Supplemental Material

Supplemental Materials and Methods

Antibodies

The following antibodies were used for western blotting: phospho-CHK1 (Cell Signaling Technology), phospho-CHK2 (Cell Signaling Technology), phospho-ATRIP (Cell Signaling Technology), γH2AX (Abcam), p21 (Santa Cruz Biotechnology), Vinculin (Santa Cruz Biotechnology), Myc (Santa Cruz Biotechnology), and rabbit polyclonal anti-PARI antibody (described in Moldovan et al, *Mol Cell* 2012 Jan 13;45(1):75-86).

Gene knockdown by siRNA

For siRNA-mediated knockdown, the following targeting sequences were used:

siPARI #1 AGGACACATGTAAAGGGATTGTCTA

siPARI #2 CATACTGTGGCAGAGATACTGTGAA

When the siPARI number is not specified, a 1:1 mixture of both oligonucleotides was used. As control, AllStars Negative Control (Qiagen) was employed. Lipofectamine RNAiMAX (Invitrogen) was used for siRNA transfection.

Histology

Xenograft biopsies were obtained and then fixed in 70% ethanol for 24 hours, followed by 3 days fixing in 10% formalin. Paraffin slides were stained with

hematoxylin/eosin (H&E). We thank Dana-Farber/Harvard Cancer Center in Boston, MA, for the use of the Rodent Histopathology Core, which provided paraffin embedding and H&E staining service. Dana-Farber/Harvard Cancer Center is supported in part by an NCI Cancer Center Support Grant # NIH 5P30CA06516.

Legends to Supplementary Figures

Supplementary Figure 1. Representative micrographs showing a decrease in IR-induced RAD51 foci in PARI-overexpressing 8988T and CAPAN2 cells. SiRNA-mediated knockdown of PARI increases RAD51 foci in 8988T cells.

Supplementary Figure 2. PARI-overexpressing pancreatic cancer cell line 8988T is hypersensitive to PARP inhibitor ABT-888. (A) Clonogenic assay showing that 8988T cells are sensitive to ABT-888. The fibroblast cell line GM6914.FA (that does not overexpress PARI –not shown) is used as control, since 293T cells do not readily form clones in our experimental setup. The average of four independent experiments is shown. Error bars represent standard deviations. (B) Survival assay showing that 8988T cells are sensitive to ABT-888. Cells were grown for three days in the presence of the drug, and viability was assayed using CellTiterGlo (Promega). FANCA-deficient cells were

used as negative control. Bars represent the average of four independent experiments. Error bars represent standard deviations.

Supplementary Figure 3. SupF-based mutagenesis assay showing increased point mutations in 293T cells overexpressing Myc-PARI at high levels. Cells were co-transfected with Myc-PARI and SupF. Bars represent the average of three independent experiments. Error bars represent standard errors.

Supplementary Figure 4. Analysis of chromosome morphology in metaphase spreads of 8988T cells, showing that PARI knockdown reduces chromosomal aberrations following exposure to 20ng/ml MMC. Bars represent the average of two to four independent experiments. Error bars represent standard errors.

Supplementary Figure 5. PARI depletion reduces viability of PARI-overexpressing cells (CAPAN2), but does not affect the cells that do not overexpress it (CAPAN1, HeLa, 293T). Cellular viability was assayed three days after acute PARI knockdown, using CellTiterGlo assay (Promega). Bars represent the average of three independent experiments. Error bars represent standard deviations.

Supplementary Figure 6. Quantification of cell cycle phases of 8988T cells following control or PARI depletion, using Modfit software. PARI knockdown leads to a significant increase in the S-phase fraction, from 31.72% to 44.82%.

Supplementary Figure 7. FACS analysis of 8988T cells treated with HU, followed by release in fresh media. The quantification was done using the Modfit software. This is an independent repeat of the experiment shown in Figure 4A.

Supplementary Figure 8. PARI depletion in CAPAN2 (pancreatic cancer cells overexpressing PARI) results in HU sensitivity. Cellular viability after incubation for three days in HU-containing media was scored using CellTiterGlo assay (Promega). The average of two independent experiments is shown. Error bars represent standard deviations.

Supplementary Figure 9. Depletion of PARI by shRNA decreases cellular proliferation when grown in the presence of 20μg/ml doxycyline. The average of two independent experiments is shown. Error bars represent standard deviations. Calculated doubling times are shown on the right hand side, -/+ standard deviation.

Supplementary Figure 10. H&E stain of paraffin-embedded slides showing increased necrosis in xenograft tumors originating from PARI-depleted 8988T cells. Upper panels show a representative increase in necrotic tissue in the shPARI xenograft tumor (marked by the arrow). Lower panels show the tumor margin. Arrow heads indicate mouse epithelial tissue, while the interior contains cells contributed by the xenograft.

Supplementary Figure 11. Model of PARI-dependent DNA repair regulation in pancreatic cancer cells. PARI overexpression in PDAC cells (left panel) ensures fast processing of DNA lesions due to reduction of slow HR and compensatory increase in faster mechanisms such as TLS and NHEJ, thus suppressing the DNA damage response. PARI knockdown (right panel) influences the balance of repair mechanisms resulting in slower clearance of DNA lesions, activation of the DDR, and ultimately - loss of viability.