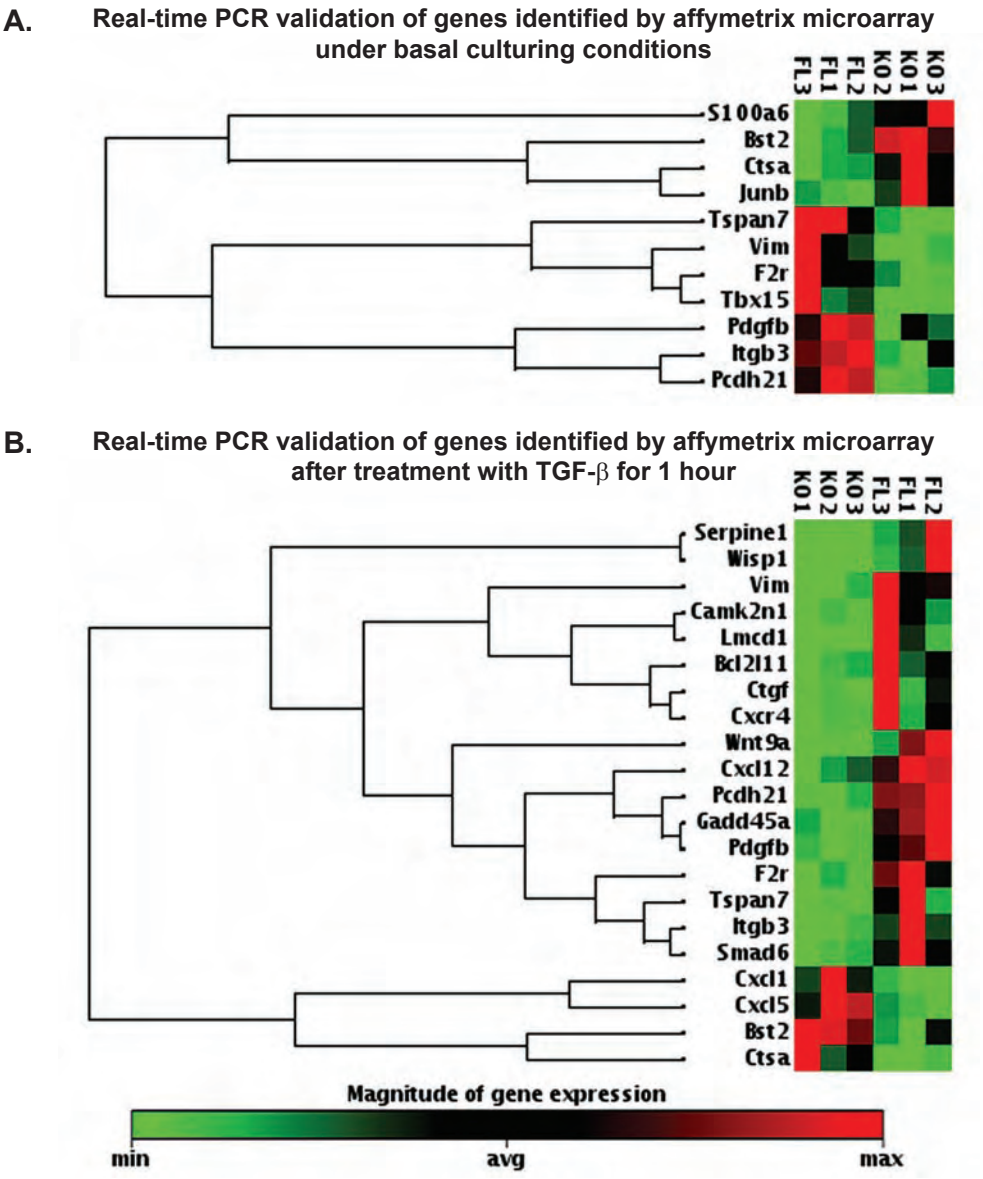
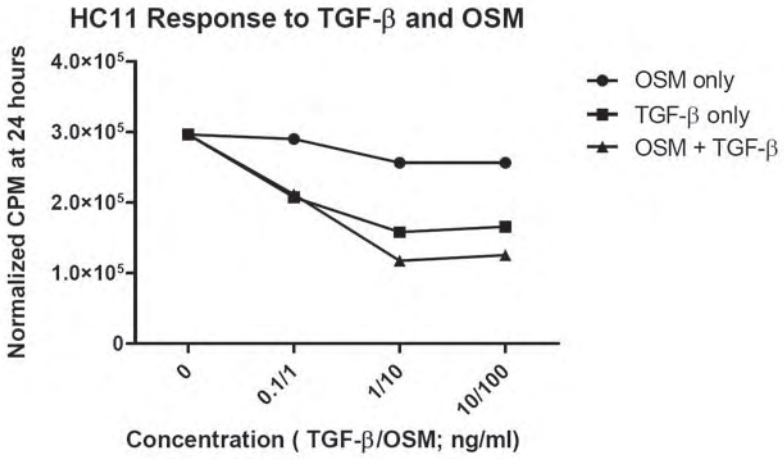
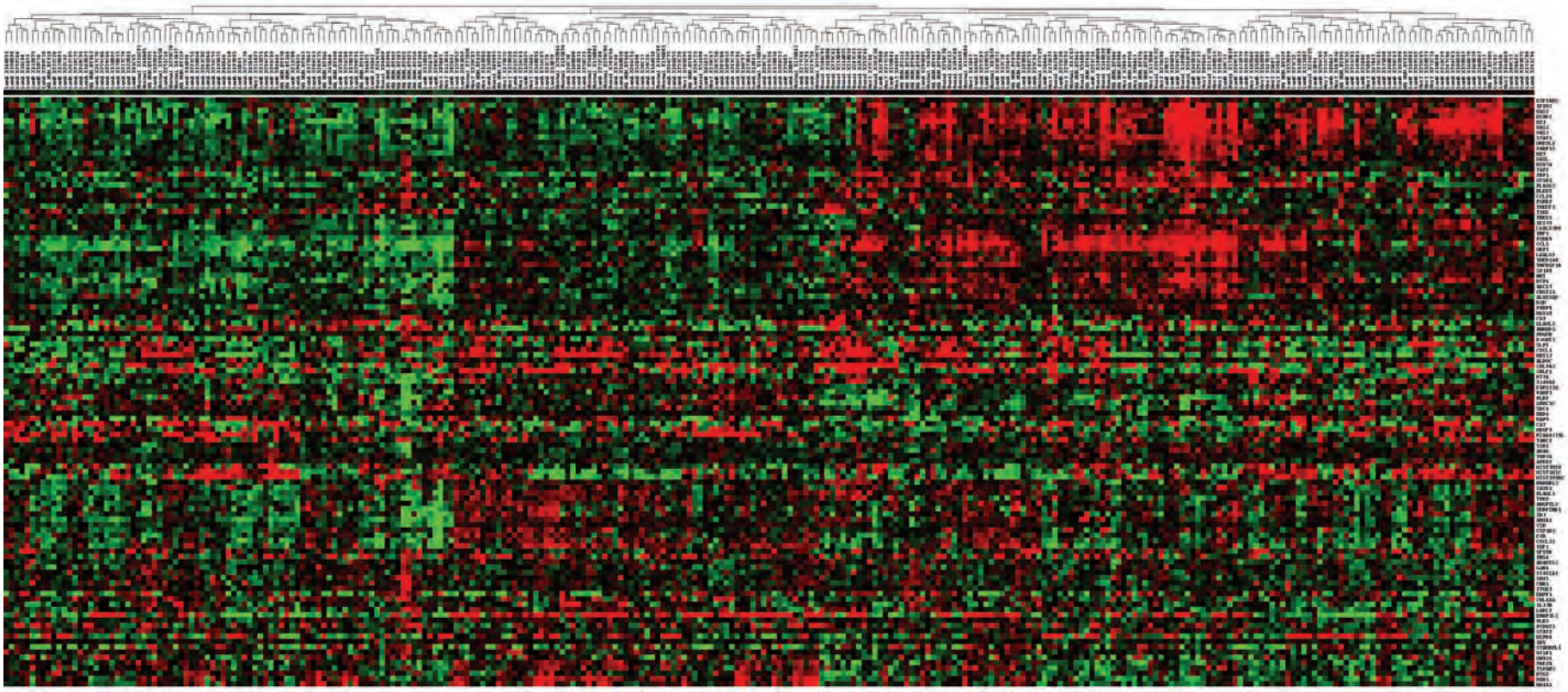


Supplementary Figure S1. Cluster analyses for genes that were validated by real-time PCR. Genes that demonstrated a significant difference in expression with a two-tailed unpaired t-test value of 0.05 or less were used for clustering. Each row represents the heatmap interpretation of $1/\Delta Ct$ values associated with a specific gene. **A.** TβRII$^{(WKO;PY)}$ and TβRII$^{(fl/fl;PY)}$ carcinoma cell lines cultured in complete medium. **B.** TβRII$^{(WKO;PY)}$ cells cultured in complete medium compared with TβRII$^{(fl/fl;PY)}$ carcinoma cells in the presence of complete medium with TGF-β at 10ng/ml one hour after stimulation. No differences in gene expression were observed when TβRII$^{(WKO;PY)}$ cells cultured in complete medium were compared with TβRII$^{(WKO;PY)}$ cells cultured in complete medium containing TGF-β ligand at 10ng/ml one hour after stimulation. FL1, FL2 and FL3, TβRII$^{(fl/fl;PY)}$; KO1, KO2 and KO3, TβRII$^{(WKO;PY)}$. Values were normalized to *Gusb*, *Hprt1*, *Hsp90ab1*, *Actb* and *PPIA*.



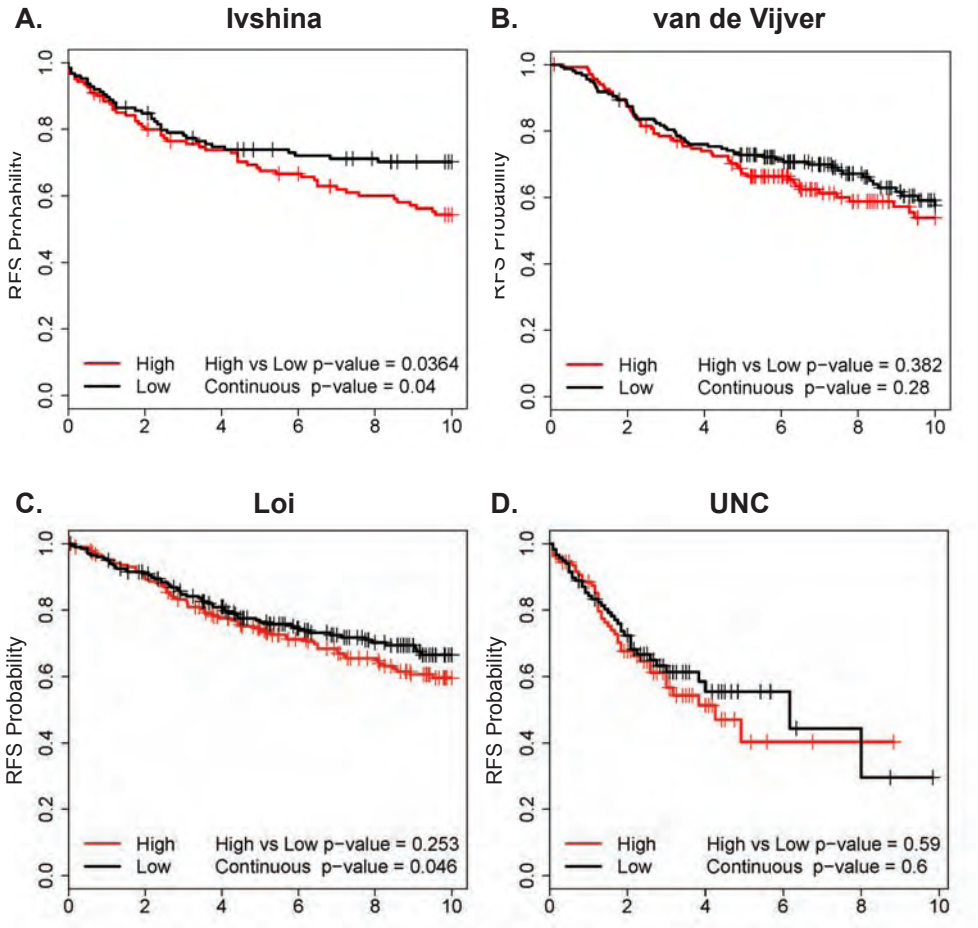
Supplementary Figure S2. TGF-β and OSM effect on HC11 cell growth. Both TGF-β and OSM stimulation at varying concentrations resulted in decreased tritiated thymidine incorporation 24 hours after treatment. TGF-β stimulation resulted in a significant decrease in tritiated thymidine uptake at 0.1, 1.0 and 10.0 ng/ml. OSM significantly decreased thymidine uptake at 10ng/ml and 100ng/ml. Results represent normalized mean counts per minute (CPM) +/- standard error of the mean. Significance was implied if the two-tailed unpaired t-test p-values were less than 0.05. The results for TGF-β and OSM appeared to be additive when both ligands were present in comparison with the values obtained from individual ligand stimulation.

Cluster analysis of the Ivshina dataset using the TβRII $^{(WKO;PY)}$ gene expression signature

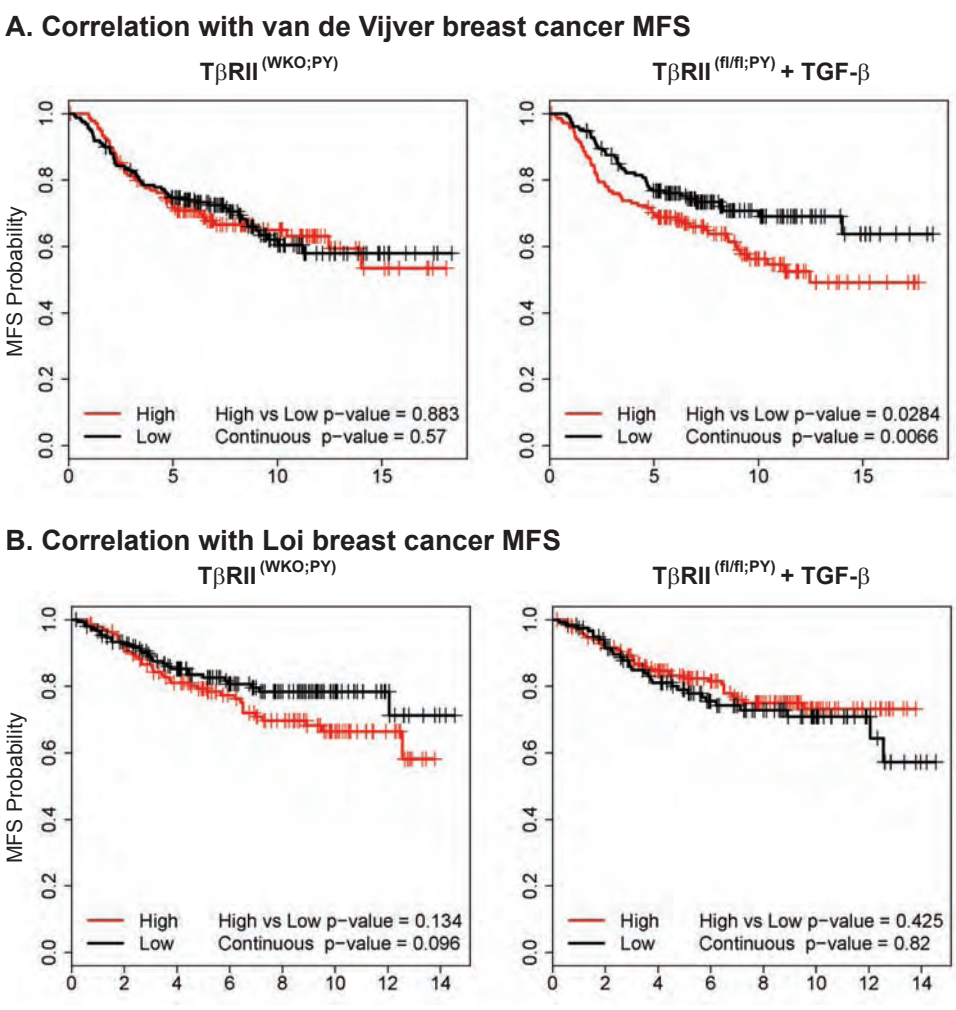


Supplementary Figure S3. Cluster analysis of the TβRII$^{(WKO;PY)}$ gene expression signature in the Ivshina dataset. The results indicated that there may be an association between the TGF-β signaling deficient TβRII$^{(WKO;PY)}$ mammary carcinoma cell signature and subtype classification in human breast cancer.

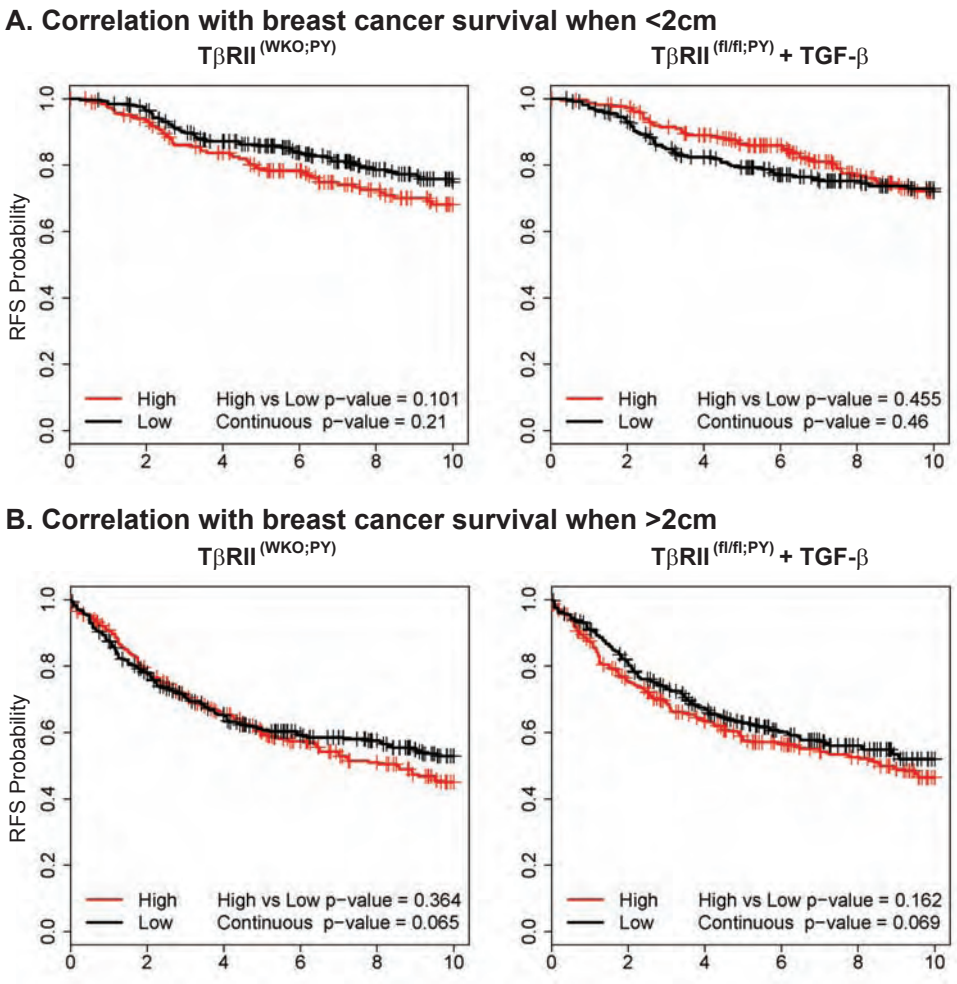
Correlation between the TβRII$^{(WKO;PY)}$ signature and breast cancer relapse-free survival



Supplementary Figure S4. Increased risk of relapse when TGF-β signaling deficient TβRII$^{(WKO;PY)}$ mammary carcinoma cell gene expression signature was correlated with samples within the Ivshina dataset. A. The TβRII$^{(WKO;PY)}$ mammary carcinoma cell signature significantly correlated with reduced relapse-free survival in the Ivshina dataset (Continuous r p-value was 0.04 and the Log Rank p-value was 0.0364). **B-D.** Although similar trends were present, no significant correlation was noted between the TβRII$^{(WKO;PY)}$ mammary carcinoma cell signature and relapse-free survival in the van de Vijver, Loi or UNC datasets respectively. Red, high correlation (r>0); Black, low correlation (r<0).

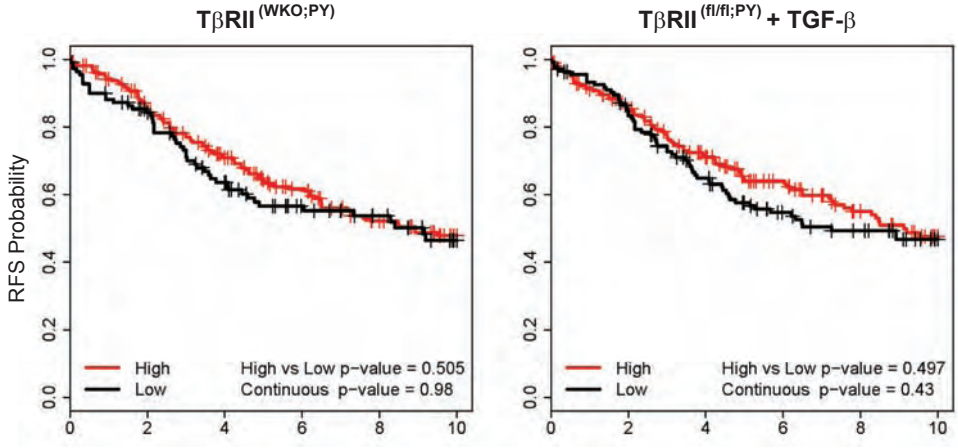


Supplementary Figure S5. Correlation between the TβRII$^{(WKO;PY)}$ and TGF-β treatment signatures with metastasis-free survival (MFS) in the van de Vijver and Loi human breast cancer datasests. A significant correlation was observed between the TGF-β treatment signature and reduced MFS in the van de Vijver dataset, however no significant difference was observed in correlation with the Loi breast cancer MFS. Red, high correlation (r>0); Black, low correlation (r<0).

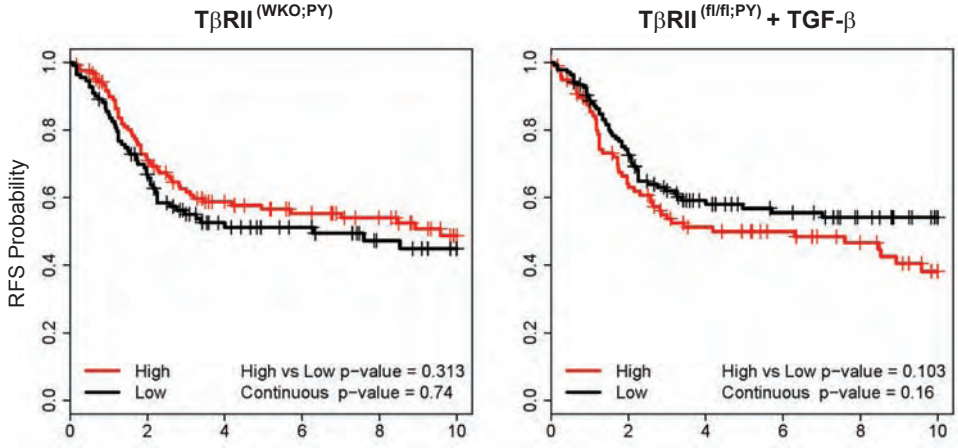


Supplementary Figure S6. Correlation between the TβRII$^{(WKO;PY)}$ and TGF-β treatment signatures with relapse-free survival in association with tumor size. A. No significant difference in relapse-free survival related to either signature when tumors were less than 2cm. **B.** In tumors that were larger than 2cm at the time of collection, no significant difference was observed. Red, high correlation (r>0); Black, low correlation (r<0).

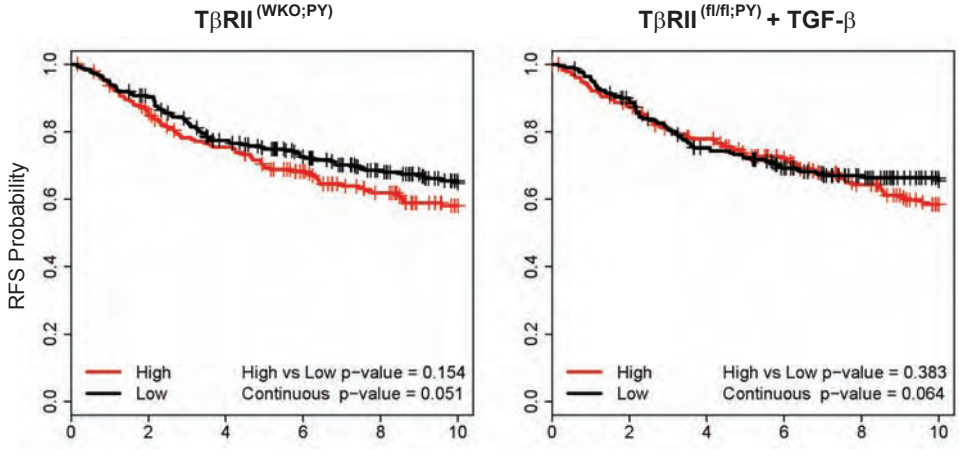
A. Correlation with luminal B subtype breast cancer survival



B. Correlation with ER- breast cancer survival

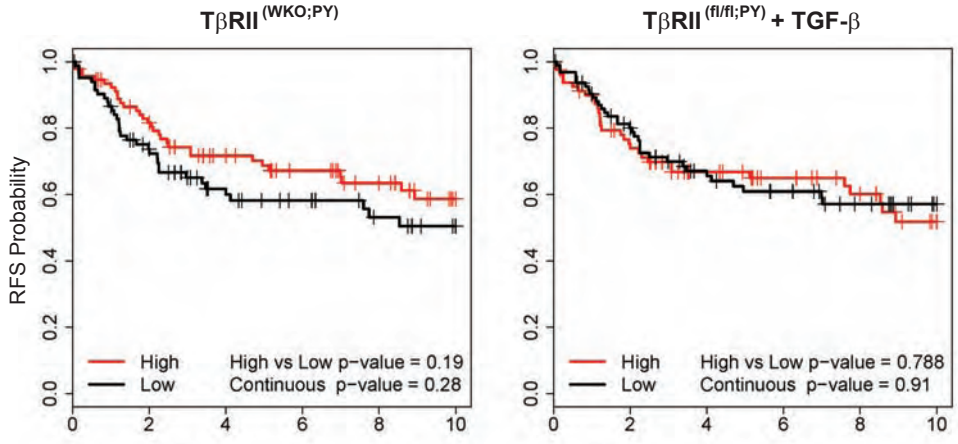


C. Correlation with no systemic adjuvent breast cancer survival

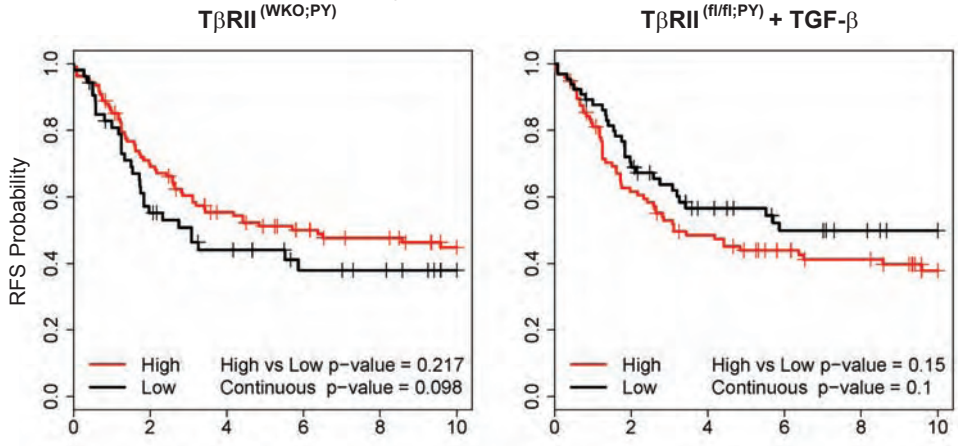


Supplementary Figure S7. Correlation between the TβRII$^{(WKO;PY)}$ and TGF-β treatment signatures with relapse-free survival in Luminal B, ER- or no adjuvant treated human breast cancer. No significant differences were observed in correlation with either signature in Luminal B (**A**) and ER- (**B**) breast cancer or in association with the patients that had not been treated with a systemic adjuvant (**C**). Red, high correlation (r>0); Black, low correlation (r<0).

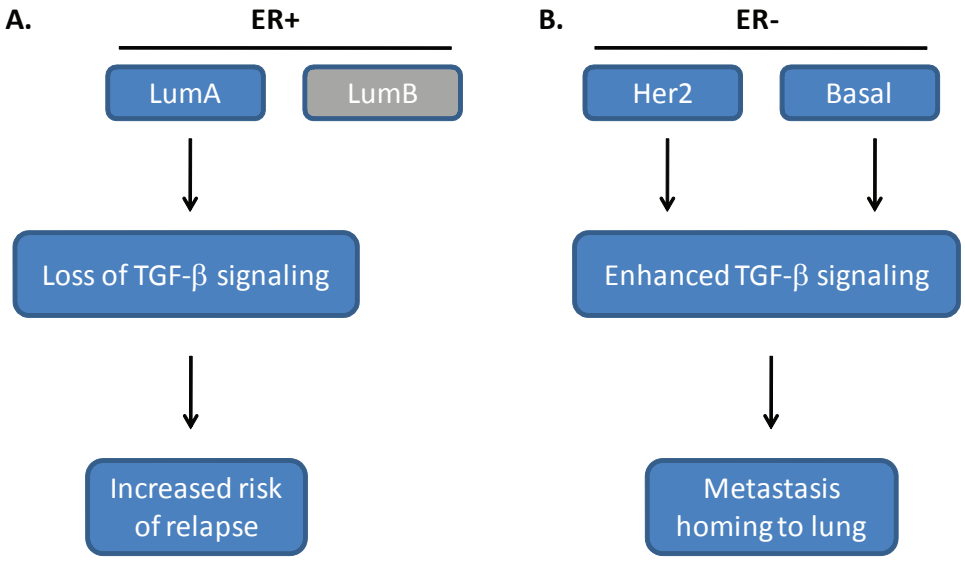
A. Correlation with Basal subtype breast cancer survival



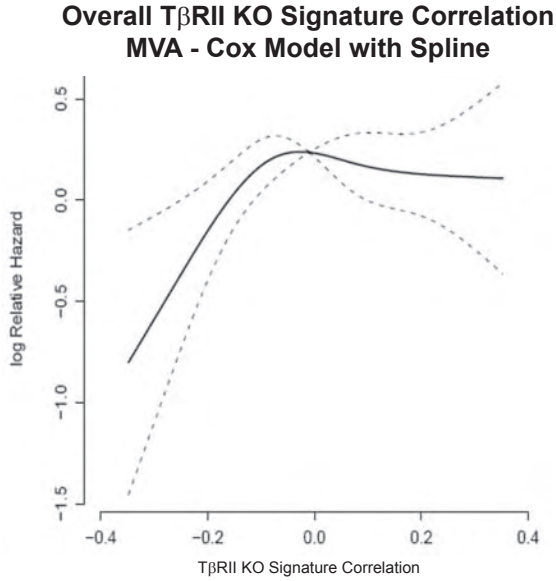
B. Correlation with Her2 subtype breast cancer survival



Supplementary Figure S8. Correlation between the TβRII$^{(WKO;PY)}$ and TGF-β treatment signatures with relapse-free survival in Basal or Her2 subtype human breast cancer. **A.** No significant differences were observed in correlation with either signature and Basal subtype tumor patient prognosis **B.** The Her2 subtype patient population also failed to demonstrate a significant correlation with RFS probability for either signature. Red, high correlation (r>0); Black, low correlation (r<0).



Supplementary Figure S9. Consolidation of our relapse-free survival data and the results recently reported by Padua, et al. regarding TGF-β dependent homing of metastatic breast cancer cells to lung tissue.



Supplementary Figure S10. Graphical representation of results obtained using a Cox model for multivariate analysis (MVA). The TβRII KO gene expression signature had a statistically significant non-linear correlation with overall survival in the combined 1,319 patient dataset.

TβRII$^{(WKO;PY)}$ Gene Expression Signature

Downregulated

Adamts3	Crlf1	Megf10	Serpine1
Adcy7	Cxcl12	Megf9	Slc35f1
Aldoc	Dpysl5	Mtap9	Sox5
Alg13	Elavl2	Myo7a	Ssr1
Alox5ap	Enpp3	Pcdh21	St8sia2
Ankrd29	F2r	Pde2a	Tbx15
Apex2	Gja7	Pdgfb	Tmeff1
Car2	Gm22	Plagl1	Tmem29
Car9	Gna14	Rgs10	Tspan7
Chst11	Igf1	Rspo3	Tyms
Cnr1	Il17b	Satb1	Vim
Col9a3	Itgb3	Sbk1	Wisp1

Upregulated

Adar	Gbp6	Lgals9	Rsad2
Angptl2	Gjb4	Lgr6	Rtp4
Ankrd1	Gprc5c	Mlstd2	S100a6
Anxa1	Gvin1	Mt1	Saa3
B2m	H2-K1	Mt2	Scara5
B3gnt3	H2-Q1	Mx1	Sdc4
Brd4	H2-Q8	Mx2	Sectm1b
Btg2	H2-T10	Nmi	Sftpd
C1qdc2	H2-T23	Nr4a1	Slfn2
C1r	Hist1h1c	Nradd	Slpi
C79267	Hist1h2bc	Nuak2	Sp100
Ccl20	Hist3h2a	Oas1g	Stambpl1
Ccl5	Hspb8	Oas2	Stat1
Clec2d	Id3	Oas3	Stat2
Col4a6	Ids	Oasl1	Syce2
Csprs	Ifi203	Parp12	Tanc2
Ctsa	Ifi35	Parp14	Tap2
Cxcl1	Ifih1	Parp3	Tmem140
Cxcl16	Ifit3	Parp8	Tnfrsf1b
Cyp1b1	Irf1	Parp9	Tns4
D11Lgp2e	Irgm	Phf11	Tor3a
Dleu2	Isgf3g	Pim3	Tpm2
Drbp1	Khdrbs3	Pla2g7	Trex1
Egr1	Krt17	Plk2	Tyki
Ehbp1l1	Lamc2	Plk3	Ube1l
Eif2ak2	Lgals3	Psmb2	Ube2l6
Gbp2	Lgals3bp	Psmb9	Zbp1

TGF-β Treatment Gene Expression Signature

Downregulated

Adamts1	Cdc42ep3	Cxcl1	Epgn
Adamts15	Cebpd	Cxcl5	Gdap10
Alcam	Chka	Cyp1b1	Ppp1r3c
Ccl20	Csn3	Dusp6	Tslp

Upregulated

Adora1	Edn2	Lmcd1	Sh3bp2
Bcl11a	Egr2	Lrig3	Slc20a1
Bcl2l11	Egr3	Lrp4	Smad6
Bhlhb2	Fos	Map3k14	Spsb1
Camk2n1	Fosb	Mfsd2	Tmem98
Ctgf	Foxq1	Myo1d	Wisp1
Ctla2a	Gadd45a	Pdgfb	Wnt9a
Ctla2b	Gadd45g	Plekhh1	Zfp750
Cxcl12	Gja3	Rasl11b	
Cxcr4	Gse1	Serpine1	
Ddit4	Junb	Sfn	

Supplementary Table S1. Differentially expressed genes identified when TβRII$^{(WKO;PY)}$ and TβRII$^{(fl/fl;PY)}$ mammary carcinoma cells were compared. Genes that had a higher level of expression in the TβRII$^{(WKO;PY)}$ samples were considered upregulated and those that were lower in the TβRII$^{(WKO;PY)}$ model were considered downregulated. Genes were selected if they met all of the following criteria: signal was consistently up- or downregulated at least 1.5 fold in all TβRII$^{(WKO;PY)}$ samples when compared to the TβRII$^{(fl/fl;PY)}$ controls, at least two of the three experimental samples represented a 2.0 fold or higher change in expression when compared to the TβRII$^{(fl/fl;PY)}$ controls, and the CV value for the TβRII$^{(fl/fl;PY)}$ group was less than 2.0.

Annotated legend for the Raybiotech Cytokine Antibody Array

Pos	Pos	Neg	Neg	Blank	Axl	Blc	Cd30 L	Cd30 T	Cd40	Cxcl10	Ccl27b	Cxcl16	Ccl11
Pos	Pos	Neg	Neg	Blank	Axl	Blc	Cd30 L	Cd30 T	Cd40	Cxcl10	Ccl27b	Cxcl16	Ccl11
Ccl24	Fasl	Cx3cl1	Gcsf	Gm-csf	Ifng	Igfbp-3	Igfbp-5	Igfbp-6	Il-1a	Il-1b	Il-2	Il-3	Il-3 Rb
Ccl24	Fasl	Cx3cl1	Gcsf	Gm-csf	Ifng	Igfbp-3	Igfbp-5	Igfbp-6	Il-1a	Il-1b	Il-2	Il-3	Il-3 Rb
Il-4	Il-5	Il-6	Il-9	Il-10	Il-12 p40/70	Il-12 p70	Il-13	Il-17	Cxcl1	Leptin R	Leptin	Cxcl5	L-Selectin
Il-4	Il-5	Il-6	Il-9	Il-10	Il-12 p40/70	Il-12 p70	Il-13	Il-17	Cxcl1	Leptin R	Leptin	Cxcl5	L-Selectin
Lymphotactin	Ccl2	Ccl12	M-csf	Cxcl9	Ccl3	Ccl9	Cxcl2	Ccl19	Ccl20	Cxcl4	P-Selectin	Ccl5	Scf
Lymphotactin	Ccl2	Ccl12	M-csf	Cxcl9	Ccl3	Ccl9	Cxcl2	Ccl19	Ccl20	Cxcl4	P-Selectin	Ccl5	Scf
Ccl12	Ccl17	Ccl1	Ccl25	Timp-1	TNFa	sTNFRI	sTNFRII	Tpo	Vcam-1	Vegf	Blank	Blank	Pos
Ccl12	Ccl17	Ccl1	Ccl25	Timp-1	TNFa	sTNFRI	sTNFRII	Tpo	Vcam-1	Vegf	Blank	Blank	Pos

Supplementary Table S2. Annotated legend for the Raybiotech Antibody Array. The antibody array description was analyzed to determine alias designations for the included antibody antigens. Chemokine ligands were listed with their respective Ccl and Cxcl designations.

Author	GEO Accession Numbers	Samples	Node+	Node- No Systemic Adjuvant Therapy	ER+ (%)	Endocrine Therapy Only
UNC	GSE10886	361	143/251 (57%)	15/178 (8.4%)	137/244 (56%)	27/228 (12%)
Ivshina, et al.	GSE4922	249	81/240 (34%)	134/203 (66%)	211/245 (86%)	66/203 (33%)
Loi, et al.	GSE6532	414	143/393 (36%)	134/393 (34.4%)	349/394 (89%)	277/414 (67%)
van de Vijver, et al.	GSE2845	295	144/295 (49%)	141/295 (47.8%)	225/295 (76%)	20/295 (7%)
	Total	1319				

Supplementary Table S3. Patient samples used for correlate analysis. A total of 1319 patient samples were included from the four annotated datasets. GEO ID, gene omnibus expression database identifier.

TGF-β Signaling Signature: Multivariate Cox Model with Spline

Variables	HR	95 CI	p-value
TβRII KO			0.015
1st tertile	1.00		
2nd tertile	1.29	1.07-1.56	
3rd tertile	1.16	0.89-1.52	
TGF-β stim			0.161
1st tertile	1.00		
2nd tertile	1.09	0.92-1.30	
3rd tertile	1.22	0.98-1.50	
T			<0.001
0	1.00		
1	2.21	1.73-2.82	
N			0.004
0	1.00		
1	1.52	1.14-2.02	
ER Status			<0.001
Negative	1.00		
Positive	0.60	0.45-0.79	
Treatment			0.236
No Adjuvant	1.00		
Chemo	0.68	0.45-1.03	
Hormone	0.76	0.54-1.06	
Chemo + Hormone	0.69	0.40-1.18	
Dataset			0.178
Ivshina	1.00		
Loi	0.88	0.65-1.20	
UNC	1.42	0.89-2.26	
van de Vijver	1.00	0.72-1.41	

Supplementary Table S5

Interaction between ER and the TβRII KO signature

Factor	Chi-Square	d.f.	P
TS	41.81	1	<.0001
N	9.83	1	0.0017
tx	5.96	3	0.1136
Dataset	4.12	3	0.2486
ER (Factor + Higher Order Factors)	17.74	4	0.0014
All Interactions	4.79	3	0.1875
TβRII KO Corr. (Factor + Higher Order Factors)	15.65	6	0.0158
All Interactions	4.79	3	0.1875
Nonlinear (Factor + Higher Order Factors)	10.59	4	0.0316
ER * TβRII KO Corr. (Factor + Higher Order Factors)	4.79	3	0.1875
Nonlinear	2.58	2	0.2751
Nonlinear Interaction : f(A,B) vs. AB	2.58	2	0.2751
TOTAL NONLINEAR	10.59	4	0.0316
TOTAL NONLINEAR + INTERACTION	12.55	5	0.0280
TOTAL	108.15	15	<.0001

Supplementary Table S6