# Supplementary Materials: Disruption of pH Dynamics Suppresses Proliferation and Potentiates Doxorubicin Cytotoxicity in Breast Cancer Cells

**Diana Tavares-Valente, Bárbara Sousa, Fernando Schmitt, Fátima Baltazar and Odília Queirós**
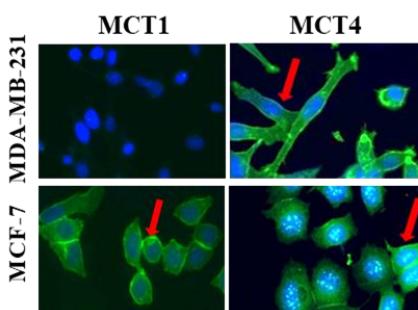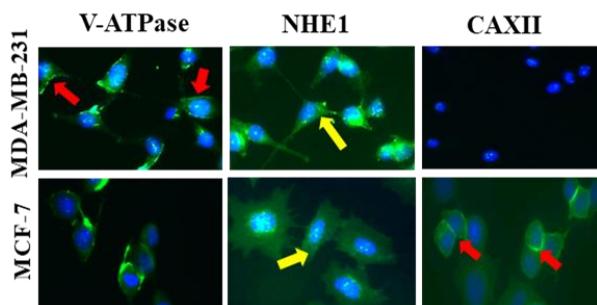
Figure S1. Cellular localization of the different pH regulators determined by immunofluorescence. The red arrows highlight PM expression and the yellow arrows cytoplasmic expression. Blue area corresponds to nuclei stained with DAPI. Images are in 100x magnification.
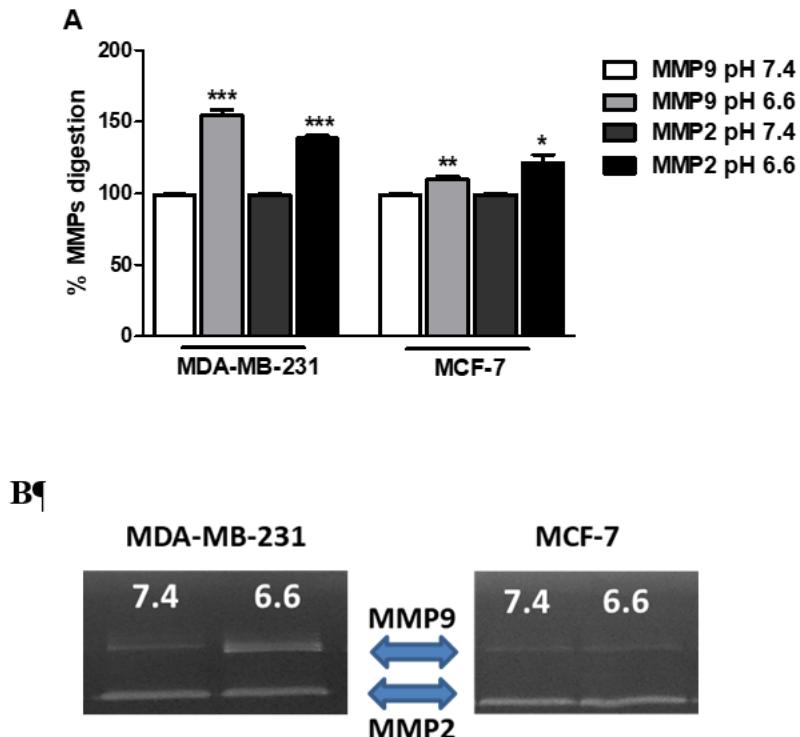
Figure S2. Effect of extracellular pH on MMP activity assay in breast cancer cell lines. (A) MMP9 and MMP2 activity in culture medium samples from breast cancer cells at different extracellular pH values, evaluated by zymography. **(B)** Representative pictures of gel digestion at different extracellular pH values. Results are expressed as mean ± SD of triplicates from three independent experiments. Significantly different between groups: *$P < 0.05$; **$P < 0.01$ compared to control (pH 7.4). ns: non significant.
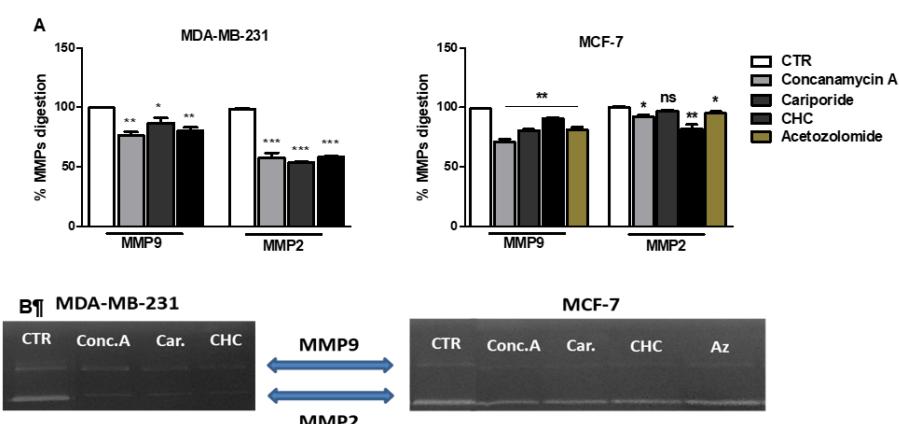
Figure S3. Effect of pH regulator inhibition on MMP activity assay in breast cancer cell lines. (A) MMP9 and MMP2 activity in culture medium samples from breast cancer cells in the presence of PRI, evaluated by zymography. **(B)** Representative pictures of gel digestion in the presence of PRI. Results are expressed as mean ± SD of triplicates from three independent experiments. Significantly different between groups: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to untreated cells (control).

Table S1. Clinical, pathological and immunohistochemical characteristics of the 473 primary invasive breast carcinomas. Characterization of the breast cancer series concerning age of the patients, tumor size, lymph-node metastasis, histological grade, molecular subtypes, ER, PgR, HER2 and Ki67 status, as well as expression of HIF-1α, GLUT1, CAIX, MCT1, MCT4 and CD147.

			Frequency (n)	Percentage (%)
Clinico-pathological and molecular characteristics	**Age (years)**	Mean	56.12	
		Min	27	
		Max	89	
		Missing	2	
	Tumor size (mm)	Mean	31.6	
		Min	5	
		Max	150	
		Missing	243	
	Lymph-node metastasis	Positive	204	51
		Negative	196	49
		Total	400	100
		Missing	73	-
	Histological grade	I	155	33.2
		II	200	42.8
		III	112	24
		Total	467	100
		Missing	6	-
	Molecular subtypes	Luminal A	262	57.3
		Luminal B	14	3.1
		HER2 OE	56	12.3
		Basal	83	18.2
		Unclassified	42	9.2
		Total	457	100
		Missing	16	-
	ER	Positive	275	58.4
		Negative	196	41.6
		Total	471	100
		Missing	2	-
	PgR	Positive	177	37.9
		Negative	290	62.1
		Total	467	100
		Missing	6	
	HER2	Positive	69	14.9
		Negative	393	85.1
		Total	462	100
		Missing	11	-
	Ki67	>20	98	39.5
		<20	150	60.5
		Total	248	100
		Missing	225	-
Biomarkers	**HIF-1α**	Positive	104	33
		Negative	211	67.0
		Total	315	100
		Missing	158	
	GLUT1	Positive	140	42.8
		Negative	187	57.2
		Total	327	100
		Missing	146	-

Biomarkers	**CAIX**	Positive	66	20.9
		Negative	250	79.1
		Total	316	100
		Missing	157	-
	MCT1	Positive	106	26
		Negative	301	74
		Total	407	100
		Missing	66	-
	MCT4	Positive	69	16.5
		Negative	350	83.5
		Total	419	100
		Missing	54	-
	CD147	Positive	24	11.1
		Negative	193	88.9
		Total	217	100
		Missing	256	-

Table S2. Association of V-ATPase and CAXII with molecular characteristics and molecular subtypes in breast carcinoma samples.

		V-ATPase			**P**	**CAXII**			**P**
		Negative (%)	**Positive (%)**	**Total**		**Negative (%)**	**Positive (%)**	**Total**	
Molecular Characteristics	**ER**				.001				.380
	Negative	47 (52.8)	8 (22.2)	55 (44.0)		20 (34.5)	26 (43.3)	46 (39.0)	
	Positive	42 (47.2)	28 (77.8)	70 (56.0)		38 (65.5)	34 (56.7)	72 (61.0)	
	Total	89 (71.2)	36 (28.8)	125 (100)		58 (49.0)	60 (51.0)	118 (100)	
	PgR				.006				.010
	Negative	61 (70.9)	16 (44.4)	77 (63.1)		33 (58.9)	37 (62.7)	70 (60.9)	
	Positive	25 (29.1)	20 (55.6)	45 (36.9)		23 (41.1)	22 (37.3)	45 (39.1)	
	Total	86 (70.5)	36 (29.5)	122 (100)		56 (48.7)	59 (51.3)	115 (100)	
	HER2				.003				.380
	Negative	62 (71.3)	33 (94.3)	95 (77.9)		38 (65.5)	48 (82.8)	91 (60.9)	
	Positive	25 (2.7)	2 (5.7)	27 (22.1)		20 (41.1)	10 (17.2)	25 (21.6)	
	Total	87 (71.3)	35 (28.7)	122 (100)		58 (50.0)	58 (50.0)	116 (100)	
	Ki67				.080				.040
	<10	52 (59.8)	16 (45.7)	68 (55.7)		28 (48.3)	37 (64.9)	65 (56.5)	
	10-20	8 (9.2)	1 (2.9)	9 (7.4)		8 (13.8)	3 (5.3)	11 (9.6)	
	>20	27 (31.0)	18 (51.4)	45 (36.9)		22 (37.9)	17 (29.8)	39 (33.9)	
	Total	87 (71.3)	35 (28.7)	122 (100)		58 (50.4)	57 (49.6)	115 (100)	
	LN invasion				.656				.010
	No	38 (46.9)	13 (52.0)	51 (48.1)		17 (34.7)	29 (61.7)	46 (47.9)	
	Yes	43 (53.1)	12 (48.0)	55 (51.9)		32 (65.3)	18 (38.3)	50 (52.1)	
	Total	81 (76.4)	25 (23.6)	106 (100)		49 (51.0)	47 (49.0)	96 (100)	
Molecular	**Luminal A**	37 (42.5)	27 (77.1)	64 (52.5)	.006	31 (54.4)	32 (55.2)	63 (54.8)	.010
	Luminal B	4 (4.6)	0 (0.0)	4 (3.3)		6 (10.5)	1 (1.7)	7 (6.1)	
	HER2 overexpressing	21 (24.1)	2 (5.7)	23 (18.9)		9 (15.8)	9 (15.5)	18 (15.7)	
	Basal	14 (16.1)	5 (14.3)	19 (15.6)		6 (10.5)	11 (19.0)	17 (14.8)	
	Unclassified	11 (12.6)	1 (2.9)	12 (9.8)		5 (8.8)	5 (8.6)	10 (8.7)	
	Total	87 (71.3)	35 (28.7)	122 (100)		57 (49.6)	58 (50.4)	115 (100)	

*It was considered only the plasma membrane expression of both pH regulators.

Table S3. Association of V-ATPase and CAXII with biomarkers in breast carcinoma samples. 　38

		V-ATPase			*P*	CAXII			*P*
		Negative (%)	Positive (%)	*Total*		Negative (%)	Positive (%)	*Total*	
	HIF1				**.687**				**.362**
	Negative	23 (36.5)	9 (32.1)	32 (35.2)		17 (37.8)	10 (28.6)	27 (33.8)	
	Positive	40 (63.5)	19 (67.9)	59 (64.8)		28 (62.2)	25 (71.4)	53 (66.3)	
	Total	63 (69.2)	28 (30.8)	91 (100)		45 (56.3)	35 (43.7)	80 (100)	
	GLUT1				**.070**				**.264**
	Negative	16 (61.5)	4 (30.8)	20 (51.3)		12 (66.7)	4 (30.8)	16 (51.6)	
	Positive	10 (38.5)	9 (69.2)	19 (48.7)		6 (33.3)	9 (69.2)	15 (48.4)	
	Total	26 (66.7)	13 (33.3)	39 (100)		18 (58.0)	13 (42.0)	31 (100)	
	CAIX				**.363**				**.213**
	Negative	19 (73.1)	7 (58.3)	26 (68.4)		15 (78.9)	7 (58.3)	22 (71.0)	
	Positive	7 (26.9)	5 (41.7)	12 (31.6)		4 (21.1)	5 (41.7)	9 (29.0)	
	Total	26 (68.4)	12 (31.6)	38 (100)		19 (61.3)	12 (38.7)	31 (100)	
	CD147				**.342**				**.301**
	Negative	78 (91.8)	31 (86.1)	109 (90.1)		52 (96.3)	53 (91.4)	105 (93.8)	
	Positive	7 (8.2)	5 (13.9)	12 (9.9)		2 (3.7)	5 (8.6)	7 (6.3)	
	Total	85 (70.2)	36 (29.8)	121 (100)		54 (48.2)	58 (51.8)	112 (100)	
	V-ATPase				-				**.030**
	Negative	-	-	-		41 (83.7)	30 (63.8)	71 (74.0)	
	Positive	-	-	-		8 (16.3)	17 (36.2)	25 (26.0)	
	Total	-	-	-		49 (51.0)	47 (49.0)	96 (100)	

(Row label at left spanning rows: **Biomarkers**)

*It was considered only the plasma membrane expression of both pH regulators. 　39

Table S4. pHe values of culture media measured after 48 hours after PRI IC$_{50}$ treatment. The ΔpH corresponds to the difference between the pHe after treatment and the pHe without treatment, after the respective time of incubation. Results represent the mean + SD of at least three independent experiments. 　40 41 42 43

	W/o compound	Conc.A	Cariporide	AZ	CHC	ΔpH
MDA-MB-231	5.4 ± 0.7	6.2 ± 0.2	6.5 ± 0.2	-	6.4 ± 0.6	Conc. A: 0.9 Cariporide: 1.1 CHC: 1.0
MCF-7	5.8 ± 0.2	6.2 ± 0.9	6.4 ± 0.2	6.6 ± 0.8	6.0 ± 0.2	Conc. A: 0.4 Cariporide: 0.6 AZ:0.8 CHC: 0.2

44