

Supplementary Material

1 Supplementary Tables

Table S1. TaqMan gene expression assays used in this study

Assay ID	Gene symbol	Gene Description
Hs00171851_m1	FOSB	FosB proto-oncogene, AP-1 transcription factor subunit
Hs04194186_s1	FOS	Fos proto-oncogene, AP-1 transcription factor subunit
Hs01114274_m1	IL24	Interleukin 24
Hs01055413_g1	IL11	Interleukin 11
Hs00985641_m1	IL6	Interleukin 6
Hs01854535_s1	MAGEC2	MAGE family member C2
Hs00152928_m1	EGR1	Early growth response 1
Hs01010736_m1	SERPINB2	Serpin family B member 2
Hs00189369_m1	DNM1	Dynamin 1
Hs00236216_m1	ITGB4	Integrin subunit beta 4
Hs00161638_m1	SCG5	Secretogranin V
Hs01084940_m1	NCF2	neutrophil cytosolic factor 2
Hs01060665_g1	ACTB*	Actin beta
Hs02786624_g1	GAPDH*	Glyceraldehyde-3-phosphate dehydrogenase

* ACTB and GAPDH were used as housekeeping gene expression controls.

Table S2. Up regulated genes at high calcium

GENE SYMBOL	GENE DESCRIPTION	FOLD CHANGE	P-VALUE
FOSB	FBJ murine osteosarcoma viral oncogene homolog B	3.1307	0.0054
FOS	FBJ murine osteosarcoma viral oncogene homolog	2.7834	0.0051
MAGEC2	Melanoma antigen family C, 2	2.5627	0.0039
SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.5328	0.0005
NCF2	Neutrophil cytosolic factor 2	2.2339	0.0010
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix	2.1861	0.0173
ACTG2	Actin, gamma 2, smooth muscle, enteric	2.1772	0.0134
SSX1	Synovial sarcoma, X breakpoint 1	2.1701	0.0208
PDE1C	Phosphodiesterase 1C, calmodulin-dependent 70kDa	2.0546	0.0206
SPANXE	SPANX family, member E	2.0388	0.0075
SNORA14B	Small nucleolar RNA, H/ACA box 14B	2.0051	0.0030
FRMD3	FERM domain containing 3	1.9955	0.0016
FRMPD4	FERM and PDZ domain containing 4	1.9895	0.0100
IL24	Interleukin 24	1.9708	0.0019
IL11	Interleukin 11	1.9645	0.0003
SCG5	Secretogranin V (7B2 protein)	1.9512	0.0118
SNORA1	Small nucleolar RNA, H/ACA box 1	1.9325	0.0004
ZNF257	Zinc finger protein 257	1.8978	0.0003
IL6	Interleukin 6 (interferon, beta 2)	1.8500	0.0447
DCAF8L1	DDB1 and CUL4 associated factor 8-like 1	1.8329	0.0229
PLEKHA7	Pleckstrin homology domain containing, family A member	1.8110	0.0047
EHF	Ets homologous factor	1.8079	0.0119
SPOCK1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testis)	1.8060	0.0003
ACTR3C	RP3 actin-related protein 3 homolog C (yeast)	1.8054	0.0131
CRLF2	Cytokine receptor-like factor 2	1.7910	0.0037
EGR1	Early growth response 1	1.7818	0.0501
GJA5	Gap junction protein, alpha 5, 40kDa	1.7779	0.0175
PSG4	Pregnancy specific beta-1-glycoprotein 4	1.7765	0.0392
COL6A3	Collagen, type VI, alpha 3	1.7606	0.0003

Table S3. Down regulated genes at high calcium

GENE SYMBOL	GENE DESCRIPTION	FOLD CHANGE	P-VALUE
C15orf51	Dynamin 1 pseudogene	-4.13153	0.00034
REXO1L1	RNA exonuclease 1 homolog (S. cerevisiae)-like 1	-2.2933	0.00670
NRK	Nik related kinase	-2.19675	0.01087
REXO1L2P	RNA exonuclease 1 homolog (S. cerevisiae)-like 2 (pseudo	-2.15397	0.00065
HLA-DRA	Major histocompatibility complex, class II, DR alpha	-2.13535	0.00013
EPB41L3	Erythrocyte membrane protein band 4.1-like 3	-2.08737	0.02883
RFPL4A	Ret finger protein-like 4A	-2.03611	0.00189
LCE2D	Late cornified envelope 2D	-2.03231	0.00019
FOLH1	Folate hydrolase (prostate-specific membrane antigen) 1	-1.88823	0.01010
DUX4L4	Double homeobox 4 like 4	-1.87542	0.03024
LOC349196	Hypothetical LOC349196	-1.84076	0.00020
LY86-AS	LY86 antisense RNA (non-protein coding)	-1.83354	0.00210
ICOSLG	Inducible T-cell co-stimulator ligand	-1.83162	0.00433
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-1.821	0.00909
TNFSF18	Tumor necrosis factor (ligand) superfamily, member 18	-1.81363	0.00013
HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	-1.81248	0.00030
LOC10013247	Hypothetical LOC100132147	-1.79995	0.01067
MUC12	Mucin 12, cell surface associated	-1.76713	0.00076
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	-1.74847	0.00033
IGHA1	Immunoglobulin heavy constant alpha 1	-1.73004	0.00147
ZFPM2	Zinc finger protein, multitype 2	-1.71818	0.01157
SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	-1.71338	0.08876
ASB2	Ankyrin repeat and SOCS box-containing 2	-1.71158	0.00926
IGHD	Immunoglobulin heavy constant delta	-1.70471	0.05360
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (Drosophila)	-1.69598	0.00043
HLA-DRB4	Major histocompatibility complex, class II, DR beta 4	-1.68976	0.00049
HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	-1.67505	0.00041
GMFG	Glia maturation factor, gamma	-1.66292	0.00452
CCNA1	Cyclin A1	-1.6413	0.00670

Table S4. Pathways modulated by high extracellular calcium

PATHWAY	PATHWAY ENTITIES	MATCHED ENTITIES	P-VALUE
Hs Senescence and Autophagy	106	5	1.69E-04
Hs Cytokines and Inflammatory Response	30	3	1.83E-04
Hs Adipogenesis	131	5	4.34E-04
Hs Dissolution of Fibrin Clot	8	2	6.41E-04
Hs Vitamin B12 Metabolism	54	3	0.001954
Hs TGF Beta Signaling Pathway	55	3	0.002428
Hs SREBF and miR33 in cholesterol and lipid homeostasis	18	2	0.003026
Hs Folate Metabolism	68	3	0.004074
Hs Glutathione metabolism	20	2	0.004187
Hs Blood Clotting Cascade	22	2	0.005059
Hs Selenium Pathway	85	3	0.007709
Hs Epigenetic Stress Regulation	1	1	0.009682
Hs MAPK targets-Nuclear events mediated by MAP kinases	8	1	0.038172
Hs Myometrial Relaxation and Contraction Pathways	156	3	0.039814
Hs Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways	66	2	0.040927
Hs Insulin Signaling	160	3	0.043075

2. Supplementary Figures

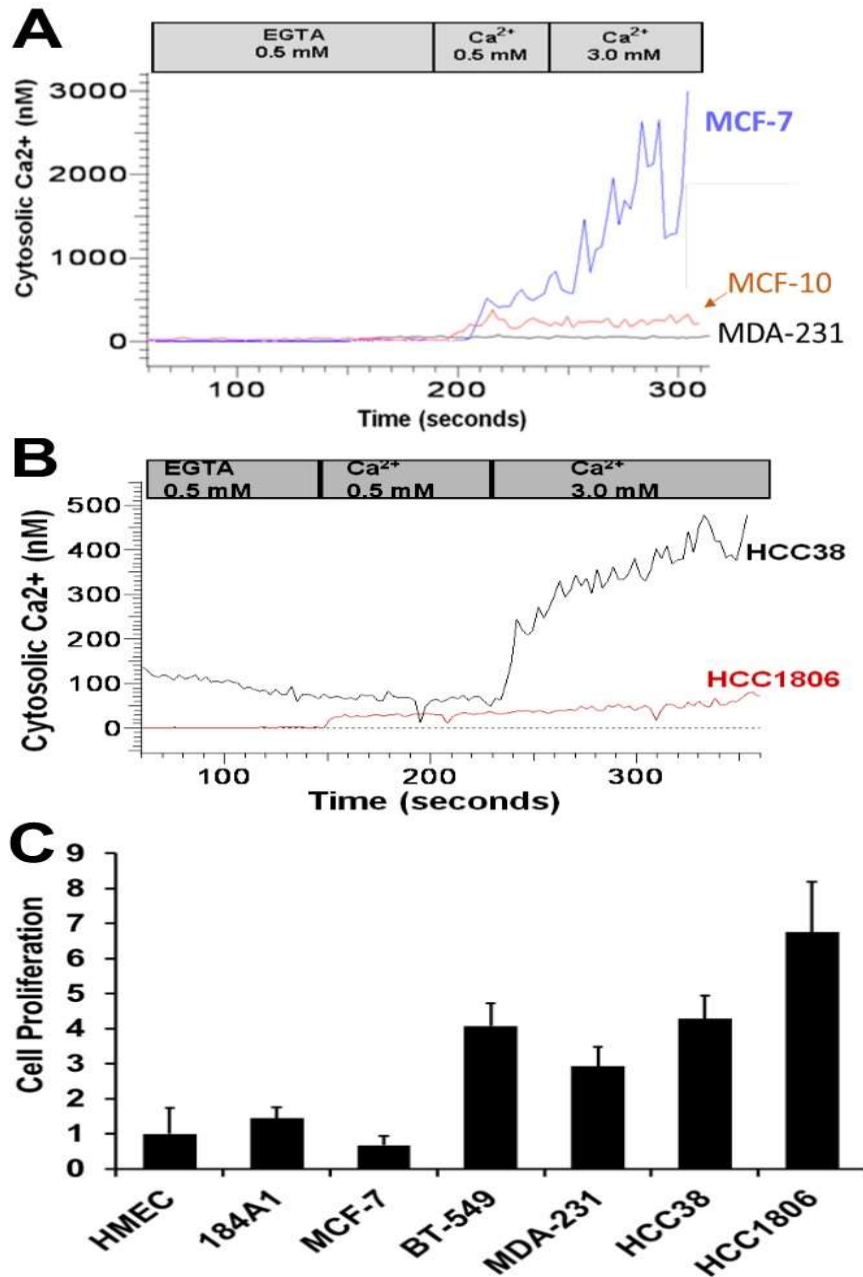


Figure S1. Differential calcium influx dynamics and survival of Breast epithelial and breast cancer cells at high Ca²⁺. (A, B) The indicated cell lines were grown to 80% confluency, harvested by trypsinization and loaded with fura-2 AM for 1 h. Cells were washed twice in Ca²⁺ and Mg²⁺-free Hanks balanced salt solution (HBSS) and intracellular Ca²⁺ levels were measured using the Hitachi F2500 spectrofluorimeter with addition of the indicated solutions. (C). Cells were cultured in complete medium supplemented with 5.0 mM Ca²⁺ and the viability/proliferation was measured as described in Fig. 1E and normalized relative to the primary human epithelial cells (HMEC).

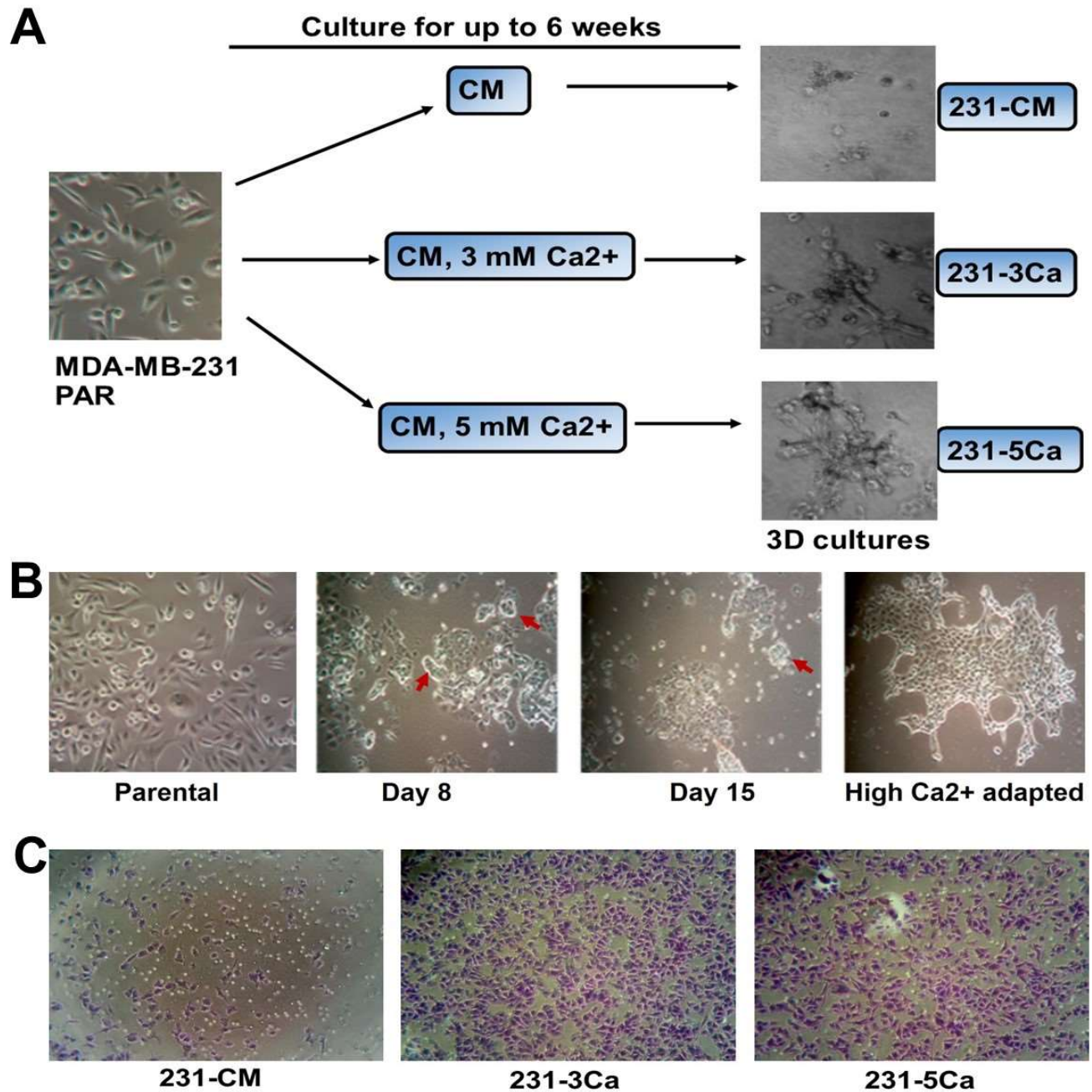


Figure S2. Adaptation of TNBC cells to high Ca^{2+} in vitro. **A)** Parental MDA-MB-231 cells were cultured in complete medium or complete medium supplemented with 3.0 or 5.0 mM Ca^{2+} for up to 6 weeks. **B)** The morphology of the cells during the continuous culture at high Ca^{2+} . Arrows indicate colonies of surviving cells by day 8 of the selection process. **C)** Migration of control and high Ca^{2+} adapted MDA-MB-231 cells. Cells were plated in Boyden chambers in serum-free medium and complete medium containing 10% FBS was used as chemoattractant. Shown are representative images from at least three fields.

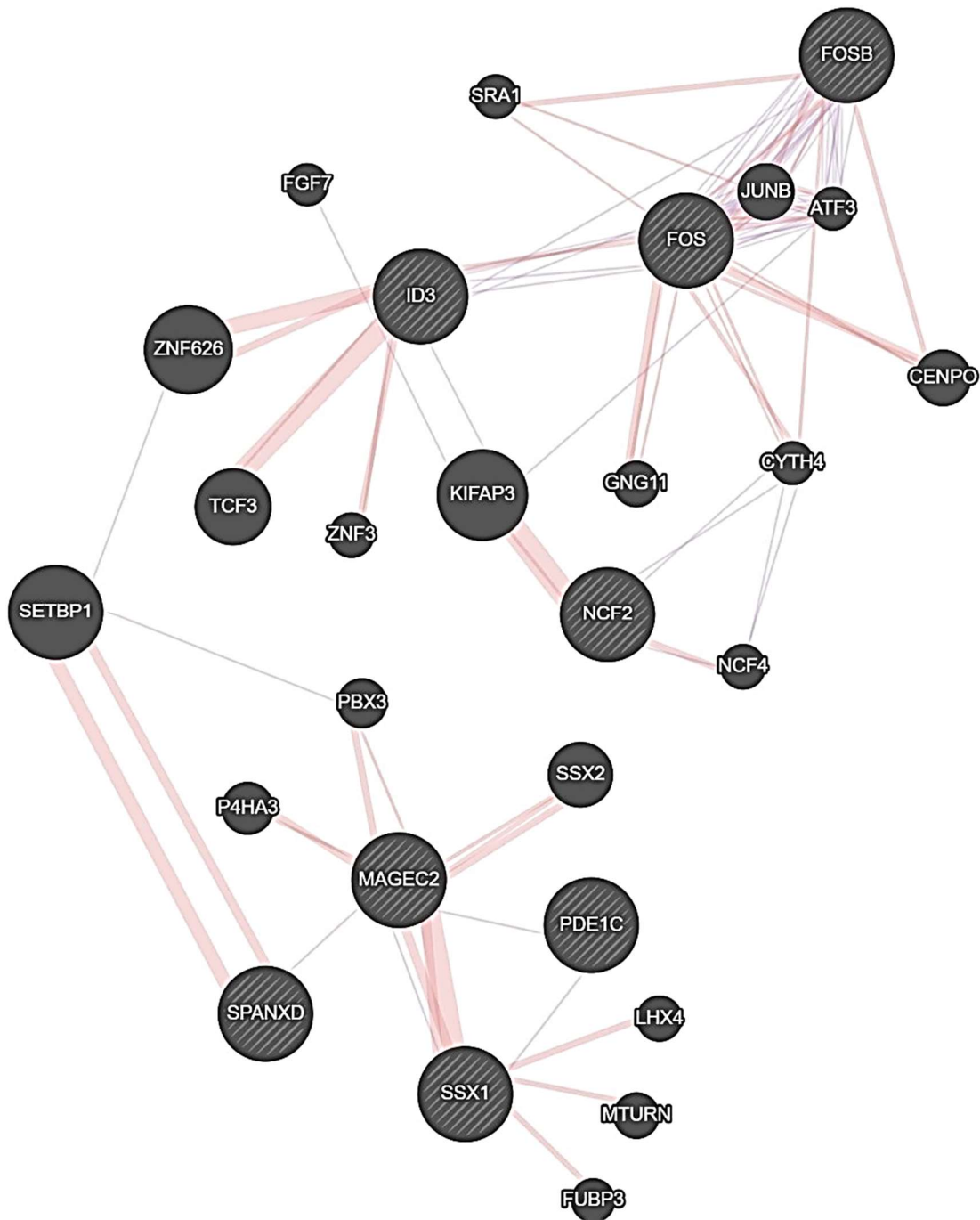


Figure S3. Biological network relationship of up regulated (high Ca^{2+} inducible) genes. The hashed nodes indicate genes identified to be up regulated at high Ca^{2+} in MDA-MB-231 TNBC cells and the physical interactions co-expression analyzed by using genemania (<https://genemania.org/>) are indicated by pink and purple connections respectively.

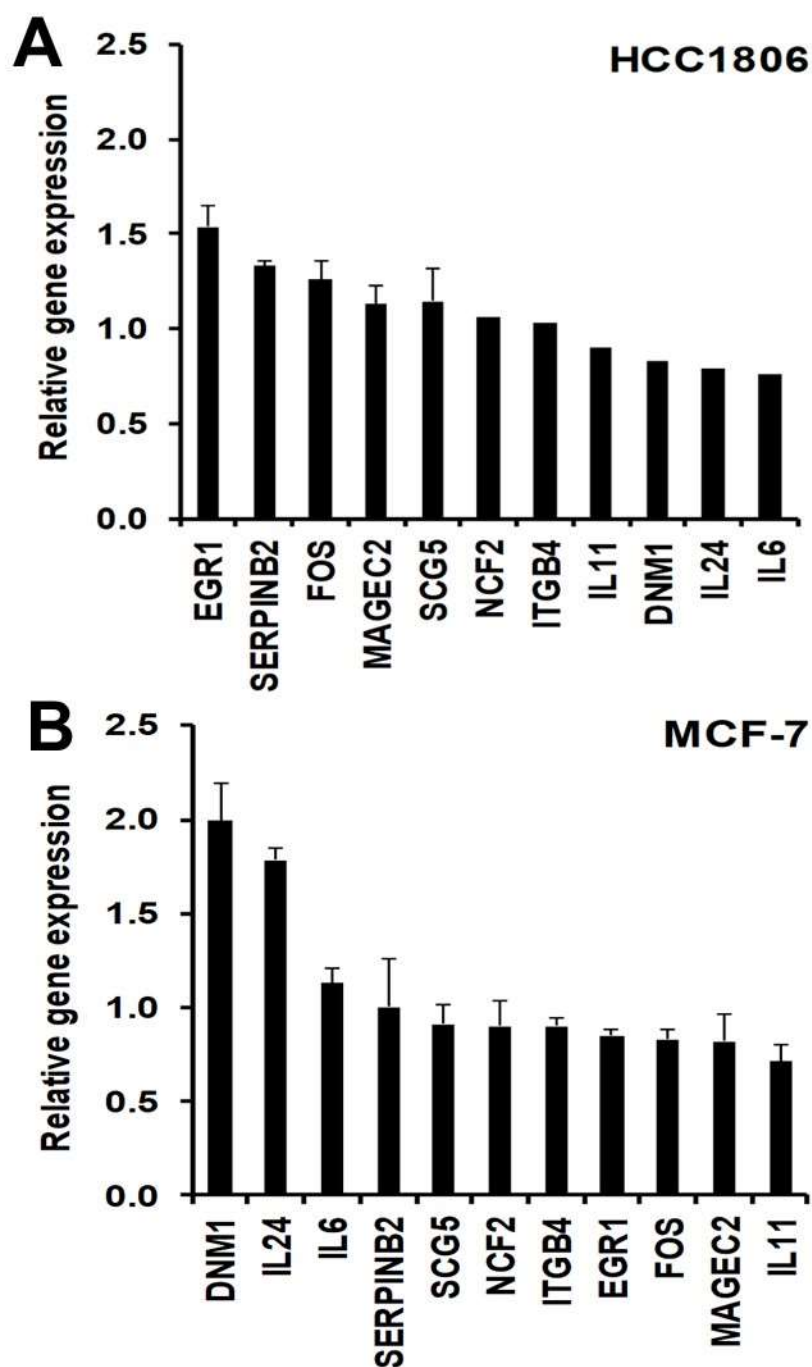


Figure S4. Validation of upregulated genes in high Ca^{2+} treated breast cancer cells. The indicated high Ca^{2+} inducible genes were validated by RT-PCR in HCC1806 (A) and MCF-7 (B) breast cancer cells. Bars represent gene expression relative to GAPDH control.

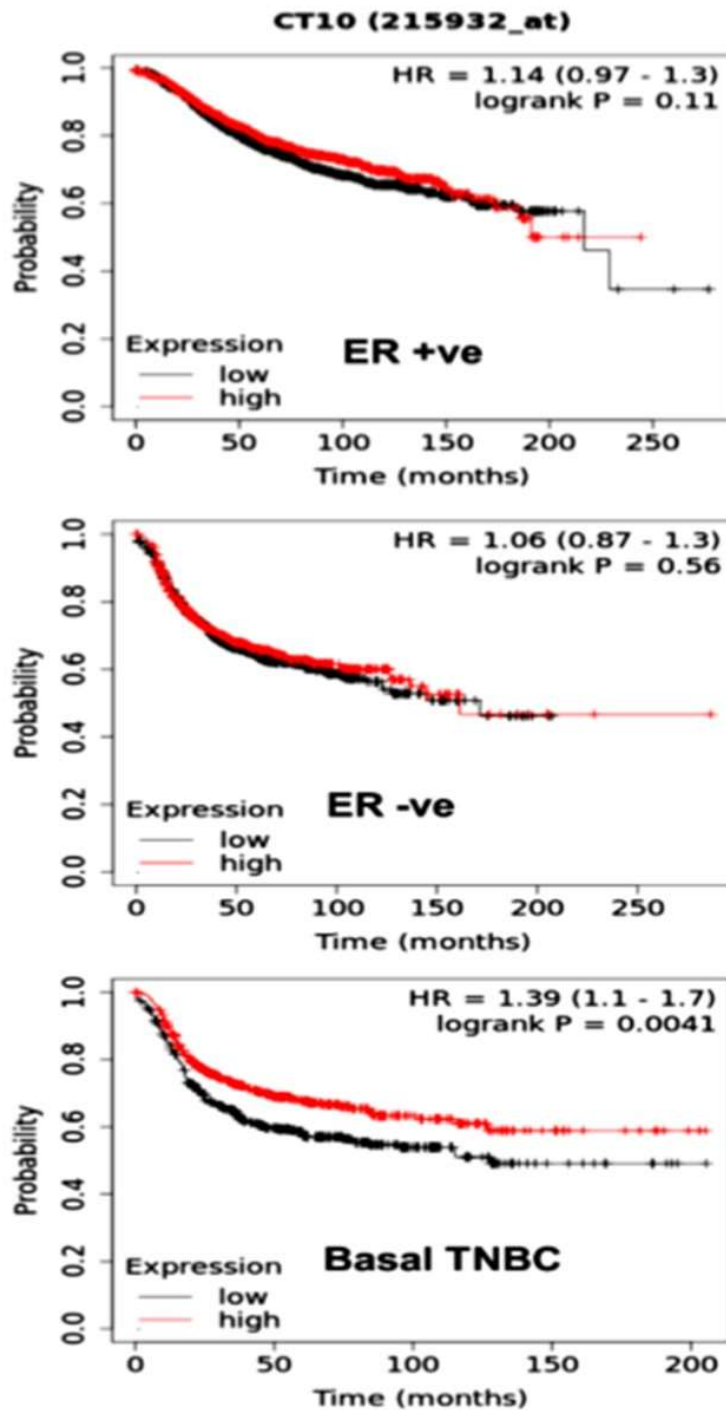


Figure S5. Clinical relevance of MAGEC2 expression in basal-like breast cancer relative to ER positive and ER negative breast cancer. The relapse-free survival of patients with ER-positive and ER-negative breast cancer as well as basal-like TNBC was analyzed as a function of high (red) and low (black) expression levels above and below the median value respectively, by using the Kaplan Meier survival analysis tool, KM plotter for breast cancer.