

Figure W1. Tamoxifen cannot prevent tumorigenesis induced by RCAS-*PyMT* or RCAS-*ErbB2*. (A) Six-week-old mice were infected with RCAS-*PyMT*. One week later, mice were randomized and divided into either vehicle (n = 23) or tamoxifen (n = 24) groups. No difference in tumor latency was detected (P = .2). (B) 10- to 12-week-old mice were infected with RCAS-*ErbB2*. One week later, mice were randomized and divided into either vehicle (n = 13) or tamoxifen (n = 15) groups. No difference in tumor latency was detected (P = .3).



Figure W2. The level of *ErbB2* expression under control of the RCAS LTR is not affected by ovariectomy. (A) Adult mice were infected with RCAS-*ErbB2*. One week later, mice were randomized, and either ovariectomy (n = 9) or sham surgery (n = 9) was performed. Two weeks later, mice were killed. ErbB2 was detected by IHC against HA. The levels of ErbB2 expression are equivalent in intact and ovariectomized mice. Scale bar, 20 μ m. (B and C) Tumor lysates from intact (n = 7) and ovariectomized mice (n = 6) were analyzed for ErbB2 by Western blot analysis for the HA tag in ErbB2 (B). The abundance of ErbB2 relative to GAPDH is shown in the scatter plot (C). There are equivalent levels of ErbB2 in tumors of intact and ovariectomized mice (P = .4). Bar indicates median value.



Figure W3. The effect of ovariectomy on downstream signaling by ErbB2 in RCAS-*ErbB2*–induced mammary tumors. (A and B) Tumor lysates from intact (n = 7) and ovariectomized mice (n = 6) were analyzed for total Erk and phospo-Erk (p-Erk) by Western blot analysis (A). The abundance of activated p-Erk relative to GAPDH is shown in the scatter plot (B). pErk is equivalent in tumors of intact and ovariectomized mice (P = .8). Bar indicates mean value. (C and D) Tumor lysates from intact (n = 7) and ovariectomized mice (n = 6) were analyzed for total AKT and phospo-AKT (p-AKT) by Western blot analysis (C). The abundance of p-AKT relative to GAPDH is shown in scatter plot (D). p-AKT is slightly reduced in tumors arising in ovariectomized mice (P = .05). Bar indicates mean value.



Figure W4. ER⁻ and ER⁺ tumors arising from somatic activation of *ErbB2* display similar proliferation rates. (A and B) ER⁻ (n = 5) and ER⁺ (n = 4) tumors were immunostained for Ki67 (A). The percentage of Ki67⁺ cells is shown in the scatter plot (B). There is no difference in the proliferation rate (P = .5). Bar indicates median value.