Vall d'Hebron
Hospital
CIBBIM - Nanomedicina

Fighting against Cancer Stem Cells with Nanomedicines

2nd Workshop on Translational Nanomedicine

CIDETEC

Donostia, September 18th-19th, 2017

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CIBBIM-Nanomedicine

www.cibbim.eu

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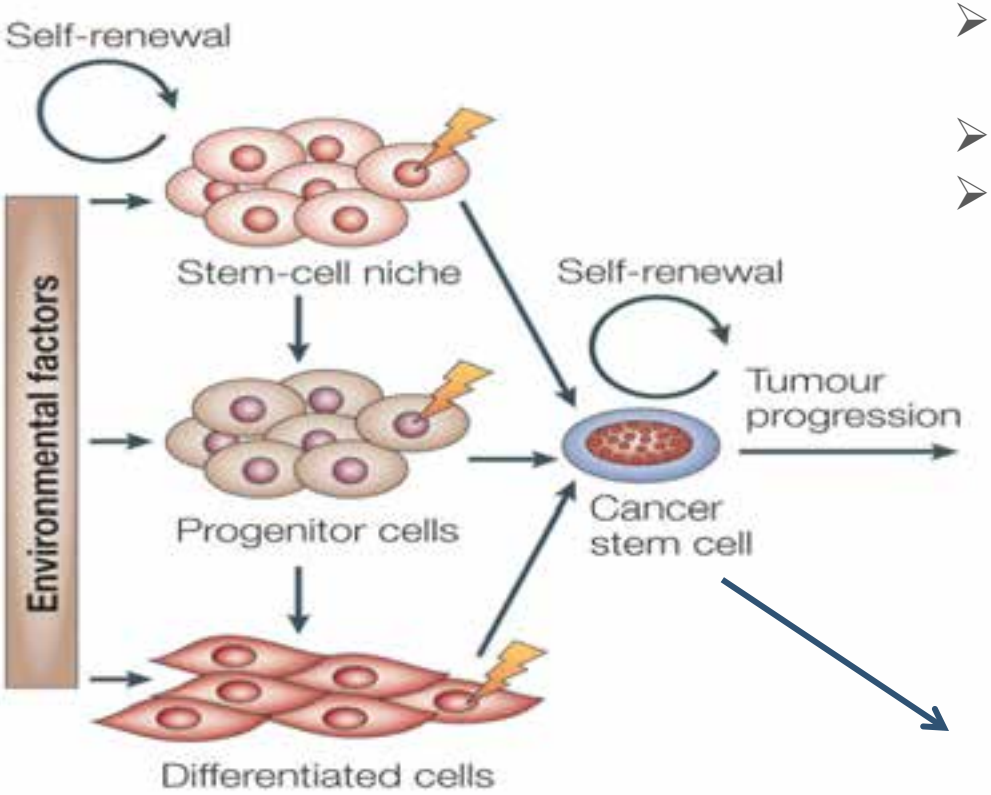
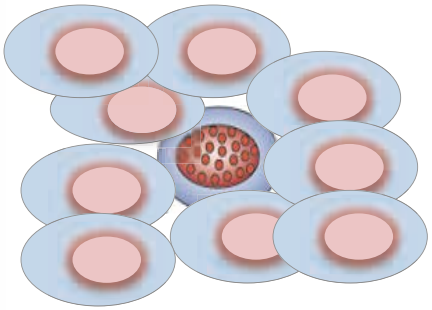
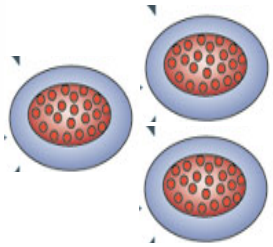
Cancer Stem Cells allow metastatic spread and tumor resistance to current therapy

- rare cells within tumors
- high tumorigenic capacity
- self-renewal ability
- give rise to the phenotypically diverse tumor cell
- resistance to therapy
- non-adhesion survival

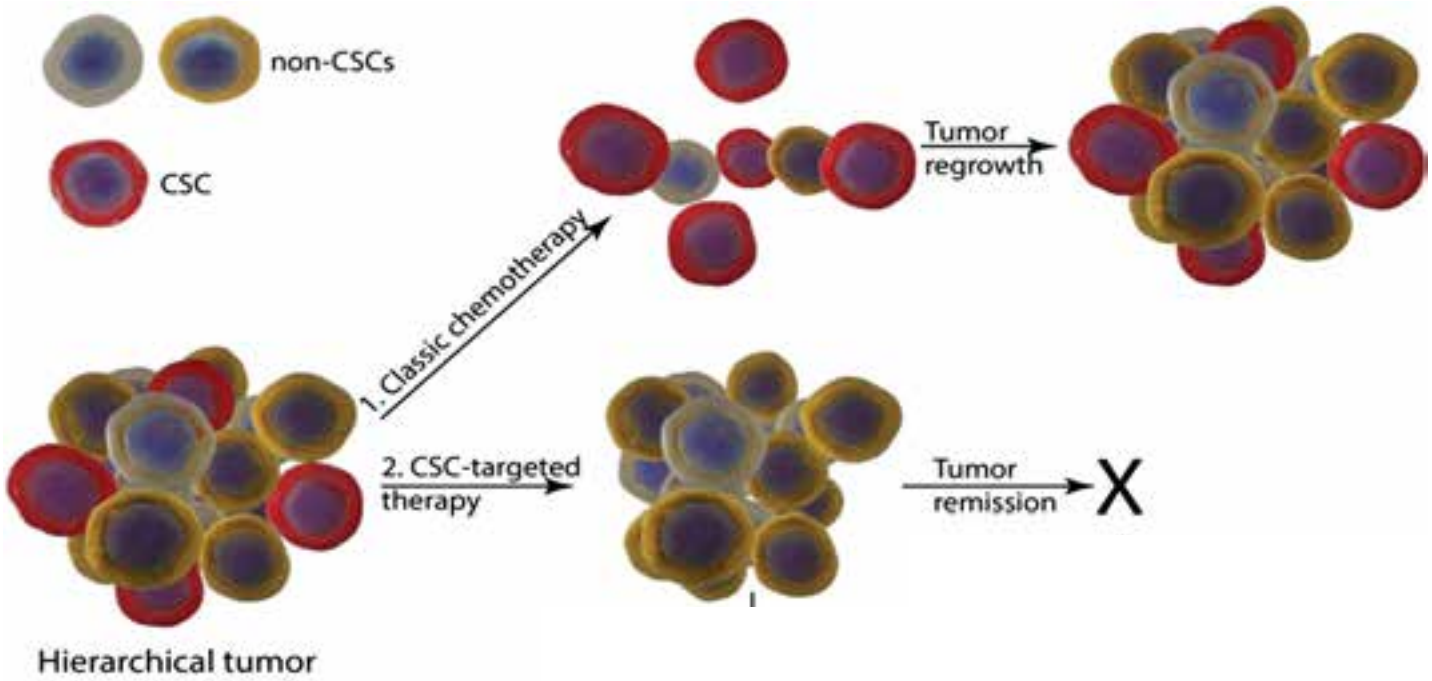






Metastatic spread

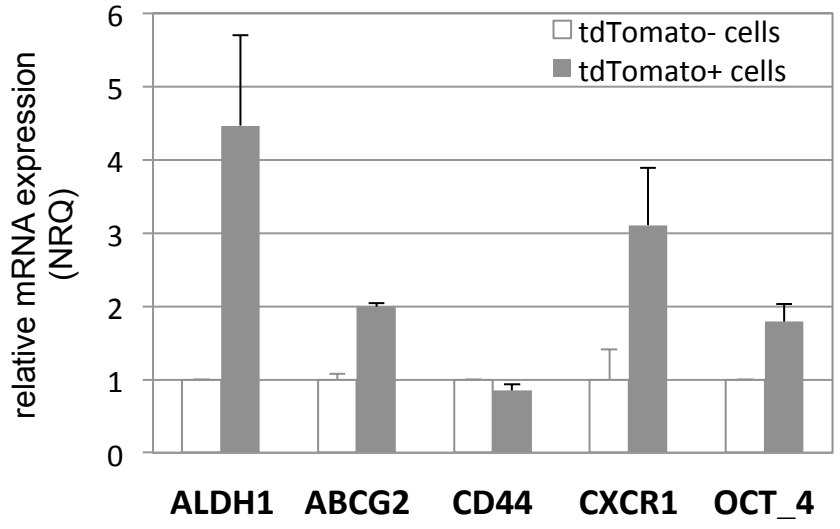
CSC therapy



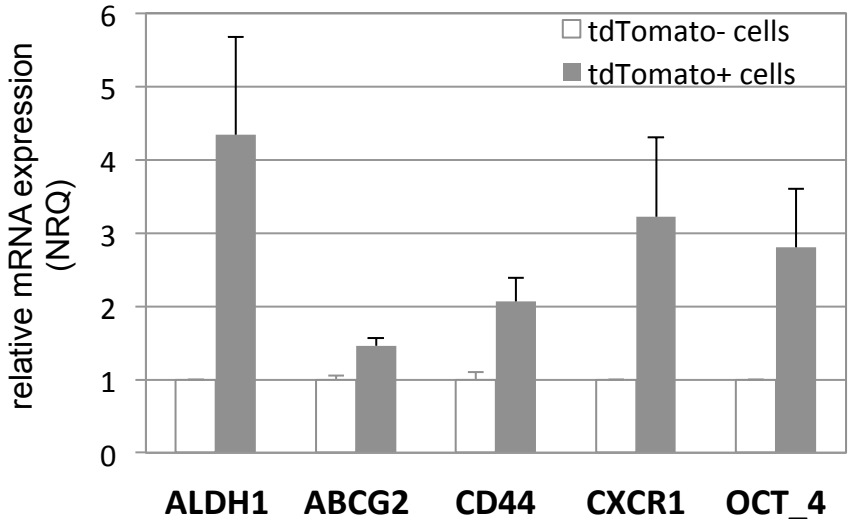
Y. Zhao et al. / Advanced Drug Delivery Reviews (2013)

CSC in vitro model: EXPRESSION OF STEMNESS MARKERS

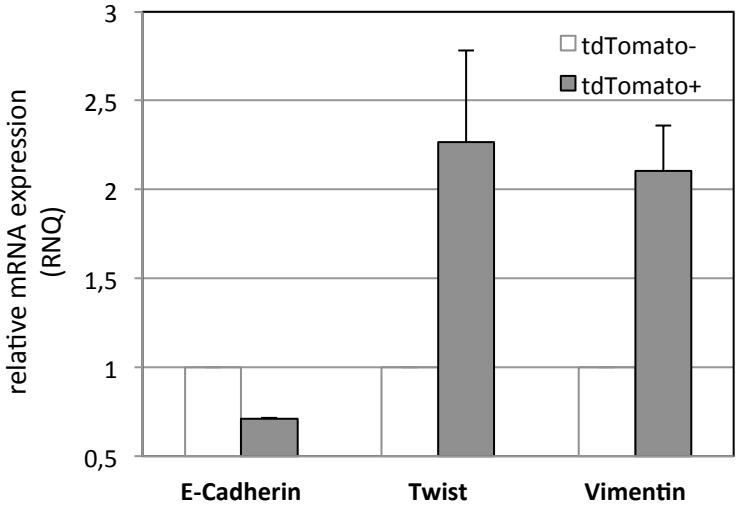
MDA-MB-231-ALDH/tdTomato



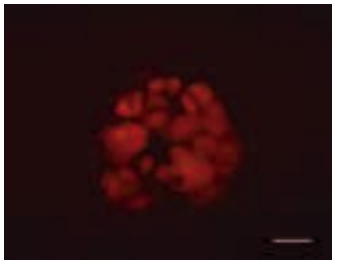
MCF7-ALDH/tdTomato



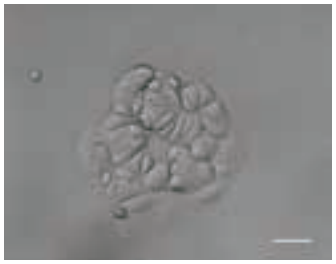
EMT transition gens



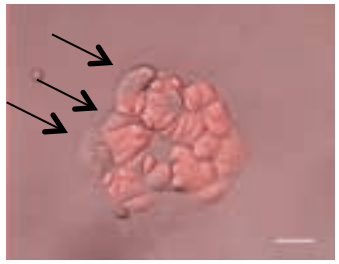
td Tomato⁺ cells

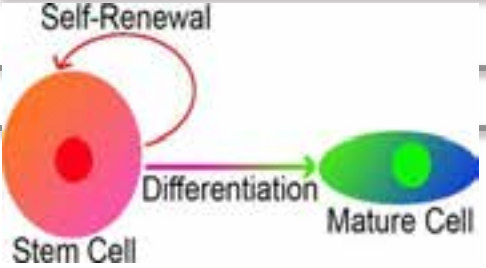


all cells

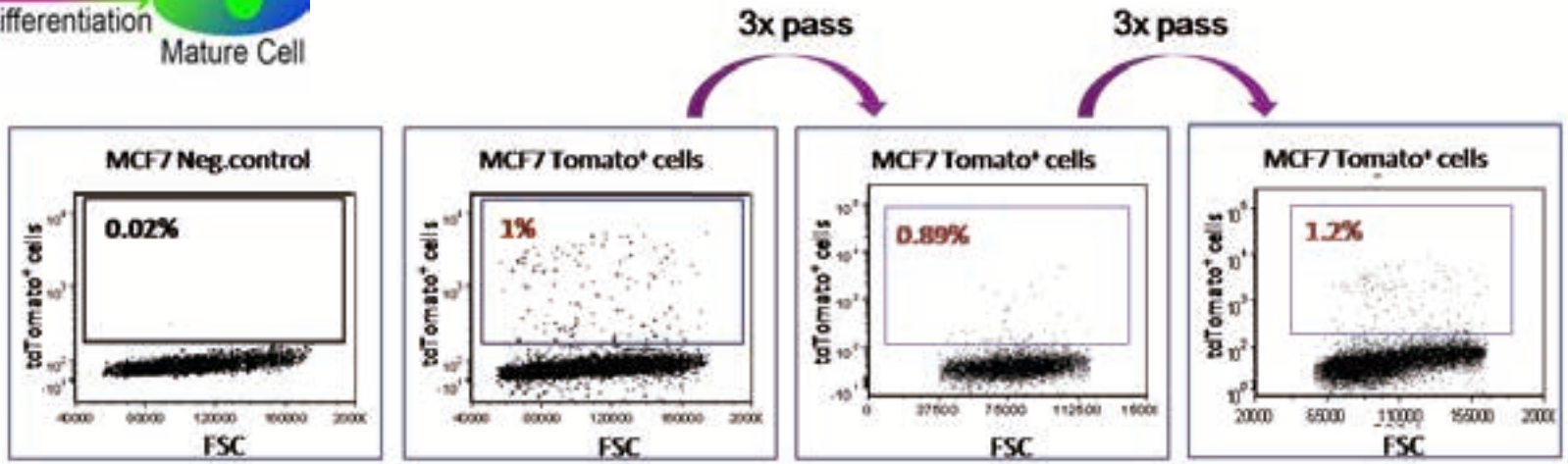


merged

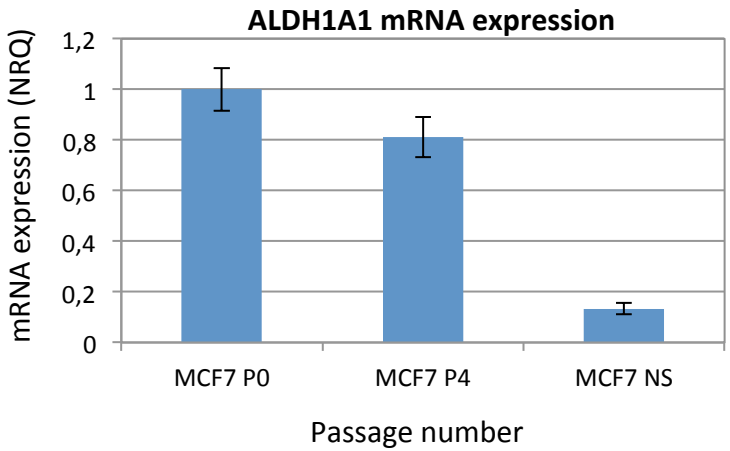


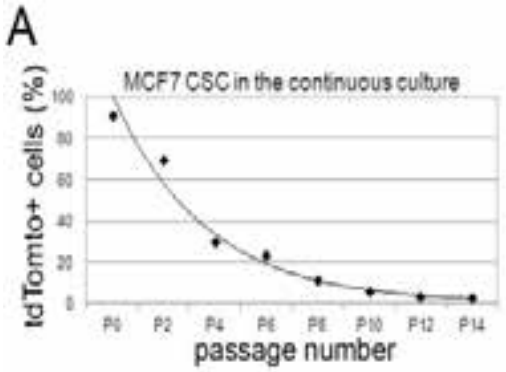


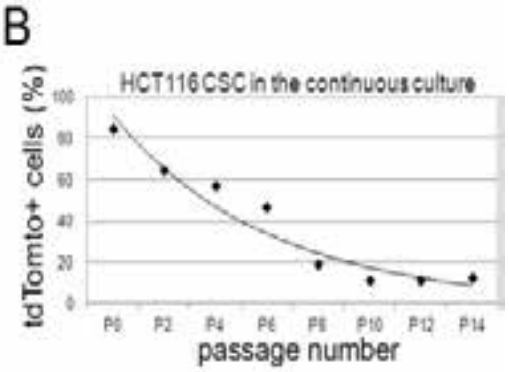
Amount of CSC markers in MCF7 CSC cell line is stable



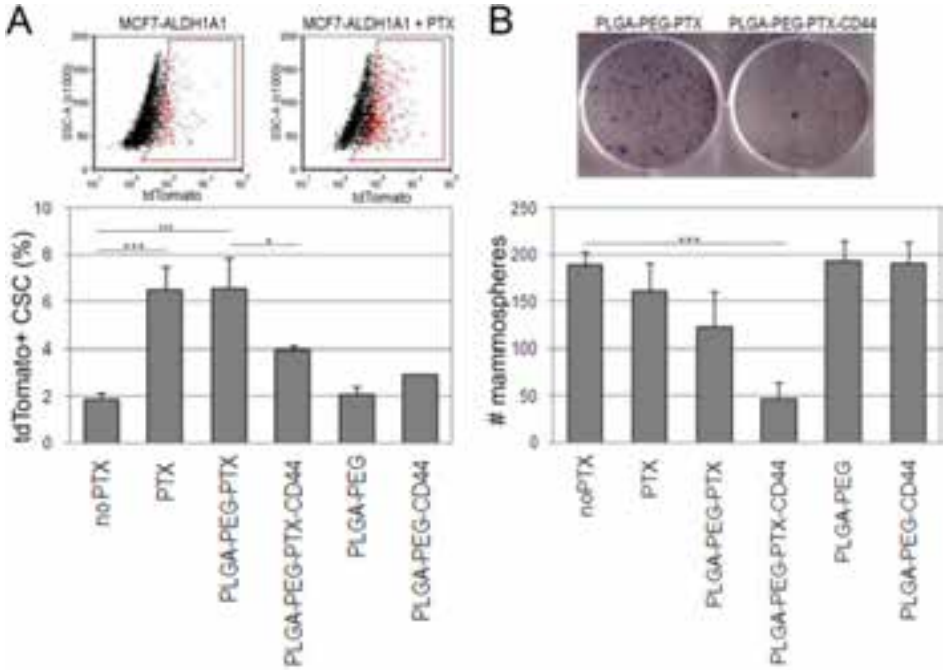
Amount of CSC markers in HCT116 CSC cell line is stable



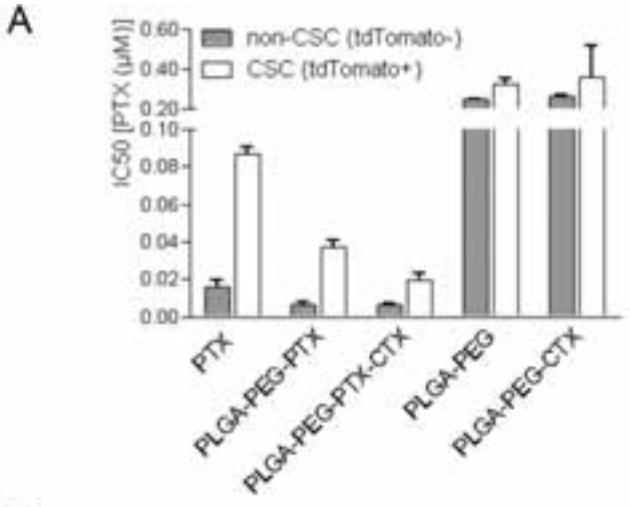




In vitro therapeutic activity of PTX-DDS in breast MCF7 tdTomato+ CSC

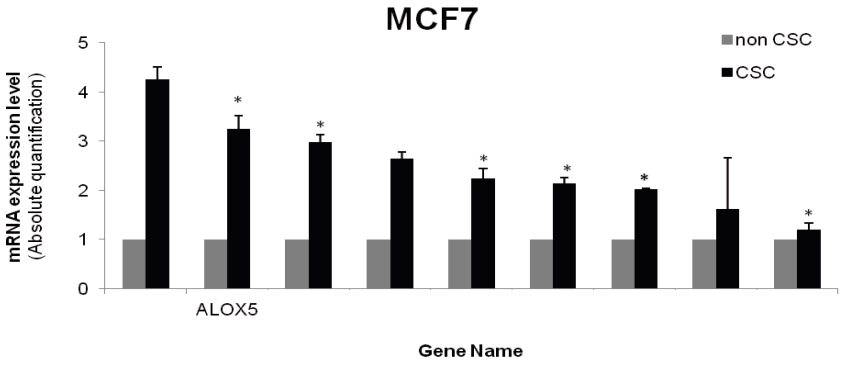


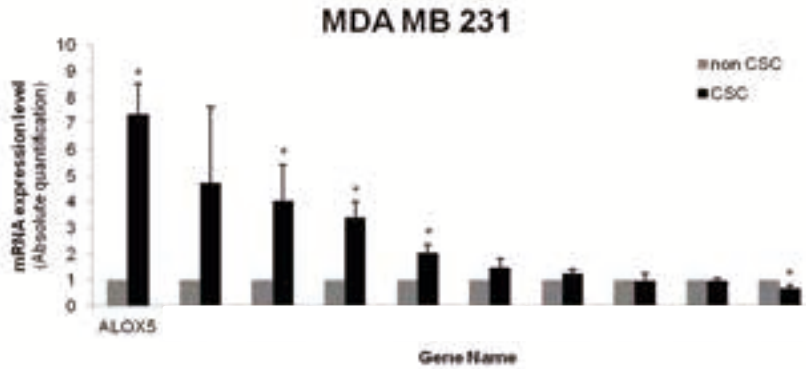
In vitro therapeutic activity of PTX-DDS in HCT116 colorectal tdTomato+ CSC

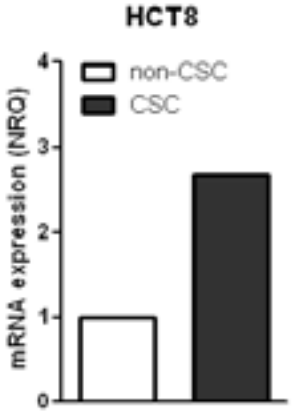


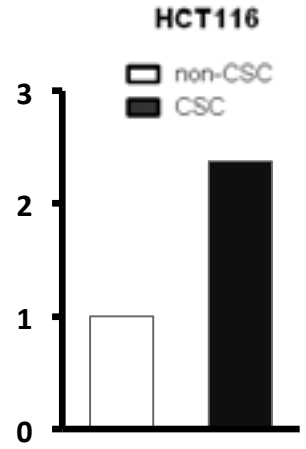
ALOX5

Gene Name	log2ratio MCF7tdT+ vs MCF7tdT- / log2ratio MDAtdT+ vs MDAtdT- / log2ratio tdT+ vs tdT-	FC MCF7tdT+ vs MCF7tdT-	FC MDAtdT+ vs MDAtdT-	FC tdT+ vs tdT-	P-Value tdT+ vs tdT-	Validated primers	Confirmed expression in MCF7	Confirmed expression in MDA MB 231
PGC		3.76	3.62	3.69	1.21E-02	—	—	—
ALOX5		5.15	2.50	3.59	4.67E-03	Yes	Yes	Yes
EGR4		3.33	2.80	3.05	3.83E-02	Yes	Yes	No*
BST2		2.60	2.62	2.61	2.55E-04	Yes	No	Yes
TGFB2		3.38	1.49	2.25	1.99E-02	Yes	No*	No
HLA-DRB5		2.34	2.15	2.24	1.32E-02	—	—	—
PTPRE		2.08	2.02	2.05	8.67E-04	Yes	No*	No
SNAI2		1.63	2.57	2.05	1.77E-02	Yes	Yes	No
IL10RA		1.23	3.21	1.99	1.01E-01	—	—	—
GRM5		1.52	1.96	1.73	3.63E-02	—	—	—
EPHA4		1.83	1.06	1.40	4.48E-01	—	—	—
LIPA		1.27	1.22	1.24	6.13E-02	Yes	Yes	No
TLR2		1.55	-1.09	1.19	4.51E-01	—	—	—
CMKLR1		-1.02	1.28	1.12	3.63E-01	Yes	No	Yes
GATA4		1.04	1.17	1.11	2.87E-01	—	—	—
SNAI1		-1.02	-1.11	-1.06	5.91E-01	Yes	Yes	Yes
SPARC		-1.03	-1.28	-1.15	3.91E-01	Yes	Yes	No

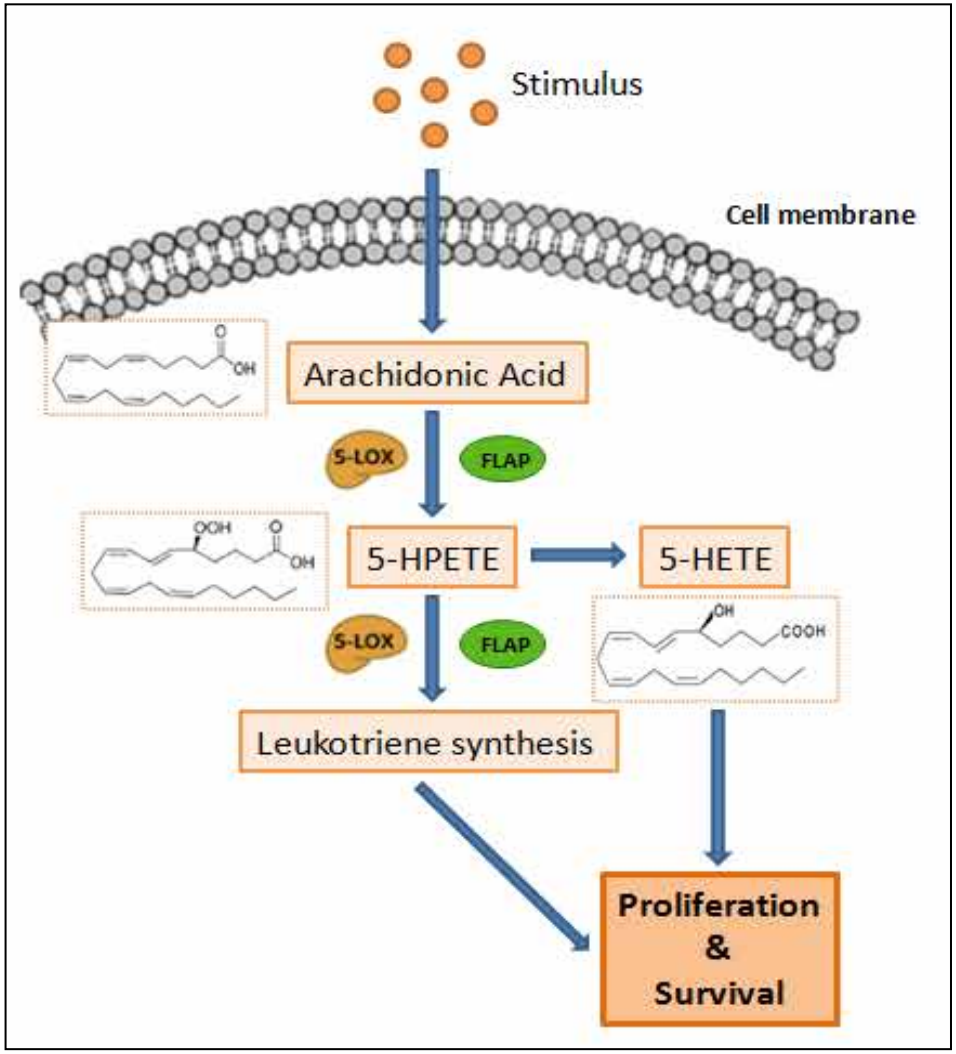








ALOX5: Inflammation, Cancer and Oxidative Lipoxygenase Activity are Intimately Linked



ALOX5 in the presence of FLAP (ALOX5 Activating Protein) catalyzes the first two steps in Leukotriene (LTB4, LTC4, LTD4, LTE4) production. The oxidized forms of 5-HETE and LTs, increase cell proliferation, leading to the inflammatory process, which are intimately related with

In Cancer:

% Patients with FC>2				
	Breast Cancer	*Ovarian Cancer*	*Lung Cancer*	*Colorectal Cancer*
ALOX5	83.55932203	80.64516129	75	50.28901734

ALOX5

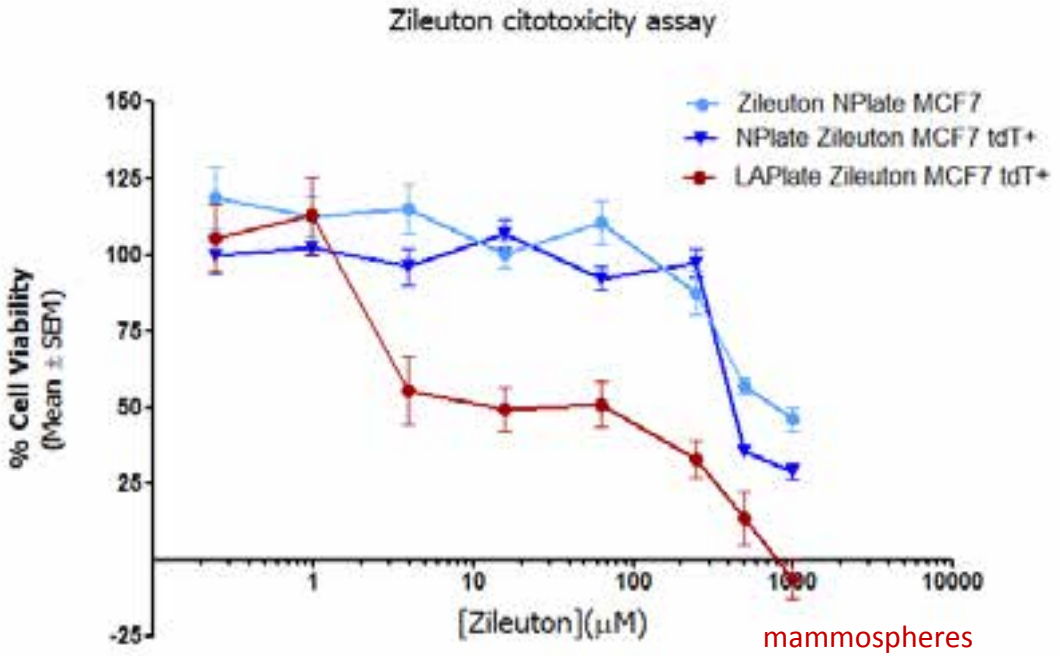
Controls CSCs survival and self-renewal in leukemia cells

ALOX5 inhibition

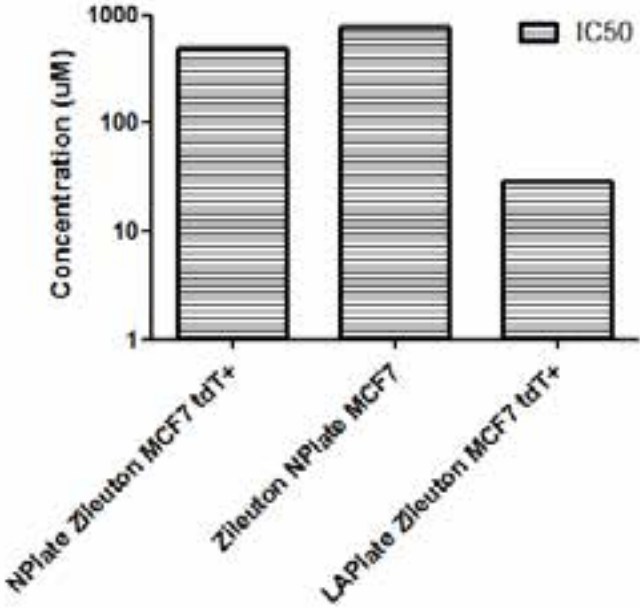
- **Do not significantly affect the functions of normal hematopoietic stem cells**

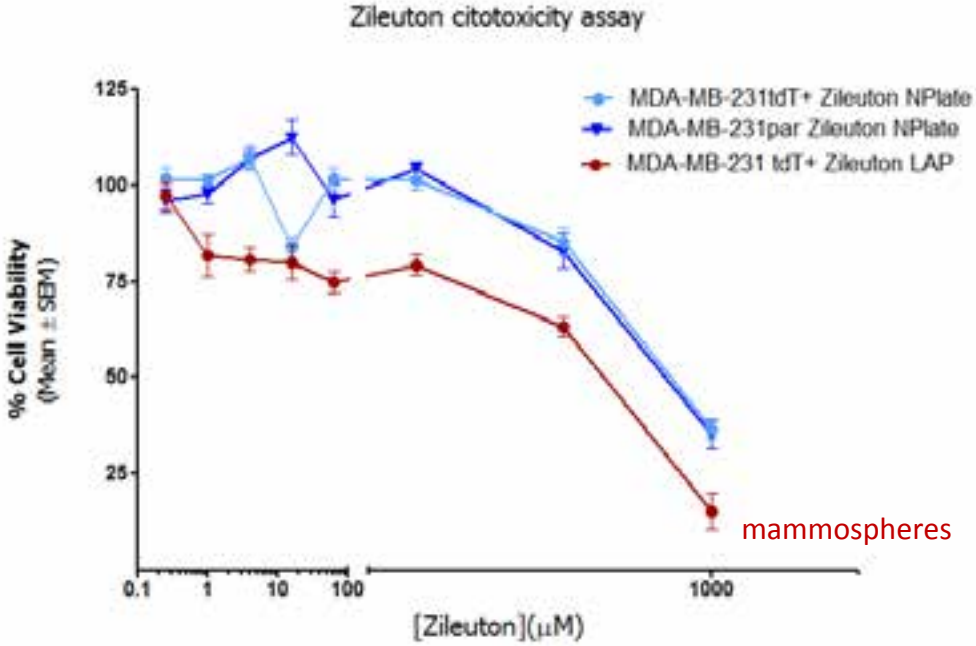
Zileuton

siRNA Alox5

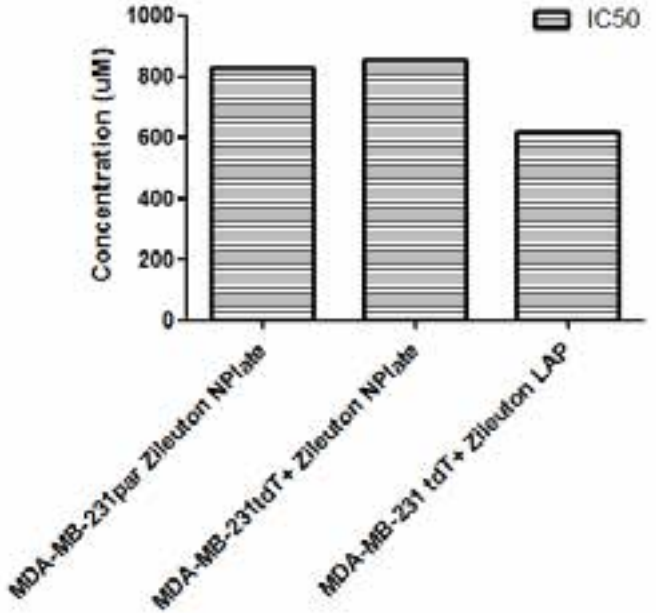


mammospheres



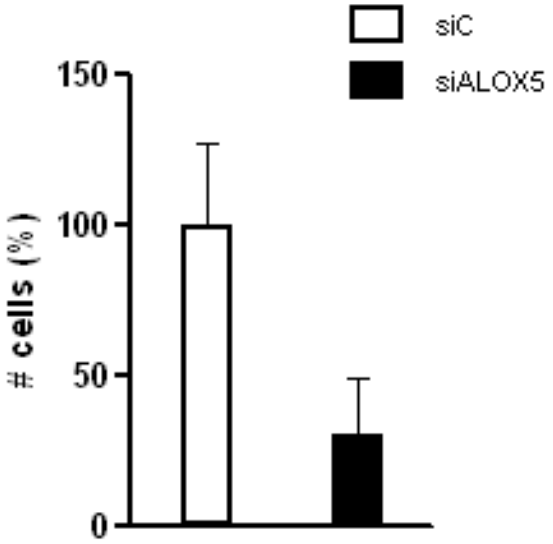


mammospheres

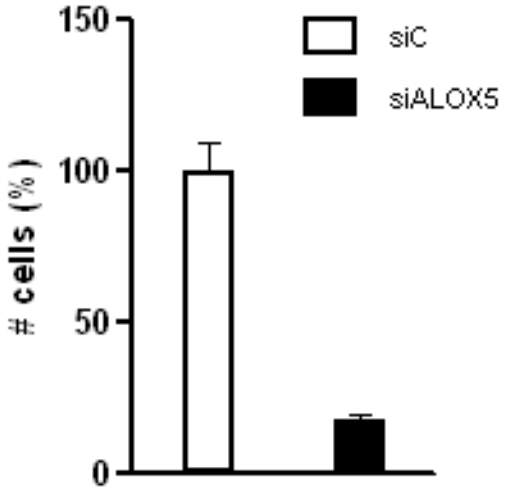


Effect of anti ALOX5-siRNA on CSC

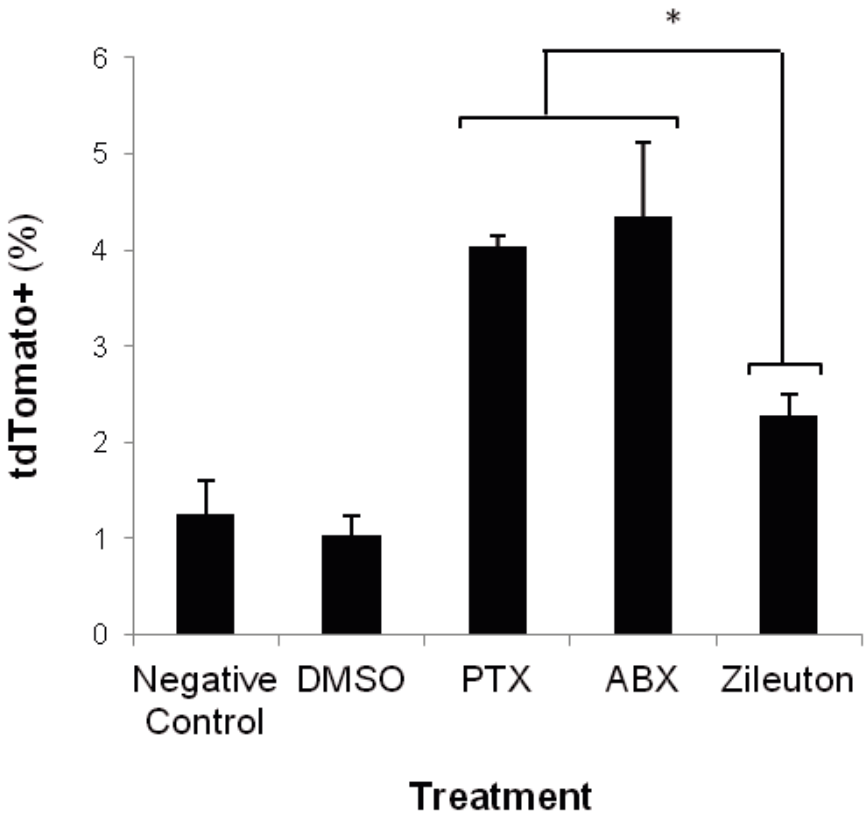
MDA-MB-231

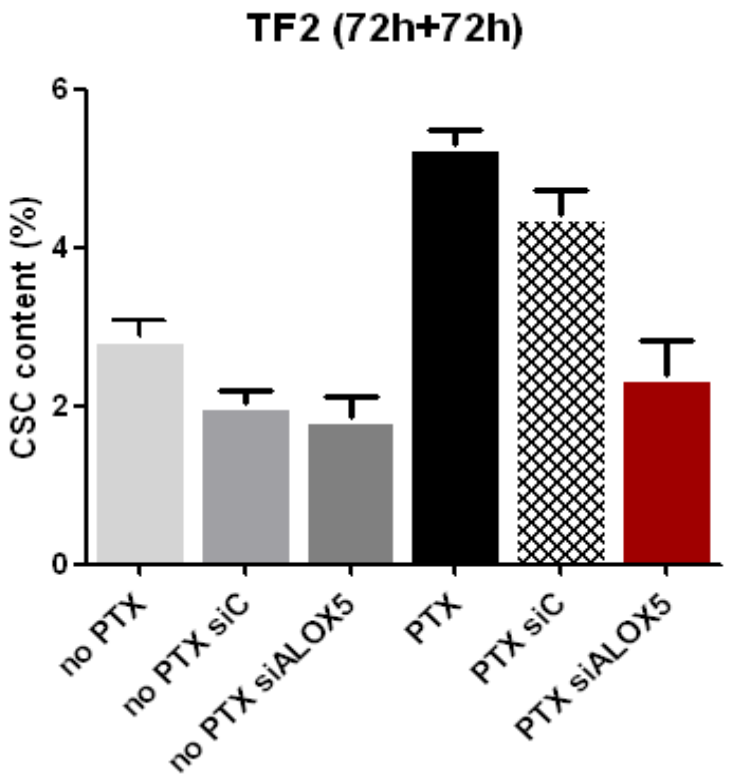


MCF7



CSC efficacy – inhibition of Alox5



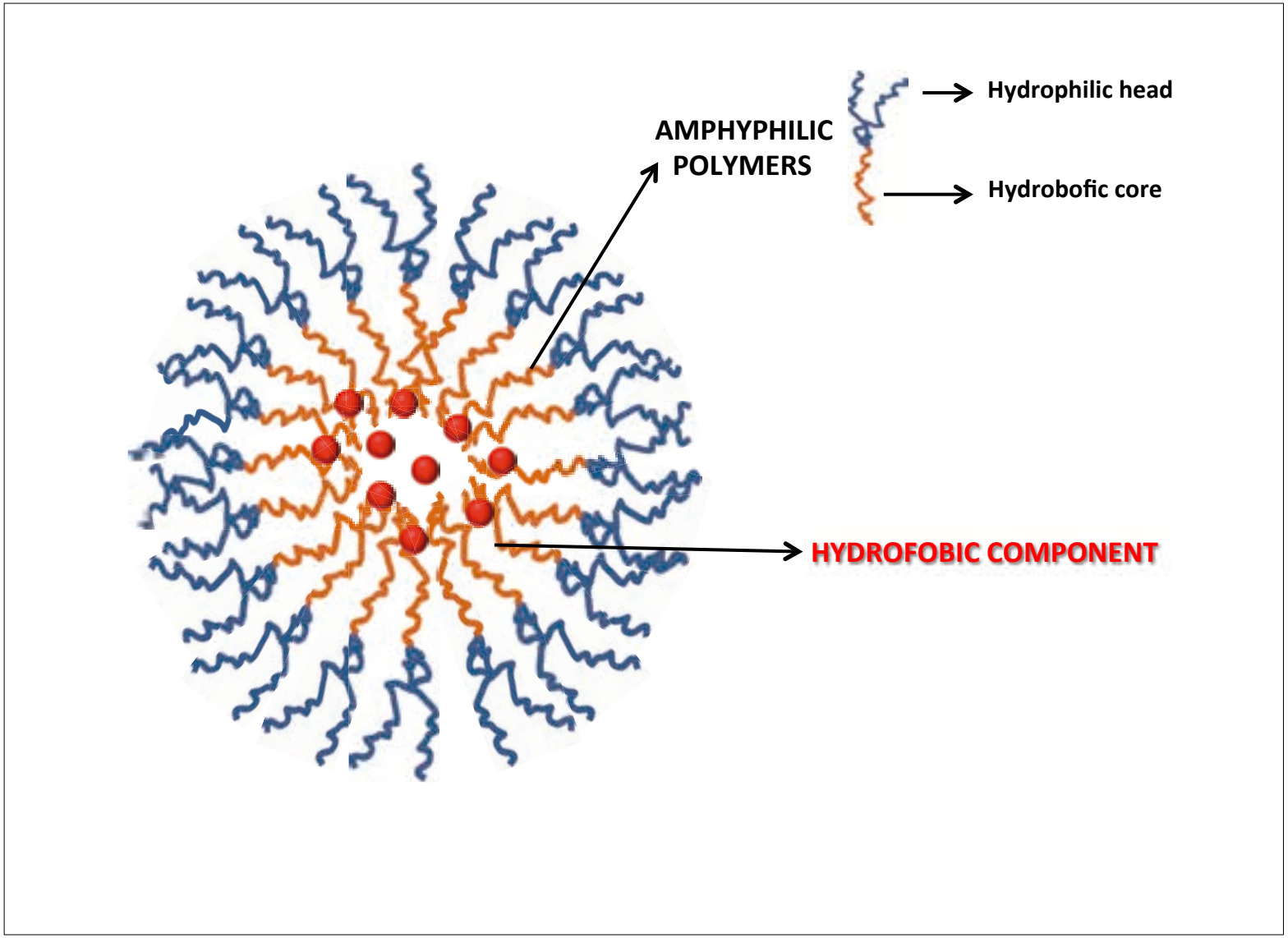


tdTomato-positive representative percentage of the overall live population, corresponding to Cancer Stem Cells (CSC).

Zileuton concentration corresponding to IC75 (500 µM). ABX and PTX concentration corresponding to IC75 (0,1 µM).

*DMSO: Dymethil sulfoxide, PTX: Paclitaxel, ABX: Abraxane. Significant results with P-value > 0,05 are marked with *.*

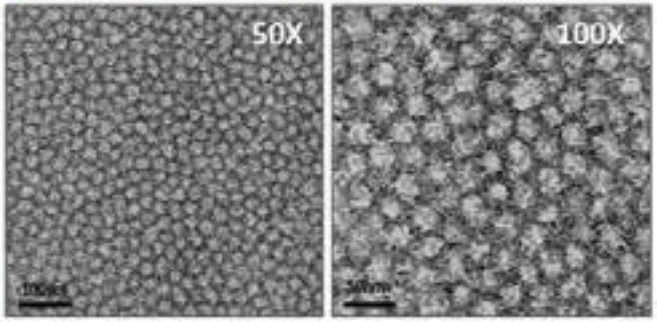
Polymeric Micelles



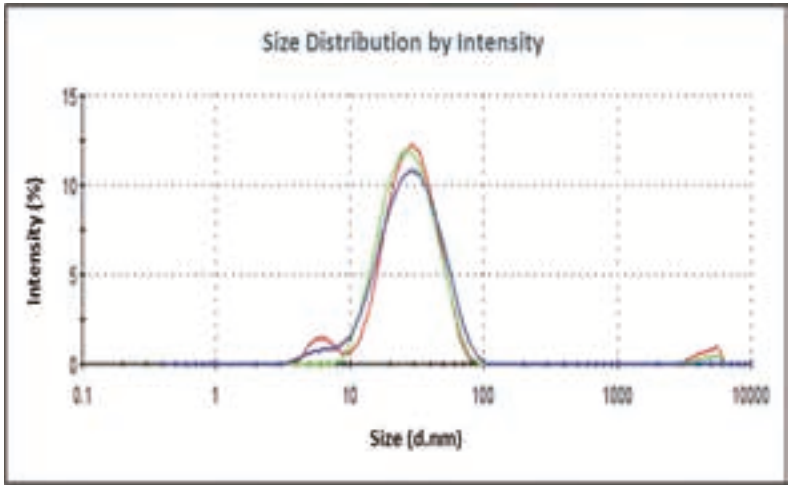
Polymeric Micelles for Drug Delivery (Zileuton)

CHARACTERIZATION

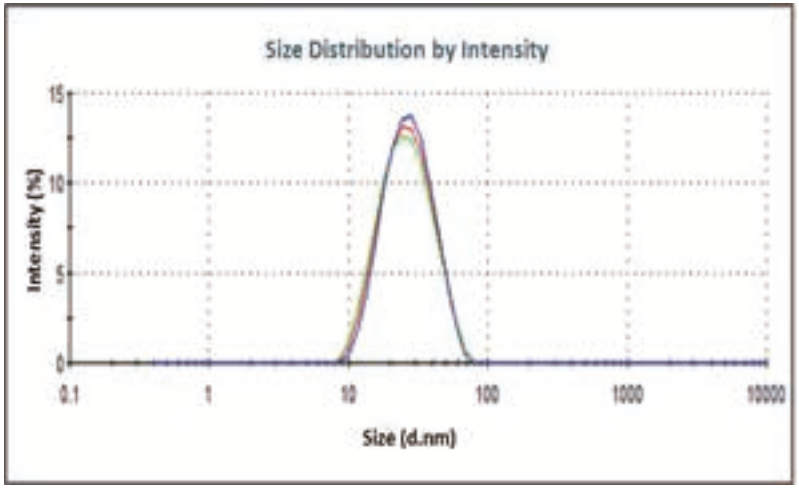
Size and Polidispersity (TEM):



PM-Zileuton Day 0



PM-Zileuton Day 30



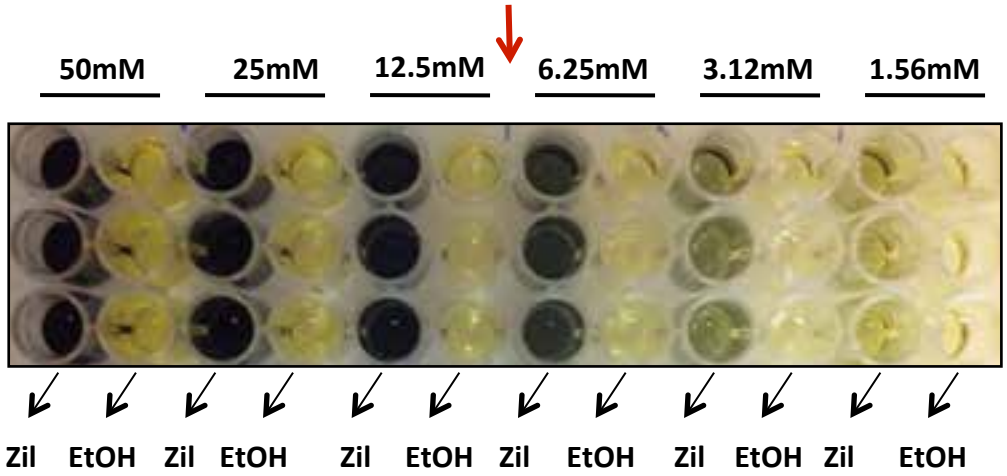
Sample	Size (nm) ± SD	Pdi (nm) ± SD
PM-Empty Day 0	23,65 ± 0,88	0,207 ± 0,012
PM-Zileuton Day 0	23,86 ± 0,89	0,226 ± 0,016

Sample	Size (nm) ± SD	Pdi (nm) ± SD
PM-Empty Day 30	23,59 ± 0,84	0,204 ± 0,029
PM-Zileuton Day 30	22,93 ± 0,89	0,176 ± 0,004

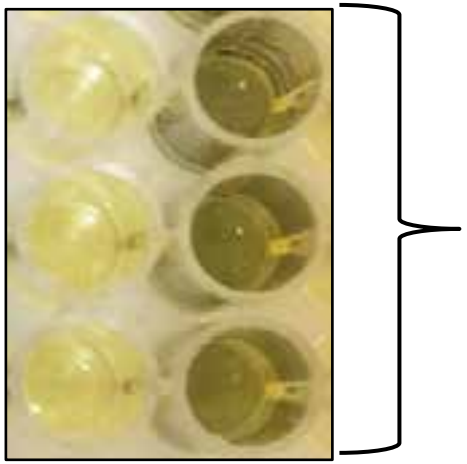
Entrapment Efficiency: 97%

Visible spectrophotometric methods for determination of zileuton in bulk drug and pharmaceutical formulation : Conjugation with (2% w/v) ferric chloride

Polymeric Micelles Zileuton Concentration = 10mM



CHARACTERIZATION



Intact PM-Zileuton

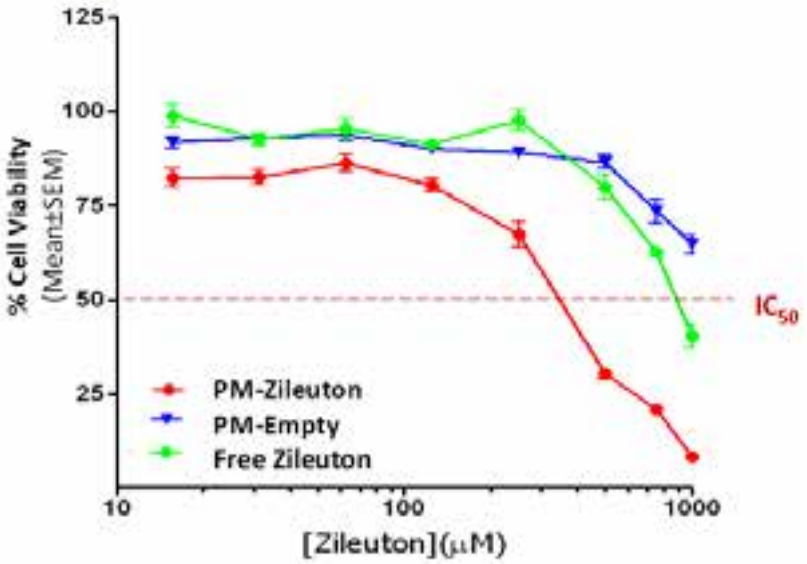
"Open"PM-Zileuton (with cycles of sonication)

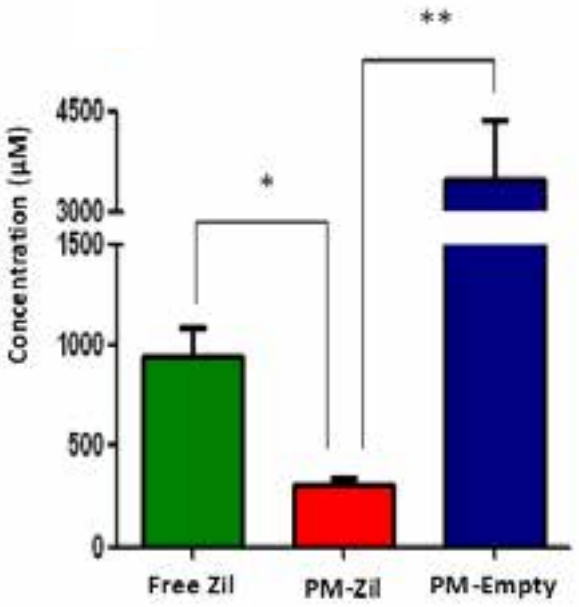
Polymeric Micelles for Drug Delivery (Zileuton)

Cell Toxicity: Presto Blue®

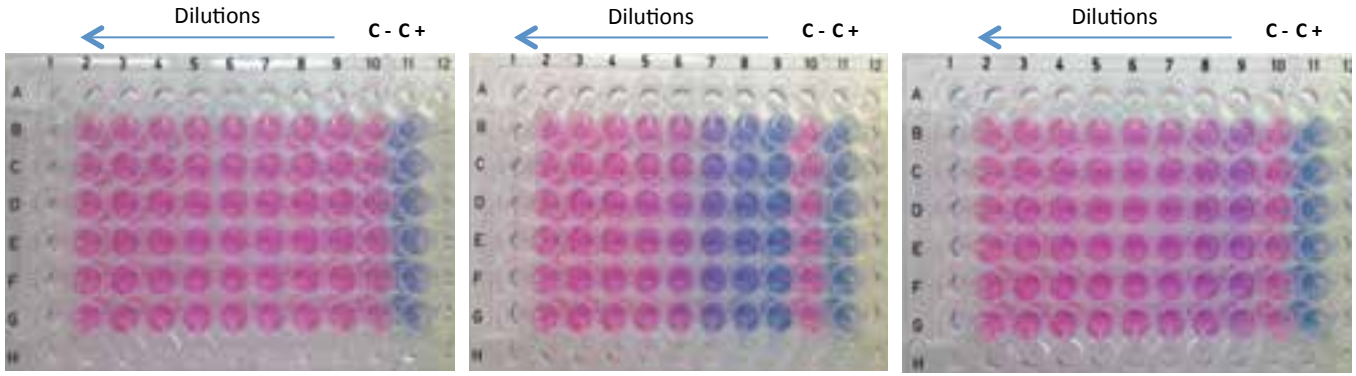
MDA-MB-231 parental

IC50





EFICACY

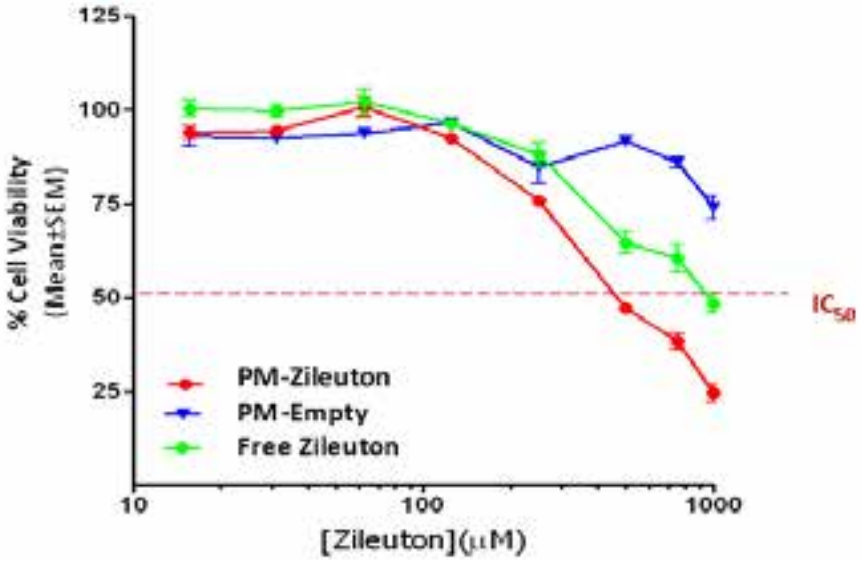


PM-Empty **PM-Zileuton** **Free-Zileuton**

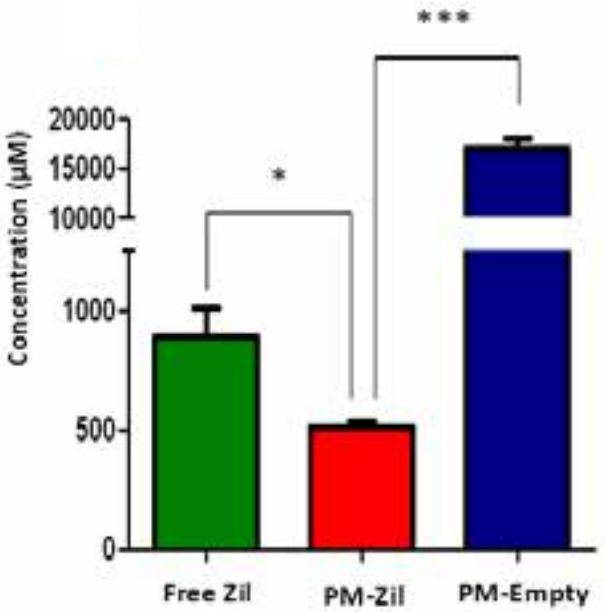
Polymeric Micelles for Drug Delivery (Zileuton)

Cell Toxicity: Presto Blue®

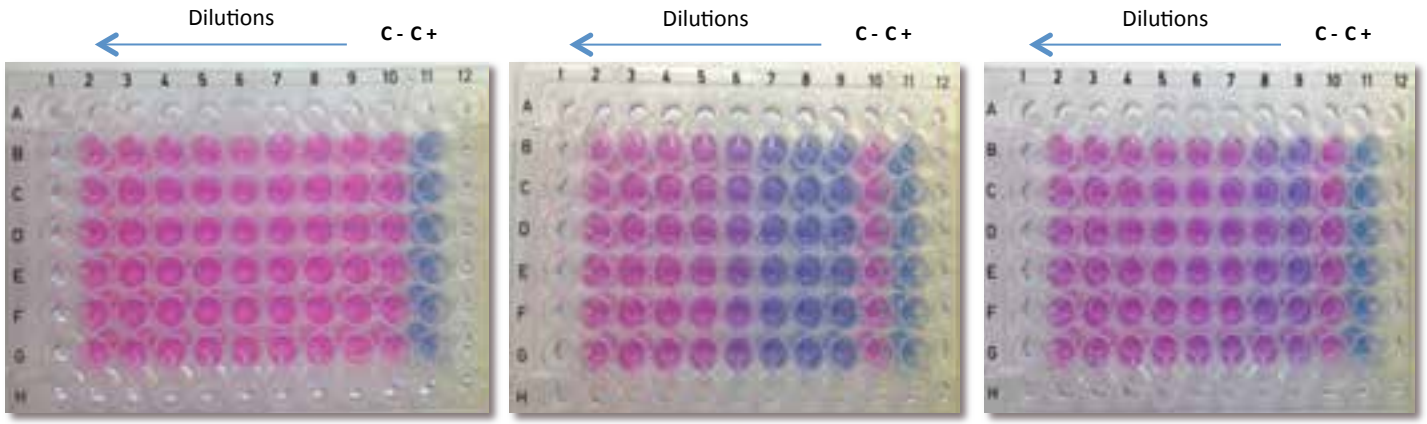
MCF7 parental



IC50



EFFICACY

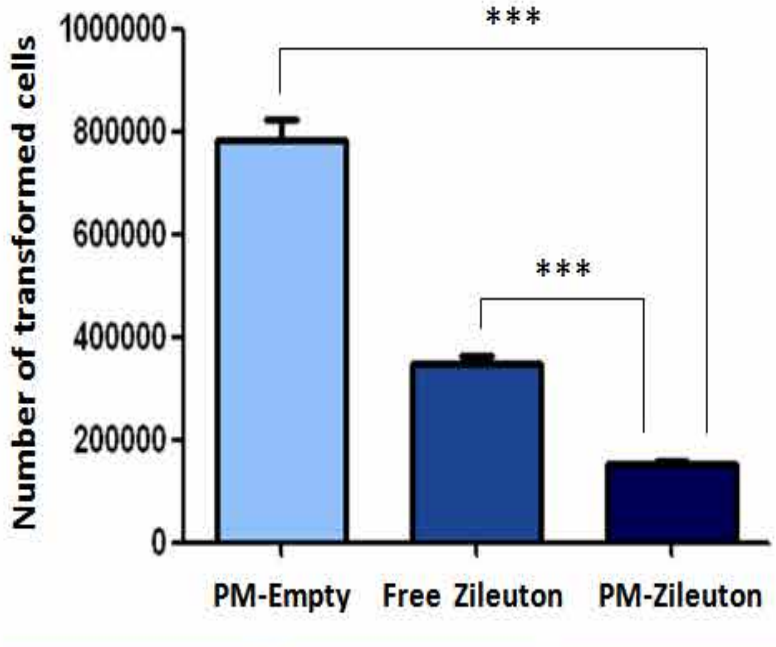


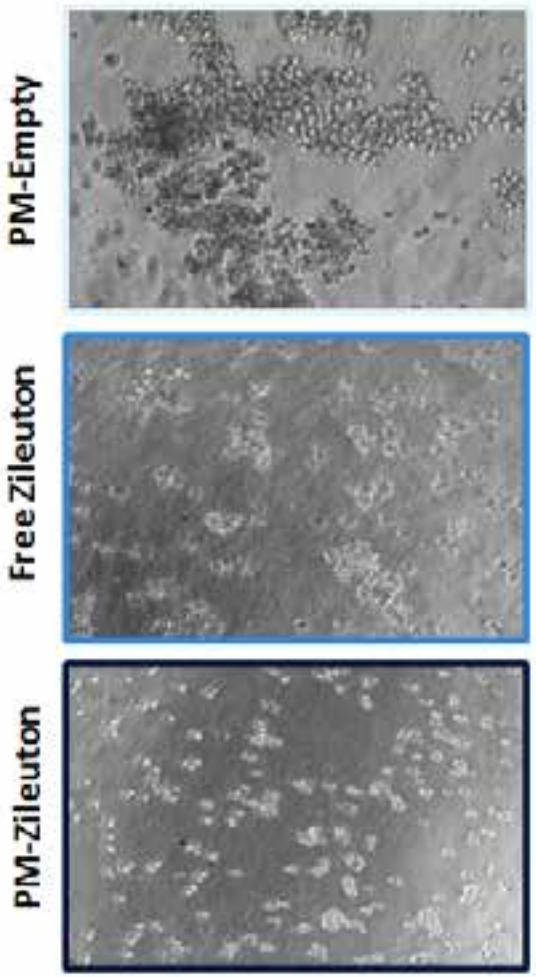
PM-Empty | PM-Zileuton | Free-Zileuton

Polymeric Micelles for Drug Delivery (Zileuton)

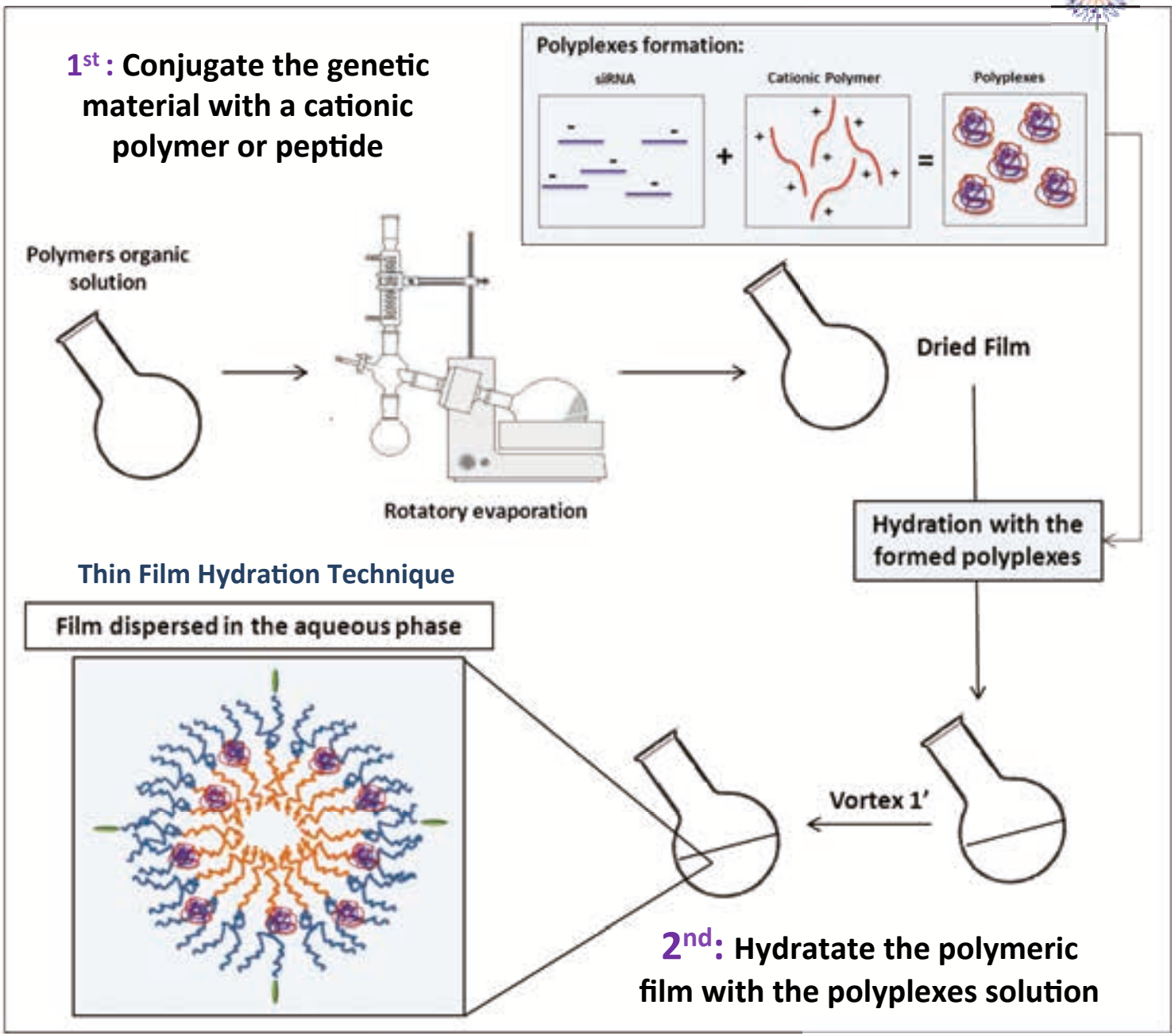
Soft Agar/Cell Transformation/ Cell Independent Growth Assay

EFFICACY



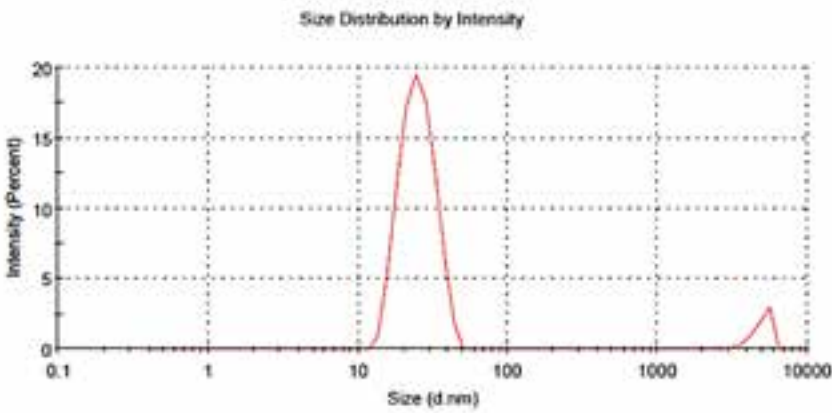


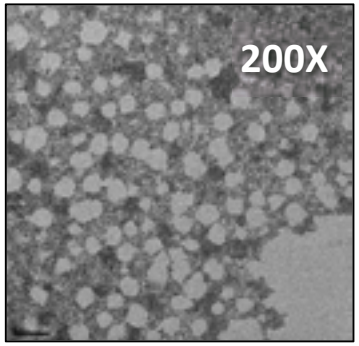
Polymeric Micelles for siRNA Delivery

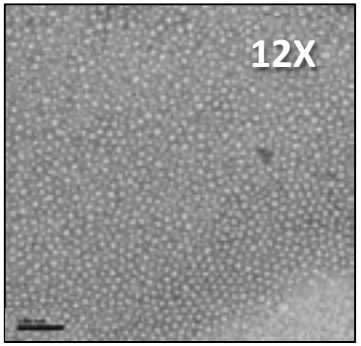


Polymeric Micelles for siRNA Delivery

CHARACTERIZATION



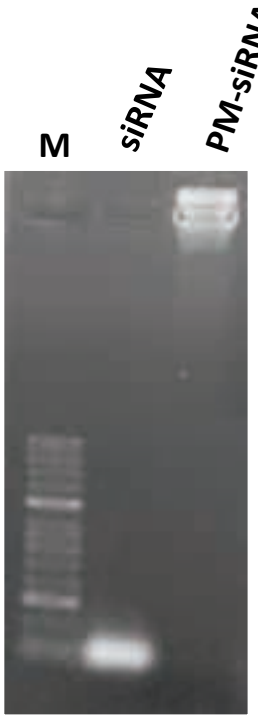




Formulations	Md ± sd (nm)	Pdi ± sd	Z ± sd (mV)
Unloaded	26.93 ± 3.61	0.20 ± 0.08	0.07 ± 0.14
siRNA-loaded	26.24 ± 4.31	0.19 ± 0.09	-0.16 ± 0.20

Formulation	[siRNA] ng/µl
siRNA solution	8.23 ± 0.15
Concentrated Micelles	8.17 ± 0.31
Micelles Supernatant	0.26 ± 0.11

Entrapment Efficiency ≈ 96.7 %



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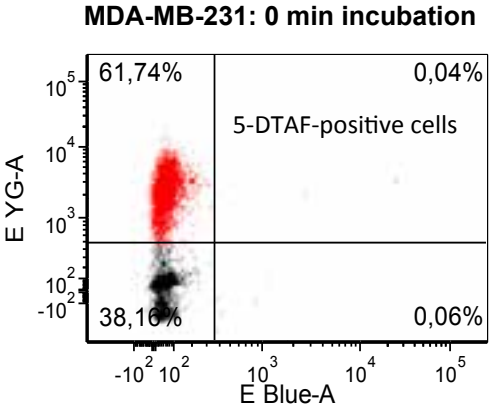

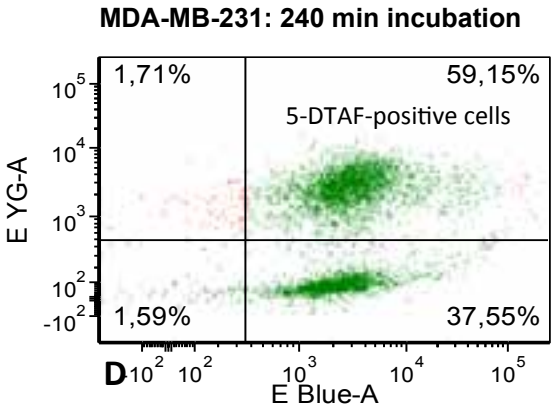

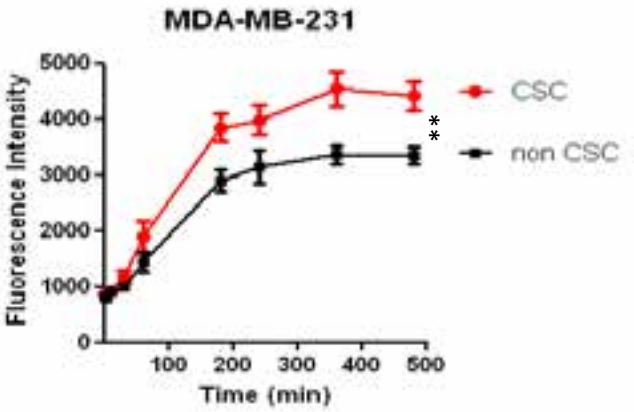

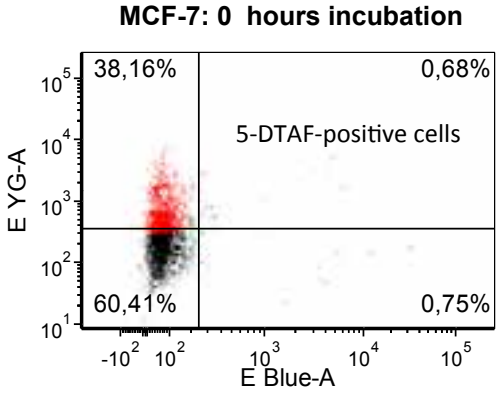

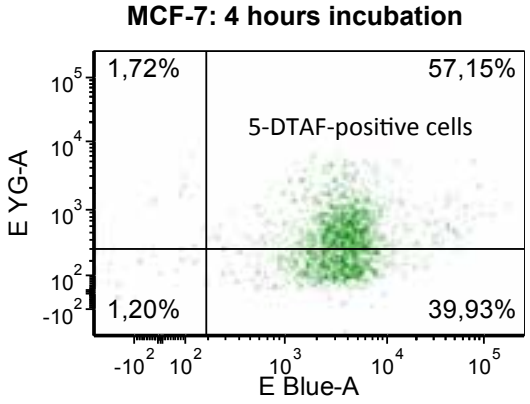

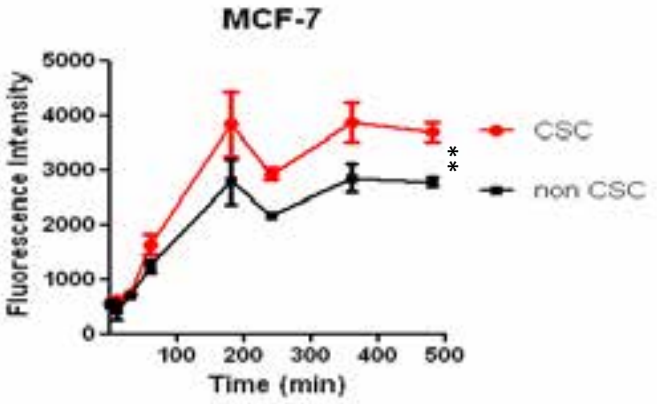

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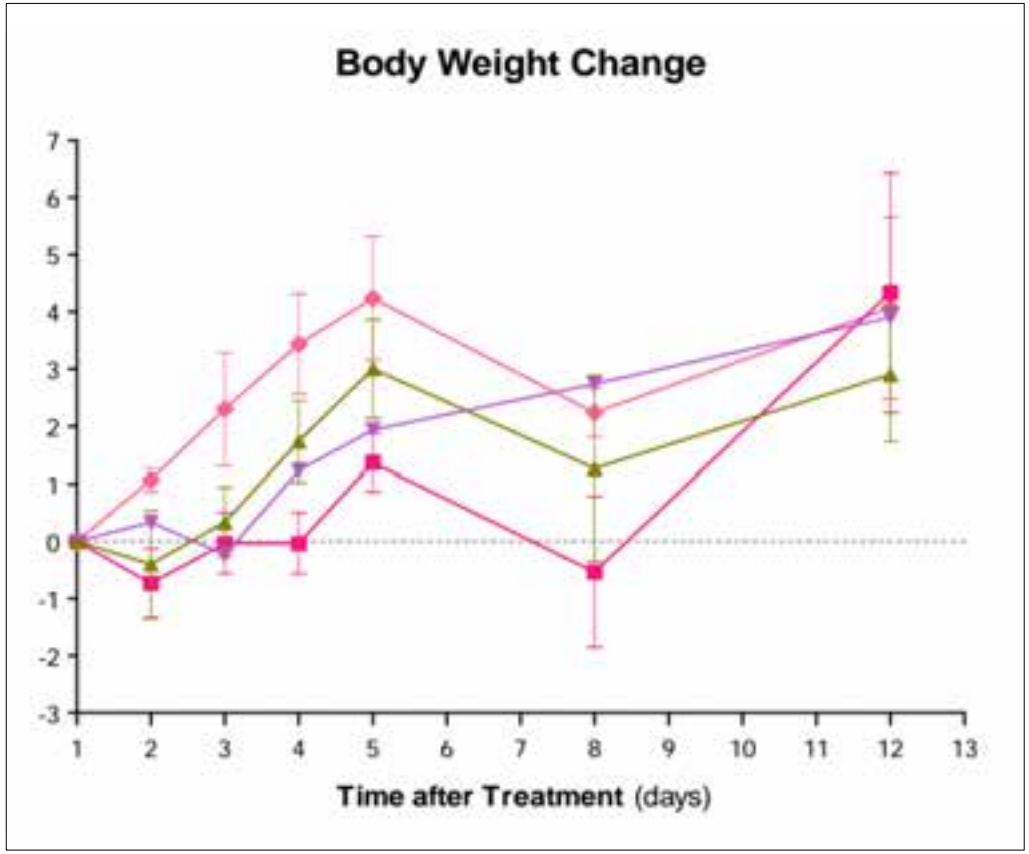
In vivo : MTD-Maximum Tolerated Dose



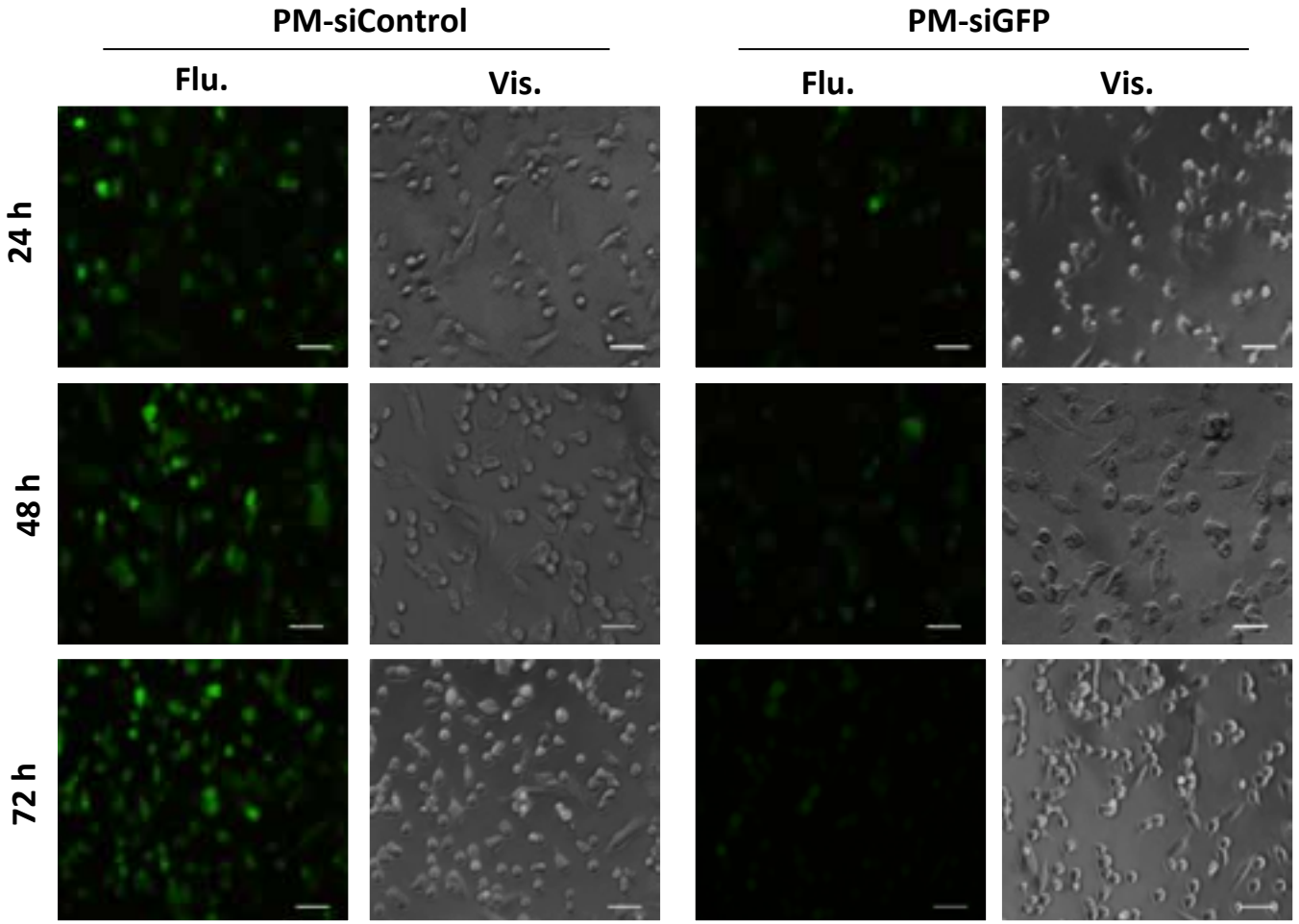
TOXIC

No side effects were detected in any animal (Day 1 -12):

- **Voluntary movement reduction**
- **Irregular breathing**
- **Motor disturbances**
- **Seizures**
- **Sudden death**
- **Flushed skin**
- **Lacrimation**
- **Dehydration**
- **Hunched position**

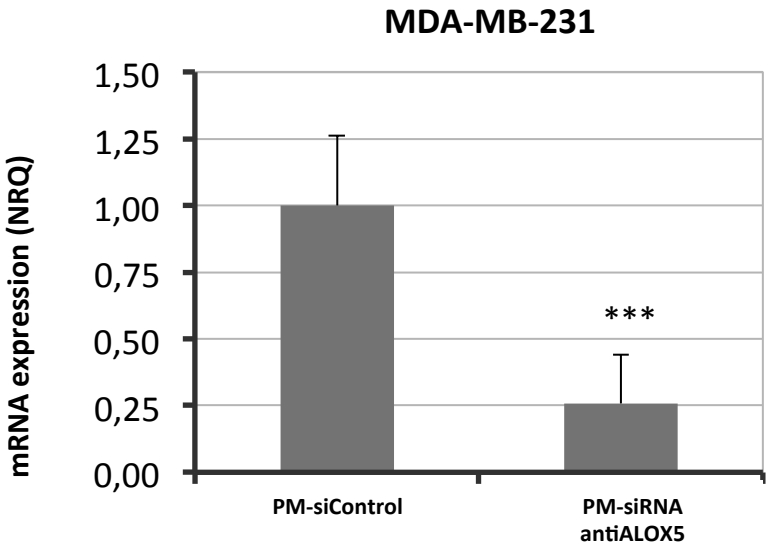
Polymeric Micelles for siRNA Delivery

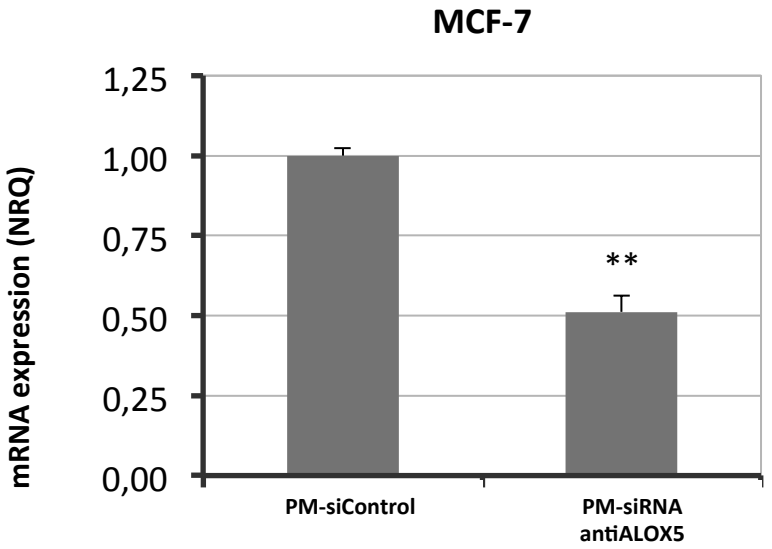
GFP silencing:

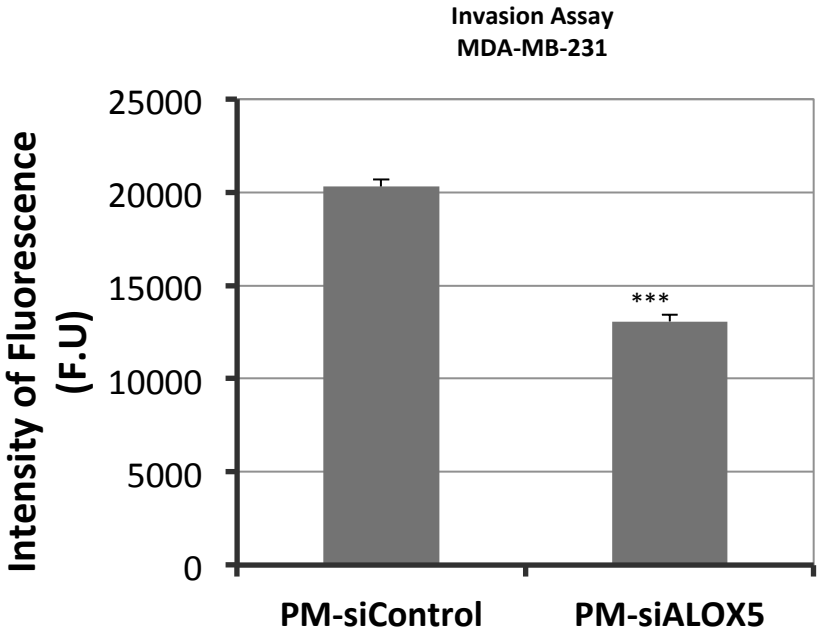


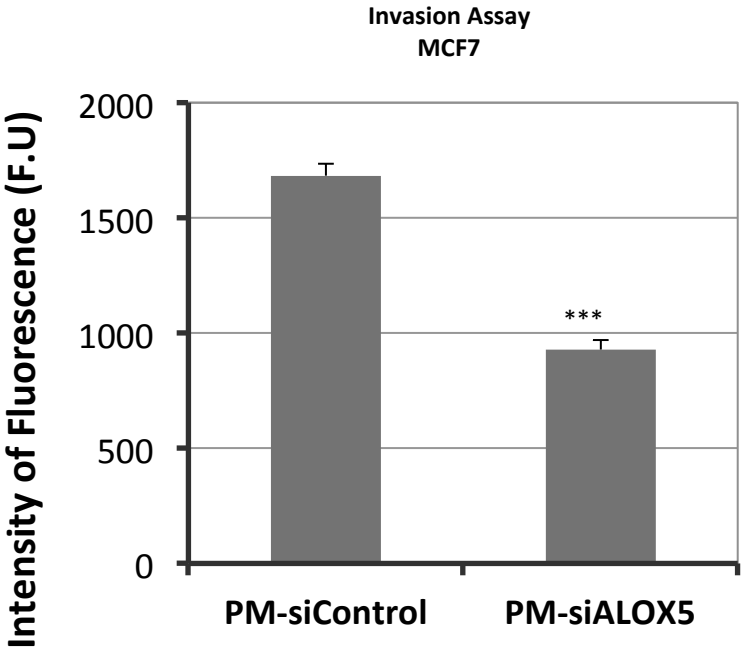
EFFICACY

PM-siALOX5


MDA-MB-231
mRNA expression (NRQ)
1,50
1,25
1,00
0,75
0,50
0,25
0,00
***
PM-siControl
PM-siRNA antiALOX5

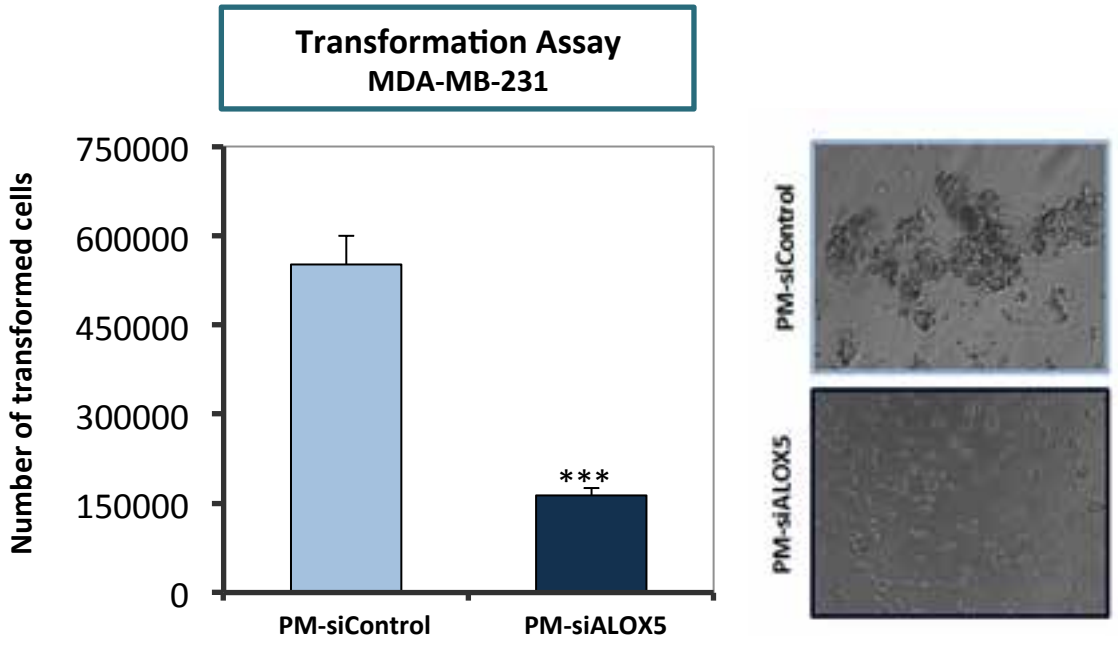

MCF-7
mRNA expression (NRQ)
1,25
1,00
0,75
0,50
0,25
0,00
**
PM-siControl
PM-siRNA antiALOX5

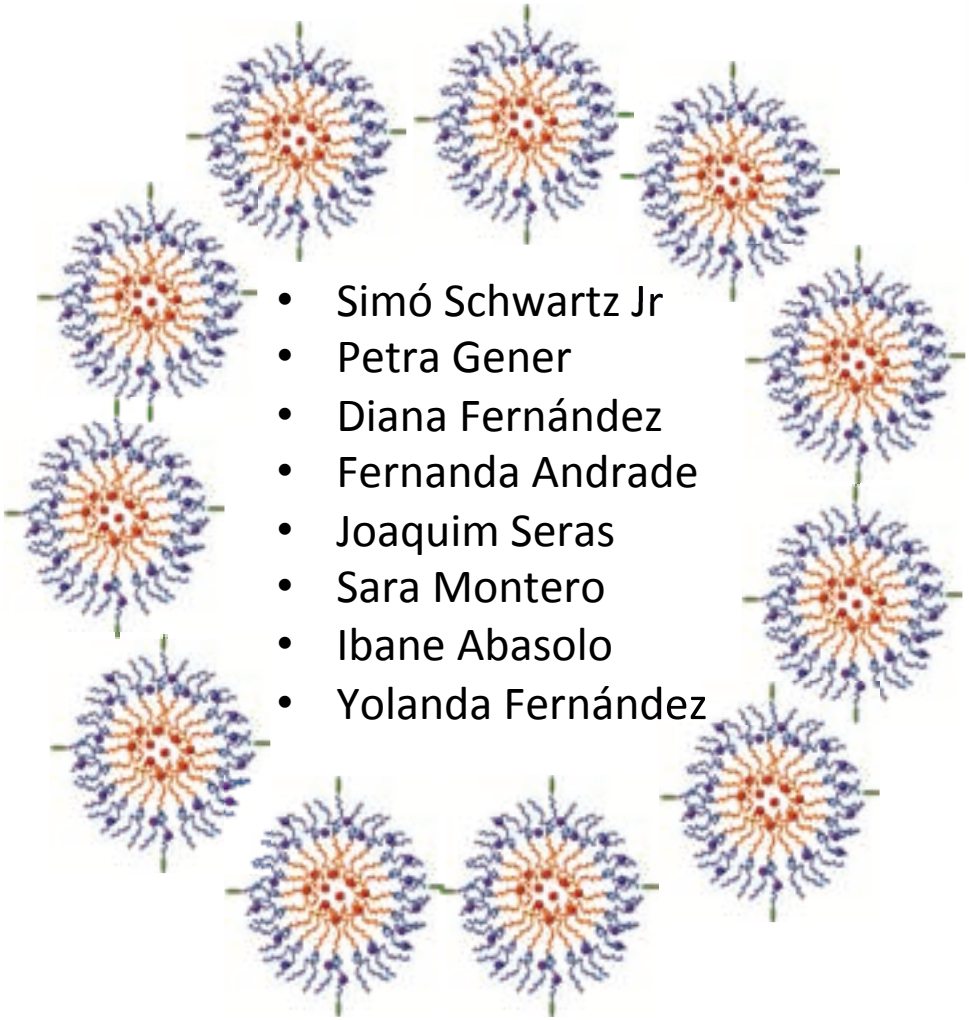

Invasion Assay
MDA-MB-231
Intensity of Fluorescence (F.U)
25000
20000
15000
10000
5000
0
***
PM-siControl
PM-siALOX5


Invasion Assay
MCF7
Intensity of Fluorescence (F.U)
2000
1500
1000
500
0
***
PM-siControl
PM-siALOX5

PM-siALOX5

Soft Agar or Transformation assay or Cell Independent anchorage Growth Assay





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- Ibane Abasolo
- Yolanda Fernández

THANK YOU FOR YOUR ATTENTION!

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