Contextual Synthetic Lethality of Cancer Cell Kill Based on the Tumor Microenvironment

Supplementary Figure Legends

Supplementary Figure S1. Hypoxia decreases RAD51 expression *in vivo* and *in vitro*. (*A*) H1299 cells treated for 72 h with varying concentrations of O_2 show that RAD51 protein expression is decreased at O_2 levels below 0.5%. (*B-D*) Representative images from (*B*) 22RV1, (*C*) RKO and (*D*) HCT116 xenografts stained for the HR protein RAD51 and the hypoxia marker EF5. Line intensity profiles show inverse association between EF5 and RAD51. Scale bar represents 100 microns. N signifies necrotic regions.

Supplementary Figure S2. PARP inhibition does not decrease RAD51 mRNA expression or MMC sensitivity. RKO cells were treated with 72 h x 0.2% O_2 with or without 2.5 μM ABT-888. (A) RAD51 mRNA expression is decreased by hypoxia but is not further decreased by PARP inhibition. (B) CA9 mRNA expression is highly induced by hypoxia independent of PARP inhibition confirming that hypoxia was achieved and that HIF-1 signaling was unaffected by PARP inhibition. (C) PARP inhibition (72 h x 2.5 μM ABT-888) had no effect on clonogenic survival following MMC treatment in H1299 cells. Knockdown of RAD51 by siRNA (48 h x 0.25 nM) is shown as a positive control. Columns, mean of 3 experiments; bars, SE.

Supplementary Figure S3. PARP inhibition kills homologous recombination defective hypoxic cancer cells. (*A*) RKO cells exposed to moderate chronic hypoxia (72 h x 0.2% O_2) are sensitized to ABT-888. (*B*) siRNA knockdown of RAD51 is toxic to cells treated with 2.5 μM ABT-888. Points and columns, mean of 3 experiments; bars, SE; *, $P<0.05$.

Supplementary Figure S4. Colocalization of hypoxia-induced PAR foci with RPA foci. Immunofluorescent staining of PAR and RPA foci in RKO cells treated with 16 h x 0.02% O_2 indicating that they occur at regions of single stranded DNA at stalled replication forks.