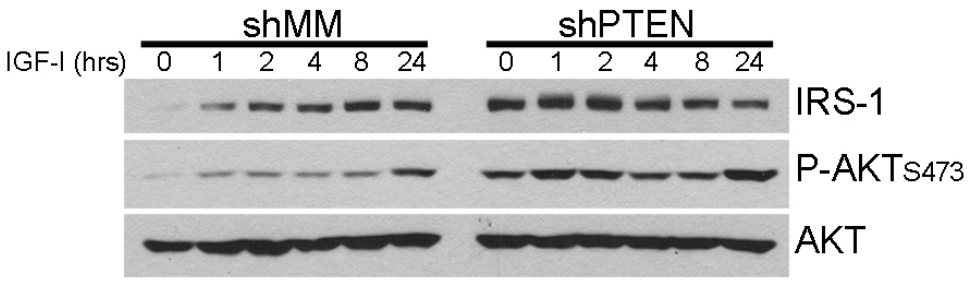

Fig. S5- PTEN loss permits modest IGF-I-induced downregulation of IRS-1. Lysates from MCF-7/shMM and MCF-7/shPTEN cells that had been incubated overnight in serum-free medium, followed by stimulation with IGF-I (100 ng/mL) for the indicated times, were analyzed by Western blotting with the indicated antibodies.

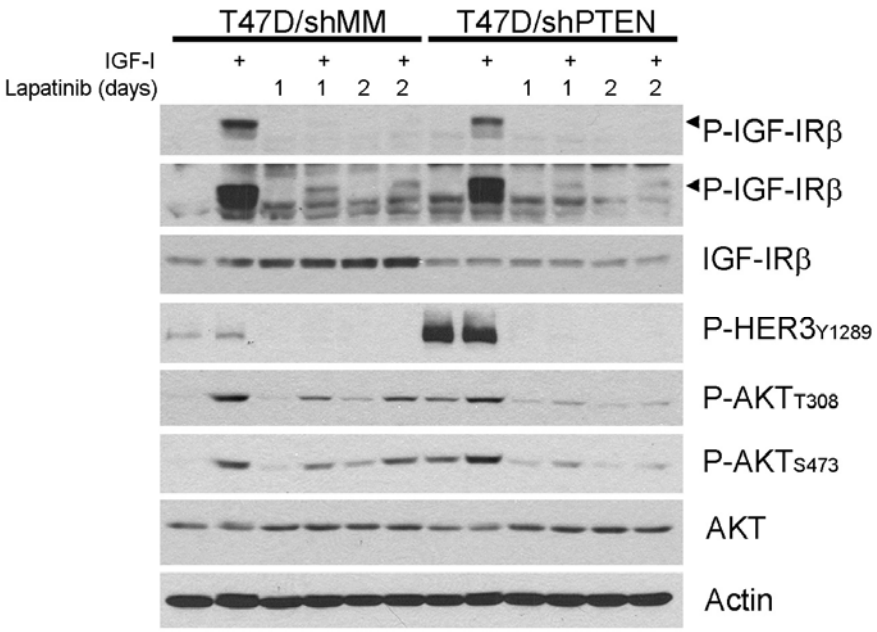

Fig. S6- ErbB kinase activity is permissive for ligand-induced activation of IGF-IR. T47D lines were treated +/- 1 μM lapatinib for 1 or 2 days. During the last day of lapatinib treatment, cells were also serum-starved. Cells were then stimulated +/- IGF-I (100 ng/mL x 15 min.) +/- lapatinib in serum-free medium. Lysates were analyzed by Western blotting with the indicated antibodies. Arrowhead indicates P-IGF-IRβ.

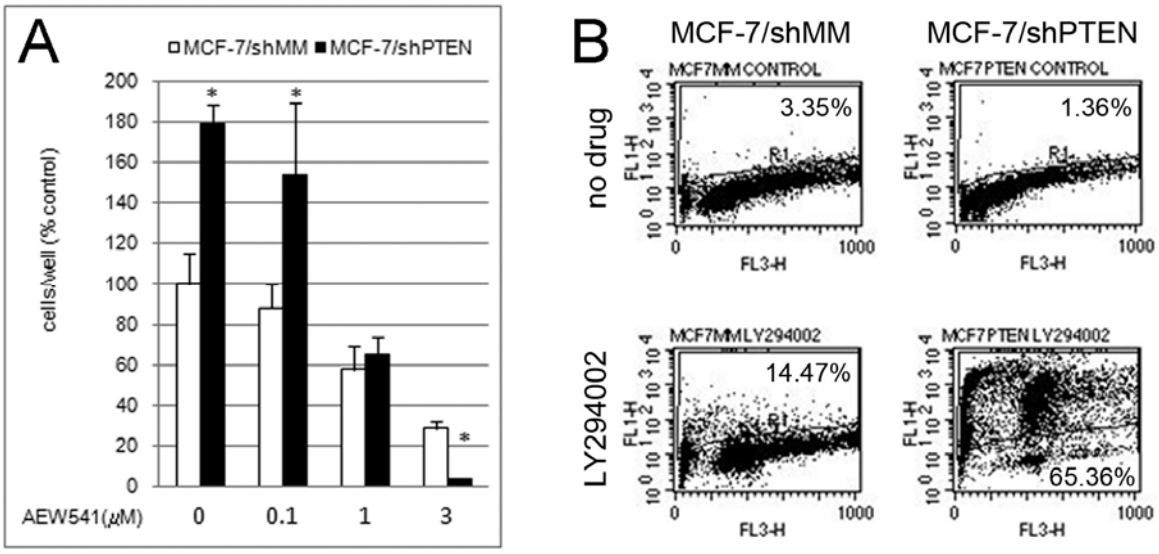

Fig. S7- PTEN loss increases sensitivity to IGF-IR and PI3K inhibitors in MCF-7 cells. A) MCF-7/shPTEN and /shMM cells were treated +/- AEW541 (0-3 μM) in DMEM + 0.1% FBS x 4 days (media were refreshed after 3 days), then trypsinized & counted. Results are presented as mean of triplicates (% untreated shMM control) +/- SD. * $p<0.05$ by *t*-test comparing shPTEN to shMM under each condition. B) MCF-7 cells were serum-starved +/- LY294002 (25 μM) x 3 days. Cells were analyzed for apoptosis with ApoBrdu kit, and analyzed by flow cytometry (10,000 events). x-axis: propidium iodide profile; y-axis: FITC-labeled fragmented DNA. % gated cells is indicative of the % of FITC-positive and/or low-PI (sub-G1) staining apoptotic cells.

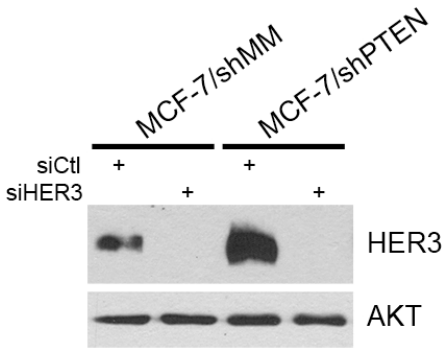

Fig. S8- siRNA against *HER3* knocked-down HER3 protein levels. MCF-7/shPTEN and /shMM cells were transfected with siRNA against *HER3* or control (siCtl). Forty-eight hrs later, whole cell lysates were analyzed by Western blotting using HER3 and total AKT (control) antibodies.