Altered Death Receptor Signaling Promotes the Epithelial-to-Mesenchymal Transition and Acquired Chemoresistance

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SUPPLEMENTAL INFORMATION

Methods

Animals

Xenograft models were performed similar to previously reported studies.[1,2] In brief, Nu/nu immune-compromised female ovariectomized mice (29-32 days old) were obtained from Charles River Laboratories (Wilmington, MA). The animals were allowed a period of adaptation in a sterile and pathogen-free environment *ad libitum*. Placebo or estradiol pellets (0.72 mg, 60-day release; Innovative Research of America, Sarasota, FL) were implanted s.c. in the lateral area of the neck in the middle point between the ear and shoulder using a precision trochar (10 gauge) 10 mice each. MCF-7 or MCF-7TN-R cells in the exponential phase of growth were harvested using PBS/EDTA solution and washed. Viable cells (5×10^6) in a 50 μL sterile PBS suspension were mixed with 100 μL Matrigel Reduced Factors (BD Biosciences, Bedford, MA). Cells were injected in the mammary fat pad through a 5 mm incision in the hypogastric region, and the incision was closed using staples. To minimize differences in tumor matrix between xenographs the same immunocompromised mouse subtype, identical cell-number, similar hormone environments, and equal amounts of Matrigel were used in each xenograft experiment.[3,4] All the procedures in animals were performed under anesthesia using a mix of isofluorane and oxygen delivered by mask. Tumor size was measured every 2 days using a digital caliper. The volume of the tumor was calculated using the following formula: $4/3\pi\ LS^2$ (L = larger radius; S = shorter radius). At necropsy on day 21, animals were euthanized by cervical dislocation after exposure to a CO_2 chamber. Tumors, uteri, livers, and lungs were removed and either frozen in liquid nitrogen or fixed in 10% formalin for further analysis. All procedures involving these animals were conducted in compliance with State and Federal laws, standards of the U.S. Department of Health and Human Services, and guidelines established by the Tulane University Animal

Care and Use Committee. The facilities and laboratory animal program of Tulane University are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Western Blot Analysis

Protein analysis was performed as described.[5,6] Briefly, Cells were plated at 50-60% confluency in 10-cm^2 culture flasks in 5% DMEM for 48 h. Cells were then treated with DMSO, SKI-II or ABC294640 and 10% DMEM added for 45 min. Following treatment, cells were detached with PBS-EDTA and centrifuged. After removing supernatant, cells were lysed in 60-100 µL lysis buffer (Mammalian Protein Extraction Reagent, MPER, and Halt protease inhibitor, Pierce, Rockford, IL; and PhoSTOP phosphatase inhibitor, Roche, Boulder, CO). Lysed cells were centrifuged for 10 min at 12,000G at 4°C to separate protein from cell debris. The supernatants were combined with loading buffer (5% 2-mercaptoethanol in 4x LDS Loading Buffer, Invitrogen, Carlsbad, CA), boiled for 5 min, and loaded onto a 4-12% Bis Tris Polyacrilamide Gels (Invitrogen) followed by polyacrylamide gel electrophoresis at 150V for 1.25 hrs. Protein was transferred to nitrocellulose membranes using the iBlot (Invitrogen) transfer unit. Nitrocellulose membranes were blocked in 5% milk (Biorad Laboratories, Hercules, CA) Tris Buffered Saline-Tween 20 (TBS-T) for 1 hr at room temperature. Cells were washed briefly with 1X TBS-T (USB, Cleveland OH) and primary antibodies were diluted in 5% BSA (Bovine Serum Albumin, Sigma-Aldrich, St. Louis, MO) TBS-T according to manufacturer's recommended dilutions. Antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Membranes were incubated in primary antibody overnight at 4°C with gentle agitation. Secondary infrared conjugated antibodies (LI-Cor Biosciences, Lincoln, NE) were diluted in 5% milk-TBST solution at 1:10,000 and membranes were incubated for 1 hr under gentle agitation at room temperature. Membranes were scanned using the LI-COR Odyssey

imager and software (LI-COR Biosciences, Lincoln, Nebraska) to detect total and phosphorylated protein levels in cell lysates.

p65 NF-κB -Luciferase assay

As previously described, the cells were seeded in 24-well plates at a density of $5x10^5$ cells/well in the same media and allowed to attach overnight.[7,8] After 18 h, cells were transfected for 5 h in serum-free DMEM with 10 ng of pFC-NF-kB-luciferase plasmid, using 6 µl of Effectene (Qiagen) per µg of DNA, using 6 µl of Effectene (Qiagen) per µg of DNA. After 5 h the transfection medium was removed and replaced with phenol red-free DMEM supplemented with 5% CS-FBS containing vehicle or indicated concentration of TNF and incubated at 37 °C. After 18 h the medium was removed, and 100 µl of lysis buffer was added per well and then incubated for 15 min at room temperature. Cell debris was pelleted by centrifugation at 15,000 x g for 5 min. Cell extracts were normalized for protein concentration using reagent according to the manufacturers protocol (BioRad Lab.). Luciferase activity for the cell extracts was determined using luciferase substrate (Promega Corp., Madison, WI) in an Autoluminat Plus luminometer (Berthhold Technologies, Bad Wildbad, Germany).

Immunofluorescence analysis of EMT markers and morphology

Immunofluorescence was performed as previously described.[9] Briefly, the expression levels of an epithelial cell marker (E-cadherin) and a mesenchymal cell marker (vimentin) were assessed by indirect immunofluorescence using specific antibodies (Ecadherin: CS-3195 (Cell Signaling Technology, Beverly, MA, USA)); vimentin: V6630 (Sigma, St. Louis, MO, USA)). The distribution of filamentous actin (F-actin) was visualized using Alexa 488 conjugated phalloidin (Invitrogen, Carlsbad, CA, USA). Briefly, MCF-7, MCF-7TN-R, and MDA-MB-231 cells were cultured in eight-well chamber slides for 48 h. The cells were fixed in 4% paraformaldhyde/PBS for 10 min followed by incubation with the primary antibodies

and phalloidin at the desired dilution (E-cadherin: 1:50 dilution; vimentin: 1:50 dilution; phalloidin: 1:100 dilution). Alexa 594 and 488 conjugated secondary antibodies (1:1,500 dilution) were used to detect E-cadherin and vimentin, respectively. The nucleus was stained using DAPI containing VectorShield mounting medium (Vector Laboratories, Burlingame, CA, USA). The digital images were captured using Nikon Eclipse 80*i* along with the accompanying program IPLab, version 3.6.5 (Nikon Inc., Melville, NY, USA).

Quantitative RT-PCR Primer Sequences:

Actin (5'-GCCAACACAGTGCTGTCT-3'; 5'- AGGAGCAATGATCTTGATCTT -3'), e-cadherin (5'- GAACGCATTGCCACATACAC -3'; 5'- GAATTCGGGCTTGTTGTCAT -3'), β-catenin (5'- CCATTCCATTGTTTGTGCAG -3'; 5'- CTTCTGCAGCTTCCTTGTCC -3'), slug (5'- CGTTTTCCAGACCCTGGTT -3'; 5'- CTGCAGATGAGCCCTCAGA -3'), snai1 (5'- GCGAGCTGCAGGACTCTAAT -3'; 5'- GGACAGAGTCCCAGATGAGC -3'), n-cadherin (5'- CTCCTATGAGTGGAACAGGAACG -3'; 5'- TTGGATCAATGTCATAATCAAGTGCTGTA -3'); ERα: (F) 5‟- GGC ATG GTG GAG ATC TTC GA - 3‟ (R) 5‟ – CCT CTC CCT GCA GAT TCA TCA - 3‟; PgR: (F) 5‟ – TAC CCG CCC TAT CTC AAC TAC C – 3‟, (R) 5‟ – TGC TTC ATC CCC ACA GAT TAA ACA - 3‟; SDF-1: (F) 5‟ – AGT CAG GTG GTG GCT TAA CAG - 3‟, (R) 5‟ – AGA GGA GGT GAA GGC AGT GG - 3‟; Cathepsin D (5'- TACCTCGTTTGACATCCAC -3'; 5'- CCTCTCCACTTTGACACC -3')

References

1 Salvo, V. A. *et al.* Antiestrogenic glyceollins suppress human breast and ovarian carcinoma tumorigenesis. *Clin Cancer Res* **12**, 7159-7164 (2006).

2 Antoon, J. W. *et al.* Anti-Estrogenic Effects of the Novel Sphingosine Kinase-2 Inhibitor ABC294640. *Endocrinology* (2010).

3 Oskarsson, T. & Massague, J. Extracellular matrix players in metastatic niches. *The EMBO journal* **31**, 254-256, doi:10.1038/emboj.2011.469 (2012).
4 Lu, P., Weaver, V. M. & Werb, Z. The extracellular matrix: a dynamic niche in cancer progression. *The Journal of cell biology* **196**, 395-406, doi:10.1083/jcb.201102147 (2012).
5 Burow, M. E. *et al.* Differences in susceptibility to tumor necrosis factor alpha-induced apoptosis among MCF-7 breast cancer cell variants. *Cancer research* **58**, 4940-4946 (1998).
6 Antoon, J. W. *et al.* Targeting NF-kB mediated breast cancer chemoresistance through selective inhibition of sphingosine kinase-2. *Cancer biology & therapy* **11**, doi:14903 [pii] (2011).
7 Bratton, M. R. *et al.* Organochlorine-mediated potentiation of the general coactivator p300 through p38 mitogen-activated protein kinase. *Carcinogenesis* **30**, 106-113, doi:bgn213 [pii]
10.1093/carcin/bgn213 (2009).
8 Boue, S. M. *et al.* Identification of the potent phytoestrogen glycinol in elicited soybean (Glycine max). *Endocrinology* **150**, 2446-2453 (2009).
9 Zhou, C. *et al.* Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype. *Breast cancer research : BCR* **10**, R105 (2008).

Supplemental Tables

Supplemental Table 1. Death Receptor Pathway Gene Expression Changes

Group	Gene Symbol	Fold Change	p-value
	TNFRSF1A	-2.32	1.08E-05
	TNFRSF19	1.80	8.49E-05
	TNFSF11	-1.12	4.64E-02
	TNFSF13B	-1.10	1.09E-01
	TNFRSF12A	-7.49	2.86E-07
	TNFRSF17	-1.04	6.51E-01
	TNFRSF17	1.03	6.68E-01
	TNFSF12-TNFSF13	-1.08	7.49E-02
	TNFRSF13B	-1.03	5.44E-01
	TNFRSF11A	1.37	9.17E-04
	TNFSF9	-1.03	5.89E-01
TNF/TNFR	TNFSF14	-1.09	1.04E-01
	TNFRSF13C	-1.01	6.82E-01
	TNFRSF21	-2.14	4.09E-07
	TNFRSF10C	-1.06	3.06E-01
	TNFRSF10B	2.00	4.07E-06
	TNFRSF10D	17.85	9.19E-10
	TNFRSF10A	-7.50	1.17E-06
	TNFRSF11B	-9.43	2.20E-09
	TNFSF15	-1.36	2.48E-03
	TNFSF8	1.02	7.32E-01
	TNF	-1.11	2.12E-01
	TNF	-1.11	2.12E-01
	TNF	-1.11	2.12E-01
TRADD	TRADD	-2.35	5.25E-07
FADD	FADD	-1.31	2.02E-03
	CASP12	-1.00	9.87E-01
	CASP4	-1.90	4.06E-05
	CASP5	-1.03	3.34E-01
	CASP1	-1.01	9.06E-01
Caspase	CASP14	1.01	8.25E-01
	CASP10	1.24	9.15E-03
	CASP8	-3.95	1.05E-06
	CASP6	-1.49	1.40E-04
	CASP3	2.81	1.34E-06
	CASP2	-1.01	9.26E-01
BID	BID	-1.71	2.91E-04

Supplemental Table 2. TRADD Transcription Factor Mediated Gene Expression

(p<0.05 for all listed genes)

Gene Symbol	*Fold Change*
AHR	1.43
ARNT	-1.3
DEAF1	2.89
E2F1	-1.35
E2F4	-1.08
EGR1	-1.1
EGR2	-2.23
EGR3	-2.26
EP300	1.28
ETF	-1.03
ETS1	3.5
GABPB1	-4.03
HES1	1.02
HIC1	-1.05
HIF	-1.14
HNF4A	-1.06
HSF1	-1.36
IKZF1	-1.08
MAX	-1.22
MED1	1.1
MYB	1.04
MYC	1.15
MAX	-1.22
MZF1	1
NFE2L1	-1.35
NFE2L2	-1.97
NFKB1	-1.68
NR2F2	-1.21
PAX4	1.05
PPARG	1.14
PRDM1	-1.05
RB1	1.02
RBPJ	1.04
SMAD3	-2.59
SP1	1.94
SP3	2.61
SPI1	-1.07
SREBF1	-3.18
STAT3	-1.23
TFAP2A	1.87
TFCP2	27.88

TFDP1	2.48
TOPORS	1.15
ZFP161	-1.05
ZIC3	1.34

Supplemental Table 3. TNFRS1A Transcription Factor Mediated Gene Expression (p < 0.05 for all listed genes)

Gene Symbol	*Fold Change*
AHR	1.43
ARNT	-1.3
BACH2	3.31
BRCA1	1.21
CAP1	1.19
CBFB	1.44
CEBPD	-1.38
CREB1	-1.3
DEAF1	2.89
E2	-1.03
E2F1	-1.35
E2F4	-1.08
E4F1	-1.26
EBF1	-3.18
EGR1	-1.1
EGR2	-2.23
EGR3	-2.26
ELF1	-3.72
ELF2	1.38
ELK1	-1.09
ELSPBP1	1.08
EP300	1.28
ETS1	3.5
ETS1	3.5
ETS2	-1.76
ETV7	1.03
FOXO1	-1.04
FOXO3	1.76
FOXO4	3.09
GABPB1	-4.03
GATA1	-1.06
GATA2	1.98
GATA2	1.98
GATA3	-9.33
GATA6	2.14
GTF2I	-1.14
HIC1	-1.05
HNF4A	-1.06
IKZF1	-1.08
IRF1	-1.88
IRF2	1.07

IRF9	-2.96
JUN	1.71
LMO2	1.16
MAF	1.51
MAFG	-1.2
MAZ	1.09
MED1	1.1
MYB	1.04
MYOG	-1.17
MZF1	1
NFATC1	1.14
NFE2	-1.04
NFE2L1	-1.35
NFE2L2	-1.97
NFKB1	-1.68
NHLH1	-1.1
NKX2-5	1.37
NR1H3	-1.46
NR2F2	-1.21
OR5I1	-1.11
PAX1	-1.06
PAX2	1.03
PAX6	11.68
PBX1	-1.65
PCBP1	-1.74
POSTN	-1.02
PPARG	1.14
PRDM1	-1.05
PTF1A	-1.11
RARA	-2.08
RB1	1.02
RBPJ	1.04
REL	4.06
RELA	-1.55
REST	-1.26
RREB1	-1.06
RUNX1	-2.13
RXRG	1.08
SMAD3	-2.59
SMAD4	2.77
SOX9	-1.19
SP1	1.94
SP3	2.61
SPI1	-1.07
SREBF1	-3.18
STAT1	-3.2

STAT3	-1.23
STAT5A	-1.06
STAT6	1.33
TAX	-2.13
TCF12	-1.09
TCF3	1.14
TEAD1	-1.1
TFAP2A	1.87
TFAP2C	-13.5
TFCP2	27.88
TFDP1	2.48
TFDP2	1.84
TGIF1	-1.38
TOPORS	1.15
TP53	2.07
TTF1	1.27
UBP1	1.27
USF1	1.03
VDR	-2.74
WT1	1.4
YY1	-1.31
ZBTB33	3.04
ZBTB7A	-1.6
ZEB1	16.35
ZNF238	4.3

Supplemental Table 4. Densitometric analysis of western blots (Fold MCF-7 normalized to actin control)

	MCF-7	MCF-7TN-R
TNFR1	1.0	0.18
TNFR2	1.0	1.28
p50	1.0	1.09
p65	1.0	0.85
IkB	1.0	0.25

Supplemental Table 5. NF-kappB Pathway Gene Expression Changes

Gene Title	Gene Symbol	Fold Change	p-value
zinc finger E-box binding homeobox 1	*ZEB1*	*12.29*	*1.70E-08*
zinc finger E-box binding homeobox 2	ZEB2	8.74	1.11E-07
NFKB activating protein	NKAP	3.77	1.01E-06
twist homolog 1 (Drosophila)	TWIST1	2.77	1.32E-05
NFKB repressing factor	NKRF	2.73	3.61E-06
superoxide dismutase 2, mitochondrial	SOD2	2.16	5.05E-06
NFKB inhibitor interacting Ras-like 2	NKIRAS2	1.67	2.63E-05
baculoviral IAP repeat-containing 2	BIRC2	1.54	4.27E-04
X-linked inhibitor of apoptosis	XIAP	1.51	5.57E-04
nuclear factor of kappa light polypeptide gene enhancer i	NFKBIE	-1.17	3.44E-03
nuclear factor of kappa light polypeptide gene enhancer i	NFKBID	-1.24	7.79E-04
TNF receptor-associated factor 6	TRAF6	-1.25	1.69E-04
nuclear factor of kappa light polypeptide gene enhancer i	NFKBIB	-1.26	5.90E-04
TNF receptor-associated factor 5	TRAF5	-1.29	3.64E-04
inhibitor of kappa light polypeptide gene enhancer in B-ce	IKBKE	-1.34	7.56E-04
transmembrane BAX inhibitor motif containing 6	TMBIM6	-1.36	3.39E-05
TNF receptor-associated factor 3	TRAF3	-1.39	8.84E-04
tumor necrosis factor (ligand) superfamily, member 10 //	TNFSF10	-1.39	1.16E-03
XIAP associated factor 1	XAF1	-1.41	3.17E-03
TNF receptor-associated factor 4	TRAF4	-1.50	2.53E-04
transmembrane BAX inhibitor motif containing 4	TMBIM4	-1.52	2.21E-03
baculoviral IAP repeat-containing 3	BIRC3	-1.59	9.52E-04
TNFAIP3 interacting protein 2	TNIP2	-1.65	1.43E-04
tumor necrosis factor, alpha-induced protein 3	TNFAIP3	-1.79	5.02E-06
C1q and tumor necrosis factor related protein 6	C1QTNF6	-2.12	6.90E-05
tumor necrosis factor receptor superfamily, member 21 /	TNFRSF21	-2.14	4.09E-07
nuclear factor of kappa light polypeptide gene enhancer in	NFKB1	-2.20	6.87E-06
nuclear factor of kappa light polypeptide gene enhancer in	NFKB2	-2.26	3.26E-09
tumor necrosis factor receptor superfamily, member 1A /	TNFRSF1A	-2.32	1.08E-05
TNFRSF1A-associated via death domain	TRADD	-2.35	5.25E-07
lipopolysaccharide-induced TNF factor	LITAF	-2.42	8.50E-07
TNF receptor-associated factor 7	TRAF7	-2.66	3.40E-06
B-cell CLL/lymphoma 2	BCL2	-3.57	2.34E-07
nuclear factor of kappa light polypeptide gene enhancer i	NFKBIA	-3.88	5.17E-08
nuclear factor of kappa light polypeptide gene enhancer i	NFKBIZ	-4.93	2.45E-07
lymphotoxin beta receptor (TNFR superfamily, member 3)	LTBR	-7.35	1.71E-07
tumor necrosis factor receptor superfamily, member 12A	TNFRSF12A	-7.49	2.86E-07
tumor necrosis factor receptor superfamily, member 10a	TNFRSF10A	-7.50	1.17E-06
tumor necrosis factor receptor superfamily, member 11b	TNFRSF11B	-9.43	2.20E-09

Supplemental Table 6. EMT Pathway Gene Expression Changes

Gene Symbol	Fold-Change	p-value
ABCA12	-28.97	8.69E-12
ABCC4	3.05	1.31E-07
ACSL4	21.62	1.05E-10
AGPAT5	1.44	5.41E-05
AGR2	-61.27	8.72E-10
AIM1	-17.51	2.82E-08
ANLN	1.2	2.86E-02
ANP32E	2.25	1.32E-05
ANXA1	1.43	1.59E-02
ANXA9	-17.41	3.99E-08
AP1S2	1.64	5.46E-04
ARL4A	1.03	3.83E-01
ARSJ	3.31	8.28E-06
ATAD4	-6.66	1.60E-07
ATCAY	-1.01	5.23E-01
BIRC3	-1.59	9.52E-04
C10orf58	-1.91	8.41E-06
C17orf28	-4.66	1.06E-06
C1orf172	-2.01	4.76E-06
C20orf151	-1.33	6.87E-03
C2orf44	1.82	1.37E-04
C6orf150	-22.38	1.00E-08
C6orf173	3.75	3.79E-06
C9orf140	-1.58	1.48E-03
CAMK4	13.55	8.67E-07
CCDC82	11.94	4.80E-08
CCDC88A	37.02	2.07E-11
CCDC99	2.2	5.19E-06
CD24	-76.29	6.62E-09
CD83	1.21	2.22E-03
CDH1	-62.42	8.07E-11
CEBPA	-1.45	1.66E-03
CELSR1	-2.11	4.09E-04
CHN1	1.81	1.45E-04
CKMT1A	-1.21	1.46E-02
CKS2	1.39	1.49E-03
CLDN3	-6.97	9.23E-07
CLDN4	-9.4	5.82E-08
CLDN7	-22.41	1.03E-08
CLDND1	1.91	1.01E-06
CLSPN	2.19	6.85E-07
COMMD8	2.09	4.66E-05
CTNNAL1	1.62	2.71E-04

DCBLD2	1.21	4.74E-04
DDX60	-2.71	4.45E-05
DDX60L	-6.77	5.86E-09
DNAJB4	3.75	2.36E-08
DNER	-1.08	2.10E-01
DPH3	1.82	7.15E-05
E2F7	1.5	4.11E-05
ECHDC1	1.5	1.31E-03
EFNA1	-3.67	1.30E-06
ELF3	-6.85	8.22E-07
ERBB3	-5.74	2.10E-07
EVPL	-1.59	8.91E-05
FABP5	2.36	1.83E-04
FAM40B	2.32	3.54E-05
FAM92A1	1.29	2.02E-02
FLJ32810	5.33	6.20E-07
FXYD3	-26.37	3.71E-08
GALNT1	1.22	4.26E-02
GALNT3	-7.33	3.09E-07
GBP1	-2.5	3.07E-05
GCA	-1.02	8.27E-01
GNAI1	31.61	7.71E-09
GRHL1	-4.42	1.44E-08
GRHL2	-43.31	8.10E-10
GULP1	-1.16	1.19E-01
HDAC9	1.49	3.73E-04
HJURP	-1.92	8.77E-07
HMGA2	3.69	1.96E-05
ICA1	-3.63	4.74E-07
IFI44	-1.52	2.38E-04
IFIT2	-4.1	7.18E-05
IGF2BP3	27.11	7.18E-06
IGSF3	-5.63	2.84E-07
IGSF9	-1.73	3.13E-05
IKIP	2.21	1.28E-05
IMPA1	3.81	9.20E-08
KIAA0020	-1.34	3.27E-03
KIAA1524	1.55	9.16E-05
KIAA1598	-1.45	3.30E-04
KIF21A	-1.21	6.62E-03
KRT8	-30.56	1.38E-09
L2HGDH	-1.05	2.07E-01
LACTB	-1.63	3.07E-04
LAD1	-9.14	5.42E-07
LIFR	3.05	8.96E-07
LLGL2	-5.39	6.15E-07

LNX2	-1.65	2.91E-05
LRRC1	-1.4	1.18E-03
LYN	6.02	2.91E-08
LYST	2.26	3.61E-06
MAK16	1.21	5.50E-02
MAL2	-21.89	2.64E-10
MAP3K1	-1.77	4.41E-05
MAP7D3	4.54	1.04E-06
MARVELD2	-4.64	1.29E-07
MCAM	1.76	3.73E-04
MGAT5B	-1.12	5.72E-03
MLPH	-15.25	1.89E-08
MPZL3	-3.59	2.10E-07
MREG	-6.2	3.87E-07
MSX2	1.39	1.47E-04
MYB	-7.54	1.40E-07
MYBL1	-2.3	2.95E-05
MYH14	-2.15	3.09E-04
NOL8	1.1	1.44E-01
OCLN	-1.92	6.58E-06
OSTM1	1.15	1.53E-01
PAG1	3.71	1.53E-05
PBK	1.74	1.03E-04
PIK3R3	-1.05	1.54E-01
PKP3	-2.73	2.46E-05
PLEKHF2	-4.69	5.25E-06
PMAIP1	5.15	1.26E-07
PNMA2	3.85	1.00E-05
POLK	2.73	2.50E-06
POT1	3.44	2.20E-06
PPARG	-1	9.72E-01
PPM1L	1.16	8.40E-03
PPM2C	-1.01	4.55E-01
PREX1	-18.11	1.09E-09
PRKCH	-3.52	3.72E-07
PROM2	-1.88	1.25E-04
PRSS22	-1.9	5.52E-05
PTTG1	2.1	2.98E-06
PTTG3	1.26	2.32E-02
RAB25	-35.18	1.80E-08
RAD18	1.42	4.88E-04
RB1CC1	1.98	1.23E-04
RBM47	-9.47	8.47E-09
RND3	-1.76	1.08E-04
RP2	1.58	8.50E-04
RP6-	1.86	3.50E-06

213H19.1		
S100A14	-11.24	5.49E-07
SACS	5.34	9.04E-08
SAMD9	-2	3.76E-04
SCML1	2.58	3.25E-06
SELENBP1	1.16	3.62E-02
SH3YL1	-2.54	2.05E-08
SLC29A2	-2.32	1.07E-06
SLC9A3R1	-5.33	3.45E-07
SMC5	1.4	6.88E-04
SOAT1	1.99	1.02E-04
SPDEF	-14.34	2.03E-08
SPINT1	-6.67	2.96E-08
SPINT2	-12.72	2.43E-08
ST14	-11.94	5.92E-08
SUSD5	2.15	5.83E-05
SYNE2	-1.52	1.46E-05
TACSTD2	-17.93	6.22E-08
TBC1D30	-5.23	8.25E-07
TOM1L1	1.04	5.33E-01
TPD52	-1.98	4.04E-04
TSPAN1	-5.52	5.75E-07
TSPAN13	-2.14	5.72E-07
TSPAN15	-3.38	1.01E-06
TTC27	-1.02	7.46E-01
TTK	2.63	3.70E-05
UBLCP1	2.49	3.20E-04
USP33	2.75	4.97E-09
VAMP8	-8.63	2.47E-07
WDR19	4.17	1.43E-06
WDR47	-1.3	2.99E-02
YBX2	-1.52	9.34E-05
ZMYM1	1.68	2.23E-05
ZNF788	-1.1	7.43E-02

Supplemental Table 7. Estrogen Receptor Target Gene Expression

Gene Symbol	Fold-Change	p-value
ABCA12	-28.97	8.69E-12
ABCC4	3.05	1.31E-07
ACSL4	21.62	1.05E-10
AGPAT5	1.44	5.41E-05
AGR2	-61.27	8.72E-10
AIM1	-17.51	2.82E-08
ANLN	1.2	2.86E-02
ANP32E	2.25	1.32E-05
ANXA1	1.43	1.59E-02
ANXA9	-17.41	3.99E-08
AP1S2	1.64	5.46E-04
ARL4A	1.03	3.83E-01
ARSJ	3.31	8.28E-06
ATAD4	-6.66	1.60E-07
ATCAY	-1.01	5.23E-01
BIRC3	-1.59	9.52E-04
C10orf58	-1.91	8.41E-06
C17orf28	-4.66	1.06E-06
C1orf172	-2.01	4.76E-06
C20orf151	-1.33	6.87E-03
C2orf44	1.82	1.37E-04
C6orf150	-22.38	1.00E-08
C6orf173	3.75	3.79E-06
C9orf140	-1.58	1.48E-03
CAMK4	13.55	8.67E-07
CCDC82	11.94	4.80E-08
CCDC88A	37.02	2.07E-11
CCDC99	2.2	5.19E-06
CD24	-76.29	6.62E-09
CD83	1.21	2.22E-03
CDH1	-62.42	8.07E-11
CEBPA	-1.45	1.66E-03
CELSR1	-2.11	4.09E-04
CHN1	1.81	1.45E-04
CKMT1A	-1.21	1.46E-02
CKS2	1.39	1.49E-03
CLDN3	-6.97	9.23E-07
CLDN4	-9.4	5.82E-08
CLDN7	-22.41	1.03E-08
CLDND1	1.91	1.01E-06
CLSPN	2.19	6.85E-07
COMMD8	2.09	4.66E-05
CTNNAL1	1.62	2.71E-04

DCBLD2	1.21	4.74E-04
DDX60	-2.71	4.45E-05
DDX60L	-6.77	5.86E-09
DNAJB4	3.75	2.36E-08
DNER	-1.08	2.10E-01
DPH3	1.82	7.15E-05
E2F7	1.5	4.11E-05
ECHDC1	1.5	1.31E-03
EFNA1	-3.67	1.30E-06
ELF3	-6.85	8.22E-07
ERBB3	-5.74	2.10E-07
EVPL	-1.59	8.91E-05
FABP5	2.36	1.83E-04
FAM40B	2.32	3.54E-05
FAM92A1	1.29	2.02E-02
FLJ32810	5.33	6.20E-07
FXYD3	-26.37	3.71E-08
GALNT1	1.22	4.26E-02
GALNT3	-7.33	3.09E-07
GBP1	-2.5	3.07E-05
GCA	-1.02	8.27E-01
GNAI1	31.61	7.71E-09
GRHL1	-4.42	1.44E-08
GRHL2	-43.31	8.10E-10
GULP1	-1.16	1.19E-01
HDAC9	1.49	3.73E-04
HJURP	-1.92	8.77E-07
HMGA2	3.69	1.96E-05
ICA1	-3.63	4.74E-07
IFI44	-1.52	2.38E-04
IFIT2	-4.1	7.18E-05
IGF2BP3	27.11	7.18E-06
IGSF3	-5.63	2.84E-07
IGSF9	-1.73	3.13E-05
IKIP	2.21	1.28E-05
IMPA1	3.81	9.20E-08
KIAA0020	-1.34	3.27E-03
KIAA1524	1.55	9.16E-05
KIAA1598	-1.45	3.30E-04
KIF21A	-1.21	6.62E-03
KRT8	-30.56	1.38E-09
L2HGDH	-1.05	2.07E-01
LACTB	-1.63	3.07E-04
LAD1	-9.14	5.42E-07
LIFR	3.05	8.96E-07
LLGL2	-5.39	6.15E-07

LNX2	-1.65	2.91E-05
LRRC1	-1.4	1.18E-03
LYN	6.02	2.91E-08
LYST	2.26	3.61E-06
MAK16	1.21	5.50E-02
MAL2	-21.89	2.64E-10
MAP3K1	-1.77	4.41E-05
MAP7D3	4.54	1.04E-06
MARVELD2	-4.64	1.29E-07
MCAM	1.76	3.73E-04
MGAT5B	-1.12	5.72E-03
MLPH	-15.25	1.89E-08
MPZL3	-3.59	2.10E-07
MREG	-6.2	3.87E-07
MSX2	1.39	1.47E-04
MYB	-7.54	1.40E-07
MYBL1	-2.3	2.95E-05
MYH14	-2.15	3.09E-04
NOL8	1.1	1.44E-01
OCLN	-1.92	6.58E-06
OSTM1	1.15	1.53E-01
PAG1	3.71	1.53E-05
PBK	1.74	1.03E-04
PIK3R3	-1.05	1.54E-01
PKP3	-2.73	2.46E-05
PLEKHF2	-4.69	5.25E-06
PMAIP1	5.15	1.26E-07
PNMA2	3.85	1.00E-05
POLK	2.73	2.50E-06
POT1	3.44	2.20E-06
PPARG	-1	9.72E-01
PPM1L	1.16	8.40E-03
PPM2C	-1.01	4.55E-01
PREX1	-18.11	1.09E-09
PRKCH	-3.52	3.72E-07
PROM2	-1.88	1.25E-04
PRSS22	-1.9	5.52E-05
PTTG1	2.1	2.98E-06
PTTG3	1.26	2.32E-02
RAB25	-35.18	1.80E-08
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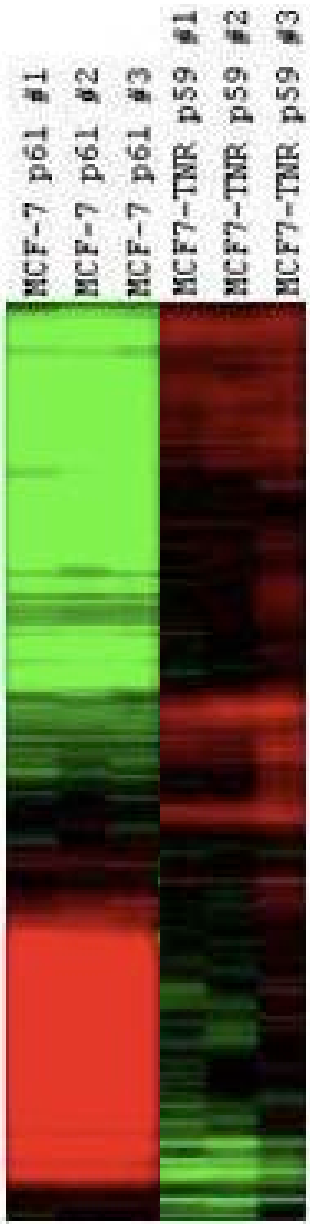
RB1CC1	1.98	1.23E-04
RBM47	-9.47	8.47E-09
RND3	-1.76	1.08E-04
RP2	1.58	8.50E-04
RP6-	1.86	3.50E-06

213H19.1		
S100A14	-11.24	5.49E-07
SACS	5.34	9.04E-08
SAMD9	-2	3.76E-04
SCML1	2.58	3.25E-06
SELENBP1	1.16	3.62E-02
SH3YL1	-2.54	2.05E-08
SLC29A2	-2.32	1.07E-06
SLC9A3R1	-5.33	3.45E-07
SMC5	1.4	6.88E-04
SOAT1	1.99	1.02E-04
SPDEF	-14.34	2.03E-08
SPINT1	-6.67	2.96E-08
SPINT2	-12.72	2.43E-08
ST14	-11.94	5.92E-08
SUSD5	2.15	5.83E-05
SYNE2	-1.52	1.46E-05
TACSTD2	-17.93	6.22E-08
TBC1D30	-5.23	8.25E-07
TOM1L1	1.04	5.33E-01
TPD52	-1.98	4.04E-04
TSPAN1	-5.52	5.75E-07
TSPAN13	-2.14	5.72E-07
TSPAN15	-3.38	1.01E-06
TTC27	-1.02	7.46E-01
TTK	2.63	3.70E-05
UBLCP1	2.49	3.20E-04
USP33	2.75	4.97E-09
VAMP8	-8.63	2.47E-07
WDR19	4.17	1.43E-06
WDR47	-1.3	2.99E-02
YBX2	-1.52	9.34E-05
ZMYM1	1.68	2.23E-05
ZNF788	-1.1	7.43E-02

Supplemental Figures

Supplemental Figure 1. Clustering analyses of EMT Signature Gene Expression. Red color indicates upregulation and green color indicates down-regulation. Trees above are sample clusters.



Supplemental Figure 2. Clustering Analyses of ER Target Gene Expression. Red color indicates upregulation and green color indicates down-regulation. Trees above are sample clusters.