Figure S4

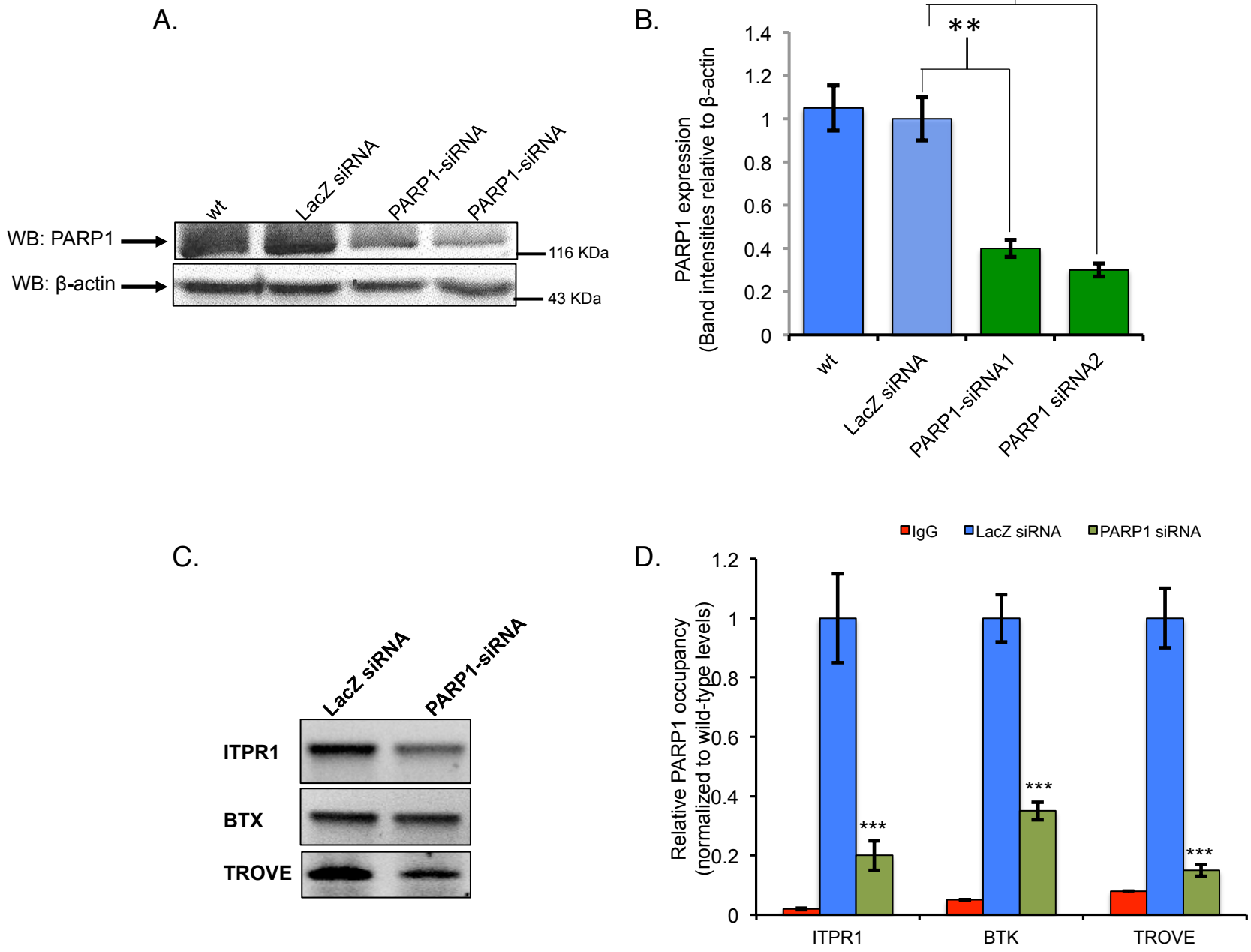
A.
wt
LacZ siRNA
PARP1-siRNA
PARP1-siRNA
WB: PARP1
WB: β-actin
116 KDa
43 KDa
B.
**
**
PARP1 expression
(Band intensities relative to β-actin
1.4
1.2
1
0.8
0.6
0.4
0.2
0
wt
LacZ siRNA
PARP1-siRNA1
PARP1 siRNA2
C.
LacZ siRNA
PARP1-siRNA
ITPR1
BTX
TROVE
D.
IgG
LacZ siRNA
PARP1 siRNA
Relative PARP1 occupancy
(normalized to wild-type levels)
1.2
1
0.8
0.6
0.4
0.2
0
***
***
***
ITPR1
BTK
TROVE

Figure S4: Evaluation of siRNA transfection efficiency in MCF7 cells. The effects of two PARP1 siRNAs - PARP1-siRNA1 and PARP1-siRNA2, targeting different regions of PARP1 gene on the levels of PARP1 were analyzed by western blot (transfection was performed at 50 nmol/L PARP1 siRNA (Dharmacon) for 48 h). Unrelated siRNA (LacZ) served as negative control. **A**. Representative western blot analyses **B**. Band intensities were quantified and measured as the percentage over β−actin, normalized to non-treated cells. **C.** Representative agarose gel images stained with Gelstar® (inverted) of PCR analyses showing the measurement of PARP1 occupancy at PARP1-target genes after PARP1-knockdown. The occupancy of PARP1 at a previously validated promoter (ITPR1 – ref. 29) was used as positive control **D.** PARP1 occupancy from quantitative real-time PCR analyses at the same PARP1-target genes as in C. Data are expressed as the mean ± S.E.M, n = 3. ** $P < 0.001$ compared to the control group by student t-test. (PARP1 occupancy in wild-type cells (treated with LacZ) was normalized to 1 and all other measurements of relative PARP1 occupancy in the various treatments were measured relative to relative PARP1 occupancy in wild-type cells).