**Supplemental Methods**

*Cell viability and clonogenic assays*

Cells were seeded at 500-1000 cells per well on 96-well plates, and treated with increasing concentrations of the drugs. After 7 days, a CellTiterGlo assay (Promega, G7570) was performed according to manufacturer’s instructions. For long-term clonogenic assay, 1000 – 10 000 cells depending on the clone were plated on 6-cm wells, and treated with increasing concentrations of Niraparib (Selleck, S2741). After 14 days, the cells were fixed with 100% methanol and stained with crystal violet. The plates were scanned and images were analyzed using Cell Profiler.

*Western blotting*

Cells were lysed with Cell Lysis Buffer Cell Lysis Buffer (Cell Signaling Technology, 9803S) supplemented with protease inhibitor (Sigma Aldrich, 11873580001) coctail and phosphatase inhibitor (Roche Diagnostics, 4906845001). The protein concentration was analyzed by DC Protein Assay Kit (Bio-Rad, DC Protein Assay Kit II, 5000112), according to the manufacturer’s instructions. 25 µg of each cell lysate was loaded to NuPAGE Novec 4-12%, or 8% Bis-Tris gels (Thermo Fisher Scientific) and then transferred to nitrocellulose membrane (Thermo Fisher Scientific). Membranes were blocked using 5% non-fat milk in 1X Tris-Buffered Saline, 0.1% Tween 20 (TBST), and incubated with primary antibodies overnight at 4°C. Signal was detected using the LICOR imaging system with secondary antibodies. All antibodies and drugs used in the study are shown in **Supplementary Table 1.**

*Immunofluoresence*

Cells were cultured in MultiSpot slides (Thermo Fisher Scientific, Cat # 9991090), and fixed with 2-10% PFA in PBS. The cells were blocked with 2-5% BSA/Goat serum, stained for primary and secondary antibodies. The slides were mounted with ProLong Gold Antifade Mountant with DAPI (Life Technologies, P36931). The wells were imaged using Nikon fluorescent microscope and signal was quantified using Cell Profiler.

*PARylation assay*

The cells were seeded on multispot microscopy slides, treated with 10% H2O2 or DMSO in PBS for 10 minutes RT, fixed with Methanol, rehydrated, and stained with anti- Poly(ADP-ribose).

*Flow cytometry*

Cell cycle profiling was performed using BD Biosciences Flow cytometry Cell cycle kit (Cat #558662) according to manufacturer’s instructions, and analyzed using FlowJo software. The differences were calculated from data from three replicate wells.

*Cytogenetics*

For chromosomal analysis, the cells in log-growth fase were treated with Niraparib 1uM or DMSO for 40 hours. To obtain metaphases, cells were cultured using DMEM/F12 medium supplemented with 10% FBS and 1% Penicillin-Streptavidin. Once the cultures were in confluence, an hour before collection, 5μg/ml of colchicine (Sigma Aldrich, St. Louis, MO) was added to the culture media to arrest cells in mitosis; cells were treated with hypotonic solution 0.075M KCl (Sigma Aldrich, St. Louis, MO) at 37 °C for 30 min and then were fixed in methanol/acetic acid, 3:1v/v (Merck, Darmstadt, Germany). Fixed cells were dropped onto cold slides and air dried, then were pretreated with 0.1 mg/mL pepsin, fixed with formaldehyde, co-denatured with WCP 21q probe Orange labelled (Vysis/Abbott, Des Plaines, IL, USA) at 73 °C for 2 min. Post-hybridization washes were performed at high stringency using a solution containing 0.4XSSC/0.3% NP40 at 70ºC ± 1ºC for two minutes and in 2XSSC/0.1% NP40 at room temperature for one minute; 10 μl of 4’,6-diamidino-2-phenylindole (DAPI)/Vectashield (Vector USA) was used for counterstaining. Metaphase spreads were analysed based on the FISH signals using Imager Zeiss-Z1 microscope with ISIS software. Digital images were captured using a ProgRes MF camera (Jenoptic Laser Optic System Camera, GmbH, Germany) coupled to and driven by ISIS (Carl Zeiss, Metasystems, GmbH, Germany). Ploidy was determined in each mitosis, and the number of normal and structurally abnormal chromosome 21was scored.

*DNA and RNA isolation, and sequencing*

Total RNA was isolated from 70% confluent 10 cm plates using Qiagen (#74104), treated with DNAse (StemCell Technologies Inc. # 07900), and the RNA quality was assessed using Bioanalyzer. RNA with RIN values 9 or 10 were used for mRNA sequencing with Illumina PE 150 platform (Novogene) in three biological replicates. DNA was isolated from 70% confluent 10cm plates, or from three distinct areas from 20x20cm plates using Qiagen kit (#51304), including RNAse treatment (Qiagen, # 19101). DNA content was measured with Nanodop, and sequenced with illumina 150 PE platform (Novogene) using Agilent SureSelect Human All Exon V6.

*Differential gene expression analysis and pathway analysis*

RNA-seq data were preprocessed as follows. First, for each sample Kallisto (31) (version 0.44.0) was used to pseudoalign paired-end sequencing reads to the transcriptome and produce estimated expression abundance for each transcript. GENCODE (32) (release 28) was used as the reference transcriptome annotation. Next, the tximport R package (33) (version 1.6) was used to aggregate the transcript-level abundance results from all samples and produce the gene-level estimated expression counts. These estimated counts were corrected for a potential bias associated with changes in average transcript length across samples. Finally, genes were filtered out if they were not expressed in most samples, by retaining genes with counts per million (CPM) of at least 0.47 (equivalent to counts of at least 10) in 2 or more samples. The multi-dimensional scaling (MDS) plots were generated using the plotMDS function of the limma R package (34). The input data were the log2-CPM values that had been normalized by the weighted trimmed mean of M-values (TMM) method of the edgeR package (35). The distances between the samples in the MDS plots corresponded to the leading log-fold-changes, defined as the average (root-mean-square) of the 500 largest absolute log-fold-changes between each pair of samples. Differential gene expression analysis was performed using the edgeR package (35). Multiple testing correction to control the false discovery rate (FDR) was performed using the Benjamini-Hochberg procedure on the *p*-values. Differentially expressed genes (DEGs) were determined using a FDR of 2.5%. The Gene Set Enrichment Analysis (GSEA) (36, 37) was performed using the GSEAPreranked module of the GSEA Java application (gsea-v3.0.jar). The input genes were ranked by the -log10-transformed *p*-values with the sign of log-fold-change. The input geneset was a collection of pathways curated by Gary Bader Biological System Laboratory at University of Toronto, Canada (<https://baderlab.org/>; release 2018-09-01).

*Whole-exome sequencing analysis*

Sequences were aligned to hg38 using BWA. Mutations were called using MuTect2 from GATK4.0.11 and CNVs using ASCAT and GATK4.1.2. Detailed investigation of the *BRCA1*-mutation in the RPE*TP53-/-BRCA1-/-* cells revealed a deletion of three base pairs leading to an early stop codon between exons 2 and 3. In the COSMIC database this particular mutation has not been described in HGSC patients. As a splicing variant, and as there is a short distance to stop codon, reversion mutations are unlikely but still possible.Evolutionary analysis for was performed from mutations and CNVs using PyClone0.13 and ClonEvol (38). The WES, mutational calls and CNVs from a *BRCA1* mutated patient was accessed from Hill et al dbGap phs001685.v1.p1. Only subclones whose frequency was above 1% and at least 5 mutations after filtering were included in the analysis. LOH score was calculated as described in (39). In mutational signature 3 analysis, counts of different types of single nucleotide variants (SNV) were analyzed to identify known patterns of mutational processes (40). The types of mutations are defined based on the reference alternate alleles and 5’ and 3’ nucleotides flanking the substitution leading to the standard 96-dimensional array format. The NNLS implementation in the SigMA R (41) package was used to determine the exposure of Sig 3. For the cell lines, the fraction of Sig3, i.e. exposure of Sig3 divided by the total number of SNVs, was calculated. Due to the variation on the number of mutations in the subclone analysis, NNLS was not used. Instead, we used CCLE dataset from breast and ovarian cancers as a reference for the cell lines, and TCGA ovarian cancer dataset as a reference for the analysis of subclones in the clinical tumor samples. First, the samples from CCLE and TCGA were classified as Sig3-positive or negative groups using SigMA. Next the mutations are subsampled randomly according to the count of mutations in each subclone. SigMA is used to calculate the likelihood of Sig3 in the simulations, as well as for the subclones. The likelihood of Sig3 for the subclonal mutations was compared to that of simulated samples. The risk ratio of Sig3-positivity was defined as the fraction of samples of Sig3-positive simulations that have likelihood that is smaller than the likelihood of the subclonal spectrum divided by the fraction of samples that have a likelihood higher than the likelihood of the subclonal spectrum.

*Image-based drug sensitivity testing*

The cells were seeded using an automated cell plater (Thermo Fisher Wellmate) into 384 well CellCarrier plates (Perkin Elmer, Waltham, MA) in log growth phase on day -1. On day 0, drugs were dispensed with a HP D300e Digital Dispenser using a randomized plate mapping. After 72 hours, the cells were pulsed for one hour with EdU (Lumiprobe, Hunt Valley, MD) and stained with 1:2000 LIVE/DEAD Far Red Dead Cell Stain (LDR) (Thermo Fisher Scientific, Waltham, MA). Cells were then fixed with 3.7% formaldehyde (Sigma Aldrich, St. Louis, MO) for 30 minutes and permeabilized with 0.5% Triton X-100 in PBS. The EdU was labeled with cy3-azide (Lumiprobe, Hunt Valley, MD) for 30 min. The cells were then blocked for one hour with Odyssey blocking buffer (LI-COR, Lincoln, NE), and stained overnight at 4˚C with 2 µg/ml Hoechst 33342 (Sigma Aldrich, St. Louis, MO) and a 1:1000 dilution of anti-phospho-histone H3 (pHH3) Alexa 488 (Ser10, clone D2C8) conjugated antibody (Cell Signaling Technologies, Danvers, MA). Fixed cells were imaged with a 10x objective using an Operetta microscope (Perkin Elmer, Waltham, MA), and nuclei were segmented using the accompanying Columbus software based on their Hoechst signal. DNA content, defined as the total Hoechst intensity within the nuclear mask, was used to identify cells in the G1 and G2 phases of the cell cycle. The average LDR, EdU and phospho-histone H3 intensities within the nuclear masks were determined, and used to classify dead, S and M phase cells respectively. Cells with intermediate DNA content and no EdU signal were classified as S phase dropout cells (ref Caitlin). The growth-rate-adjusted viabilities were calculated for each drug concentration as described previously (42). The GR50 values were calculated, normalized with respect to the B40 cell line, and visualized using hierarchical clustering heatmap with column normalization. The growth rate over 72 hours was calculated using a formula Gr=LN(N(72h)/N(0))/72h, and each cell line was compared to B40 using Dunett’s multiple comparison test. The proportions were compared using Oneway Anova followed by Dunett’s or Sidak’s multiple comparison test.

**Supplementary Figure legends**

**Supplementary Figure 1**

**(A)** Schematics for the strategy used to generate the B40 cell line resistant to PARPi. This was achieved via cyclic exposure of the cell line to increasing concentrations of the PARP inhibitors Niraparib or Olaparib. The B40 cells and resistant clones were cultured for a maximum of 3 months for performing each of the functional experiments, after which a new fresh batch was taken up.

(**B**) Propagation and timing of the cell line samples analyzed via Whole-Exome Sequencing (WES) and RNA sequencing.

**(C)** In a 7-day survival assay, the B40 cell line was more sensitive to Niraparib than the two commercially available *BRCA1-*deficient cell lines COV362 and UWB1.289.

**(D)** The B40 cell line was more sensitive to Olaparib than the two commercially available *BRCA1-*deficient cell lines COV362 and UWB1.289.

**(E)** Five of the resistant single-cell clones were also cross-resistant to cisplatinum.

(**F**) Two of the resistant clones (NA4 and OA4) were still sensitive to cisplatinum.

**Supplementary Figure 2.**

**(A)** Western blotting showing lack of BRCA1 protein expression in the B40 cells and in the resistant clones in basal conditions using an N-terminal antibody.

**(B)** Representative immunofluorescence images of the cells 6h after 5Gy IR to induce DNA damage (γH2AX) and formation of BRCA1 nuclear foci. Note the lack of BRCA1 foci in the B40 cells and in the resistant clones in IF using a C-terminal antibody.

**(C)** Representative IF images of RAD51 foci formation in Cyclin A positive cells 6h after 5Gy of IR.

**(D)** Representative IF images of 53BP1 and γH2AX foci formation 6h after 5Gy of IR.

(**E**) Representative IF images of PARP1 (left column) and PAR (middle column) in the cells. Note the increased PARP1 (left column) and PAR levels in the B40 cells, and decreased levels in the NA4, NA5 and OA5 clones. Niraparib treatment effectively abolished PARylation in all the cell lines (right column).

**Supplementary Figure 3**

**(A)** Bar graph of unique differentially expressed genes (DEGs) in the resistant cells with reference to the B40 cells.

(**B**) Photomicrographs showing the EMT phenotypes in the B40 compared to the RPE*TP53-/-*cells, and in PARPi resistant clones NA1, NA2, NA3, NB1 and OA5.

(**C**) Immunofluorescence images of Vimentin staining in the cells.

(**D**) Quantification of the Vimentin IF signal per cell in 5-15 image fields.

(**E**) Quantification of the Zeb1 IF signal per cell in 5-15 image fields.

(**F**) Average pRPA foci per nucleus in 12 image fields in B40 and NB1 cells. The groups were compared using One-way ANOVA followed by Dunnett’s test or Mann-Whitney U test \*p<0.05, \*\*p<0.01, \*\*\*p<0,001, \*\*\*\*p<0.0001.

**Supplementary Figure 4.**

Log2 copy ratios and B-allele frequency (BAF) plots for the cell lines. Log2 copy ratios show the amount of DNA captured from each segment of chromosomes. The BAF plots indicate the allelic balances. Deviation higher than 0.5 indicate imbalances.

**Supplementary Figure 5.**

**(A)** Quantification of the cells’ growth rate. Note the decreased growth rates of the B40 cells in comparison to the RPE*TP53-/-*cells, and the increased growth rates of NA2, NA3 and OA5 clones in comparison to the B40 cells. The groups were compared using Oneway Anova followed by Dunnett’s test, \*p<0.05, \*\*p<0.01, \*\*\*p<0,001, \*\*\*\*p<0.0001.

**(B)** Mutation signature 3 risk ratio (RR) of the mutational clusters detected in the cell lines.

(**C**) Heatmap of clustered mutations in the *BRCA1*-mutated HGSC tumor samples. S; Sensitive, R; Resistant.

(D) Mutation signature 3 risk ratio (RR) of the mutational clusters detected in the HGSC tumor samples.

(**E**) IC50 values for Niraparib in single-cell clones derived from the sensitive B40 cell line. Bar graphs represent the mean and error bars are the SD. The growth-rates were similar between the B40 subclones (data not shown).

(**F**) Quantification of RAD51 foci at baseline and 6 hours after 5 Gy of IR . IF showed significantly increased RAD51 foci formation in the pre-existing drug tolerant subclone C5 after IR and in comparison to the B40 cell line. Data are presented as median foci number per Cyclin A positive nucleus per image in 20 representative images, and error bars represent the SD. \* p<0.05, \*\*\*\*p<0.0001

(**G**) Quantification of pRPA (S32) foci at baseline and 6 hours after 5 Gy of IR. IF showed significantly increased pRPA foci formation after IR in all cells, but decreased pRPA foci in the pre-existing drug tolerant subclone C5, and in RPE*TP53-/-*cells in comparison to the B40 cells. Data are presented as mean foci per nucleus per image in 20 representative images, and error bars represent the SD. \* p<0.05, \*\*\*\*p<0.0001

(**H**) Quantification of γH2AX foci at baseline and at 6 hours after 5 Gy of IR. IF showed significantly increased foci formation after IR in all cells, but decreased pRPA foci formation in the pre-existing drug tolerant subclone C5, and in RPE*TP53-/-*cells in comparison to the B40 cells. Data are presented as mean foci per nucleus per image in 20 representative images, and error bars represent the SD. \* p<0.05, \*\*\*\*p<0.0001

(**I**) Cytogenetic analysis of the 21q chromosome painting in the B40 cells, B40\_C6 sensitive clone, B40\_C9 drug tolerant clone, NA3 fully resistant clone, and NA4 fully resistant clone. The analysis was consistent with the genomic evolutionary model, where increased 21q loss and triploid karyotype follows the evolutionary trajectory via the drug tolerant B40\_C9 cells. The NA4 cells that did not follow the same trajectory showed lower proportions of 21q loss and mostly diploid karyotype.

**Supplementary Figure 6.**

(**A**) Quantification of pChk1 foci at baseline (black bars) and after Niraparib (1μM for 24 hours, grey bars). IF showed baseline lower levels in the RPETP53-/- cells and in all resistant clones except for NB1 in comparison to B40. In addition, all cells showed an increase in pChk1 foci after Niraparib treatment. Data are presented as mean foci per nucleus per image in 5-10 representative images and error bars represent the SD.

(**B**) Confirmation of growth rate- normalized resistance to Niraparib in the resistant clones in the image-based drug screen.