Legends to Supplementary Figures and Tables

Supplementary Table S1. List of 99 CRC cell lines screened for sensitivity to olaparib. Mutational status for KRAS, NRAS and BRAF is indicated.

Supplementary Table S2. BRCA1 and BRCA2 status in olaparib resistant and sensitive CRC cell lines. We considered the 10 most sensitive and the 10 most resistant (olaparib 5 µM) cell lines and analyzed their BRCA1 and BRCA2 genetic profiles with a clinical-grade test. VUS: Variant of Unknown Significance.

Supplementary Table S3. Predicted functional impact of SNV in HR genes identified in CRC cell lines. For each cell line, SNVs identified by exome analysis that are not synonymous and with VAF > 10% are listed. For each mutation, we report the number of occurrences in the COSMIC database, the gene symbol and its description, the effect of variation along with nucleotide and amino acid change, the genomic coordinates, the accession number and dbSNP annotation. Finally, SIFT and PolyPhen prediction as given by the Ensembl Variant Effect Predictor (VEP) tool are reported.

Supplementary Table S4. Clinicopathological characteristics of patients from whom organoids were derived. M: Male; F: Female; MSS: Microsatellite stable; PDX: Patient-Derived Xenograft; NA: Not Available; NM: Not Metastatic; met: metastasis. *PR achieved when oxaliplatin was given both in first-line and in re-challenge setting (fifth line). PR: partial response; Beva: bevacizumab; Pani: panitumumab.

Supplementary Fig. S1. Olaparib sensitive cells are responsive to other FDA-approved PARP inhibitors. The indicated olaparib sensitive CRC cells were seeded in 24-wells plates at day 0, the following day cells were treated with serial dilutions of olaparib, niraparib or rucapaprib (0.5-15 μM). Treatment was refreshed every week. The assay was stopped when untreated cells reached confluency (from 10 days to 2 weeks of treatment). Plates were fixed with 4% paraformaldehyde (Santa Cruz) and stained with 1% crystal violet-methanol solution (Sigma-Aldrich). HROC80 and SW480 are olaparib-resistant cells that were included as negative controls.

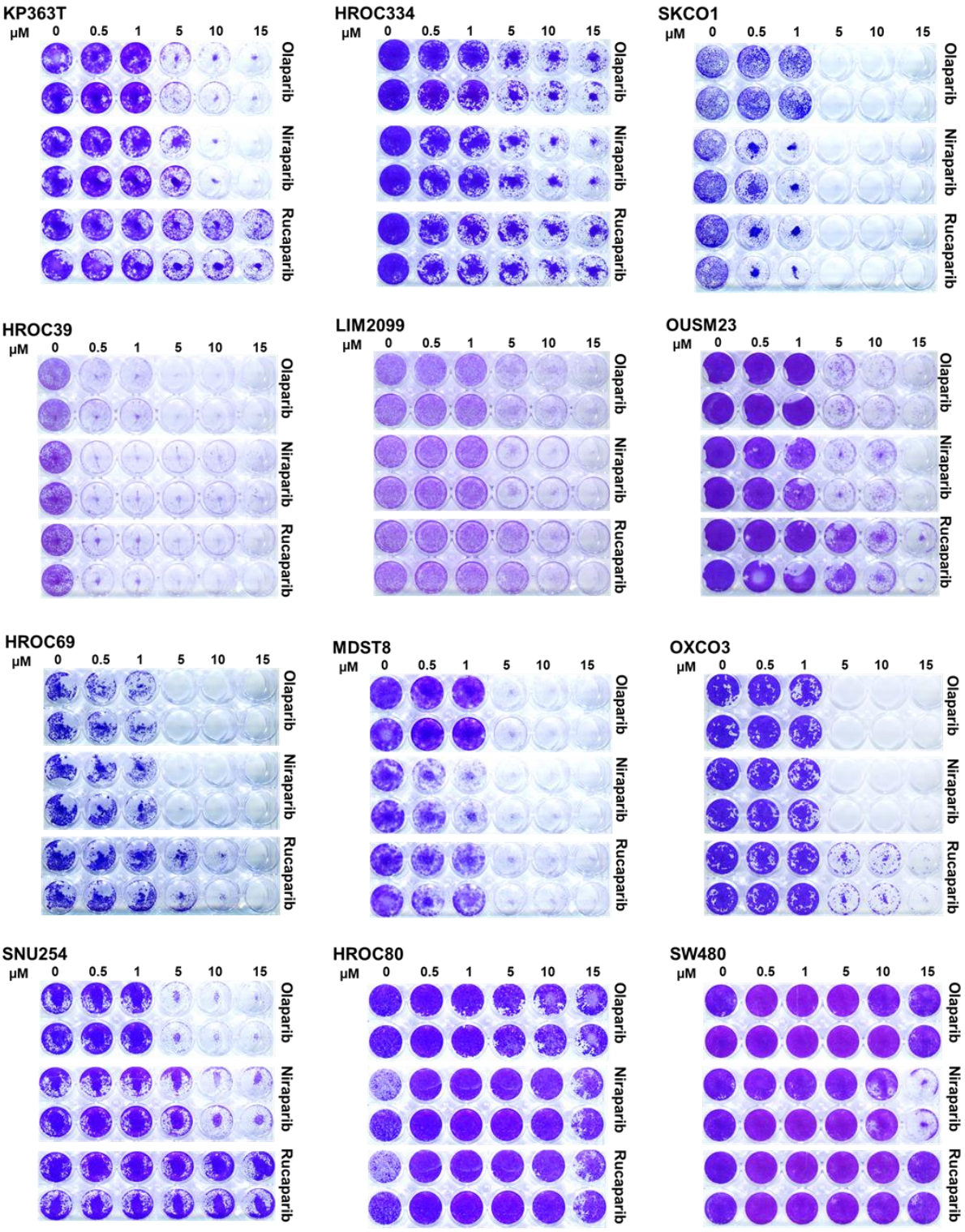
Supplementary Fig. S2. Mutational signatures and sensitivity to olaparib in CRC cells. A, Heatmap of signature contributions associated with alterations in 99 CRC cell lines. Mutational signature and CRC samples are represented on y- and x-axis respectively. Analysis were performed as reported in the "Methods" section. **B**, Swarmplot of signature contribution associated with failure of DNA double-strand break-repair by homologous recombination (S3). Each point represents the contribution of signature 3 and all points are adjusted so that they don't overlap to give a better representation of the distribution of values. In blue are represented the 13 cell lines more sensitive to olaparib, in red all the remaining 85 CRC cell lines.

Supplementary Fig. S3. Olaparib resistant and sensitive cells exhibit different homologous recombination activity in response to ionizing radiation. Immunofluorescence detection of DNA damage (γ-H2AX, **A**) and a marker of homologous recombination (RAD51, **B**) in olaparib resistant (SNU977) and sensitive (OXCO-3) cells treated as indicated. Four hours after irradiation, cells were fixed and stained. Nuclei were stained with DAPI (blue) and anti-γ-H2AX antibody (red) or anti-RAD51 antibody (green). Scale bar: 50 μm. Representative images for two resistant and two sensitive cell lines are shown.

Supplementary Fig. S4. FACS analysis on CRC cell lines tested for their HR proficiency through the pDR-GFP reporter assay. The indicated cells were initially transfected with the pDR-GFP plasmid. After puromycin selection, stably expressing cells were transfected with the pCBASce-I plasmid to generate a double strand-break damage. Fifty to sixty hours post transfection cells were analyzed by flow cytometry. A mock transfection was used as control. Numbers represent percentage of cells in thirty-thousand acquired events. Results from one representative experiment are reported.

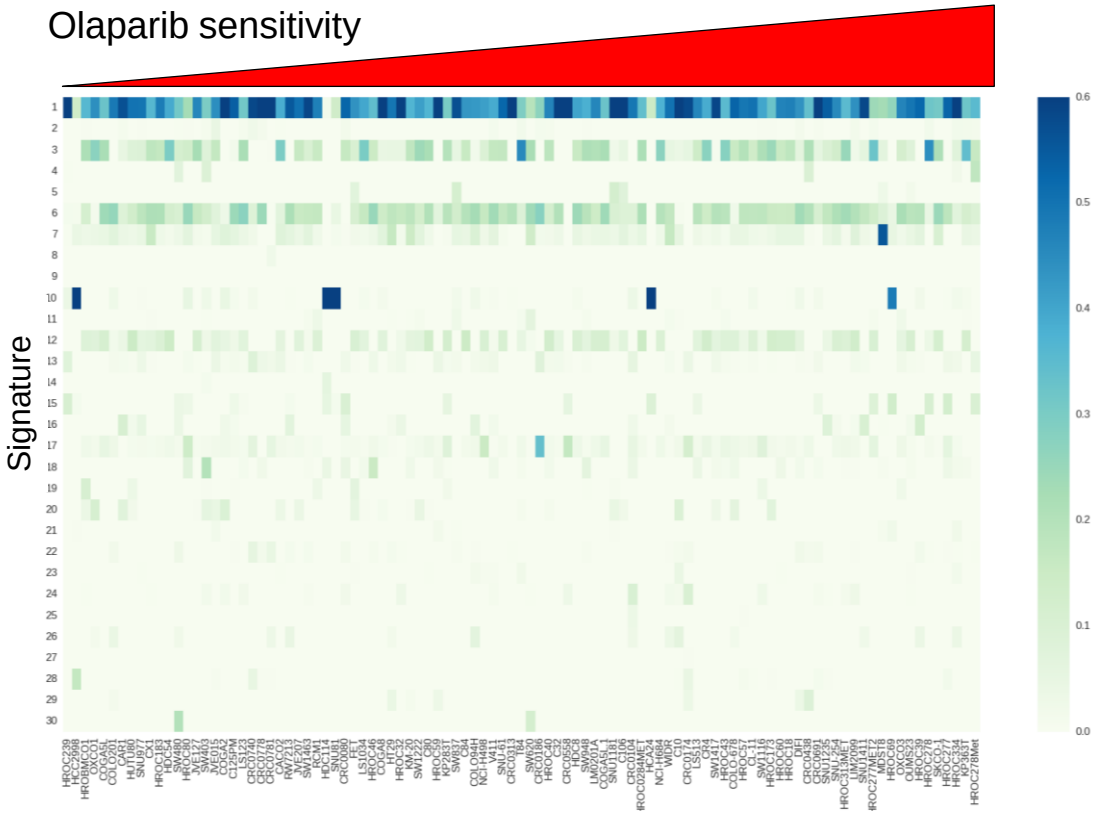
Supplementary Fig. S5. Response of Patient #4-derived organoids to panitumumab treatment. Organoid from patient #4 were seeded following procedures described in the *Materials and Methods* section. They were treated with indicated concentrations of panitumumab for one week and then viability was measured by CellTiter-Glo® (Promega) according to manufacturer's instruction with modifications. Non-linear fit with exponential growth curve (Graphpad Prism) was applied to data points to show growth kinetics. Growth curve represents the average of two independent experiments performed with technical quadruplicates. Error bars represent SD.

Supplementary Figure S1



Supplementary Figure S2

A



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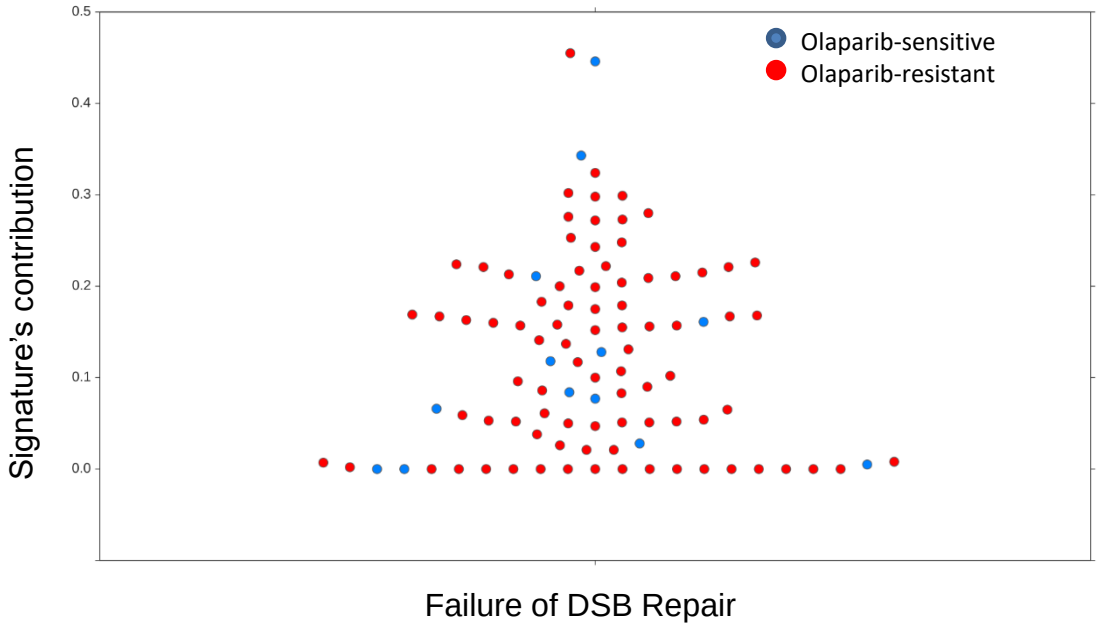
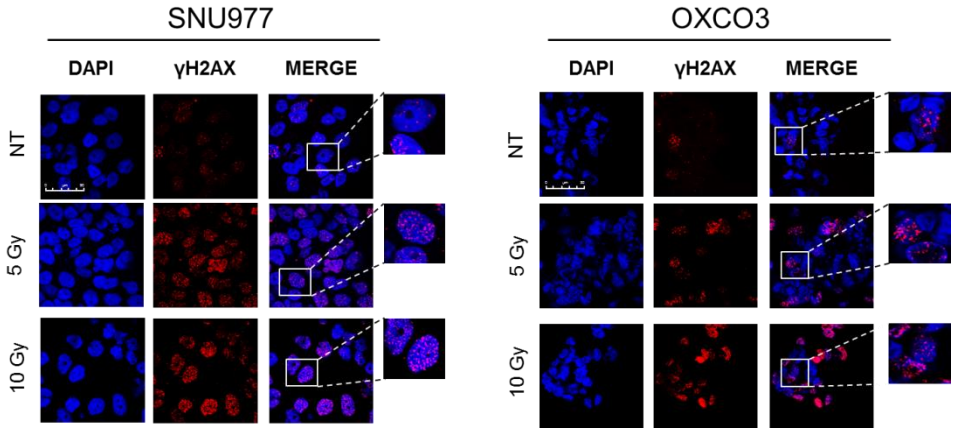


Supplementary Figure S3

A



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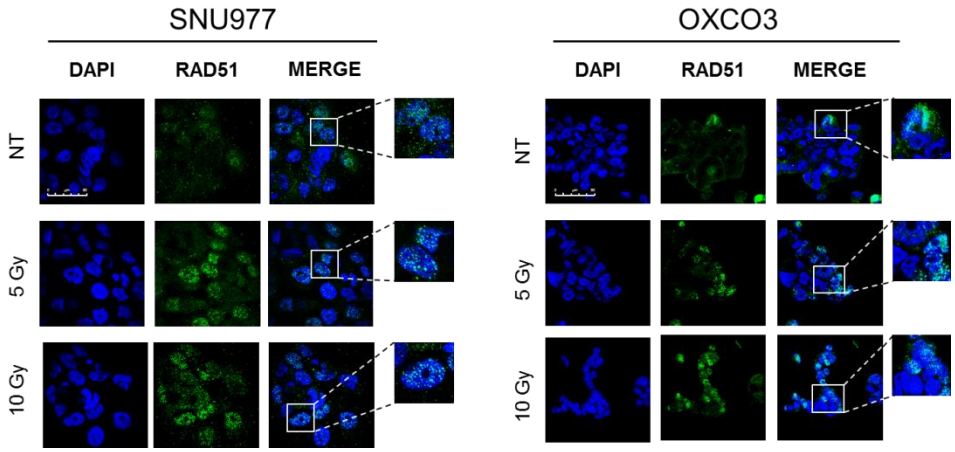
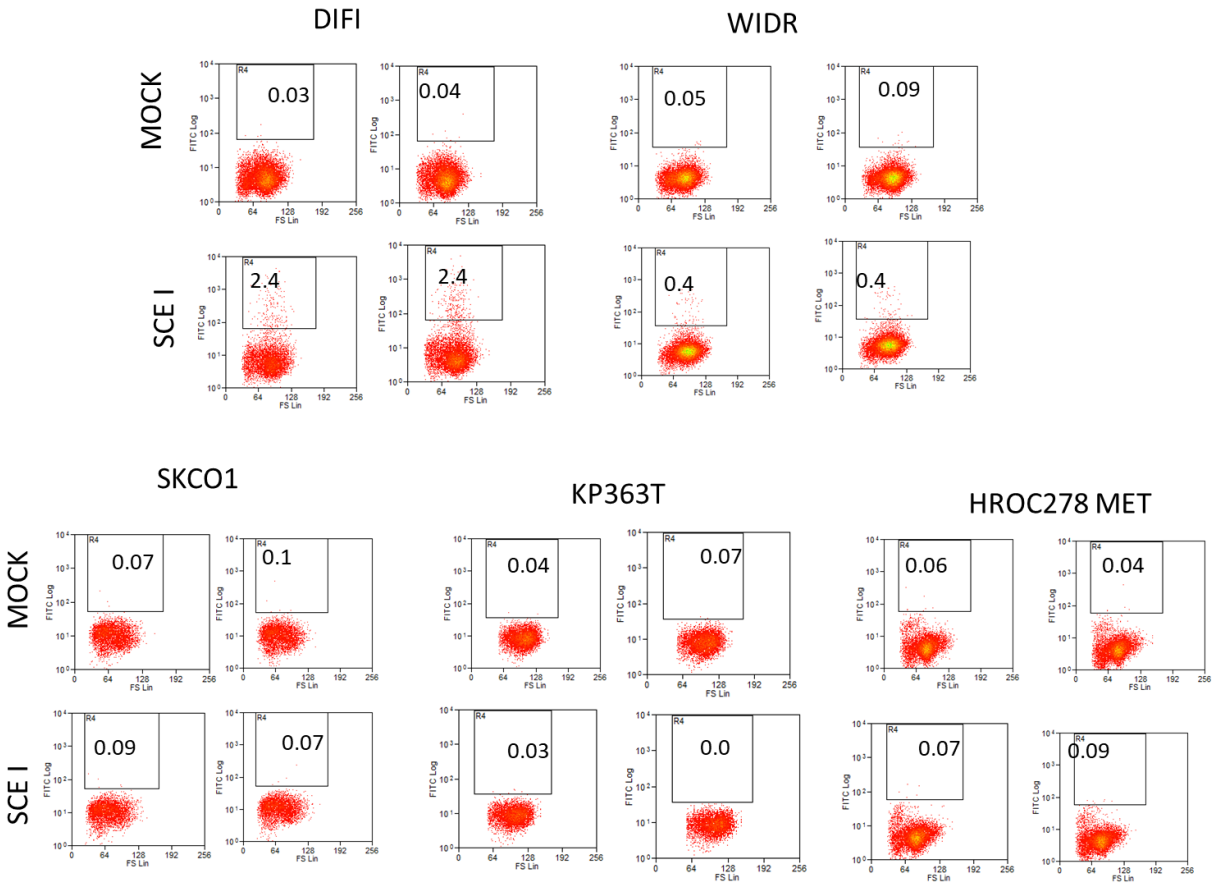


Supplementary Figure S4



Supplementary Figure S5