Institut de Recherche en Cancérologie
I R C Montpellier

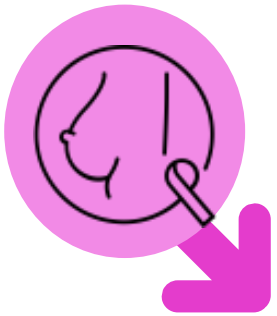
Exploring sensitivity to replication stress in BRCA-deficient Triple Negative Breast Cancer

Imene TABET
2020/2021

Genetic and phenotypic plasticity of cancer's team

Triple Negative Breast Cancer (TNBC)

- The most aggressive from all breast cancer molecular subtypes :
- ER-/PR-/HER2- :



- 15% of breast cancers
- 40% recur within 12 to 60 months
- Treatment FCE100 (5FU/Epirubicin/Cyclophosphamide), little alternative in case of recurrence

TNBC and BRCAness

30-35% of TNBC are BRCA-deficient

Defective Homologous Recombination repair (HRR)

Unrepaired DNA breaks

Sensitivity to Genotoxic drugs

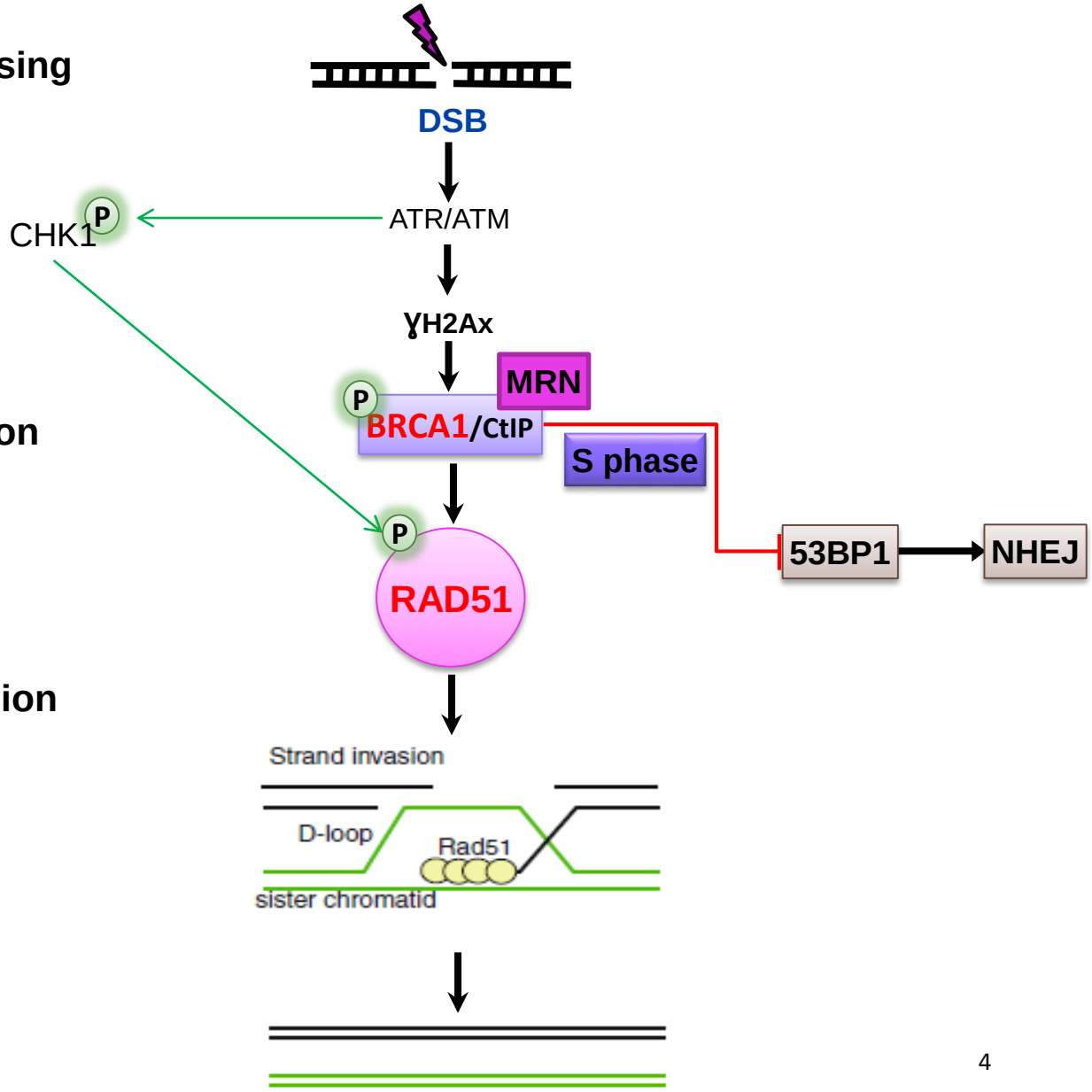
Homologous Recombination Pathway

1) Damage Sensing

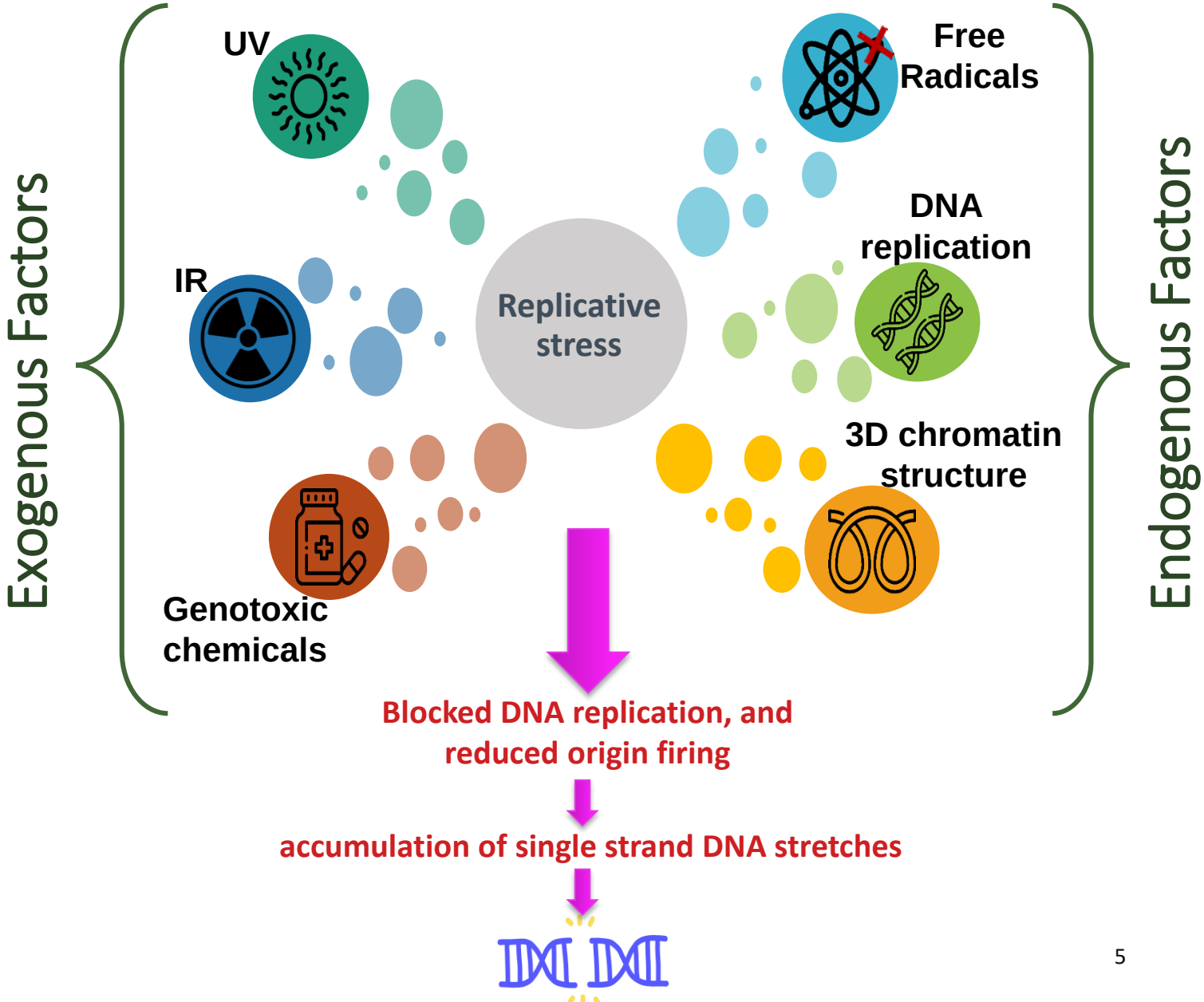
2) DNA Resection

3) Strand Invasion

4) DNA Repair



HR involvment in replication stress response

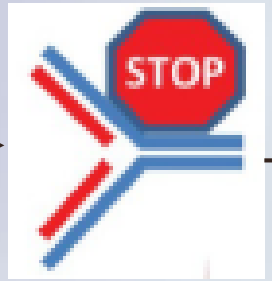


We propose :

BRCA deficient tumors might be hypersensitive to replicative stress

Replicative stress inducing drugs

BRCA deficient tumor



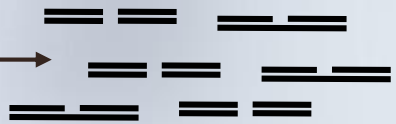
Blocked replication

No BRCA

No RAD51 recruitment ?

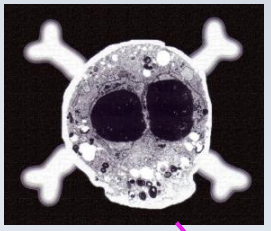
No fork stabilization

No repair ?



Accumulation of SSB and DSB

S/G2 ATR/CHK1 checkpoint

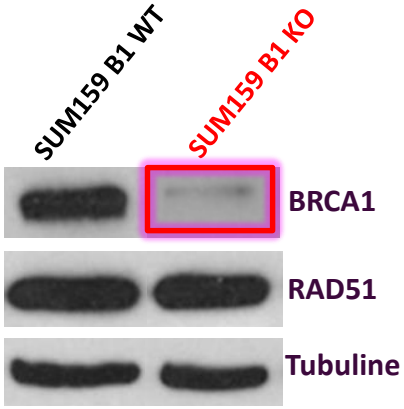


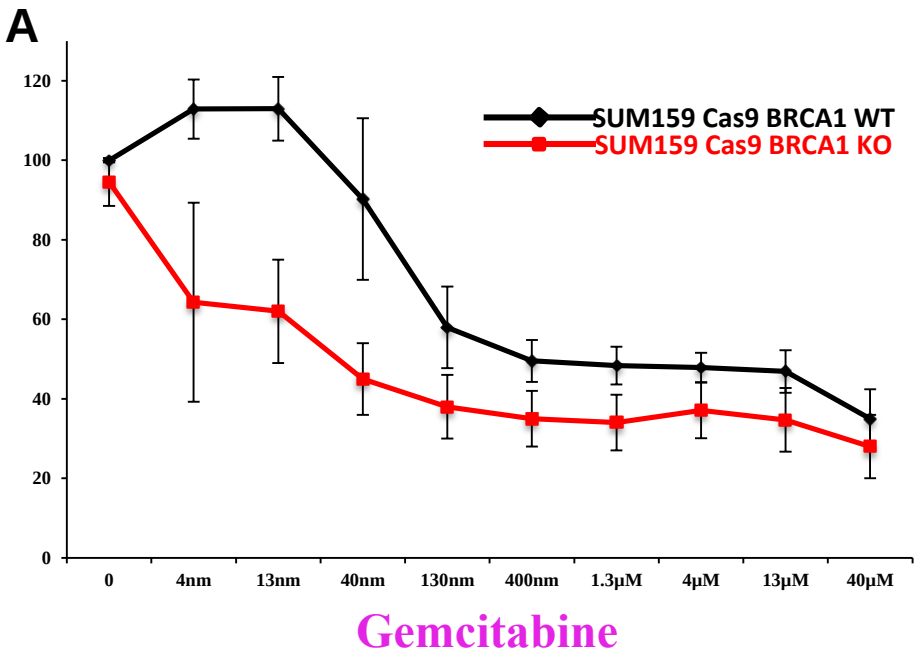
Replicative Catastrophe ?

Mitotic Catastrophe ?

Cell cycle arrest and cell death ?

Gemcitabine in SUM159 BRCA1 WT vs. SUM159 BRCA1 KO CRISPR/Cas9 isogenic models

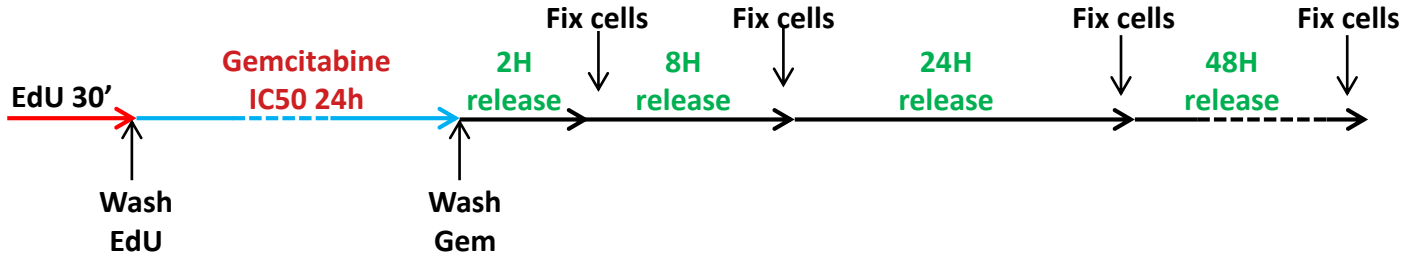


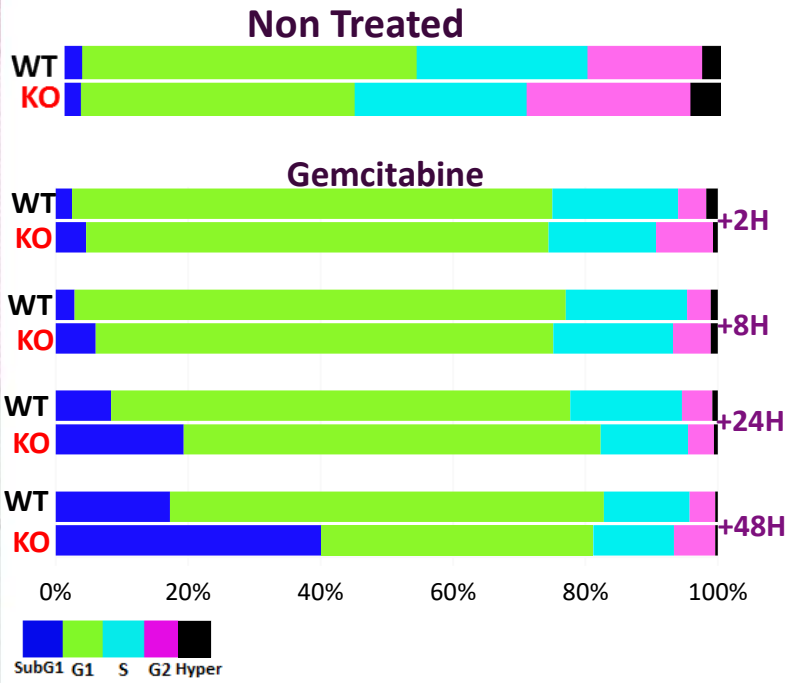


SUM159 BRCA1 KO isogenic model is more sensitive to Gemcitabine

Comparative analysis of cell cycle distribution in SUM159 BRCA1 WT Vs. SUM159 BRCA1 KO treated with Gemcitabine

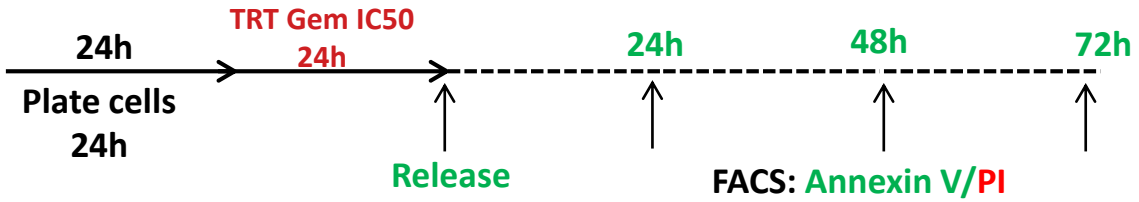
1) By Flow Cytometry :

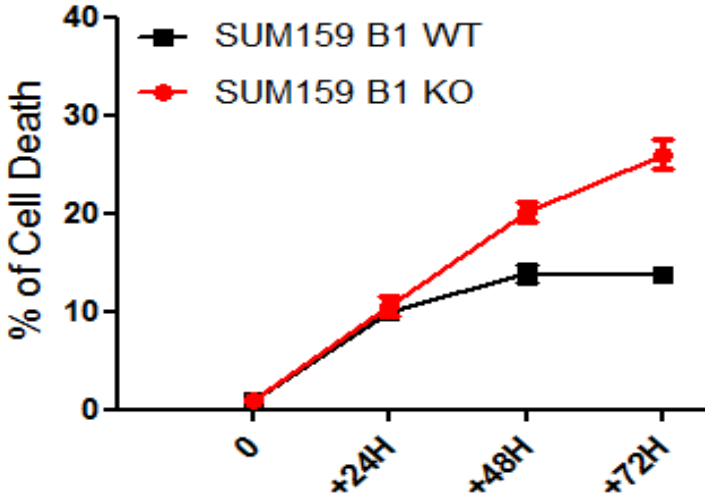




- ✓ important accumulation of cells in G1, and much less G2 than NT
- ✓ The percentage of SubG1 is twice as high in KO compared to the WT

Gemcitabine induced cell death in SUM159 BRCA1 KO confirmed



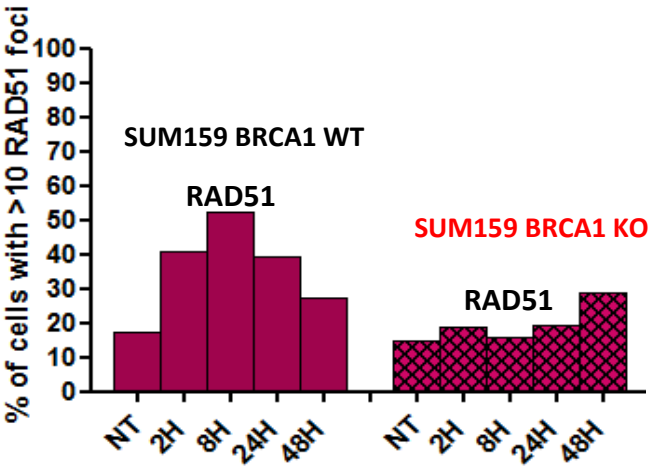


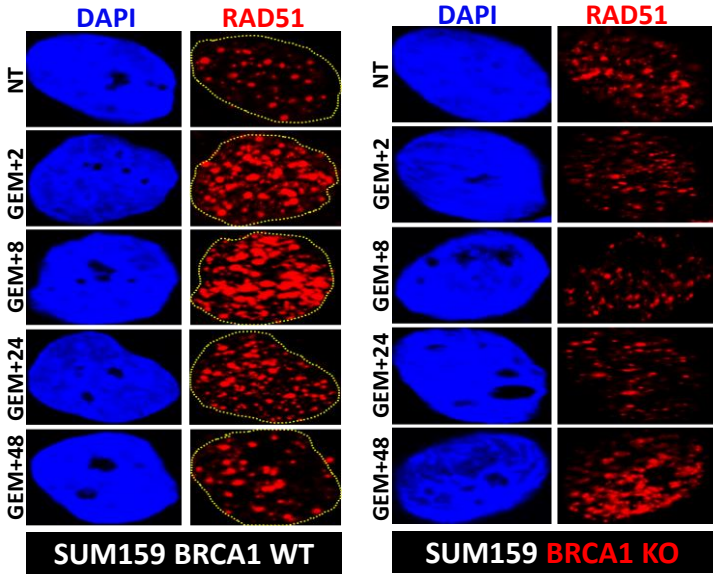
Gemcitabine IC50 24H

- ✓ Cell death continues to increase In the BRCA1 KO isogenic model at +48, and +72h

Results, *In vitro*

SUM159 BRCA1 KO display disrupted HR upon Gemcitabine treatment

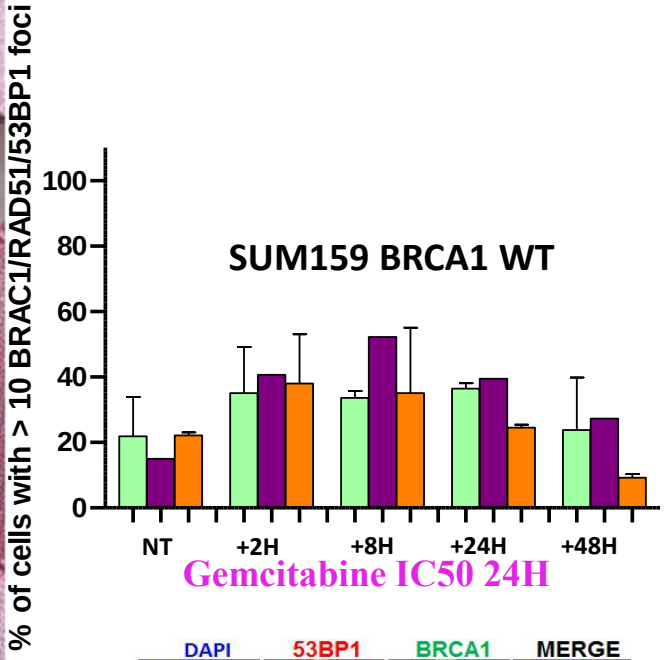


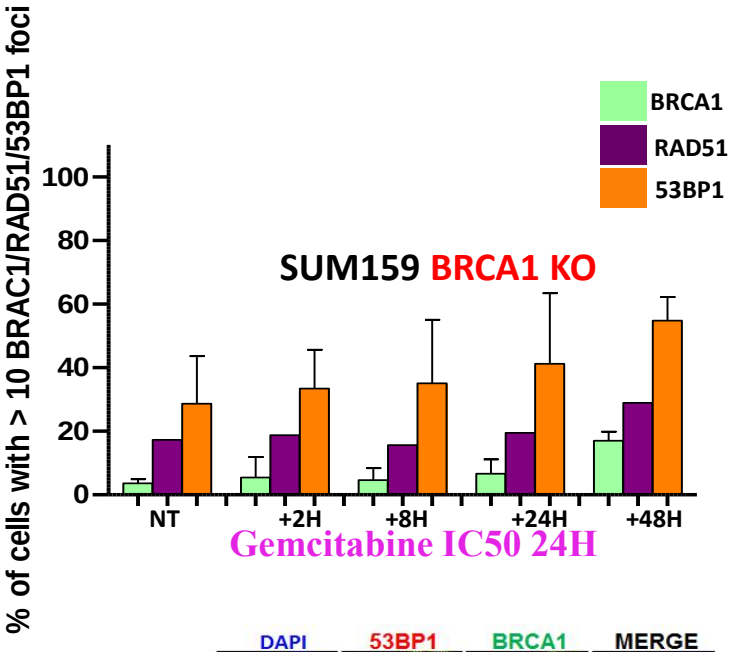


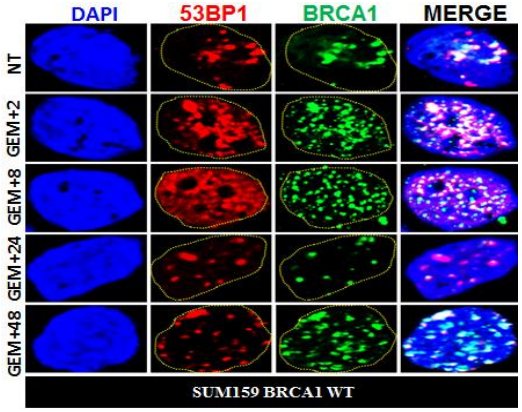
✓ Weak RAD51 foci formation in the SUM159 BRCA1 KO cells

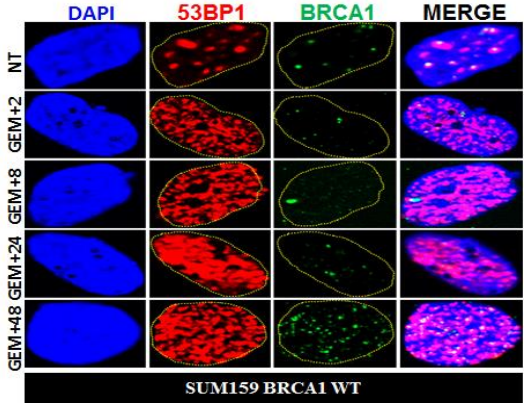
Results, *In vitro*

SUM159 BRCA1 KO seem to engage in a non homologous recombination pathway upon Gemcitabine treatment



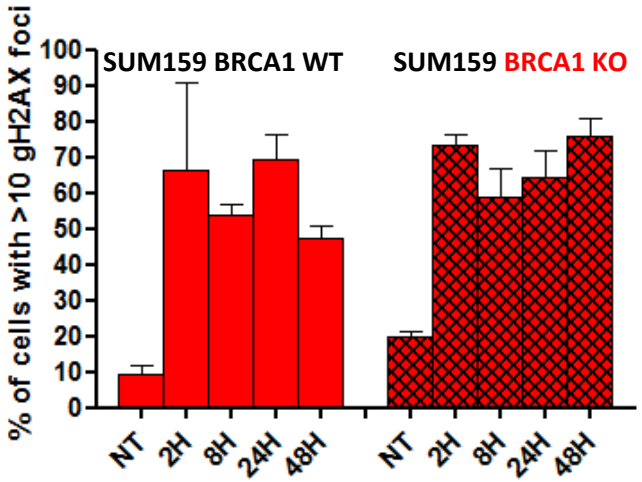


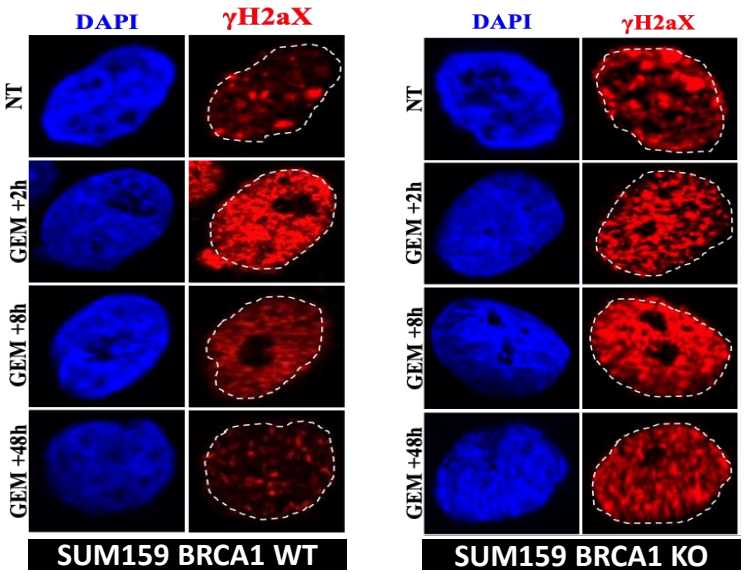




✓ Progressive increase of cells with 53BP1 foci in SUM159 BRCA1 KO

SUM159 BRCA1 KO display persistent DNA damage upon Gemcitabine treatment

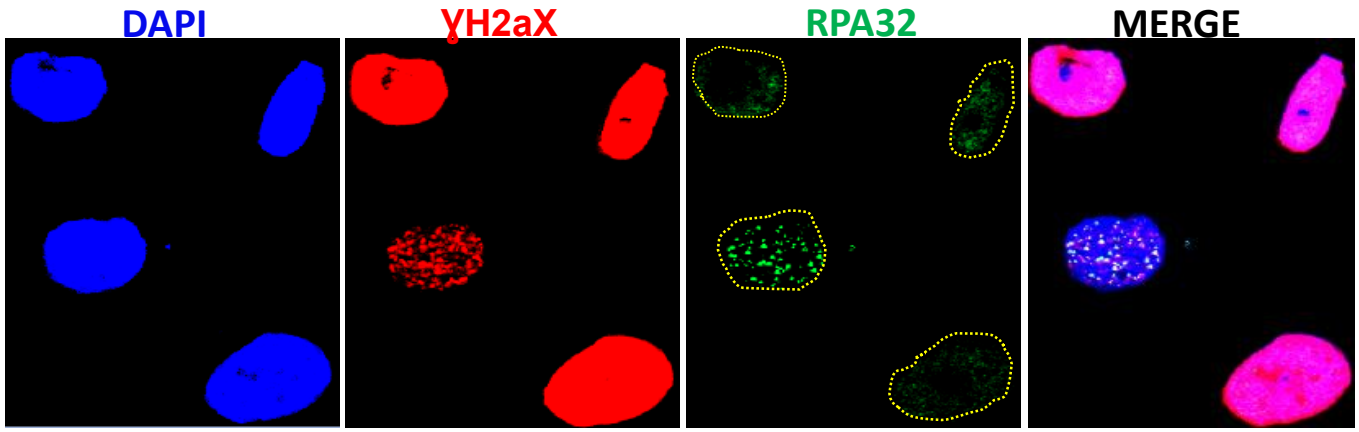


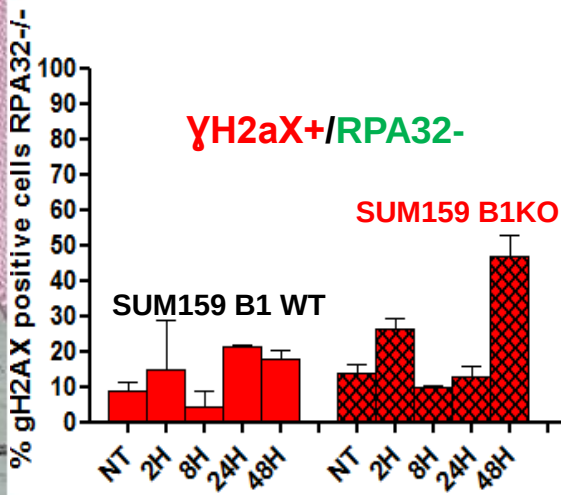


✓ % of gH2AX positive cells doesn't decrease even at +48h post release in SUM159 BRCA1 KO

Results, *In vitro*

SUM159 BRCA1 KO display more gH2AX+/RPA- cells upon Gemcitabin treatment



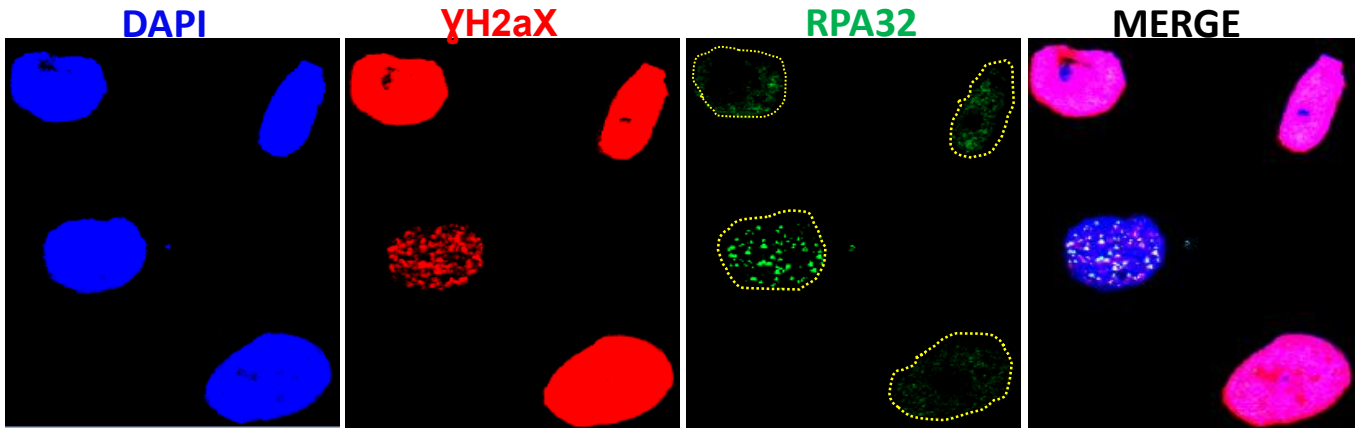


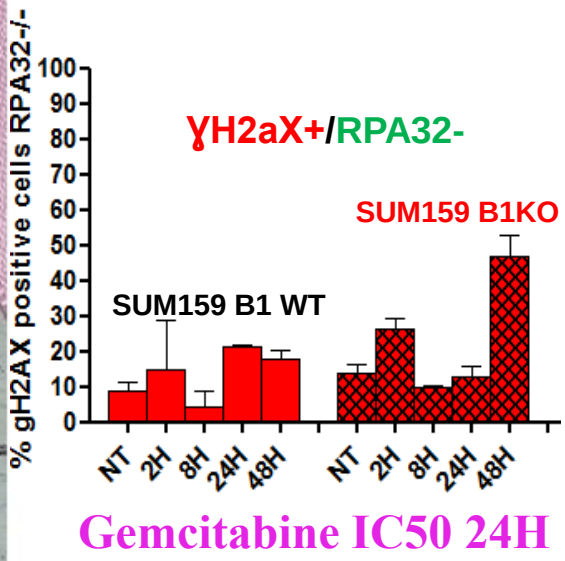
Gemcitabine IC50 24H

- **Potential Replicative Catastrophe in the SUM159 BRCA1 KO especially at +48h**

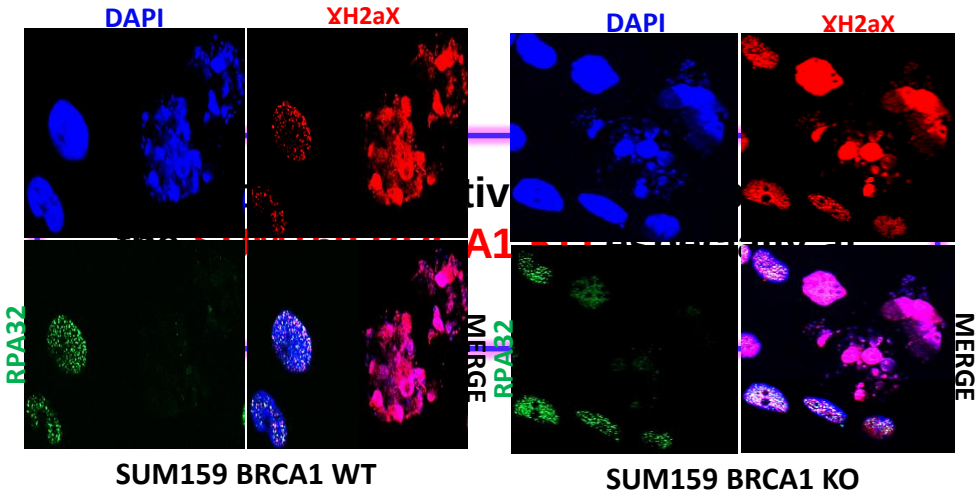
Results, *In vitro*

SUM159 BRCA1 KO display more gH2AX+/RPA- cells upon Gemcitabin treatment

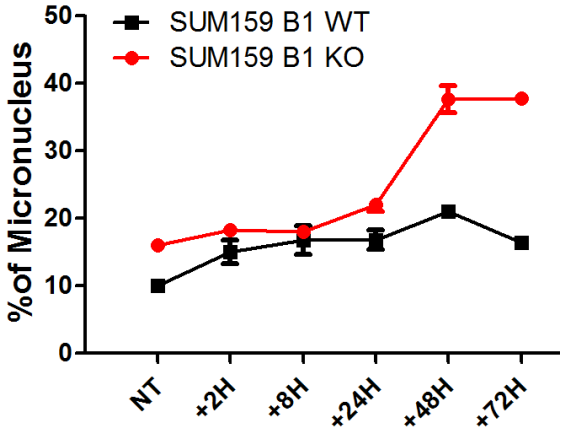




Gemcitabine IC50 24H

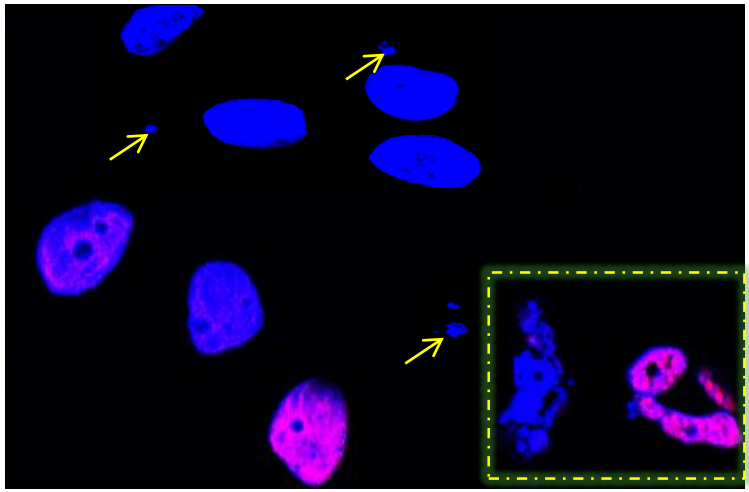


SUM159 BRCA1 KO present more mitotic aberrations upon Gemcitabine treatment

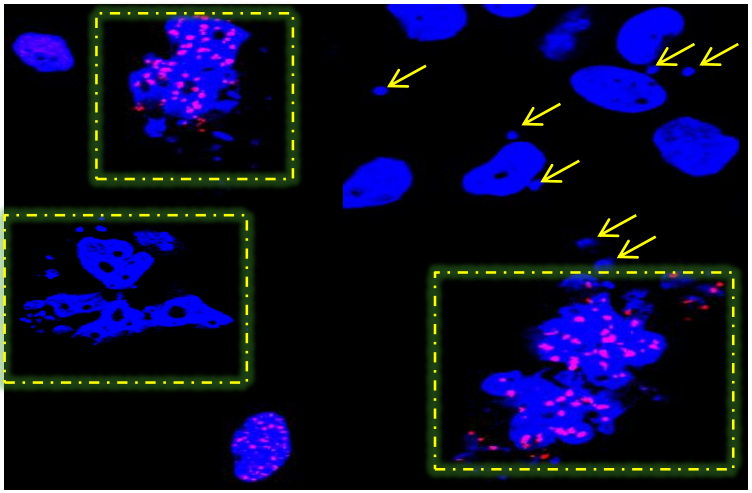


Gemcitabine IC50 24H

WT Gem+72h



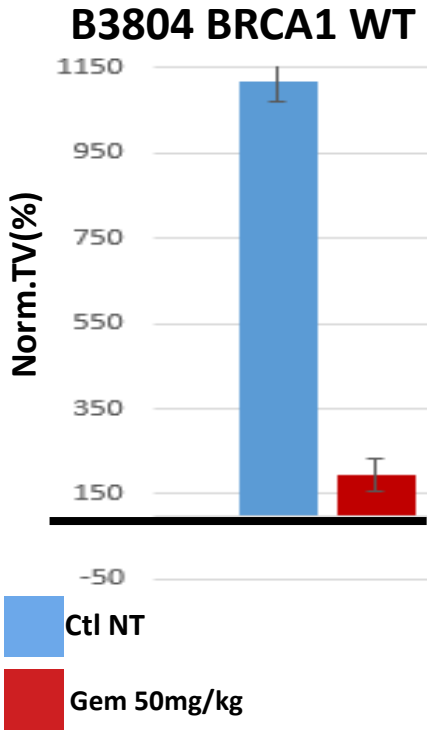
KO Gem+72h

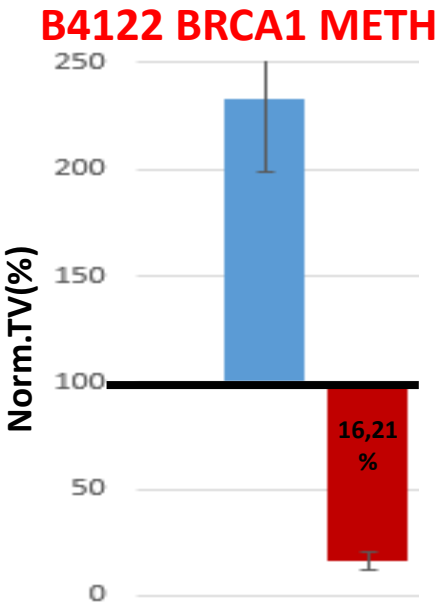


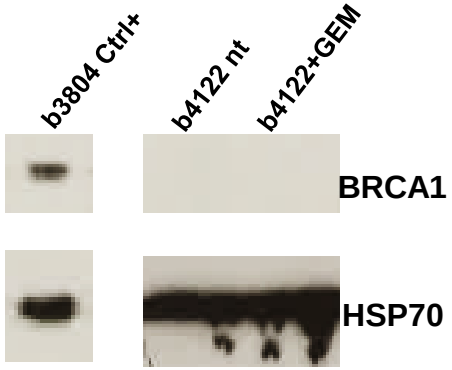
✓ % of micronuclei increases between +24h and +48h in the SUM159 BRCA1 KO

Results, *In vivo*

BRCA1 deficient (Hypermethylated) PDX's tumor volume decreases under gemcitabine treatment

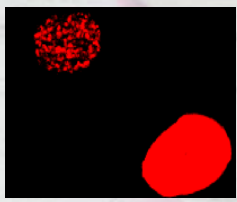






In vitro results confirmed *in vivo*

Conclusion



gH2ax accumulation = Pan-nuclear

Gemcitabine

DSB

ATR/ATM

CHK1 P

CHK2

γH2Ax

G2/M Cell cycle checkpoint

CyclineB/CDK1

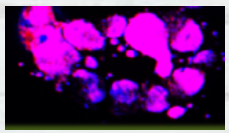
P

BR 1

Replication arrest/Stalled forks

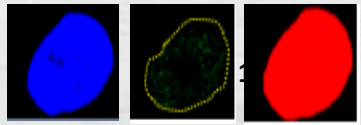
Less efficient fork protection, less efficient repair

Accumulation of unrepaired DNA breaks and single stranded DNA



Mitotic entry
Mitotic catastrophe

RPA pool exhaustion= Replicative catastrophe



Thank you for your attention

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