Supplementary Figure Legends

Supplementary Figure S1

A, genotyping PCR of genomic tail DNA from a *K14cre;Brca1$^{F/F}$;p53$^{F/F}$;Mdr1a/b$^{-/-}$* mouse (left lanes) and controls (right lanes). **B-C,** response curves of untreated tumors (left panels) and when treated with olaparib (50 mg/kg i.p., daily for 28 days). Figure **B** shows the response of 11 individual *Brca1$^{\Delta/\Delta}$;p53$^{\Delta/\Delta}$;Mdr1a/b$^{-/-}$* tumors (KB1PM) and figure **C** shows the response of 12 individual *Brca1$^{\Delta/\Delta}$;p53$^{\Delta/\Delta}$* tumors (KB1P). The treatment of 28 consecutive days with olaparib was repeated when a tumor relapsed and reached the size of 200 mm^3 (100 %).

Supplementary Figure S2

Response of three or four tumors that originate from the same KB1PM donor tumor to olaparib (50 mg/kg i.p., daily for 28 days). The treatment of 28 consecutive days with olaparib was repeated when a tumor relapsed and reached the size of 200 mm^3 (100 %).

Supplementary Figure S3

γH2AX immunohistochemistry after 7 days of PARP inhibition in control and olaparib-resistant tumors from KB1PM1 and 5. Tumors shown in the bottom row were treated daily for 7 days with olaparib (50 mg/kg i.p.) and the tumors were harvested 2 hours after the last treatment. Scale bar = 100 μm.

Supplementary Figure S4

To prevent stromal contamination we analyzed the mutations in the cell lines derived from the control and olaparib-resistant tumor of KB1PM5. **A-B,** PCR reactions on the genomic DNA of the spleen (spl), KB1PM5 cell line Control 3 (con) and Ol-res 2 (res) showing a rearrangement in *Trp53bp1* of exon 26 and intron 24 (**A**) and of exon 24 and intron 24 (**B**). **C,** PCR reactions on cDNA showed absence of the wild-type sequence in the resistant cells.

Sequencing showed an exact duplication of exons 25 and 26 (295 bp, see also **Figure 3C**). For the primers that were used, see **Supplementary Table S1**.

Supplementary Figure S5

A, genotyping PCR to confirm that the cell lines are derived from the KB1PM tumor cells and not contaminated with stromal cells. All six cell lines have deletions in *Brca1*, *p53* and *Mdr1a/b*, and have completely lost the flox bands of *Brca1* and *p53*, indicating complete Cre-mediated deletion in all cells. **B,** comparative genomic hybridization shows that the cell lines are highly similar to the tumor that they were derived from. The spleen from the *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$*;Mdr1a/b*$^{-/-}$ mouse that produced tumor KB1PM5 was used as reference DNA. **C,** the cell lines derived from olaparib-resistant tumor KB1PM5 are negative for 53BP1, whereas the cell lines from the control tumor express 53BP1. **D,** clonogenic assay for olaparib with tumor-derived cell lines. The IC50 is indicated between brackets.

Supplementary Figure S6

Images of RAD51 and 53BP1 IRIFs measured 6 hours after irradiation with 10 Gy in short-term tumor cell suspension of a KP tumor and the control (ctr) and olaparib-resistant (ol-res) tumors of 5 KB1PM donors. Quantification of the RAD51 foci is presented in **Figure 4C**.

Supplementary Figure S7

A, Quantification of RAD51 IRIFs in KP cells and two KB1P cell lines expressing a non-targeting hairpin (shNT) or two different hairpins against 53BP1 (sh53BP1 #1 and #2). Cells were fixed 6 hours after irradiation with 10 Gy. **B,** spontaneous point mutation in intron 22 of *Trp53bp1* in KB1P-3.12 cells resulting in a cryptic splice acceptor site and the resulting mRNA sequence. The extra seven base pairs are highlighted in red. The alternating codons are underlined and the premature stop codon is indicated in bold. **C,** 53BP1 protein in tumors

that grew out upon orthotopic transplantation of KB1P-B11 cells expressing either a non-targeting hairpin (shNT) or a hairpin against 53BP1 (sh53BP1 #1). Scale bar = 100 μm.

Supplementary Figure S8

Absence of 53BP1 was detected in three *Brca1*$^{\Delta/\Delta}$*;p53*$^{\Delta/\Delta}$*;Bcrp*$^{-/-}$ tumors that have acquired resistance to topotecan. Scale bar = 100 μm.

Supplementary Figure S9

A, chemical structure of AZD2461. **B,** pharmacodynamics of olaparib and AZD2461. PAR levels measured at several time points after a single administration of olaparib (50 mg/kg i.p.) or AZD2461 (100 mg/kg p.o.). At the indicated time points tumors were harvested and snap frozen. PAR levels were measured in whole tumor extracts with an ELISA. n.d. = not detectable (lower than 2*SD above background). Data are presented as mean+SD of five tumors per time point per treatment. **C,** response curves of 12 individual *Brca1*$^{\Delta/\Delta}$*;p53*$^{\Delta/\Delta}$ tumors (KB1P) tumors that were untreated (left panel) or treated with AZD2461 (100 mg/kg p.o., daily for 28 days). The treatment of 28 days with AZD2461 was repeated when the tumor relapsed and reached the size of 200 mm^3 (100 %). **D,** PCR (primers 14 and 15) on genomic DNA of the control (con) and AZD2461-resistant (res) tumors KB1P2, showing a deletion in exon 21 of *Trp53bp1* in the AZD2461-resistant tumor, which has been confirmed by Sanger sequencing (see **Figure 6E**). **E,** PCR (primers 18 and 19) on genomic DNA of the control (con) and AZD2461-resistant (res) tumors KB1P8, showing a deletion at the border of intron 24 and exon 25 in *Trp53bp1* in the AZD2461-resistant tumor, which has been confirmed by Sanger sequencing (see **Figure 6F**).

Supplementary Figure S10

A-C, response of tumors from three individual donor tumors (KB1P 10-12) to 100 days of daily treatment with AZD2461 (100 mg/kg p.o.). The treatment of 100 days was repeated

when the tumor relapsed and reached the size of 200 mm^3 (100 %). Except one tumor (KB1P12-2) that acquired resistance during the first treatment cycle, all other tumors respond to three cycles of 100 days AZD2461. **D,** histology and immunohistochemical staining of vimentin and 53BP1 of the KB1P12-2 tumor that acquired resistance to AZD2461 during the first treatment cycle of 100 days (see red curve in (**C**)). HE = hematoxylin eosin, vim = vimentin. Scale bar = 100 μm.