The *POLD1*^{R689W} variant increases the sensitivity of colorectal cancer cells to ATR and CHK1 inhibitors

Albert Job[1], Marina Tatura[1], Cora Schäfer[1], Veronika Lutz[1], Hanna Schneider[1], Brigitte Lankat-Buttgereit[1], Alexandra Zielinski[2], Kerstin Borgmann[2], Christian Bauer[1], Thomas M. Gress[1], Malte Buchholz[1], and Eike Gallmeier[1,*]

[1]Department of Gastroenterology, Endocrinology, Metabolism, and Infectiology, University Hospital of Marburg, Philipps-University Marburg, Marburg, Germany
[2]Lab of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
*eike.gallmeier@med.uni-marburg.de

Supplementary Information

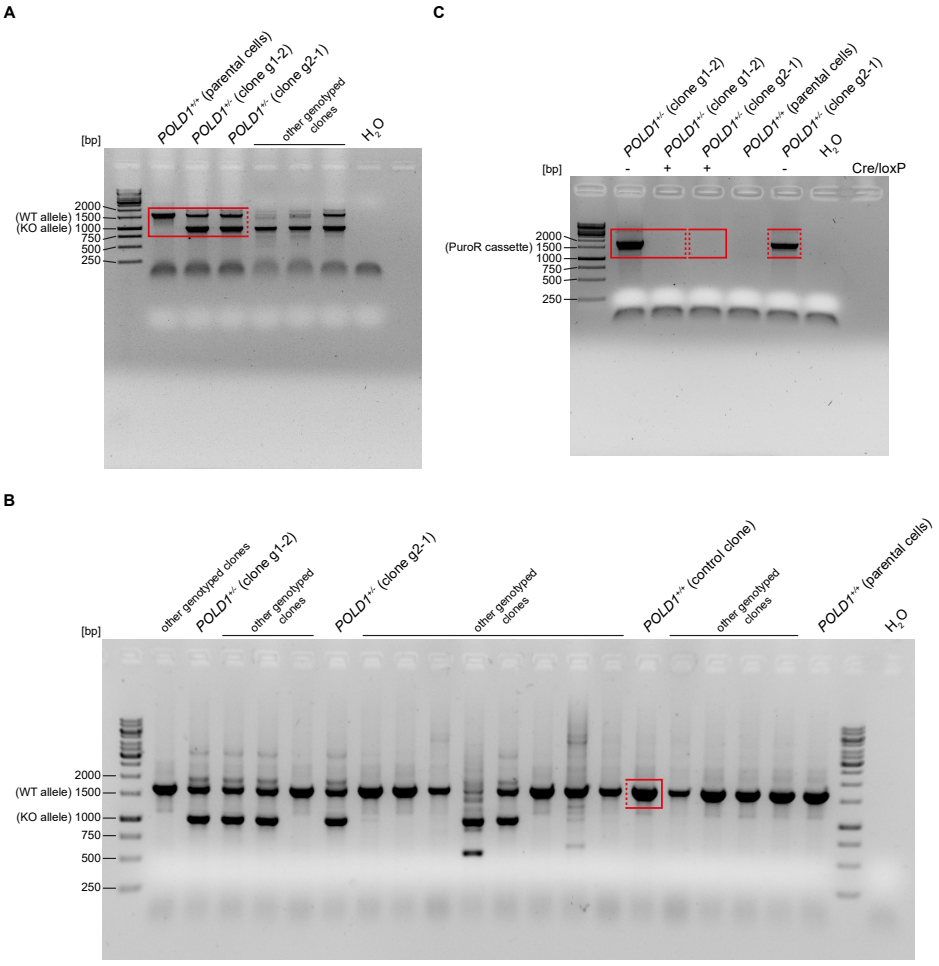

Figure S1. (**A+B**) Original gels of the PCR detecting the *POLD1*-KO and -WT alleles in the *POLD1*$^{+/+}$ parental and control clones as well as in the *POLD1*$^{+/-}$ clones g1-2 and g2-1. Data which were cropped and depicted in Figure 1B are framed red. (**C**) Original gels of the PCR detecting the Cre/loxP-mediated excision of the puromycin resistance cassette. Data which were cropped and depicted in Figure 1C are framed red.

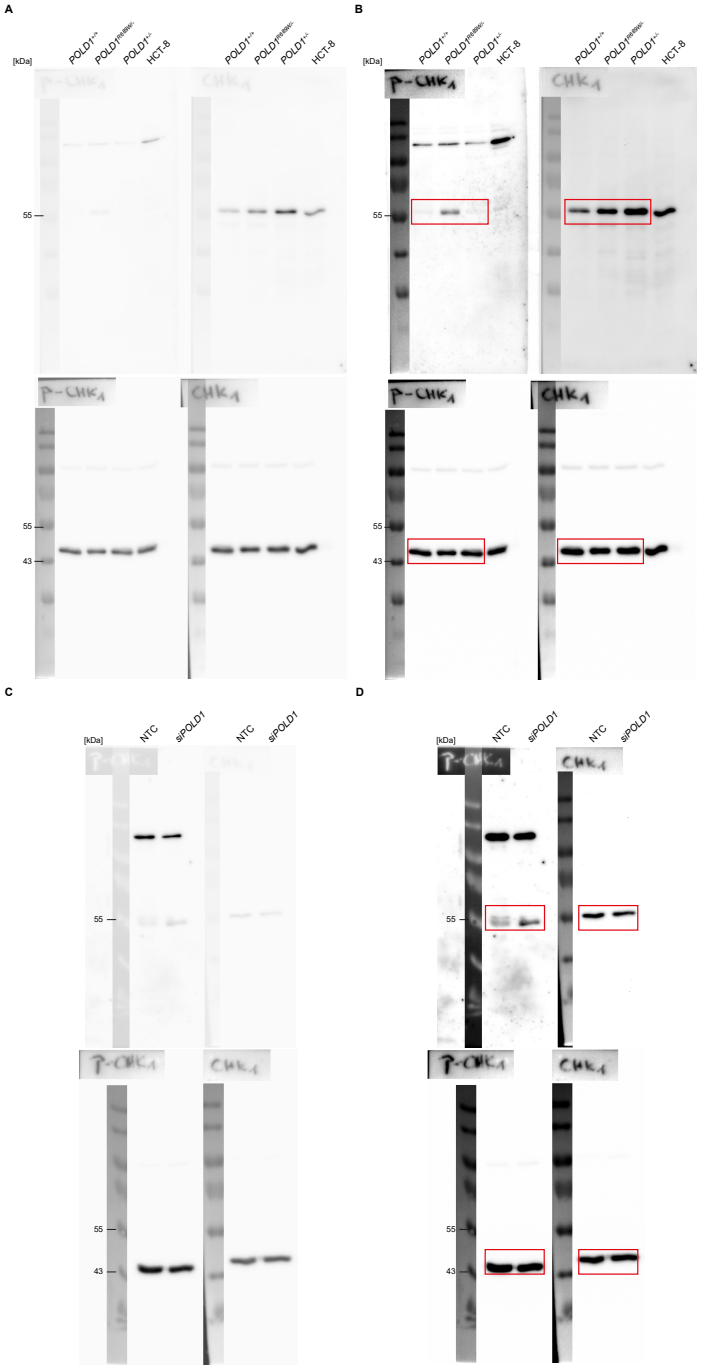

Figure S2. (**A, upper panel**) Original immunoblots and (**B, upper panel**) immunoblots after contrast modification of constitutive protein levels of pCHK1 and CHK1 in *POLD1*$^{+/+}$, *POLD1*$^{R689W/-}$ and *POLD1*$^{+/-}$ cells. (**A+B, lower panels**) Original immunoblots and immunoblots after contrast modification, respectively, of β-Actin serving as loading control. (**C, upper panel**) Original immunoblots and (**D, upper panel**) immunoblots after contrast modification of protein levels of pCHK1 and CHK1 120 hours after *siPOLD1* transfection in *POLD1*$^{+/+}$ cells. (**C+D, lower panels**) Original immunoblots and immunoblots after contrast modification, respectively, of β-Actin serving as loading control. Data which were cropped and depicted in Figure 3A are framed red.

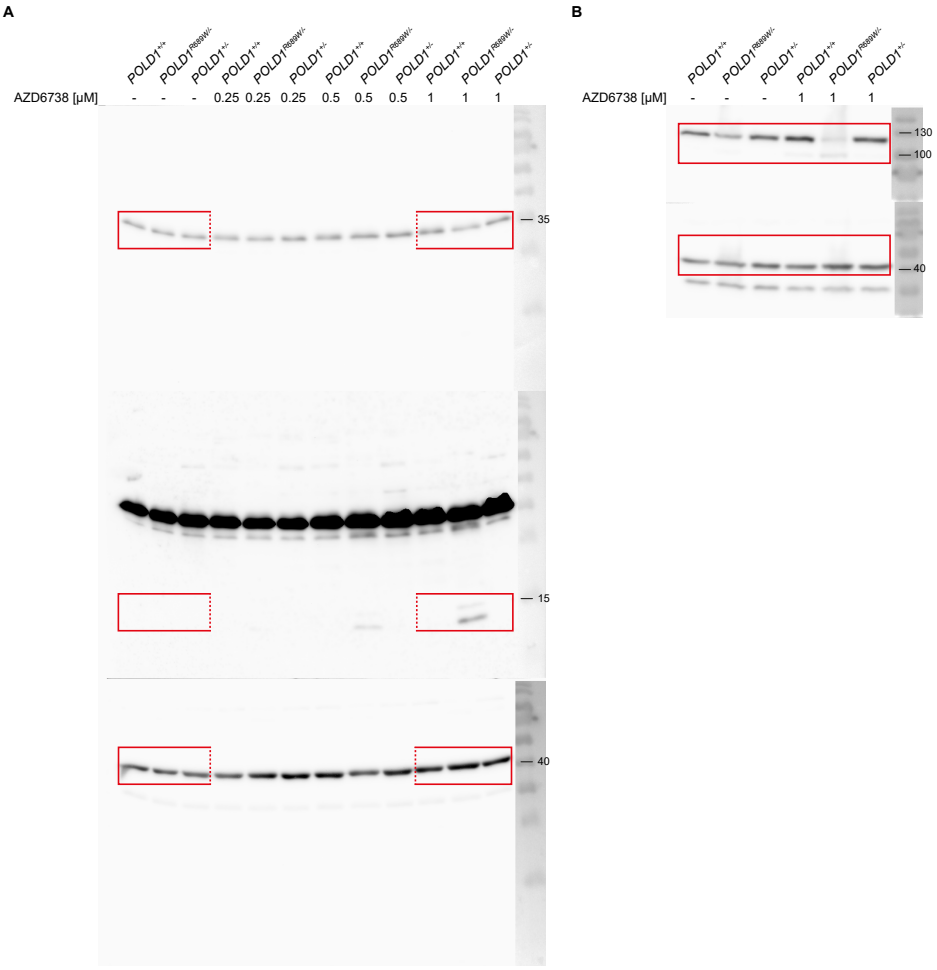

Figure S3. Original immunoblots of protein levels of (**A, upper part**) caspase 3, (**A, middle part**) cleaved caspase 3 as well as (**B, upper part**) PARP and cleaved PARP in *POLD1*$^{+/+}$, *POLD1*$^{R689W/-}$ and *POLD1*$^{+/-}$ cells 72 hours after treatment with AZD6738. (**A+B, lower parts**) Original immunoblots of β-Actin serving as loading control. Data which were cropped and depicted in Figure 4C are framed red.

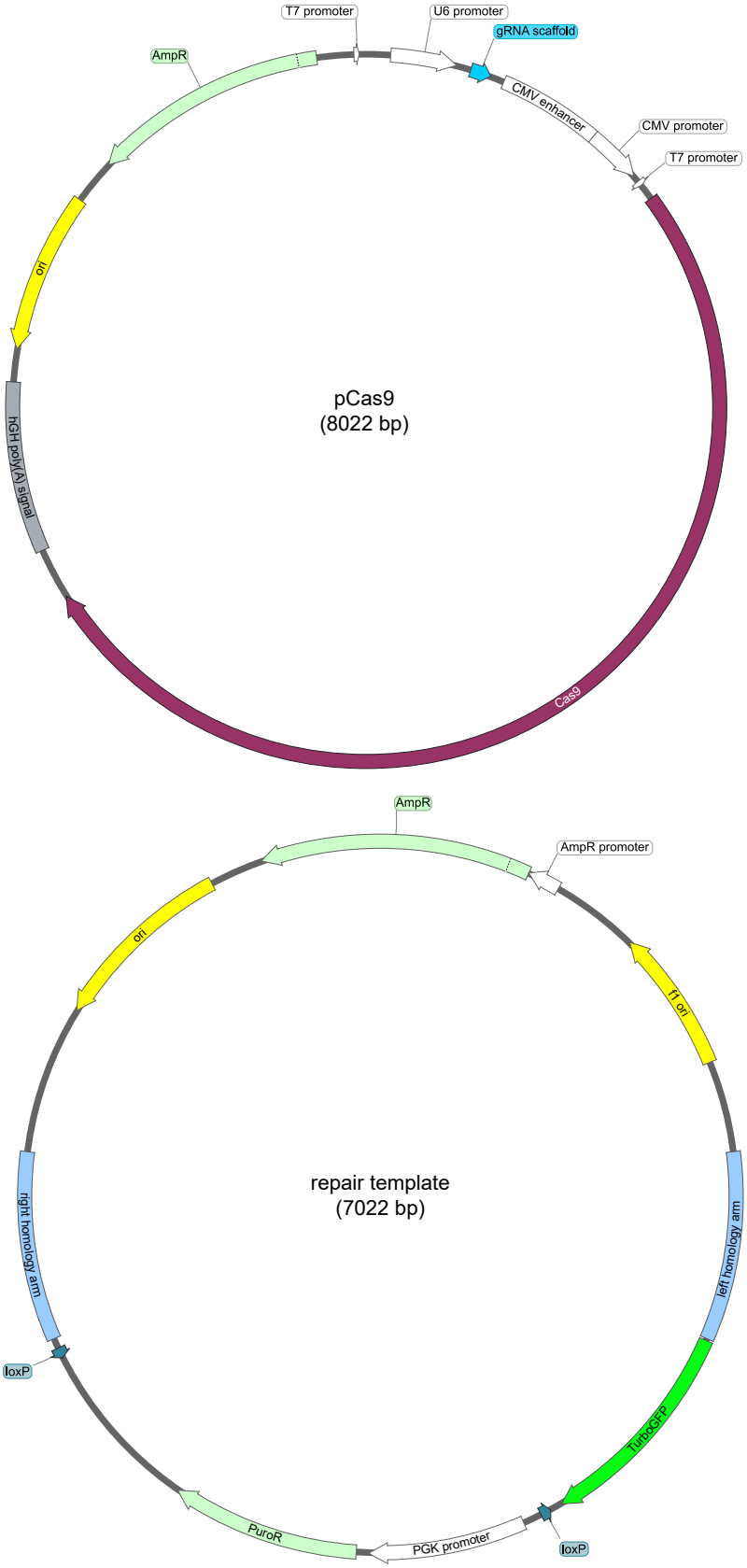

Figure S4. Maps of the pCas9 and repair template plasmids.

Table S1. Primer used in PCR

Primer	Orientation	Localization	Sequence (5' - 3')
# 1	sense	POLD1 NCS[a] upstream LHA	GTGAGAGAGCACACACACGAC
# 2	antisense	repair template, GFP	TAGGTGCCGAAGTGGTAGAAGC
# 3	sense	repair template, spacer region	TCTCTTGATTCCCACTTTGTGGT
# 4	antisense	POLD1 NCS, downstream RHA	CAGATCAACGCTCCAAGCAC
# 5	sense	POLD1, LHA	GAGGTGTCTCCGGTCAGAAC
# 6	antisense	repair template, puromycin resistance	GAGGCCTTCCATCTGTTGCT
# 7	sense	POLD1 NCS, upstream exon 13	CCCAGACCCTGACGACTTGG
# 8	antisense	POLD1 NCS, downstream exon 13	TGGGAGTGGGGAGAAAAAGTG
# 9	sense	POLD1 NCS, upstream exon 17	TGCGTGAATTAGCACAAGGC
# 10	antisense	POLD1 NCS, downstream exon 17	GGACCAATTGCTCAAGCCAC
# 11	sense	POLD1 NCS, upstream exon 18	TCCGCATGATTCTCTCCCCG
# 12	antisense	POLD1 NCS, downstream exon 18	GTGGCTAATGCCAACGGGAC
# 13	sense	POLD1, exon 2a	GGGCCTCTGGGATGATGATG
# 14	antisense	POLD1, exon 21	CTGGGAGATATCGATGCGGT
# 15	sense	POLD1, exon 10	GTATCATGGACCCCGACGTG
# 16	antisense	POLD1, exon 14/15b	CGTAGTACCCTTTGAGGGGC
# 17	sense	POLD1, exon 15b	CTGTGTTACACCACGCTCCT
# 18	antisense	POLD1, exon 20	GTGAGGCAGTGACCAGGTTG

[a] NCS: non coding sequence

Table S2. Primer used for sequencing

Primer	Localization	Sequence (5' - 3')
# S1	upstream exon 2a, NCS[a]	TCAGAACCTCCACCAAG
# S2	upstream exon 13, NCS	ACTTCCTTCTCCTGCTC
# S3	upstream exon 17, NCS	TGTGCAGTGCACAGTAC
# S4	upstream exon 18, NCS	GTTCGGACGTCAGATGATC
# S5	upstream exon 13	CTCCTACACGCTCAATG
# S6	upstream exon 17	AGATCCTGGAGAACCTG

[a] NCS: non coding sequence