Supplemental Table 1: Correlation between EGFRvIII expression and ER/PR/HER2 phenotype

	ER+/PR+/ HER2-	ER+/PR- /HER2-	ER-/PR- /HER2+	ER-/PR- /HER2-	Other Subtypes	Total
EGFRvIII+	9 (69)	1 (8)	0	3 (23)	0	13
EGFRvIII-	41 (31)	11 (8)	18 (13)	37 (28)	27 (20)	134
Total	50	12	18	40	27	147

Note—Numbers in parentheses are percentages. Percentages may not add up to 100% because of rounding.

Supplemental Table 2: EGFRvIII expression in primary breast carcinoma, corresponding adjacent normal tissue, and affected lymph node samples

	Tumor		Adjacent Tissue		Lymph Node	
	%EGFRvIII+ (flow cytometry)	EGFRvIII (RT-PCR)	%EGFRvIII+ (flow cytometry)	EGFRvIII (RT-PCR)	%EGFRvIII+ (flow cytometry)	EGFRvIII (RT-PCR)
BR1	4.7	-	3.5	-		
BR2	28.7	+	9.35	+	32.3	+
BR5	11.1	+	8.58	+	40.7	+
BR10	9.52	+	2.2	-		
BR12	2.6	+	0	-		
BR13	0	-	0	-		

Supplemental Table 3: Primer sequences utilized for qRT-PCR analyses

Gene Name	Primer Sequence
ABCC1-F	5’-AGCCGGTGAAGGTTGTGTAC-3’
ABCC1-R	5’-TGACGAAGCAGATGTGGAAG-3’
ABCG2-F	5'-CACCTTATTGGCCTCAGGAA-3'
ABCG2-R	5'-CCTGCTTGGAAGGCTCTATG-3'
AXIN2-F	5’-CCAGACTCAGTGGGAAGAGC-3’
AXIN2-R	5’-AAGAGACAGGCATGGGTTTG-3’
BMI1-F	5'-GCAGCAATGACTGTGATG-3'
BMI1-R	5'-AGTCCATCTCTCTGGTGAC-3'
Fibronectin-F	5’-ACCAACCTACGGATGACTCG-3’
Fibronectin-R	5’-GCTCATCATCTGGCCATTTT-3’
GAPDH-F	5'-GGTCTTACTCCTTGGAGGCCATGTG-3'
GAPDH-R	5'-ACCTAACTACATGGTTTACATGTT-3'
LEF1-F	5′-AATAAAGTCCCGTGGTGC-3′
LEF1-R	5′-ATGGGTAGGGTTGCCTGAATC-3′
N-Cadherin-F	5’-GACAATGCCCCTCAAGTGTT-3’
N-Cadherin-R	5’-CCATTAAGCCGAGTGATGGT-3’
NESTIN-F	5'-GGCAGCGTTGGAACAGAGGTTGGA-3'
NESTIN-R	5'-CTCTAAACTGGAGTGGTCAGGGCT-3'
OCT4-F	5'-ATTCAGCCAAACGACCATCT-3'
OCT4-R	5'-TTGCCTCTCCACTCGGTTCTC-3'
Slug1-F	5’-CTTTTTCTTGCCCTCACTGC-3’
Slug1-R	5’-GCTTCGGAGTGAAGAAATG-3’
Snail1-F	5’-ACCCCACATCCTTCTCACTG-3’
Snail1-R	5’-TACAAAAACCCACGCAGACA-3’
SOX-2-F	5'-ACACCAATCCCATCCACACT-3'
SOX-2-R	5'-GCAAACTTCCTGCAAAGCTC-3'
Twist1-F	5’-GTCCGCAGTCTTACGAGGAG-3’
Twist1-R	5’-CCAGCTTGAGGGTCTGAATC-3’

Supplemental Figure 1: Non-tumor associated normal breast tissues do not express EGFRvIII

A Fresh primary non-tumor associated human breast samples (NTB) were dissociated and analyzed for EGFRvIII (FITC labeled, x axis) expression by flow cytometry. Representative plots from NTB1 and NTB2 samples are shown. Upper panels show gating determined from the isotype control (mouse IgG1).

Supplemental Figure 2: Engineered breast cell lines express EGFRvIII under an inducible promoter

A-D SUM159-LUC (**A**), SUM159-vIII (**B**), MCF10A-LUC (**C**), and MCF10A-vIII (**D**) cells were cultured for 72 hours with or without the addition of dox at a concentration of 100 ng/ml. Cells were harvested and analyzed by flow cytometry for EGFRvIII expression. Left panels show background from the isotype control (mouse IgG1), middle panels show EGFRvIII expression in the absence of dox, and right panels show EGFRvIII expression in the presence of 100 ng/ml dox.

Supplemental Figure 3: DKK-1, FH535, and AG1478 inhibitors mediate decreases in Wnt signaling using the pTopFLASH-GFP reporter system

A-B SUM159-LUC (**A**) and SUM159-vIII (**B**) cells were infected with the pTopFLASH-GFP reporter system and GFP expression was assayed in the presence or absence of DMSO (vehicle control), human recombinant DKK1 (100ng/ml), FH535 (20 μM), or AG1478 (4 μM). Flow cytometry was used to quantify GFP expression, plots show percentages of GFP^+ cells. Left panel shows background from non-infected cells.

Supplemental Figure 4: EGFRvIII increases the percentage of $CD44^{+}/CD24^{-/low}$ cells through activation of Wnt signaling

A-B Representative flow cytometry plots showing percentages of $CD44^{+}/CD24^{-/low}$ stem-cell like cells in MCF10A-LUC (**A**) and MCF10A-vIII (**B**) cells cultured for 48 hours with or without the addition of DMSO (vehicle control), human recombinant DKK1 (100ng/ml), FH535 (20 μM), or AG1478 (4 μM). Cells were analyzed by flow cytometry for CD44 and CD24 expression.