**SUPPLEMENTARY INFORMATION**

**Supplementary Table 1: Summary of Viability of *Brca2*$^{ko/ko}$ mESC Expressing BRCA2 VUS**

| **Variant** | **Clone1** (% of viable clones) | **Clone2** (% of viable clones) | **WT** (% of viable clones) |
|---|---|---|---|
| c.1600G>A (E534K) | 19.82 | 27.42 | 28.78 |
| c.3782C>G (S1261C) | 11.84 | 9.90 | 11.71 |
| c.6322C>T (R2108C) | 21.58 | 30.06 | 17.10 |
| c.6929C>A (T2310N) | 6.42 | 7.54 | 7.85 |
| c.8356G>A (A2786T) | 15.10 | 15.75 | 15.59 |
| c.8393C>T (P2798L) | 11.76 | 11.66 | 11.71 |
| c.9104A>G (Y3035C) | 16.80 | 15.4 | 17.65 |
| c.9104A>T (Y3035F) | 12.72 | 13.07 | 14.12 |
| c.9104A>C (Y3035S) | 13.77 | 19.01 | 10.14 |
| c.9106C>G (Q3036E) | 17.0 | 13.5 | 14.76 |
| c.9344A>G (K3115R) | 7.76 | 10.68 | 12.58 |
| c.9538C>T (L3180F) | 35.02$^{*}$ | 26.09$^{**}$ | 12.94$^{*}$ and 15.52$^{**}$ |
| c.9907A>T S3303C) | 11.90 | 8.67 | 11.77 |
| c.68-7T>A (IVS2-7T>A) | 11.55 | 10.83 | 9.60 |
| c.632-10dupT (IVS7-10insT) | 13.46 | 10.2 | 11.50 |
| c.8954-5_8954-2delAACA (IVS22-5delAACA) | 13.11$^{*}$ | 31.78$^{**}$ | 5.31$^{*}$ and 12.80$^{**}$ |

$^{*}$, $^{**}$Respective WT controls

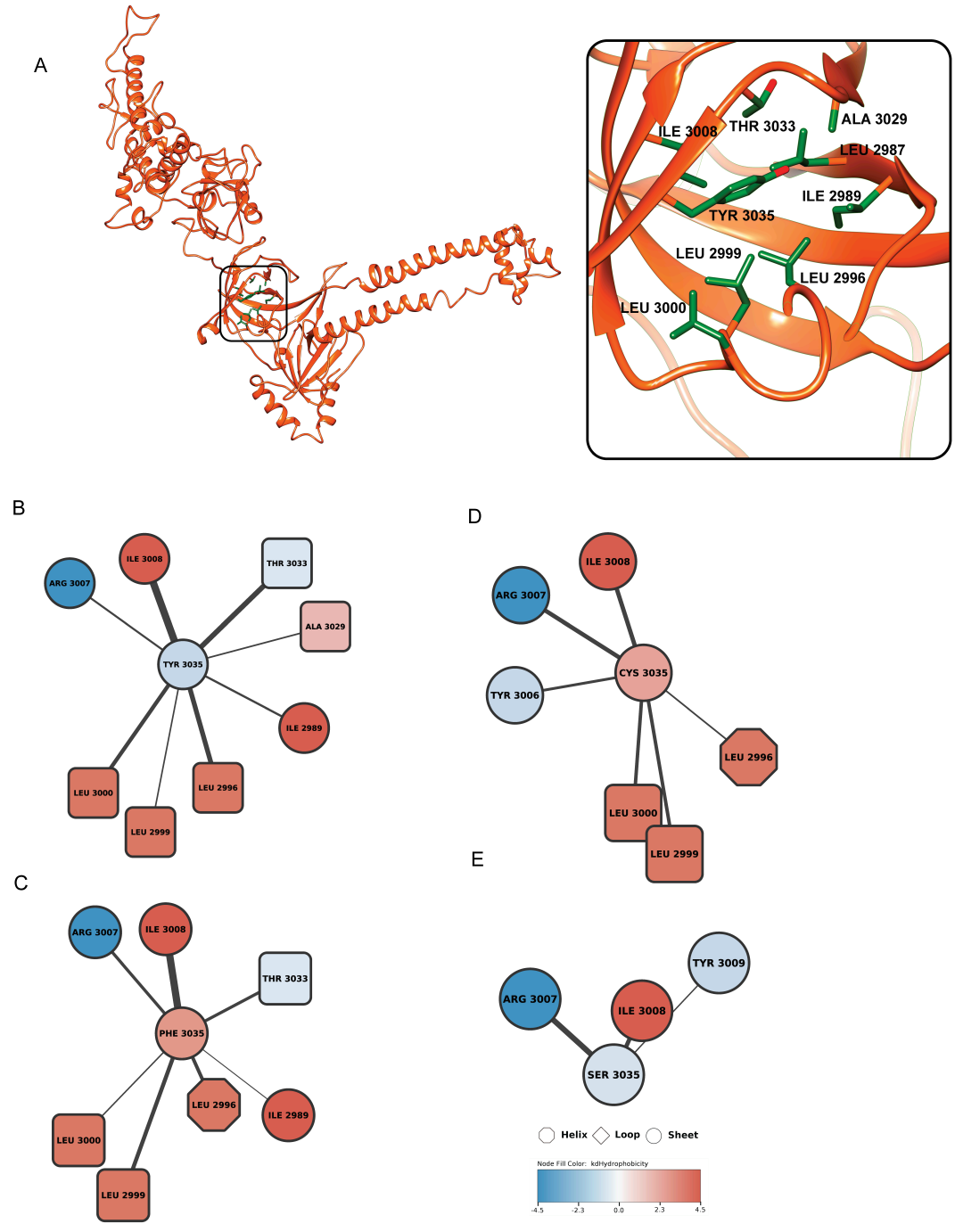
Viability was calculated by determining as the percentage of HAT resistant (HAT$^{R}$) ES cell colonies that survived after CRE-mediated recombination over the total number cells that were plated (cell grown without HAT selection). For two of the variants (L3180F and IVS22-5delAACA), the two clones were tested separately for cell viability, each with their own WT control.

**Supplementary Figure 1**

**Structural analysis of Y3035S/C/F variants**

**A.** Modelled structure of Human BRCA2. The highlighted region shows Tyr3035 variant site along with its hydrophobic interactions. Residue Interaction Network obtained from MD simulations of WT **(B)**, Y3035F **(C)**, Y3035C **(D)** and Y3035S **(E)**. The node color indicates hydrophobicity (Red-blue scale (hydrophobic to hydrophilic)). The node shape indicates the secondary structure (circle (sheet), Diamond (Loop), Octagon (helix)). The node location is proportional to the relative location on amino acids in 3D coordinates. Edge length is proportional to the distance between amino acids. Edge width/color is proportional to the number of frames in the trajectory in which the contact is present.

**Supplementary Fig. 1**



**Supplementary Figure 2**

**Effect of potential splice site BRCA2 variants on normal splicing.**

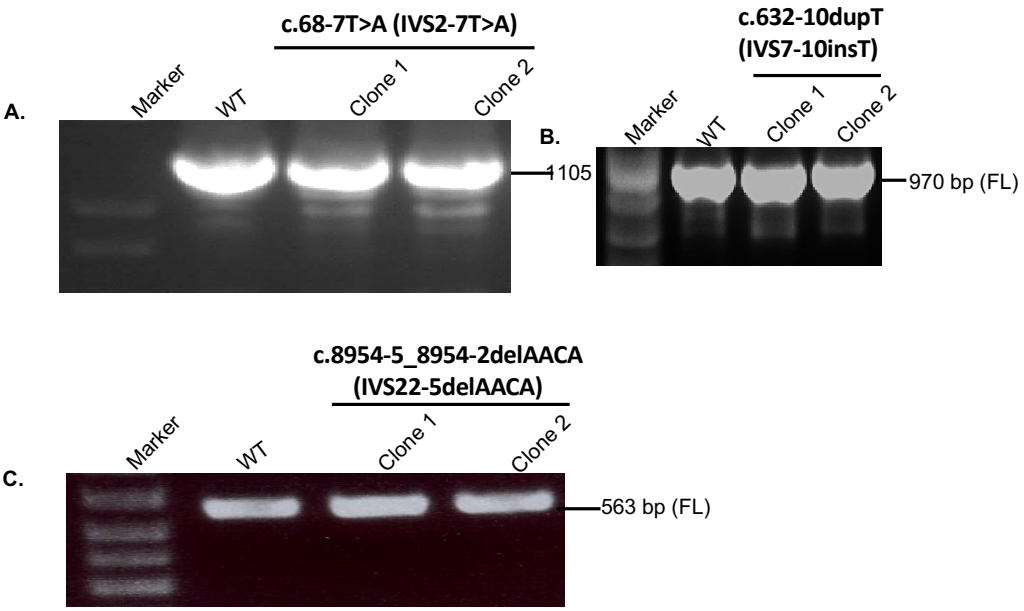
RT-PCR analysis to examine the expression of full-length *BRCA2* and alternatively spliced transcripts in mouse ES cells potential splice site variants.

**A.** IVS2-7T>A expressing mES cells were examined by using PCR primers from exons 1 and 10.

**B.** IVS7-10insT expressing mES cells were examined by using PCR primers from exons 2 and 10.

**C.** IVS22-5delAACA expressing mES cells were examined by using PCR primers from exons 21 and 25. In every case, mES cells expressing WT *BRCA2* were used as control.

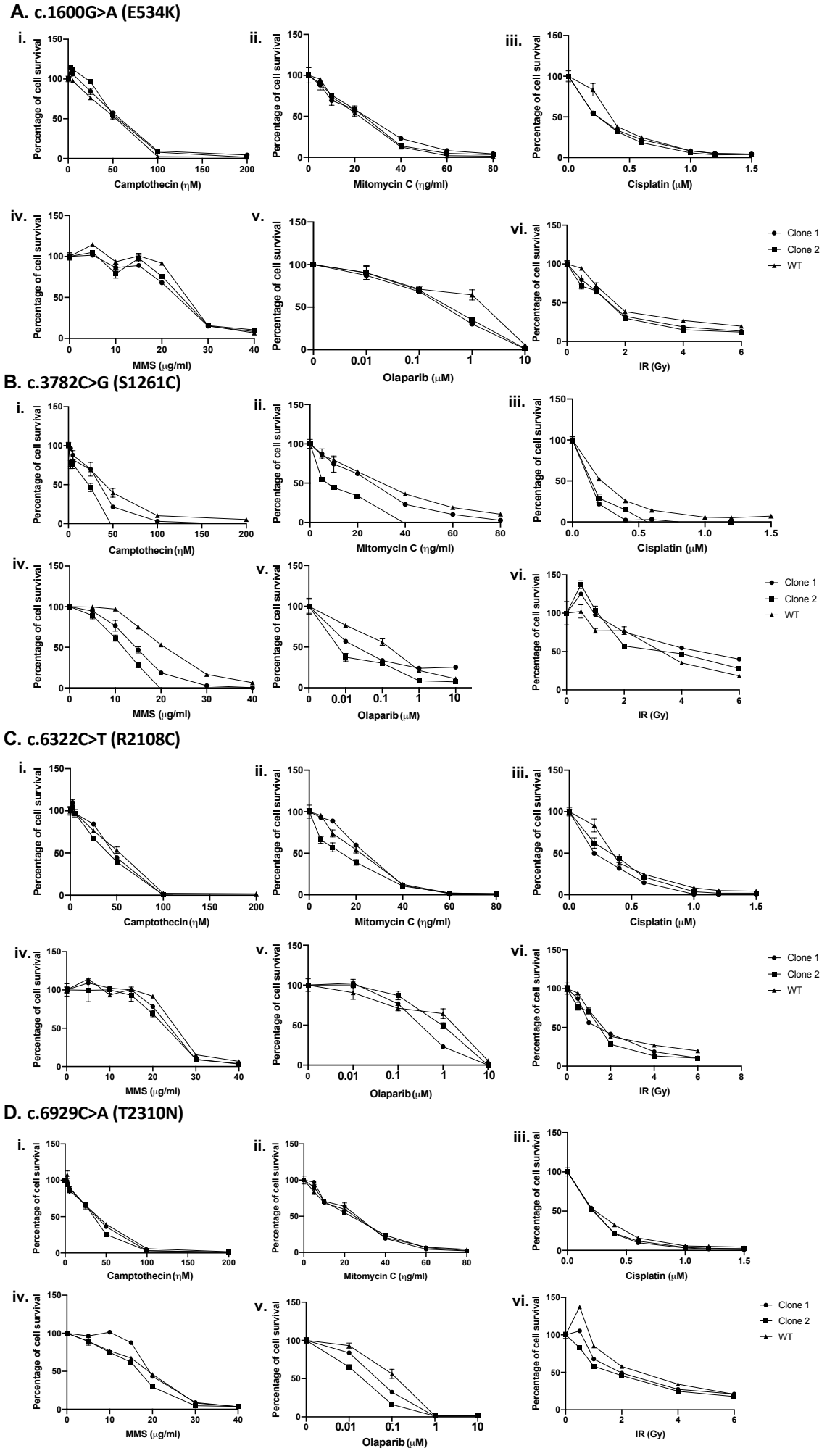
**Supplementary Fig. 2**



**Supplementary Figure 3**

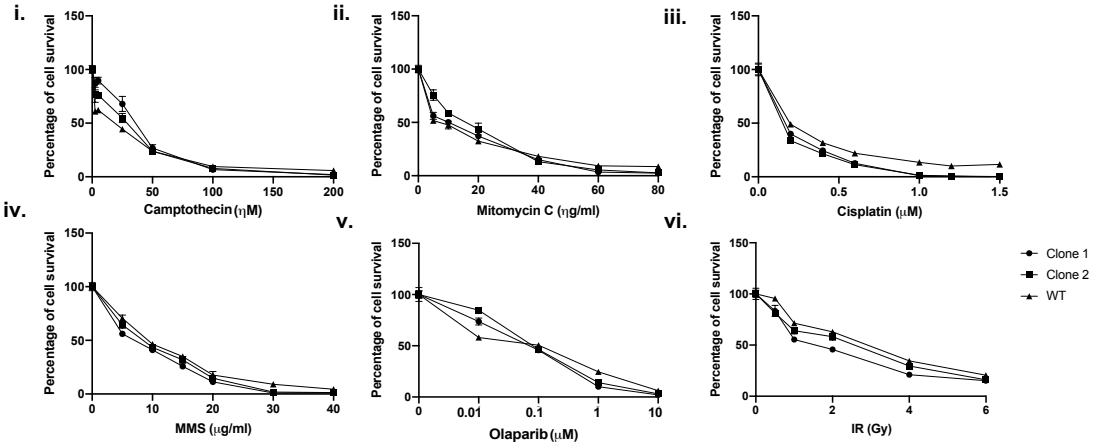
Assessment of sensitivity of *Brca2*$^{ko/ko}$ mouse ES cells expressing BRCA2 variants (**A.** E534K, **B.** S1261C, **C.** R2108C, **D.** T2310N, **E.** A2786T, **F.** P2798L, **G.** Y3035C, **H.** Y3035F, **I.** Y3035S, **J.** Q3036E, **K.** K3115R, **L.** K3180F, **M.** S3303C, **N**. IVS2-7T>A, **O**. IVS7-10insT, **P**. IVS22-5delAACA) following exposure to six DNA damaging agents. XTT assay was performed on cells following 72 hrs. of treatment with camptothecin (i) Mitomycin C (ii), cisplatin (iii), MMS (iv), olaparib (v), and γ-irradiation (IR, vi). *Brca2*$^{ko/ko}$ mouse ES cells expressing WT BRCA2 were used as control. Two independently generated mES cells were used for each variant.

**Supplementary Fig. 3**

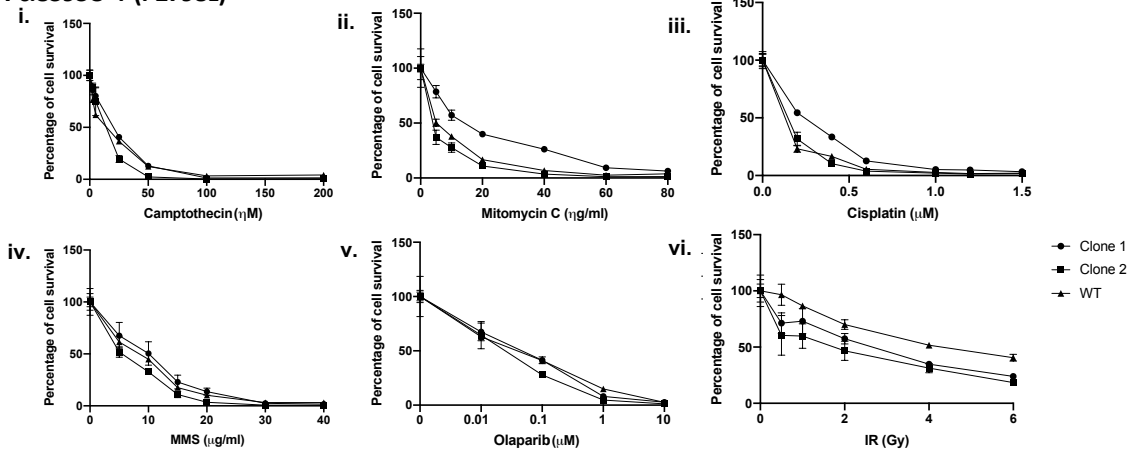


**Supplementary Fig 2 continued**

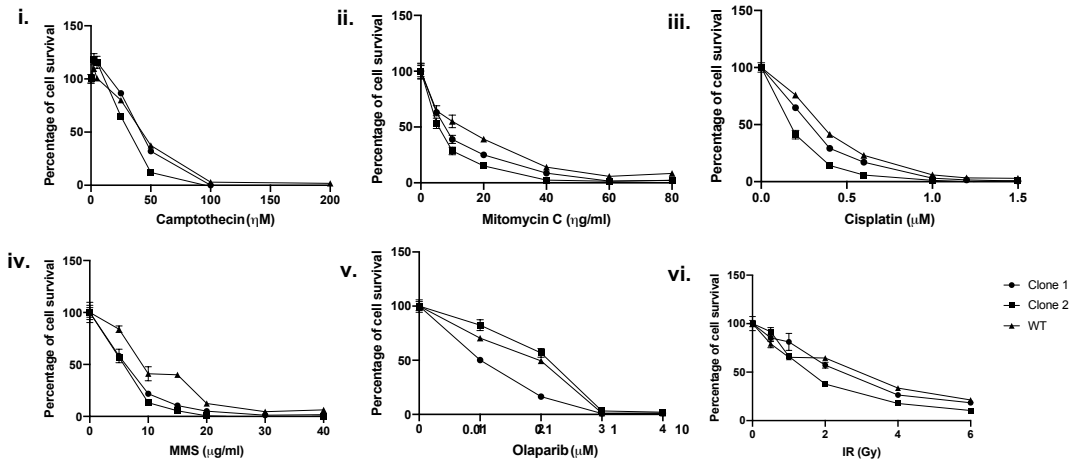
**E. c.8356G>A (A2786T)**



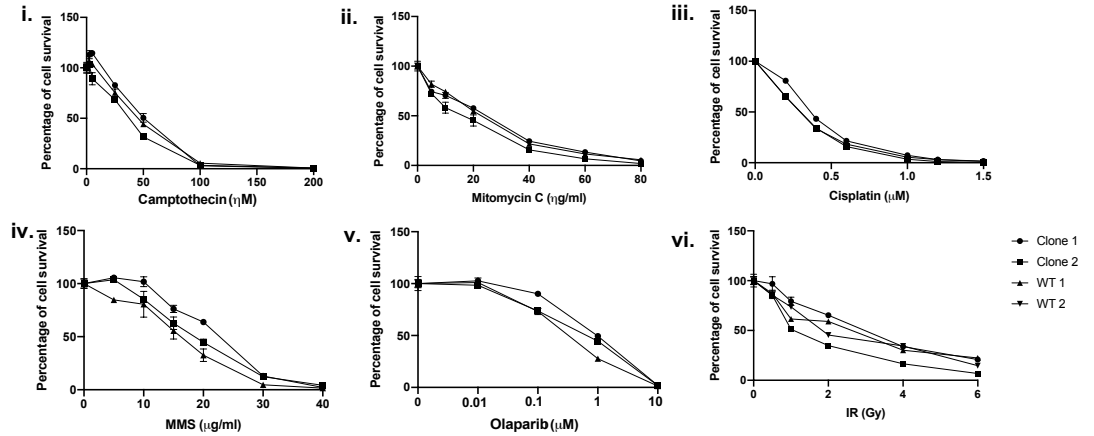

**F. c.8393C>T (P2798L)**




**G. c.9104A>G (Y3035C)**

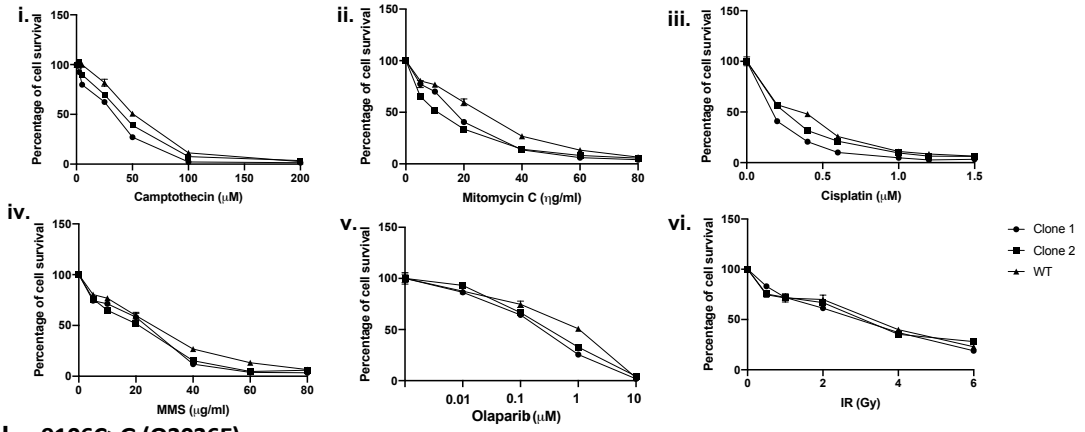



**H. c.9104A>T (Y3035F)**

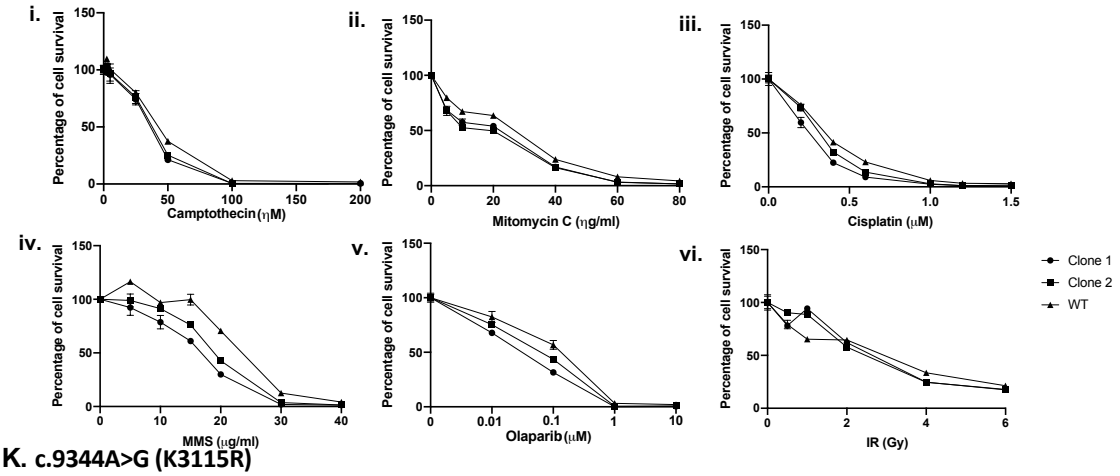


## Supplementary Fig 2 continued

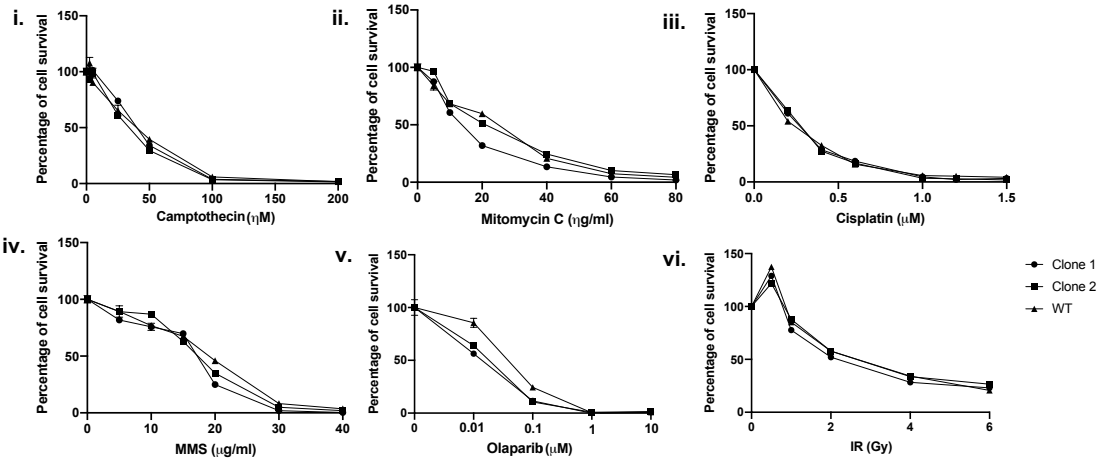
### I. c.9104A>C (Y3035S)



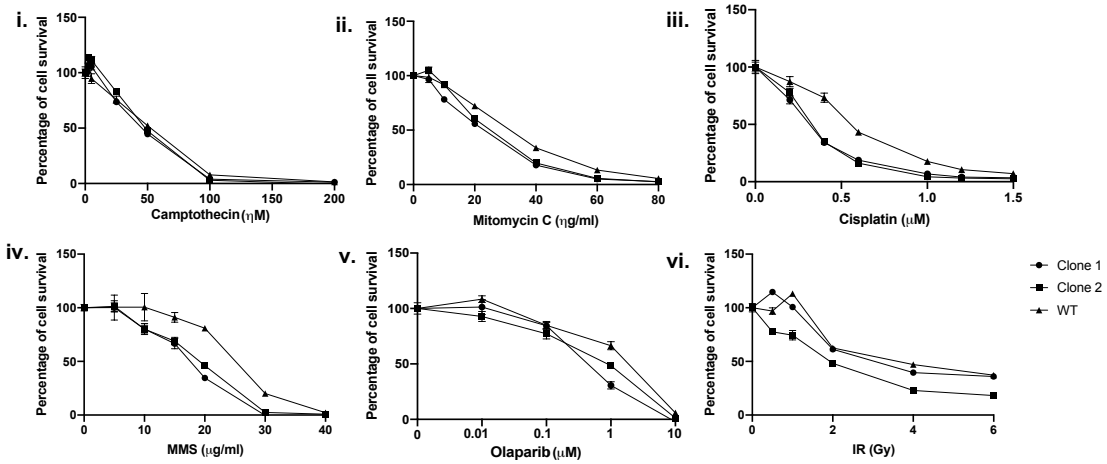

### J. c.9106C>G (Q3036E)




### K. c.9344A>G (K3115R)

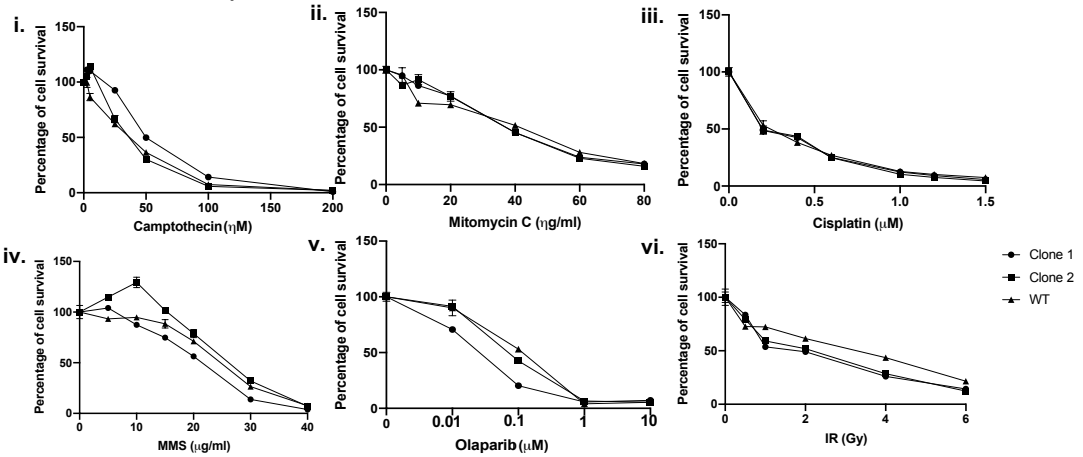



### L. c.9538C>T (L3180F)

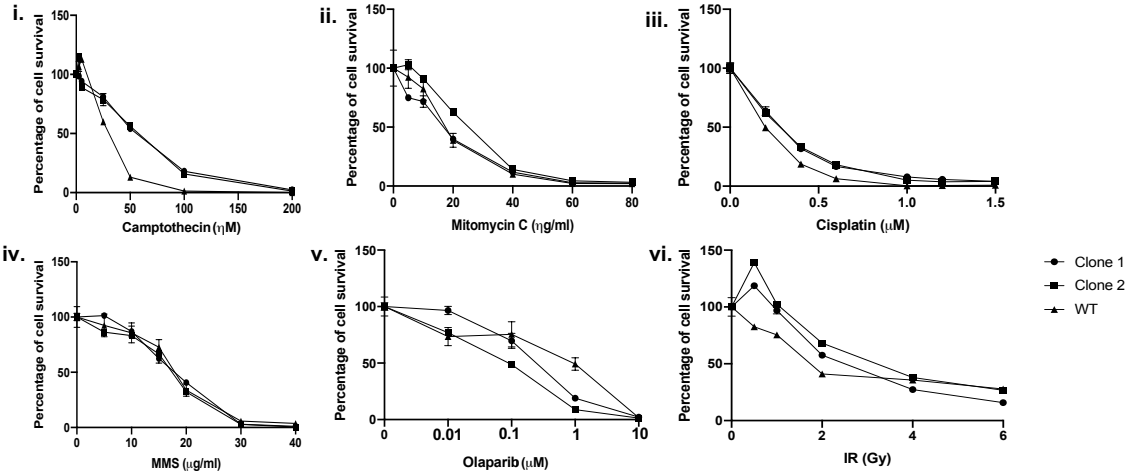


**Supplementary Fig 2 continued**

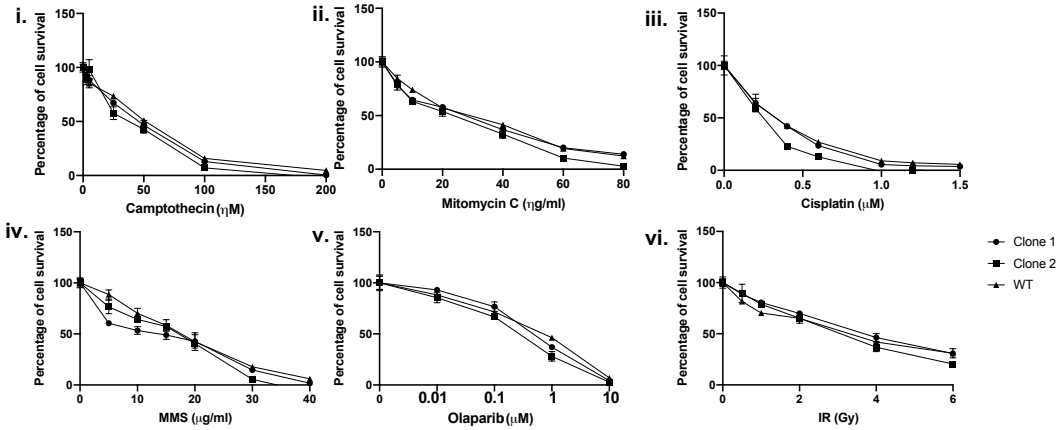
**M. c.9907A>T S3303C)**




**N. c.68-7T>A (IVS2-7T>A)**




**O. c.632-10dupT (IVS7-10insT)**




**P. c.8954-5_8954-2delAACA (IVS22-5delAACA)**