**Supplementary Data**

**BRCA2 and RAD51 promote double strand break formation and cell death in response to Gemcitabine**

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**Supplementary Materials and Methods**

**Western blotting**

Primary antibodies were anti-ATM phospho-S1981 (R&D systems, 1:1000), mouse monoclonal anti-ATM (11G12 mAb made in house, 1:500), anti-SMC1 phospho-S966 (Bethyl, 1:1000), anti-SMC1 (Bethyl, 1:1000), anti-NBS1 phospho-S343 (Abcam, 1:1000), anti-NBS1 (Genetex, 1:10000), anti-CHK1 phospho-S345 (New England Biolabs, 1:1000), anti-CHK1 (Santa Cruz, 1:1000), anti-RPA2 phospho-S4/8 (Bethyl, 1:1000), anti-RPA2 (Merck, 1:1000), anti-γH2AX (Millipore, 1:1000), anti-H2A (Millipore, 1:1000), anti-CDK phospho-T14/Y15 (Santa Cruz Biotechnology, 1:300) and anti-Chk1 phospho-S317 (Cell Signalling, 1:300)

**Supplementary Figures**



**Figure S1: VC8 cells are hypersensitive to cisplatin and RAD51 depletion in BxPC3 pancreatic cancer cells reduces sensitivity to Gemcitabine.**

**(A)** Clonogenic survival of VC8-B2 (+ BRCA2) and VC8 (- BRCA2) cells treated with cisplatin. **(B)** Clonogenic survival of BxPC3 cells ± RAD51 siRNA treated with Gemcitabine for 2 h and released into fresh medium (n=4). Error bars: SEM; Asterisks: \* p<0.05, Student’s t-test.



**Figure S2: Replication restart is not due to loss of checkpoint signalling**

**(A)** Levels of phospho-S1981-ATM, ATM, phospho-S966-SMC1, SMC1, phospho-S343-NBS1, NBS1, phospho-S4/S8-RPA2, RPA2, γH2AX, CHK1, phospho-S345-CHK1 and H2A (loading control) in U2OS and H1299 cells after release from 2 µM Gemcitabine (2 h). **(B)** Levels of phospho-S317-CHK1 and β-Actin (loading control) in U2OS and VC8-B2 cells after release from Gemcitabine. **(C)** Levels of phospho-Y15-CDK and αTubulin (loading control) in U2OS cells after release from Gemcitabine. Relative levels of phospho-CHK1 and phospho-CDK were quantified by densitometry using Image J.



**Figure S3: Pulse-field gel electrophoresis shows DSB induction after release from Gemcitabine.**

**(A)** DSB levels measured by pulsed-field gel electrophoresis (PFGE) shown in Figure 4C. Error bars: SEM; Asterisks (compare to Con): \*\* p<0.01, Student’s t-test.

**(B)** The pulse-field gel that was cropped for Figure 4D and used as part of the analysis in Figure 4E and 5B. Bands shown in Figure 4E are outlined in grey boxes.



**Figure S4: Gemcitabine-induced double strand breaks are RAD51-dependent in pancreatic cancer cell line BxPC3.**

**(A)** Protein levels of RAD51 and α tubulin (loading control) in BxPC3 cells transfected with RAD51 or NonT siRNA for 24 h, incubated with 5 µM Gemcitabine for 2 h, and released into fresh medium for times indicated. **(B)** Percentages of BxPC3 cells displaying more than 10 53BP1 foci after treatment as in (A). **(C)** Quantification of increase in cells displaying more than 10 53BP1 foci as in (B).Error bars: SEM; Asterisks: \* p<0.05, \*\* p<0.01, Student’s t-test.



**Figure S5: Gemcitabine-induced double strand breaks are RAD51-dependent in ovarian cancer cell line OVCAR3.**

**(A)** Protein levels of RAD51 and α tubulin (loading control) in OVCAR3 cells transfected with RAD51 or NonT siRNA for 24 h, incubated with 5 µM Gemcitabine for 2 h, and released into fresh medium for indicated times. **(B)** Percentages of OVCAR3 cells displaying more than 10 53BP1 foci after treatment as in (A). **(C)** Quantification of increase in cells displaying more than 10 53BP1 foci as in (B).Error bars: SEM; Asterisks: \* p<0.05, \*\* p<0.01, Student’s t-test.



**Figure S6: Gemcitabine-induced double strand breaks are RAD51-dependent in ovarian cancer cell line MCF7.**

**(A)** Protein levels of RAD51 and α tubulin (loading control) in MCF7 cells transfected with RAD51 or NonT siRNA for 24 h, incubated with 5 µM Gemcitabine for 2 h, and released into fresh medium for indicated times. **(B)** Percentages of MCF7 cells displaying more than 10 53BP1 foci after treatment as in (A). **(C)** Quantification of increase in cells displaying more than 10 53BP1 foci as in (B).Error bars: SEM; Asterisks: \* p<0.05, Student’s t-test.

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**Figure S7: Pulse-field gel electrophoresis shows higher levels of DSB induction after release from Gemcitabine compared to continuous treatment.**

**(A)** Schematic for release versus continuous treatment. Cells were either treated with 2 μM Gemcitabine for 2 h and released for 16h, or treated with 2 μM Gemcitabine for 18 h continuously before processing for PFGE. **(B)** DSB levels measured by PFGE in U2OS, VC8-B2 and VC8 cells treated as in (A).