Supporting Information

Samanta et al. 10.1073/pnas.1421438111

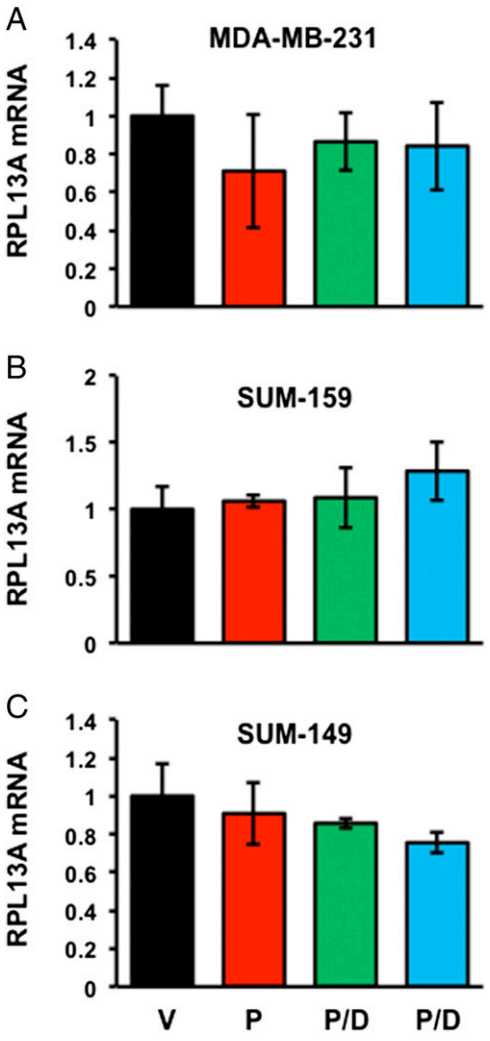

Fig. S1. RPL13A mRNA was analyzed as a representative non–HIF-regulated gene product. MDA-MB-231 (*A*), SUM-159 (*B*), and SUM-149 (*C*) cells were treated with vehicle or either 10 nM (MDA-231 and SUM-159) or 5 nM (SUM-149) paclitaxel, either alone (P) or in combination with 100 nM digoxin (P/D) or 1 μM acriflavine (P/A) for 4 d. RT-qPCR analyses were performed to assess the expression of RPL13A mRNA relative to 18S rRNA in the same sample and normalized to vehicle-treated cells (mean ± SEM; $n = 3$).

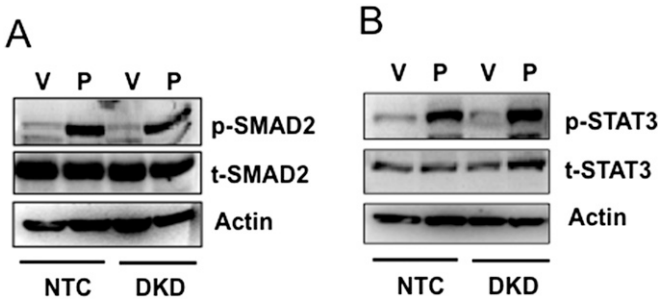

Fig. S2. SMAD2 and STAT3 are phosphorylated in response to paclitaxel in the presence or absence of HIF activity. NTC and DKD subclones of MDA-MB-231 cells were treated with either vehicle or 10 nM paclitaxel for 4 d and immunoblot assays were performed using antibodies specific for phosphorylated (p-) and total (t-) SMAD2 (*A*) or STAT3 (*B*).

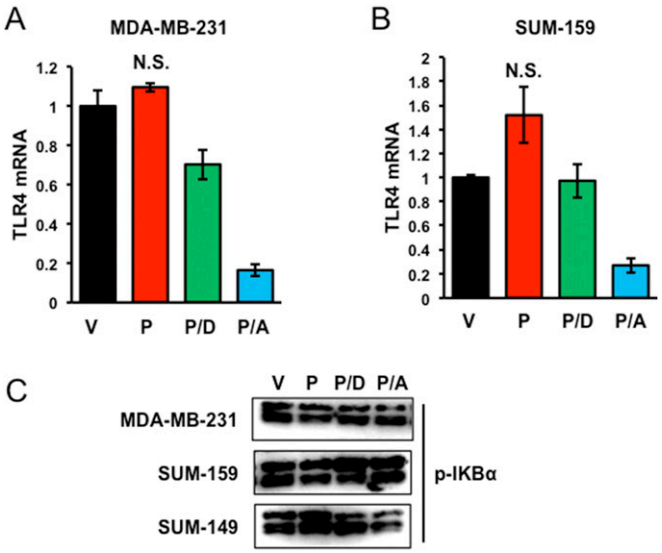

Fig. S3. TLR-4 expression and IκB phosphorylation are not affected by paclitaxel treatment of TNBC cell lines. (*A* and *B*) MDA-MB-231 (*A*) and SUM-159 (*B*) cells were treated with vehicle (V) or 10 nM paclitaxel, either alone (P) or in combination with 100 nM digoxin (P/D) or 1 μM acriflavine (P/A), for 4 d. RT-qPCR analyses were performed to quantify TLR-4 mRNA relative to 18S rRNA in the same sample and normalized to V (mean ± SEM; $n = 3$). N.S., not significant. (*C*) MDA-MB-231, SUM-159, and SUM-149 cells were treated with vehicle or either 10 nM (MDA-MB-231 and SUM-159) or 5 nM (SUM-149) paclitaxel, either alone (P) or in combination with 100 nM digoxin (P/D) or 1 μM acriflavine (P/A) for 4 d, and immunoblot assays were performed using antibodies specific for phosphorylated IκBα.

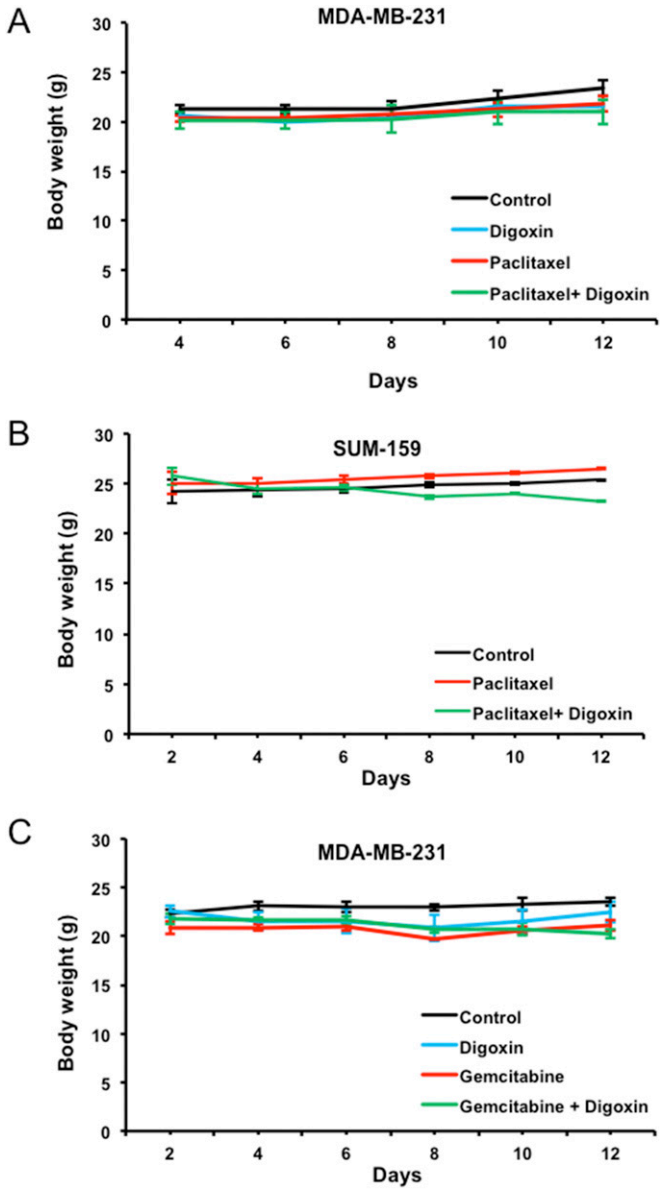

Fig. S4. Body weights of tumor-bearing mice were not affected by treatment with chemotherapy or digoxin. Scid mice received mammary fat pad injections of MDA-MB-231 cells (*A* and *C*) and athymic Nude mice received s.c. injections of SUM-159 cells (*B*). Body weights were measured every 2–3 d during the time period of treatment with saline (control), digoxin (days 1–12), chemotherapy (paclitaxel or gemcitabine, days 5 and 10), or the combination of chemotherapy and digoxin.

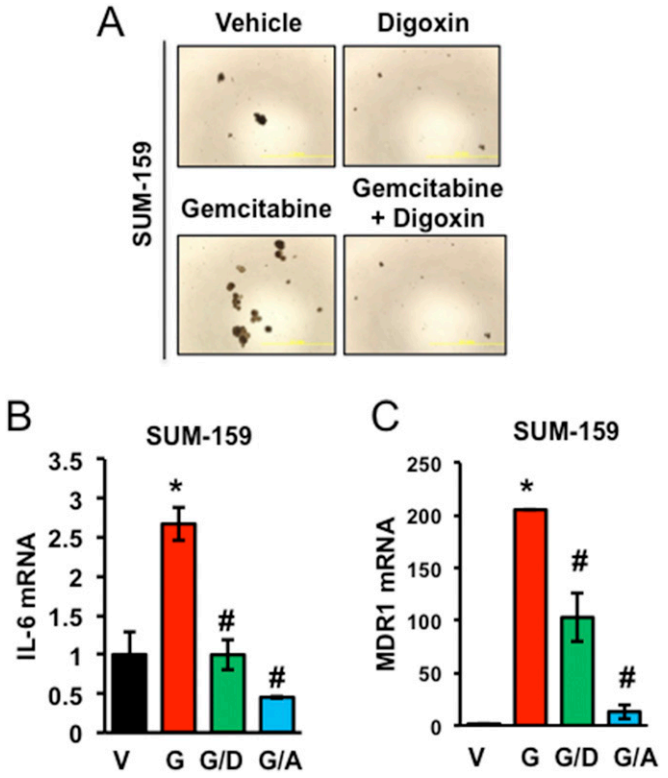

Fig. S5. Gemcitabine treatment of SUM-159 cells induced BCSC enrichment and increased expression of IL-6 and MDR1 mRNA, which were inhibited by digoxin or acriflavine. (*A*) Cells were treated with vehicle, 100 nM digoxin, 20 nM gemcitabine, or gemcitabine and digoxin for 4 d. Cells were trypsinized and subjected to mammosphere assays. Representative photomicrographs of mammospheres are shown. (Scale bar, 2 mm.) (*B* and *C*) Cells were treated with vehicle (V) or 20 nM gemcitabine, either alone (G) or in combination with 100 nM digoxin (G/D) or 1 μM acriflavine (G/A) for 4 d. RT-qPCR assays were performed for IL-6 (*B*) and MDR1 (*C*) mRNA relative to 18S rRNA, with expression normalized to vehicle-treated cells (mean ± SEM; $n = 3$). $^{*}P < 0.001$ compared with V, and $^{\#}P < 0.001$ compared with G, by Student's *t* test.

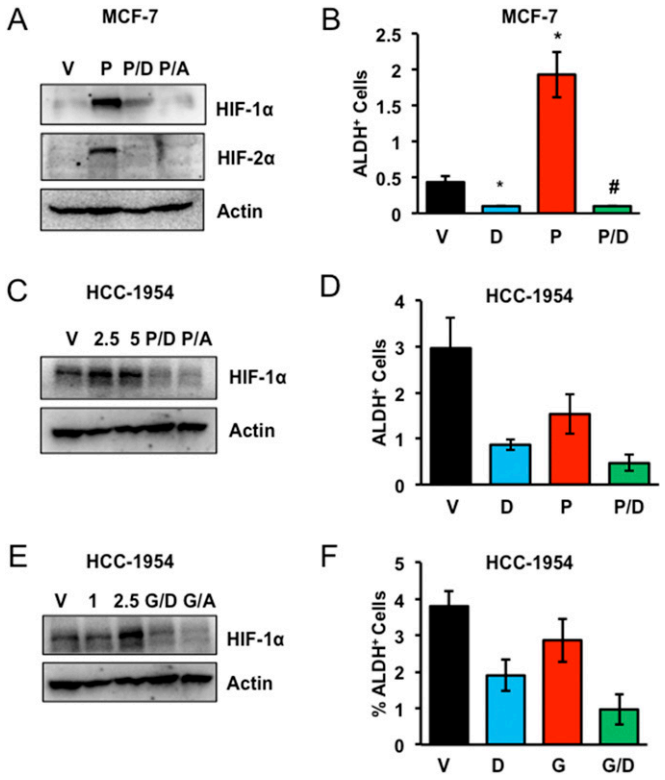

Fig. S6. Effect of paclitaxel or gemcitabine treatment of other breast cancer cell types. (*A* and *B*) MCF-7 cells were treated with vehicle (V) or 10 nM paclitaxel, either alone (P) or in combination with 100 nM digoxin (P/D) or 1 μM acriflavine (P/A) for 4 d and immunoblot (*A*) or Aldefluor (*B*) assays were performed (mean ± SEM; $n = 3$). *$P < 0.001$ compared with V, and $^{\#}P < 0.001$ compared with P, by Student's *t* test. (*C*) HCC-1954 cells were treated with vehicle (V), 2.5 or 5 nM paclitaxel alone, or 2.5 nM paclitaxel in combination with 100 nM digoxin (P/D) or 1 μM acriflavine (P/A) for 4 d, and immunoblot assays were performed. (*D*) HCC-1954 cells were treated with vehicle (V), digoxin (D; 100 nM), paclitaxel (P; 2.5 nM), or both paclitaxel and digoxin (P/D) for 4 d, and Aldefluor assays were performed (mean ± SEM; $n = 3$). (*E*) HCC-1954 cells were treated with vehicle (V), 1 or 2.5 nM gemcitabine alone, or 2.5 nM gemcitabine in combination with 100 nM digoxin (G/D) or 1 μM acriflavine (G/A) for 4 d, and immunoblot assays were performed. (*F*) HCC-1954 cells were treated with vehicle (V), digoxin (D; 100 nM), gemcitabine (G; 2.5 nM), or both gemcitabine and digoxin (G/D) for 4 d, and Aldefluor assays were performed (mean ± SEM; $n = 3$).

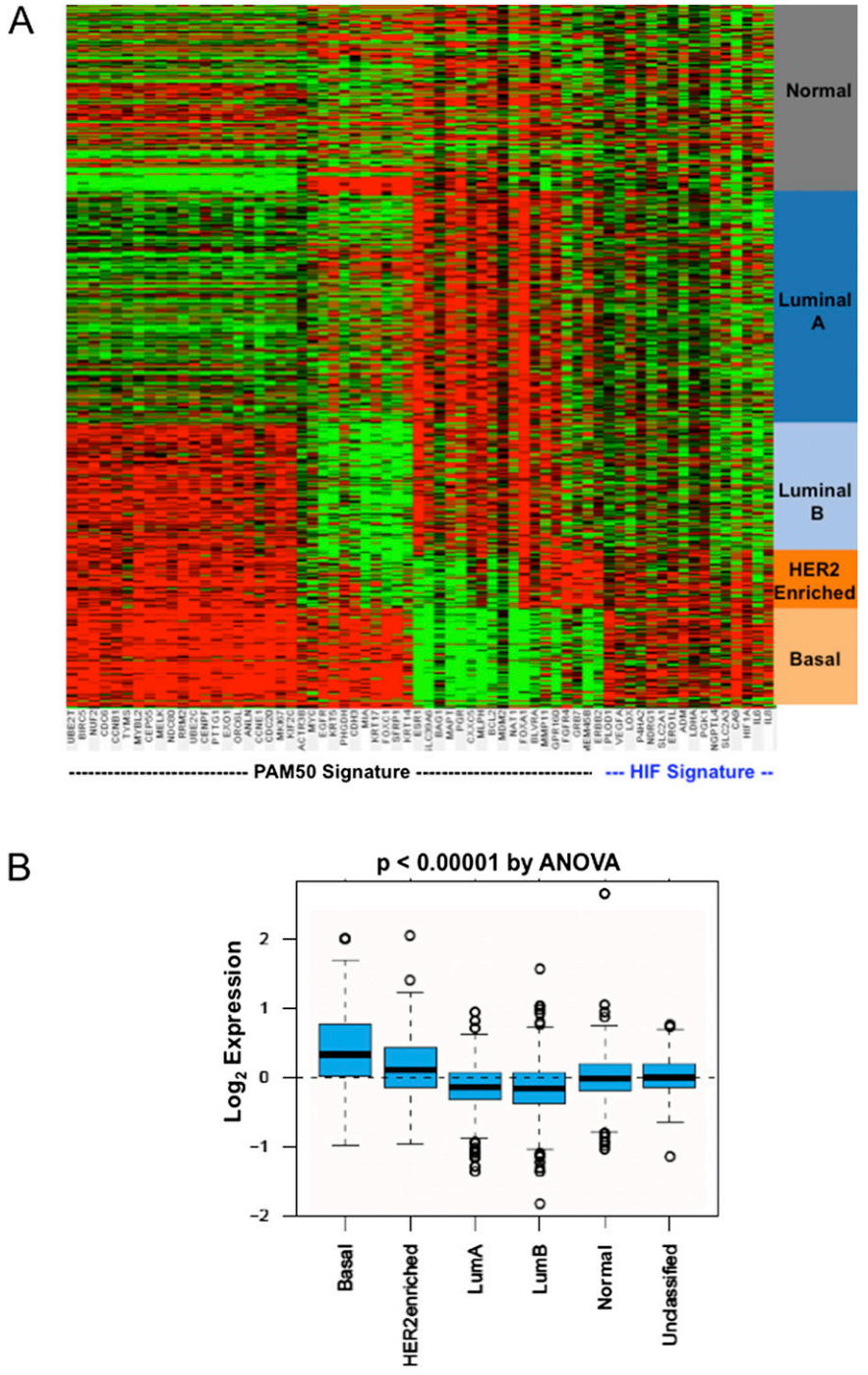

Fig. S7. Expression of HIF-1 target genes (HIF-1 signature) in primary breast cancers. (*A*) Expression of PAM50 (1) and HIF-1 signatures in 1,160 primary breast cancers was used to classify tumors into those with expression greater (red) or less (green) than the median expression level. (*B*) HIF-1 signature expression was significantly associated with basal subtype in an analysis of 1,160 breast cancers using the GOBO database (2). Lum, luminal.

1. Chia SK, et al. (2012) A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res* 18(16):4465–4472.
2. Ringnér M, Fredlund E, Häkkinen J, Borg Å, Staaf J (2011) GOBO: Gene expression-based outcome for breast cancer online. *PLoS ONE* 6(3):e17911.

Table S1. Sequence of oligonucleotide primers used for qPCR

Gene name	Oligonucleotide sequence (5′ to 3′)
HIF-1α Fwd	CCACAGGACAGTACAGGATG
HIF-1α Rev	TCAAGTCGTGCTGAATAATACC
HIF-2α Fwd	TTGCTCTGAAAACGAGTCCGA
HIF-2α Rev	GGTCACCACGGCAATGAAAC
IL-6 Fwd	ACTCACCTCTTCAGAACGAATTG
IL-6 Rev	CCATCTTTGGAAGGTTCAGGTTG
IL-8 Fwd	ACTGAGAGTGATTGAGAGTGGAC
IL-8 Rev	AACCCTCTGCACCCAGTTTTC
18S rRNA Fwd	CGGCGACGACCCATTCGAAC
18S rRNA Rev	GAATCGAACCCTGATTCCCCGTC
RPL13A Fwd	CTCAAGGTCGTGCGTCTG
RPL13A Rev	TGGCTTTCTCTTTCCTCTTCT
CA9 Fwd	TCTCGTTTCCAATGCACGTACAGC
CA9 Rev	AGTGACAGCAGCAGTTGCACAGT
ENO1 Fwd	AGATAGGACCGGTGAGCCGAACT
ENO1 Rev	AAAGTTGTCAGCAAGGTCGAGGG
MDR1 Fwd	TTGGCTGATGTTTGTGGGAAG
MDR1 Rev	CCAAAAATGAGTAGCACGCCT