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DEGLI STUDI
DI TORINO



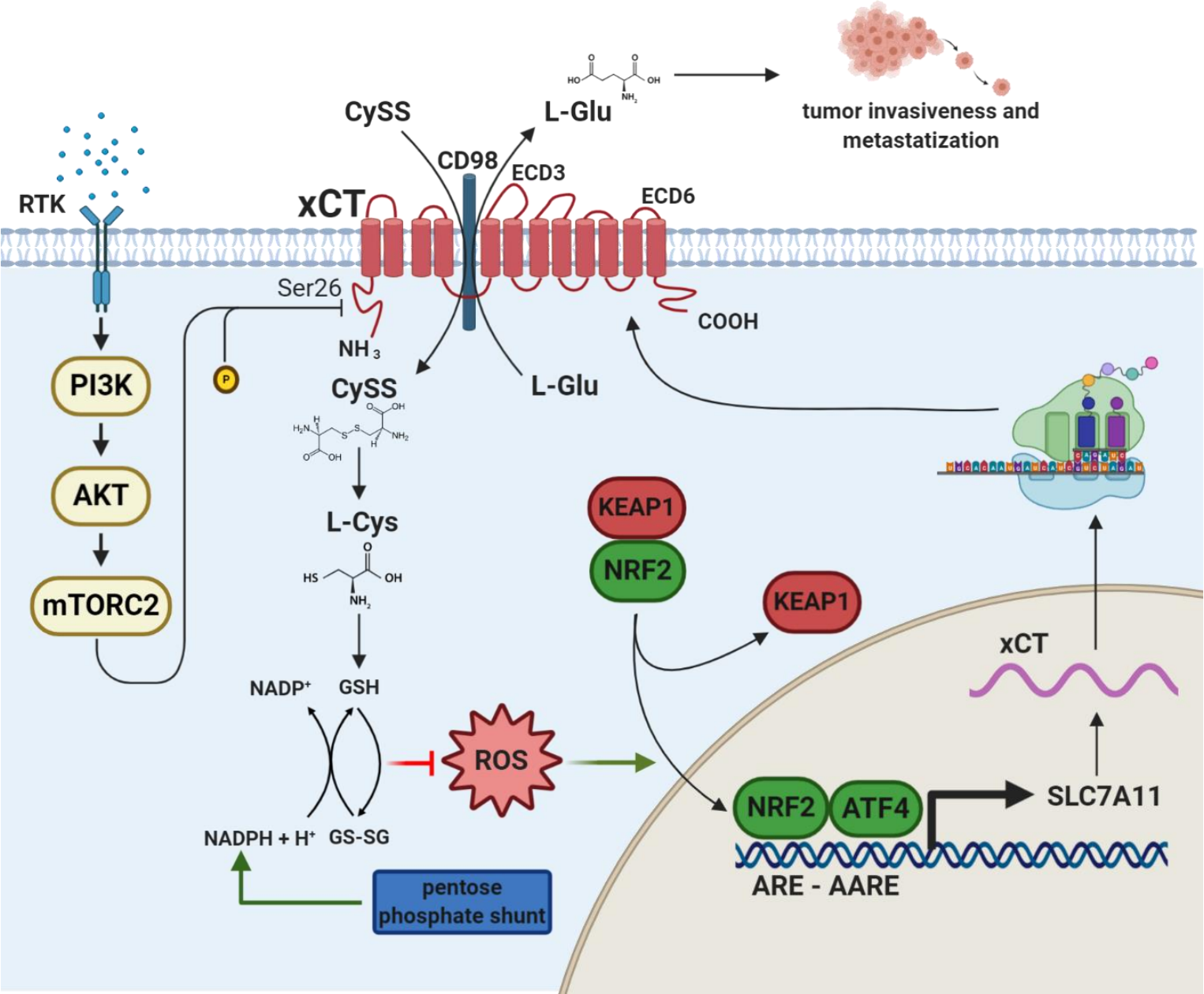
Inibizione del sistema xc- in combinazione con la somministrazione di APR-246, come nuova terapia combinata contro il tumore della mammella

Group Leader: Prof.ssa **Federica Cavallo**, PhD

Alessandro Gasparetto, MD-PhD student
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xc- pathway



xCT as
oncoantigen

Microenvironment and Immunology

Cancer
Research

Immunotargeting of Antigen xCT Attenuates Stem-like Cell Behavior and Metastatic Progression in Breast Cancer

Stefania Lanzardo¹, Laura Conti¹, Ronald Rooke², Roberto Ruii¹, Nathalie Accart³, Elisabetta Bolli¹, Maddalena Arigoni¹, Marco Macagno¹, Giuseppina Barrera⁴, Stefania Pizzimenti⁴, Luigi Aurisicchio⁵, Raffaele Adolfo Calogero¹, and Federica Cavallo¹

ONCOIMMUNOLOGY
2018, VOL. 7, NO. 3, e1408746 (14 pages)
<https://doi.org/10.1080/2162402X.2017.1408746>



ORIGINAL RESEARCH

OPEN ACCESS



A Virus-Like-Particle immunotherapy targeting Epitope-Specific anti-xCT expressed on cancer stem cell inhibits the progression of metastatic cancer *in vivo*

Elisabetta Bolli^a, John P. O'Rourke^b, Laura Conti^a, Stefania Lanzardo^a, Valeria Rolih^a, Jayne M. Christen^b, Giuseppina Barutello^a, Marco Forni^c, Federica Pericle^{b,#}, and Federica Cavallo^{a,#}

Cancer Immunology, Immunotherapy (2019) 68:131–141
<https://doi.org/10.1007/s00262-018-2185-1>

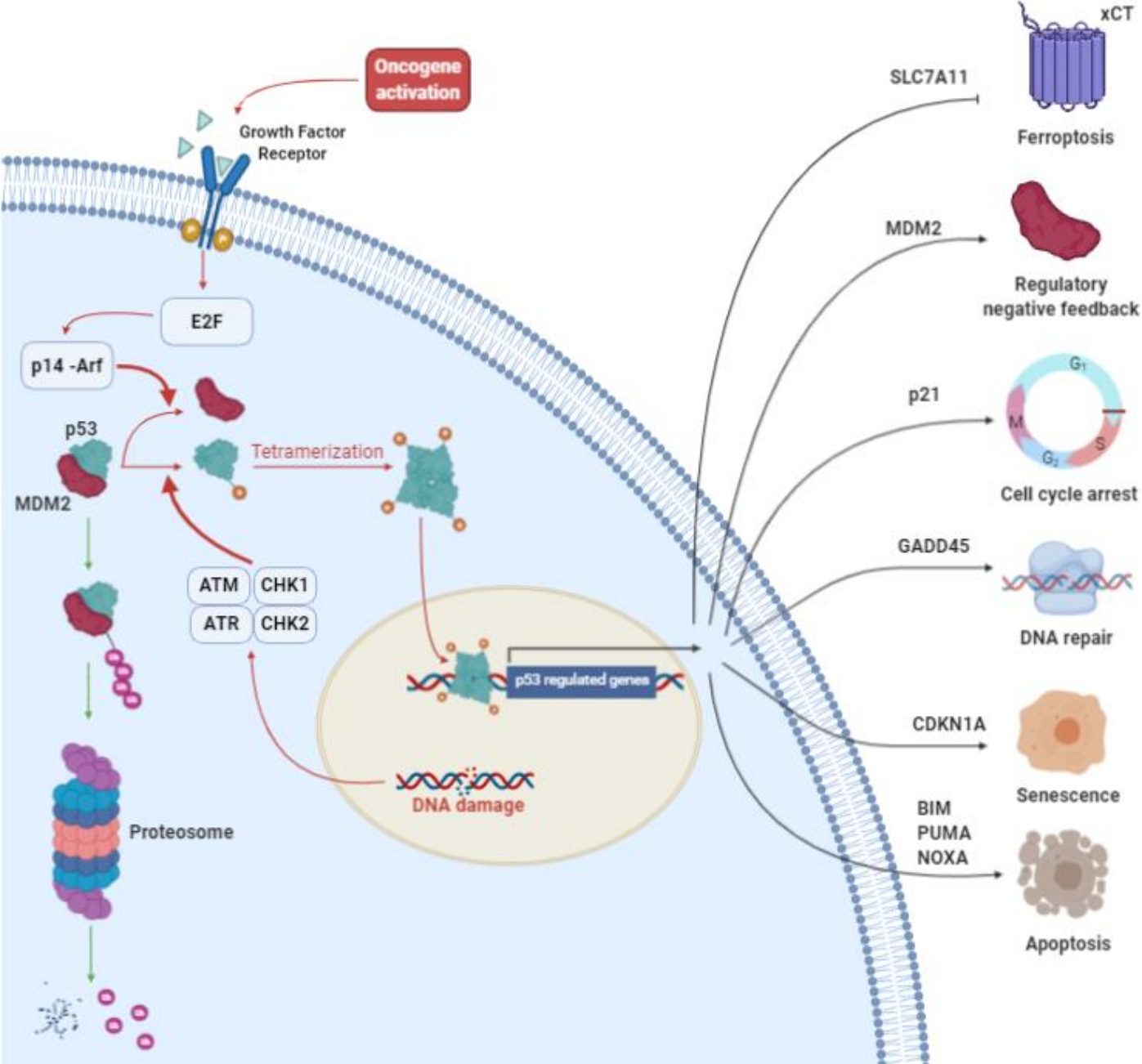
FOCUSSED RESEARCH REVIEW



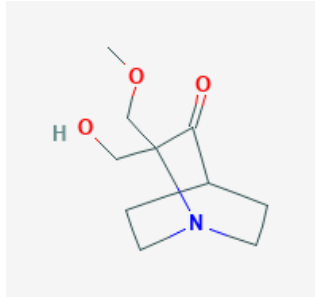
Fighting breast cancer stem cells through the immune-targeting of the xCT cystine–glutamate antiporter

Roberto Ruii¹ · Valeria Rolih¹ · Elisabetta Bolli¹ · Giuseppina Barutello¹ · Federica Riccardo¹ · Elena Quaglino¹ · Irene Fiore Merighi¹ · Federica Pericle² · Gaetano Donofrio³ · Federica Cavallo¹ · Laura Conti¹

p53 pathway



APR-246



Actions

- p53mut - dependent
- p53mut - independent

HEMATOLOGIC					
target indication	treatment line	preclinical	phase one	phase two	phase three
TP53 Mutant MDS	Frontline				
TP53 Mutant MDS/AML	Frontline (U.S.)				
	Frontline (France)				
	Post-transplant Maintenance				
TP53 Mutant AML	Frontline and Relapsed/Refractory				
TP53 Mutant CLL/MCL	Relapsed/Refractory				
SOLID TUMOR					
target indication	treatment line	preclinical	phase one	phase two	phase three
Gastric/Bladder/NSCLC	Relapsed/Refractory				



www.aprea.com

p53 reactivation
and its activity
in breast cancer

Rivlin, Genes
Cancer. 2011 Apr;
2(4), 466–474.
Synnott,
Translational
Oncology (2018) 11,
1343–1349

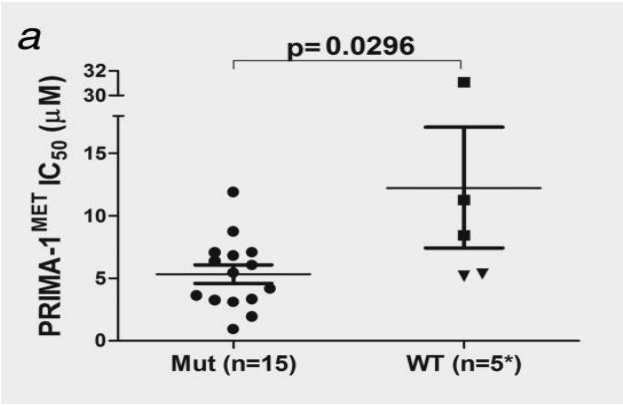
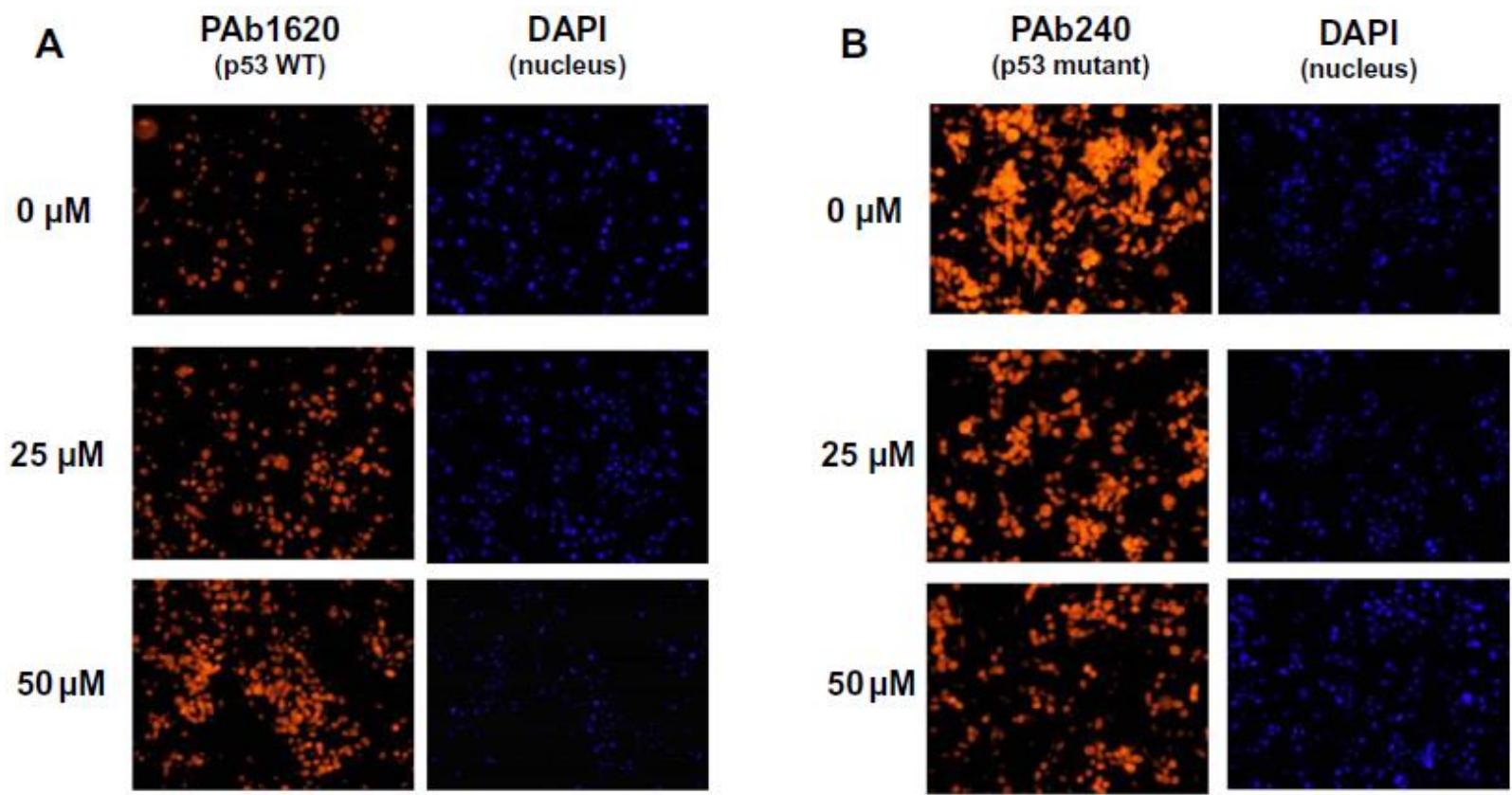
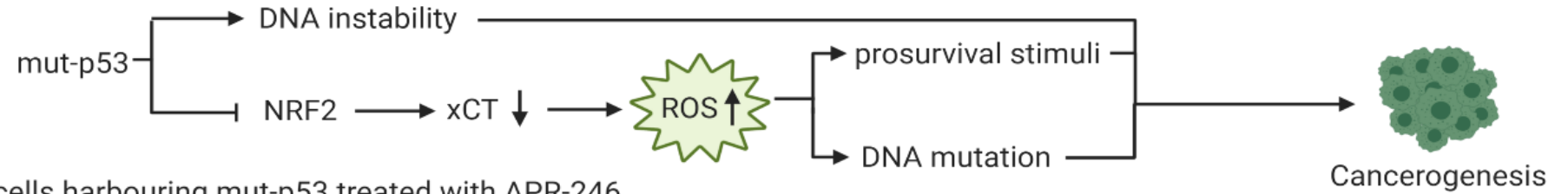


Table 1 | *TP53* mutation frequency and most common mutations in different tumour types

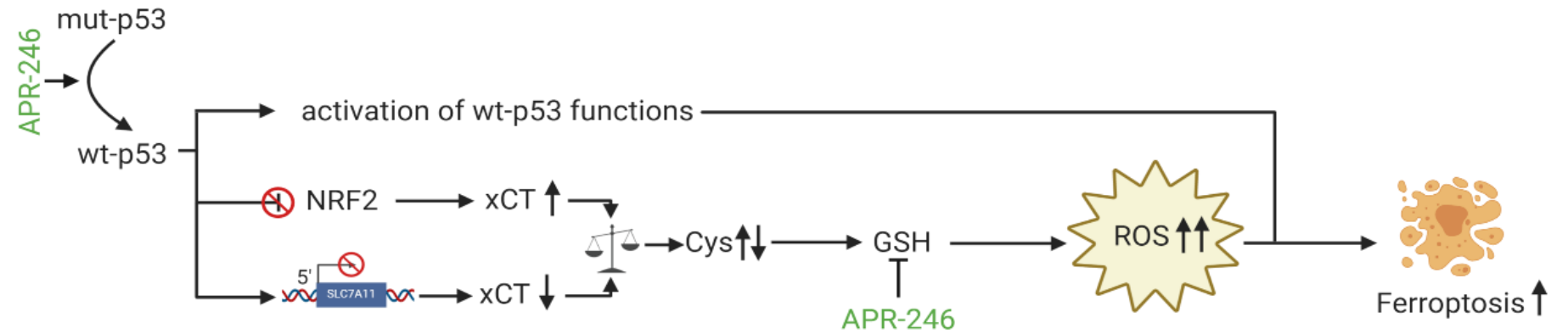
Tumour type	<i>TP53</i> mutation frequency (%)	Most common <i>TP53</i> mutations*
Ovarian serous carcinoma	94.6	R273H>Y220C>R248Q>R175H
Lung squamous cell carcinoma	79.3	R158L>R175G>V157F=R213X=T125T
Head and neck squamous cell carcinoma	69.8	R175H=R273H=R213X=R282W>R248W
Glioblastoma	28.3	R248Q>R175H>R273H=R282W
Pan-cancer [†]	42.0	R175H>R273H>R248Q=R248W>R213X>Y220C

Targeting strategy

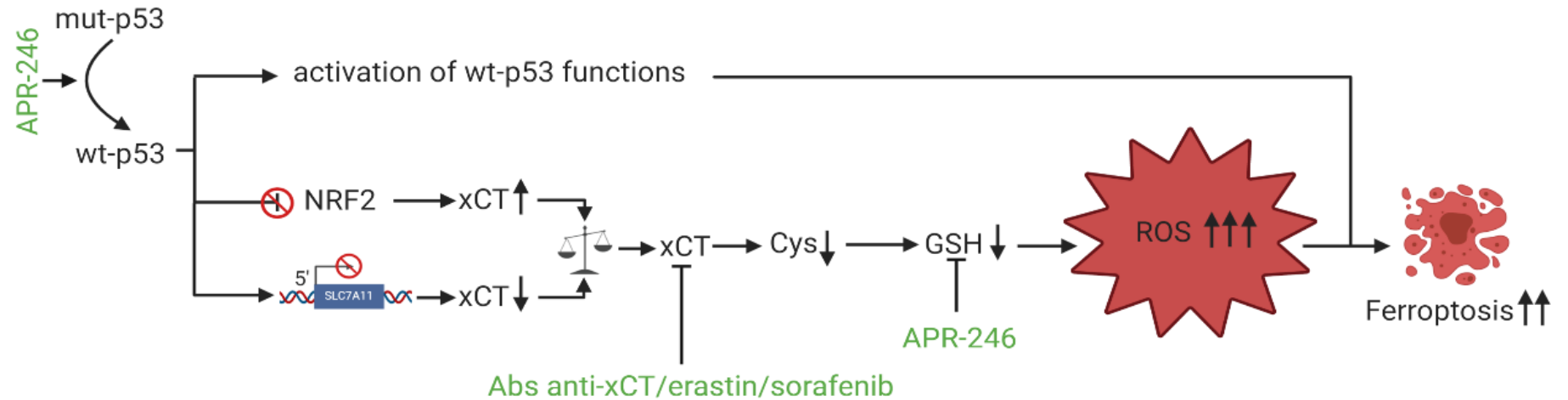
a) cells harbouring mut-p53 without any treatment



b) cells harbouring mut-p53 treated with APR-246

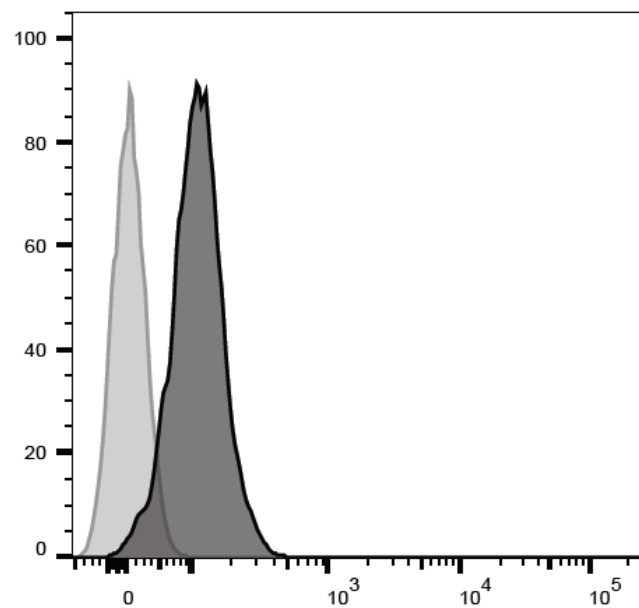


c) cells harbouring mut-p53 treated with xCT inhibitors in combination with APR-246

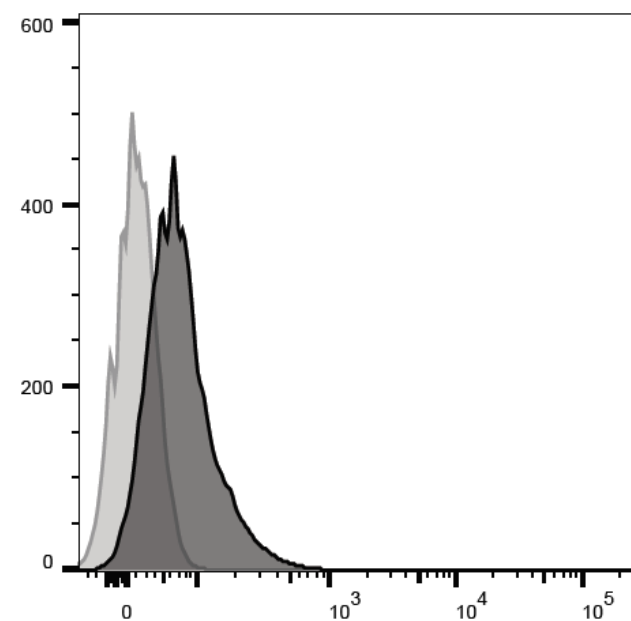


xCT expression
In breast cancer
cell lines

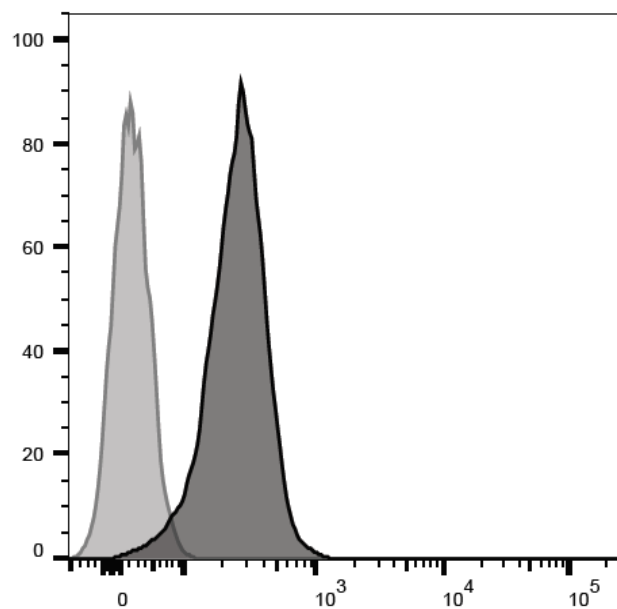
MCF7



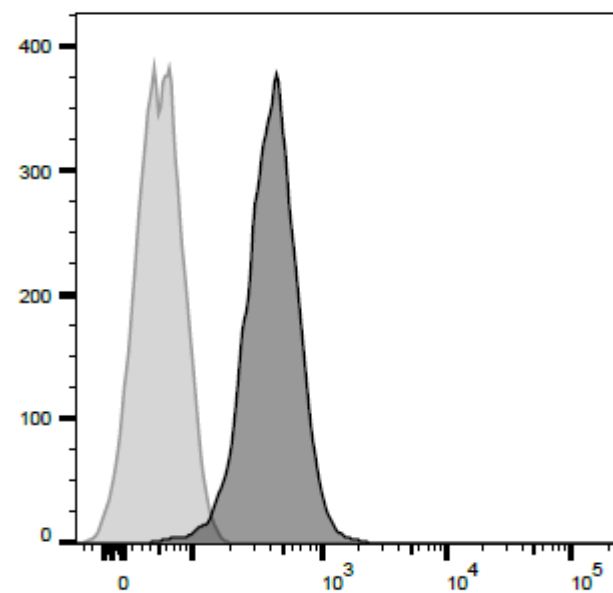
MDA-MB-231



4T1



TSA

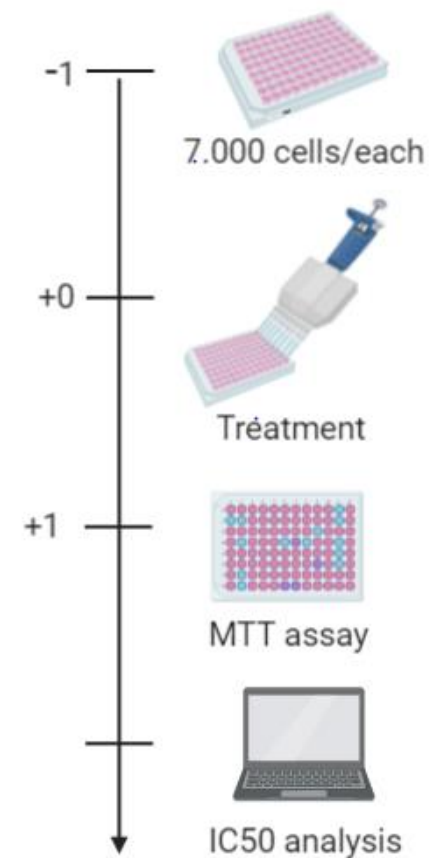
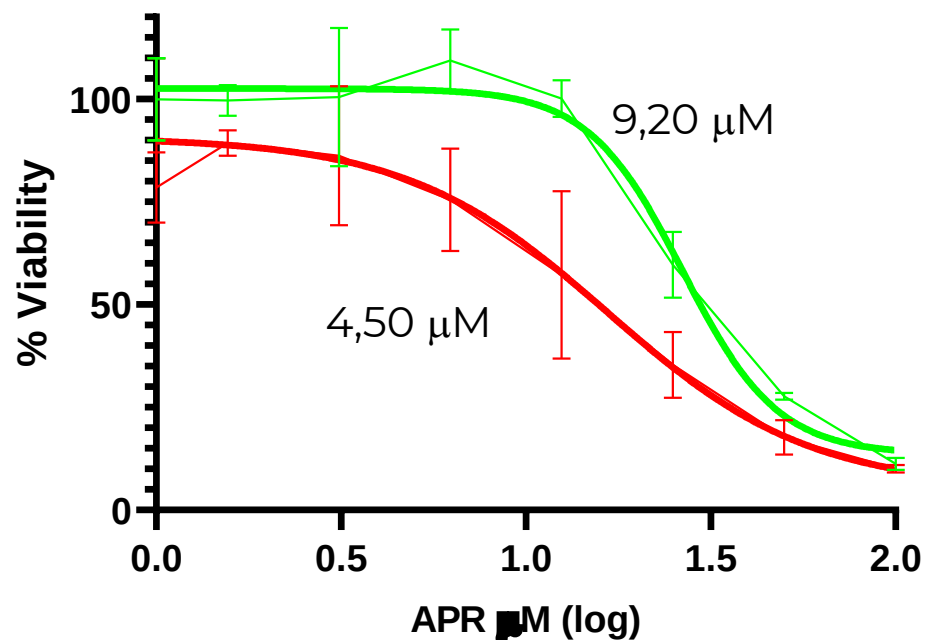
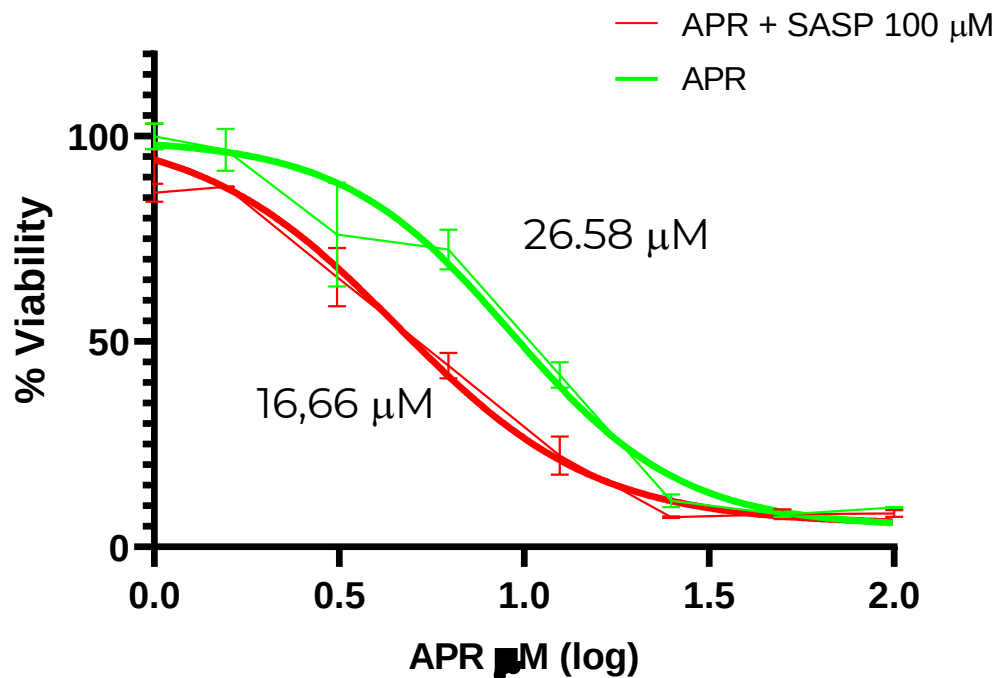


MTT Assay
Human cell lines

24h

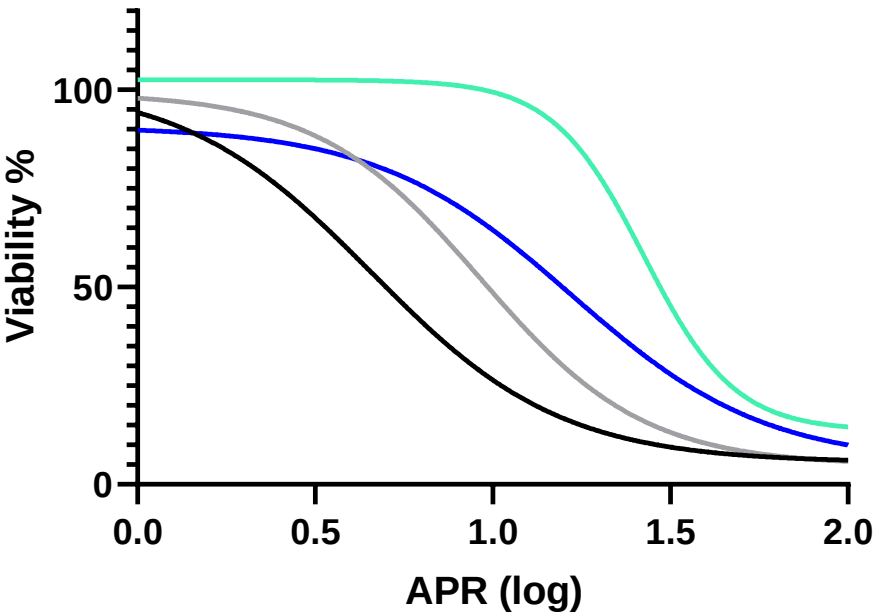
MCF7 human
(p53wt)
APR IC50
literature = 31.1 μ M

MDA-MB-231 human
(p53mut R280K)
APR IC50
literature = 4,1 μ M

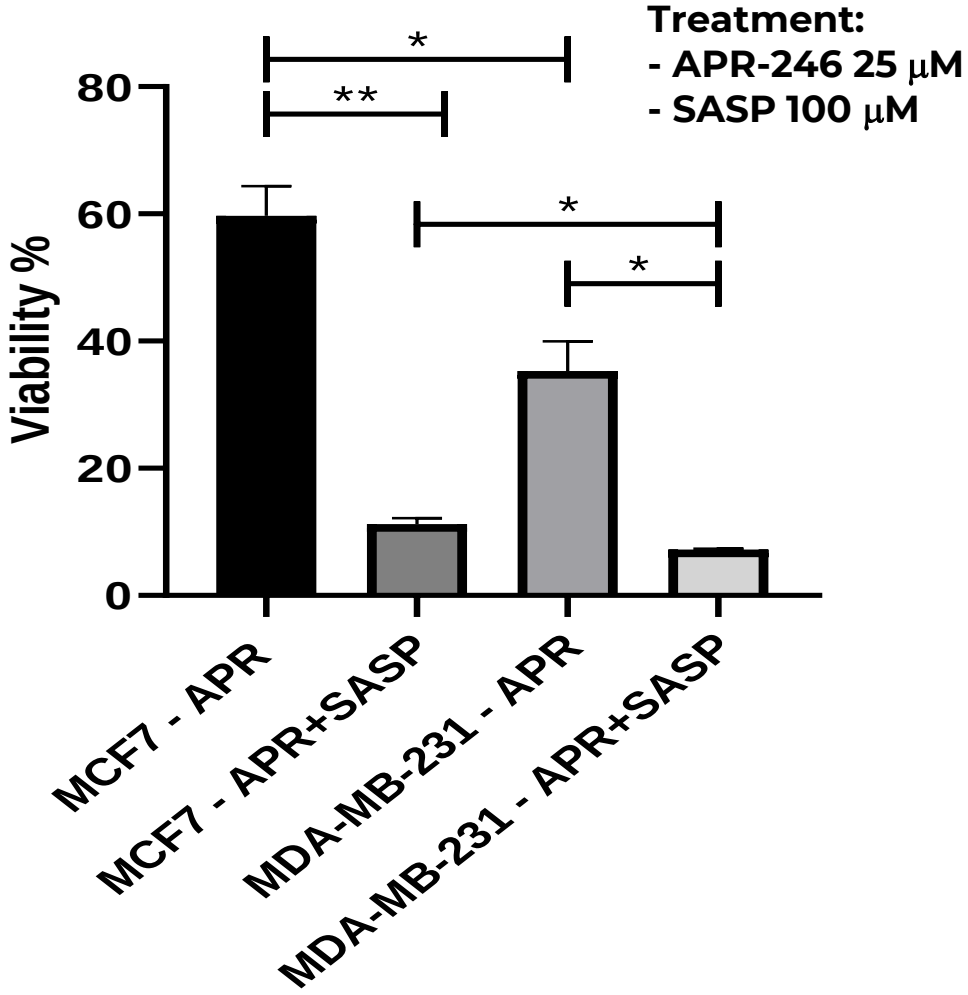


Numbers represent the IC50 of the corresponding treatment, calculated using a NonLinear Regression formula (dose-response). All values are not ambiguous.

MTT Assay
Human cell lines
24h



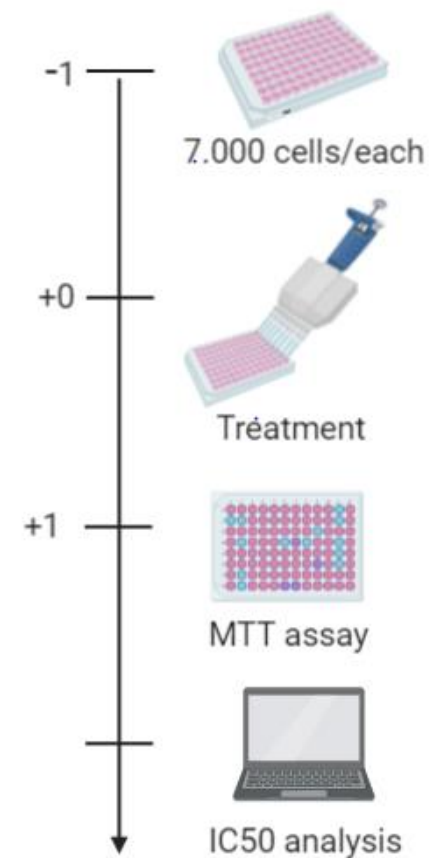
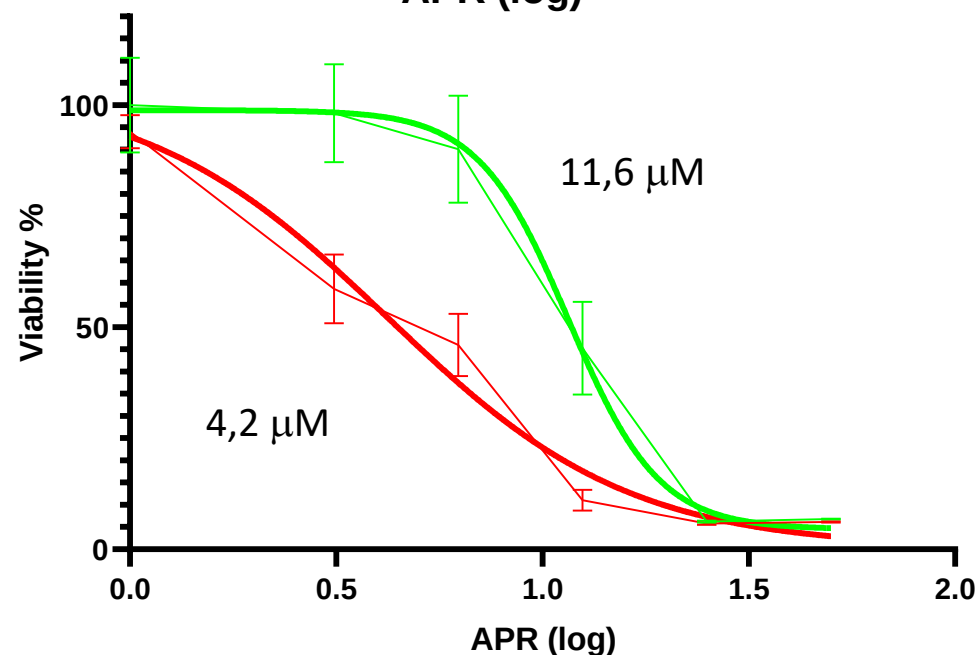
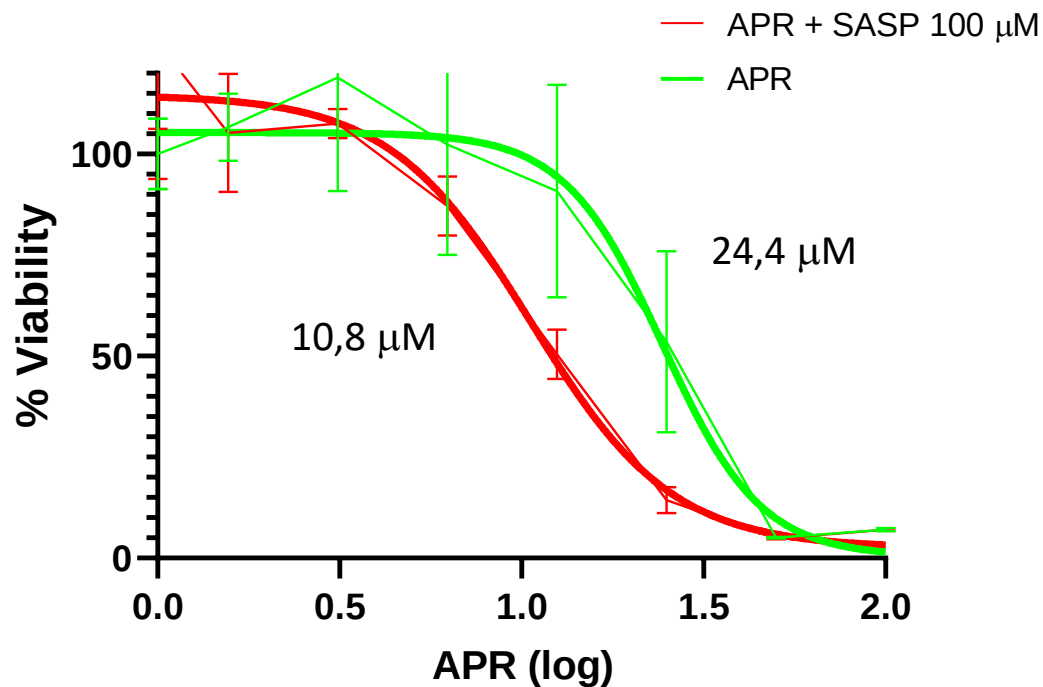
- MDA-MB-231 APR
- MDA-MB-231 APR + SASP 100 µM
- MCF7 - APR
- MCF7 - APR + SASP 100 µM



MTT Assay
Mouse cell lines
24h

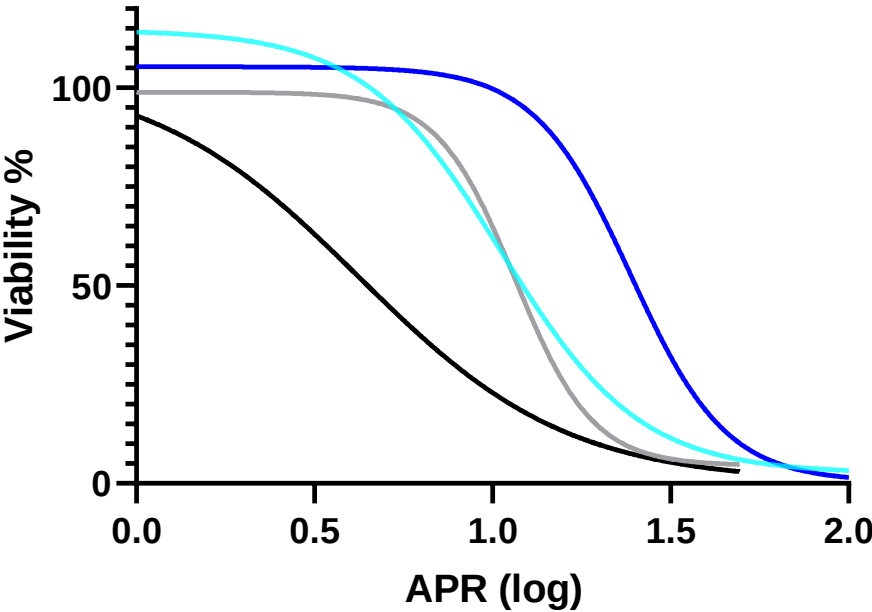
4T1 mouse (p53null)
APR IC50
literature = not found

TSA mouse
(p53mut R270H)
APR IC50
literature = not found

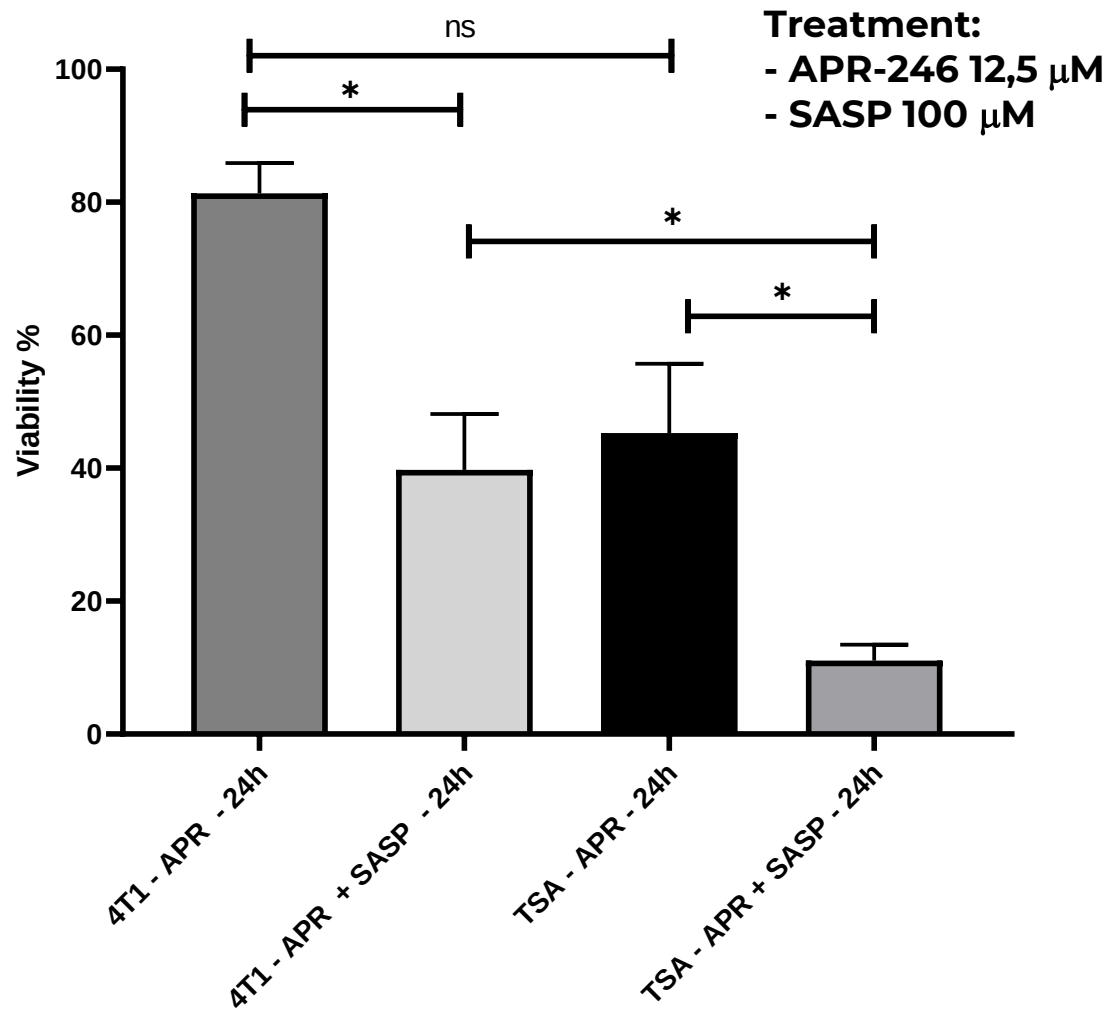


Numbers represent the IC50 of the corresponding treatment, calculated using a NonLinear Regression formula (dose-response). All values are not ambiguous.

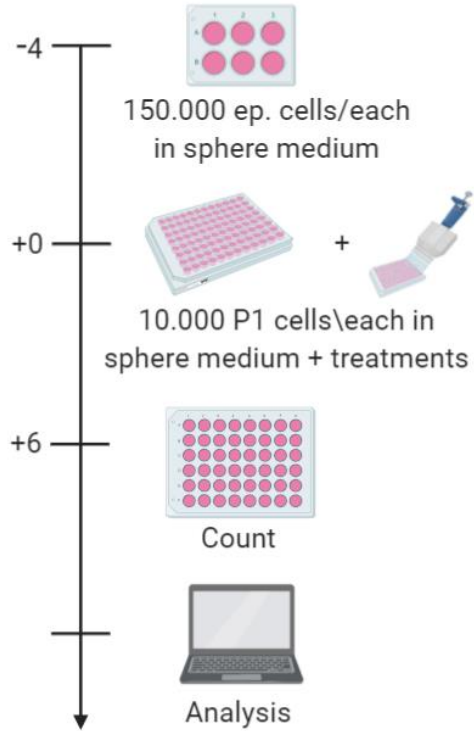
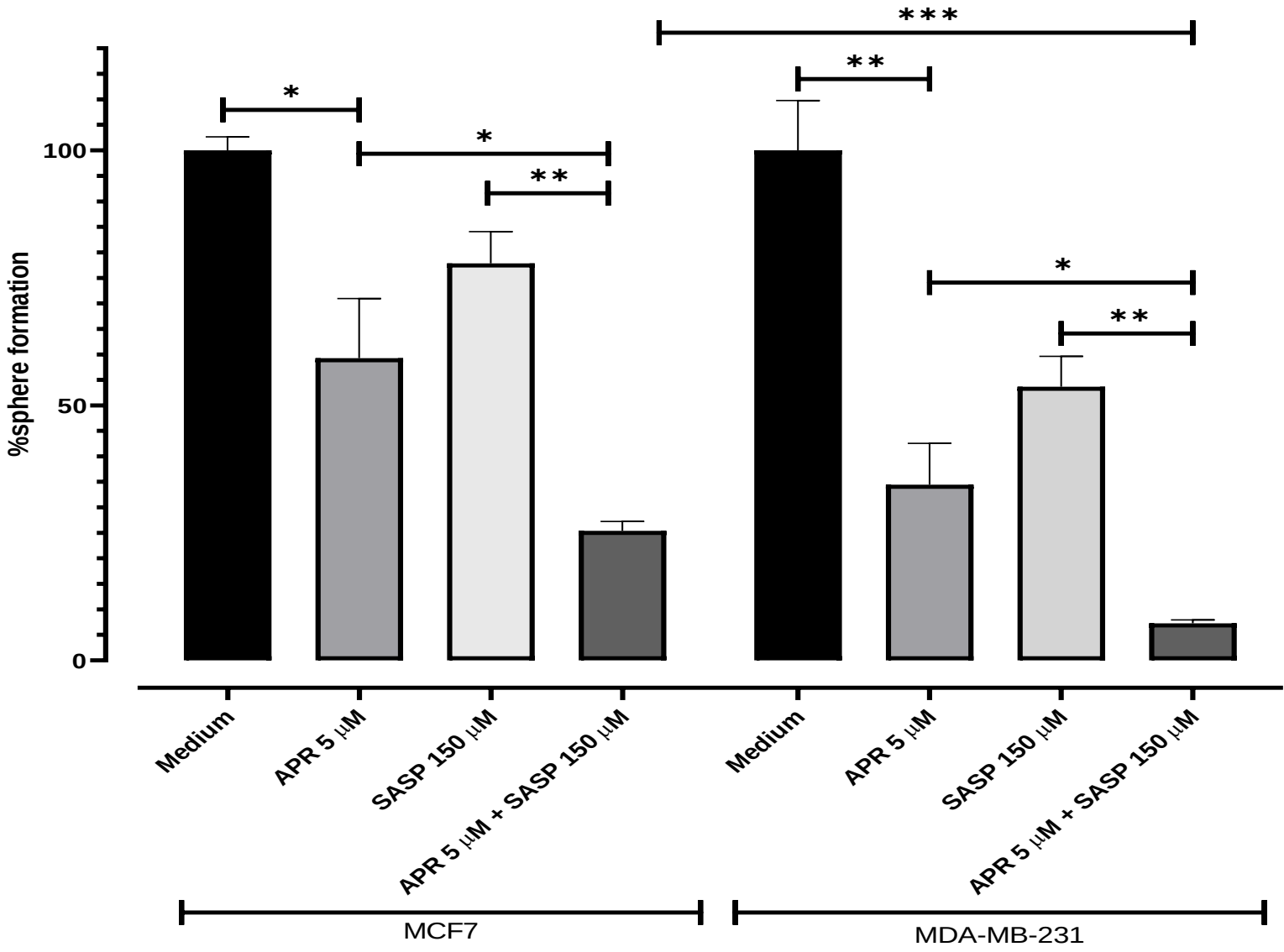
MTT Assay
Mouse cell lines
24h



- 4T1 - APR + SASP 100 μ M
- 4T1 - APR
- TSA - APR
- TSA - APR + SASP 100 μ M

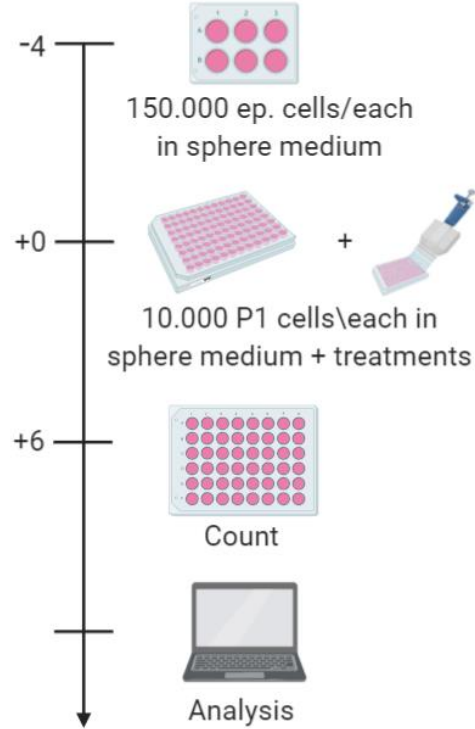
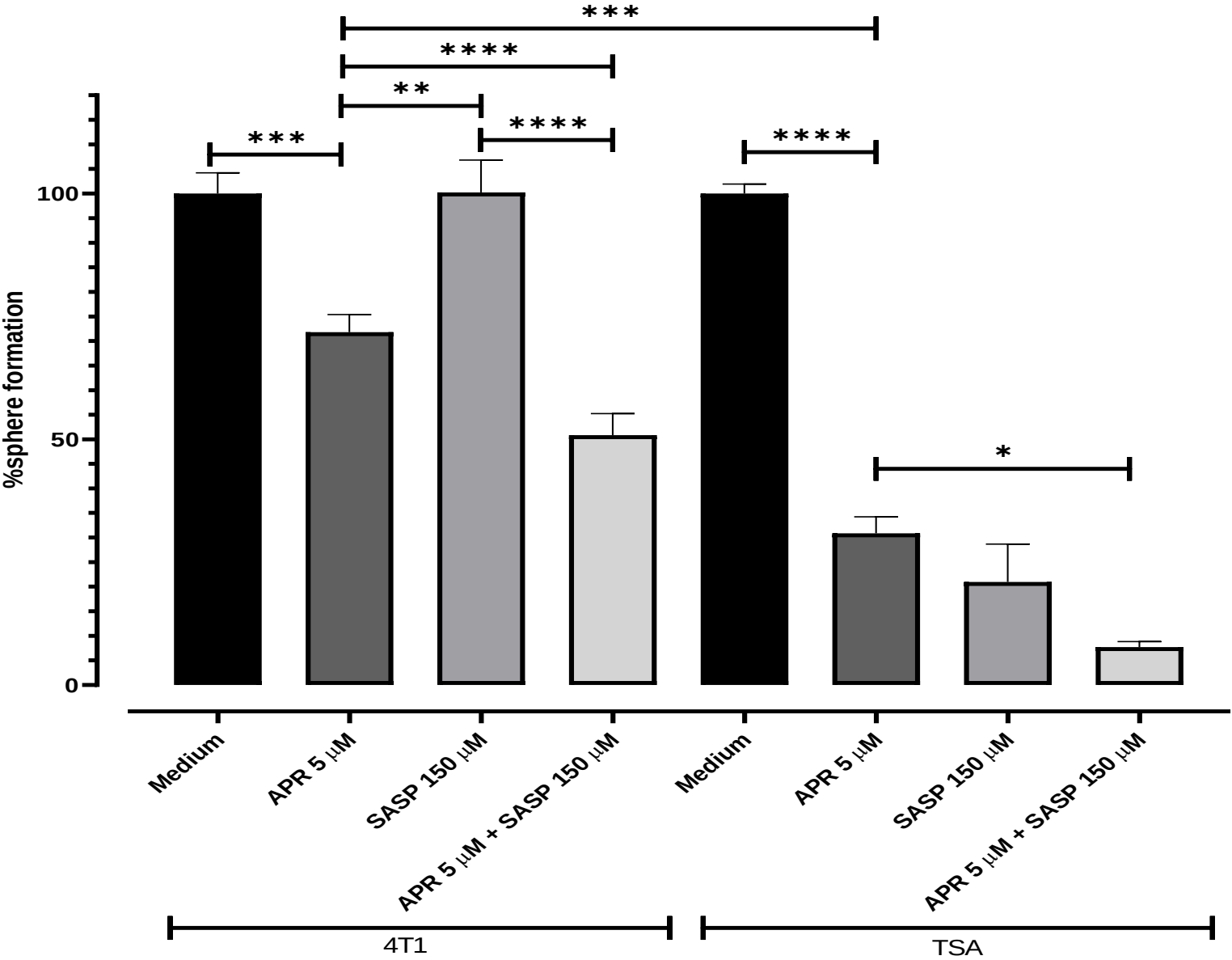


**Tumorspheres
Formation
Human cell lines
6 days**



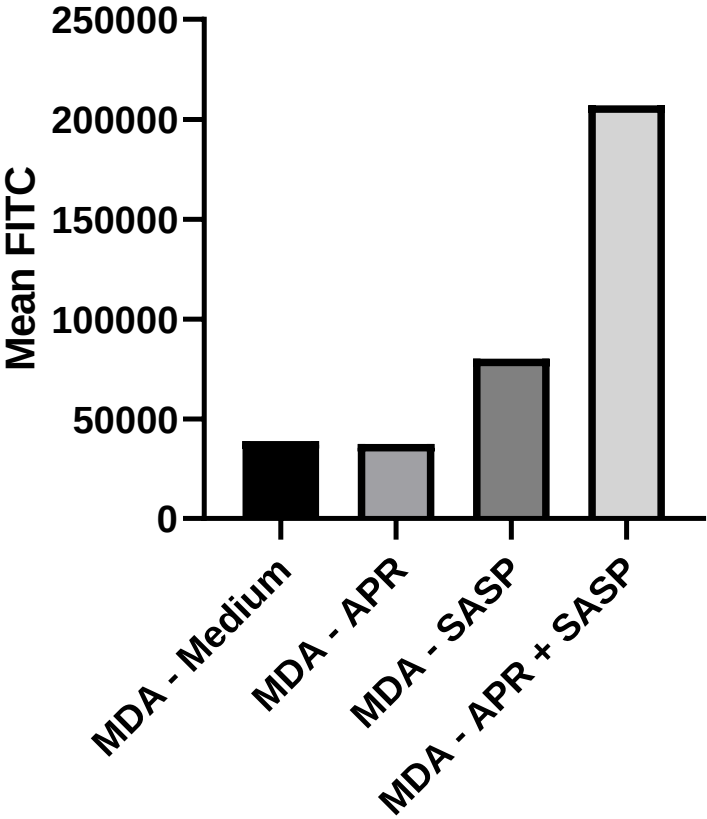
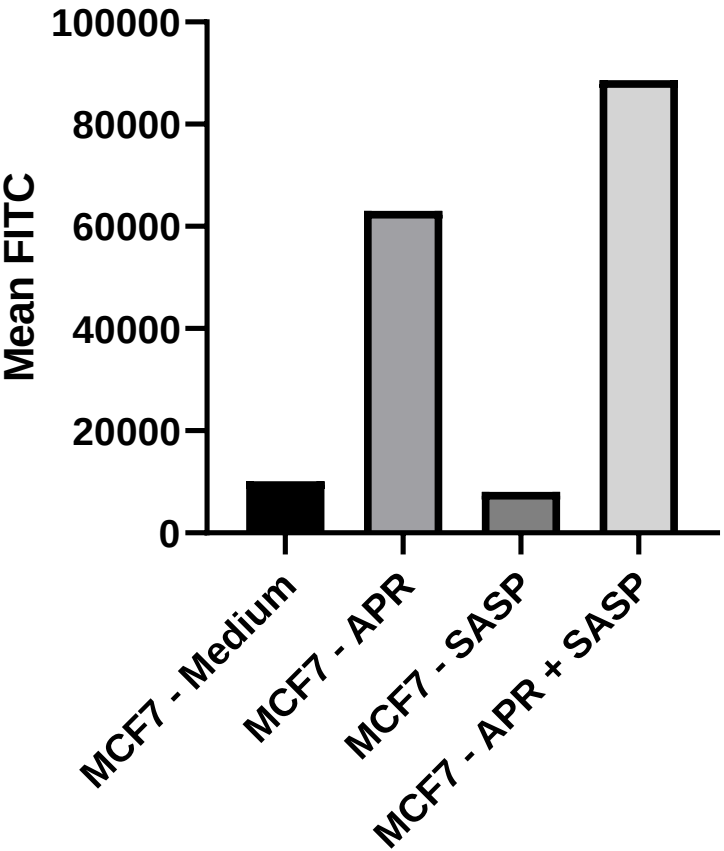
% compared to medium

**Tumorspheres
Formation
Mouse cell lines
6 days**

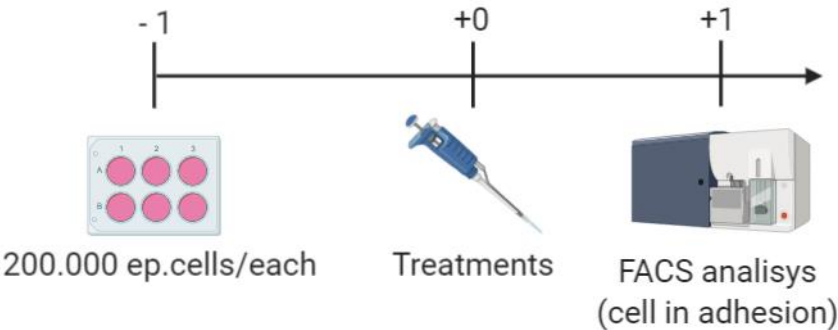


% compared to medium

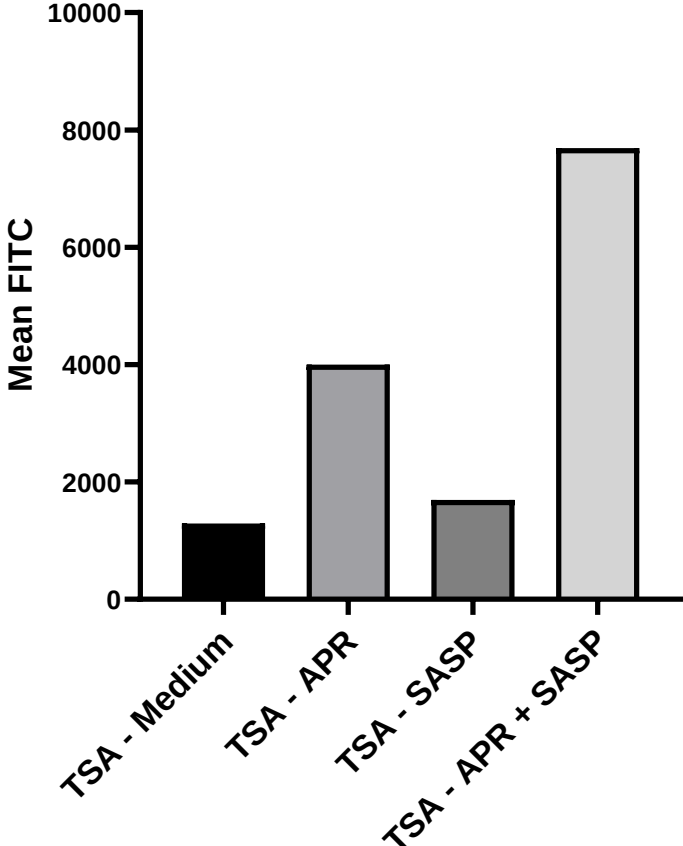
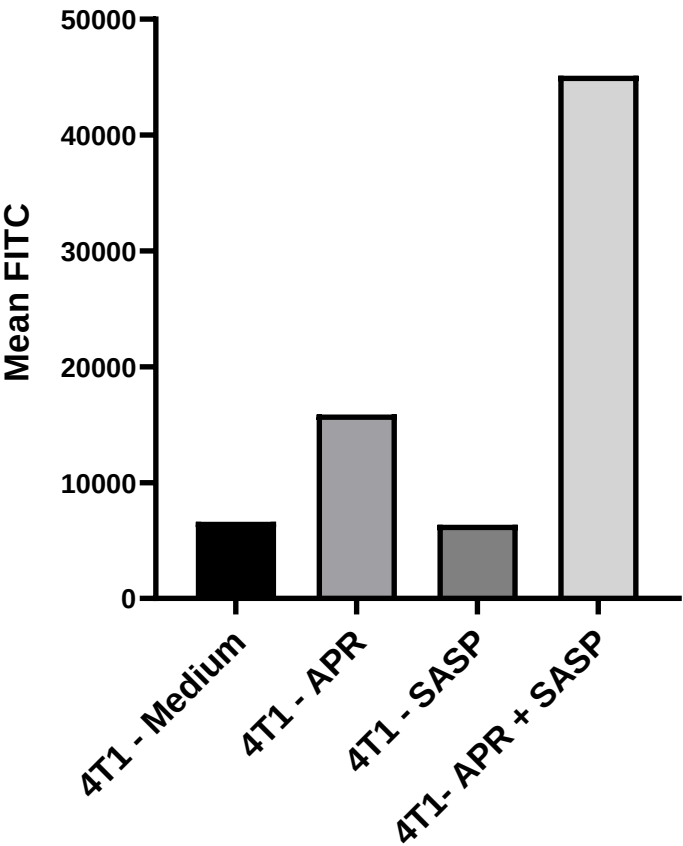
ROS
FACS assay
Human cell lines
24h



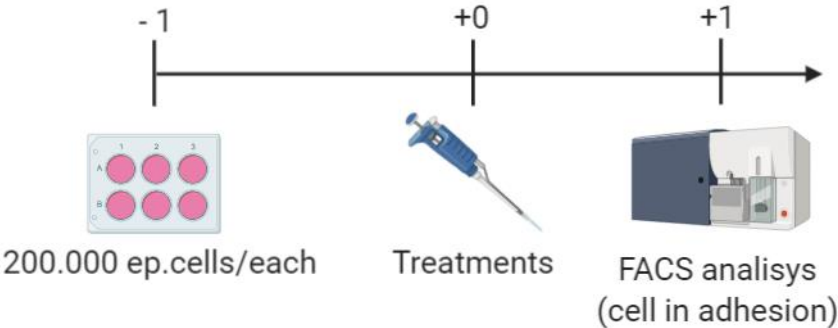
	APR-246	SAS
MCF7	25 µM	200 µM
MDA-MB-231	10 µM	200 µM



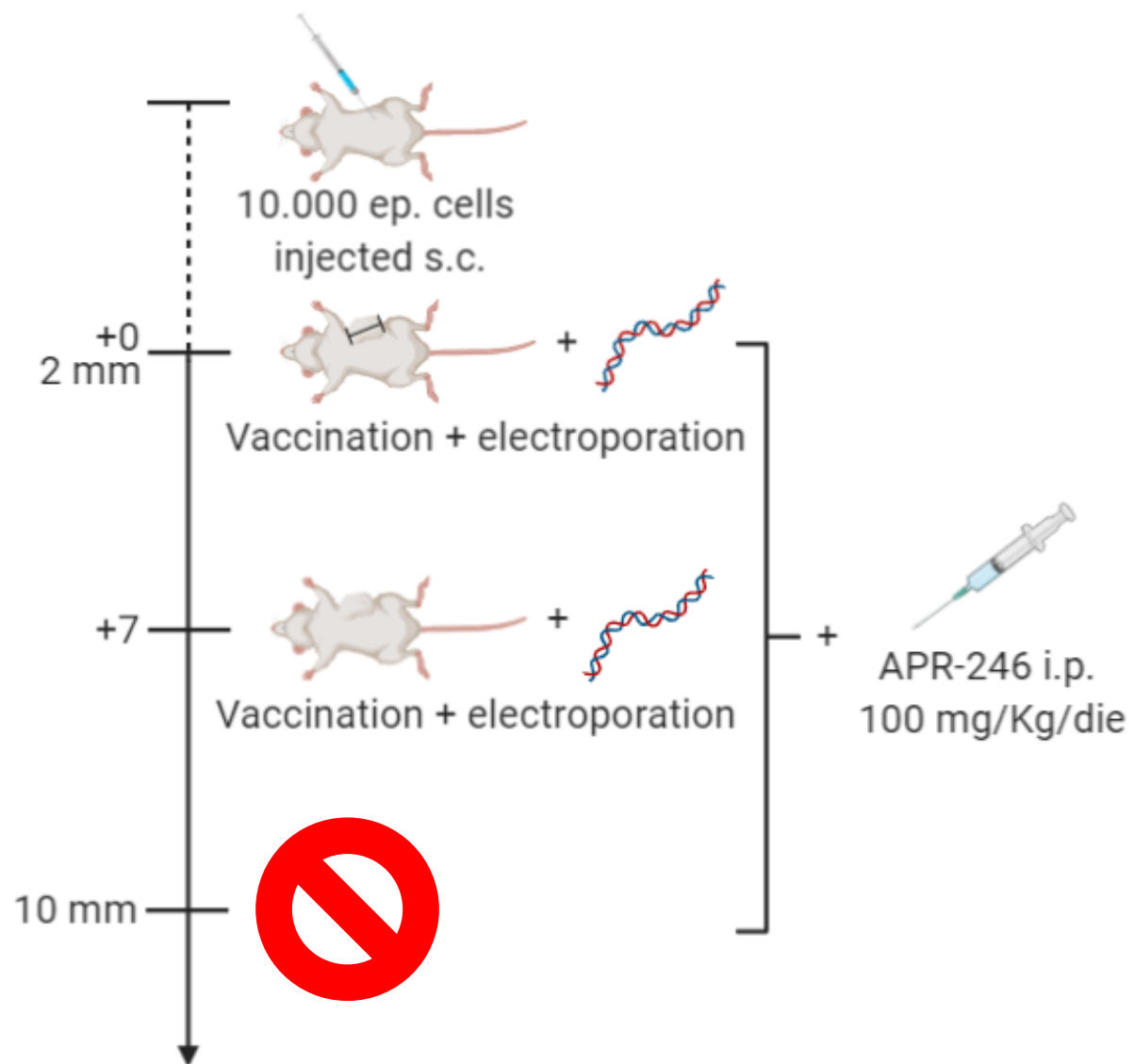
ROS
FACS assay
Mouse cell lines
24h



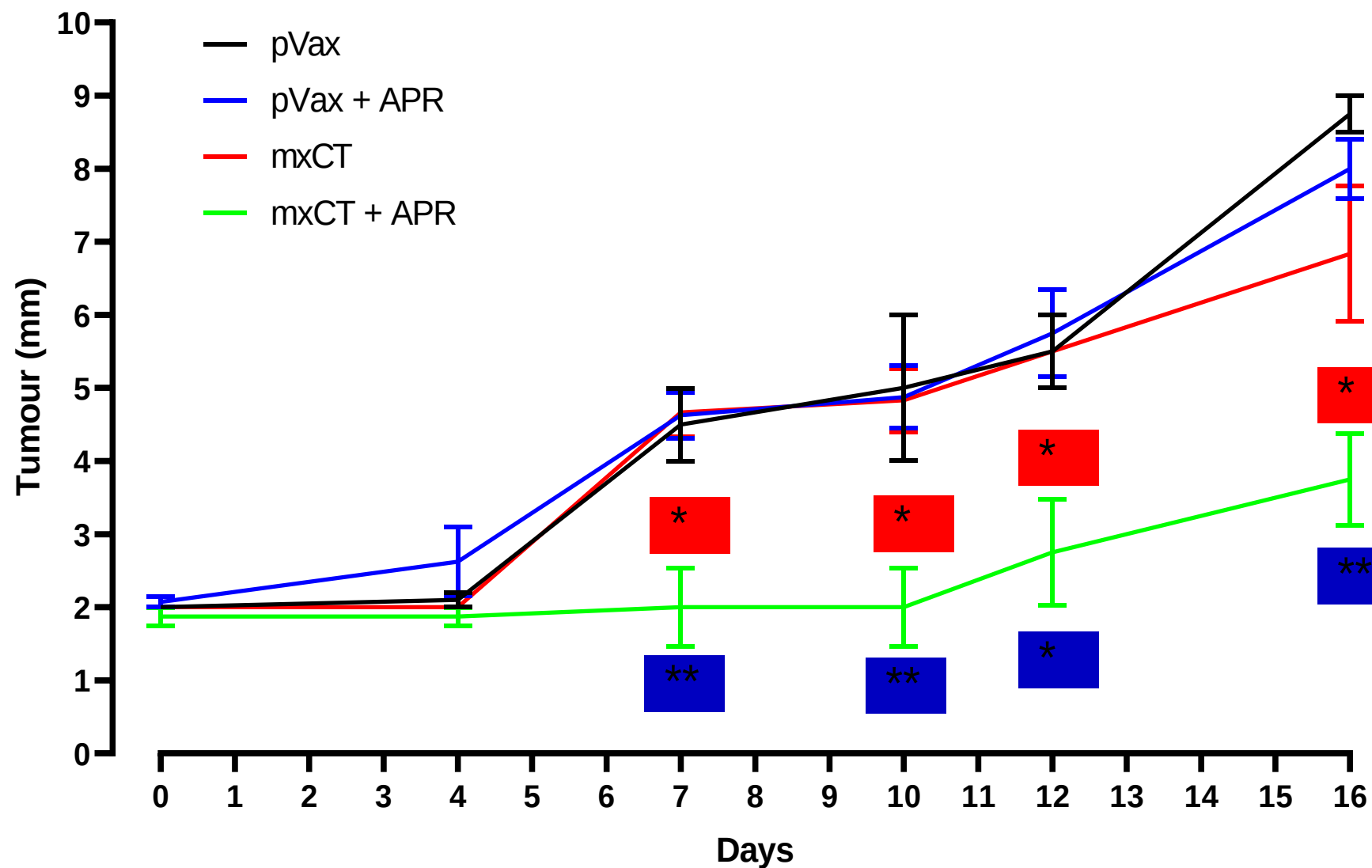
	APR-246	SAS
4T1	20 µM	150 µM
TSA	25 µM	100 µM



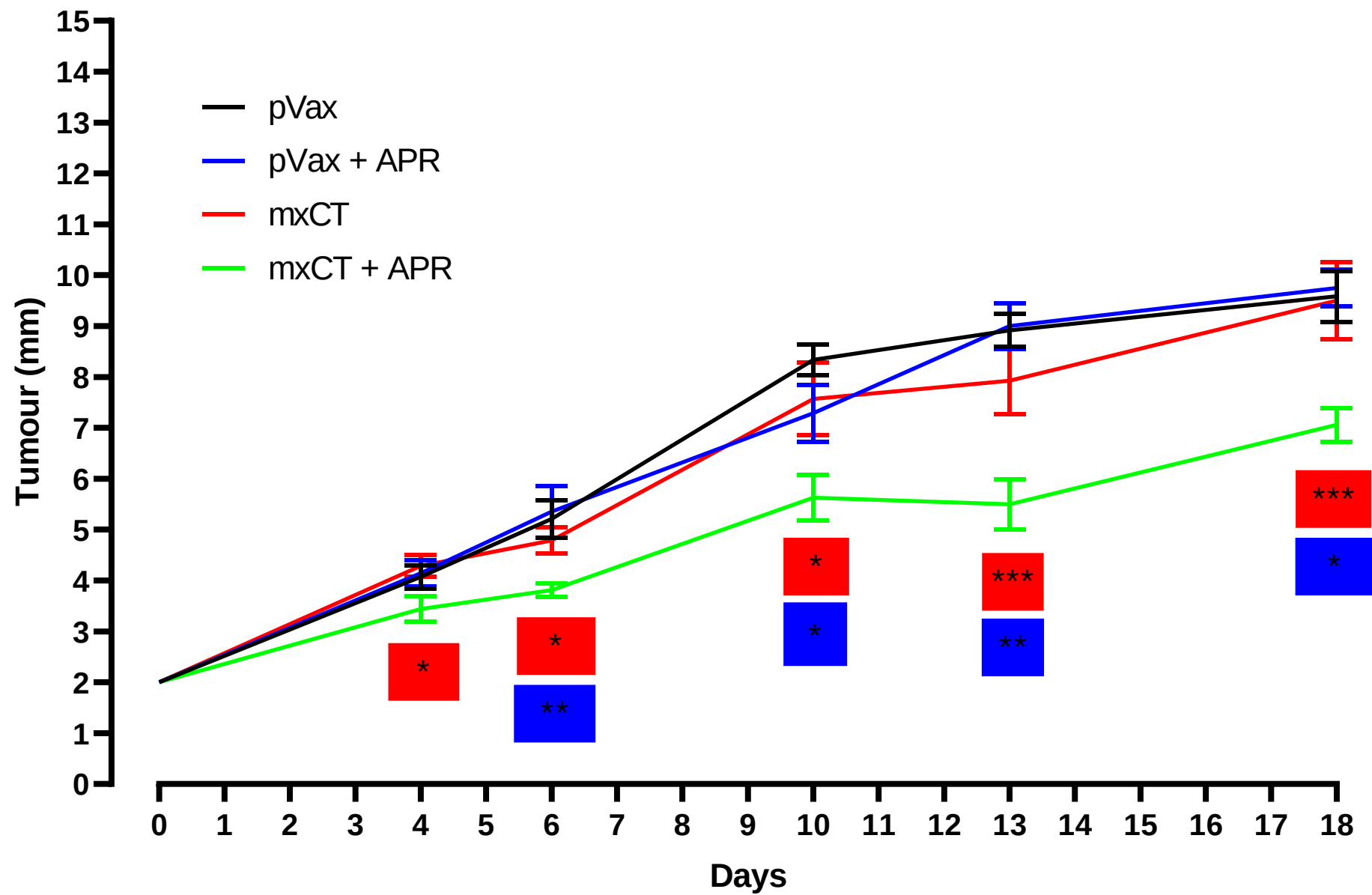
**Vaccination
protocol**



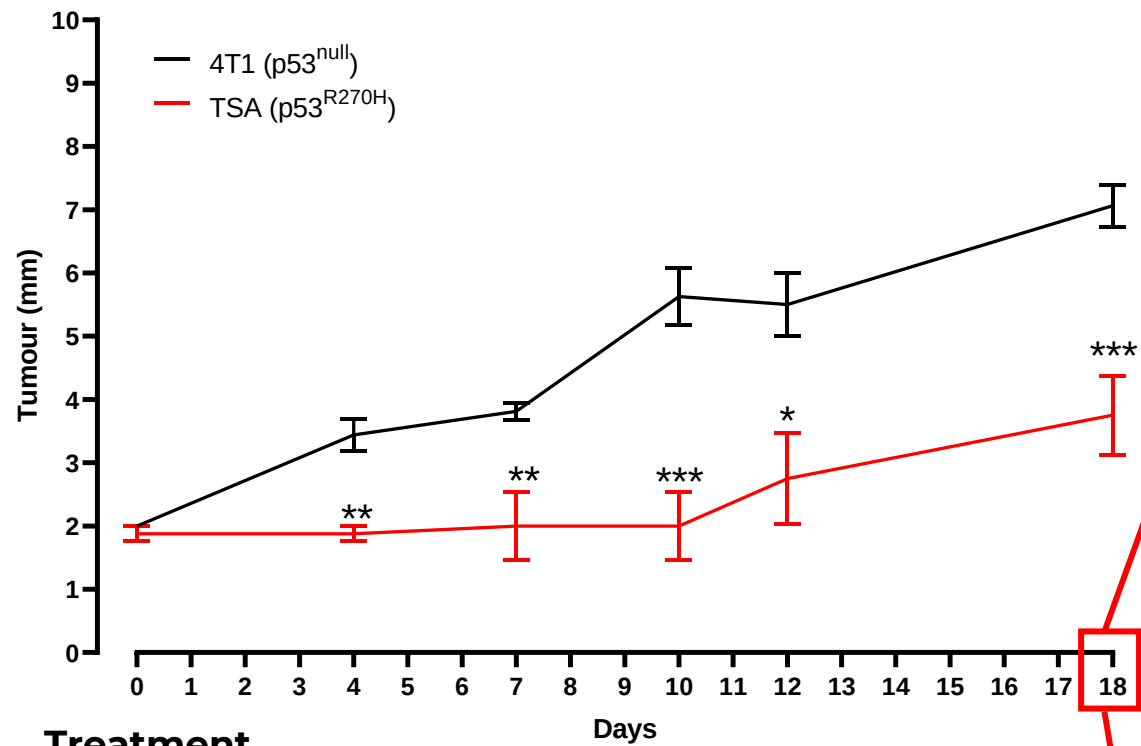
In Vivo
TSA (s.c.) cells
BALB/c mice



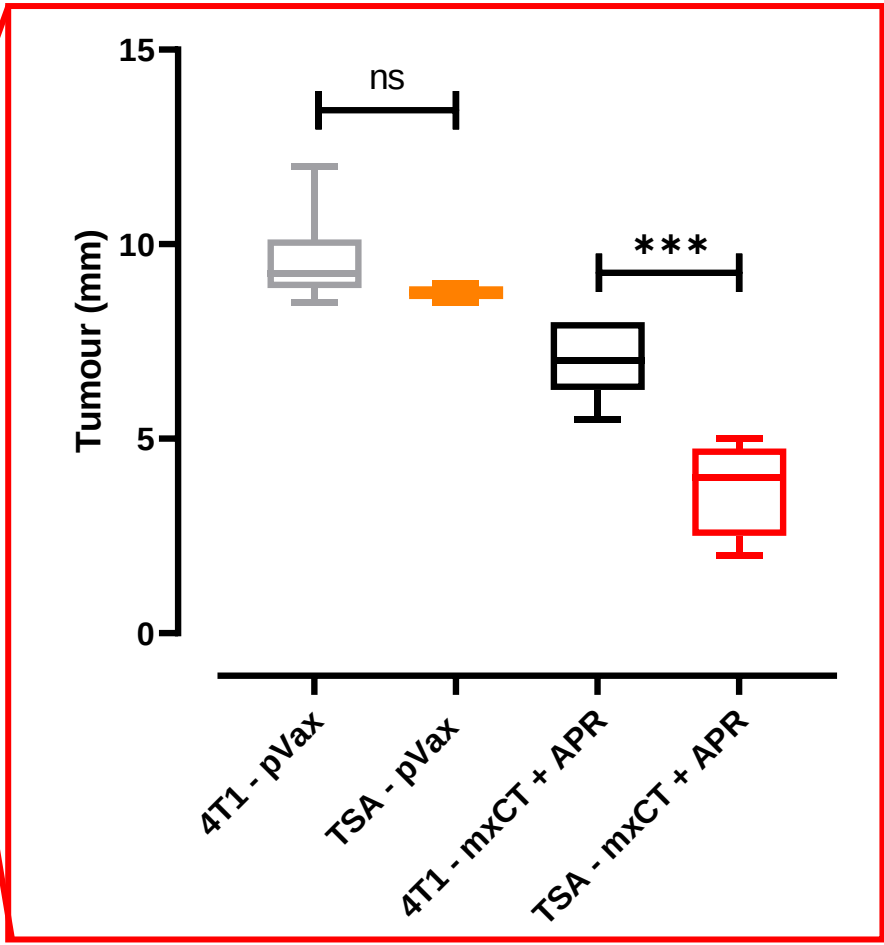
In Vivo
4T1 (s.c.) cells
BALB/c mice



In Vivo
BALB/c mice

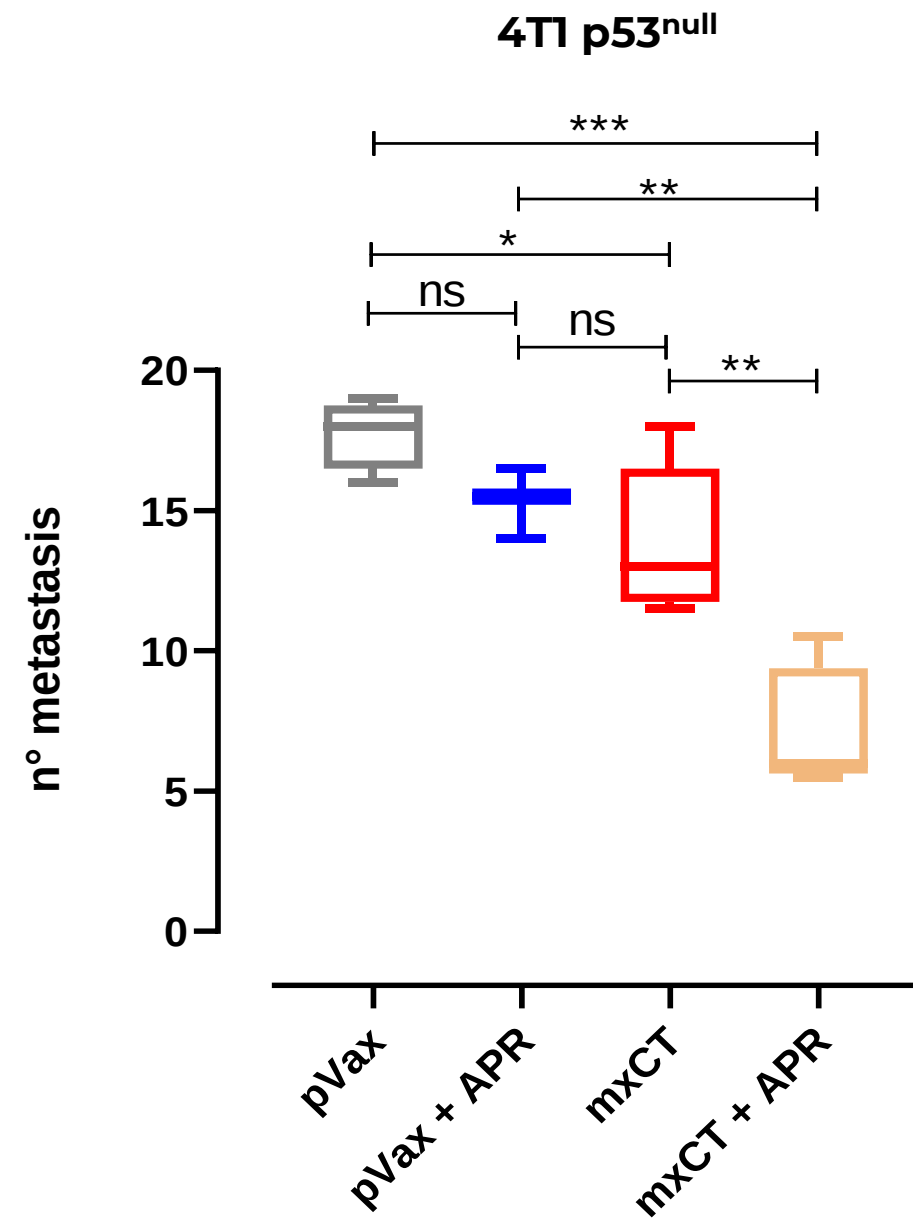
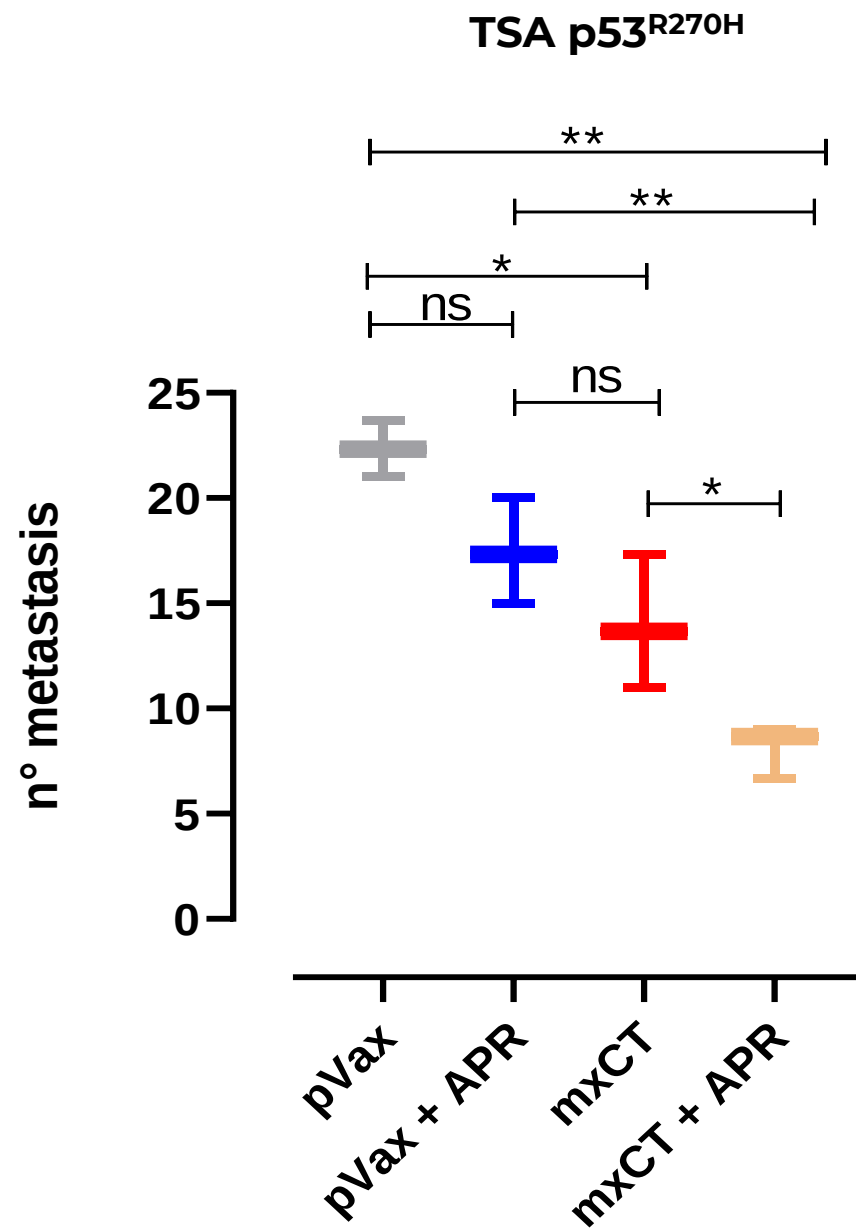


Treatment
APR-246 + mxCT



In Vivo
APR-246 + mxCT

Lung
Micrometastasis



Conclusions

- APR-246 has a cytotoxic activity both in p53 mutated cells and p53 wt cells
- Combinatorial treatment of APR-246 and xCT inhibition provide a synergistic effect
- In vivo, APR-246 in combination with DNA-based vaccination against xCT has an improved capacity in impairing tumour growth and lung metastatization, compared with the single treatments, both in a mut-p53 cancer model and p53null model

15.04.2021



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Thank you!

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