Phosphorylation of ribosomal protein S6 confers PARP inhibitor resistance in BRCA1-deficient cancers

Supplemental Information

S-Fig. 1: S6 phosphorylation is increased in BRCA1 deficient cells with 14-day olaparib treatment. SUM149 cells (BRCA1-inactive) were treated with 10 nM olaparib with indicated times. Whole-cell lysates were prepared and analyzed by Western blotting with the indicated antibodies.
**S-Fig. 2: Measurement of PAR levels and PARP activity.** HCC1937 parental cells and resistant clones were treated with 1 µM olaparib for 24 h. PAR levels and PARP activity were measured in cell lysates by Western blot with indicated antibody in the absence of activated DNA.

**Genomic DNA sequence**

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\begin{array}{ccccccc}
235 & & & & & & \\
S & S & S & S & S & S & \\
\text{Wild-type S6 DNA} & \ldots\ldots\text{TCCTCT} & \ldots\ldots\text{TCT} & \ldots\ldots\text{TCT} & \ldots\ldots\text{AGT} & \\
\text{S6P+ DNA} & \ldots\ldots\text{GCCGCT} & \ldots\ldots\text{GCT} & \ldots\ldots\text{GCT} & \ldots\ldots\text{GCT} & \\
\end{array}
\]

**S-Fig. 3: Knock-in of S6\textsuperscript{P-/-} in HCC1937 cells by ZFNs.** Exon 5 of S6 gene wild-type and S6\textsuperscript{P-/-} sequencing results.
S-Fig. 4: Decrease of $\gamma$H2AX and increase of RAD51 foci in PARP resistant cells. Immunofluorescence images show $\gamma$H2AX and RAD51 foci in HCC1937 parental and resistant cells with 2 Gy IR-treatment for 2 h. Quantitative data show the histogram of cells with $\gamma$H2AX foci ($n=200$).
S-Fig. 5: Detection of γH2AX and RAD51 foci in $S^6_{+/−}$ cells and PARP resistant cells.

Quantitative data show the histogram of cells with γH2AX and RAD51 foci ($n=200$)
S-Fig. 6: Rapamycin restores sensitivity of HCC1937 resistant clones to the PARP inhibitor. Colony formation assay was performed in HCC1937 parental and olaparib resistant clone cells (OR2 and OR3) with 1 µM rapamycin and/or 10 nM olaparib treatment (n=3, mean±SEM of colonies formed relative to DMSO-treated cells).

S-Fig. 7: Olaparib treatment does not change the level of 53BP1 protein in BRCA1 deficient cells. HCC1937 cells were treated with 10 nM olaparib with indicated times. Whole-cell lysates were prepared and analyzed by Western blotting with the indicated antibodies.