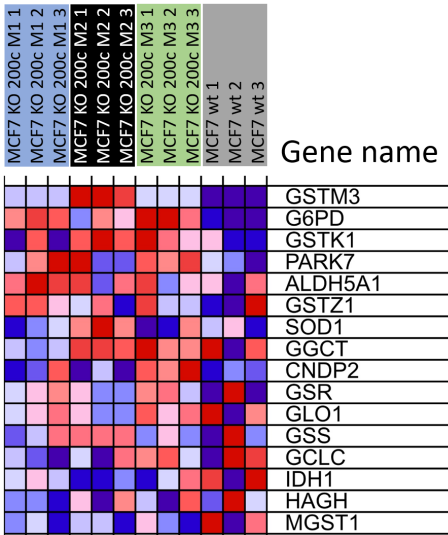


Figure S1

A



B



C

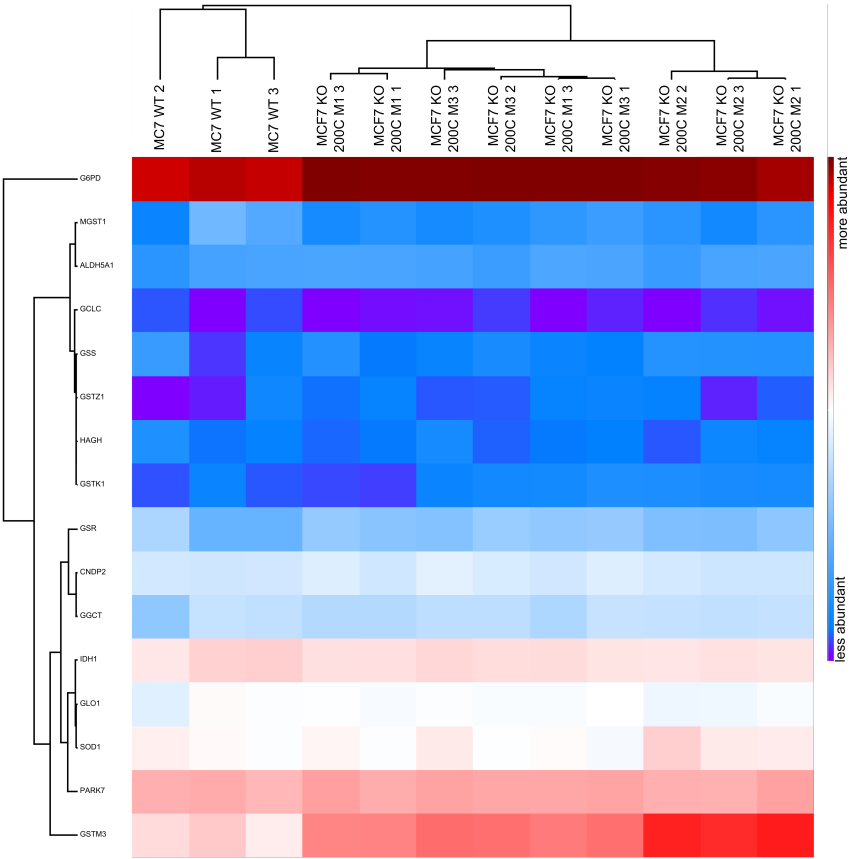


Figure S1. Proteomic analysis of MCF7 KO 200c (M1, M2, M3) vs. MCF7 wt upon doxorubicin (DXR) treatment. Differential protein expression depending on the hsa-miR-200c expression. (a) Gene set enrichment plot of the glutathione metabolic process pathway (left) and table of altered protein expression ranked upon metric score (right). **(b)** Corresponding heatmap of the glutathione metabolic process pathway (n=3). **(c)** An unsupervised hierarchical clustering of the proteins of the GO term "Glutathione Metabolic Process".

Figure S2

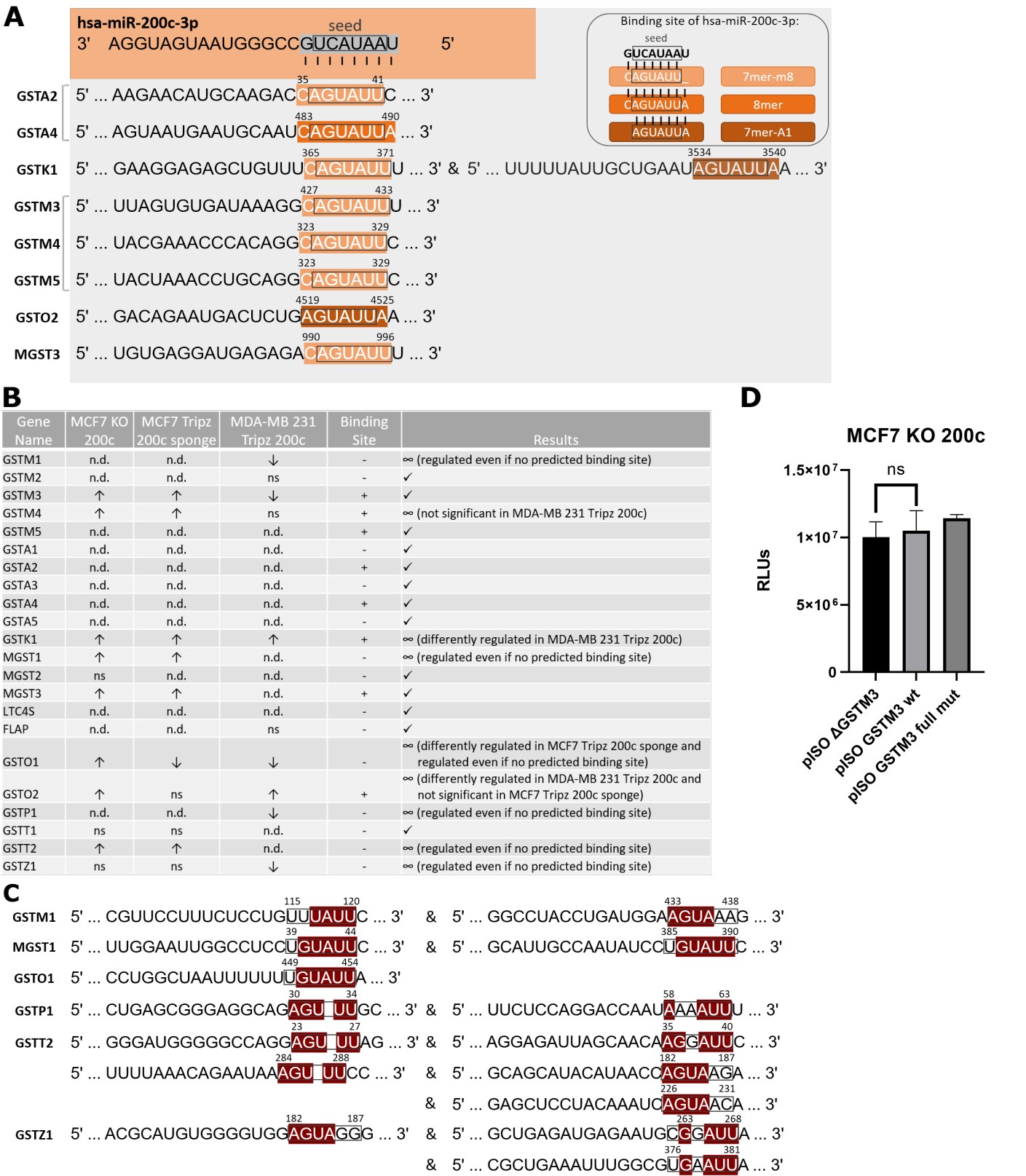
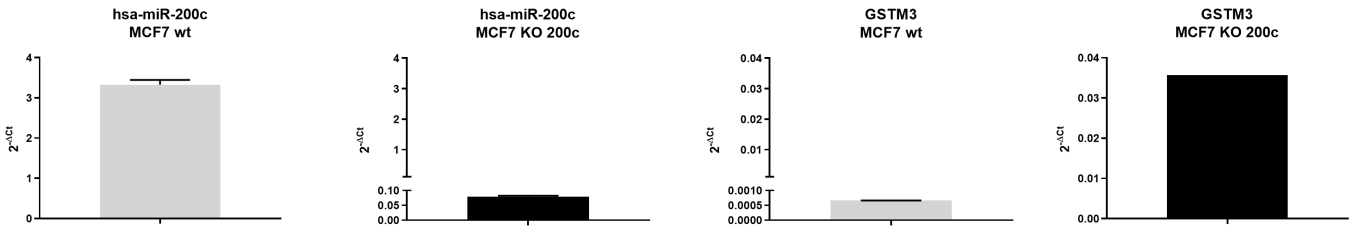


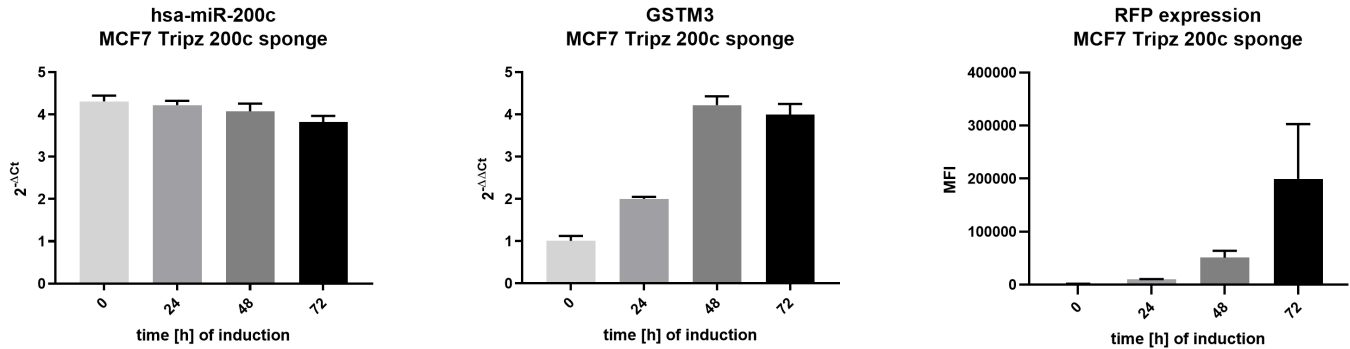
Figure S2: *In silico* miRNA-mRNA seed-site interaction. (a) 3'UTR sequences of GSTs with a predicted target site for hsa-miR-200c-3p. The seed region and complementary region are framed. Numbers indicate position within the 3'UTR sequence. Illustration of the 7mer-m8, 8mer and 7mer-A1 is shown on the right. Adapted from TargetScan. (b) Comparison of the regulation of all GSTs in the three hsa-miR-200c expression systems. Results, indicated with a check mark, verify the predicted miRNA-mRNA seed-site interaction. Further evaluation of the interaction is needed if the infinity symbol is displayed. n.d. = not detected, ns = not significant (c) Possible target sites for hsa-miR-200c-3p seed region. Framed sequences display possible complementary regions. Dark red highlighted nucleotides show a complementary match with the hsa-miR-200c-3p seed region. Sequences are adapted from TargetScan. (d) Luciferase assay of different GSTM3 3'UTR reporter plasmids co-transfected with a scrambled siRNA in MCF7 KO 200c cells. One representative diagram is shown. A two-tailed Student's t-test for pISO ΔGSTM3 and pISO GSTM3 wt was performed. Values are displayed as mean with SD.

Figure S3

A



B



C

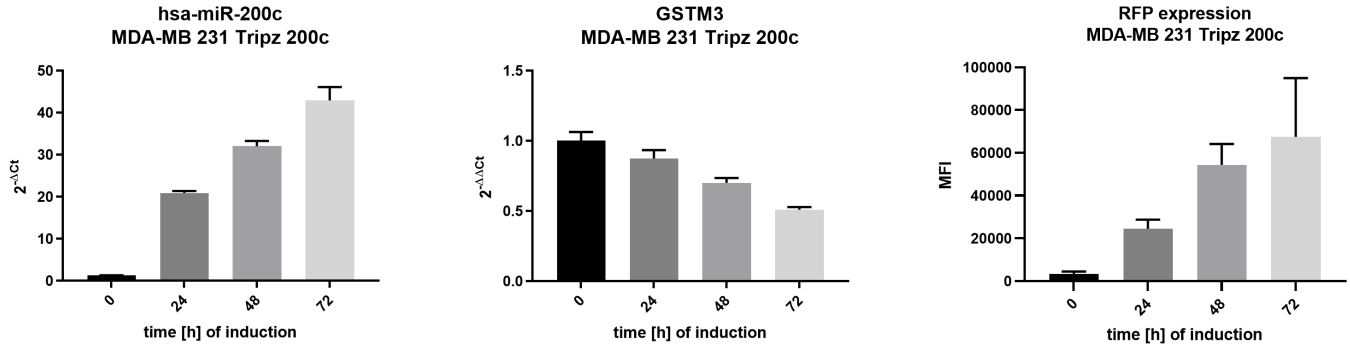


Figure S3. Characterization of hsa-miR-200c expression systems. (a) qRT-PCR analysis of hsa-miR-200c and GSTM3 expression in MCF7 wt and KO 200c cells. Quantitative RT-PCR analysis of hsa-miR-200c and GSTM3 expression in (b) MCF7 Tripz 200c sponge cells and (c) in MDA-MB 231 Tripz 200c cells, each induced with 5 μ g/ml doxycycline and FACS analysis of RFP expression of the same cells displayed as MFI. One representative replicate out of three is displayed. Values are displayed as mean with SD.

Figure S4

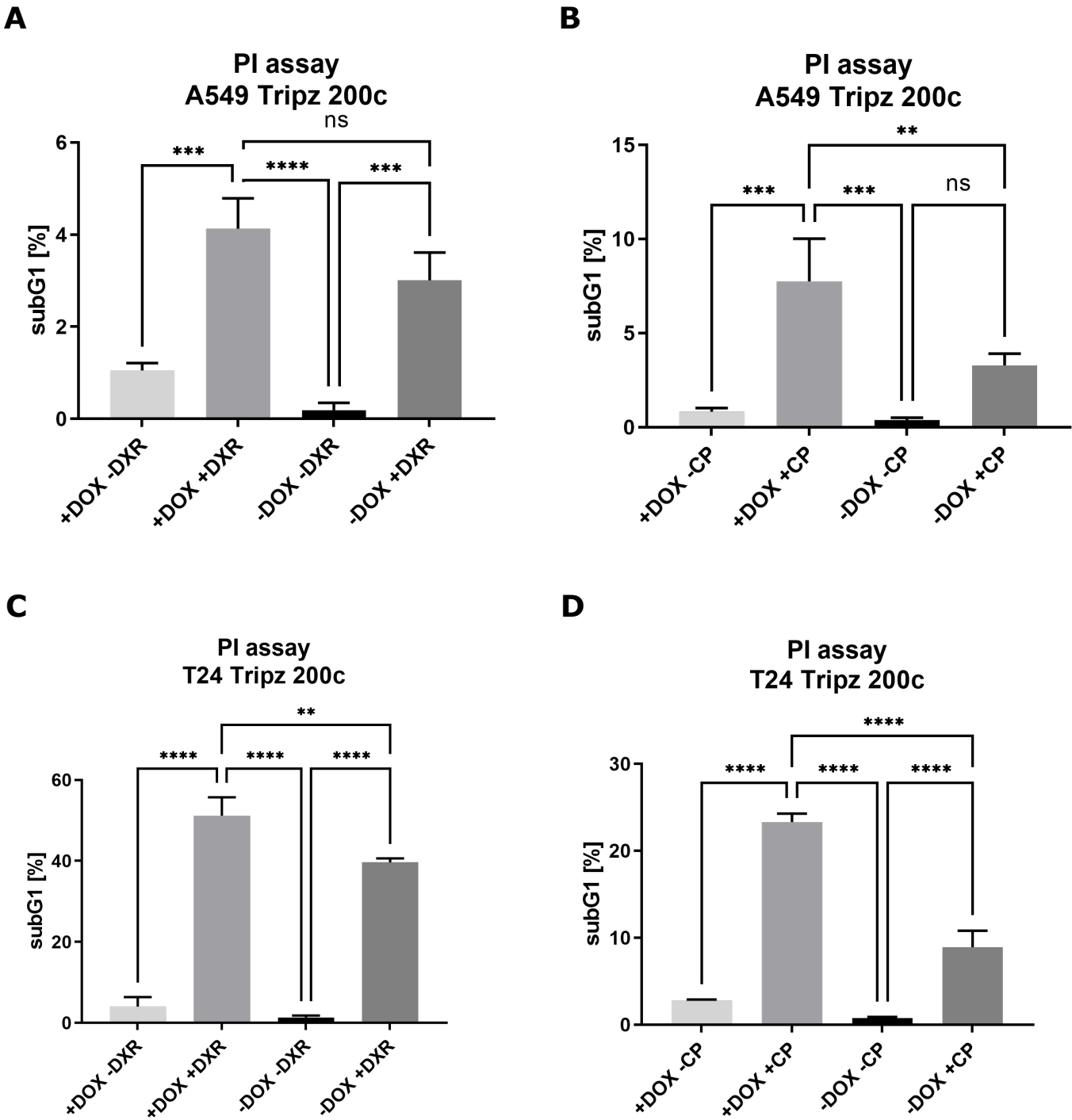


Figure S4. Effect of hsa-miR-200c on cell death in two additional cancer types in combination with chemotherapeutic treatment. Analysis of subG1 population in (a, b) A549 lung cancer cells in combination with doxorubicin (DXR) or cisplatin (CP) treatment and (c, d) T24 bladder cancer cells in combination with DXR or CP treatment using propidium iodide assay. Both cell lines were 72 hours pre-induced with DOX or not (-DOX). An ordinary one-way ANOVA with Tukey's multiple comparison test was performed for statistics. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. One representative diagram out of three is displayed. Values are displayed as mean with SD.

Figure S5

A

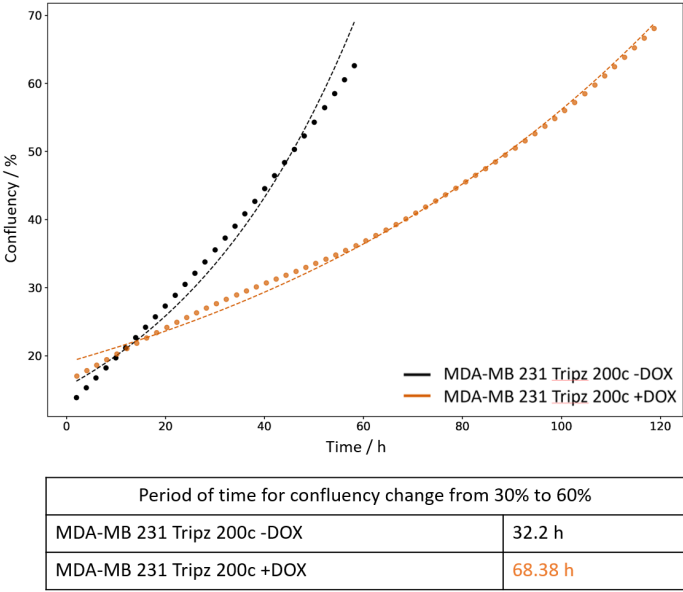
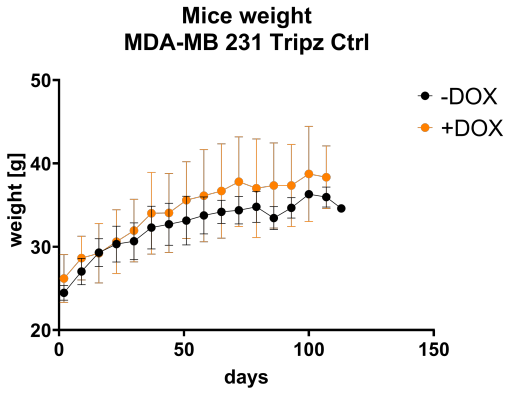


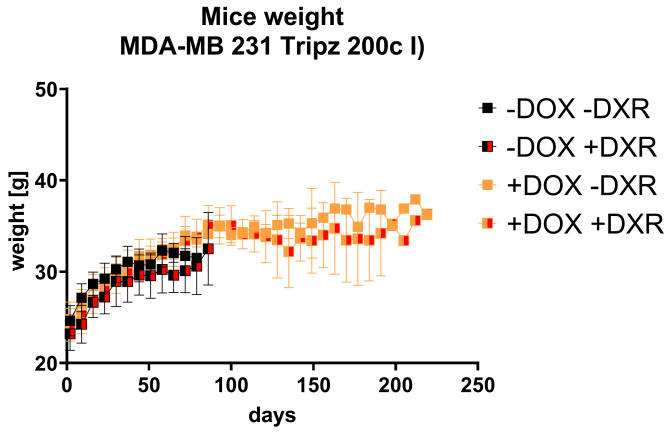
Figure S5. Proliferation curves of hsa-miR-200c positive or negative MDA-MB 231 cells. Proliferation of (a) MDA-MB 231 Tripz 200c with doxycycline induction every 48 to 72 hours. Comparison of proliferation slopes and doubling times in hsa-miR-200c expressing (+DOX, orange curve) and hsa-miR-200c depleted (-DOX, black curve) cells. One representative diagram out of three is presented.

Figure S6

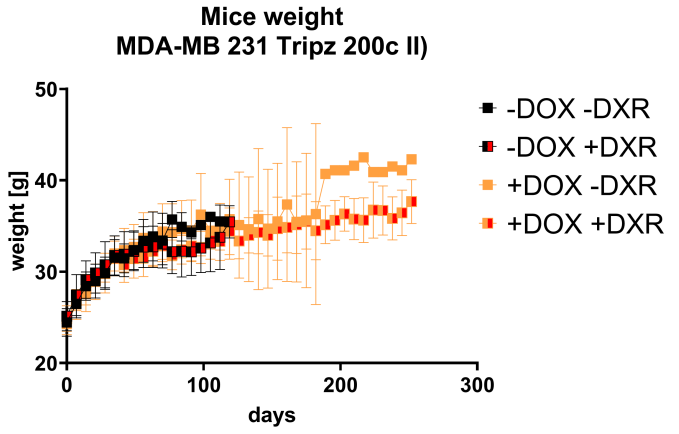
A



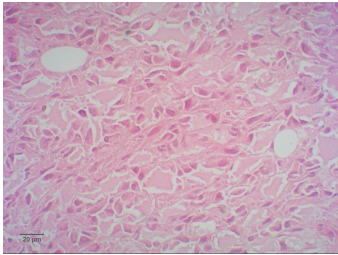
B



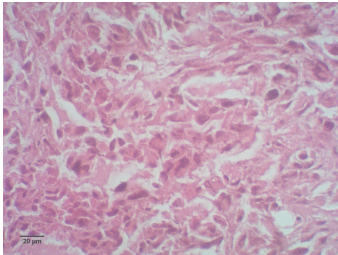
C



D



-DOX -DXR

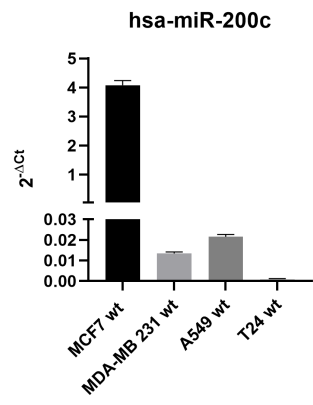


+DOX -DXR

Figure S6. Animal welfare monitoring of the different xenograft mouse models and histological analysis of hsa-miR-200c positive and negative tumors. (a) Weekly body weight mean of mice bearing control tumors (MDA-MB 231 Tripz Ctrl) and fed with regular (black curves) or doxycycline (DOX, orange curves) diet (n=5). (b) Body weight mean displayed once a week of mice with hsa-miR-200c positive (MDA-MB 231 Tripz 200 +DOX, orange curves) or negative (-DOX, black curves) tumors and additional chemotherapeutic treatment (n=5, red filled squares) related to animal experiment I) Treatment of hsa-miR-200c positive and negative tumors. (c) Mean body weight (displayed once a week) of mice with initial hsa-miR-200c negative tumors and their subsequent treatment with either doxorubicin (DXR, black curve and red filled squares) or miRNA-200c (orange curve) or their combination (orange curve and red filled squares) related to animal experiment II) Single or double treatment of hsa-miR-200c negative tumors, (n=10 per group). Body weight graphs do not terminate when mice were euthanized. (d) H&E staining of hsa-miR-200c negative (-DOX) or positive (+DOX) tumors without DXR treatment. Values are displayed as mean with SD.

Figure S7

A



B

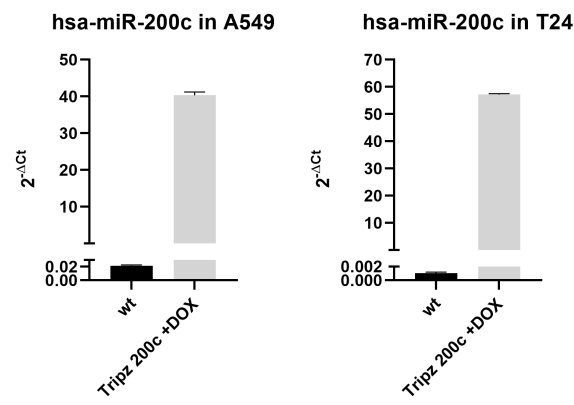


Figure S7. hsa-miR-200c expression profile. (a) qRT-PCR analysis of hsa-miR-200c expression in the wt cell lines. (b) Induction of hsa-miR-200c in A549 Tripz 200c (left) and T24 Tripz 200c (right) upon doxycycline application (5 μg/ml) compared to the corresponding wt cell line. Values are displayed as mean with SD.

Figure S8

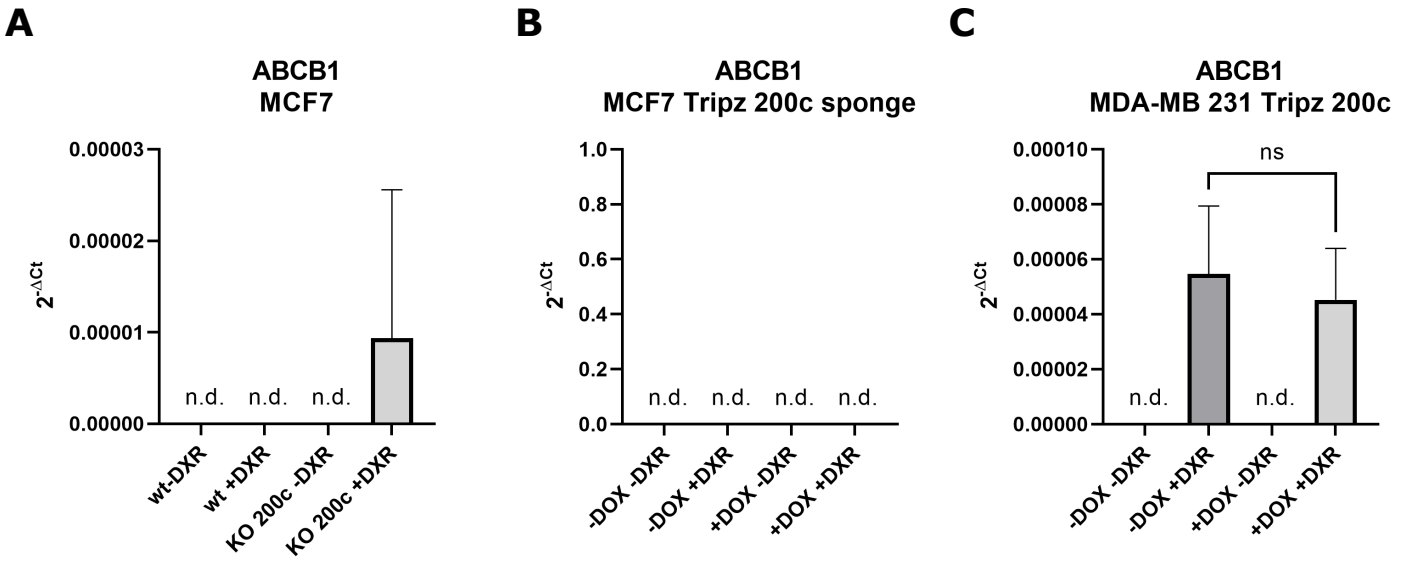


Figure S8. hsa-miR-200c expression and the abundance of ABCB1. Expression of ABCB1 analyzed with qRT-PCR in the **(a)** MCF7 wt and KO 200c (treatment with 5 μ M of DXR for 6 hours), **(b)** the MCF7 Tripz 200c sponge (72 hours DOX pre-induced and treated with 0.1 μ M DXR for 24 hours) and **(c)** the MDA-MB 231 Tripz 200c cell system (72 hours DOX pre-induced and subsequently treated with 0.6 μ M DXR for 24 hours). Ct values of 35 and more are uncertain values and therefore were excluded from the analysis and termed as not detected (n.d.). ns = not significant. Values are displayed as mean with SD.

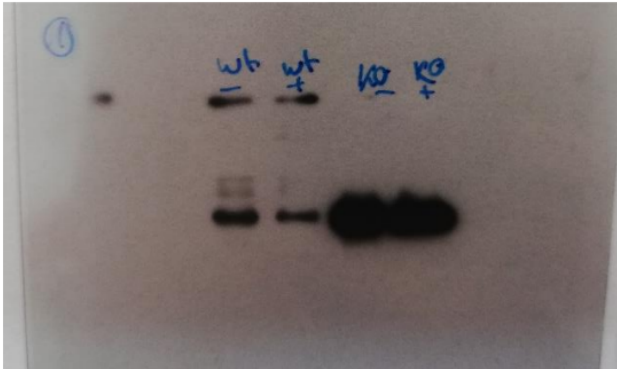
Figure S9

Kaplan-Meier plot for hsa-miR-200c	
miRNA: miRpower for breast cancer	
miRNA ID	hsa-miR-200c
Survival	Overall survival
Split patients by	auto select best cutoff
Follow up threshold	60 months, censor at threshold
Dataset	TCGA
ER status	IHC and gene chip: all
PGR status	IHC: all
HER2 status	IHC and gene chip: all
Molecular subtype	TNBC
Grade	all
Lymph node status	all
Restrict analysis to selected cohorts	do not use cohorts
Kaplan-Meier plot for GSTM3	
mRNA gene chip for breast cancer	
Affy ID	202554_s_at
Survival	Relapse free survival (RFS)
Split patients by	median
Follow up threshold	60 months, censor at threshold
Probe set option	only JetSet best probe set
ER status	IHC and array: all
PR status	IHC: all
HER2 status	array: all
Subtype - StGallen	basal
Subtype - PAM50	all
Lymph node status	all
Grade	all
TPS3 status	all
Pietenpol subtype	all
Use earlier release of the database	all
Use following dataset for the analysis	all
Quality control - remove redundant samples	checked
Quality control - array quality control	exclude biased arrays
Quality control - proportional hazards assumption	checked
Cohorts	patients with following systemic treatment
Endocrine therapy	any
Chemotherapy	neoadjuvant only

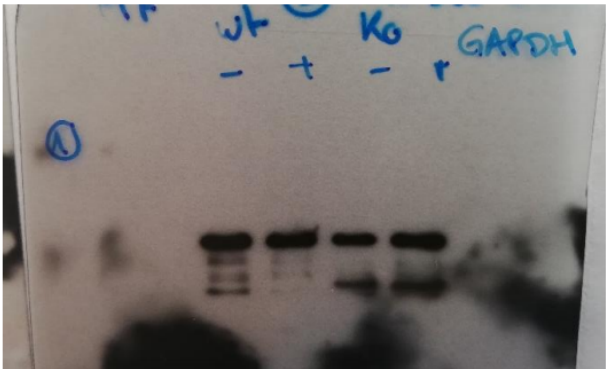
Figure S9: Overview on the parameters entered into the Kaplan-Meier Plotter for the analysis of hsa-miR-200c and GSTM3 in breast cancer patients.

Figure S10

For Figure 4B:

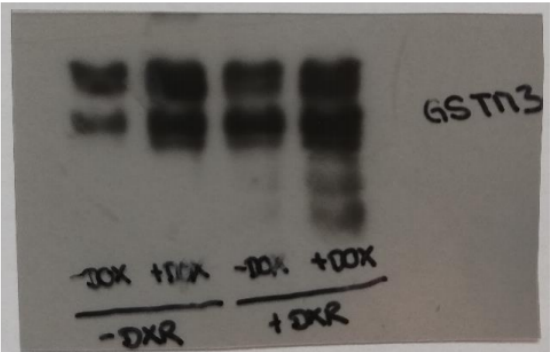


GSTM3

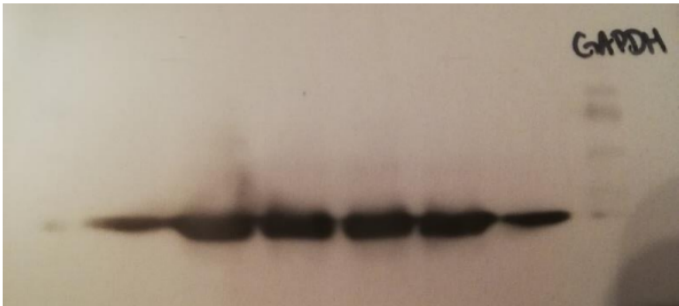


GAPDH

For Figure 4D:

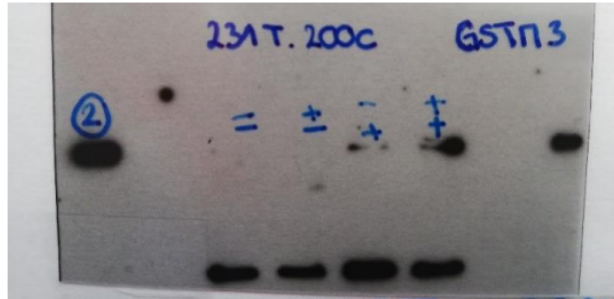


GSTM3

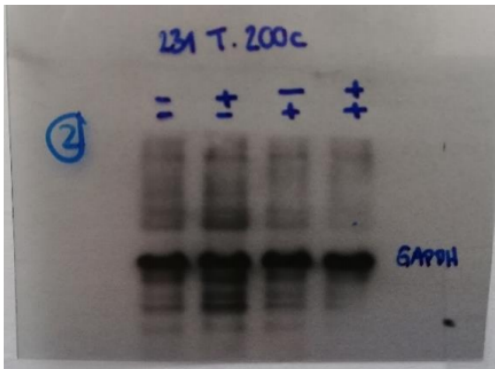


GAPDH

For Figure 4F:



GSTM3



GAPDH

Figure S10: Uncropped blots of western blots presented in Figure 4B,D,F

Table S1

MDA-MB 231 Tript 200c -DOX				MDA-MB 231 Tript 200c +DOX			
gene	Ct value 1	Ct value 2	Ct value 3	gene	Ct value 1	Ct value 2	Ct value 3
mu family				mu family			
GSTM1	26.85	26.87	26.92	GSTM1	27.51	27.53	27.62
GSTM2	28.25	28.29	28.91	GSTM2	29.05	28.86	29.12
GSTM3	27.84	27.78	27.82	GSTM3	28.37	28.28	28.25
GSTM4	25.86	26	26.03	GSTM4	26.23	26.16	26.23
GSTM5	37.65	37.11	40	GSTM5	37.61	38.18	38.18
GAPDH	19.6	19.47	19.46	GAPDH	19.74	19.68	19.66
alpha and kappa family				alpha and kappa family			
GSTA1				GSTA1			
GSTA2				GSTA2			
GSTA3	40	40	40	GSTA3	38.53	40	37.25
GSTA4	37.42	36.76	37.28	GSTA4	40	40	37.63
GSTA5	38.38	40	40	GSTA5	40	40	40
GSTK1	24.88	24.87	24.86	GSTK1	24.67	24.66	24.67
GAPDH	19.42	19.24	19.01	GAPDH	19.26	19.34	19.16
MAPEG family part 1				MAPEG family part 1			
MGST1				MGST1			
MGST2				MGST2			
MGST3				MGST3			
GAPDH	19.42	19.24	19.01	GAPDH	19.26	19.34	19.16
MAPEG family part 2				MAPEG family part 2			
LTC4S	40	40	40	LTC4S			
FLAP	31.89	31.98	31.9	FLAP	32.21	32.43	32.34
GAPDH	19.34	19.46	19.46	GAPDH	19.63	19.66	19.68
omega, pi, theta and zeta family				omega, pi, theta and zeta family			
GSTO1	29.73	29.67	29.54	GSTO1	30	30.09	30.06
GSTO2	30.8	30.79	30.76	GSTO2	29.98	30.11	30.08
GSTP1	23.23	23.2	23.25	GSTP1	23.62	23.62	23.82
GSTT1				GSTT1			
GSTT2				GSTT2			
GSTZ1	24.13	24.06	23.87	GSTZ1	24.67	24.6	24.65
GAPDH	19.42	19.24	19.01	GAPDH	19.26	19.34	19.16

MCF7 Tript 200c-sponge-DOX				MCF7 Tript 200c-sponge+DOX			
gene	Ct value 1	Ct value 2	Ct value 3	gene	Ct value 1	Ct value 2	Ct value 3
mu family				mu family			
GSTM1	36.16	36.62	36.16	GSTM1	33.96	34	33.73
GSTM2	29.36	29.33	29.34	GSTM2	28.03	28	28
GSTM3	27.21	27.17	27.25	GSTM3	26.76	26.76	26.66
GSTM4	19.13	19.25	19.24	GSTM4	19.27	19.22	19.11
GSTM5	40	40	40	GSTM5	40	40	40
GAPDH	35.8	36.58	36.84	GAPDH	35.2	36.19	35.49
alpha and kappa family				alpha and kappa family			
GSTA1	40	40	40	GSTA1	40	40	40
GSTA2	35.8	36.58	36.84	GSTA2	35.2	36.19	35.49
GSTA3	40	40	40	GSTA3	40	40	40
GSTA4	36.36	37	37.62	GSTA4	36.84	35.96	36.09
GSTA5	24.9	24.94	25	GSTA5	24.26	24.25	24.3
GSTK1	19.13	19.25	19.24	GSTK1	19.27	19.22	19.11
GAPDH	19.13	19.25	19.24	GAPDH	19.27	19.22	19.11
MAPEG family part 1				MAPEG family part 1			
MGST1	27.05	26.87	27.03	MGST1	26.54	26.67	26.35
MGST2	37.27	37.09	37.17	MGST2	36.79	37.04	36.72
MGST3	25.32	25.4	25.56	MGST3	24.95	24.87	24.79
LTC4S	19.13	19.25	19.24	LTC4S	40	38.03	40
GAPDH	19.13	19.25	19.24	GAPDH	19.27	19.22	19.11
MAPEG family part 2				MAPEG family part 2			
FLAP	18.99	19.11	19.13	FLAP	19.15	19.14	19.13
GAPDH	18.99	19.11	19.13	GAPDH	19.15	19.14	19.13
omega, pi, theta and zeta family				omega, pi, theta and zeta family			
GSTO1	33.67	33.52	33.44	GSTO1	33.94	34.05	34.37
GSTO2	25.75	25.8	25.9	GSTO2	25.9	25.84	25.87
GSTP1	35.46	35.98	37.31	GSTP1	36.74	36.3	35.94
GSTP1	25.6	25.79	25.73	GSTP1	25.13	25.9	25.49
GSTT2	35.43	35.51	35.66	GSTT2	34.95	34.66	34.93
GSTP1	23.78	23.91	23.67	GSTP1	23.64	23.68	23.73
GAPDH	18.99	19.11	19.13	GAPDH	19.15	19.14	19.13