Supplementary Information

Title:

Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters

Running title: EMT transcription factors regulate ABC transporters

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Figure S1

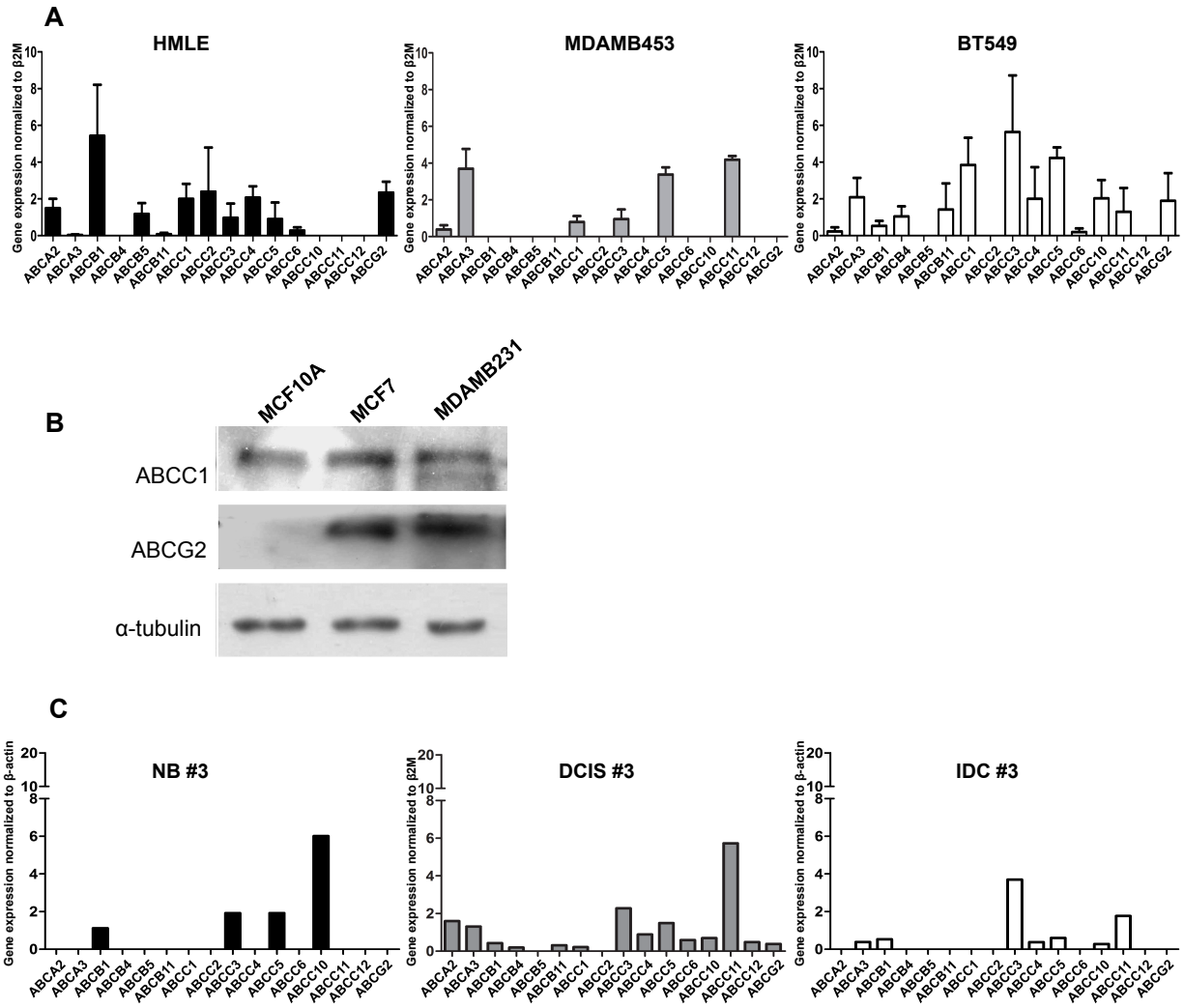

A
HMLE
MDAMB453
BT549
Gene expression normalized to β2M
ABCA2 ABCA3 ABCB1 ABCB4 ABCB5 ABCB11 ABCC1 ABCC2 ABCC3 ABCC4 ABCC5 ABCC6 ABCC10 ABCC11 ABCC12 ABCG2
B
MCF10A
MCF7
MDAMB231
ABCC1
ABCG2
α-tubulin
C
NB #3
DCIS #3
IDC #3
Gene expression normalized to β-actin
Gene expression normalized to β2M

Figure S2

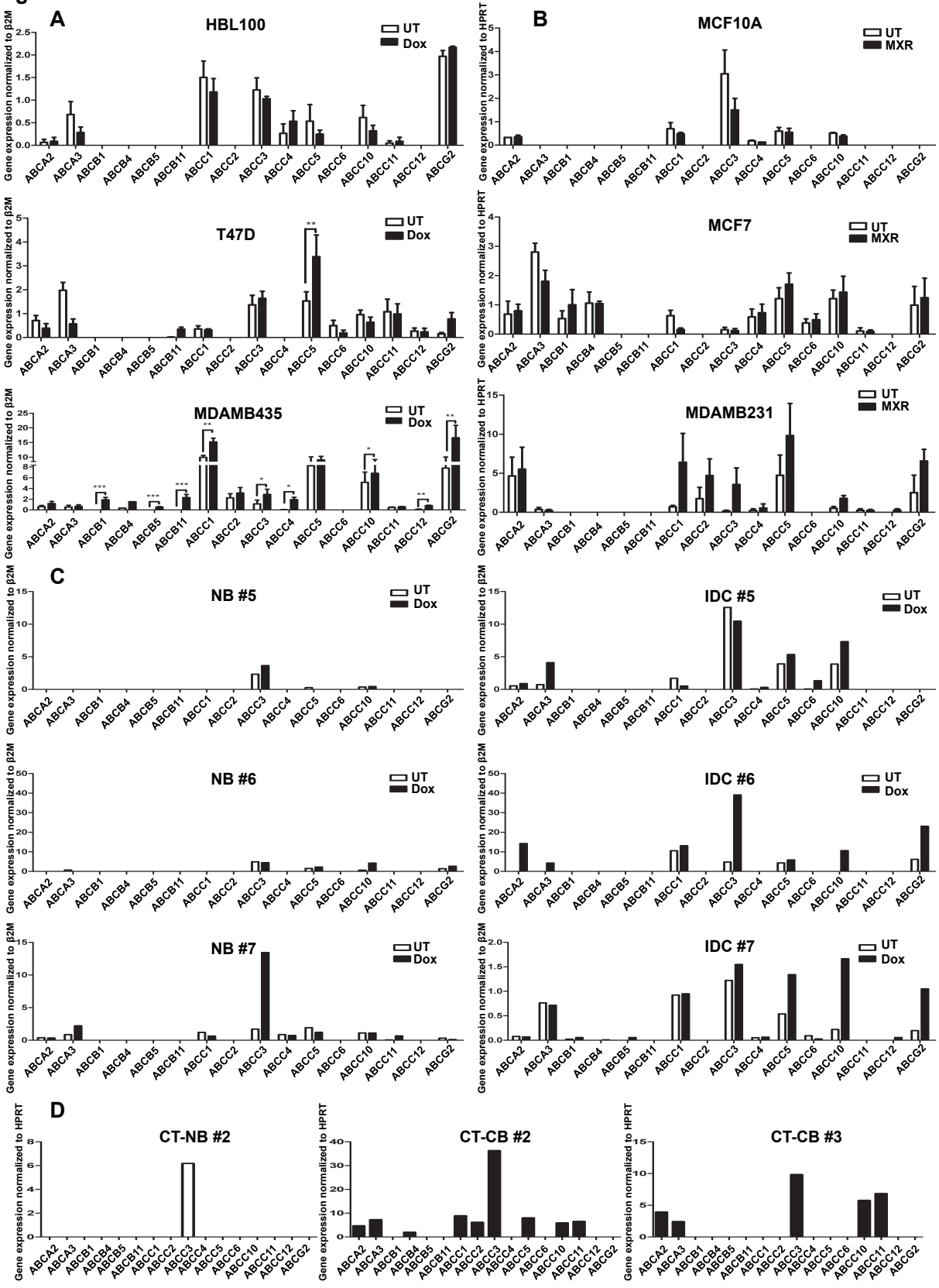
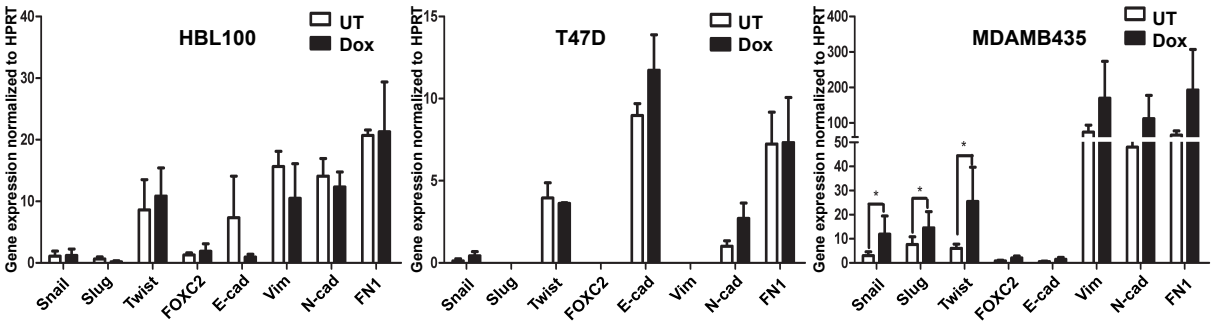

A
HBL100
UT
Dox
Gene expression normalized to β2M
B
MCF10A
UT
MXR
Gene expression normalized to HPRT
T47D
MCF7
MDAMB435
MDAMB231
C
NB #5
IDC #5
NB #6
IDC #6
NB #7
IDC #7
D
CT-NB #2
CT-CB #2
CT-CB #3
ABCA2 ABCA3 ABCB1 ABCB4 ABCB5 ABCB11 ABCC1 ABCC2 ABCC3 ABCC4 ABCC5 ABCC6 ABCC10 ABCC11 ABCC12 ABCG2

Figure S3

A


HBL100
T47D
MDAMB435
UT
Dox
Gene expression normalized to HPRT
Snail
Slug
Twist
FOXC2
E-cad
Vim
N-cad
FN1

B

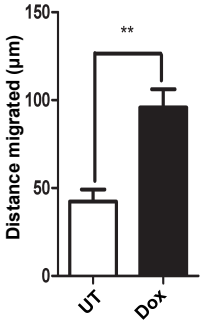

Distance migrated (µm)
**
UT
Dox

Figure S4

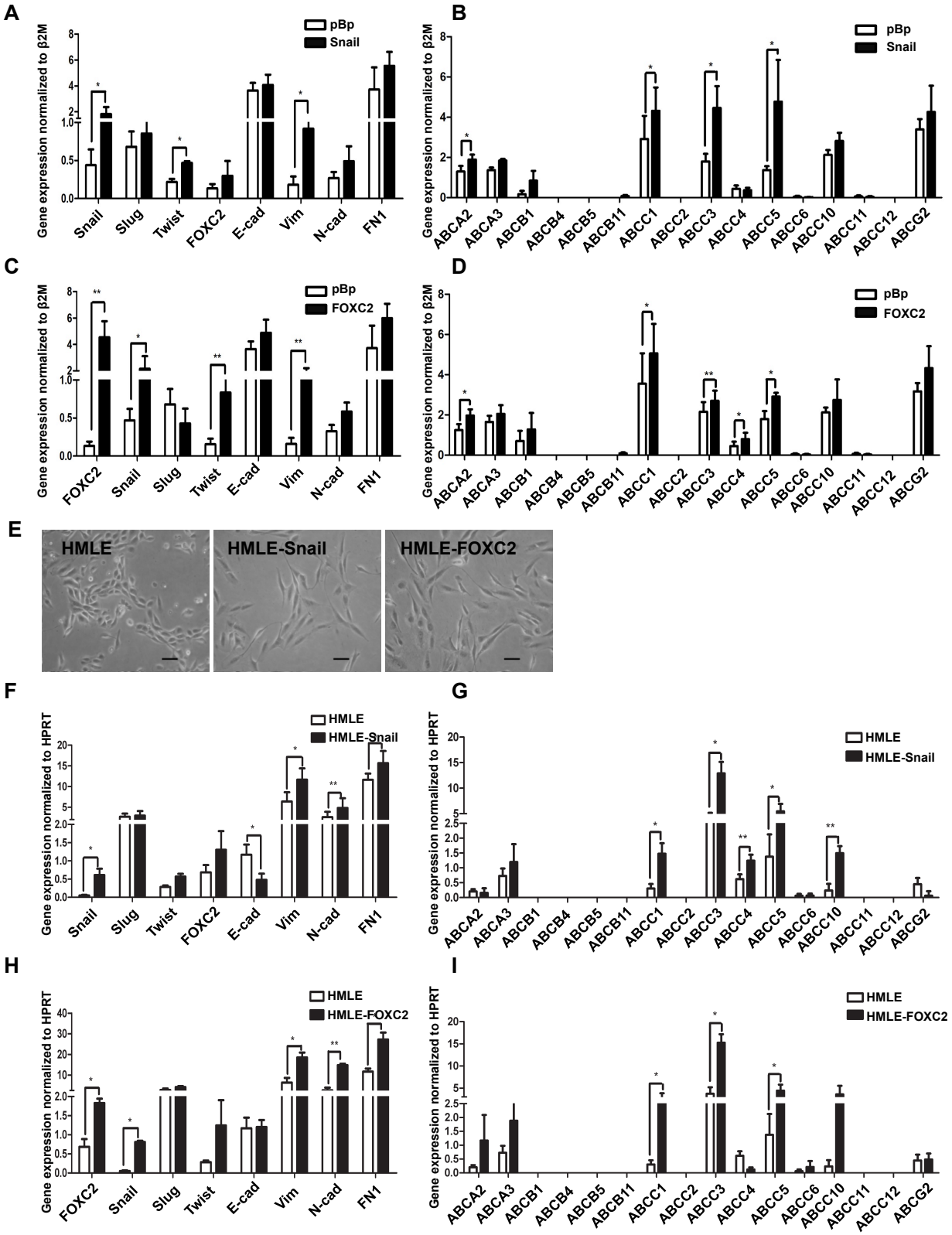

A
pBp
Snail
Gene expression normalized to β2M
Snail
Slug
Twist
FOXC2
E-cad
Vim
N-cad
FN1
B
pBp
Snail
Gene expression normalized to β2M
ABCA2
ABCA3
ABCB1
ABCB4
ABCB5
ABCB11
ABCC1
ABCC2
ABCC3
ABCC4
ABCC5
ABCC6
ABCC10
ABCC11
ABCC12
ABCG2
C
pBp
FOXC2
Gene expression normalized to β2M
FOXC2
Snail
Slug
Twist
E-cad
Vim
N-cad
FN1
D
pBp
FOXC2
Gene expression normalized to β2M
ABCA2
ABCA3
ABCB1
ABCB4
ABCB5
ABCB11
ABCC1
ABCC2
ABCC3
ABCC4
ABCC5
ABCC6
ABCC10
ABCC11
ABCC12
ABCG2
E
HMLE
HMLE-Snail
HMLE-FOXC2
F
HMLE
HMLE-Snail
Gene expression normalized to HPRT
Snail
Slug
Twist
FOXC2
E-cad
Vim
N-cad
FN1
G
HMLE
HMLE-Snail
Gene expression normalized to HPRT
ABCA2
ABCA3
ABCB1
ABCB4
ABCB5
ABCB11
ABCC1
ABCC2
ABCC3
ABCC4
ABCC5
ABCC6
ABCC10
ABCC11
ABCC12
ABCG2
H
HMLE
HMLE-FOXC2
Gene expression normalized to HPRT
FOXC2
Snail
Slug
Twist
E-cad
Vim
N-cad
FN1
I
HMLE
HMLE-FOXC2
Gene expression normalized to HPRT
ABCA2
ABCA3
ABCB1
ABCB4
ABCB5
ABCB11
ABCC1
ABCC2
ABCC3
ABCC4
ABCC5
ABCC6
ABCC10
ABCC11
ABCC12
ABCG2

Figure S5

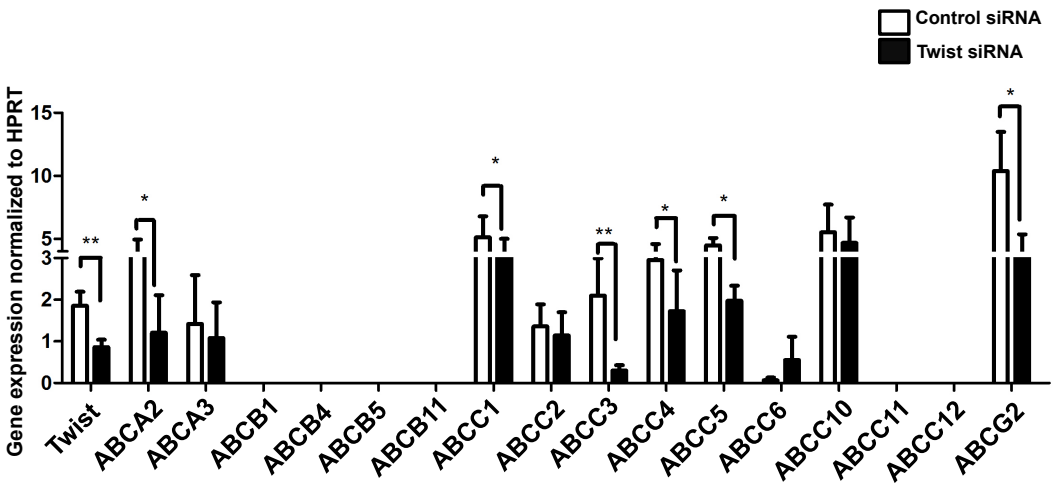
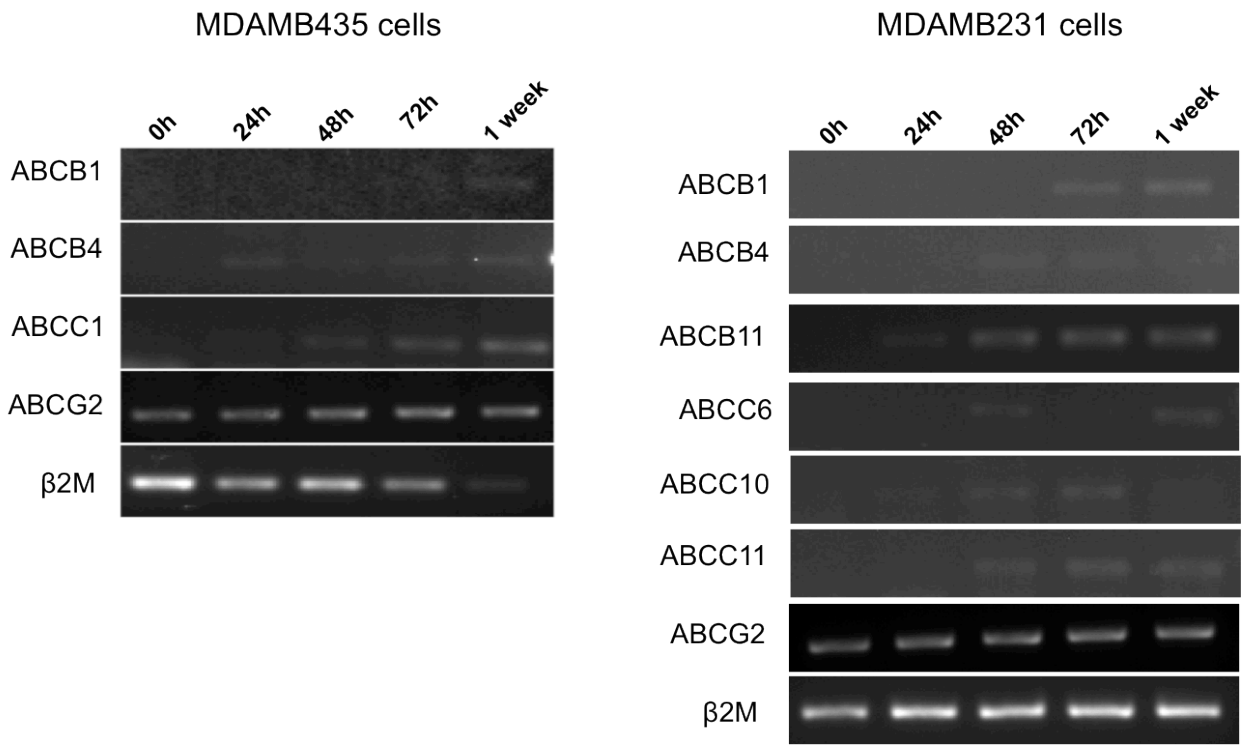

Control siRNA
Twist siRNA
Gene expression normalized to HPRT
15
10
5
3
2
1
0
**
*
*
*
**
*
*
*
Twist
ABCA2
ABCA3
ABCB1
ABCB4
ABCB5
ABCB11
ABCC1
ABCC2
ABCC3
ABCC4
ABCC5
ABCC6
ABCC10
ABCC11
ABCC12
ABCG2

Figure S6


MDAMB435 cells
0h
24h
48h
72h
1 week
ABCB1
ABCB4
ABCC1
ABCG2
β2M
MDAMB231 cells
0h
24h
48h
72h
1 week
ABCB1
ABCB4
ABCB11
ABCC6
ABCC10
ABCC11
ABCG2
β2M

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Breast epithelial cells have a heterogeneous expression of ABC transporters.

(A) Representative graphs showing relative mRNA expression of ABC transporters in immortalized HMLE, non-invasive MDAMB453 and invasive BT549 breast epithelial cell lines as analyzed by RT-PCR, n=3. (**B**) Immunoblot analysis of whole-cell lysates of MCF10A, MCF7 and MDAMB231 breast epithelial cell lines for the expression of ABCC1 and ABCG2 transporters. ABCB1 expression was not detected in any of the cell lines. α-tubulin served as the loading control. (**C**) Graphs represent relative mRNA expression of 16 ABC transporters in primary normal (NB), DCIS and IDC breast samples as analyzed by RT-PCR. n=3 for NB, n=6 for DCIS, and n=3 for IDC samples.

Figure S2. Dox treatment upregulates ABC transporter expression only in invasive cells. (**A**) Graphs represent relative mRNA expression of 16 ABC transporters after one-week Dox treatment in HBL100, T47D and MDAMB435 breast epithelial cell lines, n=3. (**B**) Graphs represent relative mRNA expression of 16 ABC transporters on one-week MXR treatment in MCF10A, MCF7 and MDAMB231 cells, n=3. (**C**) Graphs represent relative mRNA expression of 16 ABC transporters after one-week Dox treatment in multiple primary normal (NB) and tumor (IDC) derived breast epithelial cells. (**D**) Graphs represent relative mRNA expression of ABC transporters in chemotherapy treated primary normal (CT-NB) and tumor (CT-CB) breast samples.

Figure S3. Dox treatment of invasive cells leads to upregulation of EMT markers and invasiveness. (**A**) Graphs represent relative mRNA expression of EMT markers after one-week Dox treatment in HBL100, T47D and MDAMB435 breast epithelial cell lines, n=3. (**B**) Graph

represents distance migrated by untreated and Dox treated MDAMB435 cells in 24 hours in wound healing assay, n=9.

Figure S4. EMT induction upregulates ABC transporter expression.

Graphs represent relative mRNA expression of EMT markers (**A**) and ABC transporters (**B**) on transient overexpression of Snail in MCF7 cells, n=4. Graphs represent relative mRNA expression of EMT markers (**C**) and ABC transporters (**D**) on transient overexpression of FOXC2 in MCF7 cells, n=4. (**E**) Phase contrast photomicrographs of parent HMLE cells and HMLE cells stably overexpressing Snail and FOXC2. Scale bar = 100 μm. Graphs represent relative mRNA expression of EMT markers (**F**) and ABC transporters (**G**) on stable overexpression of Snail in HMLE cells, n=3. Graphs represent relative mRNA expression of EMT markers (**H**) and ABC transporters (**I**) on stable overexpression of FOXC2 in HMLE cells, n=3.

Figure S5. Reversal of EMT leads to downregulation of ABC transporters.

Graph represents relative mRNA expression of ABC transporters after 72 hours of Twist knockdown in invasive MDAMB231 cells, n=6.

Figure S6. Time-course assay with Doxorubicin.

Gel pictures depict the expression of ABC transporters in MDAMB435 and MDAMB231 cells on time-course treatment with Dox. In MDAMB435 cells, while ABCC1 and ABCG2 showed upregulation by 48 hours and 72 hours, ABCB1 induction was observed only at one-week time point. In MDAMB231 cells a time-course revealed an increase in several ABC transporters as

early as 72 hours. β2-microglobulin was used as a normalizing control.

Table S1: Breast epithelial cells have a heterogeneous expression of ABC transporters.

	ABCA2	ABCA3	ABCB1	ABCB4	ABCB5	ABCB11	ABCC1	ABCC2	ABCC3	ABCC4	ABCC5	ABCC6	ABCC10	ABCC11	ABCC12	ABCG2
MCF10A	++	-	-	-	-	-	++	-	+++	++	++	-	+	-	-	-
HBL100	++	++	-	-	-	-	+++	-	+++	+	++	-	+++	+	-	+++
HMLE	+	+	+++	-	+	+	+	++	+	+	+	+	-	-	-	++
MCF7	++	++	+	-	-	+	++	-	++	+	+++	-	++	+	-	+
T47D	++	+++	-	-	-	+	+	-	+++	-	+++	++	+++	++	++	+
MDAMB453	+	++	-	-	-	-	+	-	+	-	++	-	-	+++	-	-
MDAMB231	+	+	-	-	-	-	+++	++	+	++	+++	-	+++	-	-	+++
MDAMB435	+	+	-	+	-	-	+++	++	++	++	+++	-	+++	+	+	+++
BT549	+	+	+	+	-	+	++	-	+++	+	+++	+	+	+	-	+

Tabulated expression of 16 ABC transporters in immortalized, non-invasive and invasive breast epithelial cell lines, n = 3. mRNA expression as detected by relative RT-PCR has been normalized to β2-microglobulin levels and the quantified data (in arbitrary units, AU) has been tabulated as follows, -: expression not detected; +: 0-2 AU; ++: 2.1-4 AU; +++: 4.1-6 AU; ++++:

6.1-8 AU; +++++: 8.1-10 AU. The data for this table is represented in **Figure 1** and **Figure S1** in form of individual graphs for each cell line.

Table S2: Concentrations of drugs used in Figure 2 and 3.

The optimum concentration of drugs were chosen based on MTT assays such that 80-85% cells were viable at the end of 48 hours of drug treatment which usually resulted in at least 10-15% of viable cells at the end of one-week of treatment.

Cell line name	Doxorubicin	Mitoxantrone
MCF10A	200 nM	200 nM
HBL100	20 nM	-
HMLE	20 nM	-
MCF7	200 nM	200 nM
T47D	200 nM	-
MDAMB231	200 nM	200 nM
MDAMB435	200 nM	-

Table S3: Sequence of primers used for RT-PCR analysis in Figures 1, 2, 3, 4 and 5.

Gene name	Primer sequence (5'→ 3')
ABCA2 Fwd	ATGTCAGCCTGCAAGAGGTG
ABCA2 Rev	AGATCTGGGAGAAGAGTGCC
ABCA3 Fwd	AGGAAAGGAGGCTGAAGGAG
ABCA3 Rev	GGTGCTGACCATGAAGCTGAAG
ABCB1 Fwd	TGACAT TTATTCAAAGTTAAAAGCA
ABCB1 Rev	TAGACACTTTATGCAAAC ATTTCAA
ABCB4 Fwd	GCATCAGCAGCAAACAAAAA
ABCB4 Rev	GCAGCGACAAGGAAAAGTTC
ABCB5 Fwd	TTCATCCTCCGTGGCTTATC
ABCB5 Rev	ACGATTGCTATTTGGGAACG
ABCB11 Fwd	GTGGCCAGAAACAAAGGGTA
ABCB1 Rev	GCGATGAGCAACTGAAATGA
ABCC1 Fwd	AGCCGGTGAAGGTTGTGTAC
ABCC1 Rev	TGACGAAGCAGATGTGGAAG
ABCC2 Fwd	TGCTTCCTGGGGATAATCAG
ABCC2 Rev	ACGGATAACTGGCAAACCTG
ABCC3 Fwd	CCTTTGCCAACTTTCTCTGC
ABCC3 Rev	AGGGCACTCAGCTGTCTCAT
ABCC4 Fwd	GGTTCCCCTTGGAATCATTT
ABCC4 Rev	AATCCTGGTGTGCATCAAACAG
ABCC5 Fwd	ACCCGTTGTTGCCATCTTAG
ABCC5 Rev	GCTTTGACCCAGGCATACAT
ABCC6 Fwd	GTGGTGTTTGCTGTCCACAC
ABCC6 Rev	ACGACACCAGGGTCAACTTC
ABCC10 Fwd	ATTGCCCATAGGCTCAACAC
ABCC10 Rev	AGCAGCCAGCACCTCTGTAT
ABCC11 Fwd	GGCTGAGCTACTGGTTGGAG
ABCC11 Rev	TGGTGAAAATCCCTGAGGAG

ABCC12 Fwd	GGTGTTCATGCTGGTGTTTGG
ABCC12 Rev	GCTCGTCCATATCCTTGGAA
ABCG2 Fwd	GCAGATGCCTTCTTCGTTATG
ABCG2 Rev	TCTTCGCCAGTACATGTTGC
E-cadherin Fwd	TGCCCAGAAAATGAAAAAGG
E-cadherin Rev	GTGTATGTGGCAATGCGTTC
Slug Fwd	CTTTTTCTTGCCCTCACTGC
Slug Rev	GCTTCGGAGTGAAGAAATG
Snail Fwd	ACCCCACATCCTTCTCACTG
Snail Rev	TACAAAAACCCACGCAGACA
hTwist Fwd	GTCCGCAGTCTTACGAGGAG
hTwist Rev	CCAGCTTGAGGGTCTGAATC
mTwist1 Fwd	CCCCACTTTTTGACGAAGAA
mTwist1 Rev	GATGATTTGCAGGCCAGTTT
FOXC2 Fwd	AGTTCATCATGGACCGCTTC
FOXC2 Rev	TCTCCTTGGACACGTCCTTC
N-cadherin Fwd	GACAATGCCCCTCAAGTGTT
N-cadherin Rev	CCATTAAGCCGAGTGATGGT
Vimentin Fwd	GAGAACTTTGCCGTTGAAGC
Vimentin Rev	TCCAGCAGCTTCCTGTAGGT
Fibronectin1 Fwd	ACCAACCTACGGATGACTCG
Fibronectin1 Rev	GCTCATCATCTGGCCATTTT
β2-microglobulin Fwd	CCTGAATTGCTATGTGTCTGGG
β2-microglobulin Rev	TGATGCTGCTTACATGTCTCGA
β-actin Fwd	ACTGGAACGGTGAAGGTGAC
β-actin Rev	AGAGAAGTGGGGTGGCTTTT
HPRT Fwd	TGCTCGAGATGTGATGAAGG
HPRT Rev	TCCCCTGTTGACTGGTCATT

Table S4: Sequence of primers used for ChIP experiment (related to Figure 6).

Name of primer	**Sequence (5'→ 3')**
ABCC4 ChIP 1 Fwd	CAGGGGGCGGGCTGCTAGA
ABCC4 ChIP 1 Rev	GCAGAGCGCGCGGCAAG
ABCC5 ChIP 1 Fwd	CGGGTTAGACGCGGGCTACG
ABCC5 ChIP 1 Rev	GCTGCCCCTCTTCCCACCGA
ABCC5 ChIP Control Fwd	GAGTGGCTGCTCAAGTTTCC
ABCC5 ChIP Control Rev	GGCATAAATTGAAAAGATAAACCA