



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

Imene TABET
2020/2021

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**

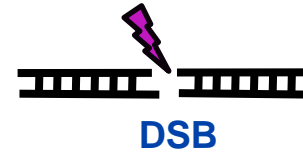
**Defective Homologous
Recombination repair (HRR)**

**Unrepaired DNA
breaks**

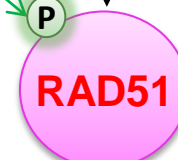
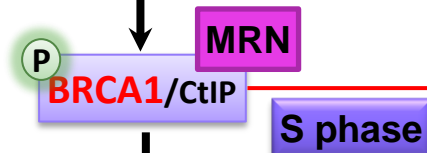
**Sensitivity to
Genotoxic drugs**

Homologous Recombination Pathway

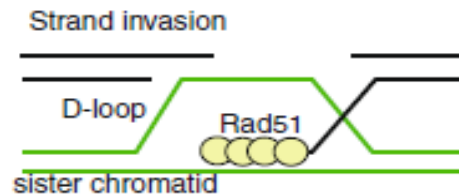
1) Damage Sensing



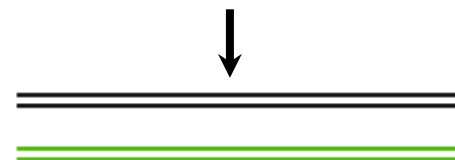
2) DNA Resection



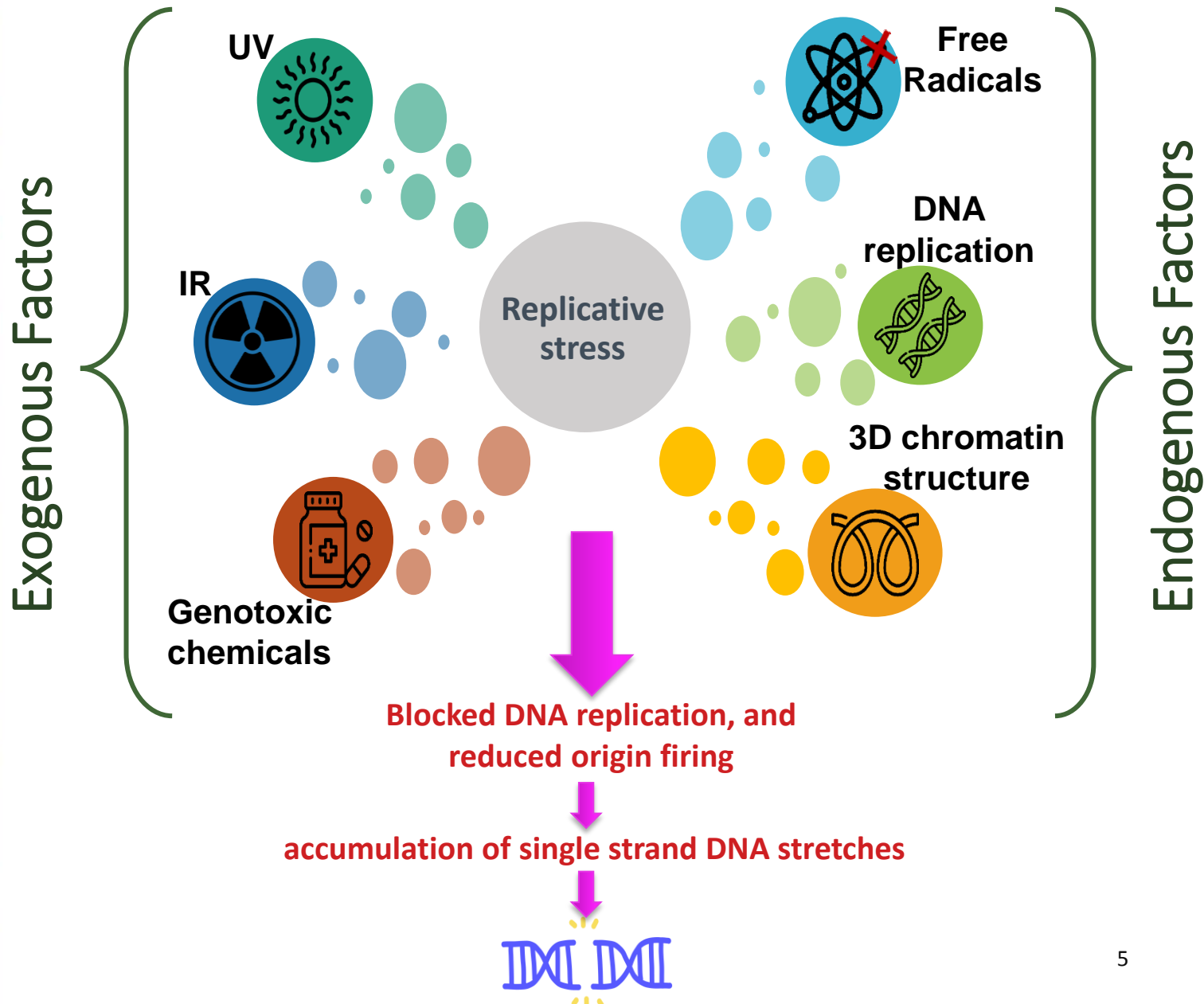
3) Strand Invasion



4) DNA Repair

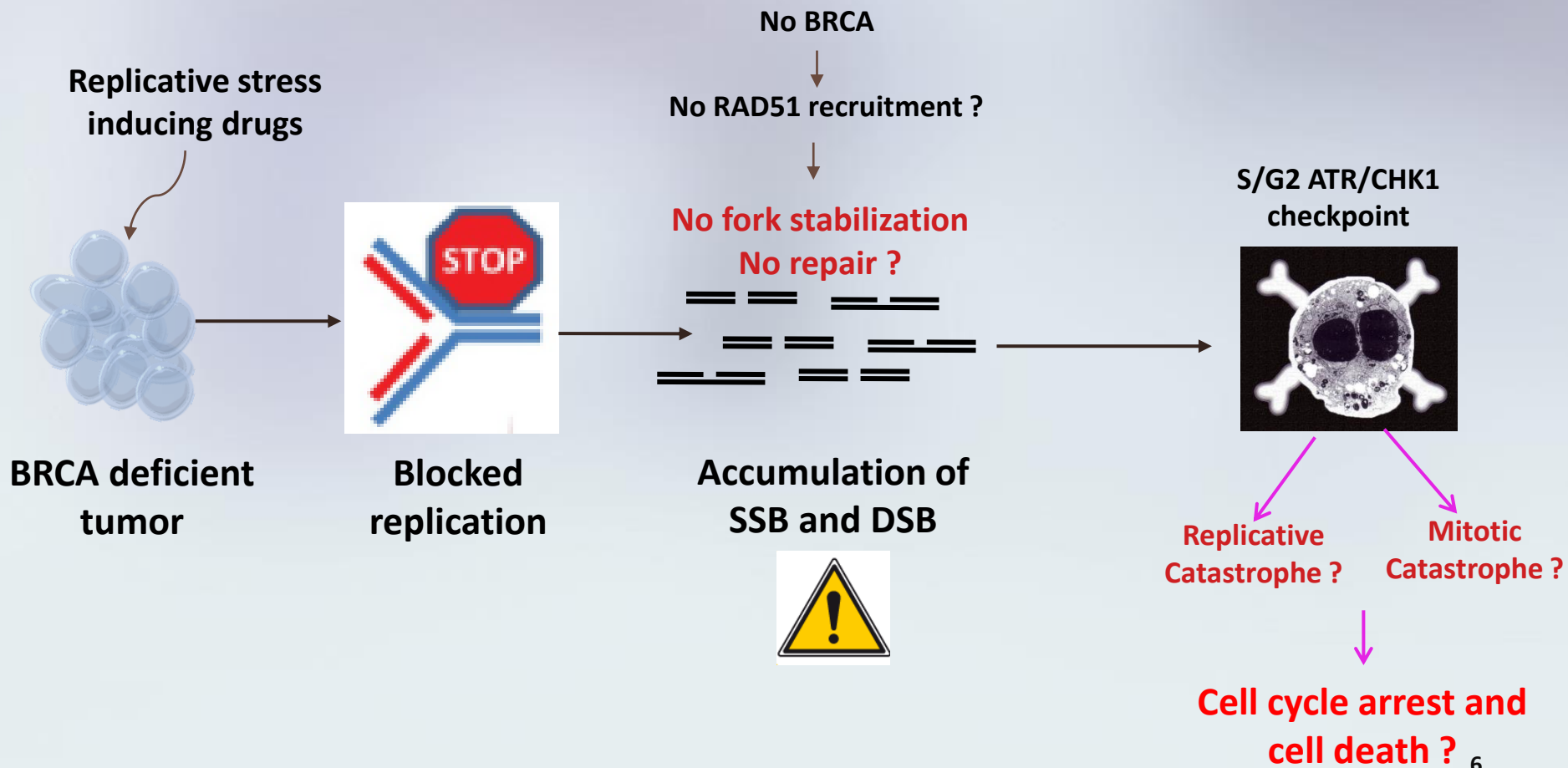


HR involmvent in replication stress response



We propose :

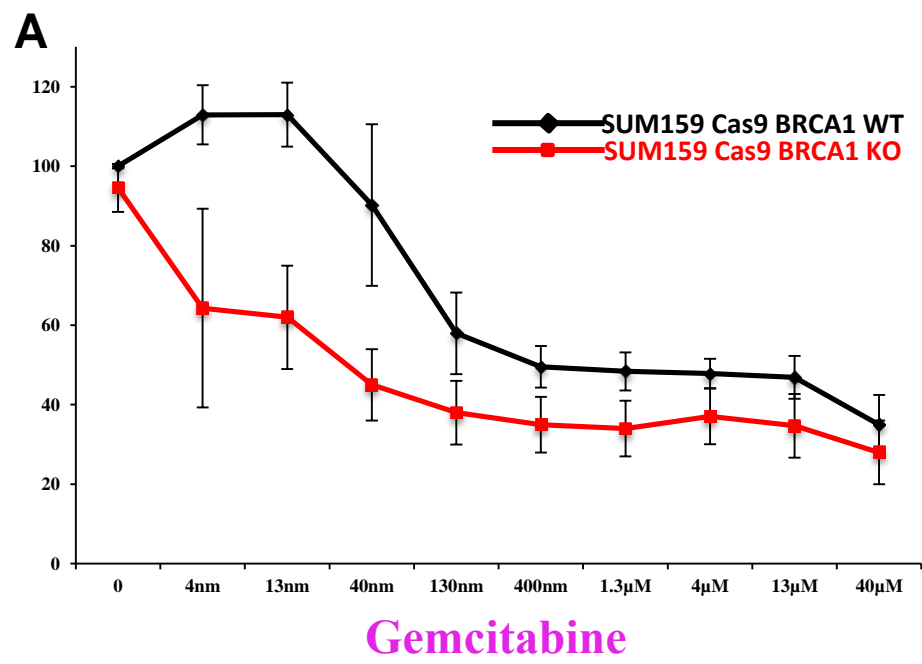
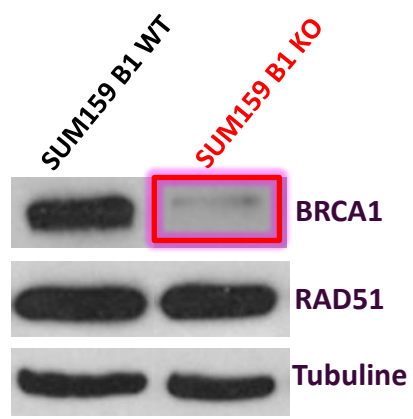
BRCA deficient tumors might be hypersensitive to replicative stress





Results, *In vitro*

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

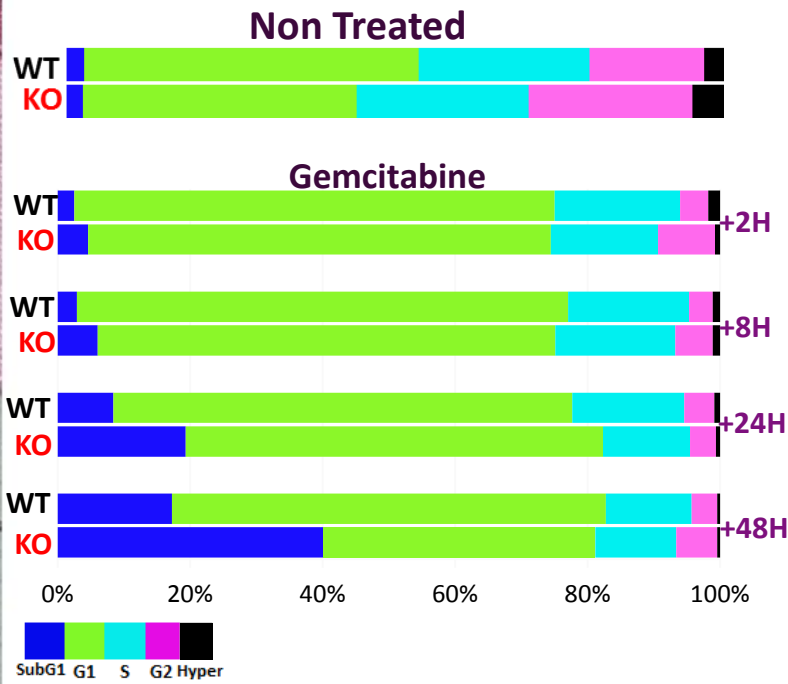
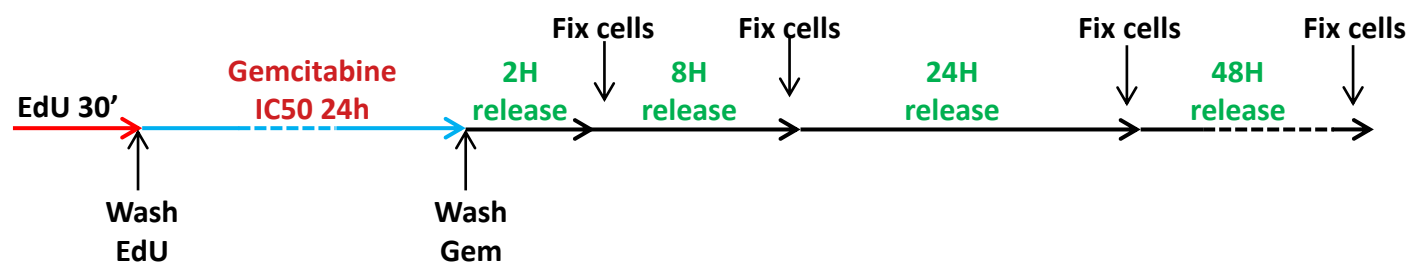


SUM159 BRCA1 KO isogenic model is more sensitive to Gemcitabine



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

1) By Flow Cytometry :

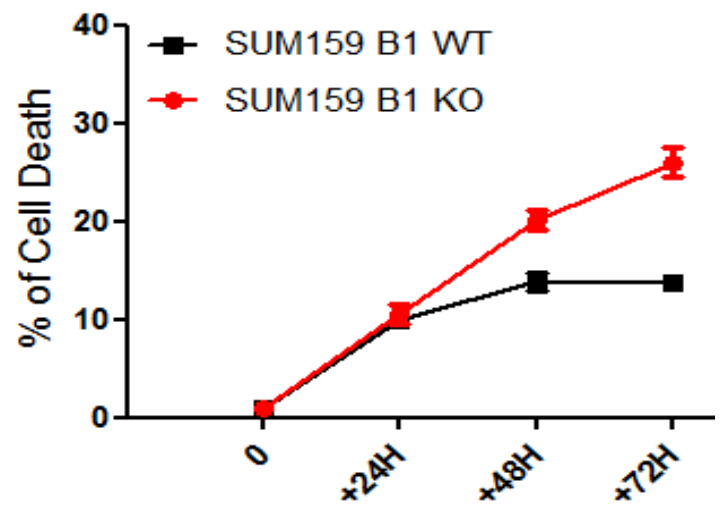
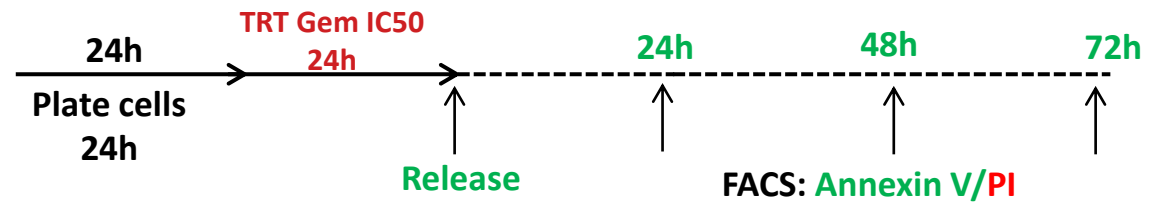


- ✓ important accumulation of cells in G1, and much less G2 than NT
- ✓ The percentage of SubG1 is twice as high in KO compared to the WT



Results, *In vitro*

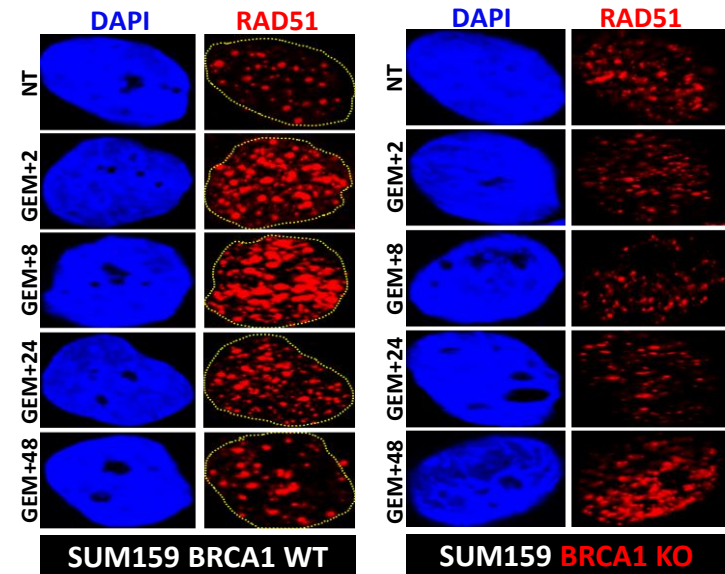
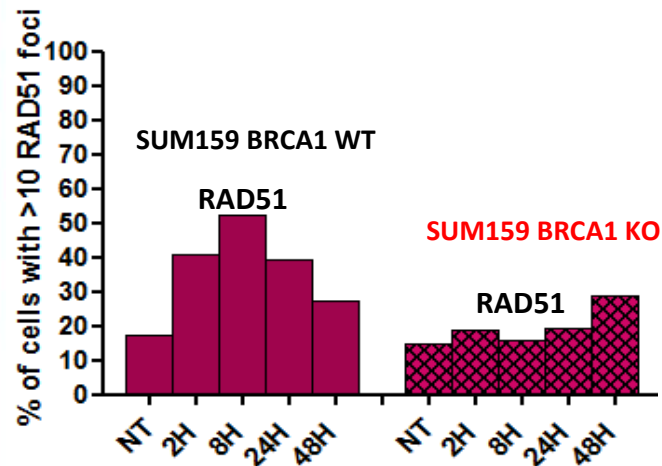
Gemcitabine induced cell death in SUM159 BRCA1 KO confirmed



Gemcitabine IC50 24H

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

SUM159 BRCA1 KO display disrupted HR upon Gemcitabine treatment

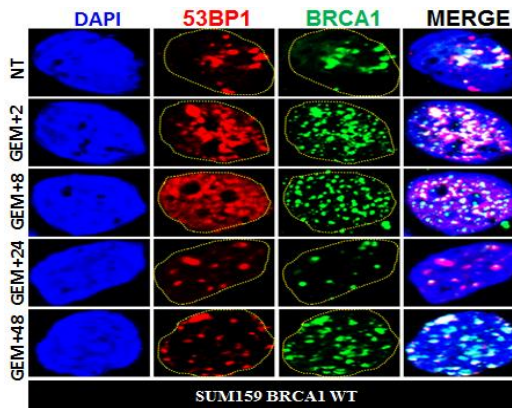
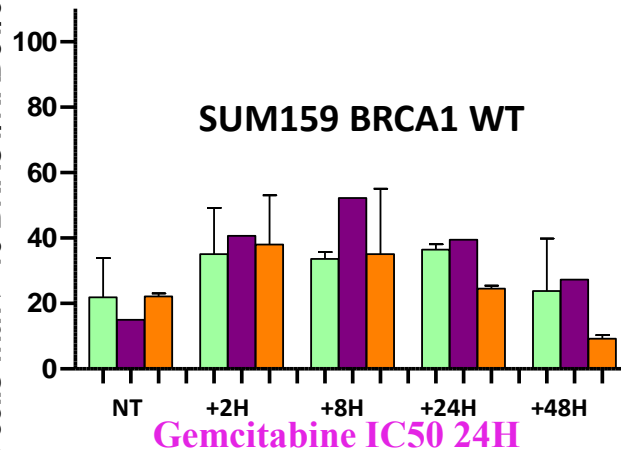


✓ Weak RAD51 foci formation in the **SUM159 BRCA1 KO** cells

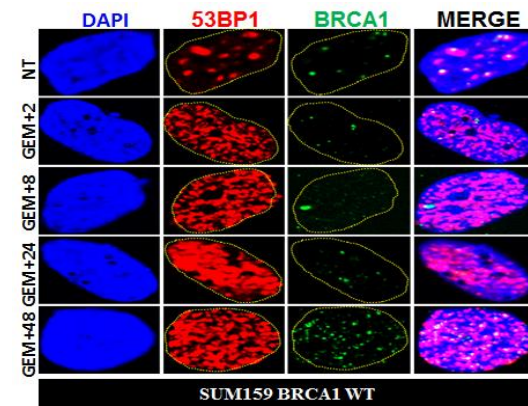
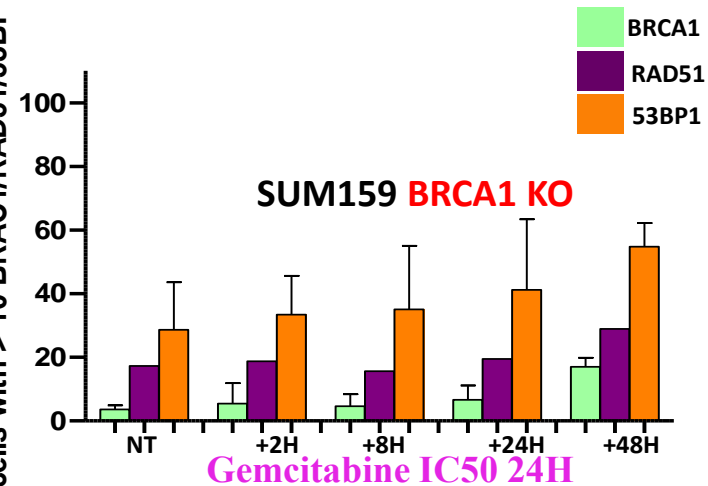
Results, *In vitro*

SUM159 BRCA1 KO isogenic model displays disrupted HR upon Gemcitabine treatment, and seem to engage in a non homologous recombination pathway

% of cells with > 10 BRAC1/RAD51/53BP1 foci

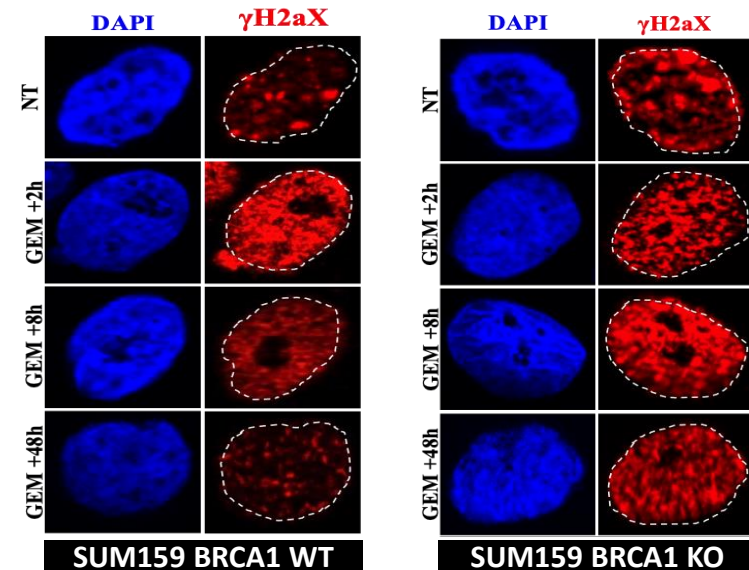
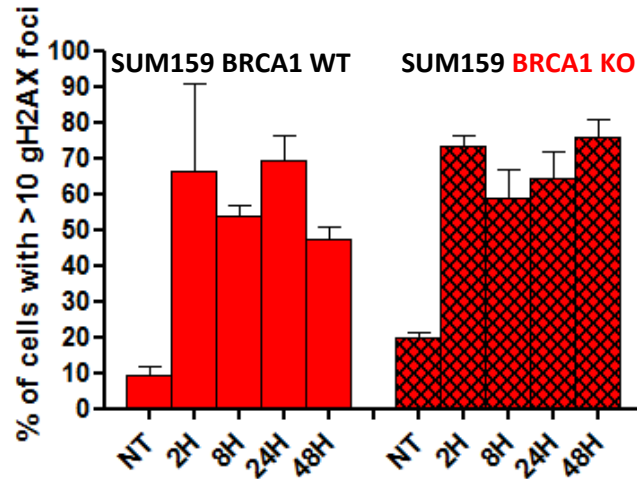


% of cells with > 10 BRAC1/RAD51/53BP1 foci



✓ Progressive increase of cells with 53BP1 foci in **SUM159 BRCA1₁KO**

SUM159 BRCA1 KO display persistent DNA damage upon Gemcitabine treatment

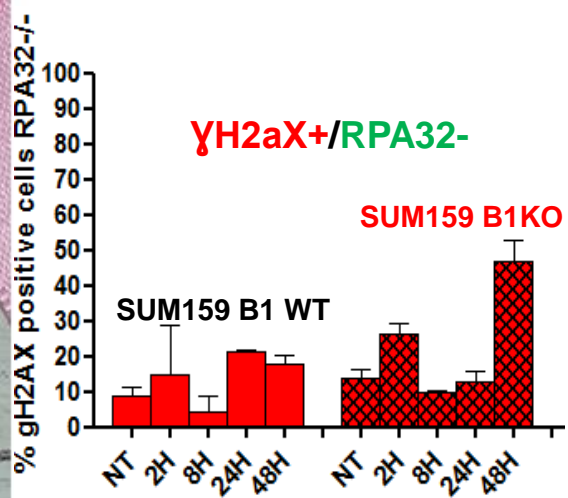
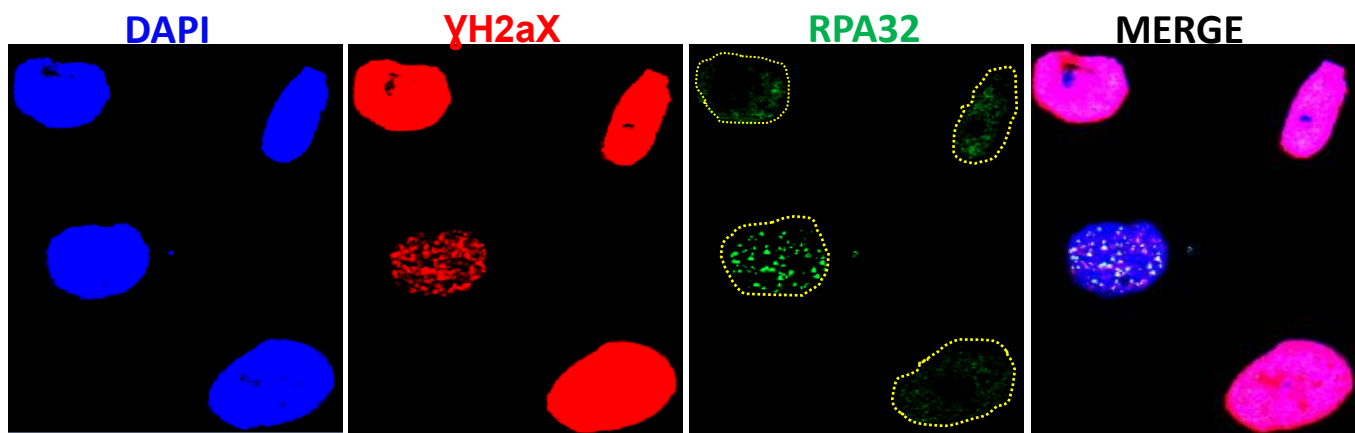


✓ % of γH2AX positive cells doesn't decrease even at +48h post release in **SUM159 BRCA1 KO**

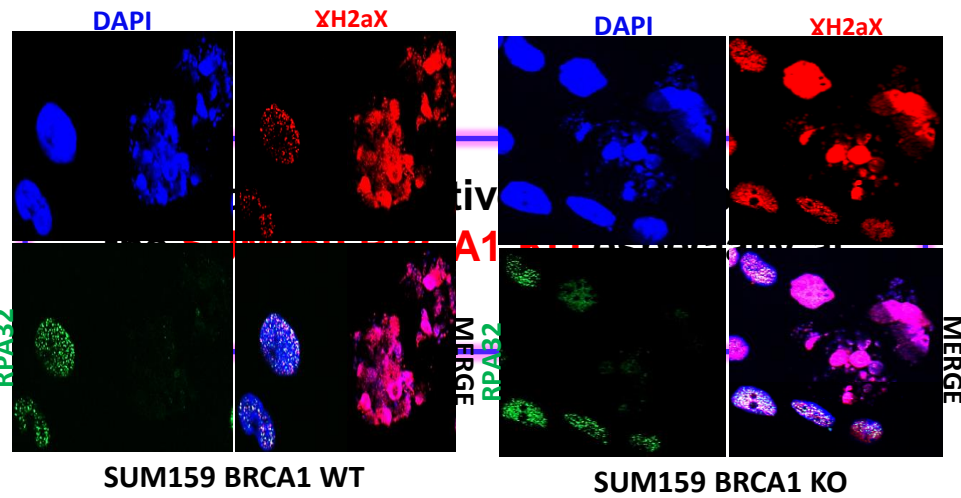


Results, *In vitro*

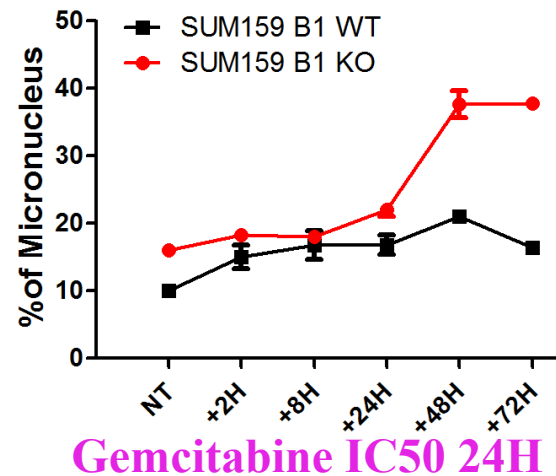
SUM159 BRCA1 KO display more gH2AX+/RPA- cells upon Gemcitabine treatment



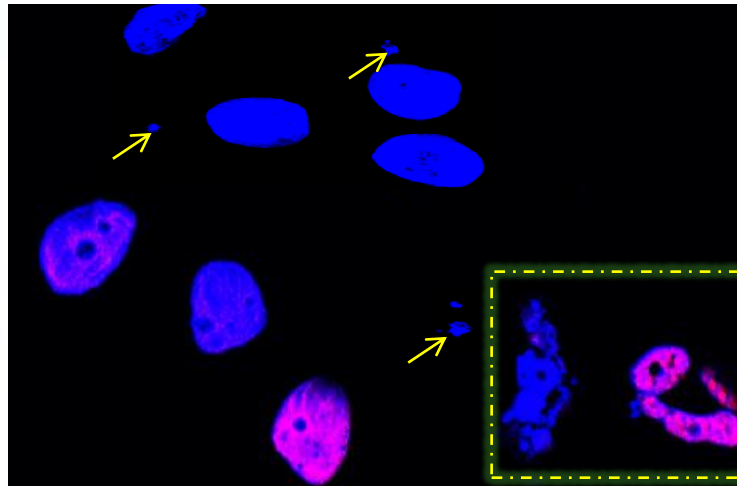
Gemcitabine IC50 24H



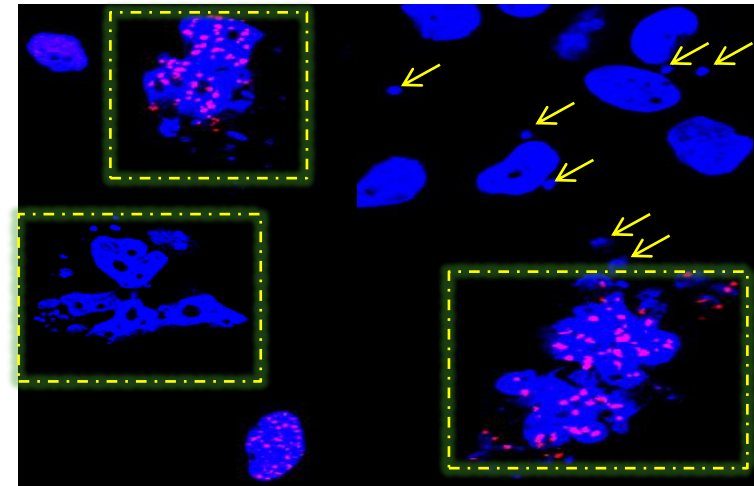
SUM159 BRCA1 KO present more mitotic aberrations upon Gemcitabine treatment



WT Gem+72h



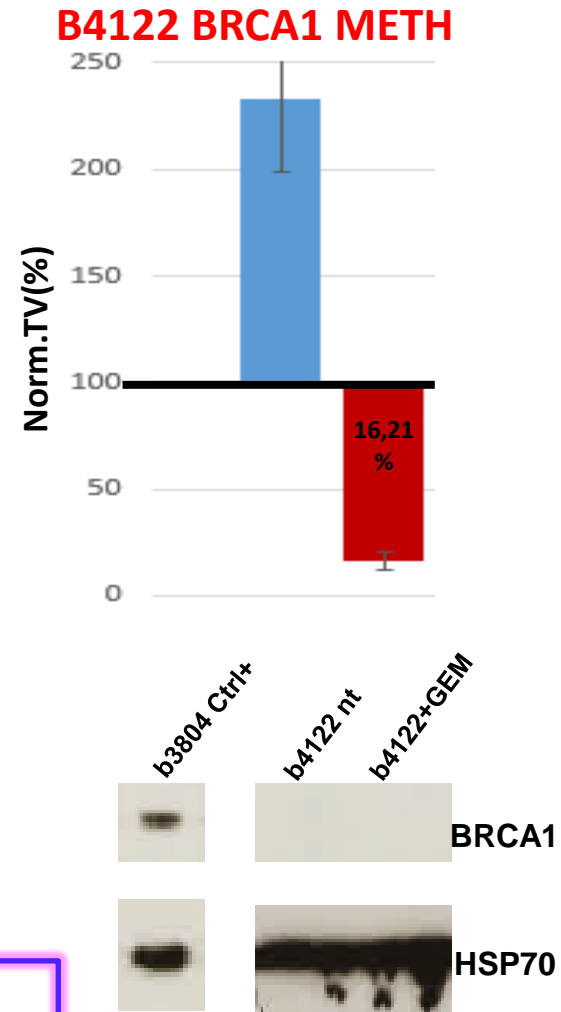
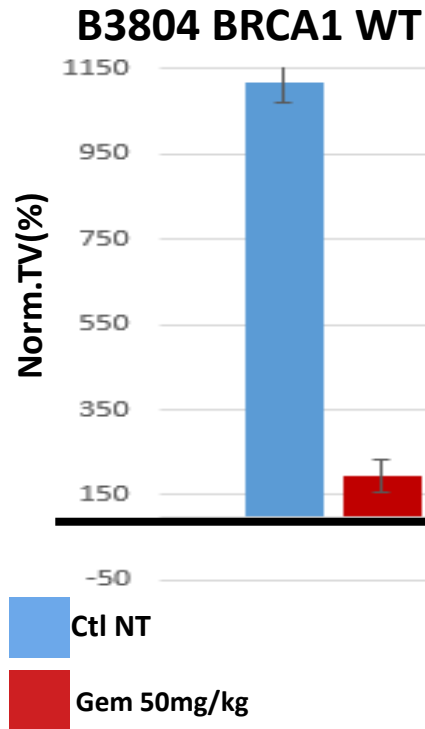
KO Gem+72h



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

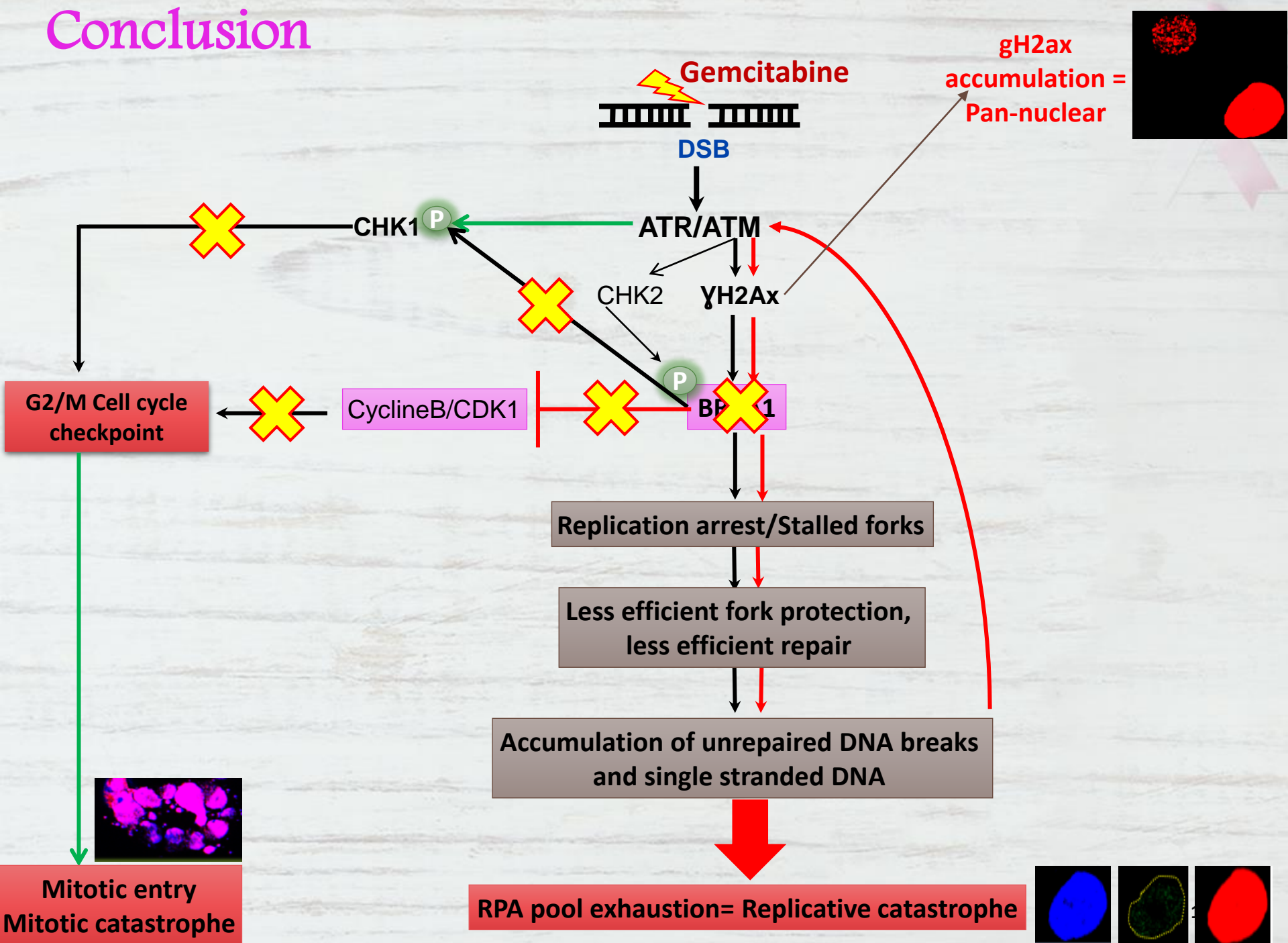
Results, *In vivo*

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



In vitro results confirmed *in vivo*

Conclusion



Thank you for your attention

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Sophie Baize
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